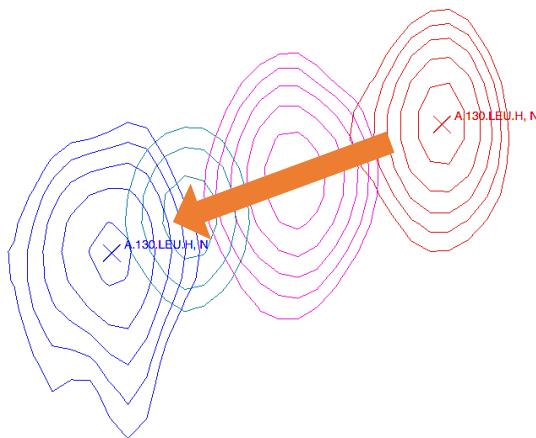


Chemical Shift Perturbation Tutorial



Introduction

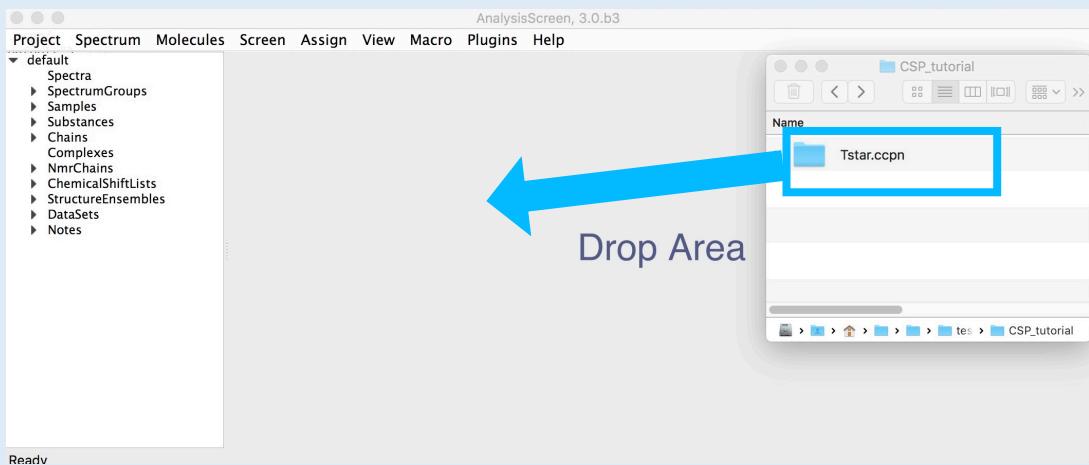
NMR is a very powerful technique to map the interaction between two biological partners. The peaks on the spectrum represent the correlation between the amide protons and the nitrogen connected to it (H – N) (^{15}N – HSQC). Each peak represents an NH of each residue (except for the first residue and the prolines), the side chain of a few amino acids (Asn, Gln, His and Trp) can also give a peak. The chemical shift (nitrogen and proton) is sensitive to the chemical environment of the two nuclei. Any chemical environment modifications of amino acids by the addition of another molecule will affect the position of the peaks in the spectrum. The analysis and the mapping of these shifts on the protein structure will give information on the binding interface. In this NMR practical, you will have to analyse the ^{15}N – HSQC spectra of a ^{15}N labelled TSTAR protein in complex with RNA and to map this interaction onto the structure of the protein.



Start CcpNmr Analysis V3

Apple users by double clicking the icon  *CcpNmrAnalysis*

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



1A Drag & drop “Tstar ccpn” into the sidebar or blank display.

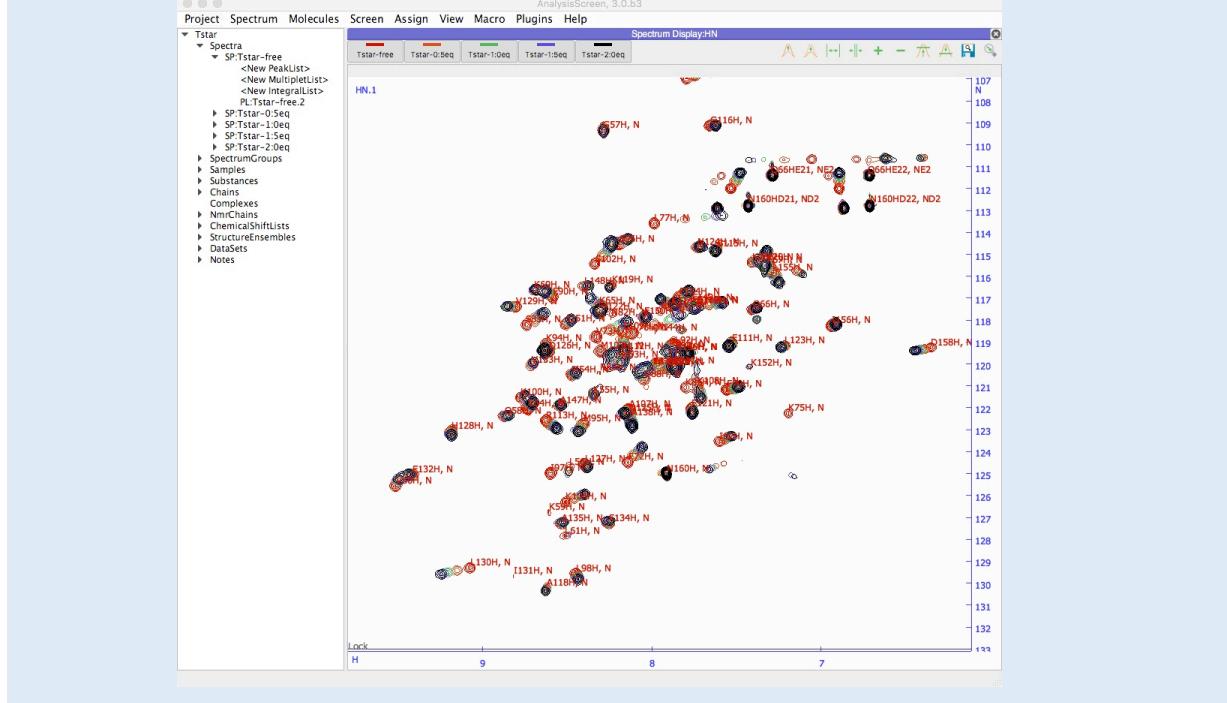
CcpNmr projects have an extension of type “filename.ccpn”. Find the project file *Tstar.ccpn* in the top directory or in AnalysisV3/data.

