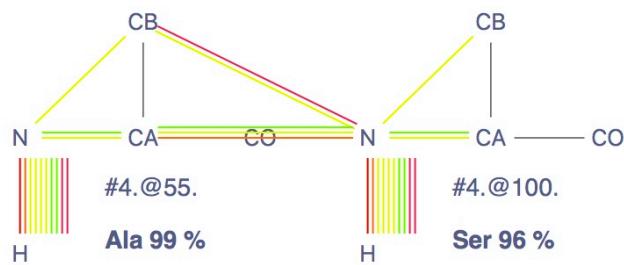
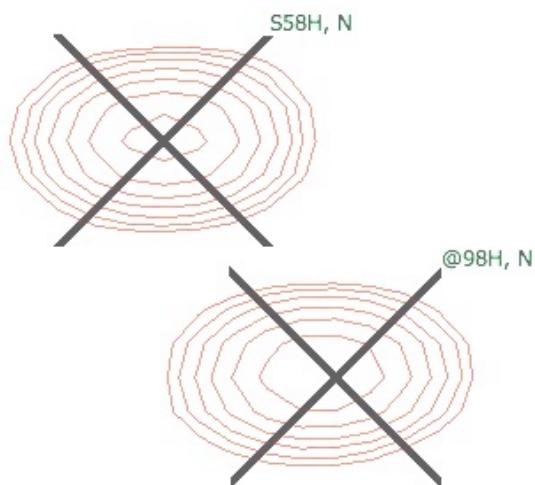


Backbone Assignment Tutorial



Introduction

These tutorials are designed to guide you through a sequential triple resonance backbone assignment using Ccpnmr AnalysisAssign Version 3.0, they are not intended to teach any theoretical aspects of NMR. For a practical guide, please visit <http://www.protein-nmr.org.uk>.

In these tutorials you will use spectra recorded on Sec5, in particular HSQC, CBCAcoNH, HNCACB, HNCA and HNcoCA spectra.

You will need four projects which are located in the directory: AnalysisV3/data/CcpnSec5BBTutorial.

The first two projects, Sec5Part1 and Sec5Part2 are used for the actual sequential backbone assignment, the others are *How-To* examples of how to do common assignment operations using different AnalysisAssign tools.

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

Start CcpNmr Analysis V3

Apple users by double clicking the icon 

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

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 **middle-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:

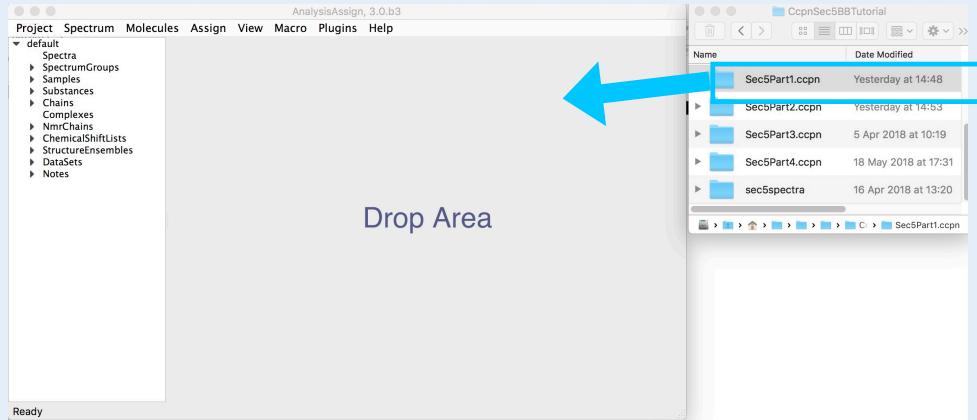
Main Menu -> *Help* -> *Tutorial (Beginners)* or *Show Shortcuts*

1

Project Setup

Sec5Part1

Open the project AnalysisV3/data/CcpnSec5BBTutorial/Sec5Part1.ccpn



1A Drag & drop “Sec5Part1.ccpn” into the sidebar or drop area.

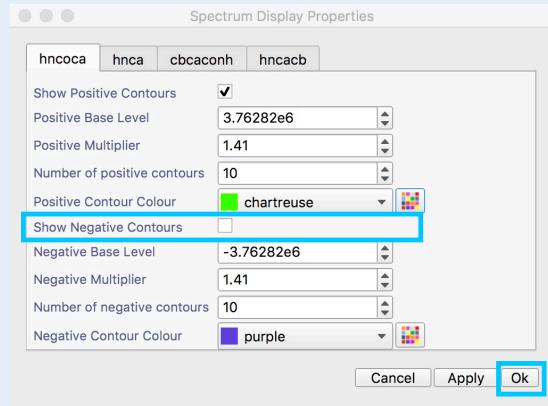
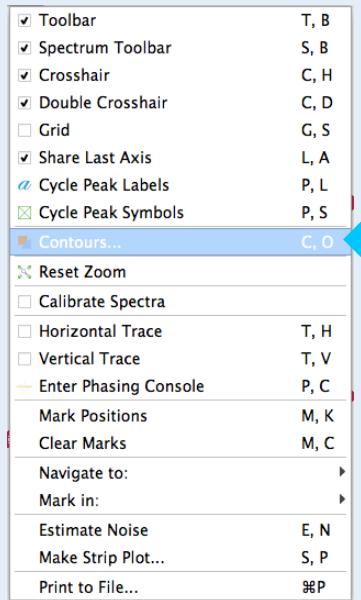
- CcpNmr projects have an extension of type “filename.ccpn”. Find the project file *Sec5Part1.ccpn* in the directory *AnalysisV3/data/CcpnSec5BBTutorial*.
- Select the file “**Sec5Part1.ccpn**”, drag and drop it into the program. The Sec5Part1 project will be loaded in a new window.

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

- hsqc (red, HN)
- cbcacnh (red, HCN)
- hnca (orange, HCN)
- hncacb (yellow, HCN)
- hncoca (green, HCN)

Set contours

Right-click on Spectrum Displays HN and HCN



or Shortcut CO

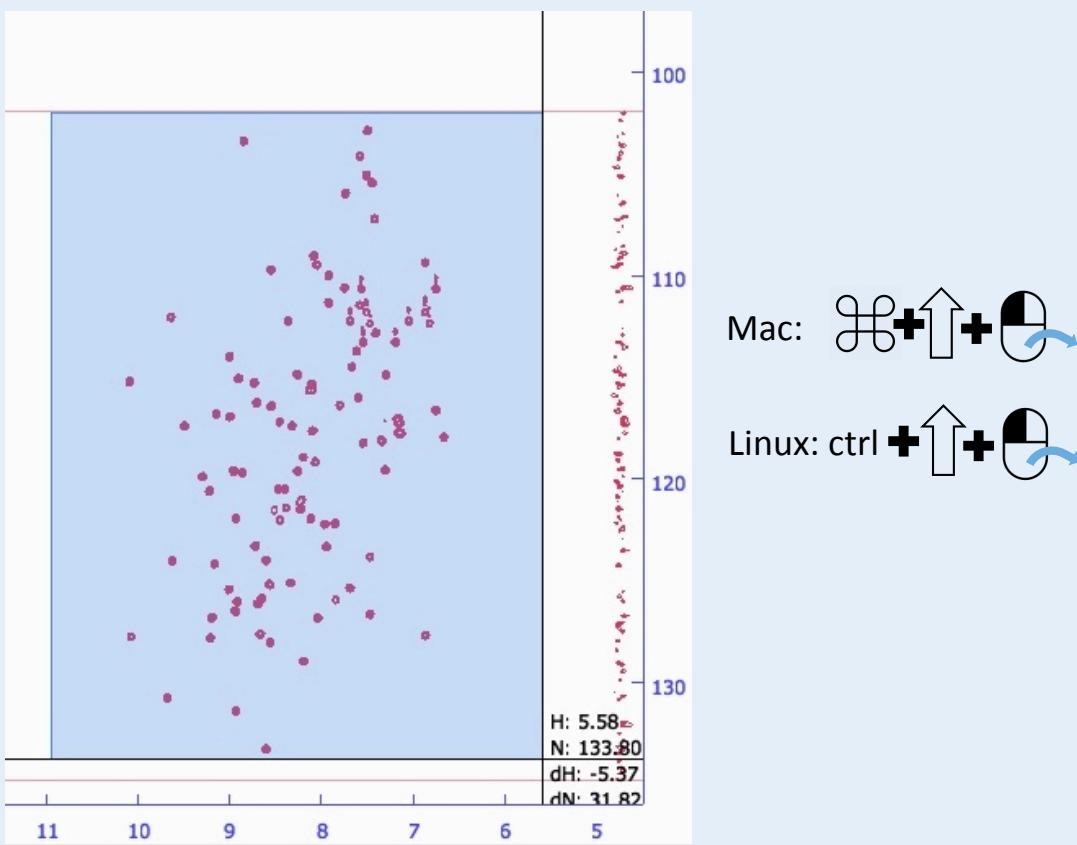
If the contours are not displayed correctly, verify that the path is set correctly in the **Spectrum Properties** popup under the **General** tab options (you can open this by **double-clicking** on the spectrum in the sidebar). Spectra are located inside the directory: AnalysisV3/data/CcpnSec5BBTutorial/sec5spectra.

