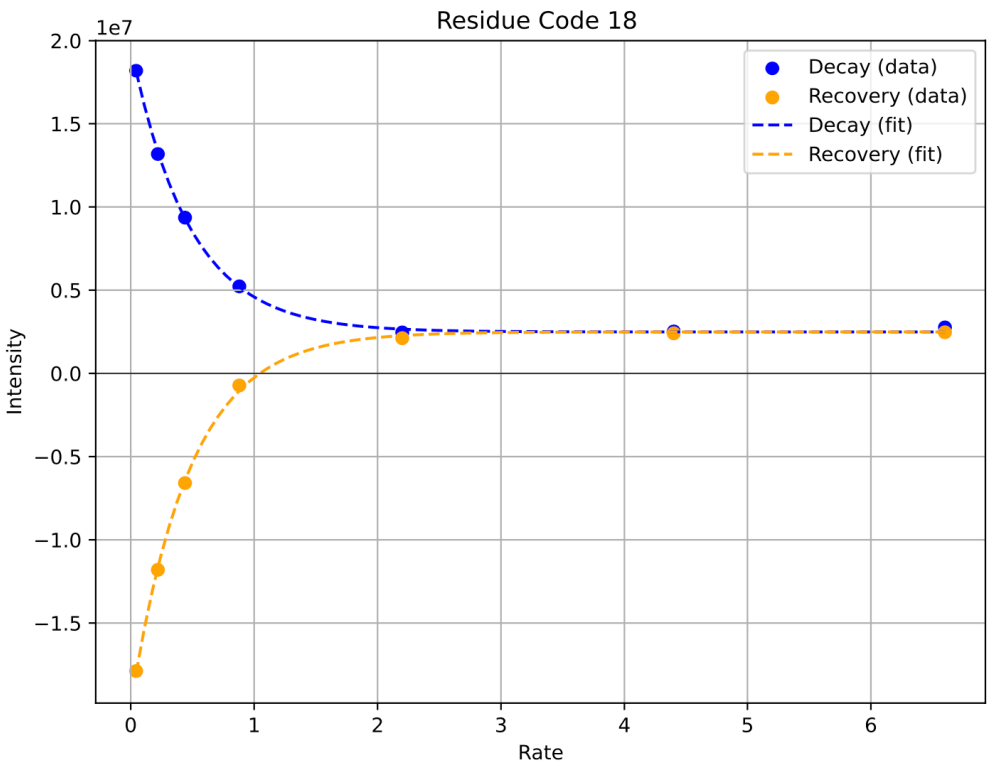
CCPN

CcpNmr Analysis Version 3

Dynamics Tutorial 2 – More rates



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 extracting R1 rates and heteronuclear NOE values from one set of experiments [1] and then determining ^{15}N CSA-dipole cross correlation rates and Rex values from another set of experiments [2-4]. 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.)

[1] O'Brien and Palmer, 2018 *JBiomolNMR* DOI: 10.1007/510858-018-0181-6

[2] Hall and Fushmann, 2003 *MRC* DOI: 10.1002/mrc.1253

[3] Wang *et al.*, 2003 *JACS* DOI: 10.1021/ja035139z

[4] Hass and Led, 2006 *MRC* DOI: 10.1002/mrc.1845

Contents:

1. Simultaneous R1 and hetNOE

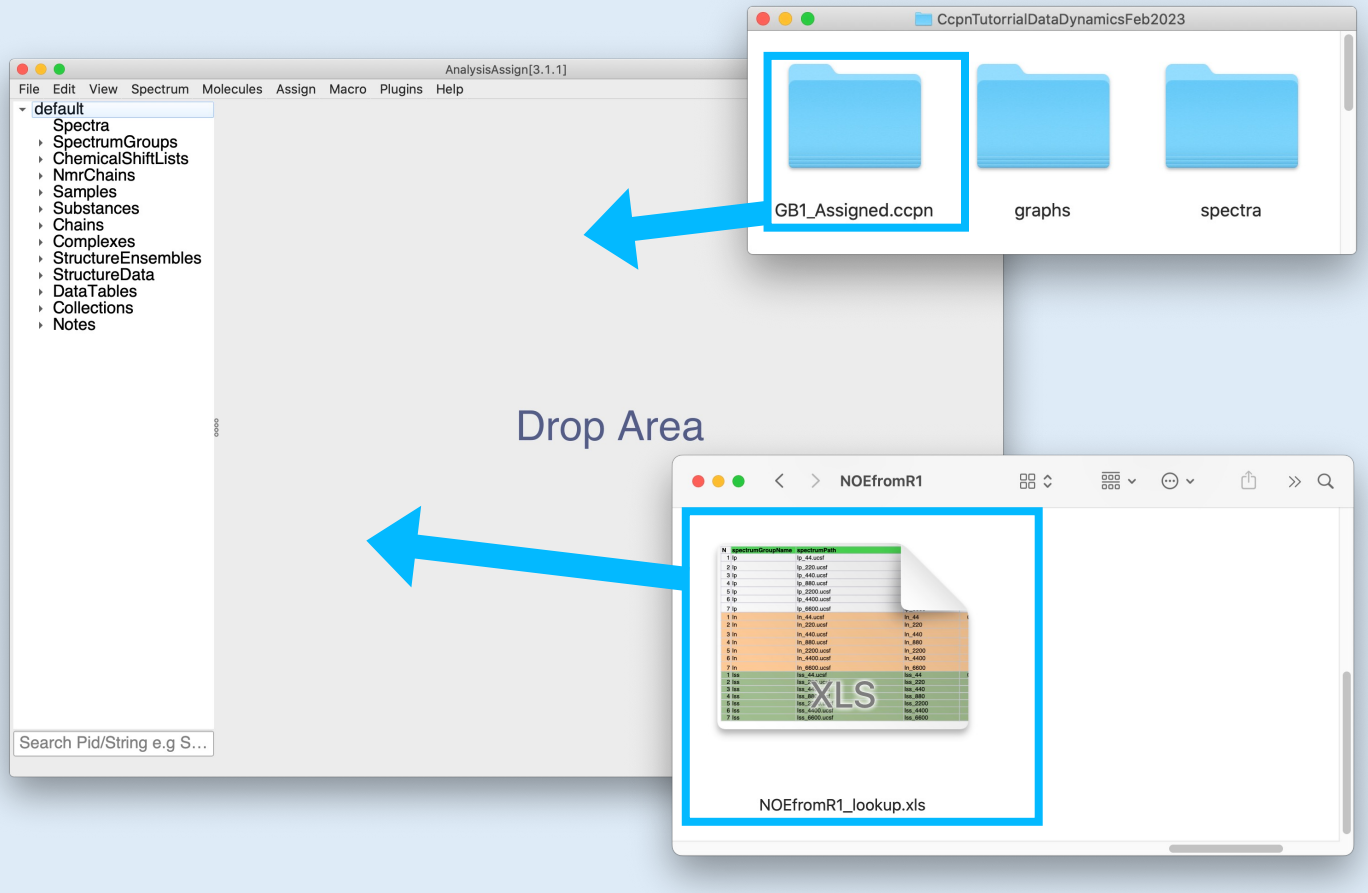
2. ^{15}N CSA-dipolar cross-correlation rates (η)

