

Magnitude COSY and Homonuclear Decoupling using Topspin

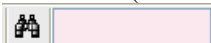
What kind of COSY experiment?

There are three types of COSY experiments implemented: Magnitude COSY, DQF-COSY, and Ecosy. Magnitude COSY is the easiest and will be sufficient for 99% of your problems. A COSY spectrum of sucrose is shown at the bottom of this handout. It provides low-resolution cross peaks between protons that are coupled. It tells you what is coupled to what. DQF-COSY is higher resolution and, for simple spin-coupling systems, can give the magnitude of H-H coupling constants, J_{HH} . Ecosy simplifies the complex structure of cross peaks and allows easy determination of J_{HH} . The disadvantage of DQF-COSY is reduced signal-to-noise (2x), increased processing complexity, and increased minimum experiment time. Ecosy is further reduced in sensitivity (another 2x-3x).

Magnitude COSY




There are two methods for obtaining a magnitude COSY spectrum: normal (rf phase cycled) and gradient. **The normal version runs on all Bruker spectrometers while the gradient version is only possible on the 400 and 500.** The gradient version is to be preferred when signal-to-noise is not a problem because it is extremely fast. For 1mg/ml or even less, an entire COSY spectrum, using gradients, can be acquired in as little as 5 minutes because only 1 scan per t_1 increment is required. If sensitivity is an issue, use the normal version.

1. *Do not spin the sample.* Lock, shim, tune the probe (wobb) and take a normal 1D spectrum. To tune the probe, type wobb. In a few seconds a tuning curve with a "dip" will be displayed. Tuning and matching rods (capacitors) are now adjusted to make the "dip" both centered and as deep as possible. This tuning information is also displayed through LEDs on the preamp box. Adjust until the LEDs show a minimum number of lights, no more than one orange light. Click return when finished.
2. Determine the spectral region to be used for the COSY – the values of **sw** and **o1p**. **sw** is the spectral width and **o1p** is the center of the spectrum. For example, if you have peaks over the range 1 to 6 ppm, the optimum region is from 0.5 to 6.5 ppm. Always leave at least 0.5 ppm on the edges of the spectrum. The value for **sw** is then 6 ppm and **o1p** is 3.5 ppm.
3. Note the file name of the 1D spectra taken above- you may wish to use it as the projection for the 2D plot. Type **new** to change the filename, or preferably, simply increment the experiment number.
4. Type **rpar cosygs.top** to read in parameters for gradient COSY or **cosy.top** for the normal version. Type **getprosol** to update pulse parameters.
5. The parameters below must be set. The F2 dimension refers to the acquisition dimension and F1 refers to the other (evolution) dimension. Click AcqPars. To find a parameter enter it search box,



O1p	center of spectrum
sw in F2	spectral width
sw in F1	equal to value for F2
td in F2	# of points in F2 dimension (multiple of 2 ⁿ)

td in F1	# of points in F1 dimension (# of FID's)
fidres	digital resolution <i>Should be about 3 Hz/pt in F2 and 4 times greater in F1</i> . Fidres is given by the ratio sw/td . Set the values of td in F1 and F2 accordingly.
ns	number of scans gradient version, ns=1 normal version, ns=4*n are permissible values

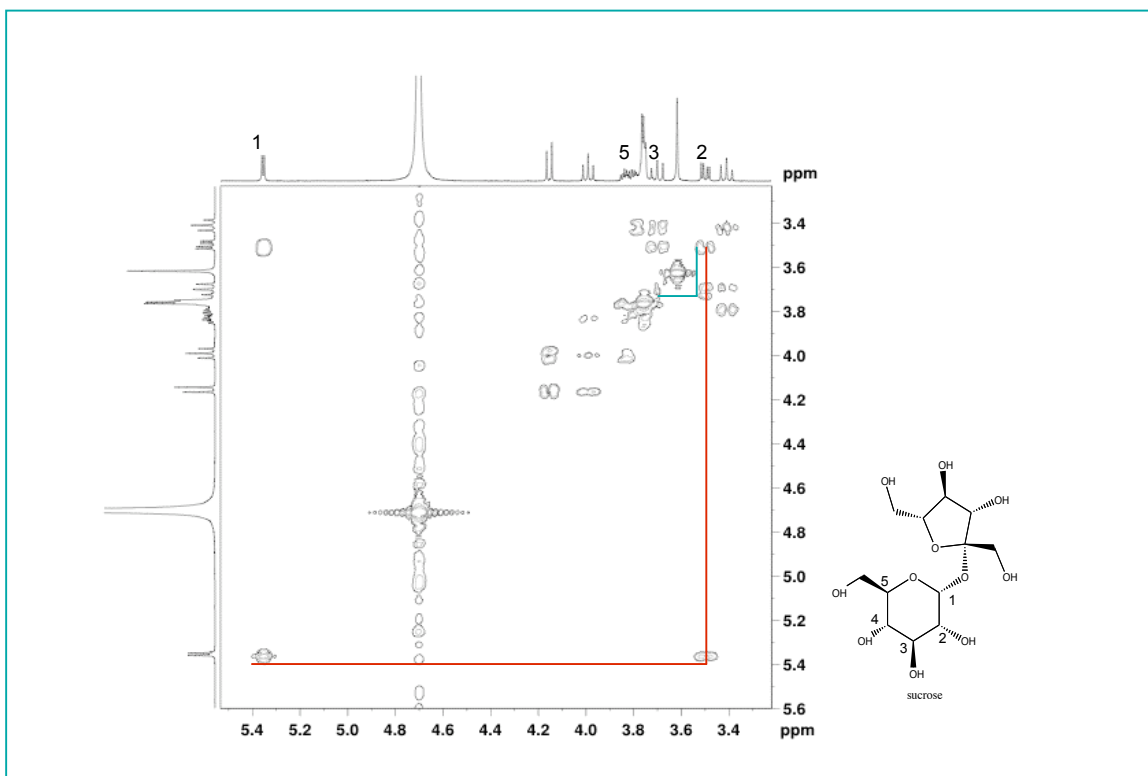
7. Type **expt** to determine the time required for the experiment.
8. Type **rga**. Wait for it to finish. Type **zg** to acquire data. You will see the lock signal fall and rise. This is normal.
9. When data acquisition is complete, type **xfb**, to transform the data. You may transform the data before the acquisition is finished but the resolution of the 2D spectrum will be reduced. 2D data acquisition is different from 1D; over the course of a 2D experiment, different resolution information is obtained. Always retransform after the experiment is finished. COSY is not phase sensitive and **no phasing is possible**.
10. Display control. To expand around a region, simply depress the left mouse button and drag the box around the desired region. To return to the full spectrum, click . To adjust the intensity, use ***2 /2 *8 /8** .
11. Calibration. To calibrate, click  and set the cross-hairs on a peak for which you know the shift in both dimensions (a diagonal peak is easiest for COSY spectra since the shift is the same in both dimensions), click the left mouse button, and enter the shifts.
12. Projections. By default, low resolution 1D projections are displayed at the top and left of your spectrum. One can replace these with the high-resolution 1D spectrum you took earlier. Place the pointer over a projection, right click the mouse, and select external projection. Enter the exact filename as noted in step 2 above (including exact experiment number, etc...)

