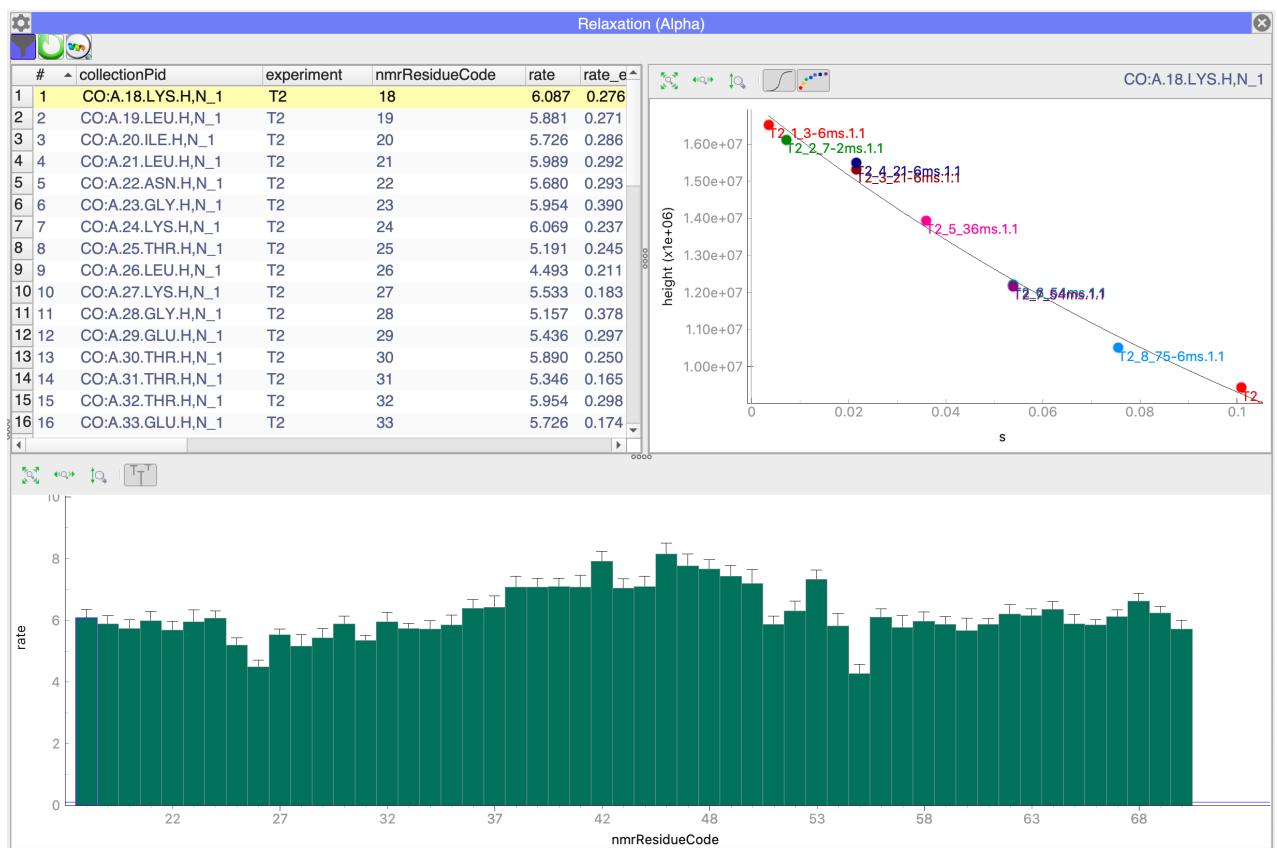


Dynamics Tutorial (Beta)



Introduction

This tutorial will take you through the analysis of some dynamics data in CcpNmr Analysis Version 3.2 using the beta 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:

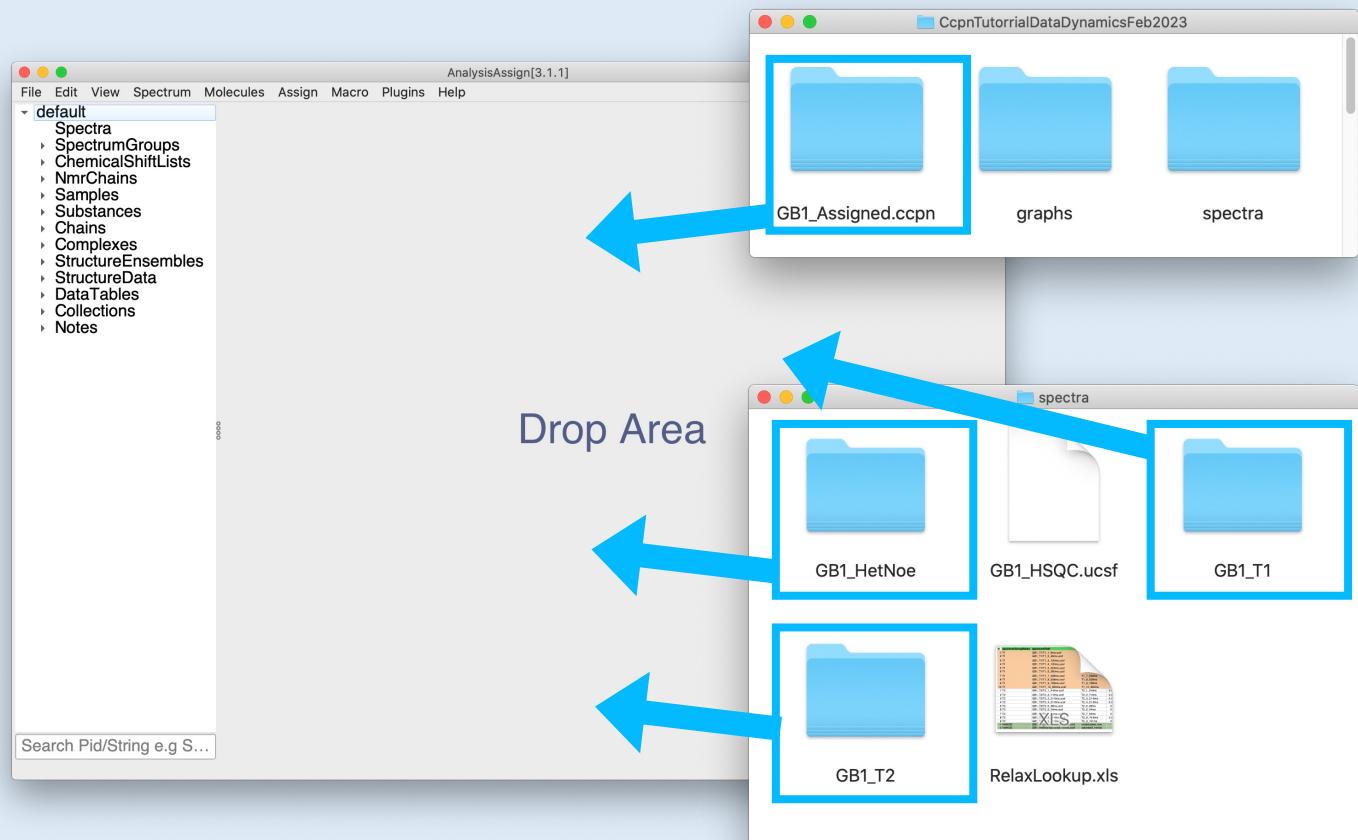
1. Loading Data
2. T1 and T2 Data
3. Heteronuclear NOE Data
4. Combined Analyses
5. Reduced Spectral Density Mapping
6. Exporting Graphs
7. Examining the Graphs

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

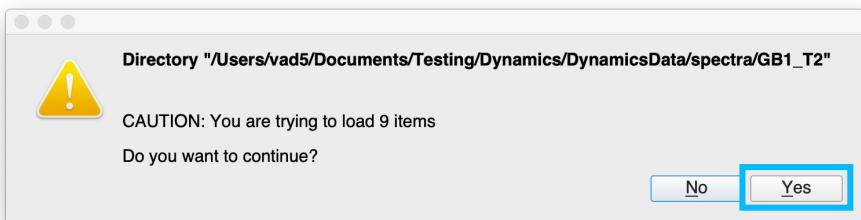
- Find the **GB1_Assigned ccpn** project folder in the Dynamics Tutorial data directory. This project contains an assigned HSQC spectrum of GB1.
- Select the folder in your file browser and drag it onto the **Sidebar** or **Drop Area**.
- Find the **spectra** directory in the Dynamics Tutorial data directory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

