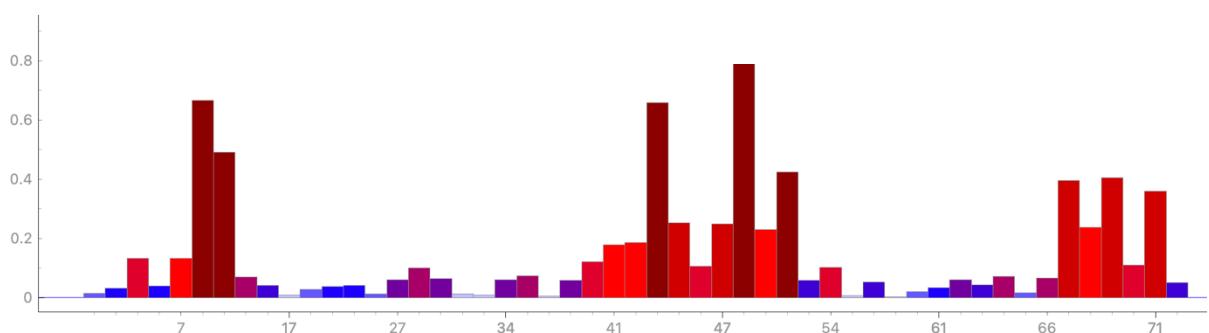
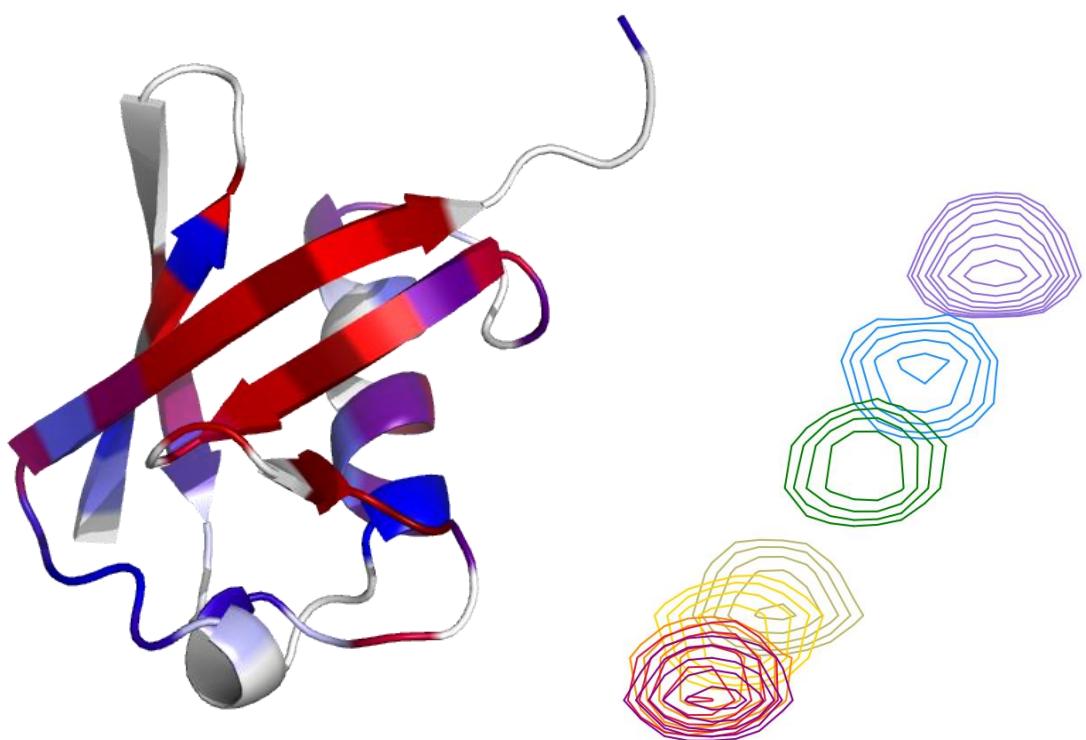


Chemical Shift Perturbation Tutorial



Introduction

This tutorial is designed to show you how to use CcpNmr Analysis Version 3.2.10 to analyse the chemical shift perturbations caused by a target molecule (in this case a protein) interacting with a ligand (in this case another protein) and map the interaction onto the structure of the protein.

The tutorial uses ^1H - ^{15}N HSQC spectra of ^{15}N labelled ubiquitin in complex with the Uba domain from ubiquilin-2. We are grateful to Dr Brian Smith for making this data available to us. The work itself is described in the thesis of Dr Bethany Waddington available at <https://theses.gla.ac.uk/83177/>.

Contents:

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

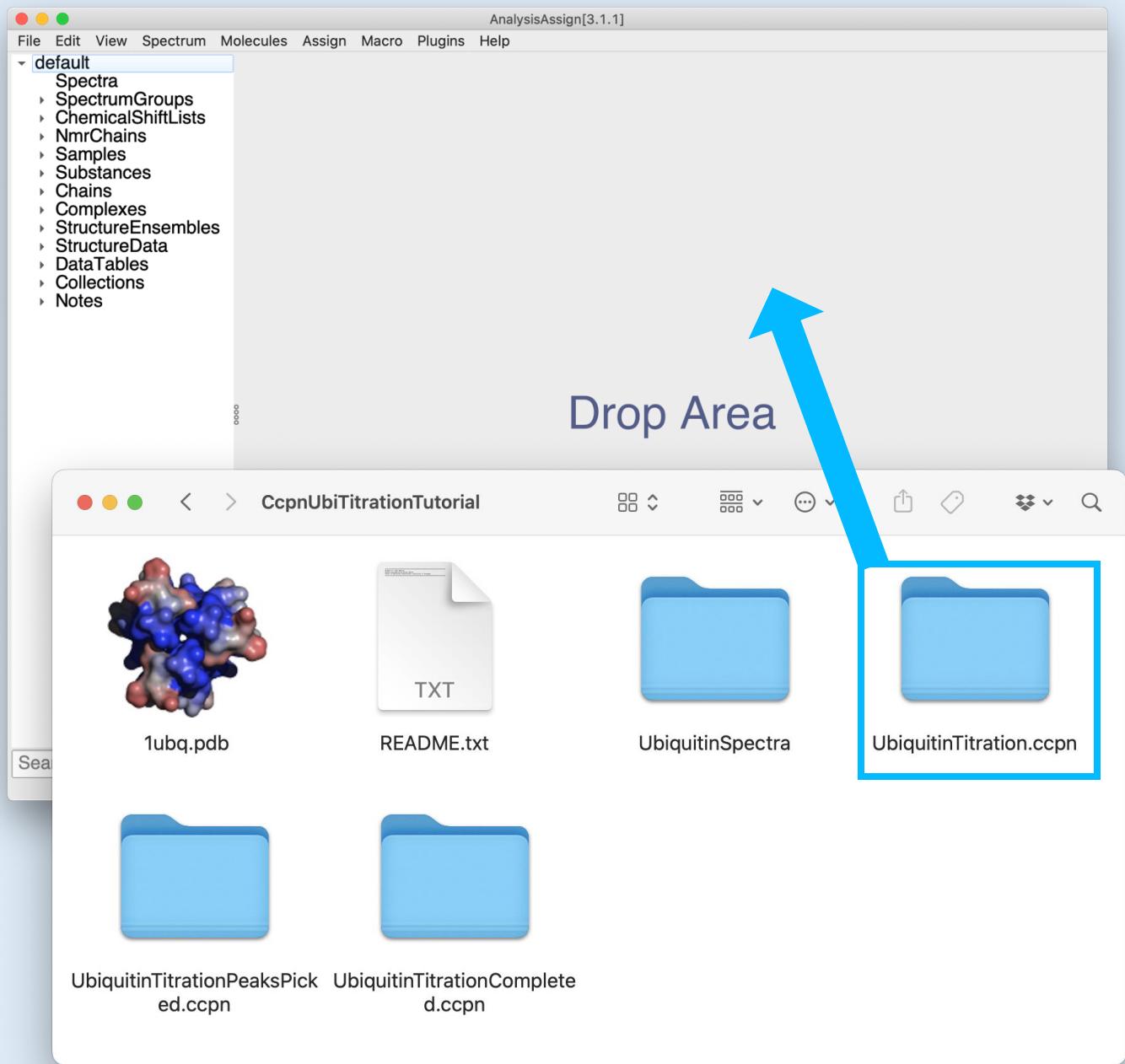
Start CcpNmr Analysis V3

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 **UbiquitinTitration.ccpn** in the Chemical Shift Perturbation Tutorial directory and drag and drop it into the program.

The UbiquitinTitration project will be loaded in a new window.

You will see eight ^1H - ^{15}N HSQC spectra:

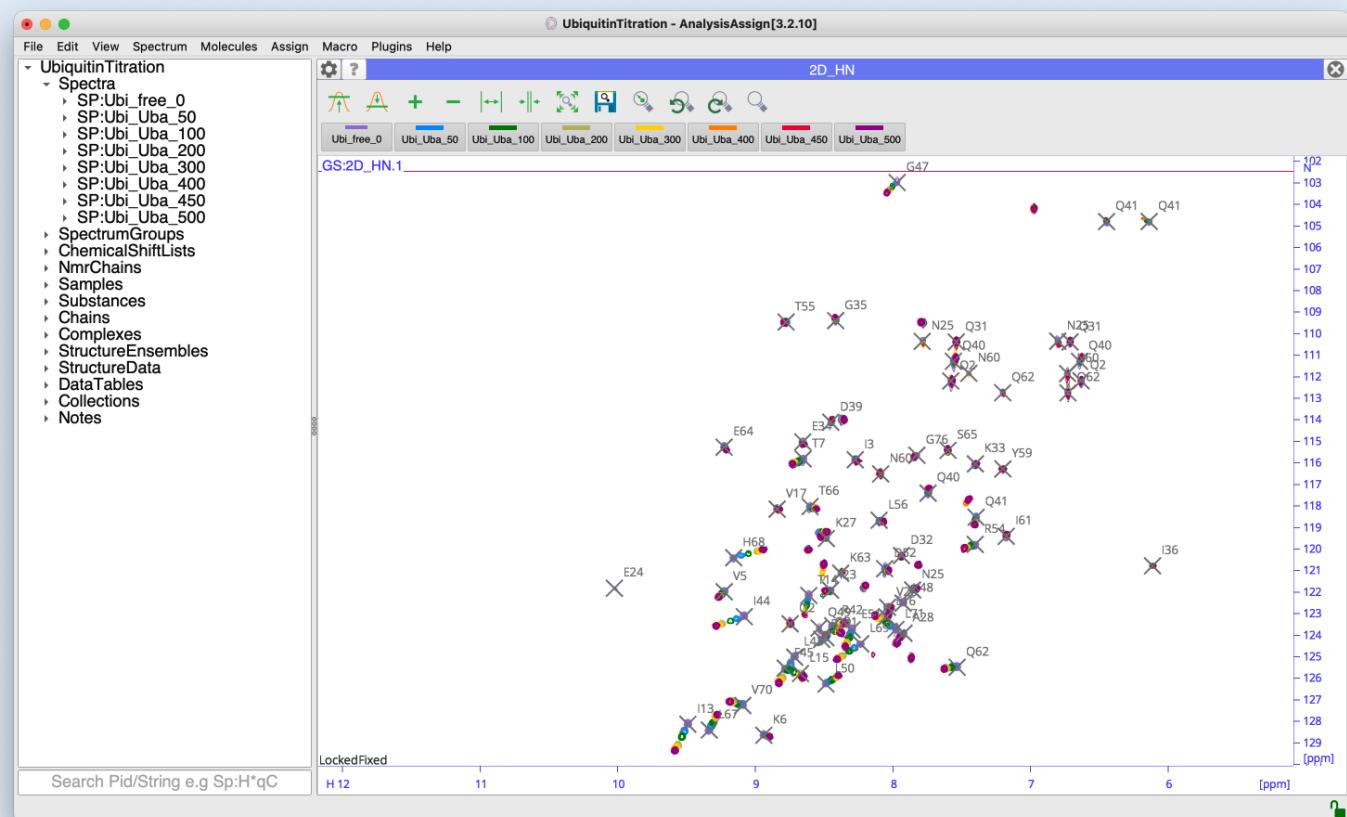
- **Ubi_free_0** (light purple)

This is a spectrum of 100 μM free ubiquitin. Most peaks are assigned.

