# How to Phase a Phase-Sensitive gHSQC

*At first, the phasing procedures seem complicated. However, after a few times it is very straightforward and fast.*

These phasing instructions are **applicable to all phase-sensitive experiments** (TROESY, NOESY, etc.).

1.  **Save and then load the spectrum** if you have not done so already. This ensures that you can go back to the original spectrum in case something goes wrong with phasing; it also ensures that the parameter *file* has the correct information.

2.  On the command line type **prochsqc** and wait for the Fourier Transformation to finish.

3.  Observe the spectrum. If the spectrum has been acquired "apt style", areas of the spectrum that contain CH3 and CH groups have similar phase (positive or negative, the choice is up to you), whereas CH2 groups have opposite phase. See Figure 1 for a well phased spectrum.
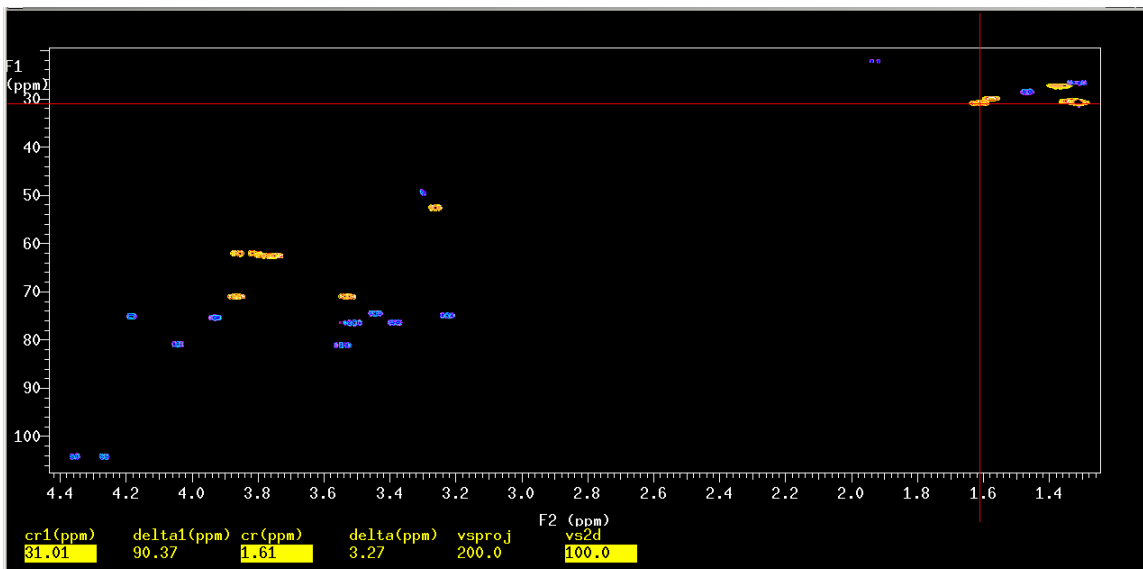


Figure 1    A well phased "apt style" gHSQC spectrum. CH3 and CH are phased negative (purple correlation peaks), CH2 groups are phased positive (orange correlation peaks).

