H-C one-bond correlations: HSQC

Preliminaries.

For both ^{1}H and ^{13}C, you need a good power-pw90 pair and you should have good values for both sw and tof for both nuclei. In short, you should have a beautiful calibrated ^{1}H spectrum in one workspace (experiment) and beautiful calibrated ^{13}C 1D conveniently loaded in another workspace. Having both these spectra loaded before you begin makes it easy to check on parameters you will need.

A good way to keep track of these is to fill out a table such as the one below

Nucleus	power	pw90	spectral width	offset
^{1}H	(→tpwr)	(→pw)	(→sw)	(→tof)
^{13}C	(→pwxlvl)	(→pwx)	(→sw1)	(→dof)

Begin with your beautiful calibrated 1H 1D.

The HSQC set up is based on the parameters of a beautiful ^{1}H 1D, so work in an experiment that has this spectrum in it. If you are using a spectrum collected on another day, it is a good precaution to reacquire it immediately before beginning the HSQC. If the pw90 is in doubt, re-determine it (30 sec !). (Also load a ^{13}C spectrum of your sample in another workspace, for reference.)

Under 'Experiments' choose 'Convert current parameters to do... > Indirect Het. Corr. (Basic) > Gradient HSQC (**Page 2**). The pulse sequence contains a number of familiar motifs. It begins with our gradient-90-gradient pre-sequence [1] to prevent any new magnetization from carrying over from the preceding scan. Next is a TANGO module [2] that will prevent ^{1}H attached to ^{12}C from contributing to the final spectrum. This eliminates artifacts that would appear to have a ^{13}C frequency of zero, because ^{12} has no magnetic moment (Larmor frequency is zero). [3] is the excitation pulse. ^{1}H magnetization is the starting point for an HSQC. Next we have a spin echo [4], note the matched delays on either side of 180° pulses. The delay is set to 1/4J, where J is the one-bond H-C coupling (for aliphatic CH, $^{1}J_{HC} \approx 146$ Hz, 1/584 = .0017 s). If you wanted to optimize the spectrum for other H-C pairs, you would modify this delay. For example aromatic CH groups have J≈ 170 Hz and the best delay would be 1/680 = .0015 s.). Instead of typing in the delay, we provide the J-coupling constant (see below). The fact that both channels have simultaneous 180° pulses means that this spin echo is accruing J-coupling between C and H.

Next there are 90° pulses on both ^{1}H and ^{13}C that transfer magnetization from H to C [5]. After that we have d2, the indirect chemical shift detection delay (also called t1) during which the ^{13}C chemical shift is encoded into amplitude [6] Then there is a module that looks like a spin-echo but serves to make the sign of CH_2 groups opposite to that of CH groups and CH_3 groups [7]. Finally, another pair of 90° pulses return magnetization from ^{13}C back to ^{1}H for detection [8]. The last item is a

gradient [9] that rephases magnetization that was dephased when on ^{13}C. This ensures that only magnetization that spent time on ^{13}C but ends up on ^{1}H will contribute to the spectrum. This filter results in a very clean baseline and thus substantially eliminates solvent, and the need for lots of phase cycling and large numbers of scans. The only reason to collect a lot of scans is if your ^{1}H signal is weak.

In Acquire>Parameters (**Page 3**), note that the ^{1}H sw [1] and tof [2] are preserved, as is the ^{1}H pw90 [3] and power [4]. However information for the ^{13}C is drawn from saved parameter sets, and is necessarily reliable. You will need to replace the ^{13}C pw90 [5], the corresponding ^{13}C power [6] and the ^{13}C offset (= carrier frequency). Go to your beautiful calibrated ^{13}C 1D and note the pw90, power and tof. Enter these in locations [5], [6] and [7]. Alternately, if you want to use the command line, these values will respectively become the pwx, pwxlvl and dof (**p**ulse **w**idth for nucleus **X** (not H), power **l**e**v**e**l** for that pulse, **d**ecoupler **of**fset (the second channel retains its traditional name of decoupler)). This is also where the ^{13}C decoupling is set up. The W40_ATB_P013 composite pulse decoupling sequence uses the 13C pw90 to calculate pulse widths and allows the use of a very modest power for decoupling (35).

