

relax

Version 4.0.3



Molecular dynamics by  
NMR data analysis

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# Contents

Preface - citing relax

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# Abbreviations

**AIC:** Akaike's Information Criteria (model selection method)

**AICc:** small sample size corrected AIC (model selection method)

**API:** application programming interface

**ANOVA:** analysis of variance (field of statistics)

**BC:** back calculation

**BIC:** Bayesian Information Criteria (model selection method)

**BFGS:** Broyden-Fletcher-Goldfarb-Shanno (optimisation method)

$C(\tau)$ : correlation function

$\chi^2$ : chi-squared function

**CG:** conjugate gradient (optimisation)

**CPMG:** the Carr-Purcell-Meiboom-Gill pulse sequence

**CR72:** the [Carver and Richards \(1972\)](#) relaxation dispersion model

**CSA:** chemical shift anisotropy

**CV:** cross validation

**CVS:** Concurrent Versions System (open source version control system)

$\mathfrak{D}$ : the set of diffusion tensor parameters

$\mathfrak{D}_{\parallel}$ : the eigenvalue of the spheroid diffusion tensor corresponding to the unique axis of the tensor

$\mathfrak{D}_{\perp}$ : the eigenvalue of the spheroid diffusion tensor corresponding to the two axes perpendicular to the unique axis

$\mathfrak{D}_a$ : the anisotropic component of the Brownian rotational diffusion tensor

$\mathfrak{D}_{iso}$ : the isotropic component of the Brownian rotational diffusion tensor

$\mathfrak{D}_r$ : the rhombic component of the Brownian rotational diffusion tensor

$\mathfrak{D}_{ratio}$ : the ratio of  $\mathfrak{D}_{\parallel}$  to  $\mathfrak{D}_{\perp}$

$\mathfrak{D}_x$ : the eigenvalue of the Brownian rotational diffusion tensor in which the corresponding eigenvector defines the x-axis of the tensor

$\mathfrak{D}_y$ : the eigenvalue of the Brownian rotational diffusion tensor in which the corresponding eigenvector defines the y-axis of the tensor

$\mathfrak{D}_z$ : the eigenvalue of the Brownian rotational diffusion tensor in which the corresponding eigenvector defines the z-axis of the tensor

**DPL94**: the [Davis et al. \(1994\)](#) relaxation dispersion model

**DQ**: double quantum

$\epsilon_i$ : elimination value

**FSF**: Free Software Foundation

**GNU**: GNU's Not Unix!

**GPG**: GNU Privacy Guard (software)

**GPL**: GNU general public licence

**GUI**: graphical user interface

**ID string**: identification string

**IT99**: the [Ishima and Torchia \(1999\)](#) relaxation dispersion model

$J(\omega)$ : spectral density function

**LM63**: the [Luz and Meiboom \(1963\)](#) relaxation dispersion model

**M61**: the [Meiboom \(1961\)](#) relaxation dispersion model

**MC**: Monte Carlo (simulations)

**MD**: molecular dynamics (simulations)

**MMQ**: proton-heteronuclear SQ, ZQ, DQ, and MQ data (multi-multiple quantum)

**MP05**: the [Miloushev and Palmer \(2005\)](#) relaxation dispersion model

**MPI**: message passing interface

**MQ**: multiple quantum

**NMR**: if you do not know this one, do not read further

**NNTP**: network news transfer protocol

**NOE**: nuclear Overhauser effect

**NS**: numeric solution

**ORD**: optical rotatory dispersion

**OS**: operating system

**PCS**: pseudocontact shift

**PDB**: Protein Data Bank

**pdf:** probability distribution function

**PRE:** paramagnetic relaxation enhancement

**$r$ :** bond length

**$R_1$ :** spin-lattice relaxation rate

**$R_2$ :** spin-spin relaxation rate

**$R_{ex}$ :** chemical exchange relaxation rate

**RDC:** residual dipolar coupling

**RMSD:** root-mean-square deviation

**ROE:** rotating-frame Overhauser effect

**RSDM:** reduced spectral density mapping

**RSS:** rich site summary (web feed format)

**$S^2$ ,  $S_f^2$ , and  $S_s^2$ :** model-free generalised order parameters

**SVN:** Apache Subversion (open source version control system)

**$\tau_e$ ,  $\tau_f$ , and  $\tau_s$ :** model-free effective internal correlation times

**$\tau_m$ :** global rotational correlation time

**TP02:** the [Trott and Palmer \(2002\)](#) relaxation dispersion model

**TAP03:** the [Trott et al. \(2003\)](#) relaxation dispersion model

**TSMFK01:** the [Tollinger et al. \(2001\)](#) relaxation dispersion model

**UI:** user interface

**XML:** extensible markup language

**ZQ:** zero quantum



# Preface - citing relax

The relax project is a large collection of work created by diverse authors. It is a community driven project created by NMR spectroscopists which supports a broad range of dynamics analyses. Care must be taken to properly cite the parts of relax that you use so that the correct authors receive the citations and credit they deserve. The following is a breakdown of all of the citations relating to relax, including the basic citations for the various analysis types. Including a link to the relax website <http://www.nmr-relax.com> in publications and other forums would also be greatly appreciated.

## The software relax

### relax references

The primary citations for relax are:

- d’Auvergne, E. J. and Gooley, P. R. (2008b). Optimisation of NMR dynamic models I. Minimisation algorithms and their performance within the model-free and Brownian rotational diffusion spaces. *J. Biomol. NMR*, **40**(2), 107–119. ([10.1007/s10858-007-9214-2](https://doi.org/10.1007/s10858-007-9214-2))
- d’Auvergne, E. J. and Gooley, P. R. (2008c). Optimisation of NMR dynamic models II. A new methodology for the dual optimisation of the model-free parameters and the Brownian rotational diffusion tensor. *J. Biomol. NMR*, **40**(2), 121–133. ([10.1007/s10858-007-9213-3](https://doi.org/10.1007/s10858-007-9213-3))

If space is at a premium, the standard rules for concatenating back-to-back papers can be used:

- d’Auvergne, E. J. and Gooley, P. R. (2008a). Optimisation of NMR dynamic models. *J. Biomol. NMR*, **40**(2), 107–133

### Graphical user interface reference

The primary citation for the GUI is:

- Bieri, M., d’Auvergne, E., and Gooley, P. (2011). relaxGUI: a new software for fast and simple NMR relaxation data analysis and calculation of ps-ns and  $\mu$ s motion of proteins. *J. Biomol. NMR*, **50**, 147–155. ([10.1007/s10858-011-9509-1](https://doi.org/10.1007/s10858-011-9509-1))

## The multi-processor reference

Although not published, if the multi-processor framework is used to run relax on multi-core systems, grids, or clusters, then please acknowledge the author of that code – Gary Thompson.

## Specific analyses

The following subsections list the citations for the individual analysis specific parts of relax.

### Model-free analysis references

If the automated analysis of the `dauvergne_protocol.py` sample script or the GUI model-free analysis which uses the same protocol has been used, then the following citations are all implicit:

- d’Auvergne, E. J. and Gooley, P. R. (2003). The use of model selection in the model-free analysis of protein dynamics. *J. Biomol. NMR*, **25**(1), 25–39. ([10.1023/a:1021902006114](https://doi.org/10.1023/a:1021902006114))
- d’Auvergne, E. J. and Gooley, P. R. (2006). Model-free model elimination: A new step in the model-free dynamic analysis of NMR relaxation data. *J. Biomol. NMR*, **35**(2), 117–135. ([10.1007/s10858-006-9007-z](https://doi.org/10.1007/s10858-006-9007-z))
- d’Auvergne, E. J. and Gooley, P. R. (2007). Set theory formulation of the model-free problem and the diffusion seeded model-free paradigm. *Mol. BioSyst.*, **3**(7), 483–494. ([10.1039/b702202f](https://doi.org/10.1039/b702202f))
- d’Auvergne, E. J. and Gooley, P. R. (2008b). Optimisation of NMR dynamic models I. Minimisation algorithms and their performance within the model-free and Brownian rotational diffusion spaces. *J. Biomol. NMR*, **40**(2), 107–119. ([10.1007/s10858-007-9214-2](https://doi.org/10.1007/s10858-007-9214-2))
- d’Auvergne, E. J. and Gooley, P. R. (2008c). Optimisation of NMR dynamic models II. A new methodology for the dual optimisation of the model-free parameters and the Brownian rotational diffusion tensor. *J. Biomol. NMR*, **40**(2), 121–133. ([10.1007/s10858-007-9213-3](https://doi.org/10.1007/s10858-007-9213-3))

Otherwise, if model-free analysis is used in relax but not via the inbuilt automated protocol, the first reference is for model selection, the second is for eliminating failed model-free models, and the forth is for the optimisation improvements (the third and fifth are for the automated protocol). All of the model-free implementation details of relax are covered by the PhD thesis (available as a PDF or as a printed version on Amazon.com) of:



- d’Auvergne, E. J. (2006). *Protein dynamics: a study of the model-free analysis of NMR relaxation data*. PhD thesis, Biochemistry and Molecular Biology, University of Melbourne. <http://eprints.infodiv.unimelb.edu.au/archive/00002799/>. ([10187/2281](#))

The reference for the hybridisation of different global diffusion models to analyse the residual inter-domain dynamics – a not very well documented feature of relax – is:

- Horne, J., d’Auvergne, E. J., Coles, M., Velkov, T., Chin, Y., Charman, W. N., Prankerd, R., Gooley, P. R., and Scanlon, M. J. (2007). Probing the flexibility of the DsbA oxidoreductase from *Vibrio cholerae*—a  $^{15}\text{N}$  -  $^1\text{H}$  heteronuclear NMR relaxation analysis of oxidized and reduced forms of DsbA. *J. Mol. Biol.*, **371**(3), 703–716. ([10.1016/j.jmb.2007.05.067](#))

The base citations for model-free theory are [Lipari and Szabo \(1982a,b\)](#); [Clore et al. \(1990\)](#).

### Consistency testing analysis references

The first is the main citation, whereas the next are the individual tests. The citation for the consistency testing of NMR relaxation as implemented in relax is:

- Morin, S. and Gagné, S. (2009a). Simple tests for the validation of multiple field spin relaxation data. *J. Biomol. NMR*, **45**, 361–372. ([10.1007/s10858-009-9381-4](#))

The base citations for the consistency testing of NMR relaxation are [Fushman et al. \(1999\)](#); [Farrow et al. \(1995\)](#); [Fushman et al. \(1998\)](#)

### N-state model analysis references

Some citations demonstrating as well as presenting the use of the N-state model for diverse analyses types are:

- Sun, H., d’Auvergne, E. J., Reinscheid, U. M., Dias, L. C., Andrade, C. K. Z., Rocha, R. O., and Griesinger, C. (2011). Bijvoet in solution reveals unexpected stereoselectivity in a michael addition. *Chem. Eur. J.*, **17**(6), 1811–1817. ([10.1002/chem.201002520](#))
- Erdelyi, M., d’Auvergne, E., Navarro-Vazquez, A., Leonov, A., and Griesinger, C. (2011). Dynamics of the glycosidic bond: Conformational space of lactose. *Chem. Eur. J.*, **17**(34), 9368–9376. ([10.1002/chem.201100854](#))

### Reduced spectral density mapping references

The base citations for reduced spectral density mapping are [Farrow et al. \(1995\)](#); [Lefevre et al. \(1996\)](#).

## Relaxation dispersion references

For the base citations for relaxation dispersion, please see chapter 11 on page 145 for a listing of the individual models. The main citation is:

- Morin, S., Linnet, T. E., Lescanne, M., Schanda, P., Thompson, G. S., Tollinger, M., Teilum, K., Gagne, S., Marion, D., Griesinger, C., Blackledge, M., and d’Auvergne, E. J. (2014). relax: the analysis of biomolecular kinetics and thermodynamics using NMR relaxation dispersion data. *Bioinformatics*, **30**(15), 2219–2220. ([10.1093/bioinformatics/btu166](https://doi.org/10.1093/bioinformatics/btu166))

## Generic parts of relax

The following subsections will list the citations for the parts of relax independent of the specific analyses.

## Model selection references

The citation for the model selection component of relax is:

- d’Auvergne, E. J. and Gooley, P. R. (2003). The use of model selection in the model-free analysis of protein dynamics. *J. Biomol. NMR*, **25**(1), 25–39. ([10.1023/a:1021902006114](https://doi.org/10.1023/a:1021902006114))

The base citations for the specific model selection techniques of AIC, AICc, and BIC are respectively [Akaike \(1973\)](#); [Hurvich and Tsai \(1989\)](#); [Schwarz \(1978\)](#)

- Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In: Petrov, B. N. and Csaki, F. (eds.): *Proceedings of the Second International Symposium on Information Theory*. Budapest, pages 267–281, Akademia Kiado
- Hurvich, C. M. and Tsai, C. L. (1989). Regression and time-series model selection in small samples. *Biometrika*, **76**(2), 297–307. ([10.1093/biomet/76.2.297](https://doi.org/10.1093/biomet/76.2.297))
- Schwarz, G. (1978). Estimating dimension of a model. *Ann. Stat.*, **6**(2), 461–464. ([10.1214/aos/1176344136](https://doi.org/10.1214/aos/1176344136))

## Other citations

If you believe that other citations should be included in this chapter, please contact the relax users mailing list ([relax-users at gna.org](mailto:relax-users@gn.org)).

# Part I

## The basics



# Chapter 1

## Introduction

The program relax is designed for the study of molecular dynamics through the analysis of experimental NMR data. Organic molecules, proteins, RNA, DNA, sugars, and other biomolecules are all supported. It was originally written for the model-free analysis of protein dynamics, though its scope has been significantly expanded. It is a community driven project created by NMR spectroscopists for NMR spectroscopists. It supports many analysis types including:

**Model-free analysis** - the Lipari and Szabo model-free analysis of NMR relaxation data

**$R_1$  and  $R_2$**  - the exponential curve fitting for the calculation of the  $R_x$  NMR relaxation rates.

**NOE** - the calculation of the steady-state NOE NMR relaxation data.

**Data consistency** - the consistency testing of multiple field NMR relaxation data.

**RSDM** - Reduced Spectral Density Mapping.

**Frame order and N-state model** - study of domain motions via the N-state model and frame order dynamics theories using anisotropic NMR parameters such as RDCs and PCSs.

**Stereochemistry** - investigations of absolute stereochemistry of flexible molecules.

**Relaxation dispersion** - the study of processes on the chemical exchange timescale.

The aim of relax is to provide a seamless and extremely flexible environment able to accept input in any format produced by other NMR software, able to faultlessly create input files, control, and read output from various programs including Modelfree and Dasha, output results in many formats, and visualise the data by controlling programs such as Grace, OpenDX, MOLMOL, and PyMOL. All data analysis tools from optimisation to model selection to Monte Carlo simulations are inbuilt into relax. Therefore the use of additional programs is optional.

The flexibility of relax arises from the choice of relax's scripting capabilities, its Python prompt interface, or its graphical user interface (GUI). Extremely complex scripts can be

created from simple building blocks to fully automate data analysis. A number of sample scripts have been provided to help understand script construction. In addition, any of Python's powerful features or functions can be incorporated as the script is executed as an arbitrary Python source file within relax's environment. The modules of relax can also be used as a vast library of dynamics related functions by your own software.

relax is free software (free as in freedom) which is licenced under the GNU General Public Licence (GPL). You are free to copy, modify, or redistribute relax under the terms of the GPL.

## 1.1 Program features

### 1.1.1 Literature

The primary references for the program relax are [d'Auvergne and Gooley \(2008b\)](#) and [d'Auvergne and Gooley \(2008c\)](#). To properly cite the various parts of relax used in your analysis, please see Chapter on page [xxvii](#).

### 1.1.2 Supported NMR theories

The following relaxation data analysis techniques are currently supported by relax:

- Model-free analysis ([Lipari and Szabo \(1982a,b\)](#); [Clore et al. \(1990\)](#) and the specific implementation of [d'Auvergne and Gooley \(2003, 2006, 2007, 2008b,c\)](#)). This includes the hybridisation of global diffusion models to study residual domain dynamics ([Horne et al., 2007](#)).
- Reduced spectral density mapping ([Farrow et al., 1995](#); [Lefevre et al., 1996](#)).
- Consistency testing – the validation of multiple field NMR relaxation data ([Morin and Gagné, 2009a](#); [Fushman et al., 1999](#)).
- Exponential curve fitting (to find the  $R_1$  and  $R_2$  relaxation rates).
- Steady-state NOE calculation.
- Determination of absolute stereochemistry of flexible molecules via the N-state model using isotropic and anisotropic NMR parameters such as NOE, ROE, and RDC combined with MD simulation or simulated annealing, and ORD ([Sun et al., 2011](#)).
- The N-state model for investigating domain motions.
- The frame order theory.
- Conformational analysis of paramagnetically tagged molecules via the N-state model ([Erdelyi et al., 2011](#)).
- Analysis and comparison of ensembles of structures using RDCs, PCSs, NOEs, etc. (the N-state model of dynamics).
- The analysis of relaxation dispersion.

## The future

Because relax is free software, if you would like to contribute addition features, functions, or modules which you have written for your own publications for the benefit of the field, almost anything relating to molecular dynamics may be accepted. Please see the Open Source chapter on page 29 for more details.

### 1.1.3 Data analysis tools

The following tools are implemented as modular components to be used by any data analysis technique:

- Numerous high-precision optimisation algorithms.
- Model selection (d’Auvergne and Gooley, 2003; Chen et al., 2004):
  - Akaike’s Information Criteria (AIC).
  - Small sample size corrected AIC (AICc).
  - Bayesian or Schwarz Information Criteria (BIC).
  - Bootstrap model selection.
  - Single-item-out cross-validation (CV).
  - Hypothesis testing ANOVA model selection (only the model-free specific technique of Mandel et al. (1995) is supported).
- Monte Carlo simulations (error analysis for all data analysis techniques).
- Model elimination – the removal of failed models prior to model selection (d’Auvergne and Gooley, 2006).

### 1.1.4 Data visualisation

The results of an analysis, or any data input into relax, can be visualised using a number of programs:

**MOLMOL** 1D data can be mapped onto a structure either by the creation of MOLMOL macros or by direct control of the program.

**PyMOL** 3D objects such as the diffusion tensor representation can be displayed with the structure.

**Grace** any 2D data can be plotted.

**OpenDX** The chi-squared space of models with three parameters can be mapped and 3D images of the space produced.

### 1.1.5 Interfacing with other programs

relax can create the input files, execute in-line, and then read the output of the following programs. These programs can be used as optimisation engines replacing the minimisation algorithms built into relax:

- Dasha (model-free analysis).
- Modelfree (model-free analysis).

### 1.1.6 The user interfaces (UI)

relax can be used through the following UIs:

**The prompt** this is the primary interface of relax. Rather than reinventing a new command language, relax’s interface is the powerful Python prompt. This gives the power user full access to a proven programming language. See Figure 1.1 for a screenshot.

**Scripting** this provides a more powerful and flexible framework for controlling the program. The script will be executed as Python code enabling advanced programming for automating data analysis. All the features available within the prompt environment are accessible to the script. See Figure 1.2 for a screenshot.

**GUI** the graphical user interface provides a sub-set of relax’s features - the automatic  $R_1$  and  $R_2$  relaxation rate curve-fitting, the NOE calculations, and the automatic model-free analysis provided by the `dauvergne_protocol` module (d’Auvergne and Gooley, 2008c). See Figure 1.3 for a screenshot.

## 1.2 How to use relax

### 1.2.1 The prompt

The primary interface of relax is the prompt. After typing “relax” within a terminal you will be presented with

```
relax>
```

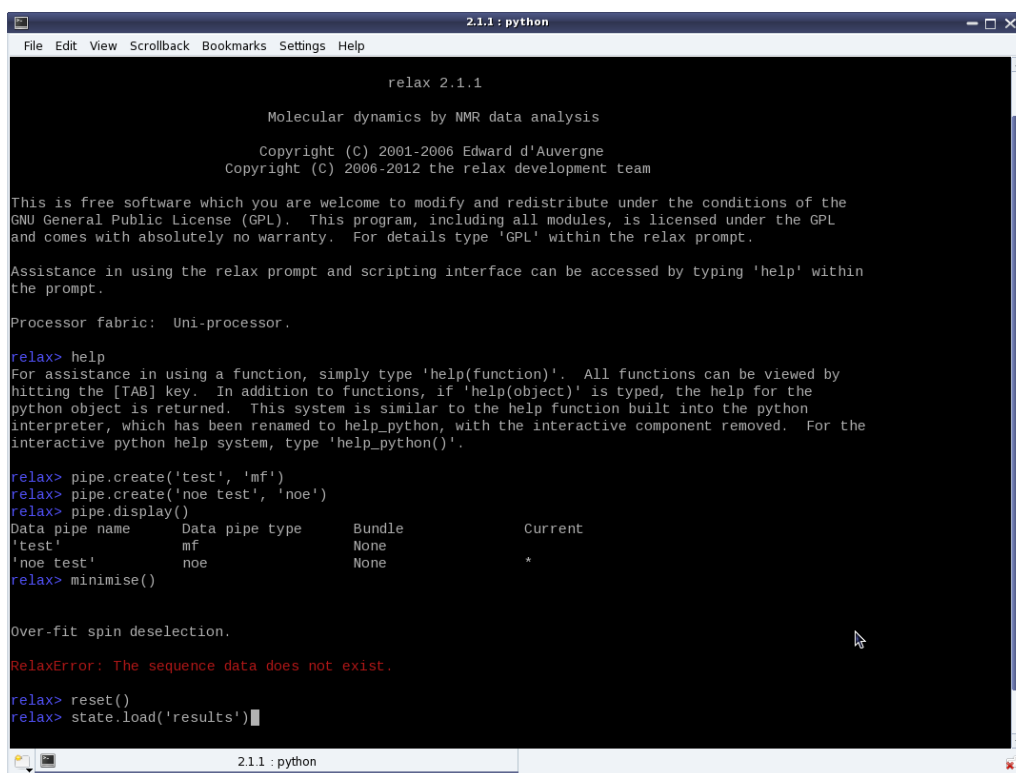
This is the Python prompt which has been tailored specifically for relax. You will hence have full access, if desired, to the power of the Python programming language to manipulate your data. You can for instance type

```
relax> print("Hello World")
```

the result being

```
relax> print("Hello World")
Hello World
relax>
```





```

2.1.1: python
File Edit View Scrollback Bookmarks Settings Help

relax 2.1.1

Molecular dynamics by NMR data analysis

Copyright (C) 2001-2006 Edward d'Auvergne
Copyright (C) 2006-2012 the relax development team

This is free software which you are welcome to modify and redistribute under the conditions of the
GNU General Public License (GPL). This program, including all modules, is licensed under the GPL
and comes with absolutely no warranty. For details type 'GPL' within the relax prompt.

Assistance in using the relax prompt and scripting interface can be accessed by typing 'help' within
the prompt.

Processor fabric: Uni-processor.

relax> help
For assistance in using a function, simply type 'help(function)'. All functions can be viewed by
hitting the [TAB] key. In addition to functions, if 'help(object)' is typed, the help for the
python object is returned. This system is similar to the help function built into the python
interpreter, which has been renamed to help_python, with the interactive component removed. For the
interactive python help system, type 'help_python()'.

relax> pipe.create('test', 'mf')
relax> pipe.create('noe test', 'noe')
relax> pipe.display()
Data pipe name      Data pipe type      Bundle      Current
'test'              mf                  None
'noe test'          noe                 None        *
relax> minimise()

Over-fit spin deselection.

RelaxError: The sequence data does not exist.

relax> reset()
relax> state.load('results')

```

Figure 1.1: A screenshot of relax being run in the primary prompt mode.

Or using relax as a calculator

```

relax> (1.0 + (2 * 3))/10
0.69999999999999996
relax>

```

## 1.2.2 Python

relax has been designed such that knowledge about Python is not required to be able to fully use the program. A few basics though will aid in understanding relax.

A number of simple programming axioms includes that of strings, integers, floating point numbers, and lists. A string is text and within Python (as well as relax) this is delimited by either single or double quotes. An integer is a number with no decimal point whereas a float is a number with a decimal point. A list in Python (called an array in other languages) is a list of anything separated by commas and delimited by square brackets, an example is `[0, 1, 2, 'a', 1.2143235]`.

Probably the most important detail is that functions in Python require brackets around their arguments. For example

```
relax> minimise.execute()
```

will commence minimisation however

```
relax> minimise.execute
```

```

1.3.15: python
[edward@localhost 1.3.15]$ ./relax simple_script.py

relax 1.3.15

Molecular dynamics by NMR data analysis

Copyright (C) 2001-2006 Edward d'Auvergne
Copyright (C) 2006-2012 the relax development team

This is free software which you are welcome to modify and redistribute under the conditions of the
GNU General Public License (GPL). This program, including all modules, is licensed under the GPL
and comes with absolutely no warranty. For details type 'GPL' within the relax prompt.

Assistance in using the relax prompt and scripting interface can be accessed by typing 'help' within
the prompt.

Processor fabric: Uni-processor.

script = 'simple_script.py'
-----
pipe.create(pipe_name='Dy test', pipe_type='N-state')
align_tensor.init(tensor='Dysprosium', params=(1.0278e-03, -1.4860e-03, 8.4778e-04, 5.7108e-04, 3.6500e-04), param_types=1)
rdc.calc_q_factors()
align_tensor.delete()
align_tensor.display()
-----

relax> pipe.create(pipe_name='Dy test', pipe_type='N-state')
relax> align_tensor.init(tensor='Dysprosium', params=(0.0010278, -0.001486, 0.00084778, 0.00057108, 0.000365), scale=1.0, angle_units='deg',
param_types=1, errors=False)
relax> rdc.calc_q_factors(spin_id=None)
RelaxWarning: No RDC data exists, Q factors cannot be calculated.
relax> align_tensor.delete(tensor=None)
Removing the 'Dysprosium' tensor.
relax> align_tensor.display(tensor=None)
RelaxError: No alignment tensor data exists.

[edward@localhost 1.3.15]$

```

Figure 1.2: A screenshot of relax being run in scripting mode.

will do nothing.

The arguments to a function are simply a comma separated list within the brackets of the function. For example to save the program’s current state type

```
relax> state.save('save', force=True)
```

Two types of arguments exist in Python – standard arguments and keyword arguments. The majority of arguments you will encounter within relax are keyword arguments however you may, in rare cases, encounter a non-keyword argument. For these standard arguments just type the values in, although they must be in the correct order. Keyword arguments consist of two parts – the key and the value. For example the key may be `file` and the value you would like to supply is “R1.out”. Various methods exist for supplying this argument. Firstly you could simply type “R1.out” into the correct position in the argument list. Secondly you can type `file='R1.out'`. The power of this second option is that argument order is unimportant. Therefore if you would like to change the default value of the very last argument, you don’t have to supply values for all other arguments. The only catch is that standard arguments must come before the keyword arguments.

### 1.2.3 User functions

For standard data analysis a large number of specially tailored functions called “user functions” have been implemented. These are accessible from the relax prompt by simply typing the name of the function. An example is `help()`. An alphabetical listing of all accessible user functions together with full descriptions is presented later in this manual.

A few special objects which are available within the prompt are not actually functions. These objects do not require brackets at their end for them to function. For example to exit relax type

```
relax> exit
```

Another special object is that of the function class. This object is simply a container which holds a number of user functions. You can access the user function within the class by typing the name of the class, then a dot “.”, followed by the name of the user function. An example is the user function for reading relaxation data out of a file and loading the data into relax. The function is called “read” and the class is called “**relax\_data**”. To execute the function, type something like

```
relax> relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',  
    res_num_col=1, data_col=3, error_col=4)
```

On first usage the relax prompt can be quite daunting. Two features exist to increase the usability of the prompt – the help system and tab completion.

### 1.2.4 The help system

For assistance in using a function simply type

```
relax> help(function)
```

In addition to functions if

```
relax> help(object)
```

is typed the help for the python object is returned. This system is similar to the help function built into the python interpreter, which has been renamed to **help\_python**, with the interactive component removed. For the standard interactive python help system type

```
relax> help_python()
```

### 1.2.5 Tab completion

Tab completion is implemented to prevent insanity as the function names can be quite long – a deliberate feature to improve usability. The behaviour of the tab completion is very similar to that of the bash prompt.

Not only is tab completion useful for preventing RSI but it can also be used for listing all available functions. To begin with if you hit the [TAB] key without typing any text all available functions will be listed (along with function classes and other python objects). This extends to the exploration of user functions within a function class. For example to list the user functions within the function class **model\_free** type

```
relax> model_free.
```

The dot character at the end is essential. After hitting the [TAB] key you should see something like

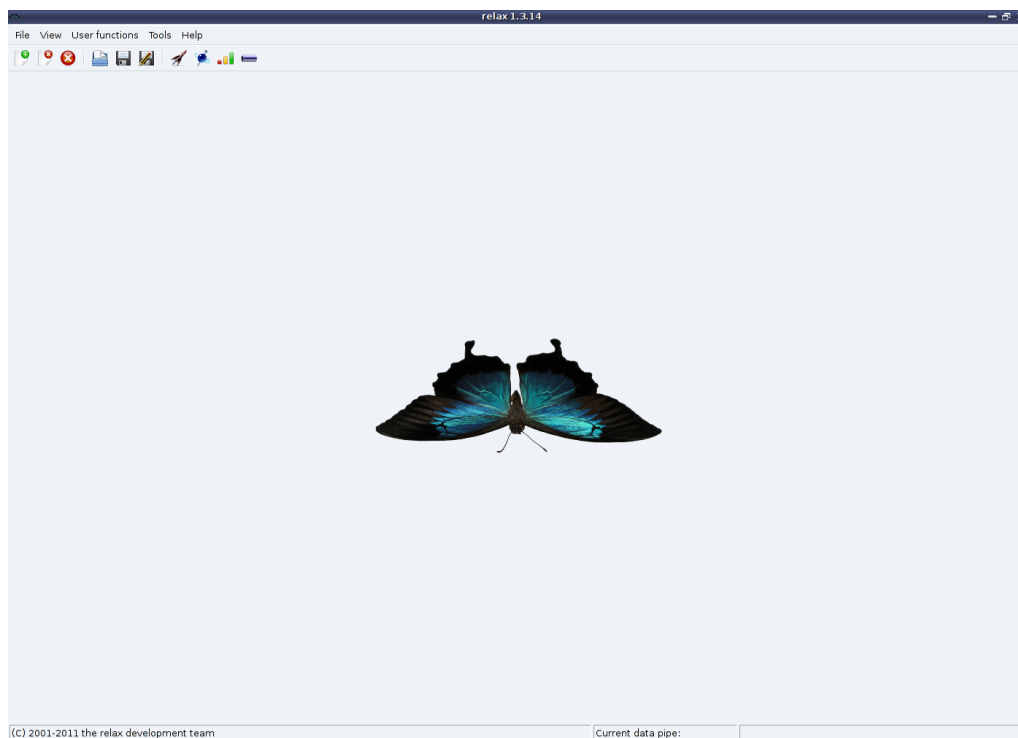


Figure 1.3: Screenshot of the relax GUI interface – the starting interface. To start one of the automated analyses, either the menu “File→New analysis” or the new analysis button in the toolbar should be selected.

```
relax| model_free.
model_free.__class__
model_free.__doc__
model_free.__init__
model_free.__module__
model_free.__relax__
model_free.__relax_help__
model_free.create_model
model_free.delete
model_free.remove_tm
model_free.select_model
relax> model_free.
```

All the objects beginning with an underscore are “hidden”, they contain information about the function class and should be ignored. From the listing the user functions `copy`, `create_model`, `delete`, `remove_tm`, and `select_model` contained within `model_free` are all visible.

### 1.2.6 The data pipe

Within relax all user functions operate on data stored within the current data pipe. This pipe stores data is input, processed, or output as user functions are called. There are different types of data pipe for different analyses, e.g. a reduced spectral density mapping pipe, a model-free pipe, an exponential curve-fitting pipe, etc. Multiple data pipes can be created within relax and various operations performed in sequence on these pipes. This is useful for operations such as model selection whereby the function `model_selection` can

operate on a number of pipes corresponding to different models and then assign the results to a newly created pipe. When running relax you choose which pipe you are currently in by using the `pipe.switch` user function to jump between pipes.

The flow of data through relax can be thought of as travelling through these pipes. User functions exist to transfer data between these pipes and other functions combine data from multiple pipes into one or vice versa. The simplest invocation of relax would be the creation of a single data pipe and with the data being processed as it is passing through this pipe.

The primary method for creating a data pipe is through the user function `pipe.create`. For example

```
relax> pipe.create('m1', 'mf')
```

will create a model-free data pipe labelled “m1”. The following is a table of all the types which can be assigned to a data pipe.

Data pipe type	Description
“ct”	Consistency testing of relaxation data
“frame order”	The Frame Order analyses of domain motions
“jw”	Reduced spectral density mapping
“hybrid”	A special hybridised data pipe
“mf”	Model-free data analysis
“N-state”	N-state model of domain motions
“noe”	Steady state NOE calculation
“relax_fit”	Relaxation curve-fitting

### 1.2.7 The spin and interatomic data containers

Any data which is not considered global for the molecule, such as diffusion tensors, alignment tensors, global minimisation statistics, etc., are stored within two special structures of the data pipes. Any NMR data or information which is specific to an isolated spin system is stored within special spin containers. This includes for example relaxation data, CSA information, nuclear isotope type, chemical element type, model-free parameters, reduced spectral density mapping values, spin specific minimisation statistics and PCS data. NMR data or information which is defined as being between two spin systems, such as the magnetic dipole-dipole interaction involved in both NMR relaxation and RDC data, interatomic vectors and NOESY data, is stored within the interatomic data containers. The spin and interatomic data containers and their associated data can be manipulated using a multitude of the relax user functions.

### 1.2.8 Scripting

What ever is done within the prompt is also accessible through scripting (Figure 1.2). First type your commands into a text file ending in `*.py`. To use this mode of relax, you will need to open up a terminal in your respective operating system:

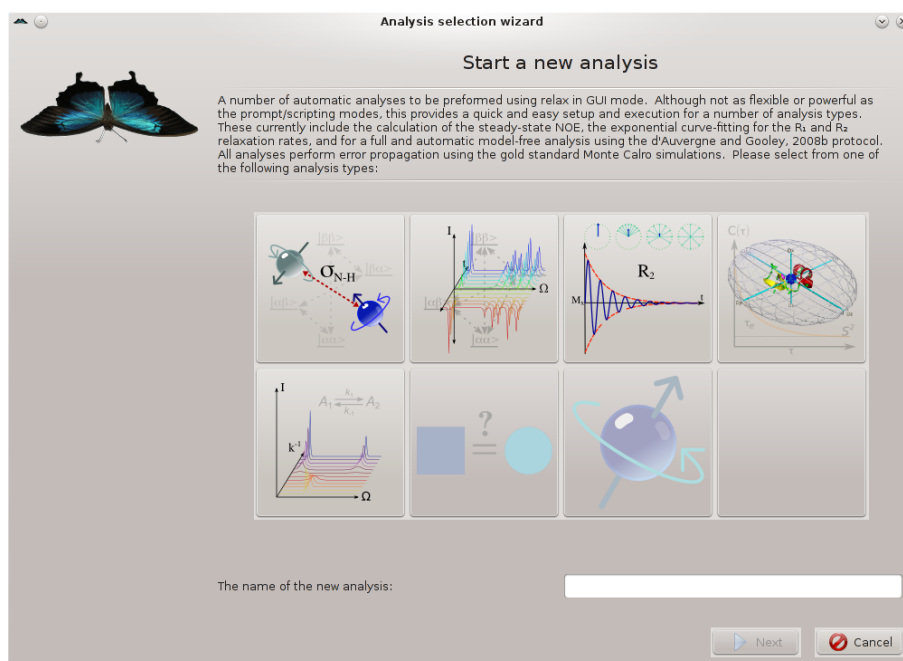


Figure 1.4: Screenshot of the relax GUI interface – the analysis selection wizard. From here, the steady-state NOE analysis, the  $R_1$  and  $R_2$  relaxation rates via exponential curve-fitting, and the automated model-free analysis can be selected.

**GNU/Linux:** Here you have an incredible number of choices. If you don't have a preferred shell already, you could try one of **Konsole**, **GNOME Terminal** or even **XTerm** if you are a masochist.

**Mac OS X:** This is as simple as in GNU/Linux – just launch **Terminal.app** from the **Utilities** folder.

**MS Windows:** If your system supports it, you should install and use **Windows PowerShell**. The alternative is the nasty **cmd** command line terminal program which comes installed by default on all Windows versions. The **PowerShell**, although no where near as powerful as the Linux and Mac terminals, is a huge improvement on the ancient **cmd** program and will make relax much better to use on MS Windows.

Once your terminal is running, go to the directory containing your script using the **cd** command (if you do not know what this is, please see the documentation for your terminal program to understand some of its basic usage). Once you are in the correct directory, within the terminal type:

```
$ relax your_script.py
```

You will need to replace **your\_script.py** with the name of your script. In most cases you would probably like to keep a log of all of the messages, warnings and errors relax produces for future reference. To active logging within relax, type:

```
$ relax --log log your_script.py
```

This will place all output (both **STDOUT** and **STDERR**) into the **log** file (you can choose any name for this log file). Alternatively you can both log the output and simultaneously see the messages in your terminal by typing:

```
$ relax --tee log your_script.py
```

These command line arguments could be replaced by IO redirection if this is a familiar concept to you, but note that these arguments are active also in the GUI mode whereby IO redirection in the terminal will have no effect. An example of a simple script which will minimise the model-free model “m4” after loading six relaxation data sets is

```
1 # Create the data pipe.
2 name = 'm4'
3 pipe.create(name, 'mf')
4
5 # Load the PDB file.
6 structure.read_pdb('1f3y.pdb')
7
8 # Set up the 15N and 1H spins.
9 structure.load_spins('@N', ave_pos=True)
10 structure.load_spins('@H', ave_pos=True)
11 spin.isotope('15N', spin_id='@N')
12 spin.isotope('1H', spin_id='@H')
13
14 # Load the relaxation data.
15 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
16                 res_num_col=1, data_col=3, error_col=4)
17 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
18                 res_num_col=1, data_col=3, error_col=4)
19 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
20                 res_num_col=1, data_col=3, error_col=4)
21 relax_data.read(ri_id='R1_500', ri_type='R1', frq=500.0*1e6, file='r1.500.out',
22                 res_num_col=1, data_col=3, error_col=4)
23 relax_data.read(ri_id='R2_500', ri_type='R2', frq=500.0*1e6, file='r2.500.out',
24                 res_num_col=1, data_col=3, error_col=4)
25 relax_data.read(ri_id='NOE_500', ri_type='NOE', frq=500.0*1e6, file='noe.500.out',
26                 res_num_col=1, data_col=3, error_col=4)
27
28 # Initialise the diffusion tensor.
29 diffusion_tensor.init((2e-8, 1.3, 60, 290), spheroid_type='prolate', param_types=2, fixed=
30                       True)
31
32 # Create all attached protons.
33 sequence.attach_protons()
34
35 # Define the magnetic dipole-dipole relaxation interaction.
36 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
37 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)
38 interatom.unit_vectors()
39
40 # Define the CSA relaxation interaction.
41 value.set(-172 * 1e-6, 'csa')
42
43 # Select a preset model-free model.
44 model_free.select_model(model=name)
45
46 # Grid search.
47 minimise.grid_search(inc=11)
48
49 # Minimise.
50 minimise.execute('newton')
51
52 # Finish.
53 results.write(file='results', force=True)
```

```
47 state.save('save', force=True)
```

Scripting is much more powerful than the prompt as advanced Python programming can be employed (see the file `relax_curve_diff.py` in the `sample_scripts` directory for an example).

## Sample scripts

A few sample scripts have been provided in the directory `sample_scripts`. These can be copied and modified for different types of data analysis.

### 1.2.9 The test suite

To test that the program functions correctly, `relax` possesses an inbuilt test suite. The suite is a collection of simple tests which execute or probe different parts of the program checking that the software runs without problem. The test suite is executed by running `relax` using the command

```
$ relax --test-suite
```

Alternatively the three components of the test suite – system tests, unit tests, and GUI tests – can be run separately with

```
$ relax --system-tests
```

```
$ relax --unit-tests
```

```
$ relax --gui-tests
```

### 1.2.10 The GUI

If the `wxPython` module is installed on your system, you will have access to the GUI interface of `relax`. To launch `relax` in GUI mode, type either

```
$ relax -g
```

or

```
$ relax --gui
```

In most cases you will probably like to have a permanent copy of all the messages, warnings, and errors `relax` produces for future reference. In such a case you could run the GUI with:

```
$ relax --gui --log log
```

This will place all of the output into the `log` file.

The GUI is currently an interface to the automated analyses, providing an easy way to perform quick analyses. The interface consists of a tab for each analysis. By clicking on the “File→New analysis” menu entry or the “New analysis” toolbar button, the analysis wizard will appear (see Figure 1.4). The following analyses can be set up using this wizard:



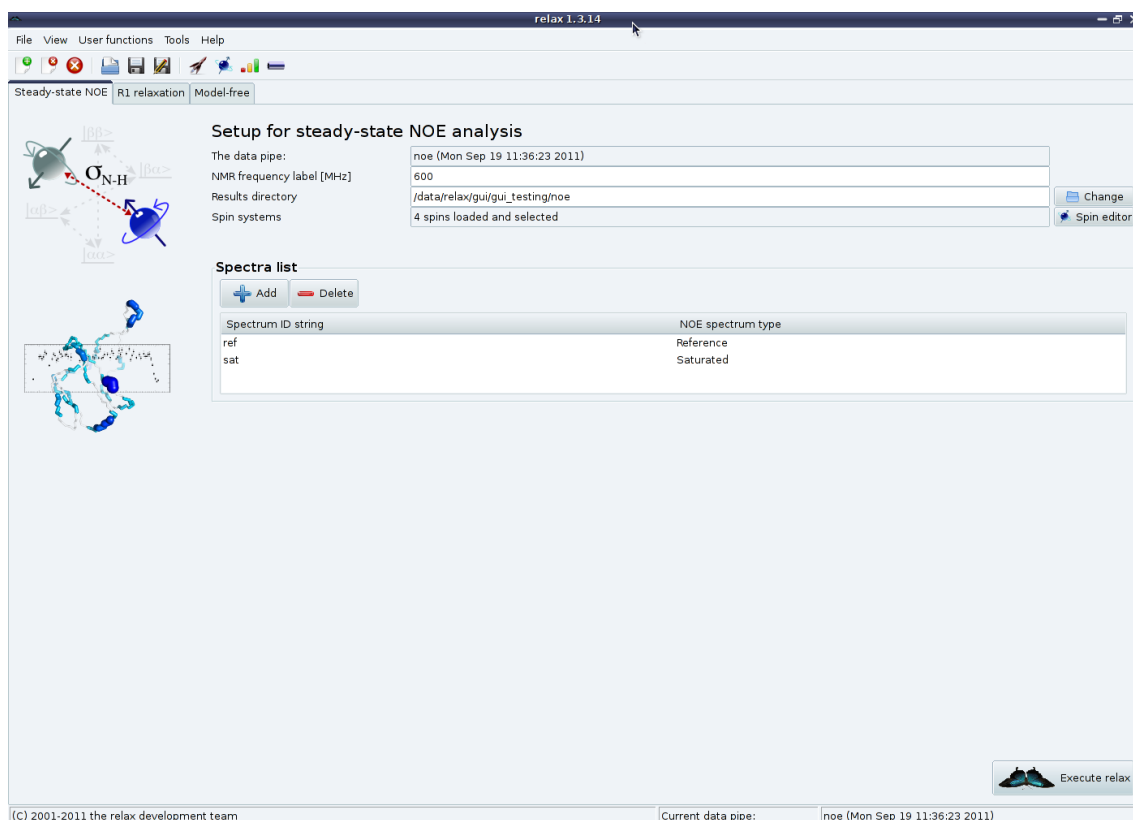


Figure 1.5: Screenshot of the relax GUI interface – the steady-state NOE analysis.

**Steady-state NOE:** this provides access to the steady-state NOE calculation with pseudo Monte Carlo simulations for error analysis (this falls back to bootstrapping as this is a calculation rather than optimisation). See Figure 1.5 on page 15.

**$R_1$  and  $R_2$  :** these provide easy access to optimisations and error analysis for the  $R_1$  and  $R_2$  relaxation rates via exponential curve-fitting (see Figures 1.6 and 1.7 on pages 16 and 17).

**Model-free analysis :** A fully automatic model-free protocol is provided in another tab. This operates via the `dauvergne_protocol` module which implements the protocol of d’Auvergne and Gooley (2008c) (see Figure 1.8 on page 18).

A number of windows in the GUI provide user feedback or allow for the viewing and editing of data. These include:

**The relax controller :** This window shows the progress of relax’s execution and displays relax’s text output for checking if the analysis has been performed correctly and has completed successfully (see Figure 1.9).

**Spin viewer window :** This is used to load spins system information into the relax data store and to see the contents of the spin containers (see Figure 1.10).

**Results viewer window :** This presents a list of the results files which can be opened by double clicking for visualisation using a text editor, Grace, PyMOL, MOLMOL, etc (see Figure 1.11).

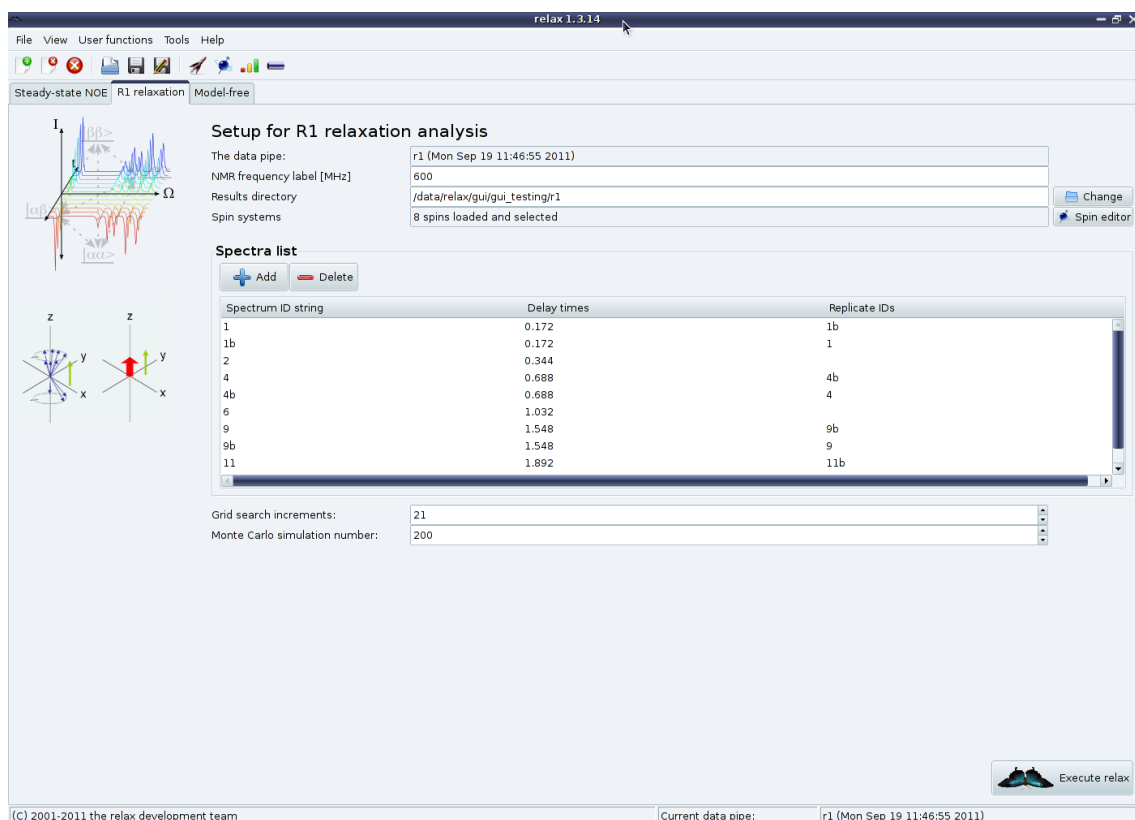


Figure 1.6: Screenshot of the relax GUI interface – the  $R_1$  analysis.

**Data pipe editor** : This window allows for easy manipulation of the data pipes of the relax data store (see Figure 1.12).

**The relax prompt** : This window gives access to the relax prompt (see Figure 1.13).

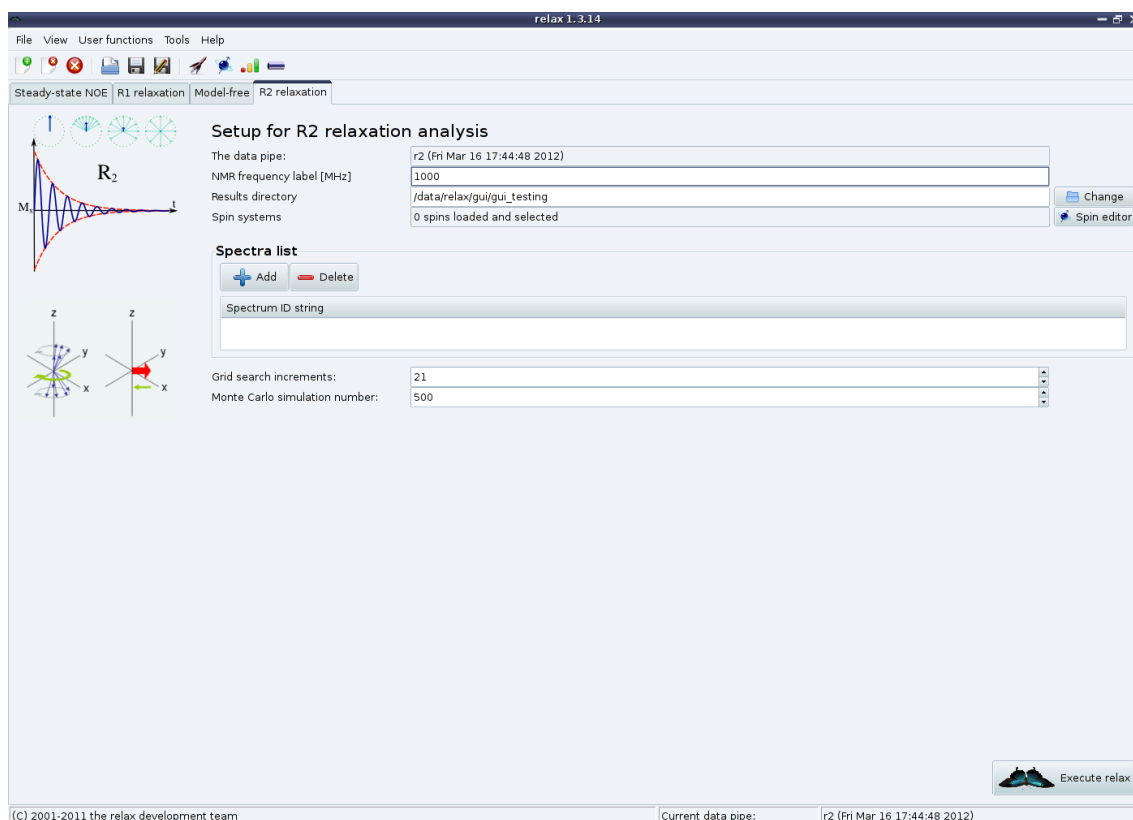
### 1.2.11 Access to the internals of relax

To enable advanced Python scripting and control, many parts of relax have been designed in an object oriented fashion. If you would like to play with internals of the program the entirety of relax is accessible by importation. For example all data is contained within the object called the relax data store which, to be able to access it, needs be imported by typing:

```
relax> from data_store import Relax_data_store; ds = Relax_data_store()
```

The `ds` object is a dictionary type which contains the multiple data pipes. All of relax's packages, modules, functions, and classes are also accessible by import statements. For example to create a rotation matrix from three Euler angles in the z-y-z notation, type:

```
relax> alpha = 0.1342
relax> beta = 1.0134
relax> gamma = 2.4747
relax> from lib.geometry.rotations import euler_to_R_zyz
relax> from numpy import float64, zeros
relax> R = zeros((3,3), float64)
```

Figure 1.7: Screenshot of the relax GUI interface – the  $R_2$  analysis.

```

relax> euler_to_R_zyz(alpha, beta, gamma, R)
relax> print(R)
[[-0.494666415429033 -0.557373756841289 -0.666813041737502]
 [ 0.219125193028791 -0.822460914570202  0.524921131013452]
 [-0.84100492699311  0.113545317776532  0.528978424497956]]
relax>

```

## 1.3 The multi-processor framework

### 1.3.1 Introduction to the multi-processor

Thanks to Gary Thompson's multi-processor framework, relax can be run on multi-core/multi-CPU systems or on clusters to speed up calculations. As most analyses are relatively quick and would not benefit from the multi-processor framework, only the model-free and frame order analyses have currently been parallelised to run within this framework. To use the multi-processor framework, the following should be installed:

**OpenMPI:** This is the most commonly used Message Passing Interface (MPI) protocol software. The rest of this manual will assume that this is the implementation in use. If another implementation is used, please see the specific documentation for that software for how to set up a program to run via MPI.

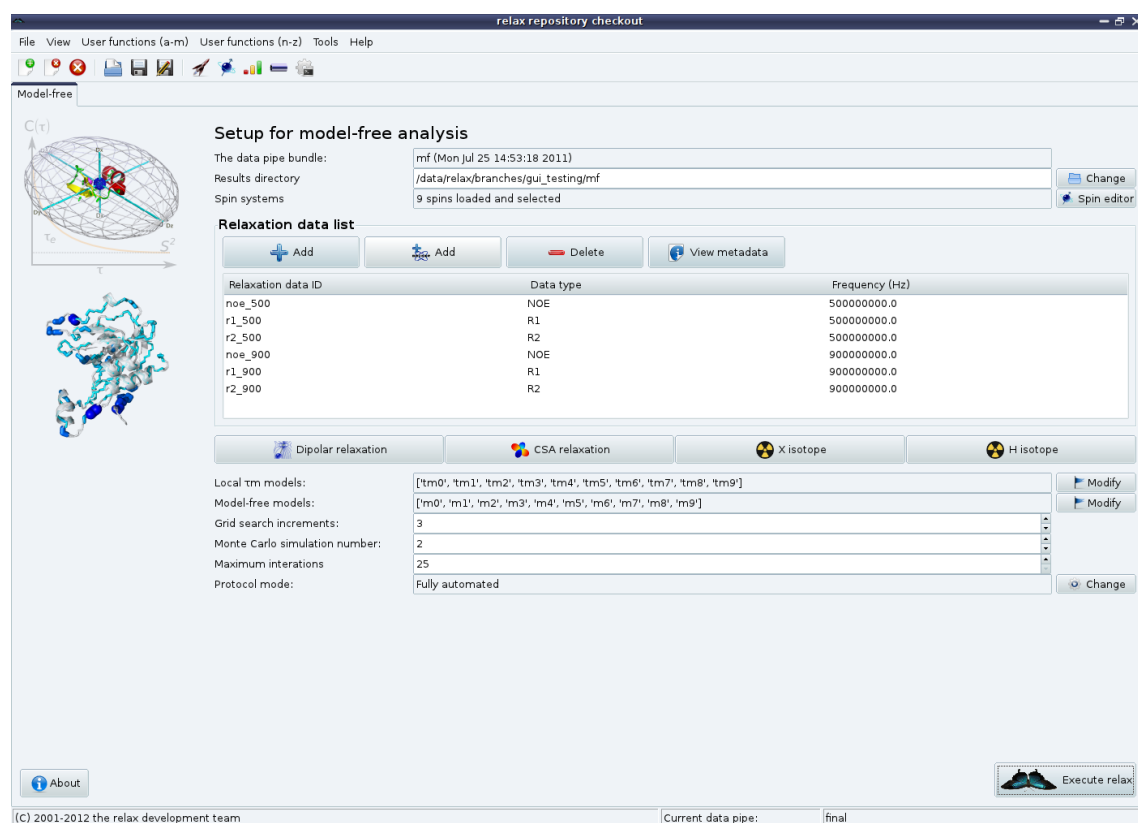


Figure 1.8: Screenshot of the relax GUI interface – the automated model-free analysis. The analysis is fully automated via a new model-free protocol as described in detail in Chapter 7. Clicking on the “About” button in the bottom left hand corner will give a full description of the protocol. For using this interface or any of the modern-day model-free protocols, data from at least two magnetic field strengths must be without question collected.

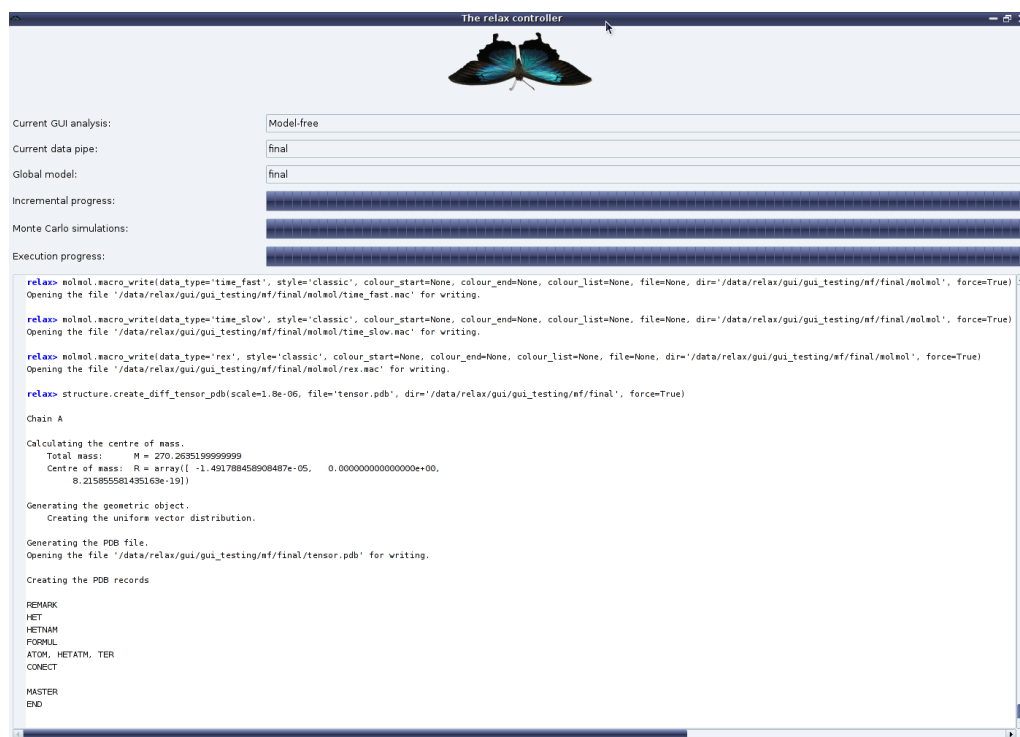


Figure 1.9: Screenshot of the relax GUI interface – the relax controller window. The purpose of the controller is for feedback. It shows the current analysis and current data pipe, a number of progress gauges, and the relax text output.

**mpi4py:** This dependency is essential for running in MPI mode in relax. If you would like to use another Python implementation to access the MPI protocol, please consider becoming a relax developer.

### 1.3.2 Usage of the multi-processor

If you have access to a 256 node cluster and can run calculations on all nodes, assuming that the `dauvergne_protocol.py` automated model-free analysis sample script will be used (after modification for the system under study), relax can be executed by typing:

```
$ mpirun -np 257 /usr/local/bin/relax --multi='mpi4py' --tee log dauvergne_protocol.py
```

Note that the argument `-np` value is one more than the number of slaves you would like to run. You should then see the following text in the initial relax printout:

```
Processor fabric: MPI 2.1 running via mpi4py with 256 slave processors & 1 master. Using
Open MPI 1.4.3.
```

### 1.3.3 Further details

For a full description of the multi-processor framework and how to use it, please see Gary Thompson's official announcement on the [relax-devel mailing list](#).

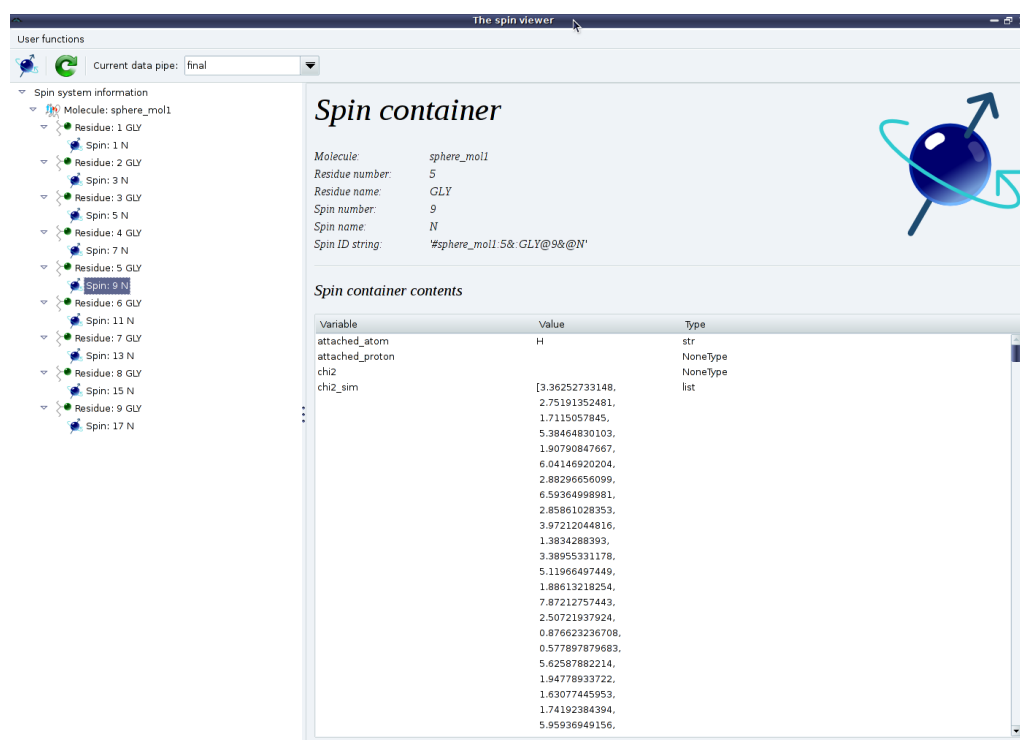


Figure 1.10: Screenshot of the relax GUI interface – the spin viewer window. This viewer is designed for easy addition and manipulation of spin systems within the relax data store. The window is accessible via the “View→Spin viewer” menu entry, typing “[Ctrl-T]”, the spin viewer button in the toolbar, or the “spin editor” button within the auto-analysis tabs.

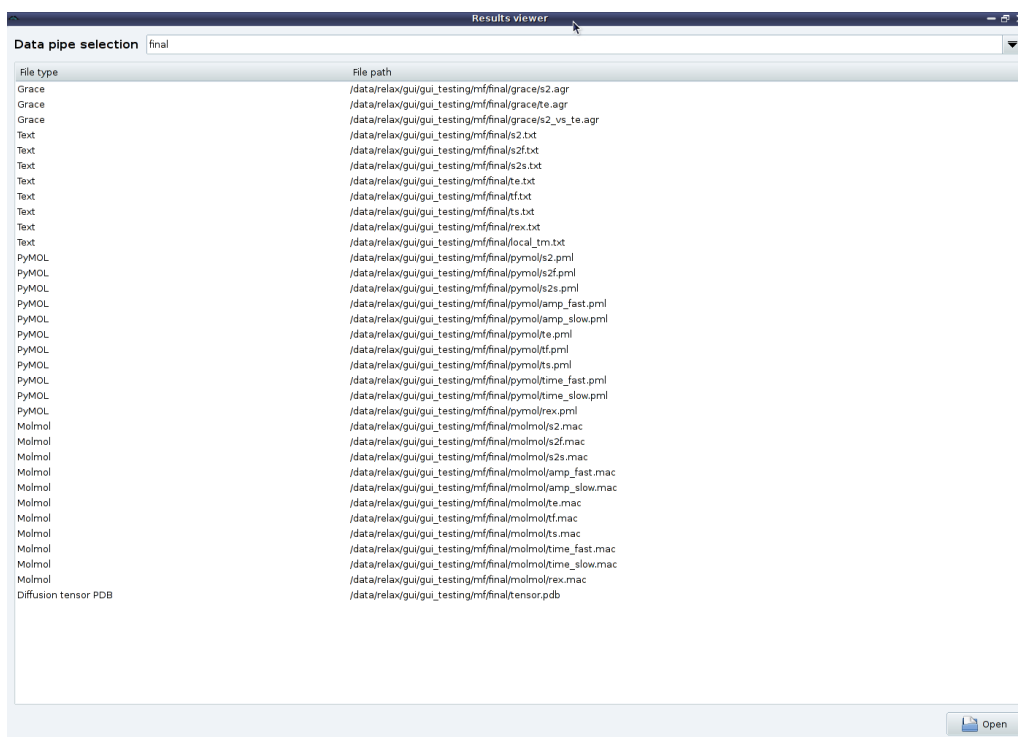


Figure 1.11: Screenshot of the relax GUI interface – the results viewer window. At the end of one of the automated analyses, a number of results files will be created. This can include text files containing the results, 2D Grace plots of the results, PyMOL and MOLMOL macros plotting the results onto the structure, diffusion tensor objects for viewing in PyMOL, etc. This window allows for easy opening of these results files.

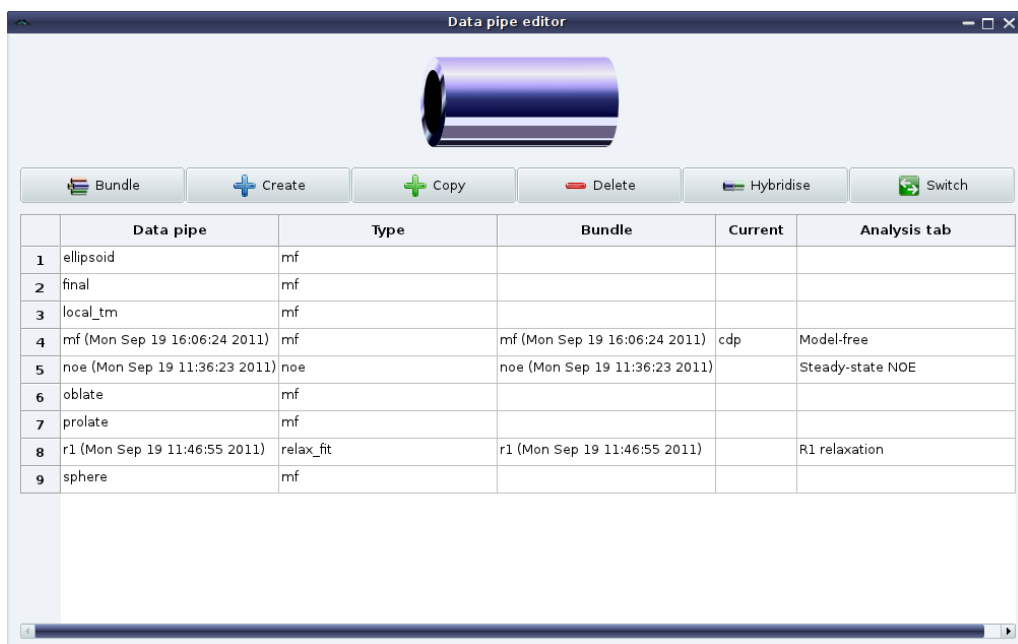
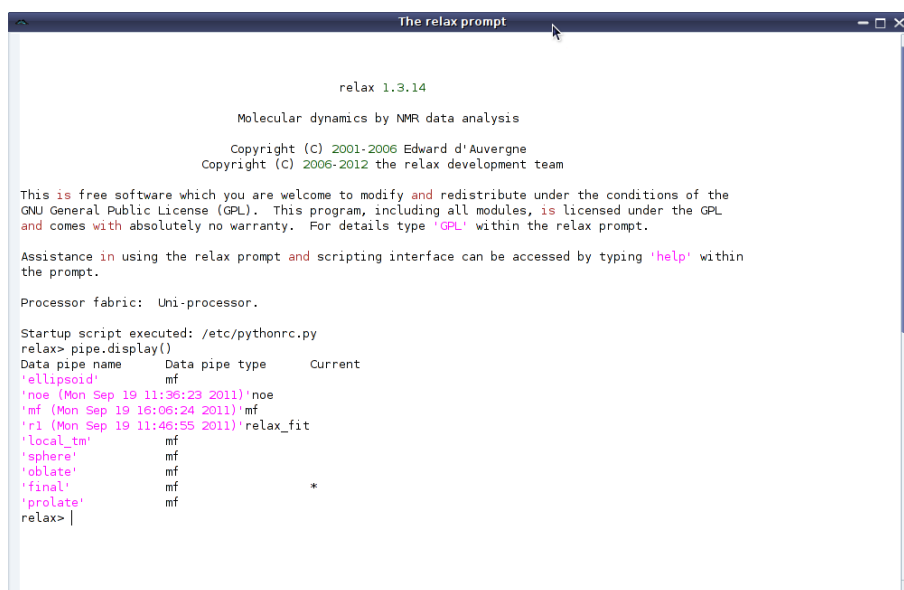


Figure 1.12: Screenshot of the relax GUI interface – the pipe editor window. One analysis may consist of one or more data pipes. And each analysis has its own unique set of data pipes. This editor allows for the easy manipulation of data pipes for advanced users.



```

relax 1.3.14

Molecular dynamics by NMR data analysis

Copyright (C) 2001-2006 Edward d'Auvergne
Copyright (C) 2006-2012 the relax development team

This is free software which you are welcome to modify and redistribute under the conditions of the
GNU General Public License (GPL). This program, including all modules, is licensed under the GPL
and comes with absolutely no warranty. For details type 'GPL' within the relax prompt.

Assistance in using the relax prompt and scripting interface can be accessed by typing 'help' within
the prompt.

Processor fabric: Uni-processor.

Startup script executed: /etc/pythonrc.py
relax> pipe.display()
Data pipe name      Data pipe type      Current
'ellipsoid'         mf
'noe (Mon Sep 19 11:36:23 2011)' noe
'mf (Mon Sep 19 16:06:24 2011)' mf
'r1 (Mon Sep 19 11:46:55 2011)' relax_fit
'local_tm'          mf
'sphere'            mf
'oblate'            mf
'final'             mf
'prolate'           mf
relax> |

```

Figure 1.13: Screenshot of the relax GUI interface – the prompt window. This window mimics relax in the prompt user interface mode, and provides the full power of the prompt/script UI modes within the GUI.

## 1.4 Usage of the name relax

The program relax is so relaxed that the first letter should always be in lower case!



## Chapter 2

# Installation instructions

### 2.1 Dependencies

The following packages need to be installed before using relax:

**Python:** Version 2.5 or higher.

**NumPy:** Version 1.6 or higher. This package is used for most of the numerical calculations within relax.

**SciPy:** Version 0.7.1 or higher. This package is optional. It is required only for the frame order theory analyses.

**wxPython:** Version 2.9 or higher. This package is also optional. It is required for the operation of the graphical user interface (GUI).

**mpi4py:** Version 1.2 or higher. This optional dependency is essential for running relax in MPI multi-processor mode.

Older versions of these packages may work, use them at your own risk. If, for older dependency versions, errors do occur please submit a bug report to the bug tracker at <https://gna.org/bugs/?group=relax>. That way a solution may be created for the next relax release.

Note that only the official Python distribution from <http://python.org> is supported. If you use the Enthought Python Distribution (EPD) or other non-official distributions you may encounter problems with the relax C modules, the graphical user interface, or other issues. These alternative distributions are to be used at your own risk. Any issues encountered will not be considered as relax bugs.

## 2.2 Installation

### 2.2.1 The source releases

Two types of software packages are available for download – the precompiled and source distribution. Currently only relaxation curve-fitting requires compilation to function and all other features of relax will be fully functional without compilation. If relaxation curve-fitting is required but no precompiled version of relax exists for your operating system or architecture then, if a C compiler is present, the C code can be compiled into the shared objects files `*.so`, `*.pyd` or `*.dylib` which are loaded as modules into relax. To build these modules the Scons system from <http://scons.org/> is required. This software requires the Python and numpy header files installed. Once Scons is installed type

```
$ scons
```

in the base directory where relax has been installed and the C modules should, hopefully, compile without any problems. Otherwise please submit a bug report to the bug tracker at <https://gna.org/bugs/?group=relax>.

### 2.2.2 Installation on GNU/Linux

To install the program relax on a GNU/Linux system download either the precompiled distribution labelled `relax-x.x.x.GNU-Linux.arch.tar.bz2` matching your machine architecture or the source distribution `relax-x.x.x.src.tar.bz2`. A number of installation methods are possible. The simplest way is to switch to the user “root”, unpack and decompress the archive within the `/usr/local` directory by typing, for instance

```
$ tar jxvf relax-x.x.x.GNU-Linux.i686.tar.bz2
```

then create a symbolic link in `/usr/local/bin` by moving to that directory and typing

```
$ ln -s ../relax/relax .
```

and finally possibly creating the byte-compiled Python `*.pyc` files to speed up the start time of relax by typing

```
$ python -m compileall .
```

in the relax base directory. Alternatively if the Scons system is installed, by typing as the root user

```
$ scons install
```

in the relax base directory, a directory in `/usr/local/` called `relax` will be created, all the uncompressed and untarred files will be copied into this directory, a symbolic link in `/usr/local/bin` to the file `/usr/local/relax/relax` will be created, and then finally the Python `*.pyc` files will be byte-compiled. To change the installation path to a non-standard location the Scons script `sconstruct` in the base relax directory should be modified by changing the variable `INSTALL_PATH` to point to the desired location.

### 2.2.3 Installation on MS Windows

In addition to the above dependencies, running relax on MS Windows requires a number of additional programs. These include:

**pyreadline:** Any version.

**ctypes:** The pyreadline package requires ctypes.

To install, simply download the pre-compiled binary distribution **relax-x.x.x.Win32.zip** or the source distribution **relax-x.x.x.src.zip** and extract the files to **C:\Program Files\relax-x.x.x**. Then add this directory to the system environment path (in Windows XP, right click on “My Computer”, go to “Properties”, click on the “Advanced” tab, and click on the “Environment Variables” button. Then double click on the “Path” system variable and add the text “;C:\Program Files\relax-x.x.x” to the end of variable value field. The Python installation must also be located on the path (add the text “;C:\Python27”, changing the text to point to the correct directory, to the field). To run the program from any directory inside the Windows command prompt (or dos prompt) type:

```
C:\> relax
```

Note that the pre-compiled binary distribution was built using a specific Python version and that that version may need to be installed for the modules to be loaded. More details are given on the [download](#) webpage.

### 2.2.4 Installation on Mac OS X

There are three ways of installing relax on a Mac. These are described at <http://www.nmr-relax.com/download.html> and are the pre-compiled relax application, the Fink or the source releases.

#### The relax application

The stand-alone relax application requires none of the dependencies listed above to be installed. It is a universal binary compiled for the i386, x86-64 and PPC CPU architectures (fat3) using the Mac OS X 10.5 framework. It should therefore run on Leopard, Snow Leopard, and Lion. This very large bundle does not require system administrator access to run.

#### Fink

Certain relax versions are available for Mac OS X within the Fink project. These can be installed for Python 2.7 with the command:

```
> fink install relax-py27
```

The relax releases packaged within Fink can be browsed at <http://pdb.finkproject.org/pdb/browse.php?name=relax>. If the desired version is

not available, please download the relevant source package below or contact the fink project using the “Maintainer” email address given in the relax fink pages.

Note that when installing via fink, all the dependencies will be automatically selected and installed as well. Although automatic, when starting from scratch that there could be well over 250 source packages that need to be compiled (to set up the full GNU compilation chain and other libraries which are then required to build Python, numpy, scipy, etc.). This may take anywhere between 2 days to over a week (don’t forget to mention this fact to your poor sys-admin).

The fink relax packages for different Python versions are [relax-py27](#), [relax-py26](#), [relax-py25](#) and [relax-py24](#).

### Source release

See Section [2.2.1](#) on page [24](#).

### 2.2.5 Installation on your OS

For all others systems, please use the source distribution files and the Scons software to build the C modules.

### 2.2.6 Running a non-compiled version

Compilation of the C code is not essential for running relax, however certain features of the program will be disabled. Currently only the exponential curve-fitting for determining the  $R_1$  and  $R_2$  relaxation rates requires compilation. To run relax without compilation install the dependencies detailed above, download the source distribution which should be named `relax-x.x.x.src.tar.bz2`, extract the files, and then run the file called `relax` in the base directory.

## 2.3 Optional programs

The following is a list of programs which can be used by relax although they are not essential for normal use.

### 2.3.1 Grace

Grace is a program for plotting two dimensional data sets in a professional looking manner. It is used to visualise parameter values. It can be downloaded from <http://plasma-gate.weizmann.ac.il/Grace/>.

### 2.3.2 OpenDX

Version 4.1.3 or compatible. OpenDX is used for viewing the output of the space mapping function and is executed by passing the command `dx` to the command line with various options. The program is designed for visualising multidimensional data and can be found at <http://www.opendx.org/>.

### 2.3.3 Molmol

Molmol is used for viewing the PDB structures loaded into the program and to display parameter values mapped onto the structure.

### 2.3.4 PyMOL

PDB structures can also be viewed using PyMOL. This program can also be used to display geometric objects generated by relax for representing physical concepts such as the diffusion tensor and certain cone diffusion models.

### 2.3.5 Dasha

Dasha is a program used for model-free analysis of NMR relaxation data. It can be used as an optimisation engine to replace the minimisation algorithms implemented within relax.

### 2.3.6 Modelfree4

Art Palmer's Modelfree4 program is also designed for model-free analysis and can be used as an optimisation engine to replace relax's high precision minimisation algorithms.



## Chapter 3

# Open source infrastructure

### 3.1 The relax web sites

The main web site for relax is <http://www.nmr-relax.com>. From these pages general information about the program, links to the latest documentation, links to the most current software releases, and information about the mailing lists are available. There are also Google search capabilities built into the pages for searching both the HTML version of the manual and the archives of the mailing lists.

The relax web site is hosted by the Gna! project (<https://gna.org/>) which is described as “a central point for development, distribution and maintenance of Libre Software (Free Software) projects”. relax is a registered Gna! project and its primary Gna! web site is <https://gna.org/projects/relax>. This site contains many more technical details than the main web site.

### 3.2 The mailing lists

A number of mailing lists have been created covering different aspects of relax. These include the announcement list, the relax users list, the relax development list, and the relax committers list.

#### 3.2.1 relax-announce

The relax announcement list “relax-announce at gna.org” is reserved for important announcements about the program including the release of new program versions. The amount of traffic on this list is relatively low. If you would like to receive information about relax you can subscribe to the list by visiting the information page at <https://mail.gna.org/listinfo/relax-announce/>. Previous announcements can be viewed at <https://mail.gna.org/public/relax-announce/>.

### 3.2.2 relax-users

If you would like to ask questions about relax, discuss certain features, receive help, or to communicate on any other subject related to relax the mailing list “relax-users at gna.org” is the place to post your message. To subscribe to the list go to the relax-users information page at <https://mail.gna.org/listinfo/relax-users/>. You can also browse the mailing list archives at <https://mail.gna.org/public/relax-users/>.

### 3.2.3 relax-devel

A second mailing list exists for posts relating to the development of relax. The list is “relax-devel at gna.org” and to subscribe go to the relax-devel information page at <https://mail.gna.org/listinfo/relax-devel/>. Feature requests, program design, or any other posts relating to relax’s structure or code should be sent to this list instead. The mailing list archives can be browsed at <https://mail.gna.org/public/relax-devel/>.

### 3.2.4 relax-commits

One last mailing list is the relax commits list. This list is reserved for automatically generated posts created by the version control software which looks after the relax source code and these web pages. If you would like to become a developer you can subscribe to the list at relax-commits information page <https://mail.gna.org/listinfo/relax-commits/>. The list can also be browsed at <https://mail.gna.org/public/relax-commits/>.

### 3.2.5 Replying to a message

When replying to a message on these lists remember to hit ‘respond to all’ so that the mailing list is included in the CC field. Otherwise your message will only be sent to the original poster and not return back to the list. Only messages to relax-users and relax-devel will be accepted. If you are using Gmail’s web based interface, please do not click on ‘Edit Subject’ as this currently mangles the email headers, creates a new thread on the mailing list, and makes it difficult to follow the thread.

## 3.3 Reporting bugs

One of the philosophies in the construction of relax is that if there is something which is not immediately obvious then that is considered a design bug. If any flaws in relax are uncovered including general design flaws, bugs in the code, or documentation issues these can be reported within relax’s bug tracker system. The link to submit a bug is <https://gna.org/bugs/?group=relax&func=additem> while the main page for browsing, submitting, viewing the statistics, or searching through the database is at <https://gna.org/bugs/?group=relax>. Please do not report bugs to personal email addresses or to the mailing lists.



When reporting a bug please include as much information as possible so that the problem can be reproduced. Include information such as the release version or the revision number if the repository sources are being used. Also include all the steps performed in order to trigger the bug. Attachment of files is allowed so scripts and subsets of the input data can be included. However please do not attach large files to the report. Prior to reporting the bug try to make sure that the problem is indeed a bug and if you have any doubts please feel free to ask on the relax-users mailing list. To avoid duplicates be sure that the bug has not already been submitted to the bug tracker. You can search the bugs from the page <https://gna.org/project/search.php?group=relax>.

Once the bug has been confirmed by one of the relax developers you may speed up the resolution of the problem by trying to fixing the bug yourself. If you do wish to play with the source code and try to fix the issue see the relax development chapter of this manual on how to check out the latest sources (Chapter 13 on page 275), how to generate a patch (which is just the output of diff in the ‘unified’ format), and the guidelines for the format of the code.

## 3.4 Latest sources – the relax repositories

relax’s source code is kept within a version control system called Subversion (<http://subversion.tigris.org/>). Subversion or SVN allows fine control over the development of the program. The repository contains all information about every change ever made to the program. To learn more about the system the Subversion book located at <http://svnbook.red-bean.com/> is a good place to start. The contents of the relax repository can be viewed on-line at <http://svn.gna.org/viewcvs/relax/>. The current sources can be downloaded using the SVN protocol by typing

```
$ svn co svn://svn.gna.org/svn/relax/trunk relax-trunk
```

however if this does not work, try the command

```
$ svn co http://svn.gna.org/svn/relax/trunk relax-trunk
```

to download using the HTTP protocol.

## 3.5 News

Summaries of the latest news on relax can be found on the relax web site <https://gna.org/projects/relax>. However more information can be found at the dedicated news page <https://gna.org/news/?group=relax>.

## 3.6 The relax distribution archives

The relax distribution archives, the files to download to install relax, can be found at <http://download.gna.org/relax/>. If a compiled binary distribution for your architecture does not exist you are welcome to create this distribution yourself and submit it for inclusion in the relax project. To do this a number of steps are required. Firstly, the code

to each relax release or version resides in the ‘tags’ directory of the relax repository. To check out version 2.1.1 for example type

```
$ svn co svn://svn.gna.org/svn/relax/tags/2.1.1 relax
```

Again the sources are available through HTTP by typing

```
$ svn co http://svn.gna.org/svn/relax/tags/2.1.1 relax
```

The binary distribution can then be created for your architecture by shifting to the main directory of the checked out sources and typing

```
$ cd relax
$ scons binary_dist
```

At the end SCons will attempt to make a GPG signature for the newly created archive. However this will fail as the current relax private GPG key is not available for security reasons. If the SCons command fails, excluding the GPG signing, please submit a bug report with as much information possible including the details described next to <https://gna.org/bugs/?group=relax&func=additem> (the python and SCons version numbers may also be useful). Once the file has been created post a message to the relax development mailing list describing the compilation and the creation of the archive, the relax version number, the machine architecture, operating system, and the name of the new file. Do not attach the file though. You will then receive a response explaining where to send the file to. For security the archive will be thoroughly checked and if the source code is identical to that in the repository and the C modules are okay, the file will be GPG signed and uploaded to <http://download.gna.org/relax/>.

## Chapter 4

# The relax data model

### 4.1 The concept of the relax data model

To begin to understand how to use relax, a basic comprehension of the relax data model is needed. The data model includes the concepts of the relax data store, the data pipes, the molecule, residue and spin data structures and the interatomic data containers. These concepts are independent of the specific analyses presented in the next chapters and are important for setting up relax.

### 4.2 The data model

#### 4.2.1 The relax data store

All permanent data handled by relax is kept in a structure known as the relax data store. This structure is initialised when relax is launched. The data store is primarily organised into a series of objects known as data pipes, and all usage of relax revolves around the flow of information in these data pipes.

#### Data pipes



The first thing one must do when relax is launched is to create a data pipe. When using the GUI, a base data pipe will be created when opening one of the automatic analyses via the analysis selection window (see figure 1.4 on page 12). This will also create a data pipe bundle for the analysis (*vide infra*). Alternatively the data pipe editor window can be

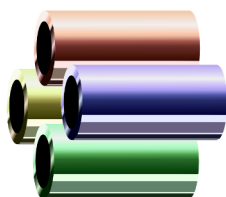
used to create data pipes (see figure 1.12 on page 21). For the prompt/scripting modes, or the “User functions→pipe→create” menu entry, a data pipe can be initialised by specifying the unique name of the data pipe and the data pipe type:

```
1 pipe.create(pipe_name='NOE 1200 MHz', pipe_type='noe')
```

A number of relax operations will also create data pipes by merging a group of pipes or branching pre-existing pipes. See section 1.2.6 on page 10 for additional details.

All data not associated with spin systems will be stored in the base data pipe. This includes information such as global optimisation statistics, diffusion tensors, alignment tensors, 3D structural data, the molecule, residue and spin container data structure and the interatomic data containers. One data pipe from the set will be defined as being the current data pipe, and all operations in relax will effect data from this pipe. The `pipe.switch` user function in all UI modes can be used to change which pipe is the current data pipe. In the GUI, switching between analysis tabs will automatically switch the current data pipe to match the analysis being displayed.

### Data pipe bundles



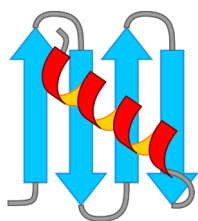
Related data pipes can be grouped into a ‘bundle’. For example if the data pipes “sphere”, “oblate spheroid”, “prolate spheroid”, and “ellipsoid” preexist, these can be grouped into a bundle called “diffusion tensors” with the following series of user function calls:

```
1 pipe.bundle(bundle='diffusion tensors', pipe='sphere')
2 pipe.bundle(bundle='diffusion tensors', pipe='oblate spheroid')
3 pipe.bundle(bundle='diffusion tensors', pipe='prolate spheroid')
4 pipe.bundle(bundle='diffusion tensors', pipe='ellipsoid')
```

The data pipe editor window of the GUI can also be used to bundle pipes together (see figure 1.12 on page 21).

### 4.2.2 Molecule, residue, and spin containers

Within a data pipe is the molecule, residue, and spin container data structure. Data which is specific to a given nucleus is stored in a special spin container structure. This includes relaxation data, model-free parameters, reduced spectral density mapping values, spin specific optimisation parameters, chemical shift tensor information, pseudo-contact shift values, etc. The spin containers can be created from 3D structural data or a sequence file, as described in the next two sections, or manually built.



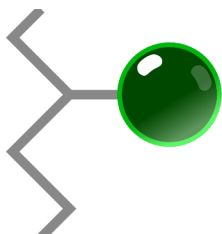
### Molecule containers

The spin containers are part of a nested set of containers, and are graphically depicted in the spin viewer window of the GUI in figure 1.10 on page 20. As can be seen from the figure, the top level holds a single molecular container. Multiple molecular containers can be present if the study is of a molecular complex. Using the GUI menus or the prompt/scripting mode, molecule containers can be manually created with the user function:

```
1 molecule.create(mol_name='glycerol', mol_type='organic molecule')
```

In the spin viewer window of the GUI, right clicking on the “Spin system information” element will pop up a menu with an entry for adding molecule containers. Right clicking on molecule containers will show a pop up menu with an entry for permanently deleting the container.

### Residue containers



Nested within the molecule containers are residue containers. These are graphically depicted in the spin viewer window (see figure 1.10 on page 20). Each molecule container can possess multiple residues. These require either a unique residue number or unique residue name. For organic molecules where the residue concept is meaningless, all spin containers can be held within a single unnamed and unnumbered residue container. Using the GUI menus or the prompt/scripting mode, residue containers can be manually created with the user function:

```
1 residue.create(res_num='-5', res_name='ASP')
```

Alternatively residues can be added in the spin viewer window from the pop up menu when right clicking on molecule containers, and can be deleted via the pop up menu when right clicking on the residue to delete.

## Spin containers



Spin containers are nested within a residue container (again graphically depicted in the spin viewer window in figure 1.10 on page 20). Multiple spin containers can exist per residue. This allows, for example, a single model-free analysis simultaneously on the backbone nitrogen spins, side-chain tryptophan indole nitrogen spins and alpha carbon spins. Or, for example, studying the pseudocontact shifts for all nitrogen, carbon and proton spins in the molecule simultaneously.

Spin containers can be manually added via the `spin.create` user function in the GUI or prompt/scripting mode:

```
1 spin.create(spin_num='200', spin_name='NE1')
```

The spin viewer window can also be used by right clicking on residue containers.

## Spin ID strings

Spins are often identified in relax using their ID strings. The spin ID strings follow the basic construct found in a number of other NMR software such as MOLMOL. The identification string is composed of three components:

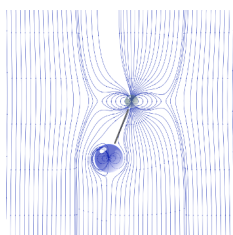
- The molecule ID token beginning with the “#” character,
- The residue ID token beginning with the “:” character,
- The atom or spin system ID token beginning with the “@” character.

Each token can be composed of multiple elements – one per spin – separated by the “,” character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the “-” character. Negative numbers are supported. The full ID string specification is “#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]”, where the token elements are “<mol\_name>”, the name of the molecule, “<res\_id>”, the residue identifier which can be a number, name, or range of numbers, “<atom\_id>”, the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the “#” character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can, in some instances, be used to select spins. For example the string “@H\*” will select the protons ‘H’, ‘H2’ and ‘H98’.

## 4.3 Interatomic data containers



Separate from the spin containers, yet strongly linked to them, are the interatomic data containers. These containers are grouped together within the same data pipe as the spins they point to. These define interactions between two spins located anywhere within the molecule, residue and spin nested data structure. These are automatically created when reading in data defined between two spins such as RDCs and NOE distance constraints. They can also be created using the `interatom.define` user function:

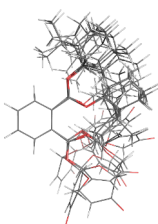
```
1 interatom.define(spin_id1=':2@N', spin_id2=':2@H')
```

As the interatomic data container concept is relatively new, how they are created and handled is likely to evolve and change in the future.

## 4.4 Setup in the prompt/script UI

Below are three different examples showing how to set up the relax data model for any analysis type requiring spin specific data.

### 4.4.1 Script mode – spins from structural data



3D structural data is stored at the level of the current data pipe. This data is completely separate from the molecule, residue and spin data structure. However the structural data can be used to generate the spin containers. For example for the nitrogen relaxation in a model-free analysis where both the nitrogen and proton are needed to define the magnetic dipole-dipole relaxation:

```
1 # Create a data pipe.
2 pipe.create(pipe_name='ellipsoid', pipe_type='mf')
3
4 # Load the PDB file.
5 structure.read_pdb('1f3y.pdb')
6
7 # Set up the 15N and 1H backbone spins.
```

```

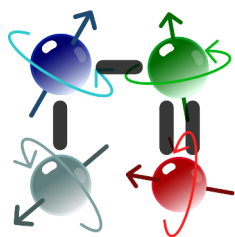
8  structure.load_spins('@N', ave_pos=True)
9  structure.load_spins('@H', ave_pos=True)
10
11 # Set up the 15N and 1H for the tryptophan indole ring.
12 structure.load_spins('@NE1', ave_pos=True)
13 structure.load_spins('@HE1', ave_pos=True)
14
15 # Define the spin isotopes.
16 spin.isotope('15N', spin_id='@N*')
17 spin.isotope('1H', spin_id='@H*')

```

The `structure.read_pdb` user function will load the structural data into the current data pipe, and the `structure.load_spins` user function will create the molecule, residue, and spin containers as needed. This will also load atomic position information into the matching spin containers. The `spin.isotope` user function is required to define the magnetic dipole-dipole interaction and is information not present in the PDB file.

Note that if structural data from the PDB is used to generate the spin containers, then all subsequent data loaded into relax must follow the exact naming convention from the PDB file. Automatic residue name matching (i.e. ‘GLY’ = ‘Gly’ = ‘gly’ = ‘G’) is currently not supported.

#### 4.4.2 Script mode – spins from a sequence file



Alternatively to setting up the molecule, residue, and spin containers via 3D structural data, a plain text columnar formatted file can be used. This is useful for when no 3D structure exists for the molecule. It also has the advantage that the residue and atom names need not conform to the PDB standard. An example for reading sequence data is:

```

1  # Create a data pipe.
2  pipe.create(pipe_name='R1 1200', pipe_type='relax_fit')
3
4  # Set up the 15N spins.
5  sequence.read(file='noe.500.out', mol_name_col=1, res_num_col=2, res_name_col=3,
6               spin_num_col=4, spin_name_col=5)
7  spin.element(element='N', spin_id='@N*')
8  spin.isotope('15N', spin_id='@N')

```

Here the molecule, residue, and spin information is extracted from the “noe.500.out” file which could look like:

#	mol_name	res_num	res_name	spin_num	spin_name	value	error
1	Ap4Aase_new_3_mol1	1	GLY	1	N	None	None
2	Ap4Aase_new_3_mol1	2	PRO	11	N	None	None
3	Ap4Aase_new_3_mol1	3	LEU	28	N	None	None
4	Ap4Aase_new_3_mol1	4	GLY	51	N	0.03892194698453	0.01903177024613



Ap4Aase_new_3_mol1	5	SER	59	N	0.31240422567912	0.01859693729836
Ap4Aase_new_3_mol1	6	MET	71	N	0.42850831873249	0.0252585632304
Ap4Aase_new_3_mol1	7	ASP	91	N	0.53054928103134	0.02799062314416
Ap4Aase_new_3_mol1	8	SER	104	N	0.56528429775819	0.02170612146773
Ap4Aase_new_3_mol1	9	PRO	116	N	None	None
Ap4Aase_new_3_mol1	40	TRP	685	N	0.65394813490548	0.03830061886537
Ap4Aase_new_3_mol1	40	TRP	698	NE1	0.67073879732046	0.01426066343831

The file can contain columns for the molecule name, the residue name and number, and the spin name and number in any order though not all are needed. For example for a single protein system, the molecule name, residue name and spin number are nonessential. Or for an organic molecule, the molecule name, residue name and number and spin number could be nonessential. The subsequent user functions in the above example are used to set up the spin containers appropriately for a model-free analysis. These are not required in the automatic analysis of GUI as these user functions will be presented to you when adding relaxation data, or when clicking on the heteronucleus and proton buttons (“X isotope” and “H isotope”).

In the GUI, the creation of molecule, residue, and spin containers from a sequence file is also available via the “Load spins” wizard within the spin viewer window (*vide supra*).

#### 4.4.3 Script mode – manual construction

For the masochists out there, the full molecule, residue and spin data structure can be manually constructed. For example:

```

1 # Manually create the molecule, residue, and spin containers.
2 molecule.create(mol_name='Ap4Aase', mol_type='protein')
3 residue.create(res_num=1, res_name='GLY')
4 residue.create(res_num=3, res_name='LEU')
5 residue.create(res_num=96, res_name='TRP')
6 spin.create(res_num=1, spin_name='N')
7 spin.create(res_num=3, spin_name='N')
8 spin.create(res_num=96, spin_name='N')
9 spin.create(res_num=96, spin_name='NE1')
```

These user functions can be repeated until the full sequence has been constructed.

## 4.5 Setup in the GUI

### 4.5.1 GUI mode – setting up the data pipe

In the GUI, the most common way to create the data pipe is to initialise one of the auto-analyses via the analysis selection wizard (see Figure 1.4 on page 12). The initialisation will create the appropriate starting data pipe. Alternatively the data pipe editor can be used (see Figure 1.12 on page 21). Or the “User functions→pipe→create” menu item can be selected for graphical access to the `pipe.create` user function.

### 4.5.2 GUI mode – spins from structural data

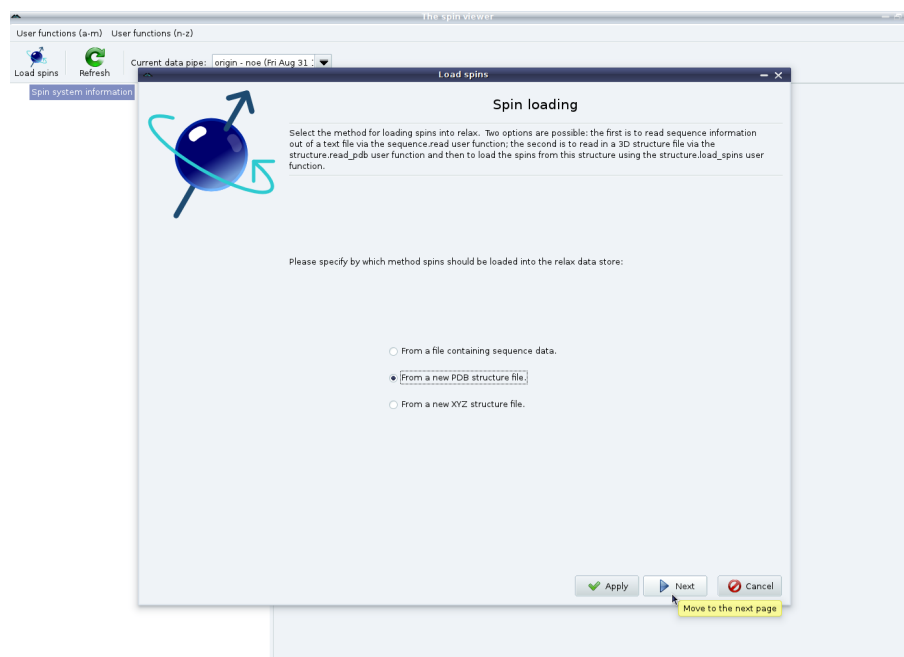
For this section, the example of protein  $^{15}\text{N}$  relaxation data will be used to illustrate how to set up the data structures. To manipulate the molecule, residue and spin data structures in the GUI, the most convenient option is to use the spin viewer window (see Figure 1.10 on page 20). This window can be opened in four ways:

- The “View→Spin viewer” menu item,
- The “[Ctrl+T]” key combination,
- The spin viewer icon in the toolbar (represented by the blue spin icon),
- The “Spin editor” button part of the “Spin systems” GUI element in the specific analysis tabs.

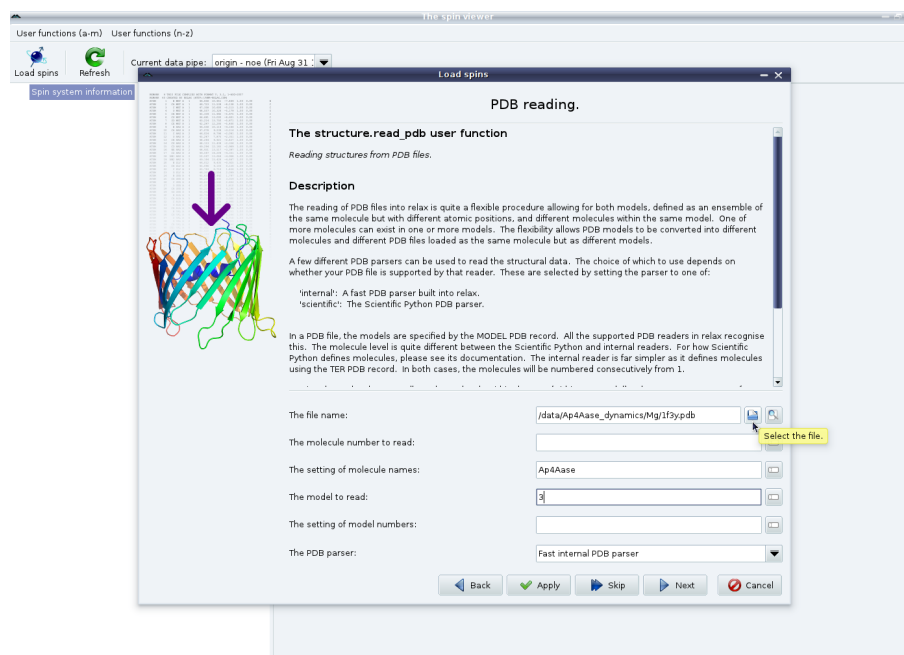
You will then see:



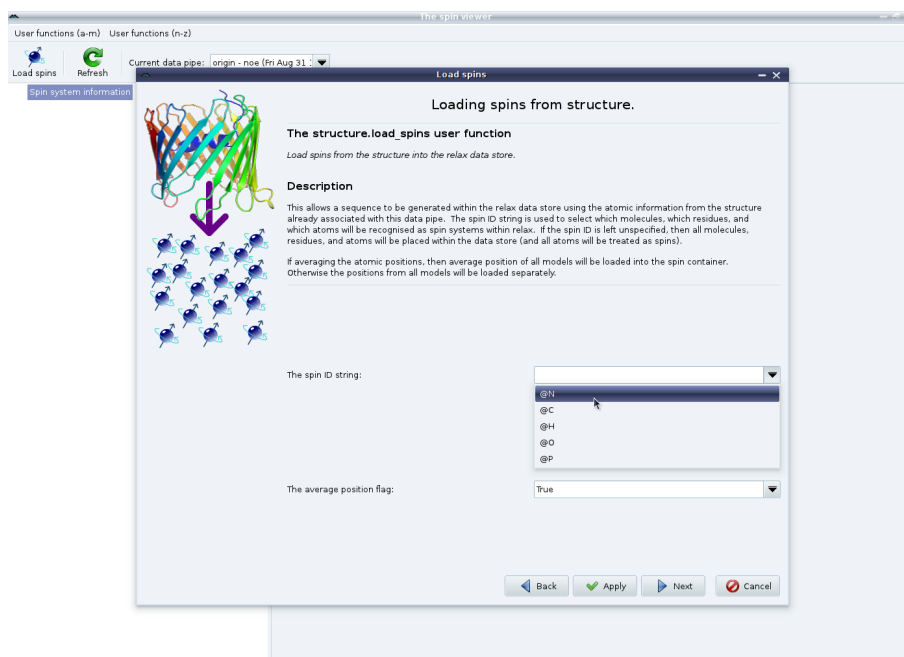
At this point, click on the “Load spins” button (or the “Load spins” menu entry from the right click pop up menu) to launch the spin loading wizard. A number of options will be presented to you:



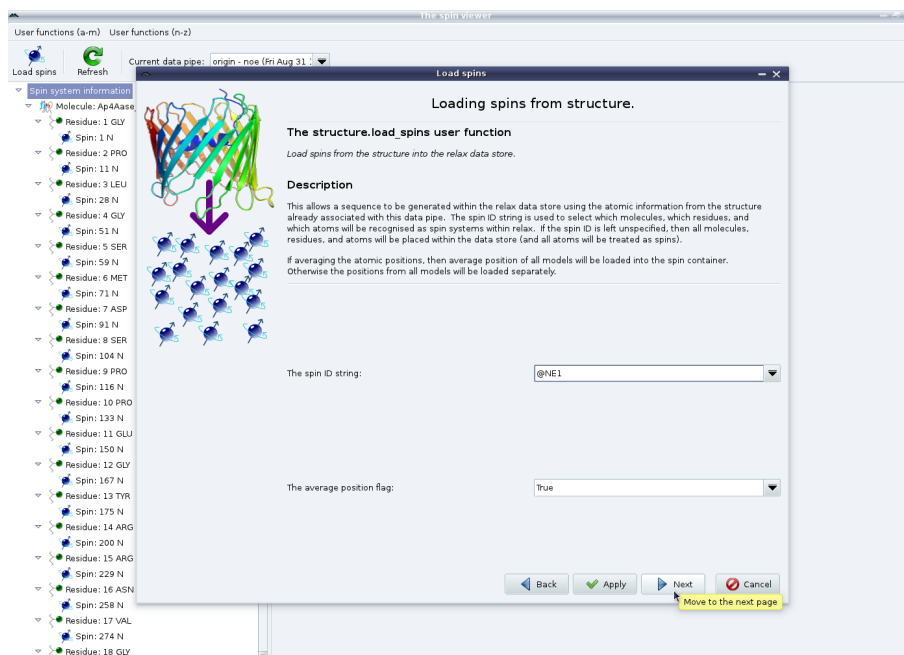
Here the spins will be loaded from a PDB file. If you do not have a 3D structure file, please see the next section. After selecting “From a new PDB structure file” and clicking on “Next”, you will see:



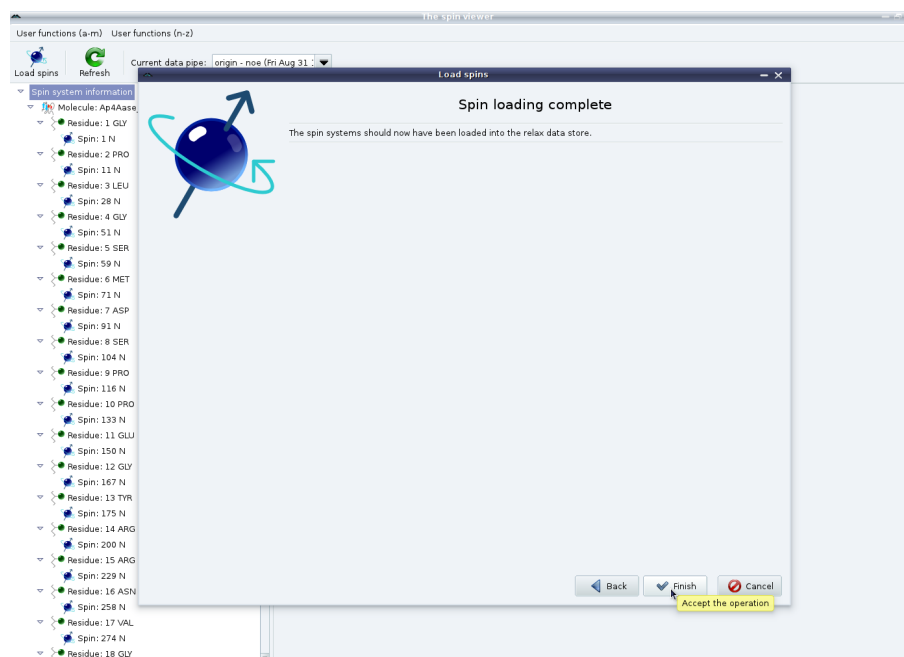
Now select the PDB file you wish to use. The other options in this screen allow you to handle NMR models and multiple molecules within a single PDB file. These options are explained in the window. Hovering the mouse over the options will give additional hints. In this example, the 3<sup>rd</sup> model from the 1F3Y PDB file will be read and the single molecule will be named “Ap4Aase” to override the default naming of “1f3y\_mol1”. Now click on “Next” to bring up the spin loading page:



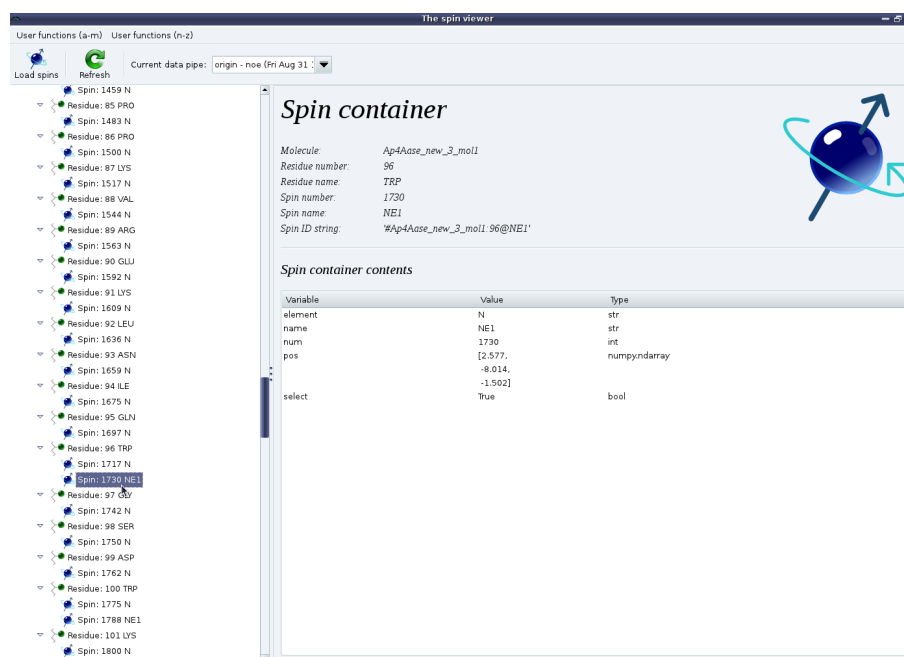
This is a bit more complicated. In this example we are studying the backbone dynamics of  $^{15}\text{N}$  spins of a protein. Therefore first set the spin ID string to “@N” (which can be selected from the pull down) and click on “Apply” to set up the backbone spins. Do not click on “Next” yet. If the current study requires the specification of the dipole-dipole interaction (for example if it involves relaxation data – model-free analyses, consistency testing, reduced spectral density mapping; or the dipolar coupling – the N-state model or ensemble analyses, the Frame Order theory) you will also need to load the  $^1\text{H}$  spins as well. Therefore set the spin ID string to “@H” and click on “Apply” again.



Now change the spin ID string to “@N1” and then click on “Next” (or “Apply” if the Trp protons “@HE1” need to be loaded as well). This will add spin containers for the tryptophan indole  $^{15}\text{N}$  spins. Finally click on “Finish” to exit the wizard:



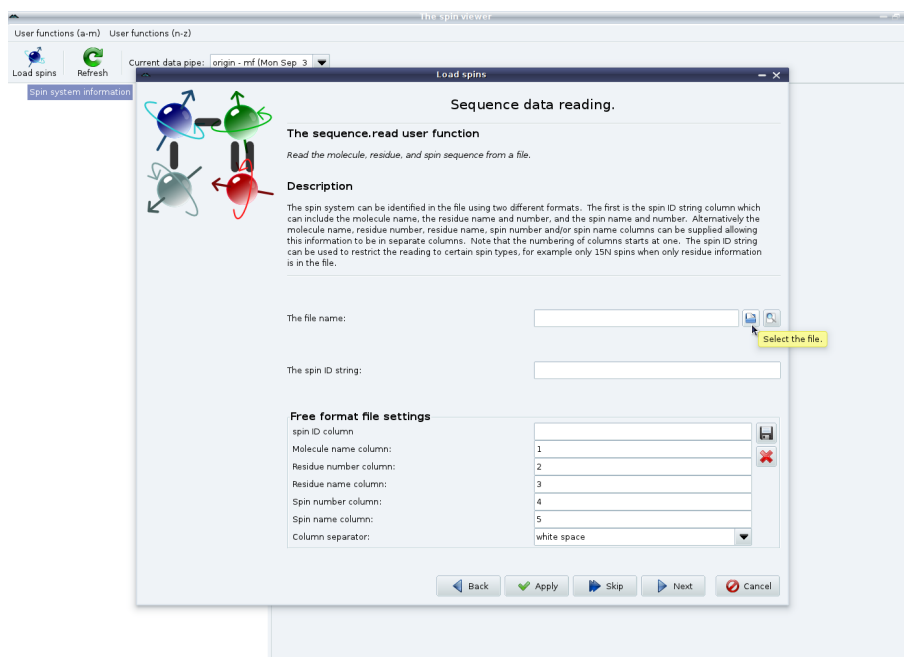
You should now see something such as:



If the  $^1\text{H}$  spins have been loaded as well, then you should see exactly twice as many spin containers as shown above.

### 4.5.3 GUI mode – spins from a sequence file

Starting from the empty spin viewer window on page 40), click on the “Load spins” button. You will then see the spin loading wizard (see page 41). Select the option for reading data from a sequence file. You should then see:



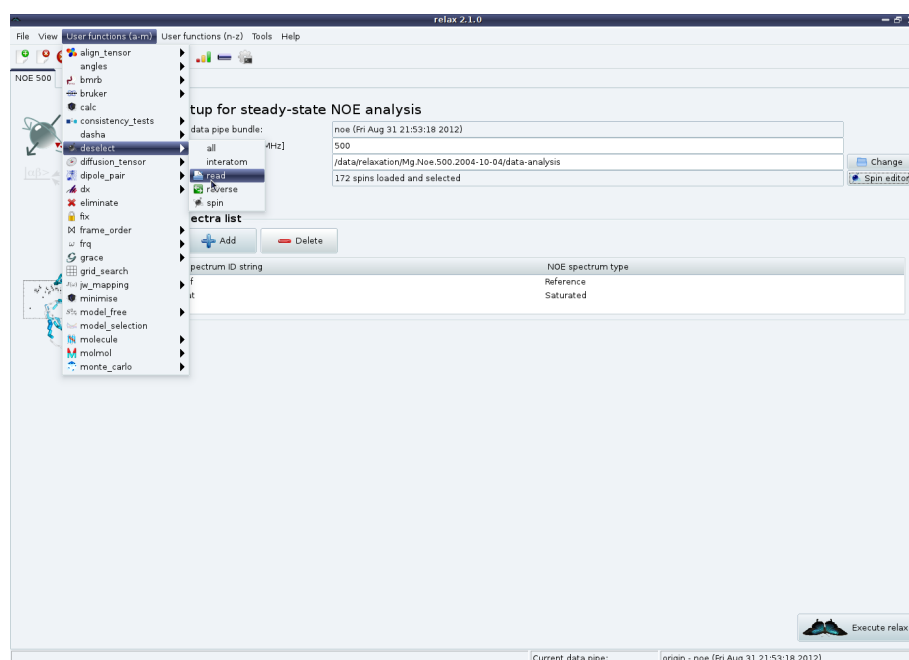
Select the file to load and change the “Free format file settings” as needed. An example of a suitable format is given on page 39. Click on “Next” to reach the wizard ending page (see 43). Finally click on “Finish” to exit the wizard.

#### 4.5.4 GUI mode – manual construction

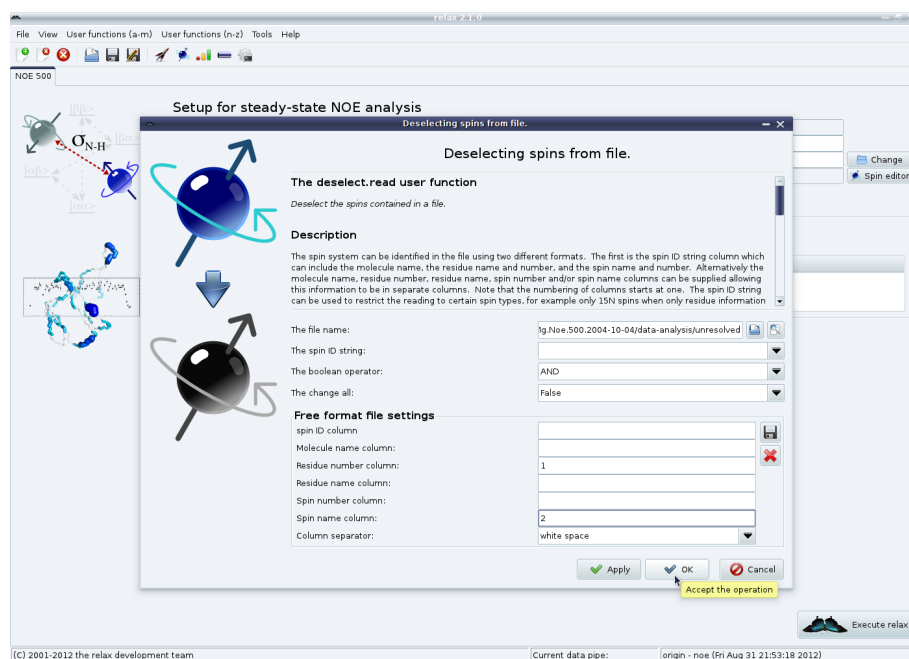
Just as in the prompt/script UI mode, the molecules, residues and spins can be manually added. First add a molecule by right clicking on the “Spin system information” element and selecting the relevant entry in the popup menu. Then right click on the newly created molecule container to add residues, and right click on residue containers to add spins.

#### 4.5.5 GUI mode – deselect spins

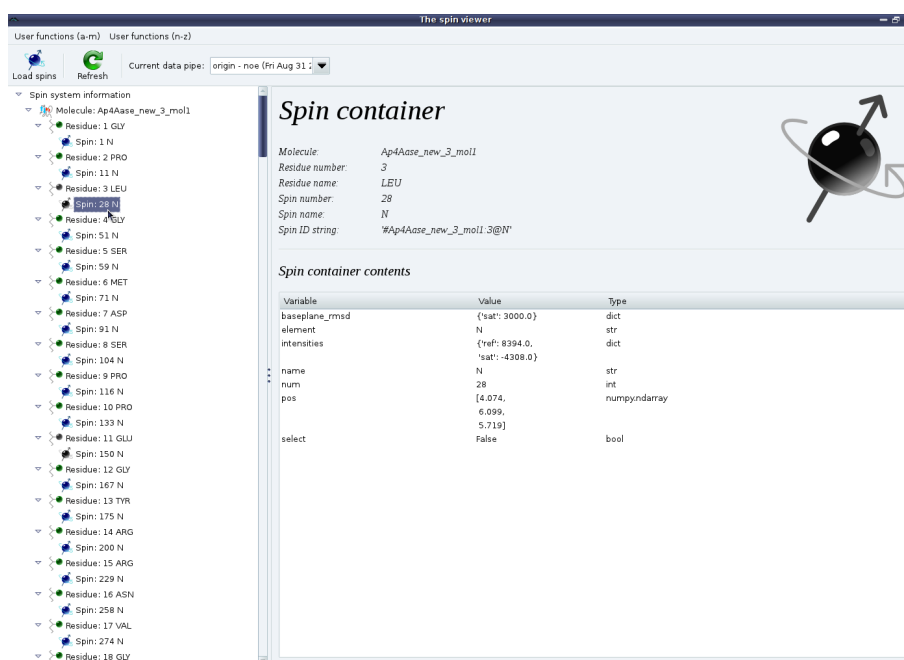
To deselect spins (for example if they are unresolved, overlapping peaks), click on the “User functions→deselect→read” menu item from the main relax window or the spin viewer window:



Select the file listing the unresolved spins and change the column numbers in the “Free format file settings” GUI element as needed:



Alternatively the spin editor window can be reopened and the spins manually deselected by right clicking on them and selecting “Deselect”. Returning to the spin editor window, you should now see certain spins coloured grey:



## 4.6 The next steps

This chapter presented the basics of setting up the relax data store, concepts which are needed for all analysis types built into relax. The next chapters will introduce specific analyses types – the steady-state NOE,  $R_1$  and  $R_2$  relaxation curve-fitting, and the automated full model-free analysis protocol of [d’Auvergne and Gooley \(2007, 2008c\)](#) – which build on the ideas introduced here.



## **Part II**

# **The specific analyses**



## Chapter 5

# The $R_1$ and $R_2$ relaxation rates – relaxation curve-fitting



### 5.1 Introduction to relaxation curve-fitting

The fitting of exponentials to relaxation curves (relaxation curve-fitting or as used throughout this chapter abbreviated simply as relax-fit) involves a number of steps including the loading of data, the calculation of both the average peak intensity across replicated spectra and the standard deviations of those peak intensities, selection of the experiment type, optimisation of the parameters of the exponential curves during the fit for each observed spin, Monte Carlo simulations to find the parameter errors, and saving and viewing the results. To simplify the process a sample script will be followed step by step as was done with the NOE calculation.

### 5.2 The exponential curve models

A number of different models are supported in this analysis. These include the two parameter exponential decay to zero, the inversion recovery experiment, and the saturation recovery experiment. These can be selected using the `relax_fit.select_model` user function.

The default is the two parameter exponential decay whereby the magnetisation starts at  $I_0$  and decays to zero. It has the parameters  $\{R_x, I_0\}$ . The formula of this function is

$$I(t) = I_0 e^{-R_x \cdot t}, \quad (5.1)$$

where  $I(t)$  is the peak intensity at any time point  $t$ ,  $I_0$  is the initial intensity, and  $R_x$  is the relaxation rate (either the  $R_1$  or  $R_2$ ).

In the inversion recovery experiment, the magnetisation starts at a negative value at  $-I_0$  and relaxes to a positive  $I_\infty$  value. This curve consists of three parameters  $\{R_x, I_0, I_\infty\}$ . The formula is

$$I(t) = I_\infty - I_0 e^{-R_x \cdot t}. \quad (5.2)$$

In the saturation recovery experiment, the magnetisation starts at zero and relaxes to a positive  $I_\infty$  value. The model consists of the two parameters  $\{R_x, I_\infty\}$  and has the formula

$$I(t) = I_\infty (1 - e^{-R_x \cdot t}). \quad (5.3)$$

## 5.3 From spectra to peak intensities for the relaxation rates

The following subsections simply contain advice on how to go from the recorded FIDs to the peak lists ready to be input into relax. This need not be followed – it is simply a set of recommendations for obtaining the highest quality relaxation rates.

### 5.3.1 Temperature control and calibration



Before starting with the spectral processing, it should be noted that proper temperature control and calibration are essential for relaxation data. Small temperature changes can have an effect on the viscosity and hence global tumbling of the molecule being studied and, as the molecular diffusion tensor is the major contributor to relaxation, any non-consistent data will likely lead to artificial motions appearing in subsequent model-free analyses.

Per-experiment temperature calibration is essential and the technique used will need to be specified for BMRB data deposition. Note that the standard MeOH/ethylene glycol calibration of a spectrometer is of no use when you are running experiments which pump in large amounts of power into the probe head. Although the  $R_1$  experiment should be about the same temperature as a HSQC and hence be close to the standard MeOH/ethylene glycol spectrometer calibration, the  $R_2$  CPMG or spin lock and, to a lesser extent, the NOE pre-saturation pump a lot more power into the probe head. The power differences can either cause the temperature in the sample to be too high or too low. This is unpredictable as the thermometer used by the VT unit is next to the coils in the probe head and not inside the NMR sample. So the VT unit tries to control the temperature inside the probe head rather than in the NMR sample. However between the thermometer and the sample is the water of the sample, the glass of the NMR tube, the air gap where the VT unit controls

air flow and the outside components of the probe head protecting the electronics. If the sample, the probe head or the VT unit is changed, this will have a different affect on the per-experiment temperature. The VT unit responds differently under different conditions and may sometimes over or under compensate by a couple of degrees. Therefore each relaxation data set from each spectrometer requires a per-experiment calibration.

Explicit temperature control techniques are also essential for relaxation data collection. Again the technique used will be asked for by relax for BMRB data deposition. A number of factors can cause significant temperature fluctuations between individual relaxation experiments. This includes the daily temperature cycle of the room housing the spectrometer, different amounts of power for the individual experiments, etc. The best methods for eliminating such problems are single scan interleaving and temperature compensation block. Single scan interleaving is the most powerful technique for averaging the temperature fluctuations not only across different experiments, but also across the entire measurement time. The application of off-resonance temperature compensation blocks at the start of the experiment is useful for the R2 and will normalise the temperature between the individual experiments, but single scan or single fid interleaving is nevertheless required for normalising the temperature across the entire measurement.

### 5.3.2 Spectral processing

For the best measurement of peak heights across the myriad of NMR spectral analysis software, it is recommend to zero fill a lot – 8k to 16k would give the best results. This does not increase the information content of the spectrum or decrease the errors, it simply interpolates. Even if the NMR spectral software performs 3-point quadratic interpolation between the highest points to determine the peak height, the additional free interpolation will make the estimation more accurate.

Additionally, care must be taken to properly scale the first point as this can cause a baseline roll which will affect peak heights. A very useful description comes directly from the [NMRPipe manual](#):

Depending on the delay, the first point of the FID should be adjusted before Fourier Transform. The first point scaling factor is selected by the window function argument `-c`.

If the required first order phase P1 for the given dimension is 0.0, the first point scaling factor should be 0.5. This is because the discrete Fourier transform does the equivalent of counting the point at  $t=0$  twice. If the first point is not scaled properly in this case, ridge-line baseline offsets in the spectrum will result.

In all other cases (P1 is not zero), this scale factor should be 1.0. This is because the first point of the FID no longer corresponds to  $t=0$ , and so it shouldn't be scaled. If the scale factor is not set correctly, it will introduce a baseline distortion which is either zero-order or sinusoidal, depending on what first-order phase is required. When possible, it is best to set up experiments with either exactly 0, 1/2, or 1-point delay. There are several reasons:

- Phase correction values can be determined easily.

Table 5.1: Summary, First Point Scaling and Phase Correction

Delay	P1	FID	Spectrum
0 point	0	Scale -c 0.5	
1/2 point	180	Scale -c 1.0	Folded peaks have opposite sign
1 point	360	Scale -c 1.0	Use “POLY -auto -ord 0”

- If the delay is not a multiple of 1/2 point, the phase of folded peaks will be distorted.
- The Hilbert transform (HT) is used, sometimes automatically, to reconstruct previously deleted imaginary data for interactive rephasing or inverse processing. But, the HT can only reconstruct imaginary data perfectly if the phase is a multiple of 1/2 point.
- Data with P1 = 360 have the first point t=0 missing (i.e. 1 point delay). Since the first point of the FID corresponds to the sum of points in the corresponding spectrum, this missing first point can be “restored” by adding a constant to the phased spectrum. This can be done conveniently by automated zero-order baseline correction, as shown in table 5.1.

Here is an example NMRPipe script designed for optimal relaxation rate extraction:

```

1  #!/bin/csh
2
3  setenv FILEROOT $1
4  set PHASE=81.4
5
6  echo "\n# Fourier Transform (nmrPipe fid/*.fid to ft/*.dat)"
7  echo "# t2 phase is set to $PHASE"
8  echo "# t1 phase is set to 0.0\n"
9
10 nmrPipe -in fid/$FILEROOT.fid \
11 | nmrPipe -fn SOL \
12 | nmrPipe -fn GM -g1 15 -g2 20 -c 0.5 \
13 | nmrPipe -fn ZF -size 8192 \
14 | nmrPipe -fn FT -auto \
15 | nmrPipe -fn PS -p0 $PHASE -p1 0.0 -di -verb \
16 | nmrPipe -fn TP \
17 | nmrPipe -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5 \
18 | nmrPipe -fn ZF -size 8192 \
19 | nmrPipe -fn FT -auto \
20 | nmrPipe -fn PS -p0 0.0 -p1 0.0 -di -verb \
21 | nmrPipe -fn TP \
22 | nmrPipe -fn POLY -auto \
23 | nmrPipe -fn EXT -left -sw \
24 | nmrPipe -out ft/$FILEROOT.dat -ov

```

The script is run by supplying the FILEROOT value as a command line option so if the script is called `nmrpipe.sh` and the `var2pipe` or `bruk2pipe` processed file `R1_ncyc4.fid` is in the `fid` directory, you would run:

```
$ ./nmrpipe.sh R1_ncyc4
```

The `ft` directory must exist for this script to execute. Different experiment specific options may be needed such as:

```
| nmrPipe -fn REV \
| nmrPipe -fn FT -neg \
| nmrPipe -fn PS -rs 2.5 \
```

The script should be changed for different phasing, first point scaling, a polynomial baseline correction added in the direct dimension or removed from the indirect dimension, solvent suppression removed or changed, and the window functions modified for optimal spectral quality. Each system and spectrum is different, so it is recommended that to find the optimal processing that each part of the script be removed and re-added one-by-one between processing and checking of the resultant spectrum. Note that the extraction at the end after the polynomial baseline correction in the indirect dimension is important as the baseline correction often displays a much better performance when the empty part of the spectrum is used in the calculation.

### 5.3.3 Measuring peak intensities

For the measurement of peak intensities, again care must be taken. A read of the paper:

- Viles, J., Duggan, B., Zaborowski, E., Schwarzing, S., Huntley, J., Kroon, G., Dyson, H. J., and Wright, P. (2001). Potential bias in NMR relaxation data introduced by peak intensity analysis and curve fitting methods. *J. Biomol. NMR*, **21**, 1–9. ([10.1023/A:1011966718826](https://doi.org/10.1023/A:1011966718826))

is highly recommended. Despite the recommendations in the discussion of this paper, a different methodology using peak heights can be used to solve the same problems. This will be discussed in a paper which is currently in preparation from the Gooley group. The steps involved are:

- For the first spectrum in the time series, shift the peak list to the tops of the peaks (for example using “pc” in Sparky on subsets of peaks).
- Copy this 1<sup>st</sup> spectrum list onto all spectra, shifting the peaks to the top as in the previous step.
- When the peak disappears into the noise, leave it at its current position and do not type “pc” or equivalent. This will add weight to the first point in the subsequent step.
- Once all spectra are shifted, calculate an average peak list.
- Copy this average peak list onto fresh copies of all spectra.
- Measure peak heights using this averaged peak list.

This will produce the most accurate peak intensity measurements until better, more robust peak shape integration comes along. This is a special technique which is designed to minimise the white-noise bias talked about in the [Viles et al. \(2001\)](#) paper. As the noise

often decreases with the decrease in total spectral power, using the tops of the peaks means that you are actually measuring the real peak height plus positive noise in all cases. This non-constant additional positive noise contribution can result in a double exponential in the measured data. The technique above eliminates this as you then measure close to real peak height with the addition of white noise centred at zero – it is both negative and positive to equal amounts – rather than the peak high with noise contribution strongly biased towards the positive. Where the peaks disappear, you then are measuring the pure baseplane noise. This is fine as these white-noise data points centred at zero will help in the subsequent exponential fit in relax.

If using Sparky then, to be sure that the peak heights are properly updated, for each spectrum type “pa” to select all peaks, “ph” to update all selected peak heights, “lt” to show the spectrum peaks window, make sure “data height” is selected in the options, and then save the peak list.

## 5.4 Relaxation curve-fitting in the prompt/script UI mode

### 5.4.1 Relax-fit script mode – the sample script

The following is a verbatim copy of the contents of the `sample_scripts/relax_fit.py` file. If your copy of the sample script is different than that below, please send an email to the relax-devel mailing list to tell the relax developers that the manual is out of date (see section 3.2.3 on page 30). You will need to first copy this script to a dedicated analysis directory containing peak lists, a PDB file and a file listing unresolved spin systems, and then modify its contents to suit your specific analysis. The script contents are:

```

1  # Script for relaxation curve-fitting.
2
3  # Create the 'rx' data pipe.
4  pipe.create('rx', 'relax_fit')
5
6  # Load the backbone amide 15N spins from a PDB file.
7  structure.read_pdb('Ap4Aase_new_3.pdb')
8  structure.load_spins(spin_id='@N')
9  structure.load_spins(spin_id='@NE1')
10
11 # Spectrum names.
12 names = [
13     'T2_ncyc1_ave',
14     'T2_ncyc1b_ave',
15     'T2_ncyc2_ave',
16     'T2_ncyc4_ave',
17     'T2_ncyc4b_ave',
18     'T2_ncyc6_ave',
19     'T2_ncyc9_ave',
20     'T2_ncyc9b_ave',
21     'T2_ncyc11_ave',
22     'T2_ncyc11b_ave'
23 ]
24
25 # Relaxation times (in seconds).
26 times = [
27     0.0176,
28     0.0176,
```



```

29     0.0352,
30     0.0704,
31     0.0704,
32     0.1056,
33     0.1584,
34     0.1584,
35     0.1936,
36     0.1936
37 ]
38
39 # Loop over the spectra.
40 for i in range(len(names)):
41     # Load the peak intensities.
42     spectrum.read_intensities(file=names[i]+'.list', dir=data_path, spectrum_id=names[i],
43                             int_method='height')
44
45     # Set the relaxation times.
46     relax_fit.relax_time(time=times[i], spectrum_id=names[i])
47
48     # Specify the duplicated spectra.
49     spectrum.replicated(spectrum_ids=['T2_ncyc1_ave', 'T2_ncyc1b_ave'])
50     spectrum.replicated(spectrum_ids=['T2_ncyc4_ave', 'T2_ncyc4b_ave'])
51     spectrum.replicated(spectrum_ids=['T2_ncyc9_ave', 'T2_ncyc9b_ave'])
52     spectrum.replicated(spectrum_ids=['T2_ncyc11_ave', 'T2_ncyc11b_ave'])
53
54     # Peak intensity error analysis.
55     spectrum.error_analysis()
56
57     # Deselect unresolved spins.
58     deselect.read(file='unresolved', mol_name_col=1, res_num_col=2, res_name_col=3,
59                 spin_num_col=4, spin_name_col=5)
60
61     # Set the relaxation curve type.
62     relax_fit.select_model('exp')
63
64     # Grid search.
65     minimise.grid_search(inc=11)
66
67     # Minimise.
68     minimise.execute('newton', constraints=False)
69
70     # Monte Carlo simulations.
71     monte_carlo.setup(number=500)
72     monte_carlo.create_data()
73     monte_carlo.initial_values()
74     minimise.execute('newton', constraints=False)
75     monte_carlo.error_analysis()
76
77     # Save the relaxation rates.
78     value.write(param='rx', file='rx.out', force=True)
79
80     # Save the results.
81     results.write(file='results', force=True)
82
83     # Create Grace plots of the data.
84     grace.write(y_data_type='chi2', file='chi2.agr', force=True)    # Minimised chi-squared
85                             value.
86     grace.write(y_data_type='i0', file='i0.agr', force=True)      # Initial peak intensity.
87     grace.write(y_data_type='rx', file='rx.agr', force=True)      # Relaxation rate.
88     grace.write(x_data_type='relax_times', y_data_type='peak_intensity', file='intensities.agr
89                 ', force=True)    # Average peak intensities.

```

```

86  grace.write(x_data_type='relax_times', y_data_type='peak_intensity', norm=True, file='
      intensities_norm.agr', force=True)    # Average peak intensities (normalised).
87
88  # Display the Grace plots.
89  grace.view(file='chi2.agr')
90  grace.view(file='i0.agr')
91  grace.view(file='rx.agr')
92  grace.view(file='intensities.agr')
93  grace.view(file='intensities_norm.agr')
94
95  # Save the program state.
96  state.save('rx.save', force=True)

```

The next sections will break this script down into its logical components and explain how these parts will be interpreted by relax. To execute this script, please see section 1.2.8 on page 11 for details.

### 5.4.2 Relax-fit script mode – initialisation of the data pipe

The data pipe is simply created by the command

```

3  # Create the 'rx' data pipe.
4  pipe.create('rx', 'relax_fit')

```

This user function will then create a relaxation exponential curve-fitting specific data pipe labelled “rx”. The second argument sets the pipe type to that of the relaxation curve-fitting. Setting the pipe type is important so that the program knows which user functions are compatible with the data pipe, for example in the steady-state NOE analysis the function `minimise.execute` (see page 493) is meaningless as the NOE values are calculated directly rather than optimised.

### 5.4.3 Relax-fit script mode – setting up the spin systems

The first thing which needs to be completed prior to any spin specific command is to generate the molecule, residue and spin data structures for storing the spin specific data. In the sample script above this is generated from a PDB file, however a plain text file with the sequence information can be used instead (see the `sequence.read` user function on page 602 for more details). In the case of the sample script, the command

```

6  # Load the backbone amide 15N spins from a PDB file.
7  structure.read_pdb(name, 'Ap4Aase_new_3.pdb')

```

will load the PDB file `Ap4Aase_new_3.pdb` into relax. Then

```

8  structure.load_spins(spin_id='@N')
9  structure.load_spins(spin_id='@NE1')

```

will generate the molecule, residue, and spin sequence for the current data pipe. In this situation there will be a single spin system per residue generated corresponding to the backbone amide nitrogens as well as  $^{15}\text{N}$  spins set up for the tryptophan indole nitrogens. Although the 3D coordinates have been loaded into the program from the PDB file, this structural information serves no purpose when calculating  $R_1$  and  $R_2$  values.

#### 5.4.4 Relax-fit script mode – loading the data

To load the peak intensities into relax the `spectrum.read_intensities` and `relax_fit.relax_time` user functions are executed. Important keyword arguments for these user functions are the file name and directory, the spectrum identification string and the relaxation time period of the experiment in seconds. By default the file format will be automatically detected. Currently Sparky, XEasy, NMRView, and generic columnar formatted peak lists are supported. To be able to import any other type of format please send an email to the relax development mailing list with the details of the format. Adding support for new formats is trivial. The following series of commands – an expansion of the `for` loop in the sample script – will load peak intensities from six different relaxation periods, four of which have been duplicated, from Sparky peak lists with the peak heights in the 10<sup>th</sup> column.

```
spectrum.read_intensities('T2_ncyc1.list', spectrum_id='1', int_col=10)
relax_fit.relax_time(spectrum_id='1', time=0.0176)
spectrum.read_intensities('T2_ncyc1b.list', spectrum_id='1b', int_col=10)
relax_fit.relax_time(spectrum_id='1b', time=0.0176)
spectrum.read_intensities('T2_ncyc2.list', spectrum_id='2', int_col=10)
relax_fit.relax_time(spectrum_id='2', time=0.0352)
spectrum.read_intensities('T2_ncyc4.list', spectrum_id='4', int_col=10)
relax_fit.relax_time(spectrum_id='4', time=0.0704)
spectrum.read_intensities('T2_ncyc4b.list', spectrum_id='4b', int_col=10)
relax_fit.relax_time(spectrum_id='4b', time=0.0704)
spectrum.read_intensities('T2_ncyc6.list', spectrum_id='6', int_col=10)
relax_fit.relax_time(spectrum_id='6', time=0.1056)
spectrum.read_intensities('T2_ncyc9.list', spectrum_id='9', int_col=10)
relax_fit.relax_time(spectrum_id='9', time=0.1584)
spectrum.read_intensities('T2_ncyc9b.list', spectrum_id='9b', int_col=10)
relax_fit.relax_time(spectrum_id='9b', time=0.1584)
spectrum.read_intensities('T2_ncyc11.list', spectrum_id='11', int_col=10)
relax_fit.relax_time(spectrum_id='11', time=0.1936)
spectrum.read_intensities('T2_ncyc11b.list', spectrum_id='11b', int_col=10)
relax_fit.relax_time(spectrum_id='11b', time=0.1936)
```

The replicated spectra a set up with the commands

```
47 # Specify the duplicated spectra.
48 spectrum.replicated(spectrum_ids=['T2_ncyc1_ave', 'T2_ncyc1b_ave'])
49 spectrum.replicated(spectrum_ids=['T2_ncyc4_ave', 'T2_ncyc4b_ave'])
50 spectrum.replicated(spectrum_ids=['T2_ncyc9_ave', 'T2_ncyc9b_ave'])
51 spectrum.replicated(spectrum_ids=['T2_ncyc11_ave', 'T2_ncyc11b_ave'])
```

Note that the relaxation time period should be calculated directly from the pulse sequence (as the sum of delays and pulses for the period), as the estimated time may not match the real time. For the Sparky peak lists, by default relax assumes that the intensity value is in the 4<sup>th</sup> column. A typical file looks like:

Assignment	w1	w2	Data Height
LEU3N-HN	122.454	8.397	129722
GLY4N-HN	111.999	8.719	422375
SER5N-HN	115.085	8.176	384180
MET6N-HN	120.934	8.812	272100
ASP7N-HN	122.394	8.750	174970
SER8N-HN	113.916	7.836	218762
GLU11N-HN	122.194	8.604	30412
GLY12N-HN	110.525	9.028	90144

By supplying the `int_col` argument to the `spectrum.read_intensities` user function, this can be changed. A typical XEasy file will look like:

No.	Color	w1	w2	ass. in w1		ass. in w2		Volume	Vol. Err.	Method	Comment
2	2	10.014	134.221	HN	21 LEU	N	21 LEU	7.919e+03	0.00e+00	m	
3	2	10.481	132.592	HE1	79 TRP	NE1	79 TRP	1.532e+04	0.00e+00	m	
17	2	9.882	129.041	HN	110 PHE	N	110 PHE	9.962e+03	0.00e+00	m	
18	2	8.757	128.278	HN	52 ASP	N	52 ASP	2.041e+04	0.00e+00	m	
19	2	10.086	128.297	HN	69 SER	N	69 SER	9.305e+03	0.00e+00	m	
20	3	9.111	127.707	HN	15 ARG	N	15 ARG	9.714e+03	0.00e+00	m	

where the peak height is in the `Volume` column. And for an NMRView file:

```
label dataset sw sf
H1 N15
cNtnc_noe0.nv
2505.63354492 1369.33557129
499.875 50.658000946
H1.L H1.P H1.W H1.B H1.E H1.J H1.U N15.L N15.P N15.W N15.B N15.E N15.J N15.U vol int stat comment flag0
0 {70.HN} 10.75274 0.02954 0.05379 ++ 0.0 {} {70.N} 116.37241 0.23155 0.35387 ++ 0.0 {} -6.88333129883 -0.1694 0 {} 0
1 {72.HN} 9.67752 0.03308 0.05448 ++ 0.0 {} {72.N} 126.41302 0.27417 0.37217 ++ 0.0 {} -5.49038267136 -0.1142 0 {} 0
2 {} 8.4532 0.02331 0.05439 ++ 0.0 {} {} 122.20137 0.38205 0.33221 ++ 0.0 {} -2.58034267191 -0.1320 0 {} 0
```

### 5.4.5 Relax-fit script mode – the rest of the setup

Once all the peak intensity data has been loaded a few calculations are required prior to optimisation. Firstly the peak intensities for individual spins needs to be averaged across replicated spectra. The peak intensity errors also have to be calculated using the standard deviation formula. These two operations are executed by the user function

```
53 # Peak intensity error analysis.
54 spectrum.error_analysis()
```

Any spins which cannot be resolved due to peak overlap were included in a file called `unresolved`. This file can consist of optional columns of the molecule name, the residue name and number, and the spin name and number. The matching spins are excluded from the analysis by the user function

```
56 # Deselect unresolved spins.
57 deselect.read(file='unresolved', mol_name_col=1, res_num_col=2, res_name_col=3,
    spin_num_col=4, spin_name_col=5)
```

Finally the experiment type is specified by the command

```
59 # Set the relaxation curve type.
60 relax_fit.select_model('exp')
```

The argument “`exp`” sets the relaxation curve to a two parameter  $\{R_x, I_0\}$  exponential which decays to zero. Changing the user function argument to “`inv`” will select the inversion recovery experiment, and changing it to “`sat`” will select the saturation recovery experiment (see section 5.2 on page 49).

### 5.4.6 Relax-fit script mode – optimisation of exponential curves

Now that everything has been setup minimisation can be used to optimise the parameter values. Firstly a grid search is applied to find a rough starting position for the subsequent optimisation algorithm. Eleven increments per dimension of the model (in this case the two dimensions  $\{R_x, I_0\}$ ) is sufficient. The user function for executing the grid search is

```
62 # Grid search.
63 minimise.grid_search(inc=11)
```

The next step is to select one of the minimisation algorithms to optimise the model parameters

```
65 # Minimise.
66 minimise.execute('newton', constraints=False)
```

### 5.4.7 Relax-fit script mode – error analysis

Only one technique adequately estimates parameter errors when the parameter values where found by optimisation – Monte Carlo simulations. In relax this can be implemented by using a series of functions from the `monte_carlo` user function class. Firstly the number of simulations needs to be set

```
68 # Monte Carlo simulations.
69 monte_carlo.setup(number=500)
```

For each simulation, randomised relaxation curves will be fit using exactly the same methodology as the original exponential curves. These randomised curves are created by back calculation from the fitted model parameter values and then each point on the curve randomised using the error values set earlier in the script

```
70 monte_carlo.create_data()
```

As a grid search for each simulation would be too computationally expensive, the starting point for optimisation for each simulation can be set to the position of the optimised parameter values of the model

```
71 monte_carlo.initial_values()
```

Then exactly the same optimisation as was used for the model can be performed

```
72 minimise.execute('newton', constraints=False)
```

The parameter errors are then determined as the standard deviation of the optimised parameter values of the simulations

```
73 monte_carlo.error_analysis()
```

### 5.4.8 Relax-fit script mode – finishing off

To finish off, the script first saves the relaxation rates together with their errors in a simple text file

```
75 # Save the relaxation rates.
76 value.write(param='rx', file='rx.out', force=True)
```

Grace plots are created and viewed

```
81 # Create Grace plots of the data.
82 grace.write(y_data_type='chi2', file='chi2.agr', force=True)    # Minimised chi-squared
    value.
83 grace.write(y_data_type='i0', file='i0.agr', force=True)        # Initial peak intensity.
84 grace.write(y_data_type='rx', file='rx.agr', force=True)        # Relaxation rate.
85 grace.write(x_data_type='relax_times', y_data_type='peak_intensity', file='intensities.agr
    ', force=True)        # Average peak intensities.
86
87 grace.write(x_data_type='relax_times', y_data_type='peak_intensity', norm=True, file='
    intensities_norm.agr', force=True)    # Average peak intensities (normalised).
```

and viewed

```
88 # Display the Grace plots.
89 grace.view(file='chi2.agr')
90 grace.view(file='i0.agr')
91 grace.view(file='rx.agr')
92 grace.view(file='intensities.agr')
93 grace.view(file='intensities_norm.agr')
```

and finally the program state is saved for future reference

```
95 # Save the program state.
96 state.save(file='rx.save', force=True)
```

## 5.5 The relaxation curve-fitting auto-analysis in the GUI

The  $R_1$  and  $R_2$  relaxation rates can be calculated using the relax GUI (see Figures 1.6 and 1.7). These auto-analyses can be selected using the analysis selection wizard (Figure 1.4 on page 12). Just as with the steady-state NOE in the next chapter, these auto-analyses are very similar in spirit to the sample script described in this chapter, though the Grace 2D visualisation is more advanced. If you have read this chapter, the usage of these analyses should be self explanatory.

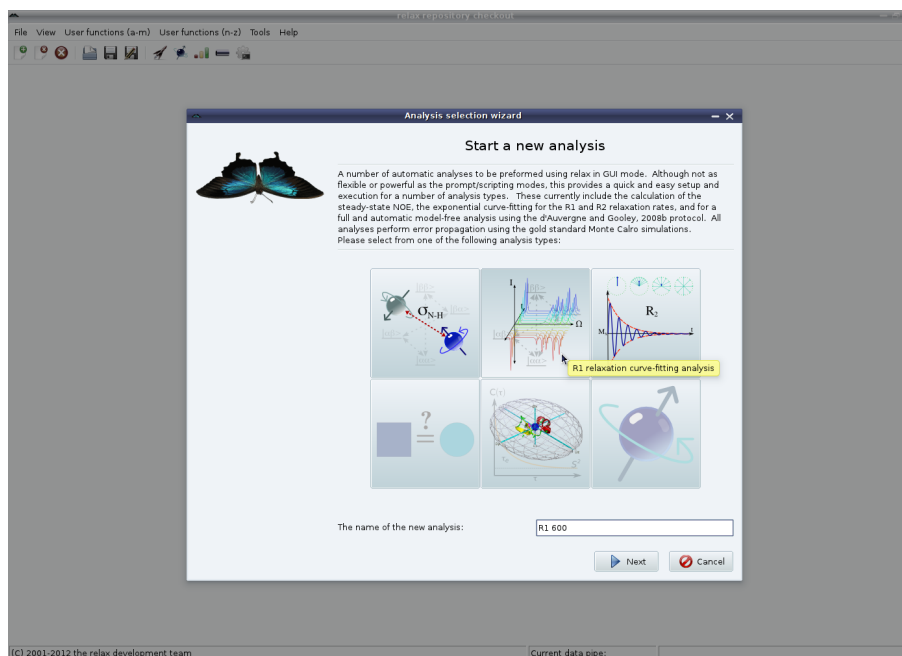
As in the script/prompt UI section above, the example of protein  $^{15}\text{N}$   $R_1$  relaxation analysis will be performed in the following sections. To keep track of all the messages relax produces for future reference, you can run the relax GUI with the following command line arguments:

```
$ relax --log log --gui
```

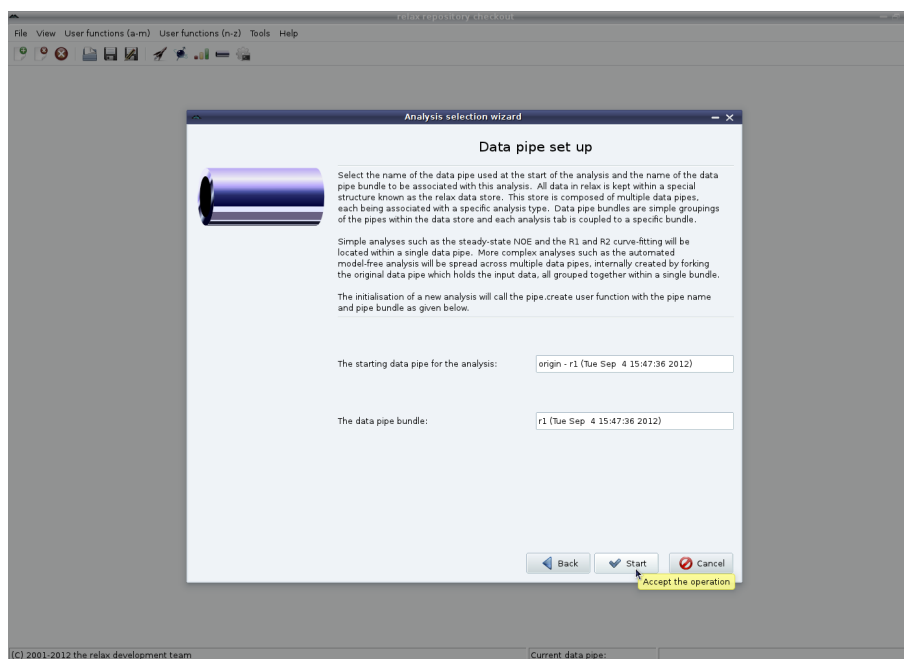
The messages will then appear both in the relax controller window (see Figure 1.9 on page 19) and in the log file.

### 5.5.1 Relax-fit GUI mode – initialisation of the data pipe

To begin the analysis, launch the analysis selection wizard (see Figure 1.4 on page 12). Select either the  $R_1$  or  $R_2$  analyses, and change the name of the analysis if you plan on running multiple analyses from different field strengths in one relax instance.

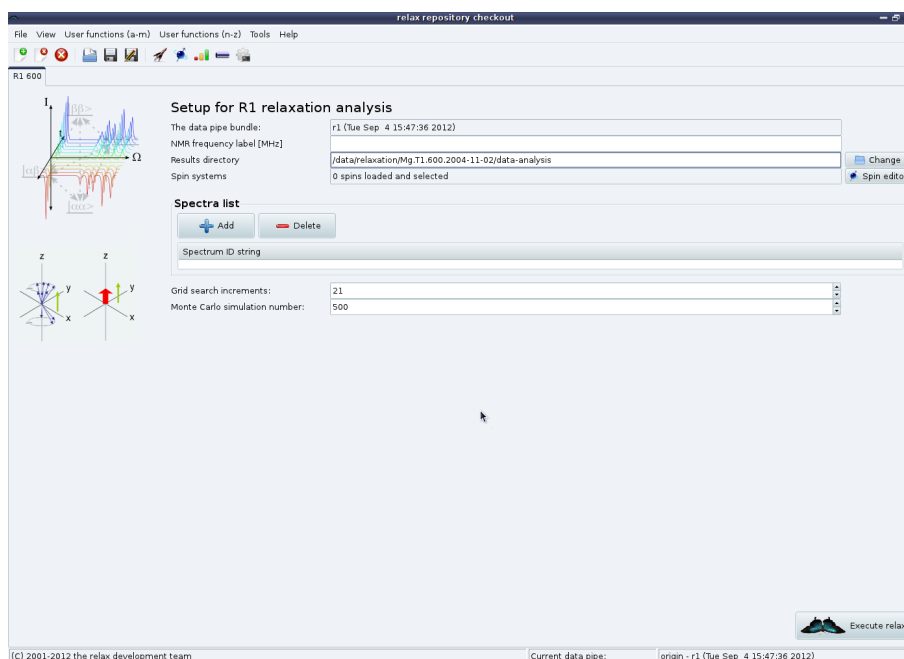


Then click on the “Next” button. On the second page click on “Start” to commence the analysis – this second part of the wizard does not need to be changed. For the  $R_1$  and  $R_2$  analyses in the GUI, a data pipe bundle containing only a single data pipe for that analysis will be created. This data pipe bundle can be safely ignored.



### 5.5.2 Relax-fit GUI mode – general setup

You will now be presented with a blank analysis tab:



Here there are two things unique to the GUI which need to be preformed:

**NMR frequency label:** First set the NMR frequency label. This is only used for the name of the output file. For example if you set the label to “1200”, the file `r1.1200.out` will be created at the end of the analysis.

**Results directory:** All of the automatically created results and Grace files will be placed into this directory. The “Results directory” can now be changed.



### 5.5.3 Relax-fit GUI mode – setting up the spin systems

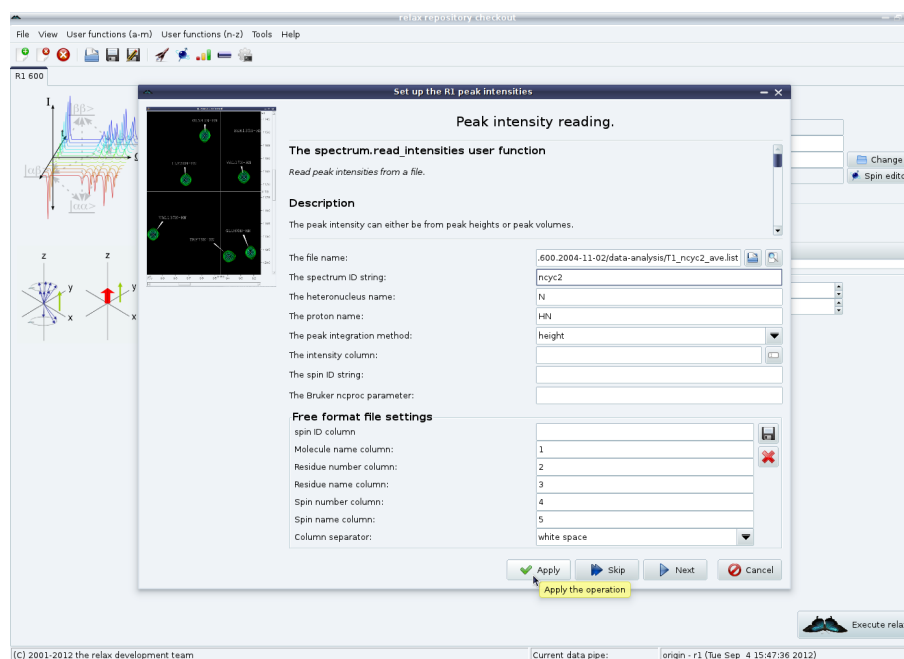
As the relaxation data is at the level of the spins, the molecule, residue and spin data structures need to be set up. In the  $R_1$  and  $R_2$  GUI analysis tabs, there is a special “Spin systems” GUI element designed for this. This will initially say “0 spins loaded and selected”. Click on the “Spin editor” button to launch the spin viewer window. The steps for setting up the spin containers using PDB files are described in section 4.5.2 on page 40 or for sequence files in section 4.5.3 on page 43.

### 5.5.4 Relax-fit GUI mode – unresolved spins

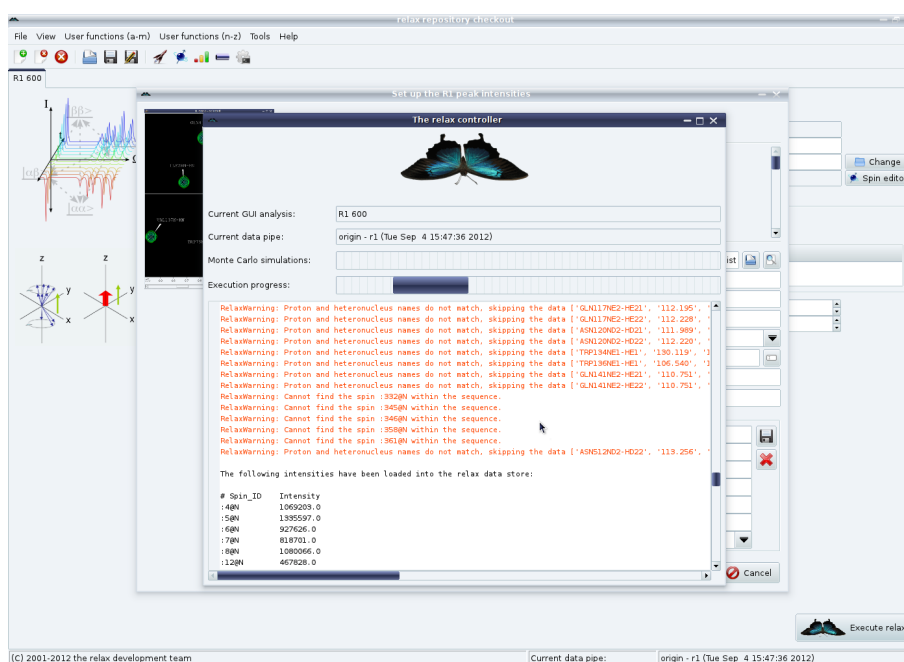
As in the prompt/script UI section 5.4.5, the spins can be deselected at this point using the same `unresolved` file. This is described in detail in section 4.5.5 on page 44.

### 5.5.5 Relax-fit GUI mode – loading the data

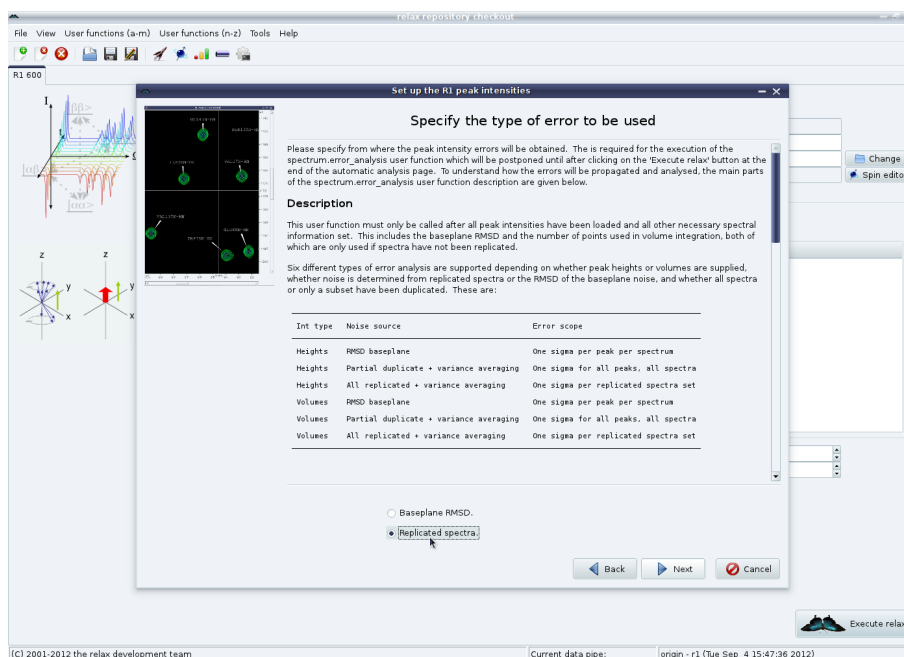
At this stage, the peak intensity data needs to be loaded. In both the  $R_1$  and  $R_2$  analysis tabs is a “Spectra list” GUI element. Click on the “Add” button to launch the peak intensity loading wizard:



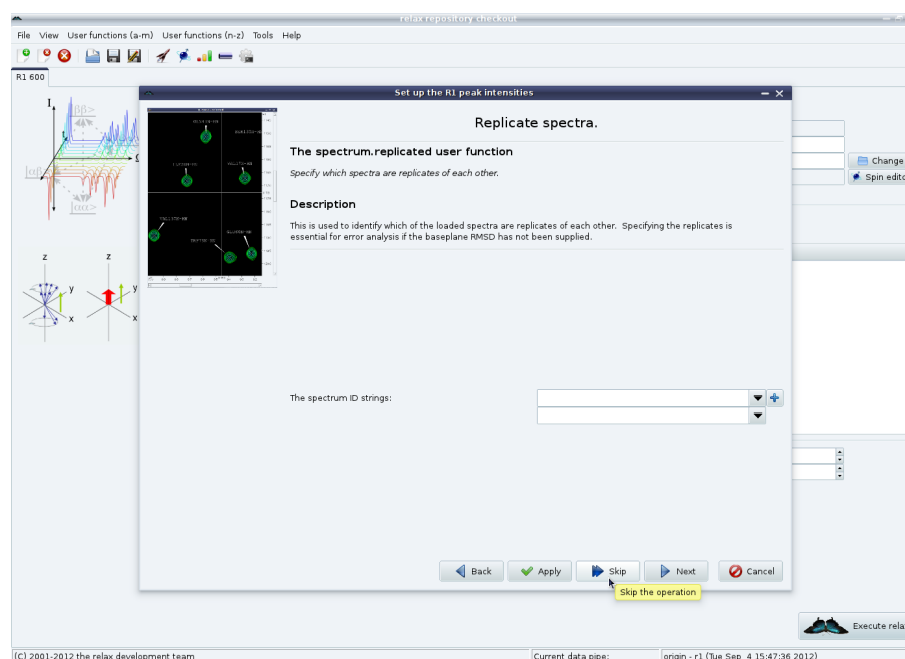
In this example, a Sparky peak list containing the peak heights determined from the averaged chemical shift positions for all spectra will be loaded. Set the spectrum ID string to a unique value. Click on “Next”. This will most likely cause a `RelaxWarning` message to appear for all peak list elements which do not correspond to any spins loaded into the relax data store:



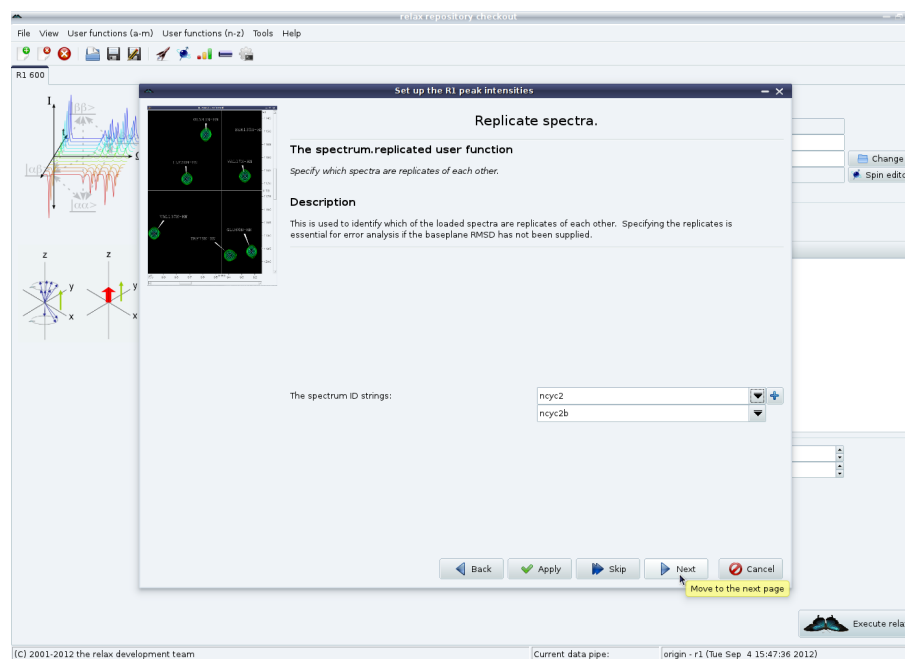
These messages must be carefully checked to be sure that the correct data has been loaded. A `RelaxError` might be thrown if the peak list is corrupted or if the dimension has been incorrectly given. In this case check the message, go “Back”, fix the problem, and click on “Next” again. Then click on “Next”. You should now see the error type page:



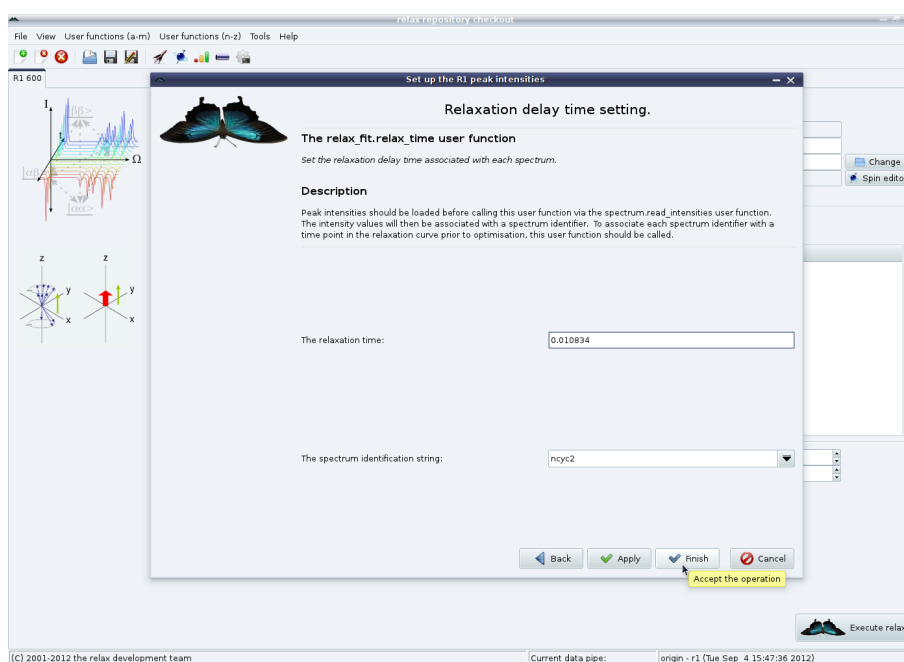
The description for this wizard page should be very carefully read – it will tell you about all of the error analysis options available and how these are implemented in relax. For the protein relaxation example, replicated spectra have been collected. Therefore the option “Replicated spectra” will be chosen. The “Baseplane RMSD” option is documented in the NOE chapter. After clicking on “Next” you will see:



For the first of the duplicate spectra, or any spectrum without a duplicate, you can click on the “Skip” button. If this is the second spectrum you have loaded from a duplicated set, select the two replicated spectra and then click on “Next”:

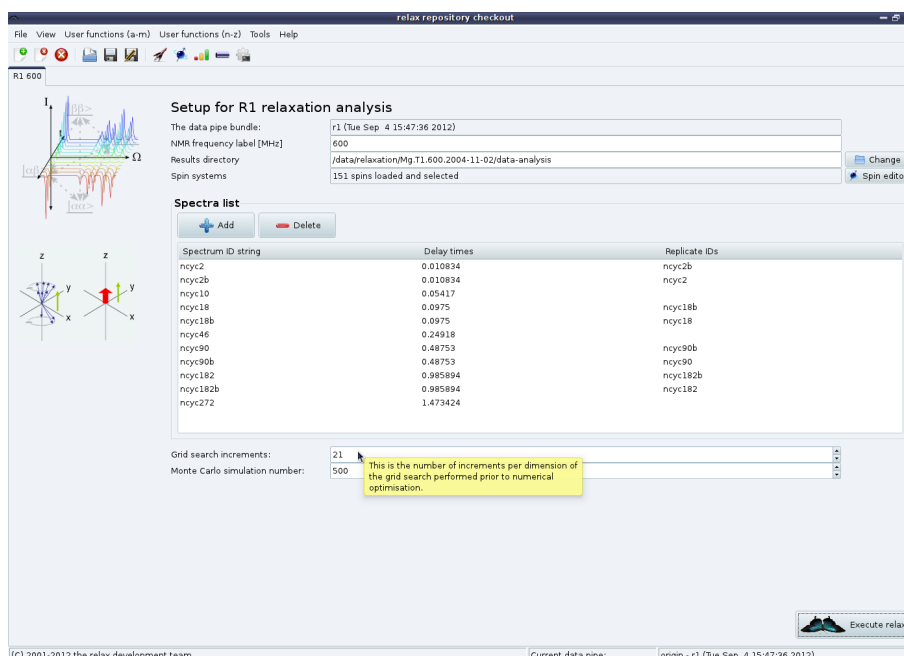


Finally set the relaxation time period for this experiment in seconds:



All delays and pulse lengths in the pulse sequence should be carefully checked to be sure that the time is exactly what you would expect – the estimated time may not match the real time. To set the time and close the wizard, click on the “Finish” button.

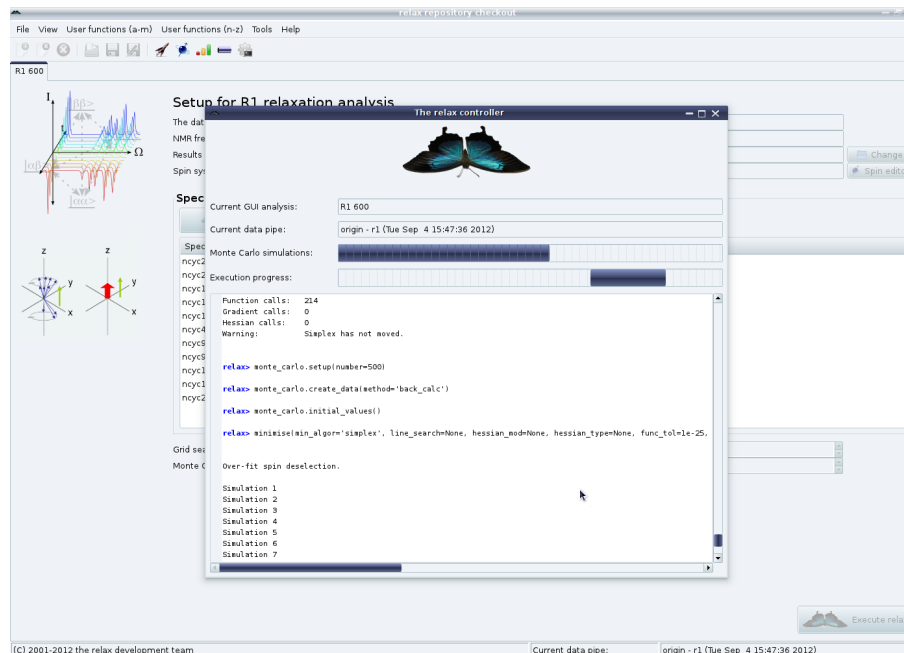
This procedure should be repeated for every experiment you have collected (you could, as an alternative, load all at the same time using the “Apply” button at each stage). In the end you should see something such as:



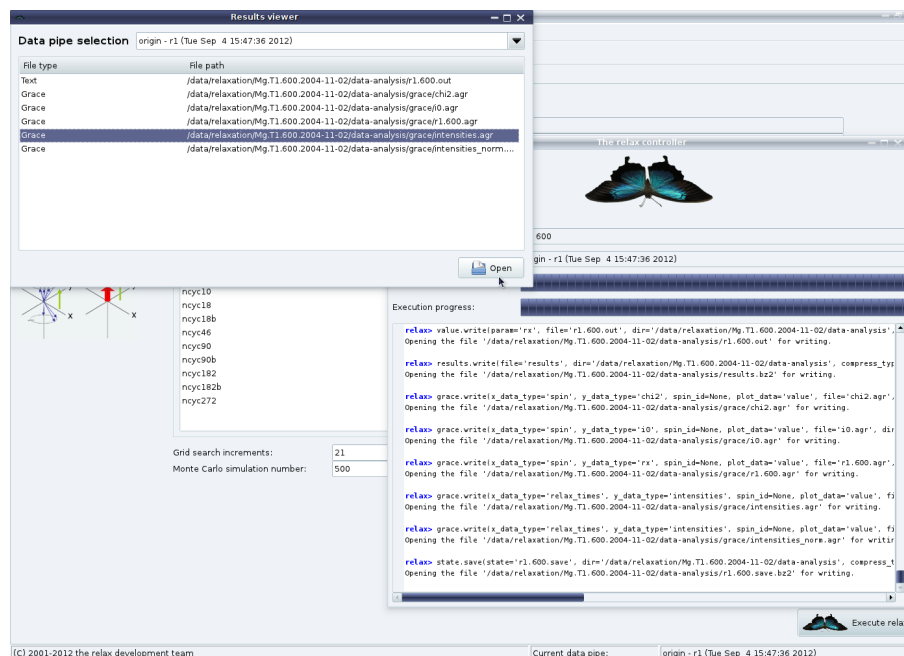
### 5.5.6 Relax-fit GUI mode – optimisation and error analysis

Back in the main  $R_1$  analysis tab, the grid search increments and number of Monte Carlo simulations can be changed. The default values of 21 grid search increments and 500 MC

simulations are optimal – lower values are not recommended. To perform the optimisation and error analysis, click on the “Execute relax” button. The relax controller will open to show you the progress of the optimisation and simulations:



Once finished, the “Results viewer” window will also appear:



This window can be used to open the text files in the default text editor for your operating system or the 2D Grace plots in *xmgrace* if available on your system.

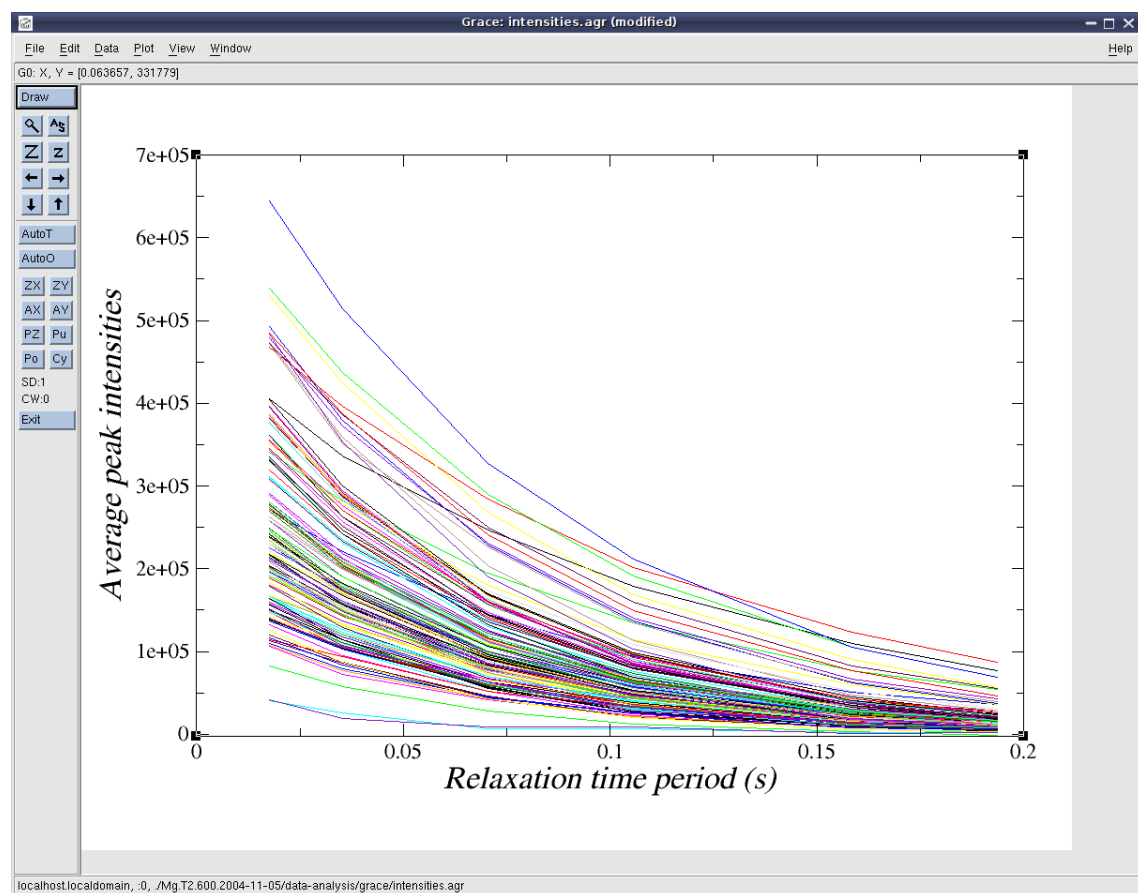


Figure 5.1: Screenshot of the 2D peak intensity plots for the exponential relaxation curves in Xmgrace.

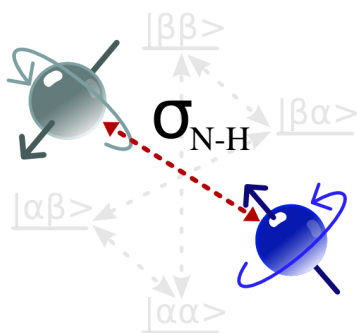
## 5.6 Final checks of the curve-fitting

To be sure that the data has been properly collected and that no instrumentation or pulse sequence timing errors have occurred, it is essential to carefully check the `intensities.agr` and `intensities_norm.agr` 2D Grace files. These are plots of the decay curves for each spin system analysed, and any non-exponential behaviour should be clearly visible (see Figure 5.1). If Xmgrace or a compatible program is not available for your operating system, the Grace files contain text representations of the curves at the end which can be opened, edited and visualised in any other 2D graphing software package.

Note that errors resulting in systematic bias in the data – for example if temperature control (single-scan interleaving or temperature compensation blocks) or per-experiment/per-spectrometer temperature calibration on MeOH or ethylene glycol have not been performed – will not be detected by looking at the decay curves. See section 5.3.1 or the `relax_data.temp_calibration` user function documentation on page 570 and the `relax_data.temp_control` user function documentation on page 571 for more details.

## Chapter 6

# Calculating the NOE



### 6.1 Introduction to the steady-state NOE

The calculation of NOE values is a straight forward and quick procedure which involves two components – the calculation of the value itself and the calculation of the errors. To understand the steps involved the execution of a sample NOE calculation script will be followed in detail. Then the same operations will be presented for the perspective of the graphical user interface.

### 6.2 From spectra to peak intensities for the NOE

For a set of recommendations for how to obtain the best quality relaxation rates, please see section 5.3 on page 50. In summary the following are important – temperature control (though the standard steady-state NOE single FID interleaved pulse sequences are fine), per-experiment temperature calibration, spectral processing with massive zero-filling and no baseplane rolling, and using an averaged peak list for determining the peak heights.

## 6.3 Calculation of the NOE in the prompt/script UI mode

### 6.3.1 NOE script mode – the sample script

This sample script can be found in the `sample_scripts` directory and will be used as the template for the next sections describing how to use relax.

```

1  # Script for calculating NOEs.
2
3  # Create the data pipe.
4  pipe.create('NOE', 'noe')
5
6  # Load the sequence from a PDB file.
7  structure.read_pdb('Ap4Aase_new_3.pdb')
8  structure.load_spins(spin_id='@N')
9  structure.load_spins(spin_id='@NE1')
10
11 # Load the reference spectrum and saturated spectrum peak intensities.
12 spectrum.read_intensities(file='ref.list', spectrum_id='ref_ave')
13 spectrum.read_intensities(file='sat.list', spectrum_id='sat_ave')
14
15 # Set the spectrum types.
16 noe.spectrum_type('ref', 'ref_ave')
17 noe.spectrum_type('sat', 'sat_ave')
18
19 # Set the errors.
20 spectrum.baseplane_rmsd(error=3600, spectrum_id='ref_ave')
21 spectrum.baseplane_rmsd(error=3000, spectrum_id='sat_ave')
22
23 # Individual residue errors.
24 spectrum.baseplane_rmsd(error=122000, spectrum_type='ref', res_num=114)
25 spectrum.baseplane_rmsd(error=8500, spectrum_type='sat', res_num=114)
26
27 # Peak intensity error analysis.
28 spectrum.error_analysis()
29
30 # Deselect unresolved spins.
31 deselect.read(file='unresolved', res_num_col=1, spin_name_col=2)
32
33 # Calculate the NOEs.
34 minimise.calculate()
35
36 # Save the NOEs.
37 value.write(param='noe', file='noe.out', force=True)
38
39 # Create Grace files.
40 grace.write(y_data_type='peak_intensity', file='intensities.agr', force=True)
41 grace.write(y_data_type='noe', file='noe.agr', force=True)
42
43 # View the Grace files.
44 grace.view(file='intensities.agr')
45 grace.view(file='noe.agr')
46
47 # Write the results.
48 results.write(file='results', dir=None, force=True)
49
50 # Save the program state.
51 state.save('save', force=True)

```



### 6.3.2 NOE script mode – initialisation of the data pipe

The start of this sample script is very similar to that of the relaxation curve-fitting calculation on page 56. The command

```
3 # Create the data pipe.
4 pipe.create('NOE', 'noe')
```

initialises the data pipe labelled “NOE”. The data pipe type is set to the NOE calculation by the argument “noe”.

### 6.3.3 NOE script mode – setting up the spin systems

The backbone amide nitrogen sequence is extracted from a PDB file using the same commands as the relaxation curve-fitting script (Chapter 5). The command

```
6 # Load the sequence from a PDB file.
7 structure.read_pdb('Ap4Aase_new_3.pdb')
```

will load the PDB file `Ap4Aase_new_3.pdb` into relax. Then the following commands will generate both the backbone amide and tryptophan indole  $^{15}\text{N}$  spins

```
8 structure.load_spins(spin_id='@N')
9 structure.load_spins(spin_id='@NE1')
```

### 6.3.4 NOE script mode – loading the data

The commands

```
11 # Load the reference spectrum and saturated spectrum peak intensities.
12 spectrum.read_intensities(file='ref.list', spectrum_id='ref_ave')
13 spectrum.read_intensities(file='sat.list', spectrum_id='sat_ave')
```

will load the peak heights of the reference and saturated NOE experiments (although the volume could be used instead). relax will automatically determine the format of the peak list. Currently only Sparky, XEasy, NMRView and a generic columnar formatted text file are supported.

In this example, relax will determine from the file contents that these are Sparky peak lists (saved after typing “lt”). The first column of the file should be the Sparky assignment string and it is assumed that the 4<sup>th</sup> column contains either the peak height or peak volume (though this can be in any column – the `int_col` argument is used to specify where the data is). Without specifying the `int_method` argument, peak heights will be assumed. See page 608 for a description of all the `spectrum.read_intensities` user function arguments. In this example, the peak list looks like:

Assignment	w1	w2	Data Height
LEU3N-HN	122.454	8.397	129722
GLY4N-HN	111.999	8.719	422375
SER5N-HN	115.085	8.176	384180

MET6N-HN	120.934	8.812	272100
ASP7N-HN	122.394	8.750	174970
SER8N-HN	113.916	7.836	218762
GLU11N-HN	122.194	8.604	30412
GLY12N-HN	110.525	9.028	90144

For subsequent usage of the data in relax, assuming a 3D structure exists, it is currently advisable to use the same residue and atom numbering as found in the PDB file.

If you have any other format you would like read by relax please send an email to the relax development mailing list detailing the software used, the format of the file (specifically where the residue number and peak intensity are located), and possibly attaching an example of the file itself.

### 6.3.5 NOE script mode – setting the errors

In this example the errors were measured from the base plain noise. The Sparky RMSD function was used to estimate the maximal noise levels across the spectrum in regions containing no peaks. For the reference spectrum the RMSD was approximately 3600 whereas in the saturated spectrum the RMSD was 3000. These errors are set by the commands

```
19 # Set the errors.
20 spectrum.baseplane_rmsd(error=3600, spectrum_id='ref_ave')
21 spectrum.baseplane_rmsd(error=3000, spectrum_id='sat_ave')
```

For the residue G114, the noise levels are significantly increased compared to the rest of the protein as the peak is located close to the water signal. The higher errors for this residue are specified by the commands

```
23 # Individual residue errors.
24 spectrum.baseplane_rmsd(error=122000, spectrum_type='ref', res_num=114)
25 spectrum.baseplane_rmsd(error=8500, spectrum_type='sat', res_num=114)
```

There are many other ways of setting the errors, for example via spectrum duplication, triplication, etc. See the documentation for the `spectrum.error_analysis` user function on page 605 for all possible options. This user function needs to be executed at this stage to correctly set up the errors for all spin systems:

```
27 # Peak intensity error analysis.
28 spectrum.error_analysis()
```

### 6.3.6 NOE script mode – unresolved spins

As the peaks of certain spins overlap to such an extent that the heights or volumes cannot be resolved, a simple text file was created called “unresolved” in which each line consists of the residue number followed by the atom name. By using the command

```
30 # Deselect unresolved spins.
31 deselect.read(name, file='unresolved', res_num_col=1, spin_name_col=2)
```

all spins in the file “unresolved” are excluded from the analysis.

### 6.3.7 NOE script mode – the NOE calculation

At this point the NOE can be calculated. The user function

```
33 # Calculate the NOEs.
34 minimise.calculate()
```

will calculate both the NOE and the errors. The NOE value will be calculated using the formula

$$NOE = \frac{I_{sat}}{I_{ref}}, \quad (6.1)$$

where  $I_{sat}$  is the intensity of the peak in the saturated spectrum and  $I_{ref}$  is that of the reference spectrum. The error is calculated by

$$\sigma_{NOE} = \sqrt{\frac{(\sigma_{sat} \cdot I_{ref})^2 + (\sigma_{ref} \cdot I_{sat})^2}{I_{ref}^2}}, \quad (6.2)$$

where  $\sigma_{sat}$  and  $\sigma_{ref}$  are the peak intensity errors in the saturated and reference spectra respectively. To create a file of the NOEs the command

```
36 # Save the NOEs.
37 value.write(param='noe', file='noe.out', force=True)
```

will create a file called `noe.out` with the NOE values and errors. The force flag will cause any file with the same name to be overwritten. An example of the format of `noe.out` is

# mol_name	res_num	res_name	spin_num	spin_name	value	error
Ap4Aase_new_3_mol1	1	GLY	1	N	None	None
Ap4Aase_new_3_mol1	2	PRO	11	N	None	None
Ap4Aase_new_3_mol1	3	LEU	28	N	None	None
Ap4Aase_new_3_mol1	4	GLY	51	N	-0.038921946984531344	0.019031770246176943
Ap4Aase_new_3_mol1	5	SER	59	N	-0.312404225679127	0.018596937298386886
Ap4Aase_new_3_mol1	6	MET	71	N	-0.42850831873249773	0.02525856323041225
Ap4Aase_new_3_mol1	7	ASP	91	N	-0.5305492810313481	0.027990623144176396
Ap4Aase_new_3_mol1	8	SER	104	N	-0.5652842977581912	0.021706121467731133
Ap4Aase_new_3_mol1	9	PRO	116	N	None	None
Ap4Aase_new_3_mol1	10	PRO	133	N	None	None
Ap4Aase_new_3_mol1	11	GLU	150	N	None	None
Ap4Aase_new_3_mol1	12	GLY	167	N	-0.7036626368123614	0.04681370194503697
Ap4Aase_new_3_mol1	13	TYR	175	N	-0.747464566367261	0.03594640051809186
Ap4Aase_new_3_mol1	14	ARG	200	N	-0.7524129557634996	0.04957018638401278

### 6.3.8 NOE script mode – viewing the results

Any two dimensional data set can be plotted in relax in conjunction with the program [Grace](#). The program is also known as Xmgrace and was previously known as ACE/gr or Xmgr. The highly flexible relax user function `grace.write` is capable of producing 2D plots of any x-y data sets. The two commands

```
39 # Create Grace files.
40 grace.write(y_data_type='peak_intensity', file='intensities.agr', force=True)
41 grace.write(y_data_type='noe', file='noe.agr', force=True)
```

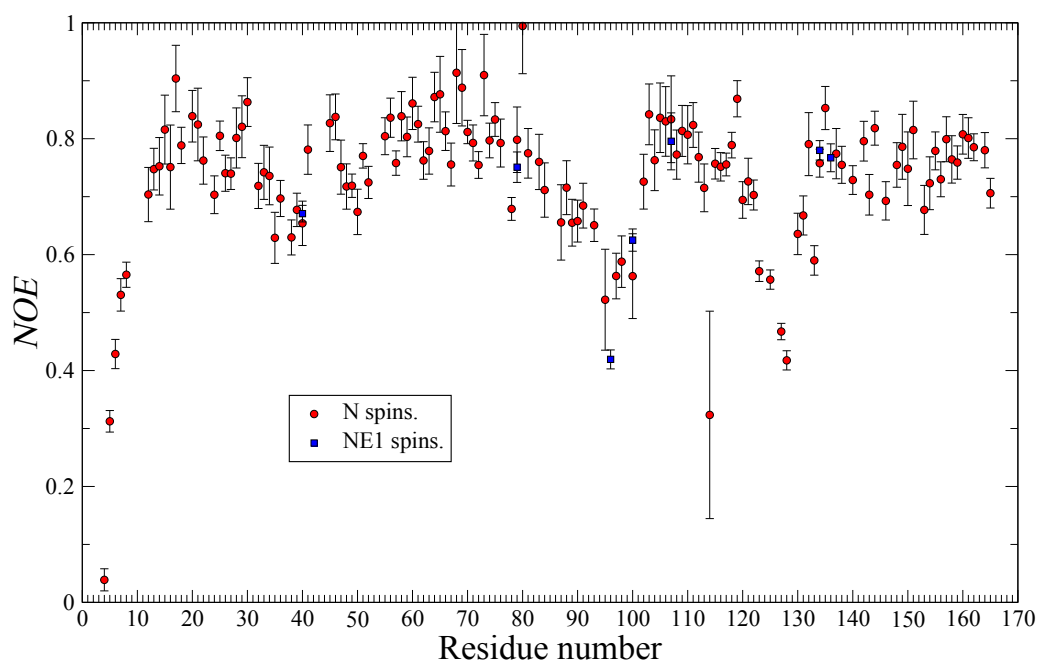


Figure 6.1: A Grace plot of the NOE value and error against the residue number. This is an example of the output of the user function `grace.write`.

will create one plot of the peak intensity of the reference and saturated spectra as different graph sets in the same plot as well as one plot for the NOE and its error. The x-axis in all three defaults to the residue number. Returning to the sample script three Grace data files are created `intensities.agr` and `noe.agr` and placed in the default directory `./grace`. These can be visualised by opening the file within Grace. However `relax` will do that for you with the commands

```

43 # View the Grace files.
44 grace.view(file='intensities.agr')
45 grace.view(file='noe.agr')
```

An example of the output after modifying the axes is shown in figure 6.1.

