

## Side-chain Assignment Tutorial

G34H, N; V33CGy

G34H, N; V33CGx

G34H, N; V33CB



# Introduction

This tutorial is designed to guide you through the assignment of the aliphatic side chain resonances of a protein using Ccpnmr AnalysisAssign Version 3.1. It is not intended to teach any theoretical aspects of NMR. A wide range of NMR experiments can be used to do such an assignment and typically each laboratory has its own "favourite" set of experiments to record. The experiments and method shown here are therefore simply "a" method, rather than "the" method. But it should be easy to transfer this to other experiments you may have available for your own protein.

This tutorial uses spectra recorded on the CW domain of ASHH2 bound to a histone tail peptide. We are grateful to Dr Olena Dobrovolska at the University of Bergen for providing these. For more information on this system see Dobrovolska et al. 2020, FEBS Journal (<https://doi.org/10.1111/febs.15256>). You can download the tutorial data from the tutorials page of the CCPN website (<https://ccpn.ac.uk/support/tutorials/>).

Please note that the images shown are only representative and you may encounter minor differences in your setup.

## Contents:

1. Assignment strategy and reference material
2. Assigning short side chains
3. Assigning long side chains

## 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

(Explanation only)

## Short side-chain amino acids

We will start by assigning those amino acids that only have alpha and beta carbons/hydrogens in the aliphatic region. These residues are:

Glycine (GLY)  
Alanine (ALA)  
Serine (SER)  
Cysteine (CYS)  
Aspartic Acid (ASP)  
Asparagine (ASN)  
Phenylalanine (PHE)  
Tyrosine (TYR)  
Tryptophane (TRP)  
Histidine (HIS)

For these residues, the CA and CB assignments are already available from the Backbone assignment. Only the HA and HB assignments are still required and can be obtained from the HBHACONH spectrum.

## Long side-chain amino acids

The remaining side-chains have additional aliphatic atoms which need to be assigned:

Threonine (THR)  
Glutamic Acid (GLU)  
Glutamine (GLN)  
Valine (VAL)  
Leucine (LEU)  
Isoleucine (ILE)  
Arginine (ARG)  
Lysine (LYS)  
Methionine (MET)  
Proline (PRO)

Assigning these amino acids requires additional spectra. We will be using the HBHACONH to assign the HA and HB atoms, the CCONH spectrum to assign all the remaining side-chain carbon atoms and the  $^{13}\text{C}$ -HSQC and hCCH-TOCSY spectra for the remaining side-chain hydrogen atom assignments.

Note that some side chains such as Arg and Lys are often highly dynamic and their resonances may overlap significantly, making assignment difficult. Met methyl groups can also be difficult to assign, as they are separated from the remaining sidechain by an NMR-silent sulphur atom.

## Aromatic side-chains

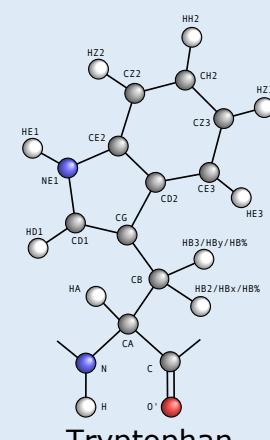
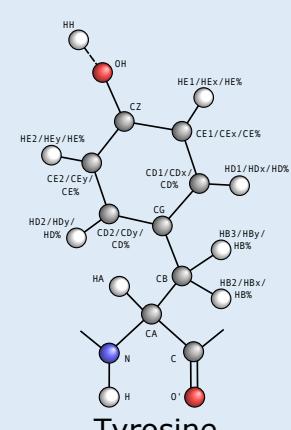
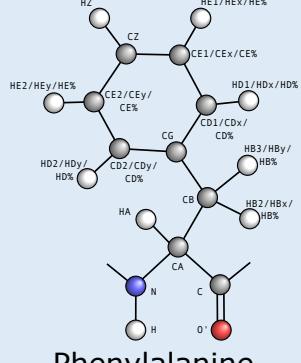
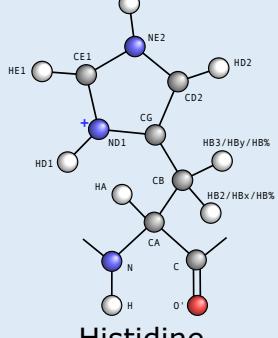
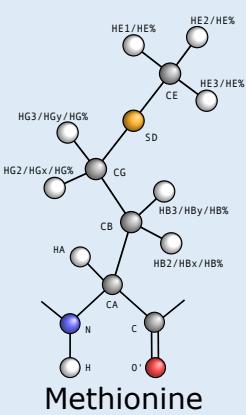
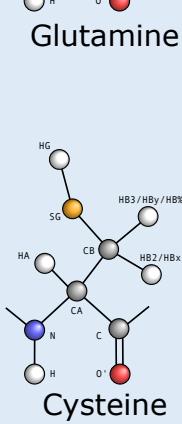
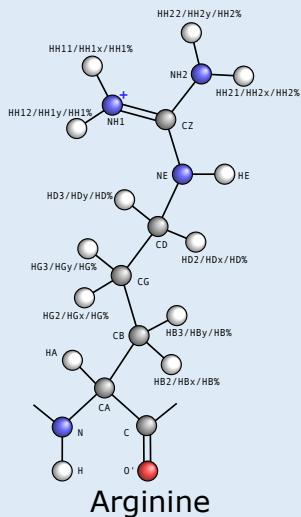
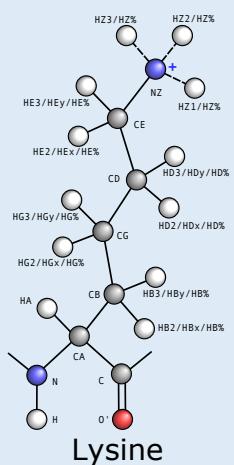
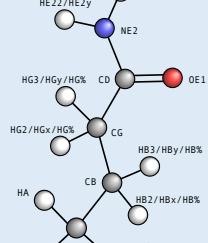
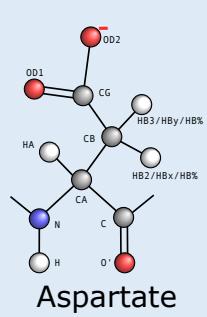
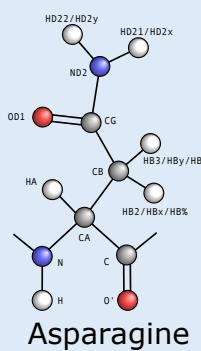
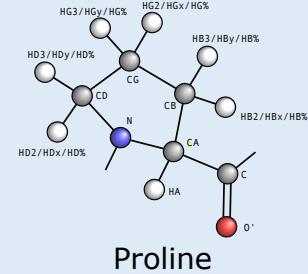
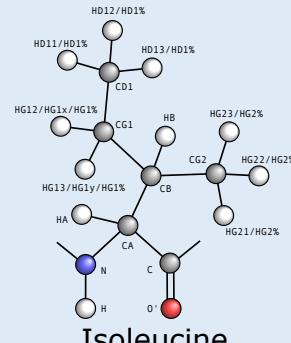
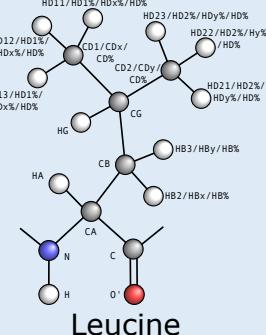
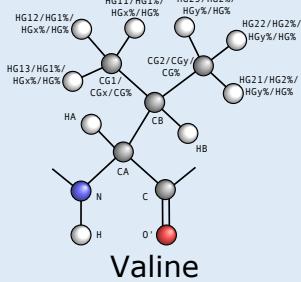
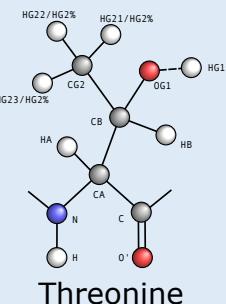
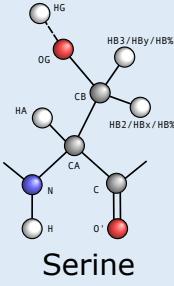
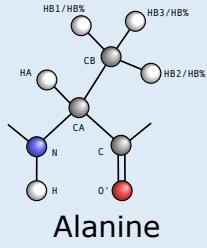
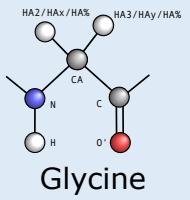
The aromatic side-chain elements of

Phenylalanine (PHE)  
Tyrosine (TYR)  
Tryptophane (TRP)  
Histidine (HIS)

need to be assigned separately using a further set of experiments, not shown here.

