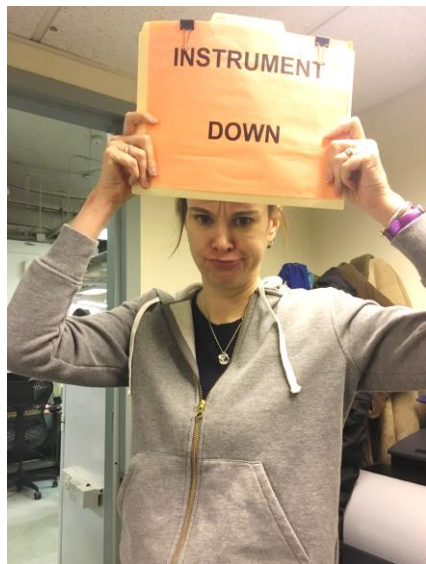


NMR 101



Anne Rachupka
October 25, 2024



DEPARTMENT OF
CHEMISTRY &
CHEMICAL BIOLOGY

What is an NMR?

- Niobium-tin-copper clad coil wound like a spool of thread. The current run through this coil, creating the magnetic field.
- This coil is submerged in liquid helium (boiling point -452.1°F , -268.9°C , 4°K).
- Liquid He chamber surrounded by liquid nitrogen (boiling point -320.5°F , -195.8°C , 77°K).
- Sample and spinner lowered using air from the top, down through the bore, until it nests in the top of the probe.

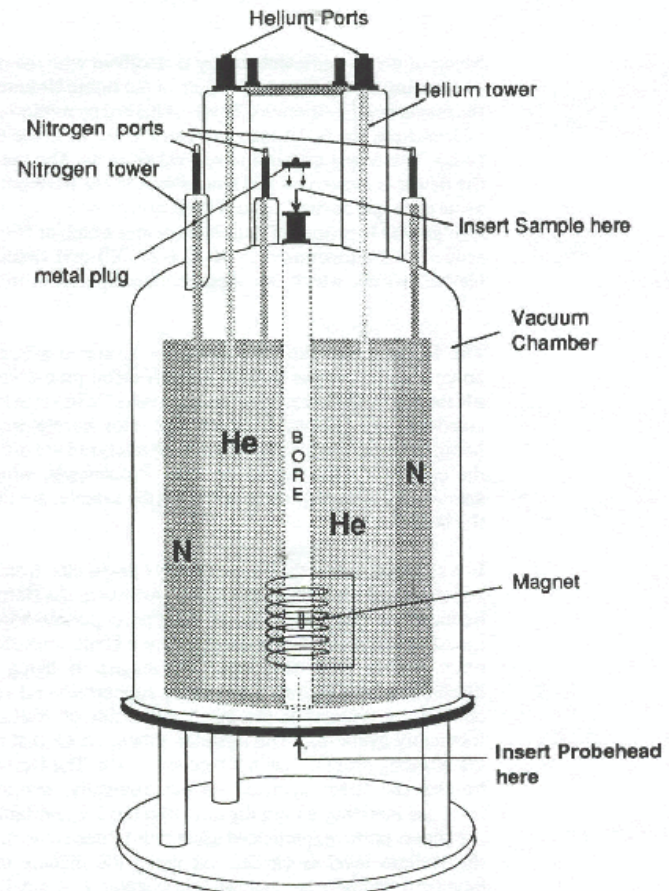


Figure 3.B Magnet and dewar

Where does the NMR signal come from?

Quantum Spin Number

- Only nuclei that possess a property called **spin** can be “seen” by a NMR.
 - If a nucleus can have more than one energy state in a magnetic field, the **quantum spin number (I)** is not 0, and energy transitions for this nucleus are possible.
 - I depends on the number of protons (Z) and neutrons (n) in a nucleus.

I=0 #Z is even #n is even mass # even

NO NMR SIGNAL!!!

¹²C and ¹⁶O are not NMR active!

I=1 #Z is odd #n is odd mass # is even

SIGNAL!!

²H and ¹⁴N

I=1/2, 3/2, 5/2.....

mass # is odd

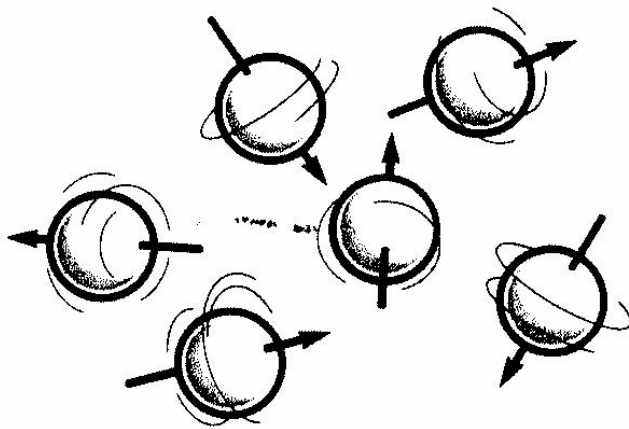
SIGNAL!!

¹H, ¹³C, ¹⁵N

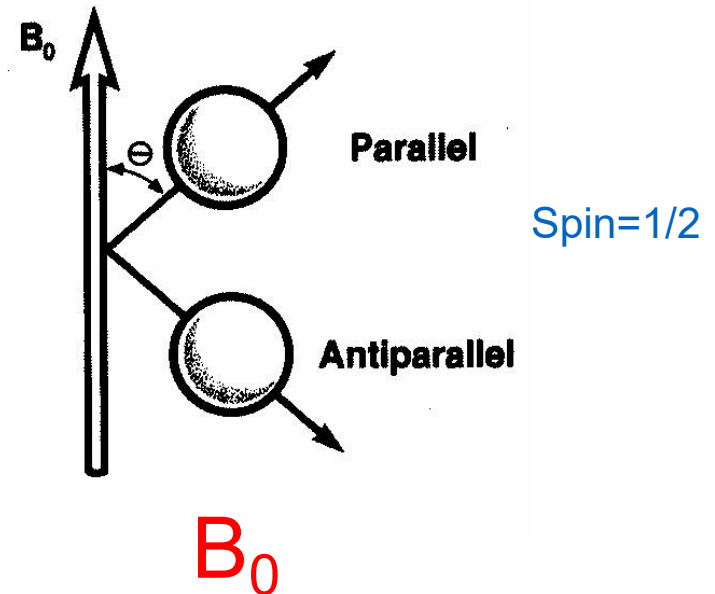
Where does the NMR signal come from?

The Zeeman Effect

- When inserted in a magnetic field (**applied field** or B_0) nuclei that possess *spin* align themselves according to their energy states.
 - This effect on their alignment is called the *Zeeman Effect*.