To set up your spectral widths you have to go to Acquire>Acquisition (**Page 4**). In The ^{13}C spectral width is entered under F1 Spectral Width [1] and the ^{1}H sw is entered under F2 [2]. . This is also where you choose ni (the number of increments) [3]. Here you confront the resolution issue for your ^{13}C dimension. The default value of 128 produces a ^{13}C resolution of 155 Hz per point (for my sw1). This sounds lousy. However this 1.54 ppm resolution is likely comparable to the separation between peaks. Look at your beautiful ^{13}C 1D and decide what resolution you need. Shoot for half or a quarter of that here, because you will be spreading signals out in a second dimension, and you will also be linear predicting. (For a real shock note that our sw1 and ni produce an at1 (acquisition time in the indirect dimension) of only 6 ms or 0.006 !!! [4]. I doubled ni to 256. You have simpler molecules, so you may be fine with **ni=128**, many people are.

The other resolution shocker is the ^{1}H at [5]. Contrary to my recommendation for COSY, TOCSY and NOESY, I do NOT recommend you double this. DO NOT. The reason is that during at we are decoupling ^{13}C. The wide sw1 of ^{13}C means the probe is carrying a lot of power during at. We cannot afford for at to be long.

Under Acquire>Pulse Sequence (**Page 5**) you see a number of options. If the TANGO option is disabled then the sequence will not actively suppress ^{1}H attached to ^{12}C and you will have weak artifacts at correct ^{1}H frequencies that have apparently a ^{13}C frequency of 0. (I recommend you run with TANGO active) [1]. Note that Page 5 shows the pulse sequence when TANGO is inactivated, and the module number 2 shown on Page 2 is now absent. This is also the panel where you input the $^{1}J_{HC}$ coupling you want to exploit, and thereby determine the delays in your spin echos

[2]. The value shown [3] is the sum of the two delays, the total duration of the spin echo.

Under X-multiplicity you have the option of XH/XH3 up and XH2 down (recommended) [4]. This will yield positive-amplitude peaks for C to which an odd number of Hs are attached. C bearing 2H will yield negative-amplitude peaks. This makes the HSQC much more valuable and can be very helpful in assigning spectra as it is not uncommon for a pair of geminal H to have very similar ^{1}H chemical shifts and therefore to fall together in the COSY and TOCSY. These cases are instantly identified in an HSQC that colour-codes peaks according to number of attached Hs.

In Acquire>Acquisition, select 'Find Gain' [1]. The spectrometer will go to a much higher gain than you had for the ordinary ^{1}H 1D. This is because the HSQC selects and detects only ^{1}H magnetization of ^{1}H bound to a ^{13}C. This is only 1.1 % of the Cs. Even if a higher gain is recommended, I don't usually bother with gains above 30.

Click on Show Time [1] (**Page 6**), modify the number of scans nt and/or ni (in Acquire> Aquisition) to reach a compromise between the quality of data and the time allowed. Click on Acquire [2] to begin acquiring. Note that typing **wft** then **dssa** [3] gives you a preview of the 1Ds being collected. **dssa(1,74,6)** shows spectra from 1 to 74 showing every 6th spectrum. We can see that signal amplitude is being modulated as the value of d2 is incremented.

Given that you left Acquire>Future Actions set to execute the 'process' routine, the software will perform automatic processing when data collection ends. All you need to do is save [1]. The 2D that results has two frequency axes. The vertical one is the ^{1}H chemical shifts [2] and the horizontal one has the chemical shifts of the ^{13}C to which ^{12}H are attached [3].

Trick for a higher-resolution HSQC

Regarding the ^{13}C spectral width, many of the ^{13}C signals may already be known to arise from C with no Hs. You see that if the full ^{13}C SW is used for sw1 in the HSQC, there will be a lot of empty space. (Page 8 shows the example of pulegone. Beware: the ^{13}C axis is not calibrated in the figure: the chemical shifts are wrong.

Instead, of using the full ^{13}C 1D spectral width for your HSQC (using dof = 1530.8 and sw1 = 25510.2 for pugelone), you could collect an HSQC with a spectral width set only for the H-bearing Cs. Obtain a new ^{13}C sw and tof by collecting second ^{13}C 1D containing only Cs coupled to H. You can identify these Cs by collecting a full-width ^{13}C with **dm = 'yyn'** (turn the decouping off). Set the cursors around the Cs that that show coupling to ^{1}H (compare with a standard decoupled ^{13}C 1D). Click 'Move SW' and collect a new narrower ^{13}C to serve as a high-resolution reference for a narrower HSQC (**Page 9**). (For pugelone, I got tof=-5931 and sw=4432.6 Hz. These then translate to dof and sw1 in the HSQC.)

