

Heteronuclear **M**ultiple **Q**uantum **C**orrelation spectroscopy is an inverse chemical shift correlation experiment that, like XHCORR, is used to determine which ^1H 's of a molecule are bonded to which ^{13}C nuclei (or other X nuclei). The advantage of HMQC over XHCORR is that in HMQC the nucleus with the highest γ (^1H) is detected, and so it is possible to obtain the highest sensitivity. The challenge of an inverse chemical shift correlation experiment, however, is that the large signals from ^1H 's not coupled directly to a ^{13}C nucleus must be suppressed in a difference experiment. This poses a dynamic range problem: the signal of interest is that of ^1H 's coupled directly to ^{13}C nuclei; however, the signal detected is dominated by the contribution of ^1H 's bonded directly to ^{12}C nuclei. HMQC, which is based on multiple-quantum NMR, minimizes this dynamic range problem while optimizing the sensitivity of the experiment. The resonance frequency of low γ spins can be detected with enhanced sensitivity by the creation and ^1H detection of ^1H - ^{13}C (or other X nucleus) multiple-quantum coherence.

In the HMQC sequence, the first ^1H pulse creates transverse magnetization, some of which evolves into anti-phase magnetization at the end of the first $1/(2J_{\text{XH}})$ delay. This anti-phase magnetization is converted into zero- and double-quantum coherence by the $(\pi/2)_{\text{X}}$ pulse. The zero- and double-quantum coherences evolve during t_1 and are exchanged by the π_{H} pulse so that single-quantum ^{13}C frequencies are observed in F1. The final $(\pi/2)_{\text{X}}$ pulse converts multiple-quantum coherence into observable ^1H transverse magnetization. In analogy with XHCORR, if a delay $1/(2J_{\text{XH}})$ is inserted between the final $(\pi/2)_{\text{X}}$ pulse and the start of acquisition, then ^{13}C decoupling can be used during acquisition. Without this delay, the ^1H magnetization components would be anti-phase at the start of acquisition and so ^{13}C decoupling would result in mutual cancellation of the ^1H signals.

Note that since it is the longitudinal ^1H magnetization present before the first $(\pi/2)_{\text{H}}$ pulse that is converted into heteronuclear multiple-quantum coherence, it is the ^1H T_1 which determines the appropriate recycle delay. Thus, it is possible to use a shorter recycle delay for HMQC than for XHCORR.

For small molecules, it is useful to use a BIRD preparation period in conjunction with the HMQC experiment. The basic idea of this preparation period is to saturate all ^1H 's not directly attached to a ^{13}C nucleus, leaving ^1H 's coupled to ^{13}C unaffected or slightly intensified by homonuclear NOE (which is the case for molecules in the fast motion limit). Then at time τ (**d7**) when the inverted magnetization changes from negative to positive (i.e., when the ^1H 's not coupled to ^{13}C are nearly saturated) the first $\pi/2$ pulse of the HMQC experiment is applied. BIRD is not recommended for proteins and other macromolecules because the negative NOE effect during the delay time τ decreases the intensity of ^1H 's coupled to ^{13}C .

In practice, since the T_1 's of various ^1H 's in the molecule vary, it is recommended to keep the delay time between experiments short: $T \sim 1.3 * T_1$ of the fastest relaxing ^1H of the molecule, where the delay time T is defined to last from the start of data acquisition in one scan to the end of the preparation period (3^{rd} ^1H pulse) in the next

scan. Similarly, it is suggested that τ be approximately $T/2.7$. To fine tune τ , choose the value that minimizes the signal obtained from a single scan preceded by 2 dummy scans (i.e., that minimizes the signal from ^1H 's bonded to ^{12}C nuclei).

HMQC is a phase-sensitive experiment, and after a 2D Fourier transform with respect to t_1 and t_2 , the 2D spectrum can be phased so that all peaks are purely absorptive.

References: A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983); A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

Sample

The sample used to demonstrate HMQC in this chapter is 50mM Gramicidin in DMSO-d₆. This is the same sample that was used to demonstrate COSY, NOESY, ROESY, and TOCSY.

Pulse Sequence Diagram

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The HMQC pulse sequence is shown in Figure 46. This version of the sequence should be used on samples consisting of proteins and other macromolecules. The HMQC pulse sequence with BIRD is shown in Figure 47. This version of the sequence is useful for smaller molecules.

