

**2d spectra II: gDQCOSY vs. DQCOSY, and TOCSY****Strychnine in CDCl<sub>3</sub> with CrAcAc (T<sub>2</sub> ≈ 90 ms, T<sub>1</sub> ≈ 200 ms)**TOCSY: total correlation spectroscopy.

In this method magnetization on each spin in a network of spins connected by J coupling can 'flow' to other spins in the network ('spin system'). This is made possible by aligning all the spins along the X (or Y) axis and holding them there with a series of pulses called a 'spin lock'. While they are there, all aligned together and held together, it is as if they all have the same chemical shift (none is precessing away from the others). Thus, the strong coupling limit applies and the spins exchange magnetization via J coupling. Complete transfer of all the magnetization from isolated spin A to coupled but otherwise isolated spin B would take 1/2J (time). In practice every spin B that receives magnetization is also passing it on (to C, D, ... and back to A), and T<sub>2</sub> relaxation\* is continuously eroding the total net magnetization on each spin, so that magnetization builds and decays (and builds again) with different delays at different spins, during the spin lock. (\* actually rotating frame T<sub>1</sub> relaxation).

To set up a TOCSY start with your nice 1D, a tof and sw that allows approximately 10% extra on each side of the last resonance (for base line correction and axial peaks), pw90, lp = 0, and type **TOCSY**. That's it !! The computer will suggest values of the mixing time, mix, the d1 delay and the number of complex points in the f1 dimension ni.

lp ≈ 0 is obtained by phasing up and typing **crof2**. The computer uses your value of lp to re-calculate rof2, a delay that follows the excitation pulse, to in effect synchronize the excitation hardware with the detection hardware, so that there will be no net spin precession in the detection frame relative to the excitation frame. You have to collect your 1D one more time to see the effect. Also query lp and alfa (another delay after the pulse).

mix, slpwr and slpw

Varian will suggest a spin-lock field strength in the form of a slpwr (spin lock power) and the corresponding slpw (spin lock pulse width). However they tend to err on the aggressive side (they recommend a 15 ppm spin lock). Your spin lock field will have to be strong enough to overcome the resonances' tendency to precess away from the X axis, so in units of Hz (cycles per second), it should be ≈ sw. Using sw as the spin lock field strength, 1/sw will be the period (cycle time) and since a cycle is 360° the corresponding pw90 will be a quarter that, or 1/(4\*sw). Make sure you calculate this slpw in μs though, not sec. This is a TARGET VALUE, not what you will actually use, because you will have to use a pulse width that really is a 90° pulse at an integer power. Use your (linear) calibrated pw90 at tpwr, and calculate the pw90s you would get at a series of lower powers, with powers extending down until you calculate pw90s as close as possible to the target slpw. Use the calculated pw90 that is closest to but smaller than the target slpw. Enter this value as slpw and enter the corresponding power as slpwr.

You should make sure that mix is close to half of 1/J for the smallest J coupling you wish to be effective in magnetization transfer. For example if you are using steps of three-bond couplings on the order of J ≈ 7 Hz, choose mix = 0.07 s. In consideration of T<sub>2</sub> relaxation however, you will have very little signal left to observe if you use a mix > 3\*T<sub>2</sub>. Signal decays by a factor of e=2.7 for every interval of T<sub>2</sub>, so after 3\*T<sub>2</sub> you can expect a FID only 5% as strong as the one you got after a simple 90° pulse. Thus how long a mix you can get away with depends on your sample concentration and T<sub>2</sub>s. It also depends on the magnitude of the spin lock power. This is rarely an issue for <sup>1</sup>H TOCSY where the power should be <50. The **max** safe spin lock is mix = 100 ms, **max** safe power is slpwr = 50.

ni and at

You may want to increase ni (or at) larger for better resolution. However the cost is that this will cause the experiment to continue increasing the indirect  $t_1$  delay (called d2 by Varian) to a maximum value of  $\text{at1} = \text{ni}/\text{sw1}$ . If this is longer than  $3 * T_2$  you will only have 5% of your starting S/N left in the latter increments of the  $2d$ , and you will be merely wasting spectrometer time. It is better to collect fewer ni and linear predict based on these (stronger) points.

d1 and sspul

Ideally d1 should be at least  $1.3 * T_1$ . Because we use an 'SS' pulse purge sequence to kill magnetization left over from one scan and shorten the delay before the next (sspul='y'), you must allow  $\text{d1} = 1.3 T_1$  for maximum sensitivity, not  $(\text{d1} + \text{at}) = 1.3 T_1$ .

