Magnitude COSY, J-Resolved, 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 400s and 500s**. 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 and take a normal 1D spectrum. You must tune the probe and the procedure depends on which NMR you are using. For new NMRs (both 500s and both 400s), type atma and the procedure is automatic. For old NMRs, such as the 300wb, 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 AcquPars. 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 ***Should be about 3 Hz/pt in F2 and 4 times greater in F1.*** **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 

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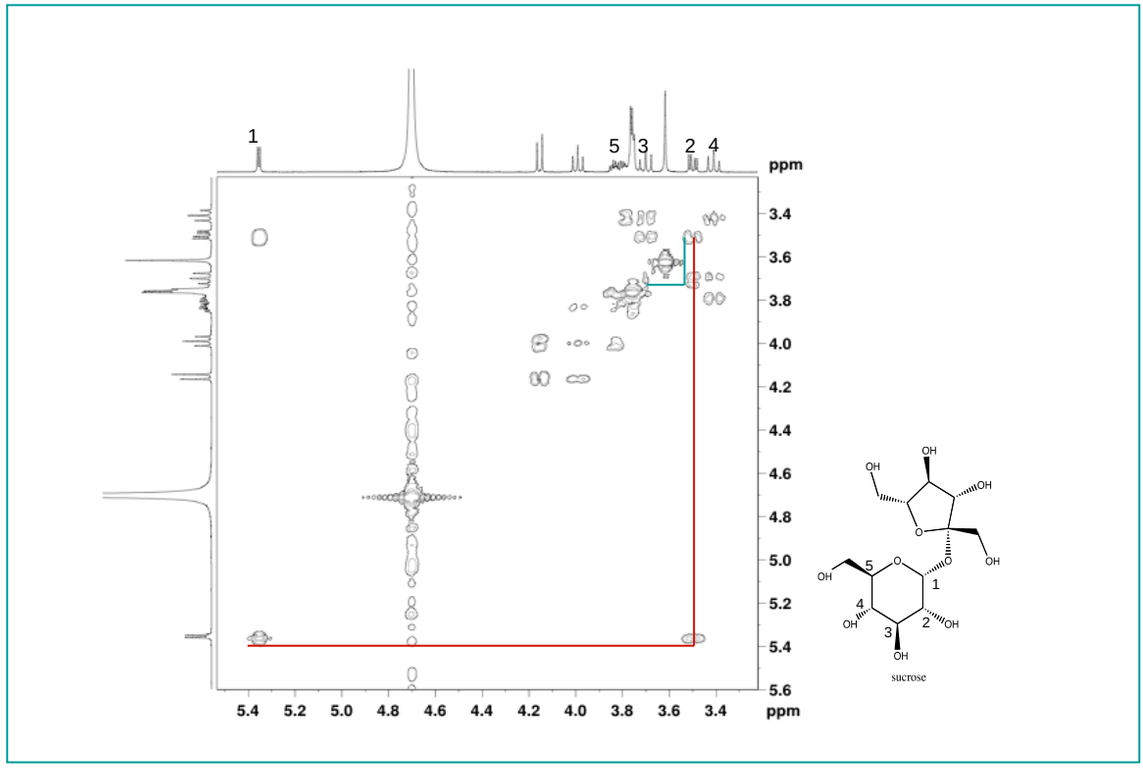
11. Calibration. To calibrate, click Process, Calib. Axis and set the cross-hairs on a peak for which you know the shift in both dimensions (a diagonal peak is easiest for COSY spectra since the shift is the same in both dimensions), click the left mouse button, and enter the shifts.

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. A) It is easiest to simply to print the active window, which gives you exactly the spectrum that is displayed. To print this way, select, print, "print active window" under  . B) To print using Topspin editor, which allows annotation and expansions, select print, print with layout – start Plot Editor, and choose layout 2Dhomo208_11x17 or other suitable layout. Sometimes, projections do not load properly. One remedy is: before you start Topspin editor, in the data browser, navigate to the file that is to be the projection and right click on it. Then select "Display as 2D Projection" and then start Topspin editor. When you print with this method, make sure the paper size and print service agree.