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 Simultaneous R1 and hetNOE



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**.
- Find the **NOEfromR1_lookup.xls** Excel file in the **spectra/NOEfromR1** folder and drag it into the project.

This will load three sets of spectra and place them into SpectrumGroups:

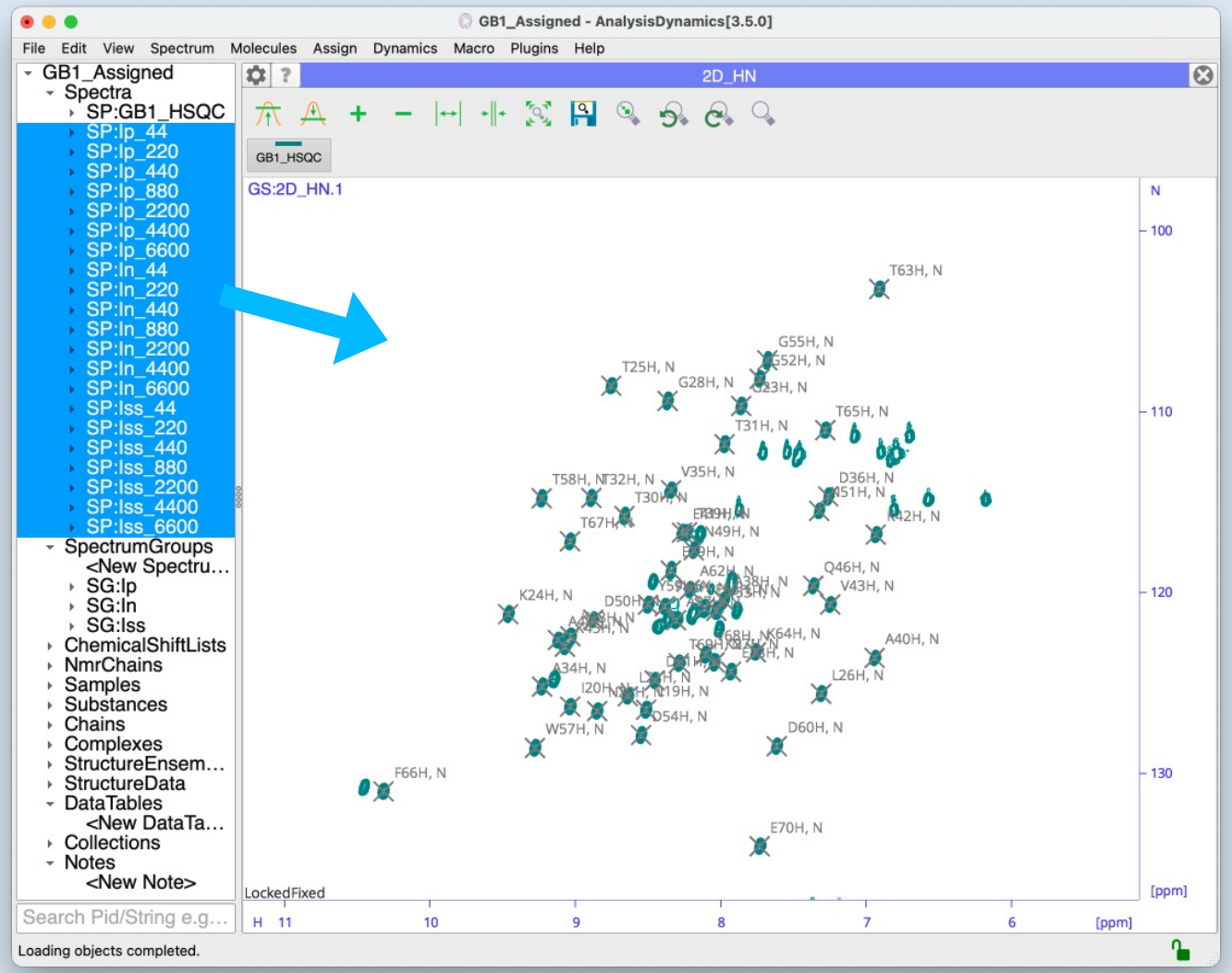
Ip: spectra starting with positive longitudinal ^{15}N magnetization

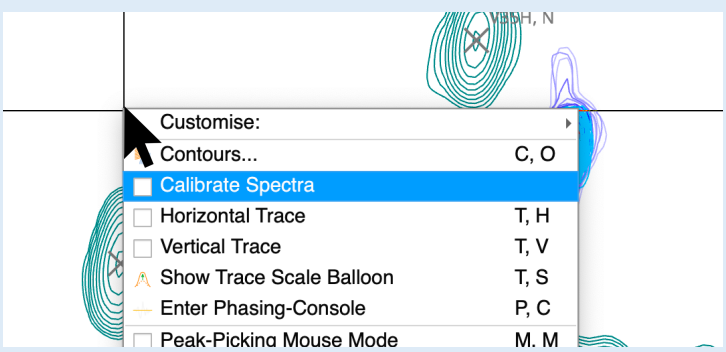
In: spectra starting with negative longitudinal ^{15}N magnetization

Iss: reference spectra starting with steady state ^{15}N magnetization

If you prefer, you can also manually load the spectra, place them into the SpectrumGroups and set the SpectrumGroup Series variables to the delay times for each spectrum.

