

HSQC/HMQC Quick Reference –

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

NOTE: HSQC picks up one bond H-C coupling (analogous to HMQC). Generally, it gives a much cleaner spectrum than the HMQC experiment...however, it can appear less sensitive than HMQC.

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 perform ATMA before running any HSQC/HMQC/HMBC experiments. Determine the optimal spectral window around your peaks of interest allowing for $\sim 0.5\text{ppm}$ 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**. If you change **SW**, be sure to put **AQ** back to **0.25s**.
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 $\sim 10\text{ppm}$ at both extremes. Example: Your ^{13}C spectrum has peaks between 20ppm and 120ppm, you should select an **sw** of 120ppm (which will span from 10ppm to 130ppm by setting *o1p* at 70ppm). If you do not yet have a ^{13}C spectrum, you can either acquire one now or after you run the HSQC, 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 **HSQC_EDITED_BROWN** (this experiment is multiplicity edited such that CH & CH₃ signals will have an opposite phase from CH₂ signals-analogous to DEPT135 result), **HSQC_BROWN** or **HMQC_BROWN** [enter], **copy all** [enter] (Click **ok** or **seen** to any boxes that pop up after the **copy all** command). 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 (Be sure **AQ** in F2 is 0.25s). If you have not done so already, perform **ATMA**. This is a gradient experiment so perform '**loopadj**' (you can find detailed instructions on this procedure in the 1D proton instructions. Finally, type **rga** [enter] and when complete, **zg** [enter] to begin the experiment.
4. Type **xfb** [enter] to process the 2-D data any time during the acquisition. NOTE: Only the HSQC experiments are phase sensitive. Consult the '2-D Phasing Guide for Topspin' if you need to phase your spectrum (each cross-peak should be a solid color if phased properly). 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.

TIPS and TRICKS FOR 2-D EXPERIMENTS

- 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**. Before you change **SW** in **F2** (the first column of parameters in the Acqui tab of 2D experiments), record the acquisition time **AQ** and return this value to its original value after you change **SW**.
- 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 24/256 steps in the indirect dimension. Increase the # of points to 96 and you may be able to stop your experiment sooner.

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

- Non-uniform Sampling (NUS) is another means of reducing experiment time. If you know your experiment will require a certain number of scans to give you the sensitivity you require, or a certain number of indirect data points to achieve a particular resolution, NUS can cut your experiment time by ~66%. Select the 2D experiment you wish to run that has the _NUS included in the name. One disadvantage to NUS is that the data doesn't look as good if you process it before it the experiment is finished. Under traditional acquisition parameters, if you type xfb prior to completion of the experiment, you may have enough

resolution/sensitivity to stop the experiment early. Typically, with NUS, you will need to allow the experiment to run to completion.