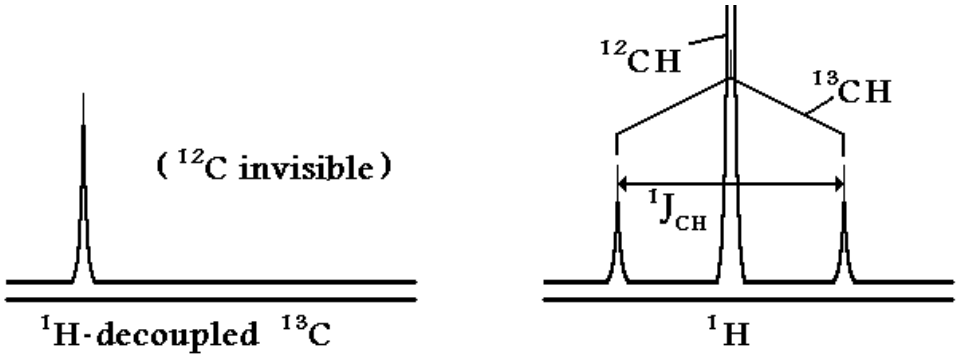
## Edited Gradient-Selected 2D HSQC

HSQC (Heteronuclear Single-Quantum Correlation) is a 2D experiment which correlates $^{13}C$ nuclei with $^{1}H$ nuclei within a molecule by means of the one-bond coupling between them. Unlike the HETCOR experiment, in which $^{1}H$ magnetization is indirectly detected ($F_1$) and converted to $^{13}C$ magnetization which is directly detected ($F_2$), in HSQC the $^{13}C$ is indirect and the $^{1}H$ is directly detected in the FID. For this reason, HSQC is called an "inverse" experiment because it is done backwards relative to the older HETCOR. "Inverse" basically means that HSQC is a proton experiment. There are numerous advantages to doing things this way, including increased sensitivity (a 1.0 mg sample is sufficient) and the ability to see long-range (2 and 3 bond) interactions between $^{13}C$ and $^{1}H$ using a variant called HMBC.

**Comparison of HETCOR and HSQC.** Both experiments yield a 2D spectrum which has $^{13}C$ chemical shifts on one axis and $^{1}H$ chemical shifts on the other axis, with crosspeaks representing the one-bond relationship between $^{13}C$ and $^{1}H$. The main difference is that the HMQC spectrum has the $^{13}C$ chemical shifts on the indirect ($F_1$) axis while the HETCOR spectrum has the $^{13}C$ chemical shifts in the directly-detected ($F_2$) dimension. Thus an HSQC spectrum looks like a HETCOR spectrum turned on its side ($90^{o}$). There are certain consequences of detecting the $^{1}H$ signal directly which become apparent if we compare a 1D $^{13}C$ spectrum with a 1D $^{1}H$ spectrum. In a 1D $^{13}C$ spectrum, the peaks arise only from the $^{13}C$ nuclei in the sample molecules, which are "dilute" - only one in every 100 carbon atoms is a $^{13}C$. The $^{12}C$ atoms are invisible to NMR so they do not in any way interfere with the detection of the $^{13}C$ signals. In a 1D $^{1}H$ spectrum, the peaks we see are due to the $^{12}C$-bound protons, which constitute the vast majority of protons (~99%). The $^{13}C$-bound protons appear as tiny "satellites", which are very wide doublets ($^{1}J_{CH}$ ~ 150 Hz or 0.3 ppm on a 500 MHz spectrometer) centered on the $^{12}C$-bound proton signal and 0.55% of its peak intensity:




In a 2D heteronuclear one-bond correlation experiment, only the $^{13}C$-bound protons are of interest because only these will be involved in magnetization transfer between $^{13}C$ and $^{1}H$ and only these will give rise to cross peaks. For HETCOR, the $^{13}C$ nuclei are directly detected ($t_2$ / $F_2$) so that the $^{12}C$ nuclei are invisible as in the 1D $^{13}C$ spectrum. With the inverse experiment (HMQC), however, the $^{13}C$-bound protons will give rise to cross peaks but the $^{12}C$-bound protons can still show up in the FID, since the directly-detected nucleus is $^{1}H$. This leads to a problem because we want to see the $^{13}C$ satellites in the $^{1}H$ spectrum, but we don't want to see the much

larger (~200X larger) central peak representing the $^{12}$C-bound protons. This leads to two significant differences between HETCOR and the HSQC experiment:

1. In HSQC, the cross peaks appear in pairs separated in the $F_2$ (horizontal) dimension by the large one-bond CH coupling (~ 150 Hz) and centered on the $^1$H chemical shift. This coupling can be eliminated by turning on a $^{13}$C decoupler during the acquisition of the FID, which operates just like the $^1$H decoupler in a $^{13}$C-detected experiment. Because the $^{13}$C range of chemical shifts is large (~200 ppm), the $^{13}$C decoupler has to put out quite a bit of power and this limits the acquisition time (**AQ**) to a maximum of about 220 ms (0.22 s). Also, by using a longer relaxation delay (**D1**) of 1.5 s, the amplifiers and the sample have a chance to "cool off" before the next FID is acquired. Acquisition time is reduced simply by acquiring fewer data points. For $^{13}$C decoupled experiments we reduce **TD** (F2) to 1024 or 512 to get the **AQ** within the 220 ms limit.

