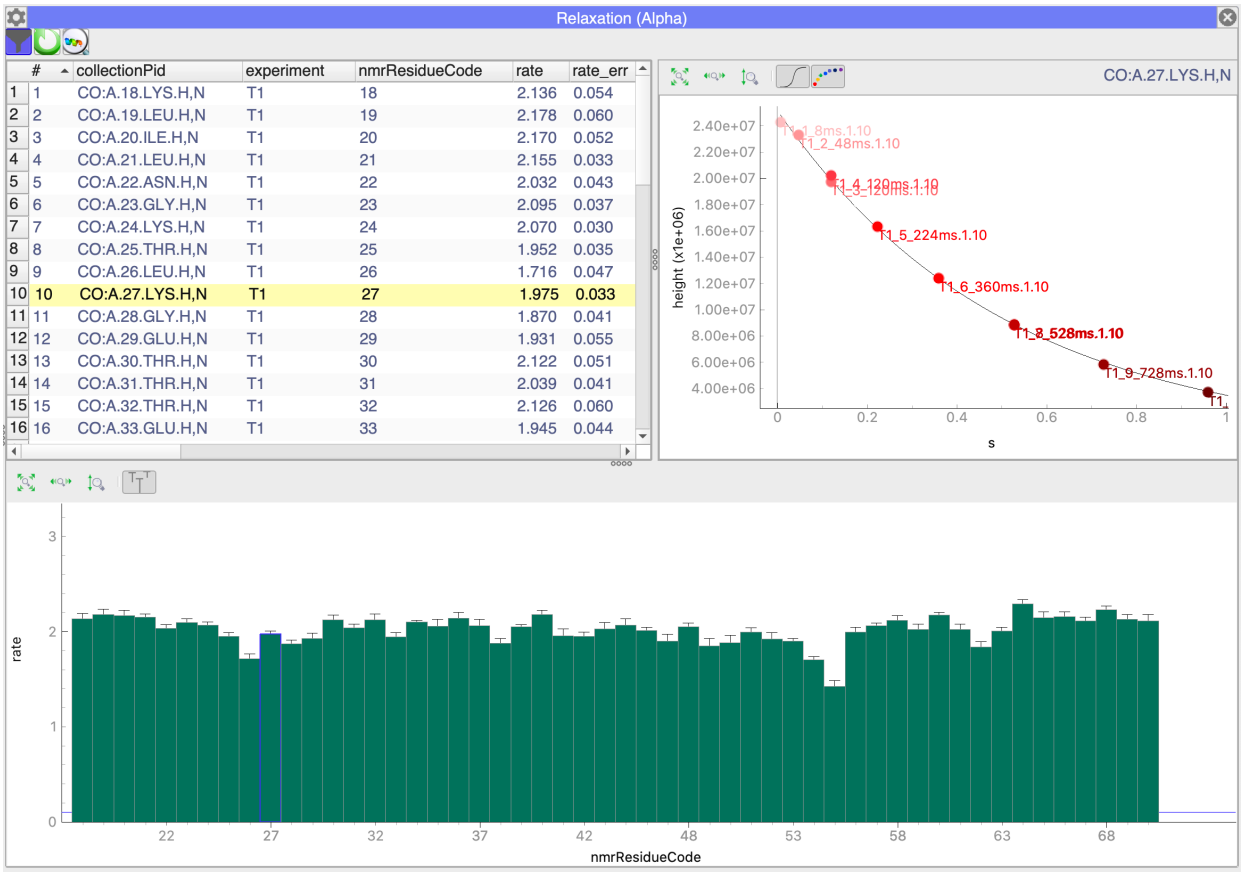
CCPN

CcpNmr Analysis Version 3

R1, R2 and heteronuclear NOE Tutorial (beta testing)



#	collectionPid	experiment	nmrResidueCode	rate	rate_err
1	CO:A.18.LYS.H,N	T1	18	2.136	0.054
2	CO:A.19.LEU.H,N	T1	19	2.178	0.060
3	CO:A.20.ILE.H,N	T1	20	2.170	0.052
4	CO:A.21.LEU.H,N	T1	21	2.155	0.033
5	CO:A.22.ASN.H,N	T1	22	2.032	0.043
6	CO:A.23.GLY.H,N	T1	23	2.095	0.037
7	CO:A.24.LYS.H,N	T1	24	2.070	0.030
8	CO:A.25.THR.H,N	T1	25	1.952	0.035
9	CO:A.26.LEU.H,N	T1	26	1.716	0.047
10	CO:A.27.LYS.H,N	T1	27	1.975	0.033
11	CO:A.28.GLY.H,N	T1	28	1.870	0.041
12	CO:A.29.GLU.H,N	T1	29	1.931	0.055
13	CO:A.30.THR.H,N	T1	30	2.122	0.051
14	CO:A.31.THR.H,N	T1	31	2.039	0.041
15	CO:A.32.THR.H,N	T1	32	2.126	0.060
16	CO:A.33.GLU.H,N	T1	33	1.945	0.044

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 T1 and T2 relaxation and heteronuclear NOE data, and then going on to do reduced spectral density mapping. We have not included any theoretical background to these experiments. Please see other publications for this information.

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.)

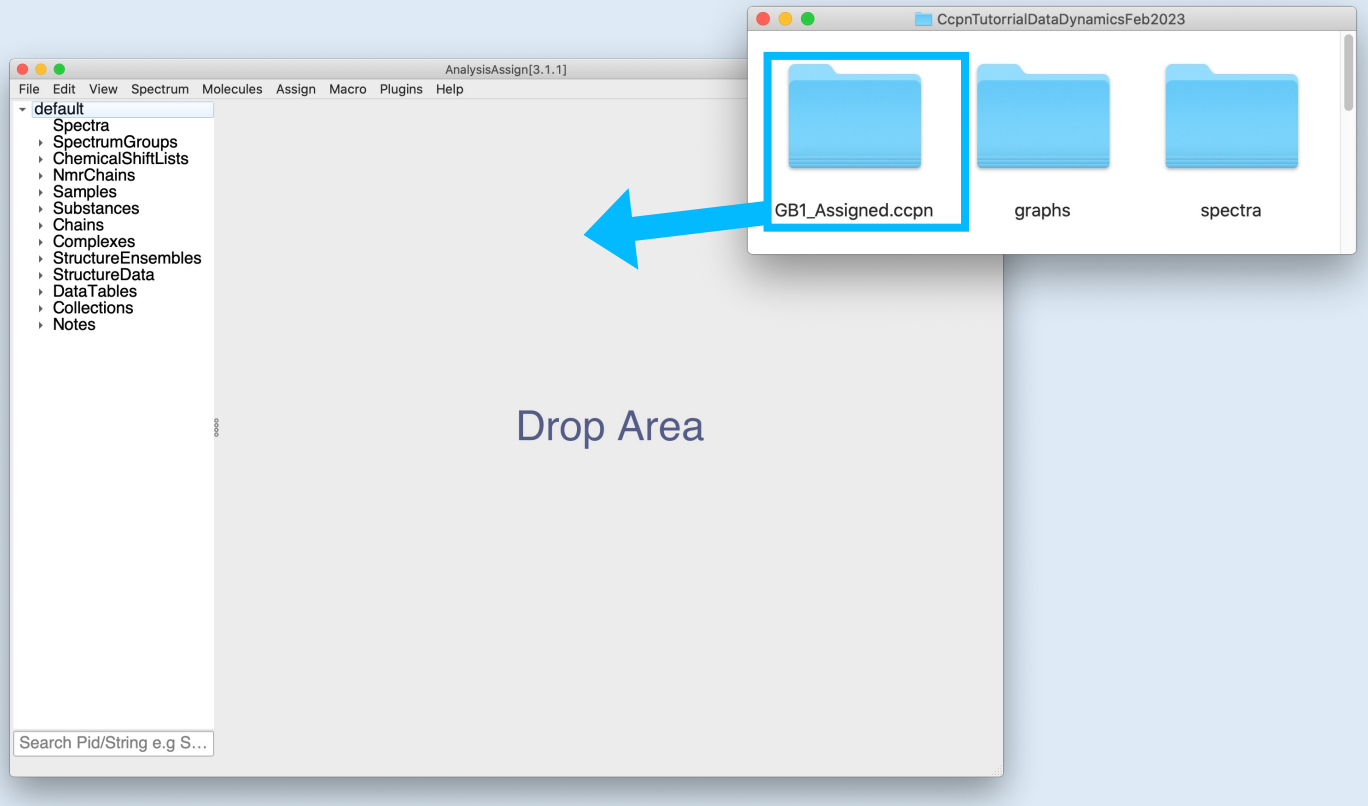
Contents:

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 Loading data

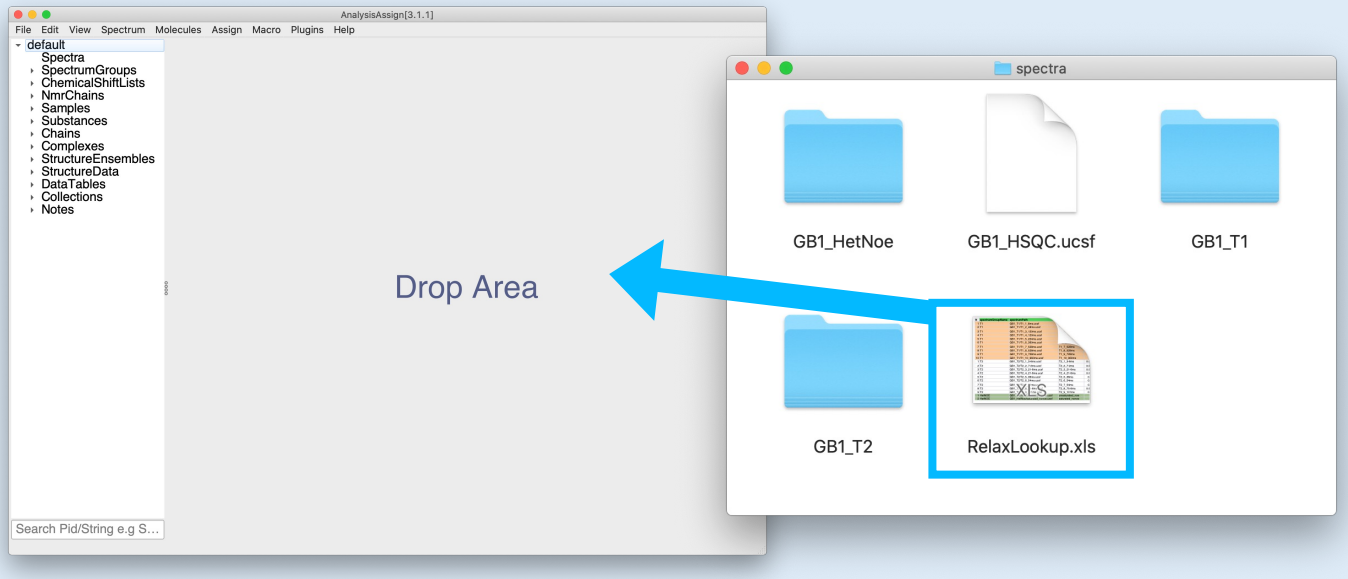


1A Open project and load spectra

- Find the **GB1_Assigned.ccpn** project folder in the Tutorial data directory. This project contains an assigned HSQC spectrum of GB1.
- Select the folder in your file browser and drag it onto the **Sidebar** or **Drop Area**.

You can load all the dynamics spectra using an Excel lookup file (Section 1B) which will also set up your SpectrumGroups at the same time.
Alternatively, you can load the spectra and create the SpectrumGroups manually (Section 1C–1E).

