

Varian HMQC help file for the INOVA 500

Last edit 09/07/2001

Heteronuclear Multiple Quantum Coherence

The HMQC is a two-dimensional heteronuclear correlation experiment developed to assist in the identification (or correlation) of proton nuclei with carbon nuclei. This experiment is ideally suited when a carbonyl moiety or other non-protonated carbon group is involved. The pulse sequence utilizes zero and double quantum coherence between J-coupled protons and carbons to 'label' each proton with the frequency of a remote carbon, thus defining the F1 dimension in this 2D experiment.

Please note that all **bold print** is input at the prompt. A more detailed description of some of the HMQC commands and parameters can be found on our 2D NMR Overview handout.

- Log into the NMR, start VNMR and join experiment #3 (type **jexp3**)
- Load the desired ^1H parameters and type **su**
- Set the cooling gas preconditioner temperature to 15° C (if you are going to regulate at 25° C, then you can leave the preconditioner at 20° C). Make sure the VT gas is flowing between 10 and 15 lpm and set the temperature to 20° C by typing **temp=20 su**
- Open acqi window, insert the sample, lock, and shim as usual. As with most 2D NMR experiments, do not spin the sample. Adjust the z, z2, x, y, xz, yz, and xy shims.
- After shimming, turn off the lock and carefully readjust (i.e. use the ± 1 button) the z0 (the field offset) to get as close to on-resonance as possible. Turn the lock back on and adjust the lock phase to maximize the signal. Repeat lock off, z0 adjust, lock on, lock phase adjust as necessary.
- Close the acqi window and tune both the ^1H (channel #1) and ^{13}C (channel #2) channels of the probe.
- As with many 2D experiments, calibrated pulses are critical for a successful experiments. At this point you might consider calibrating the 90° pulse widths. If you haven't done this before download our "How to calibrate a 90° pulse" handout or ask a staff member for assistance.
- Collect and process a 1D proton. Type **ds** and position the cursors around all the peaks in the spectrum and type **movesw** (move spectral window according to cursors). Recollect the 1D proton and set the reference (if you have trouble here please logout now).
- Type **mp(3,1)** Move parameters from experiment #3 to experiment #1.
- Type **jexp2** (join experiment #2). Load the desired ^{13}C parameters type **nt=1 ga**.
- Once completed use the cursors to select the desired sweep width and type **movesw ga**.
- Set your reference peak and then record the following values:
 - Type **tof?** and record value. (Frequency offset for observe transmitter)
 - Type **sw?** and record value. (Spectral width in directly detected dimension)
 - Type **rf1?** and record value. (Reference peak position in directly detected dimension)
 - Type **rfp?** And record the value. (Reference peak frequency in directly detected dimension)
- Type **jexp1 HMQC**. This will join experiment #1 and modify the proton parameters for use with the HMQC pulse sequence. Maximize the lock power without saturating the lock channel then adjust the lock gain to set the lock level to ~80%.

- Using the values recorded above, set the **dof** (decoupler frequency offset), **sw1** (spectral width in 1st directly detected dimension), **rf11** (reference peak position in 1st indirectly detected dimension), and **rfp1** (reference peak frequency in 1st indirectly detected dimension). Do this by typing:
 - `dof=tof #` (Use the value recorded above.)
 - `sw1=sw#` (Use the value recorded above.)
 - `rf11=rf1#` (Use the value recorded above.)
 - `rfp1=rfp#` (Use the value recorded above.)
- Set the 'null' value. The 'null' is a WEFT-like delay used to improve the suppression of protons connected to ¹²C (and not to ¹³C) that have been inverted by the preceding BIRD pulse. Type **setnull**. This macro will set up array of null values from 0.1 to 1.0. When the macro completes select the 'null' signal. For example, if the "best" null value is 0.5, type **null=0.5**. This is a judgement call but we have complete faith in your ability to select the "best" value.
- Set the HMQC gain by typing **set2dgain gain=gain-2**. This will set the 2D gain and reduce the value by 2db just for good measure.
- Select the length of experiment by typing **short2d** (macro w/ **ni=32, phase=1,2, nt=8, ss=200, gain='y', time**), **medium2d** (macro w/ **ni=128, phase=1,2, nt=16, ss=256, time**), or **long2d** (macro w/ **ni=256, phase=1,2, nt=32, ss=256, time**) Any of these will adjust nt and ni for you automatically.
- Type **d1=0.3 time** This will set the first delay to 0.3 seconds and display the acquisition time.
- Type **au** (submit experiment to acquisition and process data) to start the run.
- Once the FID number is on 2 or more, process the first FID by typing **wft(1)**
- Phase the first spectrum/slice, then integrate *every* peak in the first spectrum.
- Type **bc** and look for baseline distortion (there should be none if you integrated every peak); if the baseline is distorted, type **wft(1)** again; if you put an integral reset in the wrong place, type **cz** to clear the integral zeroes (or resets) and start over with the integration
- When the FID number in the acquisition status window has gone to 33 or greater, you can process initially with the help of linear prediction. Type **dolp wft1da bc('f2') wft2da** to process the data set (**dolp** does the following: **proc1='lp' parlp(1) lpopt1='f' lpfilt1=8 if (celem < 64) then lpfilt1=4 endif lpnupts1=(celem-1)/2 strtlp1=(celem-1)/2 lpext1=fn1/4-celem/2 strttext1=(celem-1)/2+1 dglp**) (feel free to look these up in the Command and Parameter Reference); typing **p2d** is the same as typing **dolp wft1da bc('f2') wft2da**
- If you get some cryptic error saying that the display window is too large, type **wc=wc2 dconi** This will set the width of the 'chart' equal in both the f1 and f2 dimensions as well as allow for interactive 2D data manipulation and control.
- You can readjust the vertical display by typing **vs2d=vs2d*1.2 dconi** to increase the vertical scale or **vs2d=vs2d*0.8 dconi** to reduce; there are also buttons that will increase and decrease the vertical scale of the 2D spectrum by 20%; the parameter **th** can also be adjusted (0 shows the most noise, higher values increase the cutoff threshold), the color scale on the right of the spectrum will show you the value of th; clicking with the middle mouse button on the color scale on the right of the 2D spectrum allows you to interactively adjust **th**.
- If the peaks look to be poorly phased (as you traverse the peaks along a row there will be a stripe that is yellow/orange on one-side and blue/purple on the other side), then you will have to phase by hand. See the "Phasing a 2D" handout for this procedure available on our website.
- When the experiment is finished type **dolp wft1da bc('f2') wft2da** (or type p2d). This will perform a linear prediction, weight and Fourier transform phase sensitive data in both dimensions as well as perform a background correction in the f2 dimension for all of the collected FIDs.

- To plot your data interactively, type **dcon**i and use the VNMR menu buttons. Once you have processed the data to your liking you can also use the plotting command **plhxc**or. For example if your ¹H 1D is in experiment #3 and your ¹³C 1D is in experiment #2 type **plhxc**or(**'pos',30,1.5,3,2**). For more details on plotting 2D spectra refer to the "Plotting a 2D" handout available on our website.
- Save the 2D data set with the **svf** command.
- Turn VT regulation off by typing **temp='n' su** (you may need to go in several steps to get from whatever temperature your sample is at back to 20° C – DO NOT CHANGE THE TEMPERATURE BY MORE THAN 10 DEGREES EVERY FIVE MINUTES). Set the preconditioner back to 20° C.