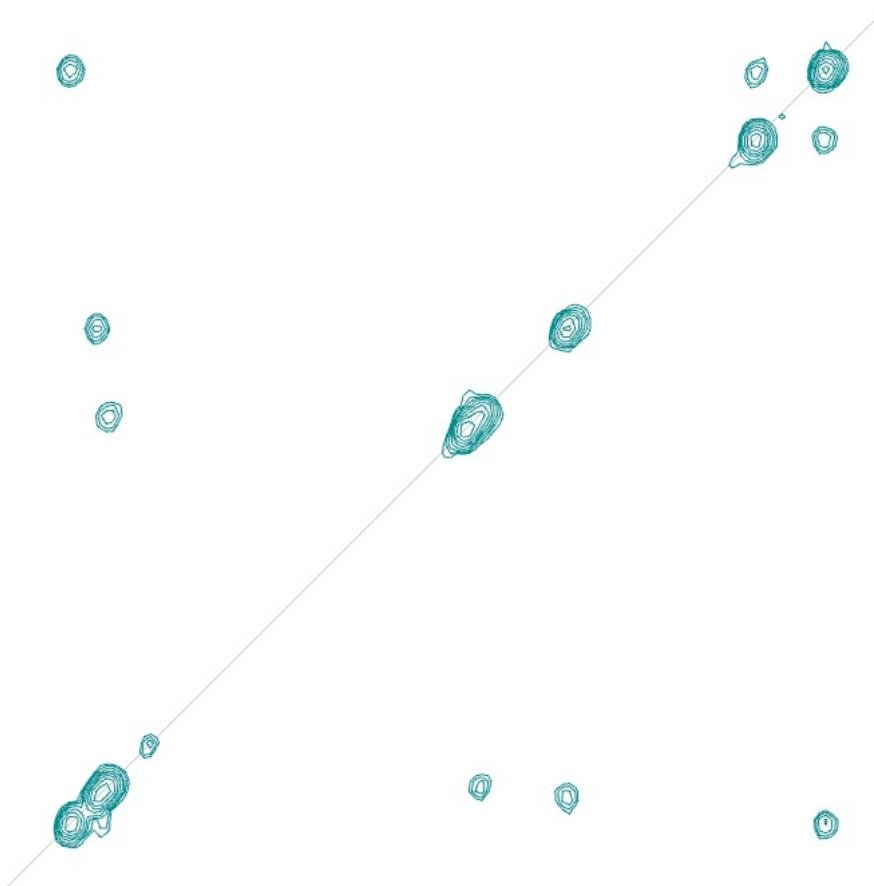


Solid State Peptide Assignment Tutorial



Sup35Peptide:A **G N N Q Q N Y**

Introduction

This tutorial is designed to introduce you to the basics of assigning carbon-detected solid-state NMR spectra using CcpNmr AnalysisAssign Version 3. It is suitable for beginners, although it does not formally teach any of the 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.

We are grateful to Prof. Patrick van der Wel for making the data used in this tutorial available to us. It consists of two sets of spectra recorded on a peptide taken from Sup35. You will find the project and data in the CCPN tutorial data directory PeptideSolidStateTutorial.

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

Contents:

1. Project setup
2. Carbon spin system identification
3. Nitrogen assignment
4. Sequential assignments
5. Sequence specific assignments
6. Further assignments
7. Other useful tools
8. ReferencedData

Start CcpNmr Analysis V3

Apple users by double clicking the icon
CcpNmrAnalysis



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

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

Getting started, basic operations

Sidebar

All data contained in a project, such as spectra and peak lists are located in the sidebar.

Spectrum Display

A Spectrum 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

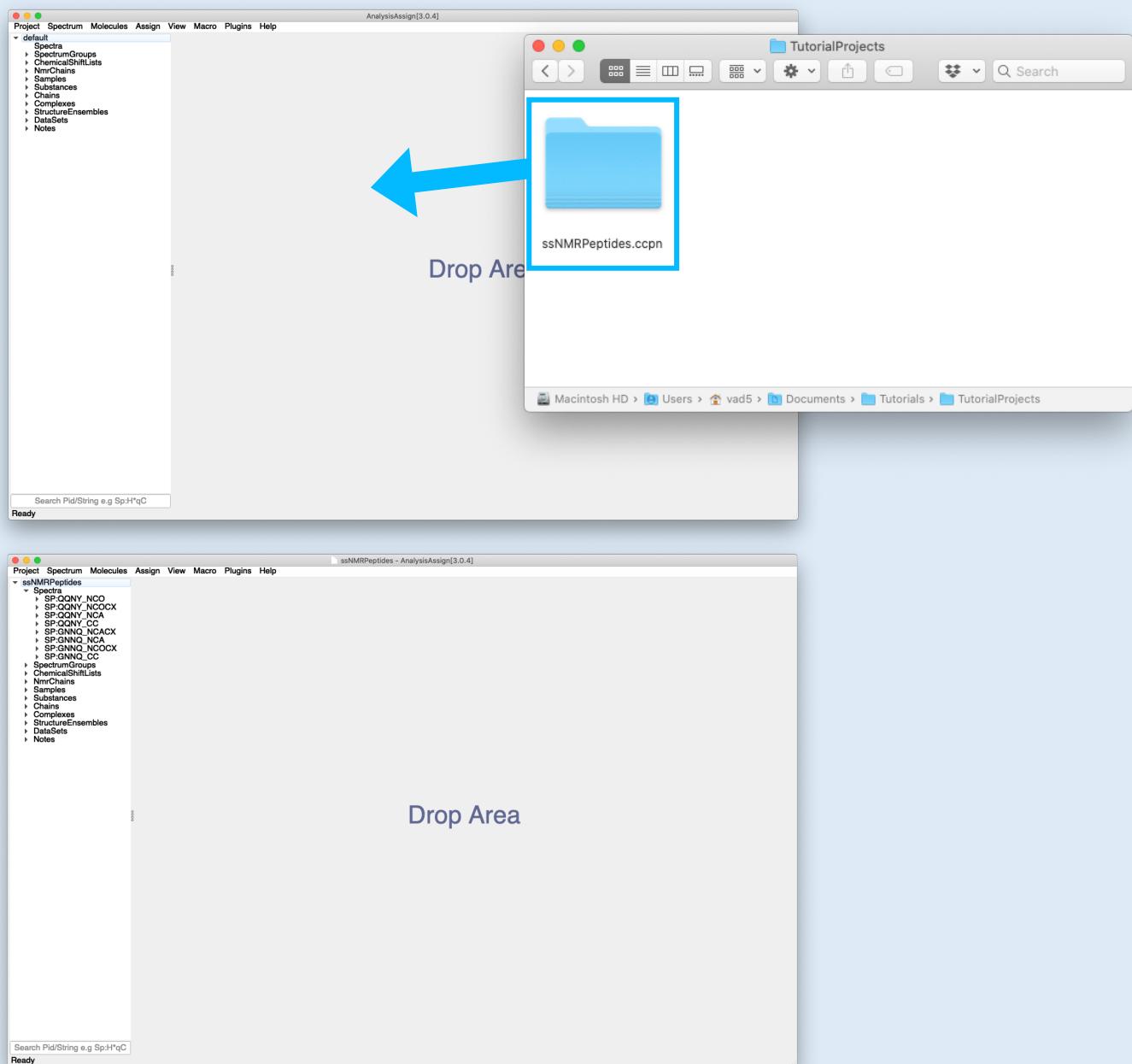
The program uses several shortcuts, for example **MK** for creating a mark at the current mouse position. You will need to press the first letter on your keyboard e.g. M, followed by the second letter, e.g. K (case insensitive). Press **Esc** to cancel the first letter.

For more commands and operations:

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

Project Setup

Open the project **ssNMRPeptides ccpn**



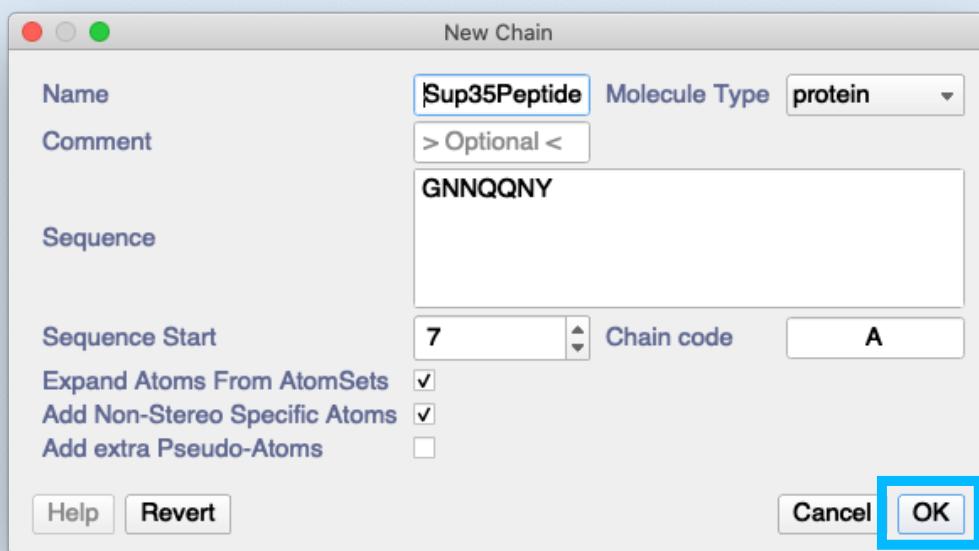
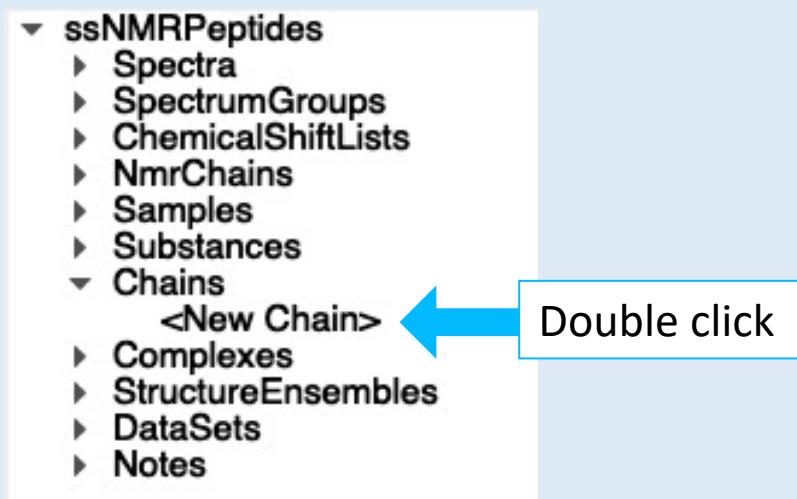
1A Drag & drop project into the sidebar or drop area

CcpNmr projects are saved as folders of type **filename ccpn**. For this tutorial we are going to use the *ssNMRPeptides ccpn* project in the PeptideSolidStateTutorial data directory.

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

Nested under **Spectra** in the sidebar, you will have eight spectra. Four were recorded on a sample in which the GNNQ amino acids were labelled and the other four on a sample in which the QQNY amino acids were labelled. The spectra are labelled accordingly. The second part of the spectrum name signifies the experiment type (CC/NCA/NCACX/NCO/NCOCX). All of them were recorded as 2D spectra.