GB1_T1

GB1_T2

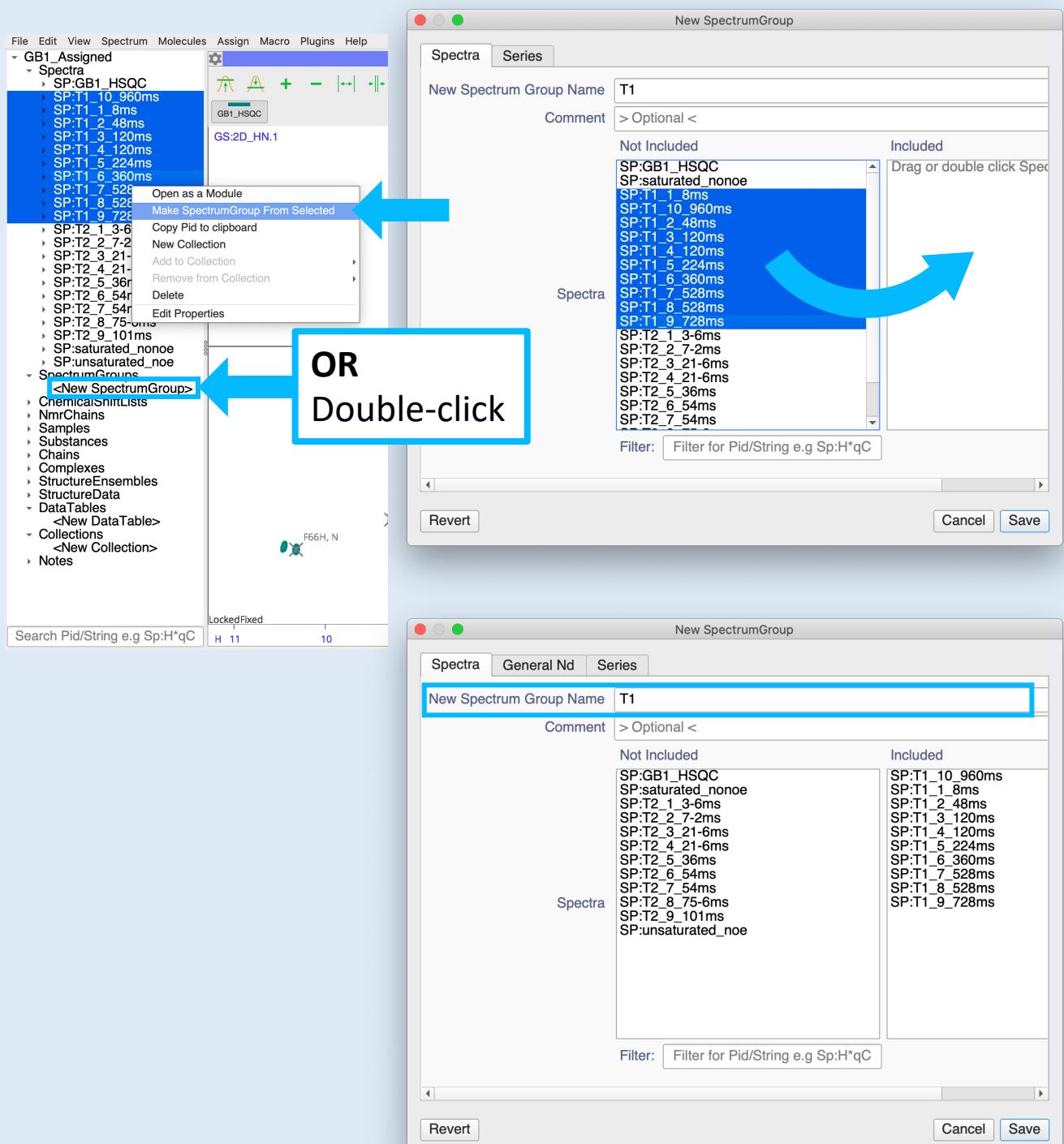
GB1_HetNOE

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



If you drop the folders into the Drop Area 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.

Loading data



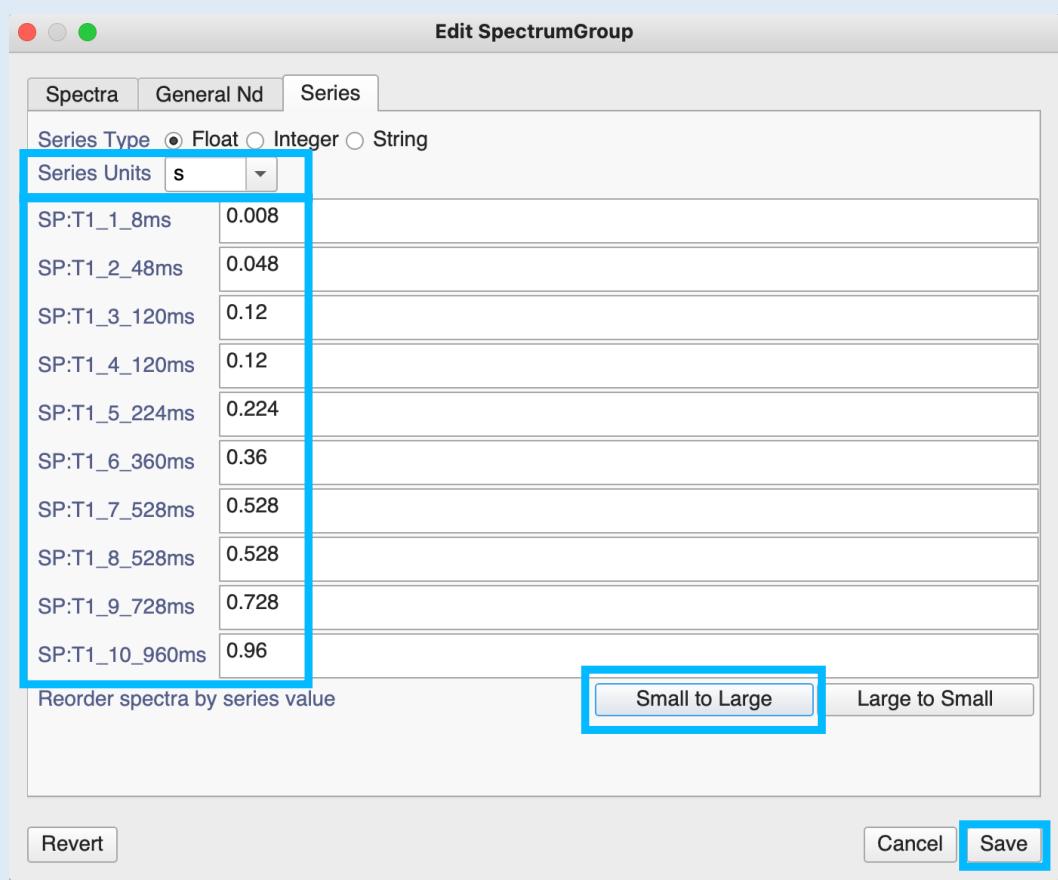
1B Create the T1 SpectrumGroup and Series

- 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 **Small to Large** 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 'Series Units' dropdown is set to 's'. 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_nono, 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'. The 'Series Units' dropdown is set to 'AU'. The table lists the spectra from the sidebar with their corresponding values: SP:saturated_nono (1.0) and SP:unsaturated_noe (0.0).

1D Create T2 and Heteronuclear NOE SpectrumGroups and Series

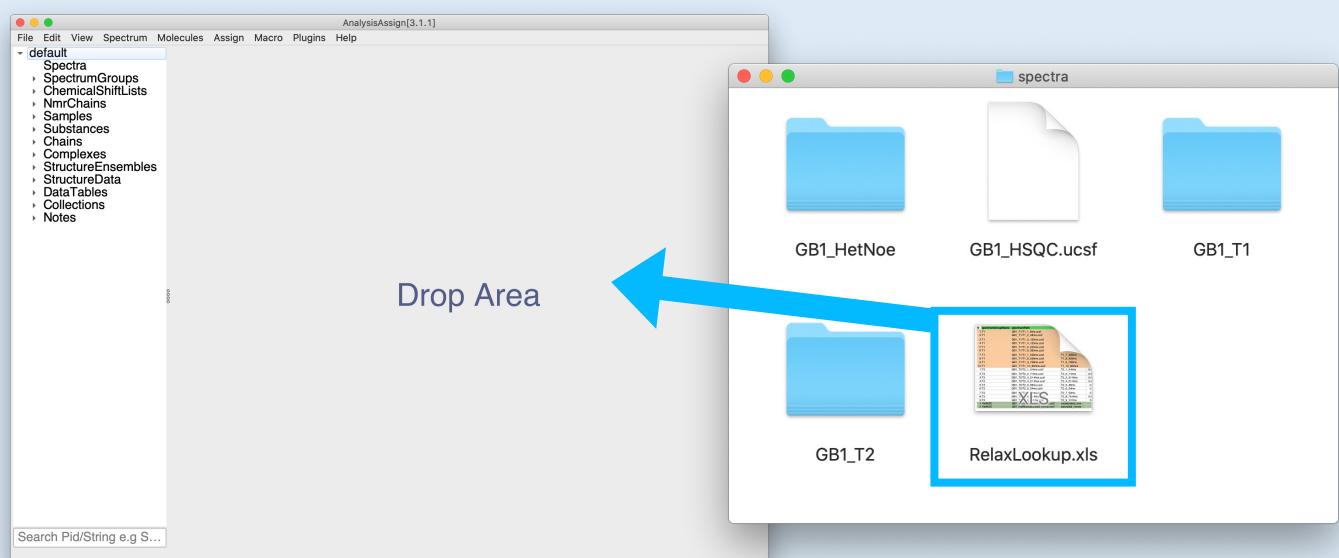
- Repeat the procedure for creating a SpectrumGroup and setting up the Series for the T2 data

