Week 6

Molecular Dynamics

6.1 Chemical Exchange and DOSY

3/11:

- Lecture outline.
  - Chemical exchange.
  - PFGs and DOSY.
  - PSet 4.
  - Final project.
- PSet 4: ROESY and NOESY for Aflatoxin B1.
  - Understand why there are three peaks in the ROESY.
  - What do they mean? Where do they come from? Should there be others?
  - This is a fairly simple, 400 ms ROESY.
  - NOESY does not look as nice.
    - But looks better after phase and baseline spectrum.
    - You can also adjust the density/level of contours. This makes peaks more defined.
- Make sure to properly phase and baseline 2D spectra, too!
  - How do you do this??
  - There are equivalents in MNova.
  - Capture a place in the spectrum in Interactive Phase Correction, look at the columns.
  - Zero-order phase correction at the pivot, first-order phase correction at the sides.
  - *Automatic* phase and baseline correction can be good, too.
- Chemical exchange and NMR timescales in *N*,*N*-dimethylacetamide (DMA).
  - Methyls are in two different chemical environments at room temperature, but they merge into one peaks at higher temperatures. It's like a high-temperature equivalent of cyclohexane ring flipping at low temperatures!
  - Proton peaks get closer together and broader at higher temperatures, before coalescing. You have a point at which the exchange rate (rotation around the bond) is basically equal to the chemical shift difference (in hertz).
  - The difference between the two signals in hertz tells you the exchange rate!
  - Glenn Facey (NMR tech at University of Ottawa) has some really good examples in his blog.
  - Two broad peaks may be different compounds, or **rotamers**; the typical test is heating up!

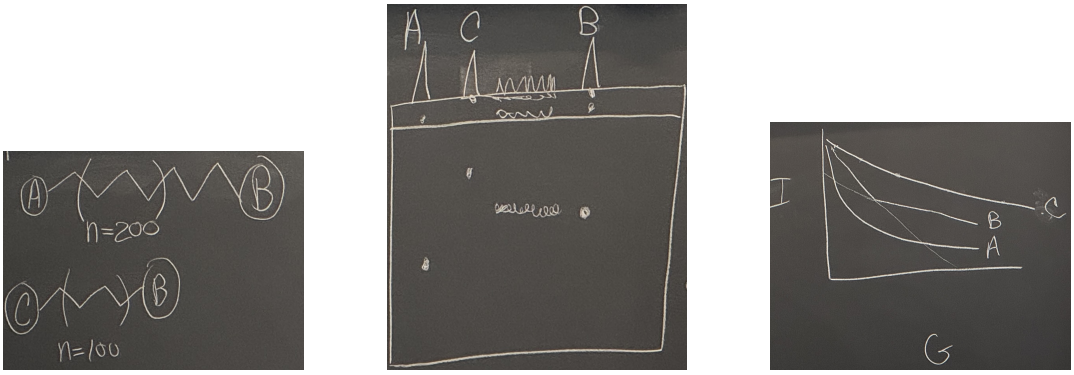
  - Coalescence happens for carbon at a higher temperature than for protons! Sometimes, your signal just goes away/disappears into the background.

- **Rotamer**: A molecule that has two forms differentiated by rotation about a chemical bond.
- If the populations are equal, the final average will be equidistant between the two; if the populations are unequal, the final average will be weighted.
- Examples of chemical exchange.
  - Often tertiary amides (restricted bond rotation).
  - Ring flipping.
  - Tautomerization (e.g., 6π electrocyclization in cyclohepta-1,3,5-trienes).
  - Center inversion (i.e., nitrogens becoming chiral at low temperatures).
  - Rearrangement reactions.
  - **Fluxionality**.
- Protonated tertiary nitrogens (with TFA vapor) may be useful for rotamers??
- **Pulsed field gradient**: Allow for the precies introduction of a linear field gradient across the sample. *Also known as* **PFG**.
  - Using molecular tumbling to figure out how big molecules are.
  - Your proton gets super spread out, e.g., over 200 ppm.
  - Instead of a Fourier transform, you apply a **Laplace transform** (or **Bayesian processing**) to figure out diffusion time and correlate that to molecular weight.
  - To correlate diffusion coefficient to weight, you have to understand the viscosity of the solvent, temperature, fluid effects, etc.
  - May need to convert data from 2D to a 1D stack, rephase, and rebaseline.
  - You can make MNova do a Bayesian transform.
  - Mixes of multiple molecules will give you two different diffusion coefficients!
    - This could help with identifying if my unknown sample in lab is multi-component or just one molecule!
    - I could also TLC/chromatograph the sample.
- PSet 4 will be assigned today, and we'll have a week to do it.
- The final project.
  - Propose a particular chemical synthesis that we're interested in, ask what I'd like to see come out at the other end, and how could I use the NMR experiments in class to distinguish between products?
- Chemical shift prediction (^{13}C, ^{15}N can guide our thought, but it shouldn't determine our assignments).
  - Aflotoxin's precisely-defined stereochemistry across the bridged ring will come in.
- PSet 3.
  - The carbons I couldn't identify are all exchange-broadened, in the 150-160 ppm.
  - Should have HMBCs to nearby protons.