1 Loading data



	A	B	C	D	E	F	G
1	N	spectrumGroupName	spectrumPath	spectrumName	series	seriesUnit	seriesQuantity
2	1	T1	GB1_T1/T1_1_8ms.ucsf	T1_1_8ms	0.008	s	Time
3	2	T1	GB1_T1/T1_2_48ms.ucsf	T1_2_48ms	0.048	s	Time
4	3	T1	GB1_T1/T1_3_120ms.ucsf	T1_3_120ms	0.12	s	Time
5	4	T1	GB1_T1/T1_4_120ms.ucsf	T1_4_120ms	0.12	s	Time
6	5	T1	GB1_T1/T1_5_224ms.ucsf	T1_5_224ms	0.224	s	Time
7	6	T1	GB1_T1/T1_6_360ms.ucsf	T1_6_360ms	0.36	s	Time
8	7	T1	GB1_T1/T1_7_528ms.ucsf	T1_7_528ms	0.528	s	Time
9	8	T1	GB1_T1/T1_8_528ms.ucsf	T1_8_528ms	0.528	s	Time
10	9	T1	GB1_T1/T1_9_728ms.ucsf	T1_9_728ms	0.728	s	Time
11	10	T1	GB1_T1/T1_10_960ms.ucsf	T1_10_960ms	0.96	s	Time
12	1	T2	GB1_T2/T2_1_3-6ms.ucsf	T2_1_3-6ms	0.0036	s	Time
13	2	T2	GB1_T2/T2_2_7-2ms.ucsf	T2_2_7-2ms	0.0072	s	Time
14	3	T2	GB1_T2/T2_3_21-6ms.ucsf	T2_3_21-6ms	0.0216	s	Time
15	4	T2	GB1_T2/T2_4_21-6ms.ucsf	T2_4_21-6ms	0.0216	s	Time

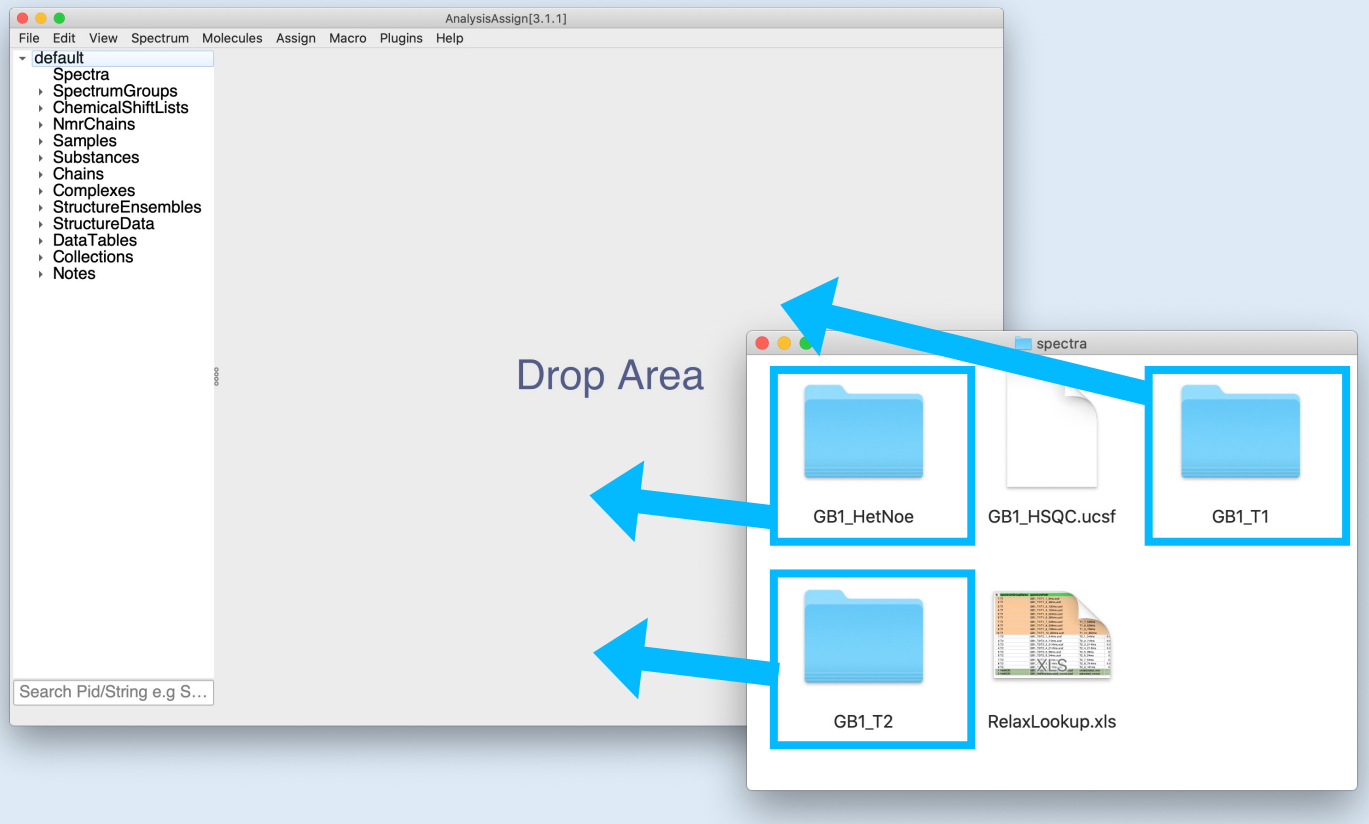
1B Import from Excel Lookup File (optional)

If you enter all the information about your spectra and the delay times into an Excel file, you can simply drag this into the program. The spectra will be automatically loaded and the SpectrumGroups and Series will be automatically created for you.

The figure above shows the column headings required for the Excel file to load correctly. SpectrumPaths are relative to the location of the Excel file.

- Find the **RelaxLookup.xls** Excel file in the tutorial data **spectra** folder and drag it into the Sidebar or Drop Area.
- Expand the **Spectra** and **SpectrumGroups** sections of the sidebar to see that the data has been imported.

1 Loading data



1c Load spectra manually

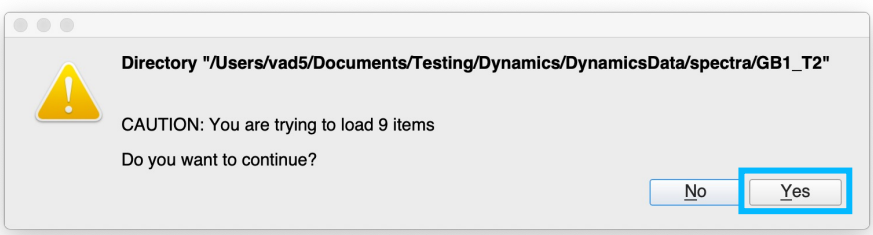
- One you have opened the **GB1_Assigned.ccpn** project, find the **spectra** directory in the Dynamics Tutorial data directory
- Select and drag the following three folders into the Sidebar or Drop Area:

  GB1_T1

  GB1_T2

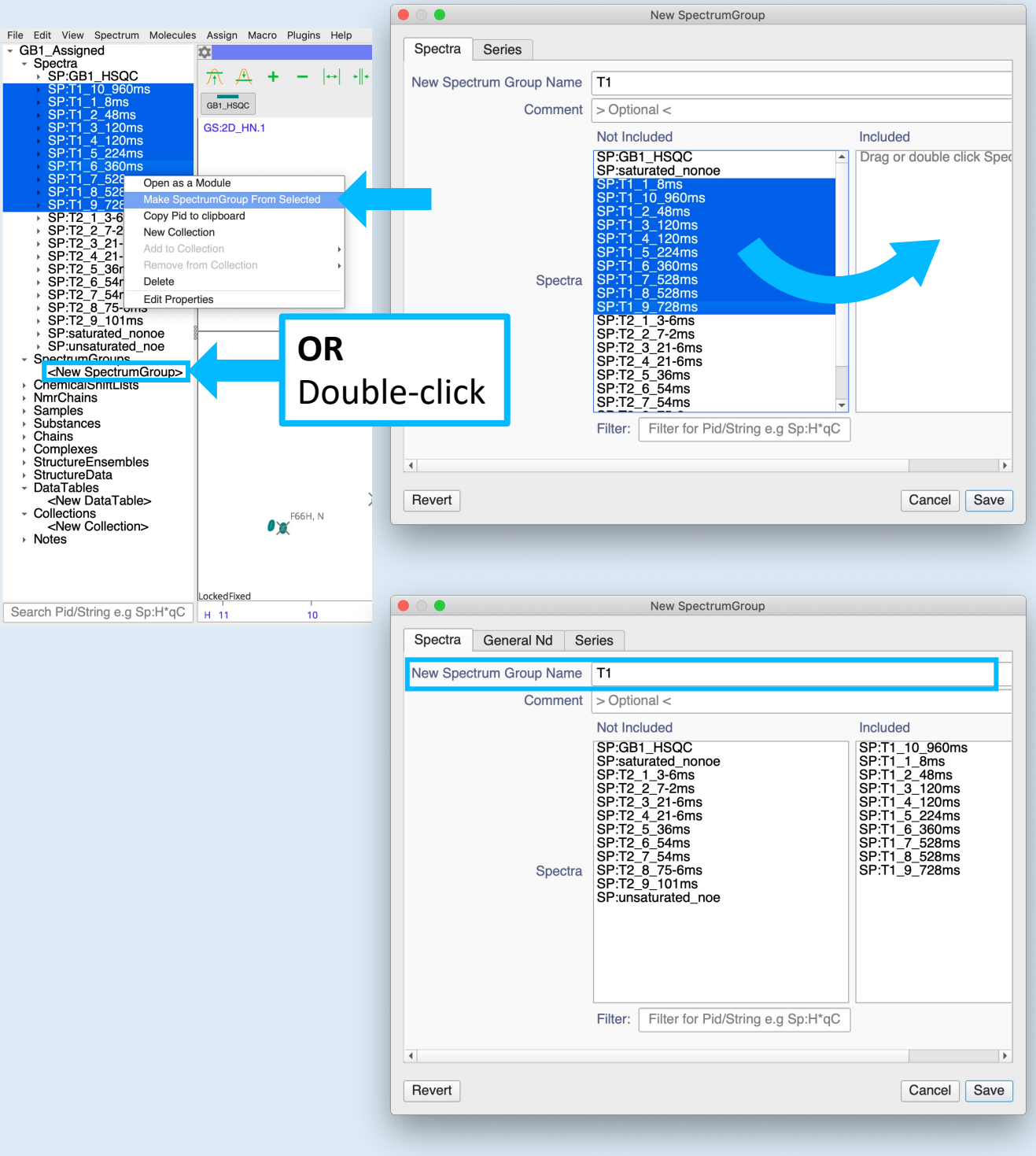
  GB1_HetNOE

  If asked whether you want to continue loading multiple items, click **Yes**.



If you drop the folders into the Drop Area the spectra will open directly. If you drop the folders on the Sidebar the spectra will simply be visible in the **Spectra** section of the sidebar and can be opened in a SpectrumDisplay module at a later stage.

1 Loading data



1D Create the T1 SpectrumGroup and Series

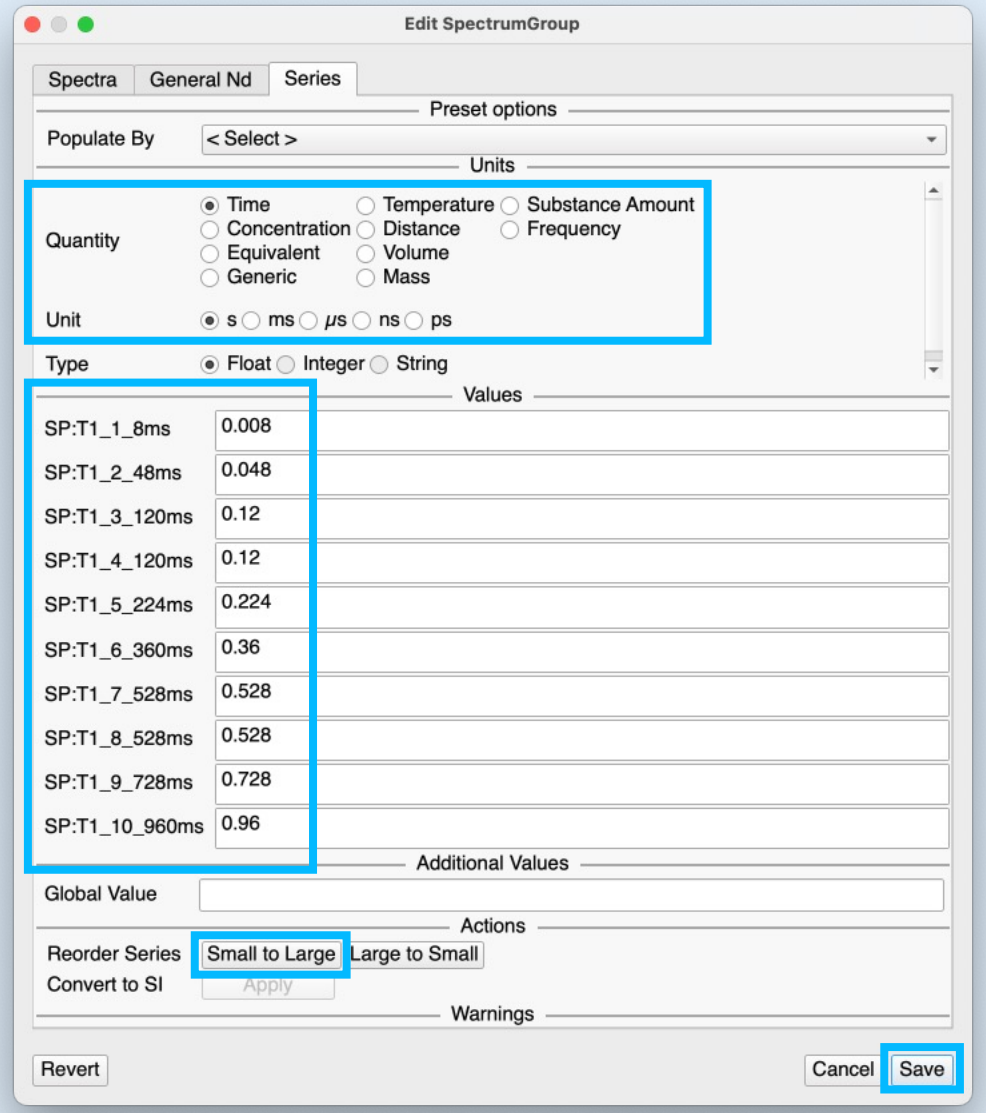
- Select all the T1 spectra in the sidebar, **right-click** and select **Make SpectrumGroup from Selected**

OR

- Expand **SpectrumGroups** in the sidebar and **double-click** on **<New SpectrumGroup>**
- In the **Edit SpectrumGroup** pop-up, **drag** all the T1 spectra from the left-hand side the right-hand side of the pop-up

- Give your SpectrumGroup a new **Name**, e.g. **T1**

Loading data

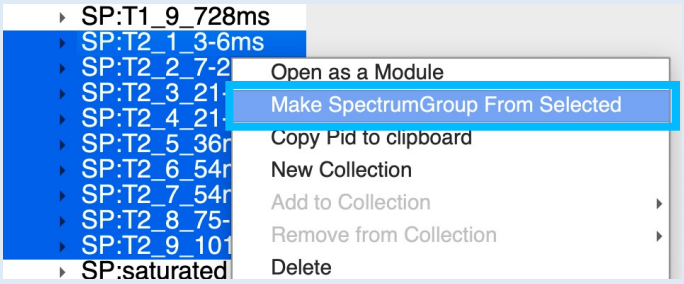


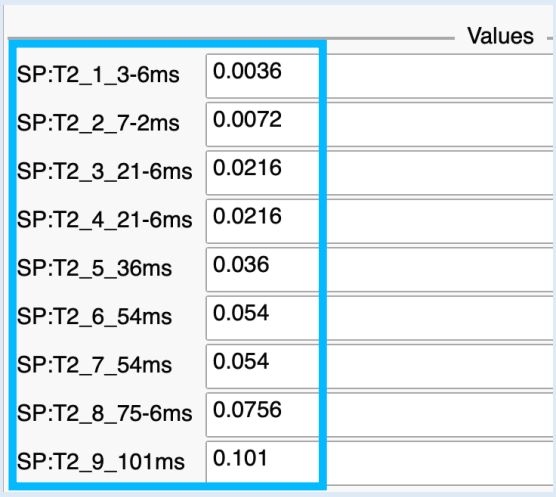
1E Create the T1 Series

- Go to the **Series** tab of the Edit SpectrumGroup pop-up
- Set the **Quantity** to **Time** and the **Unit** to **s** (seconds).
- Enter the relaxation delay (in seconds) for each spectrum. You can derive these from the spectrum names (note that these are given in ms, not s). Note that some delay times are repeated in order to give an indication of the measurement error.
- If your spectra are not ordered in ascending order, then click the **Small to Large** button to do so.
- Finally click on **Save**.

