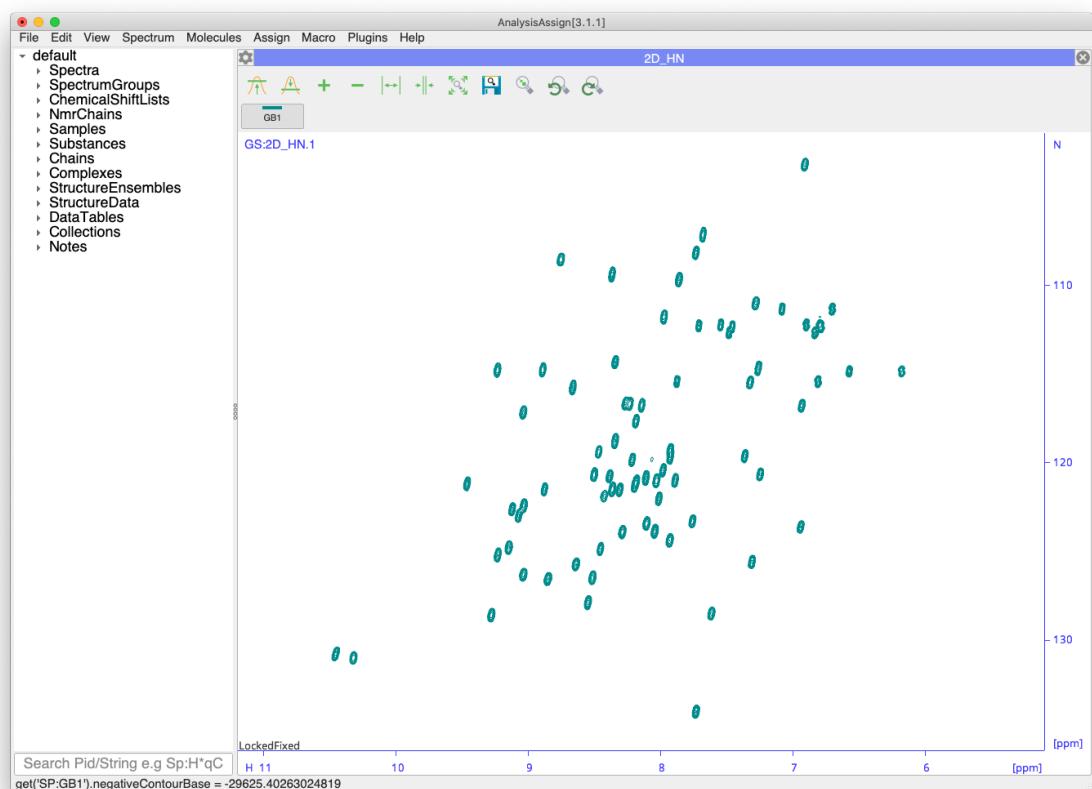


Introduction to NMR Tutorial



Introduction

This tutorial will introduce you to some basic analysis of 2D ^1H - ^{15}N HSQC spectra in CcpNmr Analysis Version 3.1.

The tutorial is divided into sections, each of them has a set of simple actions. Each page of this tutorial corresponds to a single process, 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.)

When you open the program you will see a large display area, with a sidebar to the left and a menu bar at the top. All the displays, tables, etc. which go into the display area are referred to as new “modules”. The sidebar shows the data in your project and lets you edit data items, create new ones, and drag the items into the display area to display them as a module. The menu bar lets you start actions and action modules. You can also start actions by two-key keyboard shortcuts (not case-sensitive), using the right mouse button, or from buttons and icons in the application.

We are grateful to Dr Fred Muskett for making the data available used in this tutorial. The data can be downloaded from the tutorials page of our website: <https://ccpn.ac.uk/support/tutorials/>.

Contents:

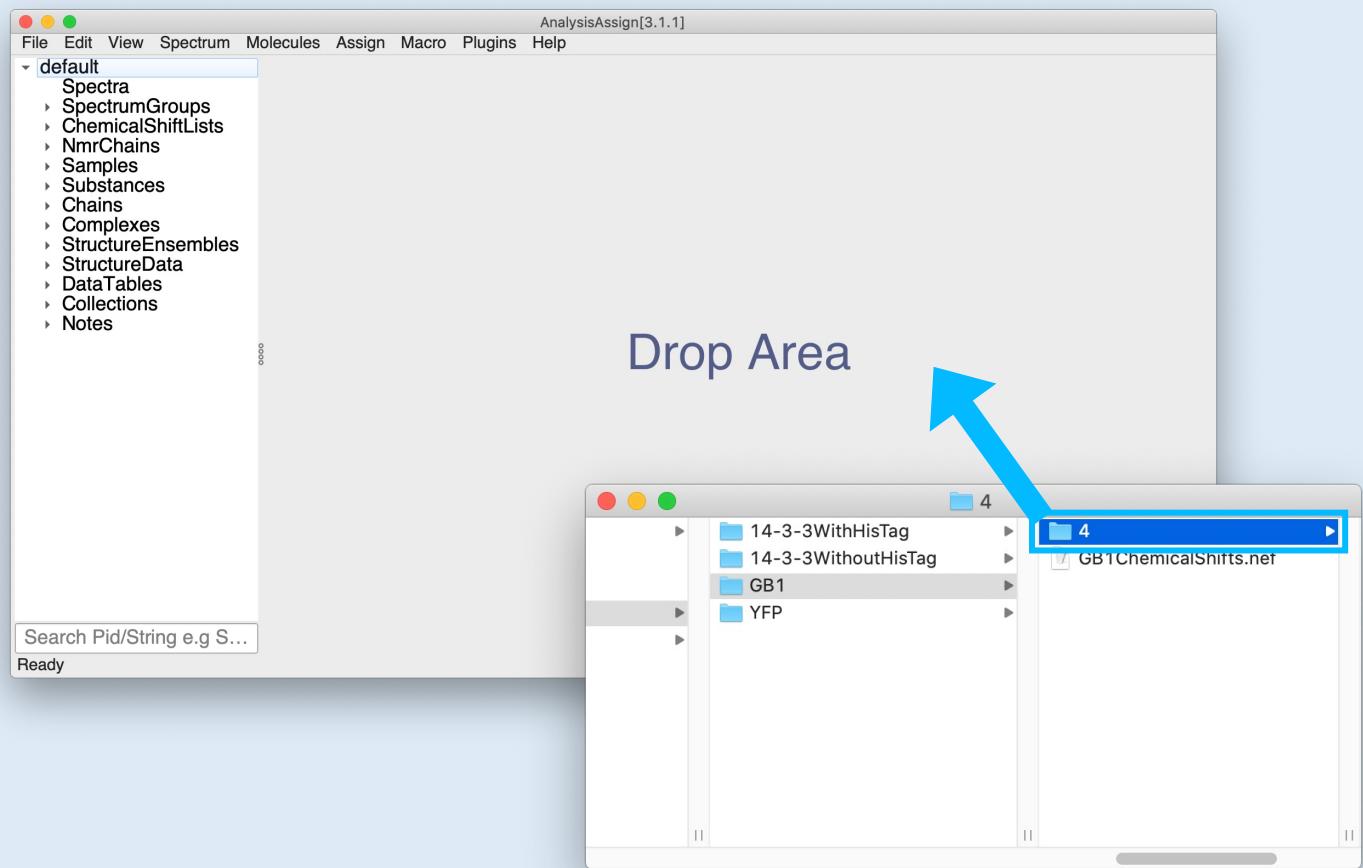
1. Loading and assessing Spectra
2. Reading in Chemical Shift Lists
3. Simulating Peak Lists from Chemical Shifts
4. Printing Spectra

Start CcpNmr Analysis V3

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

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

1 Loading and assessing Spectra



1A Drag & drop the HSQC spectra into the sidebar or drop area

- in your data folder, find the GB1/4 directory
- select it in your file browser and drag it onto the Sidebar or Drop Area.

If you drop it onto the Drop Area, the spectrum will be displayed immediately, if you drop it onto the Sidebar you will need an extra step to display it (see Section 1B).

You will also see an arrow appear next to the Spectra label in the Sidebar showing that the spectrum has been loaded.