Project Setup



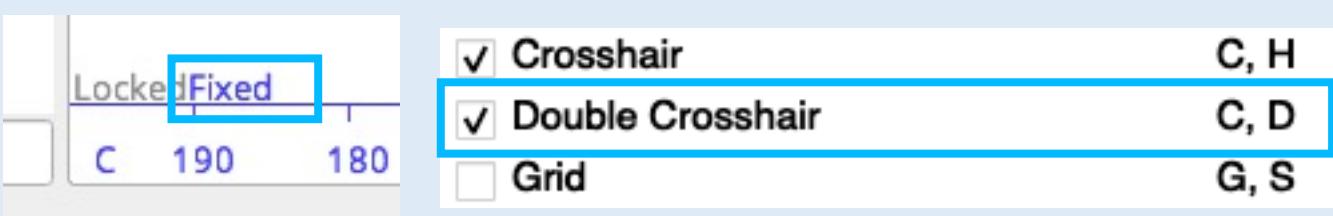
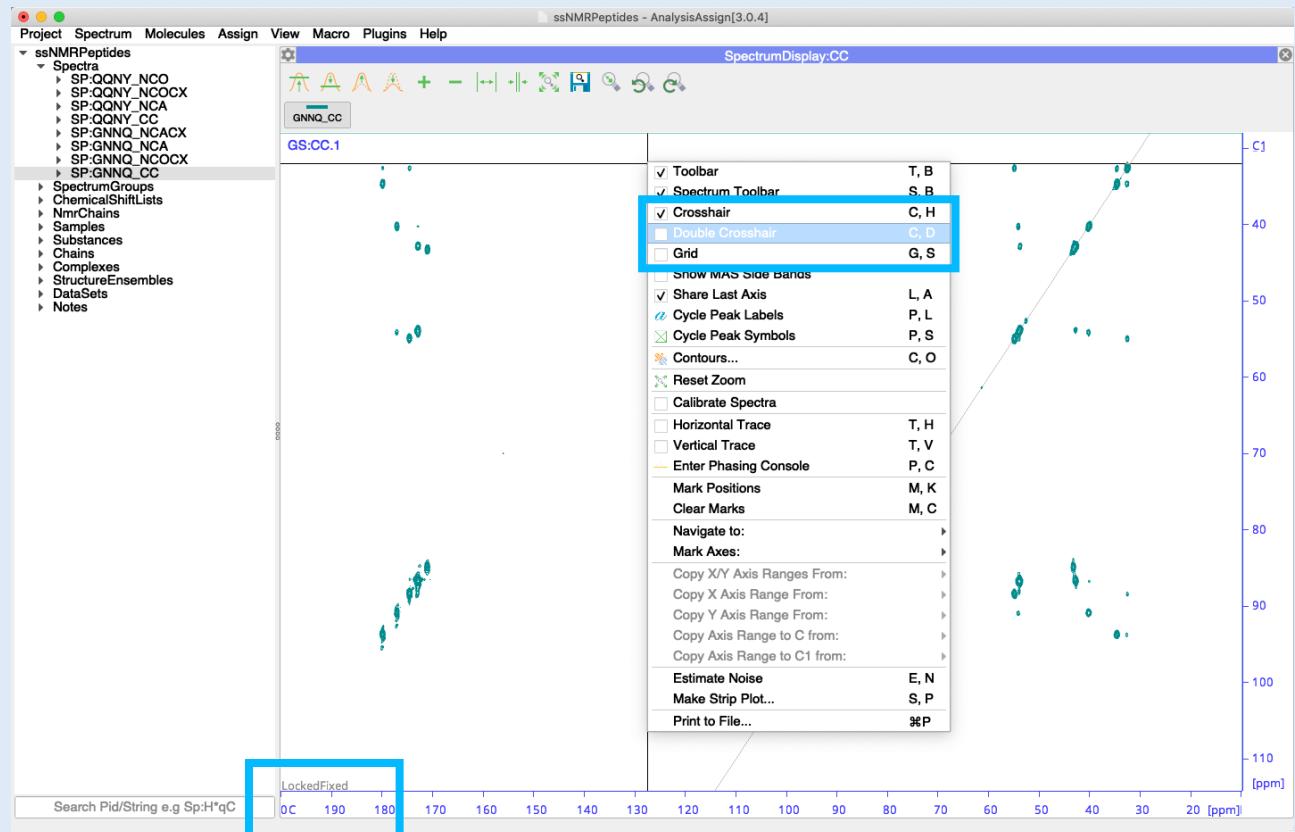
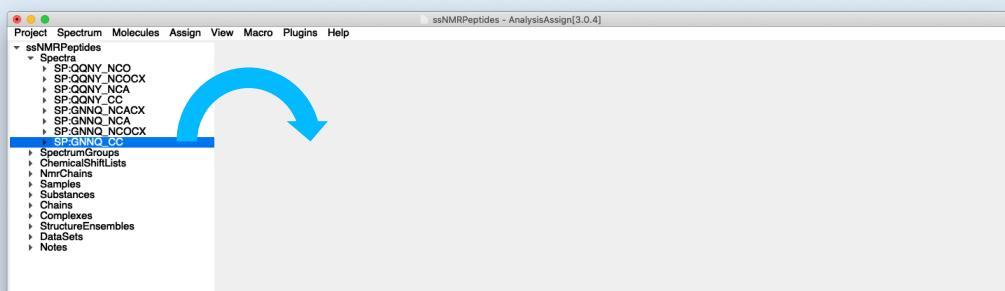
1B Create Chain

Add the peptide sequence to the project:

- Go to Sidebar → Chains → <NewChain>
- Fill in the Pop-up box with the following information:
 - Molecule Name: Sup35Peptide
 - ChainCode: A
 - Sequence: (type or copy & paste)
GNNQQNY
 - Sequence Start: 7
 - Click Ok

You can also add a Chain to your project by going to Main Menu → Molecule → Generate Chain or dropping a FASTA formatted file into the project.

Project Setup



1c Displaying spectra

- Drag the GNNQ_CC spectrum into the drop area to display it.
- Click on Fixed in the bottom left hand corner.

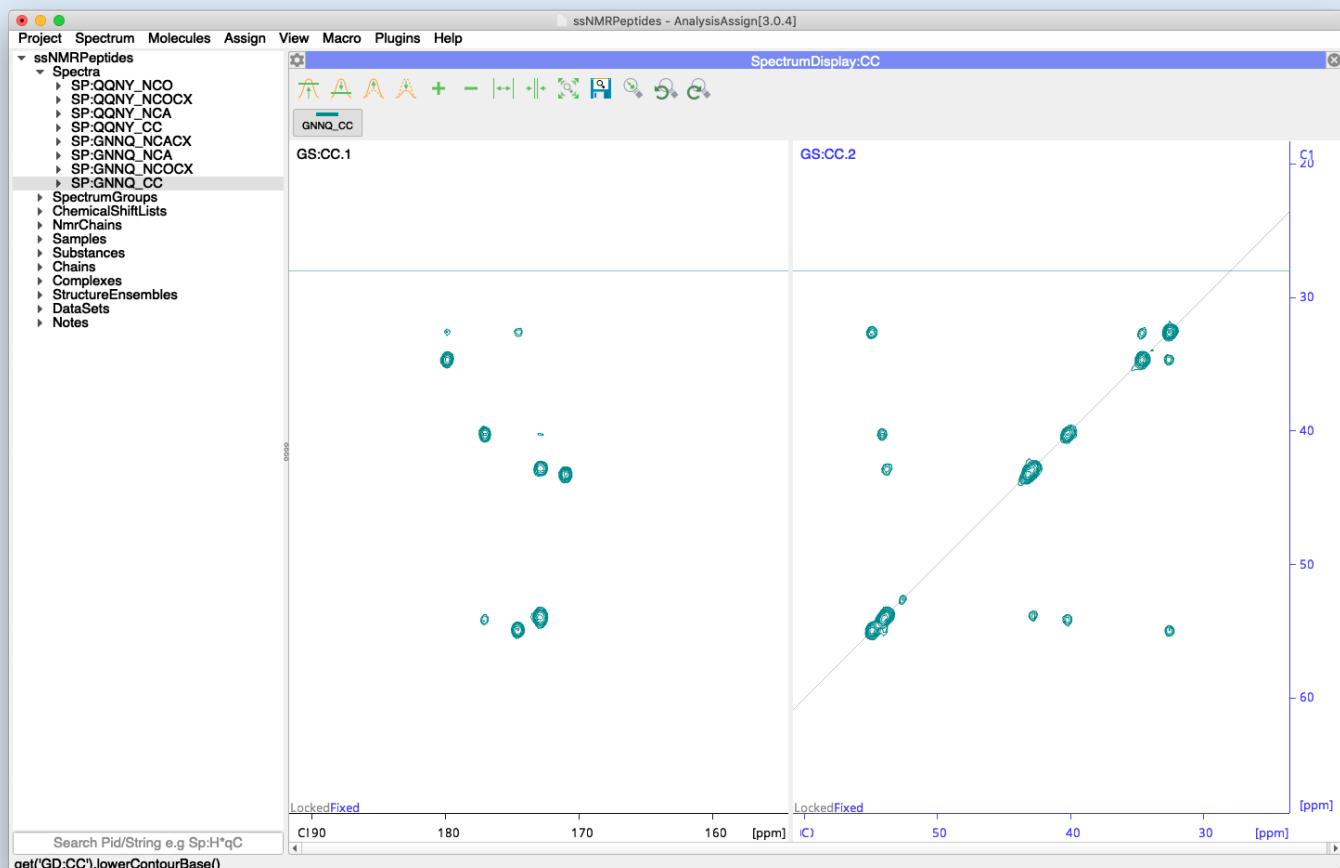
This will set the x and y axis scales the same and make sure the diagonal is at 45°.

- Right-click into the spectrum and make sure that Double Crosshair is selected.

This will mirror your mouse crosshair to the other side of the diagonal.

You can toggle the double crosshair on or off using the keyboard shortcut CD.

Project Setup



1c Add a strip

- Click on in the toolbar in order to add a second strip to your display.

Now arrange the spectra in the two strips so that you can see the carbonyl region on left hand side and the aliphatic region on the right hand side as shown above.

Assignment nomenclatures (Explanation only)

Peak labels in Analysis Assign are referred to as NmrAtoms which are grouped within NmrResidues and these in turn in NmrChains.

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

We call these strings the ‘id’ (id: identifier) of the NmrAtom. If an id matches the strings defining a molecular Atom¹, this effectively constitutes an assignment to the Atom.

If not, the NmrAtom id is a placeholder, reflecting its progress towards assignment².

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 this tutorial 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 can use 'connected' NmrChains, whose names start with '#' instead of '@'. Consequently, names with '@' (and NmrChain names starting with '#') are reserved. We won't be using such connected NmrChains in this tutorial.

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 ¹H and ¹³C so that Leu HDx% are the methyl protons bound to Leu CDx (NEF convention).

