Homework 10 Chem 636, Spring 2014

due at the beginning of lab April 15-17 updated 8 Apr 2014 (cgf)

2D NMR: TOCSY and HSQC

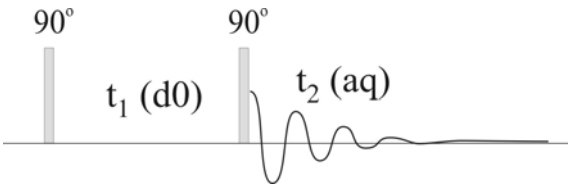
Use Artemis (Av-400) or Callisto (Av-500) for this week's HW.
Likely best to use the same sample as in HW#9 (but a change is OK).

Reading – **TOCSY in Claridge sections 5.7;**
– **HSQC in Claridge sections 6-6.3 (much of this is overly technical)**

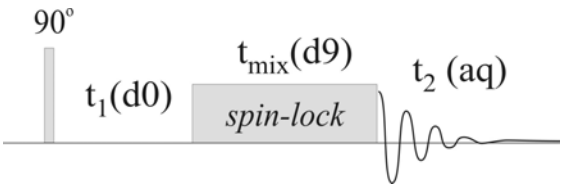
Goals – **Learn about the utility of these two experiments, more technical details about 2D experimental setup, and assignment aids in MNova.**

I. TOCSY: Total Correlation Spectroscopy

COSY experiments provide information about ^{1}H-^{1}H coupling, showing crosspeaks for proton pairs having direct J_{HH} couplings ranging 2.5 to 25 Hz for standard COSY and DQ-COSY, and smaller J_{HH} using (long-range) lr-COSY. TOCSY[1] is the last variant of homonuclear 2D experiments that we'll investigate. The standard COSY is shown below next to TOCSY:



Standard COSY sequence



TOCSY sequence

In TOCSY, the 2nd 90° pulse is replaced with a spin-lock pulse.[2] The spin-lock is *very* interesting (ya, ya, I know; maybe just to me): the spins are "fooled" into thinking that the large static magnetic field has disappeared, and interactions – like coupling and chemical shifts – now depend on the spin-lock strength, typically of about 12 kHz. That's a lot smaller than 400 or 500 MHz. J-couplings do not scale with the field strength, but chemical shifts do. All couplings during a spin-lock are therefore *strong*: $J_{H_AH_B}^{spinlock} \gg \Delta\delta_{AB}^{spinlock}$. The strong coupling enables efficient mixing of all protons in a coupled spin system. The spin system will "stop" at heteronuclear bonds (e.g., an ether or amide bond), and other areas where J_{HH} is small (e.g., aliphatic to aromatic). Magnetization transfers occur faster with larger coupling, so 2- and 3-bond coupling occurs first. But as the mixing time is increased, the magnetization transfers further out.

Acquire a TOCSY spectrum with an 80ms mix time. **D1**=2 should be fine, since TOCSY, like COSY, is fairly robust with respect to fast repetition times. If you are acquiring data on a research compound use a larger **TD1** (256 or 512). Acquiring another spectrum (or two) with shorter mix (e.g., **D9** = 0.03) is often best, and is fairly common.

[1] TOCSY is sometimes called homonuclear Hartmann-Hahn spectroscopy (HOHAHA).

[2] Two primary flavors of spinlock are used, MLEV and DIPSI. Bruker names their TOCSY parameter sets and pulses sequences by these names, and avoids the use of TOCSY. Facility generated parameters sets are named as TOCSY, located in /home/topspin3.1/uwchem/par.

Critical parameters for TOCSY, those that should always be checked, are:

D1 ≥ 1×T_1(longest)

NS ≥ 2 (for the standard experiment; many other TOCSY variants require 8)

TD1 = 128, 256, … up to perhaps 800

D9 = spinlock mixing time; it is common to acquire 2 or 3 spectra with different mix:
- = 0.015 will observe 2-3 bond couplings only (1st shell)
- = 0.03 start to see next shell (i.e., protons 2-3 bonds from 2nd
- = 0.05 2 shells, with some hints of 3
- = 0.08 3 shells, with hints of 4
- ≤ 0.12 120ms is the maximum spinlock that should be used; shows all shells

1☞ Work up the TOCSY spectrum, and compare to the COSY you acquired last week. The spectrum may need a slight amount of phase correction, but don't be surprised if some peaks simply won't phase very well. Crosspeaks as one moves to further shells will get less intense for D9=80ms. Some diagonal peaks may become weak, as magnetization transfers out.

