Chem 728 Lecture Notes – Part 1b - NMR

The following are lecture notes for Chem 728 (by C. Martin, with minor modifications by L. Thompson). Much of the material is taken directly from the indicated references (old vH, some refs updated to new). This is not intended to replace the original references, but is made available solely for the convenience of students in the class.

VH = "Principles of Physical Biochemistry," Kensal E. van Holde, W. Curtis Johnson, & P. Shing Ho, Prentice Hall, NJ, 1998 (ISBN 0-13-720459-0)

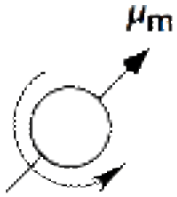
CS = "Biophysical Chemistry, Volumes I-III" Charles R. Cantor & Paul R. Schimmel, W. H. Freeman, NY, 1980 (ISBN 0-7167-1188-5, 0-7167-1190-7, 0-7167-1192-3)

Magnetic Resonance Spectroscopies (CS 9; vH 12)

Introduction - Spin

The classical / quantum mechanical spinning top

We discussed previously how a circulating charge produces an electric current. This current in turn produces a magnetic moment $\boldsymbol{\mu}_m$ with an angular momentum, **L**.



This is how most introductory NMR courses introduce *spin angular momentum*. Note, however, that this does **not** refer to an electron moving through space within its orbital - that is called *orbital angular momentum* and will come up later. The classical (not quite correct) explanation for spin angular momentum pictures the electron or nuclear proton "spinning about its own axis).

Note that for an individual particle (electron or atomic nucleus) quantum mechanics dictates that angular momentum is **quantized**. For the electron, angular momentum is quantized in units of the Bohr magneton β_e. Quantum mechanics further dictates that the magnetic moment is related to the angular momentum by a factor g_e.

For electrons: $\boldsymbol{\mu}_m = -\frac{g_e \beta_e}{\hbar} \mathbf{L} = \gamma_e \mathbf{L}$

where the Bohr magneton $\beta_e = 9.27 \times 10^{-21}$ erg gauss^{-1}

Similarly, for nuclei: $\boldsymbol{\mu}_m = -\frac{g_n \beta_n}{\hbar} \mathbf{L} = \gamma_n \mathbf{L}$

where the nuclear magneton $\beta_n = 5.05 \times 10^{-24}$ erg gauss^{-1}

Now, the gyromagnetic ratio $\gamma = \frac{\boldsymbol{\mu}_m}{\mathbf{L}} = -\frac{g_e \beta_e}{\hbar}$ (for electrons) or $-\frac{g_n \beta_n}{\hbar}$ (for nuclei)

, and can also be expressed as $\frac{ze}{2m}$ (it is also called the magnetogyric ratio in C&S) .

Quantum mechanics further tells us that **L** is quantized, having allowed values of:

$\hbar[I(I+1)]^{1/2}$ (nuclei)

or

$\hbar[S(S+1)]^{1/2}$ (electrons).

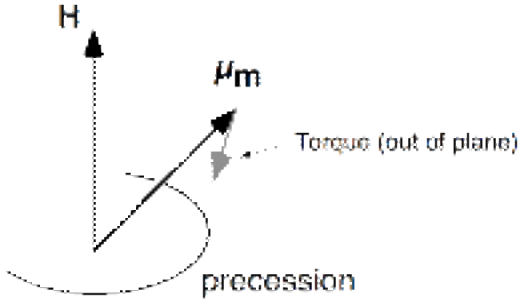
Therefore allowed values of $\boldsymbol{\mu}_m$ are $\gamma\hbar[I(I+1)]^{1/2}$ (nuclei) or $\gamma\hbar[S(S+1)]^{1/2}$ (electrons).

Magnetic moments interact with magnetic fields

The magnetic moment can interact with a magnetic field, **H**, to produce a torque perpendicular to the plane defined by **H** and $\boldsymbol{\mu}_m$.

$\boldsymbol{\tau} = \boldsymbol{\mu}_m \times \mathbf{H}$

This torque then acts to produce a change (d**L**) in the angular momentum according to



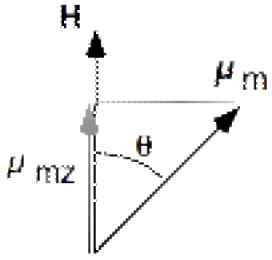
$$\frac{d\mathbf{L}}{dt} = \boldsymbol{\tau} = \boldsymbol{\mu}_m \times \mathbf{H} = \frac{ze}{2m} \mathbf{L} \times \mathbf{H} = \mathbf{L} \times \frac{ze}{2m} \mathbf{H} = \mathbf{L} \times \boldsymbol{\omega}_0$$

where ω_0 is termed the Larmour frequency. We say that the magnetic moment precesses about the applied magnetic field **H** with an angular velocity of precession of ω_0.

Nuclear magnetic moments - allowed values and quantized energy levels

The energy associated with a magnetic moment in an applied field is

$E = - \boldsymbol{\mu_m} \bullet \mathbf{H}$



Note that the dot product really means "the component along..." as shown in the diagram at right.

So that $E = - \mu_m \ H \cos \theta = - \mu_{mz} \ H$

μ_{mz} is the component of $\boldsymbol{\mu_m}$ along **H.**

But quantum mechanics dictates that μ_{mz} takes on discrete values according to

$\mu_{mz} = m_I \hbar$ where $m_I = I, I-1, I-2, ..., I-2I$

$E = - m_I \hbar H = -m_I g_N \beta_N H$

Note that the quantum mechanical restrictions on μ_{mz} require that $\boldsymbol{\mu_m}$ is always slightly off axis. Therefore, $\boldsymbol{\mu_m}$ always precesses about $\mathbf{H}_z$, as we saw before.

