17. Heteronuclear Correlation on AM Consoles

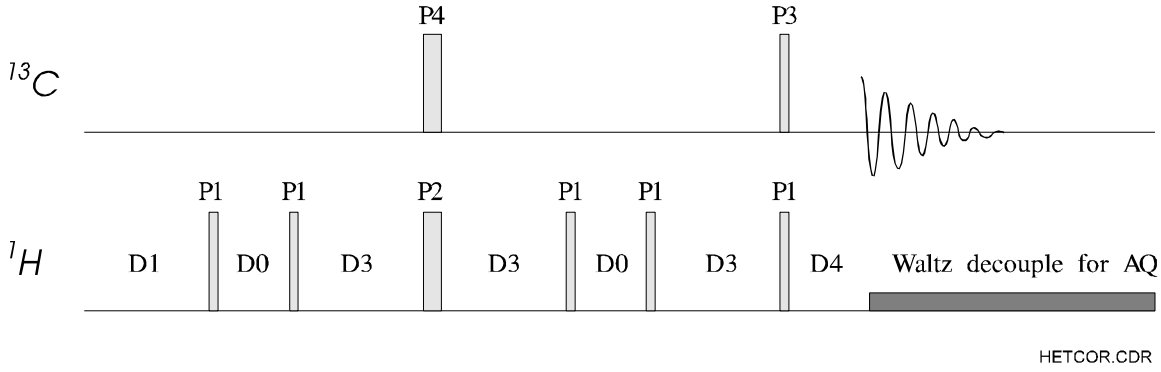
by cg fry: created 2/22/94 – revised 7/31/95

I. Discussion

Heteronuclear correlation maps interactions from an X nucleus (e.g., ^{13}C) to ^{1}H. The nominal experiment, referred to here as the HETCOR experiment, is implemented on the Bruker spectrometers, and uses X-nucleus detection with ^{1}H decoupling. The inverse experiment (i.e., HMQC), currently only possible on the Unity 500, uses ^{1}H detection with X-nucleus decoupling, and has much improved sensitivity over the standard HETCOR experiment. HETCOR will work ok down to 20 mg material in a 5 mm tube; more dilute samples should be run on the Unity with the inverse experiment.

The HETCOR experiment is relatively straightforward, requiring as a minimum running standard ^{1}H and ^{13}C spectra with optimized sweep widths as setup. It is important to have correct pulse width calibrations for both ^{1}H and ^{13}C transmitters, however, for correct optimized use of the the experiment. An understanding of filter bandwidths and folding is helpful to properly set the sweep widths.

II. Critical Parameters



D3 – $= \dfrac{1}{2J_{CH}} \sim 3.3$ ms ***change if have unusual J_{CH} couplings***

D4 – $= \dfrac{1}{4J_{CH}}$

P1 – ^{1}H 90° pulse width; see calibration sheet next to spectrometer, or recalibrate before experiment

P3 – ^{13}C 90° pulse width; see calibration sheet

S1 – 0H for full ^{1}H power

S2 – same decoupling power used for standard ^{13}C spectrum (typically 20H)

IN – increment for 2D; make sure $\boldsymbol{IN} = \dfrac{1}{4 \cdot \boldsymbol{SW1}} = \dfrac{1}{2 \cdot \left(^{1}H \text{ sweep width}\right)} = \boldsymbol{DW}$ (^{1}H dwell)

III. Acquiring 2D HETCOR Spectra

1. **Use job 1:** Obtain a routine ^{1}H spectrum. Save this spectrum using **WR**, as *name*H.001 where *name* is a seven letter or less name used for all HETCOR data for this sample. Do not use alphanumerics in the suffix for this file, to stay compatible with the DISNMR plotting routine CP2P. Note SR after setting the reference (using, e.g., EP-G command).

   Note SR(^{1}H) and save ^{1}H spectrum as *name*H.001

2. Use EP and CNTL-O command to set SW and O1 for region of interest. Take a full (e.g., SI=16 or 32K) ^{1}H spectrum with new settings, being careful that foldovers do not interfere with important ^{1}H resonances.

   Note O1(^{1}H), and DW(^{1}H)

3. **Use job 2:** Obtain a routine ^{13}C spectrum using CPD (i.e. Waltz-16) decoupling with O2 set to the O1 value used in the ^{1}H spectrum. Set the reference, and write down the value for SR.

