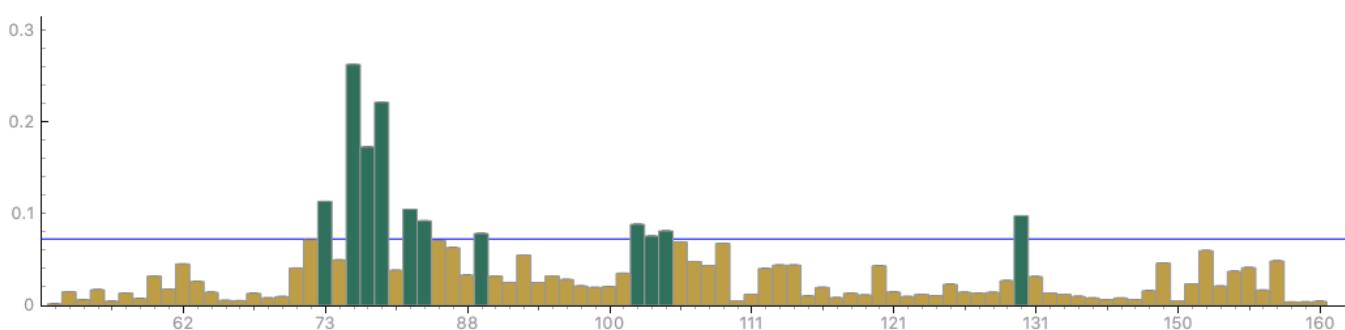
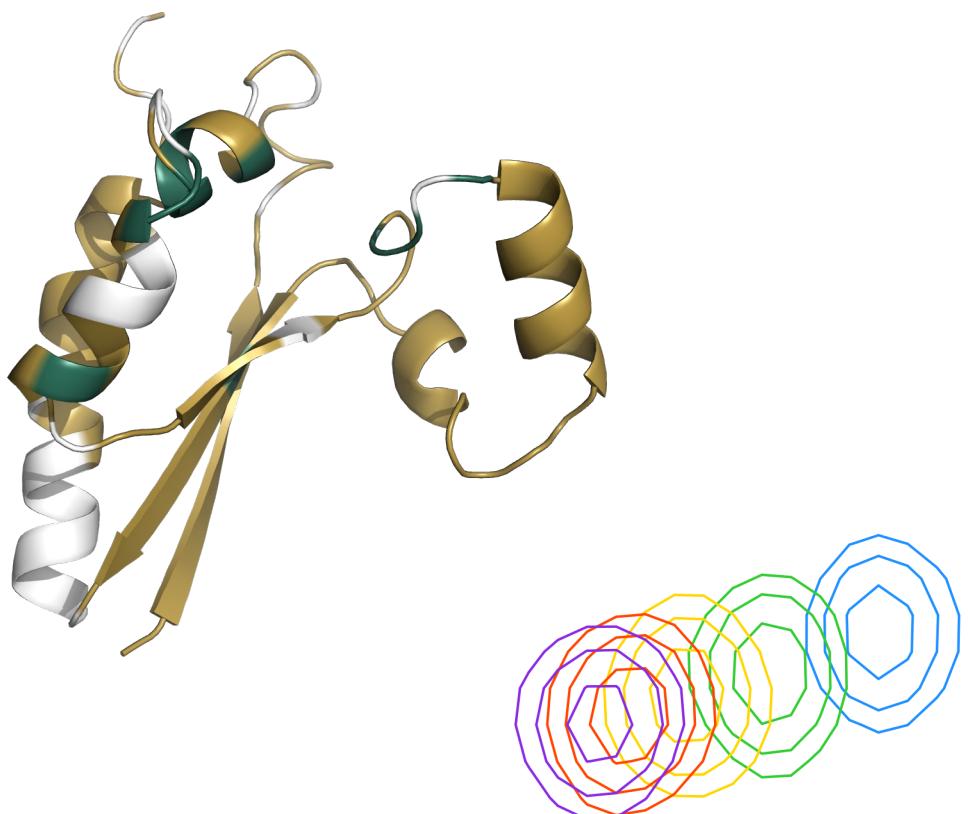


Chemical Shift Perturbation Tutorial



Introduction

This tutorial is designed to show you how to use CcpNmr Analysis Version 3.1.1 to analyse the chemical shift perturbations caused by a protein interacting with a binding partner (in this case an RNA molecule) and map the interaction onto the structure of the protein.

The tutorial uses ^1H - ^{15}N HSQC spectra of ^{15}N labelled TSTAR protein in complex with RNA. We are grateful to Dr Cyril Dominguez for making this data available to us. The original paper describing this work is [Feracci, M et al. \(2016\) Nature Communications 7, 20355](#).

Contents:

1. Open Project
2. Pick Peaks and Copy Assignments
3. Group Spectra
4. Fit and Map Peaks
5. View in PyMOL

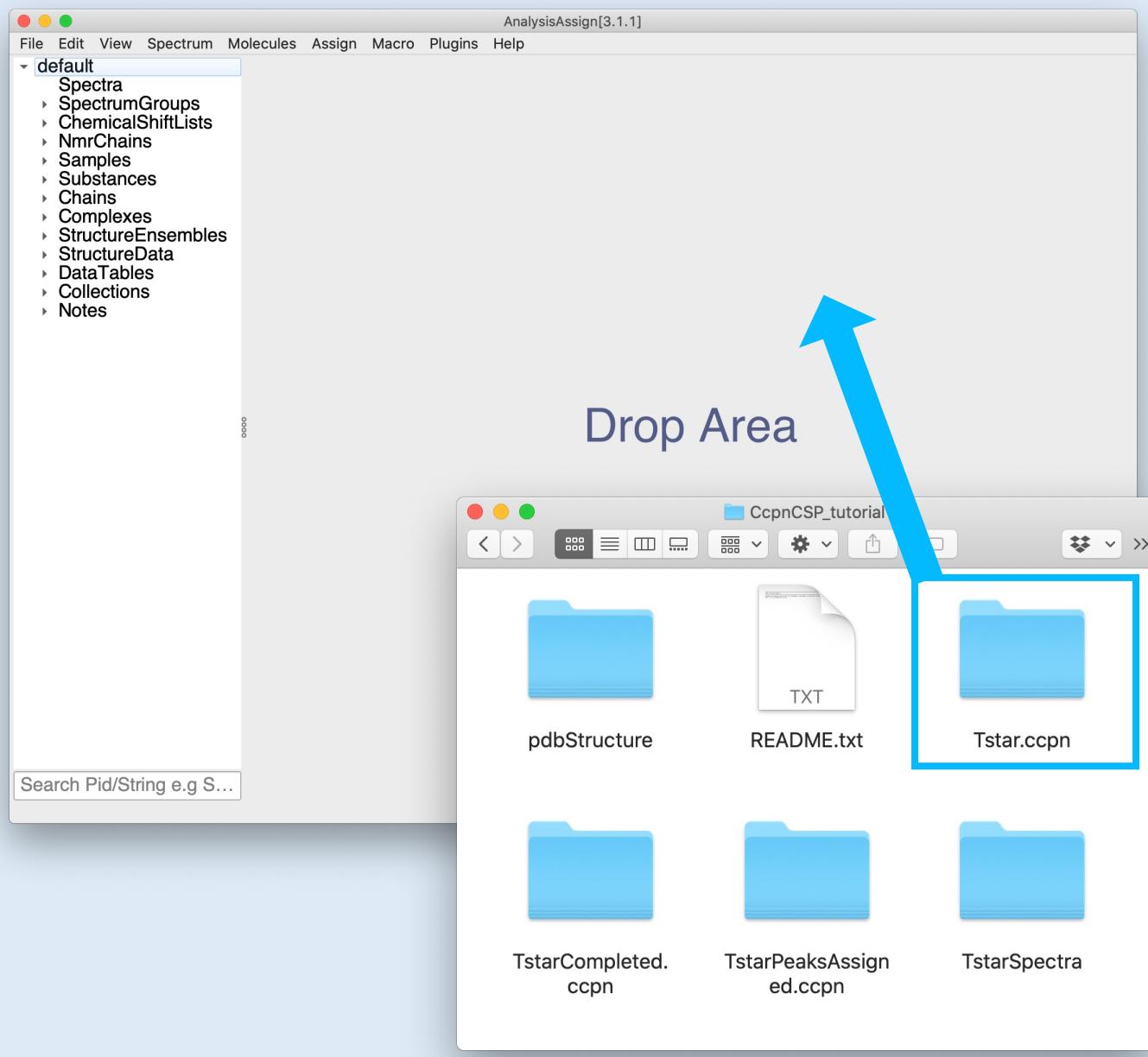
Start CcpNmr Analysis V3

Apple users by double clicking the icon  *CcpNmr Analysis*

Linux users by using the terminal command: *bin/assign*

Windows users by double-clicking on the *assign.bat* file

Open Project



1A Open Project

CcpNmr projects have an extension of type **projectName.ccpn**.

- Find the project directory **Tstar.ccpn** in the Chemical Shift Perturbation Tutorial directory and drag and drop it into the program.

The Tstar project will be loaded in a new window.

You will see five spectra, displayed as:

Tstar-free (blue)

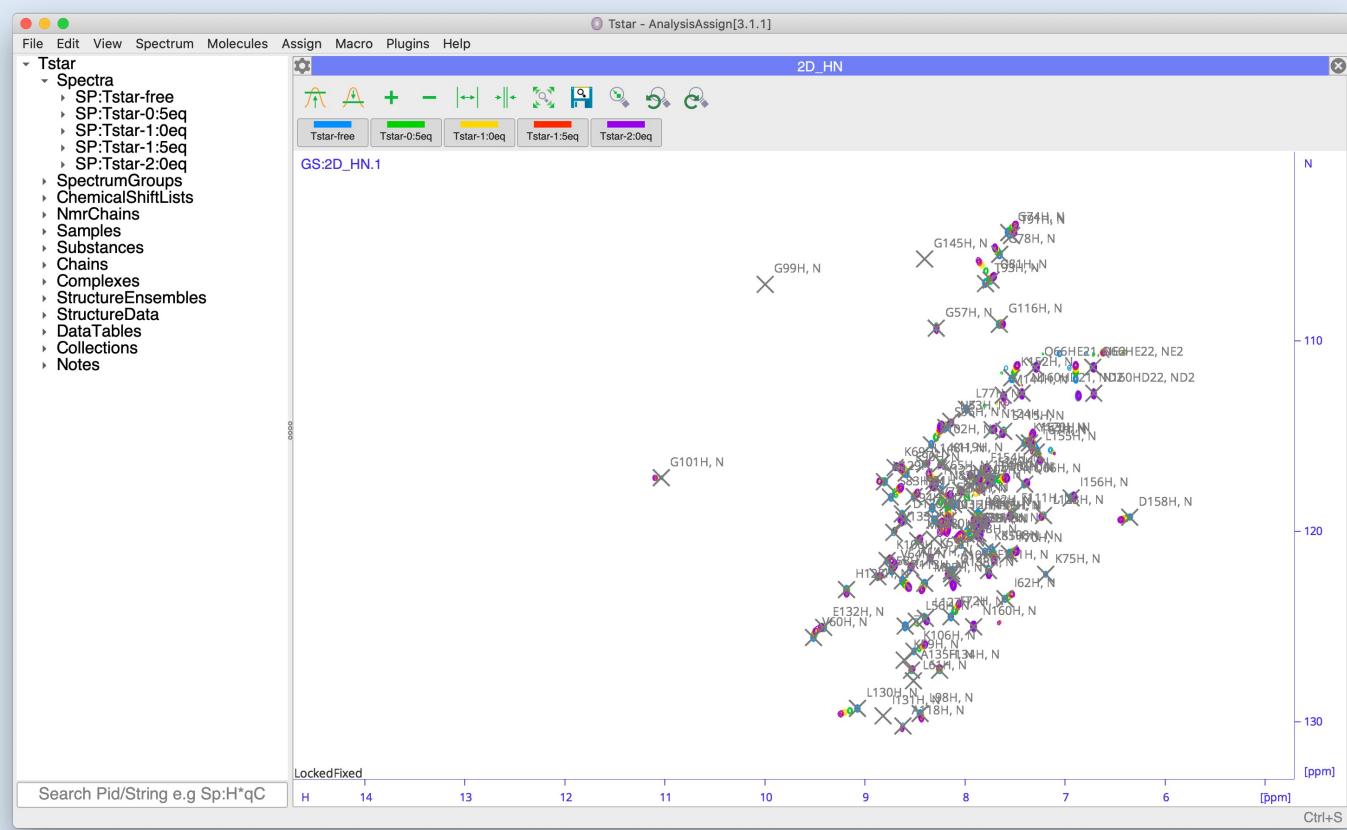
Tstar-0:50eq (green)