- Select the file “**Tstar.ccpn**”, drag and drop into the program. The Tstar project will be loaded in a new window.

You will see five spectra, displayed as:

- Tstar-free* (red)
- Tstar-0:50eq* (orange)
- Tstar-1:00eq* (green)
- Tstar-1:50eq* (blue)
- Tstar-2:00eq* (black)

Open Project



Getting started, basic operations

Sidebar

All spectra and peak lists are located in sidebar. Double click on an item will open the properties popup.

Display

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

If you close a display. You can open a new one from main menu -> view -> *New Blank Display*. Drag and drop into it the spectra from sidebar.

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 hold Middle and drag

Shortcuts

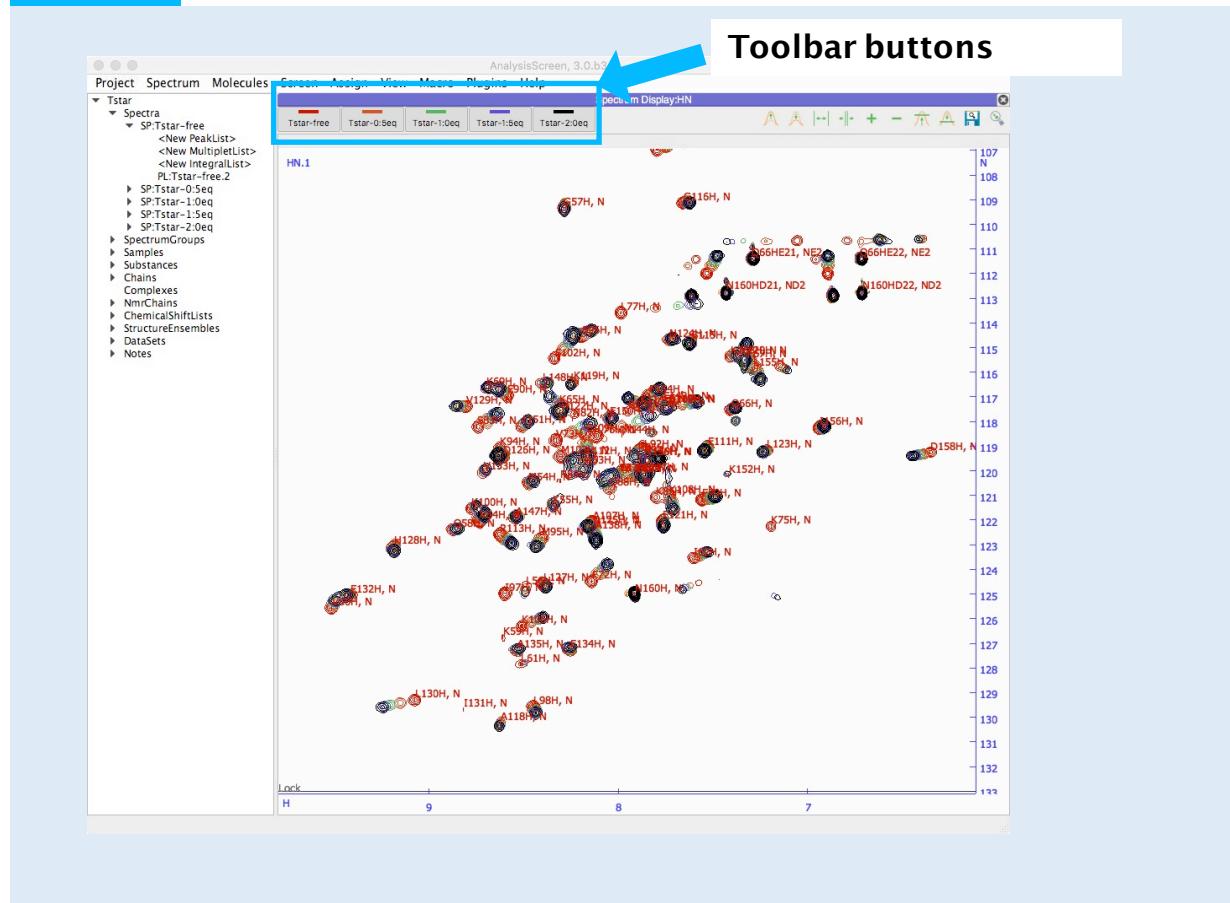
The program uses several shortcuts, example “CL” for copying a peak list.

You will need to press the first letter on your keyboard e.g. “C”, followed by the second letter, e.g. “L” .

