

BARE BONES GUIDE TO gradientHSQC or HMQC on the Inova-400&500 (w/ PFG probe installed)

This guide is written assuming proficiency in basic operation of the Varian NMR instrument. You should be experienced in performing basic 1-dimensional NMR experiments before attempting to perform 2D experiments on your own. Please ask for help the first time you perform this, to minimize your frustration (if for no other reason).

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Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<**angle brackets**> are used to designate a key to be pressed (i.e. <**Ret**> for Return/Enter)

[**square brackets**] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button

MMB will be used to designate the Middle Mouse Button

RMB will be used to designate the Right Mouse Button

Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press <**Return**> afterwards.

Important Considerations before starting:

- You can perform this experiment in either the 4-nucleus (n4n/asw) probe, or the indirect-detection (ntr/3mmID) probes; however, the sensitivity is MUCH better indirect-detect probe. If you need a probe-change on either the 400 or 500, please schedule with Dr. Shoemaker in advance, and reserve an extra 10-20 minutes.
 - Having a narrow-band ^{13}C bandpass filter on the carbon channel improves sensitivity. If the NTR probe is in use (500MHz instrument) then this filter will already be installed. If using the ASW probe in the 400, ask to learn how to install this filter.
 - Optimizing (narrowing) the width in the ^{13}C dimension is useful for improving the resolution in the indirect-carbon dimension. Instructions are provided to do this; however, the default ^{13}C window is often adequate for routine experiments.
- 1) Make sure you are working in Exp:1 (type **jexp1**<**Ret**> if necessary).
 - 2) Acquire a normal ^1H NMR spectrum, setting NT=4. REFERENCE the 1H chemical shift axis now.
 - 3) Zoom-In on the region containing peaks of interest (Note: if you zoom-in on a region with peaks outside that region, you will have *folded* peaks in your spectrum...this can be OK, or it can be a problem; depending upon where the folded peaks land).
 - 4) Type **movesw** , and this will set the spectral width and offset to match your selected window.
 - 5) Type **ga** to acquire a new spectrum, then phase it.
 - 6) Save this spectrum (if you wish) in your directory, as a 1-D trace for plotting beside your 2D contour plot.
 - 7) Optimizing the ^{13}C spectral window (this only works for solvents containing carbon).
 - a) If you don't know where to expect your protonated carbon resonances, skip this step and use the defaults.
 - b) If you DO know where your ^{13}C peaks are, you need to join an unused experiment (i.e. Exp:2) to setup the carbon window:
 - i) Type **jexp2**<**Ret**> (assuming that the ^1H data is in Exp:1, we'll use Exp:2 for ^{13}C)
 - ii) Setup for ^{13}C observe in your solvent, set nt=4, then do **ga**<**Ret**>. You will probably only see the solvent peak in the ^{13}C spectrum, but that's all you need.
 - iii) Set your ^{13}C chemical shift using the solvent peak, turn on the PPM axis (dscale), and zoom-in on the desired ^{13}C window (using the cursors and shift-axis for reference), then type **movesw**<**Ret**>... then do **ga** to collect another 4-scan ^{13}C spectrum. Be sure that the full ^{13}C spectral window contains your desired window.
 - iv) Remember the experiment number that contains this optimized ^{13}C window (i.e. Experiment #2 in this example).