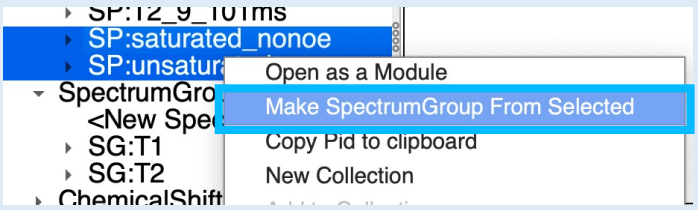
1 Loading data

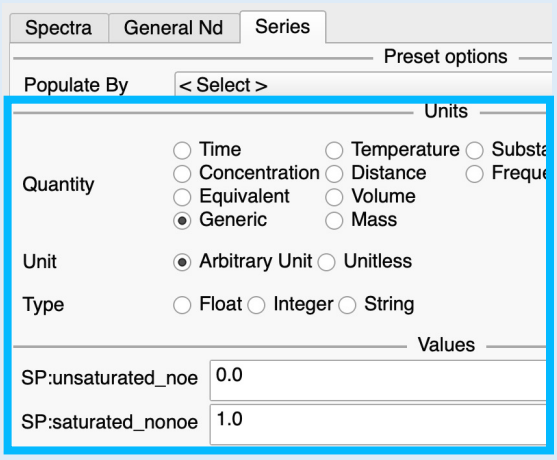
T2Data





HetNOE





1F Create T2 and Heteronuclear NOE SpectrumGroups and Series

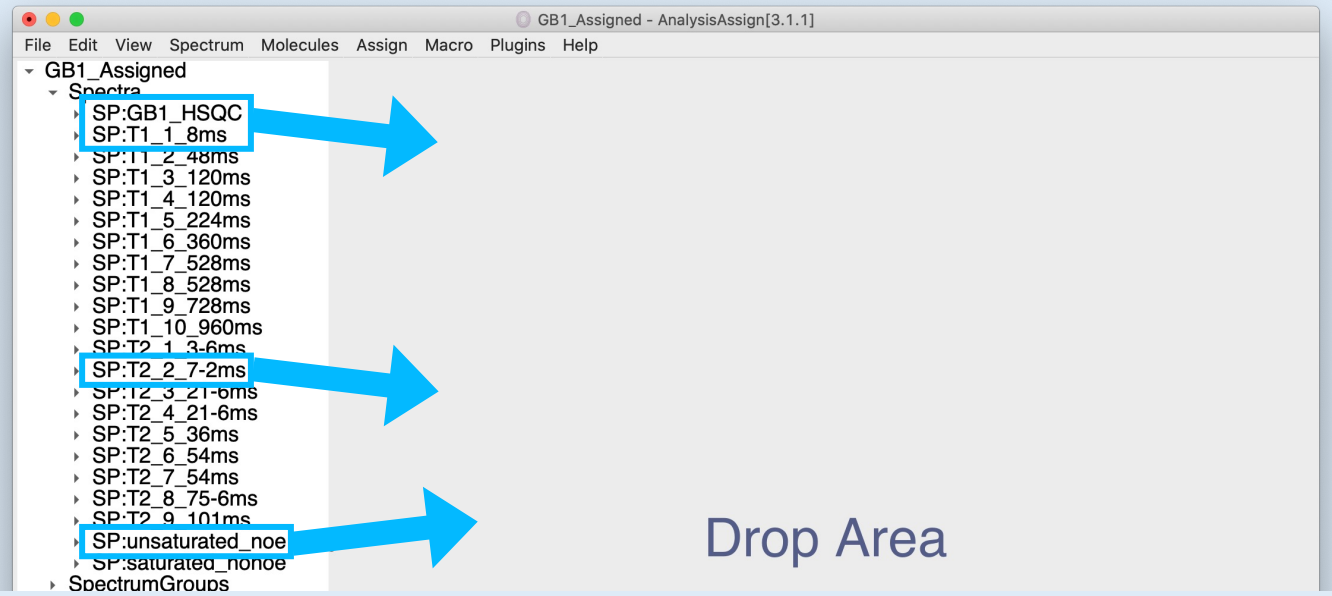
- Repeat the procedure for creating a SpectrumGroup and setting up the Series for the T2 data

Create a SpectrumGroup and Series for the Heteronuclear NOE data:

- Select the **saturated** and **unsaturated** spectra in the sidebar, right-click and select **Make SpectrumGroup from Selected.**
- Give the SpectrumGroup a **Name** (e.g. **HetNOE**)
- In the Series tab, set your **Quantity** to **Generic** and **Unit** to **Arbitrary Unit** and enter the following values:

unsaturated	**0.0**
saturated	**1.0**

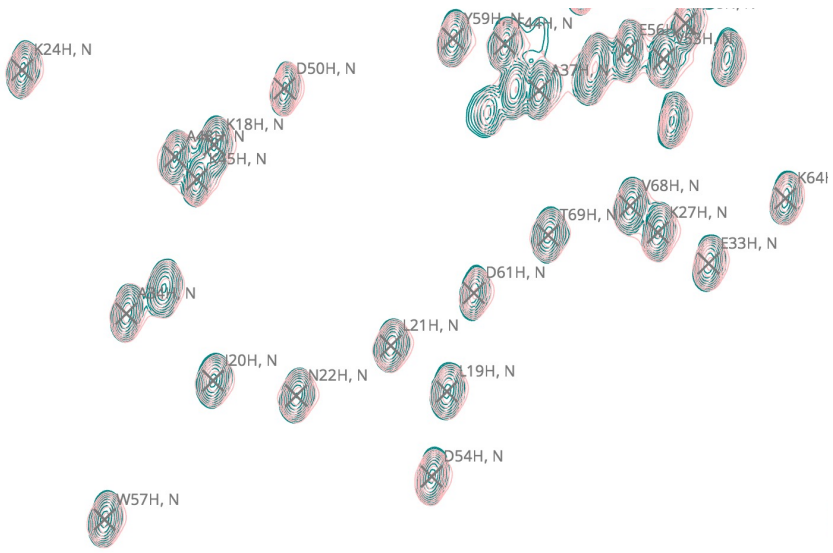
1 Loading data



1G Check alignment to HSQC

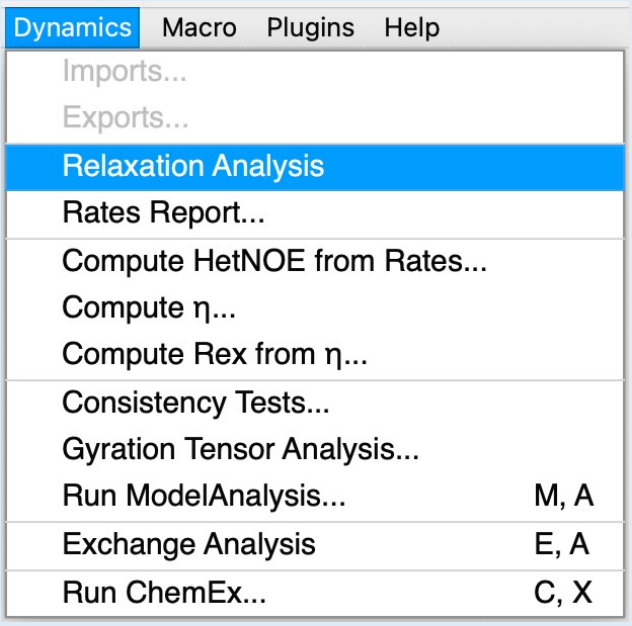
- Close any open **SpectrumDisplays** (click on the in the top right corner).
- **Drag** the **GB1_HSQC** spectrum from the sidebar into the DropArea.
- **Drag** the **T1_1_8ms** spectrum from the sidebar on top of the other spectrum.

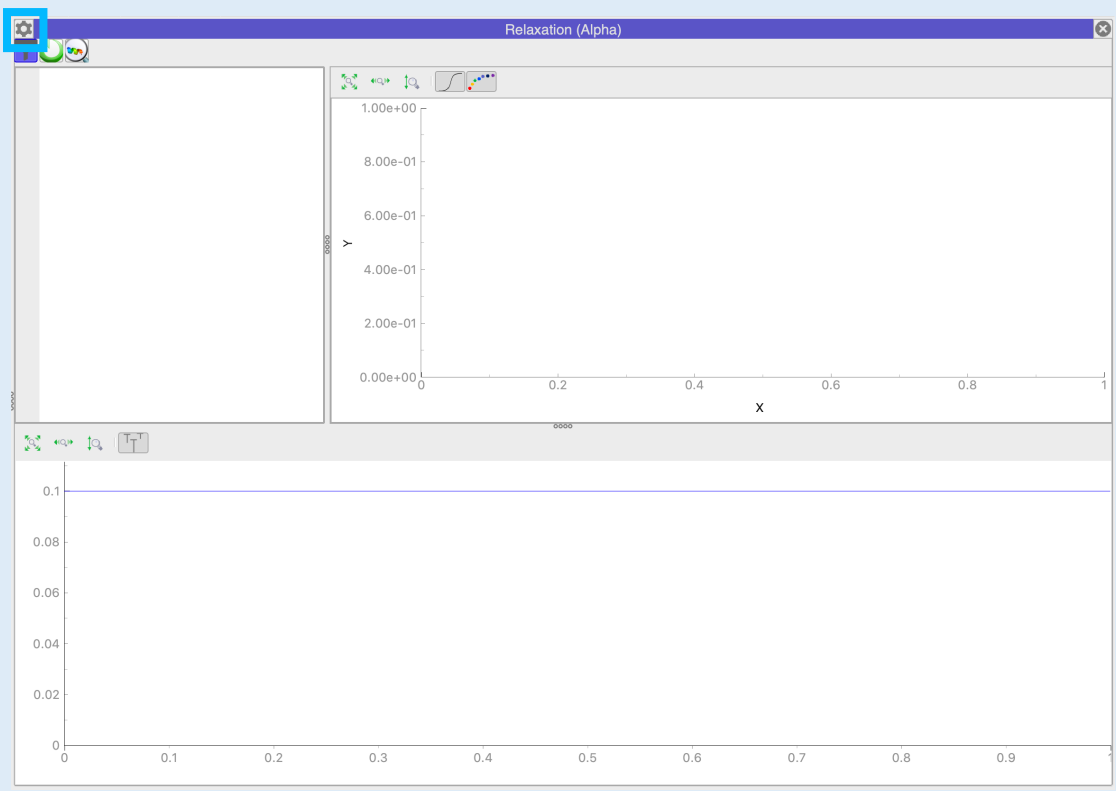
These should overlay well, meaning that you will be able copy the peaks from the **GB1_HSQC** spectrum to the T1 spectra without having to change their positions.



- **Drag** the **T2_1_3-6ms** and **unsaturated_noe** spectra onto the **GB1_HSQC** spectrum and check that these align well, too.

