

Determining C-H Connectivity: gHMQC and gHMBC (VnmrJ-2.2D Version: For use with the new Software)

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.

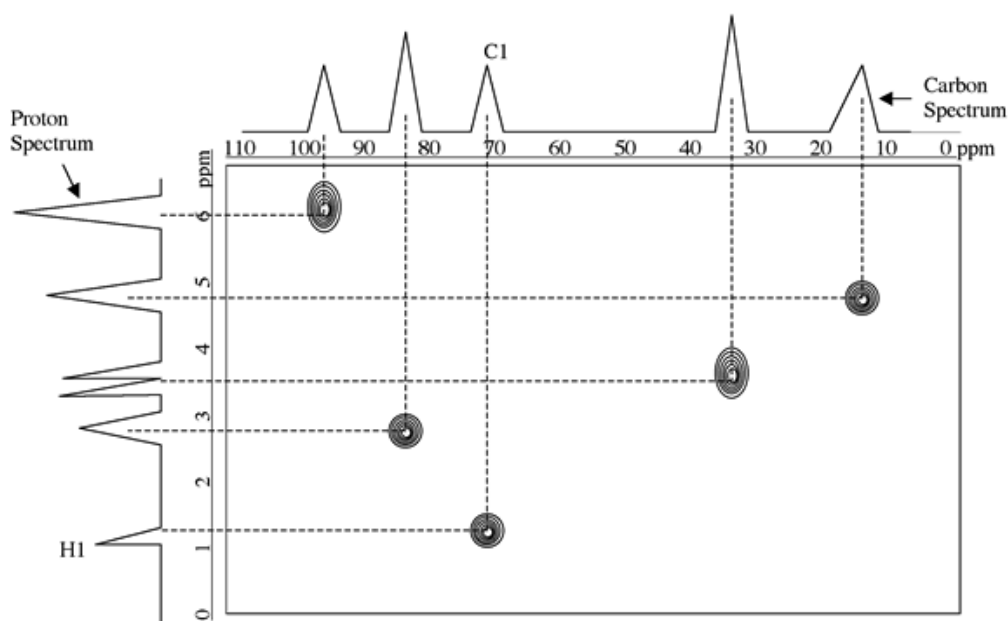


Figure 1. Representation of a gHMQC spectrum. Cross-peaks appear as contour relief plots at the intersection of directly coupled C-H peaks. Only directly bonded hydrogen and carbons will give cross-peaks (quaternary carbons are not seen in gHMQC, but are present in gHMBC), which makes interpretation rather straight forward. 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>**

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Preliminaries

NOTE: This Handout is for the new software, VnmrJ2.2D, and not for our older systems. Please refer to the other gHMQC handout for the older instruments.

IMPORTANT: To simplify use, please make sure that you have only 1 viewport enabled. Click *Edit, Viewports...* Select **1** for the *Number of Viewports*. Click *Close*.

jexp1

Acquire a standard ^{13}C spectrum in exp1 and save it. Please 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 in the F1 dimension. Run a DEPT if you are unsure of which are the quaternaries.

Note the chemical shift of the right-most peaks.

jexp2

Acquire a standard ^1H spectrum in exp2 (i.e. jexp2) and save it.

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

NOTE: The ^{13}C spectrum can be obtained earlier and retrieved for the gHMQC experiment, but you must run the ^1H spectrum at the time of the gHMQC/BC.

Running the Gradient-Selected HMQC (gHMQC)

jexp3 (If exp3 does not exist, click File, New Workspace)

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

Make sure the temperature is regulated (check that the temperature reading is 25. If not, click on the Start tab, then on Spin/Temp, set the slider to 25 and click Regulate Temp. Allow 10 minutes to regulate).

Turn off the spin

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

Set Lock Level >80%

Set the lock gain 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%.