Whereas a full-width HSQC can devote a lot of data collection time to characterizing empty space (**Page 10**), a sw1 set to the spectral region containing H-bearing Cs

permits the use of a smaller ni to get the same resolution, and thus allows you to collect the HSQC in less time. Alternately, if you retain the same ni, the narrower HSQC will have higher resolution. (compare at1.)

Gourmet Processing

Each of the F1 and F2 dimensions could need linear prediction, weighting and phasing, in that order. However we usually phase both dimensions at the very end after obtaining a 2D. Moreover the F2 dimension rarely requires linear prediction. Thus, the procedure is -1- weighting in F2, -2- weighting in F1, -3- Linear prediction in F1, -4- phasing in F1 (if needed), -5- phasing in F2 (if needed). For the HSQC, phase corrections are usually small.

1- weighting in F2
In Process > Weighting, click 'FT 1D - 1st Increment'. The phasing SHOULD be 90° off. Leave it and phase if necessary on the 2D. (**Page 11**).

Go to the right-hand side of this panel by sliding the slider to the right [1] (**Page 12**), Click on 'Interactive Weighting' [2] to produce the three panels and click with the middle mouse in the bottom panel to reveal the FID [3]. Because I have strong data right to the end of the at, I chose a squared sine-bell [4] under F2, because this is the directly-detected FID [5]. The squared sine-bell collects more of the late-time data than a Gaussian does. The minus sign ahead of the sinebell number [4] makes is a sine^2 function whereas a positive value would simply be a sine function. Shifting it by the same value [6] as the sine value (always with a negative sign on the shift) produces the cosine function in the example. The cosine function begins with a value of Cos(0) = 1 [7] (A sine function begins with a value of sin(0) = 0).

Next, click on 'Transform F2' (back on the left-side of the panel) (**page 13** [1]).

2- weighting in F1
Click in the graphical display window with the middle mouse to reveal the data. You can see the intensities oscillating as a function of t1, for example at 4.5 ppm [2]. Place the cursor on one of the streaks, and click on 'Interactive Weighting' [3] (right hand side of panel).

In the resulting display (**Page 14**), click in the lower panel with the middle mouse to reveal the interferrogram [1] . (You may have to click several times, and click well above the green horizontal line.) The suggested Gaussian [2] fits my data nicely assuming that the length of the FID will be doubled by linear prediction. If you want to alter the weighting function, choose different functions by activation the corresponding boxes under F1, click 'Interactive Weighting' again and then click in the middle panel [[2] to modify the duration of your chosen function.

3- Linear prediction in F1
Given that we have doubled ni (page 4), we also need to alter the linear prediction

parameters that apply to F1. In Process>More 2D (**Page 15**) under F1 enter the value we used as ni as the number of basis points, the number of actual data points to be used as the basis for linear prediction [1]. Also enter the same number as starting point in order to use all the ni data points you collected [2]. To double the data set size we will want to add one ni more predicted points [3]. The first predicted point will be point number ni+1 [4] because you already have ni actual data, and we want to add the predicted data right after that. (In the example, you see that my value of ni is 256.) Now that you wil have a total of 512 points as input to the Fourier transform, make the size of the Fourier transform at least twice as large (1k = 1024 points) [5]. In this example the choice of an F2 Fourier transform size of 1k is on the small side, I would use 2k (=2048 points) to allow for doubling in F2 as well, with a room to spare [6].

For perspective, Varian's default parameters assume 128 complex points and add 384 more (quadrupling the data). I am not quite that aggressive, but our data was good enough that we could have tripled it, adding 512 points instead of 256, if we had wanted to. Varian assumes 4 signals per row whereas I assumed 8 [7].

With these adjustments made, click on 'Full 2D Transform' [8]. . You will probably need to use the middle mouse again to make the vertical scale of your 2D (vs2d) smaller in order to not be looking at the noise floor. Alternately, you can type **vs2d=2000**, or another value, or type **vs2d=vs2d/2** to make the vertical scale half as tall. (**Page 16** [1])

Page 16 shows a very nice spectrum. You can see that the peaks identified as quaternary C by the DEPT are indeed absent from the HSQC, as expected since HSQC only detects ^{13}C via bound ^{1}H. (Multiple bond experiments such as HMBC or CIGAR will reveal C two bonds from H).