2 T1 and T2 Data



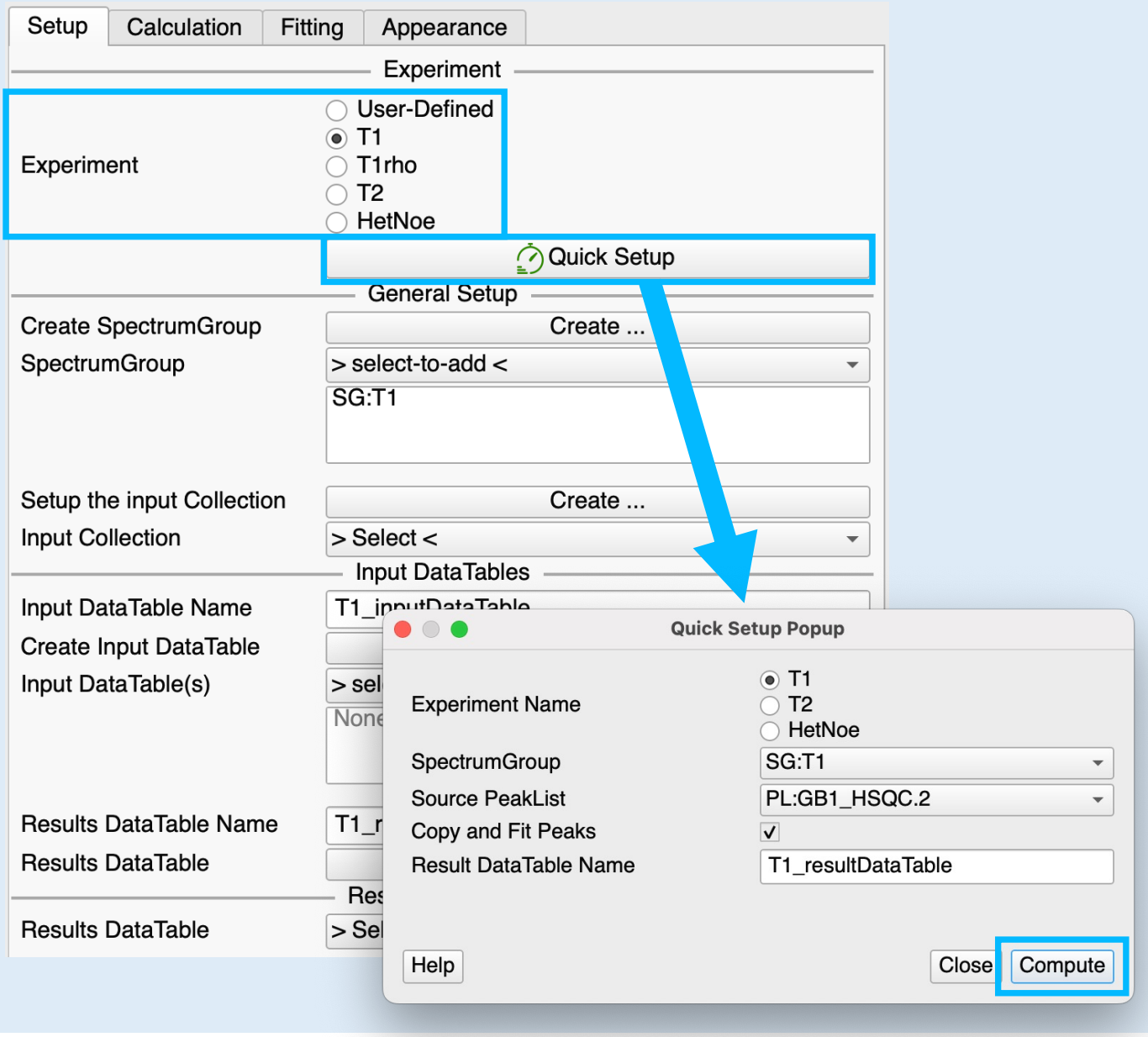


2A Open Relaxation Module

At this point you can either continue from Section 1 or start using our **1_DataLoaded.ccpn** project in the tutorial data.

- Go to **Main Menu ⟶ Dynamics ⟶ Relaxation Analysis**.
  This will open the Relaxation Analysis Module.
- Open the Settings Panel by clicking the gear icon in the top left of the module window.
  This is where you will set up your data and run all the calculations required before inspecting it in the main module.

2 T1 and T2 Data

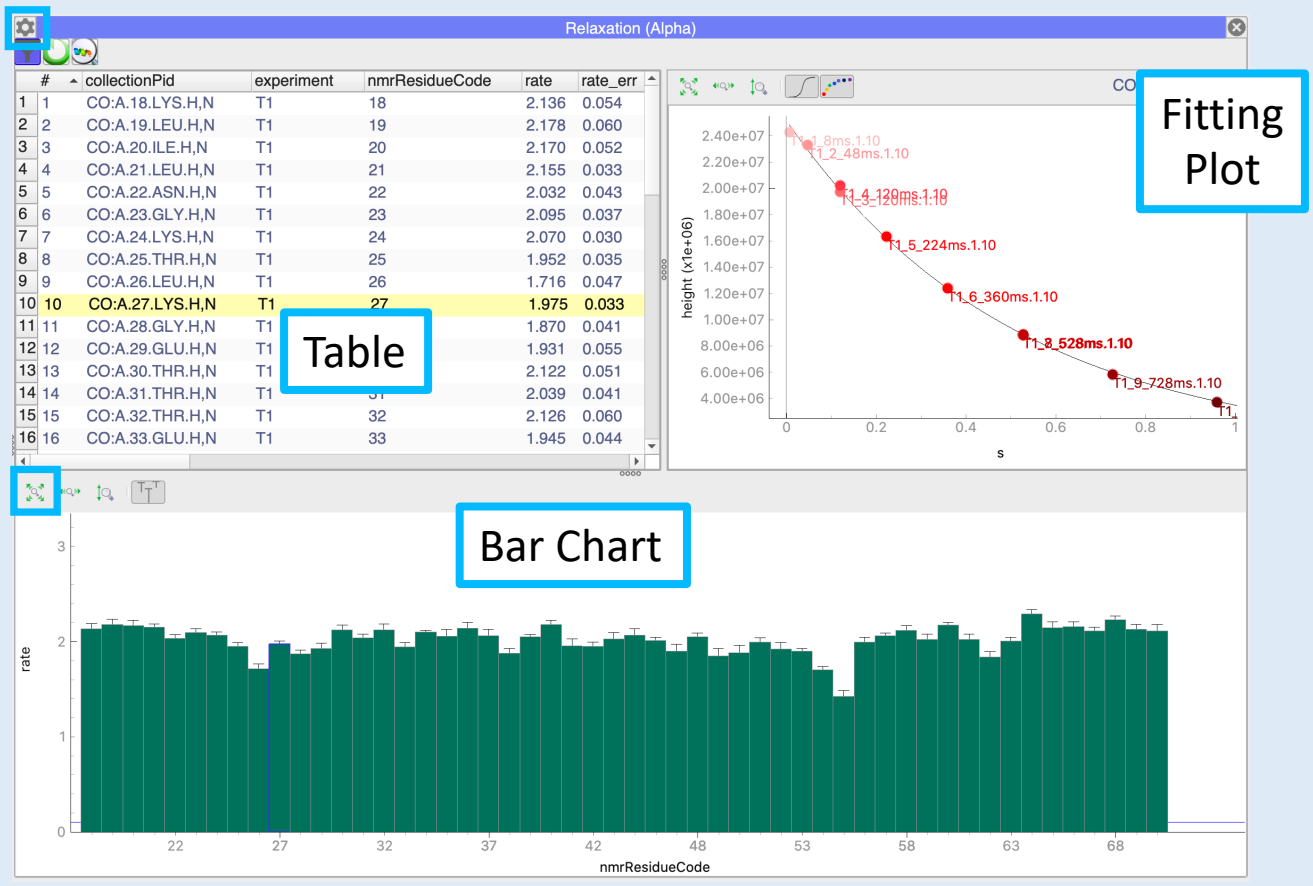


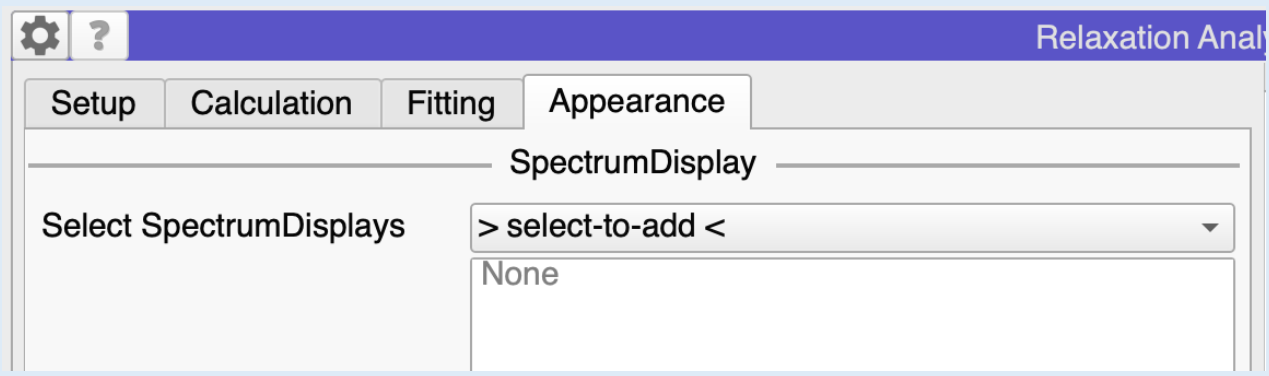
2B Copy Peaks

- Set the **Experiment** to **T1**.
  This will pre-populate some elements in the sections below and other tabs.
- Click on **Quick Setup** and check the correct SpectrumGroup and Source Peak List have been selected:
  Select SpectrumGroup: **SG:T1**
  Source PeakList: **PL:GB1_HSQC.2**
- Keep the remaining default options, as shown above and click on **Compute**.
  This will copy and group the peaks which may take a minute or so.

Note that the Peaks are copied from the assigned spectrum to the dynamics spectra and then fitted *in place*.

2 T1 and T2 Data



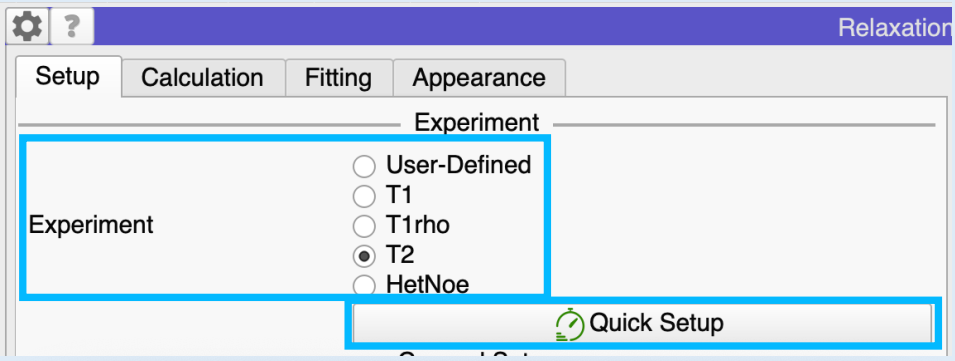


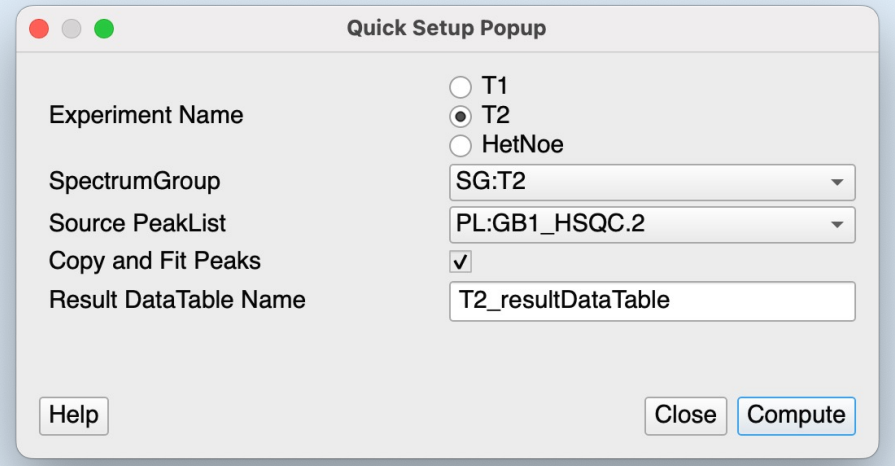
2C Inspect Results

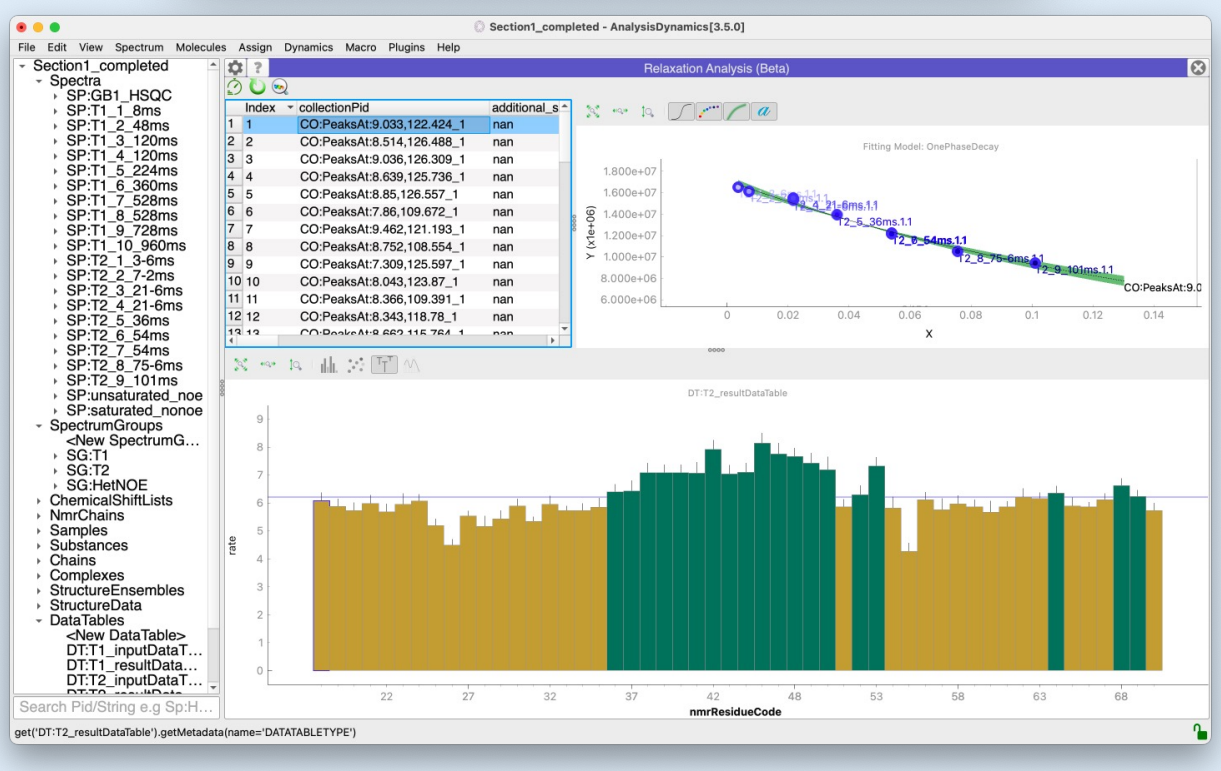
The Table, Fitting Plot and Bar Chart sections of the Relaxation Analysis module are filled with the results.

