2D NOESY and ROESY for Small Molecules (VnmrJ ChemPack)

IU NMR Facility – *September, 2010*

Summary:

The ^{1}H to ^{1}H nOe effect is through-space (as opposed to through-bond scalar coupling) and can be detected for protons up to about 5 Angstroms apart with NOESY and ROESY, providing valuable positional information that cannot otherwise be easily obtained short of using X-ray crystallography. Signal intensity is proportional to the 6^{th} power of distance so drops off quickly beyond 5 Å. NOESY gives positive peaks for small molecules (opposite of diagonal) and negative peaks for macromolecules. For mid-sized molecules (MW ~1000-3000) nOe's can approach zero and the more demanding ROESY experiment must be used, which always gives positive nOe peaks.

2D NOESY and ROESY experiments are easy to perform with VnmrJ. NOESY can be run in less than an hour on several milligrams of a small molecule with our 400 MHz instruments. The "transient" nOe detected with NOESY is not directly comparable to the "static" nOe measured with the 1D nOe difference experiments, and enhancements are summarized as strong, medium or weak rather than percentage enhancements. The selective 1D NOESY and ROESY experiments are good alternatives if you need high resolution NOESY information on just a few proposed correlations.

ROESY is a more demanding experiment than NOESY, with lower S/N and more artifacts, so you should initially try NOESY on your small molecules.

Experiment Procedure:

1) Optional: Lock, shim, setup a 1D proton experiment, choose solvent, acquire a quick 1D proton spectrum, reference and save it. This step is helpful but not required. You can also optionally determine pw90 for your sample to get best results. See the VnmrJ 2D Guide for this procedure.
2) Type "**iunoesy**" to setup the custom IU NMR Facility 2D NOESY experiment or **"iuroesy"** to setup an optimized 2D ROESY experiment.
3) For a longer or shorter experiment change **nt**, **ni** and **d1**. **nt** should be a multiple of 8 and increasing it improves signal to noise. Increasing **ni** gives better resolution in the 2D dimension and a little better signal to noise.
4) Save your data after acquisition is complete. Optionally type "**lp2d**" to setup linear prediction or from the "Process" -> "Default" template use the "Auto LP F1" button with "F1" checked. Uncheck "F1" box to turn linear prediction off. The 2D Fourier transforms can then be done using the VnmrJ "Process" -> "Basic" template or the "**wft2da**" command.

5) See the general VnmrJ 2D guide for phasing 2D spectra. You will want to phase NOESY spectra so that the diagonal is negative and NOESY peaks positive.
   Quick summary:
   a) Type **"wft(1)"** and phase this first 1D increment. This will usually set the correct phase for the F2 dimension. Check results with **"dconi".**
   b) "**trace='f1' f full dconi**" will set F1 as the active dimension.
   c) Select 1D traces with horizontal cursor and "**ds**" command and correct left and right phase with toolbar. Check results with "**dconi**" command.
   d) Repeat these steps with "**trace='f2'**".
   e) The command "**phase(180)**" will invert a 2D spectrum.
6) Use the "Process" -> "Default" template for referencing and optional baseline correction. Use the 2D toolbar on the right side to adjust the display.
7) Use the VnmrJ "Process" -> "Plot" template for plotting or type "**plot2d**" to use the IU macro for plotting that allows you to plot 1D spectra along the edges of the 2D spectrum.
8) If you like the results, you can save the data again to save your modified processing parameters with the data.

Additional Information on NOESY:

It is helpful to purify, filter and degass your sample to maximize nOe's.

Mix time can be changed using **mix**, **mixN** or **mixR**. The default NOESY mix time is **mixN=0.8** seconds, optimal for small molecules. For less than optimal situations (i.e. larger molecule, more viscous sample, faster relaxation from other sources) you can set **mixN=0.2 -0.6** seconds**.** The default ROESY mix time is **mixR=0.2** seconds. You will rarely need to change this.

You may occasionally see peaks with opposite intensities to 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.

NOESY Z-filter is off by default, and can be turned on using "**Gzqfilt='y'**" on the I500 and I400. Zero quantum filtering reduces all signal slightly but can reduce or eliminate COSY and other unwanted zero quantum correlations.

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