Homework 9 Chem 636, Spring 2014

due at the beginning of lab April 8-10 updated 8 Apr 2014 (cgf)

Intro to 2D NMR: Homonuclear correlation
COSY, lr-COSY, and DQ-COSY experiments

Use Artemis (Av-400) or Callisto (Av-500) for this week's HW.

Many choices exist for samples to be used for this and the following 2D labs:

1. **A research sample is always OK (it should be a clean compound of moderate complexity).**
2. **Your unknown compound: Many of these compounds are quite complex. High quality data will be obtained using the HW as guides. But these compounds are not good examples for first time attempts at using 2D spectra to assist with assignments.**
3. **Quinidine in C_6D_6. This compound is of good complexity, and assignments are similar (but not identical) to those for quinine, as posted on-line.**
4. **Quinine in C_6D_6. An on-line guide provides all assignments:**
   http://www.chem.wisc.edu/~cic/nmr/NMRdatab/res_cmpd/pdfs/quin_assignment_example-for-pdf.pdf

   Quinine and quinidine in C_6D_6 are available in sample positions 2 and 3 on Callisto.

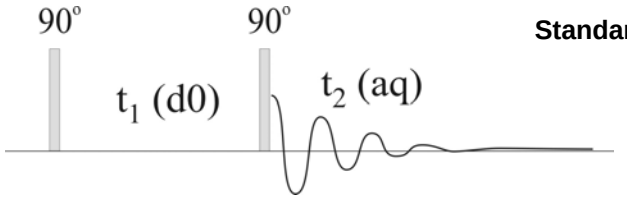
   Quinine and quinidine data are available on-line at:
   castor:\fry\public_html\Chem636\quinine-quinidine
5. **Sucrose or other samples used in previous homeworks.**

Reading – **Intro to 2D NMR: Claridge sections 5.1-5.5 (esp. 5.1)**
– **COSY flavors: Claridge sections 5.6, 5.7**

Goals – **Learn basic terminology of 2D NMR, and the utility of various major flavors of COSY (including TOCSY)**

2D spectra are acquired in a manner that is analogous to kinetic data sets that have many spectra that are often presented as ***stacked plots***. You will learn more about the second dimension in lecture. It is built experimentally by acquiring many one dimensional spectra with a delay in the pulse sequence, usually defined as t_1, being changed incrementally. The number of spectra that are acquired for the 2nd dimension is set by the parameter **TD1** (Bruker) [or **ni** (Varian)].

A COSY pulse sequence has a 2nd 90° pulse added to the standard one pulse 1D sequence:



Standard COSY sequence

The delay between the two 90° pulses is t_1; on the spectrometer it is the delay **d0** [or **d2** (Varian)]. This delay will be increased incrementally as one 1D spectrum after another is acquired. **d0**=0 for

the first spectrum, 2nd spectrum has **d0**=1/**SW1**, 3rd spectrum **d0**=2/**SW1**, and the nth spectrum **d0**=(n-1)/**SW1**. t_1 is 1st time period, and is also called the *indirect dimension*; it is also called the *evolution time*. The 2nd time period is t_2, and is the same "normal" acquisition period as used in 1D spectroscopy; remember that 2D is the acquisition of a series of 1D spectra. After two Fourier transform, one along each dimension, t_1 and t_2 are changed to F1 and F2, respectively.

Resolution takes on different requirements in 2D spectra, usually in needing to only resolve different spins (or multiplets) from each other. The need to resolve within a multiplet is removed or reduced. Lower resolution is therefore acceptable in a 2D spectrum. Information content in a 2D arises from **crosspeaks**, which are formed through evolution (during t_1; in COSY, ^{1}H-^{1}H J-coupling is the useful component that evolves) and mixing of magnetization (here done by the 2nd 90° pulse).

Fundamental questions in 2D NMR then become how do crosspeaks arise, how intense are they (what needs to be optimized to insure that they are sufficiently intense), and what else needs to be optimized (including resolution) to minimize experiment times.

I. Resolution in absolute-value COSY: (this is the most common type of COSY run in our lab)

[Any of the four samples mentioned above will work fine for section.]

In this HW, we'll explore the issues of resolution and crosspeak intensity in COSY 2D NMR. Resolution is (relatively) easy to understand: it is controlled by the acquisition time in the 2nd dimension, just as it is in 1D NMR. In the detected dimension (***$t_2 \rightarrow F2$***):

$$\mathbf{AQ} = (\text{number of points acquired}) \times (\text{time per point}) = \mathbf{TD \times DW} \qquad [1]$$

The tricky part is Nyquist's theorem:

SW = 1/(2DW) , so **AQ = TD / (2×SW)** .