- Close the **Settings** tab by clicking on the gear icon in the top left again.
- If the data aren't shown in full, then press the auto-zoom icon above the bar chart to auto-scale it.
- If you click on a row in the Table or on a bar in the Bar Chart, then the matching bar/row will be selected and in the Fitting Plot you will see a graph showing your data points (in spectrum colours) and the fit.
  The table contains both the data points and fitting parameters.
  If you have a SpectrumDisplay open this will navigate to the peaks corresponding to the residue selected.
- If you wish, make changes to the appearance of the chart in the **Appearance** tab of the **Settings** panel.

T1 and T2 Data







2D T2 Data

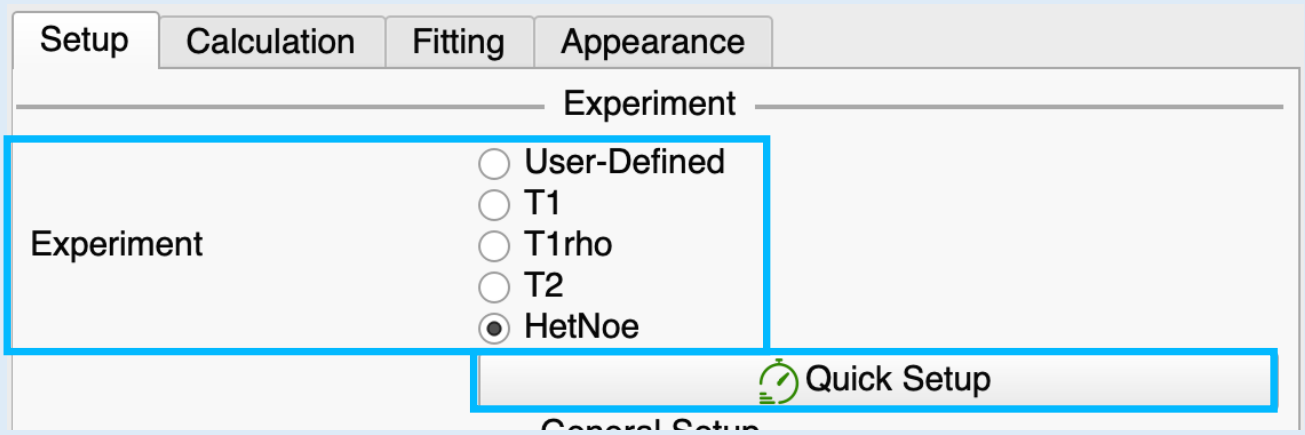
- Open the **Settings** panel with the gear icon.

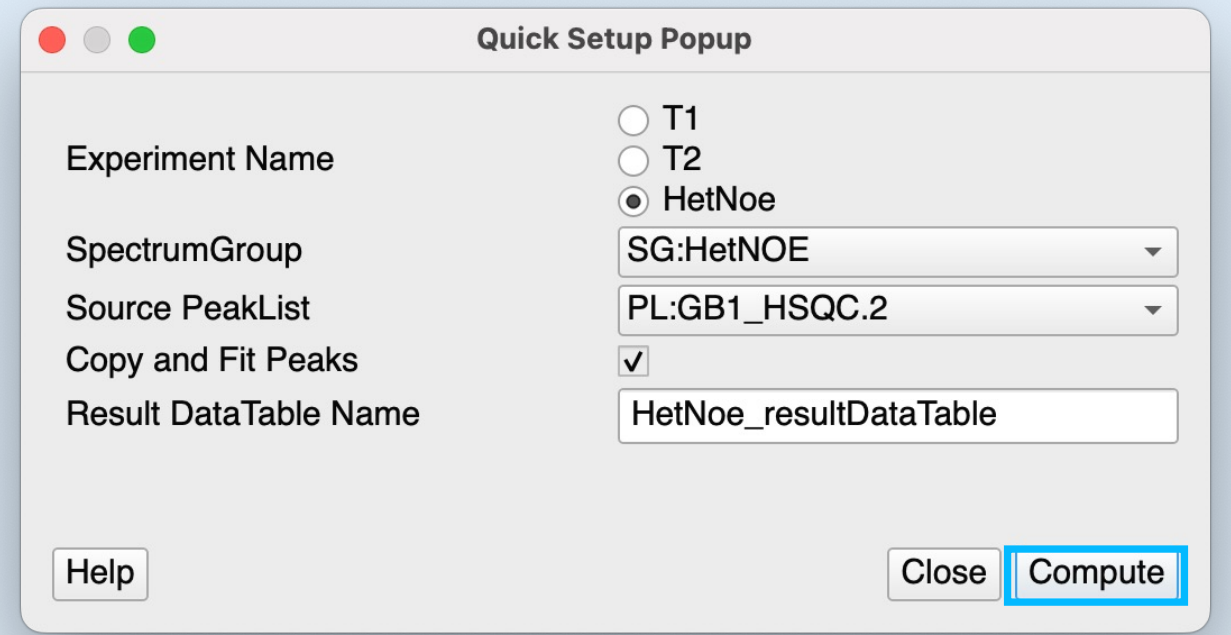
In the **Setup** tab:

- In the **Experiment** section, you will need to set the **Experiment** to **T2**.

- Repeat **Sections 2B–C** for the T2 data.

3 Heteronuclear NOE





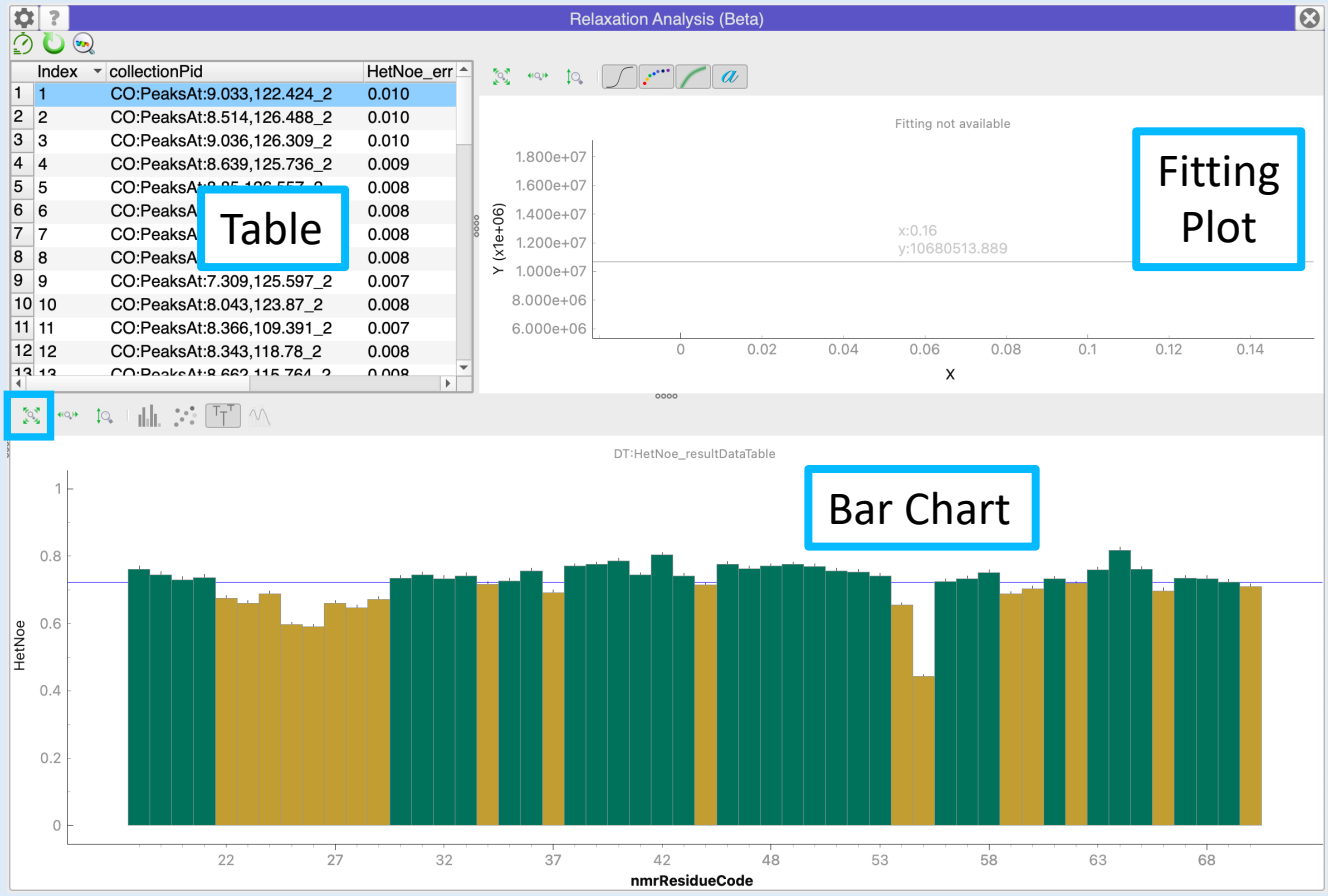
3A Heteronuclear NOE Calculation Setup

- Open the **Settings** panel with the gear icon.

In the **Setup** tab:

- Select **HetNoe** as the **Experiment**
- Click on **Quick Setup**
- Check the options and click on **Compute**

3 Heteronuclear NOE



3B Inspecting the Heteronuclear NOE data

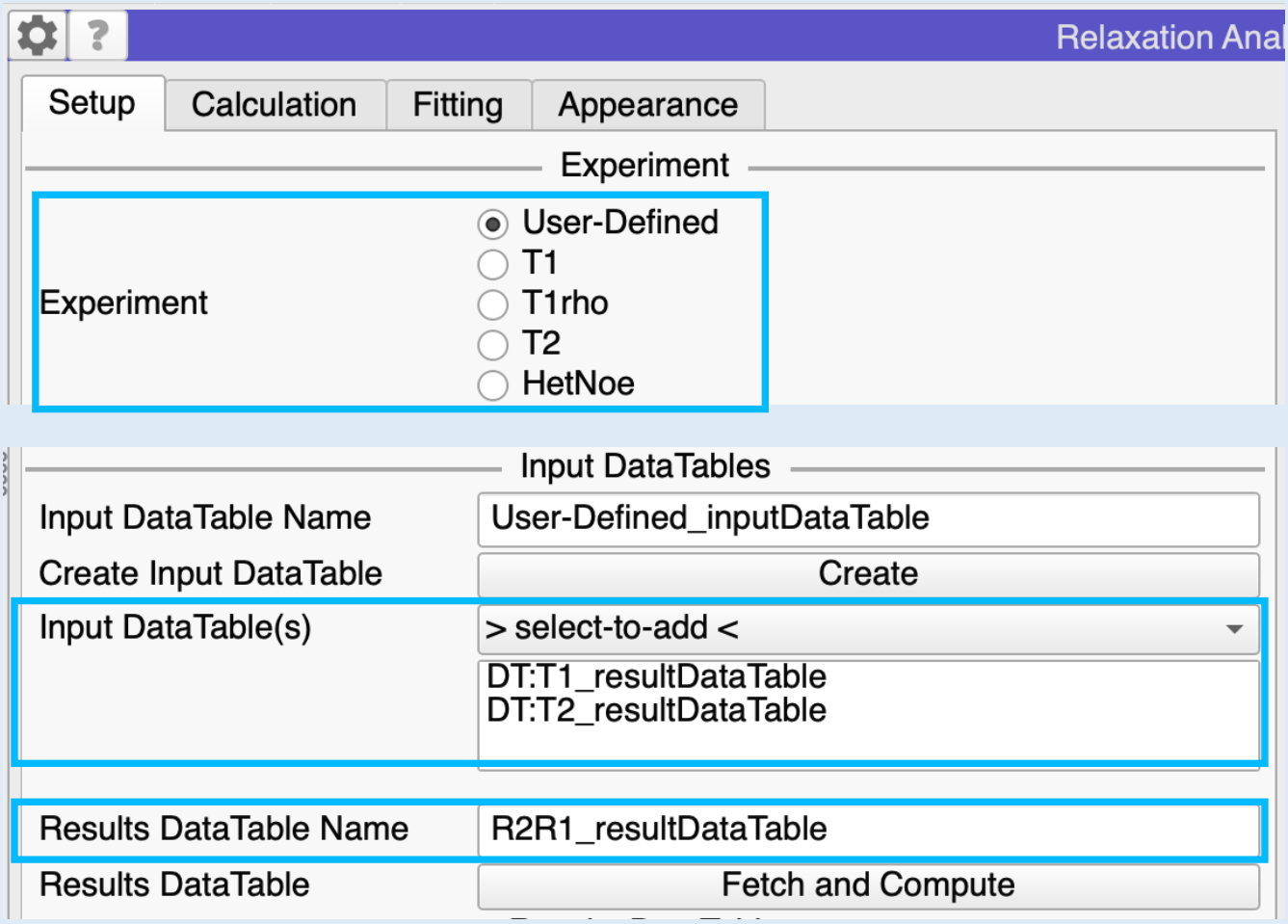
The bar chart should automatically show the Heteronuclear NOE on the Y-axis.

