5.46 (NMR Spectroscopy and Organic Structure Determination) Notes

Steven Labalme

March 12, 2025

Weeks

List of Figures

Week 1

1D NMR Principles and Practices

1.1 Underlying Principles of NMR

2/4:

- Philosophy: NMR is a complicated and useful set of tool for chemists.
- Background on Walt.
  - Training: Masters, PhD, Postdoc, and 5 jobs in NMR.
  - 20 years industry experience, 10 years academia experience, some small company experience.
  - Considers himself somewhere in the middle between knowing nothing and everything about NMR.
  - Experience in natural products discovery, drug discovery, biological NMR, etc.
- Goal of the class: Distill what's most important for us to know.
- Announcements.
  - Syllabus now posted on Canvas!
  - Everything important will be posted on Canvas.
  - MestReNova is what we should use; TopSpin is what Walt is more comfortable with.
  - Slides posted to Canvas at the end of the day.
- This week: The fundamentals.
  - Chemical shift, coupling constants, correlations (new), NMR relaxation
- The NMR periodic table.
  - ^{1}H has almost 100% spin 1/2.
  - ^{12}C is 99% abundant and has spin 0.
    - Thus, we can only do NMR with ^{13}C.
- The resonant frequency depends on an absolutely fundamental physical property called the **gyromagnetic ratio** (γ).
  - Highest gyromagnetic ratio is tritium (^{3}H), then fluorine, then a whole bunch, then phosphorus, carbon, nitrogen (with a bunch in between these three as well).
- Higher magnetic field gives more signal.
  - But 1/4 as much for ^{13}C as for ^{1}H, because γ for ^{13}C is 1/4 what it is for ^{1}H.
- The highest field NMR systems commercially available are at 1.2 GHz.
  - Walt will typically only go up to 600 MHz in this class, corresponding to a 14.1 T magnet.

- Range of chemical shifts.

Figure 1.1: Chemical shift ranges of common nuclei.

  - The range of chemical signals we'll see is tiny, though; only about 6000 Hz if we're talking about a 10 ppm window.
  - Different nuclei appear in different windows and with different ranges (think of how carbon is 0-200 ppm vs. proton -5-15!!).
  - Note that the ranges in Figure 1.1 are to scale relative to each other, but have been scaled up absolutely by 10 times.

- All atoms' spins are active as soon as we magnetize the sample in the magnet bore. Differentiating between them is now an electronics problem.

- α- and β-D-glucose's anomeric protons have significantly different chemical shifts (4.6 ppm vs. 5.1 ppm, roughly).

- Oxygen is virtually all spin 0 ^{16}O, but protons will couple to each other and to ^{13}C (giving carbon satellites).

  - 1% of the time, the proton is coupled to ^{13}C, and gets massively split.
  - All couplings exist; it's just a question of whether we can see them!
    - 170 Hz coupling for the 1-bond carbon-to-proton coupling.
    - 2-bond connection is then expected to be much smaller, maybe 25-30 Hz.
  - Protons are present in much higher concentration, though, so we see their splitting much more (but it's also smaller because they're farther away!). This is why vicinal protons couple in 4-8 Hz instead of 200 Hz.

- Sergei: Why no coupling to the alcohol protons?

  - Because the sample is in D_2O, we get exchange everywhere to OD.
  - Since deuterium is spin 1, it should split the spin 1/2 nucleus into a triplet. But it also has 1/6 the gyromagnetic ratio. Additionally, fast exchange prevents any meaningful coupling from developing.
  - Dissolving the sample in (very dry) DMSO-d_6 will *not* lead to proton exchange, and we *can* observe the couplings to the hydroxyl protons!

- Equations.

  - 5/2 exponent for gyromagnetic ratio means it *really* matters for sensitivity.

- 600 MHz denotes the resonance for protons at the set magnetic field.

  - Carbon would be at 150 MHz in this case (because 1/4 gyromagnetic ratio)!
  - 140-150 A of current in the magnetic.

- A 4 Hz coupling is on the order of parts per billion, so to discern it, we need parts per billion homogeneity in the magnetic field. This is why we need shimming.

  - Shimming is done with additional coils that impart additional magnetism to parts of the sample.