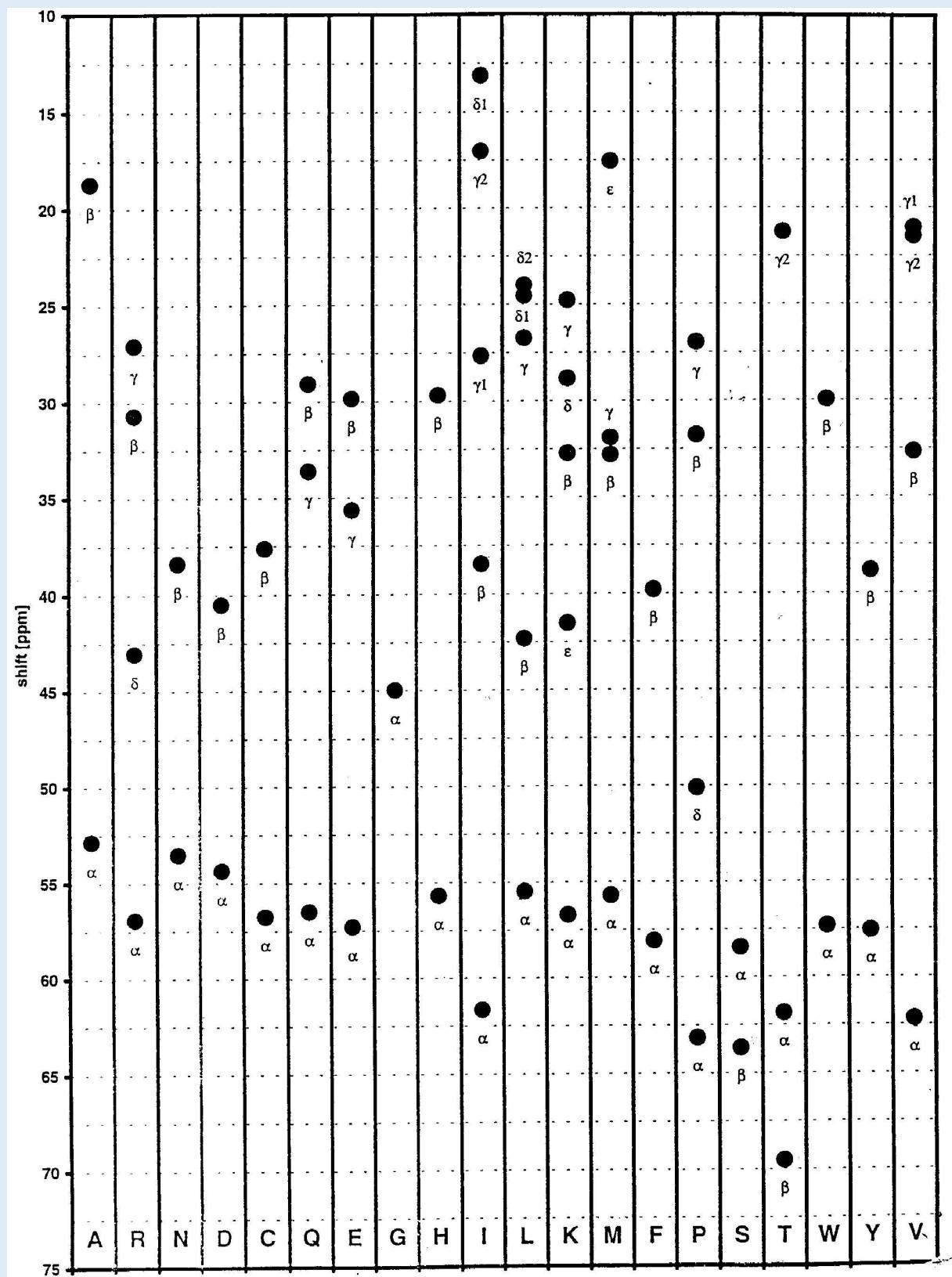
# Natural Amino Acids

## Amino Acids with NEF Atom Names (for reference)



# Standard Chemical Shift Values

Carbon chemical shifts for the 20 natural amino acids (for reference)



## Getting started, basic operations

### Sidebar

All data contained in a project, such as spectra and peak lists are located in the sidebar. **Double-clicking** 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. If you close a display, you can open a spectrum by **dragging and dropping** it into the drop area from the sidebar or **right-clicking** on a sidebar item and selecting **Open as module**.

### Mouse

- Pan → **Left-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 **right-click and drag**

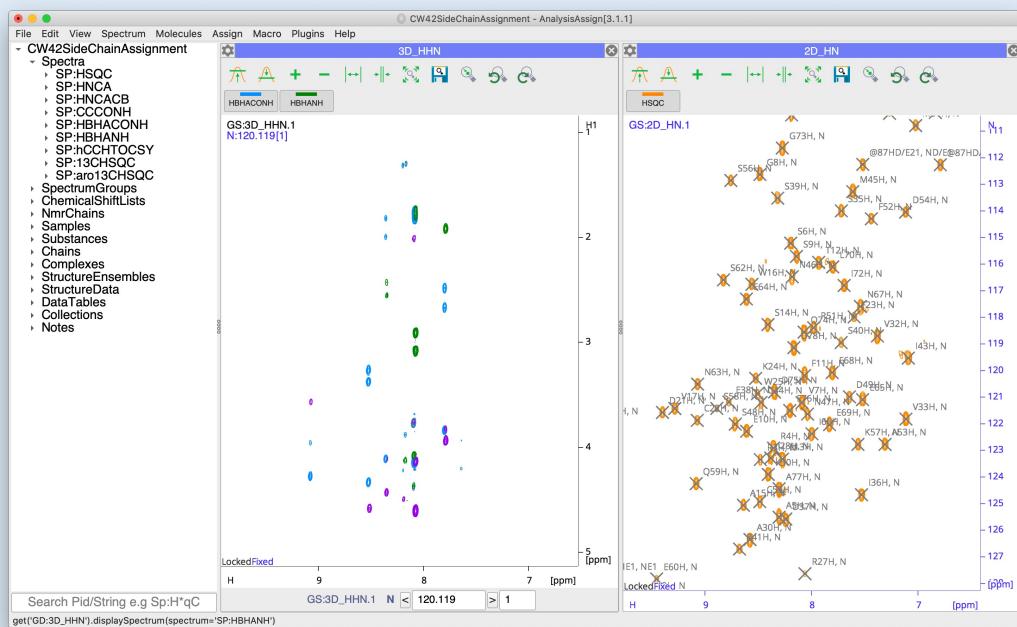
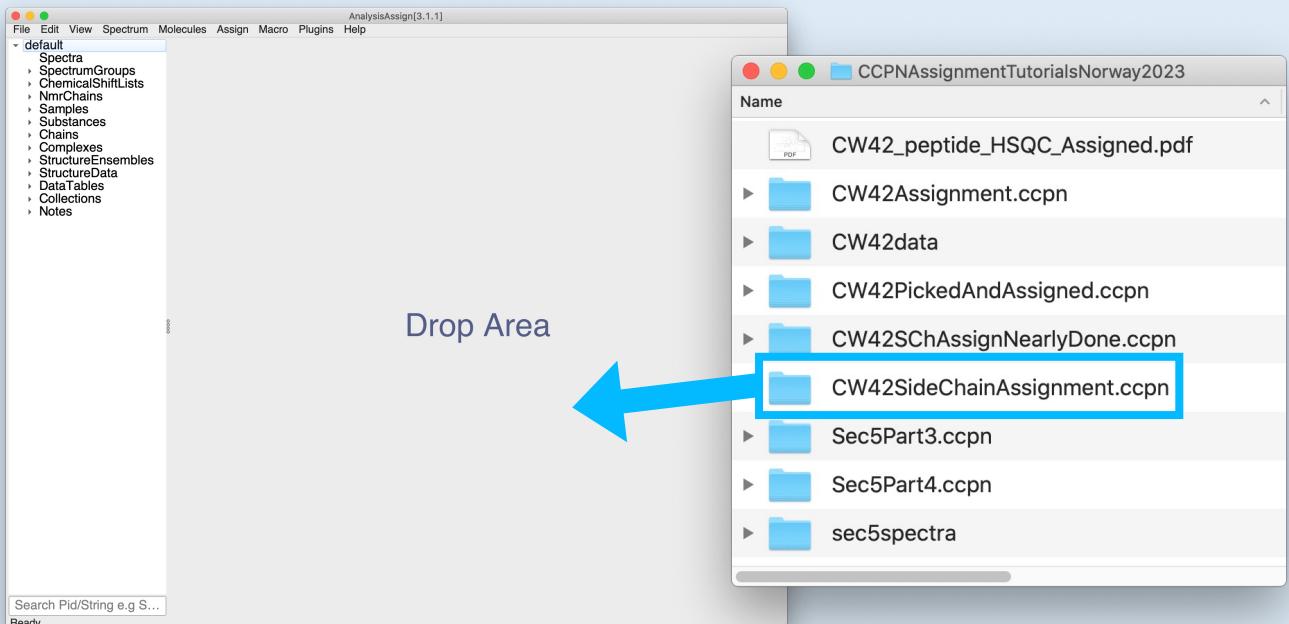
### Shortcuts

The program uses several shortcuts, for 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** (case insensitive). Press **Esc** to cancel the first letter.