## 6.4 The NOE auto-analysis in the GUI

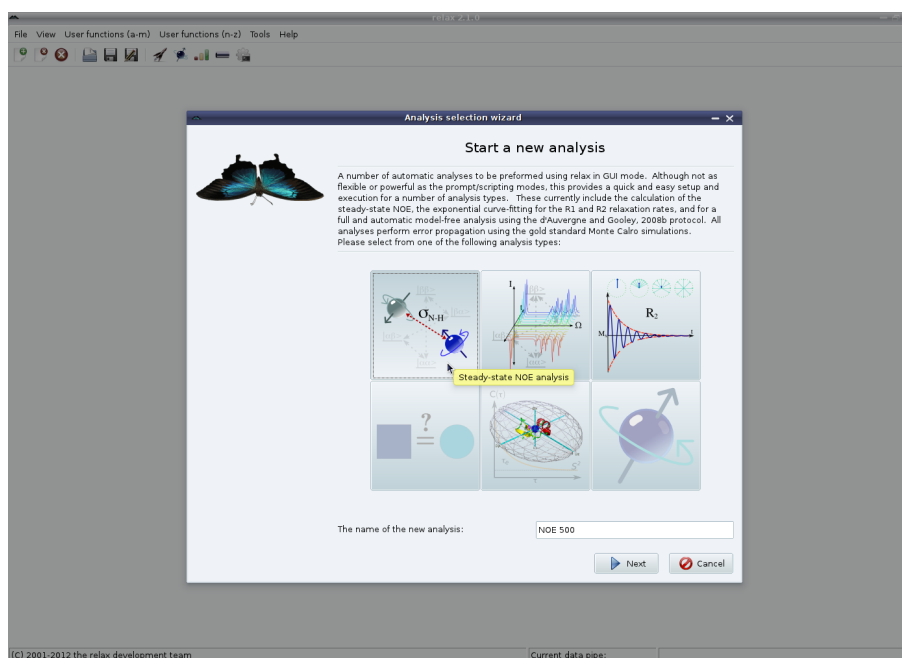
The relax graphical user interface provides access to an automated steady-state NOE analysis. This auto-analysis operates in the same way as the sample script described earlier in this chapter. In this example, relax will be launched with:

```
$ relax --log log --gui
```

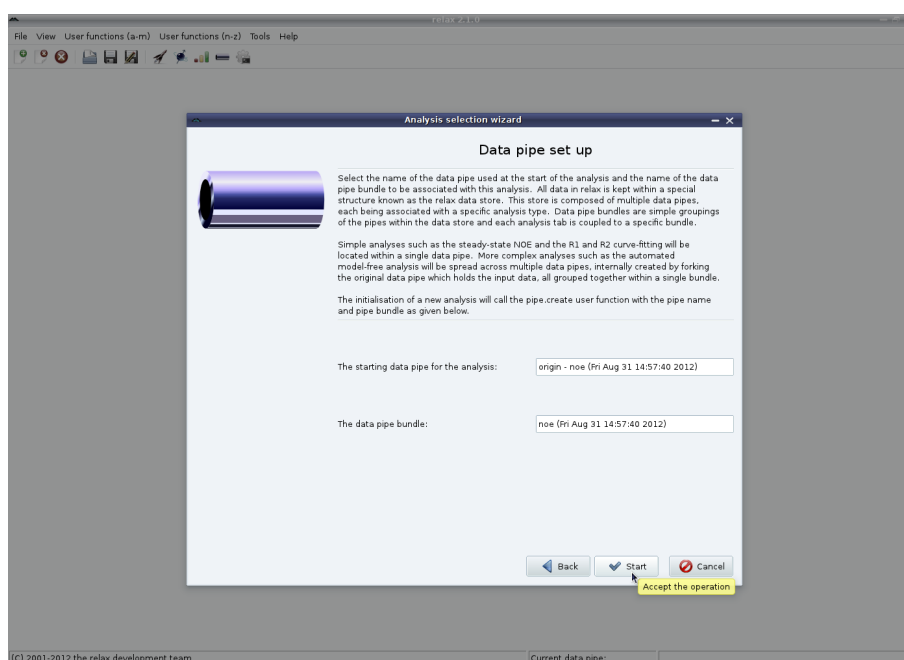
The `--log` command line argument will cause all of relax's text printouts to be placed into the `log` file which can serve as a record for later reference (the `--tee` command line argument could be used as well).

### 6.4.1 NOE GUI mode – initialisation of the data pipe

First launch the analysis selection wizard (see Figure 1.4 on page 12). Select the NOE analysis and, if you plan on running steady-state NOE analyses from multiple fields in one relax instance, change the name of the analysis:

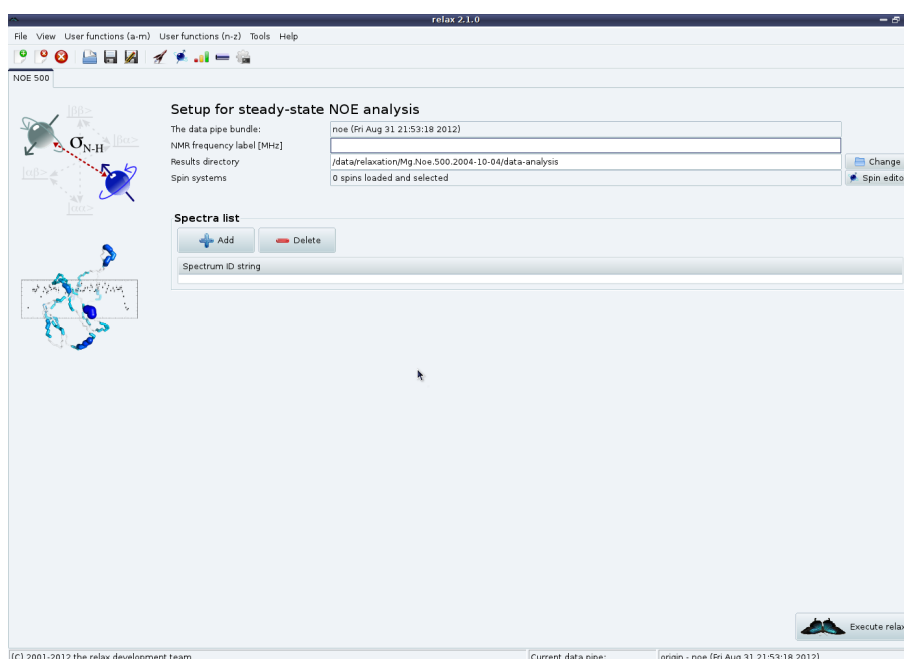


The second part of the wizard need not be modified, just click on “Start” to begin. This will create a dedicated data pipe for the analysis. A data pipe bundle will also be created, but for the steady-state NOE will only contain a single data throughout the analysis.



### 6.4.2 NOE GUI mode – general setup

You should then see the blank analysis tab:



The first thing to do now is to set the NMR frequency label. This is only used for the name of the NOE output file. For example if you set the label to “500”, the file `noe.500.out` will be created at the end of the analysis.

You can also choose to change the “Results directory” where all of the automatically created results files will be placed. These two steps are unique to the GUI mode.

### 6.4.3 NOE GUI mode – setting up the spin systems

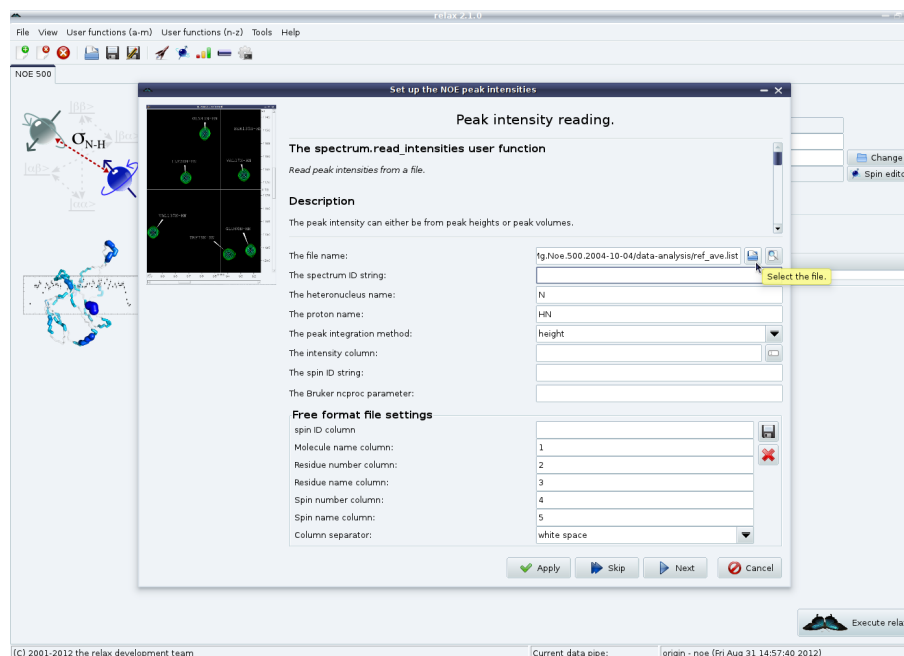
Just as in the prompt and scripting UI modes, the molecule, residue and spin data structures need to be set up prior to the loading of any spin specific data. The “Spin systems” GUI element is used for this purpose. Before any spin systems have been set up, this should say something like “0 spins loaded and selected”. To fix this, click on the “Spin editor” button and you should then see the spin viewer window. The next steps are fully described in section 4.5.2 on page 40 for PDB files or section 4.5.3 on page 43 for a sequence file. The spin viewer window can now be closed.

### 6.4.4 NOE GUI mode – unresolved spins

Using the unresolved spins file as described in the prompt/script UI sections, the same spins can be deselected at this point. See Section 4.5.5 on page 44 for the details of how to deselect the spins in the GUI.

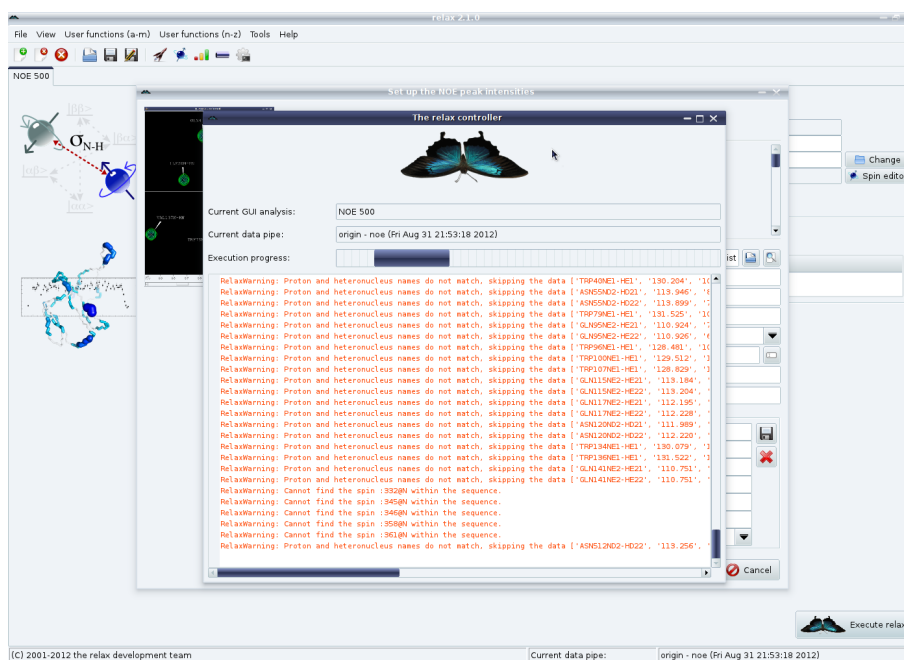
### 6.4.5 NOE GUI mode – loading the data

The next step is to load the saturated and reference NOE peak lists. From the main NOE auto-analysis tab, click on the “Add” button in the “Spectra list” GUI element. This will launch the NOE peak intensity loading wizard. From the first wizard page, select the peak list file containing the reference intensities (from the averaged shift list):

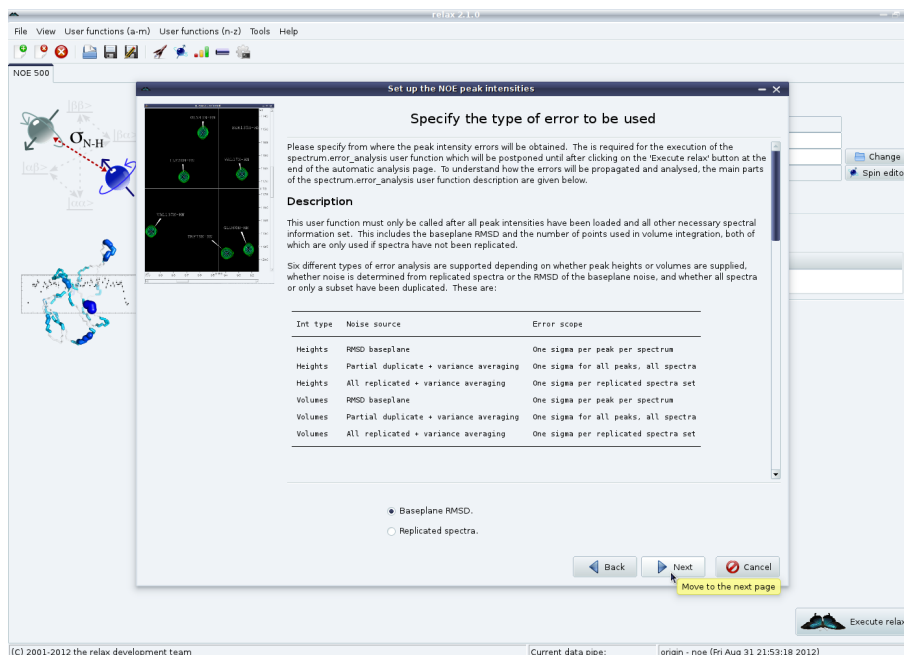


Then set the obligatory spectrum ID string to a unique value (in this case “ref”). The spectral dimension may need to be changed so that the peak intensities are associated with the correct atom of the pair. In case you have forgotten the spin names or the format of the peak list next to the file name selection button is a preview button which can be used to open the peak list in the default text editor. Set the other fields as needed. Click on “Next” Note that a `RelaxWarning` will be thrown for all peak list entries which do

not match a spin system within the relax data store. This will cause the relax controller window to appear:



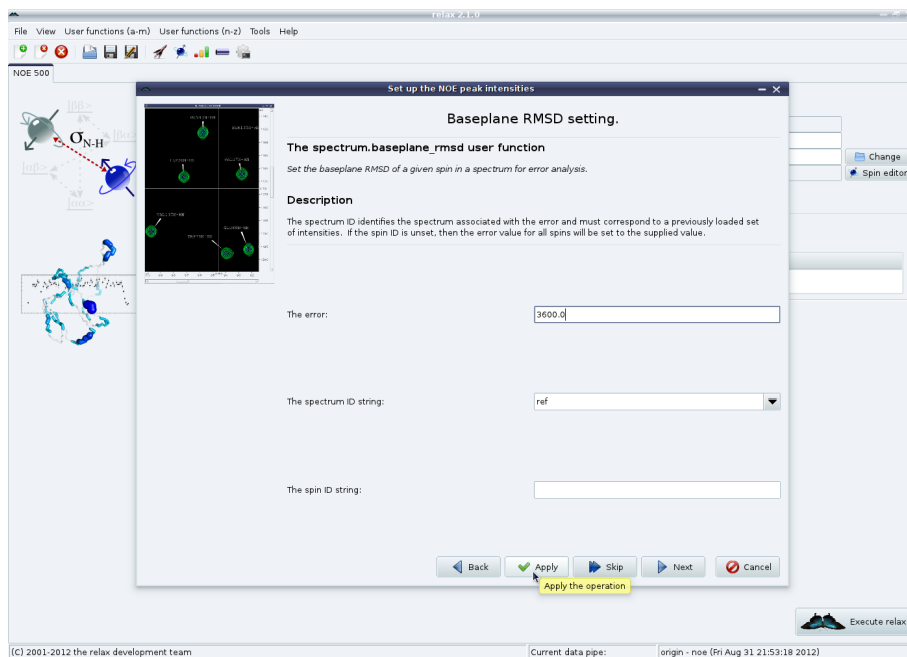
Carefully check these warnings to be sure that the data is correctly loaded and, if everything is fine, the relax controller window can be closed. If the dimension has been wrongly specified or some other setting is incorrect a **RelaxError** might appear saying that no data was loaded – you will then need to fix the settings and click on “Apply” again. The error type page should now appear.



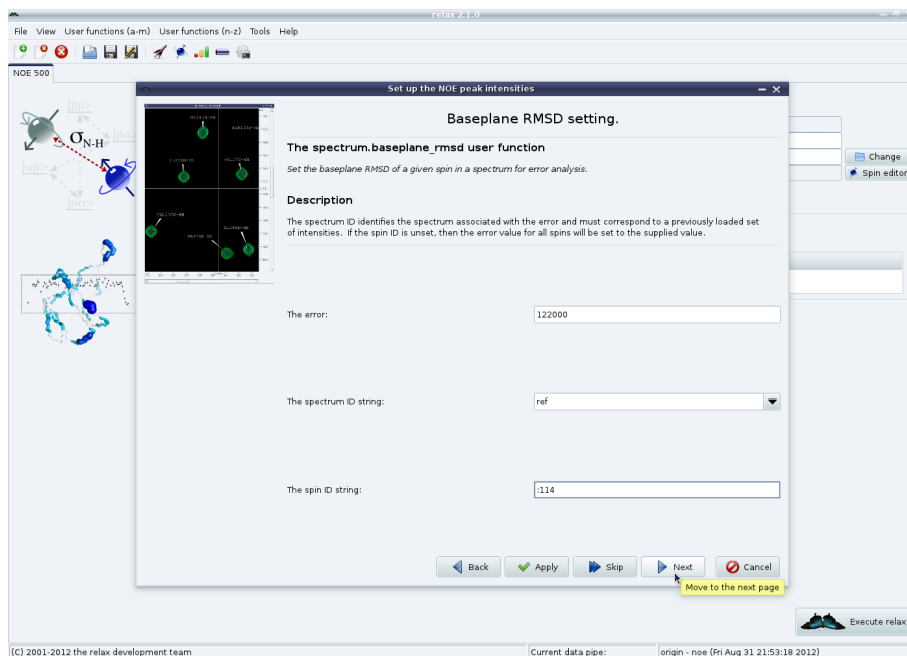
Please read the description in this window very carefully to know what to do next. In this example, we will choose “Baseplane RMSD”. For this specific example, Sparky’s “Extensions→Spectrum→Spectrum baseplane RMSD” option in the “F1” selection mode was used to measure empty regions of the spectrum (mainly in the random coil region) to determine an average



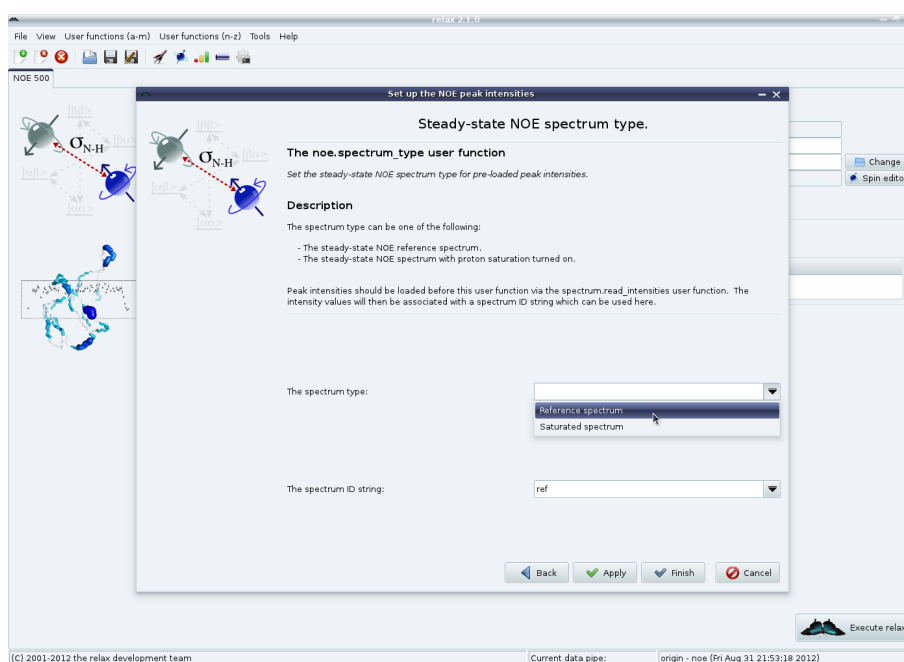
RMSD of approximately 3600. Set the value and click on “Apply”.



As glycine 114 is located close to the noise signal, its error was much higher at 122000. Individual spin errors can be set via the spin ID string (see section 4.2.2 on page 36 for information about spin IDs):

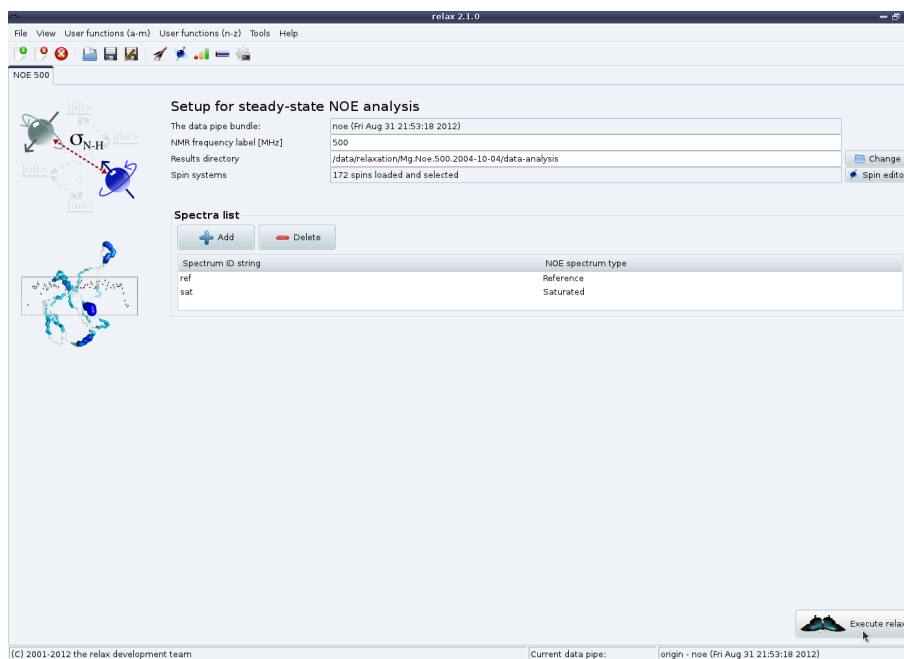


Finally select which type of spectrum this is and click on “Finish”:



The entire procedure should be repeated for the saturated spectrum (or you may have worked out that both can be loaded simultaneously by using the “Apply” button more often). For this example, the spectrum ID was set to “sat” and the baseplane RMSD to 3000 for all spins (except for G114 which had an error of 8500).

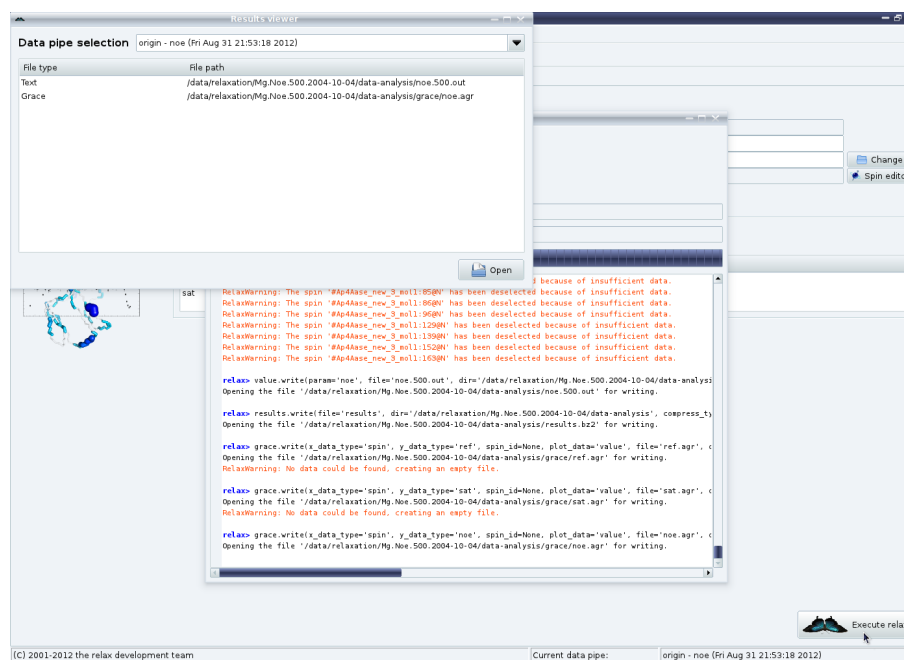
The NOE analysis tab should now look like:



#### 6.4.6 NOE GUI mode – the NOE calculation

Now that everything is set up, simply click on “Execute relax” in the NOE analysis tab. The relax controller window will appear displaying many messages. These should all be

checked very carefully to make sure that everything has executed as you expected. The “Results viewer” window will also appear:



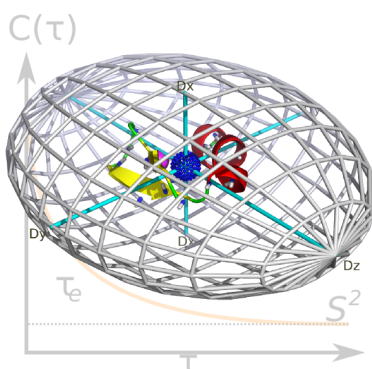
The results viewer window can be used to launch a text editor to see the NOE values and error or Grace to visualise the results (see Figure 6.1 on page 74).

As a last step, the relax state can be saved (via the “File” menu) and relax closed. Take one last look at the `noe.out` log file to be certain that there are no strange warnings or errors.



## Chapter 7

# Model-free analysis



### 7.1 Model-free theory

#### 7.1.1 The chi-squared function – $\chi^2(\theta)$

For the minimisation of the model-free models a chain of calculations, each based on a different theory, is required. At the highest level the equation which is actually minimised is the chi-squared function

$$\chi^2(\theta) = \sum_{i=1}^n \frac{(R_i - R_i(\theta))^2}{\sigma_i^2}, \quad (7.1)$$

where the index  $i$  is the summation index ranging over all the experimentally collected relaxation data of all spins used in the analysis;  $R_i$  belongs to the relaxation data set  $R$  for an individual spin, a collection of spins, or the entire macromolecule and includes the  $R_1$ ,  $R_2$ , and NOE data at all field strengths;  $R_i(\theta)$  is the back-calculated relaxation value belonging to the set  $R(\theta)$ ;  $\theta$  is the model parameter vector which when minimised is denoted by  $\hat{\theta}$ ; and  $\sigma_i$  is the experimental error.

The significance of the chi-squared equation (7.1) is that the function returns a single value which is then minimised by the optimisation algorithm to find the model-free parameter values of the given model.

### 7.1.2 The transformed relaxation equations – $R_i(\theta)$

The chi-squared equation is itself dependent on the relaxation equations through the back-calculated relaxation data  $R(\theta)$ . Letting the relaxation values of the set  $R(\theta)$  be the  $R_1(\theta)$ ,  $R_2(\theta)$ , and  $\text{NOE}(\theta)$  an additional layer of abstraction can be used to simplify the calculation of the gradients and Hessians. This involves decomposing the NOE equation into the cross relaxation rate constant  $\sigma_{\text{NOE}}(\theta)$  and the auto relaxation rate  $R_1(\theta)$ . Taking equation (7.6) below the transformed relaxation equations are

$$R_1(\theta) = R'_1(\theta), \quad (7.2a)$$

$$R_2(\theta) = R'_2(\theta), \quad (7.2b)$$

$$\text{NOE}(\theta) = 1 + \frac{\gamma_H}{\gamma_X} \frac{\sigma_{\text{NOE}}(\theta)}{R_1(\theta)}. \quad (7.2c)$$

whereas the relaxation equations are the  $R_1(\theta)$ ,  $R_2(\theta)$ ,  $\sigma_{\text{NOE}}(\theta)$ .

### 7.1.3 The relaxation equations – $R'_i(\theta)$

The relaxation values of the set  $R'(\theta)$  include the spin-lattice, spin-spin, and cross-relaxation rates at all field strengths. These rates are respectively (Abragam, 1961)

$$R_1(\theta) = d \left( J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H + \omega_X) \right) + cJ(\omega_X), \quad (7.3a)$$

$$R_2(\theta) = \frac{d}{2} \left( 4J(0) + J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H) \right. \\ \left. + 6J(\omega_H + \omega_X) \right) + \frac{c}{6} \left( 4J(0) + 3J(\omega_X) \right) + R_{ex}, \quad (7.3b)$$

$$\sigma_{\text{NOE}}(\theta) = d \left( 6J(\omega_H + \omega_X) - J(\omega_H - \omega_X) \right), \quad (7.3c)$$

where  $J(\omega)$  is the power spectral density function and  $R_{ex}$  is the relaxation due to chemical exchange. The dipolar and CSA constants are defined in SI units as

$$d = \frac{1}{4} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{(\gamma_H \gamma_X \hbar)^2}{\langle r^6 \rangle}, \quad (7.4)$$

$$c = \frac{(\omega_X \Delta\sigma)^2}{3}, \quad (7.5)$$

where  $\mu_0$  is the permeability of free space,  $\gamma_H$  and  $\gamma_X$  are the gyromagnetic ratios of the  $H$  and  $X$  spins respectively,  $\hbar$  is Plank's constant divided by  $2\pi$ ,  $r$  is the bond length, and  $\Delta\sigma$  is the chemical shift anisotropy measured in ppm. The cross-relaxation rate  $\sigma_{\text{NOE}}$  is related to the steady state NOE by the equation

$$\text{NOE}(\theta) = 1 + \frac{\gamma_H}{\gamma_X} \frac{\sigma_{\text{NOE}}(\theta)}{R_1(\theta)}. \quad (7.6)$$

#### 7.1.4 The spectral density functions – $J(\omega)$

The relaxation equations are themselves dependent on the calculation of the spectral density values  $J(\omega)$ . Within model-free analysis these are modelled by the original model-free formula (Lipari and Szabo, 1982a,b)

$$J(\omega) = \frac{2}{5} \sum_{i=-k}^k c_i \cdot \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2} \right), \quad (7.7)$$

where  $S^2$  is the square of the Lipari and Szabo generalised order parameter and  $\tau_e$  is the effective correlation time. The order parameter reflects the amplitude of the motion and the correlation time in an indication of the time scale of that motion. The theory was extended by Clore et al. (1990) by the modelling of two independent internal motions using the equation

$$J(\omega) = \frac{2}{5} \sum_{i=-k}^k c_i \cdot \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega\tau_f\tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega\tau_s\tau_i)^2} \right). \quad (7.8)$$

where  $S_f^2$  and  $\tau_f$  are the amplitude and timescale of the faster of the two motions whereas  $S_s^2$  and  $\tau_s$  are those of the slower motion.  $S_f^2$  and  $S_s^2$  are related by the formula  $S^2 = S_f^2 \cdot S_s^2$ .

If these forms of the model-free spectral density functions are unfamiliar, that is because these are the numerically stabilised forms presented in d’Auvergne and Gooley (2008b). The original model-free spectral density functions presented in Lipari and Szabo (1982a) and Clore et al. (1990) are not the most numerically stable form of these equations. An important problem encountered in optimisation is round-off error in which machine precision influences the result of mathematical operations. The double reciprocal  $\tau^{-1} = \tau_m^{-1} + \tau_e^{-1}$  used in the equations are operations which are particularly susceptible to round-off error, especially when  $\tau_e \ll \tau_m$ . By incorporating these reciprocals into the model-free spectral density functions and then simplifying the equations this source of round-off error can be eliminated, giving relax an edge over other model-free optimisation software.

#### 7.1.5 Brownian rotational diffusion

In equations (7.7) and (7.8) the generic Brownian diffusion NMR correlation function presented in d’Auvergne (2006) has been used. This function is

$$C(\tau) = \frac{1}{5} \sum_{i=-k}^k c_i \cdot e^{-\tau/\tau_i}, \quad (7.9)$$

where the summation index  $i$  ranges over the number of exponential terms within the correlation function. This equation is generic in that it can describe the diffusion of an ellipsoid, a spheroid, or a sphere.

### Diffusion as an ellipsoid

For the ellipsoid defined by the parameter set  $\{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r, \alpha, \beta, \gamma\}$  the variable  $k$  is equal to two and therefore the index  $i \in \{-2, -1, 0, 1, 2\}$ . The geometric parameters  $\{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r\}$  are defined as

$$\mathfrak{D}_{iso} = \frac{1}{3}(\mathfrak{D}_x + \mathfrak{D}_y + \mathfrak{D}_z), \quad (7.10a)$$

$$\mathfrak{D}_a = \mathfrak{D}_z - \frac{1}{2}(\mathfrak{D}_x + \mathfrak{D}_y), \quad (7.10b)$$

$$\mathfrak{D}_r = \frac{\mathfrak{D}_y - \mathfrak{D}_x}{2\mathfrak{D}_a}, \quad (7.10c)$$

and are constrained by

$$0 < \mathfrak{D}_{iso} < \infty, \quad (7.11a)$$

$$0 \leq \mathfrak{D}_a < \frac{\mathfrak{D}_{iso}}{\frac{1}{3} + \mathfrak{D}_r} \leq 3\mathfrak{D}_{iso}, \quad (7.11b)$$

$$0 \leq \mathfrak{D}_r \leq 1. \quad (7.11c)$$

The orientational parameters  $\{\alpha, \beta, \gamma\}$  are the Euler angles using the z-y-z rotation notation.

The five weights  $c_i$  are defined as

$$c_{-2} = \frac{1}{4}(d - e), \quad (7.12a)$$

$$c_{-1} = 3\delta_y^2\delta_z^2, \quad (7.12b)$$

$$c_0 = 3\delta_x^2\delta_z^2, \quad (7.12c)$$

$$c_1 = 3\delta_x^2\delta_y^2, \quad (7.12d)$$

$$c_2 = \frac{1}{4}(d + e), \quad (7.12e)$$

where

$$d = 3(\delta_x^4 + \delta_y^4 + \delta_z^4) - 1, \quad (7.13)$$

$$e = \frac{1}{\mathfrak{R}} \left[ (1 + 3\mathfrak{D}_r)(\delta_x^4 + 2\delta_y^2\delta_z^2) + (1 - 3\mathfrak{D}_r)(\delta_y^4 + 2\delta_x^2\delta_z^2) - 2(\delta_z^4 + 2\delta_x^2\delta_y^2) \right], \quad (7.14)$$

and where

$$\mathfrak{R} = \sqrt{1 + 3\mathfrak{D}_r^2}. \quad (7.15)$$

The five correlation times  $\tau_i$  are

$$1/\tau_{-2} = 6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R}, \quad (7.16a)$$

$$1/\tau_{-1} = 6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r), \quad (7.16b)$$

$$1/\tau_0 = 6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r), \quad (7.16c)$$

$$1/\tau_1 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a, \quad (7.16d)$$

$$1/\tau_2 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R}. \quad (7.16e)$$



### Diffusion as a spheroid

The variable  $k$  is equal to one in the case of the spheroid defined by the parameter set  $\{\mathfrak{D}_{iso}, \mathfrak{D}_a, \theta, \phi\}$ , hence  $i \in \{-1, 0, 1\}$ . The geometric parameters  $\{\mathfrak{D}_{iso}, \mathfrak{D}_a\}$  are defined as

$$\mathfrak{D}_{iso} = \frac{1}{3}(\mathfrak{D}_{\parallel} + 2\mathfrak{D}_{\perp}), \quad (7.17a)$$

$$\mathfrak{D}_a = \mathfrak{D}_{\parallel} - \mathfrak{D}_{\perp}. \quad (7.17b)$$

and are constrained by

$$0 < \mathfrak{D}_{iso} < \infty, \quad (7.18a)$$

$$-\frac{3}{2}\mathfrak{D}_{iso} < \mathfrak{D}_a < 3\mathfrak{D}_{iso}. \quad (7.18b)$$

The orientational parameters  $\{\theta, \phi\}$  are the spherical angles defining the orientation of the major axis of the diffusion frame within the lab frame.

The three weights  $c_i$  are

$$c_{-1} = \frac{1}{4}(3\delta_z^2 - 1)^2, \quad (7.19a)$$

$$c_0 = 3\delta_z^2(1 - \delta_z^2), \quad (7.19b)$$

$$c_1 = \frac{3}{4}(\delta_z^2 - 1)^2. \quad (7.19c)$$

The five correlation times  $\tau_i$  are

$$1/\tau_{-1} = 6\mathfrak{D}_{iso} - 2\mathfrak{D}_a, \quad (7.20a)$$

$$1/\tau_0 = 6\mathfrak{D}_{iso} - \mathfrak{D}_a, \quad (7.20b)$$

$$1/\tau_1 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a. \quad (7.20c)$$

### Diffusion as a sphere

In the situation of a molecule diffusing as a sphere either described by the single parameter  $\tau_m$  or  $\mathfrak{D}_{iso}$ , the variable  $k$  is equal to zero. Therefore  $i \in \{0\}$ . The single weight  $c_0$  is equal to one and the single correlation time  $\tau_0$  is equivalent to the global tumbling time  $\tau_m$  given by

$$1/\tau_m = 6\mathfrak{D}_{iso}. \quad (7.21)$$

This is diffusion equation presented in [Bloembergen et al. \(1948\)](#).

#### 7.1.6 The model-free models

Extending the list of models given in [Mandel et al. \(1995\)](#); [Fushman et al. \(1997\)](#); [Orekhov et al. \(1999a\)](#); [Korzhnev et al. \(2001\)](#); [Zhuravleva et al. \(2004\)](#), the models built

into relax include

$$m0 = \{\}, \quad (7.22.0)$$

$$m1 = \{S^2\}, \quad (7.22.1)$$

$$m2 = \{S^2, \tau_e\}, \quad (7.22.2)$$

$$m3 = \{S^2, R_{ex}\}, \quad (7.22.3)$$

$$m4 = \{S^2, \tau_e, R_{ex}\}, \quad (7.22.4)$$

$$m5 = \{S^2, S_f^2, \tau_s\}, \quad (7.22.5)$$

$$m6 = \{S^2, \tau_f, S_f^2, \tau_s\}, \quad (7.22.6)$$

$$m7 = \{S^2, S_f^2, \tau_s, R_{ex}\}, \quad (7.22.7)$$

$$m8 = \{S^2, \tau_f, S_f^2, \tau_s, R_{ex}\}, \quad (7.22.8)$$

$$m9 = \{R_{ex}\}. \quad (7.22.9)$$

The parameter  $R_{ex}$  is scaled quadratically with field strength in these models as it is assumed to be fast. In the set theory notation, the model-free model for the spin system  $i$  is represented by the symbol  $\mathfrak{F}_i$ . Through the addition of the local  $\tau_m$  to each of these models, only the component of Brownian rotational diffusion experienced by the spin system is probed. These models, represented in set notation by the symbol  $\mathfrak{T}_i$ , are

$$tm0 = \{\tau_m\}, \quad (7.23.0)$$

$$tm1 = \{\tau_m, S^2\}, \quad (7.23.1)$$

$$tm2 = \{\tau_m, S^2, \tau_e\}, \quad (7.23.2)$$

$$tm3 = \{\tau_m, S^2, R_{ex}\}, \quad (7.23.3)$$

$$tm4 = \{\tau_m, S^2, \tau_e, R_{ex}\}, \quad (7.23.4)$$

$$tm5 = \{\tau_m, S^2, S_f^2, \tau_s\}, \quad (7.23.5)$$

$$tm6 = \{\tau_m, S^2, \tau_f, S_f^2, \tau_s\}, \quad (7.23.6)$$

$$tm7 = \{\tau_m, S^2, S_f^2, \tau_s, R_{ex}\}, \quad (7.23.7)$$

$$tm8 = \{\tau_m, S^2, \tau_f, S_f^2, \tau_s, R_{ex}\}, \quad (7.23.8)$$

$$tm9 = \{\tau_m, R_{ex}\}. \quad (7.23.9)$$

### 7.1.7 Model-free optimisation theory

The implementation of optimisation in relax is discussed in detail in Chapter 14. To understand the concepts in this subsection, it is best to look at that chapter first.

#### The model-free space

In model-free analysis the target function  $f(\theta)$  is the chi-squared equation

$$\chi^2(\theta) = \sum_{i=1}^n \frac{(R_i - R_i(\theta))^2}{\sigma_i^2}, \quad (7.24)$$

where  $i$  is the summation index,  $R_i$  is the experimental relaxation data which belongs to the data set  $R$  and includes the  $R_1$ ,  $R_2$ , and NOE values at all field strengths,  $R_i(\theta)$  is the back calculated relaxation data belonging to the set  $R(\theta)$ , and  $\sigma_i$  is the experimental error. For the optimisation of the model-free parameters while the diffusion tensor is held fixed, the summation index ranges over the relaxation data of an individual spin. If the diffusion parameters are optimised simultaneously with the model-free parameters the summation index ranges over all relaxation data of all selected spins of the macromolecule.

Given the current parameter values the model-free function provided to the algorithm will calculate the value of the model-free spectral density function  $J(\omega)$  at the five frequencies which induce NMR relaxation by using Equations (7.7) and (7.8). The theoretical  $R_1$ ,  $R_2$ , and NOE values are then back-calculated using Equations (7.3a), (7.3b), (7.3c), and (7.6). Finally, the chi-squared value is calculated using Equation (7.24).

To produce the gradient and Hessian required for model-free optimisation a large chain of first and second partial derivatives needs to be calculated. Firstly the partial derivatives of the spectral density functions (7.7) and (7.8) are necessary. Then the partial derivatives of the relaxation equations (7.3a) to (7.3c) followed by the NOE equation (7.6) are needed. Finally the partial derivative of the chi-squared formula (7.24) is required. These first and second partial derivatives, as well as those of the components of the Brownian diffusion correlation function for non-isotropic tumbling, are presented as Chapter 15.

## Grid search

Due to the complexity of the curvature of the model-free space, the grid point with the lowest chi-squared value may in fact be on the opposite side of the space to the local minimum. Therefore the model-free space renders many optimisation algorithms ineffective (d’Auvergne and Gooley, 2008b).

## Parameter constraints

To understand this section, please see Section 14.5 on page 306. For model-free analysis, linear constraints are the most useful type of constraint as the correlation time  $\tau_f$  can be restricted to being less than  $\tau_s$  by using the inequality  $\tau_s - \tau_f \geq 0$ .

For the parameters specific to individual spins the linear constraints in the notation of

(14.18) are

$$\begin{pmatrix}
 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
 \end{pmatrix} \cdot \begin{pmatrix} S^2 \\ S_f^2 \\ S_s^2 \\ \tau_e \\ \tau_f \\ \tau_s \\ R_{ex} \\ r \\ CSA \end{pmatrix} \geq \begin{pmatrix} 0 \\ -1 \\ 0 \\ -1 \\ 0 \\ -1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0.9e^{-10} \\ 2e^{-10} \\ 300e^{-6} \\ 0 \end{pmatrix}. \quad (7.25)$$

Through the isolation of each individual element, the constraints can be seen to be equivalent to

$$0 \leq S^2 \leq 1, \quad (7.26a)$$

$$0 \leq S_f^2 \leq 1, \quad (7.26b)$$

$$0 \leq S_s^2 \leq 1, \quad (7.26c)$$

$$S^2 \leq S_f^2, \quad (7.26d)$$

$$S^2 \leq S_s^2, \quad (7.26e)$$

$$\tau_e \geq 0, \quad (7.26f)$$

$$\tau_f \geq 0, \quad (7.26g)$$

$$\tau_s \geq 0, \quad (7.26h)$$

$$\tau_s \geq 0, \quad (7.26i)$$

$$\tau_f \leq \tau_s, \quad (7.26j)$$

$$R_{ex} \geq 0, \quad (7.26k)$$

$$0.9e^{-10} \leq r \leq 2e^{-10}, \quad (7.26l)$$

$$-300e^{-6} \leq CSA \leq 0. \quad (7.26m)$$

To prevent the computationally expensive optimisation of failed models in which the internal correlation times minimise to infinity (d'Auvergne and Gooley, 2006), the constraint  $\tau_e, \tau_f, \tau_s \leq 2\tau_m$  was implemented. When the global correlation time is fixed the constraints in the matrix notation of (14.18) are

$$\begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} \tau_e \\ \tau_f \\ \tau_s \end{pmatrix} \geq \begin{pmatrix} -2\tau_m \\ -2\tau_m \\ -2\tau_m \end{pmatrix}. \quad (7.27)$$

However when the global correlation time  $\tau_m$  is one of the parameters being optimised the constraints become

$$\begin{pmatrix} 2 & -1 & 0 & 0 \\ 2 & 0 & -1 & 0 \\ 2 & 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} \tau_m \\ \tau_e \\ \tau_f \\ \tau_s \end{pmatrix} \geq \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}. \quad (7.28)$$

For the parameters of the diffusion tensor the constraints utilised are

$$0 \leq \tau_m \leq 200.0e^{-9}, \quad (7.29a)$$

$$\mathfrak{D}_a \geq 0, \quad (7.29b)$$

$$0 \leq \mathfrak{D}_r \leq 1, \quad (7.29c)$$

which in the matrix notation of (14.18) become

$$\begin{pmatrix} 1 & 0 & 0 \\ -1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} \tau_m \\ \mathfrak{D}_a \\ \mathfrak{D}_r \end{pmatrix} \geq \begin{pmatrix} 0 \\ -200.0e^{-9} \\ 0 \\ 0 \\ -1 \end{pmatrix}. \quad (7.30)$$

The upper limit of 200 ns on  $\tau_m$  prevents the parameter from heading towards infinity when model failure occurs (see d’Auvergne and Gooley (2006)). This can significantly decrease the computation time. To isolate the prolate spheroid the constraint

$$(1) \cdot (\mathfrak{D}_a) \geq (0), \quad (7.31)$$

is used whereas to isolate the oblate spheroid the constraint used is

$$(-1) \cdot (\mathfrak{D}_a) \geq (0). \quad (7.32)$$

Dependent on the model optimised, the matrix  $A$  and vector  $b$  are constructed from combinations of the above linear constraints.

## Diagonal scaling

The concept of diagonal scaling is explained in Section 14.6 on page 308.

For the model-free analysis the scaling factor of one is used for the order parameter and a scaling factor of  $1e^{-12}$  is used for the correlation times. The  $R_{ex}$  parameter is scaled to be the chemical exchange rate of the first field strength. The scaling matrix for the parameters  $\{S^2, S_f^2, S_s^2, \tau_e, \tau_f, \tau_s, R_{ex}, r, CSA\}$  of individual spins is

[illegible]

For the ellipsoidal diffusion parameters  $\{\tau_m, \mathfrak{D}_a, \mathfrak{D}_r, \alpha, \beta, \gamma\}$  the scaling matrix is

$$\begin{pmatrix} 1e^{-12} & 0 & 0 & 0 & 0 & 0 \\ 0 & 1e^7 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \quad (7.34)$$

For the spheroidal diffusion parameters  $\{\tau_m, \mathfrak{D}_a, \theta, \phi\}$  the scaling matrix is

$$\begin{pmatrix} 1e^{-12} & 0 & 0 & 0 \\ 0 & 1e^7 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}. \quad (7.35)$$

## 7.2 Optimisation of a single model-free model

### 7.2.1 Single model-free model script mode – the sample script

The sample script which demonstrates the optimisation of model-free model *m4* which consists of the parameters  $\{S^2, \tau_e, R_{ex}\}$  is `model_free/single_model.py`. The text of the script is:

```

1  # Script for model-free analysis.
2
3  # Create the data pipe.
4  name = 'm4'
5  pipe.create(name, 'mf')
6
7  # Set up the 15N spins.
8  sequence.read('noe.500.out', res_num_col=1, res_name_col=2)
9  spin.name('N')
10 spin.element(element='N', spin_id='@N')
11 spin.isotope('15N', spin_id='@N')
12
13 # Load the relaxation data.
14 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
15                res_num_col=1, data_col=3, error_col=4)
16 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
17                res_num_col=1, data_col=3, error_col=4)
18 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
19                res_num_col=1, data_col=3, error_col=4)
20 relax_data.read(ri_id='R1_500', ri_type='R1', frq=500.0*1e6, file='r1.500.out',
21                res_num_col=1, data_col=3, error_col=4)
22 relax_data.read(ri_id='R2_500', ri_type='R2', frq=500.0*1e6, file='r2.500.out',
23                res_num_col=1, data_col=3, error_col=4)
24 relax_data.read(ri_id='NOE_500', ri_type='NOE', frq=500.0*1e6, file='noe.500.out',
25                res_num_col=1, data_col=3, error_col=4)
26
27 # Initialise the diffusion tensor.
28 diffusion_tensor.init(10e-9, fixed=True)
29
30 # Create all attached protons.

```

```

25 sequence.attach_protons()
26
27 # Define the magnetic dipole-dipole relaxation interaction.
28 interatom.define(spinid1='@N', spin_id2='@H', direct_bond=True)
29 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)
30 #interatom.unit_vectors()
31
32 # Define the CSA relaxation interaction.
33 value.set(-172 * 1e-6, 'csa')
34
35 # Select the model-free model.
36 model_free.select_model(model=name)
37
38 # Grid search.
39 minimise.grid_search(inc=11)
40
41 # Minimise.
42 minimise.execute('newton')
43
44 # Monte Carlo simulations.
45 monte_carlo.setup(number=100)
46 monte_carlo.create_data()
47 monte_carlo.initial_values()
48 minimise.execute('newton')
49 eliminate()
50 monte_carlo.error_analysis()
51
52 # Finish.
53 results.write(file='results', force=True)
54 state.save('save', force=True)

```

### 7.2.2 Single model-free model script mode – explanation

The above script consists of three major sections:

**Loading of data** Firstly a data pipe called “m4” is created to hold all of the analysis data.

Then the  $^{15}\text{N}$  spin system data consisting of molecule, residue, and spin information is loaded into relax from the columns of the `noe.500.out` file, assuming that only residue numbers and names are present and are in the first and second columns respectively. The options of this `sequence.read` user function allow the molecule name, residue number, residue name, spin number, or spin name columns to be specified if desired. The  $^{15}\text{N}$  spin is then set up using the `spin` user functions. The next part is to load all of the relaxation data, to set up the initial diffusion tensor, create the  $^1\text{H}$  spins required for the magnetic dipole-dipole interaction, and to set up the magnetic dipole-dipole and CSA relaxation mechanisms. Finally the model-free model “m4” is chosen.

**Optimisation** The optimisation of model-free models requires an initial grid search to find a position close to the minimum, followed by the high precision Newton optimisation together with the Method of Multipliers constraint algorithm (d’Auvergne and Gooley, 2008b). Errors are propagated from the relaxation data to the model-free parameters via Monte Carlo simulations which is a multi-step process in relax (designed for flexibility and to teach how the simulations are constructed and carried out).

**Data output** The last stage consists of writing out the XML formatted results file which contains all of the data in the current data pipe, as well as the XML formatted save file which contains not only the current data pipe data but all of the relax data store data. Both files can be loaded back into relax later on.

## 7.3 Optimisation of all model-free models

### 7.3.1 All model-free models script mode – the sample script

The sample script which demonstrates the optimisation of all model-free models from  $m0$  to  $m9$  of individual spins is `model_free/mf_multimodel.py`. The important parts of the script are:

```

1  # Set the data pipe names (also the names of preset model-free models).
2  pipes = ['m0', 'm1', 'm2', 'm3', 'm4', 'm5', 'm6', 'm7', 'm8', 'm9']
3
4  # Loop over the pipes.
5  for name in pipes:
6      # Create the data pipe.
7      pipe.create(name, 'mf')
8
9      # Set up the 15N spins.
10     sequence.read('noe.500.out', res_num_col=1)
11     spin.name('N')
12     spin.element(element='N', spin_id='@N')
13     spin.isotope('15N', spin_id='@N')
14
15     # Load a PDB file.
16     structure.read_pdb('example.pdb')
17
18     # Load the relaxation data.
19     relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
20                     res_num_col=1, data_col=3, error_col=4)
21     relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
22                     res_num_col=1, data_col=3, error_col=4)
23     relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
24                     res_num_col=1, data_col=3, error_col=4)
25     relax_data.read(ri_id='R1_500', ri_type='R1', frq=500.0*1e6, file='r1.500.out',
26                     res_num_col=1, data_col=3, error_col=4)
27     relax_data.read(ri_id='R2_500', ri_type='R2', frq=500.0*1e6, file='r2.500.out',
28                     res_num_col=1, data_col=3, error_col=4)
29     relax_data.read(ri_id='NOE_500', ri_type='NOE', frq=500.0*1e6, file='noe.500.out',
30                     res_num_col=1, data_col=3, error_col=4)
31
32     # Set up the diffusion tensor.
33     diffusion_tensor.init(1e-8, fixed=True)
34
35     # Generate the 1H spins for the magnetic dipole-dipole relaxation interaction.
36     sequence.attach_protons()
37
38     # Define the magnetic dipole-dipole relaxation interaction.
39     interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
40     interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)
41     structure.get_pos('@N')
42     structure.get_pos('@H')
43     interatom.unit_vectors()

```



```

38
39     # Define the chemical shift relaxation interaction.
40     value.set(-172 * 1e-6, 'csa', spin_id='@N')
41
42     # Select the model-free model.
43     model_free.select_model(model=name)
44
45     # Minimise.
46     minimise.grid_search(inc=11)
47     minimise.execute('newton')
48
49     # Write the results.
50     results.write(file='results', force=True)
51
52 # Save the program state.
53 state.save('save', force=True)

```

### 7.3.2 All model-free models script mode – explanation

The above script is very similar in spirit to the previous single model script in section 7.2 on page 92. The major difference is that this script loops over all of the model-free models, saving all of the results in the `save.bz2` file.

## 7.4 Model-free model selection

### 7.4.1 Model-free model selection script mode – the sample script

The sample script which demonstrates both model-free model elimination and model-free model selection between models from  $m_0$  to  $m_9$  is `model_free/modsel.py`. The text of the script is:

```

1  # Set the data pipe names.
2  pipes = ['m0', 'm1', 'm2', 'm3', 'm4', 'm5', 'm6', 'm7', 'm8', 'm9']
3
4  # Loop over the data pipe names.
5  for name in pipes:
6      print("\n\n# " + name + " #")
7
8      # Create the data pipe.
9      pipe.create(name, 'mf')
10
11     # Reload precalculated results from the file 'm1/results', etc.
12     results.read(file='results', dir=name)
13
14 # Model elimination.
15 eliminate()
16
17 # Model selection.
18 model_selection(method='AIC', modsel_pipe='aic')
19
20 # Write the results.
21 state.save('save', force=True)
22 results.write(file='results', force=True)

```

### 7.4.2 Model-free model selection script mode – explanation

This script is designed to be used in conjunction with the `model_free/mf_multimodel.py` script in the previous section. It will load all of the results files from the previous script and then perform the following:

**Model-free model elimination** The optimisation of model-free models performed by the previous script will fail for certain data sets together with certain models. To ensure that these models are never selected, they are removed from the analysis (see [d’Auvergne and Gooley \(2006\)](#)).

**Model-free model selection** The AIC model selection as described in [d’Auvergne and Gooley \(2003\)](#) will be used to determine which model-free model best describes the relaxation data.

**Data output** Finally both a save state and result file will be created.

These three sample scripts describe the basic components of model-free analysis. However a full analysis requires the construction of a much more complex iterative procedure. The following sections will describe both the original diffusion seeded approaches as well as the new model-free protocol built into relax.

## 7.5 The methodology of Mandel et al., 1995

By presenting a systematic methodology for obtaining a consistent model-free description of the dynamics of the system, the manuscript of [Mandel et al. \(1995\)](#) revolutionised the application of model-free analysis. The full protocol is presented in Figure 7.1.

All of the data analysis techniques required for this protocol can be implemented within relax. The chi-squared distributions required for the chi-squared tests are constructed by Modelfree4 from the Monte Carlo simulations. If the optimisation algorithms and Monte Carlo simulations built into relax are utilised, then the relax script will need to construct the chi-squared distributions from the results as this is not yet coded into relax. The specific step-up hypothesis testing model selection of [Mandel et al. \(1995\)](#) is available through the `model_selection` user function. Coding the rest of the protocol into a script should be straightforward.

To implement this analysis, a number of scripts would need to be written. There is no sample script in relax for performing this analysis. The simple sample scripts from above would need to be extended. For example a starting script for determining the initial diffusion tensor estimates based on the R1/R2 ratio of [Kay et al. \(1989\)](#) would have to be written. The tensor from this script could then be feed into the `model_free/mf_multimodel.py` script, followed by the `model_free/modsel.py` script, and then a third script written to optimise the diffusion tensor. A master script could be written first run the initial diffusion tensor script, then to iteratively execute the last three scripts until convergence, and finally to select the best diffusion model (see Figure 7.1). Alternatively, these could all be combined into one super script.

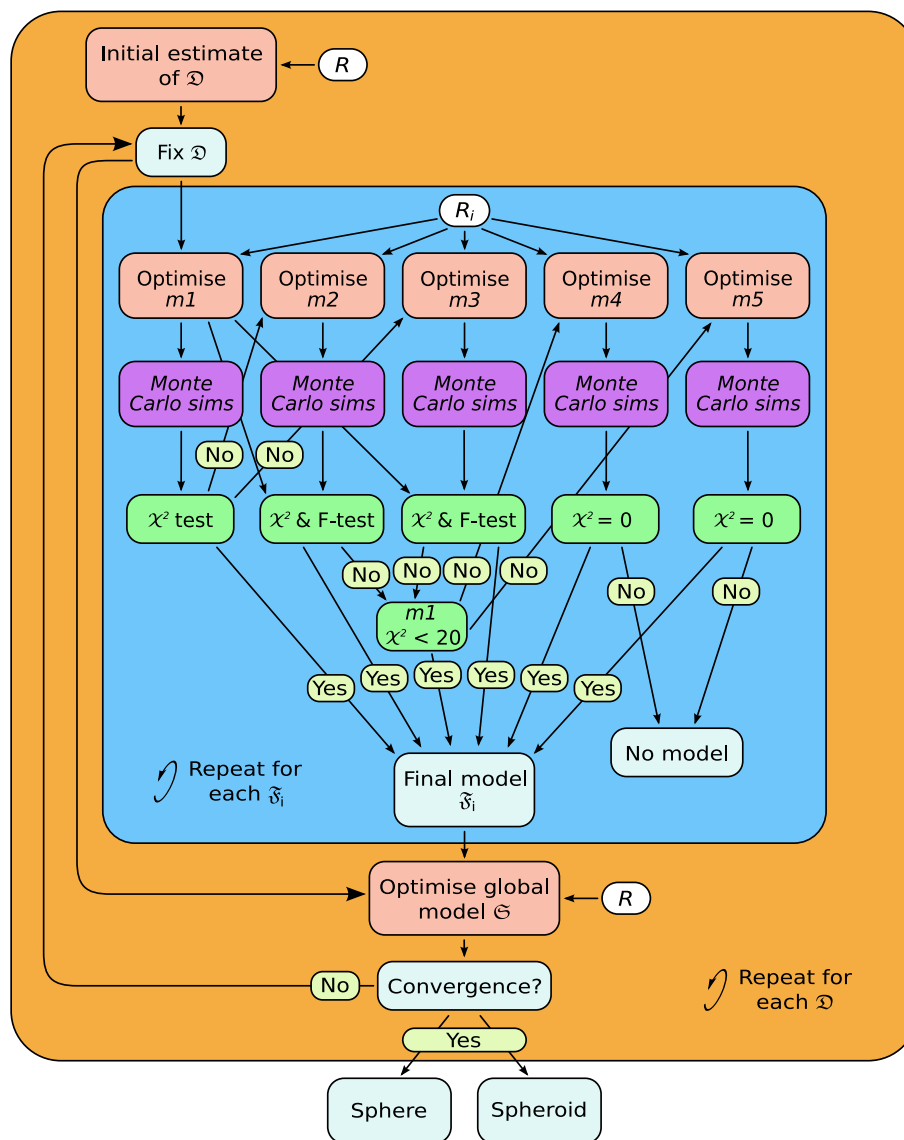


Figure 7.1: A schematic of the model-free optimisation protocol of Mandel et al. (1995). This specific protocol is for single field strength data. The initial diffusion tensor estimate is calculated using the  $R_2/R_1$  ratio. The diffusion parameters of  $\mathcal{D}$  are held constant while model-free models  $m_1$  to  $m_5$  (7.22.1–7.22.5) of the set  $\mathfrak{F}_i$  for each spin  $i$  are optimised and 500 Monte Carlo simulations executed. Using a web of ANOVA statistical tests, specifically  $\chi^2$  and F-tests, a step-up hypothesis testing model selection procedure is used to choose the best model-free model. These steps are repeated for all spins of the molecule. The global model  $\mathfrak{S}$ , the union of  $\mathcal{D}$  and all  $\mathfrak{F}_i$ , is then optimised. These steps are repeated until convergence of the global model. The iterative process is repeated for both isotropic diffusion (sphere) and anisotropic diffusion (spheroid).

## 7.6 The diffusion seeded paradigm

Ever since the original Lipari and Szabo papers (Lipari and Szabo, 1982a,b), the question of how to obtain the model-free description of the system has followed the route in which the diffusion tensor is initially estimated. Using this rough estimate, the model-free models are optimised for each spin system  $i$ , the best model selected, and then the global model  $\mathfrak{S}$  of the diffusion model  $\mathfrak{D}$  with each model-free model  $\mathfrak{F}_i$  is optimised. This procedure is then repeated using the diffusion tensor parameters of  $\mathfrak{S}$  as the initial input. Finally the global model is selected. The full protocol, when combined with AIC model selection (d’Auvergne and Gooley, 2003), is illustrated in Figure 7.2.

Again this protocol is not implemented in the relax sample scripts. This would have to be implemented in exactly the same manner as described in the previous section, but using the AIC model selection build into relax. Constructing this set of scripts, or a single master script, would be much easier than the Mandel et al. (1995) protocol as Modelfree4 would not need to be used, and the handling of F-tests and chi-squared tests is avoided.

## 7.7 The new model-free optimisation protocol

Here a new, fully automated model-free optimisation protocol will be presented. This protocol, defined in d’Auvergne and Gooley (2007) and d’Auvergne and Gooley (2008c), is significantly different from all those that came before, reversing the diffusion seeded paradigm as detailed below. Within relax it is referred to as the “new protocol” or the “d’Auvergne protocol”. The later name is to allow for more advanced protocols to be developed and added to relax by adventurous users in the future. Note that for advanced model-free analysis protocols, such as this one, that multiple field relaxation data is essential.

### 7.7.1 The new protocol – model-free models

The study of the dynamics of a macromolecule using model-free analysis to interpret the  $R_1$  and  $R_2$  relaxation rates together with the steady-state heteronuclear NOE brings two distinct, yet linked physical theories into play. The Brownian rotational diffusion of the molecule is the major contributor to relaxation. Although having less of an influence on relaxation the internal dynamics of individual nuclei within the molecule is nevertheless significant. The model-free description of the internal motion and the global diffusion of the entire molecule are theories which are linked due to their dependence on the same relaxation data. The model-free models for individual spin system constructed from the original and extended model-free theories (Lipari and Szabo, 1982a,b; Clore et al., 1990) are assembled using parametric restrictions, the dropping of insignificant parameters, and the addition of the chemical exchange parameter  $R_{ex}$ . Labelled as  $m0$  to  $m9$  (Models 7.22.0–7.22.9 on page 88) these models are an extended list of those in (Fushman et al., 1997; Orekhov et al., 1999a; Korzhnev et al., 2001; Zhuravleva et al., 2004).

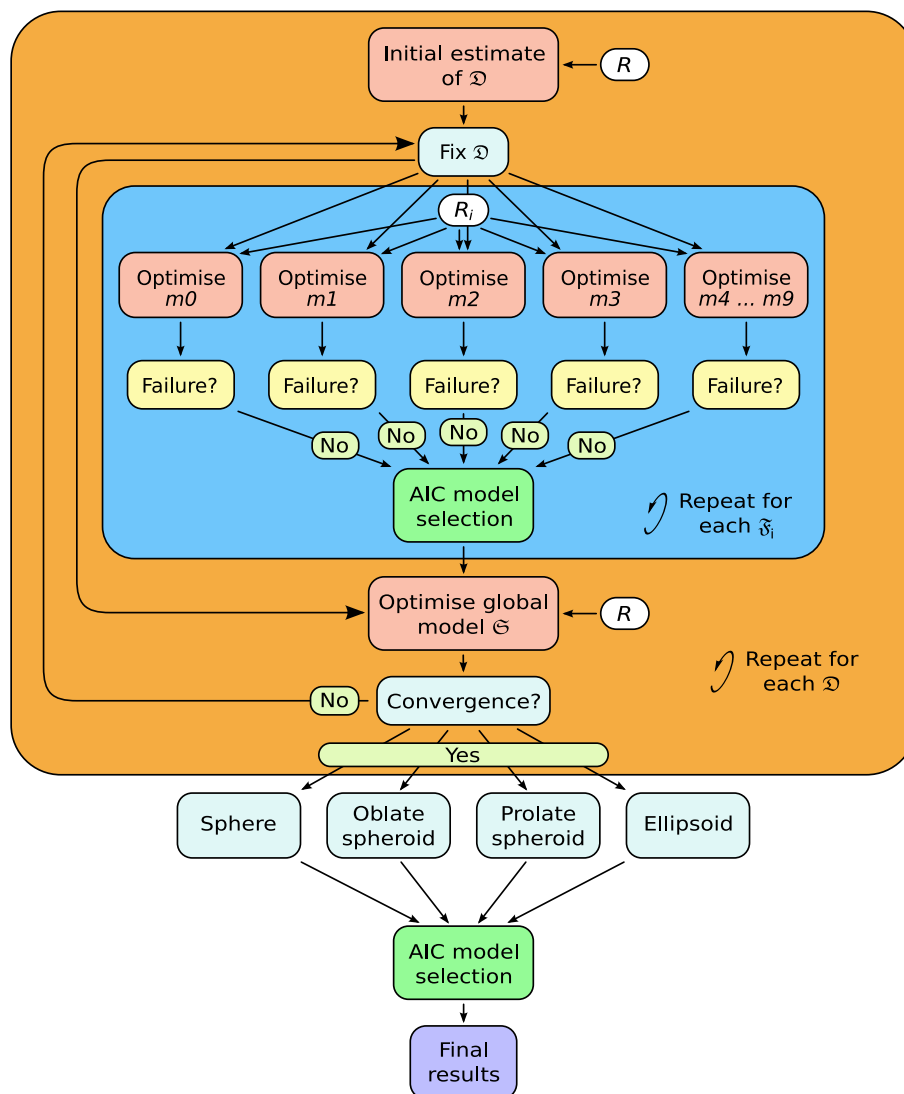


Figure 7.2: A schematic of model-free analysis using the diffusion seeded paradigm – the initial diffusion tensor estimate – together with AIC model selection and model elimination. The initial estimates of the parameters of  $\mathcal{D}$  are held constant while model-free models  $m_0$  to  $m_9$  (7.22.0–7.22.9) of the set  $\mathfrak{F}_i$  for each spin system  $i$  are optimised, model elimination applied to remove failed models, and AIC model selection used to determine the best model. The global model  $\mathcal{S}$ , the union of  $\mathcal{D}$  and all  $\mathfrak{F}_i$ , is then optimised. These steps are repeated until convergence of the global model. The entire iterative process is repeated for each of the Brownian diffusion models. Finally AIC model selection is used to determine the best description of the dynamics of the molecule by selecting between the global models  $\mathcal{S}$  including the sphere, oblate spheroid, prolate spheroid, and ellipsoid. Once the solution has been found, Monte Carlo simulations can be utilised for error analysis.

### 7.7.2 The new protocol – the diffusion tensor

#### The ellipsoid

The most general form of Brownian rotational diffusion of macromolecules is the diffusion of an ellipsoid, a diffusion also labelled as asymmetric or fully anisotropic. This diffusion tensor can be fully specified by the geometric parameters  $\mathfrak{D}_x$ ,  $\mathfrak{D}_y$ , and  $\mathfrak{D}_z$ , the eigenvalues of the tensor, as well as three orientational parameters, the Euler angles  $\alpha$ ,  $\beta$ , and  $\gamma$ . The diffusion equation for an ellipsoid was derived using the reasoning of [Einstein \(1905\)](#) in the two papers of [Perrin \(1934\)](#) and [Perrin \(1936\)](#). Following this, [Favro \(1960\)](#) unknowingly derived the same equations as presented in [Perrin \(1936\)](#) using a pseudo quantum mechanical approach. Borrowing heavily from [Perrin \(1936\)](#), [Woessner \(1962\)](#) derived the correlation function relevant for NMR relaxation of a bond vector rigidly attached to an ellipsoid. However these equations are not fully simplified and the parameter set  $\{\mathfrak{D}_x, \mathfrak{D}_y, \mathfrak{D}_z, \alpha, \beta, \gamma\}$ , the eigenvalues and Euler angles defining the tensor, is not optimally constructed for minimisation. A parameter shift to the set  $\{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r, \alpha, \beta, \gamma\}$ , whereby the three geometric parameters are respectively the isotropic, anisotropic, and rhombic components of the diffusion tensor, drastically simplifies optimisation and is how the diffusion tensor is implemented within relax.

#### The spheroid

When two of the eigenvalues of the diffusion tensor are equal the molecule diffuses as a spheroid. This is also called axially symmetric anisotropic diffusion and can be described by the two geometric parameters  $\mathfrak{D}_{iso}$  and  $\mathfrak{D}_a$  together with the polar angle  $\theta$  and azimuthal angle  $\phi$  which define the unique axis of the diffusion tensor. Two classes of spheroid can be distinguished dependent on the relative values of the eigenvalues – the prolate and oblate spheroids. By using parametric constraints, both tensor types can be optimised within relax.

#### The sphere

The simplest form of diffusion occurs when all three eigenvalues are equal and the molecule diffuses as a sphere. This isotropic rotation can be characterised by the single parameter  $\mathfrak{D}_{iso}$  which is related to the global correlation time by the formula  $1/\tau_m = 6\mathfrak{D}_{iso}$  ([Bloembergen et al., 1948](#)).

#### The local $\tau_m$ model-free models

Not only can the diffusion tensor be optimised as a global model affecting all spins of the molecule but a set of model-free models can be constructed in which each spin is assumed to diffuse independently. In these models a single local  $\tau_m$  parameter approximates the true, multiexponential description of the Brownian rotational diffusion of the molecule. Each spin of the macromolecule is treated independently. Another set of model-free models which include the local  $\tau_m$  parameter can be created and include *tm0* to *tm9* (Models 7.23.0–7.23.9 on page 88). These are simply models *m0* to *m9* with the local  $\tau_m$

parameter added. These models are an extension of the ideas introduced in [Barbato et al. \(1992\)](#) and [Schurr et al. \(1994\)](#) whereby the model *tm2*, the original Lipari and Szabo model-free equation with a local  $\tau_m$  parameter, is optimised to avoid issues with inaccurate diffusion tensor approximations.

### Determination of the diffusion tensor from the local $\tau_m$ parameter

In [Brüschweiler et al. \(1995\)](#) and further investigated in [Lee et al. \(1997\)](#), a methodology for determining the diffusion tensor from the local  $\tau_m$  parameter together with the orientation of the XH bond represented by the unit vector  $\mu_i$  was presented. A local  $\tau_m$  value was obtained for each spin  $i$  by optimising model *tm2*. The  $\tau_{m,i}$  values were approximated using the quadric model

$$(6\tau_{m,i})^{-1} = \mu_i^T Q \mu_i, \quad (7.36)$$

where the eigenvalues of the matrix  $Q$  are defined as  $Q_x = (\mathfrak{D}_y + \mathfrak{D}_z)/2$ ,  $Q_y = (\mathfrak{D}_x + \mathfrak{D}_z)/2$ , and  $Q_z = (\mathfrak{D}_x + \mathfrak{D}_y)/2$ . The diffusion tensor is then found by linear least-squares fitting.

#### 7.7.3 The universal solution $\mathfrak{U}$

The complex model-free problem, in which the motions of each spin are both mathematically and statistically dependent on the diffusion tensor and vice versa, was formulated using set theory in [d’Auvergne and Gooley \(2007\)](#). This paper is important for understanding the entire concept of the new protocol in relax and for truly grasping the complexity of the model-free problem. The solution  $\hat{\mathfrak{U}}$  to the model-free problem was derived as an element of the universal set  $\mathfrak{U}$ , the union of the diverse model-free parameter spaces  $\mathfrak{S}$ . Each set  $\mathfrak{S}$  was constructed from the union of the model-free models  $\mathfrak{F}$  for all spins and the diffusion parameter set  $\mathfrak{D}$ . A single parameter loss on a single spin shifts optimisation to a different space  $\mathfrak{S}$ . Ever since the seminal work of [Kay et al. \(1989\)](#) the model-free problem has been tackled by first finding an initial estimate of the diffusion tensor and then determining the model-free dynamics of the system (see Sections 7.5 on page 96 and 7.6 on page 98). This diffusion seeded paradigm is now highly evolved and much theory has emerged to improve this path to the solution  $\hat{\mathfrak{U}}$ . The technique can, at times, suffer from a number of issues including the two minima problem of the spheroid diffusion tensor parameter space, the appearance of artificial chemical exchange ([Tjandra et al., 1996](#)), the appearance of artificial nanosecond motions ([Schurr et al., 1994](#)), and the hiding of internal nanosecond motions caused by the violation of the rigidity assumption ([Orekhov et al., 1995, 1999a,b](#)).

#### 7.7.4 Model-free analysis in reverse

A different approach was proposed in [d’Auvergne and Gooley \(2008c\)](#) for finding the universal solution  $\hat{\mathfrak{U}}$  of the extremely complex, convoluted model-free optimisation and modelling problem ([d’Auvergne and Gooley, 2007](#)), defined as

$$\hat{\mathfrak{U}} = \hat{\theta} \in \left\{ \mathfrak{S} : \min_{\hat{\theta} \in \mathfrak{U}} \Delta_{\text{K-L}}(\hat{\theta}) \right\}, \quad \text{s.t. } \hat{\theta} = \arg \min \{ \chi^2(\theta) : \theta \in \mathfrak{S} \}. \quad (7.37)$$

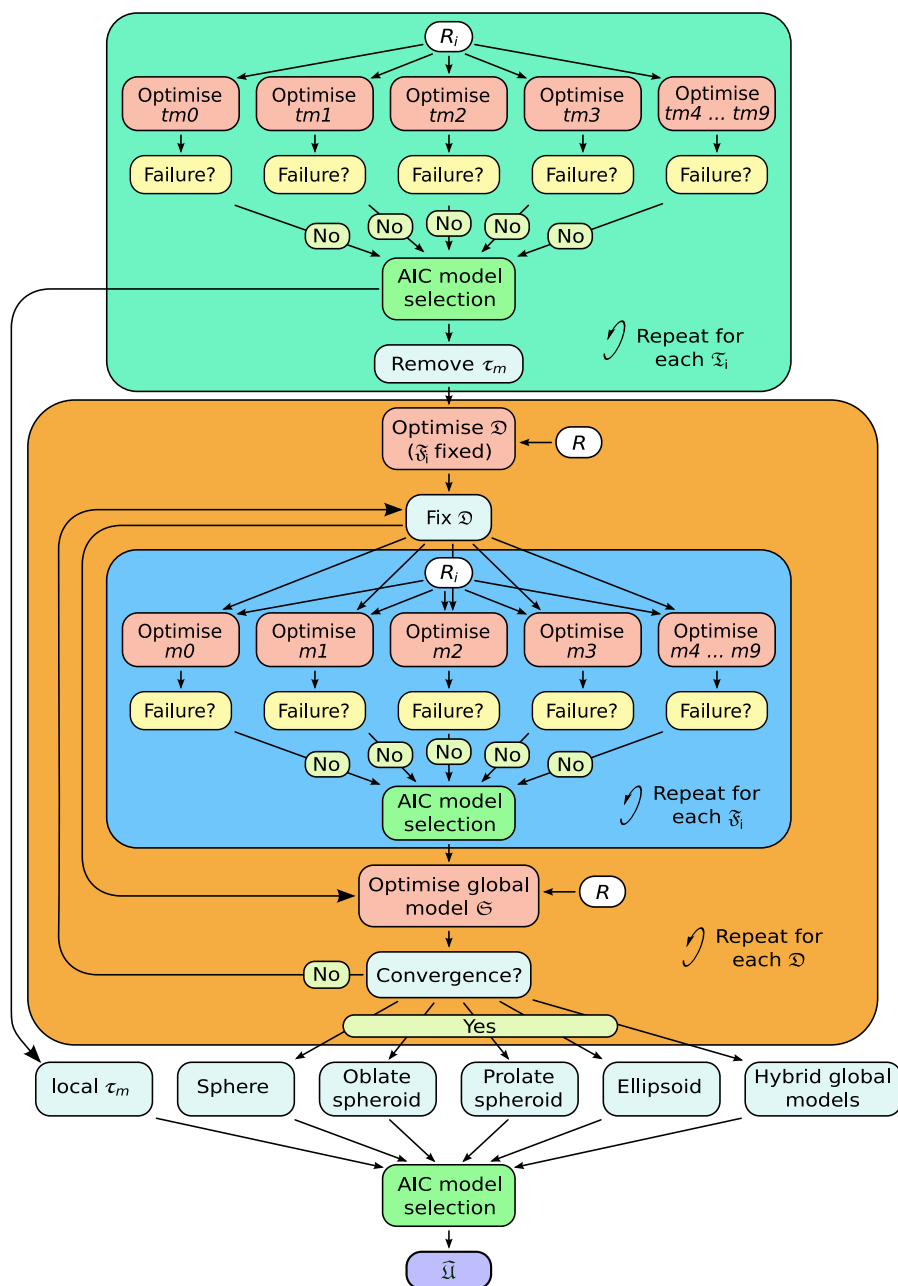


Figure 7.3: A schematic of the new model-free optimisation protocol. Initially models  $tm_0$  to  $tm_9$  (7.23.0–7.23.9) of the set  $\Xi_i$  for each spin system  $i$  are optimised, model elimination used to remove failed models, and AIC model selection used to pick the best model. Once all the  $\Xi_i$  have been determined for the system the the local  $\tau_m$  parameter is removed, the model-free parameters are held fixed, and the global diffusion parameters of  $\mathcal{D}$  are optimised. These parameters are used as input for the central part of the schematic which follows the same procedure as that of Figure 7.2. Convergence is however precisely defined as identical models  $\mathcal{S}$ , identical  $\chi^2$  values, and identical parameters  $\theta$  between two iterations. The universal solution  $\hat{\mathcal{U}}$ , the best description of the dynamics of the molecule, is determined using AIC model selection to select between the local  $\tau_m$  models for all spins, the sphere, oblate spheroid, prolate spheroid, ellipsoid, and possibly hybrid models whereby multiple diffusion tensors have been applied to different parts of the molecule.



This notation says that the minimised parameter vector within the space  $\mathfrak{S}$  which minimises the common Kullback-Leibler discrepancy  $\Delta_{\text{K-L}}$  is selected from the universal set  $\mathfrak{U}$  as the universal solution  $\hat{\mathfrak{U}}$ . The discrepancy of Kullback and Leibler (1951) is a measure of how well the model fits the data, in this case how well the global model  $\mathfrak{S}$  of the diffusion tensor together with the model-free models of all residues fits the relaxation data. This selection is subject to the condition that  $\hat{\theta}$  is the argument or specific parameter vector which minimises the chi-squared function  $\chi^2(\theta)$  such that  $\theta$  is an element of the space  $\mathfrak{S}$ . Whereas the minimisation of the continuous chi-squared function within the single space  $\mathfrak{S}$  belongs to the mathematical field of optimisation (Nocedal and Wright, 1999), the selection of the universe  $\mathfrak{S}$  which minimises the discrepancy belongs to the statistical field of model selection (Akaike, 1973; Schwarz, 1978; Linhart and Zucchini, 1986; Zucchini, 2000; d’Auvergne and Gooley, 2003).

This new model-free optimisation protocol incorporates the ideas of the local  $\tau_m$  model-free model (Barbato et al., 1992; Schurr et al., 1994) and the optimisation of the diffusion tensor using information from these models, analogously to the linear least-squares fitting of the quadric model (Brüschweiler et al., 1995; Lee et al., 1997). The protocol also follows the lead of the model-free optimisation protocol presented in Butterwick et al. (2004) whereby the diffusion seeded paradigm was reversed. Rather than starting with an initial estimation of the global diffusion tensor from the set  $\mathfrak{D}$  the protocol starts with the model-free parameters from  $\mathfrak{F}$ .

The first step of the Butterwick et al. (2004) protocol is the reduced spectral density mapping of Farrow et al. (1995). As  $R_{ex}$  has been eliminated from the analysis, three model-free models corresponding to  $tm1$ ,  $tm2$ , and  $tm5$  (Models 7.23.1, 7.23.2, and 7.23.5 on page 88) are employed. The model-free parameters are optimised using the reduced spectral density values and the best model is selected using F-tests. The spherical, spheroidal, and ellipsoidal diffusion tensors are obtained by linear least-squares fitting of the quadric model of Equation (7.36) using the local  $\tau_m$  values (Brüschweiler et al., 1995; Lee et al., 1997). The best diffusion model is selected via F-tests and refined by iterative elimination of spins systems with high chi-squared values. This tensor is used to calculate local  $\tau_m$  values for each spin system, approximating the multiexponential sum of the Brownian rotational diffusion correlation function with a single exponential, using the quadric model of Equation (7.36). In the final step of the protocol these  $\tau_m$  values are fixed and  $m1$ ,  $m2$ , and  $m5$  (Models 7.22.1, 7.22.2, and 7.22.5 on page 88) are optimised and the best model-free model selected using F-tests.

The new model-free protocol built into relax utilises the core foundation of the Butterwick et al. (2004) protocol yet its divergent implementation is designed to solve the universal equation of d’Auvergne and Gooley (2007) to find  $\hat{\mathfrak{U}}$  (Equation 7.37). Models  $tm0$  to  $tm9$  (7.23.0–7.23.9 on page 88) in which no global diffusion parameters exist are employed to significantly collapse the complexity of the problem. Model-free minimisation (d’Auvergne and Gooley, 2008b), model elimination (d’Auvergne and Gooley, 2006), and then AIC model selection (Akaike, 1973; d’Auvergne and Gooley, 2003) can be carried out in the absence of the influence of global parameters. By removing the local  $\tau_m$  parameter and holding the model-free parameter values constant these models can then be used to optimise the diffusion parameters of  $\mathfrak{D}$ . Model-free optimisation, model elimination, AIC model selection, and optimisation of the global model  $\mathfrak{S}$  is iterated until convergence. The iterations allow for sliding between different universes  $\mathfrak{S}$  to enable the collapse of model complexity, to refine the diffusion tensor, and to find the solution within the universal set

$\mathfrak{U}$ . The last step is the AIC model selection between the different diffusion models. Because the AIC criterion approximates the Kullback-Leibler discrepancy (Kullback and Leibler, 1951), central to the universal solution of Equation (7.37), it was chosen for all three model selection steps over BIC model selection (Schwarz, 1978; d’Auvergne and Gooley, 2003; Chen et al., 2004). The new protocol avoids the problem of under-fitting whereby artificial motions appear, avoids the problems involved in finding the initial diffusion tensor within  $\mathfrak{D}$ , and avoids the problem of hidden internal nanosecond motions and the inability to slide between universes to get to  $\hat{\mathfrak{U}}$  (see d’Auvergne and Gooley (2007) for more details). The full protocol is summarised in Figure 7.3.

## 7.8 The new protocol in the prompt/script UI mode

### 7.8.1 d'Auvergne protocol script mode – the sample script

The sample script for performing this new analysis is `sample_scripts/model_free/dauvergne_protocol.py`. The full script is replicated below. The docstring at the start of the script explains the practical implementation of the full protocol. If your copy of the `dauvergne_protocol.py` script taken from the same relax version as this manual does not match the text below, please contact the relax developers via the relax-devel mailing list (see section 3.2.3 on page 30). To use this script, copy it to a dedicated directory containing your PDB file and relaxation data files. The protocol will produce many files and directories, so it is best that these are placed within a dedicated and results directory. The contents of the script are:

```
1  """Script for black-box model-free analysis.
2
3  This script is designed for those who appreciate black-boxes or those who appreciate
    complex code. Importantly data at multiple magnetic field strengths is essential for
    this analysis. The script will need to be heavily tailored to the molecule in
    question by changing the variables just below this documentation. If you would like
    to change how model-free analysis is performed, the code in the class Main can be
    changed as needed. For a description of object-oriented coding in python using
    classes, functions/methods, self, etc., see the python tutorial.
4
5  If you have obtained this script without the program relax, please visit http://www.nmr-relax.com.
6
7
8  References
9  =====
10
11  The model-free optimisation methodology herein is that of:
12
13      d'Auvergne, E. J. and Gooley, P. R. (2008b). Optimisation of NMR dynamic models II. A
        new methodology for the dual optimisation of the model-free parameters and the
        Brownian rotational diffusion tensor. J. Biomol. NMR, 40(2), 121-133
14
15  Other references for features of this script include model-free model selection using
        Akaike's Information Criterion:
16
17      d'Auvergne, E. J. and Gooley, P. R. (2003). The use of model selection in the model-
        free analysis of protein dynamics. J. Biomol. NMR, 25(1), 25-39.
18
19  The elimination of failed model-free models and Monte Carlo simulations:
20
21      d'Auvergne, E. J. and Gooley, P. R. (2006). Model-free model elimination: A new step
        in the model-free dynamic analysis of NMR relaxation data. J. Biomol. NMR, 35(2),
        117-135.
22
23  Significant model-free optimisation improvements:
24
25      d'Auvergne, E. J. and Gooley, P. R. (2008a). Optimisation of NMR dynamic models I.
        Minimisation algorithms and their performance within the model-free and Brownian
        rotational diffusion spaces. J. Biomol. NMR, 40(2), 107-109.
26
27  Rather than searching for the lowest chi-squared value, this script searches for the model
        with the lowest AIC criterion. This complex multi-universe, multi-dimensional search
        is formulated using set theory as the universal solution:
```

d'Auvergne, E. J. and Gooley, P. R. (2007). Set theory formulation of the model-free problem and the diffusion seeded model-free paradigm. 3(7), 483-494.

The basic three references for the original and extended model-free theories are:

Lipari, G. and Szabo, A. (1982a). Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules I. Theory and range of validity. J. Am. Chem. Soc., 104(17), 4546-4559.

Lipari, G. and Szabo, A. (1982b). Model-free approach to the interpretation of nuclear magnetic-resonance relaxation in macromolecules II. Analysis of experimental results. J. Am. Chem. Soc., 104(17), 4559-4570.

Clore, G. M., Szabo, A., Bax, A., Kay, L. E., Driscoll, P. C., and Gronenborn, A.M. (1990). Deviations from the simple 2-parameter model-free approach to the interpretation of N-15 nuclear magnetic-relaxation of proteins. J. Am. Chem. Soc., 112(12), 4989-4991.

How to use this script

=====

The value of the variable DIFF\_MODEL will determine the behaviour of this script. The five diffusion models used in this script are:

Model I (MI) - Local tm.  
 Model II (MII) - Sphere.  
 Model III (MIII) - Prolate spheroid.  
 Model IV (MIV) - Oblate spheroid.  
 Model V (MV) - Ellipsoid.

Model I must be optimised prior to any of the other diffusion models, while the Models II to V can be optimised in any order. To select the various models, set the variable DIFF\_MODEL to the following strings:

MI - 'local\_tm'  
 MII - 'sphere'  
 MIII - 'prolate'  
 MIV - 'oblate'  
 MV - 'ellipsoid'

This approach has the advantage of eliminating the need for an initial estimate of a global diffusion tensor and removing all the problems associated with the initial estimate.

It is important that the number of parameters in a model does not exceed the number of relaxation data sets for that spin. If this is the case, the list of models in the MF\_MODELS and LOCAL\_TM\_MODELS variables will need to be trimmed.

Model I - Local tm

~~~~~

This will optimise the diffusion model whereby all spin of the molecule have a local tm value, i.e. there is no global diffusion tensor. This model needs to be optimised prior to optimising any of the other diffusion models. Each spin is fitted to the multiple model-free models separately, where the parameter tm is included in each model.

AIC model selection is used to select the models for each spin.

```

70
71
72 Model II - Sphere
73 ~~~~~
74
75 This will optimise the isotropic diffusion model. Multiple steps are required, an initial
    optimisation of the diffusion tensor, followed by a repetitive optimisation until
    convergence of the diffusion tensor. Each of these steps requires this script to be
    rerun. For the initial optimisation, which will be placed in the directory './sphere/
    init/', the following steps are used:
76
77 The model-free models and parameter values for each spin are set to those of diffusion
    model MI.
78
79 The local tm parameter is removed from the models.
80
81 The model-free parameters are fixed and a global spherical diffusion tensor is minimised.
82
83
84 For the repetitive optimisation, each minimisation is named from 'round_1' onwards. The
    initial 'round_1' optimisation will extract the diffusion tensor from the results file
    in './sphere/init/', and the results will be placed in the directory './sphere/
    round_1/'. Each successive round will take the diffusion tensor from the previous
    round. The following steps are used:
85
86 The global diffusion tensor is fixed and the multiple model-free models are fitted to each
    spin.
87
88 AIC model selection is used to select the models for each spin.
89
90 All model-free and diffusion parameters are allowed to vary and a global optimisation of
    all parameters is carried out.
91
92
93 Model III - Prolate spheroid
94 ~~~~~
95
96 The methods used are identical to those of diffusion model MII, except that an axially
    symmetric diffusion tensor with  $D_a \geq 0$  is used. The base directory containing all
    the results is './prolate/'.
97
98
99 Model IV - Oblate spheroid
100 ~~~~~
101
102 The methods used are identical to those of diffusion model MII, except that an axially
    symmetric diffusion tensor with  $D_a \leq 0$  is used. The base directory containing all
    the results is './oblate/'.
103
104
105 Model V - Ellipsoid
106 ~~~~~
107
108 The methods used are identical to those of diffusion model MII, except that a fully
    anisotropic diffusion tensor is used (also known as rhombic or asymmetric diffusion).
    The base directory is './ellipsoid/'.
109
110
111
112 Final run
113 ~~~~~

```

```

114
115 Once all the diffusion models have converged, the final run can be executed. This is done
    by setting the variable DIFF_MODEL to 'final'. This consists of two steps, diffusion
    tensor model selection, and Monte Carlo simulations. Firstly AIC model selection is
    used to select between the diffusion tensor models. Monte Carlo simulations are then
    run solely on this selected diffusion model. Minimisation of the model is bypassed as
    it is assumed that the model is already fully optimised (if this is not the case the
    final run is not yet appropriate).
116
117 The final black-box model-free results will be placed in the file 'final/results'.
118 """
119
120 # Python module imports.
121 from time import asctime, localtime
122
123 # relax module imports.
124 from auto_analyses.dauvergne_protocol import dAuvergne_protocol
125
126
127 # Analysis variables.
128 #####
129
130 # The diffusion model.
131 DIFF_MODEL = 'local_tm'
132
133 # The model-free models. Do not change these unless absolutely necessary, the protocol is
    likely to fail if these are changed.
134 MF_MODELS = ['m0', 'm1', 'm2', 'm3', 'm4', 'm5', 'm6', 'm7', 'm8', 'm9']
135 LOCAL_TM_MODELS = ['tm0', 'tm1', 'tm2', 'tm3', 'tm4', 'tm5', 'tm6', 'tm7', 'tm8', 'tm9']
136
137 # The grid search size (the number of increments per dimension).
138 GRID_INC = 11
139
140 # The optimisation technique.
141 MIN_ALGOR = 'newton'
142
143 # The number of Monte Carlo simulations to be used for error analysis at the end of the
    analysis.
144 MC_NUM = 500
145
146 # Automatic looping over all rounds until convergence (must be a boolean value of True or
    False).
147 CONV_LOOP = True
148
149
150
151 # Set up the data pipe.
152 #####
153
154 # The following sequence of user function calls can be changed as needed.
155
156 # Create the data pipe.
157 pipe_bundle = "mf (%s)" % asctime(localtime())
158 name = "origin - " + pipe_bundle
159 pipe.create(name, 'mf', bundle=pipe_bundle)
160
161 # Load the PDB file.
162 structure.read_pdb('1f3y.pdb', set_mol_name='Ap4Aase', read_model=3)
163
164 # Set up the 15N and 1H spins (both backbone and Trp indole sidechains).
165 structure.load_spins('@N', ave_pos=True)

```

```

166 structure.load_spins('@NE1', ave_pos=True)
167 structure.load_spins('@H', ave_pos=True)
168 structure.load_spins('@HE1', ave_pos=True)
169 spin.isotope('15N', spin_id='@N*')
170 spin.isotope('1H', spin_id='@H*')
171
172 # Set up the 15N spins (alternative to the structure-based approach).
173 #sequence.read(file='noe.500.out', dir=None, mol_name_col=1, res_num_col=2, res_name_col
    =3, spin_num_col=4, spin_name_col=5)
174 #spin.element(element='N', spin_id='@N*')
175 #spin.isotope('15N', spin_id='@N*')
176
177 # Generate the 1H spins for the magnetic dipole-dipole relaxation interaction (alternative
    to the structure-based approach).
178 #sequence.attach_protons()
179
180 # Load the relaxation data.
181 relax_data.read(ri_id='R1_600', ri_type='R1', frq=599.719*1e6, file='r1.600.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
182 relax_data.read(ri_id='R2_600', ri_type='R2', frq=599.719*1e6, file='r2.600.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
183 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=599.719*1e6, file='noe.600.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
184 relax_data.read(ri_id='R1_500', ri_type='R1', frq=500.208*1e6, file='r1.500.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
185 relax_data.read(ri_id='R2_500', ri_type='R2', frq=500.208*1e6, file='r2.500.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
186 relax_data.read(ri_id='NOE_500', ri_type='NOE', frq=500.208*1e6, file='noe.500.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
187
188 # Deselect spins to be excluded (including unresolved and specifically excluded spins).
189 deselect.read(file='unresolved', dir=None, spin_id_col=None, mol_name_col=1, res_num_col
    =2, res_name_col=3, spin_num_col=4, spin_name_col=5, sep=None, spin_id=None, boolean='
    AND', change_all=False)
190 deselect.read(file='exclude', spin_id_col=1)
191
192 # Define the magnetic dipole-dipole relaxation interaction.
193 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
194 interatom.define(spin_id1='@NE1', spin_id2='@HE1', direct_bond=True)
195 interatom.set_dist(spin_id1='@N*', spin_id2='@H*', ave_dist=1.02 * 1e-10)
196 interatom.unit_vectors()
197
198 # Define the chemical shift relaxation interaction.
199 value.set(-172 * 1e-6, 'csa', spin_id='@N*')
200
201
202
203 # Execution.
204 #####
205
206 # Do not change!
207 dAuvergne_protocol(pipe_name=name, pipe_bundle=pipe_bundle, diff_model=DIFF_MODEL,
    mf_models=MF_MODELS, local_tm_models=LOCAL_TM_MODELS, grid_inc=GRID_INC, min_algor=
    MIN_ALGOR, mc_sim_num=MC_NUM, conv_loop=CONV_LOOP)

```

### 7.8.2 d'Auvergne protocol script mode – analysis variables

At the start of the script you will notice a number of `Analysis variables`. Unless you know what you are doing, you should only change the `DIFF_MODEL` variable to the following:

`'local_tm'`: This is the first diffusion model which must be optimised prior to optimising any of the other diffusion models. It consists of the local  $\tau_m$  models (equations 7.23.0 to 7.23.9 on page 88).

`'sphere'`: This second diffusion model is that of isotropic Brownian diffusion.

`'prolate'`: This third diffusion model is that of the prolate axially-symmetric rotor.

`'oblate'`: This fourth diffusion model is that of the oblate axially-symmetric rotor.

`'ellipsoid'`: This fifth diffusion model is that of fully rhombic Brownian diffusion (see Perrin (1934, 1936) for the original theory).

`'final'`: This is a special value which will finalise the analysis by selecting the best diffusion model to describe your system and to perform Monte Carlo simulations for error propagation.

The `MF_MODELS` and `LOCAL_TM_MODELS` variables specify which model-free models will be used in the analysis. But, as the full protocol behind this script which is designed to find the solution of the universal set  $\mathcal{U}$  (see section 7.7.2 on page 101) expects that all these models are present, you should not change these variables. If you do remove some model-free models, you should fully expect to see artificial motions which you will not be able to distinguish from the real molecular motions.

The next variables `GRID_INC` and `MIN_ALGOR` are related to the optimisation of the model-free models. These should also not be touched unless you fully understand the consequences (and have read d'Auvergne and Gooley (2008b)). The variable `MC_NUM` specifies the number of Monte Carlo simulations. This number can be increased but, for realistic parameter errors in your publication, it should not set lower than 500 simulations.

Finally the `CONV_LOOP` variable is designed to make your life easier. If left at the value of `True`, the script will iterate until convergence (see Figure 2 in d'Auvergne and Gooley (2008c) to understand this concept). If changed to `False`, then you will need to run the script manually for the 15 or so iterations of each diffusion model, and then repeat this for all diffusion models II to V.

### 7.8.3 d'Auvergne protocol script mode – data pipe initialisation

The next part of the script between the `Analysis variables` and execution sets up a data pipe with all of the spin information and relaxation data to pass into the automated protocol. The data pipe is created in the lines:

```
156 # Create the data pipe.
157 pipe_bundle = "mf (%s)" % asctime(localtime())
158 name = "origin - " + pipe_bundle
159 pipe.create(name, 'mf', bundle=pipe_bundle)
```



Firstly a data pipe bundle name is created containing the date and time at the point the script is first executed. This pipe bundle is used to group together all of the data pipes created automatically by the protocol. See section 4.2.1 on page 34 for more details.

The data pipe name used for this initial setup is set to `origin - mf (x)` where `x` is the data and time again. This name is unique and will not clash with the data pipes created within the protocol. The `pipe.create` command will create the data pipe and add it to a new pipe bundle.

#### 7.8.4 d'Auvergne protocol script mode – setting up the spin systems

To see how to set up the spin system data in all possible situations, please see Chapter 4 for a thorough description. Here two different methods are presented. The first is by extracting the spins from a PDB file which is first loaded with:

```
161 # Load the PDB file.
162 structure.read_pdb('1f3y.pdb', set_mol_name='Ap4Aase', read_model=3)
```

This will read the 3<sup>rd</sup> model from the 1F3Y PDB file and name the single molecule as 'Ap4Aase'. The <sup>15</sup>N and <sup>1</sup>H spins for the backbone and tryptophan indole sidechain are extracted from the structure with the user functions:

```
164 # Set up the 15N and 1H spins (both backbone and Trp indole sidechains).
165 structure.load_spins('@N', ave_pos=True)
166 structure.load_spins('@NE1', ave_pos=True)
167 structure.load_spins('@H', ave_pos=True)
168 structure.load_spins('@HE1', ave_pos=True)
```

As the PDB file does not contain isotope information, this is set with the user functions:

```
169 spin.isotope('15N', spin_id='@N*')
170 spin.isotope('1H', spin_id='@H*')
```

The spin ID '@N\*' uses regular expression and will match both the 'N' and 'NE1' spins.

The alternative approach is if a structure is missing. This is the commented out code:

```
172 # Set up the 15N spins (alternative to the structure-based approach).
173 sequence.read(file='noe.500.out', dir=None, mol_name_col=1, res_num_col=2, res_name_col=3,
174               spin_num_col=4, spin_name_col=5)
175 spin.element(element='N', spin_id='@N*')
176 spin.isotope('15N', spin_id='@N*')
177 # Generate the 1H spins for the magnetic dipole-dipole relaxation interaction (alternative
178   to the structure-based approach).
179 sequence.attach_protons()
```

To use this, you will need to place comments (the # character) in front of the previous `structure.read_pdb`, `structure.load_spins` and `spin.isotope` user functions. Then uncomment the `sequence.read`, `spin.element`, `spin.isotope` and `sequence.attach_protons` user functions. The <sup>15</sup>N spins will be extracted from the `noe.500.out` file. The `spin.element` and `spin.isotope` user functions set the information required for relaxation to understand which relaxation mechanisms are active. Finally the `sequence.attach_protons` user function will automatically attach protons to all nitrogen spin systems. As

this method is devoid of atomic positional information, the N-H bonds are absent and the diffusion models requiring structural information (the spheroids and ellipsoid) must be skipped.

### 7.8.5 d’Auvergne protocol script mode – loading the data

The next step is to load the relaxation data for each spin system. The sample script assumes that the NOE,  $R_1$  and  $R_2$  data was generated using relax. One of the six user function calls is:

```
180 # Load the relaxation data.
181 relax_data.read(ri_id='R1_600', ri_type='R1', frq=599.719*1e6, file='r1.600.out',
    mol_name_col=1, res_num_col=2, res_name_col=3, spin_num_col=4, spin_name_col=5,
    data_col=6, error_col=7)
```

This pattern is repeated for all of the relaxation data files loaded. The important points are that each relaxation data set must have its own unique identification string (`ri_id`), the relaxation data type specified (`ri_type`) and the frequency in Hertz (not MHz) specified. Note that the frequency must be the exact value – see the `sfrq` parameter in the Varian procparr file or the SF01 parameter in the Bruker acquis file.

### 7.8.6 d’Auvergne protocol script mode – deselection

The sample script now presents the deselection of spins using two different files:

```
188 # Deselect spins to be excluded (including unresolved and specifically excluded spins).
189 deselect.read(file='unresolved', dir=None, spin_id_col=None, mol_name_col=1, res_num_col
    =2, res_name_col=3, spin_num_col=4, spin_name_col=5, sep=None, spin_id=None, boolean='
    AND', change_all=False)
190 deselect.read(file='exclude', spin_id_col=1)
```

The `unresolved` file contains a list of spins which are unresolved in all spectra. If relax has been used for calculating the NOE and fitting the relaxation curves, then this step is not needed as the relaxation data files will not have any data for the spins deselected in those analyses. The second file `exclude` is a list of spin ID strings (see section 4.2.2 on page 36) of spins that for which ever reason are to be excluded from the analysis.

### 7.8.7 d’Auvergne protocol script mode – relaxation interactions

The next step is to fully specify all of the relaxation interactions active on the spins of interest. Firstly the magnetic dipole-dipole interaction is defined between directly bonded nitrogens and protons:

```
192 # Define the magnetic dipole-dipole relaxation interaction.
193 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
194 interatom.define(spin_id1='@NE1', spin_id2='@HE1', direct_bond=True)
195 interatom.set_dist(spin_id1='@N*', spin_id2='@H*', ave_dist=1.02 * 1e-10)
196 interatom.unit_vectors()
```

The regular expression '@N\*' and '@H\*' cannot be used with the `dipole_pair`. define user function as otherwise @N spins will be connected to @HE1 spins of the same tryptophan residue and @H spins to @NE1 spins. The average interatomic distance is set to 1.02 Ångstrom (though the `dipole_pair.set_dist` user function expects the units of meters). The `dipole_pair.unit_vectors` is used to calculate the averaged unit vector between the two atoms.

Secondly the chemical shift anisotropy (CSA) relaxation mechanism is defined via the single command:

```
198 # Define the chemical shift relaxation interaction.
199 value.set(-172 * 1e-6, 'csa', spin_id='@N*')
```

If your system does not experience CSA relaxation, the value can be set to zero.

### 7.8.8 d'Auvergne protocol script mode – execution

Once the data is set up and you have modified your script to match your analysis needs, then the data pipe, pipe bundle and analysis variables are passed into the `dAuvergne_protocol` class. This is the final line of the script:

```
203 # Execution.
204 #####
205
206 # Do not change!
207 dAuvergne_protocol(pipe_name=name, pipe_bundle=pipe_bundle, diff_model=DIFF_MODEL,
                    mf_models=MF_MODELS, local_tm_models=LOCAL_TM_MODELS, grid_inc=GRID_INC, min_algor=
                    MIN_ALGOR, mc_sim_num=MC_NUM, conv_loop=CONV_LOOP)
```

This script needs to be executed multiple times, once for each of the diffusion models. For example if the `DIFF_MODEL` variable is set to 'ellipsoid', you can run relax with:

```
$ relax --tee log.ellipsoid dauvergne_protocol.py
```

You should use a different log file for each diffusion model, though relax will prevent you from overwriting an old log file. Note that the `log.*` files for each diffusion model may end up being a few gigabytes in size.

For a full analysis of a protein system, the analysis may require between one to two weeks to complete. This can be speed up using Gary Thompson's multi-processor code (see section 1.3 on page 17). The analysis is performed as described in the previous sections and summarised in Figure 7.3. If you are curious, the implementation is within a very large relax script called `auto_analyses/dauvergne_protocol.py` (which must never be changed). This automatic analysis script hides all of the complexity of the full protocol from the sample script.

## 7.9 The new protocol in the GUI

A model-free analysis can be performed within the GUI (see Figure 1.8 on page 18). This analysis is that of the fully automated d’Auvergne protocol which can be chosen via the analysis selection wizard (Figure 1.4 on page 12). Please see Section 7.7 on page 98 for a description of this new model-free protocol. As mentioned previously, please note that this protocol requires multiple field relaxation data.

The GUI is designed to be robust – you should be able to set up all the input data and parameters in any order with relax returning you warnings if something is missing. The analysis will only execute once everything is correctly set up. If this is not the case, clicking on the “Execute relax” button will display a warning window explaining what the issue is rather than initialising the analysis. Despite the self-explanatory nature of the GUI a tutorial on how to use the GUI, with screenshots, will be presented below.

If the “Protocol mode” field is left to the “Fully automated” setting then, after clicking on “Execute relax”, the calculation can be left to complete. It is highly recommended to check the log messages in the relax controller window, at least at the start of the analysis, to make sure that all the data is being read correctly and everything is set up as desired. All warnings should be carefully checked as these can indicate a fatal problem. If you would like to log all the messages into a file, relax can be run with:

```
$ relax -g --log log
```

Note that the size of this log file could end up being in the gigabyte range for a model-free analysis.

For the full analysis to complete, for a protein system this may take about a week. Depending on the nature of the problem and the speed of the computer, the calculation time may be significantly shorter or longer. To speed up the calculations, if you have access to multiple cores and/or hyper-threading, the GUI can be run using Gary Thompson’s multi-processor framework (see section 1.3 on page 17). For example on a dual-core, dual-CPU system, four calculations can be run simultaneously. In this case, the GUI can be launched with:

```
$ mpirun -np 5 /usr/local/bin/relax --multi='mpi4py' --gui --log log
```

This assumes that OpenMPI and the Python mpi4py module have been installed on your system, and relax is installed into the /usr/local/bin/ directory. If this is successful, you should only see a single relax GUI window (and not five windows) and in the relax controller, you should see text similar to:

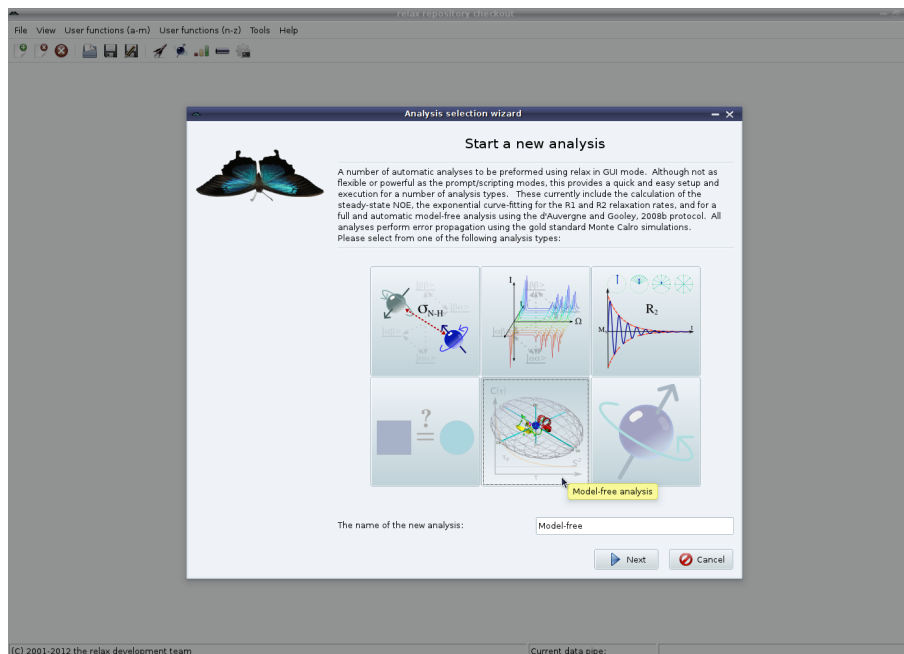
```
Processor fabric: MPI 2.1 running via mpi4py with 4 slave processors & 1 master. Using
Open MPI 1.4.3.
```

If you are using a different MPI implementation, please see the documentation of that implementation to see how to launch a program in MPI mode. Finally as the calculation takes so long, we will run the calculations at a lower priority so that the computer is not slowed down too much and remains responsive. Therefore this model-free GUI analysis tutorial will be launched with the full command:

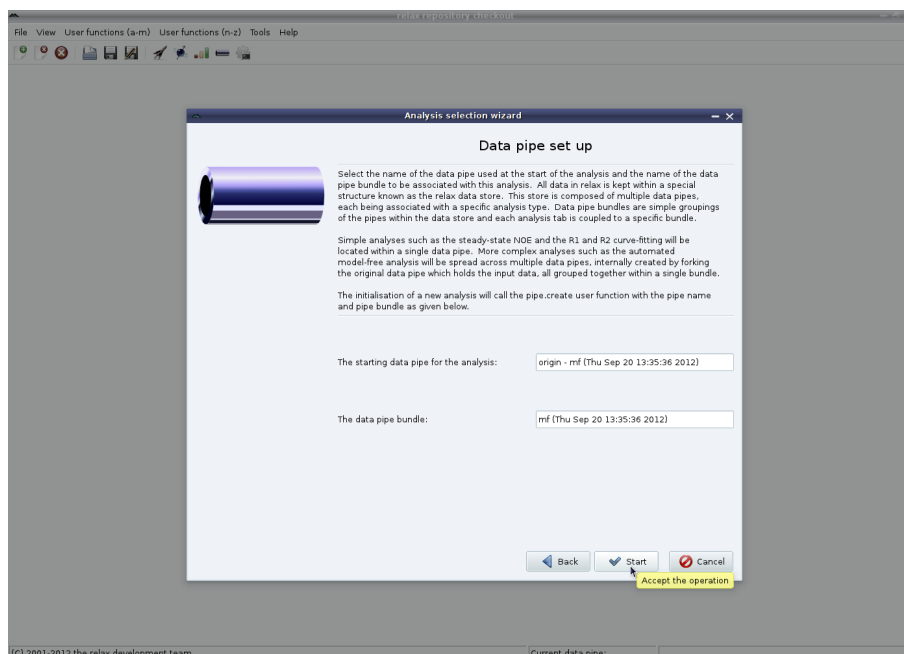
```
$ nice -n 15 mpirun -np 5 /usr/local/bin/relax --multi='mpi4py' --gui --log log
```

### 7.9.1 d'Auvergne protocol GUI mode – data pipe initialisation

First launch the analysis selection wizard (see Figure 1.4 on page 12) and click on the model-free analysis button.

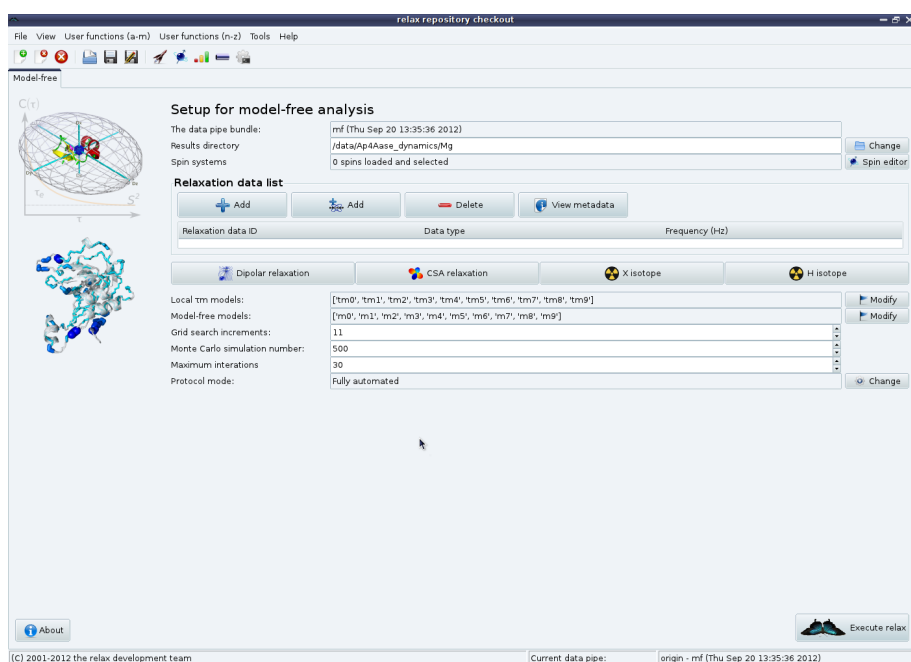


Click on the “Next” button and on the second page click on the “Start” button. The text in the second page need not be changed.

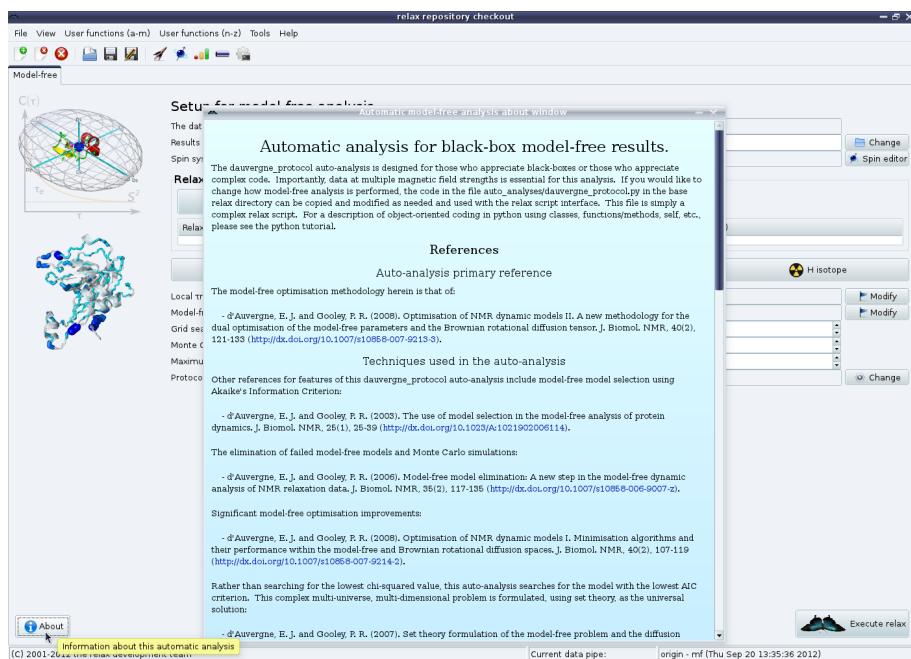


### 7.9.2 d'Auvergne protocol GUI mode – general setup

Once the analysis is initialised, the screen should look like:



The “About” button in the bottom left will bring up a window with the same description as given in the sample script:



At this point, back in the main relax window, the results directory where all of the output files and directories will be saved can be changed.

### 7.9.3 d’Auvergne protocol GUI mode – setting up the spin systems

The model-free dynamics is at the level of the spins – relaxation affects individual nuclei. In the main model-free tab you will see the “Spin systems” GUI element. Clicking on the “Spin editor” button to the right of this element will launch the spin editor window.

In this tutorial, the 3<sup>rd</sup> model of the PDB file 1f3y.pdb will be used to extract the spin system information. The molecule will be named “Ap4Aase”. For details on how to create the spin containers necessary for this analysis, please see section 4.5.2 on page 40 (or analyses lacking structural data in section 4.5.3 on page 43 for sequence files).

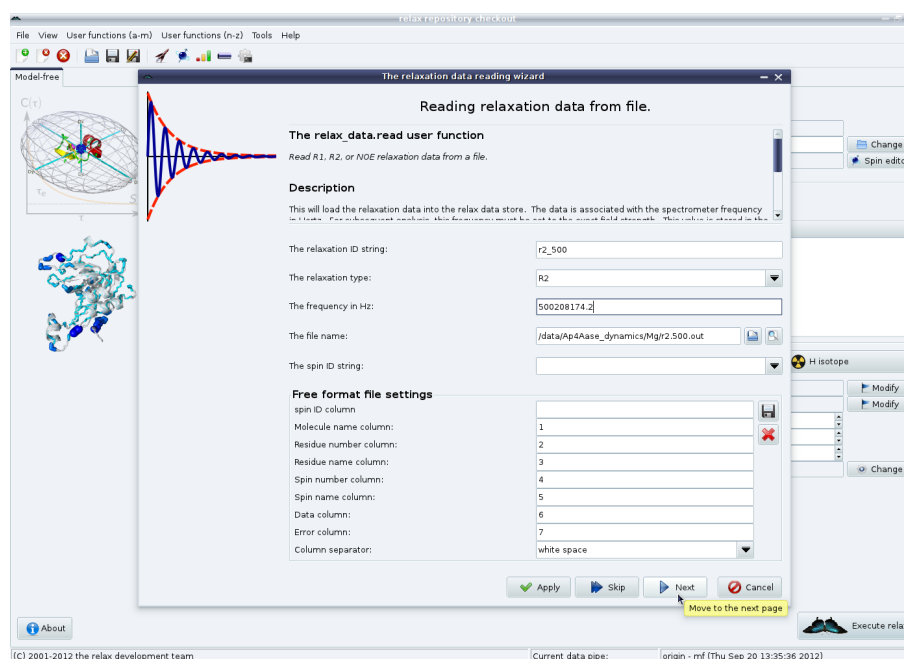
Note that for this tutorial, the protein backbone spins “@N” and “@H” as well as the tryptophan sidechain indole “@NE1” and “@HE1” spins should be loaded in the spin viewer window.

#### 7.9.4 d’Auvergne protocol GUI mode – unresolved spins

To deselect all unwanted spins, please read section 4.5.5 on page 44 for all the necessary instructions for how to do this in the GUI.

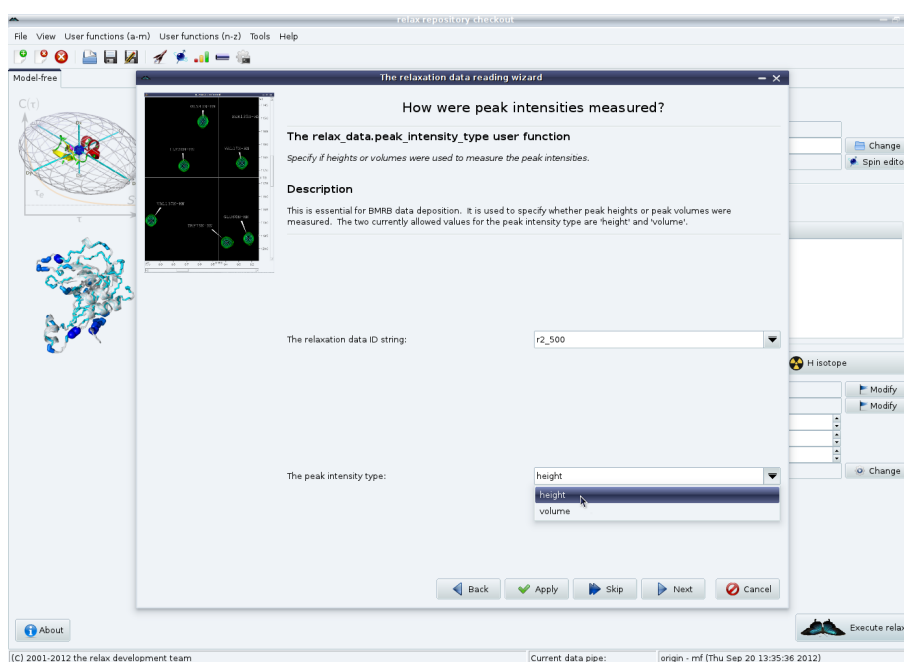
#### 7.9.5 d’Auvergne protocol GUI mode – loading the data

The relaxation data can either come from plain columnar formatted text files (such as if relax was used for the NOE,  $R_1$  and  $R_2$  analyses) or from the Bruker Dynamics Centre. For the former, click on the “Add” button in the “Relaxation data list” GUI element. This route will be used for this tutorial. For the later, click on the “Add Bruker” button. After clicking on “Add”, you will see the relaxation data loading wizard:

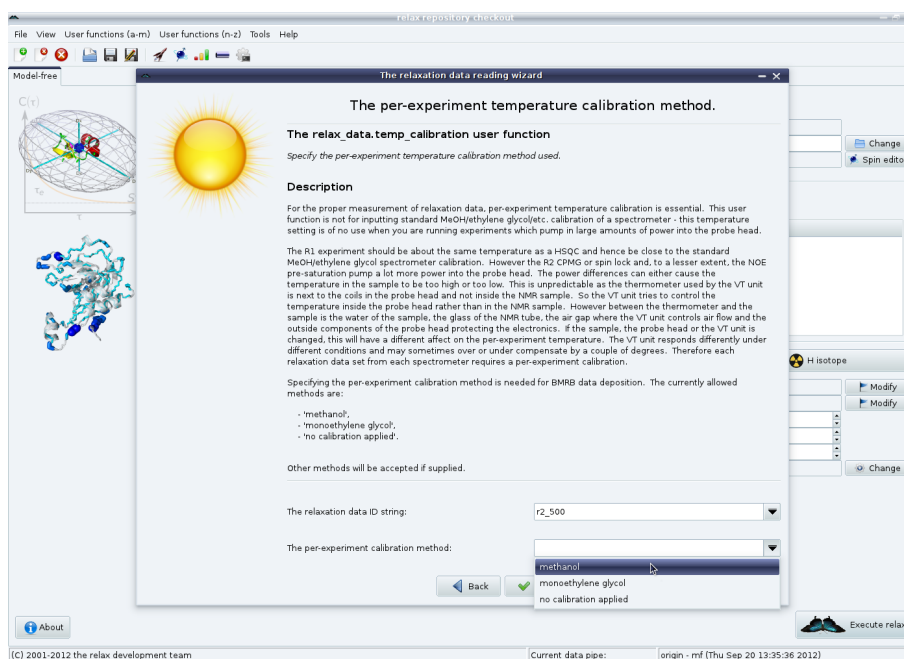


In this first page, the unique relaxation data identification string (“r2\_500”), the relaxation data type (“R2”), the frequency in Hertz (“500208174.2”) and the file (“r2.500.out”) are specified. If your data comes from another program, you may need to change the values in the “Free format file settings” element. Click on “Next” to load the data from the file.

The next wizard pages are for loading the metadata which is used in the BioMagResBank deposition of your final results. The first is how the peak intensities were measured, either peak heights or volumes. Select the appropriate value, then click on “Next”.

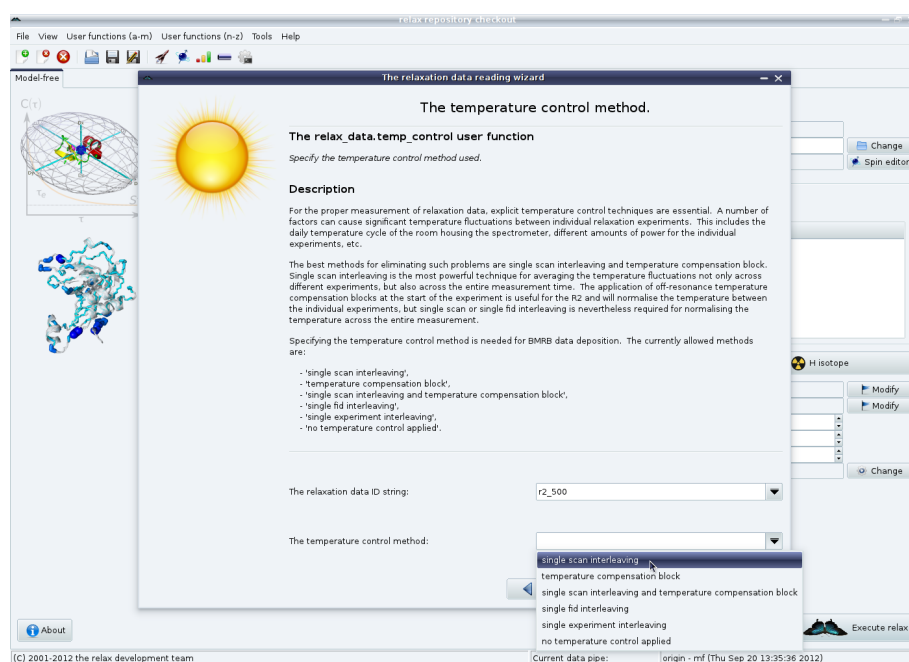


Then the temperature control method is given. For more details, please read the documentation provided in the wizard and see section 5.3.1 on page 50. Click on “Next” to continue.

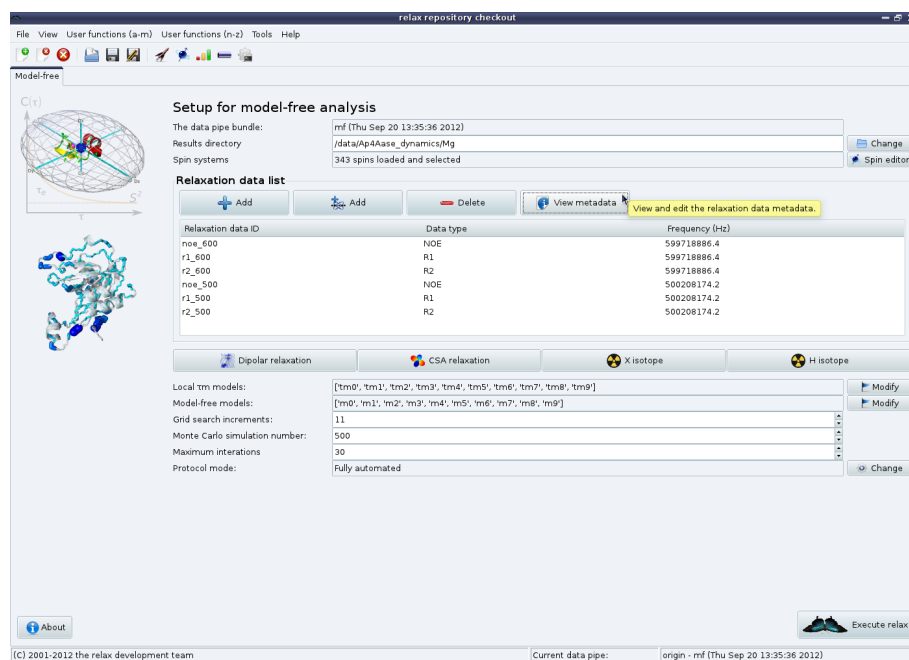


The temperature calibration method can finally be specified. Again, see section 5.3.1 on page 50 for the full details. Click on the “Finish” button to close the wizard.

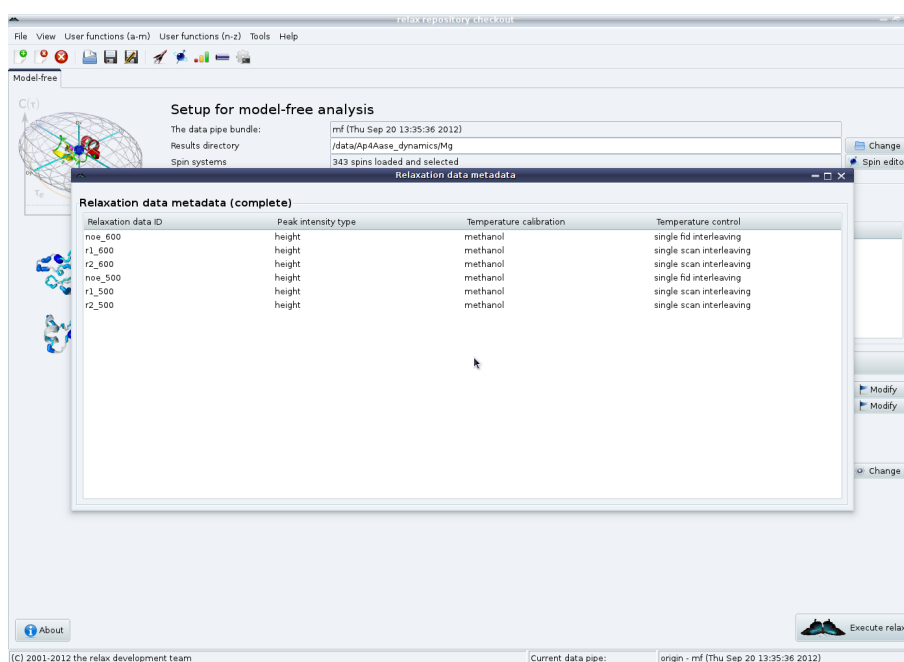




After you have repeated this for the NOE,  $R_1$  and  $R_2$  at both 500 and 600 MHz, you should now see:

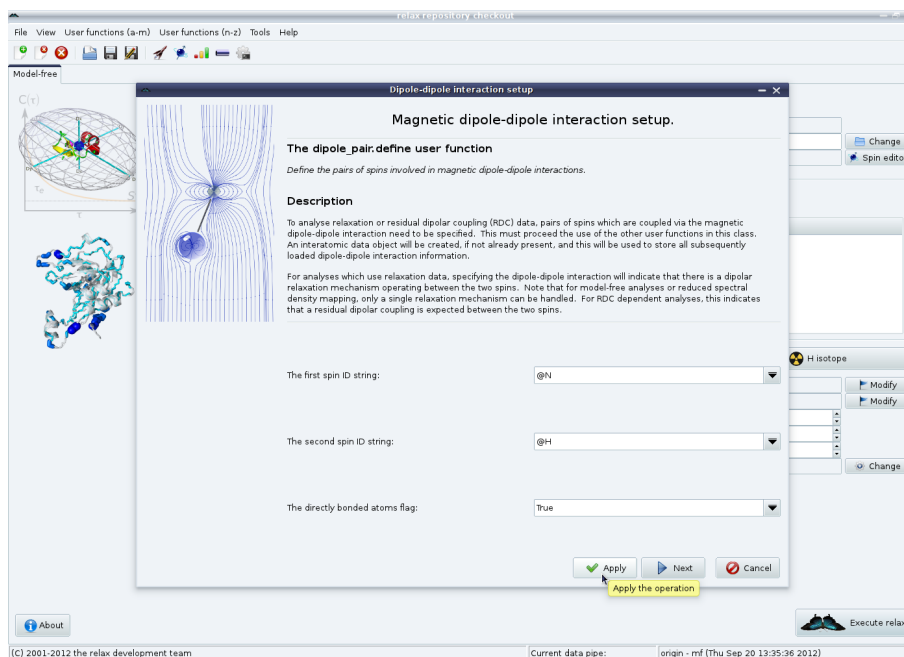


Check that the metadata has been properly entered by clicking on the “View metadata” button in the “Relaxation data list” GUI element:



### 7.9.6 d'Auvergne protocol GUI mode – relaxation interactions

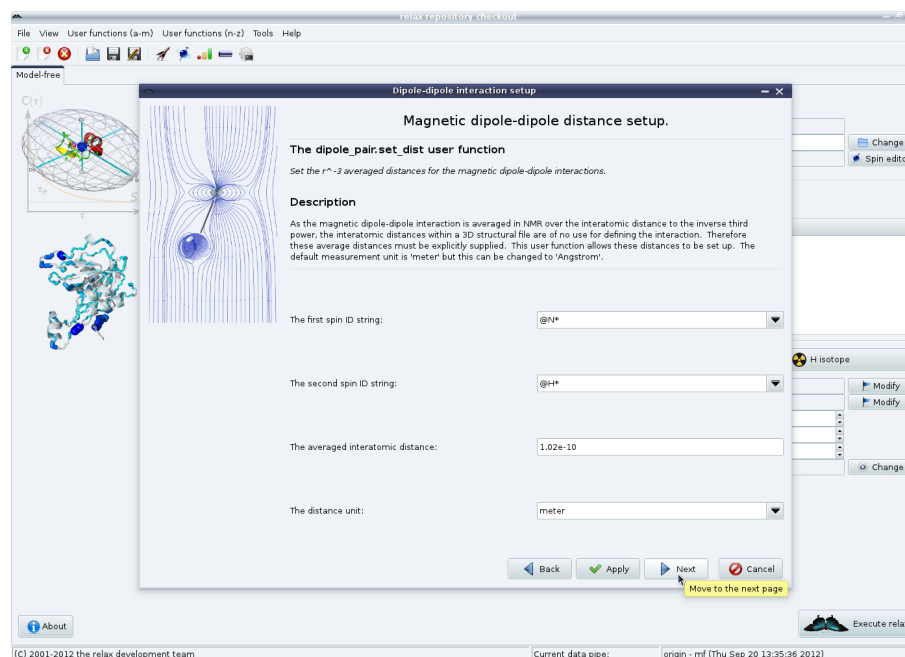
Just as in the scripting mode, the relaxation interactions need to now be defined. The first is the magnetic dipole-dipole interaction. All coupled nitrogen and proton spins should already be loaded at this point. Click on the “Dipolar relaxation” button in the model-free tab in the main relax window to launch the magnetic dipole-dipole interaction wizard:



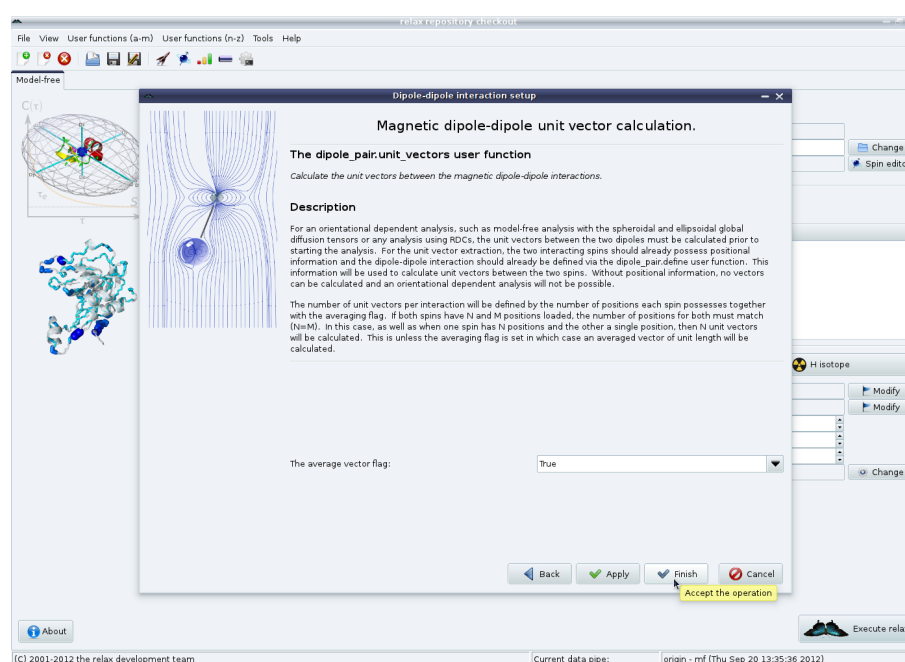
For this example, directly bonded nitrogens and protons will be analysed. To start with, the backbone NH pairs will be defined. Leave the values at “@N” and “@H” and click on the “Apply” button. Then change the two spin ID strings to “@NE1” and “@HE1” to set up the tryptophan sidechain indole NH pairs and click on the “Next” button. Note that

the regular expression “@N\*” and “@H\*” should not be used in this first wizard page as otherwise @N spins will be connected to @HE1 spins of the same tryptophan residue and @H spins to @NE1 spins.

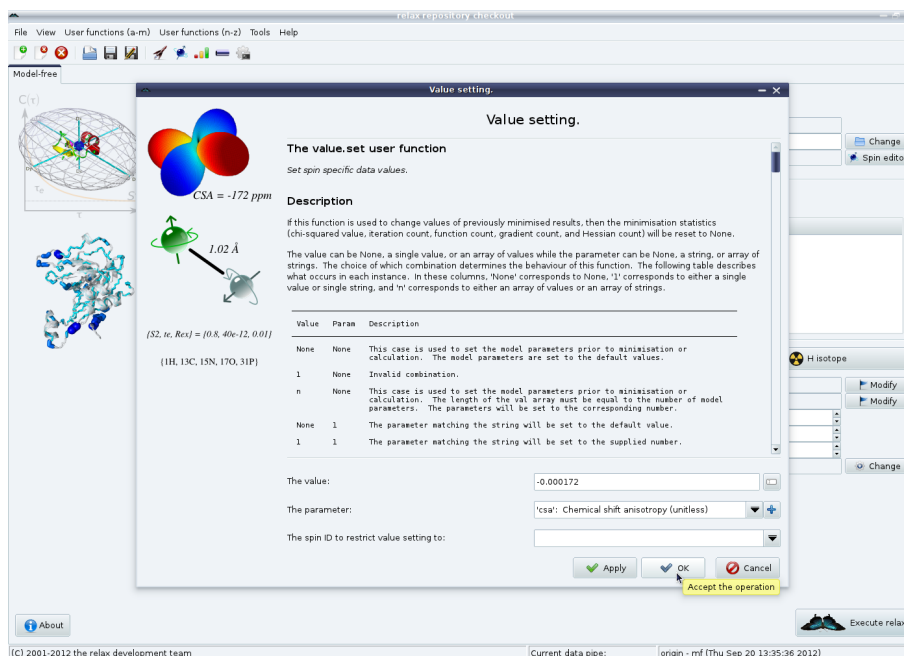
Now the  $\langle r^{-3} \rangle$  averaged distance of 1.02 Å will be set. Leave all settings as they are and click on “Next”:



If multiple models have been loaded in the previous steps, then the unit vectors between each model need to be calculated. For a model-free analysis multiple unit vectors must be averaged to a single vector – current model-free theory is based on the assumption of a single vector orientation. Therefore the averaged vector flag must be left on “True”. Click on “Finish” to terminate the set up of the magnetic dipole-dipole interactions:

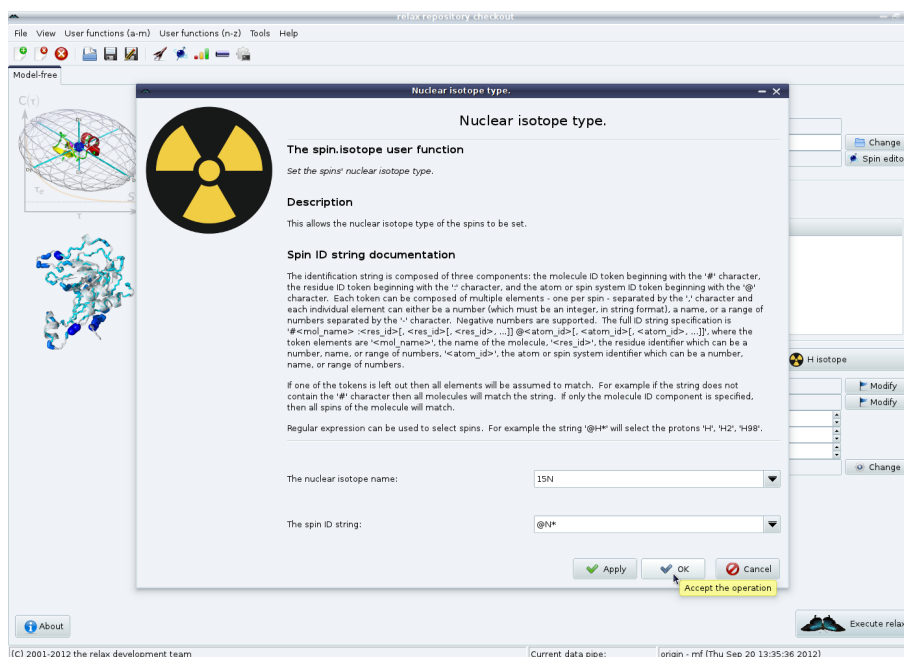


Secondly the chemical shift anisotropy (CSA) relaxation mechanism needs to be defined. Click on the “CSA relaxation” button in the model-free tab in the main relax window. An averaged CSA value of -172 ppm will be used for all spins, so simply click on “Ok” to finish.



### 7.9.7 d'Auvergne protocol GUI mode – spin isotopes

As the PDB file contains no isotope information, this needs to now be specified. First click on the “X isotope” button to set the nuclear isotope type of the heteronuclei:



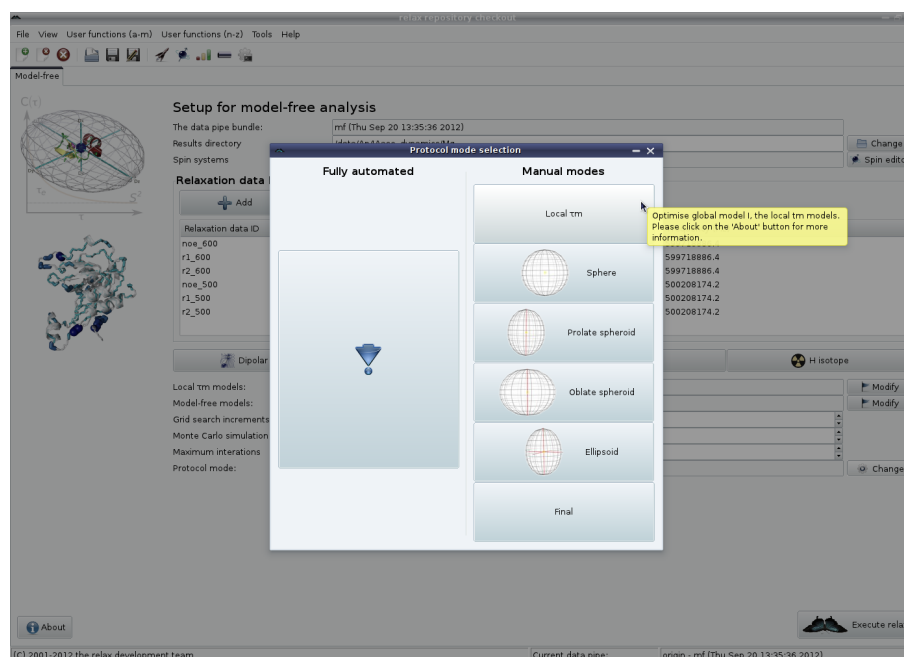
As nitrogen relaxation is being studied, the nuclear isotope name can be left as “15N” and the spin ID string to “@N\*”. Therefore simply click on the “Ok” button. Exactly the same

procedure can be used for the proton with the “H isotope” button.

### 7.9.8 d’Auvergne protocol GUI mode – the rest of the setup

The local  $\tau_m$  models and model-free models should not be modified, the reason for this is explained in section 7.8.2 on page 110. The grid search increments defaults to “11”. This is used in the optimisation of the individual model-free models for each spin. This value should also not be touched unless you know what you are doing (and have read d’Auvergne and Gooley (2008b)). The number of Monte Carlo simulations can be increased but, for accurate error estimates, it should not be less than 500 simulations. One additional setting is the “Maximum iterations”. This is a maximum number of times the protocol will iterate before terminating. This allows infinite loops to be broken. The value of 30 iterations should be fine for most analyses.

The “Protocol mode” GUI element setting of “Fully automated” will not be changed for the analysis of this tutorial. However if you are studying a system without a 3D structure, you can execute each individual component of the analysis by clicking on the “Change” button. This will make the protocol mode selection window appear:



From this you can first select the “Local  $\tau_m$ ” model, then the “Sphere” and finally the “Final” mode, clicking on “Execute relax” between each selection.

### 7.9.9 d’Auvergne protocol GUI mode – execution

Prior to executing relax, you should very carefully check the relax controller window for any strange messages, warnings or errors. You can open this window in three ways:

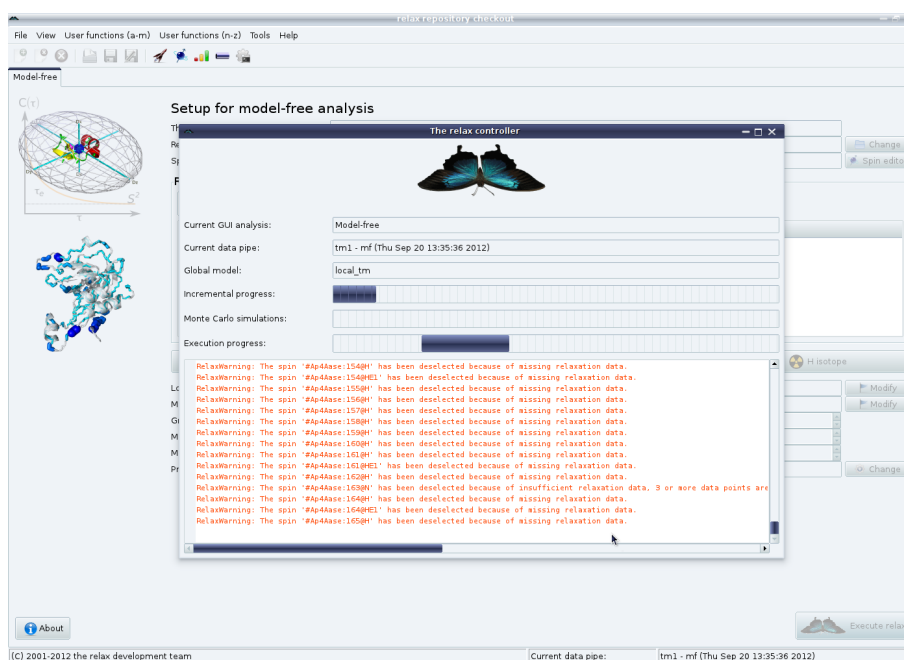
- Selecting the “View→Controller” menu item.
- Typing “[Ctrl+Z]” within the main relax window.

- Clicking on the “relax controller” button on the toolbar.

These messages are very important and will indicate to you if there are any problems prior to starting the very long model-free calculation. This information should be stored in the log file as well. As the execution of a fully iterative and complete model-free protocol takes a very long time to finish, it is advisable to save the current relax state. This will allow you restart the calculation without performing all of the steps detailed above. Just in case you cannot work out how to do this yourself, here is a list of the different ways you can do this (if this is not enough for you, please email the relax-users mailing list with your suggestions):

- Selecting the “File→Save relax state” menu item.
- Typing “[Ctrl+S]” within the main relax window.
- Clicking on the “Save relax state” button on the toolbar.
- Selecting the “File→Save as...” menu item.
- Typing “[Shift+Ctrl+S]” within the main relax window.
- Clicking on the “Save as” button on the toolbar.
- Selecting the “User functions→state→save” menu item.
- Opening up the relax prompt window with “View→relax prompt” or “[Ctrl+P]” and using the `state.save` user function.

If all the messages in the relax controller or log file appear to be fine and you have saved the current relax state, then click on “Execute relax”. This will start the calculations, freeze most of the GUI and open up the relax controller to give you feedback on the progress of the calculations:



At the start of the protocol, you should again check the messages carefully to be sure that relax is operating as you would expect. There may be very important **RelaxWarnings** that will require you to quit relax and start the analysis all over again.

### 7.9.10 d’Auvergne protocol GUI mode – completion

Upon completion of the analysis, the save and results files for the final result will be located in the **final** directory within the selected results directory. The results files will consist of text files for each of the spin specific model-free parameters, 2D Grace plots of the model-free parameters, PyMOL and MOLMOL macros for superimposing the model-free parameter values onto the 3D structure of the molecule, and a PDB representation of the final diffusion tensor.

Further visualisations of the results are possible via the “User functions” menu entry. For example to generate a 2D plot of order parameters for one of the other diffusion tensor results, the pipe editor window can be used to switch data pipes to the other diffusion models and then the “User functions→grace→write” menu item can be selected to create the plot.

### 7.9.11 d’Auvergne protocol GUI mode – BMRB deposition

Once you are ready to publish your results, the very last step of the model-free analysis is to create a NMR-STAR formatted file for [BioMagResBank](#) submission for each model-free analysis you perform. This can be accomplished using the BMRB export window. Simply select the “File→Export for BMRB deposition” menu item. You will then see the BMRB export window:

The screenshot shows the 'BMRB export window' titled 'Data export for BMRB deposition'. It includes a BMRB logo and a warning message: 'This wizard will help in executing all of the relevant user functions required to convert the contents of the selected data pipe to the NMR-STAR format for deposition within the BioMagResBank. Note that this is currently only for the deposition of model-free analysis results or simple NMR relaxation data.'

The data pipe to export is set to 'origin - mf (Thu Sep 20 13:35:36)'.

**Relaxation data metadata (complete)**

| Relaxation data ID | Peak intensity type | Temperature calibration | Temperature control      |
|--------------------|---------------------|-------------------------|--------------------------|
| noe_600            | height              | methanol                | single fid interleaving  |
| r1_600             | height              | methanol                | single scan interleaving |
| r2_600             | height              | methanol                | single scan interleaving |
| noe_500            | height              | methanol                | single fid interleaving  |
| r1_500             | height              | methanol                | single scan interleaving |
| r2_500             | height              | methanol                | single scan interleaving |

**Molecule information (incomplete)**

| ID string | Name    | Type | Thiol state |
|-----------|---------|------|-------------|
| #Ap4Aase  | Ap4Aase |      |             |

**Software**

| Program name |
|--------------|
| relax        |

**Scripts**

| Script name |
|-------------|
|-------------|

**Citations**

| Citation ID |
|-------------|
| relax_ref1  |
| relax_ref2  |

Buttons: Add, Select, Add, Add, Preview, Export, Preview the NMR-STAR formatted data.

From here you can complete the relaxation data metadata if needed, set up all the molecule information needed for a BMRB deposition, specify the software you have used running up

to the model-free analysis and any spectral processing or relax scripts you have used. You can also add as many citations relevant to your analysis as you wish. The NMR-STAR formatted file can be previewed in the relax controller window via the “Preview” button and the final file created using the “Export” button.

Once you are in the stage of writing up, simply go to the ADIT-NMR webpage at <http://deposit.bmr.b.wisc.edu/bmr-b-adit/>, create a new BMRB deposition, upload the file you have created, complete the deposition as needed, and add the BMRB deposition number to your paper.



## Chapter 8

# Reduced spectral density mapping

### 8.1 Introduction to reduced spectral density mapping

The reduced spectral density mapping analysis is often performed when the system under study is not suitable for model-free analysis, or as a last resort if a model-free analysis fails. The aim is to convert the relaxation data into three  $J(\omega)$  values for the given field strength. Interpretation of this data, although slightly less convoluted than the relaxation data, is still plagued by problems related to non-spherical diffusion and much care must be taken when making conclusions. A full understanding of the model-free analysis and the effect of diffusion tensor anisotropy and rhombicity allows for better interpretation of the raw numbers.

To understand how reduced spectral density mapping is implemented in relax, the sample script will be worked through. This analysis type is not implemented in the GUI yet, though it shouldn't be too hard if anyone would like to contribute this and have a reference added to Chapter , the citations chapter.

### 8.2 $J(\omega)$ mapping script mode – the sample script

```
1  """Script for reduced spectral density mapping."""
2
3
4  # Create the data pipe.
5  pipe.create(pipe_name='my_protein', pipe_type='jw')
6
7  # Set up the 15N spins.
8  sequence.read(file='noe.600.out', res_num_col=1, res_name_col=2)
9  spin.name(name='N')
10 spin.element(element='N')
11 spin.isotope(isotope='15N', spin_id='@N')
12
13 # Load the 15N relaxation data.
14 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
15                 res_num_col=1, data_col=3, error_col=4)
16 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
17                 res_num_col=1, data_col=3, error_col=4)
```

```

16 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
    res_num_col=1, data_col=3, error_col=4)
17
18 # Generate 1H spins for the magnetic dipole-dipole relaxation interaction.
19 sequence.attach_protons()
20
21 # Define the magnetic dipole-dipole relaxation interaction.
22 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
23 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)
24
25 # Define the chemical shift relaxation interaction.
26 value.set(val=-172 * 1e-6, param='csa')
27
28 # Select the frequency.
29 jw_mapping.set_frq(frq=600.0 * 1e6)
30
31 # Reduced spectral density mapping.
32 minimise.calculate()
33
34 # Monte Carlo simulations (well, bootstrapping as this is a calculation and not a fit!).
35 monte_carlo.setup(number=500)
36 monte_carlo.create_data()
37 minimise.calculate()
38 monte_carlo.error_analysis()
39
40 # Create grace files.
41 grace.write(y_data_type='j0', file='j0.agr', force=True)
42 grace.write(y_data_type='jwx', file='jwx.agr', force=True)
43 grace.write(y_data_type='jwh', file='jwh.agr', force=True)
44
45 # View the grace files.
46 grace.view(file='j0.agr')
47 grace.view(file='jwx.agr')
48 grace.view(file='jwh.agr')
49
50 # Write out the values.
51 value.write(param='j0', file='j0.txt', force=True)
52 value.write(param='jwx', file='jwx.txt', force=True)
53 value.write(param='jwh', file='jwh.txt', force=True)
54
55 # Finish.
56 results.write(file='results', force=True)
57 state.save('save', force=True)

```

### 8.3 J(w) mapping script mode – data pipe and spin system setup

The steps for setting up relax and the data model concept are described in full detail in Chapter 4. The first step, as for all analyses in relax, is to create a data pipe for storing all the data:

```

4 # Create the data pipe.
5 pipe.create(pipe_name='my_protein', pipe_type='jw')

```

Then, in this example, the  $^{15}\text{N}$  spins are created from one of the NOE relaxation data files (Chapter 6):

```

7 # Set up the 15N spins.

```

```

8 sequence.read(file='noe.600.out', res_num_col=1, res_name_col=2)
9 spin.name(name='N')
10 spin.element(element='N')
11 spin.isotope(isotope='15N', spin_id='@N')

```

Skipping the relaxation data loading, the next part of the analysis is to create protons attached to the nitrogens for the magnetic dipole-dipole relaxation interaction:

```

18 # Generate 1H spins for the magnetic dipole-dipole relaxation interaction.
19 sequence.attach_protons()

```

This is needed to define the magnetic dipole-dipole interaction which governs relaxation.

## 8.4 $J(\omega)$ mapping script mode – relaxation data loading

The loading of relaxation data is straight forward. This is performed prior to the creation of the proton spins so that the data is loaded only into the  $^{15}\text{N}$  spin containers and not both spins for each residue. Only data for a single field strength can be loaded:

```

13 # Load the 15N relaxation data.
14 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
    res_num_col=1, data_col=3, error_col=4)
15 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
    res_num_col=1, data_col=3, error_col=4)
16 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
    res_num_col=1, data_col=3, error_col=4)

```

The frequency of the data must also be explicitly specified:

```

28 # Select the frequency.
29 jw_mapping.set_freq(frq=600.0 * 1e6)

```

## 8.5 $J(\omega)$ mapping script mode – relaxation interactions

Prior to calculating the  $J(\omega)$  values, the physical interactions which govern relaxation of the spins must be defined. For the magnetic dipole-dipole relaxation interaction, the user functions are:

```

21 # Define the magnetic dipole-dipole relaxation interaction.
22 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
23 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)

```

For the chemical shift relaxation interaction, the user function call is:

```

25 # Define the chemical shift relaxation interaction.
26 value.set(val=-172 * 1e-6, param='csa')

```

## 8.6 $J(\omega)$ mapping script mode – calculation and error propagation

Optimisation for this analysis is not needed as this is a direct calculation. Therefore the  $J(\omega)$  values are simply calculated with the call:

```
31 # Reduced spectral density mapping.  
32 minimise.calculate()
```

The propagation of errors is more complicated. The Monte Carlo simulation framework of relax can be used to propagate the relaxation data errors to the spectral density errors. As this is a direct calculation, this collapses into the standard bootstrapping method. The normal Monte Carlo user functions can be called:

```
34 # Monte Carlo simulations (well, bootstrapping as this is a calculation and not a fit!).  
35 monte_carlo.setup(number=500)  
36 monte_carlo.create_data()  
37 minimise.calculate()  
38 monte_carlo.error_analysis()
```

In this case, the `monte_carlo.initial_values` user function call is not required.

## 8.7 $J(\omega)$ mapping script mode – visualisation and data output

The rest of the script is used to output the results to 2D Grace files for visualisation (the `grace.view` user function calls will launch Grace with the created files), and the output of the values into plain text files.

## Chapter 9

# Consistency testing

### 9.1 Introduction to the consistency testing of relaxation data

In spin relaxation, datasets are often recorded at different magnetic fields. This is especially important when  $R_2$  values are to be used since  $\mu$ s-ms motions contribute to  $R_2$ . This contribution being scaled quadratically with the strength of the magnetic field, recording at multiple magnetic fields helps extract it. Also, acquiring data at multiple magnetic fields allows over-determination of the mathematical problems, e.g. in the model-free approach.

Recording at multiple magnetic fields is a good practice. However, it can cause artifacts if those different datasets are inconsistent. Inconsistencies can originate from, inter alia, the sample or the acquisition. Sample variations can be linked to changes in temperature, concentration, pH, etc. Water suppression is the main cause of acquisition variations as it affects relaxation parameters (especially NOE) of exposed and exchangeable moieties (e.g. the NH moiety).

It is thus a good idea to assess consistency of datasets acquired at different magnetic fields. For this purpose, three tests are implemented in relax. They are all based on the same principle – calculate a field independent value and compare it from one field to another.

The three tests are:

$J(0)$  The spectral density at the zero frequency calculated using the reduced spectral density approach.

$F_\eta$  A consistency function proposed by [Fushman et al. \(1998\)](#).

$F_{R_2}$  A consistency function proposed by [Fushman et al. \(1998\)](#).

These three tests are very similar (all probing consistency of  $R_2$  data and all suffering from the same limitations) and any of them can be used for consistency testing. In the example below, the  $J(0)$  values are used for consistency testing.

Different methods exist to compare tests values calculated from one field to another. These include correlation plots and histograms, and calculation of correlation, skewness and

kurtosis coefficients. The details of how to interpret such analyses are available at the end of this chapter in Section 9.7.

For more details on the tests and their implementation within relax, see:

- Morin, S. and Gagné, S. (2009a). Simple tests for the validation of multiple field spin relaxation data. *J. Biomol. NMR*, **45**, 361–372. ([10.1007/s10858-009-9381-4](https://doi.org/10.1007/s10858-009-9381-4))

Or for the origin of the tests themselves:

- Fushman, D., Tjandra, N., and Cowburn, D. (1999). An approach to direct determination of protein dynamics from  $^{15}\text{N}$  NMR relaxation at multiple fields, independent of variable  $^{15}\text{N}$  chemical shift anisotropy and chemical exchange contributions. *J. Am. Chem. Soc.*, **121**(37), 8577–8582. ([10.1021/ja9904991](https://doi.org/10.1021/ja9904991))

In addition, see the following review which includes a discussion on how to evaluate the reliability of recorded relaxation data:

- Morin, S. (2011). A practical guide to protein dynamics from  $^{15}\text{N}$  spin relaxation in solution. *Prog. NMR Spectrosc.*, **59**(3), 245–262. ([10.1016/j.pnmrs.2010.12.003](https://doi.org/10.1016/j.pnmrs.2010.12.003))

## 9.2 Consistency testing in the prompt/script UI mode

The consistency testing analysis is only available via the prompt/script UI modes – no GUI auto-analysis has yet been built by a relax power-user.

### 9.2.1 Consistency testing script mode – the sample script

The following script can be found in the `sample_scripts` directory.

```

1  """ Script for consistency testing.
2
3  Severe artifacts can be introduced if model-free analysis is performed from inconsistent
   multiple magnetic field datasets. The use of simple tests as validation tools for the
   consistency assessment can help avoid such problems in order to extract more reliable
   information from spin relaxation experiments. In particular, these tests are useful
   for detecting inconsistencies arising from R2 data. Since such inconsistencies can
   yield artificial Rex parameters within model-free analysis, these tests should be use
   routinely prior to any analysis such as model-free calculations.
4
5  This script will allow one to calculate values for the three consistency tests J(0), F_eta
   and F_R2. Once this is done, qualitative analysis can be performed by comparing
   values obtained at different magnetic fields. Correlation plots and histograms are
   useful tools for such comparison, such as presented in Morin & Gagne (2009a) J.
   Biomol. NMR, 45: 361-372.
6
7
8  References
9  =====
10
```

```

11 The description of the consistency testing approach:
12
13     Morin & Gagne (2009a) Simple tests for the validation of multiple field spin
        relaxation data. J. Biomol. NMR, 45: 361-372. U{http://dx.doi.org/10.1007/s10858
        -009-9381-4}
14
15 The origins of the equations used in the approach:
16
17     J(0):
18         Farrow et al. (1995) Spectral density function mapping using 15N relaxation data
        exclusively. J. Biomol. NMR, 6: 153-162. U{http://dx.doi.org/10.1007/BF00211779}
19
20     F_eta:
21         Fushman et al. (1998) Direct measurement of 15N chemical shift anisotropy in
        solution. J. Am. Chem. Soc., 120: 10947-10952. U{http://dx.doi.org/10.1021/ja981686m}
22
23     F_R2:
24         Fushman et al. (1998) Direct measurement of 15N chemical shift anisotropy in
        solution. J. Am. Chem. Soc., 120: 10947-10952. U{http://dx.doi.org/10.1021/ja981686m}
25
26 A study where consistency tests were used:
27
28     Morin & Gagne (2009) NMR dynamics of PSE-4 beta-lactamase: An interplay of ps-ns order
        and us-ms motions in the active site. Biophys. J., 96: 4681-4691. U{http://dx.doi.org
        /10.1016/j.bpj.2009.02.068}
29 ""
30
31 # Create the data pipe.
32 name = 'consistency'
33 pipe.create(name, 'ct')
34
35 # Set up the 15N spins.
36 sequence.read('noe.600.out', res_num_col=1)
37 spin.name(name='N')
38 spin.element(element='N')
39 spin.isotope(isotope='15N', spin_id='@N')
40
41 # Load the relaxation data.
42 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
        res_num_col=1, data_col=3, error_col=4)
43 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
        res_num_col=1, data_col=3, error_col=4)
44 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
        res_num_col=1, data_col=3, error_col=4)
45
46 # Generate the 1H spins for the magnetic dipole-dipole interaction.
47 sequence.attach_protons()
48
49 # Define the magnetic dipole-dipole relaxation interaction.
50 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
51 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)
52
53 # Define the chemical shift relaxation interaction.
54 value.set(val=-172 * 1e-6, param='csa')
55
56 # Set the angle between the 15N-1H vector and the principal axis of the 15N chemical shift
    tensor
57 value.set(val=15.7, param='orientation')
58
59 # Set the approximate correlation time.
60 value.set(val=13 * 1e-9, param='tc')

```

```

61
62 # Set the frequency.
63 consistency_tests.set_frq(frq=600.0 * 1e6)
64
65 # Consistency tests.
66 minimise.calculate()
67
68 # Monte Carlo simulations.
69 monte_carlo.setup(number=500)
70 monte_carlo.create_data()
71 minimise.calculate()
72 monte_carlo.error_analysis()
73
74 # Create grace files.
75 grace.write(y_data_type='j0', file='j0.agr', force=True)
76 grace.write(y_data_type='f_eta', file='f_eta.agr', force=True)
77 grace.write(y_data_type='f_r2', file='f_r2.agr', force=True)
78
79 # View the grace files.
80 grace.view(file='j0.agr')
81 grace.view(file='f_eta.agr')
82 grace.view(file='f_r2.agr')
83
84 # Finish.
85 results.write(file='results', force=True)
86 state.save('save', force=True)

```

This is similar in spirit to the reduced spectral density mapping sample script (Chapter 8 on page 127).

### 9.3 Consistency testing script mode – data pipe and spin system setup

The steps for setting up relax and the data model concept are described in full detail in Chapter 4. The first step, as for all analyses in relax, is to create a data pipe for storing all the data:

```

31 # Create the data pipe.
32 name = 'consistency'
33 pipe.create(name, 'ct')

```

Then, in this example, the  $^{15}\text{N}$  spins are created from one of the NOE relaxation data files (Chapter 6):

```

35 # Set up the  $^{15}\text{N}$  spins.
36 sequence.read('noe.600.out', res_num_col=1)
37 spin.name(name='N')
38 spin.element(element='N')
39 spin.isotope(isotope='15N', spin_id='@N')

```

Skipping the relaxation data loading, the next part of the analysis is to create protons attached to the nitrogens for the magnetic dipole-dipole relaxation interaction:

```

46 # Generate the  $^1\text{H}$  spins for the magnetic dipole-dipole interaction.
47 sequence.attach_protons()

```



This is needed to define the magnetic dipole-dipole interaction which governs relaxation.

## 9.4 Consistency testing script mode – relaxation data loading

The loading of relaxation data is straight forward. This is performed prior to the creation of the proton spins so that the data is loaded only into the  $^{15}\text{N}$  spin containers and not both spins for each spin system. Note that if the relaxation data files contain spin information, then this order is not important. For this analysis, only data for a single field strength can be loaded:

```

41 # Load the relaxation data.
42 relax_data.read(ri_id='R1_600', ri_type='R1', frq=600.0*1e6, file='r1.600.out',
    res_num_col=1, data_col=3, error_col=4)
43 relax_data.read(ri_id='R2_600', ri_type='R2', frq=600.0*1e6, file='r2.600.out',
    res_num_col=1, data_col=3, error_col=4)
44 relax_data.read(ri_id='NOE_600', ri_type='NOE', frq=600.0*1e6, file='noe.600.out',
    res_num_col=1, data_col=3, error_col=4)

```

The frequency of the data must also be explicitly specified:

```

62 # Set the frequency.
63 consistency_tests.set_frq(frq=600.0 * 1e6)

```

## 9.5 Consistency testing script mode – relaxation interactions

Prior to calculating the  $J(0)$ ,  $F_\eta$ , and  $F_{R_2}$  values, the physical interactions which govern relaxation of the spins must be defined. For the magnetic dipole-dipole relaxation interaction, the user functions are:

```

49 # Define the magnetic dipole-dipole relaxation interaction.
50 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
51 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.02 * 1e-10)

```

For the chemical shift relaxation interaction, the user function call is:

```

53 # Define the chemical shift relaxation interaction.
54 value.set(val=-172 * 1e-6, param='csa')

```

For the angle in degrees between the  $^{15}\text{N}$ - $^1\text{H}$  vector and the principal axis of the  $^{15}\text{N}$  chemical shift tensor, the user function call is:

```

56 # Set the angle between the 15N-1H vector and the principal axis of the 15N chemical shift
    tensor
57 value.set(val=15.7, param='orientation')

```

## 9.6 Consistency testing script mode – calculation and error propagation

Optimisation for this analysis is not needed as this is a direct calculation. Therefore the  $J(0)$ ,  $F_\eta$ , and  $F_{R_2}$  values are simply calculated with the call:

```
65 # Consistency tests.
66 minimise.calculate()
```

The propagation of errors is more complicated. The Monte Carlo simulation framework of relax can be used to propagate the relaxation data errors to the spectral density errors. As this is a direct calculation, this collapses into the standard bootstrapping method. The normal Monte Carlo user functions can be called:

```
68 # Monte Carlo simulations.
69 monte_carlo.setup(number=500)
70 monte_carlo.create_data()
71 minimise.calculate()
72 monte_carlo.error_analysis()
```

In this case, the `monte_carlo.initial_values` user function call is not required.

## 9.7 Consistency testing script mode – visualisation and data output

The rest of the script is used to output the results to 2D Grace files for visualisation (the `grace.view` user function calls will launch Grace with the created files), and the output of the values into plain text files.

However, simply visualizing the calculated  $J(0)$ ,  $F_\eta$ , and  $F_{R_2}$  values this way does not allow proper consistency testing. Indeed, for assessing the consistency of relaxation data using these tests, different methods exist to compare values calculated from one field to another. These include correlation plots and histograms, and calculation of correlation, skewness and kurtosis coefficients.

To complete the consistency testing analysis, the following steps are needed:

- Extract the  $J(0)$  values at multiple magnetic fields.
- Join together the data from a pair of magnetic fields either by pasting them as two columns of one file (approach A), or by dividing values from a first magnetic field by values from a second magnetic field (approach B).
- Make either a correlation plot (approach A), or an histogram of the ratios (approach B).
- See if the correlation plot is centered around a perfect correlation or skewed away (approach A), or if the values are centered around 1 in the histogram (approach B). If yes, data from multiple magnetic fields is consistent from one magnetic field to another. If no, data is inconsistent. In the case where inconsistency arises, if data

from more than two magnetic fields is available, more than one pair of data can be checked and the inconsistent magnetic field data can be identified.

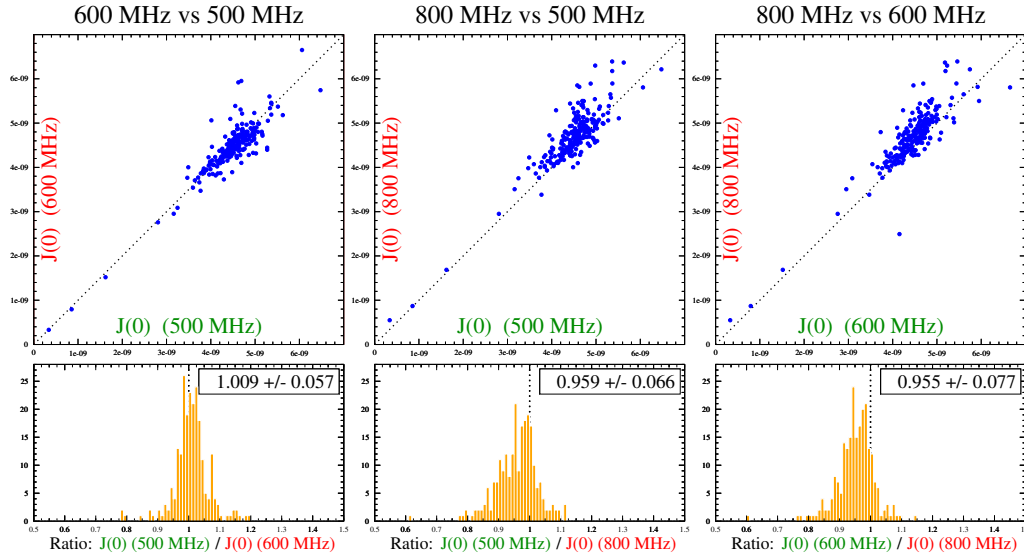


Figure 9.1: Example of consistency testing visual analysis. Relaxation data from three different magnetic fields are compared. For each pair of magnetic field, a correlation plot of the calculated  $J(0)$  values (approach A, top) as well as an histogram of the ration of calculated  $J(0)$  values (approach B, bottom) are shown. These graphs must be manually created from the output of the sample script shown in section 9.2.1. The PSE-4 data, as published in [Morin and Gagné \(2009b\)](#), has been reused for the purpose of this example.

An example of such an analysis is shown in Figure 9.1. This example displays both consistent and inconsistent data. As the figure shows, the data recorded at 500 MHz and 600 MHz are consistent with each other whereas the data recorded at 800 MHz is consistent with the neither the 500 MHz nor 600 MHz data. Since more than two magnetic fields were used, this allowed the identification of the 800 MHz data as being inconsistent allowing the authors to take special care with this data set.

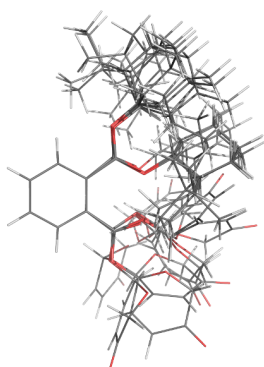
The 800 MHz data inconsistency is seen in the correlation plots (top) by a deviation from the dotted line (which represents the theoretical situation when equal  $J(0)$  values are extracted from both magnetic fields. It is also observable in the histograms (bottom) where the ratio of the data from two magnetic fields is not centered at 1.0. In fact, there seems to be a systematic shift of the calculated  $J(0)$  values at 800 MHz when compared to the two other magnetic fields. This is caused by a similar shift in the experimental  $R_2$  (transversal relaxation rate) data.

For the 500 MHz and 600 MHz data pair, the data are centered around the dotted line in the correlation plot (approach A, top left) as well as centered around a value of 1.0 in the histogram comparing the ratios of values from both magnetic fields (approach B, bottom left). Of course, there are some outlier values even in the case of consistent data. There are caused by specific dynamic characteristics of these spins and are different from systematic inconsistencies such as depicted in the example above with the data recorded at 800 MHz.



## Chapter 10

# The N-state model or ensemble analysis



### 10.1 Introduction to the N-state model

The modelling of motion in molecules using experimental data consists of either continuous or discrete distributions. These can be visualised respectively as either an infinite number of states or a limited set of  $N$  states. The N-state model analysis in relax models the molecular dynamics using an ensemble of static structures.

This analysis supports a number of data types including:

- Residual dipolar couplings (RDCs)
- Pseudo-contact shifts (PCSs)
- NOEs

The main idea is to evaluate the quality of a fixed ensemble of structures. relax will not perform structural optimisations. The evaluation includes:

- Alignment tensor optimisation for the RDCs and PCSs.

- Optional optimisation of the position of the paramagnetic centre for the PCSs.
- Calculation of NOE constraint violations.
- Q factor calculation for the RDC, PCS, and NOE.

Note that this analysis will also handle single structures. Hence you can use the N-state model in relax with N set to 1 to find, for example, a single alignment tensor for a single structure using RDCs, PCSs, or both together. This is useful for comparing an ensemble to a single structure to determine if any statistically significant motions are present.

The primary references for the N-state model analysis in relax are:

- Sun, H., d’Auvergne, E. J., Reinscheid, U. M., Dias, L. C., Andrade, C. K. Z., Rocha, R. O., and Griesinger, C. (2011). Bijvoet in solution reveals unexpected stereoselectivity in a michael addition. *Chem. Eur. J.*, **17**(6), 1811–1817. ([10.1002/chem.201002520](https://doi.org/10.1002/chem.201002520))
- Erdelyi, M., d’Auvergne, E., Navarro-Vazquez, A., Leonov, A., and Griesinger, C. (2011). Dynamics of the glycosidic bond: Conformational space of lactose. *Chem. Eur. J.*, **17**(34), 9368–9376. ([10.1002/chem.201100854](https://doi.org/10.1002/chem.201100854))

## 10.2 Experimental data support for the N-state model

### 10.2.1 RDCs in the N-state model

Residual dipolar couplings (RDCs) can be used to evaluate ensembles. The ensemble interconversion is assumed to be fast relative to timescale of the alignment process, hence a single tensor for all members of the ensemble will be used. As such, precise superimposition of structures using a logical frame of reference is very important. This can be performed in relax using the `structure.superimpose` user function. The RDCs can either be from external or internal alignment.

### 10.2.2 PCSs in the N-state model

Pseudo-contact shifts (PCSs) can also be used to evaluate ensembles. The same averaging process as described above for the RDC is assumed. Hence correct structural superimposition is essential and one alignment tensor will be optimised for the entire ensemble.

One powerful feature of relax is that the paramagnetic centre can either be fixed or be allowed to move during optimisation. This allows an unknown paramagnetic centre position to be found. Or a known position to be refined to higher accuracy than that possible with most other techniques.

### 10.2.3 NOEs in the N-state model

Another data type which can be used to evaluate dynamics ensembles is the NOE. This is not used in optimisation but rather is used to calculate NOE constraint violations. These violations are then compared to evaluate the ensemble. In the stereochemistry auto-analysis, these violations will also be converted to Q factors to allow direct comparison with RDC Q factors.

## 10.3 Determining stereochemistry in dynamic molecules

A published application of the N-state model in relax is:

- Sun, H., d’Auvergne, E. J., Reinscheid, U. M., Dias, L. C., Andrade, C. K. Z., Rocha, R. O., and Griesinger, C. (2011). Bijvoet in solution reveals unexpected stereoselectivity in a michael addition. *Chem. Eur. J.*, **17**(6), 1811–1817. ([10.1002/chem.201002520](https://doi.org/10.1002/chem.201002520))

This analysis of the stereochemistry of a small molecule consists of two steps. The first part is to determine the relative configuration. The idea is to use NMR data (consisting of RDCs and NOEs) to find the relative configuration. Ensembles of 10 members are created from molecular dynamics simulations (MD) or simulated annealing (SA). These are then ranked by the RDC Q factor and NOE violation. By converting the NOE violation into a Q factor:

$$Q_{\text{NOE}}^2 = \frac{U}{\sum_i \text{NOE}^2}, \quad (10.1)$$

where U is the quadratic flat bottom well potential, i.e. the NOE violation in Å<sup>2</sup>, and the denominator is the sum of all squared NOEs. A combined Q factor is calculated as:

$$Q_{\text{total}}^2 = Q_{\text{NOE}}^2 + Q_{\text{RDC}}^2. \quad (10.2)$$

The second step is to distinguish enantiomers. As NMR data is symmetric, it cannot distinguish enantiomers. Therefore an optical technique such as [optical rotatory dispersion](#) can be used. For molecules experiencing large amounts of motion, sampling all possible conformations, calculating the expected dispersion properties, and calculating an averaged dispersion curve is not feasible. The idea is therefore to combine NMR and ORD by taking the best NMR ensembles from step one to use for ORD spectral prediction.

### 10.3.1 Stereochemistry – the auto-analysis

Step one of the N-state model is implemented as an auto-analysis. This is located in the module `auto_analysis.stereochem_analysis` (see [http://www.nmr-relax.com/api/3.1/auto\\_analyses.stereochem\\_analysis-module.html](http://www.nmr-relax.com/api/3.1/auto_analyses.stereochem_analysis-module.html)). The auto-analysis is accessed via the `Stereochem_analysis` class, the details of which can be seen at [http://www.nmr-relax.com/api/3.1/auto\\_analyses.stereochem\\_analysis.Stereochem\\_analysis-class.html](http://www.nmr-relax.com/api/3.1/auto_analyses.stereochem_analysis.Stereochem_analysis-class.html).

### 10.3.2 Stereochemistry – the sample script

The following script was used for the analysis in [Sun et al. \(2011\)](#). It is used to complete the first step of the analysis, the determination of relative configuration, and for the generation of ensembles for the second step of the analysis. The file is located at `sample_scripts/n_state_model/stereochem_analysis.py`. The contents of the script are:

```

1  """Script for the determination of relative stereochemistry.
2
3  The analysis is preformed by using multiple ensembles of structures, randomly sampled from
   a given set of structures. The discrimination is performed by comparing the sets of
   ensembles using NOE violations and RDC Q factors.
4
5  This script is split into multiple stages:
6
7      1. The random sampling of the snapshots to generate the N ensembles (NUM_ENS, usually
        10,000 to 100,000) of M members (NUM_MODELS, usually ~10). The original snapshot
        files are expected to be named the SNAPSHOT_DIR + CONFIG + a number from SNAPSHOT_MIN
        to SNAPSHOT_MAX + ".pdb", e.g. "snapshots/R647.pdb". The ensembles will be placed
        into the "ensembles" directory.
8
9      2. The NOE violation analysis.
10
11     3. The superimposition of ensembles. For each ensemble, Molmol is used for
        superimposition using the fit to first algorithm. The superimposed ensembles will be
        placed into the "ensembles_superimposed" directory. This stage is not necessary for
        the NOE analysis.
12
13     4. The RDC Q factor analysis.
14
15     5. Generation of Grace graphs.
16
17     6. Final ordering of ensembles using the combined RDC and NOE Q factors, whereby the
        NOE Q factor is defined as::
18
19         
$$Q^2 = U / \sum(\text{NOE}_i^2),$$

20
21     where U is the quadratic flat bottom well potential - the NOE violation in Angstrom^2.
        The denominator is the sum of all squared NOEs - this must be given as the value of
        NOE_NORM. The combined Q is given by::
22
23         
$$Q_{\text{total}}^2 = Q_{\text{NOE}}^2 + Q_{\text{RDC}}^2.$$

24
25     """
26
27     # relax module imports.
28     from auto_analyses.stereochem_analysis import Stereochem_analysis
29
30     # Stage of analysis (see the docstring above for the options).
31     STAGE = 1
32
33     # Number of ensembles.
34     NUM_ENS = 100000
35
36     # Ensemble size.
37     NUM_MODELS = 10
38
39     # Configurations.
40     CONFIGS = ["R", "S"]
41

```



```
42 # Snapshot directories (corresponding to CONFIGS).
43 SNAPSHOT_DIR = ["snapshots", "snapshots"]
44
45 # Min and max number of the snapshots (corresponding to CONFIGS).
46 SNAPSHOT_MIN = [0, 0]
47 SNAPSHOT_MAX = [76, 71]
48
49 # Pseudo-atoms.
50 PSEUDO = [
51     ["Q7", ["@H16", "@H17", "@H18"]],
52     ["Q9", ["@H20", "@H21", "@H22"]],
53     ["Q10", ["@H23", "@H24", "@H25"]]
54 ]
55
56 # NOE info.
57 NOE_FILE = "noes"
58 NOE_NORM = 50 * 4**2 # The NOE normalisation factor (sum of all NOEs squared).
59
60 # RDC file info.
61 RDC_NAME = "PAN"
62 RDC_FILE = "pan_rdc"
63 RDC_SPIN_ID1_COL = 1
64 RDC_SPIN_ID2_COL = 2
65 RDC_DATA_COL = 2
66 RDC_ERROR_COL = None
67
68 # Bond length.
69 BOND_LENGTH = 1.117 * 1e-10
70
71 # Log file output (only for certain stages).
72 LOG = True
73
74 # Number of buckets for the distribution plots.
75 BUCKET_NUM = 200
76
77 # Distribution plot limits.
78 LOWER_LIM_NOE = 0.0
79 UPPER_LIM_NOE = 600.0
80 LOWER_LIM_RDC = 0.0
81 UPPER_LIM_RDC = 1.0
82
83
84 # Set up and code execution.
85 analysis = Stereochem_analysis(
86     stage=STAGE,
87     num_ens=NUM_ENS,
88     num_models=NUM_MODELS,
89     configs=CONFIGS,
90     snapshot_dir=SNAPSHOT_DIR,
91     snapshot_min=SNAPSHOT_MIN,
92     snapshot_max=SNAPSHOT_MAX,
93     pseudo=PSEUDO,
94     noe_file=NOE_FILE,
95     noe_norm=NOE_NORM,
96     rdc_name=RDC_NAME,
97     rdc_file=RDC_FILE,
98     rdc_spin_id1_col=RDC_SPIN_ID1_COL,
99     rdc_spin_id2_col=RDC_SPIN_ID2_COL,
100     rdc_data_col=RDC_DATA_COL,
101     rdc_error_col=RDC_ERROR_COL,
102     bond_length=BOND_LENGTH,
```

