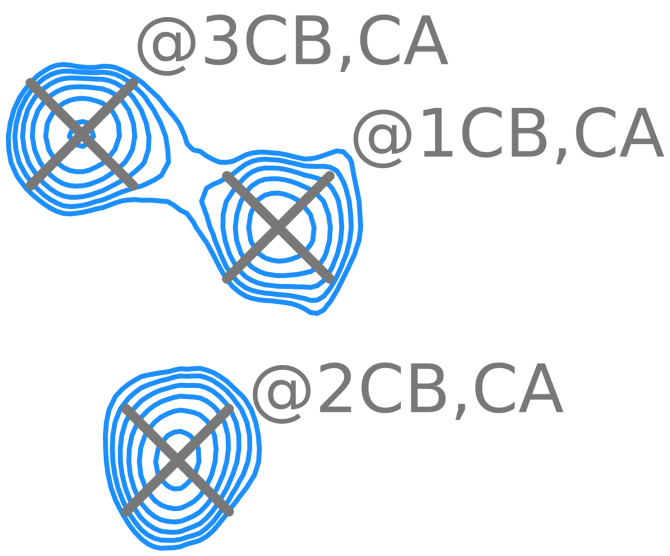
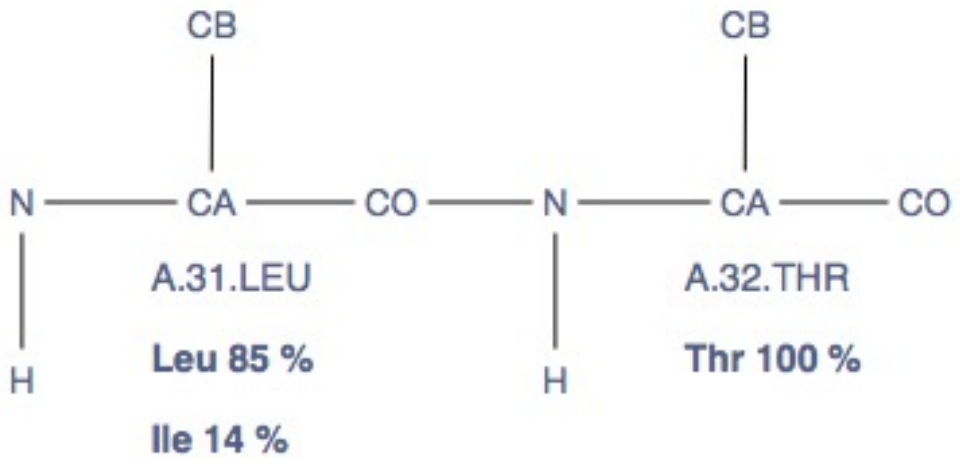
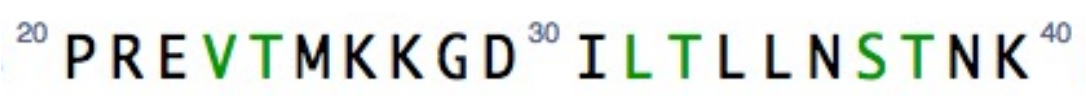
CCPN

CcpNmr Analysis Version 3

Solid State SH3 Assignment Tutorial







Introduction

This tutorial is designed to introduce you to a variety of tools and features which are useful for the assignment of carbon-detected solid-state protein NMR spectra using CcpNmr AnalysisAssign Version 3.1. It is not intended to teach any theoretical aspects of NMR assignment. For more details about procedures to assign proteins in the solid state see Higman, VA (2018) Progress in NMR Spectroscopy 106-107, 37-65.

It is assumed that you have some basic familiarity with the program, e.g. from having completed our Beginners Tutorial.

In this tutorial you will use spectra recorded on the SH3 domain of chicken alpha-spectrin, in particular PDSD, NCACX and NCOCX spectra recorded on uniformly labelled protein and protein labelled using 1,3-^{13}C and 2-^{13}C-labelled glycerol. We are grateful to Prof. Hartmut Oschkinat for making these spectra available to us. More details on the data can found in Castellani *et al.* (2002) Nature 420, 99-102.

You will need to use the project which is located in the directory: CcpnTutorialDataSolidStateNmrJune2022/ssNMRSH3AsssignmentTutorial.

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

Contents:

Start CcpNmr Analysis V3

Apple users by double clicking the icon *CcpNmrAnalysis* or using the terminal command: *bin/assign*

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

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

1

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 which share the same axes. 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**. You can also add additional spectra to a spectrum display module later on, or drag several spectra into the drop area together to open them simultaneously.

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 **middle-click and drag**

Shortcuts

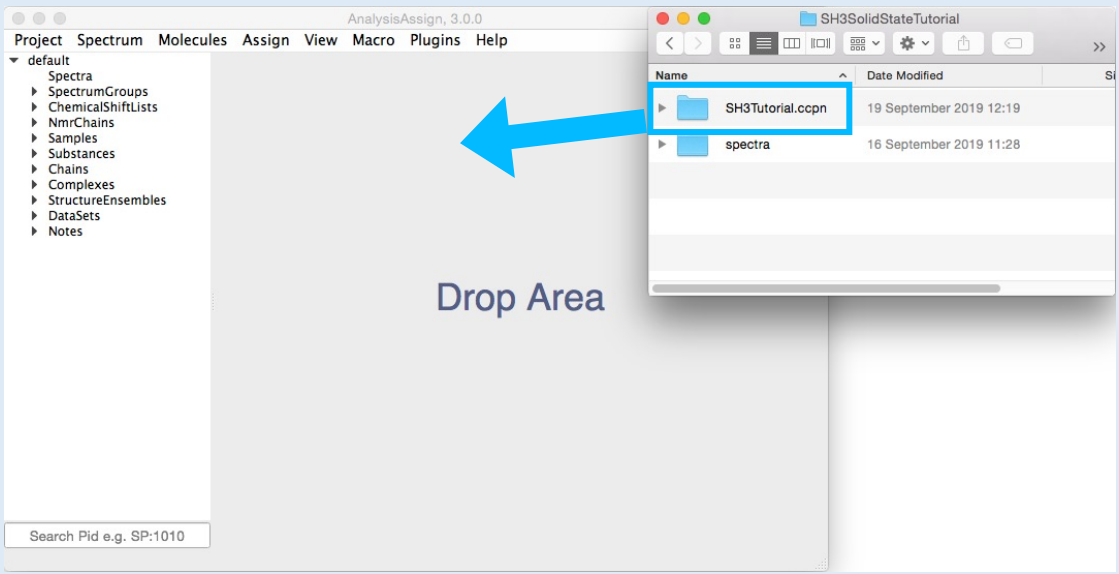
The program uses several shortcuts, for example **MK** for creating a mark at the current mouse position. You will need to press the first letter on your keyboard e.g. **M**, followed by the second letter, e.g. **K** (case insensitive). Press **Esc** to cancel the first letter.

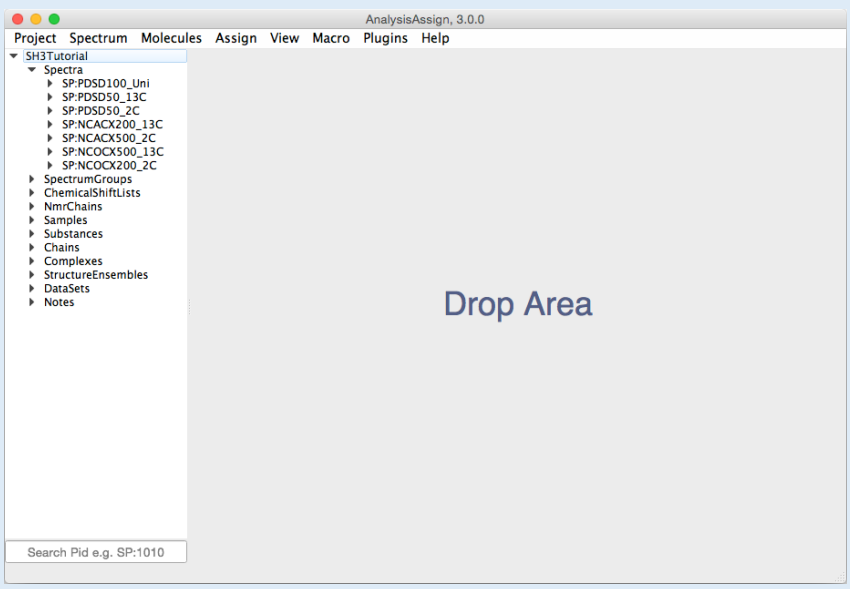
Fore more commands and operations:

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

Project Setup

Open the project AnalysisV3/data/SH3SolidStateTutorial/SH3Tutorial.ccpn





1A **Drag & drop** SH3Tutorial.ccpn **into the sidebar or drop area**

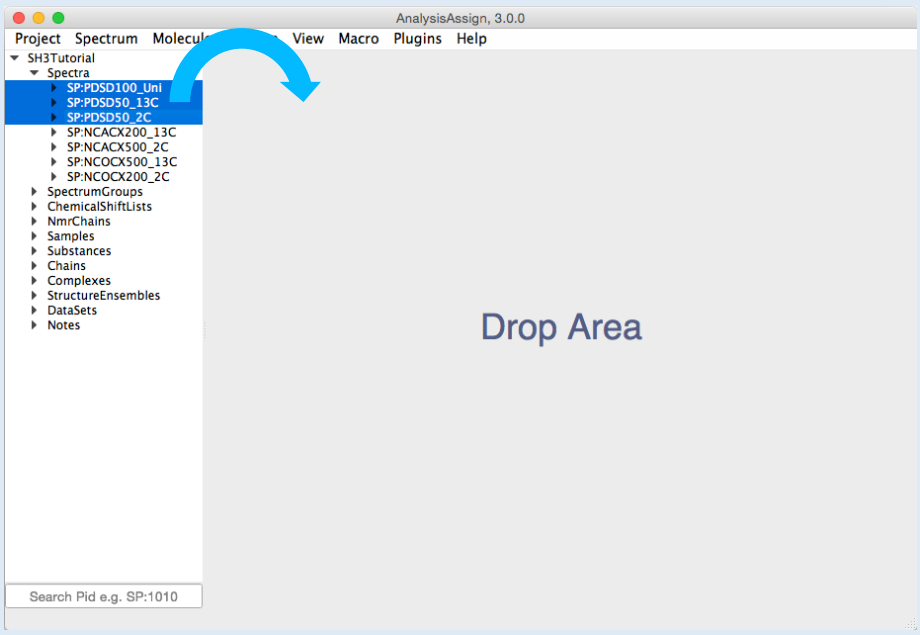
CcpNmr projects have an extension of type **filename.ccpn.** in the **CcpnTutorialDataSolidStateNmrJune2022/ssNMRSH3AsssignmentTutorial** directory, find the project **SH3Tutorial.ccpn**.

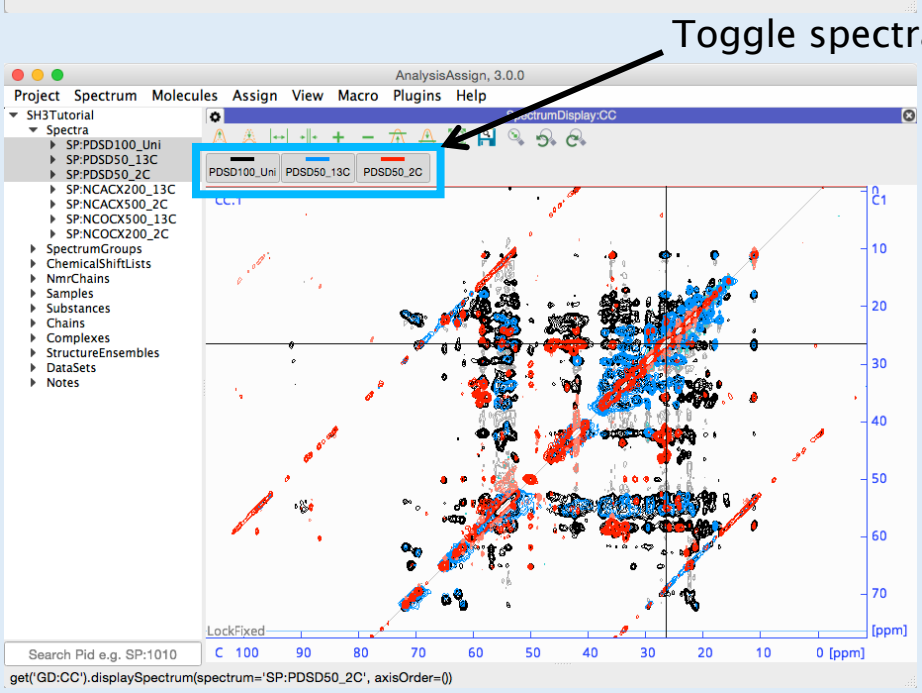
- Select the directory **SH3Tutorial.ccpn,** drag and drop it into the program. The SH3 Tutorial project will be loaded.

Nested under **Spectra** in the sidebar, you will have seven spectra. The spectrum names indicate the spectrum type, mixing time and sample labelling:

PDSD100_Uni	PDSD, 100ms mixing time, uniformly labelled sample
PDSD50_13C	PDSD, 50ms mixing time, 1,3-^{13}C glycerol sample
PDSD50_2C	PDSD, 50ms mixing time, 2-^{13}C glycerol sample
NCACX200_13C	NCACX, 200ms mixing time, 1,3-^{13}C glycerol sample
NCACX500_2C	NCACX, 500ms mixing time, 2-^{13}C glycerol sample
NCOCX500_13C	NCOCX, 500ms mixing time, 1,3-^{13}C glycerol sample
NCOCX200_2C	NCOCX, 200ms mixing time, 2-^{13}C glycerol sample

Displaying Spectra





1B Displaying spectra

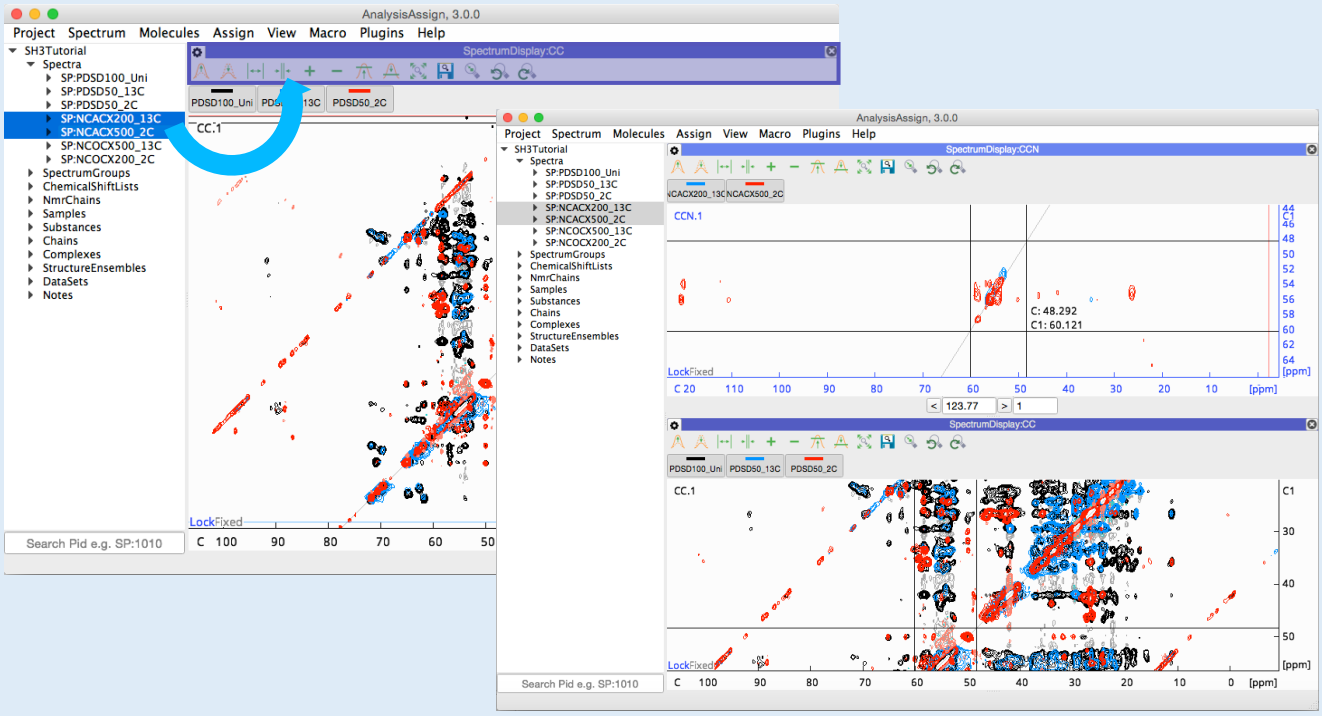
Spectra with the same axes can be shown in the same display. E.g. you can drag all PDSD spectra into the drop area together or drag further spectra into the display once you have opened one of them.