Create a SpectrumGroup and Series for the Heteronuclear NOE data:

- Select the **saturated_nono** 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:

saturated_nono	1.0
unsaturated_noe	0.0

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

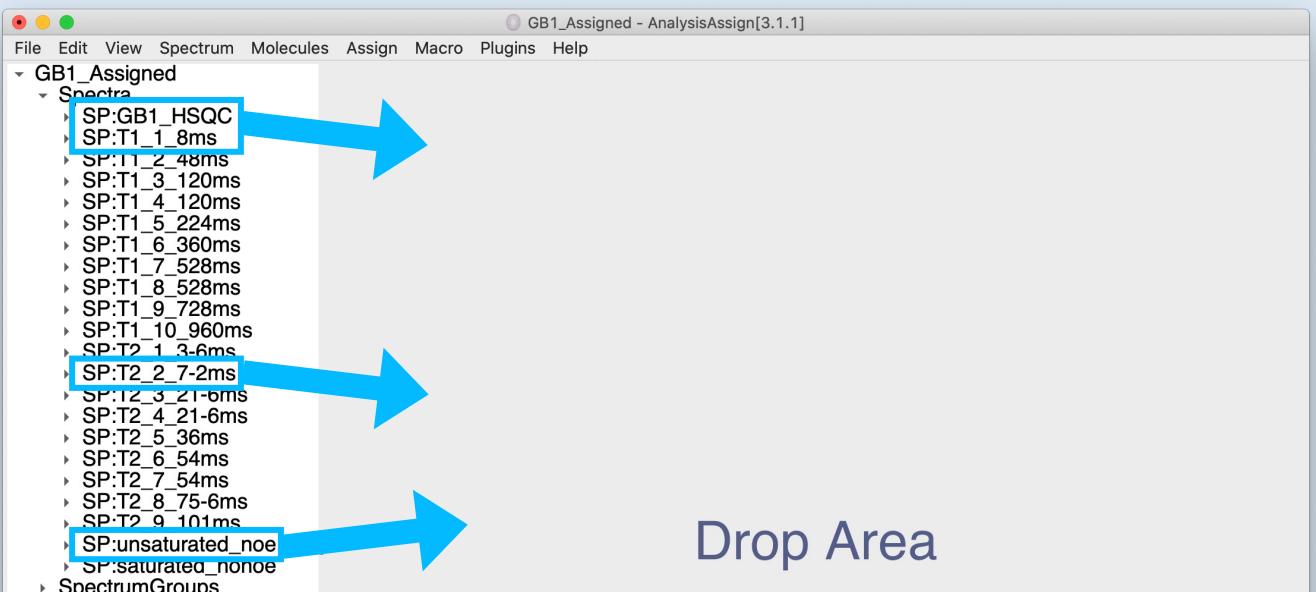
1E Import from Excel Lookup File (optional)

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.