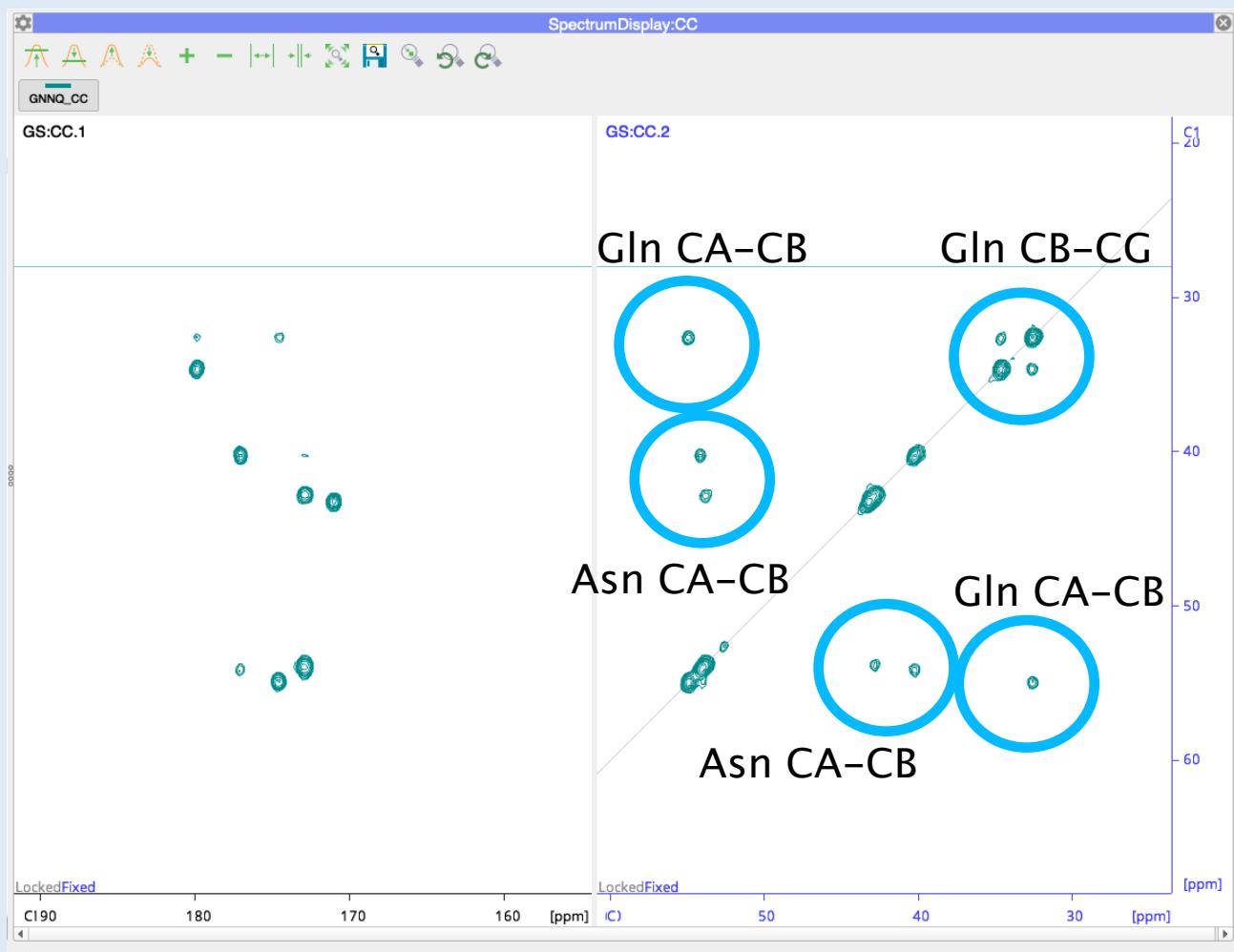
¹ Atoms reside in Residues, which reside in Chains; multiple chains can form a Complex.

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>



2A Identifying and picking peaks

The only labelled amino acids in our current spectrum are one Gly, two Asn and one Gln. Based on the typical aliphatic chemical shifts for the 20 natural amino acids, the cross peaks in the aliphatic region of the spectrum can be assigned to Asn and Gln groups.

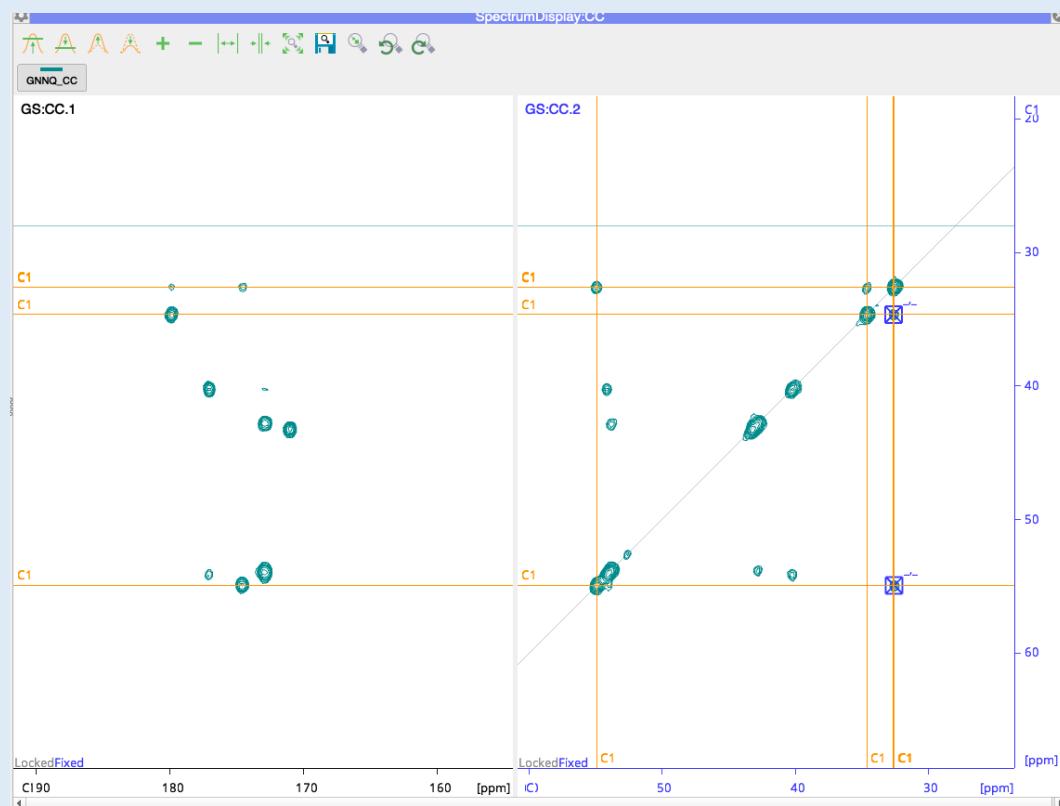
A graph showing the typical aliphatic carbon chemical shifts for the 20 natural amino acids is provided as part of the reference material in **Section 8**.

Section 7A shows how you can access this reference chemical shifts within the program.

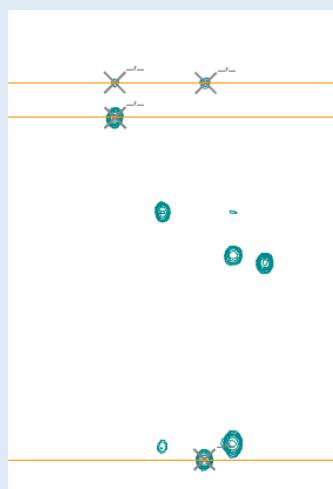
We will start by identifying the full Gln carbon spin system.

- Peak pick the Gln CA-CB and CB-CG peaks below the diagonal by pressing **Shift+Ctrl** (Shift+Cmd on a Mac) while **left-dragging** the mouse over the peaks.

Carbon spin system identification



PM
(mark selected peaks)



Other useful marking commands:

MC	clear marks
MK	mark mouse position
MX/PX	mark x axis only
MY/PY	mark y axis only

2B Marking and picking more peaks

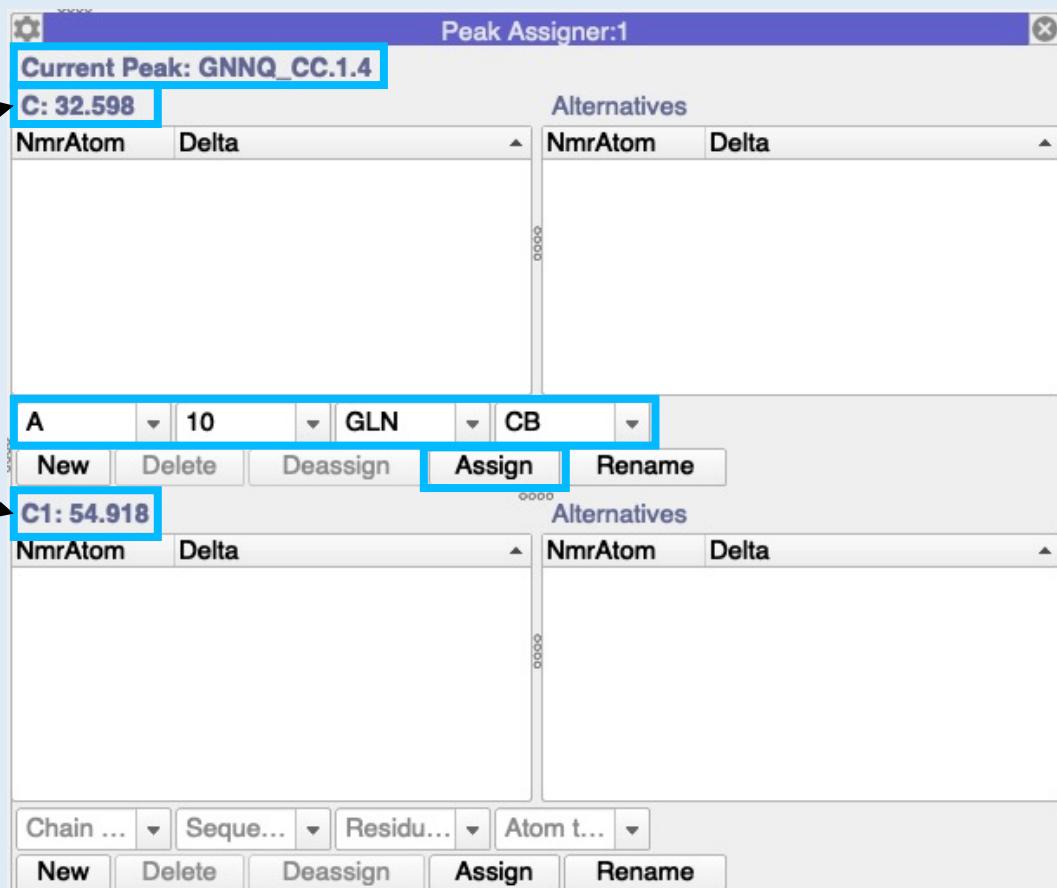
- Select your Gln CA-CB and CB-CG peaks by holding down the **Ctrl/Cmd** key while **left-clicking** on the peaks.
- Place marks through your peaks using the keyboard shortcut **PM**

You will see that these marks pass through peaks at two more chemical shift positions in the carbonyl region (170–180 ppm on the x axis). These two positions belong to the backbone and side-chain carbonyl atoms in the Gln residue. The CA only shows a link to the backbone carbonyl (C), the CG only a link to the side-chain carbonyl (CD) and the CB shows weak links to both.

- Pick all these peaks as before by pressing **Shift+Ctrl** (Shift+Cmd on a Mac) while **left-dragging** the mouse over the peaks.

Carbon spin system identification

The AxisCode and chemical shift position are shown for both dimensions



2c Assigning Peaks

As our current spectrum only contains one labelled Gln, we know that this must be **GLN 10** in the sequence and we can assign it as such.

- Select the Gln CA-CB peak at 32.6/54.9 ppm by **left-clicking** on it.
- Open the Peak Assigner with **AP** (or **Main Menu → Assign → Peak Assigner**).

