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 of interest'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 ≤ 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**.