For more commands and operations:

Main Menu → *Help* → *Tutorial (Beginners)* or *Show Shortcuts*

Open the project CW42SideChainAssignment ccpn



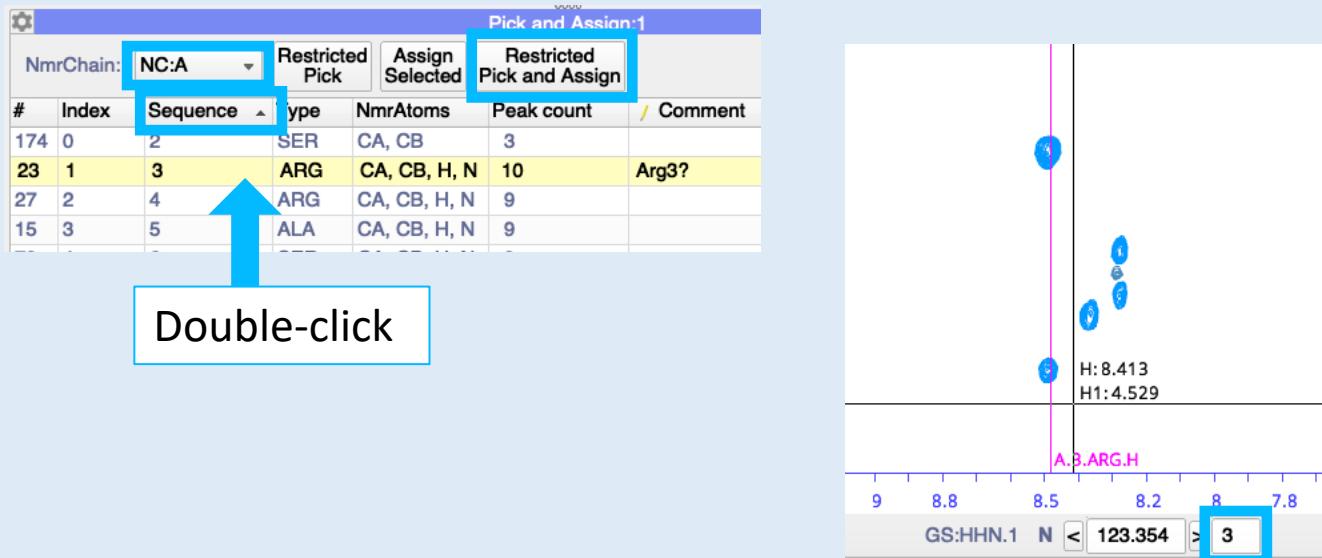
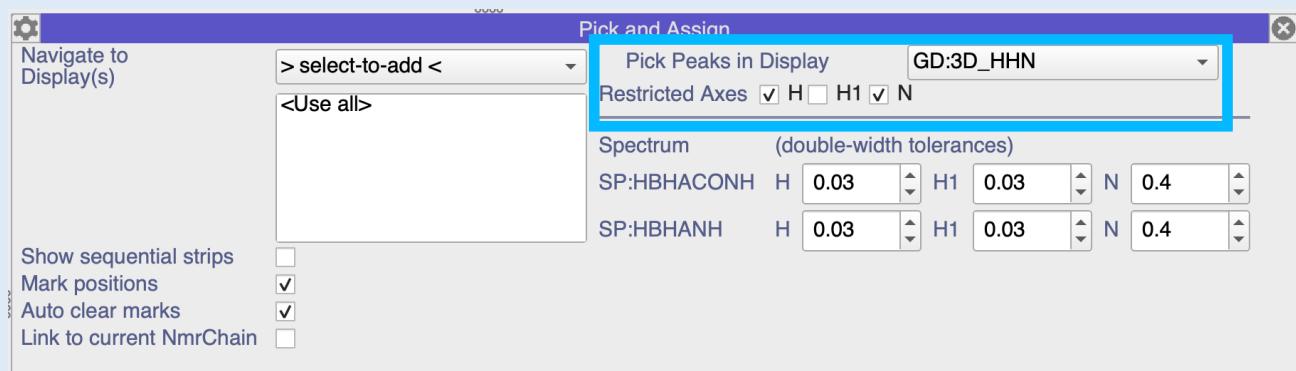
## 2A Open Project

- Drag & drop the **CW42SideChainAssignment ccpn** folder into the sidebar or drop area.

You will see two spectra, displayed in two Spectrum Displays as:

- HBACONH (orange, 2D\_HN)
- HBACONH (blue, 3D\_HHN)

with additional spectra in the sidebar.



## 2B Pick and Assign HBHACONH

Open the **Pick and Assign** module:

- Go to **Main Menu → Assign → Pick and Assign**, or shortcut PA.
- Open the Settings (gearbox icon )
  - For **Pick Peaks in Display** select **GD:3D\_HHN**
  - Make sure that only **H** and **N** are selected as **Restricted Axes**

In the main part of the **Pick and Assign** module:

- Select the NmrChain: **NC:A**.
- Click on the **Sequence** column heading to sort the table by sequence number.
- **Double-click** on a row in the table, e.g. the row for **3 ARG**:

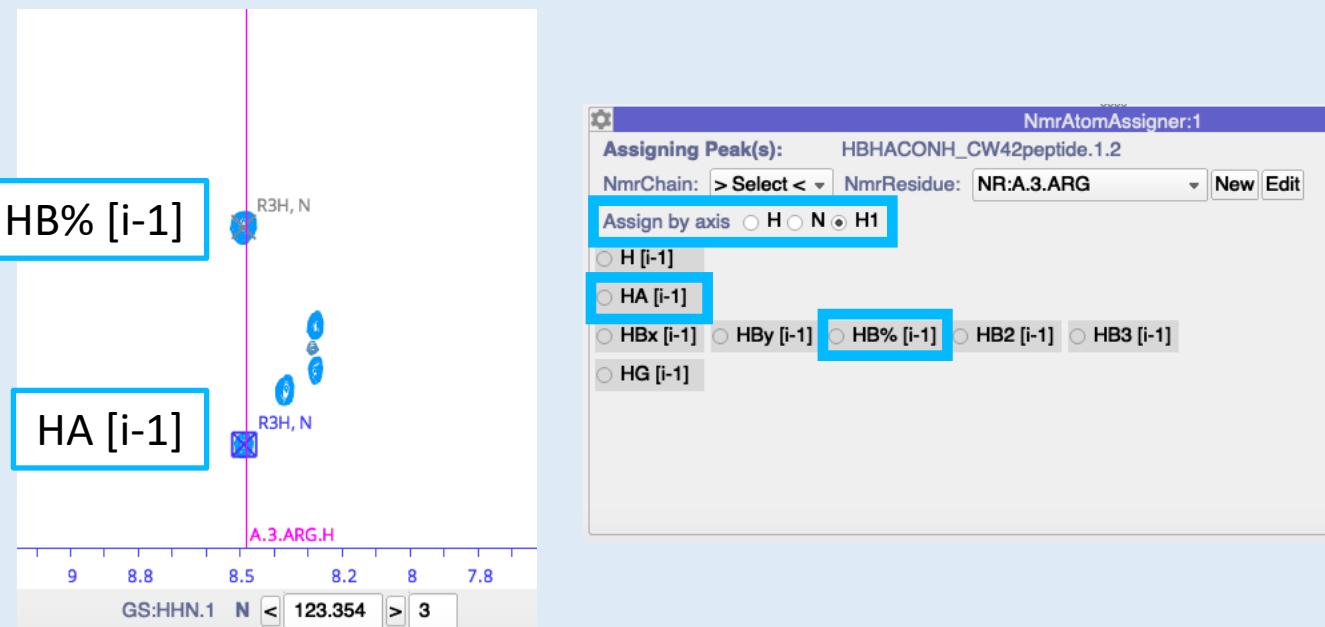
This will cause the **2D\_HN** and **3D\_HHN** SpectrumDisplays to focus on the position of the NH resonances of Arg 3 and mark their chemical shifts.

In the **3D\_HHN** Spectrum Display:

- increase the plane count from **1** to **3**
- set the contour level high enough not to show negative or noise peaks.

In the **Pick and Assign** module:

- Click on **Restricted Pick and Assign** to pick the peaks in the **HBHACONH** spectrum and assign the H and N dimensions.



## 2c Assigning HA/HB NmrAtoms

Open the NmrAtom Assigner with

- Go to Main Menu → Assign → NmrAtom Assigner, or shortcut AN.