Tstar-1:00eq (yellow)

Tstar-1:50eq (red)

Tstar-2:00eq (purple)

Open Project



Getting started, basic operations

Sidebar

All spectra and peak lists are located in the sidebar. Double-click on an item to open its Properties popup.

Display

A display can contain multiple overlaid spectra. To show/hide a single spectrum, click on its toolbar button. (See next page.)

Mouse

- Pan → Left-click and drag in display
- Zoom in/out → Scroll wheel in display
- Context menu → Right-click
- Select a peak → Left-click on a peak symbol X
- Move a peak → select first, then middle- or right-drag

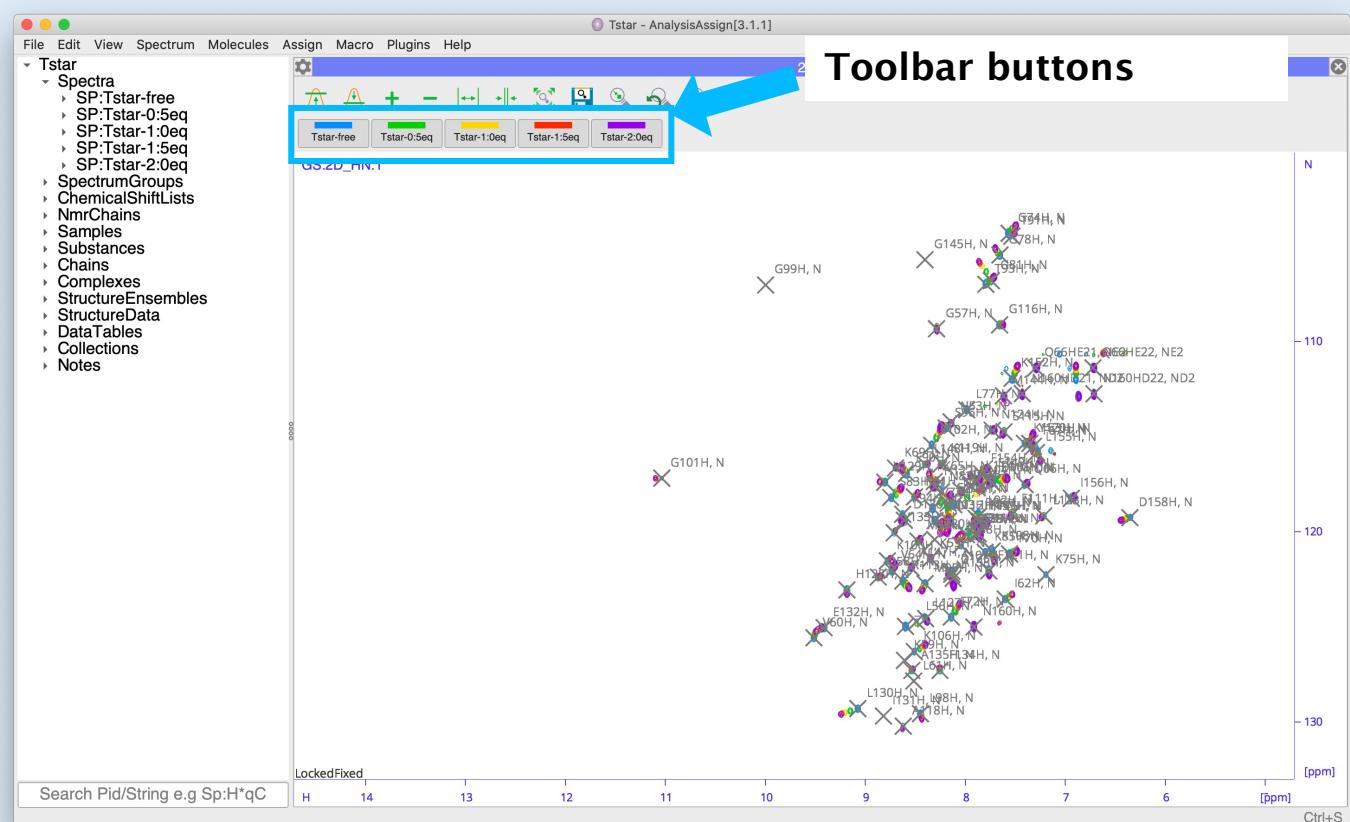
Shortcuts

The program uses several shortcuts, example **CL** for copying a peak list. You will need to press the first (lowercase) letter on your keyboard e.g. **c**, followed by the second letter, e.g. **I**.

For more commands and operations:

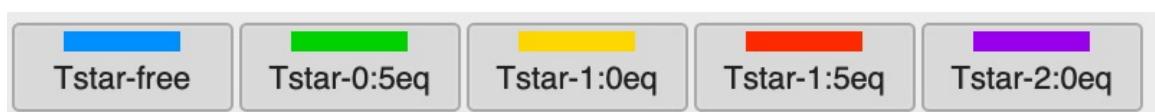
Main Menu -> *Help* -> *Tutorial (Beginners)* or *Show Shortcuts*

Open Project

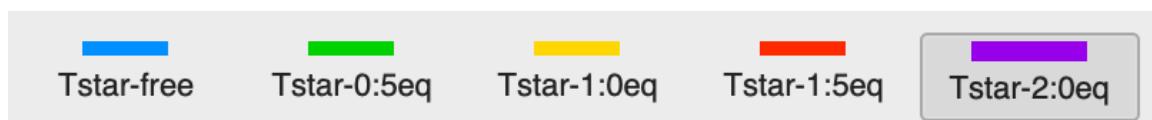


1B Toggle off all spectra apart from Tstar-2:00eq on the toolbar.

- Click on the toolbar button of the first four spectra (*a, b, c, d*).
- The buttons will change from on (spectrum visible on the display, “toggle On”) to off (spectrum hidden on the display, “toggle Off”)

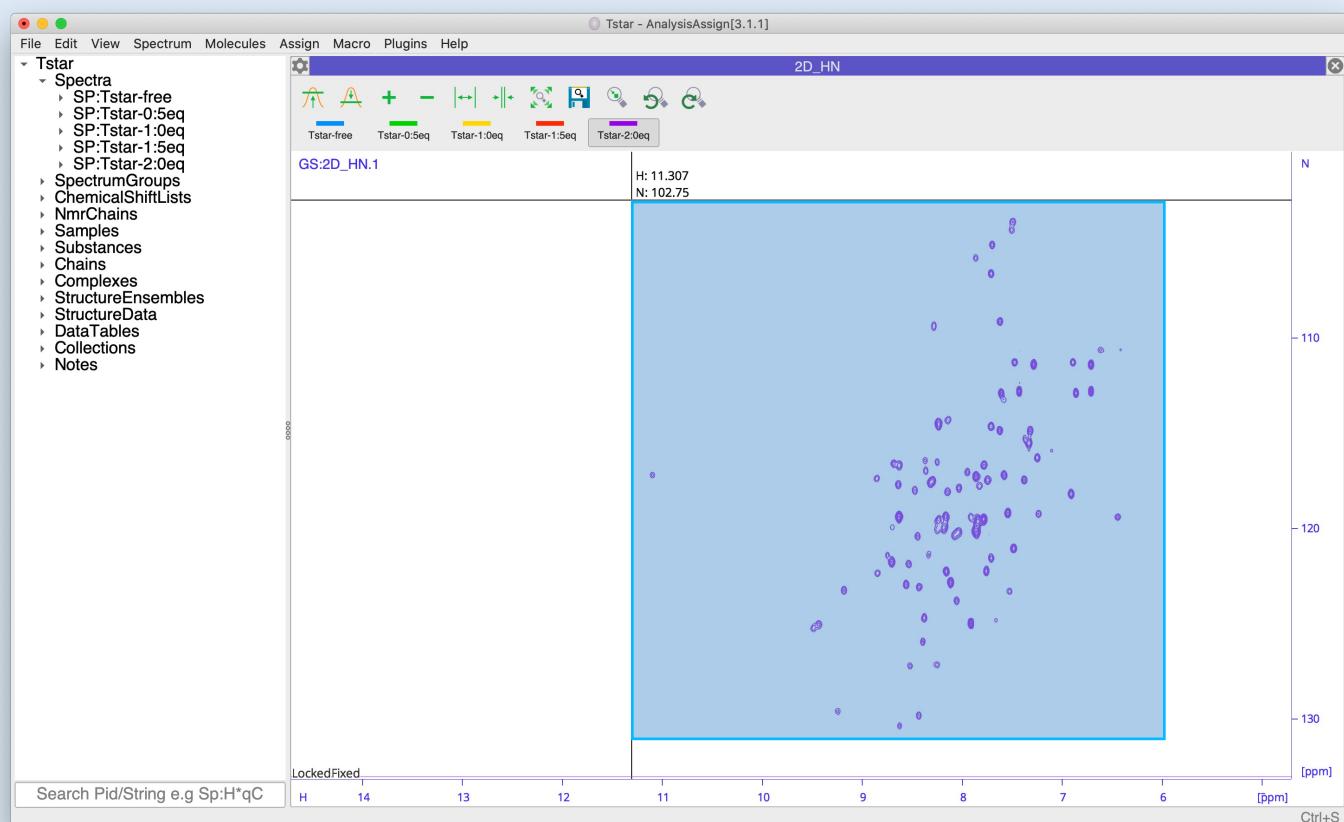


1 Click to toggle spectra on/off



Pick Peaks and Copy Assignments

Currently only the free Tstar spectrum has a peak list. The next task is to pick and assign all the peaks in the spectra containing Tstar with bound RNA. To do this, we will first pick the peaks in the other spectra and then copy the peak assignments from the free Tstar spectrum peaks over to the peaks in the other spectra.