- **Ubi_Uba_50 – 500** (in ordered rainbow colours)

These are of 100 μM ubiquitin with increasing amounts of the Uba domain of ubiquilin. The concentration of Uba (in μM) is given in the spectrum name. No peaks have been picked or assigned.

Open Project



Reminder: 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

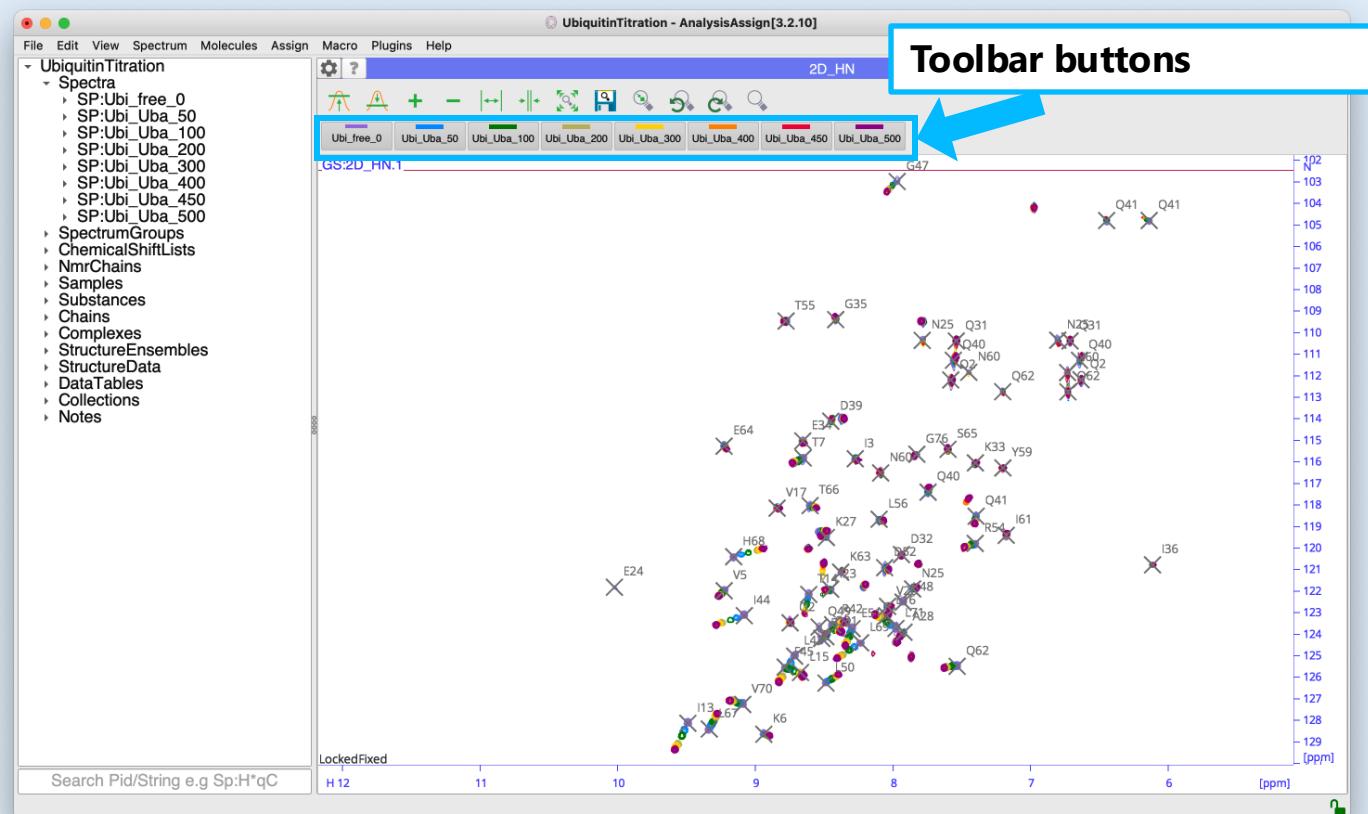
Shortcuts

The program uses several shortcuts, example **CA** to copy an assignment. You will need to press the first (lowercase) letter on your keyboard e.g. **c**, followed by the second letter, e.g. **a**.

For more commands and operations:

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

Open Project



1B Toggle Spectra on/off using the toolbar and shortcuts

- Click on the toolbar buttons of the first two spectra.

The buttons will change from on (spectrum visible on the display, "toggle On") to off (spectrum hidden on the display, "toggle Off") and vice versa.



Click to toggle spectra on/off



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

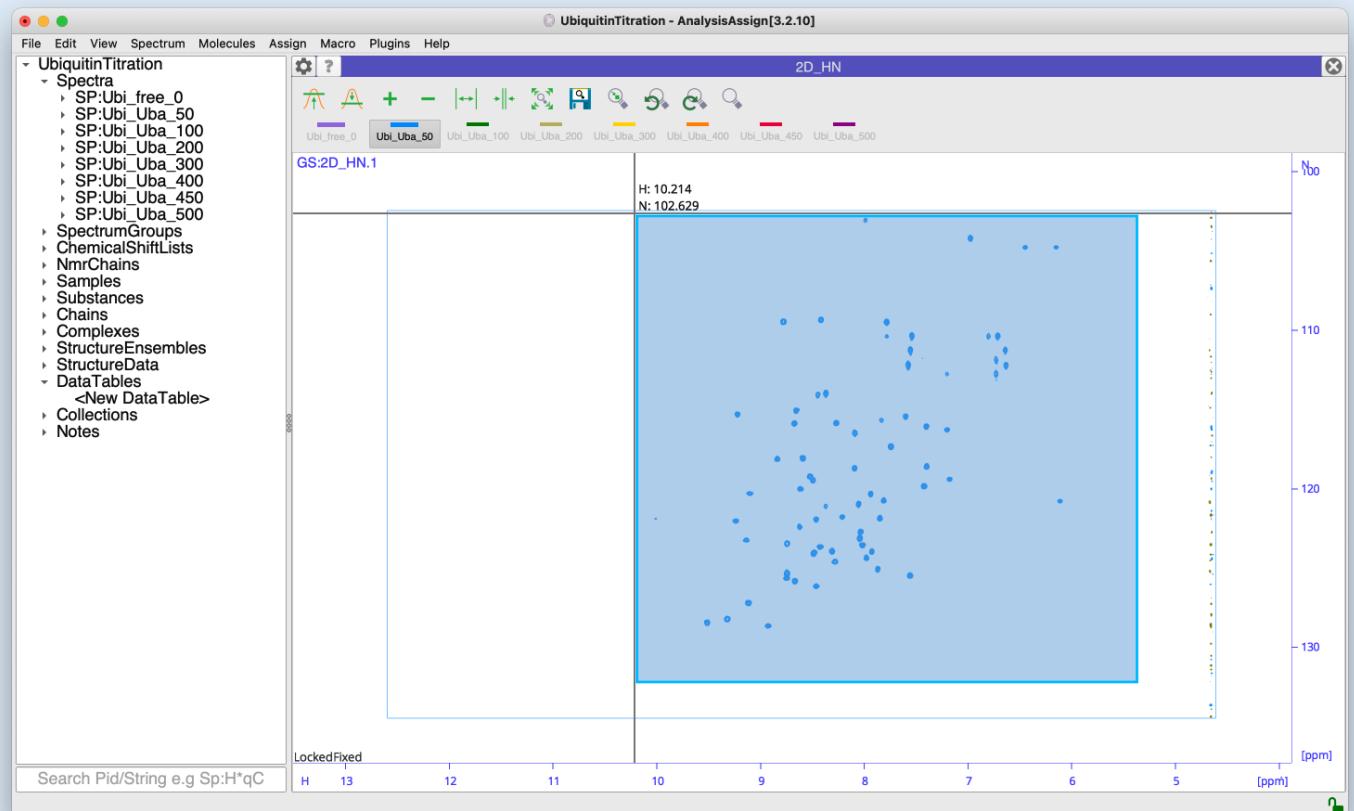
Tab, A will switch all Spectra on.

Tab, Z will switch all Spectra off.

Tab, X will reverse the on/off pattern.

