

HMBC/Selective HMBC Quick Reference – Best Sensitivity on Artemis/Hades

(See Tips and Tricks at the end of this section before starting)

NOTE: Often times one needs greater resolution in the indirect dimension for an HMBC and this is accomplished using the selective HMBC experiment that is detailed after the regular version.

1. *If you already have a ^1H spectrum and know your **sw** and **o1p**, you can skip to step 2.* Setup and obtain a 1-D proton spectrum. Be sure to check the tuning and matching of the probe – see instructions in this binder. Determine the optimal spectral window around your peaks of interest allowing for ~ 0.5ppm on either side of your peaks. If your resonances fall between 1-8 ppm, you should select a **sw** of 8 (from 0.5ppm to 8.5ppm). The center of your spectrum is called **o1p** and for the above example it would be 4.5ppm. Record **sw** and **o1p**.
2. If you already have a ^{13}C spectrum for your sample, you should select an **sw** and **o1p** that will include all the protonated carbons allowing for ~10ppm at both extremes. Example: Your ^{13}C spectrum has peaks between 20ppm and 200ppm, you should select an **sw** of 200ppm (which will span from 10ppm to 210ppm by setting **o1p** at 110ppm). If you do not yet have a ^{13}C spectrum, you can either acquire one now or after you run the HMBC, but you will not be able to optimize the **sw** and **o1p** for the ^{13}C dimension without a spectrum (use the default values unless you can predict the limits from your structure).
3. Type **edc** [enter] and change the experiment number to 3. Type **rpar** [enter] and select **HMBCGP** [enter] **copy all** [enter] (Click **ok** or **seen** to any boxes that pop up after the **copy all** command). *NOTE: On Artemis, you will need to select the versions that have the suffix **_BROWN...** if you don't see it in the list, make sure you are in the directory that ends with **/user**.* For selective HMBC, choose '**SELECTIVE_HMBC_BROWN**'. and follow the instructions at the end of this section. Type **eda** [enter] and change **sw & o1p** (in F2 column) and **sw & o2p** (in F1 column) values to those determined optimum for **sw/o1p** in the ^1H and ^{13}C experiments, respectively or use the default. Turn **off** the sample spinning (either by pushing the button on the BSMS console – top left – or in the shim panel of the bsms display). Touch up **z** and **z2**. If you have not done so already, tune and match the probe following the directions in this manual (On **Zeus** and **Hades** – just type '**ATMA**', on **Artemis**, use the '**wobb**' command). When the tuning and matching is complete, type **rga** [enter]. HMBC experiments are much less sensitive than HSQC so unless you are very concentrated, you should set the **ns** to a minimum of 8. Type **zg** [enter] to begin the experiment.
4. Type **xfb** [enter] to process the 2-D data any time during the acquisition. Type **abs1** [enter], and **abs2** [enter] to perform a baseline correction on your spectrum. You can stop your acquisition before it finishes if you have already resolved your cross peaks of interest. Just type **halt** [enter] and **xfb** [enter] to process the latest scans. The spectrum will be saved. *NOTE: If the scale is off in either dimension of your 2-D plot, type **edp** and make sure the*

offset values are correct! If they are not, change the **offset** in F2 and F1 to the values recorded for **offset** in your optimized 1-D spectrum for the ^1H and ^{13}C experiments, respectively. NOTE: Due to a bug, you may have to go in and change the offset value multiple times.

5. There is often incomplete suppression of 1-bond couplings and this will result in cross peaks in the ^1H dimension that do not line up with any protons but will precisely bisect the resonance in question (if you overlay the HMBC with the HSQC spectrum it should be clear which peaks have not filtered out completely).
6. If you do not see any meaningful correlations at all, go back and double check your o1p values in the **eda** menu or consider using a more concentrated sample with more scans.

Selective HMBC Instructions:

If you are lacking the necessary resolution in a crowded region of your spectrum, you can use a shaped pulse to excite just that region (just changing SW and o1p to select a region would result in peaks outside that area to be folded back into the spectrum and make it very difficult to interpret).

Determine the ^{13}C sweep width you would like to focus on – (example – 10ppm, or 50ppm). Determine the frequency spread – on a 400MHz, it would be ~100Hz for every ppm, on HADES – it would be ~75Hz for each ppm. Record this value. Also write down the value of P3 from an HMBC experiment after having hit the little blue test tube. RPAR SELECTIVE_HMBC (remember on Artemis it will be _BROWN). Change the SW and o1p in both the ^1H and ^{13}C dimensions to meet your needs. Type STDISP to launch the shape tool. In the main menu click on ‘Shapes’, select ‘Classical’ and select ‘Sinc’ by clicking it. Change the size of shape to 256. Click on the ‘save’ icon and change the title to ‘username_shape’, and make sure the flip angle is 90. Click ‘ok’. If a new box pops up, put the same name in there and save it again.

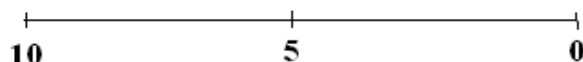
Click on the ‘Analysis’ tab at the top and select ‘Calculate Bandwidth for Excitation’. Change ‘Delta Omega’ to the value you calculated in Hz for your sweep width and hit enter (NOTE: total rotation should be 90°). Record Delta T. Hit the ‘update parameters’ button. Click ‘ok’.

Click on ‘Analysis’ again and select ‘integrate shape’. Make the following changes – Length of pulse = Delta T value [enter], Total Rotation = 90 [enter], put the value of P3 you recorded earlier into the 90° hard pulse box [enter]. Record the change of power level button. You can now exit the shape tool.

Type 'ased' and enter Delta T into the P13 box. Add the change in power level you recorded in the last step to the SP14 dB value (IMPORTANT – not the W value!!). Change SPNAM file to your saved shape. RGA, ZG. Same processing procedure for regular HMBC applies.

TIPS and TRICKS FOR 2-D EXPERIMENTS

example



SW = Spectral Width = 10ppm
o1p = 5 ppm (center of your spectrum
offset = 10ppm

- Select an **sw** and **o1p** that are easy to remember. If your ^1H spectrum has peaks from 2.5 to 7.5ppm, you would select an **sw** of 6 and an **o1p** to 5. The **offset** value in the processing parameters should always be the most downfield ppm value, in this case **8**.
- If you already have a proton and carbon spectrum for the sample on which you wish to perform a COSY, HMQC/HSQC, or HMBC, just determine the optimum sw, o1p, and offset values for those spectra and plug those values into the appropriate 2-D parameter set. You do not need to rerun the ^1H and ^{13}C spectra. Come see me if you want to learn how to incorporate the traces from old spectra into your 2-D dataset for processing.
- Linear prediction is a useful tool for improving the resolution in the indirect dimension of any 2-D experiment. To use this, click the ProcPar tab and scroll down to LPfr and increase the # of output points for LP from 0. Example: Your experiment is running and you have acquired 32/256 steps in the indirect dimension. Increase the # of points to 96 and you may be able to stop your experiment sooner. The # of output points should not exceed 3X the actual # of steps in your indirect dimension.

Fourier transform			
TDef =	0	0	# of fid data points used by ft
STSR =	0	0	First output point of strip transform
STSI =	0	0	Total # of output points of strip transform
ME_mod =	no	LPfr	Linear prediction for ft, xfb, ...
NCOEF =	0	32	# of LP coefficients
LPBIN =	0	0	# of output points for LP
TDOFF =	0	0	# of back-predicted points