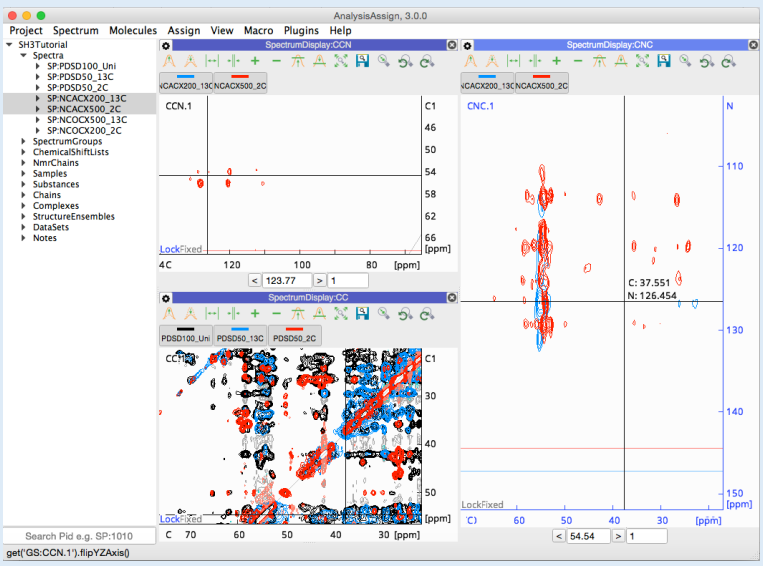
- **Drag** the PDSD spectra into the drop area.
- Use the **Spectrum Buttons** in the toolbar to toggle individual spectra on or off in the display.

The spectra are colour coded according to the isotopic labelling of the samples they were recorded on:

black	uniform ^{13}C,^{15}N labelling
blue	uniform ^{15}N labelling, 1,3-^{13}C glycerol labelling
red	uniform ^{15}N labelling, 2-^{13}C glycerol labelling

Displaying 3D Spectra





YZ

to flip the y and z-axes in the 3D spectrum

2A Displaying the 3D spectra

Your 3D spectra will have to go into a separate display to your PDSD spectra. If at least one spectrum display module is already open, then you can choose where to open a new one. The appearance of a purple box indicates possible drop positions.

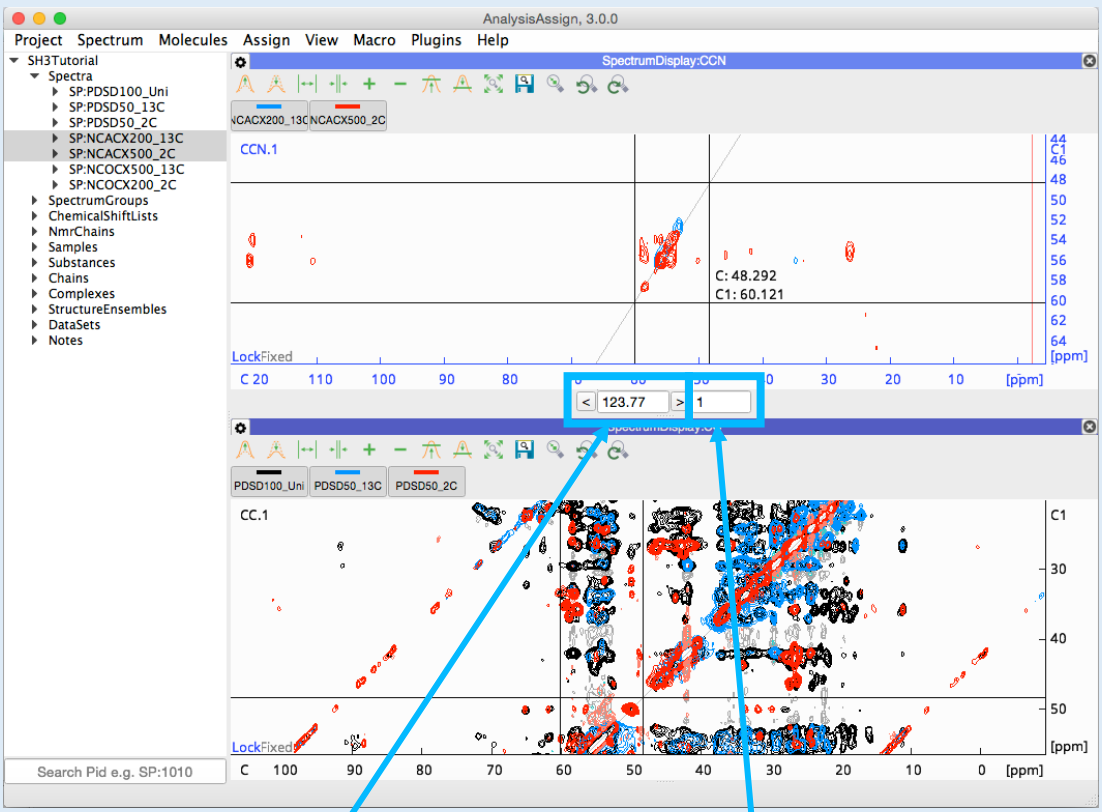
- **Drag** the NCACX spectra from the sidebar into the drop area.

By default the 3D NCC spectra are shown with ^{15}N on the z-axis. You can easily flip these to show ^{15}N on the y-axis:

- **Click** on the NCACX spectrum display you want to flip to make sure it is the active strip (its axes are then highlighted)
- Type **YZ**

This will open a new spectrum display module – close or retain the initial 3D spectrum display module as you wish and re-arrange your modules to suit you.

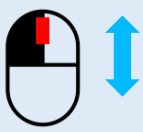
Navigating through 3D Spectra



JJ/KK

or

Ctrl/ Cmd +



use arrows to move through planes, or type a ppm value

change number of visible planes here

2B Navigating through 3D spectra

To move through the z-planes of a 3D spectrum:

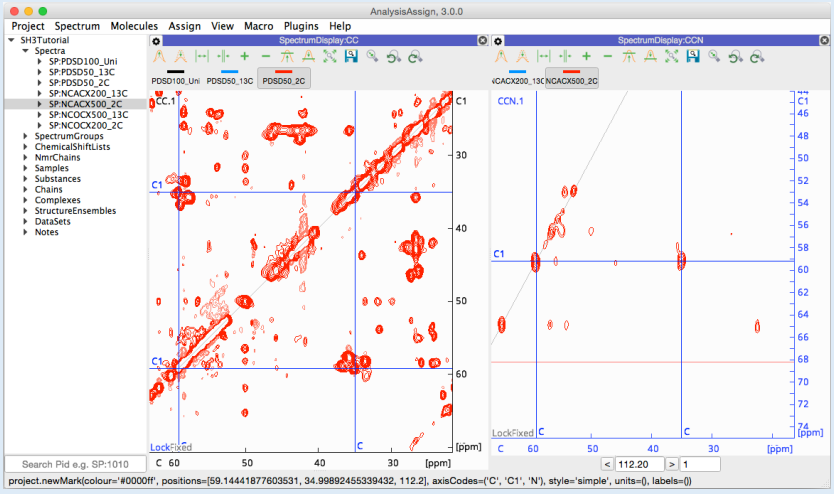
- use the **arrow buttons** either side of the z-position, **or**
- **type** a ppm value in the box to go to a specific position **or**
- use the shortcuts **JJ/KK or**
- press **Ctrl** (**Cmd** on a Mac) and use the **mouse scroll wheel**

Change the number of visible planes by

- typing a different number into the planes box

Sometimes it can be useful to move to a neighbouring plane or increase the number of visible planes when trying to pick peaks in 3D spectra.

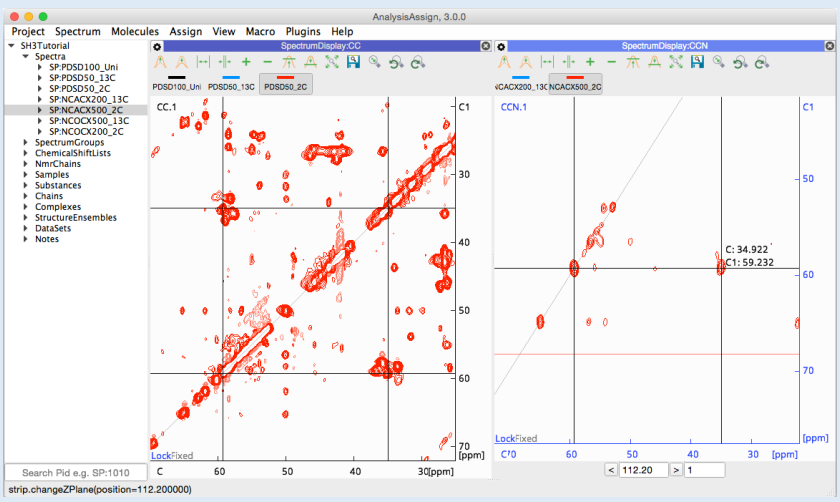
Marks and Mouse Crossharis



MK Draw mark at mouse position

PM Draw mark at selected peak position(s)

MC Clear all marks



2C Marks and crosshairs

Often it is useful to draw lines through your spectrum to check whether two peaks occur at the same chemical shift or not. A Mark is drawn through all dimensions in all displays at the position where it is placed.

- Place the mouse where you want the mark to be and type **MK**.
- You can also place a mark directly on one or more peaks by selecting the peak(s) and typing **PM**.
- Clear all your marks by typing **MC**.

Note that the mouse appears as a double crosshair which links all carbon dimensions to one another.

3 Spin System Identification

The SH3 domain of chicken α-spectrin contains three threonines. This section will show you how to identify them based on their chemical shifts, pick their peaks, generate NmrAtoms and NmrResidues for them, and assign their atom and amino acid types. Later you will use the 3D spectra and glycerol labelling pattern to sequence specifically assign the threonines.

Assignment nomenclatures (Explanation only)

Assignment in AnalysisAssign is simply a matter of setting strings that define the NmrAtoms.

We call this the 'id' (id: identifier) of the NmrAtom. If an id matches the strings defining a molecular Atom[1],this effectively constitutes an assignment to the Atom.
If not, the NmrAtom id is a placeholder, reflecting its progress towards assignment[2].

At this point, it is appropriate also to consider the relationships between Peak, ChemicalShift and NmrAtom. Each dimension of a Peak is assigned to one or more NmrAtoms. The ChemicalShift (which resides in a ChemicalShiftList) of an NmrAtom, is defined by all the peaks that have been assigned to this NmrAtom. Hence, changing an assignment for a Peak (e.g. reassigning a peak from "nmratom_1" to another "nmratom_2") has an effect on the ChemicalShift of "nmratom_1", as it is now no longer defined by the Peak. Likewise, it also affects the ChemicalShift of the "nmratom_2", as it now comes to be (also) defined by the Peak. We will see in next sections how to inspect and change the assignment(s) of a Peak.
If you change the id of an NmrAtom (or its parent NmrResidue or NmrChain), the assignment of all ChemicalShifts and Peaks are updated.

We use NmrChains and NmrResidues to keep track of the NmrAtoms during the assignment process. By default, new NmrResidues are put in NmrChain '@-', and new, temporary NmrChains are given names like '@2'. Initially, NmrChains contain no information about the sequential connections of the NmrResidues, i.e. their ordering. In this case, the NmrChain functions like a simple list with all its NmrResidues.
To store sequential stretches, i.e. lists in which the NmrResidues are ordered, the program uses 'connected' NmrChains, whose names start with '#' instead of '@'. Consequently, names with '@' (and NmrChain names starting with '#') are reserved.

NmrResidues are created with names like '@173' and with no residueType. You can add or change the residueType at any point.

NmrAtom names always start with the nucleus, and default names would be e.g. 'H@31' or 'C@88'. Some names have a special meaning:

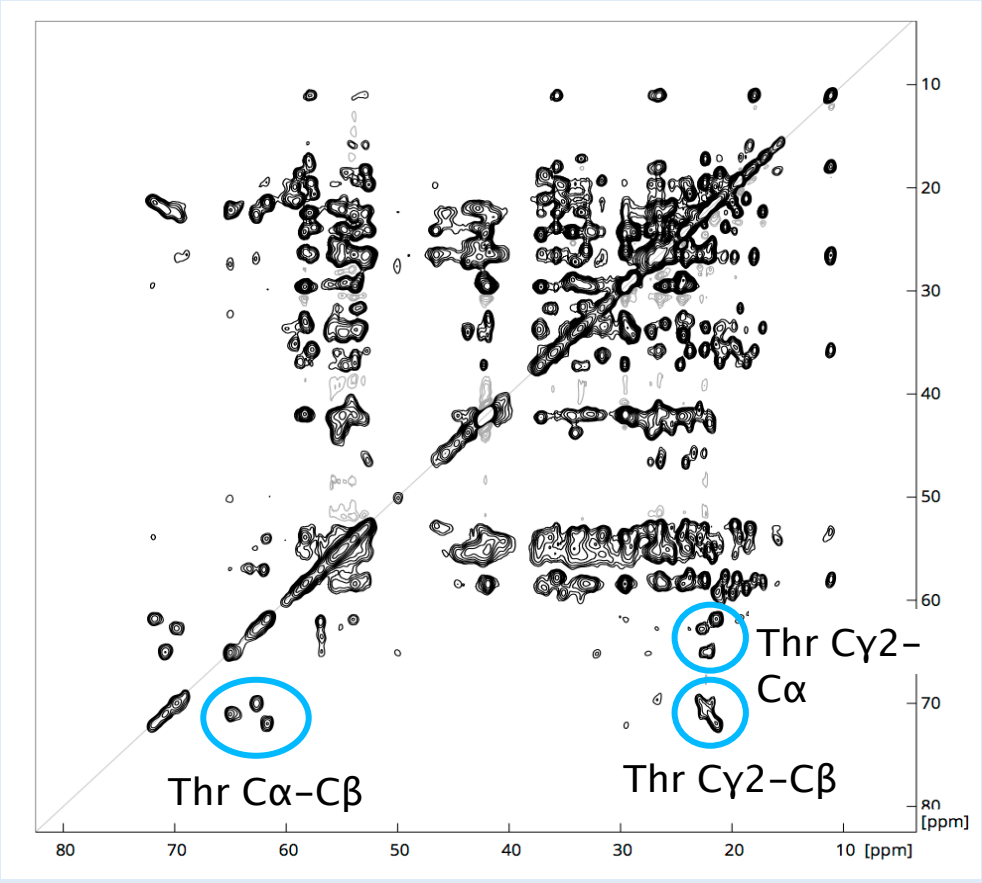
- '%' means 'any number', so 'HB%' would be a beta methylene or methyl group. 'H%' would be the backbone NH3 group.
- '*' means 'any string', so 'C*' would be 'any carbon in the residue'
- Names starting with 'M' and 'Q' are (proton) pseudoatom names
- Number suffixes follow NEF (IUPAC) convention, so serine HB2 or HB3 denote stereospecific assignments.
- Suffixes 'x' and 'y' are used for non-stereospecific pairs – the normal assignment to serine beta would use HBx and HBy. For e.g. isopropyl groups the x and y assignments match up between ^{1}H and ^{13}C so that Leu HDx% are the methyl protons bound to Leu CDx (NEF convention).

