# The Gradient-Selected COSY (gCOSY) Experiment

COrrelation **S**pectroscop**Y** (COSY) is a 2D NMR technique that gives correlations between *J*-coupled signals by incrementing the delay between two 90º-proton pulses (see pulse sequence below). The resulting 2D spectrum is generally displayed as a contour plot, which is similar to a topographical map. When looking at a contour map, you are actually looking down at a cross-section (slice) of a 3D-image of an NMR spectrum. The usual 1D spectrum is traced on the diagonal of the plot and any peaks that are not on the diagonal represent cross-peaks or rather correlation peaks that are a result of *J*-coupling. Thus, by simply tracing a rectangle using the diagonal and cross-peaks as vertices you will know which protons are coupled to each other. Standard COSY experiments require phase cycling to remove unwanted signals and thus can be quite time consuming. This can be circumvented using gradient-selected COSY (gCOSY), which utilizes pulsed field gradients to destroy unwanted z-magnetization and hence their associated signals (axial peaks). Quality gCOSY spectra can be acquired in as little as 5 minutes! All our instruments except the Gemini-300 and the Unityplus-300's are equipped to do gradient selected spectroscopy.
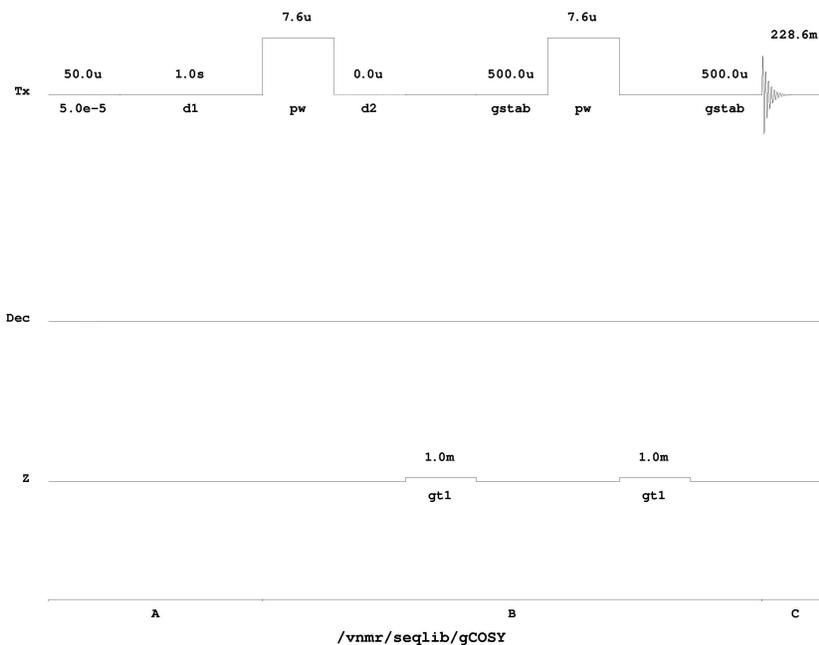
gCOSY Pulse Sequence as Implemented on a Varian Inova 500 MHz Spectrometer.

<u>Explanation of Types of Commands Found in this Handout:</u>

1. The VNMR software and the UNIX operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (*i.e.* CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.
   Example: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by a return (a Return is assumed for typed **bold** text commands).
   Example: **su**

3. Some commands are executed by clicking a mouse button with its pointer on a "button" found on the screen. The execution of these commands are indicated by a two letter designation (LC {left click}, RC {right click}, or CC {center click}) followed by a word or words in shadow or **bold** that would appear in the button.
   Example: LC **Main Menu**

   This means to click the left mouse button with its pointer on the button that says "Main Menu".

4. Some commands are executed by the mouse itself. These commands are indicated by a two letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.
   Example: LC (at 6 ppm)

   This means to click the left mouse button with the mouse cursor positioned at 6ppm.

5. Parameters are entered by typing the parameter name followed by a equal sign, the value, and a return.
   Example: **nt=16**

*   *   *   *   *

Detailed procedures including screenshots are also available at:

http://www.chemistry.msu.edu/facilities/nmr/how%20do%20i.html#COSY

If you have any problems or if you find any errors as you go through this handout, please let me know.

Daniel Holmes
Max T. Rogers NMR Facility

Running a gCOSY Experiment

Acquire a Standard ¹H Spectrum

jexp1	join experiment 1.
temp	this opens the temperature control window. Click on the slider and set to 25 degrees Celsius. If it is already 25, you do not need to do anything. If you did change it, allow temperature to equilibrate for 10 minutes prior to running experiment.

Insert your sample, **lock**, **shim** well, **acquire**, and **process** *a standard 1D Proton spectrum.*

Note the left-most and right-most peaks from your spectrum (for example, 8 and 2 ppm).

svf('filename')	save the FID.

Setup the gCOSY Experiment

jexp2	this joins experiment 2. If you get an error message, click **Workspace** => **Create New**.
mf(1,2)	this moves the FID from exp1 to exp2.

Turn off the spin *and adjust the lock level to 80% or higher using lockgain and lockpower. Be sure not to saturate your lock signal with too high lock power. The lock power is too high if the lock level has large fluctuations or begins to decrease with increased lock power.*
        NOTE: Turning off spinning is very important. You will not get good spectra if the sample is spinning because the gradients rely on the spatial stability of the nuclear spins!

gCOSY	this loads the acquisition parameters.
setwindows	this is an in-house macro that allows you to easily set the sweep width in both dimensions.

