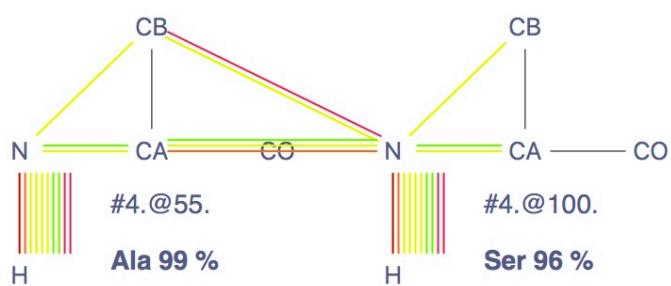
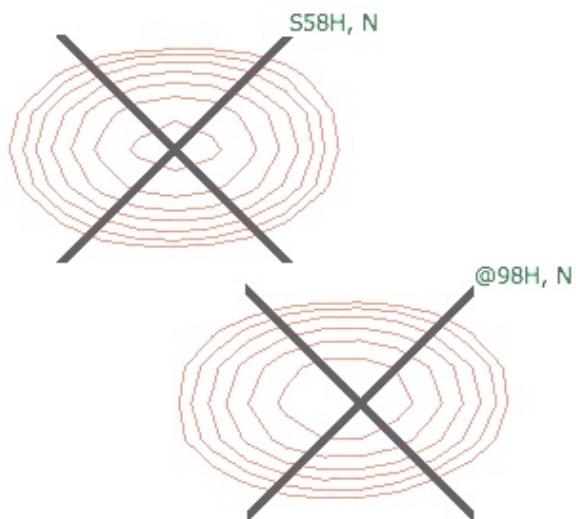


Backbone Assignment Tutorial



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, cbcacnh, hncacb, hncoca.

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 examples of *How-To* do common assignment operations using different AnalysisAssign tools.

Start CcpNmr Analysis V3

Apple users by double clicking the icon
CcpNmrAnalysis



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 sidebar. Double click 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 Drag and drop into the drop area from sidebar or right click on sidebar item and select “open as module”

Mouse

- Pan → Left Click and 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 hold Middle and drag

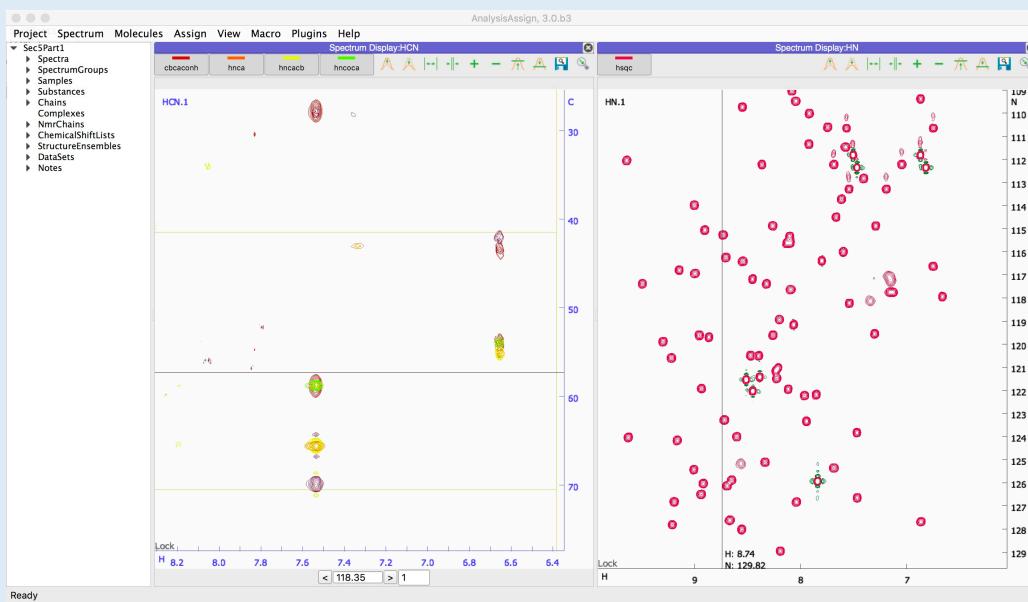
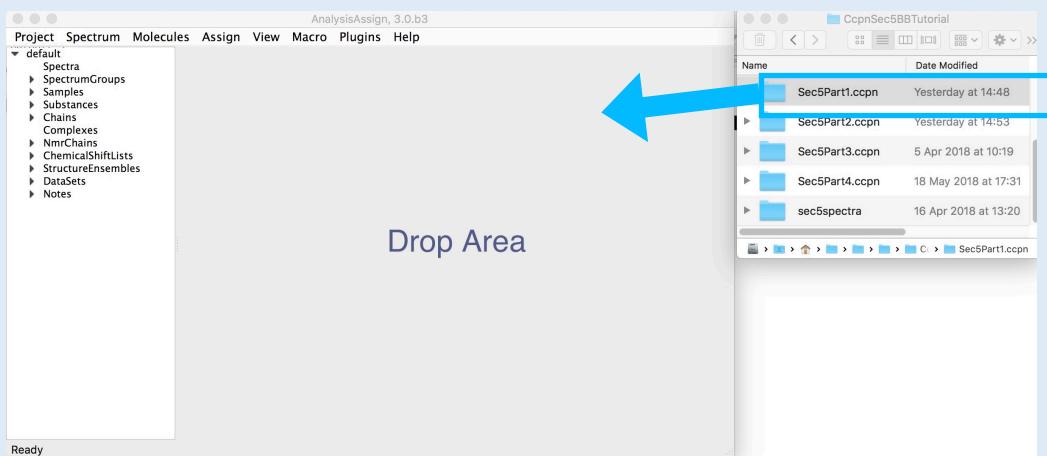
Shortcuts

The program uses several shortcuts, 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).

For more commands and operations:

main menu -> *help* -> *Tutorial (Beginners)* or *Show Shortcuts*

Open project AnalysisV3 /data/CcpnSec5BBTutorial/Sec5Part1.ccpn



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

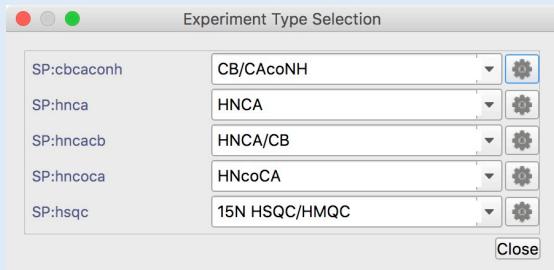
CcpNmr projects have an extension of type “filename.ccpn”. Find the project file *Sec5Part1.ccpn* in the directory *AnalysisV3/data/CcpnSec5BBTutorial*.

- Select the file “**Sec5Part1.ccpn**”, drag and drop into the program. The Sec5Part1 project will be loaded in a new window.

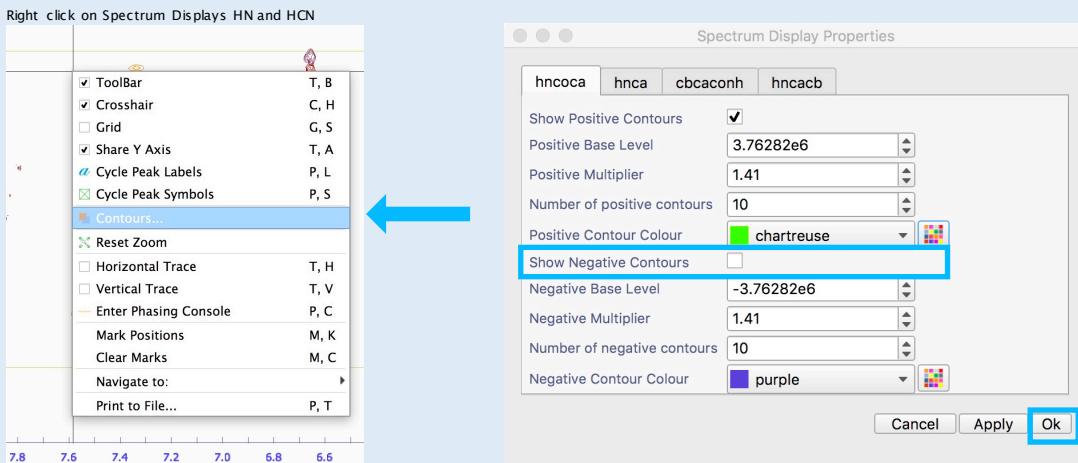
You will see five spectra, displayed in two Spectrum Displays as:

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

Set experiment types



Set contours



If the contours are not displayed correctly, verify the path is set correctly on the Spectrum properties popup under the General tab options; open it from the sidebar items. Spectra are located inside the directory: AnalysisV3 /data/CcpnSec5BBTutorial/sec5spectra.