[1] Atoms reside in Residues, which reside in Chains; multiple chains can form a Complex.
[2] The id together with the type identifier forms the so-called pid, the project-identifier. As an example for an un-assigned CA in the 123rd NmrResidue in the second NmrChain: NA:@2.@123..CA. For an assigned NmrAtom, all the fields will have been filled, yielding something like NA:A.GLU.14.CA.

For more information see our video tutorial on NmrResidues at

https://www.youtube.com/embed/DS9IZzNsBbQ

Thr peaks in the PDSD sepctrum



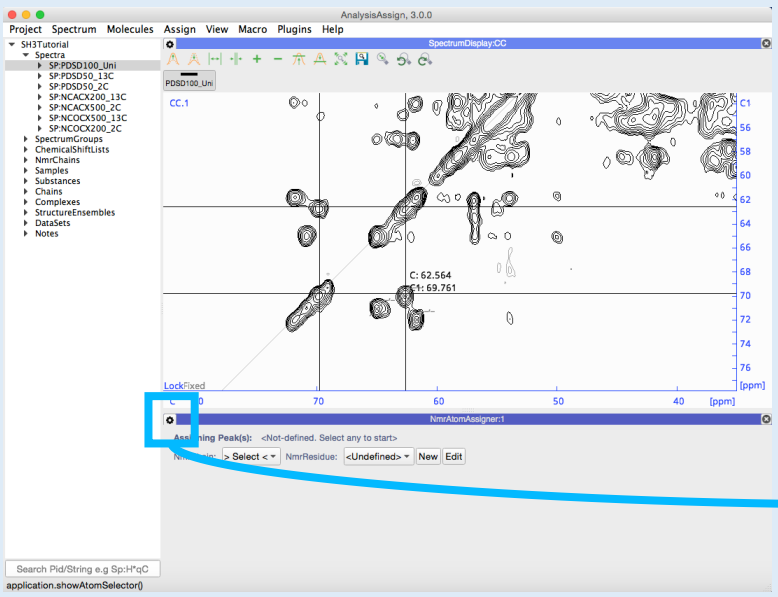
3A Identifying and peak picking the threonine residues

Based on their characteristic chemical shifts, try to identify the Cα–Cβ, Cα–Cγ2, Cβ–Cγ2, Cα–CO and Cβ–CO cross peaks of the three threonines in the uniform (black) PDSD spectrum and peak pick them (except possibly the Cβ–Cγ2 peaks which are not very well separated). Drawing marks through your peaks (**PM**) will help you connect them.

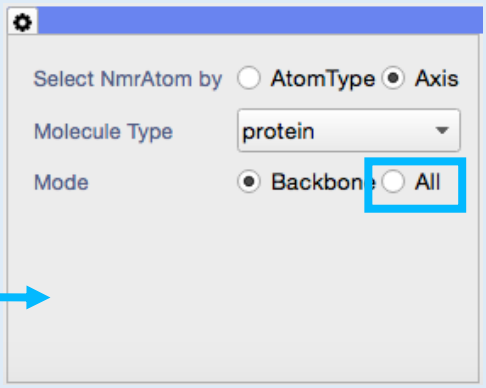
- Drag your PDSD100_Uni spectrum into the drop area and find the three Cα–Cβ cross peaks below the diagonal (x-axis ≈ 62.5 ppm; y-axis ≈ 70 ppm).
- Begin by peak picking the left hand cross peak (**Shift+Ctrl/Cmd+left-drag**).
- Place a mark through this peak (**PM**).
- Look at the threonine Cγ2 region at about 20 ppm. There is only one chemical shift (22.6 ppm) at which there are peaks which go through both marks (i.e. the Cα–Cγ2 and Cβ–Cγ2 cross peaks). Peak pick the Cα–Cγ2 cross peak (22.6 and 63.0 ppm).
- Now go to the carbonyl region of the spectrum at around 170–180ppm on the x-axis. Again there is one chemical shift (173.5 ppm) at which there are peaks which go through both marks. These are the Cα–CO and Cβ–CO cross peaks. Peak pick both of these. You have now identified one threonine spin system.

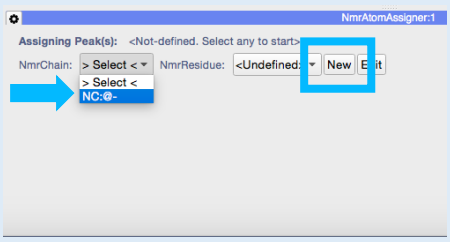
Now remove your marks (**MC**) and try the same procedure for the other two threonine spin systems.

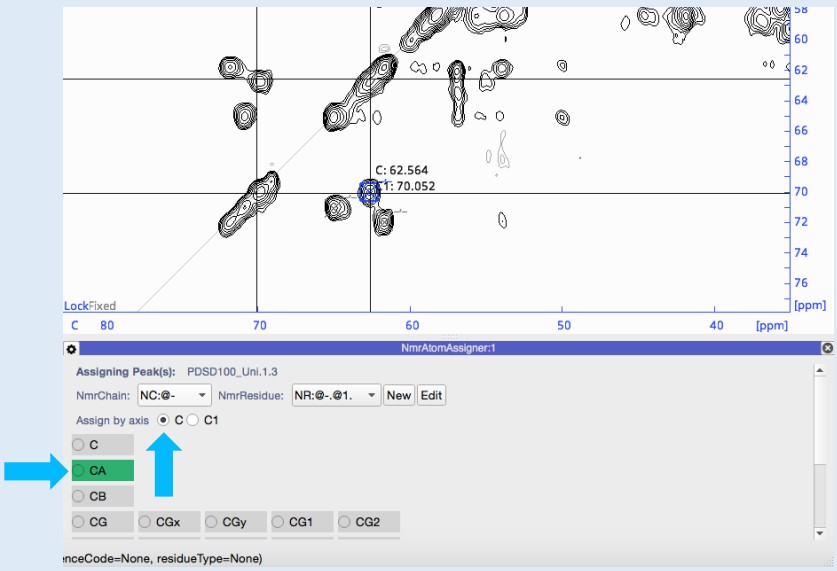
Creating and assigning NmrAtoms and NmrResidues



AN







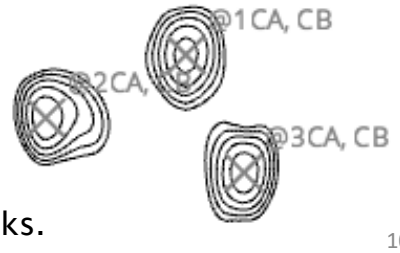
3B Assigning NmrAtoms and NmrResidues

We will create NmrAtoms and NmrResidues using the NmrAtom Assigner:

- Open the NmrAtom Assigner module with **AN** (or go to **Main Menu → Assign → NmrAtom Assigner**).
- Click on the gearbox icon (⚙) to change a setting: select Mode **All** (instead of **Backbone**). Click on the gearbox icon again to close the settings box.
- Select the NmrChain as **@-** from the pull down menu.
- Select a Cα–Cβ cross peak below the diagonal and click on **New** in the NmrAtom Assigner. This will create a new NmrResidue **@1** in NmrChain **@-**.

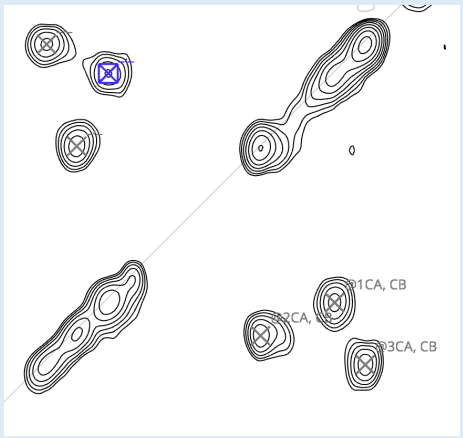
Now you need to assign each of the two dimensions of the peak to an atom type. In this case the C Axis corresponds to the Cα chemical shift, and the C1 Axis to the Cβ:

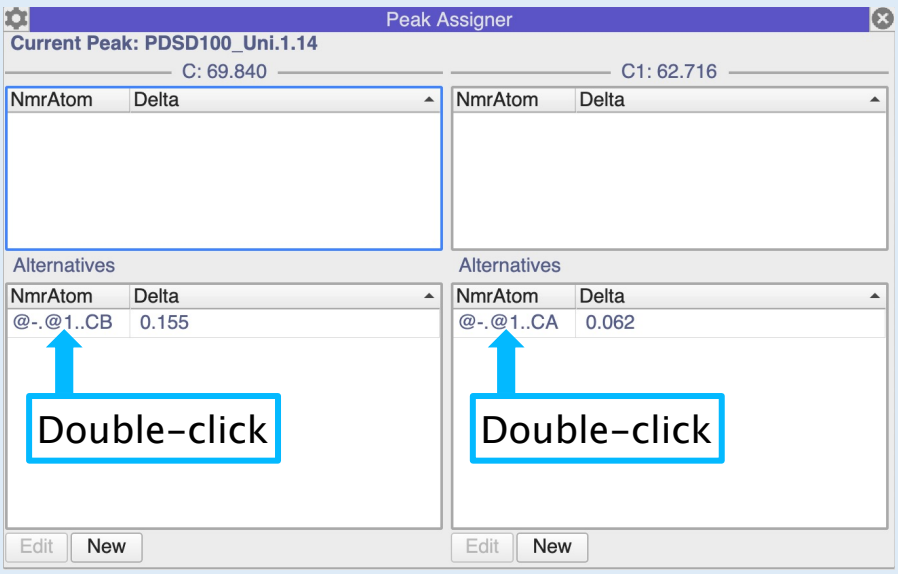
- Select **Assign by axis C** and then click on **CA**.
- Select **Assign by axis C1** and then click on **CB**.

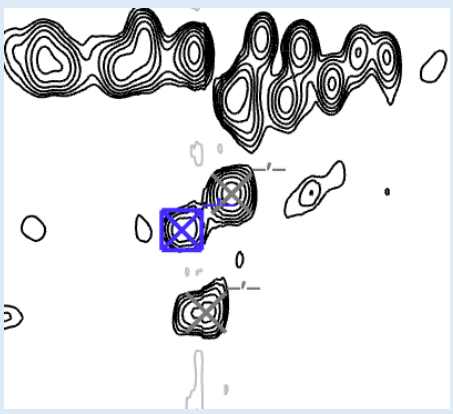


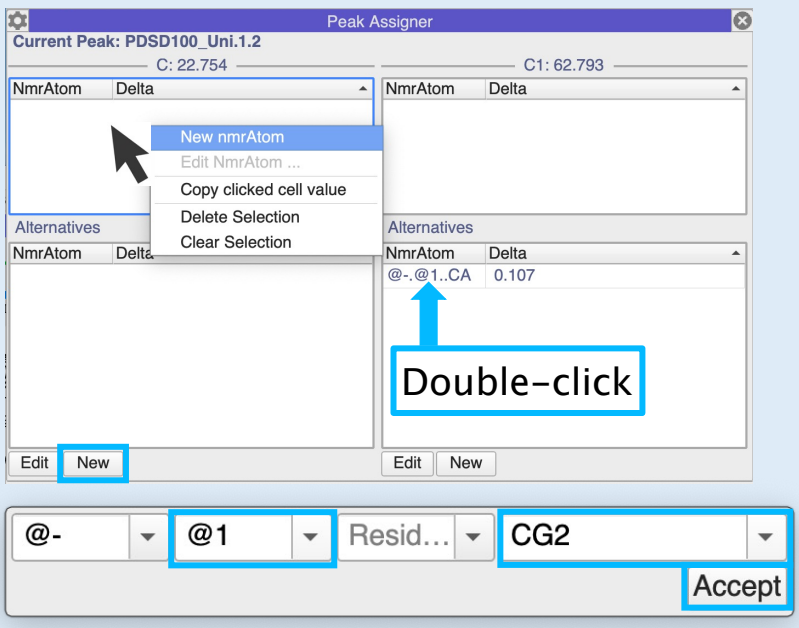
Repeat this procedure for the other two Cα–Cβ peaks.

Using the Peak Assigner









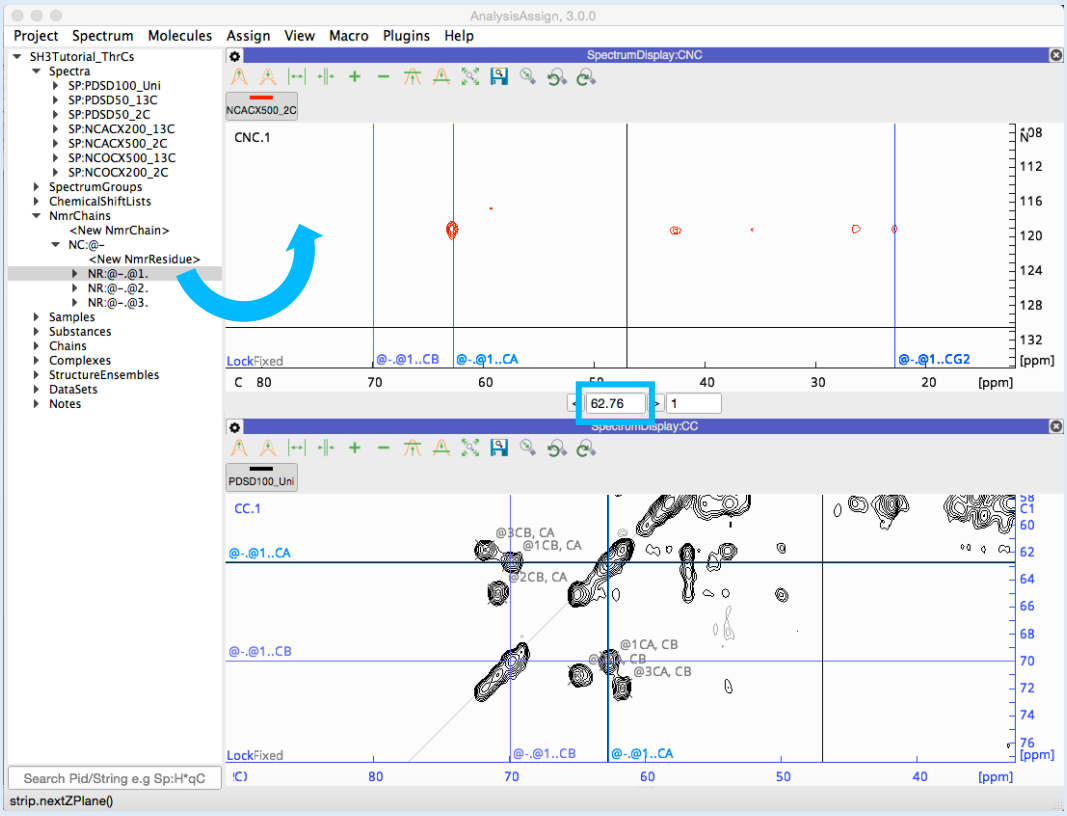
3C Assigning the remaining threonine peaks

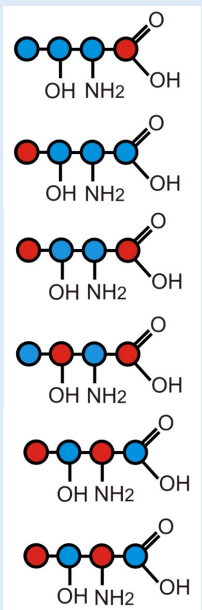
For the remaining peaks it easiest to assign them using the Peak Assigner:

- Open the Peak Assigner with **AP** (or **Main Menu → Assign → Peak Assigner**).
- In the spectrum, select a Cβ–Cα peak from above the diagonal. The Peak Assigner will now give you the CA and CB NmrAtoms as assignment options in the lower panels.
- **Double-click** both of these NmrAtoms in turn to assign them to the peak.
- Now select a Cα–Cγ2 peak.
- **Double-click** on the CA NmrAtom in the bottom right-hand panel to assign it to the peak.
- For the Cγ2 dimension (left hand panels), click on **New** or **right-click** in the top panel and select **New nmrAtom**. In the pop-up, change the NmrResidue to be the same as that of the CA NmrAtom (here **@1**) and change the atom name to **CG2**. Then press **Enter** or click on **Assign** to make the assignment.