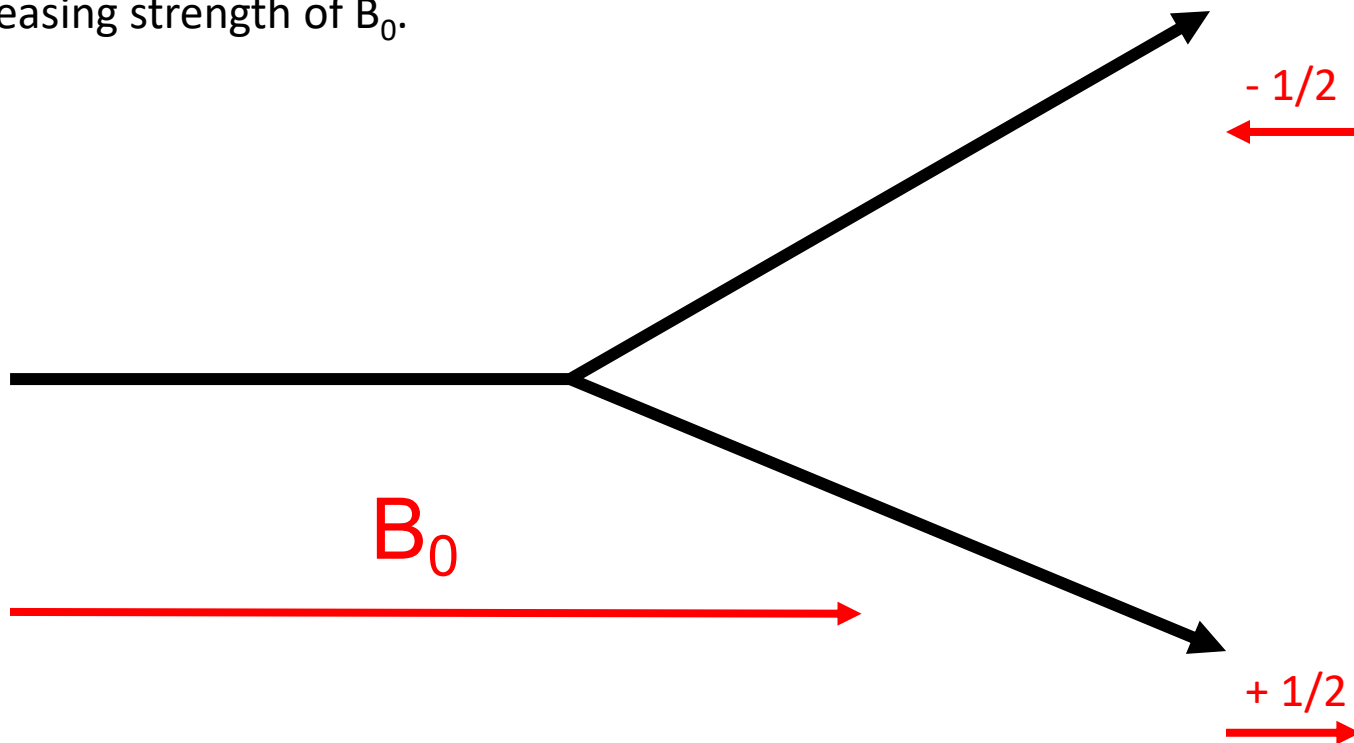
No B_0



The Zeeman Effect

Energy levels if $I=1/2$

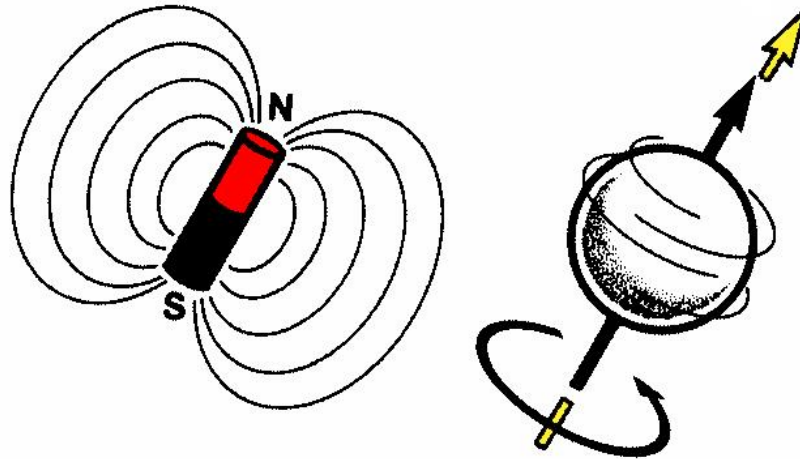
- The spins are said to be split into two populations, $-1/2$ (anti-parallel) and $+1/2$ (parallel), by B_0 .
 - $-1/2$ is aligned opposed to the direction of B_0 .
 - $+1/2$ is aligned with B_0 and is the lower energy state.
- The difference in energy between the two spin states increases with the increasing strength of B_0 .



Where does the NMR signal come from?

Magnetic Moments

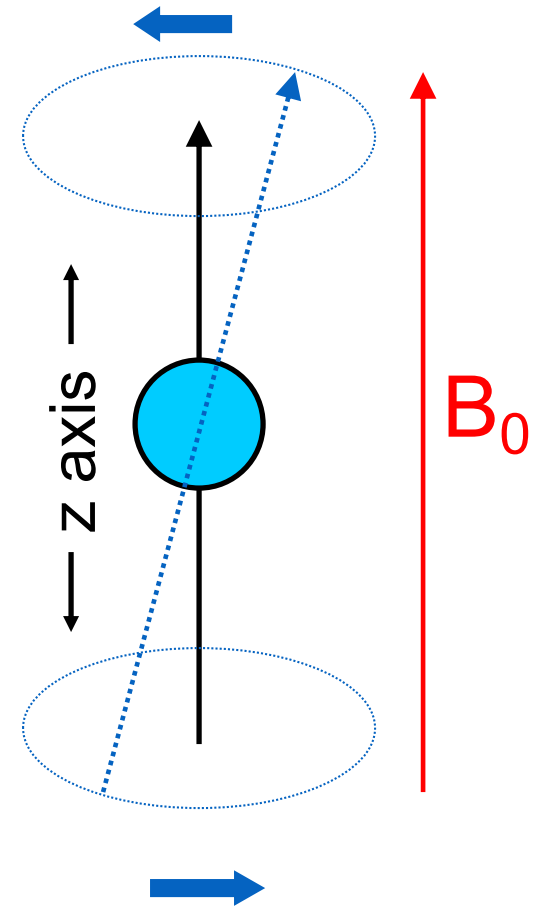
- Spinning, charged nuclei generate a magnetic field and possess a *magnetic moment* (μ).
 - This magnetic moment is a vector and is partially aligned along the magnetic field (B_0) axis (the z axis).



Where does the NMR signal come from?

Larmor Precession

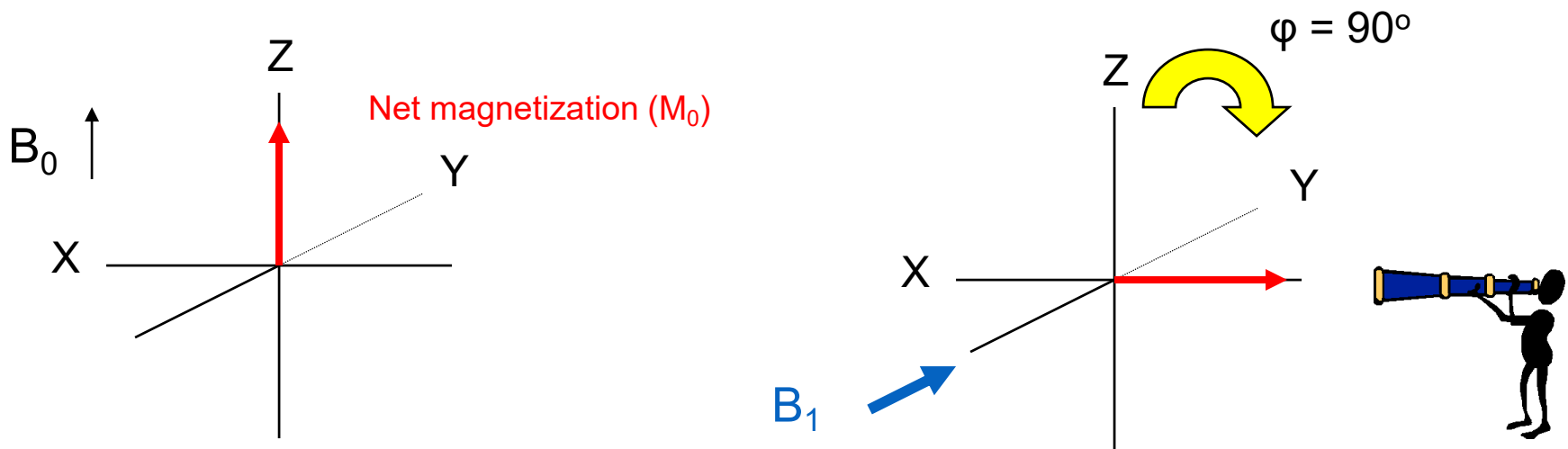
- These vectors are not exactly parallel to the z axis, they *precess*, much like a spinning top does.
 - The frequency of precession (aka Larmor frequency or ω_L) is the number of times per second that the proton precesses in a complete circle.
 - A proton's precessional frequency increases with the strength of B_0 .
- Within B_0 , there is the tendency for more spins to precess with a component aligned with the +z (+1/2 or parallel) direction.
 - **Boltzmann!** There are more spins in the lower energy state (+z direction or N+).
 - This population difference results in a **net magnetization** (M_0) vector in the +z direction.
 - There is a small component in the x and y direction, but this cancels out.



Where does the NMR signal come from?

Tip me over!

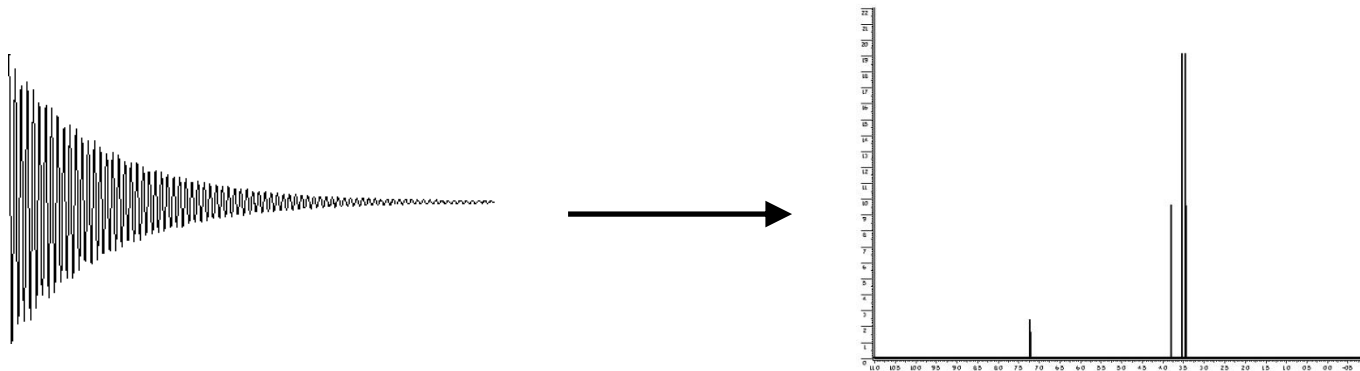
- Nuclei in B_0 .
 - Zeeman effect \rightarrow Boltzmann distribution.
- A second magnetic field is applied (B_1) at radio frequencies (a *pulse*).
 - The length and amplitude of the pulse determine how much the net magnetization vector is tipped from the z direction into the xy plane.
 - A 90° pulse tips M_0 fully into the xy plane.
- We “see” only what is tipped into the xy plane.
 - This is collected as a FID.
 - Relaxes back to ground state (along z-axis) and awaits next pulse.