▼ default
 ▼ Spectra
 ► SP:4-1
 ► SpectrumGroups
 ► ChemicalShiftLists
 ► NmrChains

- now load the other four spectra provided in the same way, by dragging these folders from the data directory into the program:

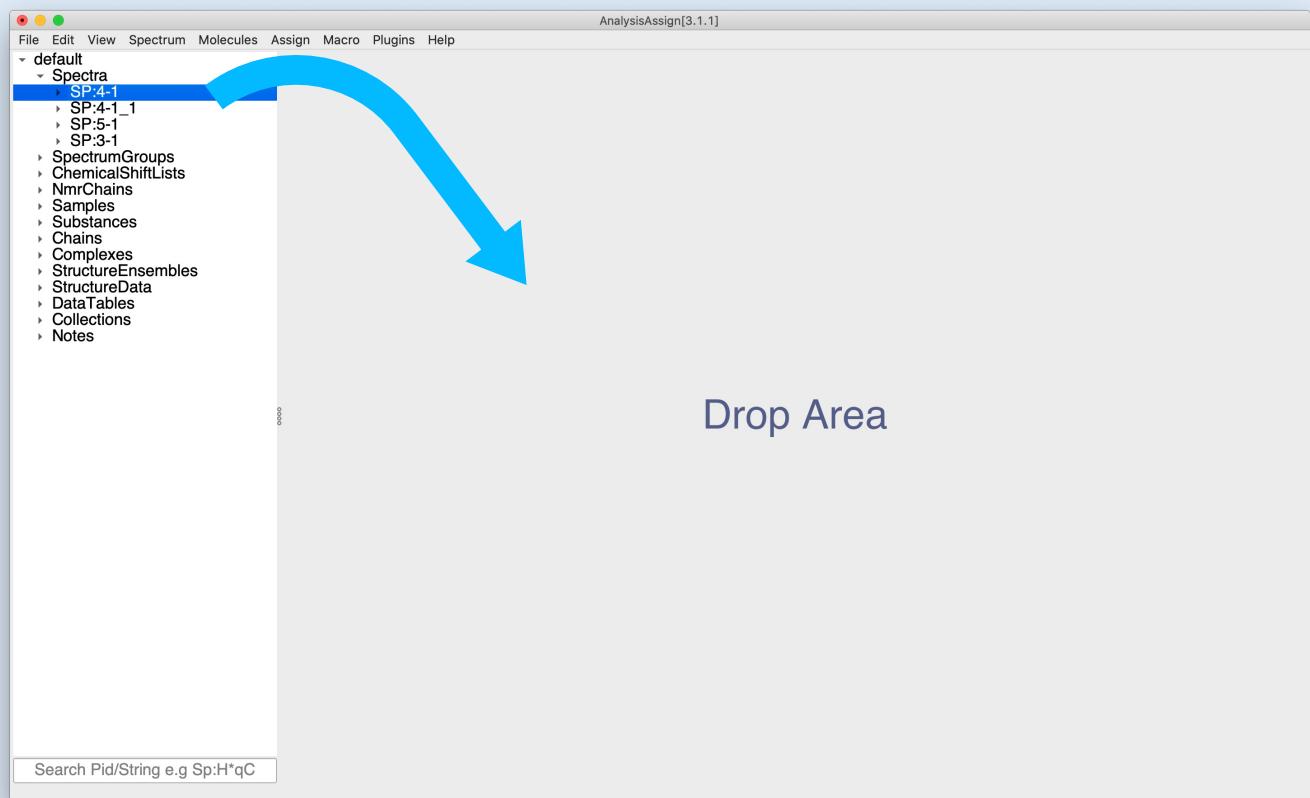
14-3-3WithHisTag/4

14-3-3WithoutHisTag/5

YFP/3

1 Loading and assessing Spectra

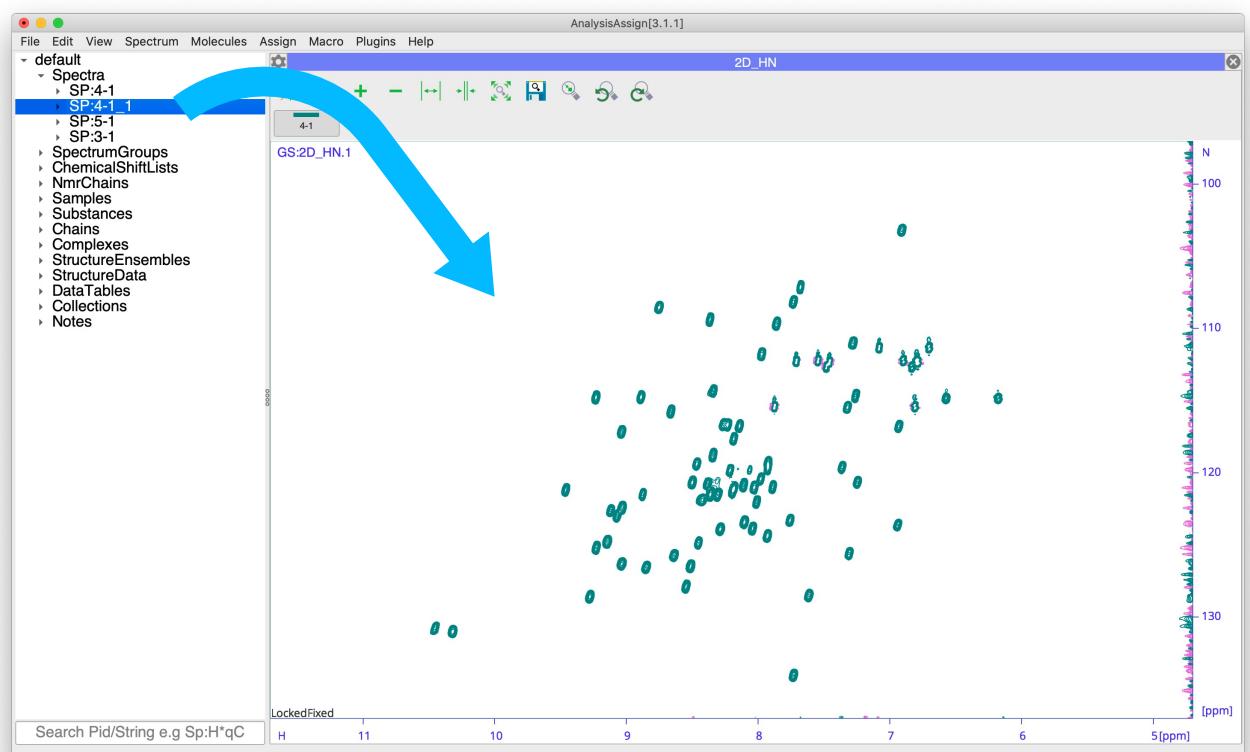
Drag & drop from Sidebar



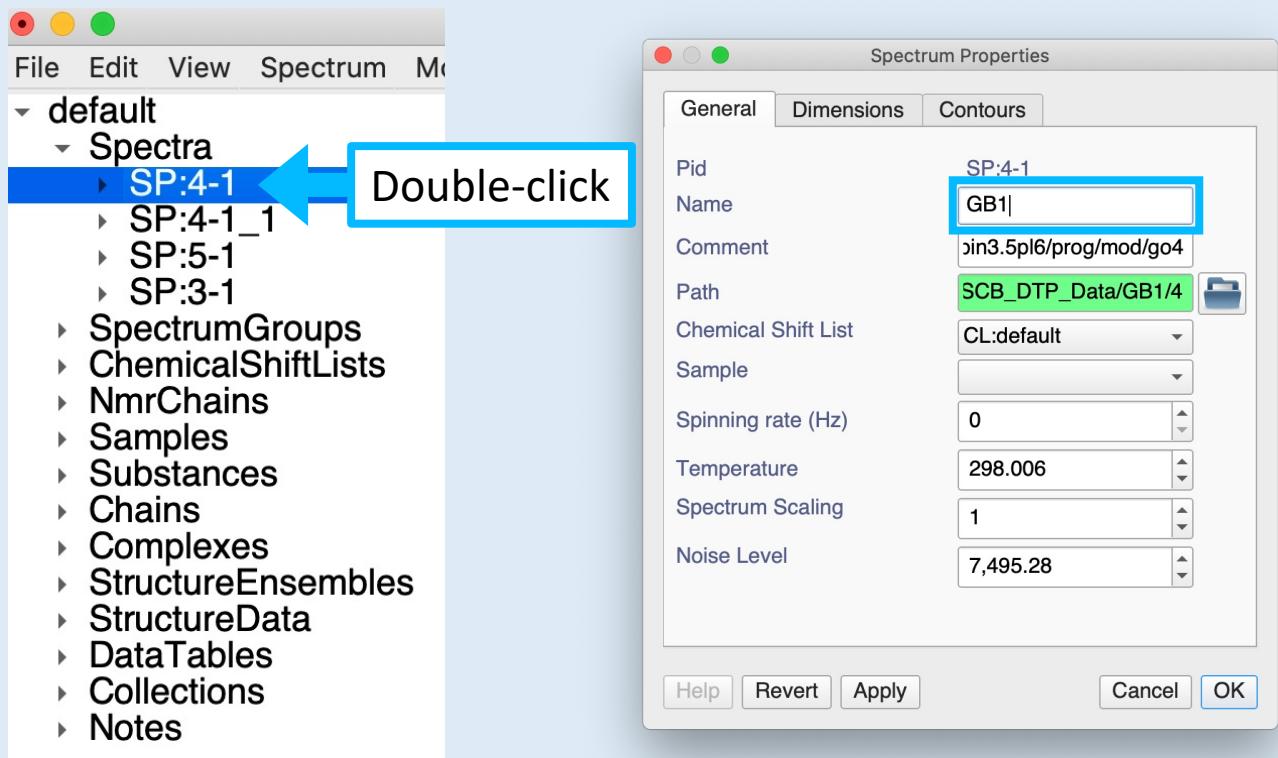
1B Drag & drop the spectra from the sidebar onto the Drop Area

- Select the spectrum (or multiple spectra) you want to display in the Sidebar
- Drag and drop them into the main Drop Area

Once one spectrum has been opened in a SpectrumDisplay module, you can also add others, by dragging them from the sidebar onto the SpectrumDisplay.