You will need to answer the following questions:

        Question 1:   'Enter the 1H left ppm limit:'

*Use a value 1 ppm greater than your left-most 1H peak. For example, if your peak is 8 ppm, then type **9**.*

        Question 2:   'Enter the 1H right ppm limit:'

*Use a value 1 ppm less than your right-most 1H peak. For example, if your peak is 2 ppm, then type **1**.*

After parameters are setup, type:

nt=# (where # is the number of scans)	sets the number of scans for each FID in the directly detected dimension (F2). Increase the number if you have a dilute sample. The minimum can be **1**, but at least **2** scans are recommended.
ni=# (where # is the number of increments)	this defines the resolution in the indirectly detected dimension (F1). The more scans, the higher resolution. ni=128, 200, or 256 are common.
time	note the value. You can increase nt and/or ni and check time again to fill your allotted instrument time.

Acquire a gCOSY spectrum

go (do not type ga)	acquires 2D spectrum.

Data Manipulation

svf('filename')	save 2D dataset.
setLP1	sets linear predication in the indirectly detected dimension.

NOTE: This will predict data to 3 times the length of your acquired data and gives apparent 'better resolution'. If, after transforming your 2-D dataset, you suspect that this has created artifacts that clutter your spectrum, you can turn it off by typing **proc1='ft'**. You will need to reprocess your data using wft2d.

sinebell wft2d	this performs a sine bell apodization and a 2-dimensional Fourier transform and the color map will be displayed. The color map is your 2-D spectrum with the levels displayed using different colors. Use the following table as a guide to color map navigation.

Printing your Spectra

Printing your gCOSY with 1-D Spectrum as Projections (preferred method):

Open your COSY Spectrum by

LC **Main Menu**   open Main Menu screen.
LC **Display**   open Display Menu.
LC **Size**   opens Size Menu.
LC **Center**   centers contour plot on screen.
jexp1   join another experiment.

Load the 1-D spectrum; Fourier transform (wft), and phase (aph).

jexp2   join the experiment with your COSY.
d2d   in-house macro (performs dconi('dpcon',25,1.2) to display contour spectrum. If this is too slow, click <Cancel Command> and type dconi.

Expand, scale, etc. the region of interest (refer to table for interacting with the color or contour map).

Interacting with the 2-D Color Map/Contour Map	
To do the following...	**You should...**
Increase/Decrease the scale	Click on either **vs+20%** or **vs-20%** or type *vs2d=vs2d*1.5* and click **Redraw**. The typed command increases the display by a factor of 1.5. You can use a larger number if you like (e.g. *vs2d=vs2d*2*, increases by a factor of 2.
Change the number of color levels	Use the middle mouse button to click on the color scale to the right of the color plot. Click on the smaller number to increase the number of colors displayed.
To expand on a region	Ensure that you are in the interactive mode; if not, click **Main Menu**=>**Display**=>**Color Map**. Click with the left mouse button on the left-most point of your desired region. Click with the right mouse button on the right-most point. Click on **Expand**.
To expand an exact region	Type *sp=#p wp=#p* (for the F2 dimension, usually vertical) and *sp1=#p wp1=#p* (for the F1 dimension, usually horizontal), where # are the numbers in ppm for the region of interest. sp designates the start of plot and wp is the width of the plot. You will need to click on **Redraw** to update the screen. For example, I want to expand the region between 1 and 4 ppm in F1 and between 2 and 4 ppm in F2, I would type *sp=2p wp=2p sp1=1p wp1=3p*, then I click **Redraw** to see the result.
To reference the 2-D spectrum	Expand the region of interest. Click **Hproj(max)** for the horizontal projection and **Vproj(max)** for the vertical projection. Place the cross-hair cursor on the diagonal position you wish to reference (the projections will help you to orient the cross-hair). Type *rl(#p) rl1(#p)*, where # is the value in ppm you want to be the reference. rl sets the F2 dimension reference and rl1 sets the F1 dimension reference.
Redisplay the spectrum	Type *d2d* or *dconi* or click on **Redraw**.
Display a projection of the 1D spectrum on the side of the 2-D plot	Click **Proj**, then click **Hproj(max)** for the horizontal projection or **Vproj(max)** for the vertical projection. Use the middle mouse to adjust the scale.
Display a trace of the 2-D plot	Click **Trace** and use the left mouse button to drag the cursor.
View the contour plot	Type *d2d* or click **Main Menu**=>**Display**=>**Contour**
Increase number of levels on	Type, for example, *dconi('dpcon',15,1.2)*. The dpcon flag is for displaying

contour plot: Interactive plot	the contours. The first number (15, in this case) is the number of contour lines (default is 4). The second number (1.2, in this case) is the relative spacing intensity (default is 2). You can input different numbers if you wish, but the second number must be greater than 1.
Increase number of levels on contour plot: Non-interactive plot	Type, for example, *dpcon(15,1.2)*. The dpcon flag is for displaying the contours. The first number (15, in this case) is the number of contour lines (default is 4). The second number (1.2, in this case) is the relative spacing intensity (default is 2). You can input different numbers if you wish, but the second number must be greater than 1.

plgcosy   follow the directions on the screen: This is
                                                    our in-house macro that prints your desired
                                                    2-D spectrum.

Autoprinting your gCOSY with Projections generated from COSY (not recommended):

Display the region of interest and
LC **Autoplot**:   this plots your COSY with the projections
                                                    generated from the 1-D data subsets. The
                                                    indirectly detected dimension will have low
                                                    resolution and thus, the projection for that
                                                    dimension (usually F1) will have broad
                                                    peaks. For better resolution projections, use
                                                    the following procedure.