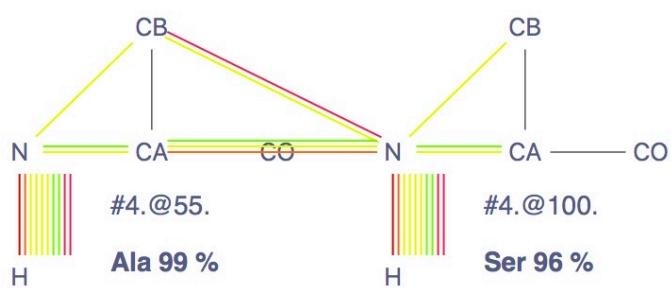
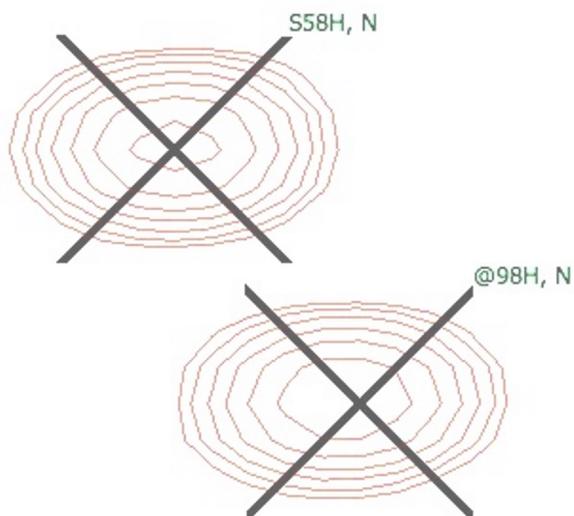


Backbone Assignment Tutorial



Introduction

These tutorials are designed to guide you through a sequential triple resonance backbone assignment using Ccpnmr AnalysisAssign Version 3.1, they are not intended to teach any theoretical aspects of NMR.

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

You will need four projects which are located in the directory:
CcpnTutorialDataSolutionNmrOct22/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.

Contents:

1. Project Setup
2. Picking Peaks
3. Setting up the Assignment
4. Peak Picking 3D Spectra
5. Sequential backbone assignment
- 6: Inspect the Assignment
- 7: Quick Assignment

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. **Double-clicking** on an item will open the properties popup. **Drag** an item into the **Drop Area** to open it as a module (Spectrum Display, Table etc.)

Spectrum Display

A Spectrum Display can contain multiple overlaid spectra. To show/hide a single spectrum, click on its toolbar button. If you close a Spectrum 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

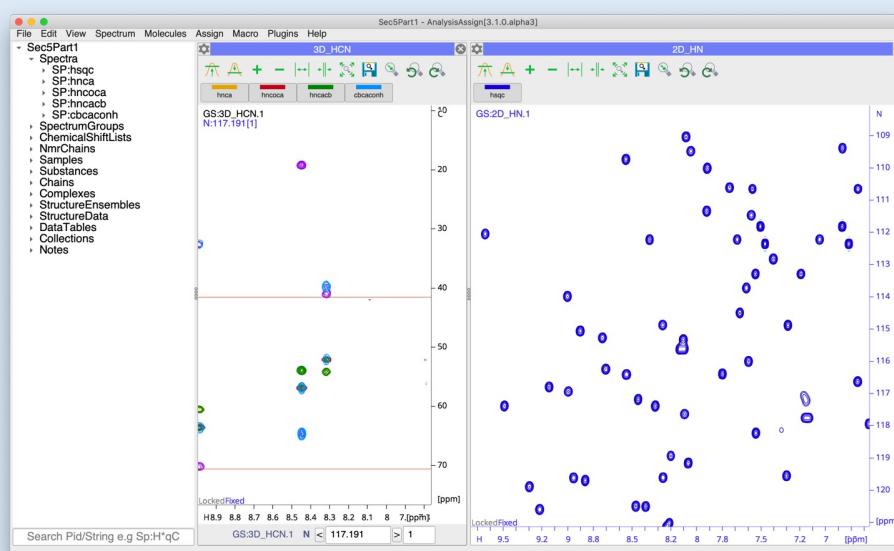
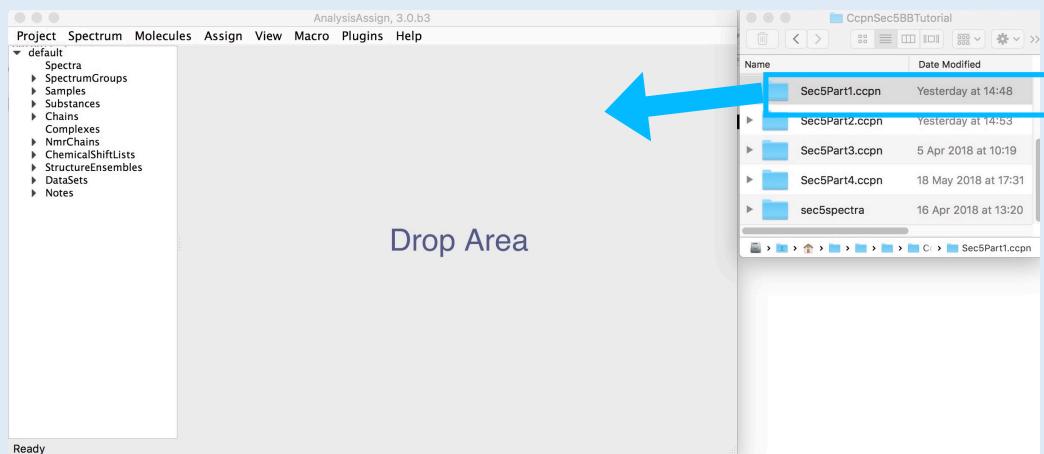
The program uses several shortcuts, for example **CL** for copying a peak list. You will need to press the first letter on your keyboard e.g. **C**, followed by the second letter, e.g. **L** (case insensitive). Press **Esc** to cancel the first letter.

For more commands and operations:

Main Menu -> *Help* -> *Tutorials* -> *Beginners Tutorial* or

Main Menu -> *Help* -> *Show Shortcuts*

Open the project CcpnTutorialDataSolutionNmrOct22/
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 CcpnTutorialDataSolutionNmrOct22/CcpnSec5BBTutorial.

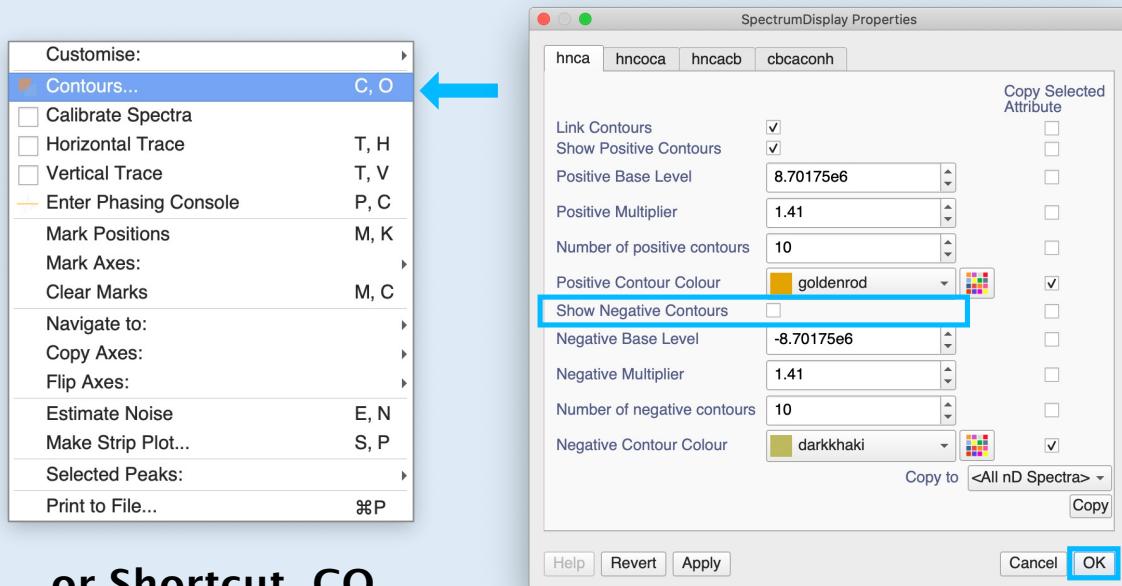
- Select the file **Sec5Part1.ccpn** and drag and drop it into the program. The Sec5Part1 project will be loaded in a new window.

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

- hsqc (dark blue, 2D_HN)
- hnca (yellow, 3D_HCN)
- hncoca (dark red, 3D_HCN)
- hncacb (green/purple, 3D_HCN)
- cbcacoh (light blue, 3D_HCN)

Set contours

Right-click on Spectrum Displays HN and HCN



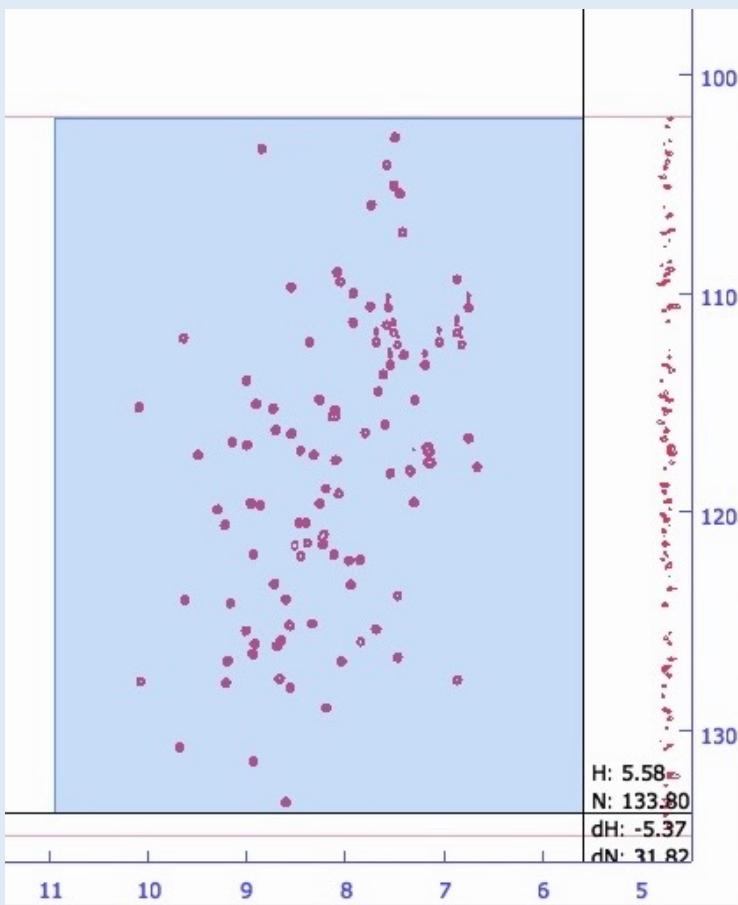
or Shortcut CO

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:
CcpnTutorialDataSolutionNmrOct22/CcpnSec5BBTutorial/sec5spectra.

