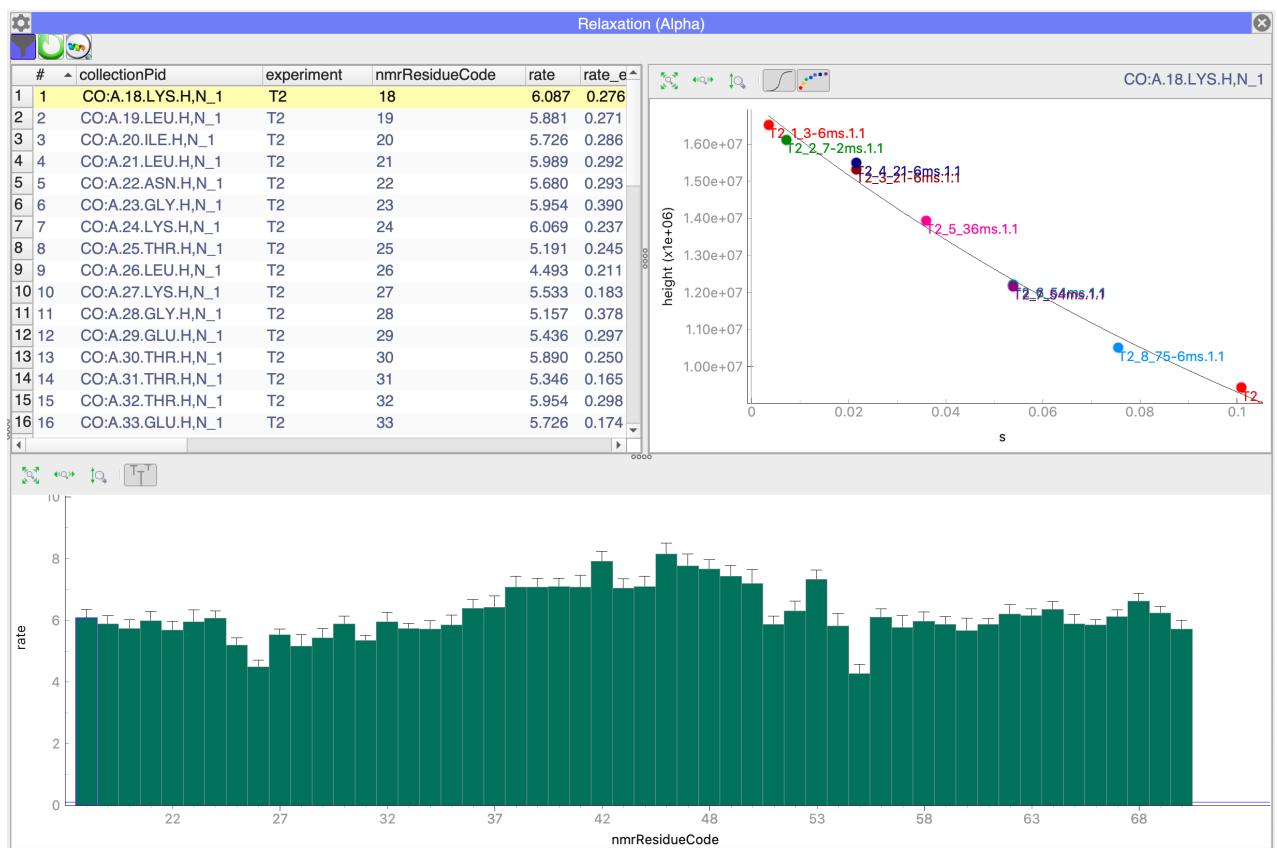


## Dynamics Tutorial (Alpha)



# Introduction

This tutorial will take you through the analysis of some dynamics data in CcpNmr Analysis Version 3.1 using the alpha-version of the Relaxation Data Analysis Module. 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 the tutorials page of our website (<https://ccpn.ac.uk/support/tutorials/>). 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:

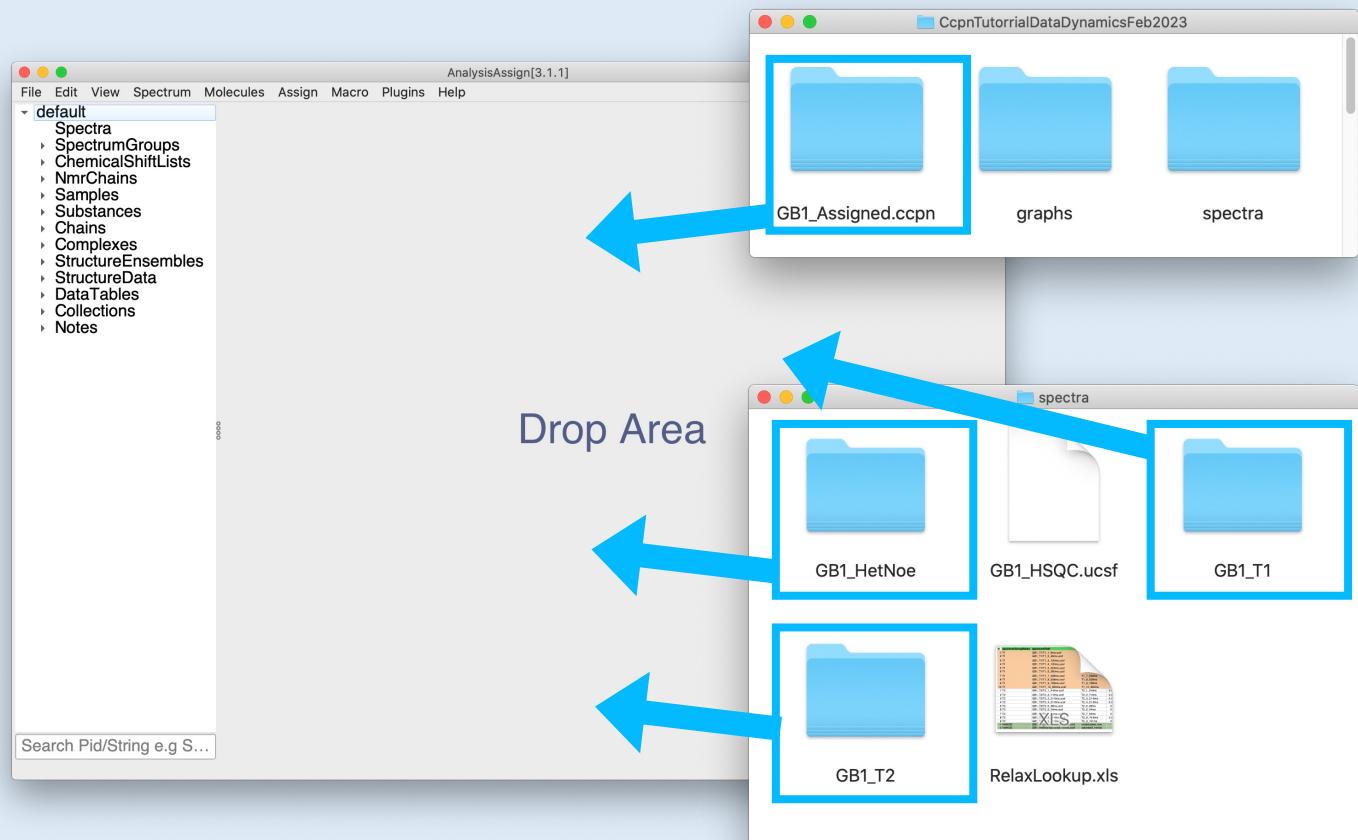
1. Loading Data
2. T1 and T2 Data
3. Heteronuclear NOE Data
4. Combined Analyses
5. Reduced Spectral Density Mapping
6. Exporting Graphs
7. Examining the Graphs

## 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

# Loading data



## 1A Open project and load spectra

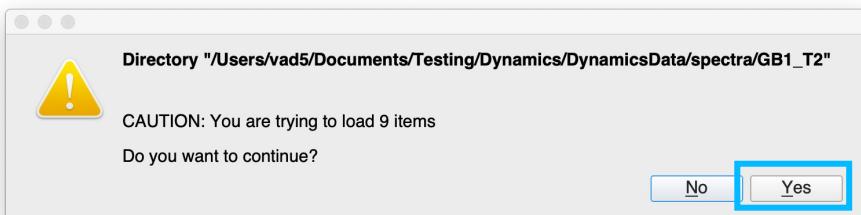
- Find the **GB1\_Assigned.ccpn** project folder in the Dynamics Tutorial data directory. This project contains an assigned HSQC spectrum of GB1.
- Select the folder in your file browser and drag it onto the **Sidebar** or **Drop Area**.
- Find the **spectra** directory in the Dynamics Tutorial data directory
- Select and drag the following three folders into the Sidebar or Drop Area:

**GB1\_T1**

**GB1\_T2**

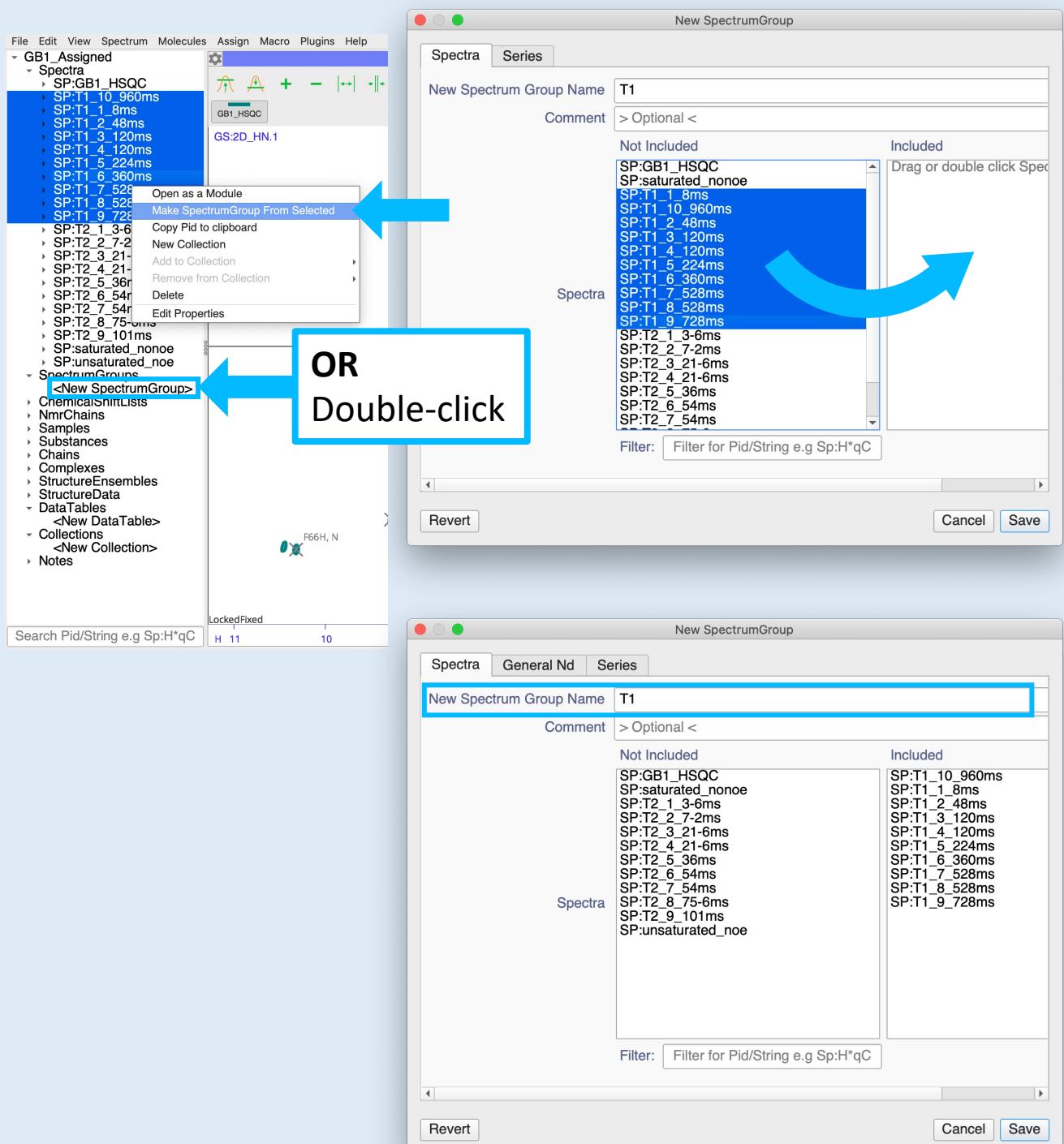
**GB1\_HetNOE**

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



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

# Loading data



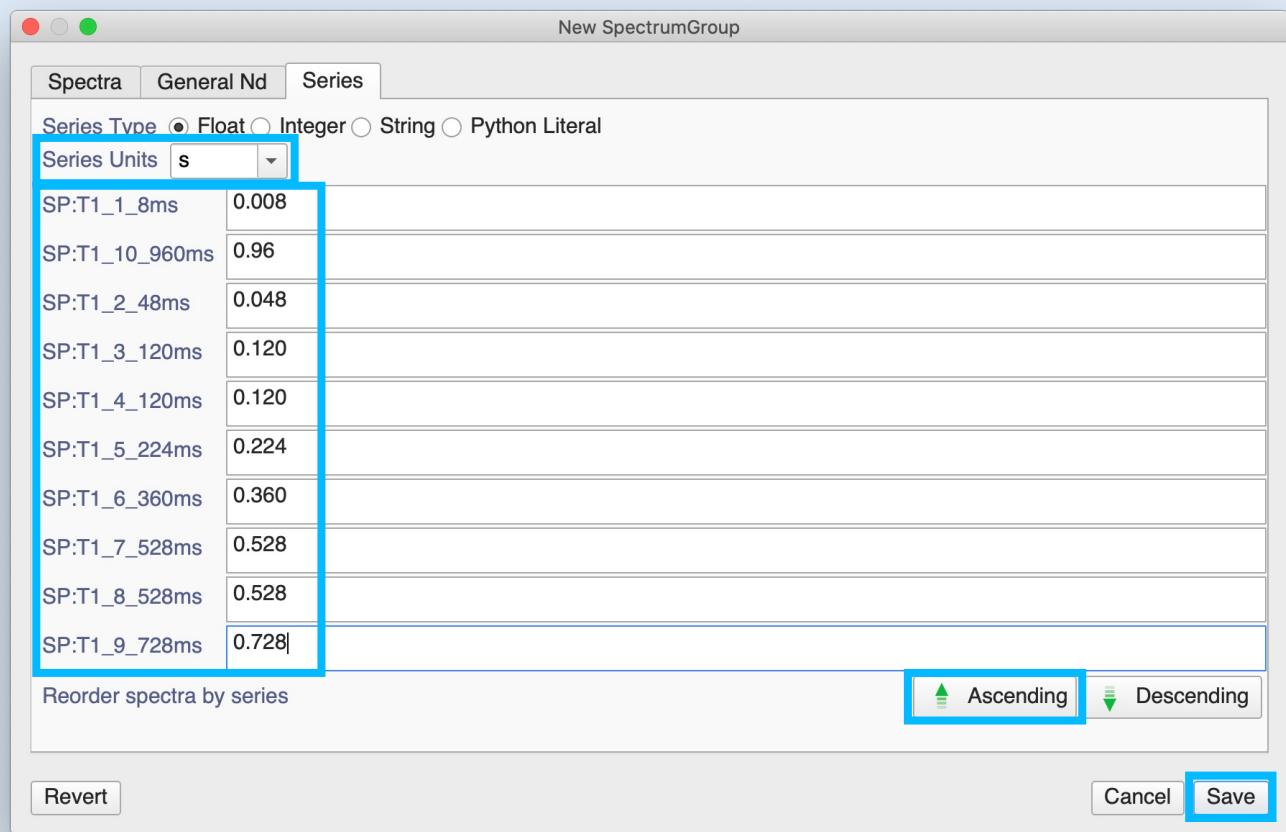
## 1B Create the T1 SpectrumGroup and Series

- 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



## 1c Create the T1 Series

- Go to the **Series** tab of the Edit SpectrumGroup pop-up
- Enter **s** (seconds) as your unit (either type it or select from the drop-down menu).
- 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 **Ascending** button to do so!
- Finally click on **Save**.

# Loading data

## T2Data

The screenshot shows the T2Data interface. On the left, a sidebar lists spectra: SP:T1\_9\_728ms, SP:T2\_1\_3-6ms, SP:T2\_2\_7-21, SP:T2\_3\_21, SP:T2\_4\_21, SP:T2\_5\_36ms, SP:T2\_6\_54ms, SP:T2\_7\_54ms, SP:T2\_8\_75ms, SP:T2\_9\_101ms, and SP:saturated. A context menu is open over the first few spectra, with 'Make SpectrumGroup From Selected' highlighted. To the right is a 'New SpectrumGroup' dialog with a 'Series' tab selected. It shows a table with 'Series Type' set to 'Float'. The table lists the spectra from the sidebar with their corresponding values: SP:T2\_1\_3-6ms (0.0036), SP:T2\_2\_7-21ms (0.0072), SP:T2\_3\_21-6ms (0.0216), SP:T2\_4\_21-6ms (0.0216), SP:T2\_5\_36ms (0.036), SP:T2\_6\_54ms (0.054), SP:T2\_7\_54ms (0.054), SP:T2\_8\_75-6ms (0.756), and SP:T2\_9\_101ms (0.101).

## HetNOE

The screenshot shows the HetNOE interface. On the left, a sidebar lists spectra: SP:T2\_9\_101ms, SP:saturated\_noe, SP:unsaturated\_noe, and a 'SpectrumGroup' section containing '<New Spec>'. A context menu is open over the spectra in the sidebar, with 'Make SpectrumGroup From Selected' highlighted. To the right is a 'New SpectrumGroup' dialog with a 'Series' tab selected. It shows a table with 'Series Type' set to 'Float' and 'Series Units' set to 'AU'. The table lists the spectra from the sidebar with their corresponding values: SP:saturated\_noe (1.0) and SP:unsaturated\_noe (0.0).