1 Simultaneous R1 and hetNOE





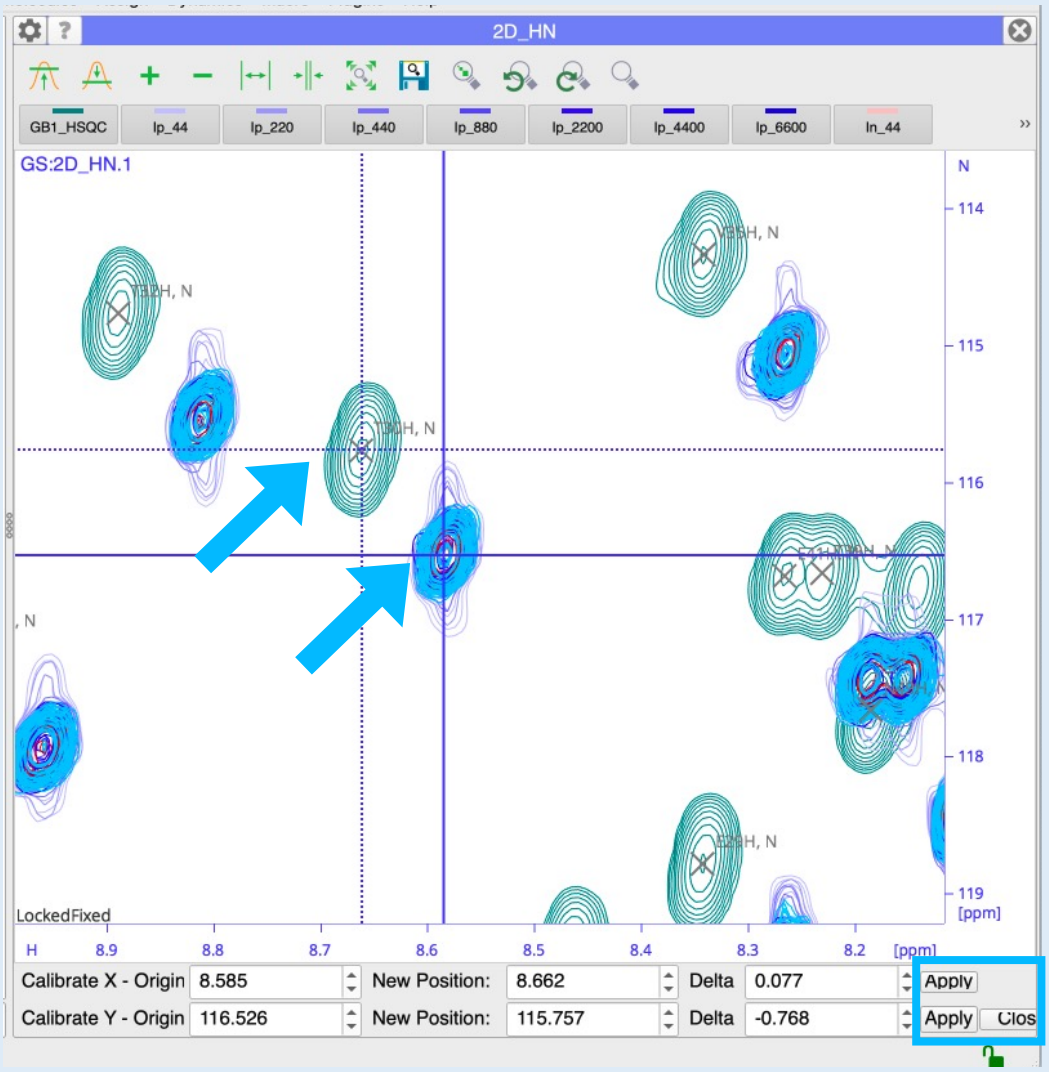
1B Check Spectrum calibration

- Select all the spectra you have just imported and drag them into the drop area.

You will notice that that the R1/NOE spectra are offset relative to the assigned GB1 spectrum.

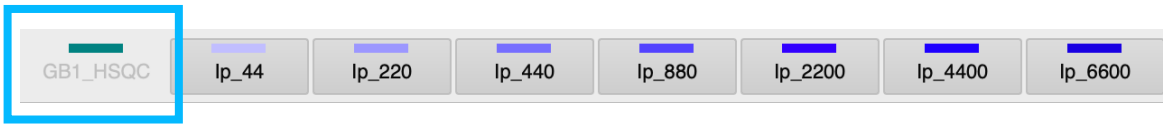
- To calibrate the spectra correctly, open up the Calibration Console by **right-clicking** on the spectrum canvas and selecting **Calibrate Spectra**

1 Simultaneous R1 and hetNOE



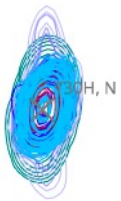
1c Calibrate spectra

- Drag the calibration lines so that the bold origin lines are on a peak in the new spectra and the dotted lines for the New Position are on an assigned peak from the reference spectrum.
- Deselect the **GB1_HSQC** spectrum in the Spectrum toolbar:

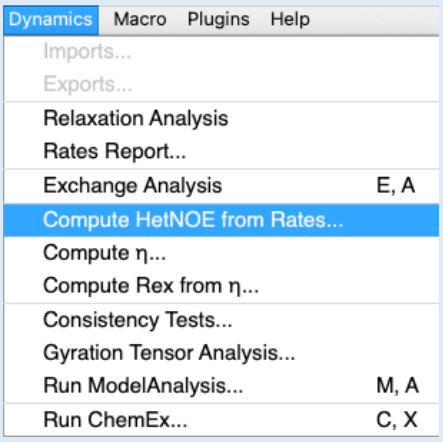


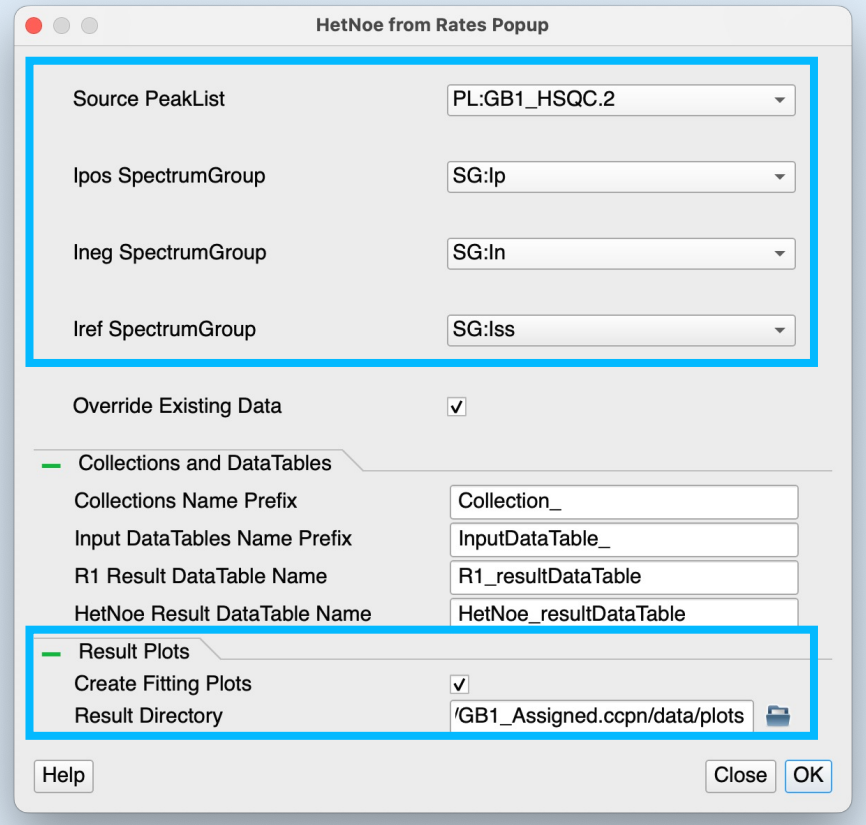
- Click **Apply** for both the X and Y dimensions in the Calibration panel.
- Click **Close** to close the Calibration panel again.
- Switch the **GB1_HSQC** spectrum back on in the Spectrum toolbar.

You will notice that that the peaks now overlap nicely:



1 Simultaneous R1 and hetNOE



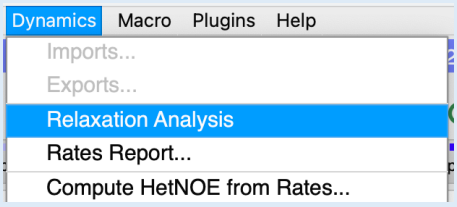


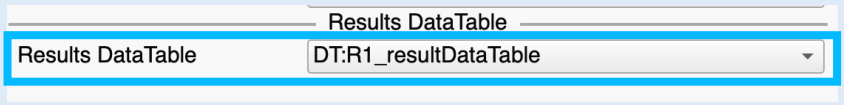
1D Calculate the rates

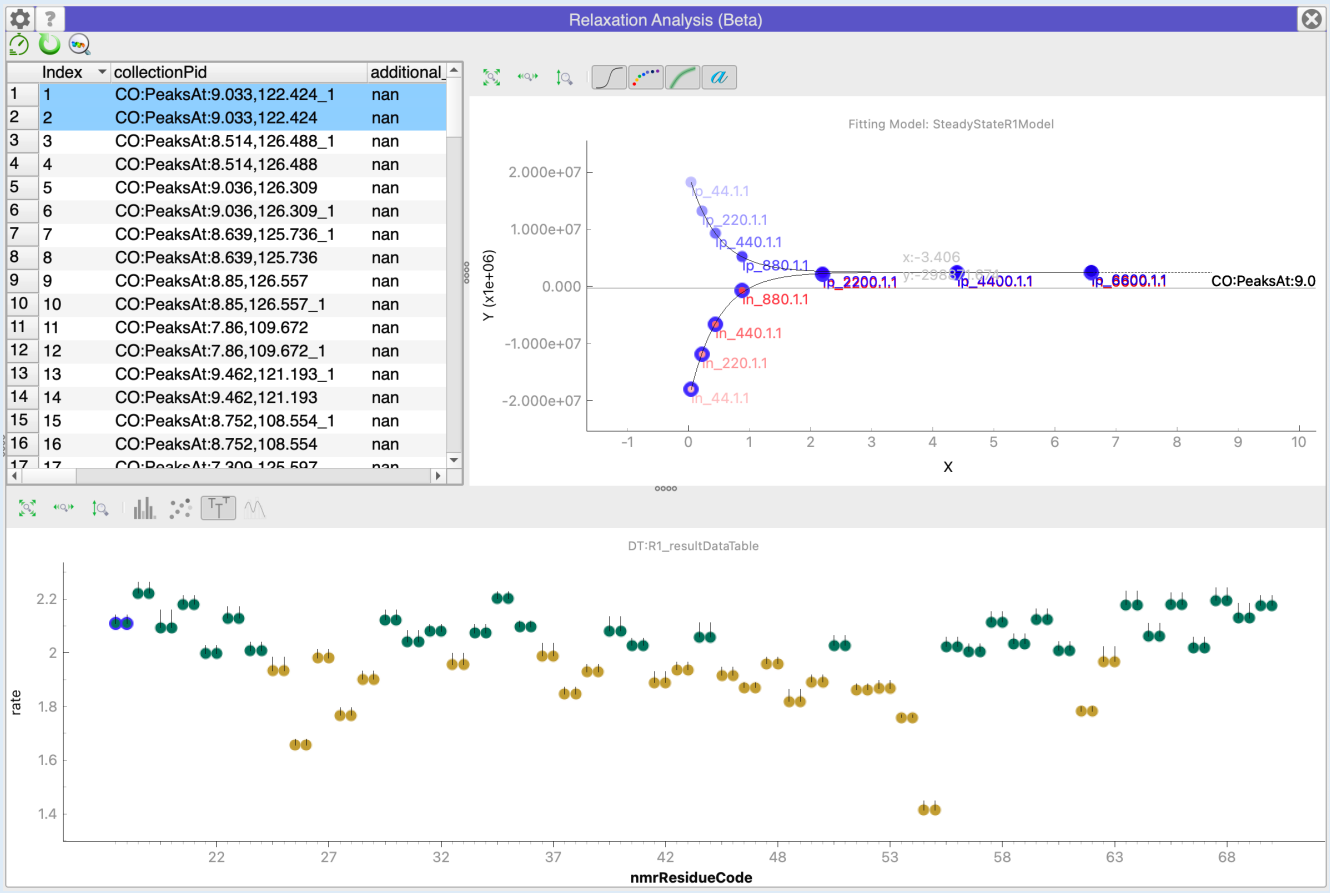
- Go to **Main Menu → Dynamics → Compute HetNOE from Rates** …
- Specify the source peak list which you want to copy over to the other spectra.
- Specify the three SpectrumGroups as shown above.
- If you wish, change the Collections and DataTables prefixes.
- Select the option to Create Fitting Plots and change the directory if you wish.
- Click **OK** to do the calculations