The peak assigner shows the currently selected peak at the top and then has two sections, one for each dimension. The AxisCode and chemical shift positions are shown for each dimension

- By either typing or using the drop-down menus, set the Chain, Sequence Code, Residue Type and Atom Type for the upper dimension at 32.6 ppm to be:

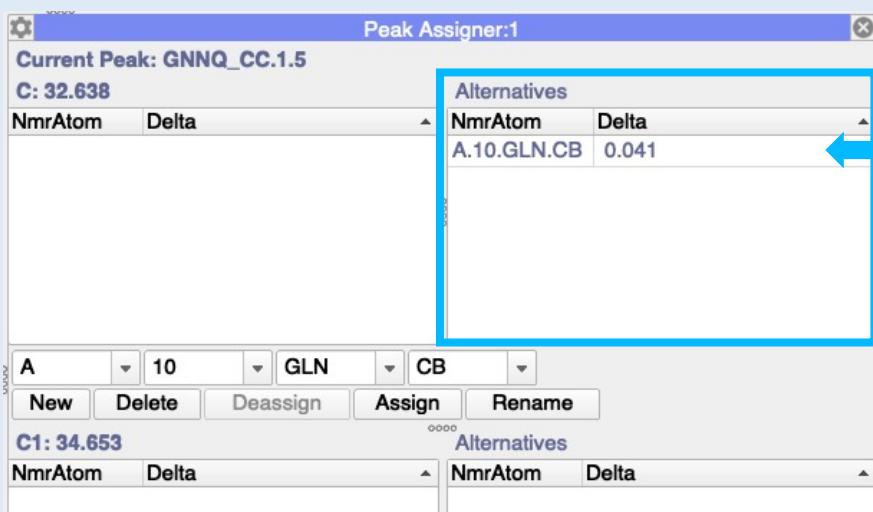
A ▾ **10** ▾ **GLN** ▾ **CB** ▾

- Click on **Assign** to make the assignment and create the **NmrAtom A.10.GLN.CB**.

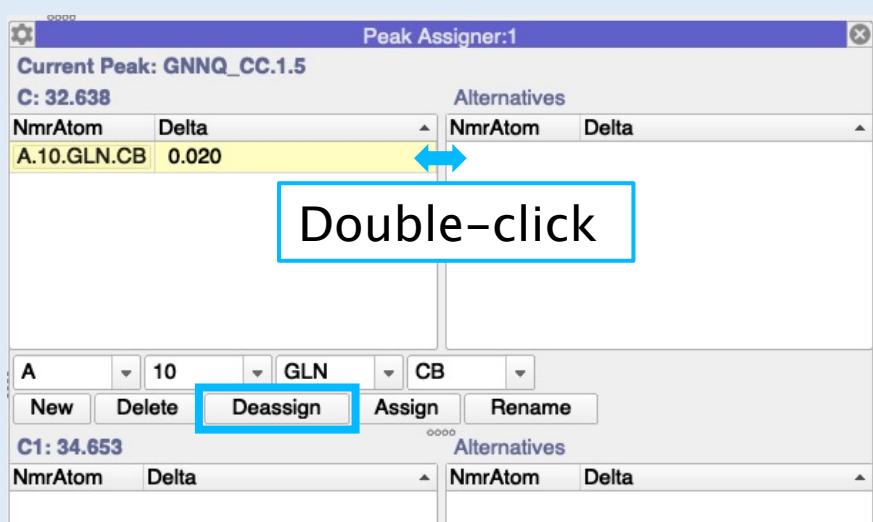
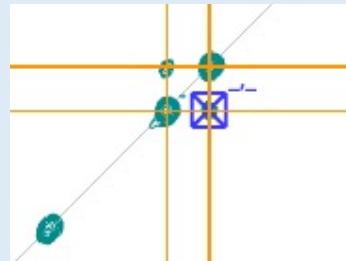


- Assign the lower dimension at 54.9 ppm to **A 10 GLN CA**.

Carbon spin system identification



Double-click



Double-click

2D Assigning more peaks

- Select the Gln CB-CG peak.

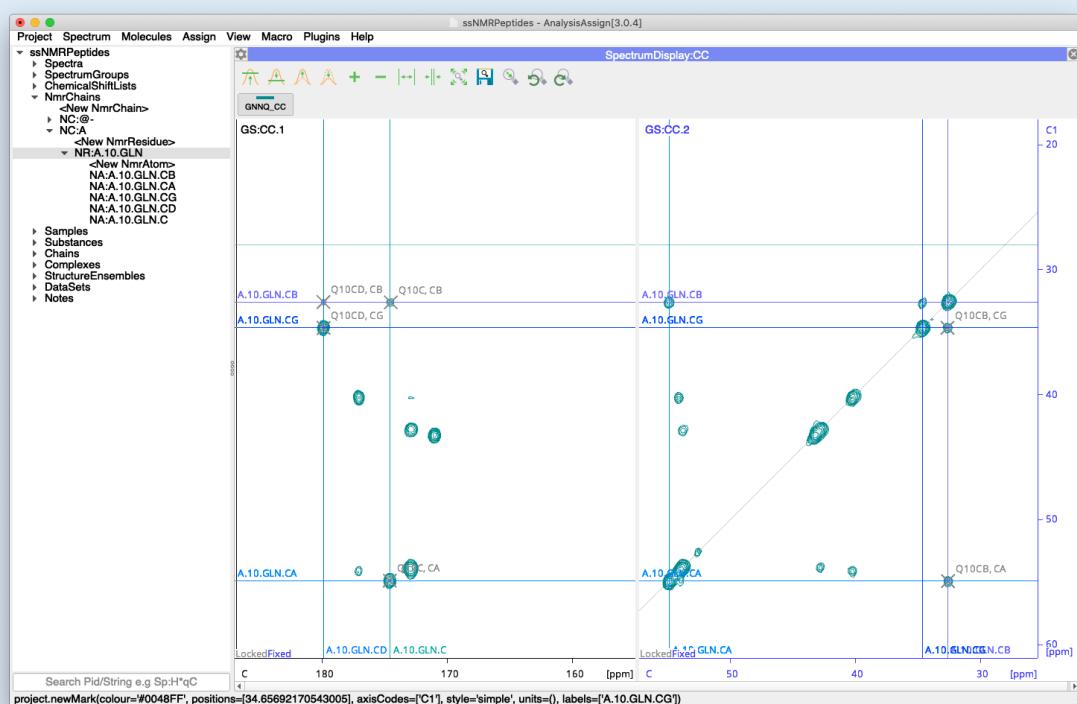
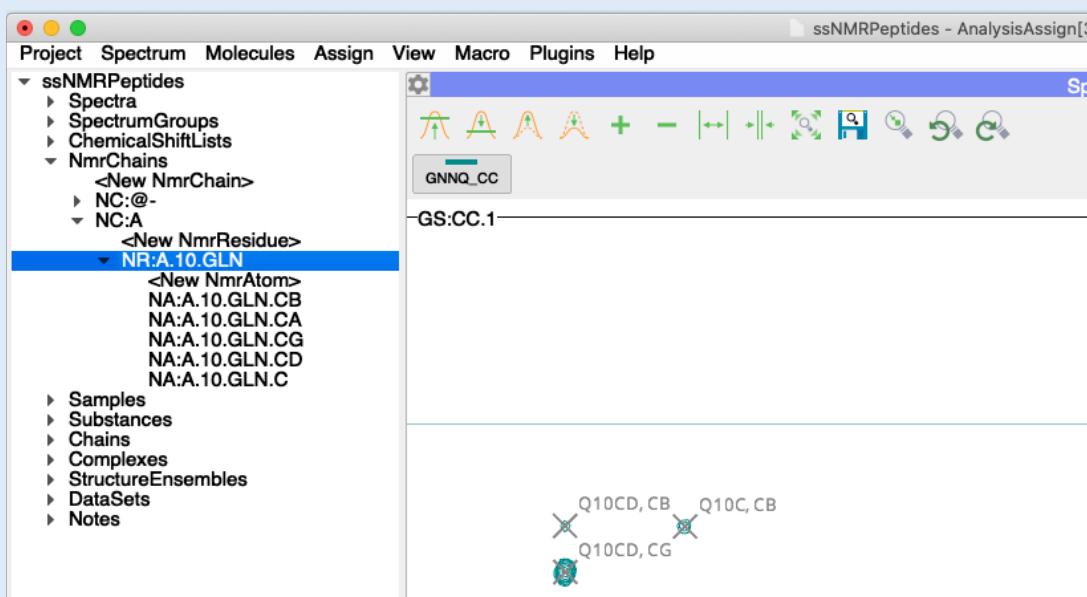
In the **Peak Assigner**, you will see that the CB dimension now has the **A.10.GLN.CB** NmrAtom as an option in the **Alternatives** box.

- Double-click** on this to move it across to the left hand side and make this the assignment for this peak dimension.
- Add the CG assignment/NmrAtom for the lower dimension at 34.6 ppm using the drop down menus and clicking **Assign**.
- Select and assign the remaining Gln 10 peaks in the same way.

Note that you can remove an assignment from a peak dimension by **double-clicking** on the assignment. Alternatively, select the assignment (it will turn yellow) and **click on Deassign**.

You can also **Rename** or **Delete** NmrAtoms in the Peak Assigner.

Carbon spin system identification



2E Mark NmrResidue A.10.GLN

- Clear your marks with shortcut MC.
- In the sidebar, expand your **NmrChains** section and **NmrChain NC:A**.
- Drag NmrResidue **NR:A.10.GLN** into your spectrum.

This will create marks at the positions of allNmrAtoms in this NmrResidue.

This can be a useful way to check a spin system assignment.

Note that you can also mark individual NmrAtoms in your spectra this way: simply drag one or more NmrAtoms from the sidebar into a spectrum to create marks.

Carbon spin system identification

The screenshot shows the Peak Assigner interface with two peaks assigned:

- Peak 1:** Current Peak: GNNQ_CC.1.10, C: 40.216. Assigned to NmrAtom @- and NmrResidue ASN.
- Peak 2:** Current Peak: GNNQ_CC.1.7, C: 54.177. Assigned to NmrAtom @-.@3.ASN.CB and NmrResidue ASN.

The sidebar on the left displays the following tree structure under ssNMRPeptides:

- ssNMRPeptides
 - Spectra
 - SpectrumGroups
 - ChemicalShiftLists
 - NmrChains
 - <New NmrChain>
 - NC:@-
 - <New NmrResidue>
 - NR:@-@.
 - NR:@-@3.ASN
 - <New NmrAtom>
 - NA:@-@3.ASN.CB
 - NC:A
 - Samples
 - Substances
 - Chains
 - Complexes
 - StructureEnsembles
 - DataSets
 - Notes

