Backbone Assignment – sec5.

This tutorial assumes that the Introductory tutorial has been completed.

This tutorial will require the spectra and projects contained in CcpnSec5BBTutorial

To open the first tutorial project, type *assign Sec5Part1* into the terminal, or open the software and drag the project directory onto the main window.

Setting spectrum paths

This project was setup on another computer, so the spectrum paths have changed. The program tries to guess the new paths starting from the new project path, but it sometimes cannot find the spectra. To fix this, double click on each spectrum in the Sidebar and the path field will show the currently specified spectrum path. This text can be manually edited or the button to the right of the text box can be clicked and a popup will appear enabling selection of the spectrum. Once all spectra have the correct path, the contours will be displayed in the spectrum modules.

Changing contour displays

For this HSQC there are a few peaks with negative contours around them and peak picking will pick using displayed contours. In this case you may want to hide the negative contours, so in the case double-click on SP:hsqc in the sidebar to display the Spectrum Properties Popup, go to contours tab and uncheck the box labelled "Show Negative Contours" to hide the negative contours. You may also want to adjust the positive contour levels of this spectrum, which can be done by changing the values in the Positive Base Level, Positive Multiplier and number of positive contours boxes in the Spectrum Properties Popup. Changing contour settings can also be achieved by using the toolbar buttons in each spectrum display.

Picking peaks

Now that we have the spectra displayed and the contour levels set, we need to pick the peaks of the HSQC spectrum to use it as a reference for backbone assignment. This can be achieved in one of two ways:

1) using the mouse shortcut: CTRL (for Linux)/CMD (for Mac) +SHIFT+Left drag.
   NB The graphics sometimes leave the operation hanging if you let go of the SHIFT key before you let go of the mouse button. If this happens, just repeat the action (and next time let go of the mouse first)

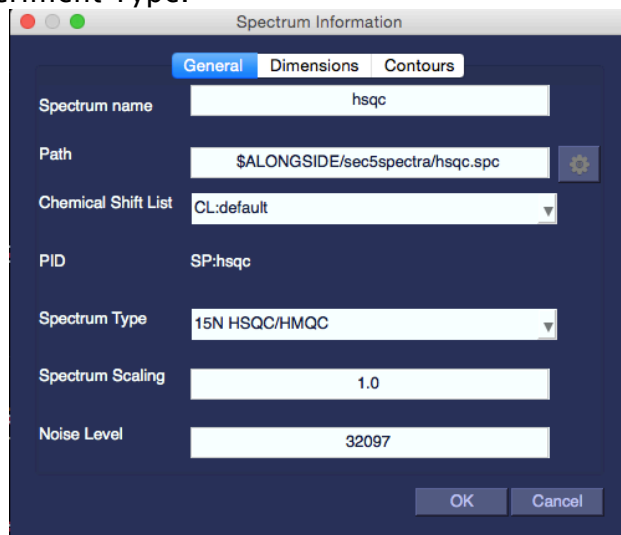2) using the Pick Peaks popup under the spectrum menu (shortcut pp).
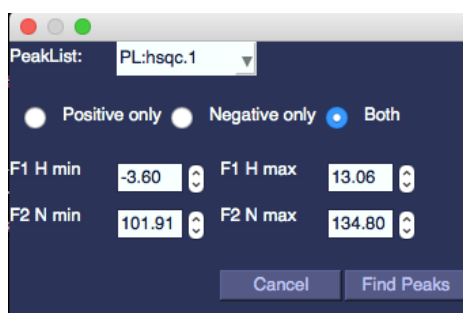
Using the mouse shortcut will pick all peaks in the region highlighted of both positive and negative contours if they are displayed. The peak picking popup allows you to choose between Positive only, Negative only or Both positive and negative peaks to be picked and more precise control over the peak picking region.