1B Set Spectrum Properties: Contours.

- Contours:

- **right-click** on each spectrum display, click **Contours...**
- uncheck **Show Negative Contours** except for the hncacb

You can also perform these actions for each individual spectra on the Spectrum properties popup; open it from their sidebar items.



The “Peak Picking Drop” parameter in the “Preferences” popup (**Main Menu → Project → Preferences, Spectrum tab**) affects peak picking. This parameter defines the percentage the intensity must drop from a local maximum (for positive peaks) in each dimension in each direction, in order for the position of the local maximum to be considered to be a peak. If this percentage is too high then some actual peaks might be missed, and if it is too low then too many peaks might be picked.

2A Picking peaks in the HSQC

You can pick peaks either manually or automatically.

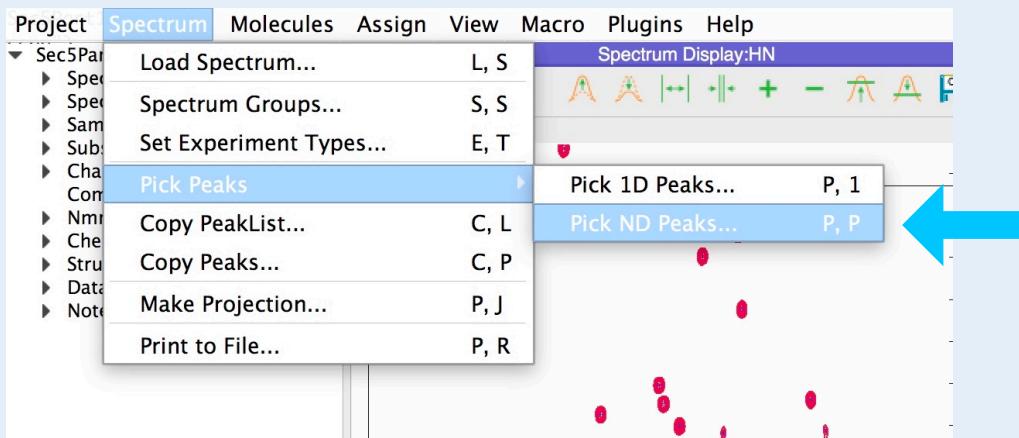
Manual Picking: Ctrl (or Cmd for Mac) + Shift + Left-drag

Click on the spectrum display, hold down **Ctrl** (or **Cmd** for Mac) + **Shift** and **left-drag** the mouse to create a blue picking box in the regions:

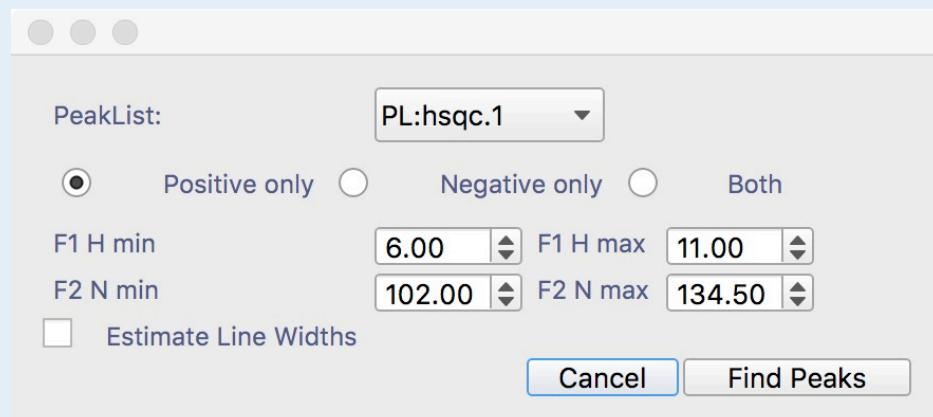
H → 11–6

N → 102–134.50

Then release the button and keys.



or Shortcut PP



2A Automatic Picking (if you haven't picked previously) :

Main Menu → Spectrum → Pick Peaks → Pick ND Peaks...

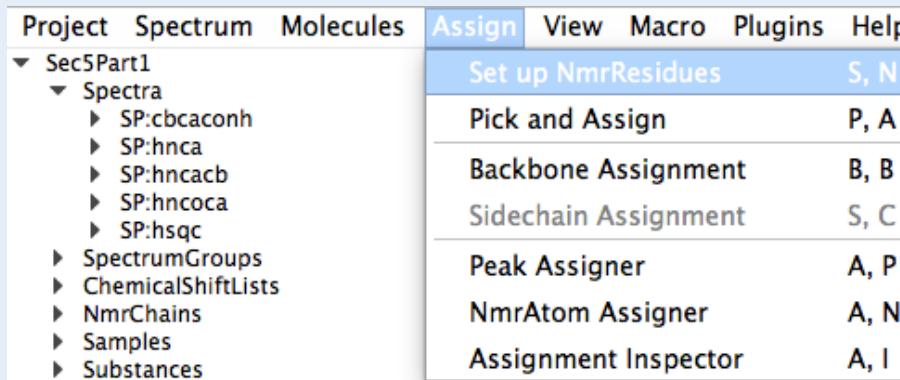
shortcut PP

- Select Positive only
- Set F1: 6-11
- set F2 102-134.5
- Click Find Peaks

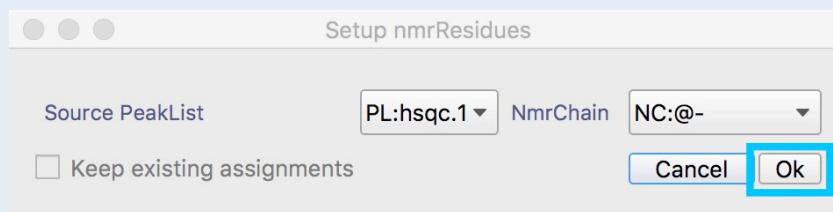
Result:

The crosses in the spectrum mark the peak positions picked. The hyphens separated by a comma indicate that the dimensions of these peaks are unassigned





or Shortcut SN



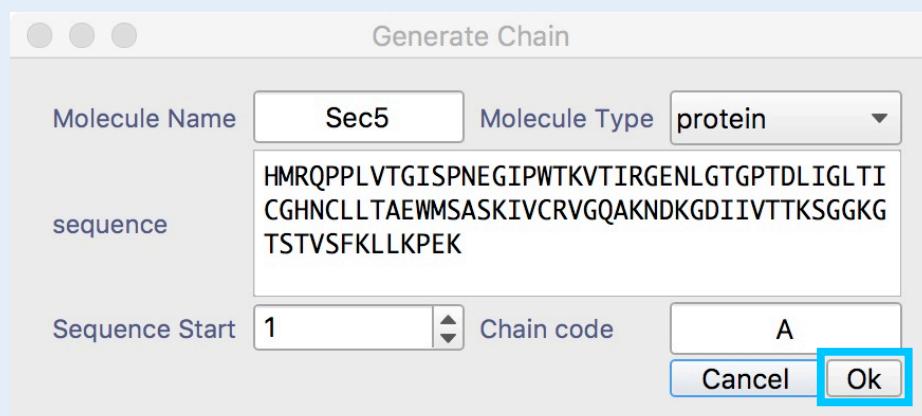
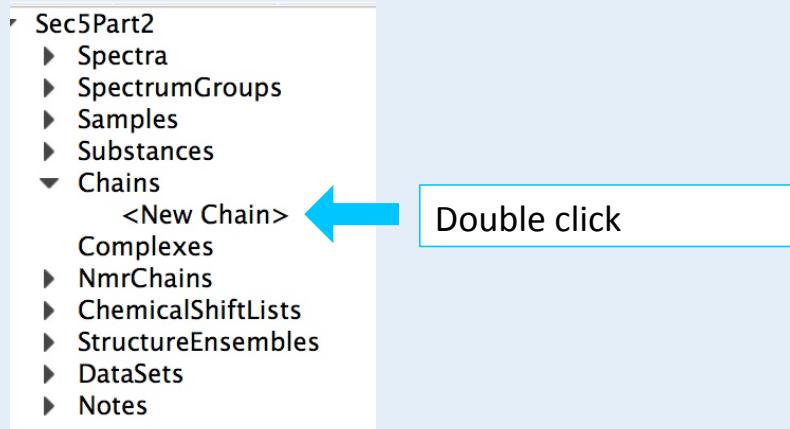
To start the backbone assignment process, we need to define 'anonymous' NmrAtoms, i.e. effectively labels, for the two dimensions of the ^{15}N - ^1H HSQC peaks, which can subsequently be linked to the 3D spectra and used as placeholders until the actual assignments are obtained. See page 9, at the end of Point 3, for a full explanation of the AnalysisAssign nomenclature.

