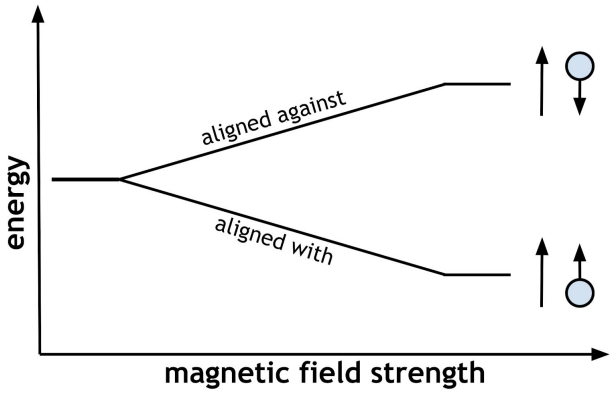
McNeil Group NMR Guide

Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy (NMR) is a technique that relies on using a magnetic field to split a population of magnetic nuclei in a sample into two groups, one aligned with the magnetic field and one aligned against the magnetic field. Outside of a magnetic field, both nuclear spin states are equal in energy and so signals from both populations of spin states cancel out. In a magnetic field, however, being aligned with the field is a lower energy state, so there will be a larger population of nuclei aligned with the field than the population aligned against the field. This difference in population means the signals do not cancel each other out, and that chemists can perform spectroscopy on these aligned nuclei.



In a magnetic field, the nuclei precess. The frequency of this precession is due to how shielded the nuclei are by their electrons. This frequency of precession is called the Larmor frequency (ω), and it depends on two variables:

$$\omega = \gamma B \qquad \text{(eq. 1)}$$

where B is the magnetic field strength and γ is the gyromagnetic ratio of the nucleus. The magnitude of γ determines the sensitivity of the nucleus to NMR measurements. When we plot a 1D NMR spectrum, the horizontal axis (chemical shift, ð) corresponds to the difference in Larmor frequency between the nuclei in our sample and the Larmor frequency of a reference compound. The value is expressed in parts per million because this difference is divided over the frequency of the reference compound:

$$ð = (\omega_{\text{observed nuclei}} - \omega_{\text{reference}})/\omega_{\text{reference}} \qquad \text{(eq. 2)}$$

The only way nuclei interact with the magnetic field is if the nuclei have a net spin. Nuclei of spin = ½, such as **^{1}H**, **^{13}C**, ^{15}N, **^{19}F**, ^{29}Si, **^{31}P,** ^{77}Se, ^{119}Sn, ^{195}Pt, ^{199}Hg, ^{207}Pb, give the easiest to interpret spectra, but any nucleus with a net spin can be analyzed via NMR spectroscopy.

NMR Instruments

The instruments available to the chemistry department are the Inova 400 (400 MHz), Inova 500 (500 MHz), Cobalt (400 MHz), Gallium (500 MHz), Tellurium (500 MHz), and Ytterbium (700 MHz). Other departments have instruments that any UMich researcher can use. Consult Biophysics, LSI, or Engineering for information on these.

The frequency next to the name of each of these magnets corresponds to the Larmor frequency of precession of the ^{1}H nuclei in tetramethylsilane. By eq. 1, the higher this Larmor frequency, the stronger the magnet. Generally, spectra of less-sensitive nuclei, like ^{13}C, will be easier to take on stronger magnets because a stronger magnet gives a larger difference in energy between the two nuclear spin states (so a larger difference in population), but the ease of taking a spectrum will also depend on the probe installed in the magnet. For example, Tellurium has the best probe for ^{13}C NMR spectroscopy, even though it doesn't have the strongest magnet. The signal-to-noise ratio in a spectrum is inversely proportional to the square of the number of scans, so if after ~256 scans (~15 minutes) your spectra still looks noisy, it likely won't improve very much with more scans unless you extend the total acquisition time to multiple hours.

When a sample is placed into the NMR instrument, three things must happen to take a spectrum. First, the instrument must be told what Larmor frequency (nucleus) to observe - this is called *tuning* the instrument. Second, the instrument must observe and follow the deuterium signal strength in the deuterated solvent. This is called *locking* the instrument and is done so that the magnet can self-correct for changes in the magnetic field during the acquisition of spectra. The last step is called *shimming* the instrument involves changing the strength of the magnetic field in a number of spatial directions so that it is homogenous over the whole sample, giving the best peak shape. After shimming, the instrument can acquire the spectrum. It acquires signal by performing a pulse sequence corresponding to excitations (changes in angle or strength of the magnetic field) over time then observing the signal of the nuclei as they align with a detector (giving the spectrum). After each excitation, the nuclei are given time to relax back to aligning with the field. The default relaxation time is usually 0.1 s for all instruments, but the proper relaxation time is on the order of seconds for some nuclei and should be adjusted according to the nuclei you are observing (see the 1D NMR section).

