CcpNMR notebook

I. Visualizing spectra in CcpNMR

A. Starting CcpNMR, opening and creating projects

- In a terminal window, enter the command `module load ccpnmr` (type the text of the command, then hit Enter). This command is specific to this tutorial (the CcpNMR environment must be activated in the system booted from USB stick).
- Then, enter the command `analysis`. A main menu window opens.
- Select the Project menu with a left mouse click. A submenu appears.
- To open an existing project, navigate to the directory containing the project (which is itself a directory) and double click on the project directory.
- Alternatively, you can also navigate to the directory containing the project in the terminal and then enter the command `analysis <project name>`, where you replace `<project name>` with the actual name of the CcpNMR project directory.
- To create a new project, click New in the Project menu. Then, enter the name of your project.

B. Example: Create a project containing the ^{15}N-HSQC of ubiquitin

Load the protein sequence

- Click New in the Project menu. Then, enter '*ubi_test*' as the name of your project.
- In the CcpNMR main menu, click Molecule > Molecules, then Add Sequence and Read File.
- Load the ubiquitin sequence file '*ubi.seq*' (in the folder '*Database_spectres/petite_proteine_structuree*').
- Click Tidy and Add Sequence.
- Accept all proposed answers in the following questions.

Load the ^{15}N-HSQC spectrum

- Select Experiment > Open Spectra.
- At the top of the new window that opens, change File format (pull-down menu at the top) to NMRPipe. Make sure that File type in the center of the window shows the NMRPipe (*.ft*) option.
- Navigate to the folder '*Database_spectres/petite_proteine_structuree/ubi*' and select the file '*hsqc_ubi.ft2*'.
- Approve the selection by clicking Open Spectrum, then click on Commit in the window that opens (after checking axis calibrations).
- The ^{15}N-HSQC spectrum will be by default associated to Expt_1.
- In the following window, you can define the spectrum as a '*15N HSQC/HMQC*' by double clicking on the empty field in the Type Synonym column of the first line in the (otherwise empty) list, and selecting '*15N HSQC/HMQC*'. As External Name, enter for example '*15NHSQC*'. Then, click Close – All Done.
- The HSQC spectrum then opens in a new window (by default window 1).

Save the project

- We will use this spectrum later in step III.B of this CcpNMR tutorial, so either keep it open or save it now.
- To save a project, in the CcpNMR main menu, go to Project > Save as and save the project (a new project directory will be created) in a location that you can write to.

II. Navigating spectra and windows

A. Overview - Shortcuts

Spectrum Manipulations

Page Up	Zoom out
Page Down	Zoom in
Up	Move spectrum up within the window
Down	Move spectrum down within the window
Left	Move spectrum left within the window
Right	Move spectrum right within the window
Home	Zoom the slice range down
End	Zoom the slice range up
c	Centre the window where the mouse is
j	Scroll left orthogonally
k	Scroll right orthogonally
i	Increase the number of contours
o	Decrease the number of contours
e	Raise the countour level
r	Lower the contour level

Marks and Rulers

h Create a horizontal ruler

v Create a vertical ruler

m Create a mark

n Clear all marks and rulers

Pop-Ups

a Bring up the Assignment pop-up

b Bring up the Browse Atoms pop-up

u Bring up the right-click Mouse Menu

s Show the selected peaks in a pop-up table

Peaks

p Move selected peak

P Automatically centre the peaks on the closest maxima/minima

q Move peak label

w Automatically set the peak label positions such that they do not overlap

W Reset the peak labels to their original positions

l Unite peak positions

s Show the selected peaks in a pop-up table

Other

S Save project

B. Switch spectra on/off

- In the top left hand corner of each spectrum window, there is a button Spectra. Click on it to show a list of all open spectra in the current project that can be displayed in this window. Each spectrum can be toggled on/off using its button in this list, so that you can display as many or as few spectra in each window as you wish.
- To open further windows, click on Window in the CcpNMR main menu. At the bottom of the pull-down menu that opens is a list of windows contained in the current project; clicking on any of them will open the corresponding window (which might have been closed / minimized and thus invisible). New windows, if needed, can be defined via New Window in the Window menu.

C. Zooming / moving around in spectra

There are several ways to zoom and move around in the spectra (note that the mouse-based ones may not work in Windows!):

- To zoom, rotate the mouse wheel; or middle-click the mouse while holding down Shift and drag the mouse; or use the Page Up / Page Down keys.
- Move to a different part of the spectrum by using the scroll bars or simply click on the middle mouse button and drag the spectrum.
- To move through the z-planes of a 3D spectrum, either use the z-plane scroll bar at the very bottom of the 3D window – or simply turn the mouse wheel while holding down Ctrl.
- If you want to go to a specific position on the z-axis of a 3D window, simply type the ppm value into the box in the bottom left hand corner.

D. Contours

- To change contour levels in a spectrum window, click the Contours button at the top and then the green up and down arrows appearing below it. Alternatively, you can use the keyboard shortcuts ‘e’ and ‘r’.
- To show negative and/or positive contours, click the ‘Pos/Neg’ button (when the Contours button is active).
- More detailed contour level settings are accessible via the More... button.

E. Using marks and rulers

Often it is useful to draw lines through your spectra to check whether two peaks occur at the same chemical shift or not.

