Gradient HMQC, HSQC and HMBC on the Bruker

HMQC (Heteronuclear Multiple Quantum Correlation) and HSQC (Heteronuclear Single Quantum Correlation) experiments correlate the chemical shift of proton with the chemical shift of the directly bonded carbon (or nitrogen). This experiment utilizes one-bond couplings.

HMBC (Heteronuclear Multiple Bond Correlation) experiment differs from the HMQC and HSQC in that multiple-bond couplings – over two or three bonds (J = 2-15Hz) are utilized. Cross peaks are between protons and carbons that are two or three bonds away. Direct one-bond cross-peaks are suppressed.

It is useful although not necessary to have already taken a carbon spectrum (use the DPX400). In terms of sensitivity, it is faster to acquire an entire 2D HMQC spectrum than a single carbon 1D.

About sample size and tubes: When sample quantity is very limited, it is advantageous to limit the amount of solvent in which it is dissolved. If a normal 5 mm tube is used, however, this cannot be less than about 500 ml without causing serious lineshape problems (shimming problems) and the attendant loss of signal-to-noise. When one reduces the solvent quantity in a normal 5mm tube, it is important that the sample be centered within the coil. To do this, center the sample about the scored line on the plastic depth gauge.

There are special tubes (made by Shigemi) and inserts (made by Wilmad), however, that can be used to restrict the active volume and, hence, reduce the amount of solvent without causing lineshape problems. These work reasonably well, with the Shigemi tubes being superior but more costly.

HMQC and HSQC

There are now two versions implemented. Both the HSQC and HMQC provide the exact same information. The differences are technical and involve signal-to-noise ratio. The HSQC has a sensitivity-enhanced version that provides a factor of two of improvement in sensitivity for methine protons (only) and amide nitrogen. If you are interested in CHs and NHs then choose the HSQC.

EXPERIMENT SETUP

1. Do not spin the sample. Lock, shim and take a normal 1D proton spectrum. Be sure to tune the proton channel of the probe (wobb). Determine the proton spectral region to be used – values of sw and olp. Recall that there is no folding in the acquisition dimension.
2. Note the file name of the 1D proton spectra taken above – you may wish to use it as the F2 projection for the 2D plot.

3. Change the data set (edc). Read in the default parameters for the 2D experiment: `rpar hmqcgs-tp` or `rpar hsqcgs-ea` for $^1$H-$^{13}$C experiments, and `hsqcgs-ea-n15` for $^1$H-$^{15}$N experiments.

4. Type `wobb` and tune the carbon or nitrogen channel of the probe on the DPX400, where the gold sliders tune the carbon or nitrogen channel. (The carbon/nitrogen channel comes up on the wobb screen when the HMQC parameters have been read – the proton channel comes up when proton parameters have been read in.) There’s no need to tune carbon and nitrogen channels on the DMX500 and 600.

5. The parameters below must be set. The F2 dimension refers to the acquisition dimension (proton) and F1 refers to the evolution (carbon/nitrogen) dimension. Type `eda` to bring up the acquisition parameter editor.
   - `o1p`: center of proton spectrum (in ppm)
   - `sw` for F2: proton spectral width (in ppm)
   - `o2p`: center of carbon/nitrogen spectrum (in ppm) on DPX400. `o2p` and `o3p` are for nitrogen and carbon respectively on DMX500 and 600.
   - `sw` for F1: carbon spectral width (in ppm)
   - `td` for F2: # of points in F2 dimension
   - `td` for F1: # of points in F1 dimension (# of FID's) (very important)
   - `fidres`: digital resolution. Should be 3-5Hz/pt in F2. Should be 20-80 Hz/pt in F1. `fidres` is given by the ratio `sw/td`. Set the values of `td` in F1 and F2 accordingly. The digital resolution should be set according to your needs, i.e., use better (lower `fidres`) resolution if you are interested in carbons that have nearly identical shifts and nearly identical proton shifts. Better digital resolution in F1 dimension requires longer experimental time. Often 80Hz/pt is sufficient, especially when combined with linear prediction (see below).
   - `ns`: number of scans. For HMQC – minimum number = 2 (ns must be a multiple of 2)
   
   For HSQC, the following is also required: `ds = ns×2` and `ds` should be no less than 16. For example, here are value that satisfy the above:
   - `ns = 2, ds = 16`
   - `ns = 4, ds = 16`
   - `ns = 8, ds = 16`
   - `ns = 16, ds = 32`
   - `ns = 32, ds = 64`
   etc...