Solvents and Sample Prep

Most NMR samples need to be prepared in a solvent in which all protons are replaced with deuterium atoms. Without this, the instrument can't lock onto the ^{2}H signal and the signal from the protons in the solvent will be so intense that you will be unable to resolve the ^{1}H or ^{13}C peaks of your compound (no matter how concentrated your compound is or how much you zoom in). A standard NMR solvent is $CDCl_3$, but if your compound is not soluble or has peaks at or near 7.26 ppm in ^{1}H NMR spectra or near 77.16 ppm in ^{13}C NMR spectra, other deuterated solvents are available on the deuterium shelf in our lab. Note that the locations of peaks can change dramatically depending on the solvent. Spectra should not be taken in CH_3OD or CH_3COOD - the protons on these will prevent you from seeing your desired peaks. If more deuterated solvents are needed, visit the Cambridge Isotopes Labs catalog. All solvents should be stored over molecular sieves when opened to reduce the water content in the solvent.

To prepare an NMR sample in a deuterated solvent, add about 10 mg of material to a clean vial, and then use ~0.5mL of deuterated solvent to dissolve it, then transfer it to a clean NMR tube. There is no upper or lower concentration limit, but if the sample is too dilute, the signal-to-noise ratio will be low, and if the sample is too concentrated, it may be difficult to lock/shim the instrument.

If you cannot dissolve your compound in any deuterated solvent, it is possible to acquire an NMR spectrum in non-deuterated solvents using solvent suppression and a ^{1}H shimming. Consult the UMich NMR guide or see the solvent suppression section below:

https://lsa.umich.edu/chem/technical-services/nmr/documentation.html

Do not take spectra of samples containing solids since the peaks will be broad, low intensity, and irreproducible.

1-Dimensional NMR Spectroscopy

1-dimensional experiments are the most common NMR experiments and can tell you about the types of nuclei present (based on the chemical shift), the number of neighbors (based off of the splitting of each peak) and other information such as hybridization and connectivity based off the coupling constant between peaks.

^{1}H NMR Spectroscopy

^{1}H NMR spectra are expected for every compound unless the compound has no protons (however, you should take a ^{1}H spectrum of these compounds to be certain that you've made something with no protons and that you've removed all residual

solvent). Since the ^{1}H nucleus is very sensitive, these spectra can be obtained using a low number of scans for small molecules - the default of 16 scans is usually enough. The ^{1}H range appropriate for most compounds is -1 to 12 ppm, and a relaxation delay of 0.1 s is usually appropriate.

For ^{1}H NMR spectra of **polymers**: spectra can be obtained as above for chemical shifts, but to integrate the polymer peaks the relaxation delay during acquisition needs to be increased from the default of 0.1 s to at least 10 s because aggregation/folding of the polymer in solution reduces the ability of nuclei to relax.

For ^{1}H NMR spectra of **any organic radical or organometallic complex**: the acquisition range should be increased to -10 ppm—60 ppm to ensure that any paramagnetic peak is captured in the spectrum (these usually appear at shifts >13 ppm).

^{13}C NMR Spectroscopy

^{13}C NMR spectra are strongly recommended for compounds that contain a small number of diagnostic protons and needed for all compounds that will be reported for publication. All instruments in the NMR facility are capable of taking ^{13}C NMR spectra, but Tellurium is most sensitive for carbon (followed by Yb, Co, I400, Ga, and least sensitive is the I500). The ^{13}C range acceptable for most compounds is 0 to 220ppm.

Generally, expect to have the instrument for at least 20 min to take a ^{13}C NMR spectrum, although if the sample is concentrated enough it can take as little time as 2 min. Change the relaxation delay from the default of 0.1 s to *at least 1 s* and the pulse angle from the default of 45° to 30° to increase the signal/noise ratio. A good looking ^{13}C spectrum will have peaks that are at least as tall as the baseline.

For spectra of compounds that have no C—H bonds, the ^{13}C peaks will be low intensity (requiring more scans). For spectra with C—F bonds, expect to see splitting of the peaks due to fluorine coupling; a CF_3 group usually appears as a quartet on ^{13}C NMR spectra.