```
103     log=LOG,  
104     bucket_num=BUCKET_NUM,  
105     lower_lim_noe=LOWER_LIM_NOE,  
106     upper_lim_noe=UPPER_LIM_NOE,  
107     lower_lim_rdc=LOWER_LIM_RDC,  
108     upper_lim_rdc=UPPER_LIM_RDC  
109 )  
110 analysis.run()
```

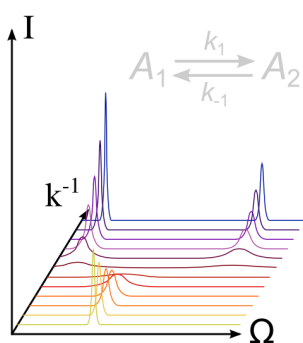
In contrast to all of the other auto-analyses, here you do not set up your own data pipe containing all of the relevant data that is then passed into the auto-analysis. This may change in the future to allow for more flexibility in how you load structures, load the RDC and NOE base data, set up pseudo-atoms and bond lengths for the RDC, etc.

Note that you need to execute this script 6 times, changing the `STAGE` variable to match. These stages are fully documented at the start of the script.

Due to the original analysis being performed prior to the addition of the `structure.superimpose` user function to relax, you will see that the auto-analysis performs superimposition of each ensemble using the external software `Molmol`. If you wish to perform this analysis without using `Molmol`, please contact the relax users mailing list “relax-users at gna.org” (see Section 3.2.2 on page 30). It would be rather straightforward for the relax developers to replace the complicated `Molmol` superimposition code with a single call to the `structure.superimpose` user function.

## Chapter 11

# The analysis of relaxation dispersion



### 11.1 Introduction to relaxation dispersion

Relaxation dispersion is the experimental modulation of chemical exchange relaxation. For the  $R_{1\rho}$ -type experiment in which the nucleus of interest is spin-locked, either the spin-lock field strength or the offset between the spin-lock pulse and the chemical shift of the spins is used to modulate the exchange. For the CPMG-type experiment, varying the time between the pulses modulates the exchange. Both experiment types are handled by relax.

The primary reference for the relaxation dispersion implemented in relax is:

- Morin, S., Linnet, T. E., Lescanne, M., Schanda, P., Thompson, G. S., Tollinger, M., Teilmann, K., Gagne, S., Marion, D., Griesinger, C., Blackledge, M., and d'Auvergne, E. J. (2014). relax: the analysis of biomolecular kinetics and thermodynamics using NMR relaxation dispersion data. *Bioinformatics*, **30**(15), 2219–2220. ([10.1093/bioinformatics/btu166](https://doi.org/10.1093/bioinformatics/btu166))

For other citations, please see the citation chapter on page [xxvii](#).

### 11.1.1 The modelling of dispersion data

For a system under the influence of chemical exchange, the evolution of the transverse magnetisation is given by the Bloch (1946) equations as modified by McConnell (1958) for chemical exchange – the Bloch-McConnell equations. For a two state exchange jumping between states A and B, the equation is:

$$\frac{d}{dt} \begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix} = \begin{bmatrix} -i\Omega_A - R_{2A}^0 - p_B k_{\text{ex}} & p_A k_{\text{ex}} \\ p_B k_{\text{ex}} & -i\Omega_B - R_{2B}^0 - p_A k_{\text{ex}} \end{bmatrix} \begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix}. \quad (11.1)$$

The analytic or closed-form frequency-domain solution for this equation however remains intractable. Solutions can nevertheless be found by either making assumptions or restrictions about the exchange process and then analytically solving 11.1 or by finding numeric solutions. The modelling of relaxation dispersion data can hence be categorised into these two distinct methodologies:

**Analytical models:** Optimisation of models based on analytical, closed-form expressions derived from the Bloch-McConnell equations subject to certain conditions (see Section 11.3 on page 157 and Section 11.7 on page 174).

**Numerical models:** Optimisation of models based on numerically solving the Bloch-McConnell equations (see Section 11.4 on page 164 and Section 11.8 on page 178).

### 11.1.2 Implemented models

A number of analytic and numeric models are supported within relax. These cover single quantum (SQ) CPMG-type, combined proton-heteronuclear single quantum (SQ), zero quantum (ZQ), double quantum (DQ) and multi quantum (MQ) CPMG-type experiments, and  $R_{1\rho}$ -type. If the model you are interested in is not available, please see Section 11.11 on page 195 for how you can add new models to relax.

Models which are independent of the experiment type include:

**‘R2eff’:** This is the model used to determine the  $R_{2\text{eff}}$  or  $R_{1\rho}$  values and errors required as the base data for all other models. See Section 11.2.1 on page 154.

**‘No Rex’:** This is the model for no chemical exchange being present. See Section 11.2.2 on page 156.

For the SQ CPMG-type experiments, the analytic models currently supported are:

**‘LM63’:** The original Luz and Meiboom (1963) 2-site fast exchange equation with parameters  $\{R_2^0, \dots, \Phi_{\text{ex}}, k_{\text{ex}}\}$ . See Section 11.3.1 on page 157.

**‘LM63 3-site’:** The original Luz and Meiboom (1963) 3-site fast exchange equation with parameters  $\{R_2^0, \dots, \Phi_{\text{ex,B}}, k_B, \Phi_{\text{ex,C}}, k_C\}$ . The equations of O’Connell et al. (2009) can be used to approximately convert the parameters  $\{\Phi_{\text{ex,B}}, k_B, \Phi_{\text{ex,C}}, k_C\}$  to more biologically relevant parameters. See Section 11.3.2 on page 158.

- ‘**CR72**’: The reduced [Carver and Richards \(1972\)](#) 2-site equation for most time scales whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.3.4 on page 160.
- ‘**CR72 full**’: The full [Carver and Richards \(1972\)](#) 2-site equation for most time scales with parameters  $\{R_{2A}^0, R_{2B}^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.3.3 on page 159.
- ‘**IT99**’: The [Ishima and Torchia \(1999\)](#) 2-site model for all time scales with  $p_A \gg p_B$  and with parameters  $\{R_2^0, \dots, p_A, \Delta\omega, \tau_{ex}\}$ . See Section 11.3.5 on page 160.
- ‘**TSMFK01**’: The [Tollinger et al. \(2001\)](#) 2-site very-slow exchange model for time scales within range of microsecond to second time scale. Applicable in the limit of slow exchange, when  $|R_{2A}^0 - R_{2B}^0| \ll k_{AB}, k_{BA} \ll 1/\tau_{CPMG}$ .  $2 * \tau_{CPMG}$  is the time between successive 180 degree pulses. Parameters are  $\{R_{2A}^0, \dots, \Delta\omega, k_{AB}\}$ . See Section 11.3.6 on page 161.
- ‘**B14**’: The reduced [Baldwin \(2014\)](#) 2-site exact solution equation for all time scales whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.3.8 on page 164.
- ‘**B14 full**’: The full [Baldwin \(2014\)](#) 2-site exact equation for all time scales with parameters  $\{R_{2A}^0, R_{2B}^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.3.7 on page 162.

For the SQ CPMG-type experiments, the numeric models currently supported are:

- ‘**NS CPMG 2-site expanded**’: A model for 2-site exchange expanded using Maple by Nikolai Skrynnikov ([Tollinger et al., 2001](#)). It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.4.1 on page 164.
- ‘**NS CPMG 2-site 3D**’: The reduced model for 2-site exchange using 3D magnetisation vectors whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.4.3 on page 167.
- ‘**NS CPMG 2-site 3D full**’: The full model for 2-site exchange using 3D magnetisation vectors with parameters  $\{R_{2A}^0, R_{2B}^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.4.2 on page 167.
- ‘**NS CPMG 2-site star**’: The reduced model for 2-site exchange using complex conjugate matrices whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.4.5 on page 168.
- ‘**NS CPMG 2-site star full**’: The full model for 2-site exchange using complex conjugate matrices with parameters  $\{R_{2A}^0, R_{2B}^0, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.4.4 on page 167.

For the combined proton-heteronuclear SQ, ZQ, DQ and MQ CPMG-type experiments (MMQ – or multi-multiple quantum), the analytic models currently supported are:

- ‘**MMQ CR72**’: The [Carver and Richards \(1972\)](#) 2-site model for most time scales expanded for MMQ CPMG data by [Korzhnev et al. \(2004a\)](#). It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, \Delta\omega^H, k_{ex}\}$ . See Section 11.5.1 on page 168.

For the combined proton-heteronuclear SQ, ZQ, DQ and MQ CPMG-type experiments (MMQ – or multi-multiple quantum), the numeric models currently supported are:

- ‘**NS MMQ 2-site**’: The model for 2-site exchange whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, \Delta\omega, \Delta\omega^H, k_{ex}\}$ . See Section 11.6.1 on page 170.
- ‘**NS MMQ 3-site linear**’: The model for 3-site exchange linearised with  $k_{AC} = k_{CA} = 0$  whereby the simplification  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, \Delta\omega_{AB}^H, \Delta\omega_{BC}^H, k_{ex}^{AB}, k_{ex}^{BC}\}$ . See Section 11.6.2 on page 172.
- ‘**NS MMQ 3-site**’: The model for 3-site exchange whereby the simplification  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0$  is assumed. It has the parameters  $\{R_2^0, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, \Delta\omega_{AB}^H, \Delta\omega_{BC}^H, k_{ex}^{AB}, k_{ex}^{BC}, k_{ex}^{AC}\}$ . See Section 11.6.3 on page 173.

For the  $R_{1\rho}$ -type experiments, the analytic models currently supported are:

- ‘**M61**’: The Meiboom (1961) 2-site fast exchange equation for on-resonance data with parameters  $\{R'_{1\rho}, \dots, \Phi_{ex}, k_{ex}\}$ . See Section 11.7.1 on page 175.
- ‘**DPL94**’: The Davis et al. (1994) extension of the ‘M61’ model for off-resonance data with parameters  $\{R'_{1\rho}, \dots, \Phi_{ex}, k_{ex}\}$ . See Section 11.7.3 on page 176.
- ‘**M61 skew**’: The Meiboom (1961) 2-site equation for all time scales with  $p_A \gg p_B$  and with parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$ . This model is disabled by default in the dispersion auto-analysis. See Section 11.7.2 on page 175.
- ‘**TP02**’: The Trott and Palmer (2002) 2-site equation for all time scales with  $p_A \gg p_B$  and with parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.7.4 on page 176.
- ‘**TAP03**’: The Trott et al. (2003) off-resonance 2-site analytic equation for all time scales with the weak condition  $p_A \gg p_B$  and with parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$ .
- ‘**MP05**’: The Miloushev and Palmer (2005) off-resonance 2-site equation for all time scales with parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.7.6 on page 178.

For the  $R_{1\rho}$ -type experiments, the numeric models currently supported are:

- ‘**NS R1rho 2-site**’: The model for 2-site exchange using 3D magnetisation vectors. It has the parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$ . See Section 11.8.1 on page 179.
- ‘**NS  $R_{1\rho}$  3-site linear**’: The model for 3-site exchange linearised with  $k_{AC} = k_{CA} = 0$  whereby the simplification  $R'_{1\rho A} = R'_{1\rho B} = R'_{1\rho C}$  is assumed. It has the parameters  $\{R'_{1\rho}, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, k_{ex}^{AB}, k_{ex}^{BC}\}$ . See Section 11.8.3 on page 181.
- ‘**NS  $R_{1\rho}$  3-site**’: The model for 3-site exchange whereby the simplification  $R'_{1\rho A} = R'_{1\rho B} = R'_{1\rho C}$  is assumed. It has the parameters  $\{R'_{1\rho}, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, k_{ex}^{AB}, k_{ex}^{BC}, k_{ex}^{AC}\}$ . See Section 11.8.2 on page 179.

### 11.1.3 Dispersion model summary

Except for ‘R2eff’ and ‘No Rex’, all models can be fit to clusterings of spins, or spin blocks. The models are described in more detail below and summarised in Table 11.1. The parameters of the models and of relaxation dispersion in general are given in Table 17.5.

#### $R_1$ parameter optimisation

For a number of models, the off-resonance  $R_1$  value can be optimised. Normally the off-resonance models will use fixed experimental  $R_1$  values for optimisation. However if the experimental values are not loaded, then the  $R_1$  values will be automatically optimised. For finer control of this optimisation behaviour, see the `relax_disp.r1_fit` user function (page 580). The models which support off-resonance  $R_1$  fitting include:

- ‘No Rex’,
- ‘DPL94’,
- ‘TP02’,
- ‘TAP03’,
- ‘MP05’,
- ‘NS R1rho 2-site’.

In the future, support for off-resonance effects in the CPMG experiments is planned (see section 11.10 on page 194).

Table 11.1: The dispersion models supported by relax.

| Model name                    | Solution | Sites | Parameters                                                                               | Restrictions                                                                   | Reference                  |
|-------------------------------|----------|-------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------|
| <b>Experiment independent</b> |          |       |                                                                                          |                                                                                |                            |
| R2eff                         | -        | -     | $\{R_{2\text{eff}}, \dots\}$                                                             | Fixed relaxation time period                                                   | -                          |
| R2eff                         | -        | -     | $\{R_{2\text{eff}}, I_0, \dots\}$                                                        | Variable relaxation time period                                                | -                          |
| No Rex                        | Closed   | 1     | $\{R_2^0, \dots\}$                                                                       | -                                                                              | -                          |
| <b>CPMG-type</b>              |          |       |                                                                                          |                                                                                |                            |
| LM63                          | Analytic | 2     | $\{R_2^0, \dots, \Phi_{\text{ex}}, k_{\text{ex}}\}$                                      | Fast exchange                                                                  | Luz and Meiboom (1963)     |
| LM63 3-site                   | Analytic | 3     | $\{R_2^0, \dots, \Phi_{\text{ex,B}}, k_{\text{B}}, \Phi_{\text{ex,C}}, k_{\text{C}}\}$   | Fast exchange, $p_{\text{A}} > p_{\text{B}}$ and $p_{\text{A}} > p_{\text{C}}$ | Luz and Meiboom (1963)     |
| CR72                          | Analytic | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$                            | $p_{\text{A}} > p_{\text{B}}$ , not very slow exchange                         | Carver and Richards (1972) |
| CR72 full                     | Analytic | 2     | $\{R_{2\text{A}}^0, R_{2\text{B}}^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$ | $p_{\text{A}} > p_{\text{B}}$ , not very slow exchange                         | Carver and Richards (1972) |
| IT99                          | Analytic | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, \tau_{\text{ex}}\}$                         | $p_{\text{A}} \gg p_{\text{B}}$                                                | Ishima and Torchia (1999)  |
| TSMFK01                       | Analytic | 2     | $\{R_{2\text{A}}^0, \dots, \Delta\omega, k_{\text{AB}}\}$                                | $p_{\text{A}} \gg p_{\text{B}}$ slow exchange                                  | Tollinger et al. (2001)    |
| B14                           | Analytic | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$                            | $p_{\text{A}} > p_{\text{B}}$ ,                                                | Baldwin (2014)             |
| B14 full                      | Analytic | 2     | $\{R_{2\text{A}}^0, R_{2\text{B}}^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$ | $p_{\text{A}} > p_{\text{B}}$ ,                                                | Baldwin (2014)             |
| NS CPMG 2-site expanded       | Numeric  | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$                            | $p_{\text{A}} > p_{\text{B}}$                                                  | Tollinger et al. (2001)    |
| NS CPMG 2-site 3D             | Numeric  | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$                            | $p_{\text{A}} > p_{\text{B}}$                                                  | -                          |
| NS CPMG 2-site 3D full        | Numeric  | 2     | $\{R_{2\text{A}}^0, R_{2\text{B}}^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$ | $p_{\text{A}} > p_{\text{B}}$                                                  | -                          |
| NS CPMG 2-site star           | Numeric  | 2     | $\{R_2^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$                            | $p_{\text{A}} > p_{\text{B}}$                                                  | -                          |
| NS CPMG 2-site star full      | Numeric  | 2     | $\{R_{2\text{A}}^0, R_{2\text{B}}^0, \dots, p_{\text{A}}, \Delta\omega, k_{\text{ex}}\}$ | $p_{\text{A}} > p_{\text{B}}$                                                  | -                          |



Table 11.1: The dispersion models supported by relax.

| Model name                 | Solution | Sites | Parameters                                                                                                                                     | Restrictions                                | Reference                   |
|----------------------------|----------|-------|------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|-----------------------------|
| <b>MMQ CPMG-type</b>       |          |       |                                                                                                                                                |                                             |                             |
| MMQ CR72                   | Analytic | 2     | $\{R_2^0, \dots, p_A, \Delta\omega, \Delta\omega^H, k_{ex}\}$                                                                                  | $p_A > p_B$                                 | Korzhnev et al. (2004a)     |
| NS MMQ 2-site              | Numeric  | 2     | $\{R_2^0, \dots, p_A, \Delta\omega, \Delta\omega^H, k_{ex}\}$                                                                                  | $p_A > p_B$                                 | Korzhnev et al. (2005a)     |
| NS MMQ 3-site linear       | Numeric  | 3     | $\{R_2^0, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, \Delta\omega_{AB}^H, \Delta\omega_{BC}^H, k_{ex}, k_{ex}^{BC}\}$              | $p_A > p_B$ and $p_B > p_C$                 | Korzhnev et al. (2005a)     |
| NS MMQ 3-site              | Numeric  | 3     | $\{R_2^0, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, \Delta\omega_{AB}^H, \Delta\omega_{BC}^H, k_{ex}, k_{ex}^{BC}, k_{ex}^{AC}\}$ | $p_A > p_B$ and $p_B > p_C$                 | Korzhnev et al. (2005a)     |
| <b>R<sub>1ρ</sub>-type</b> |          |       |                                                                                                                                                |                                             |                             |
| M61                        | Analytic | 2     | $\{R'_{1\rho}, \dots, \Phi_{ex}, k_{ex}\}$                                                                                                     | Fast exchange, on-resonance,<br>$R_1 = R_2$ | Meiboom (1961)              |
| DPL94                      | Analytic | 2     | $\{R'_{1\rho}, \dots, \Phi_{ex}, k_{ex}\}$                                                                                                     | Fast exchange                               | Davis et al. (1994)         |
| M61 skew                   | Analytic | 2     | $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                             | $p_A \gg p_B$ , on-resonance                | Meiboom (1961)              |
| TP02                       | Analytic | 2     | $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                             | $p_A \gg p_B$ , not fast exchange           | Trott and Palmer (2002)     |
| TAP03                      | Analytic | 2     | $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                             | Weak condition of $p_A \gg p_B$             | Trott et al. (2003)         |
| TP04 <sup>1</sup>          | Analytic | N     | $\{R'_{1\rho}, \dots, p_1, \dots, p_N, \bar{\omega}, k_{12}, \dots, k_{1N}\}$                                                                  | One site dominant                           | Trott and Palmer (2004)     |
| MP05                       | Analytic | 2     | $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                             | $p_A > p_B$                                 | Miloushev and Palmer (2005) |
| BK13                       | Analytic | 2     | $\{R_2^0, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                                  | $p_A > p_B$ ,                               | Baldwin and Kay (2013)      |
| BK13 full                  | Analytic | 2     | $\{R_{2A}^0, R_{2B}^0, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                     | $p_A > p_B$ ,                               | Baldwin and Kay (2013)      |
| NS R1rho 2-site            | Numeric  | 2     | $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{ex}\}$                                                                                             | $p_A > p_B$                                 | -                           |
| NS R1rho 3-site linear     | Numeric  | 3     | $\{R'_{1\rho}, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, k_{ex}^{AB}, k_{ex}^{BC}\}$                                              | $p_A > p_B$ and $p_A > p_C$                 | -                           |
| NS R1rho 3-site            | Numeric  | 3     | $\{R'_{1\rho}, \dots, p_A, p_B, \Delta\omega_{AB}, \Delta\omega_{BC}, k_{ex}^{AB}, k_{ex}^{BC}, k_{ex}^{AC}\}$                                 | $p_A > p_B$ and $p_A > p_C$                 | -                           |

<sup>1</sup>Not implemented yet

Table 11.2: The parameters of relaxation dispersion.

| Parameter            | Equation                                            | Description                                                                           | Units               |
|----------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------|---------------------|
| $\nu_{\text{CPMG}}$  | $1/(4\tau_{\text{CPMG}})$                           | CPMG frequency                                                                        | Hz                  |
| $\tau_{\text{CPMG}}$ | $1/(4\nu_{\text{CPMG}})$                            | Delay between CPMG $\pi$ pulses                                                       | s                   |
| $T_{\text{relax}}$   | -                                                   | The relaxation delay period                                                           | s                   |
| $I_0$                | -                                                   | Reference peak intensity when $T_{\text{relax}}$ is zero                              | -                   |
| $I_1$                | -                                                   | Peak intensity for a given $\nu_{\text{CPMG}}$ or spin-lock field strength $\omega_1$ | -                   |
| $R_2^0$              | -                                                   | $R_2$ relaxation rate in the absence of exchange                                      | rad.s <sup>-1</sup> |
| $R_{2A}^0$           | -                                                   | $R_2$ relaxation rate for state A in the absence of exchange                          | rad.s <sup>-1</sup> |
| $R_{2B}^0$           | -                                                   | $R_2$ relaxation rate for state B in the absence of exchange                          | rad.s <sup>-1</sup> |
| $R_{1\rho}^0$        | -                                                   | $R_{1\rho}$ relaxation rate in the absence of exchange                                | rad.s <sup>-1</sup> |
| $\bar{\omega}$       | $\bar{\omega} - \omega_{\text{rf}}$                 | The average resonance offset in the rotating frame                                    | rad.s <sup>-1</sup> |
| $\Omega_A$           | $\omega_A - \omega_{\text{rf}}$                     | The resonance offset in the rotating frame for state A                                | rad.s <sup>-1</sup> |
| $\Omega_B$           | $\omega_B - \omega_{\text{rf}}$                     | The resonance offset in the rotating frame for state B                                | rad.s <sup>-1</sup> |
| $\Omega_C$           | $\omega_C - \omega_{\text{rf}}$                     | The resonance offset in the rotating frame for state C                                | rad.s <sup>-1</sup> |
| $\omega_A$           | -                                                   | The Larmor frequency of the spin in state A                                           | rad.s <sup>-1</sup> |
| $\omega_B$           | -                                                   | The Larmor frequency of the spin in state B                                           | rad.s <sup>-1</sup> |
| $\omega_C$           | -                                                   | The Larmor frequency of the spin in state C                                           | rad.s <sup>-1</sup> |
| $\omega_A^H$         | -                                                   | The proton Larmor frequency of the spin in state A (for MMQ data)                     | rad.s <sup>-1</sup> |
| $\omega_B^H$         | -                                                   | The proton Larmor frequency of the spin in state B (for MMQ data)                     | rad.s <sup>-1</sup> |
| $\omega_C^H$         | -                                                   | The proton Larmor frequency of the spin in state C (for MMQ data)                     | rad.s <sup>-1</sup> |
| $\bar{\omega}$       | $p_A\omega_A + p_B\omega_B$                         | The population averaged Larmor frequency of the spin                                  | rad.s <sup>-1</sup> |
| $\omega_1$           | -                                                   | Spin-lock field strength, i.e. the amplitude of the rf field                          | rad.s <sup>-1</sup> |
| $\omega_e$           | $\sqrt{\bar{\omega}^2 + \omega_1^2}$                | Effective field in the rotating frame                                                 | rad.s <sup>-1</sup> |
| $\omega_{\text{rf}}$ | -                                                   | Spin-lock offset, i.e. the frequency of the rf field                                  | rad.s <sup>-1</sup> |
| $\theta$             | $\arctan\left(\frac{\omega_1}{\bar{\omega}}\right)$ | Rotating frame tilt angle                                                             | rad                 |
| $k_{AB}$             | $p_B k_{\text{ex}}$                                 | The forward exchange rate from state A to state B (2-site)                            | rad.s <sup>-1</sup> |
| $k_{BA}$             | $p_A k_{\text{ex}}$                                 | The reverse exchange rate from state B to state A (2-site)                            | rad.s <sup>-1</sup> |
| $k_{AB}$             | $p_B k_{\text{ex}}^{AB}$                            | The forward exchange rate from state A to state B (3-site)                            | rad.s <sup>-1</sup> |
| $k_{BA}$             | $p_A k_{\text{ex}}^{AB}$                            | The reverse exchange rate from state B to state A (3-site)                            | rad.s <sup>-1</sup> |
| $k_{BC}$             | $p_C k_{\text{ex}}^{BC}$                            | The forward exchange rate from state B to state C (3-site)                            | rad.s <sup>-1</sup> |
| $k_{CB}$             | $p_B k_{\text{ex}}^{BC}$                            | The reverse exchange rate from state C to state B (3-site)                            | rad.s <sup>-1</sup> |

Table 11.2: The parameters of relaxation dispersion.

| Parameter             | Equation                                    | Description                                                       | Units                                                      |
|-----------------------|---------------------------------------------|-------------------------------------------------------------------|------------------------------------------------------------|
| $k_{AC}$              | $p_C k_{ex}^{AC}$                           | The forward exchange rate from state A to state C (3-site)        | $\text{rad.s}^{-1}$                                        |
| $k_{CA}$              | $p_A k_{ex}^{AC}$                           | The reverse exchange rate from state C to state A (3-site)        | $\text{rad.s}^{-1}$                                        |
| $k_{ex}$              | $1/\tau_{ex}$                               | Chemical exchange rate constant                                   | $\text{rad.s}^{-1}$                                        |
| $k_{AB}^{ex}$         | $k_{AB} + k_{BA}$                           | Chemical exchange rate constant between sites A and B             | $\text{rad.s}^{-1}$                                        |
| $k_{BC}^{ex}$         | $k_{BC} + k_{CB}$                           | Chemical exchange rate constant between sites B and C             | $\text{rad.s}^{-1}$                                        |
| $k_{AC}^{ex}$         | $k_{AC} + k_{CA}$                           | Chemical exchange rate constant between sites A and C             | $\text{rad.s}^{-1}$                                        |
| $k_B$                 | $\approx k_{ex}^{AB}$                       | Approximate chemical exchange rate constant between sites A and B | $\text{rad.s}^{-1}$                                        |
| $k_C$                 | $\approx k_{ex}^{AC}$                       | Approximate chemical exchange rate constant between sites A and C | $\text{rad.s}^{-1}$                                        |
| $\tau_{ex}$           | $1/k_{ex}$                                  | Time of exchange                                                  | $\text{s.rad}^{-1}$                                        |
| $p_A$                 | -                                           | Population of state A                                             | -                                                          |
| $p_B$                 | $1 - p_A$                                   | Population of state B (2-site)                                    | -                                                          |
| $p_B$                 | $1 - p_A - p_C$                             | Population of state B (3-site)                                    | -                                                          |
| $p_C$                 | $1 - p_A - p_B$                             | Population of state C (3-site)                                    | -                                                          |
| $\Delta\omega$        | $\omega_B - \omega_A$                       | Chemical shift difference between sites A and B (2-site)          | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{AB}$   | $\omega_B - \omega_A$                       | Chemical shift difference between sites A and B (3-site)          | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{BC}$   | $\omega_C - \omega_B$                       | Chemical shift difference between sites B and C (3-site)          | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{AC}$   | $\Delta\omega_{AB} + \Delta\omega_{BC}$     | Chemical shift difference between sites A and C (3-site)          | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega^H$      | $\omega_B^H - \omega_A^H$                   | Proton chemical shift difference between sites A and B (2-site)   | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{AB}^H$ | $\omega_B^H - \omega_A^H$                   | Proton chemical shift difference between sites A and B (3-site)   | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{BC}^H$ | $\omega_C^H - \omega_B^H$                   | Proton chemical shift difference between sites B and C (3-site)   | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Delta\omega_{AC}^H$ | $\Delta\omega_{AB}^H + \Delta\omega_{BC}^H$ | Proton chemical shift difference between sites A and C (3-site)   | $\text{rad.s}^{-1}$ (stored as ppm)                        |
| $\Phi_{ex}$           | $p_A p_B \Delta\omega^2$                    | Fast exchange factor                                              | $\text{rad}^2.\text{s}^{-2}$ (stored as ppm <sup>2</sup> ) |
| $\Phi_{ex,B}$         | See 11.20a on page 158                      | Fast exchange factor between sites A and B                        | $\text{rad}^2.\text{s}^{-2}$ (stored as ppm <sup>2</sup> ) |
| $\Phi_{ex,C}$         | See 11.20b on page 158                      | Fast exchange factor between sites A and C                        | $\text{rad}^2.\text{s}^{-2}$ (stored as ppm <sup>2</sup> ) |

## 11.2 The base dispersion models

### 11.2.1 The R2eff model

This is the simplest of all models in that the dispersion component of the base data – the peak intensity values – is not modelled. It is used to determine either the  $R_{2\text{eff}}$  or  $R_{1\rho}$  values and errors as required for the base data for all other models. It can be selected by setting the model to ‘R2eff’. Depending on the experiment type, this model will be handled differently. The  $R_{2\text{eff}}/R_{1\rho}$  values determined can be later copied to the data pipes of the other dispersion models using the appropriate user functions.

#### Fixed relaxation period experiments

For the fixed relaxation time period CPMG-type experiments, the  $R_{2\text{eff}}/R_{1\rho}$  values are determined by direct calculation using the formula

$$R_{2\text{eff}}(\nu_{\text{CPMG}}) = -\frac{1}{T_{\text{relax}}} \cdot \ln \left( \frac{I_1(\nu_{\text{CPMG}})}{I_0} \right). \quad (11.2)$$

The values and errors are determined with a single call of the `minimise.calculate` user function. The  $R_{1\rho}$  version of the equation is essentially the same:

$$R_{1\rho}(\omega_1) = -\frac{1}{T_{\text{relax}}} \cdot \ln \left( \frac{I_1(\omega_1)}{I_0} \right). \quad (11.3)$$

Errors are calculated using the formula

$$\sigma_{R_2} = \frac{1}{T_{\text{relax}}} \sqrt{\left( \frac{\sigma_{I_1}}{I_1(\omega_1)} \right)^2 + \left( \frac{\sigma_{I_0}}{I_0} \right)^2}. \quad (11.4)$$

The derivation of this is simple enough. Rearranging [11.2](#),

$$R_2 \cdot T_{\text{relax}} = -\ln \left( \frac{I_1}{I_0} \right). \quad (11.5)$$

Using the rule

$$\ln \left( \frac{X}{Y} \right) = \ln(X) - \ln(Y), \quad (11.6)$$

where X and Y are normally distributed variables, then

$$R_2 \cdot T_{\text{relax}} = \ln(I_0) - \ln(I_1), \quad (11.7)$$

and

$$R_2 = -\frac{1}{T_{\text{relax}}} \cdot (\ln(I_0) - \ln(I_1)), \quad (11.8)$$

Using the estimate from [https://en.wikipedia.org/wiki/Propagation\\_of\\_uncertainty](https://en.wikipedia.org/wiki/Propagation_of_uncertainty) that for

$$f = a \ln(A), \quad (11.9)$$

the variance of  $f$  is

$$\sigma_f^2 = \left( a * \frac{\sigma_A}{A} \right)^2, \quad (11.10)$$

then the  $R_2$  variance is

$$\sigma_{R_2}^2 = \left( \frac{1}{T_{\text{relax}}} \cdot \frac{\sigma_{I_0}}{I_0} \right)^2 + \left( \frac{1}{T_{\text{relax}}} \cdot \frac{\sigma_{I_1}}{I_1} \right)^2. \quad (11.11)$$

Rearranging gives 11.4.

In a number of publications, the error formula from Ishima and Torchia (2005) has been used. This is the collapse of Equation 11.4 by setting  $\sigma_{I_0}$  to zero:

$$\sigma_{R_2} = \frac{\sigma_{I_1}}{T_{\text{relax}} I_1(\omega_1)}. \quad (11.12)$$

This is not implemented in relax as it can be shown by simple simulation that the formula is incorrect (see Figure 11.1). This formula significantly underestimates the real errors. The use of the same  $I_0$  value for all dispersion points does not cause a decrease in the  $R_{2\text{eff}}$  error but rather a correlation in the errors.

### Variable relaxation period experiments

For the variable relaxation time period type experiments, the  $R_{2\text{eff}}/R_{1\rho}$  values are determined by fitting to the simple two parameter exponential as in a  $R_1$  or  $R_2$  analysis. Both  $R_{2\text{eff}}/R_{1\rho}$  and the initial peak intensity  $I_0$  are optimised using the minimise user function for each exponential curve separately. Monte Carlo simulations are used to obtain the parameter errors.

### Links

More information about the  $R_{2\text{eff}}$  model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/R2eff>,
- the API documentation (for exponential curves) at [http://www.nmr-relax.com/api/3.2/specific\\_analyses.relax\\_disp.optimisation-module.html#back\\_calc\\_r2eff](http://www.nmr-relax.com/api/3.2/specific_analyses.relax_disp.optimisation-module.html#back_calc_r2eff),
- the API documentation (for the 2-point fit) at [http://www.nmr-relax.com/api/3.2/lib.dispersion.two\\_point-module.html#calc\\_two\\_point\\_r2eff](http://www.nmr-relax.com/api/3.2/lib.dispersion.two_point-module.html#calc_two_point_r2eff),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#R2eff](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#R2eff).

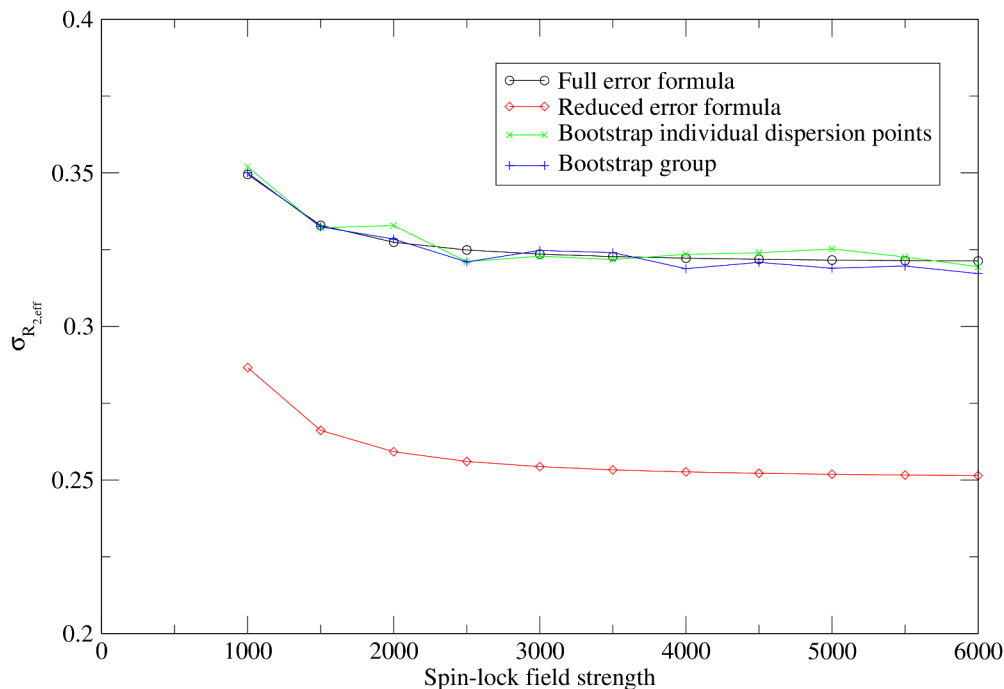


Figure 11.1: A demonstration of the inaccuracy of the error formula of Equation 11.12 from Ishima and Torchia (2005). This plot was generated using the script `test_suite/shared_data/dispersion/error_testing/simulation.py`. The bootstrapping simulation involves randomising noise-free  $I_0$  and  $I_1$  values for each dispersion data point assuming Gaussian errors. The full error formula is from Equation 11.4, the reduced error formula is from Equation 11.12, the bootstrapping using individual dispersion points estimates the errors assuming different  $I_0$  randomisations for each dispersion point and each simulation, and the bootstrapping group graph uses the same randomised  $I_0$  value for all dispersion points for each simulation.

### 11.2.2 The model for no chemical exchange relaxation

This model is provided for model selection purposes. In combination with frequentist methods, such as AIC, or Bayesian methods it can show if the presence of chemical exchange is statistically significant. Optimisation is still required as one  $R_2^0$  value per magnetic field strength will be fit to the measured data for each spin system. If off-resonance CPMG or  $R_{1\rho}$  data is being used, then one of the following equations will be fit:

$$R_{2\text{eff}} = R_1 \cos^2 \theta + R_2^0 \sin^2 \theta, \quad (11.13a)$$

$$R_{1\rho} = R_1 \cos^2 \theta + R_{1\rho}' \sin^2 \theta. \quad (11.13b)$$

The  $R_1$  value can either be fixed to loaded experimental values or optimised. It is selected by setting the model to ‘No Rex’.

More information about the No Rex model is available from the:

- the relax wiki at [http://wiki.nmr-relax.com/No\\_Rex](http://wiki.nmr-relax.com/No_Rex),

- the API documentation at [http://www.nmr-relax.com/api/3.2/target\\_functions.relax\\_disp.Dispersion-class.html#func\\_NOREX](http://www.nmr-relax.com/api/3.2/target_functions.relax_disp.Dispersion-class.html#func_NOREX),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#No\\_Rex](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#No_Rex).

## 11.3 The analytic CPMG models

These are the analytic models designed for a single data type – the single quantum CPMG-type experiment.

### 11.3.1 The LM63 2-site fast exchange CPMG model

This is the original model for 2-site fast exchange for CPMG-type experiments. It is selected by setting the model to ‘LM63’, here named after [Luz and Meiboom \(1963\)](#). The original  $n$ -site Equation (7) from their paper can be written as

$$R_{\text{ex}} = \left[ 1 - 2\tau_{\text{ex}}g \cdot \tanh(2\tau_{\text{ex}}g)^{-1} \right] \cdot \tau_{\text{ex}} \cdot \sum_{i=2}^n p_i \Delta\omega_i, \quad (11.14)$$

where  $g$  is the pulse repetition rate defined as

$$g = 2\nu_{\text{CPMG}}. \quad (11.15)$$

It can be rearranged as

$$R_{\text{ex}} = \sum_{i=2}^n \frac{\Phi_{\text{ex},i}}{k_i} \cdot \left( 1 - \frac{4\nu_{\text{CPMG}}}{k_i} \cdot \tanh\left(\frac{k_i}{4\nu_{\text{CPMG}}}\right) \right). \quad (11.16)$$

The equation for the 2-site exchange process can be expressed as

$$R_{2\text{eff}} = R_2^0 + \frac{\Phi_{\text{ex}}}{k_{\text{ex}}} \cdot \left( 1 - \frac{4\nu_{\text{CPMG}}}{k_{\text{ex}}} \cdot \tanh\left(\frac{k_{\text{ex}}}{4\nu_{\text{CPMG}}}\right) \right). \quad (11.17)$$

The reference for this equation is:

- Luz, Z. and Meiboom, S. (1963). Nuclear magnetic resonance study of protolysis of trimethylammonium ion in aqueous solution - order of reaction with respect to solvent. *J. Chem. Phys.*, **39**(2), 366–370. ([10.1063/1.1734254](https://doi.org/10.1063/1.1734254))

More information about the LM63 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/LM63>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.lm63-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#LM63](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#LM63).

### 11.3.2 The LM63 3-site fast exchange CPMG model

This is the original [Luz and Meiboom \(1963\)](#) model for 3-site fast exchange for CPMG-type experiments. It is selected by setting the model to ‘LM63 3-site’. Taking the original Equation 11.16, the equation for 3-site exchange is simply:

$$R_{\text{ex}} = \sum_{i=2}^3 \frac{\Phi_{\text{ex},i}}{k_i} \cdot \left( 1 - \frac{4\nu_{\text{CPMG}}}{k_i} \cdot \tanh \left( \frac{k_i}{4\nu_{\text{CPMG}}} \right) \right), \quad (11.18)$$

The reference for this equation is:

- Luz, Z. and Meiboom, S. (1963). Nuclear magnetic resonance study of protolysis of trimethylammonium ion in aqueous solution - order of reaction with respect to solvent. *J. Chem. Phys.*, **39**(2), 366–370. ([10.1063/1.1734254](#))

This model is only provided as a demonstration and should not be used for a normal analysis. Without data at multiple temperatures, a feature not currently supported within relax, that there are infinite lines of solutions and that the  $\Phi_{\text{ex},B}$ ,  $\Phi_{\text{ex},C}$ ,  $k_B$  and  $k_C$  parameters are all convoluted together.

This equation was made more practically relevant in the paper of [O’Connell et al. \(2009\)](#). This relies on the assumption that site 1 (or A) has a much larger equilibrium population than the other sites ( $p_A \gg p_B$  and  $p_A \gg p_C$ ). As stated, “if the different values of  $j_i$  are well-separated (by a factor of 3-10), then Eq. 3 reduces approximately to the sum of  $n - 1$  independent two-state processes for exchange between site 1 and the  $n - 1$  other sites”. In this situation, the following relationships hold

$$k_B \approx k_{\text{ex}}^{\text{AB}} = k_{\text{AB}} + k_{\text{BA}}, \quad (11.19a)$$

$$k_C \approx k_{\text{ex}}^{\text{AC}} = k_{\text{AC}} + k_{\text{CA}}, \quad (11.19b)$$

and

$$\Phi_{\text{ex},B} = \overline{\Phi_{\text{ex}}} \frac{(k_{\text{ex}}^{\text{AB}})^2 (\overline{k_{\text{ex}}} - k_{\text{ex}}^{\text{AC}})}{k_{\text{ex}}^2 (\overline{k_{\text{ex}}} - k_{\text{ex}}^{\text{AC}})}, \quad (11.20a)$$

$$\Phi_{\text{ex},C} = \overline{\Phi_{\text{ex}}} \frac{(k_{\text{ex}}^{\text{AC}})^2 (\overline{k_{\text{ex}}} - k_{\text{ex}}^{\text{AB}})}{k_{\text{ex}}^2 (\overline{k_{\text{ex}}} - k_{\text{ex}}^{\text{AB}})}, \quad (11.20b)$$

with

$$\overline{\Phi_{\text{ex},B}} \approx (p_A + p_C) p_B \Delta\omega_{\text{AB}}^2, \quad (11.21a)$$

$$\overline{\Phi_{\text{ex},C}} \approx (p_A + p_B) p_C \Delta\omega_{\text{AC}}^2. \quad (11.21b)$$

The parameter deconvolutions for this model can be performed after a relax analysis, if desired.

More information about the LM63 3-site model is available from:



- the relax wiki at [http://wiki.nmr-relax.com/LM63\\_3-site](http://wiki.nmr-relax.com/LM63_3-site),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.lm63\\_3site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.lm63_3site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#LM63\\_3-site](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#LM63_3-site).

### 11.3.3 The full CR72 2-site CPMG model

This is the model for 2-site exchange on most times scales (with the constraint that  $p_A > p_B$ ), named after [Carver and Richards \(1972\)](#). It is selected by setting the model to ‘CR72 full’. The equation is

$$R_{2\text{eff}} = \frac{1}{2} \left( R_{2A}^0 + R_{2B}^0 + k_{\text{ex}} - 2\nu_{\text{CPMG}} \cosh^{-1} (D_+ \cosh(\eta_+) - D_- \cos(\eta_-)) \right), \quad (11.22)$$

where

$$D_{\pm} = \frac{1}{2} \left( \pm 1 + \frac{\Psi + 2\Delta\omega^2}{\sqrt{\Psi^2 + \zeta^2}} \right), \quad (11.23)$$

$$\eta_{\pm} = 2^{\frac{-3}{2}} \frac{1}{\nu_{\text{CPMG}}} \left( \pm \Psi + \sqrt{\Psi^2 + \zeta^2} \right)^{\frac{1}{2}}, \quad (11.24)$$

$$\Psi = (R_{2A}^0 - R_{2B}^0 - p_A k_{\text{ex}} + p_B k_{\text{ex}})^2 - \Delta\omega^2 + 4p_A p_B k_{\text{ex}}^2, \quad (11.25)$$

$$\zeta = 2\Delta\omega (R_{2A}^0 - R_{2B}^0 - p_A k_{\text{ex}} + p_B k_{\text{ex}}). \quad (11.26)$$

Note that these equations use the numerically simplified form derived in the appendix of [Davis et al. \(1994\)](#).

This model is not accurate when the motional process is very slow. In that case, the ‘TSMFK01’ model in Section 11.3.6 on page 161 should be used instead.

The reference for this equation is:

- Carver, J. P. and Richards, R. E. (1972). General 2-site solution for chemical exchange produced dependence of T2 upon Carr-Purcell pulse separation. *J. Magn. Reson.*, **6**(1), 89–105. ([10.1016/0022-2364\(72\)90090-X](https://doi.org/10.1016/0022-2364(72)90090-X))

More information about the CR72 full model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/CR72\\_full](http://wiki.nmr-relax.com/CR72_full),
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.cr72-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#CR72\\_full](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#CR72_full).

### 11.3.4 The reduced CR72 2-site CPMG model

This is the model for 2-site exchange on most times scales (with the constraint that  $p_A > p_B$ ), named after [Carver and Richards \(1972\)](#). It is selected by setting the model to ‘CR72’. It is the same as the full CR72 model described above, but with the simplification that  $R_{2A}^0 = R_{2B}^0$ . This simplifies the equations to

$$R_{2\text{eff}} = R_2^0 + \frac{k_{\text{ex}}}{2} - \nu_{\text{CPMG}} \cosh^{-1} (D_+ \cosh(\eta_+) - D_- \cosh(\eta_-)), \quad (11.27)$$

where  $D_{\pm}$  and  $\eta_{\pm}$  are unchanged and

$$\Psi = k_{\text{ex}}^2 - \Delta\omega^2, \quad (11.28)$$

$$\zeta = -2\Delta\omega(p_A k_{\text{ex}} - p_B k_{\text{ex}}). \quad (11.29)$$

As mentioned in the ‘CR72 full’ model section, this model is not accurate when the motional process is very slow. In that case please use the ‘TSMFK01’ model in Section 11.3.6 on page 161 instead.

More information about the CR72 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/CR72>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.cr72-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#CR72](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#CR72).

### 11.3.5 The IT99 2-site CPMG model

This is the model for 2-site exchange on all times scales (with the constraint that  $p_A \gg p_B$ ), named after [Ishima and Torchia \(1999\)](#). It is selected by setting the model to ‘IT99’. The equation is:

$$R_{\text{ex}} \simeq \frac{\Phi_{\text{ex}} \tau_{\text{ex}}}{1 + \omega_a^2 \tau_{\text{ex}}^2}, \quad (11.30)$$

$$\omega_a^2 = \sqrt{\omega_{1\text{eff}}^4 + p_A^2 \Delta\omega^4}, \quad (11.31)$$

$$R_{2\text{eff}} = R_2^0 + R_{\text{ex}}. \quad (11.32)$$

The effective rotating frame field for a CPMG-type experiment is given by

$$\omega_{1\text{eff}} = 4\sqrt{3}\nu_{\text{CPMG}}, \quad (11.33)$$

and hence

$$\omega_{1\text{eff}}^4 = 2304\nu_{\text{CPMG}}^4. \quad (11.34)$$

The reference for this equation is:

- Ishima, R. and Torchia, D. A. (1999). Estimating the time scale of chemical exchange of proteins from measurements of transverse relaxation rates in solution. *J. Biomol. NMR*, **14**(4), 369–372. ([10.1023/A:1008324025406](https://doi.org/10.1023/A:1008324025406))

More information about the IT99 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/IT99>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.it99-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#IT99](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#IT99).

### 11.3.6 The TSMFK01 2-site CPMG model

This is the model for 2-site very-slow exchange model for time scales within range of microsecond to second time scale, where  $p_A \gg p_B$ , and named after [Tollinger et al. \(2001\)](#). It is selected by setting the model to ‘TSMFK01’. A particularly interesting feature of the dispersion curves is the damped oscillations, which occur at low CPMG field strengths, and is solely a function of the chemical shift difference between the two sites (i.e. independent of the rate of exchange).

The equation is:

$$R_{2\text{eff}} = R_{2A}^0 + k_{AB} - k_{AB} \frac{\sin(\Delta\omega \cdot \tau_{\text{CPMG}})}{\Delta\omega \cdot \tau_{\text{CPMG}}} \quad (11.35)$$

The reference for this equation is:

- Tollinger, M., Skrynnikov, N. R., Mulder, F. A. A., Forman-Kay, J. D., and Kay, L. E. (2001). Slow dynamics in folded and unfolded states of an sh3 domain. *J. Am. Chem. Soc.*, **123**(46), 11341–11352. ([10.1021/ja011300z](https://doi.org/10.1021/ja011300z))

More information about the TSMFK01 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/TSMFK01>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.tsmfk01-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#TSMFK01](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#TSMFK01).

### 11.3.7 The full B14 2-site CPMG model

This is the model for 2-site exchange exact analytical derivation on all time scales (with the constraint that  $p_A > p_B$ ), named after [Baldwin \(2014\)](#). It is selected by setting the model to ‘B14 full’. The equation is

$$R_{2\text{eff}} = \frac{R_{2A}^0 + R_{2B}^0 + k_{\text{ex}}}{2} - \frac{N_{\text{cyc}}}{T_{\text{relax}}} \cosh^{-1}(\nu_{1c}) - \frac{1}{T_{\text{relax}}} \ln \left( \frac{1+y}{2} + \frac{1-y}{2\sqrt{\nu_{1c}^2 - 1}} (\nu_2 + 2k_{AB}p_D) \right), \quad (11.36a)$$

$$= R_{2\text{eff}}^{\text{CR72}} - \frac{1}{T_{\text{relax}}} \ln \left( \frac{1+y}{2} + \frac{1-y}{2\sqrt{\nu_{1c}^2 - 1}} (\nu_2 + 2k_{AB}p_D) \right), \quad (11.36b)$$

where Appendix 1 in [Baldwin \(2014\)](#) lists the recipe for the exact calculation of  $R_{2\text{eff}}$ . Note that the following definitions are different to those in the original publication, but match both the reference implementation and the relax implementation. The definitions are functionally equivalent. First establish the complex free precession eigenfrequency with

$$\Delta R_2^0 = R_{2A}^0 - R_{2B}^0, \quad (11.37a)$$

$$\alpha_- = \Delta R_2^0 + k_{AB} - k_{BA}, \quad (11.37b)$$

$$\zeta = 2\Delta\omega\alpha_-, \quad (11.37c)$$

$$\Psi = \alpha_-^2 + 4k_{AB}k_{BA} - \Delta\omega^2, \quad (11.37d)$$

$$h_3 = \frac{1}{\sqrt{2}} \sqrt{\Psi + \sqrt{\zeta^2 + \Psi^2}}, \quad (11.37e)$$

$$h_4 = \frac{1}{\sqrt{2}} \sqrt{-\Psi + \sqrt{\zeta^2 + \Psi^2}}. \quad (11.37f)$$

The ground state ensemble evolution frequency  $f_{00}$  expressed in separated real and imaginary components in terms of definitions  $\zeta$ ,  $\Psi$ , and  $h_4$  is

$$f_{00} = \frac{1}{2} (R_{2A}^0 + R_{2B}^0 + k_{\text{ex}}) + \frac{i}{2} (\Delta\omega - h_4). \quad (11.38)$$

Define substitutions for ‘stay’ and ‘swap’ factors as

$$N = h_3 + ih_4, \quad (11.39a)$$

$$NN^* = h_3^2 + h_4^2, \quad (11.39b)$$

$$F_0 = (\Delta\omega^2 + h_3^2) / NN^*, \quad (11.39c)$$

$$F_2 = (\Delta\omega^2 - h_4^2) / NN^*, \quad (11.39d)$$

$$F_1^b = (\Delta\omega + h_4) (\Delta\omega - ih_3) / NN^*, \quad (11.39e)$$

$$F_1^{a+b} = (2\Delta\omega^2 + i\zeta) / NN^*. \quad (11.39f)$$

The weighting factors for frequencies  $E_{0-2}$  emerging from a single CPMG block,  $F_{0-2}$ , are

$$E_0 = 2\tau_{\text{CPMG}} \cdot h_3, \quad (11.40a)$$

$$E_2 = 2\tau_{\text{CPMG}} \cdot h_4, \quad (11.40b)$$

$$E_1 = (h_3 - ih_4) \cdot \tau_{\text{CPMG}}. \quad (11.40c)$$

Here  $\tau_{\text{CPMG}} = 1/4\nu_{\text{CPMG}}$ . The final result, with identities to assist efficient matrix exponentiation optimised for numerical calculation, is

$$\nu_{1c} = F_0 \cosh(E_0) - F_2 \cos(E_2), \quad (11.41a)$$

$$\nu_{1s} = F_0 \sinh(E_0) - \imath F_2 \sin(E_2), \quad (11.41b)$$

$$\nu_3 = \sqrt{\nu_{1c}^2 - 1}, \quad (11.41c)$$

$$\nu_4 = F_1^b(-\alpha_- - h_3) + \imath F_1^b(\Delta\omega - h_4), \quad (11.41d)$$

$$\nu_5 = (-\Delta R_2^0 + k_{\text{ex}} + \imath\Delta\omega) \nu_{1s} + 2 \left( \nu_4 + k_{\text{AB}} F_1^{a+b} \right) \sinh(E_1), \quad (11.41e)$$

$$y = \left( \frac{\nu_{1c} - \nu_3}{\nu_{1c} + \nu_3} \right)^{N_{\text{cyc}}}, \quad (11.41f)$$

$$T = \frac{1+y}{2} + \frac{1-y}{2} \frac{\nu_5}{\nu_3 N}, \quad (11.41g)$$

$$R_{2\text{eff}}^{\text{CR72}} = \frac{R_{2A}^0 + R_{2B}^0 + k_{\text{ex}}}{2} - \frac{N_{\text{cyc}}}{T_{\text{relax}}} \operatorname{arccosh}(\Re(\nu_{1c})), \quad (11.41h)$$

$$R_{2\text{eff}} = R_{2\text{eff}}^{\text{CR72}} - \frac{1}{T_{\text{relax}}} \log(\Re(T)). \quad (11.41i)$$

The advantage of these equations is that you will always obtain the correct answer provided you have 2-site exchange, in-phase magnetisation and on-resonance pulses.

The term  $p_D$  is based on product of the off diagonal elements in the CPMG propagator, see supplementary Section 3 (Baldwin, 2014).

It is interesting to consider the region of validity of the Carver and Richards result. The two results are equal when the correction is zero, which is true when

$$\sqrt{\nu_{1c}^2 - 1} \approx \nu_2 + 2k_{\text{AB}}p_D. \quad (11.42)$$

This occurs when  $k_{\text{AB}}p_D$  tends to zero, and so  $\nu_2 = \nu_3$ . Setting  $k_{\text{AB}}p_D$  to zero amounts to neglecting magnetisation that starts on the ground state ensemble and end on the excited state ensemble and vice versa. This will be a good approximation when  $p_A \gg p_B$ . In practise, significant deviations from the Carver and Richards equation can be incurred if  $p_B > 1\%$ . Incorporation of the correction term results in an improved description of the CPMG experiment over Carver and Richards (1972).

The reference for this equation is:

- Baldwin, A. J. (2014). An exact solution for  $R_{2,\text{eff}}$  in CPMG experiments in the case of two site chemical exchange. *J. Magn. Reson.*, **244**, 114–124. ([10.1016/j.jmr.2014.02.023](https://doi.org/10.1016/j.jmr.2014.02.023))

More information about the B14 full model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/B14\\_full](http://wiki.nmr-relax.com/B14_full),
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.b14-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#B14\\_full](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#B14_full).

### 11.3.8 The reduced B14 2-site CPMG model

This is the model for 2-site exchange exact analytical derivation on all time scales (with the constraint that  $p_A > p_B$ ), named after Baldwin (2014). It is selected by setting the model to ‘B14’. It is the same as the full B14 model described above, but with the simplification that  $R_{2A}^0 = R_{2B}^0$ .

More information about the B14 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/B14>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.b14-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#B14](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#B14).

## 11.4 The numeric CPMG models

These are the numeric models designed for a single data type – the single quantum CPMG-type experiment.

### 11.4.1 The NS 2-site expanded CPMG model

This is the numerical model for 2-site exchange expanded using Maple by Nikolai Skrynnikov. It is selected by setting the model to ‘NS CPMG 2-site expanded’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

This model will give the same results as the other numerical solutions whereby  $R_{2A}^0 = R_{2B}^0$ . The following is the set of equations of the expansion used in relax. It has been modified from the original for speed. See the `lib.dispersion.ns_cpmg_2site_expanded` module for more details including the original code. Further simplifications can be found in the code.

$$t_3 = \tau, \tag{11.43.1}$$

$$t_4 = t_3 \Delta\omega, \tag{11.43.2}$$

$$t_5 = k_{BA}^2, \tag{11.43.3}$$

$$t_8 = 2t_4 k_{BA}, \tag{11.43.4}$$

$$t_{10} = 2k_{BA} k_{AB}, \tag{11.43.5}$$

$$t_{11} = \Delta\omega^2, \tag{11.43.6}$$

$$t_{14} = 2t_4 k_{AB}, \tag{11.43.7}$$

$$t_{15} = k_{AB}^2, \tag{11.43.8}$$

$$t_{17} = \sqrt{t_5 - t_8 + t_{10} - t_{11} + t_{14} + t_{15}}, \tag{11.43.9}$$

$$t_{21} = \exp \left( \frac{(-k_{BA} + t_4 - k_{AB} + t_{17})\tau_{CPMG}}{2} \right), \quad (11.43.10)$$

$$t_{22} = \frac{1}{t_{17}}, \quad (11.43.11)$$

$$t_{28} = \exp \left( \frac{(-k_{BA} + t_4 - k_{AB} - t_{17})\tau_{CPMG}}{2} \right), \quad (11.43.12)$$

$$t_{31} = t_{22}k_{AB}(t_{21} - t_{28}), \quad (11.43.13)$$

$$t_{33} = \sqrt{t_5 + t_8 + t_{10} - t_{11} - t_{14} + t_{15}}, \quad (11.43.14)$$

$$t_{34} = k_{BA} + t_4 - k_{AB} + t_{33}, \quad (11.43.15)$$

$$t_{37} = \exp((-k_{BA} - t_4 - k_{AB} + t_{33})\tau_{CPMG}), \quad (11.43.16)$$

$$t_{39} = \frac{1}{t_{33}}, \quad (11.43.17)$$

$$t_{41} = k_{BA} + t_4 - k_{AB} - t_{33}, \quad (11.43.18)$$

$$t_{44} = \exp((-k_{BA} - t_4 - k_{AB} - t_{33})\tau_{CPMG}), \quad (11.43.19)$$

$$t_{47} = \frac{t_{39}}{2} (t_{34}t_{37} - t_{41}t_{44}), \quad (11.43.20)$$

$$t_{49} = k_{BA} - t_4 - k_{AB} - t_{17}, \quad (11.43.21)$$

$$t_{51} = t_{21}t_{49}t_{22}, \quad (11.43.22)$$

$$t_{52} = k_{BA} - t_4 - k_{AB} + t_{17}, \quad (11.43.23)$$

$$t_{54} = t_{28}t_{52}t_{22}, \quad (11.43.24)$$

$$t_{55} = t_{54} - t_{51}, \quad (11.43.25)$$

$$t_{60} = \frac{1}{2}t_{39}k_{AB}(t_{37} - t_{44}), \quad (11.43.26)$$

$$t_{62} = t_{31}t_{47} + t_{55}t_{60}, \quad (11.43.27)$$

$$t_{63} = \frac{1}{k_{AB}}, \quad (11.43.28)$$

$$t_{68} = \frac{t_{63}}{2} (t_{49}t_{54} - t_{52}t_{51}), \quad (11.43.29)$$

$$t_{69} = \frac{t_{62}t_{68}}{2}, \quad (11.43.30)$$

$$t_{72} = t_{37}t_{41}t_{39}, \quad (11.43.31)$$

$$t_{76} = t_{44}t_{34}t_{39}, \quad (11.43.32)$$

$$t_{78} = \frac{t_{63}}{2} (t_{41}t_{76} - t_{34}t_{72}), \quad (11.43.33)$$

$$t_{80} = \frac{1}{2}(t_{76} - t_{72}), \quad (11.43.34)$$

$$t_{82} = \frac{1}{2}(t_{31}t_{78} + t_{55}t_{80}), \quad (11.43.35)$$

$$t_{83} = \frac{t_{82}t_{55}}{2}, \quad (11.43.36)$$

$$t_{88} = \frac{t_{22}}{2} (t_{52}t_{21} - t_{49}t_{28}), \quad (11.43.37)$$

$$t_{91} = t_{88}t_{47} + t_{68}t_{60}, \quad (11.43.38)$$

$$t_{92} = t_{91}t_{88}, \quad (11.43.39)$$

$$t_{95} = \frac{1}{2}(t_{88}t_{78} + t_{68}t_{80}), \quad (11.43.40)$$

$$t_{96} = t_{95}t_{31}, \quad (11.43.41)$$

$$t_{97} = t_{69} + t_{83}, \quad (11.43.42)$$

$$t_{98} = t_{97}^2, \quad (11.43.43)$$

$$t_{99} = t_{92} + t_{96}, \quad (11.43.44)$$

$$t_{102} = t_{99}^2, \quad (11.43.45)$$

$$t_{108} = t_{62}t_{88} + t_{82}t_{31}, \quad (11.43.46)$$

$$t_{112} = \sqrt{t_{98} - 2t_{99}t_{97} + t_{102} + 2(t_{91}t_{68} + t_{95}t_{55})t_{108}}, \quad (11.43.47)$$

$$t_{113} = t_{97} - t_{99} - t_{112}, \quad (11.43.48)$$

$$t_{115} = n_{\text{CPMG}}, \quad (11.43.49)$$

$$t_{116} = \left( \frac{t_{97} + t_{99} + t_{112}}{2} \right)^{t_{115}}, \quad (11.43.50)$$

$$t_{118} = \frac{1}{t_{112}}, \quad (11.43.51)$$

$$t_{120} = t_{97} - t_{99} + t_{112}, \quad (11.43.52)$$

$$t_{122} = \left( \frac{t_{97} + t_{99} - t_{112}}{2} \right)^{t_{115}}, \quad (11.43.53)$$

$$t_{127} = \frac{1}{2t_{108}}, \quad (11.43.54)$$

$$t_{139} = \frac{1}{2(k_{\text{AB}} + k_{\text{BA}})} \left[ (t_{120}t_{122} - t_{113}t_{116})t_{118}k_{\text{BA}} + (t_{120}t_{122} - t_{116}t_{120})t_{113}t_{118}t_{127}k_{\text{AB}} \right]. \quad (11.43.55)$$

The relative peak intensities, magnetisation, and effective  $R_2$  relaxation rate are calculated as

$$I_0 = p_{\text{A}}, \quad (11.44a)$$

$$I_1 = \Re(t_{139}) \exp(-T_{\text{relax}}R_2^0), \quad (11.44b)$$

$$M_x = I_1/I_0, \quad (11.44c)$$

$$R_{2\text{eff}} = -\frac{1}{T_{\text{relax}}} \cdot \ln(M_x). \quad (11.44d)$$

In these equations  $\tau_{\text{CPMG}}$  and  $n_{\text{CPMG}}$  are numpy arrays and hence  $t_{139}$  is also a numpy array. This avoids a Python loop over the dispersion points until the very end of the calculation, required to populate the  $R_{2\text{eff}}$  data structure, resulting in very fast calculations.

The reference for this model is:

- Tollinger, M., Skrynnikov, N. R., Mulder, F. A. A., Forman-Kay, J. D., and Kay, L. E. (2001). Slow dynamics in folded and unfolded states of an sh3 domain. *J. Am. Chem. Soc.*, **123**(46), 11341–11352. ([10.1021/ja011300z](https://doi.org/10.1021/ja011300z))

More information about the NS CPMG 2-site expanded model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_CPMG\\_2-site\\_expanded](http://wiki.nmr-relax.com/NS_CPMG_2-site_expanded),



- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_cpmg\\_2site\\_expanded-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_cpmg_2site_expanded-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_CPMG\\_2-site\\_expanded](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_CPMG_2-site_expanded).

#### 11.4.2 The full NS 2-site 3D CPMG model

This is the numerical model for 2-site exchange using 3D magnetisation vectors. It is selected by setting the model to ‘NS CPMG 2-site 3D full’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

More information about the NS CPMG 2-site 3D full model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_CPMG\\_2-site\\_3D\\_full](http://wiki.nmr-relax.com/NS_CPMG_2-site_3D_full),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_cpmg\\_2site\\_3d-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_cpmg_2site_3d-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_CPMG\\_2-site\\_3D\\_full](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_CPMG_2-site_3D_full).

#### 11.4.3 The reduced NS 2-site 3D CPMG model

This is the numerical model for 2-site exchange using 3D magnetisation vectors, whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It is selected by setting the model to ‘NS CPMG 2-site 3D’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

More information about the NS CPMG 2-site 3D model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_CPMG\\_2-site\\_3D](http://wiki.nmr-relax.com/NS_CPMG_2-site_3D),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_cpmg\\_2site\\_3d-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_cpmg_2site_3d-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_CPMG\\_2-site\\_3D](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_CPMG_2-site_3D).

#### 11.4.4 The full NS 2-site star CPMG model

This is the numerical model for 2-site exchange using complex conjugate matrices. It is selected by setting the model to ‘NS CPMG 2-site star full’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

More information about the NS CPMG 2-site star full model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_CPMG\\_2-site\\_star\\_full](http://wiki.nmr-relax.com/NS_CPMG_2-site_star_full),

- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_cpmg\\_2site\\_star-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_cpmg_2site_star-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_CPMG\\_2-site\\_star\\_full](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_CPMG_2-site_star_full).

### 11.4.5 The reduced NS 2-site star CPMG model

This is the numerical model for 2-site exchange using complex conjugate matrices, whereby the simplification  $R_{2A}^0 = R_{2B}^0$  is assumed. It is selected by setting the model to ‘NS CPMG 2-site star’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

More information about the NS CPMG 2-site star model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_CPMG\\_2-site\\_star](http://wiki.nmr-relax.com/NS_CPMG_2-site_star),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_cpmg\\_2site\\_star-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_cpmg_2site_star-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_CPMG\\_2-site\\_star](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_CPMG_2-site_star).

## 11.5 The analytic MMQ CPMG models

These are the analytic models designed for handling combined proton-heteronuclear SQ, ZQ, DQ, and MQ CPMG-type experiments. This data combination is labelled as multiple multiple quantum data or MMQ.

### 11.5.1 The MMQ CR72 model

This is the analytic CR72 model for 2-site exchange on most times scales (Section 11.3.4 on page 160) extended for multiple types of multiple quantum data (MMQ) by Korzhnev et al. (2004a). It is selected by setting the model to ‘MMQ CR72’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces. The equation for the exchange process is

$$R_{2\text{eff}} = \Re(\lambda_1) - \frac{1}{T_{\text{relax}}} \ln(Q), \quad (11.45)$$

where

$$\lambda_1 = R_{2,\text{MQ}}^0 + \frac{k_{\text{ex}}}{2} - \nu_{\text{CPMG}} \cosh^{-1} (D_+ \cosh(\eta_+) - D_- \cosh(\eta_-)), \quad (11.46)$$

$$D_{\pm} = \frac{1}{2} \left( \pm 1 + \frac{\Psi + 2\Delta\omega^2}{\sqrt{\Psi^2 + \zeta^2}} \right), \quad (11.47)$$

$$\eta_{\pm} = 2^{\frac{2}{3}} \frac{1}{\nu_{\text{CPMG}}} \left( \pm \Psi + \sqrt{\Psi^2 + \zeta^2} \right)^{\frac{1}{2}}, \quad (11.48)$$

$$\Psi = (i\Delta\omega^{\text{H}} + p_{\text{A}}k_{\text{ex}} - p_{\text{B}}k_{\text{ex}})^2 - \Delta\omega^2 + 4p_{\text{A}}p_{\text{B}}k_{\text{ex}}^2, \quad (11.49)$$

$$\zeta = -2\Delta\omega (i\Delta\omega^{\text{H}} + p_{\text{A}}k_{\text{ex}} - p_{\text{B}}k_{\text{ex}}), \quad (11.50)$$

and where

$$Q = \Re \left( 1 - m_{D+}^2 + m_{D+}m_{Z+} - m_{Z+}^2 + \frac{m_{D+} + m_{Z+}}{2} \sqrt{\frac{p_{\text{B}}}{p_{\text{A}}}} \right), \quad (11.51)$$

and

$$m_{D\pm} = \pm \frac{i k_{\text{ex}} \sqrt{p_{\text{A}} p_{\text{B}}}}{d_{\pm} z_{\pm}} \left( z_{\pm} + 2\Delta\omega \frac{\sin(z_{\pm} \delta)}{\sin((d_{\pm} + z_{\pm}) \delta)} \right), \quad (11.52)$$

$$m_{Z\mp} = \pm \frac{i k_{\text{ex}} \sqrt{p_{\text{A}} p_{\text{B}}}}{d_{\pm} z_{\pm}} \left( d_{\pm} - 2\Delta\omega \frac{\sin(d_{\pm} \delta)}{\sin((d_{\pm} + z_{\pm}) \delta)} \right), \quad (11.53)$$

and

$$d_{\pm} = (\Delta\omega^{\text{H}} + \Delta\omega) \pm i k_{\text{ex}}, \quad (11.54)$$

$$z_{\pm} = (\Delta\omega^{\text{H}} - \Delta\omega) \pm i k_{\text{ex}}. \quad (11.55)$$

The symbol  $\delta$  is half of  $\tau_{\text{CPMG}}$  or

$$\delta = \frac{1}{4\nu_{\text{CPMG}}}. \quad (11.56)$$

The references for this model are:

- Korzhnev, D. M., Kloiber, K., Kanelis, V., Tugarinov, V., and Kay, L. E. (2004a). Probing slow dynamics in high molecular weight proteins by methyl-TROSY NMR spectroscopy: application to a 723-residue enzyme. *J. Am. Chem. Soc.*, **126**(12), 3964–3973. ([10.1021/ja039587i](https://doi.org/10.1021/ja039587i))
- Korzhnev, D. M., Kloiber, K., and Kay, L. E. (2004b). Multiple-quantum relaxation dispersion NMR spectroscopy probing millisecond time-scale dynamics in proteins: theory and application. *J. Am. Chem. Soc.*, **126**(23), 7320–7329. ([10.1021/ja049968b](https://doi.org/10.1021/ja049968b))
- Korzhnev, D. M., Neudecker, P., Mittermaier, A., Orekhov, V. Y., and Kay, L. E. (2005a). Multiple-site exchange in proteins studied with a suite of six NMR relaxation dispersion experiments: an application to the folding of a Fyn SH3 domain mutant. *J. Am. Chem. Soc.*, **127**(44), 15602–15611. ([10.1021/ja054550e](https://doi.org/10.1021/ja054550e))

More information about the MMQ CR72 model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/MMQ\\_CR72](http://wiki.nmr-relax.com/MMQ_CR72),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.mmq\\_cr72-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.mmq_cr72-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#MMQ\\_CR72](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#MMQ_CR72).

## 11.6 The numeric MMQ CPMG models

These are the numeric models designed for handling combined proton-heteronuclear SQ, ZQ, DQ, and MQ CPMG-type experiments. This data combination is labelled as multiple multiple quantum data or MMQ.

### 11.6.1 The NS MMQ 2-site model

This is the numerical model for 2-site exchange for proton-heteronuclear SQ, ZQ, DQ and MQ CPMG data, as derived in (Korzhnev et al., 2004a,b, 2005a). It is selected by setting the model to ‘NS MMQ 2-site’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces. Different sets of equations are used for the different data types.

#### The SQ, ZD and DQ equations

The basic evolution matrices for single, zero and double quantum CPMG-type data for this model are

$$R_{2\text{eff}} = -\frac{1}{T_{\text{relax}}} \log \frac{\mathbf{M}_A(T_{\text{relax}})}{\mathbf{M}_A(0)}, \quad (11.57)$$

where  $\mathbf{M}_A(0)$  is proportional to the vector  $[p_A, p_B]^T$  and

$$\mathbf{M}_A(T_{\text{relax}}) = (\mathbf{A}_{\pm} \mathbf{A}_{\mp} \mathbf{A}_{\mp} \mathbf{A}_{\pm})^n \mathbf{M}_A(0) \quad (11.58)$$

The evolution matrix  $\mathbf{A}$  is defined as

$$\mathbf{A}_{\pm} = e^{\mathbf{a}_{\pm} \cdot \tau_{\text{CPMG}}}, \quad (11.59)$$

where

$$\mathbf{a}_{\pm} = \begin{pmatrix} -k_{AB} - R_{2A}^0 & k_{BA} \\ k_{AB} & -k_{BA} \pm i\Delta\omega - R_{2B}^0 \end{pmatrix}. \quad (11.60)$$

For different data types  $\Delta\omega$  is defined as:  $\Delta\omega$  ( $^{15}\text{N}$  SQ-type data);  $\Delta\omega^{\text{H}}$  ( $^1\text{H}$  SQ-type data);  $\Delta\omega^{\text{H}} - \Delta\omega$  (ZQ-type data); and  $\Delta\omega^{\text{H}} + \Delta\omega$  (DQ-type data).

### The MQ equations

The equation for the exchange process for multiple quantum CPMG-type data is

$$R_{2\text{eff}} = -\frac{1}{T} \log \left\{ \text{Re} \left[ \frac{0.5}{p_A} (1 \quad 0) \cdot (\mathbf{A}\mathbf{B} + \mathbf{C}\mathbf{D}) \cdot \begin{pmatrix} p_A \\ p_B \end{pmatrix} \right] \right\}, \quad (11.61)$$

where  $T$  is the constant time interval, and the matrices  $\mathbf{A}$ ,  $\mathbf{B}$ ,  $\mathbf{C}$ , and  $\mathbf{D}$  are dually defined. When  $n$  is even, they are defined as

$$\mathbf{A} = (\mathbf{M}_1 \mathbf{M}_2 \mathbf{M}_2 \mathbf{M}_1)^{\frac{n}{2}}, \quad (11.62a)$$

$$\mathbf{B} = (\mathbf{M}_2^* \mathbf{M}_1^* \mathbf{M}_1^* \mathbf{M}_2^*)^{\frac{n}{2}}, \quad (11.62b)$$

$$\mathbf{C} = (\mathbf{M}_2 \mathbf{M}_1 \mathbf{M}_1 \mathbf{M}_2)^{\frac{n}{2}}, \quad (11.62c)$$

$$\mathbf{D} = (\mathbf{M}_1^* \mathbf{M}_2^* \mathbf{M}_2^* \mathbf{M}_1^*)^{\frac{n}{2}}. \quad (11.62d)$$

When  $n$  is odd, they are defined as

$$\mathbf{A} = (\mathbf{M}_1 \mathbf{M}_2 \mathbf{M}_2 \mathbf{M}_1)^{\frac{n-1}{2}} \mathbf{M}_1 \mathbf{M}_2, \quad (11.63a)$$

$$\mathbf{B} = (\mathbf{M}_1^* \mathbf{M}_2^* \mathbf{M}_2^* \mathbf{M}_1^*)^{\frac{n-1}{2}} \mathbf{M}_1^* \mathbf{M}_2^*, \quad (11.63b)$$

$$\mathbf{C} = (\mathbf{M}_2 \mathbf{M}_1 \mathbf{M}_1 \mathbf{M}_2)^{\frac{n-1}{2}} \mathbf{M}_2 \mathbf{M}_1, \quad (11.63c)$$

$$\mathbf{D} = (\mathbf{M}_2^* \mathbf{M}_1^* \mathbf{M}_1^* \mathbf{M}_2^*)^{\frac{n-1}{2}} \mathbf{M}_2^* \mathbf{M}_1^*. \quad (11.63d)$$

When  $n$  is zero, to avoid matrix powers of zero they are defined as

$$\mathbf{A} = \mathbf{M}_1 \mathbf{M}_2, \quad (11.64a)$$

$$\mathbf{B} = \mathbf{M}_1^* \mathbf{M}_2^*, \quad (11.64b)$$

$$\mathbf{C} = \mathbf{M}_2 \mathbf{M}_1, \quad (11.64c)$$

$$\mathbf{D} = \mathbf{M}_2^* \mathbf{M}_1^*. \quad (11.64d)$$

The  $\mathbf{M}$  matrices are defined as:

$$\mathbf{M}_j = \exp(\mathbf{m}_j \delta), \quad (11.65)$$

where  $2\delta$  is the spacing between successive  $180^\circ$  pulses and where The references for this model are:

$$\mathbf{m}_1 = \begin{pmatrix} -p_B k_{\text{ex}} - R_{2,\text{DQ}}^A & p_A k_{\text{ex}} \\ p_B k_{\text{ex}} & -p_A k_{\text{ex}} - i(\Delta\omega^H + \Delta\omega) - R_{2,\text{DQ}}^B \end{pmatrix}, \quad (11.66a)$$

$$\mathbf{m}_2 = \begin{pmatrix} -p_B k_{\text{ex}} - R_{2,\text{ZQ}}^A & p_A k_{\text{ex}} \\ p_B k_{\text{ex}} & -p_A k_{\text{ex}} - i(\Delta\omega^H - \Delta\omega) - R_{2,\text{ZQ}}^B \end{pmatrix}. \quad (11.66b)$$

For the model it is assumed that  $R_{2,\text{DQ}}^A = R_{2,\text{ZQ}}^A = R_{2A}^0$  and  $R_{2,\text{DQ}}^B = R_{2,\text{ZQ}}^B = R_{2B}^0$ . The references for this model are:

- Korzhnev, D. M., Kloiber, K., Kanelis, V., Tugarinov, V., and Kay, L. E. (2004a). Probing slow dynamics in high molecular weight proteins by methyl-TROSY NMR spectroscopy: application to a 723-residue enzyme. *J. Am. Chem. Soc.*, **126**(12), 3964–3973. ([10.1021/ja039587i](https://doi.org/10.1021/ja039587i))
- Korzhnev, D. M., Kloiber, K., and Kay, L. E. (2004b). Multiple-quantum relaxation dispersion NMR spectroscopy probing millisecond time-scale dynamics in proteins: theory and application. *J. Am. Chem. Soc.*, **126**(23), 7320–7329. ([10.1021/ja049968b](https://doi.org/10.1021/ja049968b))
- Korzhnev, D. M., Neudecker, P., Mittermaier, A., Orekhov, V. Y., and Kay, L. E. (2005a). Multiple-site exchange in proteins studied with a suite of six NMR relaxation dispersion experiments: an application to the folding of a Fyn SH3 domain mutant. *J. Am. Chem. Soc.*, **127**(44), 15602–15611. ([10.1021/ja054550e](https://doi.org/10.1021/ja054550e))

More information about the NS MMQ 2-site model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_MMQ\\_2-site](http://wiki.nmr-relax.com/NS_MMQ_2-site),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_mmq\\_2site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_mmq_2site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_MMQ\\_2-site](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_MMQ_2-site).

### 11.6.2 The NS MMQ 3-site linear model

This is the numerical model for 3-site exchange for proton-heteronuclear SQ, ZQ, DQ and MQ CPMG data, as derived in (Korzhnev et al., 2004a,b, 2005a). As this model is linear, the assumption that  $k_{AC} = k_{CA} = 0$  has been made. To simplify the optimisation space for the model, the assumption  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0 = R_2^0$  has also been made.

#### The SQ, ZD and DQ equations

The basic evolution matrices for single, zero and double quantum CPMG-type data for this model are

$$\mathbf{A}_{\pm} = e^{\mathbf{a}_{\pm} \cdot \tau_{\text{CPMG}}}, \quad (11.67)$$

where

$$\mathbf{a}_{\pm} = \begin{pmatrix} -k_{AB} & k_{BA} & 0 \\ k_{AB} & -k_{BA} - k_{BC} \pm i\Delta\omega_{AB} & k_{CB} \\ 0 & k_{BC} & -k_{CB} \pm i\Delta\omega_{AC} \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}. \quad (11.68)$$

### The MQ equations

The formulae for multiple quantum CPMG-type data are the same as for the ‘NS MMQ 2-site’ model except for the  $R_{2\text{eff}}$  calculation and the  $\mathbf{m}_j$  matrices. The rate is calculated as

$$R_{2\text{eff}} = -\frac{1}{T} \log \left\{ \text{Re} \left[ \frac{0.5}{p_A} (1 \ 0 \ 0) \cdot (\mathbf{A}\mathbf{B} + \mathbf{C}\mathbf{D}) \cdot \begin{pmatrix} p_A \\ p_B \\ p_C \end{pmatrix} \right] \right\}. \quad (11.69)$$

The  $\mathbf{m}_j$  matrices are

$$\mathbf{m}_1 = \begin{pmatrix} -k_{AB} & k_{BA} & 0 \\ k_{AB} & -k_{BA} - k_{BC} - i(\Delta\omega_{AB}^H + \Delta\omega_{AB}) & k_{CB} \\ 0 & k_{BC} & -k_{CB} - i(\Delta\omega_{AC}^H + \Delta\omega_{AC}) \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}, \quad (11.70a)$$

$$\mathbf{m}_2 = \begin{pmatrix} -k_{AB} & k_{BA} & 0 \\ k_{AB} & -k_{BA} - k_{BC} - i(\Delta\omega_{AB}^H - \Delta\omega_{AB}) & k_{CB} \\ 0 & k_{BC} & -k_{CB} - i(\Delta\omega_{AC}^H - \Delta\omega_{AC}) \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}. \quad (11.70b)$$

For the model, the assumption  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0 = R_2^0$  is made.

More information about the NS MMQ 3-site linear model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_MMQ\\_3-site\\_linear](http://wiki.nmr-relax.com/NS_MMQ_3-site_linear),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_mmq\\_3site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_mmq_3site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_MMQ\\_3-site\\_linear](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_MMQ_3-site_linear).

### 11.6.3 The NS MMQ 3-site model

This is the numerical model for 3-site exchange for proton-heteronuclear SQ, ZQ, DQ and MQ CPMG data, as derived in (Korzhnev et al., 2004a,b, 2005a). However it has been extended to allow the  $A \leftrightarrow C$  transition. To simplify the optimisation space for the model as in the ‘NS MMQ 3-site linear’ model, the assumption  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0 = R_2^0$  has been made.

### The SQ, ZD and DQ equations

The basic evolution matrices for single, zero and double quantum CPMG-type data for this model are

$$\mathbf{A}_{\pm} = e^{\mathbf{a}_{\pm} \cdot \tau_{\text{CPMG}}}, \quad (11.71)$$

where

$$\mathbf{a}_{\pm} = \begin{pmatrix} -k_{AB} - k_{AC} & k_{BA} & k_{CA} \\ k_{AB} & -k_{BA} - k_{BC} \pm i\Delta\omega_{AB} & k_{CB} \\ k_{AC} & k_{BC} & -k_{CB} - k_{CA} \pm i\Delta\omega_{AC} \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}. \quad (11.72)$$

### The MQ equations

The  $\mathbf{m}_j$  matrices for this model are

$$\mathbf{m}_1 = \begin{pmatrix} -k_{AB} - k_{AC} & k_{BA} & k_{CA} \\ k_{AB} & -k_{BA} - k_{BC} - i(\Delta\omega_{AB}^H + \Delta\omega_{AB}) & k_{CB} \\ k_{AC} & k_{BC} & -k_{CB} - k_{CA} - i(\Delta\omega_{AC}^H + \Delta\omega_{AC}) \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}, \quad (11.73a)$$

$$\mathbf{m}_2 = \begin{pmatrix} -k_{AB} - k_{AC} & k_{BA} & k_{CA} \\ k_{AB} & -k_{BA} - k_{BC} - i(\Delta\omega_{AB}^H - \Delta\omega_{AB}) & k_{CB} \\ k_{AC} & k_{BC} & -k_{CB} - k_{CA} - i(\Delta\omega_{AC}^H - \Delta\omega_{AC}) \end{pmatrix} - \begin{pmatrix} R_{2A}^0 & 0 & 0 \\ 0 & R_{2B}^0 & 0 \\ 0 & 0 & R_{2C}^0 \end{pmatrix}. \quad (11.73b)$$

More information about the NS MMQ 3-site model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_MMQ\\_3-site](http://wiki.nmr-relax.com/NS_MMQ_3-site),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_mmq\\_3site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_mmq_3site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_MMQ\\_3-site](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_MMQ_3-site).

## 11.7 The analytic $R_{1\rho}$ models

These are the analytic models designed for  $R_{1\rho}$ -type experiments.



### 11.7.1 The M61 2-site fast exchange $R_{1\rho}$ model

This is the model for 2-site fast exchange for on-resonance  $R_{1\rho}$ -type data. It is selected by setting the model to ‘M61’, here named after [Meiboom \(1961\)](#). The equation for the exchange process is

$$R_{1\rho} = R'_{1\rho} + \frac{\Phi_{\text{ex}} k_{\text{ex}}}{k_{\text{ex}}^2 + \omega_e^2}. \quad (11.74)$$

The reference for this equation is:

- Meiboom, S. (1961). Nuclear magnetic resonance study of proton transfer in water. *J. Chem. Phys.*, **34**(2), 375–388. ([10.1063/1.1700960](#))

More information about the M61 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/M61>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.m61-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#M61](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#M61).

### 11.7.2 The M61 skew 2-site fast exchange $R_{1\rho}$ model

This is the second model for 2-site fast exchange for on-resonance  $R_{1\rho}$ -type data from [Meiboom \(1961\)](#). It is selected by setting the model to ‘M61 skew’. The equation for the exchange process is

$$R_{1\rho} = R'_{1\rho} + \frac{p_A^2 p_B \Delta\omega^2 k_{\text{ex}}}{k_{\text{ex}}^2 + p_A^2 \Delta\omega^2 + \omega_1^2}. \quad (11.75)$$

Care must be taken as this model appears to have infinite lines of solutions –  $p_A$  and  $\Delta\omega$  are convoluted. Hence this model is disabled in the dispersion auto-analysis.

More information about the M61 skew model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/M61\\_skew](http://wiki.nmr-relax.com/M61_skew),
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.m61b-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#M61\\_skew](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#M61_skew).

### 11.7.3 The DPL94 2-site fast exchange $R_{1\rho}$ model

This is the model for 2-site fast exchange for  $R_{1\rho}$ -type data. It is selected by setting the model to ‘DPL94’, here named after [Davis et al. \(1994\)](#). It extends the [Meiboom \(1961\)](#) model to off-resonance data. The model collapses to the M61 model for on-resonance data. The equation for the exchange process is

$$R_{1\rho} = R_1 \cos^2 \theta + \left( R'_{1\rho} + \frac{\Phi_{\text{ex}} k_{\text{ex}}}{k_{\text{ex}}^2 + \omega_e^2} \right) \sin^2 \theta, \quad (11.76)$$

where  $\theta$  is the rotating frame tilt angle. The reference for this equation is:

- Davis, D. G., Perlman, M. E., and London, R. E. (1994). Direct measurements of the dissociation-rate constant for inhibitor-enzyme complexes via the T1rho and T2 (CPMG) methods. *J. Magn. Reson.*, **104**(3), 266–275. ([10.1006/jmrb.1994.1084](#))

More information about the DPL94 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/DPL94>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.dpl94-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#DPL94](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#DPL94).

### 11.7.4 The TP02 2-site exchange $R_{1\rho}$ model

This is the model for 2-site exchange for off-resonance  $R_{1\rho}$ -type data from [Trott and Palmer \(2002\)](#). It is selected by setting the model to ‘TP02’. The equation for the exchange process is

$$R_{1\rho} = R_1 \cos^2 \theta + R'_{1\rho} \sin^2 \theta + \frac{\sin^2 \theta p_A p_B \Delta \omega^2 k_{\text{ex}}}{\omega_{\text{Aeff}}^2 \omega_{\text{Beff}}^2 / \omega_{\text{eff}}^2 + k_{\text{ex}}^2}, \quad (11.77)$$

in which

$$\delta_A = \omega_A - \omega_{\text{rf}}, \quad (11.78a)$$

$$\delta_B = \omega_B - \omega_{\text{rf}}, \quad (11.78b)$$

$$\bar{\omega} = p_A \omega_A + p_B \omega_B, \quad (11.78c)$$

$$\bar{\Omega} = \bar{\omega} - \omega_{\text{rf}}, \quad (11.78d)$$

$$\omega_{\text{Aeff}}^2 = \omega_1^2 + \delta_A^2, \quad (11.78e)$$

$$\omega_{\text{Beff}}^2 = \omega_1^2 + \delta_B^2, \quad (11.78f)$$

$$\omega_{\text{eff}}^2 = \omega_1^2 + \bar{\Omega}^2, \quad (11.78g)$$

$$\theta = \arctan \left( \frac{\omega_1}{\bar{\Omega}} \right). \quad (11.78h)$$

The equation is accurate only when populations are highly skewed with  $p_A \gg p_B$ . And it is valid only for exchange processes which are not fast. Note that this model has been superseded by the ‘TAP03’ and ‘MP05’ models. The reference for this equation is:

- Trott, O. and Palmer, 3rd, A. G. (2002). R1rho relaxation outside of the fast-exchange limit. *J. Magn. Reson.*, **154**(1), 157–160. ([10.1006/jmre.2001.2466](https://doi.org/10.1006/jmre.2001.2466))

More information about the TP02 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/TP02>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.tp02-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#TP02](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#TP02).

### 11.7.5 The TAP03 2-site exchange $R_{1\rho}$ model

This is the model for 2-site exchange for off-resonance  $R_{1\rho}$ -type data from [Trott et al. \(2003\)](#). It is selected by setting the model to ‘TAP03’. The equation for the exchange process is

$$R_{1\rho} = R_1 \cos^2 \theta + R'_1 \sin^2 \theta + \left( \frac{1}{\gamma} \right) \frac{\sin^2 \hat{\theta} p_A p_B \Delta \omega^2 k_{\text{ex}}}{\hat{\omega}_{\text{Aeff}}^2 \hat{\omega}_{\text{Beff}}^2 / \hat{\omega}_{\text{eff}}^2 + k_{\text{ex}}^2 - 2 \sin^2 \hat{\theta} p_A p_B \Delta \omega^2 + (1 - \gamma) \omega_1^2}, \quad (11.79)$$

in which, in addition to those parameters defined above for the ‘TP02’ model,

$$\sigma = p_B \delta_A + p_A \delta_B, \quad (11.80a)$$

$$\gamma = 1 - p_A p_B \Delta \omega^2 \frac{\sigma^2 - k_{\text{ex}}^2 + \omega_1^2}{(\sigma^2 + k_{\text{ex}}^2 + \omega_1^2)^2}, \quad (11.80b)$$

$$\hat{\omega}_{\text{Aeff}}^2 = \gamma \omega_1^2 + \delta_A^2, \quad (11.80c)$$

$$\hat{\omega}_{\text{Beff}}^2 = \gamma \omega_1^2 + \delta_B^2, \quad (11.80d)$$

$$\hat{\omega}_{\text{eff}}^2 = \gamma \omega_1^2 + \bar{\Omega}^2, \quad (11.80e)$$

$$\hat{\theta} = \arctan \left( \frac{\sqrt{\gamma} \omega_1}{\bar{\Omega}} \right). \quad (11.80f)$$

The equation is accurate when populations are less skewed than the ‘TP02’ model ( $p_A \gg p_B$ ). Note that this model, as with the ‘TP02’ model, has been superseded by the ‘MP05’ model in the next section. The reference for this equation is:

- Trott, O., Abergel, D., and Palmer, A. (2003). An average-magnetization analysis of R-1 rho relaxation outside of the fast exchange. *Mol. Phys.*, **101**(6), 753–763. ([10.1080/0026897021000054826](https://doi.org/10.1080/0026897021000054826))

More information about the TAP03 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/TAP03>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.tap03-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#TAP03](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#TAP03).

### 11.7.6 The MP05 2-site exchange $R_{1\rho}$ model

This is the model for 2-site exchange for off-resonance  $R_{1\rho}$ -type data for all time scales from Miloushev and Palmer (2005). It is selected by setting the model to ‘MP05’. The equation for the exchange process is

$$R_{1\rho} = R_1 \cos^2 \theta + R'_{1\rho} \sin^2 \theta + \frac{\sin^2 \theta p_A p_B \Delta \omega^2 k_{\text{ex}}}{\omega_{\text{Aeff}}^2 \omega_{\text{Beff}}^2 / \omega_{\text{eff}}^2 + k_{\text{ex}}^2 - \sin^2 \theta p_A p_B \Delta \omega^2 \left( 1 + \frac{2k_{\text{ex}}^2 (p_A \omega_{\text{Aeff}}^2 + p_B \omega_{\text{Beff}}^2)}{\omega_{\text{Aeff}}^2 \omega_{\text{Beff}}^2 + \omega_{\text{eff}}^2 k_{\text{ex}}^2} \right)}, \quad (11.81)$$

in which the parameters are defined as in the ‘TP02’ model above. This model supersedes both the ‘TP02’ and ‘TAP03’ models. The reference for this equation is:

- Miloushev, V. Z. and Palmer, 3rd, A. G. (2005).  $R(1\rho)$  relaxation for two-site chemical exchange: general approximations and some exact solutions. *J. Magn. Reson.*, **177**(2), 221–227. ([10.1016/j.jmr.2005.07.023](https://doi.org/10.1016/j.jmr.2005.07.023))

More information about the MP05 model is available from:

- the relax wiki at <http://wiki.nmr-relax.com/MP05>,
- the API documentation at <http://www.nmr-relax.com/api/3.2/lib.dispersion.mp05-module.html>,
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#MP05](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#MP05).

## 11.8 The numeric $R_{1\rho}$ models

These are the numeric models designed for  $R_{1\rho}$ -type experiments.

### 11.8.1 The NS 2-site $R_{1\rho}$ model

This is the numerical model for 2-site exchange using 3D magnetisation vectors. It is selected by setting the model to ‘NS R1rho 2-site’. The simple constraint  $p_A > p_B$  is used to halve the optimisation space, as both sides of the limit are mirror image spaces.

For this model, the equations from [Korzhnev et al. \(2005b\)](#) have been used. The  $R_{1\rho}$  value for state A magnetisation is defined as

$$R_{1\rho} = -\frac{1}{T_{\text{relax}}} \cdot \ln \left( M_0^T \cdot e^{R \cdot T_{\text{relax}}} \cdot M_0 \right), \quad (11.82)$$

where

$$M_0 = \begin{pmatrix} \sin \theta \\ 0 \\ \cos \theta \\ 0 \\ 0 \\ 0 \end{pmatrix}, \quad (11.83)$$

$$\theta = \arctan \left( \frac{\omega_1}{\Omega_A} \right). \quad (11.84)$$

The relaxation evolution matrix is defined as

$$R = \begin{pmatrix} -R'_{1\rho} - k_{AB} & -\delta_A & 0 & k_{BA} & 0 & 0 \\ \delta_A & -R'_{1\rho} - k_{AB} & -\omega_1 & 0 & k_{BA} & 0 \\ 0 & \omega_1 & -R_1 - k_{AB} & 0 & 0 & k_{BA} \\ k_{AB} & 0 & 0 & -R'_{1\rho} - k_{BA} & -\delta_B & 0 \\ 0 & k_{AB} & 0 & \delta_B & -R'_{1\rho} - k_{BA} & -\omega_1 \\ 0 & 0 & k_{AB} & 0 & \omega_1 & -R_1 - k_{BA} \end{pmatrix}, \quad (11.85)$$

where  $\delta_{A,B}$  is defined in Equations [11.78a](#) and [11.78b](#).

More information about the NS R1rho 2-site model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_R1rho\\_2-site](http://wiki.nmr-relax.com/NS_R1rho_2-site),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_r1rho\\_2site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_r1rho_2site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_R1rho\\_2-site](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_R1rho_2-site).

### 11.8.2 The NS 3-site $R_{1\rho}$ model

This is the numerical model for 3-site exchange using 3D magnetisation vectors. It is selected by setting the model to ‘NS R1rho 3-site’. The constraints  $p_A > p_B$  and  $p_A > p_C$

is used to decrease the size of the optimisation space, as both sides of the limit are mirror image spaces.

For this model, as for the 2-site model above, the equations from [Korzhnev et al. \(2005b\)](#) have been used. These have been however rearranged to match the notation in [Palmer and Massi \(2006\)](#). The  $R_{1\rho}$  value for state A magnetisation is defined as

$$R_{1\rho} = -\frac{1}{T_{\text{relax}}} \cdot \ln \left( M_0^T \cdot e^{R \cdot T_{\text{relax}}} \cdot M_0 \right), \quad (11.86)$$

where

$$M_0 = \begin{pmatrix} \sin \theta \\ 0 \\ \cos \theta \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \quad (11.87)$$

$$\theta = \arctan \left( \frac{\omega_1}{\Omega_A} \right). \quad (11.88)$$

This assumes that the starting magnetisation has an X and Z component only for the A state. The relaxation evolution matrix is defined as

$$\begin{aligned} R = & \begin{pmatrix} -R'_{1\rho A} - k_{AB} - k_{AC} & -\delta_A & 0 & \cdots \\ \delta_A & -R'_{1\rho A} - k_{AB} - k_{AC} & -\omega_1 & \cdots \\ 0 & \omega_1 & -R_{1A} - k_{AB} - k_{AC} & \cdots \\ \vdots & \vdots & \vdots & \ddots \end{pmatrix} \\ & + \begin{pmatrix} \ddots & \vdots & \vdots & \vdots & \ddots \\ \cdots & -R'_{1\rho B} - k_{BA} - k_{BC} & -\delta_B & 0 & \cdots \\ \cdots & \delta_B & -R'_{1\rho B} - k_{BA} - k_{BC} & -\omega_1 & \cdots \\ \cdots & 0 & \omega_1 & -R_{1B} - k_{BA} - k_{BC} & \cdots \\ \ddots & \vdots & \vdots & \vdots & \ddots \end{pmatrix} \\ & + \begin{pmatrix} \ddots & \vdots & \vdots & \vdots \\ \cdots & -R'_{1\rho C} - k_{CA} - k_{CB} & -\delta_C & 0 \\ \cdots & \delta_C & -R'_{1\rho C} - k_{CA} - k_{CB} & -\omega_1 \\ \cdots & 0 & \omega_1 & -R_{1C} - k_{CA} - k_{CB} \end{pmatrix} \\ & + \begin{pmatrix} & & k_{BA} & 0 & 0 & \cdots \\ & \ddots & 0 & k_{BA} & 0 & \cdots \\ & & 0 & 0 & k_{BA} & \cdots \\ k_{AB} & 0 & 0 & & & \\ 0 & k_{AB} & 0 & & \ddots & \cdots \\ 0 & 0 & k_{AB} & & & \\ \vdots & \vdots & \vdots & & \vdots & \ddots \end{pmatrix} \end{aligned}$$

$$\begin{aligned}
& + \begin{pmatrix} & & & \cdots & k_{CA} & 0 & 0 \\ & \ddots & & \cdots & 0 & k_{CA} & 0 \\ & & & \cdots & 0 & 0 & k_{CA} \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\ k_{AC} & 0 & 0 & \cdots & & & \\ 0 & k_{AC} & 0 & \cdots & & \ddots & \\ 0 & 0 & k_{AC} & \cdots & & & \end{pmatrix} \\
& + \begin{pmatrix} \ddots & & \vdots & & \vdots & \vdots & \vdots \\ & & & & k_{CB} & 0 & 0 \\ \cdots & & \ddots & & 0 & k_{CB} & 0 \\ & & & & 0 & 0 & k_{CB} \\ \cdots & k_{BC} & 0 & 0 & & & \\ \cdots & 0 & k_{BC} & 0 & & \ddots & \\ \cdots & 0 & 0 & k_{BC} & & & \end{pmatrix}, \tag{11.89}
\end{aligned}$$

where  $\delta_{A,B,C}$  are defined as in Equations 11.78a and 11.78b. For the model, the assumptions  $R'_{1\rho A} = R'_{1\rho B} = R'_{1\rho C} = R'_{1\rho}$  and  $R_{1A} = R_{1B} = R_{1C} = R_1$  have been made.

More information about the NS R1rho 3-site model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_R1rho\\_3-site](http://wiki.nmr-relax.com/NS_R1rho_3-site),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_r1rho\\_3site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_r1rho_3site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_R1rho\\_3-site](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_R1rho_3-site).

### 11.8.3 The NS 3-site linear $R_{1\rho}$ model

This is the numerical model for 3-site linear exchange using 3D magnetisation vectors. The assumption that  $k_{AC} = k_{CA} = 0$  has been made to linearise this model. It is selected by setting the model to ‘NS R1rho 3-site linear’. The constraints  $p_A > p_B$  and  $p_A > p_C$  is used to decrease the size of the optimisation space, as both sides of the limit are mirror image spaces. To simplify the optimisation space for the model as in the ‘NS  $R_{1\rho}$  3-site’ model, the assumptions  $R_{2A}^0 = R_{2B}^0 = R_{2C}^0 = R_2^0$  and  $R_{1A} = R_{1B} = R_{1C} = R_1$  have been made.

The equations are the same as for the ‘NS R1rho 3-site’ model except for the relaxation

evolution matrix which simplifies to

$$\begin{aligned}
 R = & \begin{pmatrix} -R'_{1\rho A} - k_{AB} & -\delta_A & 0 & \cdots \\ \delta_A & -R'_{1\rho A} - k_{AB} & -\omega_1 & \cdots \\ 0 & \omega_1 & -R_{1A} - k_{AB} & \cdots \\ \vdots & \vdots & \vdots & \ddots \end{pmatrix} \\
 + & \begin{pmatrix} \ddots & \vdots & \vdots & \vdots & \vdots & \ddots \\ \cdots & -R'_{1\rho B} - k_{BA} - k_{BC} & -\delta_B & 0 & \cdots \\ \cdots & \delta_B & -R'_{1\rho B} - k_{BA} - k_{BC} & -\omega_1 & \cdots \\ \cdots & 0 & \omega_1 & -R_{1B} - k_{BA} - k_{BC} & \cdots \\ \cdots & \vdots & \vdots & \vdots & \ddots \end{pmatrix} \\
 + & \begin{pmatrix} \ddots & \vdots & \vdots & \vdots \\ \cdots & -R'_{1\rho C} - k_{CB} & -\delta_C & 0 \\ \cdots & \delta_C & -R'_{1\rho C} - k_{CB} & -\omega_1 \\ \cdots & 0 & \omega_1 & -R_{1C} - k_{CB} \end{pmatrix} \\
 + & \begin{pmatrix} & & k_{BA} & 0 & 0 & \cdots \\ & \ddots & 0 & k_{BA} & 0 & \cdots \\ & & 0 & 0 & k_{BA} & \cdots \\ k_{AB} & 0 & 0 & & & \\ 0 & k_{AB} & 0 & & \ddots & \cdots \\ 0 & 0 & k_{AB} & & & \\ \vdots & \vdots & \vdots & \vdots & & \ddots \end{pmatrix} \\
 + & \begin{pmatrix} \ddots & \vdots & \vdots & \vdots & \vdots \\ & & k_{CB} & 0 & 0 \\ \cdots & \ddots & 0 & k_{CB} & 0 \\ & & 0 & 0 & k_{CB} \\ \cdots & k_{BC} & 0 & 0 & \\ \cdots & 0 & k_{BC} & 0 & \ddots \\ \cdots & 0 & 0 & k_{BC} & \end{pmatrix}, \tag{11.90}
 \end{aligned}$$

where  $\delta_{A,B,C}$  are defined as in Equations 11.78a and 11.78b. For the model, the assumptions  $R'_{1\rho A} = R'_{1\rho B} = R'_{1\rho C} = R'_{1\rho}$  and  $R_{1A} = R_{1B} = R_{1C} = R_1$  have been made.

More information about the NS R1rho 3-site linear model is available from:

- the relax wiki at [http://wiki.nmr-relax.com/NS\\_R1rho\\_3-site\\_linear](http://wiki.nmr-relax.com/NS_R1rho_3-site_linear),
- the API documentation at [http://www.nmr-relax.com/api/3.2/lib.dispersion.ns\\_r1rho\\_3site-module.html](http://www.nmr-relax.com/api/3.2/lib.dispersion.ns_r1rho_3site-module.html),
- the relaxation dispersion page of the relax website at [http://www.nmr-relax.com/analyses/relaxation\\_dispersion.html#NS\\_R1rho\\_3-site\\_linear](http://www.nmr-relax.com/analyses/relaxation_dispersion.html#NS_R1rho_3-site_linear).



## 11.9 Relaxation dispersion optimisation theory

The implementation of optimisation in relax is discussed in detail in Chapter 14. To understand the concepts in this subsection, it is best to look at that chapter first.

### 11.9.1 The relaxation dispersion auto-analysis

In relax, optimisation can either be performed manually or one of the auto-analyses can be employed. Note that if you are using the relax GUI, you will be using the dispersion auto-analysis. The auto-analysis is a fully self-contained protocol designed to make the analysis as simple as possible. All details can be seen in the `auto_analyses/relax_disp.py` file which, in reality, is simply a large relax script.

The relaxation dispersion auto-analysis implements many of the concepts described in detail in the next sections. It can be summarised as:

**Peak intensity error analysis:** An error analysis is performed to determine the peak intensity errors, if not already calculated (see Section 17.2.212 on page 605).

**‘R2eff’ model optimisation:** Firstly the ‘R2eff’ model is either optimised (using the `minimise.execute` user function) or simply calculated (using the `minimise.calculate` user function) to find the  $R_{2\text{eff}}$  or  $R_{1\rho}$  values used as the base data for all other dispersion models (see Section 11.2.1 on page 154).

**Dispersion curve insignificance:** Spins with insignificant dispersion profiles will be de-selected with the `relax_disp.insignificance` user function, as described below, excluding the ‘No Rex’ model.

**Model optimisation:** Sequential optimisation of each of the specified dispersion models. This consists of a grid search followed by Nelder-Mead simplex optimisation constrained using the log-barrier constraint algorithm. Each model will be stored in a different data pipe. See Section 17.2.69 on page 497 for the grid search and Section 17.2.68 on page 493 for minimisation.

**Grid search avoidance:** A number of tricks are used to speed up optimisation by skipping or decreasing the size of the initial grid search:

**Pre-run directory:** If a pre-run directory is supplied – a separate directory containing the dispersion auto-analysis results from a previous run – the optimised parameters from these previous results will be used as the starting point for optimisation rather than performing a grid search. This is used in a clustered analysis whereby the pre-run directory contains results from a non-clustered analysis. This is essential for when large spin clusters are specified, as a grid search becomes prohibitively expensive with clusters of three or more spins. At some point a `RelaxError` will occur because the grid search is impossibly large. For the cluster specific parameters, i.e. the populations of the states and the exchange parameters, an average value will be used as the starting point. For all other parameters, the  $R_2^0$  values for each spin and magnetic field, as well as the parameters related to the chemical shift difference  $\Delta\omega$ , the optimised values of the previous run will be directly copied.

**Model nesting:** If two models are nested, then the parameters of the simpler will be used as the starting point for optimisation of the more complex. The currently supported nested model sets are presented in Table 11.3 on page 186. The models are optimised in the order presented in that table. In some cases, the  $R_{2A}^0$  and  $R_{2B}^0$  parameter values are set to the simpler model  $R_2^0$  value and the grid search is bypassed.

**Model equivalence:** When two models are equivalent, the optimised parameters of one model can be used as the starting point of the other rather than performing a grid search. This is used in the auto-analysis for avoiding the grid search in the numeric models. The optimised ‘CR72’ model is used for the ‘NS CPMG 2-site expanded’, ‘NS CPMG 2-site 3D’, and ‘NS CPMG 2-site star’ models. The optimised ‘MMQ CR72’ model is used for the ‘NS MMQ 2-site’ model. And the ‘MP05’ model is used for the ‘NS R1rho 2-site’ model.

**Interruption:** The optimisation procedure of the auto-analysis can read saved results files if a previous calculation was interrupted.

**Model elimination:** As it is quite common that some of the dispersion models fail to optimise to reasonable values, or will even optimise to non-physically possible values where the global minimum is located, model elimination is performed to remove these models. The relax implementation is described in d’Auvergne and Gooley (2006). This needs to be performed prior to model selection as a failed model will often provide a statistically better fit than a non-failed model.

**Per-model error analysis:** If desired, Monte Carlo simulations for error propagation can be performed for each model. This does however require far greater computation time.

**Model selection:** If more than one model is analysed, AIC model selection will be performed to judge statistical significance of the models (Akaike, 1973). This is used to determine if statistically significant  $R_{ex}$  contributions can be extracted from the data, as well as determine if one model is better than the other. Different statistical techniques such as AICc and BIC can be used when using the script UI (Hurvich and Tsai, 1989; Schwarz, 1978). The AIC, AICc and BIC equations for NMR relaxation data were derived in d’Auvergne and Gooley (2003). In most cases, the list models to choose from should be severely limited. The results will be stored in a new ‘final’ data pipe and output files placed in the `final` directory.

**Error analysis:** Monte Carlo simulations for error propagation is performed on the final data pipe (see Section 17.2.95 on page 532 as well as the descriptions for all of the other `monte_carlo` user functions). Model elimination is performed again to remove the Monte Carlo simulations which have failed.

**Output file creation:** For each of the models and the final model selection results, the `relax_disp.plot_disp_curves` (Section 17.2.169 on page 578), `relax_disp.plot_exp_curves` (Section 17.2.170 on page 579), `relax_disp.write_disp_curves` (Section 17.2.181 on page 587), `grace.write` (Section 17.2.55 on page 481) and `value.write` (Section 17.2.266 on page 656) user functions will be called to generate all the output files you would need. These generate both Grace 2D plots of the data as well as plain text files. Additional output files can be created after the analysis by using the user functions manually.

All these steps will be shown in full detail in the relax logs. You should check very carefully for any relax warnings as these can be an indication that something has not been set up correctly.

If you are a power user, you are free to use all of the relax user functions, the relax library, and the relax data store to implement your own protocol. If you wish, the protocol can be converted into a new auto-analysis and distributed as part of relax. The relax test suite will ensure the protocol remains functional for the lifetime of relax.

Table 11.3: Model nesting for the relaxation dispersion auto-analysis.

| Model                                       | Nested models <sup>1</sup>                                                                                                                 |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Base models</b>                          |                                                                                                                                            |
| $R_{2\text{eff}}/R'_{1\rho}$                | -                                                                                                                                          |
| No Rex                                      | -                                                                                                                                          |
| <b>Single quantum (SQ) CPMG-type</b>        |                                                                                                                                            |
| LM63                                        | -                                                                                                                                          |
| LM63 3-site                                 | LM63                                                                                                                                       |
| CR72                                        | NS CPMG 2-site expanded, NS CPMG 2-site 3D, NS CPMG 2-site star, B14                                                                       |
| CR72 full                                   | NS CPMG 2-site 3D full, NS CPMG 2-site star full, B14 full, NS CPMG 2-site expanded,<br>NS CPMG 2-site 3D, NS CPMG 2-site star, B14, CR72  |
| IT99                                        | -                                                                                                                                          |
| TSMFK01                                     | -                                                                                                                                          |
| B14                                         | NS CPMG 2-site expanded, NS CPMG 2-site 3D, NS CPMG 2-site star, CR72                                                                      |
| B14 full                                    | NS CPMG 2-site 3D full, NS CPMG 2-site star full, CR72 full, NS CPMG 2-site expanded,<br>NS CPMG 2-site 3D, NS CPMG 2-site star, B14, CR72 |
| NS CPMG 2-site expanded                     | NS CPMG 2-site 3D, NS CPMG 2-site star, B14, CR72                                                                                          |
| NS CPMG 2-site 3D                           | NS CPMG 2-site expanded, NS CPMG 2-site star, B14, CR72                                                                                    |
| NS CPMG 2-site 3D full                      | NS CPMG 2-site star full, B14 full, CR72 full, NS CPMG 2-site expanded, NS CPMG 2-site 3D,<br>NS CPMG 2-site star, B14, CR72               |
| NS CPMG 2-site star                         | NS CPMG 2-site expanded, NS CPMG 2-site 3D, B14, CR722                                                                                     |
| NS CPMG 2-site star full                    | NS CPMG 2-site 3D full, B14 full, CR72 full, NS CPMG 2-site expanded, NS CPMG 2-site 3D,<br>NS CPMG 2-site star, B14, CR72                 |
| <b>MMQ (SQ, ZQ, DQ, &amp; MQ) CPMG-type</b> |                                                                                                                                            |
| MMQ CR72                                    | NS MMQ 2-site                                                                                                                              |
| NS MMQ 2-site                               | MMQ CR72                                                                                                                                   |
| NS MMQ 3-site linear                        | NS MMQ 3-site, NS MMQ 2-site, MMQ CR72                                                                                                     |
| NS MMQ 3-site                               | NS MMQ 3-site linear, NS MMQ 2-site, MMQ CR72                                                                                              |

Table 11.3: Model nesting for the relaxation dispersion auto-analysis.

| Model                           | Nested models <sup>1</sup>                                             |
|---------------------------------|------------------------------------------------------------------------|
| $R_{1\rho}$ -type               |                                                                        |
| M61                             | -                                                                      |
| M61 skew                        | -                                                                      |
| DPL94                           | -                                                                      |
| DPL94 $R_1$ fit                 | -                                                                      |
| TP02                            | MP05, TAP03                                                            |
| TP02 $R_1$ fit                  | MP05 $R_1$ fit, TAP03 $R_1$ fit                                        |
| TAP03                           | MP05, TP02                                                             |
| TAP03 $R_1$ fit                 | MP05 $R_1$ fit, TP02 $R_1$ fit                                         |
| MP05                            | TAP03, TP02                                                            |
| MP05 $R_1$ fit                  | TAP03 $R_1$ fit, TP02 $R_1$ fit                                        |
| NS $R_{1\rho}$ 2-site           | MP05, TAP03, TP02                                                      |
| NS $R_{1\rho}$ 2-site $R_1$ fit | MP05 $R_1$ fit, TAP03 $R_1$ fit, TP02 $R_1$ fit                        |
| NS $R_{1\rho}$ 3-site linear    | NS $R_{1\rho}$ 2-site, MP05, TAP03, TP02                               |
| NS $R_{1\rho}$ 3-site           | NS $R_{1\rho}$ 3-site linear, NS $R_{1\rho}$ 2-site, MP05, TAP03, TP02 |

<sup>1</sup>The nested models are ordered by preference. The earliest model in the list which has been optimised in the auto-analysis will be used as the nested model. For example for the 'B14 full' model, the 'CR72 full' model is the first preference, followed by 'B14', then the final fall back is 'CR72' is neither 'CR72 full' or 'B14' have been optimised. If none of the nested models have been optimised, the grid search will be performed. In this example, 'CR72 full' is preferred as it has perfect parameter nesting – all parameters of 'B14 full' are found in 'CR72 full'. The B14 and CR72 are fallbacks, and for these R20A and R20B are copied from R20 so they start optimisation as R20A == R20B. Hence 'CR72 full' whereby R20A != R20B is a much better starting point as R20A and R20B have been optimised to different values. But because of the large model instability in the 'CR72 full' model, you may wish to instead start with 'B14'.

### 11.9.2 Dispersion curve insignificance

To avoid severe model failure due to the fitting of statistically insignificant dispersion curves, the `relax_disp.insignificance` user function can be used. This is activated by default in the auto-analysis. The user function takes a single insignificance value and if the difference between the smallest and largest  $R_{2\text{eff}}$  or  $R_{1\rho}$  value for an individual spin for all dispersion curves is less than this, then that spin will be deselected. See Section 17.2.166 on page 577 for more details.

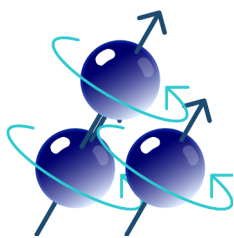
### 11.9.3 The relaxation dispersion space

In a dispersion analysis the target function  $f(\theta)$  is the chi-squared equation

$$\chi^2(\theta) = \sum_{i=1}^n \frac{(R_{2\text{eff}} - R_{2\text{eff}}(\theta))^2}{\sigma_i^2}, \quad (11.91)$$

where  $i$  is the summation index,  $R_{2\text{eff}}$  is the experimental relaxation data which belongs to the data set  $R$  and includes the  $R_{2\text{eff}}$  and  $R_{1\rho}$  values for all experiments at all magnetic field strengths,  $R_{2\text{eff}}(\theta)$  is the back calculated relaxation data belonging to the set  $R(\theta)$ , and  $\sigma_i$  is the experimental  $R_{2\text{eff}}/R_{1\rho}$  error. For standard optimisation, the summation index ranges over the relaxation data of an individual spin. However this can be changed with clustering whereby the relaxation data from a group of spin systems are optimised using a shared set of parameters.

### 11.9.4 The clustered relaxation dispersion analysis



Often in a relaxation dispersion analysis you will wish to fit a number of spin systems together using global parameters such as  $k_{\text{ex}}$ ,  $p_A$ , etc. This can be achieved through the concept of clustering. A cluster is defined by an ID string and can contain any number of spins. Multiple clusters in one analysis can be defined. Any spins not included in a cluster will be treated as a free spin whereby all parameters of the dispersion model are local to that spin. Spin clusters can be defined using the `relax_disp.cluster` user function (see Section 17.2.161 on page 574) or via the spin cluster GUI element.

For the spin clustering, the special `relax_disp.parameter_copy` user function has been designed to help avoid the impossibly large grid search. This user function will copy the parameters from one non-clustered data pipe to another pipe, taking the median of the parameters from the first data pipe. Rather than taking the median, the  $R_2^0/R_{1\rho}'$  and  $\Delta\omega$  related parameters which are independent of the clustering are simply copied.

### 11.9.5 Dispersion parameter grid search

One of the most statistically unbiased methods for determining an initial parameter estimate prior to optimisation is to perform a grid search. This is performed via the `minimise.grid_search` user function (see Section 17.2.69 on page 497).

For some dispersion models the grid search can be too computationally expensive. In this case, some tricks can be used to bypass the parts of the grid search or the whole grid search:

**Model nesting:** Using the optimised parameters of a simpler nested model as the starting point for optimisation.

**Model equivalence:** Using the optimised solution of an equivalent analytic model as the starting point for a numeric model.

These tricks are implemented in the relaxation dispersion auto-analysis protocol as described above. If you do not use the auto-analysis or the GUI, then you are free to implement your own solutions.

The grid search lower and upper bounds default to:

$$5 \leq R_2^0 \leq 20, \quad (11.92a)$$

$$5 \leq R_{2A}^0 \leq 20, \quad (11.92b)$$

$$5 \leq R_{2B}^0 \leq 20, \quad (11.92c)$$

$$0 \leq \Phi_{\text{ex}} \leq 10, \quad (11.92d)$$

$$0 \leq \Phi_{\text{ex},B} \leq 10, \quad (11.92e)$$

$$0 \leq \Phi_{\text{ex},C} \leq 10, \quad (11.92f)$$

$$0 \leq p_A \Delta\omega^2 \leq 10, \quad (11.92g)$$

$$0 \leq \Delta\omega \leq 10, \quad (11.92h)$$

$$0 \leq \Delta\omega_{AB} \leq 10, \quad (11.92i)$$

$$0 \leq \Delta\omega_{BC} \leq 10, \quad (11.92j)$$

$$0 \leq \Delta\omega^H \leq 3, \quad (11.92k)$$

$$0 \leq \Delta\omega_{AB}^H \leq 3, \quad (11.92l)$$

$$0 \leq \Delta\omega_{BC}^H \leq 3, \quad (11.92m)$$

$$0.5 \leq p_A \leq 1, \quad (11.92n)$$

$$0.0 \leq p_B \leq 0.5, \quad (11.92o)$$

$$1 \leq k_{\text{ex}} \leq 1e^4, \quad (11.92p)$$

$$1 \leq k_{\text{ex}}^{AB} \leq 1e^4, \quad (11.92q)$$

$$1 \leq k_{\text{ex}}^{BC} \leq 1e^4, \quad (11.92r)$$

$$1 \leq k_A \leq 1e^4, \quad (11.92s)$$

$$1 \leq k_B \leq 1e^4, \quad (11.92t)$$

$$0.1 \leq k_{AB} \leq 20, \quad (11.92u)$$

$$1e^{-4} \leq \tau_{\text{ex}} \leq 1. \quad (11.92v)$$

For the MMQ models, the grid bounds are slightly different with

$$-10 \leq \Delta\omega \leq 10, \quad (11.93a)$$

$$-10 \leq \Delta\omega_{AB} \leq 10, \quad (11.93b)$$

$$-10 \leq \Delta\omega_{BC} \leq 10, \quad (11.93c)$$

$$-3 \leq \Delta\omega^H \leq 3, \quad (11.93d)$$

$$-3 \leq \Delta\omega_{AB}^H \leq 3, \quad (11.93e)$$

$$-3 \leq \Delta\omega_{BC}^H \leq 3. \quad (11.93f)$$

These values can be changed when not using the auto-analysis. Linear constraints can decrease the number of grid points searched through.

### 11.9.6 Dispersion parameter optimisation

For a description of gradients and Hessians, see Section 14.3.2 on page 300 and Section 14.3.3 on page 300 respectively. The relaxation dispersion model gradients or Hessians have either not been calculated for the analytic models or cannot be calculated as the solution is not analytic. Numeric gradients and Hessians could be calculated but this is too computationally expensive, especially for the numeric models where this adds a second layer of numeric approximation.

Optimisation in relax is via the minfx package <https://gna.org/projects/minfx/>. This allows the Nelder-Mead simplex optimisation technique (see Section 14.4.5 on page 305) and the log-barrier constraint algorithm (see Section 14.5.2 on page 308) to be used. The advantage of these two techniques is that it enables extremely reliable and high precision optimisation without the use of gradients or Hessians, hence can significantly increase optimisation speeds. They however do not avoid the multiple local minimum problem present in the MMQ models – for that a highly accurate grid search is a reasonable solution.

### 11.9.7 Relaxation dispersion parameter constraints

To understand this section, please see Section 14.5 on page 306. For a dispersion analysis, linear constraints are the most useful type of constraint.

For most models, the linear constraints in the notation of (14.18) for the relaxation rates are

$$\begin{pmatrix} 1 & 0 & 0 \\ -1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} R_2^0 \\ R_{2A}^0 \\ R_{2B}^0 \end{pmatrix} \geq \begin{pmatrix} 0 \\ -200 \\ 0 \\ -200 \\ 0 \\ -200 \end{pmatrix}, \quad (11.94)$$



for the  $\Phi_{\text{ex}}$  and  $\Delta\omega$  parameters as

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} \Phi_{\text{ex}} \\ \Phi_{\text{ex,B}} \\ \Phi_{\text{ex,C}} \\ p_A \Delta\omega^2 \\ \Delta\omega \\ \Delta\omega_{\text{AB}} \\ \Delta\omega_{\text{BC}} \\ \Delta\omega^{\text{H}} \\ \Delta\omega_{\text{AB}}^{\text{H}} \\ \Delta\omega_{\text{BC}}^{\text{H}} \end{pmatrix} \geq \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \quad (11.95)$$

for the population parameters as

$$\begin{pmatrix} -1 & 0 \\ 1 & 0 \\ 1 & 0 \\ -1 & -1 \\ 1 & 2 \end{pmatrix} \cdot \begin{pmatrix} p_A \\ p_B \end{pmatrix} \geq \begin{pmatrix} -1 \\ 0.5 \\ 0.85 \\ -1 \\ 1 \end{pmatrix}, \quad (11.96)$$

and for the exchange rate and time parameters as

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \cdot \begin{pmatrix} k_{\text{ex}} \\ k_{\text{ex}}^{\text{AB}} \\ k_{\text{ex}}^{\text{BC}} \\ k_{\text{B}} \\ k_{\text{C}} \\ k_{\text{AB}} \\ \tau_{\text{ex}} \end{pmatrix} \geq \begin{pmatrix} 0 \\ -2e^6 \\ 0 \\ -2e^6 \\ 0 \\ -2e^6 \\ 0 \\ -2e^6 \\ 0 \\ -2e^6 \\ 0 \\ -100 \\ 0 \end{pmatrix}. \quad (11.97)$$

Through the isolation of each individual element, the constraints can be seen to be equivalent to

$$0 \leq R_2^0 \leq 200, \quad (11.98a)$$

$$0 \leq R_{2A}^0 \leq 200, \quad (11.98b)$$

$$0 \leq R_{2B}^0 \leq 200, \quad (11.98c)$$

$$\Phi_{\text{ex}} \geq 0, \quad (11.98d)$$

$$\Phi_{\text{ex,B}} \geq 0, \quad (11.98e)$$

$$\Phi_{\text{ex,C}} \geq 0, \quad (11.98f)$$

$$\Delta\omega \geq 0, \quad (11.98g)$$

$$\Delta\omega_{\text{AB}} \geq 0, \quad (11.98h)$$

$$\Delta\omega_{BC} \geq 0, \quad (11.98i)$$

$$\Delta\omega^H \geq 0, \quad (11.98j)$$

$$\Delta\omega_{AB}^H \geq 0, \quad (11.98k)$$

$$\Delta\omega_{BC}^H \geq 0, \quad (11.98l)$$

$$p_A \Delta\omega^2 \geq 0, \quad (11.98m)$$

$$0 \leq p_A \leq 1, \quad (11.98n)$$

$$0 \leq p_B \leq p_A, \quad (11.98o)$$

$$0 \leq p_C \leq p_A, \quad (11.98p)$$

$$p_A \geq 0.85 \quad (\text{the skewed condition, } p_A \gg p_B), \quad (11.98q)$$

$$0 \leq k_{\text{ex}} \leq 2e^6, \quad (11.98r)$$

$$0 \leq k_{\text{ex}}^{\text{AB}} \leq 2e^6, \quad (11.98s)$$

$$0 \leq k_{\text{ex}}^{\text{BC}} \leq 2e^6, \quad (11.98t)$$

$$0 \leq k_A \leq 2e^6, \quad (11.98u)$$

$$0 \leq k_B \leq 2e^6, \quad (11.98v)$$

$$0 \leq k_{AB} \leq 100, \quad (11.98w)$$

$$\tau_{\text{ex}} \geq 0. \quad (11.98x)$$

Note that the  $\Delta\omega$  and  $\Delta\omega^H$  constraints are not used for any of the MMQ-type models as sign differentiation is possible. These constraints are also turned off for the ‘NS  $R_{1\rho}$  3-site linear’ and ‘NS  $R_{1\rho}$  3-site’ models. And that the  $p_A \geq 0.85$  constraint is used instead of the  $p_A \geq 0.5$  constraint for all models which require  $p_A \gg p_B$ . When not using the auto-analysis, constraints can be modified or turned off.

### 11.9.8 Relaxation dispersion diagonal scaling

The concept of diagonal scaling is explained in Section 14.6 on page 308.

For the dispersion analysis the scaling factor of 10 is used for the relaxation rates,  $1e^4$  for the exchange rates,  $1e^{-4}$  for exchange times, and 1 for all other parameters. The scaling matrix for the parameters  $\{R_2^0, R_{2A}^0, R_{2B}^0, \Phi_{\text{ex}}, \Phi_{\text{ex},B}, \Phi_{\text{ex},C}, p_A \Delta\omega^2, \Delta\omega, \Delta\omega^H, p_A, p_B,$

$k_{\text{ex}}, k_B, k_C, k_{AB}, \tau_{\text{ex}}\}$  is

$$\begin{pmatrix} 10 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 10 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 10 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1e^4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1e^4 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1e^4 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 20 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1e^{-4} \end{pmatrix}. \quad (11.99)$$

### 11.9.9 Relaxation dispersion model elimination

Relaxation dispersion models will often fail. This may be due to data quality and quantity issues, inherent instability in certain models, or the use of analytic models outside of the range of their defined viability. Model elimination is therefore required to remove these failed models prior to model selection, as failed models will often fit the experimental data statistically better than non-failed models. The user function `eliminate` (see Section 17.2.41 on page 471) is used to remove the failed models. Model elimination was implement in relax as described in:

- d’Auvergne, E. J. and Gooley, P. R. (2006). Model-free model elimination: A new step in the model-free dynamic analysis of NMR relaxation data. *J. Biomol. NMR*, **35**(2), 117–135. ([10.1007/s10858-006-9007-z](https://doi.org/10.1007/s10858-006-9007-z))

The following hard coded rules are used to eliminate models:

$$p_A \leq 0.501, \quad (11.100a)$$

$$p_A \geq 0.999, \quad (11.100b)$$

$$\tau_{\text{ex}} \geq 1.0. \quad (11.100c)$$

If a parameter falls outside of these limits, the entire spin cluster will be deselected. When not using the auto-analysis, custom model elimination rules can be defined and used with the `eliminate` user function.

### 11.9.10 Monte Carlo simulation elimination

Just as models can fail, often Monte Carlo simulations will also experience optimisation failures (see Figure 4 of d’Auvergne and Gooley (2006) for such a failure in the model-free

optimisation space). The minimum can be warped so much by the data randomisation that a new minimum appears at an unreasonable position in the optimisation space. Even when the original model optimisation is successful, this can affect a small portion of the simulations. These must be removed prior to calculating the parameter errors otherwise the errors will be significantly over estimated. The simulation model failures are outliers which skew the error estimate, introducing a bias. This can result in parameter error estimates which are too large. The solution is the use of the `eliminate` user function when Monte Carlo simulations are turned on – this will automatically deselect simulations rather than spins using the rules from the previous section. Note that `relax` is the only software which provides this feature.

### 11.9.11 Relaxation dispersion on a computer cluster using OpenMPI

If the optimisation is too slow on a single computer, the dispersion analysis has been parallelised on the level of both the spin cluster and the Monte Carlo simulation. The scaling efficiency is very close to perfect so if you have access to a computer cluster and the OpenMPI protocol, then the calculations can be run much faster. The implementation uses Gary Thompson’s multi-processor package. See Section 1.3 on page 17 for details on how to use this.

## 11.10 To do – dispersion features yet to be implemented

The capabilities of the relaxation dispersion analysis in `relax` is expansive but it cannot be called complete. There are a number of features and models yet to be implemented. Missing features include:

- The handling of off-resonance effects in the models of the numeric solution for CPMG-type data. This is specifically the ‘NS CPMG 2-site 3D’ and ‘NS CPMG 2-site star’ models and their ‘\* full’ equivalents. The necessary infrastructure is in place, but not activated yet (mainly due to a lack of synthetic data to test against). Currently only the software CATIA can handle this effect.
- Multi-state data – the handling of data from two sets of peaks from the same spin system is not properly supported. It is currently handled by assuming each state is a separate spin system but this means that  $\Delta\omega$ ,  $\Delta\omega^H$  and related parameters are not shared as they should be.
- The van’t Hoff analysis of multi-temperature dispersion data (see [https://en.wikipedia.org/wiki/Van\\_%27t\\_Hoff\\_equation](https://en.wikipedia.org/wiki/Van_%27t_Hoff_equation)).
- The Korzhnev et al. (2005b) correction for constant-time  $R_{1\rho}$  experiments for the analytic models ( $R_{1\rho} = -\lambda_1 - 1/T_{\text{relax}} \log a_1$ , where  $a_1 = 1 - p_B \cos^2(\theta_A - \theta_B)$ , and  $\theta_A = \arctan(\omega_1/\omega_A)$  and  $\theta_B = \arctan(\omega_1/\omega_B)$ ).
- Calculation of the dispersion  $\alpha$  value from Millet et al. (2000) (see <https://gna.org/task/?7800>).
- Support for CEST-type data.

- Offset support for the ground state chemical shift rather than the observed peak position. See <http://thread.gmane.org/gmane.science.nmr.relax.devel/5414/focus=5457>. This is really only needed for  $R_{1\rho}$ -type data.

If you would like one of these features, please contact the “relax-devel at gna.org” mailing list. The most useful would be if you have synthetic data whereby you know what the true solution should be. This can then be incorporated into a relax system test and the feature implemented to allow the test to pass. Note that such data should never be emailed to a public mailing list! Synthetic data, or experimental data, can be obtained from the literature.

Some of the missing models include:

- ‘**TP04**’: The  $R_{1\rho}$ -type data [Trott and Palmer \(2004\)](#) N-site analytic equation for all time scales with parameters  $\{R'_{1\rho}, \dots, p_1, \dots, p_N, \overline{\omega}, k_{12}, \dots, k_{1N}\}$ .
- ‘\*  $R_{1\rho}$ ’: All of the 3-site and N-site models as summarised in Table 1 of [Palmer and Massi \(2006\)](#).
- ‘**BK13**’: The  $R_{1\rho}$ -type data [Baldwin and Kay \(2013\)](#) off-resonance 2-site equation for all time scales with parameters  $\{R'_{1\rho}, \dots, p_A, \Delta\omega, k_{\text{ex}}\}$ . For details and code, see <https://gna.org/support/?3155>.
- ‘**BK13 full**’: The  $R_{1\rho}$ -type data full [Baldwin and Kay \(2013\)](#) off-resonance 2-site equation for all time scales with parameters  $\{R'_{1\rho A}, R'_{1\rho B}, \dots, p_A, \Delta\omega, k_{\text{ex}}\}$ . For details and code, see <https://gna.org/support/?3155>.

Information for how these can be added is given in the next section.

## 11.11 Tutorial for adding relaxation dispersion models

As the field of NMR relaxation dispersion has a very long history, it is not possible to include all analytic and numeric relaxation dispersion models for both CPMG-type or  $R_{1\rho}$ -type experiments in relax. However it is not too difficult to add new models for your own needs if you have some Python, Matlab, Mathematica, or similar scripting skills. The steps required are detailed on the [relax wiki](http://wiki.nmr-relax.com/Tutorial_for_adding_relaxation_dispersion_models_to_relax) page [http://wiki.nmr-relax.com/Tutorial\\_for\\_adding\\_relaxation\\_dispersion\\_models\\_to\\_relax](http://wiki.nmr-relax.com/Tutorial_for_adding_relaxation_dispersion_models_to_relax).

## 11.12 Comparison of dispersion analysis software

Diverse software exists for analysing relaxation dispersion data. The following is a list of the officially released software which you can use instead of relax:

**CPMGFit** Art Palmer’s original dispersion analysis software at <http://www.palmer.hs.columbia.edu/software/cpmgfit.html>.

**cpmg\_fit** Dmitry Korzhnev’s dispersion software available upon request.

**CATIA** Flemming Hansen’s dispersion software at <http://www.biochem.ucl.ac.uk/hansen/catia/>. The reference is Hansen et al. (2008).

**NESSY** Michael Bieri’s dispersion software at <http://home.gna.org/nessy/>. The reference is Bieri and Gooley (2011).

**GUARDD** Ian Kleckner’s dispersion software at <http://code.google.com/p/guardd/>. The reference is Kleckner and Foster (2012).

**ShereKhan** See the web server at <http://sherekhan.bionmr.org/>. The reference is Mazur et al. (2013).

**GLOVE** Peter Wright’s dispersion software at <http://www.scripps.edu/wright/>. The reference is Sugase et al. (2013).

**chemex** Guillaume Bouvignies’ dispersion software which can be found at <http://code.google.com/p/chemex/>.

There is currently support in relax for generating the input files for CPMGFit, CATIA, NESSY, and ShereKhan and for running CPMGFit and CATIA from within relax.

The features of the different software are compared in Table 11.4 on page 197. Note that this table is likely to be incomplete so please see the websites of the respective software for an up to date list of features. The aim of this table is to provide a fair comparison between all of the available dispersion software. Therefore if you do find deficiencies or errors in this table please report these either to the relax users mailing list at “relax-users at gna.org”, submit a bug report (see section 3.3 on page 30), or send a private message to one of the relax developers (<http://gna.org/project/memberlist.php?group=relax>) so that the details can be corrected.

Table 11.4: Comparison of the features for the different dispersion software.

|                                                 | CPMGFit | cpmg-fit | CATIA | NESSY | GUARDD | ShereKhan | GLOVE | chemex | relax |
|-------------------------------------------------|---------|----------|-------|-------|--------|-----------|-------|--------|-------|
| <b>Dispersion models</b>                        |         |          |       |       |        |           |       |        |       |
| Base models                                     |         |          |       |       |        |           |       |        |       |
| $R_{2\text{eff}}/R_{1\rho}$                     | -       | -        | -     | ✓     | ✓      | ✓         | ✓     | -      | ✓     |
| No Rex                                          | -       | ✓        | -     | ✓     | ✓      | -         | ✓     | -      | ✓     |
| Single quantum (SQ) CPMG-type                   |         |          |       |       |        |           |       |        |       |
| LM63                                            | ✓       | -        | -     | ✓     | -      | ✓         | ✓     | -      | ✓     |
| LM63 3-site                                     | ✓       | -        | -     | ✓     | -      | -         | -     | -      | ✓     |
| CR72                                            | ✓       | -        | -     | ✓     | -      | ✓         | ✓     | -      | ✓     |
| IT99                                            | ✓       | -        | -     | -     | -      | -         | ✓     | -      | ✓     |
| TSMFK01                                         | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| B14                                             | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| NS 2-site                                       | -       | ✓        | ✓     | -     | -      | ✓         | ?     | -      | ✓     |
| MMQ (SQ, ZQ, DQ, & MQ) CPMG-type                |         |          |       |       |        |           |       |        |       |
| MMQ CR72                                        | -       | ✓        | -     | -     | ✓      | -         | ?     | -      | ✓     |
| NS MMQ 2-site                                   | -       | ✓        | -     | -     | -      | -         | ?     | -      | ✓     |
| NS MMQ 3-site linear                            | -       | ✓        | -     | -     | -      | -         | ?     | -      | ✓     |
| NS MMQ 3-site                                   | -       | ✓        | -     | -     | -      | -         | ?     | -      | ✓     |
| $R_{1\rho}$ -type                               |         |          |       |       |        |           |       |        |       |
| M61                                             | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| DPL94                                           | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| TP02                                            | -       | ✓        | -     | -     | -      | -         | -     | -      | ✓     |
| TAP03                                           | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| TP04                                            | -       | -        | -     | -     | -      | -         | -     | -      | -     |
| MP05                                            | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| BK13                                            | -       | -        | -     | -     | -      | -         | -     | -      | -     |
| NS $R_{1\rho}$ 2-site                           | -       | ✓        | -     | -     | -      | -         | ?     | -      | ✓     |
| NS $R_{1\rho}$ 3-site linear                    | -       | ✓        | -     | -     | -      | -         | -     | -      | ✓     |
| NS $R_{1\rho}$ 3-site                           | -       | ✓        | -     | -     | -      | -         | -     | -      | ✓     |
| <b>General features</b>                         |         |          |       |       |        |           |       |        |       |
| Parallelisation via MPI for running on clusters | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| Off-resonance effects (CPMG-type data)          | -       | -        | ✓     | -     | -      | -         | -     | -      | -     |
| Off-resonance effects ( $R_{1\rho}$ -type data) | -       | ✓        | -     | -     | -      | -         | -     | -      | ✓     |
| Support for TROSY-type data                     | -       | -        | ✓     | -     | -      | -         | -     | -      | -     |
| Support for CEST-type data                      | -       | -        | -     | -     | -      | -         | -     | ✓      | -     |
| Support for scalar coupling effects             | -       | -        | ✓     | -     | -      | -         | -     | -      | -     |
| Arrhenius / Van't Hoff analysis                 | -       | ✓        | -     | ✓     | ✓      | -         | -     | -      | -     |
| Dispersion data back calculation (BC)           | -       | ✓        | -     | ✓     | ✓      | -         | -     | -      | ✓     |

Table 11.4: Comparison of the features for the different dispersion software.

|                                            | CPMGfit | cpmg-fit | CATIA | NESSY | GUARDD | ShereKhan | GLOVE | chemex | relax |
|--------------------------------------------|---------|----------|-------|-------|--------|-----------|-------|--------|-------|
| <b>User interface</b>                      |         |          |       |       |        |           |       |        |       |
| Graphical user interface (GUI)             | -       | -        | -     | ✓     | ✓      | -         | -     | -      | ✓     |
| Web user interface (Web UI)                | -       | -        | -     | -     | -      | ✓         | -     | -      | -     |
| Scripting user interface (Script UI)       | -       | ✓        | ✓     | -     | -      | ✓         | ✓     | -      | ✓     |
| Shell interface                            | ✓       | -        | -     | -     | -      | -         | -     | ✓      | -     |
| Temperature and field-dependent simulator  | -       | -        | -     | -     | ✓      | -         | -     | -      | -     |
| UI for experimental planning (data BC)     | -       | -        | -     | -     | ✓      | -         | -     | -      | -     |
| <b>Optimisation</b>                        |         |          |       |       |        |           |       |        |       |
| Grid search algorithm                      | ✓       | -        | -     | ✓     | ✓      | ✓         | ✓     | -      | ✓     |
| Grid search (via scripting)                | -       | -        | ✓     | -     | -      | -         | -     | -      | -     |
| Nelder-Mead simplex algorithm              | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| Levenberg-Maquardt algorithm               | ✓       | ✓        | ✓     | ✓     | -      | ✓         | ✓     | ✓      | -     |
| Numeric gradient approximation             | ✓       | ✓        | ✓     | ✓     | -      | ✓         | ✓     | ✓      | -     |
| Logarithmic-barrier constraint algorithm   | -       | -        | -     | -     | -      | -         | ✓     | -      | ✓     |
| MATLAB interior-point black magic          | -       | -        | -     | -     | ✓      | -         | -     | -      | -     |
| Visualisation of the chi-squared space     | -       | -        | -     | -     | ✓      | -         | -     | -      | ✓     |
| <b>Error propagation</b>                   |         |          |       |       |        |           |       |        |       |
| Covariance matrix (lowest quality)         | ✓       | ✓        | ✓     | -     | ✓      | ✓         | ✓     | -      | -     |
| Jackknife simulations (for missing errors) | ✓       | -        | -     | -     | -      | -         | ✓     | -      | -     |
| Bootstrapping simulations (false errors)   | -       | -        | -     | -     | -      | -         | -     | -      | -     |
| Monte Carlo simulations (gold standard)    | ✓       | -        | -     | ✓     | ✓      | -         | ✓     | ✓      | ✓     |
| <b>Inbuilt statistical comparisons</b>     |         |          |       |       |        |           |       |        |       |
| Akaike's Information Criterion (AIC)       | -       | -        | -     | ✓     | -      | -         | -     | -      | ✓     |
| Small sample size AIC (AICc)               | -       | -        | -     | ✓     | -      | -         | -     | -      | ✓     |
| Bayesian Information Criterion (BIC)       | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| F-testing (ANOVA statistics)               | -       | -        | -     | ✓     | ✓      | -         | -     | -      | -     |
| <b>Data and model testing</b>              |         |          |       |       |        |           |       |        |       |
| Insignificant dispersion curve tests       | -       | -        | -     | ✓     | -      | -         | -     | -      | ✓     |
| Model elimination tests                    | -       | -        | -     | -     | -      | -         | -     | -      | ✓     |
| <b>Programming languages</b>               |         |          |       |       |        |           |       |        |       |
| Python                                     | -       | -        | -     | ✓     | -      | ✓         | -     | ✓      | ✓     |
| C                                          | -       | ✓        | ✓     | -     | -      | -         | ✓     | -      | ✓     |
| Perl                                       | -       | -        | -     | -     | -      | -         | ✓     | -      | -     |
| MATLAB                                     | -       | -        | -     | -     | ✓      | -         | -     | -      | -     |
| FORTRAN                                    | ✓       | -        | -     | -     | -      | -         | -     | -      | -     |
| Ruby on Rails                              | -       | -        | -     | -     | -      | ✓         | -     | -      | -     |
| <b>Open source licencing</b>               |         |          |       |       |        |           |       |        |       |
| GNU General Public Licence (version 2+)    | ✓       | -        | -     | -     | -      | -         | -     | -      | -     |
| GNU General Public Licence (version 3+)    | -       | -        | -     | ✓     | ✓      | -         | -     | ✓      | ✓     |
| Open source infrastructure                 | -       | -        | -     | ✓     | ✓      | -         | -     | ✓      | ✓     |
| Proprietary                                | -       | ✓        | ✓     | -     | -      | ✓         | ✓     | -      | -     |



## 11.13 Analysing dispersion in the prompt/script UI mode

Before reading this section, please read Chapter 4 covering the relax data model first. It will explain many of the concepts used within the following example script. For detailed information on how to run a relax script, please see section 1.2.8 on page 11. The dispersion analysis is parallelised on the level of the spin cluster and Monte Carlo simulations so, if you have access to an MPI cluster or multi-core system with OpenMPI installed, please see section 1.3 on page 17 for how to run the calculations much quicker.

### 11.13.1 Dispersion script mode – the sample script

The following is a verbatim copy of the contents of the `sample_scripts/relax_disp/cpmg_analysis.py` file. You will need to first copy this script to a dedicated analysis directory containing peak lists, a sequence or PDB file and a file listing unresolved spin systems, and then modify its contents to suit your specific analysis. The script contents are:

```

1  """Script for performing a full relaxation dispersion analysis using CPMG-type data."""
2
3
4  # Python module imports.
5  from os import sep
6
7  # relax module imports.
8  from auto_analyses.relax_disp import Relax_disp
9
10
11 # Analysis variables.
12 #####
13
14 # The dispersion models.
15 MODELS = ['R2eff', 'No Rex', 'CR72', 'N2 CPMG 2-site expanded']
16
17 # The grid search size (the number of increments per dimension).
18 GRID_INC = 11
19
20 # The number of Monte Carlo simulations to be used for error analysis at the end of the
    analysis.
21 MC_NUM = 500
22
23 # The results directory.
24 RESULTS_DIR = '.'
25
26 # The model selection technique to use.
27 MODSEL = 'AIC'
28
29 # The flag for only using numeric models in the final model selection.
30 NUMERIC_ONLY = False
31
32 # The R2eff value in rad/s by which to judge insignificance. If the maximum difference
    between two points on all dispersion curves for a spin is less than this value, that
    spin will be deselected.
```

```

33 INSIGNIFICANCE = 1.0
34
35
36
37 # Set up the data pipe.
38 #####
39
40 # Create the data pipe.
41 pipe_name = 'base pipe'
42 pipe_bundle = 'relax_disp'
43 pipe.create(pipe_name=pipe_name, bundle=pipe_bundle, pipe_type='relax_disp')
44
45 # Load the sequence.
46 sequence.read('fake_sequence.in', res_num_col=1, res_name_col=2)
47
48 # Name the spins so they can be matched to the assignments, and the isotope for field
   strength scaling.
49 spin.name(name='N')
50 spin.isotope(isotope='15N')
51
52 # The spectral data - spectrum ID, peak list file name, CPMG frequency (Hz), spectrometer
   frequency in Hertz.
53 data = [
54     ['500_reference.in',      '500_MHz'+sep+'reference.in_sparky',      None, 500e6],
55     ['500_66.667.in',        '500_MHz'+sep+'66.667.in_sparky',        66.6666, 500e6],
56     ['500_133.33.in',        '500_MHz'+sep+'133.33.in_sparky',        133.3333, 500e6],
57     ['500_133.33.in.bis',    '500_MHz'+sep+'133.33.in.bis_sparky',    133.3333, 500e6],
58     ['500_200.in',           '500_MHz'+sep+'200.in_sparky',           200.0000, 500e6],
59     ['500_266.67.in',        '500_MHz'+sep+'266.67.in_sparky',        266.6666, 500e6],
60     ['500_333.33.in',        '500_MHz'+sep+'333.33.in_sparky',        333.3333, 500e6],
61     ['500_400.in',           '500_MHz'+sep+'400.in_sparky',           400.0000, 500e6],
62     ['500_466.67.in',        '500_MHz'+sep+'466.67.in_sparky',        466.6666, 500e6],
63     ['500_533.33.in',        '500_MHz'+sep+'533.33.in_sparky',        533.3333, 500e6],
64     ['500_533.33.in.bis',    '500_MHz'+sep+'533.33.in.bis_sparky',    533.3333, 500e6],
65     ['500_600.in',           '500_MHz'+sep+'600.in_sparky',           600.0000, 500e6],
66     ['500_666.67.in',        '500_MHz'+sep+'666.67.in_sparky',        666.6666, 500e6],
67     ['500_733.33.in',        '500_MHz'+sep+'733.33.in_sparky',        733.3333, 500e6],
68     ['500_800.in',           '500_MHz'+sep+'800.in_sparky',           800.0000, 500e6],
69     ['500_866.67.in',        '500_MHz'+sep+'866.67.in_sparky',        866.6666, 500e6],
70     ['500_933.33.in',        '500_MHz'+sep+'933.33.in_sparky',        933.3333, 500e6],
71     ['500_933.33.in.bis',    '500_MHz'+sep+'933.33.in.bis_sparky',    933.3333, 500e6],
72     ['500_1000.in',          '500_MHz'+sep+'1000.in_sparky',          1000.0000, 500e6],
73     ['800_reference.in',      '800_MHz'+sep+'reference.in_sparky',      None, 800e6],
74     ['800_66.667.in',        '800_MHz'+sep+'66.667.in_sparky',        66.6666, 800e6],
75     ['800_133.33.in',        '800_MHz'+sep+'133.33.in_sparky',        133.3333, 800e6],
76     ['800_133.33.in.bis',    '800_MHz'+sep+'133.33.in.bis_sparky',    133.3333, 800e6],
77     ['800_200.in',           '800_MHz'+sep+'200.in_sparky',           200.0000, 800e6],
78     ['800_266.67.in',        '800_MHz'+sep+'266.67.in_sparky',        266.6666, 800e6],
79     ['800_333.33.in',        '800_MHz'+sep+'333.33.in_sparky',        333.3333, 800e6],
80     ['800_400.in',           '800_MHz'+sep+'400.in_sparky',           400.0000, 800e6],
81     ['800_466.67.in',        '800_MHz'+sep+'466.67.in_sparky',        466.6666, 800e6],
82     ['800_533.33.in',        '800_MHz'+sep+'533.33.in_sparky',        533.3333, 800e6],
83     ['800_533.33.in.bis',    '800_MHz'+sep+'533.33.in.bis_sparky',    533.3333, 800e6],
84     ['800_600.in',           '800_MHz'+sep+'600.in_sparky',           600.0000, 800e6],
85     ['800_666.67.in',        '800_MHz'+sep+'666.67.in_sparky',        666.6666, 800e6],
86     ['800_733.33.in',        '800_MHz'+sep+'733.33.in_sparky',        733.3333, 800e6],
87     ['800_800.in',           '800_MHz'+sep+'800.in_sparky',           800.0000, 800e6],
88     ['800_866.67.in',        '800_MHz'+sep+'866.67.in_sparky',        866.6666, 800e6],
89     ['800_933.33.in',        '800_MHz'+sep+'933.33.in_sparky',        933.3333, 800e6],
90     ['800_933.33.in.bis',    '800_MHz'+sep+'933.33.in.bis_sparky',    933.3333, 800e6],
91     ['800_1000.in',          '800_MHz'+sep+'1000.in_sparky',          1000.0000, 800e6]

```

```

92 ]
93
94 # Loop over the spectra.
95 for id, file, cpmg_frq, H_frq in data:
96     # Load the peak intensities.
97     spectrum.read_intensities(file=file, spectrum_id=id, int_method='height')
98
99     # Set the relaxation dispersion experiment type.
100     relax_disp.exp_type(spectrum_id=id, exp_type='SQ CPMG')
101
102     # Set the relaxation dispersion CPMG frequencies.
103     relax_disp.cpmg_setup(spectrum_id=id, cpmg_frq=cpmg_frq)
104
105     # Set the NMR field strength of the spectrum.
106     spectrometer.frequency(id=id, frq=H_frq)
107
108     # Relaxation dispersion CPMG constant time delay T (in s).
109     relax_disp.relax_time(spectrum_id=id, time=0.030)
110
111 # Specify the duplicated spectra.
112 spectrum.replicated(spectrum_ids=['500_133.33.in', '500_133.33.in.bis'])
113 spectrum.replicated(spectrum_ids=['500_533.33.in', '500_533.33.in.bis'])
114 spectrum.replicated(spectrum_ids=['500_933.33.in', '500_933.33.in.bis'])
115 spectrum.replicated(spectrum_ids=['800_133.33.in', '800_133.33.in.bis'])
116 spectrum.replicated(spectrum_ids=['800_533.33.in', '800_533.33.in.bis'])
117 spectrum.replicated(spectrum_ids=['800_933.33.in', '800_933.33.in.bis'])
118
119 # Peak intensity error analysis.
120 spectrum.error_analysis(subset=['500_reference.in', '500_66.667.in', '500_133.33.in', '500_133.33.in.bis', '500_200.in', '500_266.67.in', '500_333.33.in', '500_400.in', '500_466.67.in', '500_533.33.in', '500_533.33.in.bis', '500_600.in', '500_666.67.in', '500_733.33.in', '500_800.in', '500_866.67.in', '500_933.33.in', '500_933.33.in.bis', '500_1000.in'])
121 spectrum.error_analysis(subset=['800_reference.in', '800_66.667.in', '800_133.33.in', '800_133.33.in.bis', '800_200.in', '800_266.67.in', '800_333.33.in', '800_400.in', '800_466.67.in', '800_533.33.in', '800_533.33.in.bis', '800_600.in', '800_666.67.in', '800_733.33.in', '800_800.in', '800_866.67.in', '800_933.33.in', '800_933.33.in.bis', '800_1000.in'])
122
123 # Deselect unresolved spins.
124 deselect.read(file='unresolved', dir='500_MHz', res_num_col=1)
125 deselect.read(file='unresolved', dir='800_MHz', res_num_col=1)
126
127
128
129 # Auto-analysis execution.
130 #####
131
132 # Do not change!
133 Relax_disp(pipe_name=pipe_name, pipe_bundle=pipe_bundle, results_dir=RESULTS_DIR, models=
    MODELS, grid_inc=GRID_INC, mc_sim_num=MC_NUM, modsel=MODSEL, insignificance=
    INSIGNIFICANCE, numeric_only=NUMERIC_ONLY)

```

### 11.13.2 Dispersion script mode – imports

At the very start of the script are two import statements. This is simply the standard Python import system for modules. The first will import the `sep` variable which is the operating system independent directory separator:

```

4  # Python module imports.
5  from os import sep

```

This `sep` variable will be used later on in the script. The second import is that of the automated relaxation dispersion class `Relax_disp` which will be used at the very end of the script to perform the full analysis:

```

7  # relax module imports.
8  from auto_analyses.relax_disp import Relax_disp

```

### 11.13.3 Dispersion script mode – analysis variables

The next part of the script is the definition of a number of analysis variables. As the example in this section is for CPMG-type experiments, the relaxation dispersion models which will be used in the auto-analysis are:

```

14 # The dispersion models.
15 MODELS = ['R2eff', 'No Rex', 'CR72', 'N2 CPMG 2-site expanded']

```

This list can be expanded to most of the 2-site exchange models, for example as:

```
MODELS = ['R2eff', 'No Rex', 'LM63', 'CR72', 'IT99', 'TSMFK01', 'NS CPMG 2-site expanded']
```

But note that the selection of which models to use is incredibly important. Do not use models which are not suitable for the data as that will cause the final results to contain rubbish. If you have  $R_{1\rho}$ -type off-resonance data, the models could be changed to:

```
MODELS = ['R2eff', 'No Rex', 'DPL94', 'NS R1rho 2-site']
```

The next variable affects the optimisation precision:

```

17 # The grid search size (the number of increments per dimension).
18 GRID_INC = 21

```

The number of grid search increments may be decreased, but only after careful checking with a higher number of increments. Setting this value too low may place the initial optimisation too far away from the minimum. Although as-of-yet undetected and unpublished, if multiple local minima do exist then optimisation may not reach the global minimum. Too little grid search increments can also cause the total optimisation time to increase as the Nelder-Mead simplex optimisation together with the Logarithmic-barrier penalty function as used in the auto-analysis may require more time to reach the minimum.

The Monte Carlo simulation number `MC_NUM` variable affects the error estimate precision:

```

20 # The number of Monte Carlo simulations to be used for error analysis at the end of the
    analysis.
21 MC_NUM = 500

```

For accurate parameter errors this number should not be decreased. Ideally it should be increased however this will significantly increase the total analysis time. The next variable allows you to change the directory in which all results files from the auto-analysis will be saved.

```

23 # The results directory.
24 RESULTS_DIR = '.'

```

The MODSEL variable defines how the best dispersion model for the measured data is chosen:

```
26 # The model selection technique to use.
27 MODSEL = 'AIC'
```

For the automated analysis, currently only AIC, AICc, and BIC are supported. For details about these frequentist model selection techniques and their application to NMR data, see [d'Auvergne and Gooley \(2003\)](#). Post-analysis comparisons can also be preformed if desired. The NUMERIC\_ONLY variable can be used to choose if only numeric or all models will be used in the model selection for the final results:

```
29 # The flag for only using numeric models in the final model selection.
30 NUMERIC_ONLY = False
```

To only use numeric models in the model selection while allowing models such as 'CR72' to be optimised and used as the starting point for the numeric models, change this variable to:

```
NUMERIC_ONLY = True
```

The last variable allows spins with insignificant dispersion profiles to be deselected:

```
32 # The R2eff value in rad/s by which to judge insignificance. If the maximum difference
    between two points on all dispersion curves for a spin is less than this value, that
    spin will be deselected.
33 INSIGNIFICANCE = 1.0
```

This is often needed due to the errors in the dispersion curves being underestimated, hence the 'No Rex' model is not selected when clearly it should be. To use all data in the analysis, this variable should be set to 0.0.

#### 11.13.4 Dispersion script mode – initialisation of the data pipe

The data pipe is created using the lines:

```
40 # Create the data pipe.
41 pipe_name = 'base pipe'
42 pipe_bundle = 'relax_disp'
43 pipe.create(pipe_name=pipe_name, bundle=pipe_bundle, pipe_type='relax_disp')
```

The first two lines define variables for the data pipe name and the pipe bundle name. The pipe bundle is used to group together all of the data pipes created by the automated protocol. See section [4.2.1](#) on page [34](#) for more details.

The `pipe.create` user function will then create a relaxation dispersion specific data pipe labelled with the pipe and bundle names. The third argument sets the pipe type to that of relaxation dispersion. The rest of the script is used to fill this base data pipe with all of the data required for a dispersion analysis. The auto-analysis will then copy the data from this pipe as it sees fit.

### 11.13.5 Dispersion script mode – setting up the spin systems

The first thing which needs to be completed prior to any spin specific command is to generate the molecule, residue and spin data structures for storing the spin specific data. In the sample script above, this is generated from a plain text file with the sequence information, however a PDB file can be used instead (see the `structure.read_pdb` user function on page 634 for more details). In the case of the sample script, the command:

```
45 # Load the sequence.
46 sequence.read('fake_sequence.in', res_num_col=1, res_name_col=2)
```

will load residue names and numbers from the `fake_sequence.in` file into `relax`, creating one spin per residue. Then:

```
48 # Name the spins so they can be matched to the assignments, and the isotope for field
    strength scaling.
49 spin.name(name='N')
50 spin.isotope(isotope='15N')
```

will set up the spin information required for loading the peak intensity data from Sparky peak lists and for the analysis of the dispersion data.

### 11.13.6 Dispersion script mode – loading the data

To load the peak intensities into `relax`, a large data structure is first defined:

```
52 # The spectral data - spectrum ID, peak list file name, CPMG frequency (Hz), spectrometer
    frequency in Hertz.
53 data = [
54     ['500_reference.in',      '500_MHz'+sep+'reference.in_sparky',      None, 500e6],
55     ['500_66.667.in',        '500_MHz'+sep+'66.667.in_sparky',        66.6666, 500e6],
56     ['500_133.33.in',         '500_MHz'+sep+'133.33.in_sparky',         133.3333, 500e6],
57     ['500_133.33.in.bis',     '500_MHz'+sep+'133.33.in.bis_sparky',     133.3333, 500e6],
58     ['500_200.in',           '500_MHz'+sep+'200.in_sparky',           200.0000, 500e6],
59     ['500_266.67.in',         '500_MHz'+sep+'266.67.in_sparky',         266.6666, 500e6],
60     ['500_333.33.in',         '500_MHz'+sep+'333.33.in_sparky',         333.3333, 500e6],
61     ['500_400.in',           '500_MHz'+sep+'400.in_sparky',           400.0000, 500e6],
62     ['500_466.67.in',         '500_MHz'+sep+'466.67.in_sparky',         466.6666, 500e6],
63     ['500_533.33.in',         '500_MHz'+sep+'533.33.in_sparky',         533.3333, 500e6],
64     ['500_533.33.in.bis',     '500_MHz'+sep+'533.33.in.bis_sparky',     533.3333, 500e6],
65     ['500_600.in',           '500_MHz'+sep+'600.in_sparky',           600.0000, 500e6],
66     ['500_666.67.in',         '500_MHz'+sep+'666.67.in_sparky',         666.6666, 500e6],
67     ['500_733.33.in',         '500_MHz'+sep+'733.33.in_sparky',         733.3333, 500e6],
68     ['500_800.in',           '500_MHz'+sep+'800.in_sparky',           800.0000, 500e6],
69     ['500_866.67.in',         '500_MHz'+sep+'866.67.in_sparky',         866.6666, 500e6],
70     ['500_933.33.in',         '500_MHz'+sep+'933.33.in_sparky',         933.3333, 500e6],
71     ['500_933.33.in.bis',     '500_MHz'+sep+'933.33.in.bis_sparky',     933.3333, 500e6],
72     ['500_1000.in',           '500_MHz'+sep+'1000.in_sparky',          1000.0000, 500e6],
73     ['800_reference.in',      '800_MHz'+sep+'reference.in_sparky',      None, 800e6],
74     ['800_66.667.in',        '800_MHz'+sep+'66.667.in_sparky',        66.6666, 800e6],
75     ['800_133.33.in',         '800_MHz'+sep+'133.33.in_sparky',         133.3333, 800e6],
76     ['800_133.33.in.bis',     '800_MHz'+sep+'133.33.in.bis_sparky',     133.3333, 800e6],
77     ['800_200.in',           '800_MHz'+sep+'200.in_sparky',           200.0000, 800e6],
78     ['800_266.67.in',         '800_MHz'+sep+'266.67.in_sparky',         266.6666, 800e6],
79     ['800_333.33.in',         '800_MHz'+sep+'333.33.in_sparky',         333.3333, 800e6],
80     ['800_400.in',           '800_MHz'+sep+'400.in_sparky',           400.0000, 800e6],
81     ['800_466.67.in',         '800_MHz'+sep+'466.67.in_sparky',         466.6666, 800e6],
```

```

82     ['800_533.33.in',      '800_MHz'+sep+'533.33.in_sparky',      533.3333,  800e6],
83     ['800_533.33.in.bis', '800_MHz'+sep+'533.33.in.bis_sparky', 533.3333,  800e6],
84     ['800_600.in',        '800_MHz'+sep+'600.in_sparky',        600.0000,  800e6],
85     ['800_666.67.in',     '800_MHz'+sep+'666.67.in_sparky',     666.6666,  800e6],
86     ['800_733.33.in',     '800_MHz'+sep+'733.33.in_sparky',     733.3333,  800e6],
87     ['800_800.in',        '800_MHz'+sep+'800.in_sparky',        800.0000,  800e6],
88     ['800_866.67.in',     '800_MHz'+sep+'866.67.in_sparky',     866.6666,  800e6],
89     ['800_933.33.in',     '800_MHz'+sep+'933.33.in_sparky',     933.3333,  800e6],
90     ['800_933.33.in.bis', '800_MHz'+sep+'933.33.in.bis_sparky', 933.3333,  800e6],
91     ['800_1000.in',       '800_MHz'+sep+'1000.in_sparky',       1000.0000, 800e6]
92 ]

```

In Python terminology, this is a list of lists data structure. It is essentially a matrix of information which is used in the subsequent for loop. The comment explains what each element is. For  $R_{1\rho}$ -type experiments, the CPMG frequency column can be replaced with the spin-lock field strength. This data structure will need to be tailored to your data. It can be seen that the `sep` variable is now being used to specify that the Sparky files are either located in the 500\_MHz or 800\_MHz directories. It is used here to make this script independent of the operating system.

The Python for loop starts with the lines:

```

94 # Loop over the spectra.
95 for id, file, cpmg_frq, H_frq in data:

```

and includes all subsequently indented lines. This line of code takes the elements of the `data` data structure and splits it into 4 variables. Therefore for the first line, `id` will be set to '500\_reference.in', `file` will be set to '500\_MHz/reference.in\_sparky' on a Linux machine, `cpmg_frq` will be None, and `H_frq` will be 500 MHz. For  $R_{1\rho}$ -type data, you could change the `cpmg_frq` variable to `field` for example.

The first user function in the block loads the peak intensity data from the peak lists:

```

96     # Load the peak intensities.
97     spectrum.read_intensities(file=file, spectrum_id=id, int_method='height')

```

This assumes that peak heights were measured. All data will be tagged with the given ID string. For examples of peak list formats supported by `relax`, see Section 5.4.4 on page 57. The next step is to specify the dispersion experiment type for each spectrum:

```

99     # Set the relaxation dispersion experiment type.
100    relax_disp.exp_type(spectrum_id=id, exp_type='SQ CPMG')

```

This can be 'SQ CPMG', 'DQ CPMG', 'ZQ CPMG', 'MQ CPMG', '1H SQ CPMG', '1H MQ CPMG' or 'R1rho'. The next user function sets the CPMG frequencies for each spectrum:

```

102    # Set the relaxation dispersion CPMG frequencies.
103    relax_disp.cpmg_setup(spectrum_id=id, cpmg_frq=cpmg_frq)

```

For an  $R_{1\rho}$ -type experiment, these lines could be changed to:

```

    # Set the relaxation dispersion R1rho spin lock field strength.
    relax_disp.spin_lock_field(spectrum_id=id, field=field)

```

Then the NMR spectrometer field strength is set:



```

105     # Set the NMR field strength of the spectrum.
106     spectrometer.frequency(id=id, frq=H_frq)

```

And finally the relaxation time period is set with:

```

108     # Relaxation dispersion CPMG constant time delay T (in s).
109     relax_disp.relax_time(spectrum_id=id, time=0.030)

```

If exponential data has been collected rather than fixed time period data, then the data structure can have an additional column added for the relaxation times, and then this same user function can be used. The for loop will need one extra variable for the times, and this should be passed into this `relax_disp.relax_time` user function for the time argument.

Finally, once the for loop has completed, replicated spectra are defined with the commands:

```

111 # Specify the duplicated spectra.
112 spectrum.replicated(spectrum_ids=['500_133.33.in', '500_133.33.in.bis'])
113 spectrum.replicated(spectrum_ids=['500_533.33.in', '500_533.33.in.bis'])
114 spectrum.replicated(spectrum_ids=['500_933.33.in', '500_933.33.in.bis'])
115 spectrum.replicated(spectrum_ids=['800_133.33.in', '800_133.33.in.bis'])
116 spectrum.replicated(spectrum_ids=['800_533.33.in', '800_533.33.in.bis'])
117 spectrum.replicated(spectrum_ids=['800_933.33.in', '800_933.33.in.bis'])

```

### 11.13.7 Dispersion script mode – the rest of the setup

Once all the peak intensity data has been loaded a few calculations are required prior to optimisation. Firstly the peak intensities for individual spins needs to be averaged across replicated spectra. The peak intensity errors also have to be calculated using the standard deviation formula. These two operations are executed by the user functions:

```

119 # Peak intensity error analysis.
120 spectrum.error_analysis(subset=['500_reference.in', '500_66.667.in', '500_133.33.in', '500_
    _133.33.in.bis', '500_200.in', '500_266.67.in', '500_333.33.in', '500_400.in', '500_
    _466.67.in', '500_533.33.in', '500_533.33.in.bis', '500_600.in', '500_666.67.in', '500_
    _733.33.in', '500_800.in', '500_866.67.in', '500_933.33.in', '500_933.33.in.bis', '500_
    _1000.in'])
121 spectrum.error_analysis(subset=['800_reference.in', '800_66.667.in', '800_133.33.in', '800_
    _133.33.in.bis', '800_200.in', '800_266.67.in', '800_333.33.in', '800_400.in', '800_
    _466.67.in', '800_533.33.in', '800_533.33.in.bis', '800_600.in', '800_666.67.in', '800_
    _733.33.in', '800_800.in', '800_866.67.in', '800_933.33.in', '800_933.33.in.bis', '800_
    _1000.in'])

```

Here the 500 MHz and 800 MHz peak intensity errors have been calculated separately as they should not be the same.

Any spins which cannot be resolved due to peak overlap were included in a file called `unresolved`. This file can consist of optional columns of the molecule name, the residue name and number, and the spin name and number. The matching spins are excluded from the analysis by the user functions:

```

123 # Deselect unresolved spins.
124 deselect.read(file='unresolved', dir='500_MHz', res_num_col=1)
125 deselect.read(file='unresolved', dir='800_MHz', res_num_col=1)

```



### 11.13.8 Dispersion script mode – execution

Once the data has set up and you have modified your script to match your analysis needs, then the data pipe, pipe bundle and analysis variables are passed into the `Relax_disp` class. This is the final lines of the script:

```
129 # Auto-analysis execution.
130 #####
131
132 # Do not change!
133 Relax_disp(pipe_name=pipe_name, pipe_bundle=pipe_bundle, results_dir=RESULTS_DIR, models=
    MODELS, grid_inc=GRID_INC, mc_sim_num=MC_NUM, modsel=MODSEL, insignificance=
    INSIGNIFICANCE, numeric_only=NUMERIC_ONLY)
```

This will start the auto-analysis. If you are adventurous, you can replace this line with your own `minimise.grid_search`, `minimise.execute`, and `monte_carlo.*` user function calls and design your own protocol. For ideas in designing your own advanced analysis, see the `auto_analysis/relax_disp.py` file.

## 11.14 The relaxation dispersion auto-analysis in the GUI

The following demonstration of the relaxation dispersion analysis in the graphical user interface (GUI) uses the experimental single quantum (SQ) CPMG-type data from Flemming Hansen located in the relax directory `test_suite/shared_data/dispersion/Hansen`. This is the data from the paper:

- Hansen, D. F., Vallurupalli, P., Lundstrom, P., Neudecker, P., and Kay, L. E. (2008). Probing chemical shifts of invisible states of proteins with relaxation dispersion NMR spectroscopy: how well can we do? *J. Am. Chem. Soc.*, **130**(8), 2667–2675. ([10.1021/ja078337p](https://doi.org/10.1021/ja078337p))

More details can be seen in the `README` file in that directory. By using the data in this directory and following the instructions below, a complete dispersion analysis can be performed.

Note that the dispersion analysis in the GUI uses the automated protocol as implemented in the `auto_analyses/relax_disp.py` relax script. If you wish to perform a custom analysis or implement your own protocol, please use the prompt/scripting user interface instead to have access to the full flexibility and power of relax.

When running the analysis, it is best to keep a permanent log of all of the printouts, warnings, errors and messages produced by relax. Such information will be an invaluable reference when comparing the non-clustered and clustered results, as well as understanding what happened to each spin system under study. This can be achieved by running relax with the command line options:

```
$ relax --log log --gui
```

All output from relax will then be visible both in the relax controller window (see Figure 1.9 on page 19) and in the `log` file. Other relax options can be seen by running:

```
$ relax --help
```

### 11.14.1 Dispersion GUI mode – two analyses

To process this test data, two separate analyses will be performed:

- The first analysis will consist of treating all spins independently from each other. This will use model selection to determine if any statistically significant relaxation dispersion is present by comparing to the ‘No Rex’ dispersion model.
- The second analysis will consist of clustering spins with similar kinetics and thermodynamics parameters (exchange rates and populations respectively) and optimising these clusters using a common set of exchange parameters.

In this example, Gary Thompson’s multiprocessor framework (see section 11.9.11 on page 194) will be used with OpenMPI on a single four-core, hyper-threaded computer with the command:

```
$ mpirun -np 8 /data/relax/relax-trunk/relax --multi='mpi4py' --log
~/tmp/dispersion/log_non-clustered --gui
```

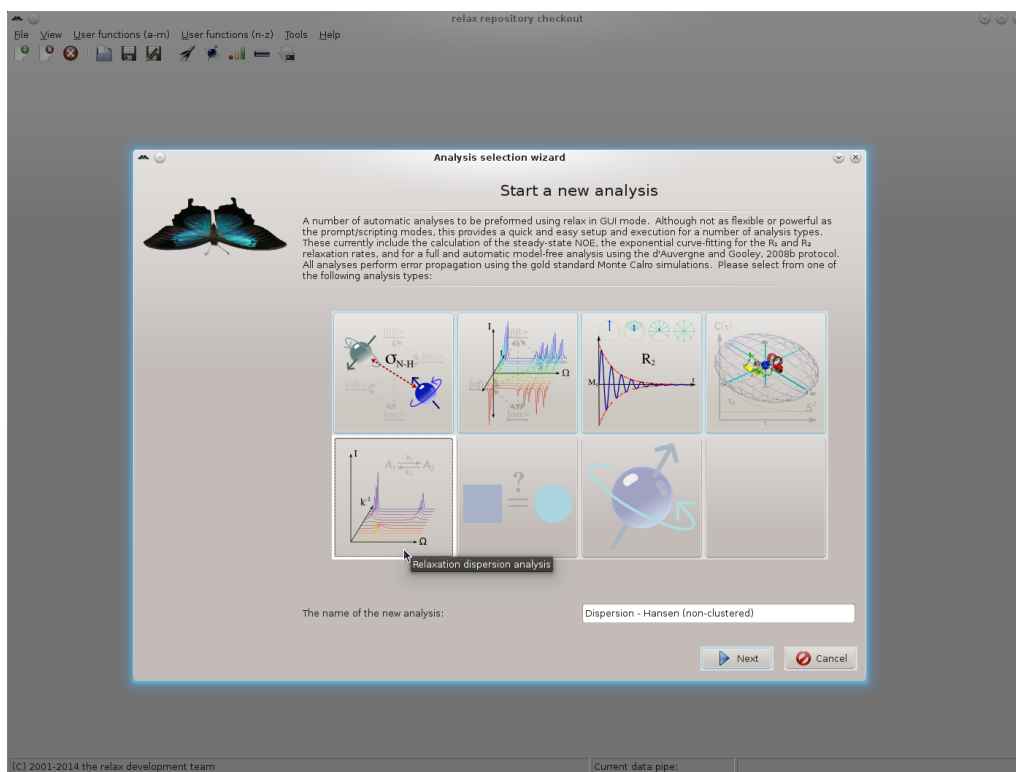
Note that the `~/tmp/dispersion` directory should be created before starting relax. If running on a system where your home directory is not defined as `~/`, MS Windows for example, the `~` part will need to be replaced with some other directory. A different command will be used for the clustered analysis to store the log messages in a separate file. The two logs will be used to compare the two analysis types at the end.

### 11.14.2 Dispersion GUI mode – computation time

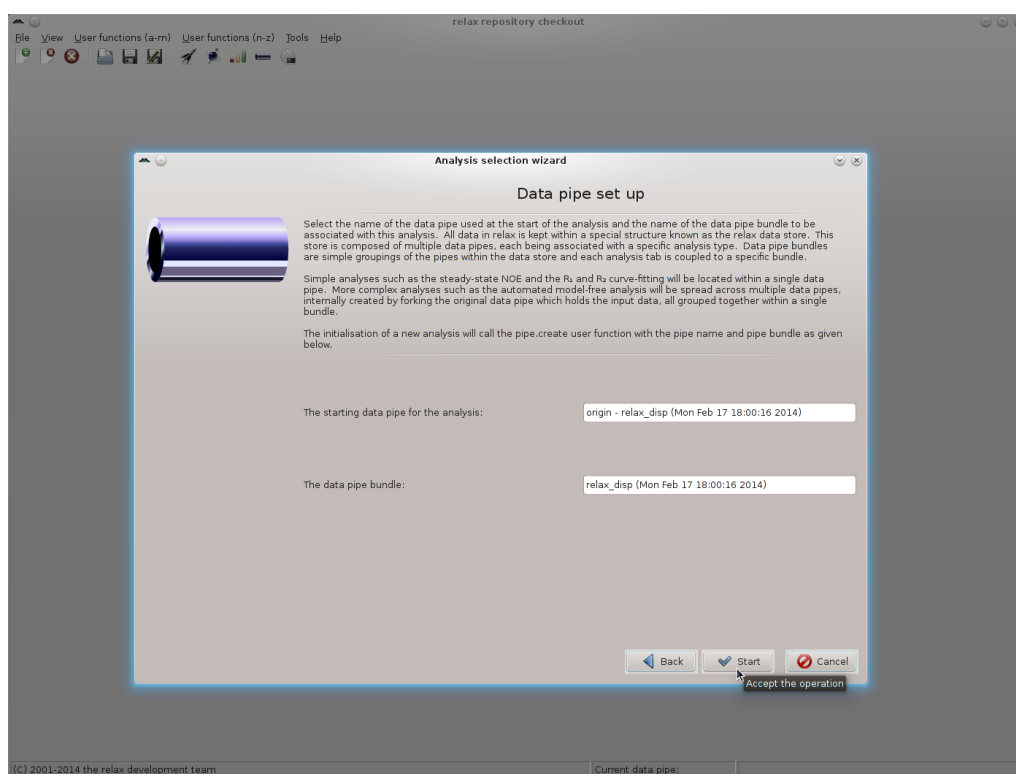
The time required to complete these two analyses is highly dependent on the computer being used as well as how many nodes can be used for running the calculations parallelised with OpenMPI. On a large cluster with many nodes both analyses should be completed in under an hour. But when running the analyses without OpenMPI on old single core computer, the analyses could take days and even up to a week.

### 11.14.3 Dispersion GUI mode – initialisation of the data pipe

After starting relax in the GUI mode, the dispersion analysis should be initialised by launching the analysis selection wizard (see Figure 1.4 on page 12). The relaxation dispersion analysis should be selected and the name changed if multiple dispersion analyses are to be performed within one relax session. In this case the name “Dispersion - Hansen (non-clustered)” will be used:



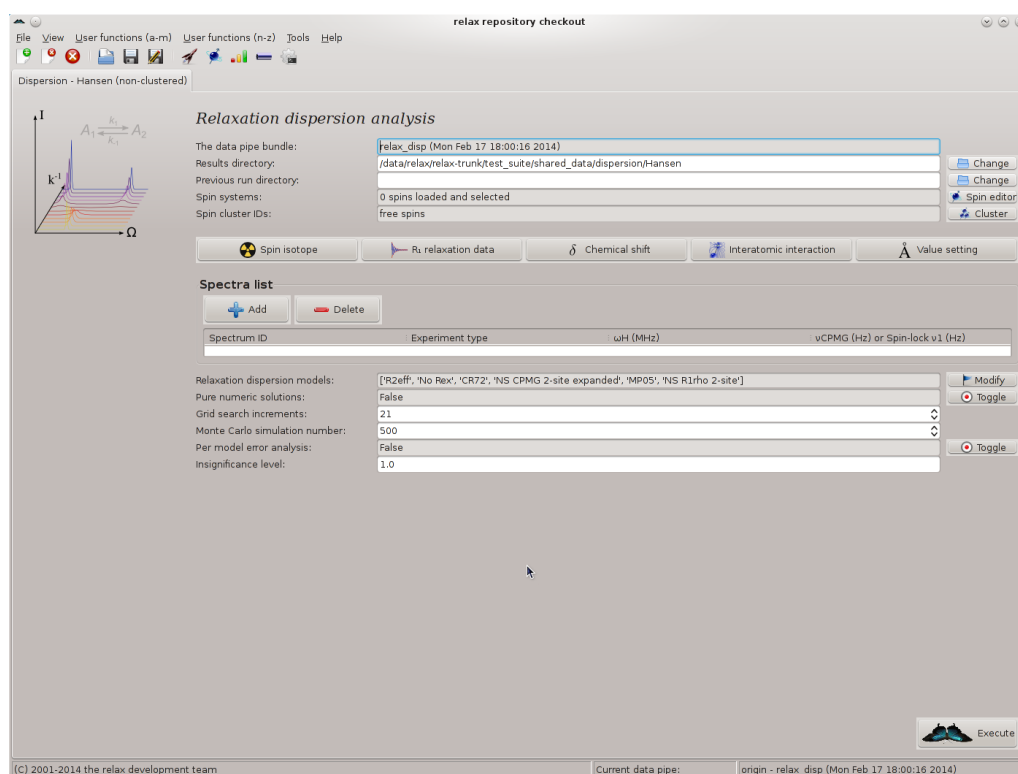
Click on the “Next” button to move to the second wizard page:



Here the values need not be changed. The data pipe bundle will be used to hold all the separate data pipes for each dispersion model type together.

#### 11.14.4 Dispersion GUI mode – general setup

A blank analysis tab should now be visible:



The first step will be to change the “Results directory” where all of the automatically created results file, 2D Grace plots, and relax state files will be saved. The directory `~/tmp/dispersion/non_clustered` will be used for this initial non-clustered analysis.

### 11.14.5 Dispersion GUI mode – setting up the spin systems

As the relaxation dispersion data is specific to individual nuclear spins, the molecule, residue and spin data structures need to be set up. For this, the special “Spin systems” GUI element can be used. The initial state will be “0 spins loaded and selected”. Click on the “Spin editor” button to launch the spin viewer window. The steps for setting up the spin containers using PDB files are described in section 4.5.2 on page 40 or for sequence files in section 4.5.3 on page 43.

In this tutorial, the sequence file `fake_sequence.in` in the `test_suite/shared_data/dispersion/Hansen` directory will be loaded. In the spin loading wizard, which can be launched by clicking on the “Load spins” button, select the “From a file containing sequence data” option and click on “Next”. In the `sequence.read` user function wizard page, select the `fake_sequence.in` file. As this file only contains residue numbers and names (click on the “preview” button to see the file contents), edit the “Free format file settings” to set the residue number and name columns to 1 and 2 respectively and all other columns to blank values. Click on “Save” to store the free format settings and close the window. Back in the `sequence.read` user function wizard page, click on “Next” to load the sequence. Finally click on “Finish” to close the wizard. Do not close the spin viewer window yet. Back in the main analysis tab, the “Spin systems” GUI element will now say “73 spins loaded and selected”.

### 11.14.6 Dispersion GUI mode – unresolved spins

As in the prompt/script UI section 11.13.7, the spins can be deselected at this point using the same `unresolved` files. This is described in detail in section 4.5.5 on page 44.

Within the open spin viewer window, click on the “User functions→deselect→read” menu item. In the `deselect.read` user function window, select the file `test_suite/shared_data/dispersion/Hansen/500_MHz/unresolved`. As this file only contains residue number, edit the “Free format file settings” to set the residue number column to 1 and all other columns to empty values. Click the “Apply” button rather than “OK” to allow a second file to be read. The relax controller window may appear and can be closed. Select the new file `test_suite/shared_data/dispersion/Hansen/800_MHz/unresolved` and click on “OK”. Now all spins from these two files will be deselected and skipped in the analysis.

### 11.14.7 Dispersion GUI mode – dispersion setup

The next step is to set up the data defining the relaxation dispersion process. This is performed by clicking on the buttons between the “Spin cluster ID” and “Spectra list” GUI elements. The buttons are as follows:

**Spin isotope:** This is needed to specify the spin isotope information for each spin in the system, for example if the data is from  $^{15}\text{N}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ , etc. The button launches the `spin.isotope` user function (see section 17.2.225 on page 616).

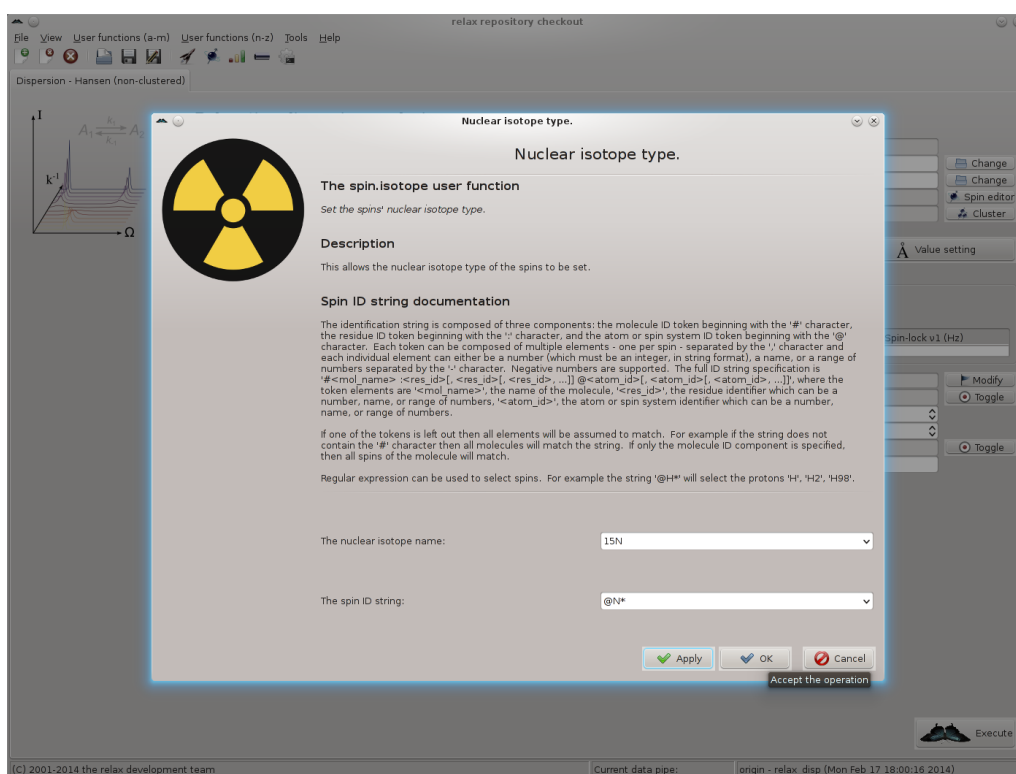
**R<sub>1</sub> relaxation data:** This is used to load a text file containing R<sub>1</sub> relaxation data for each spin of interest (see Chapter 5 for calculating R<sub>1</sub> values). The button launches the `relax_data.read` user function (see section 17.2.154 on page 569). It is currently only used for handling off-resonance effects in the R<sub>1ρ</sub>-type dispersion data but may be extended, in the future, to handle off-resonance effects in the CPMG-type experiments.

**Chemical shift:** As with the R<sub>1</sub> button, this is for properly handling off-resonance effects in the R<sub>1ρ</sub>-type dispersion data. The button launches the `chemical_shift.read` user function (see section 17.2.23 on page 455).

**Interatomic interaction:** This button launches the `interatom.define` user function (see section 17.2.57 on page 485). This is needed to link, for example,  $^1\text{H}$  to  $^{15}\text{N}$  spins into a spin system so that MMQ-type data, which relies on a multiple-spin system, can be handled.

**Value setting:** When one of the parameters of the dispersion model is already known, its value can be set by clicking on this button which launches the `value.set` user function (see section 17.2.265 on page 652). Setting a parameter value will fix the parameter in the initial grid search. However it will be free to vary in the non-linear least squares optimisation used to refine its value.

For SQ CPMG-type dispersion data, only the spin isotope information is required:



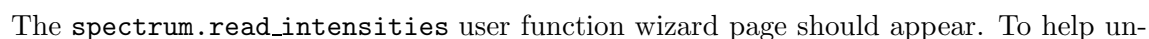
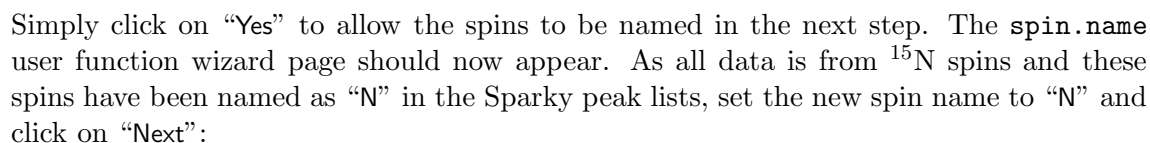
In this case, all spins are  $^{15}\text{N}$ . However as no spins are named yet, the text “@N\*” cannot be used. So simply change this to “@\*” and click on “OK”. The other buttons can be ignored.

### 11.14.8 Dispersion GUI mode – loading the data

The standard way for handling experimental NMR data for starting a relaxation dispersion analysis in the relax GUI is to load the peak intensity values (either height or volume) from a peak list. For a list of all the currently supported peak list formats, see the `spectrum.readintensities` user function documentation in section 17.2.215 on page 608.

Note that relax also accepts pre-fitted or pre-calculated  $R_{2\text{eff}}$  or  $R_{1\rho}$  values via the `relax_disp.r2eff_read` and `relax_disp.r2eff_read.spin` user functions (see section 17.2.174 on page 581 and section 17.2.175 on page 582 respectively), however this is not the standard way of using the GUI. As this is not tested, if you decide to work with pre-calculated relaxation rates please report any bugs encountered as described in section 3.3 on page 30. To access the user functions, click on “User functions→relax\_disp→r2eff\_read” or “User functions→relax\_disp→r2eff\_read.spin”.

In this tutorial, the Sparky formatted peak lists in the `test_suite/shared_data/dispersion/Hansen/500_MHz` and `test_suite/shared_data/dispersion/Hansen/800_MHz` directories will be loaded. First click on the “Add” button in the “Spectra list” GUI element. A warning message will appear as the spins have not yet been named, spin names were not present in the `fake_sequence.in` file, hence they cannot be matched to the data in the peak lists:



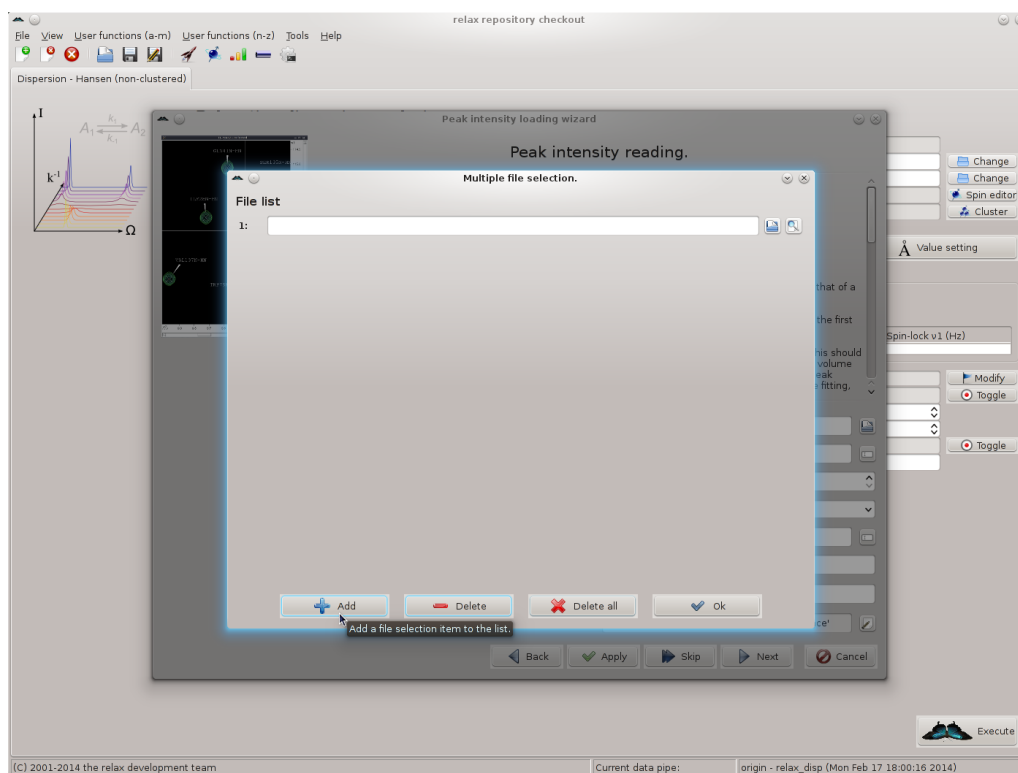


derstand the next steps of the peak intensity loading wizard, note the following table which summarises the peak list metadata for this analysis:

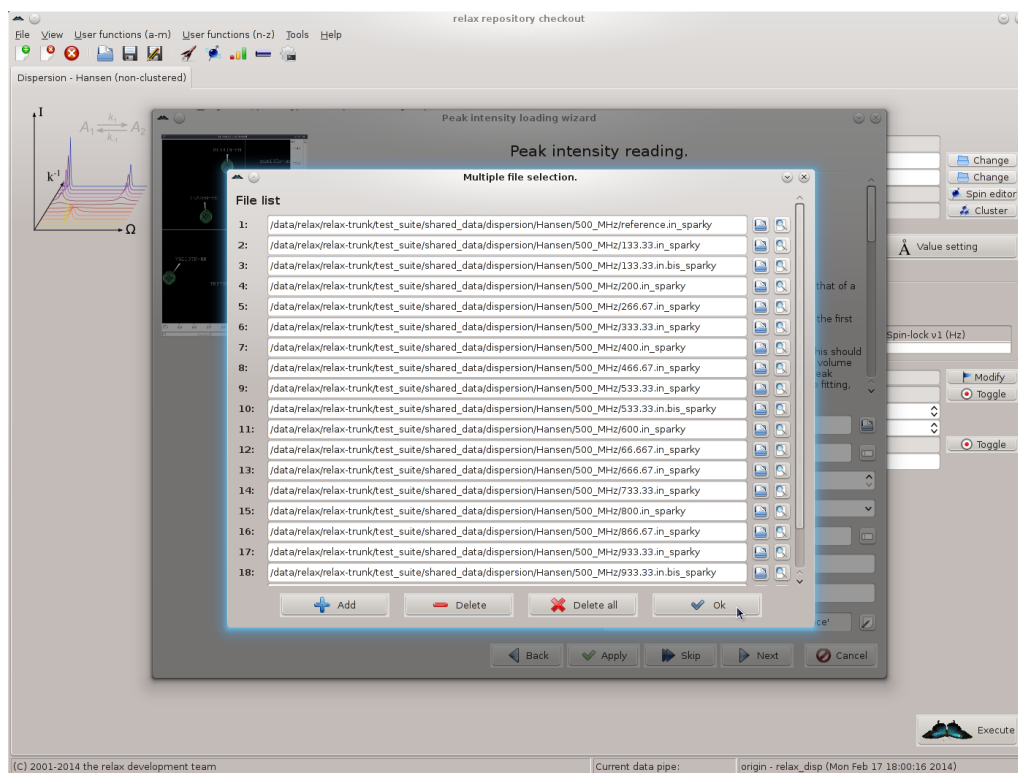
| File name                    | Spectrum ID | Spectrometer<br>frq. (MHz) | $\nu_{\text{CPMG}}$<br>(Hz) | Replicate<br>IDs |
|------------------------------|-------------|----------------------------|-----------------------------|------------------|
| 500_MHz/reference.in_sparky  | 500_ref     | 500.0                      | None                        |                  |
| 500_MHz/66.667.in_sparky     | 500_66.667  | 500.0                      | 66.6666                     |                  |
| 500_MHz/133.33.in_sparky     | 500_133.33  | 500.0                      | 133.3333                    | 500_133.33b      |
| 500_MHz/133.33.in_bis_sparky | 500_133.33b | 500.0                      | 133.3333                    | 500_133.33       |
| 500_MHz/200.in_sparky        | 500_200.00  | 500.0                      | 200.0000                    |                  |
| 500_MHz/266.67.in_sparky     | 500_266.67  | 500.0                      | 266.6666                    |                  |
| 500_MHz/333.33.in_sparky     | 500_333.33  | 500.0                      | 333.3333                    |                  |
| 500_MHz/400.in_sparky        | 500_400.00  | 500.0                      | 400.0000                    |                  |
| 500_MHz/466.67.in_sparky     | 500_466.67  | 500.0                      | 466.6666                    |                  |
| 500_MHz/533.33.in_sparky     | 500_533.33  | 500.0                      | 533.3333                    | 500_533.33b      |
| 500_MHz/533.33.in_bis_sparky | 500_533.33b | 500.0                      | 533.3333                    | 500_533.33       |
| 500_MHz/600.in_sparky        | 500_600.00  | 500.0                      | 600.0000                    |                  |
| 500_MHz/666.67.in_sparky     | 500_666.67  | 500.0                      | 666.6666                    |                  |
| 500_MHz/733.33.in_sparky     | 500_733.33  | 500.0                      | 733.3333                    |                  |
| 500_MHz/800.in_sparky        | 500_800.00  | 500.0                      | 800.0000                    |                  |
| 500_MHz/866.67.in_sparky     | 500_866.67  | 500.0                      | 866.6666                    |                  |
| 500_MHz/933.33.in_sparky     | 500_933.33  | 500.0                      | 933.3333                    | 500_933.33b      |
| 500_MHz/933.33.in_bis_sparky | 500_933.33b | 500.0                      | 933.3333                    | 500_933.33       |
| 500_MHz/1000.in_sparky       | 500_1000.0  | 500.0                      | 1000.0000                   |                  |
| 800_MHz/reference.in_sparky  | 800_ref     | 800.0                      | None                        |                  |
| 800_MHz/66.667.in_sparky     | 800_66.667  | 800.0                      | 66.6666                     |                  |
| 800_MHz/133.33.in_sparky     | 800_133.33  | 800.0                      | 133.3333                    | 800_133.33b      |
| 800_MHz/133.33.in_bis_sparky | 800_133.33b | 800.0                      | 133.3333                    | 800_133.33       |
| 800_MHz/200.in_sparky        | 800_200.00  | 800.0                      | 200.0000                    |                  |
| 800_MHz/266.67.in_sparky     | 800_266.67  | 800.0                      | 266.6666                    |                  |
| 800_MHz/333.33.in_sparky     | 800_333.33  | 800.0                      | 333.3333                    |                  |
| 800_MHz/400.in_sparky        | 800_400.00  | 800.0                      | 400.0000                    |                  |
| 800_MHz/466.67.in_sparky     | 800_466.67  | 800.0                      | 466.6666                    |                  |
| 800_MHz/533.33.in_sparky     | 800_533.33  | 800.0                      | 533.3333                    | 800_533.33b      |
| 800_MHz/533.33.in_bis_sparky | 800_533.33b | 800.0                      | 533.3333                    | 800_533.33       |
| 800_MHz/600.in_sparky        | 800_600.00  | 800.0                      | 600.0000                    |                  |
| 800_MHz/666.67.in_sparky     | 800_666.67  | 800.0                      | 666.6666                    |                  |
| 800_MHz/733.33.in_sparky     | 800_733.33  | 800.0                      | 733.3333                    |                  |
| 800_MHz/800.in_sparky        | 800_800.00  | 800.0                      | 800.0000                    |                  |
| 800_MHz/866.67.in_sparky     | 800_866.67  | 800.0                      | 866.6666                    |                  |
| 800_MHz/933.33.in_sparky     | 800_933.33  | 800.0                      | 933.3333                    | 800_933.33b      |
| 800_MHz/933.33.in_bis_sparky | 800_933.33b | 800.0                      | 933.3333                    | 800_933.33       |
| 800_MHz/1000.in_sparky       | 800_1000.0  | 800.0                      | 1000.0000                   |                  |

For all of these files, the experiment type is SQ CPMG and the relaxation delay time is 30 ms. All files are located in the `test_suite/shared_data/dispersion/Hansen` base directory.

To simplify the loading of the data, all of the 500 MHz data will be read simultaneously (followed by all of the 800 MHz data). Firstly, to select all of the 500 MHz files, click on the button at the end of the “file name(s)” GUI element. This will launch the multiple file selection window:

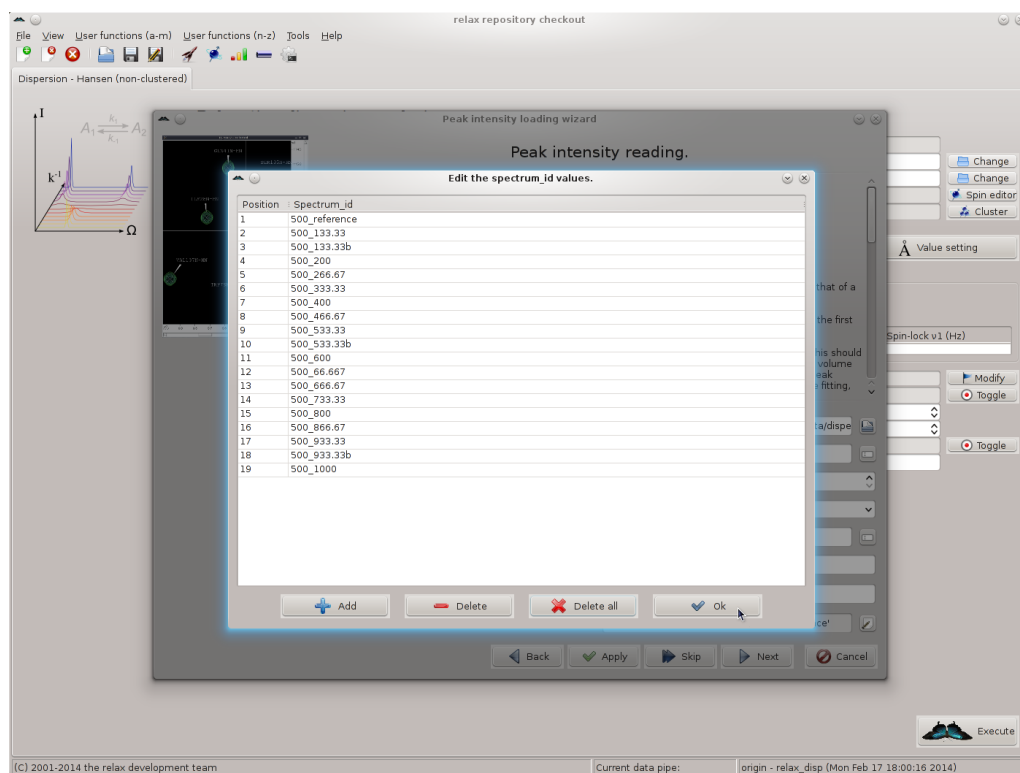


Add the first file by typing the file name or clicking on the file selection button to the right of the file input field and choosing the file. A preview button is included to allow the file to be checked. Then click on the “Add” button to insert a new file selection item, add the next file, and continue until completion. You should now see 19 file names:



Click on “OK” to return to the `spectrum.read_intensities` user function wizard page.

Next, the spectrum IDs should be set. For this, click on the button to the far right of the “spectrum ID string” GUI element. Click on “Add” and fill in the spectrum IDs corresponding to the files selected. As 19 files exist for the 500 MHz data, 19 spectrum IDs should be added. When complete you should see:



Click on “OK” to return to the `spectrum.read_intensities` user function wizard page.

There is a shortcut to these previous steps in that the file names and spectrum IDs can be manually typed into the input elements of the `spectrum.read_intensities` user function wizard page. For the file names, assuming relax is installed in the base directory `/data/relax/relax-trunk` and a Unix-like system is being used, the following text can be added:

```
[ '/data/relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/
reference.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/dispersion/
Hansen/500_MHz/133.33.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/
dispersion/Hansen/500_MHz/133.33.in.bis_sparky', '/data/relax/relax-trunk/test_suite/
shared_data/dispersion/Hansen/500_MHz/200.in_sparky', '/data/relax/relax-trunk/
test_suite/shared_data/dispersion/Hansen/500_MHz/266.67.in_sparky', '/data/relax/relax-
trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/333.33.in_sparky', '/data/
relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/400.in_sparky', '/
data/relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/466
.67.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500
_MHz/533.33.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/dispersion/
Hansen/500_MHz/533.33.in.bis_sparky', '/data/relax/relax-trunk/test_suite/shared_data/
dispersion/Hansen/500_MHz/600.in_sparky', '/data/relax/relax-trunk/test_suite/
shared_data/dispersion/Hansen/500_MHz/66.667.in_sparky', '/data/relax/relax-trunk/
test_suite/shared_data/dispersion/Hansen/500_MHz/666.67.in_sparky', '/data/relax/relax-
trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/733.33.in_sparky', '/data/
relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/800.in_sparky', '/
data/relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500_MHz/866
.67.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/dispersion/Hansen/500
_MHz/933.33.in_sparky', '/data/relax/relax-trunk/test_suite/shared_data/dispersion/
Hansen/500_MHz/933.33.in.bis_sparky', '/data/relax/relax-trunk/test_suite/shared_data/
```

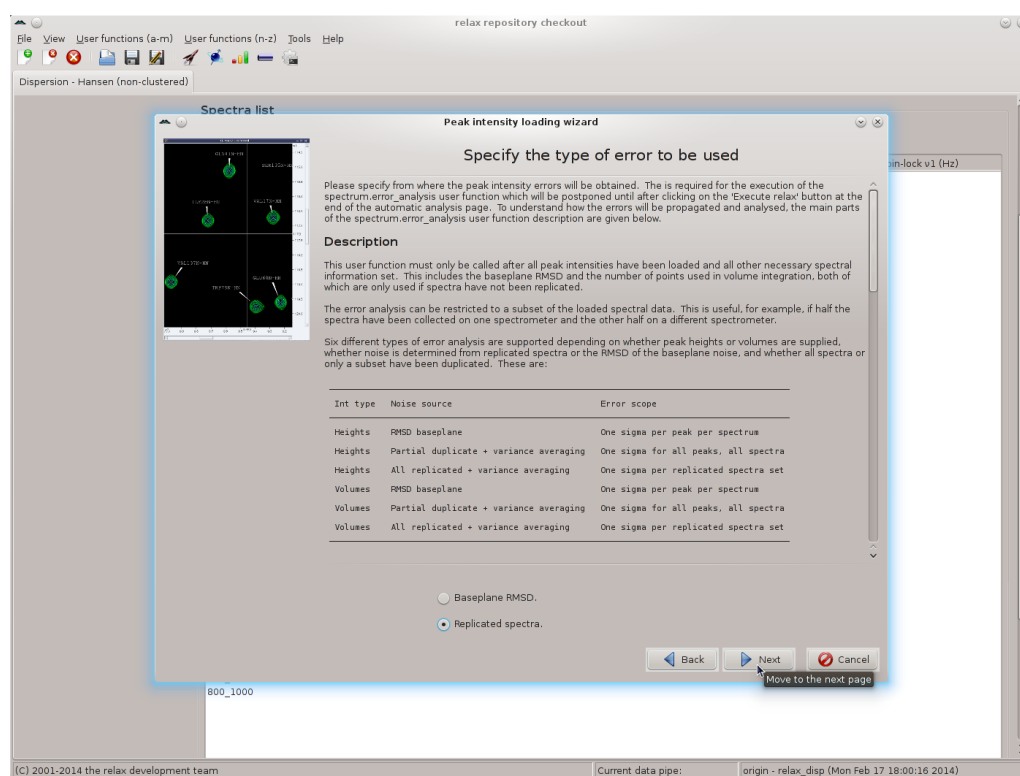
```
dispersion/Hansen/500_MHz/1000.in_sparky']
```

The spectrum IDs can be added by copying and pasting the following text, making sure all text is on one line:

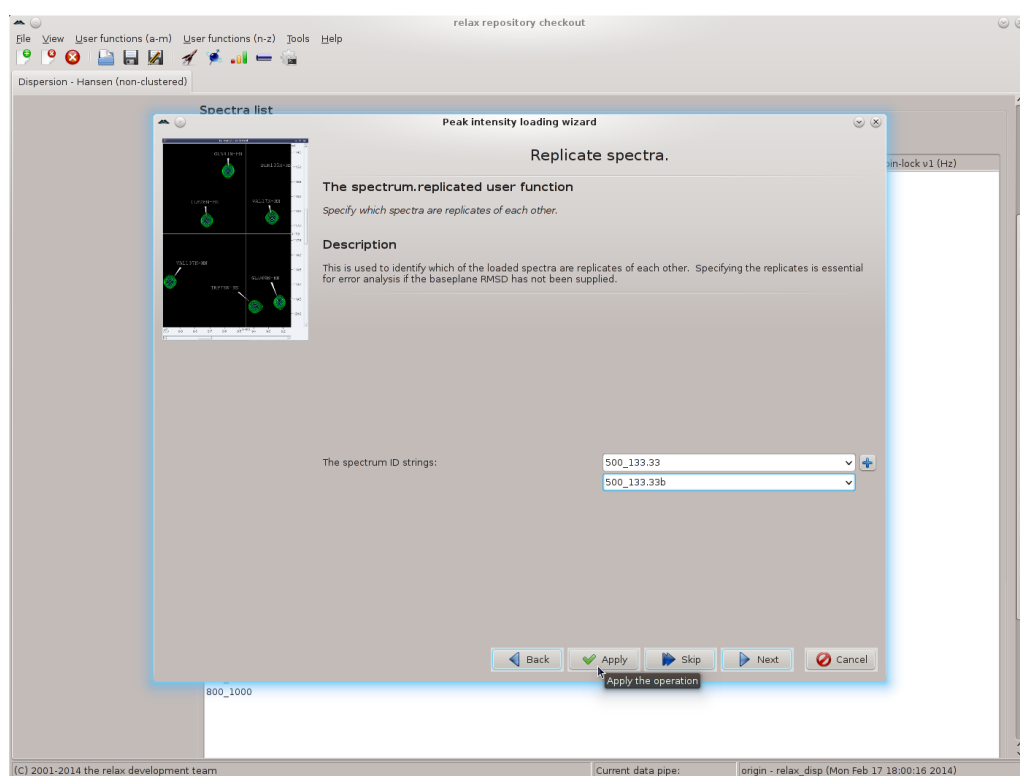
```
['500_reference', '500_133.33', '500_133.33b', '500_200', '500_266.67', '500_333.33', '500_400', '500_466.67', '500_533.33', '500_533.33b', '500_600', '500_66.667', '500_666.67', '500_733.33', '500_800', '500_866.67', '500_933.33', '500_933.33b', '500_1000']
```

To see if the data has been correctly entered, click on the buttons to the right of the GUI elements. You should see the files and spectrum IDs correctly listed in the multiple file selection window and the sequence input window respectively.

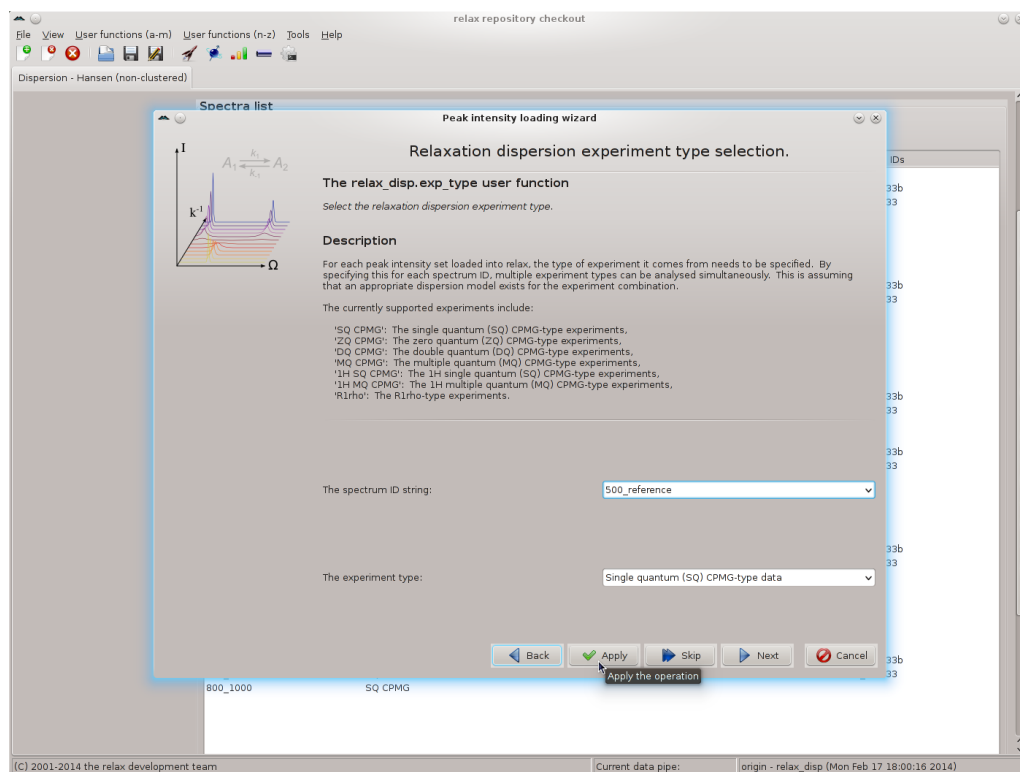
For the SQ CPMG data of this tutorial, none of the other settings in the `spectrum.read_intensities` user function wizard page need to be changed. By clicking on the “Apply” button, the 500 MHz data will be loaded but the wizard will stay on the same page. Note that the contents of the main relax window will have changed. Clicking “Apply” rather than “Next” will allow the 800 MHz data to be loaded next. Change all of the file names and spectrum IDs for the 800 MHz and click on the “Next” button to read the data and to move to the next wizard page. This will be the page for specifying the types of errors to use:



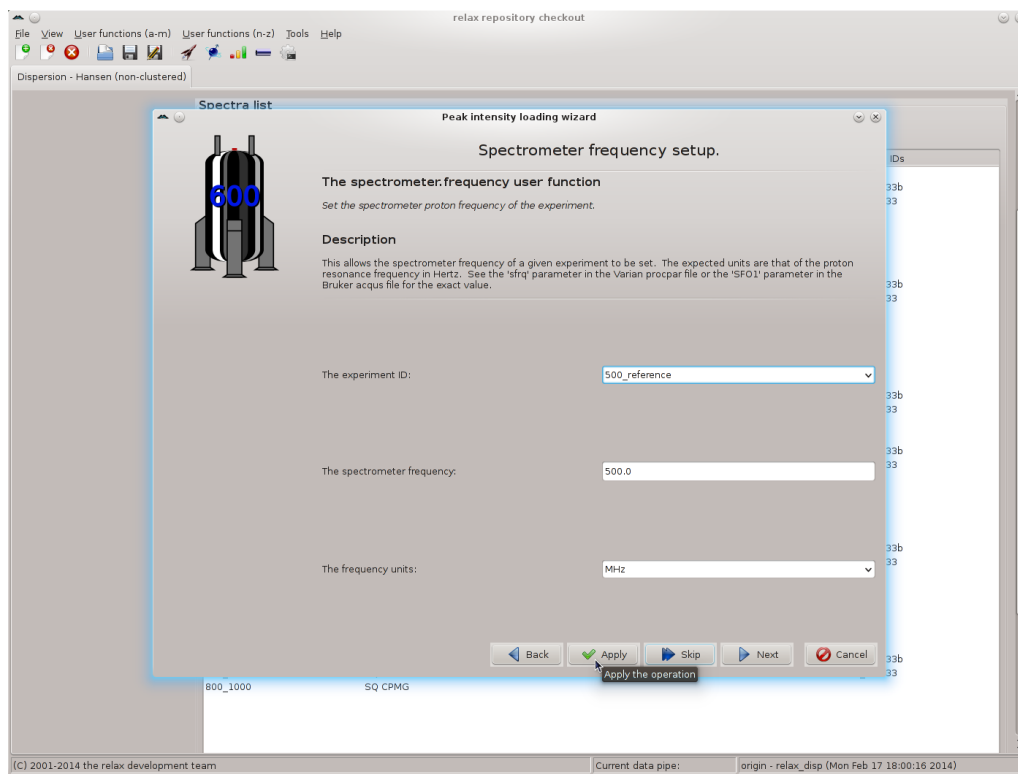
As replicated spectra have been collected, set the value to “Replicated spectra” and then click the “Next” button. Select the pairs of spectra which have been replicated and click the “Apply” button to allow all of the replicates to be specified:



Repeat for each pair of replicates, then click the “Next” button to move to the next wizard page – the `relax_disp.exp_type` user function. Here the tedious operation of labelling all spectrum IDs as being “Single quantum (SQ) CPMG-type data” must be performed. Select the spectrum ID and the SQ CPMG data type and click on the “Apply” button, repeating for each ID:



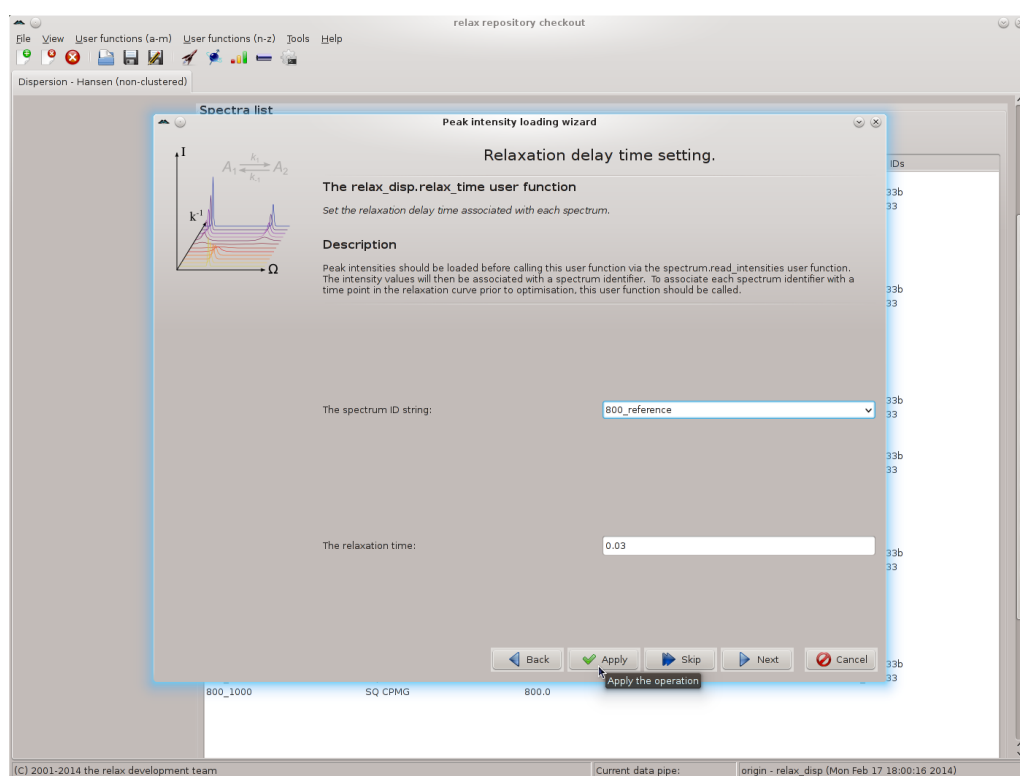
Click the “Next” button when finished. The next wizard page will be that of the `spectrometer.frequency` user function. Here the exact spectrometer frequency values should be specified. These values should be those of the “`sfrq`” parameter in the Varian `procpa` file or the “`SF01`” parameter in the Bruker `acqus` file. As the exact values are not known for the data of this tutorial, the values of 500.0 and 800.0 MHz will be used:



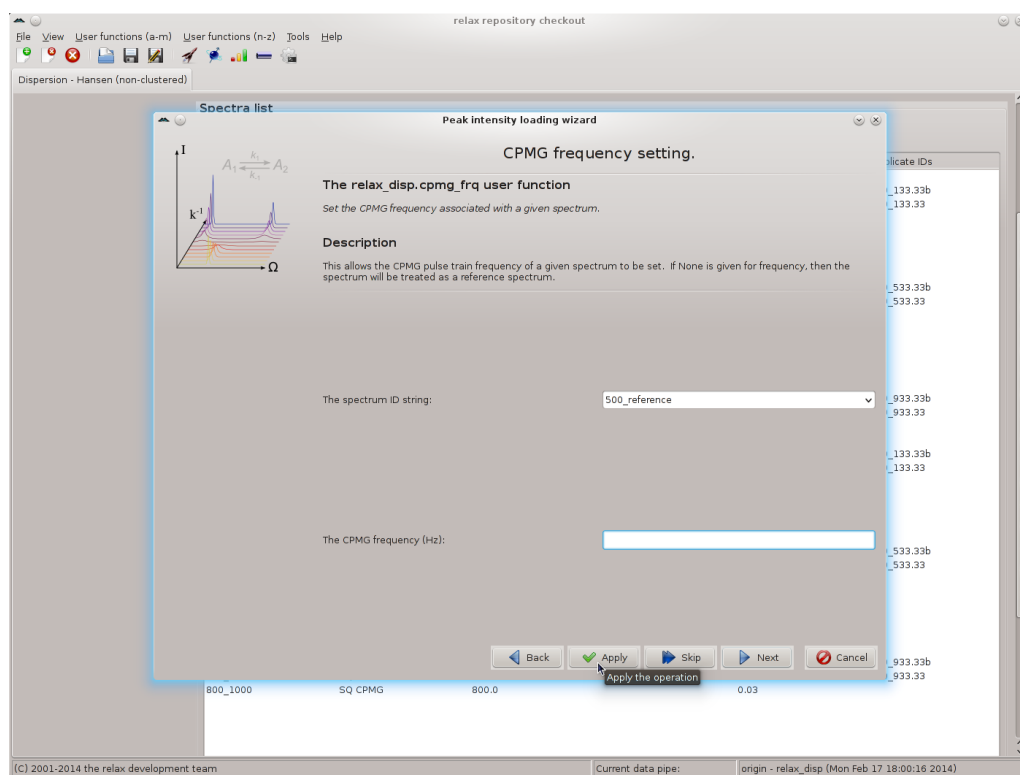
Again use the “Apply” button to set the value, repeating for all matching spectrum IDs. Change the frequency to 800.0 MHz and continue for the next set of matching spectrum IDs. Finally click on “Next” to move to the next wizard page. If you have not used the exact values from the files, the `relax` controller window will appear with the warning:

```
relax> spectrometer.frequency(id='500_reference', frq=500.0, units='MHz')
RelaxWarning: The precise spectrometer frequency should be supplied, a value such as
500000000 or 5e8 for a 500 MHz machine is not acceptable. Please see the 'sfrq'
parameter in the Varian procpa file or the 'SF01' parameter in the Bruker acqus file.
```

The controller window can be safely closed. Though, to avoid frustration when setting the frequency for all spectrum IDs, it may be better to shift the window to the side of the screen. The next wizard page is that of the `relax_disp.relax_time` user function. Set the time to 0.03 and click on the “Apply” button for all spectrum IDs, one after the other:

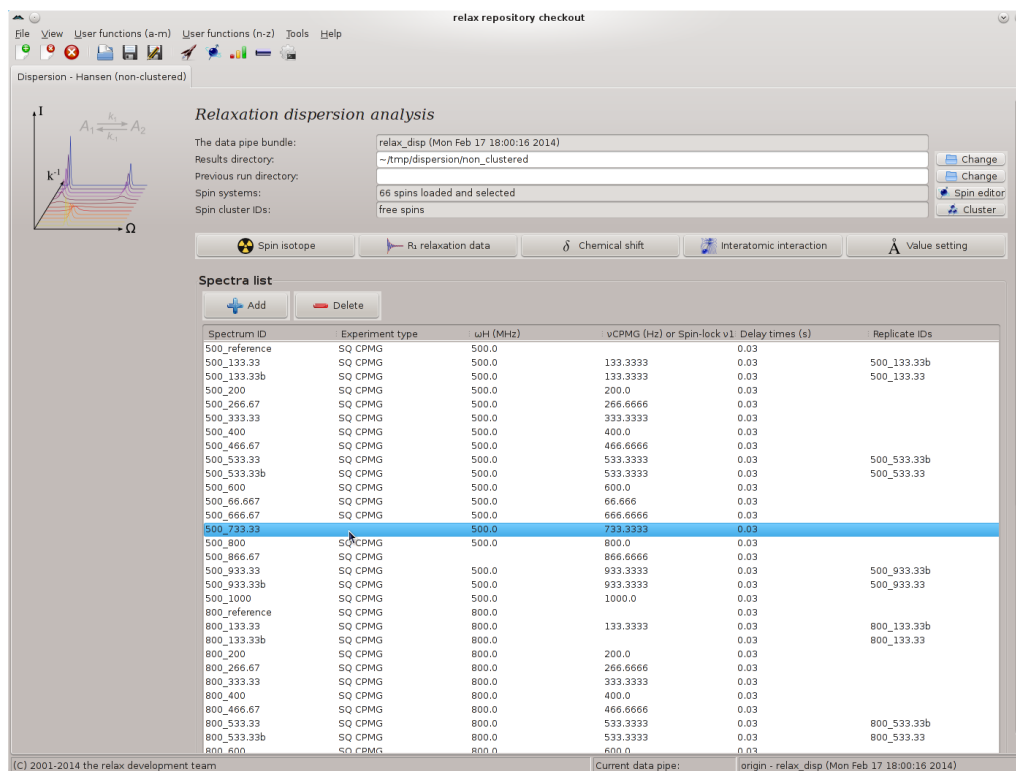


Click on “Next” to move to the `relax_disp.cpmg_freq` user function wizard page. If  $R_{1\rho}$  data has been collected, the `relax_disp.spin_lock_field` user function wizard page would appear instead. For the reference spectrum IDs, leave the CPMG frequency value blank and click on “Apply”:



This will label these spectra as the reference. For all other spectrum IDs, use the CPMG

frequencies as given in the table above, using “Apply” to execute the `relax_disp.cpmg_frq` user function while staying on the same wizard page. Click on “Next” to finish. You should now see the main relax window:



In this screenshot it can be seen that some of the metadata is missing. This often happens due to the large amounts of metadata specified in the peak intensity loading wizard and human error. The missing metadata can now easily be filled in by right clicking on the corresponding row. For example here the “500-733.33” spectrum ID does not have the experiment type set. Simply right click and select the “Set the experiment type” menu entry from the popup menu. For the missing spectrometer frequency for the “500-866.67” spectrum ID, the “Set the spectrometer frequency” menu entry can be used. For the missing  $\nu_{\text{CPMG}}$  value for the “800-133.33” spectrum ID, the “Set the CPMG pulse frequency  $\nu_{\text{CPMG}}$ ” menu entry can be used. All of the metadata should be double and triple checked and fixed where required using the popup menu. Any errors in this metadata would be catastrophic for the subsequent dispersion analysis.

#### 11.14.9 Dispersion GUI mode – choosing the models to optimise

The next step is to specify which relaxation dispersion models will be used in the analysis. The number of models used should be limited based on your knowledge of the problem. Depending on experimental information, a number of models can be ruled out. For example for the data used in this tutorial, the exchange is known to be slow. Hence all of the fast exchange models can be excluded. For the conditions under which the various dispersion models can be used, please refer to the original references. In relax many dispersion models are provided, however you should never use them all.

For this tutorial, we will only use the following dispersion models:



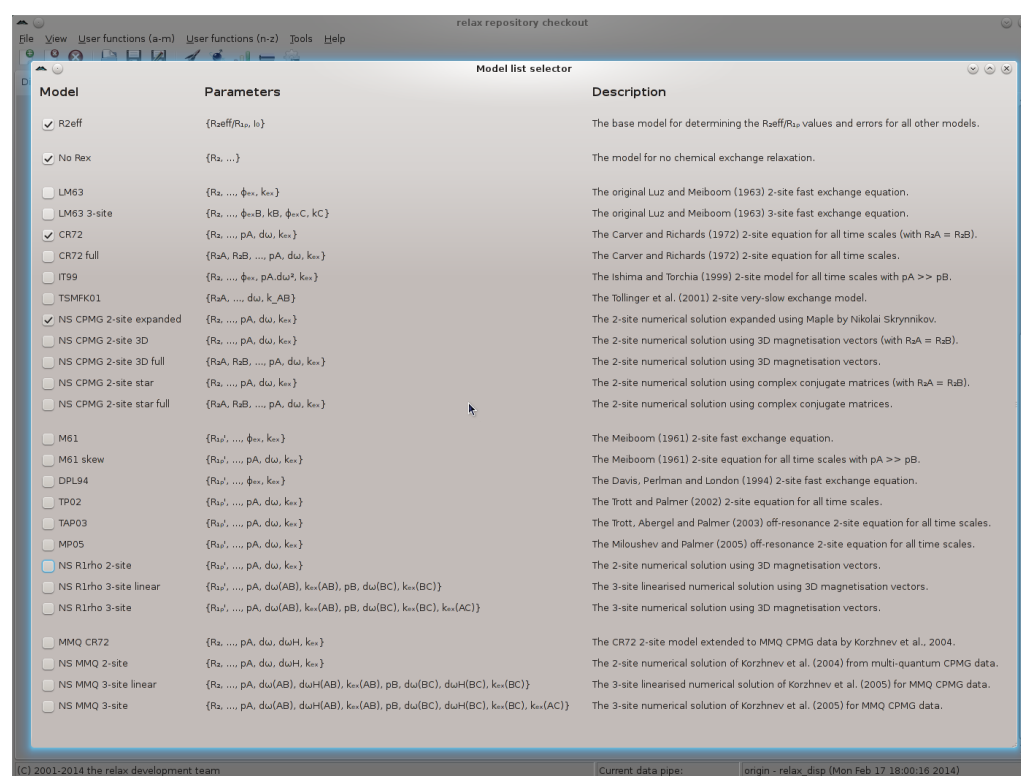
‘**R2eff**’: This is essential for calculating the  $R_{2\text{eff}}$  values used as the input data for all other models (see section 11.2.1 on page 154).

‘**No Rex**’: This model will be important in the model selection stage to determine if any statistically significant relaxation dispersion is present in the  $R_{2\text{eff}}$  data (see section 11.2.2 on page 156).

‘**CR72**’: The Carver and Richards model for most time scales (see Section 11.3.4 on page 160). This is the standard model for slow timescale CPMG-type data (for fast timescales use the ‘LM63’ model instead and for very slow exchange the ‘TSMFK01’ model).

‘**NS CPMG 2-site expanded**’: The best and fastest of the numerical models for 2-site exchange (see Section 11.4.1 on page 164).

To chose which models will be used in the analysis, click on the “Modify” button of the “Relaxation dispersion models” GUI element in the main relax window and change the model list to:



The “Pure numerical solution” flag will be left on “False”. But if you prefer to only have results from the numerical models, this can be changed to “True” by clicking on the “Toggle” button (note that the ‘CR72’ model should nevertheless be used to speed up optimisation – see section 11.9.5 on page 189 for details).

#### 11.14.10 Dispersion GUI mode – optimisation settings

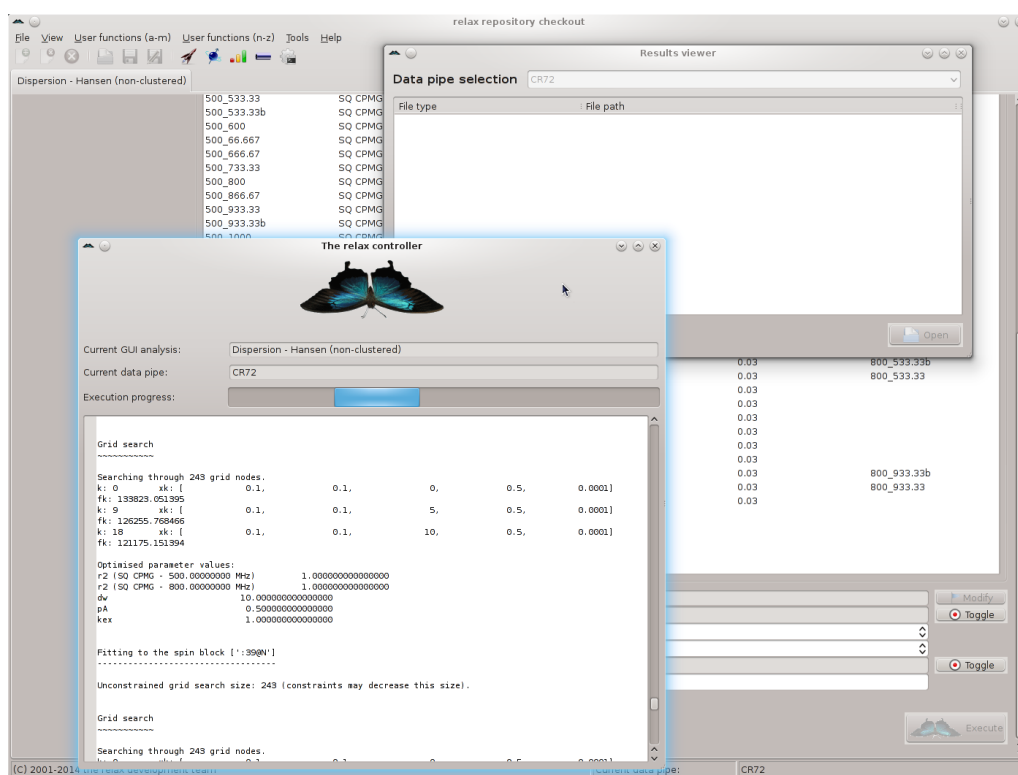
The grid search increment number of 21 might be excessive, especially if the multiprocessor framework is not used to speed up calculations (see section 11.9.11 on page 194). This

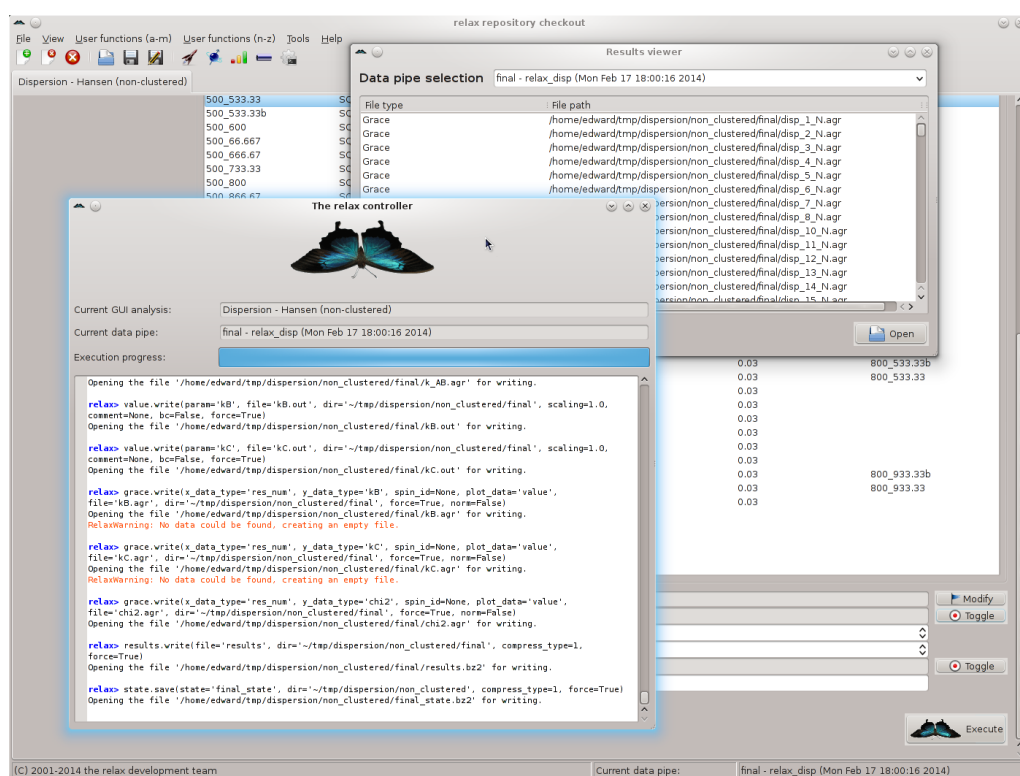
value can be changed to 11, as this number is probably sufficient for most dispersion analyses.

Also for speed, the number of Monte Carlo simulations will be set to 200. However for real analyses, the minimum number of 500 should be used for accurate parameter error estimates. The “Per model error analysis” setting will be left to “False”. And the “Insignificance level” will be left at 1.0 (see section 11.9.2 on page 188).

#### 11.14.11 Dispersion GUI mode – execution of the non-clustered analysis

To start the analysis, simply click the “Execute” button to start the analysis:





The relax controller window can be closed. At all stages of the analysis, the logs should be checked for all warnings. For example searching for the text 'RelaxWarning' in the log file `~/dispersion/log_non_clustered`, the only warnings can be seen at the end of the analysis. These can be seen to be harmless as they are indicating that certain Grace 2D plots contain no data – specifically those for dispersion parameters not present in the set of models optimised. Any other warnings should however be very carefully noted and checked as these could be pointing to a serious problem.

#### 11.14.12 Dispersion GUI mode – inspection of the results

To view the results of the analysis, the relax results window will have been automatically opened. If it was closed, click on the “View→Results viewer” menu entry or the “Results viewer window” button in the toolbar to open the window. This window will list all of the \*.out text files and \*.agr 2D Grace plots created by the auto-analysis. By double clicking on the file, these can be opened. The operating specific text editor will be launched for the text files. For the Grace files, the installed Grace version (Xmgrace, QtGrace, or GraceGTK) will be launched.

Firstly, to see which models have been chosen for the spin systems and which have no statistically significant dispersion, find the `model.out` file and double click on it. You should see the following:

```
# Parameter description: The dispersion model.
#
# mol_name    res_num    res_name    spin_num    spin_name    value    error
None          1          GLY         None        N            'NS CPMG 2-site expanded'  None
None          2          GLY         None        N            'No Rex'      None
None          3          GLY         None        N            'No Rex'      None
None          4          GLY         None        N            'No Rex'      None
None          5          GLY         None        N            'CR72'        None
```

|      |    |     |      |   |                           |      |
|------|----|-----|------|---|---------------------------|------|
| None | 6  | GLY | None | N | 'No Rex'                  | None |
| None | 7  | GLY | None | N | 'No Rex'                  | None |
| None | 8  | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 9  | GLY | None | N | None                      | None |
| None | 10 | GLY | None | N | 'No Rex'                  | None |
| None | 11 | GLY | None | N | 'No Rex'                  | None |
| None | 12 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 13 | GLY | None | N | 'No Rex'                  | None |
| None | 14 | GLY | None | N | 'CR72'                    | None |
| None | 15 | GLY | None | N | 'No Rex'                  | None |
| None | 16 | GLY | None | N | 'No Rex'                  | None |
| None | 17 | GLY | None | N | 'No Rex'                  | None |
| None | 18 | GLY | None | N | 'No Rex'                  | None |
| None | 19 | GLY | None | N | 'No Rex'                  | None |
| None | 20 | GLY | None | N | 'No Rex'                  | None |
| None | 21 | GLY | None | N | 'CR72'                    | None |
| None | 22 | GLY | None | N | 'No Rex'                  | None |
| None | 23 | GLY | None | N | 'CR72'                    | None |
| None | 24 | GLY | None | N | 'No Rex'                  | None |
| None | 25 | GLY | None | N | 'No Rex'                  | None |
| None | 26 | GLY | None | N | 'CR72'                    | None |
| None | 27 | GLY | None | N | None                      | None |
| None | 28 | GLY | None | N | None                      | None |
| None | 29 | GLY | None | N | 'No Rex'                  | None |
| None | 30 | GLY | None | N | None                      | None |
| None | 31 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 32 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 33 | GLY | None | N | 'No Rex'                  | None |
| None | 34 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 35 | GLY | None | N | 'No Rex'                  | None |
| None | 36 | GLY | None | N | 'No Rex'                  | None |
| None | 37 | GLY | None | N | 'No Rex'                  | None |
| None | 38 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 39 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 40 | GLY | None | N | 'No Rex'                  | None |
| None | 41 | GLY | None | N | 'No Rex'                  | None |
| None | 42 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 43 | GLY | None | N | 'No Rex'                  | None |
| None | 44 | GLY | None | N | 'No Rex'                  | None |
| None | 45 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 46 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 47 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 48 | GLY | None | N | 'No Rex'                  | None |
| None | 49 | GLY | None | N | 'No Rex'                  | None |
| None | 50 | GLY | None | N | 'No Rex'                  | None |
| None | 51 | GLY | None | N | 'CR72'                    | None |
| None | 52 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 53 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 54 | GLY | None | N | None                      | None |
| None | 55 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 56 | GLY | None | N | 'No Rex'                  | None |
| None | 57 | GLY | None | N | 'No Rex'                  | None |
| None | 58 | GLY | None | N | 'No Rex'                  | None |
| None | 59 | GLY | None | N | 'CR72'                    | None |
| None | 60 | GLY | None | N | 'No Rex'                  | None |
| None | 61 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 62 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 63 | GLY | None | N | 'CR72'                    | None |
| None | 64 | GLY | None | N | 'CR72'                    | None |
| None | 65 | GLY | None | N | 'CR72'                    | None |
| None | 66 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 67 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 68 | GLY | None | N | None                      | None |
| None | 69 | GLY | None | N | None                      | None |
| None | 70 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 71 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 72 | GLY | None | N | 'NS CPMG 2-site expanded' | None |
| None | 73 | GLY | None | N | 'CR72'                    | None |

Excluding the deselected spin systems with the model ‘None’ and those with no statistically significant dispersion, ‘No Rex’, it can be seen that in some cases the ‘CR72’ model is selected whereas in others the ‘NS CPMG 2-site expanded’ model is selected. The differences between the ‘CR72’ analytic model and the ‘NS CPMG 2-site expanded’ numeric model are insignificant. To see this, open the `CR72/chi2.out` and `NS CPMG 2-site expanded/chi2.out` text files in the `~/dispersion/log_non_clustered` directory and compare the optimised chi-squared values (this can be performed with the mouse by using the pipe editor window, changing the current data pipe, and double clicking on the files in the results viewer window for each data pipe – see below for more details).

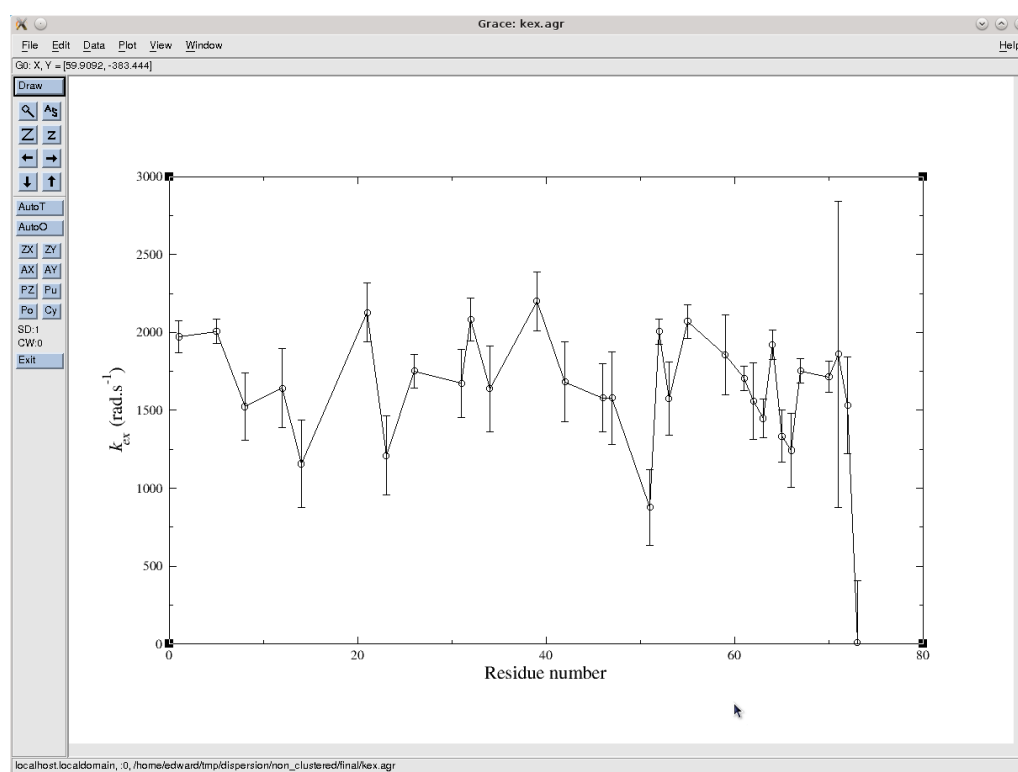
To see which spins have been assigned the model ‘No Rex’ due to the  $R_{2\text{eff}}/R_{1\rho}$  insignificance level of 1.0 rad/s, search the log messages for ‘insignificance’. You should see:

```
relax> relax_disp.insignificance(level=1.0)
Deselecting spin ':20N', the maximum dispersion curve difference for all curves is 0.772528040762 rad/s.
Deselecting spin ':30N', the maximum dispersion curve difference for all curves is 0.572686080104 rad/s.
Deselecting spin ':40N', the maximum dispersion curve difference for all curves is 0.20753288407 rad/s.
Deselecting spin ':70N', the maximum dispersion curve difference for all curves is 0.184120905625 rad/s.
Deselecting spin ':100N', the maximum dispersion curve difference for all curves is 0.746360942576 rad/s.
Deselecting spin ':110N', the maximum dispersion curve difference for all curves is 0.372702361421 rad/s.
Deselecting spin ':130N', the maximum dispersion curve difference for all curves is 0.261522940719 rad/s.
Deselecting spin ':150N', the maximum dispersion curve difference for all curves is 0.743965404051 rad/s.
Deselecting spin ':160N', the maximum dispersion curve difference for all curves is 0.198783344901 rad/s.
Deselecting spin ':170N', the maximum dispersion curve difference for all curves is 0.469568638477 rad/s.
Deselecting spin ':180N', the maximum dispersion curve difference for all curves is 0.720840385548 rad/s.
Deselecting spin ':190N', the maximum dispersion curve difference for all curves is 0.290773963568 rad/s.
Deselecting spin ':200N', the maximum dispersion curve difference for all curves is 0.983669594767 rad/s.
Deselecting spin ':220N', the maximum dispersion curve difference for all curves is 0.507488886605 rad/s.
Deselecting spin ':240N', the maximum dispersion curve difference for all curves is 0.984086643389 rad/s.
Deselecting spin ':250N', the maximum dispersion curve difference for all curves is 0.638104572082 rad/s.
Deselecting spin ':290N', the maximum dispersion curve difference for all curves is 0.525261970487 rad/s.
Deselecting spin ':330N', the maximum dispersion curve difference for all curves is 0.822112754666 rad/s.
Deselecting spin ':350N', the maximum dispersion curve difference for all curves is 0.713976877685 rad/s.
Deselecting spin ':360N', the maximum dispersion curve difference for all curves is 0.413602640091 rad/s.
Deselecting spin ':370N', the maximum dispersion curve difference for all curves is 0.302953864843 rad/s.
Deselecting spin ':400N', the maximum dispersion curve difference for all curves is 0.401535026435 rad/s.
Deselecting spin ':410N', the maximum dispersion curve difference for all curves is 0.805657060225 rad/s.
Deselecting spin ':430N', the maximum dispersion curve difference for all curves is 0.582523964429 rad/s.
Deselecting spin ':440N', the maximum dispersion curve difference for all curves is 0.325638582443 rad/s.
Deselecting spin ':450N', the maximum dispersion curve difference for all curves is 0.947956877688 rad/s.
Deselecting spin ':490N', the maximum dispersion curve difference for all curves is 0.872396631779 rad/s.
Deselecting spin ':500N', the maximum dispersion curve difference for all curves is 0.403543891199 rad/s.
Deselecting spin ':560N', the maximum dispersion curve difference for all curves is 0.468272490195 rad/s.
Deselecting spin ':570N', the maximum dispersion curve difference for all curves is 0.634215047495 rad/s.
Deselecting spin ':580N', the maximum dispersion curve difference for all curves is 0.953109267554 rad/s.
```

Search for ‘eliminate’ to see which models have been removed due to the model elimination step (see section 11.9.9 on page 193 for details). For example for the CR72 model:

```
relax> eliminate(function=None, args=None)
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50003 is less than 0.50100, eliminating the spin cluster [' :60N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50001 is less than 0.50100, eliminating the spin cluster [' :380N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50001 is less than 0.50100, eliminating the spin cluster [' :420N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50001 is less than 0.50100, eliminating the spin cluster [' :450N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50000 is less than 0.50100, eliminating the spin cluster [' :460N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50002 is less than 0.50100, eliminating the spin cluster [' :470N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50000 is less than 0.50100, eliminating the spin cluster [' :480N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50002 is less than 0.50100, eliminating the spin cluster [' :600N'].
Data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)': The pA parameter of 0.50000 is less than 0.50100, eliminating the spin cluster [' :620N'].
```

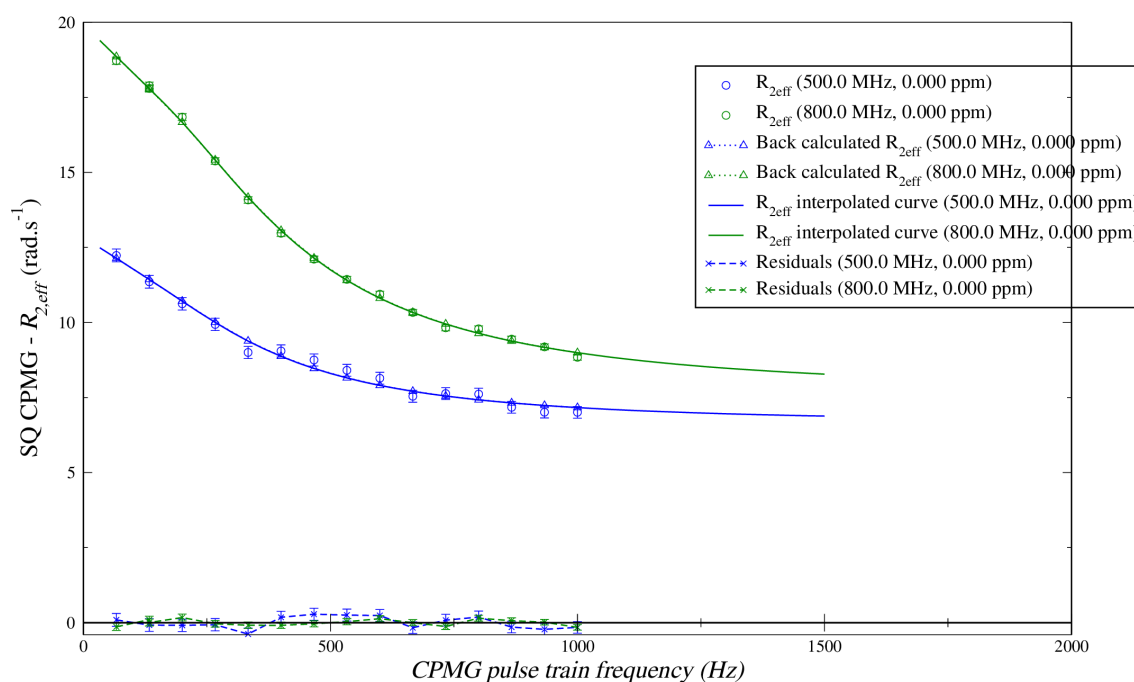
To see the optimised parameter values, double click on the `kex.agr` file to see the exchange rates. This will open the `grace.view` user function window by which the file can be opened. The default settings produces the following graph:



The exchange rate for most spins experiencing exchange is between 1000 and 2000  $\text{s}^{-1}$ . Opening the `pA.agr` file, it can be seen that the population of state A is approximately 0.98. A number of spins have much lower values than this, but their errors are huge meaning that all but 3 are statistically the same as 0.98 (note that a proper ANOVA statistics analysis would be required to make such statements).

To see one of the dispersion curves, open the `disp_64_N.agr` file in Grace. The default settings will produce the graph:

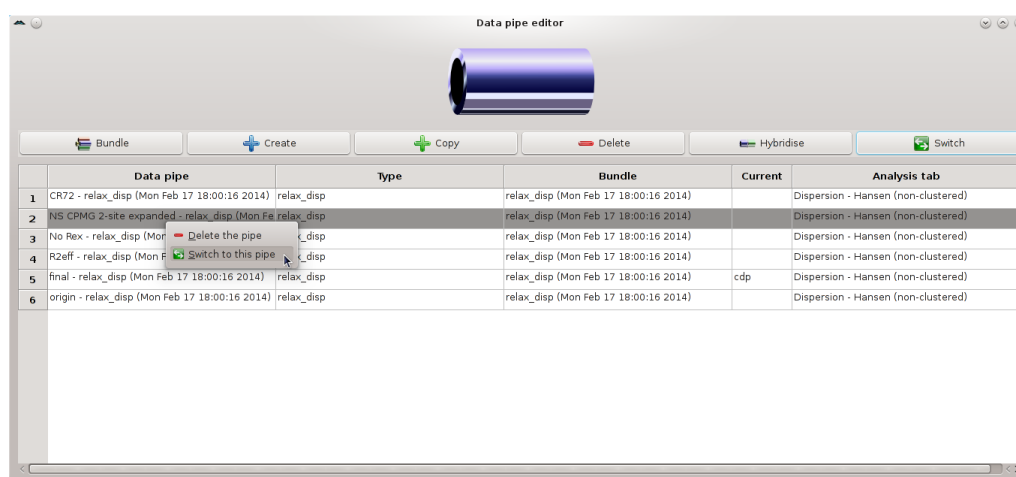
### Relaxation dispersion plot



To improve the appearance of this plot, please refer to the Grace software documentation.

#### 11.14.13 Dispersion GUI mode – comparing models

To compare the results of the different optimised models, the pipe editor window can be used to switch between the data pipes containing the results of the individual models. Select the “View→Data pipe editor” menu entry. Right click on the model of interest and select “Switch to this pipe”:



The results viewer window can then be used to open the text files and Grace plots for that model. Switch to the data pipe of another model and open the same file to compare the results.

#### 11.14.14 Dispersion GUI mode – the clustered analysis

Before the second analysis with spin clustering will be performed, the relax state will be saved in the file `state.bz2` and the program closed. To store the details of the second analysis in a separate log file, relax will be restarted with the command:

```
$ mpirun -np 8 /data/relax/relax-trunk/relax --multi='mpi4py' --log
~/tmp/dispersion/log-clustered --gui
```

When the GUI has started, load the `state.bz2` file. If relax is not restarted, all messages will be in a single log file.

For the clustered analysis, we will focus on one group of spins – those from residues :59 to :67. These can be seen to have very similar dynamics:

```
# Parameter description: The population for state A.
#
# mol_name  res_num  res_name  spin_num  spin_name  value  error
[snip]
None      59      GLY      None      N          0.991169677577733  0.0254974551085798
None      60      GLY      None      N          None      None
None      61      GLY      None      N          0.989169345780449  0.000173707304433962
None      62      GLY      None      N          0.892612114003636  0.156741312688688
None      63      GLY      None      N          0.991579380015928  0.00101555844987099
None      64      GLY      None      N          0.983519617639107  0.00141882200997569
None      65      GLY      None      N          0.993831316342131  0.00840481515172743
None      66      GLY      None      N          0.996816227018878  0.00495523034494496
None      67      GLY      None      N          0.987206586948786  0.000217099775069814
[snip]

# Parameter description: The exchange rate.
#
# mol_name  res_num  res_name  spin_num  spin_name  value  error
[snip]
None      59      GLY      None      N          1856.39029180567  258.796681611922
None      60      GLY      None      N          None      None
None      61      GLY      None      N          1706.42820099893  79.5779060629935
None      62      GLY      None      N          1560.77926730839  246.876174669559
None      63      GLY      None      N          1448.69535431372  121.940593279104
None      64      GLY      None      N          1922.2405164604  96.0557804598977
None      65      GLY      None      N          1333.8423680145  168.000554346898
None      66      GLY      None      N          1243.45993122534  238.215664971556
None      67      GLY      None      N          1753.32557147779  78.2028166128168
[snip]
```

All other spins will be deselected.

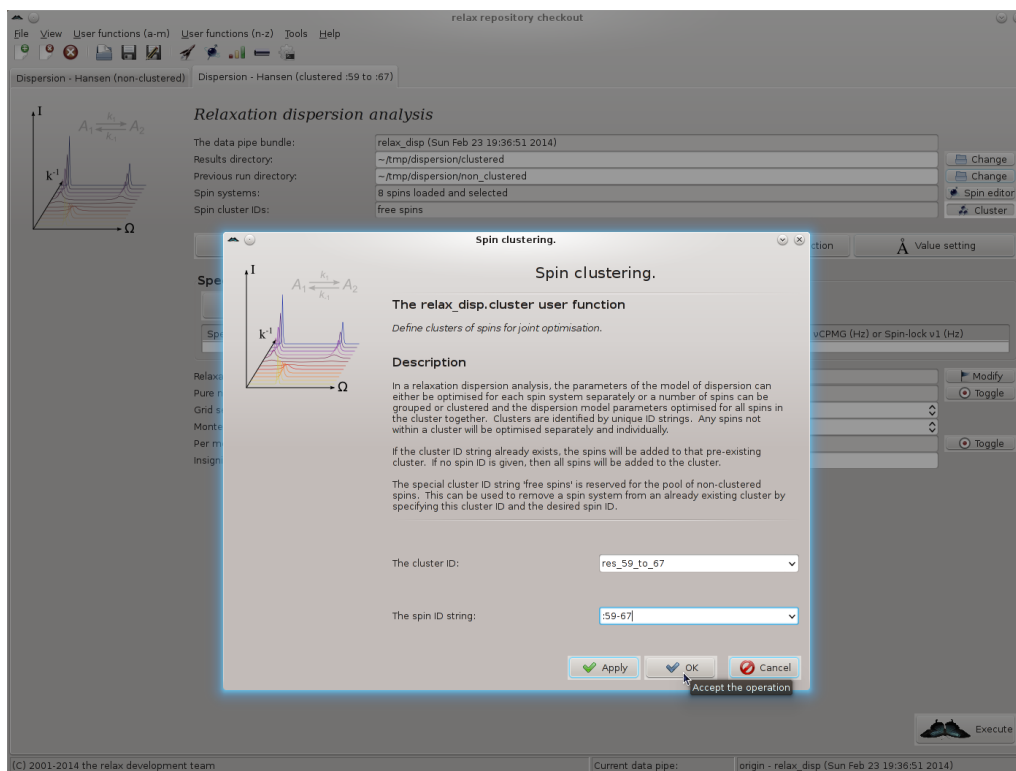
From the non-clustered results, it could be argued that all spins in the entire system experience the same dynamic process, i.e. they have the same  $p_A$  and  $k_{ex}$  values. Such an analysis could be performed at a later stage if desired. The dispersion curves for the residue :60 could also be inspected to see that dispersion is likely to be present and another clustered analysis including this spin performed. The number of clustered analyses performed is up to the user – imagination is the only limit.

To start the analysis, open the analysis selection wizard as was performed previously. Name the analysis as ‘Dispersion - Hansen (clustered :59 to :67)’. Once the analysis is initialised, change the results directory to `~/tmp/dispersion/clustered`. To use the results of the previous analysis to speed up this analysis, as clustering will cause the grid search to be impossibly long, change the “Previous run directory” value to `~/tmp/dispersion/non-clustered`.



Set up the spin systems as for the non-clustered analysis. This time however deselect all spins except for those of residues :59 to :67 (excluding :60). This can be performed by right clicking on the spins in the spin viewer window.

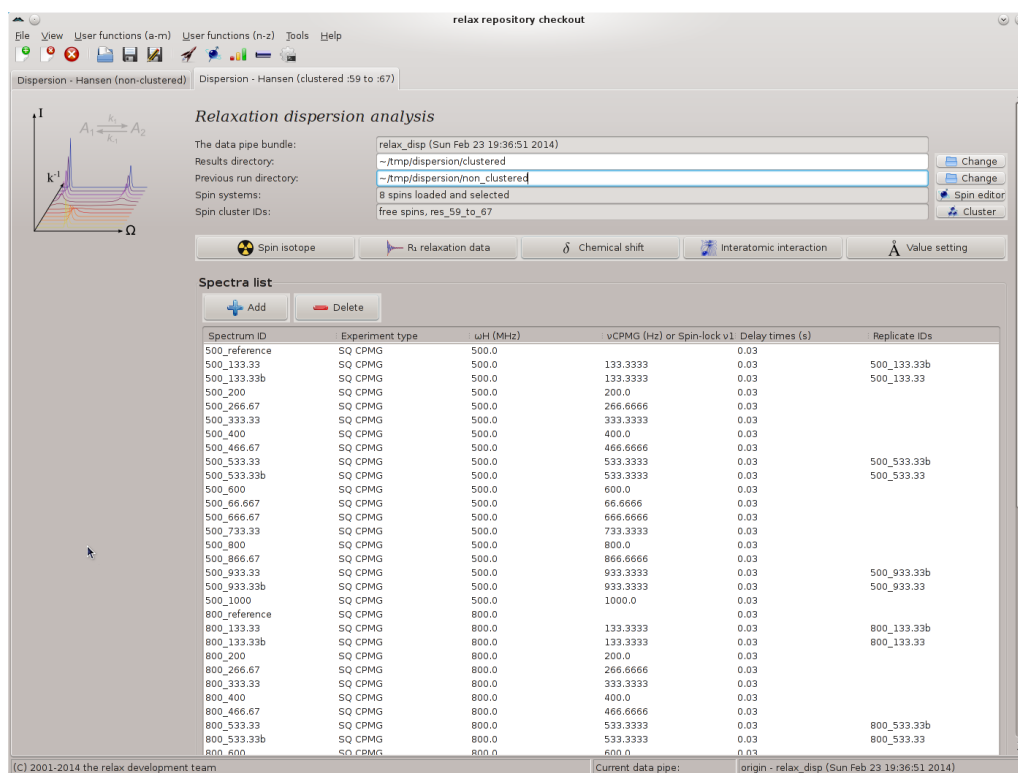
The next step is to cluster these eight spins. In the “Spin cluster IDs” GUI element, click on the “Cluster” button. This launches the `relax_disp.cluster` user function. Set the cluster ID to “res\_59\_to\_67”, for example, and the spin ID string to “:59-67” (this says all residues from :59 to :67, see section 4.2.2 on page 36 for details):



As spin :60 is deselected, that residue will be skipped in the analysis.

Then set the spin isotope and load all of the Sparky peak lists as before. Chose the models ‘R2eff’, ‘No Rex’, ‘CR72’, and ‘NS CPMG 2-site expanded’. As the ‘CR72’ and ‘NS CPMG 2-site expanded’ were seen as being statistically equivalent in the non-clustered analysis, click on the “Toggle” button of the “Pure numerical solutions” GUI element to avoid the ‘CR72’ model in the model selection step. Actually, as the initial parameters for the ‘NS CPMG 2-site expanded’ model in the dispersion auto-analysis will be taken as the average from the non-clustered analysis, the ‘CR72’ model could be completely skipped.

Ignore the grid search increment setting as this will have no effect. No grid searches will be performed because the results from the non-clustered analysis will be used as the optimisation starting point. Set the Monte Carlo simulations to 200. The main window should now look like:



Start the analysis by clicking on the “Execute” button. You should notice that the spin cluster printout in the log messages in the relax controller window now show the text:

The spin cluster ['59', '60', '61', '62', '63', '64', '65', '66', '67'].

As residue 60 is deselected, it will not be used in the optimisation or any part of the analysis. The full analysis should take a few hours to complete.

### 11.14.15 Dispersion GUI mode – comparison of the analyses

To statistically compare the non-clustered and clustered analyses, the advanced Akaike’s Information Criterion (AIC) as derived in [d’Auvergne and Gooley \(2003\)](#) can be used. This information is stored within the recorded log files. Open the `~/tmp/dispersion/log_non_clustered` file and search for the model selection section. The text for residues 59 to 67 should be:

```
The spin cluster ['59@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.
```

```
The spin cluster ['60@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'No Rex - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.
```

```
The spin cluster ['61@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.
```

```
The spin cluster ['62@N'].
```

```

# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':63@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':64@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':65@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':66@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'CR72 - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':67@N'].
# Data pipe
No Rex - relax_disp (Mon Feb 17 18:00:16 2014)
CR72 - relax_disp (Mon Feb 17 18:00:16 2014)
NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)
The model from the data pipe 'NS CPMG 2-site expanded - relax_disp (Mon Feb 17 18:00:16 2014)' has been selected.

The spin cluster [':59', ':60', ':61', ':62', ':63', ':64', ':65', ':66', ':67'].
# Data pipe
No Rex - relax_disp (Sun Feb 23 19:36:51 2014)
NS CPMG 2-site expanded - relax_disp (Sun Feb 23 19:36:51 2014)
The model from the data pipe 'NS CPMG 2-site expanded - relax_disp (Sun Feb 23 19:36:51 2014)' has been selected.

```

For the log file from the clustered analysis (the ~/tmp/dispersion/log\_clustered file), the text should be as follows:

```

The spin cluster [':59', ':60', ':61', ':62', ':63', ':64', ':65', ':66', ':67'].
# Data pipe
No Rex - relax_disp (Sun Feb 23 19:36:51 2014)
NS CPMG 2-site expanded - relax_disp (Sun Feb 23 19:36:51 2014)
The model from the data pipe 'NS CPMG 2-site expanded - relax_disp (Sun Feb 23 19:36:51 2014)' has been selected.

```

The numbers for the ‘NS CPMG 2-site expanded’ model can be directly compared. This is because the parameter number, data set number, chi-squared value and AIC value (labelled as ‘Criterion’ in the logs) can be summed for the non-clustered analysis and then compared to the clustered values.

| Analysis      | Parameter number (k) | Data set number (n) | Chi-squared value | AIC value |
|---------------|----------------------|---------------------|-------------------|-----------|
| Non-clustered | 40                   | 240                 | 388.966           | 468.966   |
| Clustered     | 26                   | 240                 | 510.966           | 562.966   |

The Akaike Information Criterion value is much less for the non-clustered analysis. Therefore this result is the most parsimonious – the result closest to Occam’s razor as defined by frequentist statistics. Therefore the non-clustered analysis is a statistically better description of the experimental data for this set of residues. For a different cluster of spins, the result may be different. If the assumptions of the same dynamics for all spins (both populations  $p_A$  and exchange rates  $k_{ex}$ ) is correct, the results of the clustered analysis are nevertheless useful as it can decrease parameter uncertainty. If the assumption is not

correct, then the decrease in parameter uncertainty will be coupled with a parameter bias – a shift of the parameter away from reality. This should be avoided at all costs.

To perform a relaxation dispersion analysis on your own system, care in the setup, model choice and design of the clustering should be taken:

- Inspect the dispersion curves of all spin systems one by one and decide if any spins should be deselected for the entire analysis (due to the data being of insufficient quality).
- Depending on the dynamics of the system, the type of data collected (SQ CPMG vs. MMQ CPMG vs.  $R_{1\rho}$ ), and personal preferences, chose which limited set of models will be used in the analysis. For this, the published literature should be consulted. Only use models for which you are sure are suited to the system being studied.
- Decide on a number of spin clustering schemes to compare to the non-clustered analysis.
- For deciding which analysis is best for representing the dynamics of the system, a balance between the statistical significance (based on modern frequentist statistics such as AIC), the decrease in parameter uncertainty, and the increase in parameter bias needs to be made.
- All results for all spins should be carefully inspected and compared.

## Chapter 12

# The ordering of frames

### 12.1 Introduction of frame ordering

#### 12.1.1 Tensors of frame ordering

The frame order theory is defined as a bridging physics theory for rigid body motions based on the statistical mechanical ordering of reference frames. It is implemented as a new analysis type in relax designed for the study of rigid body motions in molecules. The theory aims to unify all rotational molecular physics techniques via a single statistical mechanical molecular dynamics (MD) model, by defining a series of rank- $2n$  frame order tensors. These tensors encapsulate the maximum information content of a rotational molecular physics experiment, as well as what type of information is contained in the data. To use the frame order theory, two steps are required:

1. The physics of the experiment should be decomposed into statistically mechanically averaged product of rotation matrix elements.
2. The MD model should be expressed in terms of a rotation matrix which modulates the motion of the rigid body, within the reference frame matching that of the experimental data.

#### 12.1.2 $\text{Ln}^{3+}$ aligned RDC and PCS data

For the current implementation in relax, the frame order theory has been derived for molecules internally aligned using paramagnetic lanthanide ions. The data required for the analysis includes both residual dipolar couplings (RDCs) and pseudocontact shifts (PCSs). Both data sets are required as they are complementary, each carrying different dynamics information:

**RDCs:** This data is dominated by the amplitudes of the MD motions, and the orientation of the statical mechanical average structure.

**PCSs:** These mainly provide information about the directions of the MD motions, and the orientation and position of the statical mechanical average structure.

For a successful analysis, the data must be of the highest quality. In addition, multiple alignments are required, either using different lanthanide ions and/or a different attachment point for the lanthanide ion. Note that the current implementation only handles alignment of one rigid body or domain.

## 12.2 Frame order theory

### 12.2.1 Frame order introduction

#### The ordering of a vector

Let  $\mu(t)$  be a time dependent vector defined within an arbitrary fixed frame  $F$  as

$$\mu(t) = [\delta_x, \delta_y, \delta_z]^T \quad (12.1)$$

where  $\delta_i$  is the time dependent direction cosine between the unit vector and the axis  $i$  of frame  $F$ . Key for understanding the statistical mechanics of a second rank rotational process is the time dependence of the outer product

$$P(t) = \mu(t) \otimes \mu(t), \quad (12.2)$$

$$= \begin{bmatrix} \delta_x^2 & \delta_x \delta_y & \delta_x \delta_z \\ \delta_y \delta_x & \delta_y^2 & \delta_y \delta_z \\ \delta_z \delta_x & \delta_z \delta_y & \delta_z^2 \end{bmatrix}. \quad (12.3)$$

Assuming statistical mechanical ensemble averaging, the observable expected value of the matrix  $P(t)$  is a matrix which defines the ordering of the vector  $\mu(t)$  within the frame  $F$ . This order matrix is

$$S(t) \equiv \overline{P(t)}, \quad (12.4a)$$

$$= \overline{\mu(t) \otimes \mu(t)}, \quad (12.4b)$$

$$= \overline{\begin{bmatrix} \delta_x^2 & \delta_x \delta_y & \delta_x \delta_z \\ \delta_y \delta_x & \delta_y^2 & \delta_y \delta_z \\ \delta_z \delta_x & \delta_z \delta_y & \delta_z^2 \end{bmatrix}}, \quad (12.4c)$$

$$= \begin{bmatrix} S_{xx}(t) & S_{xy}(t) & S_{xz}(t) \\ S_{yx}(t) & S_{yy}(t) & S_{yz}(t) \\ S_{zx}(t) & S_{zy}(t) & S_{zz}(t) \end{bmatrix}, \quad (12.4d)$$

where

$$S_{ij}(t) = \overline{\delta_i \delta_j}. \quad (12.5)$$

Because of the symmetry  $S_{ij}(t) = S_{ji}(t)$ , the order matrix has 6 unique elements.

Assuming that the time dependent process modulating  $\mu(t)$  is much faster than the evolution period  $t_{\max}$  of the observed physical interaction, for example the weak molecular alignment process which induces residual dipolar couplings (RDCs) and pseudo-contact

shifts (PCSs) in NMR, the order matrix which gives rise to the non-isotropic effect is equation 12.4c at  $t_{\max} = \infty$ . Hence the non-zero order matrix is

$$S(\infty) = \begin{bmatrix} S_{xx}(\infty) & S_{xy}(\infty) & S_{xz}(\infty) \\ S_{yx}(\infty) & S_{yy}(\infty) & S_{yz}(\infty) \\ S_{zx}(\infty) & S_{zy}(\infty) & S_{zz}(\infty) \end{bmatrix}. \quad (12.6)$$

### The ordering of a frame

Let the frame  $C(t)$  be time dependent within an arbitrary fixed frame  $F$ . After a time period  $t$  the shift from  $C(0)$  to  $C(t)$  is given by the rotation

$$R(t) = \begin{bmatrix} c_{xx} & c_{xy} & c_{xz} \\ c_{yx} & c_{yy} & c_{yz} \\ c_{zx} & c_{zy} & c_{zz} \end{bmatrix} \equiv \begin{bmatrix} \delta_{xx} & \delta_{xy} & \delta_{xz} \\ \delta_{yx} & \delta_{yy} & \delta_{yz} \\ \delta_{zx} & \delta_{zy} & \delta_{zz} \end{bmatrix}, \quad (12.7)$$

where rotation matrix element  $c_{ij}$  is equivalent to the direction cosine  $\delta_{ij}$  between axis  $i$  of  $C(t)$  and axis  $j$  of  $C(0)$ . For second rank physical processes modulated by rotational motions, analogously to the outer product expected value of 12.4b, the time dependence of the process is governed by the outer product

$$\aleph^{(2)}(t) = \overline{R(t) \otimes R(t)} \quad (12.8)$$

This is a rank-4, three dimensional rotational tensor defining the ordering of the frame  $C(t)$  after a period  $t$  within the original frame  $C(0)$ . This is the definition of the second degree frame order tensor.

The matrix form of the second degree frame order tensor in rank-2, 9D Kronecker product notation is

$$\aleph^{(2)}(t) = \begin{bmatrix} \delta_{xx}^2 & \delta_{xx}\delta_{xy} & \delta_{xx}\delta_{xz} & \delta_{xy}\delta_{xx} & \delta_{xy}^2 & \delta_{xy}\delta_{xz} & \delta_{xz}\delta_{xx} & \delta_{xz}\delta_{xy} & \delta_{xz}^2 \\ \delta_{xx}\delta_{yx} & \delta_{xx}\delta_{yy} & \delta_{xx}\delta_{yz} & \delta_{xy}\delta_{yx} & \delta_{xy}\delta_{yy} & \delta_{xy}\delta_{yz} & \delta_{xz}\delta_{yx} & \delta_{xz}\delta_{yy} & \delta_{xz}\delta_{yz} \\ \delta_{xx}\delta_{zx} & \delta_{xx}\delta_{zy} & \delta_{xx}\delta_{zz} & \delta_{xy}\delta_{zx} & \delta_{xy}\delta_{zy} & \delta_{xy}\delta_{zz} & \delta_{xz}\delta_{zx} & \delta_{xz}\delta_{zy} & \delta_{xz}\delta_{zz} \\ \delta_{yx}\delta_{xx} & \delta_{yx}\delta_{xy} & \delta_{yx}\delta_{xz} & \delta_{yy}\delta_{xx} & \delta_{yy}\delta_{xy} & \delta_{yy}\delta_{xz} & \delta_{yz}\delta_{xx} & \delta_{yz}\delta_{xy} & \delta_{yz}\delta_{xz} \\ \delta_{yx}^2 & \delta_{yx}\delta_{yy} & \delta_{yx}\delta_{yz} & \delta_{yy}\delta_{yx} & \delta_{yy}^2 & \delta_{yy}\delta_{yz} & \delta_{yz}\delta_{yx} & \delta_{yz}\delta_{yy} & \delta_{yz}^2 \\ \delta_{yx}\delta_{zx} & \delta_{yx}\delta_{zy} & \delta_{yx}\delta_{zz} & \delta_{yy}\delta_{zx} & \delta_{yy}\delta_{zy} & \delta_{yy}\delta_{zz} & \delta_{yz}\delta_{zx} & \delta_{yz}\delta_{zy} & \delta_{yz}\delta_{zz} \\ \delta_{zx}\delta_{xx} & \delta_{zx}\delta_{xy} & \delta_{zx}\delta_{xz} & \delta_{zy}\delta_{xx} & \delta_{zy}\delta_{xy} & \delta_{zy}\delta_{xz} & \delta_{zz}\delta_{xx} & \delta_{zz}\delta_{xy} & \delta_{zz}\delta_{xz} \\ \delta_{zx}\delta_{yx} & \delta_{zx}\delta_{yy} & \delta_{zx}\delta_{yz} & \delta_{zy}\delta_{yx} & \delta_{zy}\delta_{yy} & \delta_{zy}\delta_{yz} & \delta_{zz}\delta_{yx} & \delta_{zz}\delta_{yy} & \delta_{zz}\delta_{yz} \\ \delta_{zx}^2 & \delta_{zx}\delta_{zy} & \delta_{zx}\delta_{zz} & \delta_{zy}\delta_{zx} & \delta_{zy}^2 & \delta_{zy}\delta_{zz} & \delta_{zz}\delta_{zx} & \delta_{zz}\delta_{zy} & \delta_{zz}^2 \end{bmatrix}, \quad (12.9)$$

where

$$\overline{\delta_{ij}\delta_{kl}} \equiv \overline{c_{ij}c_{kl}}. \quad (12.10)$$

This is a rank-2, 3D order matrix of rank-2, 3D order matrices. To see this, the  $T_{14}$  rank-4

matrix transpose of  $\mathbb{M}^{(2)}$  in Kronecker product notation is

$$\mathbb{M}^{T_{14}}(t) = \begin{bmatrix} \delta_{xx}^2 & \delta_{yx}\delta_{xx} & \delta_{zx}\delta_{xx} & \delta_{xy}\delta_{xx} & \delta_{yy}\delta_{xx} & \delta_{zy}\delta_{xx} & \delta_{xz}\delta_{xx} & \delta_{yz}\delta_{xx} & \delta_{zz}\delta_{xx} \\ \delta_{xx}\delta_{yx} & \delta_{yy}^2 & \delta_{zx}\delta_{yx} & \delta_{xy}\delta_{yx} & \delta_{yy}\delta_{yx} & \delta_{zy}\delta_{yx} & \delta_{xz}\delta_{yx} & \delta_{yz}\delta_{yx} & \delta_{zz}\delta_{yx} \\ \delta_{xx}\delta_{zx} & \delta_{yx}\delta_{zx} & \delta_{zz}^2 & \delta_{xy}\delta_{zx} & \delta_{yy}\delta_{zx} & \delta_{zy}\delta_{zx} & \delta_{xz}\delta_{zx} & \delta_{yz}\delta_{zx} & \delta_{zz}\delta_{zx} \\ \delta_{xx}\delta_{xy} & \delta_{yx}\delta_{xy} & \delta_{zx}\delta_{xy} & \delta_{xy}^2 & \delta_{yy}\delta_{xy} & \delta_{zy}\delta_{xy} & \delta_{xz}\delta_{xy} & \delta_{yz}\delta_{xy} & \delta_{zz}\delta_{xy} \\ \delta_{xx}\delta_{yy} & \delta_{yx}\delta_{yy} & \delta_{zx}\delta_{yy} & \delta_{xy}\delta_{yy} & \delta_{yy}^2 & \delta_{zy}\delta_{yy} & \delta_{xz}\delta_{yy} & \delta_{yz}\delta_{yy} & \delta_{zz}\delta_{yy} \\ \delta_{xx}\delta_{zy} & \delta_{yx}\delta_{zy} & \delta_{zx}\delta_{zy} & \delta_{xy}\delta_{zy} & \delta_{yy}\delta_{zy} & \delta_{zy}^2 & \delta_{xz}\delta_{zy} & \delta_{yz}\delta_{zy} & \delta_{zz}\delta_{zy} \\ \delta_{xx}\delta_{xz} & \delta_{yx}\delta_{xz} & \delta_{zx}\delta_{xz} & \delta_{xy}\delta_{xz} & \delta_{yy}\delta_{xz} & \delta_{zy}\delta_{xz} & \delta_{xz}^2 & \delta_{yz}\delta_{xz} & \delta_{zz}\delta_{xz} \\ \delta_{xx}\delta_{yz} & \delta_{yx}\delta_{yz} & \delta_{zx}\delta_{yz} & \delta_{xy}\delta_{yz} & \delta_{yy}\delta_{yz} & \delta_{zy}\delta_{yz} & \delta_{xz}\delta_{yz} & \delta_{yz}^2 & \delta_{zz}\delta_{yz} \\ \delta_{xx}\delta_{zz} & \delta_{yx}\delta_{zz} & \delta_{zx}\delta_{zz} & \delta_{xy}\delta_{zz} & \delta_{yy}\delta_{zz} & \delta_{zy}\delta_{zz} & \delta_{xz}\delta_{zz} & \delta_{yz}\delta_{zz} & \delta_{zz}^2 \end{bmatrix}. \quad (12.11)$$

The 3D matrix in the top left corner is the ordering of the x-axis with itself, the central matrix is the ordering of the y-axis with itself, and the bottom right is the ordering of the z-axis with itself. The off-diagonal 3D matrices are the cross-correlations between the three axes. Using the notation  $e_x$ ,  $e_y$  and  $e_z$  for the orthogonal axis system of the time dependent frame  $C(t)$ , the second degree frame order matrix can be written as

$$\mathbb{M}^{T_{14}}(t) = \begin{bmatrix} \overline{e_x \otimes e_x} & \overline{e_x \otimes e_y} & \overline{e_x \otimes e_z} \\ \overline{e_y \otimes e_x} & \overline{e_y \otimes e_y} & \overline{e_y \otimes e_z} \\ \overline{e_z \otimes e_x} & \overline{e_z \otimes e_y} & \overline{e_z \otimes e_z} \end{bmatrix}. \quad (12.12)$$

If the rank-2, 3D order matrix between the axes A and B is denoted as

$$S_{AB}(t) = \overline{e_A \otimes e_B}, \quad (12.13)$$

then the frame order matrix is

$$\mathbb{M}^{T_{14}}(t) = \begin{bmatrix} S_{XX}(t) & S_{XY}(t) & S_{XZ}(t) \\ S_{YX}(t) & S_{YY}(t) & S_{YZ}(t) \\ S_{ZX}(t) & S_{ZY}(t) & S_{ZZ}(t) \end{bmatrix}. \quad (12.14)$$

The frame order matrix is diagonally symmetric, as can be seen in the  $T_{14}$  transpose of the matrix in rank-2, 9D Kronecker product form (equation 12.11, hence for the second degree frame order matrix there are 45 unique elements. For the 9D Kronecker product notation of equation 12.9, this transformed diagonal symmetry can be schematically represented as

$$\mathbb{M}^{(2)}(t) = \begin{bmatrix} \cdot & \text{---} & \text{---} & \cdot \\ \cdot & \text{---} & \text{---} & \cdot \\ \cdot & \text{---} & \text{---} & \cdot \\ \cdot & \text{---} & \text{---} & \cdot \end{bmatrix}.$$





$$I_3 = \begin{bmatrix} 1 & . & . & . & . & . & . & . & . \\ . & . & . & 1 & . & . & . & . & . \\ . & . & . & . & . & . & 1 & . & . \\ . & 1 & . & . & . & . & . & . & . \\ . & . & . & . & 1 & . & . & . & . \\ . & . & . & . & . & . & . & 1 & . \\ . & . & 1 & . & . & . & . & . & . \\ . & . & . & . & . & 1 & . & . & . \\ . & . & . & . & . & . & . & . & 1 \end{bmatrix}. \quad (12.18c)$$

The identity matrices are related to each other via the rank-4 matrix transposes

$$I_2 = I_1^{T_{14}} = I_1^{T_{23}}, \quad (12.19a)$$

$$I_3 = I_1^{T_{24}} = I_1^{T_{13}}. \quad (12.19b)$$

In the case of unrestricted motions, the time limits of the frame order matrix are

$$\mathbb{M}^{(2)}(t=0) = I_1, \quad (12.20)$$

and

$$\mathbb{M}^{(2)}(t=\infty) = \frac{1}{3}I_2. \quad (12.21)$$

### Tensor power of the frame order

The rank-4, 3D frame order tensor of equation 12.8 on page 237 was derived for second order rotational physical processes. However this can be generalised for physical processes of all orders. The tensor power of the time dependent rotation matrix  $R(t)$  is defined as

$$R^{\otimes n}(t) \stackrel{\text{def}}{=} R(t) \otimes \cdots \otimes R(t), \quad (12.22)$$

where the outer product is repeated  $n$  times. Therefore let the frame order tensor be defined as

$$\mathbb{M}^{(n)}(t) = \overline{R^{\otimes n}(t)}, \quad (12.23)$$

where  $n$  is the order of the physical process. The rank of the 3D tensors is  $2n$ . The first few frame order tensors of rank-2, rank-4, rank-6, and rank-8 are

$$\mathbb{M}^{(1)}(t) = \overline{R(t)}, \quad (12.24a)$$

$$\mathbb{M}^{(2)}(t) = \overline{R(t) \otimes R(t)}, \quad (12.24b)$$

$$\mathbb{M}^{(3)}(t) = \overline{R(t) \otimes R(t) \otimes R(t)}, \quad (12.24c)$$

$$\mathbb{M}^{(4)}(t) = \overline{R(t) \otimes R(t) \otimes R(t) \otimes R(t)}. \quad (12.24d)$$

In index and direction cosine notation,

$$\mathbb{M}_{ij}^{(1)}(t) = \overline{\delta_{ij}}, \quad (12.25a)$$

$$\mathbb{M}_{ijkl}^{(2)}(t) = \overline{\delta_{ij}\delta_{kl}}, \quad (12.25b)$$

$$\mathbb{M}_{ijklmn}^{(3)}(t) = \overline{\delta_{ij}\delta_{kl}\delta_{mn}}, \quad (12.25c)$$

$$\mathbb{M}_{ijklmnop}^{(4)}(t) = \overline{\delta_{ij}\delta_{kl}\delta_{mn}\delta_{op}}. \quad (12.25d)$$

### The frame order motional eigenframe

The rotation matrices of the general frame order tensor of equation 12.23 can be decomposed into a time dependent and time independent component. The original frame  $F$  can be defined as the motional eigenframe of the system and a new arbitrary frame  $F'$  introduced. The forward rotation from the reference frame  $F'$  to the motional eigenframe  $F$  will be denoted as  $R_{\text{eigen}}$ . The rotation matrix decomposition is

$$R'(t) = R_{\text{eigen}} \cdot R(t) \cdot R_{\text{eigen}}^T. \quad (12.26)$$

Hence the second degree frame order tensor is

$$\mathbb{M}^{(2)} = \overline{R'(t) \otimes R'(t)}, \quad (12.27)$$

Using the mixed product property

$$AC \otimes BD = (A \otimes B)(C \otimes D), \quad (12.28)$$

the arbitrary frame, second degree frame order matrix is

$$\mathbb{M}^{(2)} = \overline{\left( R_{\text{eigen}} \cdot R(t) \cdot R_{\text{eigen}}^T \right) \otimes \left( R_{\text{eigen}} \cdot R(t) \cdot R_{\text{eigen}}^T \right)}, \quad (12.29a)$$

$$= \overline{\left( R_{\text{eigen}} \otimes R_{\text{eigen}} \right) \cdot \left( R(t) \cdot R_{\text{eigen}}^T \right) \otimes \left( R(t) \cdot R_{\text{eigen}}^T \right)}, \quad (12.29b)$$

$$= \overline{\left( R_{\text{eigen}} \otimes R_{\text{eigen}} \right) \cdot \left( R(t) \otimes R(t) \right) \cdot \left( R_{\text{eigen}}^T \otimes R_{\text{eigen}}^T \right)}, \quad (12.29c)$$

$$= \left( R_{\text{eigen}} \otimes R_{\text{eigen}} \right) \cdot \overline{\left( R(t) \otimes R(t) \right)} \cdot \left( R_{\text{eigen}}^T \otimes R_{\text{eigen}}^T \right). \quad (12.29d)$$

Generalising from the 2<sup>nd</sup> to the n<sup>th</sup>-order, the generalised frame order tensor rotation is

$$\mathbb{M}^{(n)}(t) = R_{\text{eigen}}^{\otimes n} \cdot \overline{R^{\otimes n}(t)} \cdot R_{\text{eigen}}^{T \otimes n}. \quad (12.30)$$

### Rotation to the average position frame of the rigid body

For the modelling aspect of the frame order theory, one more rotation is required. In equation 12.30, it is assumed that the starting position for the moving rigid body is that of its motional average. However in the initial 3D structure, this is not the case and an additional rotation to the average position  $R_{\text{ave}}$  is required. Taking this into account, the generalised frame order tensor is defined as

$$\mathbb{M}^{(n)}(t) = R_{\text{eigen}}^{\otimes n} \cdot \overline{R^{\otimes n}(t)} \cdot R_{\text{eigen}}^{T \otimes n} \cdot R_{\text{ave}}^{T \otimes n}, \quad (12.31)$$

where  $R_{\text{eigen}}$  is the eigenframe rotation matrix,  $R(t)$  is the time dependent rotation matrix,  $R_{\text{ave}}$  is the rotation from the average domain position to the motional eigenframe, and  $\otimes n$  is the n<sup>th</sup> tensor power. In applications to physical processes which require numerical integration, pre-rotating the rigid body by  $R_{\text{ave}}$  to the average position is equivalent but more numerically efficient. Therefore the  $R_{\text{ave}}$  can be dropped and equation 12.30 used instead.

### 12.2.2 Frame order and the alignment tensor

#### The RDC and PCS

For the residual dipolar coupling (RDC) and pseudo-contact shift (PCS) NMR phenomena, both effects are governed by the partial molecular alignment tensor  $A$ . For a two domain molecular system, when one domain is internally aligned with for example a paramagnetic lanthanide ion within a magnetic field, the other domain experiences a reduced alignment  $\overline{A}$  due to the interdomain motions.

**The RDC** The residual dipolar coupling is given by

$$D = d \hat{r}^T \cdot A \cdot \hat{r}, \quad (12.32)$$

where  $\hat{r}$  is the internuclear unit vector,  $d$  is the dipolar constant defined as

$$d = -\frac{3}{2\pi} \frac{\mu_0}{4\pi} \frac{\gamma_I \gamma_S \hbar}{\langle r \rangle^3}, \quad (12.33)$$

$\mu_0$  is the permeability of free space,  $\gamma_i$  is the gyromagnetic ratio of the nucleus  $i$ ,  $\hbar$  is Planck's constant divided by  $2\pi$ ,  $\langle r \rangle$  is the time averaged internuclear distance, the factor of  $\frac{1}{2\pi}$  is to convert the constant from radians per second to Hertz, and the factor of three is associated with the alignment tensor. In the presence of an alignment tensor reduction, and assuming that the fast vibrational and librational internal motions of the vector are statistically self-decoupled from the rigid body motions, the RDC is simply

$$D = d \hat{r}^T \cdot \overline{A} \cdot \hat{r}, \quad (12.34)$$

as the vector  $\hat{r}$  is considered time independent in the molecular reference frame.

**The PCS** The pseudo-contact shift equation is simply

$$\delta = \frac{\mu_0}{4\pi} \frac{15kT}{B_0^2} \frac{1}{|r|^5} \hat{r}^T \cdot A \cdot \hat{r}, \quad (12.35a)$$

$$= \frac{c}{|r|^5} \hat{r}^T \cdot A \cdot \hat{r}, \quad (12.35b)$$

$$= \frac{1}{4\pi} \frac{1}{|r|^5} \hat{r}^T \cdot \chi \cdot \hat{r}, \quad (12.35c)$$

where  $A$  is the alignment tensor,  $\chi$  is the magnetic susceptibility tensor,  $\hat{r}$  is the lanthanide to nuclear unit vector, and  $c$  is the PCS constant defined as

$$c = \frac{\mu_0}{4\pi} \frac{15kT}{B_0^2}. \quad (12.36)$$

The alignment tensor reduction process is complicated by the inverse  $|r|^5$  normalisation factor, as  $r$  is not time independent in the molecular reference frame.

### Alignment tensor reduction

The statistical mechanics behind the alignment tensor reduction can be expressed as

$$\overline{A} = \left\langle \int_0^{t_{\max}} R^{-1}(\Omega_t) \cdot A \cdot R(\Omega_t) dt \right\rangle, \quad (12.37)$$

where the angular brackets denote the ensemble averaging, the time integration is for a single molecule over the evolution period of the physical interaction,  $\Omega_t$  are the SO(3) rotational angles describing the change in position of the moving rigid body, and  $A$  is the full alignment tensor. Here the alignment tensor has been created by an averaging of the partially restricted Brownian diffusion process of the non-moving component, again both over the ensemble and time, as

$$A = \left\langle \int_0^{t_{\max}} R^{-1}(\Omega_t) \cdot F \cdot R(\Omega_t) dt \right\rangle, \quad (12.38)$$

where  $F$  is the molecular frame. It is assumed that the alignment process of the non-moving domain and the motions of the moving domain are decoupled.

Using the ergodic hypothesis, the averaging process which generates the reduced alignment tensor can be simplified as

$$\overline{A} = \overline{R^{-1} \cdot A \cdot R}, \quad (12.39a)$$

$$= \overline{R^T \cdot A \cdot R}. \quad (12.39b)$$

The index notation for a tensor rotation is

$$T'_{ij} = \sum_{kl} R_{ki} R_{lj} T_{kl}. \quad (12.40)$$

Therefore the reduced alignment tensor in index notation is

$$\overline{A}_{ij} = \sum_{kl} \overline{c_{ki} c_{lj}} A_{kl}, \quad (12.41)$$

$$= \sum_{kl} \mathfrak{M}_{kilj}^{(2)} A_{kl}, \quad (12.42)$$

where  $\mathfrak{M}^{(2)}$  is a rank-4, 3D orientational tensor which will be called the frame order tensor.

Expanding the sum,

$$\begin{aligned} \overline{A}_{ij} = & \mathfrak{M}_{xixj} A_{xx} + \mathfrak{M}_{xiyj} A_{xy} + \mathfrak{M}_{xizj} A_{xz} \\ & + \mathfrak{M}_{yixj} A_{yx} + \mathfrak{M}_{yiyj} A_{yy} + \mathfrak{M}_{yizj} A_{yz} \\ & + \mathfrak{M}_{zixj} A_{zx} + \mathfrak{M}_{ziyj} A_{zy} + \mathfrak{M}_{zizj} A_{zz}. \end{aligned} \quad (12.43)$$

As

$$A_{ij} = A_{ji}, \quad (12.44a)$$

$$A_{zz} = -A_{xx} - A_{yy}, \quad (12.44b)$$

equation 12.43 becomes

$$\begin{aligned} \overline{A_{ij}} = & \left( \mathbb{M}_{xixj} - \mathbb{M}_{zizj} \right) A_{xx} + \left( \mathbb{M}_{yiyj} - \mathbb{M}_{zizj} \right) A_{yy} \\ & + \left( \mathbb{M}_{xiyj} + \mathbb{M}_{yixj} \right) A_{xy} + \left( \mathbb{M}_{xizj} + \mathbb{M}_{zixj} \right) A_{xz} + \left( \mathbb{M}_{yizj} + \mathbb{M}_{ziyj} \right) A_{yz}. \end{aligned} \quad (12.45)$$

A single element of the reduced tensor is simply a linear combination of all elements of the original tensor multiplied by constants consisting of different combinations of frame order matrix components.

Converting from the rank-2, 3D, symmetric and traceless space of alignment tensors to the rank-1, 5D space, a non-linear frame order superoperator can be written as

$$\overline{A} = \mathbb{M}_{5D}^{(2)} \cdot A. \quad (12.46)$$

In matrix notation, this is

$$\begin{bmatrix} \overline{A_{xx}} \\ \overline{A_{yy}} \\ \overline{A_{xy}} \\ \overline{A_{xz}} \\ \overline{A_{yz}} \end{bmatrix} = \begin{bmatrix} \mathbb{M}_{xxxx} - \mathbb{M}_{zzxx} & \mathbb{M}_{xyyx} - \mathbb{M}_{zzxx} & \mathbb{M}_{xyyx} + \mathbb{M}_{yxxx} & \mathbb{M}_{xxzx} + \mathbb{M}_{zzxx} & \mathbb{M}_{yxxz} + \mathbb{M}_{zxyx} \\ \mathbb{M}_{xyxy} - \mathbb{M}_{zyzy} & \mathbb{M}_{yyyy} - \mathbb{M}_{zyzy} & \mathbb{M}_{xyyy} + \mathbb{M}_{yyxy} & \mathbb{M}_{xyzy} + \mathbb{M}_{zyxy} & \mathbb{M}_{yyzy} + \mathbb{M}_{zyyy} \\ \mathbb{M}_{xxxy} - \mathbb{M}_{zzxy} & \mathbb{M}_{xyxy} - \mathbb{M}_{zzxy} & \mathbb{M}_{xxyy} + \mathbb{M}_{yxyx} & \mathbb{M}_{xxzy} + \mathbb{M}_{zzxy} & \mathbb{M}_{yxxz} + \mathbb{M}_{zxyy} \\ \mathbb{M}_{xxzx} - \mathbb{M}_{zzxz} & \mathbb{M}_{xyxz} - \mathbb{M}_{zzxz} & \mathbb{M}_{xxyz} + \mathbb{M}_{yxxz} & \mathbb{M}_{xxzz} + \mathbb{M}_{zzxz} & \mathbb{M}_{yxxz} + \mathbb{M}_{zxyx} \\ \mathbb{M}_{xyxz} - \mathbb{M}_{zyxz} & \mathbb{M}_{yyyz} - \mathbb{M}_{zyyz} & \mathbb{M}_{xyyz} + \mathbb{M}_{yyxz} & \mathbb{M}_{xyzz} + \mathbb{M}_{zyxz} & \mathbb{M}_{yyzz} + \mathbb{M}_{zyyz} \end{bmatrix} \cdot \begin{bmatrix} A_{xx} \\ A_{yy} \\ A_{xy} \\ A_{xz} \\ A_{yz} \end{bmatrix}. \quad (12.47)$$

Let

$$\{A_0, A_1, A_2, A_3, A_4\} = \{A_{xx}, A_{yy}, A_{xy}, A_{xz}, A_{yz}\}, \quad (12.48)$$

and assuming the rank-2, 9D Kronecker product form of  $\mathbb{M}_{ij}^{(2)}$  using numerical indices where  $\{i, j\} = 0, 1, \dots, 8$ , then

$$\begin{bmatrix} \overline{A_0} \\ \overline{A_1} \\ \overline{A_2} \\ \overline{A_3} \\ \overline{A_4} \end{bmatrix} = \begin{bmatrix} \mathbb{M}_{00} - \mathbb{M}_{80} & \mathbb{M}_{40} - \mathbb{M}_{80} & \mathbb{M}_{10} + \mathbb{M}_{30} & \mathbb{M}_{20} + \mathbb{M}_{60} & \mathbb{M}_{50} + \mathbb{M}_{70} \\ \mathbb{M}_{04} - \mathbb{M}_{84} & \mathbb{M}_{44} - \mathbb{M}_{84} & \mathbb{M}_{14} + \mathbb{M}_{34} & \mathbb{M}_{24} + \mathbb{M}_{64} & \mathbb{M}_{54} + \mathbb{M}_{74} \\ \mathbb{M}_{01} - \mathbb{M}_{81} & \mathbb{M}_{41} - \mathbb{M}_{81} & \mathbb{M}_{11} + \mathbb{M}_{31} & \mathbb{M}_{21} + \mathbb{M}_{61} & \mathbb{M}_{51} + \mathbb{M}_{71} \\ \mathbb{M}_{02} - \mathbb{M}_{82} & \mathbb{M}_{42} - \mathbb{M}_{82} & \mathbb{M}_{12} + \mathbb{M}_{32} & \mathbb{M}_{22} + \mathbb{M}_{62} & \mathbb{M}_{52} + \mathbb{M}_{72} \\ \mathbb{M}_{05} - \mathbb{M}_{85} & \mathbb{M}_{45} - \mathbb{M}_{85} & \mathbb{M}_{15} + \mathbb{M}_{35} & \mathbb{M}_{25} + \mathbb{M}_{65} & \mathbb{M}_{55} + \mathbb{M}_{75} \end{bmatrix} \cdot \begin{bmatrix} A_0 \\ A_1 \\ A_2 \\ A_3 \\ A_4 \end{bmatrix}. \quad (12.49)$$

For the alignment tensor, the 81 elements of the frame order matrix have recombined into 25 unique scaling factors.

**The alignment tensor is the anisotropic part of a frame order matrix**

The alignment tensor is related to the orientational probability tensor by

$$A = P - \frac{1}{3}I. \quad (12.50)$$

The  $P$  probability tensor is the average orientation position of the molecule, hence is the average molecular frame  $\overline{F}$ . As this frame is simply the rotation matrix relative to the

laboratory frame, then

$$P = \overline{F}, \quad (12.51a)$$

$$= \overline{R}, \quad (12.51b)$$

$$= \mathbb{K}^{(1)}. \quad (12.51c)$$

Therefore the alignment tensor can then be written as the anisotropic part of the first degree frame order matrix

$$A = \mathbb{K}^{(1)} - \frac{1}{3}I. \quad (12.52)$$

### 12.2.3 Single pivoted motions

#### Atomic level mechanics of the single pivot

For the PCS, the lanthanide ion to nuclear vector is

$$r = p_N - p_{\text{Ln}^{3+}}, \quad (12.53)$$

where  $p_N$  is the Cartesian coordinates of the nucleus of interest and  $p_{\text{Ln}^{3+}}$  is the position of the aligning lanthanide ion.  $r$  is defined in the alignment frame, and  $p_{\text{Ln}^{3+}}$  is constant in this frame. After a forward rotation to the discrete state  $i$ , the new atomic position in the reference frame is

$$p'_N = R_i \cdot (p_N - p_P) + p_P. \quad (12.54)$$

where  $p_P$  is the pivot point of the rotation. Hence the transformed vector is

$$r_i = p'_N - p_{\text{Ln}^{3+}}, \quad (12.55a)$$

$$= R_i \cdot (p_N - p_P) + p_P - p_{\text{Ln}^{3+}}. \quad (12.55b)$$

The set of three vectors are defining this pivoted system are

$$r_{\text{LN}} = p_N - p_{\text{Ln}^{3+}}, \quad (12.56a)$$

$$r_{\text{PN}} = p_N - p_P, \quad (12.56b)$$

$$r_{\text{LP}} = p_P - p_{\text{Ln}^{3+}}. \quad (12.56c)$$

Let the pre-rotation vectors be

$$r_{\text{LN}}^{(0)} = r_{\text{LP}}^{(0)} + r_{\text{PN}}^{(0)}, \quad (12.57a)$$

$$r_{\text{PN}}^{(0)}, \quad (12.57b)$$

$$r_{\text{LP}}^{(0)}. \quad (12.57c)$$

The post-rotation vectors are

$$r_{\text{LN}}^{(1)} = r_{\text{LP}}^{(0)} + R_i^{(1)} \cdot r_{\text{PN}}^{(0)}, \quad (12.58a)$$

$$r_{\text{PN}}^{(1)} = R_i^{(1)} \cdot r_{\text{PN}}^{(0)}, \quad (12.58b)$$

$$r_{\text{LP}}^{(1)} = r_{\text{LP}}^{(0)}. \quad (12.58c)$$

The vector  $r_{\text{PN}}$  is independent of alignment so can be calculated once per atom, and  $r_{\text{LP}}$  is independent of alignment and atom position so can be calculate once.

### PCS and single pivoted motions

For a single state  $i$ , the PCS value when substituting 12.55b into 12.35 is

$$\delta = \frac{c}{|r_i|^5} r_i^T \cdot A \cdot r_i. \quad (12.59)$$

Expanding for the single motion of the lanthanide-atom vector  $r_{\text{LN}}^{(1)}$ , this becomes

$$\delta = \frac{c}{|r_{\text{LN}}^{(1)}|^5} r_{\text{LN}}^{(1)T} \cdot A \cdot r_{\text{LN}}^{(1)}, \quad (12.60a)$$

$$\delta = \frac{c}{|r_{\text{LN}}^{(1)}|^5} \left( r_{\text{LP}}^{(0)} + R_i^{(1)} \cdot r_{\text{PN}}^{(0)} \right)^T \cdot A \cdot \left( r_{\text{LP}}^{(0)} + R_i^{(1)} \cdot r_{\text{PN}}^{(0)} \right), \quad (12.60b)$$

$$\delta = \frac{c}{|r_{\text{LN}}^{(1)}|^5} \left( r_{\text{LP}}^{(0)T} + r_{\text{PN}}^{(0)T} \cdot R_i^{(1)T} \right) \cdot A \cdot \left( r_{\text{LP}}^{(0)} + R_i^{(1)} \cdot r_{\text{PN}}^{(0)} \right), \quad (12.60c)$$

$$\begin{aligned} \delta = \frac{c}{|r_{\text{LN}}^{(1)}|^5} & \left[ r_{\text{PN}}^{(0)T} \cdot R_i^{(1)T} \cdot A \cdot R_i^{(1)} \cdot r_{\text{PN}}^{(0)} \right. \\ & + r_{\text{LP}}^{(0)T} \cdot A \cdot r_{\text{PN}}^{(0)} + r_{\text{PN}}^{(0)T} \cdot A \cdot r_{\text{LP}}^{(0)} \\ & \left. + r_{\text{LP}}^{(0)T} \cdot A \cdot r_{\text{LP}}^{(0)} \right]. \end{aligned} \quad (12.60d)$$

Due to the distance normalisation factor in these equations, the symbolic integration for the modelling of specific motional modes is currently intractable.

#### 12.2.4 Double pivoted motions

When the motion of a multiple rigid body system can be described as two rotations about two different pivots, the modulation of the PCS becomes more complicated. Figure 12.1 shows this motion for a single lanthanide to atom vector.

#### Atomic level mechanics of the double pivot

The six vectors at the original position are

$$r_{\text{LN}}^{(0)} = r_{\text{LP}_2}^{(0)} + r_{\text{P}_2\text{P}_1}^{(0)} + r_{\text{P}_1\text{N}}^{(0)}, \quad (12.61a)$$

$$r_{\text{P}_2\text{N}}^{(0)} = r_{\text{P}_2\text{P}_1}^{(0)} + r_{\text{P}_1\text{N}}^{(0)}, \quad (12.61b)$$

$$r_{\text{P}_1\text{N}}^{(0)}, \quad (12.61c)$$

$$r_{\text{LP}_1}^{(0)} = r_{\text{LP}_2}^{(0)} + r_{\text{P}_2\text{P}_1}^{(0)}, \quad (12.61d)$$

$$r_{\text{P}_2\text{P}_1}^{(0)}, \quad (12.61e)$$

$$r_{\text{LP}_2}^{(0)}. \quad (12.61f)$$



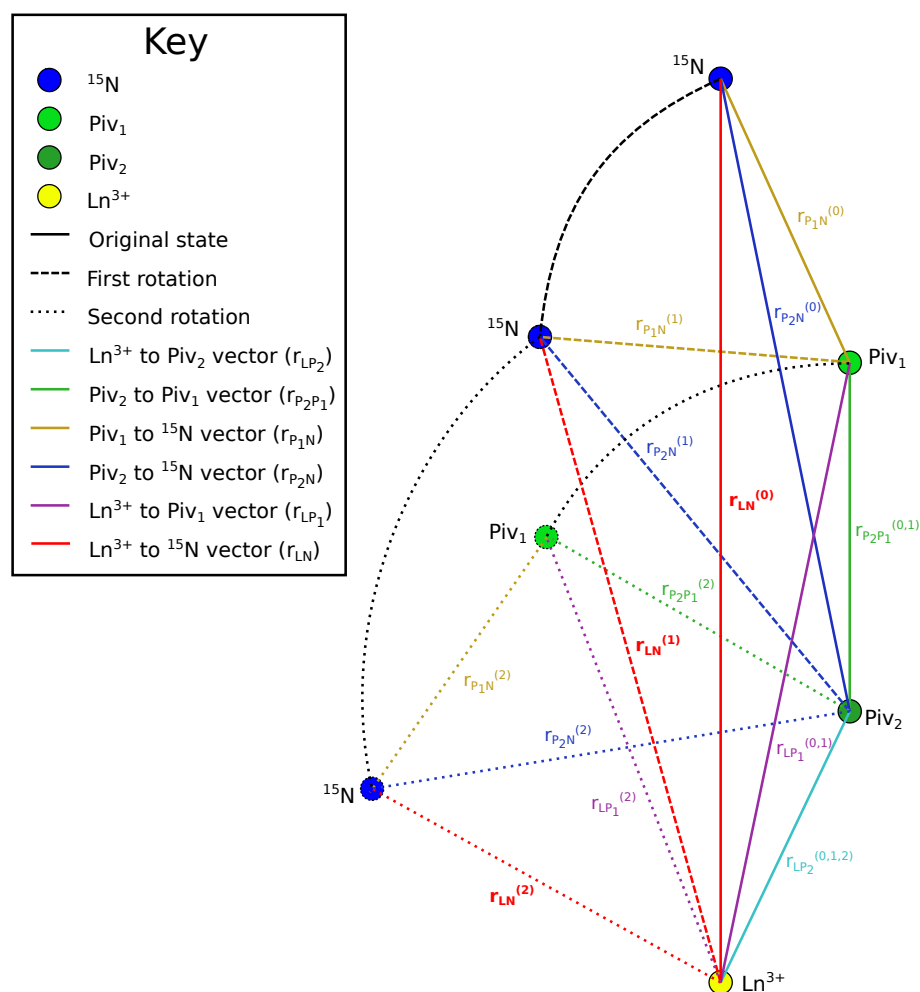


Figure 12.1: Frame order in the double pivot system. The lanthanide position is denoted by  $\text{Ln}^{3+}$  or simply L, the position of the first pivot by  $\text{Piv}_1$ , the position of the second pivot by  $\text{Piv}_2$ , and the position of the nucleus of interest by  $^{15}\text{N}$ . In the vector notation these are L, P<sub>1</sub>, P<sub>2</sub> and N. The original position is denoted by (0), the position after the first rotation by (1), and the position after the second rotation by (2).

The six vectors after the first rotation for state  $i$ ,  $R_i^{(1)}$ , are

$$r_{\text{LN}}^{(1)} = r_{\text{LP}_2}^{(0)} + r_{\text{P}_2\text{P}_1}^{(0)} + R_i^{(1)} \cdot r_{\text{P}_1\text{N}}^{(0)}, \quad (12.62\text{a})$$

$$r_{\text{P}_2\text{N}}^{(1)} = r_{\text{P}_2\text{P}_1}^{(0)} + R_i^{(1)} \cdot r_{\text{P}_1\text{N}}^{(0)}, \quad (12.62\text{b})$$

$$r_{\text{P}_1\text{N}}^{(1)} = R_i^{(1)} \cdot r_{\text{P}_1\text{N}}^{(0)}, \quad (12.62\text{c})$$

$$r_{\text{LP}_1}^{(1)} = r_{\text{LP}_2}^{(0)} + r_{\text{P}_2\text{P}_1}^{(0)}, \quad (12.62\text{d})$$

$$r_{\text{P}_2\text{P}_1}^{(1)} = r_{\text{P}_2\text{P}_1}^{(0)}, \quad (12.62\text{e})$$

$$r_{\text{LP}_2}^{(1)} = r_{\text{LP}_2}^{(0)}. \quad (12.62\text{f})$$

The six vectors after the second rotation for state  $i$ ,  $R_i^{(2)}$ , are

$$r_{\text{LN}}^{(2)} = r_{\text{LP}_2}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_1\text{N}}^{(1)}, \quad (12.63\text{a})$$

$$r_{\text{P}_2\text{N}}^{(2)} = R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_1\text{N}}^{(1)}, \quad (12.63\text{b})$$

$$r_{\text{P}_1\text{N}}^{(2)} = R_i^{(2)} \cdot r_{\text{P}_1\text{N}}^{(1)}, \quad (12.63\text{c})$$

$$r_{\text{LP}_1}^{(2)} = r_{\text{LP}_2}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)}, \quad (12.63\text{d})$$

$$r_{\text{P}_2\text{P}_1}^{(2)} = R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)}, \quad (12.63\text{e})$$

$$r_{\text{LP}_2}^{(2)} = r_{\text{LP}_2}^{(1)}. \quad (12.63\text{f})$$

### PCS and double pivoted motions

As defined in equation 12.59 on page 246, the PCS for state  $i$  is

$$\delta = \frac{c}{|r_i|^5} r_i^T \cdot A \cdot r_i. \quad (12.64)$$

For the double motion of the lanthanide-atom vector  $r_{\text{LN}}^{(2)}$ , this becomes

$$\delta = \frac{c}{|r_{\text{LN}}^{(2)}|^5} r_{\text{LN}}^{(2)T} \cdot A \cdot r_{\text{LN}}^{(2)}, \quad (12.65\text{a})$$

$$= \frac{c}{|r_{\text{LN}}^{(2)}|^5} \left( r_{\text{LP}_2}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_1\text{N}}^{(1)} \right)^T \cdot A \cdot \left( r_{\text{LP}_2}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(1)} + R_i^{(2)} \cdot r_{\text{P}_1\text{N}}^{(1)} \right), \quad (12.65\text{b})$$

$$= \frac{c}{|r_{\text{LN}}^{(2)}|^5} \left( r_{\text{LP}_2}^{(0)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(0)} + R_i^{(2)} \cdot R_i^{(1)} \cdot r_{\text{P}_1\text{N}}^{(0)} \right)^T \cdot A \cdot \left( r_{\text{LP}_2}^{(0)} + R_i^{(2)} \cdot r_{\text{P}_2\text{P}_1}^{(0)} + R_i^{(2)} \cdot R_i^{(1)} \cdot r_{\text{P}_1\text{N}}^{(0)} \right). \quad (12.65\text{c})$$

### 12.2.5 Frame order in rotational Brownian diffusion and NMR relaxation

#### Free ellipsoidal Brownian diffusion

In Perrin's equations for free ellipsoidal Brownian diffusion (Perrin, 1934, 1936), the second degree frame order matrix elements  $\overline{c_{ij}c_{kl}}$  are an essential step of the derivation. From Perrin (1936), the solution for the ellipsoidal diffusion equation is

$$\overline{c_{jj}c_{kk}} + \overline{c_{jk}c_{kj}} = e^{-(4\mathfrak{D}_i + \mathfrak{D}_j + \mathfrak{D}_k)t}, \quad (12.66)$$

$$\overline{c_{ii}^2} = \frac{1}{3} + \frac{1}{6} (2 + \mu_i) e^{-6(\mathfrak{D}_{iso} - \sqrt{\mathfrak{D}_{iso}^2 - \mathfrak{L}^2})t} + \frac{1}{6} (2 - \mu_i) e^{-6(\mathfrak{D}_{iso} + \sqrt{\mathfrak{D}_{iso}^2 - \mathfrak{L}^2})t}, \quad (12.67)$$

$$\overline{c_{jk}^2} = \frac{1}{3} - \frac{1}{6} (1 - \mu_i) e^{-6(\mathfrak{D}_{iso} - \sqrt{\mathfrak{D}_{iso}^2 - \mathfrak{L}^2})t} - \frac{1}{6} (1 + \mu_i) e^{-6(\mathfrak{D}_{iso} + \sqrt{\mathfrak{D}_{iso}^2 - \mathfrak{L}^2})t}, \quad (12.68)$$

where

$$\mu_i = \frac{\mathfrak{D}_i - \mathfrak{D}_{iso}}{\sqrt{\mathfrak{D}_{iso}^2 - \mathfrak{L}^2}}, \quad (12.69)$$

$$\mathfrak{D}_{iso} = \frac{1}{3} \sum_i \mathfrak{D}_i, \quad (12.70)$$

$$\mathfrak{L}^2 = \frac{1}{3} \sum_{i < j} \mathfrak{D}_i \mathfrak{D}_j, \quad (12.71)$$

$\mathfrak{D}_i$  are the three diffusion rates, and  $c_{ij}$  are the direction cosines in the diffusion frame. According to Perrin (1936), because of the symmetry of the rotation the averages of the double-products  $\overline{c_{ij}c_{kl}}$  where an index appears only once are zero and the second degree frame order matrix is represented by equation 12.15 on page 239. At time  $t = 0$ , the frame order matrix simplifies to

$$\mathbb{M}^{(n)}(0) = I_1, \quad (12.72)$$

and at time  $t = \infty$  the matrix decays to

$$\mathbb{M}^{(n)}(\infty) = \frac{1}{3} I_2. \quad (12.73)$$

#### NMR relaxation

The free ellipsoid Brownian diffusion equations form the base theory for interpreting NMR relaxation data - the spheroidal and spherical diffusion equations are simply parametric restrictions of the full ellipsoid equations. As they are the definition of the frame order matrix, the frame order tensor can be seen as the modulator of all NMR relaxation processes.

## 12.3 Frame order modelling

### 12.3.1 Rigid body motions for a two domain system

#### Ball and socket joint

For a molecule consisting of two rigid bodies with pivoted inter-domain or inter-segment motions, the most natural mechanical description of the motion would be that of the spherical joint. This is also known as the ball and socket joint. The mechanical system consists of a single pivot point and three rotational degrees of freedom.

#### Tilt and torsion angles from robotics

To describe the motional mechanics of a ball and socket joint, the Euler angle system is a poor representation as the angles do not correspond to the mechanical modes of motion. In the field of robotics, many different orientation parameter sets have been developed for describing three degree-of-freedom joint systems. For the spherical joint description of intra-molecular rigid body motions, an angle system for describing symmetrical spherical parallel mechanisms (SPMs), a parallel manipulator, was found to be ideal. This is the ‘tilt-and-torsion’ angle system (Huang et al., 1999; Bonev and Gosselin, 2006). These angles were derived independently a number of times to model human joint mechanics, originally as the ‘halfplane-deviation-twist’ angles (Korein, 1985), and then as the ‘tilt/twist’ angles (Crawford et al., 1999).

In the tilt-and-torsion angle system, the rigid body is first tilted by the angle  $\theta$  about the horizontal axis  $a$ . The axis  $a$ , which lies in the  $xy$ -plane, is defined by the azimuthal angle  $\phi$  (the angle is between the rotated  $z'$  axis projection onto the  $xy$ -plane and the  $x$ -axis). The tilt component is hence defined by both  $\theta$  and  $\phi$ . Finally the domain is rotated about the  $z'$  axis by the torsion angle  $\sigma$ . The resultant rotation matrix is

$$R(\theta, \phi, \sigma) = R_z(\phi)R_y(\theta)R_z(\sigma - \phi), \quad (12.74a)$$

$$= R_{zyz}(\sigma - \phi, \theta, \phi), \quad (12.74b)$$

$$= \begin{pmatrix} c_\phi c_\theta c_{\sigma-\phi} - s_\phi s_{\sigma-\phi} & -c_\phi c_\theta s_{\sigma-\phi} - s_\phi c_{\sigma-\phi} & c_\phi s_\theta \\ s_\phi c_\theta c_{\sigma-\phi} + c_\phi s_{\sigma-\phi} & -s_\phi c_\theta s_{\sigma-\phi} + c_\phi c_{\sigma-\phi} & s_\phi s_\theta \\ -s_\theta c_{\sigma-\phi} & s_\theta s_{\sigma-\phi} & c_\theta \end{pmatrix}, \quad (12.74c)$$

where  $c_\eta = \cos(\eta)$  and  $s_\eta = \sin(\eta)$  and  $R_{zyz}$  is the Euler rotation in  $zyz$  axis rotation notation where

$$\alpha = \sigma - \phi, \quad (12.75a)$$

$$\beta = \theta, \quad (12.75b)$$

$$\gamma = \phi. \quad (12.75c)$$

As  $\sigma = \alpha + \gamma$ , it can be seen that both these Euler angles influence the torsion angle, demonstrating the problem with this parameterisation.

**Modelling torsion** An advantage of this angle system is that the tilt and torsion components can be treated separately in the modelling of domain motions. The simplest model for the torsion angle would be the restriction

$$-\sigma_{\max} \leq \sigma \leq \sigma_{\max}. \quad (12.76)$$

The angle can be completely restricted as  $\sigma_{\max} = 0$  to create torsionless models. In this case, the tilt and torsion rotation matrix simplifies to

$$R(\theta, \phi, 0) = R_z(\phi)R_y(\theta)R_z(-\phi), \quad (12.77a)$$

$$= R_{zyz}(-\phi, \theta, \phi), \quad (12.77b)$$

$$= \begin{pmatrix} c_\phi^2 c_\theta + s_\phi^2 & c_\phi s_\phi c_\theta - c_\phi s_\phi & c_\phi s_\theta \\ c_\phi s_\phi c_\theta - c_\phi s_\phi & s_\phi^2 c_\theta + c_\phi^2 & s_\phi s_\theta \\ -c_\phi s_\theta & -s_\phi s_\theta & c_\theta \end{pmatrix}. \quad (12.77c)$$

**Modelling tilt** The tilt angles  $\theta$  and  $\phi$  are related to spherical angles, hence the modelling of this component relates to a distribution on the surface of a sphere. At the simplest level, this can be modelled as both isotropic and anisotropic cones of uniform distribution.

### Model list

For the modelling of the ordering of the motional frame, the tilt and torsion angle system will be used together with uniform distributions of rigid body position. For the torsion angle  $\sigma_{\max}$ , this can be modelled as being rigid ( $\sigma_{\max} = 0$ ), being a free rotor ( $\sigma_{\max} = \pi$ ), or as having a torsional restriction ( $0 < \sigma_{\max} < \pi$ ). For the  $\theta$  and  $\phi$  angles of the tilt component, the rigid body motion can be modelled as being rigid ( $\theta = 0$ ), as moving in an isotropic cone, or moving anisotropically in a pseudo-elliptic cone. Both single and double modes of motion have been modelled. The total list of models so far implemented are:

1. Rigid
2. Rotor
3. Free rotor
4. Isotropic cone
5. Isotropic cone, torsionless
6. Isotropic cone, free rotor
7. Pseudo-ellipse
8. Pseudo-ellipse, torsionless
9. Pseudo-ellipse, free rotor
10. Double rotor

The equations for these models are derived in Chapter 16 on page 371.

### 12.3.2 Frame order axis permutations

Multiple local minima exist in the optimisation space for the isotropic and pseudo-elliptic cone frame order models. In the case of the pseudo-ellipse, the eigenframes at each minimum are identical, however the  $\theta_x$ ,  $\theta_y$ , and  $\sigma_{\max}$  half-angles are permuted. Because of the constraint  $\theta_x \leq \theta_y$  in the pseudo-ellipse model, there are exactly three local minima (out of 6 possible permutations). In the isotropic cone, the  $\theta_x \equiv \theta_y$  condition collapses this to two. The multiple minima correspond to permutations of the motional system - the eigenframe x, y and z-axes as well as the cone opening angles  $\theta_x$ ,  $\theta_y$ , and  $\sigma_{\max}$  associated with these axes. But as the mechanics of the cone angles is not identical to that of the torsion angle, only one of the three local minima is the global minimum.

As the [minfx library](#) used in the frame order analysis currently only implements local optimisation algorithms, and because a global optimiser cannot be guaranteed to converge to the correct minima, a different approach is required:

- Optimise to one solution.
- Duplicate the data pipe for the model as ‘permutation A’.
- Permute the axes and amplitude parameters to jump from one local minimum to the other.
- Optimise the new permuted model, as the permuted parameters will not be exactly at the minimum.
- Repeat for the remaining ‘permutation B’ solution (only for the pseudo-ellipse models).

These steps have been incorporated into the automated analysis protocol.

The permutation step has been implemented as the `frame_order.permute_axes` user function. It is complicated by the fact that  $\theta_x$  is defined as a rotation about the y-axis and  $\theta_y$  is about the x-axis. See table 12.1 on page 255 for the pseudo-ellipse model permutations. These are also illustrated in figure 12.2 on page 253.

For the isotropic cone model, the same permutations exist but with some differences:

- The x and y axes are not defined in the x-y plane, therefore there are only two permutations (the first solution and ‘permutation A’).
- Any axis in the x-y plane can be used for the permutation, however different axes will result in different  $\chi^2$  values.
- As  $\theta_x \equiv \theta_y$ , the condition  $\theta_x \leq \sigma_{\max} \leq \theta_y$  can only exist if the torsion and cone angles are identical.
- Permutations A and B create identical cones as the x and y axes are equivalent.

The new isotropic cone angle is defined as

$$\theta' = \frac{\theta'_x + \theta'_y}{2}. \quad (12.78)$$

The isotropic cone axis permutations are shown in figure 12.3 on page 254.

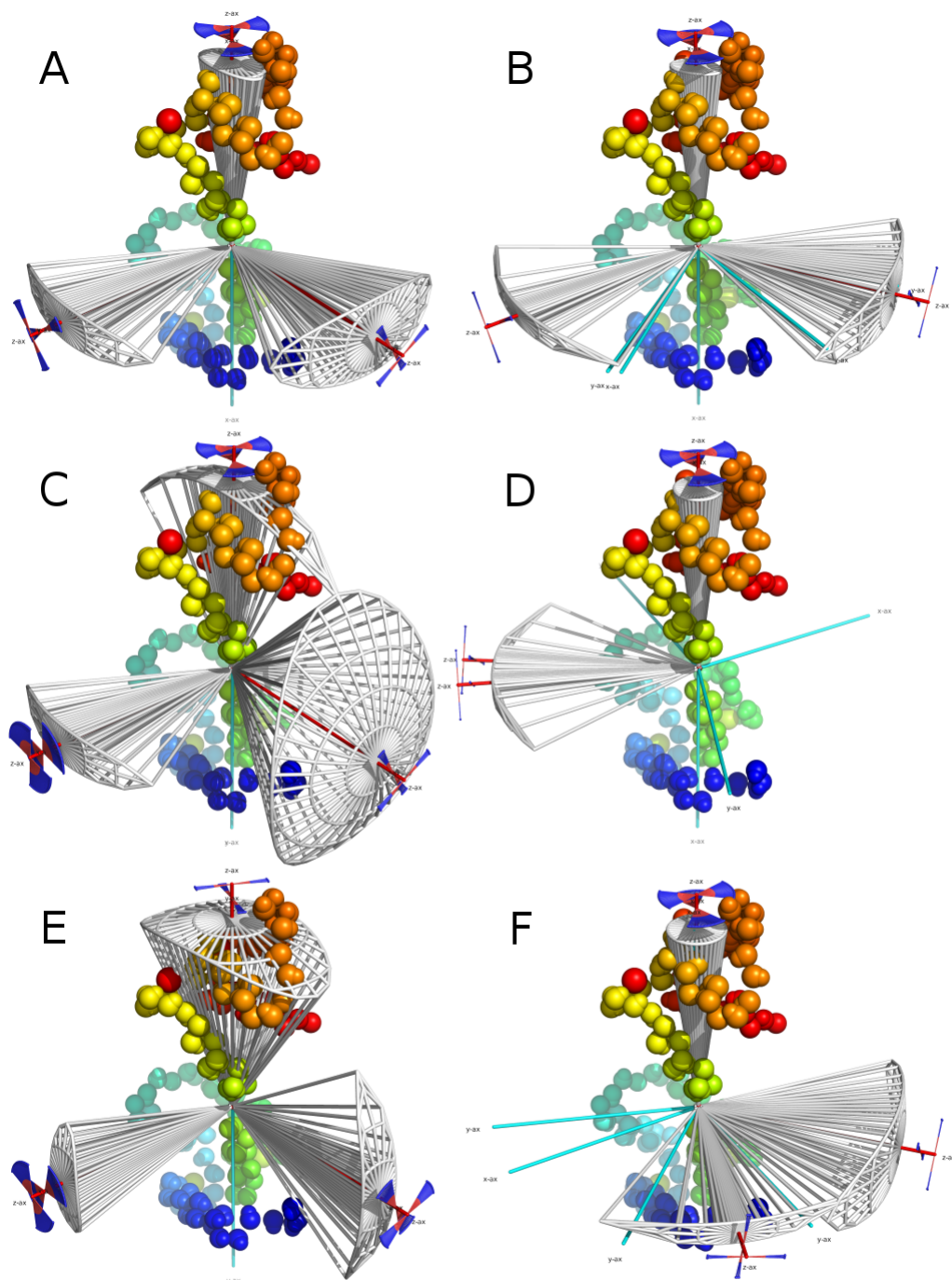


Figure 12.2: Pseudo-ellipse axis permutations. This uses synthetic data for a rotor model applied to CaM, with the rotor axis defined as being between the centre of the two helices between the domains (the centre of all cones in the figure) and the centre of mass of the C-terminal domain, and the rotor half-angle set to  $30^\circ$ . The condition  $\theta_x \leq \theta_y \leq \sigma_{\max}$  is shown in A and B. The condition  $\theta_x \leq \sigma_{\max} \leq \theta_y$  is shown in C and D. The condition  $\sigma_{\max} \leq \theta_x \leq \theta_y$  is shown in E and F. A, C, and E are the axis permutations for a set of starting half-angles and B, D, and F are the results after low quality optimisation demonstrating the presence of the multiple local minima.

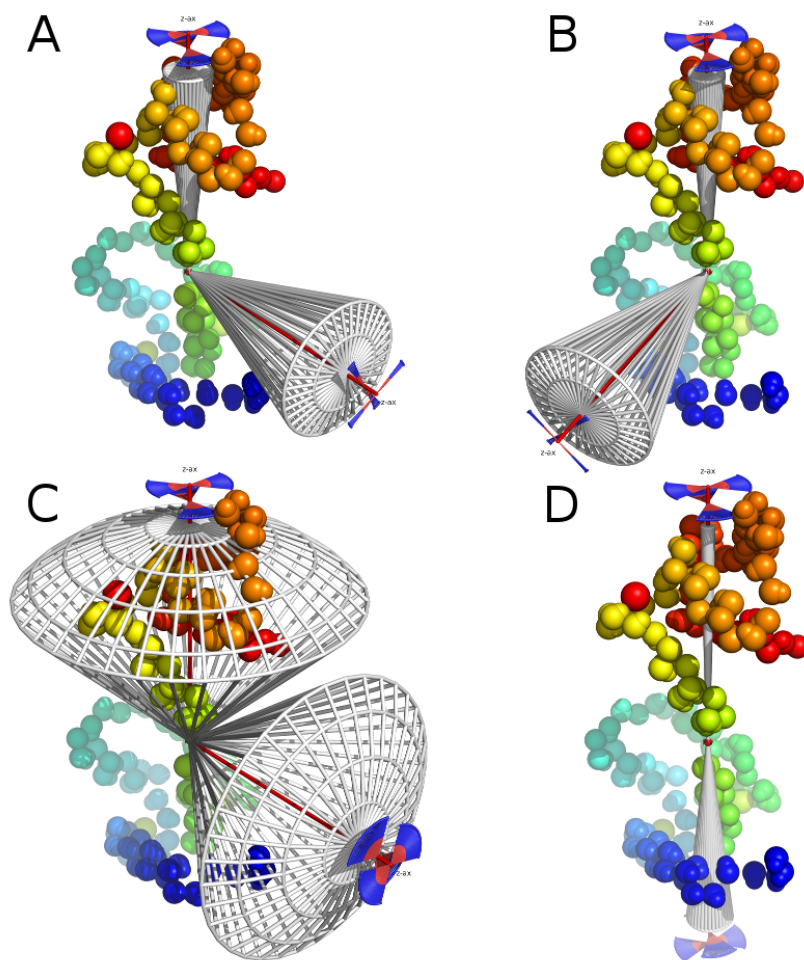


Figure 12.3: Isotropic cone axis permutations. This uses synthetic data for a rotor model applied to CaM, with the rotor axis defined as being between the centre of the two helices between the domains (the centre of all cones in the figure) and the centre of mass of the C-terminal domain, and the rotor half-angle set to  $30^\circ$ . The condition  $\theta \leq \sigma_{\max}$  is shown in A and B. The condition  $\sigma_{\max} \leq \theta$  is shown in C and D. A and C are the axis permutations for a set of starting half-angles and B and D are the results after low quality optimisation demonstrating the presence of the multiple local minima.



Table 12.1: The pseudo-ellipse motional eigenframe and half-angle permutations implemented in the `frame_order.permute_axes` user function.

| Condition                                   | Permutation name  | Cone half-angles<br>$[\theta'_x, \theta'_y, \sigma'_{\max}]$ | Axes<br>$[x', y', z']$ |
|---------------------------------------------|-------------------|--------------------------------------------------------------|------------------------|
| $\theta_x \leq \theta_y \leq \sigma_{\max}$ | Self <sup>1</sup> | $[\theta_x, \theta_y, \sigma_{\max}]$                        | $[x, y, z]$            |
|                                             | A                 | $[\theta_x, \sigma_{\max}, \theta_y]$                        | $[-z, y, x]$           |
|                                             | B                 | $[\theta_y, \sigma_{\max}, \theta_x]$                        | $[z, x, y]$            |
| $\theta_x \leq \sigma_{\max} \leq \theta_y$ | Self <sup>1</sup> | $[\theta_x, \theta_y, \sigma_{\max}]$                        | $[x, y, z]$            |
|                                             | A                 | $[\theta_x, \sigma_{\max}, \theta_y]$                        | $[-z, y, x]$           |
|                                             | B                 | $[\sigma_{\max}, \theta_y, \theta_x]$                        | $[x, -z, y]$           |
| $\sigma_{\max} \leq \theta_x \leq \theta_y$ | Self <sup>1</sup> | $[\theta_x, \theta_y, \sigma_{\max}]$                        | $[x, y, z]$            |
|                                             | A                 | $[\sigma_{\max}, \theta_x, \theta_y]$                        | $[y, z, x]$            |
|                                             | B                 | $[\sigma_{\max}, \theta_y, \theta_x]$                        | $[x, -z, y]$           |

<sup>1</sup> The first optimised solution.

### 12.3.3 Linear constraints for the frame order models

Linear constraints are implemented for the frame order models using the log-barrier constraint algorithm in [minfx](#), as this does not require the derivation of gradients.

The pivot point and average domain position parameter constraints in Ångstrom are:

$$-500 \leq P_x \leq 500, \quad (12.79a)$$

$$-500 \leq P_y \leq 500, \quad (12.79b)$$

$$-500 \leq P_z \leq 500, \quad (12.79c)$$

$$-999 \leq p_x \leq 999, \quad (12.79d)$$

$$-999 \leq p_y \leq 999, \quad (12.79e)$$

$$-999 \leq p_z \leq 999. \quad (12.79f)$$

These translation parameter restrictions are simply to stop the optimisation in the case of model failures. Converting these to the  $A \cdot x \geq b$  matrix notation required for the optimisation constraint algorithm, the constraints become

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} P_x \\ P_y \\ P_z \\ p_x \\ p_y \\ p_z \end{pmatrix} \geq \begin{pmatrix} -500 \\ -500 \\ -500 \\ -500 \\ -500 \\ -500 \\ -999 \\ -999 \\ -999 \\ -999 \\ -999 \\ -999 \end{pmatrix} \quad (12.80)$$

For the order or motional amplitude parameters of the set  $\mathfrak{S}$ , the constraints used are

$$0 \leq \theta \leq \pi, \quad (12.81a)$$

$$0 \leq \theta_x \leq \theta_y \leq \pi, \quad (12.81b)$$

$$0 \leq \sigma_{\max} \leq \pi, \quad (12.81c)$$

$$0 \leq \sigma_{\max,2} \leq \pi. \quad (12.81d)$$

These reflect the range of validity of these parameters. Converting to the  $A \cdot x \geq b$  notation, the constraints are

$$\begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 \\ 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & -1 \end{pmatrix} \cdot \begin{pmatrix} \theta \\ \theta_x \\ \theta_y \\ \sigma_{\max} \\ \sigma_{\max,2} \end{pmatrix} \geq \begin{pmatrix} 0 \\ -\pi \\ 0 \\ -\pi \\ 0 \\ 0 \\ -\pi \\ 0 \\ -\pi \\ 0 \\ -\pi \end{pmatrix} \quad (12.82)$$

The pseudo-elliptic cone model constraint  $\theta_x \geq \theta_y$  is used to simplify the optimisation space by eliminating symmetry.

## 12.4 Computation time and the numerical integration of the PCS

The numerical integration of the PCS using standard quadratic integration for the frame order models is impractical and cannot be used. A rough estimate for the computation time required for a full analysis of all the frame order models is in the order of  $1e^7$  years. Therefore a number of tricks have been implemented to speed up the calculations.

### 12.4.1 Numerical integration techniques

The numerical integration is approximated as

$$\int f \, dV \approx V \langle f \rangle \pm V \sqrt{\frac{\langle f^2 \rangle - \langle f \rangle^2}{N}}, \quad (12.83)$$

where the angular brackets are the means. As the average PCS value in the frame order models is defined as

$$\bar{\delta} = \int_S \delta \, dS \Big/ \int_S dS, \quad (12.84)$$

then

$$\bar{\delta} \approx \langle f \rangle \pm \sqrt{\frac{\langle f^2 \rangle - \langle f \rangle^2}{N}}. \quad (12.85)$$

This simply means that the average PCS value of a set of  $N$  domain positions which satisfy the constraints of the current model parameter values can be used as the back-calculated PCS value for the model.

The simplest method to calculate the PCS value would be to generate a uniform distribution of domain positions. The PCS value is calculated for each state in the distribution of  $N$  structures and then averaged. However this technique has non-ideal convergence properties, hence the number  $N$  needs to be high to obtain a reasonable estimate of the PCS value. Two techniques with better convergence properties are the Monte Carlo integration algorithm and the quasi-random integration algorithms.

### Monte Carlo numerical integration

As the convergence properties are better than that of a uniform distribution, the Monte Carlo integration algorithm is a viable option for using the PCS in the frame order analysis. Less states  $N$  are required for a reasonable estimate of the back-calculated PCS value. By randomly selecting  $N$  orientations of the domain which lie within the cone half-angle limits, back-calculating the PCS value for each state, and then averaging over all  $N$  states, the PCS value for the model can be numerically integrated. The original implementation used this technique.

### Quasi-random numerical integration – Sobol’ point sequence

Although the Monte Carlo numerical integration algorithm is a huge improvement on both the quadratic integration and the uniform distribution numerical integration techniques, it was nevertheless still too slow for optimising the frame order models. Therefore the quasi-random integration techniques were investigated, specifically using the Sobol’ point sequence (Sobol’, 1967).

To implement the numerical integration of the PCS using the quasi-random Sobol’ sequence, the LGPL licenced Sobol library of John Burkardt and Corrado Chiari from [http://people.sc.fsu.edu/~jburkardt/py\\_src/sobol/sobol.html](http://people.sc.fsu.edu/~jburkardt/py_src/sobol/sobol.html) was integrated into the relax library.

For each point coordinate  $s_i$ , the following functions were used to translate from linear space sampling to rotational space

$$\theta = \arccos(2s_i - 1), \quad (12.86a)$$

$$\phi = 2\pi s_i, \quad (12.86b)$$

$$\sigma = 2\pi(s_i - \frac{1}{2}), \quad (12.86c)$$

where  $\theta$  is the frame order tilt angle (the angle of rotation about the x-y plane rotation axis),  $\phi$  is the angle defining the x-y plane rotation axis, and  $\sigma$  is the torsion angle (the angle of rotation about the z’ axis). Each frame order model uses a different set of  $\theta$ ,  $\phi$ , and  $\sigma$  angles, therefore 1D, 2D, and 3D Sobol’ sequences are generated.

### Oversampling the Sobol' sequence points

As generating Sobol' points is computationally expensive, for speed this operation occurs during target function initialisation prior to optimisation. Hence the Sobol' points are not dynamically generated and a special algorithm is required to ensure adequate 1D, 2D and 3D sampling in the torsion-tilt angle space. The problem is that as the number of dimensions  $M$  increases, the density of a fixed  $N$  number of points in the  $M$  dimensions decreases. For example if a fixed value of  $N$  is chosen so that the pseudo-ellipse model is properly sampled, then the rotor model will be severely oversampled and take far too long to optimise. The protocol implemented to avoid this problem is:

- Generate  $N.Ov.10^M$  points, where  $N$  is the maximum number of Sobol' points to be used,  $Ov$  is the oversampling factor defaulting to 1, and  $M$  is the dimensions of the torsion-tilt angle space.
- Convert all Sobol' points into torsion-tilt angles.
- Convert all angles to rotation matrices.

During optimisation, the following two checks are implemented:

- Skip points outside of the limits of the current parameter values.
- Terminate the loop over the Sobol' points once  $N$  is reached.

For most cases,  $N$  should be reached. However if the cone or torsion half-angles are extremely small, then the points used may be less than  $N$ . This is therefore monitored and printed out after each optimisation step. For these cases,  $Ov$  can be increased for better sampling. This is implemented in the `frame_order.sobol_setup` user function.

### 12.4.2 Parallelization and running on a cluster

Four different attempts at parallelizing the calculations using the MPI 2 protocol resulted in no speeds up. In each case, the calculations were up to 2 times slower. It appears as though the data transfer of the PCS, atomics positions, vectors, etc. between nodes is slower than the calculations. As parallelization for speeding up the calculations can only achieve around two orders of magnitude faster calculations, the technique was abandoned.

### 12.4.3 Frame order model nesting

The concept of model nesting is used to hugely speed up the optimisation in the automated protocol. The most complex models have 15 independent parameters, and performing a grid search over 15 dimensions of the pseudo-ellipse frame order model is not feasible when using PCS numerical integration. The idea is to use the optimised parameters of a simpler model as the starting point for a more complex model, avoiding the need for a grid search for those copied parameters. This appears to work as the PCS value is dominated by the average domain position, hence the average domain parameters are very similar in all models.

### Model categories

The modelling of the  $\sigma$  torsion angle gives a number of categories of related models, those with no torsion, those with restricted torsion, and the free rotors.

**No torsion** When  $\sigma = 0$ , the following models are defined:

- Rigid,
- Isotropic cone, torsionless,
- Pseudo-ellipse, torsionless.

**Restricted torsion** When  $0 < \sigma < \pi$ , the following models are defined:

- Rotor,
- Isotropic cone,
- Pseudo-ellipse.

**Free rotors** When  $\sigma = \pi$ , i.e. there is no torsional restriction, the following models are defined:

- Free rotor,
- Isotropic cone, free rotor,
- Pseudo-ellipse, free rotor.

**Multiple torsion angles** This covers a single model – the double rotor.

### Parameter categories

There are three major parameter categories – the average domain position, the eigenframe of the motion, and the amplitude of the motion.

**Average domain position** Let the translational parameters be

$$\mathfrak{T} = \{P_x, P_y, P_z\}, \quad (12.87)$$

and the rotational or orientational parameters be

$$\mathfrak{D} = \{P_\alpha, P_\beta, P_\gamma\}. \quad (12.88)$$

Two full average position parameter sets used in the frame order models are

$$\mathfrak{P} = \mathfrak{T} + \mathfrak{D} = \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\}, \quad (12.89a)$$

$$\mathfrak{P}' = \{P_x, P_y, P_z, P_\beta, P_\gamma\}. \quad (12.89b)$$

Table 12.2: The nesting of frame order model parameters and the resultant grid search dimensionality. The boxes highlight parameter sets which are optimised in the initial grid search. The start of each train of arrows are the optimised parameters which will be copied for the more complex model and excluded from the grid search. The non-nested grid search dimensionality is given in brackets.

| Model                       | Parameter sets  |                                     |                  | Order parameters |            |            |                       |                         | Grid search dimensionality |
|-----------------------------|-----------------|-------------------------------------|------------------|------------------|------------|------------|-----------------------|-------------------------|----------------------------|
| Rigid                       | $\mathfrak{P}$  | -                                   | -                | -                | -          | -          | -                     | -                       | 6 (6)                      |
| Rotor                       | $\mathfrak{P}$  | $\mathfrak{E}_{\text{ax}}^{\alpha}$ | $\mathfrak{p}_1$ | -                | -          | -          | $\sigma_{\text{max}}$ | -                       | 5 (11)                     |
| Isotropic cone              | $\mathfrak{P}$  | $\mathfrak{E}_{\text{ax}}$          | $\mathfrak{p}_1$ | -                | $\theta$   | -          | $\sigma_{\text{max}}$ | -                       | 1 (13)                     |
| Pseudo-ellipse              | $\mathfrak{P}$  | $\mathfrak{E}_{\alpha\beta\gamma}$  | $\mathfrak{p}_1$ | -                | $\theta_x$ | $\theta_y$ | $\sigma_{\text{max}}$ | -                       | 4 (15)                     |
| Isotropic cone, torsionless | $\mathfrak{P}$  | $\mathfrak{E}_{\text{ax}}$          | $\mathfrak{p}_1$ | -                | $\theta$   | -          | -                     | -                       | 0 (12)                     |
| Pseudo-ellipse, torsionless | $\mathfrak{P}$  | $\mathfrak{E}_{\alpha\beta\gamma}$  | $\mathfrak{p}_1$ | -                | $\theta_x$ | $\theta_y$ | -                     | -                       | 0 (14)                     |
| Free rotor                  | $\mathfrak{P}'$ | $\mathfrak{E}_{\text{ax}}^{\alpha}$ | $\mathfrak{p}_1$ | -                | -          | -          | -                     | -                       | 5 (9)                      |
| Isotropic cone, free rotor  | $\mathfrak{P}'$ | $\mathfrak{E}_{\text{ax}}$          | $\mathfrak{p}_1$ | -                | $\theta$   | -          | -                     | -                       | 0 (11)                     |
| Pseudo-ellipse, free rotor  | $\mathfrak{P}'$ | $\mathfrak{E}_{\alpha\beta\gamma}$  | $\mathfrak{p}_1$ | -                | $\theta_x$ | $\theta_y$ | -                     | -                       | 0 (13)                     |
| Double rotor                | $\mathfrak{P}$  | $\mathfrak{E}_{\alpha\beta\gamma}$  | $\mathfrak{p}_1$ | $\mathfrak{p}_2$ | -          | -          | $\sigma_{\text{max}}$ | $\sigma_{\text{max},2}$ | 3 (15)                     |

**The motional eigenframe** This consists of either the full eigenframe or a single axis, combined with the pivot point(s) defining the origin of the frame(s) within the PDB space. The eigenframe parameters themselves are

$$\mathfrak{E}_{\alpha\beta\gamma} = \{E_{\alpha}, E_{\beta}, E_{\gamma}\}, \quad (12.90a)$$

$$\mathfrak{E}_{\text{ax}} = \{E_{\theta}, E_{\phi}\}, \quad (12.90b)$$

$$\mathfrak{E}_{\text{ax}}^{\alpha} = \{E_{\alpha}^{\text{ax}}\}. \quad (12.90c)$$

The pivot parameter sets are

$$\mathfrak{p}_1 = \{p_x, p_y, p_z\}, \quad (12.91a)$$

$$\mathfrak{p}_2 = \{p_d\}, \quad (12.91b)$$

**The rigid body ordering** The parameters of order are

$$\mathfrak{S} = \{\theta, \theta_x, \theta_y, \sigma_{\text{max}}, \sigma_{\text{max},2}\}. \quad (12.92)$$

### Frame order parameter nesting in the automated protocol

The parameter nesting used in the automated protocol is shown in table 12.2. This massively collapses the dimensionality of the initial grid search.

#### 12.4.4 PCS subset

Another trick that can be used to speed up the optimisation is to use a subset of all PCS values. The PCS data for a rigid domain can consist of hundreds of data points. Rather than using all these values, a very small subset of data points well chosen throughout the 3D structure – far apart, in rigid locations, and all nearby atoms having a similar value – can be used for the initial grid search and optimisation. As the PCS is most sensitive to the average domain position and less to the amplitude of motions, if the subset of atoms is well chosen the optimisation minimum for the subset should be very close to the optimisation minimum for the full data set. The subset minimum optimisation should be about two orders of magnitude faster as the number of PCS data points are linearly correlated with computation time. At the end of the analysis, a final stage of slower optimisation using all PCS data can be performed to find the full data set minimum.

In the case of the CaM analyses, a subset of five points was used. In the peptide bound calmodulin analyses, the H data of residues {Tyr 99, Val 108, Glu 114, Glu 119, Arg 126} was used. As data was not available for the same residues in the free calmodulin analysis, instead the proton data for residues {Tyr 99, Met 109, Lys 115, Glu 119, Glu 127} was used.

#### 12.4.5 Optimisation of the frame order models

Due to the numerical integration of the PCS, optimisation is extremely slow. A number of optimisation techniques can help speed up this part including using a low precision initial grid search, a zooming grid search, an alternating grid search, and zooming precision optimisation.

##### Low precision grid search

Using 10,000 Sobol' points appears to be the minimum while still delivering about 2-3 decimal places of accuracy for the frame order models (when values are not close to zero). But rather than using this number of points, 100 can be used in the initial grid search. This is not accurate enough from the parameter perspective, but is close enough to the real minimum (local or global, see section 12.3.2 on page 252). This speeds up the calculation by two orders of magnitude.

##### The zooming grid search

This is implemented in `relax` rather than in the [minfx optimisation library](#). As the grid search is parallelised to run on a cluster using OpenMPI, it can sometimes be advantageous to use a fine grid search to find a better optimisation starting position for the Nelder-Mead simplex algorithm. Rather than simply increasing the number of increments in the grid search, iteratively performing the grid search while zooming into the optimised parameter values is a more efficient alternative. This zooming grid search can be seen in the frame order optimisation script on page 266.

### The alternating grid search

For finding the average domain position in the rigid frame order model, an alternating grid search has been implemented in the automated analysis protocol. The idea is to speed up the slow 6D grid search by first searching over the 3D rotational space, then the 3D translational space. The grid is then zoomed by one level and alternating grid search is repeated. As this technique does not guarantee convergence, it is not turned on by default. The results from the alternation should be carefully checked and the technique avoided if the average domain position is not reasonable.

### Zooming precision optimisation

One trick is to perform an iterative optimisation whereby each iteration increases in precision. For this the function tolerance cutoff for optimisation is used. But the use of the amount of sampling through the Sobol' sequence can result in much greater speed ups. The reason is because each point in the Sobol' sequence requires a fixed amount of time to calculate the PCSs for all spins, so time is linearly correlated with the number of points.

Using synthetic CaM data found in `test_suite/shared_data/frame_order/cam/`, the ideal number of Sobol' points was found to be  $\{100, 1000, 10000\}$ . This is combined with a function tolerance cutoff of  $\{1e^{-2}, 1e^{-3}, 1e^{-4}\}$ .

## 12.4.6 Error analysis

### Low precision Monte Carlo simulations

The propagation of errors via Monte Carlo simulation, which is extremely computationally expensive, can also be sped up. By only using a small number of simulations (50 to 100), 100 Sobol' points, and a function tolerance of  $1e^{-3}$ , the computational time required for Monte Carlo simulations can be dramatically reduced. However, despite using the lower quality settings, with the current implementation of the frame order theory this error analysis is currently not computationally feasible.

## 12.5 The frame order data analysis

### 12.5.1 Introduction to frame order data analysis

The analysis consists of two parts. The first is the single structure or ensemble analysis of the combined RDC and PCS alignment tensor for the non-moving paramagnetically aligned domain or rigid body. The resultant alignment tensor and optimised paramagnetic  $\text{Ln}^{3+}$  position is then used as input into the frame order analysis.



### 12.5.2 The N-state model analysis scripts

For determining the alignment tensor and paramagnetic  $\text{Ln}^{3+}$  position from the RDC and PCS data, the N-state model or ensemble analysis should be used. The script is:

```

1  # Python imports.
2  from numpy import array
3
4  # relax imports.
5  from lib.physical_constants import NH_BOND_LENGTH_RDC
6
7
8  # Create a data pipe for all the data.
9  pipe.create('CaM N-dom', 'N-state')
10
11 # Load the CaM structure.
12 structure.read_pdb('2BE6_core_II_III.pdb', dir='.././.././structures/2BE6_superimpose/
    Ndom_II_III', set_mol_name=['CaM_A', 'IQ_A', 'Metals_A', 'CaM_B', 'IQ_B', 'Metals_B',
    'CaM_C', 'IQ_C', 'Metals_C'])
13
14 # Load the spins.
15 structure.load_spins('@N', from_mols=['CaM_A', 'CaM_B', 'CaM_C'], mol_name_target='CaM',
    ave_pos=False)
16 structure.load_spins('@H', from_mols=['CaM_A', 'CaM_B', 'CaM_C'], mol_name_target='CaM',
    ave_pos=False)
17
18 # Select only the superimposed spins (skipping mobile residues :2-4,42,56-57,76-80,
    identified from model-free order parameters).
19 select.spin(':17-41,43-55,58-67', change_all=True)
20 select.display()
21
22 # Define the magnetic dipole-dipole relaxation interaction.
23 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
24 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=NH_BOND_LENGTH_RDC)
25 interatom.unit_vectors(ave=False)
26
27 # Set the nuclear isotope and element.
28 spin.isotope(isotope='15N', spin_id='@N')
29 spin.isotope(isotope='1H', spin_id='@H')
30
31 # The alignment data.
32 align_data = [
33     ['dy', 'RDC_DY_111011_spinID.txt', 'PCS_DY_200911.txt', 900.00423401],
34     ['tb', 'RDC_TB_111011_spinID.txt', 'PCS_TB_200911.txt', 900.00423381],
35     ['tm', 'RDC_TM_111011_spinID.txt', 'PCS_TM_200911.txt', 900.00423431],
36     ['er', 'RDC_ER_111011_spinID.txt', 'PCS_ER_200911.txt', 899.90423151],
37     ['yb', 'RDC_YB_110112_spinID.txt', 'PCS_YB_211111.txt', 899.90423111],
38     ['ho', 'RDC_HO_300512_spinID.txt', 'PCS_HO_300412.txt', 899.80423481]
39 ]
40
41 # Loop over the alignments.
42 for i in range(len(align_data)):
43     # Alias the data.
44     TAG = align_data[i][0]
45     RDC_FILE = align_data[i][1]
46     PCS_FILE = align_data[i][2]
47     FRQ = align_data[i][3]
48
49     # RDCs.

```

```

50     rdc.read(align_id=TAG, file=RDC_FILE, dir='../..', data_type='D', spin_id1_col=1,
51             spin_id2_col=2, data_col=3, error_col=4)
52
53     # PCSs.
54     pcs.read(align_id=TAG, file=PCS_FILE, dir='../..', res_num_col=1, data_col=2,
55             error_col=4, spin_id='@N')
56     pcs.read(align_id=TAG, file=PCS_FILE, dir='../..', res_num_col=1, data_col=3,
57             error_col=4, spin_id='@H')
58
59     # The temperature.
60     spectrometer.temperature(id=TAG, temp=303.0)
61
62     # The frequency.
63     spectrometer.frequency(id=TAG, frq=FRQ, units='MHz')
64
65     # The paramagnetic centre (average Ca2+ position).
66     ave = array([7.608, 5.402, 16.725]) + array([7.295, 4.684, 16.168]) + array([7.338, 5.275,
67             16.086])
68     ave = ave / 3
69     paramag.centre(pos=ave)
70
71     # Set up the model.
72     n_state_model.select_model('fixed')
73
74     # Tensor optimisation.
75     print("\n\n# Tensor optimisation.\n\n")
76     minimise.grid_search(inc=5)
77     minimise.execute('newton', constraints=False)
78     state.save('tensor_only_fit', force=True)
79
80     # Optimisation of everything.
81     paramag.centre(fix=False)
82     minimise.execute('bfgs', constraints=False)
83
84     # PCS structural noise.
85     print("\n\n# Tensor optimisation with PCS structural noise.\n\n")
86     pcs.structural_noise(rmsd=0.3, sim_num=10000, file='structural_noise.agr', force=True)
87
88     # Optimisation of everything.
89     paramag.centre(fix=False)
90     minimise.execute('bfgs', constraints=False)
91
92     # Monte Carlo simulations.
93     monte_carlo.setup(number=500)
94     monte_carlo.create_data()
95     monte_carlo.initial_values()
96     minimise.execute('bfgs', constraints=False)
97     monte_carlo.error_analysis()
98
99     # Show the tensors.
100     align_tensor.display()
101
102     # Q-factors.
103     rdc.calc_q_factors()
104     pcs.calc_q_factors()
105
106     # Correlation plots.
107     rdc.corr_plot(file="rdc_corr.agr", force=True)
108     pcs.corr_plot(file="pcs_corr.agr", force=True)
109
110     # Save the program state.

```

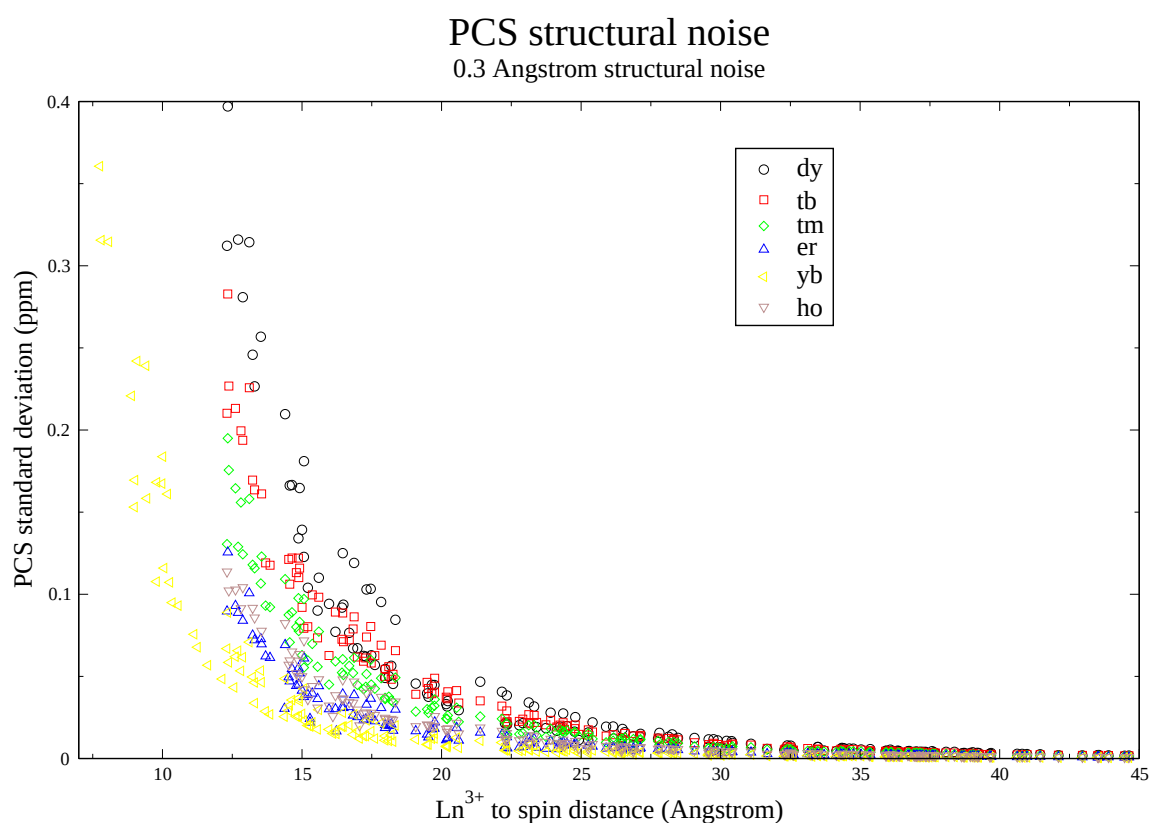


Figure 12.4: Structural noise simulation using the 2BE6 CaM-IQ X-ray ABC ensemble as an example. The simulated structural error, with the atom position uncertainty set to 0.3 Å, is an extra contribution to the measured PCS error. The PCS structural error is compared to the distance from the paramagnetic centre to illustrate the relationship between the two. The atomic positions were randomised 10,000 times with the lanthanide position assumed fixed, the PCS value back-calculated using a pre-fit alignment tensor, and the PCS standard deviations calculated.

```
107 state.save('tensor_fit', force=True)
```

The calculation of the additional PCS error due to structural noise is very important for the subsequent frame order analysis. A plot of such a calculation is shown in Figure 12.4

Statistics for the tensors, including the matrix or inter-tensor angles, the singular value decomposition (SVD) values and condition number, and a printout of the tensors for copying directly in the frame order analysis script, are obtained via the script:

```
1 # Script for determining the tensor angles and SVD values of the CaM-IQ tensors.
2
3 # Load the state.
4 state.load('tensor_fit')
5
6 # Loop over the alignment tensors, producing a string of relax user function commands.
7 print("\nTensor strings for relax input:")
8 string = ""
9 for A in cdp.align_tensors:
10     string += "align_tensor.init(tensor='%s', params=(%s, %s, %s, %s, %s), param_types=2)\n" % (A.name, A.Axx, A.Ayy, A.Axy, A.Axz, A.Ayz)
```

```

11     string += "align_tensor.init(tensor='%s', params=(%s, %s, %s, %s, %s), param_types=2,
12         errors=True)\n" % (A.name, A.Axx_err, A.Ayy_err, A.Axy_err, A.Axz_err, A.Ayz_err)
13
14 # The matrix angles.
15 align_tensor.matrix_angles(basis_set=0)
16 align_tensor.matrix_angles(basis_set=1)
17
18 # The SVD analysis.
19 align_tensor.svd(basis_set=0)
20 align_tensor.svd(basis_set=1)

```

### 12.5.3 The frame order analysis scripts

The following is the example frame order analysis script located at `sample_scripts/frame_order/full_analysis.py`:

```

1  """Script for black-box Frame Order analysis.
2
3  This is for the CaM-IQ data.
4
5  The free rotor pseudo-elliptic cone model is not used in this script as the cone X and Y
6  opening angles cannot be differentiated with simply RDC and PCS data, hence this model
7  is perfectly approximated by the free rotor isotropic cone.
8  """
9
10 # Python module imports.
11 from numpy import array
12 from time import asctime, localtime
13
14 # relax module imports.
15 from auto_analyses.frame_order import Frame_order_analysis, Optimisation_settings
16
17 # Analysis variables.
18 #####
19
20 # The frame order models to use.
21 MODELS = [
22     'rigid',
23     'rotor',
24     'iso cone',
25     'pseudo-ellipse',
26     'iso cone, torsionless',
27     'pseudo-ellipse, torsionless',
28     'double rotor'
29 ]
30
31 # The number of Monte Carlo simulations to be used for error analysis at the end of the
32 protocol.
33 MC_NUM = 10
34
35 # Rigid model optimisation setup.
36 OPT_RIGID = Optimisation_settings()
37 OPT_RIGID.add_grid(inc=21, zoom=0)
38 OPT_RIGID.add_grid(inc=21, zoom=1)
39 OPT_RIGID.add_grid(inc=21, zoom=2)
40 OPT_RIGID.add_grid(inc=21, zoom=3)

```

```

39 OPT_RIGID.add_min(min_algor='simplex')
40
41 # PCS subset optimisation setup.
42 OPT_SUBSET = Optimisation_settings()
43 OPT_SUBSET.add_grid(inc=11, zoom=0, sobol_max_points=100)
44 OPT_SUBSET.add_grid(inc=11, zoom=1, sobol_max_points=100)
45 OPT_SUBSET.add_grid(inc=11, zoom=2, sobol_max_points=100)
46 OPT_SUBSET.add_min(min_algor='simplex', func_tol=1e-2, sobol_max_points=100)
47
48 # Full data set optimisation setup.
49 OPT_FULL = Optimisation_settings()
50 OPT_FULL.add_grid(inc=11, zoom=2, sobol_max_points=100)
51 OPT_FULL.add_grid(inc=11, zoom=3, sobol_max_points=100)
52 OPT_FULL.add_min(min_algor='simplex', func_tol=1e-2, sobol_max_points=100)
53 OPT_FULL.add_min(min_algor='simplex', func_tol=1e-3, sobol_max_points=1000)
54 OPT_FULL.add_min(min_algor='simplex', func_tol=1e-4, sobol_max_points=10000)
55
56 # Monte Carlo simulation optimisation setup.
57 OPT_MC = Optimisation_settings()
58 OPT_MC.add_min(min_algor='simplex', func_tol=1e-3, sobol_max_points=100)
59
60
61 # Set up the base data pipes.
62 #####
63
64 # The data pipe bundle to group all data pipes.
65 PIPE_BUNDLE = "Frame Order (%s)" % asctime(localtime())
66
67 # Create the base data pipe containing only a subset of the PCS data.
68 SUBSET = "Data subset - " + PIPE_BUNDLE
69 pipe.create(pipe_name=SUBSET, pipe_type='frame order', bundle=PIPE_BUNDLE)
70
71 # Read the structures.
72 structure.read_pdb('2BE6_ndom_truncN.pdb', dir='../././structures/2BE6_superimpose',
73                  set_mol_name='N-dom')
74
75 structure.read_pdb('2BE6_cdom_truncC.pdb', dir='../././structures/2BE6_superimpose',
76                  set_mol_name='C-dom')
77
78 # Set up the 15N and 1H spins.
79 structure.load_spins(spin_id='@N', mol_name_target='CaM', ave_pos=False)
80 structure.load_spins(spin_id='@H', mol_name_target='CaM', ave_pos=False)
81 spin.isotope(isotope='15N', spin_id='@N')
82 spin.isotope(isotope='1H', spin_id='@H')
83
84 # Define the magnetic dipole-dipole relaxation interaction.
85 interatom.define(spin_id1='@N', spin_id2='@H', direct_bond=True)
86 interatom.set_dist(spin_id1='@N', spin_id2='@H', ave_dist=1.041 * 1e-10)
87 interatom.unit_vectors()
88
89 # Deselect mobile spins and vectors (from the CaM model-free order parameters from the
90 BMRB).
91 deselect.spin(':2-4')
92 deselect.spin(':42')
93 deselect.spin(':56-57')
94 deselect.spin(':76-82')
95 deselect.spin(':114-117')
96 deselect.spin(':129-130')
97 deselect.spin(':146-148')
98 deselect.interatom(':2-4')
99 deselect.interatom(':42')
100 deselect.interatom(':56-57')

```

```

97  deselect.interatom(':76-82')
98  deselect.interatom(':114-117')
99  deselect.interatom(':129-130')
100 deselect.interatom(':146-148')
101
102 # The lanthanides and data files.
103 ln = ['dy', 'tb', 'tm', 'er', 'yb', 'ho']
104 pcs_files = [
105     'PCS_DY_200911.txt',
106     'PCS_TB_200911.txt',
107     'PCS_TM_200911.txt',
108     'PCS_ER_200911.txt',
109     'PCS_YB_211111.txt',
110     'PCS_HO_300412.txt'
111 ]
112 pcs_files_subset = [
113     'PCS_DY_200911_subset.txt',
114     'PCS_TB_200911_subset.txt',
115     'PCS_TM_200911_subset.txt',
116     'PCS_ER_200911_subset.txt',
117     'PCS_YB_211111_subset.txt',
118     'PCS_HO_300412_subset.txt'
119 ]
120 rdc_files = [
121     'RDC_DY_111011_spinID.txt',
122     'RDC_TB_111011_spinID.txt',
123     'RDC_TM_111011_spinID.txt',
124     'RDC_ER_111011_spinID.txt',
125     'RDC_YB_110112_spinID.txt',
126     'RDC_HO_300512_spinID.txt'
127 ]
128
129 # The spectrometer frequencies for Luigi's measurements (matching the above lanthanide
    ordering, taken from the acqus SF01 parameter).
130 pcs_freq = [
131     701.2533001,    # Dy3+.
132     701.2533002,    # Tb3+.
133     701.2533005,    # Tm3+.
134     701.2533003,    # Er3+.
135     701.2533004,    # Yb3+.
136     701.2533005     # Ho3+.
137 ]
138 rdc_freq = [
139     900.00423401,   # Dy3+.
140     900.00423381,   # Tb3+.
141     900.00423431,   # Tm3+.
142     899.90423151,   # Er3+.
143     899.90423111,   # Yb3+.
144     899.80423481,   # Ho3+.
145 ]
146
147 # Loop over the alignments.
148 for i in range(len(ln)):
149     # Load the RDCs.
150     rdc.read(aligned_id=ln[i], file=rdc_files[i], dir='../.../align_data/CaM_IQ/',
        spin_id1_col=1, spin_id2_col=2, data_col=3, error_col=4)
151
152     # The 1H PCS (only a subset of ~5 spins for fast initial optimisations).
153     pcs.read(aligned_id=ln[i], file=pcs_files_subset[i], dir='../.../align_data/CaM_IQ/',
        res_num_col=1, data_col=3, error_col=4, spin_id='@H')
154

```

```

155     # The temperature and field strength.
156     spectrometer.temperature(id=ln[i], temp=303.0)
157     spectrometer.frequency(id=ln[i], frq=rdc_frq[i], units="MHz")
158
159 # Set up the tensors from the CaM-IQ ABC :6-74@N,CA,C,0 N-state model fit.
160 align_tensor.init(tensor='Dy N-dom', align_id='dy', params=(-0.000895122969134, 0
    .000200206126748, 0.000350783562498, 0.000789321179176, -0.000185956794915),
    param_types=2)
161 align_tensor.init(tensor='Dy N-dom', align_id='dy', params=(1.2293468401e-05, 1
    .74966511177e-05, 1.07296910627e-05, 1.21471537359e-05, 9.98472771055e-06),
    param_types=2, errors=True)
162 align_tensor.init(tensor='Tb N-dom', align_id='tb', params=(-0.000386773980249, -0
    .000252229451755, 0.000289345115245, 0.00077454551221, -0.000411564842864),
    param_types=2)
163 align_tensor.init(tensor='Tb N-dom', align_id='tb', params=(9.08568851197e-06, 1
    .25046911688e-05, 9.13279347879e-06, 8.66699785438e-06, 8.17084290029e-06),
    param_types=2, errors=True)
164 align_tensor.init(tensor='Tm N-dom', align_id='tm', params=(0.000138832563763, 0
    .00019276873546, -0.000401761891364, -0.00053984778662, 0.000385156710458),
    param_types=2)
165 align_tensor.init(tensor='Tm N-dom', align_id='tm', params=(7.45028293534e-06, 1
    .15087527652e-05, 7.80160598908e-06, 7.48687231235e-06, 8.44077530542e-06),
    param_types=2, errors=True)
166 align_tensor.init(tensor='Er N-dom', align_id='er', params=(0.00013266928235, 6
    .08491225722e-05, -0.000249892897607, -0.000344865388853, 0.000118692962249),
    param_types=2)
167 align_tensor.init(tensor='Er N-dom', align_id='er', params=(6.24728334522e-06, 8
    .68937486363e-06, 7.96726504939e-06, 6.43064935791e-06, 1.00354375045e-05),
    param_types=2, errors=True)
168 align_tensor.init(tensor='Yb N-dom', align_id='yb', params=(0.000150564392882, -7
    .59743643441e-05, -0.00013958907081, -0.000188379895441, 0.000102722198261),
    param_types=2)
169 align_tensor.init(tensor='Yb N-dom', align_id='yb', params=(4.42731599871e-06, 5
    .1565091874e-06, 5.18051425981e-06, 3.9225664592e-06, 4.49007020445e-06), param_types
    =2, errors=True)
170 align_tensor.init(tensor='Ho N-dom', align_id='ho', params=(-0.000307522207243, 2
    .76511812842e-05, 0.000152789357344, 0.000307999279733, -0.000235201851074),
    param_types=2)
171 align_tensor.init(tensor='Ho N-dom', align_id='ho', params=(6.56971189673e-06, 1
    .0420422445e-05, 8.05282585054e-06, 7.42469124453e-06, 7.25413636142e-06), param_types
    =2, errors=True)
172
173 # Define the domains.
174 domain(id='N', spin_id=":1-78")
175 domain(id='C', spin_id=":80-148")
176
177 # The tensor domains and reductions.
178 full = ['Dy N-dom', 'Tb N-dom', 'Tm N-dom', 'Er N-dom', 'Yb N-dom', 'Ho N-dom']
179 red = ['Dy C-dom', 'Tb C-dom', 'Tm C-dom', 'Er C-dom', 'Yb C-dom', 'Ho C-dom']
180 ids = ['dy', 'tb', 'tm', 'er', 'yb', 'ho']
181 for i in range(len(full)):
182     # Initialise the reduced tensors (fitted during optimisation).
183     align_tensor.init(tensor=red[i], align_id=ids[i], params=(0, 0, 0, 0, 0))
184
185     # Set the domain info.
186     align_tensor.set_domain(tensor=full[i], domain='N')
187     align_tensor.set_domain(tensor=red[i], domain='C')
188
189     # Specify which tensor is reduced.
190     align_tensor.reduction(full_tensor=full[i], red_tensor=red[i])
191

```

```

192 # Set the reference domain.
193 frame_order.ref_domain('N')
194
195 # Set the initial pivot point.
196 pivot = array([ 21.863, 5.270, 5.934])
197 frame_order.pivot(pivot, fix=False)
198
199 # Set the paramagnetic centre position.
200 paramag.centre(pos=[6.518, 8.520, 13.767])
201
202 # Duplicate the PCS data subset data pipe to create a data pipe containing all the PCS
    data.
203 DATA = "Data - " + PIPE_BUNDLE
204 pipe.copy(pipe_from=SUBSET, pipe_to=DATA, bundle_to=PIPE_BUNDLE)
205 pipe.switch(DATA)
206
207 # Load the complete PCS data into the already filled data pipe.
208 for i in range(len(ln)):
209     # The 15N PCS.
210     pcs.read(aligned_id=ln[i], file=pcs_files[i], dir='../.../align_data/CaM_IQ/',
        res_num_col=1, data_col=2, error_col=4, spin_id='@N')
211
212     # The 1H PCS.
213     pcs.read(aligned_id=ln[i], file=pcs_files[i], dir='../.../align_data/CaM_IQ/',
        res_num_col=1, data_col=3, error_col=4, spin_id='@H')
214
215
216 # Execution.
217 #####
218
219 # Do not change!
220 Frame_order_analysis(data_pipe_full=DATA, data_pipe_subset=SUBSET, pipe_bundle=PIPE_BUNDLE
    , results_dir=None, opt_rigid=OPT_RIGID, opt_subset=OPT_SUBSET, opt_full=OPT_FULL,
    opt_mc=OPT_MC, mc_sim_num=MC_NUM, models=MODELS)

```

Once this analysis has been completed then, a refinement step is required. This is due to the low amount of motion in the system which causes the pivot point to be less well defined and hence strongly affected by artifacts of discrete sampling of a continuous and uniform distribution. The collapse of certain cone open half-angles and torsion angles to zero also causes the number of Sobol' points  $N$  to sometimes be zero. The refinement script is:

```

1  """Script for black-box Frame Order analysis.
2
3  This is for the CaM-IQ data.
4
5  The free rotor pseudo-elliptic cone model is not used in this script as the cone X and Y
    opening angles cannot be differentiated with simply RDC and PCS data, hence this model
    is perfectly approximated by the free rotor isotropic cone.
6  """
7
8  # Python module imports.
9  from time import asctime, localtime
10
11 # relax module imports.
12 from auto_analyses.frame_order import Frame_order_analysis, Optimisation_settings
13
14
15 # Analysis variables.
16 #####

```



```

17
18 # The frame order models to use.
19 MODELS = [
20     'rigid',
21     'rotor',
22     'iso cone',
23     'pseudo-ellipse',
24     'iso cone, torsionless',
25     'pseudo-ellipse, torsionless',
26     'double rotor'
27 ]
28
29 # The number of Monte Carlo simulations to be used for error analysis at the end of the
    protocol.
30 MC_NUM = 10
31
32 # Full data set optimisation setup.
33 OPT_FULL = Optimisation_settings()
34 OPT_FULL.add_min(min_algor='simplex', func_tol=1e-4, quad_int=True)
35
36 # Monte Carlo simulation optimisation setup.
37 OPT_MC = Optimisation_settings()
38 OPT_MC.add_min(min_algor='simplex', func_tol=1e-3, quad_int=True)
39
40
41 # Set up the base data pipes.
42 #####
43
44 # The data pipe bundle to group all data pipes.
45 PIPE_BUNDLE = "Frame Order (%s)" % asctime(localtime())
46
47
48 # Execution.
49 #####
50
51 # Do not change!
52 Frame_order_analysis(pipe_bundle=PIPE_BUNDLE, results_dir='refinement', pre_run_dir='.',
    opt_full=OPT_FULL, opt_mc=OPT_MC, mc_sim_num=MC_NUM, models=MODELS)

```

#### 12.5.4 Computation times

It should be noted that computation times are currently very long, in the order of months. This depends on the models chosen, the amount and quality of the input data, and the refinement step. This is due to the numeric integration for the PCS data. Calculations can range from one to six months on a very fast computer. Unfortunately parallelization was not possible, but multiple analyses can be performed simultaneously on multiple core or multiple CPU systems. A dedicated machine with a reliable UPS system is highly recommended.



## Part III

# Power users



## Chapter 13

# relax development

This chapter is for developers or those who would like to extend the functionality of relax. It is not required for using relax. If you would like to make modifications to the relax source code please subscribe to all the relax mailing lists (see the open source infrastructure chapter for more details). Announcements are sent to “relax-announce at gna.org” whereas “relax-users at gna.org” is the list where discussions about the usage of relax should be posted. “relax-devel at gna.org” is where all discussions about the development of relax including feature requests, program design, or any other discussions relating to relax’s structure or code should be posted. Finally, “relax-commits at gna.org” is where all changes to relax’s code and documentation, as well as changes to the web pages, are automatically sent to. Anyone interested in joining the project should subscribe to all four lists.

### 13.1 Version control using Subversion

The development of relax requires the use of the Subversion (SVN) version control software downloadable from <http://subversion.tigris.org/>. The source code to relax is stored in an SVN repository located at <http://svn.gna.org/svn/relax/>. Every single change which has ever made to the program is recorded within this repository. For more information see the open source infrastructure chapter 3 on page 29.

Although the downloadable distribution archives can be modified it is best that the most current and up to date revision (the *head* revision) is modified instead. More information about the basics of version control and how this is implemented in Subversion can be found in the Subversion book located at <http://svnbook.red-bean.com/>.

If you are not currently a relax developer you can check out the head revision by typing

```
$ svn co svn://svn.gna.org/svn/relax/trunk relax-trunk
```

Otherwise if you are a developer type

```
$ svn co svn+ssh://xxxxx@svn.gna.org/svn/relax/trunk relax-trunk
```

replacing xxxxx with your Gna! login name. If your version is out of date it can be updated to the latest revision by typing

```
$ svn up
```

Modifications can be made to these sources.

## 13.2 Coding conventions

The following conventions should be followed at all times for any code to be accepted into the relax repository. A Python script which tests if code meets relax's coding conventions is distributed with relax and is located at `scripts/code_validator`. The main reason for these conventions is for readability. By using a consistent coding style and a high comment ratio, the code becomes much easier to read for non-coders and those new to Python. It significantly decreases the barrier of entry into the relax source code for NMR spectroscopists.

### 13.2.1 Indentation

Indentation should be set to four spaces rather than a tab character. This is the recommendation given in the Python style guide found at <http://www.python.org/doc/essays/styleguide.html>. Emacs should automatically set the tabstop correctly. For vi add the following lines to the `vimrc` file:

```
set tabstop=4
set shiftwidth=4
set expandtab
```

For UNIX systems, including Linux and Mac OS X, the `vimrc` file is `~/.vimrc` whereas in MS Windows the file is `$VIM/_vimrc` which is usually `C:\Program Files\vim\_vimrc`. Certain versions of vim, those within the 6.2 series, contain a bug where the tabstop value cannot be changed using the `vimrc` file (although typing `:set tabstop=4` in vim will fix it). One solution is to edit the file `python.vim` which on GNU/Linux systems is located in the path `/usr/share/vim/ftplugin/`. It contains the two lines

```
" Python always uses a 'tabstop' of 8.
setlocal ts=8
```

If these lines are deleted the bug will be removed. Another way to fix the problem is to install newer versions of the run-time files (which will do the same thing).

### 13.2.2 Doc strings

The following are relax's conventions for docstrings. Many of these are Python conventions.

- The standard Python convention of a one line description separated from a detailed description by an empty line should be adhered to. This line must start with a capital letter and end in a period. This convention is required for certain docstring parsers (see the Python docs).

- All functions should have a docstring describing in detail the function, structure, and organisation of the code.
- A docstring should be followed by an empty line.
- Indentation of the docstring should be the same as that of the first line of code, excluding indented lists, etc.

An example of a single line docstring is:

```
1     def delete(self):
2         """Function for deleting all model-free data."""
```

An example of a multiline docstring is:

```
1     def aic(chi2, k, n):
2         """Akaike's Information Criteria (AIC).
3
4         The formula is::
5
6             AIC = chi2 + 2k
7
8
9         @param chi2:    The minimised chi-squared value.
10        @type chi2:     float
11        @param k:       The number of parameters in the model.
12        @type k:        int
13        @param n:       The dimension of the relaxation data set.
14        @type n:        int
15        @return:        The AIC value.
16        @rtype:         float
17        """
18
19        return chi2 + 2.0*k
```

In addition to the text descriptions, the docstrings use the [Epydoc](http://epydoc.sourceforge.net/fields.html) markup language to describe arguments, return values, and other information about the code. See <http://epydoc.sourceforge.net/fields.html> for a listing of all the epydoc fields allowed. This mark up language is important for the creation of the [API documentation](#) and to help developers understand the purpose and operation of the code.

### 13.2.3 Variable, function, and class names

In relax a mixture of both camel case (e.g. CamelCase) and lower case with underscores is used. Despite the variability there are fixed rules which should be adhered to. These naming conventions should be observed at all times.

#### Variables and functions

For both variables and functions lower case with underscores between words is always used. This is for readability as the convention is much more fluent than camel case. A few rare exceptions exist, an example is the Brownian diffusion tensor parameter of anisotropy  $\mathfrak{D}_a$  which is referenced as `cdp.diff_tensor.Da`. As a rule though all new variable or function

names should be kept as lower case. An example of this convention for both the variable name and function name is:

```

1  def assemble_param_vector(self, spin=None, spin_id=None, sim_index=None, model_type=
    None):
2      """Assemble the model-free parameter vector (as numpy array).
3
4      If the spin argument is supplied, then the spin_id argument will be ignored.
5
6      @keyword spin:      The spin data container.
7      @type spin:         SpinContainer instance
8      @keyword spin_id:   The spin identification string.
9      @type spin_id:      str
10     @keyword sim_index:  The optional MC simulation index.
11     @type sim_index:     int
12     @keyword model_type: The optional parameter set, one of 'all', 'diff', 'mf', or
        'local_tm'.
13     @type model_type:    str or None
14     @return:             An array of the parameter values of the model-free model.
15     @rtype:             numpy array
16     """
17
18     # Initialise.
19     param_vector = []
20
21     # Determine the model type.
22     if not model_type:
23         model_type = self.determine_param_set_type()
24
25     # Diffusion tensor parameters.
26     if model_type == 'diff' or model_type == 'all':
27         # Normal parameters.
28         if sim_index == None:
29             # Spherical diffusion.
30             if cdp.diff_tensor.type == 'sphere':
31                 param_vector.append(cdp.diff_tensor.tm)

```

## Classes

For classes relax uses a mix of camel case (for example all the `RelaxError` objects) and underscores (for example `Model_free`). The first letter in all cases is always capitalised. Generally the camel case is reserved for very low level classes which are involved in the program's infrastructure. Examples include the `RelaxError` code, the threading code, and the relax data store code. All the data analysis specific code, generic code, interface code, etc. uses underscores between the words with only the first letter capitalised. One exception is the space mapping class `OpenDX`, the reason being that the program is called `OpenDX`. An example is:

```

1  class Model_free_main:
2      """Class containing functions specific to model-free analysis."""
3
4      def are_mf_params_set(self, spin):
5          """Test if the model-free parameter values are set.
6
7          @param spin:    The spin container object.
8          @type spin:     SpinContainer instance

```



```

9         @return:          The name of the first parameter in the parameter list in which the
                           corresponding parameter value is None. If all parameters are set, then None is
                           returned.
10        @rtype:           str or None
11        """
12
13        # Deselected residue.
14        if spin.select == 0:
15            return

```

## Long names

If you have a look at a few relax source files, you will notice that the variable, function, and class names can be quite long. For example the model-free function `disassemble_param_vector()` and the RelaxError class `RelaxNoSequenceError`. While this is not normal for coding, it is an important component of relax as it facilitates the reading of the source code by a non-coder or someone not familiar with the codebase. Iteration counters can be single letter variables such as `i`, `j`, `k`, etc., however for all other variables, functions, and classes please attempt to use descriptive names which are instantly identifiable. Please minimise the amount of abbreviations used and only use those which are obvious. For example naming the parameter vector `self.param_vector`, the relaxation data `relax_data`, the model selection class `class Model_selection`, the type of spheroidal diffusion `spheroid_type`, etc.

### 13.2.4 Whitespace

The following conventions are for general code cleanliness and readability:

- Trailing whitespace should be avoided, although this is not very important.
- All functions should be preceded by two empty lines. The only exception is the first function of the class definition.
- Function arguments should be separated by a comma followed by a single space.
- The assignment operator should be surrounded by spaces, for example `tm_ = 1e-8`. The exception is function arguments where for example `self.classic\_colour(res\_num=None, width=0.3)`.
- The comparison operators should also be surrounded by spaces, e.g. `<`, `>`, `==`, `<=`, `>=`, `<>`, `!=`, `is`, and `in`.

An example which shows most of these conventions is:

```

1 class Internal:
2     """The internal relax structural data object.
3
4     The structural data object for this class is a container possessing a number of
5     different arrays corresponding to different structural information. These objects are
        described in the structural container docstring.
        """

```

```

6
7 def _bonded_atom(self, attached_atom, index, mol):
8     """Find the atom named attached_atom directly bonded to the atom located at the
9     index.
10
11     @param attached_atom: The name of the attached atom to return.
12     @type attached_atom: str
13     @param index: The index of the atom which the attached atom is attached
14     to.
15     @type index: int
16     @param mol: The molecule container.
17     @type mol: MolContainer instance
18     @return: A tuple of information about the bonded atom.
19     @rtype: tuple consisting of the atom number (int), atom name (str)
20     , element name (str), and atomic position (Numeric array of len 3)
21     """
22
23     # Init.
24     bonded_found = False
25
26     # No bonded atoms, so determine the connectivities.
27     if not mol.bonded[index]:
28         # Determine the molecule type if needed.
29         if not hasattr(mol, 'type'):
30             self._mol_type(mol)

```

### 13.2.5 Comments

Comments are a very important component within relax. In the current source code the percentage of comment lines relative to lines of code ranges from 15% to over 30% for different files. The average comment density would be close to 25%. The purpose of having so many comment lines, much more than you would expect from source code, is so that the relax's code is fully self documented. It allows someone who is not familiar with the codebase to read the code and quickly understand what is happening. It simplifies the process of learning and allows NMR spectroscopists who are not coders to dive into the code. If writing code for relax, please attempt to maintain the tradition by aiming towards a 25% comment ratio. The comment should be descriptive of what the code below it is supposed to do. Most importantly the comment explains why that code exists. The script `scripts/code_validator` can be used to check the comment density.

## 13.3 Submitting changes to the relax project

### 13.3.1 Submitting changes as a patch

The preferred method for submitting fixes and improvements to the relax source code is by the creation of a patch. If your changes are a fix make sure you have submitted a bug report to the bug tracker located at <https://gna.org/bugs/?group=relax> first. See section 3.3 on page 30 for more details. Two methods can be used to generate the patch – the Unix command `diff` or the Subversion program. The resultant file `patch` of either the `diff` or `svn` command described below can be posted to the “relax-devel at gna.org”

mailing list. Please label within your post which version of relax you modified or which revision the patch is for. Also try to create a commit log message according to the format described in section 13.4.4 on page 282 for one of the relax committers to use as a template for committing the change.

### 13.3.2 Modification of official releases – creating patches with diff

If your modifications have been made to the source code of one of the official relax releases (for example 2.1.1) then the Unix command `diff` can be used to create a patch. A patch file is simply the output of the `diff` command run with the recursive flag and presented in the ‘unified’ format. Therefore two directories need to be compared. If the original sources are located in the directory `relax_orig` and the modified sources in `relax_mod` then the patch can be created by typing

```
$ diff -ur relax_orig relax_mod > patch
```

### 13.3.3 Modification of the latest sources – creating patches with Subversion

If possible changes to the latest sources is preferred. Using the most up to date sources from the relax SVN repository will significantly aid the relax developers to incorporate your changes back into the main development line. To check out the current development line see section 13.1 on page 275 for details. Prior to submitting a patch to the mailing list your sources should be updated to include the most recent changes. To do this type

```
$ svn up
```

and note the revision number to include in your post. The update may cause a conflict if changes added to the repository clash with your modifications. If this occurs see the Subversion book at <http://svnbook.red-bean.com/> for details on how to resolve the conflict or submit a message to the relax-devel list.

Once the sources are up to date your changes can be converted into the patch text file. Using SVN creating a patch is easy. Just type

```
$ svn diff > patch
```

in the base relax directory.

## 13.4 Committers

### 13.4.1 Becoming a committer

Anyone can become a relax developer and obtain commit access to the relax repository. The main criteria for selection by the relax developers is to show good judgement, competence in producing good patches, compliance with the coding and commit log conventions, comportment on the mailing lists, not producing too many bugs, only taking on challenges which can be handled, and the skill in judging your own abilities. You will also need to

have an understanding of the concepts of version control specifically those relating to Subversion. The SVN book at <http://svnbook.red-bean.com/> contains all the version control information you will need. After a number of patches have been submitted and accepted any of the relax developers can propose that you receive commit access. If a number of developers agree while no one says no then commit access will be offered.

One area where coding ability can be demonstrated is within the relax test suite. The addition of tests, especially those where the relax internal data structures of the relax data store are scrutinised, can be a good starting point for learning the structure of relax. This is because the introduction of bugs has no effect on normal program execution. The relax test suite is an ideal proving ground.

If skills in only certain areas of relax development, for example in creation of the documentation, an understanding of C but not python, an understanding of solely the code of the user interface, or an understanding of the code specific to a certain type of data analysis methodology, then partial commit access may be granted. Although you will have the ability to make modifications to any part of the repository please make modifications only those areas for which you have permission.

### 13.4.2 Joining Gna!

The first step in becoming a committer is to create a Gna! account. Go to <https://gna.org/account/register.php> and type in a login name, password, real name, and the email address you would like to use. You will then get an automatic email from Gna! which will contain a link to activate your registration.

### 13.4.3 Joining the relax project

The second step in becoming a committer is to register to become a member of the relax project. Go to the Gna! website (<https://gna.org/>) and login. Click on 'My Groups' to go to <https://gna.org/my/groups.php>. In the section 'Request for inclusion' type 'relax' and hit enter. Select relax and write something in the comments. If you have been approved (see section 13.4.1) you will be added to the project committers list.

### 13.4.4 Format of the commit logs

If you are a relax developer and you have commit access to the repository the following conventions should be followed for all commit messages.

- The length of all lines in the commit log should never exceed 100 characters. This is so that the log message viewed in either emails or by the command prompt command `svn log` is legible.
- The first line of the commit log should be a short description or synopsis of the changes. If the change relates to a bug or a task, include the bug and task number using the notation `type #num` where type is either `bug`, `task` or `support` and `num` is the id number (for example `bug #6503`). This terminology is important because the

Gna! infrastructure knows how to translate this into a link to the issue. Also include a link to the issue.

- The second line should be blank.
- If the commit is a bug fix reported by a non-committer or if the commit originates from a patch posted by a non-committer the next lines should be reserved for crediting. The name of the person and their obfuscated email address (for example `edward_at_nmr-relax_dot_com`) should be included in the message.
- Next should be another blank line.
- If the commit relates to an entry in the bug tracker or to a discussion on the mailing lists then the web address of either the bug report or the mailing list archive message should be cited in the next section (separated from the synopsis or credit section by a blank line). All relevant links should be included to allow easy navigation between the repository, mailing lists, bug tracker, etc. An example is bug #5559 which is located at [https://gna.org/bugs/?func=detailitem&item\\_id=5559](https://gna.org/bugs/?func=detailitem&item_id=5559) and the post to “relax-devel at gna.org” describing the fix to that bug which is located at <https://mail.gna.org/public/relax-devel/2006-03/msg00013.html>.
- A full description with all the details can follow. This description should follow a blank line, can itself be sectioned using blank lines, and finally the log is terminated by one blank line at the end of the message.

An example of a commit message for the closure of a bug is:

Fixing the rest of bug #7241 (<https://gna.org/bugs/?7241>).

Bug #7241 was thought to be fixed in in r2591 and r2593, the commit messages describing the solution being located at <https://mail.gna.org/public/relax-commits/2006-09/msg00064.html> (Message-id: <E1GTgBi-0000R6-4h@subversion.gna.org>) for r2591 and <https://mail.gna.org/public/relax-commits/2006-10/msg00001.html> (Message-id: <E1GTt6C-0005rk-8q@subversion.gna.org>) for r2593.

However this was not the only place that the Scientific Python PDB data structure `peptide_chains` was being accessed. The chains were being accessed in the file ‘`pipe_control/sequence.py`’ when the sequence was being read out of the PDB file. This has now been modified with changes similar to r2591 and r2593.

An example of a commit message for changes relating to a task is:

This change implements half of task #3630 (<https://gna.org/task/?3630>).

The docstring in the generic optimisation function has been modified to clear up the ambiguity caused by supplying the option ‘None’ together with Newton optimisation.

One last commit message example is:

Added the API documentation creation (using Epydoc) to the Scons scripts.

The Scons target to create the HTML API documentation is called ‘`api_manual_html`’. The documentation can be created by typing: `$ scons api_manual_html`

The function ‘`compile_api_manual_html()`’ was added to the ‘`scons/manuals.py`’ file. This function runs the ‘`epydoc`’ command. All the Epydoc options are specified in that function.

### 13.4.5 Discussing major changes

If you are contemplating major changes, either for a bug fix, to add a completely new feature or user function for your own work, to improve the behaviour of part the program, or any other potentially disruptive modifications, please discuss these ideas on the relax-devel mailing list. If the planned changes have the potential to introduce problems the creation of a private branch may be suggested.

## 13.5 Branches

### 13.5.1 Branch creation

If a change is likely to be disruptive or cause breakages in the program, the use of your own temporary branch is recommended. This private branch is a complete copy of one of the main development lines wherein you can make changes without disrupting the other developers. Although called a private branch every change is visible to all other developers and each commit will result in an automatic email to the relax-commits mailing list. Other developers are even able to check out your branch and make modifications to it. Private branches can also be used for testing ideas. If the idea does not work the branch can be deleted from the repository (in reality the branch will always exist between the revision numbers of its creation and deletion and can always be resurrected). For example to create a branch from the main development line, the ‘trunk’, called `molmol_macros` whereby new Molmol macros are to be written, type

```
$ svn cp svn+ssh://xxxxx@svn.gna.org/svn/relax/trunk \  
svn+ssh://xxxxx@svn.gna.org/svn/relax/branches/molmol_macros
```

replacing `xxxxx` with your login name. You can then check out your private branch by typing

```
$ svn co svn+ssh://xxxxx@svn.gna.org/svn/relax/branches/molmol_macros
```

which will create a directory called `molmol_macros` containing all the relax source files. To have the files placed into a different directory, type the name of that directory at the end of the last command. Modifications can be made to this copy while normal development continues on the main line.

### 13.5.2 Keeping the branch up to date using `svnmerge.py`

As you develop your branch, changes will be occurring simultaneously within the main line. These changes should be merged into your branch on a regular basis to avoid large incompatible changes from forming between the two branches. To simplify this process, the `svnmerge.py` script located at <http://www.orcaware.com/svn/wiki/Svnmerge.py> can be used. It is best to download the trunk version from that page, unless that version is non-functional. Once you have this script, the merging from the main line to your private branch must be initialised by typing, from within the checked out copy of your branch,

```
$ svnmerge.py init
```

This then needs to be committed using the automatically generated log

```
$ svn ci -F svnmerge-commit-message.txt
```

To keep up to date, simply type

```
$ svnmerge.py merge
```

If conflicts have occurred please refer to the Subversion book at <http://svnbook.red-bean.com/> for information on how to resolve the problem. Otherwise, or once fixed, the main line revisions merged into your branch can be committed using the automatically generated log file:

```
$ svn ci -F svnmerge-commit-message.txt
```

### 13.5.3 Merging the branch back into the main line

Once you have completed the modifications desired for your branch, all changes which have occurred in the main line have been merged using `svnmerge.py`, and the changes have been approved for merging back into the main line – then your branch can be merged. First check out a copy of the main line,

```
$ svn co svn+ssh://xxxxx@svn.gna.org/svn/relax/trunk relax-trunk
```

or update a previously checked out version,

```
$ svn up
```

Then `svnmerge.py` can be utilised again. First initialise the merging process by typing, from within the checked out copy of the main line,

```
$ svnmerge.py init svn+ssh://xxxxx@svn.gna.org/svn/relax/branches/molmol_macros
```

Then commit the change

```
$ svn ci -F svnmerge-commit-message.txt
```

To merge the branch and commit the changes, type

```
$ svnmerge.py merge --bidirectional
```

```
$ svn ci -F svnmerge-commit-message.txt
```

Finally the merge properties need to be removed

```
$ svnmerge.py uninit -S svn+ssh://xxxxx@svn.gna.org/svn/relax/branches/molmol_macros
```

the changes committed

```
$ svn ci -F svnmerge-commit-message.txt
```

and your private branch deleted

```
$ svn rm svn+ssh://xxxxx@svn.gna.org/svn/relax/branches/molmol_macros
```

## 13.6 The SCons build system

The **SCons** build system was chosen over other build systems including **make** as it is a cross-platform build system which can be used in Unix, GNU/Linux, Mac OS X, and even MS Windows (the correct compilers are nevertheless required). Various components of the program **relax** can be created using the SCons utility. This includes C module compilation, manual creation, distribution creation, and cleaning up and removing certain files. The file **sconstruct** in the base **relax** directory, which consists of python code, directs the operation of SCons for the various functions.

### 13.6.1 SCons help

Multiple functions have been built into the **sconstruct** script and the modules of the **scons** directory. Each of these can be selected by specifying different “targets” when running SCons. A description of each target is given by the SCons help system which is accessible by typing **scons --help** in the base **relax** directory.

### 13.6.2 C module compilation

As described in the installation chapter, typing **scons** in the base directory will create the shared objects or dll files which are imported into Python as modules.

### 13.6.3 Compilation of the user manual (PDF version)

To create the PDF version of the **relax** user manual type

```
$ scons user_manual.pdf
```

in the base directory. SCons will then run a series of shell commands to create the manual from the **L<sup>A</sup>T<sub>E</sub>X** sources located in the **docs/latex** directory. This is dependent on the programs **latex**, **makeindex**, **dvips**, and **ps2pdf** being located within the environment’s path.

### 13.6.4 Compilation of the user manual (HTML version)

The HTML version of the **relax** user manual is made by typing

```
$ scons user_manual.html
```

in the base directory. One command calling the program **latex2html** will be executed which will create HTML pages from the **L<sup>A</sup>T<sub>E</sub>X** sources.

### 13.6.5 Compilation of the API documentation (HTML version)

The HTML version of the **relax** API documentation is made by typing



```
$ scons api_manual.html
```

in the base directory. The programs Epydoc and Graphviz are required for creating this documentation. The resultant HTML pages will be located in the directory `docs/api`.

### 13.6.6 Making distribution archives

Two types of distribution archive can be created from the currently checked out sources – the source and binary distributions. To create the source distribution type

```
$ scons source_dist
```

whereas to create the binary distribution, whereby the C modules are compiled and the resultant shared objects are included in the bziped tar file, type

```
$ scons binary_dist
```

If a binary distribution does not exist for your architecture feel free to create it yourself and contribute the archive to be included on the download pages. To do this you will need to check out the appropriate tagged branch from the relax subversion repository. If the current stable release is called 1.2.3 check out that branch by typing

```
$ svn co svn+ssh://bugman@svn.gna.org/svn/relax/tags/1.2.3 relax
```

replacing `bugman` with your user name if you are a relax developer, otherwise typing

```
$ svn co svn://svn.gna.org/svn/relax/tags/1.2.3 relax
```

Then build the binary distribution and send a message to the relax development mailing list. If compilation does not work please submit a bug to the bug tracker system at <https://gna.org/bugs/?group=relax> detailing the relax version, operation system, architecture, and any other information you believe will help to solve the problem. More information about donating binary distributions to the relax project is given in the open source infrastructure chapter (Chapter 3, page 29).

### 13.6.7 Cleaning up

If the command

```
$ scons clean
```

is run in the base directory all Python byte compiled files `*.pyc`, all C object files `*.o` and `*.os`, and any backup files with the extension `*.bak` are removed from all sub-directories. In addition any temporary L<sup>A</sup>T<sub>E</sub>X compilation files are removed from the `docs/latex` directory.

The more powerful command

```
$ scons clean_all
```

will, in addition to all the files removed by the `clean` target, remove all compiled C shared object files (`*.so`, `*.dylib`, `*.pyd`) and the `build` and `dist` directories created when building the Mac OS X application.

## 13.7 The core design of relax

To enable flexibility the internal structure of relax is modular. By construction the large collection of independent data analysis tools can be used individually and relatively easily by any new code implementing other forms of relaxation data analysis or even by other programs. The core modular design of the program is shown in Figure 13.1.

### 13.7.1 The divisions of relax's source code

relax's source code can be divided into five major areas: the initialisation code, the user interface (UI) code, the functional code, the number crunching code, and the code storing the program state.

**Initialisation:** The code belonging to this section initialises the program, processes the command-line arguments, and determines what mode the program will be run in including the choice of the UI.

**UI:** The current UI modes in relax include the prompt, the script and the GUI modes. These consist of separate code paths, all sitting on top of the underlying functional code. In the future, a web-based interface may be added.

**Functional code:** This code is the main part of the program. It includes anything which does not fit into the other sections and comprises the generic code, the specific code, and the specific setup code used as an interface between the two.

**Number crunching:** The computationally expensive code belongs in this section.

**Program state:** The state of the program is contained within the relax data store which is accessible from all parts of the program as a singleton object.

### 13.7.2 The major components of relax

Each of the boxes in Figure 13.1 represents a different grouping of code.

**relax:** The top level module. This initialises the entire program, tests the dependencies, sets up the multi-processor framework and specific processor fabric, and prints the program's introduction message.

**Command line arguments:** This code processes the arguments supplied to the program and decides whether to print the help message, initialise the prompt, execute a script, initialise a different UI, run the program in test mode, or run the program as a slave thread.

**Prompt:** The main user interface consisting of a Python prompt. The namespace of the interpreter contains the various user functions which are front ends to the generic code. The user functions are simply Python functions which test the supplied arguments to make sure they are of the correct type (string, integer, list, or any other type) before sending the values to the generic code. The code for the prompt is located in the directory `prompt/`.

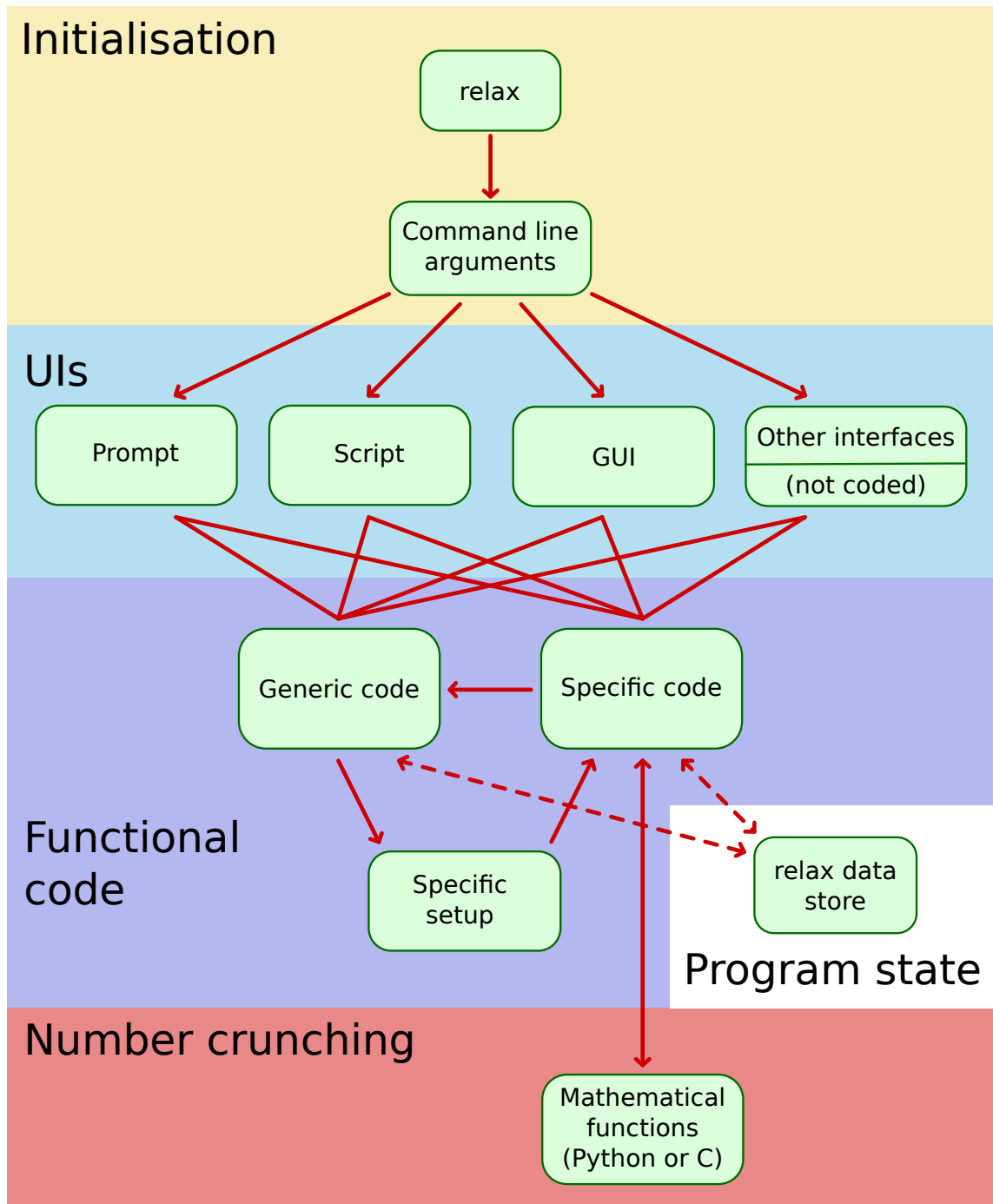


Figure 13.1: The core design of relax.

**Script:** If a script is supplied on the command line or executed within another user interface it will be run in the same namespace as that of the prompt. Hence the script has access to all the user functions available at the relax prompt. This allows commands which are typed at the prompt to be pasted directly and unmodified into a text file to be run as a script.

**GUI:** The graphical user interface code base is located in the `gui` directory.

**Other interfaces:** Any number of interfaces (for example a web-based interface or an ncurses interface) could be added to relax without modification of the current sources. This must be, without question, developed within the relax source code repository otherwise the code will not be maintainable in the future and will be almost impossible back into relax later on.

**Generic code:** This code includes classes and functions which are independent of the UI and not specific to a certain data pipe type, for example not being involved in model-free analysis, relaxation curve-fitting, the NOE calculation, and reduced spectral density mapping. All this code is located in the directory `pipe_control/`.

**Specific setup:** This code implements the internal interface between the generic and specific code. The generic code calls the specific setup asking for a specific function for the given data pipe type. For example by asking for the minimise function when the data pipe type is model-free analysis the model-free specific `minimise()` method is returned. Although the generic code accesses the specific code solely through this interface the specific code can access the generic code directly. The code is located in the file `specific_fns/specific_setup.py`.

**Specific code:** This is the code which is specific to the data pipe type – model-free analysis, relaxation curve-fitting, reduced spectral density mapping, and the NOE calculation. Each type is located in a separate file in the directory `specific_fns/`.

**Mathematical functions:** This is reserved for CPU intensive code involved in calculations. The code may be written in Python however C code can be used to significantly increase the speed of the calculations. For optimisation the code can include function evaluations, calculation of gradients, and calculation of Hessians. These functions are located in the directory `maths_fns/`.

**Data:** The program state stored in the relax data store singleton object. This class contains all the program data and is accessed by the generic and specific code. The mathematical functions may also access this data but this is not recommended. The structure is initialised by the file `data/__init__.py`.

## 13.8 The mailing lists for development

### 13.8.1 Private vs. public messages

If you would like to start a private discussion, please label your email as such. Private messages are however strongly discouraged, only start a private conversation if you really must.

If you receive a reply to a message you have sent, a bug report you have filed, etc. which has not been sent to the mailing list and has not been labelled as private, then the most likely explanation is that “reply-to-all” has not been used and hence the mailing list has not been included on the CC list. If this occurs, please ask the person if the message was meant to be private and refrain from discussing any of the comments within the post. Save these comments until after the person responds by saying that the message was private or re-sends the message to the mailing list. Try to encourage public messages if you think that the post need not be private and if you think that it would be useful for the mailing list archives.

For thread consistency, if you send a message which accidentally misses the mailing list, please do not then forward the previously sent message to the list. For better readability of the mailing list archives, it is best that you create an entirely new message responding to the original post. Just cut and paste your miss-directed message into your new message. That way the thread will be continuous – there will not be any messages missing from the middle of the thread in between the original post and your forwarded message.

To simplify the process of checking if the message was supposed to be private, you could copy-and-paste the following message (modifying it as you see fit):

```
Sorry in advance, but the following is the standard pre-composed response to a post not
sent to the relax mailing lists and not labelled as private.  If you would like to start
a private conversation about relax, please label your message as such.  If you really
must start a private exchange, please respond to this message saying so.  If your message
was meant to be sent to the relax mailing list, please send the message again.  For this,
please copy-and-paste your message, replying to the original (i.e. no forwarding), and
making sure that the mailing list is in the CC field by clicking on ‘reply-to-all’.
```

## 13.9 The bug, task, and support request trackers

relax’s infrastructure includes three different issue trackers. These are the [bug tracker](#), the [task tracker](#), and the [support request tracker](#).

### 13.9.1 Submitting a bug report

If someone reports a bug to one of the relax mailing lists, ask that person if they would like to create a bug report for that problem, pointing them to the submission web page. This is a good starting point to allow the person to become more involved in the relax project. If they do not respond or say that they would prefer not to, then you can create bug report for the issue linking to the original message and crediting the person for reporting the issue.

### 13.9.2 Assigning an issue to yourself

If you are a relax committer and see an issue which you would like to solve, please assign that issue to yourself before you start work on it. The assignment will prevent duplicated

efforts. If you can see an area where relax needs work, feel free to create a report within task tracker and then assign the task to yourself.

### 13.9.3 Closing an issue

When closing an issue (whether a bug report, a task, or a support request) a number of steps need to be taken. The tracker status should be changed to “Done” and the issue “Closed”. In addition, a message should be included which states the repository revision and the relax-commits mailing list archive link (with the message-id) in which the issue was solved. If multiple commits were required, then include all the revisions and as many links as possible (if a task required many commits, the relax-commits links could be skipped). An example is [bug #7402](#) where the closing comment was:

```
This documentation bug was fixed in r2641. The commit message is located at
https://mail.gna.org/public/relax-commits/2006-10/msg00073.html (Message-id:
<E1GYG41-0002kK-Jx@subversion.gna.org>).
```

## 13.10 Links, links, and more links

Creating links throughout the relax infrastructure is important for two major reasons – navigation and search engine indexing. When including a link to a post within the mailing list archives, please include the message-id email header. This enables subscribers to the mailing lists to search for the specific message within their local copy of the email messages.

### 13.10.1 Navigation

To be able to easily navigate between the relax infrastructure components – the bug tracker, the task tracker, the support request tracker, the relax-devel mailing list, the commit logs, and the SVN and CVS repositories – try to include as many links as possible.

For example a bug may first be reported on the relax-users mailing list, then placed within the bug tracker, discussed on relax-devel, a fix committed to the repository, and finally the bug report closed. To be able to follow this chain, links are very important (email message-ids are also important). When the bug is first added to the bug tracker, a link to the relax-users mailing list archive message and the message-id should be included. If you start a discussion on relax-devel, try to include links to the bug tracker entry and the relax-users message. When committing a fix to the repository, include links to the bug report, to the start of the thread in the mailing list archive, and the original message to relax-users. Then when the bug report is closed, include the revision number of the fix and a link to the relax-commits archive message (and message-id). By having all these links, it is then very easy for someone else to jump between the systems and follow the progression of the bug fix.

If you send a message referring to an old post which was sent the relax mailing lists, an old bug report, or any other archived information, please take the time to find that original information in the archives and include a link to it (including the message-id if relevant).

It is much more efficient for a single person to hunt down that message than for the many recipients of your post to search for the message themselves. By including the link, you decrease the overhead of following the mailing list.

### 13.10.2 Search engine indexing

Having a large web of links across relax's infrastructure aids in the search engine indexing of the mailing list archives and the <http://www.nmr-relax.com> web site. The web of links is useful for catching those Google bots. That way the Google searching of the mailing list archives located on the [communication web page](#) will be more up to date. However to increase the search engine ranking of the web site, links to <http://www.nmr-relax.com> from external sites is required. This is one reason why relax can be found at a number of sites across the web:

**Freecode:** New relax releases are announced not only on the [relax-announce](#) mailing list and on the relax [news](#) pages, but also on [Freecode](#). This used to be called Freshmeat.

**The mail archive:** This site archives all of the relax mailing lists, including [relax-announce](#), [relax-users](#), [relax-devel](#), and [relax-commits](#).

**Gmane:** Pronounced as “main”, the relax mailing lists are also archived at Gmane in numerous formats. The archived relax mailing lists include relax-announce ([thread](#), [blog](#), [NNTP](#), [RSS](#)), relax-users ([thread](#), [blog](#), [NNTP](#), [RSS](#)), relax-devel ([thread](#), [blog](#), [NNTP](#), [RSS](#)), and relax-commits ([thread](#), [blog](#), [NNTP](#), [RSS](#)).

**MARC - Mailing list ARChives:** This site archives all of the relax mailing lists, including [relax-announce](#), [relax-users](#), [relax-devel](#), and [relax-commits](#).

**CIA.vc:** This is the open source version control informant. CIA tracks open source projects in real-time. The relax real-time open source activity stats page is <http://cia.vc/stats/project/relax>. This website also has pages for each of the relax developers (in alphabetical order): [Edward d'Auvergne](#), [Michael Bieri](#), [Chris MacRaid](#), [Sébastien Morin](#), [Andrew Perry](#), [Han Sun](#), [Gary Thompson](#).

**LinuxLinks.com:** LinuxLinks.com, the Linux portal, is a website listing many Linux software projects. relax can be found on the [Software:Scientific:Biology:Proteins](#) page.

**Softpedia:** This is the encyclopedia of free software downloads. The relax page on Softpedia is <http://linux.softpedia.com/get/Science/relax-22351.shtml>. The relax developers pages are: [Edward d'Auvergne](#).

**Pro-Linux:** Diese ist eine der größten deutschsprachigen Seiten zum Thema Linux. The relax page is <http://www.pro-linux.de/cgi-bin/DBApp/check.cgi?ShowApp..10010.100>.





## Part IV

# Advanced topics



## Chapter 14

# Optimisation

### 14.1 Implementation

#### 14.1.1 The interface

Optimisation or minimisation is available in relax via the `minimise.grid_search` and `minimise.execute` user functions. The mathematical model optimised depends on the current data pipe type – it is implemented differently for each specific analysis. For analyses such as the steady state NOE (Chapter 6) or reduced spectral density mapping (Chapter 8), the solution can be found by direct calculation rather than optimisation. In these cases, the `minimise.calculate` user function should be used instead.

#### 14.1.2 The minfx package

To minimise target functions within relax, the minfx optimisation library is used (<https://gna.org/projects/minfx/>). This Python package is bundled with the official relax distribution archives. If you are using a version of relax checked out directly from the source code repository, you will need to manually install minfx as a standard Python package.

The minfx library originated as one of relax’s packages, but has been spun off as its own project for the benefit of other scientific, analytical, or numerical projects. Minfx is complete, very stable, well tested. Numerous optimisation algorithms are supported and can be clustered into three major categories – the line search methods, the trust-region methods, and the conjugate gradient methods.

The supported line search methods include:

- Steepest descent,
- Back-and-forth coordinate descent,
- Quasi-Newton BFGS,
- Newton,

- Newton-CG.

The supported trust-region methods include:

- Cauchy point,
- Dogleg,
- CG-Steihaug,
- Exact trust region.

The supported conjugate gradient methods include:

- Fletcher-Reeves,
- Polak-Ribière,
- Polak-Ribière +,
- Hestenes-Stiefel.

In addition, the following miscellaneous algorithms are implemented:

- Grid search,
- Nelder-Mead simplex,
- Levenberg-Marquardt.

The step selection subalgorithms include:

- Backtracking line search,
- Nocedal and Wright interpolation based line search,
- Nocedal and Wright line search for the Wolfe conditions,
- More and Thuente line search,
- No line search.

The Hessian modification subalgorithms include:

- Unmodified Hessian,
- Eigenvalue modification,
- Cholesky with added multiple of the identity,
- The Gill, Murray, and Wright modified Cholesky algorithm,

- The Schnabel and Eskow 1999 algorithm.

All methods can be constrained by:

- The Method of Multipliers (also known as the Augmented Lagrangian),
- The logarithmic barrier function.

These lists may be out of date, so please see the minfx website for additional information.

## 14.2 The optimisation space

The optimisation of the parameters of an arbitrary model is dependent on a function  $f$  which takes the current parameter values  $\theta \in \mathbb{R}^n$  and returns a single real value  $f(\theta) \in \mathbb{R}$  corresponding to position  $\theta$  in the  $n$ -dimensional space. For it is that single value which is minimised as

$$\hat{\theta} = \arg \min_{\theta} f(\theta), \quad (14.1)$$

where  $\hat{\theta}$  is the parameter vector which is equal to the argument which minimises the function  $f(\theta)$ . In most analyses in relax,  $f(\theta)$  is the chi-squared equation

$$\chi^2(\theta) = \sum_{i=1}^n \frac{(y_i - y_i(\theta))^2}{\sigma_i^2}, \quad (14.2)$$

where  $i$  is the summation index over all data,  $y_i$  is the experimental data,  $y_i(\theta)$  is the back calculated data, and  $\sigma_i$  is the experimental error.

## 14.3 Topology of the space

The problem of finding the minimum is complicated by the fact that optimisation algorithms are blind to the curvature of the complete space. Instead they rely on topological information about the current and, sometimes, the previous parameter positions in the space. The techniques use this information to walk iteratively downhill to the minimum.

### 14.3.1 The function value

At the simplest level all minimisation techniques require at least a function which will supply a single value for different parameter values  $\theta$ . Conceptually this is the height of the space at the current position. For certain algorithms, such as a simplex minimisation, this single value suffices.

### 14.3.2 The gradient

Most techniques also utilise the gradient at the current position. Although symbolically complex in the case of model-free analysis, for example, the gradient can simply be calculated as the vector of first partial derivatives of the chi-squared equation with respect to each parameter. It is defined as

$$\nabla = \begin{pmatrix} \frac{\partial}{\partial \theta_1} \\ \frac{\partial}{\partial \theta_2} \\ \vdots \\ \frac{\partial}{\partial \theta_n} \end{pmatrix} \quad (14.3)$$

where  $n$  is the total number of parameters in the model.

The gradient is supplied as a second function to the algorithm which is then utilised in diverse ways by different optimisation techniques. The function value together with the gradient can be combined to construct a linear or planar description of the space at the current parameter position by first-order Taylor series approximation

$$f(\theta_k + x) \approx f_k + x^T \nabla f_k, \quad (14.4)$$

where  $f_k$  is the function value at the current parameter position  $\theta_k$ ,  $\nabla f_k$  is the gradient at the same position, and  $x$  is an arbitrary vector. By accumulating information from previous parameter positions a more comprehensive geometric description of the curvature of the space can be exploited by the algorithm for more efficient optimisation.

An example of a powerful algorithm which requires both the value and gradient at current parameter values is the BFGS quasi-Newton minimisation. The gradient is also essential for the use of the Method of Multipliers constraints algorithm (also known as the Augmented Lagrangian algorithm).

### 14.3.3 The Hessian

The best and most comprehensive description of the space is given by the quadratic approximation of the topology which is generated from the combination of the function value, the gradient, and the Hessian. From the second-order Taylor series expansion the quadratic model of the space is

$$f(\theta_k + x) \approx f_k + x^T \nabla f_k + \frac{1}{2} x^T \nabla^2 f_k x, \quad (14.5)$$

where  $\nabla^2 f_k$  is the Hessian, which is the symmetric matrix of second partial derivatives of the function, at the position  $\theta_k$ . The Hessian is the matrix of second partial derivatives and is defined as

$$\nabla^2 = \begin{pmatrix} \frac{\partial^2}{\partial \theta_1^2} & \frac{\partial^2}{\partial \theta_1 \partial \theta_2} & \cdots & \frac{\partial^2}{\partial \theta_1 \partial \theta_n} \\ \frac{\partial^2}{\partial \theta_2 \partial \theta_1} & \frac{\partial^2}{\partial \theta_2^2} & \cdots & \frac{\partial^2}{\partial \theta_2 \partial \theta_n} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial^2}{\partial \theta_n \partial \theta_1} & \frac{\partial^2}{\partial \theta_n \partial \theta_2} & \cdots & \frac{\partial^2}{\partial \theta_n^2} \end{pmatrix}. \quad (14.6)$$

The order in which the partial derivatives are calculated is inconsequential, hence the Hessian is symmetric.

As the Hessian is computationally expensive a number of optimisation algorithms try to approximate it, the BFGS algorithm being a notable example. The most powerful minimisation algorithm for model-free analysis – Newton optimisation – requires the value, gradient, and Hessian at the current parameter values.

## 14.4 Optimisation algorithms

Prior to minimisation, all optimisation algorithms require a starting position within the optimisation space. This initial parameter vector is found by employing a coarse grid search – chi-squared values at regular positions spanning the space are calculated and the grid point with the lowest value becomes the starting position. The grid search itself is an optimisation technique. As it is computationally expensive the number of grid points needs to be kept to a minimum. Hence the initial parameter values are a rough and imprecise approximation of the local minimum.

Once the starting position has been determined by the grid search the optimisation algorithm can be executed. The number of algorithms developed within the mathematical field of optimisation is considerable. They can nevertheless be grouped into one of a small number of major categories based on the fundamental principles of the technique. These include the line search methods, the trust region methods, and the conjugate gradient methods. For more details on the algorithms described below see [Nocedal and Wright \(1999\)](#).

### 14.4.1 Line search methods

The defining characteristic of a line search algorithm is to choose a search direction  $p_k$  and then to find the minimum along that vector starting from  $\theta_k$  ([Nocedal and Wright, 1999](#)). The distance travelled along  $p_k$  is the step length  $\alpha_k$  and the parameter values for the next iteration are

$$\theta_{k+1} = \theta_k + \alpha_k p_k. \quad (14.7)$$

The line search algorithm determines the search direction  $p_k$  whereas the value of  $\alpha_k$  is found using an auxiliary step-length selection algorithm.

#### The steepest descent algorithm

One of the simplest line search methods is the steepest descent algorithm. The search direction is simply the negative gradient,  $p_k = -\nabla f_k$ , and hence the direction of maximal descent is always followed. This method is inefficient – the linear rate of convergence requires many iterations of the algorithm to reach the minimum and it is susceptible to being trapped on saddle points within the space.

### The coordinate descent algorithm

The coordinate descent algorithms are a simplistic group of line search methods whereby the search directions alternate between vectors parallel to the parameter axes. For the back-and-forth coordinate descent the search directions cycle in one direction and then back again. For example for a three parameter model the search directions cycle  $\theta_1, \theta_2, \theta_3, \theta_2, \theta_1, \theta_2, \dots$ , which means that each parameter of the model is optimised one by one. The method becomes less efficient when approaching the minimum as the step length  $\alpha_k$  continually decreases (ibid.).

### The BFGS algorithm

The quasi-Newton methods begin with an initial guess of the Hessian and update it at each iteration using the function value and gradient. Therefore the benefits of using the quadratic model of (14.5) are obtained without calculating the computationally expensive Hessian. The Hessian approximation  $B_k$  is updated using various formulae, the most common being the BFGS formula (Broyden, 1970; Fletcher, 1970; Goldfarb, 1970; Shanno, 1970). The search direction is given by the equation  $p_k = -B_k^{-1} \nabla f_k$ . The quasi-Newton algorithms can attain a superlinear rate of convergence, being superior to the steepest descent or coordinate descent methods.

### The Newton algorithm

The most powerful line search method when close to the minimum is the Newton search direction

$$p_k = -\nabla^2 f_k^{-1} \nabla f_k. \quad (14.8)$$

This direction is obtained from the derivative of (14.5) which is assumed to be zero at the minimum of the quadratic model. The vector  $p_k$  points from the current position to the exact minimum of the quadratic model of the space. The rate of convergence is quadratic, being superior to both linear and superlinear convergence. The technique is computationally expensive due to the calculation of the Hessian. It is also susceptible to failure when optimisation commences from distant positions in the space as the Hessian may not be positive definite and hence not convex, a condition required for the search direction both to point downhill and to be reasonably oriented. In these cases the quadratic model is a poor description of the space. This algorithm is also known as the Newton-Raphson method.

### The Newton conjugate gradient algorithm

A practical Newton algorithm which is robust for distant starting points is the Newton conjugate gradient method (Newton-CG). This line search method, which is also called the truncated Newton algorithm, finds an approximate solution to Equation (14.8) by using a conjugate gradient (CG) sub-algorithm. Retaining the performance of the pure Newton algorithm, the CG sub-algorithm guarantees that the search direction is always downhill as the method terminates when negative curvature is encountered.



### The auxiliary step-length selection algorithm

Once the search direction has been determined by the above algorithms the minimum along that direction needs to be determined. Not to be confused with the methodology for determining the search direction  $p_k$ , the line search itself is performed by an auxiliary step-length selection algorithm to find the value  $\alpha_k$ . A number of step-length selection methods can be used to find a minimum along the line  $\theta_k + \alpha_k p_k$ . One is the backtracking line search of Nocedal and Wright (1999). This method is inexact – it takes a starting step length  $\alpha_k$  and decreases the value until a sufficient decrease in the function is found. Another is the line search method of Moré and Thunberg (1994). Designed to be robust, the MT algorithm finds the exact minimum along the search direction and guarantees sufficient decrease.

#### 14.4.2 Trust region methods

In the trust region class of algorithms the curvature of the space is modelled quadratically by (14.5). This model is assumed to be reliable only within a region of trust defined by the inequality  $\|p\| \leq \Delta_k$  where  $p$  is the step taken by the algorithm and  $\Delta_k$  is the radius of the  $n$ -dimensional sphere of trust (Nocedal and Wright, 1999). The solution sought for each iteration of the algorithm is

$$\min_{p \in \mathbb{R}^n} m_k(p) = f_k + p^T \nabla f_k + \frac{1}{2} p^T B_k p, \quad \text{s.t. } \|p\| \leq \Delta_k, \quad (14.9)$$

where  $m_k(p)$  is the quadratic model,  $B_k$  is a positive definite matrix which can be the true Hessian as in the Newton model or an approximation such as the BFGS matrix, and  $\|p\|$  is the Euclidean norm of  $p$ . The trust region radius  $\Delta_k$  is modified dynamically during optimisation – if the quadratic model is found to be a poor representation of the space the radius is decreased whereas if the quadratic model is found to be reasonable the radius is increased to allow larger, more efficient steps to be taken.

### The Cauchy point algorithm

The Cauchy point algorithm is similar in concept to the steepest descent line search algorithm. The Cauchy point is the point lying on the gradient which minimises the quadratic model subject to the step being within the trust region. By iteratively finding the Cauchy point the local minimum can be found. The convergence of the technique is inefficient, being similar to that of the steepest descent algorithm.

### The dogleg algorithm

In changing the trust region radius the exact solutions to (14.9) map out a curved trajectory which starts parallel to the gradient for small radii. The end of the trajectory, which occurs for radii greater than the step length, is the bottom of the quadratic model. The dogleg algorithm attempts to follow a similar path by first finding the minimum along the gradient and then finding the minimum along a trajectory from the current point to the bottom

of the quadratic model. The minimum along the second path is either the trust region boundary or the quadratic solution. The matrix  $B_k$  of (14.9) can be the BFGS matrix, the unmodified Hessian, or a Hessian modified to be positive definite.

### Steihaug's algorithm

Another trust region algorithm is Steihaug's modified conjugate gradient approach (Steihaug, 1983). For each step  $k$  an iterative technique is used which is almost identical to the standard conjugate gradient procedure except for two additional termination conditions. The first is if the next step is outside the trust region, the second is if a direction of zero or negative curvature is encountered.

### The exact trust region

An almost exact solution to (14.9) can be found using an algorithm described in Nocedal and Wright (1999). This exact trust region algorithm aims to precisely find the minimum of the quadratic model  $m_k$  of the space within the trust region  $\Delta_k$ . Any matrix  $B_k$  can be used to construct the quadratic model. However, the technique is computationally expensive.

## 14.4.3 Conjugate gradient methods

The conjugate gradient algorithm (CG) was originally designed as a mathematical technique for solving a large system of linear equations Hestenes and Stiefel (1952), but was later adapted to solving nonlinear optimisation problems (Fletcher and Reeves, 1964). The technique loops over a set of directions  $p_0, p_1, \dots, p_n$  which are all conjugate to the Hessian (Nocedal and Wright, 1999), a property defined as

$$p_i^T \nabla^2 f_k p_j = 0, \quad \text{for all } i \neq j. \quad (14.10)$$

By performing line searches over all directions  $p_j$  the solution to the quadratic model (14.5) of the position  $\theta_k$  will be found in  $n$  or less iterations of the CG algorithm where  $n$  is the total number of parameters in the model. The technique performs well on large problems with many parameters as no matrices are calculated or stored. The algorithms perform better than the steepest descent method and preconditioning of the system is used to improve optimisation. Preconditioned techniques include the Fletcher-Reeves algorithm which was the original conjugate gradient optimisation technique (Fletcher and Reeves, 1964), the Polak-Ribière method (Polak and Ribière, 1969), a modified Polak-Ribière method called the Polak-Ribière + method (Nocedal and Wright, 1999), and the Hestenes-Stiefel algorithm which originates from a formula in Hestenes and Stiefel (1952). As a line search is performed to find the minimum along each conjugate direction both the backtracking and Moré and Thuente auxiliary step-length selection algorithms will be tested with the CG algorithms.

#### 14.4.4 Hessian modifications

The Newton search direction, used in both the line search and trust region methods, is dependent on the Hessian being positive definite for the quadratic model to be convex so that the search direction points sufficiently downhill. This is not always the case as saddle points and other non-quadratic features of the space can be problematic. Two classes of algorithms can be used to handle this situation. The first involves using the conjugate gradient method as a sub-algorithm for solving the Newton problem for the step  $k$ . The Newton-CG line search algorithm described above is one such example. The second class involves modifying the Hessian prior to, or at the same time as, finding the Newton step to guarantee that the replacement matrix  $B_k$  is positive definite. The convexity of  $B_k$  is ensured by its eigenvalues all being positive.

The first modification uses the Cholesky factorisation of the matrix  $B_k$ , initialised to the true Hessian, to test for convexity (Algorithm 6.3 of Nocedal and Wright (1999)). If factorisation fails the matrix is not positive definite and a constant  $\tau_k$  times the identity matrix  $I$  is then added to  $B_k$ . The constant originates from the Robbins norm of the Hessian  $\|\nabla^2 f_k\|_F$  and is steadily increased until the factorisation is successful. The resultant Cholesky lower triangular matrix  $L$  can then be used to find the approximate Newton direction. If  $\tau_k$  is too large the convergence of this technique can approach that of the steepest descent algorithm.

The second method is the Gill, Murray, and Wright (GMW) algorithm (Gill et al., 1981) which modifies the Hessian during the execution of the Cholesky factorisation  $\nabla^2 f_k = LIL^T$ , where  $L$  is a lower triangular matrix and  $D$  is a diagonal matrix. Only a single factorisation is required. As rows and columns are interchanged during the algorithm the technique may be slow for large problems such as the optimisation of the model-free parameters of all spins together with the diffusion tensor parameters. The rate of convergence of the technique is quadratic.

#### 14.4.5 Other methods

##### Nelder-Mead simplex

Some optimisation algorithms cannot be classified within line search, trust region, or conjugate gradient categories. For example the well known Nelder-Mead simplex optimisation algorithm. The technique is often used as the only the function value is employed and hence the derivation of the gradient and Hessian can be avoided. The simplex is created as an  $n$ -dimensional geometric object with  $n + 1$  vertices. The first vertex is the starting position. Each of the other  $n$  vertices are created by shifting the starting position by a small amount parallel to one of unit vectors defining the coordinate system of the space. Four simple rules are used to move the simplex through the space: reflection, extension, contraction, and a shrinkage of the entire simplex. The result of these movements is that the simplex moves in an ameoboid-like fashion downhill, shrinking to pass through tight gaps and expanding to quickly move through non-convoluted space, eventually finding the minimum.

Key to these four movements is the pivot point, the centre of the face created by the  $n$  vertices with the lowest function values. The first movement is a reflection – the vertex

with the greatest function value is reflected through the pivot point on the opposite face of the simplex. If the function value at this new position is less than all others the simplex is allowed to extend – the point is moved along the line to twice the distance between the current position and the pivot point. Otherwise if the function value is greater than the second highest value but less than the highest value, the reflected simplex is contracted. The reflected point is moved to be closer to the simplex, its position being half way between the reflected position and the pivot point. Otherwise if the function value at the reflected point is greater than all other vertices, then the original simplex is contracted – the highest vertex is moved to a position half way between the current position and the pivot point. Finally if none of these four movements yield an improvement, then the simplex is shrunk halfway towards the vertex with the lowest function value.

### Levenberg-Marquardt algorithm

Another algorithm is the commonly used Levenberg-Marquardt algorithm ([Levenberg, 1944](#); [Marquardt, 1963](#)). This is the algorithm used by the model-free analysis programs Modelfree4, Dasha, and Tensor2. This technique is designed for least-squares problems to which the chi-squared equation ([14.2](#)) belongs. The key to the algorithm is the replacement of the Hessian with the Levenberg-Marquardt matrix  $J^T J + \lambda I$ , where  $J$  is the Jacobian of the system calculated as the matrix of partial derivatives of the residuals,  $\lambda > 0$  is a factor related to the trust-region radius, and  $I$  is the identity matrix. The algorithm is conceptually allied to the trust region methods and its performance varies finely between that of the steepest descent and the pure Newton step. When far from the minimum  $\lambda$  is large and the algorithm takes steps close to the gradient; when in vicinity of the minimum  $\lambda$  heads towards zero and the steps taken approximate the Newton direction. Hence the algorithm avoids the problems of the Newton algorithm when non-convex curvature is encountered and approximates the Newton step in convex regions of the space.

The technique does have one weak point though which is often mentioned only in the small print. That is that the algorithm catastrophically fails when the Levenberg-Marquardt matrix is singular. This occurs whenever a parameter is undefined. For example in a model-free analysis if the order parameter is one, then the corresponding internal correlation time can take any value. Performing a grid search with such undefined points greatly amplifies the problem and is the reason why many published model-free papers contain results with order parameters fixed at one ([d’Auvergne and Gooley, 2008b](#)).

## 14.5 Constraint algorithms

To guarantee that the minimum will still be reached the implementation of constraints limiting the parameter values together with optimisation algorithms is not a triviality. For this to occur the space and its boundaries must remain smooth thereby allowing the algorithm to move along the boundary to either find the minimum along the limit or to slide along the limit and then move back into the centre of the constrained space once the curvature allows it.

### 14.5.1 Method of Multipliers algorithm

One of the most powerful approaches is the Method of Multipliers (Nocedal and Wright, 1999), also known as the Augmented Lagrangian. Instead of a single optimisation the algorithm is iterative with each iteration consisting of an independent unconstrained minimisation on a sequentially modified space. When inside the limits the function value is unchanged but when outside a penalty, which is proportional to the distance outside the limit, is added to the function value. This penalty, which is based on the Lagrange multipliers, is smooth and hence the gradient and Hessian are continuous at and beyond the constraints. For each iteration of the Method of Multipliers the penalty is increased until it becomes impossible for the parameter vector to be in violation of the limits. This approach allows the parameter vector  $\theta$  outside the limits yet the successive iterations ensure that the final results will not be in violation of the constraint.

For inequality constraints, each iteration of the Method of Multipliers attempts to solve the quadratic sub-problem

$$\min_{\theta} \mathfrak{L}_A(\theta, \lambda^k; \mu_k) \stackrel{\text{def}}{=} f(\theta) + \sum_{i \in \mathfrak{I}} \Psi(c_i(\theta), \lambda_i^k; \mu_k), \quad (14.11)$$

where the function  $\Psi$  is defined as

$$\Psi(c_i(\theta), \lambda^k; \mu_k) = \begin{cases} -\lambda^k c_i(\theta) + \frac{1}{2\mu_k} c_i^2(\theta) & \text{if } c_i(\theta) - \mu_k \lambda^k \leq 0, \\ -\frac{\mu_k}{2} (\lambda^k)^2 & \text{otherwise.} \end{cases} \quad (14.12)$$

In (14.11),  $\theta$  is the parameter vector;  $\mathfrak{L}_A$  is the Augmented Lagrangian function;  $k$  is the current iteration of the Method of Multipliers;  $\lambda^k$  are the Lagrange multipliers which are positive factors such that, at the minimum  $\hat{\theta}$ ,  $\nabla f(\hat{\theta}) = \lambda_i \nabla c_i(\hat{\theta})$ ;  $\mu_k > 0$  is the penalty parameter which decreases to zero as  $k \rightarrow \infty$ ;  $\mathfrak{I}$  is the set of inequality constraints; and  $c_i(\theta)$  is an individual constraint value. The Lagrange multipliers are updated using the formula

$$\lambda_i^{k+1} = \max(\lambda_i^k - c_i(\theta)/\mu_k, 0), \quad \text{for all } i \in \mathfrak{I}. \quad (14.13)$$

The gradient of the Augmented Lagrangian is

$$\nabla \mathfrak{L}_A(\theta, \lambda^k; \mu_k) = \nabla f(\theta) - \sum_{i \in \mathfrak{I} | c_i(\theta) \leq \mu_k \lambda_i^k} \left( \lambda_i^k - \frac{c_i(\theta)}{\mu_k} \right) \nabla c_i(\theta), \quad (14.14)$$

and the Hessian is

$$\nabla^2 \mathfrak{L}_A(\theta, \lambda^k; \mu_k) = \nabla^2 f(\theta) + \sum_{i \in \mathfrak{I} | c_i(\theta) \leq \mu_k \lambda_i^k} \left[ \frac{1}{\mu_k} \nabla^2 c_i^2(\theta) - \left( \lambda_i^k - \frac{c_i(\theta)}{\mu_k} \right) \nabla^2 c_i(\theta) \right]. \quad (14.15)$$

The Augmented Lagrangian algorithm can accept any set of three arbitrary constraint functions  $c(\theta)$ ,  $\nabla c(\theta)$ , and  $\nabla^2 c(\theta)$ . When given the current parameter values  $c(\theta)$  returns a vector of constraint values whereby each position corresponds to one of the model parameters. The constraint is defined as  $c_i \geq 0$ . The function  $\nabla c(\theta)$  returns the matrix of

constraint gradients and  $\nabla^2 c(\theta)$  is the constraint Hessian function which should return the 3D matrix of constraint Hessians.

A more specific set of constraints accepted by the Method of Multipliers are bound constraints. These are defined by the function

$$l \leq \theta \leq u, \quad (14.16)$$

where  $l$  and  $u$  are the vectors of lower and upper bounds respectively and  $\theta$  is the parameter vector. For example for model-free model *m4* to place lower and upper bounds on the order parameter and lower bounds on the correlation time and chemical exchange parameters, the vectors are

$$\begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix} \leq \begin{pmatrix} S^2 \\ \tau_e \\ R_{ex} \end{pmatrix} \leq \begin{pmatrix} 1 \\ \infty \\ \infty \end{pmatrix}. \quad (14.17)$$

The default setting in the program *relax* is to use linear constraints which are defined as

$$A \cdot \theta \geq b, \quad (14.18)$$

where  $A$  is an  $m \times n$  matrix where the rows are the transposed vectors  $a_i$  of length  $n$ ; the elements of  $a_i$  are the coefficients of the model parameters;  $\theta$  is the vector of model parameters of dimension  $n$ ;  $b$  is the vector of scalars of dimension  $m$ ;  $m$  is the number of constraints; and  $n$  is the number of model parameters.

In rearranging (14.18) the linear constraint function  $c(\theta)$  returns the vector  $A \cdot \theta - b$ . Because of the linearity of the constraints the gradient and Hessian are greatly simplified. The gradient  $\nabla c(\theta)$  is simply the matrix  $A$  and the Hessian  $\nabla^2 c(\theta)$  is zero.

### 14.5.2 Logarithmic barrier constraint algorithm

Another constraint method is that of the logarithmic barrier algorithm. As in the Method of Multipliers this method is iterative. The function being minimised is replaced with

$$\Phi(\theta) = \begin{cases} \epsilon \sum_{i=1}^m -\log(b_i - A_i^T \theta) & \text{if } A \cdot \theta < b, \\ +\infty & \text{otherwise.} \end{cases} \quad (14.19)$$

The value of  $\epsilon$  is increased with each iteration, increase the logarithmic penalty. An advantage of this method over the Method of Multipliers is that gradients are not required.

## 14.6 Diagonal scaling

Model scaling can have a significant effect on the optimisation algorithm – a poorly scaled model can cause certain techniques to fail. When two parameters of the model lie on very different numeric scales the model is said to be poorly scaled. For example in model-free analysis the order of magnitude of the order parameters is one whereas for the internal

correlation times the order of magnitude is between  $1e^{-12}$  to  $1e^{-8}$ . Most effected are the trust region algorithms – the multidimensional sphere of trust will either be completely ineffective against the correlation time parameters or severely restrict optimisation in the order parameter dimensions. Again in model-free analyses the significant scaling disparity can even cause failure of optimisation due to amplified effects of machine precision. Therefore the model parameters need to be scaled.

This can be done by supplying the optimisation algorithm with the scaled rather than unscaled parameters. When the chi-squared function, gradient, and Hessian are called the vector is then premultiplied with a diagonal matrix in which the diagonal elements are the scaling factors.





## Chapter 15

# Optimisation of relaxation data – values, gradients, and Hessians

### 15.1 Introduction to the mathematics behind the optimisation of relaxation data

A word of warning before reading this chapter, the topics covered here are quite advanced and are not necessary for understanding how to either use relax or to implement any of the data analysis techniques present within relax. The material of this chapter is intended as an in-depth explanation of the mathematics involved in the optimisation of the parameters of the model-free models, or any theory involving relaxation data. As such it contains the chi-squared equation, relaxation equations, spectral density functions, and diffusion tensor equations as well as their gradients (the vector of first partial derivatives) and Hessians (the matrix of second partial derivatives). All these equations are used in the optimisation of model-free models  $m0$  to  $m9$ ; models  $tm0$  to  $tm9$ ; the ellipsoidal, spheroidal, and spherical diffusion tensors; and the combination of the diffusion tensor and the model-free models. They also apply to all other theories involving the base  $R_1$ ,  $R_2$ , and steady-state NOE relaxation rates.

### 15.2 The four parameter combinations

In model-free analysis four different combinations of parameters can be optimised, each of which requires a different approach to the construction of the chi-squared value, gradient, and Hessian. These categories depend on whether the model-free parameter set  $\mathfrak{F}$ , the diffusion tensor parameter set  $\mathfrak{D}$ , or both sets are simultaneously optimised. The addition of the local  $\tau_m$  parameter to the model-free set  $\mathfrak{F}$  creates a fourth parameter combination.

#### 15.2.1 Optimisation of the model-free models

This is the simplest category as it involves solely the optimisation of the model-free parameters of an individual residue while the diffusion tensor parameters are held constant.

The model-free parameters belong to the set  $\mathfrak{F}_i$  of the residue  $i$ . The models include  $m0$  to  $m9$  and the dimensionality is low with

$$\dim \mathfrak{F}_i = k \leq 5 \quad (15.1)$$

for the most complex model  $m8 = \{S^2, \tau_f, S_f^2, \tau_s, R_{ex}\}$ . The relaxation data of a single residue is used to build the chi-squared value, gradient, and Hessian.

### 15.2.2 Optimisation of the local $\tau_m$ models

The addition of the local  $\tau_m$  parameter to the set  $\mathfrak{F}_i$  creates a new set of models which will be labelled  $\mathfrak{T}_i$ . These include models  $tm0$  to  $tm9$ . The local  $\tau_m$  parameter is the single member of the set  $\mathfrak{D}_i$  and in set notation

$$\mathfrak{T}_i = \mathfrak{D}_i \cup \mathfrak{F}_i. \quad (15.2)$$

Although the Brownian rotational diffusion parameter local  $\tau_m$  is optimised, this category is residue specific. As such the complexity of the optimisation is lower than the next two categories. It is slightly greater than the optimisation of the set  $\mathfrak{F}_i$  as

$$\dim \mathfrak{T}_i = 1 + k \leq 6, \quad (15.3)$$

where  $k$  is the number of model-free parameters.

### 15.2.3 Optimisation of the diffusion tensor parameters

The parameters of the Brownian rotational diffusion tensor belong to the set  $\mathfrak{D}$ . This set is the union of the geometric parameters  $\mathfrak{G} = \{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r\}$  and the orientational parameters  $\mathfrak{O}$ ,

$$\mathfrak{D} = \mathfrak{G} \cup \mathfrak{O}. \quad (15.4)$$

When diffusion is spherical solely the geometric parameter  $\mathfrak{D}_{iso}$  is optimised. When the molecule diffuses as a spheroid the geometric parameters  $\mathfrak{D}_{iso}$  and  $\mathfrak{D}_a$  and the orientational parameters  $\theta$  (the polar angle) and  $\phi$  (the azimuthal angle) are optimised. If the molecule diffuses as an ellipsoid the geometric parameters  $\mathfrak{D}_{iso}$ ,  $\mathfrak{D}_a$ , and  $\mathfrak{D}_r$  are optimised together with the Euler angles  $\alpha$ ,  $\beta$ , and  $\gamma$ .

This category is defined as the optimisation of solely the parameters of  $\mathfrak{D}$ . The model-free parameters of  $\mathfrak{F}$  are held constant. As all selected residues of the macromolecule are involved in the optimisation, this category is global and can be more complex than the optimisation of  $\mathfrak{F}_i$  or  $\mathfrak{T}_i$ . The dimensionality of the problem nevertheless low with

$$\dim \mathfrak{D} = 1, \quad \dim \mathfrak{D} = 4, \quad \dim \mathfrak{D} = 6, \quad (15.5)$$

for the diffusion as a sphere, spheroid, and ellipsoid respectively.

### 15.2.4 Optimisation of the global model $\mathfrak{S}$

The global model is defined as

$$\mathfrak{S} = \mathfrak{D} \cup \left( \bigcup_{i=1}^l \mathfrak{F}_i \right), \quad (15.6)$$

where  $i$  is the residue index and  $l$  is the total number of residues used in the analysis. This is the most complex of the four categories as both diffusion tensor parameters and model-free parameters of all selected residues are optimised simultaneously. The dimensionality of the model  $\mathfrak{S}$  is much greater than the other categories and is equal to

$$\dim \mathfrak{S} = \dim \mathfrak{D} + \sum_{i=1}^l k_i \leq 6 + 5l, \quad (15.7)$$

where  $k_i$  is the number of model-free parameters for the residue  $i$  and is equal to  $\dim \mathfrak{F}_i$ , the number six corresponds to the maximum dimensionality of  $\mathfrak{D}$ , and the number five corresponds to the maximum dimensionality of  $\mathfrak{F}_i$ .

## 15.3 Construction of the values, gradients, and Hessians

### 15.3.1 The sum of chi-squared values

For the single residue models of  $\mathfrak{F}_i$  and  $\mathfrak{T}_i$  the chi-squared value  $\chi_i^2$  which is optimised is simply Equation (15.13) on page 317 in which the relaxation data is that of residue  $i$ . However for the global models  $\mathfrak{D}$  and  $\mathfrak{S}$  in which all selected residues are involved the optimised chi-squared value is the sum of those for each residue,

$$\chi^2 = \sum_{i=1}^l \chi_i^2, \quad (15.8)$$

where  $i$  is the residue index and  $l$  is the total number of residues used in the analysis. This is equivalent to Equation (15.13) when the index  $i$  ranges over the relaxation data of all selected residues.

### 15.3.2 Construction of the gradient

The construction of the gradient is significantly different for the models  $\mathfrak{F}_i$ ,  $\mathfrak{T}_i$ ,  $\mathfrak{D}$ , and  $\mathfrak{S}$ . In Figure 15.1 the construction of the chi-squared gradient  $\nabla \chi^2$  for the global model  $\mathfrak{S}$  is demonstrated. In this case

$$\nabla \chi^2 = \sum_{i=1}^l \nabla \chi_i^2, \quad (15.9)$$

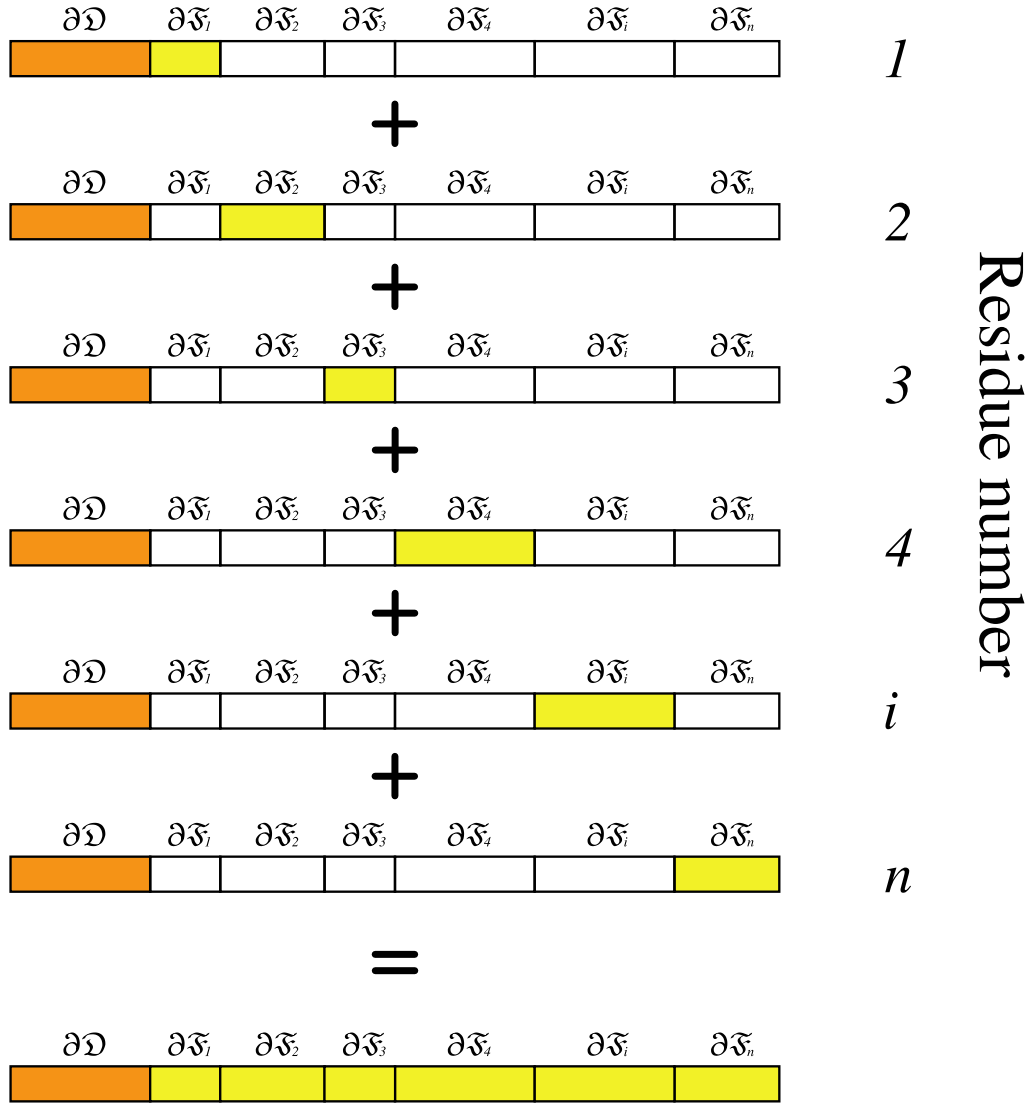


Figure 15.1: The construction of the model-free gradient  $\nabla \chi^2$  for the global model  $\mathfrak{S}$ . For each residue  $i$  a different vector  $\nabla \chi_i^2$  is constructed. The first element of the vector represented by the symbol  $\partial \mathfrak{D}$  (the orange block) is the sub-vector of chi-squared partial derivatives with respect to each of the diffusion tensor parameters  $\mathfrak{D}_j$ . The rest of the elements, grouped into blocks for each residue denoted by the symbol  $\partial \mathfrak{S}_i$ , are the sub-vectors of chi-squared partial derivatives with respect to each of the model-free parameters  $\mathfrak{S}_i^j$ . For the residue dependent vector  $\nabla \chi_i^2$  the partial derivatives with respect to the model-free parameters of  $\mathfrak{S}_j$  where  $i \neq j$  are zero. These blocks are left uncoloured. The complete gradient of  $\mathfrak{S}$  is the sum of the vectors  $\nabla \chi_i^2$ .

where  $\nabla\chi_i^2$  is the vector of partial derivatives of the chi-squared equation  $\chi_i^2$  for the residue  $i$ . The length of this vector is

$$\|\nabla\chi_i^2\| = \dim \mathfrak{S}, \quad (15.10)$$

with each position of the vector  $j$  equal to  $\frac{\partial\chi_i^2}{\partial\theta_j}$  where each  $\theta_j$  is a parameter of the model.

The construction of the gradient  $\nabla\chi^2$  for the model  $\mathfrak{D}$  is simply a subset of that of  $\mathfrak{S}$ . This is demonstrated in Figure 15.1 by simply taking the component of the gradient  $\nabla\chi_i^2$  denoted by the symbol  $\partial\mathfrak{D}$  (the orange blocks) and summing these for all residues. This sum is given by (15.9) and

$$\|\nabla\chi_i^2\| = \dim \mathfrak{D}. \quad (15.11)$$

For the parameter set  $\mathfrak{T}_i$ , which consists of the local  $\tau_m$  parameter and the model-free parameters of a single residue, the gradient  $\nabla\chi_i^2$  for the residue  $i$  is simply the combination of the single orange block and single yellow block of the index  $i$  (Figure 15.1).

The model-free parameter set  $\mathfrak{F}_i$  is even simpler. In Figure 15.1 the gradient  $\nabla\chi_i^2$  is simply the vector denoted by the single yellow block for the residue  $i$ .

### 15.3.3 Construction of the Hessian

The construction of the Hessian for the models  $\mathfrak{F}_i$ ,  $\mathfrak{T}_i$ ,  $\mathfrak{D}$ , and  $\mathfrak{S}$  is very similar to the procedure used for the gradient. The chi-squared Hessian for the global models  $\mathfrak{D}$  and  $\mathfrak{S}$  is

$$\nabla^2\chi^2 = \sum_{i=1}^l \nabla^2\chi_i^2. \quad (15.12)$$

Figure 15.2 demonstrates the construction of the full Hessian for the model  $\mathfrak{S}$ . The Hessian for the model  $\mathfrak{D}$  is the sum of all the red blocks. The Hessian for the model  $\mathfrak{T}_i$  is the combination of the single red block for residue  $i$ , the two orange blocks representing the sub-matrices of chi-squared second partial derivatives with respect to the diffusion parameter  $\mathfrak{D}_j$  and the model-free parameter  $\mathfrak{F}_i^k$ , and the single yellow block for that residue. The Hessian for the model-free model  $\mathfrak{F}_i$  is simply the sub-matrix for the residue  $i$  coloured yellow.

## 15.4 The value, gradient, and Hessian dependency chain

The dependency chain which was outlined in the model-free chapter – that the chi-squared function is dependent on the transformed relaxation equations which are dependent on the relaxation equations which themselves are dependent on the spectral density functions – combine with the values, gradients, and Hessians to create a complex web of dependencies. The relationship between all the values, gradients, and Hessians are outlined in Figure 15.3.

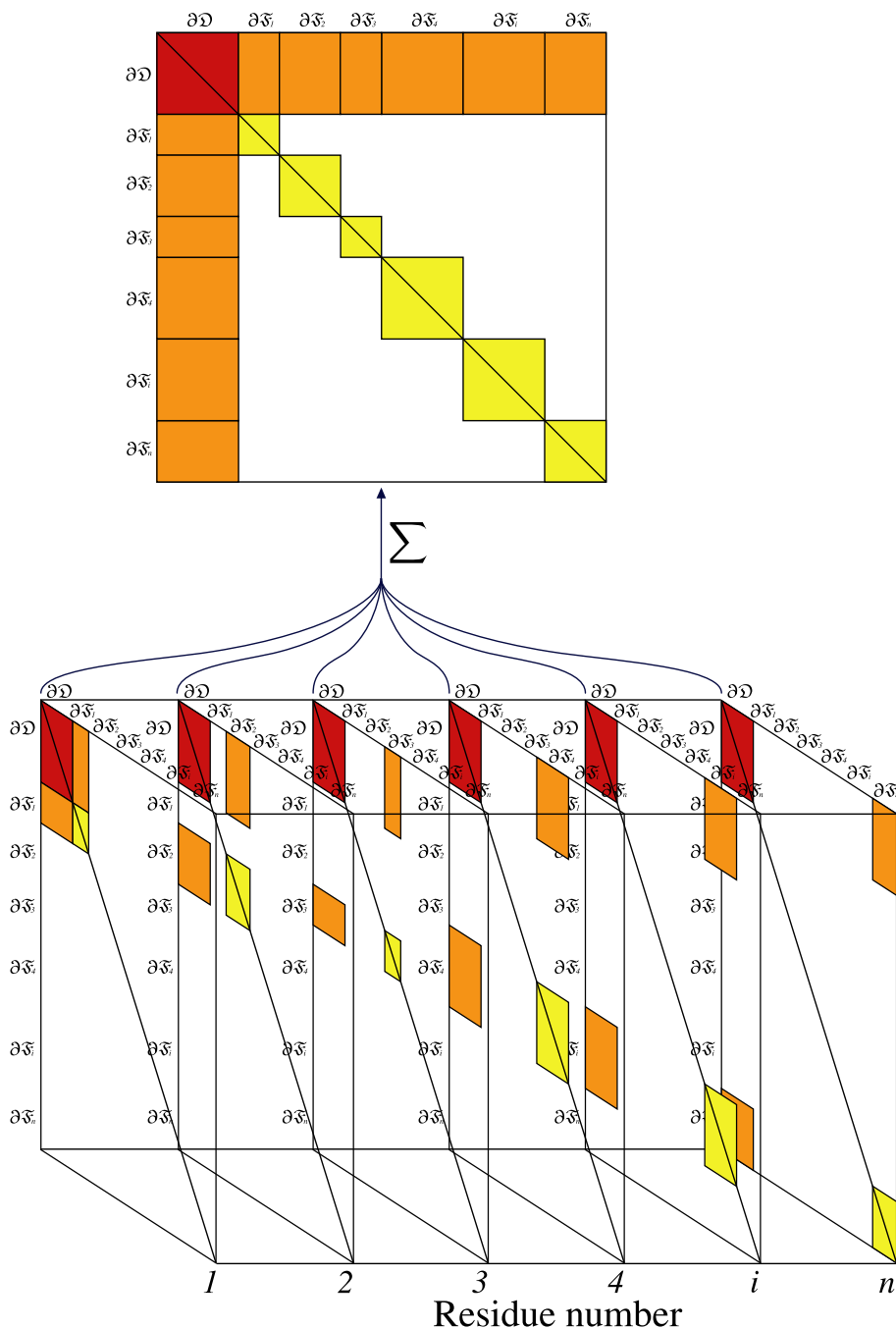


Figure 15.2: The model-free Hessian kite – a demonstration of the construction of the model-free Hessian  $\nabla^2\chi^2$  for the global model  $\mathfrak{S}$ . For each residue  $i$  a different matrix  $\nabla^2\chi_i^2$  is constructed. The first element of the matrix represented by the two symbols  $\partial^2\mathfrak{D}$  (the red block) is the sub-matrix of chi-squared second partial derivatives with respect to the diffusion tensor parameters  $\mathfrak{D}_j$  and  $\mathfrak{D}_k$ . The orange blocks are the sub-matrices of chi-squared second partial derivatives with respect to the diffusion parameter  $\mathfrak{D}_j$  and the model-free parameter  $\mathfrak{F}_i^k$ . The yellow blocks are the sub-matrices of chi-squared second partial derivatives with respect to the model-free parameters  $\mathfrak{F}_i^j$  and  $\mathfrak{F}_i^k$ . For the residue dependent matrix  $\nabla^2\chi_i^2$  the second partial derivatives with respect to the model-free parameters  $\mathfrak{F}_l^j$  and  $\mathfrak{F}_l^k$  where  $i \neq l$  are zero. In addition, the second partial derivatives with respect to the model-free parameters  $\mathfrak{F}_i^j$  and  $\mathfrak{F}_l^k$  where  $i \neq l$  are also zero. These blocks of sub-matrices are left uncoloured. The complete Hessian of  $\mathfrak{S}$  is the sum of the matrices  $\nabla^2\chi_i^2$ .

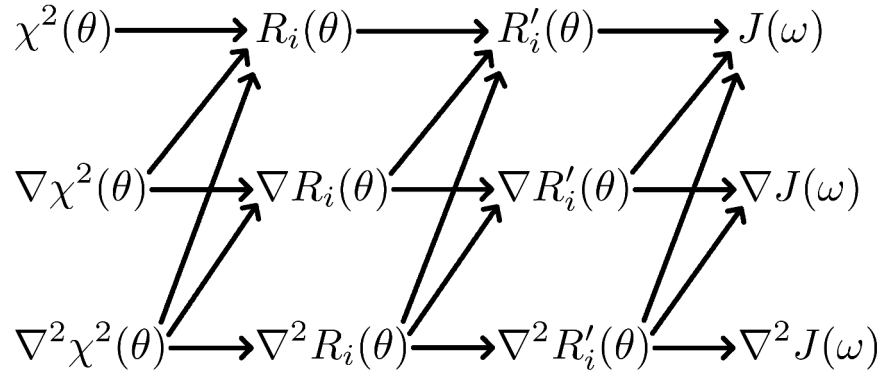


Figure 15.3: Dependencies between the  $\chi^2$ , transformed relaxation, relaxation, and spectral density equations, gradients, and Hessians.

## 15.5 The $\chi^2$ value, gradient, and Hessian

### 15.5.1 The $\chi^2$ value

The  $\chi^2$  value is defined as

$$\chi^2(\theta) = \sum_{i=1}^n \frac{(R_i - R_i(\theta))^2}{\sigma_i^2}, \quad (15.13)$$

where the summation index  $i$  ranges over all the relaxation data of all residues used in the analysis.

### 15.5.2 The $\chi^2$ gradient

The  $\chi^2$  gradient in vector notation is

$$\nabla \chi^2(\theta) = 2 \sum_{i=1}^n \frac{(R_i - R_i(\theta))^2}{\sigma_i^2} \nabla R_i(\theta). \quad (15.14)$$

### 15.5.3 The $\chi^2$ Hessian

The  $\chi^2$  Hessian in vector notation is

$$\nabla^2 \chi^2(\theta) = 2 \sum_{i=1}^n \frac{1}{\sigma_i^2} (\nabla R_i(\theta) \cdot \nabla R_i(\theta)^T - (R_i - R_i(\theta)) \nabla^2 R_i(\theta)). \quad (15.15)$$

## 15.6 The $R_i(\theta)$ values, gradients, and Hessians

### 15.6.1 The $R_i(\theta)$ values

The  $R_i(\theta)$  values are given by

$$R_1(\theta) = R'_1(\theta), \quad (15.16a)$$

$$R_2(\theta) = R'_2(\theta), \quad (15.16b)$$

$$\text{NOE}(\theta) = 1 + \frac{\gamma_H}{\gamma_X} \frac{\sigma_{\text{NOE}}(\theta)}{R_1(\theta)}. \quad (15.16c)$$

### 15.6.2 The $R_i(\theta)$ gradients

The  $R_i(\theta)$  gradients in vector notation are

$$\nabla R_1(\theta) = \nabla R'_1(\theta), \quad (15.17a)$$

$$\nabla R_2(\theta) = \nabla R'_2(\theta), \quad (15.17b)$$

$$\nabla \text{NOE}(\theta) = \frac{\gamma_H}{\gamma_X} \frac{1}{R_1(\theta)^2} \left( R_1(\theta) \nabla \sigma_{\text{NOE}}(\theta) - \sigma_{\text{NOE}}(\theta) \nabla R_1(\theta) \right). \quad (15.17c)$$

### 15.6.3 The $R_i(\theta)$ Hessians

The  $R_i(\theta)$  Hessians in vector notation are

$$\nabla^2 R_1(\theta) = \nabla^2 R'_1(\theta), \quad (15.18a)$$

$$\nabla^2 R_2(\theta) = \nabla^2 R'_2(\theta), \quad (15.18b)$$

$$\begin{aligned} \nabla^2 \text{NOE}(\theta) = \frac{\gamma_H}{\gamma_X} \frac{1}{R_1(\theta)^3} & \left[ \sigma_{\text{NOE}}(\theta) \left( 2 \nabla R_1(\theta) \cdot \nabla R_1(\theta)^T - R_1(\theta) \nabla^2 R_1(\theta) \right) \right. \\ & \left. - R_1(\theta) \left( \nabla \sigma_{\text{NOE}}(\theta) \cdot \nabla R_1(\theta)^T - R_1(\theta) \nabla^2 \sigma_{\text{NOE}}(\theta) \right) \right]. \end{aligned} \quad (15.18c)$$



## 15.7 $R'_i(\theta)$ values, gradients, and Hessians

The partial and second partial derivatives of the relaxation equations of the set  $R'(\theta)$  are different for each parameter of the vector  $\theta$ . The vector representation of the gradient  $\nabla R'_i(\theta)$  and the matrix representation of the Hessian  $\nabla^2 R'_i(\theta)$  can be reconstructed from the individual elements presented in the next section.

### 15.7.1 Components of the $R'_i(\theta)$ equations

To simplify the calculations of the gradients and Hessians the  $R'_i(\theta)$  equations have been broken down into a number of components. These include the dipolar and CSA constants as well as the dipolar and CSA spectral density terms for each of the three transformed relaxation data types  $\{R_1, R_2, \sigma_{\text{NOE}}\}$ . The segregation of these components simplifies the maths as many partial derivatives of the components are zero.

#### Dipolar constant

The dipolar constant is defined as

$$d = \frac{1}{4} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{(\gamma_H \gamma_X \hbar)^2}{\langle r^6 \rangle}. \quad (15.19)$$

This component of the relaxation equations is independent of the parameter of the spectral density function  $\theta_j$ , the chemical exchange parameter  $\rho_{ex}$ , and the CSA parameter  $\Delta\sigma$ . Therefore the partial and second partial derivatives with respect to these parameters is zero. Only the derivative with respect to the bond length  $r$  is non-zero being

$$d' \equiv \frac{dd}{dr} = -\frac{3}{2} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{(\gamma_H \gamma_X \hbar)^2}{\langle r^7 \rangle}. \quad (15.20)$$

The second derivative with respect to the bond length is

$$d'' \equiv \frac{d^2d}{dr^2} = \frac{21}{2} \left( \frac{\mu_0}{4\pi} \right)^2 \frac{(\gamma_H \gamma_X \hbar)^2}{\langle r^8 \rangle}. \quad (15.21)$$

#### CSA constant

The CSA constant is defined as

$$c = \frac{(\omega_X \cdot \Delta\sigma)^2}{3}. \quad (15.22)$$

The partial derivative of this component with respect to all parameters but the CSA parameter  $\Delta\sigma$  is zero. This derivative is

$$c' \equiv \frac{dc}{d\Delta\sigma} = \frac{2\omega_X^2 \cdot \Delta\sigma}{3}. \quad (15.23)$$

The CSA constant second derivative with respect to  $\Delta\sigma$  is

$$c'' \equiv \frac{d^2c}{d\Delta\sigma^2} = \frac{2\omega_X^2}{3}. \quad (15.24)$$

**$R_{ex}$  constant**

The  $R_{ex}$  constant is defined as

$$R_{ex} = \rho_{ex}(2\pi\omega_H)^2. \quad (15.25)$$

The partial derivative of this component with respect to all parameters but the chemical exchange parameter  $\rho_{ex}$  is zero. This derivative is

$$R'_{ex} \equiv \frac{dR_{ex}}{d\rho_{ex}} = (2\pi\omega_H)^2. \quad (15.26)$$

The  $R_{ex}$  constant second derivative with respect to  $\rho_{ex}$  is

$$R''_{ex} \equiv \frac{d^2R_{ex}}{d\rho_{ex}^2} = 0. \quad (15.27)$$

**Spectral density terms of the  $R_1$  dipolar component**

For the dipolar component of the  $R_1$  equation (7.3a) on page 84 the spectral density terms are

$$J_d^{R_1} = J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H + \omega_X). \quad (15.28)$$

The partial derivative of these terms with respect to the spectral density function parameter  $\theta_j$  is

$$J_d^{R_1'} \equiv \frac{\partial J_d^{R_1}}{\partial \theta_j} = \frac{\partial J(\omega_H - \omega_X)}{\partial \theta_j} + 3 \frac{\partial J(\omega_X)}{\partial \theta_j} + 6 \frac{\partial J(\omega_H + \omega_X)}{\partial \theta_j}. \quad (15.29)$$

The second partial derivative with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  is

$$J_d^{R_1''} \equiv \frac{\partial^2 J_d^{R_1}}{\partial \theta_j \cdot \partial \theta_k} = \frac{\partial^2 J(\omega_H - \omega_X)}{\partial \theta_j \cdot \partial \theta_k} + 3 \frac{\partial^2 J(\omega_X)}{\partial \theta_j \cdot \partial \theta_k} + 6 \frac{\partial^2 J(\omega_H + \omega_X)}{\partial \theta_j \cdot \partial \theta_k}. \quad (15.30)$$

**Spectral density terms of the  $R_1$  CSA component**

For the CSA component of the  $R_1$  equation (7.3a) on page 84 the spectral density terms are

$$J_c^{R_1} = J(\omega_X). \quad (15.31)$$

The partial derivative of these terms with respect to the spectral density function parameter  $\theta_j$  is

$$J_c^{R_1'} \equiv \frac{\partial J_c^{R_1}}{\partial \theta_j} = \frac{\partial J(\omega_X)}{\partial \theta_j}. \quad (15.32)$$

The second partial derivative with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  is

$$J_c^{R_1''} \equiv \frac{\partial^2 J_c^{R_1}}{\partial \theta_j \cdot \partial \theta_k} = \frac{\partial^2 J(\omega_X)}{\partial \theta_j \cdot \partial \theta_k}. \quad (15.33)$$

**Spectral density terms of the  $R_2$  dipolar component**

For the dipolar component of the  $R_2$  equation (7.3b) on page 84 the spectral density terms are

$$J_d^{R_2} = 4J(0) + J(\omega_H - \omega_X) + 3J(\omega_X) + 6J(\omega_H) + 6J(\omega_H + \omega_X). \quad (15.34)$$

The partial derivative of these terms with respect to the spectral density function parameter  $\theta_j$  is

$$J_d^{R_2'} \equiv \frac{\partial J_d^{R_2}}{\partial \theta_j} = 4 \frac{\partial J(0)}{\partial \theta_j} + \frac{\partial J(\omega_H - \omega_X)}{\partial \theta_j} + 3 \frac{\partial J(\omega_X)}{\partial \theta_j} + 6 \frac{\partial J(\omega_H)}{\partial \theta_j} + 6 \frac{\partial J(\omega_H + \omega_X)}{\partial \theta_j}. \quad (15.35)$$

The second partial derivative with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  is

$$J_d^{R_2''} \equiv \frac{\partial^2 J_d^{R_2}}{\partial \theta_j \cdot \partial \theta_k} = 4 \frac{\partial^2 J(0)}{\partial \theta_j \cdot \partial \theta_k} + \frac{\partial^2 J(\omega_H - \omega_X)}{\partial \theta_j \cdot \partial \theta_k} + 3 \frac{\partial^2 J(\omega_X)}{\partial \theta_j \cdot \partial \theta_k} + 6 \frac{\partial^2 J(\omega_H)}{\partial \theta_j \cdot \partial \theta_k} + 6 \frac{\partial^2 J(\omega_H + \omega_X)}{\partial \theta_j \cdot \partial \theta_k}. \quad (15.36)$$

**Spectral density terms of the  $R_2$  CSA component**

For the CSA component of the  $R_2$  equation (7.3b) on page 84 the spectral density terms are

$$J_c^{R_2} = 4J(0) + 3J(\omega_X). \quad (15.37)$$

The partial derivative of these terms with respect to the spectral density function parameter  $\theta_j$  is

$$J_c^{R_2'} \equiv \frac{\partial J_c^{R_2}}{\partial \theta_j} = 4 \frac{\partial J(0)}{\partial \theta_j} + 3 \frac{\partial J(\omega_X)}{\partial \theta_j}. \quad (15.38)$$

The second partial derivative with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  is

$$J_c^{R_2''} \equiv \frac{\partial^2 J_c^{R_2}}{\partial \theta_j \cdot \partial \theta_k} = 4 \frac{\partial^2 J(0)}{\partial \theta_j \cdot \partial \theta_k} + 3 \frac{\partial^2 J(\omega_X)}{\partial \theta_j \cdot \partial \theta_k}. \quad (15.39)$$

**Spectral density terms of the  $\sigma_{\text{NOE}}$  dipolar component**

For the dipolar component of the  $\sigma_{\text{NOE}}$  equation (7.3c) on page 84 the spectral density terms are

$$J_d^{\sigma_{\text{NOE}}} = 6J(\omega_H + \omega_X) - J(\omega_H - \omega_X). \quad (15.40)$$

The partial derivative of these terms with respect to the spectral density function parameter  $\theta_j$  is

$$J_d^{\sigma_{\text{NOE}'}} \equiv \frac{\partial J_d^{\sigma_{\text{NOE}}}}{\partial \theta_j} = 6 \frac{\partial J(\omega_H + \omega_X)}{\partial \theta_j} - \frac{\partial J(\omega_H - \omega_X)}{\partial \theta_j}. \quad (15.41)$$

The second partial derivative with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  is

$$J_d^{\sigma_{\text{NOE}''}} \equiv \frac{\partial^2 J_d^{\sigma_{\text{NOE}}}}{\partial \theta_j \cdot \partial \theta_k} = 6 \frac{\partial^2 J(\omega_H + \omega_X)}{\partial \theta_j \cdot \partial \theta_k} - \frac{\partial^2 J(\omega_H - \omega_X)}{\partial \theta_j \cdot \partial \theta_k}. \quad (15.42)$$

### 15.7.2 $R'_i(\theta)$ values

Using the components of the relaxation equations defined above the three relaxation equations can be re-expressed as

$$R_1(\theta) = dJ_d^{\text{R}_1} + cJ_c^{\text{R}_1}, \quad (15.43a)$$

$$R_2(\theta) = \frac{d}{2}J_d^{\text{R}_2} + \frac{c}{6}J_c^{\text{R}_2}, \quad (15.43b)$$

$$\sigma_{\text{NOE}}(\theta) = dJ_d^{\sigma_{\text{NOE}}}. \quad (15.43c)$$

### 15.7.3 $R'_i(\theta)$ gradients

A different partial derivative exists for the spectral density function parameter  $\theta_j$ , the chemical exchange parameter  $\rho_{ex}$ , CSA parameter  $\Delta\sigma$ , and bond length parameter  $r$ . In model-free analysis the spectral density parameters include both the parameters of the diffusion tensor and the parameters of the various model-free models.

#### $\theta_j$ partial derivative

The partial derivatives of the relaxation equations with respect to the spectral density function parameter  $\theta_j$  are

$$\frac{\partial R_1(\theta)}{\partial \theta_j} = dJ_d^{\text{R}_1'} + cJ_c^{\text{R}_1'}, \quad (15.44a)$$

$$\frac{\partial R_2(\theta)}{\partial \theta_j} = \frac{d}{2}J_d^{\text{R}_2'} + \frac{c}{6}J_c^{\text{R}_2'}, \quad (15.44b)$$

$$\frac{\partial \sigma_{\text{NOE}}(\theta)}{\partial \theta_j} = dJ_d^{\sigma_{\text{NOE}}'}. \quad (15.44c)$$

#### $\rho_{ex}$ partial derivative

The partial derivatives of the relaxation equations with respect to the chemical exchange parameter  $\rho_{ex}$  are

$$\frac{\partial R_1(\theta)}{\partial \rho_{ex}} = 0, \quad (15.45a)$$

$$\frac{\partial R_2(\theta)}{\partial \rho_{ex}} = (2\pi\omega_H)^2, \quad (15.45b)$$

$$\frac{\partial \sigma_{\text{NOE}}(\theta)}{\partial \rho_{ex}} = 0. \quad (15.45c)$$

**$\Delta\sigma$  partial derivative**

The partial derivatives of the relaxation equations with respect to the CSA parameter  $\Delta\sigma$  are

$$\frac{\partial R_1(\theta)}{\partial \Delta\sigma} = c' J_c^{R_1}, \quad (15.46a)$$

$$\frac{\partial R_2(\theta)}{\partial \Delta\sigma} = \frac{c'}{6} J_c^{R_2}, \quad (15.46b)$$

$$\frac{\partial \sigma_{\text{NOE}}(\theta)}{\partial \Delta\sigma} = 0. \quad (15.46c)$$

 **$r$  partial derivative**

The partial derivatives of the relaxation equations with respect to the bond length parameter  $r$  are

$$\frac{\partial R_1(\theta)}{\partial r} = d' J_d^{R_1}, \quad (15.47a)$$

$$\frac{\partial R_2(\theta)}{\partial r} = \frac{d'}{2} J_d^{R_2}, \quad (15.47b)$$

$$\frac{\partial \sigma_{\text{NOE}}(\theta)}{\partial r} = d' J_d^{\sigma_{\text{NOE}}}. \quad (15.47c)$$

**15.7.4  $R'_i(\theta)$  Hessians**

Again different second partial derivatives with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$ , the chemical exchange parameter  $\rho_{ex}$ , CSA parameter  $\Delta\sigma$ , and bond length parameter  $r$ . These second partial derivatives are the components of the  $R'_i(\theta)$  Hessian matrices.

 **$\theta_j - \theta_k$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the spectral density function parameters  $\theta_j$  and  $\theta_k$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \theta_j \cdot \partial \theta_k} = d J_d^{R_1''} + c J_c^{R_1''}, \quad (15.48a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \theta_j \cdot \partial \theta_k} = \frac{d}{2} J_d^{R_2''} + \frac{c}{6} J_c^{R_2''}, \quad (15.48b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \theta_j \cdot \partial \theta_k} = d J_d^{\sigma_{\text{NOE}}''}. \quad (15.48c)$$

**$\theta_j - \rho_{ex}$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the spectral density function parameter  $\theta_j$  and the chemical exchange parameter  $\rho_{ex}$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \theta_j \cdot \partial \rho_{ex}} = 0, \quad (15.49a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \theta_j \cdot \partial \rho_{ex}} = 0, \quad (15.49b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \theta_j \cdot \partial \rho_{ex}} = 0. \quad (15.49c)$$

 **$\theta_j - \Delta\sigma$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the spectral density function parameter  $\theta_j$  and the CSA parameter  $\Delta\sigma$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \theta_j \cdot \partial \Delta\sigma} = c' J_c^{\text{R}_1'}, \quad (15.50a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \theta_j \cdot \partial \Delta\sigma} = \frac{c'}{6} J_c^{\text{R}_2'}, \quad (15.50b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \theta_j \cdot \partial \Delta\sigma} = 0. \quad (15.50c)$$

 **$\theta_j - r$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the spectral density function parameter  $\theta_j$  and the bond length parameter  $r$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \theta_j \cdot \partial r} = d' J_d^{\text{R}_1'}, \quad (15.51a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \theta_j \cdot \partial r} = \frac{d'}{2} J_d^{\text{R}_2'}, \quad (15.51b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \theta_j \cdot \partial r} = d' J_d^{\sigma_{\text{NOE}}'}. \quad (15.51c)$$

 **$\rho_{ex} - \rho_{ex}$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the chemical exchange parameter  $\rho_{ex}$  twice are

$$\frac{\partial^2 R_1(\theta)}{\partial \rho_{ex}^2} = 0, \quad (15.52a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \rho_{ex}^2} = 0, \quad (15.52b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \rho_{ex}^2} = 0. \quad (15.52c)$$

**$\rho_{ex} - \Delta\sigma$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the chemical exchange parameter  $\rho_{ex}$  and the CSA parameter  $\Delta\sigma$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \rho_{ex} \cdot \partial \Delta\sigma} = 0, \quad (15.53a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \rho_{ex} \cdot \partial \Delta\sigma} = 0, \quad (15.53b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \rho_{ex} \cdot \partial \Delta\sigma} = 0. \quad (15.53c)$$

 **$\rho_{ex} - r$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the chemical exchange parameter  $\rho_{ex}$  and the bond length parameter  $r$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \rho_{ex} \cdot \partial r} = 0, \quad (15.54a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \rho_{ex} \cdot \partial r} = 0, \quad (15.54b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \rho_{ex} \cdot \partial r} = 0. \quad (15.54c)$$

 **$\Delta\sigma - \Delta\sigma$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the CSA parameter  $\Delta\sigma$  twice are

$$\frac{\partial^2 R_1(\theta)}{\partial \Delta\sigma^2} = c'' J_c^{R_1}, \quad (15.55a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \Delta\sigma^2} = \frac{c''}{6} J_c^{R_2}, \quad (15.55b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \Delta\sigma^2} = 0. \quad (15.55c)$$

 **$\Delta\sigma - r$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the CSA parameter  $\Delta\sigma$  and the bond length parameter  $r$  are

$$\frac{\partial^2 R_1(\theta)}{\partial \Delta\sigma \cdot \partial r} = 0, \quad (15.56a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial \Delta\sigma \cdot \partial r} = 0, \quad (15.56b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial \Delta\sigma \cdot \partial r} = 0. \quad (15.56c)$$

**$r - r$  partial derivative**

The second partial derivatives of the relaxation equations with respect to the bond length parameter  $r$  twice are

$$\frac{\partial^2 R_1(\theta)}{\partial r^2} = d'' J_d^{R_1}, \quad (15.57a)$$

$$\frac{\partial^2 R_2(\theta)}{\partial r^2} = \frac{d''}{2} J_d^{R_2}, \quad (15.57b)$$

$$\frac{\partial^2 \sigma_{\text{NOE}}(\theta)}{\partial r^2} = d'' J_d^{\sigma_{\text{NOE}}}. \quad (15.57c)$$



## 15.8 Optimisation equations for the model-free analysis

### 15.8.1 The model-free equations

In the original model-free analysis of [Lipari and Szabo \(1982a\)](#) the correlation function  $C(\tau)$  of the XH bond vector is approximated by decoupling the internal fluctuations of the bond vector  $C_I(\tau)$  from the correlation function of the overall Brownian rotational diffusion  $C_O(\tau)$  by the equation

$$C(\tau) = C_O(\tau) \cdot C_I(\tau). \quad (15.58)$$

The overall correlation functions of the diffusion of a sphere, spheroid, and ellipsoid are presented respectively in section 15.9.1 on page 346, section 15.10.1 on page 359, and section 15.11.1 on page 363. These three different equations can be combined into one generic correlation function which is independent of the type of diffusion. This generic correlation function is

$$C_O(\tau) = \frac{1}{5} \sum_{i=-k}^k c_i \cdot e^{-\tau/\tau_i}, \quad (15.59)$$

where  $c_i$  are the weights and  $\tau_i$  are correlation times of the exponential terms. In the original model-free analysis of [Lipari and Szabo \(1982a,b\)](#) the internal motions are modelled by the correlation function

$$C_I(\tau) = S^2 + (1 - S^2)e^{-\tau/\tau_e}, \quad (15.60)$$

where  $S^2$  is the generalised Lipari and Szabo order parameter which is related to the amplitude of the motion and  $\tau_e$  is the effective correlation time which is an indicator of the timescale of the motion, albeit being dependent on the value of the order parameter. The order parameter ranges from one for complete rigidity to zero for unrestricted motions. Model-free theory was extended by [Clare et al. \(1990\)](#) to include motions on two timescales by the correlation function

$$C_I(\tau) = S^2 + (1 - S_f^2)e^{-\tau/\tau_f} + (S_f^2 - S^2)e^{-\tau/\tau_s}, \quad (15.61)$$

where the faster of the motions is defined by the order parameter  $S_f^2$  and the correlation time  $\tau_f$ , the slower by the parameters  $S_s^2$  and  $\tau_s$ , and the two order parameter are related by the equation  $S^2 = S_f^2 \cdot S_s^2$ .

The relaxation equations of [Abragam \(1961\)](#) are composed of a sum of power spectral density functions  $J(\omega)$  at five frequencies. The spectral density function is related to the correlation function as the two are a Fourier pair. Applying the Fourier transform to the correlation function composed of the generic diffusion equation and the original model-free correlation function results in the equation

$$J(\omega) = \frac{2}{5} \sum_{i=-k}^k c_i \cdot \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2} \right). \quad (15.62)$$

The Fourier transform using the extended model-free correlation function is

$$J(\omega) = \frac{2}{5} \sum_{i=-k}^k c_i \cdot \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega\tau_f\tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega\tau_s\tau_i)^2} \right). \quad (15.63)$$

### 15.8.2 The original model-free gradient

The model-free gradient of the original spectral density function (15.62) is the vector of partial derivatives of the function with respect to the geometric parameter  $\mathfrak{G}_i$ , the orientational parameter  $\mathfrak{D}_i$ , the order parameter  $S^2$ , and the internal correlation time  $\tau_e$ . The positions in the vector correspond to the model parameters which are being optimised.

#### $\mathfrak{G}_j$ partial derivative

The partial derivative of (15.62) with respect to the geometric parameter  $\mathfrak{G}_j$  is

$$\begin{aligned} \frac{\partial J(\omega)}{\partial \mathfrak{G}_j} = \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( S^2 \frac{1 - (\omega\tau_i)^2}{(1 + (\omega\tau_i)^2)^2} + (1 - S^2) \tau_e^2 \frac{(\tau_e + \tau_i)^2 - (\omega\tau_e\tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2)^2} \right) \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2} \right) \right). \quad (15.64) \end{aligned}$$

#### $\mathfrak{D}_j$ partial derivative

The partial derivative of (15.62) with respect to the orientational parameter  $\mathfrak{D}_j$  is

$$\frac{\partial J(\omega)}{\partial \mathfrak{D}_j} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{S^2}{1 + (\omega\tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2} \right). \quad (15.65)$$

#### $S^2$ partial derivative

The partial derivative of (15.62) with respect to the order parameter  $S^2$  is

$$\frac{\partial J(\omega)}{\partial S^2} = \frac{2}{5} \sum_{i=-k}^k c_i \tau_i \left( \frac{1}{1 + (\omega\tau_i)^2} - \frac{(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2} \right). \quad (15.66)$$

#### $\tau_e$ partial derivative

The partial derivative of (15.62) with respect to the correlation time  $\tau_e$  is

$$\frac{\partial J(\omega)}{\partial \tau_e} = \frac{2}{5} (1 - S^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_e + \tau_i)^2 - (\omega\tau_e\tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega\tau_e\tau_i)^2)^2}. \quad (15.67)$$

### 15.8.3 The original model-free Hessian

The model-free Hessian of the original spectral density function (15.62) is the matrix of second partial derivatives. The matrix coordinates correspond to the model parameters which are being optimised.

#### $\mathfrak{G}_j - \mathfrak{G}_k$ partial derivative

The second partial derivative of (15.62) with respect to the geometric parameters  $\mathfrak{G}_j$  and  $\mathfrak{G}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( -2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \left( S^2 \omega^2 \tau_i \frac{3 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^3} \right. \right. \\ & \left. \left. + (1 - S^2) \tau_e^2 \frac{(\tau_e + \tau_i)^3 + 3\omega^2 \tau_e^3 \tau_i (\tau_e + \tau_i) - (\omega \tau_e)^4 \tau_i^3}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^3} \right) \right. \\ & \left. + \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_k} + \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_j} + c_i \frac{\partial^2 \tau_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \right) \left( S^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \right. \\ & \left. \left. + (1 - S^2) \tau_e^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2} \right) \right. \\ & \left. + \left( \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2} \right) \right) \right). \quad (15.68) \end{aligned}$$

#### $\mathfrak{G}_j - \mathfrak{D}_k$ partial derivative

The second partial derivative of (15.62) with respect to the geometric parameter  $\mathfrak{G}_j$  and the orientational parameter  $\mathfrak{D}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \frac{\partial c_i}{\partial \mathfrak{D}_k} \left( S^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} + (1 - S^2) \tau_e^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2} \right) \right. \\ & \left. + \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2} \right) \right). \quad (15.69) \end{aligned}$$

#### $\mathfrak{G}_j - S^2$ partial derivative

The second partial derivative of (15.62) with respect to the geometric parameter  $\mathfrak{G}_j$  and the order parameter  $S^2$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial S^2} = & \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} - \tau_e^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2} \right) \right. \\ & \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2} \right) \right). \quad (15.70) \end{aligned}$$

**$\mathfrak{G}_j - \tau_e$  partial derivative**

The second partial derivative of (15.62) with respect to the geometric parameter  $\mathfrak{G}_j$  and the correlation time  $\tau_e$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \tau_e} = \frac{2}{5}(1 - S^2) \sum_{i=-k}^k \left( 2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \tau_e \tau_i (\tau_e + \tau_i) \frac{(\tau_e + \tau_i)^2 - 3(\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^3} + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2} \right). \quad (15.71)$$

 **$\mathfrak{D}_j - \mathfrak{D}_k$  partial derivative**

The second partial derivative of (15.62) with respect to the orientational parameters  $\mathfrak{D}_j$  and  $\mathfrak{D}_k$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial^2 c_i}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S^2)(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2} \right). \quad (15.72)$$

 **$\mathfrak{D}_j - S^2$  partial derivative**

The second partial derivative of (15.62) with respect to the orientational parameter  $\mathfrak{D}_j$  and the order parameter  $S^2$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial S^2} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_e + \tau_i)\tau_e}{(\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2} \right). \quad (15.73)$$

 **$\mathfrak{D}_j - \tau_e$  partial derivative**

The second partial derivative of (15.62) with respect to the orientational parameter  $\mathfrak{D}_j$  and the correlation time  $\tau_e$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \tau_e} = \frac{2}{5}(1 - S^2) \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2}. \quad (15.74)$$

 **$S^2 - S^2$  partial derivative**

The second partial derivative of (15.62) with respect to the order parameter  $S^2$  twice is

$$\frac{\partial^2 J(\omega)}{(\partial S^2)^2} = 0. \quad (15.75)$$

**$S^2 - \tau_e$  partial derivative**

The second partial derivative of (15.62) with respect to the order parameter  $S^2$  and correlation time  $\tau_e$  is

$$\frac{\partial^2 J(\omega)}{\partial S^2 \cdot \partial \tau_e} = -\frac{2}{5} \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_e + \tau_i)^2 - (\omega \tau_e \tau_i)^2}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^2}. \quad (15.76)$$

 **$\tau_e - \tau_e$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation time  $\tau_e$  twice is

$$\frac{\partial^2 J(\omega)}{\partial \tau_e^2} = -\frac{4}{5} (1 - S^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_e + \tau_i)^3 + 3\omega^2 \tau_i^3 \tau_e (\tau_e + \tau_i) - (\omega \tau_i)^4 \tau_e^3}{((\tau_e + \tau_i)^2 + (\omega \tau_e \tau_i)^2)^3} \quad (15.77)$$

### 15.8.4 The extended model-free gradient

The model-free gradient of the extended spectral density function (15.63) is the vector of partial derivatives of the function with respect to the geometric parameter  $\mathfrak{G}_i$ , the orientational parameter  $\mathfrak{D}_i$ , the order parameters  $S^2$  and  $S_f^2$ , and the internal correlation times  $\tau_f$  and  $\tau_s$ . The positions in the vector correspond to the model parameters which are being optimised.

#### $\mathfrak{G}_j$ partial derivative

The partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  is

$$\begin{aligned} \frac{\partial J(\omega)}{\partial \mathfrak{G}_j} = \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( S^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \right. \\ \left. \left. + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \right. \right. \\ \left. \left. + (S_f^2 - S^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \end{aligned} \quad (15.78)$$

#### $\mathfrak{D}_j$ partial derivative

The partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  is

$$\frac{\partial J(\omega)}{\partial \mathfrak{D}_j} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.79)$$

#### $S^2$ partial derivative

The partial derivative of (15.63) with respect to the order parameter  $S^2$  is

$$\frac{\partial J(\omega)}{\partial S^2} = \frac{2}{5} \sum_{i=-k}^k c_i \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.80)$$

#### $S_f^2$ partial derivative

The partial derivative of (15.63) with respect to the order parameter  $S_f^2$  is

$$\frac{\partial J(\omega)}{\partial S_f^2} = -\frac{2}{5} \sum_{i=-k}^k c_i \tau_i \left( \frac{(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.81)$$

**$\tau_f$  partial derivative**

The partial derivative of (15.63) with respect to the correlation time  $\tau_f$  is

$$\frac{\partial J(\omega)}{\partial \tau_f} = \frac{2}{5}(1 - S_f^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.82)$$

 **$\tau_s$  partial derivative**

The partial derivative of (15.63) with respect to the correlation time  $\tau_s$  is

$$\frac{\partial J(\omega)}{\partial \tau_s} = \frac{2}{5}(S_f^2 - S^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.83)$$

### 15.8.5 The extended model-free Hessian

The model-free Hessian of the extended spectral density function (15.63) is the matrix of second partial derivatives. The matrix coordinates correspond to the model parameters which are being optimised.

#### $\mathfrak{G}_j - \mathfrak{G}_k$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameters  $\mathfrak{G}_j$  and  $\mathfrak{G}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( -2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \left( S^2 \omega^2 \tau_i \frac{3 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^3} \right. \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^3 + 3\omega^2 \tau_f^3 \tau_i (\tau_f + \tau_i) - (\omega \tau_f)^4 \tau_i^3}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \\ & + (S_f^2 - S^2) \tau_s^2 \frac{(\tau_s + \tau_i)^3 + 3\omega^2 \tau_s^3 \tau_i (\tau_s + \tau_i) - (\omega \tau_s)^4 \tau_i^3}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \Big) \\ & + \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_k} + \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_j} + c_i \frac{\partial^2 \tau_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \right) \left( S^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \\ & + (S_f^2 - S^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \Big) \\ & \left. + \left( \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right) \right). \end{aligned} \quad (15.84)$$

#### $\mathfrak{G}_j - \mathfrak{D}_k$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the orientational parameter  $\mathfrak{D}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \frac{\partial c_i}{\partial \mathfrak{D}_k} \left( S^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \\ & + (S_f^2 - S^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \Big) \\ & + \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \Big). \end{aligned} \quad (15.85)$$



**$\mathfrak{G}_j - S^2$  partial derivative**

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the order parameter  $S^2$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial S^2} = \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} - \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \quad (15.86) \end{aligned}$$

 **$\mathfrak{G}_j - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the order parameter  $S_f^2$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial S_f^2} = -\frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} - \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \quad (15.87) \end{aligned}$$

 **$\mathfrak{G}_j - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the correlation time  $\tau_f$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \tau_f} = \frac{2}{5} (1 - S_f^2) \sum_{i=-k}^k \left( 2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \tau_f \tau_i (\tau_f + \tau_i) \frac{(\tau_f + \tau_i)^2 - 3(\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \right). \quad (15.88) \end{aligned}$$

 **$\mathfrak{G}_j - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the correlation time  $\tau_s$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \tau_s} = \frac{2}{5} (S_f^2 - S^2) \sum_{i=-k}^k \left( 2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \tau_s \tau_i (\tau_s + \tau_i) \frac{(\tau_s + \tau_i)^2 - 3(\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right). \quad (15.89) \end{aligned}$$

**$\mathfrak{D}_j - \mathfrak{D}_k$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameters  $\mathfrak{D}_j$  and  $\mathfrak{D}_k$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{S^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(S_f^2 - S^2)(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.90)$$

 **$\mathfrak{D}_j - S^2$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the order parameter  $S^2$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial S^2} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.91)$$

 **$\mathfrak{D}_j - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the order parameter  $S_f^2$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial S_f^2} = -\frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} - \frac{(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.92)$$

 **$\mathfrak{D}_j - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \tau_f} = \frac{2}{5} (1 - S_f^2) \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.93)$$

 **$\mathfrak{D}_j - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \tau_s} = \frac{2}{5} (S_f^2 - S^2) \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.94)$$

**$S^2 - S^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S^2$  twice is

$$\frac{\partial^2 J(\omega)}{(\partial S^2)^2} = 0. \quad (15.95)$$

 **$S^2 - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameters  $S^2$  and  $S_f^2$  is

$$\frac{\partial^2 J(\omega)}{\partial S^2 \cdot \partial S_f^2} = 0. \quad (15.96)$$

 **$S^2 - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S^2$  and correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial S^2 \cdot \partial \tau_f} = 0. \quad (15.97)$$

 **$S^2 - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S^2$  and correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial S^2 \cdot \partial \tau_s} = -\frac{2}{5} \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.98)$$

 **$S_f^2 - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  twice is

$$\frac{\partial^2 J(\omega)}{(\partial S_f^2)^2} = 0. \quad (15.99)$$

 **$S_f^2 - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  and correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial S_f^2 \cdot \partial \tau_f} = -\frac{2}{5} \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.100)$$

**$S_f^2 - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  and correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial S_f^2 \cdot \partial \tau_s} = \frac{2}{5} \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.101)$$

 **$\tau_f - \tau_f$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation time  $\tau_f$  twice is

$$\frac{\partial^2 J(\omega)}{\partial \tau_f^2} = -\frac{4}{5} (1 - S_f^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^3 + 3\omega^2 \tau_i^3 \tau_f (\tau_f + \tau_i) - (\omega \tau_i)^4 \tau_f^3}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \quad (15.102)$$

 **$\tau_f - \tau_s$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation times  $\tau_f$  and  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial \tau_f \cdot \partial \tau_s} = 0. \quad (15.103)$$

 **$\tau_s - \tau_s$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation time  $\tau_s$  twice is

$$\frac{\partial^2 J(\omega)}{\partial \tau_s^2} = -\frac{4}{5} (S_f^2 - S^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^3 + 3\omega^2 \tau_i^3 \tau_s (\tau_s + \tau_i) - (\omega \tau_i)^4 \tau_s^3}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \quad (15.104)$$

### 15.8.6 The alternative extended model-free gradient

Because of the equation  $S^2 = S_f^2 \cdot S_s^2$  and the form of the extended spectral density function (15.63) a convolution of the model-free space occurs if the model-free parameters  $\{S_f^2, S_s^2, \tau_f, \tau_s\}$  are optimised rather than the parameters  $\{S^2, S_f^2, \tau_f, \tau_s\}$ . This convolution increases the complexity of the gradient. For completeness the first partial derivatives are presented below.

#### $\mathfrak{G}_j$ partial derivative

The partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  is

$$\begin{aligned} \frac{\partial J(\omega)}{\partial \mathfrak{G}_j} = & \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( S_f^2 \cdot S_s^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \\ & \left. \left. + S_f^2 (1 - S_s^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ & \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{S_f^2 \cdot S_s^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{S_f^2 (1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \end{aligned} \quad (15.105)$$

#### $\mathfrak{D}_j$ partial derivative

The partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  is

$$\frac{\partial J(\omega)}{\partial \mathfrak{D}_j} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{S_f^2 \cdot S_s^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{S_f^2 (1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.106)$$

#### $S_f^2$ partial derivative

The partial derivative of (15.63) with respect to the order parameter  $S_f^2$  is

$$\frac{\partial J(\omega)}{\partial S_f^2} = \frac{2}{5} \sum_{i=-k}^k c_i \tau_i \left( \frac{S_s^2}{1 + (\omega \tau_i)^2} - \frac{(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.107)$$

#### $S_s^2$ partial derivative

The partial derivative of (15.63) with respect to the order parameter  $S_s^2$  is

$$\frac{\partial J(\omega)}{\partial S_s^2} = \frac{2}{5} S_f^2 \sum_{i=-k}^k c_i \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.108)$$

**$\tau_f$  partial derivative**

The partial derivative of (15.63) with respect to the correlation time  $\tau_f$  is

$$\frac{\partial J(\omega)}{\partial \tau_f} = \frac{2}{5}(1 - S_f^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.109)$$

 **$\tau_s$  partial derivative**

The partial derivative of (15.63) with respect to the correlation time  $\tau_s$  is

$$\frac{\partial J(\omega)}{\partial \tau_s} = \frac{2}{5} S_f^2 (1 - S_s^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.110)$$

### 15.8.7 The alternative extended model-free Hessian

The model-free Hessian of the extended spectral density function (15.63) is also complicated by the convolution resulting from the use of the parameters  $\{S_f^2, S_s^2, \tau_f, \tau_s\}$ . The second partial derivatives with respect to these parameters are presented below.

#### $\mathfrak{G}_j - \mathfrak{G}_k$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameters  $\mathfrak{G}_j$  and  $\mathfrak{G}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( -2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \left( S_f^2 \cdot S_s^2 \omega^2 \tau_i \frac{3 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^3} \right. \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^3 + 3\omega^2 \tau_f^3 \tau_i (\tau_f + \tau_i) - (\omega \tau_f)^4 \tau_i^3}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \\ & + S_f^2 (1 - S_s^2) \tau_s^2 \frac{(\tau_s + \tau_i)^3 + 3\omega^2 \tau_s^3 \tau_i (\tau_s + \tau_i) - (\omega \tau_s)^4 \tau_i^3}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \Big) \\ & + \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_k} + \frac{\partial \tau_i}{\partial \mathfrak{G}_k} \cdot \frac{\partial c_i}{\partial \mathfrak{G}_j} + c_i \frac{\partial^2 \tau_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \right) \left( S_f^2 \cdot S_s^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \\ & + S_f^2 (1 - S_s^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \Big) \\ & \left. + \left( \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{G}_k} \tau_i \left( \frac{S_f^2 \cdot S_s^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{S_f^2 (1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right) \right). \end{aligned} \quad (15.111)$$

#### $\mathfrak{G}_j - \mathfrak{D}_k$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the orientational parameter  $\mathfrak{D}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} = & \frac{2}{5} \sum_{i=-k}^k \left( \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \frac{\partial c_i}{\partial \mathfrak{D}_k} \left( S_f^2 \cdot S_s^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} \right. \right. \\ & + (1 - S_f^2) \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \\ & + S_f^2 (1 - S_s^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \Big) \\ & + \frac{\partial^2 c_i}{\partial \mathfrak{G}_j \cdot \partial \mathfrak{D}_k} \tau_i \left( \frac{S_f^2 \cdot S_s^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{S_f^2 (1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \Big). \end{aligned} \quad (15.112)$$

### $\mathfrak{G}_j - S_f^2$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the order parameter  $S_f^2$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial S_f^2} = & \frac{2}{5} \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( S_s^2 \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} - \tau_f^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \right. \right. \\ & \left. \left. + (1 - S_s^2) \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ & \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{S_s^2}{1 + (\omega \tau_i)^2} - \frac{(\tau_f + \tau_i) \tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} \right. \right. \\ & \left. \left. + \frac{(1 - S_s^2)(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \quad (15.113) \end{aligned}$$

### $\mathfrak{G}_j - S_s^2$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the order parameter  $S_s^2$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial S_s^2} = & \frac{2}{5} S_f^2 \sum_{i=-k}^k \left( c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \left( \frac{1 - (\omega \tau_i)^2}{(1 + (\omega \tau_i)^2)^2} - \tau_s^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right) \right. \\ & \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right) \right). \quad (15.114) \end{aligned}$$

### $\mathfrak{G}_j - \tau_f$ partial derivative

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the correlation time  $\tau_f$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \tau_f} = & \frac{2}{5} (1 - S_f^2) \sum_{i=-k}^k \left( 2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \tau_f \tau_i (\tau_f + \tau_i) \frac{(\tau_f + \tau_i)^2 - 3(\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \right. \\ & \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2} \right). \quad (15.115) \end{aligned}$$



**$\mathfrak{G}_j - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the geometric parameter  $\mathfrak{G}_j$  and the correlation time  $\tau_s$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{G}_j \cdot \partial \tau_s} = \frac{2}{5} S_f^2 (1 - S_s^2) \sum_{i=-k}^k \left( 2c_i \frac{\partial \tau_i}{\partial \mathfrak{G}_j} \tau_s \tau_i (\tau_s + \tau_i) \frac{(\tau_s + \tau_i)^2 - 3(\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \right. \\ \left. + \frac{\partial c_i}{\partial \mathfrak{G}_j} \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2} \right). \quad (15.116) \end{aligned}$$

 **$\mathfrak{D}_j - \mathfrak{D}_k$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameters  $\mathfrak{D}_j$  and  $\mathfrak{D}_k$  is

$$\begin{aligned} \frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial^2 c_i}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} \tau_i \left( \frac{S_f^2 \cdot S_s^2}{1 + (\omega \tau_i)^2} + \frac{(1 - S_f^2)(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} \right. \\ \left. + \frac{S_f^2(1 - S_s^2)(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.117) \end{aligned}$$

 **$\mathfrak{D}_j - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the order parameter  $S_f^2$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial S_f^2} = \frac{2}{5} \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{S_s^2}{1 + (\omega \tau_i)^2} - \frac{(\tau_f + \tau_i)\tau_f}{(\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2} + \frac{(1 - S_s^2)(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.118)$$

 **$\mathfrak{D}_j - S_s^2$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the order parameter  $S_s^2$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial S_s^2} = \frac{2}{5} S_f^2 \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i)\tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.119)$$

 **$\mathfrak{D}_j - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{D}_j$  and the correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{D}_j \cdot \partial \tau_f} = \frac{2}{5} (1 - S_f^2) \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{D}_j} \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.120)$$

**$\mathfrak{O}_j - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the orientational parameter  $\mathfrak{O}_j$  and the correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial \mathfrak{O}_j \cdot \partial \tau_s} = \frac{2}{5} S_f^2 (1 - S_s^2) \sum_{i=-k}^k \frac{\partial c_i}{\partial \mathfrak{O}_j} \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.121)$$

 **$S_f^2 - S_f^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  twice is

$$\frac{\partial^2 J(\omega)}{(\partial S_f^2)^2} = 0. \quad (15.122)$$

 **$S_f^2 - S_s^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameters  $S_f^2$  and  $S_s^2$  is

$$\frac{\partial^2 J(\omega)}{\partial S_f^2 \cdot \partial S_s^2} = \frac{2}{5} \sum_{i=-k}^k c_i \tau_i \left( \frac{1}{1 + (\omega \tau_i)^2} - \frac{(\tau_s + \tau_i) \tau_s}{(\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2} \right). \quad (15.123)$$

 **$S_f^2 - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  and correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial S_f^2 \cdot \partial \tau_f} = -\frac{2}{5} \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^2 - (\omega \tau_f \tau_i)^2}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^2}. \quad (15.124)$$

 **$S_f^2 - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_f^2$  and correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial S_f^2 \cdot \partial \tau_s} = \frac{2}{5} (1 - S_s^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.125)$$

 **$S_s^2 - S_s^2$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_s^2$  twice is

$$\frac{\partial^2 J(\omega)}{(\partial S_s^2)^2} = 0. \quad (15.126)$$

**$S_s^2 - \tau_f$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_s^2$  and correlation time  $\tau_f$  is

$$\frac{\partial^2 J(\omega)}{\partial S_s^2 \cdot \partial \tau_f} = 0. \quad (15.127)$$

 **$S_s^2 - \tau_s$  partial derivative**

The second partial derivative of (15.63) with respect to the order parameter  $S_s^2$  and correlation time  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial S_s^2 \cdot \partial \tau_s} = -\frac{2}{5} S_f^2 \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^2 - (\omega \tau_s \tau_i)^2}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^2}. \quad (15.128)$$

 **$\tau_f - \tau_f$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation time  $\tau_f$  twice is

$$\frac{\partial^2 J(\omega)}{\partial \tau_f^2} = -\frac{4}{5} (1 - S_f^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_f + \tau_i)^3 + 3\omega^2 \tau_i^3 \tau_f (\tau_f + \tau_i) - (\omega \tau_i)^4 \tau_f^3}{((\tau_f + \tau_i)^2 + (\omega \tau_f \tau_i)^2)^3} \quad (15.129)$$

 **$\tau_f - \tau_s$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation times  $\tau_f$  and  $\tau_s$  is

$$\frac{\partial^2 J(\omega)}{\partial \tau_f \cdot \partial \tau_s} = 0. \quad (15.130)$$

 **$\tau_s - \tau_s$  partial derivative**

The second partial derivative of (15.62) with respect to the correlation time  $\tau_s$  twice is

$$\frac{\partial^2 J(\omega)}{\partial \tau_s^2} = -\frac{4}{5} S_f^2 (1 - S_s^2) \sum_{i=-k}^k c_i \tau_i^2 \frac{(\tau_s + \tau_i)^3 + 3\omega^2 \tau_i^3 \tau_s (\tau_s + \tau_i) - (\omega \tau_i)^4 \tau_s^3}{((\tau_s + \tau_i)^2 + (\omega \tau_s \tau_i)^2)^3} \quad (15.131)$$

## 15.9 Ellipsoidal diffusion tensor

### 15.9.1 The diffusion equation of the ellipsoid

The correlation function of the Brownian rotational diffusion of an ellipsoid is

$$C_O(\tau) = \frac{1}{5} \sum_{i=-2}^2 c_i e^{-\frac{\tau}{\tau_i}}. \quad (15.132)$$

where  $c_i$  are the weights of the five exponential terms which are dependent on the orientation of the XH bond vector and  $\tau_i$  are the correlation times of the five exponential terms.

### 15.9.2 The weights of the ellipsoid

#### Definitions

The three direction cosines defining the XH bond vector within the diffusion frame are

$$\delta_x = \widehat{XH} \cdot \widehat{\mathfrak{D}}_x, \quad (15.133a)$$

$$\delta_y = \widehat{XH} \cdot \widehat{\mathfrak{D}}_y, \quad (15.133b)$$

$$\delta_z = \widehat{XH} \cdot \widehat{\mathfrak{D}}_z. \quad (15.133c)$$

Let the set of geometric parameters be

$$\mathfrak{G} = \{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r\}, \quad (15.134)$$

and the set of orientational parameters be the Euler angles

$$\mathfrak{D} = \{\alpha, \beta, \gamma\}. \quad (15.135)$$

#### The weights

The five weights  $c_i$  in the correlation function of the Brownian rotational diffusion of an ellipsoid (15.132) are

$$c_{-2} = \frac{1}{4}(d - e), \quad (15.136a)$$

$$c_{-1} = 3\delta_y^2 \delta_z^2, \quad (15.136b)$$

$$c_0 = 3\delta_x^2 \delta_z^2, \quad (15.136c)$$

$$c_1 = 3\delta_x^2 \delta_y^2, \quad (15.136d)$$

$$c_2 = \frac{1}{4}(d + e), \quad (15.136e)$$

where

$$d = 3(\delta_x^4 + \delta_y^4 + \delta_z^4) - 1, \quad (15.137)$$

$$e = \frac{1}{\mathfrak{R}} \left[ (1 + 3\mathfrak{D}_r) (\delta_x^4 + 2\delta_y^2\delta_z^2) + (1 - 3\mathfrak{D}_r) (\delta_y^4 + 2\delta_x^2\delta_z^2) - 2(\delta_z^4 + 2\delta_x^2\delta_y^2) \right]. \quad (15.138)$$

The factor  $\mathfrak{R}$  is defined as

$$\mathfrak{R} = \sqrt{1 + 3\mathfrak{D}_r^2}. \quad (15.139)$$

### 15.9.3 The weight gradients of the ellipsoid

#### $\mathfrak{D}_i$ partial derivative

The partial derivatives with respect to the orientational parameter  $\mathfrak{D}_i$  are

$$\frac{\partial c_{-2}}{\partial \mathfrak{D}_i} = 3 \left( \delta_x^3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} + \delta_y^3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_z^3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \right) - \frac{\partial e}{\partial \mathfrak{D}_i}, \quad (15.140a)$$

$$\frac{\partial c_{-1}}{\partial \mathfrak{D}_i} = 6\delta_y\delta_z \left( \delta_y \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \right), \quad (15.140b)$$

$$\frac{\partial c_0}{\partial \mathfrak{D}_i} = 6\delta_x\delta_z \left( \delta_x \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right), \quad (15.140c)$$

$$\frac{\partial c_1}{\partial \mathfrak{D}_i} = 6\delta_x\delta_y \left( \delta_x \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_y \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right), \quad (15.140d)$$

$$\frac{\partial c_2}{\partial \mathfrak{D}_i} = 3 \left( \delta_x^3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} + \delta_y^3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_z^3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \right) + \frac{\partial e}{\partial \mathfrak{D}_i}, \quad (15.140e)$$

where

$$\begin{aligned} \frac{\partial e}{\partial \mathfrak{D}_i} = \frac{1}{\mathfrak{R}} & \left[ (1 + 3\mathfrak{D}_r) \left( \delta_x^3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} + \delta_y\delta_z \left( \delta_y \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \right) \right) \right. \\ & + (1 - 3\mathfrak{D}_r) \left( \delta_y^3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_x\delta_z \left( \delta_x \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right) \right) \\ & \left. - 2 \left( \delta_z^3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_x\delta_y \left( \delta_x \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_y \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right) \right) \right]. \end{aligned} \quad (15.141)$$

**$\tau_m$  partial derivative**

The partial derivatives with respect to the  $\tau_m$  geometric parameter are

$$\frac{\partial c_{-2}}{\partial \tau_m} = 0, \quad (15.142a)$$

$$\frac{\partial c_{-1}}{\partial \tau_m} = 0, \quad (15.142b)$$

$$\frac{\partial c_0}{\partial \tau_m} = 0, \quad (15.142c)$$

$$\frac{\partial c_1}{\partial \tau_m} = 0, \quad (15.142d)$$

$$\frac{\partial c_2}{\partial \tau_m} = 0. \quad (15.142e)$$

 **$\mathfrak{D}_a$  partial derivative**

The partial derivatives with respect to the  $\mathfrak{D}_a$  geometric parameter are

$$\frac{\partial c_{-2}}{\partial \mathfrak{D}_a} = 0, \quad (15.143a)$$

$$\frac{\partial c_{-1}}{\partial \mathfrak{D}_a} = 0, \quad (15.143b)$$

$$\frac{\partial c_0}{\partial \mathfrak{D}_a} = 0, \quad (15.143c)$$

$$\frac{\partial c_1}{\partial \mathfrak{D}_a} = 0, \quad (15.143d)$$

$$\frac{\partial c_2}{\partial \mathfrak{D}_a} = 0. \quad (15.143e)$$

 **$\mathfrak{D}_r$  partial derivative**

The partial derivatives with respect to the  $\mathfrak{D}_r$  geometric parameter are

$$\frac{\partial c_{-2}}{\partial \mathfrak{D}_r} = -\frac{3}{4} \frac{\partial e}{\partial \mathfrak{D}_r}, \quad (15.144a)$$

$$\frac{\partial c_{-1}}{\partial \mathfrak{D}_r} = 0, \quad (15.144b)$$

$$\frac{\partial c_0}{\partial \mathfrak{D}_r} = 0, \quad (15.144c)$$

$$\frac{\partial c_1}{\partial \mathfrak{D}_r} = 0, \quad (15.144d)$$

$$\frac{\partial c_2}{\partial \mathfrak{D}_r} = \frac{3}{4} \frac{\partial e}{\partial \mathfrak{D}_r}, \quad (15.144e)$$

where

$$\frac{\partial e}{\partial \mathfrak{D}_r} = \frac{1}{\mathfrak{R}^3} \left[ (1 - \mathfrak{D}_r) (\delta_x^4 + 2\delta_y^2 \delta_z^2) - (1 + \mathfrak{D}_r) (\delta_y^4 + 2\delta_x^2 \delta_z^2) + 2\mathfrak{D}_r (\delta_z^4 + 2\delta_x^2 \delta_y^2) \right]. \quad (15.145)$$

### 15.9.4 The weight Hessians of the ellipsoid

#### $\mathfrak{D}_i - \mathfrak{D}_j$ partial derivative

The second partial derivatives with respect to the orientational parameters  $\mathfrak{D}_i$  and  $\mathfrak{D}_j$  are

$$\begin{aligned} \frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = & 3 \left( \delta_x^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \right. \\ & + \delta_y^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\ & \left. + \delta_z^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \right) - \frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j}, \end{aligned} \quad (15.146a)$$

$$\begin{aligned} \frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = & 6\delta_y^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \\ & + 12\delta_y \delta_z \left( \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\ & + 6\delta_z^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right), \end{aligned} \quad (15.146b)$$

$$\begin{aligned} \frac{\partial^2 c_0}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = & 6\delta_x^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \\ & + 12\delta_x \delta_z \left( \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \\ & + 6\delta_z^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right), \end{aligned} \quad (15.146c)$$

$$\begin{aligned} \frac{\partial^2 c_1}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = & 6\delta_x^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\ & + 12\delta_x \delta_y \left( \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \\ & + 6\delta_y^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right), \end{aligned} \quad (15.146d)$$

$$\begin{aligned} \frac{\partial^2 c_2}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = & 3 \left( \delta_x^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \right. \\ & + \delta_y^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\ & \left. + \delta_z^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \right) + \frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j}, \end{aligned} \quad (15.146e)$$

where

$$\begin{aligned}
\frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = \frac{1}{\Re} & \left[ (1 + 3\mathfrak{D}_r) \left( \delta_x^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \right. \right. \\
& + \delta_y^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \\
& + \delta_z^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\
& + 2\delta_y \delta_z \left( \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \Big) \\
& + (1 - 3\mathfrak{D}_r) \left( \delta_y^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \right. \\
& + \delta_x^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \\
& + \delta_z^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \\
& + 2\delta_x \delta_z \left( \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \Big) \\
& - 2 \left( \delta_z^2 \left( \delta_z \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + 3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} \right) \right. \\
& + \delta_x^2 \left( \delta_y \frac{\partial^2 \delta_y}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} \right) \\
& + \delta_y^2 \left( \delta_x \frac{\partial^2 \delta_x}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} + \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \\
& \left. \left. + 2\delta_x \delta_y \left( \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_y}{\partial \mathfrak{D}_j} + \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_x}{\partial \mathfrak{D}_j} \right) \right) \right]. \tag{15.147}
\end{aligned}$$

### $\mathfrak{D}_i - \tau_m$ partial derivative

The second partial derivatives with respect to the orientational parameter  $\mathfrak{D}_i$  and the geometric parameter  $\tau_m$  are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_i \cdot \partial \tau_m} = 0, \tag{15.148a}$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_i \cdot \partial \tau_m} = 0, \tag{15.148b}$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_i \cdot \partial \tau_m} = 0, \tag{15.148c}$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_i \cdot \partial \tau_m} = 0, \tag{15.148d}$$



$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_i \cdot \partial \tau_m} = 0. \quad (15.148e)$$

### $\mathfrak{D}_i - \mathfrak{D}_a$ partial derivative

The second partial derivatives with respect to the orientational parameter  $\mathfrak{D}_i$  and the geometric parameter  $\mathfrak{D}_a$  are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_a} = 0, \quad (15.149a)$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_a} = 0, \quad (15.149b)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_a} = 0, \quad (15.149c)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_a} = 0, \quad (15.149d)$$

$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_a} = 0. \quad (15.149e)$$

### $\mathfrak{D}_i - \mathfrak{D}_r$ partial derivative

The second partial derivatives with respect to the orientational parameter  $\mathfrak{D}_i$  and the geometric parameter  $\mathfrak{D}_r$  are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = -3 \frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r}, \quad (15.150a)$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = 0, \quad (15.150b)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = 0, \quad (15.150c)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = 0, \quad (15.150d)$$

$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = 3 \frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r}, \quad (15.150e)$$

where

$$\begin{aligned} \frac{\partial^2 e}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_r} = \frac{1}{\mathfrak{R}^3} & \left[ (1 - \mathfrak{D}_r) \left( \delta_x^3 \frac{\partial \delta_x}{\partial \mathfrak{D}_i} + \delta_y \delta_z \left( \delta_y \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_y}{\partial \mathfrak{D}_i} \right) \right) \right. \\ & - (1 + \mathfrak{D}_r) \left( \delta_y^3 \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_x \delta_z \left( \delta_x \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_z \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right) \right) \\ & \left. + 2\mathfrak{D}_r \left( \delta_z^3 \frac{\partial \delta_z}{\partial \mathfrak{D}_i} + \delta_x \delta_y \left( \delta_x \frac{\partial \delta_y}{\partial \mathfrak{D}_i} + \delta_y \frac{\partial \delta_x}{\partial \mathfrak{D}_i} \right) \right) \right]. \quad (15.151) \end{aligned}$$

**$\tau_m - \tau_m$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\tau_m$  twice are

$$\frac{\partial^2 c_{-2}}{\partial \tau_m^2} = 0, \quad (15.152a)$$

$$\frac{\partial^2 c_{-1}}{\partial \tau_m^2} = 0, \quad (15.152b)$$

$$\frac{\partial^2 c_0}{\partial \tau_m^2} = 0, \quad (15.152c)$$

$$\frac{\partial^2 c_1}{\partial \tau_m^2} = 0, \quad (15.152d)$$

$$\frac{\partial^2 c_2}{\partial \tau_m^2} = 0. \quad (15.152e)$$

 **$\tau_m - \mathfrak{D}_a$  partial derivative**

The second partial derivatives with respect to the geometric parameters  $\tau_m$  and  $\mathfrak{D}_a$  are

$$\frac{\partial^2 c_{-2}}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 0, \quad (15.153a)$$

$$\frac{\partial^2 c_{-1}}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 0, \quad (15.153b)$$

$$\frac{\partial^2 c_0}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 0, \quad (15.153c)$$

$$\frac{\partial^2 c_1}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 0, \quad (15.153d)$$

$$\frac{\partial^2 c_2}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 0. \quad (15.153e)$$

 **$\tau_m - \mathfrak{D}_r$  partial derivative**

The second partial derivatives with respect to the geometric parameters  $\tau_m$  and  $\mathfrak{D}_r$  are

$$\frac{\partial^2 c_{-2}}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0, \quad (15.154a)$$

$$\frac{\partial^2 c_{-1}}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0, \quad (15.154b)$$

$$\frac{\partial^2 c_0}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0, \quad (15.154c)$$

$$\frac{\partial^2 c_1}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0, \quad (15.154d)$$

$$\frac{\partial^2 c_2}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0. \quad (15.154e)$$

**$\mathfrak{D}_a - \mathfrak{D}_a$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\mathfrak{D}_a$  twice are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_a^2} = 0, \quad (15.155a)$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_a^2} = 0, \quad (15.155b)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_a^2} = 0, \quad (15.155c)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_a^2} = 0, \quad (15.155d)$$

$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_a^2} = 0. \quad (15.155e)$$

 **$\mathfrak{D}_a - \mathfrak{D}_r$  partial derivative**

The second partial derivatives with respect to the geometric parameters  $\mathfrak{D}_a$  and  $\mathfrak{D}_r$  are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0, \quad (15.156a)$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0, \quad (15.156b)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0, \quad (15.156c)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0, \quad (15.156d)$$

$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0. \quad (15.156e)$$

 **$\mathfrak{D}_r - \mathfrak{D}_r$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\mathfrak{D}_r$  twice are

$$\frac{\partial^2 c_{-2}}{\partial \mathfrak{D}_r^2} = -\frac{3}{4} \frac{\partial^2 e}{\partial \mathfrak{D}_r^2}, \quad (15.157a)$$

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_r^2} = 0, \quad (15.157b)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_r^2} = 0, \quad (15.157c)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_r^2} = 0, \quad (15.157d)$$

$$\frac{\partial^2 c_2}{\partial \mathfrak{D}_r^2} = \frac{3}{4} \frac{\partial^2 e}{\partial \mathfrak{D}_r^2}, \quad (15.157e)$$

where

$$\begin{aligned} \frac{\partial^2 e}{\partial \mathfrak{D}_r^2} = \frac{1}{\mathfrak{R}^5} & \left[ (6\mathfrak{D}_r^2 - 9\mathfrak{D}_r - 1) (\delta_x^4 + 2\delta_y^2 \delta_z^2) \right. \\ & + (6\mathfrak{D}_r^2 + 9\mathfrak{D}_r - 1) (\delta_y^4 + 2\delta_x^2 \delta_z^2) \\ & \left. - 2(6\mathfrak{D}_r^2 - 1) (\delta_z^4 + 2\delta_x^2 \delta_y^2) \right]. \end{aligned} \quad (15.158)$$

### 15.9.5 The correlation times of the ellipsoid

The five correlation times  $\tau_i$  in the correlation function of the Brownian rotational diffusion of an ellipsoid (15.132) on page 346 are

$$\tau_{-2} = (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-1}, \quad (15.159a)$$

$$\tau_{-1} = (6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-1}, \quad (15.159b)$$

$$\tau_0 = (6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-1}, \quad (15.159c)$$

$$\tau_1 = (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-1}, \quad (15.159d)$$

$$\tau_2 = (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-1}, \quad (15.159e)$$

where  $\mathfrak{R}$  is defined in Equation (15.139) on page 347.

### 15.9.6 The correlation time gradients of the ellipsoid

#### $\tau_m$ partial derivative

The partial derivatives with respect to the geometric parameter  $\tau_m$  are

$$\frac{\partial \tau_{-2}}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-2}, \quad (15.160a)$$

$$\frac{\partial \tau_{-1}}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-2}, \quad (15.160b)$$

$$\frac{\partial \tau_0}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-2}, \quad (15.160c)$$

$$\frac{\partial \tau_1}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}, \quad (15.160d)$$

$$\frac{\partial \tau_2}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-2}. \quad (15.160e)$$

#### $\mathfrak{D}_a$ partial derivative

The partial derivatives with respect to the geometric parameter  $\mathfrak{D}_a$  are

$$\frac{\partial \tau_{-2}}{\partial \mathfrak{D}_a} = 2\mathfrak{R}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-2}, \quad (15.161a)$$

$$\frac{\partial \tau_{-1}}{\partial \mathfrak{D}_a} = (1 + 3\mathfrak{D}_r)(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-2}, \quad (15.161b)$$

$$\frac{\partial \tau_0}{\partial \mathfrak{D}_a} = (1 - 3\mathfrak{D}_r)(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-2}, \quad (15.161c)$$

$$\frac{\partial \tau_1}{\partial \mathfrak{D}_a} = -2(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}, \quad (15.161d)$$

$$\frac{\partial \tau_2}{\partial \mathfrak{D}_a} = -2\mathfrak{R}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-2}. \quad (15.161e)$$

**$\mathfrak{D}_r$  partial derivative**

The partial derivatives with respect to the geometric parameter  $\mathfrak{D}_r$  are

$$\frac{\partial \tau_{-2}}{\partial \mathfrak{D}_r} = 6 \frac{\mathfrak{D}_a \mathfrak{D}_r}{\mathfrak{K}} (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a \mathfrak{K})^{-2}, \quad (15.162a)$$

$$\frac{\partial \tau_{-1}}{\partial \mathfrak{D}_r} = 3\mathfrak{D}_a (6\mathfrak{D}_{iso} - \mathfrak{D}_a (1 + 3\mathfrak{D}_r))^{-2}, \quad (15.162b)$$

$$\frac{\partial \tau_0}{\partial \mathfrak{D}_r} = -3\mathfrak{D}_a (6\mathfrak{D}_{iso} - \mathfrak{D}_a (1 - 3\mathfrak{D}_r))^{-2}, \quad (15.162c)$$

$$\frac{\partial \tau_1}{\partial \mathfrak{D}_r} = 0, \quad (15.162d)$$

$$\frac{\partial \tau_2}{\partial \mathfrak{D}_r} = -6 \frac{\mathfrak{D}_a \mathfrak{D}_r}{\mathfrak{K}} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a \mathfrak{K})^{-2}. \quad (15.162e)$$

### 15.9.7 The correlation time Hessians of the ellipsoid

#### $\tau_m - \tau_m$ partial derivative

The second partial derivatives with respect to the geometric parameter  $\tau_m$  twice are

$$\frac{\partial^2 \tau_{-2}}{\partial \tau_m^2} = 2\tau_m^{-4}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-3} - 2\tau_m^{-3}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-2}, \quad (15.163a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \tau_m^2} = 2\tau_m^{-4}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3} - 2\tau_m^{-3}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-2}, \quad (15.163b)$$

$$\frac{\partial^2 \tau_0}{\partial \tau_m^2} = 2\tau_m^{-4}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3} - 2\tau_m^{-3}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-2}, \quad (15.163c)$$

$$\frac{\partial^2 \tau_1}{\partial \tau_m^2} = 2\tau_m^{-4}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3} - 2\tau_m^{-3}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}, \quad (15.163d)$$

$$\frac{\partial^2 \tau_2}{\partial \tau_m^2} = 2\tau_m^{-4}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-3} - 2\tau_m^{-3}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-2}. \quad (15.163e)$$

#### $\tau_m - \mathfrak{D}_a$ partial derivative

The second partial derivatives with respect to the geometric parameters  $\tau_m$  and  $\mathfrak{D}_a$  are

$$\frac{\partial^2 \tau_{-2}}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 4\mathfrak{R}\tau_m^{-2}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-3}, \quad (15.164a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 2(1 + 3\mathfrak{D}_r)\tau_m^{-2}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3}, \quad (15.164b)$$

$$\frac{\partial^2 \tau_0}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 2(1 - 3\mathfrak{D}_r)\tau_m^{-2}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3}, \quad (15.164c)$$

$$\frac{\partial^2 \tau_1}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = -4\tau_m^{-2}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3}, \quad (15.164d)$$

$$\frac{\partial^2 \tau_2}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = -4\mathfrak{R}\tau_m^{-2}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-3}. \quad (15.164e)$$

#### $\tau_m - \mathfrak{D}_r$ partial derivative

The second partial derivatives with respect to the geometric parameters  $\tau_m$  and  $\mathfrak{D}_r$  are

$$\frac{\partial^2 \tau_{-2}}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 12\frac{\mathfrak{D}_a\mathfrak{D}_r}{\mathfrak{R}}\tau_m^{-2}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{R})^{-3}, \quad (15.165a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 6\mathfrak{D}_a\tau_m^{-2}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3}, \quad (15.165b)$$

$$\frac{\partial^2 \tau_0}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = -6\mathfrak{D}_a\tau_m^{-2}(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3}, \quad (15.165c)$$

$$\frac{\partial^2 \tau_1}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = 0, \quad (15.165d)$$

$$\frac{\partial^2 \tau_2}{\partial \tau_m \cdot \partial \mathfrak{D}_r} = -12\frac{\mathfrak{D}_a\mathfrak{D}_r}{\mathfrak{R}}\tau_m^{-2}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{R})^{-3}. \quad (15.165e)$$

**$\mathfrak{D}_a - \mathfrak{D}_a$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\mathfrak{D}_a$  twice are

$$\frac{\partial^2 \tau_{-2}}{\partial \mathfrak{D}_a^2} = 8\mathfrak{K}^2(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-3}, \quad (15.166a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \mathfrak{D}_a^2} = 2(1 + 3\mathfrak{D}_r)^2(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3}, \quad (15.166b)$$

$$\frac{\partial^2 \tau_0}{\partial \mathfrak{D}_a^2} = 2(1 - 3\mathfrak{D}_r)^2(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3}, \quad (15.166c)$$

$$\frac{\partial^2 \tau_1}{\partial \mathfrak{D}_a^2} = 8(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3}, \quad (15.166d)$$

$$\frac{\partial^2 \tau_2}{\partial \mathfrak{D}_a^2} = 8\mathfrak{K}^2(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{K})^{-3}. \quad (15.166e)$$

 **$\mathfrak{D}_a - \mathfrak{D}_r$  partial derivative**

The second partial derivatives with respect to the geometric parameters  $\mathfrak{D}_a$  and  $\mathfrak{D}_r$  are

$$\frac{\partial^2 \tau_{-2}}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 24\mathfrak{D}_a\mathfrak{D}_r(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-3} + 6\frac{\mathfrak{D}_r}{\mathfrak{K}}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-2}, \quad (15.167a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 6\mathfrak{D}_a(1 + 3\mathfrak{D}_r)(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3} + 3(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-2}, \quad (15.167b)$$

$$\frac{\partial^2 \tau_0}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = -6\mathfrak{D}_a(1 - 3\mathfrak{D}_r)(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3} - 3(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-2}, \quad (15.167c)$$

$$\frac{\partial^2 \tau_1}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 0, \quad (15.167d)$$

$$\frac{\partial^2 \tau_2}{\partial \mathfrak{D}_a \cdot \partial \mathfrak{D}_r} = 24\mathfrak{D}_a\mathfrak{D}_r(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{K})^{-3} - 6\frac{\mathfrak{D}_r}{\mathfrak{K}}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{K})^{-2}. \quad (15.167e)$$

 **$\mathfrak{D}_r - \mathfrak{D}_r$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\mathfrak{D}_r$  twice are

$$\frac{\partial^2 \tau_{-2}}{\partial \mathfrak{D}_r^2} = 72 \left( \frac{\mathfrak{D}_a\mathfrak{D}_r}{\mathfrak{K}} \right)^2 (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-3} + 6\frac{\mathfrak{D}_a}{\mathfrak{K}^3}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-2}, \quad (15.168a)$$

$$\frac{\partial^2 \tau_{-1}}{\partial \mathfrak{D}_r^2} = 18\mathfrak{D}_a^2(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 + 3\mathfrak{D}_r))^{-3}, \quad (15.168b)$$

$$\frac{\partial^2 \tau_0}{\partial \mathfrak{D}_r^2} = 18\mathfrak{D}_a^2(6\mathfrak{D}_{iso} - \mathfrak{D}_a(1 - 3\mathfrak{D}_r))^{-3}, \quad (15.168c)$$

$$\frac{\partial^2 \tau_1}{\partial \mathfrak{D}_r^2} = 0, \quad (15.168d)$$

$$\frac{\partial^2 \tau_2}{\partial \mathfrak{D}_r^2} = 72 \left( \frac{\mathfrak{D}_a\mathfrak{D}_r}{\mathfrak{K}} \right)^2 (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a\mathfrak{K})^{-3} - 6\frac{\mathfrak{D}_a}{\mathfrak{K}^3}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a\mathfrak{K})^{-2}. \quad (15.168e)$$



## 15.10 Spheroidal diffusion tensor

### 15.10.1 The diffusion equation of the spheroid

The correlation function of the Brownian rotational diffusion of a spheroid is

$$C_O(\tau) = \frac{1}{5} \sum_{i=-1}^1 c_i e^{-\frac{\tau}{\tau_i}}. \quad (15.169)$$

where  $c_i$  are the weights of the three exponential terms which are dependent on the orientation of the XH bond vector and  $\tau_i$  are the correlation times of the three exponential terms.

### 15.10.2 The weights of the spheroid

#### Definitions

The direction cosine defining the XH bond vector within the spheroidal diffusion frame is

$$\delta_z = \widehat{XH} \cdot \widehat{\mathfrak{D}}_z. \quad (15.170)$$

Let the set of geometric parameters be

$$\mathfrak{G} = \{\mathfrak{D}_{iso}, \mathfrak{D}_a\}, \quad (15.171)$$

and the set of orientational parameters be the spherical angles

$$\mathfrak{D} = \{\theta, \phi\}. \quad (15.172)$$

#### The weights

The three spheroid weights  $c_i$  in the correlation function of the Brownian rotational diffusion of a spheroid (15.169) are

$$c_{-1} = \frac{1}{4}(3\delta_z^2 - 1)^2, \quad (15.173a)$$

$$c_0 = 3\delta_z^2(1 - \delta_z^2), \quad (15.173b)$$

$$c_1 = \frac{3}{4}(\delta_z^2 - 1)^2. \quad (15.173c)$$

### 15.10.3 The weight gradients of the spheroid

#### $\mathfrak{D}_i$ partial derivative

The partial derivatives with respect to the orientational parameter  $\mathfrak{D}_i$  are

$$\frac{\partial c_{-1}}{\partial \mathfrak{D}_i} = 3\delta_z(3\delta_z^2 - 1)\frac{\partial \delta_z}{\partial \mathfrak{D}_i}, \quad (15.174a)$$

$$\frac{\partial c_0}{\partial \mathfrak{D}_i} = 6\delta_z(1 - 2\delta_z^2)\frac{\partial \delta_z}{\partial \mathfrak{D}_i}, \quad (15.174b)$$

$$\frac{\partial c_1}{\partial \mathfrak{D}_i} = 3\delta_z(\delta_z^2 - 1)\frac{\partial \delta_z}{\partial \mathfrak{D}_i}. \quad (15.174c)$$

### 15.10.4 The weight Hessians of the spheroid

#### $\mathfrak{D}_i - \mathfrak{D}_j$ partial derivative

The second partial derivatives with respect to the orientational parameters  $\mathfrak{D}_i$  and  $\mathfrak{D}_j$  are

$$\frac{\partial^2 c_{-1}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = 3 \left( (9\delta_z^2 - 1) \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \delta_z(3\delta_z^2 - 1) \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} \right), \quad (15.175a)$$

$$\frac{\partial^2 c_0}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = 6 \left( (1 - 6\delta_z^2) \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \delta_z(1 - 2\delta_z^2) \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} \right), \quad (15.175b)$$

$$\frac{\partial^2 c_1}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = 3 \left( (3\delta_z^2 - 1) \frac{\partial \delta_z}{\partial \mathfrak{D}_i} \cdot \frac{\partial \delta_z}{\partial \mathfrak{D}_j} + \delta_z(\delta_z^2 - 1) \frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} \right). \quad (15.175c)$$

### 15.10.5 The correlation times of the spheroid

The three spheroid correlation times  $\tau_i$  in the correlation function of the Brownian rotational diffusion of a spheroid (15.169) are

$$\tau_{-1} = (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-1}, \quad (15.176a)$$

$$\tau_0 = (6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-1}, \quad (15.176b)$$

$$\tau_1 = (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-1}. \quad (15.176c)$$

### 15.10.6 The correlation time gradients of the spheroid

#### $\tau_m$ partial derivative

The partial derivatives with respect to the geometric parameter  $\tau_m$  are

$$\frac{\partial \tau_{-1}}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-2}, \quad (15.177a)$$

$$\frac{\partial \tau_0}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-2}, \quad (15.177b)$$

$$\frac{\partial \tau_1}{\partial \tau_m} = \tau_m^{-2} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}. \quad (15.177c)$$

#### $\mathfrak{D}_a$ partial derivative

The partial derivatives with respect to the geometric parameter  $\mathfrak{D}_a$  are

$$\frac{\partial \tau_{-1}}{\partial \mathfrak{D}_a} = 2(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-2}, \quad (15.178a)$$

$$\frac{\partial \tau_0}{\partial \mathfrak{D}_a} = (6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-2}, \quad (15.178b)$$

$$\frac{\partial \tau_1}{\partial \mathfrak{D}_a} = -2(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}. \quad (15.178c)$$

### 15.10.7 The correlation time Hessians of the spheroid

#### $\tau_m - \tau_m$ partial derivative

The second partial derivatives with respect to the geometric parameter  $\tau_m$  twice are

$$\frac{\partial^2 \tau_{-1}}{\partial \tau_m^2} = 2\tau_m^{-4} (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-3} - 2\tau_m^{-3} (6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-2}, \quad (15.179a)$$

$$\frac{\partial^2 \tau_0}{\partial \tau_m^2} = 2\tau_m^{-4} (6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-3} - 2\tau_m^{-3} (6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-2}, \quad (15.179b)$$

$$\frac{\partial^2 \tau_1}{\partial \tau_m^2} = 2\tau_m^{-4} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3} - 2\tau_m^{-3} (6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-2}. \quad (15.179c)$$

**$\tau_m - \mathfrak{D}_a$  partial derivative**

The second partial derivatives with respect to the geometric parameters  $\tau_m$  and  $\mathfrak{D}_a$  are

$$\frac{\partial^2 \tau_{-1}}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 4\tau_m^{-2}(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-3}, \quad (15.180a)$$

$$\frac{\partial^2 \tau_0}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = 2\tau_m^{-2}(6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-3}, \quad (15.180b)$$

$$\frac{\partial^2 \tau_1}{\partial \tau_m \cdot \partial \mathfrak{D}_a} = -4\tau_m^{-2}(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3}. \quad (15.180c)$$

 **$\mathfrak{D}_a - \mathfrak{D}_a$  partial derivative**

The second partial derivatives with respect to the geometric parameter  $\mathfrak{D}_a$  twice are

$$\frac{\partial^2 \tau_{-1}}{\partial \mathfrak{D}_a^2} = 8(6\mathfrak{D}_{iso} - 2\mathfrak{D}_a)^{-3}, \quad (15.181a)$$

$$\frac{\partial^2 \tau_0}{\partial \mathfrak{D}_a^2} = 2(6\mathfrak{D}_{iso} - \mathfrak{D}_a)^{-3}, \quad (15.181b)$$

$$\frac{\partial^2 \tau_1}{\partial \mathfrak{D}_a^2} = 8(6\mathfrak{D}_{iso} + 2\mathfrak{D}_a)^{-3}. \quad (15.181c)$$

## 15.11 Spherical diffusion tensor

### 15.11.1 The diffusion equation of the sphere

The correlation function of the Brownian rotational diffusion of a sphere is

$$C_O(\tau) = \frac{1}{5} e^{-\frac{\tau}{\tau_m}}, \quad (15.182)$$

$$= \frac{1}{5} \sum_{i=0}^0 c_i e^{-\frac{\tau}{\tau_i}}. \quad (15.183)$$

where  $c_i$  is the weight of the single exponential term and  $\tau_i$  is the correlation time of the single exponential term.

### 15.11.2 The weight of the sphere

#### Definitions

The entire diffusion parameter set consists of a single geometric parameter and is

$$\mathfrak{D} = \{\tau_m\}. \quad (15.184)$$

#### Summation terms

The summation indices of the correlation function of the Brownian rotational diffusion of a sphere (15.169) range from  $k = 0$  to  $k = 0$  therefore

$$i \in \{0\}. \quad (15.185)$$

#### The weights

The single weight  $c_i$  in the correlation function of the Brownian rotational diffusion of a sphere (15.169) is

$$c_0 = 1. \quad (15.186)$$

### 15.11.3 The weight gradient of the sphere

#### $\tau_m$ partial derivative

The partial derivative with respect to the geometric parameter  $\tau_m$  is

$$\frac{\partial c_0}{\partial \tau_m} = 0. \quad (15.187)$$

#### 15.11.4 The weight Hessian of the sphere

$\tau_m - \tau_m$  **partial derivative**

The second partial derivatives with respect to the geometric parameter  $\tau_m$  twice is

$$\frac{\partial^2 c_0}{\partial \tau_m^2} = 0. \quad (15.188)$$

#### 15.11.5 The correlation time of the sphere

The single correlation time  $\tau_i$  of the correlation function of the Brownian rotational diffusion of a sphere (15.169) is

$$\tau_0 = \tau_m. \quad (15.189)$$

#### 15.11.6 The correlation time gradient of the sphere

$\tau_m$  **partial derivative**

The partial derivative with respect to the geometric parameter  $\tau_m$  is

$$\frac{\partial \tau_0}{\partial \tau_m} = 1. \quad (15.190)$$

#### 15.11.7 The correlation time Hessian of the sphere

$\tau_m - \tau_m$  **partial derivative**

The second partial derivative with respect to the geometric parameter  $\tau_m$  twice is

$$\frac{\partial^2 \tau_0}{\partial \tau_m^2} = 0. \quad (15.191)$$

## 15.12 Ellipsoidal dot product derivatives

### 15.12.1 The dot product of the ellipsoid

The dot product is defined as

$$\delta_i = \widehat{XH} \cdot \widehat{\mathfrak{D}}_i, \quad (15.192)$$

where  $i$  is one of  $\{x, y, z\}$ ,  $\widehat{XH}$  is a unit vector parallel to the XH bond vector, and  $\widehat{\mathfrak{D}}_i$  is one of the unit vectors defining the diffusion frame. The three diffusion frame unit vectors can be expressed using the Euler angles  $\alpha$ ,  $\beta$ , and  $\gamma$  as

$$\widehat{\mathfrak{D}}_x = \begin{pmatrix} -\sin \alpha \sin \gamma + \cos \alpha \cos \beta \cos \gamma \\ -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma \\ \cos \alpha \sin \beta \end{pmatrix}, \quad (15.193a)$$

$$\widehat{\mathfrak{D}}_y = \begin{pmatrix} \cos \alpha \sin \gamma + \sin \alpha \cos \beta \cos \gamma \\ \cos \alpha \cos \gamma - \sin \alpha \cos \beta \sin \gamma \\ \sin \alpha \sin \beta \end{pmatrix}, \quad (15.193b)$$

$$\widehat{\mathfrak{D}}_z = \begin{pmatrix} -\sin \beta \cos \gamma \\ \sin \beta \sin \gamma \\ \cos \beta \end{pmatrix}. \quad (15.193c)$$

### 15.12.2 The dot product gradient of the ellipsoid

The partial derivative of the dot product  $\delta_i$  with respect to the orientational parameter  $\mathfrak{D}_j$  is

$$\frac{\partial \delta_i}{\partial \mathfrak{D}_j} = \frac{\partial}{\partial \mathfrak{D}_j} (\widehat{XH} \cdot \widehat{\mathfrak{D}}_i) = \widehat{XH} \frac{\partial \widehat{\mathfrak{D}}_i}{\partial \mathfrak{D}_j} + \frac{\partial \widehat{XH}}{\partial \mathfrak{D}_j} \cdot \widehat{\mathfrak{D}}_i. \quad (15.194)$$

Because  $\widehat{XH}$  is constant and not dependent on the Euler angles its derivative is zero. Therefore

$$\frac{\partial \delta_i}{\partial \mathfrak{D}_j} = \widehat{XH} \frac{\partial \widehat{\mathfrak{D}}_i}{\partial \mathfrak{D}_j}. \quad (15.195)$$

#### The $\mathfrak{D}_x$ gradient

The partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_x$  with respect to the Euler angles are

$$\frac{\partial \widehat{\mathfrak{D}}_x}{\partial \alpha} = \begin{pmatrix} -\cos \alpha \sin \gamma - \sin \alpha \cos \beta \cos \gamma \\ -\cos \alpha \cos \gamma + \sin \alpha \cos \beta \sin \gamma \\ -\sin \alpha \sin \beta \end{pmatrix}, \quad (15.196a)$$

$$\frac{\partial \widehat{\mathfrak{D}}_x}{\partial \beta} = \begin{pmatrix} -\cos \alpha \sin \beta \cos \gamma \\ \cos \alpha \sin \beta \sin \gamma \\ \cos \alpha \cos \beta \end{pmatrix}, \quad (15.196b)$$

$$\frac{\partial \widehat{\mathfrak{D}}_x}{\partial \gamma} = \begin{pmatrix} -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma \\ \sin \alpha \sin \gamma - \cos \alpha \cos \beta \cos \gamma \\ 0 \end{pmatrix}. \quad (15.196c)$$

**The  $\mathfrak{D}_y$  gradient**

The partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_y$  with respect to the Euler angles are

$$\frac{\partial \widehat{\mathfrak{D}}_y}{\partial \alpha} = \begin{pmatrix} -\sin \alpha \sin \gamma + \cos \alpha \cos \beta \cos \gamma \\ -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma \\ \cos \alpha \sin \beta \end{pmatrix}, \quad (15.197a)$$

$$\frac{\partial \widehat{\mathfrak{D}}_y}{\partial \beta} = \begin{pmatrix} -\sin \alpha \sin \beta \cos \gamma \\ \sin \alpha \sin \beta \sin \gamma \\ \sin \alpha \cos \beta \end{pmatrix}, \quad (15.197b)$$

$$\frac{\partial \widehat{\mathfrak{D}}_y}{\partial \gamma} = \begin{pmatrix} \cos \alpha \cos \gamma - \sin \alpha \cos \beta \sin \gamma \\ -\cos \alpha \sin \gamma - \sin \alpha \cos \beta \cos \gamma \\ 0 \end{pmatrix}. \quad (15.197c)$$

**The  $\mathfrak{D}_z$  gradient**

The partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_z$  with respect to the Euler angles are

$$\frac{\partial \widehat{\mathfrak{D}}_z}{\partial \alpha} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \quad (15.198a)$$

$$\frac{\partial \widehat{\mathfrak{D}}_z}{\partial \beta} = \begin{pmatrix} -\cos \beta \cos \gamma \\ \cos \beta \sin \gamma \\ -\sin \beta \end{pmatrix}, \quad (15.198b)$$

$$\frac{\partial \widehat{\mathfrak{D}}_z}{\partial \gamma} = \begin{pmatrix} \sin \beta \sin \gamma \\ \sin \beta \cos \gamma \\ 0 \end{pmatrix}. \quad (15.198c)$$



### 15.12.3 The dot product Hessian of the ellipsoid

The second partial derivative of the dot product  $\delta_i$  with respect to the orientational parameters  $\mathfrak{D}_j$  and  $\mathfrak{D}_k$  is

$$\frac{\partial^2 \delta_i}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} = \frac{\partial^2}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k} (\widehat{XH} \cdot \widehat{\mathfrak{D}}_i) = \widehat{XH} \frac{\partial^2 \widehat{\mathfrak{D}}_i}{\partial \mathfrak{D}_j \cdot \partial \mathfrak{D}_k}. \quad (15.199)$$

#### The $\mathfrak{D}_x$ Hessian

The second partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_x$  with respect to the Euler angles are

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \alpha^2} = \begin{pmatrix} \sin \alpha \sin \gamma - \cos \alpha \cos \beta \cos \gamma \\ \sin \alpha \cos \gamma + \cos \alpha \cos \beta \sin \gamma \\ -\cos \alpha \sin \beta \end{pmatrix}, \quad (15.200a)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \alpha \cdot \partial \beta} = \begin{pmatrix} \sin \alpha \sin \beta \cos \gamma \\ -\sin \alpha \sin \beta \sin \gamma \\ -\sin \alpha \cos \beta \end{pmatrix}, \quad (15.200b)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \alpha \cdot \partial \gamma} = \begin{pmatrix} -\cos \alpha \cos \gamma + \sin \alpha \cos \beta \sin \gamma \\ \cos \alpha \sin \gamma + \sin \alpha \cos \beta \cos \gamma \\ 0 \end{pmatrix}, \quad (15.200c)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \beta^2} = \begin{pmatrix} -\cos \alpha \cos \beta \cos \gamma \\ \cos \alpha \cos \beta \sin \gamma \\ -\cos \alpha \sin \beta \end{pmatrix}, \quad (15.200d)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \beta \cdot \partial \gamma} = \begin{pmatrix} \cos \alpha \sin \beta \sin \gamma \\ \cos \alpha \sin \beta \cos \gamma \\ 0 \end{pmatrix}, \quad (15.200e)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_x}{\partial \gamma^2} = \begin{pmatrix} \sin \alpha \sin \gamma - \cos \alpha \cos \beta \cos \gamma \\ \sin \alpha \cos \gamma + \cos \alpha \cos \beta \sin \gamma \\ 0 \end{pmatrix}. \quad (15.200f)$$

#### The $\mathfrak{D}_y$ Hessian

The second partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_y$  with respect to the Euler angles are

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \alpha^2} = \begin{pmatrix} -\cos \alpha \sin \gamma - \sin \alpha \cos \beta \cos \gamma \\ -\cos \alpha \cos \gamma + \sin \alpha \cos \beta \sin \gamma \\ -\sin \alpha \sin \beta \end{pmatrix}, \quad (15.201a)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \alpha \cdot \partial \beta} = \begin{pmatrix} -\cos \alpha \sin \beta \cos \gamma \\ \cos \alpha \sin \beta \sin \gamma \\ \cos \alpha \cos \beta \end{pmatrix}, \quad (15.201b)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \alpha \cdot \partial \gamma} = \begin{pmatrix} -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma \\ \sin \alpha \sin \gamma - \cos \alpha \cos \beta \cos \gamma \\ 0 \end{pmatrix}, \quad (15.201c)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \beta^2} = \begin{pmatrix} -\sin \alpha \cos \beta \cos \gamma \\ \sin \alpha \cos \beta \sin \gamma \\ -\sin \alpha \sin \beta \end{pmatrix}, \quad (15.201d)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \beta \cdot \partial \gamma} = \begin{pmatrix} \sin \alpha \sin \beta \sin \gamma \\ \sin \alpha \sin \beta \cos \gamma \\ 0 \end{pmatrix}, \quad (15.201e)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_y}{\partial \gamma^2} = \begin{pmatrix} -\cos \alpha \sin \gamma - \sin \alpha \cos \beta \cos \gamma \\ -\cos \alpha \cos \gamma + \sin \alpha \cos \beta \sin \gamma \\ 0 \end{pmatrix}. \quad (15.201f)$$

### The $\mathfrak{D}_z$ Hessian

The second partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_z$  with respect to the Euler angles are

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \alpha^2} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \quad (15.202a)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \alpha \cdot \partial \beta} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \quad (15.202b)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \alpha \cdot \partial \gamma} = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \quad (15.202c)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \beta^2} = \begin{pmatrix} \sin \beta \cos \gamma \\ -\sin \beta \sin \gamma \\ -\cos \beta \end{pmatrix}, \quad (15.202d)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \beta \cdot \partial \gamma} = \begin{pmatrix} \cos \beta \sin \gamma \\ \cos \beta \cos \gamma \\ 0 \end{pmatrix}, \quad (15.202e)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}}_z}{\partial \gamma^2} = \begin{pmatrix} \sin \beta \cos \gamma \\ -\sin \beta \sin \gamma \\ 0 \end{pmatrix}. \quad (15.202f)$$

## 15.13 Spheroidal dot product derivatives

### 15.13.1 The dot product of the spheroid

The single dot product of the spheroid is defined as

$$\delta_z = \widehat{XH} \cdot \widehat{\mathfrak{D}}_{\parallel}, \quad (15.203)$$

where  $\widehat{XH}$  is a unit vector parallel to the XH vector.  $\widehat{\mathfrak{D}}_{\parallel}$  is a unit vector parallel to the unique axis of the diffusion tensor and can be expressed using the spherical angles where  $\theta$  is the polar angle and  $\phi$  is the azimuthal angle as

$$\widehat{\mathfrak{D}}_{\parallel} = \begin{pmatrix} \sin \theta \cos \phi \\ \sin \theta \sin \phi \\ \cos \theta \end{pmatrix}. \quad (15.204)$$

### 15.13.2 The dot product gradient of the spheroid

The partial derivative of the dot product with respect to the orientational parameter  $\mathfrak{D}_i$  is

$$\frac{\partial \delta_z}{\partial \mathfrak{D}_i} = \frac{\partial}{\partial \mathfrak{D}_i} (\widehat{XH} \cdot \widehat{\mathfrak{D}}_{\parallel}) = \widehat{XH} \frac{\partial \widehat{\mathfrak{D}}_{\parallel}}{\partial \mathfrak{D}_i} + \frac{\partial \widehat{XH}}{\partial \mathfrak{D}_i} \widehat{\mathfrak{D}}_{\parallel}. \quad (15.205)$$

Because the XH bond vector is constant and not dependent on the spherical angles its derivative is zero. Therefore

$$\frac{\partial \delta_z}{\partial \mathfrak{D}_i} = \widehat{XH} \frac{\partial \widehat{\mathfrak{D}}_{\parallel}}{\partial \mathfrak{D}_i}. \quad (15.206)$$

#### The $\mathfrak{D}_{\parallel}$ gradient

The partial derivatives of the unit vector  $\widehat{\mathfrak{D}}_{\parallel}$  with respect to the spherical angles are

$$\frac{\partial \widehat{\mathfrak{D}}_{\parallel}}{\partial \theta} = \begin{pmatrix} \cos \theta \cos \phi \\ \cos \theta \sin \phi \\ -\sin \theta \end{pmatrix}, \quad (15.207a)$$

$$\frac{\partial \widehat{\mathfrak{D}}_{\parallel}}{\partial \phi} = \begin{pmatrix} -\sin \theta \sin \phi \\ \sin \theta \cos \phi \\ 0 \end{pmatrix}. \quad (15.207b)$$

### 15.13.3 The dot product Hessian of the spheroid

The second partial derivative of the single spheroidal dot product  $\delta_z$  with respect to the orientational parameters  $\mathfrak{D}_i$  and  $\mathfrak{D}_j$  is

$$\frac{\partial^2 \delta_z}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} = \frac{\partial^2}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j} (\widehat{XH} \cdot \widehat{\mathfrak{D}}_{\parallel}) = \widehat{XH} \frac{\partial^2 \widehat{\mathfrak{D}}_{\parallel}}{\partial \mathfrak{D}_i \cdot \partial \mathfrak{D}_j}. \quad (15.208)$$

**The  $\mathfrak{D}_{\parallel}$  Hessian**

The second partial derivatives of the unit vector  $\widehat{\mathfrak{D}_{\parallel}}$  with respect to the spherical angles are

$$\frac{\partial^2 \widehat{\mathfrak{D}_{\parallel}}}{\partial \theta^2} = \begin{pmatrix} -\sin \theta \cos \phi \\ -\sin \theta \sin \phi \\ -\cos \theta \end{pmatrix}, \quad (15.209a)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}_{\parallel}}}{\partial \theta \cdot \partial \phi} = \begin{pmatrix} -\cos \theta \sin \phi \\ \cos \theta \cos \phi \\ 0 \end{pmatrix}, \quad (15.209b)$$

$$\frac{\partial^2 \widehat{\mathfrak{D}_{\parallel}}}{\partial \phi^2} = \begin{pmatrix} -\sin \theta \cos \phi \\ -\sin \theta \sin \phi \\ 0 \end{pmatrix}. \quad (15.209c)$$

## Chapter 16

# The frame order models

### 16.1 The current frame order models

In this advanced topic chapter, the equations for the various frame order models will be derived and validated using simulations. The models include:

1. Rigid
2. Rotor
3. Free rotor
4. Isotropic cone
5. Isotropic cone, torsionless
6. Isotropic cone, free rotor
7. Pseudo-ellipse
8. Pseudo-ellipse, torsionless
9. Pseudo-ellipse, free rotor
10. Double rotor

For a basic introduction to the frame order concept and the modelling of the tilt and torsion components, see Section [12.3](#).

### 16.2 Simulation of the frame order models

To validate the derived frame order matrix equations for the models, the real frame order matrix values for a given parameter set can be simulated. The following script was used for simulating the isotropic cone and pseudo-ellipse frame order matrices. As the other models are parametric restrictions of these two models, the simpler models were not simulated.

The script allows for both an ‘in\_frame’ and ‘out\_of\_frame’ or ‘axis2\_1\_3’ mode to observe the components both within the motional eigenframe and in a rotated frame. The value of VAR can be set to ‘ISO’ for selecting the isotropic cone  $\theta$  parameter to vary, or ‘X’, ‘Y’, or ‘Z’ to vary the pseudo-ellipse  $\theta_x$ ,  $\theta_y$ , and  $\sigma_{\max}$  parameters respectively.

