I. COSY Experiment

Cosy Pulse Sequence:
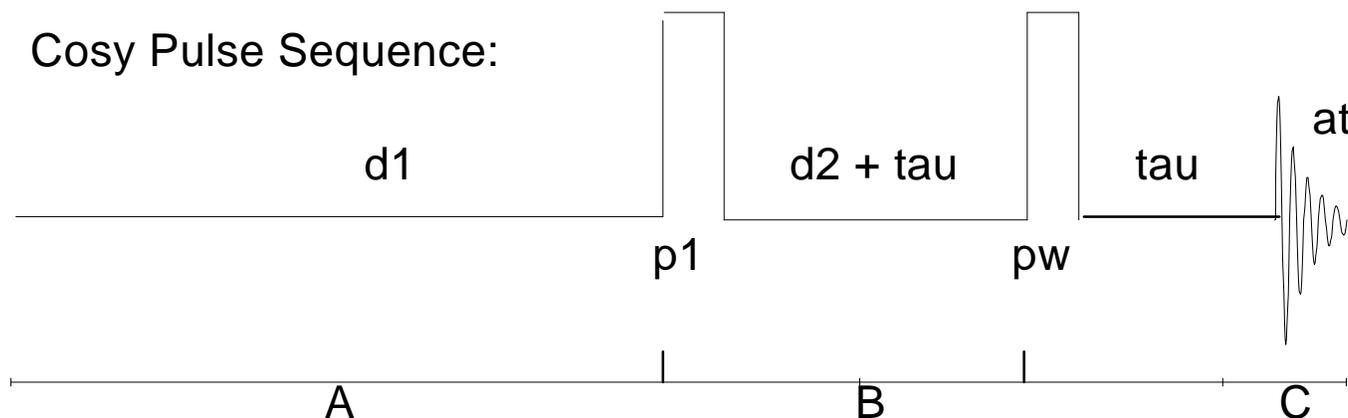
The COSY pulse sequence consists of an initial delay (**d1**) during period A that allows the magnetization to return to an equilibrium steady-state. This is followed by a pulse (**p1**) and delay (**d2**) that generates the spin-coupling information and allows that coupling to evolve. Finally, the resulting magnetization is examined with the pulse **pw** and the data acquired during time **at**. Long-range coupling information can be examined by adding a delay **tau** before and after the **pw** pulse. Additional pulses can be added to this sequence to 'relay' coupling information from one adjacent spin-coupled proton to the next in a spin system (sequence not shown). Important parameters in the COSY sequence are shown in the following table.

Observation pulse	**pw**	90 degree pulse (will be automatically set to 90 degrees).
Preparation pulse	**p1**	90 degree pulse for normal COSY. Use 45 degree for COSY45 experiment.
Relaxation Delay	**d1**	set to 1-3*T1 (typically 0.6 to 3 secs). Small **d1** values can be used to speed the experiment, but this can increase baseline artifacts.
Evolution Delay	**d2**	evolution time (t1 period). **d2** is automatically incremented with each subsequent fid and should always be equal to 0 to start. The amount by which **d2** is incremented is determined by **sw1**.
Data Acquisition Time	**at**	acquisition time (t2 period). Determined by **sw** and the number of acquired data points in the acquisition dimension (**np**).
Spectral Width	**sw, sw1**	spectral width (for COSY spectra, **sw1** should always = **sw**). **sw** determines spectral width in the acquisition dimension, while **sw1** controls the spectral width in the time-incremented dimension.
Number of Time Increments (or fids)	**ni**	number of t1 increments. Set **ni**=128 for low-resolution, 192-256 for medium resolution, 400-512 for very high resolution. **ni** may be further decreased (e.g., 64-96) if speed is essential.
Coupling delay in long-range COSY	**tau**	propagation time for long-range COSY (**relay** = 0) and for relayed COSY (**relay** > 0)
Relayed-COSY spectra	**relay**	number of relays to be performed; if **relay** = 0, a normal absolute value COSY is performed; if **relay** = 1, a **relay**-COSY is performed; if **relay** = 2, a DOUBLE-RELAY-COSY is performed.

| Calculate Experiment Time | **time** | **time** will calculate the overall time required to acquire the 2d spectral data. Allow about 5 extra minutes for disk-preparation before acquisition. |

II. Automatically Obtain a COSY Spectrum using Glide

You can aqcuire a COSY spectrum automatically under GLIDE, or you can choose to obtain the COSY spectrum manually.