  - Shimming is done for all of the few dozen coils every once in a while, and then with some of the coils for each particular sample.
  - To shim, you measure the deuterium lock signal and how broad or narrow/high the peak is and then you fiddle with the coils!
  - All you really have to look at is the height because the area is the same, so the height of the lock signal correlates to how good the shimming is.
  - Today, we do **radian shimming**, which tells us how to change the current in the coils to make the shimming better.
  - Shim coils can only be adjusted so far; if there's no sample below the coil, the shimming likely can't compensate enough to get a good spectrum.

- Administrivia.
  - There will be some kind of group project.
  - Final project is for us to use our skills to do something useful.
  - Write up our PSets independently, but we can work together on them.

1.2 Recording NMR Data

2/6:

- Deuterated solvents are used both to remove the proton background *and* for lock.
- If you run a sample automatically, everything we do in the next 20 minutes is gonna be automated.
- Locking.
  - If your sample doesn't lock in automation, the sample will fail.
  - If you've got 50:50 $CDCl_3$ to MeOD, the system may or may not lock on the chloroform signal, specifically.
  - The spectrometer is sweeping resonant frequencies across a relatively small range for deuterium.
  - x-axis is frequency, y-axis is intensity; perhaps an FID??
  - When lock is on, we're picking a deuterium frequency. If DMSO-d6 resonates at 2.49 ppm, we relate everything else back to that.
- Shimming.
  - We don't use R_f pulses, but rather magnetic gradient pulses.
  - A constant gradient across the measured window will give a broad line because samples at one part will go at one frequency, and samples at another part will go at a different frequency.
  - Various currents for various gradients that you add together properly can be added together, like Fourier analysis! Creating a straight line as the sum of nonstraight lines!
  - There's error in the machine picking the deuterium sample exactly right.
    - This is what makes the machine say $CHCl_3$ is at 7.19-7.28 ppm.
    - There's a difference between robustness and precision; the machine probably loses some precision for the sake of robustness.
- Tuning.
  - Looking at the response of the entire R_f system.
  - Is that response maximum at the frequency at which I'm looking?
  - You need to tune the system to your sample, because otherwise, your sample's response will be much weaker.

- Phasing.
  - Maximizing the real and imaginary components.
- The FID.
  - The FID goes down due to a **relaxation effect** (t_2) that we'll discuss more later.
  - Exponential multiplication of 0.5 Hz, i.e., (reciprocal) 2 s.
  - Hertz/seconds conversions are good math to practice on.
  - Getting rid of the signal after 2 seconds gives less noise, but you lose signal intensity.
  - Losing 0.5 Hz couplings is fine if you're mainly looking for 3-5 Hz couplings.
  - Zero-filling gives an increase in resolution, but it has limited advantages.
  - The further out you go in time, the more frequency discrimination you get. But lose S/N as well.
- t_2 is the relaxation to equilibrium perpendicular to the magnetic field.
- t_1 is the relaxation to equilibrium parallel to the magnetic field.
  - This determines if spins actually get back to equilibrium after you do something with them (e.g., pulses).
- Pulse length.
  - 5 µs by default.
  - What if we lengthen it to 500 µs?
    - Things get out of phase. Manual phasing allows you to see it, but you get a broad background.
  - At 5 ms, you don't get anything really interpretable, although the peaks are in roughly the same space.
  - 1/5 µs is 20 kHz, which is parts per million on a 600 MHz spectrum.
- Project #1.
  - Task: Prepare a presentation for the class, and a report for the class.
  - Purpose: Share as much information about a range of useful nuclei as we can with each other.
  - We're not gonna touch the stuff in this class for a long time, so it will be good for our future selves to have resources.
  - List of references that people can go to is really important (online, published articles, etc.).
  - Stay within the allotted time no matter what.
  - Report and slides can be the same, but just make sure that all of your references go in the slides, too.

Week 2

Project 1 Presentations

2.1 Introduction to Proton, Carbon, Nitrogen, and Phosphorus

2/11:

- Our presentation.
- ^{13}C NMR presentation (Angel, Nate).
  - Broadband decoupled ^{13}C NMR gives no coupling with protons, so the number of peaks is the number of distinct carbons.
  - Low abundance of ^{13}C gives 100 times weaker signal than ^{1}H.
  - Gyromagnetic ratio γ is 1/4 that of ^{1}H.
    - The signal intensity is proportional to γ^3, so overall, proton signal is about 6400 times stronger than ^{13}C.
  - Solution: Increase sample concentration, longer relaxation delay (d1), higher field strength NMR (600 MHz), DEPT, 2D NMR.
  - Chemical shifts: 0-220 ppm.
    - Two regions: Above and below 100 ppm.
    - Aliphatic: 0-50 ppm.
    - EWG-substituted aliphatic: 50-100 ppm.
    - Aromatic: 100-150 ppm.
    - Since carbon is more electronegative than hydrogen, adding carbon substituents shifts signals downfield.
    - Resonance structures and partial charges can help predict shifts.
    - Steric effects: Up to 10 ppm shifts from van der Waals interactions of atoms being near each other, especially in rigid molecules.
  - Impurities.
    - $CDCl_3$ has an equally heighted triplet at 77 ppm due to the spin 1 deuteron splitting the carbon peak into 3 peaks of equal height.
  - Functional groups (shifts and couplings).
    - Alkenes: 100-150 ppm.
      - ➢ One-bond coupling of about 150 Hz.
    - Alkynes: 70-90 ppm.
      - ➢ Results from differences in electronic configuration around the carbon nuclei.
      - ➢ One-bond coupling to proton in acetylene of about 249 Hz (*sp*-hybridized carbons have huge couplings; shorter bonds!).
      - ➢ Two-bond coupling to other proton of about 49 Hz.

    - Aldehydes.
    - Halides.
        - Big bulky electron density on iodine pushes shift for alkyl iodides to -20 to -40 ppm.
  - Why isn't ^{13}C NMR quantitative?
    - We'll talk about it, but it might have something to do with NOESY.
    - Polarization transfer can amplify signals *and* decouple.
    - Turning off NOE, very long relaxation delay, and can make ^{13}C NMR quantitative!
  - For ^{1}H, we don't have an issue with chemical shift anisotropy. For almost any heteroatom (and carbon), we will have this issue. And it increases with the square of the field strength, so there's an ideal field strength range for carbon NMR whereas for proton, you can go as high as they make them.

- ^{15}N and ^{31}P NMR (Natalie, Rosalind).
  - For both nuclei: Typical chemical shifts, proton-heteroatom coupling constants, and what this can look like in biomolecular NMR.
  - ^{15}N.
    - Spin 1/2.
    - 0.37% abundant.
    - Low γ.
    - >1000 ppm range of chemical shifts.
    - Most groups fall within 0-500 ppm. Metal nitrosyl (M−NO) complexes are roughly 300-1200 ppm, but this is helpful for identifying metal complexes (such as iron sulfur complexes, i.e., metalloproteins which store or transport NO)!
    - Proton-nitrogen couplings are difficult to detect. Magnitude affected by solvent used as well as intermolecular interactions (e.g., hydrogen bonding).
    - A variety of techniques be used to study biomolecules (e.g., at MIT in Mei Hong's lab). HSQC experiments, solid-state, isotopic labeling, and many more.
    - DNA is only made of four simple nitrogen-containing heterocycles, so looking at isolated nucleotides can be very helpful.
    - Shifts affected by post-translational modifications, DNA shape, protonation, etc.
    - 100-130 ppm for backbone nitrogens in proteins, varies drastistically for side chains (30-220 ppm).
    - HSQC is a protein fingerprint, as well as the gateway into deuterium exchange experiments. Can be used to study the folding of proteins.
  - ^{31}P.
    - Spin 1/2 and 100% isotopic abundance. Thus, very easy to measure!
    - 2000 ppm shift range.
        - Upfield defined by P_4 at -527 ppm.
        - Downfield defined by...
    - Proton-phosphorus J-coupling allows us to tell how far part phosphorus and hydrogen atoms are. Very useful tool!
        - Great examples in the slides.
    - Chirality determination with a chiral phosphorus reagent and ^{31}P NMR.
    - ^{31}P NMR in DNA.
        - Gives information about backbone conformation (e.g., A vs. B vs. Z).
        - Dickerson dodecamer backbone; researchers were able to correlate ^{31}P NMR shift with the percent of a certain conformation in the sample.
    - Cummins and Radosevich labs will have a lot to say on ^{31}P-^{31}P couplings!
  - These nuclei are also not often studied at higher fields; you lose stuff even at 600 MHz.

