2D NMR: TOCSY and HSQC

> **Use Artemis (Av-400) for this week's HW.**
> **Use the same sample as assigned for HW#8.**

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. <u>TOCSY</u>: Total Correlation Spectroscopy

COSY experiments provide information about ^1H-^1H coupling, producing 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**
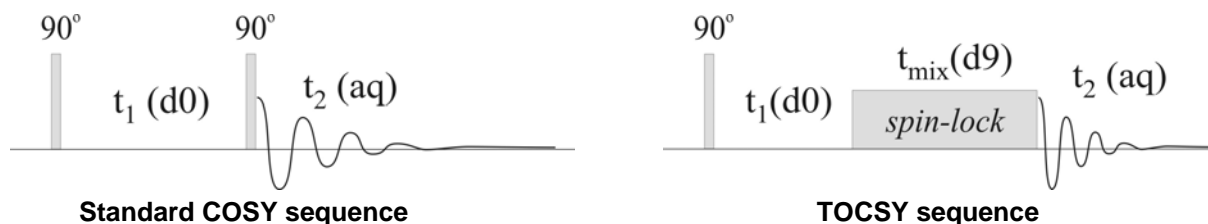
In TOCSY, the 2nd 90° pulse is replaced with a spin-lock pulse.[2] The spin-lock is *very* interesting (ya, ya, I know; maybe just to me): the spins are "fooled" into thinking that the large static magnetic field, B_0, has disappeared. Thus interactions like J-coupling and chemical shifts now depend on the spin-lock strength, which has a size of ~ 12 kHz. That's a <u>lot</u> smaller than 400 or 500 MHz! J-couplings do not scale with the field strength, but chemical shifts do, thus J-couplings during a spin-lock are *large* compared to chemical shift: $J_{H_A H_B}^{spinlock} \gg \Delta\delta_{AB}^{spinlock}$. The spins now experience "strong coupling", and that enables efficient <u>mixing</u> of all protons in a coupled spin system. The mixing of the spin system will "stop" at heteronuclear bonds (e.g., an ether or amide bond), as well as other areas where J_{HH} is small (e.g., aliphatic to aromatic). Mixing, or magnetization transfer, occurs faster with larger coupling, so 2- and 3-bond correlations will show up earlier in a TOCSY spectrum. As the mixing time is increased, the magnetization transfers further out: the magnetization seems to relay out from the starting proton.

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[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 acronym TOCSY (perhaps to avoid the contention that arose when TOCSY gained favor over HOHAHA). Facility generated parameters sets are named as TOCSY, and are located in /home/topspin3.1/uwchem/par.

Acquire two TOCSY 2D spectra with 15 and 80ms mix times.[3] **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** than the default (e.g., 512). Acquiring another spectrum (or two) with additional mix times (e.g., **D9** = 0.05) is not uncommon.

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

      D1 $\geq$ $1 \times T_1$(longest)

      NS $\geq$ 2   (for the standard experiment; other TOCSY variants may require 8)

      TD1 = 128, 256, …   up to perhaps 1600

      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
          $\leq$ 0.12   120ms is the maximum spinlock that should be used; shows all shells

1☞ Work up the TOCSY spectra, and compare them to the COSYs you acquired last week. The spectra may need slight amounts 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. <u>HSQC</u>: ^{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) <u>Choice of experiment:</u> *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 be a serious concern. In such cases, the DEPT-45 analog — all peaks positive — of HSQC 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) <u>Manual setting of ^{1}H spectral region:</u> As discussed in the previous HW, 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:

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[3] As always, look 1st in the *uwchem* folder for experiments. There you will find Tocsy2d.UW. Use that experiment/parameter set. [Oddly, Bruker does not provide a parameter set that uses the pulse sequence chosen here — mlevesgpph — which is the newest and best of their mlev-flavor TOCSY pulse sequences.

