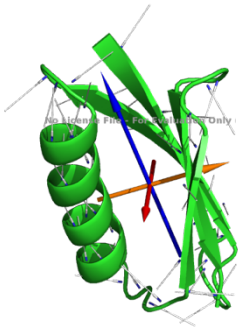
Biomolecular NMR: Advanced Tools, protein dynamics

Gothenburg 2024

CcpNmr AnalysisDynamics – Part 2
Model Analysis Practical



For the final part of the workshop, you will be using the latest version of CcpNmr, which is not yet publicly available. Please note that this is a Demo Version, and it is not for redistribution, and it should only be used for this practical session.

This practical is divided into three sections, each followed by a set of questions related to the resulting data. Please note that listening to the morning lecture will help with answering these questions.

We assume that you are using NMRbox and that you are already logged in.

You can find the software, tutorials, and data in the folder:

EVENTS/2024-biomol-nmr-goteborg/CCPN/practicals/dynamics_part2

Launch CcpNmr AnalysisDynamics

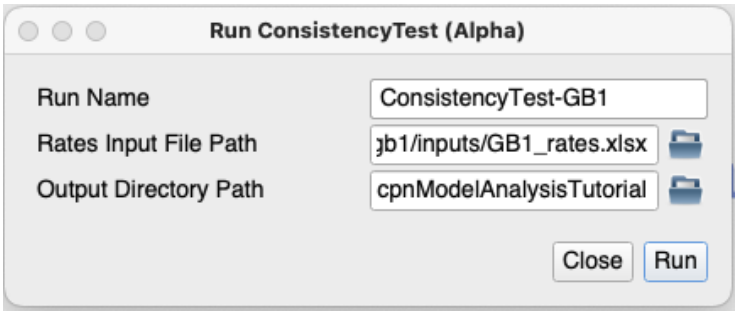
1) Double clicking the icon AnalysisDynamics
   Or
2) Open the terminal and cd to the bin directory:
   cd EVENTS/2024-biomol-nmr-goteborg/CCPN/practicals/ccpnmr_dynamics_part2/ccpnmr/bin/
   run
   ./analysisdynamics

Section I. Consistency-Tests

S 1.01 Main menus -> *Dynamics* -> *Consistency Tests…*

S 1.02 Insert the input values:

- *Run Name*: Give a name, e.g.: ConsistencyTest-GB1
- *Rates input*: Select the GB1 rates from the data directory ~/CcpnModelAnalysisTutorial/ConsistencyTestData/GB1_rates.xlsx
- *Output Directory:* Select an existing output directory or create a new one



S 1.03 Click **Run**

A popup with plots will appear.

◊ The first plot, (top left) is a J_0 vs J_0 scatter plot. J_0 points are computed using the various rates from the input data file

◊ The second plot (top right) is a Quantile-Quantile (Q-Q) plot of the J_0 values

◊ The third plot (bottom left) is a Principal Component Analysis (PCA) plot of J_0, more precisely, the first two components PC_1 vs PC_2

◊ The last plot (bottom right) is a Q-score for the PCA and the dotted line represents the 95th percentile of J_0s

S 1.04 Repeat the same steps for the bmrb15445 rates: ~/CcpnModelAnalysisTutorial/ConsistencyTestData/bmrb15445.xlsx

S 1.05 This process will generate a series of files in the specified output path

If you were unable to complete the section or encountered any issues, you can find a copy of the completed results in the *ConsistencyTestData/outputExample* directory.

Questions I. Consistency-Tests

Q 1.01 Why do we use J_0 vs J_0?

Q 1.02 Based on the J_0 and Q-Q plots, would you consider the GB1 rates consistent across the two magnetic fields?

Q 1.03 How does the scatter distribution of **GB1** compare to **bmrb15445** in the various plots?

Q 1.04 What could be the potential causes of discrepancies observed in the **bmrb15445** plots?