For more commands and operations:

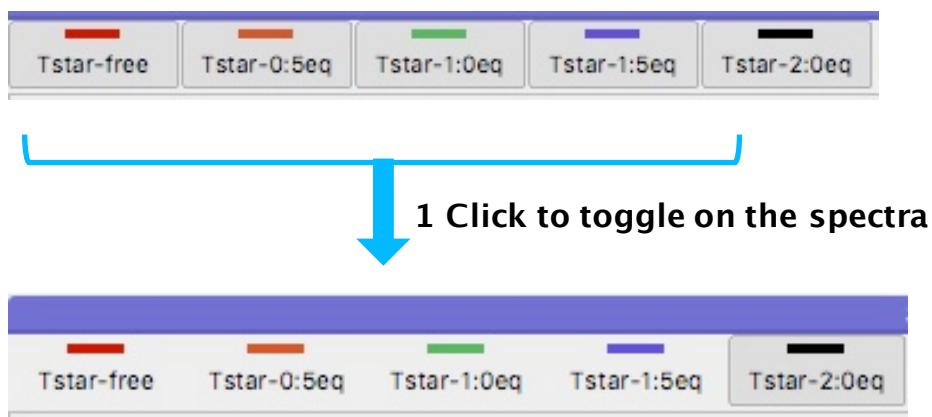
main menu -> *help* -> *Tutorial (Beginners)* or *Show Shortcuts*

Open Project

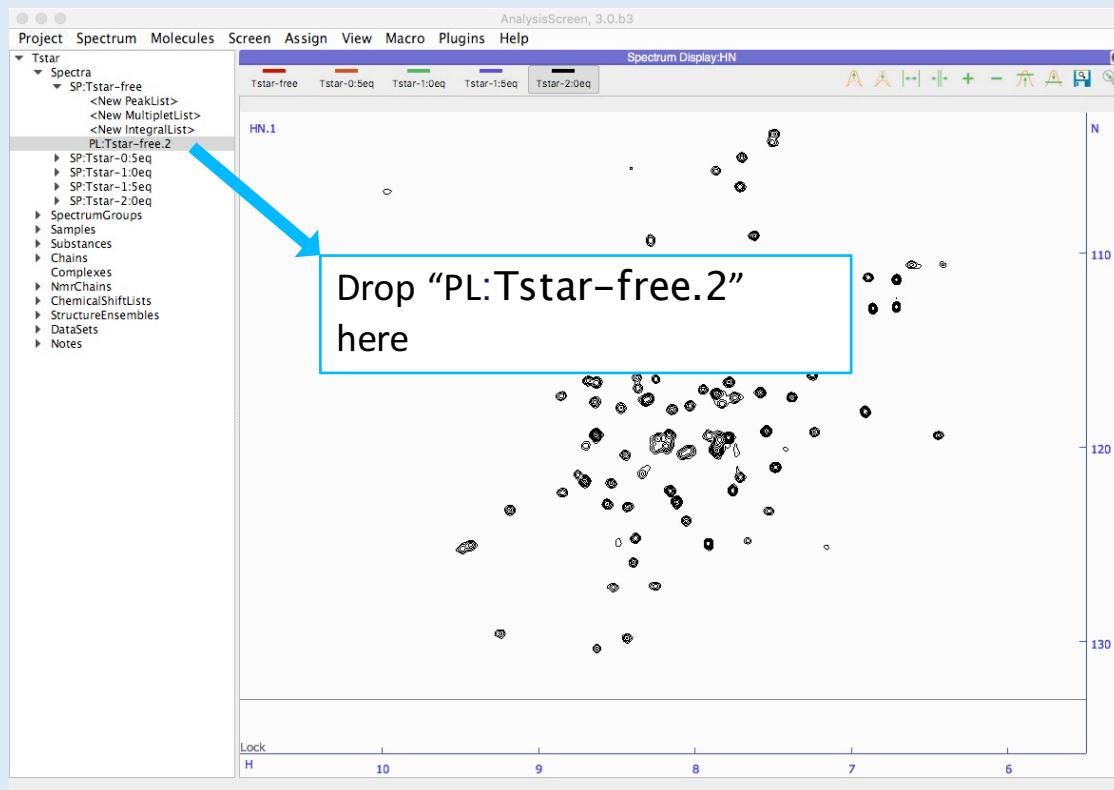


1B Toggle off all spectra apart from Tstar-2:00eq on the toolbar.

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



Copy Peak List



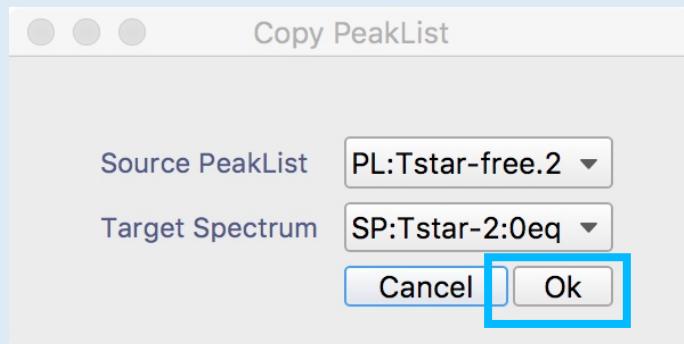
Only the Free-Form Tstar has a peak list. You will need to copy this peak list to a spectrum recorded with Tstar bound to the RNA.

2A Drag & drop the peak list tree item “PL:Tstar-free” into the spectrum display.

- On side bar, expand the tree:
 - ▼ tStar
 - ▼ Spectra
 - ▼ SP:Tstar-free
 - PL:Tstar-free.2**

- Click to select PL:a-Tstar-free.2, drag and drop the item into the spectrum display.

Copy Peak List



2B Select target Spectrum: e-200.

A new popup will open, the first entry is the peak list source you want to copy; the second entry is the target spectrum, where the peak list will be copied.