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:
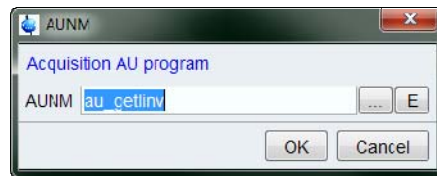
Experiment Type	**AUNM** for Automatic SW,O1p	**AUNM** for Manual SW,O1p
HSQC, HMQC, HMBC	au_getlinv	au_zg
all COSYs, TOCSY, NOESY, ROESY	au_getlcosy	au_zgcosy
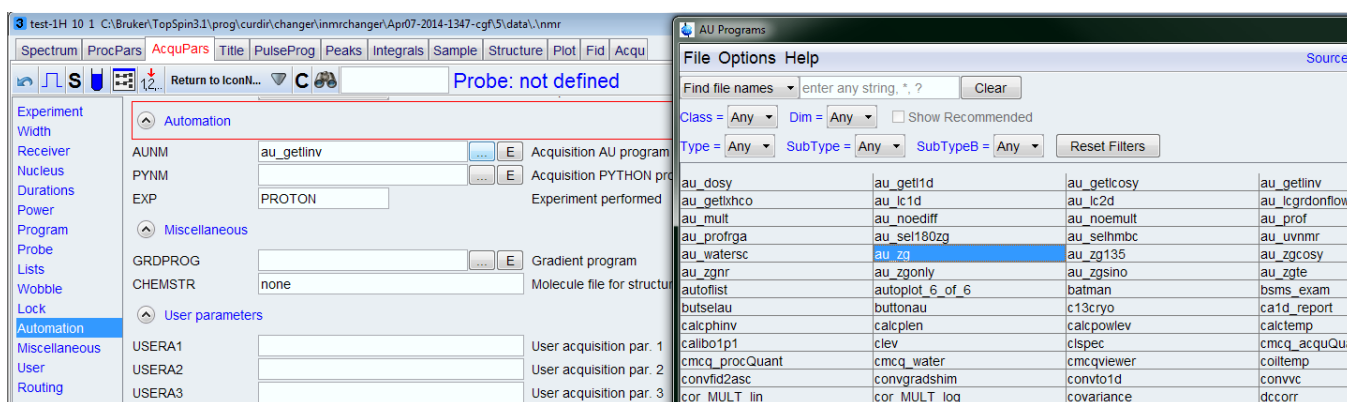
iii) Change F2 values for **SW** and **O1p** based on the ^{1}H spectrum (see the COSY lab, HW 8, for notes on how best to do this: i.e., expand the spectrum in TopSpin and click ⤓).

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 into the spectra, some proton and/or carbon that can be assigned with definiteness that show correlations in the COSY/TOCSY/HSQC spectra. Next week we'll do HMBC. The more assignments made in the HSQC, the better for that.

III. Data Assignments Using MNova

MNova setup (1st-time only)

A. The following settings are recommended for multiplet analysis in MNova:

       Peak Pick   → Options…   → Method = GSD

       Integration   → Options…   → Calculation Method = Sum

       Multiplet Analysis → Options…   → Calculation Method = Peaks

There are other options within these sets, and some are worth looking at. Most are pretty clear as to their meaning, so we won't spend more time on them here. The choice of doing Integrations as Sums rather than Peaks, however, is one that deserves more discussion. We'll discuss this in lecture later (but you can think about what that change might do).

B. In this HW, use 🎵 Multiplet Analysis in the most straightforward manner: attempt to not play around with it too much (unless you feel compelled to… ☺). We'll work with this tool more in the next HW. For now, the following recommendations should be sufficient to do some assignments:

1. ^{1}H 1D spectra: do Multiplet Analysis first (do not do integrals or peak picks), then the assignments

2. ^{13}C 1D spectra: make the assignments directly to the singlet peaks (if you have X-nucleus coupling, then do a Multiplet Analysis 1st, similar to 1H 1D spectra)

3. 2D spectra: make assignments directly to the diagonal or crosspeaks; it is often best to clean these up later by making the assignment in the respective 1D spectra (if they are available)

C. MNova provides useful tools to assist in making assignments based on sets of 1D and 2D NMR data, involving multiplet analysis 🎵 , as well as adding a compound structure to the MNova document and making assignments directly to it.

a) Use ChemDraw or ChemSketch to copy-and-paste a compound structure into your ^{1}H 1D spectrum; CNTL-C and CNTL-V work fine for this.

Note: I (cgf) have been unable to get numbering in ChemDraw to stay as done in MNova, whereas the numbering in ChemSketch is kept correct. Pasting the ChemDraw compound into ChemSketch and from there copy-and-paste into MNova will keep the original numbering from ChemDraw. Yes, this is annoying, especially when ChemSketch garps up the looks of the ChemDraw structure…. If any of you figures this out, let me know.