1B Set Spectrum Properties: Experiment types and contours.

- Set experiment types:

Main Menu → Spectrum → Set Experiment Types, shortcut 'ET'.

You might copy and paste the following:

CB/CAcoNH

HNCA

HNCA/CB

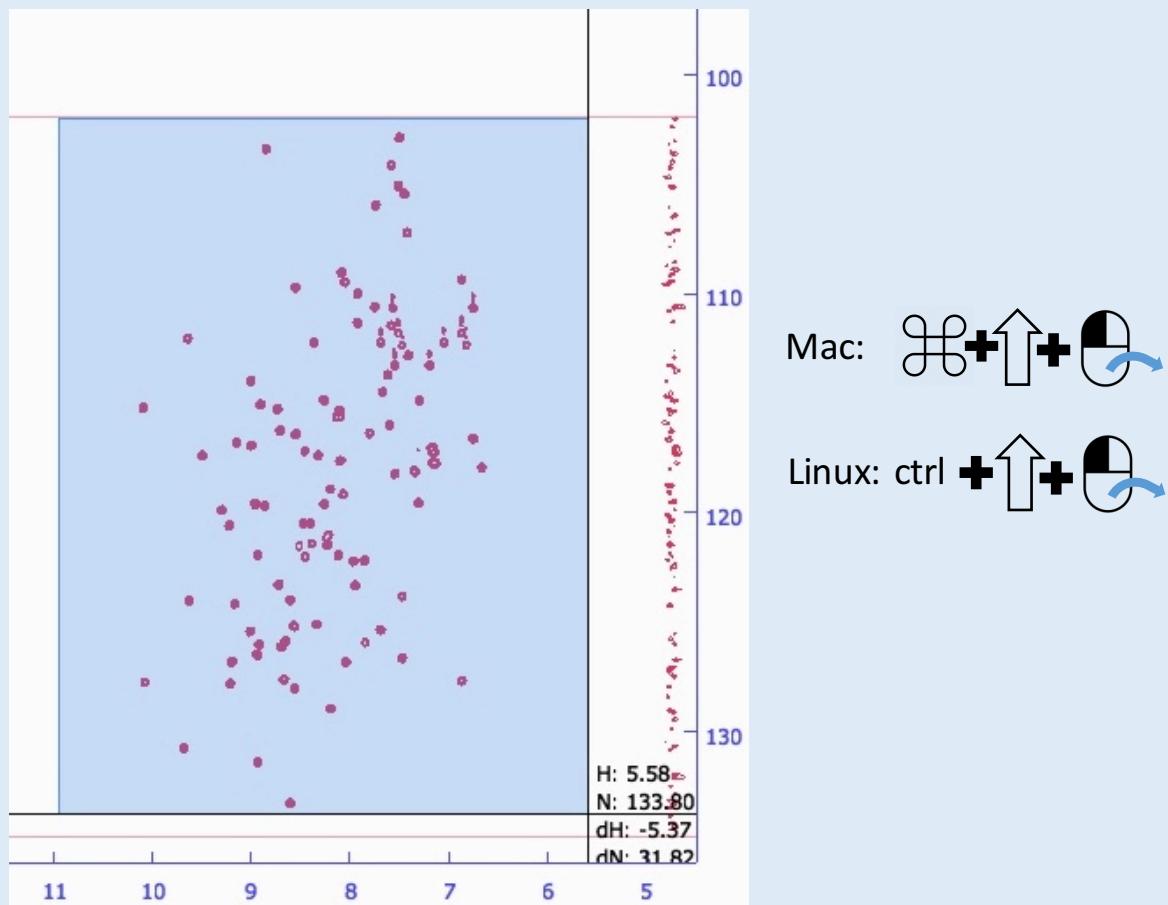
HNcoCA

15N HSQC/HMQC

- 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 (Menu: Project→Preferences, tab Spectrum) 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 on the SP:hsqc

You can pick peaks either manually or automatically.

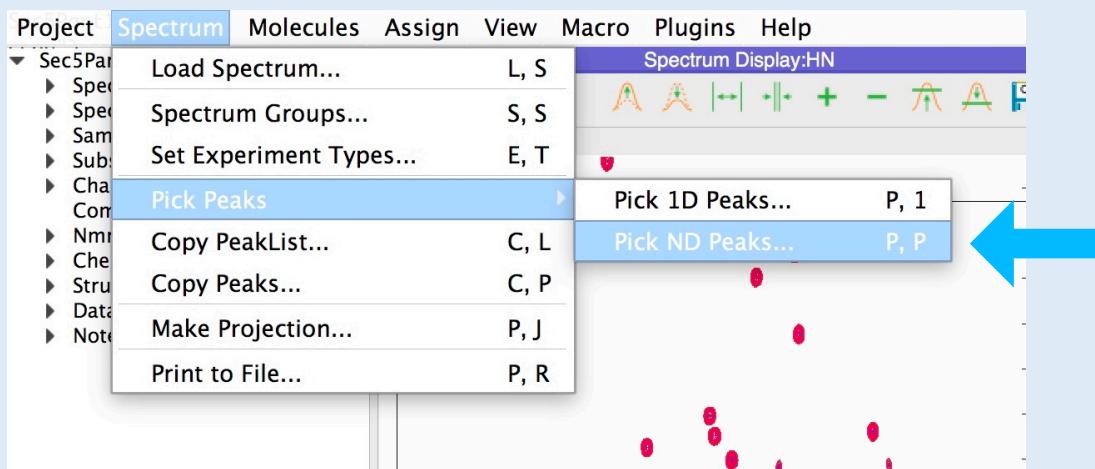
Manual Picking: CTRL or CMD (for Mac) + SHIFT + Left drag

Click on the spectrum display, hold down CTRL or CMD (for Mac) plus SHIFT and Left drag the mouse to create a blue picking box in the regions:

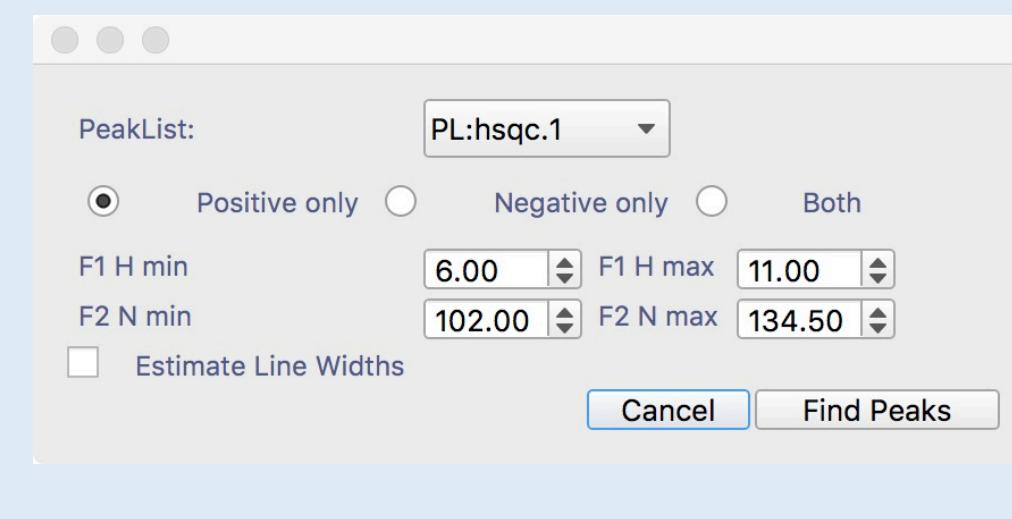
H → 11–6

N → 102–134.50

then release the button and keys



or Shortcut 'PP'