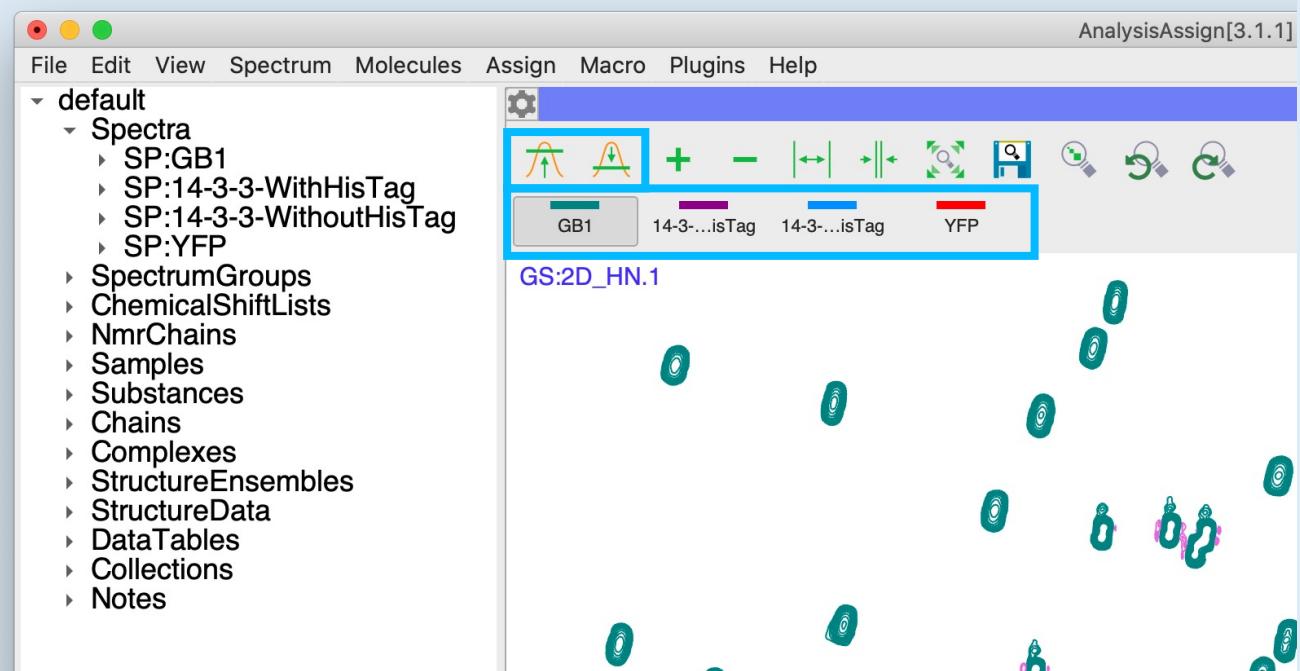
1 Loading and assessing Spectra



1C Rename spectra (optional)

- Double-click on a spectrum in the spectrum in the sidebar to bring up the Spectrum Properties box
- Go to the General tab and enter GB1 as the new name:
GB1
- Repeat for the other spectra:
 - 4-1_1: **14-3-3-WithHisTag**
 - 5-1: **14-3-3-WithoutHisTag**
 - 3-1_1: **YFP**

1 Loading and assessing Spectra



1D Show/hide spectra

- Click on the spectra in the spectrum Toolbar to show or hide individual spectra

1E Set contour levels

With only one spectrum showing:

- click on the Contour up/down buttons   until most of the noise is no longer showing
- repeat this for all your spectra



1 Loading and assessing Spectra

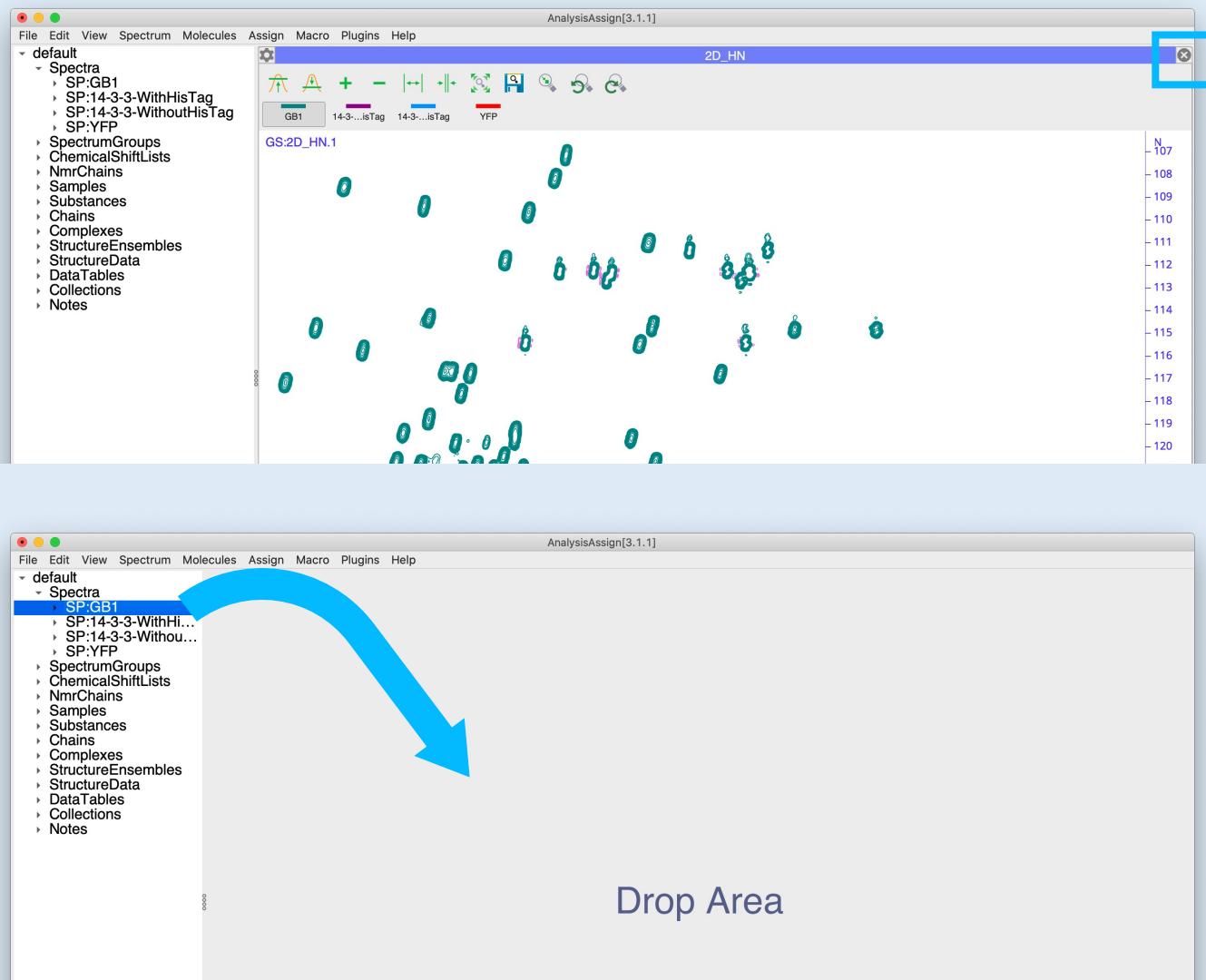
1F Assessing the spectra

Investigate each of the spectra in turn.

You can zoom in using the mouse wheel on the SpectrumDisplay canvas.

1. For GB1: For which residue types do you expect to see peaks in the ^{15}N -HSQC? How many peaks per residue?
2. What can you deduce about the different spectra?
3. What is their quality like?
4. How large are the proteins?
5. Are they folded?
6. For YFP: The HSQC also shows negative peaks; why is this and which peaks are these? (This is a difficult question as we have not given you the required prior knowledge! Try googling or ChatGPT).

Reading in Chemical Shift Lists



2A Open only the GB1 spectrum

Either continue directly with your project from **Section 1**, or start with the **Section1_completed ccpn** project provided in the data directory.