TO ACQUIRE THE SPECTRUM USING GLIDE: Click on Glide on the menu bar. Click on **Experiment & Solvent.** Choose **Run proton and Cosy** from the Experiment menu. Click **DO.** Then click the **GO** icon. That's it. The spectrometer will run a proton spectrum, optimize the sweep width, and then run a quick COSY experiment. The proton and cosy spectra will then be plotted. This will take about 25-30 minutes. **DO NOT** interfere with the spectrometer until plotting is complete. After the COSY spectrum has been plotted, you can save the data, re-process and replot as you wish, using the commands shown below.

III. Manually Setup for COSY ACQUISITION

A. Acquire a Proton Spectrum

The first step in setting up for a COSY spetrum is to acquire a proton spectrum of the sample and optimize the spectral width. Take a ^{1}H NMR spectrum of the sample in the normal way. After data acquisition is finished, display the full spectrum (all 15 PPM) and put up two cursors. Bracket the left-most and right-most peaks in the spectrum with the cursors, leaving about 0.5 PPM of noise between each cursor and the nearest peak. Next, type **movesw**. **movesw** will adjust the spectral width **SW** and the transmitter frequency to correspond to the region defined by the two cursors. **WARNING: DO NOT TYPE movesw MORE THAN ONCE IN THE SAME EXPERIMENT WITHOUT RE-ACQUIRING THE SPECTRUM.**

| Optimize Sweep Width | **movesw** | **movesw** will narrow the sweep width to the region defined by the current position of the left and right cursors. The transmitter frequency (**tof**) will be centered at the middle of the two cursors. |

After typing **movesw**, you may see a message that the number of points and sweep width will not give optimum signal to noise. This is normal on the Gemini, which lacks filters for best S/N at all sweep widths. Once the sweep width and transmitter frequencies have been set with **movesw**, you can begin to setup for a COSY experiment. The next step is to move these new acquisition parameters into the 2D workspace.

B. Move Acquisition Parameters and Setup COSY Pulse Sequence

VNMR allows you to acquire spectra in several workspaces that are called experiments. You can have up to nine on the UNIX workstations. Experiments can be used to run simple 1D spectra, arrayed spectra (T_1 measurements, NOE difference, DEPT, Kinetics, etc.), or 2-, 3-, or 4-dimensional NMR spectra.

Once you have defined the optimum spectral window for the COSY experiment by taking a simple proton spectrum and narrowing the sweep width with **movesw**, you need to move these new
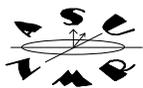
parameters from the current experiment (exp1) to a new experiment, which will be used to acquire and process the COSY spectrum. Move the acquisition parameters from the proton experiment (exp1) to experiment 2 (exp2) by typing **mp(1,2)**. This will copy the current acquisition parameters into exp2. Next, join exp2 by typing **jexp2.**

Once the 1D proton parameters have been copied into exp2, they can be transformed into the acquisition parameter set suitable for acquiring a COSY spectrum simply by typing **cosy. cosy** will load the pulse sequence required for to obtain a COSY spectrum, and will convert the optimized 1D parameters into the 2D parameter set. These commands are summarized in the following Table.

Join a different experiment	**jexp*n***	**jexp*n*,** will allow you to acquire, process and display spectra in a different experiment. *n* can be 1 to 9. The command will not work if the experiment you want to join doesn't exist.
move parameters to a different expt.	**mp(*n,m*)**	**mp** will copy all acquisition and processing parameters from expt *n* to expt. *m*., where *n* and *m* can be 1 to 9. NOTE: **mp** DOES NOT COPY THE FID!.
Setup for COSY	**cosy**	**cosy** will transform the 1D proton acquisition parameters into a parameter set suitable for acquisition of a 2D COSY spectrum.