2. At each proton peak position in $F_2$ there will be a vertical streak (parallel to the $F_1$ axis) which represents the $^{12}$C-bound proton signal. Because the $^{12}$C-bound proton signal is not associated with (correlated to) any $^{13}$C chemical shift, it just appears at all $F_1$ frequencies; that is, as a vertical streak. The advent of pulsed field gradients allows us to select the coherence pathway desired and destroy the $^{12}$C-H signal by gradient scrambling of the coherence. This leads to excellent suppression of the $^{12}$C-H artefact in a single scan. Since we are only observing the $^{13}$C-H signal, we can turn up the receiver gain dramatically, increasing selectivity. Furthermore, there is no need for a phase cycle to cancel the $^{12}$C-H signal, so we can acquire a 2D HSQC with NS=1, leading to very short time experiments provided there is an adequate concentration of sample (>10 mg for typical organic molecules). For further improvement in pathway selection, or for more dilute samples, you can increase NS to 2 or 4.

The HSQC experiment works like this: a 90$^o$ proton pulse rotates the $^1$H magnetization into the x-y plane. This magnetization is converted to $^{13}$C magnetization by applying simultaneous 90$^o$ pulses on the $^{13}$C and $^1$H channels. The $^{13}$C magnetization rotates in the x-y plane during the $t_1$ delay, which has a 180$^o$ pulse in the center to cancel the effects of J-coupling to the proton during $t_1$. The $^{13}$C magnetization is converted back into $^1$H magnetization by again applying simultaneous $^1$H and $^{13}$C pulses. Each of these magnetization jumps is due to the one-bond J-coupling between $^1$H and $^{13}$C. The proton FID is recorded, with $^{13}$C decoupling, for each value of $t_1$ and the complete set of proton FIDs is used to generate the 2D spectrum. Coherence pathway selection in the HSQC can be acheived by using two gradients on either side of the central 180$^o$ pulse. This twists the $^{13}$C magnetization in the x-y plane depending on the position of the molecule in the sample tube along the z axis. After the magnetization is converted back to $^1$H magnetization, a refocusing gradient is applied to undo this twist. The coherence levels in the three gradient periods are +1 ($^{13}$C single quantum), -1 ($^{13}$C inverted by the 180$^o$ pulse) and -4 (observable $^1$H single quantum). The total "twist" is thus zero at the end if the magnetization has passed through the desired pathway. For $^{12}$C-H, it is not possible to follow this pathway so the final gradient destroys all $^1$H magnetization.

The edited HSQC uses a further trick to invert the $CH_2$ signals, leaving these crosspeaks negative in the 2D spectrum. The CH and $CH_3$ signals should be phased to be positive.

**2D-HSQC Spectrum.** Insert the sample, lock, and run a standard 1D $^{1}H$ spectrum. Write down the range of chemical shifts desired in the 2D spectrum: for example, from 2.0 to 6.0 ppm. Remember that solvent peaks, impurities, and TMS can be ignored because the digital filter will cut these signals out if they are not within the spectral window. Now calculate O1P as the average of these two values (e.g., (2.0 + 6.0)/2 = 4.0 ppm). This is the center of the F2 spectral window. Calculate **SW**(F2) as the difference between the values (e.g. 6.0 - 2.0 = 4.0 ppm). this is the width of the spectral window in ppm.

Run a $^{13}C$ spectrum (or look at one you have already run) and write down the desired range of chemical shifts for protonated carbons. Don't count the quaternary carbons since they will not show up in the HSQC spectrum. If you have no idea about the $^{13}C$ chemical shifts, use a range of 150 ppm to 0 ppm. The average value (e.g. (150 + 0)/2 = 75) will be **O2P**, the center of the $F_1$ spectral window, and the difference (e.g. 150 - 0 = 150 ppm) will be the **SW**(F1), the width of the $F_1$ spectral window in ppm.

In a new data area, use **rpar** to get the **std-hsqc-dec-ed-n3** parameters. Enter eda and set the four values **SW**(F2), **SW**(F1), **O1**P and **O2**P. Then check **AQ** and if it is greater than 0.22 sec reduce **TD** (F2) by a factor of two. Continue to reduce **TD** (F2) until the **AQ** is 0.22 sec or shorter. Set the following:

**TD**(F1) = 512
**NS** =1 or 2 (for sample with more than 10 mg)
**AQ_mod** = DQD

Tune the probe (**wobb**) for $^{1}H$ and $^{13}C$. Use gs to adjust the receiver gain RG. Check experiment time with expt. Start the experiment with zg.

**Processing and Plotting.** In **edp** set the following parameters:

**SI**(F2) = 1024
**SI**(F1) = 512
**WDW**(F2) = **WDW**(F1) = QSINE
**SSB**(F2) = **SSB**(F1) = 2

Transform using **xfb** and phase correct. Plot the spectrum in color with negative contours red and positive contours black.