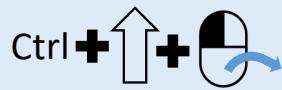
1B Set Spectrum Properties: Contours.

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

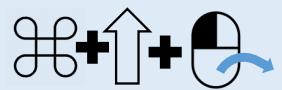
You can also perform these actions for each individual spectrum in the Spectrum properties popup; open them from their sidebar items.



Linux / Windows:



Mac:



The **Peak Picking Drop** parameter in the **Preferences** popup (Main Menu → Project → Preferences, Peaks 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.

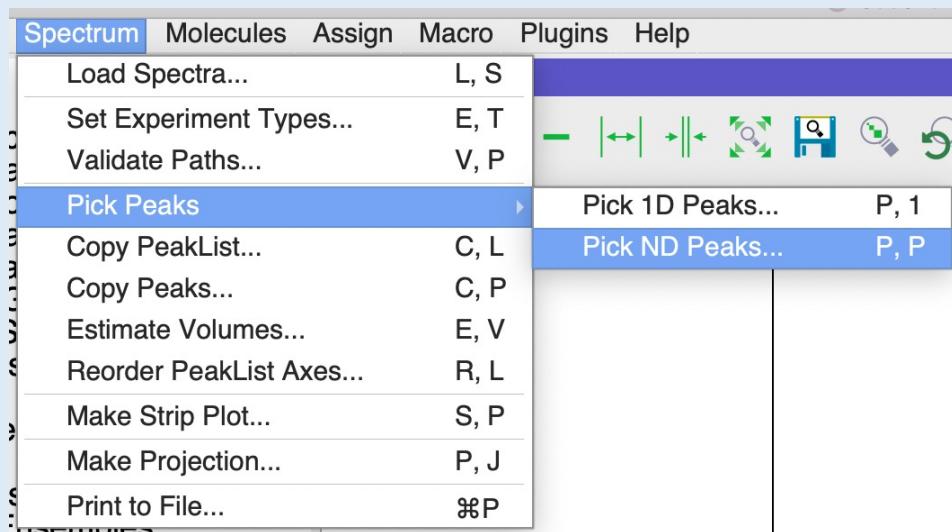
Manual Picking: Ctrl (or Cmd for Mac) + Shift + Left-drag

- 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:

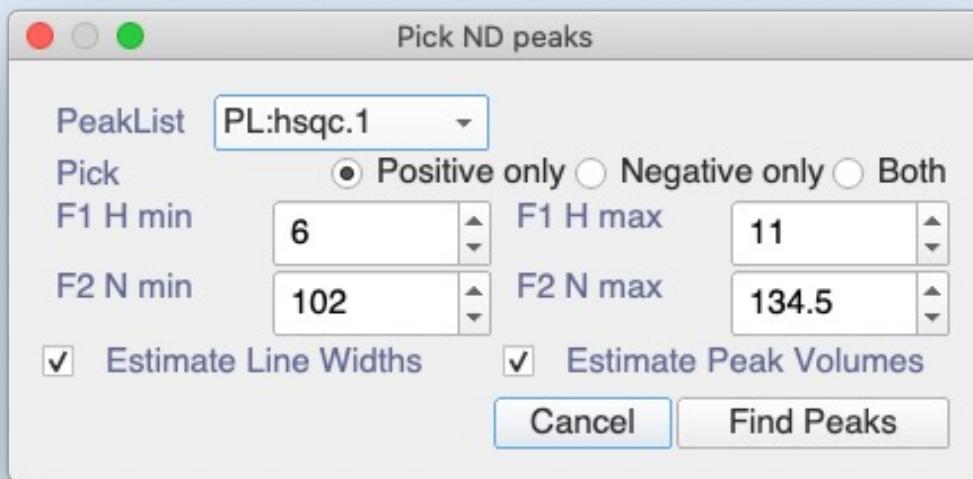
H → 11-6

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...

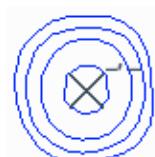
shortcut PP

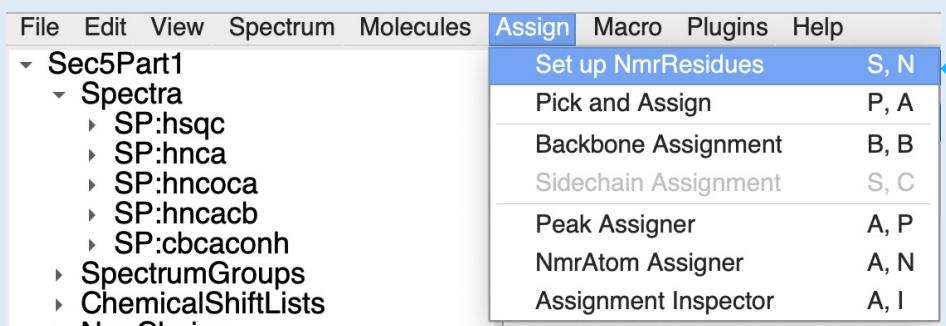
- Select **Positive** only
- Set **F1 H** : 6–11
- Set **F2 N**: 102–134.5
- Click **Find Peaks**.

Result:

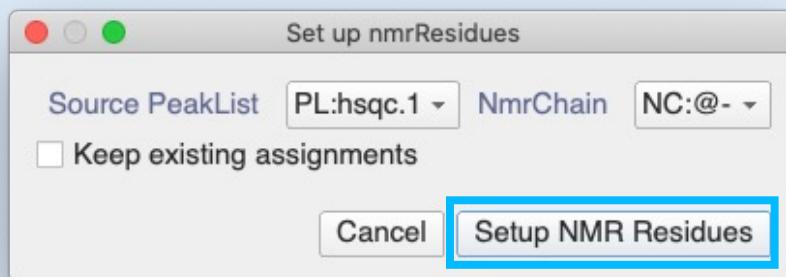
The crosses in the spectrum mark the peak positions picked.

The hyphens separated by a comma indicate that the dimensions of these peaks are unassigned





or Shortcut SN



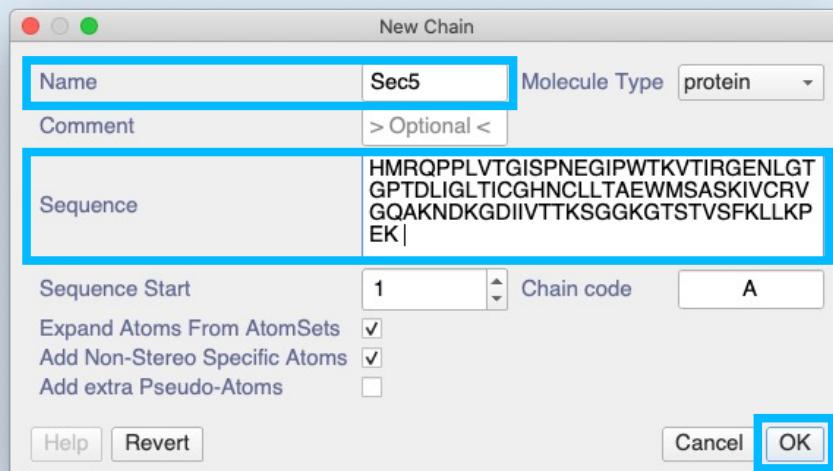
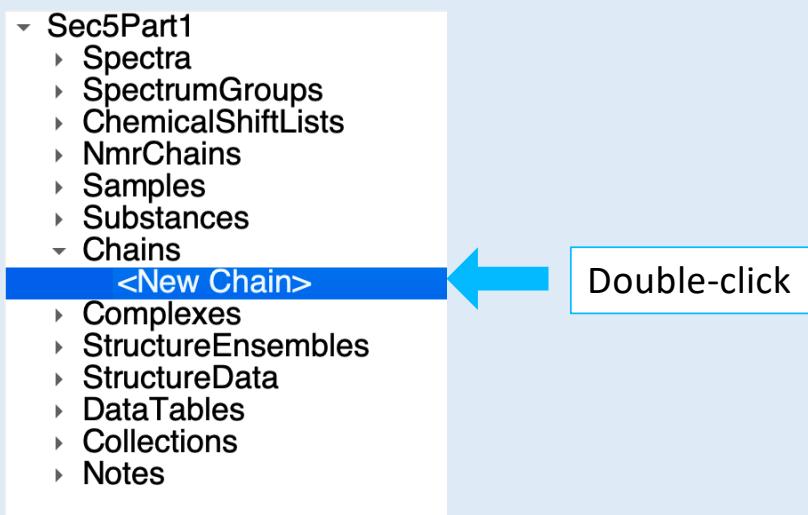
To start the backbone assignment process, we need to define ‘anonymous’ NmrAtoms, i.e. effectively random labels, for the two dimensions of the ^{15}N - ^1H HSQC peaks, which can subsequently be linked to the 3D spectra and used as placeholders until the actual assignments are obtained. See page 9, at 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 **Setup NMR Residues**

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>

Use the default settings with

- Name: Sec5
- Sequence: (copy and paste)

HMRQPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTICGHNCLLTAEWMASAKIVCRVQQAQNDKGDIIVTTKSGGKGTVSFKLLKPEK

- 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' .