The crosses mark the peak positions picked and the two hyphens separated by a comma indicate that the dimensions of these peaks are unassigned. Some of the peaks picked may be noise and should be deleted prior to proceeding further, which is done by selecting the peaks and hitting the Delete key. Selecting peaks can be done using a rubber band selection CMD/CTRL+drag or individual selection by clicking on a peak, holding CMD/CTRL and clicking on additional peaks.
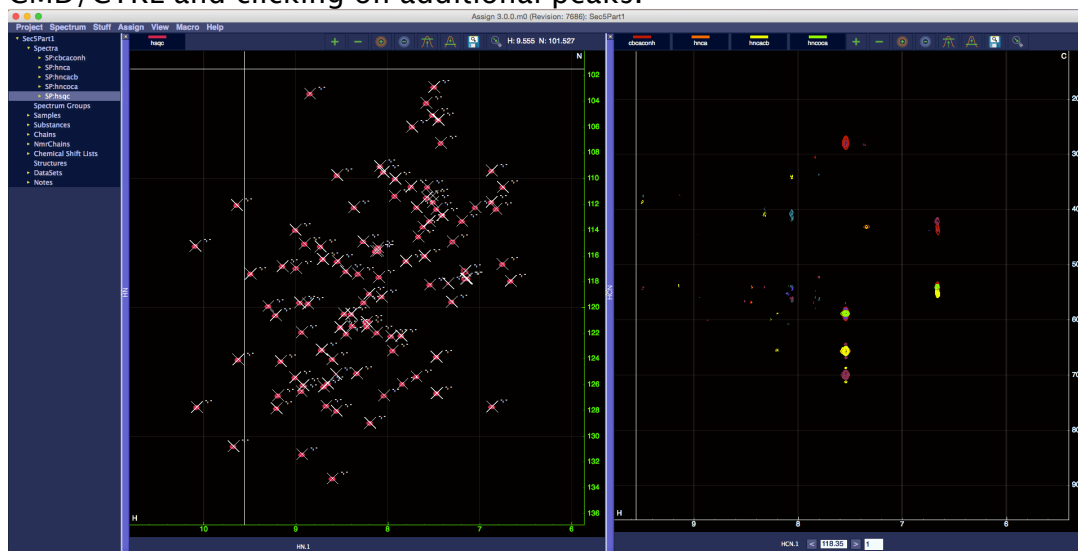
Setting up the HSQC spectrum for assignment

To start the backbone assignment process, set the experiment types of each spectrum so Assign knows how to handle them. This can be done per spectrum using the Spectrum Properties Popup accessed by double clicking the spectrum in the Sidebar. In the General tab of this popup is a dropdown list labelled Experiment Type.


Selecting the appropriate type from this list sets the experiment type. If you have multiple spectra, you can set all experiment types of all spectra using the Set Experiment Types popup under the Spectrum menu (shortcut et) and select the appropriate experiment types from the dropdown lists.
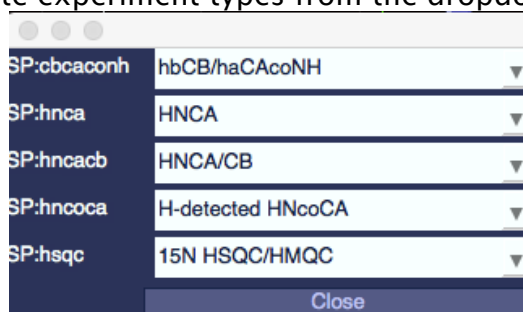
Having set all experiment types close the popup – Assign now knows what types of experiments are in the project.

Next we need to assign anonymous labels to the HSQC peaks, which can subsequently be linked to the 3D spectra and used as placeholders until the actual assignments are obtained. Setup NmrResidues under the Assign menu (shortcut sn) performs this task and will take an HSQC or an HNCO as input.
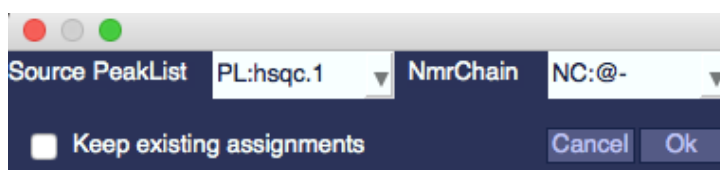
Select SP:HSQC in the Source PeakList dropdown list and leave the NmrChain as it is. There is also a checkbox to keep existing assignments, but at this point there are none so we can leave this alone. This procedure takes less

than a second and once it has finished, all the peaks of the HSQC spectrum will have the comma separated dashes replaced with something beginning @-.@, e.g. @-.@1..H, N for the peak at 8.600, 133.28. These annotations are NmrAtoms.

Assignment and names

Assignment in AnalysisAssign V3 is a matter of setting assignment strings. If an assignment string matches an Atom in one of the chains, that is an assignment to the atom. If not, it is a placeholder. In general, changing an assignment string (e.g. reassigning a peak) has no effect on anything else. NmrAtoms, Peaks, and ChemicalShifts are treated specially. If you rename an NmrAtom (or its parent NmrResidue or NmrChain), the assignment of all ChemicalShifts and Peaks assigned to it is updated as well.

We use NmrChains and NmrResidues to keep track during the assignment process. By default, new NmrResidues are put in NmrChain '@-', and new, temporary NmrChains are given names like '@2'. Normally NmrChains say nothing about the sequential connections of the NmrResidues. To store sequential stretches you use 'connected' NmrChains, whose names start with '#' instead of '@'.
NmrResidues are created with names like '@173' and with no residueType. When you want to create the '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 IUPAC, so serine HB2 or HB3 are *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, so that Leu HDX% are the methyl protons bound to Leu CDX.

Names with '@' (and NmrChain names starting with '#') are reserved.