## 1D Create T2 and Heteronuclear NOE SpectrumGroups and Series

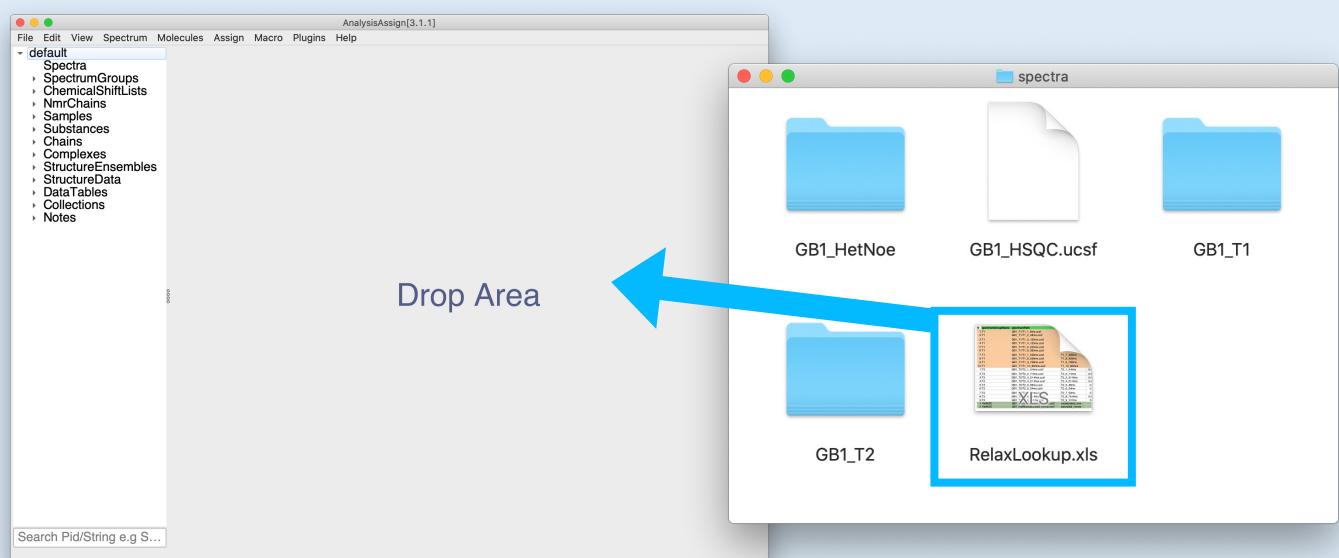
- Repeat the procedure for creating a SpectrumGroup and setting up the Series for the T2 data

Create a SpectrumGroup and Series for the Heteronuclear NOE data:

- Select the **saturated\_noe** and **unsaturated\_noe** 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 units to **AU** (arbitrary units) and enter the following values:

<b>saturated_noe</b>	<b>1.0</b>
<b>unsaturated_noe</b>	<b>0.0</b>

# Loading data



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

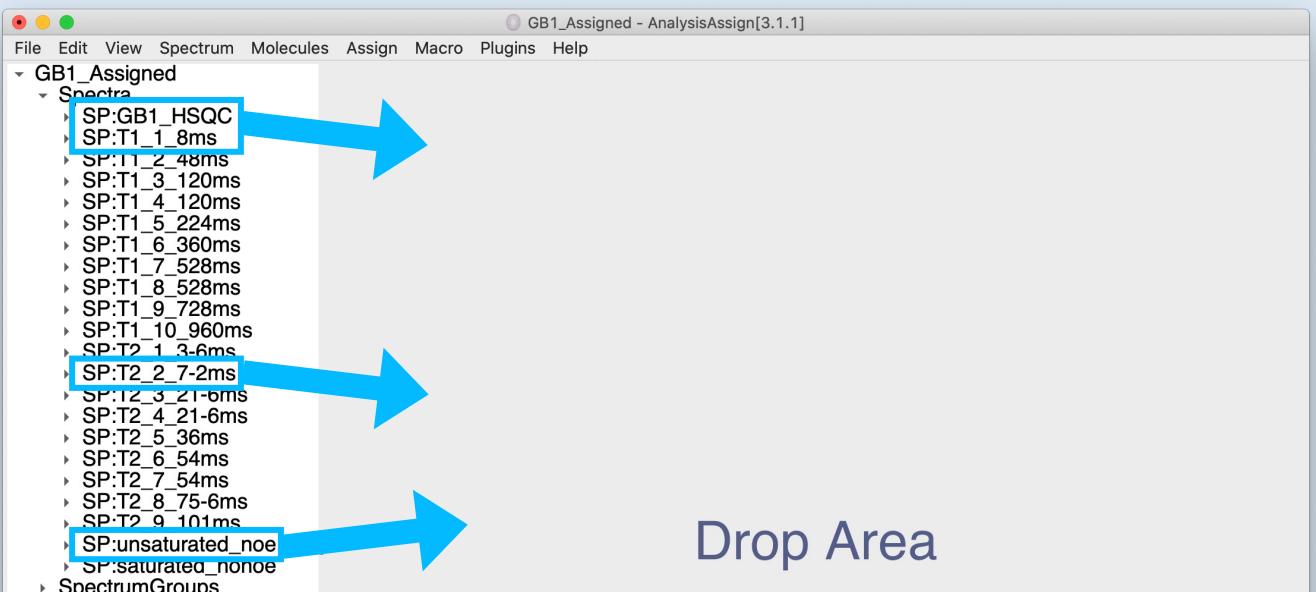
## 1E Import from Excel Lookup File (optional)

As an alternative to loading the spectra and setting up the SpectrumGroups and Series manually, you can also enter all the information into an Excel file and drag this into the program. This will automatically load the spectra and create the SpectrumGroups and Series 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 **GB1\_Assigned ccpn** project folder in the Dynamics Tutorial data directory and drag it onto the **Sidebar** or **Drop Area**.
- Select **Yes**, if asked whether you want to open a new project or not
- 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.

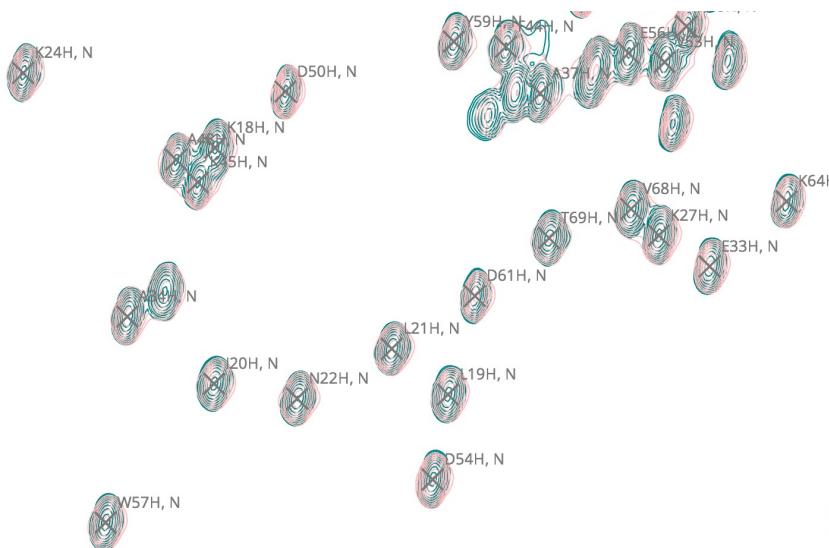
# Loading data



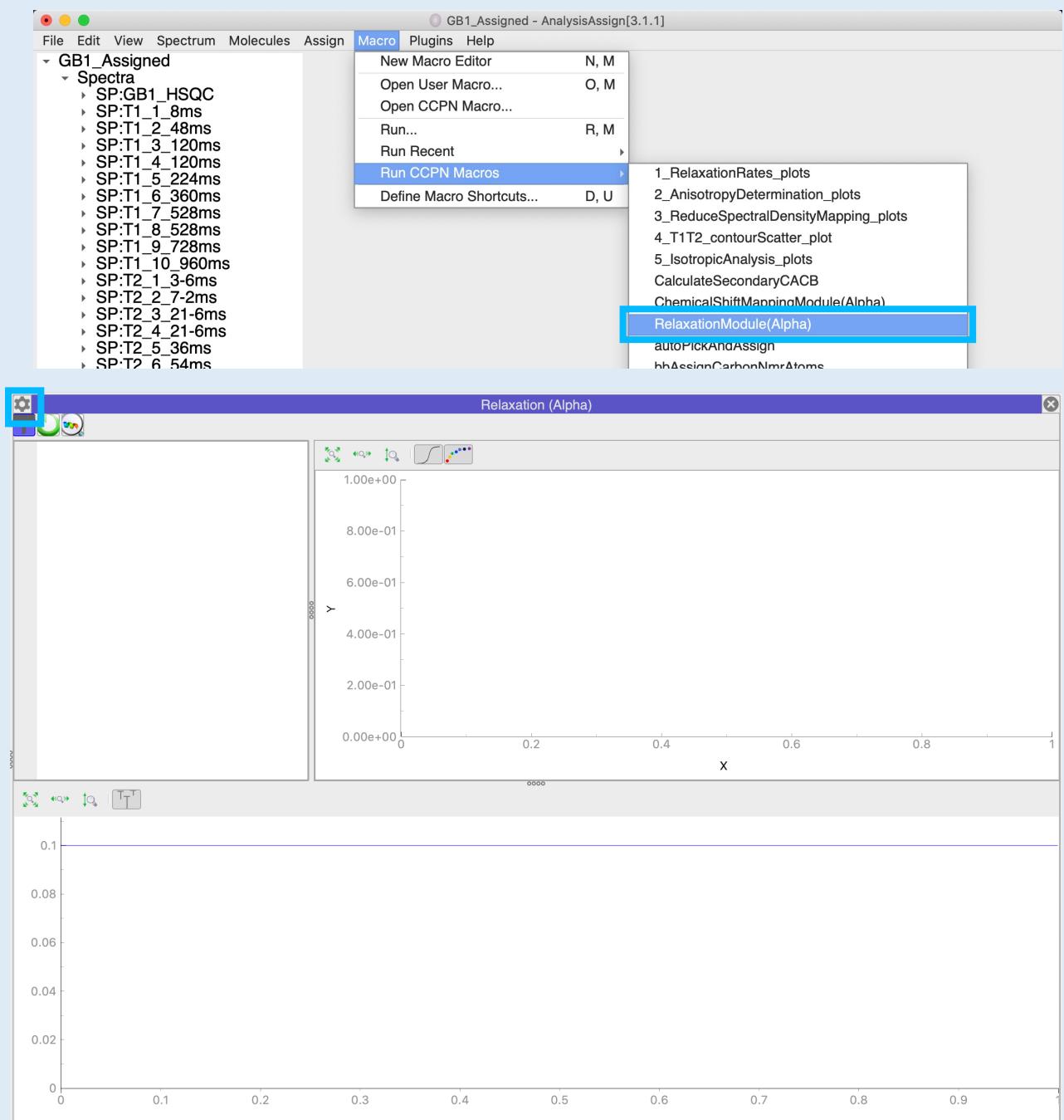
## 1F Check alignment to HSQC

- 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.