Do not expect to be able to integrate ^{13}C NMR spectra unless the relaxation delay is very long or a relaxation agent (like $Cr(acac)_3$) is used.

^{19}F NMR Spectroscopy

^{19}F NMR spectra are expected for every fluorine containing compound. ^{19}F spectra can be acquired as quickly as proton spectra and can be integrated easily to determine the ratios of inequivalent fluorines present. Most ^{19}F peaks appear as negative values relative to the reference compound, $CFCl_3$. ^{19}F has a wide range (200 to -880 ppm), but for most organic fluoride compounds, 0 to -220 ppm is an acceptable range and a relaxation delay of 0.1s is appropriate.

^{31}P NMR Spectroscopy

^{31}P NMR spectra are expected for every phosphorus containing compound. ^{31}P NMR spectroscopy has a wide range of -400 ppm to 300 ppm, and can be integrated to determine the ratio of inequivalent P atoms in a sample. The shifts of P(III) compounds, like phosphines, and P(V) compounds, like phosphine oxides, can overlap and are non-intuitive (PPh_3's shift is about -6 ppm and the oxide, $O{=}PPh_3$'s shift is higher at 27 ppm. Conversely, $P(OPh)_3$'s shift is 138 ppm while the oxide, $O{=}P(OPh)_3$, is lower at -17 ppm).

For non-symmetrical bidentate phosphine nickel compounds, the P,P coupling constants can be diagnostic of the oxidation state of Ni. To understand the effect, look up the $^2J_{p,p}$ coupling constants of compounds similar to the complex you are synthesizing (with similar oxidation state and similar backbone length (propane is not comparable to ethane)).

Other Nuclei

NMR spectroscopy of other nuclei are possible but may require specific instruments - talk to the NMR technicians if you need to do one of these experiments. For ^{10}B or ^{11}B NMR spectroscopy, spectra should be taken in quartz NMR tubes (since most NMR tubes are made from borosilicate glass and have boron in them). For ^{17}O or ^{29}Si NMR spectroscopy, spectra should be taken in PTFE NMR tubes (since quartz and borosilicate have Si and O atoms in them).

No-D NMR Spectroscopy Using Solvent Suppression

Deuteriumless NMR spectroscopy, or No-D NMR spectroscopy, is a technique that suppresses specific, user-defined peaks in the ^1H NMR spectrum. It is most useful for when an NMR spectrum cannot be obtained in a deuterated solvent for some reason

(ex. Many experiments are done in THF, but d_8-THF is incredibly expensive). Without suppression, the signal from the solvent will be so intense that you will be unable to see your desired compound no matter how many scans or how far you zoom in because the signal from those peaks make up a small fraction of the FID curve. There is a manual on the NMR homepage describing how to perform solvent suppression. To maintain high ethical integrity, you should save the spectra obtained both with and without solvent suppression because solvent suppression can be used to suppress any peak, not just the solvent. To get the best resolution on the peaks you care about, choose the solvent wisely - use benzene or trifluorotoluene as a solvent if you care about peaks below 5.0 ppm, and use THF or hexanes if you care about peaks above 6.0 ppm.

2-dimensional NMR spectroscopy

2D experiments can tell you about whether two nuclei are "correlated" - that is, whether they're close enough that their electronic environments are coupled. Three common types are COSY, HSQC, and HMBC. A more in depth source covering 2D NMR can be found here:

http://www.people.fas.harvard.edu/~ekwan/pdfs/15%20-%20NMR%20II.pdf

COSY: correlation spectroscopy

COSY experiments show correlation between atoms of the same nuclei - generally these experiments are used for proton-proton coupling and have both horizontal and vertical axes as proton spectra. Peaks that are on the diagonal of the spectrum are trivial (saying that protons are correlated with themselves) but peaks that appear off diagonal either are neighbors or share an aromatic ring. This technique is useful for showing the exact protons that are correlated (rather than relying on coupling constants alone).

HSQC: heteronuclear single bond quantum correlation spectroscopy

HSQC experiments are like COSY, showing correlation between nuclei, except that it is used to probe correlation between different nuclei. The horizontal axis is usually a proton spectrum, and the vertical axis is a different nucleus. This experiment is usually used for ^{1}H-^{13}C or ^{1}H-^{15}N coupling and it tells you exactly which protons are attached to which carbons (or nitrogens). It only gives information about pairs of nuclei separated by a single bond, so it only shows correlation for C–H and N–H groups; CR_4 and NR_3 groups do not give peaks in HSQC.

