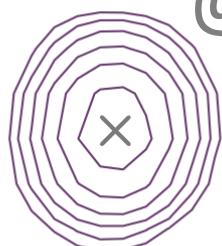


Backbone Assignment Tutorial

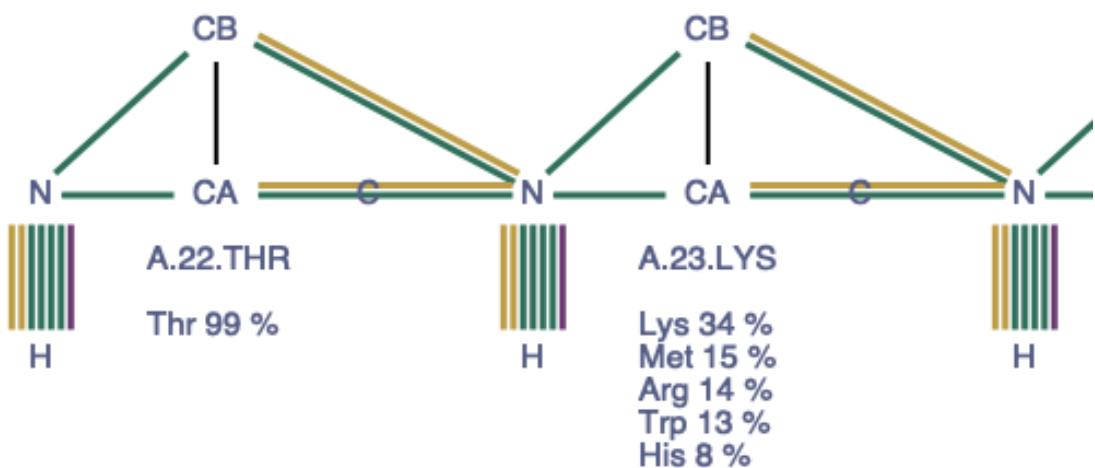
@54H,N



@55H,N



K23H,N



Introduction

These tutorials are designed to guide you through a sequential triple resonance backbone assignment using Ccpnmr AnalysisAssign Version 3.3, 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 **CcpnBBAssignTutorial** directory.

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

Reminder: Basic Operations

Reminder: basic operations in CcpNmr Analysis

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 its properties popup. **Dragging** an item into the **Drop Area** will open it as a module (Spectrum Display, Table etc.)

Spectrum Display

A Spectrum Display can contain multiple overlaid spectra which share the same axes. To show/hide a single spectrum, click on its spectrum 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 by **right-clicking** on a sidebar item and selecting **Open as module**. You can also add additional spectra to a spectrum display module or drag several spectra into the drop area together to open them simultaneously.

Mouse

- Pan -> **Left-drag** in display
- Zoom in/out -> **Scroll wheel** in display
- Context menu -> **Right-click**
- Select a peak -> **Left-click** on a peak symbol "X"
- Move a peak -> select first, then **right-click and drag**

Two-Letter Shortcuts

Press the first letter on your keyboard e.g., **M**, followed by the second letter, e.g., **K** (case insensitive). Press **Esc** to cancel the first letter.

For more commands and operations

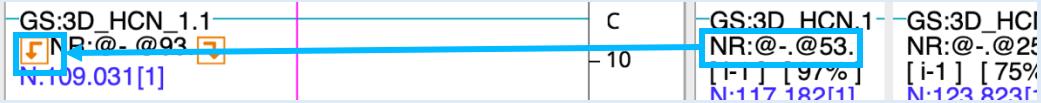
Main Menu → Help → Tutorials → Beginners Tutorial

OR

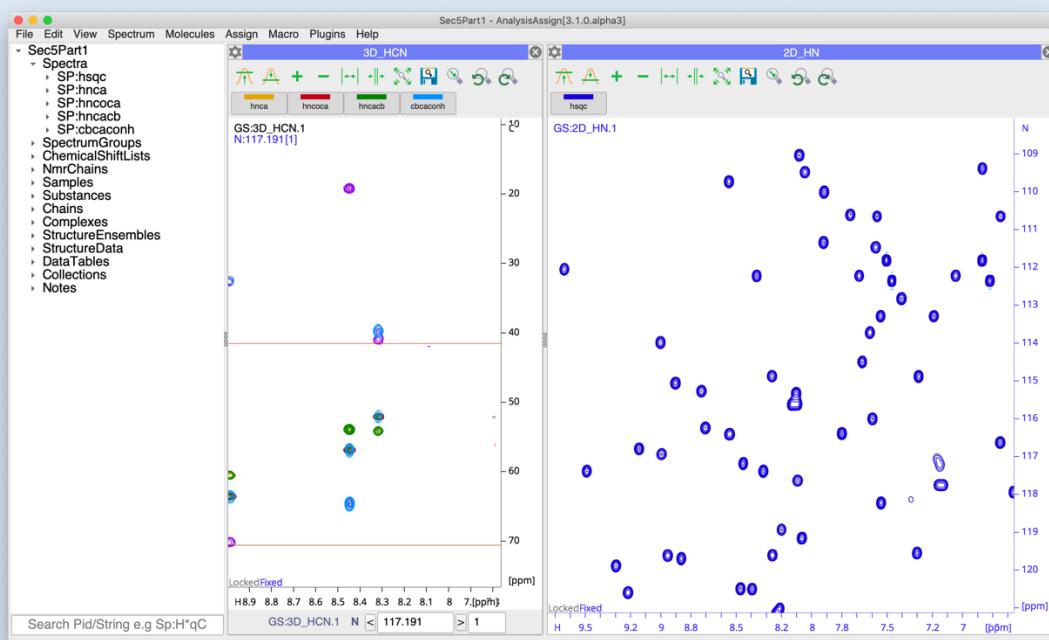
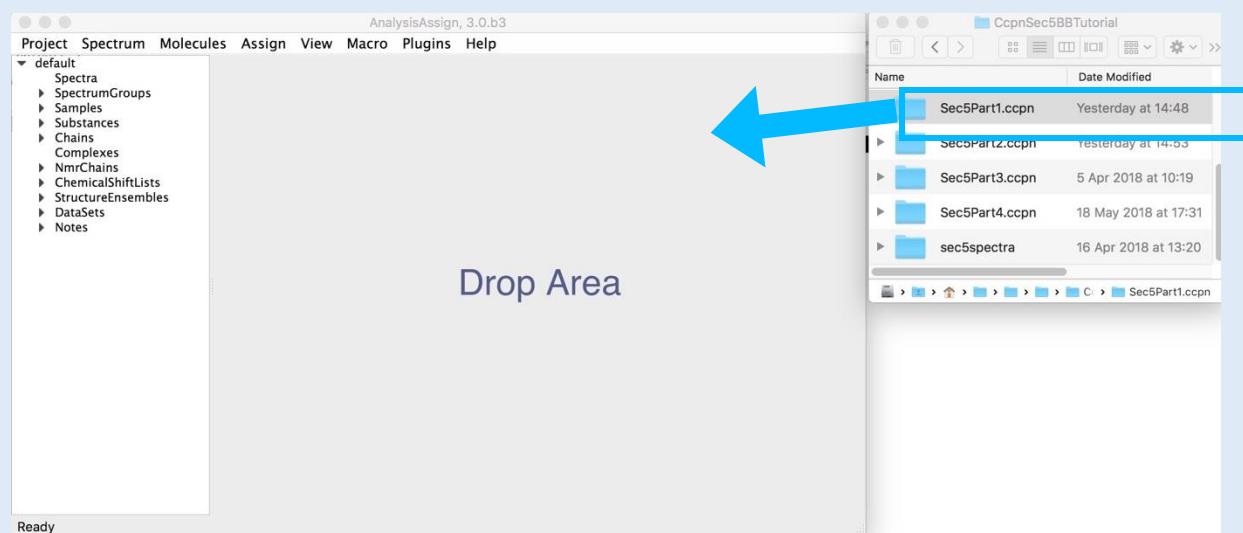
Main Menu → Help → Show Shortcuts

Tutorial Summary

Main Steps

- **Open project** 1
 - Drag in **Sec5Part1 ccpn**
- **Pick HSQC Peaks** 2
- **Set up Assignment:** 3
 - Assign > Set up NmrResidues (**SN**)
 - Sidebar > Chain > <New Chain> using protein name and sequence
 - Spectrum > Set Experiment Types (**ET**)
- **Pick and Assign 3D Spectra:** 4
 - Assign > Pick and Assign (**PA**) using Settings:
 - Tick **Automatic C/CA/CB NmrAtom assignment**
 - For **Pick Peaks** select **Display** and **3D_HCN** from drop-down
 - **Double-click** on an NmrResidue in table to navigate
 - Click **Restricted Pick and Assign** to pick peaks and assign H, N and C atom types for one or more rows at a time
 - Correct carbon atom assignments, if necessary, using Assignment > NmrAtom Assigner (**AN**)
- **Open project** Sec5Part2 ccpn 5
- **Sequential Assignment / Linking NmrResidues:**
 - Assign > Backbone Assignment (**BB**) using Settings:
 - Set **Match** and **Search** modules to different 3D Spectrum Displays
 - Double-click on NmrResidue **@93-1** in BB Assignment table
 - Drag NmrAtom of matching strip onto orange arrow in Search Spectrum Display:
 - Continue linking in i-1 direction
 - For i+1 direction double click **@93** and drag onto right hand arrow
- **Make Sequence Specific Assignment:**
 - In Sequence Graph: drag an NmrResidue onto matching Residue in Sequence (in lower part of module) to assign the whole stretch
- **Inspecting / correcting Assignments:** 6
 - Find/correct mistakes in project **Sec5Part3 ccpn** using Assignment Inspector (**AI**), Peak Assigner (**AP**) and Sequence Graph (**SG**)
- **Quick Assignments:**
 - Drag NmrAtoms or NmrResidues from sidebar onto selected peaks

