

Varian HSQC help file for the INOVA 500

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Heteronuclear Single Quantum Coherence

The HSQC is a two-dimensional heteronuclear correlation experiment developed to assist in the identification (or correlation) of proton nuclei with carbon nuclei.

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 #1 (type **jexp1**)
- Load the desired ¹H parameters and type **su**
- Set the cooling gas pre-conditioner temperature to 15° C (if you are going to regulate at 25° C, then you can leave the pre-conditioner 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**
 - Note: turn on the temperature controller **after** loading standard parameters
- Open the **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 ¹³C (channel #2) channels of the probe.
- As with many 2D experiments, calibrated pulses are critical for 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.
 - Save the file (with the **svf** command) for future plot projections.
 - **If you have any troubles up to this point please logout now!**
- Type **mp(1,4)** Move parameters from experiment #1 to experiment #4.
 - Check the pw90 (**pw90?**) to ensure the value is as you determined.
- Type **jexp2** (join experiment #2). Load the desired ¹³C parameters type **nt=1 gain=60 ga**.
 - Type **ffav** the type **setref** (automatically sets the solvent reference).
- Once completed use the cursors to select the desired sweep width (try to include ~10 ppm on each side of the peaks) then type **movesw ga**.
- One the acquisition is complete 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 (it should be 0). (Reference peak frequency in directly detected dimension)
- Type **jexp4 HSQC** (all capitals) This will join experiment #4 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 **rfl1** (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.)
 - **rfl1=rfl#** (Use the value recorded above.)
 - **rfl1=rfp#** (Use the value recorded above.)
- Type **ssb1**. This is an “in-house” macro and is the same as typing: **lb1='n' gf1='n' awc1='n' sb1=-1*fn1/sw1/4, sbs1=sb1**. Details can be found in the Varian ‘Command and Parameter Reference’.
 - Suffice it to say this will “fix” the apodization function in the F1 dimension.
- Set the HSQC 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 **at?** This will display the acquisition time. The acquisition time must be set to <= 0.1 seconds. Not checking the acquisition time could destroy the probe!
- 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 **dconi** and use the VNMR menu buttons. Once you have processed the data to your liking you can also use the plotting command plhxcor. For example if your 1H 1D is in experiment #3 and your 13C 1D is in experiment #2 type **plhxcor('pos',30,1.5,1,2)**. 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.