2A Pick Peaks

With only the **Tstar-2:0eq** spectrum showing:

- Hold down **Shift+Ctrl (Cmd on a Mac)** and drag a box across all the peaks to pick them.

Repeat this for each of the other three unpicked spectra:

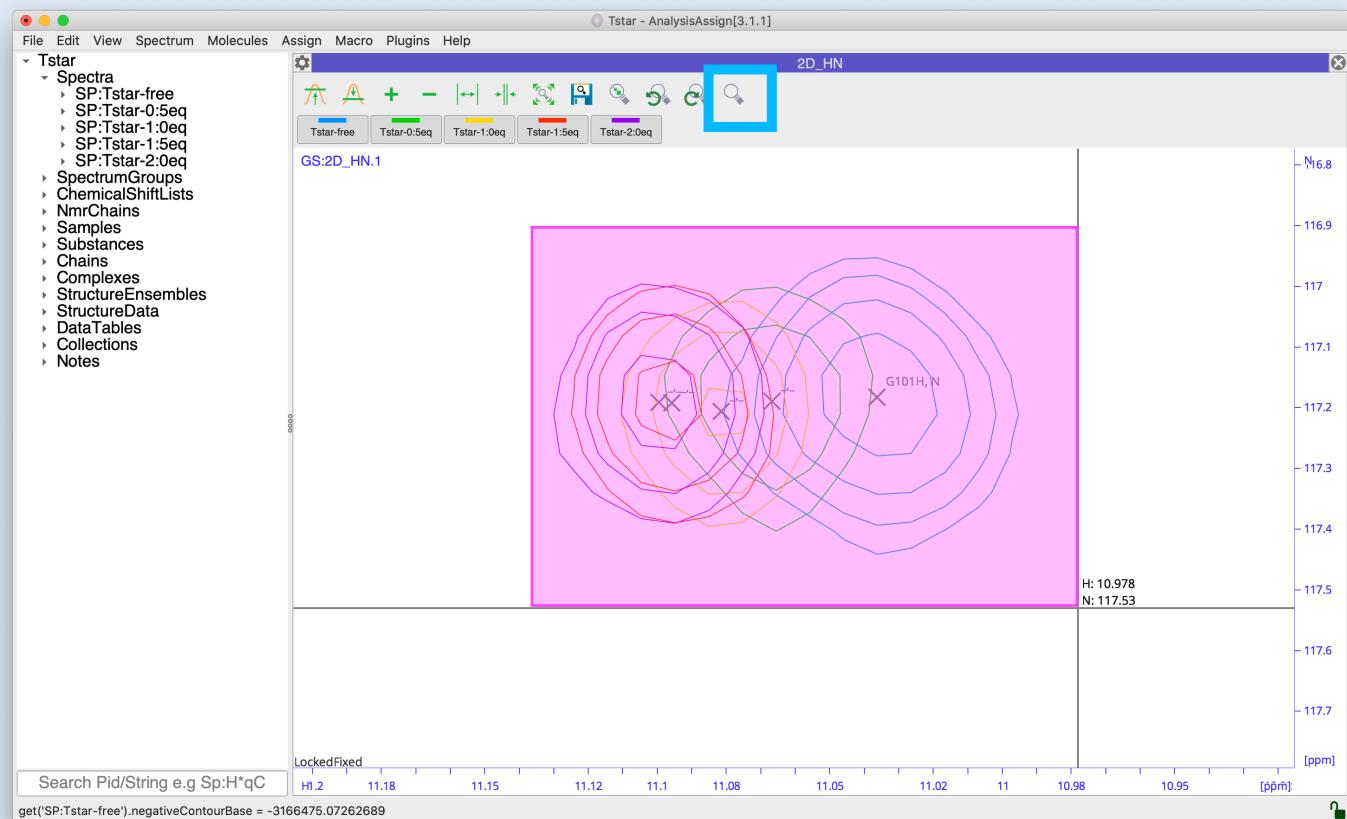
Tstar-0:5eq (green)

Tstar-1:0eq (yellow)

Tstar-1:5eq (red)

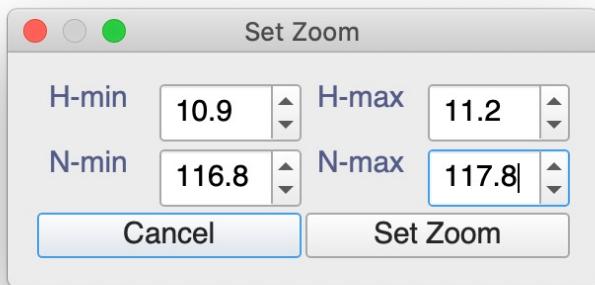
Note that using the shortcut **TabTab**, i.e. pressing the **Tab** (\rightarrow) button twice, will automatically display the next spectrum and hide all others. It is a convenient way to move between spectra in a SpectrumDisplay.

Pick Peaks and Copy Assignments



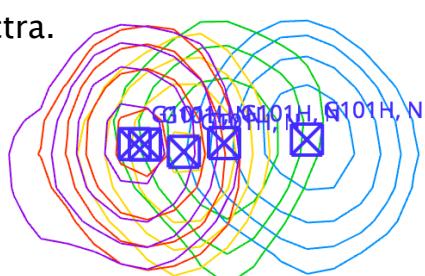
2B Find Peak Trajectories and Copy Assignments

- Use the shortcut **Tab+A** to switch all spectra back on.
- Zoom into an individual peak group, e.g. **G101** at ~ 11.0/117.2 ppm.
If you wish, you can use the SpectrumDisplay Zoom button or the shortcut **SZ** to bring up the Zoom pop-up to navigate to this region of the spectrum:

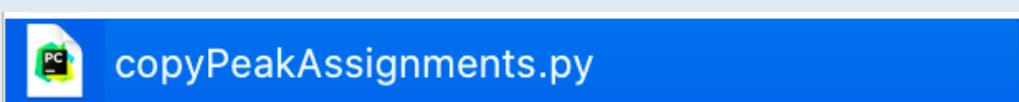
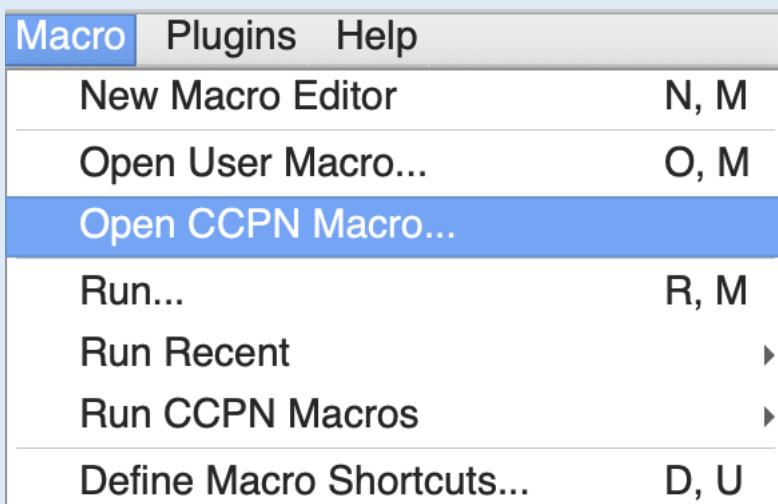


- Hold down **Ctrl** (**Cmd** on a Mac) and drag a box across all the peaks to select them.
- Go to **Main Menu -> Macro -> Run CCPN Macros -> copyPeakAssignments**

This will copy the Assignment from the peak in the Tstar-free spectrum to the peaks in all the other spectra.



Pick Peaks and Copy Assignments



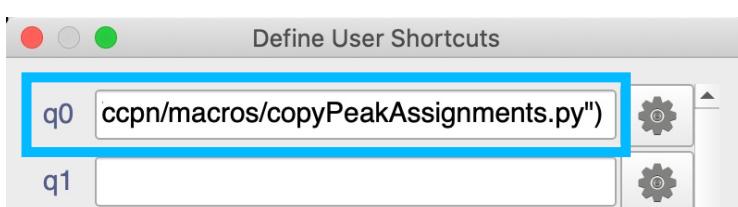
2C Associate Copy Peak Assignments macro with a shortcut

To repeat the procedure in **Section 2B** for other peak groups, it is convenient to link the macro to the keyboard shortcut.

- Go to **Main Menu → Macro → Open CCPN...**
- Select the **copyPeakAssignments.py** file and click on **Open**.

- Click on the  symbol to associate the macro with a shortcut.

The program will automatically add the path to the next available free slot:



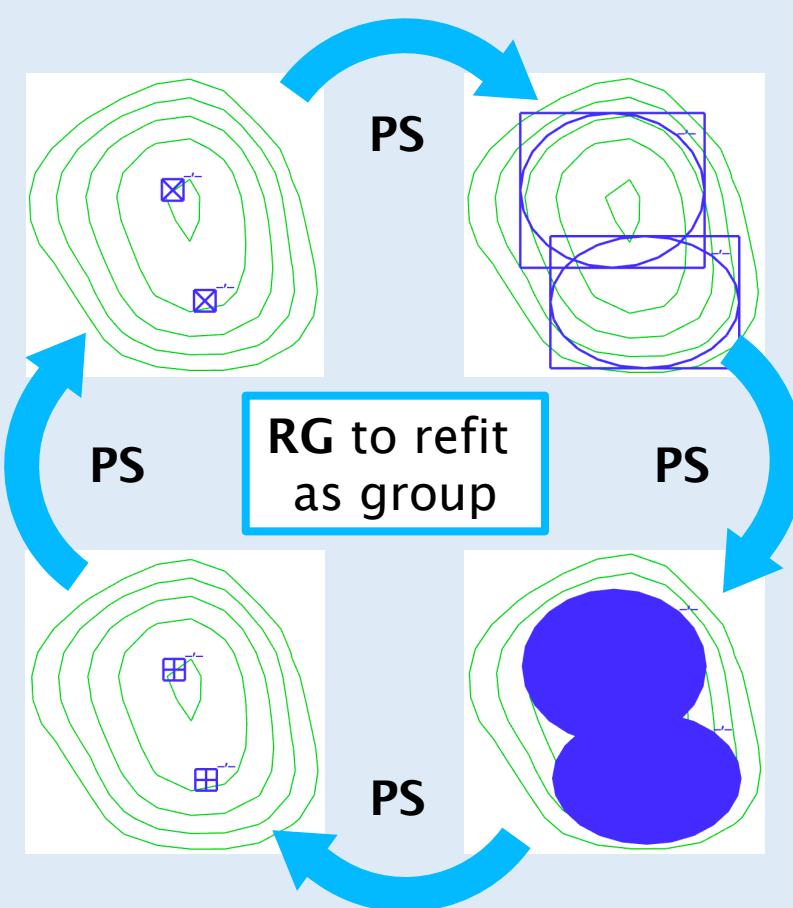
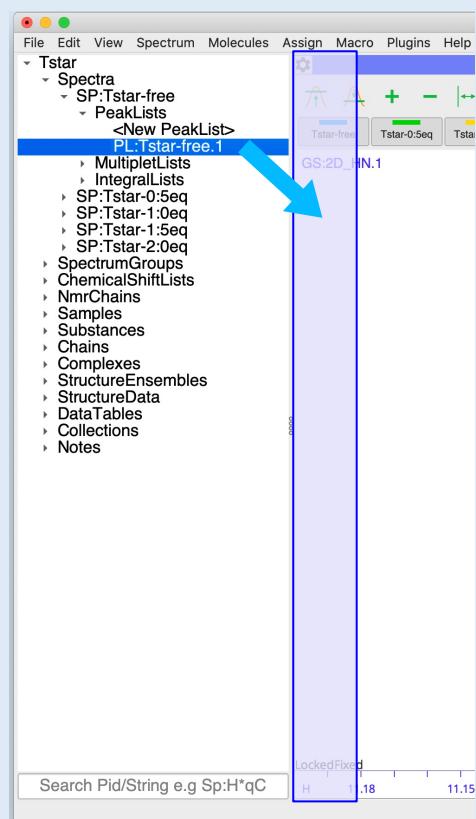
- Remember the key combination (e.g. **q0**) or choose a different one by cutting and pasting the file path elsewhere (**q1** is much easier to type than **q0!**), then click on **Save and Close** and close the **MacroEditor** module.