Magnetic properties of selected nuclei

Nucleus	I	γ (rad G^{-1} s^{-1})	% Nat. Abun	Rel. Sens.
^{1}H	1/2	26753	99.98	1.000
^{2}H	1	4107	0.016	0.0096
^{12}C	0			
^{13}C	1/2	6728	1.11	0.016
^{14}N	1	1934	99.64	0.0010
^{15}N	1/2	-2711	0.37	0.0010
^{16}O	0			
^{17}O	5/2	-3627	0.037	0.029
^{19}F	1/2	25179	100	0.834
^{23}Na	3/2	7076	100	0.093
^{31}P	1/2	10840	100	0.066
^{35}Cl	3/2	2621	75.53	0.0047
^{37}Cl	3/2	2182	24.47	0.0027

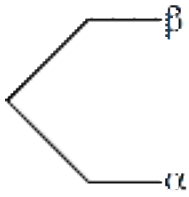
Nuclei with no net spin (I=0) are useless to us in NMR for obvious reasons. Nuclei with I>1/2, have an additional interaction known as the the nuclear quadrupole interaction, which greatly increases their relaxation rates, and therefore, as we will see later, their NMR linewidths. For this reason, NMR is most simple for nuclei with I=1/2. From the above, we see that the most useful biological nuclei are ^{1}H, ^{13}C, and ^{31}P, with ^{15}N being significantly less sensitive. Other nuclei have proven useful in special cases, notably ^{2}H, ^{19}F, ^{23}Na, ^{35}Cl, and ^{37}Cl.

The relative sensitivity above refers to the expected signal strength for samples with the same number of nuclei and is related to γ (see CS p. 489).

Spin-Spin Interactions - J Coupling

Remember the Nuclear Zeeman interaction:

$$E_{Zeeman} = m_I \hbar H = m_I g_N \beta_N H$$



A spin with $m_I = +1/2$ is said to be β, while a spin with $m_I = -1/2$ is said to be α.

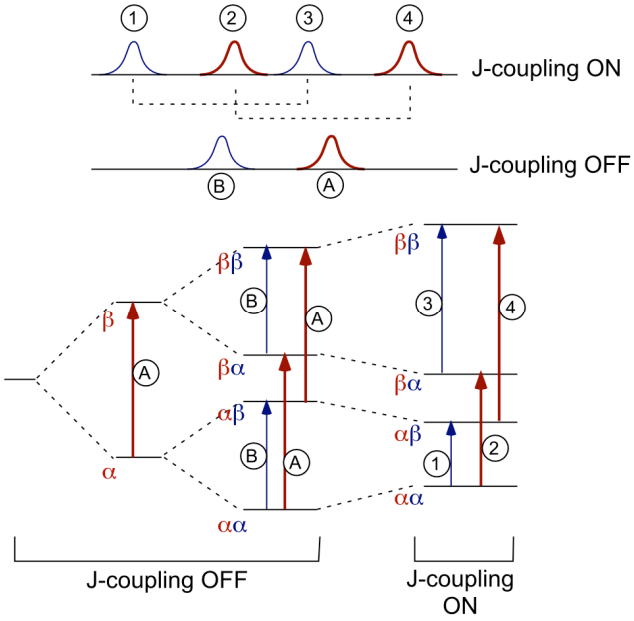
$m_I = +1/2$ (β) refers to a spin aligned *with* the field. This is a favorable interaction. Energy is lowered by this favorable interaction. Conversely for spin with $m_I = -1/2$ (α).

For the interaction between two non-equivalent spins A and B

$E_{AB} = \pm m_{I_A} m_{I_B} J_{AB}$ J_{AB} = spin-spin coupling constant

If one nuclear spin is aligned with the field, then the total field that the second nucleus feels is larger. If the second nuclear spin aligns with this field, it is stabilized more than it when aligned with the external field only. In other words, if the spins are both α (or both β), then the energy of the system decreases. However, if the spins are aligned opposite to each other, the energy of the system is increased relative to the energy for no interaction.

This yields the following for the total energy of the system



$$E_{Zeeman} = \pm m_{I_A} g_{N_A} \beta_N H \mp m_{I_B} g_{N_B} \beta_N H$$

$$E_{Zeeman+SpinSpin} = \pm m_{I_A} g_{N_A} \beta_N H \mp m_{I_B} g_{N_B} \beta_N H + m_{I_A} m_{I_B} J_{AB}$$

$$E_{Zeeman+SpinSpin} = \pm m_{I_A} g_{N_A} \beta_N H \mp m_{I_B} g_{N_B} \beta_N H + m_{I_A} m_{I_B} J_{AB}$$

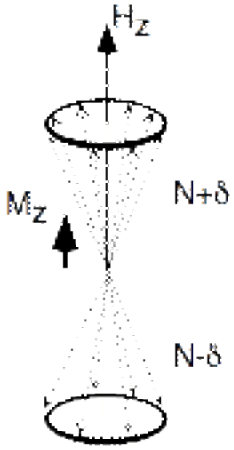
Magnetization

Many spins - bulk magnetization and the NMR experiment

N.B. The following assumes a spin system with I (or S) = 1/2. In this system, there are only two quantum mechanically allowed states (or energies). Analogous arguments hold for systems with I > 1/2, but there will be more allowed states.

Consider a collection of nuclei, each with magnetic moment $\boldsymbol{\mu_m}$. In the absence of a magnetic field, the magnetic moments are randomly oriented.

Now apply a field along the (laboratory) z-axis. The nuclei will tend to align along the axis of the field and will precess about this axis at the Larmour frequency, ω_o. They will eventually populate the allowed quantized energy levels according to the Boltzmann distribution. But how do they get there?



As with absorption energy levels seen before, the spins interchange between energy levels via thermal energy, that is, by interaction with the environment. As a consequence of these random interactions with the environment, the **total** magnetization (**bulk magnetization**) will increase with time according to

$M_z = \bar{M}_z \left(1 - e^{-t/T_1} \right)$ $M_x = M_y = 0$ (the field remains randomly oriented in the xy directions)

where T_1 is termed the "*longitudinal* relaxation time" (longitudinal refers to the direction of the field axis). Again, as we saw in absorption spectroscopy, the non-radiative mechanisms which couple the two states (and determine T_1) can be quite complicated and depend on exactly how the molecule interacts with its environment.

Note that the nuclei are precessing about the field axis, but since they have no phase relationship, the net magnetization in plane remains 0 (all contributions cancel each other in a randomly precessing group of spins).