HMBC: heteronuclear multiple bond correlation spectroscopy

HMBC is a technique that shows correlation between two nuclei that are separated by up to 3-bonds. For example, a $^{1}H/^{13}C$ HMBC shows a proton spectrum on the horizontal axis and a ^{13}C axis in the vertical, and cross peaks link protons and carbons that are separated by up to three bonds. In our lab, $^{1}H/^{31}P$ HMBC has been used to establish the formation of off-cycle complexes during CTP.

Other Experiments

NOESY: Nuclear Overhauser Effect spectroscopy

NOSEY relies on the nuclear overhauser effect in which two nuclei that are spatially close can transfer magnetization to each other, regardless of whether they are bonded or not. If you selectively excite one nucleus, and see a peak on the NOESY corresponding to another nucleus, you can assume that the nuclei are close in space.

DOSY: Diffusion ordered spectroscopy

DOSY is an experiment in which a spatial gradient in a magnetic field is applied to a sample, then is altered over time to give spatial information about the nuclei in a sample. This spatial information can then be analyzed to give information about diffusion of different species in the sample, and thus give diffusion constants. Check the UMich NMR manual on DOSY for more info.

NMR Tubes

There are three types of NMR tubes in our lab: normal, non-sealable NMR tubes, and the air free tubes: screw-capped tubes and J. Young tubes

For most samples, the non-sealable NMR tubes will be fine to use. Before using one of these, make sure that the tube is free of solvent (by drying in an oven) and free of residues and particulates (otherwise, these might show up as impurities in your spectrum).

Air Free NMR Tubes

Screw-capped NMR tubes and **J. Young tubes**

The department glassblower maintains that both types are equally air-tight.

Screw-Capped NMR Tubes

These NMR tubes are useful because the cap can be changed to suit the experiment. A **bakelite cap** (pictured above) will give you the best seal but will not enable you to add reagents outside of the box. A **septum cap** may give a worse seal but enables you to inject reagents via syringe, which can be useful for kinetics experiments.

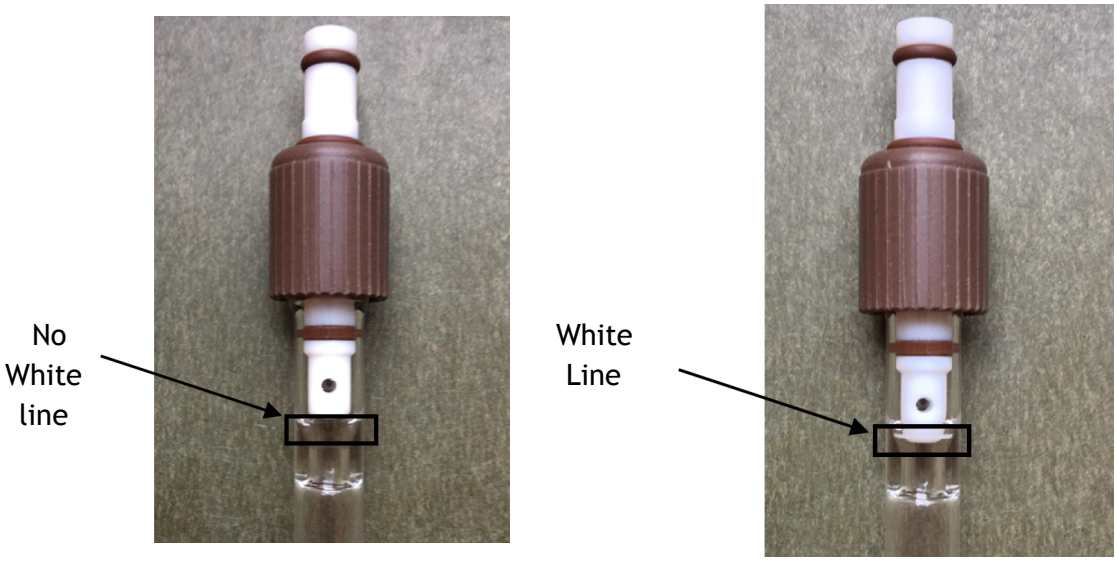
Screw cap NMR tubes should be loaded with a sample in the glovebox, sealed using either cap, then covered with electrical tape for a greater seal before taking them out of the glovebox. Removing tape too quickly can cause you to break the NMR tube, so be careful.