3A Setup NmrResidues

Main Menu → Assign → Setup NmrResidues, shortcut SN

- Source PeakList: PL:hsqc.1
- NmrChain: NC:@-
- Click Ok

Each peak will have now a label such as @1H,N. Use the shortcut PL to toggle between different ways to display the peak labels.



3B Create Chain

Sidebar → Chains → <NewChain>

- Molecule Name: Sec5
- ChainCode: A
- Sequence: (copy and paste)
HMRQPPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTCGHNCLLTAEWM...KPEK
VGQAKNDKGDIIVTTKSGGKGTVSFKLLKPEK
- click Ok

You can also use Main Menu → Molecule → Generate Chain or drop a FASTA formatted file into the project.

Assignment nomenclatures (Explanation only)

Assignment in Analysis Assign 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 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 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. When you want to create the previous, 'i-1' residue to a given residue (for backbone assignment) you give it a '-1' suffix, in this case '@173-1'. When you assign the NmrResidue to a real residue, renaming it e.g., 'A.45.GLY', the i-1 residue name updates to 'A.45-1'.

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:

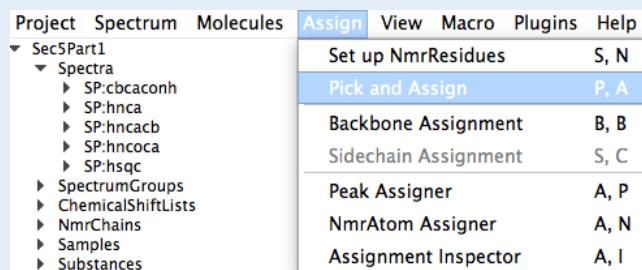
- '%' means 'any number', so 'HB%' would be a beta methylene or methyl group. 'H%' would be the backbone NH3 group.
- '*' means 'any string', so 'H*' would be 'any proton in the residue'
- Names starting with 'M' and 'Q' are (proton) pseudoatom names
- Number suffixes follow NEF (IUPAC) convention, so 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.

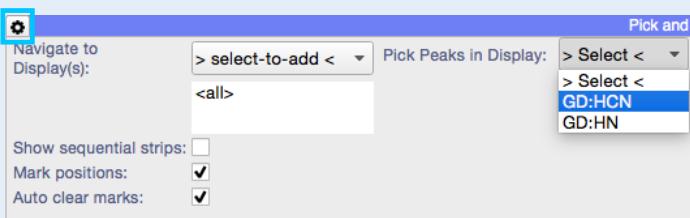
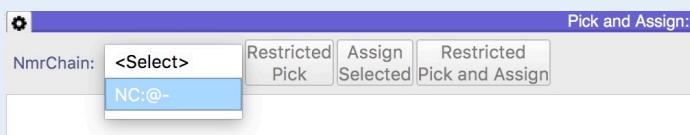
² The id together with the type identifier forms the so-called pid, the project-identifier. As an example for an un-assigned amide in the 123rd NmrResidue in the second NmrChain: NA:@2.@123..H. For an assigned NmrAtom, all the fields will have been filled, yielding something like NA:A.GLU.14.H.

For more information see our video tutorial on NmrResidues at

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



or Shortcut PA



4A Pick and Assign 3D peaks

To link the 3D spectra to the HSQC spectrum we will use the **Pick and Assign** module.

- Go to **Main Menu** → **Assign** → **Pick and Assign**, or shortcut **PA**.
- Select the **NmrChain**: NC:@-.
- Open the **Settings** (gearbox icon) and select:
 - Match **Pick Peaks in Display**: HCN
 - Leave the rest as default and close the settings
- **Double-click** on a row in the table, e.g. the row for @1.

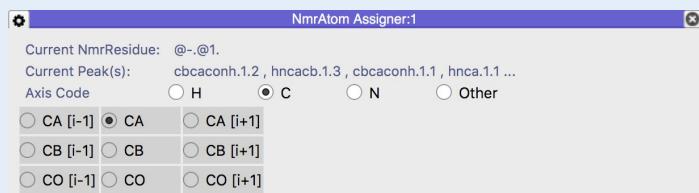
This will cause the spectrum display with the HSQC spectrum to focus on the position of the peak labelled @-@1..H, N and to mark this position with two labelled rulers corresponding to each dimension. Likewise, in the 3D module navigates to the corresponding z (i.e. N) position and marks the appropriate frequency along the proton axis with a labelled ruler.

- Click the **Restricted Pick** button.
This will pick all the peaks along a narrow region in the 3D window and these peaks will be selected.
- Inspect the 3D peaks and delete any noise peaks.
- Select the peaks to assign and click the **Assign Selected** button.

Project	Spectrum	Molecules	Assign	View	Macro	Plugins	Help
▼ Sec5Part1				Set up NmrResidues	S, N		
▼ Spectra	► SP:cbcacnh			Pick and Assign	P, A		
	► SP:hnca			Backbone Assignment	B, B		
	► SP:hncab			Sidechain Assignment	S, C		
	► SP:hncoa			Peak Assigner	A, P		
	► SP:hsqc			NmrAtom Assigner	A, N		
	► SpectrumGroups			Assignment Inspector	A, I		
	► ChemicalShiftLists						
	► NmrChains						
	► Samples						
	► Substances						



or Shortcut AN



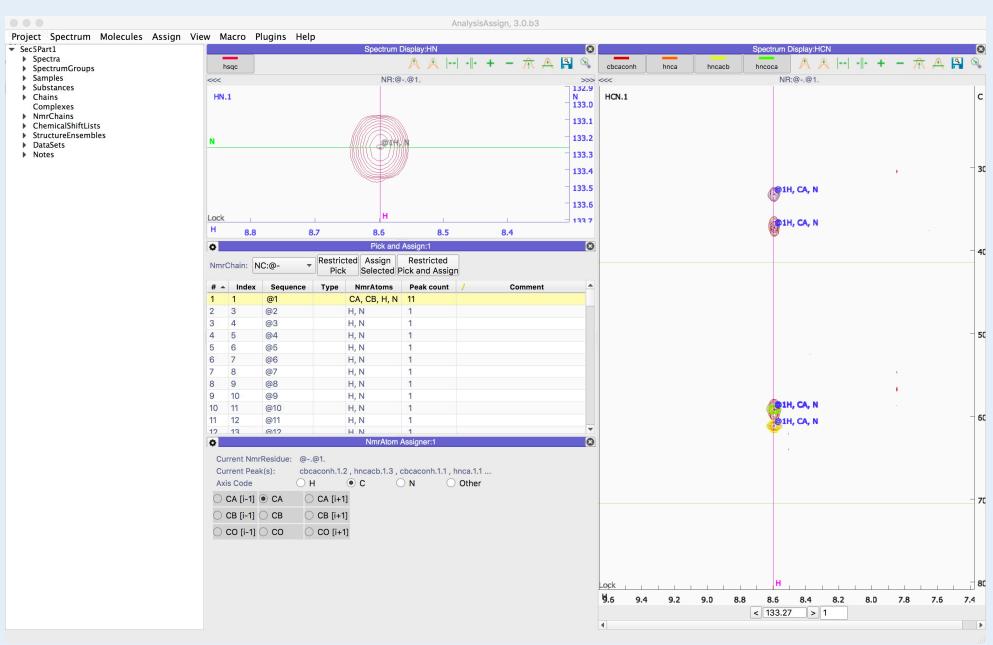
4B Complete assignment for the carbon dimension

- Go to Main Menu → View → NmrAtom Assigner or use the shortcut AN.
- Toggle any NmrAtom button to assign it to the selected peak(s).

Select a single peak in the 3D window to predict the assignment for the carbon dimension. The program uses green for likely and orange for less likely assignments.

In the event that you assign the wrong NmrAtom, you can simply select another NmrAtom or toggle it off to de-assign the peak.

Repeating this procedure for the all groups of peaks along this line yield the CAi-1, CAi, CBi-1 and CBi assignments for this NmrResidue.



4C Continue the CB*i*-1 and CB*i* assignments (Optional)

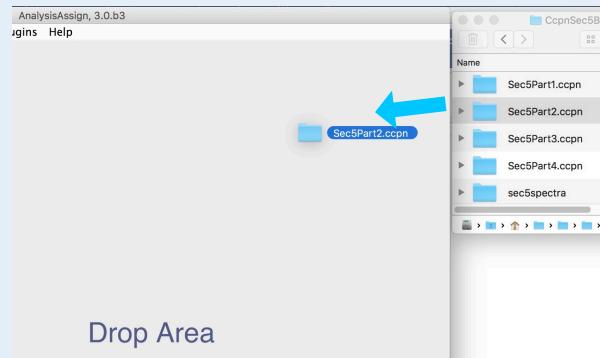
To use the sequential Backbone Assignment tools in AnalysisAssign, the *i* and *i*-1 assignments for all NmrResidues need to be provided, so the procedure described above should be carried out for all NmrResidues listed in the Pick and Assign table, where possible.