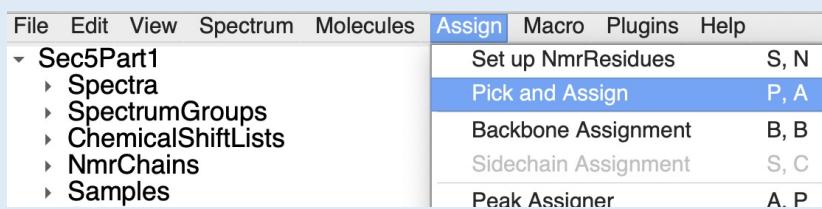
NmrAtom names always start with the nucleus, and default names would be e.g. 'H@31' or 'C@88'. Some names have a special meaning:

- '%' 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).

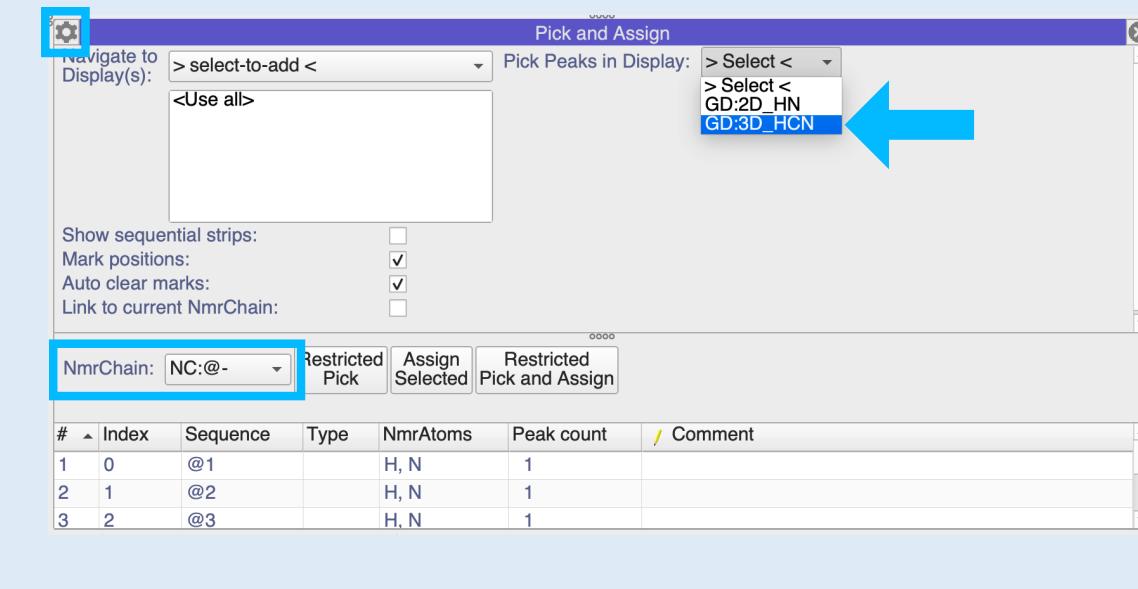
¹ Atoms reside in Residues, which reside in Chains; multiple chains can form a Complex.

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://youtu.be/DS9lZzNsBbQ>



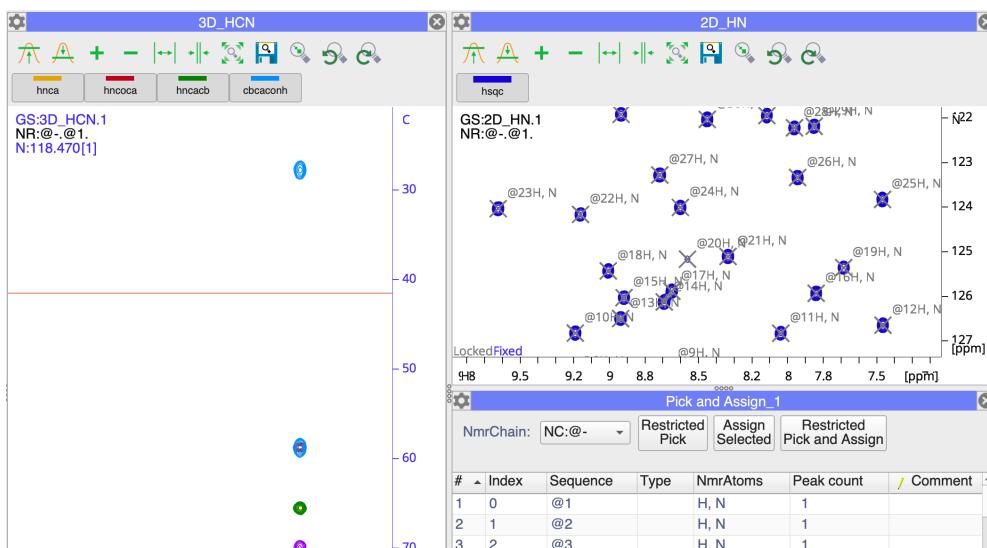
or Shortcut PA

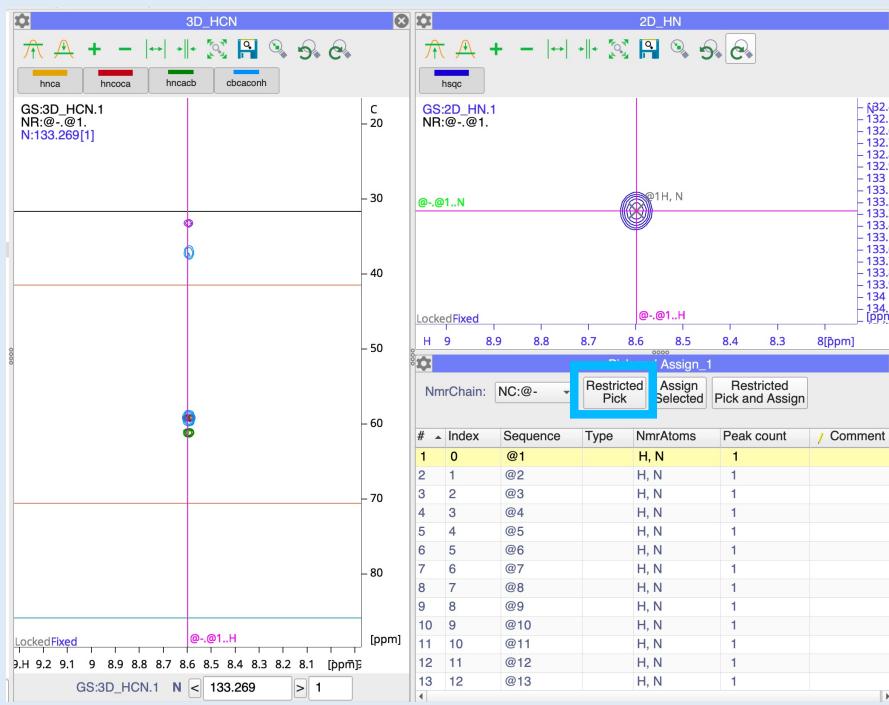


4A Pick and Assign 3D Peaks Setup

To pick the 3D spectra based on the HSQC spectrum we will use the **Pick and Assign** module.

- Go to **Main Menu → Assign → Pick and Assign**, or shortcut **PA**.
- Open the Settings (gearbox icon) and select:
 - Pick Peaks in Display 3D_HCN**
 - Leave the rest as default and close the settings
- Ensure the selected NmrChain is **NC:@-** .
- Finally, arrange your modules with the **Pick and Assign** module below or above the **2D_HN** (HSQC containing) module:





4B Pick and Assign 3D Peaks

In the Pick and Assign module:

- 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, the 3D_HCN 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 in the **Pick and Assign** module.

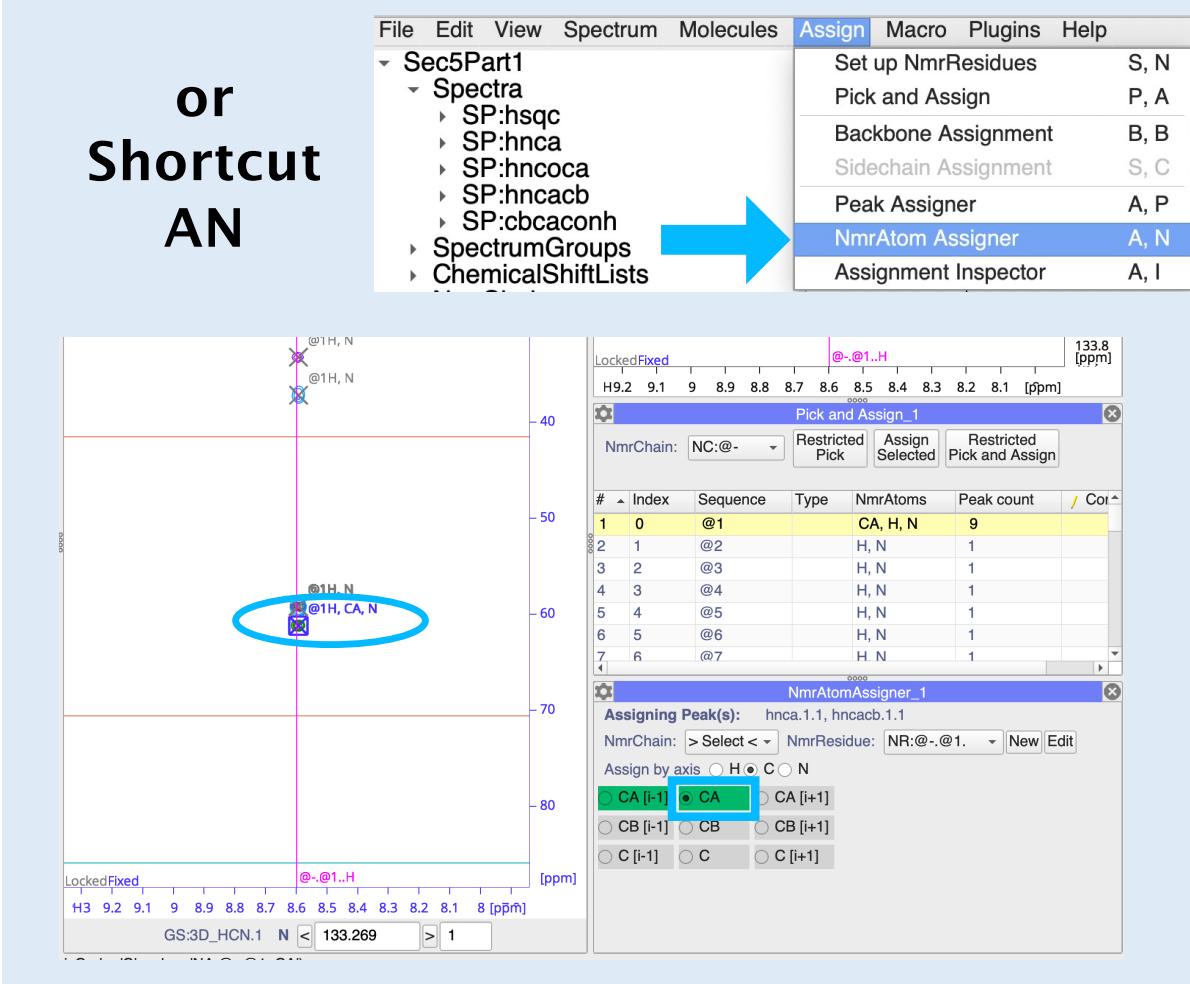
This will pick all the peaks along a narrow region in the 3D window and these peaks will be selected.