Repeat this process for the other Cα–Cγ2 peaks and then do the equivalent for the Cα–C' and Cβ–C' peaks, selecting **C** as the carbonyl atom name.

Identifying Thr peaks in the NCACX





3D Assigning the threonine nitrogen chemical shifts

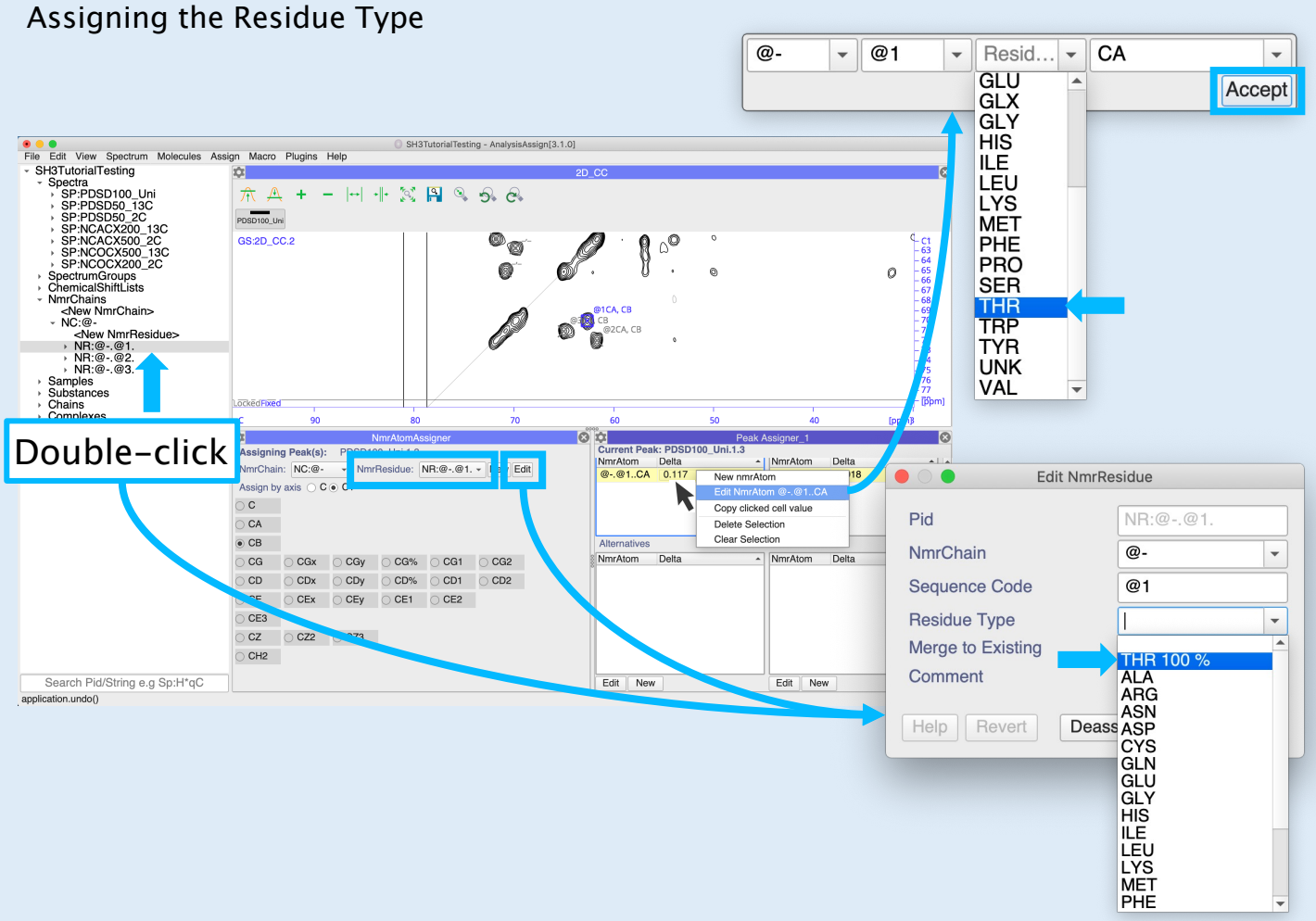
Now we will identify the threonine nitrogen chemical shifts using the NCACX500_2C spectrum:

- **Drag** the NCACX500_2C spectrum into the drop area.
- Type **YZ** to swap the Y and Z axes so that the CA dimension lies along the z-axis. You may want to rearrange your display modules as above now.
- In the sidebar, expand **NmrChains** and NmrChain **NC:@-**.
- **Drag** one of the threonine NmrResidues into the drop area. This will draw marks at the positions of this NmrResidue's NmrAtoms.
- Type the Cα chemical shift of this NmrResidue into the z-axis position of the 3D display. You should find a strip which contains a strong N-Cα-Cα 'diagonal' cross peak as well as a slightly weaker N-Cα-Cγ2 cross peak. Looking at the glycerol labelling scheme you will see that N-Cα-CO and N-Cα-Cβ cross peaks should not be observable for a threonine in the NCACX spectrum of the 2-^{13}C glycerol sample, as the Cα and CO or Cα and Cβ are never simultaneously labelled.

Peak pick your N-Cα-Cγ2 cross peaks and assign the nitrogen chemical shifts using the procedure you used previously for the carbon resonances:

- Bring up the Peak Assigner with **AP**.
- **Double-click** on the Cα and Cγ2 resonances suggested as assignment options. In the nitrogen dimension, create a new NmrAtom with the correct NmrChain and NmrResidue and **N** as the atom.
- Press **Enter** or click on **Assign** to make the assignment.

Assigning the Residue Type



3E Assigning the Residue Type

From the chemical shifts we know that the spin systems, or NmrResidues that we have assigned are threonine residues, so we can also assign the amino acid type.

There are several ways in which this can be done:

- **Double-click** on an NmrResidue in the sidebar to bring up the **Edit NmrResidue** popup.
- Select the residue type from the drop-down menu and click **OK**.

OR

- Select a threonine peak and open the Peak Assigner with **AP**.
- In one of the dimensions, right-click on the NmrAtom and select **Edit NmrAtom**
- Select the residue type (**THR**) from the drop-down menu and click **Assign** or press **Enter**.

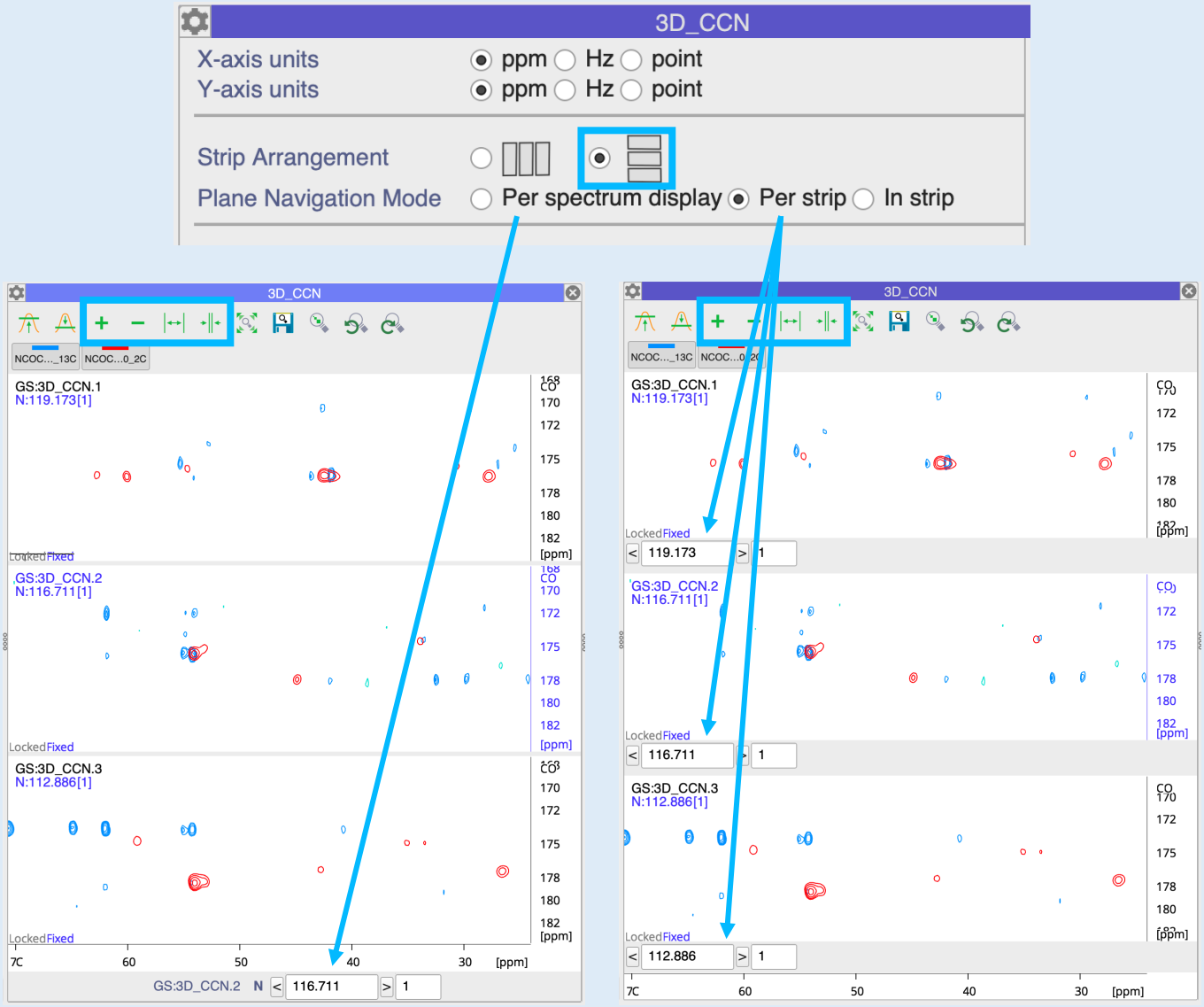
OR

- Open the NmrAtom Assigner with **AN**.
- Make sure the correct NmrResidue is selected in the drop-down menu.
- Click on **Edit** to bring up the **Edit NmrResidue** popup.
- Select the residue type from the drop-down menu and click **Okay**.

Repeat for all three threonine residues.

The assignment of the residue type can be done at any stage, i.e. you could have done this as part of one of the previous steps if you had wanted.

Setting up Horizontal Strips



4A Setting up horizontal strips

We will now use horizontal strips to look at all three threonines in parallel:

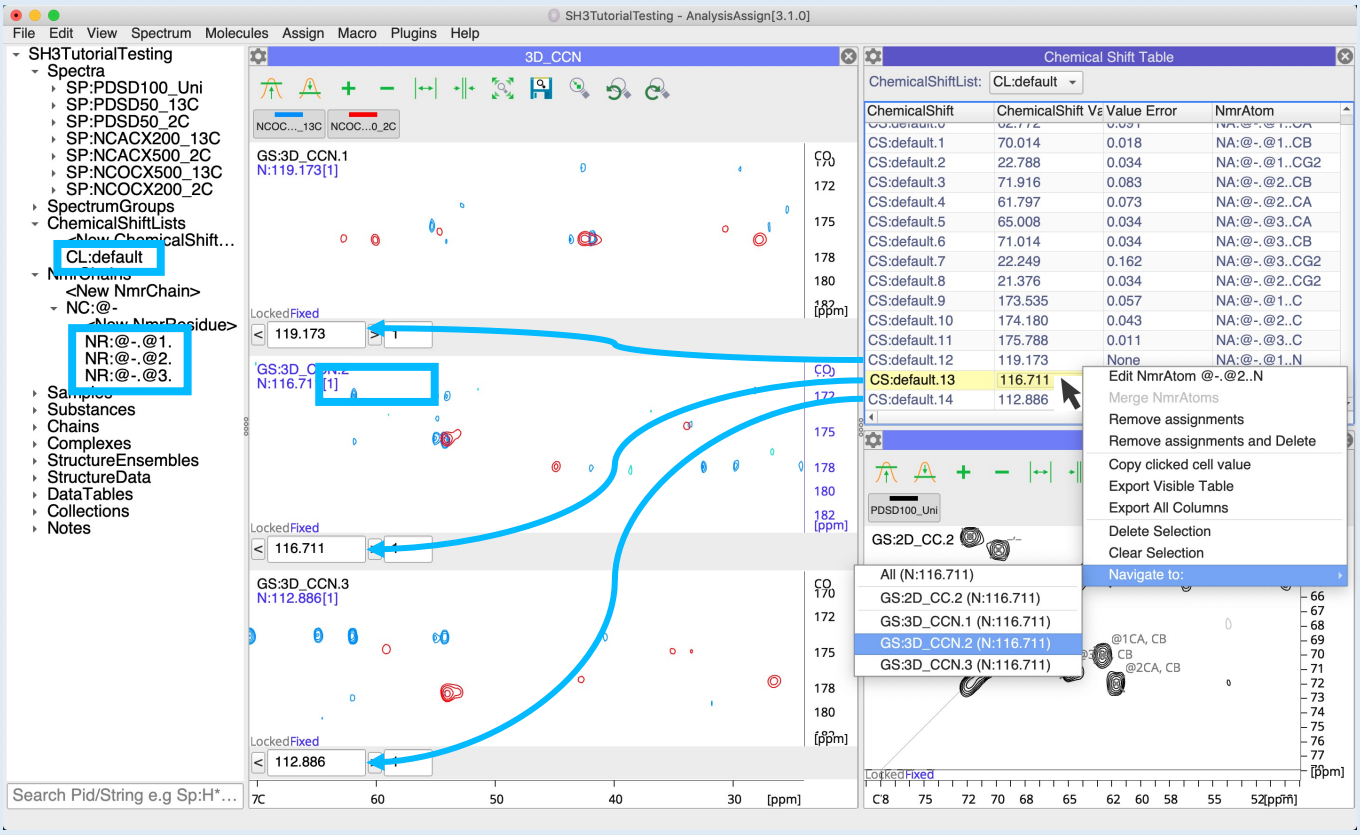
- **Drag** your NCOCX500_13C and NCOCX200_2C spectra into the drop area.
- **Click** on the gear icon ( ) to change the Settings: select **Strip Arrangement**

By default the **Plane Navigation Mode** to is set to **Per Spectrum Display**. This means only one z-plane navigation toolbar is provided. It works on the active (or most recently active) strip, i.e. strip with highlighted axes. Click into a strip to make it active.