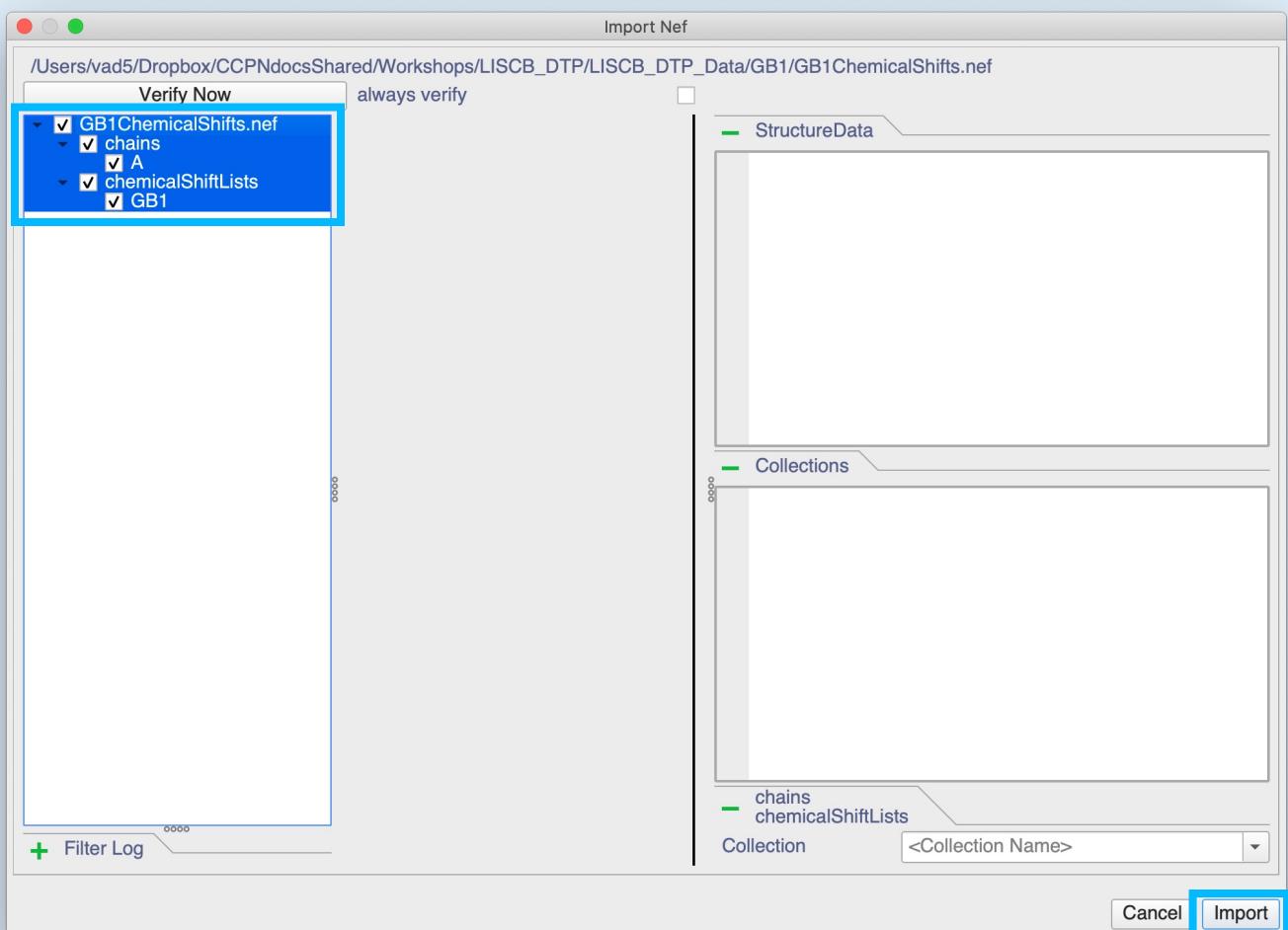
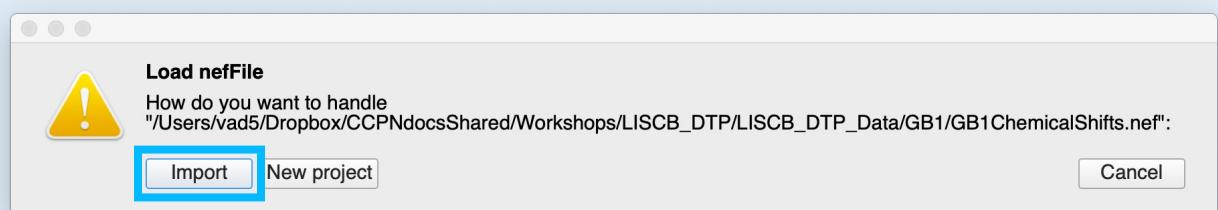
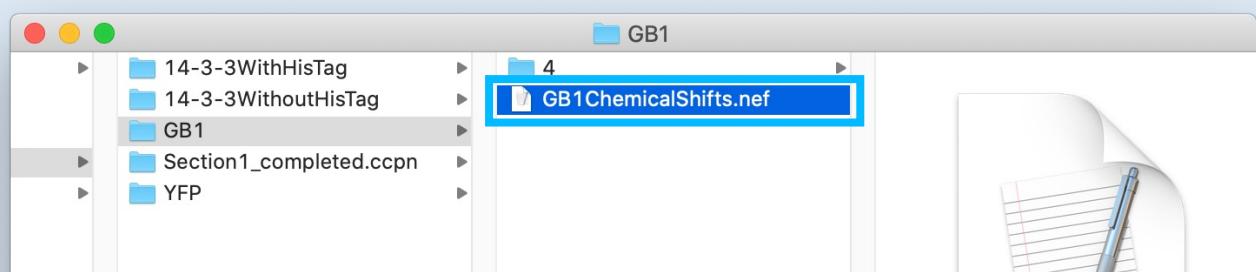
If you start with our project:

- Drag the **Section1_completed ccpn** folder into the sidebar or Drop Area to open the project.

Then continue:

- Close your SpectrumDisplay by clicking on the cross  in the top right corner.
- Open only the GB1 spectrum in a new SpectrumDisplay by dragging it from the sidebar into the DropArea.

Reading in Chemical Shift Lists

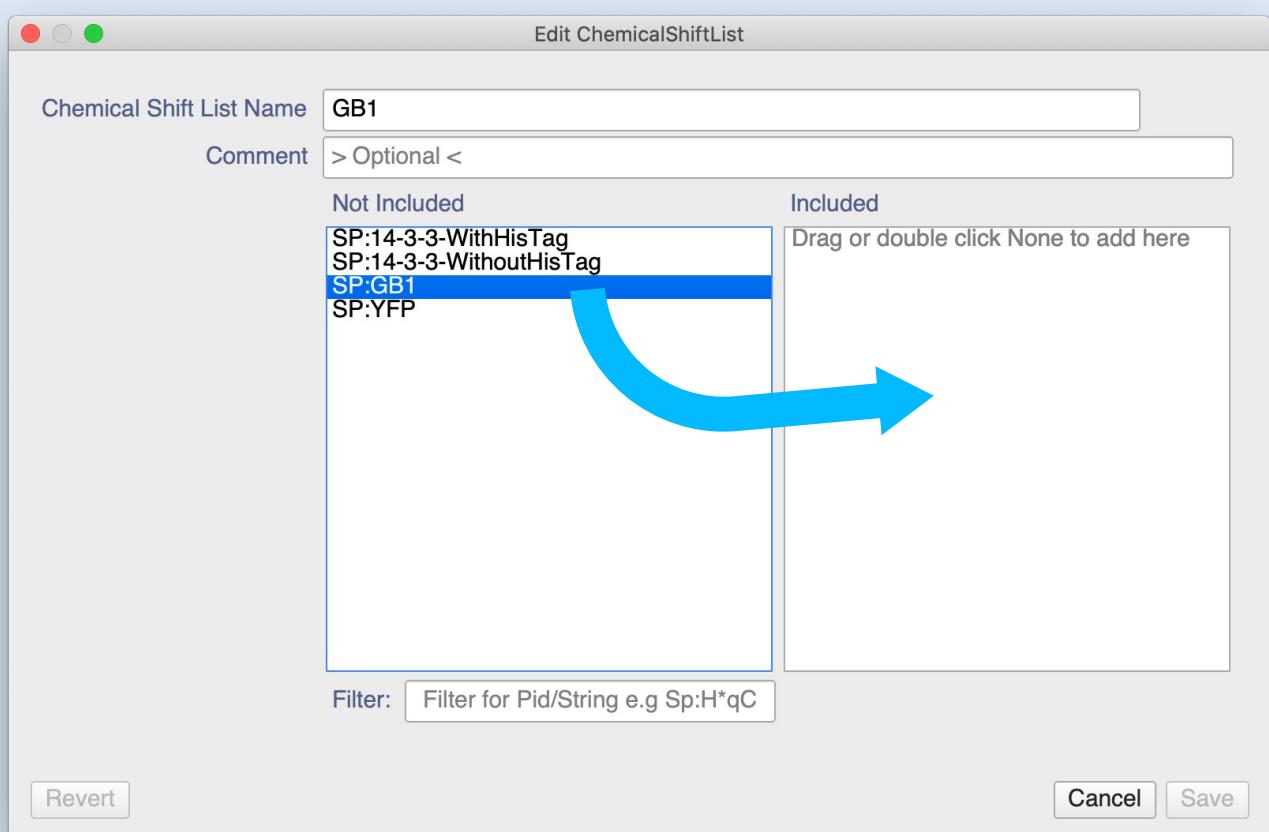
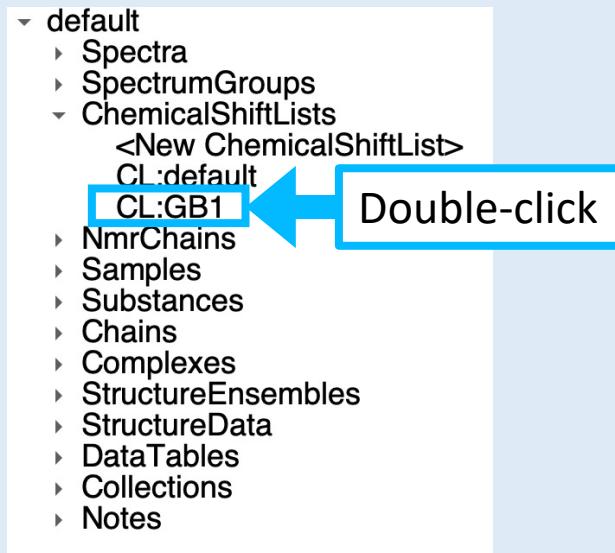


2B Read in the ChemicalShifts from a NEF file

In the **GB1** folder, find the **GB1ChemicalShifts.nef** file. This is a file containing all the NH chemical shifts for GB1 along with the sequence of GB1.

