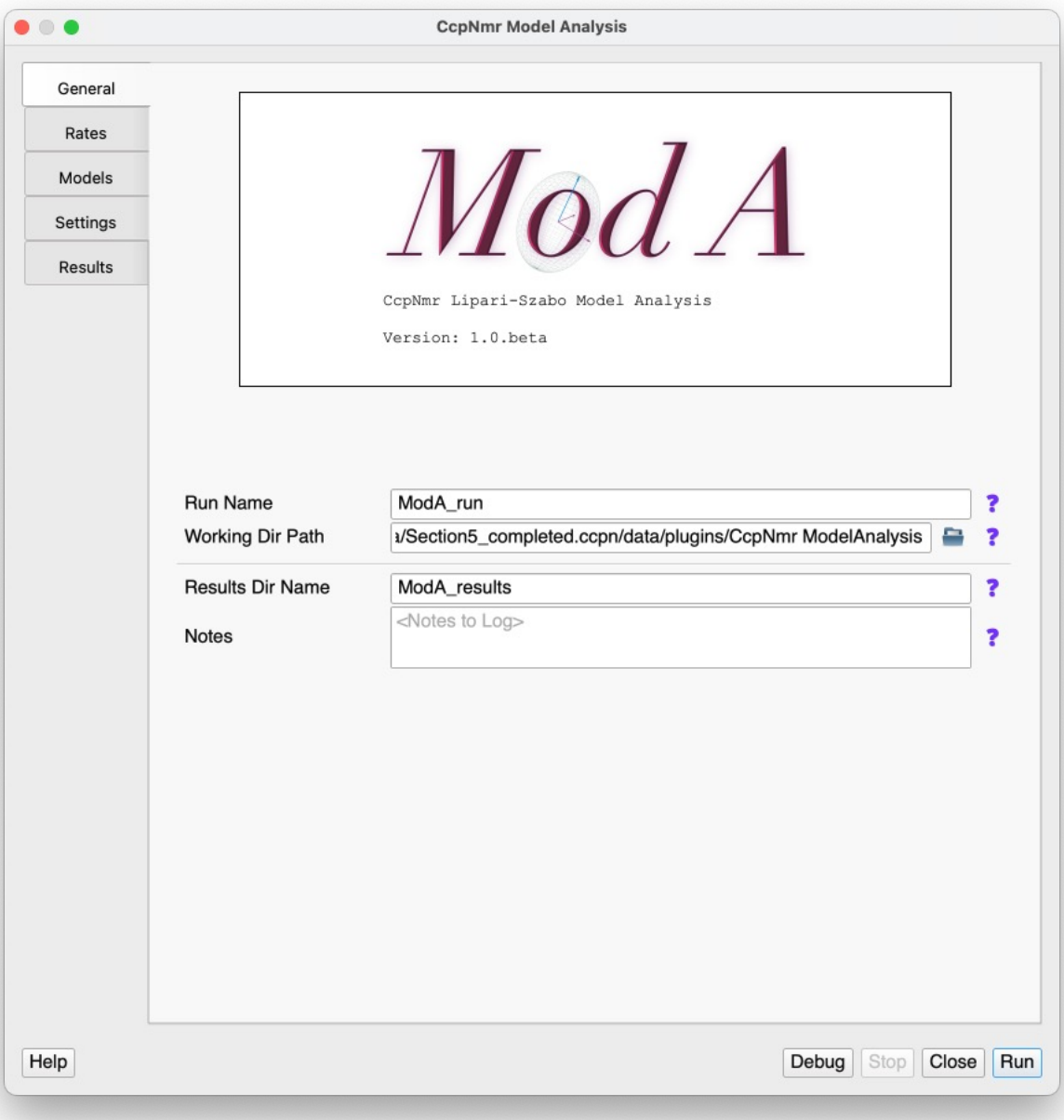
CCPN

CcpNmr Analysis Version 3

Model Free Analysis
(beta testing)



Introduction

This tutorial will take you through the analysis of some dynamics data in the CcpNmr Analysis Version 3.5 pre-release. The functionality is still in the beta testing phase, so do please let us know about any bugs at support@ccpn.ac.uk or via our forum: https://forum.ccpn.ac.uk/.

We assume that you are already familiar with the basics of how the program works, e.g. by doing our Beginner Tutorial.

You can download the example data to go with this tutorial from our website at https://ccpn.ac.uk/support/tutorials-for-analysisdynamics-pre-release/. We are grateful to Dr Fred Muskett for making the spectra of GB1 available to us for use in this tutorial. You will be analysing R1 and R2 relaxation rates and heteronuclear NOE data on which reduced spectral density mapping has already been performed. You will be checking the consistency of data across two fields, calculating a molecular gyration tensor and conducting Model Free analysis on the data.

The tutorial is divided into sections, each of them has a set of simple actions: you will see a descriptive image on top and a full description below. (Note that images are representative, and that there may be small differences between your setup and that shown in the tutorial.)

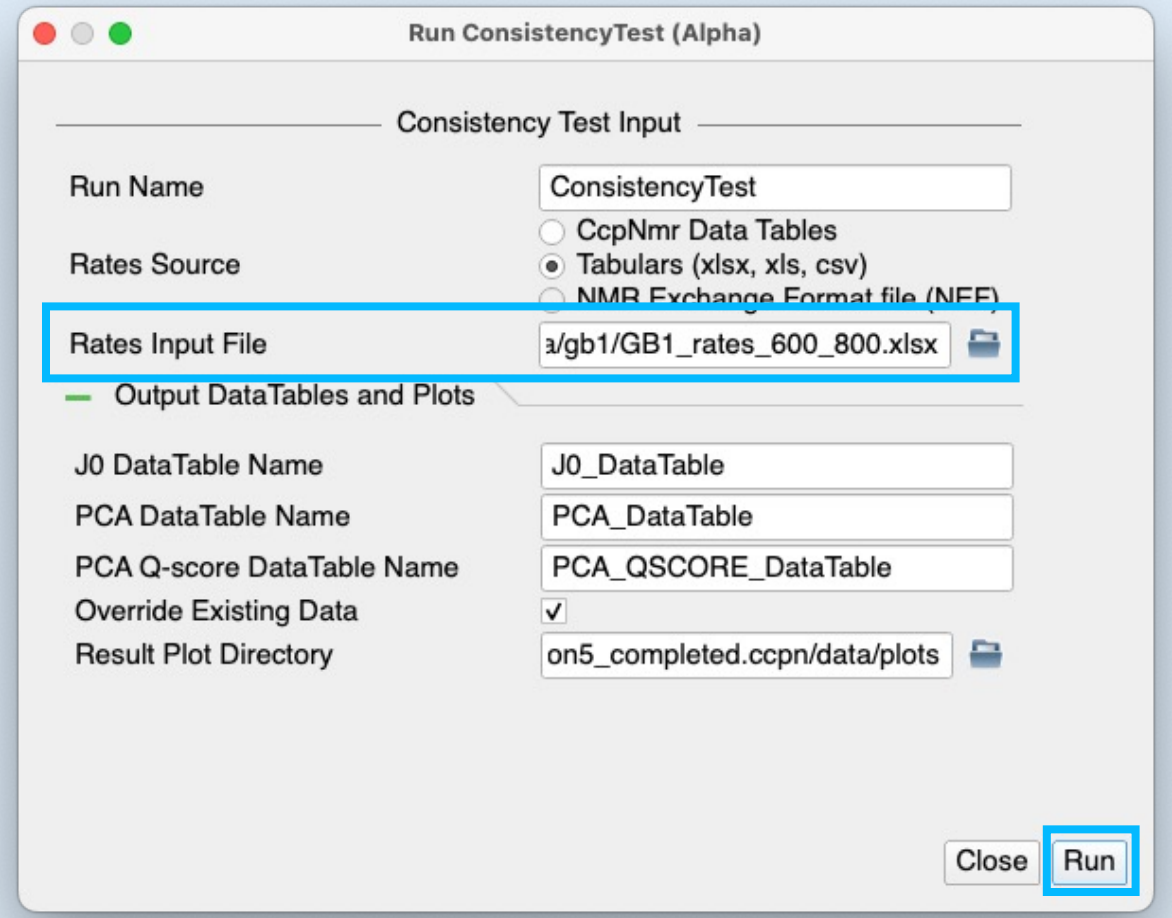
Contents:

Start CcpNmr Analysis V3

Apple/Linux users by using the terminal command *bin/assign* in the ccpnmr directory

Windows users by double-clicking on the *assign.bat* file in the bin directory of the ccpnmr directory

1 Consistency Tests

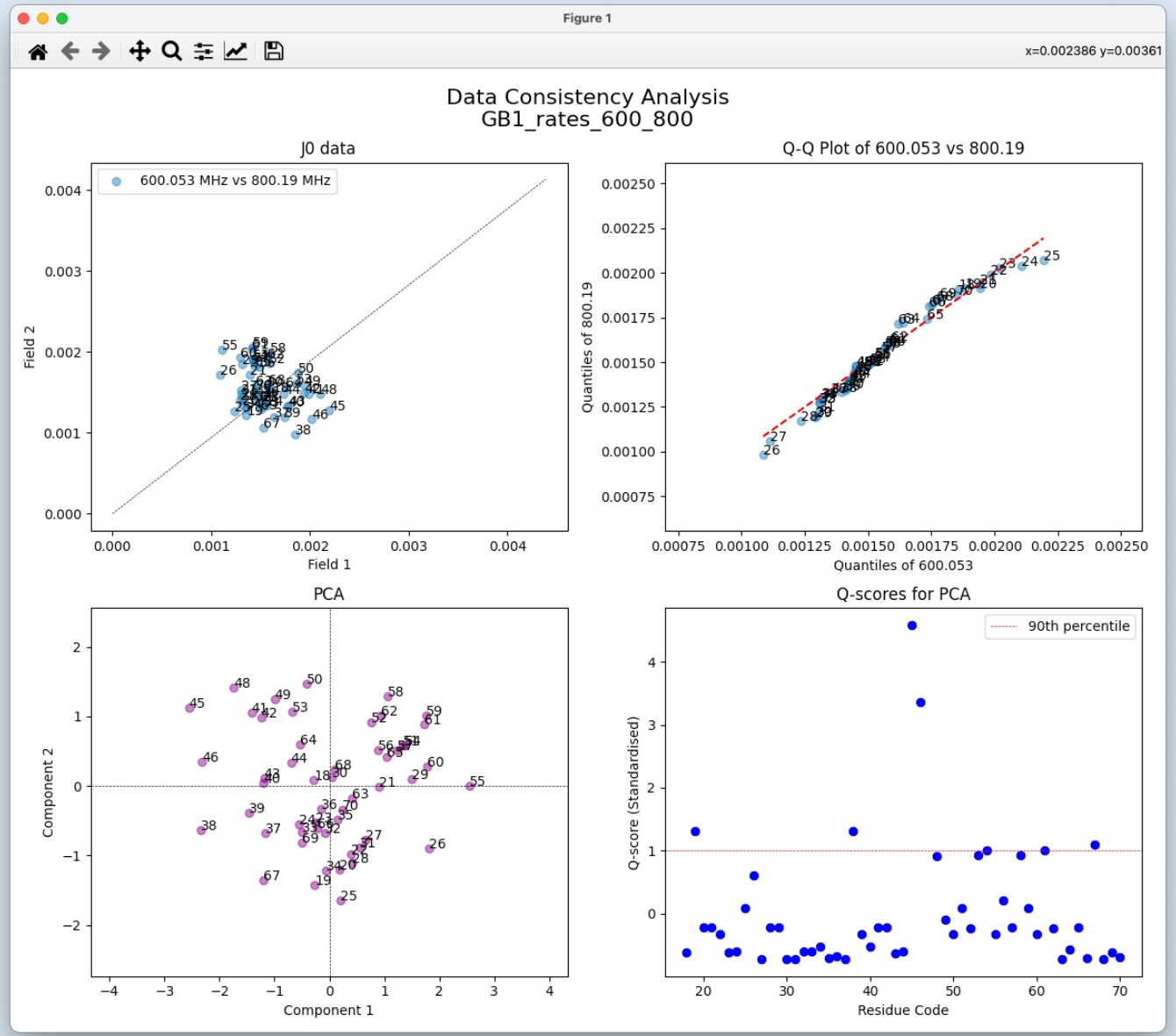


1A Run Consistency Tests

This is a way to check that your R1, R2 and heteronuclear NOE data collected at two different fields are consistent with one another. If they are not, it could be that the data were not recorded at exactly the same temperature, for example.

- Go to **Main Menu ⟶ Dynamics ⟶ Consistency Tests** …
- As your **Rates Input File** select the **GB1_rates_600_800.xlsx** file from the tutorial data folder. Either type/copy the full path, select the file via the folder icon or drag the file into the box from a file browser.
- Click **Run**

1 Consistency Tests



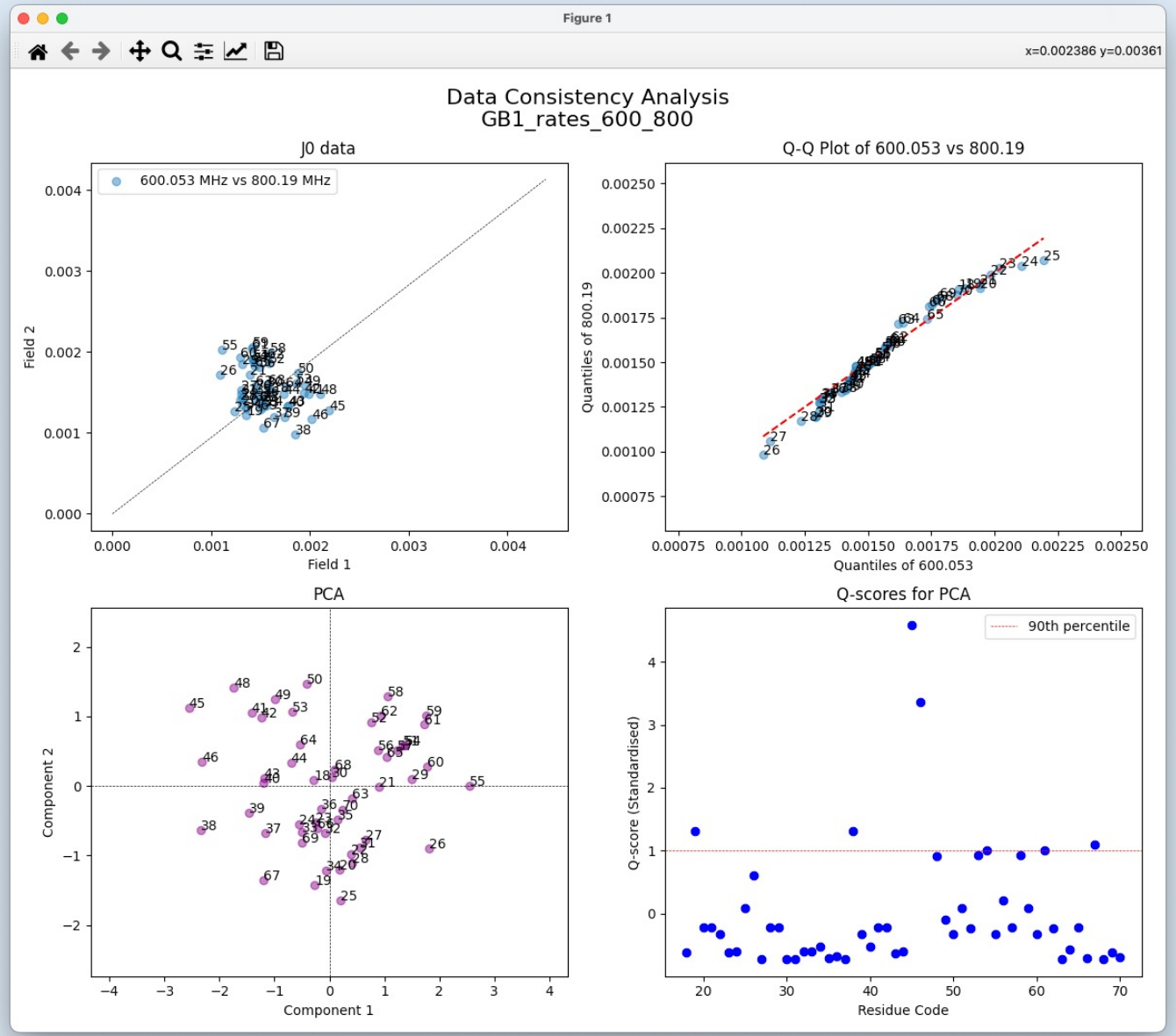
1B Consistency Test Results

A window with the results will open. The plots are also saved in pdf format in the project's data/plots folder.