Open the Settings (gearbox icon ) and adjust the settings:

- Set Mode to All
- Set Offset to -1

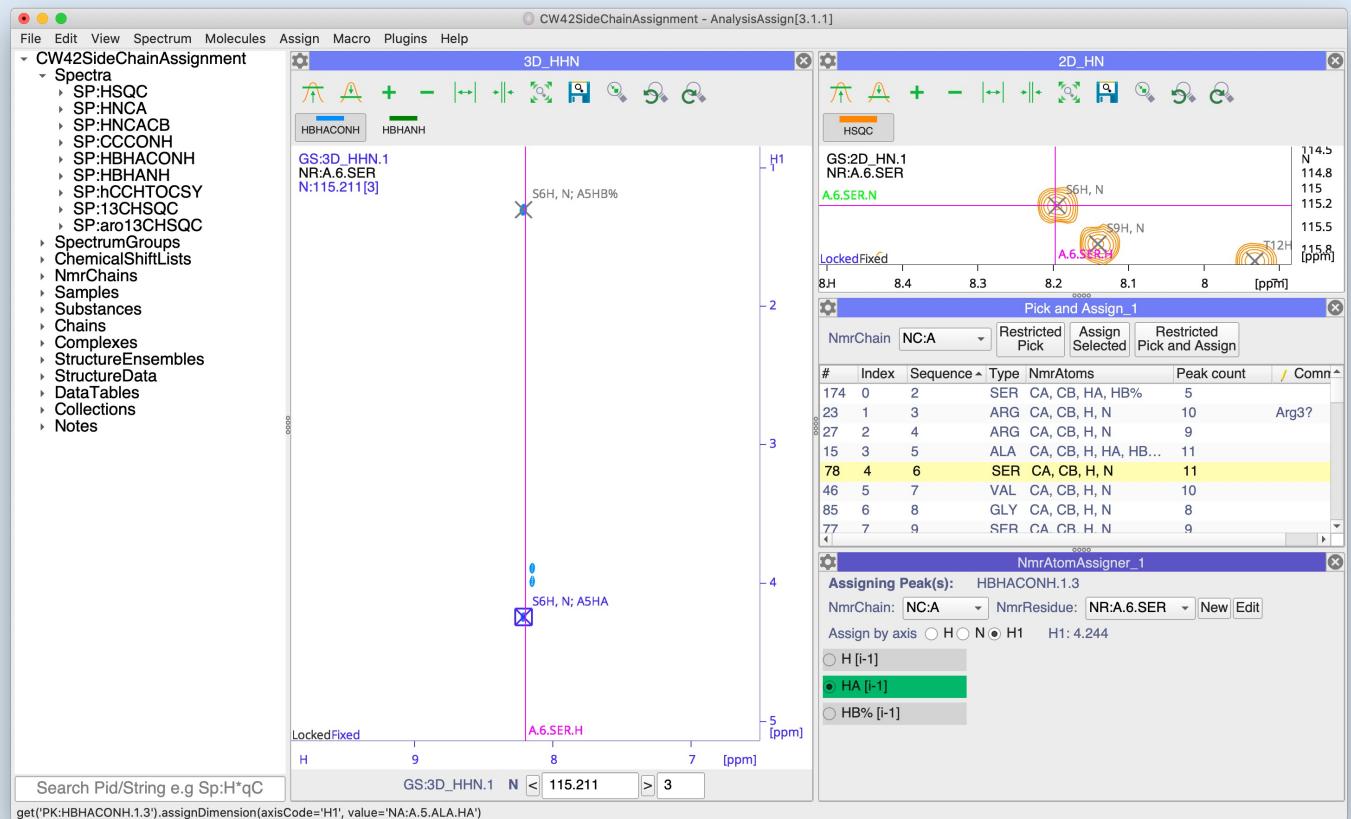
In the main module

- Make sure that Assign by axis is set to H1
- Select the lower peak and click on the HA [i-1] button
- Select the upper peak and click on the HB% [i-1] button

Note that if you have two HB peaks, you need to assign them to HBx [i-1] and HBy [i-1] (doesn't matter which way round). HB2/HB3 should only be used in the unlikely situation that you are making stereospecific assignments.

The assignment options shown in the lower part of the NmrAtom Assigner will adjust depending on the i-1 amino acid type. For each amino acid type you will only be offered its possible atom names.

See section 1 for a drawings of all amino acids and their NEF atom names.



## 2D Further assignments

Arrange your modules as shown above and repeat the procedure outlined in sections 2B and 2C for further amino acids with short aliphatic sidechains.

These are:

Glycine (GLY)

Alanine (ALA)

Serine (SER)

Cysteine (CYS)

Aspartic Acid (ASP)

Asparagine (ASN)

Phenylalanine (PHE)

Tyrosine (TYR)

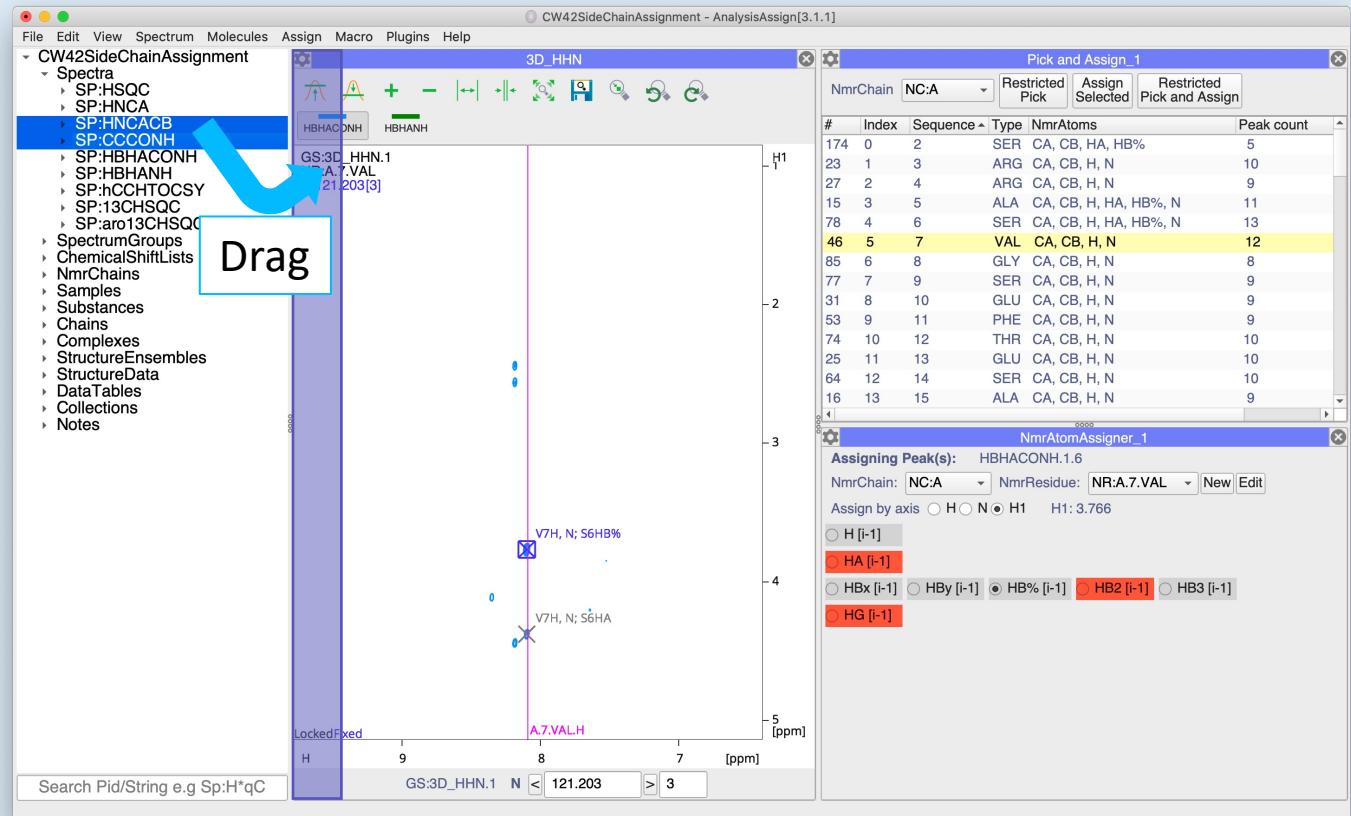
Tryptophane (TRP)

Histidine (HIS)

Note that you will need to select the i+1 residue in the **Pick and Assign** module to assign the HA/HB resonances. E.g. above, Ser 6 is selected, in order to assign the HA and HB% atoms in Ala 5.