2F Asn spin system identification

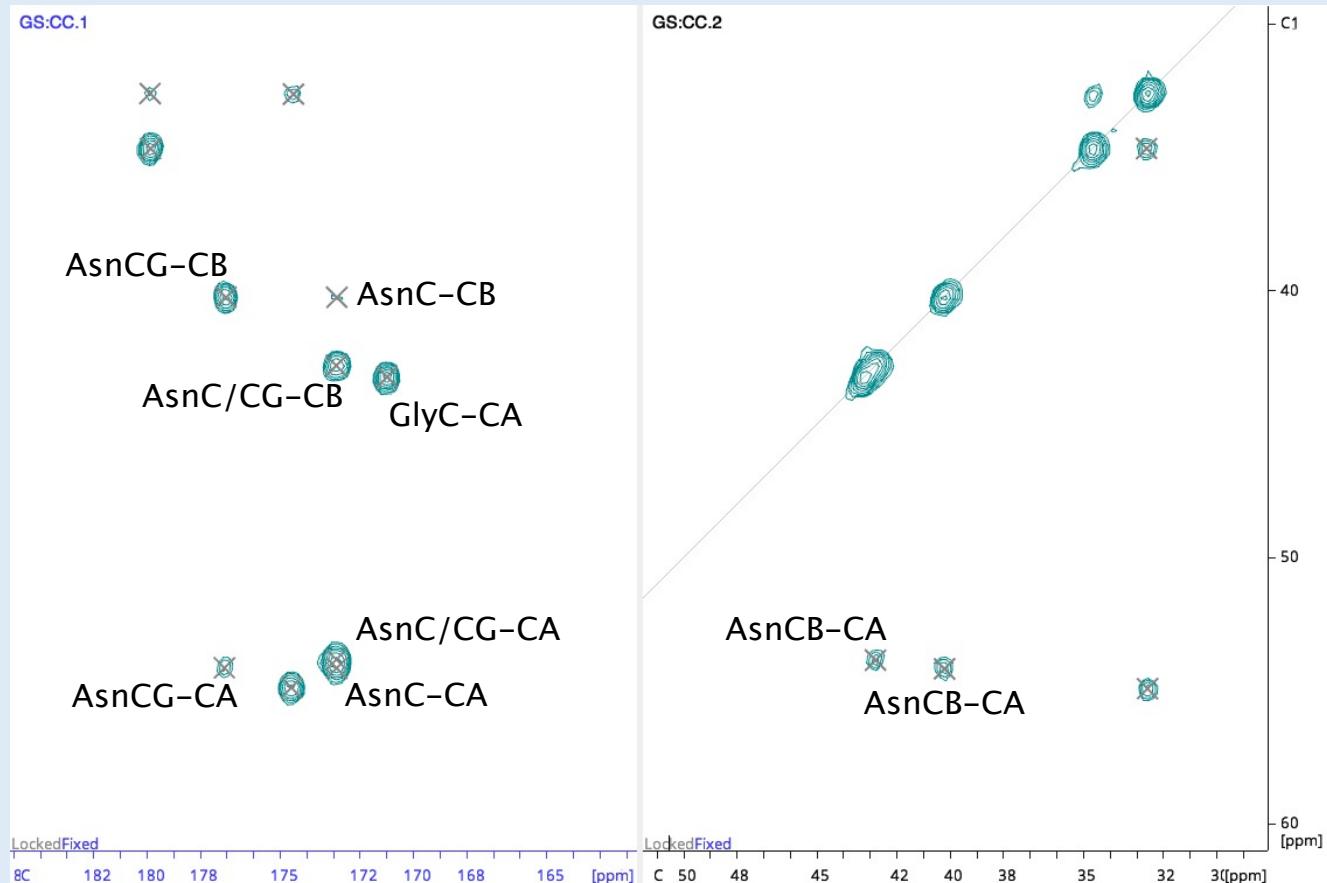
You can now repeat this same procedure for the two Asn spin systems. The only difference is that this time you do not know which Asn is which, so you have to use a random placeholder for the Sequence Code.

- Peak pick an Asn CA-CB peak.
- If not open already, open the **Peak Assigner** with shortcut AP.
- Assign the first NmrAtom choosing the default NmrChain @- as the **Chain** and leaving the **Sequence Code** blank:

@-	Seque...	ASN	CB
----	----------	-----	----

The program will allocate a random number proceeded by @ as the Sequence Code, e.g. @3.

- This new NmrAtom @-@3.ASN.CB and NmrResidue @-@3.ASN will be visible in your sidebar as shown above.



2G Asn spin system identification

- Identify, pick and assign the remaining Asn peaks.

Make sure you create a new randomly named NmrResidue for the second Asn.

Also be aware of overlapping peaks shown above:

- Both Asn Cs and one CG all have the same chemical shift and thus give rise to some overlapped peaks.

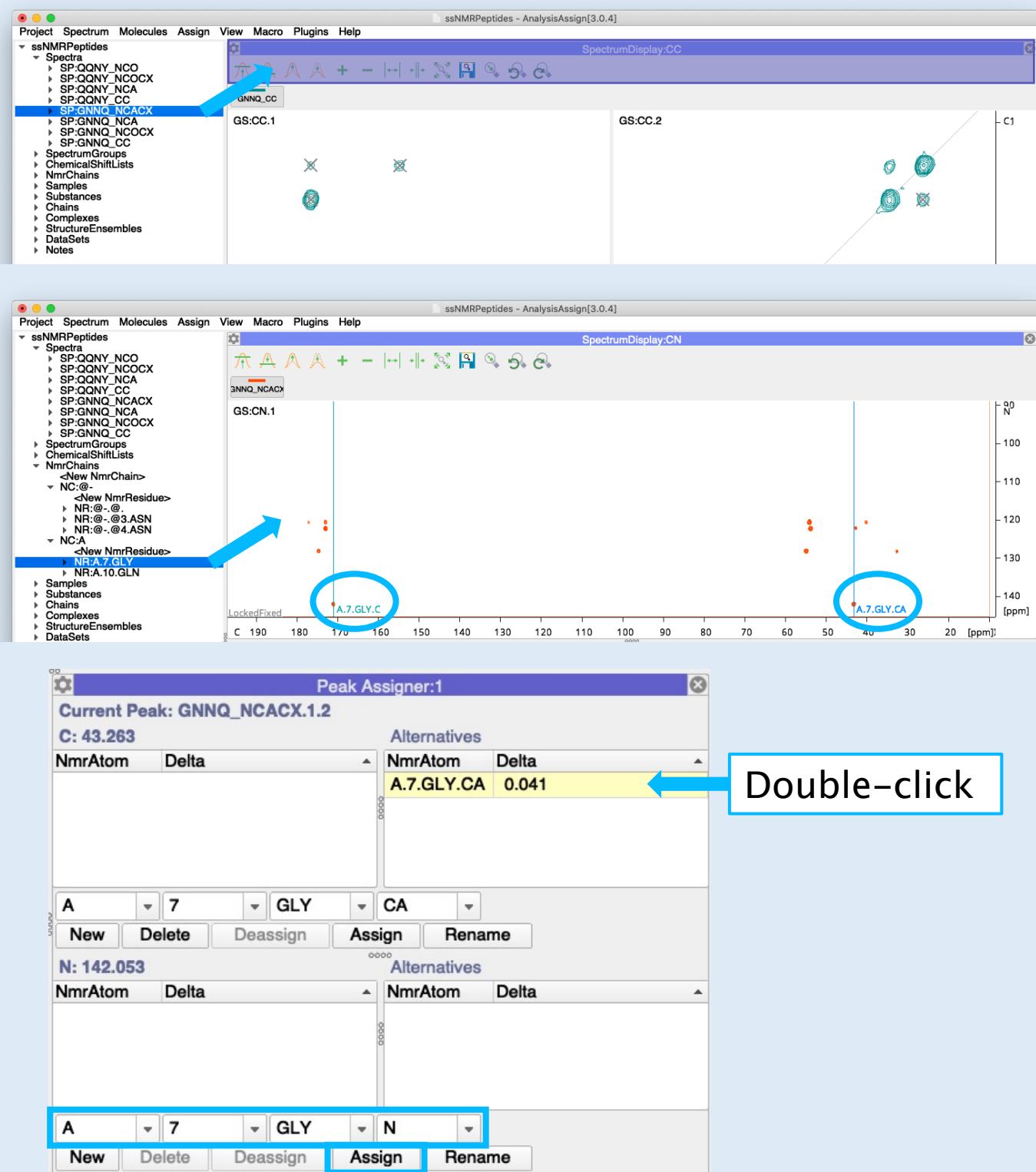
2H Gly spin system identification

The only non-diagonal Glycine peak is the CA-C peak.

- Pick this peak and assign it to Gly 7, the only glycine in this peptide.

3

Nitrogen assignments



3A Open the NCACX spectrum

- Drag the **GNNQ_NCACX** spectrum from the sidebar into the drop area.

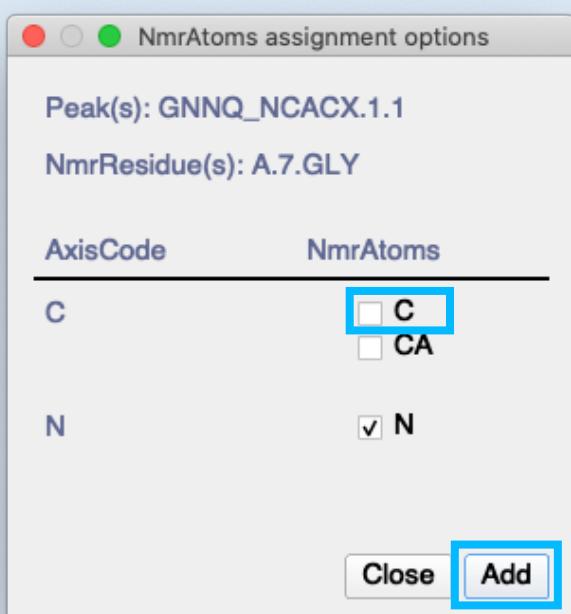
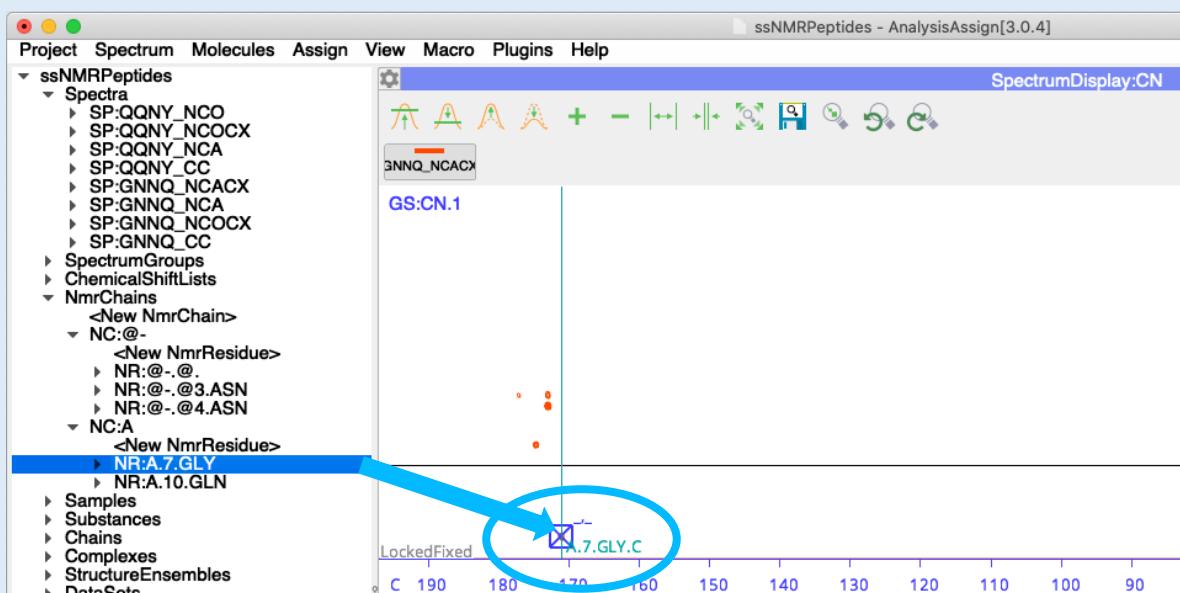
3B Mark assian Gly 7

- Drag the **NR:A.7.GLY** NmrResidue from the sidebar into a SpectrumDisplay in order to mark its chemical shifts.

You can see that these marks pass through two peaks in the GNNQ_NCACX spectrum at about 142 ppm.

- Pick the CA-N peak and assign it in the **Peak Assigner** (AP).