If you move on to assign NmrResidue @2, after double-clicking you will see no peaks in the “HCN” spectral display; this NmrResidue originates from a Tryptophan side-chain NH and hence does not display any peaks in the triple-resonance spectra. Move on to NmrResidue @3 to continue, and so on for a few more residue to get the hang of it.

You might like to re-arrange the modules to better suit your work-flow.

We suggest an arrangement as shown in the figure above.

Once all three dimensions of all 3D peaks have been assigned to the appropriate NmrAtoms, the backbone assignment can be carried out, but obviously for this tutorial we provide you with a CcpNmr project in which this has already been completed.



Sec5Part2.ccpn is a project in which all the carbon atom type assignments for the NmrResidues have been completed and thus can be used directly for the sequential backbone assignment. The project can be found in: AnalysisV3/data/testProjects/CcpnSec5BBTutorial

5A Open Sec5Part2.ccpn

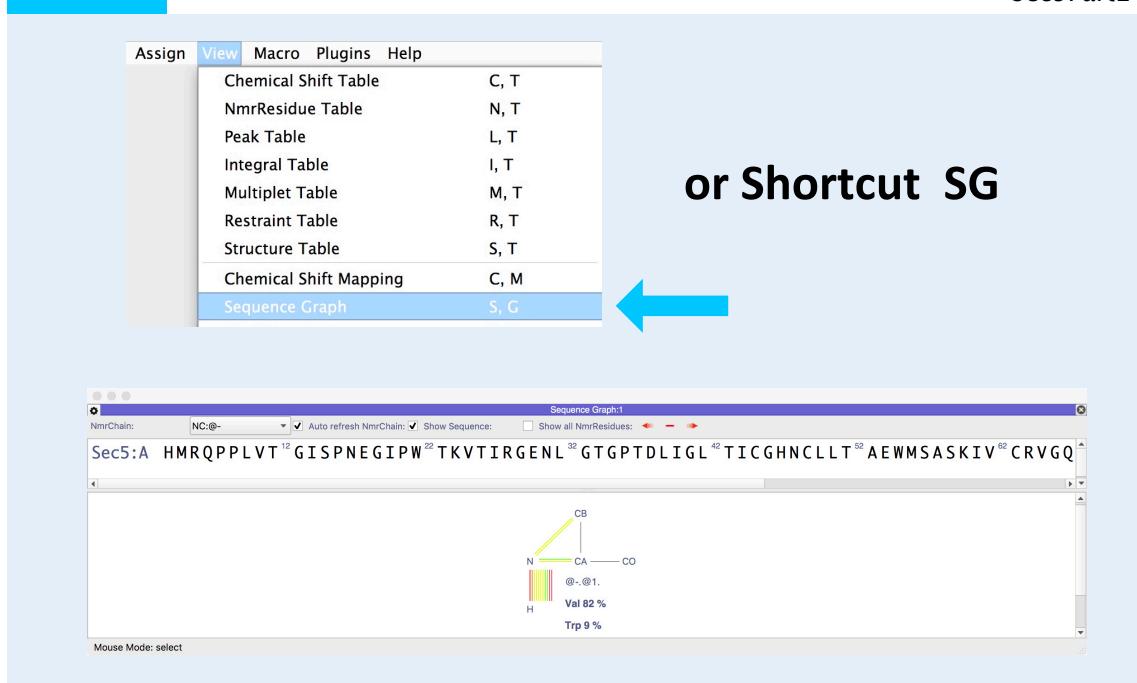
- Open the project Sec5Part2.ccpn by dragging and dropping it into the drop area or on sidebar. Quit the current Sec5Part1 project with or without saving.

The screenshot shows the AnalysisAssign software interface. The main menu bar is visible with options: Assign, View, Macro, Plugins, Help. Below the menu, there are three buttons: Set up NmrResidues (S, N), Pick and Assign (P, A), and Backbone Assignment (B, B). A blue arrow points from the 'Backbone Assignment' button to the text 'or Shortcut BB'. In the center, the 'Backbone Assignment:1' panel is open, showing a table of NmrChain assignments. On the left, there are various settings and dropdown menus, one of which is highlighted with a blue arrow pointing to the text 'Match module(s): GD:HnCANh_1'. The table data is as follows:

#	Index	Sequence	Type	NmrAtoms	Peak count
1	2	@1		CA, CB, H, N	11
2	3	@2		H, N	1
3	5	@3		CA, CB, H, N	11
4	7	@4		CA, CB, H, N	11
5	9	@5		CA, CB, H, N	10
6	11	@6		CA, CB, H, N	9
7	12	@7		H, N	1
8	14	@8		CA, CB, H, N	11
9	16	@9		CA, CB, H, N	10
10	18	@10		CA, CB, H, N	10
11	20	@11		CA, CB, H, N	8
12	22	@12		CA, CB, H, N	9

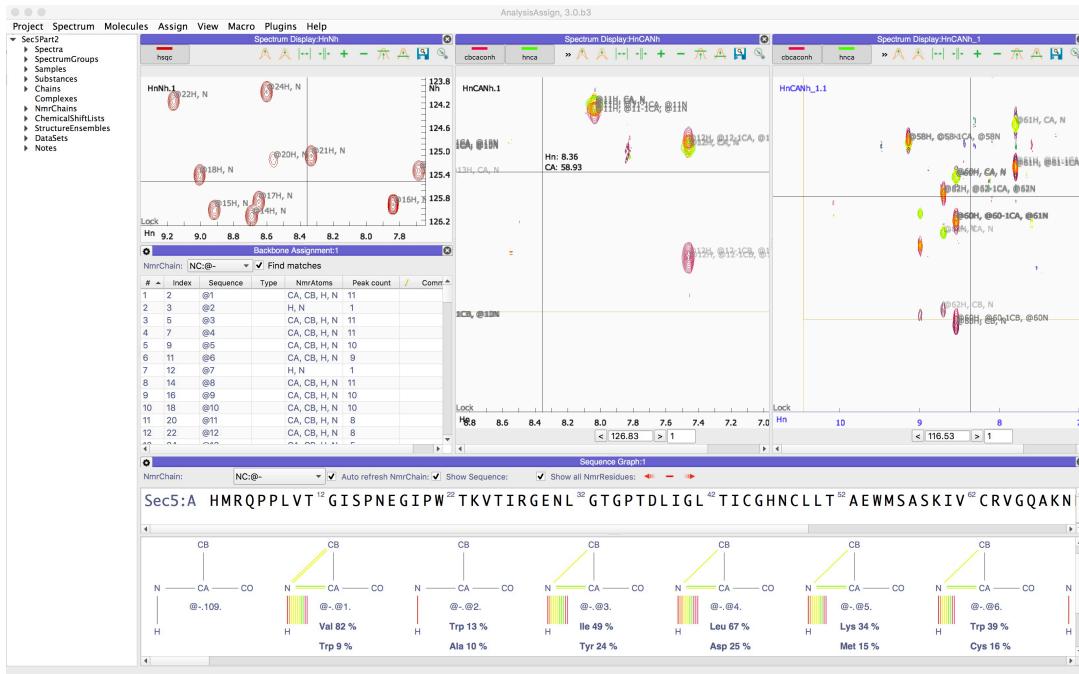
5B Open and set the backbone assignment module

- Main Menu → Assign → Backbone Assignment or shortcut BB
- Select the NmrChain: NC:@-.
- Open the settings (gearbox icon) and select:
 - Match module(s): HnCANh_1
 - Leave the rest as default and close the settings