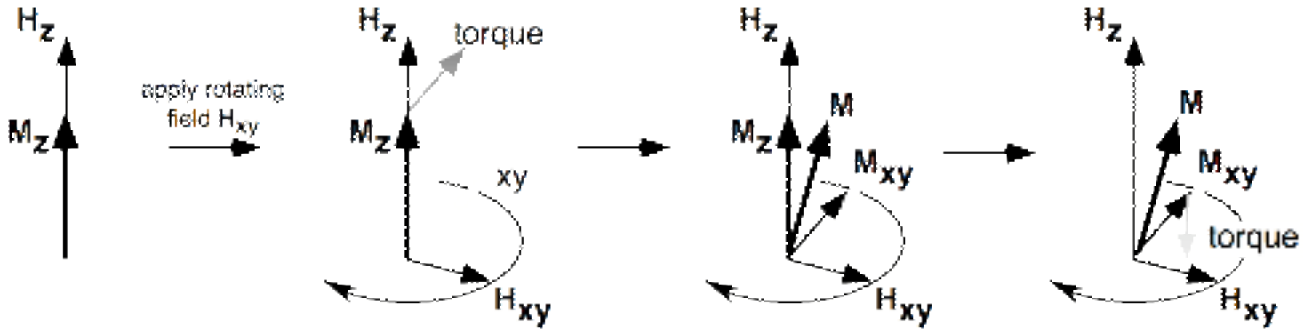
We can measure T_1 by applying a magnetic field at a given instant in time and then watching the growth of the magnetization along the axis of the field. This is not, however, how T_1 is normally measured.

The picture so far presented forms the basis for simple "continuous wave" (CW) NMR spectroscopy. For a system with I (or S) = 1/2, we have a system of two different quantized energy levels (spin up and spin down).

$\Delta E = \gamma\hbar H = \hbar\omega_o = h\nu_o$ for I (or S) = 1/2

Application of electromagnetic radiation of the appropriate energy (now in the radiofrequency region of the spectrum) can induce transitions between levels. Since there is a net population difference, we will have a net absorption of radiofrequency energy, which is measured in a manner analogous to before (now a coiled wire, an antenna, forms the basis for the generation and detection of the "light" (radiofrequency). Also note that in the absence of an applied magnetic field, there is no difference in energy between the two states (in fact, there is no direction about which to quantize the states). The energy gap between the two states is proportional to the applied magnetic field.

Transverse Magnetization - towards Fourier Transform NMR



In the previous picture, a system of spins under the influence of an applied magnetic field, H_z, gives rise to a bulk magnetization $\mathbf{M}_z$ aligned along the axis of the field $\mathbf{H}_z$. If we were now to apply a second field $\mathbf{H}_{xy}$, perpendicular to the first and rotating at the Larmour frequency, ω_o, there will be a torque exerted on the bulk magnetization vector according to

$\tau = \mathbf{M}_z \times \mathbf{H}_{xy}$

The direction of the torque will be perpendicular to the two vectors, and will tend to tip the magnetization away from the z-axis. This will, in turn, produce a net magnetization perpendicular to the z-axis, $\mathbf{M}_{xy}$. Moreover, this magnetization will be perpendicular to the field $\mathbf{H}_{xy}$. Since the in-plane field is rotating, we say that $\mathbf{M}_{xy}$ and $\mathbf{H}_{xy}$ are 90° out of phase.

But now another torque comes into play. $\mathbf{M}_{xy}$ and $\mathbf{H}_{XY}$ interact to produce a torque perpendicular to their plane (ie., along the negative z-axis). This torque acts to drive $\mathbf{M}$ along the negative z-axis. You can see that this is the mechanism whereby an applied RF field (with its oscillating magnetic field component) can induce transitions between the two quantum mechanical states (aligned with and against the applied magnetic field).

So we see that the application of a field $\mathbf{H}_{xy}$ perturbs the Boltzmann distribution of states quantized along $\mathbf{H}_z$. If we now suddenly turn off the field $\mathbf{H}_{xy}$, the system will return to its equilibrium distribution and by monitoring the return of the bulk magnetization, we can measure T_1 as before (this is a more common approach).

Note also that after the application of the field $\mathbf{H}_{xy}$, there remains a steady state component of magnetization in the xy plane, $\mathbf{M}_{xy}$. After $\mathbf{H}_{xy}$ is turned off, this

magnetization component will also decay, but now with a different time course, T_2. With time

$\mathbf{M}_{xy} = (\mathbf{M}_{xy})_0 \, e^{-t/T_2}$

The parameter T_2 is called the *transverse* relaxation time. The same interactions with the environment which give rise to T_1 relaxation contribute to T_2, but additional mechanisms exist for T_2 relaxation. Consequently, $T_2 < T_1$**.**

In order to maintain the 90° phase relationship between $\mathbf{H}_{xy}$ and $\mathbf{M}_{xy}$, the field $\mathbf{H}_{xy}$ must oscillate at the Larmour frequency ω_o. The energy of the radiofrequency radiation producing $\mathbf{H}_{xy}$ must be

$E = \hbar\omega_o$ which is the energy gap between the levels (see above).

Thus the "light" (RF) producing the transverse field $\mathbf{H}_{xy}$ must oscillate at the frequency ω_o which corresponds to the energy gap between the quantized states.

Steady-state absorption at resonance

We have mentioned above that the application of a field $\mathbf{H}_{xy}$ rotating at the Larmour frequency induces a component of the bulk magnetization in the xy plane. We have also discussed how this field can induce transitions among allowed $\mathbf{M}_z$ states, as for absorption spectroscopy. But as before, there are also thermal processes (non-radiative) which tend to drive the population of states back to the equilibrium Boltzmann distribution. These are T_1 processes.

Similarly, the equilibrium situation in the xy plane is $\mathbf{M}_{xy} = 0$. But the application of $\mathbf{H}_{xy}$ rotating at ω_o causes a net magnetization $\mathbf{M}_{xy}$ rotating 90° out of phase with $\mathbf{H}_{xy}$. Again, thermal processes (T_2) tend to drive this back to the equilibrium value of $M_{xy} = 0$.

As for absorption processes in the optical regime, as long as thermal processes can redistribute the system to near Boltzmann levels faster than you can excite them, you will continue to have a net absorption of energy (the energy is eventually being funnelled off into the thermal processes - heat).

Transverse phase

We instituted the requirement that $\mathbf{H}_{xy}$ be oscillating very near the Larmour frequency ω_o. We see from above $\hbar\omega_o$ ($h\nu$) corresponds to the energy between states. That makes perfect sense from what we've seen before.