Alternatively you can set the **Plane Navigation Mode** to **Per Strip** which will give you separate navigation toolbars for each

- Decide which Plane Navigation Mode you would like to use and then click on the gear icon again to close the settings box.

- Now add two strips by clicking on the + icon in the Spectrum Display toolbar.
- If necessary, narrow the strips with so you can see all three strips properly.



4A ...**continued: Setting up horizontal strips**

Now we need to nagivate to one of the three Threonine ^{15}N positions in each of the strips:

- Expand **Chemical Shift Lists** in the side bar and drag the chemical shift list into the drop area.

Set the z-plane Nitrogen positions of your NOCOCX strips to those of your three threonines:

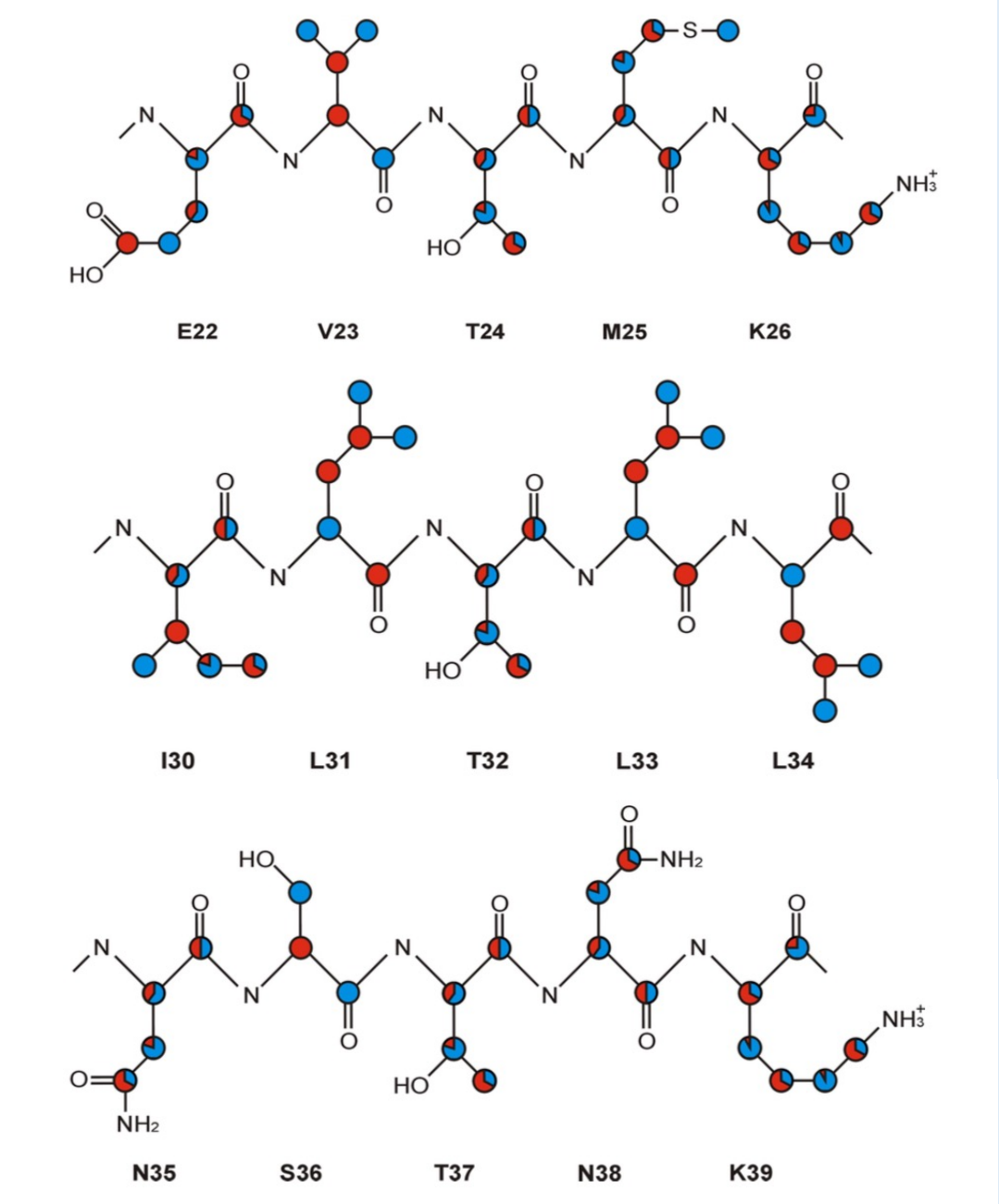
- Type the values into the z-plane toolbar by hand

OR

- Right-click on each ^{15}N chemical shift in the **Chemical Shift List** table in turn and select **Navigate to:** and then the strip which you would like to set to that nitrogen chemical shift.

- You can now mark the NmrAtoms of each threonine in turn by dragging the NmrResidue from the sidebar into the drop area. Use **MC** to clear the marks again.

Glycerol labelling pattern of Thr motifs in the SH3 domain

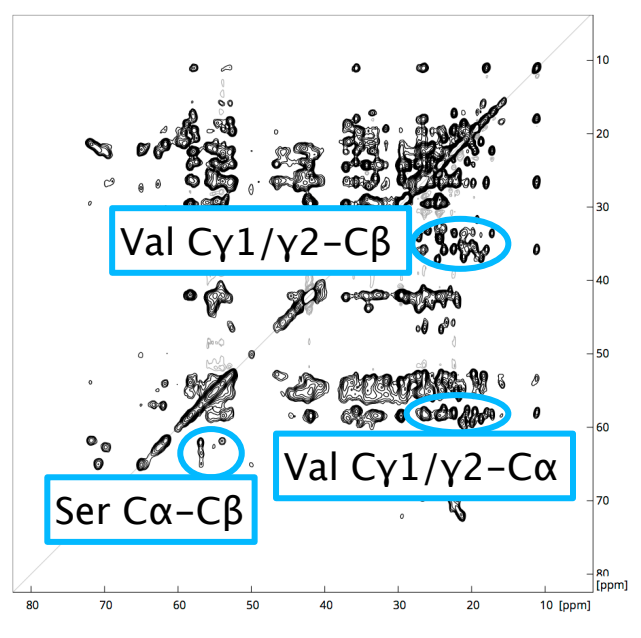


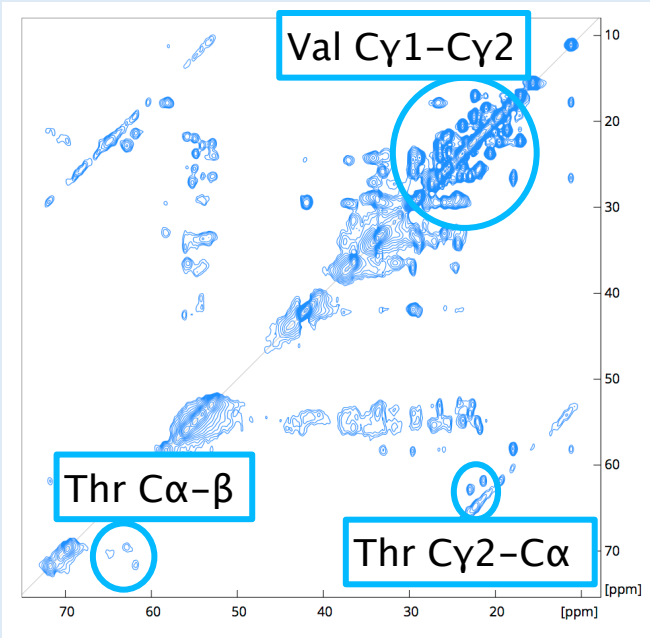
4B Identifying the residues preceding the threonines

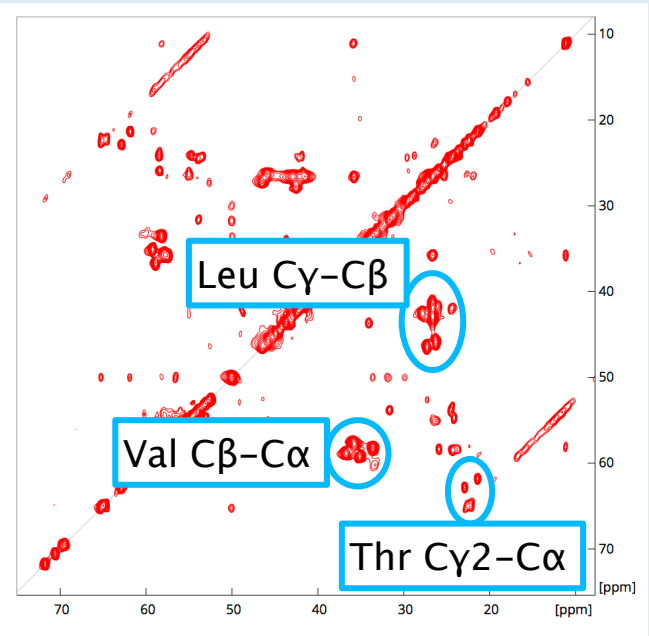
Using the NCOCX500_13C and NCOCX200_2C spectra, the glycerol labelling pattern and the standard chemical shifts it is possible to work out which threonine is preceded by a valine, which by a leucine and which by a serine:

- In the nitrogen plane of the threonine which is preceded by a valine, you should only be able to see cross peaks in the NCOCX spectrum of the 1,3-^{13}C glycerol sample. The Cγ resonances of a valine (~ 20 ppm) and the Cα, Cβ, possibly also Cγ2 resonance of your threonine should be visible.
- For the threonine preceded by the leucine, you should only see peaks in the 2-^{13}C glycerol sample: the Cβ and Cγ resonances of a leucine (~42 and ~27 ppm, respectively) and the Cα, perhaps also Cβ and Cγ2 of the threonine.
- For the threonine preceded by a serine, cross peaks will again only be visible in the 1,3-^{13}C glycerol sample: The Cβ of the serine (~ 64 ppm) and the Cα, Cβ, possibly also Cγ2 resonance of your threonine.

Val, Ser and Leu patterns in C-C spectra





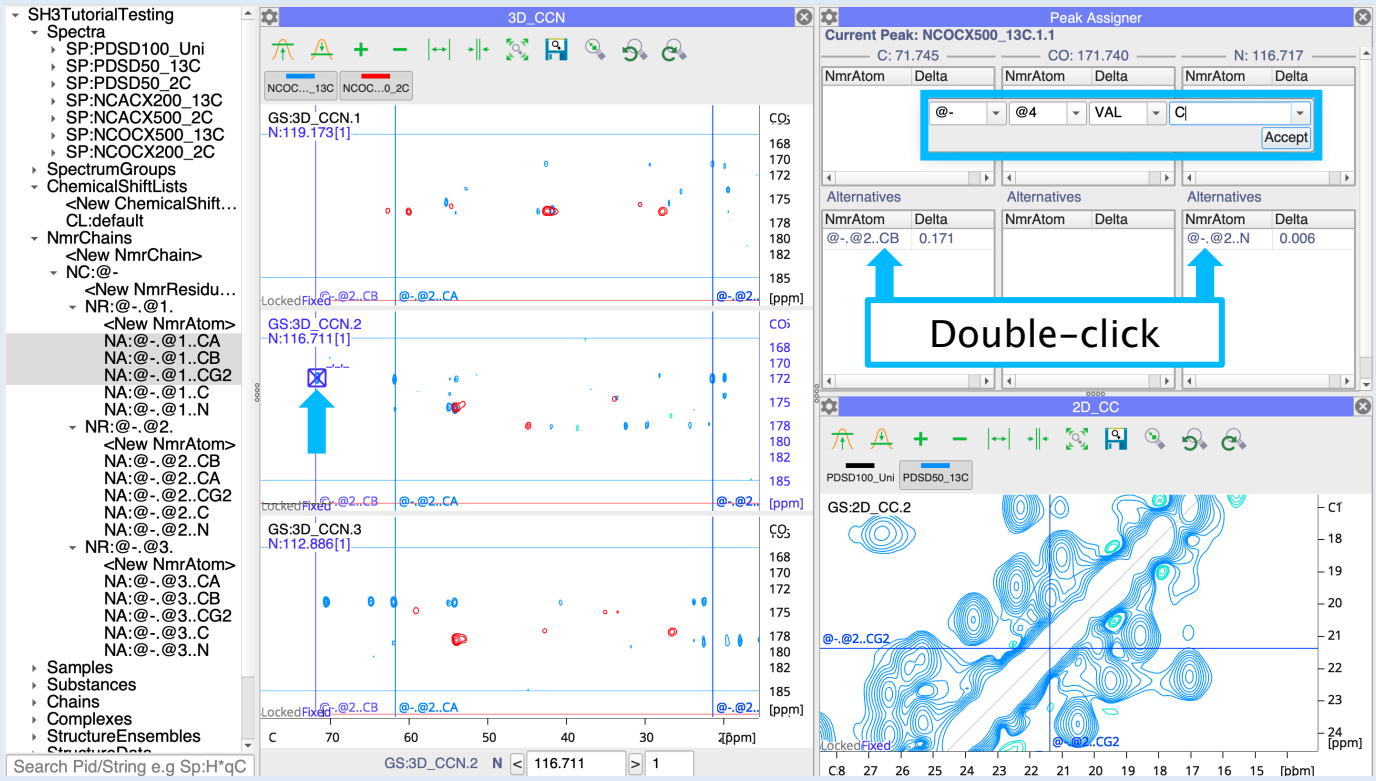


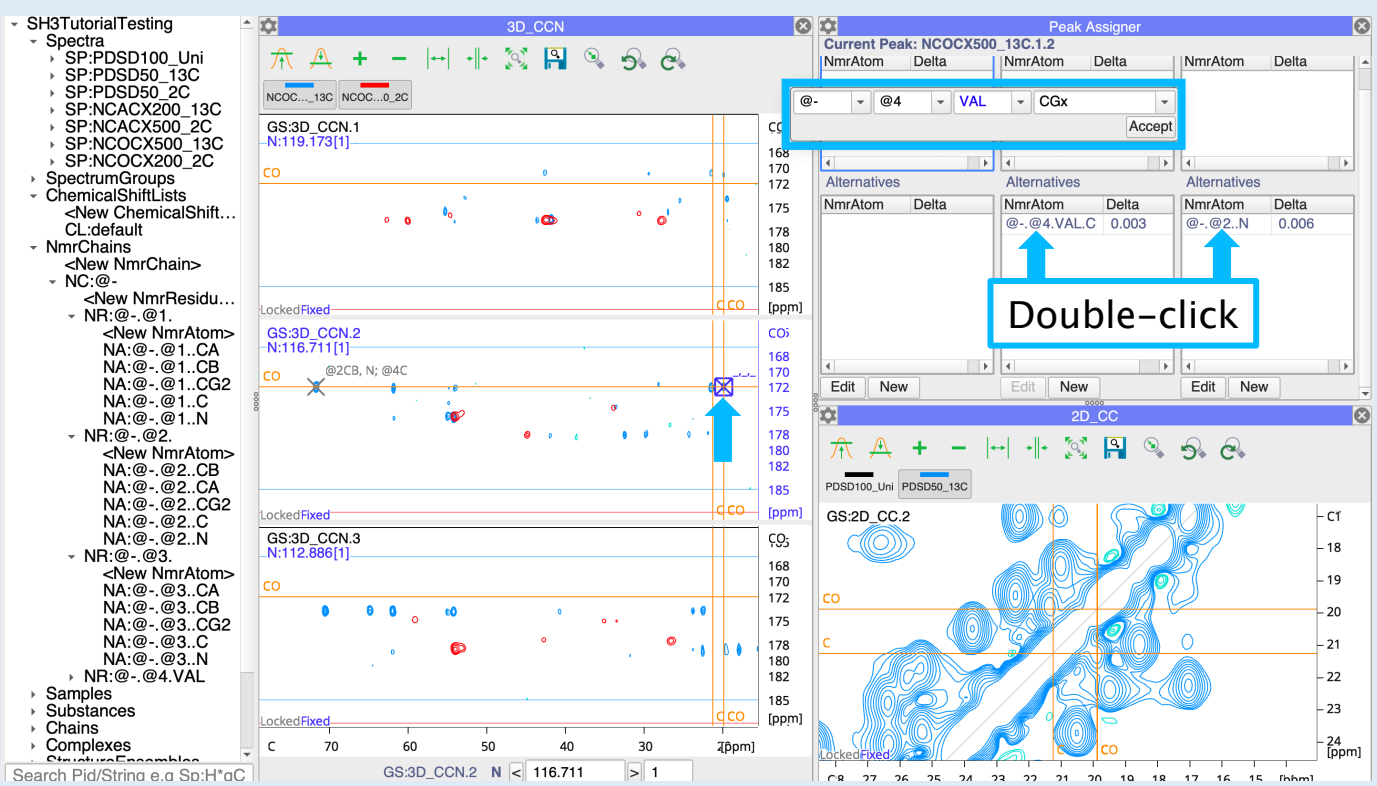
4C Confirming your assignment of the preceding residues

You can confirm whether the peaks which you think may arise from valine/leucine/serine residues really do, by marking them and checking whether you can see any corresponding cross peaks in the PDSD spectra.