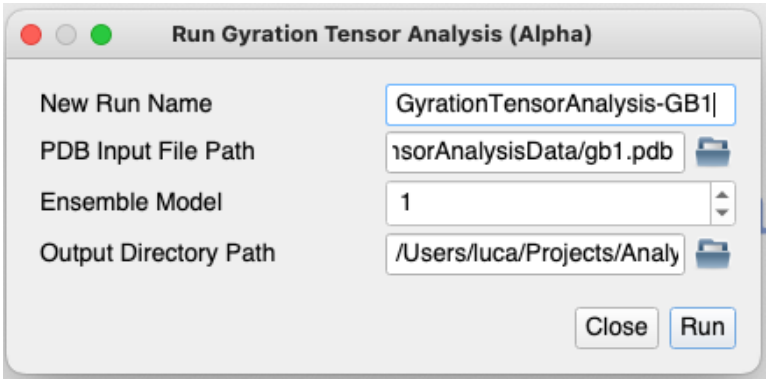
Q 1.05 Following your observations, which **bmrb15445** spectrometer data (rates) would you use to run a ModelAnalysis?

Section II. Gyration Tensor Analysis

S 2.01 Main menus -> *Dynamics* -> *Gyration Tensor Analysis…*

S 2.02 Insert the input values:

- *Run Name*: Give a name, e.g.: GyrationTensorAnalysis-GB1
- *PDB input*: Select the PDB file from the data directory, ~/CcpnModelAnalysisTutorial/GyrationTensorAnalysisData
- *Output Directory:* Select an existing output directory or create a new one



S 2.03 Click Run to run the calculation and Open PyMOL. This process will generate a series of files in the specified output path

S 2.04 Navigate to the output directory and inspect the resulting .txt file. Its syntax will be something like *runName_moleculeName_date_time.txt*

S 2.05 Open the theta angles file .csv and inspect the Principal Axis and the various NH-vectors in PyMOL

S 2.06 Optionally, repeat the same steps for the 2jnb.pdb and 2jnb_truncated.pdb files.

If you were unable to complete the section or encountered any issues, you can find a copy of the completed results in the *GyrationTensorAnalysisData/outputExample* directory, including PyMOL sessions for each molecule.

N.B if you are running the Gyration Tensor Analysis with your own PDB file, ensure the molecule is protonated. If not, you could use PyMOL to add the necessary atoms with the command:

```
cmd.h_add("name N and backbone")
```

Then save it as new file:

```
cmd.save("your_new_path.pdb")
```

Use the newly generated PDB for the Analysis.

Questions II. Gyration Tensor Analysis

Q 2.01 Why do we inspect the molecular shape using the PDB file?

Q 2.02 What insights are provided by the gyration tensor and principal axis?

Q 2.03 Why are NH-vectors and angular measurements critical to the ModelAnalysis calculations?

If you have run the calculations on 2jnb.pdb and 2jnb_truncated.pdb

Q 2.04 What are the main differences between the two conformations?

Q 2.05 How could the choice of PDB file affect the outcomes of the ModelAnalysis?

Section III. Model Analysis

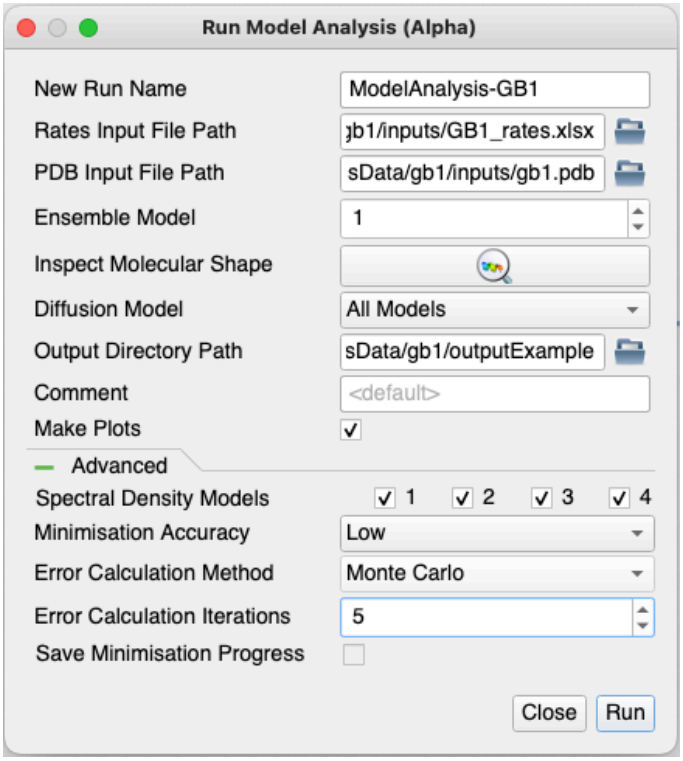
S 3.01 Main menus -> *Dynamics* -> *Model Analysis…*