Open the project **CcpnBBAssignTutorial/Sec5Part1 ccpn**



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

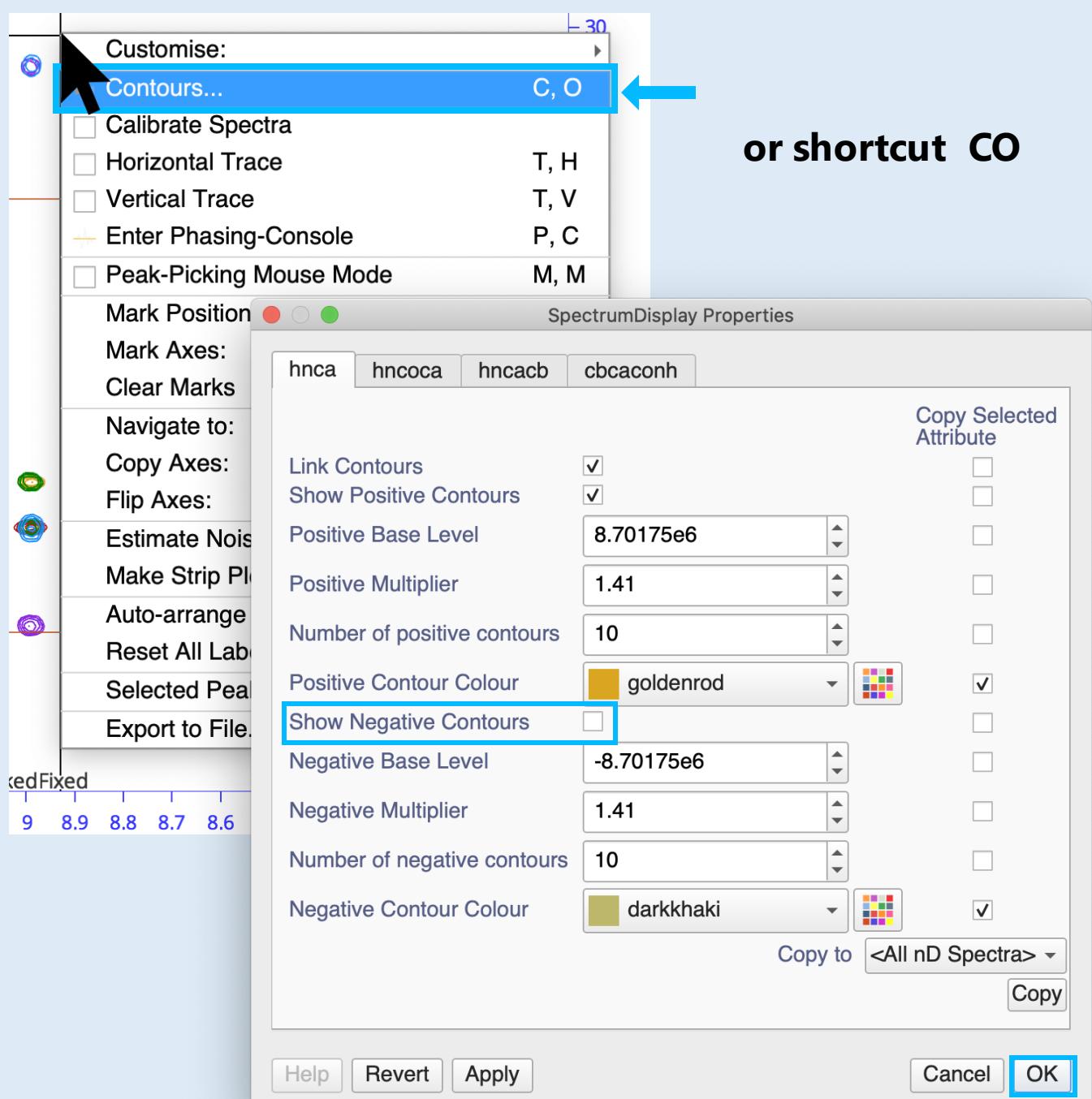
CcpNmr projects are saved as directories with the **.ccpn**.

- Find the project directory **Sec5Part1 ccpn** in the **CcpnBBAssignTutorial** directory and drag & drop it into the program.

The Sec5Part1 project will be loaded in your program window.

You will see five spectra, displayed in two SpectrumDisplays 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)

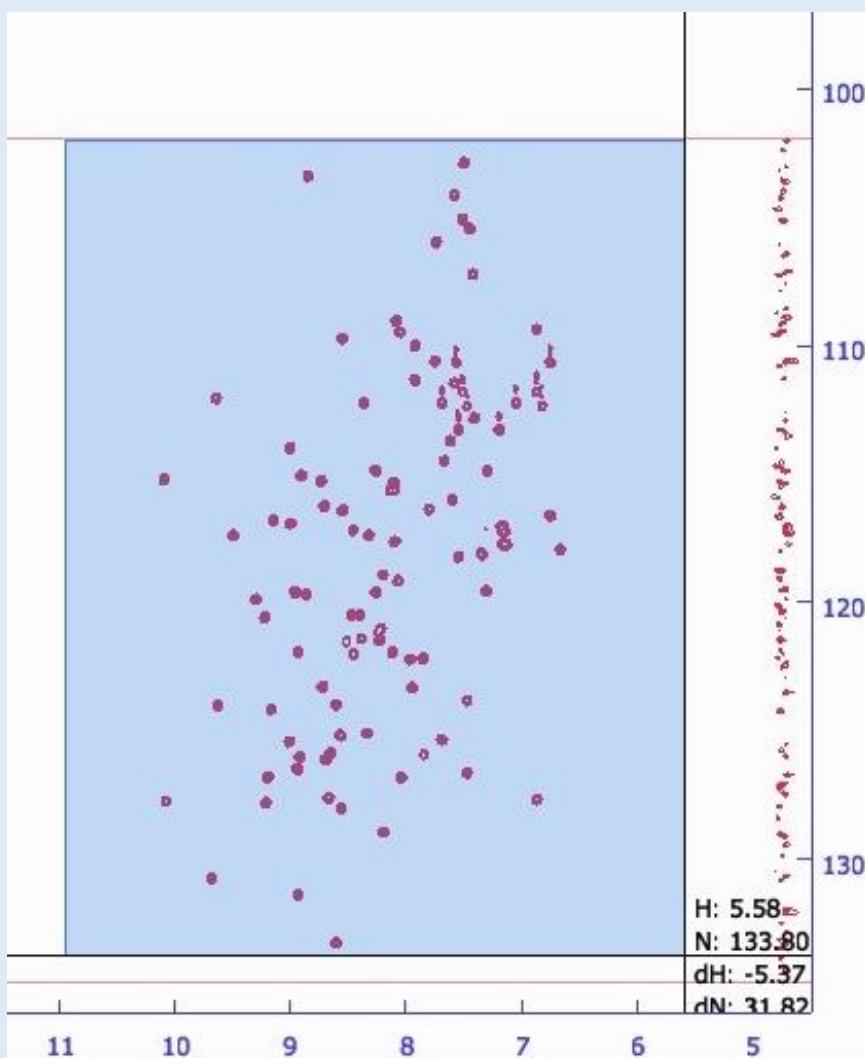


If the contours are not displayed correctly, verify that the path is set correctly in the **Spectrum Properties** popup under the **General** tab options (you can open this by **double-clicking** on the spectrum in the sidebar). Spectra are located inside the **CcpnBBAssignTutorial/sec5spectra** directory.

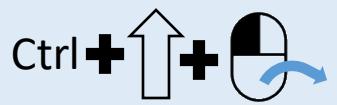
1B Set Spectrum Properties: Contours.

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

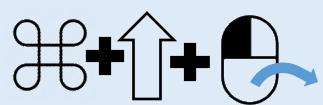
You can also perform these actions for each individual Spectrum in the **Spectrum Properties** popup; open them by **double-clicking** on the spectra in the sidebar.



Linux / Windows:



Mac:



2A Picking peaks in the HSQC

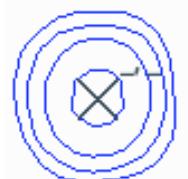
You can pick peaks either manually or automatically.

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

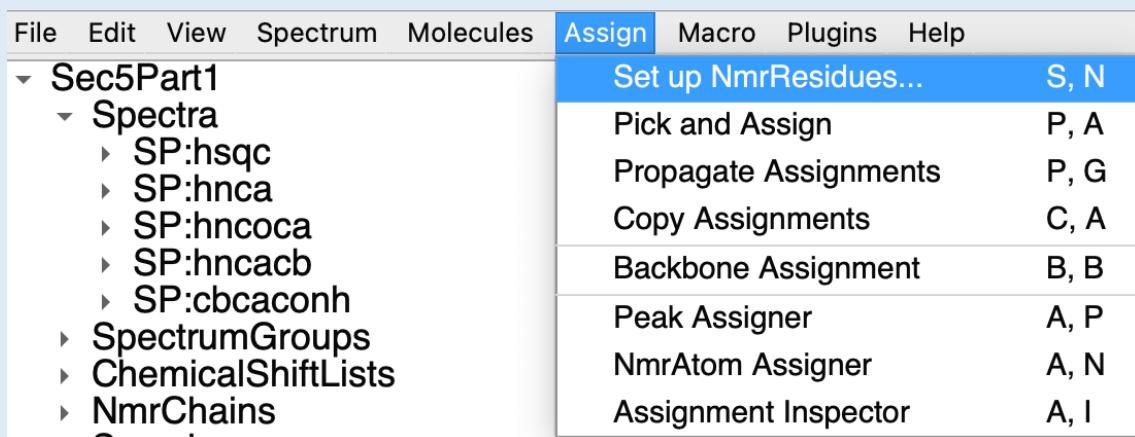
- Click on the spectrum display, hold down **Ctrl** (or **Cmd** for Mac) + **Shift** and **left-drag** the mouse to create a blue picking box in the regions:
H → 11-6
N → 102-134.50
- Then release the button and keys.

The crosses in the spectrum mark the peak positions picked.

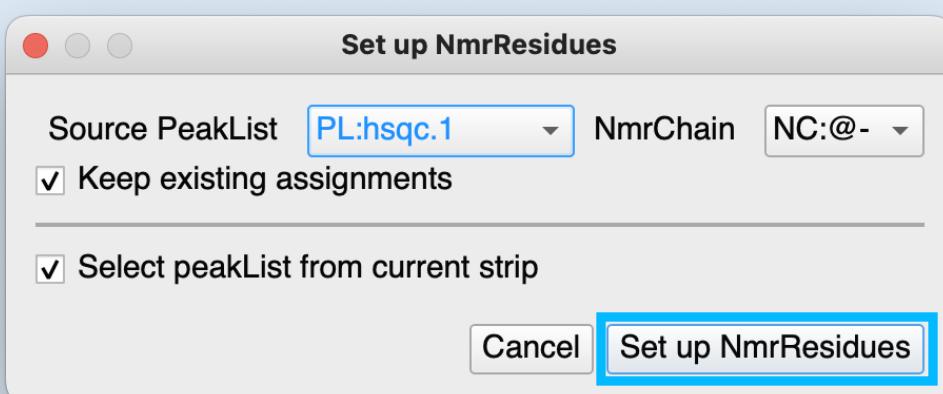
The hyphens separated by a comma indicate that the dimensions of these peaks are unassigned.



For more information about peak picking including other ways to pick your peaks, go to
<https://www.ccpn.ac.uk/manual/v3/PeakPicking.html>



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

- Go to **Main Menu** → **Assign** → **Setup NmrResidues** (or use shortcut **SN**)
- Source PeakList: **PL:hsqc.1**
- NmrChain: **NC:@-**
- Click **Set up NmrResidues**

Each peak will now have a label such as **@1H,N**. Use the shortcut **PL** to toggle between different ways to display the peak labels or go to the **SpectrumDisplay Settings** (click on the gear icon in the top left hand corner) to set the way you wish to view your PeakLabels. There are also further settings if you **double-click** on the PeakList in the sidebar.