In the indirect dimension (***$t_1 \rightarrow F1$***), we just label everything with a one:

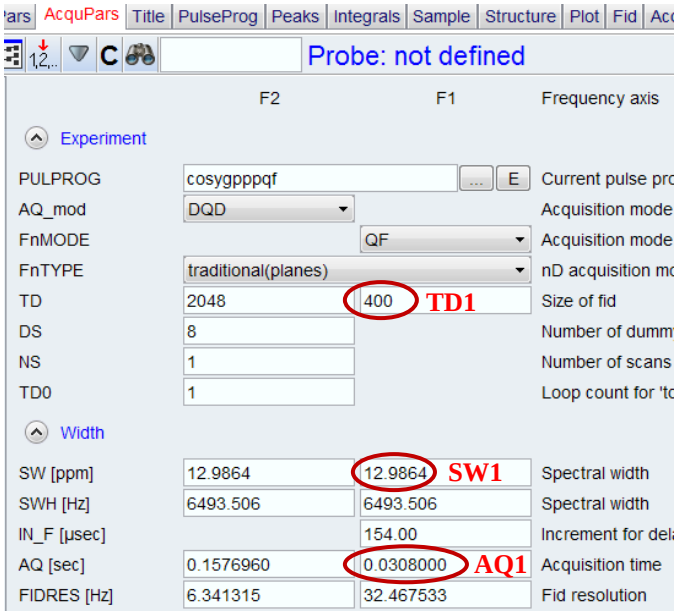
AQ1 = TD1 / (2×SW1) [2]

Annoyingly, you can't type the parameters this way into TopSpin, but they show up in columns (1 *after* 2, of course!) in the ACQUPARS (**eda**) panel.

For a 2D COSY spectrum:

digital resolution ~ 1/AQ1 [3]

Note that in normal COSY data, **SW = SW1** and **TD1 < TD**.



The digital resolution in COSY spectra is dominated by the indirect dimension parameter **TD1**. I.e., the number of ^{1}H spectra acquired to form the 2D stack is a crucial parameter during COSY experiment setups. [The other three crucial parameters are *COSY-type selected*, **D1**, and **NS**.]

We're now ready to play around with resolution. In this section, we'll use the simplest, most common form of COSY utilizing the experiment (parameter set) **COSYGPSW**.

One might wonder how small **TD1** can be: experiments get shorter in total time linearly with **TD1**.

a) Acquire a very low resolution cosy: try an experiment with 0.5 to 1 ppm resolution (comute TD1 to match this resolution from eqns 2 and 3). Assume your sweep width will be 8 ppm.

D1 Does this make any sense? One might well think not — are you kidding me; 1 ppm resolution?? — and in general that negativity would be correct. You'll of course work the data up and see what you think based on spectra. But note how fast the experiment can be done: you're asking for just a few ^{1}H spectra to be acquired. I (your puny lab director) definitely prefer **NS**=2, but **NS**=1 works ok, and is the correct choice when rapid acquisitions are required. Perhaps monitoring of a moderately fast reaction can be done with COSY spectra, providing useful information? Maybe… There are better methods for taking fast data, and we'll discuss some of those briefly in lecture. Using those other methods, I have seen useful applications of fast TOCSY (a similar experiment to COSY), as well as HSQC.

b) Now acquire a "standard" cosy (again, using **COSYGPSW**). Calculate the digital resolution for this spectrum.

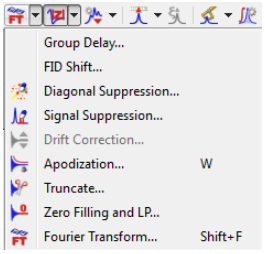
D2 If you find it amazing even this "improved" resolution works, you're not alone in your first impressions. For years after COSY was first invented, prominent NMR spectroscopists believed that **TD1** had to be larger than the value used here (128). It took much empirical evidence, and more precise understanding of the COSY mixing and evolution to convince otherwise. We'll investigate these issues further in the next section. The key requirement for digital resolution is that it resolve different multiplets. The ability to resolve coupling within the multiplet is removed by the experiment itself: *crosspeaks* tell us about the coupling, not measurement of the coupling itself. Even so, it is important to keep in mind that **TD1**=128 is not sufficient for many research situations, so **TD1** is a critical parameter to optimize.

c) Last in this section is to acquire a "high-resolution" spectrum, by improving the digital resolution by a factor of four over that for the standard setup. Running this spectrum with **NS** = 1 is acceptable (although for a research compound, you should leave **NS** ≥ 2; it gives much better data quality). In research involving complex compounds, **TD1**=512 would be fairly common. Going to **TD1**=1024 would not be unusual. **TD1**=1600 and perhaps occasionally 2048 are also used when multiplets are strongly overlapped. [NOTE: Going to such large values with HSQC is ***not*** recommended, as will be discussed in the next lab.]