Peak Picking the 3Ds and assigning them to NmrAtoms

To link the 3D spectra to the HSQC spectrum we will use the pick and assign and atom selector modules, both of which can be found under Assign in the menubar (shortcuts pa and as, respectively). You may want to rearrange the modules since the Atom Selector does not require much screen space and you want to see as much of the 3D module as possible. For example, your layout could look like this:

In the Pick and Assign module you will see a table of NmrResidues, a selector for NmrChain and two buttons labelled "Restricted Peak" and "Assign Selected." To start the pick and assign procedure double click on a row in the Nmr Residue table, e.g. the row the for @1. This will cause the module with the HSQC in it to move to the position of the peak labelled @-.@1..H, N and to mark this position with two labelled rulers corresponding to each dimension. The 3D module to navigate to the corresponding z position and to mark the set of 3D peaks along the proton frequency with a labelled ruler. Clicking the "Restricted Pick" button will pick all the peaks along the line in the 3D window and these peaks will be selected. At this point, you have two choices: either to assign all selected peaks to NmrResidue @-.@1 by clicking Assign Selected or to refine the selection of the peaks and remove any noise peaks, for example, and then clicking Assign Selected. Assign selected will only assign the dimensions corresponding to the x and z axes of the 3D spectrum display(s), where possible. If you move on to assign NmrResidue @2, you will see no peaks; this one is a Tryptophan side chain NH.

We can use the atom selector to identify the atom type of the carbon dimension, i.e. CA or CB, i or i–1. Selecting a group of peaks in the 3D window will cause the atom selector to predict the assignment for the carbon dimension as long as the peaks are close enough within the assignment tolerances. For example, if we select the group of peaks at 59.5 ppm, the CA buttons under i–1 and i in the atom selector change colour, both will be coloured green.
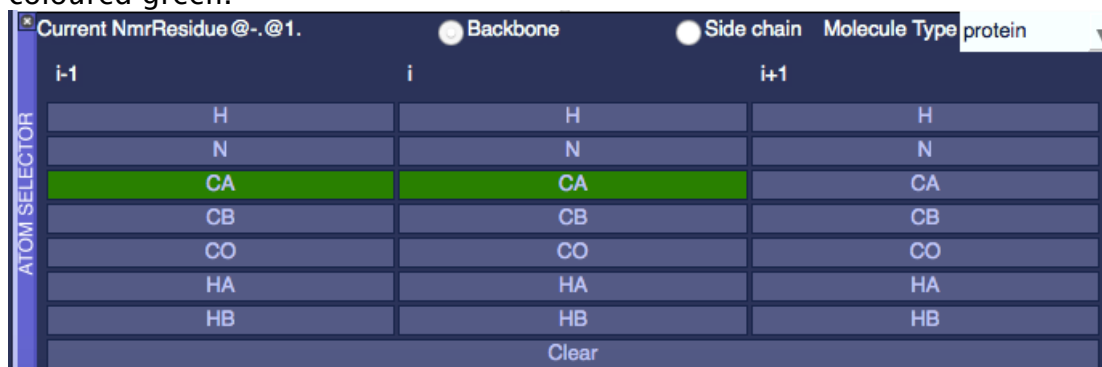
The program uses green for likely and orange for less likely assignments, but for a peak in (e.g.) an HNCA assignments to 'i' and 'i–1' are equally likely in themselves. To assign the carbon dimension, click the appropriate button in

the atom selector and the carbon dimension will be assigned. Repeating this procedure for the other groups of peaks along this line will give CAi, CBi-1 and CBi assignments for this NmrResidue. To use the Backbone Assignment tools in Assign, the i and i-1 assignments for all NmrResidues needs to be provided, so this procedure should be carried out for all items in the Pick and Assign table, where possible. Once all the 3D peak dimensions have been assigned to the appropriate NmrAtoms, the backbone assignment can be carried out.

Performing backbone assignment

Sec5Part2 is a project wherein all the carbon assignments for the NmrResidues have been completed and thus can be used directly for backbone assignment. Open up this project and set the spectrum paths as required and you will see a 2D module with the hsqc displayed and two 3D modules with the cbcaconh, hnca, hncacb and hncoca displayed.

Assign has a dedicated workflow for backbone assignment that can be accessed via the Assign menu on the menubar (shortcut bb). This workflow works off a Backbone Assignment module and the sequence graph, accessed from the View menu (shortcut sg). To setup backbone assignment, first rearrange the modules to a layout you would like to work with. Before you start the backbone assignment module you need to set the options – click on the cogwheel 'Settings' button to do that. In the settings menu, you select the modules that are used to display the strips that match the particular NmrResidue you are trying to connect. The "Selected Modules" dropdown list shows the module(s) to choose from. In this exercise, we will select GD:user.View.HnCANh_1 as the match module. Close the settings menu, open the sequence module (Show Sequence in the Molecules menu, shortcut sq) and you're ready to start assigning.