2.2 Miscellaneous Nuclei

2/13:

- Announcements.
  - We don't have class next Tuesday; only next Thursday.
- ^{19}F NMR (Yifan, Francesca).
  - Quite similar to proton!
    - Natural abundance: 100%.
    - Nuclear spin of 1/2.
    - $\gamma_F \approx \gamma_H$.
    - Reliable integration.
    - Broad range of chemical shifts.
    - Standard reference: $CFCl_3$.
  - However, shielding is more paramagnetic; proton shielding is more diamagnetic.
    - Consequence: OChem proton NMR intiution goes out the window.
    - It's harder to predict shift based on functional groups.
    - Magnetic anisotropy ring currents have less effect (overlapping aromatic and aliphatic regions).
  - Some tables of shielding and deshielding effects.
  - Steric deshielding.
  - Talking about isotope effects and satellites.
  - Not very sensitive to solvent effects, unlike proton where benzene-d6 has a big effect.
  - ^{19}F-^{19}F, ^{19}F-^{1}H, and ^{19}F-^{13}C couplings are most common.
    - Very similar to proton-proton couplings, because both nuclei have $I = 1/2$.
    - Proton NMR may couple to both nuclei! Quartet of quartets possible from 3 protons and 3 fluorines nearby (in 1,1,1,-trifluoropropane).
    - Coupling constants decrease with more electronegative substituents nearby.
    - Karplus-type effects are still there: *trans* vs. *cis* coupling constants.
    - Geminal fluorine coupling constants increase with more electronegative groups.
    - Carbon couplings can be huge.
    - Long-range couplings are especially noticable with fluorine NMR.
    - Coupling can be transferred through quadrupolar interactions with benzene.
  - Applications of ^{19}F NMR.
    - Reaction time courses, method optimization, and mechanistic investigation.
    - Deconstruction C–F bonds is really big rn.
    - Fluorine is rather bioorthogonal, so you can put fluorine-substituted amino acids into proteins!
    - Chemical shift of fluorine is very sensitive to the local chemical environment, so it can be used to reconstruct how proteins fold!
    - Confirms the presence of weakly coordinating anions (e.g., BArF).
    - Swager does a lot of PFAS sensing, especially with porous polymers and ^{19}F NMR, which can be much more reliable than the EPA's current LCMS methods.
  - There is also a fluorine NMR background from teflon in almost all NMR probes.
- Main group NMR nuclei (Sunny, Kwanwoo, Georgia).
  - ^{11}B NMR.
    - ^{11}B has a spin of 3/2, 80% abundance, higher γ, lower quadrupole moment.

- Borosilicate glass within the NMR probe gives a hump from $-30 - 30$ ppm.
  - ➢ Can do a number of things to reduce this.
- Can reduce the issue of tubes with quartz NMR tubes.
- Heizenberg uncertainty principle leads to more uncertainty and greater broadening. Strong quadrupolar moment also gives shorter relaxation time.
- Chemical shift references.
- It's most common to do proton decoupling.
- B-F couplings are difficult to see; difference in electronegativity is cause??
- zgbs pulse sequence helps decouple the probe's peaks.

– ^{14}N NMR.

- Nuclear spin number 1, hence quadrupolar and fast relaxation (so broader peaks).
- Low γ.
- Much more abundant, but more difficult to work with.
- You can monitor the progress of a relaxation, but you have to know where to expect things.
- Can be helpful for identifying heterocyclic isomers.
- Conclusion: It's not the best, but you can determine isomeric structures. Since it's so abundant, you don't have to label your molecule or have specific growth media.

– ^{29}Si NMR.

- $I = 1/2$, negative γ, 5% abudance.
- Really long relaxation time.
- Does have some uses, though.
- TMS is the reference standard for this.
- Components of the probe and glass and other materials have silicon, so there's a large background peak around 100 ppm.
  - ➢ You can computationally subtract the background, or do some other things.
- Example from soil science.
  - ➢ Dipolar decoupling and magic angle spinning in the solid state helped identify imogolite in different horizons of the soil.

– ^{27}Al NMR.

- 100% natural abundance.
- $I = 5/2$, quadrupolar (interacts with not only the external magnetic field, but lso the electric field gradient generated by its surrounding environment).
- Highly sensitive.
- Wide chemical shift range, and references.
- p-character explains why more electronegative atoms lead to *lower* chemical shifts.
- You can monitor formation and degradation of a polyanion.
- Solid-state aluminum NMR can study aluminum coordination in zeolites.

– ^{77}Se NMR.