123☞ Work up the 3 COSY spectra acquired above. Spend some time with the processing, using the following steps as a guide:

a) In properties (double-left-click the spectrum background), I prefer 40 contours (positive and negative), and Scaling=1.2 as defaults for 2D spectra. This will slow down some operations, so you may need to back off to fewer contours on occasion if things get too slow.

b) The Fourier transform sizes in both dimensions need to be identical in order to "symmetrize" the data (see step f below). This can be checked using Zero Filling and LP… within the FT button.

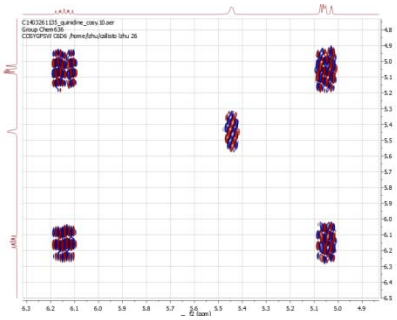


c) The apodization (**W**) should be set to Sine Square at 0° in both dimensions; click f1 f2 to switch between the two dimensions. For F1, the First Point = 0.5 is normal.

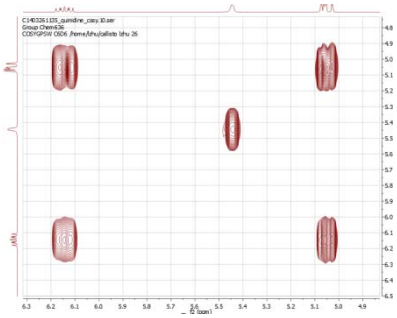
d) Set traces (as done in an earlier lab) by loading the proton spectrum with the COSY into the same document. Then click on  and Setup.

e) The COSY should look fine at this point. But occasionally MNova does not put the data into magnitude mode. Expansion on a section of the spectrum will then show many positive-to-negative transitions, as shown below as Phased. If so, click  and choose "Magnitude along F2". Your display will then look as shown in the middle (as long as you're displaying a Contour Plot (in ).

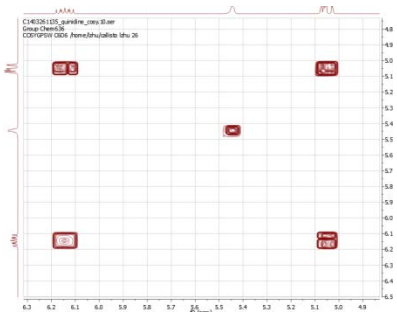
f) Often the data is improved by symmetrizing it (be wary of symmetrization artifacts; compare spectra prior and after processing). Perform by implementing thru the menu selections: **Processing → Symmetrize → Cosy-Like** .



Phased



Magnitude Mode



Symmetrized

Plot each spectrum as .pdf and .mnova and upload.

II. Crosspeak intensities — Long-Range COSY:

So what's the deal with the crosspeaks? You should have observed that most crosspeaks are least intense in the poorly resolved (first) spectrum you acquired above. But they won't consistently get larger when going from **TD1**=128 to **TD1**=512; some will, but some will not. Crosspeaks arise from transfer of magnetization from one spin in the first time period to a coupled spin in the 2nd time period, e.g.: $I_Y^A(t_1) \rightarrow I_Y^B(t_2)$. The transfer is sinusoidal in character: during t_1, the magnetization evolves as:

$$I_Y^A \xrightarrow{t_1} I_Y^A \cos(\pi J t_1) + I_Y^A I_Z^B \sin(\pi J t_1) \qquad [4]$$

During the acquisition (and ignoring chemical shift), this magnetization is detected as:

$$\xrightarrow{t_2} I_Y^A \cos(\pi J t_1)\cos(\pi J t_2) + I_Y^B \sin(\pi J t_1)\sin(\pi J t_2)$$

The $\cos^2$ term (starting as I_Y^A) forms the diagonal in the COSY spectrum. The $\sin^2$ term (starting as $I_Y^A I_Z^B$ forms the crosspeaks. The larger the $\sin^2$ term gets, the larger the crosspeak at $\delta_A(t_1,\text{F1}),\delta_B(t_2,\text{F2})$ will be. Now we can see that as $t_1 \rightarrow 1/2J$, crosspeaks intensity grows since $\sin(\pi J t_1) \rightarrow 1$. Once past 1/2J, the crosspeaks will get smaller. At t_1 = 1/J, the crosspeak will vanish: $\sin(\pi J t_1) = 0$.