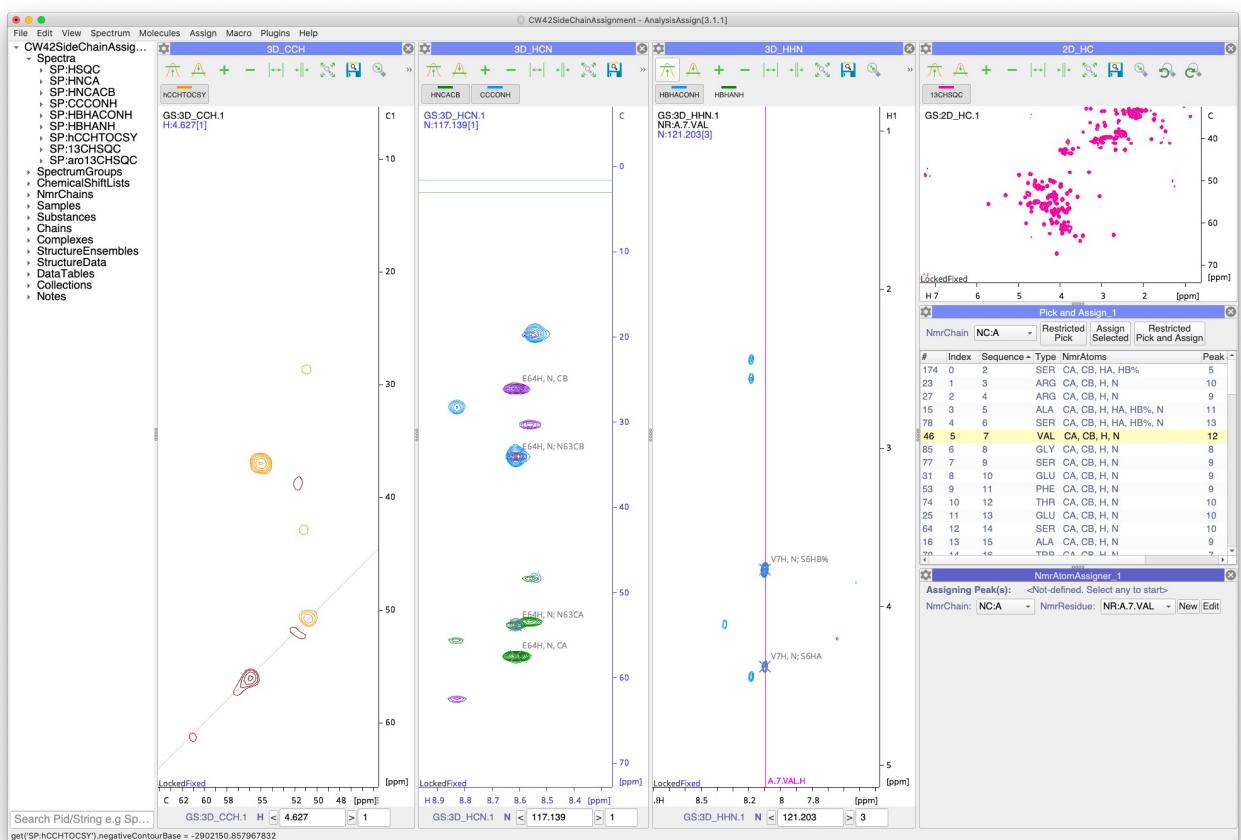
# Assigning Long Side Chains

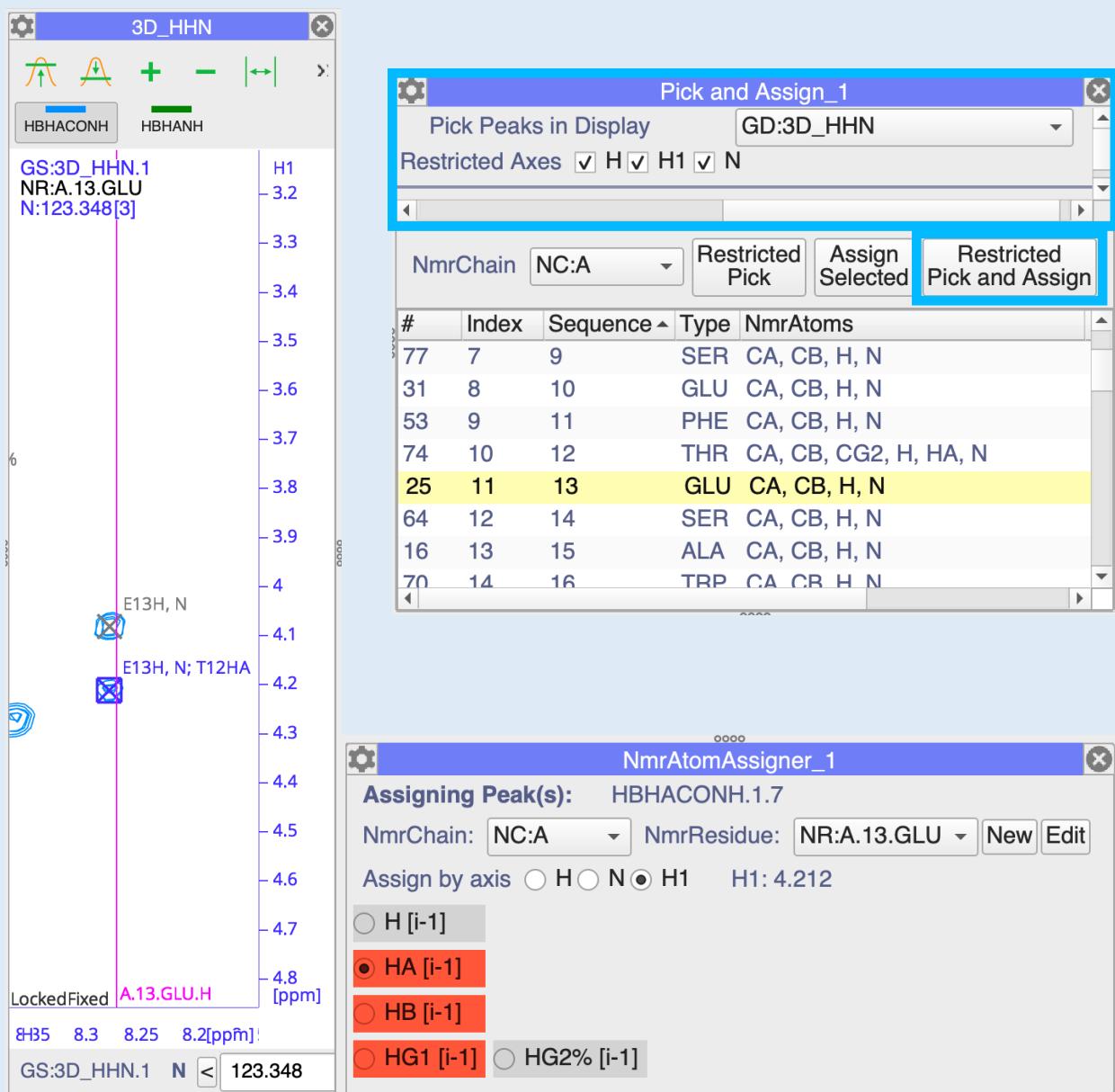
CW42SideChainAssignment



## 3C Adding additional spectra

- Close your 2D\_HN SpectrumDisplay using the X in the top right hand corner
- Drag the **CCCONH** and **HNCACB** spectra from the sidebar into the drop area so as to open them in a new SpectrumDisplay.
- Drag the **hCCHTOCSY** spectrum into a new SpectrumDisplay.
- Drag the **13CHSQC** spectrum into a new SpectrumDisplay so that your modules are arranged roughly like this:



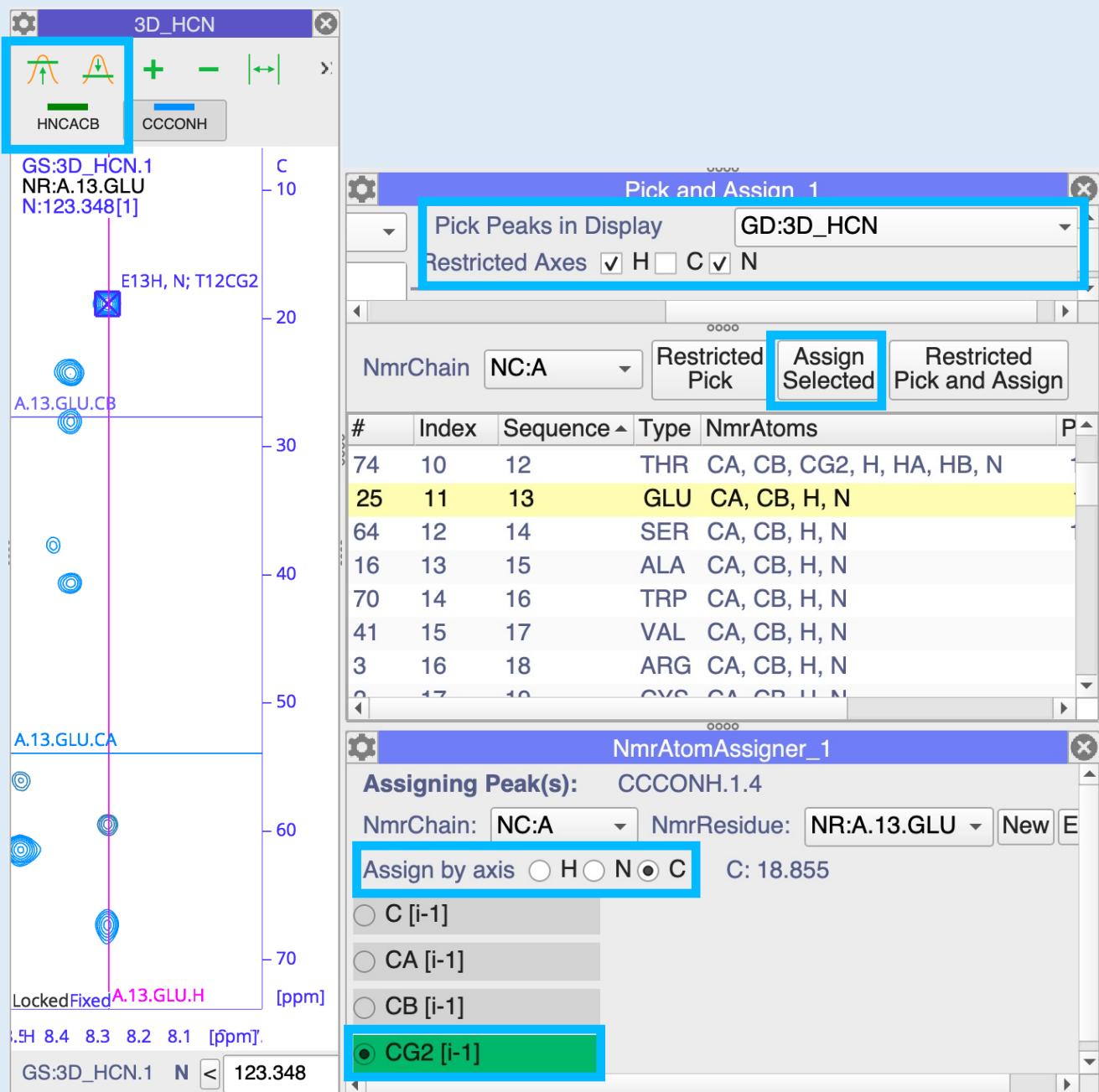


### 3B Picking and assigning HBHACONH peaks

- Open the **Pick and Assign** module **Settings**.
- For **Pick Peaks in Display** select the **GD:3D\_HHN** SpectrumDisplay.
- Make sure that the **Restricted Axes** are only set to **H** and **N**.
- Leave the **Settings** open, so you can see this part them (and later easily switch to a different SpectrumDisplay) as shown above.
- Double-click** on **13 GLU** in the NmrResidues table of the **Pick and Assign** module.

The SpectrumDisplays will now navigate to the GLU 13 NH location.

- Make sure the **HBHACONH** spectrum is at a suitable contour level for peak picking.
- Click on the **Restricted Pick and Assign** button in the **Pick and Assign** module.
- Now Assign the HA and HB peaks for THR 12 using the NmrAtom Assigner, as in Section 2.



### 3C Picking and assigning CCCONH peaks

The **CCCONH** spectrum should show the THR 12 CA and CB peaks (overlapped with these same peaks in the HNCACB) as well as a new CG peak (at ~19 ppm).

- Switch the HNCACB spectrum off using its button in the Spectrum Toolbar.
- Make sure the **CCCONH** spectrum is at a suitable contour level.
- Pick the CG peak in the **CCCONH** manually with **Shift+Ctrl/Cmd+right-drag**.
- In the **Pick and Assign** module **Settings** switch the **Pick Peaks in Display** option to the **GD:3D\_HCN** SpectrumDisplay (containing the **CCCONH** spectrum).
- Click on **Assign Selected** in the **Pick and Assign** module to assign the H and N atoms to Glu 13.

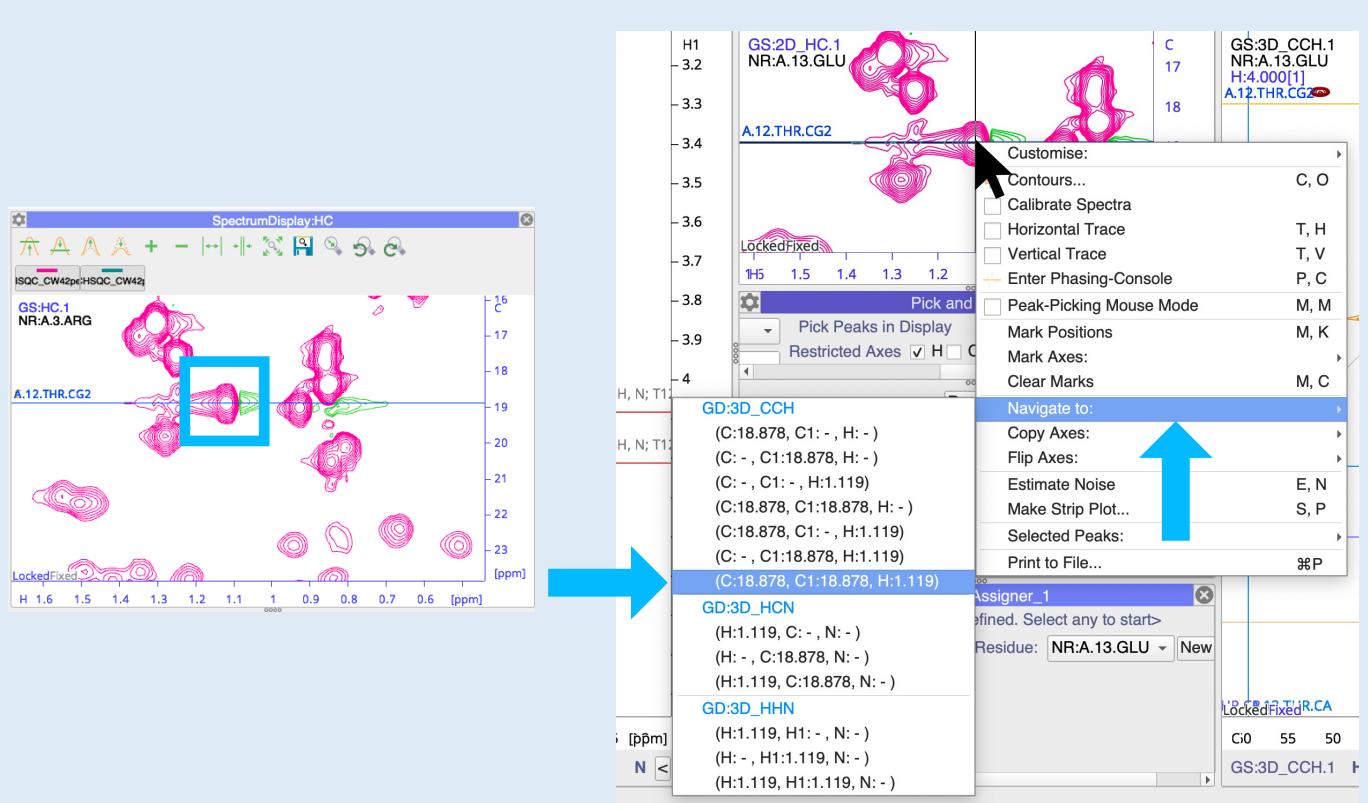
Assign the CG carbon in the **NmrResidue Assigner**:

- Select the **C** for **Assign by Axis**
- Select **CG2**.