For long 2Ds, get trained to use variable temperature control, set temp = 25, shim up well and have a strong lock signal but without too much lock power (lock level  $\approx 60$ ). Reduce ALL instabilities.

## Last but not least

Before actually launching ANY 2D, please first check that it will not be hard on the hardware by doing a trial mini-run. set ss=0, ni=1, phase=1, and retain the nt you think you will need for the real deal (this is a good time to check it, rather than learn at the end that your data are not quite strong enough to support your analysis). **ga** and watch for rcvr overflow, ADC overflow etc. If you are a real keener (and/or a member of the Miller group) set ss = 128, **ga**, and immediately upon completion of the run open acqi and optimize z1 before the sample cools. Thus your shims will be better suited to the steady state level of heating produced by the spin lock (or decoupling for heteronuclear experiments).

If all is well, set up ss = 64 (or 128), phase = 1,2 (so you get both real and imaginary numbers in  $t_1$ , in support of a full hypercomplex data set), ni = whatever-it-was-you-chose. Check **da** to make sure you are getting all the data you intend. Type **time** to have the computer calculate how long your run will take, and adjust ni and nt to conform to the time you have allocated. **ga**

**Relaxation during internal delays:**

$T_2$  relaxation is operative during the mix time of a TOCSY (actually rotating frame  $T_1$ ). The benefit of a longer mix is longer-range couplings and more magnetization steps linking more distant Hs in a spin system. (Note that the short-range TOCSY transfer cross peaks may get weaker and even disappear when mix is long.)

$T_1$  relaxation is operative during the mix time of a NOESY. The benefit of a longer mix is strong long-distance nOes, because these take longer to build up than short-range nOes.

$T_2$  relaxation is operative during the mix time of a ROESY (actually rotating frame  $T_1$ ). The benefit of a longer mix is strong long-distance nOes, because these take longer to build up than short-range nOes.

**Processing and linear prediction**

Type **wft(1)** and set up the weighting and linear prediction you want to use for the first dimension, as if it were a 1d. If you want to linear predict in  $f_2$  (normally not necessary) and no linear prediction parameters are in the data set, create them by typing **parlp**. You will then also have to set proc = 'lp', set up the linear prediction, and modify the window function accordingly. These operations are described below for the  $f_1$  dimension, which is where linear prediction is most often used.

Process the whole first dimension: **wft1da**. Display the resulting interferogram with the  $t_1$  axis along the bottom: **trace='f1' dconi**. Place the cross hair on the line corresponding to a signal of interest's behaviour in  $t_1$ . Display this signal's  $t_1$  time dependence with **ds**. This is that signal's FID in  $t_1$  (d2). Use **wti** to choose

processing parameters as usual. Note that the window function parameters for f1 are identified with '1's as belonging to the indirect dimension (gf1, lb1 . . .). The window function you have just made is based on actual data only and will have to be scaled to accommodate linear prediction. Now set up linear prediction (COSY was discussed in Practicum 6). For TOCSY, NOESY, HSQC etc, linear prediction is often used in the f1 dimension to increase resolution. To double your data set **lpnupts1=ni**, **strtlp1=ni**, **lpext1=ni**, **strtext1=ni+1**. Choose lpfilt1 based on the number of strong signals you expect in an individual row of the finished 2d, this number will be much less than the number of signals in the whole spectrum, e.g. 8, in which case you might use lpfilt1 = 12. How would you triple your data? Double or triple the corresponding processing parameters gf1, lb1 etc consistent with the extension of your FID (if you are going to double your number of data points in f1 (i.e. using lpext1 = ni) then you should double gf1 or sb1 and sbs1). Make sure that fn1 is large enough to allow for 2 or 3\* ni points, times two, plus zero filling. Eg. for ni = 200 and lpext1=400 (tripling) fn1=2048. You could now go ahead and type **wft2da** to complete the 2-dimensional Fourier transformation, if your spectra have no further artifacts.