We use Q-Q plots in the consistency test to assess whether J(0) values derived from different spectrometer fields follow the same distribution. If the fields are consistent, the quantiles should fall close to the diagonal; systematic deviations from the diagonal indicate field-dependent differences in the relaxation data.

When more than two spectrometer fields are available, PCA provides a compact way to compare the multi-field J(0) profiles across residues. Instead of inspecting each field pair separately, PCA represents the field-dependent variation in a single multivariate space, helping to identify residues or datasets that show inconsistent behaviour across fields.

1 Consistency Tests



1B Consistency Test Results continued…

The Q-score measures how poorly each residue is reconstructed by the PCA model; high Q-scores indicate residues with unusual multi-field J(0) behaviour. The dashed threshold marks the 90th percentile of the standardised Q-score distribution, highlighting residues that deviate most strongly from the overall multi-field pattern.

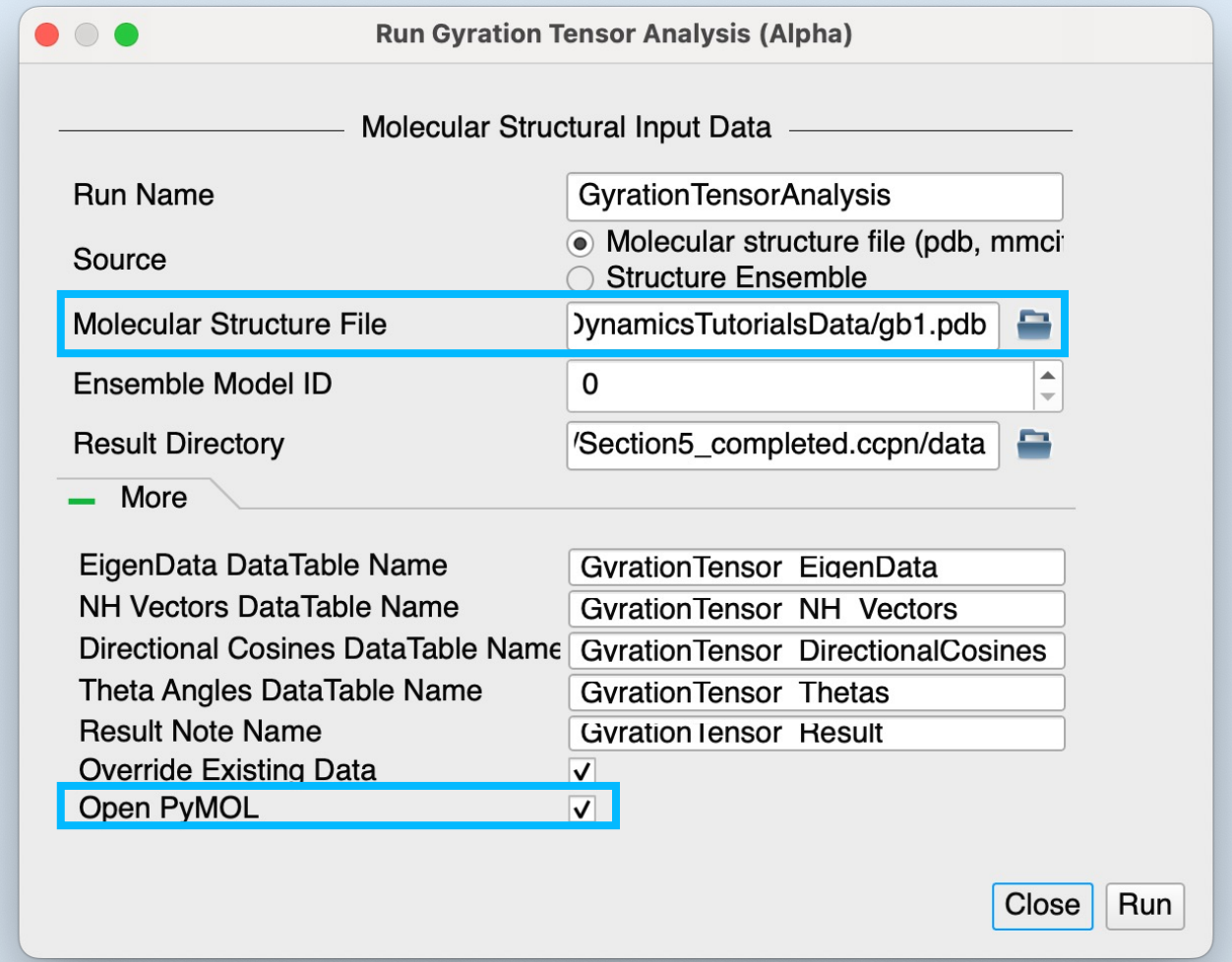
References:

Morin and Gagné, JBiomolNMR (2009) DOI 10.1007/s10858-009-9381-4

Mureddu *et al.*, (2026) Pre-print

DOI https://doi.org/10.64898/2026.06.19.733360

2 Gyration Tensor

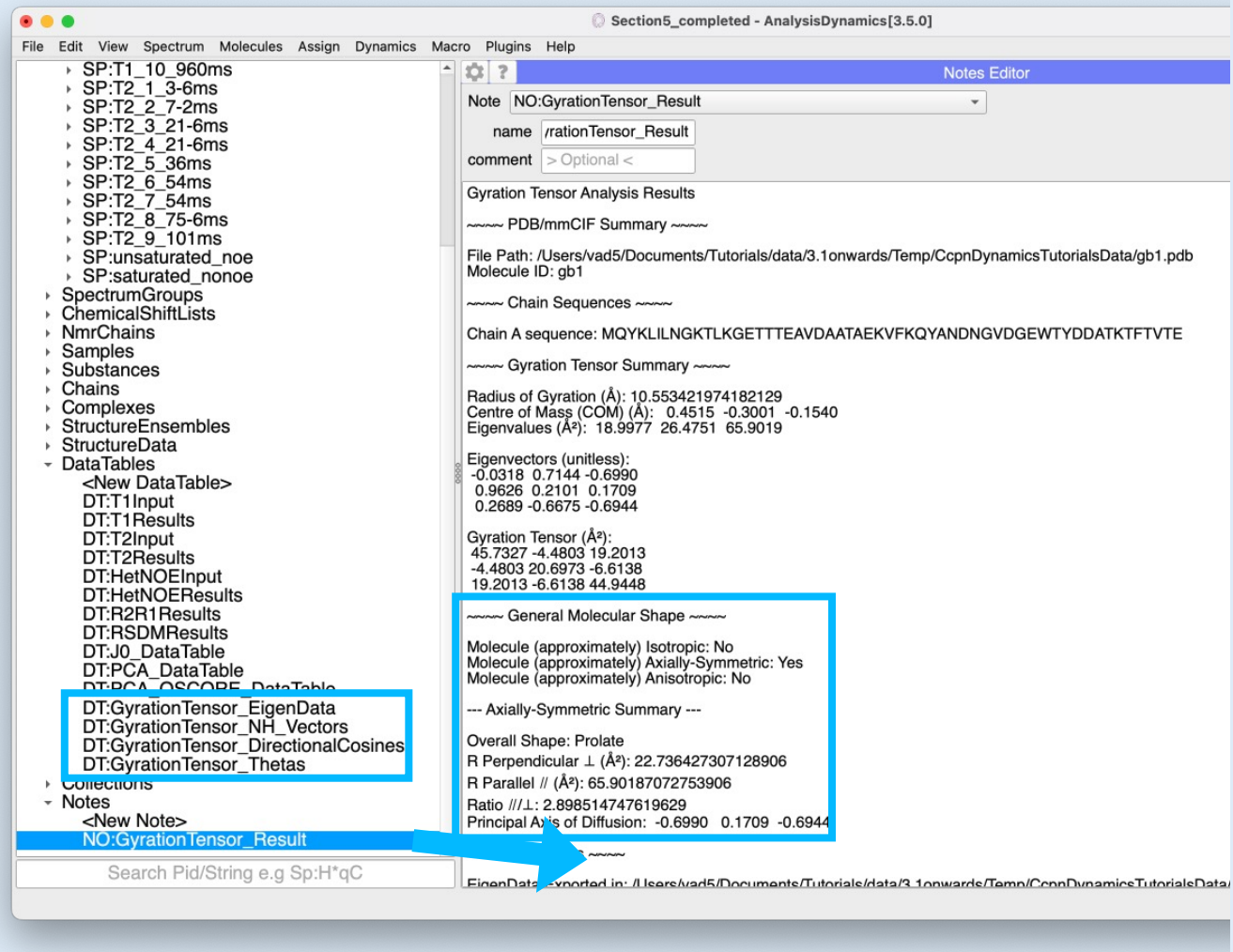


2A Gyration Tensor Analysis

Using a pdb or mmCIF file of a protein structure file, you can calculate its gyration tensor and determine whether the shape is isotropic, .