If phase correction is necessary, proceed as for TOCSY, selecting red peaks (positive amplitude).

referencing the spectrum

For 1D spectra, we reference the ^{13}-C and 1H axes vs. internal TMS ideally, or vs. solvent if no TMS can be seen. However the HSQC spectrum will not likely contain a resonance from TMS. The ^{1}H calibration should carry over from your beautiful calibrated 1D.

To transfer your ^{13}C calibration to the HSQC, go to your referenced ^{13}C1D (loaded in another workspace, **Page 17**) and locate a line that is well separated from its neighbours and can easily be recognized by its context. Put the cursor on it [1] and note the value cr [2] at the bottom of the screen (use the nearest line button to get the cursor right on the highest point of the line). Return to the workspace containing your HSQC experiment (**Page 18**) and place cursors around the corresponding resonance and zoom in to make it easier to put your cursor on the

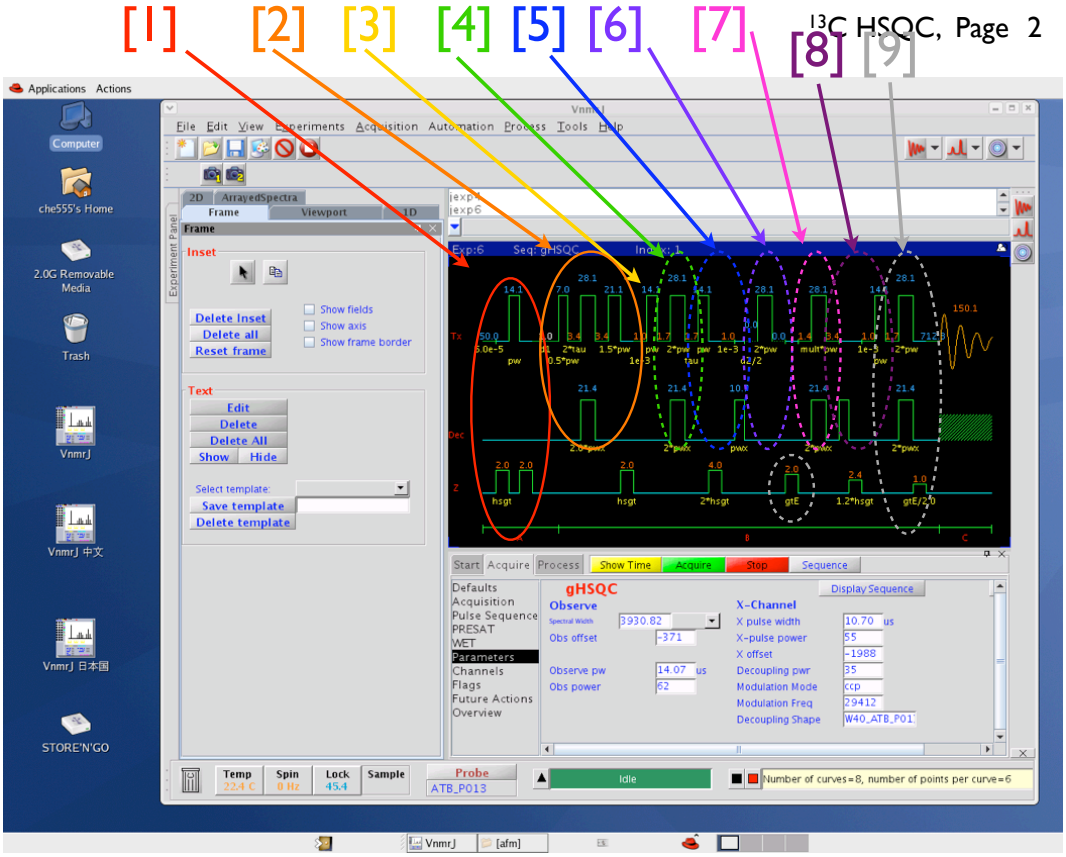
exact centre of the peak as far as the ^{13}C axis is concerned (the horizontal axis here [1]). then type **rl1(26.44d)** where 26.44 is the chemical shift of that line, based on the calibrated ^{13}C 1D, the 'd' specifies that the instrument is to use the MHz frequency of the second channel (the X-channel) to convert frequencies to ppm, and the rl1 means reference line on F1 axis. Display the spectrum [2] one more time to confirm that the ^{13}C axis is correctly adjusted.