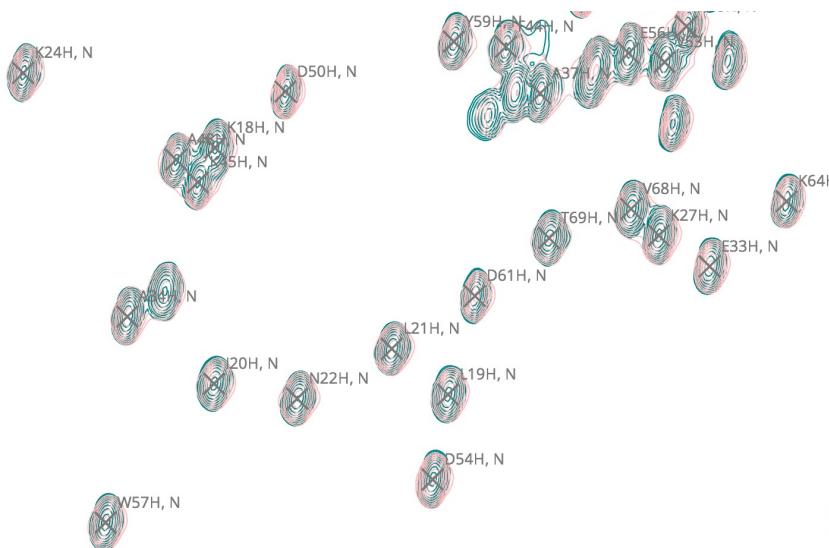
Loading data



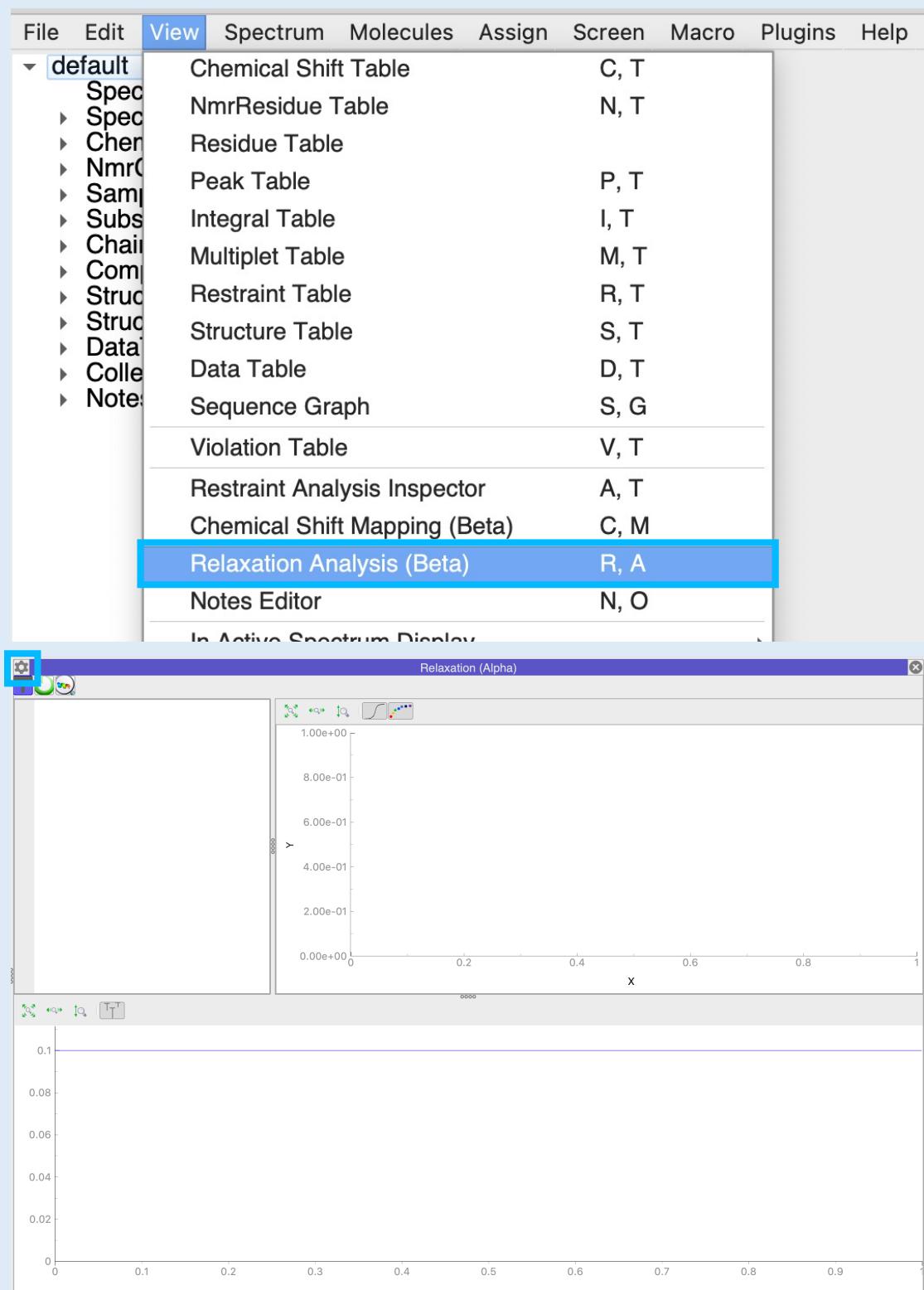
1F Check alignment to HSQC

- 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

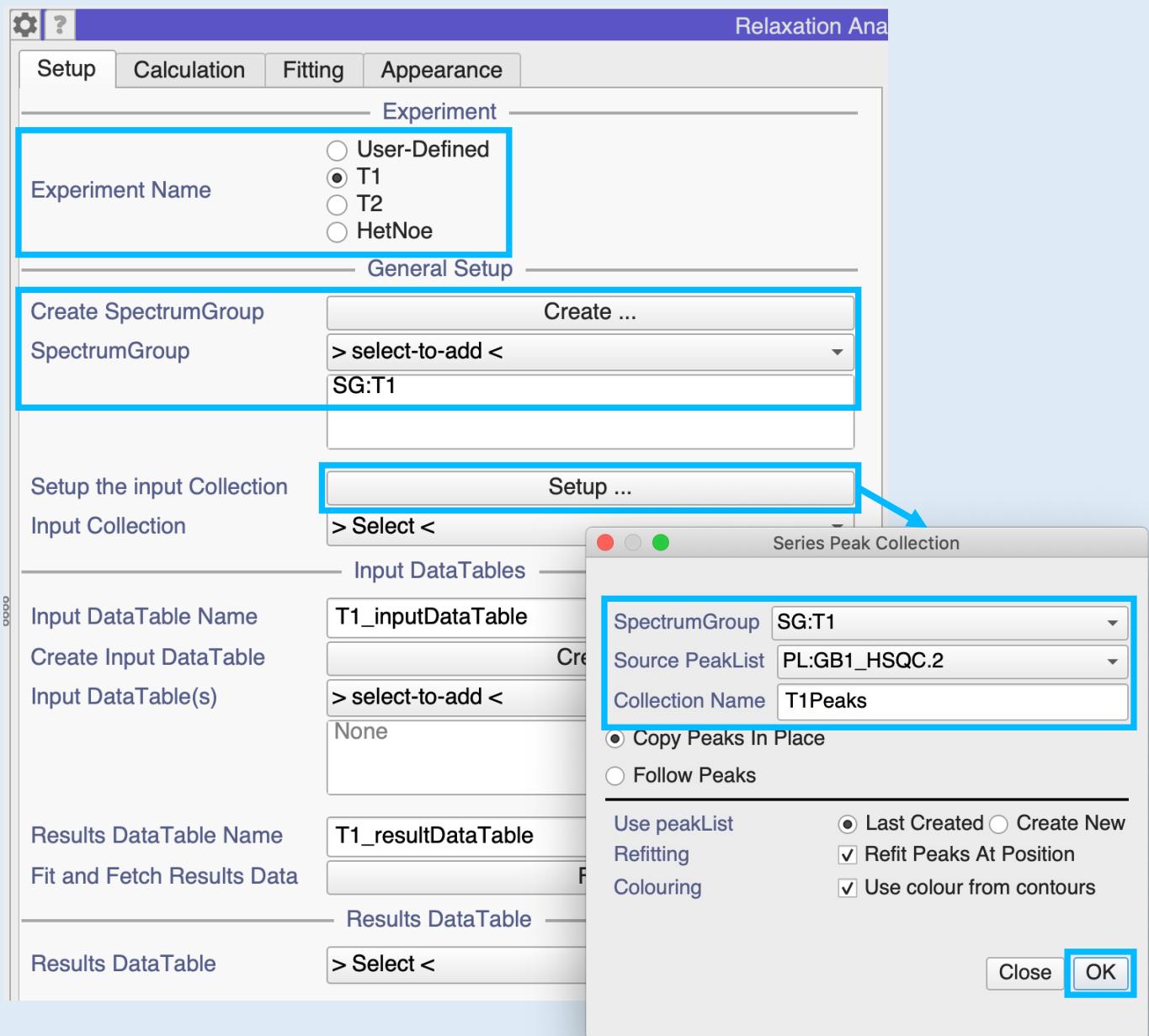


2A Open Relaxation Module

At this point you can either continue from Section 1 or start using our **Section1_completed ccpn** project.

- Go to Main Menu → View → Relaxation Analysis (Beta).
This will open the 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

- Set the **Experiment Name** to **T1**.

This will pre-populate some elements in the sections below and other tabs.

In the **General Setup** the **SpectrumGroup** specifies which spectra you want to use.

The **Input Collection** is a collection of peak groups across the spectra.

We have already created our **SpectrumGroups**:

- Ensure the **T1** SpectrumGroup from the drop-down menu is selected.

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**

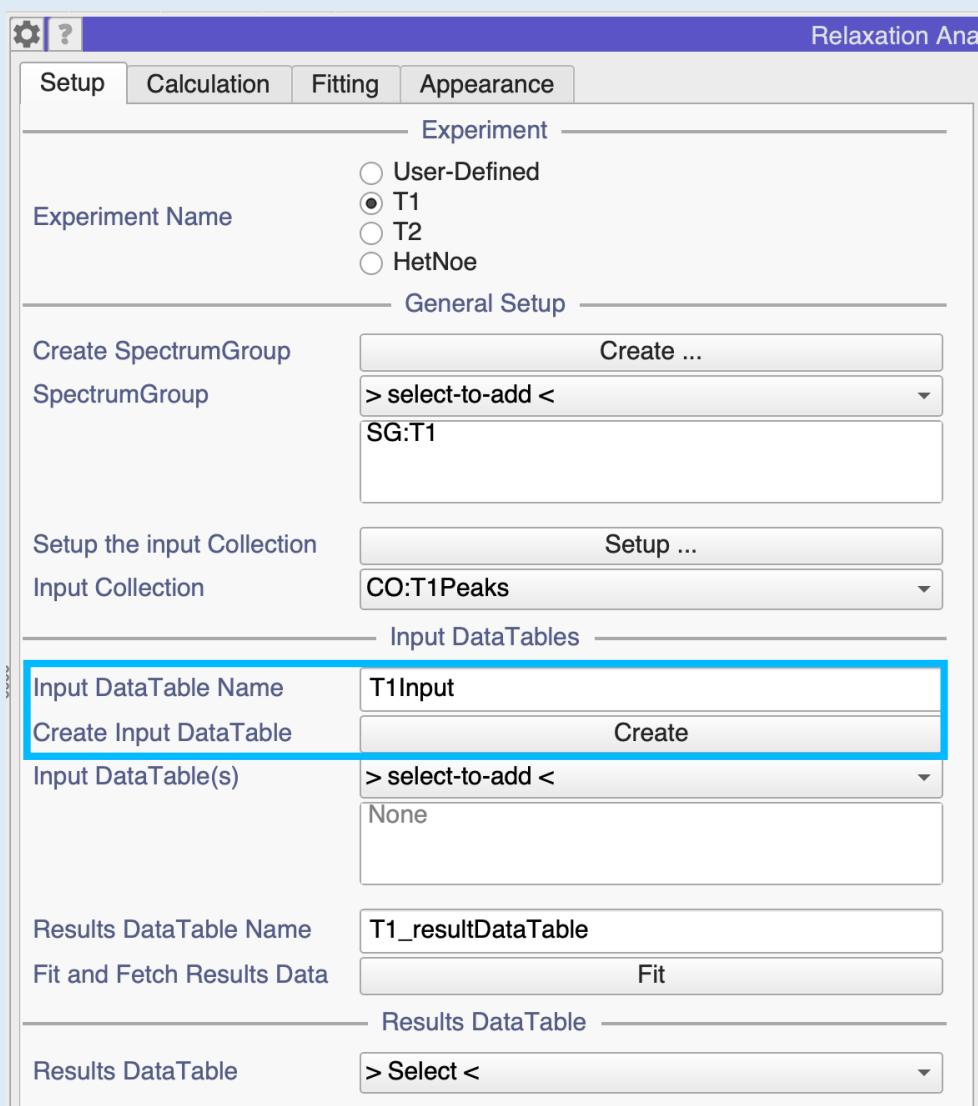
Collection Name: **T1Peaks**

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.

T1 and T2 Data



2C Create Input DataTable

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

In the **Input DataTables** Section:

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

The screenshot shows the 'Input DataTables' section with the 'Input DataTable(s)' dropdown highlighted by a blue border. The dropdown shows 'DT:T1Input'.

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

T1 and T2 Data

Setup	Calculation	Fitting	Appearance
Peak Property	height		
Calculation Options <div style="border: 2px solid blue; padding: 5px;"> <input checked="" type="radio"/> Blank <input type="radio"/> HetNoe <input type="radio"/> R2/R1 <input type="radio"/> ETAs Ratio <input type="radio"/> Reduced_Spectral_Density_Mapping </div>			
Setup	Calculation	Fitting	Appearance
Optimiser Options <div style="border: 2px solid blue; padding: 5px;"> Optimiser Method: leastsq Fitting Error Method: Default </div>			
Fitting Options <div style="border: 2px solid blue; padding: 5px;"> Fitting Model: OnePhaseDecay (selected) <input type="radio"/> Blank <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery </div>			
Setup	Calculation	Fitting	Appearance
Input DataTables <div style="border: 2px solid blue; padding: 5px;"> Input DataTable Name: T1Input Create Input DataTable: Create Input DataTable(s): > select-to-add < DT:T1Input </div>			
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.