- Drag the **GB1ChemicalShifts.nef** file into the program
 - Select the option to **Import** the data
 - In the Import Nef pop-up, tick the selection box belonging to **GB1ChemicalShifts.nef** on the left hand side.
- This will also select all the other elements nested below it.
- Click **Import** to import the data.

Reading in Chemical Shift Lists

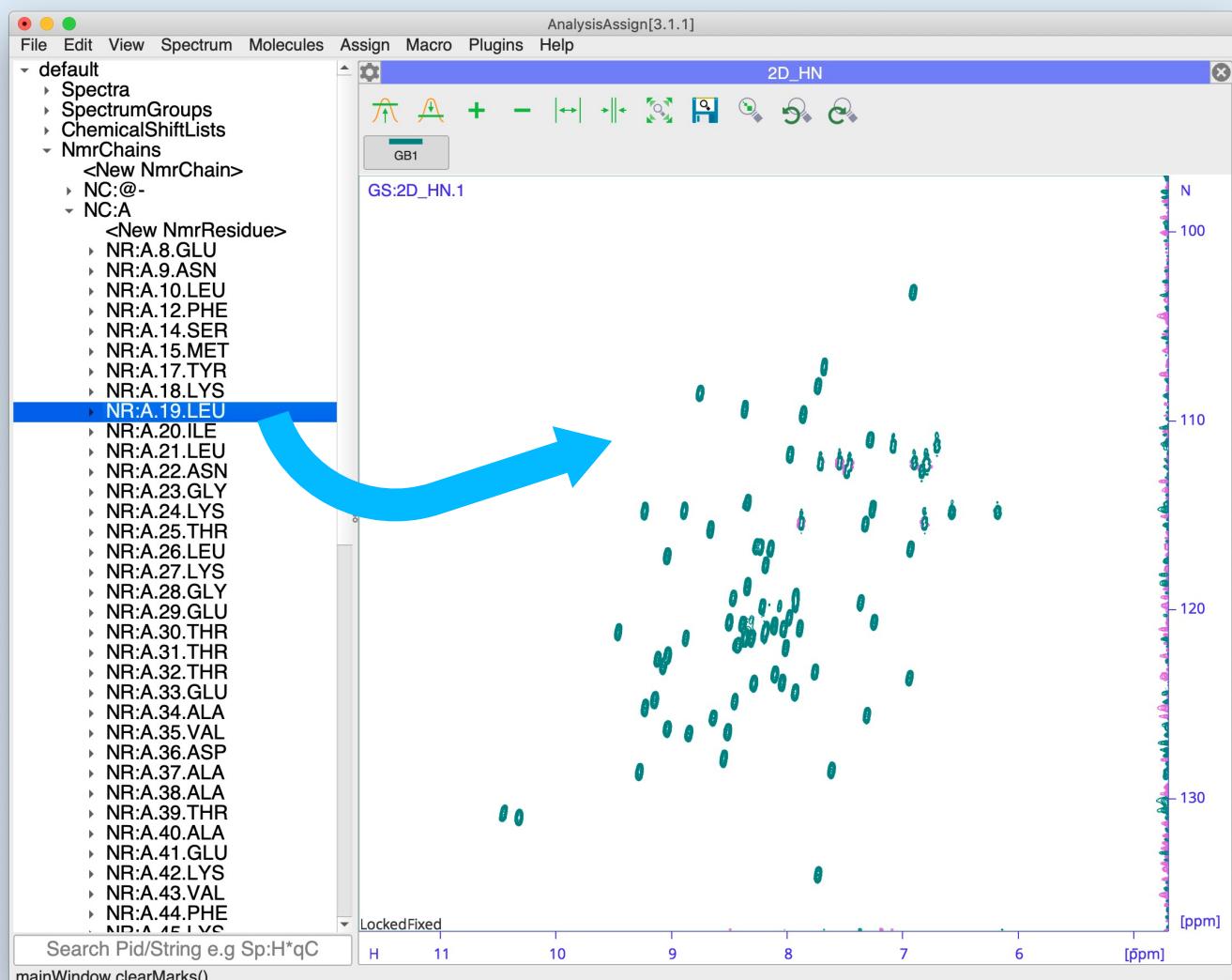


2c Associate the GB1 spectrum with the new Chemical Shift List

Nested below the ChemicalShiftLists in the sidebar, you will now see a new Chemical Shift List called GB1.

- Double-click on **CL:GB1** to open up the **Edit ChemicalShiftList** pop-up.
- Drag the **SP:GB1** spectrum from the left-hand **Not Included** side to the right-hand **Included** box.
- Click **Save**.

Reading in Chemical Shift Lists



2D Marking Peaks in the Spectrum

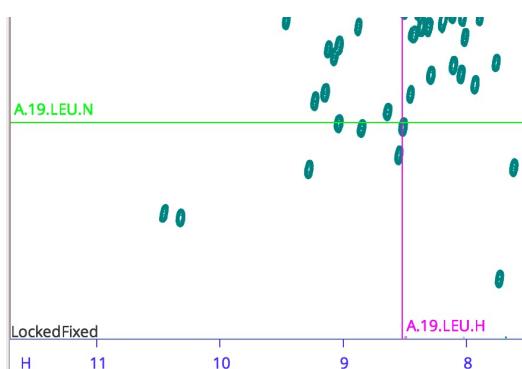
Nested below NmrChains in the sidebar, you will see an NmrChain called NC:A. This was created when we imported the GB1 Chemical Shifts. This NmrChain contains a list of NmrResidues and these in turn each contain two NmrAtoms (one H and N). These are the peak labels we can now use to mark and later label our peaks with.

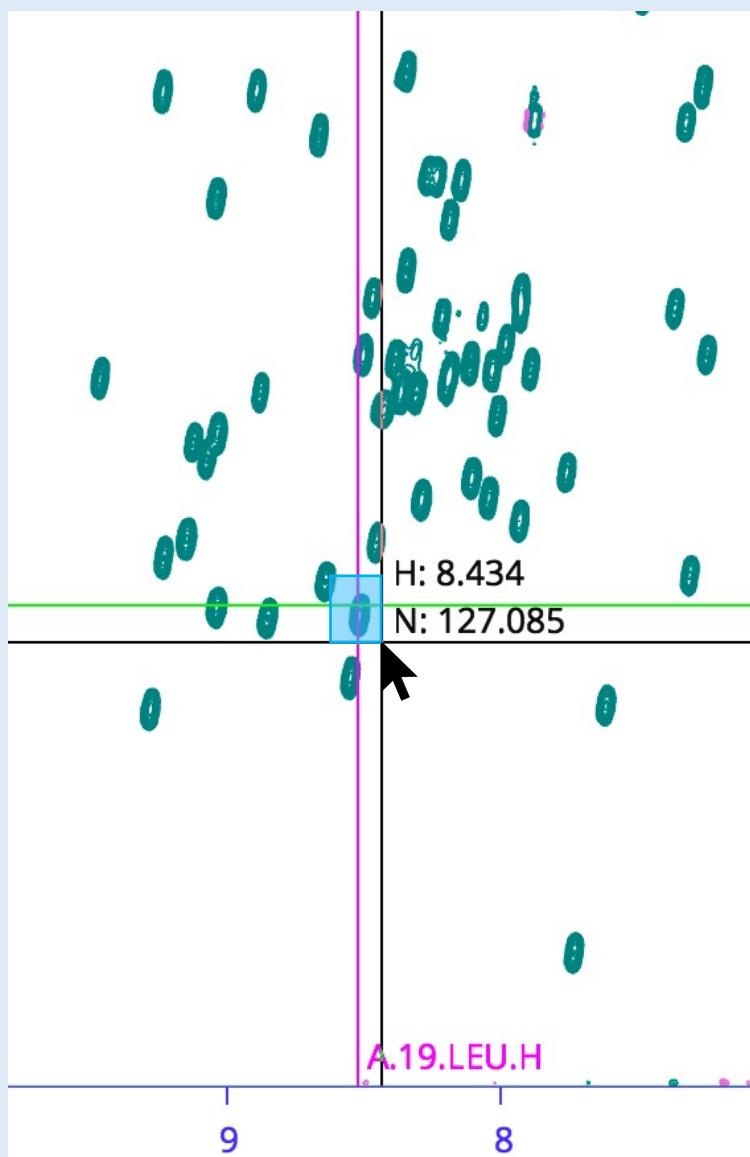
- Expand the NC:A NmrChain so that you can see the Nmr Residues
- Choose an NmrResidue and drag it onto the SpectrumDisplay

This will mark the chemical shifts of the N and H atoms of this residue and will hopefully lie on top of one your peaks. You now know which residue in GB1 this peak belongs to.