5c Open and set the Sequence Graph module

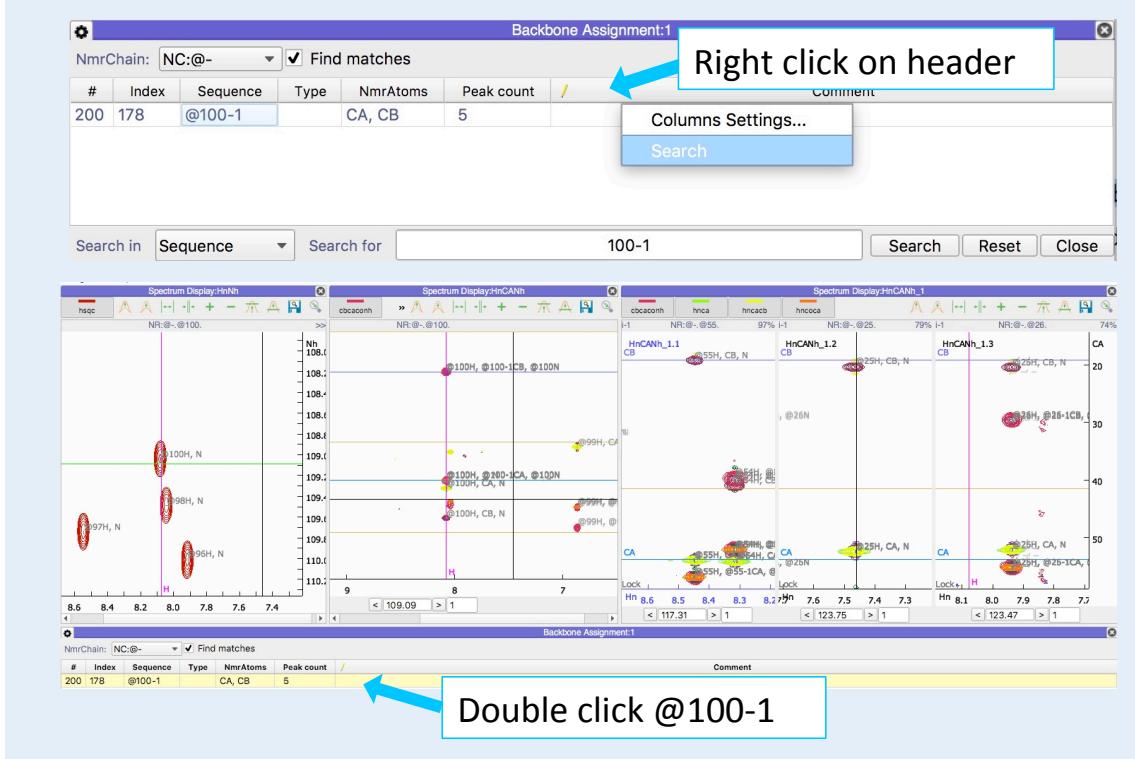
- Main Menu → View → Sequence Graph or shortcut SG
- Select the NmrChain: NC:@-
- unchecked: Show all NmrResidues
- Rearrange the modules to a layout like the Figure below.



5

Sequential backbone assignment

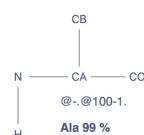
Sec5Part2

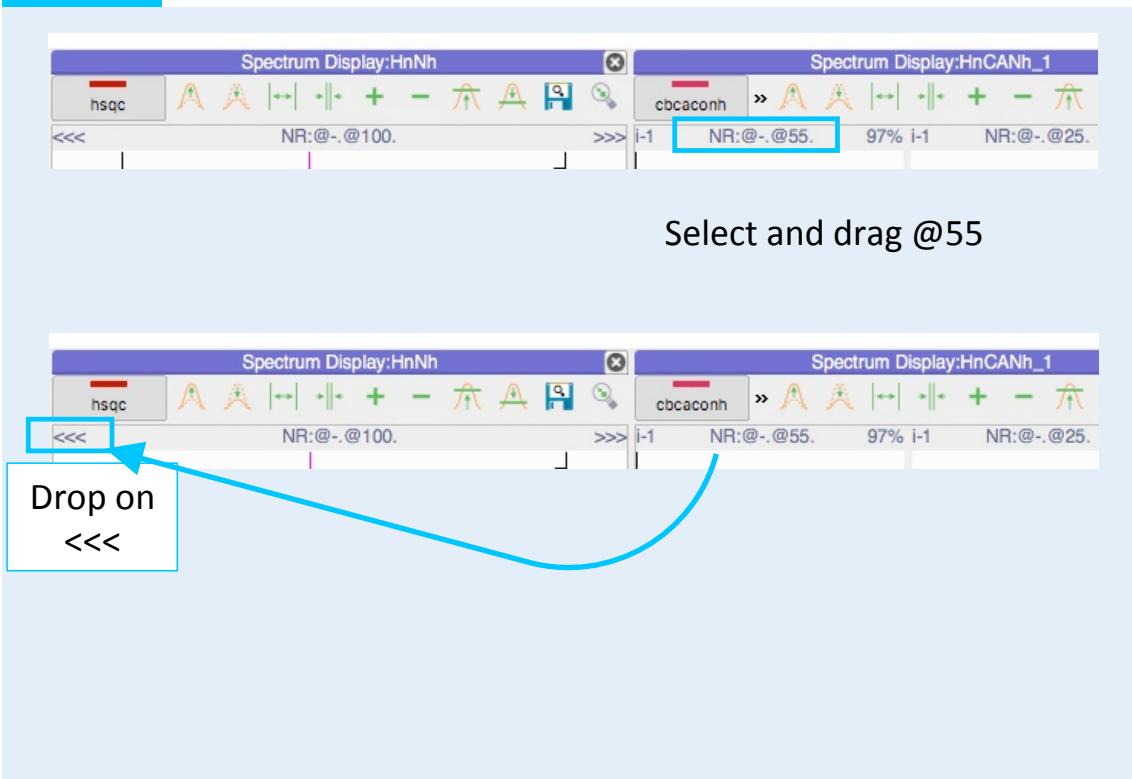


5D Assign in the i-1 direction

- Right-click on the Backbone Assignment module table header and click on Search
- search for 100-1
- Double click the row. You will see a series of changes in the GUI. The HnCANh module will navigate to the appropriate plane containing the assignment for the NmrResidue @100; the relevant frequencies of @100 are marked in the spectra.

The Sequence Graph will have a schematic residue drawn, labelled with the NmrResidue name (@100) and predictions of the possible residue type(s) below it. The match module (HnCANh_1) will display three strips in order (left to right) that the AnalysisAssign algorithm thinks best match the i-1 chemical shifts of @100. (C-terminal of the residue @100)

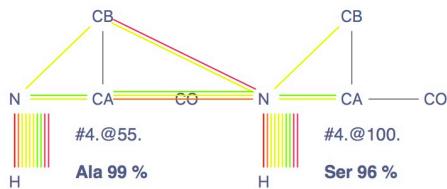




5_E Connecting NmrResidues

Upon examination, the first strip in module HnCANh_1 shows NmrResidue @55; the H, CA and CB marks align with the peaks of this residue, we can therefore see that is the best match for @100-1.

- Select the strip label NR:@-55 on the spectrum display HnCANh_1
- Drag and drop the label to the <<< icon in the Spectrum display HnCANh
- Check **Show all NmrResidues** in the **Sequence Graph** module to see the new connection



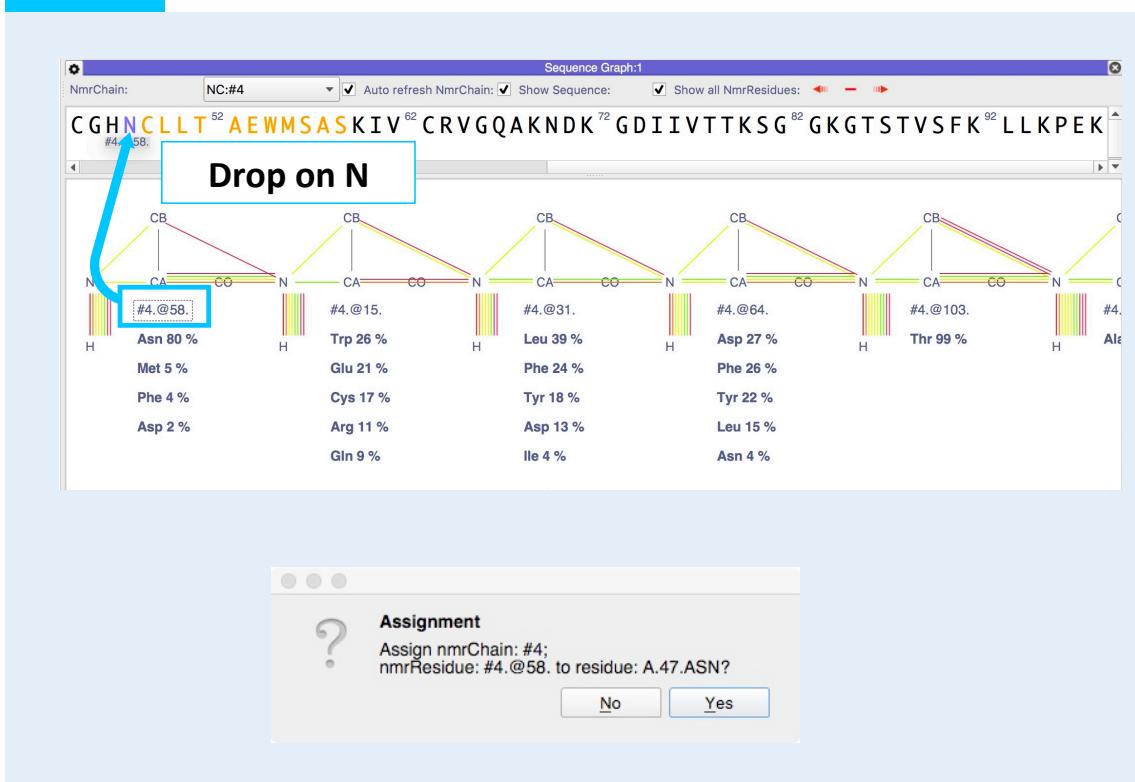
At this point @100 and @55 have been put into a so-called connected stretch (cf. section 3) and they are in a new NmrChain called #4, which means that their names have changed to #4.@100. and #4.@55. The NmrChain pulldown in the module is automatically set to #4.