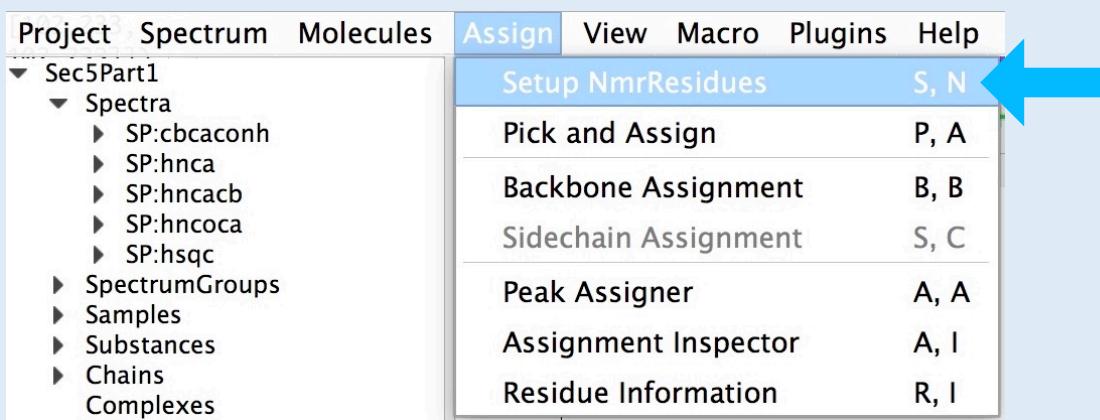
2A Automatic Picking (if you haven't picked previously) :

Main Menu → Spectrum → Pick Peaks → Pick ND Peaks... , shortcut 'PP'

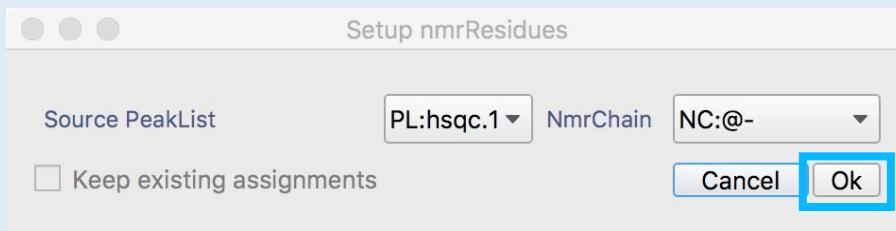
- Select Positive only
- Set F1: 6-11
- set F2 102-134.5
- Click Find Peaks

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 -HSQC peaks, which can subsequently be linked to the 3D spectra and used as placeholders until the actual assignments are obtained. See 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 like @1Hn,Nh. Use the shortcut ‘PL’ to switch how to display the peak labels.

The screenshot shows the Chimera X software interface. On the left, a sidebar menu is open under 'Sec5Part2'. The 'Chains' section is expanded, showing options like '<New Chain>', 'Complexes', 'NmrChains', etc. A blue arrow points from a 'Double click' callout box to the '<New Chain>' option. Below the sidebar is a 'Generate Chain' dialog box. It contains fields for 'Molecule Name' (set to 'Sec5'), 'Molecule Type' (set to 'protein'), and a sequence text area containing the amino acid sequence 'HMRQPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTICGHNCLLTAEWMASASKIVCRVGQAKNDKGDIIVTTKSGGKG TSTVSFKLLKPEK'. There are also 'Sequence Start' (set to '1') and 'Chain code' (set to 'A') fields, along with 'Cancel' and 'Ok' buttons. The 'Ok' button is highlighted with a blue border.

3B Create Chain

Sidebar → Chains → <NewChain>

- Molecule Name: Sec5
- ChainCode: A
- Sequence: (copy and paste)
HMRQPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTICGHNCLLTAEWMASASKIVCRVGQAKNDKGDIIVTTKSGGKG TSTVSFKLLKPEK
- press Ok

You can also use Main Menu: Molecule->Generate Chain or drop a Fasta formatted file onto the Sidebar

Setting up Assignment

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 to also consider the relationships between Peak, ChemicalShift and NmrAtom. Each dimension of a Peak is assigned to one or more NmrAtom. The ChemicalShift (which resides in a ChemicalShiftList) of an NmrAtom, is defined by all the peaks that have assigned to this NmrAtom assignment. 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 the Peak. Likewise, it also affects the ChemicalShift of the "nmratom_2", as it is now becomes 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..

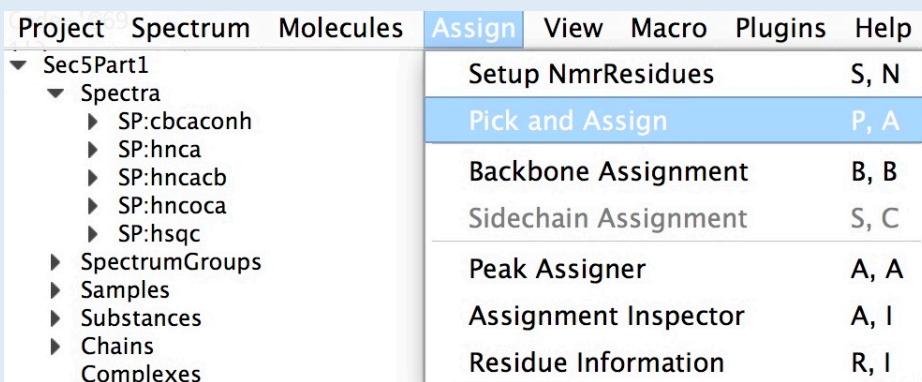
NmrAtom names always start with the nucleus, and default names would be e.g. 'H@31' or 'C@88'. Some names have a special meaning:

- '%' 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).

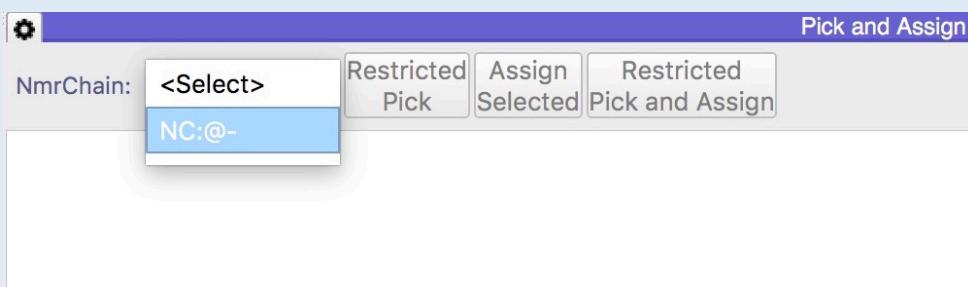
¹ Atoms reside in Residues, which reside in Chains; multiple chains can form a Complex.

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.



or Shortcut ‘ PA ’



The “Pick and Assign” module has a settings panel (click to toggle) that gives access to the parameters that determine in which spectra and with what tolerances the program tries to pick the peaks

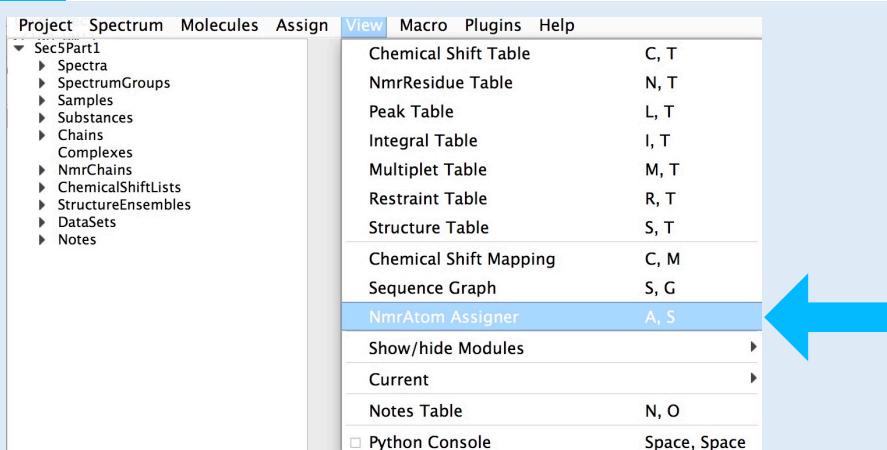
4A Pick and Assign Nd peaks

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

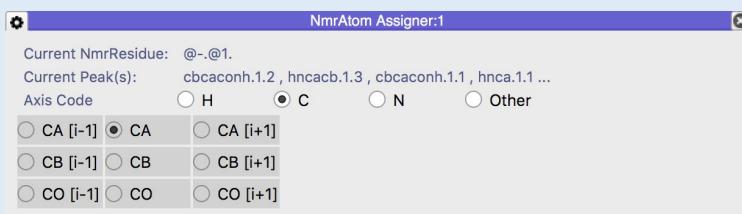
- Go to the main menu: Assign→Pick and Assign module or use the shortcut “PA”.
- Select the NmrChain: NC:@-.
- Double click on a row in the table, e.g. the row for @1.