<New Chain>

Double-click

New Chain

Chain Name	A
Substance Name	Sec5
Molecule Type	protein
Comment	> Optional <
Sequence Start	1
Sequence	HMRQPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTICGHNCLLAEWMSASKIVCRVGQA KNDKGDIIVTTKSGGKGTSTVSFKLLKPEK

Add NMR equivalent atoms

Add non-stereospecific atoms

Add extra pseudo-atoms

Cyclic Polymer

Help Revert Cancel OK

3B Create Chain

- Go to **Sidebar** → **Chains** → **<NewChain>**

Use the default settings with

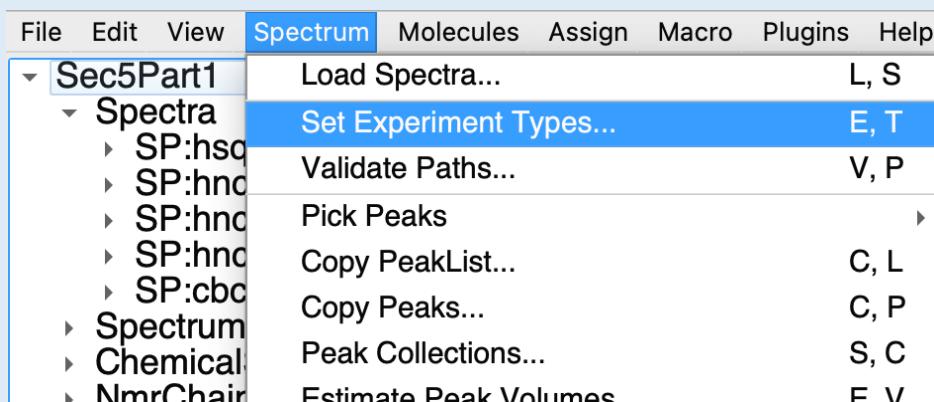
- Substance Name:** Sec5

- Sequence:** (copy and paste)

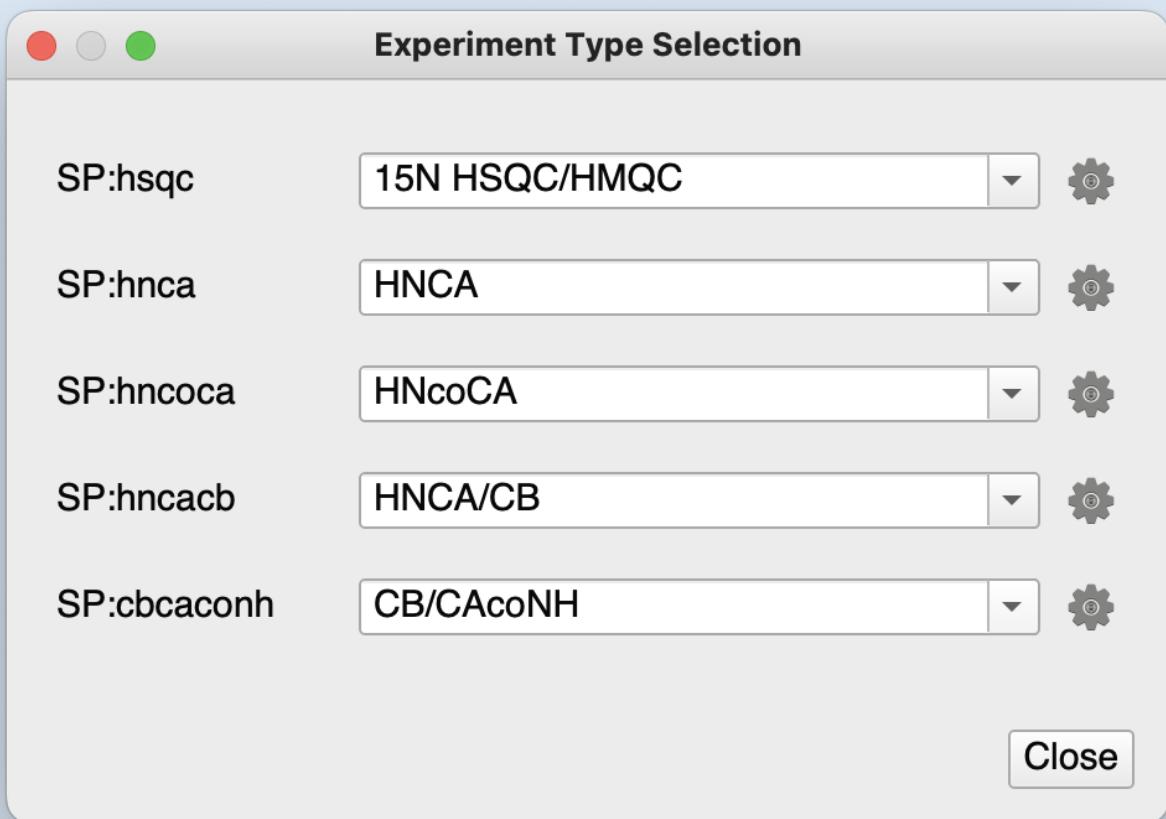
HMRQPPLVTGISPNEGIPWTKVTIRGENLGTGPTDLIGLTICGHNCLLAEWMSASKIVCRVGQA
KNDKGDIIVTTKSGGKGTSTVSFKLLKPEK

- Click **OK**

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



or shortcut ET



3c Set Experiment types

It is possible to tell the programme what kind of experiment each spectrum is.

Although this is not strictly necessary in order to assign you spectra, it will allow you to make use of several helpful features later on.

- 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**.

Setting up the 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 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..

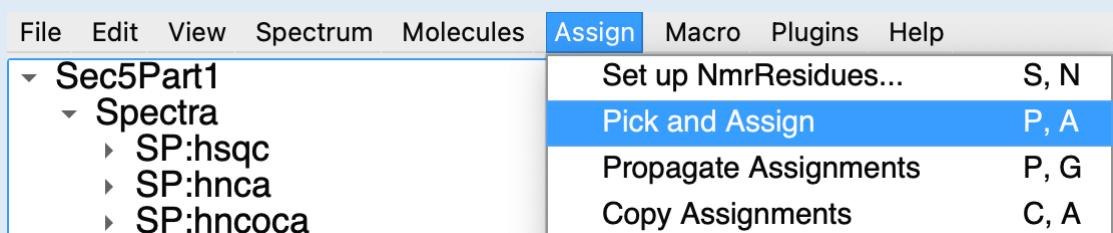
NmrAtom names always start with the nucleus, and default names would be e.g. 'H@31' or 'C@88'. Some names have a special meaning:

- '%' 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).

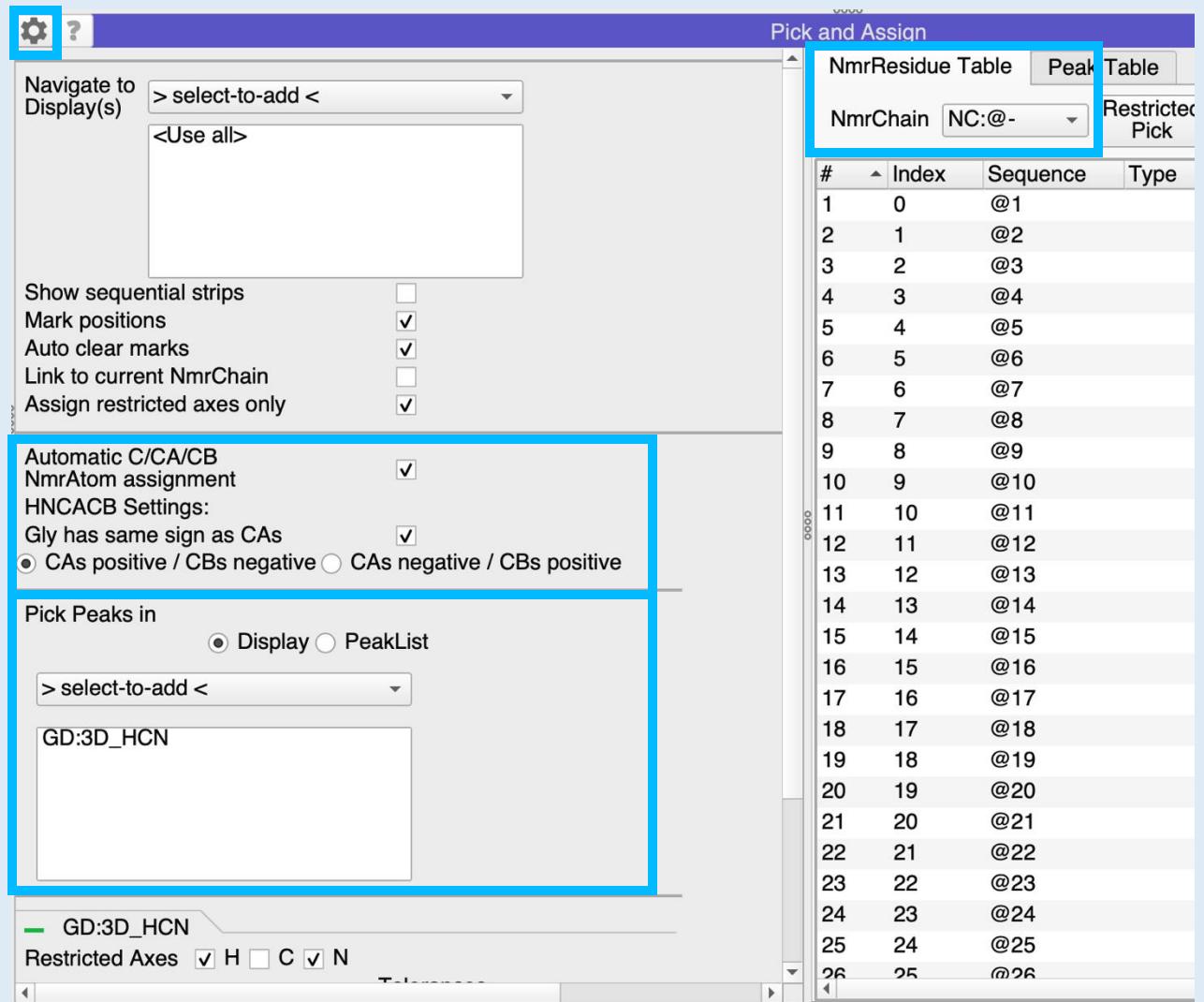
¹ Atoms reside in Residues, which reside in Chains; multiple chains can form a Complex.