```

1  # relax script.
2
3  # Python module imports.
4  from math import cos, pi, sin, sqrt
5  from numpy import array, cross, dot, eye, float64, transpose, zeros
6  from numpy.linalg import norm
7  from random import uniform
8  from string import lower
9  import sys
10
11 # relax module imports.
12 from lib.errors import RelaxError
13 from lib.geometry.angles import wrap_angles
14 from lib.geometry.rotations import axis_angle_to_R, R_random_hypersphere, R_to_euler_zyz,
    tilt_torsion_to_R
15 from lib.linear_algebra.kronecker_product import kron_prod
16 from lib.text.progress import progress_meter
17
18
19 # Variables.
20 #MODEL = 'rotor'
21 #MODEL = 'free_rotor'
22 #MODEL = 'iso_cone'
23 #MODEL = 'iso_cone_torsionless'
24 #MODEL = 'iso_cone_free_rotor'
25 #MODEL = 'pseudo-ellipse'
26 #MODEL = 'pseudo-ellipse_torsionless'
27 #MODEL = 'pseudo-ellipse_free_rotor'
28 MODEL = 'double_rotor'
29 #MODEL_TEXT = 'Rotor frame order model'
30 #MODEL_TEXT = 'Free rotor frame order model'
31 #MODEL_TEXT = 'Isotropic cone frame order model'
32 #MODEL_TEXT = 'Torsionless isotropic cone frame order model'
33 #MODEL_TEXT = 'Free rotor isotropic cone frame order model'
34 #MODEL_TEXT = 'Pseudo-ellipse frame order model'
35 #MODEL_TEXT = 'Torsionless pseudo-ellipse frame order model'
36 #MODEL_TEXT = 'Free rotor pseudo-ellipse frame order model'
37 MODEL_TEXT = 'Double rotor frame order model'
38 SAMPLE_SIZE = 1000000
39 TAG = 'in_frame'
40 #TAG = 'out_of_frame'
41 #TAG = 'axis2_1_3'
42
43 # Angular restrictions.
44 THETA_X = pi / 4
45 THETA_Y = 3 * pi / 8
46 THETA_Z = pi / 6
47 INC = 18
48 VAR = 'X'
49
50 # The frame order eigenframe - I.
51 if TAG == 'in_frame':
52     EIG_FRAME = eye(3)
53
54 # The frame order eigenframe - rotated.

```

```

55 if TAG == 'out_of_frame':
56     EIG_FRAME = array([[ 2, -1,  2],
57                        [ 2,  2, -1],
58                        [-1,  2,  2]], float64) / 3.0
59
60 # The frame order eigenframe (and tag) - original isotropic cone axis [2, 1, 3].
61 elif TAG == 'axis2_1_3':
62     # Generate 3 orthogonal vectors.
63     vect_z = array([2, 1, 3], float64)
64     vect_x = cross(vect_z, array([1, 1, 1], float64))
65     vect_y = cross(vect_z, vect_x)
66
67     # Normalise.
68     vect_x = vect_x / norm(vect_x)
69     vect_y = vect_y / norm(vect_y)
70     vect_z = vect_z / norm(vect_z)
71
72     # Build the frame.
73     EIG_FRAME = zeros((3, 3), float64)
74     EIG_FRAME[:, 0] = vect_x
75     EIG_FRAME[:, 1] = vect_y
76     EIG_FRAME[:, 2] = vect_z
77
78
79
80 class Frame_order:
81     def __init__(self):
82         """Calculate the frame order at infinity.
83
84         This is when the starting positions are random.
85         """
86
87         # The file name.
88         file_name = '_%s_%s_theta_%s_ens%s.agr' % (MODEL, TAG, lower(VAR), SAMPLE_SIZE)
89
90         # Set the initial storage structures.
91         self.init_storage()
92
93         # Init.
94         index = 0
95         self.torsion_check = True
96
97         # Pre-transpose the eigenframe for speed.
98         self.eig_frame_T = transpose(EIG_FRAME)
99
100        # Generate the angle data structures.
101        self.angles = []
102        self.angles_deg = []
103        for i in range(INC):
104            # The angle of one increment.
105            inc_angle = pi / INC
106
107            # The angle of the increment.
108            self.angles.append(inc_angle * (i+1))
109
110            # In degrees for the graphs.
111            self.angles_deg.append(self.angles[-1] / (2.0*pi) * 360.0)
112
113        # Alias the bound checking methods.
114        if MODEL == 'rotor':
115            self.inside = self.inside_rotor

```

```

116         self.rotation = self.rotation_z_axis
117     elif MODEL == 'free_rotor':
118         self.inside = self.inside_free_rotor
119         self.rotation = self.rotation_z_axis
120     elif MODEL == 'iso_cone':
121         self.inside = self.inside_iso_cone
122         self.rotation = self.rotation_hypersphere
123     elif MODEL == 'iso_cone_torsionless':
124         self.inside = self.inside_iso_cone
125         self.rotation = self.rotation_hypersphere_torsionless
126     elif MODEL == 'iso_cone_free_rotor':
127         self.inside = self.inside_iso_cone
128         self.rotation = self.rotation_hypersphere
129         self.torsion_check = False
130     elif MODEL == 'pseudo-ellipse':
131         self.inside = self.inside_pseudo_ellipse
132         self.rotation = self.rotation_hypersphere
133     elif MODEL == 'pseudo-ellipse_torsionless':
134         self.inside = self.inside_pseudo_ellipse
135         self.rotation = self.rotation_hypersphere_torsionless
136     elif MODEL == 'pseudo-ellipse_free_rotor':
137         self.inside = self.inside_pseudo_ellipse
138         self.rotation = self.rotation_hypersphere
139         self.torsion_check = False
140     elif MODEL == 'double_rotor':
141         self.inside = self.inside_double_rotor
142         self.rotation = self.rotation_double_xy_axes
143     else:
144         raise RelaxError("Unknown model '%s'." % MODEL)
145
146     # Loop over random starting positions.
147     while True:
148         # Printout.
149         progress_meter(index, a=1000, b=100000)
150
151         # Generate the random rotation.
152         theta, phi, sigma = self.rotation()
153
154         # Pre-calculate the R Kronecker outer product for speed.
155         Rx2 = kron_prod(self.rot, self.rot)
156
157         # Loop over the angle incs.
158         for i in range(INC):
159             # The new limits.
160             max_theta_x, max_theta_y, max_theta_z = self.limits(i)
161
162             # Inside the cone.
163             if not self.full[i] and self.inside(i=i, theta=theta, phi=phi, sigma=sigma
, max_theta_x=max_theta_x, max_theta_y=max_theta_y, max_theta_z=max_theta_z):
164
165                 # Sum of rotations and cross products.
166                 self.first_frame_order[i] += self.rot
167                 self.second_frame_order[i] += Rx2
168
169                 # Increment the counter.
170                 self.count[i] += 1
171
172                 # Full.
173                 if self.count[i] == SAMPLE_SIZE:
174                     sys.stdout.write("\b"*100 + "The angle restriction of %s deg is
complete.\n" % self.angles_deg[i])

```



```

175         self.full[i] = 1
176
177         # Increment the global index.
178         index += 1
179
180         # Break out.
181         if sum(self.full) == INC:
182             break
183
184         # Average.
185         self.first_frame_order = self.first_frame_order / float(SAMPLE_SIZE)
186         self.second_frame_order = self.second_frame_order / float(SAMPLE_SIZE)
187
188         # Write the data.
189         self.write_data(file_name=file_name)
190
191         # Final printout.
192         sys.stdout.write("Random rotations required: %i\n\n" % index)
193
194
195     def init_storage(self):
196         """Initialise the storage structures."""
197
198         # Create the average rotation matrix (first order).
199         self.first_frame_order = zeros((INC, 3, 3), float64)
200
201         # Create the frame order matrix (each element is ensemble averaged and corresponds
202         # to a different time step).
203         self.second_frame_order = zeros((INC, 9, 9), float64)
204
205         # Init the rotation matrix.
206         self.rot = zeros((3, 3), float64)
207         self.rot2 = zeros((3, 3), float64)
208
209         # Some data arrays.
210         self.full = zeros(INC)
211         self.count = zeros(INC)
212
213         # Axes.
214         self.x_axis = array([1, 0, 0], float64)
215         self.y_axis = array([0, 1, 0], float64)
216         self.z_axis = array([0, 0, 1], float64)
217
218     def inside_double_rotor(self, i=None, theta=None, phi=None, sigma=None, max_theta_x=
219     None, max_theta_y=None, max_theta_z=None):
220         """Determine if the frame is inside the limits."""
221
222         # Alias the angles.
223         sigma1, sigma2 = theta, phi
224
225         # Check for torsion angle violations.
226         if sigma1 < -max_theta_y or sigma1 > max_theta_y:
227             return False
228         if sigma2 < -max_theta_x or sigma2 > max_theta_x:
229             return False
230
231         # Inside.
232         return True
233

```

```

234 def inside_free_rotor(self, i=None, theta=None, phi=None, sigma=None, max_theta_x=None
    , max_theta_y=None, max_theta_z=None):
235     """Determine if the frame is inside the limits, which for the free rotor is always
        true."""
236
237     # Inside.
238     return True
239
240
241 def inside_iso_cone(self, i=None, theta=None, phi=None, sigma=None, max_theta_x=None,
    max_theta_y=None, max_theta_z=None):
242     """Determine if the frame is inside the limits."""
243
244     # Check for a torsion angle violation.
245     if self.torsion_check and (sigma < -max_theta_z or sigma > max_theta_z):
246         return False
247
248     # Check for a tilt angle violation.
249     if theta > max_theta_x:
250         return False
251
252     # Inside.
253     return True
254
255
256 def inside_pseudo_ellipse(self, i=None, theta=None, phi=None, sigma=None, max_theta_x=
    None, max_theta_y=None, max_theta_z=None):
257     """Determine if the frame is inside the limits."""
258
259     # Check for a torsion angle violation.
260     if self.torsion_check and (sigma < -max_theta_z or sigma > max_theta_z):
261         return False
262
263     # Check for a tilt angle violation.
264     max_theta = 1.0 / sqrt(cos(phi)**2 / max_theta_x**2 + sin(phi)**2 / max_theta_y
        **2)
265     if theta > max_theta:
266         return False
267
268     # Inside.
269     return True
270
271
272 def inside_rotor(self, i=None, theta=None, phi=None, sigma=None, max_theta_x=None,
    max_theta_y=None, max_theta_z=None):
273     """Determine if the frame is inside the limits."""
274
275     # Check for a torsion angle violation.
276     if sigma < -max_theta_z or sigma > max_theta_z:
277         return False
278
279     # Inside.
280     return True
281
282
283 def limits(self, i):
284     """Determine the angular restrictions for the increment i."""
285
286     # Alias the angle for the increment.
287     theta = self.angles[i]
288

```

```

289     # The different angles to vary.
290     if VAR == 'X':
291         return theta, THETA_Y, THETA_Z
292     elif VAR == 'Y':
293         return THETA_X, theta, THETA_Z
294     elif VAR == 'Z':
295         return THETA_X, THETA_Y, theta
296
297
298 def rotation_double_xy_axes(self):
299     """Random double rotation around the x- and y-axes and return of torsion-tilt
300     angles"""
301
302     # First a random angle between -pi and pi for the y-axis.
303     sigma1 = uniform(-pi, pi)
304     axis_angle_to_R(self.y_axis, sigma1, self.rot)
305
306     # Second a random angle between -pi and pi for the x-axis.
307     sigma2 = uniform(-pi, pi)
308     axis_angle_to_R(self.x_axis, sigma2, self.rot2)
309
310     # Construct the frame.
311     frame = dot(self.rot2, self.rot)
312
313     # Rotate the frame.
314     self.rot = dot(EIG_FRAME, dot(frame, self.eig_frame_T))
315
316     # Return the two torsion angles, and zero.
317     return sigma1, sigma2, 0.0
318
319 def rotation_hypersphere(self):
320     """Random rotation using 4D hypersphere point picking and return of torsion-tilt
321     angles."""
322
323     # Generate a random rotation.
324     R_random_hypersphere(self.rot)
325
326     # Rotate the frame.
327     frame = dot(self.eig_frame_T, dot(self.rot, EIG_FRAME))
328
329     # Decompose the frame into the zyz Euler angles.
330     alpha, beta, gamma = R_to_euler_zyz(frame)
331
332     # Convert to tilt and torsion angles (properly wrapped) and return them.
333     theta = beta
334     phi = wrap_angles(gamma, -pi, pi)
335     sigma = wrap_angles(alpha + gamma, -pi, pi)
336     return theta, phi, sigma
337
338 def rotation_hypersphere_torsionless(self):
339     """Random rotation using 4D hypersphere point picking and return of torsion-tilt
340     angles."""
341
342     # Obtain the random torsion-tilt angles from the random hypersphere method.
343     theta, phi, sigma = self.rotation_hypersphere()
344
345     # Reconstruct a rotation matrix, setting the torsion angle to zero.
346     tilt_torsion_to_R(phi, theta, 0.0, self.rot)

```

```

347     # Rotate the frame.
348     self.rot = dot(EIG_FRAME, dot(self.rot, self.eig_frame_T))
349
350     # Return the angles.
351     return theta, phi, 0.0
352
353
354 def rotation_z_axis(self):
355     """Random rotation around the z-axis and return of torsion-tilt angles"""
356
357     # Random angle between -pi and pi.
358     angle = uniform(-pi, pi)
359
360     # Generate the rotation matrix.
361     axis_angle_to_R(self.z_axis, angle, self.rot)
362
363     # Decompose the rotation into the zyz Euler angles.
364     alpha, beta, gamma = R_to_euler_zyz(self.rot)
365
366     # Rotate the frame.
367     self.rot = dot(EIG_FRAME, dot(self.rot, self.eig_frame_T))
368
369     # Convert to tilt and torsion angles (properly wrapped) and return them.
370     theta = beta
371     phi = wrap_angles(gamma, -pi, pi)
372     sigma = wrap_angles(alpha + gamma, -pi, pi)
373     return theta, phi, sigma
374
375
376 def write_data(self, file_name=None):
377     """Dump the data to files.
378
379     @keyword file_name:      The end part of the files to create. This will be
380     prepended by either 'Sij' or 'Sijkl'.
381     @type file_name:        str
382     """
383
384     # Open the files.
385     file_1st = open("Sij" + file_name, 'w')
386     file_2nd = open("Sijkl" + file_name, 'w')
387     files = [file_1st, file_2nd]
388
389     # The headers.
390     for i in range(2):
391         # Alias the file.
392         file = files[i]
393
394         # The titles.
395         file.write("@with g0\n")
396         if i == 0:
397             file.write("@ world 0, -0.2, 180, 1\n")
398         else:
399             file.write("@ world 0, -0.7, 180, 1\n")
400         file.write("@ title \"Simulated frame order matrix elements\\\"\\n")
401         if i == 0:
402             file.write("@ subtitle \"%s, 1\\Sst\\N degree matrix, %i simulations\\\"\\n" % (MODEL_TEXT, SAMPLE_SIZE))
403         else:
404             file.write("@ subtitle \"%s, 2\\Snd\\N degree matrix, %i simulations\\\"\\n" % (MODEL_TEXT, SAMPLE_SIZE))

```

```

405         # Legend.
406         if i == 0:
407             file.write("@    legend 0.23, 0.55\n")
408         else:
409             file.write("@    legend off\n")
410
411         # Plot data.
412         file.write("@    xaxis  bar linewidth 0.5\n")
413         file.write("@    xaxis  label \"Cone half-angle \\xq\\f{\\s%s\\N (deg.)\"\\n"
% VAR)
414         file.write("@    xaxis  label char size 1.000000\n")
415         file.write("@    xaxis  tick major 45\n")
416         file.write("@    xaxis  tick major linewidth 0.5\n")
417         file.write("@    xaxis  tick minor ticks 3\n")
418         file.write("@    xaxis  tick minor linewidth 0.5\n")
419         file.write("@    yaxis  bar linewidth 0.5\n")
420         if i == 0:
421             file.write("@    yaxis  label \"Order parameter \\qS\\sij\"\\n")
422         else:
423             file.write("@    yaxis  label \"Order parameter \\qS\\sijkl\"\\n")
424         file.write("@    yaxis  label char size 1.000000\n")
425         file.write("@    yaxis  tick major 0.2\n")
426         file.write("@    yaxis  tick major linewidth 0.5\n")
427         file.write("@    yaxis  tick minor ticks 1\n")
428         file.write("@    yaxis  tick minor linewidth 0.5\n")
429
430         # Header for first order matrix.
431         graph_num = 0
432         for i in range(3):
433             for j in range(3):
434                 file_1st.write("@    s%i legend \"<\\q<\\s%s\\N>\"\\n" % (graph_num, i+1,
j+1))
435                 file_1st.write("@    s%i linewidth 0.5\n" % graph_num)
436                 graph_num += 1
437
438         # Header for second order matrix.
439         graph_num = 0
440         for i in range(3):
441             for j in range(3):
442                 for k in range(3):
443                     for l in range(3):
444                         file_2nd.write("@    s%i legend \"<\\q<\\s%s\\N.c\\s%s\\N>\"\\n"
" % (graph_num, i+1, j+1, k+1, l+1))
445                         file_2nd.write("@    s%i linewidth 0.5\n" % graph_num)
446                         graph_num += 1
447
448         # Loop over the first rotation matrix index.
449         graph_num = 0
450         for i in range(3):
451             # Loop over the second rotation matrix index.
452             for j in range(3):
453                 # Header.
454                 file_1st.write("@target G0.S%i\n" % graph_num)
455                 file_1st.write("@type xy\n")
456
457                 # Loop over each time point.
458                 for k in range(INC):
459                     file_1st.write("%s %s\n" % (self.angles_deg[k], self.first_frame_order
[k, i, j]))
460
461                 # Footer.

```

```

462         file_1st.write("&\n")
463
464         # Inc.
465         graph_num += 1
466
467         # Loop over the first frame order index.
468         graph_num = 0
469         for i in range(9):
470             # Loop over the second frame order index.
471             for j in range(9):
472                 # Header.
473                 file_2nd.write('@target G0.S%i\n' % graph_num)
474                 file_2nd.write('@type xy\n')
475
476                 # Loop over each time point.
477                 for k in range(INC):
478                     file_2nd.write('%s %s\n' % (self.angles_deg[k],
self.second_frame_order[k, i, j]))
479
480                 # Footer.
481                 file_2nd.write('&\n')
482
483                 # Inc.
484                 graph_num += 1
485
486         # No autoscaling.
487         file_1st.write("@autoscale onread none\n")
488         file_2nd.write("@autoscale onread none\n")
489
490         # Close the files.
491         file_1st.close()
492         file_2nd.close()
493
494
495         # Calculate the frame order.
496         Frame_order()

```

## 16.3 Rigid frame order model

### 16.3.1 Rigid model parameterisation

This model consists solely of the six parameters of translation and rotation of the ‘moving’ domain to its average position. The parameter set is therefore

$$\mathfrak{M} = \mathfrak{P} = \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\}, \quad (16.1)$$

where  $P_i$  are the average domain position translations and rotations.

### 16.3.2 Rigid model equations

#### Rigid model rotation matrices

For the rigid model, there is no motion so the rotation matrix is simply the identity matrix

$$R = \begin{pmatrix} 1 & \cdot & \cdot \\ \cdot & 1 & \cdot \\ \cdot & \cdot & 1 \end{pmatrix}. \quad (16.2)$$

#### Rigid frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = R^{\otimes n}, \quad (16.3)$$

where surface normalisation factor is 1.

**Rigid 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is the identity matrix

$$\mathbb{M}^{(1)} = R^{\otimes 1}, \quad (16.4a)$$

$$= \begin{pmatrix} 1 & \cdot & \cdot \\ \cdot & 1 & \cdot \\ \cdot & \cdot & 1 \end{pmatrix}. \quad (16.4b)$$

**Rigid 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 is the identity matrix

$$\mathbb{M}^{(2)} = R^{\otimes 2}, \quad (16.5a)$$

$$= \begin{pmatrix} 1 & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & 1 & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & 1 & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & 1 & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & 1 & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & 1 & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 1 & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 1 & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 1 \end{pmatrix}. \quad (16.5b)$$

## 16.4 Rotor frame order model

The rotor model is defined by its rotation axis and a pivot point to position the center of the rotation.

### 16.4.1 Rotor parameterisation

The natural way to parameterise the rotation axis of rotor frame order model is to use a 3D point, the pivot point, and a unit vector using the spherical angle basis set. This would result in the parameter set

$$\mathfrak{M} = \mathfrak{E}_{\text{ax}} + \mathbf{p}_1, \quad (16.6a)$$

$$= \{E_\theta, E_\phi\} + \{p_x, p_y, p_z\}. \quad (16.6b)$$

However this is an overparameterisation as the point  $\{p_x, p_y, p_z\}$  can lie anywhere on the line defined by itself and the unit vector. Due to computational truncation artifacts, this results in the pivot point shooting out to infinity along the line during optimisation.

The minimal set of independent parameters for the rotor model is four. There are many parameterisation of lines in 3D using this minimal set of four parameters, however many of these suffer from singularity problems which can be fatal for optimisation. By using geometry of the model together with information about the molecular system, a parameterisation can be constructed which avoid singularities:

- A point of reference is the PDB frame close to the molecule is chosen, in this case the centre of mass (CoM) of the total system.
- The point on the line defining the rotation axis closest to the reference point defines an orthogonal system of the rotation axis to the vector of the CoM to closest point. This point can be optimised with the parameters  $\mathbf{p}_1 = \{p_x, p_y, p_z\}$  with no singularity problems and minimising truncation artifacts in the numerical PCS calculation.
- As the rotation axis is perpendicular to the CoM- $\mathbf{p}_1$  vector, it can be defined using a single angle of rotation ( $E_\alpha^{\text{ax}}$ ). The reference starting point for the angle is arbitrarily set to the xy-plane. The rotation of a unit vector about the CoM- $\mathbf{p}_1$  centred at  $\mathbf{p}_1$  is smooth, hence singularities are also avoided.

The full parameter set for the rotor model implementation is therefore

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E}_{\text{ax}}^\alpha + \mathbf{p}_1 + \mathfrak{S}, \quad (16.7a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\alpha^{\text{ax}}\} + \{p_x, p_y, p_z\} + \{\sigma_{\text{max}}\}, \quad (16.7b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_\alpha^{\text{ax}}$  is the single angle defining the rotation axis,  $p_i$  are the coordinates of the pivot point, and  $\sigma_{\text{max}}$  is the torsion half-angle of the rotor motion.

The rotation axis vector in the system is calculated as

$$\hat{r}_{ax} = |R_\alpha \cdot (\hat{r}_{\text{CoM}-\mathbf{p}_1} \times \hat{z})|. \quad (16.8)$$

The angle  $E_\alpha^{\text{ax}}$  is obtained from  $\hat{r}_{ax}$  as

$$E_\alpha^{\text{ax}} = \frac{\pi}{2} - \arccos(\hat{r}_{ax} \cdot \hat{z}), \quad (16.9a)$$

$$= \arcsin(\hat{r}_{ax} \cdot \hat{z}), \quad (16.9b)$$



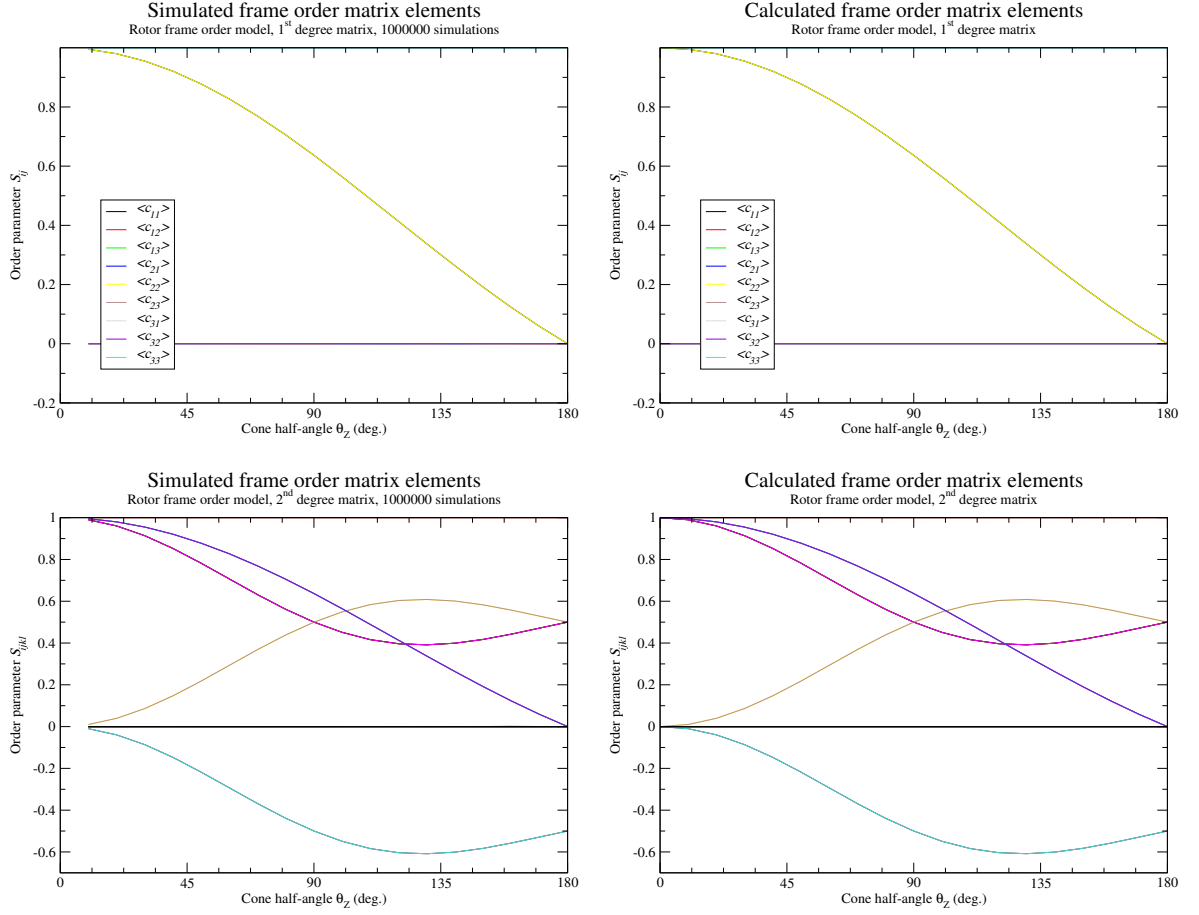


Figure 16.1: The rotor model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots,  $\theta_z$  corresponds to the torsion half-angle  $\sigma_{\max}$ . Frame order matrix values have been calculated every 10 degrees.

### 16.4.2 Rotor equations

The only motion is in the torsion angle about the rotation axis.

#### Rotor rotation matrices

Assuming the rotation axis is the z-axis, the rotation matrix is defined as

$$R(\sigma) = \begin{pmatrix} \cos \sigma & -\sin \sigma & 0 \\ \sin \sigma & \cos \sigma & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad (16.10)$$

#### Rotor frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.11a)$$

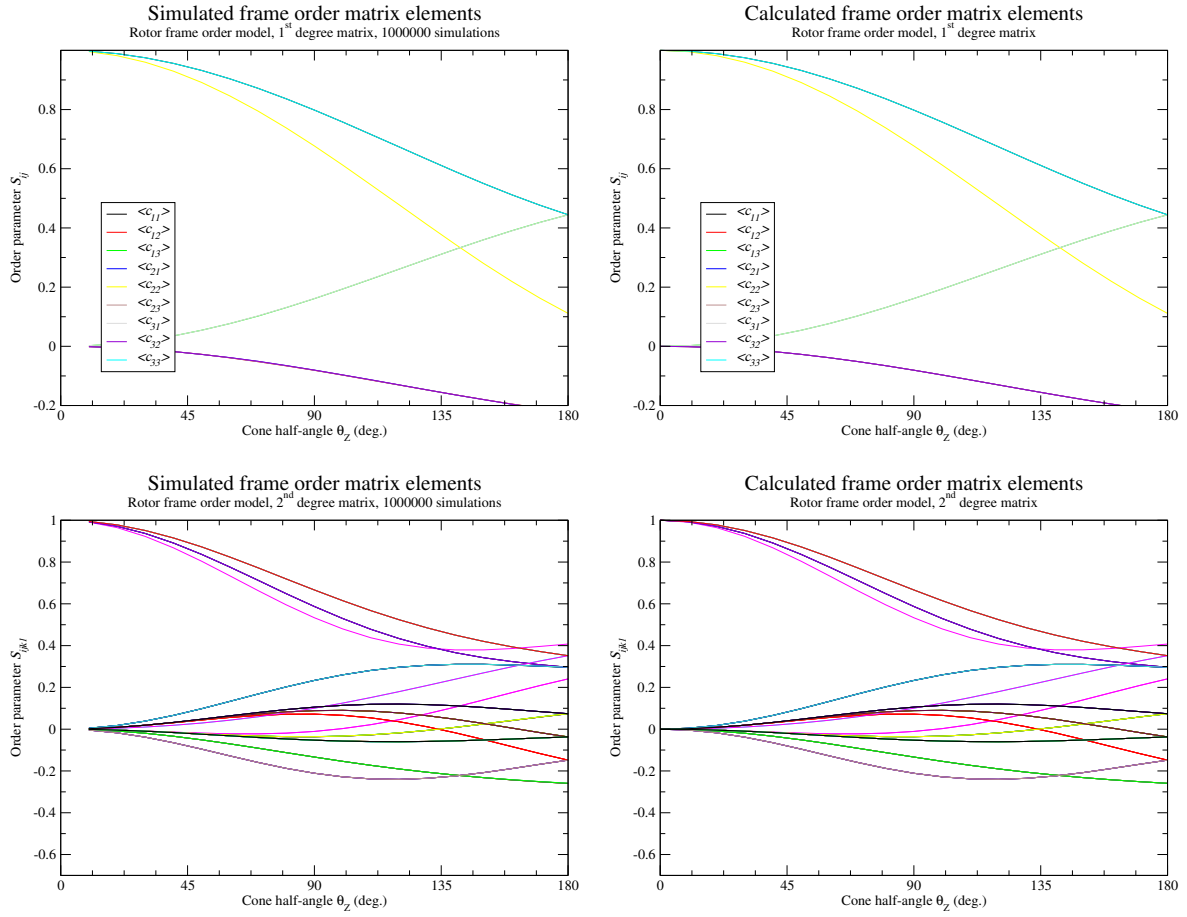


Figure 16.2: The rotor model simulated and calculated out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots,  $\theta_z$  corresponds to the torsion half-angle  $\sigma_{\max}$ . Frame order matrix values have been calculated every 10 degrees.

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} R^{\otimes n} d\sigma \Big/ \int_S dS. \quad (16.11b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\sigma_{\max}}^{\sigma_{\max}} d\sigma, \quad (16.12a)$$

$$= 2\sigma_{\max}. \quad (16.12b)$$

**Rotor 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.13a)$$

$$= \int_S R dS \Big/ 2\sigma_{\max}, \quad (16.13b)$$

$$= \begin{pmatrix} \text{sinc } \sigma_{\max} & \cdot & \cdot \\ \cdot & \text{sinc } \sigma_{\max} & \cdot \\ \cdot & \cdot & 1 \end{pmatrix}. \quad (16.13c)$$

**Rotor 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 is

$$\mathbb{M}^{(2)} = \int_S R^{\otimes 2} dS \Big/ \int_S dS, \quad (16.14a)$$

$$= \int_S R \otimes R dS \Big/ 2\sigma_{\max}, \quad (16.14b)$$

$$= \frac{1}{2} \begin{pmatrix} s_{2\sigma} + 1 & \cdot & \cdot & \cdot & -s_{2\sigma} + 1 & \cdot & \cdot & \cdot & \cdot \\ \cdot & s_{2\sigma} + 1 & \cdot & s_{2\sigma} - 1 & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & 2s_{\sigma} & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & s_{2\sigma} - 1 & \cdot & s_{2\sigma} + 1 & \cdot & \cdot & \cdot & \cdot & \cdot \\ -s_{2\sigma} + 1 & \cdot & \cdot & \cdot & s_{2\sigma} + 1 & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & 2s_{\sigma} & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 2s_{\sigma} & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 2s_{\sigma} & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 1 \end{pmatrix}, \quad (16.14c)$$

where  $s_{\sigma} = \text{sinc } \sigma_{\max}$  and  $s_{2\sigma} = \text{sinc}(2\sigma_{\max})$ . The active matrix elements which are not

zero due to symmetries, in Kronecker product double indices from 0 to 8, are

$$\mathbb{M}_{00} = \frac{1}{2} \text{sinc}(2\sigma_{\max}) + \frac{1}{2}, \quad (16.15a)$$

$$\mathbb{M}_{11} = \mathbb{M}_{00}, \quad (16.15b)$$

$$\mathbb{M}_{22} = \text{sinc } \sigma_{\max}, \quad (16.15c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{00}, \quad (16.15d)$$

$$\mathbb{M}_{44} = \mathbb{M}_{00}, \quad (16.15e)$$

$$\mathbb{M}_{55} = \mathbb{M}_{22}, \quad (16.15f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.15g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{22}, \quad (16.15h)$$

$$\mathbb{M}_{88} = 1, \quad (16.15i)$$

$$\mathbb{M}_{04} = -\frac{1}{2} \text{sinc}(2\sigma_{\max}) + \frac{1}{2}, \quad (16.15j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{04}, \quad (16.15k)$$

$$\mathbb{M}_{08} = 0, \quad (16.15l)$$

$$\mathbb{M}_{80} = 0, \quad (16.15m)$$

$$\mathbb{M}_{48} = 0, \quad (16.15n)$$

$$\mathbb{M}_{84} = 0, \quad (16.15o)$$

$$\mathbb{M}_{13} = -\mathbb{M}_{04}, \quad (16.15p)$$

$$\mathbb{M}_{31} = -\mathbb{M}_{04}, \quad (16.15q)$$

$$\mathbb{M}_{26} = 0, \quad (16.15r)$$

$$\mathbb{M}_{62} = 0, \quad (16.15s)$$

$$\mathbb{M}_{57} = 0, \quad (16.15t)$$

$$\mathbb{M}_{75} = 0. \quad (16.15u)$$

**Rotor frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.13 and 16.15 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.1. The out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.2.

## 16.5 Free rotor frame order model

This is similar to the rotor model but with no torsional restriction ( $\sigma_{\max} = \pi$ ).

### 16.5.1 Free rotor parameterisation

The full parameter set for the free-rotor model implementation is

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E}_{\text{ax}}^{\alpha} + \mathfrak{p}_1, \quad (16.16a)$$

$$= \{P_x, P_y, P_z, P_{\alpha}, P_{\beta}, P_{\gamma}\} + \{E_{\alpha}^{\text{ax}}\} + \{p_x, p_y, p_z\}, \quad (16.16b)$$

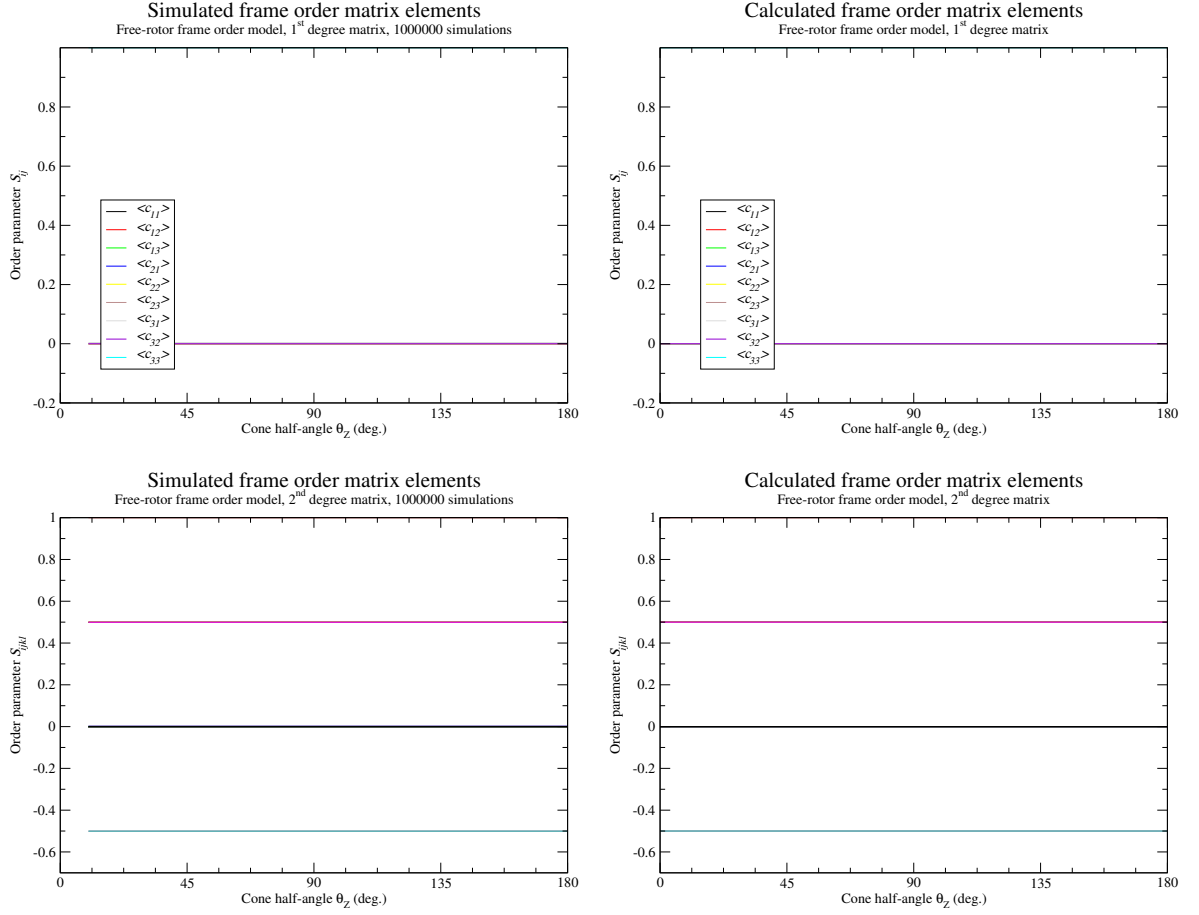


Figure 16.3: The free rotor model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots, as no motional order parameters exist for the model,  $\theta_z$  corresponds to nothing. Frame order matrix values have been calculated every 10 degrees.

where  $P_i$  are the average domain position translations and rotations,  $E_\alpha^{\text{ax}}$  is the single angle defining the rotation axis, and  $p_i$  are the coordinates of the pivot point.

### 16.5.2 Free rotor equations

#### Free rotor rotation matrices

The rotation matrix is defined in equation 16.10.

#### Free rotor frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.17a)$$

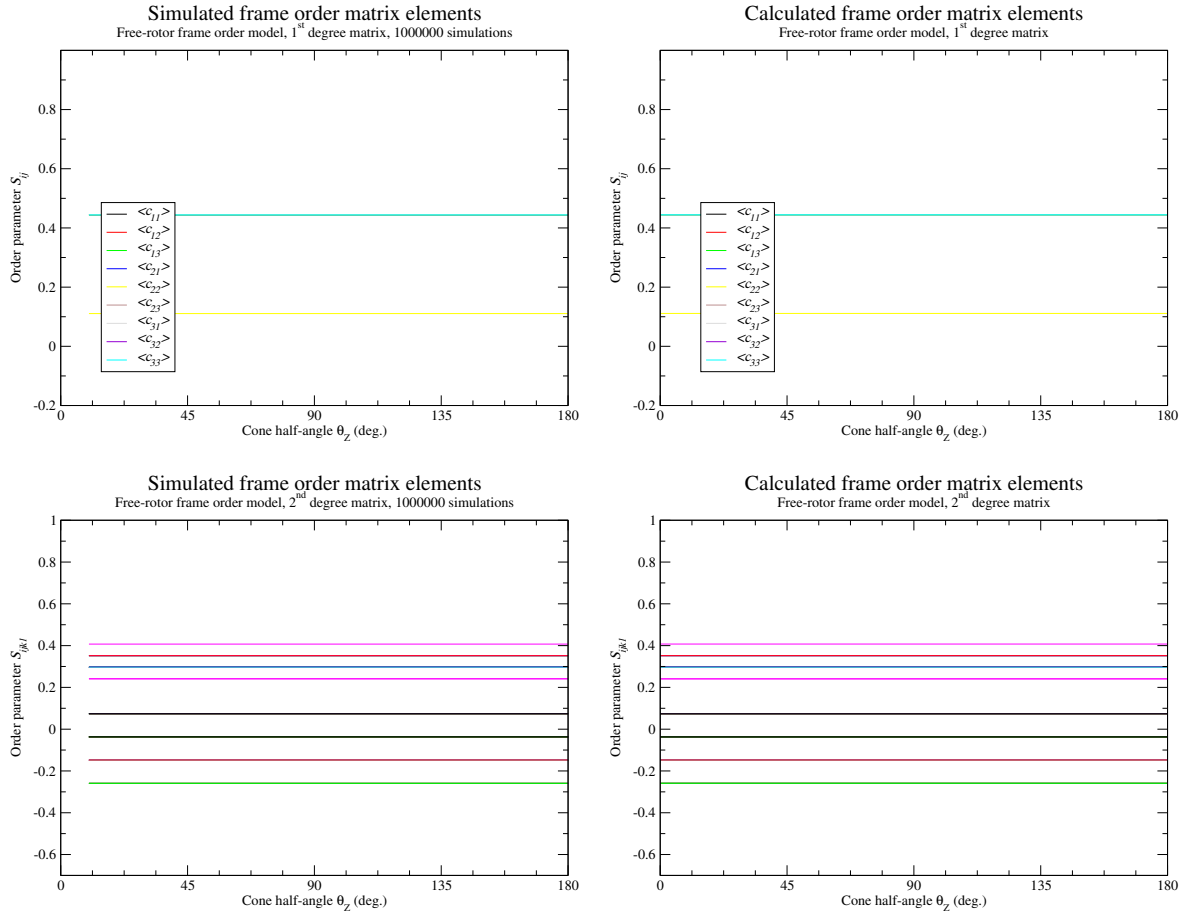


Figure 16.4: The free rotor model simulated and calculated out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots, as no motional order parameters exist for the model,  $\theta_z$  corresponds to nothing. Frame order matrix values have been calculated every 10 degrees.

$$= \int_{-\pi}^{\pi} R^{\otimes n} d\sigma \Big/ \int_S dS. \quad (16.17b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\pi}^{\pi} d\sigma, \quad (16.18a)$$

$$= 2\pi. \quad (16.18b)$$

**Free rotor 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.19a)$$

$$= \int_S R dS \Big/ 2\pi, \quad (16.19b)$$

$$= \begin{pmatrix} \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & 1 \end{pmatrix}. \quad (16.19c)$$

**Free rotor 2<sup>nd</sup> degree frame order** The frame order matrix in Kronecker product notation is fixed as

$$\mathbb{M}^{(2)} = \begin{pmatrix} \frac{1}{2} & \cdot & \cdot & \cdot & \frac{1}{2} & \cdot & \cdot & \cdot & \cdot \\ \cdot & \frac{1}{2} & \cdot & -\frac{1}{2} & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & -\frac{1}{2} & \cdot & \frac{1}{2} & \cdot & \cdot & \cdot & \cdot & \cdot \\ \frac{1}{2} & \cdot & \cdot & \cdot & \frac{1}{2} & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & 1 \end{pmatrix}. \quad (16.20)$$

**Free rotor frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.19 and 16.20 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.3. The out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.4.

## 16.6 Isotropic cone frame order model

### 16.6.1 Isotropic cone parameterisation

In this model, the tilt component of the tilt and torsion angle system is modelled simply as

$$\theta \leq \theta_{\max}. \quad (16.21)$$

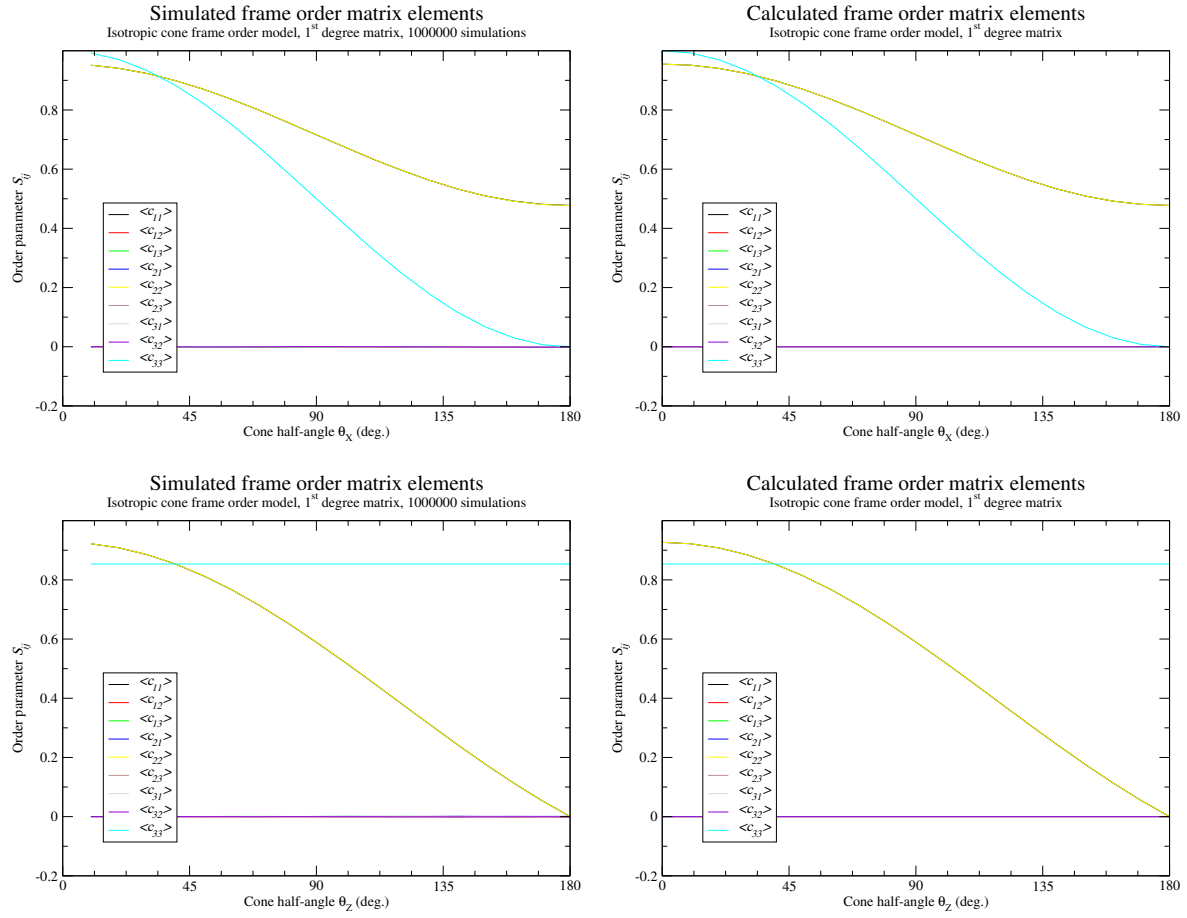


Figure 16.5: The isotropic cone model simulated and calculated in-frame  $\mathfrak{M}^{(1)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$  and  $\theta_Z$  to the torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta = \pi/4$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees.

The torsion angle restriction of 12.76 on page 251 is used for the modelling of the torsion component. A uniform distribution of rigid body positions within these bounds is assumed.

The isotropic cone model of the ball and socket joint consists of a single pivot point, a single unit z-vector of the motional eigenframe defining the cone axis, and the maximum cone opening and torsion half-angles. Including the average domain position, the parameter set is therefore

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E}_{\text{ax}} + \mathfrak{p}_1 + \mathfrak{S}, \quad (16.22a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\theta, E_\phi\} + \{p_x, p_y, p_z\} + \{\theta_{\max}, \sigma_{\max}\}, \quad (16.22b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the spherical angles defining the cone axis,  $p_i$  are the coordinates of the pivot point, and  $\theta_{\max}$  and  $\sigma_{\max}$  are the maximum cone opening and torsion half-angles respectively.



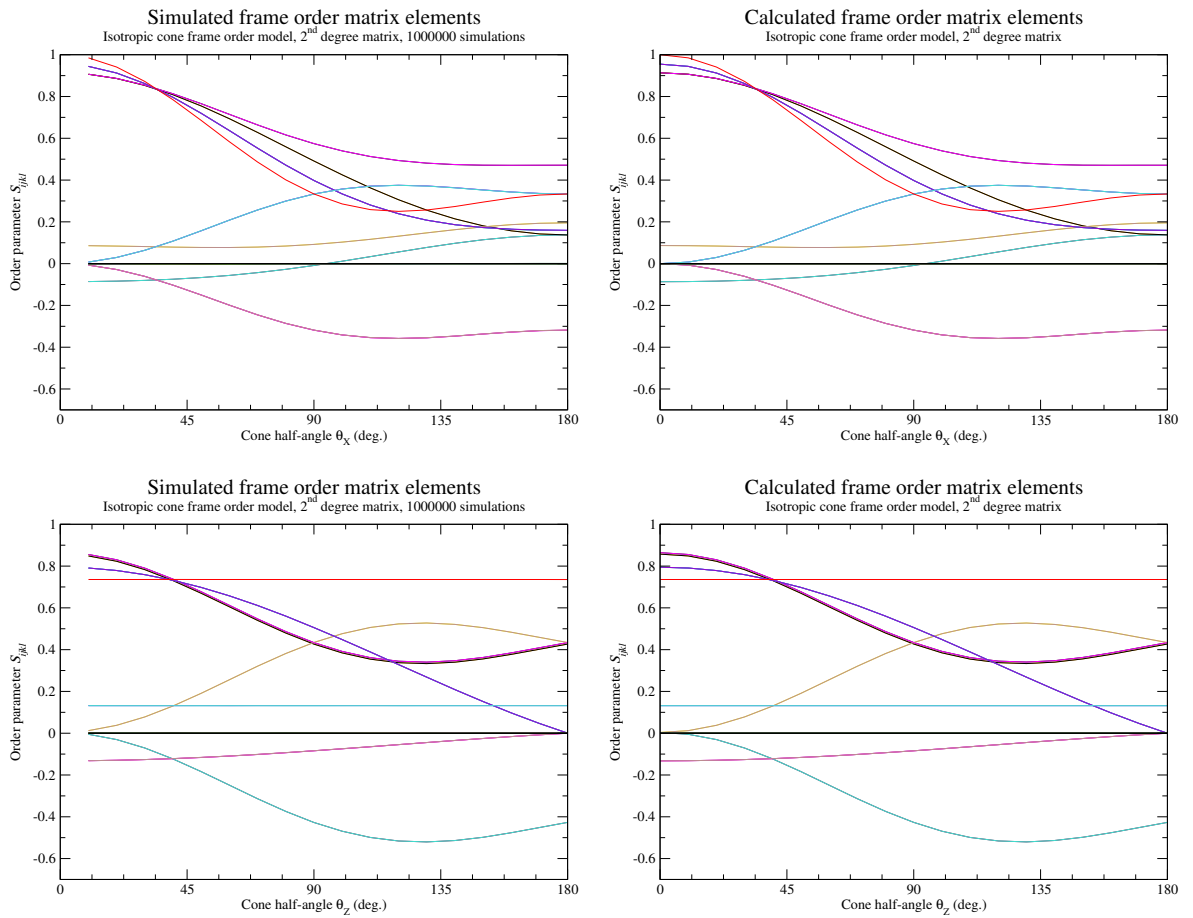


Figure 16.6: The isotropic cone model simulated and calculated in-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$  and  $\theta_Z$  to the torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta = \pi/4$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees.

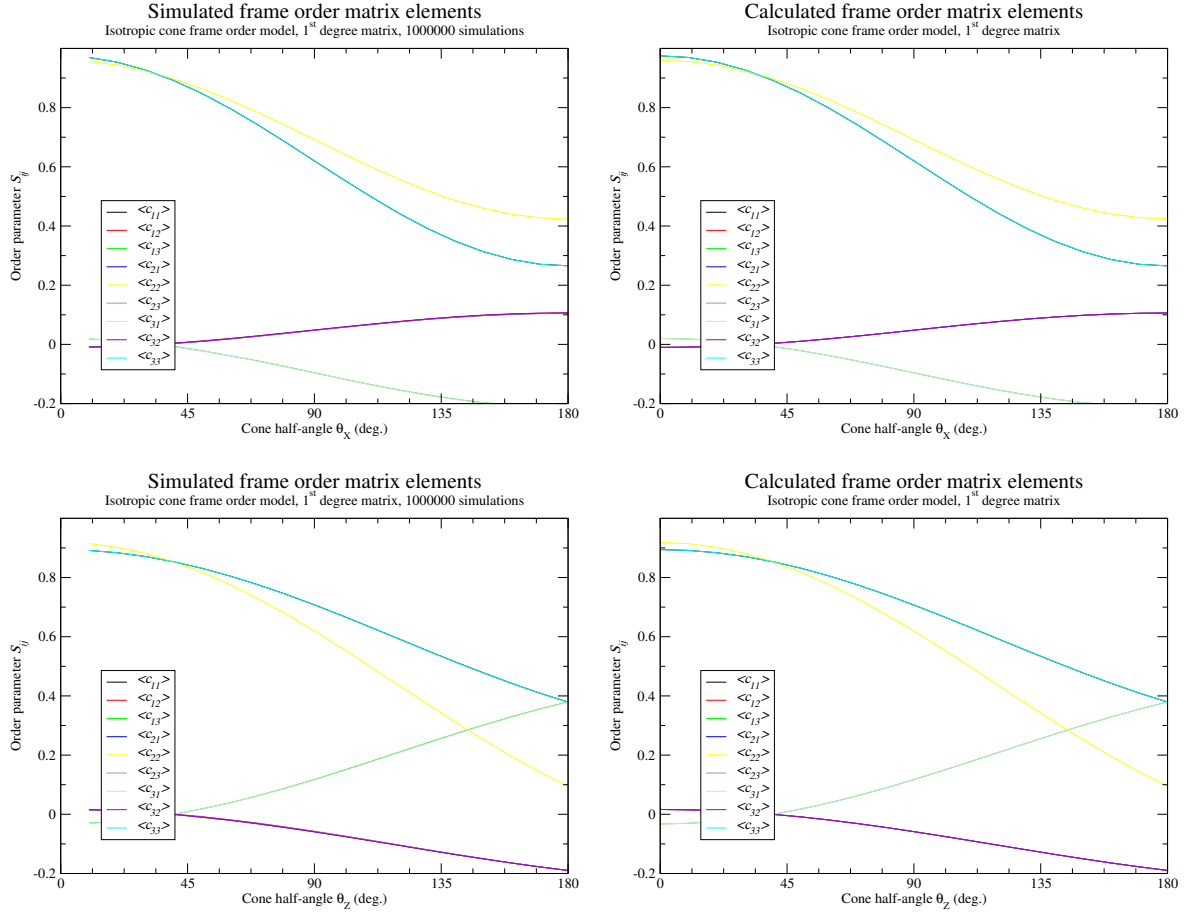


Figure 16.7: The isotropic cone model simulated and calculated out-of-frame  $\mathbb{N}^{(1)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$  and  $\theta_Z$  to the torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta = \pi/4$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees.

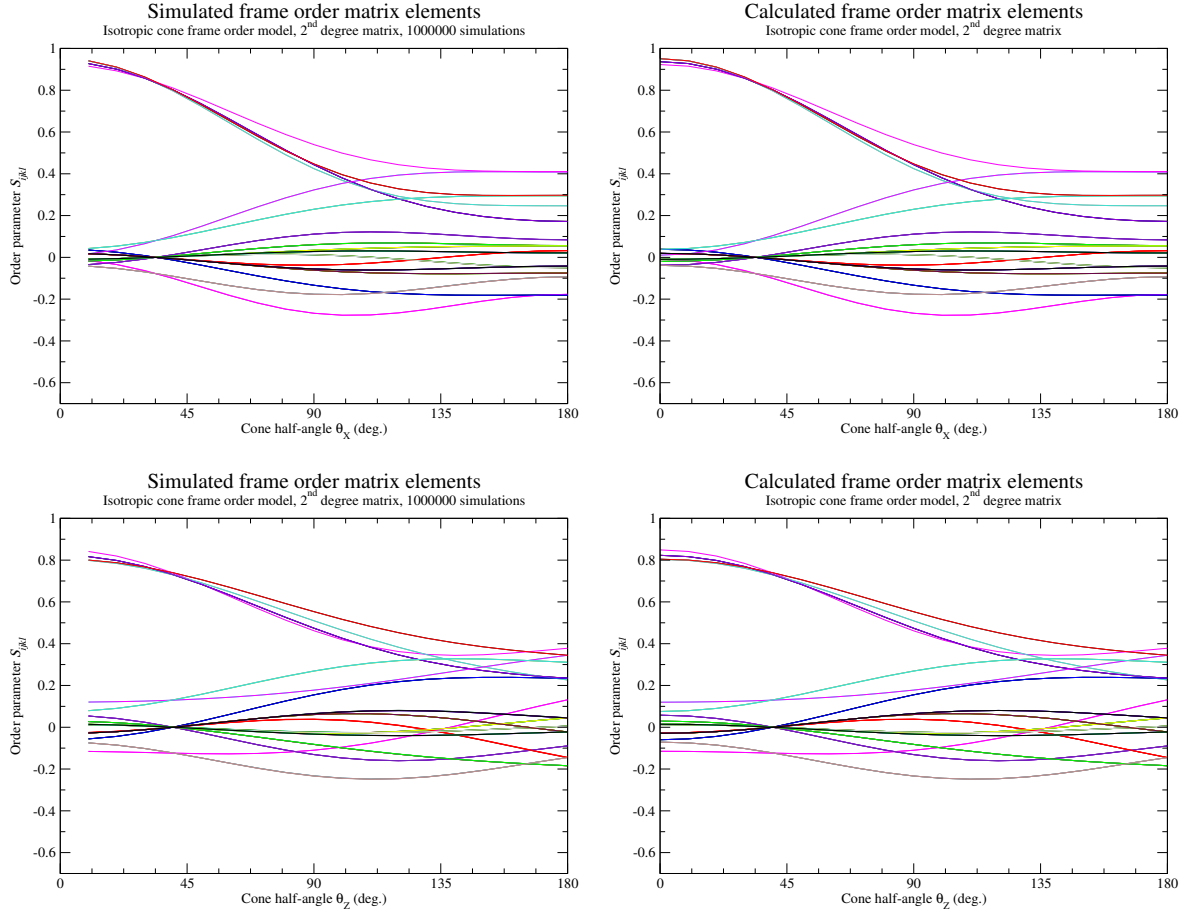


Figure 16.8: The isotropic cone model simulated and calculated out-of-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$  and  $\theta_Z$  to the torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta = \pi/4$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees.

### 16.6.2 Isotropic cone equations

#### Isotropic cone rotation matrices

The rotation matrix is the full torsion-tilt rotation matrix of equation 12.74c on page 250.

#### Isotropic cone frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.23a)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta d\theta d\phi d\sigma \Big/ \int_S dS. \quad (16.23b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta d\theta d\phi d\sigma, \quad (16.24a)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi d\sigma, \quad (16.24b)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} 2\pi (1 - \cos \theta_{\max}) d\sigma, \quad (16.24c)$$

$$= 4\pi\sigma_{\max} (1 - \cos \theta_{\max}). \quad (16.24d)$$

**Isotropic cone 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.25a)$$

$$= \int_S R dS \Big/ 4\pi\sigma_{\max} (1 - \cos \theta_{\max}), \quad (16.25b)$$

$$= \frac{1}{4} \begin{pmatrix} \text{sinc } \sigma_{\max} (\cos \theta_{\max} + 3) & \cdot & \cdot \\ \cdot & \text{sinc } \sigma_{\max} (\cos \theta_{\max} + 3) & \cdot \\ \cdot & \cdot & 2 \cos \theta_{\max} + 2 \end{pmatrix}. \quad (16.25c)$$

**Isotropic cone 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices

from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{24} \text{sinc}(2\sigma_{\max}) (\cos^2 \theta_{\max} + 4 \cos \theta_{\max} + 7) + \frac{1}{12} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 4), \quad (16.26a)$$

$$\mathbb{M}_{11} = \frac{1}{24} \text{sinc}(2\sigma_{\max}) (\cos^2 \theta_{\max} + 4 \cos \theta_{\max} + 7) + \frac{1}{4} (\cos \theta_{\max} + 1), \quad (16.26b)$$

$$\mathbb{M}_{22} = \frac{1}{12} \text{sinc}(2\sigma_{\max}) (2 \cos^2 \theta_{\max} + 5 \cos \theta_{\max} + 5), \quad (16.26c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.26d)$$

$$\mathbb{M}_{44} = \mathbb{M}_{00}, \quad (16.26e)$$

$$\mathbb{M}_{55} = \mathbb{M}_{22}, \quad (16.26f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.26g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{22}, \quad (16.26h)$$

$$\mathbb{M}_{88} = \frac{1}{3} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 1), \quad (16.26i)$$

$$\mathbb{M}_{04} = -\frac{1}{24} \text{sinc}(2\sigma_{\max}) (\cos^2 \theta_{\max} + 4 \cos \theta_{\max} + 7) + \frac{1}{12} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 4), \quad (16.26j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{04}, \quad (16.26k)$$

$$\mathbb{M}_{08} = -\frac{1}{6} (\cos^2 \theta_{\max} + \cos \theta_{\max} - 2), \quad (16.26l)$$

$$\mathbb{M}_{80} = \mathbb{M}_{08}, \quad (16.26m)$$

$$\mathbb{M}_{48} = \mathbb{M}_{08}, \quad (16.26n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{08}, \quad (16.26o)$$

$$\mathbb{M}_{13} = \frac{1}{24} \text{sinc}(2\sigma_{\max}) (\cos^2 \theta_{\max} + 4 \cos \theta_{\max} + 7) - \frac{1}{4} (\cos \theta_{\max} + 1), \quad (16.26p)$$

$$\mathbb{M}_{31} = \mathbb{M}_{13}, \quad (16.26q)$$

$$\mathbb{M}_{26} = \frac{1}{6} \text{sinc}(2\sigma_{\max}) (\cos^2 \theta_{\max} + \cos \theta_{\max} - 2), \quad (16.26r)$$

$$\mathbb{M}_{62} = \mathbb{M}_{26}, \quad (16.26s)$$

$$\mathbb{M}_{57} = \mathbb{M}_{26}, \quad (16.26t)$$

$$\mathbb{M}_{75} = \mathbb{M}_{26}. \quad (16.26u)$$

**Isotropic cone frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.25 and 16.26 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.5 and  $\mathbb{M}^{(2)}$  in figure 16.6. The out-of-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.7 and  $\mathbb{M}^{(2)}$  in figure 16.8.

## 16.7 Torsionless isotropic cone frame order model

### 16.7.1 Torsionless isotropic cone parameterisation

As this is simply the isotropic cone model with the torsion angle set to zero,  $\sigma_{\max} = 0$ , the models parameters are

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E}_{\text{ax}} + \mathfrak{p}_1 + \mathfrak{S}, \quad (16.27a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\theta, E_\phi\} + \{p_x, p_y, p_z\} + \{\theta_{\max}\}, \quad (16.27b)$$

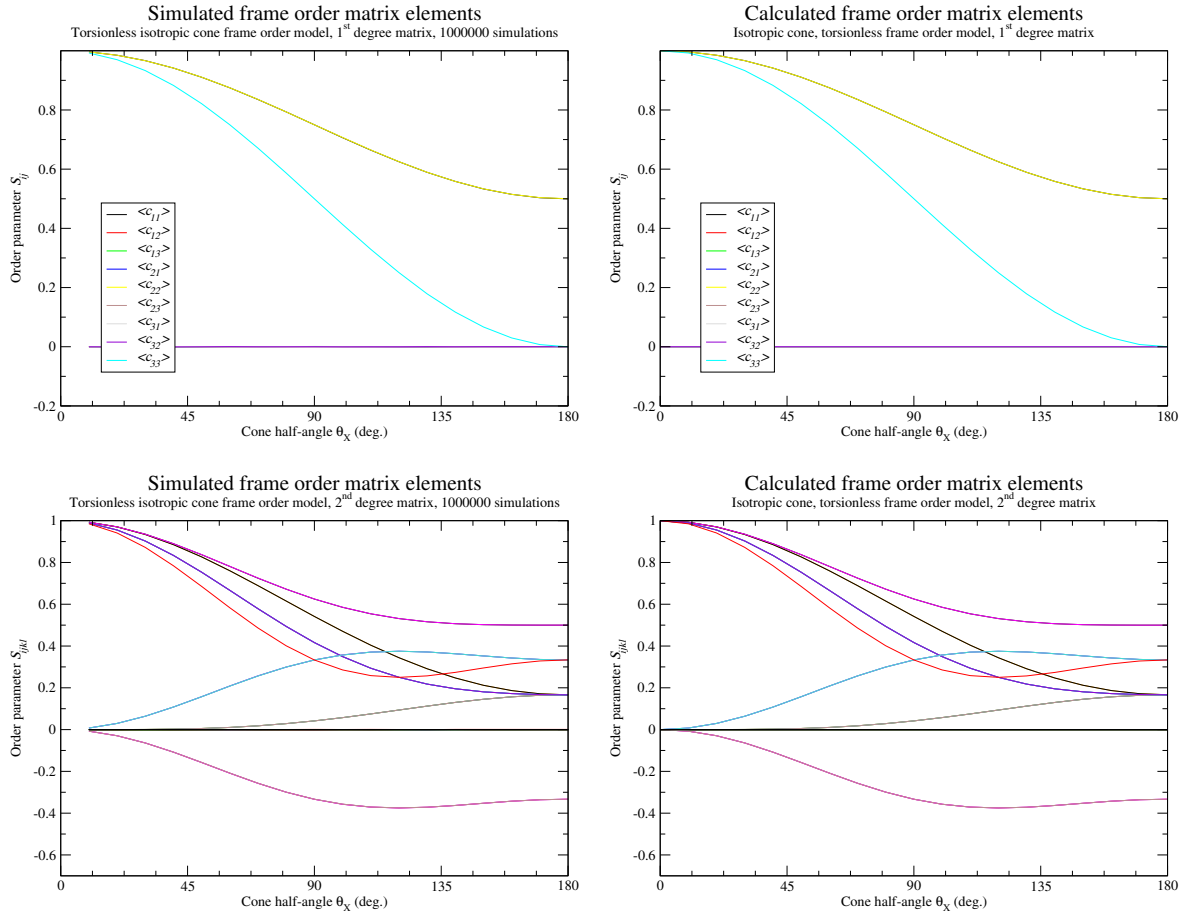


Figure 16.9: The torsionless isotropic cone model simulated and calculated in-frame  $\mathbb{N}^{(1)}$  and  $\mathbb{N}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{N}^{(1)}$  and the bottom to  $\mathbb{N}^{(2)}$ . In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$ . Frame order matrix values have been calculated every 10 degrees.

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the spherical angles defining the cone axis,  $p_i$  are the coordinates of the pivot point, and  $\theta_{\max}$  is the maximum cone opening half-angle.

### 16.7.2 Torsionless isotropic cone equations

#### Torsionless isotropic cone rotation matrices

The torsionless rotation matrix is defined in equation 12.77c.

#### Torsionless isotropic cone frame order matrix

The frame order matrix is

$$\mathbb{N}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.28a)$$

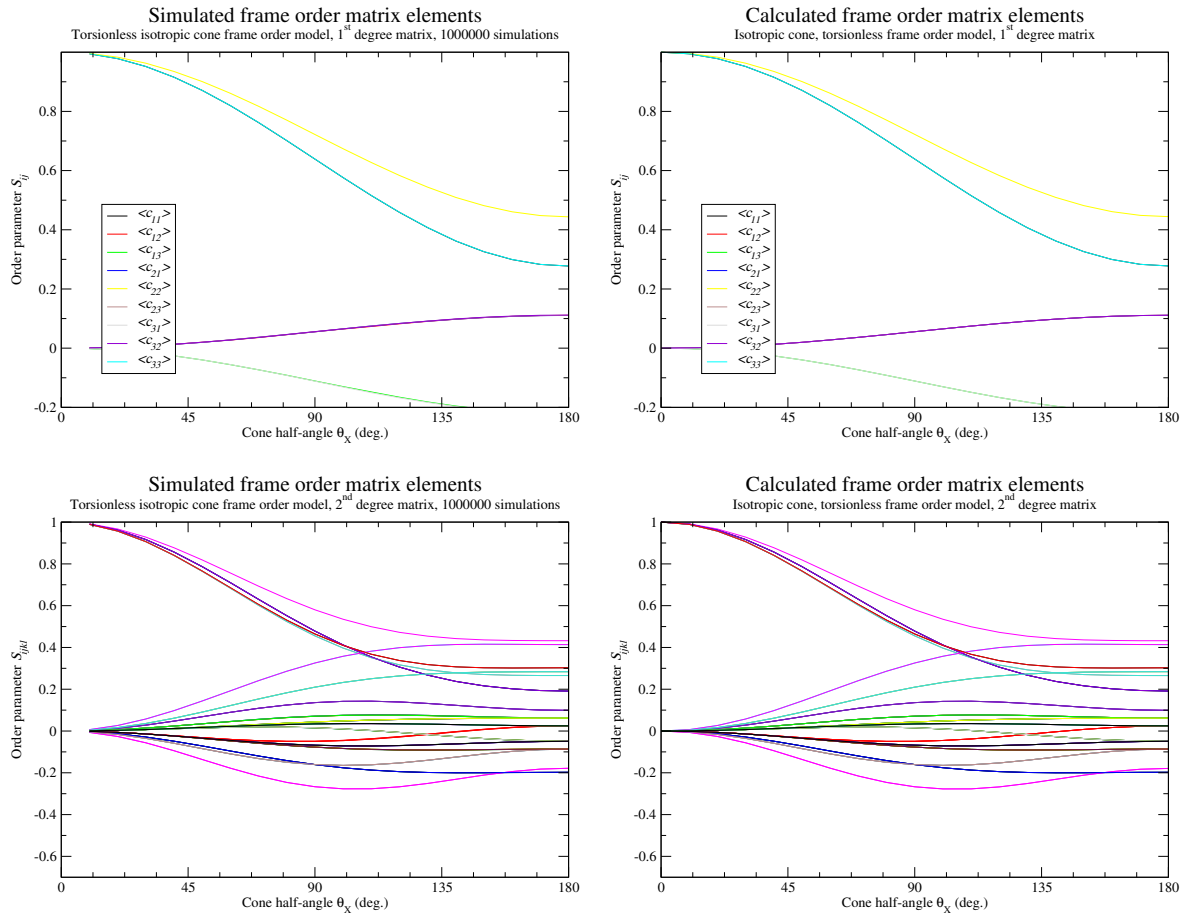


Figure 16.10: The torsionless isotropic cone model simulated and calculated out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$ . Frame order matrix values have been calculated every 10 degrees.

$$= \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta \, d\theta \, d\phi \Big/ \int_S dS. \quad (16.28b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta \, d\theta \, d\phi, \quad (16.29a)$$

$$= \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) \, d\phi, \quad (16.29b)$$

$$= 2\pi (1 - \cos \theta_{\max}). \quad (16.29c)$$

**Torsionless isotropic cone 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.30a)$$

$$= \int_S R dS \Big/ 2\pi (1 - \cos \theta_{\max}), \quad (16.30b)$$

$$= \frac{1}{4} \begin{pmatrix} \cos \theta_{\max} + 3 & \cdot & \cdot \\ \cdot & \cos \theta_{\max} + 3 & \cdot \\ \cdot & \cdot & 2(\cos \theta_{\max} + 1) \end{pmatrix}. \quad (16.30c)$$

**Torsionless isotropic cone 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{24} (3 \cos^2 \theta_{\max} + 6 \cos \theta_{\max} + 15), \quad (16.31a)$$

$$\mathbb{M}_{11} = \frac{1}{24} (\cos^2 \theta_{\max} + 10 \cos \theta_{\max} + 13), \quad (16.31b)$$

$$\mathbb{M}_{22} = \frac{1}{24} (4 \cos^2 \theta_{\max} + 10 \cos \theta_{\max} + 10), \quad (16.31c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.31d)$$

$$\mathbb{M}_{44} = \mathbb{M}_{00}, \quad (16.31e)$$

$$\mathbb{M}_{55} = \mathbb{M}_{22}, \quad (16.31f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.31g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{22}, \quad (16.31h)$$

$$\mathbb{M}_{88} = \frac{1}{3} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 1), \quad (16.31i)$$

$$\mathbb{M}_{04} = \frac{1}{24} (\cos^2 \theta_{\max} - 2 \cos \theta_{\max} + 1), \quad (16.31j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{04}, \quad (16.31k)$$

$$\mathbb{M}_{08} = -\frac{1}{6} (\cos^2 \theta_{\max} + \cos \theta_{\max} - 2), \quad (16.31l)$$

$$\mathbb{M}_{80} = \mathbb{M}_{08}, \quad (16.31m)$$

$$\mathbb{M}_{48} = \mathbb{M}_{08}, \quad (16.31n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{08}, \quad (16.31o)$$

$$\mathbb{M}_{13} = \mathbb{M}_{04}, \quad (16.31p)$$

$$\mathbb{M}_{31} = \mathbb{M}_{04}, \quad (16.31q)$$



$$\mathbb{M}_{26} = -\mathbb{M}_{08}, \quad (16.31r)$$

$$\mathbb{M}_{62} = -\mathbb{M}_{08}, \quad (16.31s)$$

$$\mathbb{M}_{57} = -\mathbb{M}_{08}, \quad (16.31t)$$

$$\mathbb{M}_{75} = -\mathbb{M}_{08}. \quad (16.31u)$$

After factorisation, the equations are

$$\mathbb{M}_{00} = \frac{1}{8} (\cos \theta_{\max} + 1)^2 + \frac{1}{2}, \quad (16.32a)$$

$$\mathbb{M}_{11} = \frac{1}{24} (\cos \theta_{\max} + 5)^2 - \frac{1}{2}, \quad (16.32b)$$

$$\mathbb{M}_{22} = \frac{1}{96} \left( (4 \cos \theta_{\max} + 5)^2 + 15 \right), \quad (16.32c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.32d)$$

$$\mathbb{M}_{44} = \mathbb{M}_{00}, \quad (16.32e)$$

$$\mathbb{M}_{55} = \mathbb{M}_{22}, \quad (16.32f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.32g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{22}, \quad (16.32h)$$

$$\mathbb{M}_{88} = \frac{1}{12} (2 \cos \theta_{\max} + 1)^2 + \frac{1}{4}, \quad (16.32i)$$

$$\mathbb{M}_{04} = \frac{1}{24} (\cos \theta_{\max} - 1)^2, \quad (16.32j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{04}, \quad (16.32k)$$

$$\mathbb{M}_{08} = -\frac{1}{6} (\cos \theta_{\max} + 2) (\cos \theta_{\max} - 1), \quad (16.32l)$$

$$\mathbb{M}_{80} = \mathbb{M}_{08}, \quad (16.32m)$$

$$\mathbb{M}_{48} = \mathbb{M}_{08}, \quad (16.32n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{08}, \quad (16.32o)$$

$$\mathbb{M}_{13} = \mathbb{M}_{04}, \quad (16.32p)$$

$$\mathbb{M}_{31} = \mathbb{M}_{04}, \quad (16.32q)$$

$$\mathbb{M}_{26} = -\mathbb{M}_{08}, \quad (16.32r)$$

$$\mathbb{M}_{62} = -\mathbb{M}_{08}, \quad (16.32s)$$

$$\mathbb{M}_{57} = -\mathbb{M}_{08}, \quad (16.32t)$$

$$\mathbb{M}_{75} = -\mathbb{M}_{08}. \quad (16.32u)$$

**Torsionless isotropic cone frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.30 and 16.32 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.9. The out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.10.

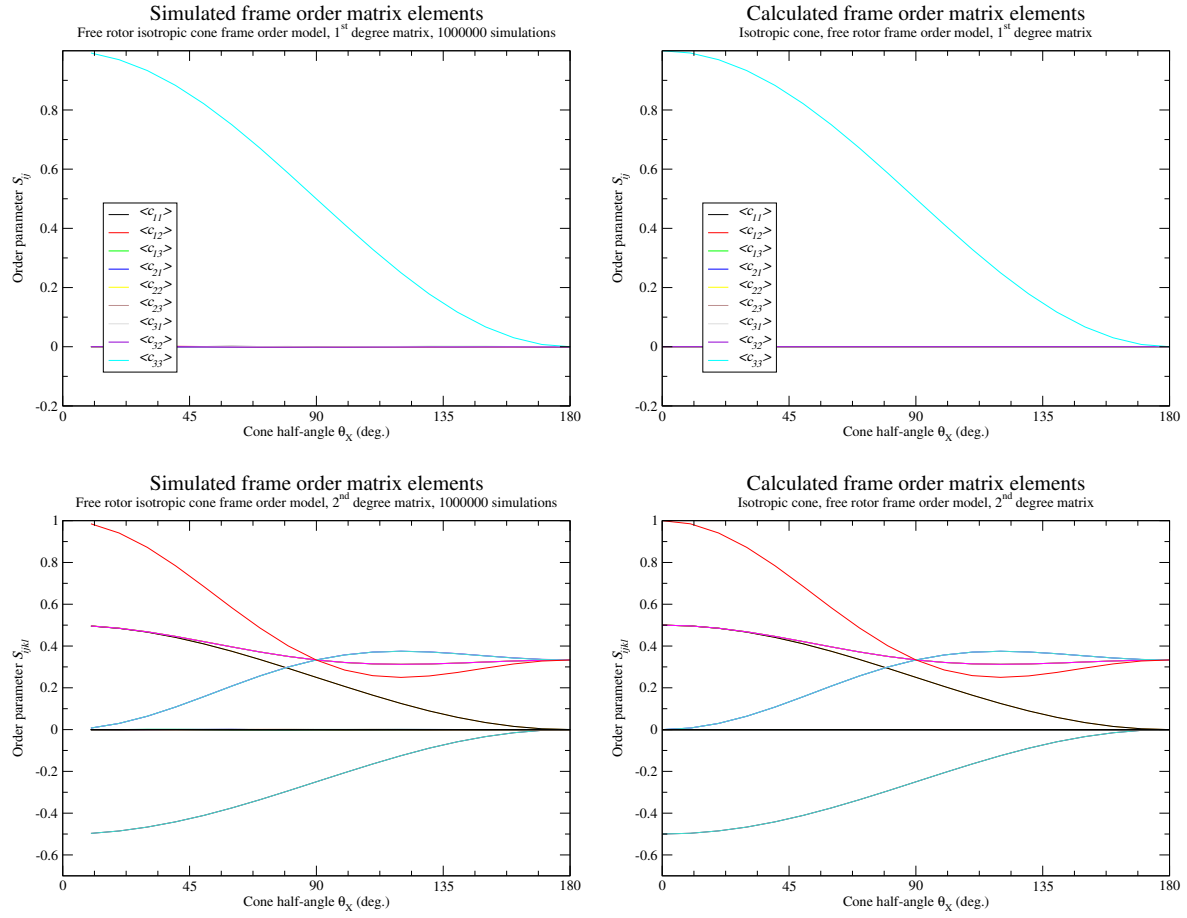


Figure 16.11: The free rotor isotropic cone model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$ . Frame order matrix values have been calculated every 10 degrees.

## 16.8 Free rotor isotropic cone frame order model

### 16.8.1 Free rotor isotropic cone parameterisation

For the free rotor variation of the isotropic cone model, the torsion angle restriction is absent and the model parameters are

$$\mathbb{M} = \mathbb{P} + \mathbb{E}_{\text{ax}} + \mathbb{p}_1 + \mathbb{S}, \quad (16.33a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\theta, E_\phi\} + \{p_x, p_y, p_z\} + \{\theta_{\text{max}}\}, \quad (16.33b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the spherical angles defining the cone axis,  $p_i$  are the coordinates of the pivot point, and  $\theta_{\text{max}}$  is the maximum cone opening half-angle.

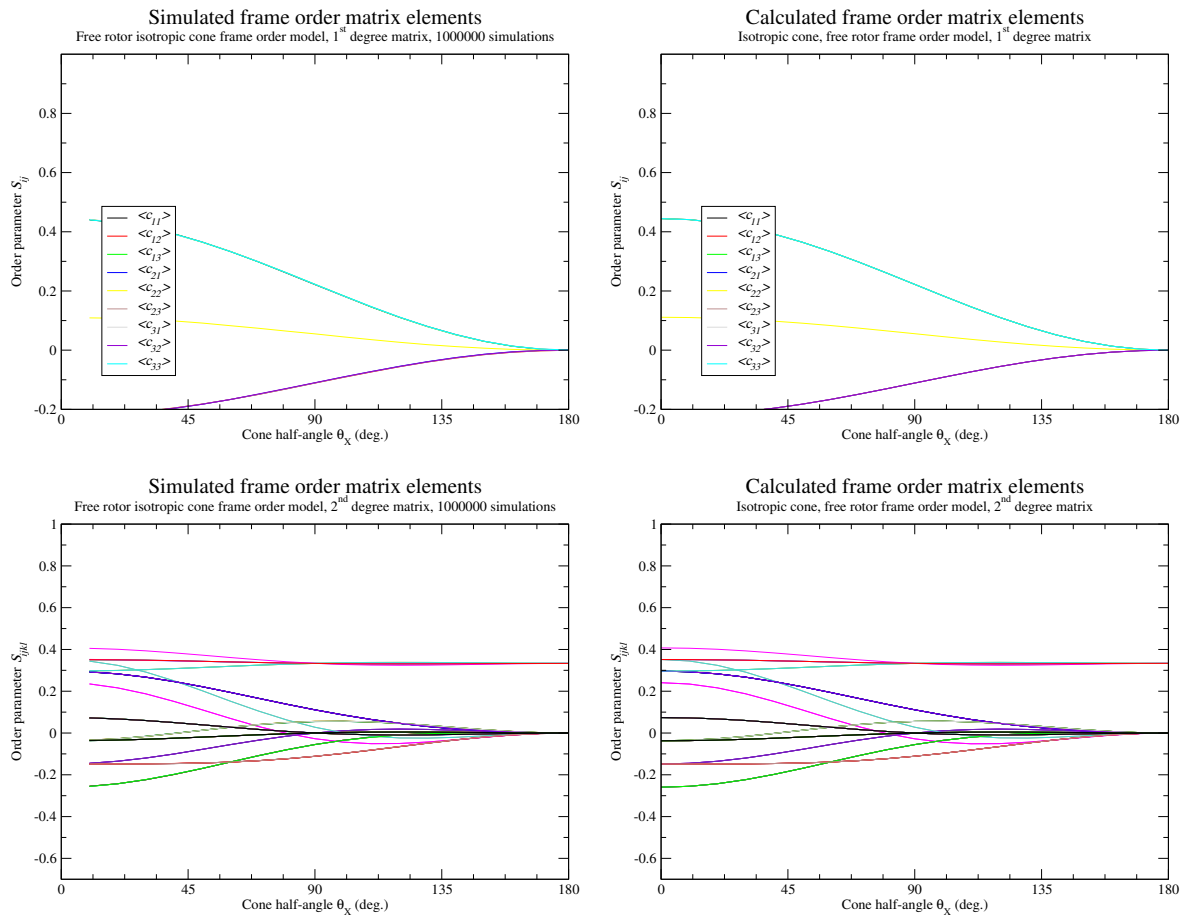


Figure 16.12: The free rotor isotropic cone model simulated and calculated out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  frame order matrix elements. The top row corresponds to  $\mathbb{M}^{(1)}$  and the bottom to  $\mathbb{M}^{(2)}$ . In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta$ . Frame order matrix values have been calculated every 10 degrees.

### 16.8.2 Free rotor isotropic cone equations

#### Free rotor isotropic cone rotation matrices

The rotation matrix is the full torsion-tilt rotation matrix of equation 12.74c on page 250.

#### Free rotor isotropic cone frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.34a)$$

$$= \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta d\theta d\phi d\sigma \Big/ \int_S dS. \quad (16.34b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta d\theta d\phi d\sigma, \quad (16.35a)$$

$$= \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi d\sigma, \quad (16.35b)$$

$$= \int_{-\pi}^{\pi} 2\pi (1 - \cos \theta_{\max}) d\sigma, \quad (16.35c)$$

$$= 4\pi^2 (1 - \cos \theta_{\max}). \quad (16.35d)$$

**Free rotor isotropic cone 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.36a)$$

$$= \int_S R dS \Big/ 2\pi (1 - \cos \theta_{\max}), \quad (16.36b)$$

$$= \frac{1}{2} \begin{pmatrix} \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \cos \theta_{\max} + 1 \end{pmatrix}. \quad (16.36c)$$

**Free rotor isotropic cone 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product

double indices from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{12} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 4), \quad (16.37a)$$

$$\mathbb{M}_{11} = \frac{1}{4} (\cos \theta_{\max} + 1), \quad (16.37b)$$

$$\mathbb{M}_{22} = 0, \quad (16.37c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.37d)$$

$$\mathbb{M}_{44} = \mathbb{M}_{00}, \quad (16.37e)$$

$$\mathbb{M}_{55} = 0, \quad (16.37f)$$

$$\mathbb{M}_{66} = 0, \quad (16.37g)$$

$$\mathbb{M}_{77} = 0, \quad (16.37h)$$

$$\mathbb{M}_{88} = \frac{1}{3} (\cos^2 \theta_{\max} + \cos \theta_{\max} + 1), \quad (16.37i)$$

$$\mathbb{M}_{04} = \mathbb{M}_{00}, \quad (16.37j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{00}, \quad (16.37k)$$

$$\mathbb{M}_{08} = -\frac{1}{6} (\cos^2 \theta_{\max} + \cos \theta_{\max} - 2), \quad (16.37l)$$

$$\mathbb{M}_{80} = \mathbb{M}_{08}, \quad (16.37m)$$

$$\mathbb{M}_{48} = \mathbb{M}_{08}, \quad (16.37n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{08}, \quad (16.37o)$$

$$\mathbb{M}_{13} = -\mathbb{M}_{11}, \quad (16.37p)$$

$$\mathbb{M}_{31} = -\mathbb{M}_{11}, \quad (16.37q)$$

$$\mathbb{M}_{26} = 0, \quad (16.37r)$$

$$\mathbb{M}_{62} = 0, \quad (16.37s)$$

$$\mathbb{M}_{57} = 0, \quad (16.37t)$$

$$\mathbb{M}_{75} = 0. \quad (16.37u)$$

**Free rotor isotropic cone frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.36 and 16.37 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.11. The out-of-frame  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values are shown in figure 16.12.

## 16.9 Pseudo-ellipse frame order model

### 16.9.1 Pseudo-ellipse parameterisation

To extend to the next level of motional complexity above the isotropic cone models, an anisotropic cone can be modelled. This cone is defined via the ball and socket joint pivoted mechanics with an angular restriction in all three angles. The simplest anisotropic distribution would be to create an ellipse using the standard quadric surface formula for an elliptic cone

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} - \frac{z^2}{c^2} = 0. \quad (16.38)$$

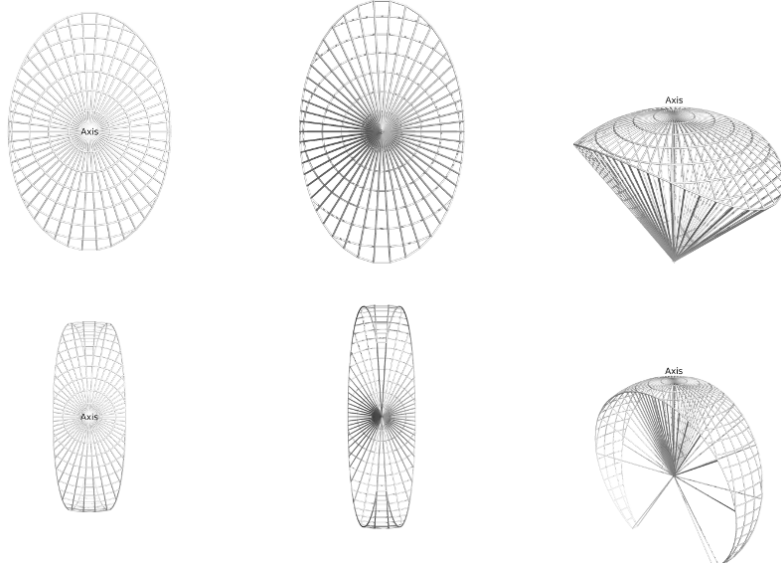


Figure 16.13: The pseudo-elliptic cone. The top three representations are for  $\theta_x = 30^\circ$  and  $\theta_y = 50^\circ$ . The bottom three representations are for  $\theta_x = 20^\circ$  and  $\theta_y = 160^\circ$ .

Let the two cone opening half-angles of the ellipse be  $\theta_x$  and  $\theta_y$ . For a sphere of radius  $z = 1$  and using the tilt angles  $\theta$  and  $\phi$ , a boundary polar angle  $\theta_{\max}$  can be modelled as

$$\frac{1}{\theta_{\max}^2} = \frac{\cos^2 \phi}{\theta_x^2} + \frac{\sin^2 \phi}{\theta_y^2}. \quad (16.39)$$

As the quadric constants  $a$ ,  $b$  and  $c$  are angles rather than axis lengths, this is not a true ellipse. It will therefore instead be called a pseudo-ellipse. The form of this pseudo-elliptic cone is shown in figure 16.13.

The model consists of the average domain position, a single pivot point, the full motional eigenframe, and the maximum cone opening and torsion half-angles

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E} + \mathfrak{p}_1 + \mathfrak{S}, \quad (16.40a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\alpha, E_\beta, E_\gamma\} + \{p_x, p_y, p_z\} + \{\theta_x, \theta_y, \sigma_{\max}\}, \quad (16.40b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the Euler angles defining the motional eigenframe,  $p_i$  are the coordinates of the pivot point,  $\theta_x$  and  $\theta_y$  are the maximum cone opening half-angles, and  $\sigma_{\max}$  is the torsion half-angle.

### 16.9.2 Derivation of a 2D trigonometric function - the pseudo-elliptic cosine

For the surface normalisation factor of the pseudo-elliptic cone, the integral from equation 16.51b on page 407 is

$$\int_S dS = \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi d\sigma. \quad (16.41)$$

When combined with the pseudo-ellipse of equation 16.39, this becomes the intractable integral

$$\int_S dS = \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} \left( 1 - \cos \left( \frac{1}{\sqrt{\frac{\cos^2 \phi}{\theta_x^2} + \frac{\sin^2 \phi}{\theta_y^2}}} \right) \right) d\phi d\sigma, \quad (16.42)$$

Instead the cosine series expansion will be used

$$\cos \theta_{\max} = 1 - \frac{\theta_{\max}^2}{2!} + \frac{\theta_{\max}^4}{4!} - \frac{\theta_{\max}^6}{6!} + \frac{\theta_{\max}^8}{8!} - \frac{\theta_{\max}^{10}}{10!} + \dots, \quad (16.43)$$

$$= \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n)!} \theta_{\max}^{2n}. \quad (16.44)$$

Integrating each element of the sum over the  $\phi$  parameter and using the assumption that  $\theta_x, \theta_y \geq 0$  gives

$$\begin{aligned} \int_{-\pi}^{\pi} \frac{\theta_{\max}^2}{2!} d\phi &= \pi \theta_x \theta_y, \\ \int_{-\pi}^{\pi} \frac{\theta_{\max}^4}{4!} d\phi &= \frac{\pi \theta_x \theta_y}{24} (\theta_x^2 + \theta_y^2), \\ \int_{-\pi}^{\pi} \frac{\theta_{\max}^6}{6!} d\phi &= \frac{\pi \theta_x \theta_y}{2880} (3\theta_x^4 + 2\theta_x^2 \theta_y^2 + 3\theta_y^4), \\ \int_{-\pi}^{\pi} \frac{\theta_{\max}^8}{8!} d\phi &= \frac{\pi \theta_x \theta_y}{322560} (5\theta_x^6 + 3\theta_x^4 \theta_y^2 + 3\theta_x^2 \theta_y^4 + 5\theta_y^6), \\ \int_{-\pi}^{\pi} \frac{\theta_{\max}^{10}}{10!} d\phi &= \frac{\pi \theta_x \theta_y}{232243200} (35\theta_x^8 + 20\theta_x^6 \theta_y^2 + 18\theta_x^4 \theta_y^4 + 20\theta_x^2 \theta_y^6 + 35\theta_y^8), \\ &\dots \end{aligned} \quad (16.45)$$

Therefore a new two dimension trigonometric function, the pseudo-elliptic cosine, can be defined as

$$\text{pec}(\theta_x, \theta_y) = \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi, \quad (16.46a)$$

$$= 2\pi \theta_x \theta_y \sum_{n=0}^{\infty} \frac{(-1)^n}{4^n (2n+2)!} f_n(\theta_x, \theta_y). \quad (16.46b)$$

Let

$$\begin{aligned} a &= \theta_x^2, \\ b &= \theta_y^2, \end{aligned} \quad (16.47)$$

then the first of the  $f_n(\theta_x, \theta_y)$  equations are

$$\begin{aligned}
f_0 &= 1, \\
f_1 &= 2a + 2b, \\
f_2 &= 6a^2 + 4ab + 6b^2, \\
f_3 &= 20a^3 + 12a^2b + 12ab^2 + 20b^3, \\
f_4 &= 70a^4 + 40a^3b + 36a^2b^2 + 40ab^3 + 70b^4, \\
f_5 &= 252a^5 + 140a^4b + 120a^3b^2 + 120a^2b^3 + 140ab^4 + 252b^5, \\
f_6 &= 924a^6 + 504a^5b + 420a^4b^2 + 400a^3b^3 + 420a^2b^4 + 504ab^5 + 924b^6, \\
f_7 &= 3432a^7 + 1848a^6b + 1512a^5b^2 + 1400a^4b^3 + 1400a^3b^4 + 1512a^2b^5 + 1848ab^6 + 3432b^7, \\
&\dots
\end{aligned} \tag{16.48}$$

Or

$$\begin{aligned}
f_0 &= 1, \\
f_1 &= 2(a + b), \\
f_2 &= 6(a^2 + b^2) + 4ab, \\
f_3 &= 20(a^3 + b^3) + 12(a^2b + ab^2), \\
f_4 &= 70(a^4 + b^4) + 40(a^3b + ab^3) + 36a^2b^2, \\
f_5 &= 252(a^5 + b^5) + 140(a^4b + ab^4) + 120(a^3b^2 + a^2b^3), \\
f_6 &= 924(a^6 + b^6) + 504(a^5b + ab^5) + 420(a^4b^2 + a^2b^4) + 400a^3b^3, \\
f_7 &= 3432(a^7 + b^7) + 1848(a^6b + ab^6) + 1512(a^5b^2 + a^2b^5) + 1400(a^4b^3 + a^3b^4), \\
f_8 &= 12870(a^8 + b^8) + 6864(a^7b + ab^7) + 5544(a^6b^2 + a^2b^6) + 5040(a^5b^3 + a^3b^5) + 4900a^4b^4, \\
f_9 &= 48620(a^9 + b^9) + 25740(a^8b + ab^8) + 20592(a^7b^2 + a^2b^7) + 18480(a^6b^3 + a^3b^6) + 17640(a^5b^4 + a^4b^5), \\
f_{10} &= 184756(a^{10} + b^{10}) + 97240(a^9b + ab^9) + 77220(a^8b^2 + a^2b^8) + 68640(a^7b^3 + a^3b^7) + 64680(a^6b^4 + a^4b^6) + 63504a^5b^5, \\
&\dots
\end{aligned} \tag{16.49}$$

This series expansion up to  $n = 10$  is sufficient for writing a fast and accurate pec function implementation in computer code. The numerical representation of this function is shown in figure 16.14.

### 16.9.3 Pseudo-ellipse equations

#### Pseudo-ellipse rotation matrices

For the pseudo-ellipse model, the full torsion-tilt system is used. The full rotation matrix is given in equation 12.74c on page 250.



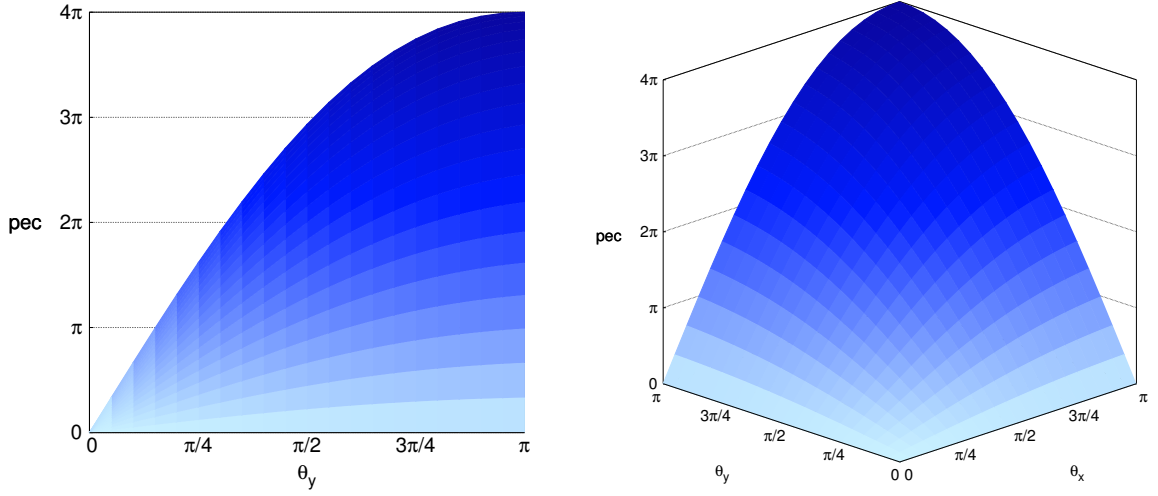


Figure 16.14: The pseudo-ellipse cosine 2D trigonometric function. This is the surface area on a unit sphere bounded by the pseudo-elliptic cone.

### Pseudo-ellipse frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.50a)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta d\theta d\phi d\sigma \Big/ \int_S dS. \quad (16.50b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta d\theta d\phi d\sigma, \quad (16.51a)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi d\sigma, \quad (16.51b)$$

$$= \int_{-\sigma_{\max}}^{\sigma_{\max}} \text{pec}(\theta_x, \theta_y) d\sigma, \quad (16.51c)$$

$$= 2\sigma_{\max} \text{pec}(\theta_x, \theta_y). \quad (16.51d)$$

**Pseudo-ellipse 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 consists of the following elements

$$\mathbb{M}_{00} = \frac{\text{sinc } \sigma_{\max}}{2 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \left( \cos^2 \phi \sin^2 \theta_{\max} - 2 \sin^2 \phi \cos \theta_{\max} \right) d\phi \right], \quad (16.52a)$$

$$\mathbb{M}_{11} = \frac{\text{sinc } \sigma_{\max}}{2 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \left( \sin^2 \phi \sin^2 \theta_{\max} - 2 \cos^2 \phi \cos \theta_{\max} \right) d\phi \right], \quad (16.52b)$$

$$\mathbb{M}_{22} = \frac{1}{2\sigma_{\max} \text{pec}(\theta_x, \theta_y)} \int_{-\pi}^{\pi} \sin^2 \theta_{\max} d\phi. \quad (16.52c)$$

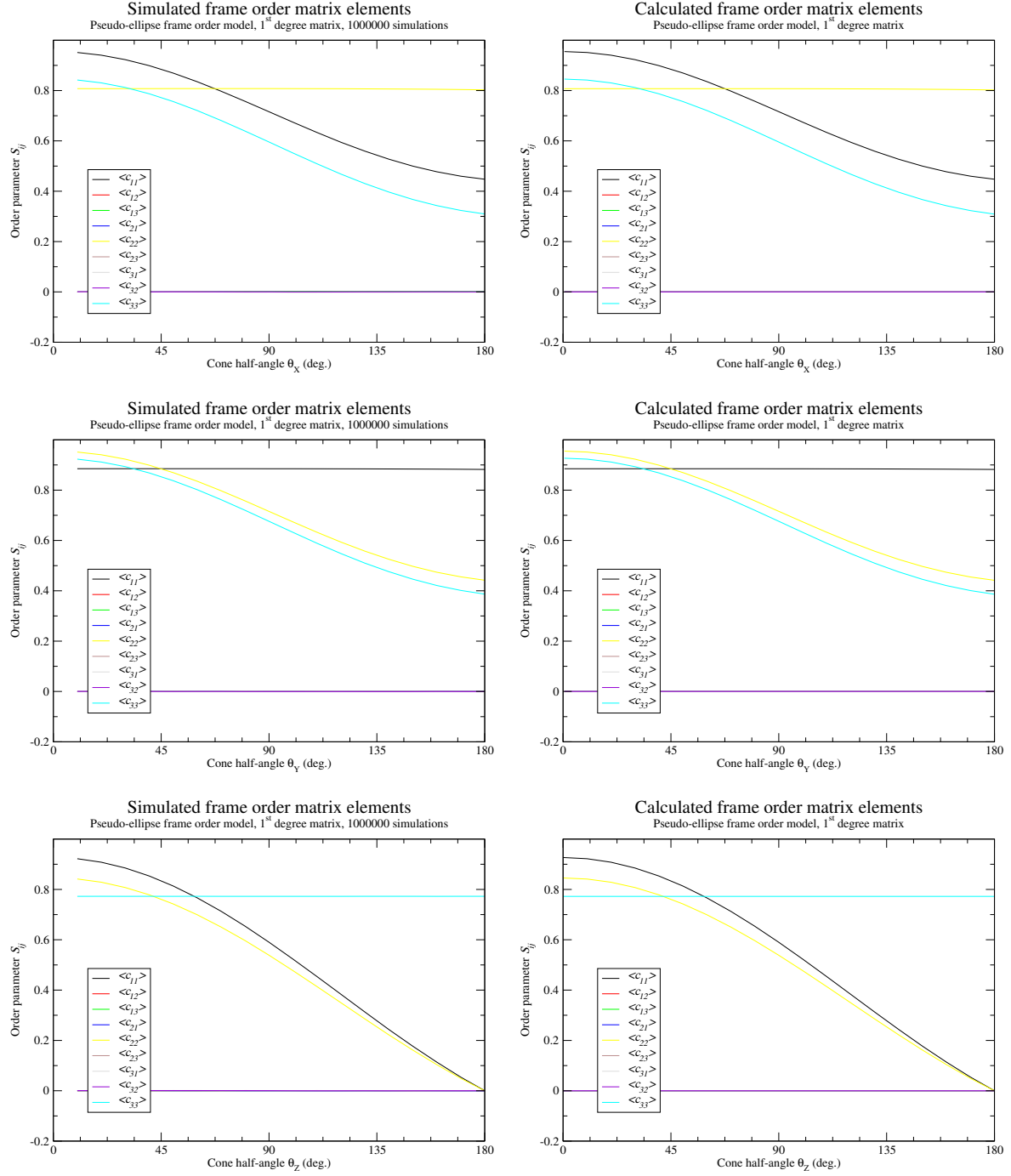


Figure 16.15: The pseudo-ellipse model simulated and calculated in-frame  $\mathfrak{N}^{(1)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$ ,  $\theta_y$  to the cone opening half-angle  $\theta_y$  and  $\theta_z$  to torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$ ,  $\theta_y = 3\pi/8$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated  $\theta_x$  and  $\theta_y$  graphs is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

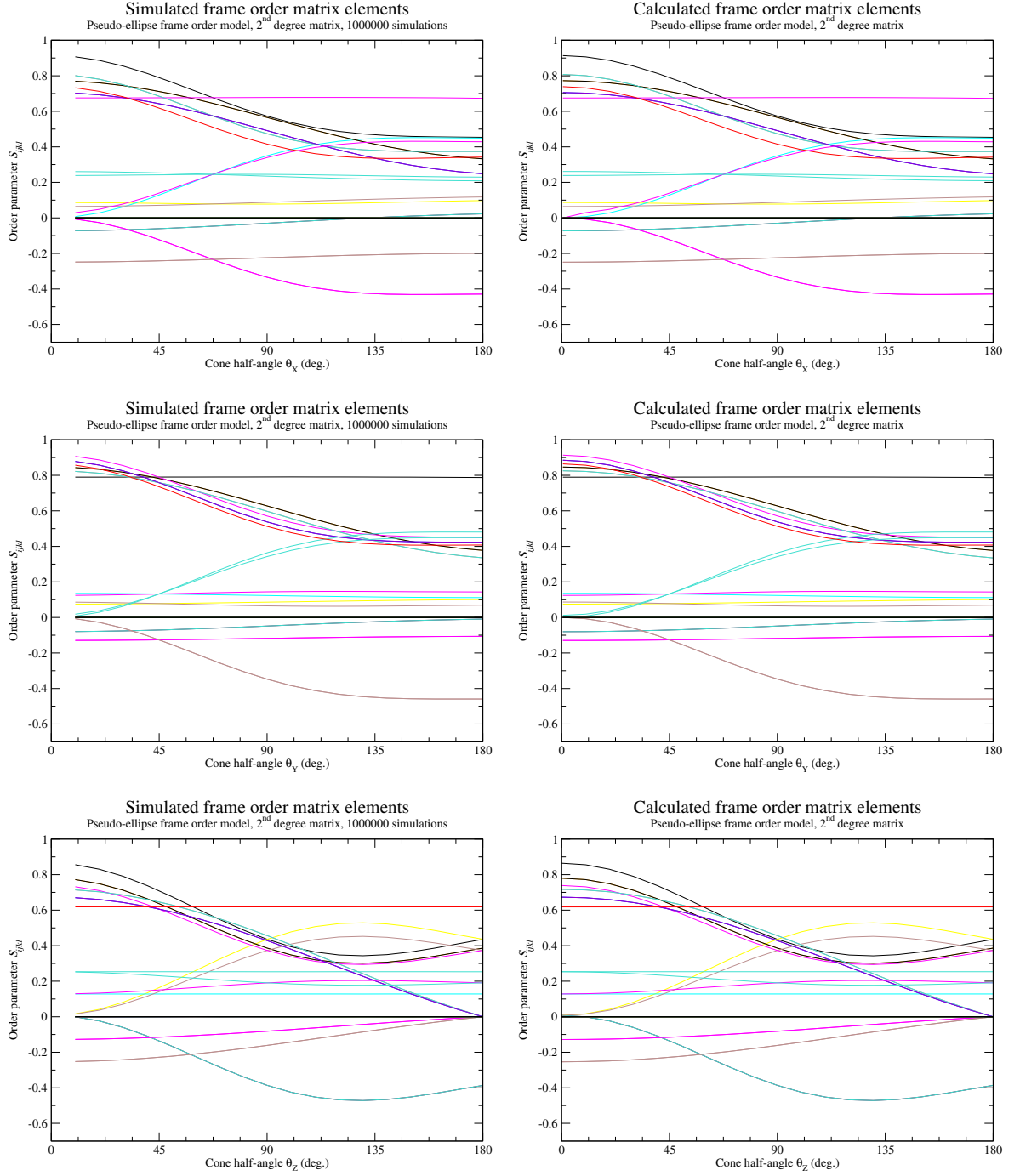


Figure 16.16: The pseudo-ellipse model simulated and calculated in-frame  $\mathbb{N}^{(2)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$ ,  $\theta_y$  to the cone opening half-angle  $\theta_y$  and  $\theta_z$  to torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$ ,  $\theta_y = 3\pi/8$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated  $\theta_x$  and  $\theta_y$  graphs is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

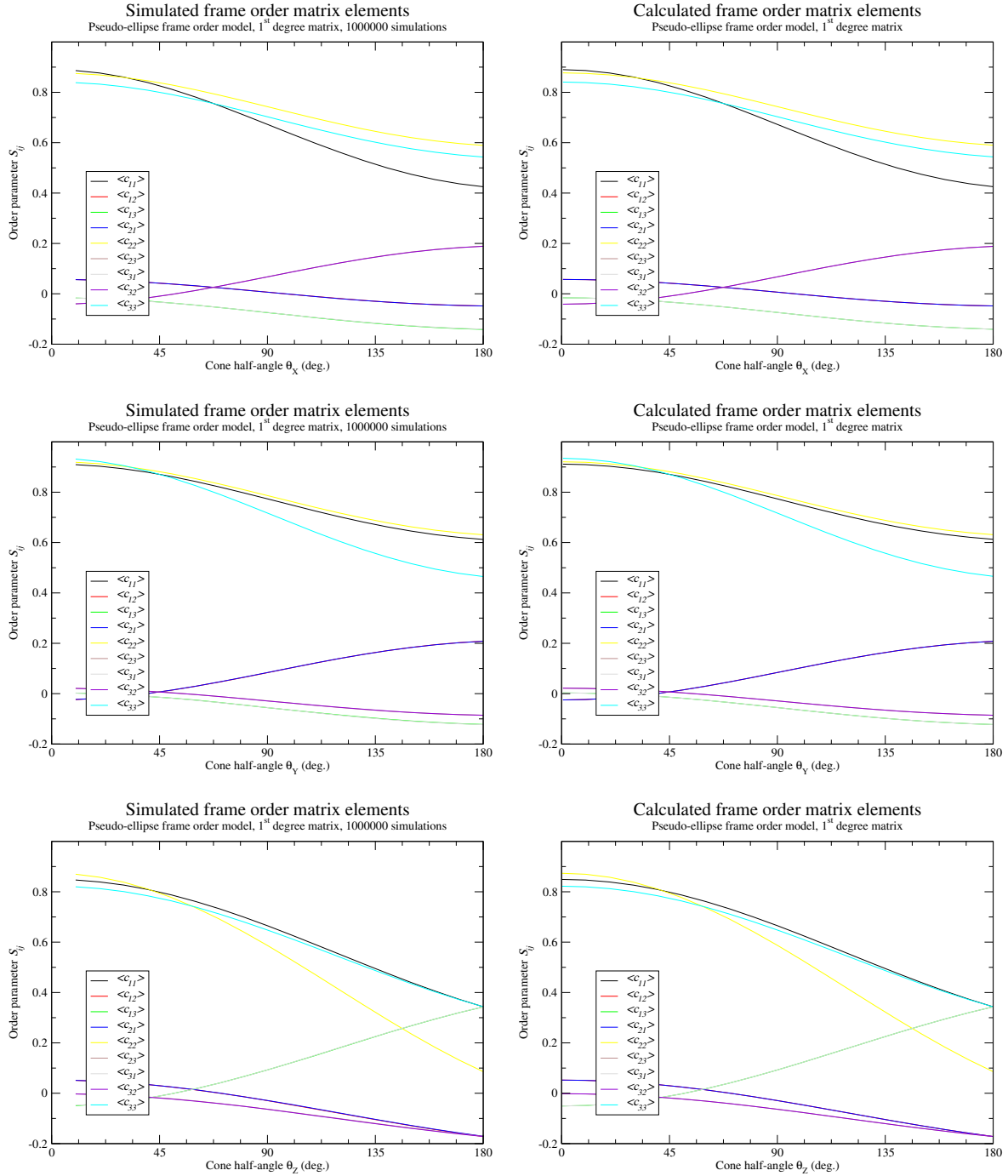


Figure 16.17: The pseudo-ellipse model simulated and calculated out-of-frame  $\mathbb{M}^{(1)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$ ,  $\theta_y$  to the cone opening half-angle  $\theta_y$  and  $\theta_z$  to torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$ ,  $\theta_y = 3\pi/8$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated  $\theta_x$  and  $\theta_y$  graphs is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

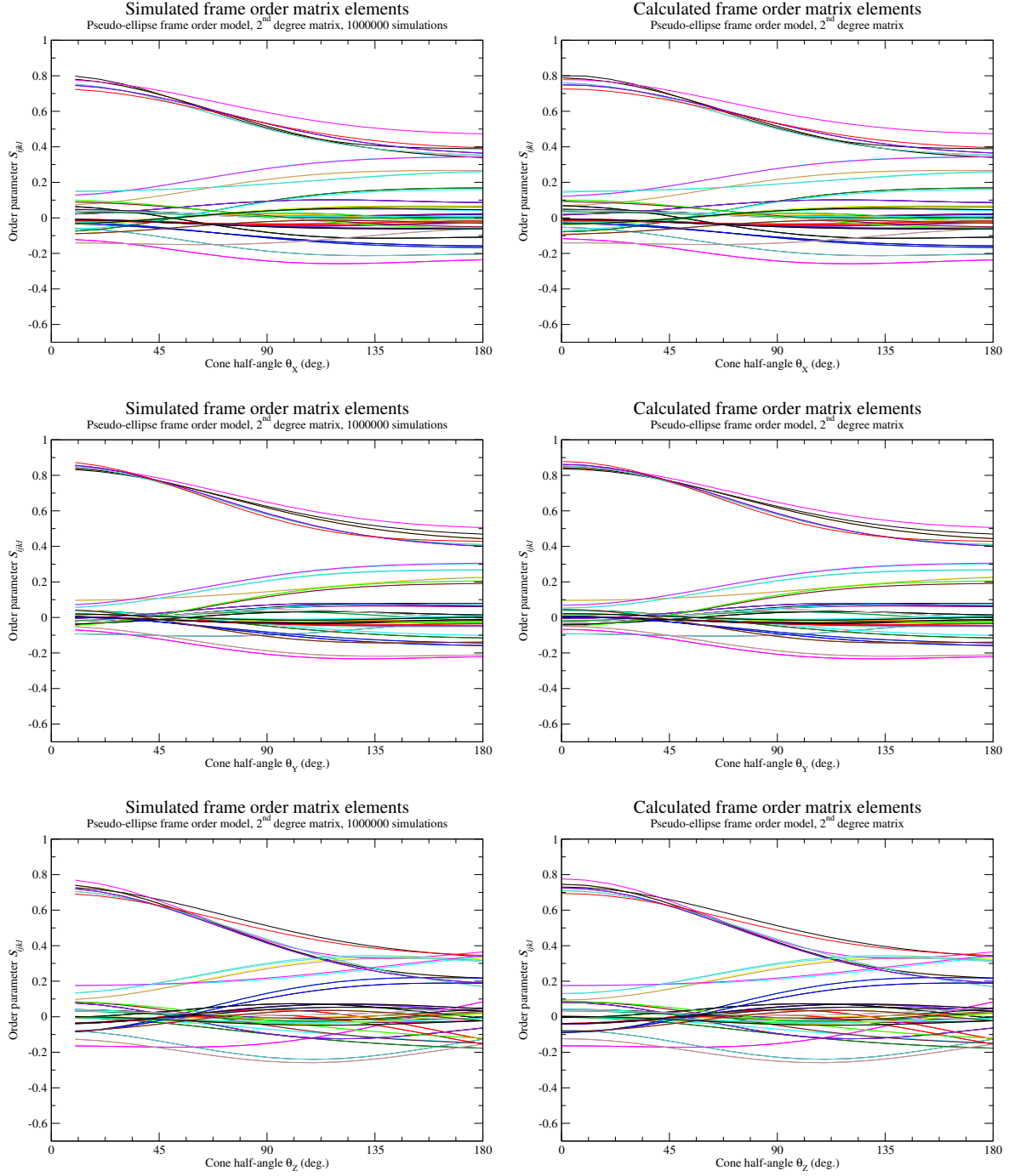


Figure 16.18: The pseudo-ellipse model simulated and calculated out-of-frame  $\mathfrak{N}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta_x$ ,  $\theta_Y$  to the cone opening half-angle  $\theta_y$  and  $\theta_Z$  to torsion half-angle  $\sigma_{\max}$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$ ,  $\theta_y = 3\pi/8$  or  $\sigma_{\max} = \pi/6$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated  $\theta_X$  and  $\theta_Y$  graphs is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

As the trigonometric functions of  $\theta_{\max}$  cannot be integrated, these components must be numerically integrated.

**Pseudo-ellipse 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices from 0 to 8

$$\begin{aligned} \mathbb{M}_{00} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi (\text{sinc}(2\sigma_{\max}) + 2) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 \sin^2 \theta_{\max} \cos^2 \phi \left( (2 \cos^2 \phi - 1) \cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. + 6 \sin^2 \phi \right) - 2 \cos \theta_{\max} \left( 2 \cos^2 \phi (4 \cos^2 \phi - 5) + 3 \right) \right) \right. \\ & \left. \left. + 2 \cos^2 \phi \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) - 6 \cos \theta_{\max} d\phi \right] \right], \end{aligned} \quad (16.53a)$$

$$\begin{aligned} \mathbb{M}_{11} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi \text{sinc}(2\sigma_{\max}) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( \sin^2 \theta_{\max} \left( 4 \cos^2 \phi \sin^2 \phi (\cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. - 3) + 3 \right) - 16 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} \right) \right. \\ & \left. \left. + 3 \sin^2 \theta_{\max} d\phi \right] \right], \end{aligned} \quad (16.53b)$$

$$\mathbb{M}_{22} = \frac{\text{sinc}(\sigma_{\max})}{6 \text{pec}(\theta_x, \theta_y)} \left[ 5\pi - \int_{-\pi}^{\pi} 2 \cos^2 \phi \cos^3 \theta_{\max} + 3 \sin^2 \phi \cos^2 \theta_{\max} d\phi \right], \quad (16.53c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.53d)$$

$$\begin{aligned} \mathbb{M}_{44} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi (\text{sinc}(2\sigma_{\max}) + 2) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 \sin^2 \theta_{\max} \sin^2 \phi \left( (2 \sin^2 \phi - 1) \cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. + 6 \cos^2 \phi \right) - 2 \cos \theta_{\max} \left( 2 \sin^2 \phi (4 \sin^2 \phi - 5) + 3 \right) \right) \right. \\ & \left. \left. + 2 \sin^2 \phi \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) - 6 \cos \theta_{\max} d\phi \right] \right], \end{aligned} \quad (16.53e)$$

$$\mathbb{M}_{55} = \frac{\text{sinc}(\sigma_{\max})}{6 \text{pec}(\theta_x, \theta_y)} \left[ 5\pi - \int_{-\pi}^{\pi} 2 \sin^2 \phi \cos^3 \theta_{\max} + 3 \cos^2 \phi \cos^2 \theta_{\max} d\phi \right], \quad (16.53f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.53g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{55}, \quad (16.53h)$$

$$\mathbb{M}_{88} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi - \int_{-\pi}^{\pi} \cos^3 \theta_{\max} d\phi \right], \quad (16.53i)$$

$$\begin{aligned} \mathbb{M}_{04} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi (2 - \text{sinc}(2\sigma_{\max})) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 \sin^2 \theta_{\max} \cos^2 \phi \left( (2 \sin^2 \phi - 1) \cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. - 6 \sin^2 \phi \right) + 2 \cos \theta_{\max} \left( 2 \cos^2 \phi (4 \cos^2 \phi - 5) + 3 \right) \right) \right. \\ & \left. + 2 \cos^2 \phi \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) - 6 \cos \theta_{\max} d\phi \right], \end{aligned} \quad (16.53j)$$

$$\begin{aligned} \mathbb{M}_{40} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi (2 - \text{sinc}(2\sigma_{\max})) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 \sin^2 \theta_{\max} \sin^2 \phi \left( (2 \cos^2 \phi - 1) \cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. - 6 \cos^2 \phi \right) + 2 \cos \theta_{\max} \left( 2 \sin^2 \phi (4 \sin^2 \phi - 5) + 3 \right) \right) \right. \\ & \left. + 2 \sin^2 \phi \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) - 6 \cos \theta_{\max} d\phi \right], \end{aligned} \quad (16.53k)$$

$$\mathbb{M}_{08} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi - \int_{-\pi}^{\pi} \cos \theta_{\max} \cos^2 \phi (\sin^2 \theta_{\max} + 2) d\phi \right], \quad (16.53l)$$

$$\begin{aligned} \mathbb{M}_{80} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 8\pi + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 (1 - 2 \cos^2 \phi) \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) \right) \right. \\ & \left. + 2 \cos^3 \theta_{\max} - 6 \cos \theta_{\max} d\phi \right], \end{aligned} \quad (16.53m)$$

$$\mathbb{M}_{48} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi - \int_{-\pi}^{\pi} \cos \theta_{\max} \sin^2 \phi (\sin^2 \theta_{\max} + 2) d\phi \right], \quad (16.53n)$$

$$\begin{aligned} \mathbb{M}_{84} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 8\pi - \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( 2 (1 - 2 \cos^2 \phi) \cos \theta_{\max} (\sin^2 \theta_{\max} + 2) \right) \right. \\ & \left. - 2 \cos^3 \theta_{\max} + 6 \cos \theta_{\max} d\phi \right], \end{aligned} \quad (16.53o)$$

$$\begin{aligned} \mathbb{M}_{13} = \frac{1}{12 \text{pec}(\theta_x, \theta_y)} & \left[ 4\pi \text{sinc}(2\sigma_{\max}) + \int_{-\pi}^{\pi} \text{sinc}(2\sigma_{\max}) \left( \sin^2 \theta_{\max} \left( 4 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} \right. \right. \right. \\ & \left. \left. \left. - 12 \cos^2 \phi \sin^2 \phi + 3 \right) - 16 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} \right) \right. \\ & \left. - 3 \sin^2 \theta_{\max} d\phi \right], \end{aligned} \quad (16.53p)$$

$$\mathbb{M}_{31} = \mathbb{M}_{13}, \quad (16.53q)$$

$$\mathbb{M}_{26} = -\frac{\text{sinc}(\sigma_{\max})}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \cos^2 \phi (\cos^3 \theta_{\max} - 3 \cos \theta_{\max}) d\phi \right], \quad (16.53r)$$

$$\mathbb{M}_{62} = \mathbb{M}_{26}, \quad (16.53s)$$

$$\mathbb{M}_{57} = -\frac{\text{sinc}(\sigma_{\max})}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \sin^2 \phi (\cos^3 \theta_{\max} - 3 \cos \theta_{\max}) d\phi \right], \quad (16.53t)$$

$$\mathbb{M}_{75} = \mathbb{M}_{57}. \quad (16.53u)$$

As the trigonometric functions of  $\theta_{\max}$  cannot be integrated, these components must be numerically integrated. All other frame order matrix elements can be numerically shown to be zero.

**Pseudo-ellipse frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.52 and 16.53 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.15 and  $\mathbb{M}^{(2)}$  in figure 16.16. The out-of-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.17 and  $\mathbb{M}^{(2)}$  in figure 16.18.

## 16.10 Torsionless pseudo-ellipse frame order model

The first simplification of the pseudo-ellipse frame order model, which can be very useful for restricted motion systems such as CaM complexed with target peptides, would be to have no torsional motions.



### 16.10.1 Torsionless pseudo-ellipse parameterisation

This model is the pseudo-ellipse model with the torsion angle set to zero,  $\sigma_{\max} = 0$ . The model parameters are therefore

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E} + \mathfrak{p}_1 + \mathfrak{S}, \quad (16.54a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\alpha, E_\beta, E_\gamma\} + \{p_x, p_y, p_z\} + \{\theta_x, \theta_y\}, \quad (16.54b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the Euler angles defining the motional eigenframe,  $p_i$  are the coordinates of the pivot point, and  $\theta_x$  and  $\theta_y$  are the maximum cone opening half-angles.

### 16.10.2 Torsionless pseudo-ellipse equations

#### Torsionless pseudo-ellipse rotation matrices

Setting the torsion angle  $\sigma$  to zero in the full torsion-tilt rotation matrix of equation 12.74c, the matrix becomes

$$R(\theta, \phi) = \begin{pmatrix} \cos^2 \phi \cos \theta + \sin^2 \phi & \cos \phi \sin \phi \cos \theta - \cos \phi \sin \phi & \cos \phi \sin \theta \\ \cos \phi \sin \phi \cos \theta - \cos \phi \sin \phi & \sin^2 \phi \cos \theta + \cos^2 \phi & \sin \phi \sin \theta \\ -\cos \phi \sin \theta & -\sin \phi \sin \theta & \cos \theta \end{pmatrix}. \quad (16.55)$$

#### Torsionless pseudo-ellipse frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.56a)$$

$$= \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta d\theta d\phi \Big/ \int_S dS. \quad (16.56b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta d\theta d\phi, \quad (16.57a)$$

$$= \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi, \quad (16.57b)$$

$$= \text{pec}(\theta_x, \theta_y). \quad (16.57c)$$

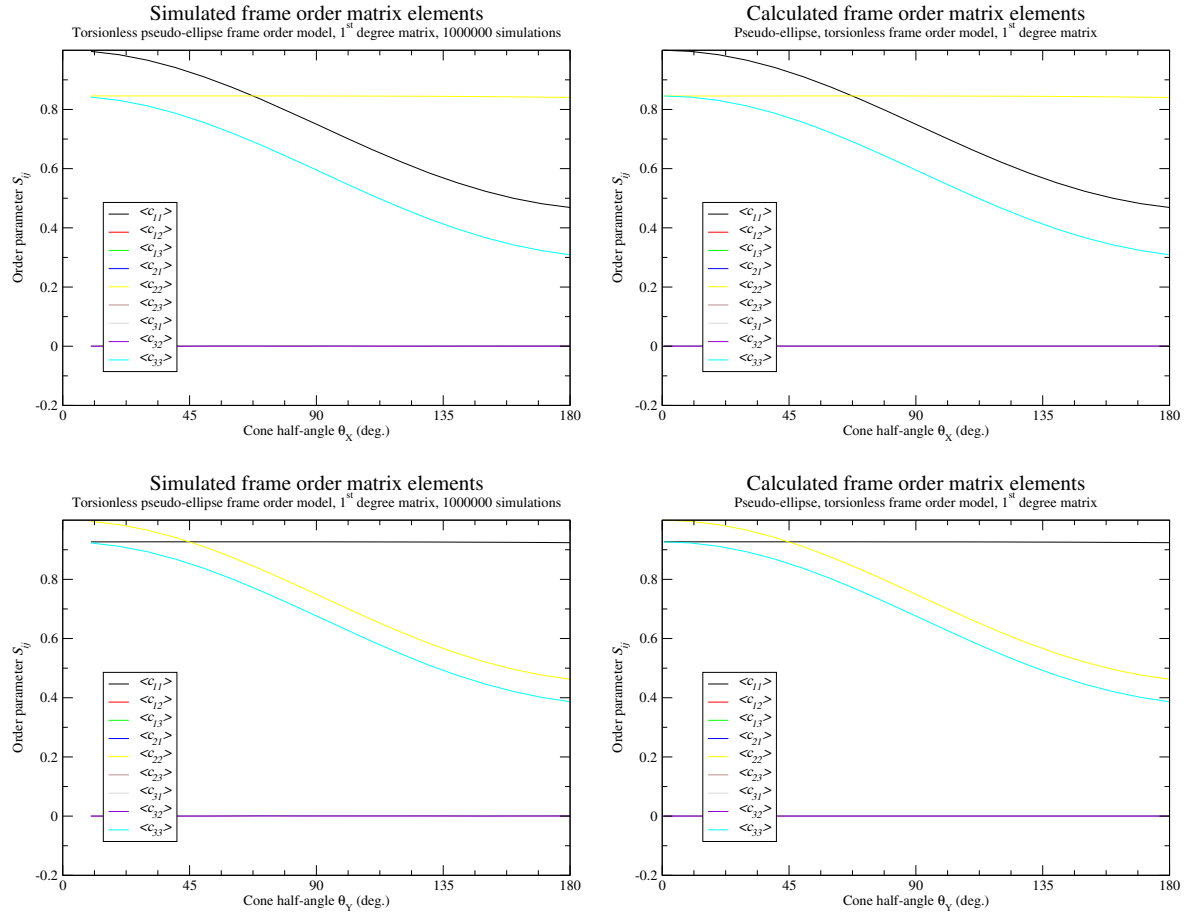


Figure 16.19: The torsionless pseudo-ellipse model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_Y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

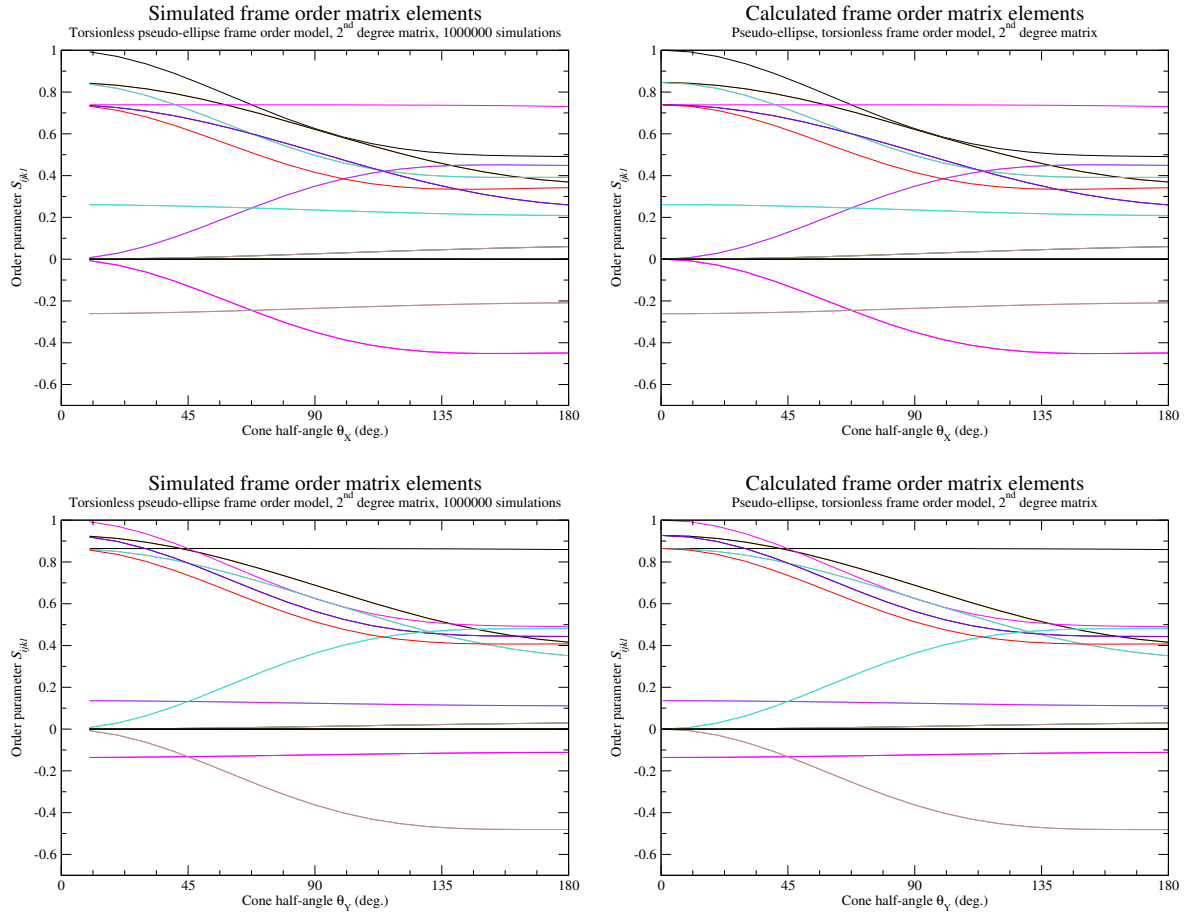


Figure 16.20: The torsionless pseudo-ellipse model simulated and calculated in-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

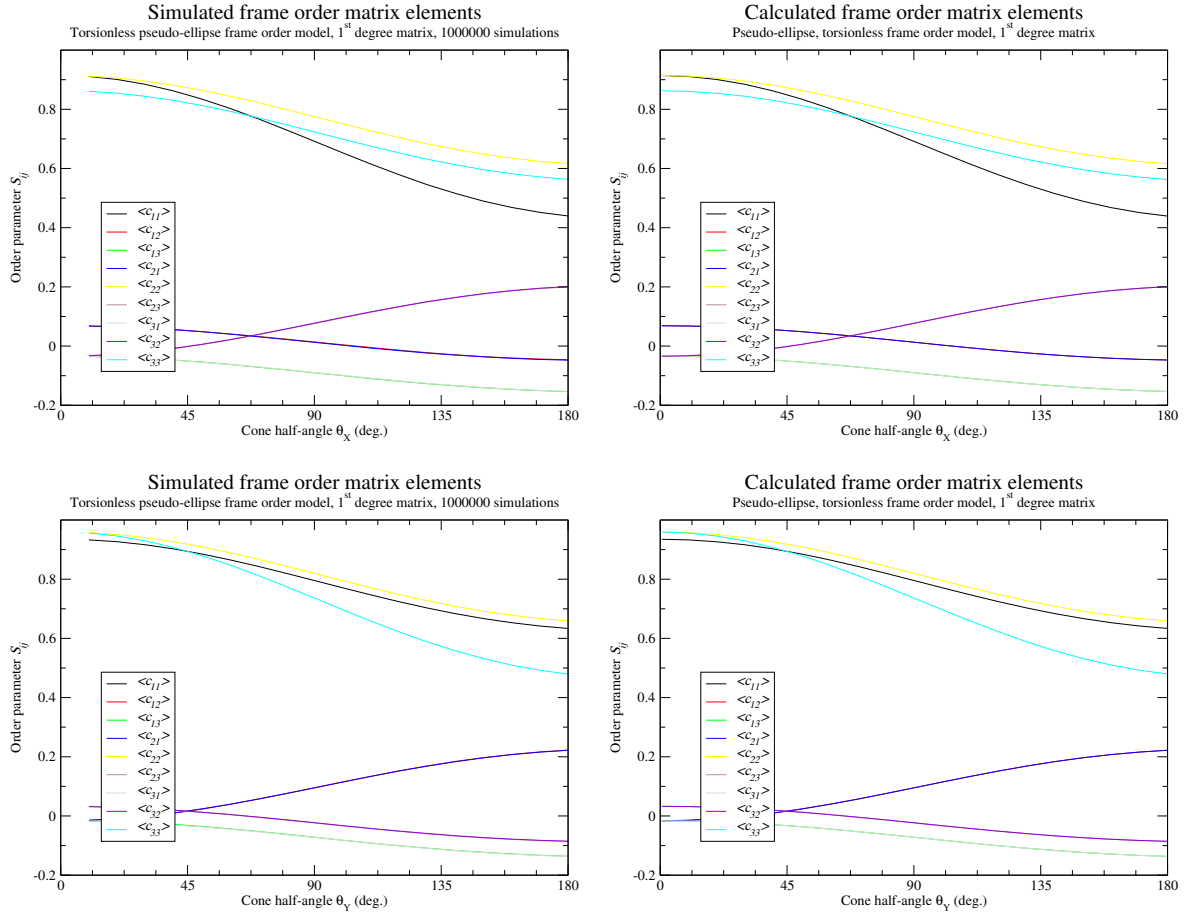


Figure 16.21: The torsionless pseudo-ellipse model simulated and calculated out-of-frame  $\mathbb{N}^{(1)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

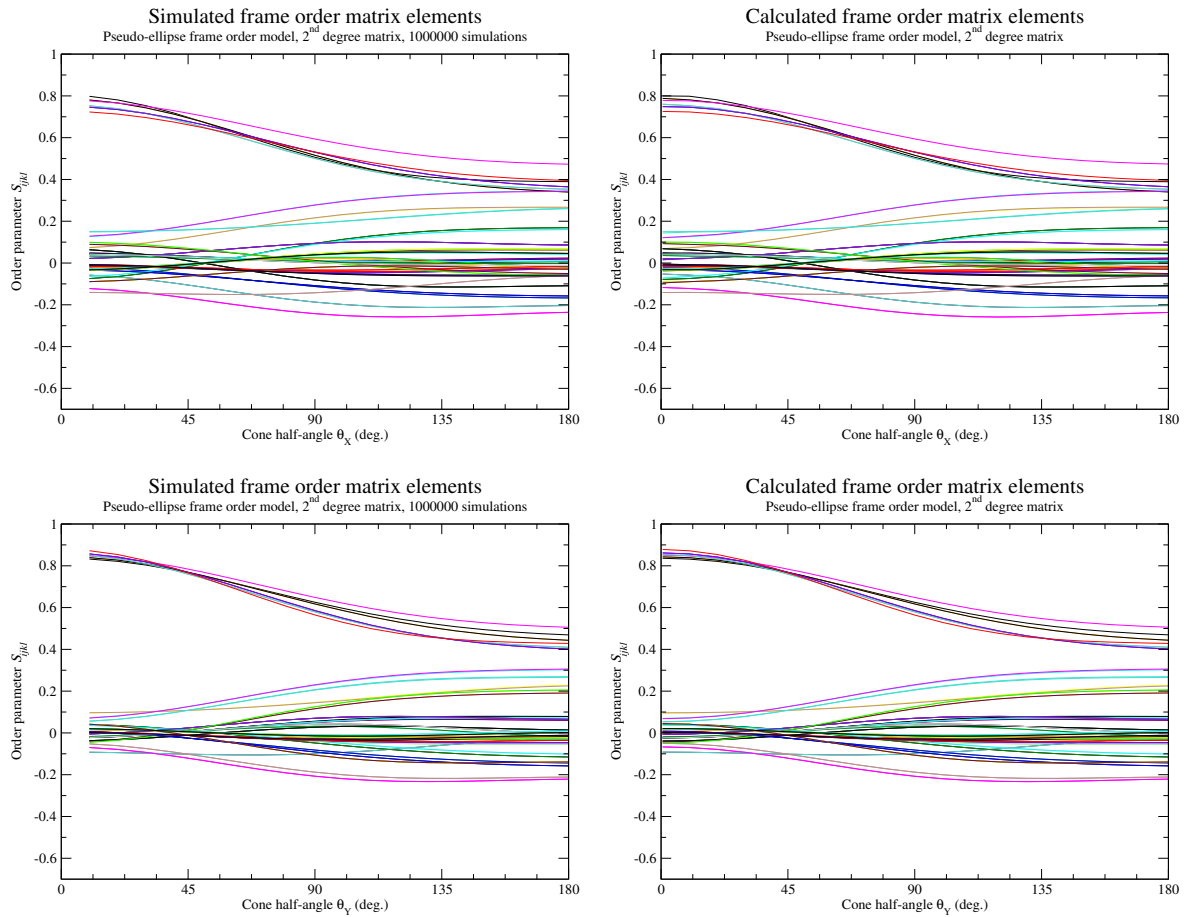


Figure 16.22: The torsionless pseudo-ellipse model simulated and calculated out-of-frame  $\mathbb{N}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_Y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

**Torsionless pseudo-ellipse 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 consists of the following elements

$$\mathbb{M}_{00} = \frac{1}{2 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \left( \cos^2 \phi \sin^2 \theta_{\max} - 2 \sin^2 \phi \cos \theta_{\max} \right) d\phi \right], \quad (16.58a)$$

$$\mathbb{M}_{11} = \frac{1}{2 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \left( \sin^2 \phi \sin^2 \theta_{\max} - 2 \cos^2 \phi \cos \theta_{\max} \right) d\phi \right], \quad (16.58b)$$

$$\mathbb{M}_{22} = \frac{1}{2 \text{pec}(\theta_x, \theta_y)} \int_{-\pi}^{\pi} \sin^2 \theta_{\max} d\phi. \quad (16.58c)$$

As the trigonometric functions of  $\theta_{\max}$  cannot be symbolically integrated, these components must be numerically integrated.

**Torsionless pseudo-ellipse 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 3\pi + \int_{-\pi}^{\pi} \left( \cos^4 \phi \cos \theta_{\max} + 3 \cos^2 \phi \sin^2 \phi \right) \sin^2 \theta_{\max} \right. \\ \left. - \left( 3 \sin^4 \phi + \cos^4 \phi \right) \cos \theta_{\max} d\phi \right], \quad (16.59a)$$

$$\mathbb{M}_{11} = \frac{1}{6 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \left( 2 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} + 3 \sin^4 \phi + 3 \cos^4 \phi \right) \sin^2 \theta_{\max} \right. \\ \left. - 8 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.59b)$$

$$\mathbb{M}_{22} = \frac{1}{6 \text{pec}(\theta_x, \theta_y)} \left[ 5\pi - \int_{-\pi}^{\pi} 2 \cos^2 \phi \cos^3 \theta_{\max} + 3 \sin^2 \phi \cos^2 \theta_{\max} d\phi \right], \quad (16.59c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.59d)$$

$$\mathbb{M}_{44} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 3\pi + \int_{-\pi}^{\pi} \left( \sin^4 \phi \cos \theta_{\max} + 3 \cos^2 \phi \sin^2 \phi \right) \sin^2 \theta_{\max} \right. \\ \left. - \left( \sin^4 \phi + 3 \cos^4 \phi \right) \cos \theta_{\max} d\phi \right], \quad (16.59e)$$

$$\mathbb{M}_{55} = \frac{1}{6 \text{pec}(\theta_x, \theta_y)} \left[ 5\pi - \int_{-\pi}^{\pi} 2 \sin^2 \phi \cos^3 \theta_{\max} + 3 \cos^2 \phi \cos^2 \theta_{\max} d\phi \right], \quad (16.59f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.59g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{55}, \quad (16.59h)$$

$$\mathbb{M}_{88} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi - \int_{-\pi}^{\pi} \cos^3 \theta_{\max} d\phi \right], \quad (16.59i)$$

$$\mathbb{M}_{04} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ \pi + \int_{-\pi}^{\pi} (\cos^2 \phi \sin^2 \phi \cos \theta_{\max} - 3 \cos^2 \phi \sin^2 \phi) \sin^2 \theta_{\max} \right. \\ \left. - 4 \cos^2 \phi \sin^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.59j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{04}, \quad (16.59k)$$

$$\mathbb{M}_{08} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \cos^2 \phi \cos^3 \theta_{\max} - 3 \cos^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.59l)$$

$$\mathbb{M}_{80} = \mathbb{M}_{08}, \quad (16.59m)$$

$$\mathbb{M}_{48} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \sin^2 \phi \cos^3 \theta_{\max} - 3 \sin^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.59n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{48}, \quad (16.59o)$$

$$\mathbb{M}_{13} = \mathbb{M}_{04}, \quad (16.59p)$$

$$\mathbb{M}_{31} = \mathbb{M}_{04}, \quad (16.59q)$$

$$\mathbb{M}_{26} = -\mathbb{M}_{80}, \quad (16.59r)$$

$$\mathbb{M}_{62} = -\mathbb{M}_{80}, \quad (16.59s)$$

$$\mathbb{M}_{57} = -\mathbb{M}_{48}, \quad (16.59t)$$

$$\mathbb{M}_{75} = -\mathbb{M}_{48}. \quad (16.59u)$$

**Torsionless pseudo-ellipse frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.58 and 16.59 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.19 and  $\mathbb{M}^{(2)}$  in figure 16.20. The out-of-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.21 and  $\mathbb{M}^{(2)}$  in figure 16.22.

## 16.11 Free rotor pseudo-ellipse frame order model

### 16.11.1 Free rotor pseudo-ellipse parameterisation

The free rotor model is the pseudo-ellipse model with the torsion angle restriction absent. Its parameters are

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E} + \mathfrak{p}_1 + \mathfrak{S}, \quad (16.60a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\alpha, E_\beta, E_\gamma\} + \{p_x, p_y, p_z\} + \{\theta_x, \theta_y\}, \quad (16.60b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the Euler angles defining the motional eigenframe,  $p_i$  are the coordinates of the pivot point, and  $\theta_x$  and  $\theta_y$  are the maximum cone opening half-angles.

### 16.11.2 Free rotor pseudo-ellipse equations

#### Free rotor pseudo-ellipse rotation matrices

The rotation matrix is the full torsion-tilt rotation matrix of equation 12.74c on page 250.

#### Free rotor pseudo-ellipse frame order matrix

The frame order matrix is

$$\mathfrak{M}^{(n)} = \int_S R^{\otimes n} dS \Big/ \int_S dS, \quad (16.61a)$$

$$= \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} R^{\otimes n} \sin \theta d\theta d\phi d\sigma \Big/ \int_S dS. \quad (16.61b)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} \int_0^{\theta_{\max}} \sin \theta d\theta d\phi d\sigma, \quad (16.62a)$$

$$= \int_{-\pi}^{\pi} \int_{-\pi}^{\pi} (1 - \cos \theta_{\max}) d\phi d\sigma, \quad (16.62b)$$

$$= \int_{-\pi}^{\pi} \text{pec}(\theta_x, \theta_y) d\sigma, \quad (16.62c)$$

$$= 2\pi \text{pec}(\theta_x, \theta_y). \quad (16.62d)$$



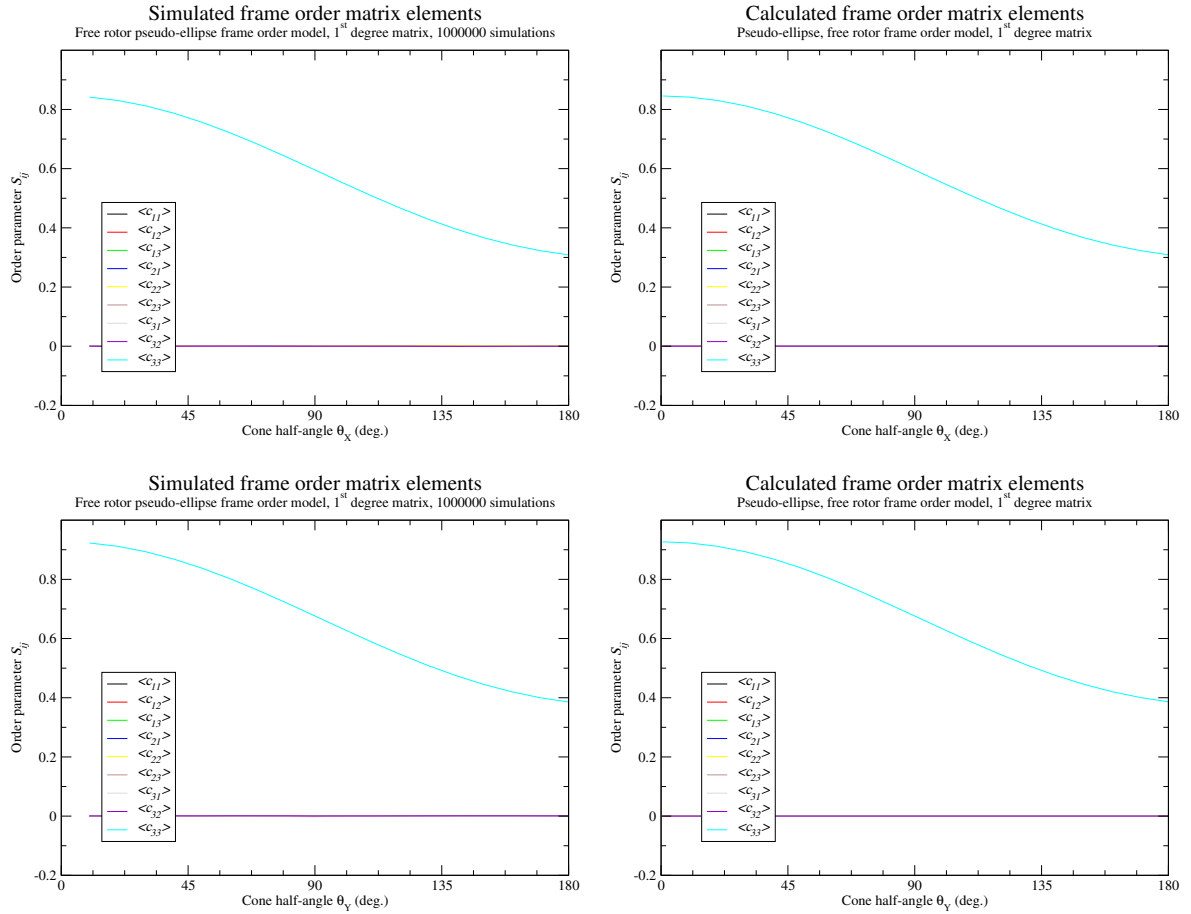


Figure 16.23: The free rotor pseudo-ellipse model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

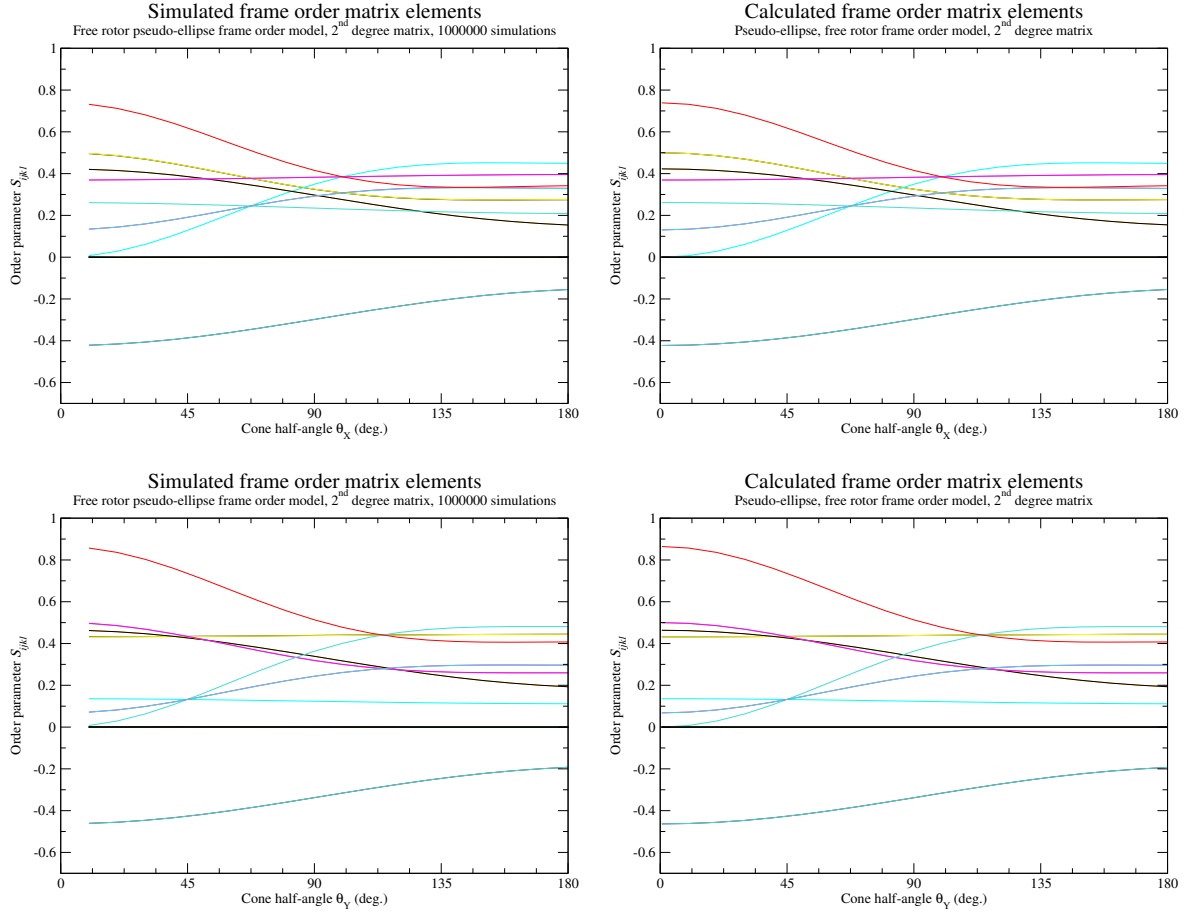


Figure 16.24: The free rotor pseudo-ellipse model simulated and calculated in-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_Y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

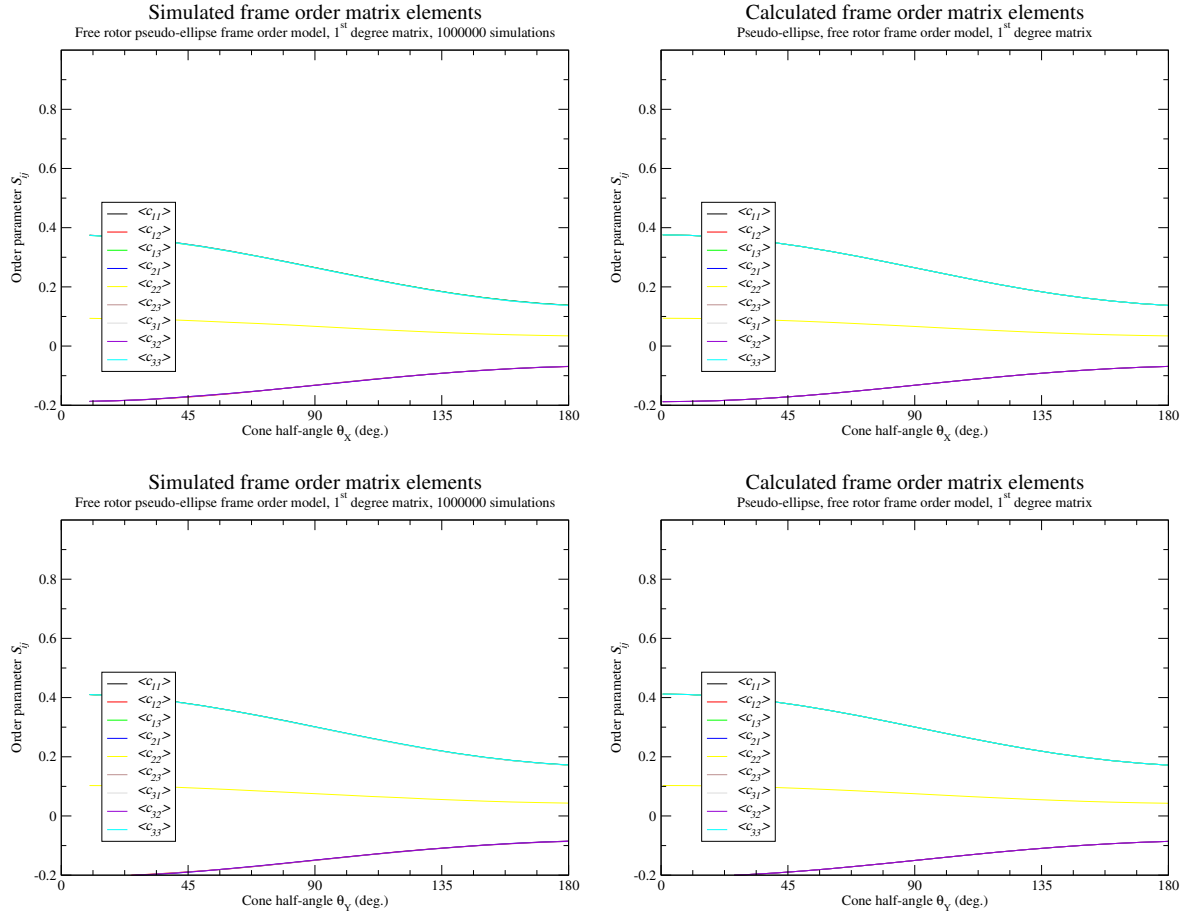


Figure 16.25: The free rotor pseudo-ellipse model simulated and calculated out-of-frame  $\mathbb{N}^{(1)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

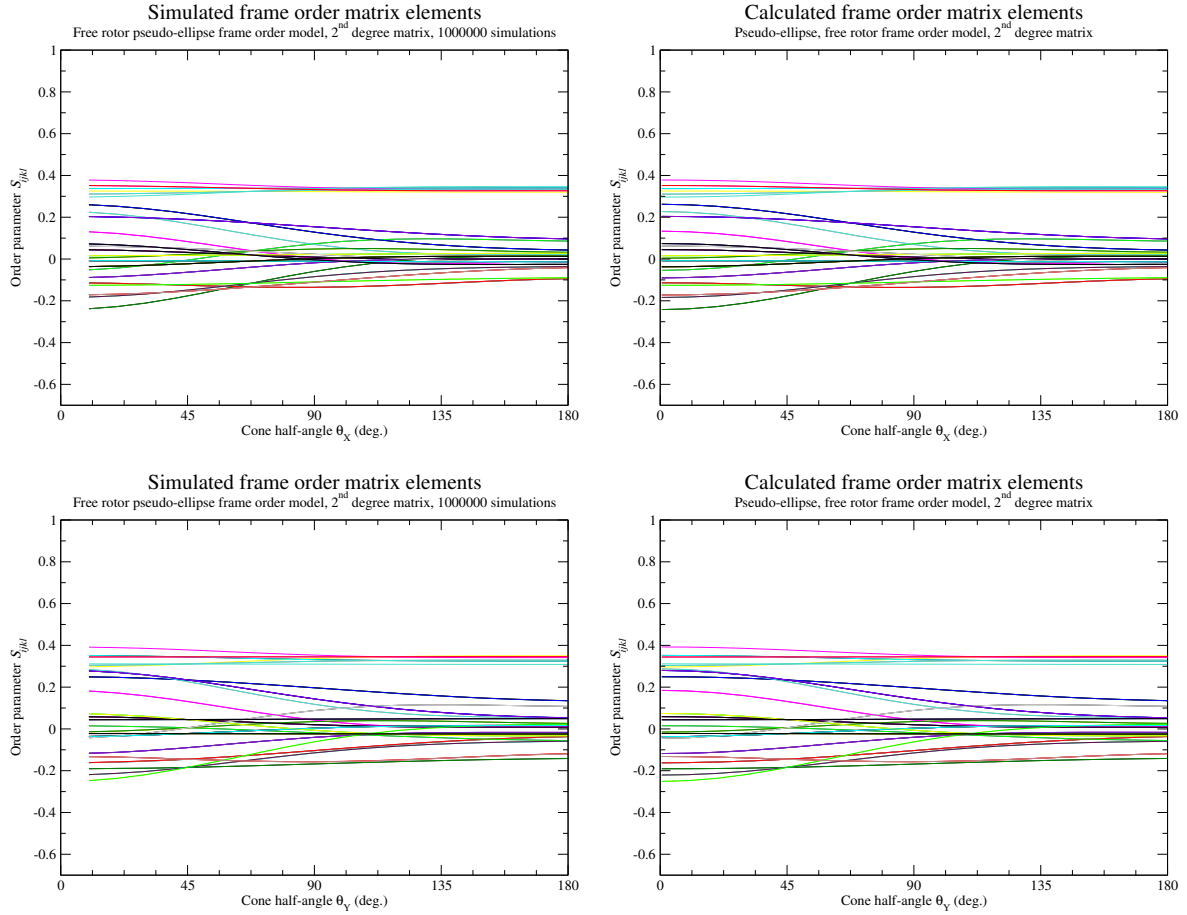


Figure 16.26: The free rotor pseudo-ellipse model simulated and calculated out-of-frame  $\mathbb{N}^{(2)}$  frame order matrix elements. In these plots,  $\theta_x$  corresponds to the cone opening half-angle  $\theta_x$  and  $\theta_y$  to the cone opening half-angle  $\theta_y$ . When the half-angle is not varied, the angle is fixed to either  $\theta_x = \pi/4$  or  $\theta_y = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees. The first angle for the calculated elements is set to 0.01 degrees as a pseudo-ellipse cone opening angle of 0.0 cannot be correctly handled by the numerical integration.

**Free rotor pseudo-ellipse 1<sup>st</sup> degree frame order** The 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)} = \int_S R^{\otimes 1} dS \Big/ \int_S dS, \quad (16.63a)$$

$$= \int_S R dS \Big/ 2\pi \text{pec}(\theta_x, \theta_y), \quad (16.63b)$$

$$= \frac{1}{2\text{pec}(\theta_x, \theta_y)} \int_{-\pi}^{\pi} \begin{pmatrix} \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot \\ \cdot & \cdot & \sin^2 \theta_{\max} \end{pmatrix} d\phi. \quad (16.63c)$$

**Free rotor pseudo-ellipse 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{6\text{pec}(\theta_x, \theta_y)} \left[ 4\pi - \int_{-\pi}^{\pi} \cos^2 \phi \cos^3 \theta_{\max} + 3 \sin^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.64a)$$

$$\mathbb{M}_{11} = \frac{1}{4\text{pec}(\theta_x, \theta_y)} \int_{-\pi}^{\pi} \sin^2 \theta_{\max} d\phi, \quad (16.64b)$$

$$\mathbb{M}_{22} = 0, \quad (16.64c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.64d)$$

$$\mathbb{M}_{44} = \frac{1}{6\text{pec}(\theta_x, \theta_y)} \left[ 4\pi - \int_{-\pi}^{\pi} \sin^2 \phi \cos^3 \theta_{\max} + 3 \cos^2 \phi \cos \theta_{\max} d\phi \right], \quad (16.64e)$$

$$\mathbb{M}_{55} = 0, \quad (16.64f)$$

$$\mathbb{M}_{66} = 0, \quad (16.64g)$$

$$\mathbb{M}_{77} = 0, \quad (16.64h)$$

$$\mathbb{M}_{88} = \frac{1}{3\text{pec}(\theta_x, \theta_y)} \left[ 2\pi - \int_{-\pi}^{\pi} \cos^3 \theta_{\max} d\phi \right], \quad (16.64i)$$

$$\mathbb{M}_{04} = \mathbb{M}_{00}, \quad (16.64j)$$

$$\mathbb{M}_{40} = \mathbb{M}_{44}, \quad (16.64k)$$

$$\mathbb{M}_{08} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \cos^2 \phi (\cos^3 \theta_{\max} - 3 \cos \theta_{\max}) d\phi \right], \quad (16.64l)$$

$$\mathbb{M}_{80} = \frac{1}{6 \text{pec}(\theta_x, \theta_y)} \left[ 4\pi + \int_{-\pi}^{\pi} \cos^3 \theta_{\max} - 3 \cos \theta_{\max} d\phi \right], \quad (16.64m)$$

$$\mathbb{M}_{48} = \frac{1}{3 \text{pec}(\theta_x, \theta_y)} \left[ 2\pi + \int_{-\pi}^{\pi} \sin^2 \phi (\cos^3 \theta_{\max} - 3 \cos \theta_{\max}) d\phi \right], \quad (16.64n)$$

$$\mathbb{M}_{84} = \mathbb{M}_{80}, \quad (16.64o)$$

$$\mathbb{M}_{13} = -\mathbb{M}_{11}, \quad (16.64p)$$

$$\mathbb{M}_{31} = -\mathbb{M}_{11}, \quad (16.64q)$$

$$\mathbb{M}_{26} = 0, \quad (16.64r)$$

$$\mathbb{M}_{62} = 0, \quad (16.64s)$$

$$\mathbb{M}_{57} = 0, \quad (16.64t)$$

$$\mathbb{M}_{75} = 0. \quad (16.64u)$$

**Free rotor pseudo-ellipse frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.63 and 16.64 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.23 and  $\mathbb{M}^{(2)}$  in figure 16.24. The out-of-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.25 and  $\mathbb{M}^{(2)}$  in figure 16.26.

## 16.12 Double rotor frame order model

The eigenframe of the motion of the double rotor model is characterised by two pivot points and two rotor axes. To simplify the modelling, the two axes are assumed to be orthogonal. Due to the nature of the RDC and PCS data used, it may not be possible to distinguish deviations from orthogonality from the noise.

### 16.12.1 Double rotor parameterisation

Assuming the axes are orthogonal for the model, the size of the set of non-redundant parameters is 15. To eliminate the redundant parameters, the geometry of the system can be used to construct a 3D eigenframe of the motion consisting of three Euler angles:

**x-axis** This axis of the eigensystem can be defined as a vector parallel to the 1<sup>st</sup> rotor axis.

**y-axis** This axis of the eigensystem can be defined as a vector parallel to the 2<sup>nd</sup> rotor axis.

**z-axis** This can be defined as a vector parallel to the line of shortest distance connecting the two rotor axes. As x and y are orthogonal by definition of the model, the line of shortest distance will be orthogonal to both x and y.

The two pivot points defining the position in space of the two rotor axes define the system. Using the above eigenframe, these can be parameterised using only four parameters:

**1<sup>st</sup> pivot point** This is defined using three coordinates and is located at the intersection of the 1<sup>st</sup> rotor axis and the line of shortest distance between the axes.

**2<sup>nd</sup> pivot point** This is defined as the intersection of the 2<sup>nd</sup> rotor axis and the line of shortest distance. Using the z-axis of the eigenframe and the 1<sup>st</sup> pivot, the 3D position can be defined as a simple displacement,  $p_d$ .

The set of all parameters of the system is therefore

$$\mathfrak{M} = \mathfrak{P} + \mathfrak{E} + \mathfrak{p}_1 + \mathfrak{p}_2 + \mathfrak{S}, \quad (16.65a)$$

$$= \{P_x, P_y, P_z, P_\alpha, P_\beta, P_\gamma\} + \{E_\alpha, E_\beta, E_\gamma\} + \{p_x, p_y, p_z\} + \{p_d\} + \{\sigma_{\max}, \sigma_{\max,2}\}, \quad (16.65b)$$

where  $P_i$  are the average domain position translations and rotations,  $E_i$  are the eigenframe Euler angles,  $p_i$  are the coordinates of the 1<sup>st</sup> pivot point,  $p_d$  is the displacement for the 2<sup>nd</sup> pivot point, and  $\sigma_{\max,i}$  are the two torsion half-angles of the rotors.

### 16.12.2 Double rotor equations

The double rotor model consists of two standard rotations, the first about the x-axis and the second about the y-axis. Hence the frame order matrix is simply the integration over both torsion angles of the Kronecker product of the product of the  $R_x$  and  $R_y$  rotation matrices, divided by the surface area normalisation factor.

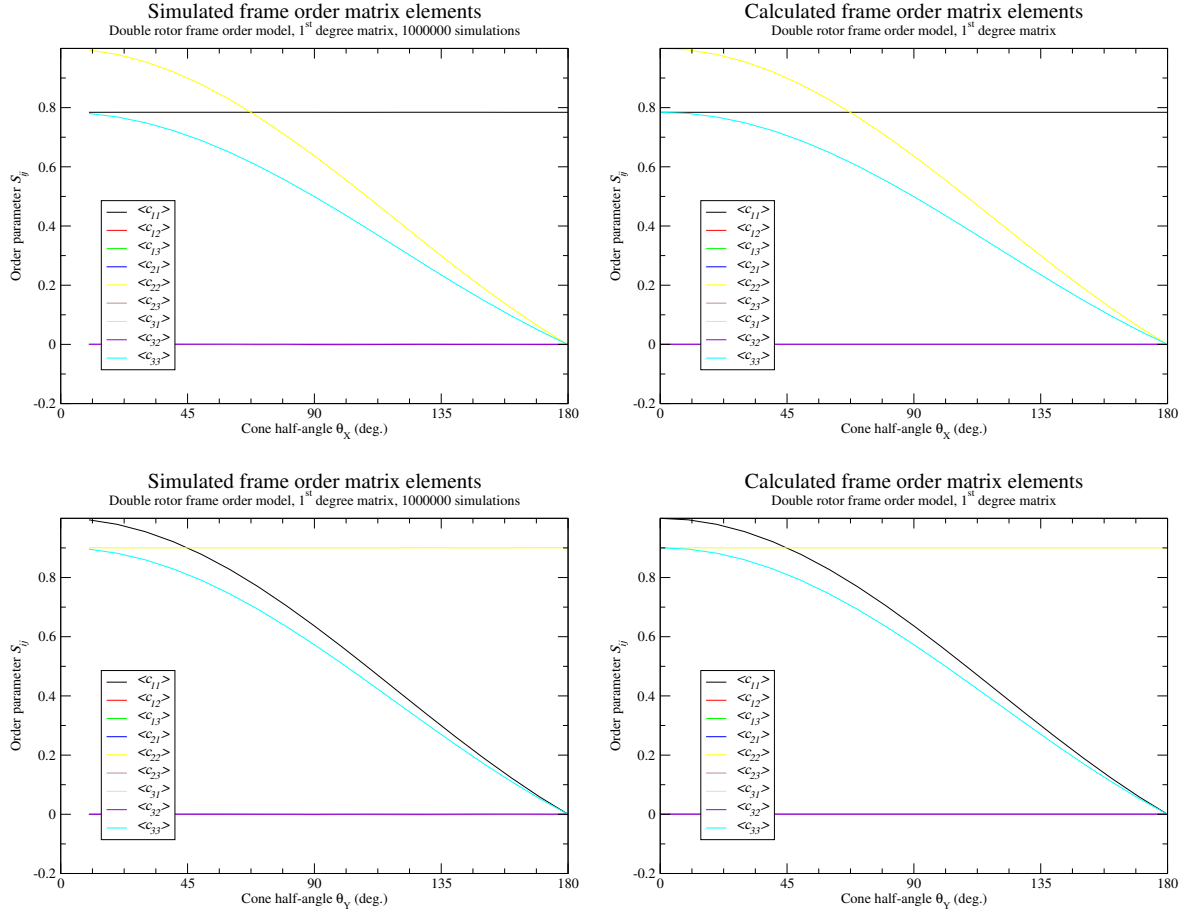


Figure 16.27: The double rotor model simulated and calculated in-frame  $\mathbb{M}^{(1)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the torsion half-angle  $\sigma_{\max,1}$  and  $\theta_Y$  to the torsion half-angle  $\sigma_{\max,2}$ . When the half-angle is not varied, the angle is fixed to either  $\sigma_{\max,1} = \pi/4$  or  $\sigma_{\max,2} = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees.



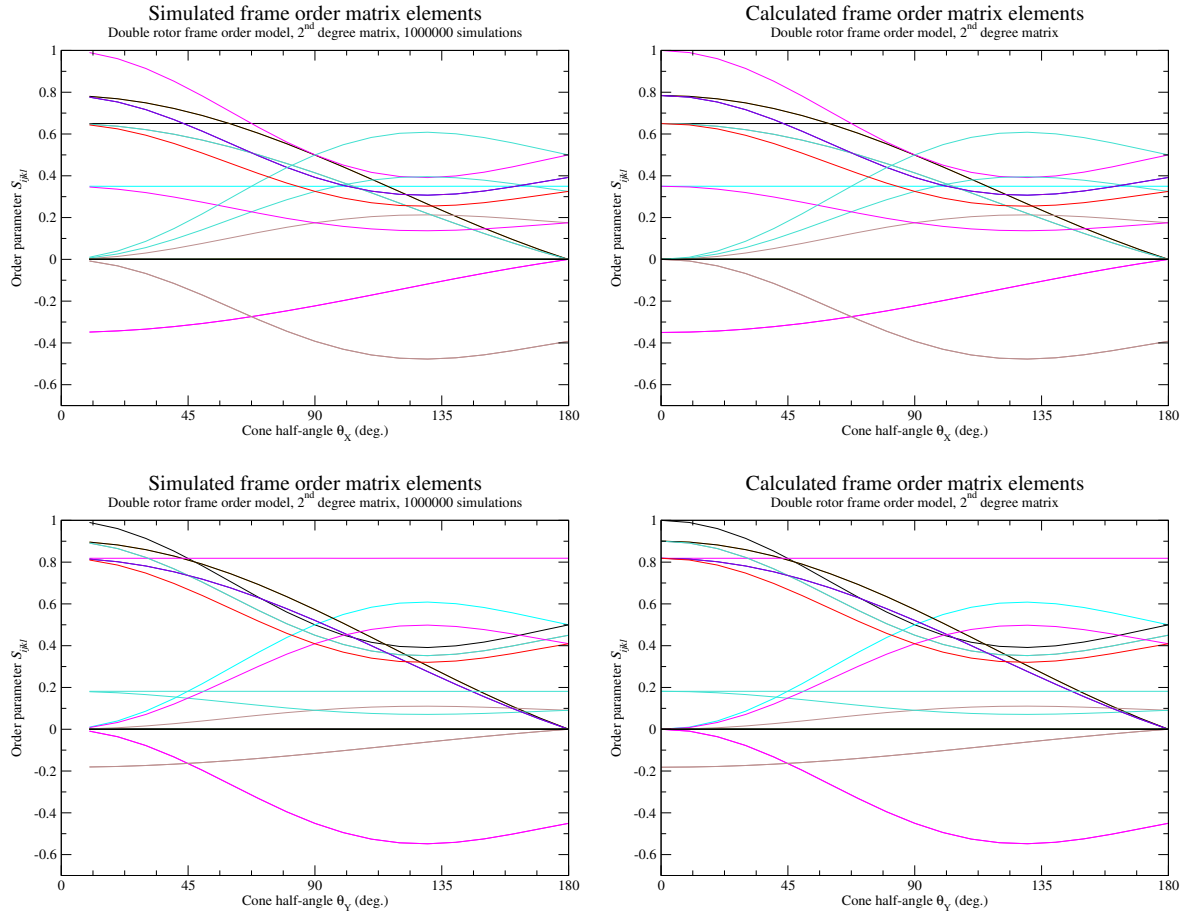


Figure 16.28: The double rotor model simulated and calculated in-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the torsion half-angle  $\sigma_{\max,1}$  and  $\theta_Y$  to the torsion half-angle  $\sigma_{\max,2}$ . When the half-angle is not varied, the angle is fixed to either  $\sigma_{\max,1} = \pi/4$  or  $\sigma_{\max,2} = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees.

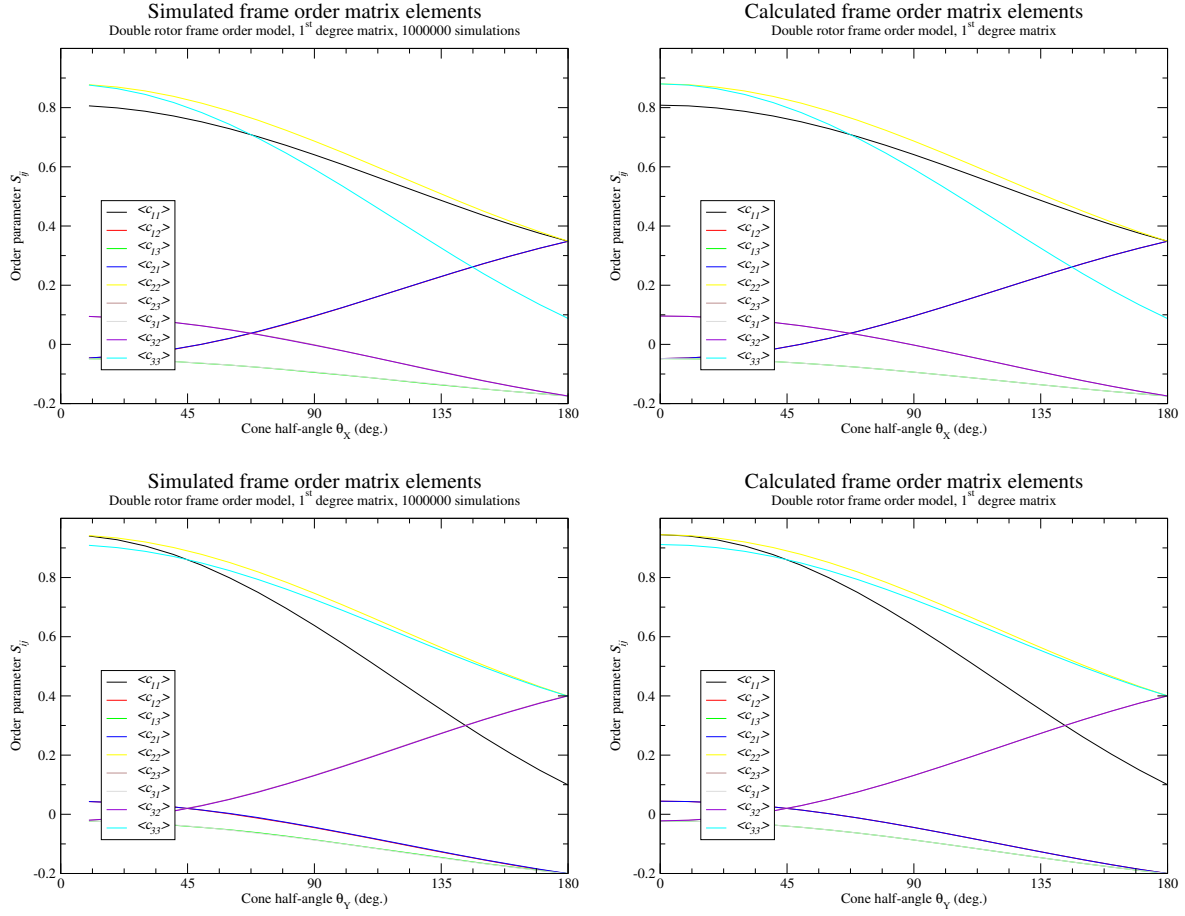


Figure 16.29: The double rotor model simulated and calculated out-of-frame  $\mathbb{N}^{(1)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the torsion half-angle  $\sigma_{\max,1}$  and  $\theta_Y$  to the torsion half-angle  $\sigma_{\max,2}$ . When the half-angle is not varied, the angle is fixed to either  $\sigma_{\max,1} = \pi/4$  or  $\sigma_{\max,2} = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees.

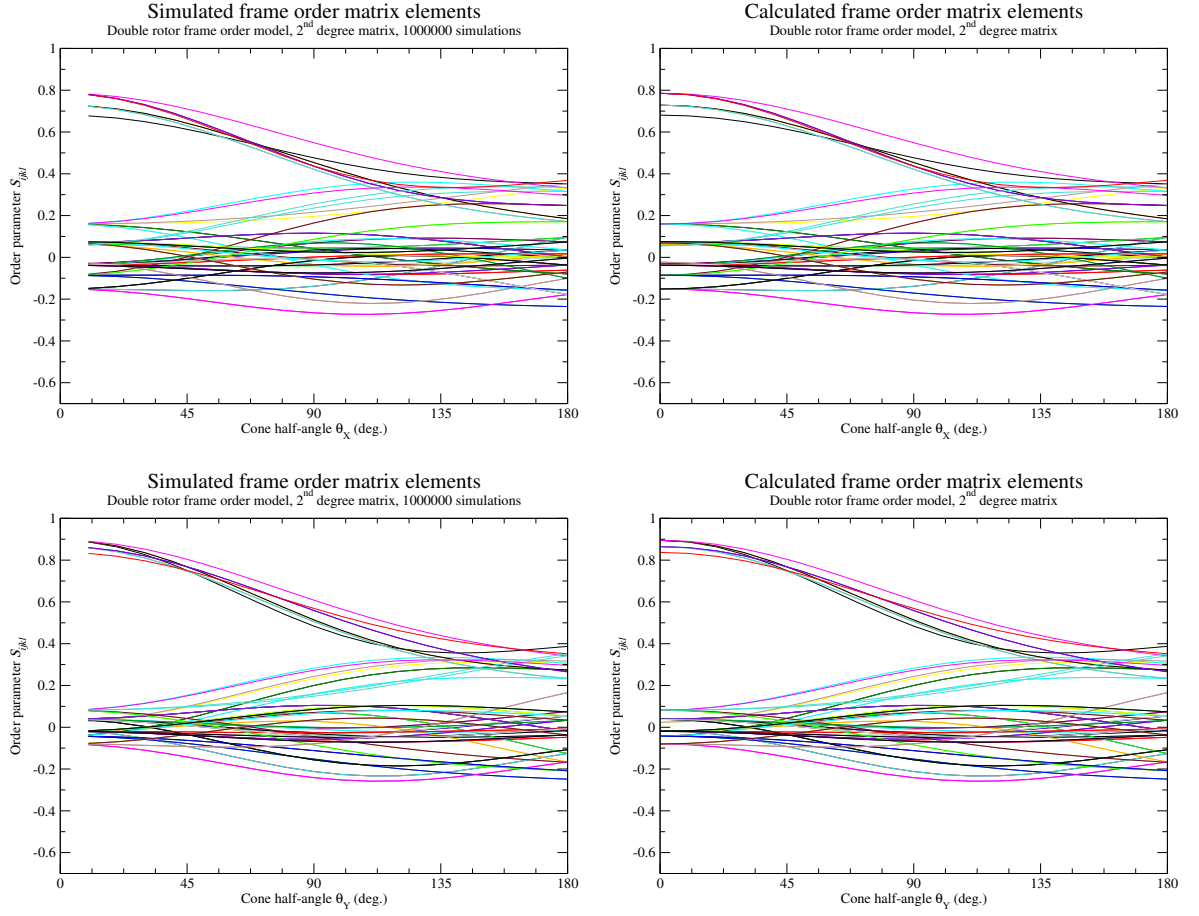


Figure 16.30: The double rotor model simulated and calculated out-of-frame  $\mathbb{M}^{(2)}$  frame order matrix elements. In these plots,  $\theta_X$  corresponds to the torsion half-angle  $\sigma_{\max,1}$  and  $\theta_Y$  to the torsion half-angle  $\sigma_{\max,2}$ . When the half-angle is not varied, the angle is fixed to either  $\sigma_{\max,1} = \pi/4$  or  $\sigma_{\max,2} = 3\pi/8$ . Frame order matrix values have been calculated every 10 degrees.

### Double rotor rotation matrices

The individual rotations are

$$R_y(\sigma_1) = \begin{pmatrix} \cos \sigma_1 & 0 & \sin \sigma_1 \\ 0 & 1 & 0 \\ -\sin \sigma_1 & 0 & \cos \sigma_1 \end{pmatrix}, \quad (16.66a)$$

$$R_x(\sigma_2) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos \sigma_2 & -\sin \sigma_2 \\ 0 & \sin \sigma_2 & \cos \sigma_2 \end{pmatrix}. \quad (16.66b)$$

The full rotation is then

$$R(\sigma_1, \sigma_2) = R_x(\sigma_2) \cdot R_y(\sigma_1), \quad (16.67a)$$

$$= \begin{pmatrix} \cos \sigma_1 & 0 & \sin \sigma_1 \\ \sin \sigma_1 \sin \sigma_2 & \cos \sigma_2 & -\cos \sigma_1 \sin \sigma_2 \\ -\sin \sigma_1 \cos \sigma_2 & \sin \sigma_2 & \cos \sigma_1 \cos \sigma_2 \end{pmatrix}. \quad (16.67b)$$

### Double rotor frame order matrix

The frame order matrix is

$$\mathbb{M}^{(n)} = \int_S R(\sigma_1, \sigma_2)^{\otimes n} dS \Big/ \int_S dS, \quad (16.68)$$

$$= \int_{-\sigma_{\max,2}}^{\sigma_{\max,2}} \int_{-\sigma_{\max,1}}^{\sigma_{\max,1}} R(\sigma_1, \sigma_2)^{\otimes n} d\sigma_{\max,1} d\sigma_{\max,2} \Big/ \int_S dS. \quad (16.69)$$

The surface normalisation factor is

$$\int_S dS = \int_{-\sigma_{\max,2}}^{\sigma_{\max,2}} \int_{-\sigma_{\max,1}}^{\sigma_{\max,1}} d\sigma_{\max,1} d\sigma_{\max,2}, \quad (16.70a)$$

$$= 2\sigma_{\max,1} \int_{-\sigma_{\max,2}}^{\sigma_{\max,2}} d\sigma_{\max,2}, \quad (16.70b)$$

$$= 4\sigma_{\max,1}\sigma_{\max,2}. \quad (16.70c)$$

**Double rotor 1<sup>st</sup> degree frame order** The un-normalised 1<sup>st</sup> degree frame order matrix with tensor rank-2 is

$$\mathbb{M}^{(1)'} = \int_S R(\sigma_1, \sigma_2)^{\otimes 1} dS, \quad (16.71a)$$

$$= \int_{-\sigma_{\max,2}}^{\sigma_{\max,2}} \int_{-\sigma_{\max,1}}^{\sigma_{\max,1}} R(\sigma_1, \sigma_2) d\sigma_{\max,1} d\sigma_{\max,2}, \quad (16.71b)$$

$$= \begin{pmatrix} 4\sin(\sigma_{\max,1})\sigma_{\max,2} & \cdot & \cdot \\ \cdot & 4\sin(\sigma_{\max,2})\sigma_{\max,1} & \cdot \\ \cdot & \cdot & 4\sin \sigma_{\max,1} \sin \sigma_{\max,2} \end{pmatrix}. \quad (16.71c)$$

After normalisation, the full frame order matrix is

$$\mathbb{M}^{(1)} = \begin{pmatrix} \text{sinc } \sigma_{\max,1} & \cdot & \cdot \\ \cdot & \text{sinc } \sigma_{\max,2} & \cdot \\ \cdot & \cdot & \text{sinc } \sigma_{\max,1} \text{ sinc } \sigma_{\max,2} \end{pmatrix}. \quad (16.72)$$

**Double rotor 2<sup>nd</sup> degree frame order** The 2<sup>nd</sup> degree frame order matrix with tensor rank-4 consists of the following elements, using Kronecker product double indices from 0 to 8

$$\mathbb{M}_{00} = \frac{1}{2} (\text{sinc}(2\sigma_{\max,1}) + 1), \quad (16.73a)$$

$$\mathbb{M}_{11} = \text{sinc } \sigma_{\max,1} \text{ sinc } \sigma_{\max,2}, \quad (16.73b)$$

$$\mathbb{M}_{22} = \frac{1}{2} \text{sinc } \sigma_{\max,2} (\text{sinc}(2\sigma_{\max,1}) + 1), \quad (16.73c)$$

$$\mathbb{M}_{33} = \mathbb{M}_{11}, \quad (16.73d)$$

$$\mathbb{M}_{44} = \frac{1}{2} (\text{sinc}(2\sigma_{\max,2}) + 1), \quad (16.73e)$$

$$\mathbb{M}_{55} = \frac{1}{2} \text{sinc } \sigma_{\max,1} (\text{sinc}(2\sigma_{\max,2}) + 1), \quad (16.73f)$$

$$\mathbb{M}_{66} = \mathbb{M}_{22}, \quad (16.73g)$$

$$\mathbb{M}_{77} = \mathbb{M}_{55}, \quad (16.73h)$$

$$\mathbb{M}_{88} = \frac{1}{4} (\text{sinc}(2\sigma_{\max,1}) + 1) (\text{sinc}(2\sigma_{\max,2}) + 1), \quad (16.73i)$$

$$\mathbb{M}_{04} = 0, \quad (16.73j)$$

$$\mathbb{M}_{40} = \frac{1}{4} (\text{sinc}(2\sigma_{\max,1}) - 1) (\text{sinc}(2\sigma_{\max,2}) - 1), \quad (16.73k)$$

$$\mathbb{M}_{08} = -\frac{1}{2} (\text{sinc}(2\sigma_{\max,1}) - 1), \quad (16.73l)$$

$$\mathbb{M}_{80} = -\frac{1}{4} (\text{sinc}(2\sigma_{\max,1}) - 1) (\text{sinc}(2\sigma_{\max,2}) + 1), \quad (16.73m)$$

$$\mathbb{M}_{48} = -\frac{1}{4} (\text{sinc}(2\sigma_{\max,1}) + 1) (\text{sinc}(2\sigma_{\max,2}) - 1), \quad (16.73n)$$

$$\mathbb{M}_{84} = -\frac{1}{2} (\text{sinc}(2\sigma_{\max,2}) - 1), \quad (16.73o)$$

$$\mathbb{M}_{13} = 0, \quad (16.73p)$$

$$\mathbb{M}_{31} = 0, \quad (16.73q)$$

$$\mathbb{M}_{26} = \frac{1}{2} \text{sinc } \sigma_{\max,2} (\text{sinc}(2\sigma_{\max,1}) - 1), \quad (16.73r)$$

$$\mathbb{M}_{62} = \mathbb{M}_{26}, \quad (16.73s)$$

$$\mathbb{M}_{57} = \frac{1}{2} \text{sinc } \sigma_{\max,1} (\text{sinc}(2\sigma_{\max,2}) - 1), \quad (16.73t)$$

$$\mathbb{M}_{75} = \mathbb{M}_{57}. \quad (16.73u)$$

**Double rotor frame order matrix simulation and calculation** The frame order matrix element simulation script from Section 16.2, page 371 was used to compare the implementation of equations 16.72 and 16.73 above. Frame order matrix  $\mathbb{M}^{(1)}$  and  $\mathbb{M}^{(2)}$  values were both simulated and calculated, both within and out of the motional eigenframe. The in-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.27 and  $\mathbb{M}^{(2)}$  in figure 16.28. The out-of-frame  $\mathbb{M}^{(1)}$  values are shown in figure 16.29 and  $\mathbb{M}^{(2)}$  in figure 16.30.



## Part V

# Reference





## Chapter 17

# Alphabetical listing of user functions

The following is a listing with descriptions of all the user functions available within the relax prompt and scripting environments. These are simply an alphabetical list of the docstrings which can normally be viewed in prompt mode by typing `help(function)`.

### 17.1 A warning about the formatting

The following documentation of the user functions has been automatically generated by a script which extracts and formats the docstring associated with each function. There may therefore be instances where the formatting has failed or where there are inconsistencies.

### 17.2 The list of functions

Each user function is presented within it's own subsection with the documentation broken into multiple parts: the synopsis, the default arguments, and the sections from the function's docstring.

#### 17.2.1 The synopsis

The synopsis presents a brief description of the function. It is taken as the first line of the docstring when browsing the help system.

#### 17.2.2 Defaults

This section lists all the arguments taken by the function and their default values. To invoke the function type the function name then in brackets type a comma separated list of arguments.

The first argument printed is always ‘self’ but you can safely ignore it. ‘self’ is part of the object oriented programming within Python and is automatically prefixed to the list of arguments you supply. Therefore you can’t provide ‘self’ as the first argument even if you do try.

### **17.2.3 Docstring sectioning**

All other sections are created from the sectioning of the user function docstring.

## 17.2.4 align\_tensor.copy



### Synopsis

Copy alignment tensor data.

### Defaults

`align_tensor.copy(tensor_from=None, pipe_from=None, tensor_to=None, pipe_to=None)`

### Keyword arguments

**tensor\_from:** The identification string of the alignment tensor to copy the data from.

**pipe\_from:** The name of the data pipe to copy the alignment tensor data from.

**tensor\_to:** The identification string of the alignment tensor to copy the data to.

**pipe\_to:** The name of the data pipe to copy the alignment tensor data to.

### Description

This will copy the alignment tensor data to a new tensor or a new data pipe. The destination data pipe must not contain any alignment tensor data corresponding to the `tensor_to` label. If the source or destination data pipes are not supplied, then both will default to the current data pipe. Both the source and destination tensor IDs must be supplied.

### Prompt examples

To copy the alignment tensor data corresponding to 'Pf1' from the data pipe 'old' to the current data pipe, type one of:

```
relax> align_tensor.copy('Pf1', 'old')
```

```
relax> align_tensor.copy(tensor_from='Pf1',
    pipe_from='old')
```

To copy the alignment tensor data corresponding to 'Otting' from the current data pipe to the data pipe new, type one of:

```
relax> align_tensor.copy('Otting', pipe_to='
    new')
```

```
relax> align_tensor.copy(tensor_from='Otting',
    pipe_to='new')
```

To copy the alignment tensor data of 'Otting' to that of 'Otting new', type one of:

```
relax> align_tensor.copy('Otting', tensor_to='
    Otting new')
```

```
relax> align_tensor.copy(tensor_from='Pf1',
    tensor_to='Otting new')
```

---

### 17.2.5 `align_tensor.delete`



#### Synopsis

Delete alignment tensor data from the relax data store.

#### Defaults

`align_tensor.delete(tensor=None)`

#### Keyword arguments

**tensor:** The alignment tensor identification string.

#### Description

This will delete the specified alignment tensor data from the current data pipe. If no tensor is specified, all tensors will be deleted.

### 17.2.6 `align_tensor.display`



#### Synopsis

Display the alignment tensor information in full detail.

#### Defaults

`align_tensor.display(tensor=None)`

#### Keyword arguments

**tensor:** The alignment tensor identification string.

#### Description

This will show all information relating to the alignment tensor, including the different tensor forms:

Probability tensor.

Saupe order matrix.

Alignment tensor.

Magnetic susceptibility tensor.

All possible tensor parameters and information will also be shown (Eigensystem, GDO, Aa, Ar,  $\mathfrak{R}$ , eta, `chi_ax`, `chi_rh`, etc). The printout will be extensive.

If no tensor is specified, all tensors will be displayed.

---

### 17.2.7 align\_tensor.fix



#### Synopsis

Fix all alignment tensors so that they do not change during optimisation.

#### Defaults

**align\_tensor.fix**(id=None, fixed=True)

#### Keyword arguments

**id**: The alignment tensor identification string.

**fixed**: The flag specifying if the tensors should be fixed or variable.

#### Description

If the ID string is left unset, then all alignment tensors will be fixed.

### 17.2.8 align\_tensor.init



#### Synopsis

Initialise an alignment tensor.

#### Defaults

**align\_tensor.init**(tensor=None, align\_id=None, domain=None, params=None, scale=1.0, angle\_units='deg', param\_types=2, errors=False)

#### Keyword arguments

**tensor**: The optional alignment tensor ID string, required if multiple tensors exist per alignment.

**align\_id**: The alignment ID string that the tensor corresponds to.

**domain**: The optional domain ID string that the tensor corresponds to.

**params**: The alignment tensor data.

**scale**: The alignment tensor eigenvalue scaling value.

**angle\_units**: The units for the angle parameters.

**param\_types**: A flag to select different parameter combinations.

**errors**: A flag which determines if the alignment tensor data or its errors are being input.

#### Description

The tensor ID is only required if there are multiple unique tensors per alignment. An example is if internal domain motions cause multiple parts of the molecule to align differently. The tensor ID is optional and in the case of only a single tensor per alignment, the tensor can be identified using the alignment ID instead.

The alignment tensor parameters should be a tuple of floating point numbers (a list surrounded by round brackets). These correspond to the parameters of the tensor which can be specified by the parameter types whereby the values correspond to:

**0** – {Sxx, Syy, Sxy, Sxz, Syz} (unitless),

**1** – {Szz, Sxx-yy, Sxy, Sxz, Syz} (Pales default format),

**2** – {Axx, Ayy, Axy, Axz, Ayz} (unitless),

- 3 – {Azz, Axx-yy, Axy, Axz, Ayz} (unitless),  
 4 – {Axx, Ayy, Axy, Axz, Ayz} (units of Hertz),  
 5 – {Azz, Axx-yy, Axy, Axz, Ayz} (units of Hertz),  
 6 – {Pxx, Pyy, Pxy, Pxz, Pyz} (unitless),  
 7 – {Pzz, Pxx-yy, Pxy, Pxz, Pyz} (unitless).

Other formats may be added later. The relationship between the Saupe order matrix  $S$  and the alignment tensor  $A$  is

$$S = 3/2 A.$$

The probability matrix  $P$  is related to the alignment tensor  $A$  by

$$A = P - 1/3 I,$$

where  $I$  is the identity matrix. For the alignment tensor to be supplied in Hertz, the bond vectors must all be of equal length.

## Prompt examples

To set a rhombic tensor for the domain labelled 'domain 1' with the alignment named 'super media', type one of:

```
relax> align_tensor.init('domain 1', 'super
media', (-8.6322e-05, -5.5786e-04, -3
.1732e-05, 2.2927e-05, 2.8599e-04),
param_types=1)

relax> align_tensor.init(tensor='domain 1',
align_id='super media', params=(-8
.6322e-05, -5.5786e-04, -3.1732e-05, 2
.2927e-05, 2.8599e-04), param_types=1)
```

## 17.2.9 align\_tensor.matrix\_angles



### Synopsis

Calculate the angles between all alignment tensors.

### Defaults

**align\_tensor.matrix\_angles**(basis\_set='matrix', tensors=None, angle\_units='deg', precision=1)

### Keyword arguments

**basis\_set**: The basis set to operate with.

**tensors**: A list of the tensors to apply the calculation to. If None, all tensors are used.

**angle\_units**: The units for the angle parameters, either 'deg' or 'rad'.

**precision**: The precision of the printed out angles. The number corresponds to the number of figures to print after the decimal point.

### Description

This will calculate the inter-matrix angles between all loaded alignment tensors for the current data pipe. For the vector basis sets, the matrices are first mapped to vector form and then the inter-vector angles rather than inter-matrix angles are calculated. The angles are dependent upon the basis set - linear maps produce identical results whereas non-linear maps result in different angles. The basis set can be one of:

**'matrix'** – The standard inter-matrix angles. This default option is a linear map, hence angles are preserved. The angle is calculated via the arccos of the Euclidean inner product of the alignment matrices in rank-2, 3D form divided by the Frobenius norm  $\sqrt{A:F}$  of the matrices.

**'irreducible 5D'** – The inter-tensor vector angles for the irreducible spherical tensor 5D basis set {A-2, A-1, A0, A1, A2}. This is a linear map, hence angles are preserved. These are the spherical harmonic decomposition coefficients.

**'unitary 9D'** – The inter-tensor vector angles for the unitary 9D basis set {Sxx, Sxy, Sxz, Syx, Syy, Syz, Szx, Szy, Szz}. This is a linear map, hence angles are preserved.

‘unitary 5D’ – The inter-tensor vector angles for the unitary 5D basis set {Sxx, Syy, Sxy, Sxz, Syz}. This is a non-linear map, hence angles are not preserved.

‘geometric 5D’ – The inter-tensor vector angles for the geometric 5D basis set {Szz, Sxxyy, Sxy, Sxz, Syz}. This is a non-linear map, hence angles are not preserved. This is also the Pales standard notation.

The full matrix angle via the Euclidean inner product is defined as

$$\theta = \arccos \frac{\langle A1, A2 \rangle}{\|A1\| \cdot \|A2\|},$$

where  $\langle a, b \rangle$  is the Euclidean inner product and  $\|a\|$  is the Frobenius norm of the matrix. For the irreducible spherical tensor 5D basis set, the  $A_m$  components are defined as

$$\begin{aligned} A_0 &= \frac{1}{\sqrt{5}} S_{zz}, \\ A_{\pm 1} &= \frac{1}{\sqrt{15}} (S_{xz} \pm i S_{yz}), \\ A_{\pm 2} &= \frac{1}{\sqrt{15}} (S_{xx} - S_{yy} \pm 2i S_{xy}) \end{aligned}$$

and, for this complex notation, the angle is

$$\theta = \arccos \frac{\text{Re}(\langle A1 | A2 \rangle)}{\|A1\| \cdot \|A2\|},$$

where the inner product is defined as

$$\langle A1 | A2 \rangle = \sum_{m=-2,2}^2 A_m^* \cdot A_m,$$

and where  $A_m^* = (-1)^m A_{-m}$ , and the norm is defined as  $\|A1\| = \sqrt{\sum_i |A1_i|^2}$ . For all other basis sets whereby the map is real matrix  $-i$  real vector, the inter-tensor angle is defined as

$$\theta = \arccos \frac{\langle A1 | A2 \rangle}{\|A1\| \cdot \|A2\|},$$

where the inner product  $\langle A1 | A2 \rangle$  is simply the vector dot product and  $\|A1\|$  is the vector length.

## 17.2.10 align\_tensor.reduction



### Synopsis

Specify that one tensor is a reduction of another.

### Defaults

`align_tensor.reduction`(full\_tensor=None, red\_tensor=None)

### Keyword arguments

`full_tensor`: The full alignment tensor.

`red_tensor`: The reduced alignment tensor.

### Description

Prior to optimisation of the N-state model and Frame Order theories using alignment tensors, which tensor is a reduction of which other tensor must be specified through this user function.

### Prompt examples

To state that the alignment tensor loaded as ‘chi3 C-dom’ is a reduction of ‘chi3 N-dom’, type:

```
relax> align_tensor.reduction(full_tensor='
    chi3 N-dom', red_tensor='chi3 C-dom')
```

## 17.2.11 align\_tensor.set\_domain



## Synopsis

Set the domain label for the alignment tensor.

## Defaults

`align_tensor.set_domain(tensor=None, domain=None)`

## Keyword arguments

**tensor:** The alignment tensor to assign the domain label to.

**domain:** The domain label.

## Description

Prior to optimisation of the N-state model or Frame Order theories, the domain to which each alignment tensor belongs must be specified.

## Prompt examples

To link the alignment tensor loaded as 'chi3 C-dom' to the C-terminal domain 'C', type:

```
relax> align_tensor.set_domain(tensor='chi3
    C-dom', domain='C')
```

## 17.2.12 align\_tensor.svd



## Synopsis

Calculate the singular values and condition number for all alignment tensors.

## Defaults

`align_tensor.svd(basis_set='irreducible 5D', tensors=None, precision=4)`

## Keyword arguments

**basis\_set:** The basis set to operate with.

**tensors:** A list of the tensors to apply the calculation to. If None, all tensors are used.

**precision:** The precision of the printed out singular values and condition numbers. The number corresponds to the number of figures to print after the decimal point.

## Description

This will perform a singular value decomposition for all alignment tensors and calculate the condition number. The singular values and condition number are dependent on the basis set - linear maps produce identical results whereas non-linear maps result in different values. The basis set can be one of:

**'irreducible 5D'** – The irreducible spherical tensor 5D basis set {A-2, A-1, A0, A1, A2}. This is a linear map, hence angles, singular values, and condition number are preserved. These are the spherical harmonic decomposition coefficients.

**'unitary 9D'** – The unitary 9D basis set {Sxx, Sxy, Sxz, Syx, Syy, Syz, Szx, Szy, Szz}. This is a linear map, hence angles, singular values, and condition number are preserved.

**'unitary 5D'** – The unitary 5D basis set {Sxx, Syy, Sxy, Sxz, Syz}. This is a non-linear map, hence angles, singular values, and condition number are not preserved.

**'geometric 5D'** – The geometric 5D basis set {Szz, Sxxyy, Sxy, Sxz, Syz}. This is a non-linear map, hence angles, singular values, and condition number are not preserved. This is also the Pales standard notation.



If the selected basis set is the default of 'irreducible 5D', the matrix on which SVD will be performed will be:

$$\begin{vmatrix} A-2(1) & A-1(1) & A0(1) & A1(1) & A2(1) \\ A-2(2) & A-1(2) & A0(2) & A1(2) & A2(2) \\ A-2(3) & A-1(3) & A0(3) & A1(3) & A2(3) \\ . & . & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ A-2(N) & A-1(N) & A0(N) & A1(N) & A2(N) \end{vmatrix}$$

$$A_{+/-2} = \frac{1}{\sqrt{15}} \frac{2\pi}{\sqrt{15}} \begin{vmatrix} . & . & . \\ . & . & . \\ . & . & . \end{vmatrix} (S_{xx} - S_{yy} \pm 2iS_{xy}) .$$

The relationships between the geometric and unitary basis sets are

$$\begin{aligned} S_{zz} &= -S_{xx} - S_{yy}, \\ S_{xxyy} &= S_{xx} - S_{yy}. \end{aligned}$$

If the selected basis set is 'unitary 9D', the matrix on which SVD will be performed will be:

$$\begin{vmatrix} S_{xx1} & S_{xy1} & S_{xz1} & S_{yx1} & S_{yy1} & S_{yz1} & S_{zx1} & S_{zy1} & S_{zz1} \\ S_{xx2} & S_{xy2} & S_{xz2} & S_{yx2} & S_{yy2} & S_{yz2} & S_{zx2} & S_{zy2} & S_{zz2} \\ S_{xx3} & S_{xy3} & S_{xz3} & S_{yx3} & S_{yy3} & S_{yz3} & S_{zx3} & S_{zy3} & S_{zz3} \\ . & . & . & . & . & . & . & . & . \\ . & . & . & . & . & . & . & . & . \\ . & . & . & . & . & . & . & . & . \\ S_{xxN} & S_{xyN} & S_{xzN} & S_{yxN} & S_{yyN} & S_{yzN} & S_{zxN} & S_{zyN} & S_{zzN} \end{vmatrix}$$

Otherwise if the selected basis set is 'unitary 5D', the matrix for SVD is:

$$\begin{vmatrix} S_{xx1} & S_{yy1} & S_{xy1} & S_{xz1} & S_{yz1} \\ S_{xx2} & S_{yy2} & S_{xy2} & S_{xz2} & S_{yz2} \\ S_{xx3} & S_{yy3} & S_{xy3} & S_{xz3} & S_{yz3} \\ . & . & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ S_{xxN} & S_{yyN} & S_{xyN} & S_{xzN} & S_{yzN} \end{vmatrix}$$

Or if the selected basis set is 'geometric 5D', the stretching and skewing parameters  $S_{zz}$  and  $S_{xx-yy}$  will be used instead and the matrix is:

$$\begin{vmatrix} S_{zz1} & S_{xxyy1} & S_{xy1} & S_{xz1} & S_{yz1} \\ S_{zz2} & S_{xxyy2} & S_{xy2} & S_{xz2} & S_{yz2} \\ S_{zz3} & S_{xxyy3} & S_{xy3} & S_{xz3} & S_{yz3} \\ . & . & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ S_{zzN} & S_{xxyyN} & S_{xyN} & S_{xzN} & S_{yzN} \end{vmatrix}$$

For the irreducible spherical tensor basis set, the  $A_m$  components are defined as

$$\begin{aligned} A_0 &= \frac{1}{\sqrt{5}} \frac{4\pi}{\sqrt{5}} \begin{vmatrix} . & . & . \\ . & . & . \\ . & . & . \end{vmatrix} S_{zz} , \\ A_{+/-1} &= \frac{1}{\sqrt{5}} \frac{8\pi}{\sqrt{5}} \begin{vmatrix} . & . & . \\ . & . & . \\ . & . & . \end{vmatrix} (S_{xz} \pm iS_{yz}) , \end{aligned}$$

17.2.13 `angles.diff_frame`

## Synopsis

Calculate the angles defining the XH bond vector within the diffusion frame.

## Defaults

`angles.diff_frame()`

## Description

If the diffusion tensor is isotropic, then nothing will be done.

If the diffusion tensor is axially symmetric, then the angle  $\alpha$  will be calculated for each XH bond vector.

If the diffusion tensor is asymmetric, then the three angles will be calculated.

17.2.14 `bmr.b.citation`

## Synopsis

Specify a citation to be added the BMRB data file.

## Defaults

`bmr.b.citation(cite_id=None, authors=None, doi=None, pubmed_id=None, full_citation=None, title=None, status='published', type='journal', journal_abbrev=None, journal_full=None, volume=None, issue=None, page_first=None, page_last=None, year=None)`

## Keyword arguments

`cite_id`: The citation ID string.

`authors`: The list of authors. Each author element is a list of four elements (the first name, last name, first initial, and middle initials).

`doi`: The DOI number, e.g. '10.1000/182'.

`pubmed_id`: The identification code assigned to the publication by PubMed.

`full_citation`: The full citation as given in a reference list.

`title`: The title of the publication.

`status`: The status of the publication. This can be a value such as 'published', 'submitted', etc.

`type`: The type of publication, for example 'journal'.

`journal_abbrev`: The standard journal abbreviation.

`journal_full`: The full journal name.

`volume`: The volume number.

`issue`: The issue number.

`page_first`: The first page number.

`page_last`: The last page number.

`year`: The publication year.

## Description

The full\_citation should be in a format similar to that used in a journal article by either cutting and pasting from another document or by typing. Please include author names, title, journal, page numbers, and year or equivalent information for the type of publication given.

The journal status can only be one of:

```
'preparation',
'in_press',
'published',
'retracted',
'submitted'.
```

The citation type can only be one of:

```
'abstract',
'BMRB only',
'book',
'book chapter',
'internet',
'journal',
'personal communication',
'thesis'.
```

The standard journal abbreviation is that defined by the Chemical Abstract Services for the journal where the data are or will be published. If the data in the deposition are related to a J. Biomol. NMR paper, the value must be 'J. Biomol. NMR' to alert the BMRB annotators so that the deposition is properly processed. If the depositor truly does not know the journal, a value of 'not known' or 'na' is acceptable.

## Prompt examples

To add the citation "d'Auvergne E. J., Gooley P. R. (2007). Set theory formulation of the model-free problem and the diffusion seeded model-free paradigm. Mol. Biosyst., 3(7), 483-494.", type:

```
relax> bmrbl.citation(authors=[["Edward", "d'
  Auvergne", "E.", "J."], ["Paul", "
  Gooley", "P.", "R."]], doi="10.1039/
  b702202f", pubmed_id="17579774",
  full_citation="d'Auvergne E. J., Gooley
  P. R. (2007). Set theory formulation
  of the model-free problem and the
  diffusion seeded model-free paradigm.
  Mol. Biosyst., 3(7), 483-494.", title="
```

```
Set theory formulation of the model-
free problem and the diffusion seeded
model-free paradigm.", status="
published", type="journal",
journal_abbrev="Mol. Biosyst.",
journal_full="Molecular Biosystems",
volume=3, issue=7, page_first=483,
page_last=498, year=2007)
```

---

### 17.2.15 `bmrbr.display`



#### Synopsis

Display the BMRB data in NMR-STAR format.

#### Defaults

`bmrbr.display(version='3.1')`

#### Keyword arguments

**version:** The version of the BMRB NMR-STAR format to display.

#### Description

This will print the BMRB NMR-STAR formatted data to STDOUT.

### 17.2.16 `bmrbr.read`



#### Synopsis

Read BMRB files in the NMR-STAR format.

#### Defaults

`bmrbr.read(file=None, dir=None, version=None, sample_conditions=None)`

#### Keyword arguments

**file:** The name of the BMRB NMR-STAR formatted file to read.

**dir:** The directory where the file is located.

**version:** The version of the BMRB NMR-STAR format to read. This is not necessary as the version is normally auto-detected.

**sample\_conditions:** The sample conditions label in the NMR-STAR file to restrict loading to.

#### Description

This will allow most of the data from a BMRB NMR-STAR formatted file to be loaded into the relax data store. Note that an empty data pipe should be created for storing the data, and that currently only model-free data pipes can be used. Also, only one sample condition can be read per relax data pipe. Therefore if one of the sample conditions is not specified and multiple conditions exist in the NMR-STAR file, an error will be raised.

## 17.2.17 bmr.script



### Synopsis

Specify the scripts used in the analysis.

### Defaults

**bmr.script**(file=None, dir=None, analysis\_type=None, model\_selection=None, engine='relax', model\_elim=False, universal\_solution=False)

### Keyword arguments

**file**: The name of the script file.

**dir**: The directory where the file is located.

**analysis\_type**: The type of analysis performed.

**model\_selection**: The model selection technique used, if relevant. For example 'AIC' model selection.

**engine**: The software engine used in the analysis.

**model\_elim**: A model-free specific flag specifying if model elimination was performed.

**universal\_solution**: A model-free specific flag specifying if the universal solution was sought after.

### Description

This user function allows scripts used in the analysis to be included in the BMRB deposition. The following addition information may need to be specified with the script.

The analysis type must be set. Allowable values include all the data pipe types used in relax, ie:

'frame\_order' – The Frame Order theories,

'jw' – Reduced spectral density mapping,

'mf' – Model-free analysis,

'N-state' – N-state model of domain motions,

'noe' – Steady state NOE calculation,

'relax\_fit' – Relaxation curve fitting,

The model selection technique only needs to be set if the script selects between different mathematical models. This can be anything, but the following are recommended:

'AIC' – Akaike's Information Criteria.

'AICc' – Small sample size corrected AIC.

'BIC' – Bayesian or Schwarz Information Criteria.

'Bootstrap' – Bootstrap model selection.

'CV' – Single-item-out cross-validation.

'Expect' – The expected overall discrepancy (the true values of the parameters are required).

'Farrow' – Old model-free method by Farrow et al., 1994.

'Palmer' – Old model-free method by Mandel et al., 1995.

'Overall' – The realised overall discrepancy (the true values of the parameters are required).

The engine is the software used in the calculation, optimisation, etc. This can be anything, but those recognised by relax (automatic program info, citations, etc. added) include:

'relax' – hence relax was used for the full analysis.

'modelfree4' – Art Palmer's Modelfree4 program was used for optimising the model-free parameter values.

'dasha' – The Dasha program was used for optimising the model-free parameter values.

'curvefit' – Art Palmer's curvefit program was used to determine the  $R_1$  or  $R_2$  values.

The model\_elim flag is model-free specific and should be set if the methods from "d'Auvergne, E. J. and Gooley, P. R. (2006). Model-free model elimination: A new step in the model-free dynamic analysis of NMR relaxation data. J. Biomol. NMR, 35(2), 117-135." were used. This should be set to True for the full\_analysis.py script.

The universal\_solution flag is model-free specific and should be set if the methods from "d'Auvergne E. J., Gooley P. R. (2007). Set theory formulation of the model-free problem and the diffusion seeded model-free paradigm. Mol. Biosyst., 3(7), 483-494." were used. This should be set to True for the full\_analysis.py script.

## Prompt examples

For BMRB deposition, to specify that the `full_analysis.py` script was used, type one of:

```
relax> bmr.script('full_analysis.py', '
    model-free', 'AIC', 'relax', True, True
    )
```

```
relax> bmr.script(file='full_analysis.py',
    dir=None, analysis_type='model-free',
    model_selection='AIC', engine='relax',
    model_elim=True, universal_solution=
    True)
```

## 17.2.18 bmr.software



### Synopsis

Specify the software used in the analysis.

### Defaults

**bmr.software**(name=None, version=None, url=None, vendor\_name=None, cite\_ids=None, tasks=None)

### Keyword arguments

**name:** The name of the software program utilised.

**version:** The version of the software, if applicable.

**url:** The web address of the software.

**vendor\_name:** The name of the company or person behind the program.

**cite\_ids:** A list of the BMRB citation ID numbers.

**tasks:** A list of all the tasks performed by the software.

### Description

This user function allows the software used in the analysis to be specified in full detail.

For the tasks list, this should be a python list of strings (e.g. `['spectral processing']`). Although not restricted to these, the values suggested by the BMRB are:

```
'chemical shift assignment',
'chemical shift calculation',
'collection',
'data analysis',
'geometry optimization',
'peak picking',
'processing',
'refinement',
'structure solution'
```

## Prompt examples

For BMRB deposition, to say that Sparky was used in the analysis, type:

```
relax> cite_id = bmr.b.citation(authors=[["
    Tom", "Goddard", "T.", "D."], ["D", "
    Kneller", "D.", "G."]], title="Goddard,
    T. D. and Kneller, D. G., SPARKY 3,
    University of California, San
    Francisco.")

relax> bmr.b.software("Sparky", version="3
    .110", url="http://www.cgl.ucsf.edu/
    home/sparky/", vendor_name="Goddard, T.
    D.", cite_ids=[cite_id], tasks=["
    spectral analysis"])
```

## 17.2.19 bmr.b.software\_select



### Synopsis

Select the software used in the analysis.

### Defaults

`bmr.b.software_select(name=None, version=None)`

### Keyword arguments

`name`: The name of the software program utilised.

`version`: The version of the software, if applicable.

### Description

Rather than specifying all the information directly, this user function allows the software packaged used in the analysis to be selected by name. The programs currently supported are:

‘NMRPipe’ – <http://spin.niddk.nih.gov/NMRPipe/>

‘Sparky’ – <http://www.cgl.ucsf.edu/home/sparky/>

More can be added if all relevant information (program name, description, website, original citation, purpose, etc.) is emailed to [relax-users@gna.org](mailto:relax-users@gna.org).

Note that relax is automatically added to the BMRB file.

## Prompt examples

For BMRB deposition, to say that both NMRPipe and Sparky were used prior to relax, type:

```
relax> bmr.b.software_select('NMRPipe')

relax> bmr.b.software_select('Sparky',
    version='3.113')
```

### 17.2.20 bmr.b.thiol\_state



#### Synopsis

Select the thiol state of the system.

#### Defaults

**bmr.b.thiol\_state**(state=None)

#### Keyword arguments

**state**: The thiol state.

#### Description

The thiol state can be any text, though the BMRB suggests the following:

```
'all disulfide bound',
'all free',
'all other bound',
'disulfide and other bound',
'free and disulfide bound',
'free and other bound',
'free disulfide and other bound',
'not available',
'not present',
'not reported',
'unknown'.
```

Alternatively the pure states 'reduced' or 'oxidised' could be specified.

#### Prompt examples

For BMRB deposition, to say that the protein studied is in the oxidised state, type one of:

```
relax> bmr.b.thiol_state('oxidised')
```

```
relax> bmr.b.thiol_state(state='oxidised')
```

### 17.2.21 bmr.b.write



#### Synopsis

Write the results to a BMRB NMR-STAR formatted file.

#### Defaults

**bmr.b.write**(file=None, dir='pipe\_name', version='3.1', force=False)

#### Keyword arguments

**file**: The name of the BMRB file to output results to. Optionally this can be a file object, or any object with a write() method.

**dir**: The directory name.

**version**: The NMR-STAR dictionary format version to create.

**force**: A flag which if True will cause the any pre-existing file to be overwritten.

#### Description

This will create a NMR-STAR formatted file of the data in the current data pipe for BMRB deposition.

In the prompt/script UI modes, to place the BMRB file in the current working directory, set dir to None. If dir is set to the special name 'pipe\_name', then the results file will be placed into a directory with the same name as the current data pipe.



### 17.2.22 `bruker.read`



#### Synopsis

Read a Bruker Dynamics Center (DC) relaxation data file.

#### Defaults

`bruker.read(ri_id=None, file=None, dir=None)`

#### Keyword arguments

`ri_id`: The relaxation data ID string. This must be a unique identifier.

`file`: The name of the Bruker Dynamics Center file containing the relaxation data.

`dir`: The directory where the file is located.

#### Description

This user function is used to load all of the data out of a Bruker Dynamics Center (DC) relaxation data file for subsequent analysis within `relax`. Currently the  $R_1$  and  $R_2$  relaxation rates and steady-state NOE data is supported.

### 17.2.23 `chemical_shift.read`

$\delta$



#### Synopsis

Read chemical shifts from a file.

#### Defaults

`chemical_shift.read(file=None, dir=None, spin_id_col=None, mol_name_col=None, res_num_col=None, res_name_col=None, spin_num_col=None, spin_name_col=None, sep=None, spin_id=None)`

#### Keyword arguments

`file`: The name of the peak list of generic formatted file containing the chemical shifts.

`dir`: The directory where the file is located.

`spin_id_col`: The spin ID string column used by the generic file format (an alternative to the `mol`, `res`, and `spin name` and number columns).

`mol_name_col`: The molecule name column used by the generic file format (alternative to the `spin ID` column).

`res_num_col`: The residue number column used by the generic file format (alternative to the `spin ID` column).

`res_name_col`: The residue name column used by the generic file format (alternative to the `spin ID` column).

`spin_num_col`: The spin number column used by the generic file format (alternative to the `spin ID` column).

`spin_name_col`: The spin name column used by the generic file format (alternative to the `spin ID` column).

`sep`: The column separator used by the generic format (the default is white space).

`spin_id`: The spin ID string used to restrict the loading of data to certain spin subsets.

#### Description

This will read chemical shifts from a peak list or a generic column formatted file.

#### Prompt examples

The following commands will read the chemical shifts out of the Sparky peak list `'10ms.list'`:

```
relax> chemical_shift.read('10ms.list')
```

### 17.2.24 consistency\_tests.set\_frq


 $\omega$ 

#### Synopsis

Select which relaxation data to use in the consistency tests by NMR spectrometer frequency.

#### Defaults

`consistency_tests.set_frq(frq=None)`

#### Keyword arguments

**frq:** The spectrometer frequency in Hz. This must match the currently loaded data to the last decimal point. See the 'sfrq' parameter in the Varian procar file or the 'SF01' parameter in the Bruker acquis file.

#### Description

This will select the relaxation data to use in the consistency tests corresponding to the given frequencies. The data is selected by the spectrometer frequency in Hertz, which should be set to the exact value (see the 'sfrq' parameter in the Varian procar file or the 'SF01' parameter in the Bruker acquis file). Note though that the  $R_1$ ,  $R_2$  and NOE are all expected to have the exact same frequency in the  $J(\omega)$  mapping analysis (to the last decimal point).

#### Prompt examples

```
relax> consistency_tests.set_frq(600.0 * 1e6
    )
relax> consistency_tests.set_frq(frq=600.0 *
    1e6)
```

### 17.2.25 dasha.create



#### Synopsis

Create the Dasha script.

#### Defaults

`dasha.create(algor='LM', dir=None, force=False)`

#### Keyword arguments

**algor:** The minimisation algorithm.

**dir:** The directory to place the files.

**force:** A flag which if set to True will cause the results file to be overwritten if it already exists.

#### Description

The script file created is called 'dir/dasha\_script'.

#### Optimisation algorithms

The two minimisation algorithms within Dasha are accessible through the algorithm which can be set to:

'LM' – The Levenberg-Marquardt algorithm,

'NR' – Newton-Raphson algorithm.

For Levenberg-Marquardt minimisation, the function 'lmin' will be called, while for Newton-Raphson, the function 'min' will be executed.

---

**17.2.26** `dasha.execute`**Synopsis**

Perform a model-free optimisation using Dasha.

**Defaults**

`dasha.execute(dir=None, force=False, binary='dasha')`

**Keyword arguments**

`dir`: The directory to place the files.

`force`: A flag which if set to True will cause the results file to be overwritten if it already exists.

`binary`: The name of the executable Dasha program file.

**Description**

Dasha will be executed as

```
$ dasha < dasha_script | tee dasha_results
```

If you would like to use a different Dasha executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, include the full path in front of the binary file name.

---

**17.2.27** `dasha.extract`**Synopsis**

Extract data from the Dasha results file.

**Defaults**

`dasha.extract(dir=None)`

**Keyword arguments**

`dir`: The directory where the file 'dasha\_results' is found.

**Description**

The model-free results will be extracted from the Dasha results file 'dasha\_results' located in the given directory.

---

### 17.2.28 `deselect.all`



#### Synopsis

Deselect all spins in the current data pipe.

#### Defaults

`deselect.all()`

#### Description

This will deselect all spins, irregardless of their current state.

#### Prompt examples

To deselect all spins, simply type:

```
relax> deselect.all()
```

---

### 17.2.29 `deselect.interatom`



#### Synopsis

Deselect specific interatomic data containers.

#### Defaults

`deselect.interatom(spin_id1=None, spin_id2=None, boolean='AND', change_all=False)`

#### Keyword arguments

**spin\_id1:** The spin ID string of the first spin of the interatomic data container.

**spin\_id2:** The spin ID string of the second spin of the interatomic data container.

**boolean:** The boolean operator specifying how interatomic data containers should be selected.

**change\_all:** A flag specifying if all other interatomic data containers should be changed.

#### Description

This is used to deselect specific interatomic data containers which store information about spin pairs such as RDCs, NOEs, dipole-dipole pairs involved in relaxation, etc. The '**change\_all**' flag default is False meaning that all interatomic data containers currently either selected or deselected will remain that way. Setting this to True will cause all interatomic data containers not specified by the spin ID strings to be deselected.

#### Boolean operators

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

Table 17.1: Boolean operators and their effects on selections

| Spin system or interatomic data container | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|-------------------------------------------|---|---|---|---|---|---|---|---|---|
| Original selection                        | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| New selection                             | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| OR                                        | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| NOR                                       | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| AND                                       | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| NAND                                      | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| XOR                                       | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| XNOR                                      | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 |

## Prompt examples

To deselect all N-H backbone bond vectors of a protein, assuming these interatomic data containers have been already set up, type one of:

```
relax> deselect.interatom('@N', '@H')
```

```
relax> deselect.interatom(spin_id1='@N',
    spin_id2='@H')
```

To deselect all H-H interatomic vectors of a small organic molecule, type one of:

```
relax> deselect.interatom('@H*', '@H*')
```

```
relax> deselect.interatom(spin_id1='@H*',
    spin_id2='@H*')
```

## 17.2.30 deselect.read



### Synopsis

Deselect the spins contained in a file.

### Defaults

**deselect.read**(file=None, dir=None, spin\_id\_col=None, mol\_name\_col=None, res\_num\_col=None, res\_name\_col=None, spin\_num\_col=None, spin\_name\_col=None, sep=None, spin\_id=None, boolean='AND', change\_all=False)

### Keyword arguments

**file**: The name of the file containing the list of spins to deselect.

**dir**: The directory where the file is located.

**spin\_id\_col**: The spin ID string column (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col**: The molecule name column (alternative to the spin\_id\_col).

**res\_num\_col**: The residue number column (alternative to the spin\_id\_col).

**res\_name\_col**: The residue name column (alternative to the spin\_id\_col).

**spin\_num\_col**: The spin number column (alternative to the spin\_id\_col).

**spin\_name\_col**: The spin name column (alternative to the spin\_id\_col).

**sep**: The column separator (the default is white space).

**spin\_id:** The spin ID string to restrict the loading of data to certain spin subsets.

**boolean:** The boolean operator specifying how spins should be selected.

**change\_all:** A flag specifying if all other spins should be changed.

## Description

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number, residue name, spin number and/or spin name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID string can be used to restrict the reading to certain spin types, for example only 15N spins when only residue information is in the file.

Empty lines and lines beginning with a hash are ignored.

The 'change all' flag default is False meaning that all spins currently either selected or deselected will remain that way. Setting this to True will cause all spins not specified in the file to be selected.

## Boolean operators

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

## Prompt examples

To deselect all overlapped residues listed with residue numbers in the first column of the file 'unresolved', type one of:

```
relax> deselect.read('unresolved',
    res_num_col=1)
```

```
relax> deselect.read(file='unresolved',
    res_num_col=1)
```

To deselect the spins in the second column of the relaxation data file 'r1.600' while selecting all other spins, for example type:

```
relax> deselect.read('r1.600', spin_num_col
    =2, change_all=True)
```

```
relax> deselect.read(file='r1.600',
    spin_num_col=2, change_all=True)
```

## 17.2.31 deselect.reverse



## Synopsis

Reversal of the spin selection for the given spins.

## Defaults

`deselect.reverse(spin_id=None)`

## Keyword arguments

**spin\_id:** The spin ID string.

## Description

By supplying the spin ID string, a subset of spins can have their selection status reversed.

## Description

To deselect all currently selected spins and select those which are deselected type:

```
relax> deselect.reverse()
```

### 17.2.32 deselect.sn\_ratio



#### Synopsis

Deselect spins with signal to noise ratio higher or lower than the given ratio.

#### Defaults

**deselect.sn\_ratio**(ratio=10.0, operation='i', all\_sn=False)

#### Keyword arguments

**ratio**: The signal to noise ratio to compare to.

**operation**: The comparison operation by which to deselect the spins.

**all\_sn**: A flag specifying if all the signal to noise ratios per spin should match the comparison operator, or if just a single comparison match is enough.

#### Description

The comparison operation is the method which to deselect spins according to: operation(sn\_ratio, ratio).

The possible operations are: '<':strictly less than, '<=':less than or equal, '>':strictly greater than, '>=':greater than or equal, '==':equal, '!=':not equal.

The '**all\_sn**' flag default is False, meaning that if any of the spin's signal to noise levels evaluates to True in the comparison, the spin is deselected.

#### Prompt examples

To deselect all spins with a signal to noise ratio lower than 10.0:

```
relax> deselect.sn_ratio(ratio=10.0,
                        operation='<')
```

```
relax> deselect.sn_ratio(ratio=10.0,
                        operation='<', all_sn=True)
```

### 17.2.33 deselect.spin



#### Synopsis

Deselect specific spins.

#### Defaults

**deselect.spin**(spin\_id=None, boolean='AND', change\_all=False)

#### Keyword arguments

**spin\_id**: The spin ID string.

**boolean**: The boolean operator specifying how spins should be deselected.

**change\_all**: A flag specifying if all other spins should be changed.

#### Description

The '**change all**' flag default is False meaning that all spins currently either selected or deselected will remain that way. Setting this to True will cause all spins not specified by the spin ID string to be deselected.

#### Boolean operators

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

#### Prompt examples

To deselect all glycines and alanines, type:

```
relax> deselect.spin(spin_id=':GLY|:ALA')
```

To deselect residue 12 MET type:

```
relax> deselect.spin(':12')
```

```
relax> deselect.spin(spin_id=':12')
```

```
relax> deselect.spin(spin_id=':12&:MET')
```

**17.2.34 diffusion\_tensor.copy****Synopsis**

Copy diffusion tensor data from one data pipe to another.

**Defaults**

`diffusion_tensor.copy(pipe_from=None, pipe_to=None)`

**Keyword arguments**

**pipe\_from:** The name of the data pipe to copy the diffusion tensor data from.

**pipe\_to:** The name of the data pipe to copy the diffusion tensor data to.

**Description**

This will copy the diffusion tensor data between data pipes. The destination data pipe must not contain any diffusion tensor data. If the source or destination data pipes are not supplied, then both will default to the current data pipe (hence specifying at least one is essential).

**Prompt examples**

To copy the diffusion tensor from the data pipe 'm1' to the current data pipe, type:

```
relax> diffusion_tensor.copy('m1')
```

```
relax> diffusion_tensor.copy(pipe_from='m1')
```

To copy the diffusion tensor from the current data pipe to the data pipe 'm9', type:

```
relax> diffusion_tensor.copy(pipe_to='m9')
```

To copy the diffusion tensor from the data pipe 'm1' to 'm2', type:

```
relax> diffusion_tensor.copy('m1', 'm2')
```

```
relax> diffusion_tensor.copy(pipe_from='m1',
                             pipe_to='m2')
```

**17.2.35 diffusion\_tensor.delete****Synopsis**

Delete the diffusion tensor data from the relax data store.

**Defaults**

`diffusion_tensor.delete()`

**Description**

This will delete all diffusion tensor data from the current data pipe.



17.2.36 `diffusion_tensor.display`

## Synopsis

Display the diffusion tensor information.

## Defaults

`diffusion_tensor.display()`

## Description

This will display all of the diffusion tensor information of the current data pipe.

17.2.37 `diffusion_tensor.init`

## Synopsis

Initialise the diffusion tensor.

## Defaults

`diffusion_tensor.init(params=None, time_scale=1.0, d_scale=1.0, angle_units='deg', param_types=0, spheroid_type=None, fixed=True)`

## Keyword arguments

**params:** The diffusion tensor data.

**time\_scale:** The correlation time scaling value.

**d\_scale:** The diffusion tensor eigenvalue scaling value.

**angle\_units:** The units for the angle parameters.

**param\_types:** A flag to select different parameter combinations.

**spheroid\_type:** A string which, if supplied together with spheroid parameters, will restrict the tensor to either being 'oblate' or 'prolate'.

**fixed:** A flag specifying whether the diffusion tensor is fixed or can be optimised.

## The sphere (isotropic diffusion)

When the molecule diffuses as a sphere, all three eigenvalues of the diffusion tensor are equal,  $\mathfrak{D}_x = \mathfrak{D}_y = \mathfrak{D}_z$ . In this case, the orientation of the XH bond vector within the diffusion frame is inconsequential to relaxation, hence, the spherical or Euler angles are undefined. Therefore solely a single geometric parameter, either  $\tau_m$  or  $\mathfrak{D}_{iso}$ , can fully and sufficiently parameterise the diffusion tensor. The correlation function for the global rotational diffusion is

$$C(\tau) = \frac{1}{5} e^{-\tau / \tau_m},$$

To select isotropic diffusion, the parameter should be a single floating point number. The number is the value of the isotropic global correlation time,  $\tau_m$ , in seconds. To specify the time in nanoseconds, set the time scale to 1e-9. Alternative parameters can be used by changing the 'param\_types' flag to the following integers

0 – {tm} (Default),

1 – {Diso},

where

$$1 / \tau_m = 6\mathfrak{D}_{iso}.$$

### The spheroid (axially symmetric diffusion)

When two of the three eigenvalues of the diffusion tensor are equal, the molecule diffuses as a spheroid. Four pieces of information are required to specify this tensor, the two geometric parameters,  $\mathfrak{D}_{iso}$  and  $\mathfrak{D}_a$ , and the two orientational parameters, the polar angle  $\theta$  and the azimuthal angle  $\phi$  describing the orientation of the axis of symmetry. The correlation function of the global diffusion is

$$C(\tau) = \frac{1}{5} \sum_{i=-1}^1 \sum_{j=-1}^1 \langle \mathbf{e}_i \cdot \mathbf{e}_j \rangle \exp(-\tau / \tau_{ij}),$$

where

$$c_{-1} = 1/4 (3 \delta_z^2 - 1)^2,$$

$$c_0 = 3 \delta_z^2 (1 - \delta_z^2),$$

$$c_1 = 3/4 (\delta_z^2 - 1)^2,$$

and

$$1 / \tau_{-1} = 6\mathfrak{D}_{iso} - 2\mathfrak{D}_a,$$

$$1 / \tau_0 = 6\mathfrak{D}_{iso} - \mathfrak{D}_a,$$

$$1 / \tau_1 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a.$$

The direction cosine  $\delta_z$  is defined as the cosine of the angle  $\alpha$  between the XH bond vector and the unique axis of the diffusion tensor.

To select axially symmetric anisotropic diffusion, the parameters should be a tuple of floating point numbers of length four. A tuple is a type of data structure enclosed in round brackets, the elements of which are separated by commas. Alternative sets of parameters, 'param\_types', are

0 – { $\tau_m$ ,  $\mathfrak{D}_a$ ,  $\theta$ ,  $\phi$ } (Default),

1 – { $\mathfrak{D}_{iso}$ ,  $\mathfrak{D}_a$ ,  $\theta$ ,  $\phi$ },

2 – { $\tau_m$ ,  $\mathfrak{D}_{ratio}$ ,  $\theta$ ,  $\phi$ },

3 – { $\mathfrak{D}_{||}$ ,  $\mathfrak{D}_{\perp}$ ,  $\theta$ ,  $\phi$ },

4 – { $\mathfrak{D}_{iso}$ ,  $\mathfrak{D}_{ratio}$ ,  $\theta$ ,  $\phi$ },

where

$$\tau_m = 1 / 6\mathfrak{D}_{iso},$$

$$\mathfrak{D}_{iso} = 1/3 (\mathfrak{D}_{||} + 2\mathfrak{D}_{\perp}),$$

$$\mathfrak{D}_a = \mathfrak{D}_{||} - \mathfrak{D}_{\perp},$$

$$\mathfrak{D}_{ratio} = \mathfrak{D}_{||} / \mathfrak{D}_{\perp}.$$

The spherical angles  $\{\theta, \phi\}$  orienting the unique axis of the diffusion tensor within the PDB frame are defined between

$$0 \leq \theta \leq \pi,$$

$$0 \leq \phi \leq 2\pi,$$

while the angle  $\alpha$  which is the angle between this axis and the given XH bond vector is defined between

$$0 \leq \alpha \leq 2\pi.$$

The spheroid type should be 'oblate', 'prolate', or None. This will be ignored if the diffusion tensor is not axially symmetric. If 'oblate' is given, then the constraint  $\mathfrak{D}_a \leq 0$  is used while if 'prolate' is given, then the constraint  $\mathfrak{D}_a \geq 0$  is used. If nothing is supplied, then  $\mathfrak{D}_a$  will be allowed to have any values. To prevent minimisation of diffusion tensor parameters in a space with two minima, it is recommended to specify which tensor is to be minimised, thereby partitioning the two minima into the two subspaces along the boundary  $\mathfrak{D}_a = 0$ .

### The ellipsoid (rhombic diffusion)

When all three eigenvalues of the diffusion tensor are different, the molecule diffuses as an ellipsoid. This diffusion is also known as fully anisotropic, asymmetric, or rhombic. The full tensor is specified by six pieces of information, the three geometric parameters  $\mathfrak{D}_{iso}$ ,  $\mathfrak{D}_a$ , and  $\mathfrak{D}_r$  representing the isotropic, anisotropic, and rhombic components of the tensor, and the three Euler angles  $\alpha$ ,  $\beta$ , and  $\gamma$  orienting the tensor within the PDB frame. The correlation function is

$$C(\tau) = \frac{1}{5} \sum_{i=-2}^{-2} \frac{\tau_i}{\tau_{iso}} e^{-\tau_i \tau},$$

where the weights on the exponentials are

$$c_2 = 1/4 (d + e),$$

$$c_1 = 3 \delta_y^2 \delta_z^2,$$

$$c_0 = 3 \delta_x^2 \delta_z^2,$$

$$c_1 = 3 \delta_x^2 \delta_y^2,$$

$$c_2 = 1/4 (d + e).$$

Let

$$\mathfrak{R} = \sqrt{1 + 3\mathfrak{D}_r},$$

then

$$d = 3 (\delta_x^4 + \delta_y^4 + \delta_z^4) - 1,$$

$$e = -1 / \mathfrak{R} ((1 + 3\mathfrak{D}_r)(\delta_x^4 + 2\delta_y^2 \delta_z^2) + (1 - 3\mathfrak{D}_r)(\delta_y^4 + 2\delta_x^2 \delta_z^2) - 2(\delta_z^4 + 2\delta_x^2 \delta_y^2)).$$

The correlation times are

$$1 / \tau_2 = 6\mathfrak{D}_{iso} - 2\mathfrak{D}_a \cdot \mathfrak{R},$$

$$1 / \tau_1 = 6\mathfrak{D}_{iso} - \mathfrak{D}_a (1 + 3\mathfrak{D}_r),$$

$$1 / \tau_0 = 6\mathfrak{D}_{iso} - \mathfrak{D}_a (1 - 3\mathfrak{D}_r),$$

$$1 / \tau_1 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a,$$

$$1 / \tau_1 = 6\mathfrak{D}_{iso} + 2\mathfrak{D}_a \cdot \mathfrak{R}.$$

The three direction cosines  $\delta_x$ ,  $\delta_y$ , and  $\delta_z$  are the coordinates of a unit vector parallel to the XH bond vector. Hence the unit vector is  $[\delta_x, \delta_y, \delta_z]$ .

To select fully anisotropic diffusion, the parameters should be a tuple of length six. A tuple is a type of data structure enclosed in round brackets, the elements of which are separated by commas. Alternative sets of parameters, 'param\_types', are

$$0 - \{\tau_m, \mathfrak{D}_a, \mathfrak{D}_r, \alpha, \beta, \gamma\} \text{ (Default),}$$

$$1 - \{\mathfrak{D}_{iso}, \mathfrak{D}_a, \mathfrak{D}_r, \alpha, \beta, \gamma\},$$

$$2 - \{\mathfrak{D}_x, \mathfrak{D}_y, \mathfrak{D}_z, \alpha, \beta, \gamma\},$$

$$3 - \{D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz}, D_{yz}\},$$

where

$$\tau_m = 1 / 6\mathfrak{D}_{iso},$$

$$\mathfrak{D}_{iso} = 1/3 (\mathfrak{D}_x + \mathfrak{D}_y + \mathfrak{D}_z),$$

$$\mathfrak{D}_a = \mathfrak{D}_z - (\mathfrak{D}_x + \mathfrak{D}_y)/2,$$

$$\mathfrak{D}_r = (\mathfrak{D}_y - \mathfrak{D}_x)/2\mathfrak{D}_a.$$

The angles  $\alpha$ ,  $\beta$ , and  $\gamma$  are the Euler angles describing the diffusion tensor within the PDB frame. These angles are defined using the z-y-z axis rotation notation where  $\alpha$  is the initial rotation angle around the z-axis,  $\beta$  is the rotation angle around the y-axis, and  $\gamma$  is the final rotation around the z-axis again. The angles are defined between

$$0 \leq \alpha \leq 2\pi,$$

$$0 \leq \beta \leq \pi,$$

$$0 \leq \gamma \leq 2\pi.$$

Within the PDB frame, the XH bond vector is described using the spherical angles  $\theta$  and  $\phi$  where  $\theta$  is the polar angle and  $\phi$  is the azimuthal angle defined between

$$0 \leq \theta \leq \pi,$$

$$0 \leq \phi \leq 2\pi.$$

When param\_types is set to 3, then the elements of the diffusion tensor matrix defined within the PDB frame can be supplied.

## Units

The correlation time scaling value should be a floating point number. The only parameter affected by this value is  $\tau_m$ .

The diffusion tensor eigenvalue scaling value should also be a floating point number. Parameters affected by this value are  $\mathfrak{D}_{iso}$ ,  $\mathfrak{D}_{||}$ ,  $\mathfrak{D}_{\perp}$ ,  $\mathfrak{D}_a$ ,  $\mathfrak{D}_x$ ,  $\mathfrak{D}_y$ , and  $\mathfrak{D}_z$ . Significantly,  $\mathfrak{D}_r$  is not affected.

The units for the angle parameters should be either 'deg' or 'rad'. Parameters affected are  $\theta$ ,  $\phi$ ,  $\alpha$ ,  $\beta$ , and  $\gamma$ .

## Prompt examples

To set an isotropic diffusion tensor with a correlation time of 10 ns, type:

```
relax> diffusion_tensor.init(10e-9)
```

```
relax> diffusion_tensor.init(params=10e-9)
```

```
relax> diffusion_tensor.init(10.0, 1e-9)
```

```
relax> diffusion_tensor.init(params=10.0,
    time_scale=1e-9, fixed=True)
```

To select axially symmetric diffusion with a  $\tau_m$  value of 8.5 ns,  $\mathfrak{D}_{ratio}$  of 1.1,  $\theta$  value of 20 degrees, and  $\phi$  value of 20 degrees, type:

```
relax> diffusion_tensor.init((8.5e-9, 1.1,
    20.0, 20.0), param_types=2)
```

To select a spheroid diffusion tensor with a  $\mathfrak{D}_{\parallel}$  value of 1.698e7,  $\mathfrak{D}_{\perp}$  value of 1.417e7,  $\theta$  value of 67.174 degrees, and  $\phi$  value of -83.718 degrees, type one of:

```
relax> diffusion_tensor.init((1.698e7, 1
    .417e7, 67.174, -83.718), param_types
    =3)
```

```
relax> diffusion_tensor.init(params=(1.698e7
    , 1.417e7, 67.174, -83.718),
    param_types=3)
```

```
relax> diffusion_tensor.init((1.698e-1, 1
    .417e-1, 67.174, -83.718), param_types
    =3, d_scale=1e8)
```

```
relax> diffusion_tensor.init(params=(1.698e
    -1, 1.417e-1, 67.174, -83.718),
    param_types=3, d_scale=1e8)
```

```
relax> diffusion_tensor.init((1.698e-1, 1
    .417e-1, 1.1724, -1.4612), param_types
    =3, d_scale=1e8, angle_units='rad')
```

```
relax> diffusion_tensor.init(params=(1.698e
    -1, 1.417e-1, 1.1724, -1.4612),
    param_types=3, d_scale=1e8, angle_units
    ='rad', fixed=True)
```

To select ellipsoidal diffusion, type:

```
relax> diffusion_tensor.init((1.340e7, 1
    .516e7, 1.691e7, -82.027, -80.573, 65
    .568), param_types=2)
```

## 17.2.38 domain

### Synopsis

Definition of structural domains.

### Defaults

`domain(id=None, spin_id=None)`

### Keyword arguments

`id`: The ID string used to identify molecular domains.

`spin_id`: The spin ID string of all atomic members of the domain.

### Description

This is used to define structural domains. Multiple domains can be defined, and these can overlap. Rather than labelling the currently loaded spins with the ID string, the spin ID string is stored for later use. This allows new spins to be loaded later and still be included within the same domain.

### 17.2.39 dx.execute



#### Synopsis

Execute an OpenDX program.

#### Defaults

**dx.execute**(file\_prefix='map', dir='dx', dx\_exe='dx',  
vp\_exec=True)

#### Keyword arguments

**file\_prefix**: The file name prefix. For example if file is set to 'temp', then the OpenDX program temp.net will be loaded.

**dir**: The directory to change to for running OpenDX. If this is set to None, OpenDX will be run in the current directory.

**dx\_exe**: The OpenDX executable file.

**vp\_exec**: A flag specifying whether to execute the visual program automatically at start-up. The default of True causes the program to be executed.

#### Description

This will execute OpenDX to display the space maps created previously by the  $\delta_x$ .map user function. This will work for any type of OpenDX map.

### 17.2.40 dx.map



#### Synopsis

Create a map of the given space in OpenDX format.

#### Defaults

**dx.map**(params=None, map\_type='Iso3D', spin\_id=None,  
inc=20, lower=None, upper=None, axis\_incs=5,  
file\_prefix='map', dir='dx', point=None, point\_file=  
'point', chi\_surface=None, create\_par\_file=False)

#### Keyword arguments

**params**: The parameters to be mapped. This should be an array of strings, the meanings of which are described below.

**map\_type**: The type of map to create. For example the default, a 3D isosurface, the type is 'Iso3D'. See below for more details.

**spin\_id**: The spin ID string.

**inc**: The number of increments to map in each dimension. This value controls the resolution of the map.

**lower**: The lower bounds of the space. If you wish to change the lower bounds of the map then supply an array of length equal to the number of parameters in the model. A lower bound for each parameter must be supplied. If nothing is supplied then the defaults will be used.

**upper**: The upper bounds of the space. If you wish to change the upper bounds of the map then supply an array of length equal to the number of parameters in the model. An upper bound for each parameter must be supplied. If nothing is supplied then the defaults will be used.

**axis\_incs**: The number of increments or ticks displaying parameter values along the axes of the OpenDX plot.

**file\_prefix**: The file name. All the output files are prefixed with this name. The main file containing the data points will be called the value of 'file'. The OpenDX program will be called 'file.net' and the OpenDX import file will be called 'file.general'.

**dir**: The directory to output files to. Set this to 'None' if you do not want the files to be placed in subdirectory. If the directory does not exist, it will be created.

**point**: This argument allows specific points in the optimisation space to be displayed as coloured spheres. This can be used to highlight a minimum or other any other feature of the space. Either a single point or a list of

points can be supplied. Each point is a list of floating point numbers in the form  $[x, y, z]$

**point\_file:** The name of that the point output files will be prefixed with.

**chi\_surface:** A list of 4 numbers, setting the level for the 4 isosurfaces. Useful in scripting if you create a set of OpenDX maps with all the same contour levels. Ideal for comparisons.

**create\_par\_file:** A flag specifying whether to create a file with parameters and associated chi2 value. The default of False causes the file not to be created.

## Description

This will map the space corresponding to the spin identifier and create the OpenDX files. The map type can be changed to one of the following supported map types:

Please see Table 17.2 on page 469.

## Model-free parameters

Please see Table 17.3 on page 469.

## N-state model parameters

Please see Table 17.4 on page 469.

## Relaxation dispersion parameters

Please see Table 17.5 on page 470.

## Frame order parameters

Please see Table 17.6 on page 470.

## Prompt examples

The following commands will generate a map of the extended model-free space for model 'm5' consisting of the parameters  $\{S^2, S_f^2, \tau_s\}$ . Files will be output into the directory 'dx' and will be prefixed by 'map'. In this case, the system is a protein and residue number 6 will be mapped.