Fourier Transform

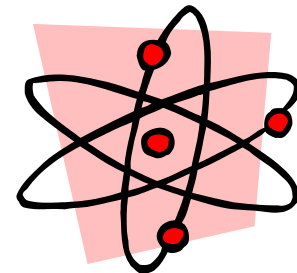
Time \rightarrow Frequency

- FID (free induction decay) – sinusoidal, exponential function modulated by a decay function.
 - Net magnetization (M_0) was tipped into xy plane, and allowed to relax back to the z direction. FID collected during this time.
 - Must translate the time domain into the frequency domain
 - Amplitude vs. time \rightarrow amplitude vs. frequency
 - A computer *Fourier transforms* (lots of math!) the FID into what we know as a NMR spectrum.



What kind of information does a 1D spectrum give us?

- **Chemical shift** (δ) – chemical environment.
- **Scalar coupling** (J) – through bond coupling, provides conformational info. Good shimming allows measurement of small J -couplings.
- **Integration** – area under a curve, provides ratio of number of atoms in a particular chemical environment. Flat baseline + well phased spectrum = good integration (assuming sufficient relaxation delay).
- **NOE** (nuclear Overhauser effect) – through space coupling – who is next to who? Spatial orientation of groups. NOE experiments are experiments in which ‘cross relaxation’ occurs due to dipole-dipole interaction.
 - In decoupled carbon spectra, the NOE ‘enhances’ the carbon signal through the irradiation of protons near to carbons, and ‘collapsing’ multiplets into singlets. The enhancement varies from carbon to carbon, and thus decoupled carbon spectra are NOT quantitative.



Changes in the Resonance Frequency

Why everyone resonates at a different frequency

- Which nucleus
- Field of magnet
- Electron Density
- Scalar coupling



Bruker 1.2 GHz NMR

Installed January 2024 at Ohio State

Which Nucleus

Sensitivity and the Gyromagnetic Ratio (γ)

	I		Abundance (%)	γ
^2H	1	Q	0.015	4.11
^{14}N	1	Q	99.63	1.93
^1H	$\frac{1}{2}$		99.98	26.76
^{13}C	$\frac{1}{2}$		1.11	6.73
^{15}N	$\frac{1}{2}$		0.37	-2.71*
^{19}F	$\frac{1}{2}$		100	25.18
^{31}P	$\frac{1}{2}$		100	10.84
^{29}Si	$\frac{1}{2}$		4.7	-5.32*
^{51}V	$7/2$		99.76	7
^{117}Sn	$\frac{1}{2}$		7.61	-9.5*

Q - Quadrupole moment. Happens when the charge not evenly distributed across nucleus. Can cause broad lines.
 γ - (gamma) is a set value.

* - A negative gyromagnetic ratio means that the -1/2 spin state is the lower energy level compared to the +1/2 (reverse the Zeeman Diagram).

You can see why ^1H is easy to “see” in NMR – high abundance and large γ

Which Nucleus

Sensitivity of ^1H vs ^{13}C

$$\frac{^1\text{H}}{^{13}\text{C}} = \frac{\gamma^3\text{H}}{\gamma^3\text{C}} = \frac{(26.7519)^3}{(6.7283)^3} = 63$$

Factor in abundance....

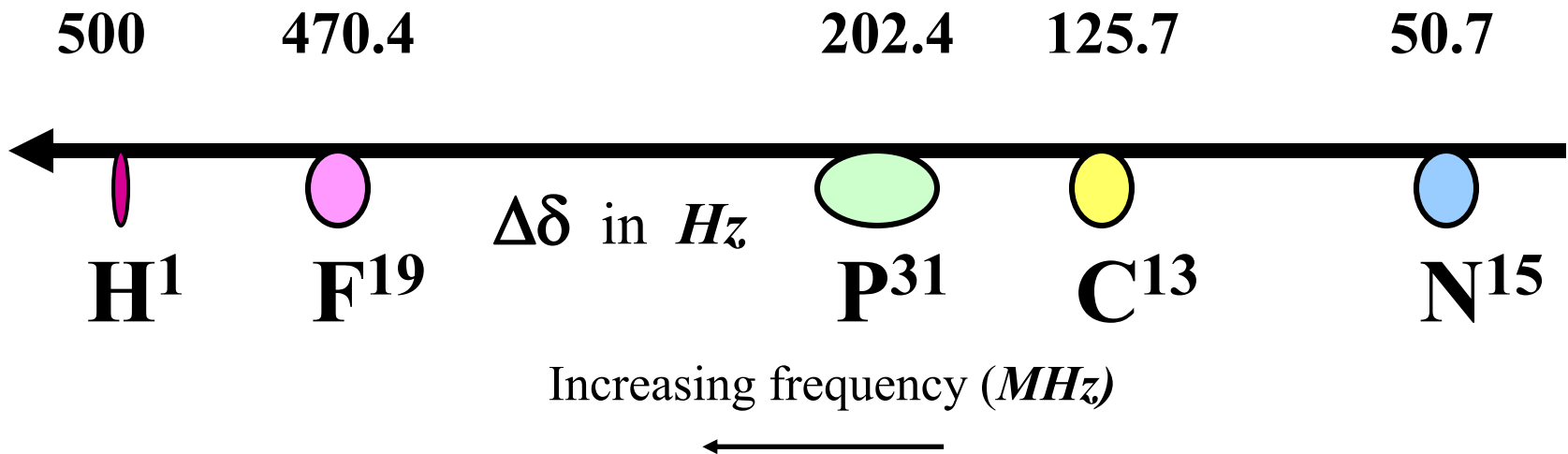
$$\frac{^1\text{H}}{^{13}\text{C}} = \frac{(99.98)}{(1.108)} \times 63 = 5672$$

^1H is 5672 times more sensitive than ^{13}C . That's why you need many more ^{13}C scans to get a decent spectrum.

Which Nucleus

Different nucleus means different frequency

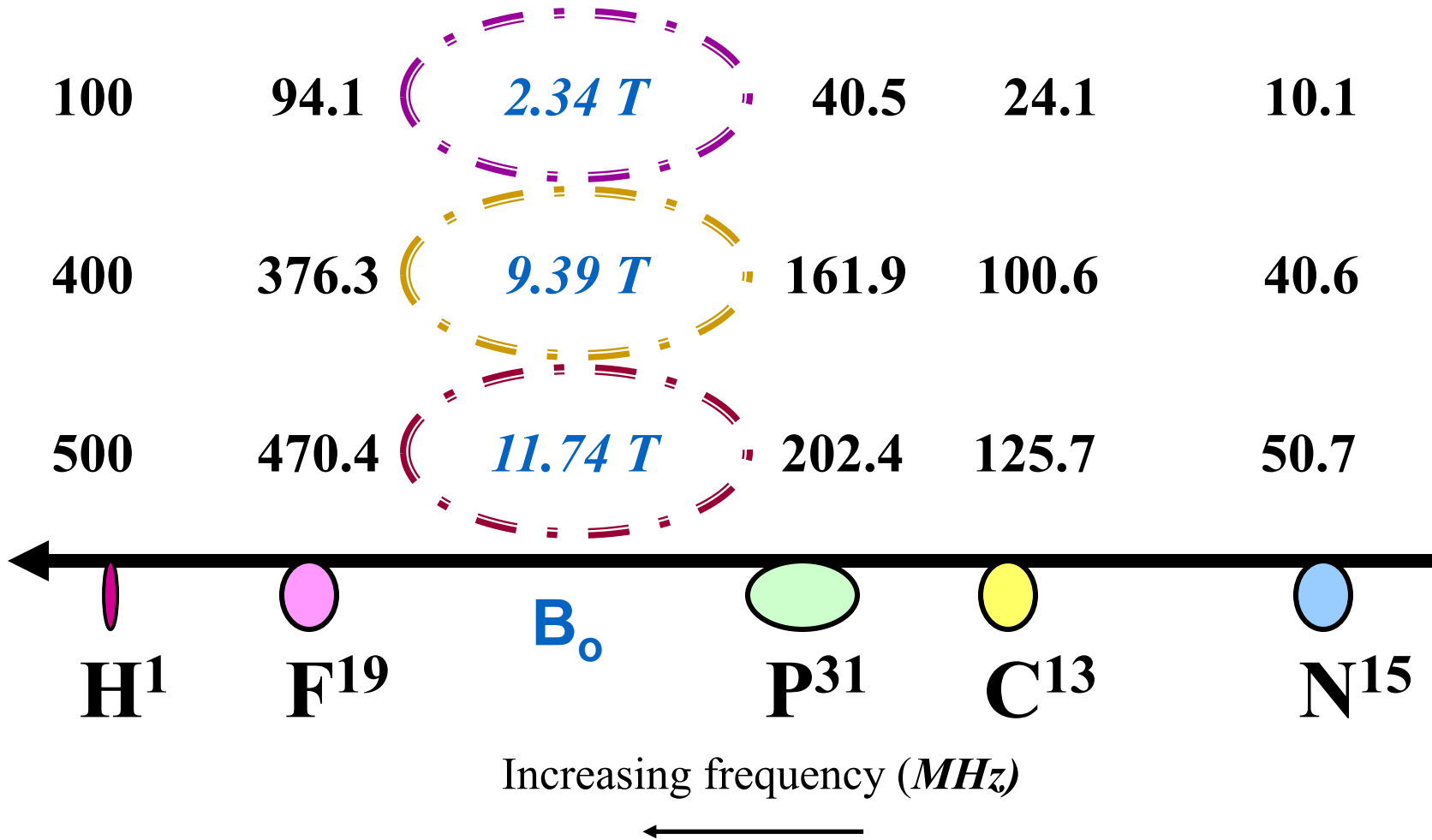
- Different nuclei resonate at different frequencies in MHz.
 - When talking about 500 MHz NMR, we are referring to the resonance frequency of proton in that particular strength magnetic field.
 - **This is why probes need to be tuned** in MHz when switching between certain nuclei. ^1H and ^{19}F are typically tuned on one channel, while ^{31}P , ^{13}C , and ^{15}N on another.