Pick Peaks and Copy Assignments

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



2A Pick Peaks

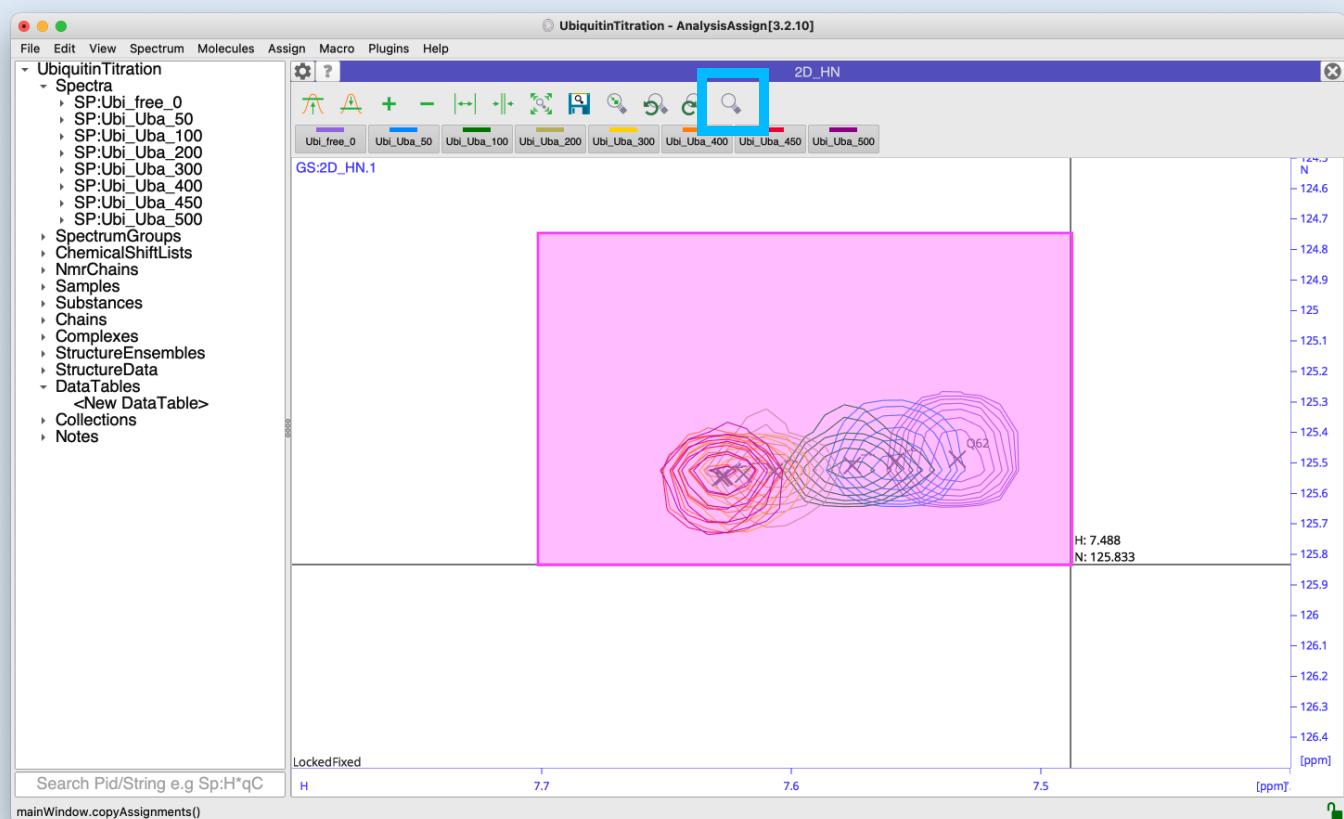
With only the **Ubi_Uba_50** spectrum showing:

- 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 six unpicked spectra.

OR

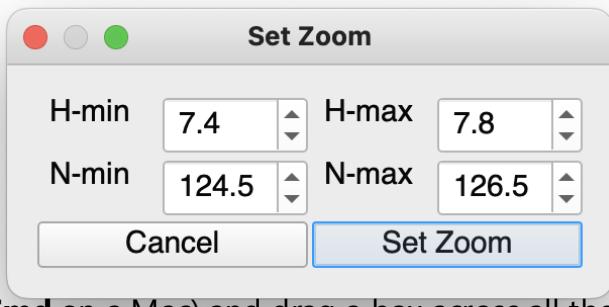
- Toggle all unpicked spectra to on.
- Hold down **Shift+Ctrl (Cmd on a Mac)** and drag a box across all the peaks to pick them together

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. **Q62** at ~ 7.6/125.5 ppm.
If you wish, you can use the SpectrumDisplay Zoom button or shortcut **SZ** to bring up the Zoom pop-up to navigate to this region of the spectrum:

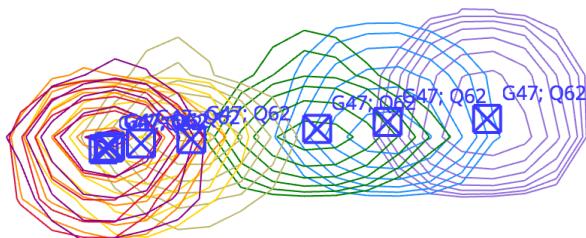


- Hold down **Ctrl** (**Cmd** on a Mac) and drag a box across all the peaks to select them.
- Go to **Main Menu -> Assign -> Copy Assignments**

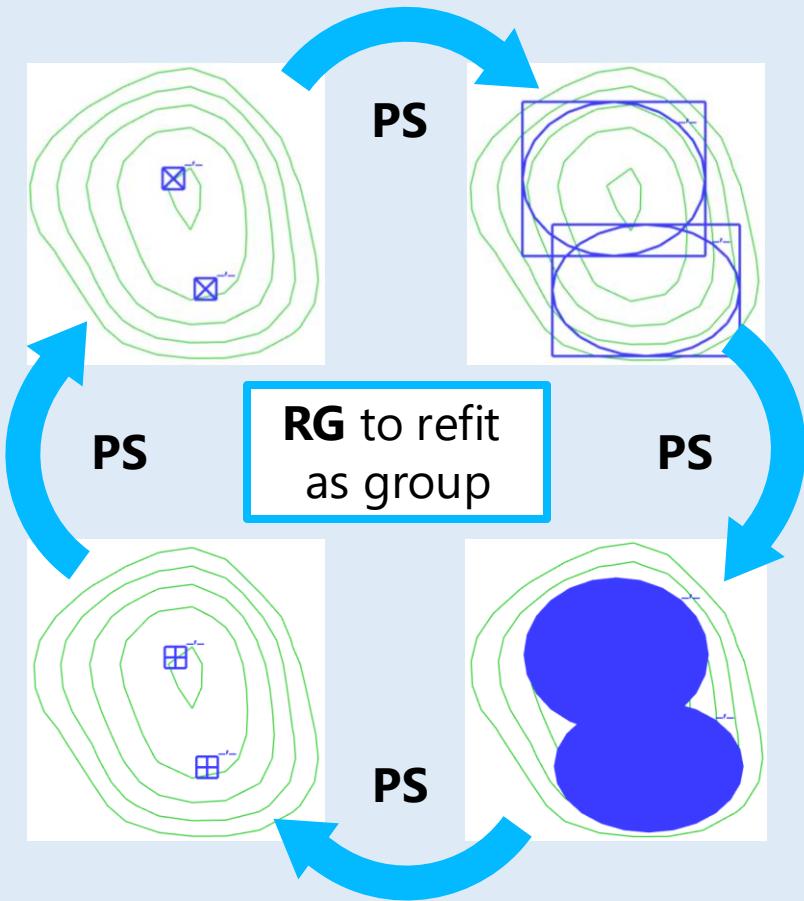
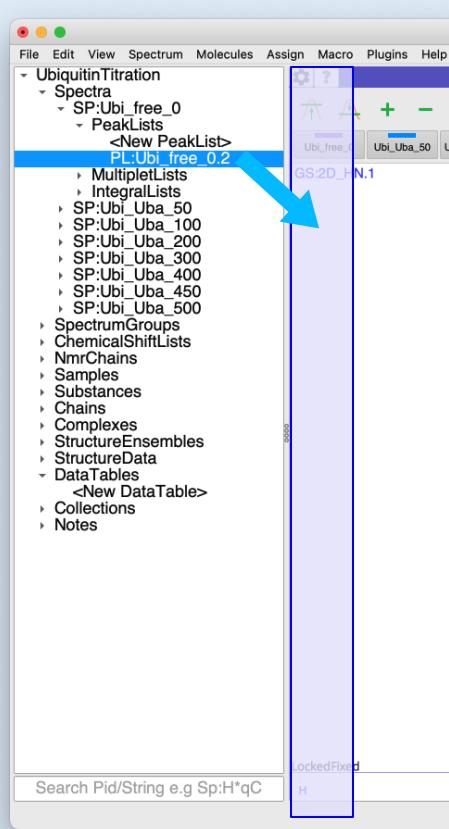
or use shortcut **CA**

This will copy the Assignment from the peak in the **Ubi_free_0** spectrum to the peaks in all the other spectra.

- Repeat for all the other peak groups. See **Section 2C** 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



2C Tips and Tricks for picking peaks and copying assignments

- Drag the **PL:Ubi_free_0.2** 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.

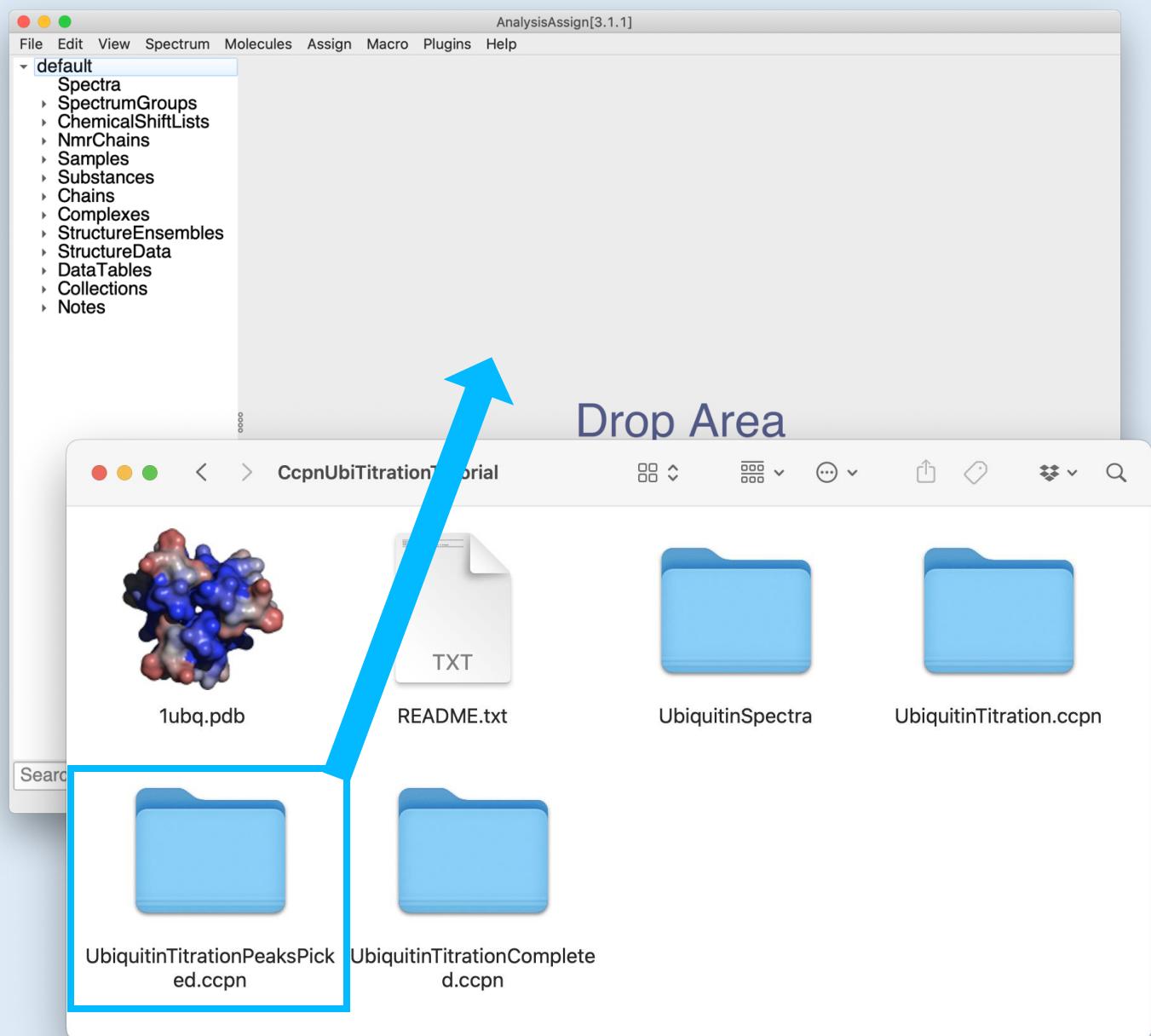
Sometimes it is most useful to place the peaks by hand:

- 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 : Reverses displayed spectra
- Tab-Z : Toggles all spectra off

Group Spectra



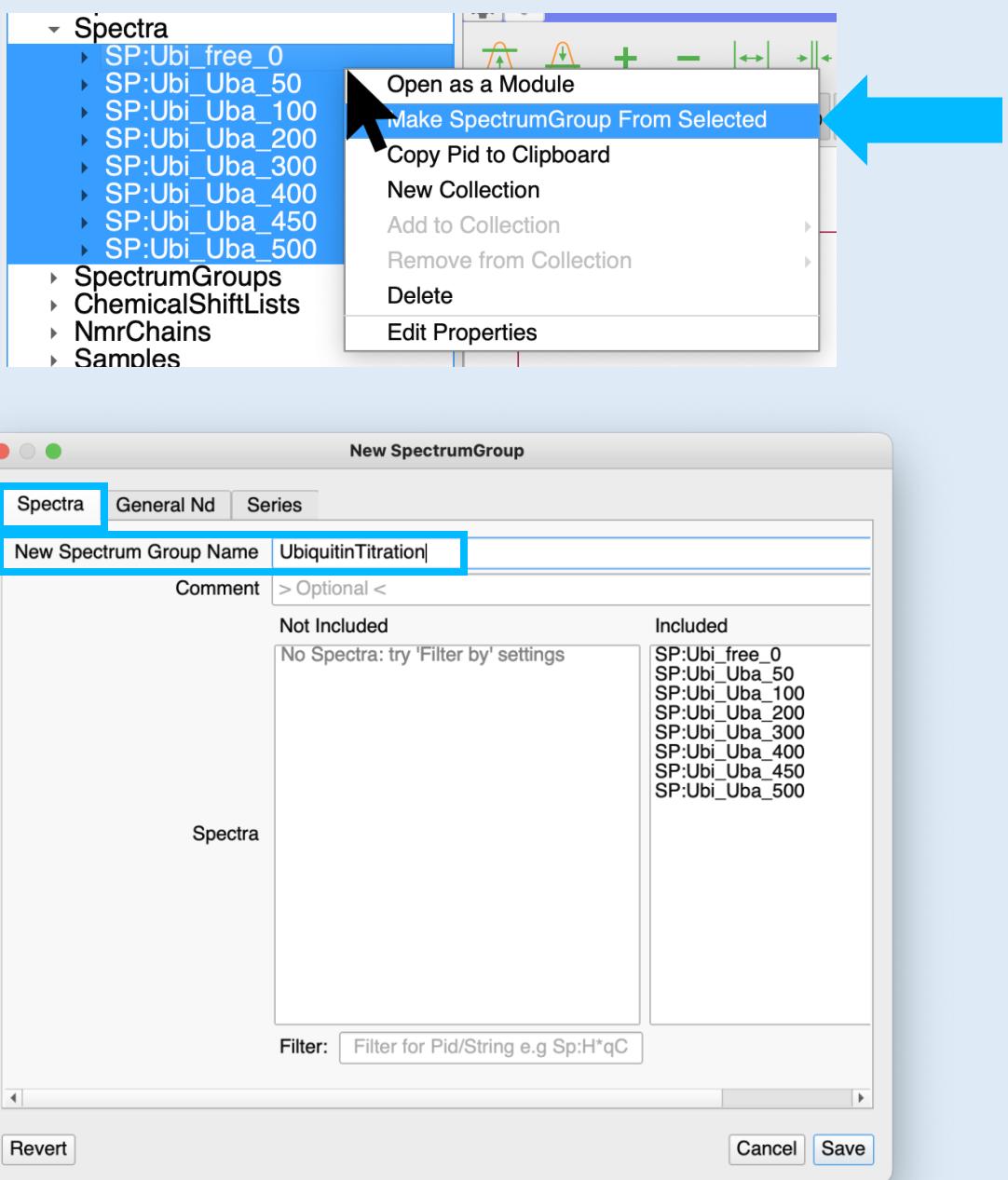
3A Open Project

If you haven't identified all the peak trajectories and copied the peak assignments to all spectra, then please continue with our prepared project,

UbiquitinTitrationPeaksPicked ccpn.

- Find the project directory **UbiquitinTitrationPeaksPicked 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

New SpectrumGroup

Spectra General Nd Series

Populate By < Select >

< Select >
Enumeration
Project Entry Order
Pid Number

Quantity Temperature
Noise Level
Clear All

Unit

Units

Quantity Time Temperature Substance Amount
 Concentration Distance Frequency
 Equivalent Volume
 Generic Mass

Unit M mM μM nM pM

Type Float Integer String

Values

SP:Ubi_free_0	0.0
SP:Ubi_Uba_50	50.0
SP:Ubi_Uba_100	100.0
SP:Ubi_Uba_200	200.0
SP:Ubi_Uba_300	300.0
SP:Ubi_Uba_400	400.0
SP:Ubi_Uba_450	450.0
SP:Ubi_Uba_500	500.0

Additional Values

Global Value

Revert Cancel **Save**

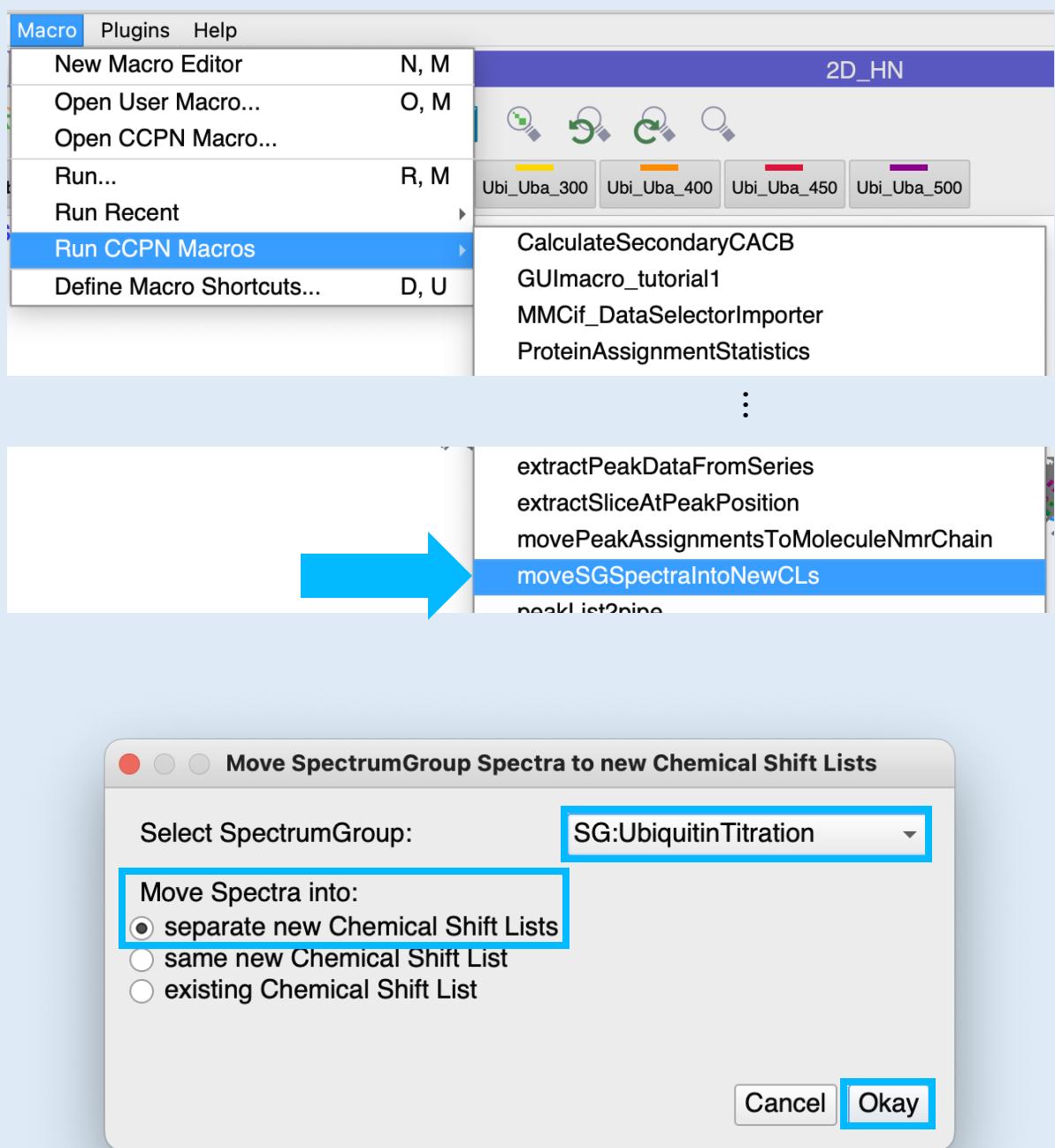
3C Enter Series information

- Move to the **Series** tab
- In the **Populate By** drop-down select **Pid Number**. This will take the final number of the Spectrum names (the Uba concentration in μM) to populate the spectrum variables in the **Values** table below. (Alternatively, enter the values by hand.)
- Select the following other options:

Quantity	Concentration
Unit	μM

- In the **Additional Values** section set the **Global Value** to **100**. This is the ubiquitin concentration (also in μM as above).
- Click **Save**.