When strips are dragged and dropped through this procedure, AnalysisAssign will subsequently look for i-1 matches for @55. The algorithm thinks that @72 is a good match for @55-1 and on inspection it is a match, so selecting and dragging @72 onto the <<< of HnCANh will continue the assignment.

5

Sequential backbone assignment

Sec5Part2



5F Link NmrChain to Chain

If you continue assigning in the i-1 direction, you should end up with a stretch consisting of:

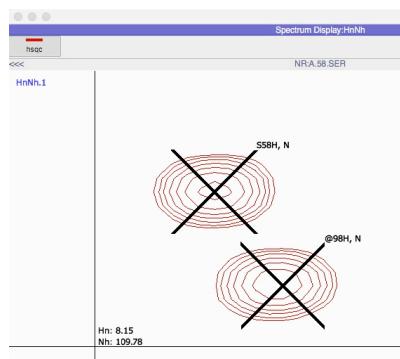
@58 @15 @31 @64 @103 @25 @23 @6 @19 @72 @55 @100

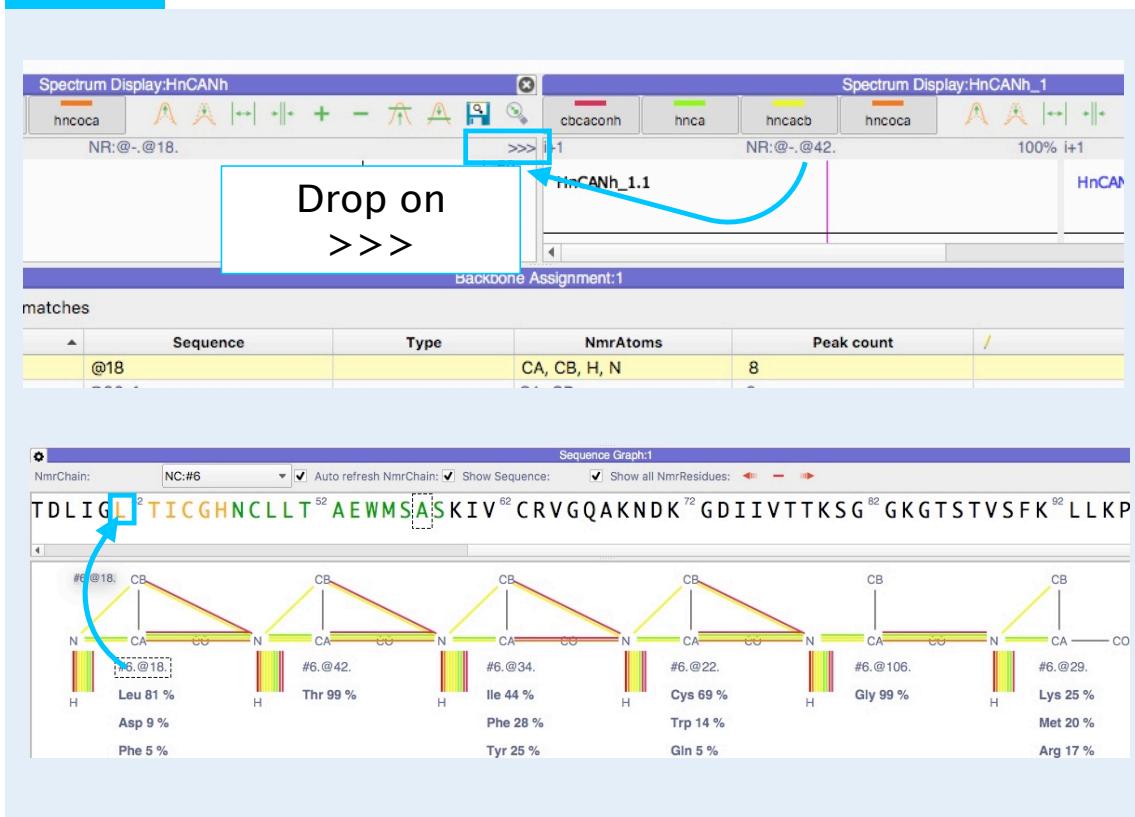
and NCLLTAEWMSAS highlighted in the sequence.

- Select the label #4.@@58 in the Sequence Graph and drag it onto the left-hand residue (N) in the highlighted sequence
- Click Yes in the confirmation popup

You may notice a new NmrChain “A” has been automatically created and is linked to the chain “A” for the above NmrResidues–Residues.

Now the relevant hsqc peaks for the selected stretch are fully assigned. You can inspect this in the hsqc spectrum display.





5G Assign in the i+1 direction

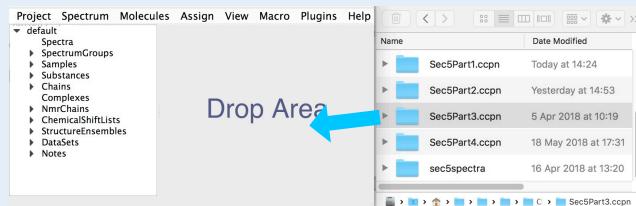
The Sequential backbone assignment in the i+1 direction is fully analogous to the 'i-1' direction except you will need to drop the strip label on the >>> icon.

- Go to the Backbone Assignment module table
- Select chain @- (to display all nmrResidues again)
- Double click on @18
- Go to the Spectrum Display:HnCaNh_1
- Select the best match @42, drag and drop it onto the HnCaNh >>> icon
- Continue in this direction to have a stretch consisting of:
@18 @42 @34 @22 @106 @29
- Select the @18 from the Sequence Graph, drag and drop onto the L of stretch LTICGH in the sequence above it
- Click Yes in the confirmation popup

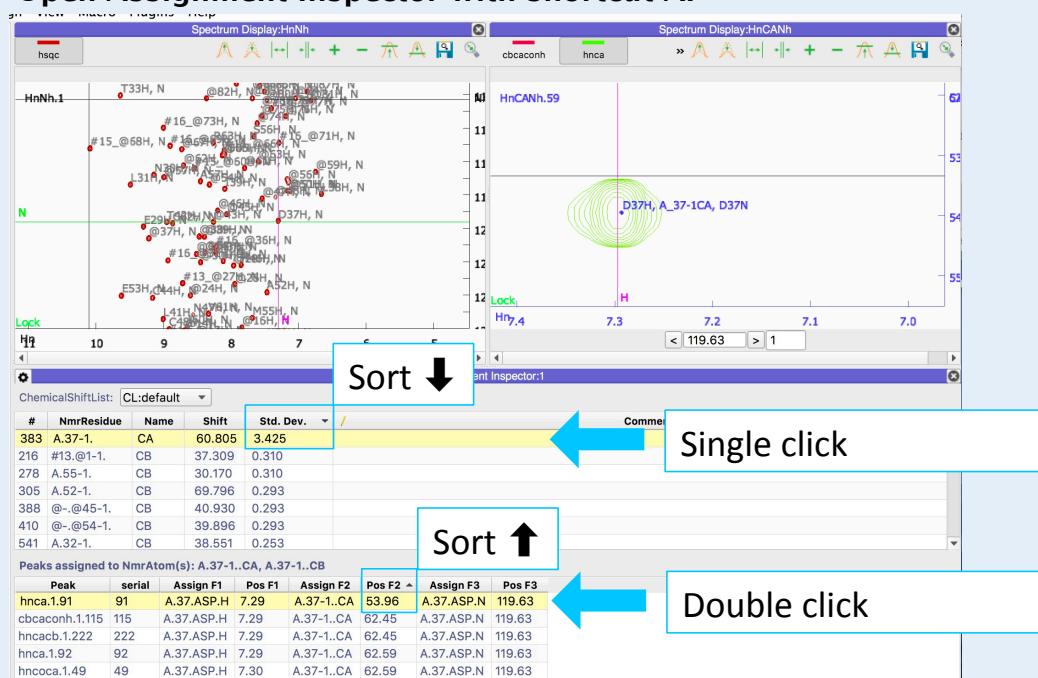
This completes the steps required for a Sequential Backbone Assignment.

The following parts of this tutorial are *How To's* and cover the usage of other Backbone Assignment tools, in particular how to inspect the assignment and edit it.

Open Sec5Part3



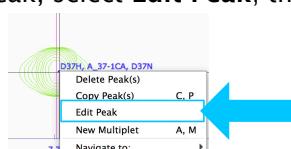
Open Assignment Inspector with shortcut AI



Sec5Part3 ccpn is a project that contains a rather obvious assignment error; the NmrResidue 37-1 has a very high standard deviation (as calculated from all peaks). We will correct this mistake using Assignment Inspector and Peak Assigner modules.