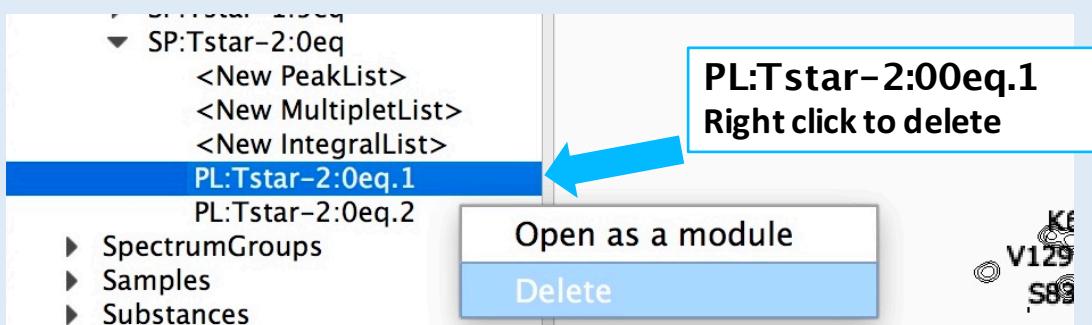
Select:

- *Source PeakList:* PL: Tstar-free
- *Target Spectrum:* SP:Tstar-2:00eq
- Press Ok

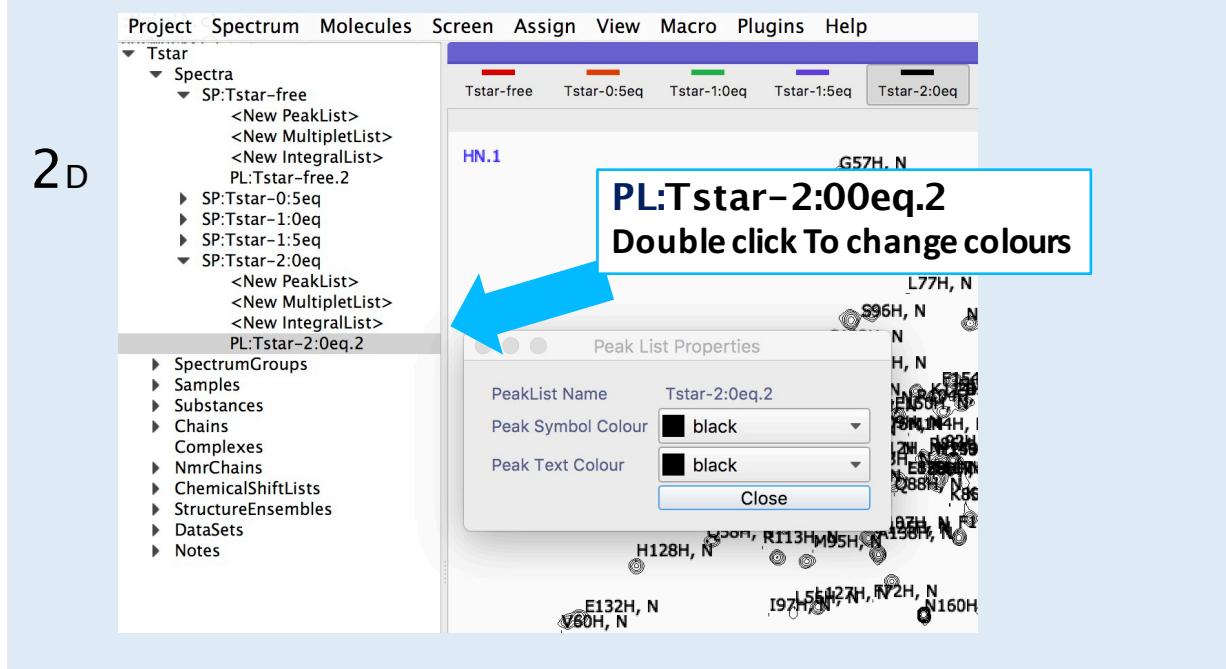
2

Copy Peak List (Optional)

2C



2D



2C Delete “PL:Tstar-2:0eq.1” from side bar.

The default peak list for the spectrum Tstar-200 is empty. For simplicity delete it from side bar. Right click on the item Tstar-2:0eq.1 then click delete.

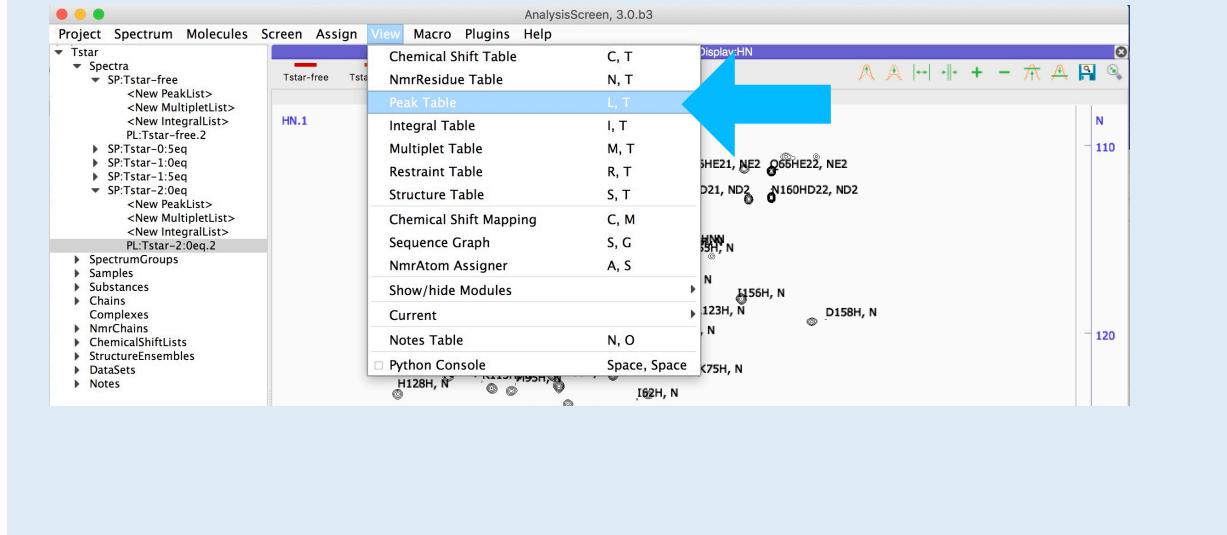
2D

Double Click on sidebar item “PL:Tstar-2:0eq.2 ” to change colours.