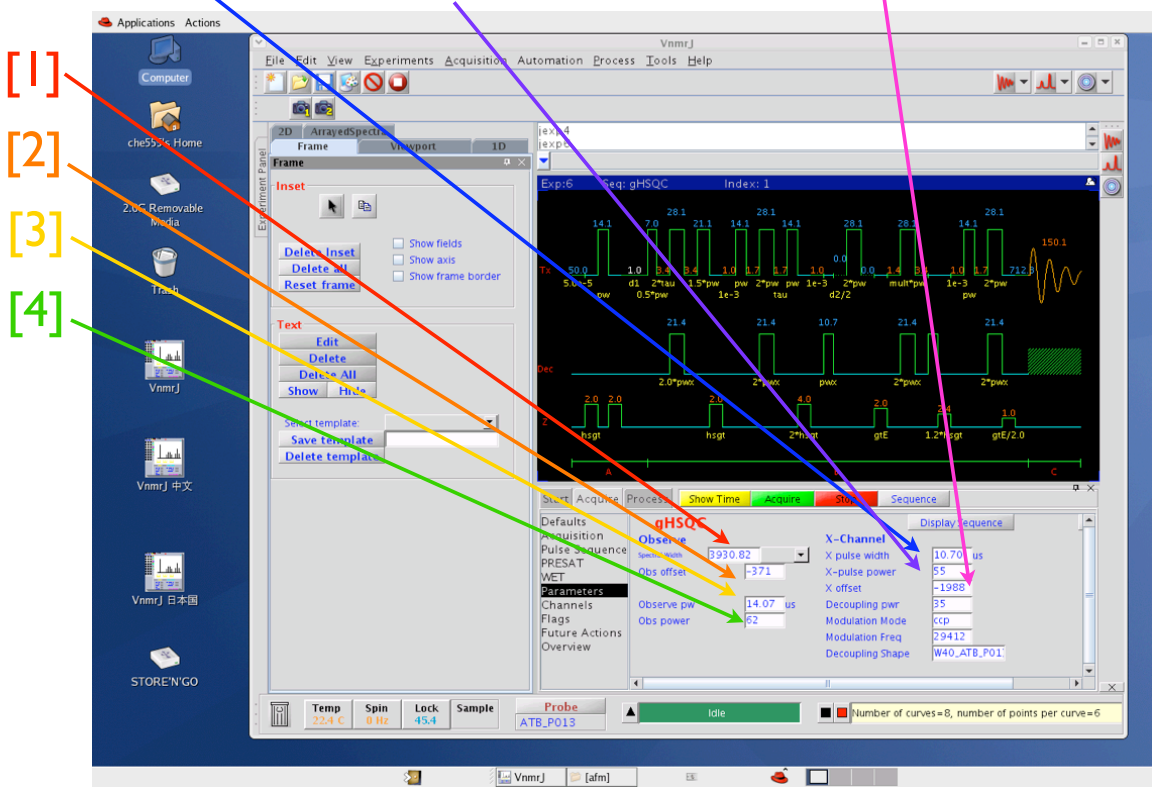
In the event that your ^{1}H chemical shifts in the HSQC do not match those of the ^{1}H1D, and the ^{1}H1D is properly referenced vs. TMS, use the ^{1}H1D to reference the chemical shift axis of the HSQC by an analogous procedure to the one described above for ^{13}C. Go to your referenced ^{1}H1D loaded in another workspace, identify a well isolated and recognizable line [1], zoom in on it and put the cursor on its centre (**Page 19**). Note the value of cr [2].