- Inspect the 3D peaks and delete any noise peaks.

Be aware that the peak picking occurs on any visible peaks, so it can be helpful to adjust the contours levels to something suitable before doing the **Restricted Pick**.

- Select the peaks to assign and click the **Assign Selected** button in the **Pick and Assign** module.

or
Shortcut
AN

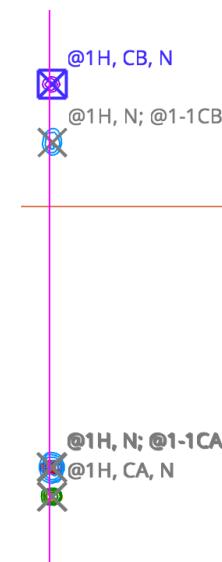


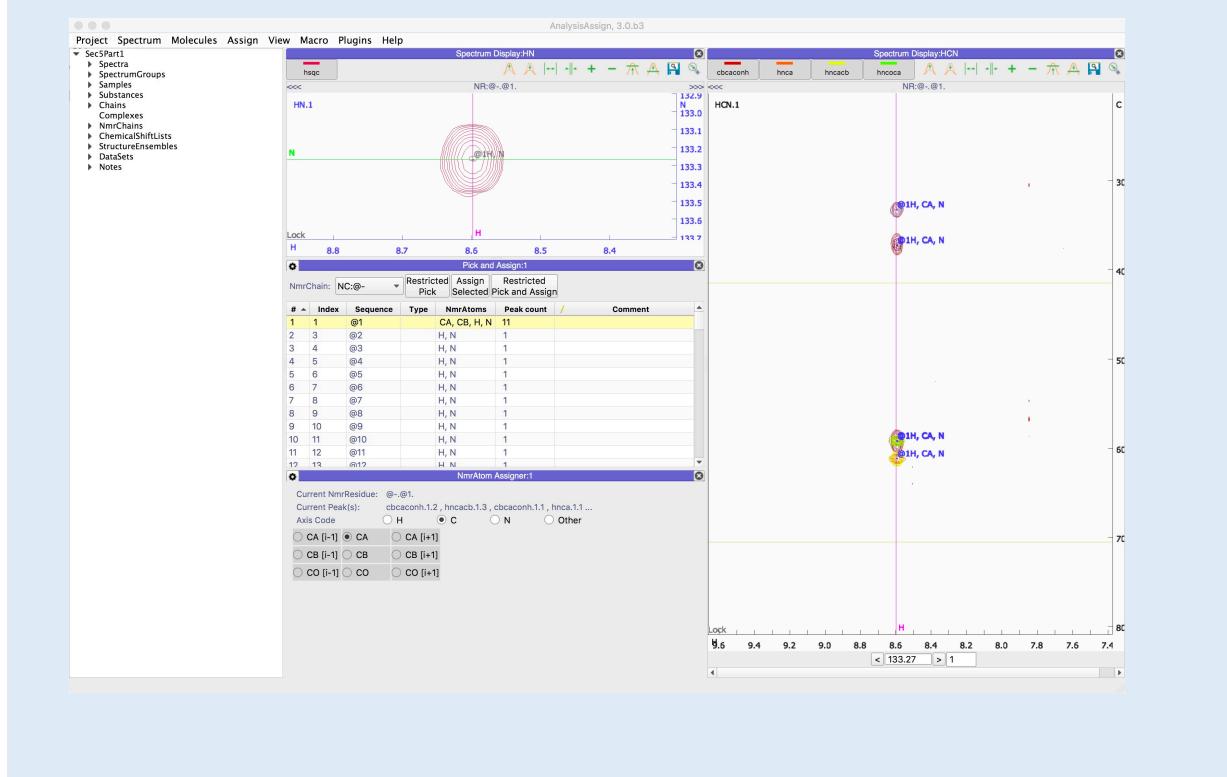
4C Make the carbon dimension atom type assignment

- Go to Main Menu → View → NmrAtom Assigner or use the shortcut AN.
- Select one or more peaks at a single position in the 3D_HCN window to predict the assignment for the carbon dimension. The program uses green for likely and orange for less likely assignments.
- Toggle any NmrAtom button to assign it to the selected peak(s).

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 all groups of peaks along this line until all the CA_{i-1} , CA_i , CB_{i-1} and CB_i assignments for this NmrResidue have been added.





4D Continue to Pick and Assign the 3D spectra

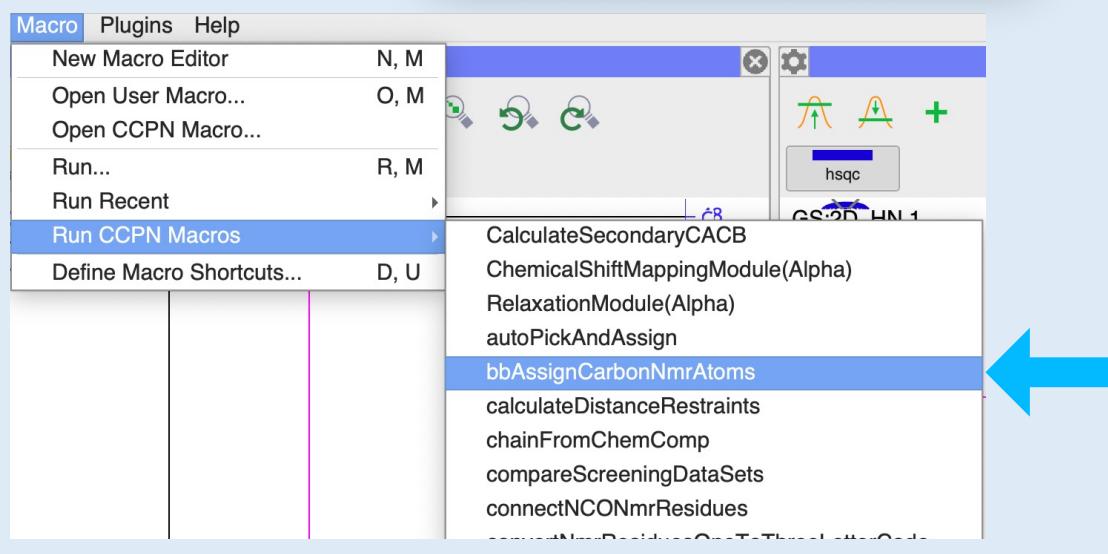
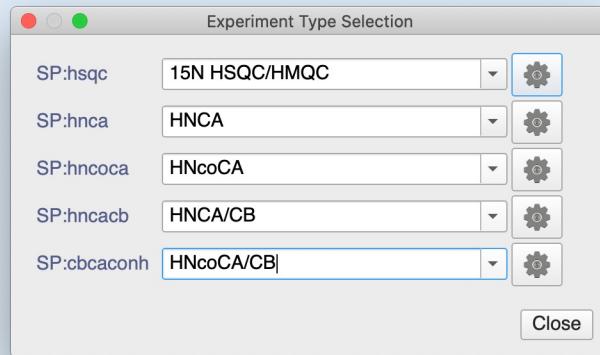
To use the sequential Backbone Assignment tools in AnalysisAssign, the i and i-1 assignments for all NmrResidues need to be provided, so the procedure described above should be carried out for all NmrResidues listed in the Pick and Assign table, where possible.

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

Since the assignment of the carbon NmrAtoms is relatively predictable, it is also possible to automate this and speed this up with a macro. See step 4E on how to do this.

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.

**Shortcut
ET**



4E Use a macro to assign the carbon dimension atom types (optional)

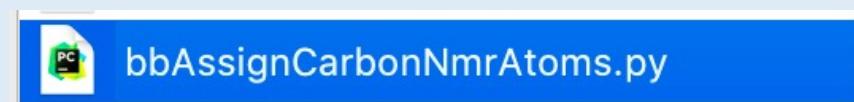
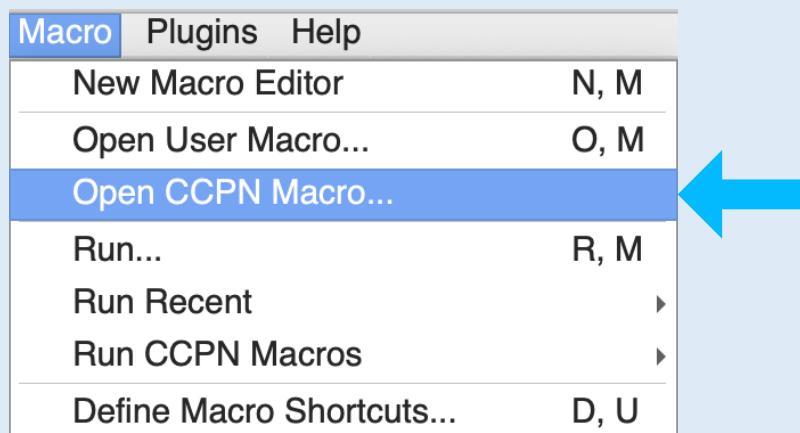
Begin by setting the experiment types for your spectra:

- Go to **Main Menu → Spectrum → Set Experiment Types...** or shortcut **ET**
- Set the Experiment Types from the drop-down menus as shown above and then click **Close**.
- **Double-click** on the next row in the **Pick and Assign** module table, e.g. the row for @3.
- Click the **Restricted Pick and Assign** button to do the peak picking and HN assignment in one go (make sure you remove any noise peaks).