Nitrogen assignments



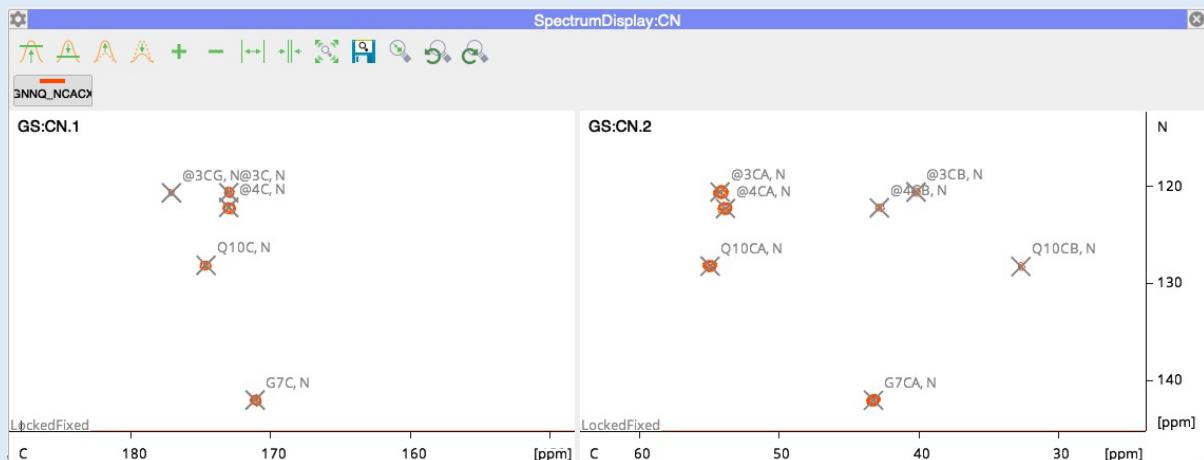
3c Drag NmrResidue to assign

As you are now no longer having to add additional NmrAtoms to your NR:A.7.GLY NmrResidue, you can assign the peaks using an alternative method if you like:

- Pick the Gly 7 C-N peak and make sure it stays selected
- Drag the NR:A.7.GLY NmrResidue from the sidebar onto the peak
- Select the C NmrAtom and click on Add.

Alternatively, assign the peak using the Peak Assigner.

Nitrogen assignments

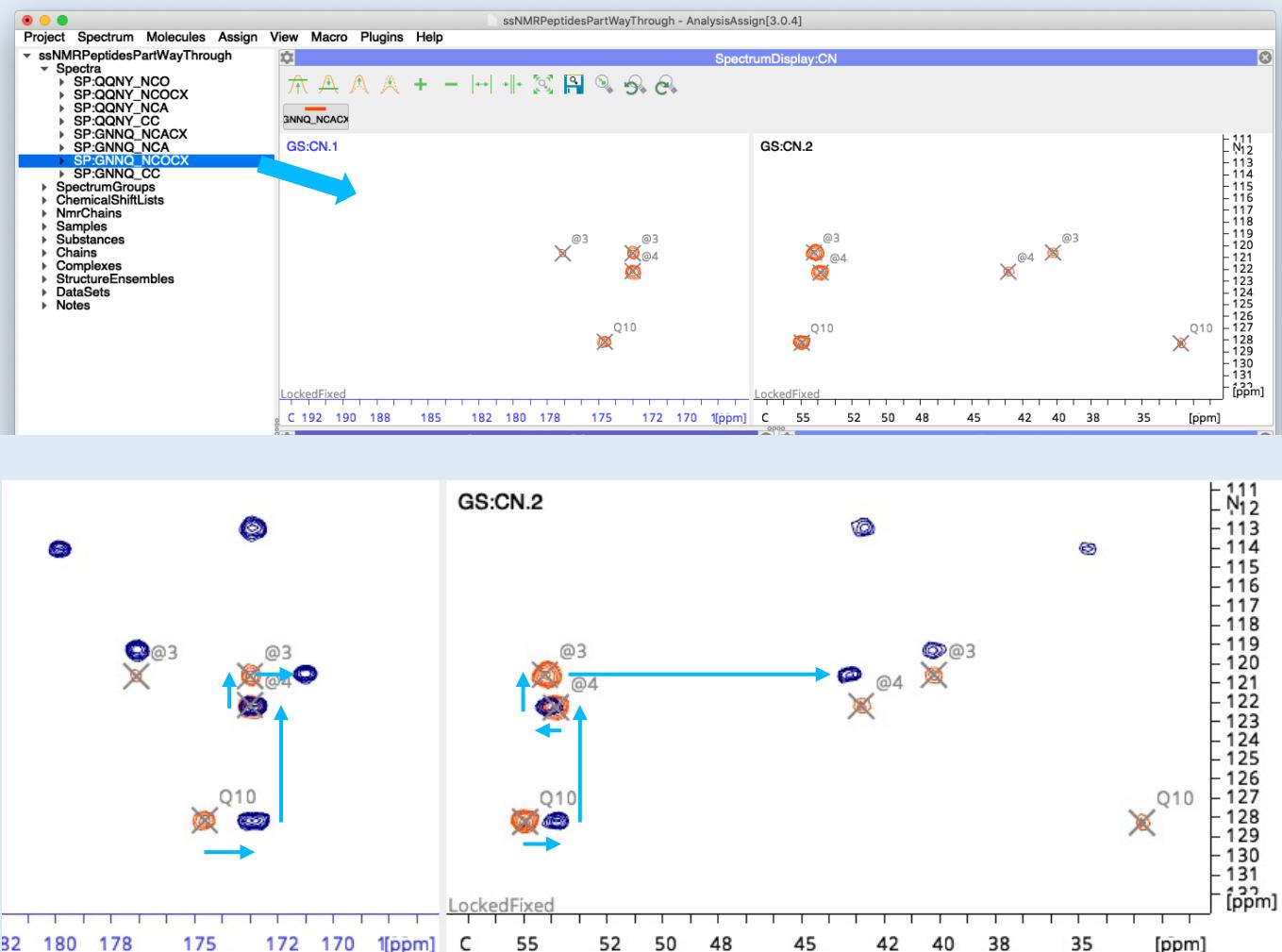


3D Assign remaining Nitrogens

Assign the other three nitrogen atoms using the same procedure:

- Clear Marks with **MC**.
- **Drag** the **NmrResidue** into a **Spectrum Display** to create marks.
- Pick the peaks.
- Assign the Nitrogen dimension using the **Peak Assigner (AP)**.
- Pick and assign any remaining peaks belonging to that residue, if desired – either using the Peak Assigner, or by dragging the **NmrResidue** onto the peaks.

Sequential Assignment



The NCACX correlates N(i) with C(i) and CA(i), while the NCOCX correlates N(i) with C(i-1) and CA(i-1). Starting with Gln 10, it is possible to “walk” through the spectra, identifying the sequential order of the residues.

4A Add NCOCX to NC SpectrumDisplay

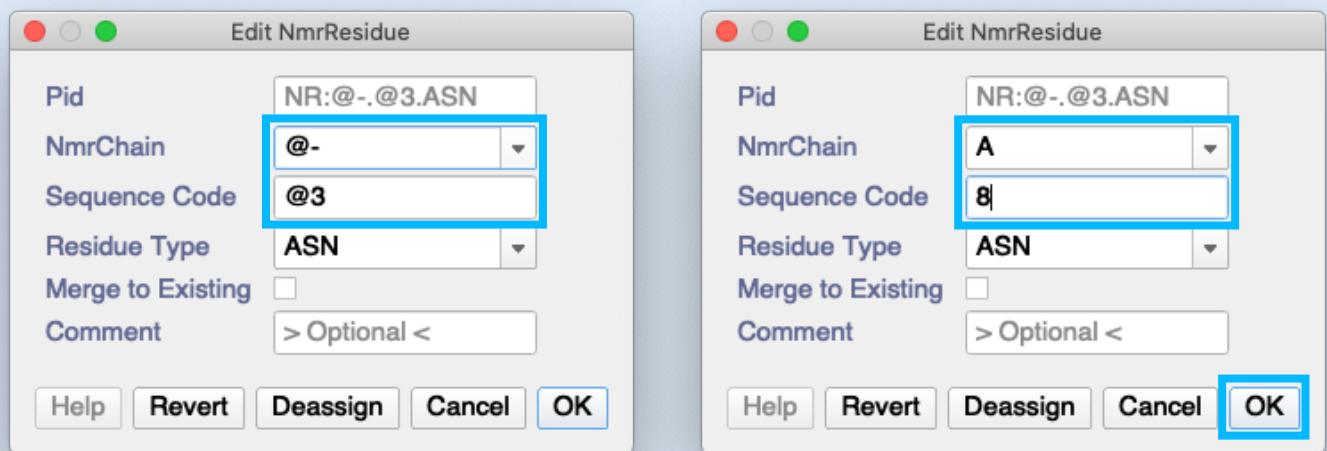
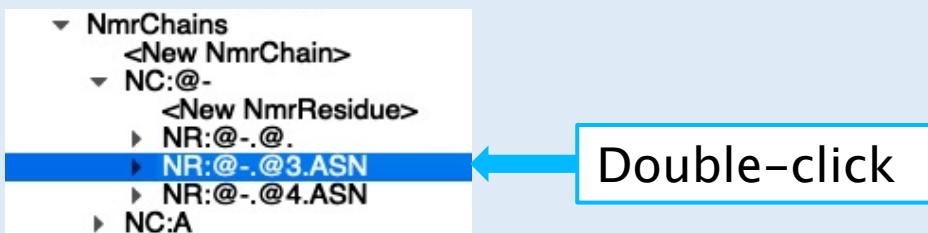
- Drag the GNNQ_NCOCX spectrum from the sidebar into the NC Spectrum Display.

4B Assign NCOCX peaks

The peaks in the NCACX and NCOCX can be correlated to form a “backbone walk”. This shows that Asn @4 above is in fact Asn 9 and Asn @3 above is Asn 8.

- Pick the NCOCX peaks and assign them using the Peak Assigner (AP). (For the time being you can ignore the peaks at 112, 113 and 119 ppm. These belong to the side-chain nitrogen atoms. Assign them at the end if you like.)

5 Sequence specific assignment

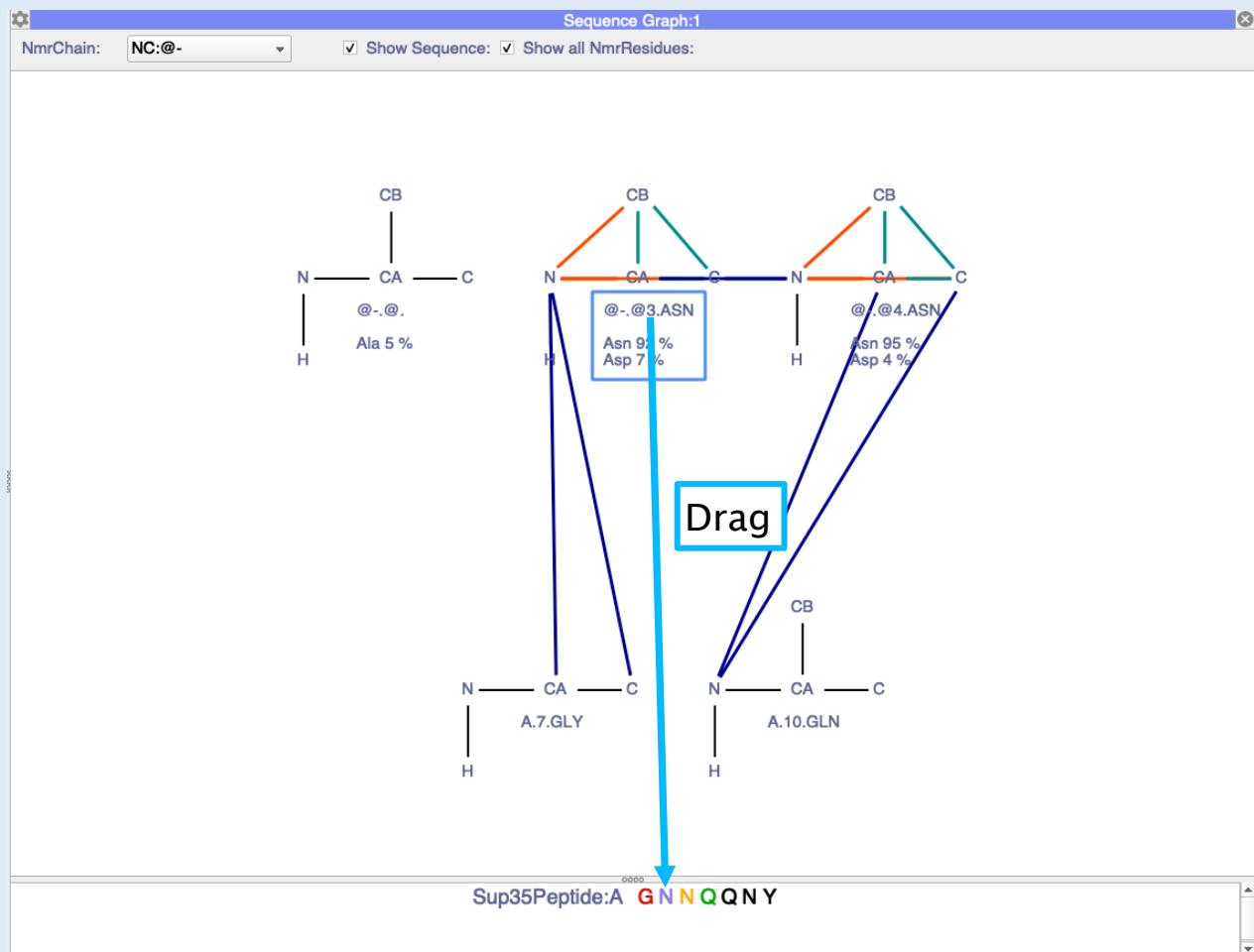


5A Sequence specific assignments via Edit NmrResidue

- Double-click on the unassigned NmrResidue **NR:@-.@3 ASN** in the sidebar to bring up the **Edit NmrResidue** popup.
- Change the NmrChain and Sequence Code from @- and @3 to A and 8, respectively.