- Go to **Main Menu → Dynamics → Gyration Tensor Analysis** …
- As your **Molecular Structure File** select the **gb1.pdb** file from the tutorial data folder. Either type/copy the full path, select the file via the folder icon or drag the file into the box from a file browser.
- In the More section you can select whether or not to Open PyMOL with the structure.
- Click **Run**

2 Gyration Tensor

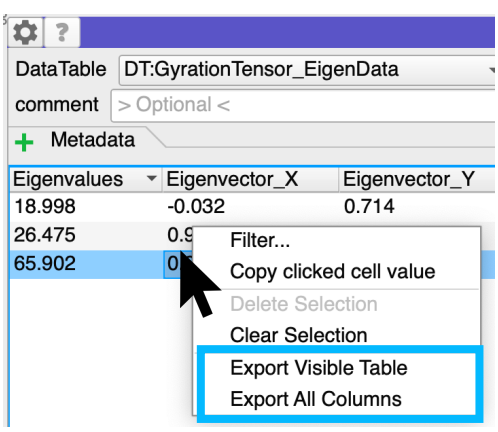


2B Inspecting the Gyration Tensor analysis results

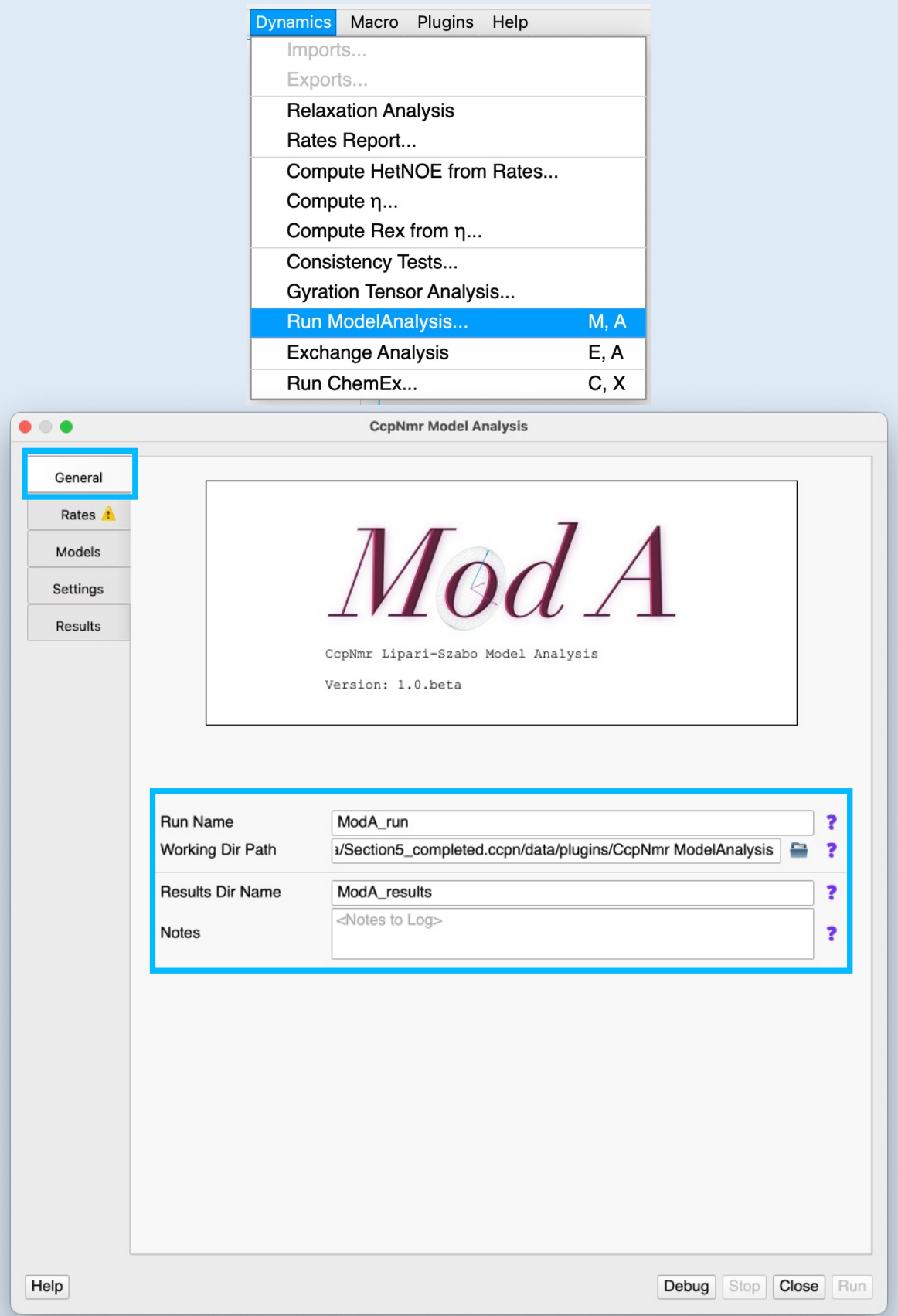
The results are written as four DataTables and one Note.

- Open the Note by dragging it into the Drop Area.
  It provides you with a variety of information about your structure, including information about the general molecular shape.

The DataTables contain various vectors which you may want to use for other calculations. Remember that DataTables can be opened as modules and then exported (as .csv or .xls) by right-clicking on them.



3 Model Free Analysis



3A Open Model Analysis Popup

- Open the **RSDMCompleted.cppn** project.
- Go to **Main Menu → Dynamics → Run Model Analysis** …