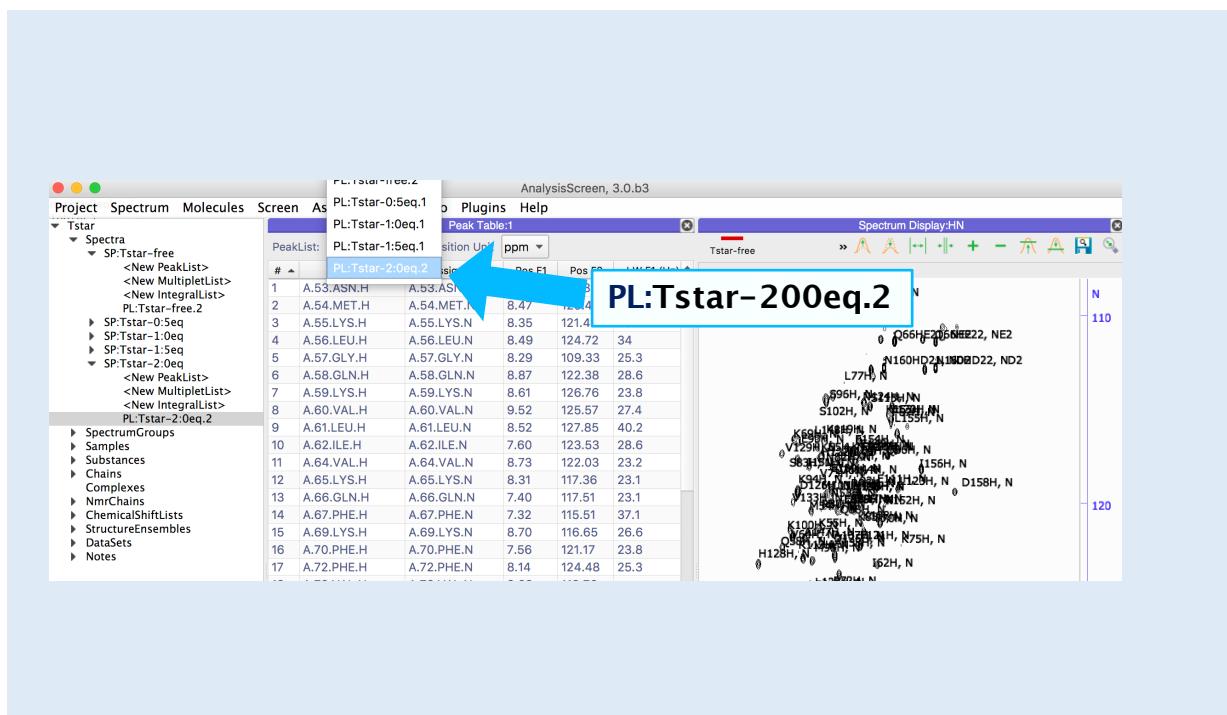
- Expand the spectrum item tree *SP:e-tStar-200* on side bar, like previously for the *SP:free-tStar*
 - Double click on “PL:Tstar-2:0eq.2
- A new popup will open.
- Select the desired colours for text labels and symbols. Close the popup.

Tip: use the same colour as the spectrum contours.

Move Peaks



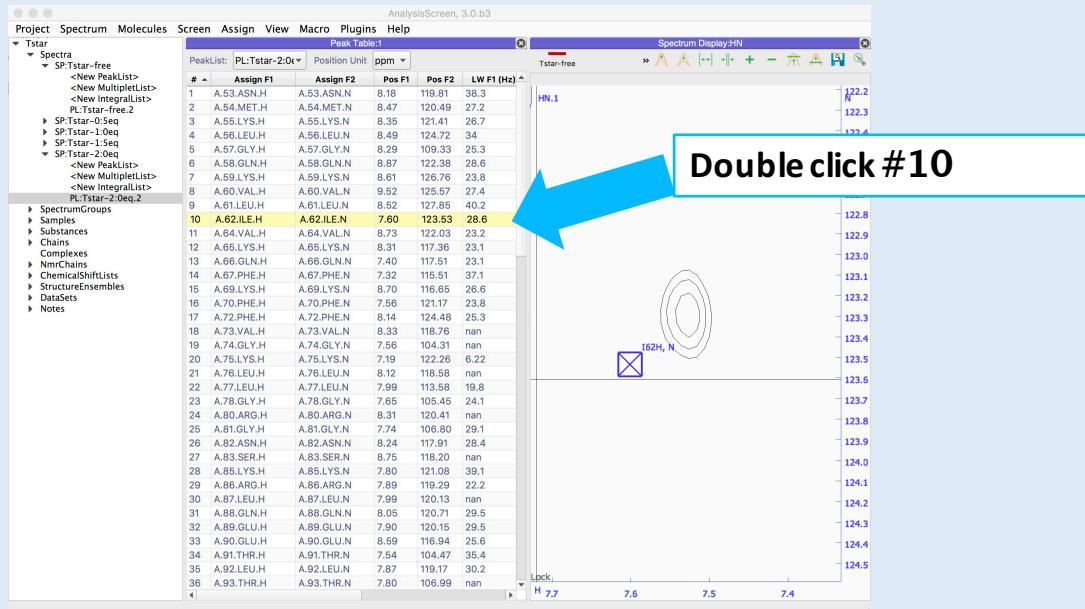
3B Open peak table, shortcut “LT”. Open a peak table with the shortcut "LT" or from the main menu -> view-> Peak Table.



3C Select PL:e-Tstar-200.2.

On the top left corner of the peak table there is a pulldown menu, select the newly created peak list: PL:Tstar-2:00eq.2.

