

## NOESY and ROESY

As for TOCSY, the most important determinant of the quality of your 2d is the quality of your reference 1d, one that is well phased, has good sw and tof and is associated with a good pw90 and knowledge of the  $T_1$  and  $T_2$ .

Once you have such a calibrated 1d, save it (svf) and move the parameters, or the parameters plus data to other experiments in which you plan to set up the NOESY and ROESY. Type **mp(destination expt #)** if you want to move the parameters from the experiment you are currently in. Alternately **mp(from expt #, to expt #)**. For example mp(2) or mp(1,2), or mf(4). jexp2 (or jexp4) to go into experiment 2 and type NOESY to convert your parameter set to a set for a NOESY.

As for TOCSY, you can watch the buildup and decay of NOESY magnetization transfer from a resonance of interest, using **NOESY1D**, this time via  $T_1$  cross relaxation, as mix is extended.

### NOESY spectrum.

In an experiment containing your good 1d, type **NOESY**. Your sw will be used for sw1 too (to generate a square spectrum).

The value of at is a compromise between resolution (obtained with a long at) and loss of signal due to  $T_2$  relaxation.

ni will determine the length of  $at1 = ni/sw1$  = maximum value  $t_1$  (d2) will have. For better resolution in the indirect dimension you will need to pay against losses due to  $T_2$  relaxation, with a large ni. However you probably need less resolution than you think in F1, because you have two dimensions. Thus it is better to collect high resolution in F2 with a long at and save time and disk space with a lower ni. You can always linear predict more indirect dimension data. I advise against  $ni/sw1 \geq 2T_2$ .

Choose nt depending on the nt needed for a good 1d. The same value or half that value are both good choices. Make sure that **sspul=y**, and leave a pretty generous d1 because NOESY is a cross relaxation method, each scan should start with a relaxed, or at the very least, equally relaxed.

For quantitative distance measurements of distances based on nOe cross peaks d1 should be  $\approx 3T_1$  and the mix should be  $< T_1$ , often as short as 15 ms (macromolecules). However for qualitative information, such as which H is close to which, mix  $\approx T_1$  works well. For your first look at a molecule, this is a good choice.

### ROESY spectrum.

This experiment, like NOESY, measures cross relaxation through space, but it does so in the rotating frame. Spins are trapped in the rotating frame by a spin lock. Artfactual peaks due to TOCSY magnetization transfer (through bonds) are a pitfall to be avoided in ROESY. ROESY and TOCSY cross peaks can be distinguished by their signs, unless you have the severe bad luck to have them perfectly cancel each other. TOCSY cross peaks have the same sign as the diagonal. ROESY cross peaks have the opposite sign. ROESY is useful for intermediate sized molecules which may have nOes close to zero because of cancellation between stimulated emission and stimulated absorptive mechanisms (see the textbook). Molecular weights on the order of 1000 are a size range where things might be complicated, and a ROESY might be a better choice than a NOESY.

As for NOESY, in an experiment containing your good 1d, type **ROESY**.

In order to do this we use a weaker spin lock. We want  $\gamma B_1 \approx sw/2$  (not sw).  $\gamma B_1$  is set by your choice of slpwr and slpw, as for TOCSY. We will also implement a parameter called ratio which introduces delays between the pulses in the string used to achieve spin locking. When ratio=1 the duration of the delays is equal to the length of the pulses, so the spin lock is effectively diluted by a factor of two. We will test a range of ratio values and choose one that is large enough that TOCSY transfer is avoided. The duration of the spin lock has to

be sufficient for cross relaxation to occur, i.e.  $\underline{\text{mix}} \approx T_2$  for small molecules. For large molecules (proteins) rOes build up twice as fast as in a NOESY so  $\underline{\text{mix}}$  should be  $\approx T_2/2$  for qualitative information. For quantitative distances, a series of  $\underline{\text{mix}}$  values are used, all shorter than  $T_2$ , to sample the 'nOe buildup' period. This is beyond our current consideration.

Process data as for TOCSY. Note that the in-phase nature of NOESY and ROESY cross peaks greatly simplifies the spectrum, and its processing. However, for small molecules the cross peaks will have the opposite sign from those of the diagonal, opposite to the case in TOCSY. For large molecules NOESY cross peaks have the same sign as the diagonal but ROESY cross peaks have the opposite sign.

**2Ds with solvent suppression**

Aside: for those who will need to suppress solvent, a set of 2Ds with the wet presequence are available as wetdqcosy, wetgcosy, wetghmqc, wetghsqc, wetnoesy, wettntocsy to name the most popular. These are good for cases where there are multiple resonances to be suppressed (mixed solvent systems) or protons that exchange quickly with solvent and therefore will become saturated during long solvent saturation periods due to exchange. If neither of the former hold, you can also use presaturation which is robust and often easier to interleave into some sequences. For example solvent saturation can be continued during the t1 and mix times in some sequences.

For the case of water suppression (one solvent peak) you need to keep tof on the water resonance in order that quadrature artifacts will lie under the resonance itself. Therefore, you should first calibrate pw90 on water using gain = 0. Then use a small tip angle pulse and nt = 4 (to eliminate quadrature artifacts), centre the cursor on water then type **movetof** (NOT **movesw**). This will make the tof choice the overriding consideration and you will then have to do some simple calculations to optimize sw (without changing tof).

Note that if you are using presaturation, you will have to set **presat = 'y'**, and you may have the option of setting satfrq (saturation frequency) to something other than tof. Array satfrq over tof ± 10 Hz to get the best value, as the water line is often not symmetric, so that tof (the top of the line) is not the centre of mass of the line. (The better your shims are, the closer tof generally is to satfrq.) For successful presaturation, excellent shimming is crucial.

For the example of wettntocsy, under mainmenu, click on file, setdirectory, parent, parent ... until you see vnmr6.1B2. select that and Set Directory, select parlib and Set Directory. Click on 'Click HERE for more elements' and select wettntocsy.par. Then load these parameters.

Note that **p1lv** is the high power pulse, enter the value you had in tpwr when you first calibrated pw90. Similarly enter pw90 for **p1**. The spin lock is implemented with tpwr and pw. tpwr should be around 44 and no higher than 50. Recall that the spin lock field should be approximately two times as strong as the biggest shift from carrier frequency in your spectrum (i.e. ≈ sw). To 'lock' a resonance 2000 Hz from tof use  $4 * \underline{pw} = 2 / 2000$  or  $\underline{pw} = 1 / 4000$ , = 62.5 μs. This is clearly nothing like what is in the standard data set. The latter knows nothing about your prior calibrations, solvent, sweep width or anything. Once you get an experiment to run well, save your own parameter set !!

Also, you will have to put in your solvent selective 90 pulse as the wetshape with its pulse width (us) and power (db) as pwwet and wetpwr. Turn down gzlvw from 32k to 20k, please.

Set **c13wet='n'**.

Recall that for a 2D you will need **ni** increments (eg. ni=256) and **phase = 1,2**. **time** calculates an estimate of the experiment duration.

**Displaying 2Ds**, your best reference is the manual.

**Problem Assignment.**

On your own sample (or one of the course's samples), collect a DQCOSY, a short-mix TOCSY, a long mix TOCSY, a NOESY and a ROESY. Use identical spectral parameters such as sweep width, ni, nt, tpwr and pw90. Process them each appropriately and turn in a printout of each along with one short paragraph