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/DS9IZzNsBbQ>



or shortcut PA

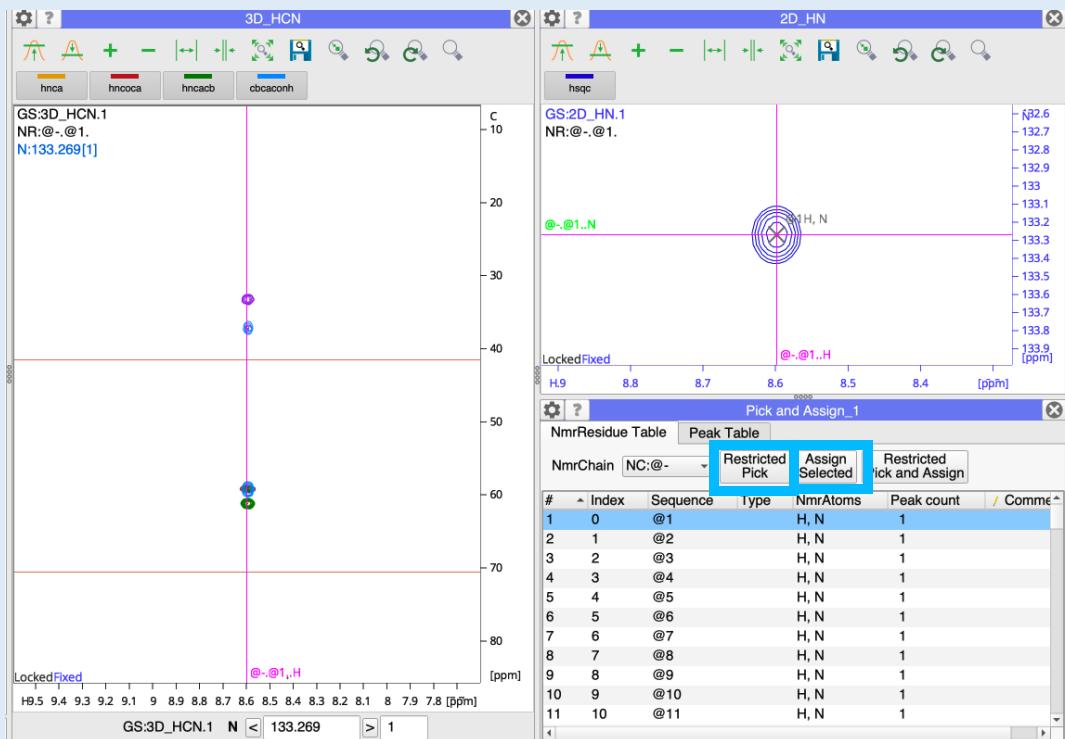


4A Pick and Assign 3D Peaks Setup

We will pick the 3D spectra based on the HSQC spectrum using the **Pick and Assign** module:

- Go to **Main Menu** → **Assign** → **Pick and Assign**, or shortcut **PA**.
- Open the Settings (gearbox icon) and select:
 - **Automatic C/CA/CB NmrAtom assignment** (using the options shown above)
 - **Pick Peaks in Display 3D_HCN**
(if required, remove **<Use All>** by **right-clicking** and selecting **Remove**)
 - Leave the remaining options as default and close the settings
- Ensure the selected NmrChain is **NC:@-**.

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.

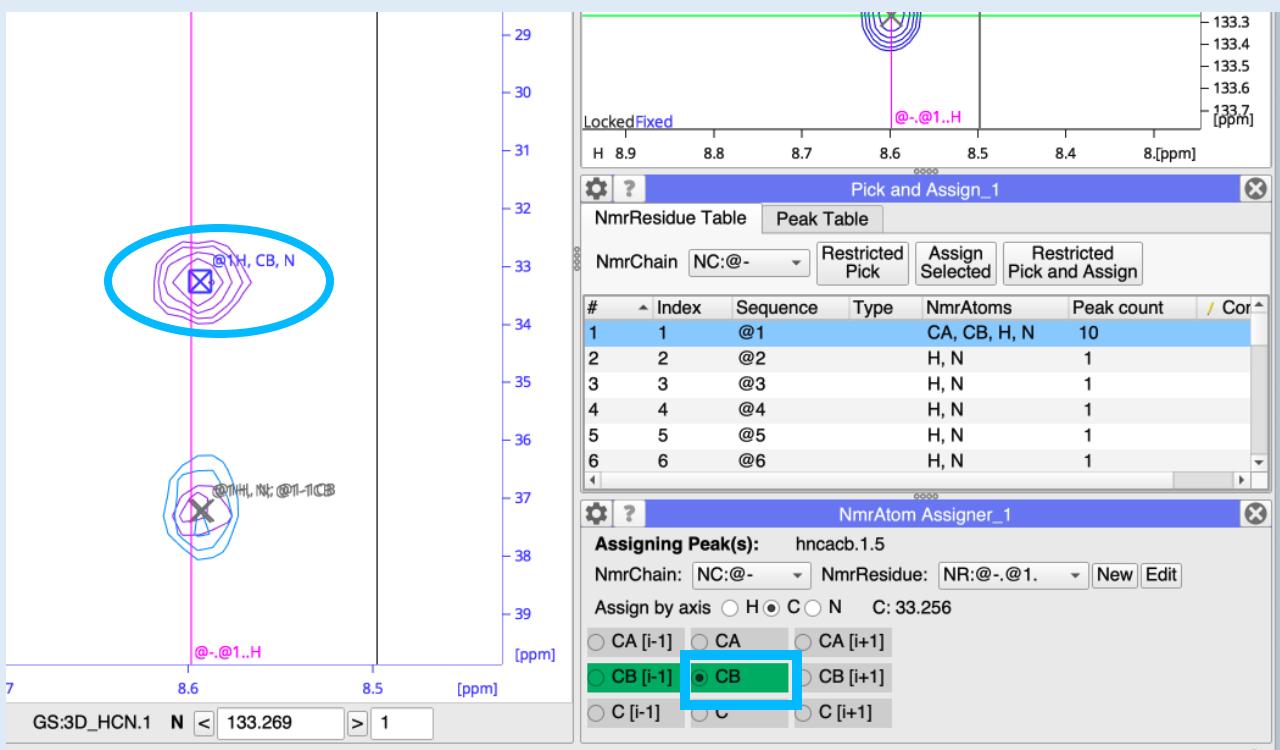
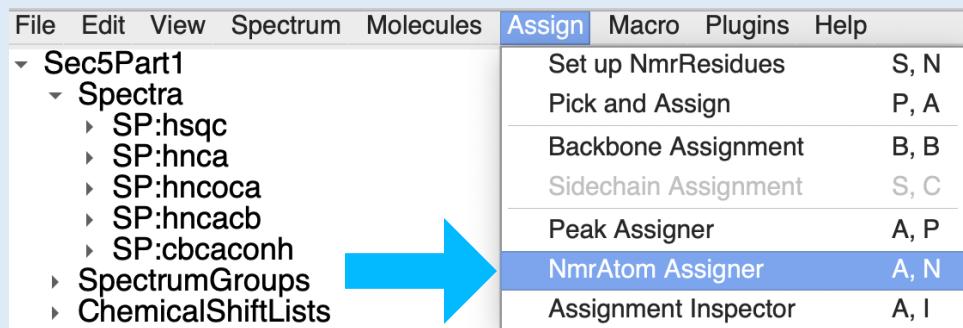
- Inspect the 3D peaks and delete any noise peaks.

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.

This transfers the H and N atom assignments from the 2D spectrum to the 3D spectra and assigns the Carbon NmrAtoms.

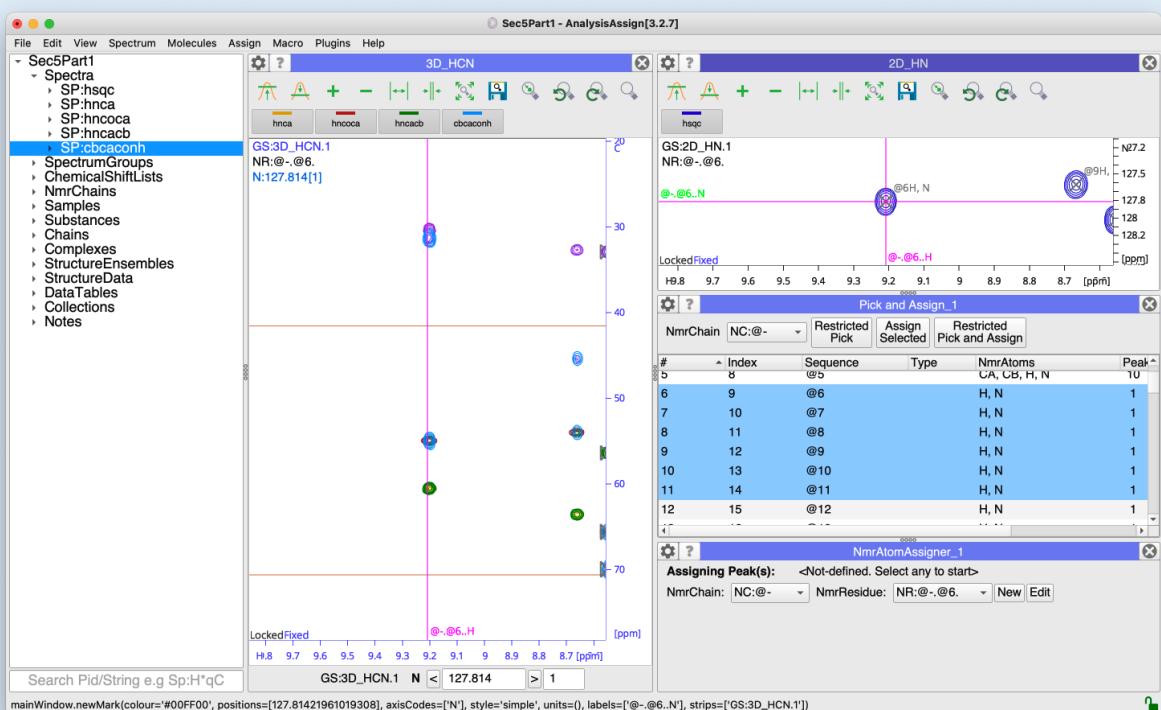
or
shortcut
AN



4C Correcting carbon atom type assignments

Occasionally you may find that the Carbon atom types have been assigned incorrectly (e.g. if peaks are overlapped, too many peaks have been picked or the peak intensities are unusual). In this case you can make corrections using the **NmrAtom Assigner** module:

- 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
- The NmrAtom Assigner will predict the assignment for the carbon dimension, using green for likely and orange for less likely assignments.
- Toggle any NmrAtom button to assign/deassign it to the selected peak(s).



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.

You can also Pick and Assign multiple rows (even the whole table) in one go:

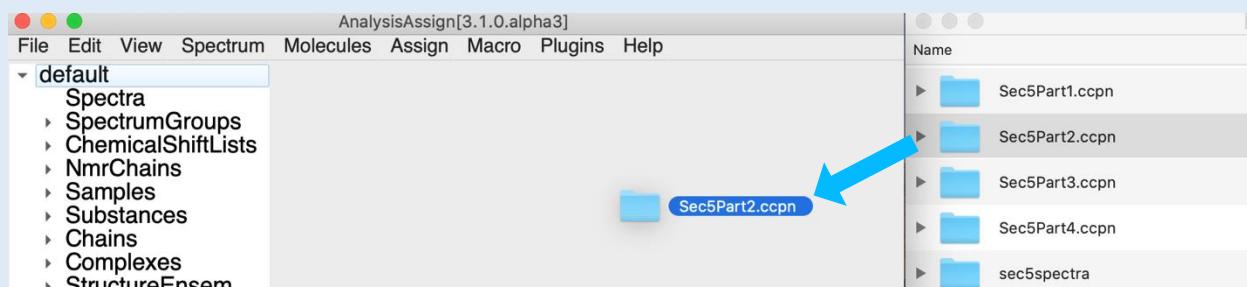
- Use the **Shift** or **Ctrl/Cmd** key to select multiple rows.
- Click on **Restricted Pick** or **Restricted Pick and Assign** to pick and/or assign the peaks for all selected NmrResidues in one go.

It is a matter of personal preference whether you want to go through each strip and make sure it has been correctly picked and assigned, or whether you just pick up on any errors later as you make your assignments.

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.

5 Sequential backbone assignment

Sec5Part2



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 the **CcpnBBAssignTutorial** directory.

