

Determining C-H Connectivity: gHMQC and gHMBC

Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) are 2-dimensional inverse H,C correlation techniques that allow for the determination of carbon (or other heteroatom) to hydrogen connectivity. HMQC is selective for direct C-H coupling and HMBC will give longer-range couplings (2-4 bond coupling). Our facility implements both standard and gradient-selected versions of both HMQC (gHMQC) and HMBC (gHMBC), the latter of which improves the acquired spectra by significantly reducing unwanted signal artifacts.

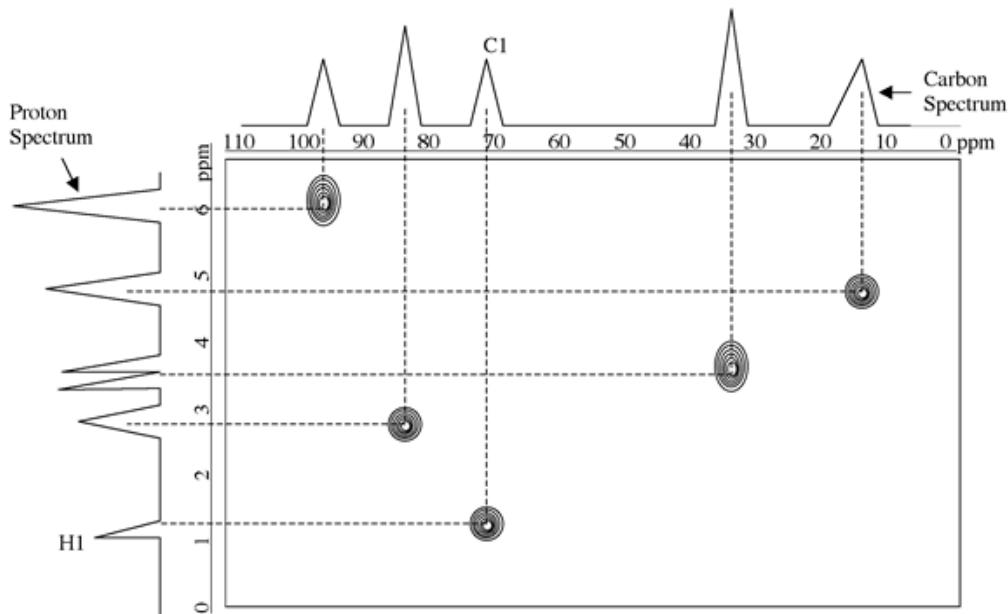


Figure 1. Representation of a gHMQC spectrum. Cross-peaks appear as contour relief plots at the intersection of directly coupled C-H peaks. Only directly bonded hydrogen and carbons will give cross-peaks (quaternary carbons are not seen in gHMQC, but are present in gHMBC), which makes interpretation rather straight foreword. As seen in the simulated spectrum above, assignment is made by drawing two lines at a right angle from the ^1H spectrum to the ^{13}C spectrum through the cross-peak, which looks like a series of concentric ellipses.

Explanation of Types of Commands Found in this Handout:

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 hitting the RETURN key.

EXAMPLE: **su**

Hitting the RETURN key is assumed for all bold text.

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 **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 the 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 at 6 ppm.

5. Parameters are entered by typing the parameter name followed by an equal sign, the value, and a return.

EXAMPLE: **nt=16 <rtn>**

* * * * *

Preliminaries

Acquire a standard ^{13}C spectrum in exp1 and save it. If you are running on the 500 MHz spectrometer, be sure to tune the probe.

Note the chemical shift of the left-most non-quaternary peaks. The quaternaries will have no correlations and hence add nothing to the experiment. In fact, including them will lead to an unnecessary reduction in resolution. Run a DEPT if you are unsure of which are the quaternaries.

Note the chemical shift of the right-most peaks.

Acquire a standard ^1H spectrum in exp2 and save it.

Note the chemical shift of the left-most and right-most peaks.

NOTE: The above spectra can be obtained earlier and retrieved for the gHMQC experiment.

Determination of a 90° proton pulse (pw90) (OPTIONAL. Go to Page 5 if skipping)

The following procedure is optional. The default 90° proton pulse will be adequate for most samples. It is advisable to calibrate the proton pulse if your sample is low concentration, buffered, has a high salt content, or is in a solvent other than CDCl_3 .