Group Spectra



3D Move Spectra into separate Chemical Shift Lists

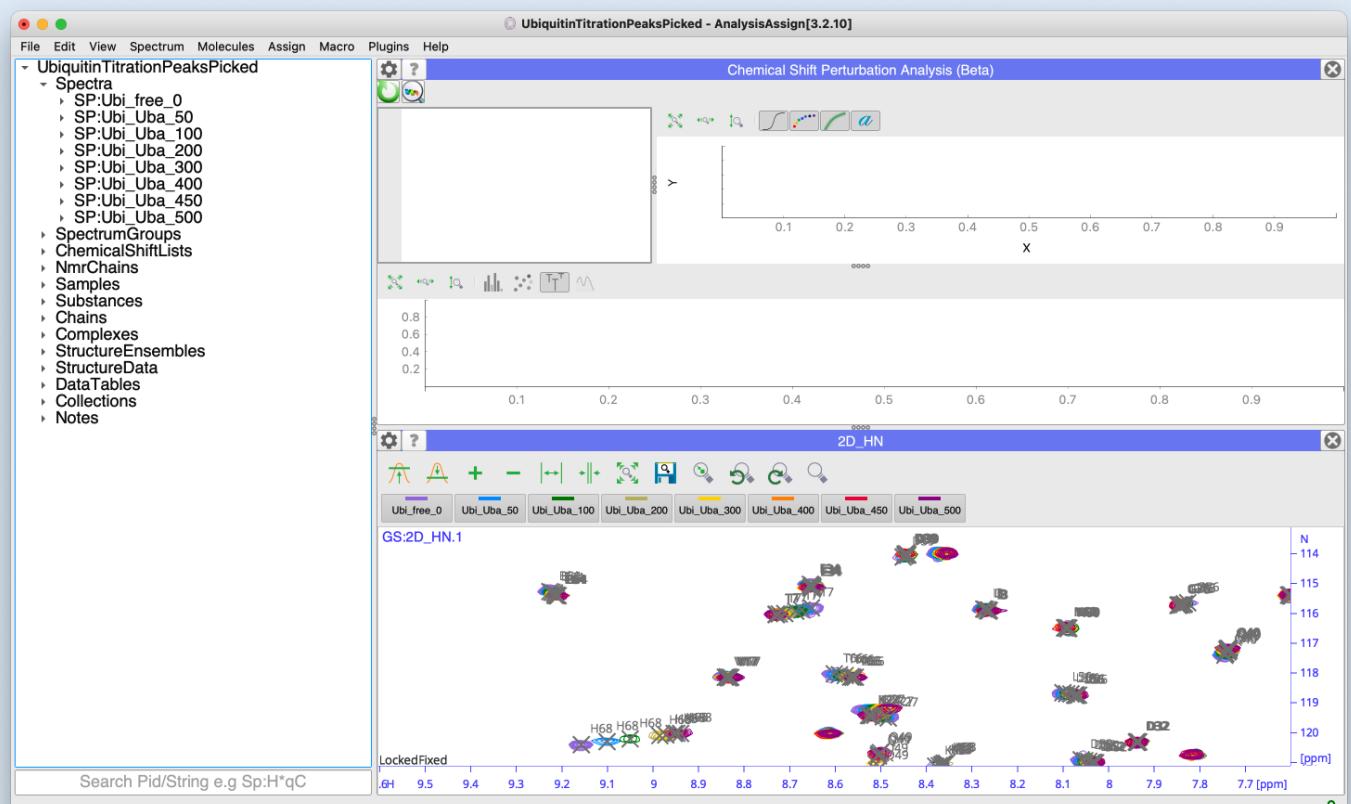
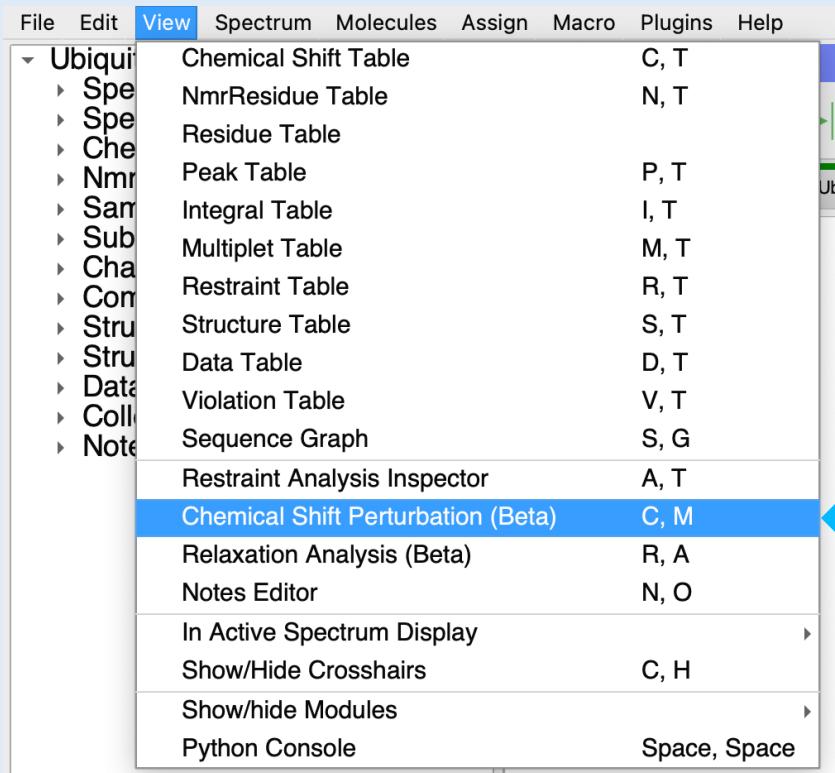
Because each of the Spectra in a titration was recorded under different conditions and the Chemical Shifts therefore vary, each Spectrum should be associated with a different Chemical Shift List. This can be done easily using a CCPN macro:

- Go to **Main Menu -> Macro -> Run CCPN Macros ->**
- moveSGSpectralIntoNewCLs**

- Select your Titration SpectrumGroup
- Select to move the Spectra into **separate new Chemical Shift Lists**
- Click **Okay**

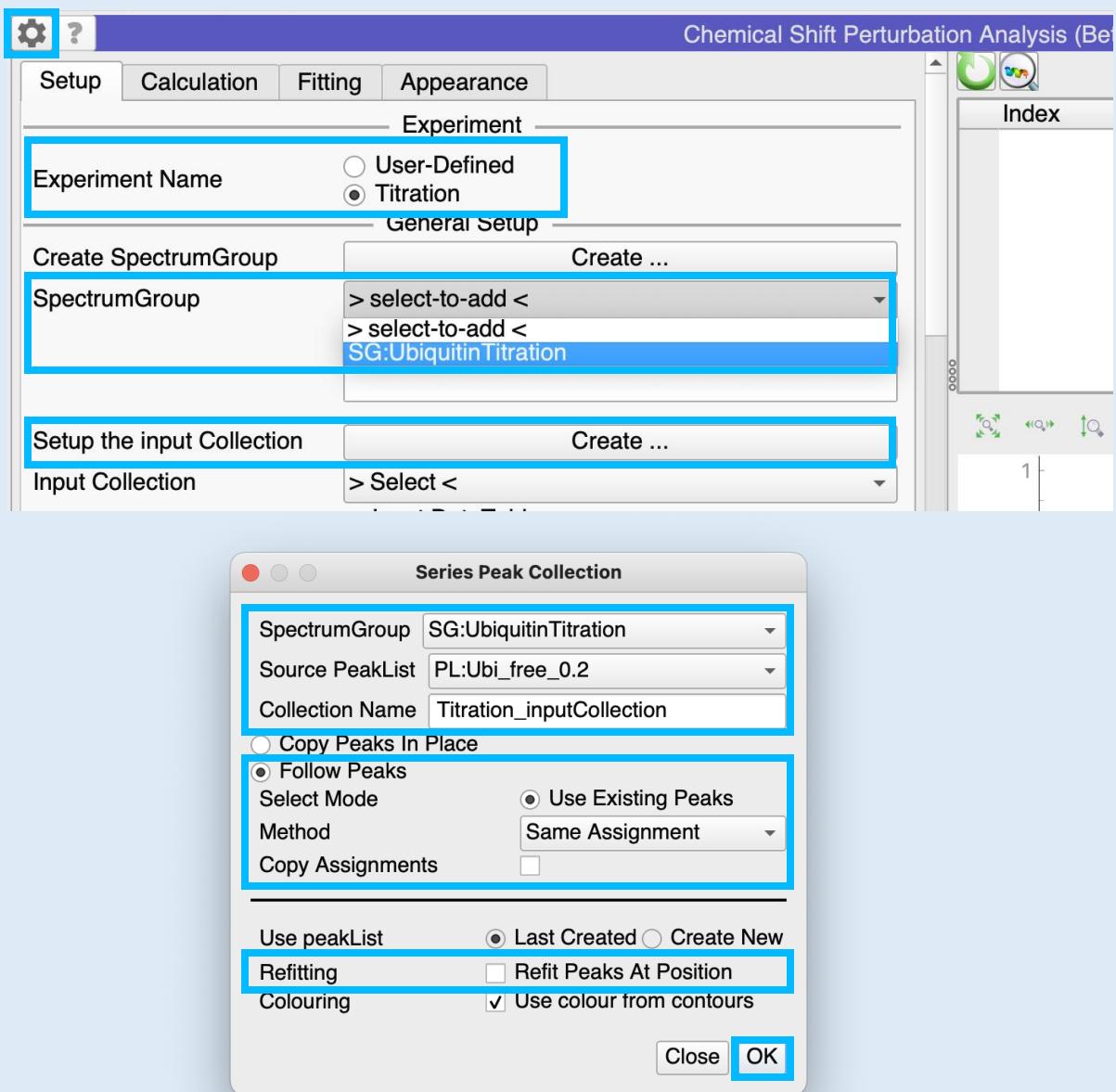
ChemicalShiftLists
<New ChemicalShiftList>
CL:default
CL:Ubi_free_0
CL:Ubi_Uba_50
CL:Ubi_Uba_100
CL:Ubi_Uba_200
CL:Ubi_Uba_300
CL:Ubi_Uba_400
CL:Ubi_Uba_450
CL:Ubi_Uba_500

Fit and Map Peaks



4A Open the Chemical Shift Mapping module

- Go to **Main Menu → View Chemical Shift Perturbation**
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 **Create ...** button to **Set up the 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:UbiquitinTitration
Source PeakList	PL:Ubi_free_0.2
Collection Name	Titration_inputCollection
Follow Peaks	
Method	Same Assignment (select from drop-down menu)
Refitting	Deselect Refit Peaks At Position

- Click **OK**.

Setup	Calculation	Fitting	Appearance
Experiment			
Experiment Name	<input type="radio"/> User-Defined <input checked="" type="radio"/> Titration		
General Setup			
Create SpectrumGroup	<input type="button" value="Create ..."/>		
SpectrumGroup	<input type="button" value="> select-to-add <"/> SG:UbiquitinTitration		
Setup the input Collection			
Input Collection	<input type="button" value="Create ..."/> CO:Titration_inputCollection		
Input DataTables			
Input DataTable Name	Titration_inputDataTable		
Create Input DataTable	<input type="button" value="Create"/>		
Input DataTable(s)	<input type="button" value="> select-to-add <"/> None		
Results DataTable Name	Titration_resultDataTable		
Results DataTable	<input type="button" value="Fetch and Compute"/>		
Results DataTable			
Results DataTable	<input type="button" value="> 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)	<input type="button" value="> select-to-add <"/> DT:Titration_inputDataTable
--------------------	---

The screenshot shows the CCPN Fit and Map Peaks software interface. The top navigation bar has tabs for Setup, Calculation, Fitting, and Appearance. The Calculation tab is currently selected and highlighted with a blue border.

Calculation Options: A dropdown menu labeled "ppmPosition" is open, showing "Calculation Options". Under this, "Euclidean Distance" is selected (radio button is checked).

Calculation Model Equation: A mathematical equation is displayed: $\sqrt{\frac{1}{N} \sum_{i=0}^N (c_i - \bar{c})^2}$.

Alpha Factors: Four input fields for "1H Alpha Factor", "15N Alpha Factor", "13C Alpha Factor", and "Other Alpha Factor" are shown, each containing the value 1.