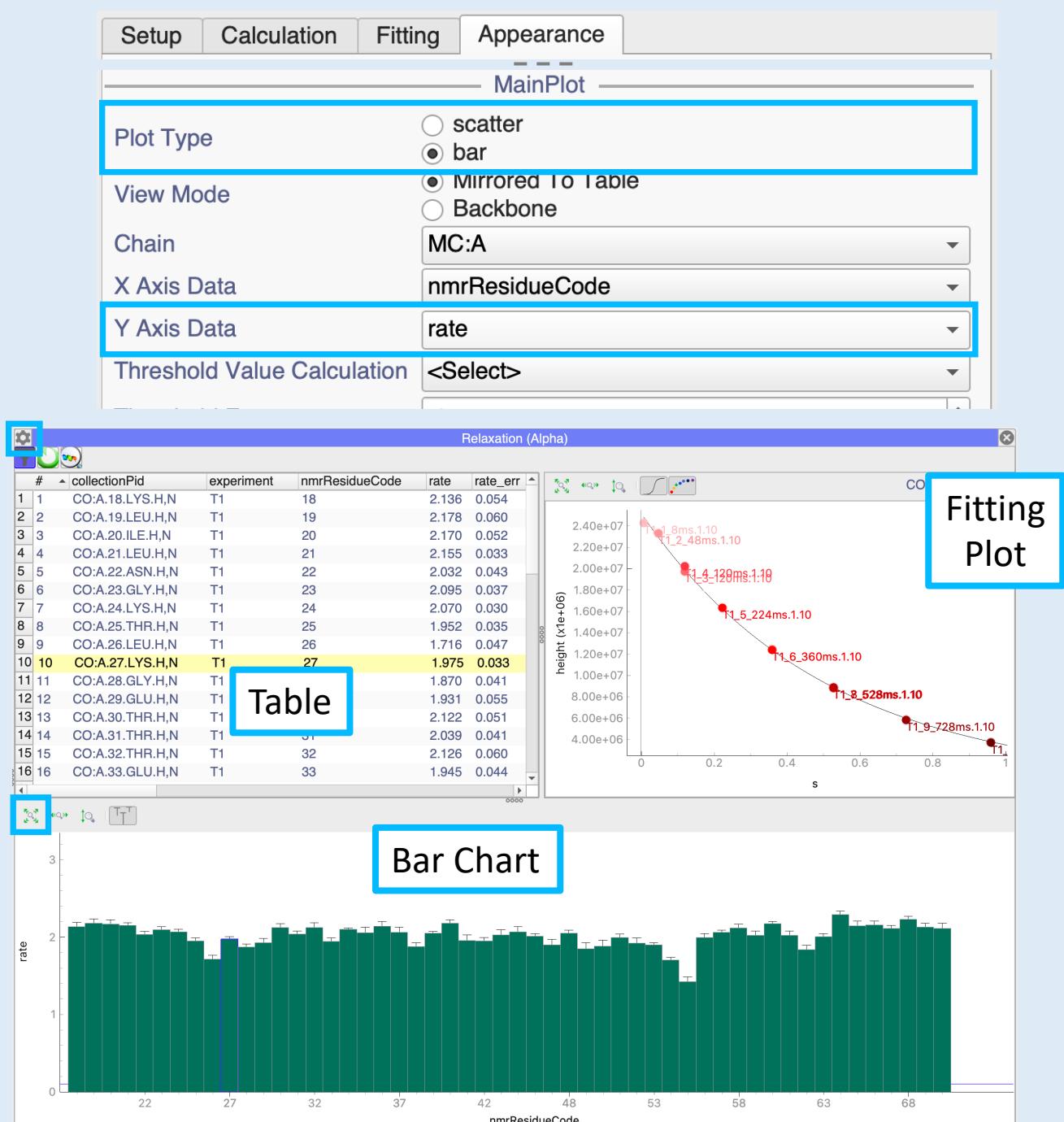
Move back to the **Setup** tab:

- Enter a **Results DataTable Name**, e.g. **T1Results** or leave the default name
- 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.

Results DataTable	
Results DataTable	DT:T1Results

T1 and T2 Data

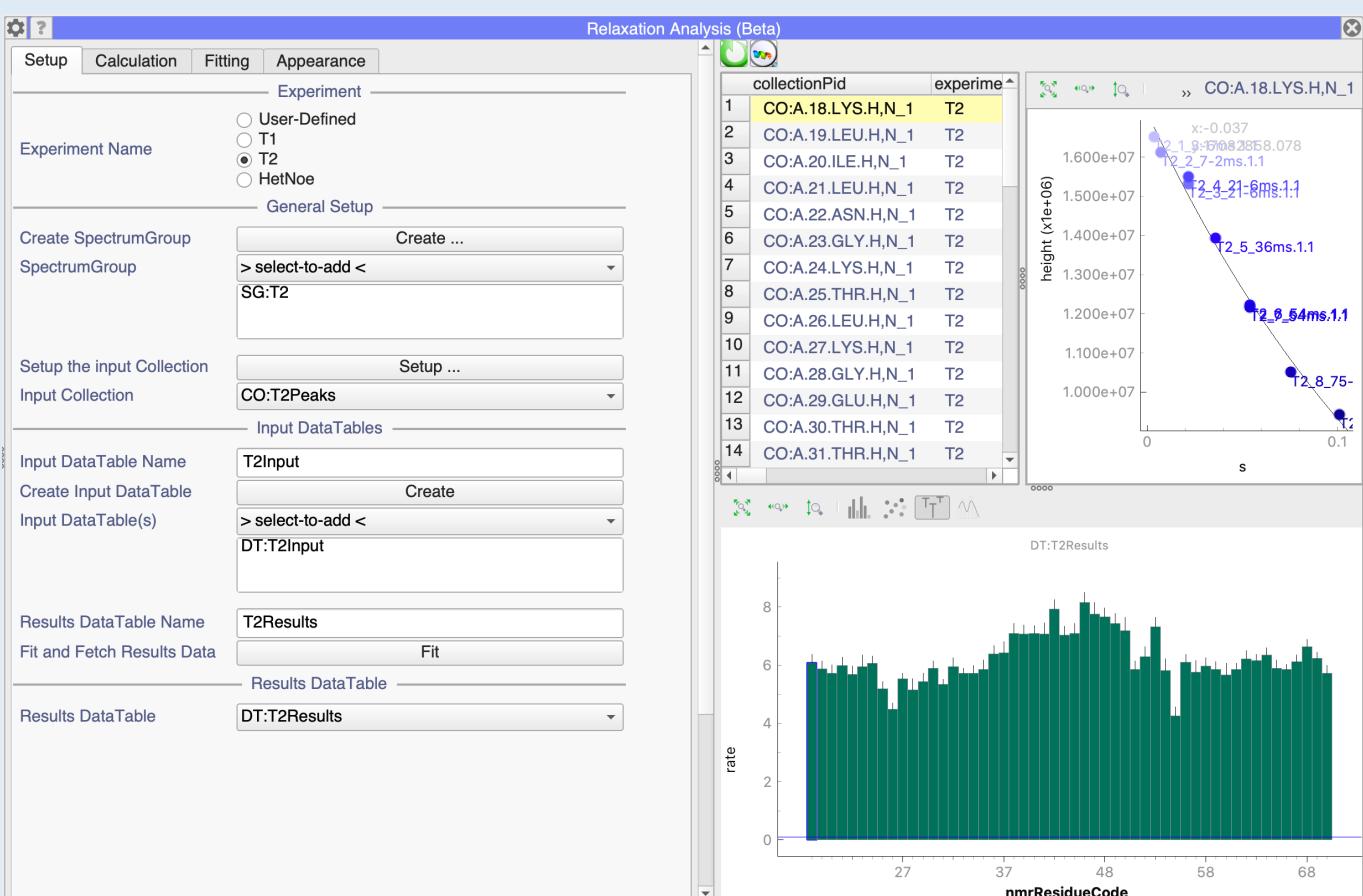
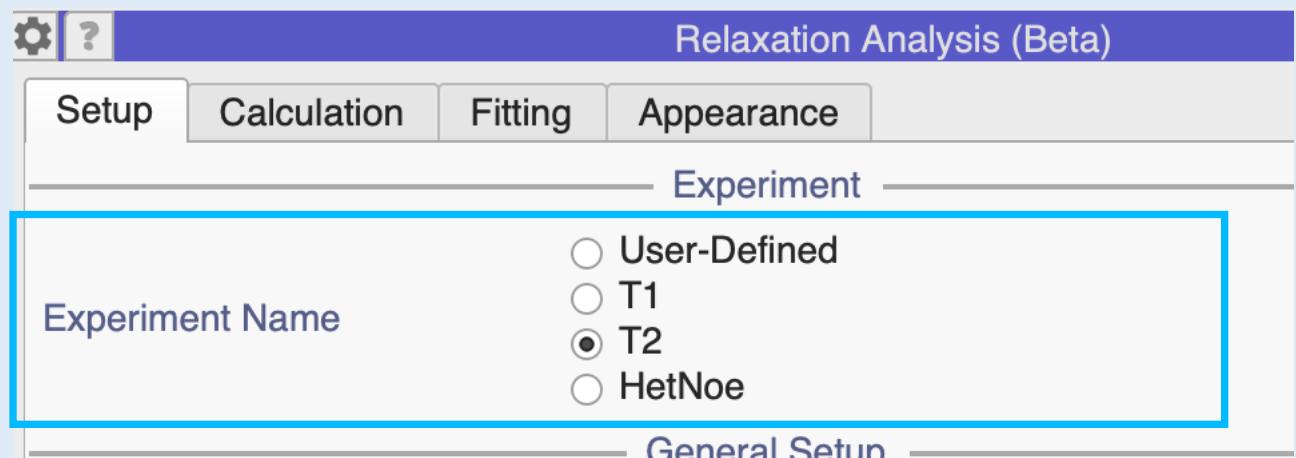