5A 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.

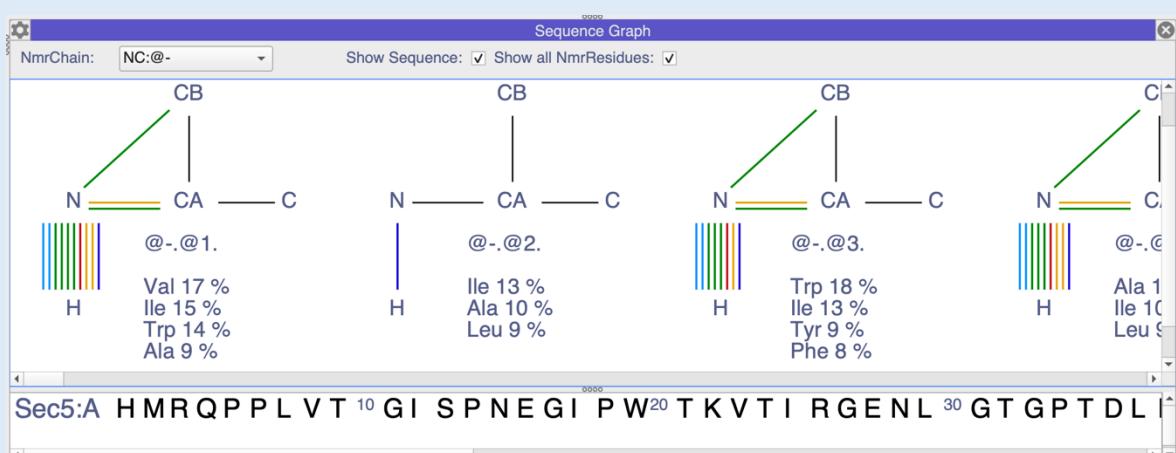
The screenshot shows the AnalysisAssign software interface. At the top is a menu bar with 'Assign' (highlighted in blue), 'Macro', 'Plugins', and 'Help'. Below the menu is a toolbar with buttons for 'Set up NmrResidues' (S, N), 'Pick and Assign' (P, A), 'Backbone Assignment' (B, B) (which is highlighted in blue), and 'Sidechain Assianment' (S, C). To the right of the toolbar is a large window titled 'Backbone Assignment' with a table of NmrChain assignments. The table has columns: #, Index, Sequence, Type, NmrAtoms, and Peak count. The first few rows show assignments for residues 1 through 18. At the bottom of the table is a 'Find matches' button. On the left side of the main window are several settings panels with dropdown menus. Three specific dropdown menus are highlighted with blue arrows: 'Match SpectrumDisplay' set to 'GD:3D_HCN', 'i-1 Matches to show:' set to '3', and 'Search SpectrumDisplay' set to 'GD:3D_HCN_1'.

5B 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

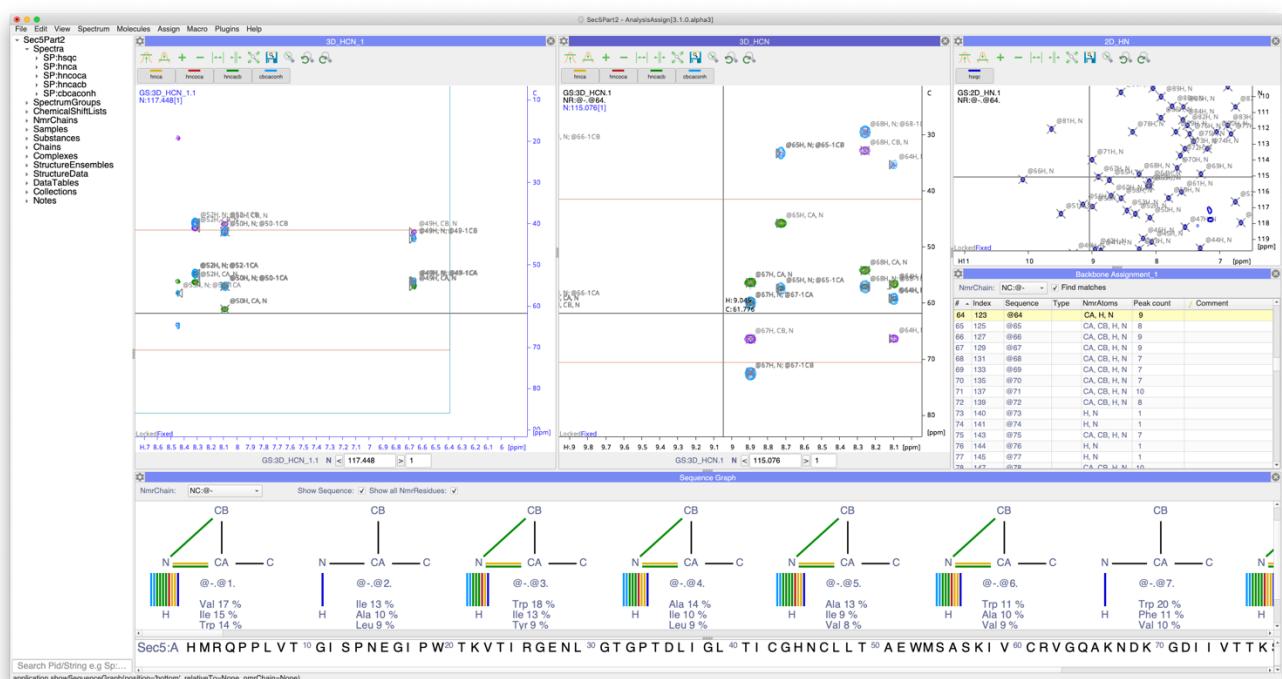
View	Spectrum	Molecules	Assign	Macro	Plugins
Chemical Shift Table			C, T		
NmrResidue Table			N, T		
Residue Table					
Peak Table			P, T		
Integral Table			I, T		
Multiplet Table			M, T		
Restraint Table			R, T		
Structure Table			S, T		
Data Table			D, T		
Sequence Graph			S, G		
Violation Table		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

or Shortcut SG



5c Open and set the Sequence Graph module

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



Note: If, using your own spectra, you do not see the coloured lines in the Sequence Graph, you will need to go to your **Spectrum Properties** and make sure your **Reference Experiment** and **Reference Experiment Dimensions** have been correctly set in the **Dimensions** tab.

5 Sequential backbone assignment

Sec5Part2

The screenshot shows the 'Backbone Assignment_1' module window. A context menu is open over the second row of the table, which corresponds to NmrResidue index 2. The menu items are: Edit NmrResidue @-.@2., Merge NmrResidues, Mark Position, Filter..., and Copy clicked cell value. The 'Filter...' option is highlighted with a blue selection bar. A callout box labeled 'Right click in table' points to the context menu.

#	Index	Sequence	Type	NmrAtoms
1	1	@1		CA, CB, H, N
2	2	@2		H, N
3	4			CA, CB, H, N
4	6			CA, CB, H, N
5	8			CA, CB, H, N
6	10			CA, CB, H, N
7	11			CA, CB, H, N

or

shortcut FT

The screenshot shows the 'Backbone Assignment_1' module window. A blue arrow points from the text 'Double-click on row @93-1' to the row in the table where Index is 169 and Sequence is @93-1. This row is highlighted with a blue selection bar. The rest of the table rows are grayed out. A callout box labeled 'Double-click on row @93-1' points to the highlighted row.

#	Index	Sequence	Type	NmrAtoms
180	169	@93-1		CA, CB
1				

Filter controls at the bottom:

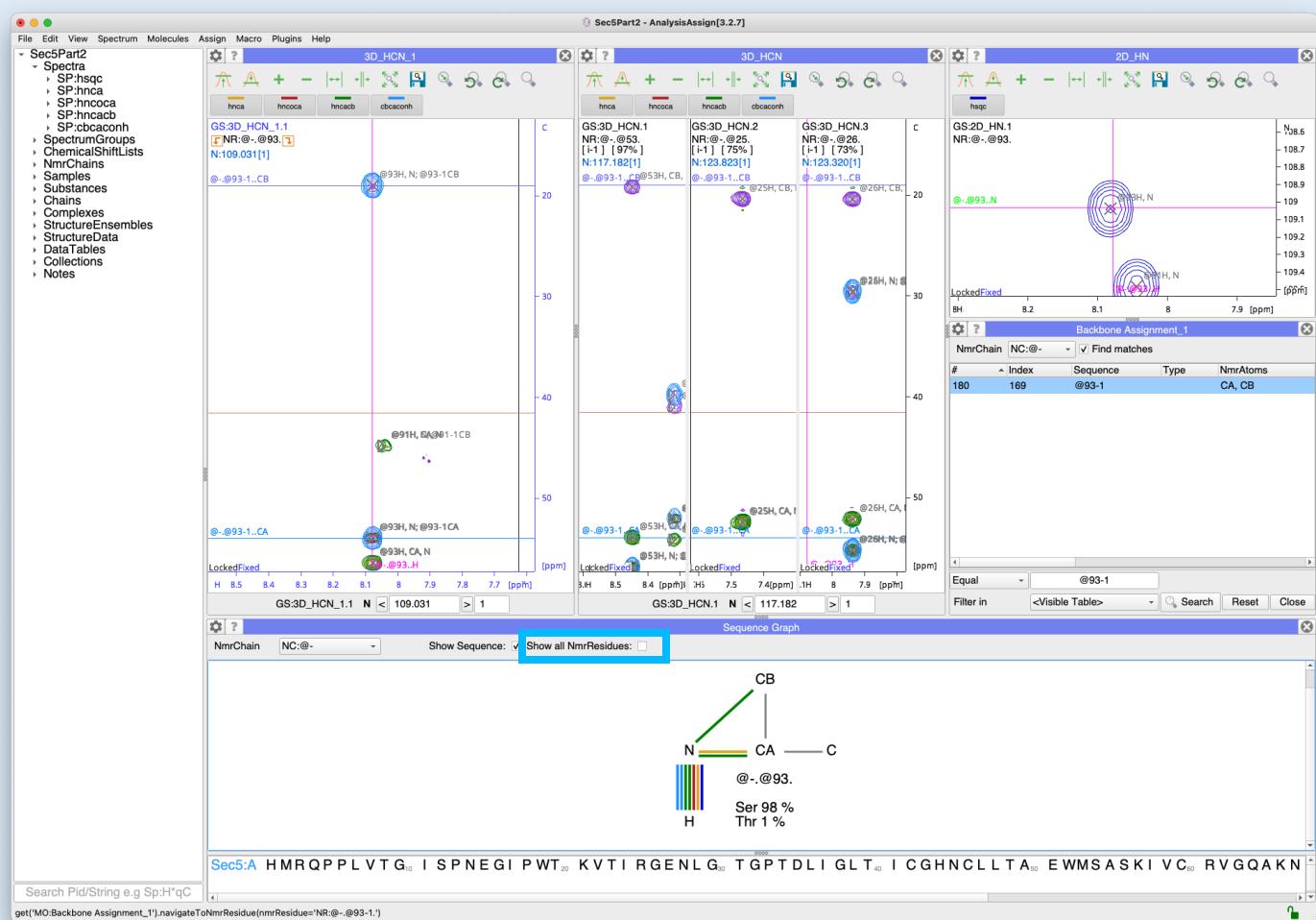
- Equal dropdown set to '@93-1'
- Filter in dropdown set to '<Visible Table>' with a blue selection bar
- Search button (highlighted with a blue selection bar)
- Reset button
- Close button

5D Assign in the i-1 direction ...