```
relax> dx.map(['s2', 's2f', 'ts'], spin_id=':6')
```

```
relax> dx.map(['s2', 's2f', 'ts'], spin_id=':6', file_prefix='map', dir='dx')
```

```
relax> dx.map(params=['s2', 's2f', 'ts'],
              spin_id=':6', inc=20, file_prefix='map',
              dir='dx')
```

```
relax> dx.map(params=['s2', 's2f', 'ts'],
              spin_id=':6', map_type='Iso3D', inc=20,
              file_prefix='map', dir='dx')
```

To map the model-free space 'm4' for residue 2, spin N6 defined by the parameters  $\{S^2, \tau_e, R_{ex}\}$ , name the results 'test', and to place the files in the current directory, use one of the following commands:

```
relax> dx.map(['s2', 'te', 'rex'], spin_id=':2@N6', file_prefix='test', dir=None)
```

```
relax> dx.map(params=['s2', 'te', 'rex'],
              spin_id=':2@N6', inc=100, file_prefix='test', dir=None)
```

Table 17.2: OpenDx mapping types.

| Surface type  | Name    |
|---------------|---------|
| 3D isosurface | 'Iso3D' |

Table 17.3: Model-free parameters.

| Name     | Description                                                                            |
|----------|----------------------------------------------------------------------------------------|
| tm       | Global correlation time                                                                |
| Diso     | Isotropic component of the diffusion tensor                                            |
| Dx       | Eigenvalue associated with the x-axis of the diffusion tensor                          |
| Dy       | Eigenvalue associated with the y-axis of the diffusion tensor                          |
| Dz       | Eigenvalue associated with the z-axis of the diffusion tensor                          |
| Dpar     | Diffusion coefficient parallel to the major axis of the spheroid diffusion tensor      |
| Dper     | Diffusion coefficient perpendicular to the major axis of the spheroid diffusion tensor |
| Da       | Anisotropic component of the diffusion tensor                                          |
| Dr       | Rhombic component of the diffusion tensor                                              |
| Dratio   | Ratio of the parallel and perpendicular components of the spheroid diffusion tensor    |
| alpha    | The first Euler angle of the ellipsoid diffusion tensor                                |
| beta     | The second Euler angle of the ellipsoid diffusion tensor                               |
| gamma    | The third Euler angle of the ellipsoid diffusion tensor                                |
| theta    | The polar angle defining the major axis of the spheroid diffusion tensor               |
| phi      | The azimuthal angle defining the major axis of the spheroid diffusion tensor           |
| s2       | $S^2$ , the model-free generalised order parameter ( $S^2 = S_f^2 \cdot S_{2s}$ )      |
| s2f      | $S_f^2$ , the faster motion model-free generalised order parameter                     |
| s2s      | $S_s^2$ , the slower motion model-free generalised order parameter                     |
| local_tm | The spin specific global correlation time (seconds)                                    |
| te       | Single motion effective internal correlation time (seconds)                            |
| tf       | Faster motion effective internal correlation time (seconds)                            |
| ts       | Slower motion effective internal correlation time (seconds)                            |
| rex      | Chemical exchange relaxation ( $\sigma_{ex} = R_{ex} / \omega^2$ )                     |
| csa      | Chemical shift anisotropy (unitless)                                                   |

Table 17.4: N-state model parameters.

| Name                | Description                                                | Type  |
|---------------------|------------------------------------------------------------|-------|
| Axx                 | The Axx component of the alignment tensor                  | float |
| Ayy                 | The Ayy component of the alignment tensor                  | float |
| Axy                 | The Axy component of the alignment tensor                  | float |
| Axz                 | The Axz component of the alignment tensor                  | float |
| Ayz                 | The Ayz component of the alignment tensor                  | float |
| probs               | The probabilities of each state                            | list  |
| alpha               | The $\alpha$ Euler angles (for the rotation of each state) | list  |
| beta                | The $\beta$ Euler angles (for the rotation of each state)  | list  |
| gamma               | The $\gamma$ Euler angles (for the rotation of each state) | list  |
| paramagnetic_centre | The paramagnetic centre                                    | list  |

Table 17.5: Relaxation dispersion parameters.

| Name     | Description                                                                                                          | Type  |
|----------|----------------------------------------------------------------------------------------------------------------------|-------|
| r2eff    | The effective transversal relaxation rate                                                                            | dict  |
| i0       | The initial intensity                                                                                                | dict  |
| r1       | The longitudinal relaxation rate                                                                                     | dict  |
| r2       | The transversal relaxation rate                                                                                      | dict  |
| r2a      | The transversal relaxation rate for state A in the absence of exchange                                               | dict  |
| r2b      | The transversal relaxation rate for state B in the absence of exchange                                               | dict  |
| pA       | The population for state A                                                                                           | float |
| pB       | The population for state B                                                                                           | float |
| pC       | The population for state C                                                                                           | float |
| phi_ex   | The $\phi_{\text{ex}} = pA \cdot pB \cdot dw^{*2}$ value (ppm <sup>2</sup> )                                         | float |
| phi_ex_B | The fast exchange factor between sites A and B (ppm <sup>2</sup> )                                                   | float |
| phi_ex_C | The fast exchange factor between sites A and C (ppm <sup>2</sup> )                                                   | float |
| padw2    | The $pA \cdot dw^{*2}$ value (ppm <sup>2</sup> )                                                                     | float |
| dw       | The chemical shift difference between states A and B (in ppm)                                                        | float |
| dw_AB    | The chemical shift difference between states A and B for 3-site exchange (in ppm)                                    | float |
| dw_AC    | The chemical shift difference between states A and C for 3-site exchange (in ppm)                                    | float |
| dw_BC    | The chemical shift difference between states B and C for 3-site exchange (in ppm)                                    | float |
| dWH      | The proton chemical shift difference between states A and B (in ppm)                                                 | float |
| dWH_AB   | The proton chemical shift difference between states A and B for 3-site exchange (in ppm)                             | float |
| dWH_AC   | The proton chemical shift difference between states A and C for 3-site exchange (in ppm)                             | float |
| dWH_BC   | The proton chemical shift difference between states B and C for 3-site exchange (in ppm)                             | float |
| kex      | The exchange rate                                                                                                    | float |
| kex_AB   | The exchange rate between sites A and B for 3-site exchange with $kex_{AB} = k_{AB} + k_{BA}$ (rad.s <sup>-1</sup> ) | float |
| kex_AC   | The exchange rate between sites A and C for 3-site exchange with $kex_{AC} = k_{AC} + k_{CA}$ (rad.s <sup>-1</sup> ) | float |
| kex_BC   | The exchange rate between sites B and C for 3-site exchange with $kex_{BC} = k_{BC} + k_{CB}$ (rad.s <sup>-1</sup> ) | float |
| kB       | Approximate chemical exchange rate constant between sites A and B (rad.s <sup>-1</sup> )                             | float |
| kC       | Approximate chemical exchange rate constant between sites A and C (rad.s <sup>-1</sup> )                             | float |
| tex      | The time of exchange ( $tex = 1/kex$ )                                                                               | float |
| k_AB     | The exchange rate from state A to state B                                                                            | float |
| k_BA     | The exchange rate from state B to state A                                                                            | float |

Table 17.6: Frame order parameters.

| Name             | Description                                                                                    |
|------------------|------------------------------------------------------------------------------------------------|
| pivot_x          | The pivot point position $x$ coordinate                                                        |
| pivot_y          | The pivot point position $y$ coordinate                                                        |
| pivot_z          | The pivot point position $z$ coordinate                                                        |
| pivot_disp       | The 2 <sup>nd</sup> pivot point displacement - the minimum distance between the two rotor axes |
| ave_pos_x        | The average position $x$ translation                                                           |
| ave_pos_y        | The average position $y$ translation                                                           |
| ave_pos_z        | The average position $z$ translation                                                           |
| ave_pos_alpha    | The average position $\alpha$ Euler angle                                                      |
| ave_pos_beta     | The average position $\beta$ Euler angle                                                       |
| ave_pos_gamma    | The average position $\gamma$ Euler angle                                                      |
| eigen_alpha      | The Eigenframe $\alpha$ Euler angle                                                            |
| eigen_beta       | The Eigenframe $\beta$ Euler angle                                                             |
| eigen_gamma      | The Eigenframe $\gamma$ Euler angle                                                            |
| axis_theta       | The cone axis polar angle (for the isotropic cone model)                                       |
| axis_phi         | The cone axis azimuthal angle (for the isotropic cone model)                                   |
| axis_alpha       | The rotor axis $\alpha$ angle (the rotation angle out of the $xy$ plane)                       |
| cone_theta_x     | The pseudo-ellipse cone opening half-angle for the $x$ -axis                                   |
| cone_theta_y     | The pseudo-ellipse cone opening half-angle for the $y$ -axis                                   |
| cone_theta       | The isotropic cone opening half-angle                                                          |
| cone_sigma_max   | The torsion angle                                                                              |
| cone_sigma_max_2 | The torsion angle of the 2 <sup>nd</sup> motional mode                                         |



## 17.2.41 eliminate



### Synopsis

Elimination or rejection of models.

### Defaults

`eliminate(function=None, args=None)`

### Keyword arguments

**function:** An optional user supplied function for model elimination.

**args:** A tuple of arguments used by the optional function for model elimination.

### Description

This is used for model validation to eliminate or reject models prior to model selection. Model validation is a part of mathematical modelling whereby models are either accepted or rejected.

Empirical rules are used for model rejection and are listed below. However these can be overridden by supplying a function in the prompt and scripting modes. The function should accept five arguments, a string defining a certain parameter, the value of the parameter, the minimisation instance (ie the residue index if the model is residue specific), and the function arguments. If the model is rejected, the function should return True, otherwise it should return False. The function will be executed multiple times, once for each parameter of the model.

The function arguments should be a tuple, a list enclosed in round brackets, and will be passed to the user supplied function or the inbuilt function. For a description of the arguments accepted by the inbuilt functions, see below.

Once a model is rejected, the select flag corresponding to that model will be set to False so that model selection, or any other function, will then skip the model.

### Local $\tau_m$ model elimination rule

The local  $\tau_m$ , in some cases, may exceed the value expected for a global correlation time. Generally the  $\tau_m$  value will be stuck at the upper limit defined for the parameter. These models are eliminated using the rule:

$$\tau_m \geq c$$

The default value of  $c$  is 50 ns, although this can be overridden by supplying the value (in seconds) as the first element of the args tuple.

### Internal correlation times $\tau_e$ , $\tau_f$ , $\tau_s$ model elimination rules

These parameters may experience the same problem as the local  $\tau_m$  in that the model fails and the parameter value is stuck at the upper limit. These parameters are constrained using the formula ( $\tau_e, \tau_f, \tau_s \leq 2\tau_m$ ). These failed models are eliminated using the rule:

$$\tau_e, \tau_f, \tau_s \geq c \cdot \tau_m.$$

The default value of  $c$  is 1.5. Because of round-off errors and the constraint algorithm, setting  $c$  to 2 will result in no models being eliminated as the minimised parameters will always be less than  $2\tau_m$ . The value can be changed by supplying the value as the second element of the tuple.

### Arguments

The 'args' argument must be a tuple of length 2, the elements of which must be numbers. For example, to eliminate models which have a local  $\tau_m$  value greater than 25 ns and models with internal correlation times greater than 1.5 times  $\tau_m$ , set 'args' to (25 \* 1e-9, 1.5).

### 17.2.42 `error_analysis.covariance_matrix`

#### Synopsis

Parameter error estimation via the covariance matrix.

#### Defaults

`error_analysis.covariance_matrix(epsrel=0.0, verbosity=1)`

#### Keyword arguments

**epsrel:** The parameter to remove linear-dependent columns when J is rank deficient.

**verbosity:** The higher the value, the greater the verbosity.

#### Description

This is a new experimental feature from version 3.3.

This will estimate parameter errors by using the exponential decay Jacobian matrix 'J' to compute the covariance matrix of the best-fit parameters.

This can be used to for comparison to Monte-Carlo simulations.

This method is inspired from the GNU Scientific Library (GSL).

The covariance matrix is given by:  $\text{covar} = Q_{xx} = (J^T W J)^{-1}$ , where the weight matrix W is constructed by the multiplication of an Identity matrix I and a weight array w. The weight array is  $1/\text{errors}^2$ , which then gives  $W = I \cdot w = I \cdot 1/\text{errors}^2$ .

$Q_{xx}$  is computed by QR decomposition,  $J^T W J = QR$ ,  $Q_{xx} = R^{-1} \cdot Q^T$ . The columns of R which satisfy  $|R_{kk}| \leq \text{epsrel} \cdot |R_{11}|$  are considered linearly-dependent and are excluded from the covariance matrix (the corresponding rows and columns of the covariance matrix are set to zero).

The parameter '**epsrel**' is used to remove linear-dependent columns when J is rank deficient.

### 17.2.43 `fix`



#### Synopsis

Fix or allow parameter values to change during optimisation.

#### Defaults

`fix(element=None, fixed=True)`

#### Keyword arguments

**element:** Which element to fix.

**fixed:** A flag specifying if the parameters should be fixed or allowed to change.

#### Description

The element can be any of the following:

**'diff'** – The diffusion tensor parameters. This will allow all diffusion tensor parameters to be toggled.

**'all\_spins'** – Using this keyword, all parameters from all spins will be toggled.

**'all'** – All parameters will be toggled. This is equivalent to combining both '**diff**' and '**all\_spins**'.

The flag '**fixed**', if set to True, will fix parameters during optimisation whereas a value of False will allow parameters to vary.

### 17.2.44 `frame_order.count_sobol_points`



#### Synopsis

Count the number of Sobol' points used for the current parameter values.

#### Defaults

`frame_order.count_sobol_points()`

#### Description

This allows the number of Sobol' integration points used during the Frame Order target function optimisation to be counted. This uses the current parameter values to determine how many are used for the PCS calculation compared to the total number.

### 17.2.45 `frame_order.distribute`



#### Synopsis

Structural distribution of the frame order motions.

#### Defaults

`frame_order.distribute(file='distribution.pdb.gz', dir=None, atom_id=None, total=1000, max_rotations=100000, model=1, force=False)`

#### Keyword arguments

**file:** The PDB file for storing the frame order motional distribution. The compression is determined automatically by the file extensions `'*.pdb'`, `'*.pdb.gz'`, and `'*.pdb.bz2'`.

**dir:** The directory where the files are to be located.

**atom\_id:** The atom identification string to allow the distribution to be a subset of all atoms.

**total:** The total number of structures to include in the uniform distribution.

**max\_rotations:** The maximum number of rotations to generate the distribution from. This prevents the user function from executing for an infinite amount of time. This occurs whenever a frame order amplitude parameter (cone opening angle or torsion angle) is zero so that the subset of all rotations within the motional distribution is also zero.

**model:** Only one model from an analysed ensemble of structures can be used for the distribution, as the distribution PDB file consists of one model per state.

**force:** A flag which, if set to True, will overwrite the any pre-existing file.

#### Description

To visualise the frame order motions, this user function generates a distribution of structures randomly within the bounds of the uniform distribution of the frame order model. The original structure is rotated randomly and only accepted for the distribution if it is within the bounds. This is a more faithful representation of the dynamics than the pseudo-Brownian simulation user function.

Note that the RDC and PCS data does not contain information about all parts of the real distribution of structures. Therefore the structures in this distribution only

represent the components of the distribution present in the data, as modelled by the frame order models.

As the distribution consists of one model per state, if an ensemble of structures has been analysed, only one model from the ensemble can be used for the representation.

---

## 17.2.46 frame\_order.pdb\_model



### Synopsis

Create a PDB file representation of the frame order dynamics.

### Defaults

```
frame_order.pdb_model(ave_pos='ave_pos', rep=  
'frame_order', dir=None, compress_type=0, size=30.0,  
inc=36, model=1, force=False)
```

### Keyword arguments

**ave\_pos:** The file root of the 3D structure PDB file for the molecular structure with the moving domains shifted to the average position.

**rep:** The file root of the PDB file for the geometric object representation of the frame order dynamics.

**dir:** The directory where the files are to be located.

**compress\_type:** The type of compression to use when creating the files.

**size:** The size of the geometric object in Å.

**inc:** The number of increments used to create the geometric object.

**model:** Only one model from an analysed ensemble can be used for the PDB representation of the Monte Carlo simulations of the average domain position, as these consists of one model per simulation.

**force:** A flag which, if set to True, will overwrite the any pre-existing files.

### Description

This function creates a set of PDB files for representing the frame order cone models. This includes a file for the average position of the molecule and a file containing a geometric representation of the frame order motions.

The three files are specified via the file root whereby the extensions `‘.pdb’`, `‘.pdb.gz’`, etc. should not be provided. This is important for the geometric representation whereby different files are created for the positive and negative representations (due to symmetry in the NMR data, these cannot be differentiated), and for the Monte Carlo simulations. For example if the file root is `‘frame_order’`, the positive and negative representations will be placed in the `‘frame_order_pos.pdb.gz’`

and 'frame\_order\_neg.pdb.gz' files and the Monte Carlo simulations in the 'frame\_order\_sim\_pos.pdb.gz' and 'frame\_order\_sim\_neg.pdb.gz' files. For models where there is no difference in representation between the positive and negative directions, the files 'frame\_order.pdb.gz' and 'frame\_order\_sim.pdb.gz' will be produced.

There are four different types of residue within the PDB. The pivot point is represented as a single carbon atom of the residue 'PIV'. The cone consists of numerous H atoms of the residue 'CON'. The cone axis vector is presented as the residue 'AXE' with one carbon atom positioned at the pivot and the other  $x$  Å away on the cone axis (set by the geometric object size). Finally, if Monte Carlo have been performed, there will be multiple 'MCC' residues representing the cone for each simulation, and multiple 'MCA' residues representing the multiple cone axes.

To create the diffusion in a cone PDB representation, a uniform distribution of vectors on a sphere is generated using spherical coordinates with the polar angle defined by the cone axis. By incrementing the polar angle using an arccos distribution, a radial array of vectors representing latitude are created while incrementing the azimuthal angle evenly creates the longitudinal vectors. These are all placed into the PDB file as H atoms and are all connected using PDB CONECT records. Each H atom is connected to its two neighbours on the both the longitude and latitude. This creates a geometric PDB object with longitudinal and latitudinal lines representing the filled cone.

The PDB representation of the Monte Carlo simulations consists of one model per simulation. Therefore if an ensemble of structures has been analysed, only one model from the ensemble can be used for the representation. This defaults to model number 1, but this can be changed.

## 17.2.47 frame\_order.permute\_axes



### Synopsis

Permute the axes of the motional eigenframe to switch between local minima.

### Defaults

`frame_order.permute_axes(permutation='A')`

### Keyword arguments

**permutation:** Which of the two permutations 'A' or 'B' to create. Three permutations are possible, and 'A' and 'B' select those which are not the starting combination.

### Description

The isotropic and pseudo-elliptic cone frame order models consist of multiple solutions as the optimisation space contains multiple local minima. Because of the constraint  $\text{cone\_theta\_x} \leq \text{cone\_theta\_y}$  in the pseudo-ellipse model, there are exactly three local minima (out of 6 possible permutations). However the  $\text{cone\_theta\_x} == \text{cone\_theta\_y}$  condition of the isotropic cone collapses this to two minima. The multiple minima correspond to permutations of the motional system - the eigenframe  $x$ ,  $y$  and  $z$ -axes as well as the cone opening angles  $\text{cone\_theta\_x}$ ,  $\text{cone\_theta\_y}$ , and  $\text{cone\_sigma\_max}$  associated with these axes. But as the mechanics of the cone angles is not identical to that of the torsion angle, only one of the three local minima is the global minimum.

When optimising the pseudo-elliptic models, specifically the 'pseudo-ellipse' and 'pseudo-ellipse, torsionless' model, any of the three local minima can be found. Convergence to the global minimum is not guaranteed. Therefore this user function can be used to permute the motional system to jump from one local minimum to the other. Optimisation will be required as the permuted parameters will not be exactly at the minimum.

Please see Table 17.7 on page 476.

In this table, the condition and cone angle values  $[x, y, z]$  correspond to  $\text{cone\_theta\_x}$ ,  $\text{cone\_theta\_y}$ , and  $\text{cone\_sigma\_max}$ .

### Prompt examples

For combination 'A', simply type:

```
relax> frame_order.permute_axes('A')
```

Table 17.7: The motional eigenframe axis permutations for the frame order models.

| Condition   | Permutation name | Cone angles | Axes         |
|-------------|------------------|-------------|--------------|
| $x < y < z$ | Self             | $[x, y, z]$ | $[x, y, z]$  |
|             | A                | $[x, z, y]$ | $[-z, y, x]$ |
|             | B                | $[y, z, x]$ | $[z, x, y]$  |
| $x < z < y$ | Self             | $[x, y, z]$ | $[x, y, z]$  |
|             | A                | $[x, z, y]$ | $[-z, y, x]$ |
|             | B                | $[z, y, x]$ | $[x, -z, y]$ |
| $z < x < y$ | Self             | $[x, y, z]$ | $[x, y, z]$  |
|             | A                | $[z, x, y]$ | $[y, z, x]$  |
|             | B                | $[z, y, x]$ | $[x, -z, y]$ |

## Prompt examples

### 17.2.48 `frame_order.pivot`



To set the pivot point, type one of:

```
relax> frame_order.pivot([12.067, 14.313, -3
                          .2675])
```

```
relax> frame_order.pivot(pivot=[12.067, 14
                              .313, -3.2675])
```

### Synopsis

Set the pivot points for the two body motion in the structural coordinate system.

To change an already set and fixed pivot point so that it can now be optimised, type:

```
relax> frame_order.pivot(fix=False)
```

### Defaults

```
frame_order.pivot(pivot=None, order=1, fix=False)
```

### Keyword arguments

**pivot:** The pivot point for the motion (e.g. the position between the 2 domains in PDB coordinates).

**order:** The ordinal number of the pivot point. The value of 1 is for the first pivot point, the value of 2 for the second pivot point, and so on.

**fix:** A flag specifying if the pivot point should be fixed during optimisation.

### Description

This will set the pivot points for the two domain system within the PDB coordinate system. This is required for interpreting PCS data as well as for the generation of cone or other PDB representations of the domain motions.

This user function can also be used to change the optimisation status of an already set pivot point. By simply providing the fixed flag and not the pivot point values, the pivot can be changed to be either fixed during optimisation or that it will be optimised.

---

**17.2.49 frame\_order.quad\_int****Synopsis**

Turn the high precision quadratic integration on or off.

**Defaults**

`frame_order.quad_int(flag=True)`

**Keyword arguments**

**flag:** The flag with if True will perform high precision numerical integration via the `scipy.integrate.quad()`, `dblquad()` and `tplquad()` integration methods rather than the rough quasi-random numerical integration.

**Description**

This allows the high precision numerical integration of the Scipy `quad()` and related functions to be used instead of the lower precision quasi-random Sobol' sequence integration. This is for the optimisation of the Frame Order target functions. The quadratic integration is orders of magnitude slower than the Sobol' sequence integration, but the precision is much higher.

---

**17.2.50 frame\_order.ref\_domain****Synopsis**

Set the reference non-moving domain for the 2-domain frame order theories.

**Defaults**

`frame_order.ref_domain(ref=None)`

**Keyword arguments**

**ref:** The non-moving domain which will act as the frame of reference.

**Description**

Prior to optimisation of the frame order model, the frame of reference non-moving domain must be specified. This is essential for determining which spins will be used in the analysis, which will be shifted to the average position, etc.

**Prompt examples**

To set up the isotropic cone frame order model with 'centre' domain being the frame of reference, type:

```
relax> frame_order.ref_domain(ref='centre')
```

### 17.2.51 `frame_order.select_model`



#### Synopsis

Select and set up the Frame Order model.

#### Defaults

`frame_order.select_model(model=None)`

#### Keyword arguments

`model`: The name of the preset Frame Order model.

#### Description

Prior to optimisation, the Frame Order model should be selected. These models consist of three parameter categories:

The average domain position. This includes the parameters `ave_pos_alpha`, `ave_pos_beta`, and `ave_pos_gamma`. These Euler angles rotate the tensors from the arbitrary PDB frame of the moving domain to the average domain position.

The frame order eigenframe. This includes the parameters `eigen_alpha`, `eigen_beta`, and `eigen_gamma`. These Euler angles define the major modes of motion. The cone central axis is defined as the z-axis. The pseudo-elliptic cone  $x$  and  $y$ -axes are defined as the  $x$  and  $y$ -axes of the eigenframe.

The cone parameters. These are defined as the tilt-torsion angles `cone_theta_x`, `cone_theta_y`, and `cone_sigma_max`. The `cone_theta_x` and `cone_theta_y` parameters define the two cone opening angles of the pseudo-ellipse. The amount of domain torsion is defined as the average domain position, plus and minus `cone_sigma_max`. The isotropic cones are defined by setting `cone_theta_x = cone_theta_y` and converting the single parameter into a 2<sup>nd</sup> rank order parameter.

The list of available models are:

**‘pseudo-ellipse’** – The pseudo-elliptic cone model. This is the full model consisting of the parameters `ave_pos_alpha`, `ave_pos_beta`, `ave_pos_gamma`, `eigen_alpha`, `eigen_beta`, `eigen_gamma`, `cone_theta_x`, `cone_theta_y`, and `cone_sigma_max`.

**‘pseudo-ellipse, torsionless’** – The pseudo-elliptic cone with the torsion angle `cone_sigma_max` set to zero.

**‘pseudo-ellipse, free rotor’** – The pseudo-elliptic cone with no torsion angle restriction.

**‘iso cone’** – The isotropic cone model. The cone is defined by a single order parameter `s1` which is related to the single cone opening angle `cone_theta_x = cone_theta_y`. Due to rotational symmetry about the cone axis, the average position  $\alpha$  Euler angle `ave_pos_alpha` is dropped from the model. The symmetry also collapses the eigenframe to a single z-axis defined by the parameters `axis_theta` and `axis_phi`.

**‘iso cone, torsionless’** – The isotropic cone model with the torsion angle `cone_sigma_max` set to zero.

**‘iso cone, free rotor’** – The isotropic cone model with no torsion angle restriction.

**‘rotor’** – The only motion is a rotation about the cone axis restricted by the torsion angle `cone_sigma_max`.

**‘rigid’** – No domain motions.

**‘free rotor’** – The only motion is free rotation about the cone axis.

**‘double rotor’** – Restricted motions about two independent but orthogonal rotor axes. The first rotation is about the  $y$ -axis and the second is about the  $x$ -axis.

#### Prompt examples

To select the isotropic cone model, type:

```
relax> frame_order.select_model(model='iso
cone')
```



### 17.2.52 `frame_order.simulate`



#### Synopsis

Pseudo-Brownian dynamics simulation of the frame order motions.

#### Defaults

`frame_order.simulate`(file='simulation.pdb.gz', dir=None, step\_size=2.0, snapshot=10, total=1000, model=1, force=False)

#### Keyword arguments

**file:** The PDB file for storing the frame order pseudo-Brownian dynamics simulation. The compression is determined automatically by the file extensions `*.pdb`, `*.pdb.gz`, and `*.pdb.bz2`.

**dir:** The directory where the files are to be located.

**step\_size:** The rotation will be of a random direction but with this fixed angle. The value is in degrees.

**snapshot:** The number of steps in the simulation when snapshots will be taken.

**total:** The total number of snapshots to take before stopping the simulation.

**model:** Only one model from an analysed ensemble of structures can be used for the pseudo-Brownian simulation, as the simulation and corresponding PDB file consists of one model per simulation.

**force:** A flag which, if set to True, will overwrite the any pre-existing file.

The simulation algorithm is as follows. The current state is initially defined as the identity matrix  $I$ . The maximum opening angle  $\theta$  or the torsion angle  $\sigma$  are defined by the parameter values of the frame order model. The algorithm for one step of the simulation is:

- 1 – Generate a random vector in 3D.
- 2 – Construct a rotation matrix from the random vector and the fixed rotation angle.
- 3 – Pre-multiply the current state by the rotation matrix.
- 4 – Decompose the new state into the torsion-tilt angles.
- 5 – If  $\theta$  or  $\sigma$  are greater than model parameter values, set them to these maximum values.
- 6 – Back convert the modified torsion-tilt angles to a rotation matrix - this is the current state.
- 7 – Store a snapshot if the correct number of iterations has been reached. This consists of rotating a new model about the pivot(s), as defined by the frame order model.
- 8 – Terminate the loop if the maximum number of snapshots has been reached.

The setting of the steps outside of the distribution to the maximum parameter values is specifically to allow for models with parameter values close to zero. Without this, the simulation would take a huge amount of time to complete.

As the simulation consists of one model per snapshot, if an ensemble of structures has been analysed, only one model from the ensemble can be used for the representation. This defaults to model number 1, but this can be changed.

#### Description

To visualise the frame order motions, this user function performs a type of simulation whereby structures are randomly rotated by a fixed angle within the bounds of the uniform distribution of the frame order model. This can be thought of as a pseudo-Brownian dynamics simulation. It is in no way a real molecular or Brownian dynamics simulation.

Note that the RDC and PCS data does not contain information about all parts of the real distribution of structures. Therefore the snapshots in this simulation only represent the components of the distribution present in the data, as modelled by the frame order models.

### 17.2.53 `frame_order.sobol_setup`



#### Synopsis

Set up the quasi-random Sobol' sequence points for numerical PCS integration.

#### Defaults

`frame_order.sobol_setup(max_num=200, oversample=1)`

#### Keyword arguments

**max\_num:** The maximum number of integration points to use in the Sobol' sequence during optimisation. This can be considered as the number of molecular structures in an ensemble used form a uniform distribution of the dynamics.

**oversample:** The generation of the Sobol' sequence oversamples as  $N * Ov * 10^{**}M$ , where  $N$  is the maximum number of points,  $Ov$  is the oversampling value, and  $M$  is the number of dimensions or torsion-tilt angles used in the system.

#### Description

This allows the maximum number of integration points  $N$  used during the frame order target function optimisation to be specified. This is used in the quasi-random Sobol' sequence for the numerical integration of the PCS. The formula used to find the total number of Sobol' points is:

$$\text{total\_num} = N * Ov * 10^{**}M,$$

where:

$N$  is the maximum number of Sobol' integration points,

$Ov$  is the oversampling factor.

$M$  is the number of dimensions or torsion-tilt angles used in the system.

The aim of the oversampling is to try to reach the maximum number of points. However if the system is not very dynamic, the maximum number of points may not be reached. In this case, simply increase the oversampling factor. The algorithm used for uniformly sampling the motional space is:

Generate the Sobol' sequence for the total number of points.

Convert all points to the torsion-tilt angle system.

Skip all Sobol' points with angles greater than the current parameter values.

Terminate the loop over the Sobol' points once the maximum number of points has been reached.

### 17.2.54 `grace.view`



#### Synopsis

Visualise the file within Grace.

#### Defaults

`grace.view(file=None, dir='grace', grace_exe='xmgrace')`

#### Keyword arguments

`file`: The name of the file.

`dir`: The directory name.

`grace_exe`: The Grace executable file.

#### Description

This can be used to view the specified Grace `*.agr` file by opening it with the Grace program.

#### Prompt examples

To view the file `s2.agr` in the directory `grace`, type:

```
relax> grace.view(file='s2.agr')

relax> grace.view(file='s2.agr', dir='grace'
)
```

### 17.2.55 `grace.write`



#### Synopsis

Create a grace `.agr` file to visualise the 2D data.

#### Defaults

`grace.write(x_data_type='res_num', y_data_type=None, spin_id=None, plot_data='value', norm_type='first', file=None, dir='grace', force=False, norm=False)`

#### Keyword arguments

`x_data_type`: The data type for the X-axis (no regular expression is allowed).

`y_data_type`: The data type for the Y-axis (no regular expression is allowed).

`spin_id`: The spin ID string.

`plot_data`: The data to use for the plot.

`norm_type`: How the graph should be normalised, if the norm flag is set.

`file`: The name of the file.

`dir`: The directory name.

`force`: A flag which, if set to True, will cause the file to be overwritten.

`norm`: A flag which, if set to True, will cause all graphs to be normalised to 1. This is for the normalisation of series type data. The point for normalisation is set with the `norm_type` argument.

#### Description

This is designed to be as flexible as possible so that any combination of data can be plotted. The output is in the format of a Grace plot (also known as ACE/gr, Xmgr, and xmgrace) which only supports two dimensional plots. Three types of information can be used to create various types of plot. These include the x-axis and y-axis data types, the spin ID string, and the type of data plot.

The x-axis and y-axis data types should be plain strings, regular expression is not allowed. The two axes of the Grace plot can be any of the data types listed in the tables below. The only limitation is that the data must belong to the same data pipe.

If the x-axis data type is not given, the plot will default to having the residue numbering along the x-axis. Two special data types for the axes are:

**'res\_num'** – The axis will consist of the residue numbering.

**'spin\_num'** – The axis will consist of the spin numbering.

The spin ID string can be used to limit which spins are used in the plot. The default is that all spins will be used, however, the ID string can be used to select a subset of all spins, or a single spin for plots of Monte Carlo simulations, etc.

The property which is actually plotted can be controlled by the plot data setting. This can be one of the following:

**'value'** – Plot values (with errors if they exist).

**'error'** – Plot errors.

**'sims'** – Plot the simulation values.

Normalisation is only allowed for series type data, for example the  $R_2$  exponential curves, and will be ignored for all other data types. If the norm flag is set to True then the y-value of the first point of the series will be set to 1. This normalisation is useful for highlighting errors in the data sets.

## Relaxation curve fitting parameters

Please see Table 17.8 on page 483.

## Steady-state NOE parameters

Please see Table 17.9 on page 483.

## Model-free parameters

Please see Table 17.10 on page 483.

## Reduced spectral density mapping parameters

Please see Table 17.11 on page 483.

## Consistency testing parameters

Please see Table 17.12 on page 484.

## Relaxation dispersion parameters

Please see Table 17.13 on page 484.

## Prompt examples

To write the NOE values for all spins to the Grace file 'noe.agr', type one of:

```
relax> grace.write('res_num', 'noe', file='noe.agr')
```

```
relax> grace.write(y_data_type='noe', file='noe.agr')
```

```
relax> grace.write(x_data_type='res_num', y_data_type='noe', file='noe.agr')
```

```
relax> grace.write(y_data_type='noe', file='noe.agr', force=True)
```

To create a Grace file of 's2' vs. 'te' for all spins, type one of:

```
relax> grace.write('s2', 'te', file='s2_te.agr')
```

```
relax> grace.write(x_data_type='s2', y_data_type='te', file='s2_te.agr')
```

```
relax> grace.write(x_data_type='s2', y_data_type='te', file='s2_te.agr', force=True)
```

To create a Grace file of the Monte Carlo simulation values of 'rex' vs. 'te' for residue 123, type one of:

```
relax> grace.write('rex', 'te', spin_id=':123', plot_data='sims', file='s2_te.agr')
```

```
relax> grace.write(x_data_type='rex', y_data_type='te', spin_id=':123', plot_data='sims', file='s2_te.agr')
```

By plotting the peak intensities, the integrity of exponential relaxation curves can be checked and anomalies searched for prior to model-free analysis or reduced spectral density mapping. For example the normalised average peak intensities can be plotted versus the relaxation time periods for the relaxation curves of all residues of a protein. The normalisation, whereby the initial peak intensity of each residue  $I(0)$  is set to 1, emphasises any problems. To produce this Grace file, type:

```
relax> grace.write(x_data_type='relax_times', y_data_type='ave_int', file='intensities_norm.agr', force=True, norm=True)
```

Table 17.8: Relaxation curve fitting parameters and minimisation statistics.

| Name    | Description                               |
|---------|-------------------------------------------|
| rx      | Either the $R_1$ or $R_2$ relaxation rate |
| i0      | The initial intensity                     |
| iinf    | The intensity at infinity                 |
| chi2    | Chi-squared value                         |
| iter    | Optimisation iterations                   |
| f_count | Number of function calls                  |
| g_count | Number of gradient calls                  |
| h_count | Number of Hessian calls                   |
| warning | Optimisation warning                      |

Table 17.9: Steady-state NOE parameters.

| Name | Description                |
|------|----------------------------|
| noe  | The steady-state NOE value |

Table 17.10: Model-free parameters and minimisation statistics.

| Name     | Description                                                              |
|----------|--------------------------------------------------------------------------|
| s2       | $S^2$ , the model-free generalised order parameter ( $S^2 = S_f^2.S2s$ ) |
| s2f      | $S_f^2$ , the faster motion model-free generalised order parameter       |
| s2s      | $S_s^2$ , the slower motion model-free generalised order parameter       |
| local_tm | The spin specific global correlation time (seconds)                      |
| te       | Single motion effective internal correlation time (seconds)              |
| tf       | Faster motion effective internal correlation time (seconds)              |
| ts       | Slower motion effective internal correlation time (seconds)              |
| rex      | Chemical exchange relaxation ( $\sigma_{ex} = R_{ex} / \omega^2$ )       |
| csa      | Chemical shift anisotropy (unitless)                                     |

Table 17.11: Reduced spectral density mapping parameters.

| Name | Description                                                                  |
|------|------------------------------------------------------------------------------|
| j0   | Spectral density value at 0 MHz - $J(0)$                                     |
| jwx  | Spectral density value at the frequency of the heteronucleus - $J(\omega_X)$ |
| jwh  | Spectral density value at the frequency of the proton - $J(\omega_H)$        |
| csa  | Chemical shift anisotropy (unitless)                                         |

Table 17.12: Consistency testing parameters.

| Name        | Description                                                                                                                 |
|-------------|-----------------------------------------------------------------------------------------------------------------------------|
| j0          | Spectral density value at 0 MHz (from Farrow et al. (1995) JBNMR, 6: 153-162)                                               |
| f_eta       | Eta-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                |
| f_r2        | R2-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                 |
| csa         | Chemical shift anisotropy (unitless)                                                                                        |
| orientation | Angle between the $^{15}\text{N}$ - $^1\text{H}$ vector and the principal axis of the $^{15}\text{N}$ chemical shift tensor |
| tc          | The single global correlation time estimate/approximation                                                                   |

Table 17.13: Relaxation dispersion parameters and minimisation statistics.

| Name     | Description                                                                                                           |
|----------|-----------------------------------------------------------------------------------------------------------------------|
| r2eff    | The effective transversal relaxation rate                                                                             |
| i0       | The initial intensity                                                                                                 |
| r1       | The longitudinal relaxation rate                                                                                      |
| r2       | The transversal relaxation rate                                                                                       |
| r2a      | The transversal relaxation rate for state A in the absence of exchange                                                |
| r2b      | The transversal relaxation rate for state B in the absence of exchange                                                |
| pA       | The population for state A                                                                                            |
| pB       | The population for state B                                                                                            |
| pC       | The population for state C                                                                                            |
| phi_ex   | The $\phi_{\text{ex}} = pA \cdot pB \cdot dw^2$ value ( $\text{ppm}^2$ )                                              |
| phi_ex_B | The fast exchange factor between sites A and B ( $\text{ppm}^2$ )                                                     |
| phi_ex_C | The fast exchange factor between sites A and C ( $\text{ppm}^2$ )                                                     |
| padw2    | The $pA \cdot dw^2$ value ( $\text{ppm}^2$ )                                                                          |
| dw       | The chemical shift difference between states A and B (in ppm)                                                         |
| dw_AB    | The chemical shift difference between states A and B for 3-site exchange (in ppm)                                     |
| dw_AC    | The chemical shift difference between states A and C for 3-site exchange (in ppm)                                     |
| dw_BC    | The chemical shift difference between states B and C for 3-site exchange (in ppm)                                     |
| dwh      | The proton chemical shift difference between states A and B (in ppm)                                                  |
| dwh_AB   | The proton chemical shift difference between states A and B for 3-site exchange (in ppm)                              |
| dwh_AC   | The proton chemical shift difference between states A and C for 3-site exchange (in ppm)                              |
| dwh_BC   | The proton chemical shift difference between states B and C for 3-site exchange (in ppm)                              |
| kex      | The exchange rate                                                                                                     |
| kex_AB   | The exchange rate between sites A and B for 3-site exchange with $kex_{AB} = k_{AB} + k_{BA}$ ( $\text{rad.s}^{-1}$ ) |
| kex_AC   | The exchange rate between sites A and C for 3-site exchange with $kex_{AC} = k_{AC} + k_{CA}$ ( $\text{rad.s}^{-1}$ ) |
| kex_BC   | The exchange rate between sites B and C for 3-site exchange with $kex_{BC} = k_{BC} + k_{CB}$ ( $\text{rad.s}^{-1}$ ) |
| kB       | Approximate chemical exchange rate constant between sites A and B ( $\text{rad.s}^{-1}$ )                             |
| kC       | Approximate chemical exchange rate constant between sites A and C ( $\text{rad.s}^{-1}$ )                             |
| tex      | The time of exchange ( $tex = 1/kex$ )                                                                                |
| k_AB     | The exchange rate from state A to state B                                                                             |
| k_BA     | The exchange rate from state B to state A                                                                             |
| chi2     | Chi-squared value                                                                                                     |
| iter     | Optimisation iterations                                                                                               |
| f_count  | Number of function calls                                                                                              |
| g_count  | Number of gradient calls                                                                                              |
| h_count  | Number of Hessian calls                                                                                               |
| warning  | Optimisation warning                                                                                                  |

17.2.56 `interatom.copy`

## Synopsis

Copy all data associated with a interatomic data container.

## Defaults

`interatom.copy`(pipe\_from=None, pipe\_to=None, spin\_id1=None, spin\_id2=None)

## Keyword arguments

**pipe\_from:** The data pipe containing the interatomic data container from which the data will be copied. This defaults to the current data pipe.

**pipe\_to:** The data pipe to copy the interatomic data container to. This defaults to the current data pipe.

**spin\_id1:** The spin ID of the first spin.

**spin\_id2:** The spin ID of the first spin.

## Description

This will copy all the data associated with the identified interatomic data container to a different data pipe. The new interatomic data container must not already exist.

## Prompt examples

To copy the interatomic data container between `:20C` and `:20H`, from the `'orig'` data pipe to the current data pipe, type one of:

```
relax> interatom.copy('orig', spin_id1=':20C', spin_id2=':20H')
```

```
relax> interatom.copy(pipe_from='orig', spin_id1=':20C', spin_id2=':20H')
```

17.2.57 `interatom.define`

## Synopsis

Define interatomic interactions between pairs of spins.

## Defaults

`interatom.define`(spin\_id1='@N', spin\_id2='@H', direct\_bond=True, spin\_selection=True, pipe=None)

## Keyword arguments

**spin\_id1:** The spin ID string for the first spin of the interatomic interaction.

**spin\_id2:** The spin ID string for the second spin of the interatomic interaction.

**direct\_bond:** This is a flag which if True means that the two spins are directly bonded. This flag is useful to simplify the set up of the main heteronuclear relaxation mechanism or one-bond residual dipolar couplings.

**spin\_selection:** Define the interatomic data container selection based on the spin selection. If either spin is deselected, the interatomic container will also be deselected. Otherwise the container will be selected.

**pipe:** The data pipe to create the interatomic data container for. This defaults to the current data pipe if not supplied.

## Description

To analyse relaxation or residual dipolar coupling (RDC) data, for example, pairs of spins which are coupled need to be defined. This can be via the magnetic dipole-dipole interaction or scalar coupling interaction. This function will create an interatomic data object connecting two existing spins. This data container will be used to store all information about the interatomic interaction including interatomic vectors and distances.

For analyses which use relaxation data, simply defining the interatomic interaction will indicate that there is a dipolar relaxation mechanism operating between the two spins. Note that for model-free analyses or reduced spectral density mapping, only a single relaxation mechanism can be handled. For RDC dependent analyses, the presence of the interatomic interaction indicates that dipolar coupling is expected between the two spins.

If the spin selection flag is set, then the newly created interatomic data container will be selected based on the

current selection status of the two spins defining the interaction. If either of the spins are deselected, then the new interatomic data container will also be deselected. If both spins are selected, then the interatomic data container will also be selected.

## Prompt examples

To connect the spins `:1@N` to `:1@H`, type one of:

```
relax> interatom.define(':1@N', ':1@H')
```

```
relax> interatom.define(spin_id1=':1@N',
                        spin_id2=':1@H')
```

To define the protein <sup>15</sup>N heteronuclear relaxation mechanism for a model-free analysis, type one of the following:

```
relax> interatom.define('@N', '@H', True)
```

```
relax> interatom.define(spin_id1='@N',
                        spin_id2='@H', direct_bond=True)
```

## 17.2.58 interatom.read\_dist



### Synopsis

Read inter-spin distances from a file.

### Defaults

**interatom.read\_dist**(file=None, dir=None, unit='meter', spin\_id1\_col=1, spin\_id2\_col=2, data\_col=3, sep=None)

### Keyword arguments

**file**: The name of the file containing the averaged distance data.

**dir**: The directory where the file is located.

**unit**: The unit of distance. The default is meter, but Å can also be specified.

**spin\_id1\_col**: The spin ID string column for the first spin.

**spin\_id2\_col**: The spin ID string column for the second spin.

**data\_col**: The distance data column.

**sep**: The column separator (the default is white space).

### Description

This allows interatomic distances to be read from a file. This is useful in the case when the distances vary, avoiding having to tediously use the `interatom.set_dist` user function for each spin-pair separately. The format of the file should be columnar, with the two spin ID strings in two separate columns and the distances in any other. The default measurement unit is meter but this can be changed to Å.

For RDC and relaxation based analyses, as the magnetic dipole-dipole interaction is averaged in NMR over the interatomic distance to the inverse third power, the interatomic distances within a 3D structural file are of no use for defining the interaction. Therefore these  $r^{-3}$  average distances must be explicitly defined.



## Prompt examples

To load the distances in meters from the fifth column of the 'distances' file, and where the spin IDs are in the first and second columns, type one of the following:

```
relax> interatom.read_dist('distances', 1,
    2, 5)
```

```
relax> interatom.read_dist(file='distances',
    unit='meter', spin_id1_col=1,
    spin_id2_col=2, data_col=5)
```

## 17.2.59 interatom.set\_dist



### Synopsis

Set the inter-spin distances.

### Defaults

```
interatom.set_dist(spin_id1='@N', spin_id2='@H',
ave_dist=1.0200000000000001e-10, unit='meter')
```

### Keyword arguments

**spin\_id1:** The spin identification string for the first spin of the dipole pair.

**spin\_id2:** The spin identification string for the second spin of the dipole pair.

**ave\_dist:** The  $r^{-3}$  averaged distance between the two spins to be used in the magnetic dipole constant, defaulting to meters.

**unit:** The unit of distance (the default is 'meter').

### Description

For many NMR interactions, the distance between the spin of interest and another spin or atom must be defined. This information can be extracted from a 3D structure but, in many cases, these distances are not of interest. For example the empirical or fixed distance calculation of proton positions in X-ray crystallographic structures will often not correspond to the real interatomic distances.

Another example is the magnetic dipole-dipole interaction which is averaged over the interatomic distance to the inverse third power. In this case, the interatomic distances from any 3D structural file can be of no use for defining the interaction. The average distances must be explicitly supplied. This user function allows these distances to be set up. The default measurement unit is meter but this can be changed to Å. Alternatively the distances can be read from a file using other user functions in this class.

## Prompt examples

To set the N-H distance for protein the <sup>15</sup>N heteronuclear relaxation mechanism to 1.02 Å, type one of the following:

```
relax> interatom.set_dist('@N', '@H', 1.02 *
    1e-10)
```

```
relax> interatom.set_dist(spin_id1='@N',
    spin_id2='@H', ave_dist=1.02 * 1e-10,
    unit='meter')
```

```
relax> interatom.set_dist(spin_id1='@N',
    spin_id2='@H', ave_dist=1.02, unit='
    Angstrom')
```

---

## 17.2.60 interatom.unit\_vectors



### Synopsis

Calculate the unit vectors for all interatomic interactions.

### Defaults

**interatom.unit\_vectors**(ave=True)

### Keyword arguments

**ave**: A flag which if True will cause the bond vectors from all models to be averaged. If vectors from only one model is extracted, this will have no effect.

### Description

For an orientational dependent analysis, such as model-free analysis with the spheroidal and ellipsoidal global diffusion tensors or any analysis using RDCs, the unit vectors between the two dipoles must be calculated prior to starting the analysis. For the unit vector extraction, the two interacting spins should already possess positional information and the dipole-dipole interaction should already be defined via the `interatom.define` user function. This information will be used to calculate unit vectors between the two spins. Without positional information from a 3D structure, no vectors can be calculated and an orientational dependent analysis will not be possible.

The number of unit vectors per interaction will be defined by the number of positions each spin possesses together with the averaging flag. If both spins have N and M positions loaded, the number of positions for both must match (N=M). In this case, as well as when one spin has N positions and the other a single position, then N unit vectors will be calculated. This is unless the averaging flag is set in which case an averaged vector of unit length will be calculated.

### Prompt examples

To calculate the unit vectors prior to a model-free analysis, type one of the following:

```
relax> interatom.unit_vectors(True)
```

```
relax> interatom.unit_vectors(ave=True)
```

---

**17.2.61 j\_coupling.copy****Synopsis**

Copy J coupling data from one data pipe to another.

**Defaults**

`j_coupling.copy(pipe_from=None, pipe_to=None)`

**Keyword arguments**

**pipe\_from:** The name of the pipe to copy the J coupling data from.

**pipe\_to:** The name of the pipe to copy the J coupling data to.

**Description**

This function will copy J coupling data from one pipe to another.

**Prompt examples**

To copy all J coupling data from pipe 'DMSO' to pipe 'CDC13', type one of:

```
relax> j_coupling.copy('DMSO', 'CDC13')
```

```
relax> j_coupling.copy(pipe_from='DMSO',  
    pipe_to='CDC13')
```

**17.2.62 j\_coupling.delete****Synopsis**

Delete the J coupling values.

**Defaults**

`j_coupling.delete()`

**Description**

This will delete all J coupling data in the current data pipe.

**Prompt examples**

To delete all J coupling data, type:

```
relax> j_coupling.delete()
```

---

### 17.2.63 j\_coupling.display



#### Synopsis

Display the J coupling data in the current data pipe.

#### Defaults

`j_coupling.display()`

#### Description

This will display all of the J coupling data in the current data pipe.

#### Prompt examples

To display all J coupling data, type:

```
relax> j_coupling.display()
```

### 17.2.64 j\_coupling.read



#### Synopsis

Read the J coupling data from file.

#### Defaults

`j_coupling.read(file=None, dir=None, spin_id1_col=1, spin_id2_col=2, data_col=None, error_col=None, sign_col=None, sep=None)`

#### Keyword arguments

**file:** The name of the file containing the J coupling data.

**dir:** The directory where the file is located.

**spin\_id1\_col:** The spin ID string column for the first spin.

**spin\_id2\_col:** The spin ID string column for the second spin.

**data\_col:** The J coupling data column.

**error\_col:** The experimental error column.

**sign\_col:** A special column holding the sign of the J coupling, being either 1 or -1, in case this data is obtained separately.

**sep:** The column separator (the default is white space).

#### Description

This will read J coupling data from a file. If the sign of the J coupling has been determined by a different experiment, this information can be present in a different column having either the value of 1 or -1.

#### Prompt examples

The following commands will read the J coupling data out of the file 'J.txt' where the columns are separated by the symbol ',':

```
relax> j_coupling.read('J.txt', sep=',')
```

If the individual spin J coupling errors are located in the file 'j\_err.txt' in column number 5 then, to read these values into relax, type one of:

```
relax> j_coupling.read('j_err.txt',  
    error_col=5)  
  
relax> j_coupling.read(file='j_err.txt',  
    error_col=5)
```

---

## 17.2.65 j\_coupling.write



### Synopsis

Write the J coupling data to file.

### Defaults

`j_coupling.write(file=None, dir=None, force=False)`

### Keyword arguments

`file`: The name of the file.

`dir`: The directory name.

`force`: A flag which if True will cause the file to be overwritten.

### Description

This will write the J coupling values to file. If no directory name is given, the file will be placed in the current working directory.

### 17.2.66 jw\_mapping.set\_frq

 $J(\omega)$ 
 $\omega$ 

#### Synopsis

Select which relaxation data to use in the  $J(\omega)$  mapping by NMR spectrometer frequency.

#### Defaults

```
jw_mapping.set_frq(frq=None)
```

#### Keyword arguments

**frq:** The spectrometer frequency in Hz. This must match the currently loaded data to the last decimal point. See the 'sfrq' parameter in the Varian procpa file or the 'SF01' parameter in the Bruker acqui file.

#### Description

This will select the relaxation data to use in the reduced spectral density mapping corresponding to the given frequency. The data is selected by the spectrometer frequency in Hertz, which should be set to the exact value (see the 'sfrq' parameter in the Varian procpa file or the 'SF01' parameter in the Bruker acqui file). Note though that the  $R_1$ ,  $R_2$  and NOE are all expected to have the exact same frequency in the  $J(\omega)$  mapping analysis (to the last decimal point).

#### Prompt examples

```
relax> jw_mapping.set_frq(600.0 * 1e6)
```

```
relax> jw_mapping.set_frq(frq=600.0 * 1e6)
```

### 17.2.67 minimise.calculate



#### Synopsis

Calculate the model parameters or the current target function value.

#### Defaults

```
minimise.calculate(verbosity=1)
```

#### Keyword arguments

**verbosity:** The amount of information to print to screen. Zero corresponds to minimal output while higher values increase the amount of output. The default value is 1.

#### Description

The operation of this user function is two-fold and depends on whether the solution for the models of the current analysis are found by direct calculation or by optimisation. The dual operations are:

**Direct calculation models** – For these models, the parameters will be directly calculated from the base data. This will be the exact solution and the user function will store the parameter values. The grid search and optimisation user functions are not implemented for this analysis type.

**Optimised models** – This will call the target function normally used for optimisation for each model using the current parameter values. This can be used to manually find the chi-squared value for different parameter values. The parameter values will not be affected.

## 17.2.68 `minimise.execute`



### Synopsis

Perform an optimisation.

### Defaults

`minimise.execute(min_algor='newton', line_search=None, hessian_mod=None, hessian_type=None, func_tol=1e-25, grad_tol=None, max_iter=10000000, constraints=True, scaling=True, verbosity=1)`

### Keyword arguments

**min\_algor:** The optimisation algorithm to use.

**line\_search:** The line search algorithm which will only be used in combination with the line search and conjugate gradient methods. This will default to the More and Thunten line search.

**hessian\_mod:** The Hessian modification. This will only be used in the algorithms which use the Hessian, and defaults to Gill, Murray, and Wright modified Cholesky algorithm.

**hessian\_type:** The Hessian type. This will only be used in a few trust region algorithms, and defaults to BFGS.

**func\_tol:** The function tolerance. This is used to terminate minimisation once the function value between iterations is less than the tolerance. The default value is 1e-25.

**grad\_tol:** The gradient tolerance. Minimisation is terminated if the current gradient value is less than the tolerance. The default value is None.

**max\_iter:** The maximum number of iterations. The default value is 1e7.

**constraints:** A boolean flag specifying whether the parameters should be constrained. The default is to turn constraints on (constraints=True).

**scaling:** The diagonal scaling boolean flag. The default that scaling is on (scaling=True).

**verbosity:** The amount of information to print to screen. Zero corresponds to minimal output while higher values increase the amount of output. The default value is 1.

### Description

This will perform an optimisation starting from the current parameter values. This is only suitable for data pipe types which have target functions and hence support optimisation.

### Diagonal scaling

Diagonal scaling is the transformation of parameter values such that each value has a similar order of magnitude. Certain minimisation techniques, for example the trust region methods, perform extremely poorly with badly scaled problems. In addition, methods which are insensitive to scaling such as Newton minimisation may still benefit due to the minimisation of round off errors.

In Model-free analysis for example, if  $S^2 = 0.5$ ,  $\tau_e = 200$  ps, and  $R_{ex} = 15$  1/s at 600 MHz, the unscaled parameter vector would be  $[0.5, 2.0e-10, 1.055e-18]$ .  $R_{ex}$  is divided by  $(2 * \pi * 600,000,000)**2$  to make it field strength independent. The scaling vector for this model may be something like  $[1.0, 1e-9, 1/(2 * \pi * 6e8)**2]$ . By dividing the unscaled parameter vector by the scaling vector the scaled parameter vector is  $[0.5, 0.2, 15.0]$ . To revert to the original unscaled parameter vector, the scaled parameter vector and scaling vector are multiplied.

### Minimisation algorithms

A minimisation function is selected if the minimisation algorithm matches a certain pattern. Because the python regular expression 'match' statement is used, various strings can be supplied to select the same minimisation algorithm. Below is a list of the minimisation algorithms available together with the corresponding patterns.

This is a short description of python regular expression, for more information, see the regular expression syntax section of the Python Library Reference. Some of the regular expression syntax used in this function is:

'[]' – A sequence or set of characters to match to a single character. For example, '[Nn]ewton' will match both 'Newton' and 'newton'.

'^' – Match the start of the string.

'\$' – Match the end of the string. For example, '^ [Ll] [Mm] \$' will match 'lm' and 'LM' but will not match if characters are placed either before or after these strings.

To select a minimisation algorithm, use a string which matches one of the following patterns given in the tables.

Unconstrained line search methods:

Please see Table 17.14 on page 494.

Unconstrained trust-region methods:

Please see Table 17.15 on page 494.

Table 17.14: Minimisation algorithms – unconstrained line search methods.

| Minimisation algorithm            | Patterns                                                                 |
|-----------------------------------|--------------------------------------------------------------------------|
| Back-and-forth coordinate descent | <code>'^[Cc][Dd]'</code> or <code>'^[Cc]oordinate[-][Dd]escent\$'</code> |
| Steepest descent                  | <code>'^[Ss][Dd]'</code> or <code>'^[Ss]teepest[-][Dd]escent\$'</code>   |
| Quasi-Newton BFGS                 | <code>'^[Bb][Ff][Gg][Ss]'</code>                                         |
| Newton                            | <code>'^[Nn]ewton\$'</code>                                              |
| Newton-CG                         | <code>'^[Nn]ewton[-][Cc][Gg]'</code> or <code>'^[Nn][Cc][Gg]'</code>     |

Table 17.15: Minimisation algorithms – unconstrained trust-region methods.

| Minimisation algorithm | Patterns                                                              |
|------------------------|-----------------------------------------------------------------------|
| Cauchy point           | <code>'^[Cc]auchy'</code>                                             |
| Dogleg                 | <code>'^[Dd]ogleg'</code>                                             |
| CG-Steihaug            | <code>'^[Cc][Gg][-][Ss]teihaug'</code> or <code>'^[Ss]teihaug'</code> |
| Exact trust region     | <code>'^[Ee]xact'</code>                                              |

Table 17.16: Minimisation algorithms – unconstrained conjugate gradient methods.

| Minimisation algorithm | Patterns                                                                  |
|------------------------|---------------------------------------------------------------------------|
| Fletcher-Reeves        | <code>'^[Ff][Rr]'</code> or <code>'^[Ff]letcher[-][Rr]eeves\$'</code>     |
| Polak-Ribiere          | <code>'^[Pp][Rr]'</code> or <code>'^[Pp]olak[-][Rr]ibiere\$'</code>       |
| Polak-Ribière +        | <code>'^[Pp][Rr]\+\$'</code> or <code>'^[Pp]olak[-][Rr]ibiere\+\$'</code> |
| Hestenes-Stiefel       | <code>'^[Hh][Ss]'</code> or <code>'^[Hh]estenes[-][Ss]tiefel\$'</code>    |



Unconstrained conjugate gradient methods:

Please see Table 17.16 on page 494.

Miscellaneous unconstrained methods:

Please see Table 17.17 on page 496.

Global minimisation methods:

Please see Table 17.18 on page 496.

## Minimisation options

The minimisation options can be given in any order.

Line search algorithms. These are used in the line search methods and the conjugate gradient methods. The default is the Backtracking line search. The algorithms are:

Please see Table 17.19 on page 496.

Hessian modifications. These are used in the Newton, Dogleg, and Exact trust region algorithms:

Please see Table 17.20 on page 496.

Hessian type, these are used in a few of the trust region methods including the Dogleg and Exact trust region algorithms. In these cases, when the Hessian type is set to Newton, a Hessian modification can also be supplied as above. The default Hessian type is Newton, and the default Hessian modification when Newton is selected is the GMW algorithm:

Please see Table 17.21 on page 496.

For Newton minimisation, the default line search algorithm is the More and Thuente line search, while the default Hessian modification is the GMW algorithm.

```
relax> minimise.execute('newton', func_tol=1e-25)
```

```
relax> minimise.execute('newton', func_tol=1e-25, grad_tol=None)
```

```
relax> minimise.execute('newton', max_iter=1e7)
```

```
relax> minimise.execute('newton', constraints=True, max_iter=1e7)
```

```
relax> minimise.execute('newton', verbosity=1)
```

To use constrained Simplex minimisation with a maximum of 5000 iterations, type:

```
relax> minimise.execute('simplex', constraints=True, max_iter=5000)
```

## Prompt examples

To apply Newton minimisation together with the GMW81 Hessian modification algorithm, the More and Thuente line search algorithm, a function tolerance of 1e-25, no gradient tolerance, a maximum of 10,000,000 iterations, constraints turned on to limit parameter values, and have normal printout, type any combination of:

```
relax> minimise.execute('newton')
```

```
relax> minimise.execute('Newton')
```

```
relax> minimise.execute('newton', 'gmw')
```

```
relax> minimise.execute('newton', 'mt')
```

```
relax> minimise.execute('newton', 'gmw', 'mt')
```

```
relax> minimise.execute('newton', 'mt', 'gmw')
```

Table 17.17: Minimisation algorithms – miscellaneous unconstrained methods.

| Minimisation algorithm | Patterns                                                              |
|------------------------|-----------------------------------------------------------------------|
| Simplex                | <code>^[Ss]implex\$</code>                                            |
| Levenberg-Marquardt    | <code>^[Ll][Mm]\$</code> or <code>^[Ll]evenburg-[Mm]arquardt\$</code> |

Table 17.18: Minimisation algorithms – global minimisation methods.

| Minimisation algorithm | Patterns                                                              |
|------------------------|-----------------------------------------------------------------------|
| Simulated Annealing    | <code>^[Ss][Aa]\$</code> or <code>^[Ss]imulated [Aa]nnealing\$</code> |

Table 17.19: Minimisation sub-algorithms – line search algorithms.

| Line search algorithm                                   | Patterns                                                                          |
|---------------------------------------------------------|-----------------------------------------------------------------------------------|
| Backtracking line search                                | <code>^[Bb]ack\$</code>                                                           |
| Nocedal and Wright interpolation based line search      | <code>^[Nn][Ww][Ii]</code> or <code>^[Nn]ocedal[ _][Ww]right[ _][Ii]nt\$</code>   |
| Nocedal and Wright line search for the Wolfe conditions | <code>^[Nn][Ww][Ww]</code> or <code>^[Nn]ocedal[ _][Ww]right[ _][Ww]olfe\$</code> |
| More and Thuente line search                            | <code>^[Mm][Tt]</code> or <code>^[Mm]ore[ _][Tt]huent\$</code>                    |
| No line search                                          | <code>^[Nn]o [Ll]ine [Ss]earch\$</code>                                           |

Table 17.20: Minimisation sub-algorithms – Hessian modifications.

| Hessian modification                                     | Patterns                                |
|----------------------------------------------------------|-----------------------------------------|
| Unmodified Hessian                                       | <code>^[Nn]o [Hh]essian [Mm]od\$</code> |
| Eigenvalue modification                                  | <code>^[Ee]igen\$</code>                |
| Cholesky with added multiple of the identity             | <code>^[Cc]hol\$</code>                 |
| The Gill, Murray, and Wright modified Cholesky algorithm | <code>^[Gg][Mm][Ww]\$</code>            |
| The Schnabel and Eskow 1999 algorithm                    | <code>^[Ss][Ee]99\$</code>              |

Table 17.21: Minimisation sub-algorithms – Hessian type.

| Hessian type      | Patterns                         |
|-------------------|----------------------------------|
| Quasi-Newton BFGS | <code>^[Bb][Ff][Gg][Ss]\$</code> |
| Newton            | <code>^[Nn]ewton\$</code>        |

## 17.2.69 `minimise.grid_search`



### Synopsis

Perform a grid search to find an initial non-biased parameter set for optimisation.

### Defaults

`minimise.grid_search(lower=None, upper=None, inc=21, verbosity=1, constraints=True, skip_preset=True)`

### Keyword arguments

**lower:** An array of the lower bound parameter values for the grid search. The length of the array should be equal to the number of parameters in the model.

**upper:** An array of the upper bound parameter values for the grid search. The length of the array should be equal to the number of parameters in the model.

**inc:** The number of increments to search over. If a single integer is given then the number of increments will be equal in all dimensions. Different numbers of increments in each direction can be set if '**inc**' is set to an array of integers of length equal to the number of parameters.

**verbosity:** The amount of information to print to screen. Zero corresponds to minimal output while higher values increase the amount of output. The default value is 1.

**constraints:** A boolean flag specifying whether the parameters should be constrained. The default is to turn constraints on (`constraints=True`).

**skip\_preset:** This argument, when True, allows any parameter which already has a value set to be skipped in the grid search. This value will be overridden and turned off when a zooming grid search is active.

### Description

The optimisation of a mathematical model normally consists of two parts - a coarse grid search across the parameter space to find an initial set of parameter values followed by the use of a high precision optimisation algorithm to exactly find the local or global solution. The grid search is an essential tool as it allows a non-biased initial optimisation position to be found. It avoids the statistical bias and preconditioning introduced by using a self chosen initial parameter set. The high computational cost of the grid search is almost always favourable to the statistical bias of a user defined starting position.

The region of the parameter space that the grid search covers is defined by the lower and upper grid bounds. These will generally default to the entire parameter space except for when the parameter is non-bounded, for example a 3D position in the PDB space. This user function will print out the grid bounds used and, if the default bounds are deemed to be insufficient, then the lower, upper or both bounds can be supplied. This only works if all active models have the same parameters. The coarseness or fineness of the grid is defined by the number of increments to search across between the bounds. For an alternative to using large numbers of increments, see the zooming grid search.

It is possible to decrease the dimensionality of the grid search, and hence drop the computational cost by orders of magnitude, if certain parameter values are known a priori. For example if the values are determined via a different experiment. Such parameters can be set with the value setting user function. Then, when the skip preset flag is set, these parameters will be skipped in the grid search. This feature should not be abused and statistical bias should be avoided at all cost.

The parameter skipping logic is as follows. Firstly setting the increments argument to a list with None elements causes the corresponding parameters to be skipped in the grid search, or an error to be raised if no preset parameter is present. This overrides all other settings. Secondly the preset skipping flag only allows parameters to be skipped if the zooming grid search is non-active and a value is preset.

17.2.70 `minimise.grid_zoom`

## Synopsis

Activate the zooming grid search by setting the zoom level.

## Defaults

`minimise.grid_zoom(level=0)`

## Keyword arguments

**level:** The zooming grid search level. This can be any number, positive or negative.

## Description

The optimisation of a mathematical model normally consists of two parts - a coarse grid search to find an initial set of parameter values followed by the use of a high precision optimisation algorithm to exactly find the local or global solution. But in certain situations, for example where a parallelised grid search is advantageous, a finer grid may be desired. The zooming grid search provides a more efficient alternative to simply increasing the number of increments used in the initial grid search. Note that in most situations, standard optimisation algorithms will be by far computationally less expensive.

The zooming grid search can be activated via this user function. After setting the desired zoom level, the original grid search user function should be called again. The zoom level is used to decrease the total area of the grid search. The grid width for each dimension of the parameter space will be divided by  $2^{\text{zoom\_level}}$ . So a level of 1 will halve all dimensions, a level of 2 will quarter the widths, a level of 3 will be an eighth of the widths, etc.

The zooming algorithm proceeds as follows. The new zoomed grid will be centred at the current parameter values. However if the new grid is outside of the bounds of the original grid, the entire grid will be translated so that it lies entirely within the original bounds. This is to avoid grid points lying within undefined regions of the space. An exception is when the zoom factor is negative, hence the new grid will be larger than the original.

An example of using the zooming grid search is to first perform a standard initial grid search, then set the zoom level to 1 and perform a second grid search. Continue for zoom levels 2, 3, etc. until the desired fineness is obtained. Note that convergence is not guaranteed - as the zoom level is increased to infinity, the parameter values do not necessarily converge to the local minimum. Therefore performing standard optimisation is recommended after completing a zooming grid search.

17.2.71 `model_free.create_model`
 $S^2, \tau_e$ 


## Synopsis

Create a model-free model.

## Defaults

`model_free.create_model(model=None, equation=None, params=None, spin_id=None)`

## Keyword arguments

**model:** The new name of the model-free model.

**equation:** The model-free equation.

**params:** The array of parameter names of the model.

**spin\_id:** The spin identification string.

## Description

This user function should almost never be used. It is provided for academic reasons for the study of old analyses and published results. If you are looking for a normal model-free model, use the `model_free.select_model` user function instead.

## Model-free equation

The model-free equation can be one of the following:

**'mf\_orig'** selects the original model-free equations with parameters  $\{S^2, \tau_e\}$ .

**'mf\_ext'** selects the extended model-free equations with parameters  $\{S_f^2, \tau_f, S^2, \tau_s\}$ .

**'mf\_ext2'** selects the extended model-free equations with parameters  $\{S_f^2, \tau_f, S_s^2, \tau_s\}$ .

## Model-free parameters

The following parameters are accepted for the original model-free equation:

**'s2'** – The square of the generalised order parameter.

‘te’ – The effective correlation time.

The following parameters are accepted for the extended model-free equation:

‘s2f’ – The square of the generalised order parameter of the faster motion.

‘tf’ – The effective correlation time of the faster motion.

‘s2’ – The square of the generalised order parameter  $S^2 = S_f^2 * S_s^2$ .

‘ts’ – The effective correlation time of the slower motion.

The following parameters are accepted for the extended 2 model-free equation:

‘s2f’ – The square of the generalised order parameter of the faster motion.

‘tf’ – The effective correlation time of the faster motion.

‘s2s’ – The square of the generalised order parameter of the slower motion.

‘ts’ – The effective correlation time of the slower motion.

The following parameters are accepted for all equations:

‘rex’ – The chemical exchange relaxation.

‘r’ – The average bond length  $\langle r \rangle$ .

‘csa’ – The chemical shift anisotropy.

The following commands will create the model-free model ‘large\_model’ which is based on the extended model-free equation and contains the seven parameters ‘s2f’, ‘tf’, ‘s2’, ‘ts’, ‘rex’, ‘csa’, ‘r’.

```
relax> model_free.create_model('large_model'
    , 'mf_ext', ['s2f', 'tf', 's2', 'ts', '
    rex', 'csa', 'r'])
```

```
relax> model_free.create_model(model='
    large_model', params=['s2f', 'tf', 's2'
    , 'ts', 'rex', 'csa', 'r'], equation='
    mf_ext')
```

## Spin identification string

If ‘spin\_id’ is supplied then the model will only be created for the corresponding spins. Otherwise the model will be created for all spins.

## Prompt examples

The following commands will create the model-free model ‘m1’ which is based on the original model-free equation and contains the single parameter ‘s2’.

```
relax> model_free.create_model('m1', '
    mf_orig', ['s2'])
```

```
relax> model_free.create_model(model='m1',
    params=['s2'], equation='mf_orig')
```

---

**17.2.72 model\_free.delete** $S^2\tau_e$ **Synopsis**

Delete all model-free data from the current data pipe.

**Defaults**

`model_free.delete()`

**Description**

This will delete all of the model-free data - parameters, model, etc. - from the current data pipe.

**Prompt examples**

To delete all model-free data, type:

```
relax> model_free.delete()
```

---

**17.2.73 model\_free.remove\_tm** $S^2\tau_e$ **Synopsis**

Remove the local  $\tau_m$  parameter from a model.

**Defaults**

`model_free.remove_tm(spin_id=None)`

**Keyword arguments**

`spin_id`: The spin identification string.

**Description**

This function will remove the local  $\tau_m$  parameter from the model-free parameter set. If there is no local  $\tau_m$  parameter within the set nothing will happen.

If no spin identification string is given, then the function will apply to all spins.

**Prompt examples**

The following command will remove the parameter 'tm':

```
relax> model_free.remove_tm()
```

## 17.2.74 model\_free.select\_model

 $S^2, \tau_e$ 


### Synopsis

Select a preset model-free model.

### Defaults

**model\_free.select\_model**(model=None, spin\_id=None)

'm11' – {CSA,  $S^2$ },

'm12' – {CSA,  $S^2$ ,  $\tau_e$ },

'm13' – {CSA,  $S^2$ ,  $R_{ex}$ },

'm14' – {CSA,  $S^2$ ,  $\tau_e$ ,  $R_{ex}$ },

'm15' – {CSA,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ },

'm16' – {CSA,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ },

'm17' – {CSA,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm18' – {CSA,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm19' – {CSA,  $R_{ex}$ }.

The preset model-free models with optimisation of the bond length are

### Keyword arguments

**model**: The name of the preset model.

**spin\_id**: The spin identification string.

'm20' – {r},

'm21' – {r,  $S^2$ },

'm22' – {r,  $S^2$ ,  $\tau_e$ },

'm23' – {r,  $S^2$ ,  $R_{ex}$ },

'm24' – {r,  $S^2$ ,  $\tau_e$ ,  $R_{ex}$ },

'm25' – {r,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ },

'm26' – {r,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ },

'm27' – {r,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm28' – {r,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm29' – {r, CSA,  $R_{ex}$ }.

### Description

This allows a standard model-free model to be selected from a long list of models.

### The preset models

The standard preset model-free models are

'm0' – {},

'm1' – {S2},

'm2' – { $S^2$ ,  $\tau_e$ },

'm3' – { $S^2$ ,  $R_{ex}$ },

'm4' – { $S^2$ ,  $\tau_e$ ,  $R_{ex}$ },

'm5' – { $S_f^2$ ,  $S^2$ ,  $\tau_s$ },

'm6' – { $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ },

'm7' – { $S_f^2$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm8' – { $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm9' – {Rex}.

The preset model-free models with both optimisation of the bond length and CSA are

'm30' – {r, CSA},

'm31' – {r, CSA,  $S^2$ },

'm32' – {r, CSA,  $S^2$ ,  $\tau_e$ },

'm33' – {r, CSA,  $S^2$ ,  $R_{ex}$ },

'm34' – {r, CSA,  $S^2$ ,  $\tau_e$ ,  $R_{ex}$ },

'm35' – {r, CSA,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ },

'm36' – {r, CSA,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ },

'm37' – {r, CSA,  $S_f^2$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm38' – {r, CSA,  $S_f^2$ ,  $\tau_f$ ,  $S^2$ ,  $\tau_s$ ,  $R_{ex}$ },

'm39' – {r, CSA,  $R_{ex}$ }.

The preset model-free models with optimisation of the CSA value are

'm10' – {CSA},

Warning: The models in the thirties range fail when using standard R<sub>1</sub>, R<sub>2</sub>, and NOE relaxation data. This is due to the extreme flexibility of these models where a change in the parameter 'r' is compensated by a corresponding change in the parameter 'csa' and vice versa.

## The preset local tm models

Additional preset model-free models, which are simply extensions of the above models with the addition of a local  $\tau_m$  parameter are:

‘tm0’ –  $\{\tau_m\}$ ,  
 ‘tm1’ –  $\{\tau_m, S^2\}$ ,  
 ‘tm2’ –  $\{\tau_m, S^2, \tau_e\}$ ,  
 ‘tm3’ –  $\{\tau_m, S^2, R_{ex}\}$ ,  
 ‘tm4’ –  $\{\tau_m, S^2, \tau_e, R_{ex}\}$ ,  
 ‘tm5’ –  $\{\tau_m, S_f^2, S^2, \tau_s\}$ ,  
 ‘tm6’ –  $\{\tau_m, S_f^2, \tau_f, S^2, \tau_s\}$ ,  
 ‘tm7’ –  $\{\tau_m, S_f^2, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm8’ –  $\{\tau_m, S_f^2, \tau_f, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm9’ –  $\{\tau_m, R_{ex}\}$ .

The preset model-free models with optimisation of the CSA value are

‘tm10’ –  $\{\tau_m, CSA\}$ ,  
 ‘tm11’ –  $\{\tau_m, CSA, S^2\}$ ,  
 ‘tm12’ –  $\{\tau_m, CSA, S^2, \tau_e\}$ ,  
 ‘tm13’ –  $\{\tau_m, CSA, S^2, R_{ex}\}$ ,  
 ‘tm14’ –  $\{\tau_m, CSA, S^2, \tau_e, R_{ex}\}$ ,  
 ‘tm15’ –  $\{\tau_m, CSA, S_f^2, S^2, \tau_s\}$ ,  
 ‘tm16’ –  $\{\tau_m, CSA, S_f^2, \tau_f, S^2, \tau_s\}$ ,  
 ‘tm17’ –  $\{\tau_m, CSA, S_f^2, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm18’ –  $\{\tau_m, CSA, S_f^2, \tau_f, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm19’ –  $\{\tau_m, CSA, R_{ex}\}$ .

The preset model-free models with optimisation of the bond length are

‘tm20’ –  $\{\tau_m, r\}$ ,  
 ‘tm21’ –  $\{\tau_m, r, S^2\}$ ,  
 ‘tm22’ –  $\{\tau_m, r, S^2, \tau_e\}$ ,  
 ‘tm23’ –  $\{\tau_m, r, S^2, R_{ex}\}$ ,  
 ‘tm24’ –  $\{\tau_m, r, S^2, \tau_e, R_{ex}\}$ ,  
 ‘tm25’ –  $\{\tau_m, r, S_f^2, S^2, \tau_s\}$ ,

‘tm26’ –  $\{\tau_m, r, S_f^2, \tau_f, S^2, \tau_s\}$ ,  
 ‘tm27’ –  $\{\tau_m, r, S_f^2, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm28’ –  $\{\tau_m, r, S_f^2, \tau_f, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm29’ –  $\{\tau_m, r, CSA, R_{ex}\}$ .

The preset model-free models with both optimisation of the bond length and CSA are

‘tm30’ –  $\{\tau_m, r, CSA\}$ ,  
 ‘tm31’ –  $\{\tau_m, r, CSA, S^2\}$ ,  
 ‘tm32’ –  $\{\tau_m, r, CSA, S^2, \tau_e\}$ ,  
 ‘tm33’ –  $\{\tau_m, r, CSA, S^2, R_{ex}\}$ ,  
 ‘tm34’ –  $\{\tau_m, r, CSA, S^2, \tau_e, R_{ex}\}$ ,  
 ‘tm35’ –  $\{\tau_m, r, CSA, S_f^2, S^2, \tau_s\}$ ,  
 ‘tm36’ –  $\{\tau_m, r, CSA, S_f^2, \tau_f, S^2, \tau_s\}$ ,  
 ‘tm37’ –  $\{\tau_m, r, CSA, S_f^2, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm38’ –  $\{\tau_m, r, CSA, S_f^2, \tau_f, S^2, \tau_s, R_{ex}\}$ ,  
 ‘tm39’ –  $\{\tau_m, r, CSA, R_{ex}\}$ .

## Spin identification string

If ‘spin\_id’ is supplied then the model will only be selected for the corresponding spins. Otherwise the model will be selected for all spins.

## Prompt examples

To pick model ‘m1’ for all selected spins, type:

```
relax> model_free.select_model('m1')
```

```
relax> model_free.select_model(model='m1')
```



### 17.2.75 model\_selection



## Synopsis

Select the best model from a set of optimised models.

## Defaults

```
model_selection(method='AIC', model_pipe=None,  
bundle=None, pipes=None)
```

## Keyword arguments

method: The model selection technique (see below).

**model\_pipe:** The name of the new data pipe which will be created by this user function by the copying of the selected data pipe.

**bundle:** The optional pipe bundle is a special grouping or clustering of data pipes. If this is specified, the newly created data pipe will be added to this bundle.

**pipes:** An array containing the names of all data pipes to include in model selection.

### Description

The following model selection methods are supported:

**AIC** – Akaike’s Information Criteria.

**AICc** – Small sample size corrected AIC.

**BIC** – Bayesian or Schwarz Information Criteria.

**Bootstrap** – Bootstrap model selection.

**CV** – Single-item-out cross-validation.

**Expect** – The expected overall discrepancy (the true values of the parameters are required).

**Farrow** – Old model-free method by Farrow et al., 1994.

**Palmer** – Old model-free method by Mandel et al., 1995.

**Overall** – The realised overall discrepancy (the true values of the parameters are required).

For the methods ‘**Bootstrap**’, ‘**Expect**’, and ‘**Overall**’, the Monte Carlo simulations should have previously been executed with the `monte.carlo.create_data` method set to `Bootstrapping` to modify its behaviour.

If the data pipes have not been specified, then all data pipes will be used for model selection.

## Prompt examples

For model-free analysis, if the preset models 1 to 5 are minimised and loaded into the program, the following commands will carry out AIC model selection and to place the selected results into the ‘mixed’ data pipe, type one of:

```
relax> model_selection('AIC', 'mixed')
```

```
relax> model_selection(method='AIC',  
                      modsel_pipe='mixed')
```

```
relax> model_selection('AIC', 'mixed', ['m1',
    , 'm2', 'm3', 'm4', 'm5'])
```

[illegible]

### 17.2.76 molecule.copy



#### Synopsis

Copy all data associated with a molecule.

#### Defaults

```
molecule.copy(pipe_from=None, mol_from=None,
               pipe_to=None, mol_to=None)
```

#### Keyword arguments

**pipe\_from**: The data pipe containing the molecule from which the data will be copied. This defaults to the current data pipe.

**mol\_from**: The name of the molecule from which to copy data from.

**pipe\_to**: The data pipe to copy the data to. This defaults to the current data pipe.

**mol\_to**: The name of the new molecule. If left blank, the new molecule will have the same name as the old. This needs to be a molecule ID string, starting with '#'.

#### Description

This will copy all the data associated with a molecule to a second molecule. This includes all residue and spin system information. The new molecule name must be unique in the destination data pipe.

#### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is '**#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]'**', where the token elements are '**<mol\_name>**', the name of the molecule, '**<res\_id>**', the residue identifier which can be a number, name, or range of numbers, '**<atom\_id>**', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

#### Prompt examples

To copy the molecule data from the molecule 'GST' to the new molecule 'wt-GST', type:

```
relax> molecule.copy('#GST', '#wt-GST')
```

```
relax> molecule.copy(mol_from='#GST', mol_to=
                    '#wt-GST')
```

To copy the molecule data of the molecule 'Ap4Aase' from the data pipe 'm1' to 'm2', assuming the current data pipe is 'm1', type:

```
relax> molecule.copy(mol_from='#ApAase',
                    pipe_to='m2')
```

```
relax> molecule.copy(pipe_from='m1',
                    mol_from='#ApAase', pipe_to='m2',
                    mol_to='#ApAase')
```

---

17.2.77 molecule.create

## Synopsis

Create a new molecule.

## Defaults

`molecule.create(mol_name=None, mol_type=None)`

## Keyword arguments

`mol_name`: The name of the new molecule.

`mol_type`: The type of molecule.

## Description

This adds a new molecule data container to the relax data storage object. The same molecule name cannot be used more than once. The molecule type need not be specified. However, if given, it should be one of 'protein', 'DNA', 'RNA', 'organic molecule', or 'inorganic molecule'.

## Prompt examples

To create the molecules 'Ap4Aase', 'ATP', and 'MgF4', type:

```
relax> molecule.create('Ap4Aase')
```

```
relax> molecule.create('ATP')
```

```
relax> molecule.create('MgF4')
```

---

17.2.78 molecule.delete

## Synopsis

Deleting molecules from the relax data store.

## Defaults

`molecule.delete(mol_id=None)`

## Keyword arguments

`mol_id`: The molecule ID string.

## Description

This can be used to delete a single or sets of molecules from the relax data store. The molecule will be deleted from the current data pipe.

## Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is '#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]', where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

## 17.2.79 molecule.display



## Synopsis

Display the molecule information.

## Defaults

`molecule.display(mol_id=None)`

## Keyword arguments

`mol_id`: The molecule ID string.

## Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

## 17.2.80 molecule.name



## Synopsis

Name a molecule.

## Defaults

`molecule.name(mol_id=None, name=None, force=False)`

## Keyword arguments

`mol_id`: The molecule ID string corresponding to one or more molecules.

`name`: The new molecule name.

`force`: A flag which if True will cause the molecule to be renamed.

## Description

This simply allows molecules to be named (or renamed).

## Prompt examples

To rename the molecule 'Ap4Aase' to 'Inhib Ap4Aase', type one of:

```
relax> molecule.name('#Ap4Aase', 'Inhib
Ap4Aase', True)
```

```
relax> molecule.name(mol_id='#Ap4Aase', name
='Inhib Ap4Aase', force=True)
```

This assumes the molecule 'Ap4Aase' already exists.

## Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`.

...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]', where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

---

## 17.2.81 molecule.type



### Synopsis

Set the molecule type.

### Defaults

**molecule.type**(mol\_id=None, type=None, force=False)

### Keyword arguments

**mol\_id**: The molecule ID string corresponding to one or more molecules.

**type**: The molecule type.

**force**: A flag which if True will cause the molecule to type to be overwritten.

### Description

This allows the type of the molecule to be specified. It can be one of:

```
'protein',  
'DNA',  
'RNA',  
'organic molecule',  
'inorganic molecule'.
```

### Prompt examples

To set the molecule 'Ap4Aase' to the 'protein' type, type one of:

```
relax> molecule.type('#Ap4Aase', 'protein',  
                      True)
```

```
relax> molecule.type(mol_id='#Ap4Aase', type  
                    ='protein', force=True)
```

## Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

---

### 17.2.82 molmol.clear\_history



#### Synopsis

Clear the Molmol command history.

#### Defaults

`molmol.clear_history()`

#### Description

This will clear the Molmol history from memory.

---

**17.2.83 molmol.command**

---

**17.2.84 molmol.macro\_apply****Synopsis**

Execute a user supplied Molmol command.

**Defaults**

**molmol.command**(command=None)

**Keyword arguments**

**command**: The Molmol command to execute.

**Description**

This allows Molmol commands to be passed to the program. This can be useful for automation or scripting.

**Prompt examples**

To reinitialise the Molmol instance, type:

```
relax> molmol.command("InitAll yes")
```

**Synopsis**

Execute Molmol macros.

**Defaults**

**molmol.macro\_apply**(data\_type=None, style='classic', colour\_start\_name=None, colour\_start\_rgb=None, colour\_end\_name=None, colour\_end\_rgb=None, colour\_list=None)

**Keyword arguments**

**data\_type**: The data type to map to the structure.

**style**: The style of the macro.

**colour\_start\_name**: The name of the starting colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the starting colour RGB colour array cannot be given.

**colour\_start\_rgb**: The starting colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the starting colour name cannot be given.

**colour\_end\_name**: The name of the ending colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the ending colour RGB colour array cannot be given.

**colour\_end\_rgb**: The ending colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the ending colour name cannot be given.

**colour\_list**: The colour list to search for the colour names. This can be either 'molmol' or 'x11'.

**Description**

This allows spin specific values to be mapped to a structure through Molmol macros. Currently only the 'classic' style, which is described below, is available.

## Colour

The values are coloured based on a linear colour gradient which is specified through starting and ending colours. These can either be a string to identify one of the RGB (red, green, blue) colour arrays listed in the tables below, or you can give the RGB vector itself. For example, `'white'` and `[1.0, 1.0, 1.0]` both select the same colour. Leaving both colours unset will select the default colour gradient which for each type of analysis is described below.

When supplying the colours as strings, two lists of colours can be selected from which to match the strings. These are the default Molmol colour list and the X11 colour list, both of which are described in the tables below. The default behaviour is to first search the Molmol list and then the X11 colour list, raising an error if neither contain the name. To explicitly select these lists, set the colour list to either `'molmol'` or `'x11'`.

## Model-free classic style

Creator: Edward d'Auvergne

Argument string: `"classic"`

Description: The classic style draws the backbone of a protein in a cylindrical bond style. Rather than colouring the amino acids to which the NH bond belongs, the three covalent bonds of the peptide bond from Ca to Ca in which the NH bond is located are coloured. Deselected residues are shown as black lines.

Supported data types:

Please see Table [17.22](#) on page [511](#).

## Molmol RGB colour arrays

The following table is a list of colours used in Molmol and their corresponding RGB colour values ranging from 0 to 1.

Please see Table [17.23](#) on page [512](#).

## X11 RGB colour arrays

The following table is the list of X11 colour names and their corresponding RGB colour values ranging from 0 to 255.

Please see Table [17.24](#) on page [513](#).



Table 17.22: The model-free classic style for mapping model spin specific data onto 3D molecular structures using either PyMOL or Molmol.

| Data type                  | String      | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|----------------------------|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| $S^2$ .                    | 's2'        | The standard model-free order parameter, equal to $S_f^2$ .S2s for the two timescale models. The default colour gradient starts at 'yellow' and ends at 'red'.                                                                                                                                                                                                                                                                                                                                                                                                              |
| $S_f^2$ .                  | 's2f'       | The order parameter of the faster of two internal motions. Residues which are described by model-free models m1 to m4, the single timescale models, are illustrated as white neon bonds. The default colour gradient is the same as that for the $S^2$ data type.                                                                                                                                                                                                                                                                                                           |
| $S_s^2$ .                  | 's2s'       | The order parameter of the slower of two internal motions. This functions exactly as $S_f^2$ except that $S_s^2$ is plotted instead.                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Amplitude of fast motions. | 'amp_fast'  | Model independent display of the amplitde of fast motions. For residues described by model-free models m5 to m8, the value plotted is that of $S_f^2$ . However, for residues described by models m1 to m4, what is shown is dependent on the timescale of the motions. This is because these single timescale models can, at times, be perfect approximations to the more complex two timescale models. Hence if $\tau_e$ is less than 200 ps, $S^2$ is plotted. Otherwise the peptide bond is coloured white. The default colour gradient is the same as that for $S^2$ . |
| Amplitude of slow motions. | 'amp_slow'  | Model independent display of the amplitde of slow motions, arbitrarily defined as motions slower than 200 ps. For residues described by model-free models m5 to m8, the order parameter $S^2$ is plotted if $\tau_s > 200$ ps. For models m1 to m4, $S^2$ is plotted if $\tau_e > 200$ ps. The default colour gradient is the same as that for $S^2$ .                                                                                                                                                                                                                      |
| $\tau_e$ .                 | 'te'        | The correlation time, $\tau_e$ . The default colour gradient starts at 'turquoise' and ends at 'blue'.                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| $\tau_f$ .                 | 'tf'        | The correlation time, $\tau_f$ . The default colour gradient is the same as that of $\tau_e$ .                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| $\tau_s$ .                 | 'ts'        | The correlation time, $\tau_s$ . The default colour gradient starts at 'blue' and ends at 'black'.                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Timescale of fast motions  | 'time_fast' | Model independent display of the timescale of fast motions. For models m5 to m8, only the parameter $\tau_f$ is plotted. For models m2 and m4, the parameter $\tau_e$ is plotted only if it is less than 200 ps. All other residues are assumed to have a correlation time of zero. The default colour gradient is the same as that of $\tau_e$ .                                                                                                                                                                                                                           |
| Timescale of slow motions  | 'time_slow' | Model independent display of the timescale of slow motions. For models m5 to m8, only the parameter $\tau_s$ is plotted. For models m2 and m4, the parameter $\tau_e$ is plotted only if it is greater than 200 ps. All other residues are coloured white. The default colour gradient is the same as that of $\tau_s$ .                                                                                                                                                                                                                                                    |
| Chemical exchange          | 'rex'       | The chemical exchange, $R_{ex}$ . Residues which experience no chemical exchange are coloured white. The default colour gradient starts at 'yellow' and finishes at 'red'.                                                                                                                                                                                                                                                                                                                                                                                                  |

Table 17.23: Molmol colour names and corresponding RGB colour values (from 0 to 1)

| Name           | Red   | Green | Blue  |
|----------------|-------|-------|-------|
| 'black'        | 0.000 | 0.000 | 0.000 |
| 'navy'         | 0.000 | 0.000 | 0.502 |
| 'blue'         | 0.000 | 0.000 | 1.000 |
| 'dark green'   | 0.000 | 0.392 | 0.000 |
| 'green'        | 0.000 | 1.000 | 0.000 |
| 'cyan'         | 0.000 | 1.000 | 1.000 |
| 'turquoise'    | 0.251 | 0.878 | 0.816 |
| 'royal blue'   | 0.255 | 0.412 | 0.882 |
| 'aquamarine'   | 0.498 | 1.000 | 0.831 |
| 'sky green'    | 0.529 | 0.808 | 0.922 |
| 'dark violet'  | 0.580 | 0.000 | 0.827 |
| 'pale green'   | 0.596 | 0.984 | 0.596 |
| 'purple'       | 0.627 | 0.125 | 0.941 |
| 'brown'        | 0.647 | 0.165 | 0.165 |
| 'light blue'   | 0.678 | 0.847 | 0.902 |
| 'grey'         | 0.745 | 0.745 | 0.745 |
| 'light grey'   | 0.827 | 0.827 | 0.827 |
| 'violet'       | 0.933 | 0.510 | 0.933 |
| 'light coral'  | 0.941 | 0.502 | 0.502 |
| 'khaki'        | 0.941 | 0.902 | 0.549 |
| 'beige'        | 0.961 | 0.961 | 0.863 |
| 'red'          | 1.000 | 0.000 | 0.000 |
| 'magenta'      | 1.000 | 0.000 | 1.000 |
| 'deep pink'    | 1.000 | 0.078 | 0.576 |
| 'orange red'   | 1.000 | 0.271 | 0.000 |
| 'hot pink'     | 1.000 | 0.412 | 0.706 |
| 'coral'        | 1.000 | 0.498 | 0.314 |
| 'dark orange'  | 1.000 | 0.549 | 0.000 |
| 'orange'       | 1.000 | 0.647 | 0.000 |
| 'pink'         | 1.000 | 0.753 | 0.796 |
| 'gold'         | 1.000 | 0.843 | 0.000 |
| 'yellow'       | 1.000 | 1.000 | 0.000 |
| 'light yellow' | 1.000 | 1.000 | 0.878 |
| 'ivory'        | 1.000 | 1.000 | 0.941 |
| 'white'        | 1.000 | 1.000 | 1.000 |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name              | Red | Green | Blue |
|-------------------|-----|-------|------|
| snow              | 255 | 250   | 250  |
| ghost white       | 248 | 248   | 255  |
| white smoke       | 245 | 245   | 245  |
| gainsboro         | 220 | 220   | 220  |
| floral white      | 255 | 250   | 240  |
| old lace          | 253 | 245   | 230  |
| linen             | 250 | 240   | 230  |
| antique white     | 250 | 235   | 215  |
| papaya whip       | 255 | 239   | 213  |
| blanched almond   | 255 | 235   | 205  |
| bisque            | 255 | 228   | 196  |
| peach puff        | 255 | 218   | 185  |
| navajo white      | 255 | 222   | 173  |
| moccasin          | 255 | 228   | 181  |
| cornsilk          | 255 | 248   | 220  |
| ivory             | 255 | 255   | 240  |
| lemon chiffon     | 255 | 250   | 205  |
| seashell          | 255 | 245   | 238  |
| honeydew          | 240 | 255   | 240  |
| mint cream        | 245 | 255   | 250  |
| azure             | 240 | 255   | 255  |
| alice blue        | 240 | 248   | 255  |
| lavender          | 230 | 230   | 250  |
| lavender blush    | 255 | 240   | 245  |
| misty rose        | 255 | 228   | 225  |
| white             | 255 | 255   | 255  |
| black             | 0   | 0     | 0    |
| dark slate grey   | 47  | 79    | 79   |
| dim grey          | 105 | 105   | 105  |
| slate grey        | 112 | 128   | 144  |
| light slate grey  | 119 | 136   | 153  |
| grey              | 190 | 190   | 190  |
| light grey        | 211 | 211   | 211  |
| midnight blue     | 25  | 25    | 112  |
| navy              | 0   | 0     | 128  |
| cornflower blue   | 100 | 149   | 237  |
| dark slate blue   | 72  | 61    | 139  |
| slate blue        | 106 | 90    | 205  |
| medium slate blue | 123 | 104   | 238  |
| light slate blue  | 132 | 112   | 255  |
| medium blue       | 0   | 0     | 205  |
| royal blue        | 65  | 105   | 225  |
| blue              | 0   | 0     | 255  |
| dodger blue       | 30  | 144   | 255  |
| deep sky blue     | 0   | 191   | 255  |
| sky blue          | 135 | 206   | 235  |
| light sky blue    | 135 | 206   | 250  |
| steel blue        | 70  | 130   | 180  |
| light steel blue  | 176 | 196   | 222  |
| light blue        | 173 | 216   | 230  |
| powder blue       | 176 | 224   | 230  |
| pale turquoise    | 175 | 238   | 238  |
| dark turquoise    | 0   | 206   | 209  |
| medium turquoise  | 72  | 209   | 204  |
| turquoise         | 64  | 224   | 208  |
| cyan              | 0   | 255   | 255  |
| light cyan        | 224 | 255   | 255  |
| cadet blue        | 95  | 158   | 160  |
| medium aquamarine | 102 | 205   | 170  |
| aquamarine        | 127 | 255   | 212  |
| dark green        | 0   | 100   | 0    |
| dark olive green  | 85  | 107   | 47   |
| dark sea green    | 143 | 188   | 143  |
| sea green         | 46  | 139   | 87   |
| medium sea green  | 60  | 179   | 113  |
| light sea green   | 32  | 178   | 170  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name                   | Red | Green | Blue |
|------------------------|-----|-------|------|
| pale green             | 152 | 251   | 152  |
| spring green           | 0   | 255   | 127  |
| lawn green             | 124 | 252   | 0    |
| green                  | 0   | 255   | 0    |
| chartreuse             | 127 | 255   | 0    |
| medium spring green    | 0   | 250   | 154  |
| green yellow           | 173 | 255   | 47   |
| lime green             | 50  | 205   | 50   |
| yellow green           | 154 | 205   | 50   |
| forest green           | 34  | 139   | 34   |
| olive drab             | 107 | 142   | 35   |
| dark khaki             | 189 | 183   | 107  |
| khaki                  | 240 | 230   | 140  |
| pale goldenrod         | 238 | 232   | 170  |
| light goldenrod yellow | 250 | 250   | 210  |
| light yellow           | 255 | 255   | 224  |
| yellow                 | 255 | 255   | 0    |
| gold                   | 255 | 215   | 0    |
| light goldenrod        | 238 | 221   | 130  |
| goldenrod              | 218 | 165   | 32   |
| dark goldenrod         | 184 | 134   | 11   |
| rosy brown             | 188 | 143   | 143  |
| indian red             | 205 | 92    | 92   |
| saddle brown           | 139 | 69    | 19   |
| sienna                 | 160 | 82    | 45   |
| peru                   | 205 | 133   | 63   |
| burlywood              | 222 | 184   | 135  |
| beige                  | 245 | 245   | 220  |
| wheat                  | 245 | 222   | 179  |
| sandy brown            | 244 | 164   | 96   |
| tan                    | 210 | 180   | 140  |
| chocolate              | 210 | 105   | 30   |
| firebrick              | 178 | 34    | 34   |
| brown                  | 165 | 42    | 42   |
| dark salmon            | 233 | 150   | 122  |
| salmon                 | 250 | 128   | 114  |
| light salmon           | 255 | 160   | 122  |
| orange                 | 255 | 165   | 0    |
| dark orange            | 255 | 140   | 0    |
| coral                  | 255 | 127   | 80   |
| light coral            | 240 | 128   | 128  |
| tomato                 | 255 | 99    | 71   |
| orange red             | 255 | 69    | 0    |
| red                    | 255 | 0     | 0    |
| hot pink               | 255 | 105   | 180  |
| deep pink              | 255 | 20    | 147  |
| pink                   | 255 | 192   | 203  |
| light pink             | 255 | 182   | 193  |
| pale violet red        | 219 | 112   | 147  |
| maroon                 | 176 | 48    | 96   |
| medium violet red      | 199 | 21    | 133  |
| violet red             | 208 | 32    | 144  |
| magenta                | 255 | 0     | 255  |
| violet                 | 238 | 130   | 238  |
| plum                   | 221 | 160   | 221  |
| orchid                 | 218 | 112   | 214  |
| medium orchid          | 186 | 85    | 211  |
| dark orchid            | 153 | 50    | 204  |
| dark violet            | 148 | 0     | 211  |
| blue violet            | 138 | 43    | 226  |
| purple                 | 160 | 32    | 240  |
| medium purple          | 147 | 112   | 219  |
| thistle                | 216 | 191   | 216  |
| snow 1                 | 255 | 250   | 250  |
| snow 2                 | 238 | 233   | 233  |
| snow 3                 | 205 | 201   | 201  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name             | Red | Green | Blue |
|------------------|-----|-------|------|
| snow 4           | 139 | 137   | 137  |
| seashell 1       | 255 | 245   | 238  |
| seashell 2       | 238 | 229   | 222  |
| seashell 3       | 205 | 197   | 191  |
| seashell 4       | 139 | 134   | 130  |
| antique white 1  | 255 | 239   | 219  |
| antique white 2  | 238 | 223   | 204  |
| antique white 3  | 205 | 192   | 176  |
| antique white 4  | 139 | 131   | 120  |
| bisque 1         | 255 | 228   | 196  |
| bisque 2         | 238 | 213   | 183  |
| bisque 3         | 205 | 183   | 158  |
| bisque 4         | 139 | 125   | 107  |
| peach puff 1     | 255 | 218   | 185  |
| peach puff 2     | 238 | 203   | 173  |
| peach puff 3     | 205 | 175   | 149  |
| peach puff 4     | 139 | 119   | 101  |
| navajo white 1   | 255 | 222   | 173  |
| navajo white 2   | 238 | 207   | 161  |
| navajo white 3   | 205 | 179   | 139  |
| navajo white 4   | 139 | 121   | 94   |
| lemon chiffon 1  | 255 | 250   | 205  |
| lemon chiffon 2  | 238 | 233   | 191  |
| lemon chiffon 3  | 205 | 201   | 165  |
| lemon chiffon 4  | 139 | 137   | 112  |
| cornsilk 1       | 255 | 248   | 220  |
| cornsilk 2       | 238 | 232   | 205  |
| cornsilk 3       | 205 | 200   | 177  |
| cornsilk 4       | 139 | 136   | 120  |
| ivory 1          | 255 | 255   | 240  |
| ivory 2          | 238 | 238   | 224  |
| ivory 3          | 205 | 205   | 193  |
| ivory 4          | 139 | 139   | 131  |
| honeydew 1       | 240 | 255   | 240  |
| honeydew 2       | 224 | 238   | 224  |
| honeydew 3       | 193 | 205   | 193  |
| honeydew 4       | 131 | 139   | 131  |
| lavender blush 1 | 255 | 240   | 245  |
| lavender blush 2 | 238 | 224   | 229  |
| lavender blush 3 | 205 | 193   | 197  |
| lavender blush 4 | 139 | 131   | 134  |
| misty rose 1     | 255 | 228   | 225  |
| misty rose 2     | 238 | 213   | 210  |
| misty rose 3     | 205 | 183   | 181  |
| misty rose 4     | 139 | 125   | 123  |
| azure 1          | 240 | 255   | 255  |
| azure 2          | 224 | 238   | 238  |
| azure 3          | 193 | 205   | 205  |
| azure 4          | 131 | 139   | 139  |
| slate blue 1     | 131 | 111   | 255  |
| slate blue 2     | 122 | 103   | 238  |
| slate blue 3     | 105 | 89    | 205  |
| slate blue 4     | 71  | 60    | 139  |
| royal blue 1     | 72  | 118   | 255  |
| royal blue 2     | 67  | 110   | 238  |
| royal blue 3     | 58  | 95    | 205  |
| royal blue 4     | 39  | 64    | 139  |
| blue 1           | 0   | 0     | 255  |
| blue 2           | 0   | 0     | 238  |
| blue 3           | 0   | 0     | 205  |
| blue 4           | 0   | 0     | 139  |
| dodger blue 1    | 30  | 144   | 255  |
| dodger blue 2    | 28  | 134   | 238  |
| dodger blue 3    | 24  | 116   | 205  |
| dodger blue 4    | 16  | 78    | 139  |
| steel blue 1     | 99  | 184   | 255  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name               | Red | Green | Blue |
|--------------------|-----|-------|------|
| steel blue 2       | 92  | 172   | 238  |
| steel blue 3       | 79  | 148   | 205  |
| steel blue 4       | 54  | 100   | 139  |
| deep sky blue 1    | 0   | 191   | 255  |
| deep sky blue 2    | 0   | 178   | 238  |
| deep sky blue 3    | 0   | 154   | 205  |
| deep sky blue 4    | 0   | 104   | 139  |
| sky blue 1         | 135 | 206   | 255  |
| sky blue 2         | 126 | 192   | 238  |
| sky blue 3         | 108 | 166   | 205  |
| sky blue 4         | 74  | 112   | 139  |
| light sky blue 1   | 176 | 226   | 255  |
| light sky blue 2   | 164 | 211   | 238  |
| light sky blue 3   | 141 | 182   | 205  |
| light sky blue 4   | 96  | 123   | 139  |
| slate grey 1       | 198 | 226   | 255  |
| slate grey 2       | 185 | 211   | 238  |
| slate grey 3       | 159 | 182   | 205  |
| slate grey 4       | 108 | 123   | 139  |
| light steel blue 1 | 202 | 225   | 255  |
| light steel blue 2 | 188 | 210   | 238  |
| light steel blue 3 | 162 | 181   | 205  |
| light steel blue 4 | 110 | 123   | 139  |
| light blue 1       | 191 | 239   | 255  |
| light blue 2       | 178 | 223   | 238  |
| light blue 3       | 154 | 192   | 205  |
| light blue 4       | 104 | 131   | 139  |
| light cyan 1       | 224 | 255   | 255  |
| light cyan 2       | 209 | 238   | 238  |
| light cyan 3       | 180 | 205   | 205  |
| light cyan 4       | 122 | 139   | 139  |
| pale turquoise 1   | 187 | 255   | 255  |
| pale turquoise 2   | 174 | 238   | 238  |
| pale turquoise 3   | 150 | 205   | 205  |
| pale turquoise 4   | 102 | 139   | 139  |
| cadet blue 1       | 152 | 245   | 255  |
| cadet blue 2       | 142 | 229   | 238  |
| cadet blue 3       | 122 | 197   | 205  |
| cadet blue 4       | 83  | 134   | 139  |
| turquoise 1        | 0   | 245   | 255  |
| turquoise 2        | 0   | 229   | 238  |
| turquoise 3        | 0   | 197   | 205  |
| turquoise 4        | 0   | 134   | 139  |
| cyan 1             | 0   | 255   | 255  |
| cyan 2             | 0   | 238   | 238  |
| cyan 3             | 0   | 205   | 205  |
| cyan 4             | 0   | 139   | 139  |
| dark slate grey 1  | 151 | 255   | 255  |
| dark slate grey 2  | 141 | 238   | 238  |
| dark slate grey 3  | 121 | 205   | 205  |
| dark slate grey 4  | 82  | 139   | 139  |
| aquamarine 1       | 127 | 255   | 212  |
| aquamarine 2       | 118 | 238   | 198  |
| aquamarine 3       | 102 | 205   | 170  |
| aquamarine 4       | 69  | 139   | 116  |
| dark sea green 1   | 193 | 255   | 193  |
| dark sea green 2   | 180 | 238   | 180  |
| dark sea green 3   | 155 | 205   | 155  |
| dark sea green 4   | 105 | 139   | 105  |
| sea green 1        | 84  | 255   | 159  |
| sea green 2        | 78  | 238   | 148  |
| sea green 3        | 67  | 205   | 128  |
| sea green 4        | 46  | 139   | 87   |
| pale green 1       | 154 | 255   | 154  |
| pale green 2       | 144 | 238   | 144  |
| pale green 3       | 124 | 205   | 124  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name               | Red | Green | Blue |
|--------------------|-----|-------|------|
| pale green 4       | 84  | 139   | 84   |
| spring green 1     | 0   | 255   | 127  |
| spring green 2     | 0   | 238   | 118  |
| spring green 3     | 0   | 205   | 102  |
| spring green 4     | 0   | 139   | 69   |
| green 1            | 0   | 255   | 0    |
| green 2            | 0   | 238   | 0    |
| green 3            | 0   | 205   | 0    |
| green 4            | 0   | 139   | 0    |
| chartreuse 1       | 127 | 255   | 0    |
| chartreuse 2       | 118 | 238   | 0    |
| chartreuse 3       | 102 | 205   | 0    |
| chartreuse 4       | 69  | 139   | 0    |
| olive drab 1       | 192 | 255   | 62   |
| olive drab 2       | 179 | 238   | 58   |
| olive drab 3       | 154 | 205   | 50   |
| olive drab 4       | 105 | 139   | 34   |
| dark olive green 1 | 202 | 255   | 112  |
| dark olive green 2 | 188 | 238   | 104  |
| dark olive green 3 | 162 | 205   | 90   |
| dark olive green 4 | 110 | 139   | 61   |
| khaki 1            | 255 | 246   | 143  |
| khaki 2            | 238 | 230   | 133  |
| khaki 3            | 205 | 198   | 115  |
| khaki 4            | 139 | 134   | 78   |
| light goldenrod 1  | 255 | 236   | 139  |
| light goldenrod 2  | 238 | 220   | 130  |
| light goldenrod 3  | 205 | 190   | 112  |
| light goldenrod 4  | 139 | 129   | 76   |
| light yellow 1     | 255 | 255   | 224  |
| light yellow 2     | 238 | 238   | 209  |
| light yellow 3     | 205 | 205   | 180  |
| light yellow 4     | 139 | 139   | 122  |
| yellow 1           | 255 | 255   | 0    |
| yellow 2           | 238 | 238   | 0    |
| yellow 3           | 205 | 205   | 0    |
| yellow 4           | 139 | 139   | 0    |
| gold 1             | 255 | 215   | 0    |
| gold 2             | 238 | 201   | 0    |
| gold 3             | 205 | 173   | 0    |
| gold 4             | 139 | 117   | 0    |
| goldenrod 1        | 255 | 193   | 37   |
| goldenrod 2        | 238 | 180   | 34   |
| goldenrod 3        | 205 | 155   | 29   |
| goldenrod 4        | 139 | 105   | 20   |
| dark goldenrod 1   | 255 | 185   | 15   |
| dark goldenrod 2   | 238 | 173   | 14   |
| dark goldenrod 3   | 205 | 149   | 12   |
| dark goldenrod 4   | 139 | 101   | 8    |
| rosy brown 1       | 255 | 193   | 193  |
| rosy brown 2       | 238 | 180   | 180  |
| rosy brown 3       | 205 | 155   | 155  |
| rosy brown 4       | 139 | 105   | 105  |
| indian red 1       | 255 | 106   | 106  |
| indian red 2       | 238 | 99    | 99   |
| indian red 3       | 205 | 85    | 85   |
| indian red 4       | 139 | 58    | 58   |
| sienna 1           | 255 | 130   | 71   |
| sienna 2           | 238 | 121   | 66   |
| sienna 3           | 205 | 104   | 57   |
| sienna 4           | 139 | 71    | 38   |
| burlywood 1        | 255 | 211   | 155  |
| burlywood 2        | 238 | 197   | 145  |
| burlywood 3        | 205 | 170   | 125  |
| burlywood 4        | 139 | 115   | 85   |
| wheat 1            | 255 | 231   | 186  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name           | Red | Green | Blue |
|----------------|-----|-------|------|
| wheat 2        | 238 | 216   | 174  |
| wheat 3        | 205 | 186   | 150  |
| wheat 4        | 139 | 126   | 102  |
| tan 1          | 255 | 165   | 79   |
| tan 2          | 238 | 154   | 73   |
| tan 3          | 205 | 133   | 63   |
| tan 4          | 139 | 90    | 43   |
| chocolate 1    | 255 | 127   | 36   |
| chocolate 2    | 238 | 118   | 33   |
| chocolate 3    | 205 | 102   | 29   |
| chocolate 4    | 139 | 69    | 19   |
| firebrick 1    | 255 | 48    | 48   |
| firebrick 2    | 238 | 44    | 44   |
| firebrick 3    | 205 | 38    | 38   |
| firebrick 4    | 139 | 26    | 26   |
| brown 1        | 255 | 64    | 64   |
| brown 2        | 238 | 59    | 59   |
| brown 3        | 205 | 51    | 51   |
| brown 4        | 139 | 35    | 35   |
| salmon 1       | 255 | 140   | 105  |
| salmon 2       | 238 | 130   | 98   |
| salmon 3       | 205 | 112   | 84   |
| salmon 4       | 139 | 76    | 57   |
| light salmon 1 | 255 | 160   | 122  |
| light salmon 2 | 238 | 149   | 114  |
| light salmon 3 | 205 | 129   | 98   |
| light salmon 4 | 139 | 87    | 66   |
| orange 1       | 255 | 165   | 0    |
| orange 2       | 238 | 154   | 0    |
| orange 3       | 205 | 133   | 0    |
| orange 4       | 139 | 90    | 0    |
| dark orange 1  | 255 | 127   | 0    |
| dark orange 2  | 238 | 118   | 0    |
| dark orange 3  | 205 | 102   | 0    |
| dark orange 4  | 139 | 69    | 0    |
| coral 1        | 255 | 114   | 86   |
| coral 2        | 238 | 106   | 80   |
| coral 3        | 205 | 91    | 69   |
| coral 4        | 139 | 62    | 47   |
| tomato 1       | 255 | 99    | 71   |
| tomato 2       | 238 | 92    | 66   |
| tomato 3       | 205 | 79    | 57   |
| tomato 4       | 139 | 54    | 38   |
| orange red 1   | 255 | 69    | 0    |
| orange red 2   | 238 | 64    | 0    |
| orange red 3   | 205 | 55    | 0    |
| orange red 4   | 139 | 37    | 0    |
| red 1          | 255 | 0     | 0    |
| red 2          | 238 | 0     | 0    |
| red 3          | 205 | 0     | 0    |
| red 4          | 139 | 0     | 0    |
| deep pink 1    | 255 | 20    | 147  |
| deep pink 2    | 238 | 18    | 137  |
| deep pink 3    | 205 | 16    | 118  |
| deep pink 4    | 139 | 10    | 80   |
| hot pink 1     | 255 | 110   | 180  |
| hot pink 2     | 238 | 106   | 167  |
| hot pink 3     | 205 | 96    | 144  |
| hot pink 4     | 139 | 58    | 98   |
| pink 1         | 255 | 181   | 197  |
| pink 2         | 238 | 169   | 184  |
| pink 3         | 205 | 145   | 158  |
| pink 4         | 139 | 99    | 108  |
| light pink 1   | 255 | 174   | 185  |
| light pink 2   | 238 | 162   | 173  |
| light pink 3   | 205 | 140   | 149  |



Table 17.24: X11 colour names and corresponding RGB colour values

| Name              | Red | Green | Blue |
|-------------------|-----|-------|------|
| light pink 4      | 139 | 95    | 101  |
| pale violet red 1 | 255 | 130   | 171  |
| pale violet red 2 | 238 | 121   | 159  |
| pale violet red 3 | 205 | 104   | 137  |
| pale violet red 4 | 139 | 71    | 93   |
| maroon 1          | 255 | 52    | 179  |
| maroon 2          | 238 | 48    | 167  |
| maroon 3          | 205 | 41    | 144  |
| maroon 4          | 139 | 28    | 98   |
| violet red 1      | 255 | 62    | 150  |
| violet red 2      | 238 | 58    | 140  |
| violet red 3      | 205 | 50    | 120  |
| violet red 4      | 139 | 34    | 82   |
| magenta 1         | 255 | 0     | 255  |
| magenta 2         | 238 | 0     | 238  |
| magenta 3         | 205 | 0     | 205  |
| magenta 4         | 139 | 0     | 139  |
| orchid 1          | 255 | 131   | 250  |
| orchid 2          | 238 | 122   | 233  |
| orchid 3          | 205 | 105   | 201  |
| orchid 4          | 139 | 71    | 137  |
| plum 1            | 255 | 187   | 255  |
| plum 2            | 238 | 174   | 238  |
| plum 3            | 205 | 150   | 205  |
| plum 4            | 139 | 102   | 139  |
| medium orchid 1   | 224 | 102   | 255  |
| medium orchid 2   | 209 | 95    | 238  |
| medium orchid 3   | 180 | 82    | 205  |
| medium orchid 4   | 122 | 55    | 139  |
| dark orchid 1     | 191 | 62    | 255  |
| dark orchid 2     | 178 | 58    | 238  |
| dark orchid 3     | 154 | 50    | 205  |
| dark orchid 4     | 104 | 34    | 139  |
| purple 1          | 155 | 48    | 255  |
| purple 2          | 145 | 44    | 238  |
| purple 3          | 125 | 38    | 205  |
| purple 4          | 85  | 26    | 139  |
| medium purple 1   | 171 | 130   | 255  |
| medium purple 2   | 159 | 121   | 238  |
| medium purple 3   | 137 | 104   | 205  |
| medium purple 4   | 93  | 71    | 139  |
| thistle 1         | 255 | 225   | 255  |
| thistle 2         | 238 | 210   | 238  |
| thistle 3         | 205 | 181   | 205  |
| thistle 4         | 139 | 123   | 139  |
| grey 0            | 0   | 0     | 0    |
| grey 1            | 3   | 3     | 3    |
| grey 2            | 5   | 5     | 5    |
| grey 3            | 8   | 8     | 8    |
| grey 4            | 10  | 10    | 10   |
| grey 5            | 13  | 13    | 13   |
| grey 6            | 15  | 15    | 15   |
| grey 7            | 18  | 18    | 18   |
| grey 8            | 20  | 20    | 20   |
| grey 9            | 23  | 23    | 23   |
| grey 10           | 26  | 26    | 26   |
| grey 11           | 28  | 28    | 28   |
| grey 12           | 31  | 31    | 31   |
| grey 13           | 33  | 33    | 33   |
| grey 14           | 36  | 36    | 36   |
| grey 15           | 38  | 38    | 38   |
| grey 16           | 41  | 41    | 41   |
| grey 17           | 43  | 43    | 43   |
| grey 18           | 46  | 46    | 46   |
| grey 19           | 48  | 48    | 48   |
| grey 20           | 51  | 51    | 51   |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name    | Red | Green | Blue |
|---------|-----|-------|------|
| grey 21 | 54  | 54    | 54   |
| grey 22 | 56  | 56    | 56   |
| grey 23 | 59  | 59    | 59   |
| grey 24 | 61  | 61    | 61   |
| grey 25 | 64  | 64    | 64   |
| grey 26 | 66  | 66    | 66   |
| grey 27 | 69  | 69    | 69   |
| grey 28 | 71  | 71    | 71   |
| grey 29 | 74  | 74    | 74   |
| grey 30 | 77  | 77    | 77   |
| grey 31 | 79  | 79    | 79   |
| grey 32 | 82  | 82    | 82   |
| grey 33 | 84  | 84    | 84   |
| grey 34 | 87  | 87    | 87   |
| grey 35 | 89  | 89    | 89   |
| grey 36 | 92  | 92    | 92   |
| grey 37 | 94  | 94    | 94   |
| grey 38 | 97  | 97    | 97   |
| grey 39 | 99  | 99    | 99   |
| grey 40 | 102 | 102   | 102  |
| grey 41 | 105 | 105   | 105  |
| grey 42 | 107 | 107   | 107  |
| grey 43 | 110 | 110   | 110  |
| grey 44 | 112 | 112   | 112  |
| grey 45 | 115 | 115   | 115  |
| grey 46 | 117 | 117   | 117  |
| grey 47 | 120 | 120   | 120  |
| grey 48 | 122 | 122   | 122  |
| grey 49 | 125 | 125   | 125  |
| grey 50 | 127 | 127   | 127  |
| grey 51 | 130 | 130   | 130  |
| grey 52 | 133 | 133   | 133  |
| grey 53 | 135 | 135   | 135  |
| grey 54 | 138 | 138   | 138  |
| grey 55 | 140 | 140   | 140  |
| grey 56 | 143 | 143   | 143  |
| grey 57 | 145 | 145   | 145  |
| grey 58 | 148 | 148   | 148  |
| grey 59 | 150 | 150   | 150  |
| grey 60 | 153 | 153   | 153  |
| grey 61 | 156 | 156   | 156  |
| grey 62 | 158 | 158   | 158  |
| grey 63 | 161 | 161   | 161  |
| grey 64 | 163 | 163   | 163  |
| grey 65 | 166 | 166   | 166  |
| grey 66 | 168 | 168   | 168  |
| grey 67 | 171 | 171   | 171  |
| grey 68 | 173 | 173   | 173  |
| grey 69 | 176 | 176   | 176  |
| grey 70 | 179 | 179   | 179  |
| grey 71 | 181 | 181   | 181  |
| grey 72 | 184 | 184   | 184  |
| grey 73 | 186 | 186   | 186  |
| grey 74 | 189 | 189   | 189  |
| grey 75 | 191 | 191   | 191  |
| grey 76 | 194 | 194   | 194  |
| grey 77 | 196 | 196   | 196  |
| grey 78 | 199 | 199   | 199  |
| grey 79 | 201 | 201   | 201  |
| grey 80 | 204 | 204   | 204  |
| grey 81 | 207 | 207   | 207  |
| grey 82 | 209 | 209   | 209  |
| grey 83 | 212 | 212   | 212  |
| grey 84 | 214 | 214   | 214  |
| grey 85 | 217 | 217   | 217  |
| grey 86 | 219 | 219   | 219  |

Table 17.24: X11 colour names and corresponding RGB colour values

| Name         | Red | Green | Blue |
|--------------|-----|-------|------|
| grey 87      | 222 | 222   | 222  |
| grey 88      | 224 | 224   | 224  |
| grey 89      | 227 | 227   | 227  |
| grey 90      | 229 | 229   | 229  |
| grey 91      | 232 | 232   | 232  |
| grey 92      | 235 | 235   | 235  |
| grey 93      | 237 | 237   | 237  |
| grey 94      | 240 | 240   | 240  |
| grey 95      | 242 | 242   | 242  |
| grey 96      | 245 | 245   | 245  |
| grey 97      | 247 | 247   | 247  |
| grey 98      | 250 | 250   | 250  |
| grey 99      | 252 | 252   | 252  |
| grey 100     | 255 | 255   | 255  |
| dark grey    | 169 | 169   | 169  |
| dark blue    | 0   | 0     | 139  |
| dark cyan    | 0   | 139   | 139  |
| dark magenta | 139 | 0     | 139  |
| dark red     | 139 | 0     | 0    |
| light green  | 144 | 238   | 144  |

## Prompt examples

To map the order parameter values,  $S^2$ , onto the structure using the classic style, type:

```
relax> molmol.macro_apply('s2')

relax> molmol.macro_apply(data_type='s2')

relax> molmol.macro_apply(data_type='s2',
                           style="classic")
```

---

## 17.2.85 molmol.macro\_run



### Synopsis

Open and execute the Molmol macro file.

### Defaults

**molmol.macro\_run**(file=None, dir='molmol')

### Keyword arguments

**file**: The name of the Molmol macro file.

**dir**: The directory name.

### Description

This user function is for opening and running a Molmol macro located within a text file.

## Prompt examples

To execute the macro file 's2.mac' located in the directory 'molmol', type:

```
relax> molmol.macro_run(file='s2.mac')

relax> molmol.macro_run(file='s2.mac', dir='
molmol')
```

## 17.2.86 molmol.macro\_write




### Synopsis

Create Molmol macros.

### Defaults

```
molmol.macro_write(data_type=None, style='classic',
colour_start_name=None, colour_start_rgb=None,
colour_end_name=None, colour_end_rgb=None,
colour_list=None, file=None, dir='molmol', force=False)
```

### Keyword arguments

**data\_type:** The data type to map to the structure.

**style:** The style of the macro.

**colour\_start\_name:** The name of the starting colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the starting colour RGB colour array cannot be given.

**colour\_start\_rgb:** The starting colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the starting colour name cannot be given.

**colour\_end\_name:** The name of the ending colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the ending colour RGB colour array cannot be given.

**colour\_end\_rgb:** The ending colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the ending colour name cannot be given.

**colour\_list:** The colour list to search for the colour names. This can be either 'molmol' or 'x11'.

**file:** The optional name of the file.

**dir:** The optional directory to save the file to.

**force:** A flag which, if set to True, will cause the file to be overwritten.

### Description

This allows residues specific values to be mapped to a structure through the creation of a Molmol '\*.mac' macro which can be executed in Molmol by clicking on 'File, Macro, Execute User...'. Currently only the 'classic' style, which is described below, is available.

### Colour

The values are coloured based on a linear colour gradient which is specified through starting and ending colours. These can either be a string to identify one of the RGB (red, green, blue) colour arrays listed in the tables below, or you can give the RGB vector itself. For example, 'white' and [1.0, 1.0, 1.0] both select the same colour. Leaving both colours unset will select the default colour gradient which for each type of analysis is described below.

When supplying the colours as strings, two lists of colours can be selected from which to match the strings. These are the default Molmol colour list and the X11 colour list, both of which are described in the tables below. The default behaviour is to first search the Molmol list and then the X11 colour list, raising an error if neither contain the name. To explicitly select these lists, set the colour list to either 'molmol' or 'x11'.

### Model-free classic style

Creator: Edward d'Auvergne

Argument string: "classic"

**Description:** The classic style draws the backbone of a protein in a cylindrical bond style. Rather than colouring the amino acids to which the NH bond belongs, the three covalent bonds of the peptide bond from Ca to Ca in which the NH bond is located are coloured. Deselected residues are shown as black lines.

Supported data types:

Please see Table 17.22 on page 511.

### Molmol RGB colour arrays

The following table is a list of colours used in Molmol and their corresponding RGB colour values ranging from 0 to 1.

Please see Table 17.23 on page 512.

### X11 RGB colour arrays

The following table is the list of X11 colour names and their corresponding RGB colour values ranging from 0 to 255.

Please see Table 17.24 on page 513.

### Prompt examples

To create a Molmol macro mapping the order parameter values,  $S^2$ , onto the structure using the classic style, type:

```
relax> molmol.macro_write('s2')
```

```
relax> molmol.macro_write(data_type='s2')  
  
relax> molmol.macro_write(data_type='s2',  
    style="classic", file='s2.mac', dir='  
    molmol')
```

---

## 17.2.87 molmol.ribbon



### Synopsis

Apply the Molmol ribbon style.

### Defaults

**molmol.ribbon()**

### Description

This applies the Molmol ribbon style which is equivalent to clicking on ‘ribbon’ in the Molmol side menu. To do this, the following commands are executed:

CalcAtom ‘H’

CalcAtom ‘HN’

CalcSecondary

XMacStand ribbon.mac

### Prompt examples

To apply the ribbon style to the PDB file loaded, type:

```
relax> molmol.ribbon()
```

## 17.2.88 molmol.tensor\_pdb



### Synopsis

Display the diffusion tensor PDB geometric object over the loaded PDB.

### Defaults

`molmol.tensor_pdb(file=None)`

### Keyword arguments

`file`: The name of the PDB file containing the tensor geometric object.

### Description

In executing this user function, a PDB file must have previously been loaded, a geometric object or polygon representing the Brownian rotational diffusion tensor will be overlain with the loaded PDB file and displayed within Molmol. The PDB file containing the geometric object must be created using the complementary `structure.create_diff_tensor_pdb` user function.

To display the diffusion tensor, the multiple commands will be executed. To overlay the structure with the diffusion tensor, everything will be selected and reoriented and moved to their original PDB frame positions:

```
SelectAtom ''
SelectBond ''
SelectAngle ''
SelectDist ''
SelectPrim ''
RotateInit
MoveInit
```

Next the tensor PDB file is read in, selected, and the covalent bonds of the PDB CONECT records calculated:

```
ReadPdb file
SelectMol '@file'
CalcBond 1 1 1
```

Then only the atoms and bonds of the geometric object are selected and the 'ball/stick' style applied:

```
SelectAtom 'O'
SelectBond 'O'
SelectAtom ':TNS'
SelectBond ':TNS'
XMacStand ball_stick.mac
```

The appearance is finally touched up:

```
RadiusAtom 1
SelectAtom ':TNS@C*'
RadiusAtom 1.5
```

---

### 17.2.89 molmol.view



#### Synopsis

View the collection of molecules from the loaded PDB file.

#### Defaults

`molmol.view()`

#### Description

This will simply launch Molmol.

#### Prompt examples

```
relax> molmol.view()
```

---

### 17.2.90 monte\_carlo.create\_data



#### Synopsis

Create the Monte Carlo simulation data.

#### Defaults

`monte_carlo.create_data(method='back_calc',  
distribution='measured', fixed_error=None)`

#### Keyword arguments

`method`: The simulation method.

`distribution`: The error distribution method.

`fixed_error`: The fixed value to use when distribution is set to 'fixed'.

#### Description

The method can either be set to back calculation (Monte Carlo) or direct (bootstrapping), the choice of which determines the simulation type. If the values or parameters are calculated rather than minimised, this option will have no effect. Errors should only be propagated via Monte Carlo simulations if errors have been measured.

For error analysis, the method should be set to back calculation which will result in proper Monte Carlo simulations. The data used for each simulation is back calculated from the minimised model parameters and is randomised using Gaussian noise where the standard deviation is from the original error set. When the method is set to back calculation, this function should only be called after the model is fully minimised.

The simulation type can be changed by setting the method to direct. This will result in bootstrapping simulations which cannot be used in error analysis (and which are no longer Monte Carlo simulations). However, these simulations are required for certain model selection techniques (see the documentation for the model selection user function for details), and can be used for other purposes. Rather than the data being back calculated from the fitted model parameters, the data is generated by taking the original data and randomising using Gaussian noise with the standard deviations set to the original error set.

The errors generated per simulation can either be generated individual per datapoint and drawn from a gauss distribution described by the standard deviation of the individual point, or it can be generated from an overall gauss distribution described by the standard deviation of the goodness of fit, where  $SD_{fit} = \sqrt{\chi^2/(N-p)}$ . The



last possibility is to supply a fixed value of the standard deviation, from which gauss distribution to draw errors from.

## Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.
- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).
- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.
- 7 – Failed simulations are removed using the techniques of model elimination.
- 8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
# Step 2.

relax> minimise.execute('newton')
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc')
# Step 4.

relax> monte_carlo.initial_values()
# Step 5.

relax> minimise.execute('newton')
# Step 6.

relax> eliminate()
# Step 7.

relax> monte_carlo.error_analysis()
# Step 8.

An example for reduced spectral density mapping is:

relax> minimise.calculate()
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc')
# Step 4.

relax> minimise.calculate()
# Step 6.

relax> monte_carlo.error_analysis()
# Step 8.
```

### 17.2.91 monte\_carlo.error\_analysis



#### Synopsis

Calculate parameter errors from the Monte Carlo simulations.

#### Defaults

`monte_carlo.error_analysis()`

#### Description

Parameter errors are calculated as the standard deviation of the distribution of parameter values. This function should never be used if parameter values are obtained by minimisation and the simulation data are generated using the method 'direct'. The reason is because only true Monte Carlo simulations can give the true parameter errors.

#### Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.
- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).

- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.
- 7 – Failed simulations are removed using the techniques of model elimination.
- 8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
# Step 2.

relax> minimise.execute('newton')
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc')
# Step 4.

relax> monte_carlo.initial_values()
# Step 5.

relax> minimise.execute('newton')
# Step 6.

relax> eliminate()
# Step 7.

relax> monte_carlo.error_analysis()
# Step 8.
```

An example for reduced spectral density mapping is:

```
relax> minimise.calculate()
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc')
# Step 4.

relax> minimise.calculate()
# Step 6.

relax> monte_carlo.error_analysis()
# Step 8.
```

## 17.2.92 monte\_carlo.initial\_values



### Synopsis

Set the initial simulation parameter values.

### Defaults

`monte_carlo.initial_values()`

### Description

This only effects where minimisation occurs and can therefore be skipped if the values or parameters are calculated rather than minimised. However, if accidentally run in this case, the results will be unaffected. It should only be called after the model or run is fully minimised. Once called, the user functions `minimise.grid_search` and `minimise.execute` will only effect the simulations and not the model parameters.

The initial values of the parameters for each simulation is set to the minimised parameters of the model. A grid search can be undertaken for each simulation instead, although this is computationally expensive and unnecessary. The minimisation function should be executed for a second time after running this function.

### Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.

- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).
- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.
- 7 – Failed simulations are removed using the techniques of model elimination.
- 8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
# Step 2.

relax> minimise.execute('newton')
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc')
# Step 4.

relax> monte_carlo.initial_values()
# Step 5.

relax> minimise.execute('newton')
# Step 6.

relax> eliminate()
# Step 7.

relax> monte_carlo.error_analysis()
# Step 8.
```

An example for reduced spectral density mapping is:

```
relax> minimise.calculate()
# Step 2.
```

```

relax> monte_carlo.setup(number=500)
                                # Step 3.

relax> monte_carlo.create_data(method='
    back_calc')                  # Step 4.

relax> minimise.calculate()
                                # Step 6.

relax> monte_carlo.error_analysis()
                                # Step 8.

```

---

## 17.2.93 monte\_carlo.off



### Synopsis

Turn the Monte Carlo simulations off.

### Defaults

**monte\_carlo.off()**

### Description

This will turn off the Monte Carlo simulations so that subsequent optimisation will operate directly on the model parameters and not on the simulations.

### Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.
- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).
- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.

7 – Failed simulations are removed using the techniques of model elimination.

8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
# Step 2.

relax> minimise.execute('newton')
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc') # Step 4.

relax> monte_carlo.initial_values()
# Step 5.

relax> minimise.execute('newton')
# Step 6.

relax> eliminate()
# Step 7.

relax> monte_carlo.error_analysis()
# Step 8.
```

An example for reduced spectral density mapping is:

```
relax> minimise.calculate()
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc') # Step 4.

relax> minimise.calculate()
# Step 6.

relax> monte_carlo.error_analysis()
# Step 8.
```

## 17.2.94 monte\_carlo.on



### Synopsis

Turn the Monte Carlo simulations on.

### Defaults

**monte\_carlo.on()**

### Description

This will turn on the Monte Carlo simulations so that subsequent optimisation will operate on the simulations rather than on the real model parameters.

### Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.
- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).
- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.

- 7 – Failed simulations are removed using the techniques of model elimination.
- 8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
           # Step 2.

relax> minimise.execute('newton')
           # Step 2.

relax> monte_carlo.setup(number=500)
           # Step 3.

relax> monte_carlo.create_data(method='
back_calc')           # Step 4.

relax> monte_carlo.initial_values()
           # Step 5.

relax> minimise.execute('newton')
           # Step 6.

relax> eliminate()
           # Step 7.

relax> monte_carlo.error_analysis()
           # Step 8.
```

An example for reduced spectral density mapping is:

```
relax> minimise.calculate()
           # Step 2.

relax> monte_carlo.setup(number=500)
           # Step 3.

relax> monte_carlo.create_data(method='
back_calc')           # Step 4.

relax> minimise.calculate()
           # Step 6.

relax> monte_carlo.error_analysis()
           # Step 8.
```

## 17.2.95 monte\_carlo.setup



### Synopsis

Set up the Monte Carlo simulations.

### Defaults

**monte\_carlo.setup**(number=500)

### Keyword arguments

number: The number of Monte Carlo simulations.

### Description

This must be called prior to any of the other Monte Carlo functions. The effect is that the number of simulations will be set and that simulations will be turned on.

### Monte Carlo Simulation Overview

For proper error analysis using Monte Carlo simulations, a sequence of function calls is required for running the various simulation components. The steps necessary for implementing Monte Carlo simulations are:

- 1 – The measured data set together with the corresponding error set should be loaded into relax.
- 2 – Either minimisation is used to optimise the parameters of the chosen model, or a calculation is run.
- 3 – To initialise and turn on Monte Carlo simulations, the number of simulations,  $n$ , needs to be set.
- 4 – The simulation data needs to be created either by back calculation from the fully minimised model parameters from step 2 or by direct calculation when values are calculated rather than minimised. The error set is used to randomise each simulation data set by assuming Gaussian errors. This creates a synthetic data set for each Monte Carlo simulation.
- 5 – Prior to minimisation of the parameters of each simulation, initial parameter estimates are required. These are taken as the optimised model parameters. An alternative is to use a grid search for each simulation to generate initial estimates, however this is extremely computationally expensive. For the case where values are calculated rather than minimised, this step should be skipped (although the results will be unaffected if this is accidentally run).

- 6 – Each simulation requires minimisation or calculation. The same techniques as used in step 2, excluding the grid search when minimising, should be used for the simulations.
- 7 – Failed simulations are removed using the techniques of model elimination.
- 8 – The model parameter errors are calculated from the distribution of simulation parameters.

Monte Carlo simulations can be turned on or off using functions within this class. Once the function for setting up simulations has been called, simulations will be turned on. The effect of having simulations turned on is that the functions used for minimisation (grid search, minimise, etc) or calculation will only affect the simulation parameters and not the model parameters. By subsequently turning simulations off using the appropriate function, the functions used in minimisation will affect the model parameters and not the simulation parameters.

An example for model-free analysis using the prompt UI mode which includes only the functions required for implementing the above steps is:

```
relax> minimise.grid_search(inc=11)
# Step 2.

relax> minimise.execute('newton')
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc') # Step 4.

relax> monte_carlo.initial_values()
# Step 5.

relax> minimise.execute('newton')
# Step 6.

relax> eliminate()
# Step 7.

relax> monte_carlo.error_analysis()
# Step 8.
```

An example for reduced spectral density mapping is:

```
relax> minimise.calculate()
# Step 2.

relax> monte_carlo.setup(number=500)
# Step 3.

relax> monte_carlo.create_data(method='
back_calc') # Step 4.

relax> minimise.calculate()
# Step 6.

relax> monte_carlo.error_analysis()
# Step 8.
```

## 17.2.96 n\_state\_model.CoM



### Synopsis

The defunct centre of mass (CoM) analysis.

### Defaults

**n\_state\_model.CoM**(pivot\_point=[0.0, 0.0, 0.0], centre=None)

### Keyword arguments

**pivot\_point**: The pivot point of the motions between the two domains.

**centre**: Manually specify the CoM of the initial position prior to the N rotations to the positions of the N states. This is optional.

### Description

WARNING: This analysis is now defunct!

This is used for analysing the domain motion information content of the N states from the N-state model. The states do not correspond to physical states, hence nothing can be extracted from the individual states. This analysis involves the calculation of the pivot to centre of mass (pivot-CoM) order parameter and subsequent cone of motions.

For the analysis, both the pivot point and centre of mass must be specified. The supplied pivot point must be a vector of floating point numbers of length 3. If the centre of mass is supplied, it must also be a vector of floating point numbers (of length 3). If the centre of mass is not supplied, then the CoM will be calculated from the selected parts of a previously loaded structure.

### Prompt examples

To perform an analysis where the pivot is at the origin and the CoM is set to the N-terminal domain of a previously loaded PDB file (the C-terminal domain has been deselected), type:

```
relax> n_state_model.CoM()
```

To perform an analysis where the pivot is at the origin (because the real pivot has been shifted to this position) and the CoM is at the position [0, 0, 1], type one of:

```
relax> n_state_model.CoM(centre=[0, 0, 1])
```



```
relax> n_state_model.CoM(centre=[0.0, 0.0, 1
    .0])
```

```
relax> n_state_model.CoM(pivot_point=[0.0, 0
    .0, 0.0], centre=[0.0, 0.0, 1.0])
```

## 17.2.97 n\_state\_model.cone\_pdb



### Synopsis

Create a PDB file representing the cone models from the centre of mass (CoM) analysis.

### Defaults

**n\_state\_model.cone\_pdb**(cone\_type=None, scale=1.0, file='cone.pdb', dir=None, force=False)

### Keyword arguments

**cone\_type**: The type of cone model to represent.

**scale**: Value for scaling the pivot-CoM distance which the size of the cone defaults to.

**file**: The name of the PDB file.

**dir**: The directory where the file is located.

**force**: A flag which, if set to True, will overwrite the any pre-existing file.

### Description

WARNING: This analysis is now defunct!

This creates a PDB file containing an artificial geometric structure to represent the various cone models. These models include:

'diff in cone'

'diff on cone'

The model can be selected by setting the cone type to one of these values. The cone is represented as an isotropic cone with its axis parallel to the average pivot-CoM vector, the vertex placed at the pivot point of the domain motions, and the length of the edge of the cone equal to the pivot-CoM distance multiplied by the scaling factor. The resultant PDB file can subsequently read into any molecular viewer.

There are four different types of residue within the PDB. The pivot point is represented as a single carbon atom of the residue 'PIV'. The cone consists of numerous H atoms of the residue 'CON'. The average pivot-CoM vector is presented as the residue 'AVE' with one carbon atom positioned at the pivot and the other at the head of the



vector (after scaling by the scaling factor). Finally, if Monte Carlo have been performed, there will be multiple 'MCC' residues representing the cone for each simulation, and multiple 'MCA' residues representing the varying average pivot-CoM vector for each simulation.

To create the diffusion in a cone PDB representation, a uniform distribution of vectors on a sphere is generated using spherical coordinates with the polar angle defined from the average pivot-CoM vector. By incrementing the polar angle using an arccos distribution, a radial array of vectors representing latitude are created while incrementing the azimuthal angle evenly creates the longitudinal vectors. These are all placed into the PDB file as H atoms and are all connected using PDB CONECT records. Each H atom is connected to its two neighbours on the both the longitude and latitude. This creates a geometric PDB object with longitudinal and latitudinal lines representing the filled cone.

---

## 17.2.98 n\_state\_model.elim\_no\_prob



### Synopsis

Eliminate the structures or states with no probability.

### Defaults

`n_state_model.elim_no_prob()`

### Description

This will simply remove the structures from the N-state analysis which have an optimised probability of zero.

### Prompt examples

Simply type:

```
relax> n_state_model.elim_no_prob(N=8)
```

---

**17.2.99** `n_state_model.number_of_states`**Synopsis**

Set the number of states in the N-state model.

**Defaults**

`n_state_model.number_of_states(N=1)`

**Keyword arguments**

`N`: The number of states.

**Description**

Prior to optimisation, the number of states in the N-state model can be specified. If the number of states is not set, then this parameter will be equal to the number of loaded structures - the ensemble size.

**Prompt examples**

To set up an 8-state model, type:

```
relax> n_state_model.number_of_states(N=8)
```

---

**17.2.100** `n_state_model.ref_domain`**Synopsis**

Set the reference domain for the '2-domain' N-state model.

**Defaults**

`n_state_model.ref_domain(ref=None)`

**Keyword arguments**

`ref`: The domain which will act as the frame of reference. This is only valid for the '2-domain' N-state model.

**Description**

Prior to optimisation of the '2-domain' N-state model, which of the two domains will act as the frame of reference must be specified. The N-states will be rotations of the other domain, so to switch the frame of reference to the other domain simply transpose the rotation matrices.

**Prompt examples**

To set up a 5-state model with 'C' domain being the frame of reference, type:

```
relax> n_state_model.ref_domain(ref='C')
```

### 17.2.101 `n_state_model.select_model`



#### Synopsis

Select the N-state model type and set up the model.

#### Defaults

```
n_state_model.select_model(model='population')
```

#### Keyword arguments

`model`: The name of the preset N-state model.

#### Description

Prior to optimisation, the N-state model type should be selected. The preset models are:

**‘population’** – The N-state model whereby only populations are optimised. The structures loaded into relax are assumed to be fixed, *i.e.* the orientations are not optimised, or if two domains are present the Euler angles for each state are fixed. The parameters of the model include the weight or probability for each state and the alignment tensors - {p0, p1, ..., pN, Axx, Ayy, Axy, Axz, Ayz, ...}.

**‘fixed’** – The N-state model whereby all motions are fixed and all populations are fixed to the set probabilities. The parameters of the model are simply the parameters of each alignment tensor {Axx, Ayy, Axy, Axz, Ayz, ...}.

**‘2-domain’** – The N-state model for a system of two domains, where one domain experiences a reduced tensor.

#### Prompt examples

To analyse populations of states, type:

```
relax> n_state_model.select_model(model='populations')
```

### 17.2.102 `noe.read_restraints`



#### Synopsis

Read NOESY or ROESY restraints from a file.

#### Defaults

```
noe.read_restraints(file=None, dir=None, proton1_col=None, proton2_col=None, lower_col=None, upper_col=None, sep=None)
```

#### Keyword arguments

`file`: The name of the file containing the restraint data.

`dir`: The directory where the file is located.

`proton1_col`: The column containing the first proton of the NOE or ROE cross peak.

`proton2_col`: The column containing the second proton of the NOE or ROE cross peak.

`lower_col`: The column containing the lower NOE bound.

`upper_col`: The column containing the upper NOE bound.

`sep`: The column separator (the default is white space).

#### Description

The format of the file will be automatically determined, for example Xplor formatted restraint files. A generically formatted file is also supported if it contains minimally four columns with the two proton names and the upper and lower bounds, as specified by the column numbers. The proton names need to be in the spin ID string format.

#### Prompt examples

To read the Xplor formatted restraint file ‘NOE.xpl’, type one of:

```
relax> noe.read_restraints('NOE.xpl')
```

```
relax> noe.read_restraints(file='NOE.xpl')
```

To read the generic formatted file ‘noes’, type one of:

```
relax> noe.read_restraints(file='NOE.xpl', proton1_col=0, proton2_col=1, lower_col=2, upper_col=3)
```

17.2.103 `noe.spectrum_type`

## Synopsis

Set the steady-state NOE spectrum type for pre-loaded peak intensities.

## Defaults

**noe.spectrum\_type**(spectrum\_type=None, spectrum\_id=None)

## Keyword arguments

**spectrum\_type**: The type of steady-state NOE spectrum, one of 'ref' for the reference spectrum or 'sat' for the saturated spectrum.

**spectrum\_id**: The spectrum ID string.

## Description

The spectrum type can be one of the following:

The steady-state NOE reference spectrum.

The steady-state NOE spectrum with proton saturation turned on.

Peak intensities should be loaded before this user function via the `spectrum.read_intensities` user function. The intensity values will then be associated with a spectrum ID string which can be used here.

17.2.104 `palmer.create`

## Synopsis

Create the Modelfree4 input files.

## Defaults

**palmer.create**(dir=None, force=False, binary='modelfree4', diff\_search='none', sims=0, sim\_type='pred', trim=0, steps=20, constraints=True, heteronuc\_type='15N', atom1='N', atom2='H', spin\_id=None)

## Keyword arguments

**dir**: The directory to place the files.

**force**: A flag which if set to True will cause the results file to be overwritten if it already exists.

**binary**: The name of the executable Modelfree program file.

**diff\_search**: See the Modelfree4 manual for 'diffusion\_search'.

**sims**: The number of Monte Carlo simulations.

**sim\_type**: See the Modelfree4 manual.

**trim**: See the Modelfree4 manual.

**steps**: See the Modelfree4 manual.

**constraints**: A flag specifying whether the parameters should be constrained. The default is to turn constraints on (constraints=True).

**heteronuc\_type**: A three letter string describing the heteronucleus type, ie '15N', '13C', etc.

**atom1**: The symbol of the X heteronucleus in the PDB file.

**atom2**: The symbol of the H nucleus in the PDB file.

**spin\_id**: The spin identification string.

## Description

The following files are created

'dir/mfin'

'dir/mfdata'

```
'dir/mfpar'
'dir/mfmodel'
'dir/run.sh'
```

The file 'dir/run.sh' contains the single command,

```
'modelfree4 -i mfin -d mfddata -p mfpar -m
mfmodel -o mfout -e out',
```

which can be used to execute modelfree4.

If you would like to use a different Modelfree executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, include the full path in front of the binary file name.

---

## 17.2.105 palmer.execute



### Synopsis

Perform a model-free optimisation using Modelfree4.

### Defaults

**palmer.execute**(dir=None, force=False, binary='modelfree4')

### Keyword arguments

**dir**: The directory to place the files.

**force**: A flag which if set to True will cause the results file to be overwritten if it already exists.

**binary**: The name of the executable Modelfree program file.

### Description

Modelfree 4 will be executed as

```
$ modelfree4 -i mfin -d mfddata -p mfpar -m
mfmodel -o mfout -e out
```

If a PDB file is loaded and non-isotropic diffusion is selected, then the file name will be placed on the command line as '**-s pdb\_file\_name**'.

If you would like to use a different Modelfree executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, include the full path in front of the binary file name.

---

**17.2.106 palmer.extract****Synopsis**

Extract data from the Modelfree4 ‘mfout’ star formatted file.

**Defaults**

**palmer.extract**(dir=None)

**Keyword arguments**

**dir**: The directory where the file ‘mfout’ is found.

**Description**

The model-free results will be extracted from the Modelfree4 results file ‘mfout’ located in the given directory.

---

**17.2.107 paramag.centre****Synopsis**

Specify which atom is the paramagnetic centre.

**Defaults**

**paramag.centre**(pos=None, atom\_id=None, pipe=None, verbosity=1, fix=True, ave\_pos=True, force=False)

**Keyword arguments**

**pos**: The atomic position of the paramagnetic centre.

**atom\_id**: The atom ID string.

**pipe**: The data pipe containing the structures to extract the centre from.

**verbosity**: The amount of information to print out.

**fix**: A flag specifying if the paramagnetic centre should be fixed during optimisation.

**ave\_pos**: A flag specifying if the position of the atom is to be averaged across all models.

**force**: A flag which if True will cause the current paramagnetic centre to be overwritten.

**Description**

This is required for specifying where the paramagnetic centre is located in the loaded structure file. If no structure number is given, then the average atom position will be calculated if multiple structures are loaded.

A different set of structures than those loaded into the current data pipe can also be used to determine the position, or its average. This can be achieved by loading the alternative structures into another data pipe, and then specifying that pipe.

If the average position flag is set to True, the average position from all models will be used as the position of the paramagnetic centre. If False, then the positions from all structures will be used. If multiple positions are used, then a fast paramagnetic centre motion will be assumed so that PCSs for a single tensor will be calculated for each position, and the PCS values linearly averaged.

## Prompt examples

If the paramagnetic centre is the lanthanide Dysprosium which is labelled as  $\text{Dy}$  in a loaded PDB file, then type one of:

```
relax> paramag.centre('Dy')
```

```
relax> paramag.centre(atom_id='Dy')
```

If the carbon atom 'C1' of residue '4' in the PDB file is to be used as the paramagnetic centre, then type:

```
relax> paramag.centre(':4@C1')
```

To state that the  $\text{Dy}^{3+}$  atomic position is [0.136, 12.543, 4.356], type one of:

```
relax> paramag.centre([0.136, 12.543, 4.356  
    ])
```

```
relax> paramag.centre(pos=[0.136, 12.543, 4  
    .356])
```

To find an unknown paramagnetic centre, type:

```
relax> paramag.centre(fix=False)
```

---

### 17.2.108 pcs.back\_calc



## Synopsis

Back calculate the pseudo-contact shifts.

## Defaults

`pcs.back_calc(align_id=None)`

## Keyword arguments

`align_id`: The alignment ID string.

## Description

This will back calculate the pseudo-contact shifts if the paramagnetic centre, temperature and magnetic field strength has been specified, an alignment tensor is present, and atomic positions have been loaded into the relax data store.

---

17.2.109 pcs.calc\_q\_factors

## Synopsis

Calculate the PCS Q factor for the selected spins.

## Defaults

`pcs.calc_q_factors(spin_id=None, verbosity=1)`

## Keyword arguments

**spin\_id:** The spin ID string for restricting to subset of all selected spins.

**verbosity:** The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

## Description

For this to work, the back-calculated PCS data must first be generated by the analysis specific code. Otherwise a warning will be given.

## Prompt examples

To calculate the PCS Q factor for only the spins '@H26', '@H27', and '@H28', type one of:

```
relax> pcs.calc_q_factors('@H26 & @H27 &
                           @H28')
```

```
relax> pcs.calc_q_factors(spin_id='@H26 &
                           @H27 & @H28')
```

## 17.2.110 pcs.copy



## Synopsis

Copy PCS data from one data pipe to another.

## Defaults

`pcs.copy(pipe_from=None, pipe_to=None, align_id=None, back_calc=True)`

## Keyword arguments

**pipe\_from:** The name of the pipe to copy the PCS data from.

**pipe\_to:** The name of the pipe to copy the PCS data to.

**align\_id:** The alignment ID string.

**back\_calc:** A flag which if True will cause any back-calculated PCSs present to also be copied with the real values and errors.

## Description

This function will copy PCS data from 'pipe\_from' to 'pipe\_to'. If align\_id is not given then all PCS data will be copied, otherwise only a specific data set will be.

## Prompt examples

To copy all PCS data from pipe 'm1' to pipe 'm9', type one of:

```
relax> pcs.copy('m1', 'm9')
```

```
relax> pcs.copy(pipe_from='m1', pipe_to='m9'
                 )
```

```
relax> pcs.copy('m1', 'm9', None)
```

```
relax> pcs.copy(pipe_from='m1', pipe_to='m9'
                 , align_id=None)
```

To copy only the 'Th' PCS data from 'm3' to 'm6', type one of:

```
relax> pcs.copy('m3', 'm6', 'Th')
```

```
relax> pcs.copy(pipe_from='m3', pipe_to='m6'
                 , align_id='Th')
```



**17.2.111 pcs.corr\_plot****Synopsis**

Generate a correlation plot of the measured vs. the back-calculated PCSs.

**Defaults**

```
pcs.corr_plot(format='grace', title=None, subtitle=None,
file='pcs_corr_plot.agr', dir=None, force=False)
```

**Keyword arguments**

**format:** The format of the plot data.

**title:** The title for the plot, overriding the default.

**subtitle:** The subtitle for the plot, overriding the default.

**file:** The name of the Grace file to create.

**dir:** The directory name.

**force:** A flag which if True will cause the file to be overwritten.

**Description**

Two formats are currently supported. If format is set to 'grace', then a Grace plot file will be created. If the format is not set then a plain text list of the measured and back-calculated data will be created.

**Prompt examples**

To create a Grace plot of the data, type:

```
relax> pcs.corr_plot()
```

To create a plain text list of the measured and back-calculated data, type one of:

```
relax> pcs.corr_plot(None)
```

```
relax> pcs.corr_plot(format=None)
```

**17.2.112 pcs.delete****Synopsis**

Delete the PCS data corresponding to the alignment ID.

**Defaults**

```
pcs.delete(align_id=None)
```

**Keyword arguments**

**align\_id:** The alignment ID string of the data to delete.

**Description**

This will delete all PCS data associated with the alignment ID in the current data pipe.

**Prompt examples**

To delete the PCS data corresponding to align\_id='PH\_gel', type:

```
relax> pcs.delete('PH_gel')
```

---

**17.2.113 pcs.display****Synopsis**

Display the PCS data corresponding to the alignment ID.

**Defaults**

`pcs.display(align_id=None, bc=False)`

**Keyword arguments**

`align_id`: The alignment ID string.

`bc`: A flag which if set will display the back-calculated rather than measured RDCs.

**Description**

This will display all of the PCS data associated with the alignment ID in the current data pipe.

**Prompt examples**

To display the ‘phage’ PCS data, type:

```
relax> pcs.display('phage')
```

---

**17.2.114 pcs.read****Synopsis**

Read the PCS data from file.

**Defaults**

`pcs.read(align_id=None, file=None, dir=None, spin_id_col=None, mol_name_col=None, res_num_col=None, res_name_col=None, spin_num_col=None, spin_name_col=None, data_col=None, error_col=None, sep=None, spin_id=None)`

**Keyword arguments**

`align_id`: The alignment ID string.

`file`: The name of the file containing the PCS data.

`dir`: The directory where the file is located.

`spin_id_col`: The spin ID string column (an alternative to the mol, res, and spin name and number columns).

`mol_name_col`: The molecule name column (alternative to the spin\_id\_col).

`res_num_col`: The residue number column (alternative to the spin\_id\_col).

`res_name_col`: The residue name column (alternative to the spin\_id\_col).

`spin_num_col`: The spin number column (alternative to the spin\_id\_col).

`spin_name_col`: The spin name column (alternative to the spin\_id\_col).

`data_col`: The PCS data column.

`error_col`: The experimental error column.

`sep`: The column separator (the default is white space).

`spin_id`: The spin ID string to restrict the loading of data to certain spin subsets.

**Description**

This will read PCS data from a file and associate it with an alignment ID, either a new ID or a preexisting one with no PCS data.

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number and name, and spin number and name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID can be used to restrict the reading to certain spin types, for example only  $^{15}\text{N}$  spins when only residue information is in the file.

### Prompt examples

The following commands will read the PCS data out of the file 'Tb.txt' where the columns are separated by the symbol ',', and store the PCSs under the ID 'Tb'.

```
relax> pcs.read('Tb', 'Tb.txt', sep=',')
```

To read the  $^{15}\text{N}$  and  $^1\text{H}$  PCSs from the file 'Eu.txt', where the  $^{15}\text{N}$  values are in the 4<sup>th</sup> column and the  $^1\text{H}$  in the 9<sup>th</sup>, type both the following:

```
relax> pcs.read('Tb', 'Tb.txt', spin_id='@N',  
               , res_num_col=1, data_col=4)
```

```
relax> pcs.read('Tb', 'Tb.txt', spin_id='@H',  
               , res_num_col=1, data_col=9)
```

---

## 17.2.115 pcs.set\_errors



### Synopsis

Set the errors for the PCSs.

### Defaults

```
pcs.set_errors(aligned_id=None, spin_id=None, sd=0.1)
```

### Keyword arguments

`align_id`: The optional alignment ID string.

`spin_id`: The optional spin ID string.

`sd`: The PCS standard deviation value in ppm.

### Description

If the PCS errors have not already been read from a PCS data file or if they need to be changed, then the errors can be set via this user function.

### 17.2.116 pcs.structural\_noise



#### Synopsis

Determine the PCS error due to structural noise via simulation.

#### Defaults

**pcs.structural\_noise**(align\_id=None, rmsd=0.2, sim\_num=1000, file=None, dir=None, force=False)

#### Keyword arguments

**align\_id**: The optional alignment ID string.

**rmsd**: The atomic position RMSD, in Å, to randomise the spin positions with for the simulations.

**sim\_num**: The number of simulations, N, to perform to determine the structural noise component of the PCS errors.

**file**: The optional name of the Grace file to plot the structural errors verses the paramagnetic centre to spin distances.

**dir**: The directory name to place the Grace file into.

**force**: A flag which if True will cause the file to be overwritten.

#### Description

The analysis of the pseudo-contact shift is influenced by two significant sources of noise - that of the NMR experiment and structural noise from the 3D molecular structure used. The closer the spin to the paramagnetic centre, the greater the influence of structural noise. This distance dependence is governed by the equation:

$$\text{sigma\_dist} = \frac{\sqrt{3} * \text{abs}(\text{delta}) * \text{RMSD}}{r},$$

where sigma\_dist is the distance component of the structural noise as a standard deviation, delta is the PCS value, RMSD is the atomic position root-mean-square deviation, and  $r$  is the paramagnetic centre to spin distance. When close to the paramagnetic centre, this error source

can exceed that of the NMR experiment. The equation for the angular component of the structural noise is more complicated. The PCS error is influenced by distance, angle in the alignment frame, and the magnetic susceptibility tensor.

For the simulation the following must already be set up in the current data pipe:

The position of the paramagnetic centre.

The alignment and magnetic susceptibility tensor.

The protocol for the simulation is as follows:

The lanthanide or paramagnetic centre position will be fixed. Its motion is assumed to be on the femto- to pico- and nanosecond timescales. Hence the motion is averaged over the evolution of the PCS and can be ignored.

The positions of the nuclear spins will be randomised N times. For each simulation a random unit vector will be generated. Then a random distance along the unit vector will be generated by sampling from a Gaussian distribution centered at zero, the original spin position, with a standard deviation set to the given RMSD. Both positive and negative displacements will be used.

The PCS for the randomised position will be back calculated.

The PCS standard deviation will be calculated from the N randomised PCS values.

The standard deviation will both be stored in the spin container data structure in the relax data store as well as being added to the already present PCS error (using variance addition). This will then be used in any optimisations involving the PCS.

If the alignment ID string is not supplied, the procedure will be applied to the PCS data from all alignments.

---

**17.2.117 pcs.weight****Synopsis**

Set optimisation weights on the PCS data.

**Defaults**

**pcs.weight**(align\_id=None, spin\_id=None, weight=1.0)

**Keyword arguments**

**align\_id**: The alignment ID string.

**spin\_id**: The spin ID string.

**weight**: The weighting value.

**Description**

This can be used to force the PCS to contribute more or less to the chi-squared optimisation statistic. The higher the value, the more importance the PCS will have.

**17.2.118 pcs.write****Synopsis**

Write the PCS data to file.

**Defaults**

**pcs.write**(align\_id=None, file=None, dir=None, bc=False, force=False)

**Keyword arguments**

**align\_id**: The alignment ID string.

**file**: The name of the file.

**dir**: The directory name.

**bc**: A flag which if set will write out the back-calculated rather than measured RDCs.

**force**: A flag which if True will cause the file to be overwritten.

**Description**

If no directory name is given, the file will be placed in the current working directory. The alignment ID is required for selecting which PCS data set will be written to file.

---

**17.2.119 pipe.bundle****Synopsis**

The grouping of data pipes into a bundle.

**Defaults**

`pipe.bundle(bundle=None, pipe=None)`

**Keyword arguments**

**bundle:** The pipe bundle is a special grouping or clustering of data pipes.

**pipe:** The name of the data pipe to add to the bundle.

**Description**

Data pipes can be grouped or clustered together through special structures known as pipe bundles. If the specified data pipe bundle does not currently exist, it will be created.

**Prompt examples**

To add the data pipes ‘test 1’, ‘test 2’, and ‘test 3’ to the bundle ‘first analysis’, type the following:

```
relax> pipe.bundle('first analysis 1', 'test 1')
```

```
relax> pipe.bundle('first analysis 1', 'test 2')
```

```
relax> pipe.bundle('first analysis 1', 'test 3')
```

---

**17.2.120 pipe.change\_type****Synopsis**

Change the type of the current data pipe.

**Defaults**

`pipe.change_type(pipe_type=None)`

**Keyword arguments**

**pipe\_type:** The type of data pipe.

**Description**

The data pipe type must be one of the following:

‘ct’ – Consistency testing.

‘frame order’ – Frame Order theories.

‘jw’ – Reduced spectral density mapping.

‘hybrid’ – Special hybrid pipe.

‘mf’ – Model-free analysis.

‘N-state’ – N-state model or ensemble analysis.

‘noe’ – Steady state NOE calculation.

‘relax\_disp’ – Relaxation dispersion.

‘relax\_fit’ – Relaxation curve fitting.

**Prompt examples**

To change the type of the current ‘frame order’ data pipe to the N-state model, type one of:

```
relax> pipe.change_type('N-state')
```

```
relax> pipe.change_type(pipe_type='N-state')
```

---

**17.2.121 pipe.copy****Synopsis**

Copy a data pipe.

**Defaults**

**pipe.copy**(pipe\_from=None, pipe\_to=None, bundle\_to=None)

**Keyword arguments**

**pipe\_from**: The name of the source data pipe to copy the data from.

**pipe\_to**: The name of the target data pipe to copy the data to.

**bundle\_to**: If given, the new data pipe will be grouped into this bundle.

**Description**

This allows the contents of a data pipe to be copied. If the source data pipe is not set, the current data pipe will be assumed. The target data pipe must not yet exist.

The optional bundling allows the newly created data pipe to be placed into either a new or existing data pipe bundle. If not specified, then the copied data pipe will not be associated with a bundle.

**Prompt examples**

To copy the contents of the 'm1' data pipe to the 'm2' data pipe, type:

```
relax> pipe.copy('m1', 'm2')
```

```
relax> pipe.copy(pipe_from='m1', pipe_to='m2',
                '')
```

If the current data pipe is 'm1', then the following command can be used:

```
relax> pipe.copy(pipe_to='m2')
```

---

**17.2.122 pipe.create****Synopsis**

Add a new data pipe to the relax data store.

**Defaults**

**pipe.create**(pipe\_name=None, pipe\_type=None, bundle=None)

**Keyword arguments**

**pipe\_name**: The name of the data pipe.

**pipe\_type**: The type of data pipe.

**bundle**: The optional pipe bundle is a special grouping or clustering of data pipes. If this is specified, the newly created data pipe will be added to this bundle.

**Description**

The data pipe name can be any string however the data pipe type can only be one of the following:

'ct' – Consistency testing,

'frame order' – The Frame Order theories,

'jw' – Reduced spectral density mapping,

'hybrid' – A special hybrid pipe,

'mf' – Model-free analysis,

'N-state' – N-state model or ensemble analysis,

'noe' – Steady state NOE calculation,

'relax\_disp' – Relaxation dispersion curve fitting,

'relax\_fit' – Relaxation curve fitting,

The pipe bundling concept is simply a way of grouping data pipes together. This is useful for a number of purposes:

The grouping or categorisation of data pipes, for example when multiple related analyses are performed.

In the auto-analyses, as all the data pipes that they spawn are bound together within the original bundle.

In the graphical user interface mode as analysis tabs are linked to specific pipe bundles.

## Prompt examples

To set up a model-free analysis data pipe with the name 'm5', type:

```
relax> pipe.create('m5', 'mf')
```

---

### 17.2.123 pipe.current



## Synopsis

Print the name of the current data pipe.

## Defaults

`pipe.current()`

## Prompt examples

To run the user function, type:

```
relax> pipe.current()
```



---

**17.2.124 pipe.delete****Synopsis**

Delete a data pipe from the relax data store.

**Defaults**

`pipe.delete(pipe_name=None)`

**Keyword arguments**

`pipe_name`: The name of the data pipe to delete.

**Description**

This will permanently remove the data pipe and all of its contents from the relax data store. If the pipe name is not given, then all data pipes will be deleted.

**17.2.125 pipe.display****Synopsis**

Print a list of all the data pipes.

**Defaults**

`pipe.display()`

**Prompt examples**

To run the user function, type:

`relax> pipe.display()`

**17.2.126 pipe.hybridise****Synopsis**

Create a hybrid data pipe by fusing a number of other data pipes.