# T1 and T2 Data



## 2A Open Relaxation Module

At this point you can either continue from Section 1 or start using our [Section1\\_completed ccpn](#) project.

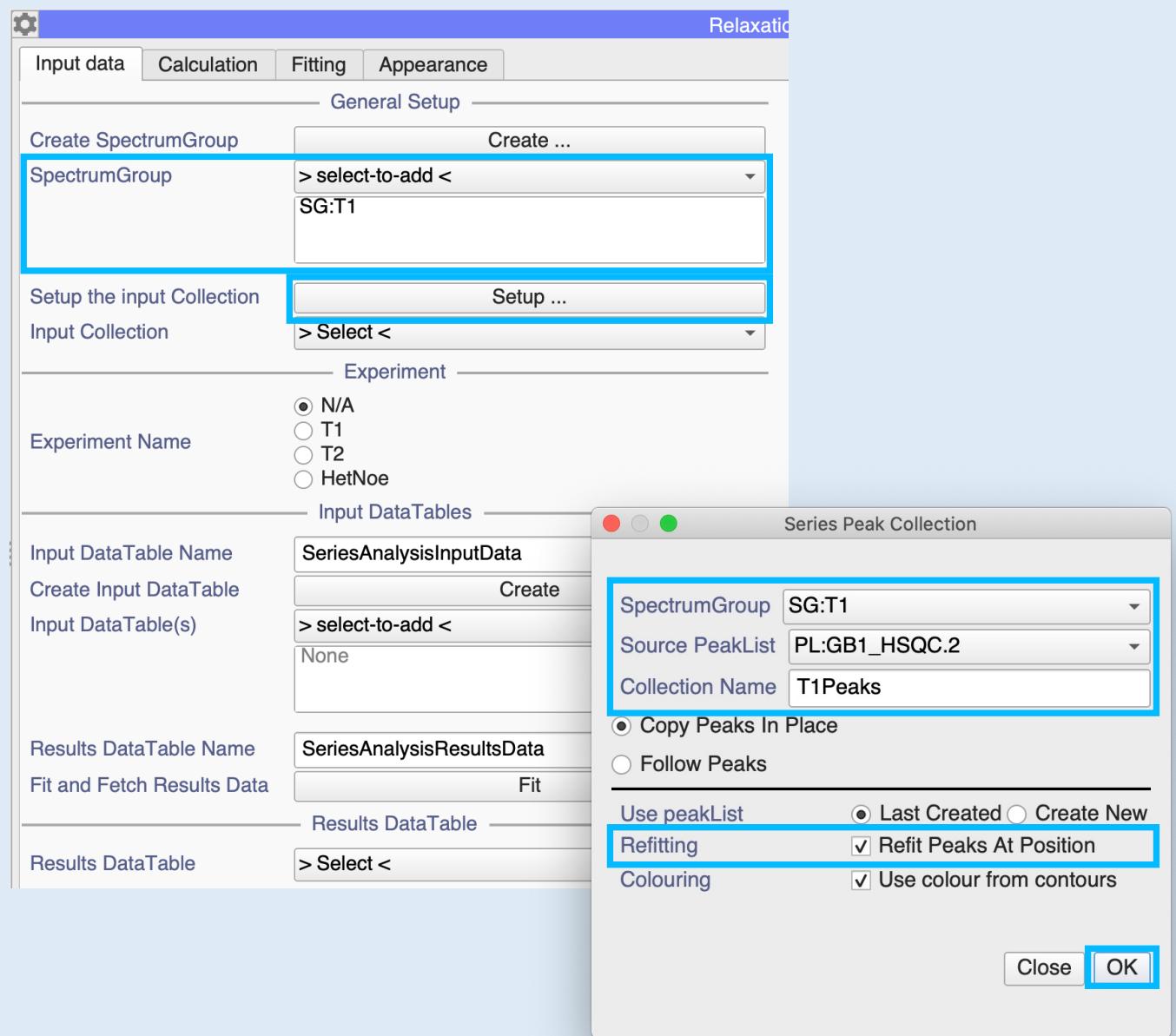
- Go to Main Menu → Macro → Run CCPN Macros → **RelaxationModule(Alpha)**.

This will open the alpha-version Relaxation 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.

# T1 and T2 Data



## 2B Copy Peaks

In the **General Setup** section you will need to select a **SpectrumGroup** (which specifies which spectra you want to use in your analysis) and a **Collection** (which is a collection of peak groups across the spectra).

We have already created our SpectrumGroups:

- Select the **T1 SpectrumGroup** from the drop-down menu.

You will need to set up the Collection, as we don't have this yet:

- Click on the **Setup...** button to set up your Collection
- In the Series Peak Collection pop-up set:

Select SpectrumGroup: SG:T1

Source PeakList: PL:GB1\_HSQC.2

CollectionName: T1Peaks

Select Refit Peaks At Position

And keep the remaining default options, as shown above.

- Click **Okay** which will start the creation of the Peak Collections. This will probably take a minute or so.

Relaxation Analysis

**Input data**   **Calculation**   **Fitting**   **Appearance**

**General Setup**

Create SpectrumGroup   **Create ...**

SpectrumGroup   > select-to-add <

SG:T1

Setup the input Collection   **Setup ...**

Input Collection   CO:T1Peaks

**Experiment**

Experiment Name    T1    N/A    T2    HetNoe

**Input DataTables**

Input DataTable Name	T1Input
Create Input DataTable	<b>Create</b>
Input DataTable(s)	> select-to-add <
	None
Results DataTable Name	SeriesAnalysisResultsData
Fit and Fetch Results Data	Fit
<b>Results DataTable</b>	
Results DataTable	> Select <

## 2C Create Input DataTable

You will now create your Input DataTable, a table containing all the information needed to fit your data.

In the **Experiment** Section:

- Select T1

In the **Input DataTables** Section:

- Enter a **Input DataTable Name**, e.g. T1Input
- Click on the **Create** button to create your Input DataTable
- Check that this new Input Data Table is the only one shown in the **Input Data Table(s)** box.

**Input DataTables**

Input DataTable Name	T1Input
Create Input DataTable	<b>Create</b>
Input DataTable(s)	> select-to-add <
	DT:T1Input

# T1 and T2 Data

Input data	Calculation	Fitting	Appearance
Peak Property	height		
<b>Calculation Options</b> <input checked="" type="radio"/> Blank <input type="radio"/> HetNoe <input type="radio"/> R2/R1 <input type="radio"/> Reduced_Spectral_Density_Mapping			
<b>Optimiser Options</b> Optimiser Method: leastsq			
Fitting Error Method: Default			
<b>Fitting Options</b> <input type="radio"/> Blank <input checked="" type="radio"/> OnePhaseDecay <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery			
<b>Input DataTables</b> Input DataTable Name: T1Input			
Create Input DataTable: Create			
Input DataTable(s): > select-to-add <			
DT:T1Input			
Results DataTable Name: T1Results			
Fit and Fetch Results Data: Fit			

## 2D Fitting

Move to the **Calculation** tab:

- Check that **Calculation Options** is set to **Blank**

Now move to the **Fitting** tab:

- Make sure the **Fitting Model** is set to **OnePhaseDecay**. Hover over this option to see information on the equation used).

A model to describe the rate of a decay.  
 Model:  

$$Y = \text{amplitude} \cdot \exp(-\text{rate} \cdot X)$$
  
 X: the various time values  
 amplitude: the Y value when X (time) is zero. Same units as Y  
 rate: the rate constant, expressed in reciprocal of the X axis time units, e.g.: Second-1.

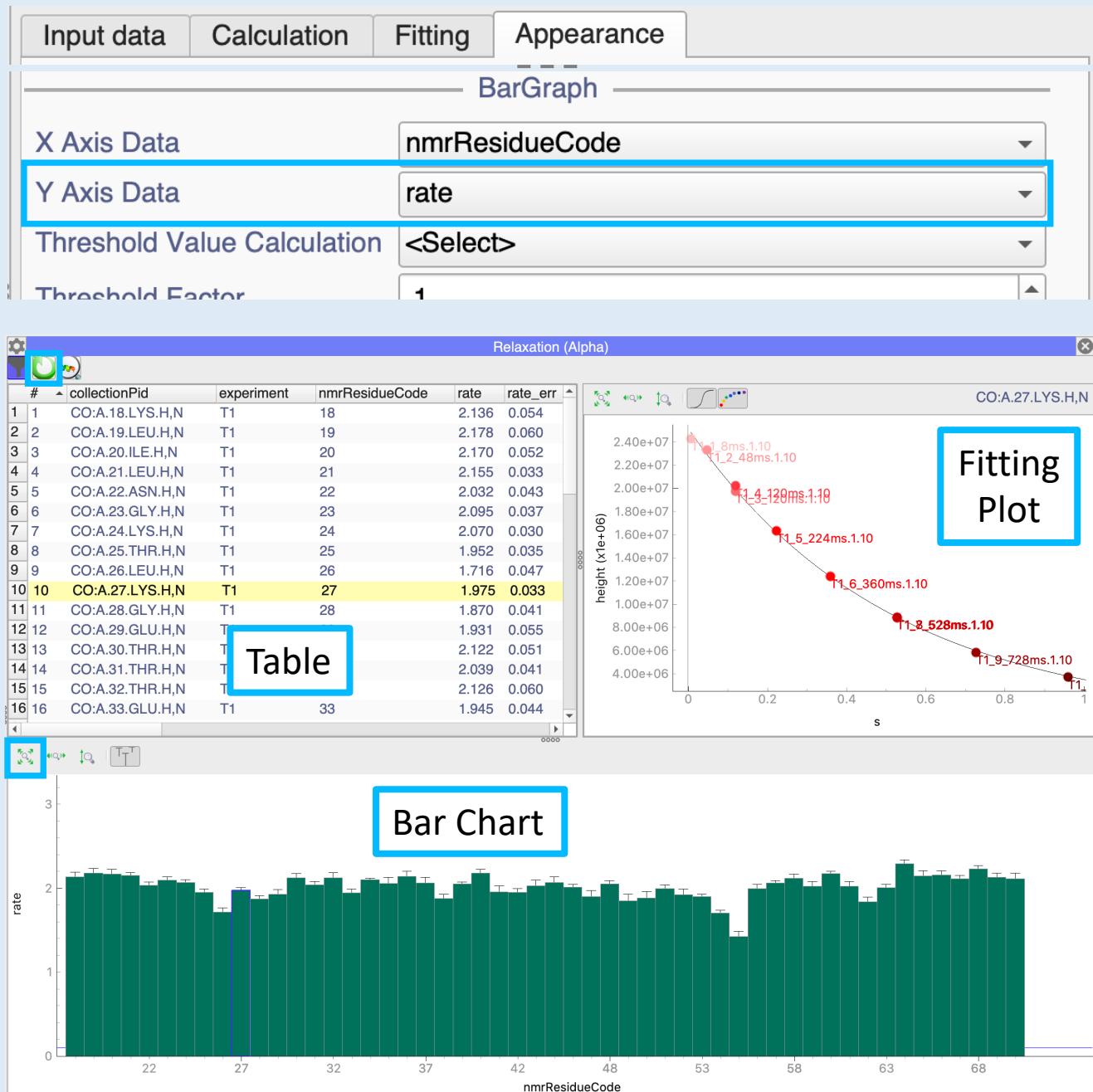
Move back to the **Input data** tab:

- Enter a **Results DataTable Name**, e.g. **T1Results**
- Click on the **Fit** button to run the fitting routine.

Your **Results DataTable** will then be automatically set to your new **T1Results** table. The table and graphs in the main module will be filled with this data.

<b>Results DataTable</b>	
Results DataTable	DT:T1Results

# T1 and T2 Data



## 2E Inspect Results

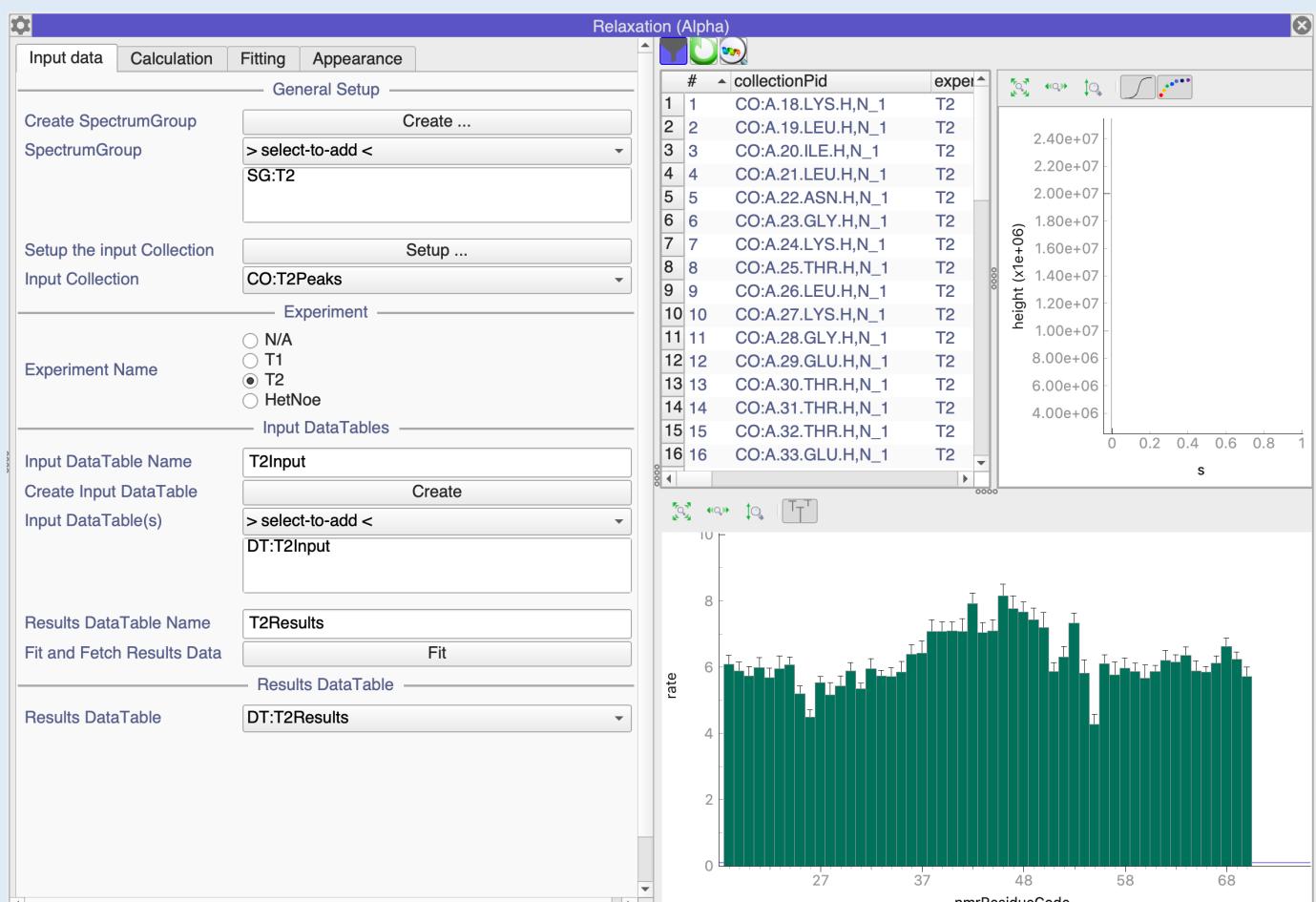
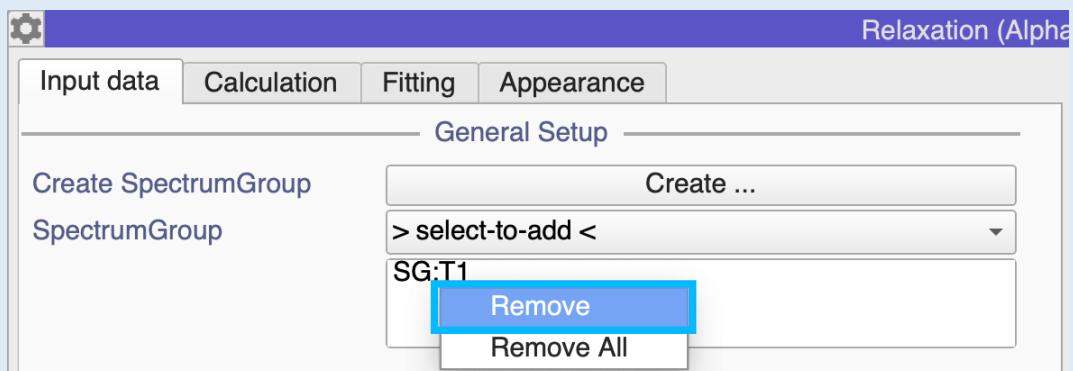
Now move to the Appearance tab:

- Make sure the Y Axis Data is set to **rate** from the drop-down menu.
- Close the **Settings** tab by clicking on the gear icon in the top left again.
- If the Refresh button has gone orange , then press this to refresh the graphs and table.
- 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.