Note: It is difficult to not have the compound structure get in the way of spectral features when pasted into spectra. A way around this is to use **View → Tables → Compounds** to display the structure, as shown in the upper portion of the left panel in the figure. You can now delete the structure in the spectrum by clicking on the upper handlebar. An unresolved problem, however, is how to get that spectrum with assignments back into the spectra for printing. Sigh… If you like the **View → Tables → Compounds** , keep one copy in one spectrum (e.g., the ^{1}H 1D). It will have all the assignments, so it can be copied later into other spectra for printing.
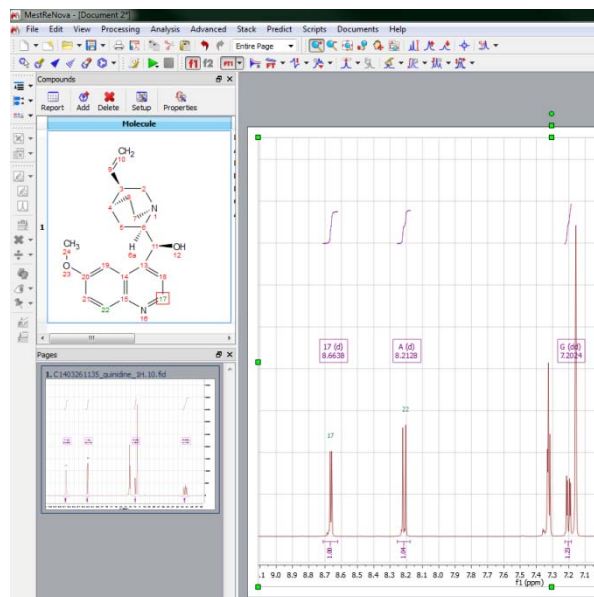
If you prefer having the compound in all spectra, copy and paste it into each new spectrum as you add them.

b) It is best to use multiplet assignments 🎵 in ^{1}H 1D spectra. [This is not required; one can make assignments based on peak picks; holding the SHIFT key down while doing this allows selection of a "peak" in the center of an overlapping doublet, as one example.]

→ Enter assignments mode by using the shortcut key **A**, or **Analysis → Assignments → Manual Assignment** . You will see a naphthalene ring attached to the mouse pointer (hard to capture, here's best I could get: 🔺). Click on an atom to be assigned, then on the multiplet box it is to be assigned to.

   Note: If you have selected a compound that is pasted in a spectrum, typing A will replace the atom label with A. Undo, click on the background, then A again to get into assignment mode.

c) Assignments of carbons in a ^{13}C 1D spectrum are best done with simple peak picks. You don't have to do peak picks first; when in Assignment mode, the cursor will automatically choose peaks as you hover over them.

   Note: ^{13}C chemical shift predictions can be quite useful for making (tentative) assignments. These should be confirmed with prior (literature) knowledge when available, but data is always best, made using spectra such as COSY and HSQC/HMBC.

d) COSY spectra are assigned by clicking the atom, then the center of diagonal peak to be assigned. Use only the top line for the assignment; i.e., uncheck the "Assign f2" box (the chemical shifts will not exactly match between the two). Crosspeaks can also be used, but be careful to uncheck the axis that doesn't match the assignment.

   Recommended: Always add the 1D traces that are available to your 2D spectra when making assignments. If you don't have a ^{13}C 1D (which you should for this HW unless you're using a research compound), that's ok, and leave the f1 trace undisplayed.

e) Assignments of HSQC crosspeaks require only clicking on the atom to be assigned, and then clicking in the center of the crosspeak. If the ^{1}H or ^{13}C is already assigned, remove it from the selection. In the example on the right, the ^{1}H assignment had been made already from the 1D spectrum, and is therefore "Assign f2" has been deselected. Only the ^{13}C chemical shift will be added.
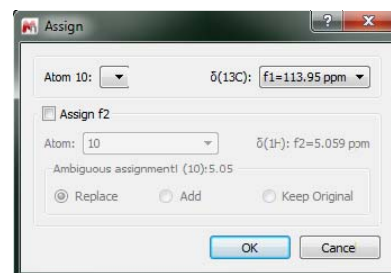
   Note: HSQC provides the simplest method for identifying non-equivalent CH$_2$ protons. In these cases choose the down-arrow box for Atom: above under Assign f2 and select 10', or 10". You will see other possibilities, e.g., ax or eq, and should make the choice that makes sense.

f) Assignments from HMBC crosspeaks will be similar to HSQC. It is a common method for determining quaternary carbons chemical shifts, and for confirming or making other tentative or unknown assignments. We'll go into more detail next HW using this experiment.

   Make a reasonable number of assignments using the data sets you have obtained: ^{1}H 1D, ^{13}C 1D, COSY, HSQC, and TOCSY. Save the assignments as a .mnova file and upload.

**Plot the TOCSY and HSQC spectra. Provide at least a few assignments via the assignments tool in MNova. Put a ? after tentative assignments. Upload 3 plots as .mnova and .pdf files, and turn in a paper copy if you've hand written the assignments.**