The program will create a Collection for each SpectrumGroup containing the peaks from those spectra. Input DataTables will be created for each SpectrumGroup. Two Result DataTables containing the R1 relaxation rates and heteronuclear NOE data will be created.

1 Simultaneous R1 and hetNOE







1E Inspect rates

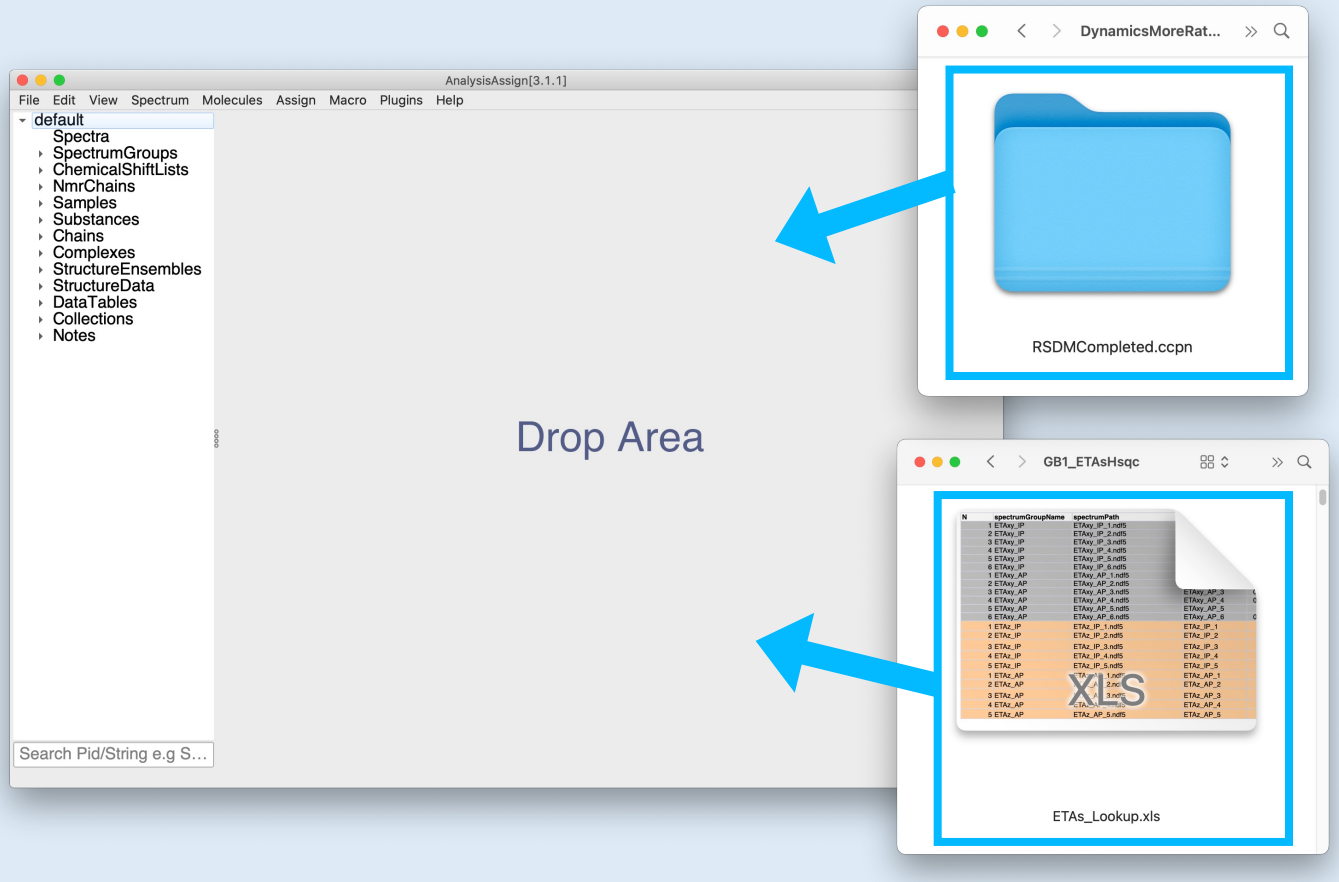
- If you wish to look at the rates or fits, open the Relaxation Analysis module with by going to **Menu → Dynamics → Relaxation Analysis**
- Select one of the Results DataTables in the **Settings Setup** tab.
- Refine the graph appearance in the **Appearance** tab if you wish (e.g. you can switch from a scatter to a bar chart etc.).
- Double-clicking on an item in the table or graph will select the peak in the spectrum but not (yet) navigate to it.