4.  It is very likely that your spectrum looks like the one in Figure 2, i.e. you need to adjust the phase of the spectrum.
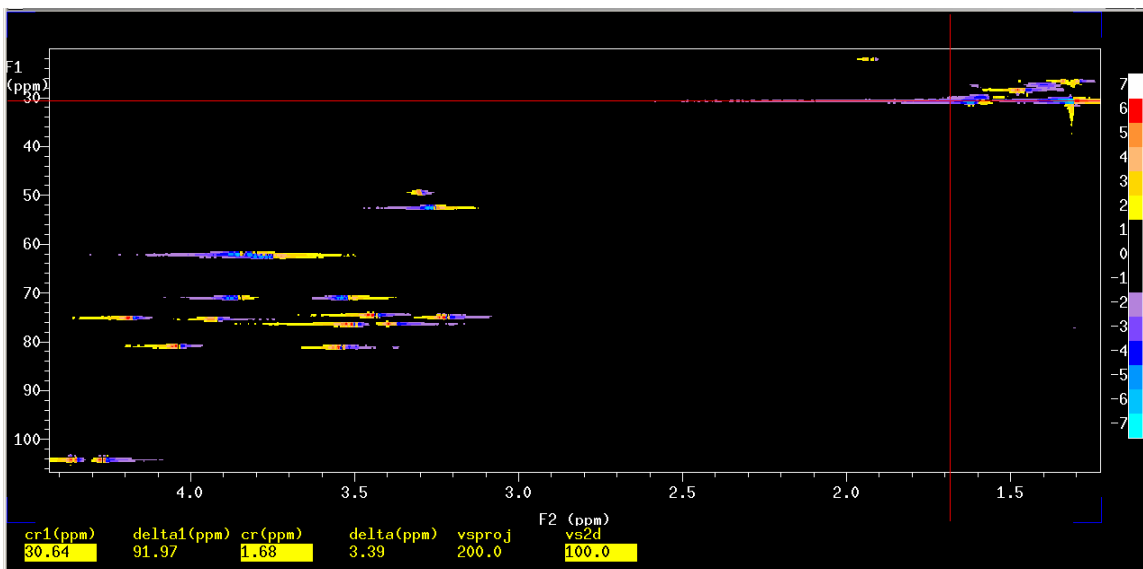


Figure 2    A poorly phased "apt style" gHSQC spectrum.

Choose an area of the spectrum, parallel to F2, through a series of correlation peaks that have a dispersive lineshape; CH3 group(s) work well for this but so do CH2 groups like the ones chosen in Figure 2; place the cursor along these correlation peaks and type

**ds**

on the VNMR command line to display the 1D spectrum (trace) parallel to F2 through these correlation peaks, see Figure 3.
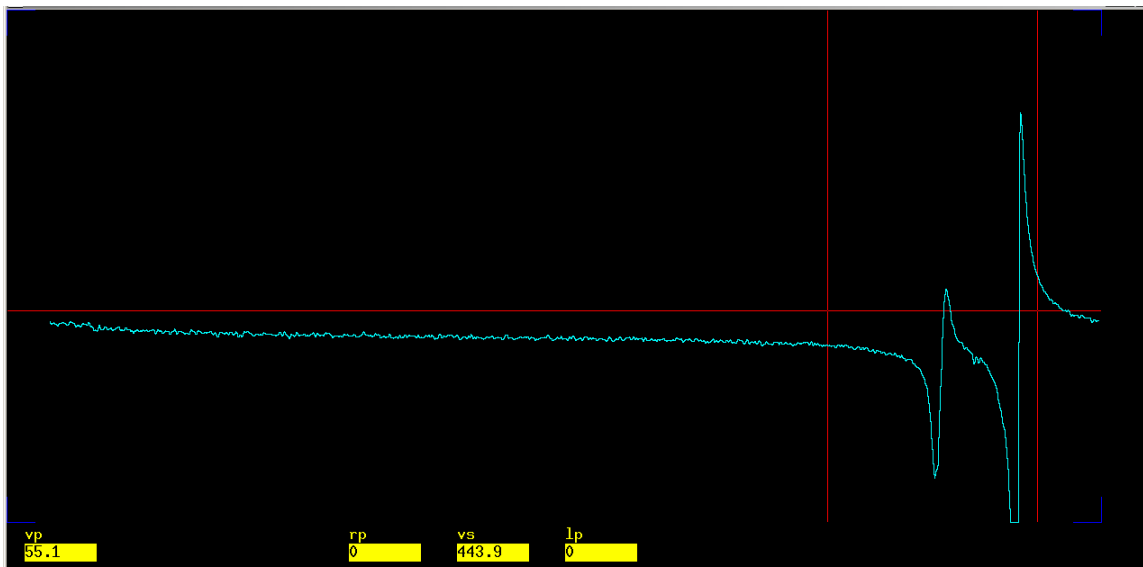


Figure 3    A trace parallel to F2 through two CH2 correlation peaks.