- Find the row for NmrResidue **@93-1**

You can do this by filtering for the string **@93-1** if you wish:

- Right-click on the Backbone Assignment module table and click on **Filter...** or use **shortcut FT**
- search for **@93-1**
- Double-click the **@93-1** row. You will see a series of changes in the GUI.



5D ... Assign in the i-1 direction continued

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 SpectrumDisplays.

- If you deselect **Show all NmrResidues** in the SequenceGraph:

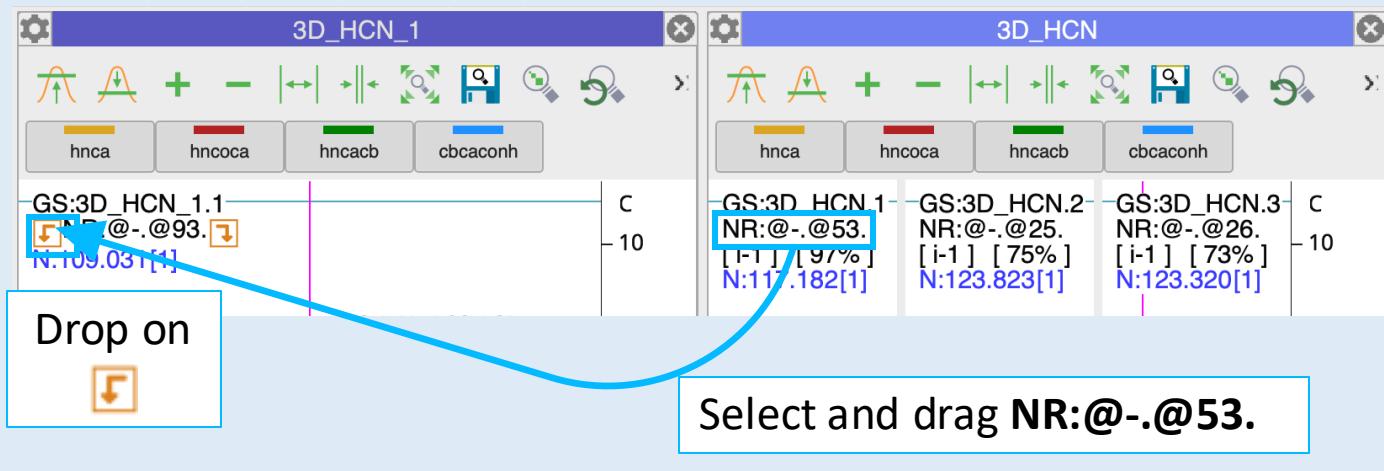
Then **Sequence Graph** will draw a schematic residue, labelled with the NmrResidue name (**@93**) and predictions of the possible residue type(s) below it. The match module (**3D_HCN**) will display three strips in order (left to right) that the AnalysisAssign algorithm thinks best match the i-1 chemical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

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

Locked Fixed

5 Sequential backbone assignment

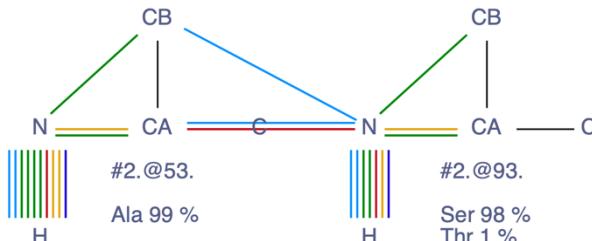
Sec5Part2



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 SpectrumDisplay
- Drag and drop the label on to the in the Search SpectrumDisplay
- 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.

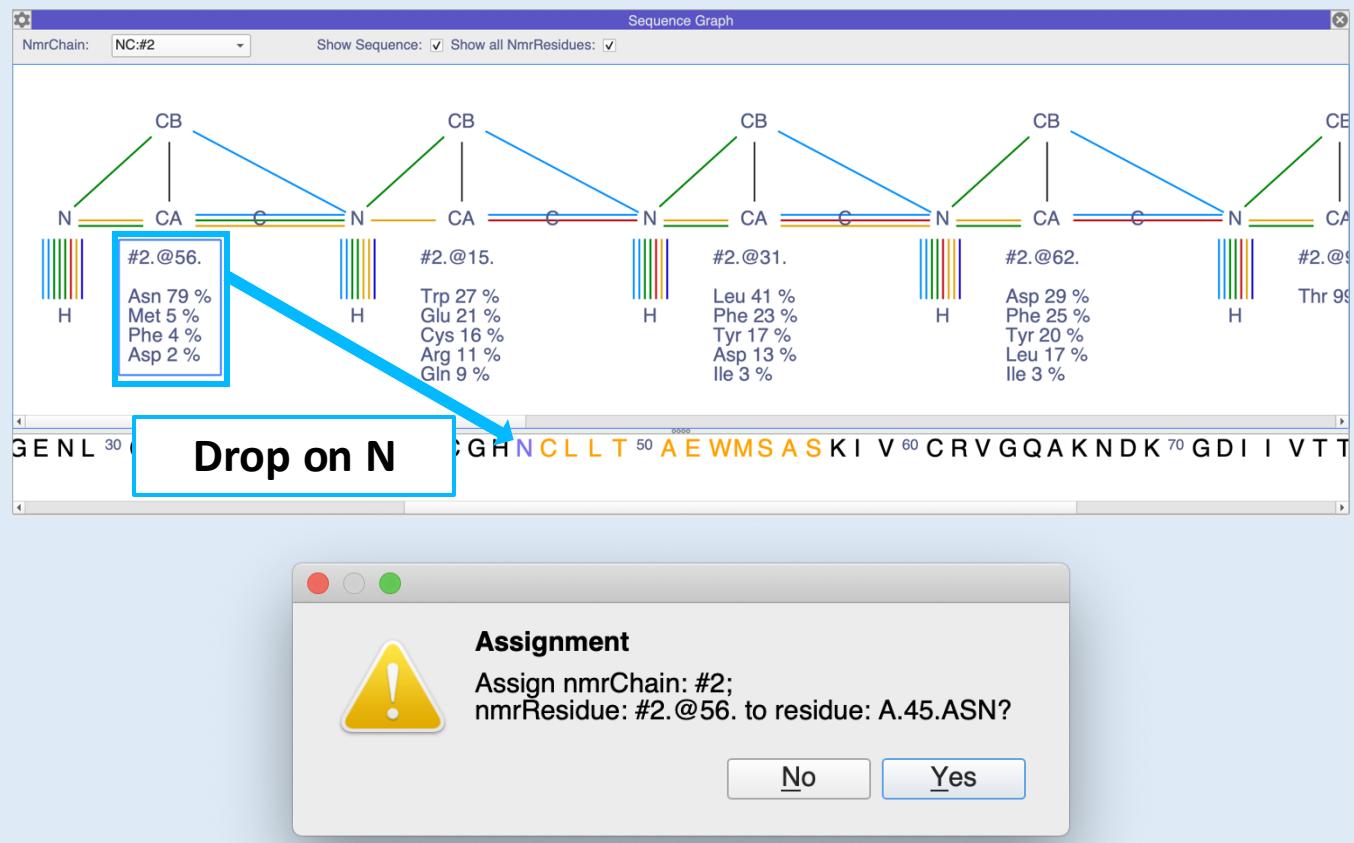
Note: The Settings panels of both the **Sequence Graph** and **Backbone Assignment** modules contain a setting **Link to current NmrChain**. If this is checked, then selecting a new NmrChain in one module will automatically update that used the other module. You can switch this on or off as suits you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

Link to current NmrChain



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:

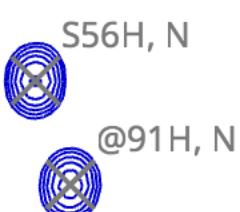
@40 @56 @15 @31 @62 @96 @25 @23 @6 @19 @70 @53 @93

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

- Select e.g. 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.



5 Sequential backbone assignment

Sec5Part2

SpectrumDisplay:HCN

SpectrumDisplay:HnCANH

Drop on

Sequence	Type	NmrAtoms	Peak count
@18	CA, CB, H, N		8

Sequence Graph

NmrChain NC:#4

Show Sequence: Show all NmrResidues:

#4. @18. Leu 81 % Asp 9 % Phe 5 % Tyr 3 %

#4. @42. Thr 100 %

#4. @34. Ile 17 % Val 13 % Cys 8 %

#4. @22. Cys 70 % Trp 13 % Gln 5 % Glu 4 % His 3 %

#4. @99. Gly 99 %

GEN L G₃₀ T G P T D L I C L₄₀ I C G H N C L L T A₅₀ E W M S A S K I V C₆₀ R V G Q A K N D K G₇₀ D I I V T T K S G G₈₀ K G T S T V S F K L₉₀ L K P E K

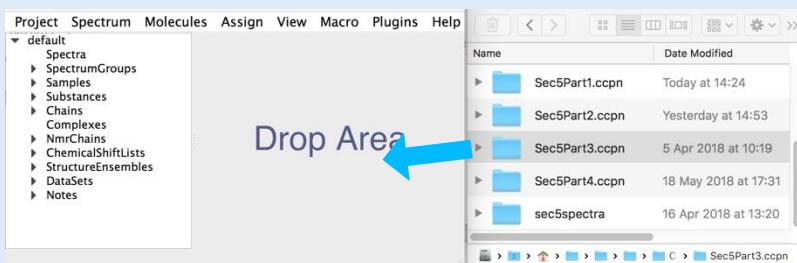
5G Assign in the i+1 direction

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 **LTICG** 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 the Assignment Inspector with shortcut AI

The screenshot shows the Assignment Inspector module. At the top, there's a dropdown menu set to 'ChemicalShiftList CL:default'. Below it is a table with columns: Value (ppm), Value Error (ppm), NmrAtom, ResidueType, AtomName, Peak Count, and Total Peak Count. The first row has a value of 27.927 and a value error of 0. The second row has a value of 29.667 and a value error of 0. The third row has a value of 59.188 and a value error of 3.346. The fourth row has a value of 66.475 and a value error of 1.780. A blue arrow points to the 'Value Error (ppm)' column header. In the bottom half, there's a table titled 'Peaks assigned to NmrAtom(s): A.25-1..CA' with columns: #, Assign F1, Assign F2, Assign F3, Pos F1, Pos F2, Pos F3, and LW F1. There are several rows of data. A blue arrow points to the '#76' row. Another blue arrow points to the 'Pos F2' column header. A callout box labeled 'Single click' points to the 'Pos F2' column header, and a callout box labeled 'Double click' points to the '#76' row.

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.