Still in exp2,
gain='y'
d1=3

turns automatic gain control off
set recycle delay to 3 seconds. d1 +
at should be about 1 to 1.5 times
your longest T_1 . If your T_1 's are
long, you can try d1=3. If you set d1
for less than the recommended time,
you will sacrifice sensitivity.
However, if your sample
concentration is adequate, the
savings in time will more than
balance this loss.

f full

Expand around any non-water peaks.

array

setup an arrayed experiment

You will need to answer the following questions, which will appear above the command line:

parameter to be arrayed:

pw

*enter number of steps in array:
10*

*enter starting value:
31*

*enter array increment:
0.5*

pw[1]=1

da
ga
ai

dssh

pulse width. You want to find the 360° pulse width, which gives null peak heights.

this is the number of different points you will check for the 360° pulse

this is approximately 4 times the 90° pulse -1 [($\text{pw90}=\sim 8\mu\text{s}$), so $4*8-1=31$]. NOTE that this starting value is dependent upon the salt concentration of the sample. If it is hard to determine the pw90, try arraying the pw in 20 steps with a starting value of 5 and increment by 5.

this is the step increase between each point

this will replace the first array element with 1. A $1\mu\text{s}$ pulse will be positive and will aid in phasing the arrayed data.

displays the array
start the arrayed experiment
absolute intensity (i.e. no peaks heights are scaled)

display stacked plots horizontally

The first spectrum will be positive and the second should be negative. If this is not the case, rerun the array starting with a lower starting value (e.g. 28).

dssl

pw90=your determined value/4
pw=pw90

pw?**ga**

Look for the spectrum that has the lowest peak intensities. Note the value: _____

for example, **pw90=33.5/4**
reset pulse width to equal the 90° pulse

Returns the pulse width. Should be $\sim 8.4\mu\text{s}$

acquire a spectrum

You will need this new pw for the gHMQC.

Running the Gradient-Selected HMQC (gHMQC)

In exp3 load your ^1H FID or move it from exp2 [i.e. type **mf(2,3)**], transform, and phase the spectrum.

Make sure the temperature is regulated:

temp=25 su

this sets the VT controller to 25 degrees Celsius. It is very important to have a stable temperature in order to minimize distortions due to convection.

Turn off the spin

Spinning will lead to significant noise in the final spectrum. In fact, never spin for any gradient or 2D experiments.

Lock and shim your sample

Set the lock power so that the lock level is about 70% or higher. Make sure that the signal is not saturated. A saturated signal is usually unstable or if you decrease the lock level and the lock level increases, you are saturating the lock. Decrease the lock power just below saturation. Increase lock gain if the lock level is below 40%.

gHMQC

this loads the gradient selected HMQC experiment with the standard parameters

tn?

IMPORTANT: make sure **tn='H1'**. This will be properly set if you typed **gHMQC** from a proton experiment. If tn is not **H1**, you must move start over from a proton experiment.

pfgon='nny'
setwindows

turns on pulse field gradient.
this is our in-house macro to adjust the ^1H and ^{13}C spectral windows.

You will need to respond to the following requests: (please allow for 1 ppm extra width on each side).

Enter the ^1H left ppm limit:

Enter the ^1H right ppm limit:

Do you wish to change the ^{13}C spectral window? <y=yes or n=no>

The default setting is 160 ppm. Since carbonyls will not give peaks in the HMQC, a narrower sweep width is advisable to improve resolution and shorten your acquisition time. Only choose no if you have no idea about the ^{13}C spectrum.

**If you responded with ‘yes’ to the above, you will need to respond to the following:
(allow about 10 ppm extra on each side.)**

Enter the ^{13}C left ppm limit:

Enter the ^{13}C right ppm limit:

nt vs. ni, which should I increase?

The short answer is increase **nt** for dilute samples and increase **ni** to get better resolution in the indirectly detected dimension (carbon).

The longer answer:

The number of transients (**nt**) determines the Signal-to-Noise (S/N) for your cross-peaks. For gHMQC, if you have sufficient sample, you should use a minimum of 2 scans. If your sample is dilute, increase nt to 8 or 16. You may want to consider using non-gradient methods, HSQC or HMQC, for very dilute sample because they will give better S/N with the same number of scans (for these methods, you must set nt=8 or higher). Non-gradient methods, however, will have larger T1 noise.

The number of increments (**ni**) determines the resolution in the indirectly detected dimension. For the traditional C-H experiment, the indirect dimension is carbon. Thus, the larger the ni, the better the resolution. The default value for ni is 128, which is more than adequate for most small molecules. Furthermore, we typically apply zero-filling and linear prediction to the indirectly detected dimension (F1), which further improves resolution.

nt=2 or desired value

sets number of transients. See above.

time

this will display the total time required for your experiment. If you have more time, you can increase ni to 200 (i.e. type *ni=200*).