Another way to look at this requirement is to examine the *phase* relationship between the involved vectors. When $\mathbf{H}_{xy}$ is applied, it generates a torque on $\mathbf{M}_z$ to create $\mathbf{M}_{xy}$ in plane and at 90° to $\mathbf{H}_{xy}$. $\mathbf{M}_{xy}$ will continue to precess at ω_o. As long as $\mathbf{H}_{xy}$ also rotates at ω_o, the two will remain at 90°. If $\mathbf{H}_{xy}$ is rotating at $\omega \neq \omega_o$, then the angle between them will slowly drift from 90°. But $\mathbf{H}_{xy}$ will continue to exert a torque on $\mathbf{M}_z$ and so will create a new $\mathbf{M}_{xy}$ at 90° to $\mathbf{H}_{xy}$. But this new $\mathbf{M}_{xy}$ will of course not align with the previously produced $\mathbf{M}_{xy}$ (no longer at 90° to $\mathbf{H}_{xy}$). You can see that the magnetization generated in the xy-plane will be random and will cancel itself out. The *net* magnetization $\mathbf{M}_{xy}$ will be 0.

The Bloch Equations Condensed

Cantor & Schimmel discuss an approach to understanding NMR first proposed by Bloch. We will skip over this formalism although students interested in seriously applying NMR will do well to read this chapter carefully (p. 493-498).

From this approach we have a formula for NMR signal strength:

$$\text{Signal} \propto N \frac{\mu_{mz}^2 H_z^2}{kT} \frac{\gamma^2 H_{xy} T_2}{1 + \gamma^2 T_2^2 (\omega_o - \omega)^2 + \gamma^2 H_{xy}^2 T_1 T_2} \qquad \text{I=1/2 only}$$

Note that, as expected, the signal strength is directly proportional to the number of spins in the sample, *i.e.* to the sample concentration. Also note that the signal increases as H_z^2, such that a doubling of field strength quadruples the signal.

Brief aside: "rates" (k) vs. "lifetimes" or "half-lives" (T_1 and T_2)

It is important to note that lifetimes are inversely proportional to rate constants $k = \frac{1}{T}$

Thus, faster (larger) relaxation rate = shorter (smaller) relaxation time (and vice versa).

T_1 - Longitudinal Relaxation - a.k.a. Spin-Lattice Relaxation Time

What happens when T_1 is "Large" ("*long*")? From the above equation: signal $1/\tau_1$

When the longitudinal relaxation time is long, thermal processes do not reequilibrate the levels efficiently so that application of $\mathbf{H}_{xy}$ causes the two levels to be more equally populated and we have fewer **net** transitions in an absorption direction. Signal decreases. This effect is called *saturation* and will prove very important in the future.

What is the basis of T_1 relaxation?

We know that non-radiative processes are due to interactions with the environment. For optical absorption, the mechanism of non-radiative mixing of the ground and excited states is often the interaction of the molecule with fluctuating dipoles in the environment. In the case of T_1, the mechanism of mixing is via interaction with fluctuating (randomly oriented) magnetic fields in the medium. To the extent that a neighboring field has a magnetic component along $\mathbf{M}_{xy}$ which is oscillating at the Larmour frequency ω_o, it can act just like $\mathbf{H}_{xy}$ to induce a transition between the quantized m_z states.

We call the environment the "**lattice**" (hence "spin-lattice relaxation) and its nature strongly effects T_1. In liquids or gases there is substantial molecular motion such that the local magnetic fields produced by neighbor molecules have a wide frequency distribution. This means that there will be a number of oscillating fields at ω_o and T_1 can therefore be small ("fast"), typically <10 sec. In a solid, those motions are severely restricted so that the low frequency fluctuations can be $<< \omega_o$. In this case T_1 can be hours.

N.B. - the rotation of neighboring molecules giving rise to fluctuating magnetic fields is *relative*, that is, it doesn't matter which of the two molecules is doing the rotating. Thus, if we are measuring T_1 of methyl group protons, even though the environmental magnetic fields may be rotating slowly with respect to the molecule (protein) to which the methyl group is attached, the methyl group itself may be rotating quite readily. Thus from its point of view, the environment is rotating rapidly - the magnetic fields that it "sees" are fluctuating at a high rate. Thus different atoms within the same molecule may have very different relaxation rates.

T_2 - Transverse Relaxation - a.k.a. Spin-Spin Relaxation Time

The frequency ω_o with which spins precess about the applied field is a function of that field $\mathbf{H}_z$. But the effective field that an individual spin "sees" is influenced by the environment, and so can be written $\mathbf{H}_z + \Delta\mathbf{H}_{loc}$. Just as the field will be heterogeneous due to local environement, so too will be ω_o. This means that spins in the xy-plane will precess at individual frequencies, $\omega_o \pm \omega_{loc}$.

In addition, this group of spins will not retain their phase relationship and M_{xy} will decay once the tipping field is turned off.

This loss of phase occurs via two mechanisms:

1) $\Delta\mathbf{H}_{loc}$ due to small time-dependent fluctuating fields from the local environment. In other words, immediately after the spin "packet" is tipped into the xy-plane, the spins all have the same phase. But with time, one spin may have its $\Delta\mathbf{H}_{loc}$ altered, changing

at the same time, its phase. Even if it returns to its original $\Delta\mathbf{H}_{loc}$, its phase "memory" is lost.

2) spin exchange with neighboring nuclei. In this latter case, an two neighboring nuclei of opposite spin can exchange the sign of their spins (analogous to energy transfer we've seen before). But in the process of this exchange, the phase relationship that each spin had is lost. Similarly T_1 transitions between m_z levels also cause a change in spin and contribute to T_2 dephasing - T_2 will therefore always be at least as fast as T_1.