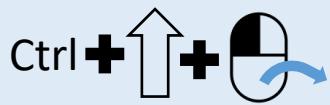
```

- NmrChains
  <New NmrChain>
  ▶ NC:@-
  ▶ NC:A
    <New NmrResidue>
    ▶ NR:A.8.GLU
      <New NmrAtom>
      NA:A.8.GLU.H
      NA:A.8.GLU.N
    ▶ NR:A.9.ASN
    ▶ NR:A.10.LEU
    ▶ NR:A.12.PHE
    ▶ NR:A.14.SER
    ▶ NR:A.15.MET
  
```

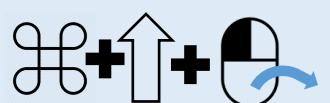




Linux / Windows:



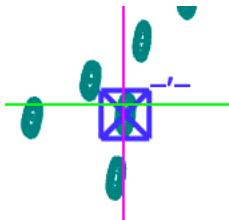
Mac:



2E Peak Picking

- Click on the SpectrumDisplay, hold down **Ctrl** (or **Cmd** on a Mac) plus **Shift** and **left-drag** the mouse to create a blue peak-picking box around the peak you want to pick.

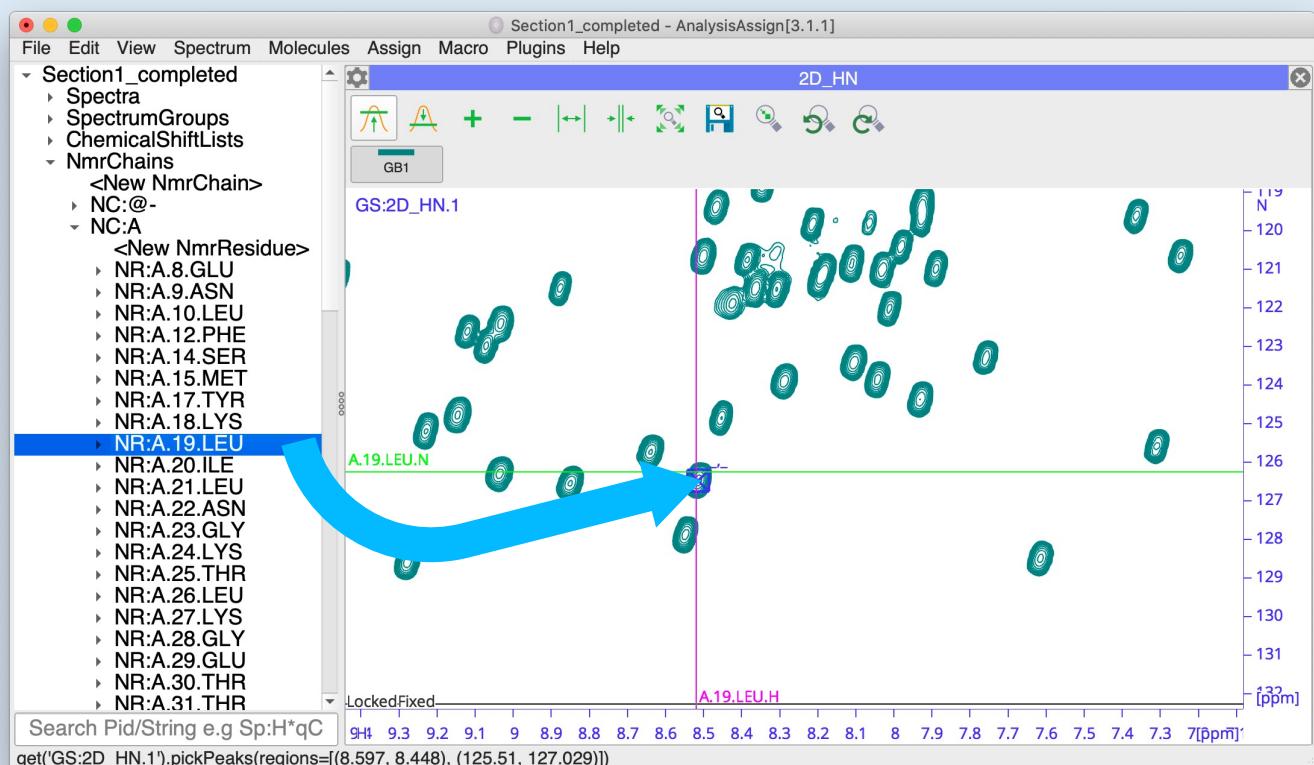
This should place a peak on all the maxima in the area you have selected. Peaks are indicated by a X and are highlighted in blue with a box around them when they are selected.



You can also place a single peak at a position of your choice by holding down **Ctrl** (**Cmd** on a Mac) plus **Shift** and **left-clicking** the mouse.

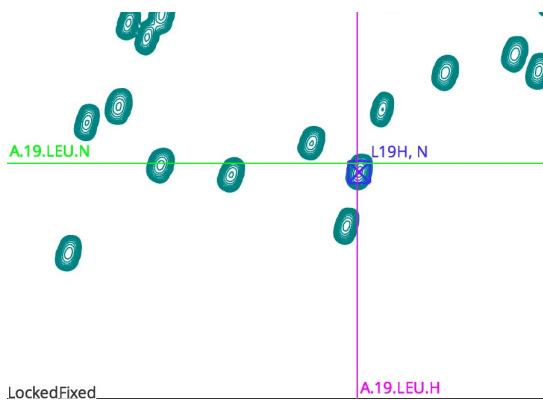
Typing the letters **SE** will snap the selected peak to the nearest extremum.

Reading in Chemical Shift Lists



2F Labelling Peaks

- Drag the same NmrResidue you used to mark the peak onto the peak. This will label it as belonging to that residue.

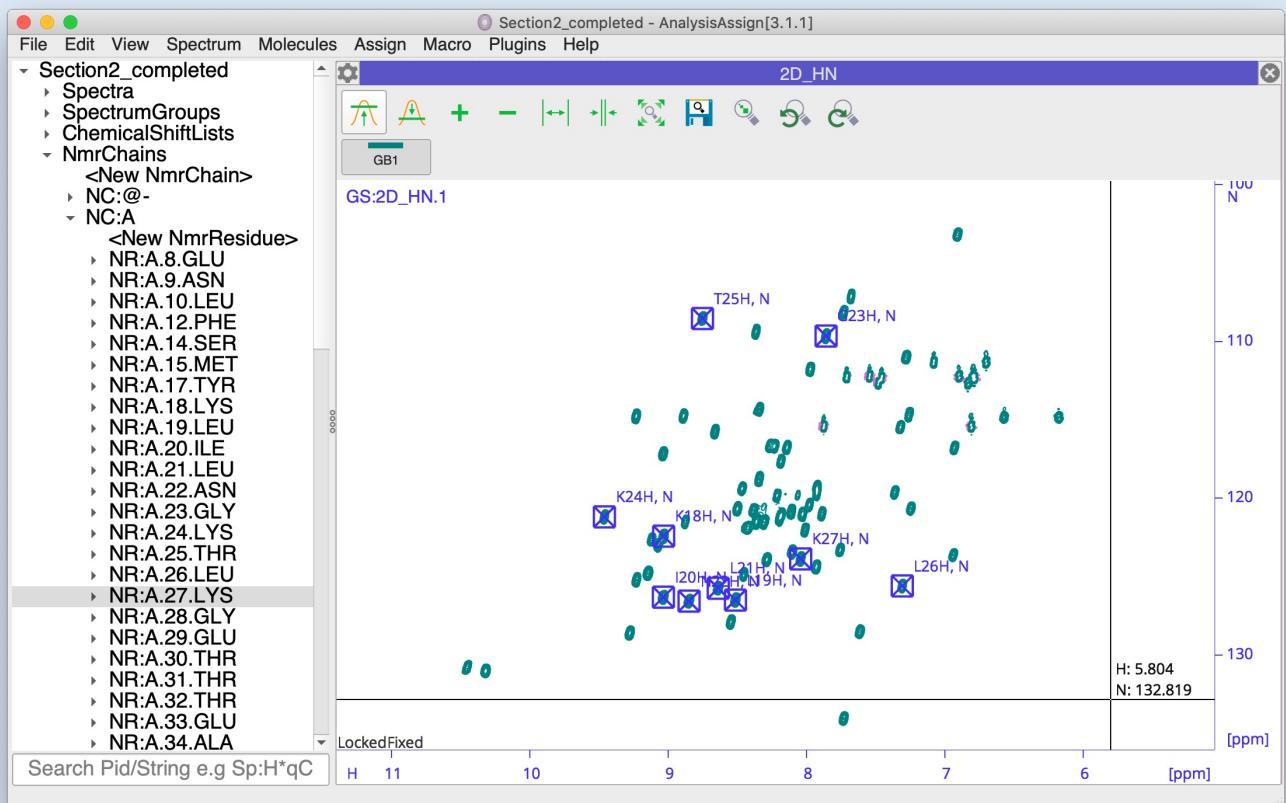


- Remove your mark by typing the shortcut MC (you can use lowercase letters).

Repeat the peak marking, picking and labelling for a few other residues. You may notice that a few of the peaks have moved slightly from their marked position. But it should still be possible to work out which belongs where.

Note that you can select a peak by left-clicking on it. Clicking on the blank canvas will deselect the peak again.

Simulating Peak Lists



3A Delete your peaks

Either continue directly with your project from **Section 2**, or start with the **Section2_completed ccpn** project provided in the data directory.