You can now repeat this for the **NR:@-.@3 ASN** NmrResidue, or you can try the alternative method for making sequence specific assignments in **Section 5B**.

5 Sequence specific assignment



SG

5B Sequence specific assignments via SequenceGraph

Bring up the Sequence Graph:

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

You will see 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.

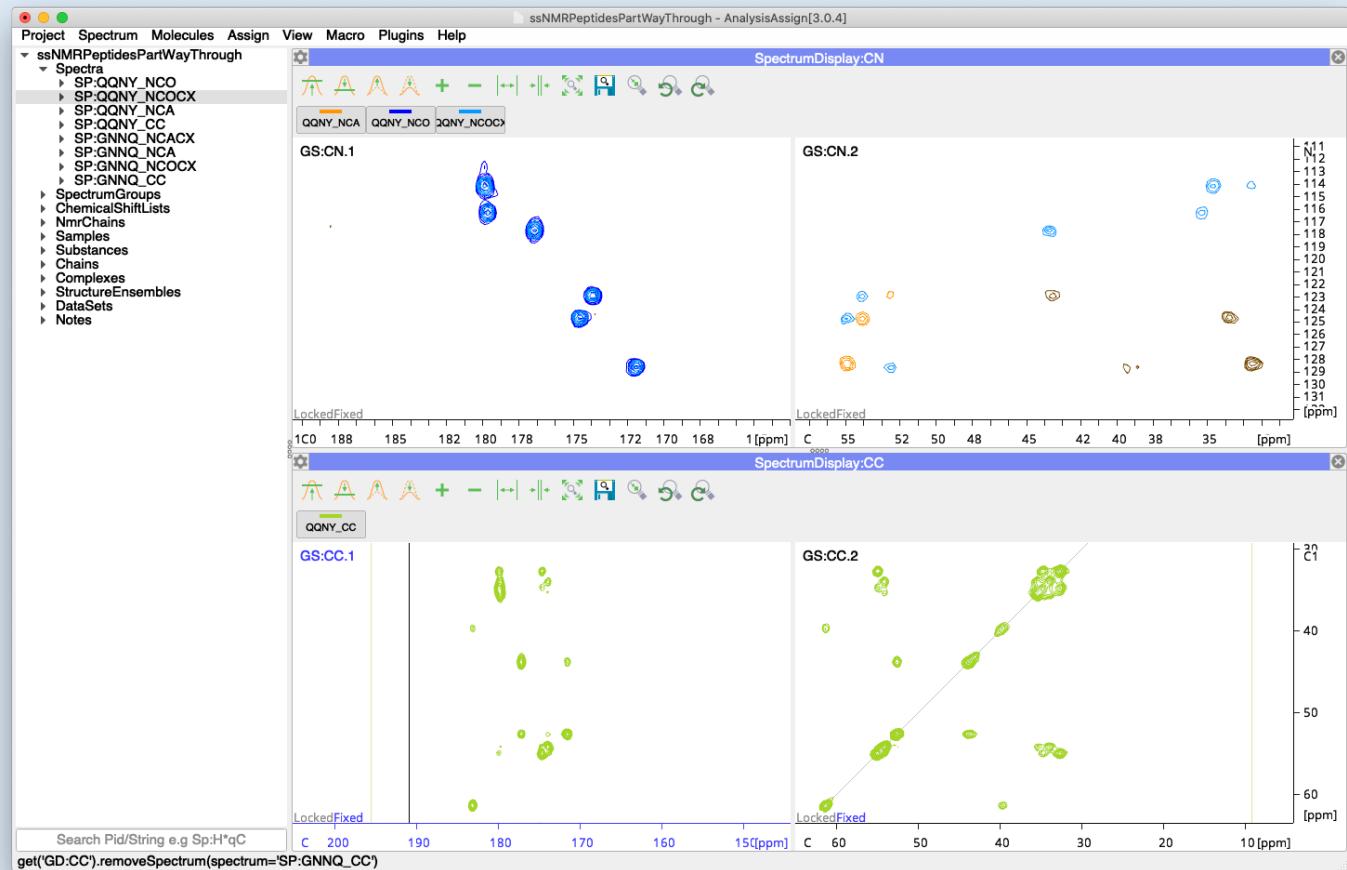
The assigned NmrResidue is placed into NmrChain A (also reflected in the sidebar).

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.

Further Assignments



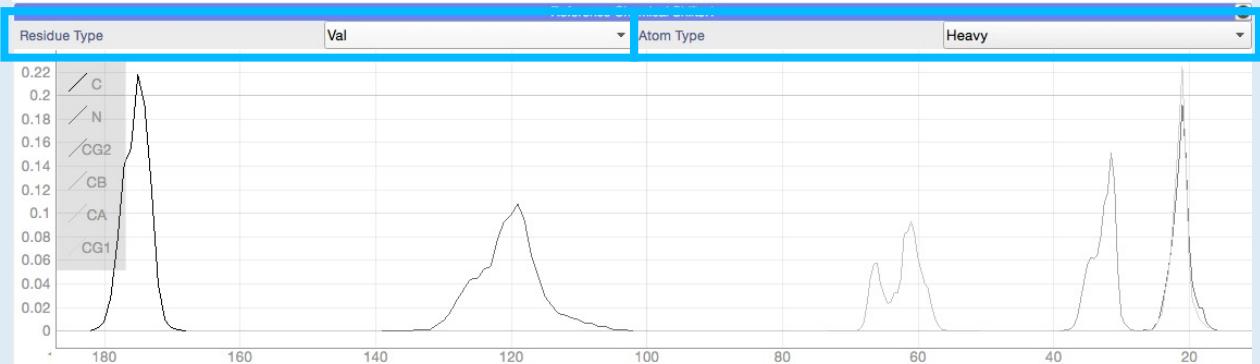
6A Assigning the QQNY motif

Now have a go at assigning the **QQNY** motif using the principles outlined in sections 1–5.

- Find, peak pick and assign the carbon spin systems in the **QQNY_CC** spectrum.
- Find, peak pick and assign the nitrogen resonances in the **QQNY_NCA** and **QQNY_NCO** spectra.
- Find the sequential correlations in the **QQNY_NCOCX** spectrum. Pick the peaks, assign them and make any sequence-specific assignments you were missing.

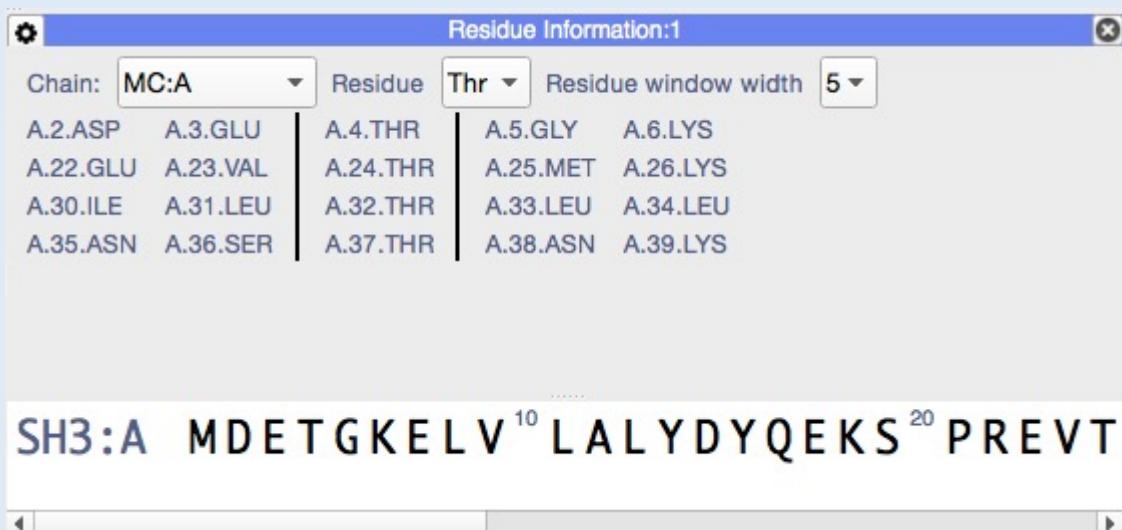
Other Useful Tools

Reference Chemical Shifts



RC

Residue Information



RI

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**.
- Select the **Residue Type** and **Atom Type** of your choice.