While the picked peaks are selected:

- Go to **Main Menu → Macro → Run CCPN Macros → BBAssignCarbonNmrAtoms**

This should assign all carbon NmrAtoms for you. Check that they correct and what you would expect, making any manual changes, if necessary.



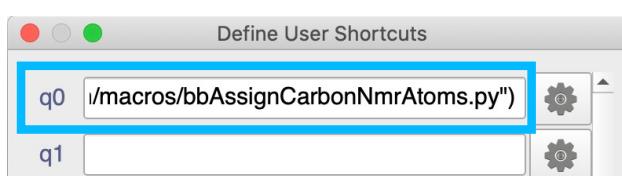
4F Create a two key shortcut for your macro (optional)

To really speed up the Pick and Assign process, it is easiest to have this macro assigned with a keyboard shortcut:

- Go to **Main Menu → Macro → Open CCPN...**
- Select the **BBAssignCarbonNmrAtoms.py** file and click on **Open**.

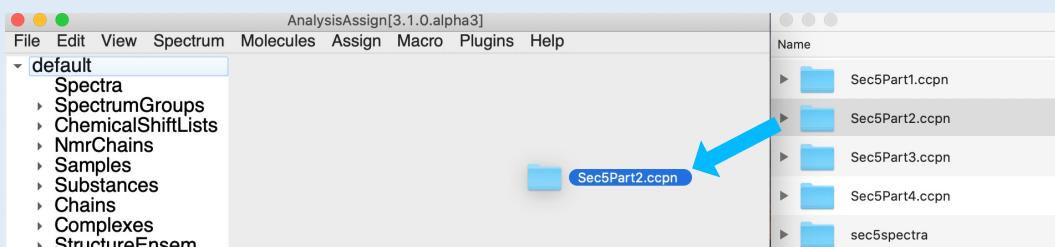
- Click on the symbol to associate the macro with a shortcut.

The program will automatically add the path to the next available free slot:



- Remember the key combination (e.g. **q0**) or choose a different one by cutting and pasting the file path elsewhere, then click on **Save and Close**.

Now you can continue with Picking and Assigning your 3D spectra for a bit, using **q0** (or whichever shortcut you chose) to assign the carbon NmrAtoms. Remember to make sure you check these assignments as overlapped peaks or unusual chemical shifts may mean the assignments are not made correctly by the macro.



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:

CcpnTutorialDataSolutionNmrOct22/CcpnSec5BBTutorial

5 A Open Sec5Part2.ccpn

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

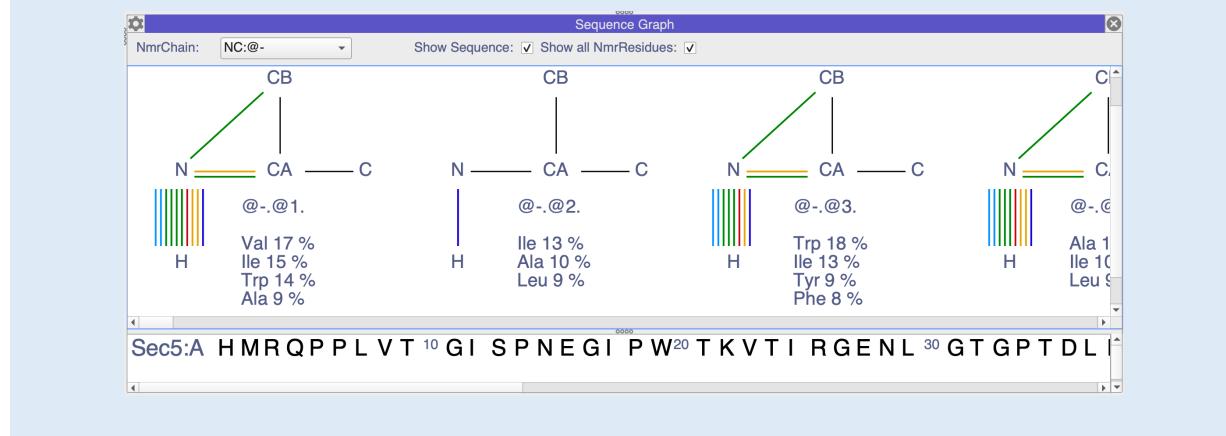
The screenshot shows the AnalysisAssign software interface. The main menu bar includes Assign, Macro, Plugins, and Help. The Assign menu has options: Set up NmrResidues (S, N), Pick and Assign (P, A), **Backbone Assignment (B, B)** (highlighted with a blue box and arrow), and Sidechain Assianment (S, C). Below the menu is a toolbar with a settings icon. The main workspace shows the 'Backbone Assignment' module. It includes a 'Display(s)' section with a dropdown set to '> select-to-add <' and '<Use all>'. There are checkboxes for 'Mark positions', 'Auto clear marks', 'Link to current NmrChain', and 'Focus Y-Axis'. Under 'Match SpectrumDisplay', the dropdown is set to 'GD:3D_HCN'. Under 'Search SpectrumDisplay', the dropdown is set to 'GD:3D_HCN_1'. On the right, there is a table titled 'Backbone Assignment' with columns: #, Index, Sequence, Type, NmrAtoms, and Peak count. The table lists 18 rows of data, with rows 10, 11, 12, 13, 14, 15, 16, 17, and 18 highlighted in yellow. The last row shows CA, CB, CG, H, N with a peak count of 8.

5 B Open and set up 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: 3D_HCN (or similar 3D SpectrumDisplay)
 - Search module: 3D_HCN_1 (a different 3D SpectrumDisplay)
 - Leave the rest as default and close the settings

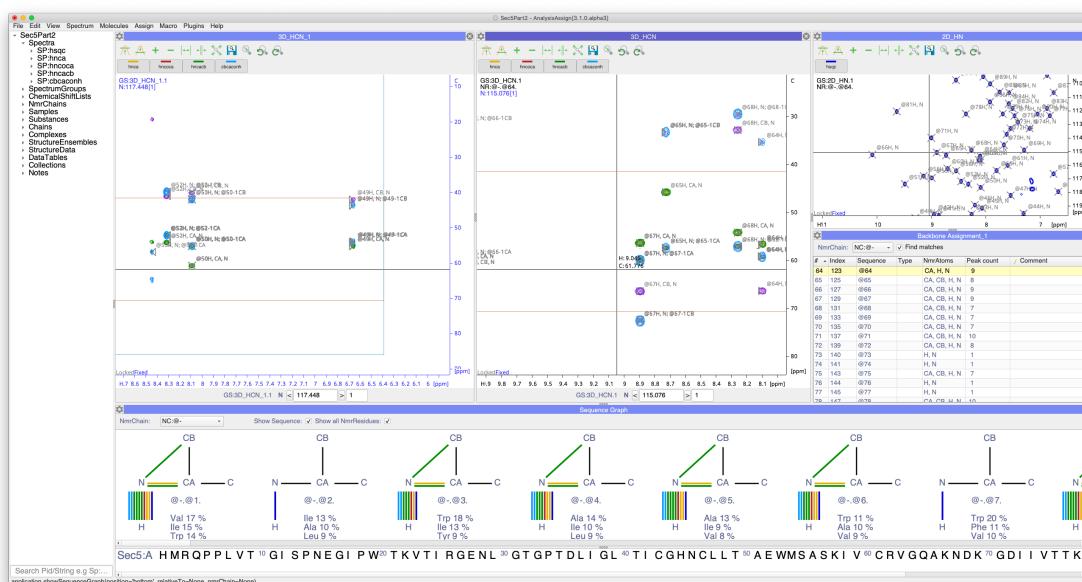
View	Spectrum	Molecules	Assign	Macro	Plugins
Chemical Shift Table			C, T		
NmrResidue Table			N, T		
Residue Table			P, T		
Peak Table			I, T		
Integral Table			M, T		
Multiplet Table			R, T		
Restraint Table			S, T		
Structure Table			D, T		
Data Table			V, T		
Sequence Graph			S, G		
Violation Table					

or Shortcut SG



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.



Backbone Assignment

NmrChain: NC:@- Find matches

#	Index	Sequence	Type	NmrAtoms	Peak count	Comment
180	169	@93-1	CA, CB	3		Column Settings... Filter...

Equal @93-1

Filter in <Visible Table> Search Reset Close

Spectrum Display:HnN

Spectrum Display:HnCANH

Spectrum Display:HnCANH_1

NR:@- @100. NR:@- @100. NR:@- @25.