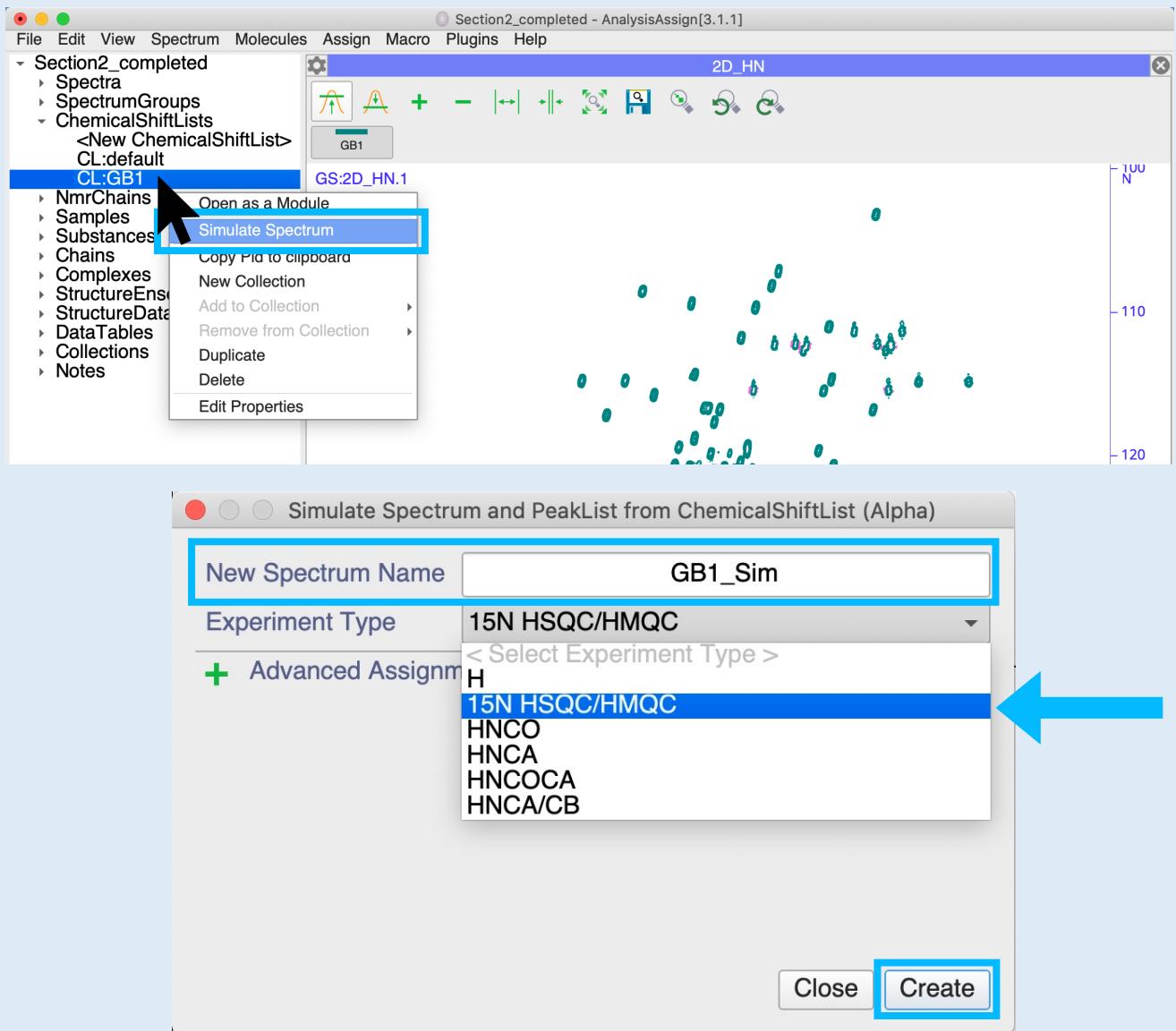
If you start with our project:

- Drag the **Section2_completed ccpn** folder into the sidebar or Drop Area to open the project.

Then continue:

- Click on the SpectrumDisplay and press **Ctrl+A** (**Cmd+A** on a Mac) to select all peaks.
- Press the **Delete** or **Backspace** key on your keyboard to delete all the peaks.

Simulating Peak Lists



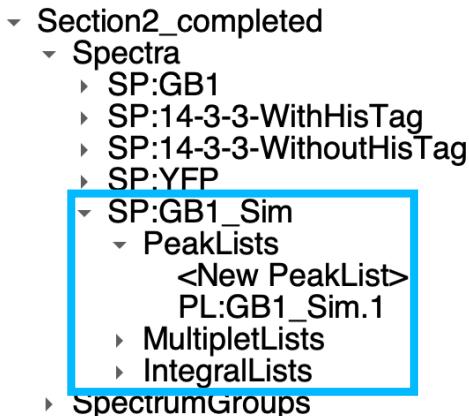
3B Simulate Spectrum Peak List (Using Known Chemical Shifts)

- Right-click on the CL:GB1 Chemical Shift List in the sidebar and select Simulate Spectrum.

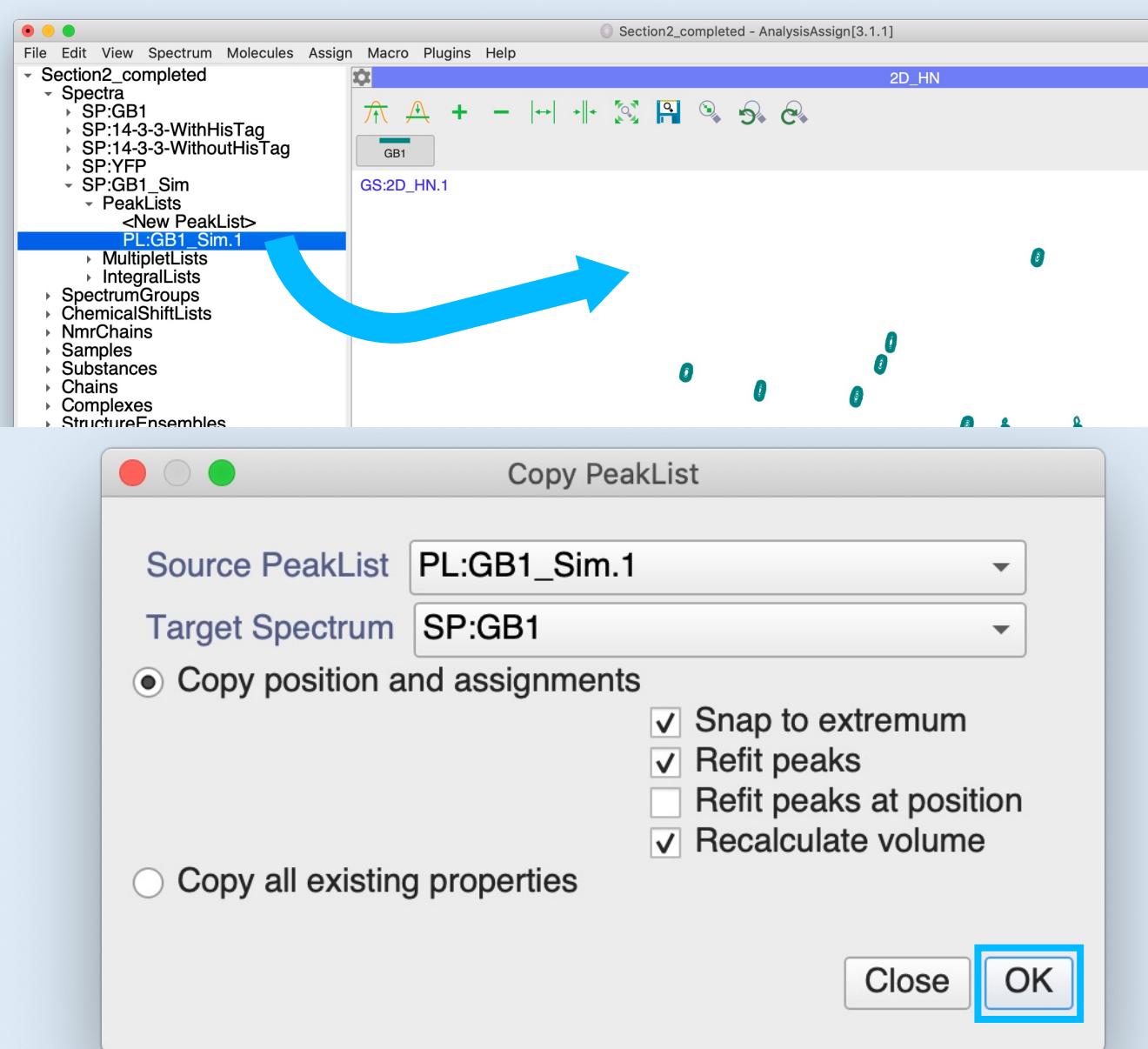
In the pop-up:

- Give your new spectrum a name, e.g. **GB1_Sim**
- For the **Experiment Type**, select **15N HSQC/HMQC** from the drop-down menu
- Click on **Create**.