- $I = 1/2$, 7.63% abundance, relatively low γ.
- Sunny has worked with this recently!
- Very broad chemical shift range.
- Selenium-proton coupling is a thing.
- Clear oxidation state shift.
- Selanocysteine can be used in biology.

– ^{129}Xe and ^{131}Xe NMR.

- Huge chemical shift range.
- You'll probably never use it, but it's cool.
- Biological applications, but drawbacks in terms of practicality.

- More nuclei.
  - ^{2}H NMR.
    - You have to pump the system with an excessive amount of deuterium if you want to do it.
    - Low quadrupole moment, so poor resolution.
    - $I = 1$.
    - Chemical shifts comparable for proton NMR, so you can use this side-by-side with proton NMR to really see what's going on in your reaction/molecule.
    - Good for deuterium labeling studies.
    - Example: Adamantanone homo-enolization.
      - ➢ *exo*- vs. *endo*-hydrogen abstraction determined by comparing ^{1}H and ^{2}H NMR.
      - ➢ Shifting reagents make proton and deuterium have very similar chemical shift ranges.
    - Example: Chemical biology.
      - ➢ Study of the lipid bilayer with deuterated lipids.
      - ➢ Used to study the order of the molecules.
  - ^{6}Li NMR.
    - $I = 1$, 8% abundant.
    - Chemical shift range of about 28 ppm; some inorganic species have dramatically different shifts.
    - Coupling with proton, carbon, or nitrogen can be used.
    - Can be used to understand the behavior of organolithium species.
      - ➢ Reveals monomeric and dimeric phenyl lithiates!
      - ➢ Isotopically labeling a nearby nitrogen reveals several possible dimer conformations.
  - ^{7}Li NMR.
    - $I = 3/2$, 92% abundant.
    - Broad peaks and very little coupling.
  - ^{23}Na NMR.
    - $I = 3/2$, 100% abundant.
    - 110 ppm range in solution; varies greatly in the solid state.
    - Implications in biology.
    - Sodium contamination is common in empty NMR tubes!
    - Application: Electrochemistry.
      - ➢ Characterizing sodium ion battery degradation mechanisms.
    - Application: Frozen seawater and how large bodies of water freeze.
      - ➢ Studied brine freezing.
      - ➢ $NaCl_{(s)}$ has a characteristic broad peak, becomes thin when dissolved in water, and gets messier when you go to lower temperatures.
  - ^{35}Cl NMR.
    - $I = 3/2$, 75.5% abundant.
    - Resolution isn't as bad as deuterium labeling, but not great due to quadrupole moment.
    - Fairly big chemical shift range.
    - Solvents give broad peaks; inorganic/salt phase is better.
    - Application: Solid-state ^{35}Cl NMR for hydrochloride salt concentration determination of pharmaceuticals.
      - ➢ Salt structure can be characterized.
      - ➢ Much better for solid-state dynamics than ^{13}C NMR.
    - Proton decoupling is important for chlorine NMR.

Week 3

Where NMR Spectra Come From

3.1 The Basic NMR Experiment

2/20:

- Announcements.
  - PSet 1 feedback should be back to us by the end of the weekend.
  - What Walt is really looking for is that we understand the material well enough to take it home with us and use it in the lab.
- Today: The most complicated/boring part of the class.
  - Going over the highlights of the basic NMR experiment. This is how we go from the sample in the spectrometer to generating an FID.
  - Many terms will be used that we may or may not know. If we don't know anything, ask Walt questions. These terms will be on the PSet!
  - After this, we'll get back to doing chemistry problems.
- Most simple experiment: 1D acquisitions of a single nucleus.
  - We rest for a while (where we're at equilibrium).
  - Radiofrequency pulse translated through probe/detector.
  - Get a signal that we can digitize.
  - We'll discuss some of the parameters we get to set and how they help us.
- Two properties of a spectrum that we care about: **Sensitivity** and **resolution**.
  - These are *not* interchangeable.
  - *Don't* say: "I'm not getting enough resolution. Do I need more sample in my tube?"
- **Sensitivity**: The amount of signal we get over the noise background.
  - I.e., how *big* is the signal.
  - The amount of noise is determined by the hardware, particularly the NMR probe. We can't do anything about this.
  - The signals that we generate are on the order of µV. So to be able to see these, the noise has to be much lower than even that!
  - The total signal we have is proportional to the number of scans.
    - We can't have a prime number of scans, for reasons we'll discuss later.