6.2 Suppressing Unwanted Signals

3/13:

- DOSY?
  - Can be run in automation with Walt's approximate parameters on 500 and 600.
  - If I want to learn how to optimize the parameters for DOSY, ask Bridget (the DOSY expert) to schedule a training time. We'll optimize it on 501 (manual), and then I can run the optimized parameters on any automation system.
  - DOSY is not good at separating peaks "vertically," i.e., different compounds that have something with the same chemical shift. Though there are ways around this.
  - Yuzhe and Eddy also are pretty good at DOSY, per Yuzhe.
- Lecture outline.
  - Filtering experiments.
  - Composite experiments.
- Everything that's been talked about in this class can be done in an automated fashion, as long as we know which parameters to dial in. Everythign that's been talked about can also be done manually, again as long as we know what to dial in.
- More on DOSY.



(a) Species in mixture. (b) DOSY spectrum. (c) Gradient plot.

Figure 6.1: DOSY with overlapping peaks.

  - Imagine you have two different polymer species (100 repeat units, 200 repeat units; A-B end groups vs. C-B end groups).
  - All chemical shift is summed along the chemical shift axis (^{1}H NMR spectrum of A-B plus C-B).
  - Signals for the middle stuff goes to a weighted average, B goes to weighted average, C is up top, and A is at bottom.
  - Can also anaylze the data as a plot of integrated intensity vs. gradient strength, which is also the way the experiment is actually done.
    - Bigger molecule's intensity decays more rapidly at higher gradients.
    - For B, one part maps with A and one with C, so you can do careful analysis and better estimate what the middle signals are telling you.
    - You need to do careful analysis in MNova and learn from Bridget.
  - To reiterate: There's a sense that DOSY is *great* for MW measurement. But in reality, DOSY is just *good* for *estimating* molecular weight; really getting accuracy requires mass spec.

- Spin echo.
  - Very good for NMR filtering and separating signals from each other.
  - Let all the noise from the receiver pulse die away, then do a 180° pulse and get your signal cleanly.
  - 1-2 Nobel prizes in the 1950s for developing this idea.
- Filtering experiments: CPMG.
  - Instead of just one spin echo, you can use two or more.
  - The 600's probe has a big carbon background, but using a double echo can filter out the broad part.
  - We can see the background in PSet 4, experiment 12.
    - Related to 1012, and subtraction.
    - Double echo carbon and simple carbon.
- Presaturation.
  - Sometimes, you can saturate the water signal in MeOD, because if exchange is fast enough, this will saturate the MeOH protons, too, when exchanged.
- Excitation sculpting.
  - zgesgp on the NMR systems.
  - If it's really important to your advisor (e.g., Mo) to suppress the $CDCl_3$ residual signal when you publish the spectrum, do something very gentle like this.
- "Wet" experiment.
  - Here, you can look at solutes in LC-NMR.
  - Pretty good at preserving the signals overlapped with the solvent peak.
- VNC Viewer allows you to remote into the spectrometers.
- HSQC correlates protons to carbons, but wouldn't it be great if we could attach that proton to other protons, too? This is the HSQC-TOCSY.
  - This connects a carbon to its proton(s), and also some nearby protons.
  - This helps with proton chemical shift overlaps, which a real TOCSY can't help with much.
  - This vs. HMBC?
    - Can give different peaks from HMBC.
    - In a perfect world, where all coupling constants are big enough to measure, they give the same data.
    - Doesn't run much faster than an HMBC.
  - You can also do HSQC-NOESY's, etc.
  - How do you run these in the DCIF??
- HSQC, no decoupling.
  - Everything is centered on the HSQC peaks and split by the $^1J_{CH}$ coupling constant.
- HSQC-DOSY.
  - Overlapping proton chemical shift, but non-overlapping carbon chemical shift.