- A *mark* is drawn through all dimensions at the position where it is placed. To draw a mark, place the mouse where you want it to be and press '*m*'. If you are close to a peak, then the mark will automatically be drawn through the peak.
- *Rulers* only go through one dimension. To draw a ruler, place the mouse where you want it to be and press '*v*' for a vertical ruler or '*h*' for a horizontal ruler.
- You can remove all marks and rulers by pressing '*n*'. Alternatively, you can right-click the mouse and go to Markers – here you can place marks and rulers, or you can selectively remove only marks or only rulers.

III. Peak picking

A. How to pick peaks

- To identify, label, and assign peaks in spectra, they have to be "picked", i.e. marked by a cross. CcpNMR stores information about picked peaks, notably their chemical shifts, in a database where it can be accessed for further analysis.
- The best way to pick peaks is to let the program find the correct peak maximum for you. To do this, simply *left-click and drag* the mouse over a peak (or several peaks) *while holding down Ctrl and Shift*. You can do this both in 2D and 3D spectra.
- Sometimes several peaks are overlapped and it is better to place the peaks manually. Simply place the mouse where you want to have your peak and then *left-click while holding down Ctrl*. Alternatively, *right-click* the mouse and select Peak and then Add New Peak.

- A peak is *selected* when a box is drawn around it. To select a peak either click on it, or drag the mouse over it. To deselect your peaks just left-click the mouse somewhere in a spectrum window. To select several peaks at the same time, drag the mouse over several peaks in one go, or keep the Shift button pressed down while you select each peak individually.
- To delete a peak, select it, then press Delete.

B. Picking the peaks of the ubiquitin ^{15}N-HSQC spectrum

- In the project 'ubi_test' created under I.B above, go into window 1.
- Try to select an appropriate contour level in order to visualize only 'real peaks' without displaying noise. (Of course, one might not know at this stage what is real and what is noise!)
- In the main menu, go to Peak > Peak Finding.
- In the tab Find Parameters, check that the option positive only is selected. Only positive peaks will be picked.
- In the tab Region Peak Find, you can adjust the size of the region used for peak picking, if needed, to prevent the picking of undesired signals (e. g. water signals near 4.7 ppm ^{1}H chemical shift in ^{15}N-HSQC spectra).
- Click on Find peaks!. Alternatively, *left-click and drag* across the entire spectrum while *keeping Ctrl and Shift pressed* down.
- Inspect the quality of peak picking. Check the peak table (main menu: Peak > Peak Lists and then the Peak Table tab).

IV. Specifics for manipulation of [^{13}C,^{13}C] and [^{13}C,^{15}N] solid-state NMR spectra

A. Set double cross-hair mouse pointer

- By default, your mouse will form a single crosshair with one vertical and one horizontal line. However, when you have two axes belonging to the same type of nucleus (e.g. in a carbon-carbon correlation spectrum), it is really useful to have a double crosshair (with two vertical and two horizontal lines) which will trace equivalent points on either side of the diagonal.
- In order to set a double crosshair mouse, go to Window in the main menu, select Windows and there the Windows & Axes tab. When you click on a window in the upper part of this pop-up, you will see the axes displayed in the lower part. One of the columns is titled Panel Type. By default, these are all set to be different, so for a ^{13}C-^{13}C window, they will be called C1 and C2. If you set the panel types to be the same, then you will obtain a double crosshair mouse. We find it useful to go through all windows and make sure that all panel types are C1, N1 or H1 for ^{13}C, ^{15}N and ^{1}H axes, respectively. This way, you will always see a double crosshair mouse in diagonal spectra, and you will always see equivalent mouse crosshair lines in all other windows as well.

B. Identifying sideband peaks

- Open the project '*ccpnmr_cristaux_b*' in the '*ssNMR/cristaux*' folder.
- In the ^{13}C-^{13}C spectra of window 1, diagonal lines are drawn at regular intervals from the spectrum diagonal. These indicate the locations of (potential) *spinning sidebands* of the diagonal. These lines are drawn if the MAS spinning speed at

which an experiment was recorded is entered in the Experimental Details tab (main menu > Experiment > Experiments). You can also use '*v*', '*h*' and '*m*' to draw vertical rulers, horizontal rulers or marks, which will be repeated at sideband intervals. This is a convenient way to identify whether, for instance, a peak in the aromatic region of the spectrum is actually a sideband peak from the carbonyls. If marks and rulers are not visible, check Window > Marks and Rulers and set mark/ruler color to something other than white.

- If you have identified a peak as being a sideband peak, you may want to mark it in some way, in order to remember that it is a sideband peak. There are two ways to do this. Either, *right-click*, go to Peak and then Set merit and choose 0.0. Alternatively, *right-click*, go to Peak and then Set details – you can then type something like "sideband" in the comment box. Now go to the Peak menu of the main menu and select Draw Parameters. Make sure that Merit Symbol or Details are selected in the Annotation Style tab, depending on which one you opted to use. If you are using the Merit Symbol, then go to the Merit Symbols tab and enter a symbol such as * or ! into the Poor merit box and click on Set symbols. Now your sideband peak should be marked either with a symbol or a comment to remind you that it is not a real peak.
- It is likely that a rather more sophisticated system of properly identifying sideband peaks will be introduced some time in the future. But in the meantime, this is a reasonably good fix.