    - The amount of signal is proportional to the number of scans. The amount of noise is proportional to the square root of the number of scans (that's a statistical thing; the noise signals will not add up, while the signals will constructively interfere when we add them together).
    - Thus,
$$\text{Signal-to-noise ratio} = \text{S/N} = \text{SNR} \propto \frac{n}{\sqrt{n}} = \sqrt{n}$$
  - Consider a 2 mM sample in a 5 mm tube with 8 scans. To double the SNR, either double the concentration to 4 mM or quadruple the scans to 32.

- **Resolution**: How close together we can observe different peaks. *Given by*
$$\text{Resolution} := 2 \cdot \left(\frac{\text{SW}}{\text{NP}}\right)$$
  - I.e., how *sharp* is the signal (how close can the peaks be and we can still tell them apart).
  - Depends on two parameters: The number of points (NP) that we acquire for each spectrum, and the spectral width (SW) we wish to observe.
  - Now we have to get into the whole Fourier transform business.
  - With an analog oscilliscope, we could measure continuous data. We acquire digitally by taking various points. The time between points is called the **dwell**.
    - Preview: In 2D NMR, we have a dwell in the **direct dimension** and **indirect dimension**.
    - Usually on the order of µs.
    - The spectral width and dwell are related to each other: Larger spectral width requires acquiring more points per time.
  - Resolution: Intensity in frequency comes from intensity in time.
  - Walt gives a brief explanation of how the Fourier transform works.
  - In order to get decent resolution in the frequency axis, we need enough points in the time axis to differentiate.
  - With many fewer points, it's harder to identify complex behavior.
    - Note: There is a way to view the individual points composing the NMR spectrum!
  - Long acquisition time and normal spectral width leads to more resolution.
    - Total **acquisition time** is NP · dwell.
  - Acquisition time, dwell, spectral width, and number of points are all related to each other. Changing one necessarily changes the others. If you fix spectral width and increase number of points, you will necessarily increase the acquisition time but also increase resolution??
    - Smaller hertz per point means higher resolution.

- NMR economics.
  - When we do an NMR experiment, our advisors pay for the amount of time that our sample is in the system.
  - A 5 minute experiment costs $1 on a $12/hour machine.
  - If we have to buy more material, it's less expensive to just acquire longer. If we have material in a bucket somewhere, it's less expensive to just put more material in the tube (unless our time is worth something).
  - This matters more when we are doing 1D carbon of 1 mg of 800 MW sample (e.g., in a natural product lab). Then you have to put it on the 600 for 20 hours and hope you get something, only because the reviewers asked for it.

- FID plots are in voltage vs. time.

- ppm vs. Hz.
  - Goes over the calculation.
    - Example: 1 ppm at 400 MHz is 400 Hz.
  - Hertz is the currency with which we think about pulses.
- Radiofrequency (RF) pulses.
  - Pulses at the resonance frequency of the nucleus in which we're interested.
  - So on a 400 MHz spectrometer, we need a 400 MHz pulse to affect protons and a 100 MHz pulse to affect carbon.
    - Nice thing: Since pulses are orthogonal, we can use a pulse sequence to make nuclei talk to each other!
  - RF pulses are defined by their **frequency**, **tip angle**, **power**, **RF bandwidth**, and **phase**.
  - When we apply a strong pulse, it excites a wide bandwidth. Weak pulses, on the contrary, excite a narrow bandwidth.
  - Relaxation and frequency are inversely proportional: When we pulse a system in the ultraviolet or visible, it relaxes in ns, ps, fs. When we pulse a system with RF, it decays in the ms to s range.
    - Thus, we have time to do a whole bunch of stuff that we can't do, unless we're in the Schlau-Cohen lab and have really fancy equipment.
  - You can do solvent suppression by dialing in a pulse of the certain power that you need.
- **Tip angle**: The amount that the magnetization is moved away from the equilibrium position.
- **Power**: The strength of the RF pulse. *Units* W, dB.
- **RF bandwidth**: The range of frequencies that the RF pulse can affect.
- **Flip angle**: The angle between where a magnitized spin started, and where it ends.
- Next topic: Extrapolation from 1D to 2D acquisition. This will lead into COSY, TOCSY, etc. spectra.