Note that each T1 rate is determined twice.

- Select both rates together in the table or graph to see the joint fit in the fitting graph.

You can also view these double fit graphs in pdf format in the *MyProject.ccpn*/data/plots directory of your project.

2 ^{15}N CSA-dipolar cross-correlation rates (η)



2A Load the data

- Open the **RSDMCompleted.ccpn** project in the tutorial data directory.
- Drag the **ETAs_Lookup.xls** Excel lookup file in the **spectra/GB1_ETAsHsqc** folder into the project.

This will add the ^{15}N CSA-dipolar cross relaxation rate (η) experiments. Both the transverse (η_{xy}) and longitudinal (η_z) rates have been measured using IPAP spectra. The spectra are therefore grouped into four SpectrumGroups:

ETAxy_IP: the inphase component of the transverse (η_{xy}) rate

ETAxy_AP: the antiphase component of the transverse (η_{xy}) rate

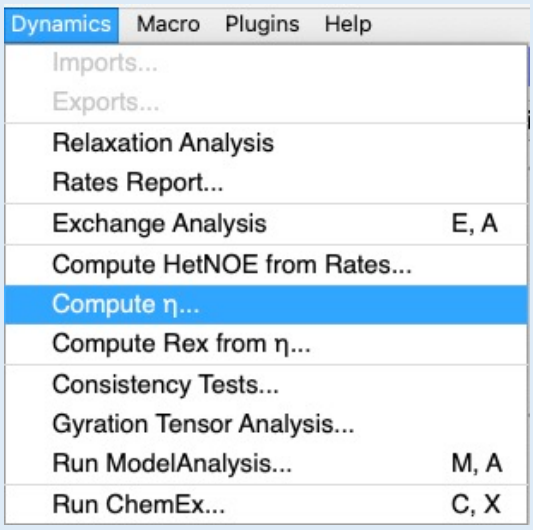
ETAz_IP: the inphase component of the longitudinal (η_z) rate

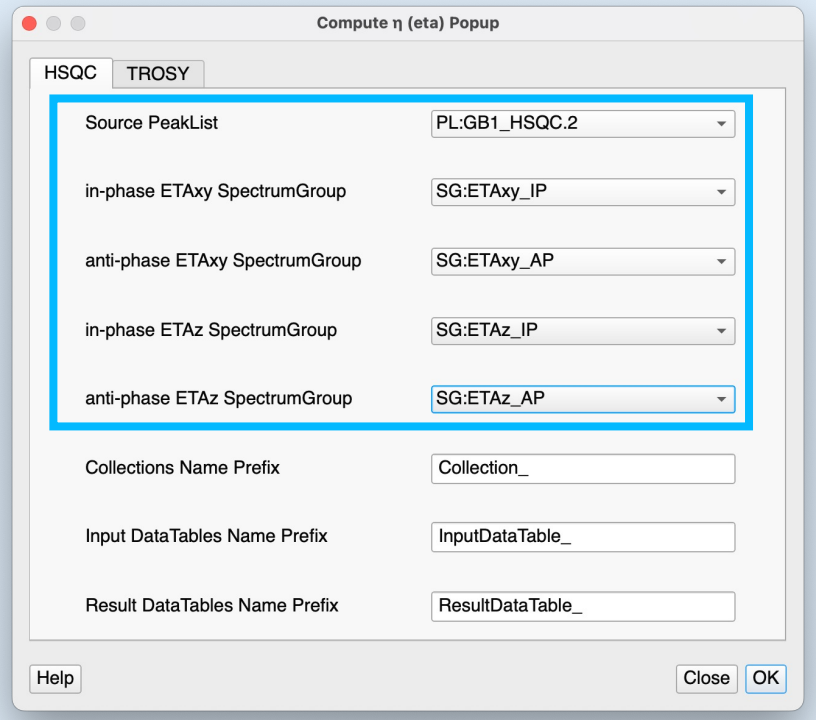
ETAz_AP: the antiphase component of the longitudinal (η_z) rate

You can see the delay times in the SpectrumGroup popups or the Excel lookup file.

A set of Trosy-based spectra are also available (**spectra/GB1_ETAsTrosy**) should you want to try these out.

2 ^{15}N CSA-dipolar cross-correlation rates (η)