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 noisy ones.
- Select the peaks to assign and Click the “Assign Selected ” button.



or Shortcut ‘AS’

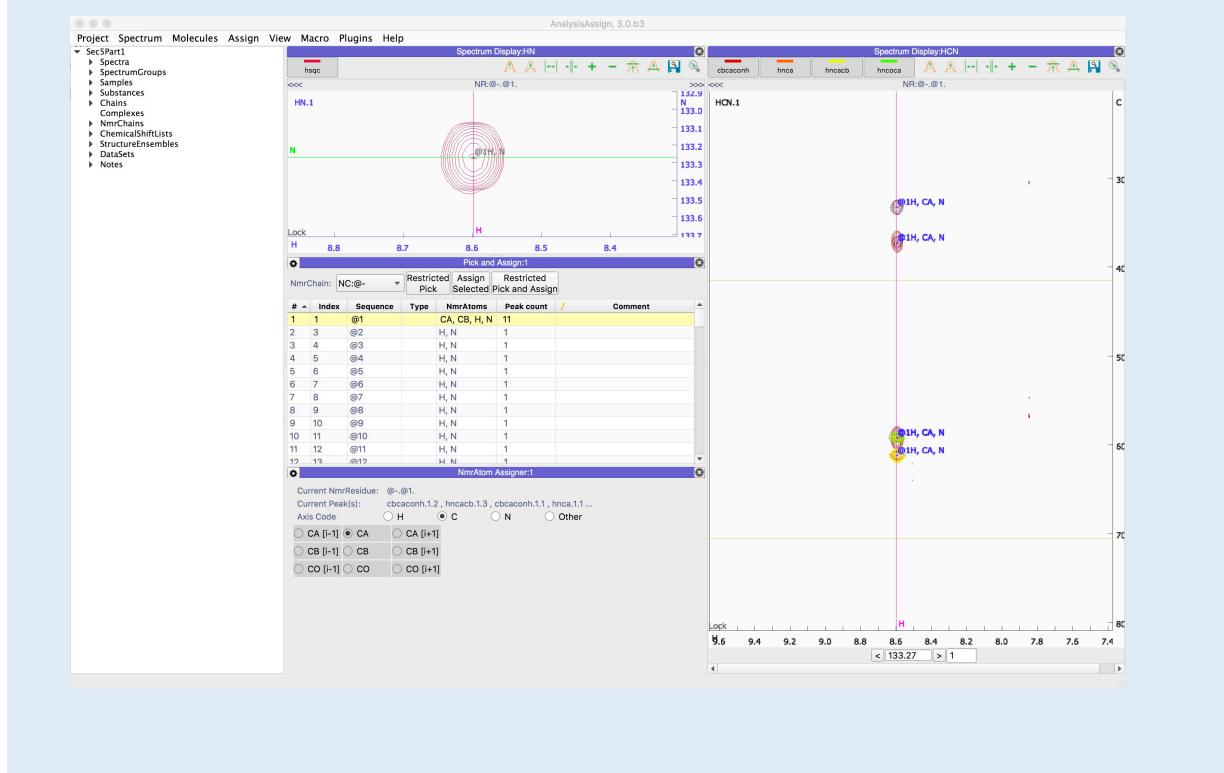


4B Complete assignment for the carbon dimension

- Go to the main menu: View → NmrAtom Assigner module or use the shortcut “AS”.
- 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 you assign the wrong nmrAtom, you can simply toggle another nmrAtom or deselect it to de-assign the peak.

Repeating this procedure for the other groups of peaks along this line will also yield the CBi-1 and CBi assignments for this NmrResidue.



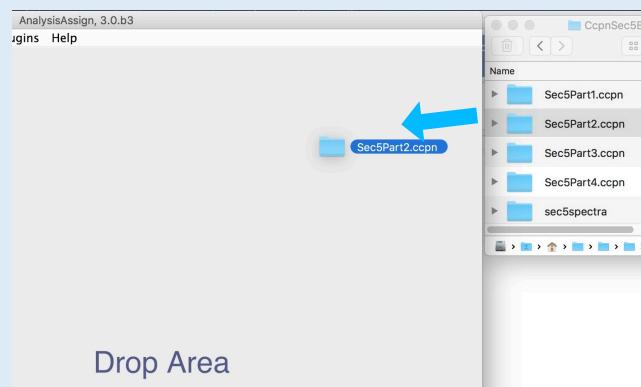
4C Continue the CBi-1 and CBi assignments (Optional)

To use the sequential Backbone Assignment tools in AnalysisAssign, the i and i-1 assignments for all NmrResidues needs to be provided, so the earlier described procedure 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 the triple-resonance peaks. Move on to NmrResidue @3 to continue, and so on for a few more residue to get the hang of it.

You might re-arrange the modules to better suit your work-flow. We suggest an arrangement as shown in figure the above.

Once all the 3D peak dimensions 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 was already completed.

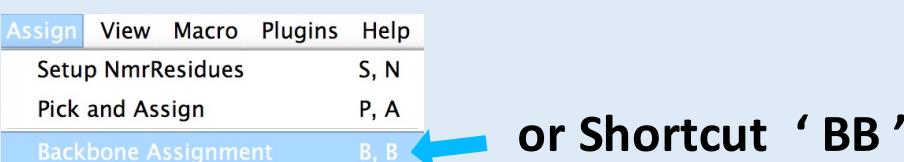


Sec5Part2.ccpn is a project wherein all the carbon atom type assignments for the NmrResidues have been completed and thus can be used directly for the sequential backbone assignment. The project can be found in:

AnalysisV3/data/testProjects/CcpnSec5BBTutorial

5A Open Sec5Part2.ccpn

- Open the project Sec5Part2.ccpn, drag and drop on the drop area or sidebar. Quit with or without saving the current Sec5Part1.

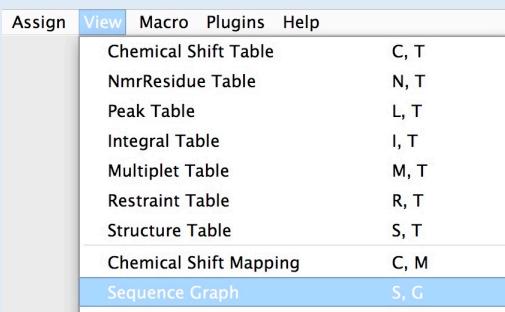


The screenshot shows the 'Backbone Assignment' module. On the left, there are settings for 'Display(s)', 'Show sequential strips', 'Mark positions', 'Auto clear marks', 'Matches to show' (set to 3), 'Match module(s)' (with 'GD:HnCANh_1' selected), and 'ChemicalShiftList'. On the right, there is a table titled 'Backbone Assignment:1' showing a list of NmrChains. The first few rows of the table are:

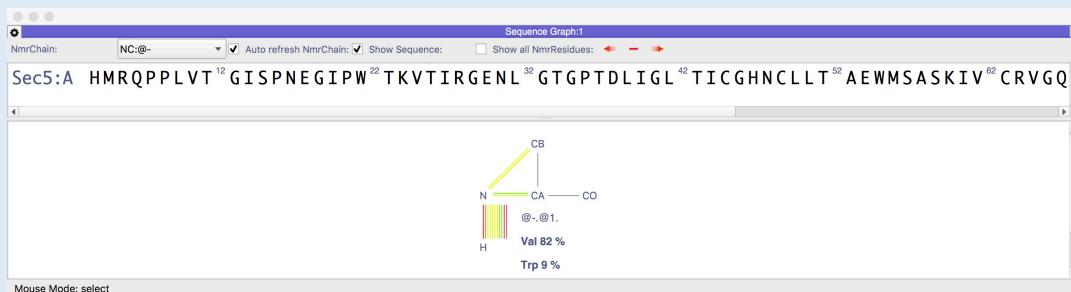
#	Index	Sequence	Type	NmrAtoms	Peak count
1	2	@1	CA, CB, H, N	11	
2	3	@2	H, N	1	
3	5	@3	CA, CB, H, N	11	
4	7	@4	CA, CB, H, N	11	
5	9	@5	CA, CB, H, N	10	
6	11	@6	CA, CB, H, N	9	
7	12	@7	H, N	1	
8	14	@8	CA, CB, H, N	11	
9	16	@9	CA, CB, H, N	10	
10	18	@10	CA, CB, H, N	10	
11	20	@11	CA, CB, H, N	8	
12	22	@12	CA, CB, H, N	0	