- For valine you should see a Cγ1-Cγ2 cross peak (near the diagonal at ~ 20 ppm) in the 1,3-^{13}C glycerol spectrum and Cγ1/2-Cα (~ 20/62 ppm) and Cγ1/2-Cβ (~ 20/33 ppm) cross peaks in the uniformly labelled spectrum and a strong Cα-Cβ cross peak (~ 62/33 ppm) in the 2-^{13}C glycerol spectrum.
- For leucine your Cβ and Cγ should form a strong cross peak in the 2-^{13}C glycerol spectrum (~ 42/27 ppm).
- For serine you would expect a Cα-Cβ cross peak (~ 58/64 ppm) in the uniformly labelled spectrum.

Assigning NCOCX peaks





4D Assigning the NCOCX peaks

In order to assign the cross peaks in the 3D NCOCX spectra,

- bring up the Peak Assigner by typing **AP** and select each peak in turn.

For the N and CX dimensions your Thr NmrAtoms should be provided as options.

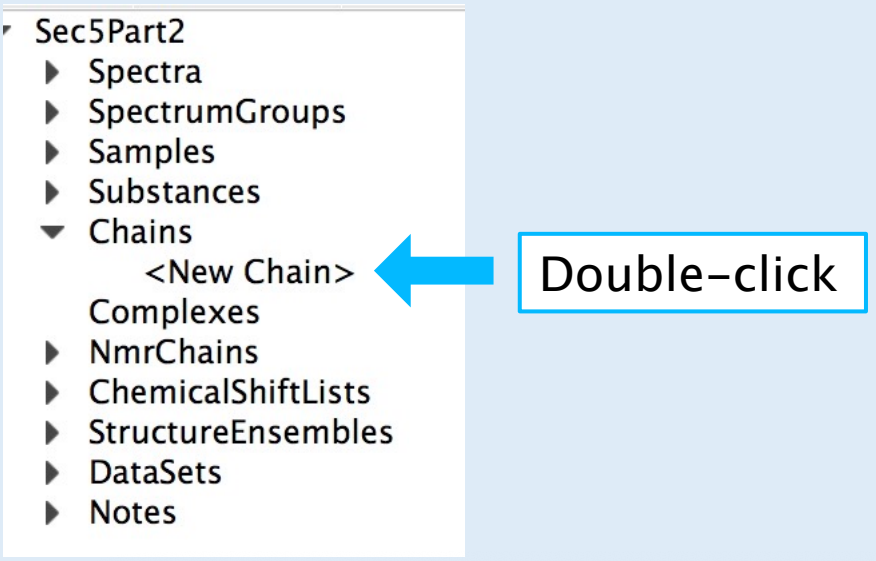
- **Double-click** these to assign them to your peak.

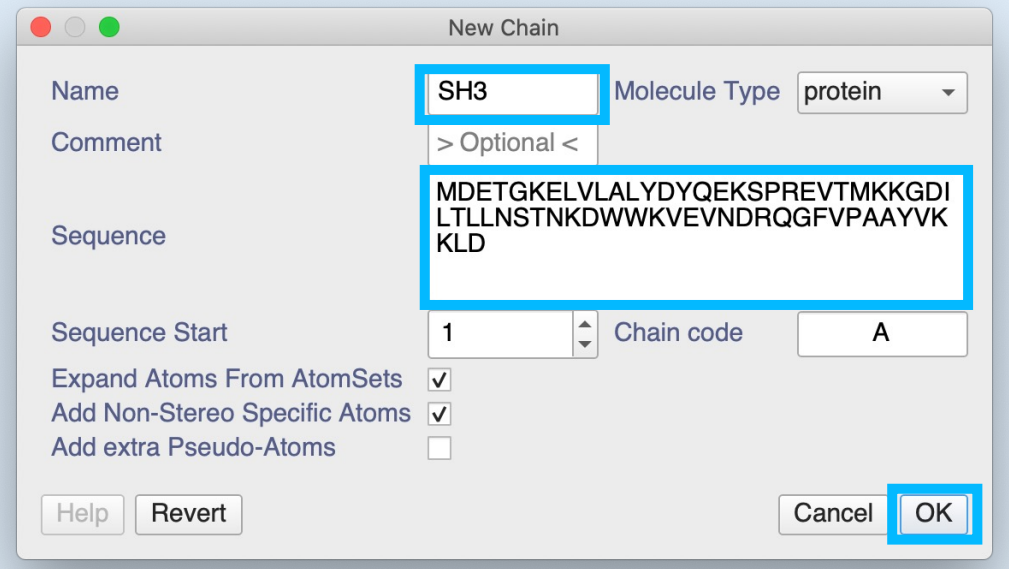
This leaves the C' dimension which belongs to the previous residue.

- Create a new NmrAtom (**right-click** / **New nmrAtom**) with a new default Sequence Code (e.g. **@4**) and AtomName **C**, then click **Assign**.

Use this same new NmrResidue when assigning further peaks. When assigning the Val Cγ peaks, use CGx and CGy as the atom names, as the assignment is non-stereo specific. You can also assign the Val, Leu and Ser residue types.

Add a new Protein Chain





5A Create chain

To make sequence-specific assignments you will need to add the sequence of the SH3 domain to your project:

- **Sidebar → Chains → <NewChain>**

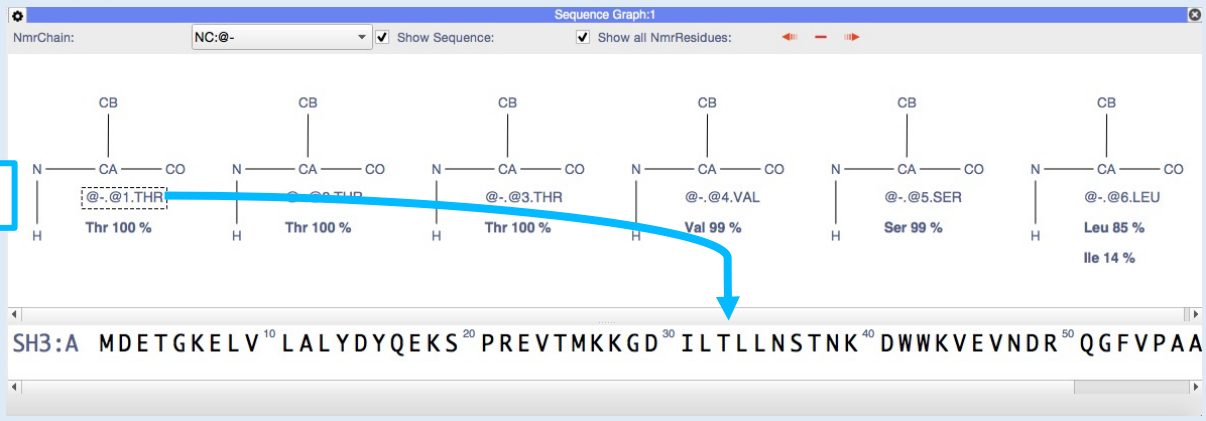
followed by

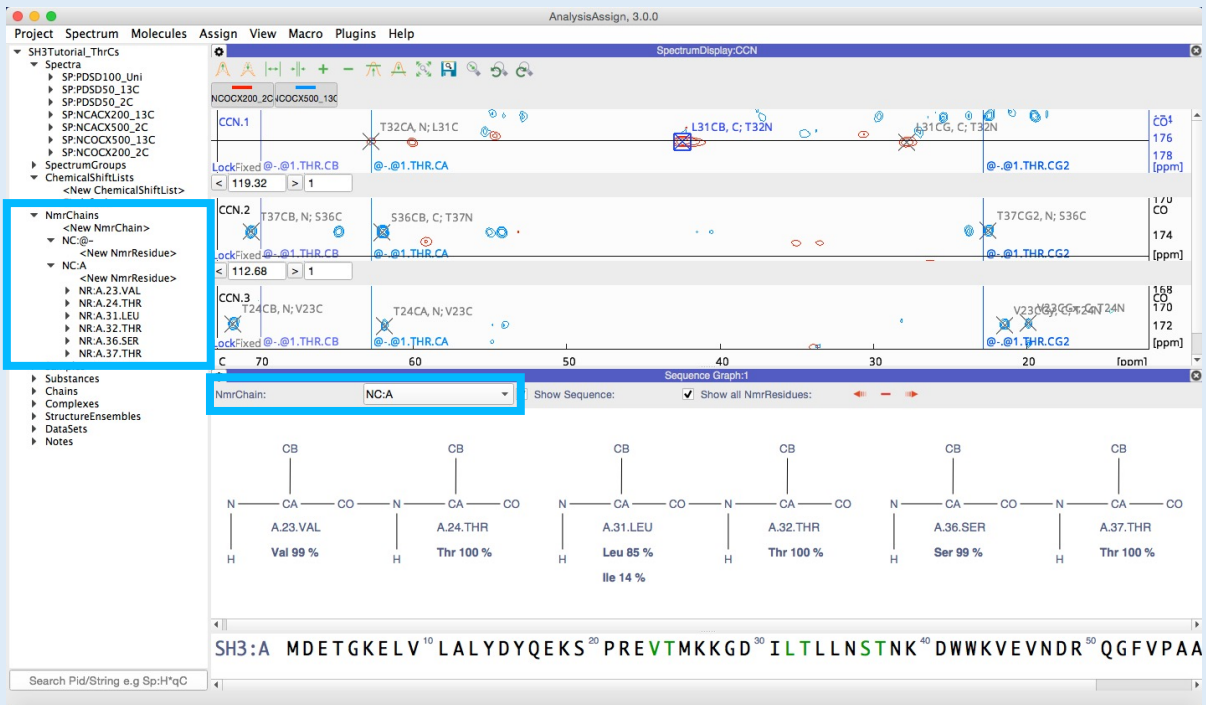
- Molecule Name: SH3
- ChainCode: A
- Sequence: (copy and paste)
  MDETGKELVLALYDYQEKSPREVTMKKGDILTLLNSTNKDWWKVEVNDRQGFVPAAYV
  KKLD
- click **OK**

You can also use **Main Menu → Molecules → New Chain**... or drop a FASTA formatted file into the project. A FASTA file for SH3 is included in the **CcpnTutorialDataSolidStateNmrJune2022/ssNMRSH3AsssignmentTutorial** directory.

Sequence-specific Assignment

SG





5B Making sequence-specific assignments

Bring up the Sequence Graph:

- Go to **Main Menu → View → Sequence Graph** or type **SG**.

You will see all your NmrResidues in the upper panel and the protein sequence below.

- **Drag** an NmrResidue from the upper panel onto the residue in the sequence below that you want to assign it to.

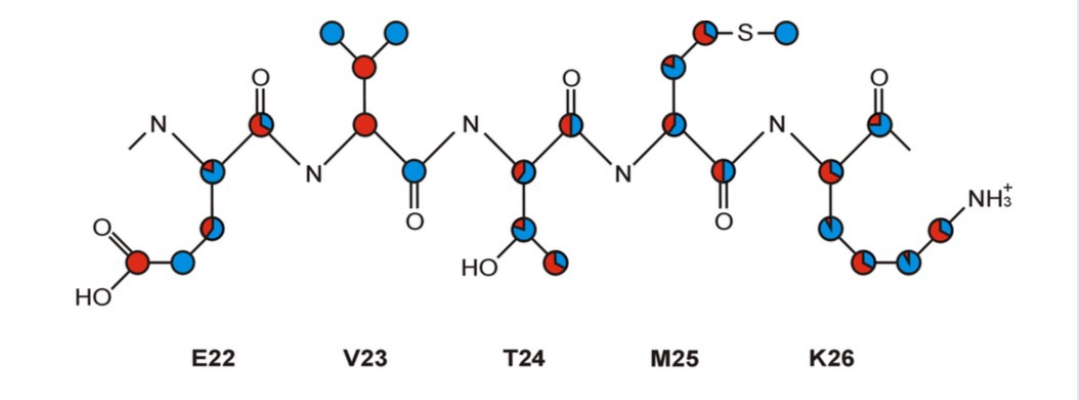
This residue in the sequence will now turn green to show that it is assigned. The assigned NmrResidues are placed into a new NmrChain called **A** to show that they are assigned to the Residues in Chain **A**. This is reflected in the sidebar.

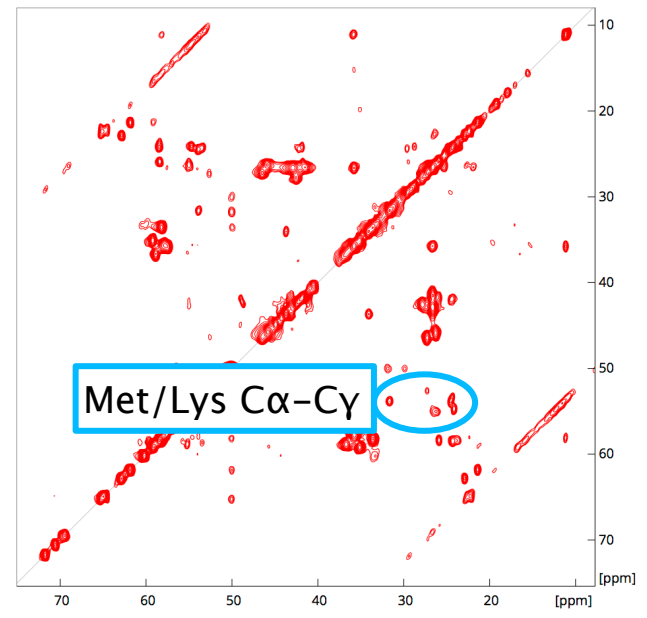
When you have assigned all your NmrResidues,

- select NmrChain **NC:A** in the Sequence Graph.

You will see all your NmrResidues again and can see that sequential residues are shown as being linked.