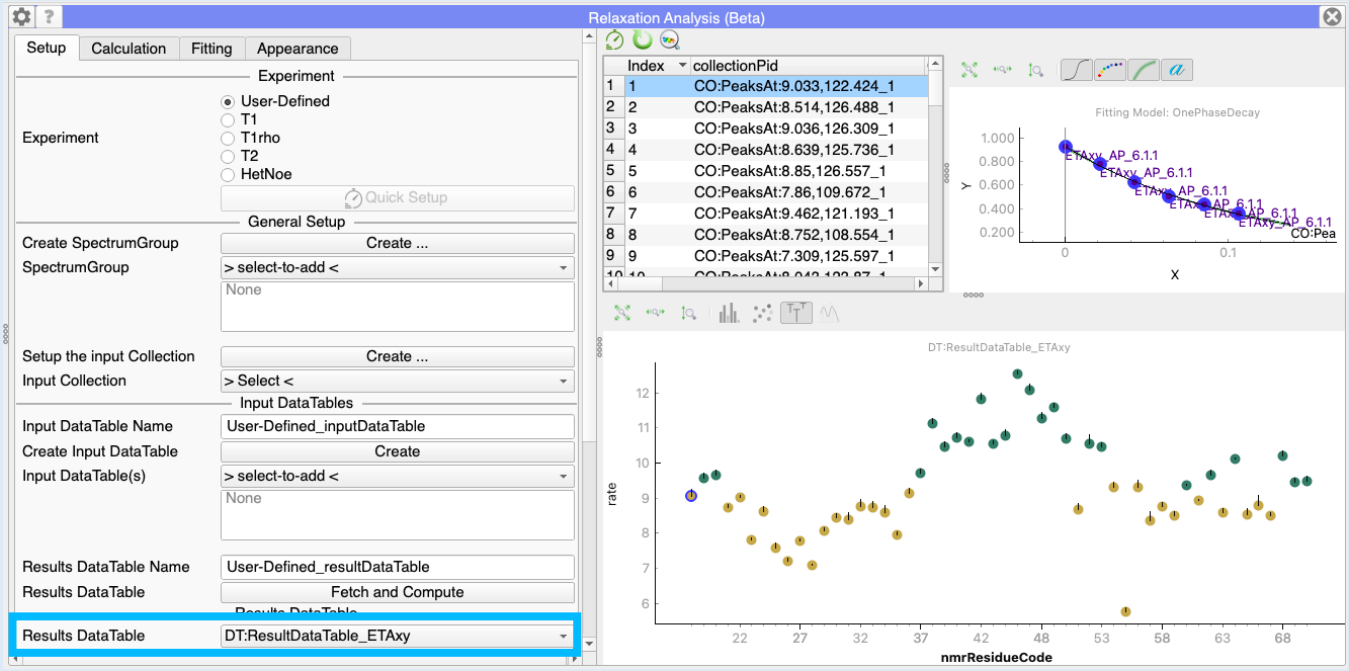


2B Calculate the rates

- Go to **Main Menu → Dynamics → Compute η …**
- Specify the source peak list which you want to copy over to the other spectra.
- Specify the four η/ETA experiment SpectrumGroups as shown above.
- If you wish, change the Collections and DataTables prefixes.
- Click **OK** to do the calculations

The program will create a Collection for each SpectrumGroup containing the peaks from those spectra. Input DataTables will be created for each SpectrumGroup. Two Result DataTables contain the transverse and longitudinal ^{15}N CSA-dipolar cross correlation rates.

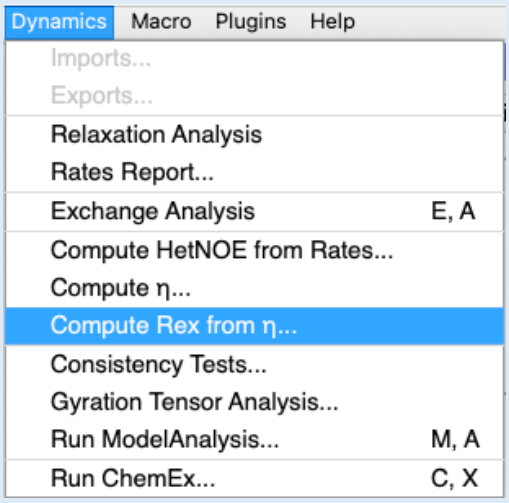
2 ^{15}N CSA-dipolar cross-correlation rates (η)

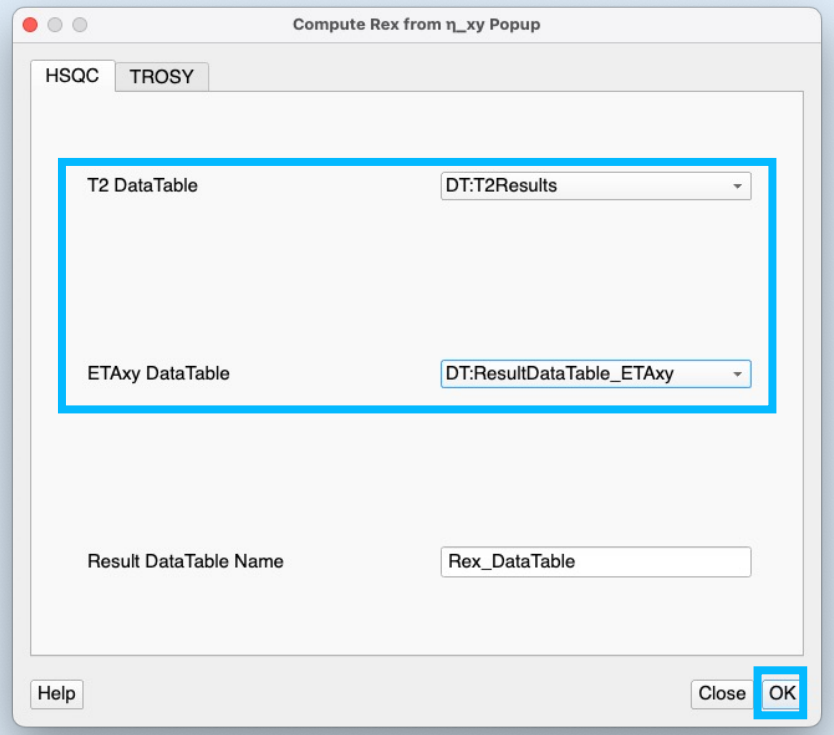


2C Inspect rates

- If you wish to look at the rates or fits, open the Relaxation Analysis module with **RA** or by going to **Menu → Dynamics → Relaxation Analysis**
- Select one of the ETA Results DataTables in the Settings **Setup** tab.
- Refine the graph appearance in the **Appearance** tab if you wish (e.g. you can switch from a scatter to a bar chart).
- Go through the table to look at the individual fits if you wish.