C. Adjust COSY Parameters for Desired Resolution and Time

Bear in mind that all users of the Gemini-300 are limited to one-hour. The Gemini can sometimes perform an adequate COSY experiment in this time. The Gemini will setup COSY acquisitions that require from 15 minutes to an hour or more. Processing of the spectra can have a tremendous influence on the quality of the result, i.e., a 10 min COSY spectrum, appropriately processed, may look far better than a 2-hour COSY spectrum that was not properly transformed.

Recognize that appropriate POST-processing of even marginal spectra can result in dramatic (orders of magnitude) improvements in sensitivity and resolution. Hence, long acquisitions are often not necessary to obtain the required information. Indeed, a long acquisition may represent a real waste of valuable instrument time. Second, the experiment time can often be reduced without any loss of resolution or sensitivity by judicious adjustment of the acquisition parameters shown in the next table. Here is a basic approach to obtaining **acceptable** COSY spectra in a reasonable time on the Gemini300:

1. Reduce **nt** to 8, or even 4, if possible. If you can obtain acceptable S/N in 256 scans or less in the 1D spectrum, **nt**=8 will work fine. If you can accept a higher level of baseline artifacts and have sufficient sample, you can use **nt**=4. *Reducing* **nt** *from 16 to 8 will cut the experiment time in half.* A 2 hr experiment is then a 1 hr experiment.

2. Reduce **ni** (number of 2d fids) to the lowest acceptable level (400-512 for very high resolution, 128-256 for medium to high resolution, 64-128 for low **but often adequate resolution**. *Reducing* **ni** *from 256 to 128 will cut the experiment time in half once again.* The 1 hr experiment is now only 30 min.

3. Reduce **d1** to NO MORE THAN 1 to 1.5 times T_1. If you are using D_2O or DMSO-d_6, relaxation can be quite short (0.2 to 0.5 sec) and a **D1** of 0.3 to 0.6 may work quite well. Acetone-d_6 or CD_3OD may require larger **d1** values (0.6 to 1.5 sec) for the best artifact reduction. Use the shortest possible **d1**. You can always redo the experiment if a quick 2D spectrum shows severe problems. You may decide at this point that the 400 or 500 might be needed to give better results. Even if you rerun the spectrum on the Gemini with a longer **d1** value, you will have the information needed to make an appropriate choice. *A short* **d1** *can further reduce overall acquisition time by 25 to 50%.*
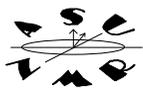
4. **ALWAYS, ALWAYS** type **time.** Check to see how long the experiment will take before you proceed!

TO SUMMARIZE:

1) Acquire a 1d-proton spectrum and optimize the spectral width (**movesw**).

2) Move parameters to experiment 2, join experiment 2, and setup for the COSY pulse-sequence (**mp(1,2), jexp2, cosy**)

3) Adjust COSY parameters as needed to optimize time and resolution (adjust **d1, nt, ni,** and check **time**).

IV. Acquire the COSY Spectrum and Save it for Processing

At this point, simply acquire the COSY spectrum by typing **go. WARNING: DO NOT TYPE ga**. **ga** will acquire the spectrum and then immediately transforms each fid as they are acquired. This makes it impossible to transform the 2d spectrum on the fly.

After acquisition of the COSY spectrum is complete, save the data to disk by typing **svf** (or click **Main Menu**, and then **7:files** on the menu bar). You can process and plot the 2D spectrum on the Gemini300 if no one is waiting. Otherwise, move to the workstations at this point. Remember, the use of the workstations frees up sorely needed time on the Gemini300 and makes possible acquisition of 2D spectra in our walk-up environment.

Acquire COSY	**go**	**go** will clear the 2D space on the disk for the COSY spectrum and will acquire the COSY
Save COSY spectrum	**svf**	**svf** (or **Main Menu, 7:files**) will save the COSY spectrum on the disk in a location that can be easily accessed by the UNIX workstations.