6. Type `expt` to determine the time required for the experiment. Values of `td1` and `ns` greatly affect the time. Type `zg` to acquire data. Do not type `start` or `rga`.

**PROCESSING**

To transform the data, type `xfb`. You may transform the data before the acquisition is
Phasing

The HMQC and HSQC spectra that you have just run are phase sensitive. You must phase it. The idea is to look at and phase individual traces through the 2D spectrum. The columns and the rows must both be phased and they must be phased separately.

You may need to click on the +- button to toggle through the various display modes in order to see both positive and negative peaks.

Click on phase. Click on row and position the cursor on a row that contains peaks. Click the middle mouse button to select the row and then the left button to release the cursor. Click on mov 1 to move the trace to window 1. Select another row and move it to window 2 by clicking on mov 2. Repeat again into window 3, if desired.

To phase, click on either big 1, big 2 or big 3. This selects the biggest peak from the chosen window as the phasing reference. Use ph0 and ph1 to phase the rows. Once satisfied, click on return and then save & return. To the question “start xf2p”, click OK, and wait for it to re transform the spectrum.

Repeat the above steps for the columns.

Linear Prediction (optional but recommended)

Linear prediction is a powerful method of improving the resolution of 2D spectra. Normally the FID in the F1 dimension is not fully sampled – it is cut off. To sample it more completely requires more points (greater TD in F1) and a longer experiment time. Each doubling of TD (in F1) doubles the number of FIDs and thus requires a doubling of experimental time. Linear prediction is a processing method that predicts these cut-off points. It improves resolution in the F1 dimension without any increase in experimental time. It is done after the data is collected and can be optimized by varying the extent of prediction and the NCOEF parameter. It can greatly improve the resolution of HMQC, HSQC and NOESY spectra (or any phase sensitive spectrum). It is by default turned off and must be turned on.

Crucial parameters are based on the value of TD (in F1) that was used for the experiment. Type “eda” and get this value. The following parameters must be set and are found within the edp editor. Type “edp” and set the following:

ME_mod: set to LPfr
NCOEF: set to 3 times the number of cross peaks in your spectrum. For NOESY spectra, count symmetric peaks as two. Values that are incorrect by more than a factor of two can degrade the resolution.
LPBIN: set to 2*TD (in F1)
SI (in F1): set to 2*TD (in F1) or 4*TD (in F1) SI is the number of points actually transformed and determines the amount of zero-filling. Caution: large values of will creat huge data sets. SI should never be smaller than TD (in F1).

To execute linear prediction, you must re-transform the spectrum with xfb. Larger values of LPBIN and SI MAY give even higher resolution. It is possible to use 4*TD for LPBIN and 4*TD for SI. If you make LPBIN larger, also increase the value of SI. Trial and error is necessary to get the optimum spectrum. Linear prediction is normally done only in the F1 dimension. In the F2 dimension, it is better to adjust the digital resolution (fidres) to the appropriate value before acquiring the spectrum.

**PLOTTING**

**Shift scaling**

The carbon shift scale will not necessarily be correct. There are two ways to set it: a) if you know the shift of a cross-peak, simply click on calib and use the cursor and middle mouse button to set it; b) the second method is appropriate when you don’t know the shift of any peak. The parameter offset is the chemical shift of the down field edge on each dimension. Type “1 offset” and enter the shift of the up field edge of the carbon window that can be calculated by \( O2P + \frac{SW}{2} \) where SW is the carbon spectral width.