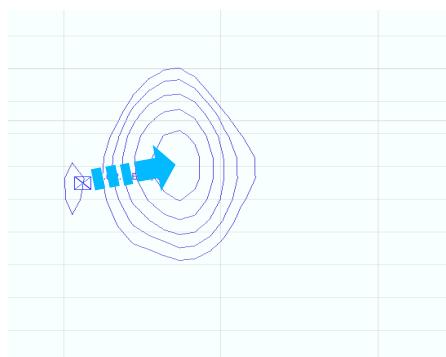
Move Peaks



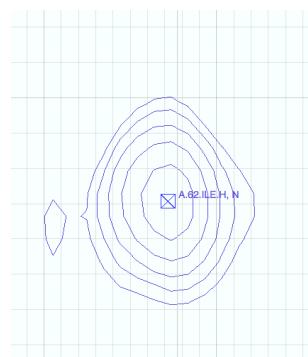
This peak list was copied from Tstar without ligand, therefore, some peak will have changed position upon titration with the ligand. You will have to find the shifted peaks and correct their position.

3D Move peaks manually.

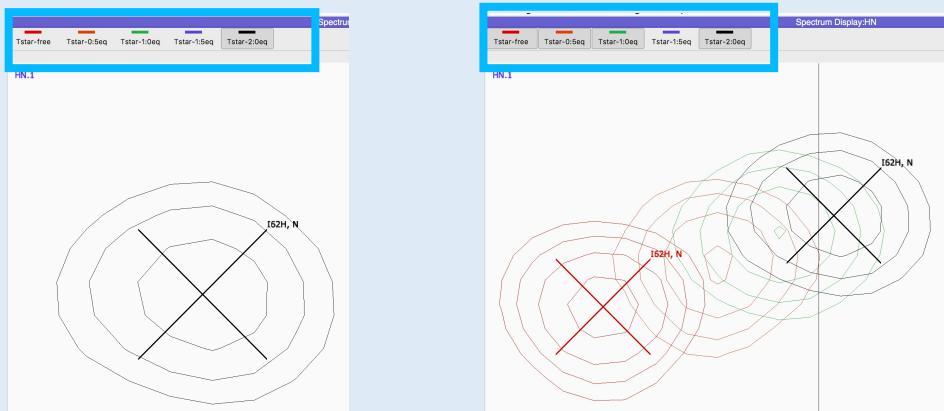
- Locate a single peak and zoom in using the middle mouse wheel, until it's in the middle of the display. Hold down the left mouse button and drag to pan the spectrum.
- On the peak table, double click on a row, e.g. # 10 | A.62.ILE. The spectrum display will navigate to the selected peak coordinates.
- Move the mouse cursor on top the selected peak [X]: hold down the **middle mouse wheel button and drag** across the selected peak. The peak will start to move, when you are in the correct position, release the mouse button.
- Use the shortcut "SE" to centre the peak to its extremum.



hold middle mouse and drag



Release



To change the order how spectra are displayed: Middle mouse on the toolbar button and drag and drop the button to a new positon in the toolbar

3E Map the trajectory.

To map the trajectory of the peak shifts it is important to check the other spectra too.

- toggle on and off manually the spectra on the toolbar to see any differences in the peak positions across the different spectra.

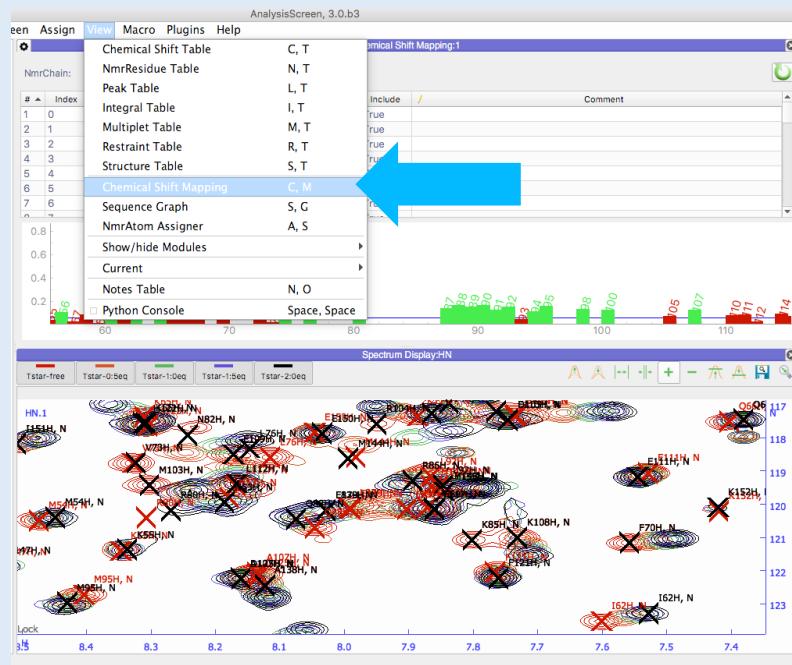
Useful shortcuts:

- “Tab–Tab” (press twice →→)
- Displays the next spectrum and hides all others
- “Tab–A” : Displays all spectra
- “Tab–x” : Reverts displayed spectra

Repeat points 3D and 3E for all other peaks in the project. In short:

- Double click on a peak table row
- View the peak perturbation across the different spectra toggling on/off the spectra on the toolbar
- Move the Tstar-2:00eq peak (you can move multiple peaks at the same time and use the shortcut “SE” on all selected peaks)
- Save the project frequently

Map Peaks



The **Chemical Shift Mapping module** includes a bar chart plot, a residue table and a settings panel.