Field of Magnet

Changing the field of the magnet

Different strength fields (in *Tel*sa) mean different resonance frequencies.

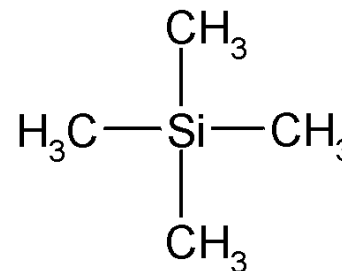


Field of Magnet ppm and Hz

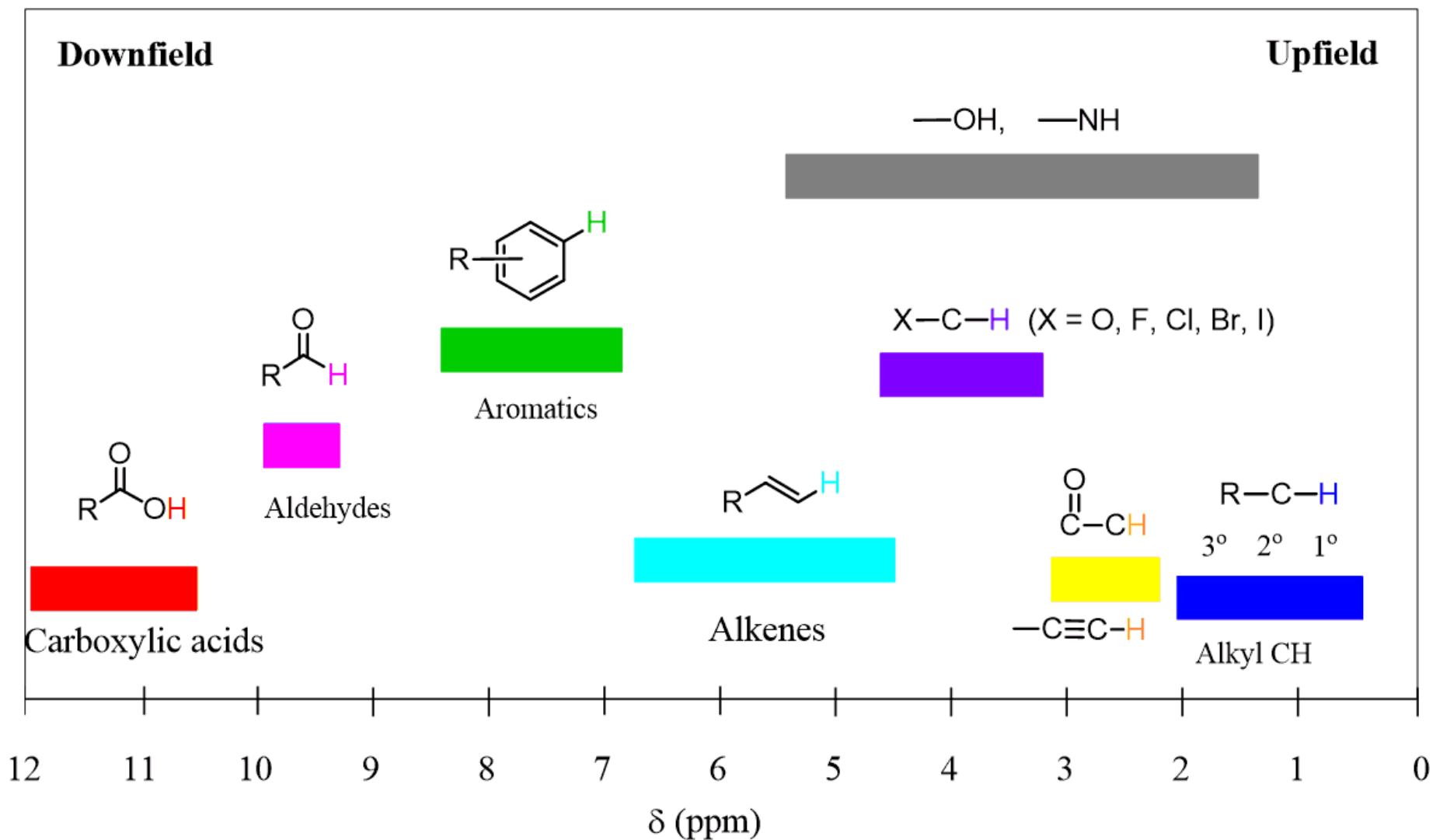
- If chemical shift was measured in Hz, it would be field dependent. This is a problem. Not everyone has the same frequency magnet. (e.g. 300 MHz NMR vs a 400 MHz NMR)
 - However, coupling is measured in Hz and is NOT field dependent. Therefore, the larger the field, the less overlap of resonances.
- Chemical shift (δ) is measured in ppm because ppm is field independent.
 - ppm is parts per million. It is a ratio, so no units.

$$\delta = \frac{\nu_{sample} - \nu_{reference}}{\nu_{reference}} \times 10^6$$

- The energy axis is called the δ (delta) axis.
 - ν_{sample} is the absolute frequency of the sample.
 - $\nu_{reference}$ is the absolute frequency of a standard reference compound, measured in the same applied magnetic field B_0 .
-
- ^1H and ^{13}C referenced to TMS (tetramethylsilane) = 0ppm.
 - In D_2O , TMSP (Trimethylsilyl propanoic acid) is used instead of TMS.
 - ^{31}P generally references to H_3PO_4 (85% phosphoric acid) = 0ppm.
 - ^{15}N generally referenced to HNO_3 (nitric acid) = 0ppm.
 - ^{19}F generally referenced to CCl_3F (trichlorofluoromethane) = 0ppm.



Field of Magnet ppm and Hz



Field of Magnet

Boltzmann revisited

- If we can use ppm, why are bigger magnets often more desirable?
 - If spin = $\frac{1}{2}$, then each spin will align itself in one of 2 possible orientations (Remember Zeeman???).
 - At room temp, the number of spins in the lower energy level (N+) is slightly greater than the number in the upper level (N-).

$$N_-/N_+ = e^{-\Delta E/kT}$$

k is Boltzmann's Constant

- The NMR signal depends on this difference in population.

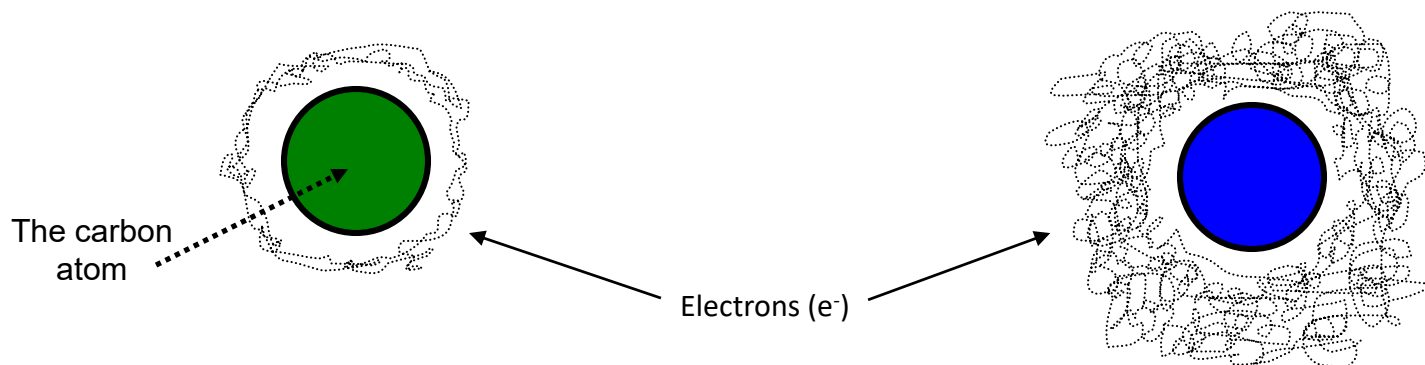
The greater the field B_0 = the greater the population difference.