5B Open and set the backbone assignment module

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

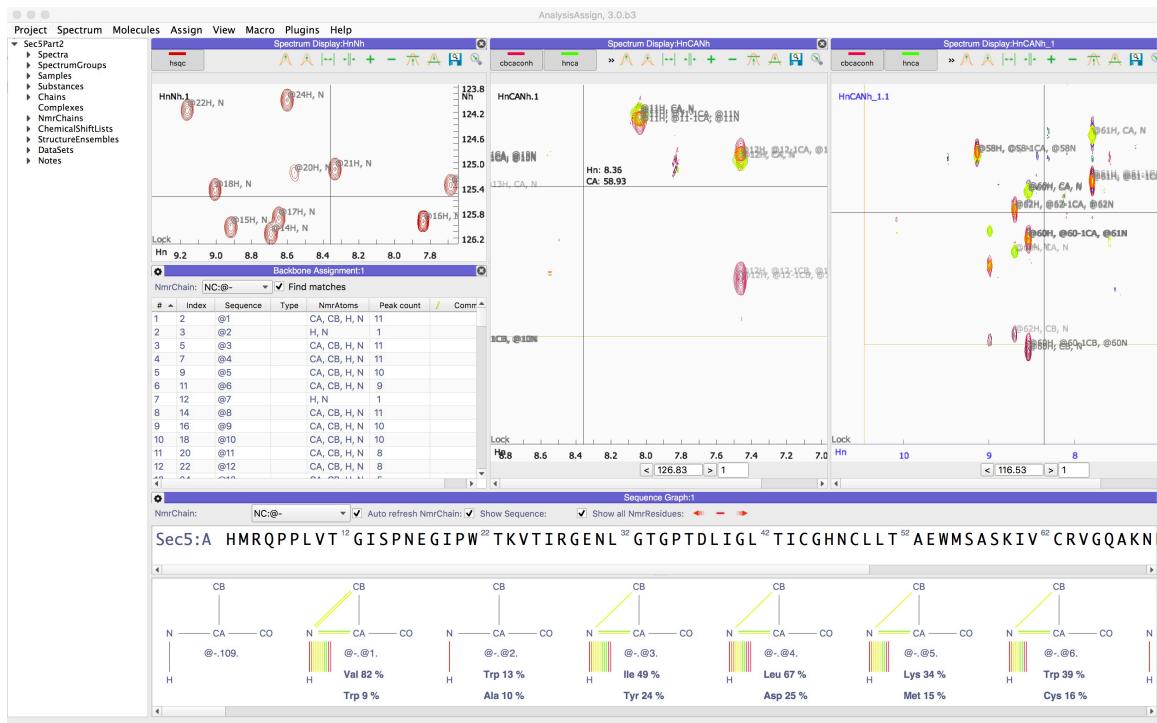


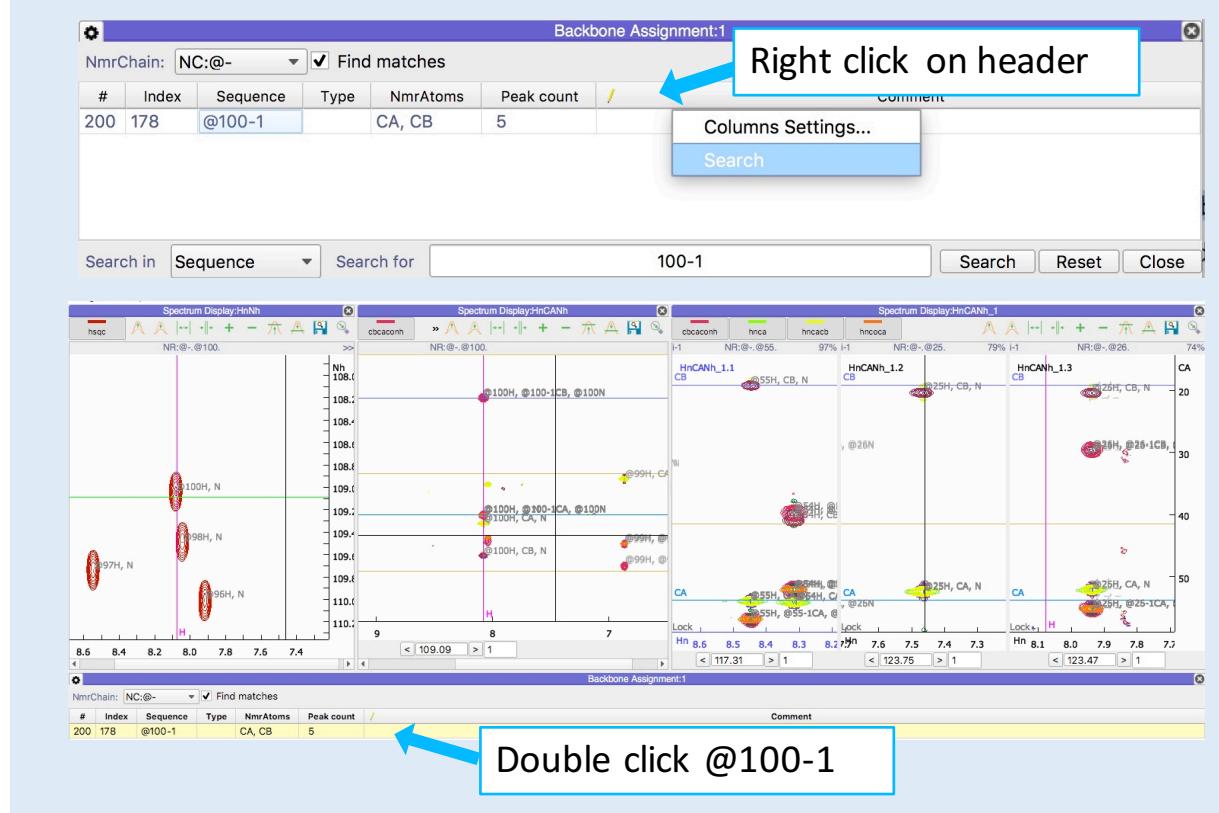
or Shortcut 'SG'



5c Open and set the Sequence Graph module

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

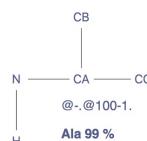


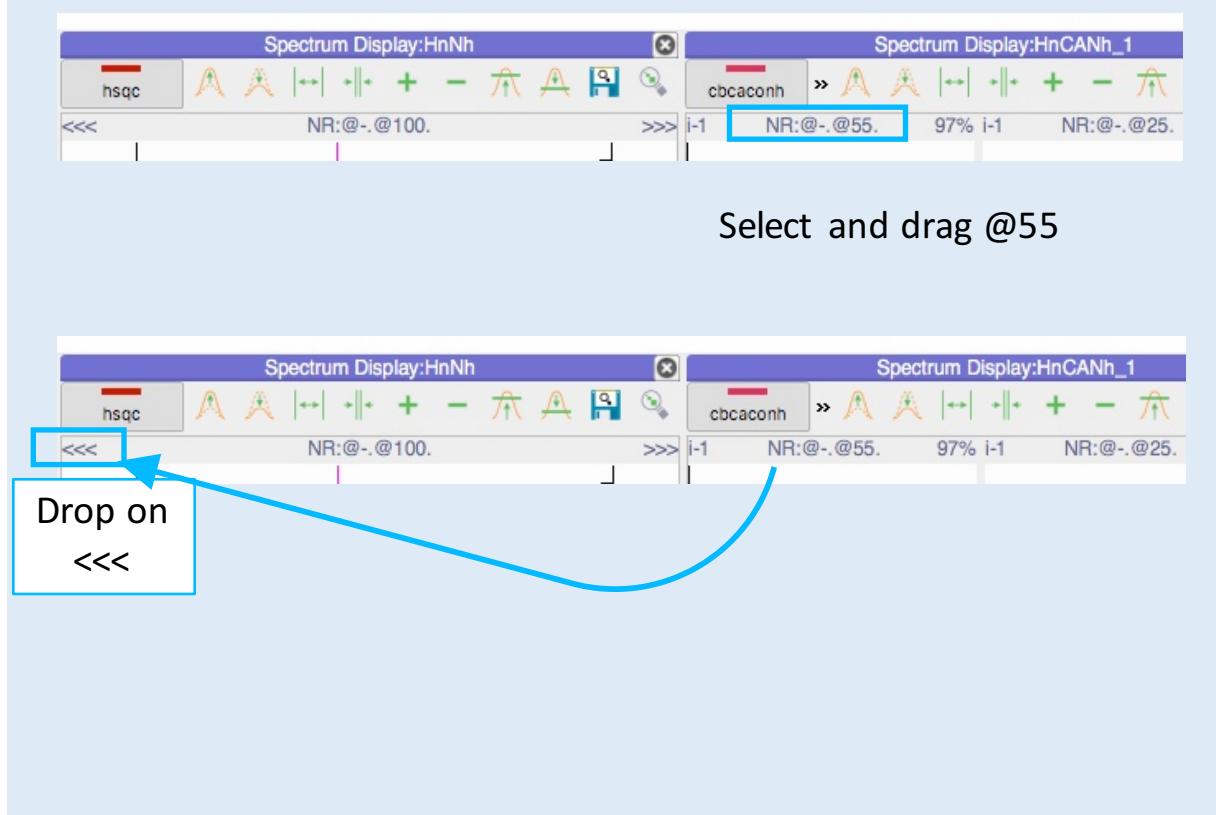


5D Assign in the i-1 direction

- Right click on the Backbone Assignment module table header and click on search
- search for the @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)