# T1 and T2 Data



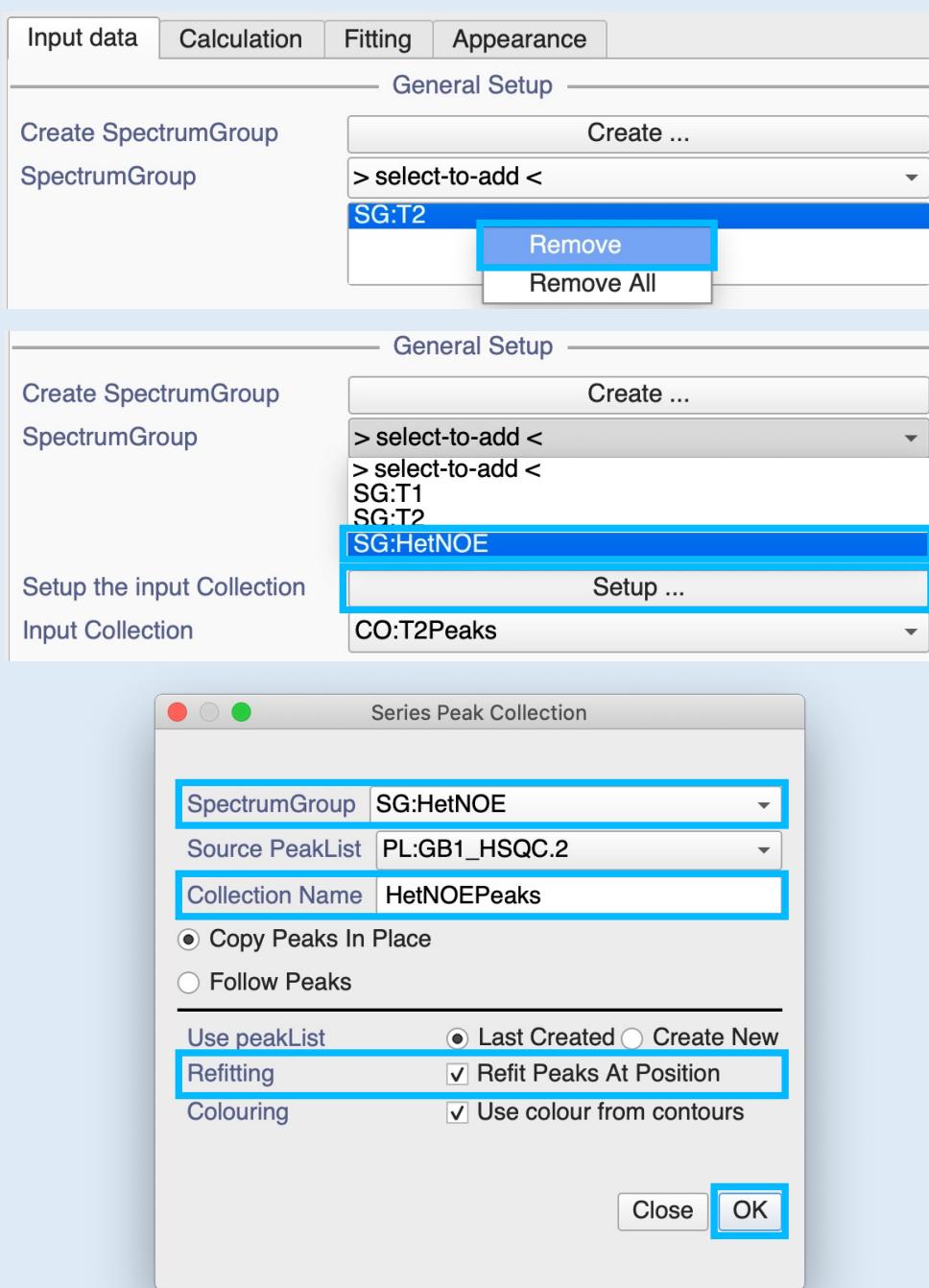
## 2F T2 Data

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

In the **Input data** tab:

- In the **General Setup** section, right-click on your **SG:T1** SpectrumGroup and select **Remove** or **Remove all**.
- Repeat sections **2B-2E** for the T2 data. Use the same options adjusting T1 to T2 in each instance and selecting **T2** as your **Experiment Name**.

# Heteronuclear NOE



## 3A Heteronuclear NOE Calculation Setup

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

In the **Input data** tab:

- In the **General Setup** section, right-click on your **SG:T1 SpectrumGroup** and select **Remove** or **Remove all**.
- Select **SG:HetNOE** as your **SpectrumGroup** from the drop-down menu
- Click on **Setup ...** to set up your Input Collection

In the Series Peak Collection pop-up:

- Select the **SG:HetNOE** SpectrumGroup
- Give your Collection a Name, e.g. **HetNOEPeaks**
- Select to **Refit Peaks At Position**
- Click **OK**

# Heteronuclear NOE

**Experiment**

Experiment Name	<input type="radio"/> N/A <input type="radio"/> T1 <input type="radio"/> T2 <input checked="" type="radio"/> HetNoe
-----------------	--

**Input DataTables**

Input DataTable Name	HetNOEInput
Create Input DataTable	<b>Create</b>

**Input data   Calculation   Fitting   Appearance**

Peak Property: height

**Calculation Options**

Calculation Options	<input type="radio"/> Blank <input checked="" type="radio"/> HetNoe <input type="radio"/> R2/R1 <input type="radio"/> Reduced_Spectral_Density_Mapping
---------------------	---

**Input data   Calculation   Fitting   Appearance**

**Optimiser Options**

Optimiser Method	leastsq
Fitting Error Method	Default

**Fitting Options**

Fitting Model	<input checked="" type="radio"/> Blank <input type="radio"/> OnePhaseDecay <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery
---------------	--

**Results DataTable Name**: HetNOEResults

**Fit and Fetch Results Data**: **Fit**

**Results DataTable**: DT:HetNOEResults

## 3B Heteronuclear NOE Calculation

In the Settings **Input data** tab:

- Set the **Experiment Name** to **HetNoe**
- Give your Input DataTable a name, e.g. **HetNOEInput**
- Click on **Create**

In the Settings **Calculation** tab:

- Select **HetNoe**

In the Settings **Fitting** tab:

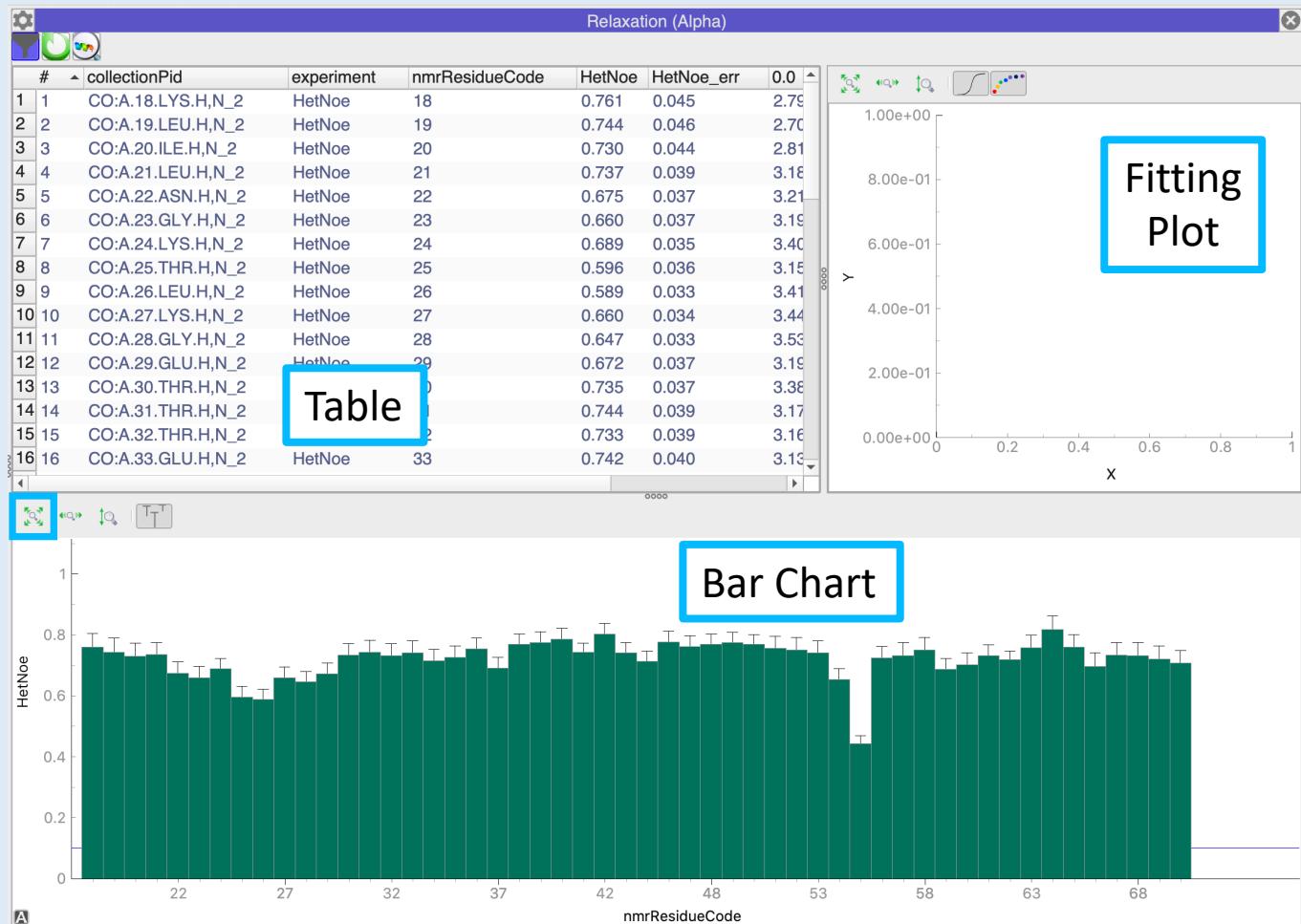
- Select **Blank**

In the **Input data** tab:

- Give your Results DataTable a name, e.g. **HetNOEResults**
- Click on the **Fit** button to run the calculation.

Your **Results DataTable** will then be automatically set to your new **HetNOEResults** table. The table and graphs in the main module will be filled with this data.

# 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.

# Combined Data Analyses

Input DataTables	
Input DataTable Name	<default>
Create Input DataTable	Create
Input DataTable(s)	> select-to-add < DT:T1Results DT:T2Results
Results DataTable Name	R2R1Results
Fit and Fetch Results Data	Fit

Input data	Calculation	Fitting	Appearance
Peak Property	height	Calculation Options	
Calculation Options	<input type="radio"/> Blank <input type="radio"/> HetNoe <input checked="" type="radio"/> R2/R1 <input type="radio"/> Reduced_Spectral_Density_Mapping		