NMR Linewidths

Returning to the equation for signal strength, we can simplify it somewhat to reveal the effects of T_1 and T_2 on the NMR signal.

$$\text{Signal} \quad \frac{T_2}{1+T_2^2(\omega_o-\omega)^2+\gamma^2 H_{xy}^2 T_1 T_2}$$

$$\text{Signal}_{max} \quad \frac{T_2}{1+\gamma^2 H_{xy}^2 T_1 T_2} \quad \text{maximum signal}$$

at resonance, $\omega = \omega_o$ (the "peak" of the signal)

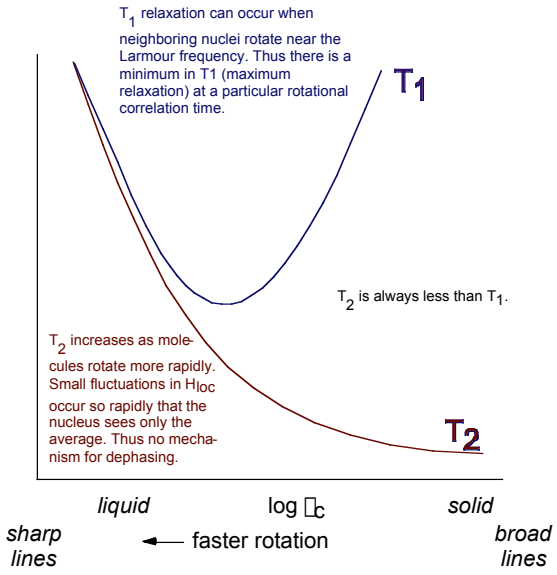
We can combine the two equations above to determine the linewidth $\Delta\omega = \omega_o - \omega$ at which the signal is half of its maximum value.

$$\Delta\omega_{1/2} = \frac{2}{T_2}(1 + \gamma^2 H_{xy}^2 T_1 T_2)^{1/2}$$

From the above, we can see that:

As T_2 gets small (solids), the linewidth goes as $1/T_2$: the linewidth *increases* (signal *broadens*) for decreasing values of T_2.

As T_2 increases (liquids; note that T_1 must also be increasing), The linewidth *decreases* (signal *narrows*).



Also note that the linewidth is independent of H_z.

Finally, note that when $\gamma^2 H_{xy}^2 T_1 T_2 << 1$, $\Delta\omega_{1/2} = \frac{2}{T_2}$ and Signal_{max} T_2

Molecular Rotation

If a molecule (or a molecular substituent) is rotating quickly enough in space, then during the time course of these measurements, a nuclear spin will only "see" an average environment (just like the rotational averaging we saw for Förster energy transfer). In this case, $\Delta\mathbf{H}_{loc}$ is the same for all nuclei of that type and T_2 dephasing is much less. Similarly neighboring nuclei spend too little time near each other to exchange spin. In this *extreme narrowing limit*

$1/T_2 \approx 1/(2\ T_1)$

A factor which often limits NMR of proteins is in fact the slow rate at which the macromolecule (and therefore the fixed nuclei within it) tumble randomly in solution. Again, note that even for a very slowly tumbling protein, protons on a surface methyl group may rotate at a much higher rate (and show sharp resonances). In solids we have the extreme limit of limited rotation, and consequently we generally see very broad spectra for solids. However, it is now possible to mechanically spin the sample at a frequency greater than T_2^{-1}. Actually, this is not completely true. To average out ^{1}H-^{1}H and ^{1}H-^{13}C interactions would require prohibitively fast spinning. These are averaged out by high power decoupling.

NMR - Properties of Molecules

Chemical Shift

So far, we have implicitely dealt with groups of identical nuclei. They may have been *randomly* in slightly different environments (thus giving rise to relaxational effects), but on average they all felt the same field. But in a real molecule with multiple nuclei, we know that some nuclei are different environments than others *in a well-defined way*.

For example, methyl protons are in a different environment than are amide protons. And in a given molecule or protein, certain amide protons will be in different environments than are other amide protons (interacting with solvent in a random coil, H-bonded to a amide carbonyl in an α-helix, etc.).

For this reason, not all protons feel the same net H_z. We can write:

$$H_z' = H_z - H_z\sigma = H_z(1-\sigma) \qquad \sigma = \frac{H_z - H_z'}{H_z}$$

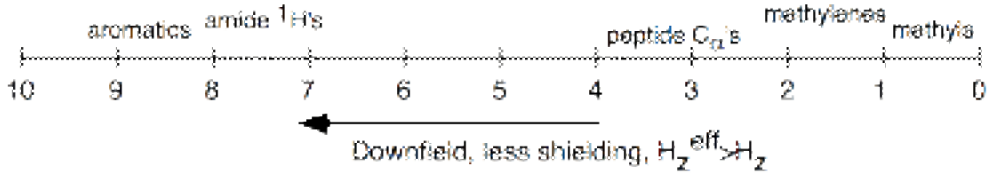
σ describes how much the local environment adds to (or subtracts from) the applied field H_z.

More generally, the additional field produced by the environment is compared to the effective field in a reference standard sample. In this case

$$\sigma = \frac{H_{ref} - H_{samp}}{H_{ref}} \times 10^6 = \frac{\nu_{ref} - \nu_{samp}}{\nu_{ref}} \times 10^6$$ expressed in *parts per million*.

where H_{ref} is the effective field felt by the reference nucleus and H_{samp} is that felt by the sample nucleus. Alternatively, one can speak about resonance frequencies, ν, at constant field.

Thus we have an NMR *spectrum*, most often plotted as a function of frequency or ppm. Individual protons in a molecule will resonate at individual frequencies in the spectrum.



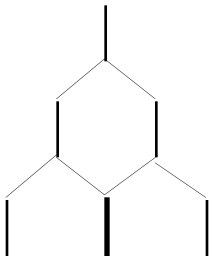
At a field strength of ≈100,000 guass, most protons resonate over a range of ≈5,000 Hz, centered around 500 MHz. Values of σ range between 0 and 10 ppm (relative to the protons of sodium 2,2-dimethyl-2-silapentane-5-sulfonate, DSS, or tetramethylsilane, TMS).

Ring current shifts - aromatic amino acids

We will not go into a detailed analysis of which groups show what type of shifts, but one effect can be very important in the study of proteins. For an aromatic compound such as benzene, we have seen that electrons reside in delocalized, circular π systems. In the presence of an applied magnetic field, these electrons are driven in a circular motion within these orbitals. This circulating charge produces an inductive magnetic moment. The resulting field adds to the applied field *outside* of the aromatic ring, and opposes the applied field within (above and below) the ring. Thus ring protons, which protrude on the outside edge of aromatic rings found in Phe, Tyr, etc, feel a field larger than applied magnetic field. This leads to a shift in σ of as much as 8-10 ppm.