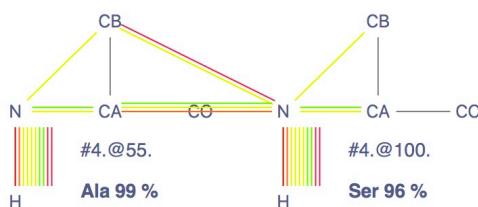




5E Connecting nmrResidues

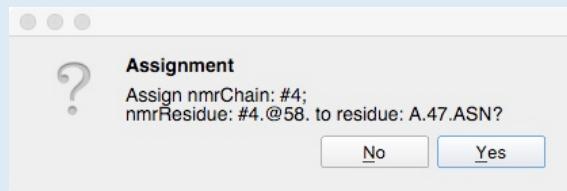
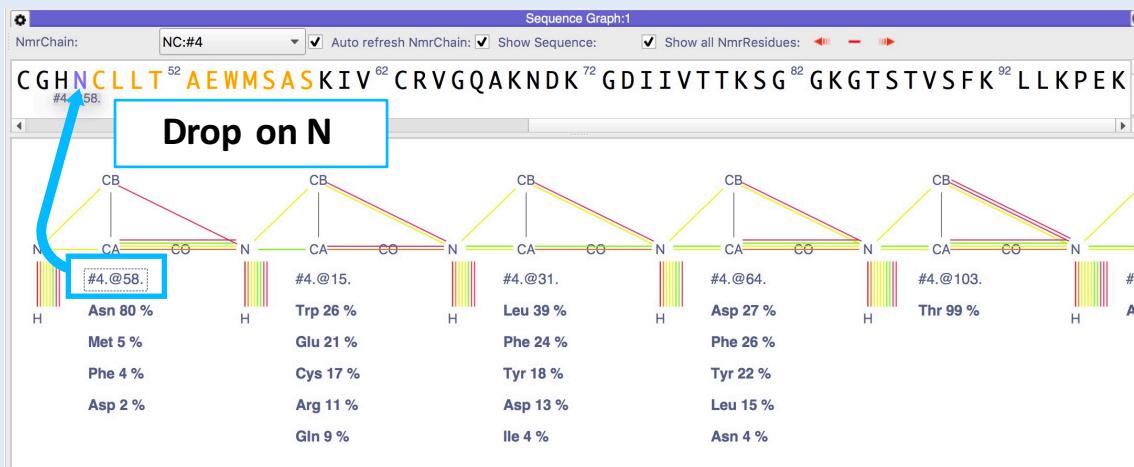
Upon examination, the first strip in module HnCANh_1 shows nmrResidue @55; the H, CA and CB marks align with the peaks of this residue, we can therefore see that is the best match for @100-1.

- Select the strip label NR:@-55 on the spectrum display HnCANh_1
- Drag and drop the label to the <<< icon on the Spectrum display HnCANh
- Check Show all NmrResidues on 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 carry the assignment on.



5F Link NmrChain to Chain

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

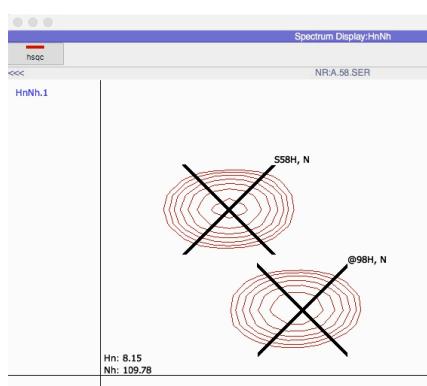
@58 @15 @31 @64 @103 @25 @23 @6 @19 @72 @55 @100

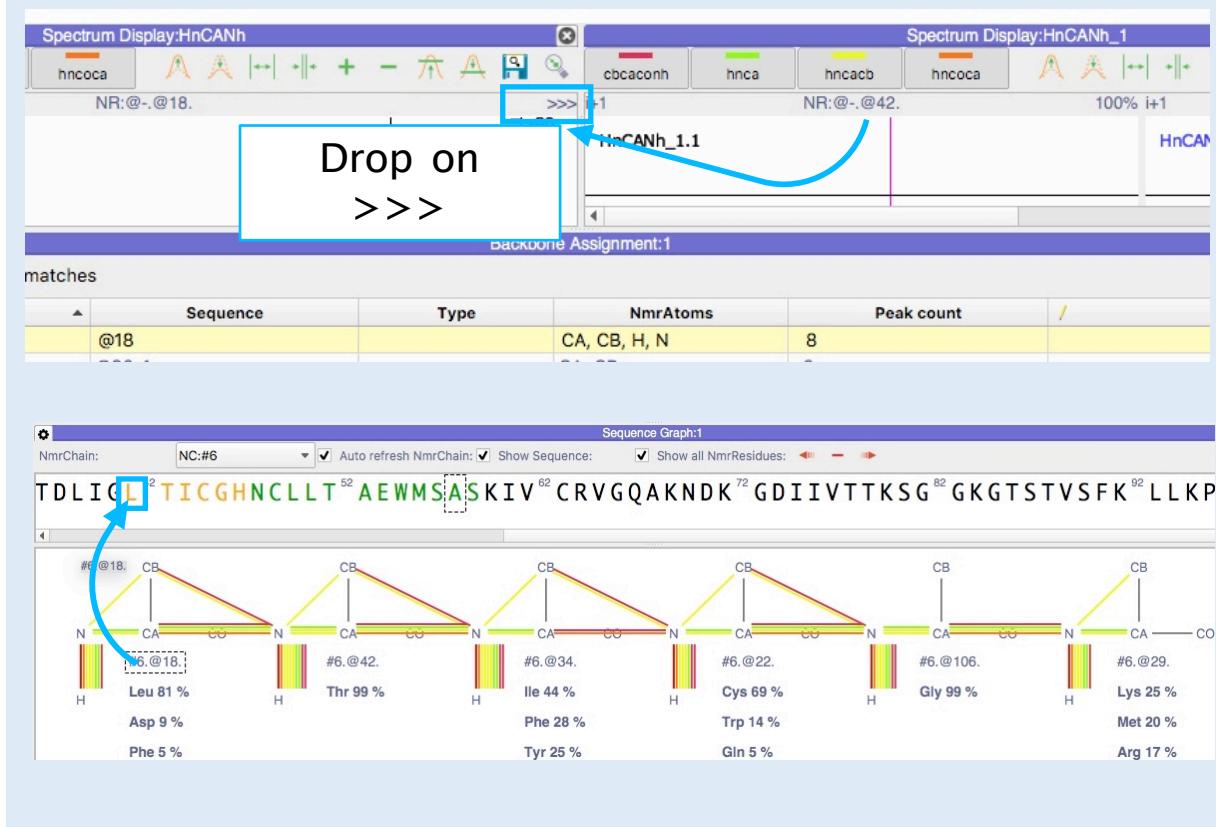
and NCLLTAEWSAS highlighted in the sequence.

- Select the label #4.@58 from the Sequence Graph and drop it to the left foremost residue in the highlighted sequence above it
- 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 on the hsqc spectrum display.





5G Assign in the i+1 direction

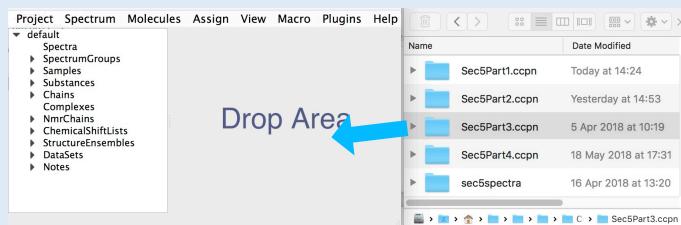
The Sequential backbone assignment in the i+1 direction is fully analogous to the 'i-1' direction except you will need to drop the strip label on the >>> icon.