You may, however, need to correct the FID's dc offsets with the t2dc option in the **wft2da** command (type **wft2da('t2dc','t1dc')**). A first point correction is often required for the Fourier transformation of the f1 dimension. Either set fpmult based on the first increment: (type **wft(1)**, **dc** place the cursor at the right edge of the base line, **cdc**, if the spectrum rises try a lower fpmult, if it drops try a higher one, repeat until you have an fpmult value that produces a spectrum with no dc offset.). Alternately, type **wft(1)** and then just type **cfpmult** and VNMR will calculate fpmult for you. A good value of fpmult reduces the amount of T1 noise in the spectrum. Alternately perform a baseline correction on each spectrum produced in the first FT before doing the second one, by using **wft2da('bc')**. \*\*You must have already chosen the regions of the spectrum to be treated as baseline, using **cz** followed by the resets button.\*\*

You can deal with ugly baselines after the fact too, using **bc2d('f1')** or **bc2d('f2')**.

### **Phasing the spectrum.**

If you want to be able to correct phases in your 2d, it must have been processed with **pmode** = 'full'. If you are good at phasing the first increment (obtained by typing **wft(1)**) you can then set **pmode** = 'partial' and only phase the f1 dimension in the 2d itself.

Unlike gDQCOSY, which produces peaks consisting of two positive and two negative components, paired across the diagonal, TOCSY cross peaks are in-phase absorptive, and this greatly simplifies the spectrum as well as its processing. Work with the diagonal (strong and known to not be artifactual). With trace = 'f2', place the crosshairs on a diagonal peak at the right-hand side of the spectrum and type **ds** to generate the corresponding 1d slice. Phase ONLY the rp (do not click a second time), based on the diagonal peak. Then return to the 2D with **dconi**. Place the crosshairs on a diagonal peak at the left-hand side and type **ds**. Phase lp. To do this go into phase mode, click once on the right-hand side to step past rp optimization (which you have already done) then move the mouse over to the diagonal peak and hold down the left or right mouse button while phasing it up (the usual way). Just do this once, do not move the mouse and click again to rephase elsewhere in the spectrum. **dconi** to return to the 2d, then change trace to turn the spectrum on its side and phase the other dimension (**trace='f1'** **dconi** if you just did f2 and now want to do f1). As before, once your phases are optimized, set **pmode** = '' and **wft2da** once more time so you will not be carrying around all your imaginary data.

### **TOCSY1D**

To view a buildup curve showing how magnetization decays from a source position, builds and then decays at destination positions, or simply to observe the spin system to which a chosen resonance belongs, load

parameters for TOCSY1D onto your nice proton 1d by typing (you guessed it) **TOCSY1D**. As always, temper the strength of the spin lock. Shoot for  $1/(4*\underline{slpw}) \approx \underline{sw}$  (although Varian recommends at 15 ppm spin lock regardless of  $\underline{sw}$ ). You should know the origin of this rule of thumb by now. You may want a longer-than-suggested  $\underline{}$ . Take your T2 into account in choosing the maximum duration of spin lock (which is often called 'mix'), Take your T<sub>1</sub> into account in choosing d1. For clean results with this sequence, I suggest nt = 8 or multiples thereof.

The selectivity of the TOCSY1D sequence is based on a pair of gradient echoes employing selective inversion pulses that will invert the resonance of interest, so that it is rephased by the second of each pair of gradients. All other resonances will experience accumulating dephasing by the sum of all four gradients. Make a selective inversion pulse using pbox, based on your gourmet 1d. Insert its name, power, pulse width into TOCSY1D 's parameter set. Confirm that the desired selectivity is achieved by setting mix = 0 and collecting 8 scans. You should see ONLY the desired resonance. Now array mix to watch the time course of magnetization transfer to other resonances in the spin system. Note that different mix times are optimal for different transfers.