Fitting Options: A dropdown menu labeled "Fitting Options" is open, showing various fitting models: Blank, Fraction Binding with Fixed Target Concentration (selected), Fraction Binding with Variable Target Concentration, Cooperative Binding, Monomer-Dimer Binding, Two Site Binding, and One Site with Allosteric Binding.

Model Name: An input field containing "Model Name".

Model Equation: A mathematical equation is displayed: $Y = B_{\max} \frac{(T + x + K_d - \sqrt{(T + x + K_d)^2 - 4Tx})}{2T}$.

Initial Values: A table showing initial values for parameters BMax, Kd, and T. All three rows have "Value" set to "Auto" and "Fixed" checked.

Input DataTable(s): A dropdown menu labeled "> select-to-add <" with "None" selected.

Results DataTable Name: An input field containing "Titration_resultDataTable".

Results DataTable: A dropdown menu labeled "Fetch and Compute" which is currently selected (highlighted with a blue border). Below it is a "Results DataTable" section with a dropdown menu labeled "> 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.

Here you can see the equations, variables and optimisation methods available for selection. Hover one of the Fitting Options will show you a ToolTip with references etc.

See also our background information about Titrations at

<https://www ccpn.ac.uk/manual/v3/Titrations.html> for further information and derivations.

- Click on **Fetch and Compute** 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.

Appearance

SpectrumDisplay

Select SpectrumDisplays: > select-to-add < (dropdown)

<Use active>

Single Click (radio button)

Double Click (radio button)

Disabled (radio button)

Plot Type: bar (radio button)

MainPlot: Mirrored To Table (radio button)

View Mode: Backbone (radio button)

Chain: MC:A (dropdown)

X Axis Data: nmrResidueCode (dropdown)

Y Axis Data: DeltaDelta (dropdown)

Threshold Value Calculation: Mean (dropdown)

SD Factor: 1 (input)

Threshold Value: 0.1987 (input)

4E Appearance Settings

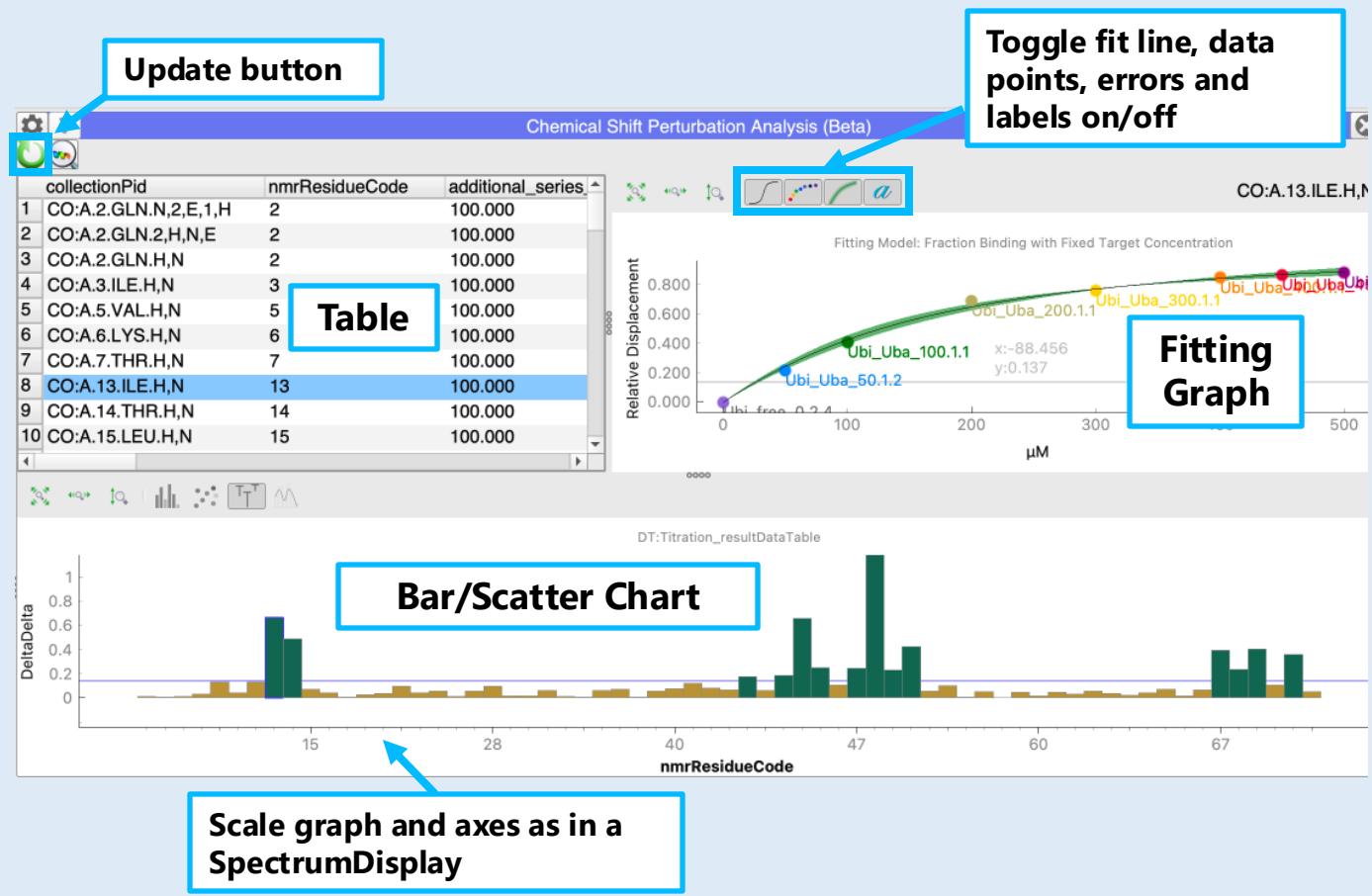
Select which data you would like to plot along the Y-axis of your graph. Options include:

- $\Delta\delta$ the measured Euclidean distances averaged over all but the first point (which is always 0)
- K_d the dissociation constant, a fitted parameter
- B_{max} the maximum chemical shift change ($\Delta\delta_{max}$), given by $\delta_{bound} - \delta_{free}$, a fitted parameter

You can select to show your data as a scatter or bar chart. Please note that for very small values (e.g. your K_d s) you may find that it is better to select a scatter plot initially, as this will autoscale better, before switching to a bar chart (a Qt bug which is difficult for us to code around!).

You can set your Threshold value automatically, manually in the Settings or interactively on the graph.

Fit and Map Peaks



4F 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

The screenshot shows a software interface for peak fitting and mapping. At the top left is a toolbar with icons for file operations. Below it is a table with columns: collectionPid, DeltaDelta, Kd, Kd_err, BMax, and BMax_err. A cursor is hovering over the Kd column header. To the right of the table is a histogram titled 'DeltaDelta' with a y-axis from 0 to 2 and an x-axis from 47 to 54. The histogram bars are dark green, with the highest peak at approximately 47.5.

	collectionPid	DeltaDelta	Kd	Kd_err	BMax	BMax_err
1	CO:A.49.GLN.H,N	1.805	73.269	9.500	2.500e-04	6.980e-06
2	CO:A.13.ILE.H,N	0.666	96.758	15.407	1.093e-04	4.485e-06
3	CO:A.43.LEU.H,N	0.659	82.34			
4	CO:A.14.THR.H,N	0.491	82.68			
5	CO:A.51.GLU.H,N	0.423	95.83			
6	CO:A.69.LEU.H,N	0.405	88.29			
7	CO:A.67.LEU.H,N	0.395	87.35			
8	CO:A.71.LEU.H,N	0.359	68.96			
9	CO:A.44.ILE.H,N	0.253	95.53			
10	CO:A.47.GLY.H,N	0.249	125.7			

A 'Column Settings' dialog box is open, showing a list of columns to display or hide. The 'nmrResidueCode' and 'additional series Step X' checkboxes are checked. Buttons for 'Check All' and 'Uncheck All' are at the bottom, and a 'Close' button is on the right.

Display Columns

collectionPid
 ROW_UID
 collectionId
 nmrResiduePid
 peakPid
 nmrChainName
 nmrResidueCode
 nmrResidueType
 nmrAtomNames
 seriesUnit
 series_Step_X
 series_Step_Y
 additional series Step X

A context menu is open over the table, with the 'Filter...' option highlighted. Other options include 'Copy clicked cell value' and 'Delete Selection'. Below the table is a detailed view of the data for the first five rows, showing more columns like DeltaDelta, Kd, Kd_err, BMax, BMax_err, and global.

	collectionPid	DeltaDelta	Kd	Kd_err	BMax
1	CO:A.49.GLN.H,N	1.805	73.269	9.500	2.500e-04
2	CO:A.13.ILE.H,N	0.666	96.758	15.407	1.093e-04
3	CO:A.43.LEU.H,N	0.659	82.34	8.797	1.036e-04
4	CO:A.14.THR.H,N	0.491	82.68	5.813	7.724e-05
5	CO:A.51.GLU.H,N	0.423	95.83		

	collectionPid	DeltaDelta	Kd	Kd_err	BMax	BMax_err	global
1	CO:A.2.GLN.H,N	0.015	0.001	0.320	1.561e-06	2.654e-07	nan
2	CO:A.3.ILE.H,N	0.032	136.961	35.905	5.823e-06	4.727e-07	nan
3	CO:A.5.VAL.H,N	0.132	162.134	34.732	2.542e-05	1.838e-06	nan
4	CO:A.6.LYS.H,N	0.040	214.719	78.206	8.583e-06	1.211e-06	nan
5	CO:A.7.THR.H,N	0.133	57.419	12.234	1.930e-05	7.932e-07	nan
6	CO:A.13.ILE.H,N	0.666	96.758	15.407	1.093e-04	4.485e-06	nan
7	CO:A.14.THR.H,N	0.491	82.681	5.813	7.724e-05	1.284e-06	nan

Include	.H,N
Filter in	<Visible Table>
<input type="button" value="Search"/> <input type="button" value="Reset"/> <input type="button" value="Close"/>	