Greater difference = more signal



Electron Density

How chemical shift works



Carbonyl (C=O)

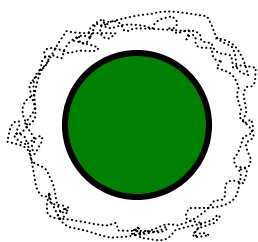
- Less e⁻ density
- Nucleus 'deshielded' from B₀
- Feels the field (B_{eff}) more

Methyl (CH₃)

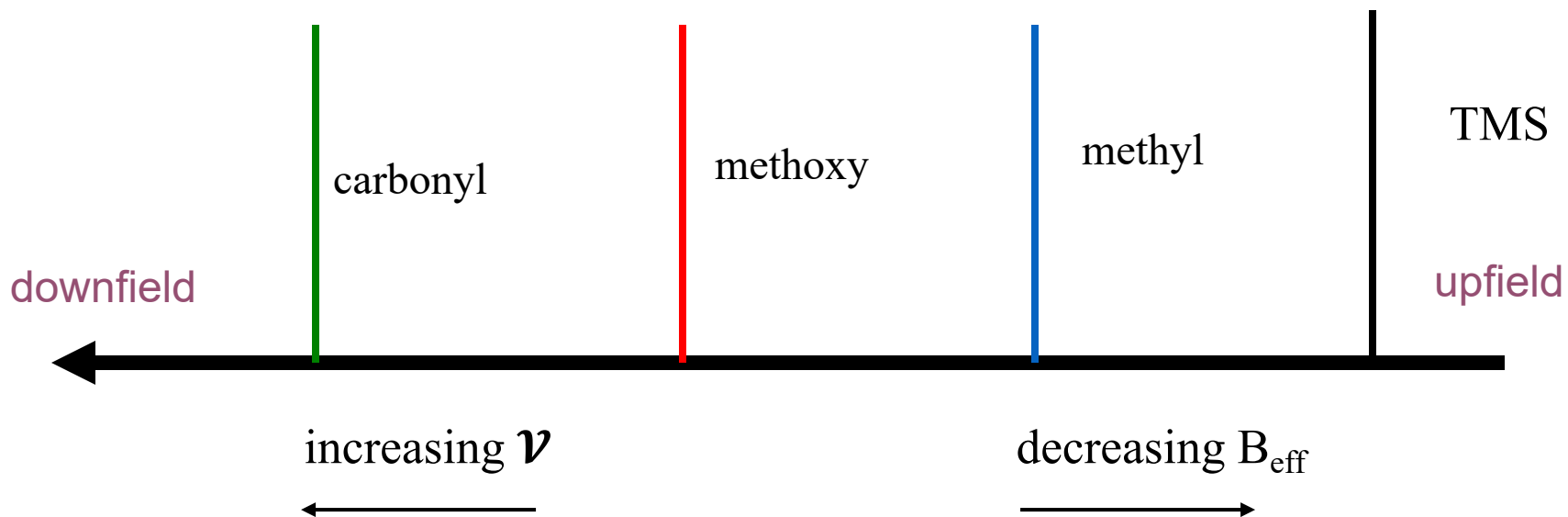
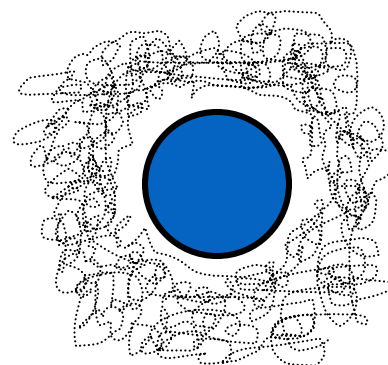
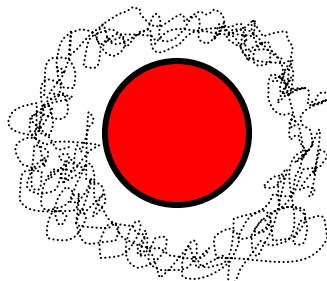
- More e⁻ density
- Nucleus 'shielded' from B₀ by e⁻
- Feels the field (B_{eff}) less

Electron Density

How chemical shift works



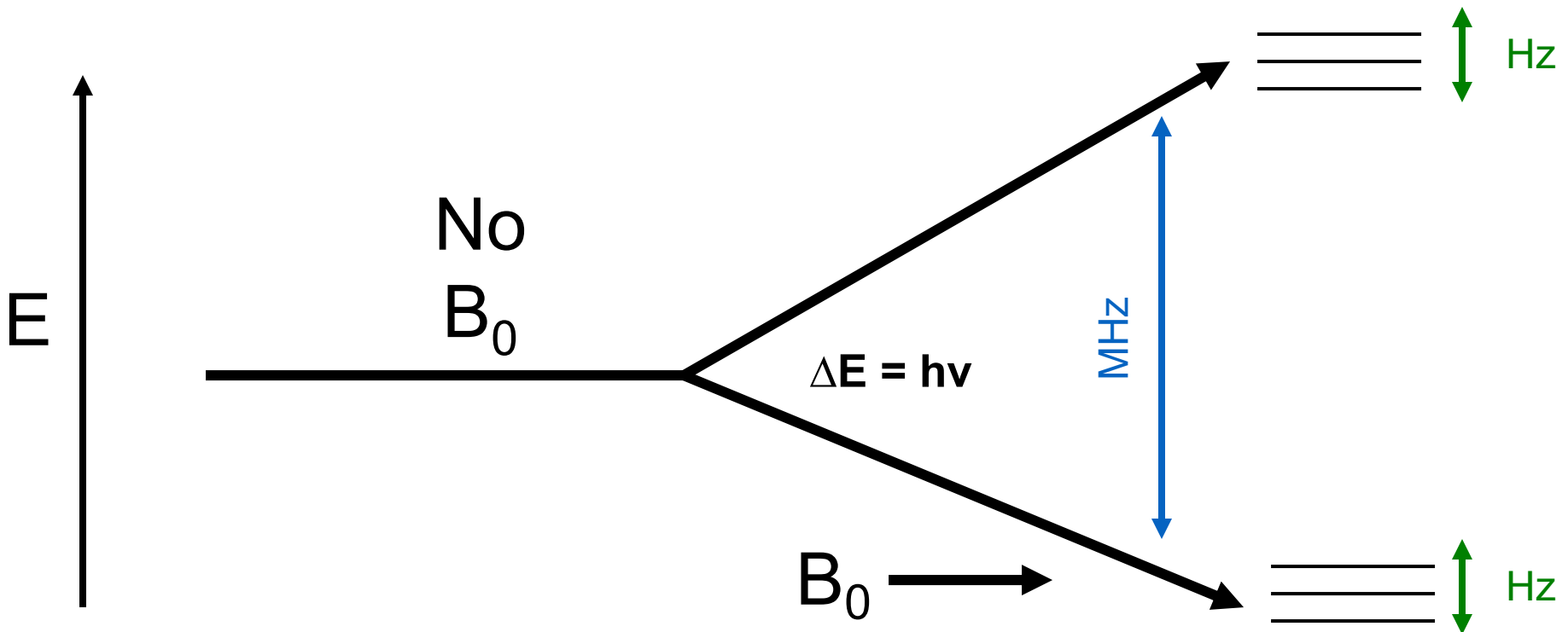
Electron density



Electron Density

How chemical shift works

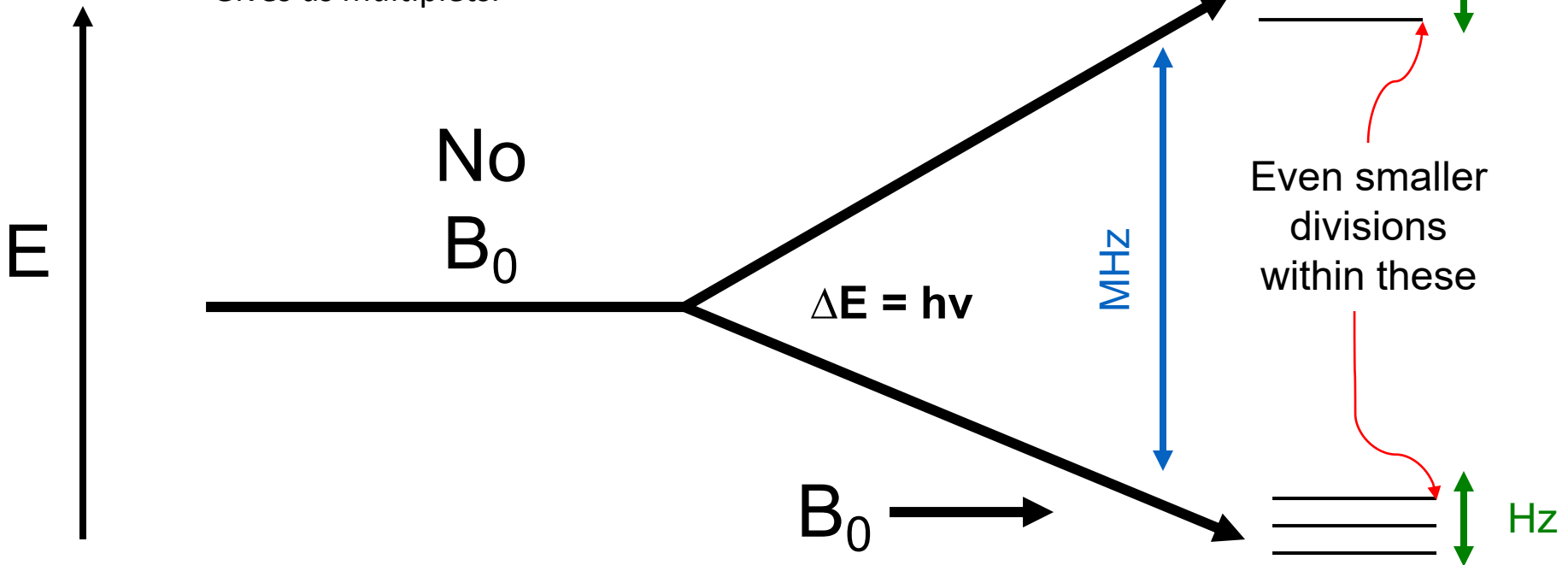
- Electron density gives us small (0-20,000 Hz) changes in energy levels.
 - Gives us chemical shift (δ) in Hz.