Week 4

2D NMR for Peak Assignments

4.1 HSQC, HMBC, and COSY

2/25:

- Questions.
  - Getting different answer consistently for PSet 2 SNR values between MNova methods?
    - That's fine; just be consistent.
  - JEOL 502 and Bruker 600 training?
    - The JEOL 502 can do simultaneous ^{1}H, ^{19}F, and ^{13}C, but no one uses it for that. It's just another autosampler machine with low queues.
    - The 600 also just has a low queue.
  - SampleJet caps?
    - Walt will show me the product ID next time I'm in the DCIF.
- Announcements.
  - PSet 1 answers posted, but grades not.
  - A note on PSet 1: Coupling constants have to be the same when we increase the quantity of ^{13}C.
  - Also, β-anomer coupling constant is almost exactly the same. Why doesn't the β-anomer couple to the anomeric carbon, but the α-anomer does?
    - It's coupling to a carbon 3-bonds away at the correct dihedral angle!
    - Dihedral angle wouldn't affect a 2-bond coupling.
- Today: Chemical shift assignment from scratch (adenosine).
  - We'll also start talking about the utility of 2D acquisition.
- Adenosine.
  - Nucleotide base in DNA, component of ATP, and occasionally used in chemical transformations.
  - Nice test system because soluble in DMSO and then will stay the same for a while.
  - 2 aromatic protons, 3 aromatic carbons that are not protonated, 5 non-aromatic carbons and associated protons, 3 hydroxyls, and amino group.
  - Dissolving it in D_2O removes the exchangeable protons; dissolving in DMSO-d6 shows us all protons.
  - As DMSO picks up H_2O, it will begin to broaden peaks for exchangeable protons.
- Assigning adenosine peaks (^{1}H spectrum).

  - Begin by integrating the spectrum.
  - 2 aromatic protons.
  - One 2H in the middle (that's probably aniline).
  - And 8 aliphatic protons (one of the 9 is hiding in the water signal).
  - Water in DMSO is 3.2-3.4 ppm depending on how many exchangeable protons there are in your solute.
  - Water in $CHCl_3$ can be between 1-5 ppm, depending on acidic protons in solution.

- Assigning adenosine peaks (^{13}C spectrum).
  - In general, you should start with the 1D carbon *before* going to the 1D proton!
    - Sensitivity is lower, but 1D chemical shift resolution is a big bonus!
    - 2 chemical shift overlaps in proton is an issue.
    - Carbon chemical shifts *can* overlap, but they do so much less often.
  - There are 10 carbons in the molecule, and we clearly see 10 carbon peaks.
  - Walt has integrated the carbons here. Quantitative carbon can be done at MIT, but this is not that??
  - Aside: Proton decoupling.
    - Before we apply the carbon pulse, we apply a moderately weak proton RF pulse that allows the proton-carbon NOE to build up. Thus, any proton will transfer its magnetization to the carbon. This enhances carbon signals by about threefold.
  - The two protonated carbons (because of NOE magnetization transfer) integrate a bit more.
  - For moderately accurate carbon integration, integrate close to the peak! It's not like proton where you can just integrate as far out as you want.
  - Aside: Quantitative carbon.
    - Have a longer t_1 relaxation delay so that the spins can all get back to equilibrium before the next pulse.
    - Use a 30° pulse instead of a 90° pulse so that it takes less time to relax back to equilibrium.
    - Don't use the NOE because you want quantitation, not maximal signal.
    - Long aquisition time as well, so you can digitize the signal as best as possible.

- **Heteronuclear single-bond quantum correlation**: An NMR experiment that shows you one-bond proton-carbon couplings in order to connect protons and carbons. *Also known as* **HSQC**.

- Assigning adenosine peaks (^{1}H-^{13}C HSQC).
  - 2 aromatic signals (proton around 8, carbon around 140).
  - 6 aliphatic signals.
    - 4 blue and 2 green.
    - This is a phase-sensitive and multiplicity-edited mode, allowing us to distinguish CH's, CH_2's, and CH_3's (analogous to DEPT experiments!).
    - DCIF has DEPT 90, 135, *and* 45!
  - Signals at the top and bottom are mirrored about the center.
    - Artifacts are pre-ordained by phase cycling, receiver gain, etc.
    - These are from the water in this case. The really strong signals can often be mirrored; this is because we didn't let the waters relax long enough between scans.
  - Gets us a labeling of the protons based on the carbon chemical shift.
    - To reiterate, carbon chemical shift is much more determining than proton chemical shifts.