Spin-spin interactions - splitting of resonance lines.

The environmental effects discussed above are due to interactions between nuclear spins and other factors in the environment - the magnetic field produced by an oscillating electric dipole or the magnetic moment produced by circulating electrons. Another very important interaction is that between two nearby spins. The magnetic moment associated with one spin adds to the effect magnetic field felt by the other and vice versa. From the point of view of one nuclear spin, the neighbor spin can be either aligned with or against the applied field (remember that it is quantized). So the field felt by the first spin is $H_z \pm 0.5J$, where J is called the (spin-spin) coupling constant and the *neighbor* spin has I=1/2. In the spectrum, this results in two absorbance lines centered at the original frequency and split by J.



You can readily see that for the interaction of one spin with two *identical* neighboring spins (I=1/2), the single transition will be split into two and each of those again split into two. Since the splittings are identical, we get the familiar 1:2:1 pattern. If the two neighboring spins are not identical, then the resulting pattern will be a doublet of doublets. Try this one for yourself.

Spin-spin splitting is very useful in NMR in that it tells us who is next to whom in the molecule. Such information is absolutely essential in assigning protein spectra. As you will see, the detailed mechanism of this interaction can be either through-space or through-bond.

Spin-spin splitting can sometimes get in our way as well. We have seen before that if a particular contributor to a spin's environment is fluctuating in its properties faster than the time course of the measurement, then the spin "sees" only the average of that fluctuation. In the case of one spin splitting another (different) spin, if we were to apply a second oscillating field at the resonance frequency of the second spin, then the first spin will see only an average - in this case since the two choices are spin up and spin down, the average will be zero. In this way, then the first spin is no longer split by the presence of the second spin. This general phenomenon is called **decoupling**.

Chemical Exchange

We have seen above that if a nucleus is flipping its spin very rapidly, then its neighbors will see an average of the flipping states (no spin, in this case). This averaging is a general effect. In fact, if a nucleus is jumping back and forth between two (or more) environments, the NMR properties of that nucleus will be an average of the two states (specifically, an average *weighted* by the proportion of time spent in each state).

Slow exchange

Consider a nucleus which can be in two chemical environments: A or B. Designate the lifetime in state A as τ_A and the lifetime in state B as τ_B. Also let the resonant frequencies of the nucleus in the two environments be ω_A and ω_B.

For conditions of "slow exchange," $\tau_A >> \frac{1}{\omega_A - \omega_B}$ AND $\tau_B >> \frac{1}{\omega_A - \omega_B}$

two distinct lines are seen in the NMR.

Moreover, their T_2 relaxation times are given by $\frac{1}{T_{2i}} = \frac{1}{T_{2i}^0} + \frac{1}{\tau_i}$

where $\frac{1}{T_{2i}^0}$ is the relaxation time of the nucleus in site i in the absence of exchange.

Fast exchange

If however the lifetime of the nucleus in each state is shorter than the precession time, then the lines will merge into a single line. Under these conditions, the T_2 relaxation time is

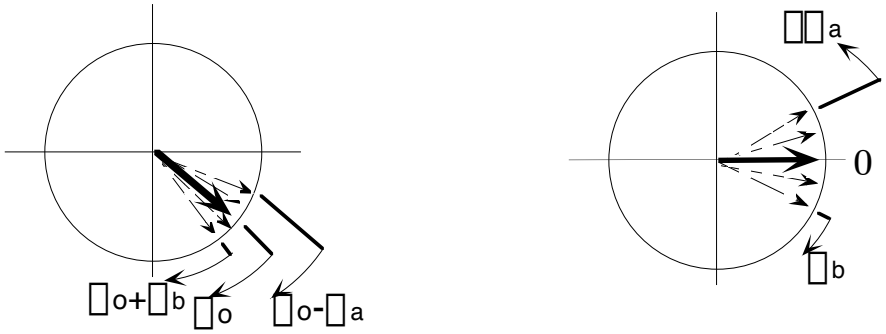
$$\frac{1}{T_2} = p_A \frac{1}{T_{2A}^0} + p_B \frac{1}{T_{2B}^0} + p_A^2 p_B^2 (\omega_A - \omega_B)^2 (\tau_A + \tau_B)$$

where p_A and p_B are the fractions of the nuclei in states A and B, respectively. This effect is called **exchange broadening**.

This phenomenon can often be used to measure *dynamics* in biological systems.