Coupling

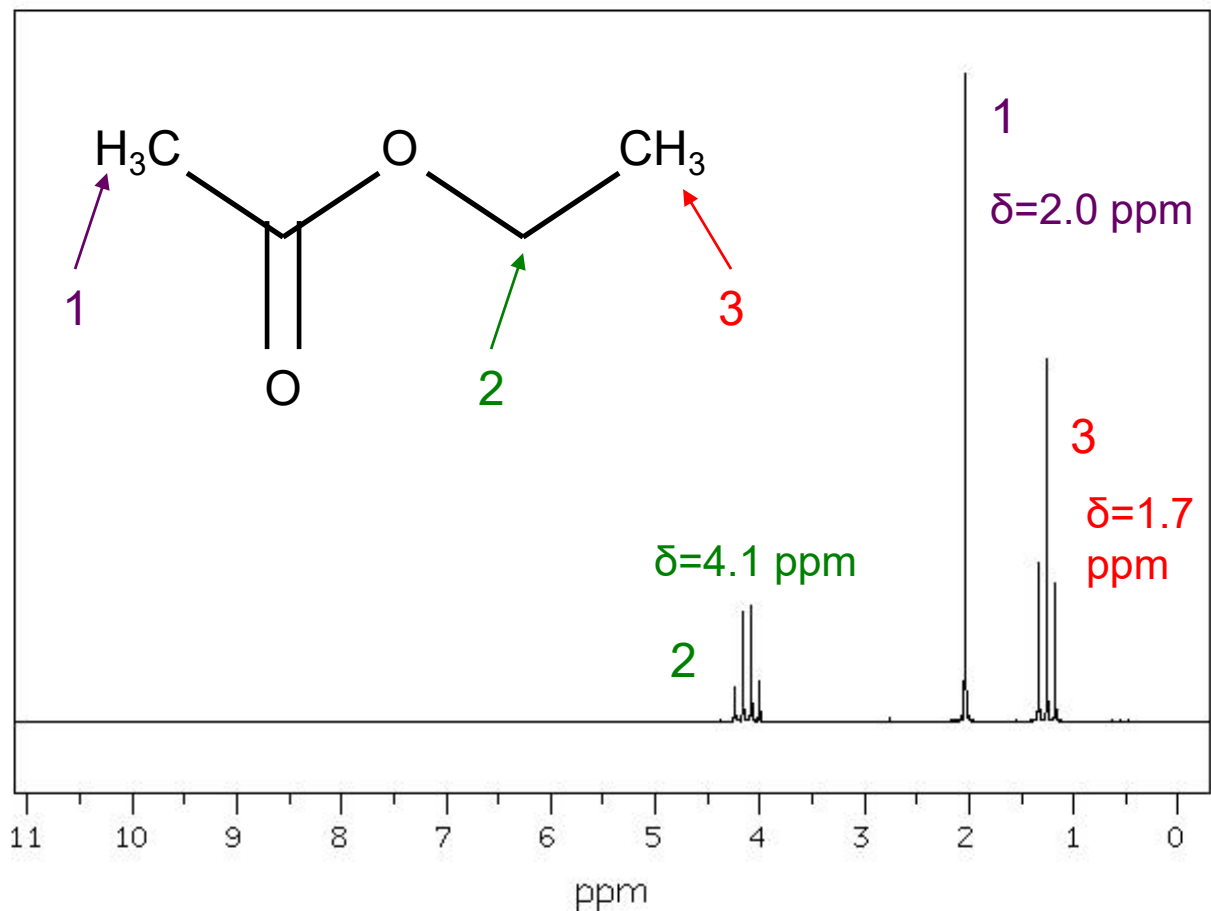
Scalar (J) Couplings

- J couplings – through bond coupling, gives us small changes (0-500 Hz) in energy levels.
 - The spin state (+ or -, if spin = $\frac{1}{2}$) of one nucleus can create a slightly different B_{eff} in a neighboring nucleus.
 - Gives us multiplets.



Coupling

Ethyl acetate



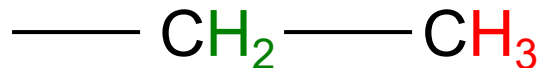
Spin-Spin Splitting patterns

- 1. Singlet.** 3 equivalent protons. Not coupled to any neighboring protons.
- 2. Quartet.** 2 equivalent protons. Split (1:3:3:1) because coupled to the 3 ¹Hs at the 2 position.
- 3. Triplet.** 3 equivalent protons. Split (1:2:1) because coupled to the 2 ¹Hs at the 3 position.

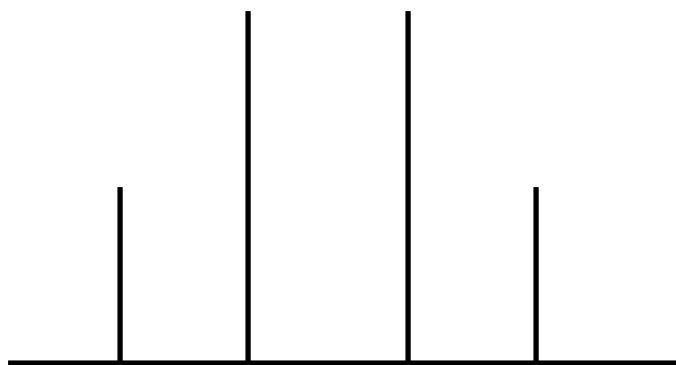
$$\text{Multiplicity} = N (\text{\# of neighbors}) + 1$$

Coupling

Spin-Spin Splitting



CH₂ Resonance
Quartet (1:3:3:1)



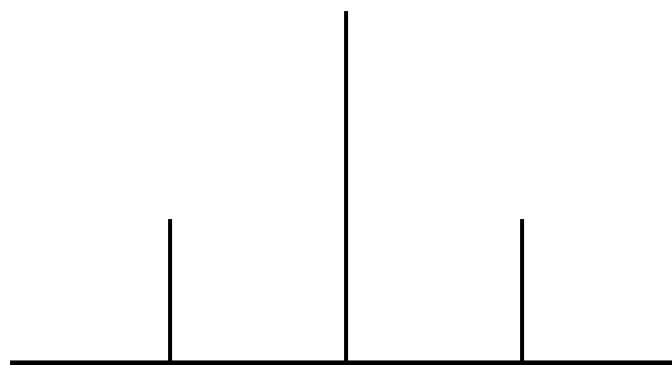
- - +
- + +
+ + +

- + -
+ - +

+ - -
+ + -

Spin states of neighboring
CH₃ protons

CH₃ Resonance
Triplet (1:2:1)