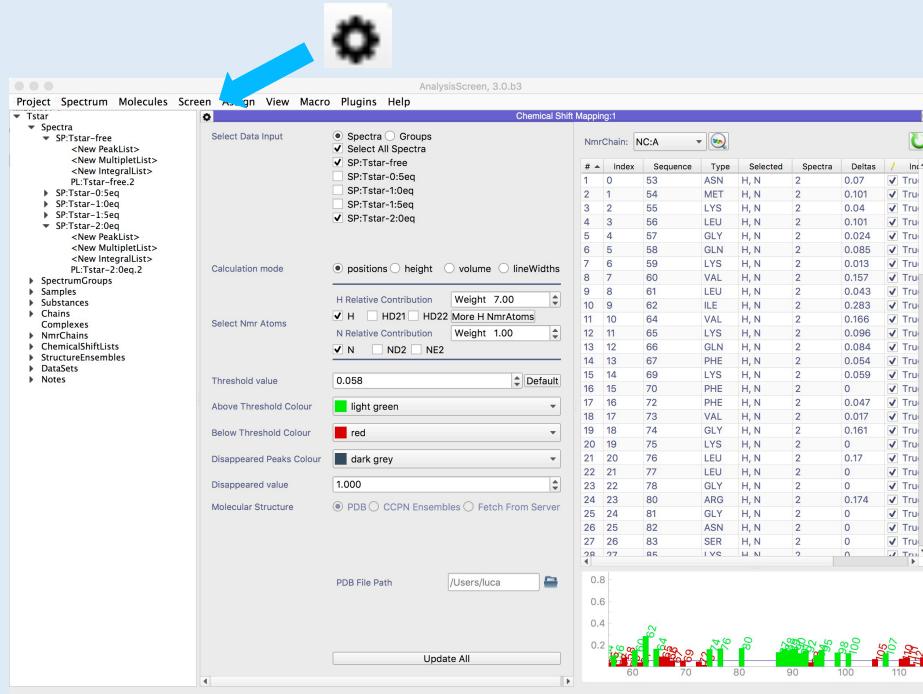
As previously stated, each peak represents a single residue in the protein (apart from the sidechain peaks). From now on, we will refer as *NmrResidue* for a residue assigned to its spectrum peak. In green are plotted the NmrResidues that have moved above a threshold of 0.1 ppm; in red the others.

4A Open the Chemical Shift Mapping module, shortcut “CM”.

Once you have moved all peaks correctly, inspect the chemical shift perturbations using the Chemical Shift Mapping module.

- Go to the main menu -> *View* -> *Chemical Shift Mapping* module or use the shortcut “CM”.

Map Peaks

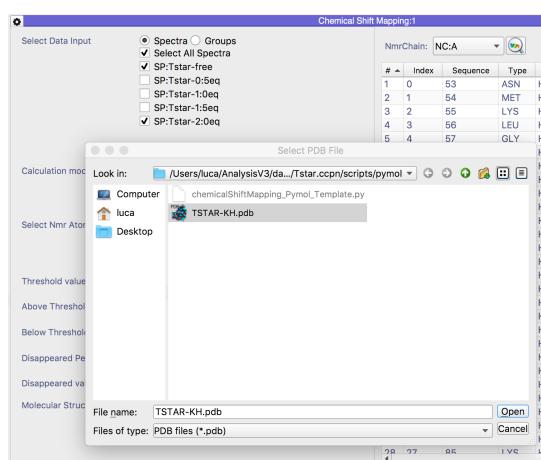


4B Changing the default values.

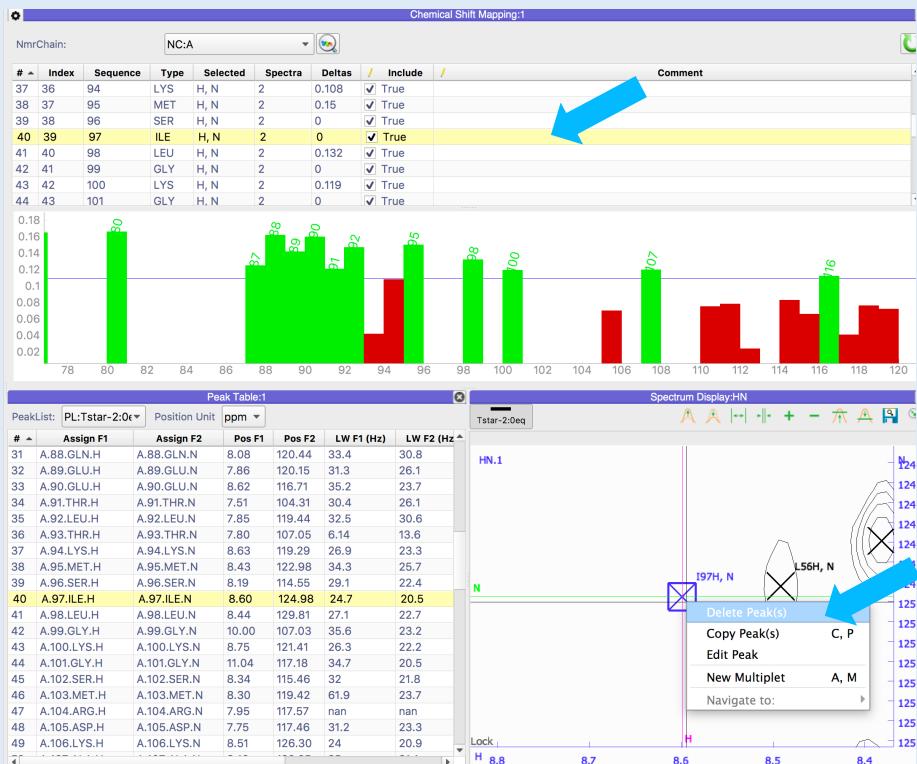
Click the “gear icon” in the top left corner of the module

Set:

- *Relative Contribution*: H = 6.5, N = 1
- *Threshold line*: 0.200 ppm
- Leave Select Atoms as the default
- You might want to change the plot colours
- *PDB file Path*: click the folder icon and select TSTAR-KH.pdb in the pymol directory. Full path tStar ccpn/scripts/pymol/TSTAR-KH.pdb
- Click the *Update All* button.