5.    Phase the spectrum by clicking on the phase button and then clicking on or near the peaks of interest, Figure 3; use the left and right mouse buttons to phase the spectrum (left click and hold - course phase; right click and hold - fine phase).

6.    Display the 2D spectrum by typing

**dconi**

on the VNMR command line. The spectrum should look similar to the example shown in Figure 4.
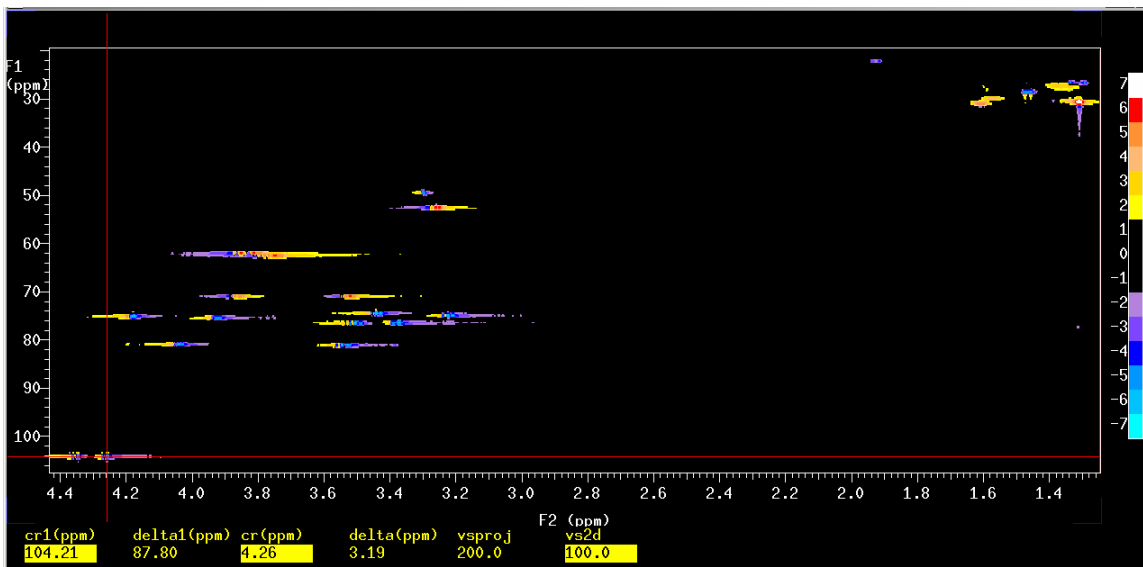


Figure 4    A partially phased "apt style" gHSQC spectrum.

7. Choose another region of the spectrum, preferably a CH group(s), as was chosen in this example (see cursor placement in Figure 4) and type

**ds**

on the VNMR command line to display a trace through the peak(s) of interest, Figure 5.

<u>Note</u>: the two areas that are used to phase the spectrum should be well separated, e.g. a normal alkane CH3 and an aromatic CH group (if present) would be most suitable for phasing.
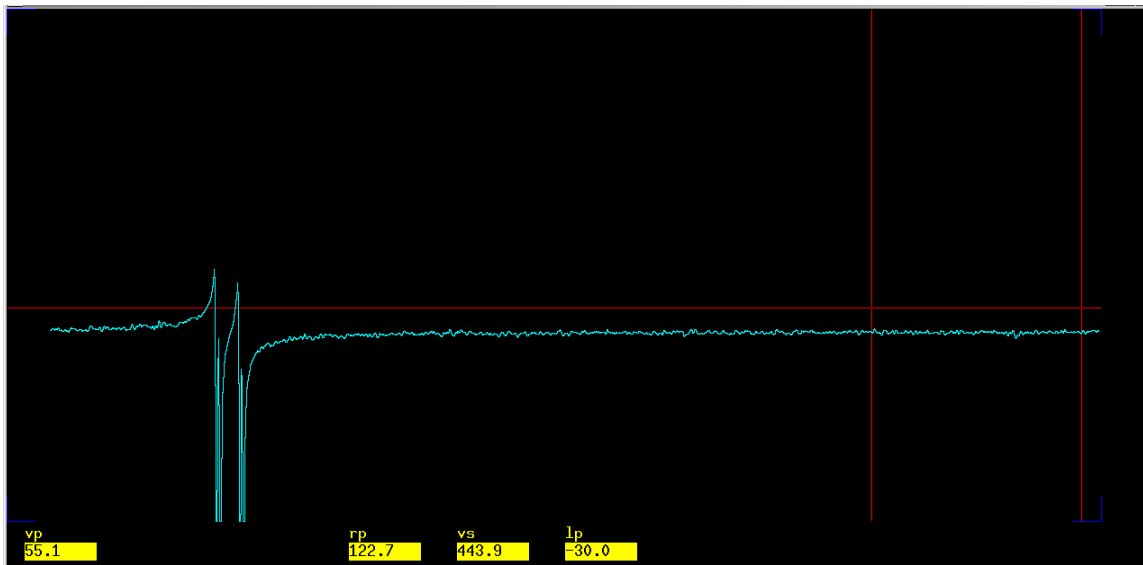