Fourier Transform NMR

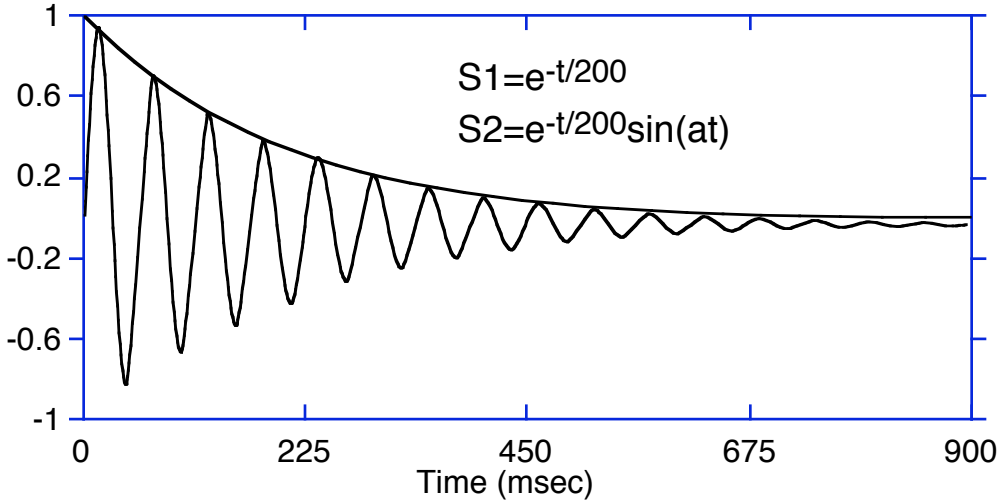
The Rotating Frame



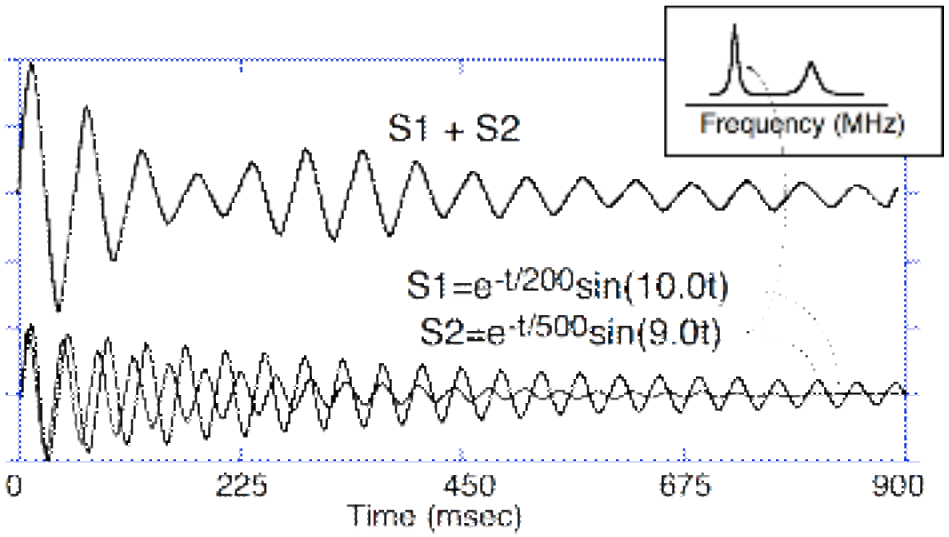
We have now seen that in a real NMR sample, there is a magnetization vector for each nucleus in the sample and that each of these vectors precesses about the applied field at a slightly different rate due to differences in environment. The figure at left illustrates several precessing spins in the xy-plane. If we fix our reference system on the central spin (*i.e.*, rotate our coordinate system at a frequency ω_o, then the rotating spins appear as at right. The spin that was precessing at exactly ω_o is now stationary. Spins precessing more slowly, now precess in a negative direction and those precessing at a higher frequency precesses in a positive direction. Note immediately following the initial 90° pulse which rotated the magnetization away from H_z, all spins are aligned. With time those that precess faster get away from those that precess more slowly. The actual precession angle as a function of time is given by $\omega_i t$.

Free Induction Decay

We saw before that if we tip the magnetization into the xy-plane, a component of the magnetization in the xy-plane precesses about the field. If we place a radio receiver antenna ("coil") in the xy-plane, this precessing magnetization will induce a sinusoidal current in the receiver, corresponding to its precession. We also saw that the *net* magnetization will slowy decay, as T_2 processes lead to a gradual dephasing of the spins. The resulting free induction decay, or **FID**, may look something like the figure below.



If there is more than one spin, then the signal will be a combination of more than one sine wave. Each with its own characteristic frequency and relaxational properties. The FID for two spins might look something like the one below.



Via a mathematical transformation of the data known as a Fourier transform, one can decompose the above FID into a frequency domain spectrum in which each peak in the NMR spectrum corresponds to a spin-generated sine wave above, and the linewidth of the signal reflects the relaxational properties we examined earlier (from the Bloch equation result above).

This is the simplest basis for an NMR experiment. But it gets more complicated... And the information gets richer...

First, let's look more at the Fourier transform. Note in the above figure that the sine wave which decays more rapidly (ie. the one with the shorter T_2), gives rise to a more broad signal in the resulting Fourier transform frequency domain spectrum. This is what the Bloch equations told us should happen.

We can manipulate FID's (and often do) in the computer to artificially alter the apparent linewidths of the NMR resonances. We saw before that it is an inherent property of the Fourier transform that fast decay in the time domain gives rise to broad resonances in the frequency domain, and of course the opposite is true. Indeed, if our spectrum is characterized by broad lines and we want to be able to distinguish peaks better, we can artifically multiply our expermental FID to decrease the early time signals and increase the later ones. This is typically done by applying a Gaussian muliplication to the FID. The result is an FID which decays more slowly. When we Fourier transform this, we get a spectrum with sharper peaks!

Why don't we just keep doing this more and more to get infinitely resolved peaks?! Notice that the beginning of the FID has more "information" in terms of the sine wave. The end of the FID has much less (typically we collect an FID until the signal has decayed completely). In the manipulation we did above, we decreased the early (signal-rich) part of the FID and increased the late (noise rich) part of the FID. Consequently, as we do this, we decrease the signal-to-noise ratio of our final spectrum. This limits how much we can artificially increase the resolution. There is no free lunch.

In fact, if we have a spectrum with good resolution but poor signal to noise, we can do the opposite. We can apply a exponential decay weighting function to our FID. This increases the signal-rich part of our FID and decreases the noise-rich part. The signal-to-noise ratio of the final spectrum increases (but our peaks broaden somewhat).

90° / 180° Pulses

90° PULSE - If we apply a very intense field H_{xy} for a very short (but specific) period of time, we can tip essentially all of the magnetization away from M_z. The angle by which the magnetization is tipped is given by $\theta = \gamma H_{xy} \Delta t$ (this can be readily derived from what we learned about torque on magnetic moments above). So we see that the angle is a function of both the intensity of the field and the time for which it is applied. If we tip

the magnetization 90° (a 1-200 μsec pulse under typical conditions), after the transverse field is gone, there will be magnetization in the xy-plane rotating at ω_o. As the T_2 phase decays, so too will the net magnetization in the plane (and so then will our signal). This signal is known as the **free induction decay**.

