Week 5

Measuring Spin Effects

5.1 NMR Relaxation

3/4:

- While we're acquiring our fid, neighboring protons' magnetic fields interact with each other.
  - While we're RF pulsing, the instrument's magnetism is far stronger. But when we stop RF pulsing and collect, the protons can interact with each other.
  - We want to be at equilbrium in the z-axis every time we pulse the system.
  - Thus, relaxation time must be $\geq t_1$.
    - If the acquisition time and relaxation delay do not add up to the right amount, the spins could be upside down when we pulse, and then the signals (positive and negative) will add to 0.
- t_1 effect: Spin-lattice relaxation, which is in the direction of the magnetic field.
- t_2 effect: Spin-spin relaxation, which is perpendicular to the direction of the magnetic field.
  - It is t_2 relaxation causes the fid to decay away!
  - If our spins were coherent forever (if they stayed knocked over forever), we would get a constant signal (instead of a decayed one) when we turn the RF off.
  - t_2 value: The reciprocal of the FWHM (width of a peak at the middle) in hertz. Hence, shorter t_2's mean broader lines.
    - Acquisition time should be double the t_2.
    - Small molecules have t_2's in the range of a quarter of a second to 1 second.
    - t_2's much smaller for bigger molecules (e.g., polymers, proteins, etc.)
- Analogous protons on different molecules are in slightly different magnetic environments due to variations in the neighboring protons' magnetism.
- The longer the FID, the sharper the signal.
- We more often use 30° or 45° pulses in ^{1}H NMR because it's much faster for the signal to recover to equilibrium.
  - However, we do not get maximal signal in these cases.
  - By trigonometry, 30° gives us 50% of the maximum signal and 45° gives us 71% of the signal, which is a good compromise.
  - Note that since ^{13}C t_1's are longer, we usually only go to 30°.
  - Lengthening $d1$ (the relaxation delay) from a tenth of a second to 10 seconds causes almost no difference in sensitivity/resolution. For some protons, it does, though.

- DMSO-d6 samples tend to relax relatively fast compared to $CDCl_3$ or MeOD samples.
- Goes over spin echos as t_2-filtering techniques.
- Using NMR to detect exchange broadening.
- Good drug molecules have "compositional or chemical heterogeneity." I.e., they can be in multiple ionization states, labile protons, etc. This tends to increase the probability that it will go from the source all the way that it should.

5.2 Band-Selective Experiments, TOCSY, IR, NOE, and ROE

3/6:

- Announcements.
  - More time provided on PSet 3.
    - Additional experiments also available in the Dropbox now!
    - There is a ^{1}H-^{15}N HMBC, and a few band-specific HSQC/HMBC's.
- 2D experiments.
  - Almost always proton or fluorine on the horizontal, and carbon or nitrogen on the vertical. We pulse the proton/fluorine, and observe carbon/nitrogen correlations.
  - Highest frequency nucleus is the observed nucleus, always: Because that's where the majority of the signal is.
  - We modulate things in the **direct** (horizontal) dimension with things that we do in the **indirect** (vertical) dimension.
  - We put filters outside of the spectral width, so we don't see signal there. This helps stop noise from regions with no signal.
    - Always make sure your proton spectral range encompasses all signals you're looking for!
    - Any proton/carbon pairs that show up within the two limits will show up fine.
    - Any proton/carbon pairs with carbon outside the limits will show up folded back in the carbon dimension: SW stops at 160 ppm and peak at 165 ppm means reflection at 155 ppm or 5 ppm.
    - So you're balancing resolution (because wider SW means lower resolution) and the ability to interpret what you see.
    - If something appears in the indirect dimension but doesn't line up with any carbons, it's probably folded back in.
  - That being said, symmetric stuff around the bottom and top is just artifacts — not folded in stuff.
  - What about two carbons very close together that we wanna tell the connections apart?
    - PSet 3 carbons at 104.6 and 104.3, for instance. You can take a guess and line it up.
    - Or, you can really increase the resolution of the HMBC to make the cross-peak in the carbon dimension much sharper. You do this simply by making the SW very small. But how do you get rid of the folding? Do a **band-selective** HMBC.
- **Band-selective** (HMBC): Instead of using regular, **hard** pulses with wide excitation bandwidths (e.g., -30 through 300), add in a selective pulse last. This only excites carbons in a 10 ppm bandwidth, so that it doesn't matter what's happened before because the only carbons that survive are ±5 around the center.
  - How do we set up one of these on the DCIF instruments?
  - Walt did HSQC/HMBC pairs for both places with close-together carbons. These experiments don't take very long to do.

  - Can you do this in the proton dimension, too?

- TOCSY (80 ms) experiment.
  - COSY shows nearest neighbors; TOCSY allows you to get to subsequent protons connected in a chain.
  - This shows not only not just vicinal proton couplings, but protons 2, 3, and even 4 carbons away.
  - This makes things more complicated, but can be helpful for confirmation.
  - Useful for connecting protons through overlapping peaks! If two carbons have the same chemical shift but differing neighbors, we can TOCSY the neighbors directly instead of having to go through the COSY.
  - When you make the mixing period less (20 ms or 10 ms), you get fewer transfers of magnetization and see nearer neighbors only.

- t_1 **inversion recovery** experiment.
  - Making the time longer allows the signals to relax more.
  - The signal will go from completely inverted, along an exponential to regular.
  - Can estimate t_1 with a 1D, a certain relaxation delay, and then double the relaxation. Keep doubling until you see that you're getting most of your magnetization back.
  - Can do the same thing with a series of individual t_2 spin echo experiments: This is the **CPMG experiment**.

- **Nuclear overhauser effect**: The interaction of individual spins directly with each other through space. *Also known as* **NOE**.
  - Maybe you already know what the assignments are, but you want to know something about the conformation of the molecule.
  - Standard question for adenosine: Is the nucleotide base oriented with the 5-membered ring pointing left, or the 6-membered ring.
  - Strength of the interaction is proportional to r^{-6}.
  - 2 Å distance is very strong NOE; 4 Å distance is imperceptible.
    - Very strong measure of distance and conformation, but at a local level.
  - **Correlation time** τ_0 nominally tells us about molecular motion in solution.
    - Multiply by ω_0, the frequency of the nucleus in the spectrometer (e.g., 500 MHz for a proton on a 500).
    - Takeaway: If you're molecule is in the middle of this unfortuante regime, your NOE is going to be zero. You have to look out for that!
    - Positive NOE: Saturate one nucleus, transfer its magnetization to another nucleus, and then revert and the other one flips.
    - Example: Saturating adenosine's $1'$ proton correlates it to the $2'$ proton and base 5-membered ring's proton. The sample used was extremely concentrated.
    - When interpreting an NOE experiment, focus on the strong signals, not the weak; the weak ones are either incomplete subtraction or not our primary determination. Don't anticipate a bump 3-4 carbons away and then see a tiny one and say, "look, an NOE!"
    - Comparing NOEs on both diastereomers is a better idea than just interpreting one of them.

- **ROE**: The NOE in a rotating frame.
  - Difference: The ROE doesn't go to zero! Similar theoretical effect for small molecules, worse for very big, much better in the middle.
  - Now, magnetization transfer is the same sign as the peak that we've saturated.

  - This can give a rough sense of molecular size, just by looking at the sign of the NOE.
  - Distinguish monomer from polymer using NOEs!!

- Final project: Think about something relevant to our research, and do experiments to characterize it.
  - More on this next Tuesday.

- 3 deliverables between now and the end of the course: PSet 4, final project, and review of the class.