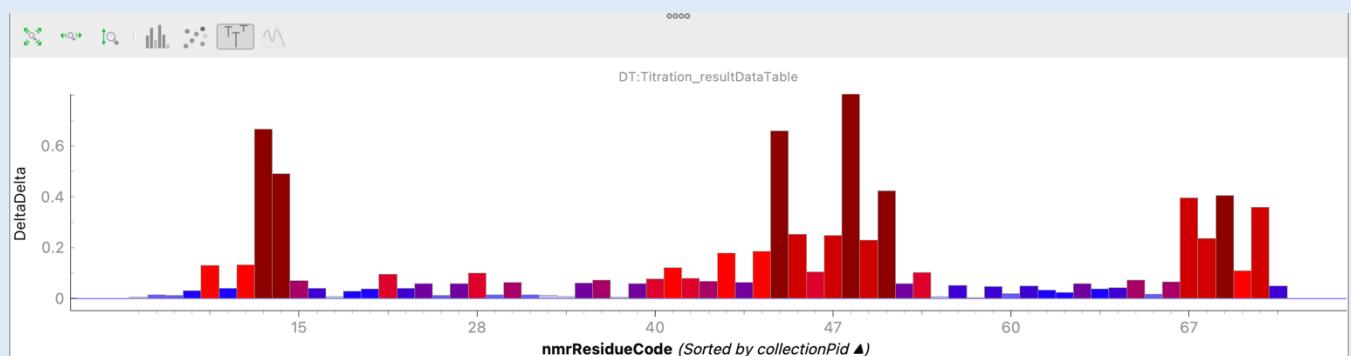
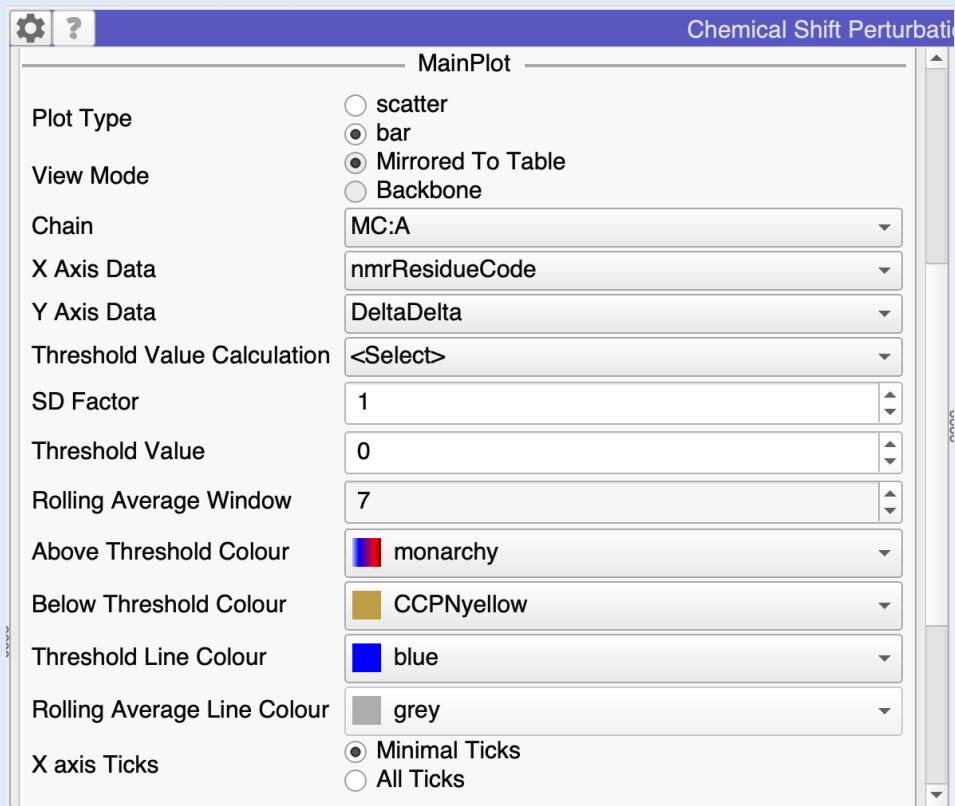
4G Inspect Results – Hints and Tips

- Click on a column heading to sort the table by the column, e.g. **collectionPid**, **nmrResidueCode**, $\Delta\delta$, K_d , B_{max}

Note: the graph mirrors the table, so this will also re-order!

- Right-click** on a column heading and select **Column Settings...** to select which columns to show/hide
- Right-click** on the table and select **Filter** to filter the table, e.g. so you only see backbone or side-chain data

Fit and Map Peaks



4G Inspect Results – Hints and Tips continued...

- In the **Appearance Settings**, set **Threshold Value Calculation** to **<Select>**,

Threshold Value to **0** and

select a gradient colour scheme for the **Above Threshold Colour**.

This will colour the bar chart on a gradient according to the height of the bars.

This is particularly useful if you want to go on to view your results on a structure in PyMol (see **Section 5**).

Fit and Map Peaks

Chemical Shift Perturbation Analysis (Beta)

collectionPid	DeltaDelta	Kd	Kd_err	BMax	BMax_err	globalFitt
12 CO:A.30.LEU.H,N	0.230	57.037	10.440	3.342e...	1.17e-06	nan
13 CO:A.42.ARG.H,N	0.187	66.800	9.407	2.791e...	7.718e-07	nan
14 CO:A.41.GLN.H,N	0.179	119.846	16.024	3.109e...	1.201e-06	nan
15 CO:A.7.THR.H,N	0.133	57.419	12.234	1.930e...	7.932e-07	nan
16 CO:A.5.VAL.H,N	0.132	162.134	34.732	2.542e...	1.838e-06	nan
17 CO:A.40.GLI	Filter...		11.990	2.093e...	6.037e-07	nan
18 CO:A.70.VAL.H,N	Copy clicked cell value		12.457	1.594e	6.470e-07	nan
19 CO:A.45.PHE.H,N	Delete Selection					
20 CO:A.54.AR	Clear Selection					
21 CO:A.28.AL	Export Visible Table					
22 CO:A.25.AS	Export All Columns					
23 CO:A.40.GLI	Edit Collection					
24 CO:A.10.GLI	Refit Collection(s) Individually...					
	Refit Collections Globally...					
	Exclude NmrResidue(s)					
	Include NmrResidue(s)					

Right-click context menu options: Filter..., Copy clicked cell value, Delete Selection, Clear Selection, Export Visible Table, Export All Columns, Edit Collection, Refit Collection(s) Individually..., Refit Collections Globally..., Exclude NmrResidue(s), Include NmrResidue(s).

Blue arrow points to the "Refit Collections Globally..." option in the context menu.

Refit Collections Globally dialog:

- Selected residues: CO:A.44.ILE.H,N, CO:A.54.ARG.H,N, CO:A.40.GLN.H,N, CO:A.5.VAL.H,N, CO:A.45.PHE.H,N, CO:A.42.ARG.H,N, CO:A.51.GLU.H,N, CO:A.43.LEU.H,N, CO:A.47.GLY.H,N, CO:A.49.GLN.H,N, CO:A.71.LEU.H,N, CO:A.13.ILE.H,N, CO:A.7.THR.H,N
- Fitting Model: Fraction Binding with Fixe
- Minimiser Method: leastsq
- Initial Fitting Params: +
- Buttons: Close, Refit

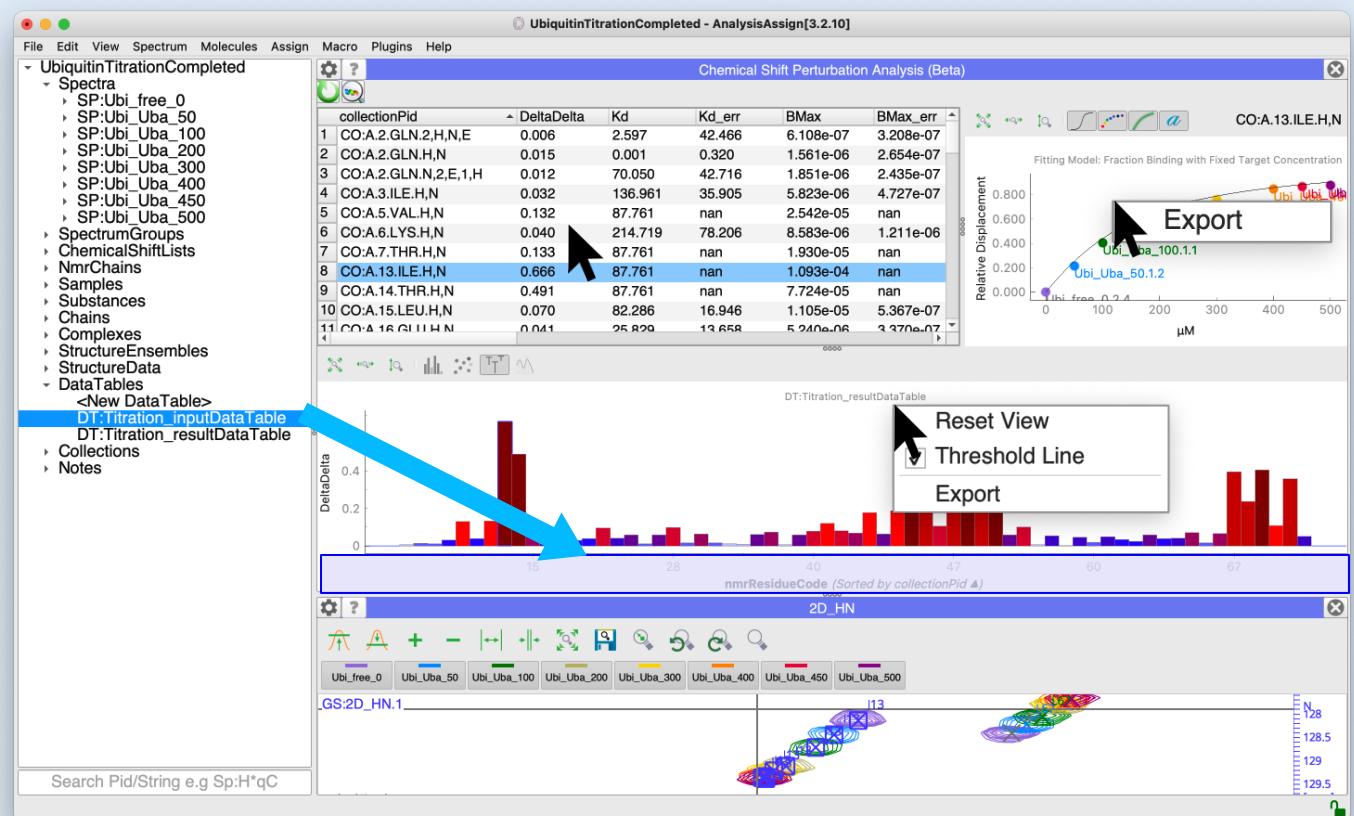
Chemical shift perturbation plot showing peaks for Ubi_Uba_200.1.24, Ubi_Uba_300.1.26, Ubi_Uba_100.1.73, and Ubi_Uba_400.1.06. The x-axis is labeled μM.

4H Global Fitting

- In the table, select all the rows which you would like to fit together.
- Right-click** on the table and select **Refit Collections Globally...**
- Confirm the settings and click on **Refit**.

There are several different ways in which you might decide which residues to fit together. See Hobbs et al. 2023 (<https://doi.org/10.1007/s10858-022-00402-3>) for a helpful discussion on this.

Fit and Map Peaks



4| Export Results

- Right-click in any of the the areas of the Chemical Shift Perturbation 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.

The screenshot shows a 'DataTable' window with the title 'DT:Titration_inputDataTable'. The table has columns: dimension, isotopeCode, series_Step_X, seriesUnit, experiment, ppmPosition, height, lineWidth, and volume. The data consists of multiple rows of experimental measurements. A context menu is open over the cell at row 2, column 2 ('15N'). The menu options are: Filter..., Copy clicked cell value, Delete Selection, Clear Selection, Export Visible Table, and Export All Columns. The cell is highlighted with a yellow background.