2E Inspect Results

Now move to the Appearance tab:

- Set the Plot Type to bar and 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



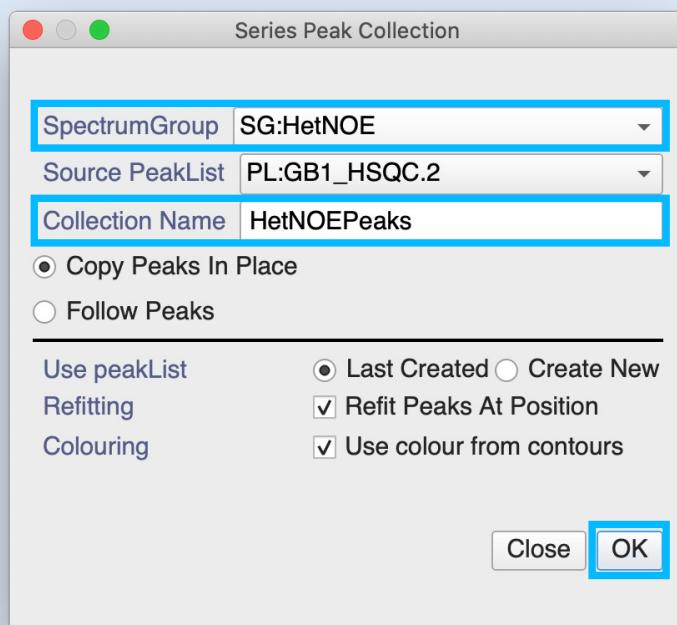
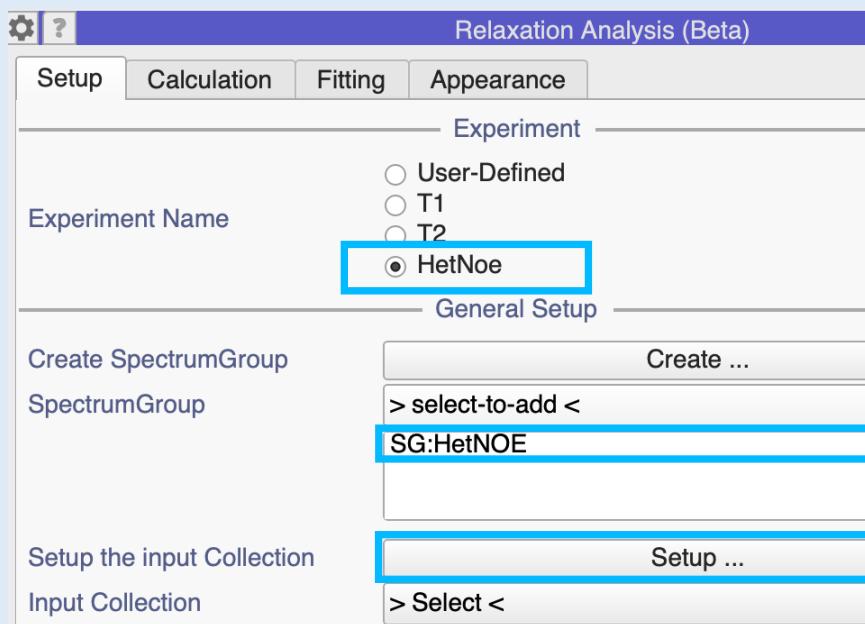
2F T2 Data

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

In the **Setup** tab:

- In the **Experiment** section, you will need to set the **Experiment Name** to **T2**.
- 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 **Setup** tab:

- In the **Experiment** section, set the **Experiment Name** to **HetNoe**.
- 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**
- 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	Create

Setup **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"/> ETAs Ratio <input type="radio"/> Reduced_Spectral_Density_Mapping

Setup **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 **Setup** tab:

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

In the Settings **Calculation** tab:

- Check **HetNoe** has been selected

In the Settings **Fitting** tab:

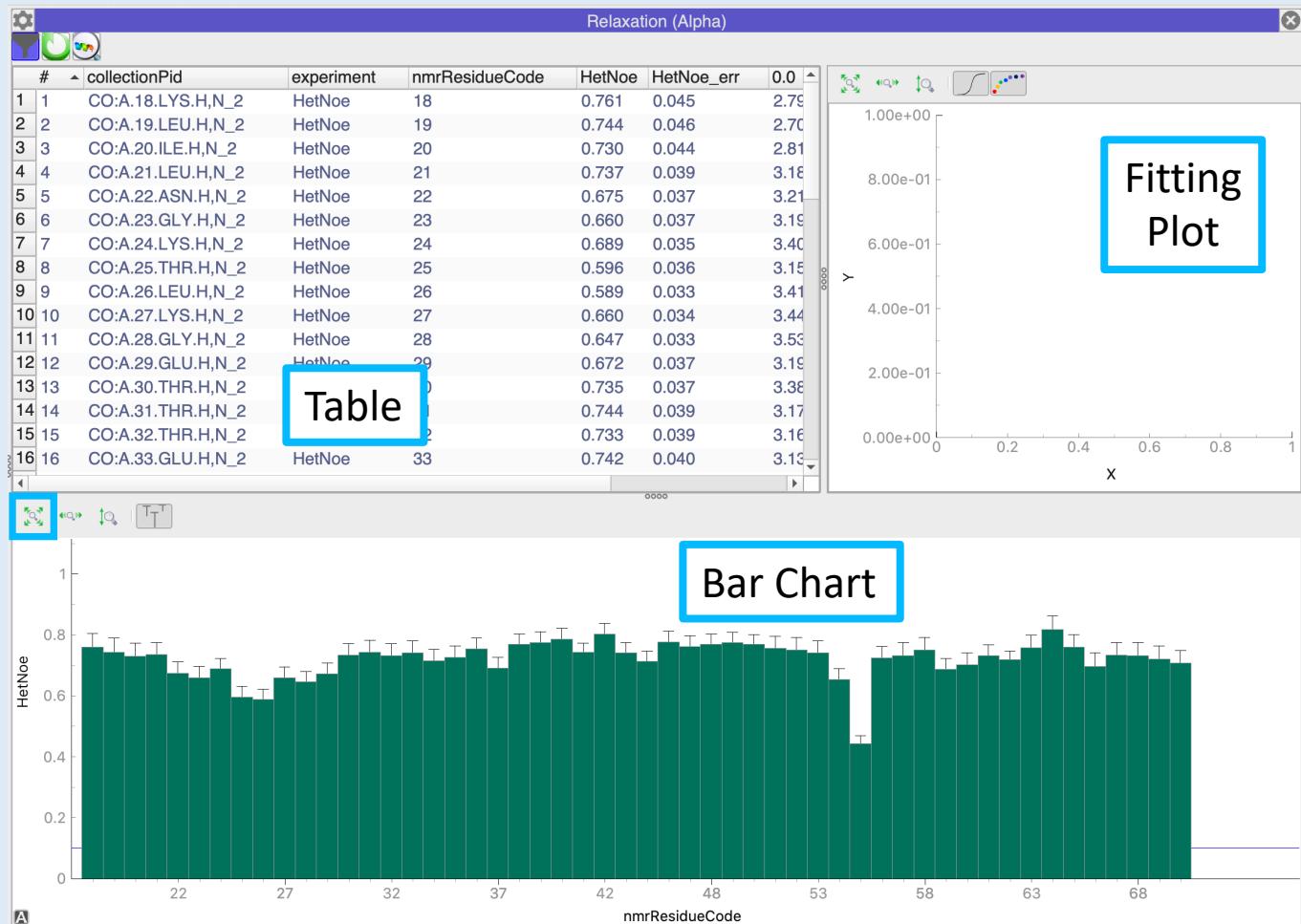
- Check **Blank** has been selected

In the **Setup** 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

Setup 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"/> ETAs Ratio <input type="radio"/> Reduced_Spectral_Density_Mapping