- 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

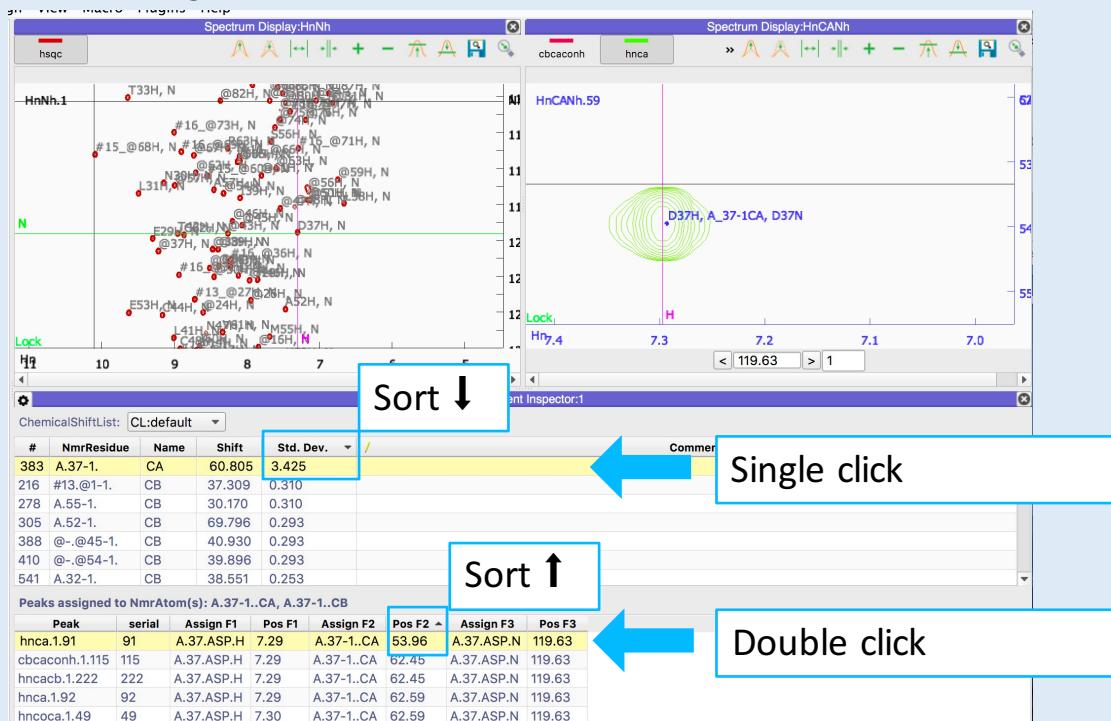
This completes the steps required for a Sequential Backbone Assignment.

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



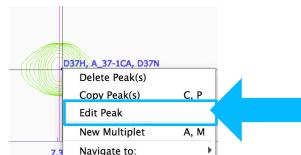
Open Assignment Inspector 'AI'



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
- sort high-low the top table by Std. Dev
- select nmrResidue 37-1; the peak table below will populate
- sort the peak table by Pos F2
- double click on peak hnca.1.91
- zoom in to the peak on 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

CA: 53.956

NmrAtom	Delta
A.37-1..CA	6.849

NH: 119.632

NmrAtom	Delta
A.37.ASP.N	0.005

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

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

Alternatives

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

Alternatives

NmrAtom	Delta
#16,@11-1..CA	
#16,@36..CA	
@-@9-1..CA	
@-@14..CA	
@-@28-1..CA	0.085
@-@35..CA	0.481

New Delete Deassign Assign

Double click to assign

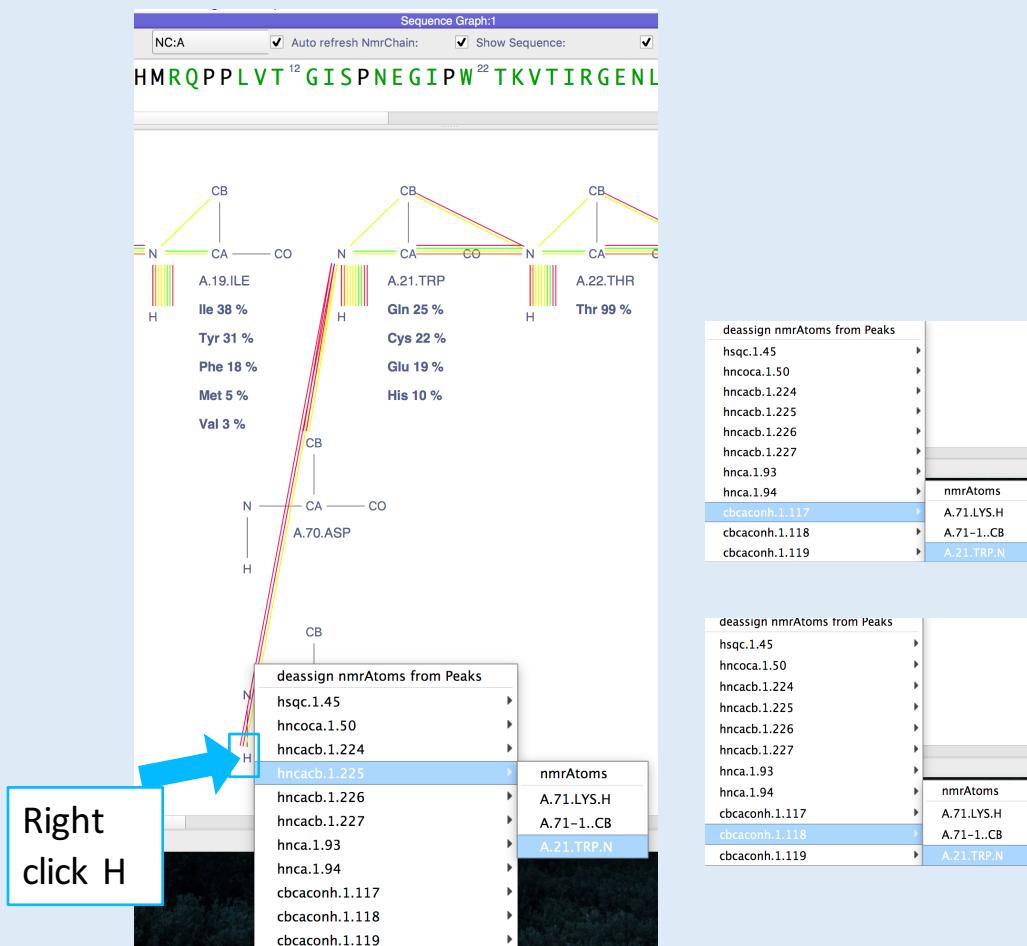
Double click to de-assign

The **Peak Assigner** is very versatile and it has a number of options to guide its behaviour. Use the gear box 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 “AA”)

1B Edit Assignment

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

Open Sec5Part4



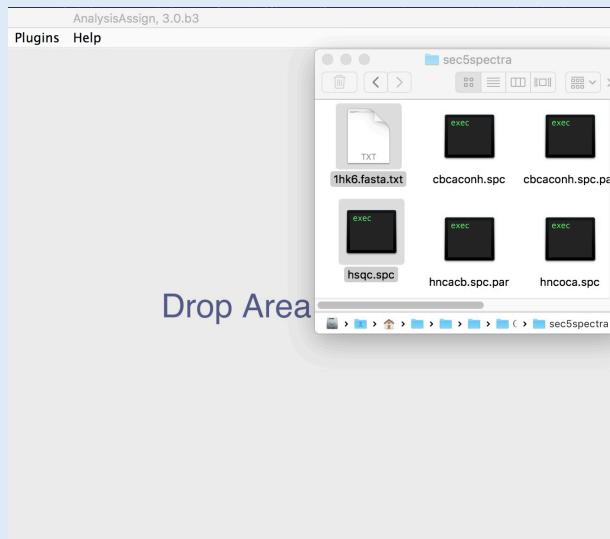
Sec5Part4.ccpn is a project that contains a assignment error; To identify assignment errors you can use the sequence graph. In this project, scrolling through the sequence graph of nmrChain A, you will notice multiple lines connecting the N atom of A.21.TRP to atoms much further down the sequence; in 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 assignment mistake