Notice that the pulses **p1** and **p3** must be set to the appropriate 90° times found in Chapter 5 'Pulse Calibration'. Also, the cpd sequence used is GARP, which requires the calibrated 90° time **pcpd2**. The 180° pulse lengths **p2** and **p4** are determined by the pulse program itself. In the HMQC sequence with BIRD, the delay **d7** should be optimized to minimize signal from ^1H 's bonded directly to ^{12}C 's.

Figure 46: HMQC Pulse Sequence

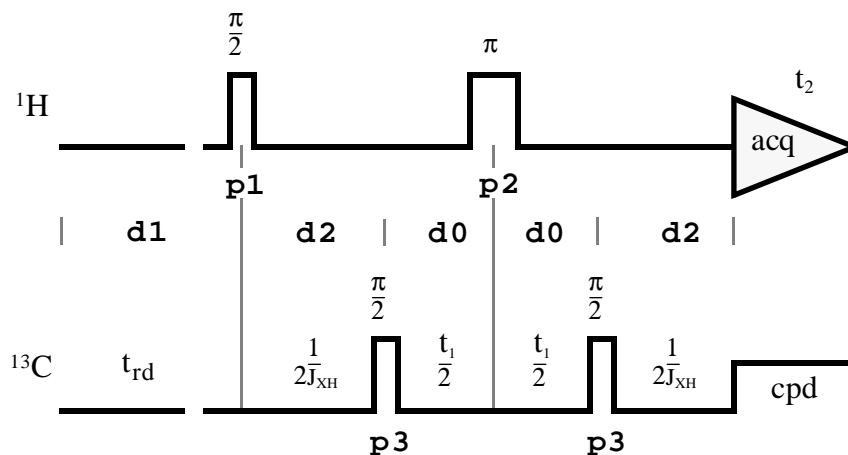
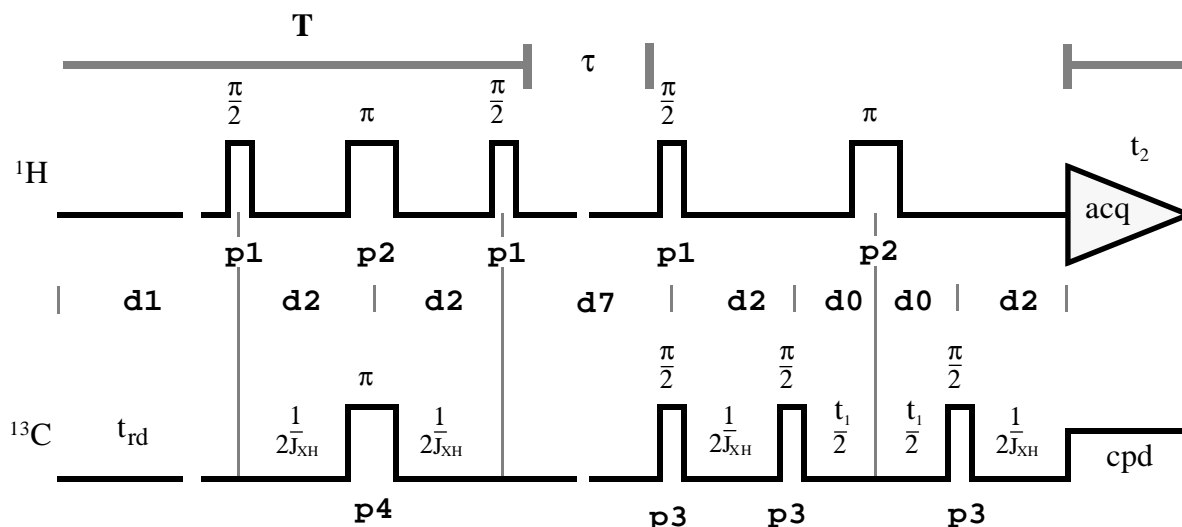


Figure 47: HMQC with BIRD Pulse Sequence



Acquisition and Processing

16.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z² shims until the lock level is optimized. Tune and match the probehead for ¹H observation ¹³C decoupling.

It is generally recommended that HMQC, like all 2D experiments, be run without sample spinning.

¹H reference spectrum

Since HMQC is a ¹H-observe, ¹³C-decouple experiment, the first step is to obtain a reference ¹H spectrum of the sample. This reference spectrum will be used to determine the correct **o1** for ¹H, the correct **sw** for the F2 dimension, and can also be used as the F2 projection of the HMQC spectrum.

A ¹H reference spectrum of this sample was already created for the magnitude COSY experiment. This spectrum is found in the data set proton/5/1.