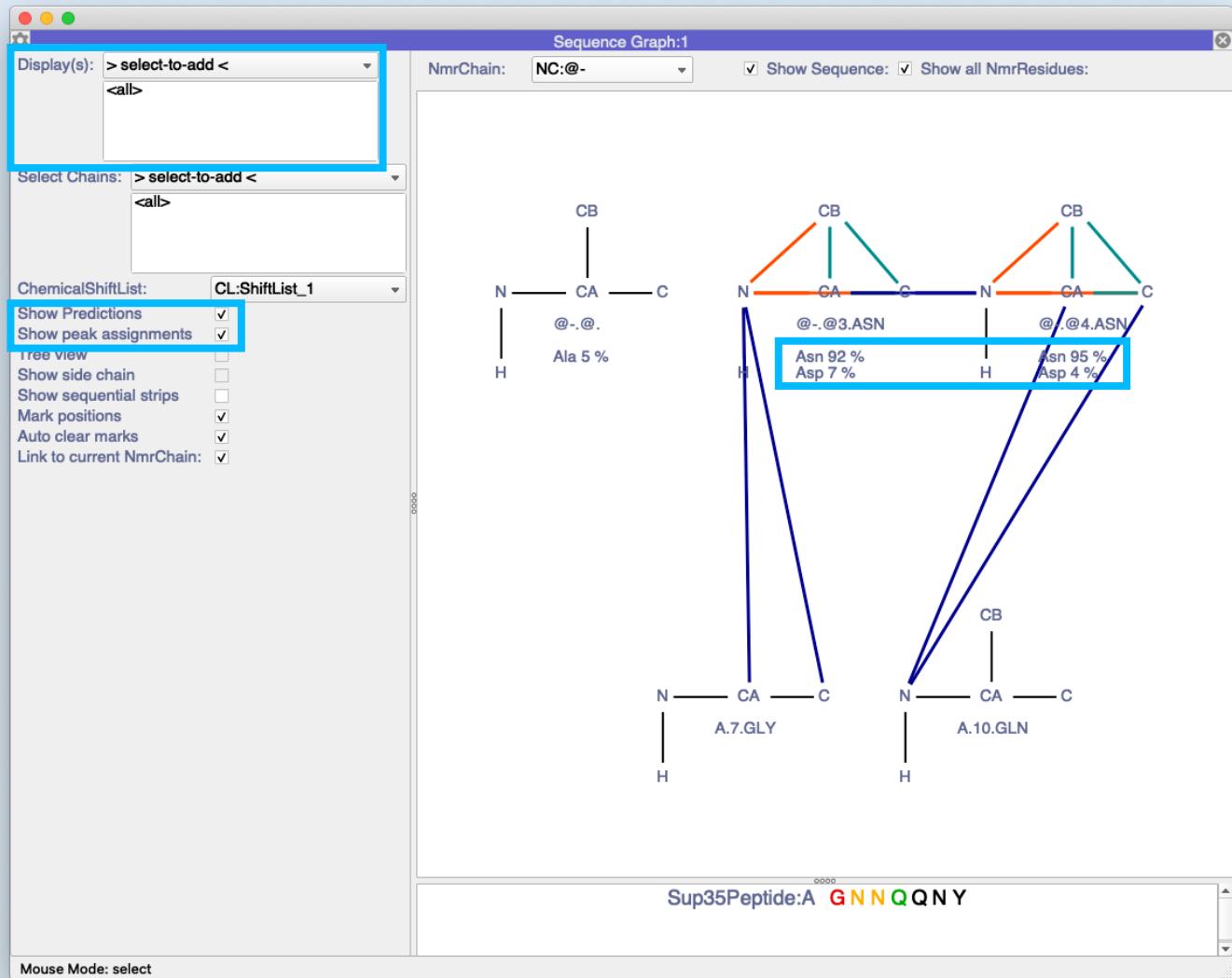
7B Residue Information

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.

Sequence Graph



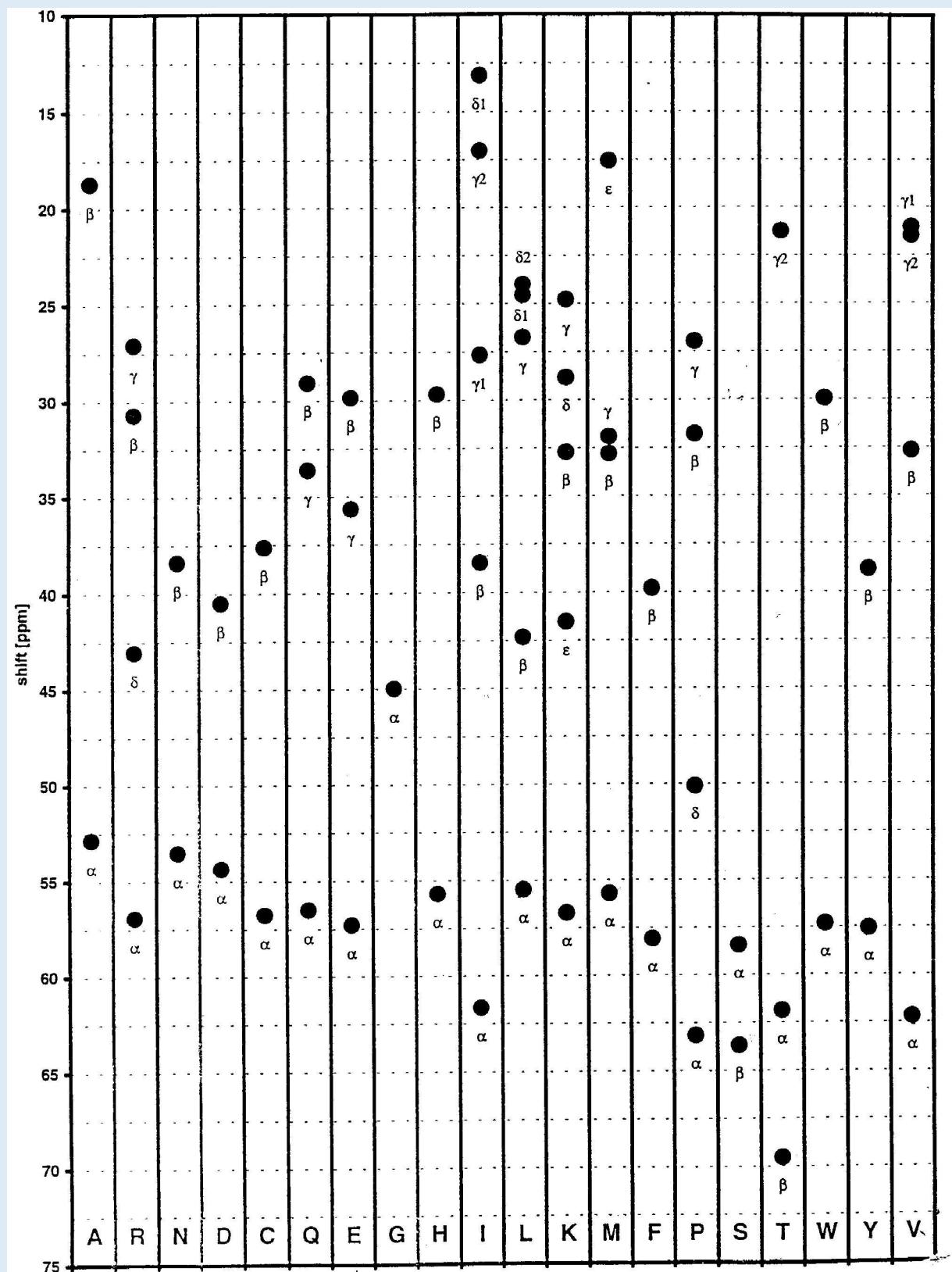
SG

7C Sequence Graph

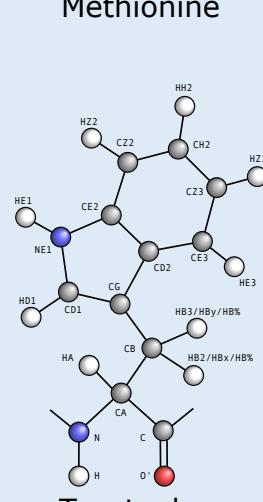
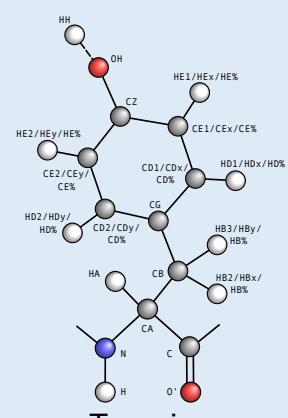
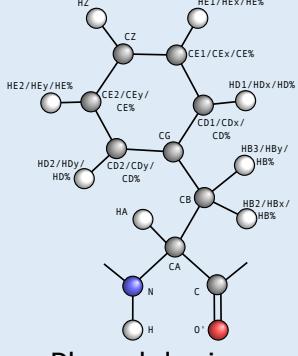
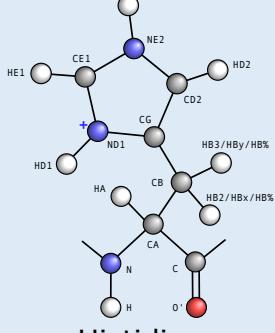
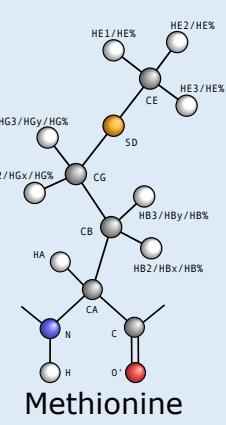
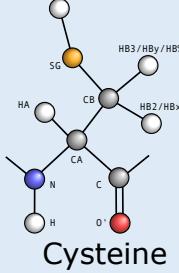
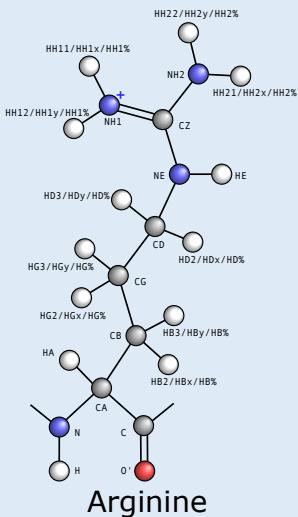
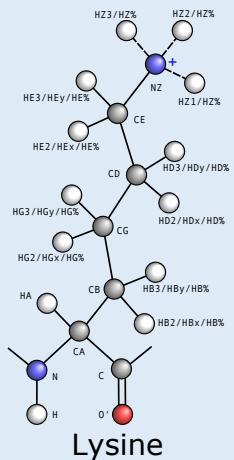
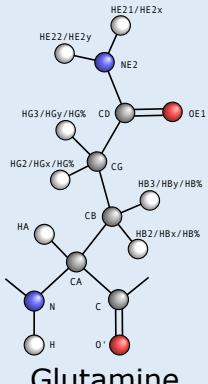
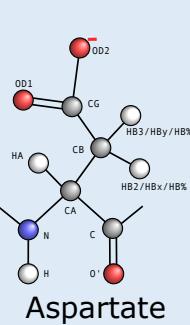
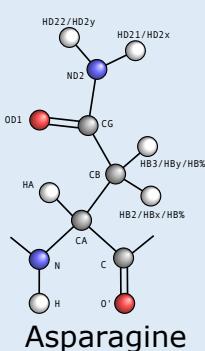
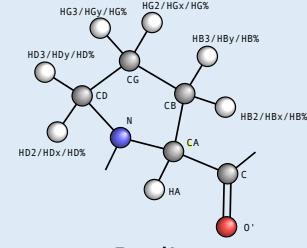
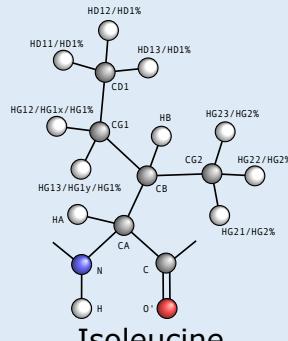
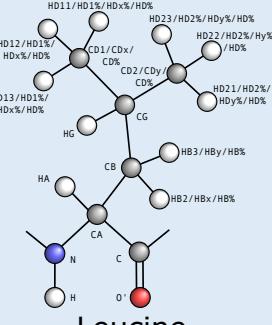
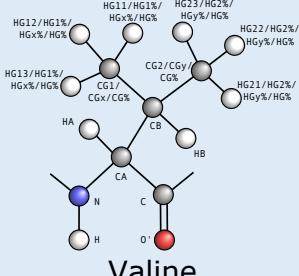
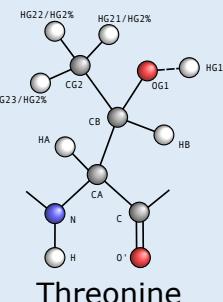
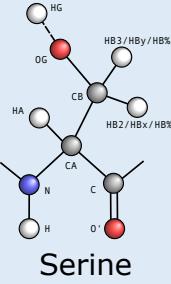
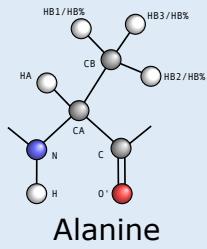
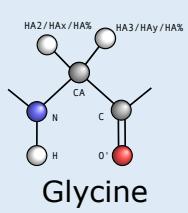
As well as being able to use the Sequence Graph to make sequence specific assignments, it also includes other information:

- Coloured lines show links between NmrAtoms from peaks. 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**). Please note that this feature relies on Experiment Types having been set with **ET**. In this tutorial project this has already been done.
- 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.
- 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.

Carbon chemical shifts for the 20 natural amino acids



20 natural amino acid structures with NEF atom names



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