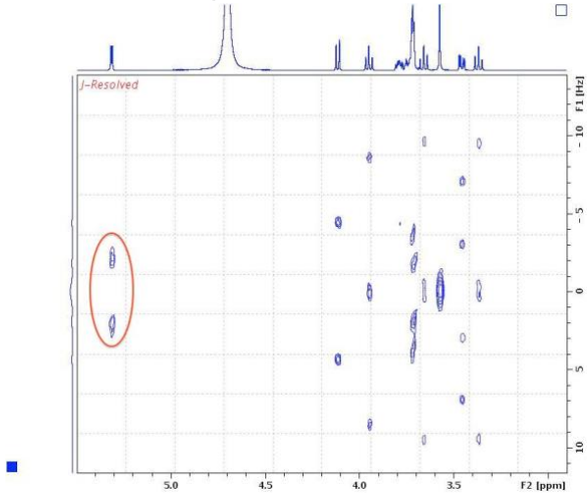


J-Resolved 2D Spectra

J-Resolved spectra is one of the original 2D experiments. It suffered from poor lineshape and sensitivity but processing improvements (backwards linear prediction) have made this experiment very useful. In a 2D J-Resolved spectrum, the X axis is a normal 1D proton, while the Y axis gives a multiplet with the separation equal to the coupling. Shown below is J-Resolved spectrum for sucrose. The red oval shows the doublet for the anomeric proton. The J coupling can be read directly off the Y axis.

To run a J-resolved (set up on 400s and 500s):

1. Type rpar jresolved.top to read in all parameters. Proceed as you would for COSY but with following differences. SW (F1) need only be 50 Hz and TD (F1) can also be small such as 64 or 128 points. Using the defaults is fine.
2. In procpar, there are processing differences: TDoff= -TD(F1) so if TD(F1)=128, then TDoff= -128. Also ME_mod=LPbr. You only need to change these processing parameters if you have altered acquisition parameter TD(F1).
3. Type xfb and tilt to process.



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 or plw24. 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.

1. Lock, shim and take a normal 1D spectrum. Tuning the probe (atma) is necessary.

2. Determine the multiplets to be decoupled. Click  and position the red line on the multiplet to be decoupled and left-click. Select 02. Type o2 to get the value. (On older NMRs, use this procedure: 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.

5. Take a spectrum. One can change the decoupler position, by clicking  , as above, or entering a different 02 value.

7. If decoupling was not complete, you may increase the decoupling power. How one adjusts the power depends on the version of Topspin and NMR you are using.

For Topspin 1 (300wb) the parameter is pl24. Type pl24. See the table below for appropriate values that depend on the NMR. Do NOT enter a number much smaller (higher power) than 30 as damage could result. Values are logarithmic and that each decrease of 3 means the power is doubled. That is a smaller number means greater power:

Instrument	power (pl24) for 5 Hz multiplet	high power (pl24) for 20 Hz multiplet
300wb	60	50

For Topspin 3 (500, 500 Ascend, 400SL, 300nb) the decoupling power is controlled by the parameter PLW24. Values are entered directly in Watts. Higher power number means MORE power:

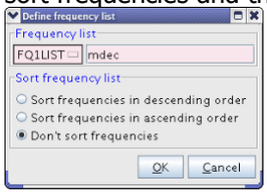
	Power (plw24)	high power (plw24)

Instrument	for 5 Hz multiplet	for 20 Hz multiplet
500, Ascend, 400L, 400SL, 300	0.0003	0.003

MULTI-FREQUENCY DECOUPLING

Decoupling two or three protons simultaneously is now possible on the new Bruker NMRs (currently implemented on the 400s and 500s) Follow this procedure.

1. Read parameters: rpar mdec.top. Type start to take a standard 1D spectrum. Type ef and phase.
2. Expand a region around the peaks to be decoupled. Click  to begin the process of creating a list of frequencies to be decoupled.
3. The following box appears. Use mdec for the name of the frequency list, FQ1LIST. Select Don't sort frequencies and then click OK. Click "overwrite", if necessary.



4. Use the cursor and left mouse to select two or three peaks and then click save.
5. Type mdec. Click OK and then when asked for the frequency list filename, enter mdec. This step creates a special pulse that irradiates the chosen frequencies.
6. Type zg or start to acquire. Then ef to process and phase.
7. If decoupling is poor, the pulse power may be adjusted. Type pldb24 and adjust by +-5 to get good decoupling. Lower numbers indicate greater power. THE LOWEST VALUE is 20! Repeat step 7.