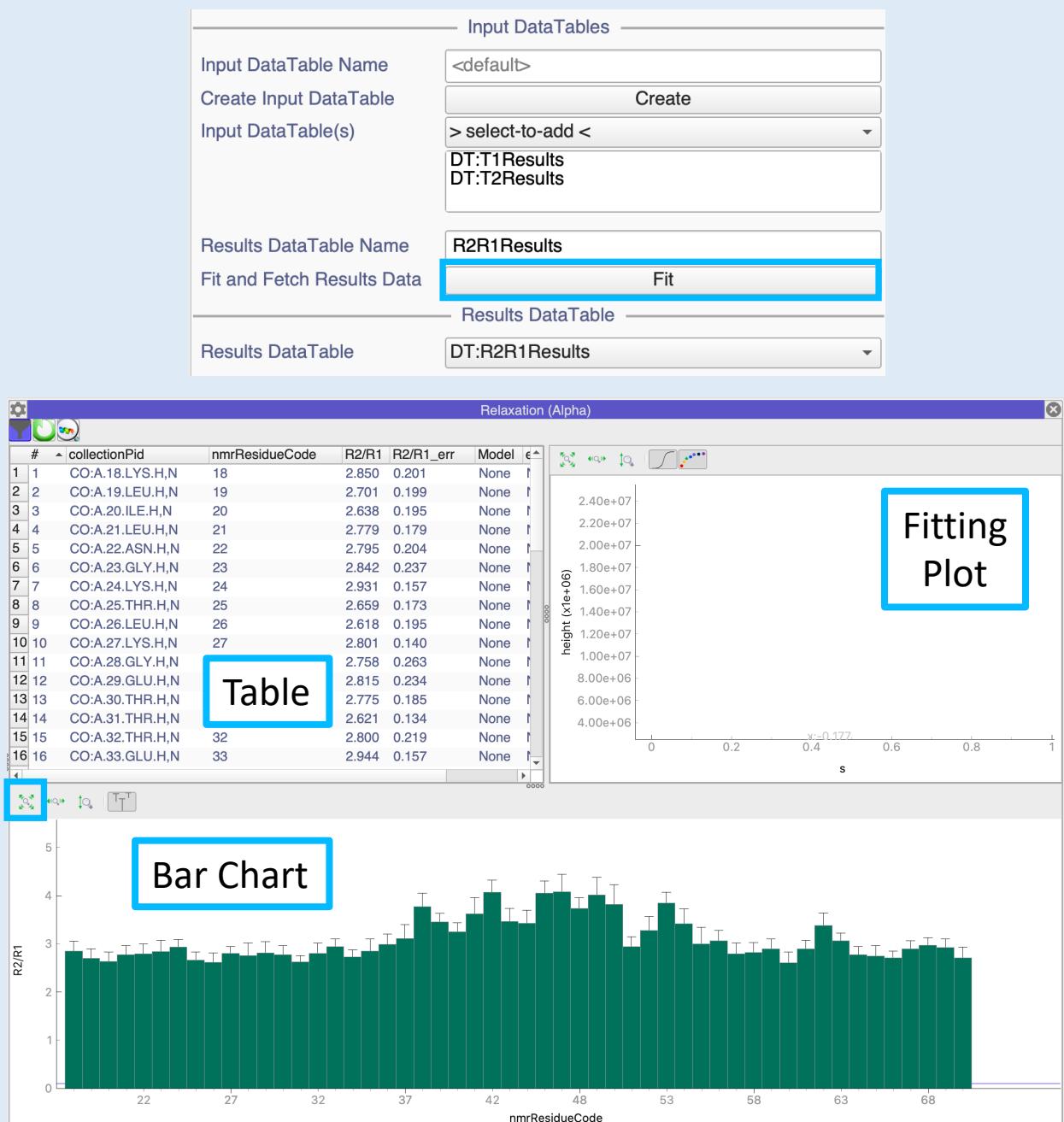
Setup Calculation Fitting Appearance	
Optimiser Options	
Optimiser Method	leastsq
Fitting Error Method	Default
Fitting Options	
Fitting Model	<input checked="" type="radio"/> Blank <input type="radio"/> OnePhaseDecay <input type="radio"/> ExponentialDecay <input type="radio"/> InversionRecovery

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 **Setup** 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 **Appearance** tab:
- Make sure the **Fitting Model** is set to **Blank**.

Combined Data Analyses



4B R2/R1 Calculation

In the **Settings Setup** 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	SeriesAnalysisInputData
Create Input DataTable	Create
Input DataTable(s)	> select-to-add < DT:T1Results DT:T2Results DT:HetNOEResults
Results DataTable Name	RSDMResults
Fit and Fetch Results Data	Fit

Fitting

Peak Property	height
Calculation Options	
Calculation Options	<input type="radio"/> Blank <input type="radio"/> HetNoe <input type="radio"/> R2/R1 <input type="radio"/> ETAs Ratio <input checked="" type="radio"/> Reduced_Spectral_Density_Mapping

Fitting

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

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 **Setup** 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

The screenshot shows the software interface for Reduced Spectral Density Mapping. It has two main sections: 'Input DataTables' and 'Fitting'.

Input DataTables:

- Input DataTable Name: SeriesAnalysisInputData
- Create Input DataTable: Create
- Input DataTable(s): > select-to-add < (dropdown menu)
 - DT:T1Results
 - DT:T2Results
 - DT:HetNOEResults
- Results DataTable Name: RSDMResults
- Fit and Fetch Results Data: Fit (button)

Fitting Tab (selected):

- Setup, Calculation, Fitting, Appearance (tab bar)
- SpectrumDisplay:
 - Select SpectrumDisplays: > select-to-add < (dropdown menu)
 - <Use active>
 - Single Click (radio button selected)
 - Double Click
 - Disabled
- MainPlot:
 - Plot Type: scatter (radio button selected)
 - View Mode: Mirrored To Table (radio button selected)
 - Chain: MC:A (dropdown menu)
 - X Axis Data: nmrResidueCode (dropdown menu)
 - Y Axis Data: Rsqr (dropdown menu)
 - Others --
 - Index
 - J0 (radio button selected)
 - JwX
 - JwH
 - J0_err
 - JwX_err
 - JwH_err
 - R1
 - R1_err

5B Reduced Spectral Density Mapping Calculation

In the **Settings Setup** tab:

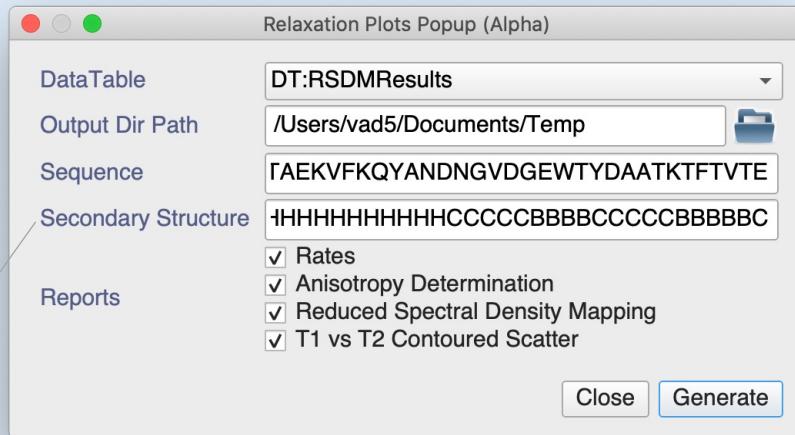
- Click on the **Fit** button.

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 ω_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.