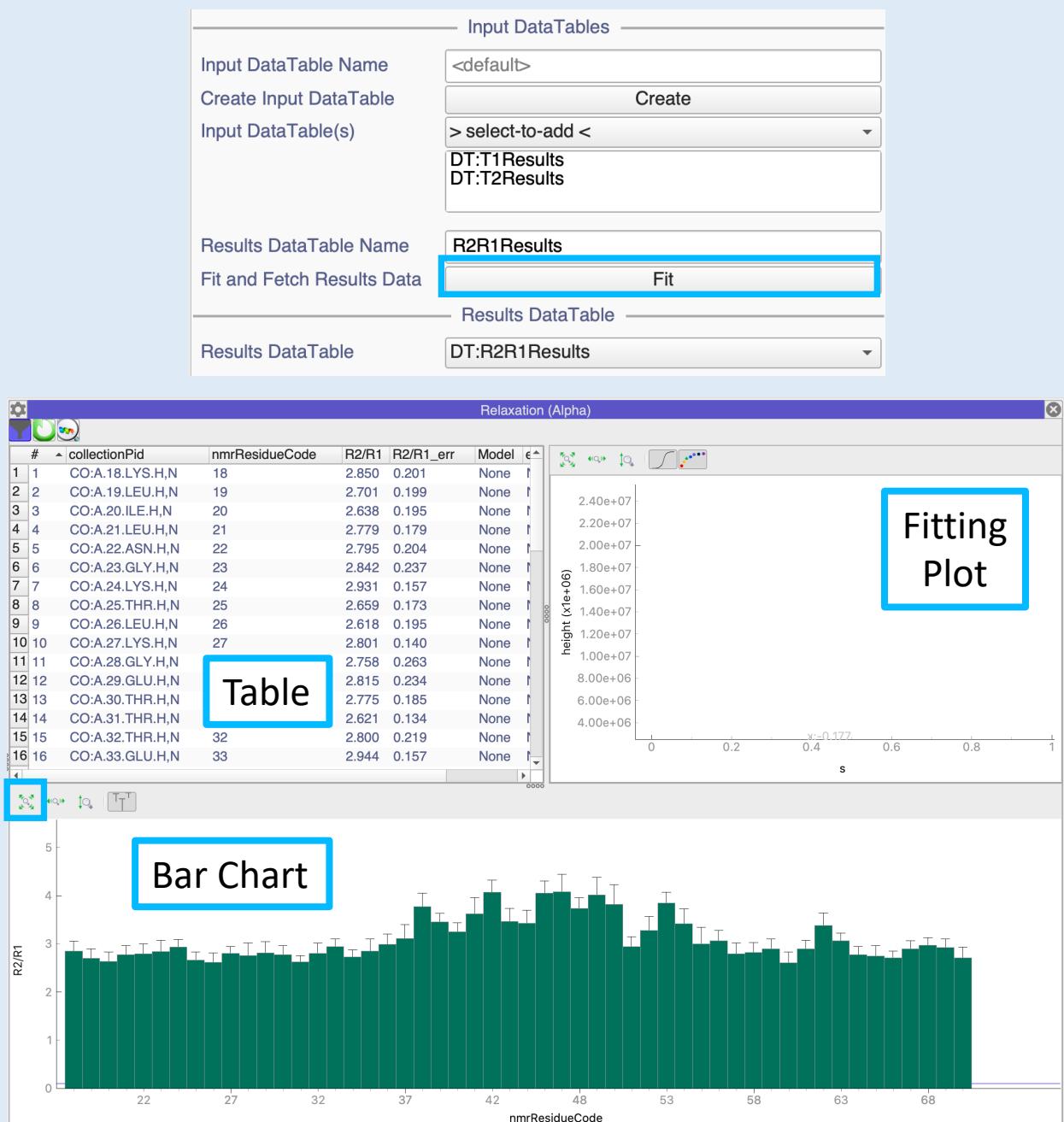
Input data	Calculation	Fitting	Appearance
Optimiser Options			
Optimiser Method	leastsq		
Fitting Error Method	Default		
Fitting Options			
Fitting Model	<input checked="" type="radio"/> Blank <input type="radio"/> OnePhaseDecay <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery		

## 4A R2/R1 Calculation Setup

At this point you can either continue from Section 3 or start using our **Section3\_completed ccpn** project.

- Open the **Relaxation Module Settings** panel with the gear icon.
  - In the **Input data** tab, 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**.
  - Provide a **Results DataTable Name**, e.g. **R2R1Results**
- In the **Fitting** tab:
- Set your **Calculation Options** to be **R2/R1**.
- In the **Optimiser** tab:
- Make sure the **Fitting Model** is set to **Blank**.

# Combined Data Analyses



## 4B R2/R1 Calculation

In the **Settings Input data** tab:

- Click on the **Fit** 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.

# Reduced Spectral Density Mapping

Input DataTables	
Input DataTable Name	<default>
Create Input DataTable	<input type="button" value="Create"/>
Input DataTable(s)	<input type="button" value="&gt; select-to-add &lt;"/> DT:T1Results DT:T2Results DT:HetNOEResults
Results DataTable Name	RDSMResults
Fit and Fetch Results Data	<input type="button" value="Fit"/>

Input data		Calculation	Fitting	Appearance
Peak Property	<input type="text" value="height"/> Calculation Options			
Calculation Options	<input type="radio"/> Blank <input type="radio"/> HetNoe <input type="radio"/> R2/R1 <input checked="" type="radio"/> Reduced_Spectral_Density_Mapping			

Input data		Calculation	Fitting	Appearance
Optimiser Options				
Optimiser Method	<input type="text" value="leastsq"/>			
Fitting Error Method	<input type="text" value="Default"/>			
Fitting Options				
Fitting Model	<input checked="" type="radio"/> Blank <input type="radio"/> OnePhaseDecay <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery			

## 5A Reduced Spectral Density Mapping Setup

At this point you can either continue from Section 4 or start using our **Section4\_completed ccpn** project.

- Open the Relaxation Module Settings panel with the gear icon.
  - In the **Input data** tab, select the **Input DataTables** to be
    - DT:T1Results**
    - DT:T2Results**
    - DT:HetNOEResults**
  - Provide a **Results DataTable Name**, e.g. **RSDMResults**
- 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**.

# Reduced Spectral Density Mapping

Input DataTables	
Input DataTable Name	<default>
Create Input DataTable	<input type="button" value="Create"/>
Input DataTable(s)	<input type="button" value="&gt; select-to-add &lt;"/> DT:T1Results DT:T2Results DT:HetNOEResults
Results DataTable Name	RDSMResults
Fit and Fetch Results Data	<input type="button" value="Fit"/>
<input type="button" value="Input data"/> <input type="button" value="Calculation"/> <input type="button" value="Fitting"/> <input type="button" value="Appearance"/>	
SpectrumDisplay	
Select SpectrumDisplays	<input type="button" value="&gt; select-to-add &lt;"/> <input type="button" value="&lt;Use active&gt;"/>
Navigate trigger	<input checked="" type="radio"/> Single Click <input type="radio"/> Double Click <input type="radio"/> Disabled
BarGraph	
X Axis Data	nmrResidueCode
Y Axis Data	<input type="button" value="J0"/> <input checked="" type="button" value="J0"/> JwX JwH argA argB Rsqr chisqr redchi aic bic
Threshold Value Calculation	
Threshold Factor	
Threshold Value	
Above Threshold Colour	
Below Threshold Colour	
Intraceable Observation Col	 CCPNpurple

## 5\_B Reduced Spectral Density Mapping Calculation

In the **Settings Input data** tab:

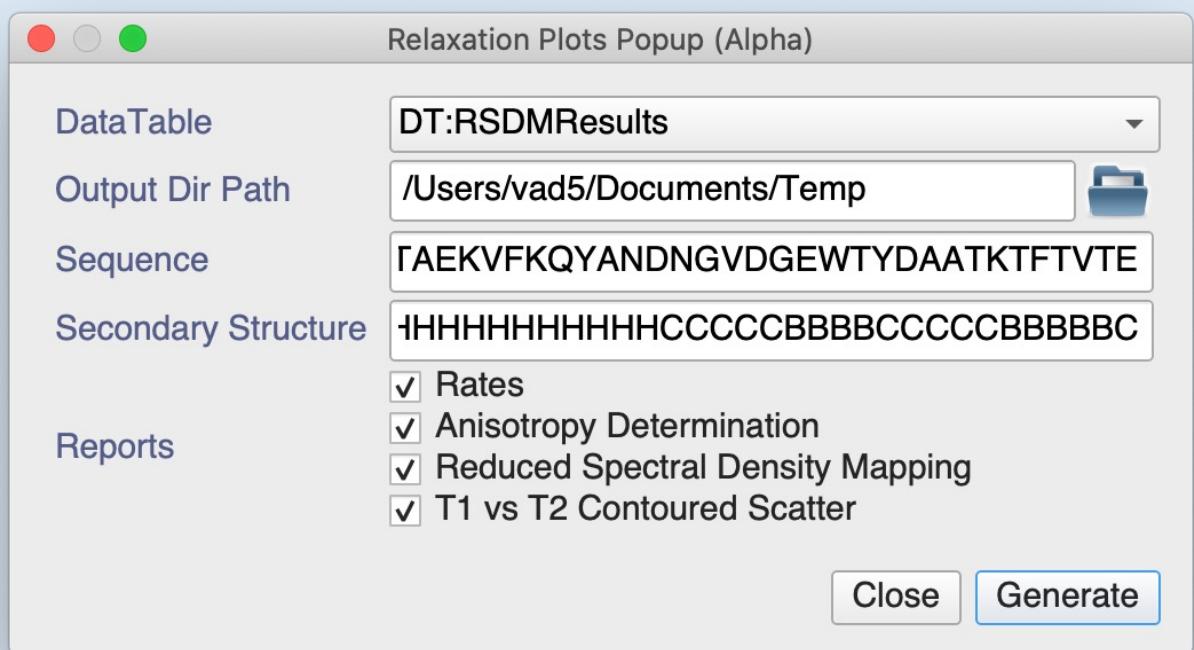
- Click on the Fit button.

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  $\omega_N$ ,  $\omega_N$  and  $\omega_H$ , respectively.
- If necessary, press the orange Update button  or auto-zoom button  to view the data on the bar chart.

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

# Exporting Graphs



## 6A Export predefined plots in pdf format

At this point you can either continue from Section 5 or start using our **Section5\_completed ccpn** project.