EVTMK Motif in the SH3 domain





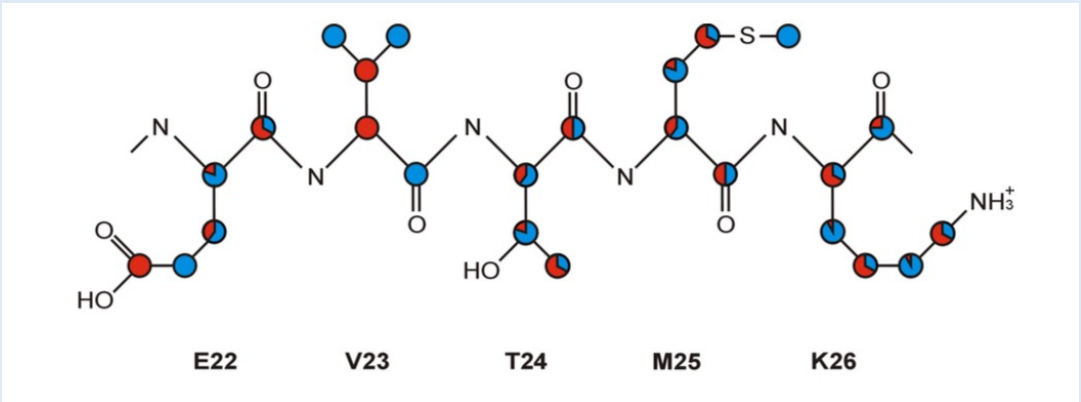
6A Further assignment of the EVTMK motif – linking to the methionine

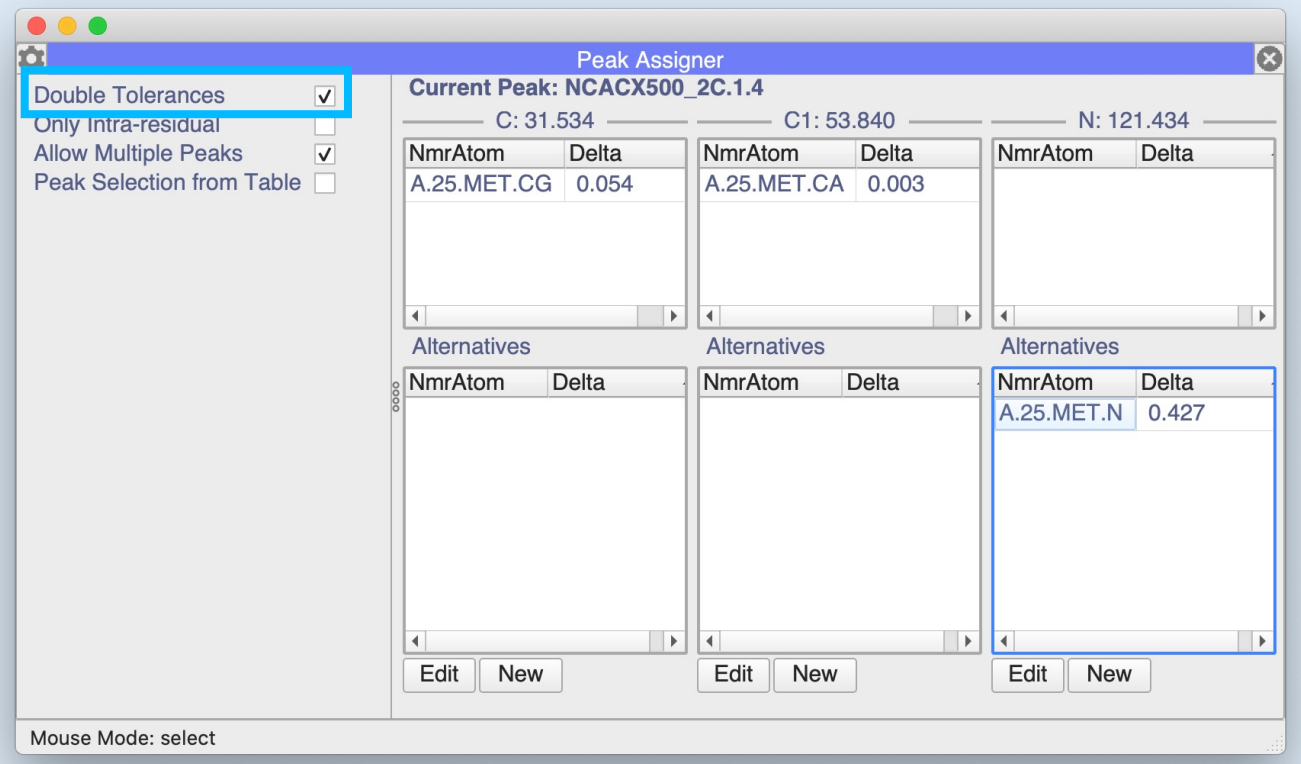
Try to identify the methionine which follows this threonine.

Use the NCOCX200_2C spectrum with C' in the Z-dimension and go to the plane of the threonine C'. You should be able to see links to the threonine Cβ and methionine Cα. To confirm that the Cα you have found really is compatible as a methionine Cα – see if it matches up with a possible Cα-Cγ cross peak in the 2-^{13}C glycerol PDSD spectrum. You should be able to confirm the nitrogen chemical shift of the methionine residue by looking for the N-Cα-Cγ peak in the 2-^{13}C glycerol NCACX.

At each stage, pick your peaks and assign your NmrAtoms and NmrResidues.

EVTMK Motif in the SH3 domain





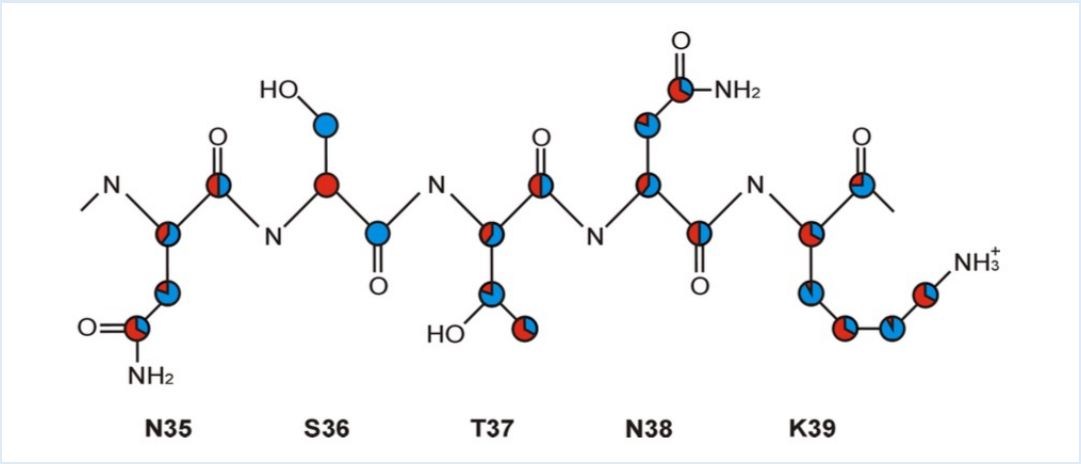
6B Further Assignment of the EVTMK motif – linking to the Lysine

Now see if you can find links from the methionine to the neighbouring lysine. First find the Met C' and Cβ chemical shifts via the Cγ–C' and Cγ–Cβ peaks in the PDSD100_Uni spectrum. Then find the Lys N and Cα in the NCOCX200_2C spectrum. You can confirm that you have identified the correct resonances/peaks (and eliminate incorrect resonances/peaks) by looking for a matching Lys Cα–Cγ peak in the PDSD50_2C spectrum and a Lys N–Cα–Cγ peak in the NCACX500_2C spectrum.

If you are expecting to see an NmrAtom as an option in the Peak Assigner and it is now shown, try extending the matching tolerance:

- Go the **Settings** box of the **Peak Assigner** by clicking on the gear icon
- Tick the **Double Tolerances** box

NSTNK Motif in the SH3 domain

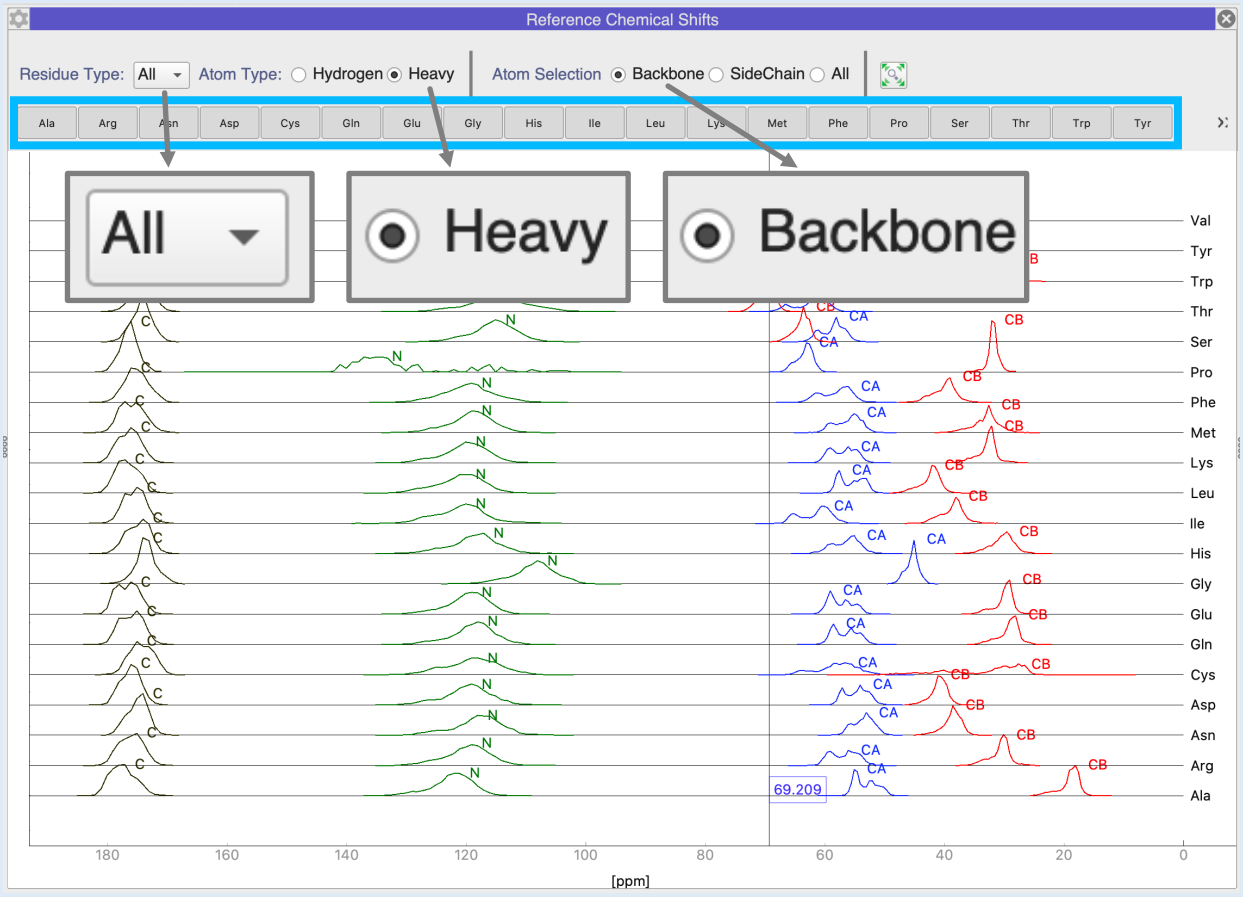


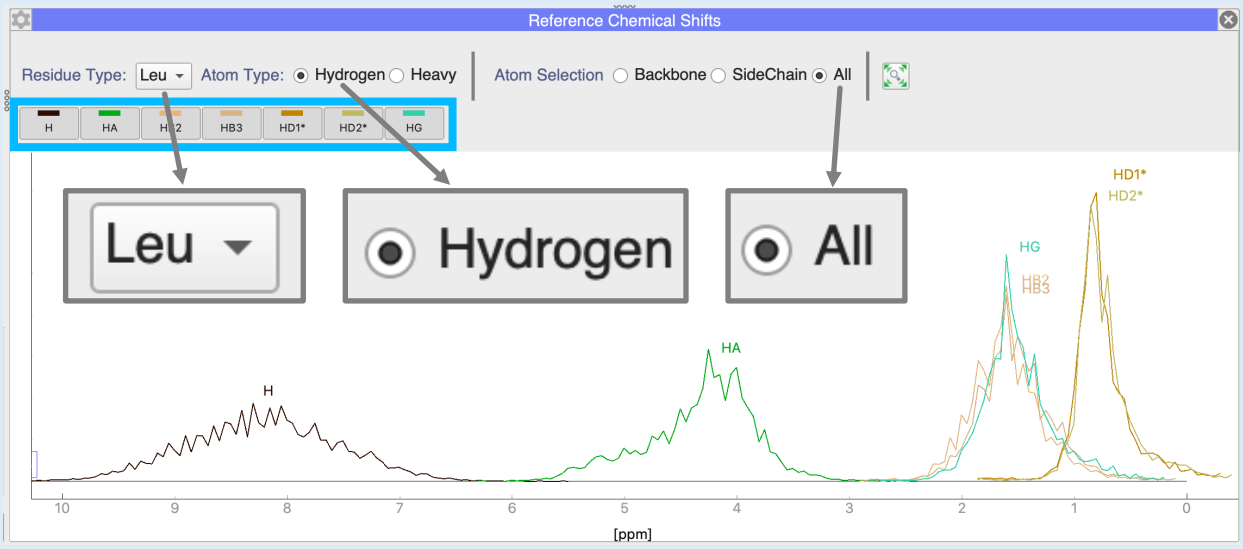
6C Further Assignment of the NSTNK motif

If you have got to this stage, then you have probably got the hang of things. Try identifying the remaining resonances of the serine spin system and see if you can find links to the preceding asparagine.

Use the tools in section 7 to help you find the serine and asparagine reference chemical shifts and correct mistakes.

7 Other Useful Tools





RC

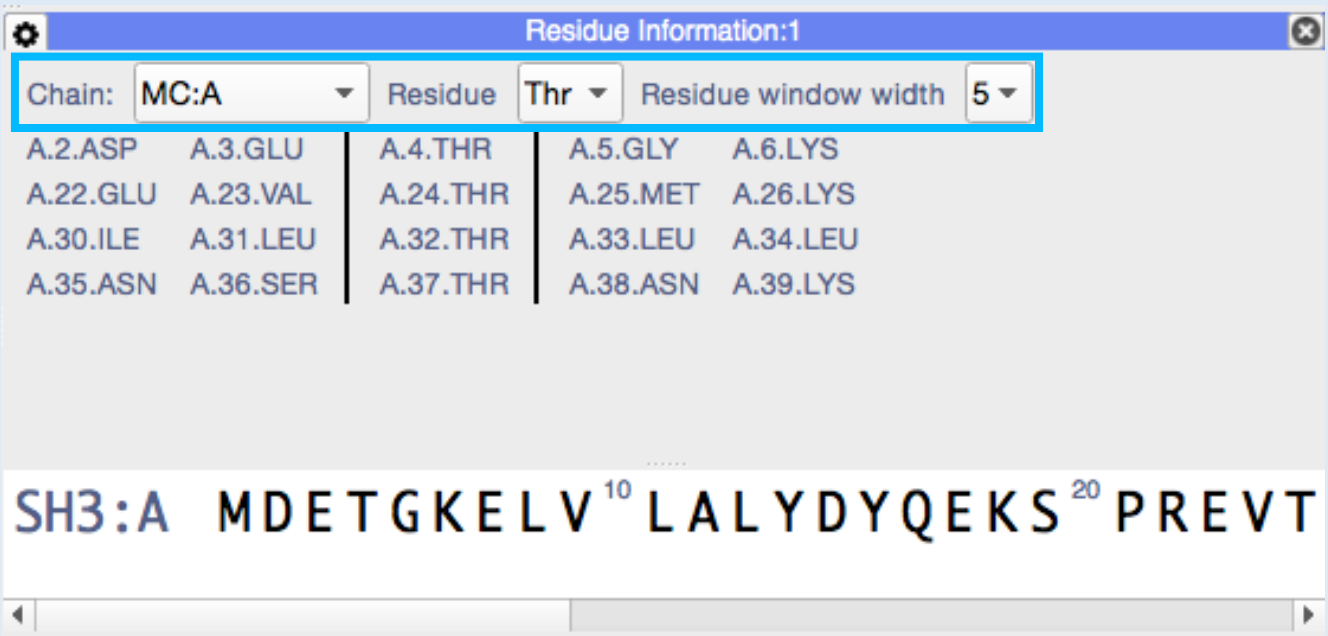
7A Reference Chemical Shifts

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 and **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.