Now you can continue to identify further peak trajectories, selecting the peaks which belong together and using **q0** (or whichever shortcut you chose) to copy the peak assignments from the free Tstar spectrum to the other spectra.

Sometimes, when peaks are close or overlap it can be hard to work out exactly which peak moves where – see **Section 2D** for some tips and tricks.

No need to do all the peaks – we have a pre-prepared project for the next stage!

Pick Peaks and Copy Assignments



2D Tips and Tricks for picking peaks and copying assignments

- Drag the **PL:Tstar-free.1** PeakList into the Drop Area
- Now double-click on each peak one by one to find their trajectories across the spectra and copy the assignments over.

Note that some peaks simply disappear in the bound spectra.

If two or more peaks are overlapped or very close together:

- Place any missing peaks with **Shift+Ctrl (Cmd on Mac)** + right-click
- Refit a group of overlapped/close peaks with shortcut **RG**. (**RP** refits single peaks)
- Cycle between different peak symbols with **PL** to be able to view filled linewidths. This gives an indication of how well the fit has worked. Repeat **RG** if necessary.

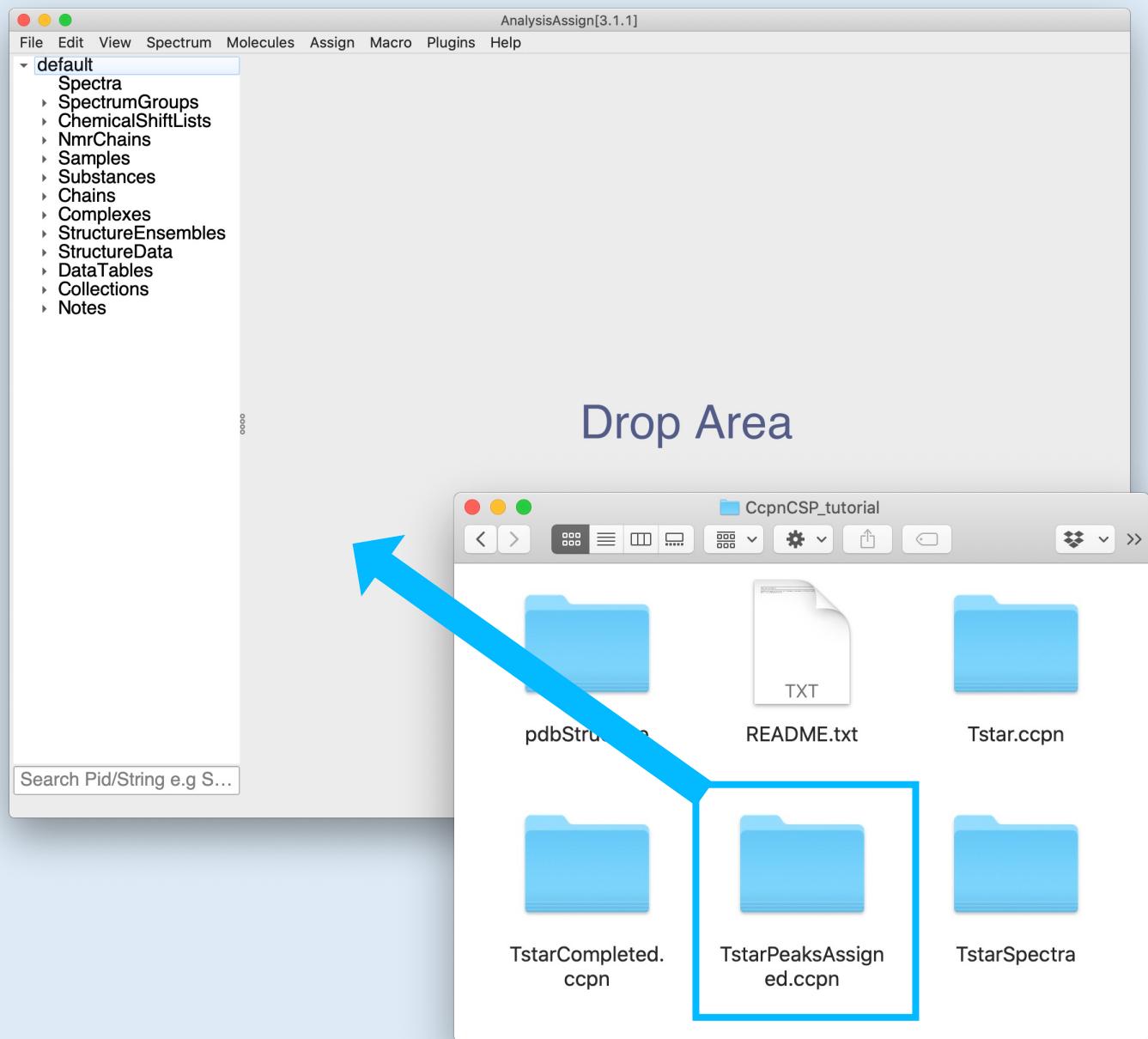
Sometimes it is most useful to place the peaks by hand:

- Move a peak by selecting it and the middle- or right-dragging it.

Useful shortcuts to navigate between spectra:

- Tab-Tab (press →→ twice): Displays the next spectrum and hides all others
- Tab-A : Displays all spectra
- Tab-X : Reverts displayed spectra
- Tab-Z : Toggles all spectra off

Group Spectra

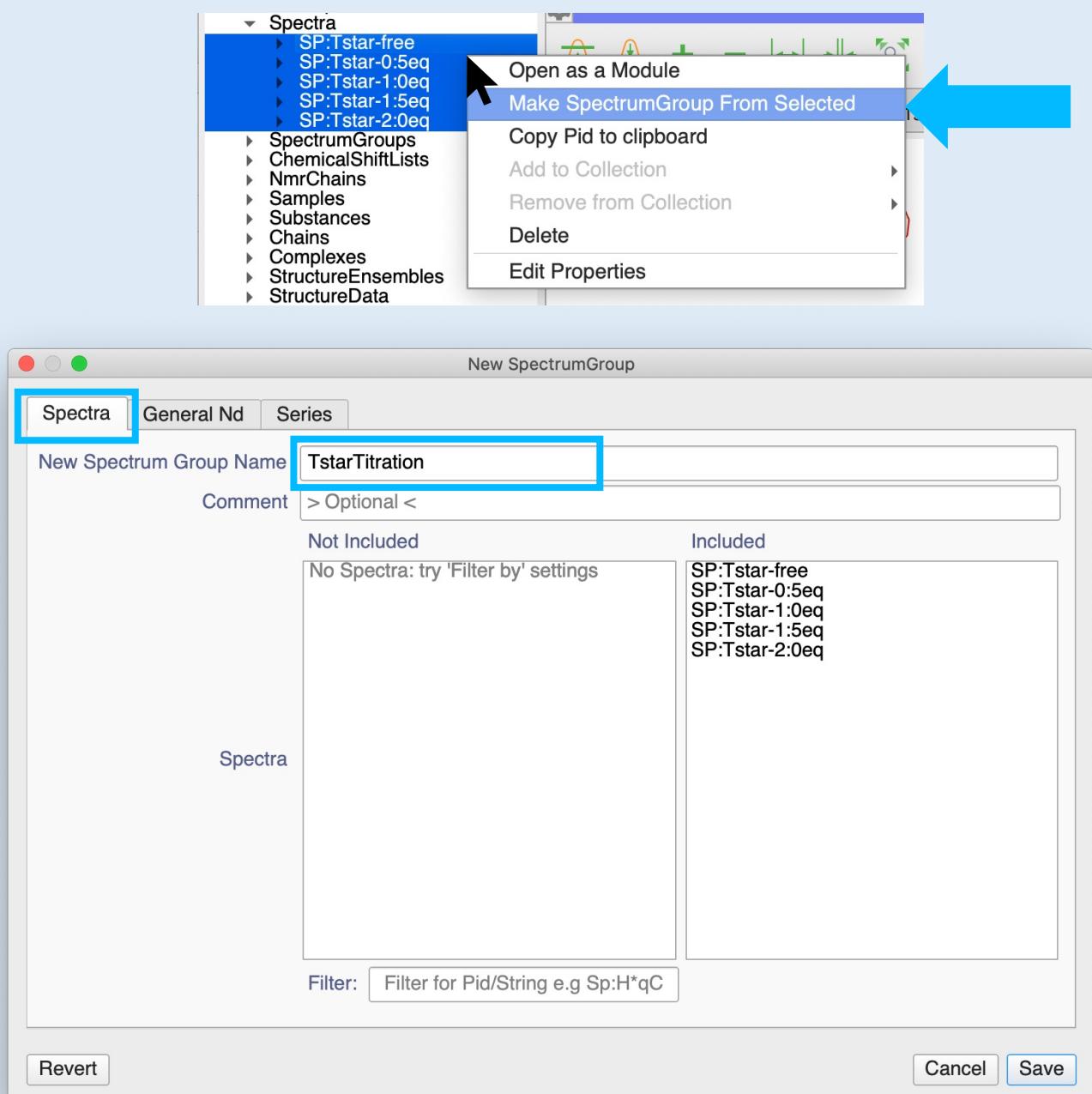


3A Open Project

If you haven't identified all the peak trajectories and copied the peak assignments to all spectra, then please continue with your prepared project, **TstarPeaksAssigned.ccpn**.

- Find the project directory **TstarPeaksAssigned.ccpn** in the Tutorial directory and drag and drop it into the program.