   Note SR(^{13}C)

4. Now obtain DEPT-45 to remove quats. Use EP and CNTL-O command to set SW and O1 to include only protonated ^{13}C plus ~10%. Take full (e.g., SI=32 or 64K) ^{13}C spectrum with new settings. Make sure you have sufficient S/N to observe all carbons of interest; S/N~3 is minimal, S/N~8 should be ok (i.e. decrease/increase NS until S/N~8). Save a good S/N ^{13}C Dept-45 spectrum as *name*C.001

   Note DW(^{13}C) and O1(^{13}C) and save ^{13}C Dept-45 spectrum as *name*C.001

5. **In job 3:** enter **PJ XHCORR.1DJ**

   RJ2D XHCORR.2DJ

6. **Return to job 2**, and transfer carbon parameters to job 3: **TR <ret> 3 <ret>**

8. Set **SI**=2K (larger or smaller depending on ^{13}C SW; 4k for SW > 150 ppm, 1k < 70 ppm) and retake the ^{13}C Dept-45 spectrum. Make sure you have sufficient S/N to observe all carbons of interest; S/N~3 is minimal, S/N~8 should be OK (i.e. decrease/increase NS until S/N~8).

7. Enter the following parameters

   NE=128 (increase if want better ^{1}H resolution, and have sufficient spectrometer time)

   ND0=2

   IN=[DW(^{1}H); see step 2]; enter IN as number followed by **U**; e.g.,

   IN <ret> 120U for DW(^{1}H)=120

8. Enter

   ST2D

   and make sure that:

   TD1=NE=128 (or if enough time, multiple of 128)

   SI1=TD1×2

   TD2=**SI2**=**TD**=**SI**=2K

   SW2=**SW**

SW1= (SW from ^{1}H spectrum)/2

SF1= 500.13 AM-500 Brutus
299.87 Athena
250.13 Phoenix

O11= (O1 from ^{1}H spectrum)

SR=(SR from ^{13}C step 3)

SR2=SR

SR1=(SR from ^{1}H step 1)

9. Set up automation routine (only typical values shown below; most should be ok from ^{13}C DEPT):

AS XHCORR.AU (or XHCORRD.AU to remove J_{HH})

D1=1 sec	**S1**=0H	**P1**=^{1}H 90° in µs
D0=3E-6	**P4**=^{13}C 180° in µs	**D3**=0.5 J_{XH} ~ 3.3 ms
P3=^{13}C 90° in µs	**D4**=0.25/J_{XH}~1.65µs	**S2**=20H (as used for ^{13}C)
RD=0	**PW**=0	**DE** set by computer
NS=# scans used for ^{13}C (e.g. 8)	**DS**=2	**P9**=^{1}H 90° at S2 power
NE=TD1	**IN**=0.25/SW1	

10. Save job parameters using command: **WJ2D *name*.J2D**
WJ *name*.J1D

11. Do not use **EXPT**!! Check experimental time as $t_{exp} = (NS + DS) \times D1 \times NE \times 1.2$ where the factor 1.2 is approximate to account for disk I/O. If t_{exp} is too long, reschedule more time later, or decrease *NS* (keep as multiple of 8).

12. Start acquisition: **AU**

use filename: ***name*.SER** *you must give .SER suffix!*

IV. HETCOR 2D Processing

[If processing directly after taking data—if ^{1}H in job1, ^{13}C Dept-45 in job2, XHCORR in job3—goto step IV.4]

1. Goto job1: **RE *name*H.001**

Reference ^{1}H spectrum and set CY=14 (18 for 11×17 plots); **WR <ret> <ret>** to rewrite data

2. Goto job2: **RE *name*C.001**

Reference Dept-45 spectrum (use written down SR if needed), set CY=14 and **WR <ret><ret>**

3. Goto job3: **PJ *name*.J1D**

RJ2D *name*.J2D

Check that SR=SR2=SR(^{13}C) and SR1=SR(^{1}H)

4. Enter: **XFB**

   and be patient. 2k×256w will take ~8 min on DataStation.

5. Enter: **EP2D** (DataStation/AM's) or **AP2D** (AC's)

   Set contour levels appropriately, and **ESC-X**

6. Enter: **CP2P**

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FILENAME IN F1:  nameH.001
FILENAME IN F2:  nameC.001
NO. OF PENS:  1 (or 2 if have red pen)
NO. OF CONTOUR LEVELS:  4 (1-7 levels)
OUTLINE BOX:  Y
GRID:  N  or  Y
```

7. Enter: **NP** to feed in new paper to plotter or printer