XIV.HMQC and HSQC – 2d Heteronuclear Spectroscopy ("inverse" experiment with ^1H detection)

(20-Jul-01)

A. Discussion

- Two sequences for measuring ^1H-X chemical shift correlations, gHSQC and gHMQC are very similar, and both will work fine for most organic compounds.
- HSQC will give sharper lines, especially for $-CH_2-$ since ^1H–^1H homonuclear coupling in the F1 axis is not suppressed in HMQC. HSQC is more sensitive to pulse width calibrations (has more π-pulses), but HMQC can suffer T_2 sensitivity losses in viscous and high-MW compounds.
- Gradient sequences are typically superior in S/N, especially for long-range HMBC.

B. Critical Parameters

- **pw** – ^1H 90° pulse width at power **tpwr**
- **pwx** – X 90° pulse width at power **pwxlvl**
- **j1xh** – J-coupling for correlations of interest, set to 140Hz for 1-bond HSQC and HMQC experiments (see HMBC for long-range experiments)
- **ni** – fixes the *digital resolution in F1, dres1 = sw1/(2ni)*; because of the large J-coupling in 1-bond experiments, *dres1* need only provide sufficient resolution for ^{13}C chem shift, and is therefore set larger than typical in a COSY experiment. For COSY, *dres1* = 4 Hz/pt, where in HSQC/HMQC *dres1* ≥ 30 Hz/pt is usually sufficient.
- **d1** – best set ≥ $2\times T_1$, but not uncommon to run at ~$1.5\times T_1$
- **sw1, dof** – sets the ^{13}C F1 spectral axis; set the next section as to how to set

C. HMQC Acquisition

- for long HMQC runs, or lossy samples, calibrate the ^{13}C decoupler using a ^{13}C-enriched sample and PWXDEC90 sequence (see section D below)
- tune the probe at ^{13}C then ^1H for your sample
- acquire a standard ^1H spectrum and optimize **sw** using **movesw**
- calibrate **pw90** by arraying **pw**; make certain **d1** is not too small (~$1.5\times T_1$)
- optimize the **gain** for **pw=pw90** and acquire a ^1H high resolution spectrum at the optimized sw
- **mp(3)** ; assuming started in exp1
- **jexp2** and obtain just enough ^{13}C (X cable to observe port) to see the solvent peaks (nt=1 usually)
  - if in D2O, you can rely on **setref** for approximate referencing, or use **decref** if the ^1H spectrum has a good reference
- optimize sw of ^{13}C by estimating where protonated carbons will show for your compound and using **movesw ga**
- **jexp3** and type **gHMQC** or **gHSQC**; enter **2** when asked where ^{13}C parameters are
- check that **pwx, pwxlvl, dmf** and **dpwr** are set correctly
- check **j** and **d1**
- check **ni** and **nt**
- if running **hmqc** instead of gradient sequences, optimize **null** (set **ni** = 1 and array **null ~ T_1**) or use **null=0.7*T1** if you know T1 (especially important for solvent if working in non-deuterated solvent)
- **au**

D. Calibration

- general rule of thumb for these calibrations is:
  - short runs, check proton **pw90**, for **pwx,pwxlvl** use facility numbers
  - if probe problems are suspected, check pw90's of *X* and ^{1}H observe (not decouple)
    - if numbers are close to facility values, probe is likely OK
    - if pw90 is much less than facility value, you are doing something wrong (figure it out! : -)
    - if pw90 is much longer (>1μs) than facility value, find TA or facility staff
  - perform calibrations for overnight or longer runs for HMQC/HSQC experiments
- tune probe at ^{13}C then ^{1}H for ^{13}C enriched sample ($^{13}CH_3I$—Indirect 1—or NaOAc-^{13}C)
- calibrate **pw90** for ^{1}H as normal
  - set **tpwr** to desired setting; see /vnmr/probes/*probe*/*probe* for most current
  - make sure you are on resonance: set cursor on $^{12}CH_3{}^{*}I$ singlet (surrounded by $^{13}CH_3{}^{*}I$, so looks somewhat like a triplet) , then **movetof**
  - set **d1=1 at=1** for Indirect 1 samplpe
  - use 360° pulse for final checks always!!
- once observe **pw90** is obtained, assuming in exp1 then **jexp2**
- obtain ^{13}C in exp2 (move *X* cable to observe port) and place $^{13}C^{*}H_3I$ quartet on resonance (place cursor in middle or quartet and **movetof**)
- **jexp3 mf(1,3) wft;** put ^{13}C on resonance by setting **dof** = ^{13}C tof from above
  - setup decoupler calibration experiment with UW macro **pwxdec90** ; check **tof** and **dof**
  - check **pwxlvl = 58** and find **pwx** where antiphase doublet nulls; this is decoupler hard 90
  - set **pwxlvl** (~46) appropriately for decoupling (probe dependent; see Table on wall or /vnmr/probes/*probe*/*probe*) and find **pwx** where antiphase doublet nulls; set **dpwr = pwxlvl** and **dmf = 1/(pwx*1e-6)**

E. Data Workup and Plotting

- work-up essentially identically to dqcosy (see end of DQCOSY section for phase-sensitive work-up)