- Close the **Settings** tab by clicking on the gear icon.

- If necessary, press the auto-zoom icon above the bar chart to auto-scale it.

- If you click on a row in the Table or on a bar in the Bar Chart, then the matching bar/row will be selected.
  If you have a SpectrumDisplay open this will navigate to the peaks corresponding to the residue selected.
  The Fitting Plot area in the top right-hand corner of the Relaxation module, will remain empty, as no fitting is required when analysing the Heteronuclear NOE data.

4 Combined Data Analyses



4A R2/R1 Settings

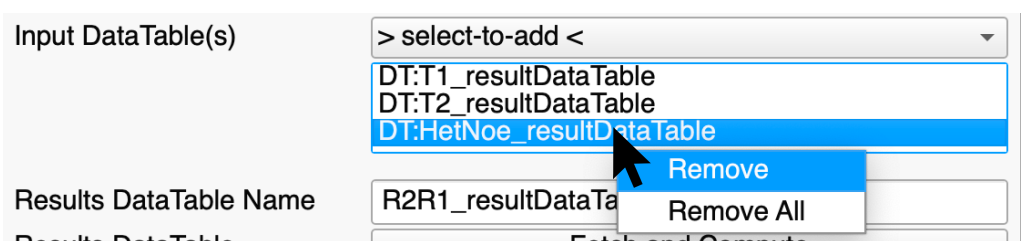
At this point you can either continue from Section 3 or start using our **2_R1R2HetNOECompleted.ccpn** project.

- Open the **Relaxation Module Settings** panel with the gear icon.
- In the **Setup** tab, set the **Experiment** to **User-Defined**
- Select the **Input DataTables** to be

  DT:T1Results

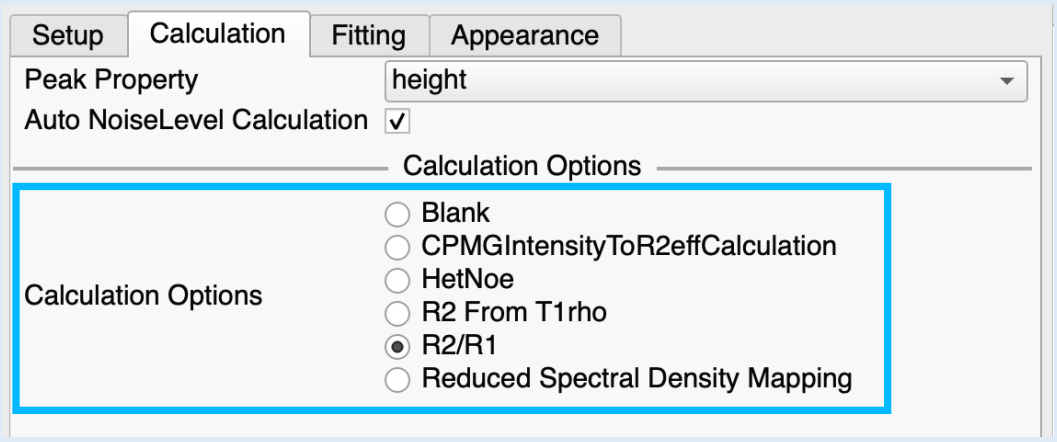
  DT:T2Results

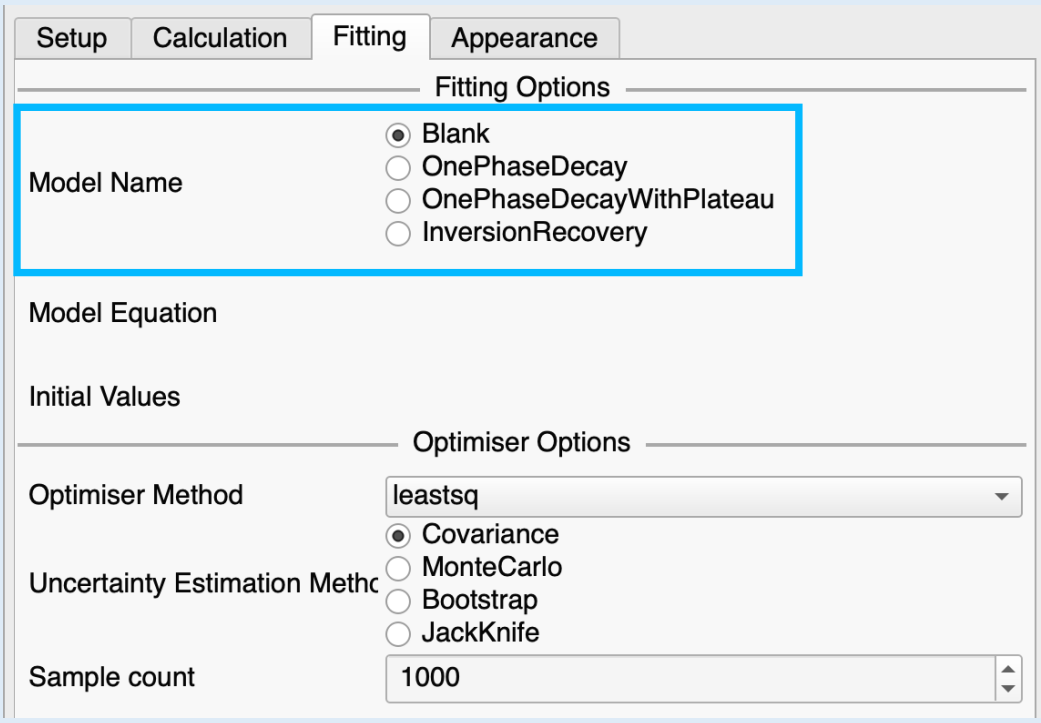
  If need be, remove the **DT:HetNOEResults** DataTable by **right-clicking** on it and selecting **Remove**.



- Adjust the **Results DataTable Name** if you wish, e.g. to **R2R1_result DataTable**

4 Combined Data Analyses





4B R2/R1 Calculation and Fitting Settings

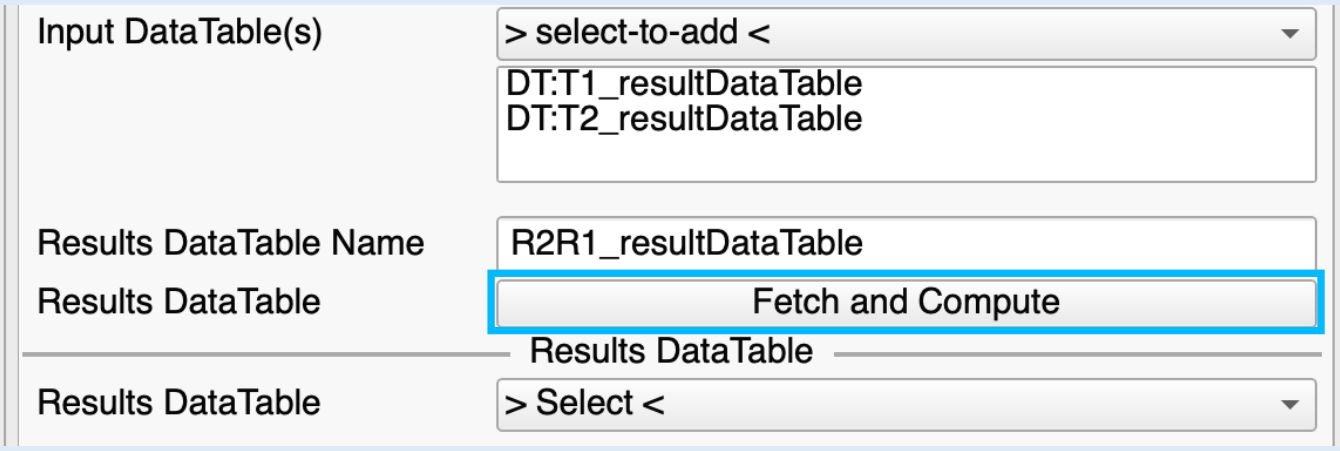
In the **Calculation** tab:

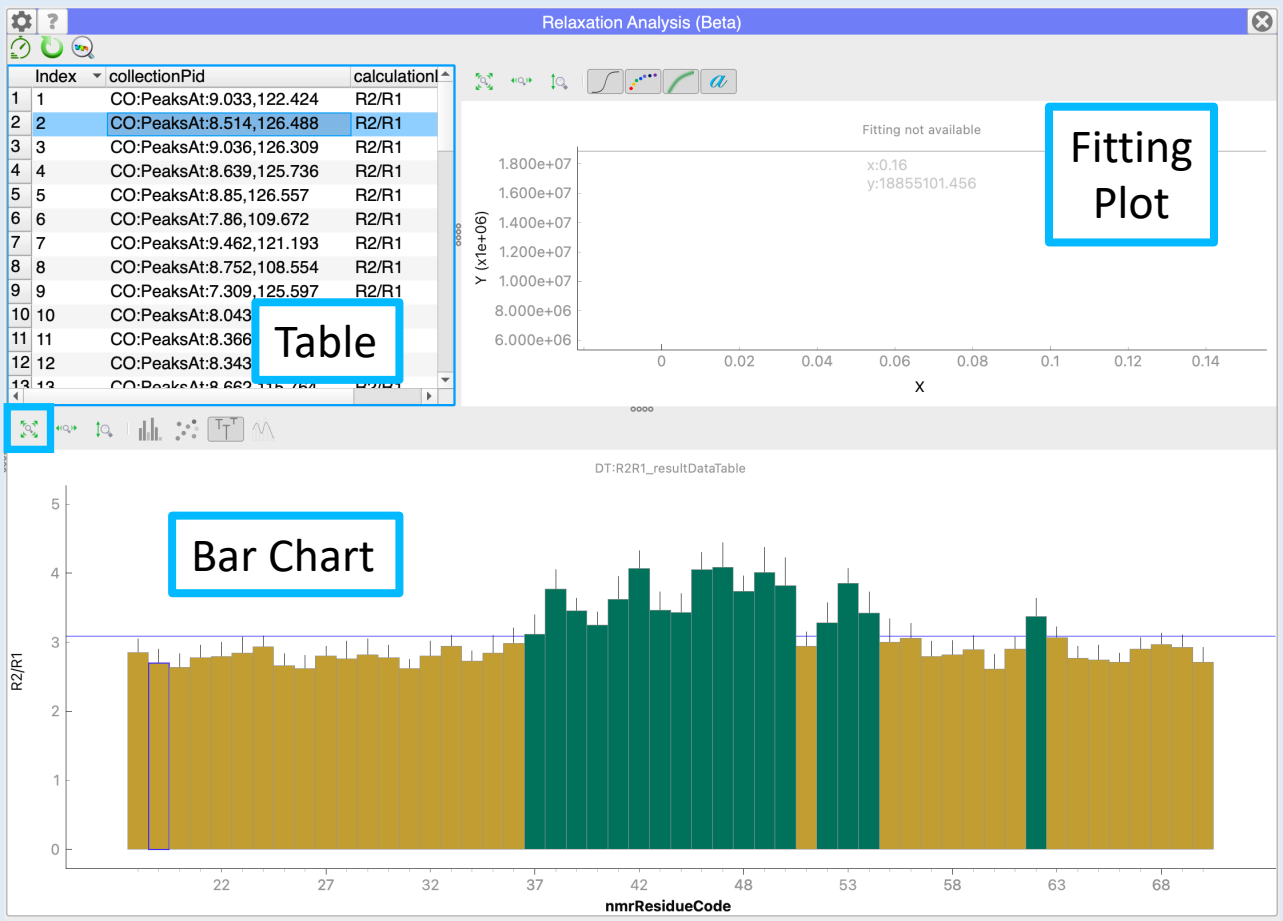
- Set your **Calculation Options** to be **R2/R1.**

In the **Fitting** tab:

- Make sure the **Model Name** is set to **Blank.**

4 Combined Data Analyses



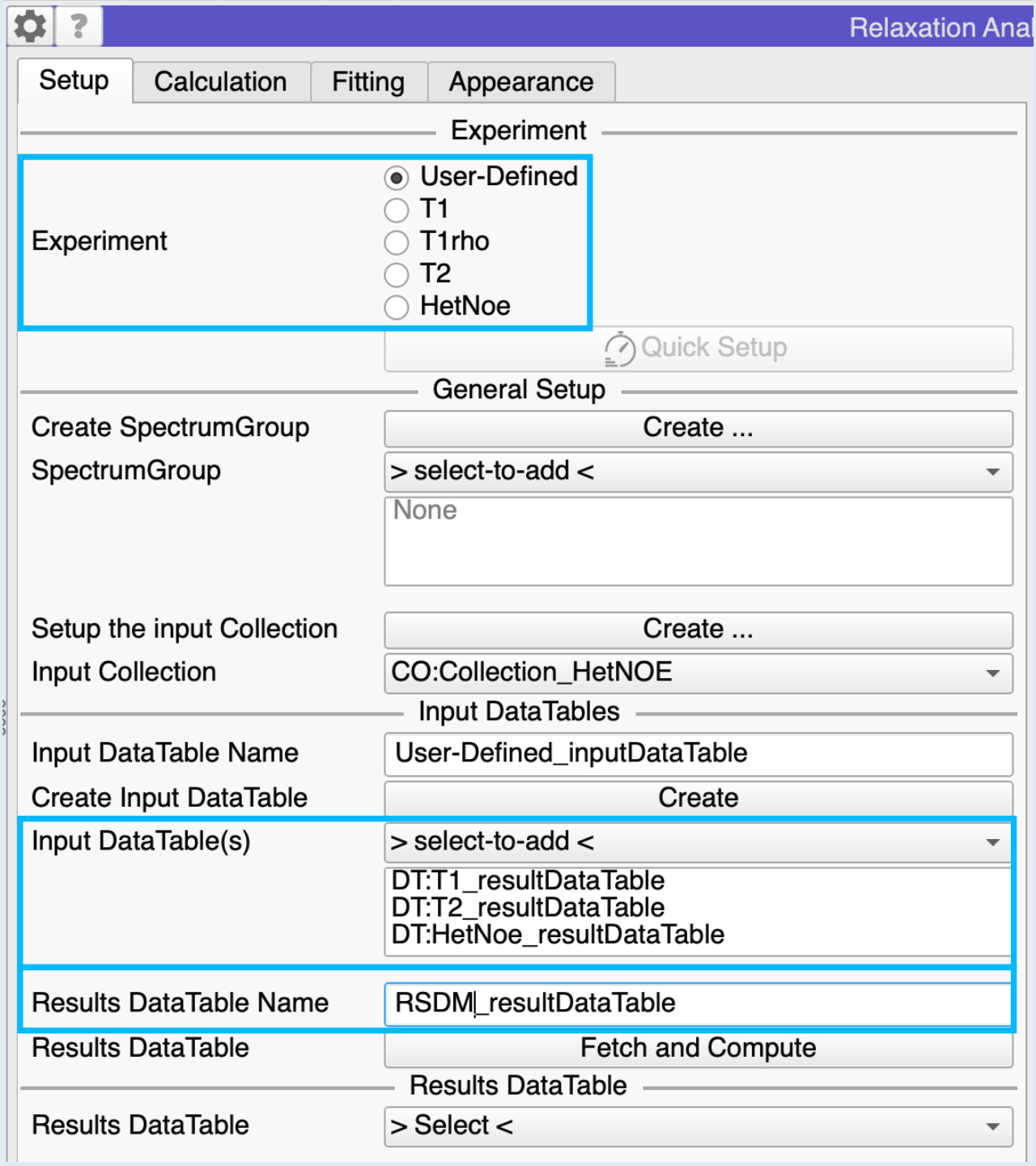


4c R2/R1 Calculation

In the **Settings Setup** tab:

- Click on the **Fetch and Compute** button.
- Close the **Settings** tab with the gear icon and inspect your results in the main part of the module.
- If necessary, press the auto-zoom icon above the bar chart to auto-scale it.
- If you click on a row in the Table or on a bar in the Bar Chart, then the matching bar/row will be selected.
  If you have a SpectrumDisplay open this will navigate to the peaks corresponding to the residue selected.
  The Fitting Plot area in the top right-hand corner of the Relaxation module, will remain empty, as no fitting was required for this calculation.

5 Reduced Spectral Density Mapping



5A Reduced Spectral Density Mapping Setup

At this point you can either continue from Section 4 or start using our **3_R2R1RatioCalculated.ccpn** project.

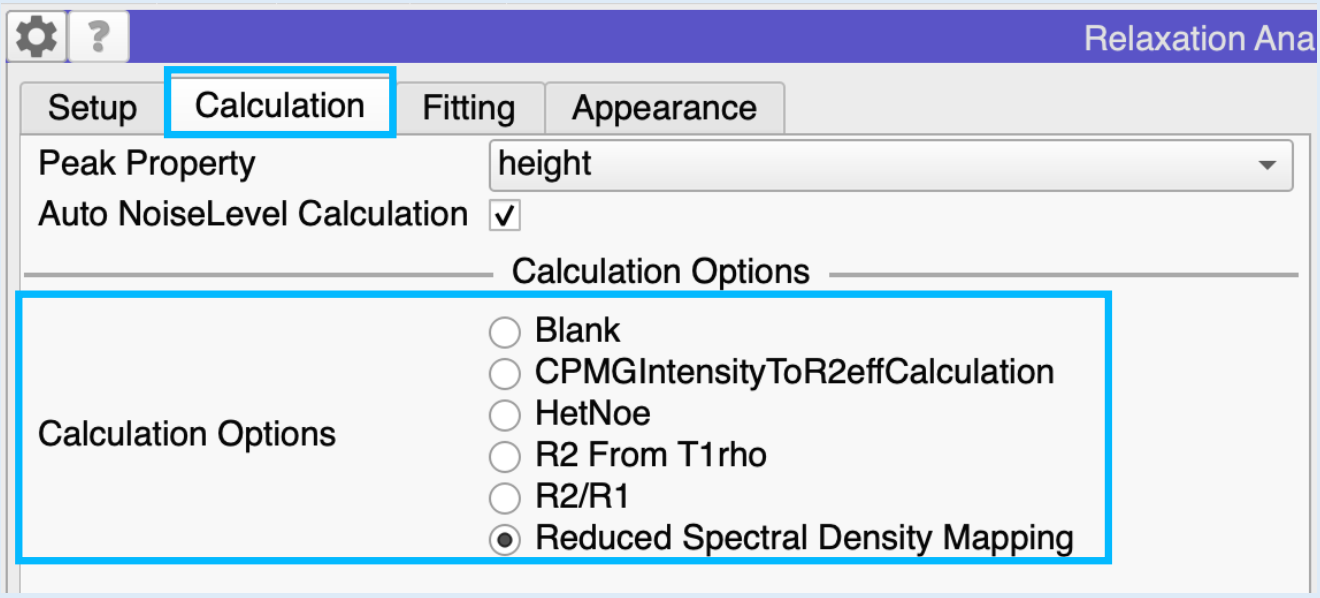
- Open the **Relaxation Module Settings** panel with the gear icon.
- In the **Setup** tab, set the **Experiment** to **User-Defined**
- Select the **Input DataTables** to be

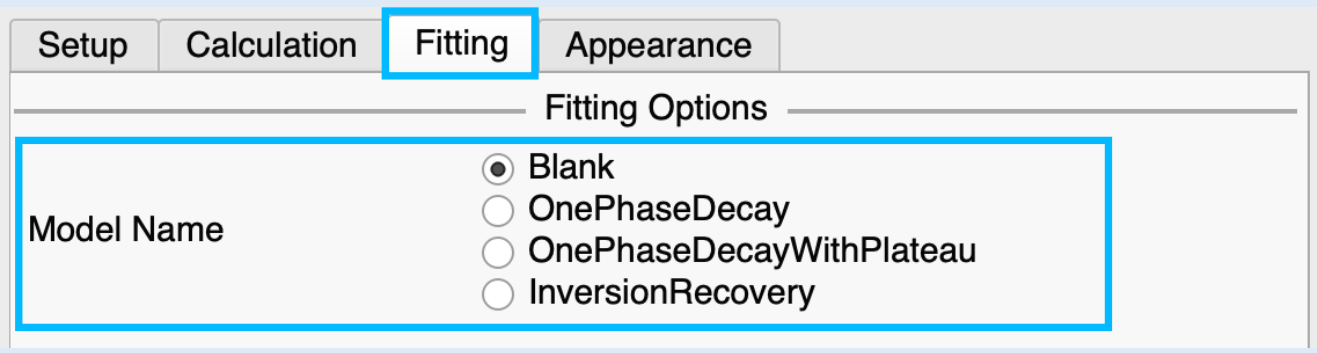
  DT:T1Results

  DT:T2Results

  DT:HetNOEResults
- Provide a **Results DataTable Name**, e.g. **RSDMResults**

5 Reduced Spectral Density Mapping





5B Reduced Spectral Density Mapping Calculation and Fitting Settings

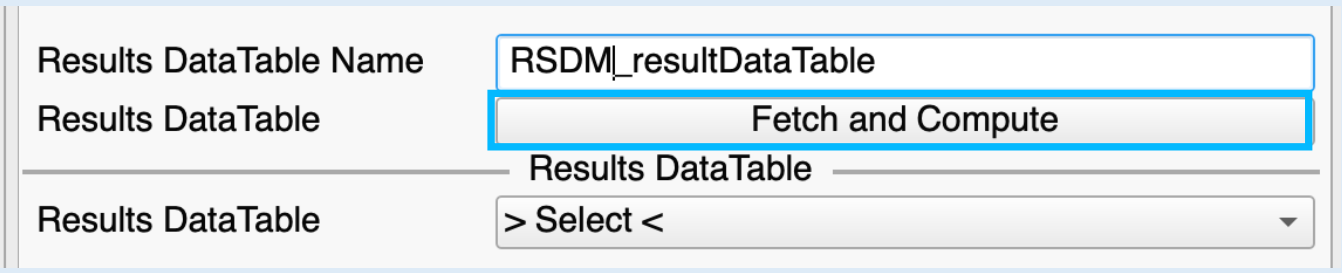
In the **Calculation** tab:

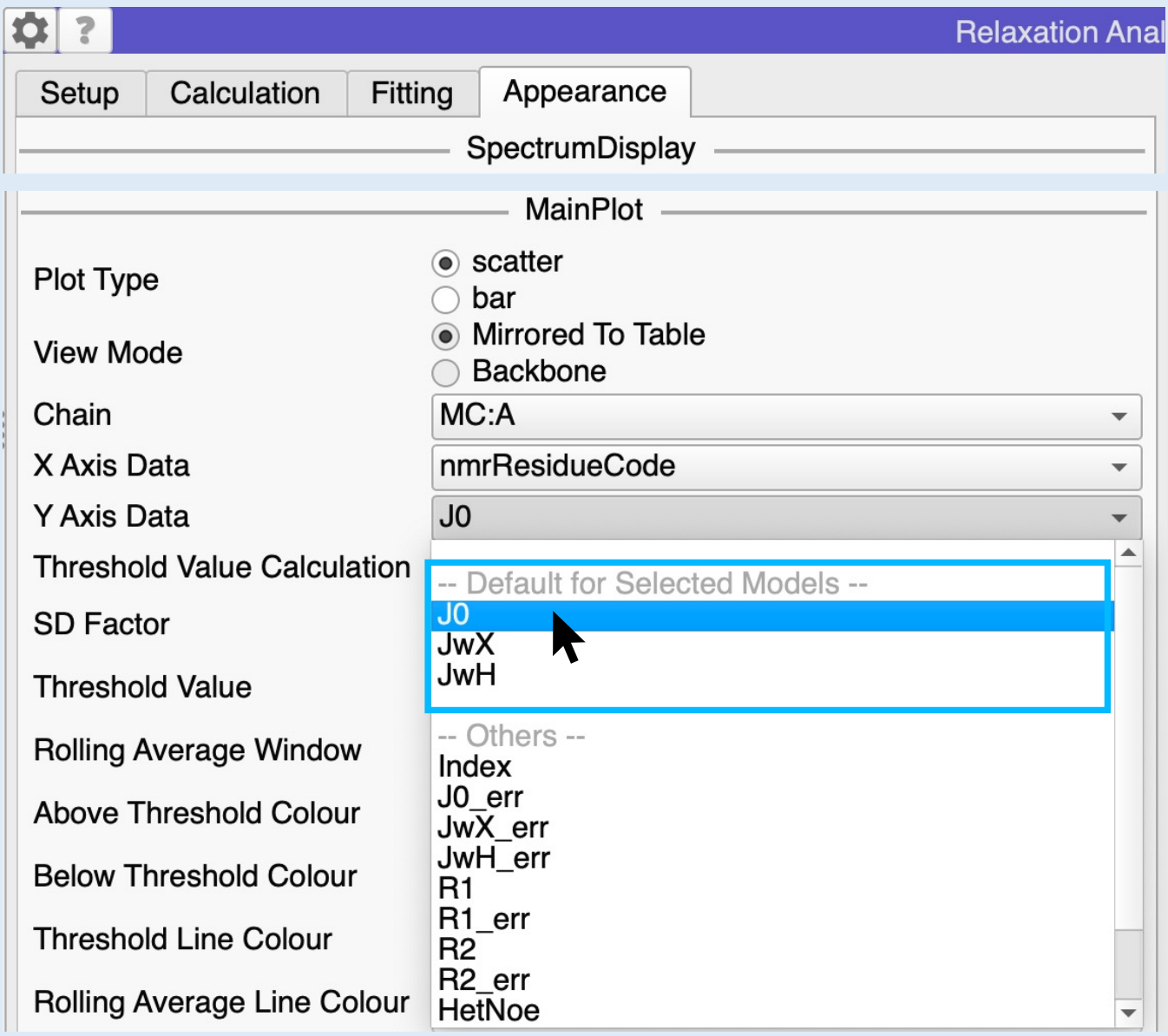
- Set your **Calculation Options** to be **Reduced Spectral Density Mapping**

In the **Fitting** tab:

- Make sure the **Fitting Model** is set to **Blank.**

5 Reduced Spectral Density Mapping





5C Reduced Spectral Density Mapping Calculation

In the **Settings Setup** tab:

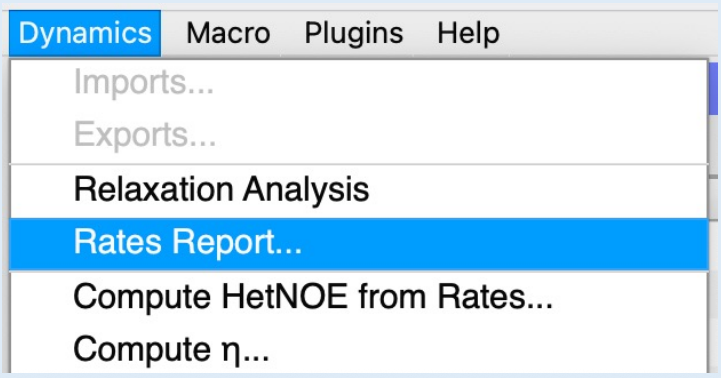
- Click on the **Fit** button.