Recalculate the time required.

(OPTIONAL) pw=your determined value from page 3.

Optimizing for Specific Heteronuclear Coupling Values

You may wish to change the parameter, *j1xh*, which is a delay to filter for optimum signal at specific *J*-values. The default *j1xh* is 140, which is an average value for the one-bond C-H coupling constant ($^1\text{J}_{\text{C},\text{H}} \sim 120\text{-}230$ Hz). In general, the more s-character the bond, the larger the coupling constant. Thus, alkynes have quite large coupling constants and the default *j1xh* would not be optimum for alkynes. To set *j1xh*, type ***j1xh=desired value***. It may be necessary to perform several experiments to get best results especially when you have alkyne and aliphatic carbons in one compound.

go

start acquiring. DO NOT USE **ga**.

Data Manipulation

(NOTE: For a detailed description of processing and plotting including phasing the contour plot please refer to the handout titled ‘Processing, Phasing, and Plotting 2D Phase Sensitive Data’)

setLP1 gaussian wft2da**d2d**

this performs linear prediction in the indirectly detected dimension, a Gaussian multiplication, and the 2-dimensional Fourier transform. The color map will be displayed following the transform.

This will display a contour map, which will be identical to what is printed.

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) r11(#p*dfrq/sfrq) , where # is the value in ppm you want to be the reference. rl sets the F2 dimension reference and r11 sets the F1 dimension reference.
Redisplay the spectrum	Click on Redraw or type dconi
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	Click Main Menu => Display => Contour
Increase number of levels on contour plot: Interactive plot	Type, for example, dconi('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.
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.

Printing your gHMQC with High resolution 1-D Spectra as Projections

If it isn't already, open your gHMQC Spectrum (preferably in exp3).

LC Main Menu	opens main menu
LC Display	opens display menu
LC Size	opens size menu
LC Full with Traces	gives full screen color map
jexp1	or other experiment where your ^{13}C resides

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

jexp2	or other experiment where your ^1H resides
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Load the 1-D proton spectrum; Fourier transform (wft), and phase (aph).

jexp3	or join the experiment number where your gHMQC resides
d2d	displays contour plot ready for printing

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

plghmqc	this is our in-house macro that prints your desired 2-D spectrum with high-resolution spectra as the projections. You will be required to respond to the following:
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Enter experiment number containing the ^1H spectrum: (2 in this case)

Enter experiment number containing the ^{13}C spectrum <0=none>: (1 in this case)

Enter experiment number containing contour spectrum: (3 in this case)

Running a Gradient Selected HMBC

The gHMBC is used to help establish the carbon skeleton through the multiple bond carbon to hydrogen connectivity. This experiment is relatively insensitive as compared to gHMQC because multiple bond correlations are less efficient than one-bond correlations. Typical one-bond coupling constants are around 150 Hz whereas multiple-bond coupling

constants fall in the range of 5-15 Hz, which is similar to the range for H, H-homo-coupling.

The experimental protocol is similar to gHMQC, just type **gHMBC** and start with the default values. As with gHMQC, you want to consider calibrating the proton 90° pulse. The default values are nt=8 and ni=400. This will require about 1 hour 10 minutes. Increase nt and ni, if you want better S/N or carbon resolution, respectively.

j1xh and j1nh

As with gHMQC, there is the parameter *j1xh*, but in this experiment this parameter sets the one-bond filter to remove one-bond couplings from the HMBC contour map. If your one-bond couplings differ significantly from the default value (as would be expected for alkynes), these one-bond couplings will show on the HMBC as a doublet with a coupling constant on the order of 150-220 Hz centered on the corresponding proton resonance. It is best to start with the default value and compare the HMBC with the HMQC. If it looks like there are one-bond couplings breaking through that cause interpretation problems, you may want to consider changing *j1xh*. We suggest talking to us prior to making such a change.

Another parameter, *j1nh*, sets the filter for the multiple bond correlation. The default value for *j1nh* is 8 Hz. Again, as described previously, the long-range coupling constants fall in the range of 5-15 Hz. Therefore, the default value will give adequate correlation for a range of coupling constants. This parameter can be changed to emphasize certain couplings, but it is not advisable to make the value too small as this makes the associated delay longer thereby reducing the overall S/N.

Perform data manipulation and plotting as with gHMQC except use **setLP1 sinebell wft2d** in place of *wtf2da*. Since this is a magnitude spectrum, phasing the 2D plot is not necessary.