NR:@- @55. 97% i-1 NR:@- @25. 79% i-1 NR:@- @26. 74%

NR:@- @100-1CA, @100N HnCANH_1.1 CB HnCANH_1.2 CB HnCANH_1.3 CB

100H, N 100H, @100-1CB, @100N 100H, N 100H, CB, N 100H, CB, N 100H, CB, N

99H, N 99H, @100-1CA, @100N 99H, N 99H, CB, N 99H, CB, N 99H, CB, N

98H, N 98H, @100-1CB, @100N 98H, N 98H, CB, N 98H, CB, N 98H, CB, N

97H, N 97H, @100-1CB, @100N 97H, N 97H, CB, N 97H, CB, N 97H, CB, N

8.6 8.4 8.2 8.0 7.8 7.6 7.4 8.6 8.4 8.2 8.0 7.8 7.6 7.4 8.6 8.4 8.2 8.0 7.8 7.6 7.4

8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7

110. 109. 108. 107. 106. 105. 104. 103. 102. 101. 100. 99. 98. 97. 96. 95. 94. 93. 92. 91. 90. 89. 88. 87. 86. 85. 84. 83. 82. 81. 80. 79. 78. 77. 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7

110. 109. 108. 107. 106. 105. 104. 103. 102. 101. 100. 99. 98. 97. 96. 95. 94. 93. 92. 91. 90. 89. 88. 87. 86. 85. 84. 83. 82. 81. 80. 79. 78. 77. 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7

110. 109. 108. 107. 106. 105. 104. 103. 102. 101. 100. 99. 98. 97. 96. 95. 94. 93. 92. 91. 90. 89. 88. 87. 86. 85. 84. 83. 82. 81. 80. 79. 78. 77. 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7

Lock Lock Lock Lock

117.31 123.75 123.47

Backbone Assignment:1

NmrChain: NC:@- Find matches

| # | Index | Sequence | Type | NmrAtoms | Peak count | Comment |
|-----|-------|----------|--------|----------|------------|---------|
| 200 | 178 | @100-1 | CA, CB | 5 | | |

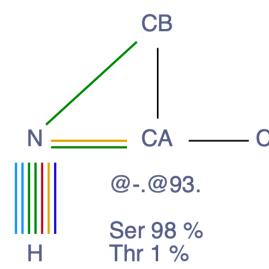
5D Assign in the i-1 direction

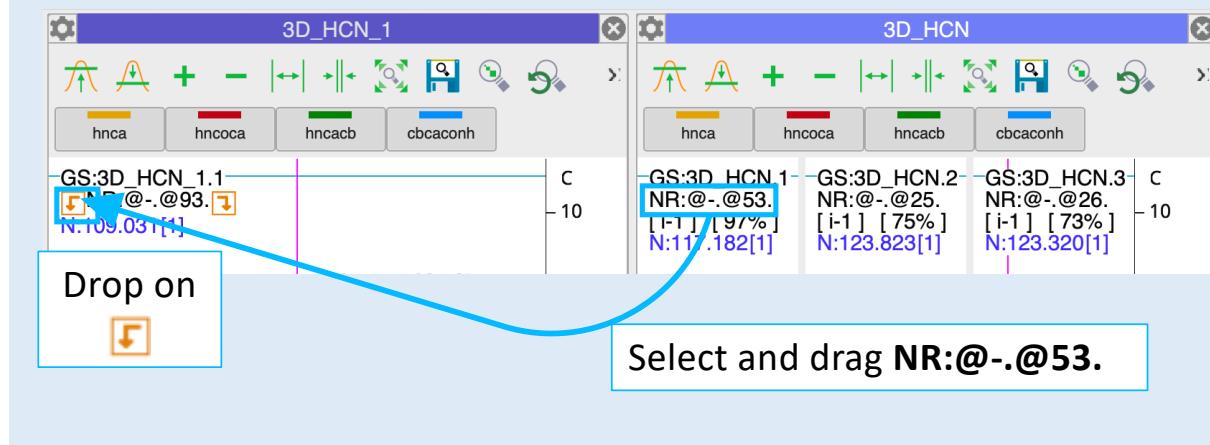
- Right-click on the Backbone Assignment module table header and click on Filter... or use shortcut FT
- search for @93-1
- Double click the row. You will see a series of changes in the GUI. The 3D_HCN_1 module will navigate to the appropriate plane containing the assignment for the NmrResidue @93; the relevant frequencies of @93 H and N and @93-1 C atoms are marked in all Spectrum Displays.

The **Sequence Graph** will have a schematic residue drawn, labelled with the NmrResidue name (@93) and predictions of the possible residue type(s) below it. The match module (**3D_HCN**) will display three strips in order (left to right) that the AnalysisAssign algorithm thinks best match the i-1 chemical shifts of @93 (C-terminal of the residue @93).

Tip: Setting the 3D spectrum displays as **Fixed** or **Locked** will keep your peaks in a nice shape.

Locked Fixed

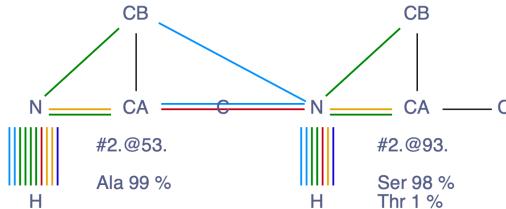




5E Connecting NmrResidues

Upon examination, the first strip in the Match module Spectrum Display (3D_HCN) shows NmrResidue @53; the H, CA and CB marks align with the peaks of this residue, we can therefore see that is the best match for @93-1.

- Select the strip label **NR:@-.@53.** on the Match spectrum display
- Drag and drop the label on to the icon in the Search Spectrum Display
- Check **Show all NmrResidues** in the **Sequence Graph** module (and make sure NmrChain #2 is selected) to see the new connection



At this point @93 and @53 have been put into a so-called connected stretch (cf. section 3) and they are in a new NmrChain called #2, which means that their names have changed to #2.@93. and #2.@53. The NmrChain pulldown in the module should automatically be set to #2.

When strips are dragged and dropped through this procedure, AnalysisAssign will subsequently look for i-1 matches for @53. The algorithm thinks that @70 is a good match for @53-1 and on inspection it is a match, so selecting and dragging @70 onto the of the Search spectrum display will continue the assignment.

Note: The Settings panels of both the **Sequence Graph** and **Backbone Assignment** modules contain a setting **Link to current NmrChain**. If this is checked, then selecting a new NmrChain in one module will automatically update that used in the other module. You can switch this on or off as suits you best.

Link to current NmrChain



The screenshot shows the Sequence Graph window with the following details:

- NmrChain:** NC:#2
- Show Sequence:** checked
- Show all NmrResidues:** checked
- Sequence:** GENL 30 G H N C L L T 50 A E W M S A S K I V 60 C R V G Q A K N D K 70 G D I I V T T
- Assignment Labels:**
 - #2. @56. (highlighted with a blue box)
 - #2. @15.
 - #2. @31.
 - #2. @62.
 - #2. @45.
- Assignment Data:**
 - #2. @56. Asn 79 % Met 5 % Phe 4 % Asp 2 %
 - #2. @15. Trp 27 % Glu 21 % Cys 16 % Arg 11 % Gln 9 %
 - #2. @31. Leu 41 % Phe 23 % Tyr 17 % Asp 13 % Ile 3 %
 - #2. @62. Asp 29 % Phe 25 % Tyr 20 % Leu 17 % Ile 3 %
 - #2. @45. Thr 9 %
- Action Bar:** Drop on N
- Confirmation Dialog:**

Assignment

Assign nmrChain: #2;
nmrResidue: #2. @56. to residue: A.45.ASN?

5F Link NmrChain to Chain

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

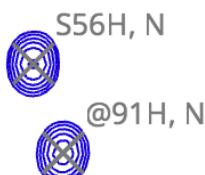
@56 @15 @31 @62 @96 @25 @23 @6 @19 @70 @53 @93

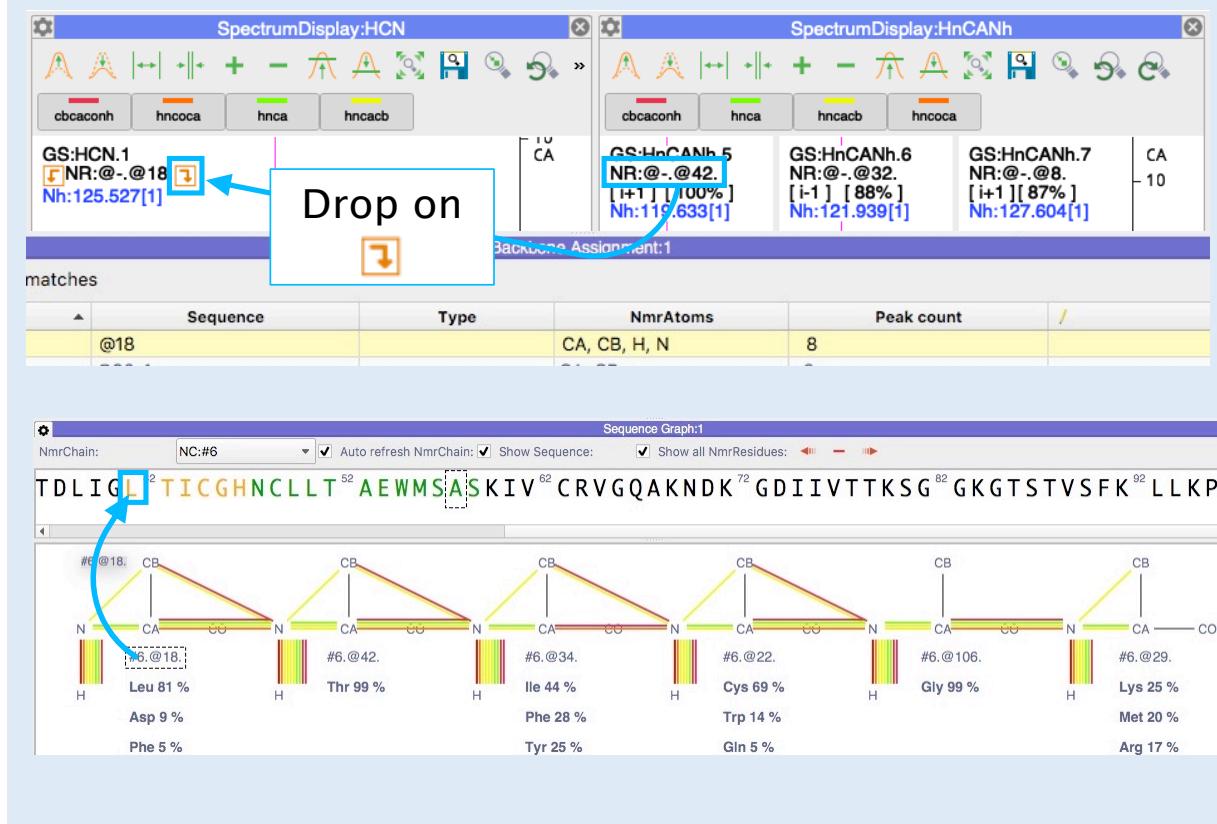
and NCLLTAEWSAS will be highlighted in the sequence because this is the best match in the sequence based on the chemical shifts.