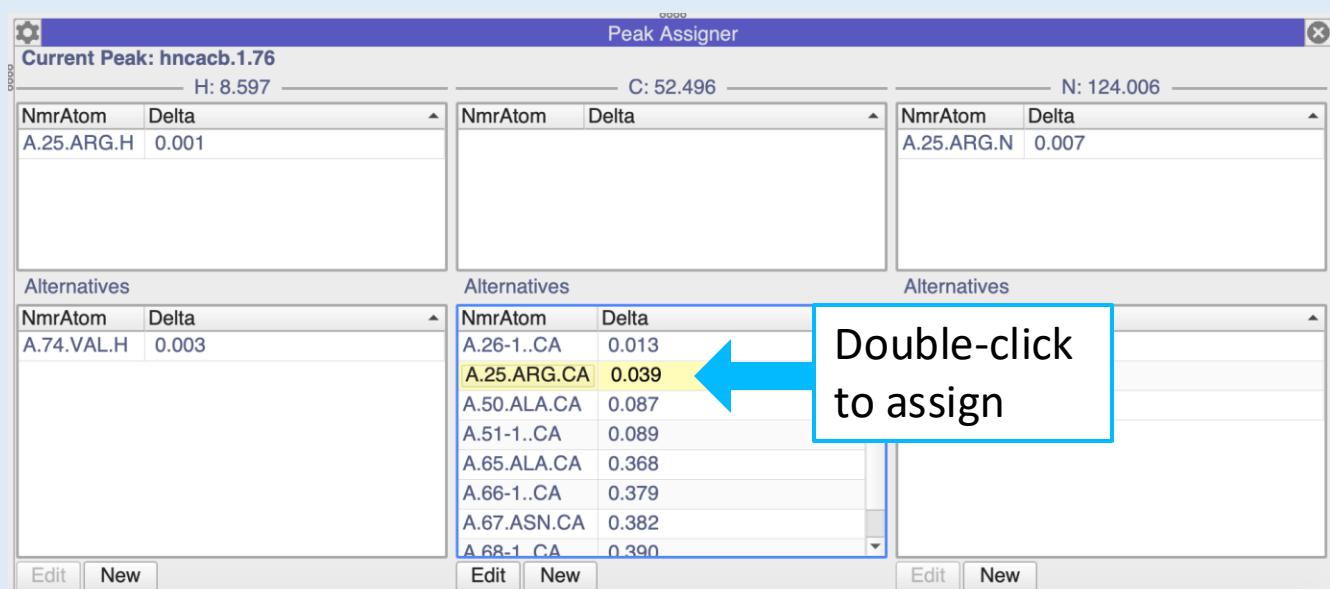
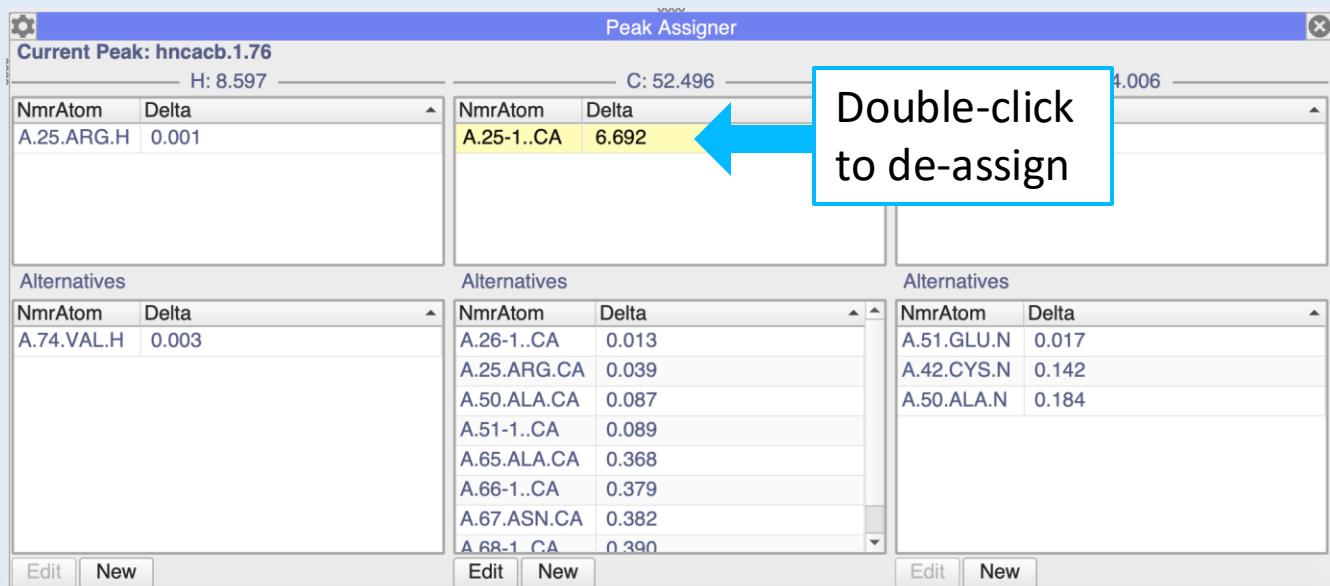
6A Correcting assignment mistakes

- 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 row #76 in the peak table.

The SpectrumDisplay modules will automatically navigate to this peak 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** tab of the **Spectrum Properties** popups of the respective spectra (open by double-clicking on a spectrum in the sidebar).

6B Edit the Assignment

While the peak which should be reassigned is selected in the table:

- Bring up the **Peak Assigner** module either by going to **Main Menu → View → Peak Assigner**, with shortcut **AP** or by **right-clicking** on your peak and selecting **Edit Peak Assignments**.

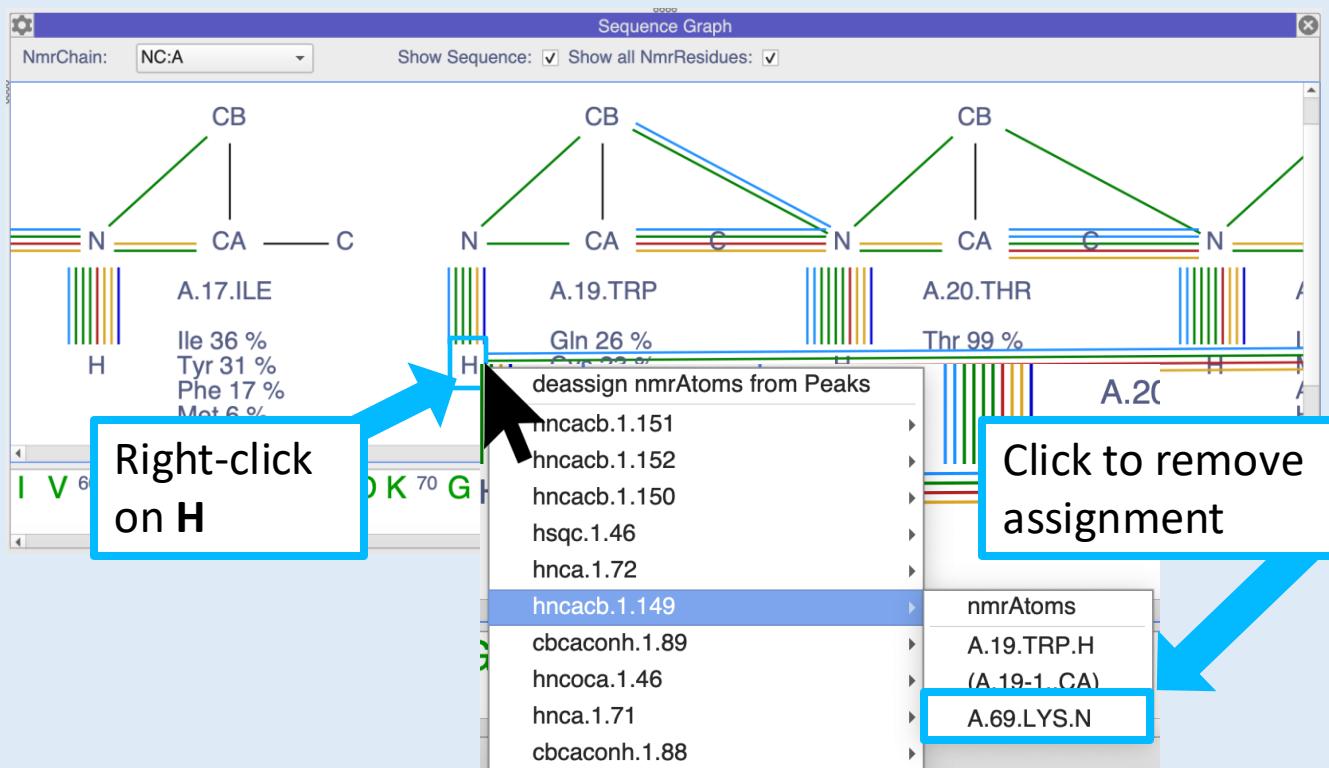
The upper panels show the peak assignments, the lower ones show NmrAtoms with Chemical Shifts close the peak position.

- 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

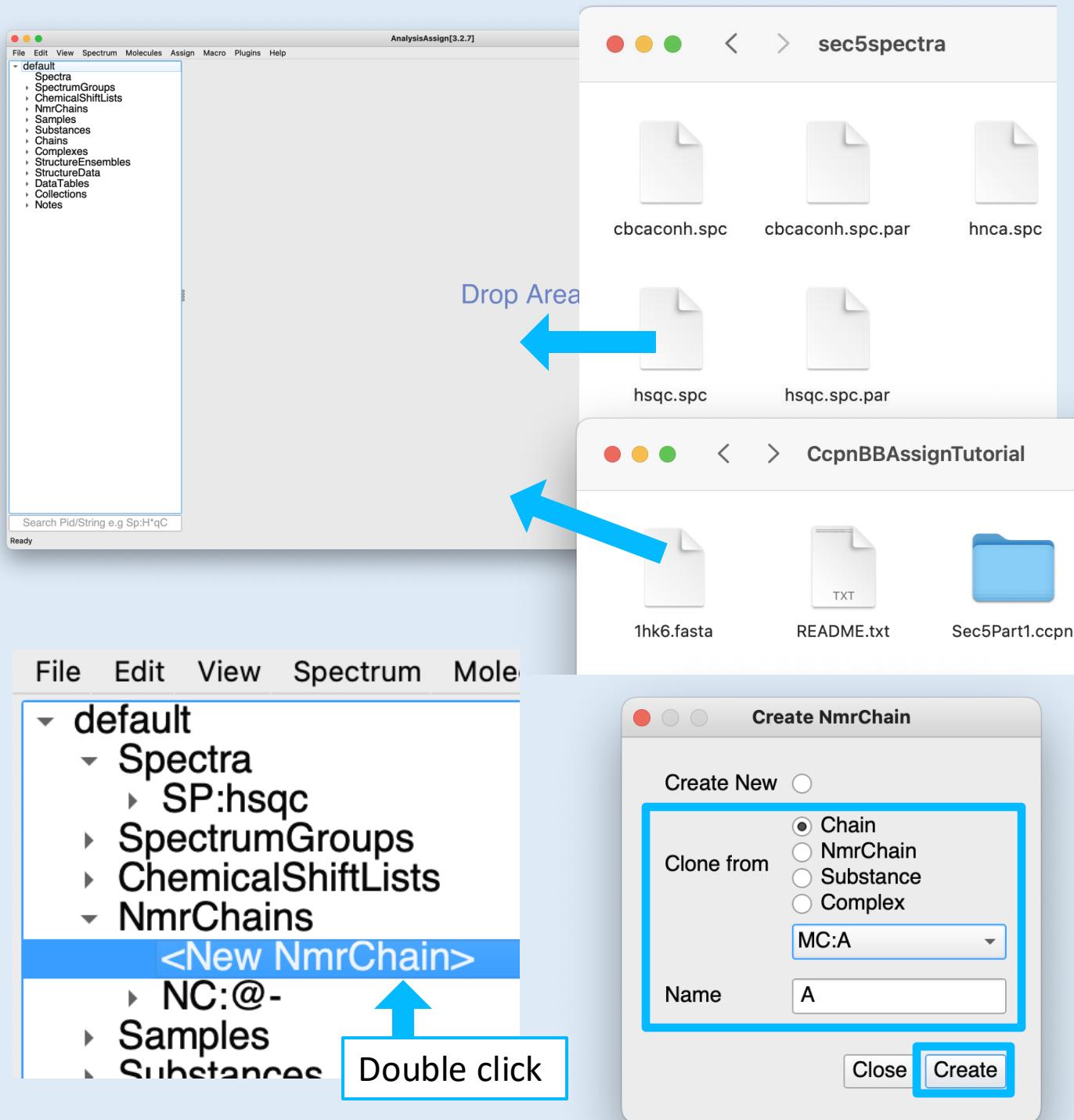
Correcting an assignment mistake

- 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**. You will see immediately, that it has connections to an NmrResidue far away.
If you follow these you will see that the connections are 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**).