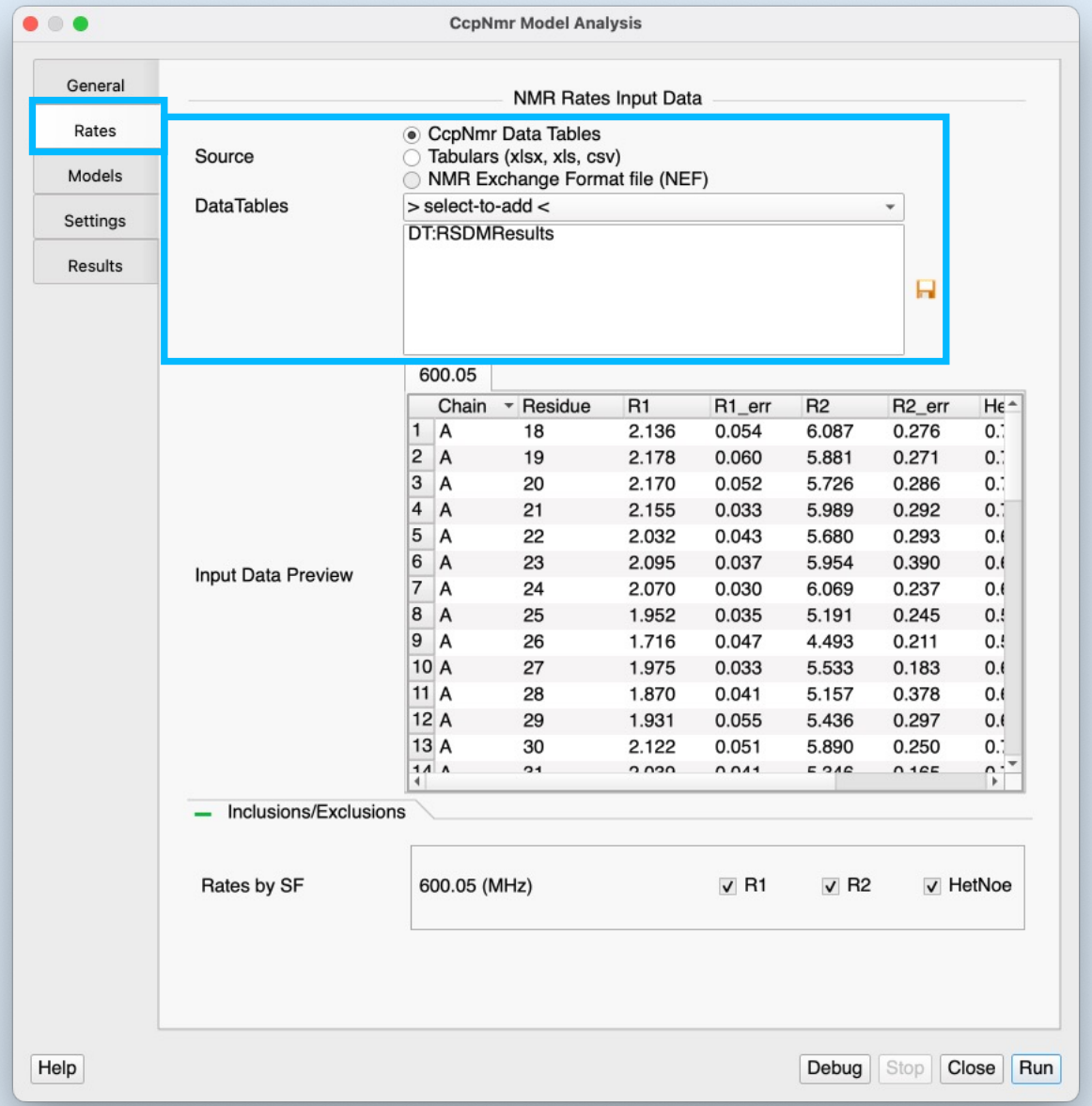
In the **General** tab:

- Change the Run Name, Working Directory Path, Results Directory Name and add any Notes if you wish.

  By default the Working path is set to

  YourProject.ccpn/data/plugins/CcpNmr ModelAnalysis

3 Model Free Analysis



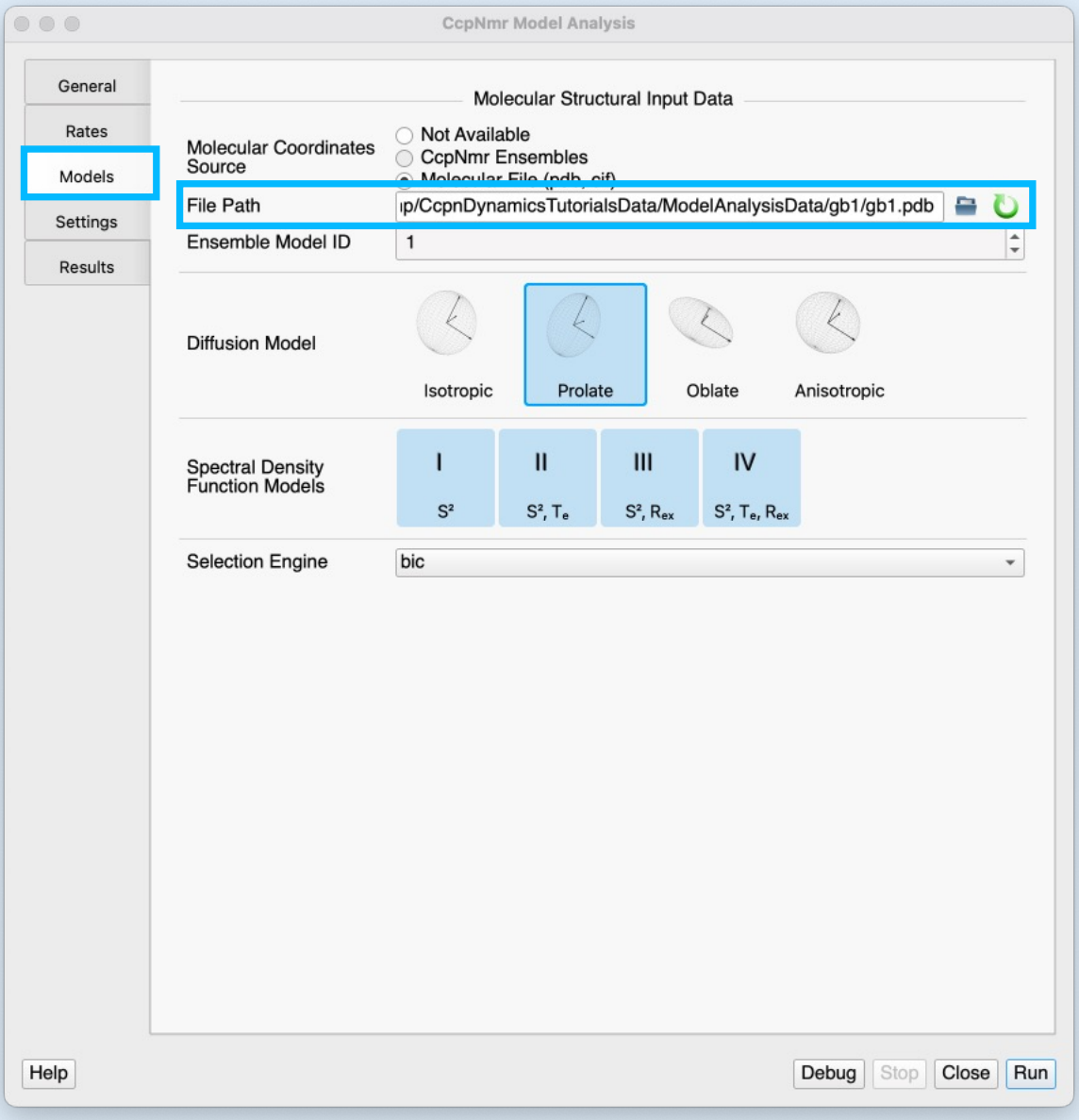
3B Select Input Data

In the **Rates** tab:

- Select **CcpNmr Data Tables** as the Source
- Select the **DT:RSDMResults** DataTable.

  This contains the R1, R2 and heteronuclear NOE data for GB1.

Model Free Analysis



3C Select Models

In the **Models** tab:

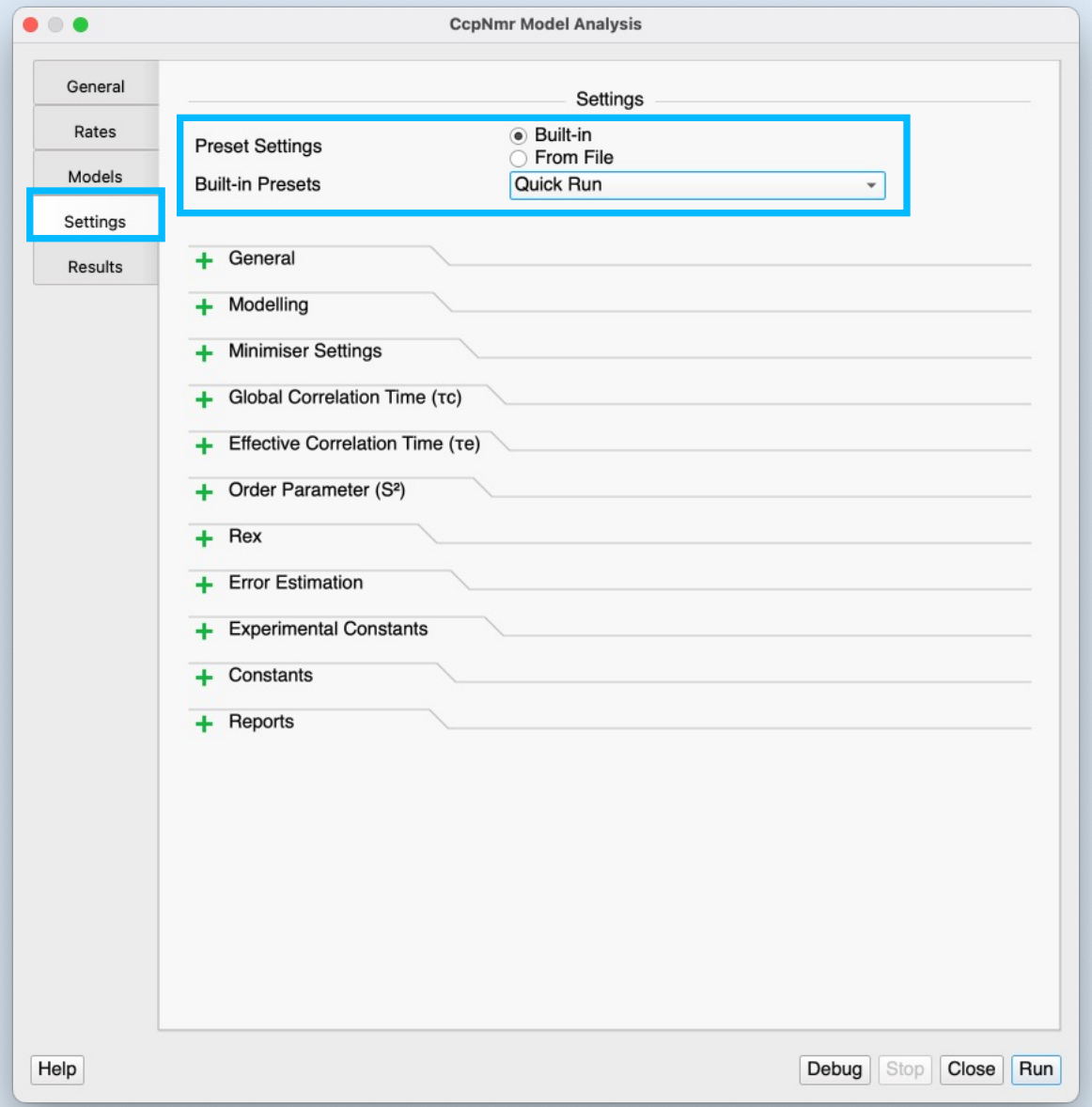
- If you wish, enter the path to the **gb1.pdb** file provided in the tutorial data.

The program will calculate this to be prolate and use that as the **Diffusion Model**. But you can add additional models if you wish (though this will make the calculation longer).

By default, all **Spectral Density Function Models** are selected. Click on any to remove them as options.

The default **Selection Engine** is **bic**, the Bayesian Information Criterion. This will give you a good indication whether a particular model is sufficiently well supported by the data, given the known errors.

3 Model Free Analysis

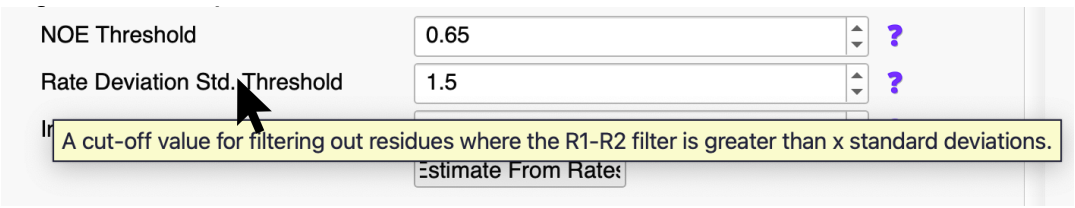


3D Settings

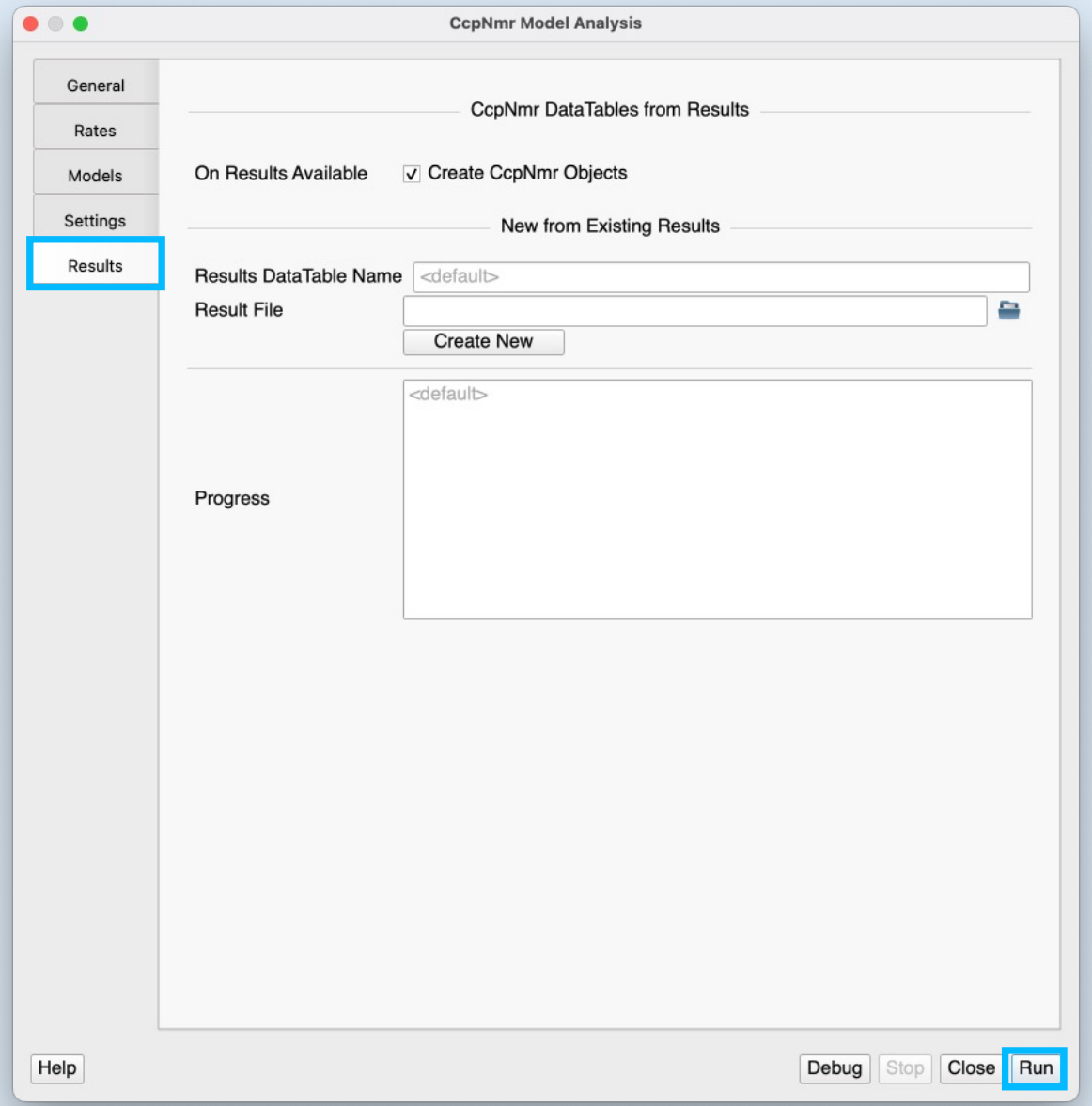
In the **Settings** tab:

- From the Built-in Presets tab you can select **Quick Run** – this will do the calculations in about 5-10 minutes. This will give you a quick view of the data, but not be very accurate. Alternatively select **Standard Run**, but be prepared for this to take an hour or more.

