Data Processing using Topspin

Sarah Neuenwander (saneuen@ku.edu) – KU NMR Labs v1 9/29/10

When processing, regardless of the software you are using, you will want to always follow the same steps in the same order. Below is a checklist of the steps to follow:

- Fourier Transform
- Phase Correction
- Baseline Correction
- Spectrum Reference (optional)
- Peak Labels
- Integrations

Fourier Transform – Type *ef*

Phase- (1) Automatic – Type *apk,* or

(2) Manually-Click on  icon. A red line will appear over the biggest peak. This is the zero order peak you will phase. Left click and drag on 0 in the menu just above the spectrum to make the baseline of this peak as flat as possible. Then look at the peak farthest from the zero order peak, left click on 1 on the menu and make the same adjustment. When done click the save and return icon  to exit the phase mode.

**In lieu of running *ef* and *apk* separately, you can choose to run the composite command *efp* (Exponential multiplication + Fourier transform + phase correction). **

Baseline Correction- Type abs n

Spectrum Reference – Click on  icon. Place red cursor on top of peak to be referenced and left click. Type in the value.

Peak Labels- Click on  icon. Highlight  icon. Left click and drag to draw a box around the peaks you want labeled. The top of the peak must be inside the box for it to be marked. You will not want to have the bottom part of the box touching the baseline as it will label all of the noise as well. If you make an error when drawing your boxes click on  icon. This will make arrows appear when you move the mouse near the box which enables you to make necessary adjustments. When finished, click the save and return icon  to exit the peak labeling mode.

Integrations – Click on  icon. Highlight  icon. Left click and drag to cut the integrals. If you need to zoom in on a region, click on  to toggle off, then left click and drag to zoom in on region. Highlight icon again to make integration function active and resume cutting integrals. If you know the integration of one of the peaks you can calibrate it and the rest of the peaks will be adjusted relatively to that peak. For example, you can adjust the methyl peak at 1.5 ppm to 3. The CH2 peaks will then show an integration of 2. To calibrate the integration, right click on the desired peak and select “Calibrate Current Integral”. Place the value in the box. When finished, click the save and return icon  to exit the integration mode.

Measuring J-Values – Label the desired peaks as described above. Type in the command ‘mana’ to enter the Multiplet Analysis mode. If you click on the  icon it will automatically define the multiplets. You can also manually define multiplets by region if you click on  . By placing the cursor and left clicking on the peaks, you can see the multiplet information in the upper right hand corner. You can generate a report of all peak and multiplet information by choosing  . When finished, click the save and return icon  to exit the multiplet analysis mode.

2D Processing

Type xfb instead of ef to do the fourier transform.

COSY and HMBC experiments do not need to be phased. To phase any other 2D spectrum click on  . You will need to select at least 2 peaks to phase. It is best to select two peaks furthest from one another in opposite corners. Right click on the desired peaks for phase correction and select “Add”. Once peaks have been selected click  to begin phasing on the rows. Phase as you would a 1D spectrum as described above, starting with 0 order and finishing with 1 order. When finished select  . You will now select  to phase on the columns. Again phase as you would a 1D spectrum and when finished select  . The 2D phasing is complete and you will need to select  to exit the phase mode.

MestReNova

Importing Spectra – Go to 'File/Open' or click on  and select appropriate file. Note: To import a 1D spectrum you will need the FID file. To import a 2D spectrum you will need the ser file. The spectrum will automatically open already processed and with the phase corrected.

Baseline Correction – Click on 'Baseline Correction'  to correct the baseline.

Spectrum Reference – Click on 'Reference'  and select the peak you want to be a reference point.

Peak Labels - Click on 'Peak Picking'  to obtain automatic peaks. To see a list of Peak Picking Tools and Options click on the arrow next to the icon.

Integrations – Click on 'Integration'  to automatically integrate spectrum. You can manually integrate by clicking on the arrow next to the icon and selecting manual. You will then get a cursor that looks like an integration trail with a red cursor next to it. Place the red cursor on the left side of the peak to be integrated, left click and drag. To calibrate an integral, place the cursor on the desired integral and right click. Select 'Edit Integral' from the menu. Place the value in the 'Normalized' box. When finished close out the window with the box in the upper right hand corner. You can turn off the integration function by either clicking on manual again to toggle off under the integration icon or by choosing another function.

Measuring J Values – Click on 'Multiplet Analysis'  to apply an automatic multiplet analysis. You can manually select peaks as well by clicking on the icon arrow and selecting 'Manual'. Place the red cursor on the left side of the peak(s), left click and drag. You can create a report by clicking on the icon arrow and selecting 'Report'. It usually places the report in the upper left hand portion of the spectrum, but you can resize and move the report anywhere you like.

Other Useful Icons –

Zoom In  - Click on icon and left click and drag in spectrum to zoom in on specific region. This icon is a toggle switch.

Full Spectrum  - Click on icon to return to original spectrum.

Increase Intensity  - Click on icon to increase the intensity of the spectrum.

Decrease Intensity  - Click on icon to decrease the intensity of the spectrum.

Crosshair  - Click on icon to toggle on and off. When on, place cursor on desired peak and it will give spectral location in ppm and Hz.

Please see Help/Contents for more detailed instructions in processing.