2 ^{15}N CSA-dipolar cross-correlation rates (η)



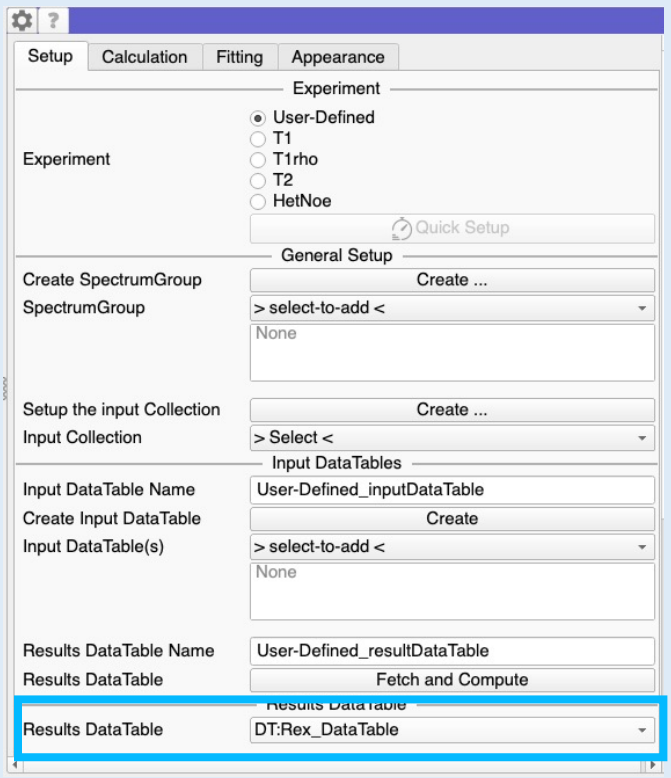


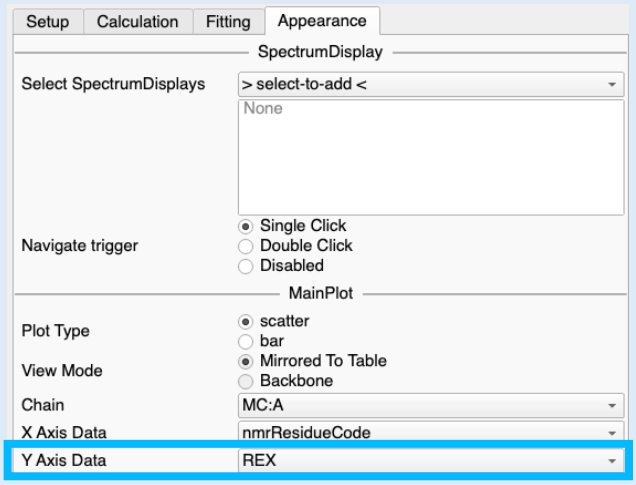
2D Calculate R_{ex}

Using the T2 and η_{xy} rates you calculate R_{ex}.

- Go to Main Menu ⟶ **Dynamics ⟶ Compute Rex from η …**
- Select the T2 and η_{xy} Results DataTables and click **OK**.

2 ^{15}N CSA-dipolar cross-correlation rates (η)

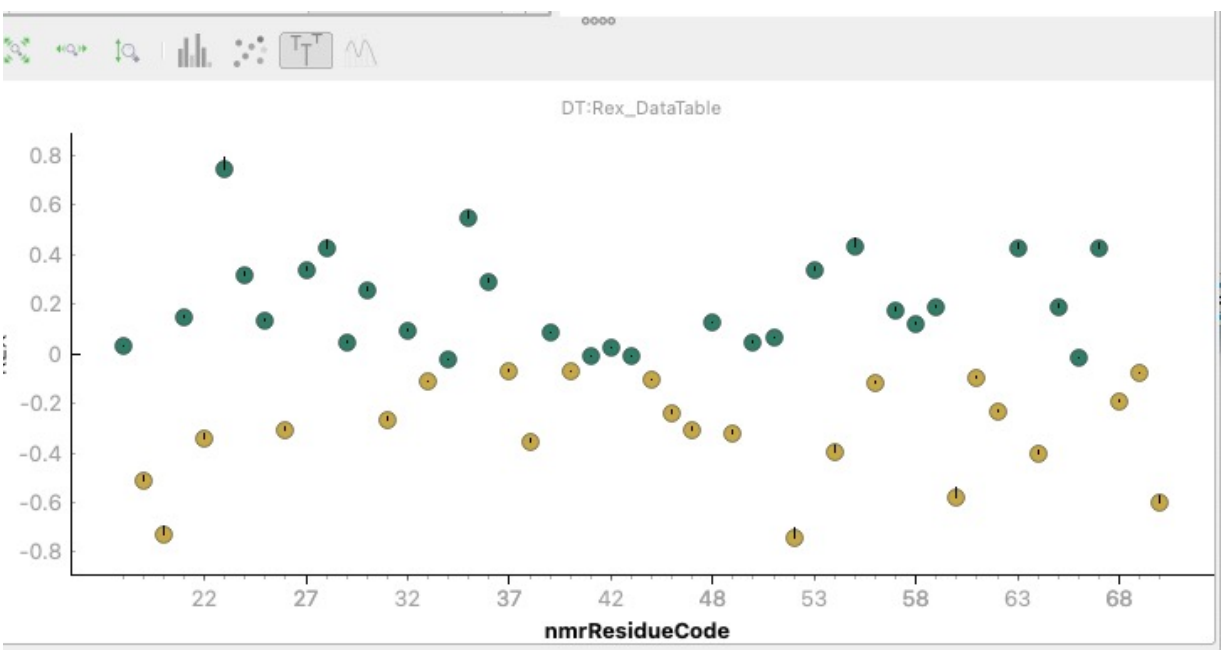




2E Inspect R_{ex}

- If you wish to look at the rates or fits, open the Relaxation Analysis module with **RA** or by going to **Menu → Dynamics → Relaxation Analysis**
- Select the Rex Results DataTable in the Settings **Setup** tab.
- Select **Rex** for the **Y axis Data** in the **Appearance** tab.

Note that the results for GB1 shows random scatter around 0. This suggests that there GB1 does not undergo exchange.



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

Cite Us

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