

## Varian VNMR5 500 MHz NMR: 1-D Selective Pulse Experiments

Questions that can be answered using 1-D Selective Pulse Experiments:

Who is coupled to whom?

Who is close, through space, to whom?

"Who is coupled to whom" experiments allow you to see which multiplets in your spectrum are involved in mutual spin-spin splitting. These selective pulse experiments are 1-D analogs to 2-D COSY and TOCSY experiments. "Who is close to whom" experiments allow you to see protons that are close to each other. These "through space" experiments use the nuclear Overhauser effect, or nOe. In nOe spectra, peaks occur for protons that are up to about 5 Å apart.

In selective pulse experiments, you set the selective pulse frequency to a peak or multiplet of interest, for example the A protons in Figure 1. In the resulting selective COSY spectrum this peak will appear along with any coupled multiplets.

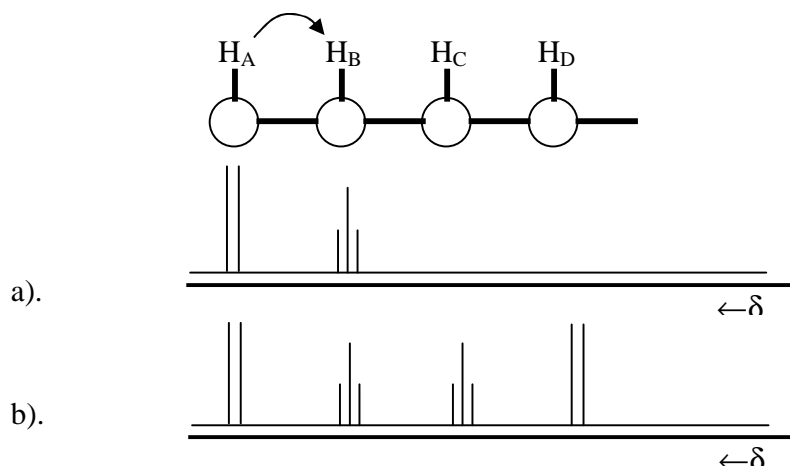


Figure 1. Selective COSY: a) selective COSY with the selective pulse set at the chemical shift of proton A. b) the normal 1-D spectrum.

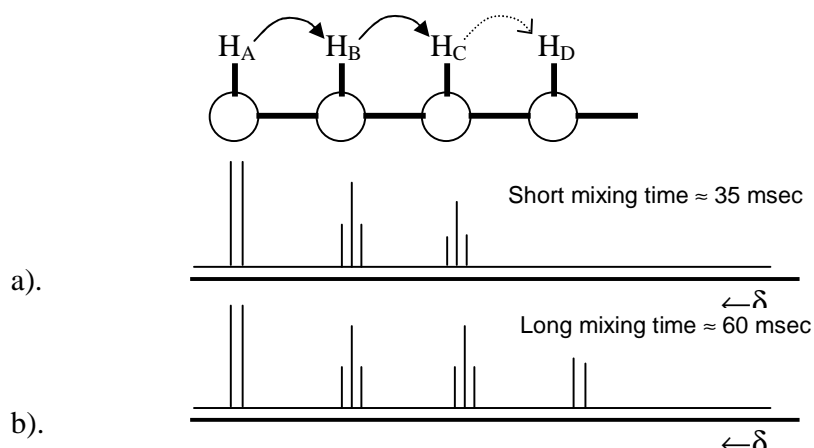


Figure 2. Selective TOCSY: a) selective TOCSY with the selective pulse set at the chemical shift of proton A and a short mixing pulse. b) selective TOCSY with the selective pulse set at the chemical shift of proton A and a long mixing pulse.

In a TOCSY experiment, peaks occur from successive relays; A is coupled to B, which is coupled to C, etc. The number of relays is controlled by the length of a mixing pulse. For a mixing pulse of about 15 msec a COSY type spectrum results, A→B. A mixing pulse of 35 msec is sufficient for a single relay or more: A→B→C. The default mixing pulse of 60 msec results in three relays or so: A→B→C→D→E. A long mixing time allows four or five relays. The long mixing times allow you to determine complete coupled subsystems in your spectra. Multiple relays are very useful for very complex spectra.

These instructions will take you through the step-by-step process using the automation system. Running selective TOCSY and nOe experiments are very similar.

### **1-D Selective TOCSY:**

1. Start by running an automated 1-D PROTON spectrum, as you would normally, but make sure to select the Tune option as well as Auto Lock and Gradient Shim. Display the spectrum with the full chemical shift range.
2. Get help to check the probe tuning, if you haven't tuned the probe before. The probe must be tuned well for these experiments to work properly. Please, don't try to tune the probe on your own unless you have been checked out by one of the faculty members. After tuning, remember to click on Stop Probe Tune and QUIT. Redisplay your spectrum from Step 1.
4. Switch the Sel1D tab in the Experiment list and click on Tocsy1D. Double click on the green Tocsy1D line in the queue and click on the sample number in the autosampler display (as usual to begin the experiment set-up).
5. Deselect the AutoLock, Tune, and Gradient Shim options in the Start tab in the Study page. (The sample should already be locked, tuned, and shimmed).
6. Display the spectrum. Switch to the Acquire tab and choose the Defaults page. Make sure the spectral width is set to the same value as your 1D spectrum in step 1. For small molecules choose a 5 sec relaxation delay (subject to the available spectrometer time). Choose the TOCSY mixing time; longer times give more relays. A relay of 60 msec gives several relays and is a good starting point. A relay of 15-20 msec is comparable to a COSY. Use the double cursor to highlight the multiplet that you wish to selectively excite. Keep the excitation width as broad as possible (~0.3 ppm if possible). The excitation band need not be symmetrical about the multiplet. Click on the SELECT button and then PROCEED. Submit the run to the automation queue.
7. To work up your spectrum, click on the Display 1D Spectrum icon in the top menu bar. The spectrum of the excited multiplet will be displayed first. Click on the green → arrow at the top of the graphics tool palette to switch to the second spectrum, which is the TOCSY. Work up this spectrum as normal for a 1D-spectrum.

**1D-Selective Pulse nOe:**

The selective-nOe experiment is quite analogous to the Tocsy1D, except that you choose the nOe mixing time instead of the TOCSY mixing time. Most nOe experiments are done with degassing in valved tubes, but you can try doing the experiment without degassing and see if it works. Do control the temperature and avoid unnecessary vibrations (door slamming, and see note below).

**Note 1:**

It is usually best to do nOe experiments non-spinning. But to do non-spinning experiments you will need to shim the X and Y shims manually.