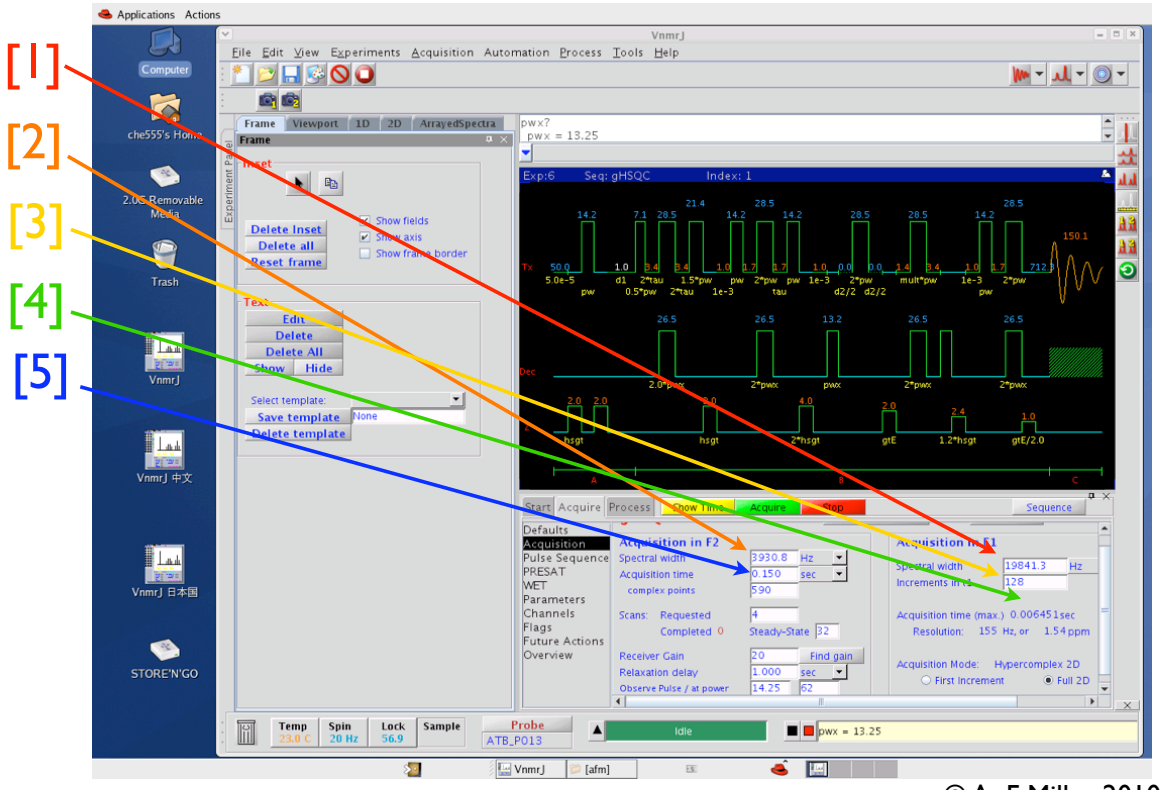
Now return to the workspace containing your HSQC, zoom in on the corresponding resonance (**Page 20**) and place your cursor so that it lies right across the centre of the peak as far as the ^{1}H axis is concerned (vertical axis) [1]. Type **rl(5.95p)** for reference line 5.95 ppm. Click 'Display Spectrum' and confirm that the chemical shift axes are now correctly positioned with respect to the resonances.

After clicking on the 'Full Spectrum' icon to see the whole HSQC (**Page 21**) you can go to the '2D' tab on the left-hand side [1] and activate the 'Print Screen' [2] to save pdf or ps files of your spectrum. See the earlier notes regarding editing display parameters to get a white background etc. Be SURE to return all settings to the state in which you found them. **Page 22** shows a sample figure I assembled by hand from a .ps file of my HSQC and files of the two corresponding high-resolution 1Ds. Varian software will also do this for you (more later ...).