Group Spectra



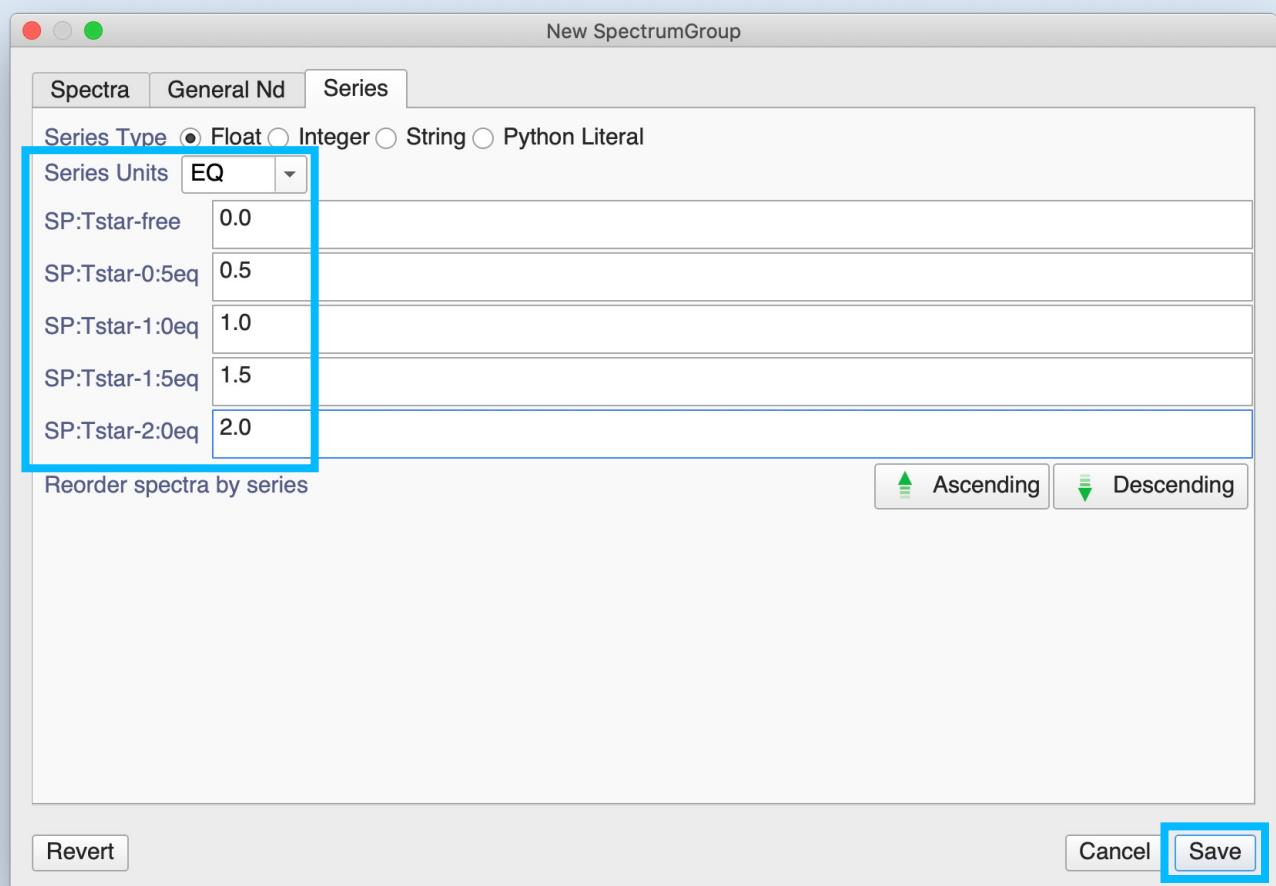
3B Create SpectrumGroup

For further analysis the spectra have to be organised in a SpectrumGroup.

- Select all spectra in the project.
- Right-click and go to **Make SpectrumGroup From Selected**.
- In the **Spectra** tab of the pop-up window type a name for your SpectrumGroup.

The **GeneralNd** tab will let you make changes to the contours across the series of spectra, but that shouldn't be necessary for this project.

Group Spectra



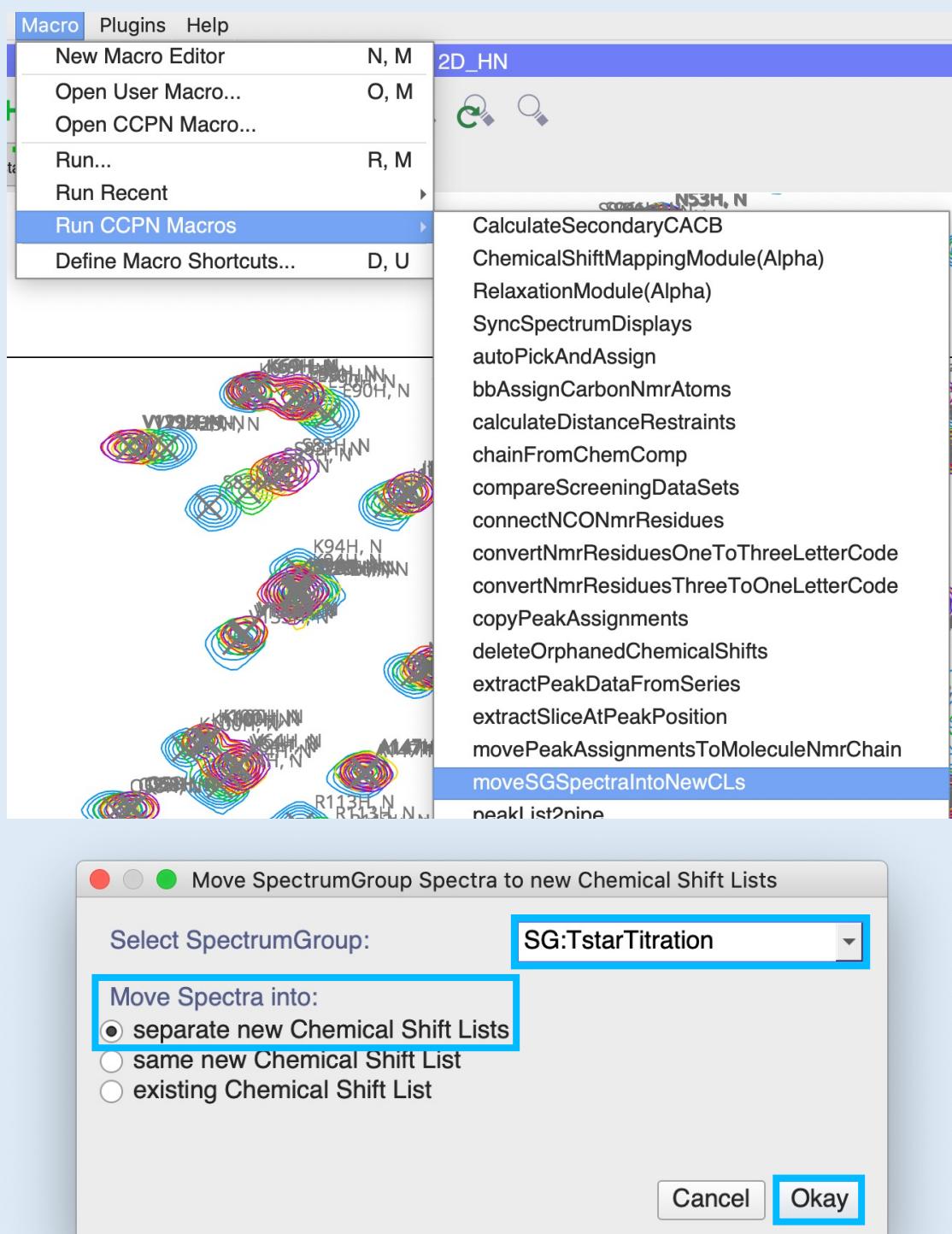
3C Enter Series information

- Move to the **Series** tab
- Set the **Series Units** to **EQ** (equivalents)
- Enter the amount of ligand equivalent present for each of the spectra (as included in the spectrum name):

SP:Tstar-free	0.0
SP:Tstar-0:5eq	0.5
SP:Tstar-1:0eq	1.0
SP:Tstar-1:5eq	1.5
SP:Tstar-2:0eq	2.0

- Click **Save**.

Group Spectra

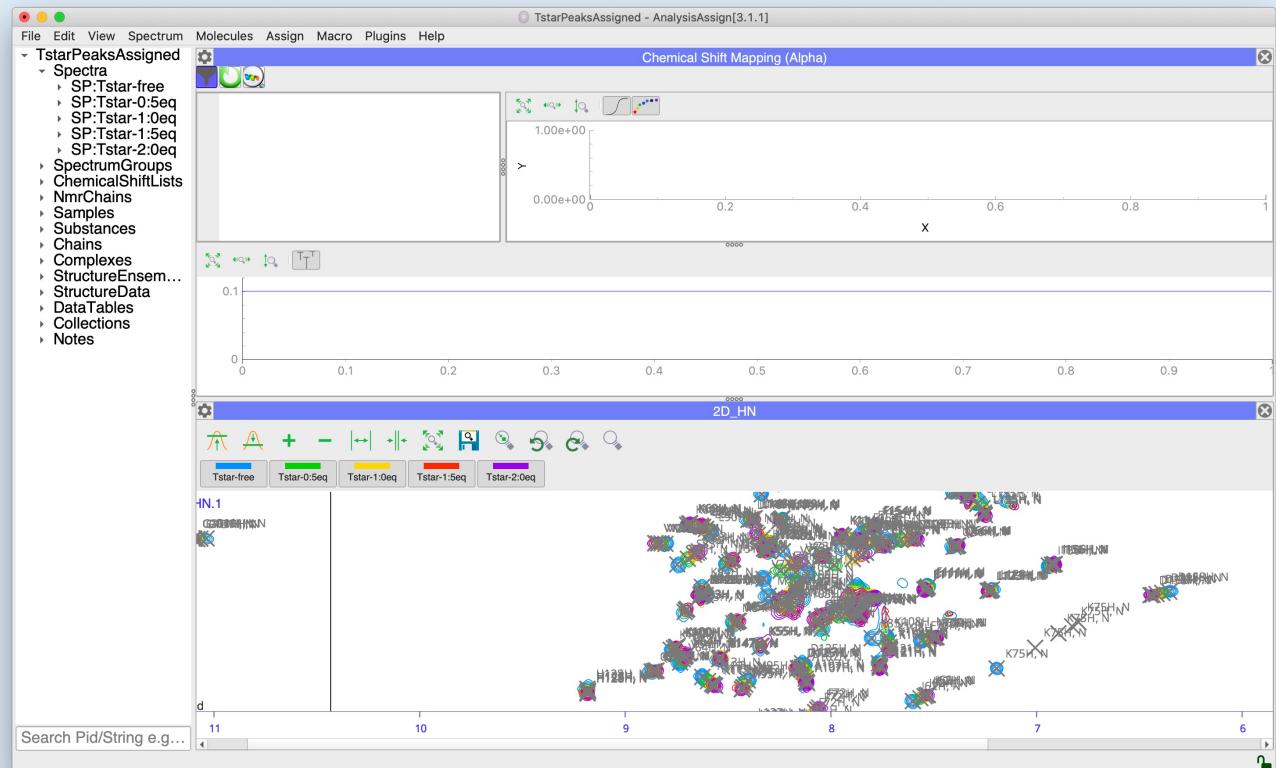
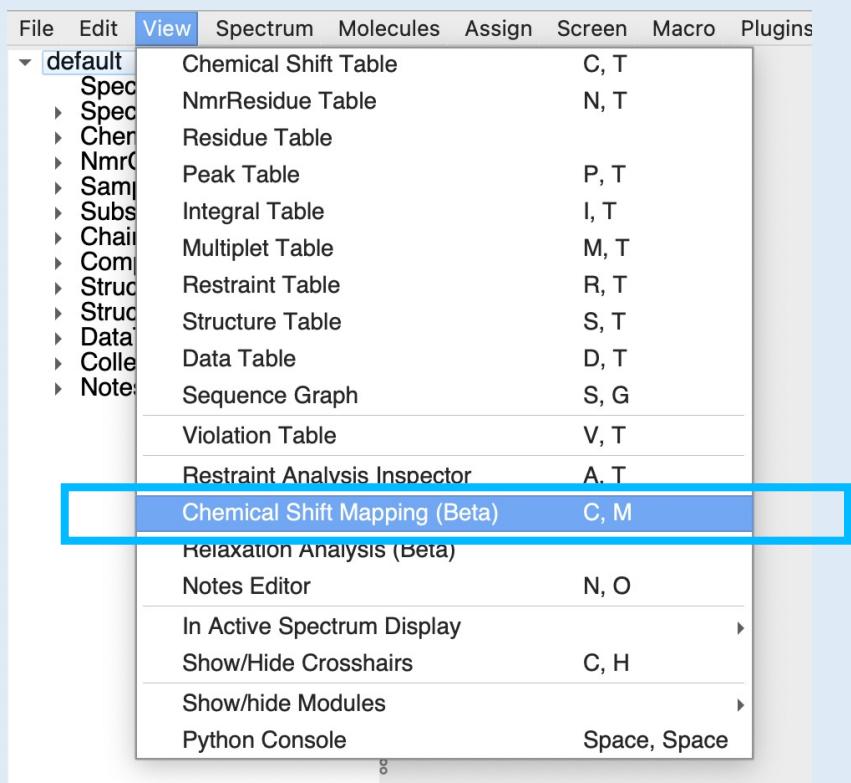


