HOWTO

Set up a fast HSQC (fHSQC) experiment on Bruker AVANCE III instruments

Tatyana Igumenova Texas A&M 04/18/2015

(1) Familiarize yourself with the following paper to understand what is being done in the pulse sequence:

Mori et al, Journal of Magnetic Resonance B 108, 94-98 (1995)

If you do not understand how the experiment works, you should not be running the NMR instrument by yourself. If this is the case, get help from Yuan, Mandar, or me.

(2) Use the following pulse sequence: 0ti.fhsqcf3gpph

The updated version can be copied from the following 800 directory:
/opt/home/tatyanai/lists/pp

(3) Adjust d19 according to the center of amide region and field strength. Look at the pulse sequence header for description on how to calculate d19. NOTE: failure to set d19 correctly will result in severe distortion of peak intensities/poor water suppression/unusable data.

(4) Calibrate the following parameters:
^{1}H: O1, p1@pl1 (hard H90)
^{15}N: p3@pl3 (hard N90)
90-degree pw @pl16 (this would be your pcpd3 value, i.e. soft N90).
Set the O3 value to the center of the ^{15}N amide region (typically 118 ppm).

For the 3-9-19 binomial pulse:

Set p27 and p0 to the calibrated value of p1.
Set pl18 to the same vale as pl1.

16 dummy scans (*ds*) is usually sufficient. The number of scans per t1 increment should be n*8, where 8 is the length of the phase cycle in this particular pulse program (convince yourself that this is the case by looking at the text of the pulse program).

Note: the excitation profile of the binomial 3-9-19 pulse falls off sharply, resulting in significant attenuation of the downfield resonances of Trp sidechains. If the Trp's are functionally important and respond to metal ion binding, consider moving the ^{1}H carrier downfield to e.g. 8.7-8.9 ppm (be very careful if you decide to do that – make sure that the upfield amides are not being wiped out as a result!)

(5) Using ***popt*** to array a parameter is fine. However, more often than not ***popt*** DOES NOT give you a correct zero estimate. Inspect the data, decide where the zero is and then run a finer optimization or "single-point" tests manually if necessary. Proper calibration is absolutely essential for getting the best S/N data.

(6) Adjust *td* for the ^{1}H dimension such that the total acquisition time (*aq*) is between 70 and 80 ms (we typically use 75 ms). It is advisable -- although not required -- to use the number of points that is = 2^{N} because of the FFT algorithm. You can tweak the spectral width to get the proper aq. Remember that the digital resolution in NMR is 1/*aq*, i.e. it depends on both *td* and *sw*.

(7) Adjust the spectral width in the indirect dimension such that you do not collect empty noise regions but at the same time have enough base-plane to phase the spectra. Moreover, some peaks may shift significantly as a result of binding – you do not want those to be aliased!

(8) Make sure that aliased Arg sidechain peaks do not overlap with backbone amide peaks. If they do, tweak the spectral width in the indirect dimension.

(9) *pcpd3* should be between 180 and 200 us (DO NOT use ^{15}N pulses longer than 200 us for decoupling – it is too weak!)

(10) Set all gradient durations to 1 ms and gradient levels to 25 % (gpz1), 7% (gpz2), and 55% (gpz3).

DO NOT ramp up the gradient power if you get adequate water suppression with the above settings (you should be able to – works fine on the 800). High-power gradients are disruptive – that's why we give our NMR probes 200 us to recover after each gradient!

(11) The number of points in the indirect dimension should typically be 256 (128 re + 128 im). If high-resolution spectra are needed, then use 512.

(12) DO NOT use the output of the **rga** command to set the receiver gain. When you get the *rg* value, back down to a "safe" value to avoid potential receiver overflow issues during your experiment.

(13) If you have trouble keeping track of all parameters, make a checklist for yourself and then check each parameter off once you adjust it. Thorough preparation for all experiments and careful optimization of all parameters is a requirement in my lab. Also remember that it takes the Department a lot of money to maintain and run these instruments, i.e. NMR time is not "free".

Titration- (binding-) specific considerations:

For the titration experiments, decide on the duration. Make sure that all of your experiments have the same duration and receiver gain. For the C2A of Syt1, use Lauren's data to guide you in the parameter selection. You should be able to fit in several titration points in a day.