2-D NOESY for Small Molecules

IU NMR Facility – August 4, 2004

Summary:

2-D NOESY is easy to set up, run and process with VNMR. It can be more efficient time-wise than multiple 1-D nOe difference experiments and you obtain all nOe correlations in one experiment. The experiment can often be run in less than an hour on several milligrams or more of a small molecule on the I400. The "transient" nOe detected with NOESY is not directly comparable to the "static" nOe measured with the 1-D difference experiment that you might be used to, and enhancements are usually summarized as strong, medium or weak rather than reporting percentage enhancements.

The ^{1}H to ^{1}H nOe effect is through-space (as opposed to through-bond) and can be detected for protons up to about 5 Angstroms apart with NOESY, providing valuable positional information that cannot otherwise be easily obtained short of using X-ray crystallography. NOESY gives positive peaks for small molecules (opposite of diagonal) and negative peaks for macromolecules, however for mid-sized molecules (MW ~1500-3000) the enhancements can approach zero and the more demanding ROESY experiment must be used, which always gives positive nOe peaks.

Experiment Overview:

1) Setup is very similar to other 2-D homonuclear experiments such as COSY.
2) Acquire a 1H spectrum with a 90 degree pulse width, phase and reference it. Do not spin the sample and autogain must be off (set gain = 'y'). Optionally copy the parameters to another experiment. Type **phase(180)** to invert the 1H spectrum. This can be done later if you forget.
3) Type **iunoesy** or use the Other/2D menu and select **noesy**. They are the same. For a longer or shorter experiment vary either **ni**, **nt** or both. **nt** should be a multiple of 8. Type **go** or **ga** to begin the experiment.
4) After acquiring the data, process with any of these options: 1) Type **iunoesyproc** or use the Other/2D menu under Processing and select **noesy** – both are the same. 2) Use the general 2-D processing command **wft2da**. Use **dconi** for 2-D display.

Procedure:

1) Set up ^{1}H experiment as normal in EXP1. Verify that pw = 90 degree pulse. This is a critical parameter and will typically be 20-25 μs for quad and broadband probes and 8-10 μs for proton detection probes.
2) Lock, shim and obtain ^{1}H spectrum as normal except DO NOT SPIN THE SAMPLE. As the sample will not be spun it is usually worth checking the non-spin shims (x,y, etc.) also.

3) Set the cursors just outside either ends of the spectrum and type **movesw**. This command adjusts **sw** and **tof** so that only the region between the cursors will be observed.

4) Obtain another ^{1}H spectrum with the new **sw** and **tof**. Autogain is not allowed with 2-D experiments. A good way to set the gain is obtain a 1-D 1H spectrum with set gain='n' (autogain on), then type **gain='y'**.

5) It is convenient to keep the 1-D spectrum in EXP1 and run the 2-D experiment in EXP2. To do this type **mp(1,2) jexp2** to transfer the 1-D info then set up the noesy experiment by typing **iunoesy** or use the Other/2D menu under Setup and select **noesy** to run the same macro.

6) There are quite a few parameters but in general they will be set up by the **iunoesy** macro. You can verify that some important ones are similar to:
ss=8 nt=8 bs=8 d1=1.5 ni=96 np=2048 mix=0.8 (for small molecules)

With these settings the experiment takes about 70 minutes. Adjust **nt** (multiple of 8) and **ni** for longer experiments. With **mix=.8 nt=32 ni=128** the experiment takes about 6 hours. Type **time** to get an estimate of how long the experiment will take.

For a quick check of the experiment type phase=1 and ni=1 to obtain just the first increment of the 2-D experiment. It should look much like the 1-D ^{1}H spectrum.

7) Type **ga** to begin the experiment.

Processing:

1) Type **iunoesyproc** or use the Other/2D menu under processing and select **noesy**. You can do the same by typing **fn=2k   fn1=2k   sb=at   sbs=-at   lp2d(512) wft2da**. You may have to make sure no other window functions are active (i.e. set **lb='n'**). lp2d is a macro that sets up linear prediction and a window function for the F1 dimension. For the **iunoesyproc** macro you will usually want to answer **y** to use linear prediction. Steps 2-4 below are more detailed instructions for obtaining better and properly phased NOESY spectra.

2) Type **wft(1)** to make sure the first increment is phased to be upside down. If not, phase it to be right side up then type **phase(180)** to invert it. The inversion is desirable to have NOESY peaks appear positive and the diagonal negative. This will be more convenient for display, analysis and plotting.

3) Type **wft2da** to do (or redo) the full 2-D transform after phase correction.

4) Severe baseline curvature and features may causes problems in discerning the peaks. An option to correct the 2-D baseline is with **trace='f2' abc**.

5) If the 2-D spectrum appears weak or not at all (black screen) right click anywhere in the spectrum to increase the 2-D vertical scale (**vs2d**). Once you can see peaks you will usually want to right click on a medium strength crosspeak to set **vs2d**, or you can always adjust it manually. There are menu options to adjust **vs2d** as well and most other 2-D display options can be found in the display menus.

6) The macros **plot2d** or **iuplcosy** can be used to plot the 2-D data with the 1-D spectrum on the sides. Type **plot2d** and follow the prompts. Typical inputs for

iuplcosy are something like **iuplcosy(12,1.4,1,3,3) iupage** which will plot up to 12 positive contour levels (1 negative level by default) with a spacing of 1.4 and plot the spectrum in EXP1 on the sides with a scaling factor of 3. Learn more about either with **man('noesy')** or **man('plot2d')** help files.

7) The easiest way to get volume integrals for 2-D NMR peaks (for comparison) is use the cursors to form a box around the peak of interest and type **mark**. You will need to manually record the volume shown.

Notes:

It is helpful to degass your sample to maximize nOe's.

The default mix time is 0.8 sec. This is a good value for well behaved clean samples of small molecules. For less than optimal situations (i.e. larger molecule, more viscous sample, faster relaxation from other sources) you can vary this from 0.4 sec to 0.8 sec.

You may occasionally see peaks with opposite signs as the NOESY peaks (same as the diagonal). These can be indirect NOESY peaks (looks like A->C but is really from A->B->C), residual zero quantum COSY-like peaks or can be from chemical exchange.

It is often useful to save your data again after processing (overwrite the original data) to save the processing parameters so that the next time you call up the data you can simply type **wft2da** to view it.

If you need to use solvent presaturation (with samples in D_2O) for example, it is best to establish parameters in a 1-D experiment and use the **man('noesy')** help file.

It is sometime useful to view the absolute value 2-D spectrum. Type **av av1 dconi** to do this. To return to the phase sensitive display type **ph ph1 dconi**.