3D Move Spectra into separate Chemical Shift Lists

Because each of the Spectra in a titration was recorded under different conditions and the Chemical Shifts therefore vary, each Spectrum should be associated with a different Chemical Shift List. This can be done easily using a CCPN macro:

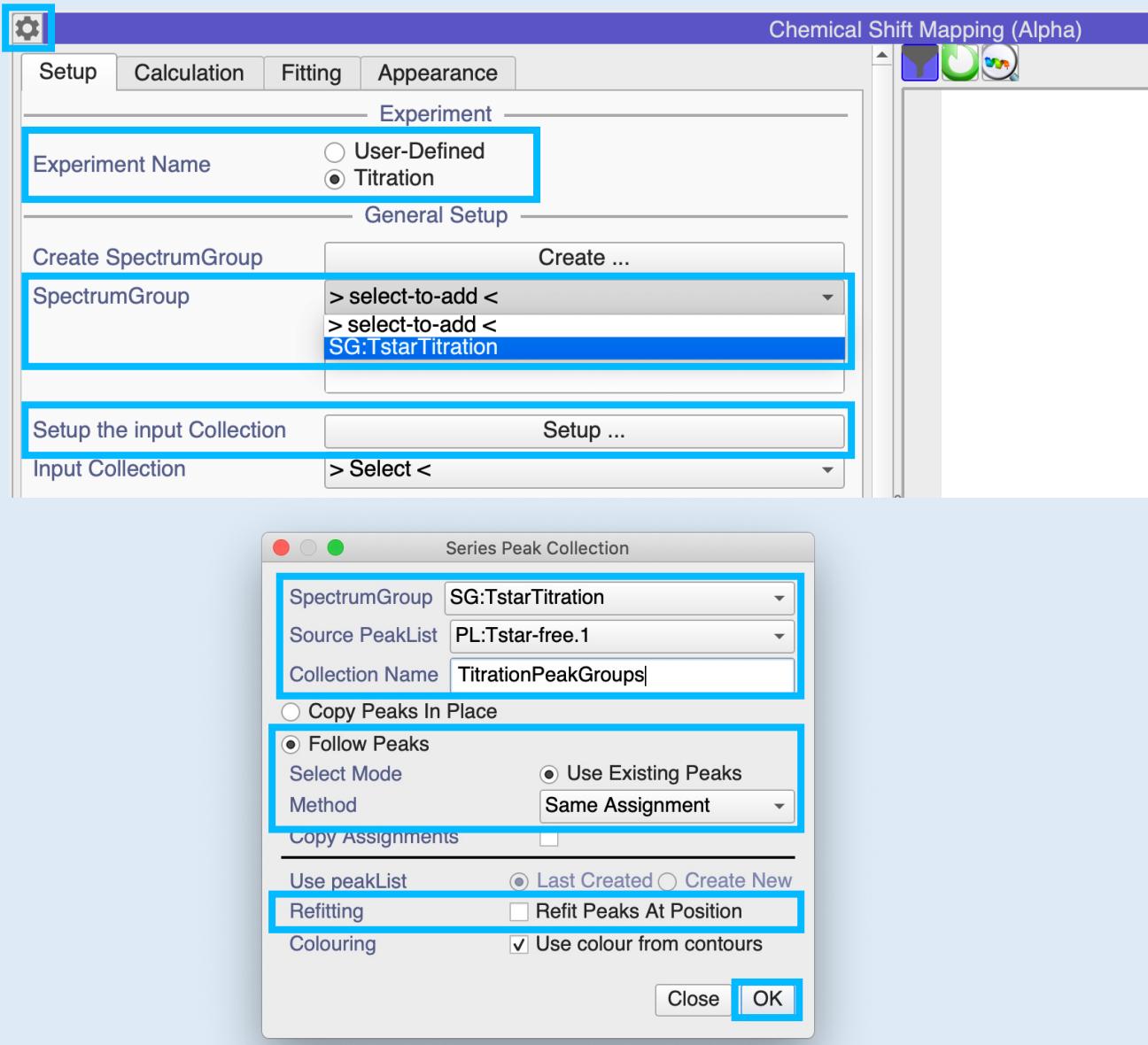
- Go to Main Menu -> Macro -> Run CCPN Macros -> **moveSGSpectraIntoNewCLs**
 - Select your Titration SpectrumGroup
 - Select to move the Spectra into **separate new Chemical Shift Lists**
 - Click **Okay**
- ChemicalShiftLists
<New ChemicalShiftList>
CL:default
CL:Tstar-free
CL:Tstar-0:5eq
CL:Tstar-1:0eq
CL:Tstar-1:5eq
CL:Tstar-2:0eq

Fit and Map Peaks



4A Open the Chemical Shift Mapping module

- Go to Main Menu → View ChemicalShift MappingModule (Beta) or use the shortcut CM



4B Set up Titration input data

- Click the gear icon in the top left corner to open the module Settings.
- Set the **Experiment Name** to **Titration**.
- Select your newly created **TstarTitration** SpectrumGroup from the SpectrumGroup drop-down menu.
- Click on the **Setup...** button to setup your Input Collection

The Input Collection is a Collection of all your peaks sorted into groups by their assignment.

- In the pop-up set the values as shown above:

SpectrumGroup	SG:TstarTitration
Source PeakList	PL:Tstar-free.1
Collection Name	TitrationPeakGroups
Follow Peaks	
Method	Same Assignment (select from drop-down menu)
Refitting	Deselect Refit Peaks At Position

- Click **OK**.

Fit and Map Peaks

Setup	Calculation	Fitting	Appearance
Experiment			
Experiment Name	<input type="radio"/> User-Defined <input checked="" type="radio"/> Titration		
General Setup			
Create SpectrumGroup	Create ...		
SpectrumGroup	> select-to-add < SG:TstarTitration		
Setup the input Collection	Setup ...		
Input Collection	CO:TitrationPeakGroups		
Input DataTables			
Input DataTable Name	Titration_inputDataTable		
Create Input DataTable	Create		
Input DataTable(s)	> select-to-add < None		
Results DataTable Name	Titration_resultDataTable		
Fit and Fetch Results Data	Fit		
Results DataTable			
Results DataTable	> Select <		

4C Create Input DataTable

- In the **InputDataTables** section of the **Settings**, click on **Create** to create the Input DataTable.

This DataTable will then be automatically selected as your **Input DataTable(s)**:

Input DataTable(s)	> select-to-add < DT:Titration_inputDataTable
--------------------	--

Fit and Map Peaks

The screenshot shows the PeakFit software interface with two main panels. The left panel is titled 'Calculation Options' and includes fields for '1H Alpha Factor' (0.142), '13C Alpha Factor' (0.25), 'Other Alpha Factor' (1), and 'Δδ for Untraceable Observations' (1). The right panel is titled 'Fitting Options' and includes fields for 'Optimiser Method' (leastsq) and 'Fitting Error Method' (Default). Below these are sections for 'Fitting Model' and 'Fitting Options' (One-Site (Specific) Binding selected). At the bottom, there are input and results tables: 'Input DataTable(s)' set to 'DT:Titration_inputDataTable', 'Results DataTable Name' set to 'Titration_resultDataTable', 'Fit and Fetch Results Data' set to 'Fit' (which is highlighted with a blue border), and 'Results DataTable' set to '> Select <'.

4D Fit Data and create Results DataTable

We will use the default Calculation and Fitting options. If you wish to see or change these:

- Go to the **Calculation** and **Fitting** tabs.

Hovering over one of the Fitting Options will show you a ToolTip with the equation and reference.

Fit data to using the One-Site Specific Binding model in a saturation binding experiment analysis.
This simple model can be used when a small fraction of the ligand binds to the target, in this state, the bound concentration is ~ equal to the unbound.