**Defaults**

`pipe.hybridise(hybrid=None, pipes=None)`

**Keyword arguments**

**hybrid:** The name of the hybrid data pipe to create.

**pipes:** An array containing the names of all data pipes to hybridise.

**Description**

This user function can be used to construct hybrid models. An example of the use of a hybrid model could be if the protein consists of two independent domains. These two domains could be analysed separately, each having their own optimised diffusion tensors. The N-terminal domain data pipe could be called 'N\_sphere' while the C-terminal domain could be called 'C\_ellipsoid'. These two data pipes could then be hybridised into a single data pipe. This hybrid data pipe can then be compared via model selection to a data pipe whereby the entire protein is assumed to have a single diffusion tensor.

The requirements for data pipes to be hybridised is that the molecules, sequences, and spin systems for all the data pipes is the same, and that no spin system is allowed to be selected in two or more data pipes. The selections must not overlap to allow for rigorous statistical comparisons.

**Prompt examples**

The two data pipes 'N\_sphere' and 'C\_ellipsoid' could be hybridised into a single data pipe called 'mixed model' by typing:

```
relax> pipe.hybridise('mixed model', ['
    N_sphere', 'C_ellipsoid'])
```

```
relax> pipe.hybridise(hybrid='mixed model',
    pipes=['N_sphere', 'C_ellipsoid'])
```

**17.2.127 pipe.switch****Synopsis**

Switch between the data pipes of the relax data store.

**Defaults**

`pipe.switch(pipe_name=None)`

**Keyword arguments**

**pipe\_name:** The name of the data pipe.

**Description**

This will switch between the various data pipes within the relax data store.

**Prompt examples**

To switch to the 'ellipsoid' data pipe, type:

```
relax> pipe.switch('ellipsoid')
```

```
relax> pipe.switch(pipe_name='ellipsoid')
```

---

**17.2.128**   `pymol.cartoon`**Synopsis**

Apply the PyMOL cartoon style and colour by secondary structure.

**Defaults**

`pymol.cartoon()`

**Description**

This applies the PyMOL cartoon style which is equivalent to hiding everything and clicking on show cartoon. It also colours the cartoon with red helices, yellow strands, and green loops. The following commands are executed:

```
cmd.hide('everything', file)
cmd.show('cartoon', file)
util.cbss(file, 'red', 'yellow', 'green')
```

where file is the file name without the '.pdb' extension.

**Prompt examples**

To apply this user function, type:

```
relax> pymol.cartoon()
```

---

**17.2.129**   `pymol.clear_history`**Synopsis**

Clear the PyMOL command history.

**Defaults**

`pymol.clear_history()`

**Description**

This will clear the Pymol history from memory.

---

**17.2.130 pymol.command****Synopsis**

Execute a user supplied PyMOL command.

**Defaults**

`pymol.command(command=None)`

**Keyword arguments**

`command`: The PyMOL command to execute.

**Description**

This allows PyMOL commands to be passed to the program. This can be useful for automation or scripting.

**Prompt examples**

To reinitialise the PyMOL instance, type:

```
relax> pymol.command("reinitialise")
```

**17.2.131 pymol.cone\_pdb****Synopsis**

Display the cone PDB geometric object.

**Defaults**

`pymol.cone_pdb(file=None)`

**Keyword arguments**

`file`: The name of the PDB file containing the cone geometric object.

**Description**

The PDB file containing the geometric object must be created using the complementary `n_state_model.cone_pdb` user function.

The cone PDB file is read in using the command:

```
load file
```

The average CoM-pivot point vector, the residue 'AVE' is displayed using the commands:

```
select resn AVE  
show sticks, 'sele'  
color blue, 'sele'
```

The cone object, the residue 'CON', is displayed using the commands:

```
select resn CON  
hide ('sele')  
show sticks, 'sele'  
color white, 'sele'
```

---

### 17.2.132 pymol.frame\_order



The user function will not only search for these files, but also all \*.gz and \*.bz2 versions of the average position and frame order representations. This is to support all output files from the frame\_order.pdb\_model user function.

#### Synopsis

Display the frame order results from the frame\_order.pdb\_model and frame\_order.simulate user functions.

#### Defaults

**pymol.frame\_order**(ave\_pos='ave\_pos', rep='frame\_order', sim='simulation.pdb.gz', dir=None)

#### Keyword arguments

**ave\_pos**: The file root of the 3D structure PDB file for the molecular structure with the moving domains shifted to the average position.

**rep**: The file root of the PDB file for the geometric object representation of the frame order dynamics.

**sim**: The full name the Brownian simulation PDB file.

**dir**: The directory where the files are located.

#### Description

This user function is designed to be combined with the frame\_order.pdb\_model and frame\_order.simulate user functions. It will take the two PDB representations created by frame\_order.pdb\_model, the molecular structure with the averaged domain positions and the frame order dynamics representation files, and the Brownian simulation PDB file and display them in PyMOL. Rather than loading the three representations into PyMOL manually, this user function will change the representation to improve visualisation.

For the PDB files, if the file roots are left to the defaults then the following files will be loaded:

**Average position** – The default is to load the 'ave\_pos.pdb' and 'ave\_pos\_sim.pdb' files.

**Frame order motional representation** – The is to load the 'frame\_order.pdb', 'frame\_order\_A.pdb', 'frame\_order\_B.pdb', 'frame\_order\_sim.pdb', 'frame\_order\_sim\_A.pdb' and 'frame\_order\_sim\_B.pdb' files, if present.

**Brownian simulation** – The default is to load the 'simulation.pdb.gz' file.

### 17.2.133 pymol.macro\_apply



#### Synopsis

Execute PyMOL macros.

#### Defaults

```
pymol.macro_apply(data_type=None, style='classic',
colour_start_name=None, colour_start_rgb=None,
colour_end_name=None, colour_end_rgb=None,
colour_list=None)
```

#### Keyword arguments

**data\_type:** The data type to map to the structure.

**style:** The style of the macro.

**colour\_start\_name:** The name of the starting colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the starting colour RGB colour array cannot be given.

**colour\_start\_rgb:** The starting colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the starting colour name cannot be given.

**colour\_end\_name:** The name of the ending colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the ending colour RGB colour array cannot be given.

**colour\_end\_rgb:** The ending colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the ending colour name cannot be given.

**colour\_list:** The colour list to search for the colour names. This can be either 'molmol' or 'x11'.

#### Description

This allows spin specific values to be mapped to a structure through PyMOL macros. Currently only the 'classic' style, which is described below, is available.

#### Colour

The values are coloured based on a linear colour gradient which is specified through starting and ending colours. These can either be a string to identify one of the RGB (red, green, blue) colour arrays listed in the tables below, or you can give the RGB vector itself. For example, 'white' and [1.0, 1.0, 1.0] both select the same colour. Leaving both colours unset will select the default colour gradient which for each type of analysis is described below.

When supplying the colours as strings, two lists of colours can be selected from which to match the strings. These are the default Molmol colour list and the X11 colour list, both of which are described in the tables below. The default behaviour is to first search the Molmol list and then the X11 colour list, raising an error if neither contain the name. To explicitly select these lists, set the colour list to either 'molmol' or 'x11'.

#### Model-free classic style

Creator: Edward d'Auvergne

Argument string: "classic"

Description: The classic style draws the backbone of a protein in a cylindrical bond style. Rather than colouring the amino acids to which the NH bond belongs, the three covalent bonds of the peptide bond from Ca to Ca in which the NH bond is located are coloured. Deselected residues are shown as black lines.

Supported data types:

Please see Table 17.22 on page 511.

#### Molmol RGB colour arrays

The following table is a list of colours used in Molmol and their corresponding RGB colour values ranging from 0 to 1.

Please see Table 17.23 on page 512.

#### X11 RGB colour arrays

The following table is the list of X11 colour names and their corresponding RGB colour values ranging from 0 to 255.

Please see Table 17.24 on page 513.

#### Prompt examples

To map the order parameter values,  $S^2$ , onto the structure using the classic style, type:

```
relax> pymol.macro_apply('s2')
```

```
relax> pymol.macro_apply(data_type='s2')  
  
relax> pymol.macro_apply(data_type='s2',  
                          style="classic")
```

---

### 17.2.134 pymol.macro\_run



#### Synopsis

Open and execute the PyMOL macro file.

#### Defaults

`pymol.macro_run(file=None, dir='pymol')`

#### Keyword arguments

`file`: The name of the PyMOL macro file.

`dir`: The directory name.

#### Description

This user function is for opening and running a PyMOL macro located within a text file.

#### Prompt examples

To execute the macro file 's2.pml' located in the directory 'pymol', type:

```
relax> pymol.macro_run(file='s2.pml')
```

```
relax> pymol.macro_run(file='s2.pml', dir='pymol')
```

### 17.2.135 pymol.macro\_write



#### Synopsis

Create PyMOL macros.

#### Defaults

```
pymol.macro_write(data_type=None, style='classic',
colour_start_name=None, colour_start_rgb=None,
colour_end_name=None, colour_end_rgb=None,
colour_list=None, file=None, dir='pymol', force=False)
```

#### Keyword arguments

**data\_type:** The data type to map to the structure.

**style:** The style of the macro.

**colour\_start\_name:** The name of the starting colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the starting colour RGB colour array cannot be given.

**colour\_start\_rgb:** The starting colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the starting colour name cannot be given.

**colour\_end\_name:** The name of the ending colour of the linear colour gradient. This can be either one of the X11 or one of the Molmol colour names listed in the description. If this is set, then the ending colour RGB colour array cannot be given.

**colour\_end\_rgb:** The ending colour of the linear colour gradient. This is an RGB colour array with values ranging from 0 to 1. If this is set, then the ending colour name cannot be given.

**colour\_list:** The colour list to search for the colour names. This can be either 'molmol' or 'x11'.

**file:** The optional name of the file.

**dir:** The optional directory to save the file to.

**force:** A flag which, if set to True, will cause the file to be overwritten.

#### Description

This allows residues specific values to be mapped to a structure through the creation of a PyMOL macro which can be executed in PyMOL by clicking on 'File, Macro, Execute User...'. Currently only the 'classic' style, which is described below, is available.

#### Colour

The values are coloured based on a linear colour gradient which is specified through starting and ending colours. These can either be a string to identify one of the RGB (red, green, blue) colour arrays listed in the tables below, or you can give the RGB vector itself. For example, 'white' and [1.0, 1.0, 1.0] both select the same colour. Leaving both colours unset will select the default colour gradient which for each type of analysis is described below.

When supplying the colours as strings, two lists of colours can be selected from which to match the strings. These are the default Molmol colour list and the X11 colour list, both of which are described in the tables below. The default behaviour is to first search the Molmol list and then the X11 colour list, raising an error if neither contain the name. To explicitly select these lists, set the colour list to either 'molmol' or 'x11'.

#### Model-free classic style

Creator: Edward d'Auvergne

Argument string: "classic"

Description: The classic style draws the backbone of a protein in a cylindrical bond style. Rather than colouring the amino acids to which the NH bond belongs, the three covalent bonds of the peptide bond from Ca to Ca in which the NH bond is located are coloured. Deselected residues are shown as black lines.

Supported data types:

Please see Table 17.22 on page 511.

#### Molmol RGB colour arrays

The following table is a list of colours used in Molmol and their corresponding RGB colour values ranging from 0 to 1.

Please see Table 17.23 on page 512.

#### X11 RGB colour arrays

The following table is the list of X11 colour names and their corresponding RGB colour values ranging from 0 to 255.

Please see Table 17.24 on page 513.

#### Prompt examples

To create a PyMOL macro mapping the order parameter values,  $S^2$ , onto the structure using the classic style, type:

```
relax> pymol.macro_write('s2')
```



```
relax> pymol.macro_write(data_type='s2')

relax> pymol.macro_write(data_type='s2',
    style="classic", file='s2.pml', dir='
    pymol')
```

---

## 17.2.136 pymol.tensor\_pdb



### Synopsis

Display the diffusion tensor PDB geometric object over the loaded PDB.

### Defaults

**pymol.tensor\_pdb**(file=None)

### Keyword arguments

**file**: The name of the PDB file containing the tensor geometric object.

### Description

In executing this user function, a PDB file must have previously been loaded into this data pipe a geometric object or polygon representing the Brownian rotational diffusion tensor will be overlain with the loaded PDB file and displayed within PyMOL. The PDB file containing the geometric object must be created using the complementary `structure.create_diff_tensor_pdb` user function.

The tensor PDB file is read in using the command:

```
load file
```

The centre of mass residue 'COM' is displayed using the commands:

```
select resn COM
show dots, 'sele'
color blue, 'sele'
```

The axes of the diffusion tensor, the residue 'AXS', is displayed using the commands:

```
select resn AXS
hide ('sele')
show sticks, 'sele'
color cyan, 'sele'
```

label 'sele', name

The simulation axes, the residues 'SIM', are displayed using the commands:

select resn SIM

colour cyan, 'sele'

---

### 17.2.137 pymol.vector\_dist



#### Synopsis

Display the PDB file representation of the XH vector distribution.

#### Defaults

**pymol.vector\_dist**(file='XH.dist.pdb')

#### Keyword arguments

**file**: The name of the PDB file containing the vector distribution.

#### Description

A PDB file of the macromolecule must have previously been loaded as the vector distribution will be overlain with the macromolecule within PyMOL. The PDB file containing the vector distribution must be created using the complementary `structure.create_vector_dist` user function.

The vector distribution PDB file is read in using the command:

load file

---

**17.2.138** `pymol.view`**Synopsis**

View the collection of molecules from the loaded PDB file.

**Defaults**

`pymol.view()`

**Description**

This will simply launch Pymol.

**Prompt examples**

```
relax> pymol.view()
```

---

**17.2.139** `rdc.back_calc`**Synopsis**

Back calculate the residual dipolar couplings.

**Defaults**

`rdc.back_calc(align_id=None)`

**Keyword arguments**

`align_id`: The alignment ID string.

**Description**

This will back calculate the residual dipolar couplings (RDCs) if an alignment tensor is present and inter-dipole vectors have been loaded into the relax data store.

**17.2.140 rdc.calc\_q\_factors****Synopsis**

Calculate the RDC Q factor for the selected spins.

**Defaults**

**rdc.calc\_q\_factors**(spin\_id=None, verbosity=1)

**Keyword arguments**

**spin\_id**: The spin ID string for restricting to subset of all selected spins.

**verbosity**: The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

**Description**

For this to work, the back-calculated RDC data must first be generated by the analysis specific code. Otherwise a warning will be given.

**Prompt examples**

To calculate the RDC Q factor for only the spins '@H26', '@H27', and '@H28', type one of:

```
relax> rdc.calc_q_factors('@H26 & @H27 &
                        @H28')
```

```
relax> rdc.calc_q_factors(spin_id='@H26 &
                        @H27 & @H28')
```

**17.2.141 rdc.copy****Synopsis**

Copy RDC data from one data pipe to another.

**Defaults**

**rdc.copy**(pipe\_from=None, pipe\_to=None, align\_id=None, back\_calc=True)

**Keyword arguments**

**pipe\_from**: The name of the pipe to copy the RDC data from.

**pipe\_to**: The name of the pipe to copy the RDC data to.

**align\_id**: The alignment ID string.

**back\_calc**: A flag which if True will cause any back-calculated RDCs present to also be copied with the real values and errors.

**Description**

This function will copy RDC data from 'pipe\_from' to 'pipe\_to'. If align\_id is not given then all RDC data will be copied, otherwise only a specific data set will be.

**Prompt examples**

To copy all RDC data from pipe 'm1' to pipe 'm9', type one of:

```
relax> rdc.copy('m1', 'm9')
```

```
relax> rdc.copy(pipe_from='m1', pipe_to='m9'
                )
```

```
relax> rdc.copy('m1', 'm9', None)
```

```
relax> rdc.copy(pipe_from='m1', pipe_to='m9'
                , align_id=None)
```

To copy only the 'Th' RDC data from 'm3' to 'm6', type one of:

```
relax> rdc.copy('m3', 'm6', 'Th')
```

```
relax> rdc.copy(pipe_from='m3', pipe_to='m6'
                , align_id='Th')
```

---

**17.2.142 rdc.corr\_plot****Synopsis**

Generate a correlation plot of the measured vs. the back-calculated RDCs.

**Defaults**

```
rdc.corr_plot(format='grace', title=None, subtitle=None,
file='rdc.corr_plot.agr', dir=None, force=False)
```

**Keyword arguments**

**format**: The format of the plot data.

**title**: The title for the plot, overriding the default.

**subtitle**: The subtitle for the plot, overriding the default.

**file**: The name of the Grace file to create.

**dir**: The directory name.

**force**: A flag which if True will cause the file to be overwritten.

**Description**

Two formats are currently supported. If format is set to 'grace', then a Grace plot file will be created. If the format is not set then a plain text list of the measured and back-calculated data will be created.

**Prompt examples**

To create a Grace plot of the data, type:

```
relax> rdc.corr_plot()
```

To create a plain text list of the measured and back-calculated data, type one of:

```
relax> rdc.corr_plot(None)
```

```
relax> rdc.corr_plot(format=None)
```

**17.2.143 rdc.delete****Synopsis**

Delete the RDC data corresponding to the alignment ID.

**Defaults**

```
rdc.delete(align_id=None)
```

**Keyword arguments**

**align\_id**: The alignment ID string of the data to delete.

**Description**

This will delete all RDC data associated with the alignment ID in the current data pipe.

**Prompt examples**

To delete the RDC data corresponding to align\_id='PH\_gel', type:

```
relax> rdc.delete('PH_gel')
```

**17.2.144 rdc.display****Synopsis**

Display the RDC data corresponding to the alignment ID.

**Defaults**

**rdc.display**(align\_id=None, bc=False)

**Keyword arguments**

**align\_id**: The alignment ID string.

**bc**: A flag which if set will display the back-calculated rather than measured RDCs.

**Description**

This will display all of the RDC data associated with the alignment ID in the current data pipe.

**Prompt examples**

To display the ‘phage’ RDC data, type:

```
relax> rdc.display('phage')
```

**17.2.145 rdc.read****Synopsis**

Read the RDC data from file.

**Defaults**

**rdc.read**(align\_id=None, file=None, dir=None, data\_type='D', spin\_id1\_col=1, spin\_id2\_col=2, data\_col=None, error\_col=None, sep=None, neg\_g\_corr=False, absolute=False)

**Keyword arguments**

**align\_id**: The alignment ID string.

**file**: The name of the file containing the RDC data.

**dir**: The directory where the file is located.

**data\_type**: Specify if the RDC data is in the D or 2D format, or the  $T = J + D$  format.

**spin\_id1\_col**: The spin ID string column for the first spin.

**spin\_id2\_col**: The spin ID string column for the second spin.

**data\_col**: The RDC data column.

**error\_col**: The experimental error column.

**sep**: The column separator (the default is white space).

**neg\_g\_corr**: A flag which is used to correct for the negative gyromagnetic ratio of  $^{15}\text{N}$ . If set to True, all RDC values will be inverted prior to being stored in the relax data store.

**absolute**: A flag which indicates that the loaded RDCs are are signless.

**Description**

This will read RDC data from a file and associate it with an alignment ID, either a new ID or a preexisting one with no RDC data.

The data type is used to specify how the RDC is defined. It can be set to a number of values:

‘D’ means that the splitting in the aligned sample was taken as  $J + D$ .

‘2D’ means that the splitting in the aligned sample was assumed to be  $J + 2D$ .

‘T’ means that the file contains  $T = J + D$  values.

Internally, relax uses the D notation. Therefore if set to ‘2D’, the values will be doubled when read in. If the ‘T’ data type is specified, then J couplings must be present for this data to be of any use.

If the negative gyromagnetic ratio correction flag is set, a sign inversion will be applied to all RDC values to be loaded. This is sometimes needed for  $^{15}\text{N}$  if the data is not compensated for the negative gyromagnetic ratio.

The absolute RDCs flag is used for RDCs in which the sign is unknown. All absolute RDCs loaded will be converted to positive values.

### Prompt examples

The following commands will read the RDC data out of the file ‘Tb.txt’ where the columns are separated by the symbol ‘,’ and store the RDCs under the ID ‘Tb’:

```
relax> rdc.read('Tb', 'Tb.txt', sep=',')
```

If the individual spin RDC errors are located in the file ‘rdc\_err.txt’ in column number 5, then to read these values into relax, assuming  $J + D$  was measured, type one of:

```
relax> rdc.read('phage', 'rdc_err.txt',
               data_type='D', error_col=5)
```

```
relax> rdc.read(aligned_id='phage', file='
               rdc_err.txt', data_type='D', error_col
               =5)
```

## 17.2.146 rdc.set\_errors



### Synopsis

Set the errors for the RDCs.

### Defaults

```
rdc.set_errors(aligned_id=None, spin_id1=None, spin_id2=
None, sd=1.0)
```

### Keyword arguments

**aligned\_id**: The optional alignment ID string.

**spin\_id1**: The optional spin ID string of the first spin.

**spin\_id2**: The optional spin ID string of the second spin.

**sd**: The RDC standard deviation value in Hertz.

### Description

If the RDC errors have not already been read from a RDC data file or if they need to be changed, then the errors can be set via this user function.

---

**17.2.147 rdc.weight****Synopsis**

Set optimisation weights on the RDC data.

**Defaults**

**rdc.weight**(align\_id=None, spin\_id=None, weight=1.0)

**Keyword arguments**

**align\_id**: The alignment ID string.

**spin\_id**: The spin ID string.

**weight**: The weighting value.

**Description**

This can be used to force the RDC to contribute more or less to the chi-squared optimisation statistic. The higher the value, the more importance the RDC will have.

**17.2.148 rdc.write****Synopsis**

Write the RDC data to file.

**Defaults**

**rdc.write**(align\_id=None, file=None, dir=None, bc=False, force=False)

**Keyword arguments**

**align\_id**: The alignment ID string.

**file**: The name of the file.

**dir**: The directory name.

**bc**: A flag which if set will write out the back-calculated rather than measured RDCs.

**force**: A flag which if True will cause the file to be overwritten.

**Description**

If no directory name is given, the file will be placed in the current working directory. The alignment ID is required for selecting which RDC data set will be written to file.



### 17.2.149 relax\_data.back\_calc



## Synopsis

Back calculate the relaxation data at the given frequency.

## Defaults

```
relax_data.back_calc(ri_id=None, ri_type=None, frq=
None)
```

## Keyword arguments

ri\_id: The relaxation data ID string.

ri\_type: The relaxation data type, ie 'R1', 'R2', or 'NOE'.

frq: The spectrometer frequency in Hz.

## Description

This allows relaxation data of the given type and frequency to be back calculated from the model parameter values. If the relaxation data ID, type and frequency are not given, then relaxation data matching that currently loaded in the relax data store will be back-calculated.



### 17.2.150 relax\_data.copy

## Synopsis

Copy relaxation data from one pipe to another.

## Defaults

```
relax_data.copy(pipe_from=None, pipe_to=None, ri_id=None)
```

## Keyword arguments

pipe\_from: The name of the pipe to copy the relaxation data from.

**pipe\_to:** The name of the pipe to copy the relaxation data to.

ri\_id: The relaxation data ID string.

## Description

This will copy relaxation data from one data pipe to another. If the relaxation ID data string is not given then all relaxation data will be copied, otherwise only a specific data set will be copied.

### Prompt examples

To copy all relaxation data from pipe 'm1' to pipe 'm9', type one of:

```
relax> relax_data.copy('m1', 'm9')
```

```
relax> relax_data.copy(pipe_from='m1',
                        pipe_to='m9')
```

```
relax> relax_data.copy('m1', 'm9', None)
```

```
relax> relax_data.copy(pipe_from='m1',
                        pipe_to='m9', ri_id=None)
```

To copy only the NOE relaxation data with the ID string of 'NOE\_800' from 'm3' to 'm6', type one of:

```
relax> relax_data.copy('m3', 'm6', 'NOE_800'
```

```
relax> relax_data.copy(pipe_from='m3',  
                        pipe_to='m6', ri_id='NOE_800')
```

---

**17.2.151** `relax_data.delete`**Synopsis**

Delete the data corresponding to the relaxation data ID string.

**Defaults**

`relax_data.delete(ri_id=None)`

**Keyword arguments**

`ri_id`: The relaxation data ID string.

**Description**

The relaxation data corresponding to the given relaxation data ID string will be removed from the current data pipe.

**Prompt examples**

To delete the relaxation data corresponding to the ID 'NOE\_600', type:

```
relax> relax_data.delete('NOE_600')
```

---

**17.2.152** `relax_data.display`**Synopsis**

Display the data corresponding to the relaxation data ID string.

**Defaults**

`relax_data.display(ri_id=None)`

**Keyword arguments**

`ri_id`: The relaxation data ID string.

**Description**

This will display the relaxation data corresponding to the given ID.

**Prompt examples**

To display the NOE relaxation data at 600 MHz with the ID string 'NOE\_600', type:

```
relax> relax_data.display('NOE_600')
```

### 17.2.153 relax\_data.peak- \_intensity\_type



#### Synopsis

Specify if heights or volumes were used to measure the peak intensities.

#### Defaults

**relax\_data.peak\_intensity\_type**(ri\_id=None, type='height')

#### Keyword arguments

**ri\_id**: The relaxation data ID string.

**type**: The peak intensity type.

#### Description

This is essential for BMRB data deposition. It is used to specify whether peak heights or peak volumes were measured. The two currently allowed values for the peak intensity type are 'height' and 'volume'.

### 17.2.154 relax\_data.read



#### Synopsis

Read R<sub>1</sub>, R<sub>2</sub>, NOE, or R<sub>2</sub>eff relaxation data from a file.

#### Defaults

**relax\_data.read**(ri\_id=None, ri\_type=None, frq=None, file=None, dir=None, spin\_id\_col=None, mol\_name\_col=None, res\_num\_col=None, res\_name\_col=None, spin\_num\_col=None, spin\_name\_col=None, data\_col=None, error\_col=None, sep=None, spin\_id=None)

#### Keyword arguments

**ri\_id**: The relaxation data ID string. This must be a unique identifier.

**ri\_type**: The relaxation data type, i.e. 'R1', 'R2', 'NOE', or 'R2eff'.

**frq**: The exact proton frequency of the spectrometer in Hertz. See the 'sfrq' parameter in the Varian procpur file or the 'SF01' parameter in the Bruker acquis file.

**file**: The name of the file containing the relaxation data.

**dir**: The directory where the file is located.

**spin\_id\_col**: The spin ID string column (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col**: The molecule name column (alternative to the spin\_id\_col).

**res\_num\_col**: The residue number column (alternative to the spin\_id\_col).

**res\_name\_col**: The residue name column (alternative to the spin\_id\_col).

**spin\_num\_col**: The spin number column (alternative to the spin\_id\_col).

**spin\_name\_col**: The spin name column (alternative to the spin\_id\_col).

**data\_col**: The relaxation data column.

**error\_col**: The experimental error column.

**sep**: The column separator (the default is white space).

**spin\_id**: The spin ID string to restrict the loading of data to certain spin subsets.

## Description

This will load the relaxation data into the relax data store. The data is associated with the spectrometer frequency in Hertz. For subsequent analysis, this frequency must be set to the exact field strength. This value is stored in the 'sfrq' parameter in the Varian procar file or the 'SF01' parameter in the Bruker acquis file.

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number, residue name, spin number and/or spin name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID string can be used to restrict the reading to certain spin types, for example only <sup>15</sup>N spins when only residue information is in the file.

## Prompt examples

The following commands will read the protein NOE relaxation data collected at 600 MHz out of a file called 'noe.600.out' where the residue numbers, residue names, data, errors are in the first, second, third, and forth columns respectively.

```
relax> relax_data.read('NOE_600', 'NOE', 599
    .7 * 1e6, 'noe.600.out', res_num_col=1,
    res_name_col=2, data_col=3, error_col
    =4)
```

```
relax> relax_data.read(ri_id='NOE_600',
    ri_type='NOE', frq=600.0 * 1e6, file='
    noe.600.out', res_num_col=1,
    res_name_col=2, data_col=3, error_col
    =4)
```

The following commands will read the R<sub>2</sub> data out of the file 'r2.out' where the residue numbers, residue names, data, errors are in the second, third, fifth, and sixth columns respectively. The columns are separated by commas.

```
relax> relax_data.read('R2_800', 'R2', 8.0 *
    1e8, 'r2.out', res_num_col=2,
    res_name_col=3, data_col=5, error_col
    =6, sep=',')
```

```
relax> relax_data.read(ri_id='R2_800',
    ri_type='R2', frq=8.0*1e8, file='r2.out
    ', res_num_col=2, res_name_col=3,
    data_col=5, error_col=6, sep=',')
```

The following commands will read the R<sub>1</sub> data out of the file 'r1.out' where the columns are separated by the symbol '%'

```
relax> relax_data.read('R1_300', 'R1', 300.1
    * 1e6, 'r1.out', sep='%')
```

## 17.2.155 relax\_data.temp- \_calibration



## Synopsis

Specify the per-experiment temperature calibration method used.

## Defaults

`relax_data.temp_calibration(ri_id=None, method=None)`

## Keyword arguments

`ri_id`: The relaxation data ID string.

`method`: The per-experiment temperature calibration method.

## Description

For the proper measurement of relaxation data, per-experiment temperature calibration is essential. This user function is not for inputting standard MeOH/ethylene glycol/etc. calibration of a spectrometer - this temperature setting is of no use when you are running experiments which pump in large amounts of power into the probe head.

The R<sub>1</sub> experiment should be about the same temperature as a HSQC and hence be close to the standard MeOH/ethylene glycol spectrometer calibration. However the R<sub>2</sub> CPMG or spin lock and, to a lesser extent, the NOE pre-saturation pump a lot more power into the probe head. The power differences can either cause the temperature in the sample to be too high or too low. This is unpredictable as the thermometer used by the VT unit is next to the coils in the probe head and not inside the NMR sample. So the VT unit tries to control the temperature inside the probe head rather than in the NMR sample. However between the thermometer and the sample is the water of the sample, the glass of the NMR tube, the air gap where the VT unit controls air flow and the outside components of the probe head protecting the electronics. If the sample, the probe head or the VT unit is changed, this will have a different affect on the per-experiment temperature. The VT unit responds differently under different conditions and may sometimes over or under compensate by a couple of degrees. Therefore each relaxation data set from each spectrometer requires a per-experiment calibration.

Specifying the per-experiment calibration method is needed for BMRB data deposition. The currently allowed methods are:

```
'methanol',  
'monoethylene glycol',  
'no calibration applied'.
```

Other methods will be accepted if supplied.

---

## 17.2.156 relax\_data.temp\_control



### Synopsis

Specify the temperature control method used.

### Defaults

**relax\_data.temp\_control**(ri\_id=None, method=None)

### Keyword arguments

**ri\_id**: The relaxation data ID string.

**method**: The control method.

### Description

For the proper measurement of relaxation data, explicit temperature control techniques are essential. A number of factors can cause significant temperature fluctuations between individual relaxation experiments. This includes the daily temperature cycle of the room housing the spectrometer, different amounts of power for the individual experiments, etc.

The best methods for eliminating such problems are single scan interleaving and temperature compensation block. Single scan interleaving is the most powerful technique for averaging the temperature fluctuations not only across different experiments, but also across the entire measurement time. The application of off-resonance temperature compensation blocks at the start of the experiment is useful for the  $R_2$  and will normalise the temperature between the individual experiments, but single scan or single fid interleaving is nevertheless required for normalising the temperature across the entire measurement.

Specifying the temperature control method is needed for BMRB data deposition. The currently allowed methods are:

```
'single scan interleaving',  
'temperature compensation block',  
'single scan interleaving and temperature  
compensation block',  
'single fid interleaving',  
'single experiment interleaving',  
'no temperature control applied'.
```

---

**17.2.157 relax\_data.type****Synopsis**

Set the type of relaxation data.

**Defaults**

**relax\_data.type**(ri\_id=None, ri\_type=None)

**Keyword arguments**

**ri\_id**: The relaxation data ID string of the data to set the frequency of.

**ri\_type**: The relaxation data type, *i.e.* 'R1', 'R2', or 'NOE'.

**Description**

This allows the type associated with the relaxation data to be either set or reset. This type must be one of 'R1', 'R2', or 'NOE'.

**17.2.158 relax\_data.write****Synopsis**

Write relaxation data to a file.

**Defaults**

**relax\_data.write**(ri\_id=None, file=None, dir=None, bc=False, force=False)

**Keyword arguments**

**ri\_id**: The relaxation data ID string.

**file**: The name of the file.

**dir**: The directory name.

**bc**: A flag which if True will cause the back-calculated data to be written to the file.

**force**: A flag which if True will cause the file to be overwritten.

**Description**

If no directory name is given, the file will be placed in the current working directory. The relaxation data ID string is required for selecting which relaxation data to write to file.

**17.2.159 relax\_disp.catia\_execute****Synopsis**

Perform a relaxation dispersion optimisation using Flemming Hansen's CATIA.

**Defaults**

**relax\_disp.catia\_execute**(dir=None, binary='catia')

**Keyword arguments**

**dir**: The directory containing all of the CATIA input files.

**binary**: The name of the executable CATIA program file.

**Description**

CATIA will be executed as

```
$ catia < Fit.catia
```

If you would like to use a different CATIA executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, include the full path in front of the binary file name.

**17.2.160 relax\_disp.catia\_input****Synopsis**

Create the input files for Flemming Hansen's CATIA program.

**Defaults**

**relax\_disp.catia\_input**(dir='catia', force=False)

**Keyword arguments**

**dir**: The directory to place the CATIA input files, output directory, etc.

**force**: A flag which if set to True will cause the files to be overwritten if they already exist.

**Description**

This will create all of the input file required for CATIA as well as the CATIA results output directory.

**17.2.161 relax\_disp.cluster****Synopsis**

Define clusters of spins for joint optimisation.

**Defaults**

`relax_disp.cluster(cluster_id=None, spin_id=None)`

**Keyword arguments**

`cluster_id`: The cluster identification string.

`spin_id`: The spin identifier string for the spin or group of spins to add to the cluster.

**Description**

In a relaxation dispersion analysis, the parameters of the model of dispersion can either be optimised for each spin system separately or a number of spins can be grouped or clustered and the dispersion model parameters optimised for all spins in the cluster together. Clusters are identified by unique ID strings. Any spins not within a cluster will be optimised separately and individually.

If the cluster ID string already exists, the spins will be added to that pre-existing cluster. If no spin ID is given, then all spins will be added to the cluster.

The special cluster ID string ‘free spins’ is reserved for the pool of non-clustered spins. This can be used to remove a spin system from an already existing cluster by specifying this cluster ID and the desired spin ID.

**Prompt examples**

To add the spins ‘:1@N’ and ‘:3@N’ to a new cluster called ‘cluster’, type one of:

```
relax> relax_disp.cluster('cluster', ':1,3@N')
```

```
relax> relax_disp.cluster(cluster_id='cluster', spin_id=':1,3@N')
```

**17.2.162 relax\_disp.cpmg\_setup****Synopsis**

Set the CPMG pulse sequence information associated with a given spectrum.

**Defaults**

`relax_disp.cpmg_setup(spectrum_id=None, cpmg_frq=None, ncyc_even=True)`

**Keyword arguments**

`spectrum_id`: The spectrum ID string to associate the CPMG pulse sequence information to.

`cpmg_frq`: The frequency, in Hz, of the CPMG pulse train.

`ncyc_even`: A flag which if True means that the number of CPMG blocks must be even. This is pulse sequence dependant.

**Description**

This allows all information about CPMG pulse sequence required for a relaxation dispersion analysis to be specified. This includes:

‘cpmg\_frq’ allows the CPMG pulse train frequency of a given spectrum to be set. If None is given for frequency, then the spectrum will be treated as a reference spectrum.

‘ncyc\_even’ specifies if an even number of CPMG blocks are required for the pulse sequence.

**Prompt examples**

To identify the reference spectrum called ‘reference\_spectrum’, type:

```
relax> relax_disp.cpmg_setup(spectrum_id='reference_spectrum', cpmg_frq=None)
```

To set a frequency of 200 Hz for the spectrum ‘200\_Hz\_spectrum’, type:

```
relax> relax_disp.cpmg_setup(spectrum_id='200_Hz_spectrum', cpmg_frq=200)
```



### 17.2.163 relax\_disp.cpmgfit-\_execute



#### Synopsis

Optimisation of the CPMG data using Art Palmer's CPMGFit program.

#### Defaults

**relax\_disp.cpmgfit\_execute**(dir=None, force=False, binary='cpmgfit')

#### Keyword arguments

**dir**: The directory containing all of the CPMGFit input files. If not given, this defaults to the model name in lower case.

**force**: A flag which if set to True will cause the results files to be overwritten if they already exist.

**binary**: The name of the executable CPMGFit program file.

#### Description

CPMGFit will be executed once per spin as:

```
$ cpmgfit -grid -xmgr -f dir/spin_x.in | tee
  dir/spin_x.out
```

where *x* is replaced by each spin ID string. If you would like to use a different CPMGFit executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, be sure to include the full path in front of the binary file name so it can be found.

### 17.2.164 relax\_disp.cpmgfit\_input



#### Synopsis

Create the input files for Art Palmer's CPMGFit program.

#### Defaults

**relax\_disp.cpmgfit\_input**(dir=None, force=False, binary='cpmgfit', spin\_id=None)

#### Keyword arguments

**dir**: The directory to place the files. If not given, this defaults to the model name in lower case.

**force**: A flag which if set to True will cause the files to be overwritten if they already exist.

**binary**: The name of the executable CPMGFit program file.

**spin\_id**: The spin identification string.

#### Description

The following files are created:

```
'dir/spin_x.in',
'dir/run.sh'.
```

One CPMGFit input file is created per spin and named '**dir/spin\_x.in**', where *x* is the spin ID string. The file '**dir/run.sh**' is a batch file for executing CPMGFit for all of the spin input files. If you would like to use a different CPMGFit executable file, change the binary name to the appropriate file name. If the file is not located within the environment's path, be sure to include the full path in front of the binary name so it can be found.

## 17.2.165 relax\_disp.exp\_type



### Synopsis

Select the relaxation dispersion experiment type.

### Defaults

`relax_disp.exp_type(spectrum_id=None, exp_type='SQ CPMG')`

### Keyword arguments

`spectrum_id`: The spectrum ID string to associate the spin-lock field strength to.

`exp_type`: The type of relaxation dispersion experiment performed.

### Description

For each peak intensity set loaded into relax, the type of experiment it comes from needs to be specified. By specifying this for each spectrum ID, multiple experiment types can be analysed simultaneously. This is assuming that an appropriate dispersion model exists for the experiment combination.

The currently supported experiments include:

‘SQ CPMG’ – The single quantum (SQ) CPMG-type experiments,

‘ZQ CPMG’ – The zero quantum (ZQ) CPMG-type experiments,

‘DQ CPMG’ – The double quantum (DQ) CPMG-type experiments,

‘MQ CPMG’ – The multiple quantum (MQ) CPMG-type experiments,

‘1H SQ CPMG’ – The 1H single quantum (SQ) CPMG-type experiments,

‘1H MQ CPMG’ – The 1H multiple quantum (MQ) CPMG-type experiments,

‘R1rho’ – The R1rho-type experiments.

### Prompt examples

To set the experiment type to ‘SQ CPMG’ for the spectrum ID ‘nu\_4500.0\_800MHz’, type one of:

```
relax> relax_disp.exp_type('nu_4500.0_800MHz', 'SQ CPMG')
```

```
relax> relax_disp.exp_type(spectrum_id='nu_4500.0_800MHz', exp_type='SQ CPMG')
```

**17.2.166 relax\_disp.insignificance****Synopsis**

Deselect all spins with insignificant dispersion.

**Defaults**

`relax_disp.insignificance(level=2.0)`

**Keyword arguments**

**level:** The  $R_{2eff}/R_{1\rho}$  value in rad/s by which to judge insignificance. If the maximum difference between two points on all dispersion curves for a spin is less than this value, that spin will be deselected.

**Description**

This can be used to deselect all spins which have insignificant dispersion profiles. The insignificance value is the  $R_{2eff}/R_{1\rho}$  value in rad/s by which to judge the dispersion curves by. If the maximum difference between two points on all dispersion curves for a spin is less than this value, that spin will be deselected.

**17.2.167 relax\_disp.nessy\_input****Synopsis**

Create the input files for Michael Bieri's NESSY program.

**Defaults**

`relax_disp.nessy_input(dir=None, force=False, spin_id=None)`

**Keyword arguments**

**dir:** The directory to place the file and to use as the NESSY project directory. If not given, this defaults to the current directory.

**force:** A flag which if set to True will cause the files to be overwritten if they already exist.

**spin\_id:** The spin identification string.

**Description**

This will create a single NESSY save file called 'save.NESSY'. This will contain all of the dispersion data currently loaded in the relax data store. If the directory name is not supplied, this will default to the current directory.

## 17.2.168 relax\_disp.parameter\_copy



### Synopsis

Copy dispersion specific parameters values from one data pipe to another.

### Defaults

**relax\_disp.parameter\_copy**(pipe\_from=None, pipe\_to=None)

### Keyword arguments

**pipe\_from**: The name of the pipe to copy from.

**pipe\_to**: The name of the pipe to copy to.

### Description

This is a special function for copying relaxation dispersion parameters from one data pipe to another. It is much more advanced than the `value.copy` user function, in that clustering is taken into account. When the destination data pipe has spin clusters defined, then the new parameter values, when required, will be taken as the median value.

For the cluster specific parameters, *i.e.* the populations of the states and the exchange parameters, a median value will be used as the starting point. For all other parameters, the R20 values for each spin and magnetic field, as well as the parameters related to the chemical shift difference  $\Delta\nu$ , the optimised values of the previous run will be directly copied.

### Prompt examples

To copy the CSA values from the data pipe 'm1' to 'm2', type:

```
relax> value.parameter_copy('m1', 'm2', 'csa')
```

## 17.2.169 relax\_disp.plot\_disp\_curves



### Synopsis

Create 2D Grace plots of the dispersion curves for each spin system.

### Defaults

**relax\_disp.plot\_disp\_curves**(dir='grace', y\_axis='r2\_eff', x\_axis='disp', num\_points=1000, extend\_hz=500.0, extend\_ppm=500.0, interpolate='disp', force=False)

### Keyword arguments

**dir**: The directory name to place all of the spin system files into.

**y\_axis**: Option can be either 'r2\_eff' which plot 'r2\_eff' for CPMG experiments or 'r1\_rho' for R1rho experiments or option can be 'r2\_r1\_rho', which for R1rho experiments plot R2.

**x\_axis**: Option can be either 'disp' which plot 'CPMG frequency (Hz)' for CPMG experiments or 'Spin-lock field strength (Hz)' for R1rho experiments or option can be either 'w\_eff' or 'theta' for R1rho experiments, which plot 'Effective field in rotating frame (rad/s)' or 'Rotating frame tilt angle  $\theta$  (rad)'

**num\_points**: The total number of points to generate the interpolated dispersion curves with. This value has no effect for the numeric CPMG-based models.

**extend\_hz**: How far to extend the interpolated dispersion curves beyond the last dispersion point, *i.e.* the nu-CPMG frequency or spin-lock field strength value, in Hertz.

**extend\_ppm**: How far to extend the interpolated dispersion curves beyond the last dispersion point, *i.e.* the spin-lock offset value, in ppm.

**interpolate**: Either by option 'disp' which interpolate CPMG frequency or spin-lock field strength, or by option 'offset' which interpolate over spin-lock offset.

**force**: A flag which, if set to True, will cause the files to be overwritten.

## Description

This is used to create 2D Grace plots of the dispersion curves of the nu-CPMG frequencies or spin-lock field strength verses the R2eff/R1rho values. One file will be created per spin system with the name 'disp\_x.agr', where x is related to the spin ID string. For each file, one Grace graph will be produced for each experiment.

Four sets of curves of R2eff/R1rho values will be produced per experiment and per magnetic field strength. These are the experimental values, the fitted values, the interpolated dispersion curves for the fitted solution, and the residuals. Different dispersion models result in different interpolated dispersion curves. For the numeric models which use CPMG-type data, the maximum interpolation resolution is constrained by the frequency of a single CPMG block for the entire relaxation period. For all other models, the interpolation resolution is not constrained and can be as fine as desired by setting the total number of interpolation points. Interpolated curves are not produced for the 'R2eff' model as they are not necessary.

For R1rho models, graphs can be interpolated against Spin-lock offset, but this feature is not available for CPMG experiment types. It is also possible to select values on X-axis of 'Effective field in rotating frame w\_eff (rad/s)' or 'Rotating frame tilt angle  $\theta$  (rad)'.

For R1rho models, special Y-value  $R_2$  R1rho can for example be plotted as function of w\_eff.  $R_2$  is calculated as:  $R_2 = (R1rho - R1 \cos^2(\theta)) / \sin^2(\theta)$ .

## 17.2.170 relax\_disp.plot\_exp\_curves



### Synopsis

Create 2D Grace plots of the exponential curves.

### Defaults

**relax\_disp.plot\_exp\_curves**(file=None, dir='grace', force=False, norm=False)

### Keyword arguments

**file:** The name of the file.

**dir:** The directory name.

**force:** A flag which, if set to True, will cause the file to be overwritten.

**norm:** A flag which, if set to True, will cause all graphs to be normalised to a starting value of 1. This is for the normalisation of series type data.

### Description

This is used to create 2D Grace plots of the individual exponential curves used to find the R2eff or R1rho values. This supplements the grace.write user function which is not capable of generating these curves in a reasonable format.

**17.2.171 relax\_disp.r1\_fit****Synopsis**

Switch between fixed or fitted  $R_1$  values for optimisation.

**Defaults**

`relax_disp.r1_fit(fit=True)`

**Keyword arguments**

**fit:** The flag specifying if  $R_1$  values should be optimised or if loaded  $R_1$  values should be fixed during optimisation.

**Description**

This user function allows the optimisation of  $R_1$  values to be turned on or off for the relaxation dispersion dispersion models. If turned off, the current values of  $R_1$  will be fixed. Otherwise the  $R_1$  values will be added to the model parameter set. For models which do not support the  $R_1$  parameter for off-resonance effects, this setting will have no effect. Only the models ['No  $R_{ex}$ ', 'DPL94', 'TP02', 'TAP03', 'MP05', 'NS  $R_1\rho$  2-site'] support  $R_1$  optimisation.

**17.2.172 relax\_disp.r20\_from\_min\_r2eff****Synopsis**

Set the  $R_{20}$  parameter values to that of the minimum  $R_{2eff}$  value.

**Defaults**

`relax_disp.r20_from_min_r2eff(force=True)`

**Keyword arguments**

**force:** A flag which if set to True will cause the  $R_{20}$  values to be overwritten if they already exist.

**Description**

Set the  $R_{20}$  parameter values to that of the minimum  $R_{2eff}$  value. This user function will look through all  $R_{2eff}$  values per magnetic field strength, find the minimum value, and set the  $R_{20}$ ,  $R_{20A}$ ,  $R_{20B}$ , and  $R_1\rho$  parameters of the model to this value. This can serve a number of purposes including using the values for the chi-squared space mapping via the  $\delta_x.map$  user function, speeding up optimisation by avoiding the grid search for these parameters, and as initial parameter values for other dispersion software.

Instead of finding the initial values for the  $R_{20}$  parameter using the grid search, the minimum for the  $R_{2eff}$  points can be used instead. This is often a good initial position for minimisation. For example for a two field CPMG experiment with model CR72, that would drop the number of uniform grid search points from 5D to 3D, i.e. two orders of magnitude faster. When using the standard 21 grid increments per dimension, it would allow the grid search to be 441 times faster. Note that the relaxation dispersion auto-analysis will take all pre-set parameter values into account and will automatically exclude these from the grid search.

Note that for optimisation, that this is an experimental and unpublished feature of the dispersion analysis. If  $R_{20} \ll \min(R_{2eff})$ , the grid search will be performed in a region of the optimisation space quite distant from the true minimum. If unsure, do not activate this option, and let the grid search find a better starting value.

17.2.173 `relax_disp.r2eff_err-  
_estimate`

## Synopsis

Estimate R2eff errors by the Jacobian matrix.

## Defaults

`relax_disp.r2eff_err_estimate`(spin\_id=None, epsrel=0.0, verbosity=1)

## Keyword arguments

**spin\_id**: The spin ID string to restrict value setting to.

**epsrel**: The parameter to remove linear-dependent columns when J is rank deficient.

**verbosity**: The higher the value, the greater the verbosity.

## Description

This is a new experimental feature from version 3.3.

This will estimate R2eff errors by using the exponential decay Jacobian matrix 'J' to compute the covariance matrix of the best-fit parameters.

This can be an huge time saving step, when performing model fitting in R1rho. Errors of R2eff values, are normally estimated by time-consuming Monte-Carlo simulations.

This method is inspired from the GNU Scientific Library (GSL).

The covariance matrix is given by:  $\text{covar} = Q_{xx} = (J^T W J)^{-1}$ , where the weight matrix W is constructed by the multiplication of an Identity matrix I and a weight array w. The weight array is  $1/\text{errors}^2$ , which then gives  $W = I \cdot w = I \cdot 1/\text{errors}^2$ .

$Q_{xx}$  is computed by QR decomposition,  $J^T W J = QR$ ,  $Q_{xx} = R^{-1}$ .  $Q^T$ . The columns of R which satisfy:  $|R_{kk}| \leq \text{epsrel} |R_{11}|$  are considered linearly-dependent and are excluded from the covariance matrix (the corresponding rows and columns of the covariance matrix are set to zero).

The parameter 'epsrel' is used to remove linear-dependent columns when J is rank deficient.

17.2.174 `relax_disp.r2eff_read`

## Synopsis

Read R2eff/R1rho values and errors from a file.

## Defaults

`relax_disp.r2eff_read`(id=None, file=None, dir=None, disp\_freq=None, spin\_id\_col=None, mol\_name\_col=None, res\_num\_col=None, res\_name\_col=None, spin\_num\_col=None, spin\_name\_col=None, data\_col=None, error\_col=None, sep=None)

## Keyword arguments

**id**: The partial experiment ID string to identify this data with. The full ID string will be constructed as this ID followed by an underscore and then the dispersion point value from the file.

**file**: The name of the file.

**dir**: The directory name.

**disp\_freq**: For CPMG-type data, the frequency of the CPMG pulse train. For R1rho-type data, the spin-lock field strength nu1. The units must be Hertz

**spin\_id\_col**: The spin ID string column used by the generic file format (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col**: The molecule name column used by the generic file format (alternative to the spin ID column).

**res\_num\_col**: The residue number column used by the generic file format (alternative to the spin ID column).

**res\_name\_col**: The residue name column used by the generic file format (alternative to the spin ID column).

**spin\_num\_col**: The spin number column used by the generic file format (alternative to the spin ID column).

**spin\_name\_col**: The spin name column used by the generic file format (alternative to the spin ID column).

**data\_col**: The RDC data column.

**error\_col**: The experimental error column.

**sep**: The column separator used by the generic format (the default is white space).

## Description

This will read R2eff/R1rho data directly from a file. The data will be associated with an experiment ID string. A partial ID is to be supplied and then the full ID string will be constructed as this ID followed by an underscore and then the dispersion point value from the file (as '%s\_%s' % (id, disp\_point)). The full IDs must already exist and have been used to set the type of dispersion experiment the data is from, spectrometer proton frequency of the data, and if needed the time of the relaxation period.

The format of this text file must be that each row corresponds to a unique spin system and that there is one file per dispersion point (i.e. per CPMG frequency nu\_CPMG or per spin-lock field strength nu1). The file must be in columnar format and information to identify the spin must be in columns of the file.

### 17.2.175 relax\_disp.r2eff\_read\_spin



#### Synopsis

Read R2eff/R1rho values and errors for a single spin from a file.

#### Defaults

**relax\_disp.r2eff\_read\_spin**(id=None, spin\_id=None, file=None, dir=None, disp\_point\_col=None, offset\_col=None, data\_col=2, error\_col=3, sep=None)

#### Keyword arguments

**id**: The experiment ID string to identify this data with.

**spin\_id**: The spin identification string.

**file**: The name of the file.

**dir**: The directory name.

**disp\_point\_col**: The column containing the CPMG frequency or spin-lock field strength (Hz).

**offset\_col**: The column containing the offset information for R1rho-type data.

**data\_col**: The column containing the R2eff or R1rho data.

**error\_col**: The column containing the R2eff or R1rho error.

**sep**: The column separator (the default is white space).

#### Description

This will read R2eff/R1rho data for a single spin directly from a file. The data will be associated with an experiment ID string. This ID can be used for setting the type of dispersion experiment the data is from, spectrometer proton frequency of the data, and the time of the relaxation period.

The format of this text file must be that each row corresponds to a dispersion point (i.e. per CPMG frequency nu\_CPMG or per spin-lock field strength nu1) and that there is one file per unique spin system. The file must be in columnar format. For R1rho data, the dispersion point column can be substituted for the offset values in Hertz.



**17.2.176 relax\_disp.relax\_time****Synopsis**

Set the relaxation delay time associated with each spectrum.

**Defaults**

`relax_disp.relax_time(spectrum_id=None, time=0.0)`

**Keyword arguments**

`spectrum_id`: The spectrum ID string.

`time`: The time, in seconds, of the relaxation period.

**Description**

Peak intensities should be loaded before calling this user function via the `spectrum.read_intensities` user function. The intensity values will then be associated with a spectrum identifier. To associate each spectrum identifier with a time point in the relaxation curve prior to optimisation, this user function should be called.

**17.2.177 relax\_disp.select\_model****Synopsis**

Select the relaxation dispersion model.

**Defaults**

`relax_disp.select_model(model='R2eff')`

**Keyword arguments**

`model`: The type of relaxation dispersion model to fit.

**Description**

A number of different dispersion models are supported. This includes both analytic models and numerical models. Models which are independent of the experimental data type are:

**'R2eff'** – This is the model used to determine the  $R_{2eff}/R_{1\rho}$  values and errors required as the base data for all other models,

**The no chemical exchange models**

**'No  $R_{ex}$ '** – This is the model for no chemical exchange being present.

**The SQ CPMG-type experiments**

The currently supported analytic models are:

**'LM63'** – The original Luz and Meiboom (1963) 2-site fast exchange equation with parameters  $\{R_{20}, \dots, \phi_{ex}, k_{ex}\}$ ,

**'LM63 3-site'** – The original Luz and Meiboom (1963) 3-site fast exchange equation with parameters  $\{R_{20}, \dots, \phi_{ex}, k_{ex}, \phi_{ex2}, k_{ex2}\}$ ,

**'CR72'** – The reduced Carver and Richards (1972) 2-site equation for most time scales whereby the simplification  $R_{20A} = R_{20B}$  is assumed. The parameters are  $\{R_{20}, \dots, pA, dw, k_{ex}\}$ ,

**'CR72 full'** – The full Carver and Richards (1972) 2-site equation for most time scales with parameters  $\{R_{20A}, R_{20B}, \dots, pA, dw, k_{ex}\}$ ,

‘IT99’ – The Ishima and Torchia (1999) 2-site model for all time scales with  $pA \gg pB$  and with parameters  $\{R20, \dots, pA, dw, kex\}$ ,

‘TSMFK01’ – The Tollinger, Kay et al. (2001) 2-site very-slow exchange model, range of microsecond to second time scale. Applicable in the limit of slow exchange, when  $\frac{1}{R20A} \gg \frac{1}{R20B} \gg \frac{1}{k_{AB}}$ .  $R20A$  is the transverse relaxation rate of site A in the absence of exchange.  $2\tau_{CP}$  is the time between successive 180 deg. pulses. The parameters are  $\{R20A, \dots, dw, k_{AB}\}$ .

‘B14’ – The Baldwin (2014) 2-site exact solution model for all time scales, whereby the simplification  $R20A = R20B$  is assumed. The parameters are  $\{R20, \dots, pA, dw, kex\}$ ,

‘B14 full’ – The Baldwin (2014) 2-site exact solution model for all time scales with parameters  $\{R20A, R20B, \dots, pA, dw, kex\}$ ,

The currently supported numeric models are:

‘NS CPMG 2-site 3D’ – The reduced numerical solution for the 2-site Bloch-McConnell equations using 3D magnetisation vectors whereby the simplification  $R20A = R20B$  is assumed. Its parameters are  $\{R20, \dots, pA, dw, kex\}$ ,

‘NS CPMG 2-site 3D full’ – The full numerical solution for the 2-site Bloch-McConnell equations using 3D magnetisation vectors. Its parameters are  $\{R20A, R20B, \dots, pA, dw, kex\}$ ,

‘NS CPMG 2-site star’ – The reduced numerical solution for the 2-site Bloch-McConnell equations using complex conjugate matrices whereby the simplification  $R20A = R20B$  is assumed. It has the parameters  $\{R20, \dots, pA, dw, kex\}$ ,

‘NS CPMG 2-site star full’ – The full numerical solution for the 2-site Bloch-McConnell equations using complex conjugate matrices with parameters  $\{R20A, R20B, \dots, pA, dw, kex\}$ ,

‘NS CPMG 2-site expanded’ – The numerical solution for the 2-site Bloch-McConnell equations expanded using Maple by Nikolai Skrynnikov. It has the parameters  $\{R20, \dots, pA, dw, kex\}$ .

## The MMQ CPMG-type experiments

The currently supported models are:

‘MMQ CR72’ – The the Carver and Richards (1972) 2-site model for most time scales expanded for MMQ CPMG data by Korzhnev et al., 2004, whereby the simplification  $R20A = R20B$  is assumed. Its parameters are  $\{R20, \dots, pA, dw, dwH, kex\}$ .

‘NS MMQ 2-site’ – The numerical solution for the 2-site Bloch-McConnell equations for combined proton-heteronuclear SQ, ZQ, DQ, and MQ CPMG data whereby the simplification  $R20A = R20B$  is assumed. Its parameters are  $\{R20, \dots, pA, dw, dwH, kex\}$ .

‘NS MMQ 3-site linear’ – The numerical solution for the 3-site Bloch-McConnell equations linearised with  $kAC = kCA = 0$  for combined proton-heteronuclear SQ, ZQ, DQ, and MQ CPMG data whereby the simplification  $R20A = R20B = R20C$  is assumed. Its parameters are  $\{R20, \dots, pA, dw(AB), dwH(AB), kex(AB), pB, dw(BC), dwH(BC), kex(BC)\}$ .

‘NS MMQ 3-site’ – The numerical solution for the 3-site Bloch-McConnell equations for combined proton-heteronuclear SQ, ZQ, DQ, and MQ CPMG data whereby the simplification  $R20A = R20B = R20C$  is assumed. Its parameters are  $\{R20, \dots, pA, dw(AB), dwH(AB), kex(AB), pB, dw(BC), dwH(BC), kex(BC), kex(AC)\}$ .

## The R1rho-type experiments

The currently supported analytic models are:

On-resonance models are:

‘M61’ – The Meiboom (1961) 2-site fast exchange equation with parameters  $\{R1rho', \dots, \phi_{ex}, kex\}$ ,

‘M61 skew’ – The Meiboom (1961) 2-site equation for all time scales with  $pA \gg pB$  and with parameters  $\{R1rho', \dots, pA, dw, kex\}$ ,

Off-resonance models are:

‘DPL94’ – The Davis, Perlman and London (1994) 2-site fast exchange equation with parameters  $\{R1rho', \dots, \phi_{ex}, kex\}$ ,

‘TP02’ – The Trott and Palmer (2002) 2-site equation for all time scales with parameters  $\{R1rho', \dots, pA, dw, kex\}$ .

‘TAP03’ – The Trott, Abergel and Palmer (2003) off-resonance 2-site equation for all time scales with parameters  $\{R1rho', \dots, pA, dw, kex\}$ .

‘MP05’ – The Miloushev and Palmer (2005) 2-site off-resonance equation for all time scales with parameters  $\{R1rho', \dots, pA, dw, kex\}$ .

The currently supported numeric models are:

‘NS R1rho 2-site’ – The numerical solution for the 2-site Bloch-McConnell equations using 3D magnetisation vectors whereby the simplification  $R20A = R20B$  is assumed. Its parameters are  $\{R1rho', \dots, pA, dw, kex\}$ .

‘NS R1rho 3-site linear’ – The numerical solution for the 3-site Bloch-McConnell equations using 3D magnetisation vectors whereby the simplification  $R20A = R20B = R20C$  is assumed and linearised with  $kAC = kCA = 0$ . Its parameters are  $\{R1rho', \dots, pA, dw(AB), kex(AB), pB, dw(BC), kex(BC)\}$ .

‘NS R1rho 3-site’ – The numerical solution for the 3-site Bloch-McConnell equations using 3D magnetisation vectors. Its parameters are {R1rho’, ..., pA, dw(AB), kex(AB), pB, dw(BC), kex(BC), kex(AC)}.

## Prompt examples

To pick the 2-site fast exchange model for all selected spins, type one of:

```
relax> relax_disp.select_model('LM63')
```

```
relax> relax_disp.select_model(model='LM63')
```

## 17.2.178 relax\_disp.sherekhan\_input



### Synopsis

Create the input files for Adam Mazur’s ShereKhan program.

### Defaults

**relax\_disp.sherekhan\_input**(force=False, spin\_id=None, dir=None)

### Keyword arguments

**force**: A flag which if set to True will cause the files to be overwritten if they already exist.

**spin\_id**: The spin identification string.

**dir**: The directory name to place ShereKhan cluster folders into.

### Description

This creates the files required for the ShereKhan server located at <http://sherekhan.bionmr.org/>. One file per spin cluster per field strength will be created. These will be placed in the directory ‘cluster $x$ ’ and named ‘sherekhan\_frq $y$ .in’, where  $x$  is the cluster index starting from 1 and  $y$  is the magnetic field strength index starting from 1.

**17.2.179 relax\_disp.spin\_lock\_field****Synopsis**

Set the relaxation dispersion spin-lock field strength (nu1).

**Defaults**

**relax\_disp.spin\_lock\_field**(spectrum\_id=None, field=None)

**Keyword arguments**

**spectrum\_id**: The spectrum ID string to associate the spin-lock field strength to.

**field**: The spin-lock field strength, nu1, in Hz.

**Description**

This sets the spin-lock field strength, nu1, for the specified R1rho spectrum in Hertz.

**Prompt examples**

To set a spin-lock field strength of 2.1 kHz for the spectrum 'nu1\_2.1kHz\_relaxT\_0.010', type one of:

```
relax> relax_disp.spin_lock_field(2100, '
    nu1_2.1kHz_relaxT_0.010')
```

```
relax> relax_disp.spin_lock_field(field
    =2100, spectrum_id='
    nu1_2.1kHz_relaxT_0.010')
```

**17.2.180 relax\_disp.spin\_lock\_offset****Synopsis**

Set the relaxation dispersion spin-lock offset (omega\_rf).

**Defaults**

**relax\_disp.spin\_lock\_offset**(spectrum\_id=None, offset=None)

**Keyword arguments**

**spectrum\_id**: The spectrum ID string to associate the spin-lock offset to.

**offset**: The spin-lock offset, omega\_rf, in ppm.

**Description**

This sets the spin-lock offset, omega\_rf, for the specified R1rho spectrum in ppm.

**Prompt examples**

To set a spin-lock offset of 110.0 ppm for the spectrum 'nu1\_2.1kHz\_relaxT\_0.010', type one of:

```
relax> relax_disp.spin_lock_offset('
    nu1_2.1kHz_relaxT_0.010', 110.0)
```

```
relax> relax_disp.spin_lock_offset(
    spectrum_id='nu1_2.1kHz_relaxT_0.010',
    offset=110.0)
```

**17.2.181 relax\_disp.write\_disp-  
\_curves****Synopsis**

Create text files of the dispersion curves for each spin system.

**Defaults**

**relax\_disp.write\_disp\_curves**(dir=None, force=False)

**Keyword arguments**

**dir**: The directory name to place all of the spin system files into.

**force**: A flag which, if set to True, will cause the files to be overwritten.

**Description**

This is used to create text files of the dispersion curves of R2eff/R1rho values, both measured and back calculated from the optimised dispersion model. The columns of the text file will be the experiment name, the magnetic field strength (as the proton frequency in MHz), dispersion point (nu\_CPMG or the spin-lock field strength), the experimental R2eff value, the back-calculated R2eff value, and the experimental R2eff error. One file will be created per spin system with the name 'disp\_x.out', where *x* is the spin ID string.

**17.2.182 relax\_fit.relax\_time****Synopsis**

Set the relaxation delay time associated with each spectrum.

**Defaults**

**relax\_fit.relax\_time**(time=0.0, spectrum\_id=None)

**Keyword arguments**

**time**: The time, in seconds, of the relaxation period.

**spectrum\_id**: The spectrum identification string.

**Description**

Peak intensities should be loaded before calling this user function via the spectrum.read\_intensities user function. The intensity values will then be associated with a spectrum identifier. To associate each spectrum identifier with a time point in the relaxation curve prior to optimisation, this user function should be called.

**17.2.183 relax\_fit.select\_model****Synopsis**

Select the relaxation curve type.

**Defaults**

**relax\_fit.select\_model**(model='exp')

**Keyword arguments**

**model**: The type of relaxation curve to fit.

**Description**

A number of relaxation experiments are supported and include:

The **'exp'** model. This is the default two parameter exponential fit. The magnetisation starts at  $I_0$  and decays to zero. The parameters are  $[R_x, I_0]$  and the equation is  $I(t) = I_0 \exp(-R_x t)$ .

The **'inv'** model. This is the inversion recovery experiment (IR). The magnetisation starts at a negative value at  $-I_0$  and relaxes to a positive  $I_{inf}$  value. The parameters are  $[R_x, I_0, I_{inf}]$  and the equation is  $I(t) = I_{inf} - I_0 \exp(-R_x t)$ .

The **'sat'** model. This is the saturation recovery experiment (SR). The magnetisation starts at zero and relaxes to a positive  $I_{inf}$  value. The parameters are  $[R_x, I_{inf}]$  and the equation is  $I(t) = I_{inf} (1 - \exp(-R_x t))$ .

**17.2.184 reset****Synopsis**

Reinitialise the relax data storage object.

**Defaults**

**reset**()

**Description**

All of the data of the relax data storage object will be erased and hence relax will return to its initial state.

**17.2.185** residue.copy**Synopsis**

Copy all data associated with a residue.

**Defaults**

**residue.copy**(pipe\_from=None, res\_from=None, pipe\_to=None, res\_to=None)

**Keyword arguments**

**pipe\_from**: The data pipe containing the residue from which the data will be copied. This defaults to the current data pipe.

**res\_from**: The residue ID string of the residue to copy the data from.

**pipe\_to**: The data pipe to copy the data to. This defaults to the current data pipe.

**res\_to**: The residue ID string of the residue to copy the data to. If left blank, the new residue will have the same name as the old.

**Description**

This will copy all the data associated with the identified residue to the new, non-existent residue. The new residue cannot currently exist.

**Prompt examples**

To copy the residue data from residue 1 to the new residue 2, type:

```
relax> residue.copy(res_from=':1', res_to=':2')
```

To copy residue 1 of the molecule 'Old mol' to residue 5 of the molecule 'New mol', type:

```
relax> residue.copy(res_from='#Old mol:1', res_to='#New mol:5')
```

To copy the residue data of residue 1 from the data pipe 'm1' to 'm2', assuming the current data pipe is 'm1', type:

```
relax> residue.copy(res_from=':1', pipe_to='m2')
```

```
relax> residue.copy(pipe_from='m1', res_from=':1', pipe_to='m2', res_to=':1')
```

**17.2.186** residue.create**Synopsis**

Create a new residue.

**Defaults**

**residue.create**(res\_num=None, res\_name=None, mol\_name=None)

**Keyword arguments**

**res\_num**: The residue number.

**res\_name**: The name of the residue.

**mol\_name**: The name of the molecule to add the residue to.

**Description**

Using this, a new sequence can be generated without using the sequence user functions. However if the sequence already exists, the new residue will be added to the end of the residue list (the residue numbers of this list need not be sequential). The same residue number cannot be used more than once. A corresponding single spin system will be created for this residue. The spin system number and name or additional spin systems can be added later if desired.

**Prompt examples**

The following sequence of commands will generate the sequence 1 ALA, 2 GLY, 3 LYS:

```
relax> residue.create(1, 'ALA')
```

```
relax> residue.create(2, 'GLY')
```

```
relax> residue.create(3, 'LYS')
```

---

**17.2.187** `residue.delete`**Synopsis**

Delete residues from the current data pipe.

**Defaults**

`residue.delete(res_id=None)`

**Keyword arguments**

`res_id`: The residue ID string.

**Description**

This can be used to delete a single or sets of residues. See the ID string documentation for more information. If spin system/atom ids are included a `RelaxError` will be raised.

**Spin ID string documentation**

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string `'@H*'` will select the protons 'H', 'H2', 'H98'.

---

**17.2.188** `residue.display`**Synopsis**

Display information about the residue(s).

**Defaults**

`residue.display(res_id=None)`

**Keyword arguments**

`res_id`: The residue ID string.

**Description**

This will display the residue data loaded into the current data pipe.

**Spin ID string documentation**

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string `'@H*'` will select the protons 'H', 'H2', 'H98'.



## 17.2.189 residue.name



### Prompt examples

The following sequence of commands will rename the sequence {1 ALA, 2 GLY, 3 LYS} to {1 XXX, 2 XXX, 3 XXX}:

```
relax> residue.name(':1', 'XXX', force=True)
```

```
relax> residue.name(':2', 'XXX', force=True)
```

```
relax> residue.name(':3', 'XXX', force=True)
```

Alternatively:

```
relax> residue.name(':1,2,3', 'XXX', force=True)
```

### Synopsis

Name the residues.

### Defaults

```
residue.name(res.id=None, name=None, force=False)
```

### Keyword arguments

**res.id:** The residue ID string corresponding to one or more residues.

**name:** The new name.

**force:** A flag which if True will cause the residue to be renamed.

### Description

This simply allows residues to be named (or renamed).

### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is '#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]', where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

## 17.2.190 `residue.number`



### Prompt examples

The following sequence of commands will renumber the sequence {1 ALA, 2 GLY, 3 LYS} to {101 ALA, 102 GLY, 103 LYS}:

```
relax> residue.number(':1', 101, force=True)
```

```
relax> residue.number(':2', 102, force=True)
```

```
relax> residue.number(':3', 103, force=True)
```

### Synopsis

Number the residues.

### Defaults

`residue.number(res_id=None, number=None, force=False)`

### Keyword arguments

**res\_id:** The residue ID string corresponding to a single residue.

**number:** The new residue number.

**force:** A flag which if True will cause the residue to be renumbered.

### Description

This simply allows residues to be numbered. The new number cannot correspond to an existing residue.

### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

---

**17.2.191 results.display****Synopsis**

Display the results.

**Defaults**

**results.display()**

**Description**

This will print to screen (STDOUT) the results contained within the current data pipe.

---

**17.2.192 results.read****Synopsis**

Read the contents of a relax results file into the relax data store.

**Defaults**

**results.read(file='results', dir=None)**

**Keyword arguments**

**file:** The name of the file to read results from.

**dir:** The directory where the file is located.

**Description**

This is able to handle uncompressed, bzip2 compressed files, or gzip compressed files automatically. The full file name including extension can be supplied, however, if the file cannot be found the file with '.bz2' appended followed by the file name with '.gz' appended will be searched for.

---

**17.2.193 results.write****Synopsis**

Write the results to a file.

**Defaults**

**results.write**(file='results', dir='pipe\_name',  
compress\_type=1, force=False)

**Keyword arguments**

**file**: The name of the file to output results to. The default is 'results'. Optionally this can be a file object, or any object with a write() method.

**dir**: The directory name.

**compress\_type**: The type of compression to use when creating the file.

**force**: A flag which if True will cause the results file to be overwritten.

**Description**

This will write the entire contents of the current data pipe into an XML formatted file. This results file can then be read back into relax at a later point in time, or transferred to another machine. This is in contrast to the state.save user function whereby the entire data store, including all data pipes, are saved into a similarly XML formatted file.

To place the results file in the current working directory in the prompt and scripting modes, leave the directory unset. If the directory is set to the special name 'pipe\_name', then the results file will be placed into a directory with the same name as the current data pipe.

The default behaviour of this function is to compress the file using bzip2 compression. If the extension '.bz2' is not included in the file name, it will be added. The compression can, however, be changed to either no compression or gzip compression. This is controlled by the compression type which can be set to

- 0 – No compression (no file extension),
- 1 – bzip2 compression ('.bz2' file extension),
- 2 – gzip compression ('.gz' file extension).

The complementary read function will automatically handle the compressed files.

**17.2.194 script****Synopsis**

Execute a relax script.

**Defaults**

**script**(file=None, dir=None)

**Keyword arguments**

**file**: The name of the file containing the relaxation data.

**dir**: The directory where the file is located.

**Description**

This will execute a relax or any ordinary Python script.

---

**17.2.195 select.all****Synopsis**

Select all spins in the current data pipe.

**Defaults**

`select.all()`

**Description**

This will select all spins, irregardless of their current state.

**Prompt examples**

To select all spins, simply type:

```
relax> select.all()
```

**17.2.196 select.display****Synopsis**

Display the current spin selection status.

**Defaults**

`select.display()`

**Description**

This simply prints out the current spin selections.

**Prompt examples**

To show the current selections, type:

```
relax> select.display()
```

### 17.2.197 select.domain



To select all spins of the domain 'N-dom', preserving the current selections, simply type one of:

```
relax> select.domain('N-dom', 'AND', True)
```

```
relax> select.domain(domain_id='N-dom',  
                     boolean='AND', change_all=True)
```

## Synopsis

Select all spins and interatomic data containers of a domain.

## Defaults

```
select.domain(domain_id=None, boolean='AND',  
change_all=True)
```

## Keyword arguments

**domain\_id:** The domain ID string of the domain to select.

**boolean:** The boolean operator specifying how interatomic data containers should be selected.

**change\_all:** A flag specifying if all non-matching spin and interatomic data containers should be deselected.

## Description

This will select all spins and interatomic data containers of a given domain. This is defined by the domain ID string as specified by the previously executed domain-related user functions.

## Boolean operators

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

## Prompt examples

To select all spins of the domain 'N-dom', simply type one of:

```
relax> select.domain('N-dom', change_all=  
True)
```

```
relax> select.domain(domain_id='N-dom',  
                     change_all=True)
```

**17.2.198 select.interatom****Synopsis**

Select specific interatomic data containers.

**Defaults**

**select.interatom**(spin\_id1=None, spin\_id2=None, boolean='OR', change\_all=False)

**Keyword arguments**

**spin\_id1**: The spin ID string of the first spin of the interatomic data container.

**spin\_id2**: The spin ID string of the second spin of the interatomic data container.

**boolean**: The boolean operator specifying how interatomic data containers should be selected.

**change\_all**: A flag specifying if all other interatomic data containers should be changed.

**Description**

This is used to select specific interatomic data containers which store information about spin pairs such as RDCs, NOEs, dipole-dipole pairs involved in relaxation, etc. The 'change\_all' flag default is False meaning that all interatomic data containers currently either selected or deselected will remain that way. Setting this to True will cause all interatomic data containers not specified by the spin ID strings to be selected.

**Boolean operators**

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

**Prompt examples**

To select all N-H backbone bond vectors of a protein, assuming these interatomic data containers have been already set up, type one of:

```
relax> select.interatom('@N', '@H')
```

```
relax> select.interatom(spin_id1='@N',
                        spin_id2='@H')
```

To select all H-H interatomic vectors of a small organic molecule, type one of:

```
relax> select.interatom('@H*', '@H*')
```

```
relax> select.interatom(spin_id1='@H*',
                        spin_id2='@H*')
```

## 17.2.199 select.read



### Synopsis

Select the spins contained in a file.

### Defaults

`select.read(file=None, dir=None, spin_id_col=None, mol_name_col=None, res_num_col=None, res_name_col=None, spin_num_col=None, spin_name_col=None, sep=None, spin_id=None, boolean='OR', change_all=False)`

### Keyword arguments

**file:** The name of the file containing the list of spins to select.

**dir:** The directory where the file is located.

**spin\_id\_col:** The spin ID string column (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col:** The molecule name column (alternative to the spin\_id\_col).

**res\_num\_col:** The residue number column (alternative to the spin\_id\_col).

**res\_name\_col:** The residue name column (alternative to the spin\_id\_col).

**spin\_num\_col:** The spin number column (alternative to the spin\_id\_col).

**spin\_name\_col:** The spin name column (alternative to the spin\_id\_col).

**sep:** The column separator (the default is white space).

**spin\_id:** The spin ID string to restrict the loading of data to certain spin subsets.

**boolean:** The boolean operator specifying how spins should be selected.

**change\_all:** A flag specifying if all other spins should be changed.

spin number and/or spin name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID string can be used to restrict the reading to certain spin types, for example only  $^{15}\text{N}$  spins when only residue information is in the file.

Empty lines and lines beginning with a hash are ignored.

The 'change\_all' flag default is False meaning that all spins currently either selected or deselected will remain that way. Setting this to True will cause all spins not specified in the file to be deselected.

### Boolean operators

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

### Prompt examples

To select all residues listed with residue numbers in the first column of the file 'isolated\_peaks', type one of:

```
relax> select.read('isolated_peaks',
                  res_num_col=1)
```

```
relax> select.read(file='isolated_peaks',
                  res_num_col=1)
```

To select the spins in the second column of the relaxation data file 'r1.600' while deselecting all other spins, for example type:

```
relax> select.read('r1.600', spin_num_col=2,
                  change_all=True)
```

```
relax> select.read(file='r1.600',
                  spin_num_col=2, change_all=True)
```

### Description

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number, residue name,



---

**17.2.200 select.reverse****Synopsis**

Reversal of the spin selection for the given spins.

**Defaults**

`select.reverse(spin_id=None)`

**Keyword arguments**

`spin_id`: The spin ID string.

**Description**

By supplying the spin ID string, a subset of spins can have their selection status reversed.

**Prompt examples**

To select all currently deselected spins and deselect those which are selected type:

```
relax> select.reverse()
```

---

**17.2.201 select.sn\_ratio****Synopsis**

Select spins with signal to noise ratio higher or lower than the given ratio.

**Defaults**

`select.sn_ratio(ratio=10.0, operation='<', all_sn=True)`

**Keyword arguments**

`ratio`: The signal to noise ratio to compare to.

`operation`: The comparison operation by which to select the spins.

`all_sn`: A flag specifying if all the signal to noise ratios per spin should match the comparison operator, or if just a single comparison match is enough.

**Description**

The comparison operation is the method which to select spins according to: `operation(sn_ratio, ratio)`.

The possible operations are: '<':strictly less than, '<=':less than or equal, '>':strictly greater than, '>=':greater than or equal, '==':equal, '!=':not equal.

The '`all_sn`' flag default is True, meaning that if all of the spin's signal to noise levels evaluates to True in the comparison, the spin is selected.

**Prompt examples**

To select all spins with a signal to noise ratio higher than 10.0:

```
relax> select.sn_ratio(ratio=10.0, operation
                      = '>')
```

```
relax> select.sn_ratio(ratio=10.0, operation
                      = '>', all_sn=False)
```

**17.2.202 select.spin****Synopsis**

Select specific spins.

**Defaults**

`select.spin(spin_id=None, boolean='OR', change_all=False)`

**Keyword arguments**

**spin\_id**: The spin ID string.

**boolean**: The boolean operator specifying how spins should be selected.

**change\_all**: A flag specifying if all other spins should be changed.

**Description**

The 'change all' flag default is False meaning that all spins currently either selected or deselected will remain that way. Setting this to True will cause all spins not specified by the spin ID string to be selected.

**Boolean operators**

The boolean operator can be used to change how spin systems or interatomic data containers are selected. The allowed values are: 'OR', 'NOR', 'AND', 'NAND', 'XOR', 'XNOR'. The following table details how the selections will occur for the different boolean operators.

Please see Table 17.1 on page 459.

**Prompt examples**

To select only glycines and alanines, assuming they have been loaded with the names GLY and ALA, type one of:

```
relax> select.spin(spin_id=':GLY|:ALA')
```

To select residue 5 CYS in addition to the currently selected residues, type one of:

```
relax> select.spin(':5')
```

```
relax> select.spin(':5&:CYS')
```

```
relax> select.spin(spin_id=':5&:CYS')
```

**17.2.203 sequence.attach\_protons****Synopsis**

Attach protons to all heteronuclei.

**Defaults**

`sequence.attach_protons()`

**Description**

This can be used to attach protons to all the heteronuclei in the current data pipe. For each proton, a spin container will be created. This should be used when the sequence information is not being extracted from a 3D structure. Note that the proton spin containers will not possess any positional information, so for analyses which require this position or vectors from one atom to this proton, it should not be used.

**Prompt examples**

To attach protons, simply type:

```
relax> sequence.attach_protons()
```

---

**17.2.204 sequence.copy****Synopsis**

Copy the molecule, residue, and spin sequence data from one data pipe to another.

**Defaults**

`sequence.copy(pipe_from=None, pipe_to=None, empty=True)`

**Keyword arguments**

**pipe\_from:** The name of the data pipe to copy the sequence data from.

**pipe\_to:** The name of the data pipe to copy the sequence data to.

**empty:** A flag which if True will create a molecule, residue, and spin sequence in the target pipe lacking all of the spin data of the source pipe. If False, then the spin data will also be copied.

**Description**

This will copy the sequence data between data pipes. The destination data pipe must not contain any sequence data. If the source and destination pipes are not specified, then both will default to the current data pipe (hence providing one is essential).

**Prompt examples**

To copy the sequence from the data pipe 'm1' to the current data pipe, type:

```
relax> sequence.copy('m1')
```

```
relax> sequence.copy(pipe_from='m1')
```

To copy the sequence from the current data pipe to the data pipe 'm9', type:

```
relax> sequence.copy(pipe_to='m9')
```

To copy the sequence from the data pipe 'm1' to 'm2', type:

```
relax> sequence.copy('m1', 'm2')
```

```
relax> sequence.copy(pipe_from='m1', pipe_to='m2')
```

---

**17.2.205 sequence.display****Synopsis**

Display sequences of molecules, residues, and/or spins.

**Defaults**

`sequence.display(sep=None, mol_name_flag=True, res_num_flag=True, res_name_flag=True, spin_num_flag=True, spin_name_flag=True)`

**Keyword arguments**

**sep:** The column separator (the default of None corresponds to white space).

**mol\_name\_flag:** A flag which if True will cause the molecule name column to be shown.

**res\_num\_flag:** A flag which if True will cause the residue number column to be shown.

**res\_name\_flag:** A flag which if True will cause the residue name column to be shown.

**spin\_num\_flag:** A flag which if True will cause the spin number column to be shown.

**spin\_name\_flag:** A flag which if True will cause the spin name column to be shown.

**Description**

This will print out the sequence information of all loaded spins in the current data pipe.

## 17.2.206 sequence.read



### Synopsis

Read the molecule, residue, and spin sequence from a file.

### Defaults

`sequence.read(file=None, dir=None, spin_id_col=None, mol_name_col=None, res_num_col=None, res_name_col=None, spin_num_col=None, spin_name_col=None, sep=None, spin_id=None)`

### Keyword arguments

**file:** The name of the file containing the sequence data.

**dir:** The directory where the file is located.

**spin\_id\_col:** The spin ID string column (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col:** The molecule name column (alternative to the spin\_id\_col).

**res\_num\_col:** The residue number column (alternative to the spin\_id\_col).

**res\_name\_col:** The residue name column (alternative to the spin\_id\_col).

**spin\_num\_col:** The spin number column (alternative to the spin\_id\_col).

**spin\_name\_col:** The spin name column (alternative to the spin\_id\_col).

**sep:** The column separator (the default is white space).

**spin\_id:** The spin ID string to restrict the loading of data to certain spin subsets.

### Description

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number, residue name, spin number and/or spin name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID string can be used to restrict the reading to certain spin types, for example only <sup>15</sup>N spins when only residue information is in the file.

### Prompt examples

The following commands will read protein backbone <sup>15</sup>N sequence data out of a file called 'seq' where the residue numbers and names are in the first and second columns respectively:

```
relax> sequence.read('seq')
```

```
relax> sequence.read('seq', res_num_col=1,
                    res_name_col=2)
```

```
relax> sequence.read(file='seq', res_num_col=
                    =1, res_name_col=2, sep=None)
```

The following commands will read the residue sequence out of the file 'noe.out' which also contains the NOE values:

```
relax> sequence.read('noe.out')
```

```
relax> sequence.read('noe.out', res_num_col=
                    =1, res_name_col=2)
```

```
relax> sequence.read(file='noe.out',
                    res_num_col=1, res_name_col=2)
```

The following commands will read the sequence out of the file 'noe.600.out' where the residue numbers are in the second column, the names are in the sixth column and the columns are separated by commas:

```
relax> sequence.read('noe.600.out',
                    res_num_col=2, res_name_col=6, sep=',')
```

```
relax> sequence.read(file='noe.600.out',
                    res_num_col=2, res_name_col=6, sep=',')
```

The following commands will read the RNA residues and atoms (including C2, C5, C6, C8, N1, and N3) from the file '500.NOE', where the residue number, residue name, spin number, and spin name are in the first to fourth columns respectively:

```
relax> sequence.read('500.NOE', res_num_col=
                    =1, res_name_col=2, spin_num_col=3,
                    spin_name_col=4)
```

```
relax> sequence.read(file='500.NOE',
                    res_num_col=1, res_name_col=2,
                    spin_num_col=3, spin_name_col=4)
```

---

17.2.207 sequence.write

## Synopsis

Write the molecule, residue, and spin sequence to a file.

## Defaults

**sequence.write**(file=None, dir=None, sep=None, mol\_name\_flag=True, res\_num\_flag=True, res\_name\_flag=True, spin\_num\_flag=True, spin\_name\_flag=True, force=False)

## Keyword arguments

**file**: The name of the file.

**dir**: The directory name.

**sep**: The column separator (the default of None corresponds to white space).

**mol\_name\_flag**: A flag which if True will cause the molecule name column to be shown.

**res\_num\_flag**: A flag which if True will cause the residue number column to be shown.

**res\_name\_flag**: A flag which if True will cause the residue name column to be shown.

**spin\_num\_flag**: A flag which if True will cause the spin number column to be shown.

**spin\_name\_flag**: A flag which if True will cause the spin name column to be shown.

**force**: A flag which if True will cause the file to be overwritten.

## Description

Write the sequence data to file. If no directory name is given, the file will be placed in the current working directory.

---

17.2.208 spectrometer.frequency $\omega$ 

## Synopsis

Set the spectrometer proton frequency of the experiment.

## Defaults

**spectrometer.frequency**(id=None, frq=None, units='Hz')

## Keyword arguments

**id**: The experiment identification string to set the frequency of.

**frq**: The spectrometer frequency. See the 'sfrq' parameter in the Varian procpa file or the 'SF01' parameter in the Bruker acqu file.

**units**: The units of frequency.

## Description

This allows the spectrometer frequency of a given experiment to be set. The expected units are that of the proton resonance frequency in Hertz. See the 'sfrq' parameter in the Varian procpa file or the 'SF01' parameter in the Bruker acqu file for the exact value.

### 17.2.209 spectrometer- .temperature



#### Synopsis

Specify the temperature of an experiment.

#### Defaults

`spectrometer.temperature(id=None, temp=None)`

#### Keyword arguments

**id**: The experiment identification string.

**temp**: The temperature of the experiment in Kelvin.

#### Description

This allows the temperature of an experiment to be set. This value should be in Kelvin. In certain analyses, for example those which use pseudocontact shift data, knowledge of the temperature is essential. For the pseudocontact shift, the experiment ID string should match one of the alignment IDs.

### 17.2.210 spectrum.baseplane- \_rmsd



#### Synopsis

Set the baseplane RMSD of a given spin in a spectrum for error analysis.

#### Defaults

`spectrum.baseplane_rmsd(error=0.0, spectrum_id=None, spin_id=None)`

#### Keyword arguments

**error**: The baseplane RMSD error value.

**spectrum\_id**: The spectrum ID string.

**spin\_id**: The spin ID string.

#### Description

The spectrum ID identifies the spectrum associated with the error and must correspond to a previously loaded set of intensities. If the spin ID is unset, then the error value for all spins will be set to the supplied value.

**17.2.211 spectrum.delete****Synopsis**

Delete the spectral data corresponding to the spectrum ID string.

**Defaults**

`spectrum.delete(spectrum_id=None)`

**Keyword arguments**

`spectrum_id`: The unique spectrum ID string.

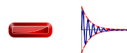
**Description**

The spectral data corresponding to the given spectrum ID string will be removed from the current data pipe.

**Prompt examples**

To delete the peak height data corresponding to the ID 'R1 ncyc5', type:

```
relax> spectrum.delete('R1 ncyc5')
```

**17.2.212 spectrum.error\_analysis****Synopsis**

Perform an error analysis for peak intensities.

**Defaults**

`spectrum.error_analysis(subset=None)`

**Keyword arguments**

`subset`: The list of spectrum ID strings to restrict the error analysis to.

**Description**

This user function must only be called after all peak intensities have been loaded and all other necessary spectral information set. This includes the baseplane RMSD and the number of points used in volume integration, both of which are only used if spectra have not been replicated.

The error analysis can be restricted to a subset of the loaded spectral data. This is useful, for example, if half the spectra have been collected on one spectrometer and the other half on a different spectrometer.

Six different types of error analysis are supported depending on whether peak heights or volumes are supplied, whether noise is determined from replicated spectra or the RMSD of the baseplane noise, and whether all spectra or only a subset have been duplicated. These are:

Please see Table 17.25 on page 606.

**Peak heights with baseplane noise RMSD**

When none of the spectra have been replicated, then the peak height errors are calculated using the RMSD of the baseplane noise, the value of which is set by the `spectrum.baseplane_rmsd` user function. This results in a different error per peak per spectrum. The standard deviation error measure for the peak height, `sigma_I`, is set to the RMSD value.

Table 17.25: The six peak intensity error analysis types.

| Int type | Noise source                           | Error scope                          |
|----------|----------------------------------------|--------------------------------------|
| Heights  | RMSD baseplane                         | One sigma per peak per spectrum      |
| Heights  | Partial duplicate + variance averaging | One sigma for all peaks, all spectra |
| Heights  | All replicated + variance averaging    | One sigma per replicated spectra set |
| Volumes  | RMSD baseplane                         | One sigma per peak per spectrum      |
| Volumes  | Partial duplicate + variance averaging | One sigma for all peaks, all spectra |
| Volumes  | All replicated + variance averaging    | One sigma per replicated spectra set |

### Peak heights with partially replicated spectra

When spectra are replicated, the variance for a single spin at a single replicated spectra set is calculated by the formula

$$\sigma^2 = \text{sum}(\{I_i - I_{av}\}^2) / (n - 1),$$

where  $\sigma^2$  is the variance,  $\sigma$  is the standard deviation,  $n$  is the size of the replicated spectra set with  $i$  being the corresponding index,  $I_i$  is the peak intensity for spectrum  $i$ , and  $I_{av}$  is the mean over all spectra *i.e.* the sum of all peak intensities divided by  $n$ .

As the value of  $n$  in the above equation is always very low since normally only a couple of spectra are collected per replicated spectra set, the variance of all spins is averaged for a single replicated spectra set. Although this results in all spins having the same error, the accuracy of the error estimate is significantly improved.

If there are in addition to the replicated spectra loaded peak intensities which only consist of a single spectrum, *i.e.* not all spectra are replicated, then the variances of replicated replicated spectra sets will be averaged. This will be used for the entire experiment so that there will be only a single error value for all spins and for all spectra.

### Peak heights with all spectra replicated

If all spectra are collected in duplicate (triplicate or higher number of spectra are supported), the each replicated spectra set will have its own error estimate. The error for a single peak is calculated as when partially replicated spectra are collected, and these are again averaged to give a single error per replicated spectra set. However as all replicated spectra sets will have their own error estimate, variance averaging across all spectra sets will not be performed.

### Peak volumes with baseplane noise RMSD

The method of error analysis when no spectra have been replicated and peak volumes are used is highly dependent

on the integration method. Many methods simply sum the number of points within a fixed region, either a box or oval object. The number of points used,  $N$ , must be specified by another user function in this class. Then the error is simply given by the sum of variances:

$$\sigma_{vol}^2 = \sigma_i^2 * N,$$

where  $\sigma_{vol}$  is the standard deviation of the volume,  $\sigma_i$  is the standard deviation of a single point assumed to be equal to the RMSD of the baseplane noise, and  $N$  is the total number of points used in the summation integration method. For a box integration method, this converts to the Nicholson, Kay, Baldissari, Arango, Young, Bax, and Torchia (1992) Biochemistry, 31: 5253-5263 equation:

$$\sigma_{vol} = \sigma_i * \sqrt{n*m},$$

where  $n$  and  $m$  are the dimensions of the box. Note that a number of programs, for example peakint ([http://hugin.ethz.ch/wuthrich/software/xeasy/xeasy\\_m15.html](http://hugin.ethz.ch/wuthrich/software/xeasy/xeasy_m15.html)) does not use all points within the box. And if the number  $N$  can not be determined, this category of error analysis is not possible.

Also note that non-point summation methods, for example when line shape fitting is used to determine peak volumes, the equations above cannot be used. Hence again this category of error analysis cannot be used. This is the case for one of the three integration methods used by Sparky (<http://www.cgl.ucsf.edu/home/sparky/manual/peaks.html#Integration>). And if fancy techniques are used, for example as Cara does to deconvolute overlapping peaks (<http://www.cara.ethz.ch/Wiki/Integration>), this again makes this error analysis impossible.

### Peak volumes with partially replicated spectra

When peak volumes are measured by any integration method and a few of the spectra are replicated, then the intensity errors are calculated identically as described in the 'Peak heights with partially replicated spectra' section above.



## Peak volumes with all spectra replicated

With all spectra replicated and again using any integration methodology, the intensity errors can be calculated as described in the ‘Peak heights with all spectra replicated’ section above.

---

### 17.2.213 spectrum.error\_analysis\_per\_field



#### Synopsis

Use spectrum IDs per each field strength for an error analysis for peak intensities.

#### Defaults

`spectrum.error_analysis_per_field()`

#### Description

Please see the `spectrum.error_analysis` user function documentation.

This user function will collect all spectrum IDs for each field strength separately, and call the `spectrum.error_analysis` with these.

This function is meant as a short-cut for the `spectrum.error_analysis` function.

#### Prompt examples

To collect all spectrum IDs per field strength, and perform peak intensity error analysis:

```
relax> spectrum.error_analysis_per_field()
```

---

**17.2.214 spectrum.integration-  
\_points****Synopsis**

Set the number of summed points used in volume integration of a given spin in a spectrum.

**Defaults**

**spectrum.integration\_points**(N=None, spectrum\_id=None, spin\_id=None)

**Keyword arguments**

**N**: The number of points used by the summation volume integration method.

**spectrum\_id**: The spectrum ID string.

**spin\_id**: Restrict setting the number to certain spins.

**Description**

For a complete description of which integration methods and how many points N are used for different integration techniques, please see the `spectrum.error_analysis` user function documentation.

The spectrum ID identifies the spectrum associated with the value of N and must correspond to a previously loaded set of intensities. If the spin ID is unset, then the number of summed points for all spins will be set to the supplied value.

---

**17.2.215 spectrum.read-  
\_intensities****Synopsis**

Read peak intensities from a file.

**Defaults**

**spectrum.read\_intensities**(file=None, dir=None, spectrum\_id=None, dim=1, int\_method='height', int\_col=None, spin\_id\_col=None, mol\_name\_col=None, res\_num\_col=None, res\_name\_col=None, spin\_num\_col=None, spin\_name\_col=None, sep=None, spin\_id=None, ncproc=None)

**Keyword arguments**

**file**: The name of the file or the list of files containing the intensity data.

**dir**: The directory where the file is located.

**spectrum\_id**: The unique spectrum ID string or list of strings to associate with the peak intensity values. If multiple files are given, then each file should have a corresponding spectrum ID string. If 'auto' is provided for a NMRPipe seriesTab formatted file, the IDs are auto generated in form of Z\_A{i}.

**dim**: Associate the data with the spins of any dimension in the peak list. This defaults to w1, the heteronucleus in HSQC type experiments.

**int\_method**: The method by which peaks were integrated.

**int\_col**: The optional column containing the peak intensity data (used by the generic intensity file format, or if the intensities are in a non-standard column).

**spin\_id\_col**: The spin ID string column used by the generic intensity file format (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col**: The molecule name column used by the generic intensity file format (alternative to the spin ID column).

**res\_num\_col**: The residue number column used by the generic intensity file format (alternative to the spin ID column).

**res\_name\_col**: The residue name column used by the generic intensity file format (alternative to the spin ID column).

**spin\_num\_col:** The spin number column used by the generic intensity file format (alternative to the spin ID column).

**spin\_name\_col:** The spin name column used by the generic intensity file format (alternative to the spin ID column).

**sep:** The column separator used by the generic intensity format (the default is white space).

**spin\_id:** The spin ID string used to restrict the loading of data to certain spin subsets.

**ncproc:** The Bruker specific FID intensity scaling factor.

## Description

The peak intensity can either be from peak heights or peak volumes.

The spectrum ID is a label which is subsequently utilised by other user functions. If this identifier matches that of a previously loaded set of intensities, then this indicates a replicated spectrum.

The spectral dimension is used to specify if the intensity data should be loaded into the spins identified by the first dimension w1, second dimension w2, etc.

The integration method is required for the subsequent error analysis. When peak heights are measured, this should be set to **'height'**. Volume integration methods are a bit varied and hence two values are accepted. If the volume integration involves pure point summation, with no deconvolution algorithms or other methods affecting peak heights, then the value should be set to **'point sum'**. All other volume integration methods, e.g. line shape fitting, the value should be set to **'other'**.

If a series of intensities extracted from Bruker FID files processed in Topspin or XWinNMR are to be compared, the ncproc parameter may need to be supplied. This is because this FID is stored using integer representation and is scaled using ncproc to avoid numerical truncation artifacts. If two spectra have significantly different maximal intensities, then ncproc will be different for both. The intensity scaling is binary, i.e.  $2^{**ncproc}$ . Therefore if spectrum A has an ncproc of 6 and spectrum B a value of 7, then a reference intensity in B will be double that of A. Internally, relax stores the intensities scaled by  $2^{**ncproc}$ .

## File formats

The peak list or intensity file will be automatically determined.

**Sparky peak list:** The file should be a Sparky peak list saved after typing the command **'lt'**. The default is to assume that columns 0, 1, 2, and 3 (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup>) contain the Sparky assignment, w1, w2, and peak intensity data respectively. The frequency data w1 and w2 are ignored while the peak intensity data can either be the peak height or volume displayed by changing the

window options. If the peak intensity data is not within column 3, set the integration column to the appropriate number (column numbering starts from 0 rather than 1).

**XEasy peak list:** The file should be the saved XEasy text window output of the list peak entries command, **'tw'** followed by **'le'**. As the columns are fixed, the peak intensity column is hardwired to number 10 (the 11<sup>th</sup> column) which contains either the peak height or peak volume data. Because the columns are fixed, the integration column number will be ignored.

**NMRView:** The file should be a NMRView peak list. The default is to use column 16 (which contains peak heights) for peak intensities. To use peak volumes (or evolumes), int\_col must be set to 15.

**NMRPipe seriesTab:** The file should be a NMRPipe-format Spectral Series list. If the spectrum\_id=**'auto'**, the IDs are auto generated in form of Z\_A{i}.

**Generic intensity file:** This is a generic format which can be created by scripting to support non-supported peak lists. It should contain in the first few columns enough information to identify the spin. This can include columns for the molecule name, residue number, residue name, spin number, and spin name. Alternatively a spin ID string column can be used. The peak intensities can be placed in another column specified by the integration column number. Intensities from multiple spectra can be placed into different columns, and these can then be specified simultaneously by setting the integration column value to a list of columns. This list must be matched by setting the spectrum ID to a list of the same length. If columns are delimited by a character other than whitespace, this can be specified with the column separator. The spin ID can be used to restrict the loading to specific spin subsets.

## Multiple files

The data from multiple files can be loaded simultaneously if a list of files is supplied. In this case, a list of spectrum ID strings of equal length must be supplied.

## Prompt examples

To read the reference and saturated spectra peak heights from the Sparky formatted files **'ref.list'** and **'sat.list'**, type:

```
relax> spectrum.read_intensities(file='
    ref.list', spectrum_id='ref')
```

```
relax> spectrum.read_intensities(file='
    sat.list', spectrum_id='sat')
```

To read the reference and saturated spectra peak heights from the XEasy formatted files **'ref.text'** and **'sat.text'**, type:

```
relax> spectrum.read_intensities(file='
    ref.text', spectrum_id='ref')
```

```
relax> spectrum.read_intensities(file='
    sat.text', spectrum_id='sat')
```

## 17.2.216 spectrum.read\_spins



### Synopsis

Read peak assignments from a file and create spins.

### Defaults

```
spectrum.read_spins(file=None, dir=None, dim=1,
spin_id_col=None, mol_name_col=None, res_num_col=
None, res_name_col=None, spin_num_col=None,
spin_name_col=None, sep=None, spin_id=None)
```

### Keyword arguments

**file:** The name of the file containing the intensity data.

**dir:** The directory where the file is located.

**dim:** Associate the data with the spins of any dimension in the peak list. This defaults to w1, the heteronucleus in HSQC type experiments.

**spin\_id\_col:** The spin ID string column used by the generic intensity file format (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col:** The molecule name column used by the generic intensity file format (alternative to the spin ID column).

**res\_num\_col:** The residue number column used by the generic intensity file format (alternative to the spin ID column).

**res\_name\_col:** The residue name column used by the generic intensity file format (alternative to the spin ID column).

**spin\_num\_col:** The spin number column used by the generic intensity file format (alternative to the spin ID column).

**spin\_name\_col:** The spin name column used by the generic intensity file format (alternative to the spin ID column).

**sep:** The column separator used by the generic intensity format (the default is white space).

**spin\_id:** The spin ID string used to restrict the loading of data to certain spin subsets.

### Description

The spectral dimension is used to specify if the intensity data should be loaded into the spins identified by the first dimension w1, second dimension w2, etc.

### File formats

The peak list or intensity file will be automatically determined.

**Sparky peak list:** The file should be a Sparky peak list saved after typing the command 'lt'. The default is to assume that columns 0, 1, 2, and 3 (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup>) contain the Sparky assignment, w1, w2, and peak intensity data respectively. The frequency data w1 and w2 are ignored while the peak intensity data can either be the peak height or volume displayed by changing the window options. If the peak intensity data is not within column 3, set the integration column to the appropriate number (column numbering starts from 0 rather than 1).

**XEasy peak list:** The file should be the saved XEasy text window output of the list peak entries command, 'tw' followed by 'le'. As the columns are fixed, the peak intensity column is hardwired to number 10 (the 11<sup>th</sup> column) which contains either the peak height or peak volume data. Because the columns are fixed, the integration column number will be ignored.

**NMRView:** The file should be a NMRView peak list. The default is to use column 16 (which contains peak heights) for peak intensities. To use peak volumes (or evolumes), int\_col must be set to 15.

**NMRPipe seriesTab:** The file should be a NMRPipe-format Spectral Series list. If the spectrum\_id='auto', the IDs are auto generated in form of Z\_A{i}.

**Generic intensity file:** This is a generic format which can be created by scripting to support non-supported peak lists. It should contain in the first few columns enough information to identify the spin. This can include columns for the molecule name, residue number, residue name, spin number, and spin name. Alternatively a spin ID string column can be used. The peak intensities can be placed in another column specified by the integration column number. Intensities from multiple spectra can be placed into different columns, and these can then be specified simultaneously by setting the integration column value to a list of columns. This list must be matched by setting the spectrum ID to a list of the same length. If columns are delimited by a character other than whitespace, this can be specified with the column separator. The spin ID can be used to restrict the loading to specific spin subsets.

### Prompt examples

To read the spin assignments from the Sparky formatted files 'ref.list' and 'sat.list', type:

```
relax> spectrum.read_spins(file='ref.list')
```

```
relax> spectrum.read_spins(file='sat.list')
```

To read the spin assignments from the XEasy formatted files 'ref.text' and 'sat.text', type:

```
relax> spectrum.read_spins(file='ref.text')
```

```
relax> spectrum.read_spins(file='sat.text')
```

---

**17.2.217 spectrum.replicated****Synopsis**

Specify which spectra are replicates of each other.

**Defaults**

**spectrum.replicated**(spectrum\_ids=None)

**Keyword arguments**

**spectrum\_ids**: The list of replicated spectra ID strings.

**Description**

This is used to identify which of the loaded spectra are replicates of each other. Specifying the replicates is essential for error analysis if the baseplane RMSD has not been supplied.

**Prompt examples**

To specify that the NOE spectra labelled 'ref1', 'ref2', and 'ref3' are the same spectrum replicated, type one of:

```
relax> spectrum.replicated(['ref1', 'ref2',
    'ref3'])
```

```
relax> spectrum.replicated(spectrum_ids=['
    ref1', 'ref2', 'ref3'])
```

To specify that the two R<sub>2</sub> spectra 'ncyc2' and 'ncyc2b' are the same time point, type:

```
relax> spectrum.replicated(['ncyc2', 'ncyc2b
    '])
```

---

**17.2.218 spectrum.sn\_ratio****Synopsis**

Calculate the signal to noise ratio for all selected spins.

**Defaults**

**spectrum.sn\_ratio**()

**Description**

This user function will per spin calculate the signal to noise ratio: S/N.

**Prompt examples**

To calculate the Signal to Noise ratio per spin.

```
relax> spectrum.sn_ratio()
```

**17.2.219 spin.copy****Synopsis**

Copy all data associated with a spin.

**Defaults**

**spin.copy**(pipe\_from=None, spin\_from=None, pipe\_to=None, spin\_to=None)

**Keyword arguments**

**pipe\_from**: The data pipe containing the spin from which the data will be copied. This defaults to the current data pipe.

**spin\_from**: The spin identifier string of the spin to copy the data from.

**pipe\_to**: The data pipe to copy the data to. This defaults to the current data pipe.

**spin\_to**: The spin identifier string of the spin to copy the data to. If left blank, the new spin will have the same name as the old.

**Description**

This will copy all the data associated with the identified spin to the new, non-existent spin. The new spin must not already exist.

**Prompt examples**

To copy the spin data from spin 1 to the new spin 2, type:

```
relax> spin.copy(spin_from='@1', spin_to='@2')
'
```

To copy spin 1 of the molecule 'Old mol' to spin 5 of the molecule 'New mol', type:

```
relax> spin.copy(spin_from='#Old mol@1',
                 spin_to='#New mol@5')
```

To copy the spin data of spin 1 from the data pipe 'm1' to 'm2', assuming the current data pipe is 'm1', type:

```
relax> spin.copy(spin_from='@1', pipe_to='m2')
'
```

```
relax> spin.copy(pipe_from='m1', spin_from='@1',
                 pipe_to='m2', spin_to='@1')
```

**17.2.220 spin.create****Synopsis**

Create a new spin.

**Defaults**

**spin.create**(spin\_name=None, spin\_num=None, res\_name=None, res\_num=None, mol\_name=None)

**Keyword arguments**

**spin\_name**: The name of the spin.

**spin\_num**: The spin number.

**res\_name**: The name of the residue to add the spin to.

**res\_num**: The number of the residue to add the spin to.

**mol\_name**: The name of the molecule to add the spin to.

**Description**

This will add a new spin data container to the relax data storage object. The same spin number cannot be used more than once.

**Prompt examples**

The following sequence of commands will add the spins 1 C4, 2 C9, 3 C15 to residue number 10:

```
relax> spin.create('C4', 1, res_num=10)
```

```
relax> spin.create('C9', 2, res_num=10)
```

```
relax> spin.create('C15', 3, res_num=10)
```

---

### 17.2.221 spin.create\_pseudo



contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

#### Synopsis

Create a spin system representing a pseudo-atom.

#### Prompt examples

The following will create the pseudo-atom named 'Q9' consisting of the protons '@H16', '@H17', '@H18':

```
relax> spin.create_pseudo('Q9', members=['  
@H16', '@H17', '@H18'])
```

#### Defaults

`spin.create_pseudo(spin_name=None, spin_num=None, res_id=None, members=None, averaging='linear')`

#### Keyword arguments

**spin\_name:** The name of the pseudo-atom spin.

**spin\_num:** The spin number.

**res\_id:** The molecule and residue ID string identifying the position to add the pseudo-spin to.

**members:** A list of the atoms (as spin ID strings) that the pseudo-atom is composed of.

**averaging:** The positional averaging technique.

#### Description

This will create a spin data container representing a number of pre-existing spin containers as a pseudo-atom. The optional spin number must not already exist.

#### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is '#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]', where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not

---

**17.2.222 spin.delete****Synopsis**

Delete spins.

**Defaults**

`spin.delete(spin_id=None)`

**Keyword arguments**

`spin_id`: The spin identifier string.

**Description**

This can be used to delete a single or sets of spins. See the identification string documentation below for more information.

**Spin ID string documentation**

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string `'@H*'` will select the protons 'H', 'H2', 'H98'.

---

**17.2.223 spin.display****Synopsis**

Display information about the spin(s).

**Defaults**

`spin.display(spin_id=None)`

**Keyword arguments**

`spin_id`: The spin identification string.

**Description**

This will display the spin data loaded into the current data pipe.

**Spin ID string documentation**

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string `'@H*'` will select the protons 'H', 'H2', 'H98'.



---

### 17.2.224 spin.element



#### Prompt examples

The set all spins of residue 1 to be carbons, type one of:

```
relax> spin.element('@1', 'C', force=True)
```

```
relax> spin.element(spin_id='@1', element='C', force=True)
```

#### Synopsis

Set the element type of the spin.

#### Defaults

```
spin.element(element=None, spin_id=None, force=False)
```

#### Keyword arguments

**element:** The IUPAC element name.

**spin\_id:** The spin identification string corresponding to one or more spins.

**force:** A flag which if True will cause the element to be changed.

#### Description

This allows the element type of the spins to be set.

#### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

## 17.2.225 spin.isotope



### Synopsis

Set the spins' nuclear isotope type.

### Defaults

`spin.isotope(isotope=None, spin_id=None, force=False)`

### Keyword arguments

**isotope:** The nuclear isotope name in the AE notation - the atomic mass number followed by the element symbol.

**spin\_id:** The spin identification string corresponding to one or more spins.

**force:** A flag which if True will cause the nuclear isotope to be changed.

### Description

This allows the nuclear isotope type of the spins to be set.

### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

### Prompt examples

The set all spins of residue 1 to the '13C' nuclear isotope, type one of:



```
relax> spin.isotope('@1', '13C', force=True)
```

```
relax> spin.isotope(spin_id='@1', isotope='13C', force=True)
```

---

### 17.2.226 spin.name



Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

#### Prompt examples

The following sequence of commands will rename the sequence {1 C1, 2 C2, 3 C3} to {1 C11, 2 C12, 3 C13}:

```
relax> spin.name('@1', 'C11', force=True)
```

```
relax> spin.name('@2', 'C12', force=True)
```

```
relax> spin.name('@3', 'C13', force=True)
```

#### Synopsis

Name the spins.

#### Defaults

```
spin.name(name=None, spin_id=None, force=False)
```

#### Keyword arguments

**name:** The new name.

**spin\_id:** The spin identification string corresponding to one or more spins.

**force:** A flag which if True will cause the spin to be renamed.

#### Description

This simply allows spins to be named (or renamed). Spin naming often essential. For example when reading Sparky peak list files, then the spin name must match that in the file.

#### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is '#<mol\_name> :<res\_id>[, <res\_id>[, <res\_id>, ...]] @<atom\_id>[, <atom\_id>[, <atom\_id>, ...]]', where the token elements are '<mol\_name>', the name of the molecule, '<res\_id>', the residue identifier which can be a number, name, or range of numbers, '<atom\_id>', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

## 17.2.227 spin.number



### Prompt examples

The following sequence of commands will renumber the sequence {1 C1, 2 C2, 3 C3} to {-1 C1, -2 C2, -3 C3}:

```
relax> spin.number('@1', -1, force=True)
```

```
relax> spin.number('@2', -2, force=True)
```

```
relax> spin.number('@3', -3, force=True)
```

### Synopsis

Number the spins.

### Defaults

`spin.number(spin_id=None, number=None, force=False)`

### Keyword arguments

**spin\_id:** The spin identification string corresponding to a single spin.

**number:** The new spin number.

**force:** A flag which if True will cause the spin to be renumbered.

### Description

This simply allows spins to be numbered. The new number cannot correspond to an existing spin number.

### Spin ID string documentation

The identification string is composed of three components: the molecule ID token beginning with the '#' character, the residue ID token beginning with the ':' character, and the atom or spin system ID token beginning with the '@' character. Each token can be composed of multiple elements - one per spin - separated by the ',' character and each individual element can either be a number (which must be an integer, in string format), a name, or a range of numbers separated by the '-' character. Negative numbers are supported. The full ID string specification is `'#<mol_name> :<res_id>[, <res_id>[, <res_id>, ...]] @<atom_id>[, <atom_id>[, <atom_id>, ...]]'`, where the token elements are '`<mol_name>`', the name of the molecule, '`<res_id>`', the residue identifier which can be a number, name, or range of numbers, '`<atom_id>`', the atom or spin system identifier which can be a number, name, or range of numbers.

If one of the tokens is left out then all elements will be assumed to match. For example if the string does not contain the '#' character then all molecules will match the string. If only the molecule ID component is specified, then all spins of the molecule will match.

Regular expression can be used to select spins. For example the string '@H\*' will select the protons 'H', 'H2', 'H98'.

**17.2.228 state.load****Synopsis**

Load a saved program state.

**Defaults**

**state.load**(state='state.bz2', dir=None, force=False)

**Keyword arguments**

**state**: The file name, which can be a string or a file descriptor object, of a saved program state.

**dir**: The name of the directory in which the file is found.

**force**: A boolean flag which if True will cause the current program state to be overwritten.

**Description**

This is able to handle uncompressed, bzip2 compressed files, or gzip compressed files automatically. The full file name including extension can be supplied, however, if the file cannot be found, this function will search for the file name with `'.bz2'` appended followed by the file name with `'.gz'` appended.

For more advanced users, file descriptor objects are supported. If the force flag is set to True, then the relax data store will be reset prior to the loading of the saved state.

**Prompt examples**

The following commands will load the state saved in the file `'save'`.

```
relax> state.load('save')
```

```
relax> state.load(state='save')
```

Use one of the following commands to load the state saved in the bzip2 compressed file `'save.bz2'`:

```
relax> state.load('save')
```

```
relax> state.load(state='save')
```

```
relax> state.load('save.bz2')
```

```
relax> state.load(state='save.bz2', force=
    True)
```

**17.2.229 state.save****Synopsis**

Save the program state.

**Defaults**

**state.save**(state='state.bz2', dir=None, compress\_type=1, force=False)

**Keyword arguments**

**state**: The file name, which can be a string or a file descriptor object, to save the current program state in.

**dir**: The name of the directory in which to place the file.

**compress\_type**: The type of compression to use when creating the file.

**force**: A boolean flag which if set to True will cause the file to be overwritten.

**Description**

This will place the program state - the relax data store - into a file for later reloading or reference. The default format is an XML formatted file.

The default behaviour of this function is to compress the file using bzip2 compression. If the extension `'.bz2'` is not included in the file name, it will be added. The compression can, however, be changed to either no compression or gzip compression. This is controlled by the compression type which can be set to

0 – No compression (no file extension).

1 – bzip2 compression (`'.bz2'` file extension).

2 – gzip compression (`'.gz'` file extension).

**Prompt examples**

The following commands will save the current program state, uncompressed, into the file `'save'`:

```
relax> state.save('save', compress_type=0)
```

```
relax> state.save(state='save',
    compress_type=0)
```

The following commands will save the current program state into the bzip2 compressed file 'save.bz2':

```
relax> state.save('save')
```

```
relax> state.save(state='save')
```

```
relax> state.save('save.bz2')
```

```
relax> state.save(state='save.bz2')
```

If the file 'save' already exists, the following commands will save the current program state by overwriting the file.

```
relax> state.save('save', force=True)
```

```
relax> state.save(state='save', force=True)
```

---

## 17.2.230 statistics.aic



### Synopsis

Calculate and store Akaike's Information Criterion (AIC) for each model.

### Defaults

`statistics.aic()`

### Description

This will perform a calculation to obtain the chi-squared statistic for the current parameter values for each model, count the number of parameters per model and calculate Akaike's Information Criterion (AIC) using the formula  $AIC = \chi^2 + 2k$ . The AIC values, chi-squared values, and number of parameters will be stored in the appropriate location for the model in the relax data store.

---

17.2.231 statistics.model

## Synopsis

Calculate and store the model statistics.

## Defaults

**statistics.model()**

## Description

This will perform a back-calculation to obtain the chi-squared statistic for the current parameter values, count the number of parameters and data points per model, and place all the values in the relax data store.

---

17.2.232 structure.add\_atom

## Synopsis

Add an atom.

## Defaults

**structure.add\_atom**(mol\_name=None, atom\_name=None, res\_name=None, res\_num=None, pos=None, element=None, atom\_num=None, chain\_id=None, segment\_id=None, pdb\_record=None)

## Keyword arguments

**mol\_name:** The name of molecule container to create or add the atom to.

**atom\_name:** The atom name.

**res\_name:** The residue name.

**res\_num:** The residue number.

**pos:** The atomic coordinates. For specifying different coordinates for each model of the ensemble, a list of lists can be supplied.

**element:** The element name.

**atom\_num:** The optional atom number.

**chain\_id:** The optional chain ID string.

**segment\_id:** The optional segment ID string.

**pdb\_record:** The optional PDB record name, e.g. 'ATOM' or 'HETATM'.

## Description

This allows atoms to be added to the internal structural object. To use the same atomic coordinates for all models, the atomic position can be an array of 3 values. Alternatively different coordinates can be used for each model if the atomic position is a rank-2 array where the first dimension matches the number of models currently present.

---

**17.2.233 structure.add\_model****Synopsis**

Add a new model.

**Defaults**

**structure.add\_model**(model\_num=None)

**Keyword arguments**

**model\_num**: The number of the new model.

**Description**

This allows new models to be added to the internal structural object. Note that no structural information is allowed to be present.

**17.2.234 structure.atomic\_fluctuations****Synopsis**

Create an interatomic distance fluctuation correlation matrix.

**Defaults**

**structure.atomic\_fluctuations**(pipes=None, models=None, molecules=None, atom\_id=None, measure='distance', file=None, format='text', dir=None, force=False)

**Keyword arguments**

**pipes**: The data pipes to generate the interatomic distance fluctuation correlation matrix for.

**models**: The list of models for each data pipe to generate the interatomic distance fluctuation correlation matrix for. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules**: The list of molecules for each data pipe to generate the interatomic distance fluctuation correlation matrix for. This allows differently named molecules in the same or different data pipes to be superimposed. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id**: The atom identification string of the coordinates of interest. This can be used to restrict the correlation matrix to one atom per residue, for example.

**measure**: The type of fluctuation to investigate. This allows for both interatomic distance and vector angle fluctuations to be calculated.

**file**: The name of the text file to create.

**format**: The output format. For all formats other than the text file, a second file will be created with the same name as the text file but with the appropriate file extension added.

**dir**: The directory to save the file to.

**force**: A flag which if set to True will cause any pre-existing files to be overwritten.



## Description

This is used to visualise the interatomic fluctuations between different structures. By setting the measure argument, different categories of fluctuations can be seen:

**‘distance’** – The interatomic distance fluctuations is the default option. The corrected sample standard deviation (SD) is calculated for the distances between all atom pairs, resulting in a pairwise matrix of SD values. This is frame independent and hence is superimposition independent.

**‘angle’** – The interatomic vector angle fluctuations. The corrected sample standard deviation (SD) is calculated for the angles between the inter atom vectors all atom pairs to an average vector. This also produces a pairwise matrix of SD values.

**‘parallax shift’** – The interatomic parallax shift fluctuations. The corrected sample standard deviation (SD) is calculated for the parallax shift between the inter atom vectors all atom pairs to an average vector. This also produces a pairwise matrix of SD values. The parallax shift is calculated as the dot product of the interatomic vector and the unit average vector, times the unit average vector. It is a frame and superimposition dependent measure close to orthogonal to the interatomic distance fluctuations. It is similar to the angle measure however, importantly, it is independent of the distance between the two atoms.

For the output file, the currently supported formats are:

**‘text’** – This is the default value and will result in a single text file being created.

**‘gnuplot’** – This will create a both a text file with the data and a script for visualising the correlation matrix using gnuplot. The script will have the same name as the text file, however the file extension will be changed to \*.gnu.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to ‘@N,C,CA,O’, assuming those are the names of the atoms in the 3D structural file.

## Prompt examples

To create the interatomic distance fluctuation correlation matrix for the models 1, 3, and 5, type:

```
relax> structure.atomic_fluctuations(models
    =[[1, 3, 5]], file='
    atomic_fluctuation_matrix')
```

To create the interatomic distance fluctuation correlation matrix for the molecules ‘A’, ‘B’, ‘C’, and ‘D’, type:

```
relax> structure.atomic_fluctuations(
    molecules=[['A', 'B', 'C', 'D']], file=
    'atomic_fluctuation_matrix')
```

---

**17.2.235 structure.com****Synopsis**

Calculate the centre of mass (CoM) for all structures.

**Defaults**

`structure.com(model=None, atom_id=None)`

**Keyword arguments**

**model:** The optional structural model number to restrict the calculation of the centre of mass to.

**atom\_id:** The atom identification string to restrict the CoM calculation to.

**Description**

This user function will calculate the centre of mass (CoM) for all loaded structures, printing out the position and storing it in the current data pipe.

**Prompt examples**

To determine the centre of mass of all structure, simply type:

```
relax> structure.com()
```

---

**17.2.236 structure.connect\_atom****Synopsis**

Connect two atoms.

**Defaults**

`structure.connect_atom(index1=None, index2=None)`

**Keyword arguments**

**index1:** The global index of the first atom.

**index2:** The global index of the second atom.

**Description**

This allows atoms to be connected in the internal structural object. The global index is normally equal to the PDB atom number minus 1.

### 17.2.237 structure.create\_diff\_tensor\_pdb



#### Synopsis

Create a PDB file to represent the diffusion tensor.

#### Defaults

**structure.create\_diff\_tensor\_pdb**(scale=1.8e-06, file='tensor.pdb', dir=None, force=False)

#### Keyword arguments

**scale**: Value for scaling the diffusion rates.

**file**: The name of the PDB file.

**dir**: The directory to place the file into.

**force**: A flag which, if set to True, will overwrite the any pre-existing file.

#### Description

This creates a PDB file containing an artificial geometric structure to represent the diffusion tensor. A structure must have previously been read into relax. The diffusion tensor is represented by an ellipsoidal, spheroidal, or spherical geometric object with its origin located at the centre of mass (of the selected residues). This diffusion tensor PDB file can subsequently read into any molecular viewer.

There are four different types of residue within the PDB. The centre of mass of the selected residues is represented as a single carbon atom of the residue 'COM'. The ellipsoidal geometric shape consists of numerous H atoms of the residue 'TNS'. The axes of the tensor, when defined, are presented as the residue 'AXS' and consist of carbon atoms: one at the centre of mass and one at the end of each eigenvector. Finally, if Monte Carlo simulations were run and the diffusion tensor parameters were allowed to vary then there will be multiple 'SIM' residues, one for each simulation. These are essentially the same as the 'AXS' residue, representing the axes of the simulated tensors, and they will appear as a distribution.

As the Brownian rotational diffusion tensor is a measure of the rate of rotation about different axes - the larger the geometric object, the faster the diffusion of a molecule. For example the diffusion tensor of a water molecule is much larger than that of a macromolecule.

The effective global correlation time experienced by an XH bond vector, not to be confused with the Lipari and

Szabo parameter  $\tau_e$ , will be approximately proportional to the component of the diffusion tensor parallel to it. The approximation is not exact due to the multiexponential form of the correlation function of Brownian rotational diffusion. If an XH bond vector is parallel to the longest axis of the tensor, it will be unaffected by rotations about that axis, which are the fastest rotations of the molecule, and therefore its effective global correlation time will be maximal.

To set the size of the diffusion tensor within the PDB frame the unit vectors used to generate the geometric object are first multiplied by the diffusion tensor (which has the units of inverse seconds) then by the scaling factor (which has the units of second Å and has the default value of 1.8e-6 s.Å). Therefore the rotational diffusion rate per Å is equal the inverse of the scale value (which defaults to 5.56e5 s<sup>-1</sup>Å). Using the default scaling value for spherical diffusion, the correspondence between global correlation time,  $\mathcal{Q}_{iso}$  diffusion rate, and the radius of the sphere for a number of discrete cases will be:



Please see Table 17.26 on page 626.

The scaling value has been fixed to facilitate comparisons within or between publications, but can be changed to vary the size of the tensor geometric object if necessary. Reporting the rotational diffusion rate per Å within figure legends would be useful.

To create the tensor PDB representation, a number of algorithms are utilised. Firstly the centre of mass is calculated for the selected residues and is represented in the PDB by a C atom. Then the axes of the diffusion are calculated, as unit vectors scaled to the appropriate length (multiplied by the eigenvalue  $\mathcal{Q}_x$ ,  $\mathcal{Q}_y$ ,  $\mathcal{Q}_z$ ,  $\mathcal{Q}_{||}$ ,  $\mathcal{Q}_{\perp}$ , or  $\mathcal{Q}_{iso}$  as well as the scale value), and a C atom placed at the position of this vector plus the centre of mass. Finally a uniform distribution of vectors on a sphere is generated using spherical coordinates. By incrementing the polar angle using an arccos distribution, a radial array of vectors representing latitude are created while incrementing the azimuthal angle evenly creates the longitudinal vectors. These unit vectors, which are distributed within the PDB frame and are of 1 Å in length, are first rotated into the diffusion frame using a rotation matrix (the spherical diffusion tensor is not rotated). Then they are multiplied by the diffusion tensor matrix to extend the vector out to the correct length, and finally multiplied by the scale value so that the vectors reasonably superimpose onto the macromolecular structure. The last set of algorithms place all this information into a PDB file. The distribution of vectors are represented by H atoms and are all connected using PDB CONECT records. Each H atom is connected to its two neighbours on the both the longitude and latitude. This creates a geometric PDB object with longitudinal and latitudinal lines.

Table 17.26: Diffusion tensor PDB representation sizes using the default scaling for different diffusion tensors

| $\tau_m$ (ns) | $\mathcal{D}_{iso}$ ( $s^{-1}$ ) | Radius ( $\text{\AA}$ ) |
|---------------|----------------------------------|-------------------------|
| 1             | 1.67e8                           | 300                     |
| 3             | 5.56e7                           | 100                     |
| 10            | 1.67e7                           | 30                      |
| 30            | 5.56e6                           | 10                      |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                            |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                     | <b>Description</b>                                                                                                                                                                                                                                         |
| <p><b>17.2.238</b> <code>structure.create_rotor_pdb</code></p> <div style="display: flex; align-items: center; margin-top: 10px;">   </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p>This creates a PDB file representation of a rotor motional model. The model axis is defined by a vector and a single point on the axis. The centre of the representation will be taken as the point on the rotor axis closest to the given centre position. The size of the representation is defined by the span, which is the distance from the central point to the rotors, and the length of the blades.</p> |                                                                                                                                                                                                                                                            |
| <p><b>Synopsis</b></p> <p>Create a PDB file representation of a rotor.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                     | <p><b>Prompt examples</b></p> <p>The following is a synthetic example:</p> <pre>relax&gt; structure.create_rotor_pdb(file='rotor.pdb', rotor_angle=20.0, axis=[0., 0., 1.], axis_pt=[1., 1., 0.], centre=[0., 0., 2.], span=2e-9, blade_length=1e-9)</pre> |
| <p><b>Defaults</b></p> <p><code>structure.create_rotor_pdb</code>(file='rotor.pdb', dir=None, rotor_angle=0.0, axis=None, axis_pt=None, centre=None, span=2e-09, blade_length=5e-10, force=False, staggered=False)</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                            |
| <p><b>Keyword arguments</b></p> <p><b>file:</b> The name of the PDB file.</p> <p><b>dir:</b> The directory to place the file into.</p> <p><b>rotor_angle:</b> The angle of the rotor motion in degrees.</p> <p><b>axis:</b> The vector defining the rotor axis.</p> <p><b>axis_pt:</b> A point lying anywhere on the rotor axis. This is used to define the position of the axis in 3D space.</p> <p><b>centre:</b> The central point of the representation. If this point is not on the rotor axis, then the closest point on the axis will be used for the centre.</p> <p><b>span:</b> The distance from the central point to the rotor blades (meters).</p> <p><b>blade_length:</b> The length of the representative rotor blades.</p> <p><b>force:</b> A flag which if True will overwrite the file if it already exists.</p> <p><b>staggered:</b> A flag which if True will cause the rotor blades to be staggered. This is used to avoid blade overlap.</p> |                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                            |

**17.2.239 structure.create\_vector\_dist****17.2.240 structure.delete****Synopsis**

Create a PDB file representation of the distribution of XH bond vectors.

**Defaults**

**structure.create\_vector\_dist**(length=2e-09, file='XH\_dist.pdb', dir=None, symmetry=True, force=False)

**Keyword arguments**

**length**: The length of the vectors in the PDB representation (meters).

**file**: The name of the PDB file.

**dir**: The directory to place the file into.

**symmetry**: A flag which if True will create a second chain with reversed XH bond orientations.

**force**: A flag which if True will overwrite the file if it already exists.

**Description**

This creates a PDB file containing an artificial vectors, the length of which default to 20 Å. A structure must have previously been read into relax. The origin of the vector distribution is located at the centre of mass (of the selected residues). This vector distribution PDB file can subsequently be read into any molecular viewer.

Because of the symmetry of the diffusion tensor reversing the orientation of the XH bond vector has no effect. Therefore by setting the symmetry flag two chains 'A' and 'B' will be added to the PDB file whereby chain 'B' is chain 'A' with the XH bonds reversed.

**Synopsis**

Delete structural information.

**Defaults**

**structure.delete**(atom\_id=None, model=None, verbosity=1, spin\_info=True)

**Keyword arguments**

**atom\_id**: The atom identification string.

**model**: Individual structural models from a loaded ensemble can be deleted by specifying the model number.

**verbosity**: The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

**spin\_info**: A flag which if True will cause all structural information in the spin containers and interatomic data containers to be deleted as well. If False, then only the 3D structural data will be deleted.

**Description**

This will delete structural information from the current data pipe. All spin and sequence information loaded from these structures will be preserved - this only affects the structural data. The atom ID argument can be used to restrict deletion to parts of the loaded molecules, or the model argument can be used to delete individual structural models from an ensemble.

**Prompt examples**

To delete everything, simply type:

```
relax> structure.delete()
```

To delete residues 50 to 100 of the molecule called 'Ap4Aase', type one of:

```
relax> structure.delete(':50-100')
```

```
relax> structure.delete(atom_id=':50-100')
```

---

**17.2.241 structure.delete\_ss****Synopsis**

Delete secondary structure information.

**Defaults**

**structure.delete\_ss()**

**Description**

This will delete all secondary structure information from the current data pipe.

**Prompt examples**

To delete all secondary structure, simply type:

```
relax> structure.delete_ss()
```

---

**17.2.242 structure.displacement****Synopsis**

Determine the rotational and translational displacement between a set of models or molecules.

**Defaults**

**structure.displacement**(pipes=None, models=None, molecules=None, atom\_id=None, centroid=None)

**Keyword arguments**

**pipes:** The data pipes to determine the displacements for.

**models:** The list of models for each data pipe to determine the displacements for. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to determine the displacements for. This allows differently named molecules in the same or different data pipes to be superimposed. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest.

**centroid:** The alternative position of the centroid.

**Description**

This user function allows the rotational and translational displacement between different models or molecules to be calculated. The information will be printed out in various formats and held in the relax data store. This is directional, so there is a starting and ending position for each displacement. Therefore the displacements in all directions between all models and molecules will be calculated.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to 'C,N,C,CA,O', assuming those are the names of the atoms in the 3D structural file.

By supplying the position of the centroid, an alternative position than the standard rigid body centre is used as the focal point of the motion. This allows, for example, a pivot of a rotational domain motion to be specified. This is not a formally correct algorithm, all translations will be zero, but does give an indication to the amplitude of the pivoting angle.

### Prompt examples

To determine the rotational and translational displacements between all sets of models, type:

```
relax> structure.displacement()
```

---

## 17.2.243 structure.find\_pivot



### Synopsis

Find the pivot point of the motion of a set of structures.

### Defaults

**structure.find\_pivot**(pipes=None, models=None, molecules=None, atom\_id=None, init\_pos=None, func\_tol=1e-05, box\_limit=200)

### Keyword arguments

**pipes:** The data pipes to use in the motional pivot algorithm.

**models:** The list of models for each data pipe to use in the motional pivot algorithm. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to use in the motional pivot algorithm. This allows differently named molecules in the same or different data pipes to be used. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest.

**init\_pos:** The initial position of the pivot.

**func\_tol:** The function tolerance. This is used to terminate minimisation once the function value between iterations is less than the tolerance. The default value is 1e-5.

**box\_limit:** The pivot point is constrained within a box of +/-  $x$  Å using the logarithmic barrier function together with simplex optimisation. This argument is the value of  $x$ .

### Description

This is used to find pivot point of motion between a set of structural models. If the list of models is not supplied, then all models will be used.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different

atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to '@N,C,CA,O', assuming those are the names of the atoms in the 3D structural file.

By supplying the position of the centroid, an alternative position than the standard rigid body centre is used as the focal point of the superimposition. This allows, for example, the superimposition about a pivot point.

---

## 17.2.244 structure.get\_pos



### Synopsis

Extract the atomic positions from the loaded structures for the given spins.

### Defaults

**structure.get\_pos**(spin\_id=None, ave\_pos=True)

### Keyword arguments

**spin\_id**: The spin identification string.

**ave\_pos**: A flag specifying if the position of the atom is to be averaged across models.

### Description

This allows the atomic positions of the spins to be extracted from the loaded structures. This is automatically performed by the `structure.load_spins` user function, but if the sequence information is generated in other ways, this user function allows the structural information to be obtained.

If averaging the atomic positions, then average position of all models will be loaded into the spin container. Otherwise the positions from all models will be loaded separately.

### Prompt examples

For a model-free backbone amide nitrogen analysis whereby the N spins have already been created, to obtain the backbone N positions from the file '1F3Y.pdb' (which is a single protein), type the following two user functions:

```
relax> structure.read_pdb('1F3Y.pdb')
```

```
relax> structure.get_pos(spin_id='@N')
```



## 17.2.245 structure.load\_spins



### Synopsis

Load spins from the structure into the relax data store.

### Defaults

`structure.load_spins(spin_id=None, from_mols=None, mol_name_target=None, ave_pos=True, spin_num=True)`

### Keyword arguments

**spin\_id:** The spin identification string for the selective loading of certain spins into the relax data store.

**from\_mols:** The list of similar, but not necessarily identical molecules to load spin information from.

**mol\_name\_target:** The name of target molecule container, overriding the name of the loaded structures.

**ave\_pos:** A flag specifying if the position of the atom is to be averaged across models.

**spin\_num:** A flag specifying if the spin number should be loaded.

### Description

This allows a sequence to be generated within the relax data store using the atomic information from the structure already associated with this data pipe. The spin ID string is used to select which molecules, which residues, and which atoms will be recognised as spin systems within relax. If the spin ID is left unspecified, then all molecules, residues, and atoms will be placed within the data store (and all atoms will be treated as spins).

As an alternative to using structural models, by specifying the list of molecules to load spins from similar though not necessarily identical molecules will be combined. In this case, the target molecule name must be supplied to create a single combined molecule. And only a single model can be loaded in the current data pipe. The spin numbering will be dropped to allow for sequential atom numbering in the PDB and other formats. Therefore only the residue number and name and atom name will be preserved for creating the spin containers. If the spin is only present in a subset of the structures, then the positional information will only be taken from that subset and hence the number of positions might be different for different spins.

If averaging the atomic positions, then average position of all models or molecules will be loaded into the spin container. Otherwise the positions from all models or molecules will be loaded separately.

### Prompt examples

For a model-free backbone amide nitrogen analysis, to load just the backbone N sequence from the file '1F3Y.pdb' (which is a single protein), type the following two user functions:

```
relax> structure.read_pdb('1F3Y.pdb')
```

```
relax> structure.load_spins(spin_id='@N')
```

For an RNA analysis of adenine C8 and C2, guanine C8 and N1, cytidine C5 and C6, and uracil N3, C5, and C6, type the following series of commands (assuming that the PDB file with this atom naming has already been read):

```
relax> structure.load_spins(spin_id=':A@C8')
```

```
relax> structure.load_spins(spin_id=':A@C2')
```

```
relax> structure.load_spins(spin_id=':G@C8')
```

```
relax> structure.load_spins(spin_id=':G@N1')
```

```
relax> structure.load_spins(spin_id=':C@C5')
```

```
relax> structure.load_spins(spin_id=':C@C6')
```

```
relax> structure.load_spins(spin_id=':U@N3')
```

```
relax> structure.load_spins(spin_id=':U@C5')
```

```
relax> structure.load_spins(spin_id=':U@C6')
```

Alternatively using some Python programming:

```
relax> for id in [":A@C8", ":A@C2", ":G@C8",
                  ":G@N1", ":C@C5", ":C@C6", ":U@N3", ":U@C5", ":U@C6"]:
    structure.load_spins(spin_id=id)
```

## 17.2.246 structure.mean



## Synopsis

Calculate the mean structure from all loaded models.

## Defaults

**structure.mean**(pipes=None, models=None, molecules=None, atom\_id=None, set\_mol\_name=None, set\_model\_num=None)

## Keyword arguments

**pipes:** The data pipes containing structures to average.

**models:** The list of models for each data pipe containing structures to average. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to average. This allows differently named molecules in the same or different data pipes to be averaged. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest. This can be used to restrict the averaged structure to one atom per residue, for example.

**set\_mol\_name:** Set the optional name of the averaged molecule.

**set\_model\_num:** Set the optional model number of the averaged molecule.

## Description

This will calculate and store the mean structure from a collection of related molecules. If a new molecule name or model number is not supplied, the mean structure will replace all the models in the internal structural object. This is provided as a structural aid, specifically for superimposition purposes.

## 17.2.247 structure.pca



## Synopsis

Principle component analysis (PCA) of the motions in an ensemble of structures.

## Defaults

**structure.pca**(pipes=None, models=None, molecules=None, obs\_pipes=None, obs\_models=None, obs\_molecules=None, atom\_id=None, algorithm='eigen', num\_modes=4, format='grace', dir=None)

## Keyword arguments

**pipes:** The data pipes to perform the PC analysis on.

**models:** The list of models for each data pipe to perform the PC analysis on. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to perform the PC analysis on. The PCA will only be calculated for atoms with identical residue name and number and atom name. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**obs\_pipes:** The data pipes in the PC analysis which will have zero weight. These structures are for comparison.

**obs\_models:** The list of models for each data pipe in the PC analysis which will have zero weight. These structures are for comparison. The number of elements must match the pipes argument. If no models are given, then all will be used.

**obs\_molecules:** The list of molecules for each data pipe in the PC analysis which will have zero weight. These structures are for comparison. The PCA will only be calculated for atoms with identical residue name and number and atom name. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest.

**algorithm:** The PCA algorithm used to find the principle components of. This can be either 'eigen' for an eigenvalue/eigenvector decomposition, or 'svd' for a singular value decomposition.

**num\_modes:** The number of PCA modes to calculate.

**format:** The format of the plot data.

**dir:** The directory to save the graphs into.

## Description

Perform a principle component analysis (PCA) for all the chosen structures. 2D graphs of the PC projections will be generated and placed in the specified directory.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

A subset of the structures can be set as 'observing'. This means that they will have a weight of zero when constructing the covariance matrix and determining its eigenvectors. Therefore the structures will not contribute to the principle components, but will be present and compared to structures used in the analysis.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to '@N,C,CA,O', assuming those are the names of the atoms in the 3D structural file.

## Prompt examples

To determine the PCA modes of all models in the current data pipe, simply type:

```
relax> structure.pca()
```

## 17.2.248 structure.read\_gaussian



## Synopsis

Reading structures from Gaussian log files.

## Defaults

**structure.read\_gaussian**(file=None, dir=None, set\_mol\_name=None, set\_model\_num=None, verbosity=1)

## Keyword arguments

**file:** The name of the Gaussian log file.

**dir:** The directory where the file is located.

**set\_mol\_name:** Set the names of the read molecules. If unset, then the molecules will be automatically labelled based on the file name or other information. This can either be a single name or a list of names.

**set\_model\_num:** Set the model numbers of the loaded molecules. This can be a single number or list of numbers.

**verbosity:** The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

## Description

The atomic positions from a Gaussian log file can be read into relax. If optimisation has been performed, the last set of atomic coordinates from the log will be read to obtain the final structure. The log file can be Gzip or Bzip2 compressed.

The setting of molecule names is used to name the molecules within the Gaussian file. If not set, then the molecules will be named after the file name, with the molecule number appended if more than one exists. By setting the molecule name or setting the model number, the loaded structure can be stored as a specific model or as a different molecule.

## Prompt examples

To load all structures from the Gaussian file 'taxol.log' in the directory '~/logs', including all models and all molecules, type one of:

```
relax> structure.read_gaussian('taxol.log',  
                               '~/logs')
```

```
relax> structure.read_gaussian(file='  
taxol.log', dir=logs')
```

---

## 17.2.249 structure.read\_pdb



### Synopsis

Reading structures from PDB files.

### Defaults

**structure.read\_pdb**(file=None, dir=None, read\_mol=None, set\_mol\_name=None, read\_model=None, set\_model\_num=None, alt\_loc=None, verbosity=1, merge=False)

### Keyword arguments

**file**: The name of the PDB file.

**dir**: The directory where the file is located.

**read\_mol**: If set, only the given molecule(s) will be read. The molecules are numbered consecutively from 1. If unset, then all molecules will be loaded. By providing a list of numbers such as [1, 2], multiple molecules will be read.

**set\_mol\_name**: Set the names of the read molecules. If unset, then the molecules will be automatically labelled based on the file name or other information. This can either be a single name or a list of names.

**read\_model**: If set, only the given model number(s) from the PDB file will be read. Otherwise all models will be read. This can be a single number or list of numbers.

**set\_model\_num**: Set the model numbers of the loaded molecules. If unset, then the PDB model numbers will be preserved if they exist. This can be a single number or list of numbers.

**alt\_loc**: The PDB ATOM record 'Alternate location indicator' field value.

**verbosity**: The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

**merge**: A flag which if set to True will try to merge the PDB structure into the currently loaded structures.

### Description

The reading of PDB files into relax is quite a flexible procedure allowing for both models, defined as an ensemble of the same molecule but with different atomic positions, and different molecules within the same model. One of more molecules can exist in one or more models. The

flexibility allows PDB models to be converted into different molecules and different PDB files loaded as the same molecule but as different models.

In a PDB file, the models are specified by the MODEL PDB record. All the supported PDB readers in relax recognise this. The internal reader defines molecules using the TER PDB record. In both cases, the molecules will be numbered consecutively from 1.

Setting the molecule name allows the molecule within the PDB (within one model) to have a custom name. If not set, then the molecules will be named after the file name, with the molecule number appended if more than one exists.

Note that relax will complain if it cannot work out what to do.

This is able to handle uncompressed, bzip2 compressed files, or gzip compressed files automatically. The full file name including extension can be supplied, however, if the file cannot be found, this function will search for the file name with '.bz2' appended followed by the file name with '.gz' appended.

If a PDB file contains alternative atomic locations, then the alternate location indicator must be specified to allow one of the multiple coordinate sets to be selected.

## Prompt examples

To load all structures from the PDB file 'test.pdb' in the directory '~/pdb', including all models and all molecules, type one of:

```
relax> structure.read_pdb('test.pdb', '~/pdb')
```

```
relax> structure.read_pdb(file='test.pdb',
    dir='pdb')
```

To load the 10<sup>th</sup> model from the file 'test.pdb' and naming it 'CaM', use one of:

```
relax> structure.read_pdb('test.pdb',
    read_model=10, set_mol_name='CaM')
```

```
relax> structure.read_pdb(file='test.pdb',
    read_model=10, set_mol_name='CaM')
```

To load models 1 and 5 from the file 'test.pdb' as two different structures of the same model, type one of:

```
relax> structure.read_pdb('test.pdb',
    read_model=[1, 5], set_model_num=[1,
    1])
```

```
relax> structure.read_pdb('test.pdb',
    set_mol_name=['CaM_1', 'CaM_2'],
    read_model=[1, 5], set_model_num=[1,
    1])
```

To load the files 'lactose\_MCMM4\_S1.1.pdb', 'lactose\_MCMM4\_S1.2.pdb', 'lactose\_MCMM4\_S1.3.pdb' and 'lactose\_MCMM4\_S1.4.pdb' as models, type the following sequence of commands:

```
relax> structure.read_pdb('
    lactose_MCMM4_S1.1.pdb', set_mol_name='
    lactose_MCMM4_S1', set_model_num=1)
```

```
relax> structure.read_pdb('
    lactose_MCMM4_S1.2.pdb', set_mol_name='
    lactose_MCMM4_S1', set_model_num=2)
```

```
relax> structure.read_pdb('
    lactose_MCMM4_S1.3.pdb', set_mol_name='
    lactose_MCMM4_S1', set_model_num=3)
```

```
relax> structure.read_pdb('
    lactose_MCMM4_S1.4.pdb', set_mol_name='
    lactose_MCMM4_S1', set_model_num=4)
```

### 17.2.250 structure.read\_xyz



#### Synopsis

Reading structures from XYZ files.

#### Defaults

**structure.read\_xyz**(file=None, dir=None, read\_mol=None, set\_mol\_name=None, read\_model=None, set\_model\_num=None, verbosity=1)

#### Keyword arguments

**file**: The name of the XYZ file.

**dir**: The directory where the file is located.

**read\_mol**: If set, only the given molecule(s) will be read. The molecules are numbered consecutively from 1. If unset, then all molecules will be loaded. By providing a list of numbers such as [1, 2], multiple molecules will be read.

**set\_mol\_name**: Set the names of the read molecules. If unset, then the molecules will be automatically labelled based on the file name or other information. This can either be a single name or a list of names.

**read\_model**: If set, only the given model number(s) from the PDB file will be read. Otherwise all models will be read. This can be a single number or list of numbers.

**set\_model\_num**: Set the model numbers of the loaded molecules. If unset, then the PDB model numbers will be preserved if they exist. This can be a single number or list of numbers.

**verbosity**: The amount of information to print out. Set to zero to silence the user function, or one to see all messages.

#### Description

The XYZ files with different models, which defined as an ensemble of the same molecule but with different atomic positions, can be read into relax. If there are several molecules in one xyz file, please separate them into different files and then load them individually. Loading different models and different molecules is controlled by specifying the molecule number read, setting the molecule names, specifying which model to read, and setting the model numbers.

The setting of molecule names is used to name the molecules within the XYZ (within one model). If not set,

then the molecules will be named after the file name, with the molecule number appended if more than one exists.

Note that relax will complain if it cannot work out what to do.

#### Prompt examples

To load all structures from the XYZ file 'test.xyz' in the directory '~/xyz', including all models and all molecules, type one of:

```
relax> structure.read_xyz('test.xyz', '~/xyz')
```

```
relax> structure.read_xyz(file='test.xyz',
                           dir='xyz')
```

To load the 10<sup>th</sup> model from the file 'test.xyz' and naming it 'CaM', use one of:

```
relax> structure.read_xyz('test.xyz',
                           read_model=10, set_mol_name='CaM')
```

```
relax> structure.read_xyz(file='test.xyz',
                           read_model=10, set_mol_name='CaM')
```

To load models 1 and 5 from the file 'test.xyz' as two different structures of the same model, type one of:

```
relax> structure.read_xyz('test.xyz',
                           read_model=[1, 5], set_model_num=[1,
1])
```

```
relax> structure.read_xyz('test.xyz',
                           set_mol_name=['CaM_1', 'CaM_2'],
                           read_model=[1, 5], set_model_num=[1,
1])
```

To load the files 'test\_1.xyz', 'test\_2.xyz', 'test\_3.xyz' and 'test\_4.xyz' as models, type the following sequence of commands:

```
relax> structure.read_xyz('test_1.xyz',
                           set_mol_name='test_1', set_model_num=1)
```

```
relax> structure.read_xyz('test_2.xyz',
                           set_mol_name='test_2', set_model_num=2)
```

```
relax> structure.read_xyz('test_3.xyz',
                           set_mol_name='test_3', set_model_num=3)
```

```
relax> structure.read_xyz('test_4.xyz',
                           set_mol_name='test_4', set_model_num=4)
```

---

### 17.2.251 structure.rmsd



#### Synopsis

Determine the RMSD between structures.

#### Defaults

**structure.rmsd**(pipes=None, models=None, molecules=None, atom\_id=None, atomic=False)

#### Keyword arguments

**pipes:** The data pipes to determine the RMSD for.

**models:** The list of models for each data pipe to determine the RMSD for. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to determine the RMSD for. The RMSD will only be calculated for atoms with identical residue name and number and atom name. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest.

**atomic:** A flag which if True will allow for per-atom RMSDs to be additionally calculated.

#### Description

This allows the root mean squared deviation (RMSD) between all structures to be calculated. The RMSDs for individual structures to the mean structure will be calculated and reported. These values averaged to produce a global RMSD stored in the structural object of the current data pipe. If the **'atomic'** argument is set, per-atom RMSDs will additionally be calculated stored in spin containers.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to **'@N,C,CA,O'**, assuming those are the names of the atoms in the 3D structural file.

#### Prompt examples

To determine the RMSD of all models in the current data pipe, simply type:

```
relax> structure.rmsd()
```

For the backbone heavy atom RMSD of all models in the current data pipe, simply type:

```
relax> structure.rmsd(atom_id='@N,C,CA,O')
```

To calculate the C-alpha backbone RMSDs of all models in the current data pipe, type:

```
relax> structure.rmsd(atom_id='CA', atomic=True)
```

**17.2.252 structure.rotate****Synopsis**

Rotate the internal structural object about the given origin by the rotation matrix.

**Defaults**

**structure.rotate**(R=array([[ 1., 0., 0.], [ 0., 1., 0.], [ 0., 0., 1.]]), origin=None, model=None, atom\_id=None)

**Keyword arguments**

**R**: The rotation matrix in forwards rotation notation.

**origin**: The origin or pivot of the rotation.

**model**: The model to rotate (which if not set will cause all models to be rotated).

**atom\_id**: The atom identification string.

**Description**

This is used to rotate the internal structural data by the given rotation matrix. If the origin is supplied, then this will act as the pivot of the rotation. Otherwise, all structural data will be rotated about the point [0, 0, 0]. The rotation can be restricted to one specific model.

**17.2.253 structure.sequence\_alignment****Synopsis**

Multiple sequence alignment (MSA) of structural data.

**Defaults**

**structure.sequence\_alignment**(pipes=None, models=None, molecules=None, msa\_algorithm='Central Star', pairwise\_algorithm=None, matrix=None, gap\_open\_penalty=10.0, gap\_extend\_penalty=1.0, end\_gap\_open\_penalty=0.0, end\_gap\_extend\_penalty=0.0)

**Keyword arguments**

**pipes**: The data pipes to use in the sequence alignment.

**models**: The list of models for each data pipe to use in the sequence alignment. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules**: The list of molecules for each data pipe to use in the sequence alignment. This allows differently named molecules in the same or different data pipes to be superimposed. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**msa\_algorithm**: The multiple sequence alignment (MSA) algorithm used to align all the primary sequence of all structures of interest.

**pairwise\_algorithm**: The pairwise alignment algorithm to align each pair of sequences.

**matrix**: The substitution matrix to use in the pairwise sequence alignment algorithm.

**gap\_open\_penalty**: The penalty for introducing gaps, as a positive number.

**gap\_extend\_penalty**: The penalty for extending a gap, as a positive number.

**end\_gap\_open\_penalty**: The optional penalty for opening a gap at the end of a sequence.

**end\_gap\_extend\_penalty**: The optional penalty for extending a gap at the end of a sequence.



## Description

To find the atoms in common between different molecules, a MSA of the primary sequence of the molecules is required. This sequence alignment will then subsequently be used by any other user function which operates on multiple molecules. The following MSA algorithms can be selected:

**‘Central Star’** – This is a heuristic, progressive alignment method using pairwise alignments to construct a MSA. It consists of four major steps - pairwise alignment between all sequence pairs, finding the central sequence, iteratively aligning the sequences to the gapped central sequence, and introducing gaps in previous alignments during the iterative alignment.

**‘residue number’** – This will simply align the molecules based on residue number.

For the MSA algorithms which require pairwise alignments, the following subalgorithms can be used:

**‘NW70’** – The Needleman-Wunsch alignment algorithm. This has been modified to use the logic of the EMBOSS software for handling gap opening and extension penalties, as well as end penalties.

For the MSAs or pairwise alignments which require a substitution matrix, one of the following can be used:

**‘BLOSUM62’** – The BLOcks SUBstitution Matrix for proteins with a cluster percentage  $\geq 62\%$ .

**‘PAM250’** – The point accepted mutation matrix for proteins with  $n = 250$  evolutionary distance.

**‘NUC 4.4’** – The nucleotide 4.4 matrix for DNA/RNA.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

## Prompt examples

To superimpose the structures in the ‘A’ data pipe onto the structures of the ‘B’ data pipe using backbone heavy atoms, type:

```
relax> structure.sequence_alignment(pipes=['B', 'A'], atom_id='@N,C,CA,O')
```

## 17.2.254 structure.superimpose



## Synopsis

Superimpose a set of structures.

## Defaults

**structure.superimpose**(pipes=None, models=None, molecules=None, atom\_id=None, displace\_id=None, method='fit to mean', centre\_type='centroid', centroid=None)

## Keyword arguments

**pipes:** The data pipes to use in the superimposition.

**models:** The list of models for each data pipe to use in the superimposition. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to use in the superimposition. This allows differently named molecules in the same or different data pipes to be superimposed. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest. This allows a subset of all residues or atoms to be used in the superimposition. For example for protein backbone heavy atoms, this can be set to '@N,C,CA,O'.

**displace\_id:** The atom identification string for restricting the displacement to a subset of all atoms. If not set, then all atoms will be translated and rotated. If supplied as a list of IDs, then the number of items must match the number of structures.

**method:** The superimposition method.

**centre\_type:** The type of centre to user for the superimposition, *i.e.* either the standard centroid superimposition or a superimposition using the centre of mass (CoM).

**centroid:** The alternative position of the centroid.

## Description

This allows a set of related structures to be superimposed to each other. If a multiple sequence alignment (MSA) of the molecules has already been performed with the structure.sequence\_alignment user function, this will

allow residues with different numbering to be superimposed. Otherwise only residues with the same numbering will be used in the superimposition. Two superimposition methods are currently supported:

**‘fit to mean’** – All models are fit to the mean structure. This is the default and most accurate method for an ensemble description. It is an iterative method which first calculates a mean structure and then fits each model to the mean structure using the Kabsch algorithm. This is repeated until convergence.

**‘fit to first’** – This is quicker but is not as accurate for an ensemble description. The Kabsch algorithm is used to rotate and translate each model to be superimposed onto the first model of the first data pipe.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to ‘@N,C,CA,O’, assuming those are the names of the atoms in the 3D structural file.

The displacement ID string, which is similar to the atom ID, gives finer control over which atoms are translated and rotated by the algorithm. When not set this allows, for example, to align structures based on a set of backbone heavy atoms and the backbone protons and side-chains are displaced by default. Or if set to the same as the atom ID, if a single domain is aligned, then just that domain will be displaced.

By supplying the position of the centroid, an alternative position than the standard rigid body centre is used as the focal point of the superimposition. This allows, for example, the superimposition about a pivot point.

## Prompt examples

To superimpose all sets of models, type one of:

```
relax> structure.superimpose()
```

```
relax> structure.superimpose(method='fit to mean')
```

To superimpose the models 1, 2, 3, 5 onto model 4, type:

```
relax> structure.superimpose(models=[[4, 1, 2, 3, 5]], method='fit to first')
```

To superimpose an ensemble of protein structures using only the backbone heavy atoms, type one of:

```
relax> structure.superimpose(atom_id='@N,C,CA,O')
```

```
relax> structure.superimpose(method='fit to mean', atom_id='@N,C,CA,O')
```

To superimpose the structures in the ‘A’ data pipe onto the structures of the ‘B’ data pipe using backbone heavy atoms, type one of:

```
relax> structure.superimpose(['B', 'A'], None, 'fit to first', '@N,C,CA,O')
```

```
relax> structure.superimpose(pipes=['B', 'A'], method='fit to first', atom_id='@N,C,CA,O')
```

---

**17.2.255 structure.translate****Synopsis**

Laterally displace the internal structural object by the translation vector.

**Defaults**

**structure.translate**(T=None, model=None, atom\_id=None)

**Keyword arguments**

**T:** The translation vector.

**model:** The model to translate (which if not set will cause all models to be translate).

**atom\_id:** The atom identification string.

**Description**

This is used to translate the internal structural data by the given translation vector. The translation can be restricted to one specific model.

---

**17.2.256 structure.web\_of\_motion****Synopsis**

Create a PDB representation of motion between structures using a web of interconnecting lines.

**Defaults**

**structure.web\_of\_motion**(pipes=None, models=None, molecules=None, atom\_id=None, file=None, dir=None, force=False)

**Keyword arguments**

**pipes:** The data pipes to generate the web between.

**models:** The list of models for each data pipe to generate the web between. The number of elements must match the pipes argument. If no models are given, then all will be used.

**molecules:** The list of molecules for each data pipe to generate the web between. This allows differently named molecules in the same or different data pipes to be superimposed. The number of elements must match the pipes argument. If no molecules are given, then all will be used.

**atom\_id:** The atom identification string of the coordinates of interest.

**file:** The name of the PDB file.

**dir:** The directory to save the file to.

**force:** A flag which if set to True will cause any pre-existing files to be overwritten.

**Description**

This will create a PDB representation of the motion between the atoms of a given set of structures. Identical atoms of the structures are concatenated into one model, within a temporary internal structural object, linked together using PDB CONECT records, and then written to the PDB file.

Support for multiple structures is provided by the data pipes, model numbers and molecule names arguments. Each data pipe, model and molecule combination will be treated as a separate structure. As only atomic coordinates with the same residue name and number and atom name will be assembled, structures with slightly different atomic structures can be compared. If the list of models is not supplied, then all models of all data pipes will be

used. If the optional molecules list is supplied, each molecule in the list will be considered as a separate structure for comparison between each other.

The atom ID string, which uses the same notation as the spin ID, can be used to restrict the coordinates compared to a subset of molecules, residues, or atoms. For example to only use backbone heavy atoms in a protein, set the atom ID to '@N,C,CA,O', assuming those are the names of the atoms in the 3D structural file.

## Prompt examples

To create a web of motion for the models 1, 3, and 5, type:

```
relax> structure.web_of_motion(models=[[1,
    3, 5]], file='web.pdb')
```

To create a web of motion for the molecules 'A', 'B', 'C', and 'D', type:

```
relax> structure.web_of_motion(molecules=[['
    A', 'B', 'C', 'D']], file='web.pdb')
```

## 17.2.257 structure.write\_pdb



### Synopsis

Writing structures to a PDB file.

### Defaults

```
structure.write_pdb(file=None, dir=None, model_num=
None, compress_type=0, force=False)
```

### Keyword arguments

**file:** The name of the PDB file.

**dir:** The directory where the file is located.

**model\_num:** Restrict the writing of structural data to a single model in the PDB file.

**compress\_type:** The type of compression to use when creating the file.

**force:** A flag which if set to True will cause any pre-existing files to be overwritten.

### Description

This will write all of the structural data loaded in the current data pipe to be converted to the PDB format and written to file. Specifying the model number allows single models to be output.

The default behaviour of this function is to not compress the file. The compression can, however, be changed to either bzip2 or gzip compression. If the '.bz2' or '.gz' extension is not included in the file name, it will be added. This behaviour is controlled by the compression type which can be set to

0 – No compression (no file extension).

1 – bzip2 compression ('.bz2' file extension).

2 – gzip compression ('.gz' file extension).

## Prompt examples

To write all models and molecules to the PDB file 'ensemble.pdb' within the directory '~/pdb', type one of:

```
relax> structure.write_pdb('ensemble.pdb', '  
    ~/pdb')
```

```
relax> structure.write_pdb(file='  
    ensemble.pdb', dir='pdb')
```

To write model number 3 into the new file 'test.pdb', use one of:

```
relax> structure.write_pdb('test.pdb',  
    model_num=3)
```

```
relax> structure.write_pdb(file='test.pdb',  
    model_num=3)
```

---

## 17.2.258 system.cd



### Synopsis

Change the current working directory to the specified path.

### Defaults

`system.cd(path=None)`

### Keyword arguments

`path`: The path to the new current working directory.

### Description

The equivalent of python module `os.chdir(path)`. Change the current working directory to the specified path.

To change the current working directory, type:

```
relax> system.cd("/path/to/dir")
```

**17.2.259 system.pwd****Synopsis**

Display the current working directory.

**Defaults**

`system.pwd()`

**Description**

This will display the current working directory.

The directory can be changed with the `system.cd(path)` user function.

```
relax> system.pwd()
```

```
relax> system.cd("/path/to/dir")
```

**17.2.260 system.sys\_info****Synopsis**

Display all system information relating to this version of relax.

**Defaults**

`system.sys_info()`

**Description**

This will display all of the relax, Python, python package and hardware information currently being used by relax. This is useful for seeing if all packages are up to date and if the correct software versions are being used. It is also very useful information for reporting relax bugs.

**17.2.261 system.time****Synopsis**

Display the current time.

**Defaults**

**system.time()**

**Description**

This user function will display the current time which can be useful for timing long calculations by having time information in any saved log files.

**17.2.262 value.copy****Å****Synopsis**

Copy parameters from one data pipe to another.

**Defaults**

**value.copy**(pipe\_from=None, pipe\_to=None, param=None, force=False)

**Keyword arguments**

**pipe\_from**: The name of the pipe to copy from.

**pipe\_to**: The name of the pipe to copy to.

**param**: The parameter to copy. Only one parameter may be selected.

**force**: A flag which, if set to True, will cause the destination parameter to be overwritten.

**Description**

If this is used to change values of previously minimised parameters, then the minimisation statistics (chi-squared value, iteration count, function count, gradient count, and Hessian count) will be reset.

**Relaxation curve fitting parameters**

Please see Table 17.27 on page 646.

**Model-free parameters**

Please see Table 17.28 on page 646.

Setting a parameter value may have no effect depending on which model-free model is chosen. For example if  $S_f^2$  values and  $S_s^2$  values are set but the data pipe corresponds to the model-free model 'm4' then because these data values are not parameters of the model they will have no effect.

Note that the  $R_{ex}$  values are scaled quadratically with field strength and should be supplied as a field strength independent value. Use the following formula to obtain the correct value:

Table 17.27: Relaxation curve fitting parameters.

| Name | Description                               |
|------|-------------------------------------------|
| rx   | Either the $R_1$ or $R_2$ relaxation rate |
| i0   | The initial intensity                     |
| iinf | The intensity at infinity                 |

Table 17.28: Model-free parameters.

| Name     | Description                                                              |
|----------|--------------------------------------------------------------------------|
| s2       | $S^2$ , the model-free generalised order parameter ( $S^2 = S_f^2.S2s$ ) |
| s2f      | $S_f^2$ , the faster motion model-free generalised order parameter       |
| s2s      | $S_s^2$ , the slower motion model-free generalised order parameter       |
| local_tm | The spin specific global correlation time (seconds)                      |
| te       | Single motion effective internal correlation time (seconds)              |
| tf       | Faster motion effective internal correlation time (seconds)              |
| ts       | Slower motion effective internal correlation time (seconds)              |
| rex      | Chemical exchange relaxation ( $\sigma_{ex} = R_{ex} / \omega^2$ )       |
| csa      | Chemical shift anisotropy (unitless)                                     |

`value = rex / (2.0 * pi * frequency) ** 2`

where:

rex is the chemical exchange value for the current frequency.

frequency is the proton frequency corresponding to the data.

Setting parameters for the N-state model is a little different from the other type of analyses as each state has a set of parameters with the same names as the other states. To set the parameters for a specific state c (ranging from 0 for the first to N-1 for the last, the number c should be given as the index argument. So the Euler angle  $\gamma$  of the third state is specified using the parameter name '`gamma`' and index of 2.

## Reduced spectral density mapping parameters

Please see Table 17.29 on page 647.

In reduced spectral density mapping, the CSA value must be set prior to the calculation of spectral density values.

## Consistency testing parameters

Please see Table 17.30 on page 647.

In consistency testing, the CSA value, angle Theta ('`orientation`') and global correlation time must be set prior to the calculation of consistency functions.

## N-state model parameters

Please see Table 17.31 on page 647.

## Relaxation dispersion parameters

Please see Table 17.32 on page 648.

Any of the relaxation dispersion parameters which are of the '`float`' type can be set. Note that setting values for parameters which are not part of the model will have no effect.

## Frame order parameters

Please see Table 17.6 on page 470.

## Prompt examples

To copy the CSA values from the data pipe '`m1`' to '`m2`', type:

```
relax> value.copy('m1', 'm2', 'csa')
```



Table 17.29: Reduced spectral density mapping parameters.

| Name | Description                                                                  |
|------|------------------------------------------------------------------------------|
| j0   | Spectral density value at 0 MHz - $J(0)$                                     |
| jwx  | Spectral density value at the frequency of the heteronucleus - $J(\omega_X)$ |
| jwh  | Spectral density value at the frequency of the proton - $J(\omega_H)$        |
| csa  | Chemical shift anisotropy (unitless)                                         |

Table 17.30: Consistency testing parameters.

| Name        | Description                                                                                                                 |
|-------------|-----------------------------------------------------------------------------------------------------------------------------|
| j0          | Spectral density value at 0 MHz (from Farrow et al. (1995) JBNMR, 6: 153-162)                                               |
| f_eta       | Eta-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                |
| f_r2        | R2-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                 |
| csa         | Chemical shift anisotropy (unitless)                                                                                        |
| orientation | Angle between the $^{15}\text{N}$ - $^1\text{H}$ vector and the principal axis of the $^{15}\text{N}$ chemical shift tensor |
| tc          | The single global correlation time estimate/approximation                                                                   |

Table 17.31: N-state model parameters.

| Name                | Description                                                | Type  |
|---------------------|------------------------------------------------------------|-------|
| Axx                 | The Axx component of the alignment tensor                  | float |
| Ayy                 | The Ayy component of the alignment tensor                  | float |
| Axy                 | The Axy component of the alignment tensor                  | float |
| Axz                 | The Axz component of the alignment tensor                  | float |
| Ayz                 | The Ayz component of the alignment tensor                  | float |
| probs               | The probabilities of each state                            | list  |
| alpha               | The $\alpha$ Euler angles (for the rotation of each state) | list  |
| beta                | The $\beta$ Euler angles (for the rotation of each state)  | list  |
| gamma               | The $\gamma$ Euler angles (for the rotation of each state) | list  |
| paramagnetic_centre | The paramagnetic centre                                    | list  |

Table 17.32: Relaxation dispersion parameters.

| Name     | Description                                                                                                       | Type  |
|----------|-------------------------------------------------------------------------------------------------------------------|-------|
| r2eff    | The effective transversal relaxation rate                                                                         | dict  |
| i0       | The initial intensity                                                                                             | dict  |
| r1       | The longitudinal relaxation rate                                                                                  | dict  |
| r2       | The transversal relaxation rate                                                                                   | dict  |
| r2a      | The transversal relaxation rate for state A in the absence of exchange                                            | dict  |
| r2b      | The transversal relaxation rate for state B in the absence of exchange                                            | dict  |
| pA       | The population for state A                                                                                        | float |
| pB       | The population for state B                                                                                        | float |
| pC       | The population for state C                                                                                        | float |
| phi_ex   | The $\phi_{\text{ex}} = pA \cdot pB \cdot dw^{*2}$ value (ppm <sup>2</sup> )                                      | float |
| phi_ex_B | The fast exchange factor between sites A and B (ppm <sup>2</sup> )                                                | float |
| phi_ex_C | The fast exchange factor between sites A and C (ppm <sup>2</sup> )                                                | float |
| padw2    | The $pA \cdot dw^{*2}$ value (ppm <sup>2</sup> )                                                                  | float |
| dw       | The chemical shift difference between states A and B (in ppm)                                                     | float |
| dw_AB    | The chemical shift difference between states A and B for 3-site exchange (in ppm)                                 | float |
| dw_AC    | The chemical shift difference between states A and C for 3-site exchange (in ppm)                                 | float |
| dw_BC    | The chemical shift difference between states B and C for 3-site exchange (in ppm)                                 | float |
| dwH      | The proton chemical shift difference between states A and B (in ppm)                                              | float |
| dwH_AB   | The proton chemical shift difference between states A and B for 3-site exchange (in ppm)                          | float |
| dwH_AC   | The proton chemical shift difference between states A and C for 3-site exchange (in ppm)                          | float |
| dwH_BC   | The proton chemical shift difference between states B and C for 3-site exchange (in ppm)                          | float |
| kex      | The exchange rate                                                                                                 | float |
| kex_AB   | The exchange rate between sites A and B for 3-site exchange with $kex\_AB = k\_AB + k\_BA$ (rad.s <sup>-1</sup> ) | float |
| kex_AC   | The exchange rate between sites A and C for 3-site exchange with $kex\_AC = k\_AC + k\_CA$ (rad.s <sup>-1</sup> ) | float |
| kex_BC   | The exchange rate between sites B and C for 3-site exchange with $kex\_BC = k\_BC + k\_CB$ (rad.s <sup>-1</sup> ) | float |
| kB       | Approximate chemical exchange rate constant between sites A and B (rad.s <sup>-1</sup> )                          | float |
| kC       | Approximate chemical exchange rate constant between sites A and C (rad.s <sup>-1</sup> )                          | float |
| tex      | The time of exchange ( $tex = 1/kex$ )                                                                            | float |
| k_AB     | The exchange rate from state A to state B                                                                         | float |
| k_BA     | The exchange rate from state B to state A                                                                         | float |

**17.2.263 value.display****Synopsis**

Display spin specific parameter values.

**Defaults**

`value.display(param=None, scaling=1.0)`

**Keyword arguments**

**param:** The parameter to display. Only one parameter may be selected.

**scaling:** The factor to scale parameters by.

**Description**

The values corresponding to the given parameter will be displayed. The scaling argument can be used to scale the parameter values. This can be useful for example in the case of the model-free  $R_{ex}$  parameter to obtain the spectrometer dependent value from the `omega_ex` field strength independent internal value. Or to scale correlation times from seconds down to nanosecond or picosecond timescales.

**Relaxation curve fitting parameters**

Please see Table 17.33 on page 650.

**Steady-state NOE parameters**

Please see Table 17.9 on page 483.

**Model-free parameters**

Please see Table 17.34 on page 650.

For model-free theory it is assumed that  $R_{ex}$  values are scaled quadratically with field strength. The values will be very small as they will be written out as a field strength independent value. Hence use the following formula to convert the value to that expected for a given magnetic field strength:

$$R_{ex} = \text{value} * (2.0 * \pi * \text{frequency}) ** 2$$

The frequency is that of the proton in Hertz.

**Reduced spectral density mapping parameters**

Please see Table 17.11 on page 483.

**Consistency testing parameters**

Please see Table 17.12 on page 484.

**Relaxation dispersion parameters**

Please see Table 17.5 on page 470.

**Prompt examples**

To show all CSA values, type:

```
relax> value.display('csa')
```

To display the model-free  $R_{ex}$  values scaled to 600 MHz, type one of:

```
relax> value.display('rex', scaling=(2.0*pi*600e6)**2)
```

```
relax> value.display(param='rex', scaling=(2.0*pi*600e6)**2)
```

Table 17.33: Relaxation curve fitting parameters.

| Name | Description                               |
|------|-------------------------------------------|
| rx   | Either the $R_1$ or $R_2$ relaxation rate |
| i0   | The initial intensity                     |
| iinf | The intensity at infinity                 |

Table 17.34: Model-free parameters.

| Name     | Description                                                              |
|----------|--------------------------------------------------------------------------|
| s2       | $S^2$ , the model-free generalised order parameter ( $S^2 = S_f^2.S2s$ ) |
| s2f      | $S_f^2$ , the faster motion model-free generalised order parameter       |
| s2s      | $S_s^2$ , the slower motion model-free generalised order parameter       |
| local_tm | The spin specific global correlation time (seconds)                      |
| te       | Single motion effective internal correlation time (seconds)              |
| tf       | Faster motion effective internal correlation time (seconds)              |
| ts       | Slower motion effective internal correlation time (seconds)              |
| rex      | Chemical exchange relaxation ( $\sigma_{ex} = R_{ex} / \omega^2$ )       |
| csa      | Chemical shift anisotropy (unitless)                                     |

## 17.2.264 value.read



### Synopsis

Read spin specific parameter values from a file.

### Defaults

**value.read**(param=None, scaling=1.0, file=None, dir=None, spin\_id\_col=None, mol\_name\_col=None, res\_num\_col=None, res\_name\_col=None, spin\_num\_col=None, spin\_name\_col=None, data\_col=None, error\_col=None, sep=None, spin\_id=None)

### Keyword arguments

**param**: The parameter. Only one parameter may be selected.

**scaling**: The factor to scale parameters by.

**file**: The name of the file containing the values.

**dir**: The directory where the file is located.

**spin\_id\_col**: The spin ID string column (an alternative to the mol, res, and spin name and number columns).

**mol\_name\_col**: The molecule name column (alternative to the spin\_id\_col).

**res\_num\_col**: The residue number column (alternative to the spin\_id\_col).

**res\_name\_col**: The residue name column (alternative to the spin\_id\_col).

**spin\_num\_col**: The spin number column (alternative to the spin\_id\_col).

**spin\_name\_col**: The spin name column (alternative to the spin\_id\_col).

**data\_col**: The RDC data column.

**error\_col**: The experimental error column.

**sep**: The column separator (the default is white space).

**spin\_id**: The spin ID string to restrict the loading of data to certain spin subsets.

### Description

The spin system can be identified in the file using two different formats. The first is the spin ID string column which can include the molecule name, the residue name and number, and the spin name and number. Alternatively the molecule name, residue number, residue name, spin number and/or spin name columns can be supplied allowing this information to be in separate columns. Note that the numbering of columns starts at one. The spin ID string can be used to restrict the reading to certain spin types, for example only  $^{15}\text{N}$  spins when only residue information is in the file.

If this is used to change values of previously minimised parameters, then the minimisation statistics (chi-squared value, iteration count, function count, gradient count, and Hessian count) will be reset.

### Relaxation curve fitting parameters

Please see Table 17.27 on page 646.

### Model-free parameters

Please see Table 17.28 on page 646.

Setting a parameter value may have no effect depending on which model-free model is chosen. For example if  $S_f^2$  values and  $S_s^2$  values are set but the data pipe corresponds to the model-free model 'm4' then because these data values are not parameters of the model they will have no effect.

Note that the  $R_{ex}$  values are scaled quadratically with field strength and should be supplied as a field strength independent value. Use the following formula to obtain the correct value:

$$\text{value} = \text{rex} / (2.0 * \pi * \text{frequency}) ** 2$$

where:

rex is the chemical exchange value for the current frequency.

frequency is the proton frequency corresponding to the data.

### Reduced spectral density mapping parameters

Please see Table 17.29 on page 647.

In reduced spectral density mapping, the CSA value must be set prior to the calculation of spectral density values.

### Consistency testing parameters

Please see Table 17.30 on page 647.

In consistency testing, the CSA value, angle Theta ('orientation') and global correlation time must be set prior to the calculation of consistency functions.

### Relaxation dispersion parameters

Please see Table 17.32 on page 648.

Any of the relaxation dispersion parameters which are of the 'float' type can be set. Note that setting values for parameters which are not part of the model will have no effect.

### Prompt examples

To load 15N CSA values from the file 'csa\_values' in the directory 'data', where spins are only identified by residue name and number, type one of the following:

```
relax> value.read('csa', 'data/csa_value',
                 spin_id='@N')
```

```
relax> value.read('csa', 'csa_value', dir='
                 data', spin_id='@N')
```

```
relax> value.read(param='csa', file='
                 csa_value', dir='data', res_num_col=1,
                 res_name_col=2, data_col=3, error_col
                 =4, spin_id='@N')
```

## 17.2.265 value.set

Å

## Synopsis

Set parameter values.

## Defaults

`value.set(val=None, param=None, index=0, spin_id=None, error=False, force=True)`

## Keyword arguments

**val:** The value(s).

**param:** The parameter(s).

**index:** The list index for when the parameter is a list of values. This is ignored in all other cases.

**spin\_id:** The spin ID string to restrict value setting to.

**error:** A flag which if True will cause the error rather than parameter to be set.

**force:** A flag which, if set to True, will cause the destination parameter to be overwritten.

## Description

If this function is used to change values of previously minimised results, then the minimisation statistics (chi-squared value, iteration count, function count, gradient count, and Hessian count) will be reset.

The value can be None, a single value, or an array of values while the parameter can be None, a string, or array of strings. The choice of which combination determines the behaviour of this function. The following table describes what occurs in each instance. In these columns, 'None' corresponds to None, '1' corresponds to either a single value or single string, and 'n' corresponds to either an array of values or an array of strings.

Please see Table 17.35 on page 653.

## Spin ID string

For spin-specific parameters, the spin ID string can be used to restrict the value setting to a specific spin system or group of spins. It has no effect for global parameters such as in the N-state model and frame order analyses.

## Relaxation curve fitting parameters

Please see Table 17.36 on page 653.

## Model-free parameters

Please see Table 17.37 on page 653.

Setting a parameter value may have no effect depending on which model-free model is chosen. For example if  $S_f^2$  values and  $S_s^2$  values are set but the data pipe corresponds to the model-free model 'm4' then because these data values are not parameters of the model they will have no effect.

Note that the  $R_{ex}$  values are scaled quadratically with field strength and should be supplied as a field strength independent value. Use the following formula to obtain the correct value:

$$\text{value} = \text{rex} / (2.0 * \pi * \text{frequency}) ** 2$$

where:

rex is the chemical exchange value for the current frequency.

frequency is the proton frequency corresponding to the data.

## Reduced spectral density mapping parameters

Please see Table 17.38 on page 653.

In reduced spectral density mapping, the CSA value must be set prior to the calculation of spectral density values.

## Consistency testing parameters

Please see Table 17.39 on page 654.

In consistency testing, the CSA value, angle Theta ('orientation') and global correlation time must be set prior to the calculation of consistency functions.

## N-state model parameters

Please see Table 17.40 on page 654.

Setting parameters for the N-state model is a little different from the other type of analyses as each state has a set of parameters with the same names as the other states. To set the parameters for a specific state c (ranging from 0 for the first to N-1 for the last, the number c should be given as the index argument. So the Euler angle  $\gamma$  of the third state is specified using the parameter name 'gamma' and index of 2.

Table 17.35: The value and parameter combination options for the value.set user function.

| Value | Param | Description                                                                                                                                                                                                              |
|-------|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| None  | None  | This case is used to set the model parameters prior to minimisation or calculation. The model parameters are set to the default values.                                                                                  |
| 1     | None  | Invalid combination.                                                                                                                                                                                                     |
| n     | None  | This case is used to set the model parameters prior to minimisation or calculation. The length of the val array must be equal to the number of model parameters. The parameters will be set to the corresponding number. |
| None  | 1     | The parameter matching the string will be set to the default value.                                                                                                                                                      |
| 1     | 1     | The parameter matching the string will be set to the supplied number.                                                                                                                                                    |
| n     | 1     | Invalid combination.                                                                                                                                                                                                     |
| None  | n     | Each parameter matching the strings will be set to the default values.                                                                                                                                                   |
| 1     | n     | Each parameter matching the strings will be set to the supplied number.                                                                                                                                                  |
| n     | n     | Each parameter matching the strings will be set to the corresponding number. Both arrays must be of equal length.                                                                                                        |

Table 17.36: Relaxation curve fitting parameter value setting.

| Name | Description                                                 | Default |
|------|-------------------------------------------------------------|---------|
| rx   | Either the R <sub>1</sub> or R <sub>2</sub> relaxation rate | 8.0     |
| i0   | The initial intensity                                       | 10000.0 |
| iinf | The intensity at infinity                                   | 0.0     |

Table 17.37: Model-free parameter value setting.

| Name     | Description                                                              | Default   |
|----------|--------------------------------------------------------------------------|-----------|
| s2       | $S^2$ , the model-free generalised order parameter ( $S^2 = S_f^2.S2s$ ) | 0.8       |
| s2f      | $S_f^2$ , the faster motion model-free generalised order parameter       | 0.8       |
| s2s      | $S_s^2$ , the slower motion model-free generalised order parameter       | 0.8       |
| local_tm | The spin specific global correlation time (seconds)                      | 1e-08     |
| te       | Single motion effective internal correlation time (seconds)              | 1e-10     |
| tf       | Faster motion effective internal correlation time (seconds)              | 1e-11     |
| ts       | Slower motion effective internal correlation time (seconds)              | 1e-09     |
| rex      | Chemical exchange relaxation ( $\sigma_{ex} = R_{ex} / \omega^2$ )       | 0.0       |
| csa      | Chemical shift anisotropy (unitless)                                     | -0.000172 |

Table 17.38: Reduced spectral density mapping parameter value setting.

| Name | Description                                                                  | Default   |
|------|------------------------------------------------------------------------------|-----------|
| j0   | Spectral density value at 0 MHz - $J(0)$                                     | None      |
| jwx  | Spectral density value at the frequency of the heteronucleus - $J(\omega_X)$ | None      |
| jwh  | Spectral density value at the frequency of the proton - $J(\omega_H)$        | None      |
| csa  | Chemical shift anisotropy (unitless)                                         | -0.000172 |

Table 17.39: Consistency testing parameter value setting.

| Name        | Description                                                                                                                 | Default   |
|-------------|-----------------------------------------------------------------------------------------------------------------------------|-----------|
| j0          | Spectral density value at 0 MHz (from Farrow et al. (1995) JBNMR, 6: 153-162)                                               | None      |
| f_eta       | Eta-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                | None      |
| f_r2        | R2-test (from Fushman et al. (1998) JACS, 120: 10947-10952)                                                                 | None      |
| csa         | Chemical shift anisotropy (unitless)                                                                                        | -0.000172 |
| orientation | Angle between the $^{15}\text{N}$ - $^1\text{H}$ vector and the principal axis of the $^{15}\text{N}$ chemical shift tensor | 15.7      |
| tc          | The single global correlation time estimate/approximation                                                                   | 1.3e-08   |

Table 17.40: N-state model parameter value setting.

| Name                | Description                                                | Default | Type  |
|---------------------|------------------------------------------------------------|---------|-------|
| Axx                 | The Axx component of the alignment tensor                  | None    | float |
| Ayy                 | The Ayy component of the alignment tensor                  | None    | float |
| Axy                 | The Axy component of the alignment tensor                  | None    | float |
| Axz                 | The Axz component of the alignment tensor                  | None    | float |
| Ayz                 | The Ayz component of the alignment tensor                  | None    | float |
| probs               | The probabilities of each state                            | 0.0     | list  |
| alpha               | The $\alpha$ Euler angles (for the rotation of each state) | 0.0     | list  |
| beta                | The $\beta$ Euler angles (for the rotation of each state)  | 0.0     | list  |
| gamma               | The $\gamma$ Euler angles (for the rotation of each state) | 0.0     | list  |
| paramagnetic_centre | The paramagnetic centre                                    | None    | list  |

## Relaxation dispersion parameters

Please see Table 17.41 on page 655.

Any of the relaxation dispersion parameters which are of the 'float' type can be set. Note that setting values for parameters which are not part of the model will have no effect.

To set the CSA value of all spins to the default value, type:

```
relax> value.set(param='csa')
```

To set the CSA value of all spins to -172 ppm, type:

```
relax> value.set(-172 * 1e-6, 'csa')
```

```
relax> value.set(val=-172 * 1e-6, param='csa')
)
```

## Frame order parameters

Please see Table 17.6 on page 470.

To set the NH bond length of all spins to 1.02 Å, type:

```
relax> value.set(1.02 * 1e-10, 'r')
```

## Prompt examples

To set the parameter values for the current data pipe to the default values, for all spins, type:

```
relax> value.set()
```

To set the parameter values of residue 10, which is in the current model-free data pipe 'm4' and has the parameters  $\{S^2, \tau_e, R_{ex}\}$ , the following can be used.  $R_{ex}$  term is the value for the first given field strength.

```
relax> value.set([0.97, 2.048*1e-9, 0.149],
    spin_id=':10')
```

```
relax> value.set(val=[0.97, 2.048*1e-9, 0
    .149], spin_id=':10')
```

To set both the bond length and the CSA value to the default values, type:

```
relax> value.set(param=['r', 'csa'])
```

To set both  $\tau_f$  and  $\tau_s$  to 100 ps, type:

```
relax> value.set(100e-12, ['tf', 'ts'])
```

```
relax> value.set(val=100e-12, param=['tf', 'ts'])
```



Table 17.41: Relaxation dispersion parameter value setting.

| Name     | Description                                                                                                          | Default | Type  |
|----------|----------------------------------------------------------------------------------------------------------------------|---------|-------|
| r2eff    | The effective transversal relaxation rate                                                                            | 10.0    | dict  |
| i0       | The initial intensity                                                                                                | 10000.0 | dict  |
| r1       | The longitudinal relaxation rate                                                                                     | 2.0     | dict  |
| r2       | The transversal relaxation rate                                                                                      | 10.0    | dict  |
| r2a      | The transversal relaxation rate for state A in the absence of exchange                                               | 10.0    | dict  |
| r2b      | The transversal relaxation rate for state B in the absence of exchange                                               | 10.0    | dict  |
| pA       | The population for state A                                                                                           | 0.9     | float |
| pB       | The population for state B                                                                                           | 0.5     | float |
| pC       | The population for state C                                                                                           | 0.5     | float |
| phi_ex   | The $\phi_{ex} = pA.pB.dw^{*2}$ value (ppm <sup>2</sup> )                                                            | 5.0     | float |
| phi_ex_B | The fast exchange factor between sites A and B (ppm <sup>2</sup> )                                                   | 5.0     | float |
| phi_ex_C | The fast exchange factor between sites A and C (ppm <sup>2</sup> )                                                   | 5.0     | float |
| padw2    | The $pA.dw^{*2}$ value (ppm <sup>2</sup> )                                                                           | 1.0     | float |
| dw       | The chemical shift difference between states A and B (in ppm)                                                        | 1.0     | float |
| dw_AB    | The chemical shift difference between states A and B for 3-site exchange (in ppm)                                    | 1.0     | float |
| dw_AC    | The chemical shift difference between states A and C for 3-site exchange (in ppm)                                    | 1.0     | float |
| dw_BC    | The chemical shift difference between states B and C for 3-site exchange (in ppm)                                    | 1.0     | float |
| dwH      | The proton chemical shift difference between states A and B (in ppm)                                                 | 1.0     | float |
| dwH_AB   | The proton chemical shift difference between states A and B for 3-site exchange (in ppm)                             | 1.0     | float |
| dwH_AC   | The proton chemical shift difference between states A and C for 3-site exchange (in ppm)                             | 1.0     | float |
| dwH_BC   | The proton chemical shift difference between states B and C for 3-site exchange (in ppm)                             | 1.0     | float |
| kex      | The exchange rate                                                                                                    | 1000.0  | float |
| kex_AB   | The exchange rate between sites A and B for 3-site exchange with $kex_{AB} = k_{AB} + k_{BA}$ (rad.s <sup>-1</sup> ) | 1000.0  | float |
| kex_AC   | The exchange rate between sites A and C for 3-site exchange with $kex_{AC} = k_{AC} + k_{CA}$ (rad.s <sup>-1</sup> ) | 1000.0  | float |
| kex_BC   | The exchange rate between sites B and C for 3-site exchange with $kex_{BC} = k_{BC} + k_{CB}$ (rad.s <sup>-1</sup> ) | 1000.0  | float |
| kB       | Approximate chemical exchange rate constant between sites A and B (rad.s <sup>-1</sup> )                             | 1000.0  | float |
| kC       | Approximate chemical exchange rate constant between sites A and C (rad.s <sup>-1</sup> )                             | 1000.0  | float |
| tex      | The time of exchange ( $tex = 1/kex$ )                                                                               | 0.001   | float |
| k_AB     | The exchange rate from state A to state B                                                                            | 2.0     | float |
| k_BA     | The exchange rate from state B to state A                                                                            | 1000.0  | float |

To set the  $S^2$  and  $\tau_e$  parameter values of residue 126, Ca spins to 0.56 and 13 ps, type:

```
relax> value.set([0.56, 13e-12], ['s2', 'te'], ':126@Ca')
```

```
relax> value.set(val=[0.56, 13e-12], param=['s2', 'te'], spin_id=':126@Ca')
```

```
relax> value.set(val=[0.56, 13e-12], param=['s2', 'te'], spin_id=':126@Ca')
```

## 17.2.266 value.write



### Synopsis

Write spin specific parameter values to a file.

### Defaults

**value.write**(param=None, file=None, dir=None, scaling=1.0, comment=None, bc=False, force=False)

### Keyword arguments

**param**: The parameter.

**file**: The name of the file.

**dir**: The directory name.

**scaling**: The factor to scale parameters by.

**comment**: Text which will be added to the start of the file as comments. All lines will be prefixed by '# '.

**bc**: A flag which if True will cause the back calculated values to be written to file rather than the actual data.

**force**: A flag which, if set to True, will cause the file to be overwritten.

### Description

The values corresponding to the given parameter will be written to file. The scaling argument can be used to scale the parameter values. This can be useful for example in the case of the model-free  $R_{ex}$  parameter to obtain the spectrometer dependent value from the omega\_ex field strength independent internal value. Or to scale correlation times from seconds down to nanosecond or picosecond timescales.

### Relaxation curve fitting parameters

Please see Table 17.33 on page 650.

### Steady-state NOE parameters

Please see Table 17.9 on page 483.

## Model-free parameters

Please see Table 17.34 on page 650.

For model-free theory it is assumed that  $R_{ex}$  values are scaled quadratically with field strength. The values will be very small as they will be written out as a field strength independent value. Hence use the following formula to convert the value to that expected for a given magnetic field strength:

$$R_{ex} = \text{value} * (2.0 * \pi * \text{frequency}) ** 2$$

The frequency is that of the proton in Hertz.

## Reduced spectral density mapping parameters

Please see Table 17.11 on page 483.

## Consistency testing parameters

Please see Table 17.12 on page 484.

## Relaxation dispersion parameters

Please see Table 17.5 on page 470.

## Prompt examples

To write the CSA values to the file 'csa.txt', type one of:

```
relax> value.write('csa', 'csa.txt')

relax> value.write(param='csa', file='
    csa.txt')
```

To write the NOE values to the file 'noe', type one of:

```
relax> value.write('noe', 'noe.out')

relax> value.write(param='noe', file='
    noe.out')

relax> value.write(param='noe', file='
    noe.out')

relax> value.write(param='noe', file='
    noe.out', force=True)
```

To write the model-free  $R_{ex}$  values scaled to 600 MHz to the file 'rex\_600', type one of:

```
relax> value.write('rex', 'rex_600', scaling
    =(2.0*pi*600e6)**2)

relax> value.write(param='rex', file='
    rex_600', scaling=(2.0*pi*600e6)**2)
```

## 17.2.267 vmd.view

### Synopsis

View the structures loaded into the relax data store using VMD.

### Defaults

`vmd.view()`

### Description

This will launch VMD with all of the structures loaded into the relax data store.

### Prompt examples

```
relax> vmd.view()
```



## Chapter 18

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```

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```

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You should also get your employer (if you work as a programmer) or school, if any, to sign a “copyright disclaimer” for the program, if necessary. For more information on this, and how to apply and follow the GNU GPL, see <http://www.gnu.org/licenses/>.

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