Exporting Graphs



* DSSP secondary structure coding:

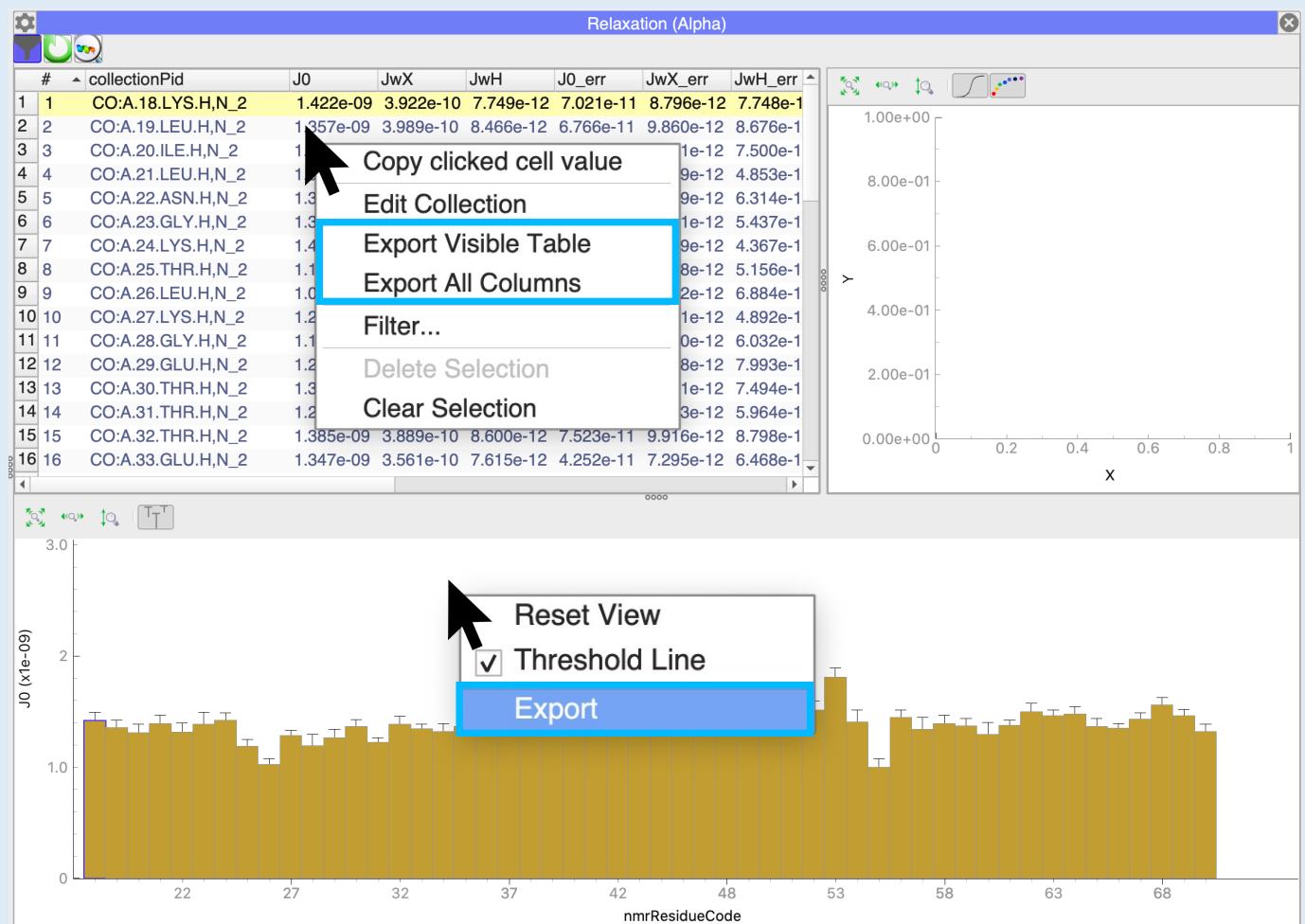
<i>H</i> : <i>a</i> -helix	<i>G</i> : β_{10} helix
<i>I</i> : π -helix	<i>E</i> : extended strand
<i>B</i> : residue in isolated β -bridge	<i>S</i> : bend
<i>T</i> : <i>H</i> -bonded turn	<i>C</i> : no secondary structure

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 → **RelaxationPlotsPopup**
- 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**

Exporting Graphs



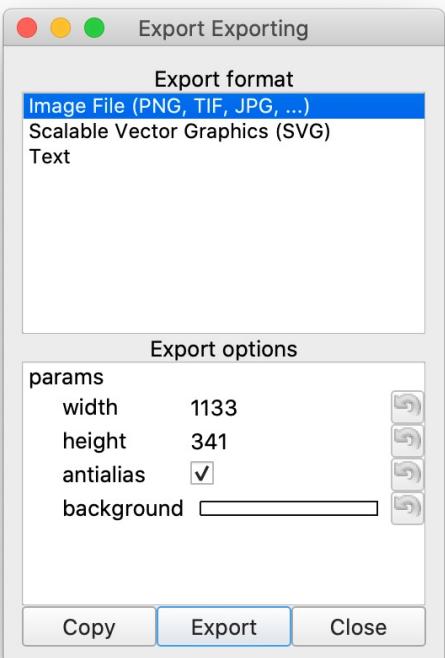
6B Exporting data for use in external graphing programs

- 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.

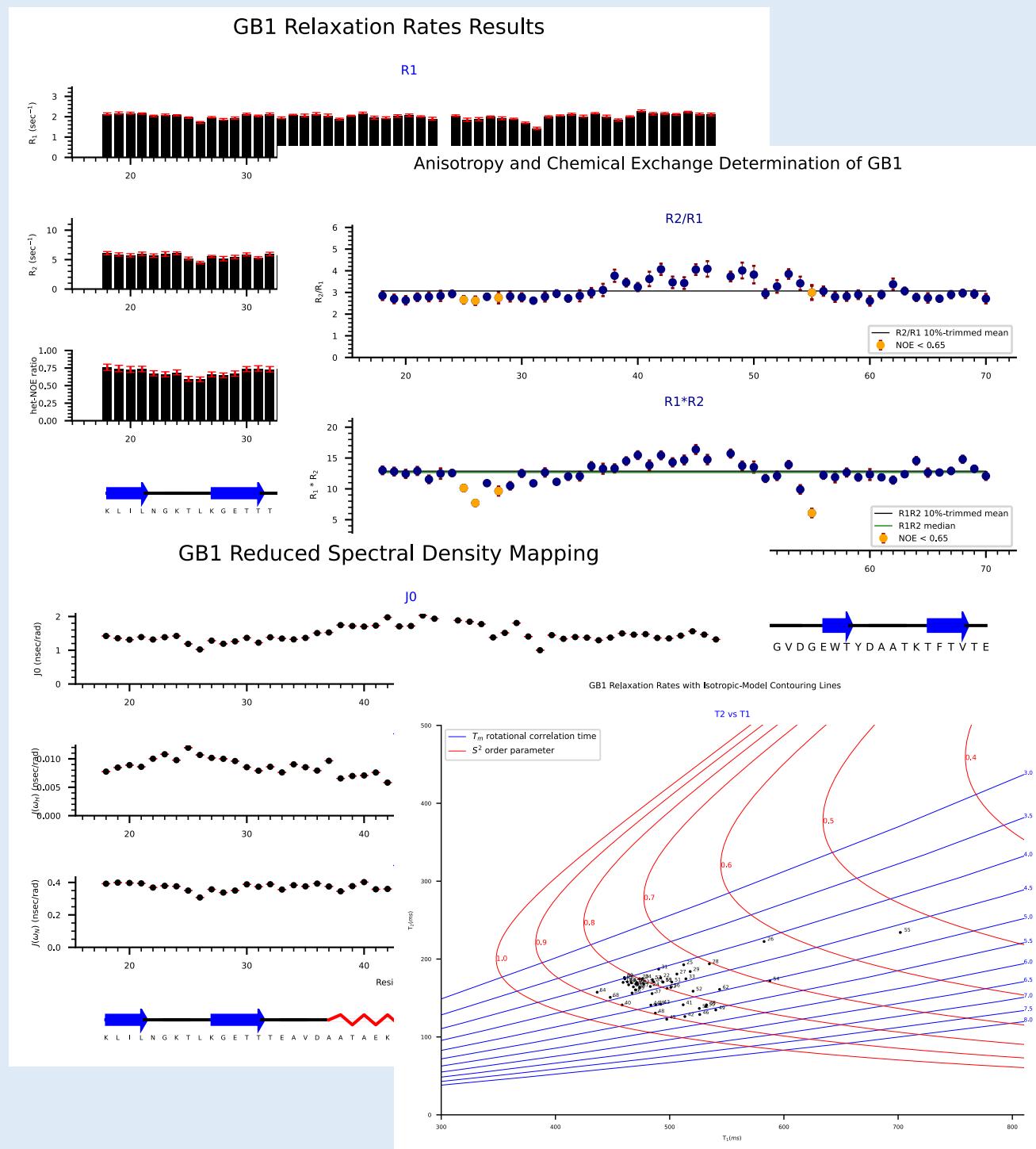
6C Exporting individual charts

- 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

Suggestions and comments:

support@ccpn.ac.uk

Issues and bug report:

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

Cite Us

Skinner, S. P. et al. CcpNmr AnalysisAssign: a flexible platform for integrated NMR analysis. *J. Biomol. NMR* (2016). doi:10.1007/s10858-016-0060-y