CcpNMR notebook

I. How to visualize spectra in CcpNmr

A. Create a project

- To run CcpNmr, in a terminal add in the command line : analysis↵. A main menu window opens.
- Select the Project menu with a left mouse. A submenu appears. Click New
- Enter the name of your project ('ubi_test').

B. Load the sequence of the protein

- Load the ubiquitin sequence:
  - Molecule>Molecules then Add Sequence and ReadFile
  - Load the ubiquitin sequence file 'ubi.seq'.
  - Click Tidy and Add Sequence
  - Approve all questions.

C. Load the ^{15}N-HSQC

- Select Experiment>Open Spectra.
- In the new window that opens, change the format of the file to the right format NMRPIPE or BRUKER.
- Select the file hsqc_3.ft2 (in the upper left corner select File format: NMRPipe, et and at the bottom on File type : NMRPipe (*.ft*)).
- Approve the selection by (Open Spectrum) then click on Commit in the New window after checking the calibrations.
- The ^{15}N-HSQC spectrum will be by default associated to s Expt_1.
- In the 'Type synonym' line, select '15N HSQC/HMQC' and in External Name, enter for example '15NHSQC'.
- Then click on 'Close / All done'
- The HSQC spectrum then opens in a window (by default window 1).
- To change contour levels in the window, select the tab Contours and then the up and down arrows (green arrows) or use the keyboard shortcuts 'e' and 'r'.
- To show negative and positive contours, in the contour tab select 'Pos/Neg'.

D. How to navigate in the window

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

II. Peakpicking of ^{15}N-HSQC spectra

- Go into window 1.
- Select the right contour level in order to only visualize 'true peaks' without seeing the noise.
- Go to the menu 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 finding', adjust the box size to prevent peaking water on the side of ^{15}N-HSQC spectra (100 to 129 ppm)
- Click on 'Find peaks'
- Inspect the quality of peak picking. Check the table. (>Peak>Peak list and then Peak Table)

III. Manipulation of [^{13}C,^{13}C] and [^{13}C,^{15}N]- ss-NMR spectra

- **Open a CcpNmr project**
  - Open a terminal window and go to the folder containing the CcpNmr project.
  - Open the ccpnmr project (for example analysis ccpnmr_cristaux_b).
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- **Switch spectra on/off**
  - In the top left hand corner of each window there is a Spectra button. Toggle this on/off to see all spectra, which can be displayed in each window. Each spectrum can be toggled on/off using its button, so that you can display as many or as few spectra in each window as you wish.
  - Open further windows by going to the Windows menu and selecting any of the windows in the lower section of the pull-down menu. Note the nomenclature of the spectrum names – they indicate spectrum type, mixing time and MAS frequency.

- **Zooming / moving around in spectra**
  - There are several ways to zoom (note that the mouse-based ones may not work with Windows!)
    - Rotate the mouse wheel
    - Middle-click the mouse while holding down Shift and drag the mouse to zoom in/out
    - Use the Page Up / Page Down keys
  - Move to a different part of the spectrum 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 or rotate 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.

- **Set double cross-hair mouse**
  - 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 atom type (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 and select Windows. 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 headed 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 and H1 and then you will always see a double crosshair mouse in diagonal spectra and you will always see equivalent mouse lines in all other windows, too.

- **Picking 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 Peaks 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.

- **Using marks and rulers**
  - Often it is useful to draw lines through your spectrum 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.

- **Identifying sideband peaks**
  - In the CC 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 Experiment Details tab (Experiment -> Experiments). You can also use *v*, *h* and *m* to draw vertical rulers, horizontal rulers or marks, which are repeated at sideband intervals. This is a convenient way to identify whether for instance a peak in the aromatic region of the spectrum is in fact a sideband peak from the carbonyls or not. 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 so that you do not forget that it is a sideband peak. There are two ways to do this. Either, right-click, go to Peak and then Set merit and 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 and select Draw Parameters. Make sure that Merit Symbol or Details are selected, depending on which one you opted to go with. 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 mean time this is a reasonably good fix.