- Select the label #4. @56 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 **2D_HN** Spectrum Display.





5G Assign in the i+1 direction

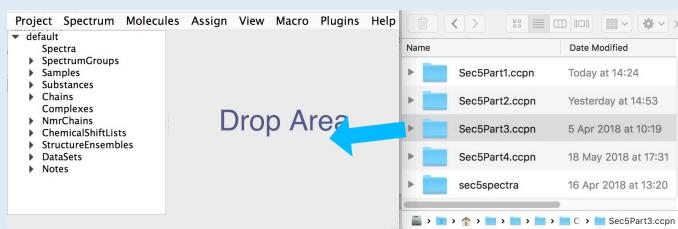
The Sequential backbone assignment in the i+1 direction is fully analogous to the 'i-1' direction except you will need to drop the strip label on the icon.

- Go to the Backbone Assignment module table
- Select chain @- (to display all nmrResidues again)
- Double-click on @18
- Go to the Match Spectrum Display 3D_HCN
- Select the best match @42, drag & drop it onto the 3D_HCN_1 icon
- Continue in this direction to have a stretch consisting of:
@18 @42 @34 @22 @99
- Select the @18 from the Sequence Graph, drag and drop onto the L of stretch LTICGH highlighted in orange in the sequence below 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.ccpn



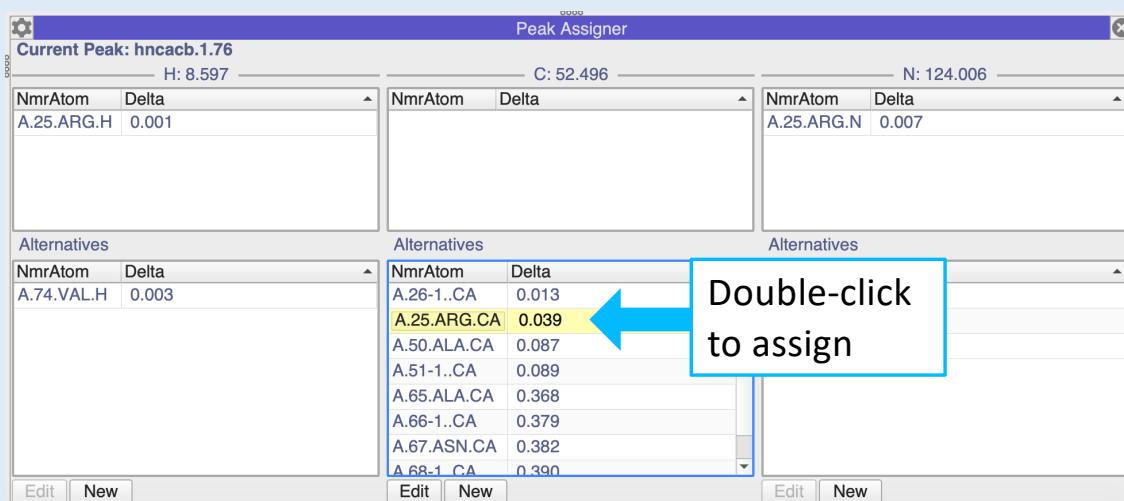
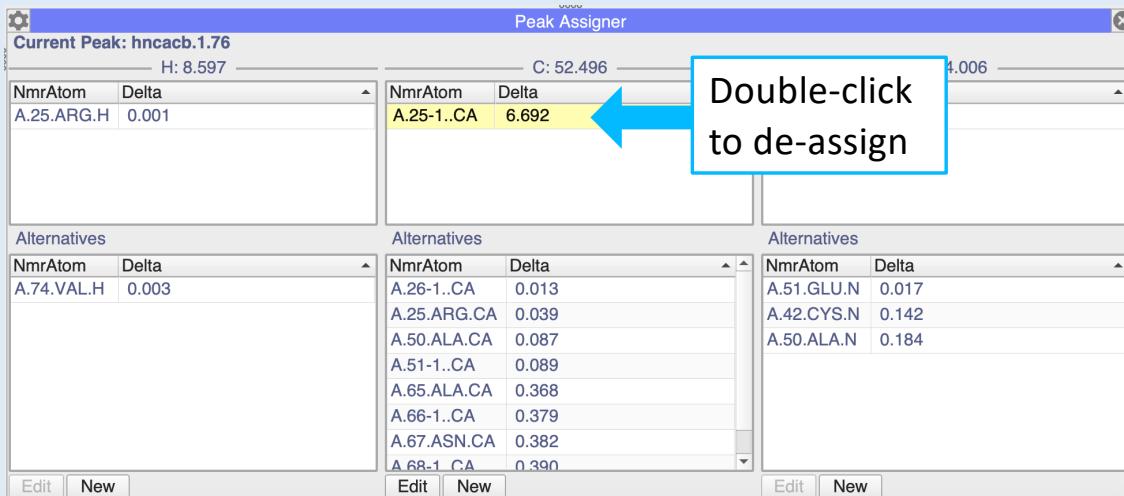
Open Assignment Inspector with shortcut AI

The screenshot shows the Assignment Inspector module. At the top, there is a dropdown menu labeled 'ChemicalShiftList: CL:default'. Below it is a table with columns: ChemicalShift, ChemicalShift Value (ppm), Value Error, NmrAtom, ResidueType, AtomName, and Peak Count. The rows show data for peaks CS:default.376, CS:default.416, CS:default.284, CS:default.212, and CS:default.505. A blue box highlights the 'Value Error' column, and a blue arrow points to it with the text 'Single click'. Below this table is a search bar: 'Filter by NmrAtom(s): Peaks assigned to NmrAtom(s): A.25-1..CA'. Underneath is another table with columns: Peak, Pid, serial, Assign F1, Pos F1, Assign F2, Pos F2, Assign F3, and Pos F3. The rows list peaks hncacb.1.76, hncacb.1.75, cbcacoh.1.42, hncoa.1.22, and hnca.1.37. A blue box highlights the 'Pos F2' column, and a blue arrow points to it with the text 'Double click'.

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.

6A Correcting assignment mistakes

- Open the project **Sec5Part3.ccpn**.
- Open the Assignment Inspector module:
 - Main Menu → Assign → Assignment Inspector or shortcut AI**
 - Make sure the ChemicalShiftList **CL:default** is selected.
 - Click on Value Error** to sort the top table by the Chemical Shift error. Chemical Shifts with unusually high errors should be investigated.
 - Select the row with NmrAtom **NA:A.25-1..CA**. This will populate the peak table below with all peaks belonging to that NmrResidue (not NmrAtom!).
 - Filter the peak table by clicking on **NA:A.25-1..CA** on the left hand side.
 - Click on Pos F2** to sort the peak table by the carbon chemical shift and find the outlier. It looks as though this peak must be incorrectly assigned.
 - Double-click on peak hncacb.1.76**. The spectrum display modules will automatically navigate to this position.



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.

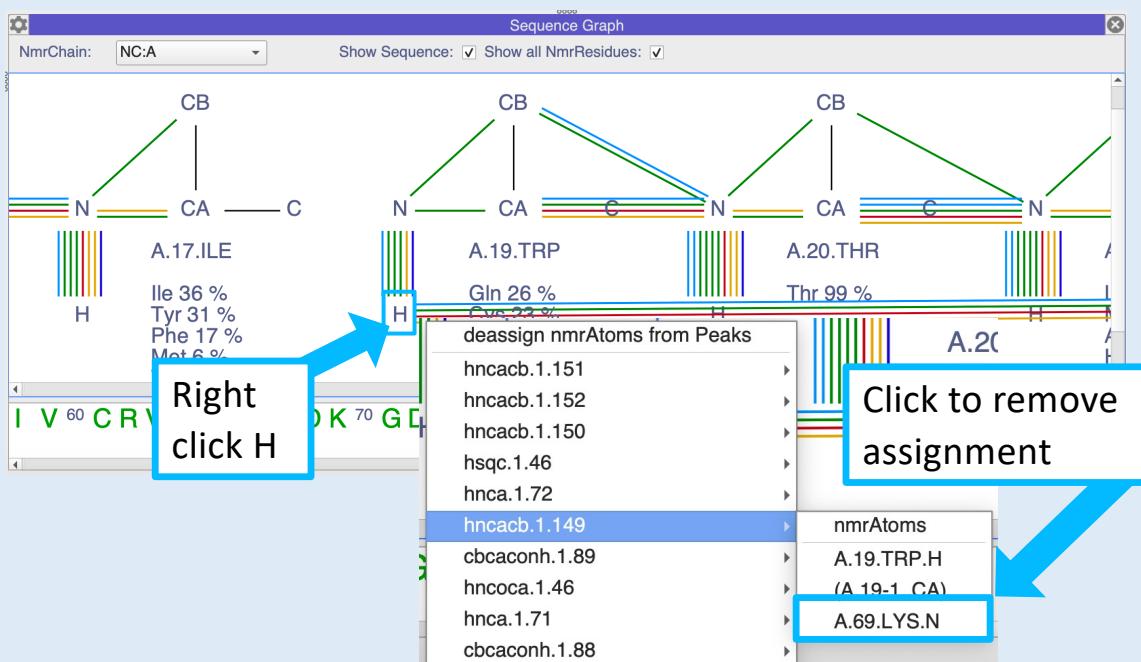
6B Edit the Assignment

- Select the peak which should be reassigned (it may be easiest if you turn the hnca, hncoca and hncacb spectra off in the Spectrum Toolbar)
- Bring up the **Peak Assigner** module either by going to **Main Menu → View → Peak Assigner**, with shortcut PI or by right-clicking on your peak and selecting **Edit Peak**.

The upper panels show the peak assignments, the lower ones show NmrAtoms with Chemical Shifts close the peak position.

- Double-click on A.25-1..CA in the top middle panel. This will remove the incorrect assignment from the selected peak. The correct assignment is likely to be A.25.AR.G.CA.
- Double-click on A.25.AR.G.CA in the lower middle panel to assign this NmrAtom to the selected peak.