Have a look for other incorrect assignments and fix these, too, if you wish.

Quick Assignment

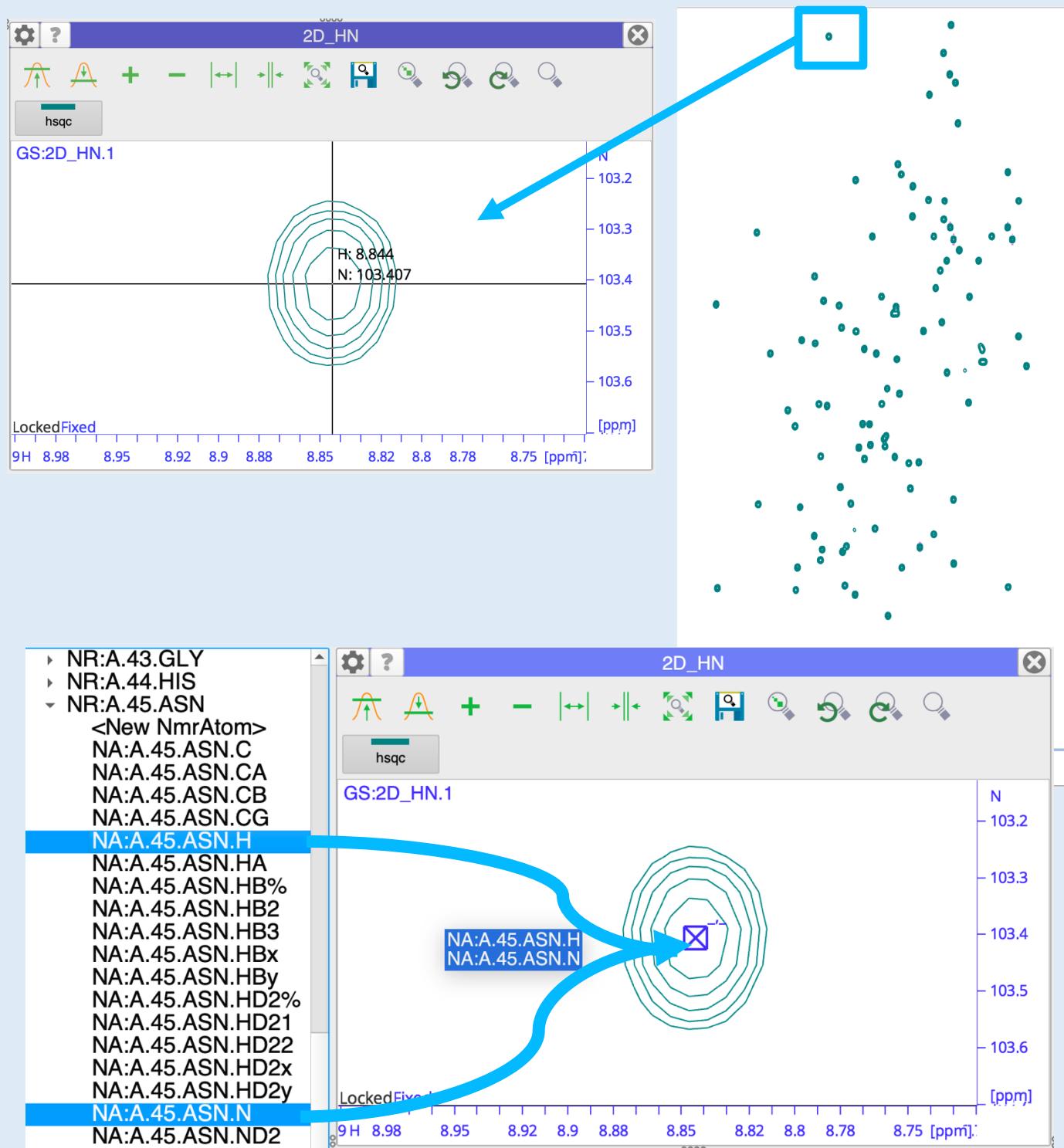


Assuming you have pre-knowledge of a peak assignments, this HOW-TO will show you how to assign residues and atoms to any dimensionality peaks in few simple steps.

7A Create a Chain and an NmrChain

- Open a new project.
- **Drag and drop** the **hsqc.spc** spectrum from the **sec5spectra** folder into the Drop Area.
- **Drag and drop** the Fasta file **1hk6.fasta** into the Drop Area; this will automatically create a new Chain.
- In the sidebar, expand the **NmrChains** section and **double-click** on **<New NmrChain>**
- Select **Clone from: Chain**
- Select **Chain A** from the pulldown
- Name: A
- Click **Create**
- Open the hsqc in a SpectrumDisplay (**drag** into the Drop Area).

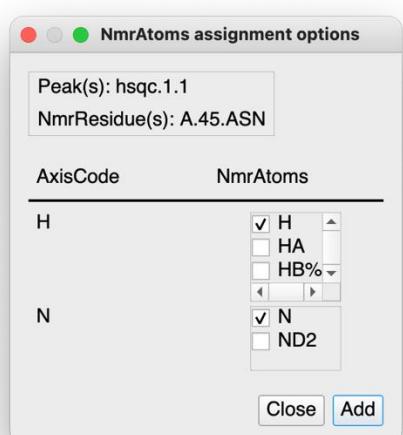
Quick Assignment



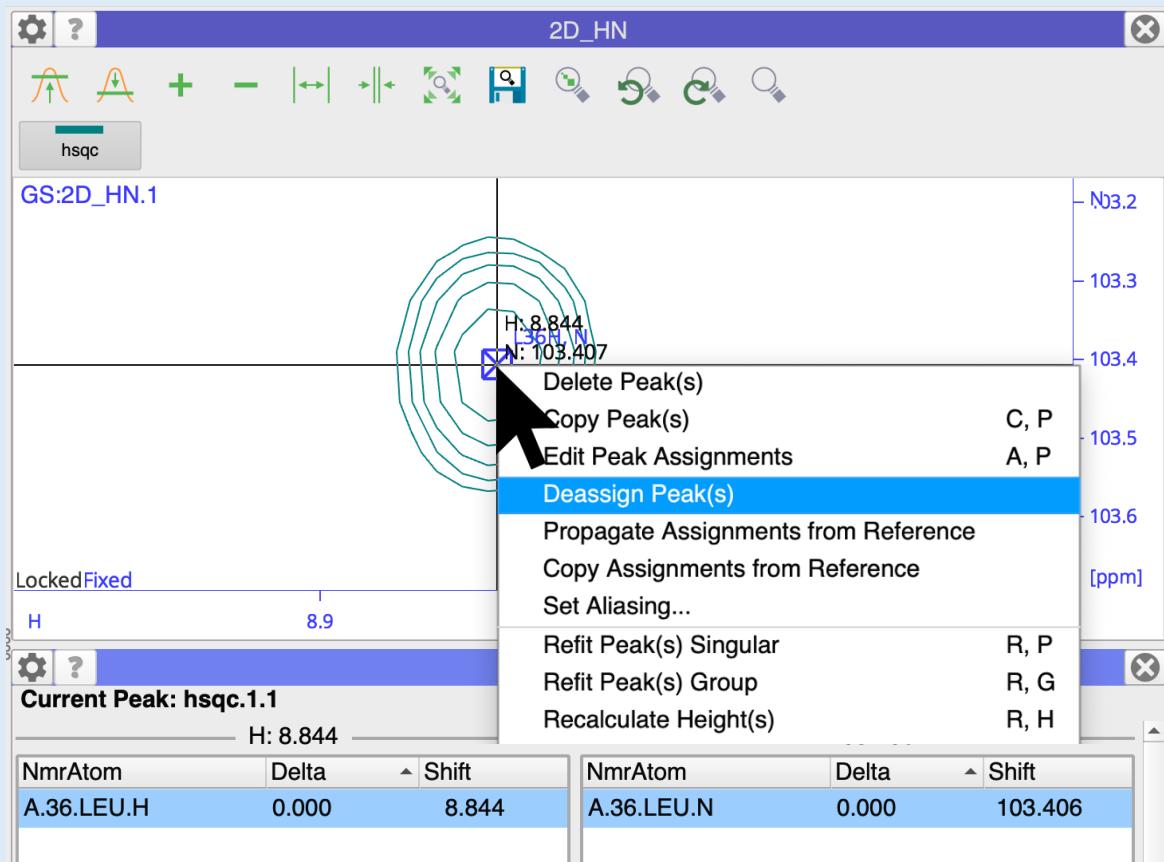
7B Drop the NmrAtoms or NmrResidue onto a selected peak

- Zoom at positions: 8.84 / 103.40
- Pick the peak with **Ctrl (Cmd on Mac) + Shift (↑) + right-click**
- In the sidebar expand the NmrChain **NC:A**
- Search for NmrResidue 45 (**NR:A.45.GLY**)
- Expand it and drag the H and N NmrAtoms onto the selected peak

You can also drag the NmrResidue **NR:A.45.GLY** and then select which atoms to assign to the peak:



Quick Assignment



You can assign any NmrAtoms to any dimensionality peaks as long as the NmrAtom's IsotopeCode matches that of one of the dimensions in the spectrum. E.g., you may assign **45.GLY.HA2** to any peak in an HN spectrum but you cannot assign the **45.GLY.O** to any of them.

7c Quick assignment edit

If you have made a mistake, then:

- You can deassing the peak by right-clicking on it and going to **Deassign Peak(s)**.
- Then reassign the peak or use the Peak Assigner to reassign it correctly.

Note: Dragging NmrAtoms or NmrResidues onto a Peak which has already been assigned will add additional assignments to the peak, not replace them.

Contact Us

Website:

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Suggestions and comments:

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Issues and bug reports:

<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