LC Experiments,
then **1H Detected Proton-Carbon 2D**,
then *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 setup from a proton experiment. If tn is not **H1**, you must start over from a proton experiment.

setwindows

this is our in-house macro to adjust the ¹H and ¹³C 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 13C 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 ¹³C 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. For gHMBC, include all carbons, not just protonated.)

Enter the 13C left ppm limit:

Enter the 13C right ppm limit:

nt vs. ni, which should I increase?

The short answer is that both **nt** and **ni** have an effect on S/N. It is most straightforward to 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 each of the FID's you collect in your 2D array. The number of increments (**ni**) sets the number of FID's that are used to construct the 2nd dimension and determines the resolution in that dimension (default is 128). For each successive **ni**, a delay time is increased in the pulse sequence. This means that if you have a sample with short T_1 's (usually observed as broader peaks), then having a larger **ni** will not be favorable because the later increments will only contribute noise and hurt overall S/N. The converse is true for long T_1 's. Thus, getting the best S/N depends on the sample.

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. If you notice your proton has broad peaks, set **ni**=64 and **nt**=16.

Keep in mind, however, that the larger the **ni**, the better the resolution. A reduced **ni** will reduce resolution, but for broad peaks this will not be an issue. 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=4 or desired value
time**

sets number of transients. See above.
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.

Optimizing for Specific Heteronuclear Coupling Values

You may wish to change the parameter, *jlxh*, which is a delay to filter for optimum signal at specific *J*-values. The default *jlxh* is 140, which is an average value for the one-bond C-H coupling constant ($^1J_{C,H} \sim 120-230$ Hz). In general, the more s-character the bond, the larger the coupling constant. Thus, alkynes have quite large coupling constants and the default *jlxh* would not be optimum for alkynes. To set *jlxh*, type ***jlxh=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

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.

Click on the icon: 

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  (vertical scale +20%) or  (vertical scale-20%).
Change the number of contour levels	Click on View => Viewport . (If the viewport window is blank or does not have a contour option at the bottom, click on Edit => Viewports... and then click Close .) Type in the number of contour levels you want and hit Enter (default is 25). Spacing Factor is best at 1.2.
To expand on a region and pan/scan through the 2D	Click on the  icon. Click and drag around the region you wish to expand. To move the expanded region, click on  , then click on the spectrum and drag to desired region.
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 Pan & Stretch	Click  . Click on the spectrum and drag to move to new area. Clicking and holding the right mouse button will allow you to expand or contract the spectrum.
To reference the 2-D spectrum	Expand the region of interest. Place the red cross-hair cursor on the diagonal position you wish to reference. Type $rl(\#p)$ $rl1(\#p*dfrq/sfrq)$, where # is the value in ppm you want to be the reference. rl sets the F2 dimension reference (^1H) and $rl1$ sets the F1 dimension reference (^{13}C).
Redisplay full spectrum	Click on 

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

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

Click *View, Parameter Panel*

opens Parameter Panel.

Click the *Process* Tab

opens Process Panel.

Click *Display*

opens Display options.

Under Screen Position, click *Projections*.

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

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).

plgrid(0.5) (optional) prints a grid on the spectrum with 0.5cm increments.

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:

Enter experiment number containing the 1H spectrum: (2 in this case)

Enter experiment number containing the 13C 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 LC **Experiments**, then **1H Detected Proton-Carbon 2D**, then **Ghmqc** and start with the default values. **IMPORTANT:** Be sure to include quaternary carbons when deciding your carbon limits. 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.

jlxh and jnxh

As with gHMQC, there is the parameter *jlxh*, 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 *jlxh*. We suggest talking to us prior to making such a change.

Another parameter, *jnxh*, sets the filter for the multiple bond correlation. The default value for *jnxh* 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 sqsinebell('f2') sqcosine('f1') wft2da**. Phasing this 2D plot is not necessary. Plot your gHMBC using the same command as for gHMQC (i.e. `plgrid(0.5) plghmqc`).