Model:

$$Y = B_{max} \cdot X / (K_d + X)$$

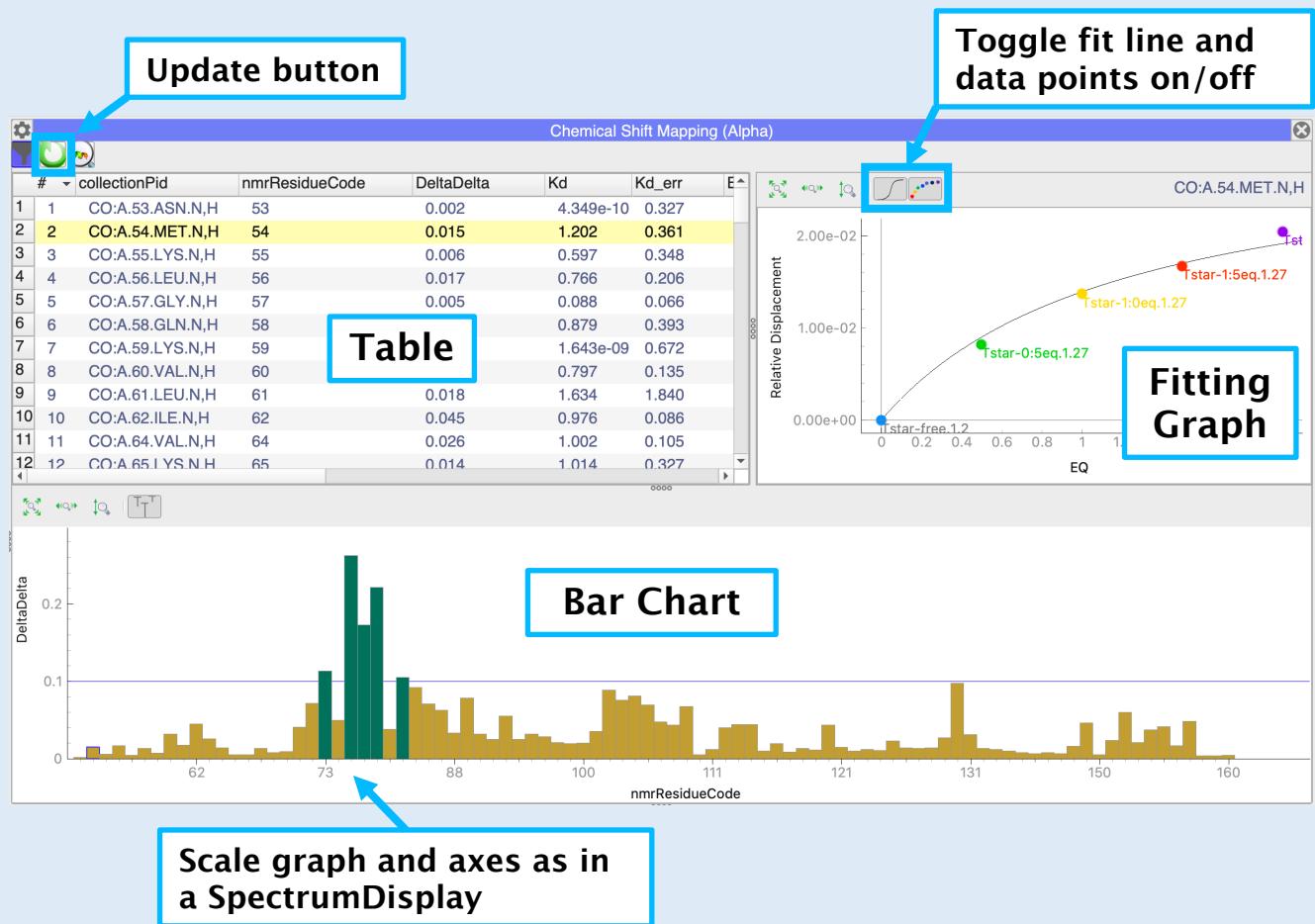
B_{max}: is the maximum specific binding and in the CSM is given by the Relative displacement (Deltas among ChemicalShifts).
K_d: is the (equilibrium) dissociation constant in the same unit as the Series.
The K_d represents the [ligand] required to get a half-maximum binding at equilibrium.
X: is the Series steps.

References:
1) Model derived from E.q. 13. Receptor and Binding Studies. Hein et al. 2005. https://doi.org/10.1007/3-540-26574-0_37

- Click on **Fit** to fit your data and create the Results DataTable.

The newly created Results DataTable will be automatically selected and the Chemical Shift Mapping module will be updated with the data from this.

- Click the gear icon to close the **Settings** panel again.



4E Inspect Results

The top left Table provides you with the results for each group of peaks. It includes the fitting parameters (K_d , B_{Max}), the Euclidean distance at each point and $\Delta\delta$, the Euclidean distance averaged over all but the first point (which is always 0).

- Select a row in the table and move up/down using the arrow keys

As you move, you will notice the following changes:

- The Fitting Graph is updated
- The relevant bar on the Bar Chart is selected (blue outline)
- The SpectrumDisplay will select and navigate to the peaks associated with this row.

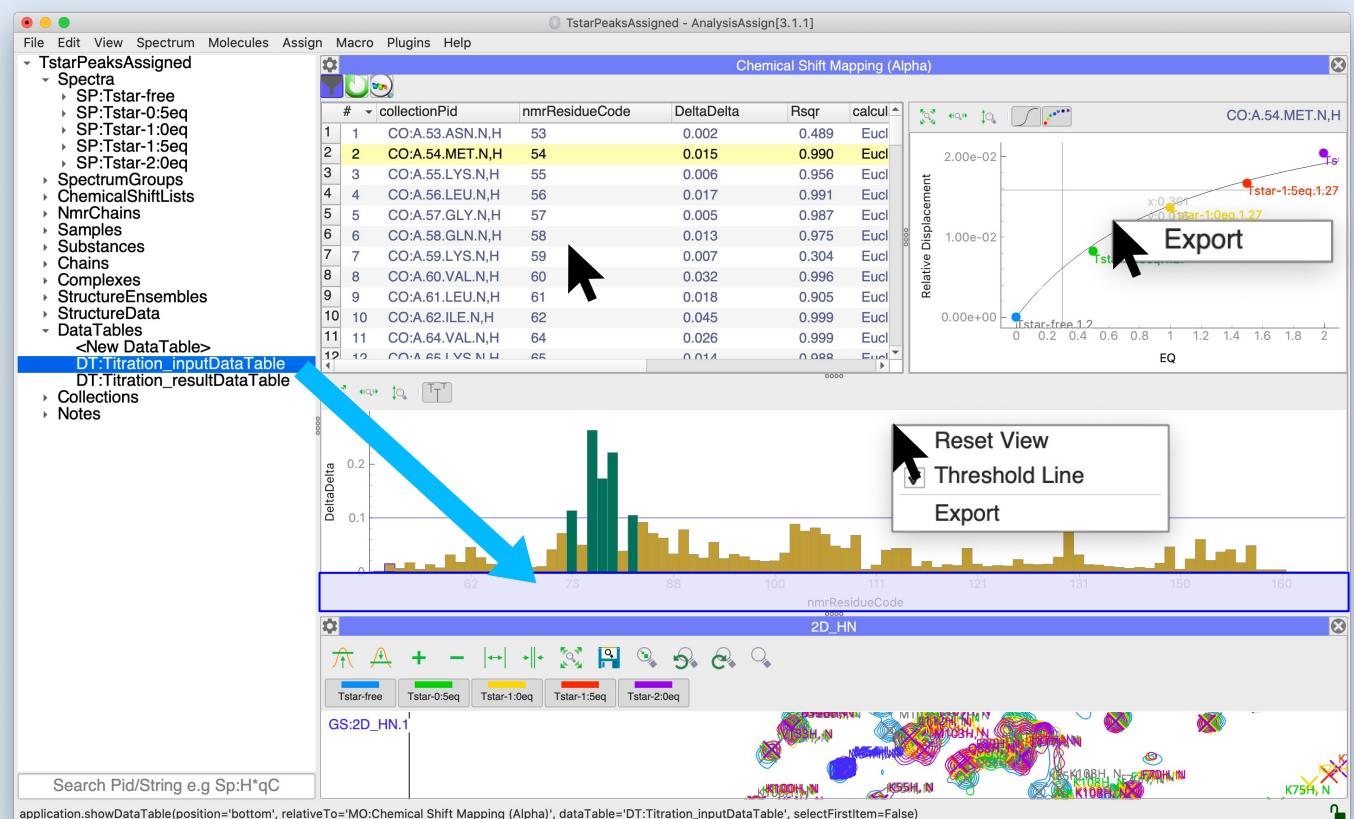
- In the Bar Chart, move the blue threshold line up/down.

You will notice that the number of bars coloured green (those above the threshold) will change.

If you move or delete a peak, the Update button will turn orange . Pressing it, will update the tables and graphs and turn the Update button green again.

- Open the **Settings** panel with the gear icon and go to the **Appearance** tab. Here you can change a variety of **SpectrumDisplay**, **BarGraph** and **Table** Options. If the Update button goes orange, press it to apply your changes.

Fit and Map Peaks



4F Export Results

- Right-click in any of the the areas of the ChemicalShiftMapping module to export the table, graph or bar chart in a variety of formats.

You can also export the raw input or results data which are contained in the DataTables:

- Drag the Titration_inputDataTable into the DropArea to open it as a module
- Right-click in the DataTable to see the usual Table Export options.

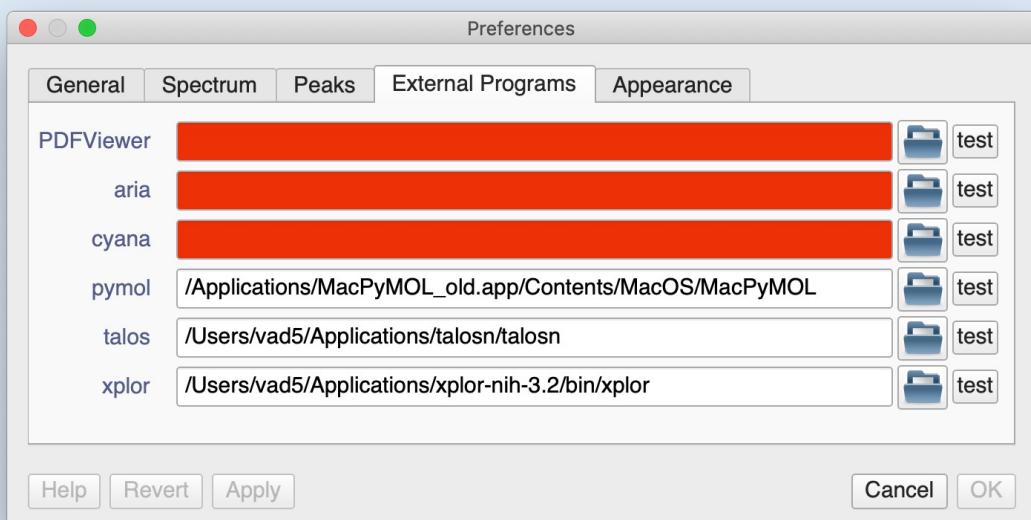
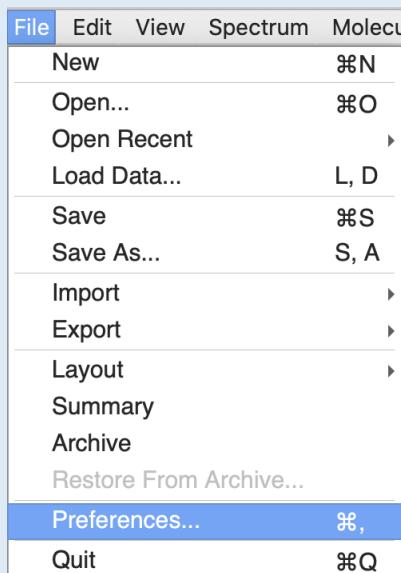
dimension	isotopeCode	series_Step_X	seriesUnit	experiment	ppmPosition	height	lineWidth	volume
1.000	1H	0.000	EQ	Titration	8.142	2.426e+07	nan	nan
2.000	15N	0.000	EQ	Titration	114.311	2.426e+07	nan	nan
1.000	1H	0.000	EQ	Titration	8.474	2.456e+07	nan	nan
2.000	15N	0.000	EQ	Titration	120.490	2.456e+07	nan	nan
1.000	1H	0.000	EQ	Titration	8.347	1.743e+07	nan	nan
2.000	15N	0.000	EQ	Titration	121.413	1.743e+07	nan	nan
1.000	1H	0.000	EQ	Titration	8.488	1.132e+07	nan	nan
2.000	15N	0.000	EQ	Titration	124.722	1.132e+07	nan	nan
1.000	1H	0.000	EQ	Titration	8.288	2.227e+07	nan	nan
2.000	15N	0.000	EQ	Titration	109.328	2.227e+07	nan	nan

View in PyMOL

PyMOL is a popular Python-enhanced molecular graphics tool. It is good for the 3D visualisation of proteins, small molecules, densities and surfaces.

