

2-D HMQC/gHMQC

IU NMR Facility - October 14, 2004

Summary:

HMQC, HMBC and COSY are arguably the most useful 2-D NMR experiments. HMQC gives correlation peaks for ^1H atoms directly connected (through bond) to ^{13}C atoms and has become the standard for determining H-C correlations. As a ^1H detected (also called indirect or inverse detected) experiment it is dependent on ^1H relaxation (instead of ^{13}C) and has better sensitivity than ^{13}C detected correlation experiments such as HETCOR.

The major alternative to HMQC/gHMQC is HSQC/gHSQC, which typically offers better resolution but lower signal to noise and is widely used for biomolecular NMR. Also, methylenes typically give distorted peaks with HMQC/gHMQC unlike HSQC/gHSQC.

gHMQC (gradient version) theoretically has less signal to noise than HMQC but is recommended as it gives much cleaner results, you can set the gain higher and typically use less transients per increment.

Experiment Overview:

- 1) Set up is similar to other 2-D indirect detection experiments.
- 2) Acquire a ^1H spectrum with a 90 degree pulse width, phase and reference it. Optionally copy the parameters to another experiment. Do not spin the sample.
- 3) Type **iuhmqc** or use the Other/2D menu and select **hmqc**. They are the same. For a longer or shorter experiment vary either **ni**, **nt** or both. **nt** should be a multiple of 8. For the gradient version use **iughmqc** or select **gHMQC** under the Setup->Other/2D->More PFG menu. For gHMQC set **nt**=multiple of 4.
- 4) After acquiring the data, process with any of these options: 1) Type **iuhmqcproc** or use the Other/2D menu under Processing and select **hmqc** – both are the same. 2) Use the general 2-D processing command **wft2da**.
- 5) The default HMQC is obtained in phase sensitive mode. It can also be run in absolute value mode for maximum signal to noise. The only changes to do this are set **phase=1** (instead of 1,2) and you will use **wft2d** to process the data.

Procedure:

- 1) Set up the ^1H 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 ^1H spectrum as normal except DO NOT SPIN THE SAMPLE. 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** to observe just the region between the cursors.

- 4) Obtain another ^1H spectrum with the new **sw** and **tof**. Autogain is not allowed with 2-D experiments. A good way to set the gain is set **gain='n'** and obtain a 1-D ^1H spectrum with autogain, 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 **hmqc** in EXP2 by typing **iuhmqc** or use the Other/2D menu under Setup and select **hmqc** to run the same macro. For the gradient version use **iughmqc**.
- 6) With the default parameters of **nt=8 ni=96** and **d1=1** the experiment will take about 32 minutes. 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 resemble the 1-D ^1H spectrum.
- 7) Type **ga** to begin the experiment.

Processing:

- 1) Type **iuhmqcproc** or use the Other/2D menu under processing and select **hmqc**. You can do nearly the same by typing **fn=2k fn1=2k 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. You will usually want to answer **y** to use linear prediction.
- 2) If phase correction is necessary you will need to select individual traces and phase them as you do with 1-D spectra. You might have to reprocess with **pmode='full'** to phase along F1. See the General 2-D Guide for details on phasing 2-D spectra.
- 3) 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.
- 4) The macros **plot2d** or **iuplhxcor** can be used to plot the 2-D data with the 1-D spectrum on the sides. We recommend typing **plot2d** and following the prompts. Learn more about either with **man('plot2d')** or **man('iuplhxcor')** help files.

Notes:

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.

The ^{13}C decoupling used with HMQC can heat your sample. If you see the sample temperature drifting up during acquisition you get help from the NMR staff, lower **dpwr** or turn it off or acquire a ^{13}C coupled HMQC.

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**.