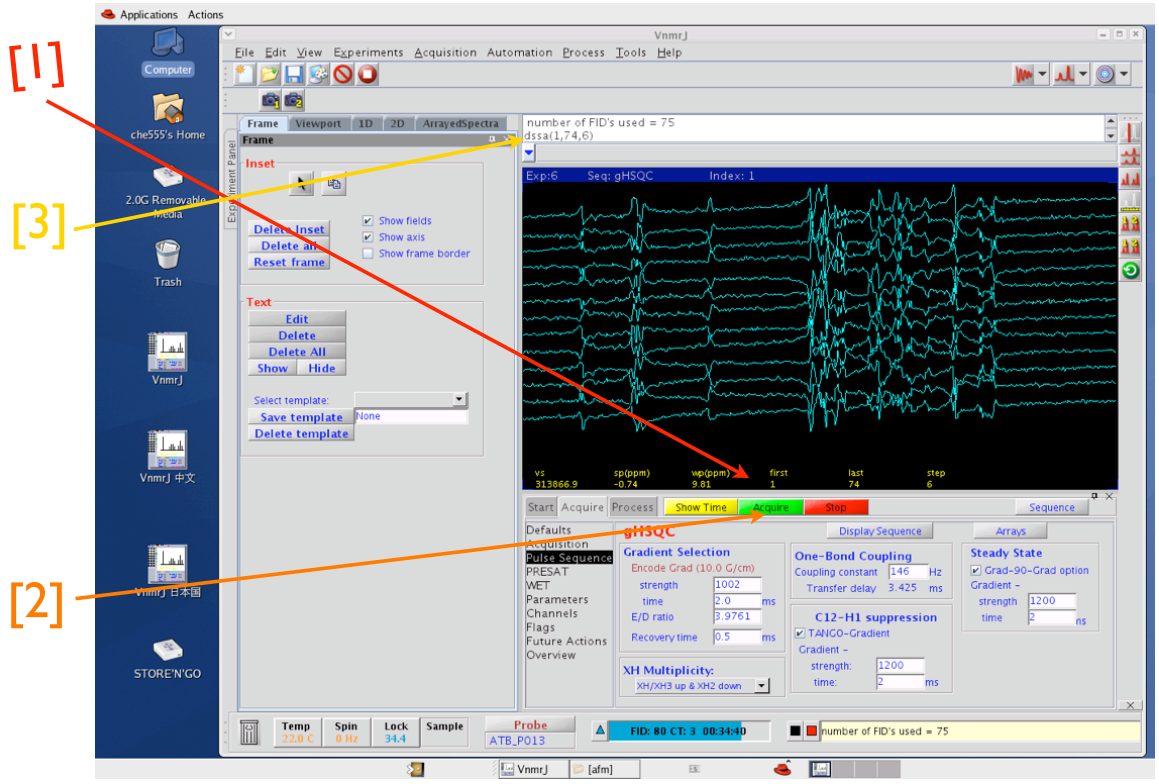
^{13}C Heteronuclear Single Quantum Coherence spectrum: HSQC