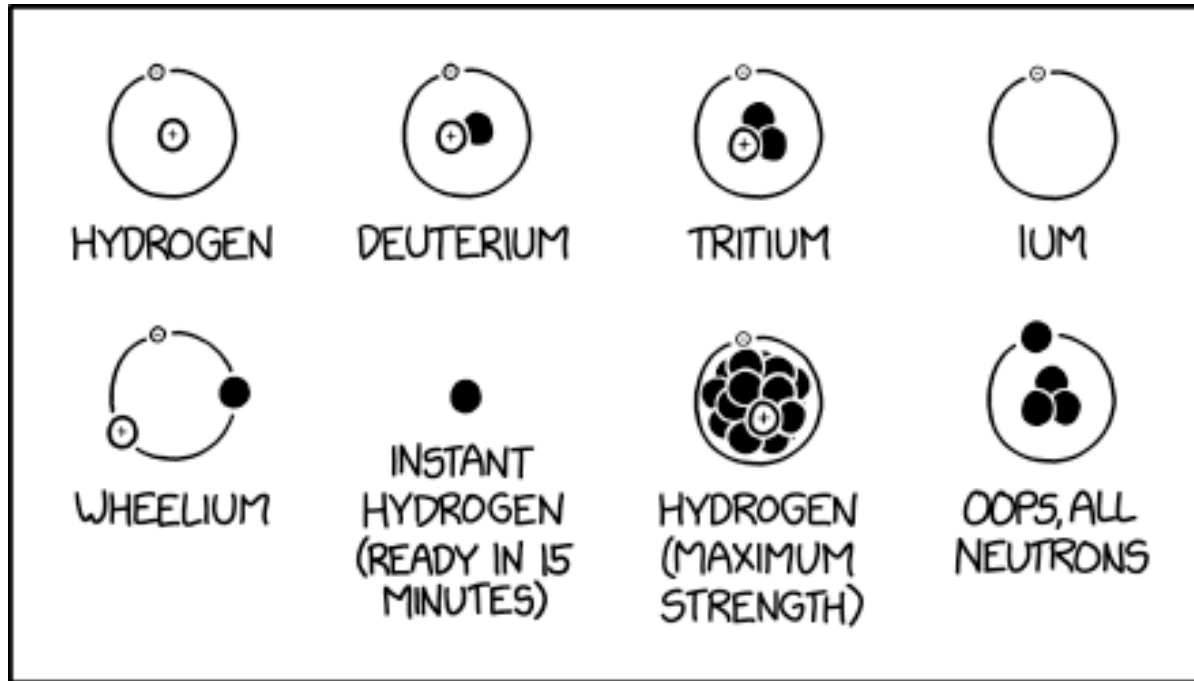
--
+ -
++

- +

Spin states of neighboring
CH₂ protons

Things to remember

Interesting tidbits and practical info

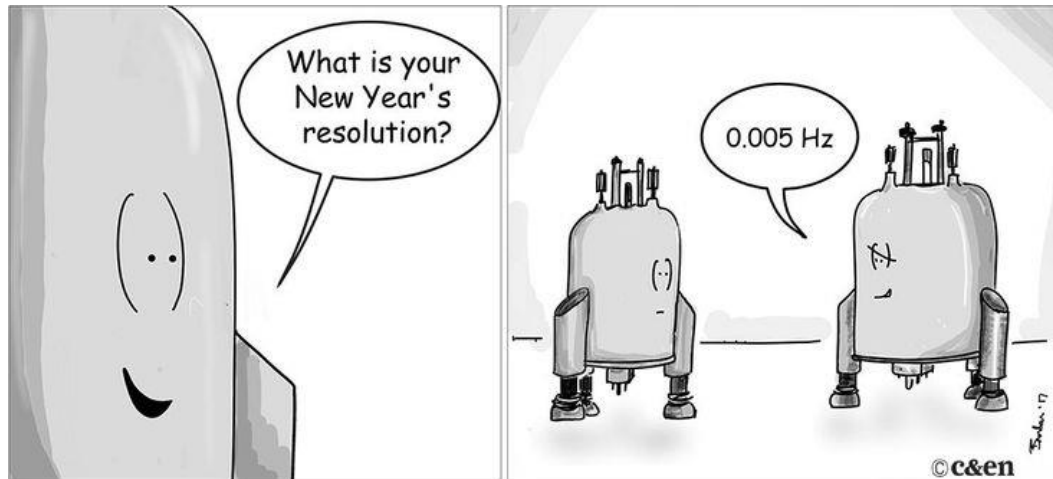


Locking

Why we use a deuterated solvent



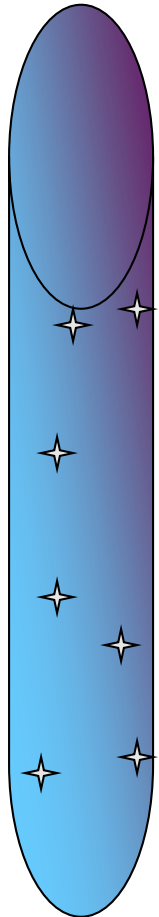
- Magnetic fields drift over time.
 - Modern NMRs drift maybe a few Hertz per hour (only about 0.01ppm in a 500 MHz NMR), but this can add up over time.
 - Important that the field remains stable during an acquisition.
- **Locking** allows for continuous adjustments to the the deuterium lock field (Z_0) so that the deuterium signal is centered on a predefined lock frequency.
 - Locking lets us track the drift and compensate all signals accordingly.
 - Can run unlocked but run the risk of broad peaks



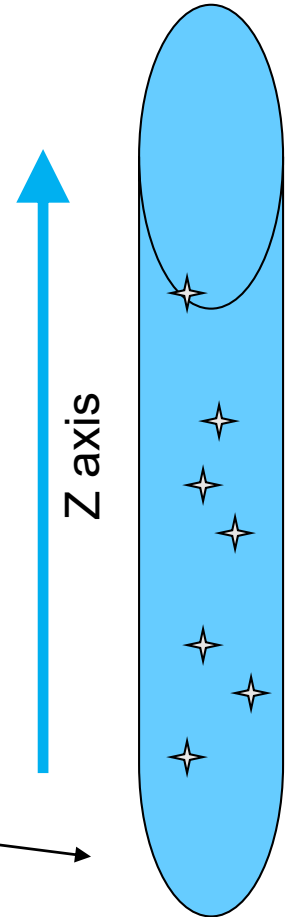
Shimming

Making the field homogeneous

ΔB_0 large



ΔB_0 small



Shim coils are small wires around the probe.

When a current flows through these wires, a small magnetic field is generated.

Current is adjusted in all three dimensions to make the applied field homogenous.

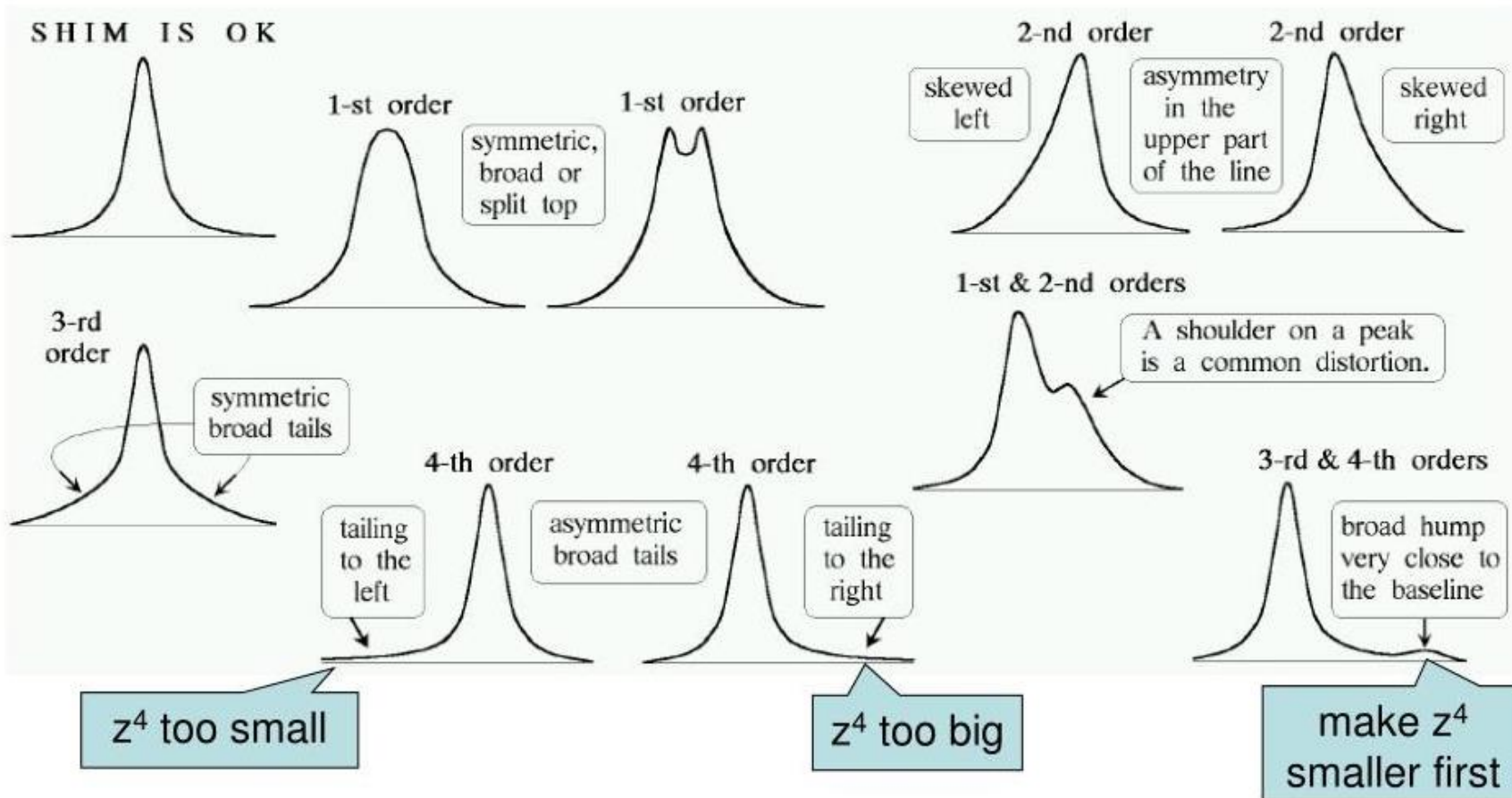
Sample tube

An aldehyde ^1H at the top of the tube will resonate at the same frequency as an aldehyde ^1H at the bottom of the tube.

Shimming

Making the field homogeneous

Plot made by G. Pearson, U. Iowa, 1991



If all your peaks have the same asymmetrical shape and/or are broad, then the shimming is bad.