¹³C reference spectrum

It can be assumed that the sample used for an inverse experiment such as HMQC has too small a ¹³C signal to make it practical to obtain a ¹³C reference spectrum. Thus, the user will need to make an educated guess as to the appropriate values of **o2** and **sw** for the F1 dimension. Actually, it is easier to use **o2p** (in ppm) rather than **o2** (in Hz). This is because the XWIN-NMRlock routine was used to lock the magnetic field, and so 0 ppm (for a given nucleus) is at the same absolute frequency regardless of the lock solvent.

In the HMQC experiment, no quaternary carbons lead to detected signal, so it is usually sufficient to select a ¹³C spectral width covering the range -10 ppm to 180 ppm. This corresponds to an **o2p** value of 85 ppm and an **sw** value of 190 ppm.

Create a new file directory for the 2D data set

Enter **re proton 5 1** to return to the optimized ^1H spectrum. From this data set, enter **edc** and change the following parameters:

NAME	hmqc
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set hmqc/1/1. By creating the HMQC data set from data set of the ^1H reference spectrum, many of the F2 parameters for HMQC are already set .

Enter **edsp** and set NUC2 to ^{13}C and OFSH1 to **o1** of the ^1H reference spectrum proton/5/1. The parameter OFSX1 should have the value of **o2** corresponding to **o2p** = 85ppm, but the best way to set this is simply to set **o2p** correctly in the main UXNMR window.

Change to 2D parameter mode

Enter **eda** and set PARMODE = 2D. Click on **SAVE** and ok the message “Delete ‘meta.ext’ files?”. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 49. Use the values determined in Chapter 5 ‘Pulse Calibration’ for the parameters **p11** and **p1** (^1H observe high power level and 90° pulse time), **p12** and **p3** (^{13}C decouple high power level and 90° pulse time), and **p112** and **pcpd2** (^{13}C decouple low power level and 90° pulse time). Note that the pulse program invbtp calls an include file in which **cnst2** is used to calculate **d2** ($d2 = 1/(2 * cnst2)$). Thus, it is only necessary for the user to set the value of **cnst2**. Similarly, the 180° pulse lengths **p2** and **p4** are calculated from the corresponding 90° pulse lengths **p1** and **p3**, so the user need only set the values of **p1** and **p3**.

The F2 parameters **o1** and **sw** (not shown in the table) should be identical to the values used in the optimized ^1H reference spectrum (proton/5/1). Make sure to set **o2p** to 85ppm as discussed above. The F1 parameter **sw** should also be set to 190ppm as discussed above.

Finally, notice that **in0** and sw(F1) are not independent. A convenient way to set **in0** is to set the F1 parameters **nuc1** by clicking on **NUCLEI** for F1 parameters, **nd0**, and **sw** correctly. This automatically sets **in0** to the correct value.

Table 49. HMQC with BIRD Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	invbtp	HMQC with BIRD; see Figure 47 for pulse sequence diagram; for HMQC without BIRD choose inv4tp.
TD	1k	
NS	8	the number of scans should be 4*n in order for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
PL2		high power level on f2 channel (see “An Important Note on Power Levels” on page 7).
PL12		power level for cpd on f2 channel.
P1		90° ¹ H high power pulse on f1 channel.
P2		180° ¹ H high power pulse on f1 channel; calculated internally.
P3		90° ¹³ C high power pulse on f2 channel.
P4		180° ¹³ C high power pulse on f2 channel; calculated internally.
PCPD2		90° ¹³ C pulse for cpd sequence.
D0	3 μsec	incremented delay (t ₁ /2); predefined.
D1	1.5 sec	relaxation delay; should be about 1.25*T ₁ (¹ H).
D2	3.45 msec	delay for creation of anti-phase magnetization (1/(2J _{XH})); calculated internally.
D7		delay for inversion recovery (optimize).
D13	3 μsec	short delay; predefined.
CNST2	145 Hz	one-bond heteronuclear J-coupling (J _{XH}); used to calculate d2; 145 Hz is a good intermediate value for ¹³ C.
CPDPRG2	garp	composite pulse decoupling sequence.
F1 Parameters		
Parameter	Value	Comments
TD	256	number of experiments.
ND0	4	there are two d0 periods per cycle and MC2 = TPPI.