- 8) Type **mp(1,100) jexp100** (remember, your narrowed ^1H spectrum is located in Exp: 1). You should now be in Exp:100 (note the top of the VNMR window). This copies ^1H parameters from Exp1 to Exp100, then joins Exp100.
- 9) Type **gHSQC** or **gHMQC**, or **HSQC**(non-gradient expt.) depending upon which experiment you wish to run.
 - a) For gHSQC only, you have two options, "Edited" mode or "Non-Edited" mode. Edited mode gives you phase-editing depending upon CH_n multiplicity (i.e. CH_2 s have opposite phase of CH and CH_3 signals). Non-Edited mode give you better sensitivity for weak signals. Processing/plotting can be more complicated in Edited Mode.
 - i) For gHSQC in edited mode, do nothing...this is the default (mult=2).
 - ii) For gHSQC in non-edited mode, type **mult=0 <Ret>**.
- 10) If you skipped step #7, skip this step also (use the default ^{13}C spectral window). If you have acquired an optimized ^{13}C spectral window (in step #7 above), type **setcarbonsw <Ret>**. You will be prompted to enter the experiment number containing the narrowed & referenced ^{13}C spectrum (Exp:2 in this example); therefore, enter **2 <Ret>**. (this macro sets "sw1, dof, rfl1, & rfp1" to match "sw, tof, rfl, & rfp" in the referenced & narrowed ^{13}C spectrum acquired in step #7).
- 11) Set nt (# transients):
 - a) Usually **nt=2** works fine if you have a concentrated sample (this takes the minimum amount of time).
 - b) Use **nt=4** for more dilute samples (will take 2x as long).
 - c) For very dilute samples (< 5mg) you may need as many as 8 or 16 scans; however, 16 scans per block, with ni=512 will require an overnight run (and you might consider using the non-gradient, phase-cycled experiment).
- 12) Set the number of increments in the *t1* dimension:
 - a) Type **ni?**, and note the current value for ni... ni=128 is the default, and usually works well for routine spectra.
 - b) To improve resolution in the ^{13}C dimension, increase ni: type **ni=256** (or larger if you have more time).
 - i) This experiment limits resolution in the ^{13}C dimension. If you wish to improve resolution in the indirect (^{13}C) dimension you must increase "ni", which will increase the total experiment time linearly.
- 13) Type **time** to see how long the experiment will take...adjust parameters (i.e. ni, or nt) to optimize time-utilization.
- 14) Type **go** to start the experiment.
 - a) After a block is completed, type **gaussian** (or sqcosine...see below for explanation), followed by **wft(1)**, and you should see some peaks (probably will be noisy). Do not attempt to phase this 1D spectrum.
 - i) After ~40 or more FIDs in *t1*, you can type **setLP1** then **gaussian** then **pgHSQC** (or **pgHMQC** if you are running gHMQC, or **wft2da** if doing a non-gradient HSQC) to see if your spectrum is coming along nicely.
 - b) If you must stop the acquisition before it is done (i.e., you run out of time), always stop the 2D experiment by typing: **sa('nt')**. This will stop the experiment at the end of the current FID.
 - c) When the acquisition is done, you should process the spectrum to be sure the results are acceptable.
 - i) The two most common *window functions* used for these experiments is either a COS^2 or Gaussian window. Generally gaussian improves S:N, and sqcosine improves resolution (less S:N). Choose the one that best fits your data.
 - ii) Type **setLP1**, then **gaussian** or **sqcosine** to set the apodization window function then type **pgHSQC** (or **pgHMQC**). Click the **[Full]** button if necessary. You should see your C-H cross-peaks, and they should be positively phased. If not, you might need to perform manual 2D-phasing (see processing below).
- 15) Save your spectrum for offline processing: Type **svf**, followed by your filename, to save the data for processing. Please DO NOT use the instrument computer for processing, plotting, and analysis of 2D data! There are many ways you can crash the software, and it's best to crash a workstation vs. the spectrometer computer!
- 16) Quitting:
 - a) Type **jexp1**, eject your sample, and re-insert the reference.
 - b) Be sure to turn the spinner back on in **[Acqi]**.
 - c) If you inserted the ^{13}C filter, please remove it and return the filter configuration to normal. This is especially important when using the N4N or ASW probes because ^{31}P experiments will not work when the filter is connected.
 - d) Lock and shim on the reference, and sign-out in the logbook.

Processing and Plotting the 2D-HSQC or 2D-HMQC spectrum:

As of January 18, 2005 the instructions for processing and plotting the data have been moved to a separate manual. Please see the manual on Processing & Plotting gHSQC or gHMQC NMR for further instructions.

-R.Shoemaker