You will see that there are many settings that can be changed. Our standard settings have been chosen to give good and reliable results across a wide range of proteins and data sets. But obviously you can change settings. Hovering over a variable will give you fairly detailed



Model Free Analysis



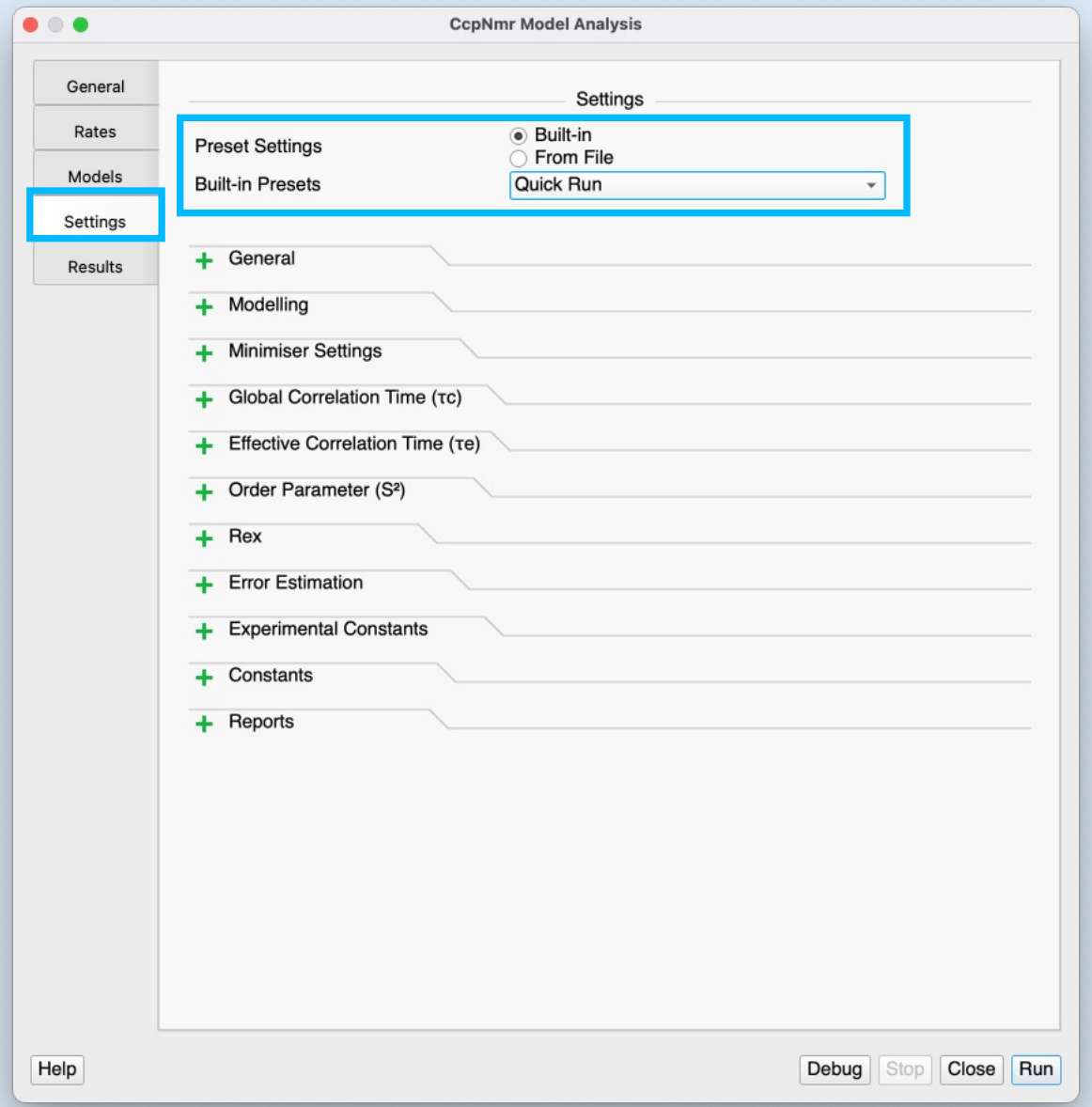
3E Settings

In the **Results** tab:

- Click **Run** to start the calculation

In the **Progress** window you will see how the calculation progresses. Note that there will be calculations for each residue, but not necessarily in order (due to parallel processing).

3 Model Free Analysis



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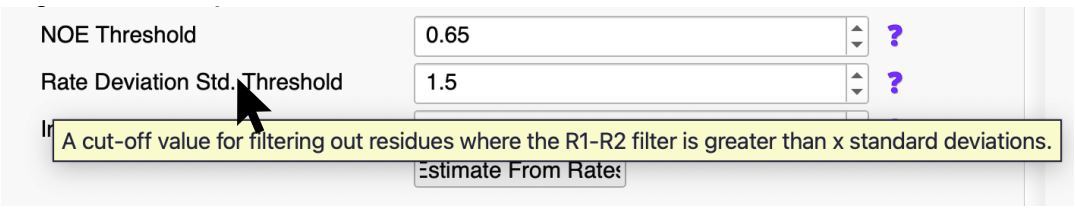
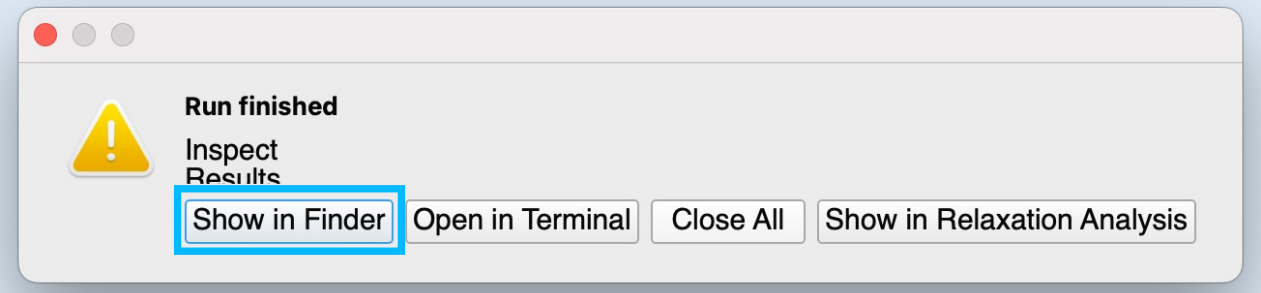


