2-D HMBC / CIGAR

IU NMR Facility - *August 31, 2004*

Summary:

HMBC and its variants are some of the most powerful 2-D NMR experiments for structure elucidation. HMBC is related to HMQC but one bond H-C correlations are suppressed and a much longer delay is used to yield only long range H-C correlations - primarily 2 and 3 bond.

As a ^{1}H detected (also called indirect or inverse detected) experiment it is dependent on 1H relaxation and has better sensitivity than ^{13}C detected long range correlation experiments such as COLOC.

The CIGAR experiment is a variant of the gradient HMBC experiment that uses a variable HMBC delay to help avoid missing correlations. For detailed work however it is better to run multiple gHMBCs with different long range delays (jnxh). CIGAR with accord='n' is a standard gHMBC experiment.

Running HMBC experiments in phase sensitive mode (phase=1,2) and processing in mixed mode (av ph1) will give a square root of 2 advantage in signal to noise over pure absolute value (phase=0 or 1). In addition the phase cycled HMBC has a square root 2 advantage in signal to noise over the gradient version. So the best signal to noise is obtained by running the mixed mode phase cycled HMBC, but usually the cleanliness of the gHMBC or CIGAR more than makes up for the difference in signal to noise.

Experiment Overview:

1) Set up is similar to other 2D indirect detection experiments.
2) Acquire a ^{1}H spectrum with a 90 degree pulse width, phase and reference it. Optionally copy the parameters to another experiment. Do not spin the sample and autogain must be off (gain='y' or a number).
3) For the phase cycled experiment type **iuhmbc** or use the Other/2D menu and select **hmbc**. They are the same. For the gradient version type **iucigar** or select **cigar** from the Other/2D menu. For a longer or shorter experiment vary either **ni**, **nt** or both. **nt** should be a multiple of 8 for the phase cycled experiment.
4) For cigar make sure pfgon='nny' and gain can usually be set to 60.
5) After acquiring the data, process with any of these options: 1) Type **iuhmbcproc** or use the Other/2D menu under Processing and select **hmbc** – both are the same and also work for cigar. The macro assumes phase=1,2. 2) Use the general 2D processing command **wft2da** (**wft2d** for absolute value).

Procedure:

1) Set up the ^{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. 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 ^{1}H spectrum with the new **sw** and **tof**. Autogain is not allowed with 2D experiments. A good way to set the gain is set gain='n' and obtain a 1D 1H spectrum with autogain, then type **gain='y'**.
5) It is convenient to keep the 1D spectrum in EXP1 and run the 2D experiment in EXP2. To do this type **mp(1,2) jexp2** to transfer the 1D info then set up the hmbc in EXP2 by typing **iuhmbc** or use the Other/2D menu under Setup and select **hmbc** to run the same macro. For cigar use **iucigar** or select **cigar** from the menu.
6) With the default parameters of **nt=8 ni=96** and **d1=1 phase=1,2** 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 look like kind of an outline of the 1-D ^{1}H spectrum.
7) Type **ga** to begin the experiment.

Processing:

1) Type **iuhmbcproc** or use the Other/2D menu under processing and select **hmbc** (for any phase cycled or gradient HMBC variant). You can do nearly the same by typing **fn=2k fn1=2k sinebell lp2d(512) wft2da** for phase=1,2. 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 you are not using the processing macro for absolute value experiments make sure **pmode='partial'** and type **av av1 wft2d**. For mixed mode processing **use pmode='full'** and **av ph1 wft2da**.
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 or wft2d** to view it.