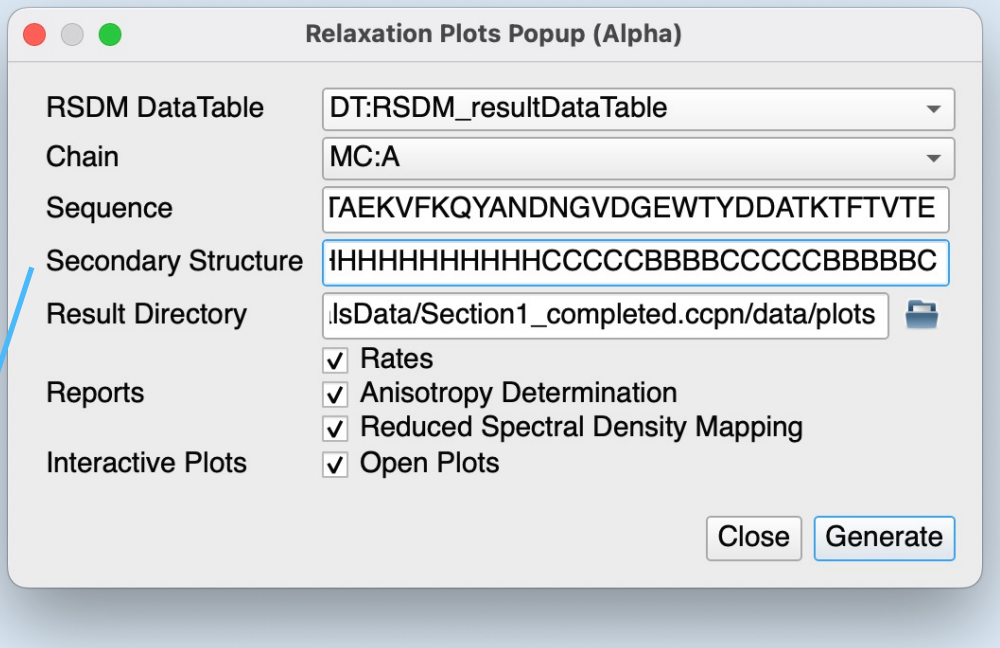
In the **Appearance** tab:

- From the **Y Axis Data** drop-down menu select **J0**, **JwX** and **JwH** in turn to view the spectral densities at 0, ω_N and ω_H, respectively.
- If necessary, press the orange Update button or auto-zoom button to view the data on the bar chart.

The Table, Chart and Spectrum Display are dynamically linked as usual and no fits are present.

6 Exporting Graphs





*** DSSP secondary structure coding:**

H: a-helix

I: π-helix

B: residue in isolated β-bridge

T: H-bonded turn

G: 3_{10} helix

E: extended strand

S: bend

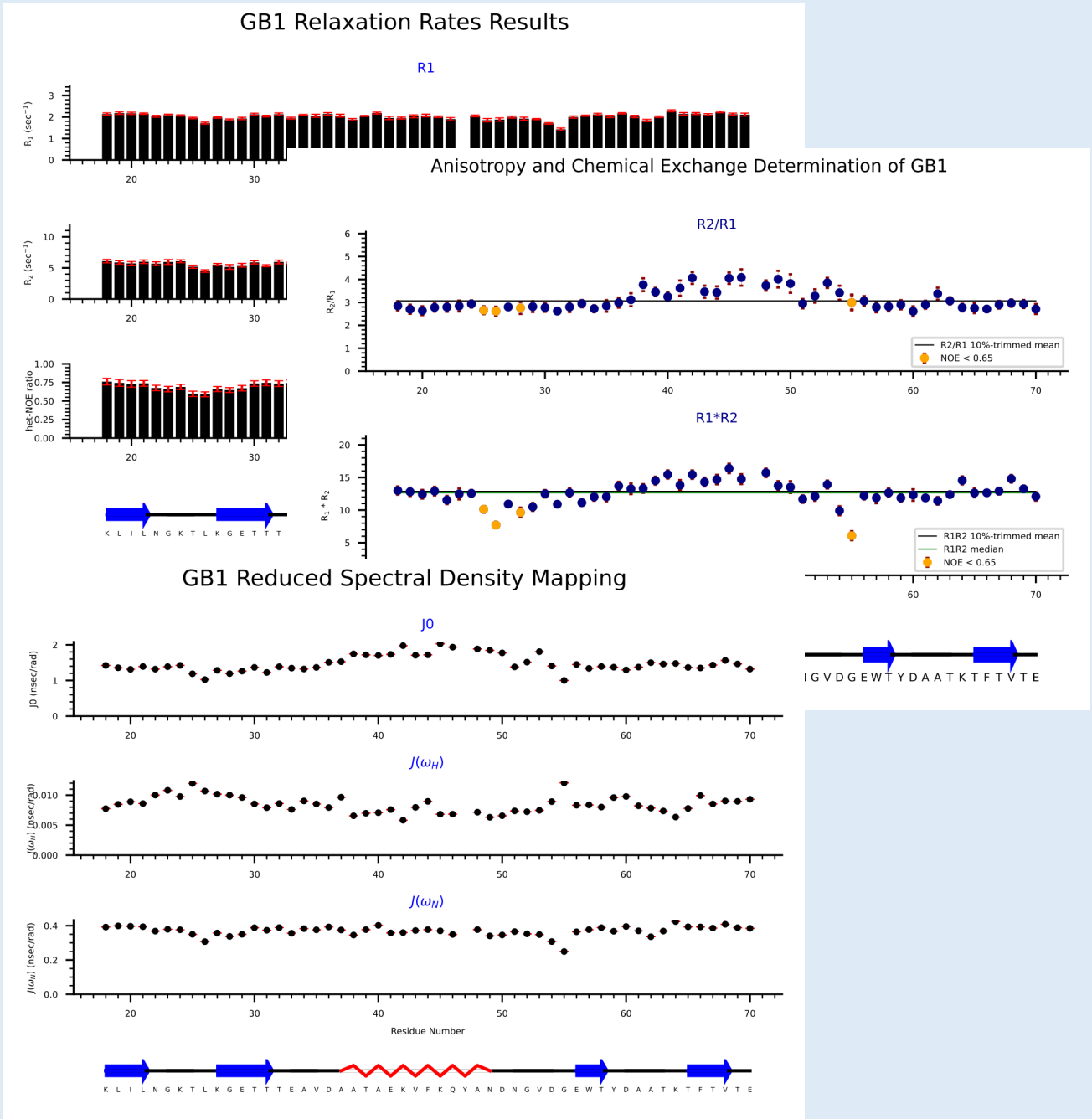
C: no secondary structure

6A Export predefined plots in pdf format

At this point you can either continue from Section 5 or start using our **4_RSDMCompleted.ccpn** project.

- Go to **Main Menu ⟶ Dynamics ⟶ Rates Report**…
- As your **RSDM DataTable**, select your Reduced Spectral Density Mapping Results table (e.g **DT:RSDM_resultDataTable**)
- Select Chain MC:A which will fill the Sequence
- If you wish, add the GB1 Secondary Structure (in DSSP code*) for GB1 (copy and paste from **GB1_SecStruct.txt** in the tutorial data)
- Select a Directory where you want to save your plots
- Keep all **Reports** selected
- Click on **Generate**

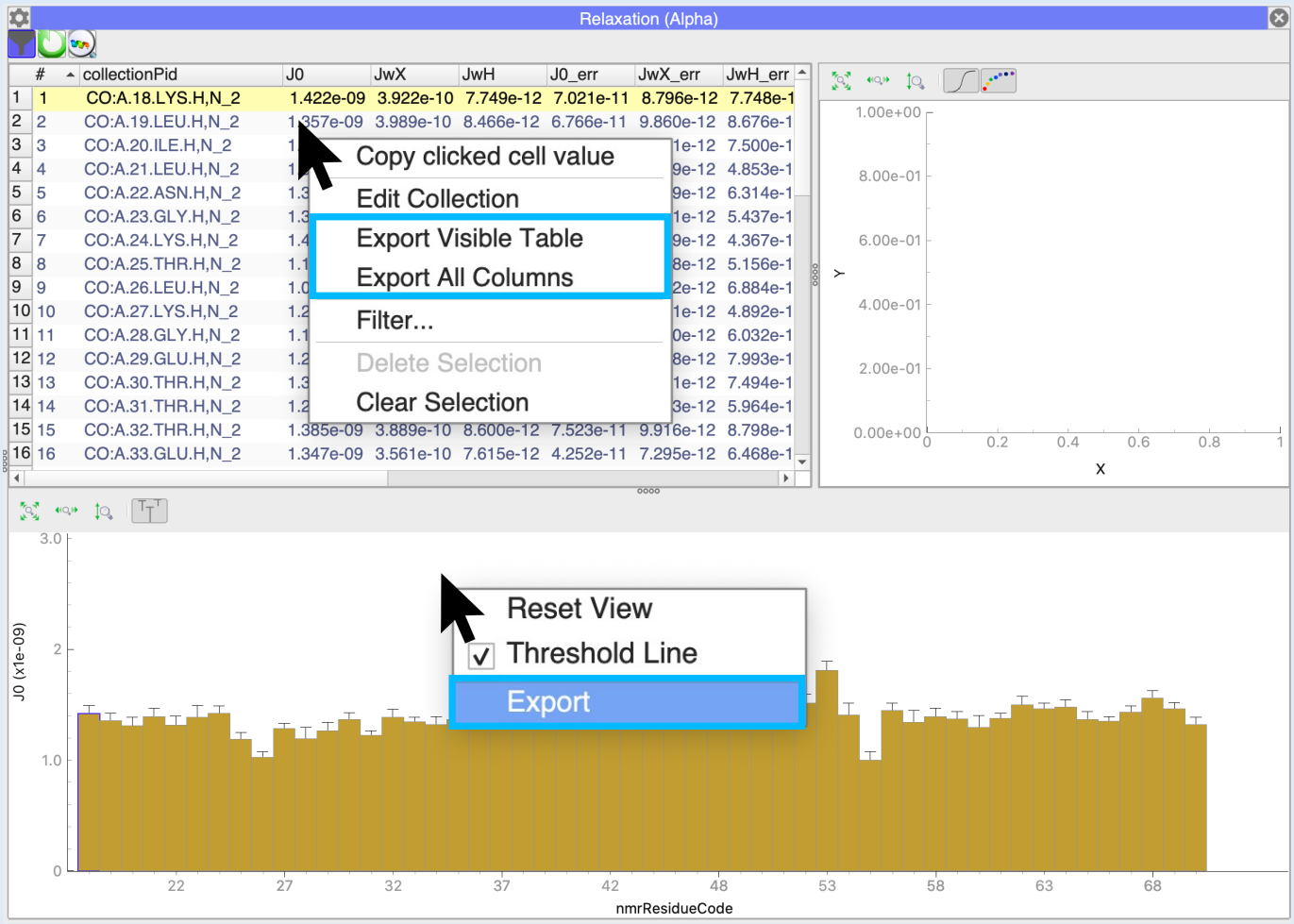
6 Exporting Graphs



6B Examine the graphs

The graphs are saved in pdf format in the Result Directory specified, which by default is the *YourProject.ccpn*/data/plots directory. They show all the different rates, spectral densities and other values calculated. If the sequence and secondary structure have been specified these are added to the graphs.

6 Exporting Graphs



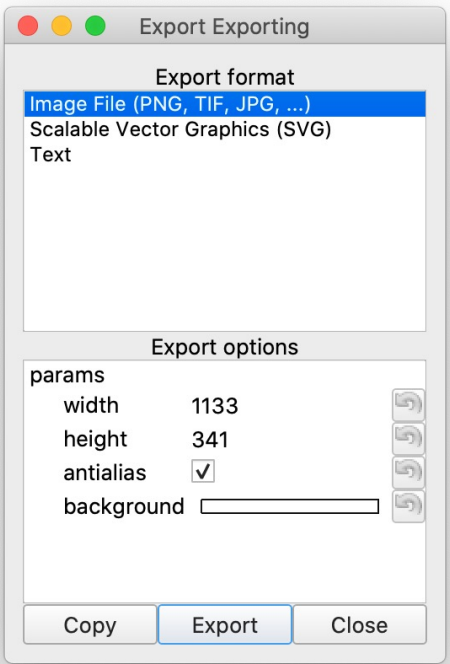
6C Exporting data for use in external graphing programs

- **Right-click** on the table and select either **Export Visible Table** or **Export All Columns**. The former is sensitive to any filtering you might have done on the table while the latter is not.

6D Exporting individual charts

- **Right-click** on the bar chart or fitting graph and select Export.

You will be presented with a small pop-up offering you several options, including the ability to export in an image or a scalable vector graphics format.



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)**
https://doi.org/10.64898/2026.06.19.733360
Pre-print BIORXIV