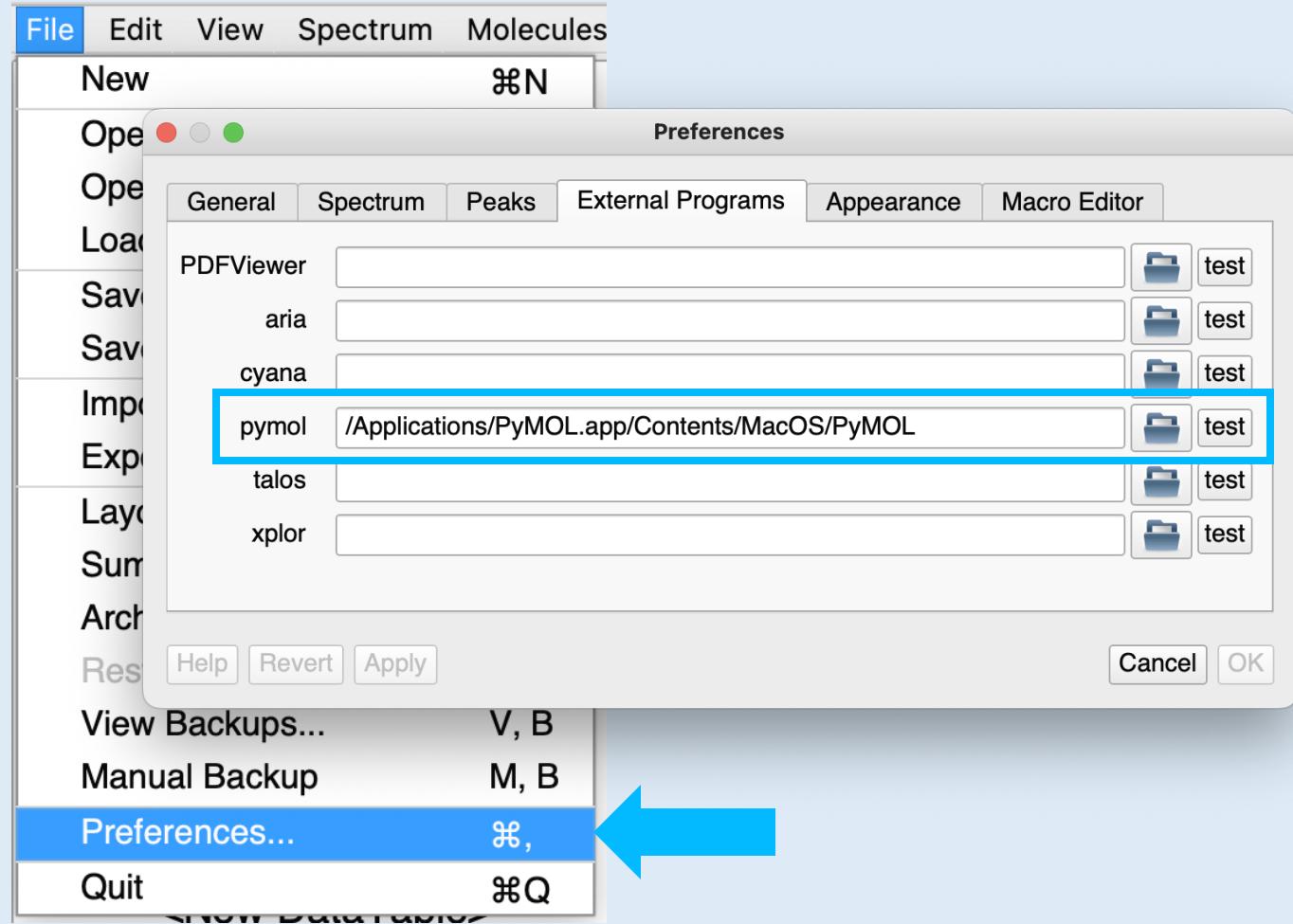
Note that the input DataTable lists all the data per dimension. In our online Macro Library we also have a macro which will allow you to create a DataTable which lists the data per peak. This is useful if you wish to export the data to another program. See <https://ccpn.ac.uk/macro-library/getting-titration-data-on-a-per-peak-basis/>

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 to 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 Perturbat

Setup Calculation Fitting Appearance

Molecular Structure File: 1ubq.pdb

Chemical Shift Perturbation Analysis (Beta)

CO:A.13.ILE.H,N

Fitting Model: Fraction Binding with Fixed Target Concentration

Relative Displacement

0.000 0.200 0.400 0.600 0.800

0 100 200 300 400 500 μM

Ubi_Uba_free

Ubi_Uba_50.1.2

Ubi_Uba_100.1.2

Ubi_Uba_200.1.1

Ubi_Uba_300.1.1

Ubi_Uba_400.1.1

Ubi_Uba_450.1.1

DT:Titration_resultDataTable

DeltaDelta

nmrResidueCode (Sorted by collectionPid ▲)

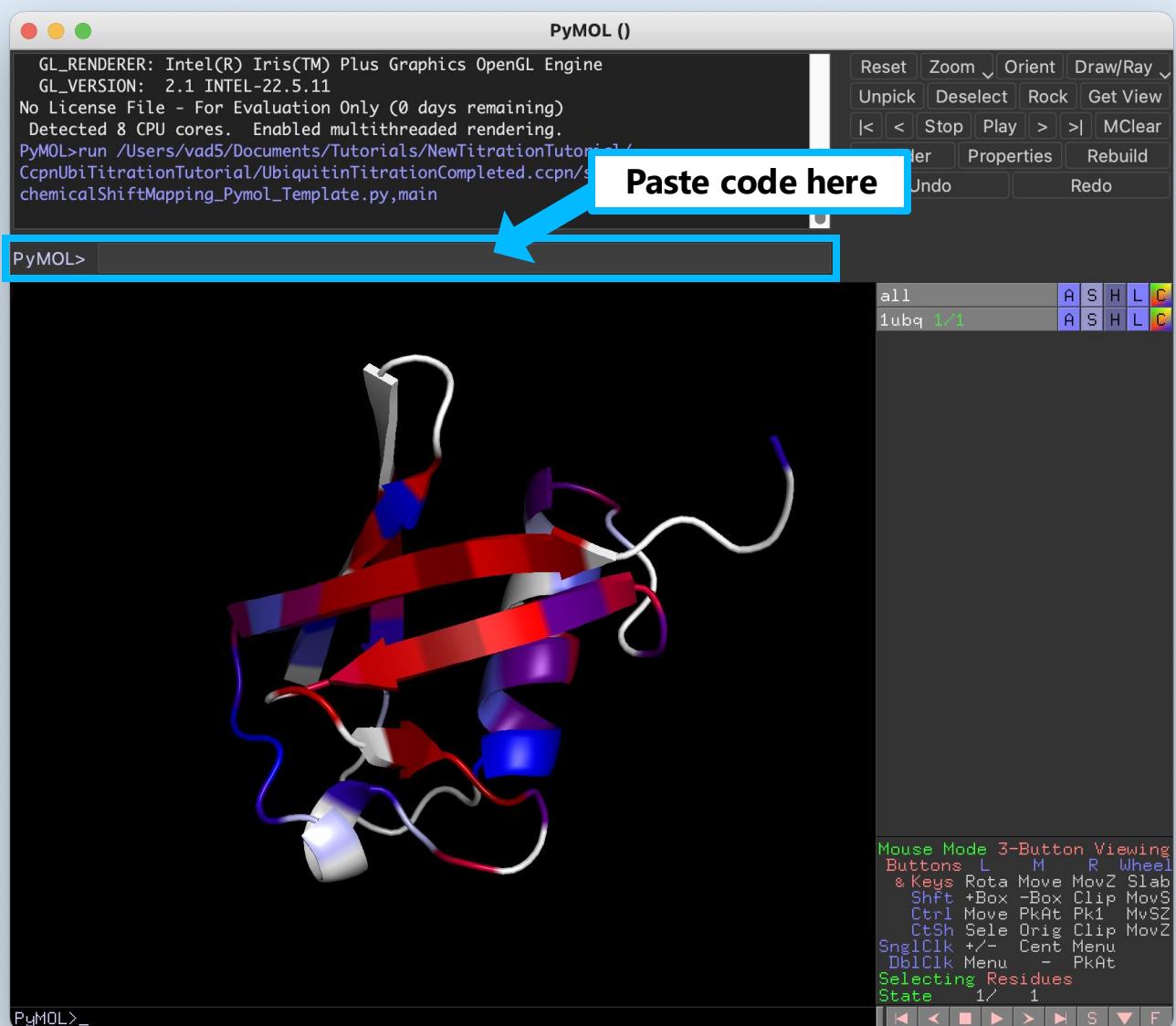
collectionPid	DeltaDelta	Kd	Kd_err	BMax	BMax_err
1 CO:A.2.GLN.2,H,N,E	0.006	2.597	42.466	6.108e-07	3.208e-0
2 CO:A.2.GLN.H,N	0.015	0.001	0.320	1.561e-06	2.654e-0
3 CO:A.2.GLN.N,2,E,1,H	0.012	70.050	42.716	1.851e-06	2.435e-0
4 CO:A.3.ILE.H,N	0.032	136.961	35.905	5.823e-06	4.727e-0
5 CO:A.5.VAL.H,N	0.132	87.761	nan	2.542e-05	nan
6 CO:A.6.LYS.H,N	0.040	214.719	78.206	8.583e-06	1.211e-0
7 CO:A.7.THR.H,N	0.133	87.761	nan	1.930e-05	nan
8 CO:A.13.ILE.H,N	0.666	87.761	nan	1.093e-04	nan
9 CO:A.14.THR.H,N	0.491	87.761	nan	7.724e-05	nan
10 CO:A.15.LEU.H,N	0.070	82.286	16.946	1.105e-05	5.367e-0
11 CO:A.16.GLU.H,N	0.041	25.829	13.658	5.240e-06	3.370e-0

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 **1ubq.pdb** file in the tutorial data directory.
- Close the Settings panel again.
- Click on the Molecular Viewer button open PyMOL with the ubiquitin 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



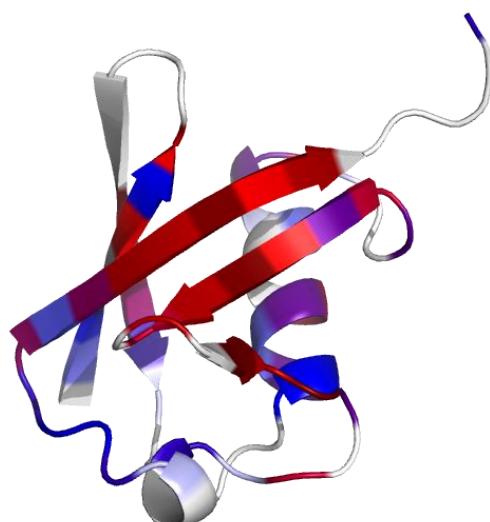
5C Export PyMOL image with transparent background (optional)

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/ubiquitin.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)