Open Sec5Part4 ccpn



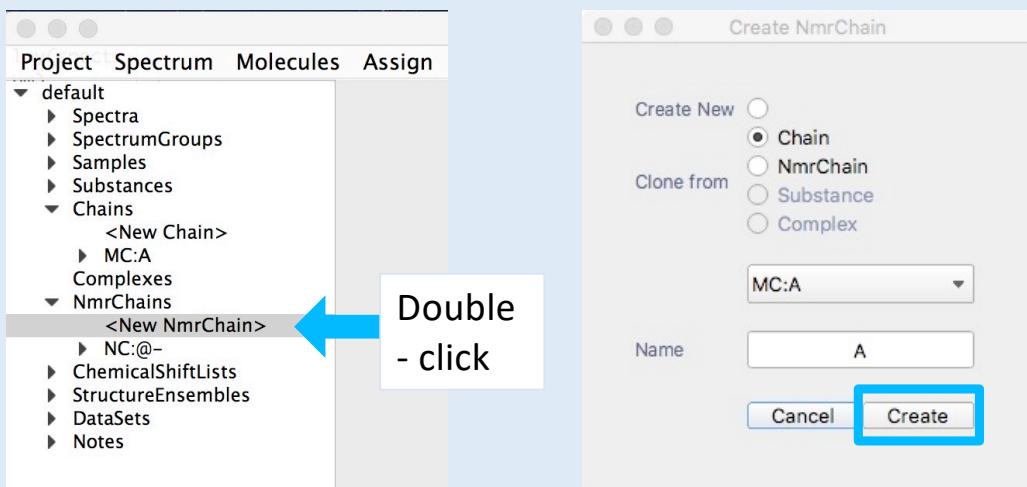
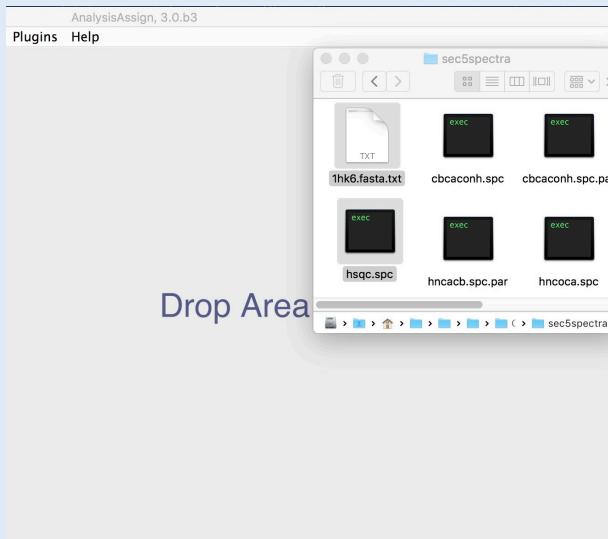
Sec5Part4 ccpn is a project that contains an assignment error; to identify assignment errors you can use the sequence graph. In this project, scrolling through the sequence graph of NmrChain A, you will notice multiple lines connecting the H atom of A.19.TRP to atoms much further down the sequence; in fact they go to the N of A.69.LYS. These are clearly mistakes in the assignment and should be corrected.

6c Correcting an assignment mistake

- Open the project **Sec5Part4 ccpn**.
- Open the **Sequence Graph** with shortcut **SG**.
- Select NmrChain **NC:A**.
- Scroll to the right and search for **A.19.TRP**. This has connections to
- You can follow the connections to **A.69.LYS**.
- Select the **H**, right-click on it and look through each of the peaks assigned to this NmrAtom.
- You will see all the assignments in the submenus. Find the incorrect assignments to **A.69.LYS.N**.
- Click on these incorrect assignments to remove them.

The wrong connections have now been removed, and the peaks have been de-assigned from the incorrect NmrAtoms. You can further edit the peak assignments to add the correct assignments using other tools like the Peak Assigner module (**AP**).

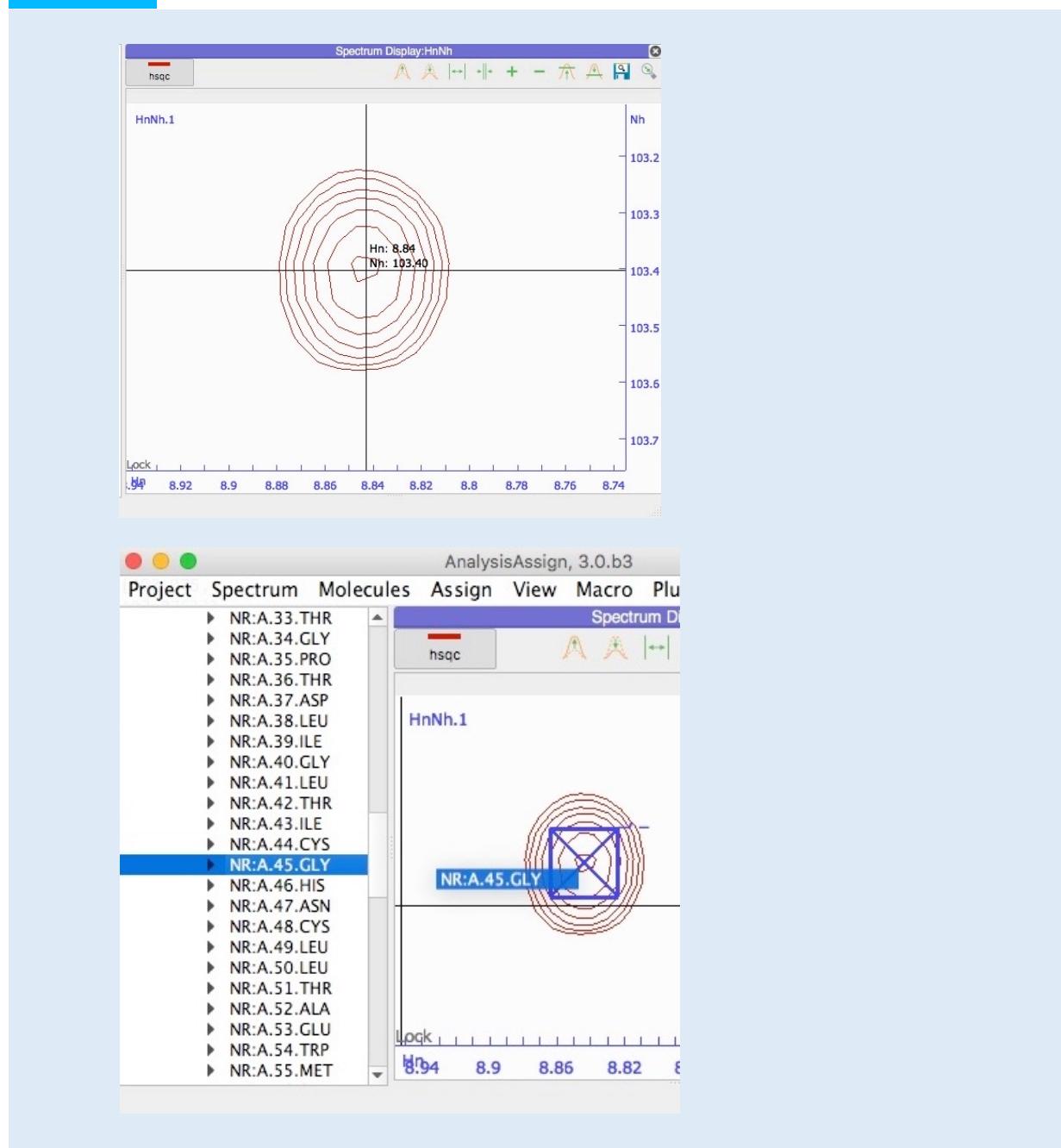
Quick Assignment



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.

7A Create a Chain and an NmrChain

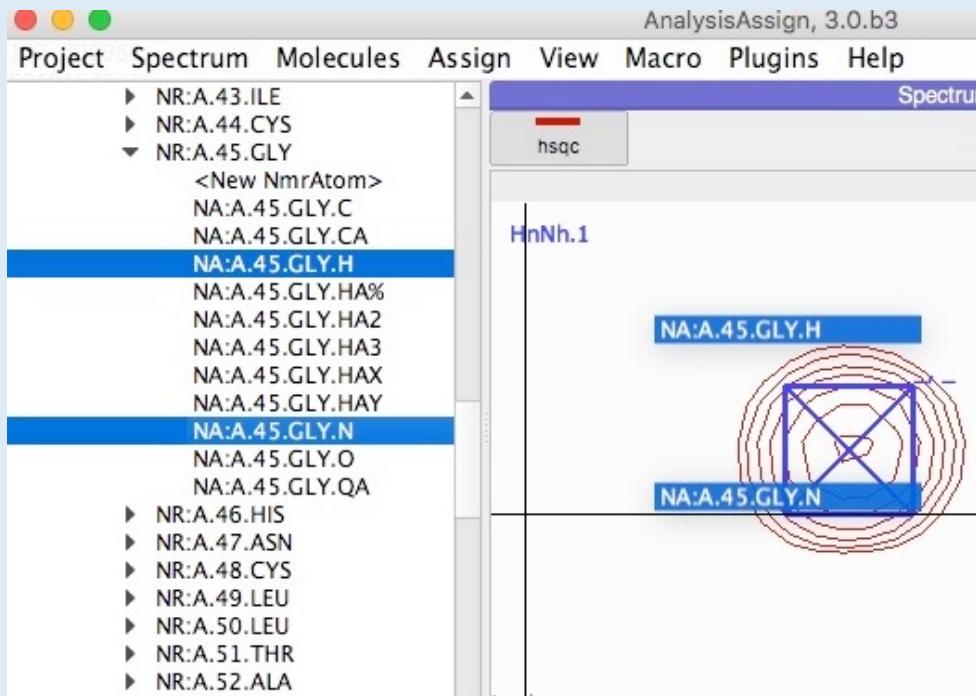
- Open a new project
- **Drag and drop** the `hsqc` spectrum from the `Sec5Spectra` folder into the Drop Area
- **Drag and drop** the Fasta file `1hk6.fasta.txt` into the Drop Area; this will automatically create a new Chain
- **Double-click** on `<New NmrChain>`
- Select **Clone from: Chain**
- Select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



7B 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.



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.

7c 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://forum.ccpn.ac.uk/>

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

V3 (VAH): Minor changes