S 3.02 Insert the input values:

- *Run Name*: Give a name, e.g.: ModelAnalysis-GB1
- *Rates input*: Select the GB1 rates from the data directory, ~/CcpnModelAnalysisTutorial/ModelAnalysisData/gb1/inputs/GB1_rates.xlsx
- *PDB input*: Select the PDB file from the data directory: ~/CcpnModelAnalysisTutorial/ModelAnalysisData/gb1/inputs/gb1.pdb
- *Diffusion Model:* select *All Model*
- *Output Directory:* Select an existing output directory or create a new one

Expand the Advanced panel:

- *Minimisation Accuracy:* select *Low (quicker)*
- Error Calculation Iterations: select 5 (quicker)



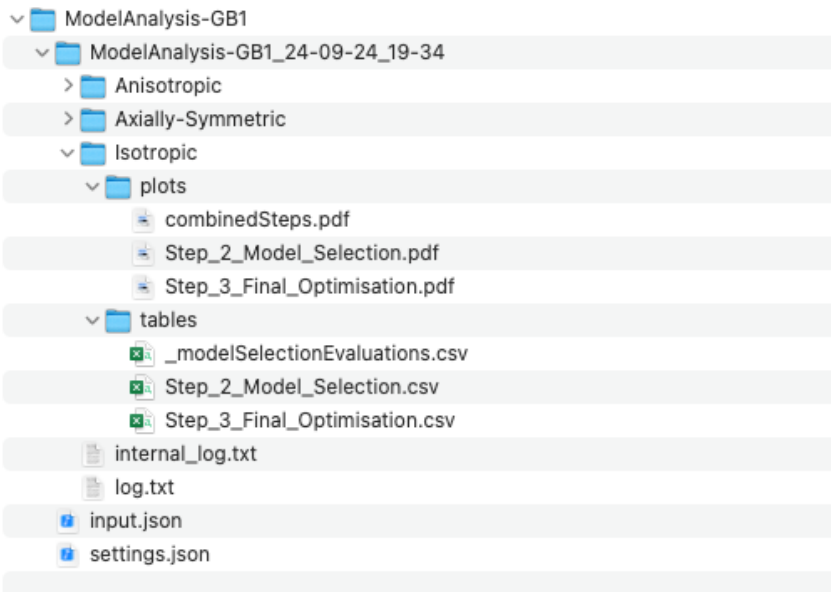
S 3.03 Click ***Run***

This process will generate a series of directories and files in the specified output path.
The top-level directory will be named after the Run Name.
Inside this directory, two .json files will be created: input.json and settings.json.
These files contain the information provided in the popup and are used to automatically execute the ModelAnalysis script, located in the bin directory.

You can run the ModelAnalysis from the terminal with the command:

```
./modelAnalysis -i input.json -s settings.json
```

Additionally, another directory will be created to store the outputs, named using the format RunName_date_time. This directory will contain the logs and a diffusion model directory. Each model will include tables and plots for the various stages of the process. See below.



S 3.04 The calculations will take several minutes. You can track the progress from the log file(s).

S 3.05 Once the calculations are complete, the plots and result tables can be found in their respective folders.

If you were unable to complete the section or encountered any issues, you can find a copy of the completed results in the *outputExample* directory.

Questions III. Model Analysis

Q 3.01 Why do we run both Diffusion Models and multiple Spectral Density models?

Q 3.02 What is the Total Correlation time for the two models?

If not already opened, review the Step_3_Final_Optimisation.pdf for both Diffusion models (located in the Isotropic and Axially Symmetric directories).

Q 3.03 How do the S^2 values reflect the internal flexibility or rigidity of specific regions of the molecule across the two Diffusion models?

Q 3.04 Which specific residues or regions show elevated R_{ex} values? Compare how these regions differ between the two Diffusion models.

Q 3.05 Based on the final BICc values, which Diffusion model provides a better fit for the relaxation rates?

End of the practical.