Sample concentration Recommendations

- For the 300 (Tardis)
 - 16 scan ^1H : 1mM
 - 256 scan ^{13}C : 20-50mM
 - 256 scan ^{31}P : 10mM
- For the 500 (Kepler)
 - Has a nitrogen cooled cryoprobe, so S/N is about 2-3 times better than a room temperature (RT) probe.
 - 16 scan ^1H : 1mM
 - 256 scan ^{13}C : 10mM
- Sample volume (7" or 8" tube length)
 - 3mm tube: 170-200uL
 - 5mm tube: 500uL

Signal to Noise

S/N ratio and Number of Scans

- S/N ratio increases with the number of scans.
 - In order to double the current S/N, you need to quadruple the number of scans.

8 scans S/N=10/1

32 scans S/N=20/1

128 scans S/N=40/1

Notice that the longer you run, the less you gain over time. For example, there is not a huge gain in S/N between 3000 and 4000 scans.



2D experiments

The Essentials

- **COSY – CO**rrelation **S**pectroscop**Y**
 - What proton is coupled to what proton.
 - Peak intensity related to the magnitude of the coupling.
- **NOESY – Nuclear Overhauser Effect SpectroscopY**
 - Which proton are near each other in space but not bonded.
 - Best for small molecules (MW < 600)
- **ROESY – Rotational Overhauser Effect SpectroscopY**
 - Similar to **NOESY**, but better for medium sized molecules (700 < MW < 1200).

2D experiments

The Essentials, cont.

- **HMBC** – **H**eteronuclear **M**ultiple **B**ond **C**orrelation
 - Long-range heteronuclear (C or N typically) correlations using proton observation, typically over 2-3 bonds.
 - One bond correlations suppressed.
 - ^1H on the observe axis (F2) and ^{13}C on the F1 axis.
- **HMQC** – **H**eteronuclear **M**ultiple **Q**uantum **C**orrelation
 - Proton-carbon single bond correlations.
 - ^1H on the observe axis (F2) and ^{13}C on the F1 axis.
 - Signals usually multiplets.
- **HSQC** – **H**eteronuclear **S**ingle **Q**uantum **C**orrelation
 - provides the same information as **HMQC**.
 - Better resolution than the HMQC in the indirect dimension (F1).
 - Signals are singlets.

2D experiments

How many scans do I need?

Best way to figure out how many scans you should run to get a decent S/N

- Load 2D HSQC parameters (pulse program [hsqcedetgpsisp2.3](#))
 - AcqPar tab, convert to 1D
 - Set dummy scans to 4
 - Remember to tune!
 - Run **pulsecal**
 - Set **ns=4** and run 1 scan. Adjust **ns** as needed.
- 1D HSQC x 4 = scans you need for your HMBC
- Hours to run your HMBC x 1.5 = how long you should run your ¹³C

Software

For data analysis

- Mnova: <https://mestrelab.com/main-product/nmr>
 - Can analyze Bruker, JEOL, and Varian/Agilent NMR data
- Free copy of Topspin from Bruker
 - More info here: <https://store.bruker.com/products/topspin-for-processing-academic-government>
 - <https://www.bruker.com/en/products-and-solutions/mr/nmr-software/topspin.html>



General Safety

Rules for the NMR Lab

- **Only authorized users will be permitted to use the spectrometers.**
- **Users may not train other users.** All new users must be trained by a NMR Facility staff member.
- **No sample preparation in the NMR Facility.** There is no hood, sink, or benchtop available for use. Please use your research lab.
- **NMR samples should be properly labeled and in a secondary container.** Samples must be removed when your experiment is finished. Any samples left sitting around will be disposed of properly. Please do not leave unlabeled NMR tubes in the Facility.
- **No food or drink is allowed in the NMR Facility.**
- **Please respect instrument reservations.** If you are more than 15 minutes late for your reservation, you forfeit your time and others may use the instrument. Please do not run into other user's time.
- **If you break something, please tell a NMR Facility staff member ASAP.** You won't be in trouble (accidents happen). The only way for things to get fixed is to let a staff member know, and many problems can and will get worse if left unaddressed.
- **Please keep all ferromagnetic objects away from the spectrometers.** This includes staples, paperclips, and most tools. It's also a good idea to keep credit cards, cellphones, and laptop computers away from the spectrometers.
- **No open toed shoes and shorts are discouraged.**
- **Please clean up after yourself.** Thank you!

Reminders

Lab Rules

- Secondary containers are now required in the NMR Lab.
 - If you need a container, I have free ones you can decorate.
- Please use **FACES** to make reservations
 - If you have reserved an instrument, it is yours during that time.
 - If someone has an instrument reserved, you **MAY NOT** interrupt or remove their sample.
 - Any problems, come see me.



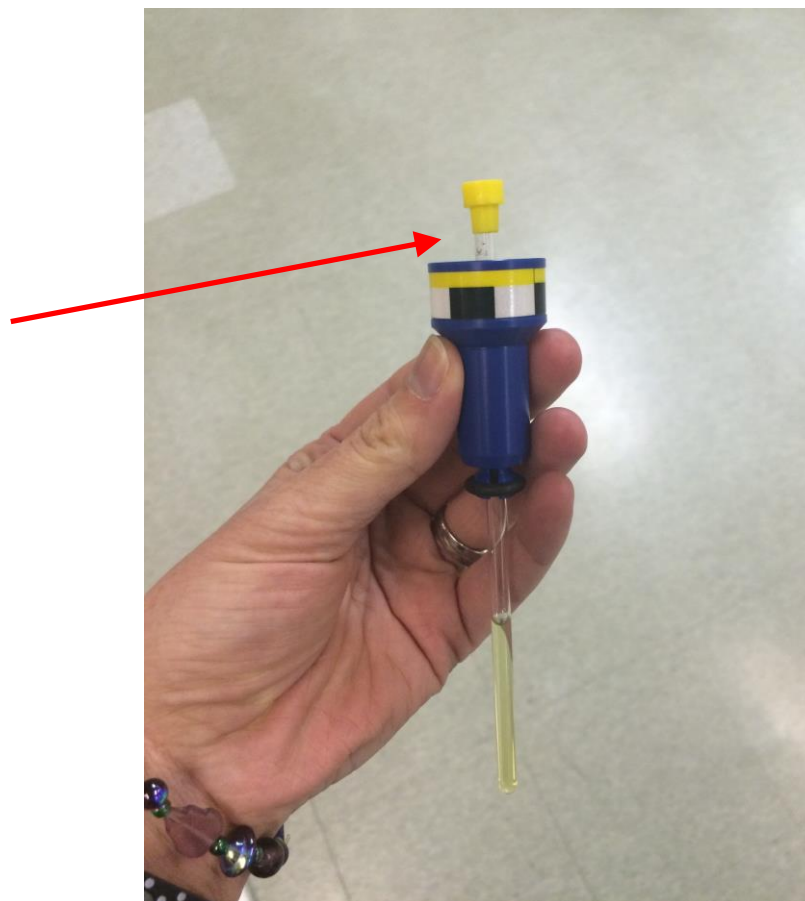
Pro-tip

Don't reuse broken NMR tubes

Notice how short the NMR tube is in the depth gauge. The top had been broken off and a cap was crammed over the broken end.

Caps will NOT properly seal if the tube is broken. Loose caps can pop off inside a magnet.

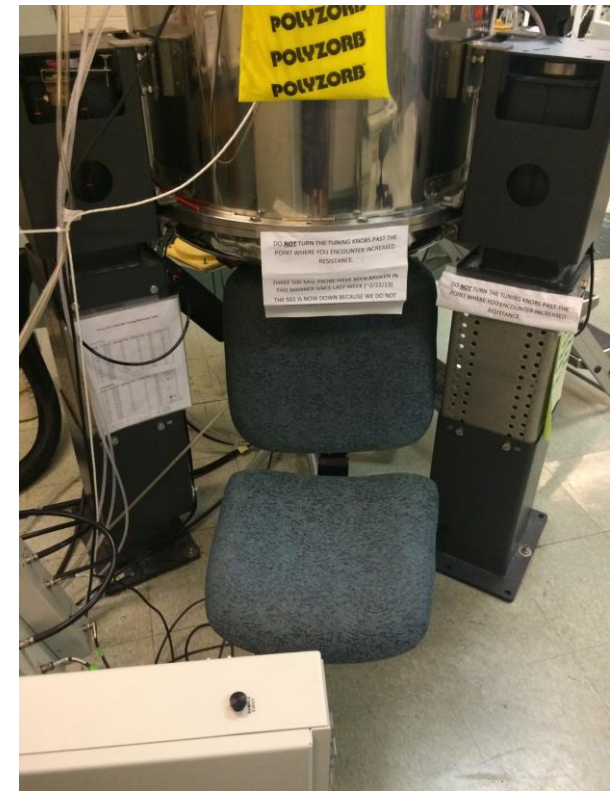
Loose caps inside the magnet are not good. Probe will have to be removed and that can take time.



What do you mean, there is a chair under the magnet?



Chair stuck under a 500.
How it got there remains a
mystery to this day.



Replacing Vibration Legs On an Oxford 500 MHz magnet



Old leg

Temporary support



New leg



Credits

Special thanks to.....

Kathleen Gallagher, University of New Hampshire.

Jeremy N. S. Evans - BIOMOLECULAR NMR SPECTROSCOPY, Oxford Press, 1995

Bruker

Varian Associates, Inc. (now part of Agilent, NMR business closed 2014)

Joseph P. Hornak – Rensselaer Institute of Technology

<http://www.cis.rit.edu/htbooks/nmr/bnmr.htm>

