

Varian TOCSY help file for the INOVA 500

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Total Correlation Spectroscopy

In contrast to the COSY experiment, a fundamentally different method for the transfer of coupling information is utilized in the TOCSY experiment. The TOCSY (also called HOHAHA for HOmonuclear Hartmann- HAHn) experiment correlates protons that are in the same spin system (i.e., along a peptide chain) but in reality protons are never more than 2 or 3 bonds apart from another proton. Mixing is achieved while a spin-lock field is applied, and ideally the effect of chemical shift is completely removed. This means, that basically all energy levels are equivalent or “degenerate”, which leads to strongly coupled systems. The spin-lock pulses are termed “isotropic mixing” or Hartmann-Hahn mixing. Isotropic mixing leads to spectra with mixed phases (phase modulation), whereas using Hartmann-Hahn mixing pure phases are obtained. The multiplets, even when generated over different mixing times, give rise to combinations of different mirror-symmetric multiplet patterns. Ideally cross peaks are formed between all nuclei in a spin system.

Please note that all **bold print** is input at the prompt

- Log into the NMR, start VNMR and join experiment #1 (type **jexp1**)
- 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 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 acqi window and tune the ¹H channel of the probe (channel #1).
- 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).
- Turn autogain off by typing **gain='y'** and recollect the 1D proton.
- Move the fid from the current experiment #1 to a new experiment (say #2) by typing **mf(1,2)**; if experiment #2 does not exist, type **cexp(2)**
- Join experiment #2 and reprocess the data by typing **jexp2 wft**
- Convert the 1D parameter set to the TOCSY 2D parameter set by typing **TOCSY** (all capitals).
- Adjust the TOCSY mix time between mix=.03 (short), and mix=.12 (long). A value of **mix=.05** is typical. The longer the mix time, the more cross peaks will be generated.
 - You can now perform the optional step of calibrating your pulses. Note tpwr (type **tpwr?**), pw90 (type **pw90?**), slpwr (type **slpwr?**), and slpw (type **slpw?**) before joining experiment #3. Type **jexp3** you may need to create experiment #3 by typing **cexp(3)**. Refer to the “calibrating pulses” handout for this procedure (available on our website).
- Set the receiver gain for the TOCSY by typing **set2dgain** (this is the same as typing **ni=1 nt=1 ss=1 phase=1 gain='n' ga**, then after the experiment finishes, typing **gain='y'**).

- Set the number of steady-state scans (also known as dummy scans) to 256 by typing **ss=256** (this will pulse for five minutes before any data is saved – if mix is short, you can cut ss down to 64 or 128, but having ss too short will give you artifacts in the 2D that will produce useless data).
- Set the number of increments to 64 by typing **ni=64** – the number of increments determines how many FIDs will be collected; collecting more FIDs will give you better resolution in the second (or indirectly detected f1) dimension.
- Set the number of scans per slice to 4. This is “2D” for set **nt=4**
- Set the phase parameter to allow for phase-sensitive data collection in the second (f1) dimension by typing **phase=1,2**
- Type **time**. The experiment duration time will be displayed.
- Adjust ni or nt (minimum value for nt is 2) to adjust the experiment time based on your needs.
 - Increasing ni will increase your resolution in the second (f1) dimension
 - Increasing nt will increase the signal-to-noise of the overall spectrum.
- 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**) will adjust nt and ni for you automatically.
- Set the apodization (window function, e.g., line broadening) for the directly detected (f2) dimension to use a shifted, squared sine bell by typing **ssb** (same as typing **sb=-1*at sbs=sb**); to set a similar apodization for the indirectly detected (f1) dimension, type **ssb1** (same as typing **sb1=-0.5*at sbs1=sb1**); note that you can use an other function like a Lorentzian (lb) or a Gaussian (gf)
- Type **au** (submit experiment to acquisition and process data) to start the run.
- After ss number of scans have been done, the data will start to accumulate (the FID counter in the acquisition status window will go past 1); 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
- Type **cfpmult** (calculate first point multiplier for 2D experiments).
 - If **cfpmult** fails, type **wft(1)** and then **dc** and look to see if the first slice moves up or down. If it does move, adjust **fpmult**, type **wft(1)** and do the **dc** test again to see if the spectrum moves up or down on the display. When you have **fpmult** set correctly, typing **wft(1)** and then typing **dc** will not move the spectrum at all.
- 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**
- 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 finished, type **p2d** to reprocess with all of the FIDs collected.
- To plot, 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.