- Go to Main Menu → Macro → Run CCPN Macros → **Relaxation Plots Popup**
- As your **DataTable**, select your Reduced Spectral Density Mapping Results table (**DT:RSDMResults**)
- Select a Directory where you want to save your plots  
The Sequence and Secondary Structure (in DSSP code\*) for GB1 are already provided in the pop-up.
- Keep all **Reports** selected
- Click on **Generate**

\* DSSP secondary structure coding:

H: a-helix

G:  $\text{3}_{10}$  helix

I:  $\pi$ -helix

E: extended strand

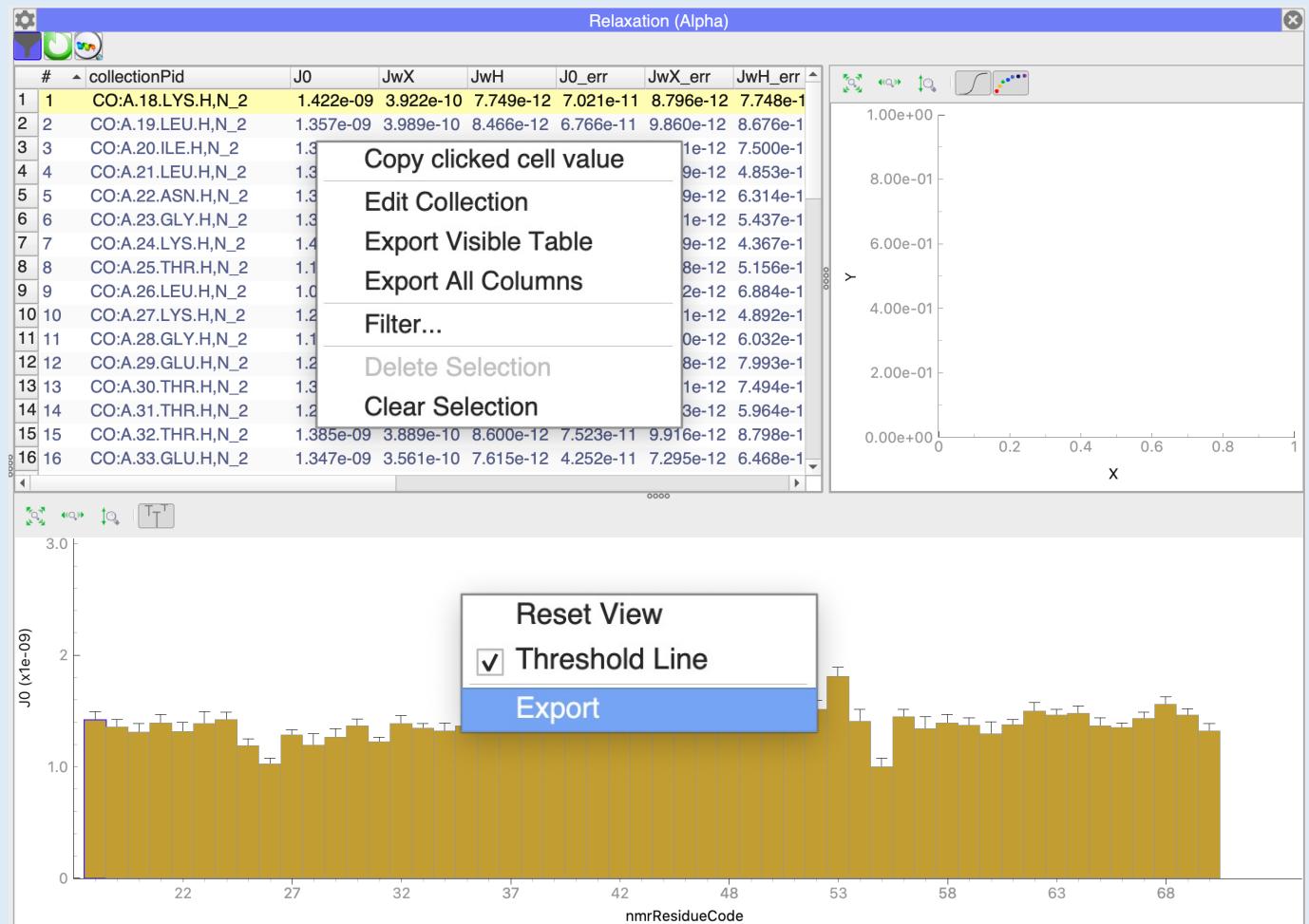
B: residue in isolated  $\beta$ -bridge

S: bend

T: H-bonded turn

C: no secondary structure

# Exporting Graphs



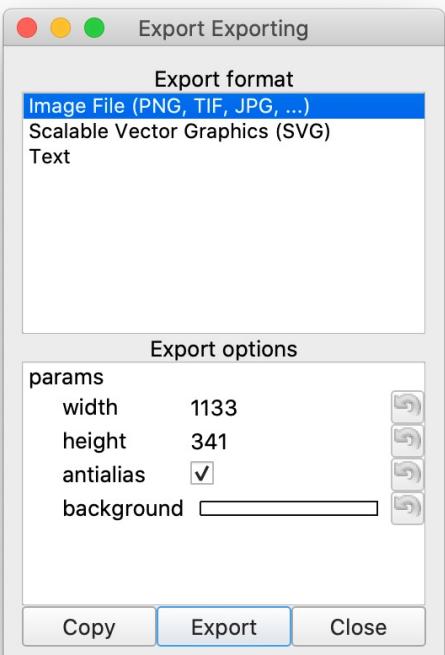
## 6B Exporting data for use in external graphing programs

- 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.

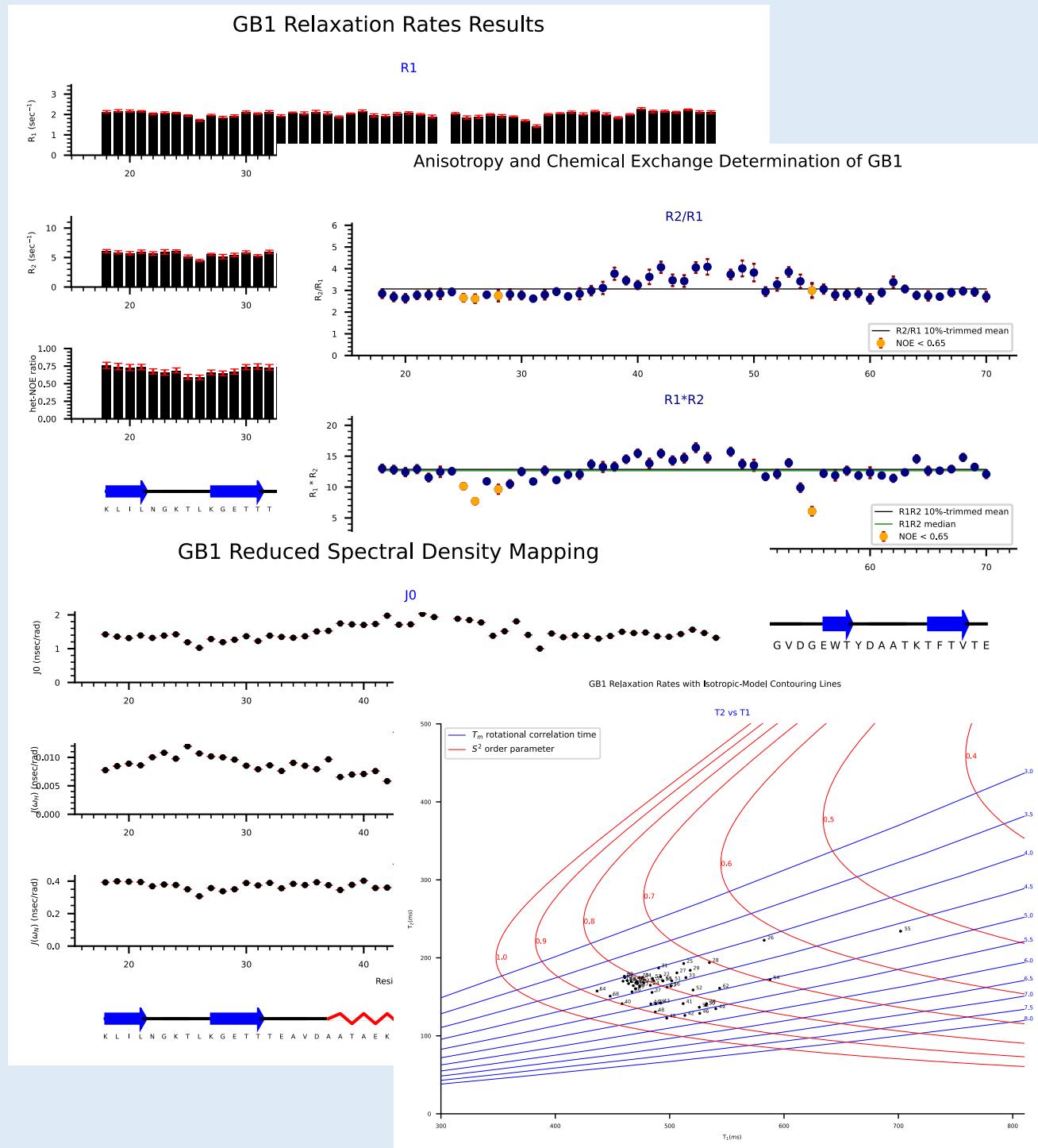
## 6C Exporting individual charts

- 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.



# Examining the Graphs



## 7

## Examine the graphs

The graphs are also available in the **graphs** folder of the tutorial data directory.

- Examine the various graph that you have produced
- **For each graph**, summarise what kind of data they display and what you can learn from them.
- What is your overall assessment of the dynamics of GB1? Is it adequately described by a small sphere tumbling in solution?

## Contact Us

**Website:**

[www ccpn ac uk](http://www ccpn ac uk)

**Suggestions and comments:**

[support@ccpn.ac.uk](mailto:support@ccpn.ac.uk)

**Issues and bug report:**

<https://forum.ccpn.ac.uk/>

## Cite Us

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