Let's start assigning in the i-1 direction by double clicking @100-1 in the list in the Backbone Assignment module (you can sort the columns by clicking on the column header). 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 and this is shown in the bottom of the strip, the Assigner Module will have a residue drawn in it with the NmrResidue name and predictions of the residue type below it. The match module will also

display five strips that the algorithm thinks match the i-1 chemical shifts of @100.

Upon examination, @55 is the best match for @100-1, so to place this i-1 of @100, hold SHIFT and drag the strip label: HnCAHN_1.1 and drop it onto the HnCANH.1 label. At this point @-.@100. and @-.@55. have been put into a connected stretch, which means that their names #4.@100. and #4.@55. and this is reflected in the Assigner and in the labels on the strips. Change the NmrChain Pulldown in the Backbone Assigner to <All> to see where the assigned residues went.

When strips are dragged and dropped in the procedure, Assign will to look for i-1 matches for @55 and place another residue in the Assigner Module labelled @55. The algorithm thinks that @72 is a good match for @55-1 and on inspection it is a match, so SHIFT left dragging HnCANH_1.1 onto HnCANh.2 will move the assignment on.

At this point, we have @72 – @55 – @100 as an assignment stretch and the residue type predictions show this to be Ser – Ala – Ser. In the sequence module at the top of the screen, you will see SAS highlighted in yellow indicating that this short stretch be this part of the sequence. If you continue assigning in the i-1 direction, you should end up with a stretch consisting of:

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

and NCLLTAEWMSAS highlighted in the sequence module:
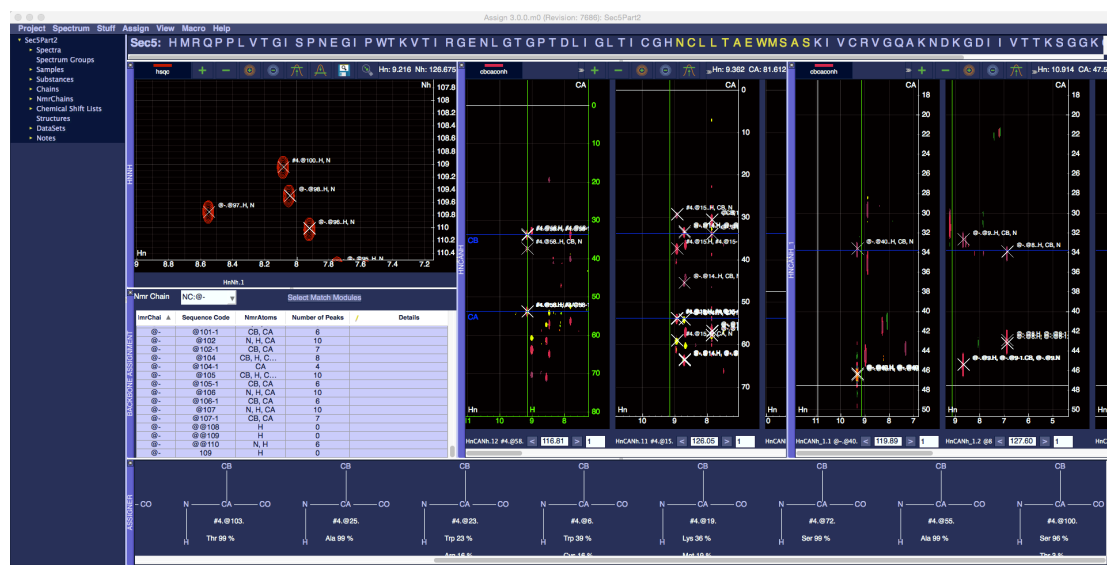
This is a very confident assignment prediction and therefore we should commit it. To do this, click on any of the residues in the stretch in the assigner and perforated box will appear around it to indicate that it is selected. Then, hold SHIFT and click and drag this box to the N coloured yellow in the sequence module and release the mouse. This will cause the sequence highlighted in yellow to become white and bold. This shows that this part of the sequence has been assigned. In addition, the names of the residues on the peak annotations, in the assigner and in the strips will change to reflect the fact that these NmrResidues have been assigned.

Double click @18 in the Backbone assignment module and the match module will show @42 as a good match, **without** using shift, drag the HnCANh_1.1 label onto HNCaNH.1 and two residues will now be shown in the Assigner module and @34 appears in the match module as the next match.

Continuing in this direction you should end up with a stretch consisting of:

@18 @42 @34 @22 @106 @29.

This corresponds to LTICGH and can be assigned using drag and drop by selecting one of NmrResidue names in the assigner and dropping on the beginning of the sequence to be assigned, i.e. on the L.

Repeat this procedure for the remaining NmrResidues in the table will give you a complete backbone assignment for this protein.

This concludes the Backbone assignment tutorial.