You should now see an additional Spectrum called **GB1_Sim** in the **Spectra** section of the sidebar:



Simulating Peak Lists



3C Copy Peak List

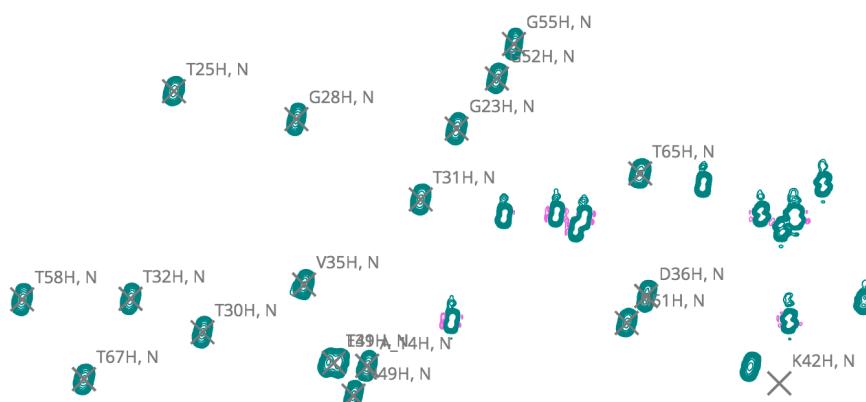
- In the sidebar, expand the **SP:GB1_Sim** Spectrum and it's **PeakLists**.

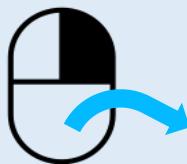
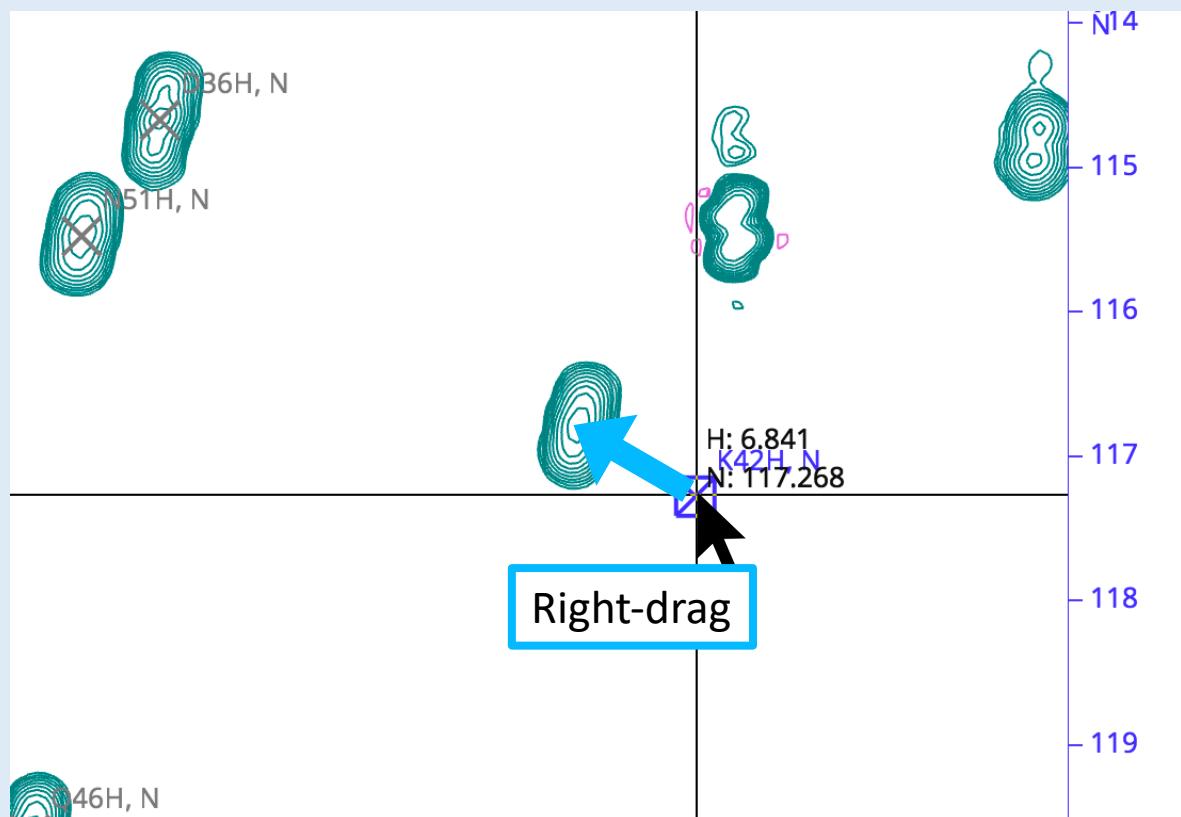
The **PL:GB1_Sim.1** PeakList is the one we have just created from our **CL:GB1** Chemical Shift List.

Drag the **PL:GB1_Sim.1** PeakList from the sidebar onto your spectrum
In the pop-up:

- keep all the default options as shown above
- Click on **OK**.

It will take a few moments for the peaks to be copied, fitted and labelled:



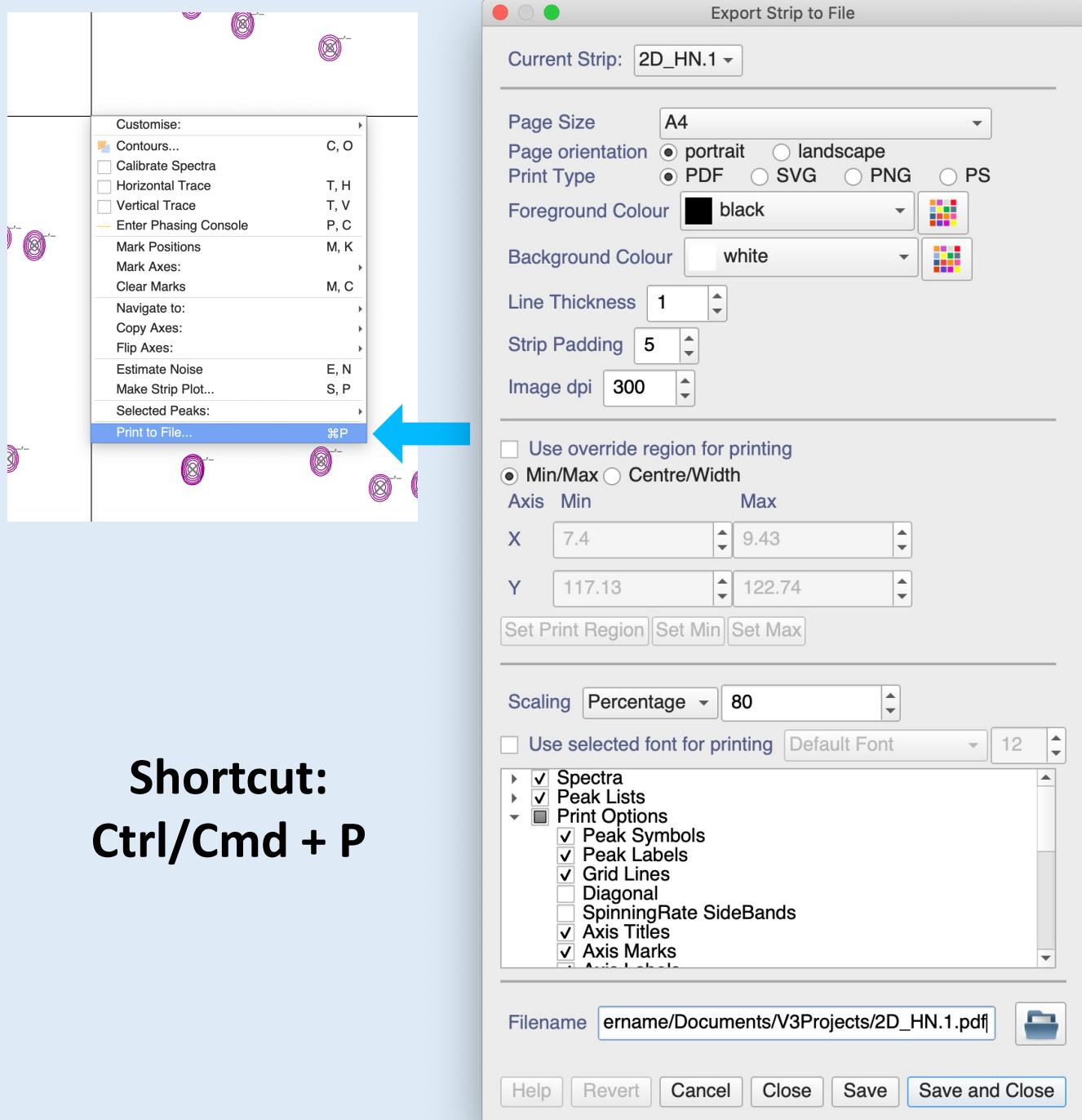


3D Refit any incorrect peaks

You will find that there were a few peaks that are not correctly positioned on top of the peak maxima. You will need to move these peaks and refit these manually in turn:

- Select an incorrectly fitted peak by **left-clicking** on it.
- **Right-drag** the peak to the closest maximum.
- Snap the peak to the exact maximum with the shortcut **SE**.
- Refit the peak with the shortcut **RP**.

Printing spectra



Shortcut:
Ctrl/Cmd + P

4A Printing spectra to file

- Right-click on the Spectrum Display you want to print
- Click on Print to File...

OR

- Use shortcut Ctrl (Cmd on a Mac) + P

The popup will appear.

- Select the Strip/SpectrumDisplay you want to print
- Select/deselect any other options as desired.
- Select a file name and path for the print file using the folder icon.
- Click Save or Save and Close.

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