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IN0	$1/(4*SW_X)$ $= (1/2)DW_X$	t_1 increment.
SW		sw of the ^{13}C spectrum (here typically 190ppm).
NUC1		select ^{13}C frequency for F1

Optimize d7 (HMQC with BIRD only)

Set the acquisition parameters as shown above and choose a starting value of 400msec for **d7** (note that it is important to select a large enough starting value for **d7** since the **gs** routine will only allow the user to vary **d7** from 0 to twice the starting value). Enter **acqu** to enter the acquisition window. Enter **gs** to start the go setup routine. Click the left mouse button to fix the “acquisition-gs” window somewhere on the screen, and then click on the box in the upper right hand corner of the window to send it away as an icon. While monitoring the intensity of the time domain data, adjust the value of **d7** (simply enter **d7** and then a new value at the prompt). The optimum value of **d7** corresponds to the *minimum* intensity. Once the optimum value of **d7** is found and stored, enter **rga** to optimize the receiver gain for this minimum signal.

Acquire the 2D data set

Enter **zg** to start the HMQC experiment. With the acquisition parameters shown above, the approximate experiment time is 1.2 hours.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 50.

Table 50. HMQC with BIRD Processing Parameters


F2 Parameters		
Parameter	Value	Comments
SI	1k	
SF		spectrum reference frequency (^1H).
WDW	QSINE	multiply data by phase-shifted sine-squared function.
SSB	2	choose pure cosine-squared wave.
PH_mod	pk	apply 0- and 1 st -order phase correction determined by phase correcting the first row.
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	

F1 Parameters		
Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^{13}C).
WDW	SINE	multiply data by phase-shifted sine function.
SSB	2	choose pure cosine-squared wave.
PH_mod	pk	apply 0- and 1 st -order phase correction determined by automation program <code>calcphinv</code> .
BC_mod	no	
MC2	TPPI	determines type of FT in F1; TPPI results in a forward single real FT.

Process the 2D data set

Enter **xfb** to multiply the time domain data by the window functions and also perform the 2D Fourier transform.

Adjust the contour levels

The threshold level can be adjusted by placing the cursor on the  button, holding down the left mouse button, and moving the mouse up and down.

Since this is a phase-sensitive spectrum, click on **+/-** with the left mouse button until both positive and negative peaks are displayed.

The optimum display (both the threshold and which peaks are displayed) may be saved by clicking on **DefPlot**.

Phase correct the spectrum

To simplify the phasing of the 2D HMQC spectrum, it helps first to phase correct the first row. Enter **rser 1** to transfer the first row to the 1D data set `~TEMP/1/1`. Enter **sinm** to apply the sine-bell windowing function, and enter **ft** to Fourier transform the data. Manually phase correct the spectrum as you would any 1D spectrum *except* that when you are finished, click **Return** and select **Save as 2D & return** to save the corrections **phc0** and **phc1** to the corresponding F2 parameters in the 2D data file `hmqc/1/1`. Then click **2D** with the left mouse button to return to the 2D data set `hmqc/1/1`.

Next, it is convenient to use the automation program **calcphinv** to determine the F1 phase correction. From the data set `hmqc/1/1`, simply enter **xau calcphinv**. Note that this automation program is designed specifically for HMQC-type experiments.

Now enter **xfb** to Fourier transform the HMQC spectrum again, this time applying the appropriate phase correction to F1 and F2. At this point, the spectrum should be phased correctly and all peaks should be positive. Further adjustments can be made in the 2D phase subroutine, as described in previous chapters.

Plot the spectrum

Read in the plot parameter file standard2D, e.g., enter **rpar standard2D plot**. This sets most of the plotting parameters to values which are appropriate for this 2D spectrum, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file standard2D can be found in Appendix C '1D and 2D Plotting Parameters'.

To set the region (full or expanded), threshold, and peak type (positive and/or negative), to be used in plotting the spectrum, first make sure the spectrum appears as desired on the screen, and then click **DefPlot** and answer the following questions.

```
Change levels?           y
Please enter number of positive levels?    6
Please enter number of negative levels?    3
Display contours?       n .
```

Enter **edg** to edit the plotting parameters.

Click the **ed** next to the parameter EDAXIS to enter the axis parameters submenu. Change the value of the parameter X1TICD from 0.1 to 2.5. Click **SAVE** to save this change and return to the **edg** menu.

Since there is no ^{13}C reference spectrum of this sample, the user may choose not to plot an F1 projection for the HMQC spectrum. To do this, simply click the YES adjacent to PROJ1 in the **edg** menu to toggle it to NO.

Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters from PF2DU to PF2PROC as follows:

```
PF2DU           u
PF2USER         (name of user for file proton/5/1)
PF2NAME         proton
PF2EXP          5
PF2PROC         1 .
```

Click **SAVE** to save these changes and return to the **edg** menu.

Click **SAVE** to save all the above changes and exit the **edg** menu.

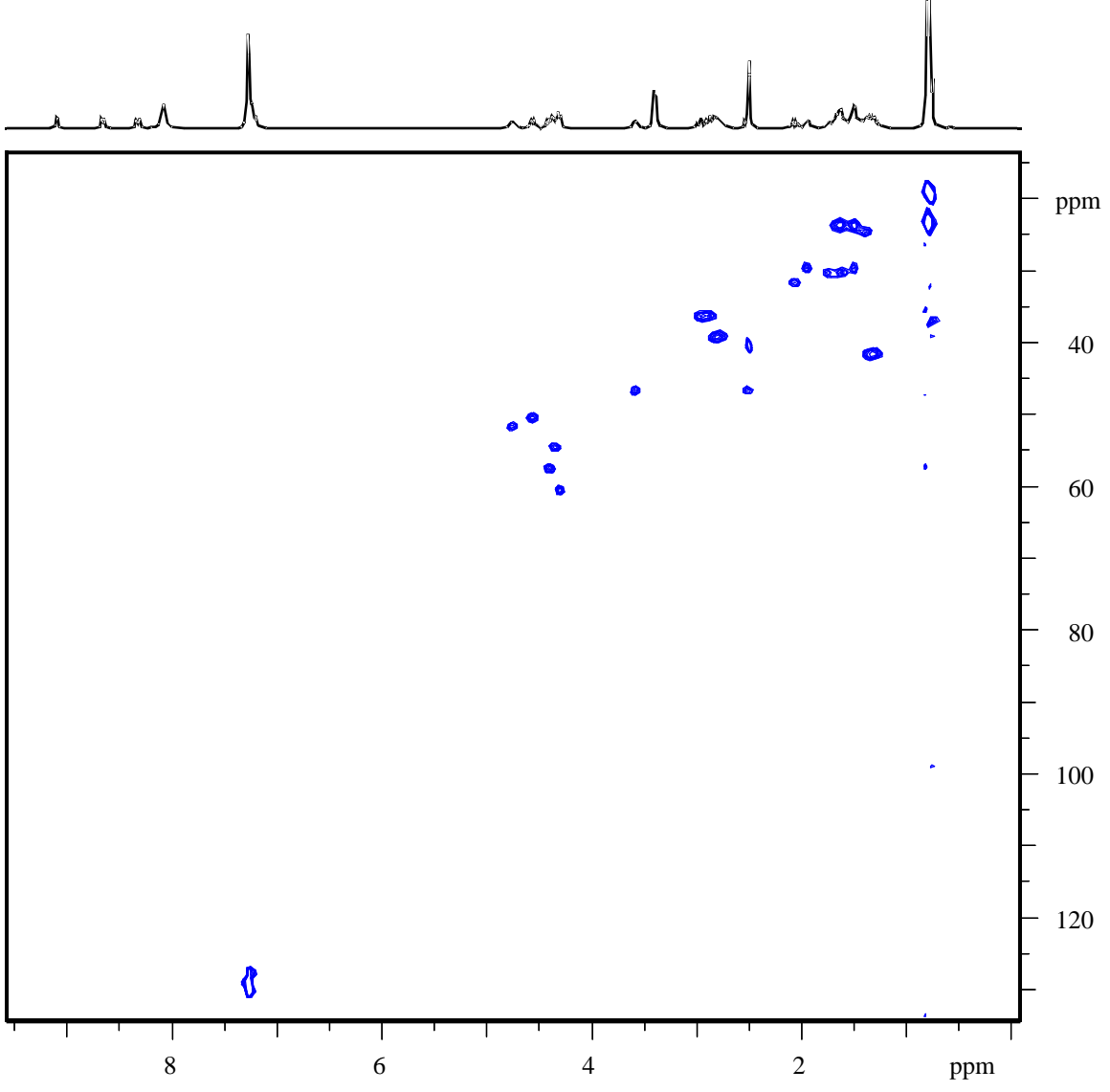
Next create a title for the spectrum. Enter **setti** to use the editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **plot** (provided the correct plotter is selected in **edo**).

An HMQC spectrum of 50 mM Gramicidin in DMSO-d₆ is shown in Figure 48.

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Figure 48: HMQC Spectrum of 50 mM Gramicidin in DMSO-d6



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