<u>Figure 5</u>  A trace parallel to F2 through two CH correlation peaks.

Click on the phase button, move the cursor approximately to the region of the spectrum that was phased in step 5 (this region is now empty, see Figure 5), click the left mouse button and then **WITHOUT CLICKING ON THE PHASE BUTTON** move the cursor adjacent to or on top of the peaks of interest.  Click the left mouse button once more and phase the spectrum as described in step 5.

The two mouse clicks described above are performed directly after each other WITHOUT CLICKING ON THE PHASE BUTTON.  This action is performed to add first order phasing to the spectrum (the parameters lp and rp change) whereas a single mouse click adds zero order phasing to the spectrum (only the parameter rp changes).

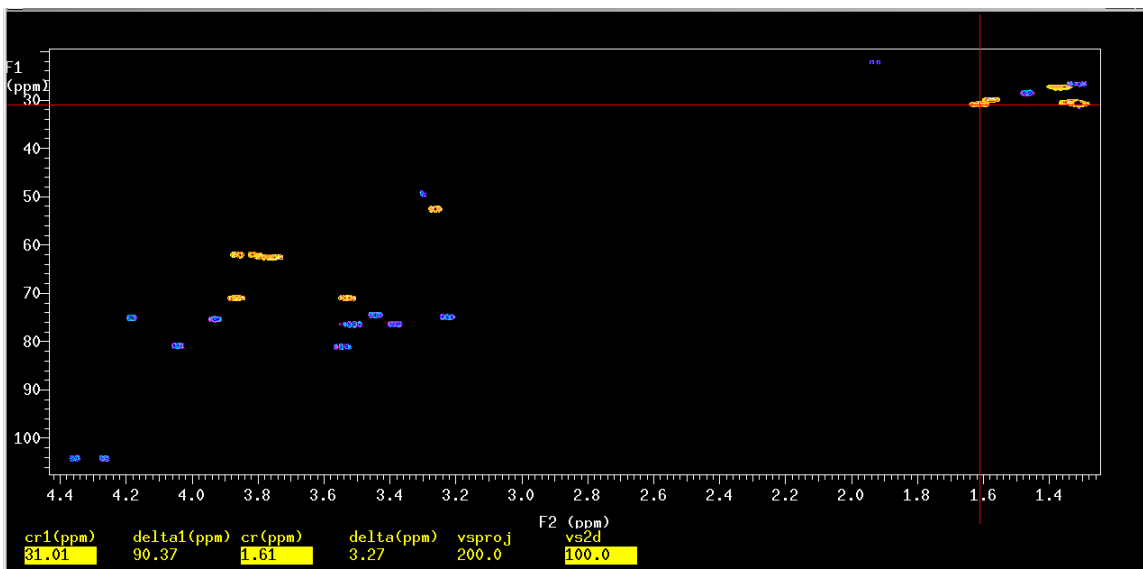Display the 2D spectrum by typing, on the VNMR command line,

**dconi**



<u>Figure 6</u>  The result of proper phasing of an "apt style" gHSQC spectrum.