1A Correcting assignment mistakes

- Open the project Sec5Part3
- Open the Assignment Inspector module:
Main Menu → Assign → Assignment Inspector or shortcut AI
- select chemicalShiftList: CL:default
- Click on Std. Dev.** to sort the top table high-low by the standard deviation
- select nmrResidue 37-1; the peak table below will populate
- Click on Pos F2** to sort the peak table by the carbon chemical shift
- double-click** on peak hnca.1.91; the spectrum display modules will navigate to this position
- zoom in to the peak in the hnca spectrum display
- right-click** on the peak, select **Edit Peak**; the **Peak Assigner** module will appear



Peak Assigner:1

Peak: hnca.1.91

Hn: 7.293

NmrAtom	Delta
A.37.ASP.H	0.004

Alternatives

NmrAtom	Delta
#16.@71..H	0.006

Chain... Sequ... Resid... Atom ...

New Delete Deassign Assign

CA: 53.956

NmrAtom	Delta
A.37-1..CA	6.849

Alternatives

NmrAtom	Delta
A.37.ASP.CA	0.197
A.38-1..CA	0.021
A.47 ASN.CA	0.197
A.48-1..CA	0.184
A.57 ALA.CA	0.057
A.58-1..CA	0.103
A.62 ASP.CA	0.170

A 37 ASP CA

New Delete Deassign Assign

Nh: 119.632

NmrAtom	Delta
A.37.ASP.N	0.005

Alternatives

NmrAtom	Delta
@-@43..N	0.005
A.29.GLU.N	0.258
A.42.THR.N	0.001
A.62.CYS.N	0.007

Chain... Sequ... Resid... Atom ...

New Delete Deassign Assign

CA: 53.956

NmrAtom	Delta
A.37-1..CA	6.849
A.37.ASP.CA	0.098

Alternatives

NmrAtom	Delta
#16.@11-1..CA	0.085
#16.@36..C	0.181
@-@9-1..C	0.181
@-@14..C	0.181
@-@28-1..CA	0.181
@-@35..CA	0.181

A 37-1 CA

New Delete Deassign Assign

Double-click to assign

Double-click to de-assign

The **Peak Assigner** is very versatile and it has a number of options to guide its behaviour. Use the gear box icon to show/hide these. Crucial parameters are the assignment tolerances along each dimension for the different spectra. These are set to (sensible) default values but can also be modified by using the “dimensions” panel of the spectral properties popups of the respective spectra. You can also open the module from the **Main menu** → **Assign** → **Peak Assigner** (shortcut AP).

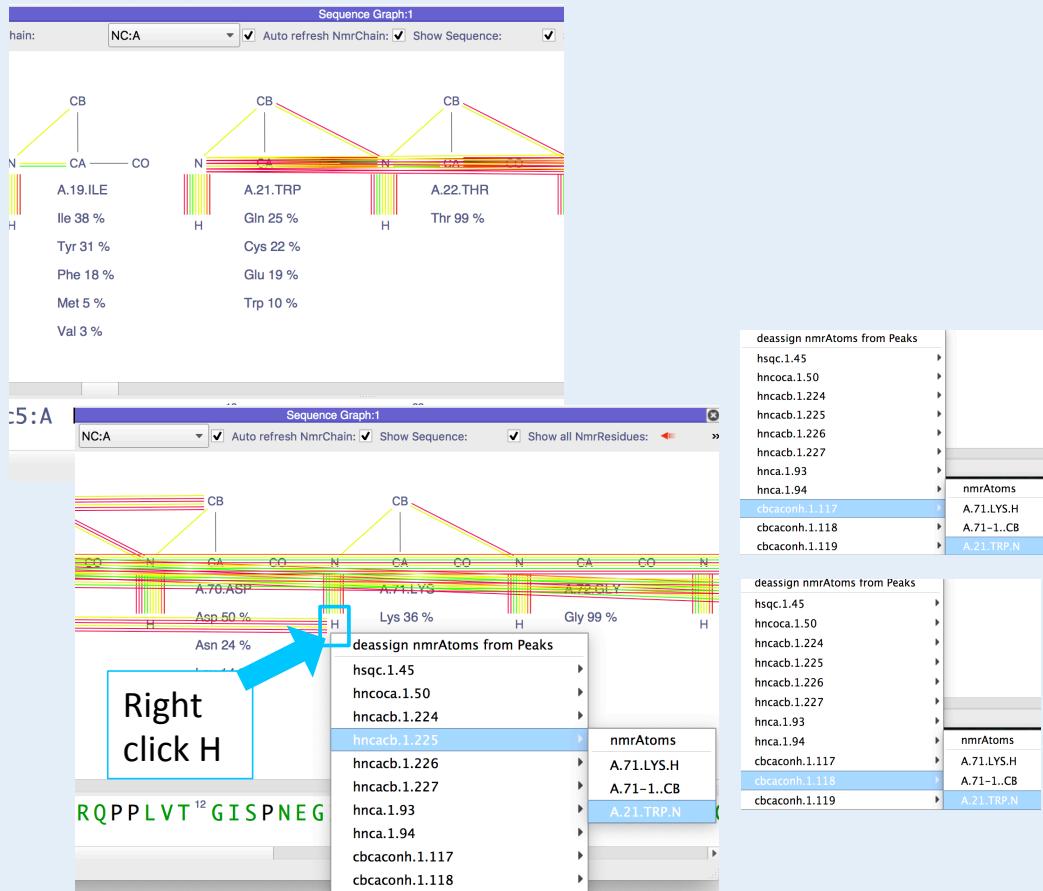
1B Edit Assignment

- **Double-click on A.37.ASP.CA** in the right-hand table located in the middle panel; this will assign the atom to the selected peak
- **Double-click on A.37-1..CA** in the left-hand table; this will delete the atom from the selected peak

Inspect the Assignment

Sec5Part4

Open Sec5Part4



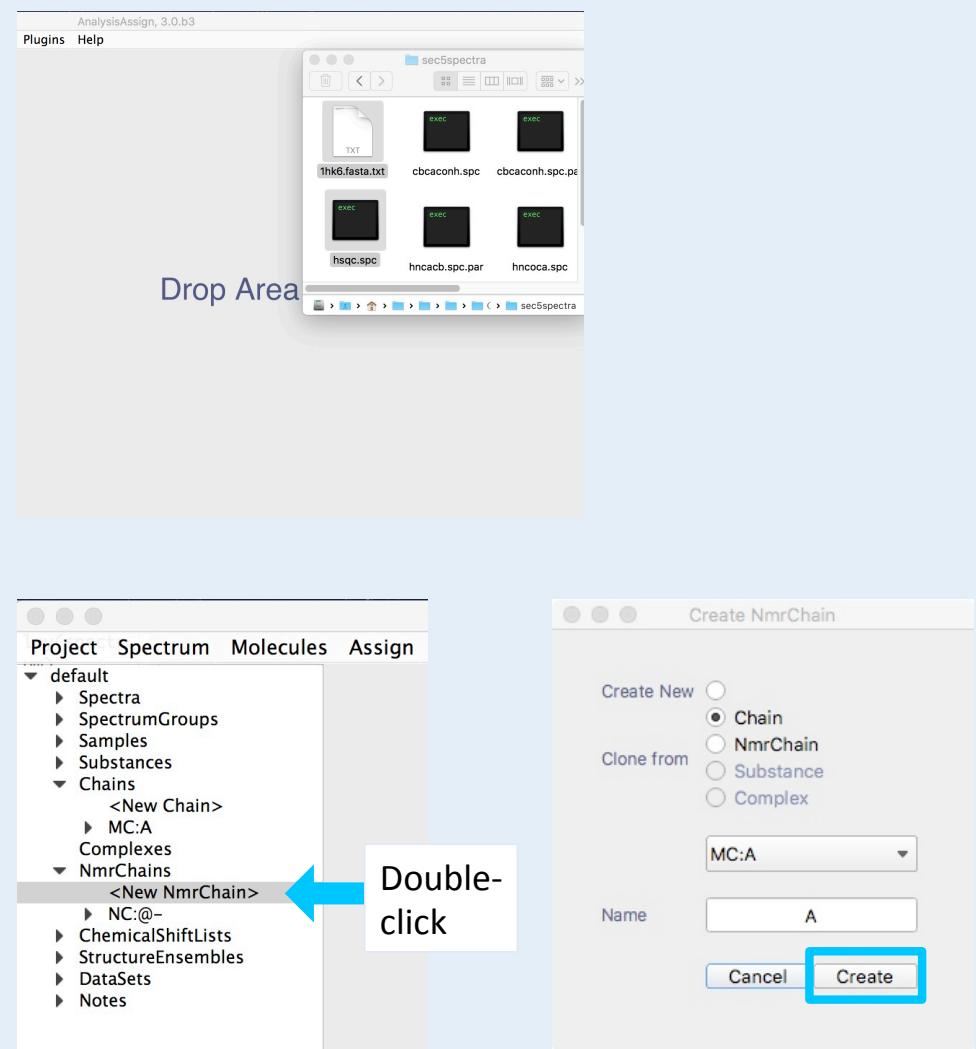
Sec5Part4.ccpn is a project that contains an assignment error; to identify assignment errors you can use the sequence graph. In this project, scrolling through the sequence graph of NmrChain A, you will notice multiple lines connecting the N atom of A.21.TRP to atoms much further down the sequence; in fact they go to the H of A.71.LYS and the CB of A.70.ASP. These are clearly mistakes in the assignment and should be corrected.