II. HSQC: ^{1}H-^{13}C 1-bond heterocorrelation

You have acquired HSQC spectra in an earlier HW. Here we'll examine a few additional setup issues.

a) Choice of experiment: *Multiplicity-edited* HSQC — analogous to DEPT-135 with –CH_2– inverted from –CH_3 and –CH< moieties — is the most common experiment run for small molecules. As samples get more complex, being comprised of mixtures of compounds or simply by increased molecular weight, partial overlap of two oppositely phased peaks may become a serious concern. In such cases, the DEPT-45 analog — all peaks positive — of the experiment may be better. There are two primary experiments covering these choices:

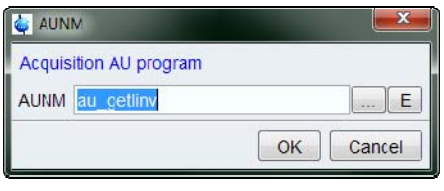
HSQCEDETGPSISP:	edited HSQC, DEPT-135 analog
HSQCETGP:	non-edited HSQC, DEPT-45 analog

b) Manual setting of ^{1}H spectral region: If your sample has large dynamic range in peak intensities — usually arising from protonated solvents, but also from tert-butyl or when trying to identify impurities or other minor constituents in a mixture — the automatic spectra region determination used for any 2D experiment may fail: e.g., the spectral region cuts out the aromatic region, rendering the acquired spectrum useless.

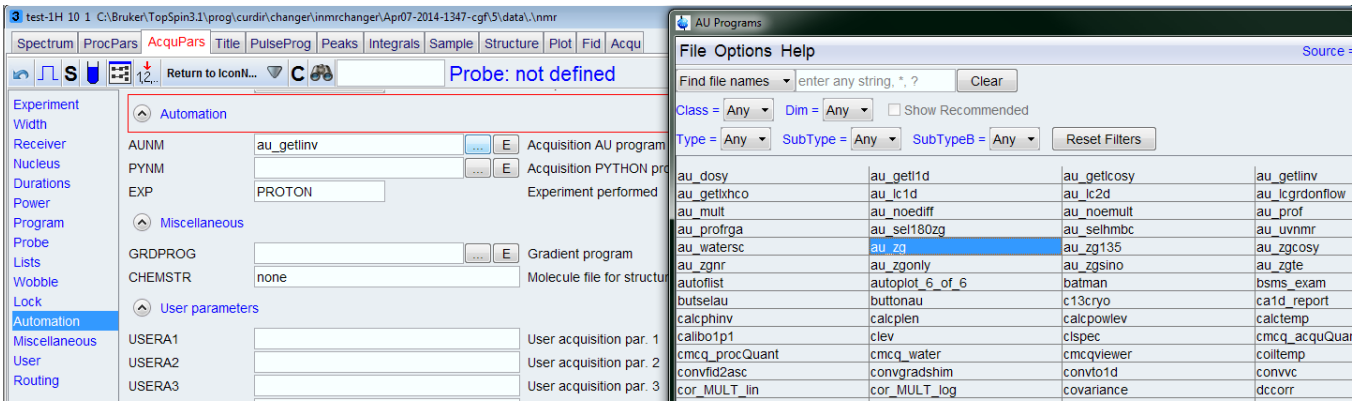
The following procedure for manually setting the spectral region is correct for any composite experiment (experiments having a C, rather than N, prefix) in IconNMR:

i) Go into **Parameters → Edit all Acquisition Parameters** .

ii) When viewing **A**ll parameters, click on Automation in lefthand links; or simpler might be to type **AUNM** in TopSpin, which will open a panel like that on the right. Open the **Au Programs** listing using the … button and then select the following, or type the au name in directly:



Experiment Type	**AUNM** for Automatic SW,O1p	**AUNM** for Manual SW,O1p
HSQC, HMQC, HMBC	au_getlinv	au_zg
all COSYs, TOCSY, NOESY, ROESY	au_getlcosy	au_zgcosy



iii) Change F2 values for **SW** and **O1p** based on the ^{1}H spectrum (see the COSY lab, HW 9, for notes on how best to do this: i.e., expand the spectrum in TopSpin and click).

iv) Check **AQ**, **TD**, and **SI** .

2☞ Choose the experiment that best matches your compound, and acquire a relatively high-resolution (e.g., **TD1** = 400) and good quality (**NS**=2) HSQC. Manually set the ^{1}H spectra region. Make some assignments of your compound based on COSY, TOCSY and HSQC data. Look in particular for entry points in the spectra, some proton and/or carbon that can be assigned with definiteness that show correlations in the COSY/TOCSY spectra. Next week we'll do HMBC. The more assignments made in the HSQC, the better for that.

Plot the TOCSY and HSQC spectra. Provide as many assignments as you can via annotations (or just write on paper if you prefer). Put a ? after tentative assignments. Upload 2 plots as .mnova and .pdf files, and turn in a paper copy if you've hand written the assignments.