Map Peaks (Optional)



4C Find the disappeared Peak.

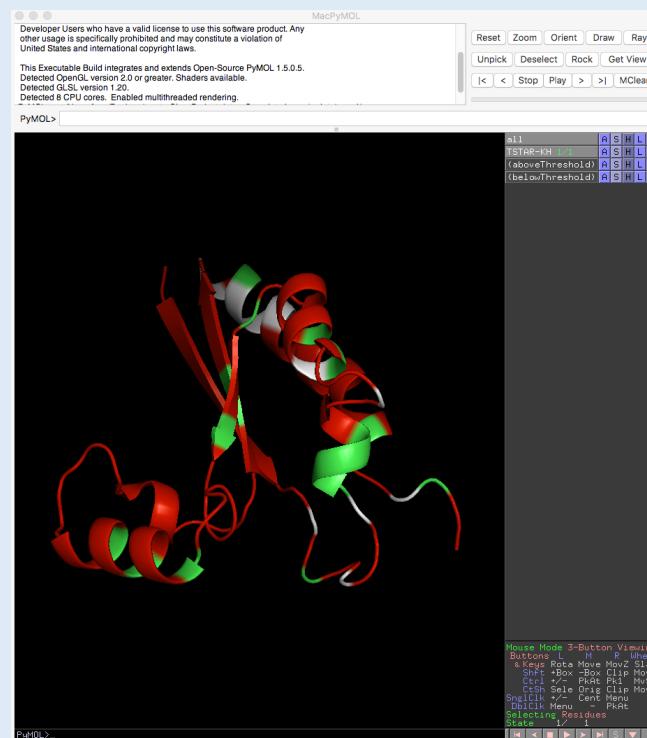
In this protein a peak has disappeared after the titration with the ligand.

- If present in the plot, double click on the residue bar 97 to navigate to the residue coordinates on the spectrum display, otherwise double click on the peak table #40 | A.97ILE.H | A.97ILE.N.
- Right click and Delete the peak from the spectrum Tstar-2:00eq. You can add a comment on the *Comment* column Chemical Shift Mapping table cell by double clicking inside the cell.
- The plot will automatically refresh and mark the residue as "missing"

4D Export plot.

- Right click in the plot in an empty space
- Click *Show Label Above Threshold*
- Click *Export*
- On the Export popup Select Image File, then click export
- Save the file





PyMOL is a Python-enhanced molecular graphics tool. It excels at 3D visualisation of proteins, small molecules, density, surfaces. The visualisation through PyMOL of the perturbed residues on the surface's protein can be started using CcpNmr.
Download and install from https://pymolwiki.org/index.php/MAC_Install

5A Link PyMol to CcpNmr Analysis

- Main menu → Project → Preferences...
- click on External Programs tab
- on PyMol, click on the folder icon,
- search on you disk the executable PyMol file, on Mac will be on:
`/Applications/MacPyMOL.app/Contents/MacOS/MacPyMOL`
- click on Test, Pymol will start if has been linked successfully
- click Ok

5

PyMOL

5B Click “Show on Molecular Viewer”.

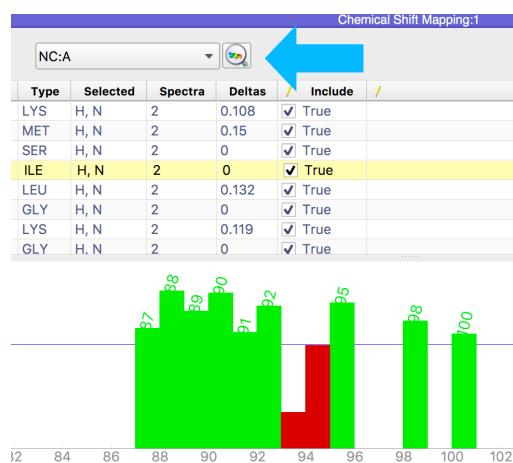
By clicking the button



on the Chemical Shift mapping inside CcpNmr Analysis, PyMol will be launched 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*) , same colour as the bars in CcpNmr

You will be able now to analyse graphically the residues that are involved in the interaction with the ligand.

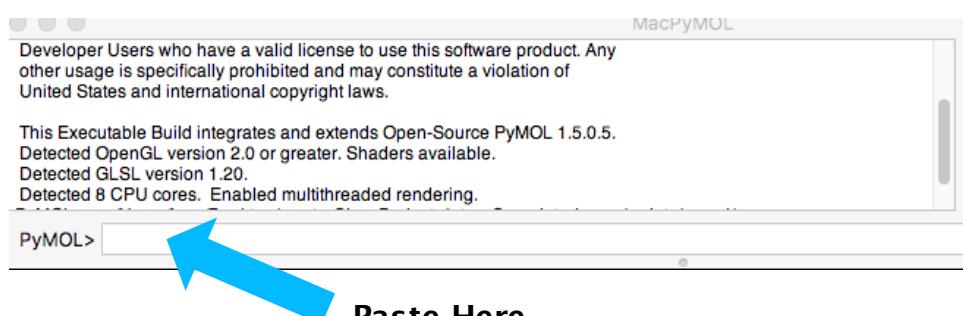


5c (Optional) Export image with transparent background

Copy and paste these commands on the Pymol terminal:

- *set ray_opaque_background, 0*
 - *png ~/Desktop/tStar.png, width=1000, dpi=300, ray=1*
 - press enter

Find the image on your desktop



Contact Us

Website:

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Suggestions and comments:

ccpnmr3@google.com

Issues and bug report:

<https://bitbucket.org/ccpnmr/issue-tracker/>

Cite Us

Simple High-Resolution NMR Spectroscopy as A Tool in Molecular Biology. Mureddu and Vuister, 2018.
(submitted on FEBS)

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