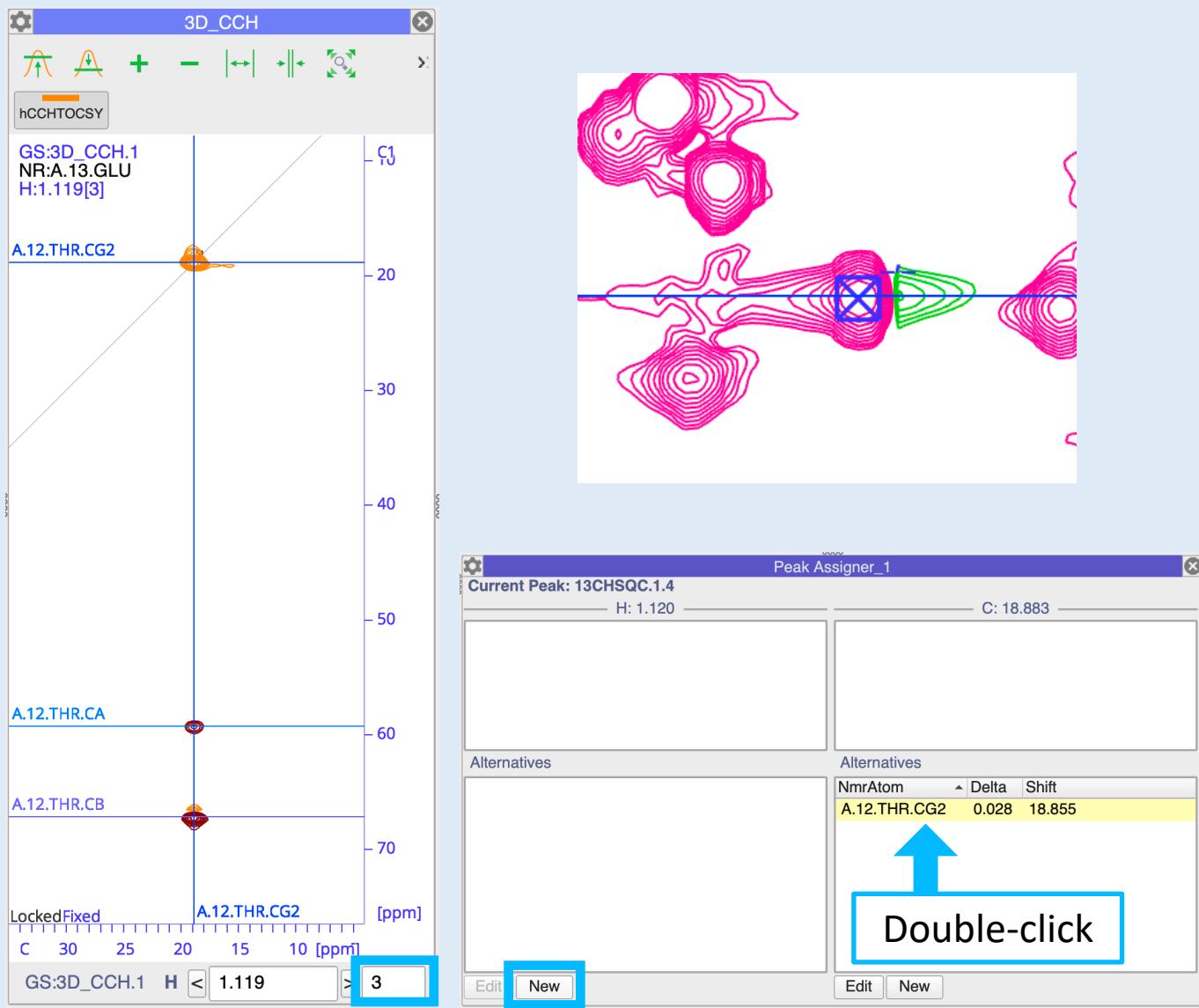
### 3D Placing marks

- Right-click in a Spectrum Display
- Clear all marks with MC.
- Drag the NR:A.12.THR NmrResidue from the sidebar into a Spectrum Display to mark all other Thr12 chemical shifts.



### 3E Navigating to the $^{13}\text{C}$ -HSQC and hCCH-TOCSY

- In the  $^{13}\text{CHSQC}$  spectrum, find the peak which the A.12.THR.CG2 mark goes through.
- Right-click on this peak and go to **Navigate to:** and then in the **GD:3D\_CCH** section select the final options which will navigate to all three dimensions. The **hCCH-TOCSY** spectrum in your **CCH SpectrumDisplay** will now navigate to this position.



### 3F Picking and assigning peaks

- Set the visible plan count in the 3D\_CCH SpectrumDisplay to 3.

The peaks at the intersection points of the Thr12 marks in the hCCHTOCSY spectrum confirm that these peaks belong to the Thr12 spin system and that the HG2% chemical shift is 1.12 ppm.

- Start by picking the CG2 peak in the **13CHSQC** spectrum.
- Open the **Peak Assigner** module by going to **Main Menu → Assign → Peak Assigner** or using the shortcut **AP**.

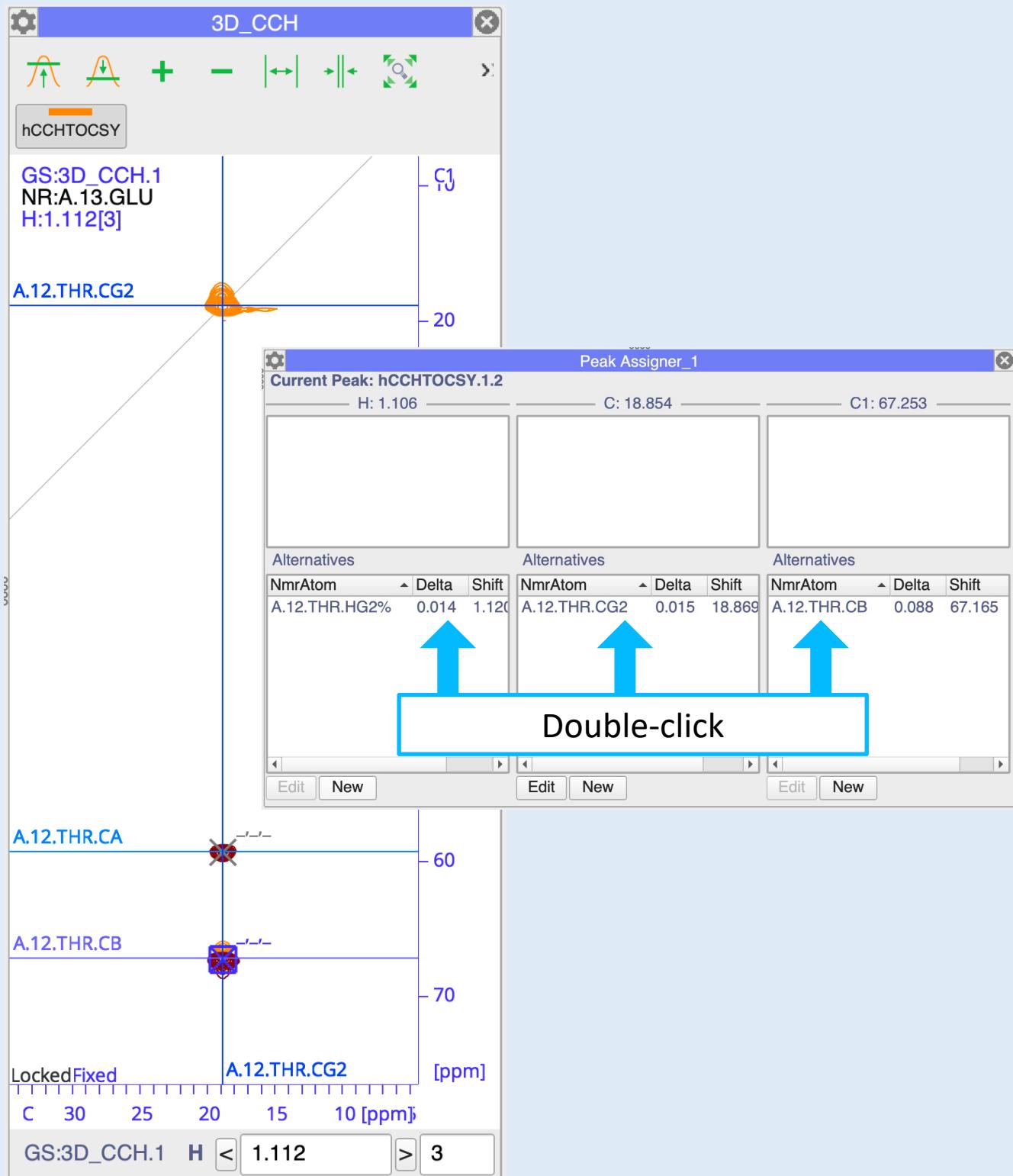
The left and right hand columns are for the H and C dimensions, respectively; the upper and lower section for the assignments and assignment options.

- Double-click** on **A.12.THR.CG2** in the lower right section, to assign it to the C dimension of this peak. It will move to the upper right section.

For the H dimension we still need to the create the Thr 12 HG2% NmrAtom:

- Click on **New** on the left hand side.
- Fill in the fields as shown and click **Accept**.

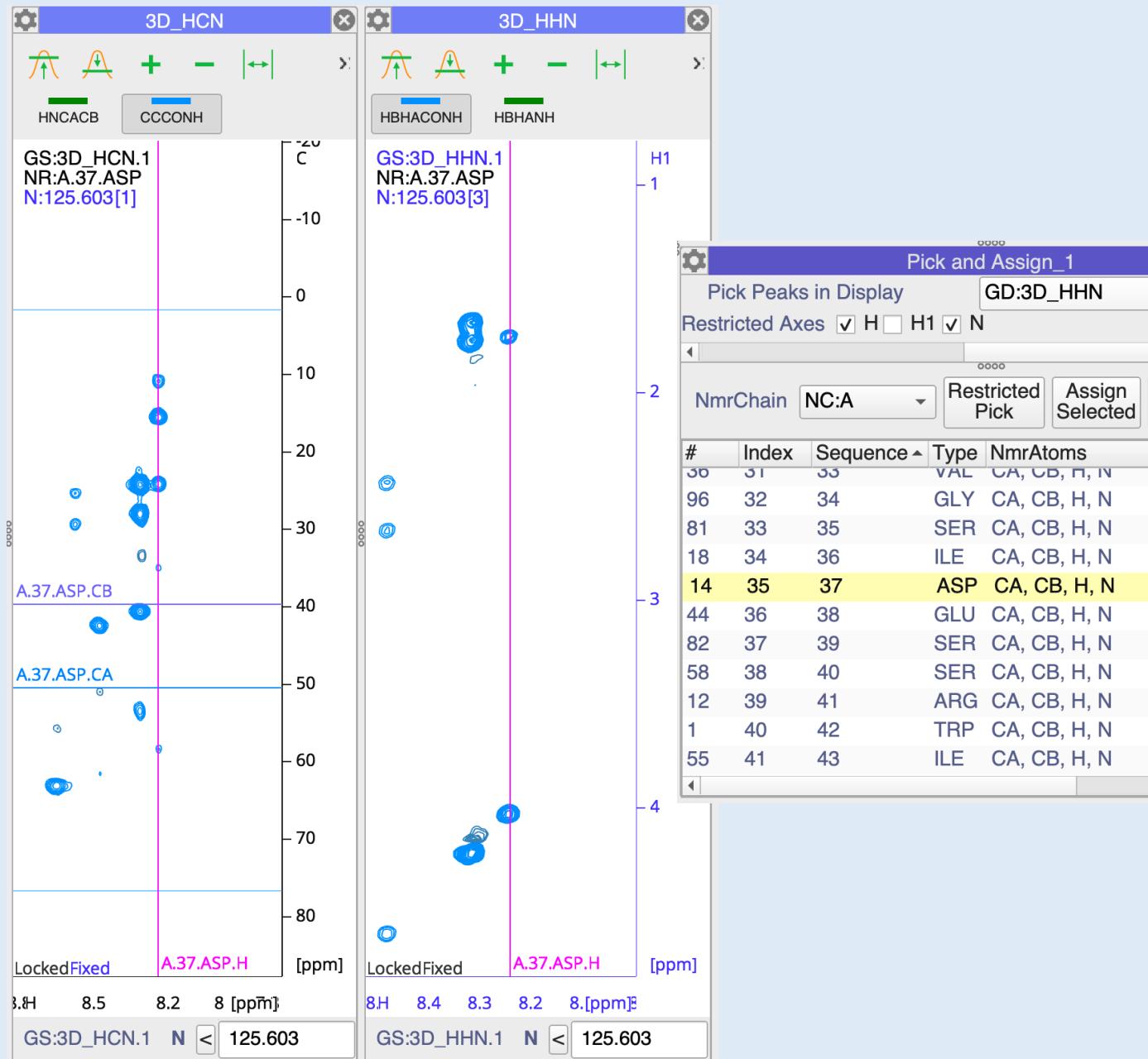
A	12	THR	HG2%	Accept
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### 3G Picking and Assigning peaks contd.