8.  If further adjustments are needed repeat steps 4 to 7. It may take several iterations to achieve best results.

9.  Phase the indirectly detected F1 (=13C) dimension. Type

    **f1**

    on the VNMR command line to rotate the spectrum such that the F1 axis is parallel to the bottom of the monitor. Phase the spectrum as indicated in steps 4 to 7.

**Note**: Phasing a phase-sensitive spectrum is an iterative process: adjustments on one side of the spectrum affect other portions of the spectrum. Be prepared to repeat steps 4-7 several times.
It may not always be possible to obtain perfect phasing for all regions of the spectrum but likely you will be able to obtain phasing that suits most regions of the spectrum.

**Additional information**

A.  Phasing parameters (rp, lp, rp1 and lp1) should be within the limits shown below (type *dg1* to display values):

| Directly detected dimension (F2) | Indirectly detected dimension (F1) |
|---|---|
| -360 < rp < 360 | -360 < rp1 < 360 |
| -360 < lp < 360 | -360 < lp1 < 360 |

Values outside these limits may be in error; it would be best to reset the parameter(s) to zero and try again.

**resetph dconi** (*resetphase* and *rstph* have the same effect)

will reset all phase constants (F1 and F2) to zero while

**resetphf1 dconi** (*resetphasef1* and *rstphf1* do the same)

will reset only the F1 phase constants. Repeat steps 4 to 8 or 4 to 9 depending on what was reset.

B.  To invert your spectrum, type

    **inv dconi**

    on the VNMR command line. The resulting spectrum is shown in Figure 7, compare with Figure 1!
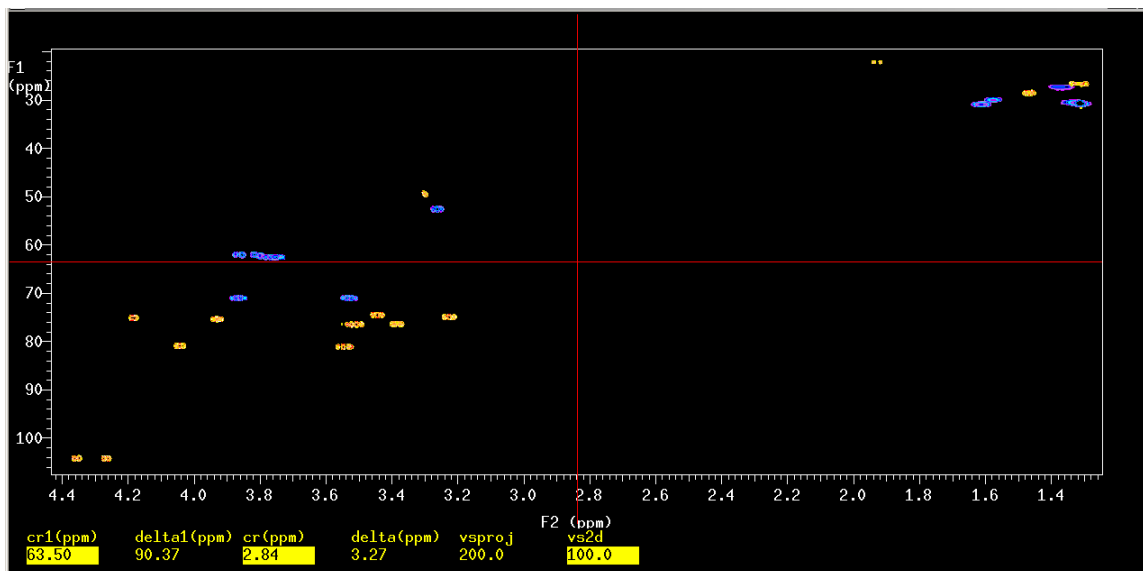


Figure 7    The inverted gHSQC spectrum: CH3 and CH groups are now phased up (orange contours) and CH2 groups are phased down (purple contours), compare with Figure 1.

C.  Resave the spectrum after phasing so that the phasing results will be present the next time you load the experiment.  To resave a spectrum with the exact same filename type

    **svf(file)**

    on the VNMR command line. The command is entered exactly as typed above.