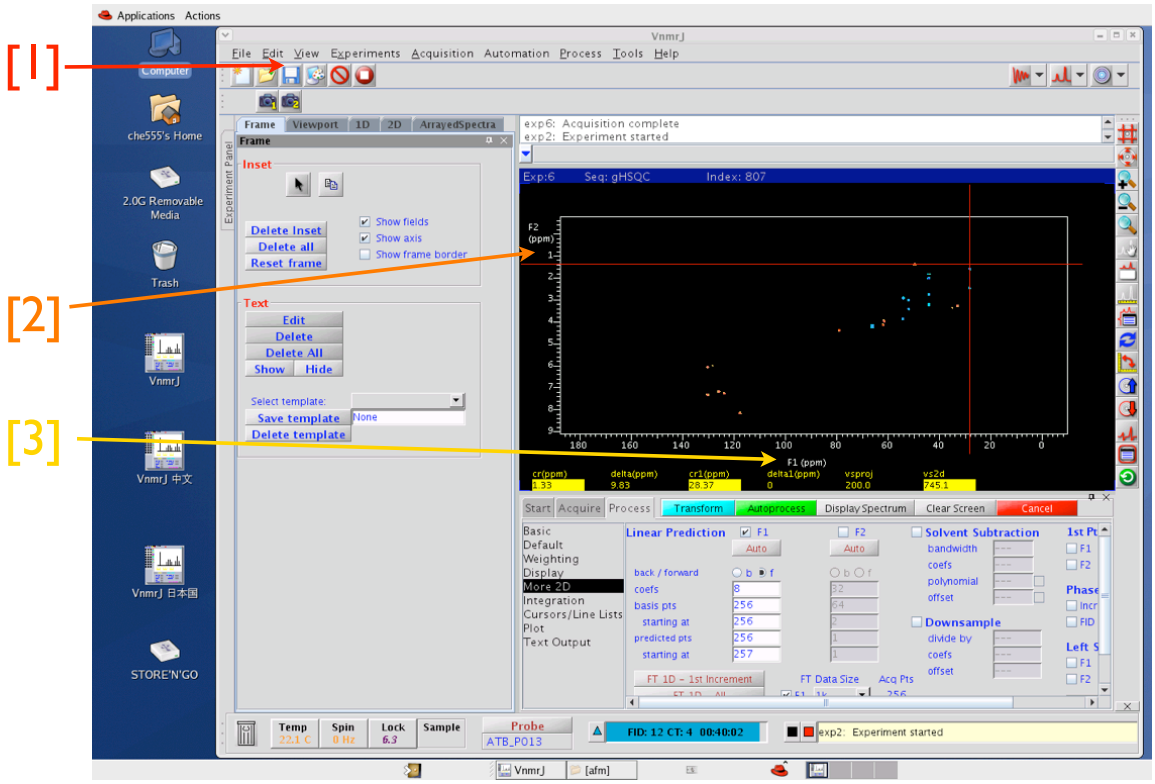


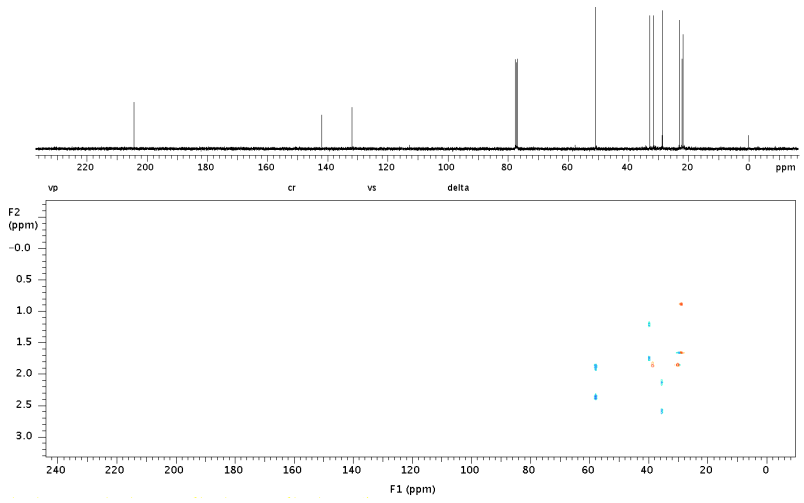




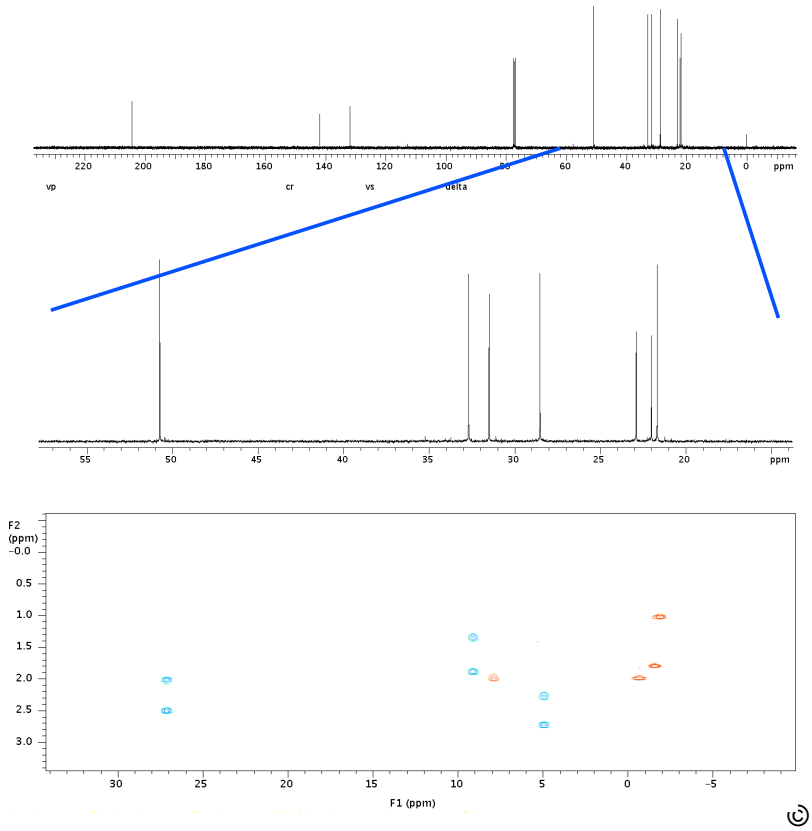
During acquisition of the 2D







^{13}C HSQC, Page 9



^{13}C HSQC, Page 10