7 Other Useful Tools



RI

7B Residue Information

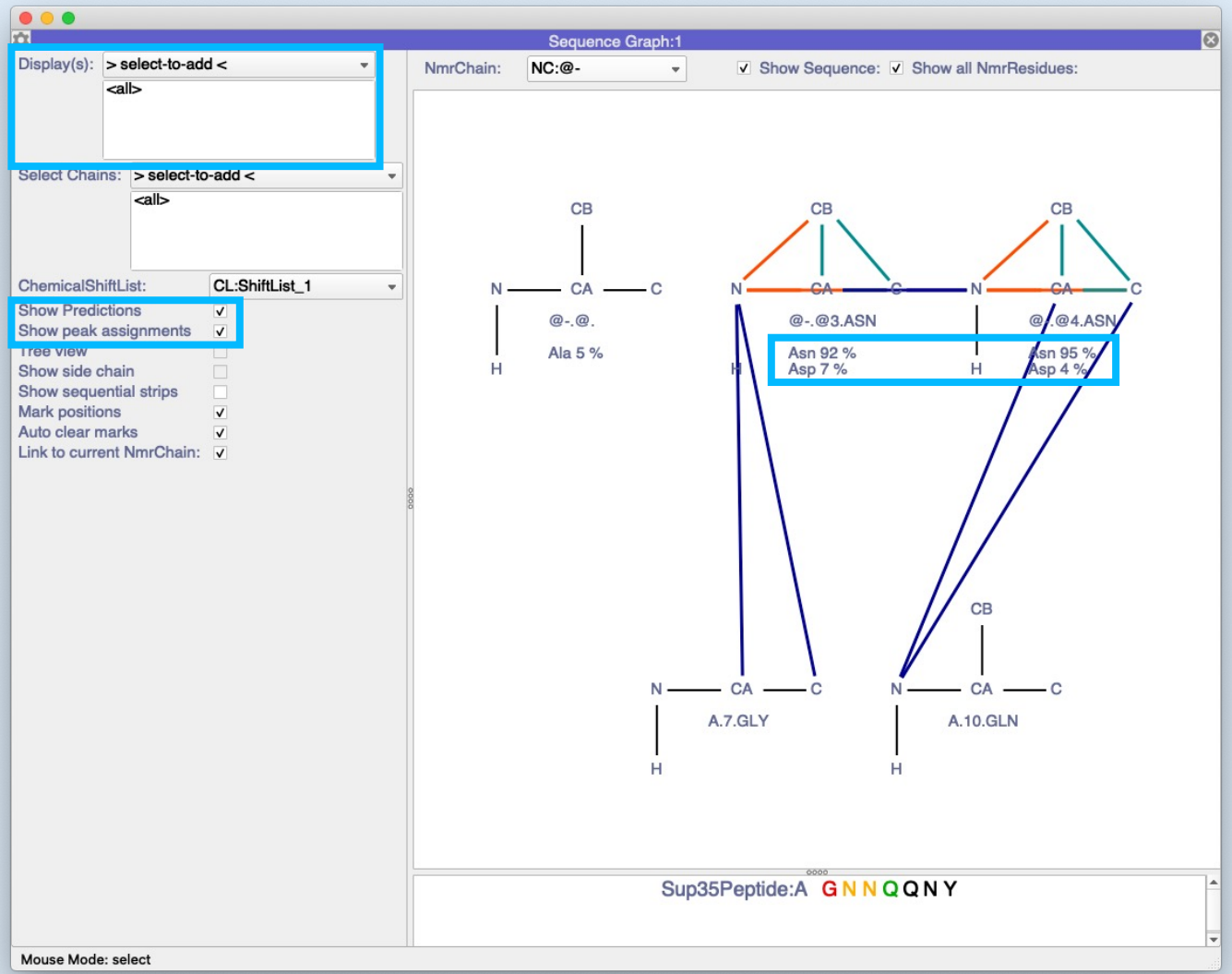
You can look at different residue types in your sequence and the motifs they are contained in:

- Go to **Main Menu → Molecules → Residue Information**, or type **RI**.
- Select the **Chain**, **Residue Type** and **Residue Window Width** of your choice.

The full sequence is shown below and if you have made any sequence specific assignments, then these residues will be highlighted in green.

7 Other Useful Tools

Sequence Graph



SG

7C Sequence Graph

As well as being able to use the Sequence Graph to make sequence specific assignments, it also includes other information:

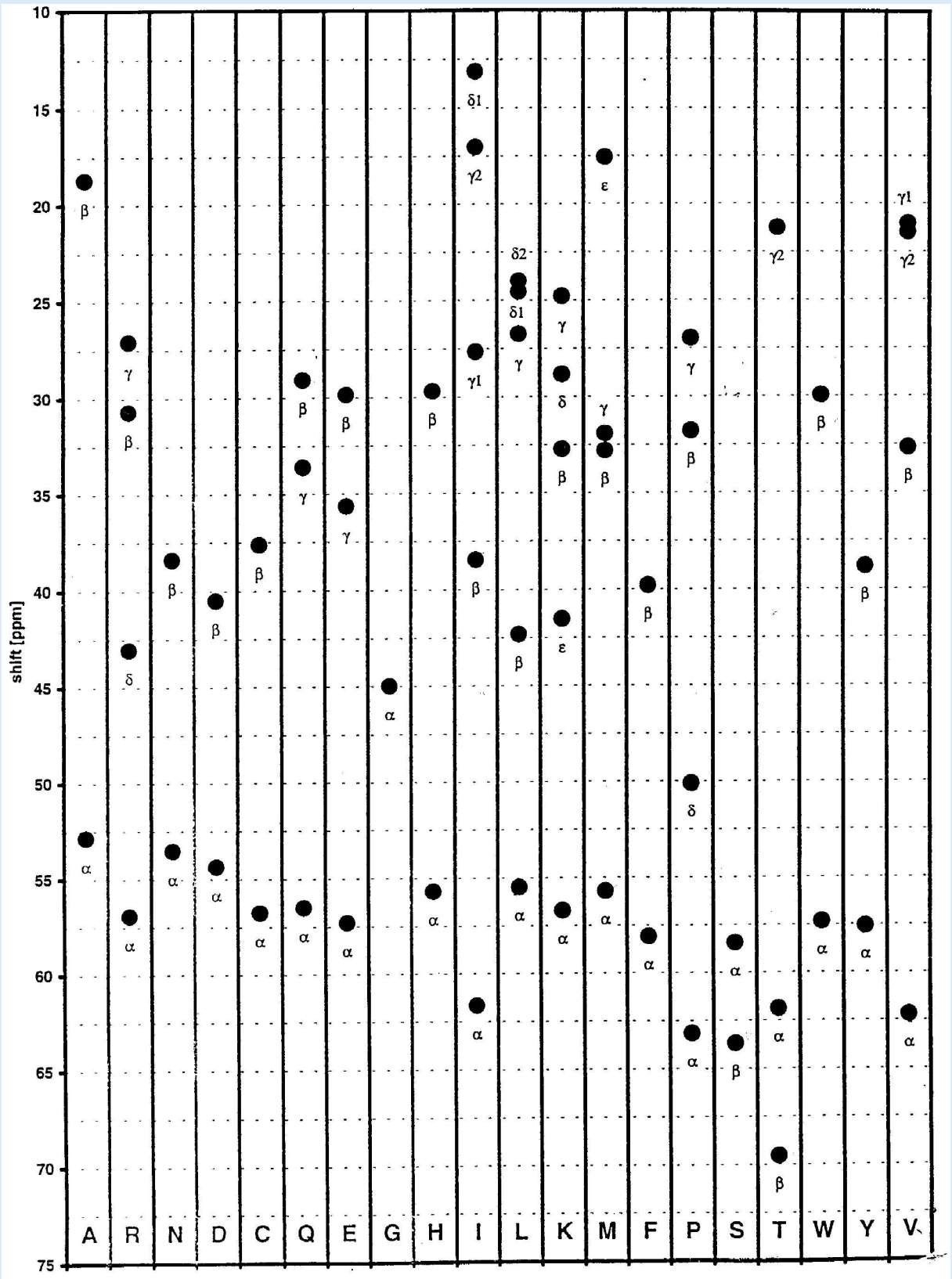
- Coloured lines show links between NmrAtoms from peak assignments. The colours of the lines reflect the contour colour of the spectra in which the peaks are found.

  You can switch this feature off in the settings (uncheck **Show peak assignments**).
- Below each NmrResidue you will see predictions for the amino acid type. These are based on the chemical shifts and atom types of the NmrAtoms in the NmrResidues. The more information there is, the more accurate the prediction will be.

  You can switch this feature off in the settings (uncheck **Show Predictions**).
- In the Settings panel you can choose (Spectrum) Displays. If at least one Spectrum Display is selected, then **double-clicking** on an NmrResidue in the Sequence Graph will place marks for that NmrResidue and navigate to its positions in the selected Spectrum Displays.

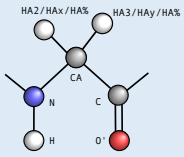
Reference Information

Carbon chemical shifts for the 20 natural amino acids

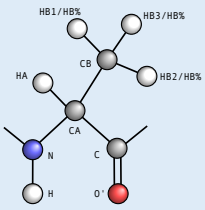


Reference Information

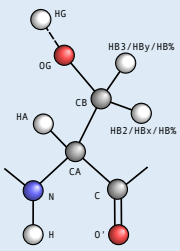
20 natural amino acid structures with NEF atom names



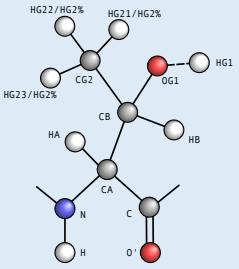
Glycine



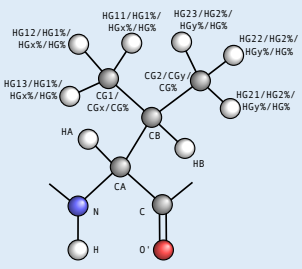
Alanine



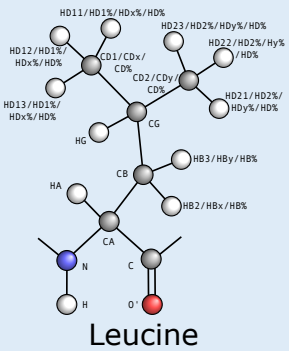
Serine



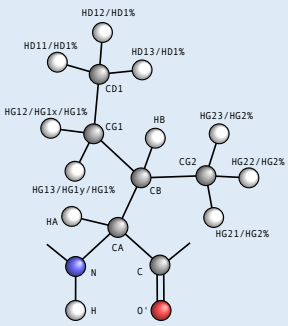
Threonine



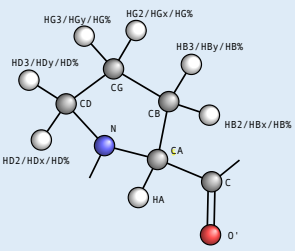
Valine



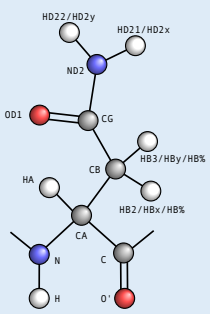
Leucine



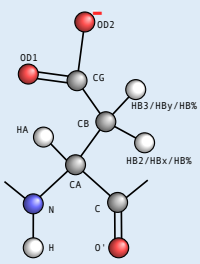
Isoleucine



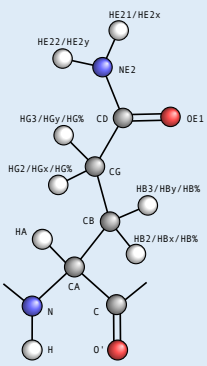
Proline



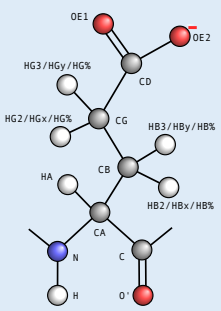
Asparagine



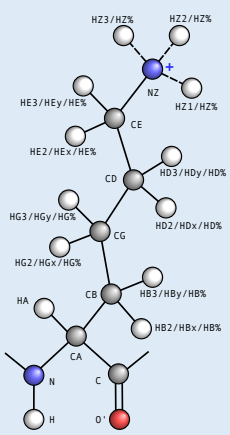
Aspartate



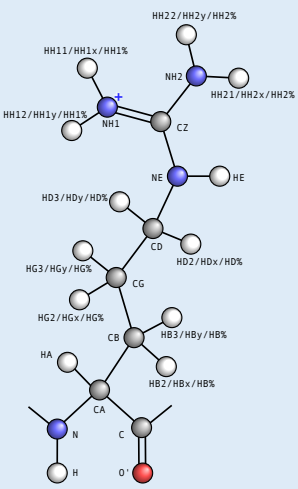
Glutamine



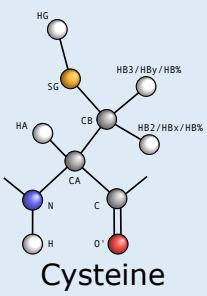
Glutamate



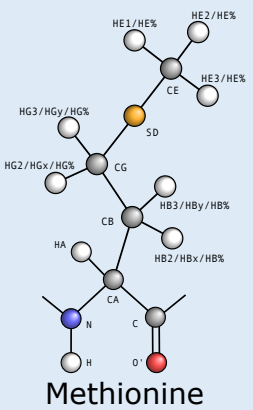
Lysine



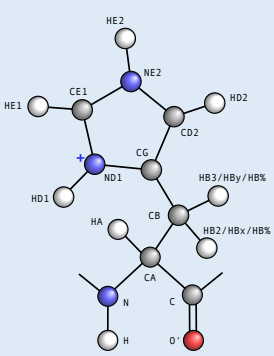
Arginine



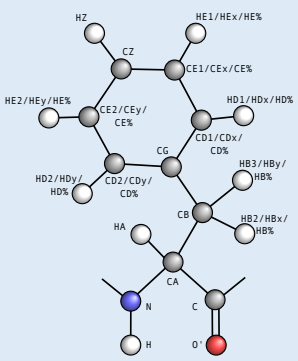
Cysteine



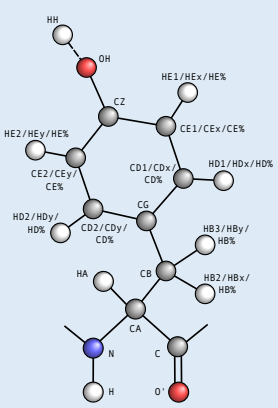
Methionine



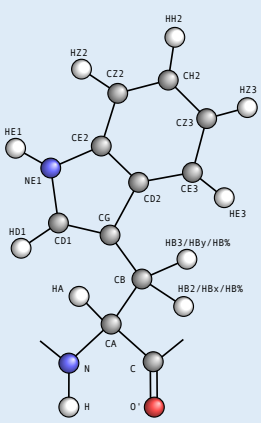
Histidine



Phenylalanine



Tyrosine



Tryptophan

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Cite Us

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Tutorial Version History:

3.0 (VAH): First version