J. Young NMR Tubes

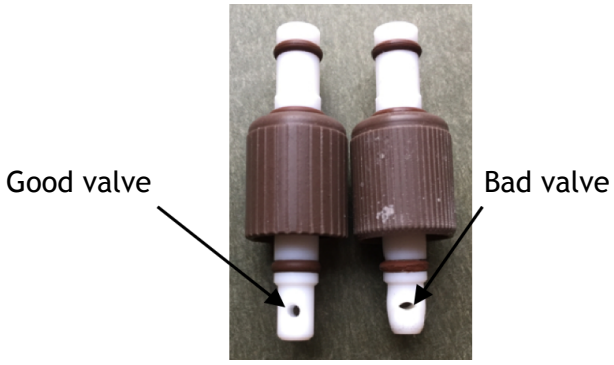
J. Young refers to the glassblower who invented the valve on the top of the tube. These NMR tubes are used to keep samples under nitrogen at normal pressure, but the valve that seals the tube can be connected to a Schlenk line using a Schleck-to-J. Young adapter to:

1. keep samples under vacuum or reduced pressure
2. freeze-pump-thaw solvents for NMR-scale studies
3. keep samples under an increased pressure atmosphere of nitrogen or other gases (i.e. H_2, ethylene, CO_2, etc.).

After loading the tube with your sample, the valve should be screwed on with two fingers only (*vide infra*). You will know that the tube is sealed when a small white line about 1mm thick appears at the point where the Teflon touches the glass of the tube:



We've had a problem with over-tightening the Teflon screw valves. You can tell if a Teflon valve has been over tightened by the shape of the hole at the bottom of the valve – if it's a circle, it's fine to use. If it's an oval, it's been crushed through over tightening at some point in the past, and may not seal the tube as well.



Note: if you are using a J. Young tube on the 700 MHz Ytterbium instrument for a ^{19}F NMR study, poly(tetrafluoroethylene) in the cap can cause the baseline to curve up in the -60 ppm to -100 ppm region.

Storing NMR Tubes

We store both types of air-free NMR tubes in the gloveboxes. If there are none in your glovebox, check the ovens. To bring tubes into the glovebox:

1. Place the tube in an oven set to >130 ºC overnight, and any caps or valves into a desiccator overnight
2. Quickly walk the tubes from the oven (and caps/valves from the dessicator) to a glovebox antechamber, and place them under vacuum in the antechamber for at least five min. Refill the chamber with nitrogen, and repeat for at least two more cycles.
3. Bring the tubes into the glovebox, and place the tubes and caps into the place horizontally in the designated bin in the glovebox

Feel free to use as many as needed, but return them to the box as soon as possible keeping in mind that there are a limited number of air-free NMR tubes in the lab.

Cleaning NMR Tubes

No seal is perfect, so air and moisture will get into the sample slowly. As such, air-free NMR tubes should be cleaned as soon as the sample is no longer needed. Otherwise, elemental nickel could deposit onto the sides, Grignard and organolithium reagents could eat at the glass, or phosphines and other organics could get absorbed by the cap and o-rings to contaminate future samples.

To clean an air-free NMR tube:

1. use an NMR tube cleaner with any solvent that can remove your sample, followed by water then acetone.
2. If there is still some residue, add at least 1.5 mL of concentrated hydrochloric acid to the tube and let it sit overnight, then rinse the tube out with water in the NMR tube cleaner.
3. If this does not get rid of the residue, soak it in a stronger acid such as sulfuric acid overnight, and rinse with water in the NMR tube cleaner.
4. If this does not work, ask the glassblower for help. Other methods that clean glassware might end up destroying the tube.

Any NMR tube (including normal, non-sealable NMR tubes) should **never** be sonicated, heated with a torch, or placed high temp oven (>300ºC) as these can change the thickness of the glass and can ruin the NMR tube. Additionally, NMR tubes should never come in contact with strong bronsted bases, fluoride bases, or hydrofluoric acid. Borosilicate glass is slightly acidic and the silicon centers in the glass can be

attacked by fluoride, so these reagents can eat away at the surface of the glass and destroy the tube or make the tube more fragile.

Broken NMR Tubes

As of January 2019, a J. Young tube costs about $60 for the glassblower to make. These are expensive and most labs have less than 10 of them. To prevent breaking an NMR tube, you should avoid applying force to the NMR tube perpendicular to its length. During transport, you should carry your tubes in a 250mL Erlenmeyer flask that is cushioned with paper towel. If you've broken an NMR tube, save the threaded glass part (this is the most expensive part of the tube, about $50) clean it, and bring it to the glassblower so a new tube can be attached.

Written by Matt Hannigan for the McNeil group in January 2019