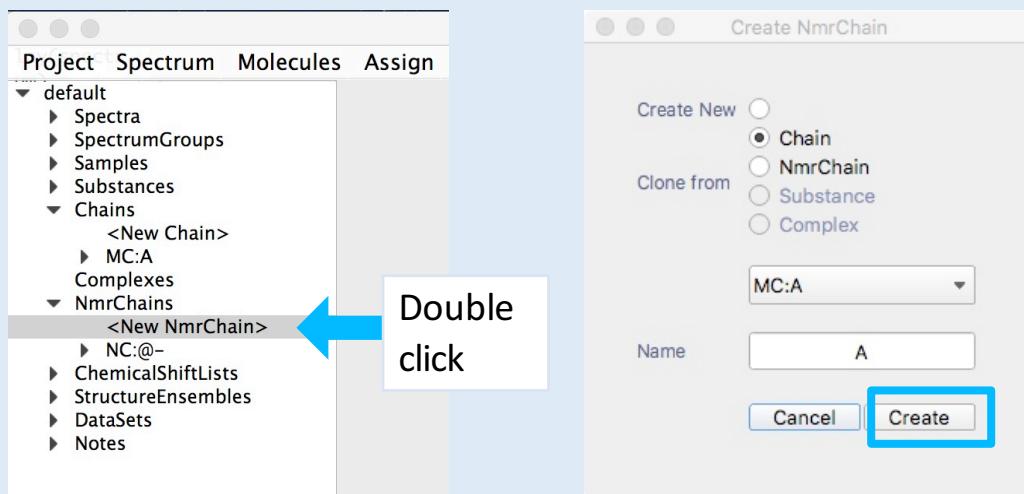
- Open the project Sec5Part4
- Open Sequence Graph with shortcut “SG”
- Select NmrChain NC:A
- Toggle settings, check Show Tree
- Scroll to the right and search for A.21.TRP
- Select the H, Right click on it
- search and click all A.21.TRP peaks from the submenus so to remove the unwanted connections

The wrong connections have been removed, which means the peaks have been de-assign from that NmrAtom. You can further edit the NmrAtom using the other tools of Assign like the Peak Assigner module.

Quick Assignment



Drop Area

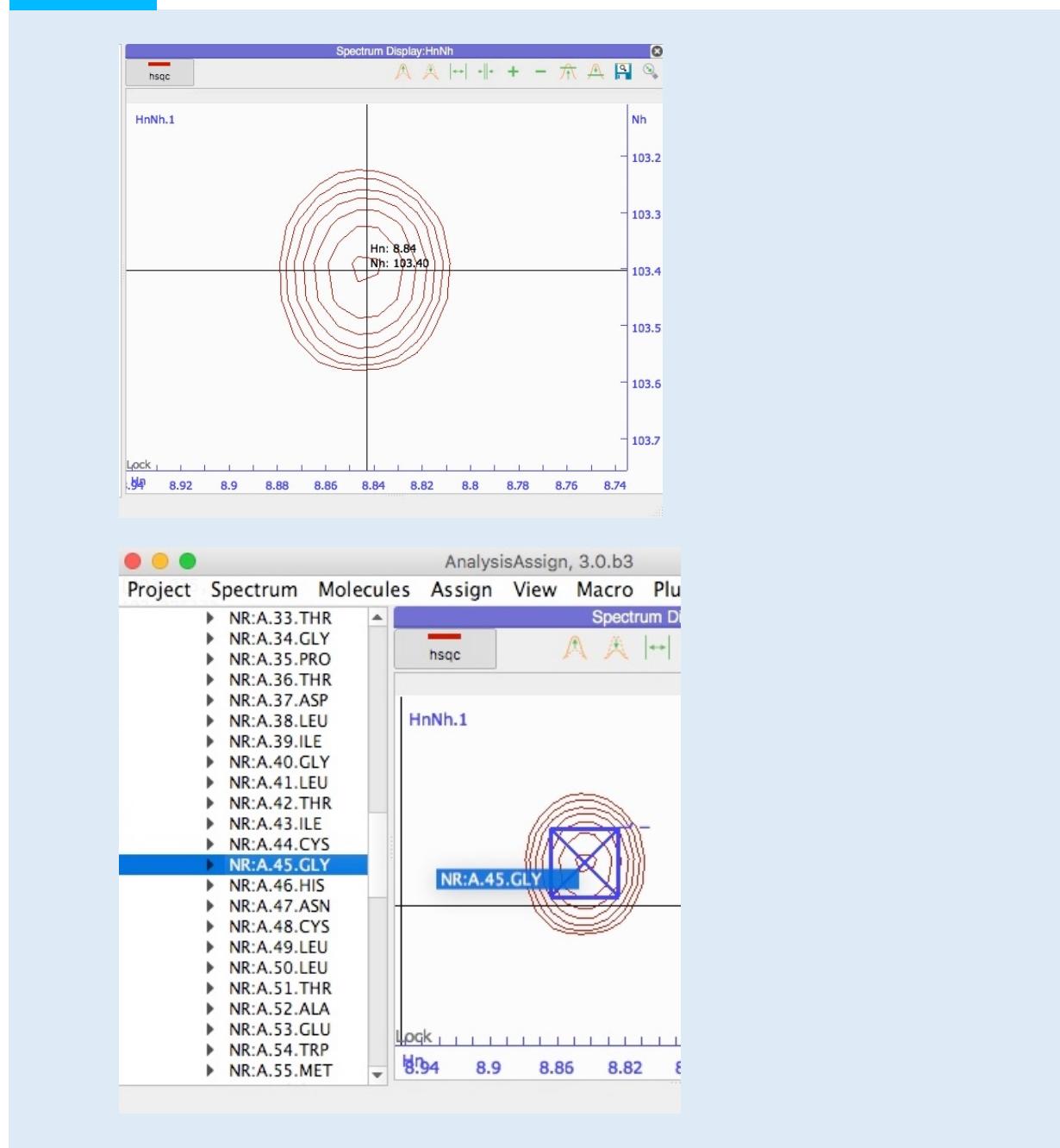


Assuming you have pre-knowledge of a peak assignment, this HOW-TO will show how to assign residues and atoms to any dimensionality peaks in few simple steps.

2A Create Chain and NmrChain

- Open a new project
- Drop the hsqc spectrum from the Sec5Spectra folder
- Drop the Fasta file *1hk6.fasta.txt*
- Double click on *<New NmrChain>*
- Select Clone from: Chain
- Select Chain A on the pulldown
- Name: A
- click Create
- Open the hsqc from sidebar

Quick Assignment

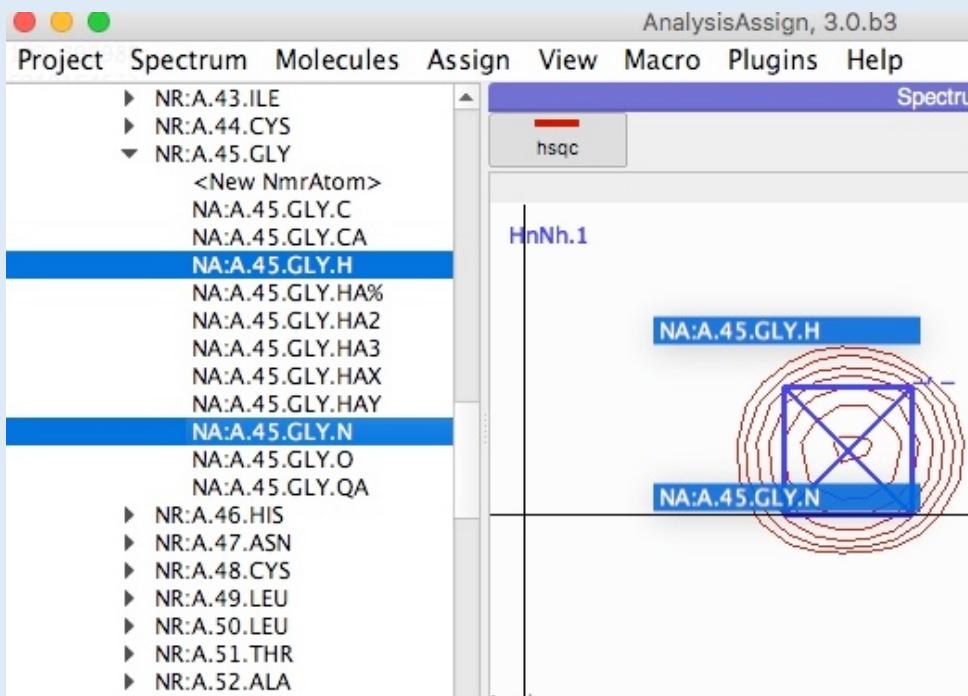


2B Drop the NmrResidue onto a selected peak

- Zoom at positions: 8.84 – 103.40
- Pick the peak with Ctrl (cmd) + shift (↑) + right click
- On sidebar expand the NmrChain A
- Search nmrResidue 45
- Drag and drop the item on the selected peak

The peak is now fully assigned for to the NmrAtoms H and N.

Quick Assignment



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

2c Quick assignment edit

- On 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 on the selected peak. Multiple selection with cmd + 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