Sometimes, the chemical shifts of the 1D projection do not align with the 2D spectrum. See the above calibration procedure if this is true. Or, you may need to calibrate the 1D spectrum.

13. Plotting. To print using Topspin editor, select print, print with layout – start Plot Editor, and choose layout 2Dhomo208_11x17 or other suitable layout. This automatically places the projections defined in the above step into the spectrum. Right click the mouse over the spectrum and select 1D/2D edit to adjust the intensity of both 2D spectrum contour levels and 1D projection intensity. These buttons control the



projections: To print, select file, print.



Homonuclear Decoupling

Homonuclear decoupling refers to selectively irradiating, or decoupling, a proton during the acquisition of the FID. When a particular proton is irradiated, the multiplet of its coupling partner is simplified because the coupling to the irradiated proton is removed. Homonuclear decoupling can give two kinds of information: the identity of coupling partners and the magnitude of coupling constants.

The COSY experiment, however, also gives the identity of coupling partners, but is a superior method and is to be preferred over homonuclear decoupling. The only reason to use homonuclear decoupling, then, is when the removal of a coupling from a multiplet allows other couplings to be measured. ECOSY is a powerful alternative to homonuclear decoupling.

One must compare decoupled spectra and normal, coupled spectra with caution. With high levels of decoupler power, the observed chemical shifts move, regardless of whether they are coupled or not. The closer the signals are to the decoupler position, the more they will move away from it. Even singlets will move when the decoupler is turned on. This change is on the order of Hz. This effect is called the Bloch-Seigert Shift and is a major drawback of homonuclear decoupling.

The operator needs to set two parameters: the position of the decoupler for the multiplet to be irradiated and the power of the decoupler. The position is defined by o2 (the letter o), and the power is defined by pl24. The amount of power required depends on the breadth of the multiplet and the size of the coupling constants. More power is needed for broader multiplets. Decoupler power is given in decibels of attenuation. Smaller numbers mean more power. Appropriate power levels for the different instruments are given below.

1. Lock, shim and take a normal 1D spectrum. Tuning the probe is necessary.
2. Determine the multiplets to be decoupled. (Make sure the display info is turned. Right click over the spectrum and select Display properties. Make sure the cursor info box is checked.) Position the cursor on the peak and note the frequency in Hz. Repeat for each multiplet to be decoupled.

3. Type **new** and change the data set. Type **rpar homodec.top** to read in the parameters for homonuclear decoupling.

4. Type o2 and enter a value that was recorded above. Type pl24 and enter a value for the power that is appropriate to the instrument. See the table below. Do NOT enter a number much smaller (higher power) than those listed because damage may result. Different instruments need different power levels because their amplifiers are different. Adjust other normal acquisition parameters, if desired.

5. Take a spectrum. One can change the decoupler position, by clicking on utilities and then o2 and using the cursor, as above.

6. If decoupling was not complete, you may increase the power by entering a smaller number for pl24. Remember that the scale is logarithmic and that each decrease of 3 means the power is doubled. Numbers less than 20 may destroy the probe.

<u>Instrument</u>	<u>power for 5 Hz multiplet</u>	<u>high power for 20 Hz multiplet</u>
300nb	50	40
300wb	60	50
400	55	45
500	45	35