- 1D vs. 2D experiments.
  - 1D is relaxation delay ($d1$), pulse, and aquisition.
  - 2D/fancy can be relaxation delay, a bunch of pulses, and *then* acquisition.
    - $d1$'s are on the order of seconds.
    - Pulses are on the order of milliseconds.
  - Two Fourier transforms.
- HMBC allows us to find 2- and 3-bond connections.
  - Proton-carbon has 120-170 Hz coupling for 2-bond, and 3-10 Hz couplings for 3-bond.
  - HMBC essentially optimizes for a different range of coupling constants, and filters out 1-bond couplings.
- **Homonuclear correlation spectroscopy**: A proton-proton 2D experiment. *Also known as* **COSY**.
  - A symmetric experiment that gives us the same cross-peaks on both sides of the diagonal.
- Assigning adenosine peaks (^{1}H-^{1}H COSY).
  - Connects nearby proton peaks.
  - The diagonal corresponds to correlation to between a proton and itself.
  - Off-diagonal elements give us what we want: 6-8, 8-9, 9-7, and 7-10.
  - 10 is the unique diastereotopic pair, so working backwards, we then get 7, 9, 8, and 6.
  - We can then assign the hydroxyl protons.
  - At this point, we've assigned the entire sugar but not the aromatic stuff.
- COSY is easier to interpret, but is there a reason we couldn't just measure coupling constants in the 1D ^{1}H NMR?
  - We could do that, but we are resolution-limited and there is much more overlap.
- Next time: Assigning the aromatic stuff with HMBC, nitrogen 2D experiments to figure out which nitrogen is which.

4.2 Heteroatom Correlation Experiments

2/27:
- Today: Finish the complete assignment of adenosine.
- More on COSY.
  - $\boldsymbol{t_1}$ **stripes**.
  - The spectrum can also be displayed in 3D, instead of as a contour/topological plot.
- $\boldsymbol{t_1}$ **stripe**: A stripe in a COSY spectrum.
  - These are the product of not waiting long enough between scans or between the increments in the indirect dimension.
  - They are just noise, and we have to go down to a point where the spectrum looks rational.
- More on HSQC.
  - Even if a mirror reflection lines up with a carbon peak, we have to be able to spot that it's not.
  - If we're still unsure, we can change the specral width or center of the spectrum. If the point moves with the spectrum, it's a reflection; if the point has the same apparent chemical shift, it's real.

- Assigning the hydroxyl peaks.
  - Three-bond ^{1}H-^{1}H couplings will be slightly different magnitudes through C−C vs. C−O bonds.
  - COSY (in DMSO-d6) does the trick.
  - Spiking DMSO-d6 with a bit of D_2O and running two consecutive experiments (with and without spiking) can also be helpful.
- A pitch for DMSO in NMR.
  - We don't like to use it because it's hard to get rid of.
  - But if you use a gentle stream of dry nitrogen on your 0.5 mL sample, it will get rid of the DMSO in about half a day!
- ^{1}H-^{13}C HMBC.
  - Can't routinely detect signal up vs. signal down (as in DEPT), so all of the signals are just magnitude (blue).
  - Carbon 6 should be correlated to proton 8, proton 9, and the hydroxyl on 8.
  - HMBC's are very rich in information, but too rich to make an absolute assignment. That's why you need COSY, too!
  - What's up with the signals that don't line up with a proton?
    - The center of the pair of signals line up with a proton; that's the breakthrough of the one-bond coupling.
  - Three-bond connections are bigger than two-bond connections in every case except 5-membered nitrogeneous heterocycles.
  - Account for the big signals first, and then go after the little ones if you want.
- Don't try to do direct observe ^{15}N unless it's fully labeled; only do correlation spectroscopy.
  - Only do direct observe if we're doing ^{14}N spectroscopy.
- ^{1}H-^{15}N HSQC.
  - Only one peak, corresponding to the NH_2 protons.
  - The nitrogen peak is measured indirectly, by projecting the nitrogen correlation over. This is as good as a direct result, though!
  - If a reviewer asks for a direct ^{15}N spectrum, argue with them!
- ^{1}H-^{15}N HMBC.
  - One nitrogen sees one aromatic proton, and two sugar protons. This is the bridging nitrogen!
- Walt pitches MestReNova's multiplet tool and automatic peak reporting.
- Next week: NMR tools for stereochemical assignments, gradient tools, final project.

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.

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.