- Now pick the two non-diagonal peaks in the **hCCHTOCSY** spectrum (at the CA and CB chemical shifts).
- In the **Peak Assigner**, assign all three dimensions of both peaks by **double-clicking** the correct alternatives on the right hand side.

If you ever accidentally select an incorrect option from the **Alternatives** below (e.g. if more than one is available), simply **double-click** on it in the upper box and it will move back down to the **Alternatives** again.



### 3H Assigning further long side chains

Try assigning the side-chains of

Ile36

Glu60

Pro29

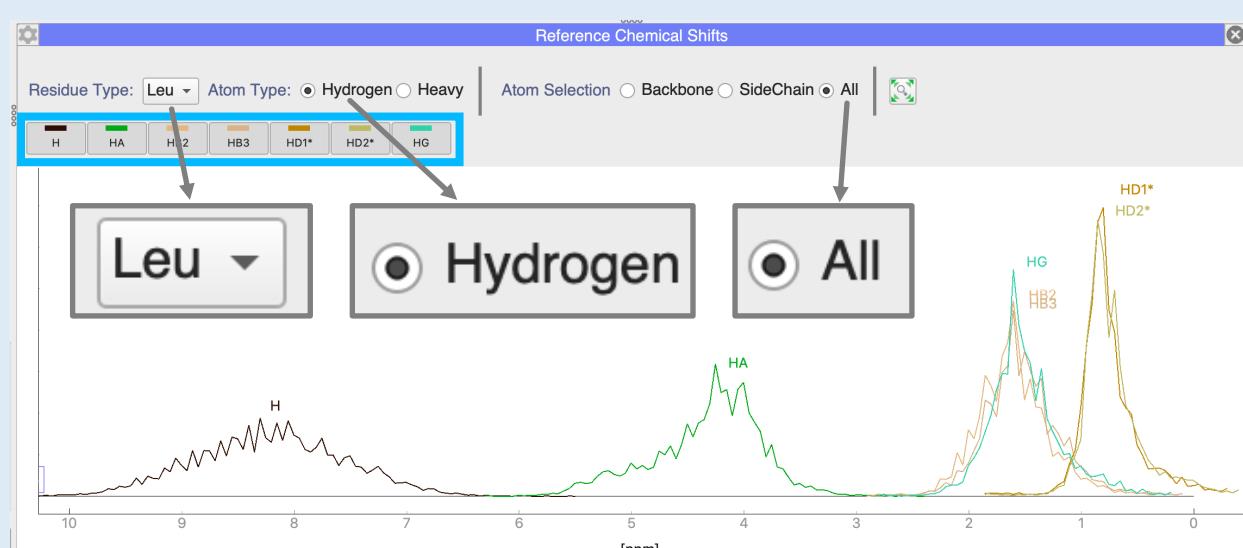
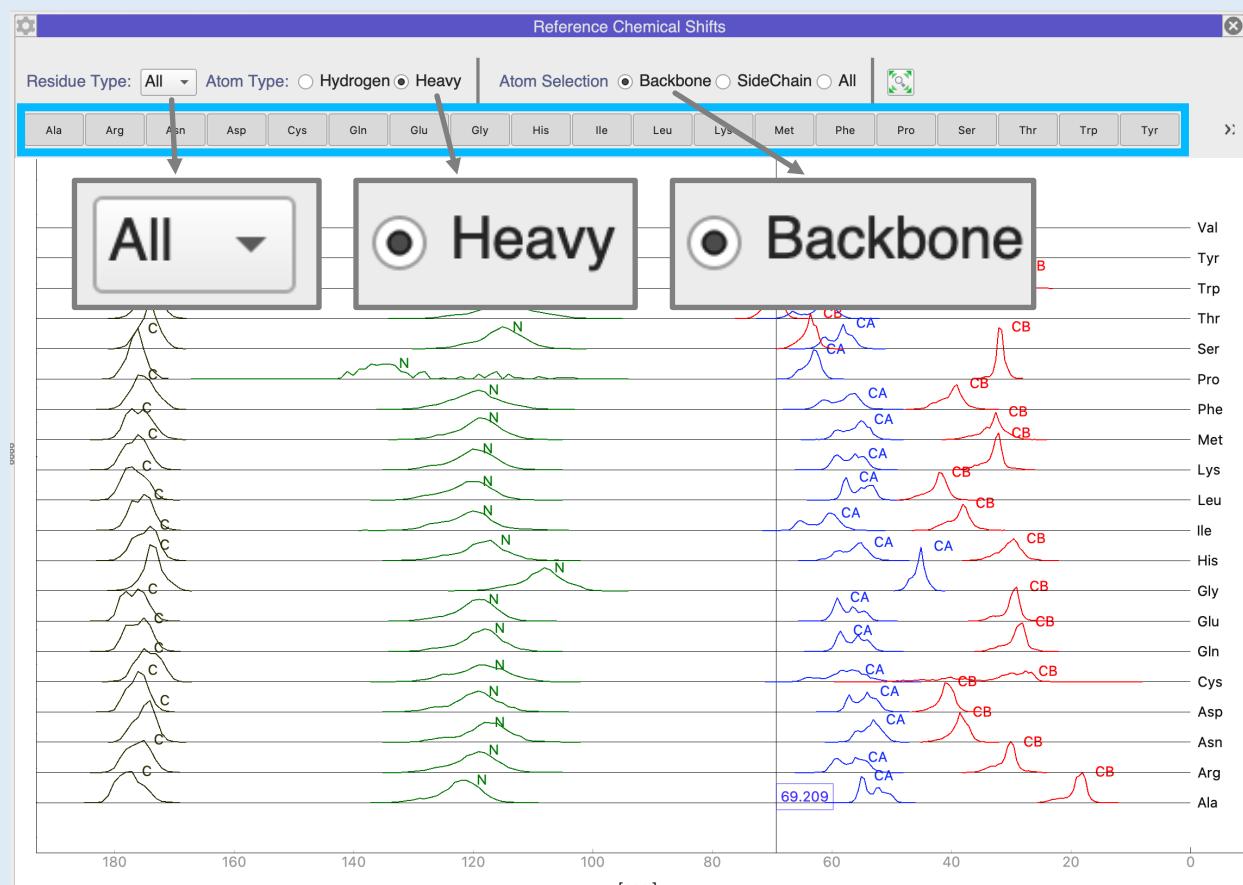
Gln74

Leu70

You may wish to refer to some of the reference material at the start of the tutorial, e.g. the typical carbon chemical shifts for the 20 natural amino acids and their IUPAC/NEF atom names.

The next page shows how you can access reference chemical shifts from within the program.

# Assigning Long Side Chains



**RC**

## 31 Reference Chemical Shifts

You can check the standard chemical shifts for protein amino acids within CcpNmr Analysis:

- Go to **Main Menu → Molecules → Reference Chemical Shifts**, or type **RC**.
- For the **Residue Type** select either **All** or an individual amino acid, e.g. **Leu**.
- For the **Atom Type** select either **Hydrogen** or **Heavy**.
- For the **Atom Selection** select **Backbone**, **SideChain** or **All**.
- Switch off particular amino acid or atom types in the toolbar.

A mouse cursor correlates the ppm position with that in your SpectrumDisplays.

You can move the graph or zoom with the mouse wheel on axes or in the main graph area like in a SpectrumDisplay.

## Contact Us

**Website:**

[www ccpn.ac.uk](http://www ccpn.ac.uk)

**Suggestions and comments:**

[support@ccpn.ac.uk](mailto:support@ccpn.ac.uk)

**Issues and bug report:**

<https://forum.ccpn.ac.uk/>

## Cite Us

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