NOESY and 1-D Gradient NOE

1. Setup and obtain a 1-D proton spectrum (If you already have a $^1$H spectrum and know your optimized SW and o1p, skip to step 2). Be sure to perform ‘loopadj’ (instructions for this are in the simple instructions for 1H/13C online). Optimize the spectral window around your peaks of interest allowing for ~ 0.5ppm on either side of your peaks. If your resonances fall between 1-8 ppm, you should select a sw of 8 (from 0.5ppm to 8.5ppm). Type sw [enter] and record the sw that applies to your optimized area. You need to reset o1p for your new sw. To do this, divide the value of your new sw by 2. Add this to the lower limit of your new sw (in the example above, you would add 4 (8/2) to 0.5 giving you an o1p of 4.5. Type o1p [enter] and enter your new value. Type aq [enter] and enter 2s or set it to the length of signal in your fid. Reacquire the proton spectrum with your new sw.

2. Type ‘iexpno’ [enter]. Type ‘eda’ [enter] and change the pulse program to t1ir_1d. Type ‘d7’ [enter] and type ‘0 ms’ [enter]. Type ‘ns 1’ [enter]. Type ‘phmod pk’ [enter]. Type ‘zz’ [enter]. Phase your spectrum so all the peaks are negative. Now, type ‘iexpno’ [enter]. Type ‘d7’ [enter] and type ‘0.35s’ [enter]. Type ‘zz’ [enter]. Some of your peaks may still be negative, some may be positive, some may be null. Your goal is find the first resonance’s null point by changing d7. T1 is approximately [d7 for null * 1.443]. Your mixing time (D8) for the NOE experiments should be no more than the d7 null value. Typical NOE mixing times are $\leq$ 500ms so provided no peaks you are interested in obtaining NOE information for have reached a null by ~350ms, you can move on to step 3. If you have a peak that is already null or positive at 350ms, reduce d7 and rerun to determine your mixing time limit. If you have peaks of interest with very short T1, you may want to do a few rounds of ‘freeze/degas/thaw’ to remove dissolved oxygen in your sample and then measure T1s again. A mixing time of less than 200ms may yield inconclusive NOE results.

3. Type edc [enter] (or type iexpno [enter]) and change the experiment number to 3. Type rpar [enter] and select NOESY_BROWN, or NOESY_NUS_BROWN (for the non-uniform sampling experiment). Type eda [enter] and change sw in F1 and F2 and o1p and o2p to the values recorded in your optimized 1-D proton (o2p should be set to the same value obtained for o1p). AQ should be set to ~0.5s. Make sure the appropriate solvent is selected and click the little blue test tube button. If you know your sample is not concentrated, you may want to increase the number of scans, ns (default should be 4 scans, and 16 dummy scans) in the eda window. Type d8 [enter] and either use 500ms, or whatever your fastest d7 null value was from step 2. NOTE: d1 should be no less than 2 X d8. Type d1 [enter] to change this value if need be. The default is 2s.

4. Turn off the sample spinning (either by pushing the button on the BSMS console – top left – or in the shim panel of the bsms display). Repeat topshim, or manually touch up z and z2 on Zeus and Ares. Type rga [enter]. Type zg [enter].

5. Any time during the acquisition, you can type xfb [enter] to process the 2-D data (except if you are using the NUS version in which case you need to wait til the experiment is finished). You can click the +/- button to remove the diagonal and any exchange peaks, leaving only
the negative NOE peaks (most small molecules will show negative NOEs….opposite sign from the diagonal). You can stop your acquisition before it finishes if you have already resolved your cross peaks of interest. Just type halt and xfb to process the latest scans. You now need to phase your spectrum (NOTE: for NUS, you will need to type xht2 and xht1 prior to phasing. See Topspin 2-D phasing Guide. It is often helpful to perform and ‘abs1’ and ‘abs2’ and ‘symt’ – select phase sensitive in the ‘symt’ menu.

6. For 1-D gradient NOE, you should still do steps 1 and 2. Make a new experiment and rpar 1D_GRAD_NOE_BROWN. Change SW, o1p, and AQ as optimized in step 1. If you have not yet turned off sample spinning, do this now and redo topshim. Measure the frequency difference in Hz from your o1p value and the peak you wish to irradiate (this is easily achieved by moving the cursor to o1p, left clicking the mouse and dragging to the peak you wish to saturate (record the measured value). Bear in mind, that selective irradiation requires that there are no other peaks within ~0.1ppm of the peak you intend to saturate otherwise you may see NOEs from partial saturation of the neighboring peak. If your selected peak is upfield of your o1p, your offset will be negative, while downfield peaks from o1p will be positive. Type ased and change the SPOFFs box about 2/3 of the way down the parameter boxes to your measured Hz value and remember to use the correct sign +/- . This is not a very sensitive experiment so it should be run with a minimum of 32 scans, 16 dummy scans, and a 2Hz line broadening. These should all be default values. Do rga and zz.