Week 7

Bridge to 5.55

7.1 Solid-State NMR

3/18:

- Do NOESY's always take like 3h30?
  - Depends on how much signal you want!
  - If you have very little compound, sure. But if you've got 20 mg of a small molecule, you can get a NOESY in 20 minutes.
  - So perhaps even less time for my 300 mg sample!
  - You can decrease the number of scans for the direct dimension from 32 down to 4, 2, or 1.
    - Will get progressively messier, but if you have a ton of signal, it may not matter.
  - You can also get rid of the phase cycling.
  - You can also decrease the number of **increments** in the indirect dimension to balance time vs. resolution.
    - Can cut down increments from 256 to 128 or something, too.
- PSet 4: ROE/NOE similar?
  - Yep! No worries.
- PSet 4: No absolute stereochemistry without chiral resolving agent?
  - Yep!
  - Just explain the relative stereochemistry.
- PSet 4: Proton-carbon couplings to interpret?
  - Hard to do because it's a very strained system, so things go in weird ways you might not expect.
  - Some explanation to that effect is good.
- Do you have to run 1D experiments ahead of 2D?
  - You never have to.
  - Sometimes, 1D experiments can capture things that 2D won't. For example, if you're taking a ^{1}H-^{13}C HSQC, the 1D projection of the 2D dots will not encompass any X–H bonds (e.g., exchangeable hydroxyl/amine protons). Thus, if you don't want to miss anything, it might make sense to take a 1D, too.
  - But it doesn't help with phasing or anything.
- ^{19}F-^{13}C HSQC didn't work?

  - Check the chemical shift ranges, check the default parameters.
  - Let Walt know if I still can't make it work.

- This week:
  - Miscellaneous experiments, transitioning into the biological structural assignment course (5.55).
  - Solids.

- Show and tell: A 3.2 mm solid-state rotor.
  - There's a couple of caps, too. These caps go into the rotor, and that's how we spin it.
  - The fins on the rotor accept a stream of nitrogen and get spun very smoothly.

- Rotors help in solid-state NMR, because you've got to do **magic-angle spinning** at 54.7°.
  - This angle solves the equation
$$3\cos^2\theta - 1 = 0$$
  - This averages out **dipolar couplings**.
  - Smaller and smaller rotors can be spun faster and faster. When you get to spinning at 32 kHz, you can just take a normal proton spectrum as if you were in liquid. Requires a 0.6-0.7 mm rotor; trying to cap this is like trying to cap a grain of sand.
  - First ultra-small rotors were developed on Albany St. by Robert Guy Griffin!

- **Dipolar coupling**: Two spins that are close enough together to interact.
  - This is an effect that is significant in a solid in a way that it's not in a liquid. Tumbling and diffusion in the liquid phase naturally decouples dipolar interactions.

- Solid-state NMR.
  - Side-bands occur at the rotor frequency (like satellites).
  - Change the spinning speed by a few kilohertz and watch the side bands move to determine what's real and what's fake.
  - You take solid-state NMR because you've got a polymer or something you can't get to go into solution, and you have to get data on it somehow.
  - In the solid state, each molecule has a different orientation with respect to the magnetic field; there is significant *anisotropy*.

- Cross-polarization.
  - Analogous to carbon-proton NOEs.
  - Transfers magnetization from a sensitive nucleus to an insensitive nucleus.
  - Occurs best when you hit the **Hartman-Hahn match**.
  - This means tht ^{13}C is much more useful than ^{1}H for solids.

- **Hartman-Hahn match**: The ratio of the gyromagnetic ratios...

- Solvent-swollen gels can be spun at lower speeds (≈ 5 kHz) to get spectra.

- Another big thing in solid-state NMR is **dynamic nuclear polarization**.
  - Can amplify signals by factors of 50-100 instead of the 2-3 you get with cross polarization.
  - Instead of transferring magnetization from one nuclear spin to another nuclear spin, you transfer it from an electron to a nuclear spin.

- Switching gears to special topics in biological NMR.

- Key things to keep in mind.
  - Biopolymers are polymers, but they're **heteropolymers** made of a small number of building blocks.
  - The building blocks are known.
  - Higher-order structure is important; we care less about connectivity and more about this.
- Proton chemical shifts and coupling patterns are often enough to make amino-acid assignments.
  - Intra-residue COSY/TOCSY connectivities extend from the backbone NH through the sidechain.
  - Sequential assignments require NOESY connectivities between adjacent amino acids.
    - Be careful with folded structures! Nearest neighbor may not be next in sequence.
- Larger proteins require uniform ^{13}C and ^{15}N labeling.
  - 3D experiments (HNCO, HNCA, HNCOCA) trace the backbone, and CBCACO-type experiments extend from the backbone to the side-chain.
- Amide protons need water in order to be seen.
  - You often have to carefully modulate the pH.

7.2 Techniques for Biomolecules

3/20:

- NMR dynamics.
  - Hydrogen bonding can be probed with H/D exchange or saturation transfer with water.
  - Structures near to solvent tend to be less stable than internal structures.
  - Thus, NMR peaks will change in magnitude over time differentially based on where they are in the molecule; this can give us additional information!
- **EXSY** (experiment): Analogous to an NOE experiment.
- T_1 vs. $T_{1\rho}$.
  - Useful for properties of solids.
  - Some solids have properties on the order of the Larmor frequency (measured by T_1), but others have much slower ones (measured by $T_{1\rho}$).
  - Used heavily in the characterization of gel polymers.
- NMR assays.
  - Typically used for measuring binding.
  - Solvent suppression is often necessary. Deuterated solvents are often too expensive, and some (like D_2O) may exchange in or alter the kinetics (e.g., inadvertent KIEs).
  - Certain signals from a ligand will broaden out as you get binding.
  - You can get a qualitative measure of binding (e.g., "I have tight binding"), but you can't quantitate it because concentration is too low.
    - Medium binding or loose binding can be quantitated with NMR.
- Saturation transfer difference.
  - You can saturate your macromolecule, which will also saturate the bound ligand. However, the unbound ligand will be left untouched.