1C Correcting an assignment mistake

- Open the project **Sec5Part4**
- Open the **Sequence Graph** with shortcut **SG**
- Select NmrChain NC:A
- Scroll to the right and search for A.21.TRP
- Follow the connections by scrolling further to the right to A.70.ASP
- Select the **H**, right-click on it
- search and click all **A.21.TRP** peaks from the submenus so to remove the unwanted connections

The wrong connections have been removed, which means the peaks have been de-assigned from that NmrAtom. You can further edit the NmrAtom using the other tools in Assign like the Peak Assigner module (**AP**).

Quick Assignment

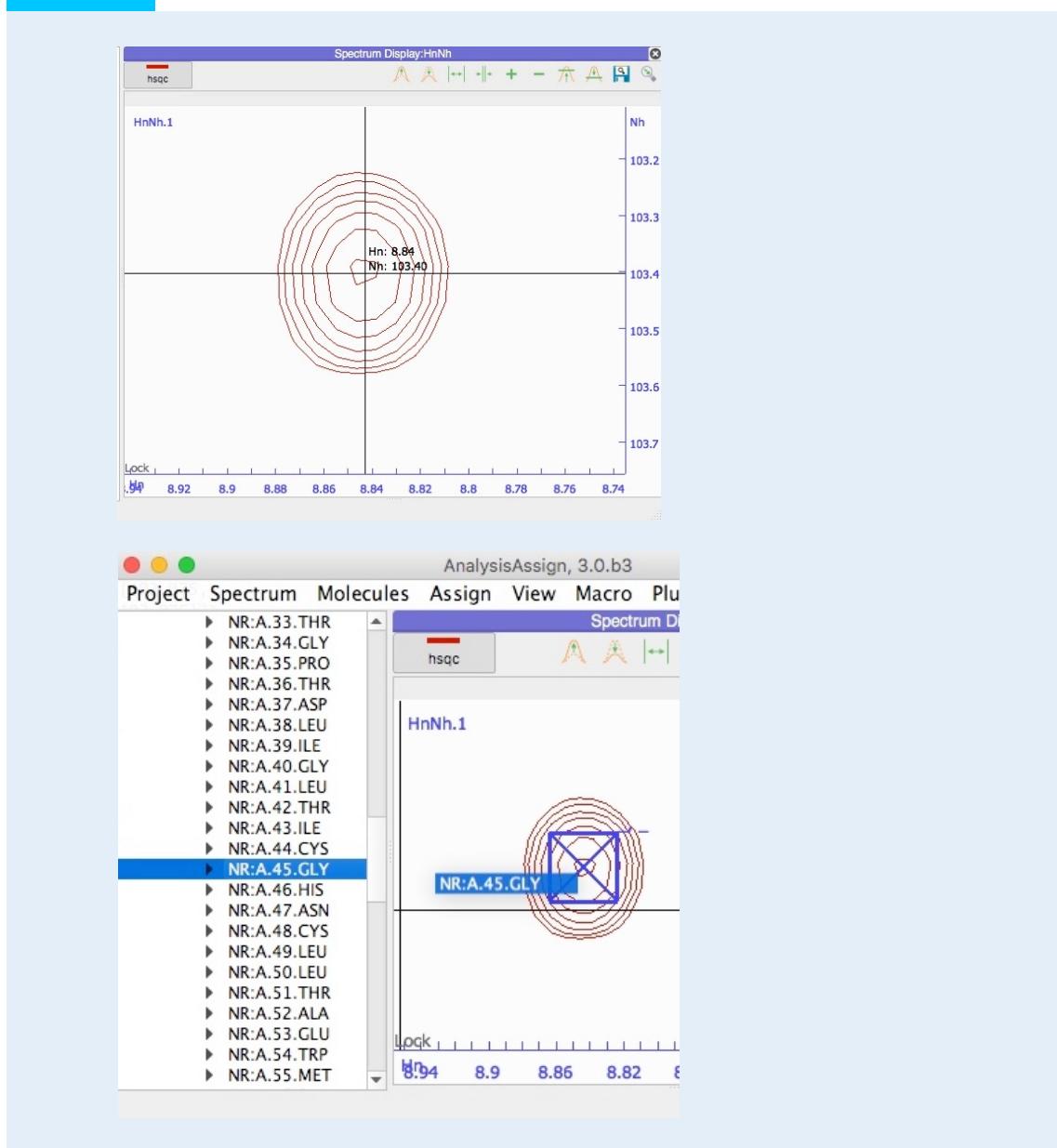


Assuming you have pre-knowledge of a peak assignments, this HOW-TO will show you how to assign residues and atoms to any dimensionality peaks in few simple steps.

2A Create a Chain and an NmrChain

- Open a new project
- **Drag and drop** the hsqc spectrum from the Sec5Spectra folder into the Drop Area
- **Drag and drop** the Fasta file *1hk6.fasta.txt* into the Drop Area; this will automatically create a new Chain
- **Double-click** on <New NmrChain>
- Select **Clone from:** Chain
- Select **Chain A** from the pulldown
- Name: A
- Click **Create**
- Open the hsqc from the sidebar

Quick Assignment

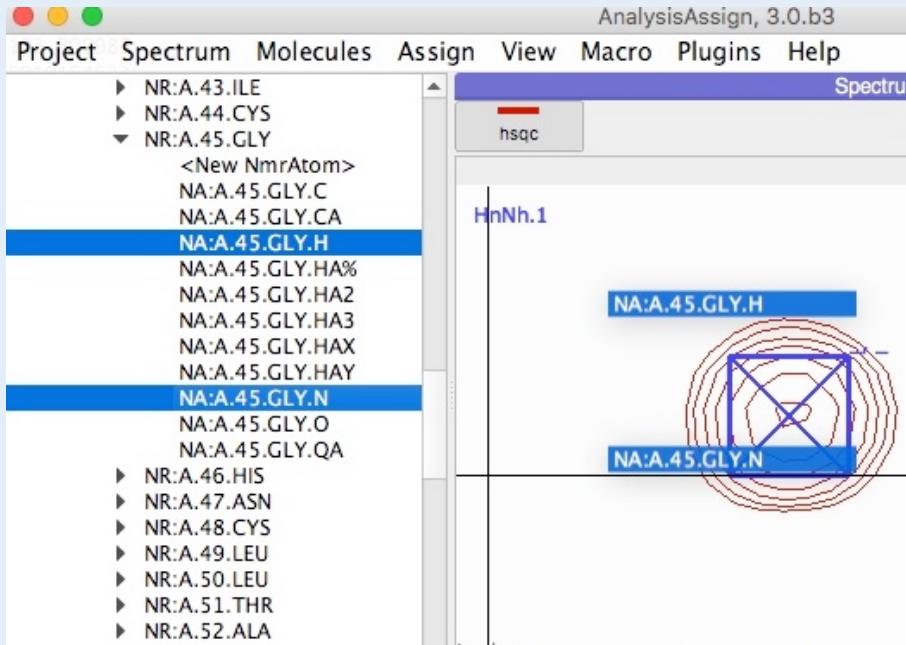


2B Drop the NmrResidue onto a selected peak

- Zoom at positions: 8.84 / 103.40
- Pick the peak with **Ctrl (Cmd on Mac) + Shift (↑) + right-click**
- In the sidebar expand the NmrChain A
- Search for NmrResidue 45
- Drag and drop this NmrResidue onto the selected peak

The peak is now fully assigned to the NmrAtoms H and N of NmrResidue 45.

Quick Assignment



You can assign any NmrAtoms to any dimensionality peaks if the NmrAtoms and spectrum axis Codes match (at least partially). E.g., you may assign 45.GLY.HA2 to any peaks in the HnNh spectrum but you cannot assign the 45.GLY.O to any of them.

2c Quick assignment edit

- In the sidebar expand the NmrChain A
- Simulate a mistake by dropping a random NmrResidue on the selected peak
- Expand NmrResidue 45
- Drag and drop the NmrAtoms H and N onto the selected peak. (You can multiple selection with **Shift + right-click**).

The peak is now fully assigned to the new NmrAtoms.

Contact Us

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Issues and bug report:

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

Cite Us

Skinner, S. P. et al. CcpNmr AnalysisAssign: a flexible platform for integrated NMR analysis. *J. Biomol. NMR* 66, (2016)

Tutorial Version History:

beta1 (SS): First version

beta2 (GWV): Minor changes

beta3 (LGM): Re-designed, re-written, added several steps

beta5 (VAH): Minor changes