Academic PyMOL licences are available from <https://pymol.org/edu/>.

If you have PyMOL installed on your computer, then you can directly start it from CcpNmr Analysis to show the most highly perturbed residues on the protein structure.



5A Link PyMOL to CcpNmr Analysis

- Go to **Main menu → Project → Preferences...**
- Go the **External Programs** tab.

In the **pymol** row either:

- Click on the folder icon and select your PyMOL executable.

or:

- Directly enter the PyMOL executable path.

The path should look something like this:

/Applications/MacPyMOL.app/Contents/MacOS/MacPyMOL (Mac)

C:\Program Files\PyMOL\ (Windows)

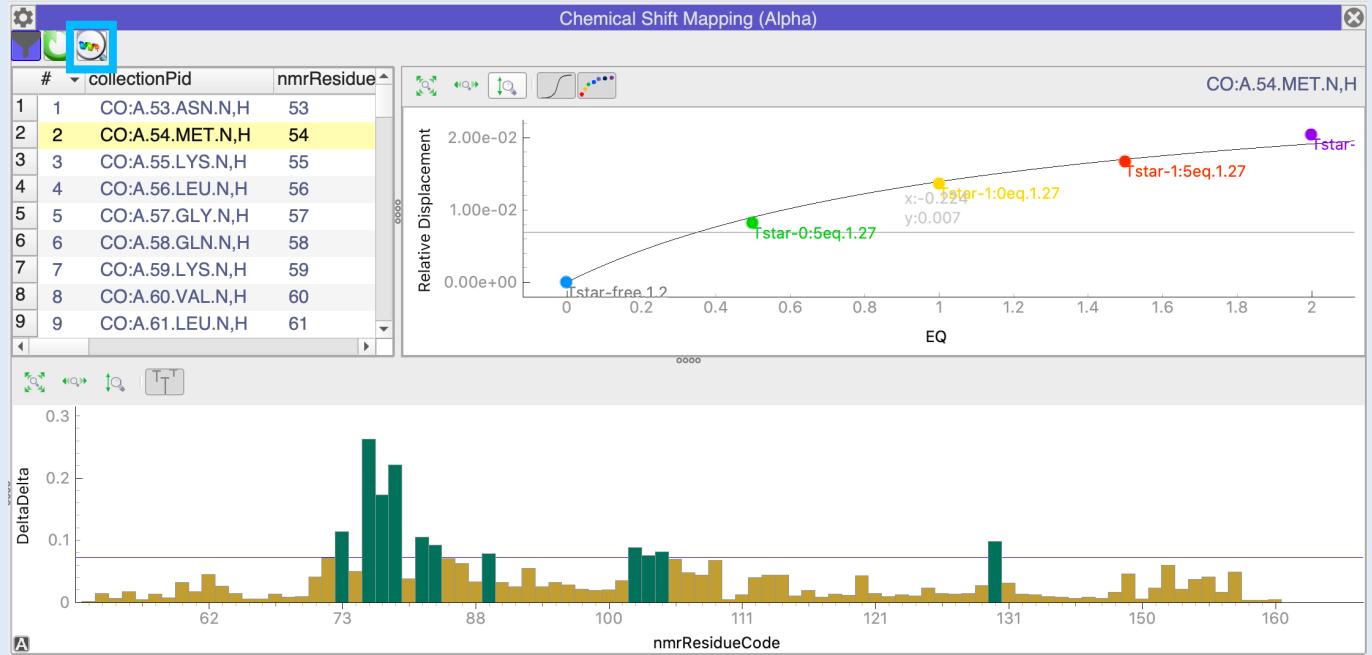
/usr/lib/python2.7/dist-packages/pymol (Linux)

- Click on **Test**, PyMOL will start if has been linked successfully.
- Click **OK**.

Chemical Shift Map

Setup Calculation Fitting Appearance

Molecular Structure File nCSP_tutorial/pdbStructure/TSTAR-KH.pdb

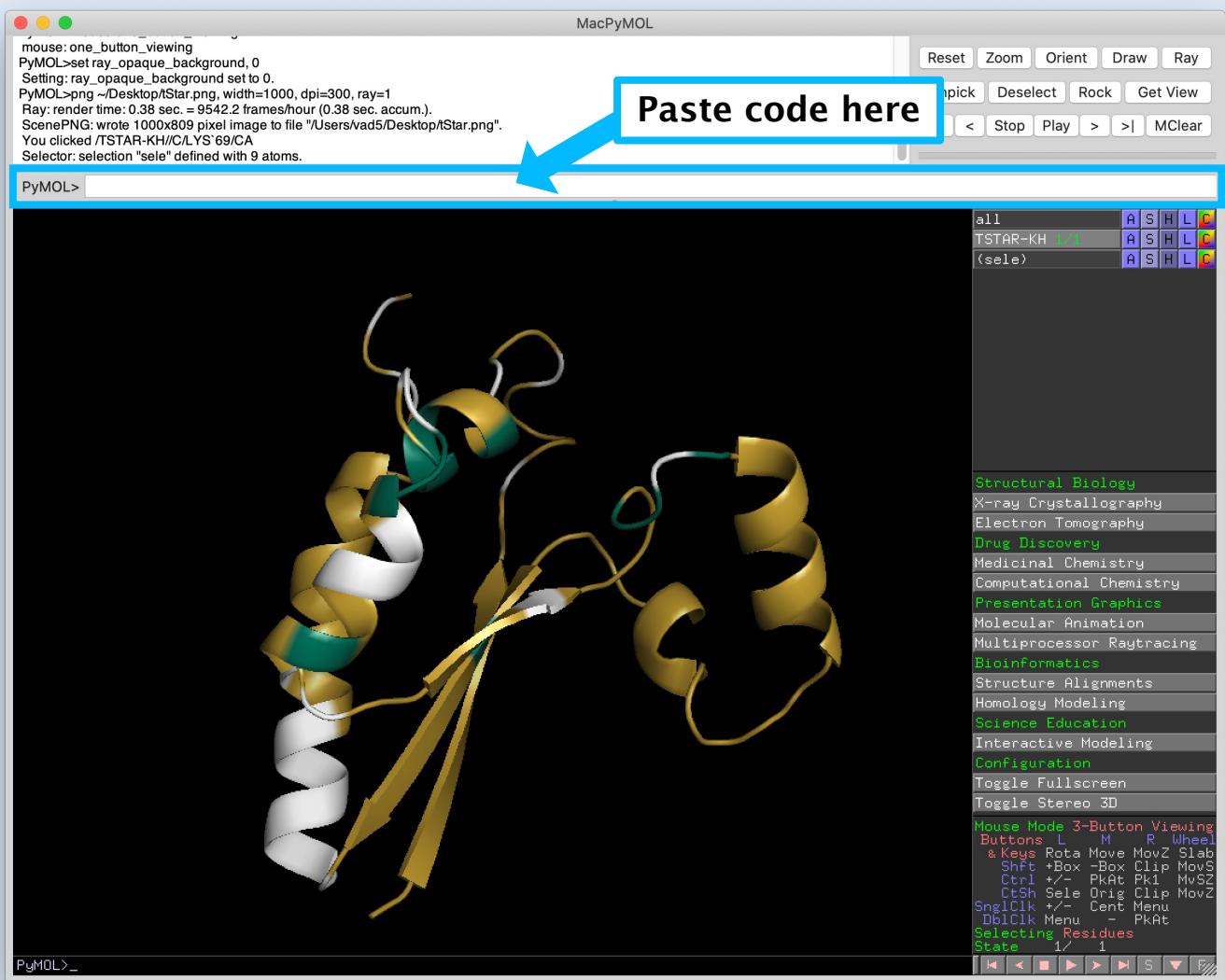


5B Select PDB file and launch PyMOL

- Open the ChemicalShiftMapping module **Settings** panel by clicking on the gear icon.
- Go to the **Appearances** tab
- In the Molecular View section at the bottom, select the **TSTAR-KH.pdb** file which should be in the **pdbStructure** directory of the tutorial data.
- Close the Settings panel again.
- Click on the Molecular Viewer button  to open PyMOL with the Tstar structure already loaded in. In PyMOL two new selections will be created:
 - (*aboveThreshold*) , same colour as the bars in CcpNmr
 - (*belowThreshold*) , same colour as the bars in CcpNmr
 - (*missing*) , light grey

You can now analyse the residues that are involved in the interaction with the ligand graphically.

View in PyMOL



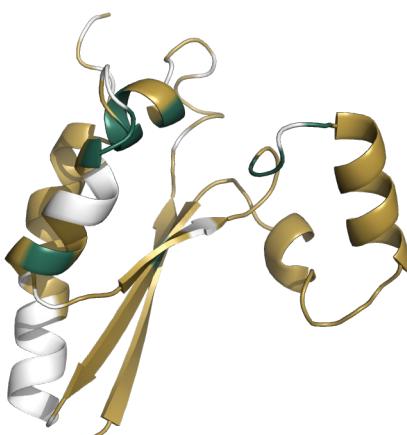
5C Export PyMOL image with transparent background (optional)

If you wish to export an image of your structure from PyMOL:

- Copy and paste these commands into the PyMOL terminal:

```
set ray_opaque_background, 0
png ~/Desktop/tStar.png, width=1000, dpi=300, ray=1
```

- Press **Enter**.
- Locate the image on your Desktop.



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

Simple High-Resolution NMR Spectroscopy as A Tool in Molecular Biology. LG Mureddu and GW Vuister. *FEBS Journal* (2019). [doi:10.1111/febs.14771](https://doi.org/10.1111/febs.14771)

CcpNmr AnalysisAssign: a flexible platform for integrated NMR analysis. SP Skinner *et al.* *J. Biomol. NMR* (2016). [doi:10.1007/s10858-016-0060-y](https://doi.org/10.1007/s10858-016-0060-y)