For targeting 1 signal:

1. Measure the decoupled resonance position and $^{1}J_{HC}$ coupling of targets signal using standard methods.

2. For the target signal, set the measured ^{1}H resonance positions as pulse sequence variable *cnst10* and ^{13}C resonance position as *cnst20*. Enter the $^{1}J_{HC}$ as *cnst4* (which will set τ_{cp}). These parameters should not need to be further optimized.

3. The sequence will automatically calculate the ^{1}H and ^{13}C power levels, it is recommended to note the values given. As the calculated values assumes linearity of both amplifiers in the low power regime, it will likely be necessary to manually calibrate the power levels. To do so, comment out the lines setting the ^{1}H and ^{13}C CP power and then enter the pre-calculated values (in dB) directly as *pldb10* and *pldb20*. Using the 'popt' command, array the *pldb20* parameter about the calculated value to achieve the null crossing of the target signal. For coarse grain, do ~ +/- 5 dB in 1 dB increments about the estimated level and for fine grain, do ~ +/- 1 dB in 0.2 dB increments. If the null condition is not achieved, set *pldb20* to value at signal minimum and repeat 'popt' for the *pldb10*. Repeat iteratively until null crossing is achieved.

4. Make sure *l3* is set to 1.

5. Once parameters SIERRA are set, set up and run 2D as normal.

For multiple targets:

1. Enter the specific ^{1}H and ^{13}C resonance positions in the 'define list' *fH* and *fC* section of the code (currently set to *cnst10* and *cnst20*).

2. Optimize the CP power levels as above for a single target.

3. Set *l3* to the total # of targets.

Other Parameters

- The sequence has a flag for selective or standard HSQC. The parameter file will default to selective mode, with ^{13}C selective parameters optimized for mAbs. To run a s astandard HSQC remove the –DSELECTIVE flag in AcquPars window.

- Gradients pulses p17/p18 may need to be adjusted to achieve optimal water suppression on a given system.

Important SIERRA parameters:

^{1}H Frequency Offset = CNST10

^{13}C Frequency Offset = CNST20

^{1}H CP Power = pl10

^{13}CP Power = pl20

τ_{cp} = d10 (automatically set to $1/^{1}J_{HC}$)

$^{1}J_{HC}$ = CNST4

Loop counter = l3