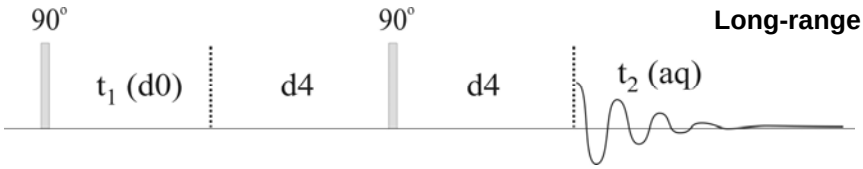
The evolution time t_1 is therefore critical to crosspeak intensity. Remember that t_1 is the incrementally increasing separation between the two 90° pulses in a COSY. It takes another

spectrum to increment t_1 another value of 1/**SW1**. Getting to large t_1 values costs in increased experiment time. What we want is just enough $\sin(\pi J t_1)$ to get the crosspeak well out of the baseline noise of the 2D spectrum, but not by too much.

It would take a lot of experimentation, and more detailed analysis to go further with this (already overly wordy) discussion, but hopefully you get the idea. For a range of normal proton-proton couplings, 3-15 Hz, **TD1** = 128 to 512 works nicely. For smaller **TD1** values (and therefore faster experiments), crosspeaks involving smaller J-couplings may be difficult to unambiguously identify. In such cases, increasing **NS** would help, but increasing **TD1** another option. Larger **NS** gives somewhat better overall signal-to-noise; larger **TD1** definitely improves resolution.

D3 What about couplings smaller than 3 Hz? Suppose you're interested in verifying a W coupling of 0.8 Hz. From the analysis above, what would t_1 need to equal? If SW=SW1=8ppm, what would **TD1** have to equal? [Note: Since we're applying sinebell apodization, the 1/2J point needs to be in the *middle* of the acquisition, not at the end. That means you need **AQ**=1/J for maximum crosspeak intensity. *ouch*] For a 2 scan COSY, how long would the experiment take? (You could use the spectrometer to estimate this, or realize that **D1**×**NS**×**TD1** is a good estimate.) The key to all this is the rather extreme value you should have arrived at for **TD1** (see eqn 2 above). [**TD1**=12,000 on a 600 when the sinebell apodization correction is included!]

One way around this issue is simple: acquire spectra when t_1 is closer to 1/2J. This is the essence of the long-range COSY experiment. If we put in a delay **D4**, typically ranging 50 to 200ms (for small molecules, up to 500ms works fine), crosspeaks arising from small J-couplings will get larger.



Long-range COSY sequence

4☞ Acquire a long-range COSY with **D4**=0.2s, **TD1**=128, and **NS** ≥ 2. Process, symmetrize, and plot. Identify a couple crosspeaks that increased in size from the standard COSY.

III. Phase-sensitive, double-quantum filtered COSY:

D4 Let's take this in two bites, *phase sensitive* first. To prove that phase sensitive is better, go to your ^{1}H spectrum in MNova, and put the spectrum into magnitude mode: each complex point $a + ib$ in the spectrum is replaced by $\sqrt{a^2+b^2}$. You'll see that that is not good for resolution. So why do magnitude processing at all? Well, we might be lazy and not want to have to phase the spectra. But the result seems to be awful. We can improve it by applying a sinebell apodization. Try it on the 1D ^{1}H spectrum: using **W**, switch the apodization to sinebell at 0°. You'll see that this greatly improves the spectrum: the sinebell narrows the long peak tails arising from the magnitude processing. Unhappily, the ability to quantify the data is also seriously degraded. Such processing is therefore not done, and the (relatively simple) process of phasing 1D spectra is done to achieve good resolution and quantitative spectra.

D5 Why then do COSY as absolute value (magnitude mode)? Turns out it's not so simple to get a phased 2D spectrum of this type. Claridge provides more detail on this if you are interested. But

eqn 4 above tells the tale: the diagonal (arising from the I_Y^A term) has $\cos^2$ phase, whereas the crosspeaks (arising the $I_Y^A I_Z^B$ term) are $\sin^2$. The two sets of peaks in a COSY spectrum will always be 90° out-of-phase: no way to phase a this simple form of COSY spectra. The sinebell apodization recovers much of the lost resolution, and magnitude mode processing removes the issue of having to phase. Everyone say Yah!

There is a more complex version of COSY that gets diagonals and crosspeaks in-phase by implementing a double-quantum filter (cool spin-physics! ☺). This improves resolution, and adds two additionally useful features:

DQ-COSY: 1) The coupling *actively* involved in forming a crosspeak can be identified as being *anti-phase* (plus-minus). Other couplings involved in the two multiplets are inactive, and are in-phase. See discussion and examples in Claridge Figs 5.45 thru 5.51.

2) The double-quantum filter removes large singlets. To create double-quantum magnetization, two coupled spins are *required*. tert-butyl groups, a large HOD peak, etc., will be removed in this experiment.

5☞ Acquire a DQ-COSY spectrum with **TD1**=512, and minimum phase cycle (remember to consult the end of the pulse sequence listing). Process, phase (should be close to correct initially), and plot. Zoom in and identify active versus inactive couplings for at least one coupled pair.

Upload 5 plots as .mnova and .pdf files, and come to lecture ready to discuss questions raised here.