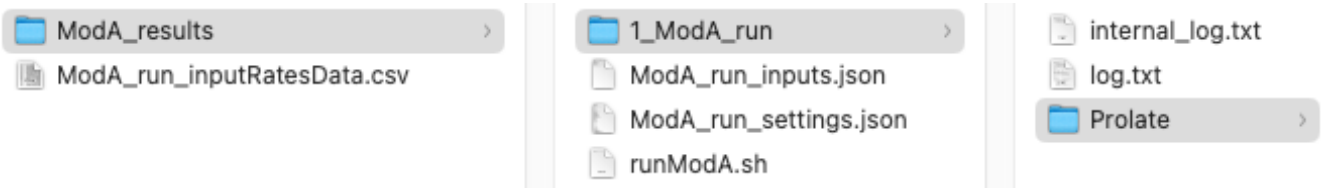
4 Model Free Analysis Results



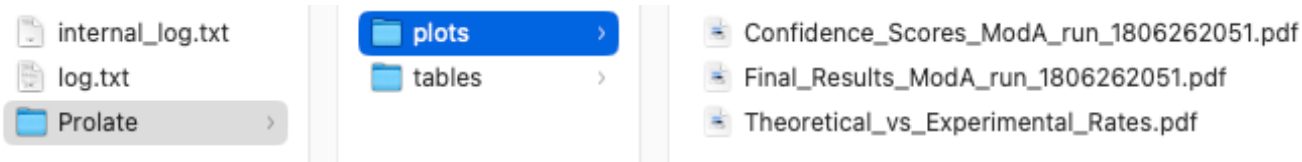
4A View results

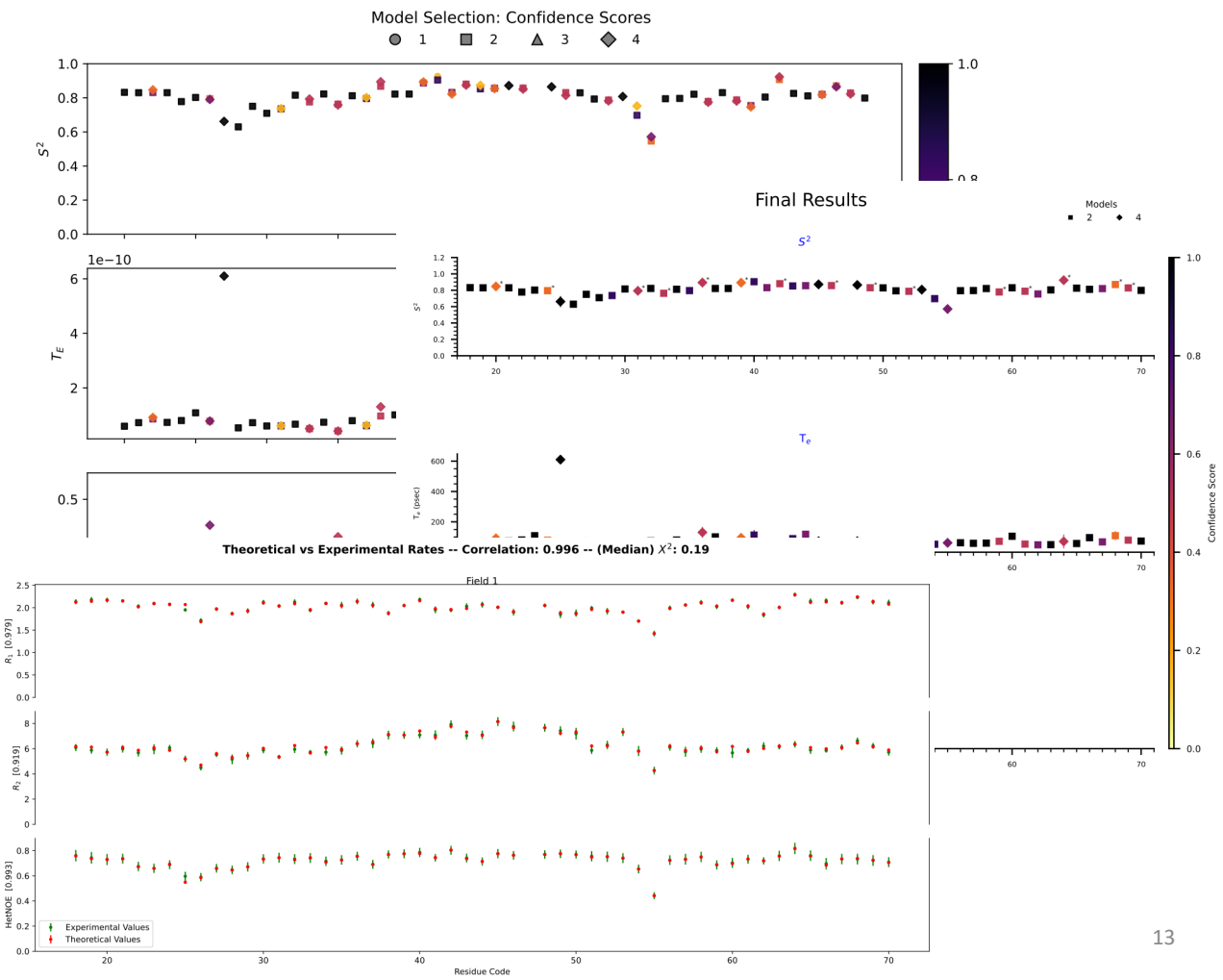
- After the run has finished, select **Show in Finder**

Your results folder will contain a folder for that run with subfolders for each Diffusion Model:



Within that folder you will find several plots showing the confidence scores for different models and the final selection based on the selection engine, as well a comparison of back calculated and experimental rates.





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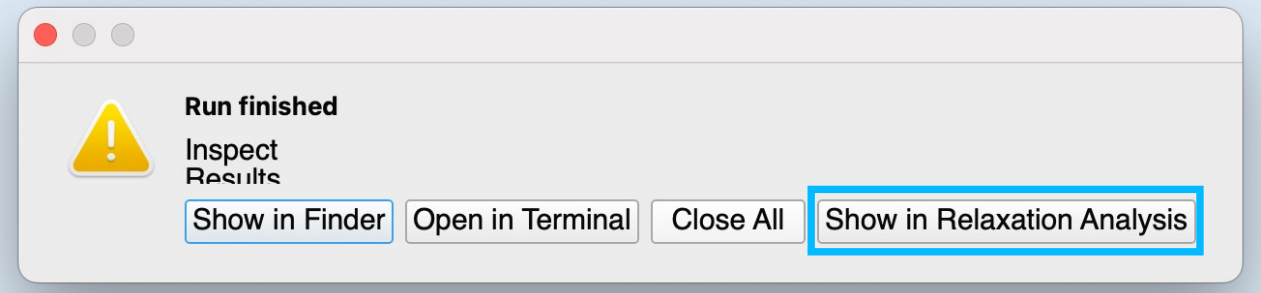

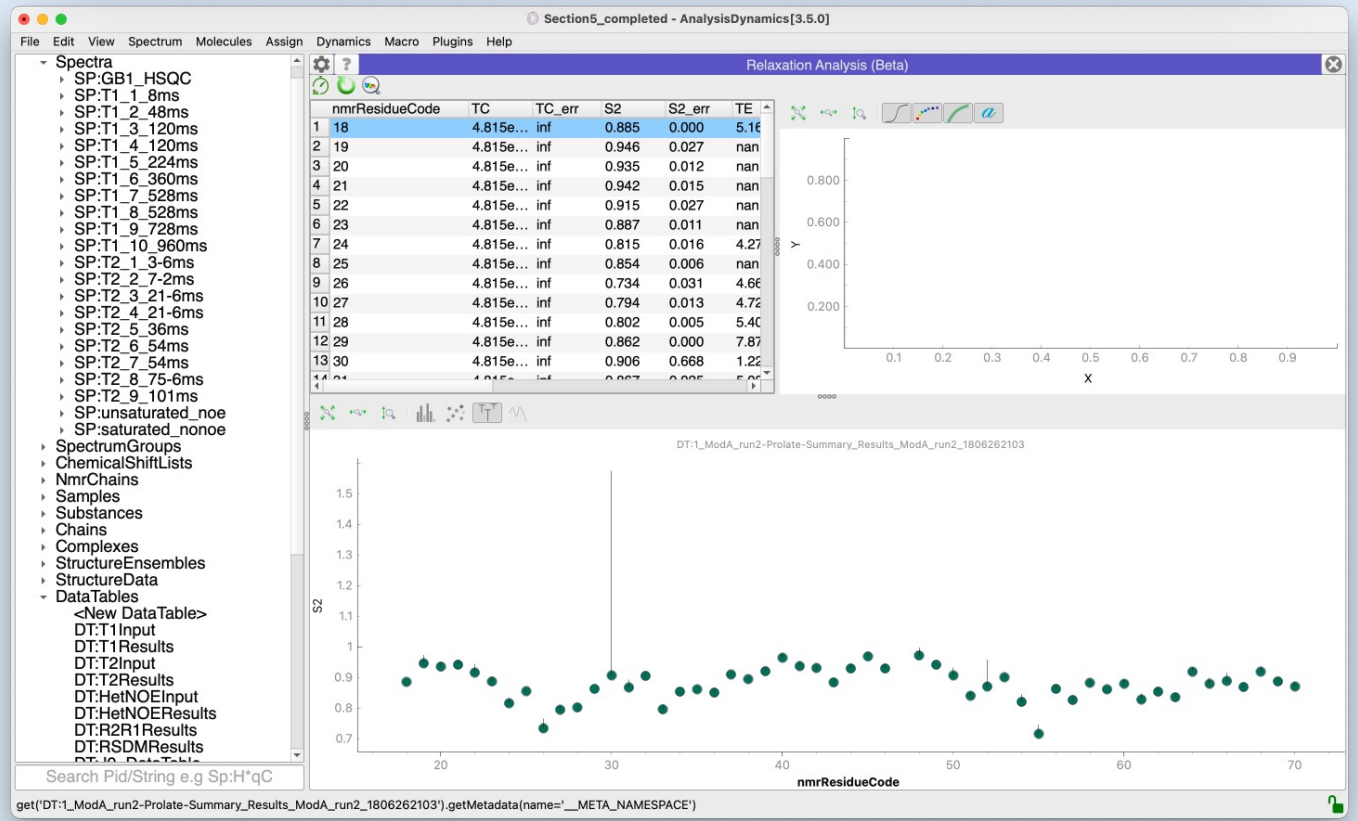

4B View results in Relaxation Analysis module

- After the run has finished, select **Show in Relaxation Analysis**

The order parameters will be shown in the Relaxation Analysis

Unfortunately, This feature not yet fully functional, so at the moment the data cannot be examined interactively yet.

Contact Us

Website:
www.ccpn.ac.uk

Suggestions and comments:
support@ccpn.ac.uk

Issues and bug report:
https://forum.ccpn.ac.uk/

Cite Us

Mureddu L.G. et al. CcpNmr AnalysisDynamics: a unified framework for NMR dynamics data analysis. **(2026)**
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