2D HMQC and HSQC (VnmrJ ChemPack)

IU NMR Facility - *September, 2010*

Summary:

HMQC, HSQC, HMBC and COSY are arguably the most useful 2D NMR experiments. HMQC and HSQC give correlation peaks for ^{1}H atoms directly connected (through bond) to heteronuclear atoms and are the standards for determining ^{1}H – ^{13}C one bond correlations. As a ^{1}H detected (also called indirect or inverse detected) experiments they are dependent on ^{1}H relaxation instead of ^{13}C and have better sensitivity than ^{13}C detected correlation experiments such as HETCOR.

The major alternative to HMQC experiments are HSQC experiments, which typically offer better resolution but lower signal to noise (S/N) and are widely used for biomolecular NMR. It is also important to note that methylene correlation peaks are often distorted with HMQC, unlike with HSQC. It is also easy to run DEPT-HSQC experiments that give methylene correlations reverse phase from methyl and methine correlations. An advantage of HMQC is that very fast 2D HMQC experiments can be run (especially absolute value with phase=1) for kinetics or other dynamic experiments.

We use gradient HMQC and HSQC experiments by default. These will often be referred to as gHMQC and gHSQC. The I500 versions have 13C broadband shaped pulses. The VXR400 can run only non-gradient experiments. The non-gradient HMQC is good but if you want to run HSQC you should use the gradient versions on the I400 and I500.

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 (^{1}H) and pwx (^{13}C) for your sample to get best results. See the VnmrJ 2D Guide for these procedures.
2) Type **"iuhmqc"** or **"iuhsqc"** to setup an optimized experiment for that spectrometer. Or from VnmrJ pulldown menu, Experiments -> Setup NEW Parameters to do … -> 1H-13C Indirect Het. Corr. (Basic) -> Gradient HMQC or Gradient HSQC.
3) Optional: You can run a test 1D HMQC or HSQC by setting **ni=1** and **phase=1**. Phasing this spectrum will preset the F2 phase for the 2D spectrum. You can also array **pw** and/or **pwx** to calibrate these values for maximum signal using the 1D HMQC. Reset **phase=1,2** and **ni=64-200** for a 2D experiment.
4) 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.

5) 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.
6) See the general VnmrJ 2D guide for phasing 2D spectra. 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**" to display full spectrum
   c) Select 1D traces with horizontal cursor and "**ds**" command and correct left and right phase with toolbar. Check with "**dconi**" command.
   d) Repeat these steps with "**trace='f2'**".
   e) The command "**phase(180)**" will quickly invert a 2D spectrum.
7) Use the "Process" -> "Default" template for referencing and optional baseline correction. Use the 2D toolbar on the right side to adjust the display.
8) 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.
9) If you like the results, you can save the data again (overwrite) to save your modified processing parameters with the data.

Notes:

HMQC is generally preferred over HSQC for small molecules.

Set "**mult=2**" for HSQC to run a "DEPT-HSQC", where methylene correlations are opposite phase to methyl and methine correlations.

For a "fast" gHMQC, set d1=0.2 and nt=4.

The high power ^{13}C decoupling used with HMQC and HSQC can heat your sample. If you see the sample temperature drifting up during acquisition, get help from the NMR staff or lower **dpwr** in increments of 1.

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

Varian has chosen to make their gradient HSQC first 2D increment not phase correct, so it cannot be used to phase the 2D spectrum.