**Plot regions**

To make expansions, click the left mouse on the button that looks like a box. Place the cursor at one edge of the region to be expanded, click the left mouse, and drag to the other edge of the desired region. Click the right mouse to activate the expansion. Click on all to return to the full spectrum. Set the plot limits and contour level with the limits button (always do this just before plotting).

**Projections**

Type edg if you want to change the plotting options. To use the 1D proton spectrum, taken above, as the projection for the 2D plot, type edg, click on edit parameters of f2 projection. A sub-editor is invoked. Click on type of projection, and select external. You must then specify the exact filename (path) of the spectrum you want to be plotted as your projection. Click on save. If you have a carbon/nitrogen spectrum, repeat the above but click on edit parameters of f1 projection.

Sometimes, the y-scaling of the projection is not as desired. To change the y-scaling of the projection, within edg and within edit parameters of f2 projection (or f1), there is
an entry for spectrum y scaling, PF1CY. Change this to some large number, e.g., 20 cm. If there is a large peak in the 1D spectrum that is outside the HMQC spectral limits, then the scaling is on this largest peak. It can be easier to exclude this peak from how the scaling is determined. To remedy this particular problem, go to the data set containing the 1D spectrum, and type pscal, and select preg. This causes the y-scaling to be based on the COSY spectral limits.

Type view to see exactly how the spectrum will be plotted. Type plot to plot.

**HMBC**

There are now two versions of HMBC implemented. One of problems with traditional HMBC is that one must pick a delay (d6) based on the value of the long-range carbon-proton coupling. But three bond couplings vary with dihedral angle (Karplus relationship) and a delay value that maximizes the intensity of cross peaks from 5 Hz couplings will minimize the intensity cross peaks from 10 Hz couplings. One had to run two HMBC experiments, with two different delay values, in order to be sure that all cross peaks were observed. The new experiment, HMBC accordion, samples a range of delay values and thus, a range of couplings and avoids this problem. It is now the preferred method. An exception might be if you know the coupling is small and you want to optimize for that particular value.

The HMBC experiment is experimentally very similar to the HMQC. To run a HMBC, follow the above procedure for HMQC with the differences pointed out below.

Type rpar hmbcgs or hmbcgs-accrd to read in the correct parameters for HMBC.

**Parameters for HMBC only**

- **cnst12, cnst13** – for accordion version only: lower and upper range of JCH couplings. Usual value is from 5 to 14 Hz but the lower can be reduced to 2 Hz at the expense of a loss of signal to noise. Start with the default values of 5 and 14. Cnst12 must be less than cnst13.
- **d6** – old version of HMBC only: Its value = 1/2J where J is the two or three bond coupling constant. Default value is a compromise value for J=8Hz, i.e., 0.0625 s. This can be optimized (ask).
- **SW in F1** (carbon): Since the HMBC experiment correlates carbons and protons that are 2 or 3 bonds apart, there are usually cross peaks between carbonyls (if present) and aliphatics. Use a carbon SW large enough to include both (e.g., 200ppm)
- **ns - minimum number = 8*n (must be a multiple of 8):** the number of scans depends upon sample concentration. See table below.

The HMBC experiment is not phase sensitive. Simply type xfb to process (skip the phasing section above). Linear prediction will likely not be successful with HMBC spectra.
**EXPERIMENTAL TIME REQUIRED**

The following table indicates the approximate experimental time for each technique based upon a sample of strychnine (MW=334, natural abundance) using the standard 5mm probe. (A 2.5mm microprobe is also available which would lower the absolute detection limits.)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sample concentration (mg/ml)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMQC ($^1$H-$^{13}$C)</td>
<td>&gt; 5</td>
<td>5 min</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>20 min</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>12 hrs (estimate)</td>
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<tr>
<td>HMBC ($^1$H-$^{13}$C)</td>
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<td>40 min</td>
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<tr>
<td></td>
<td>0.8</td>
<td>12 hrs (estimate)</td>
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