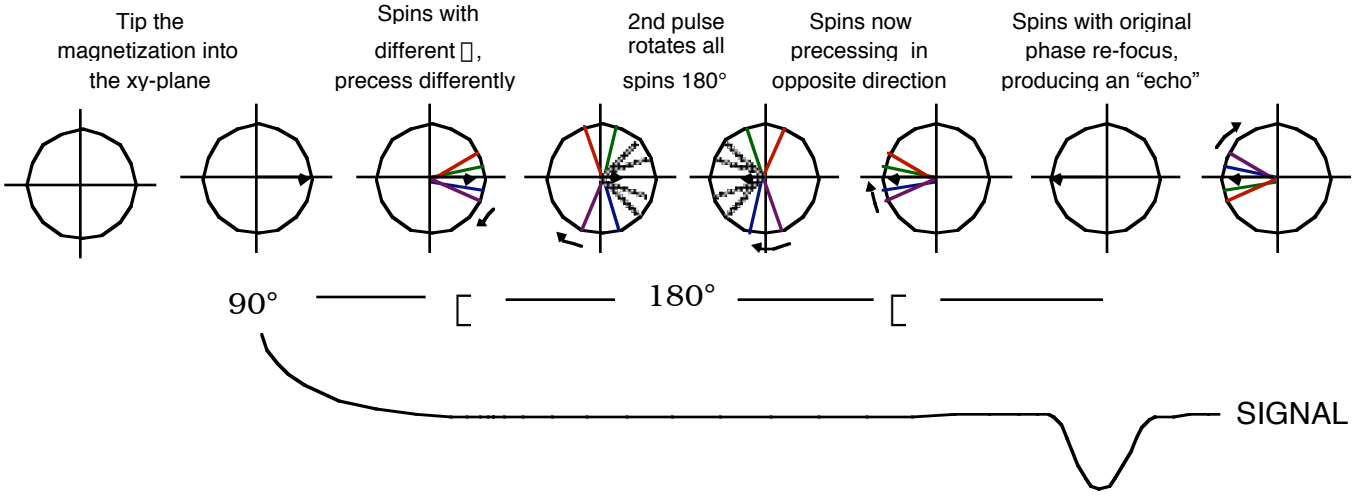
180° PULSE - In a similar fashion, we can apply twice the field strength (or the same field for twice the time) and tip the magnetization all the way around 180°. Note that in this experiment, we have taken the whole system from its Boltzmann distribution producing the original M_z to one with a net $-M_z$. At the end of the pulse, the system will slowly decay back to its Boltzmann value of $+M_z$ according to T_1.

Pulse Sequences: 180° - τ - 90° measures T_1

We can exploit a combination of pulses above, to measure T_1 and T_2. We just say that following the application of a 180° pulse, the net magnetization is changed from $+M_z$ to $-M_z$ and that with time this decays back to $+M_z$ according to T_1. But our detection scheme has relied on detecting a rotating magnetization in the xy-plane. At no time in this sequence, is there net magnetization in the xy-plane.

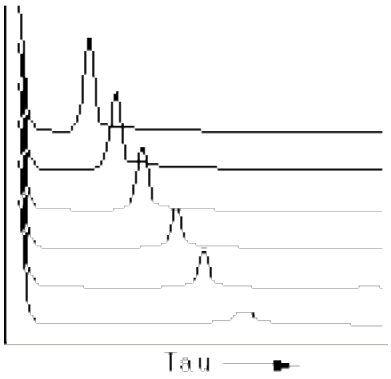
Now look at what happens if we apply a 180° pulse, followed immediately by a 90° pulse. This is essentially the same as a 270° pulse. All of the magnetization will be sent to the xy-plane and we will see a large signal in our detector. If however, we apply a 180° pulse and wait a short period of time (τ), the magnetization $-\mathbf{M}_z$ will decrease according to e^{-t/T_1}. If we then apply a 90° pulse, we will tip the remaining magnetization into the xy-plane and see a signal (now smaller, reflecting the fact that the tipped M_z was smaller). This is called a 180-τ-90 pulse sequence.

Pulse Sequences: 90° - τ - 180° - The "Spin Echo"



The above "pulse sequence" is the basis for much of modern FT NMR. It is very important to examine what is happening during the time course of this pulse sequence. We first apply a 90° pulse to tip the bulk magnetization into the xy-plane. As we have seen, the spins will precess about the field, but each at a slightly different ω due to slight differences in the chemical shift of each spin. If we wait a period of time τ the bulk magnetization will have spread out in the plane, reducing the overall signal, but in the absence of dephasing, each spin is precessing at a different, but *well-defined* frequency ω. If we then apply a 180° pulse, each spin will be rotated about y as shown above. Each spin is still precessing about the applied field at its own frequency, but now in the opposite direction that it had been. These means that each spin will retrace its steps and after a time (exactly) τ, they will all end up together (rotated 180° from where they were placed by the initial 90° pulse). Thus the spins will coalesce, returning the signal to its maximum value and producing an *echo*.

But we know that in a real system, T_2 processes will occur, leading some spins to lose their original phasing. Such spins will not coalesce with the rest to produce the echo at time 2□. Thus, if we plot the echo intensity as a function of □, the resultant trace will decay as $e^{-t/2\tau}$ as shown at right.



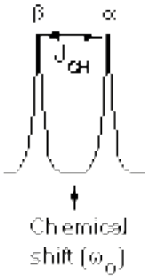
Field Inhomogeneity

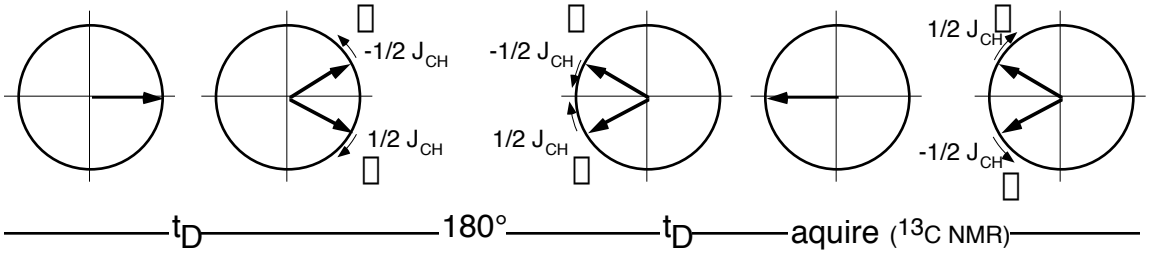
This pulse sequence is useful for another reason. NMR magnets are not perfect and it is impossible to design one such that every spin in the sample sees exactly the same H_z. One part of the tube will have a slightly different H_z (remember it only takes one part in a million to mess things up...). This means that protons which are chemically the same (eg. methyl group protons at the "3" position of our molecule) *should* precess at the same □$_o$ in fact will have different precession frequencies. This means that they no longer precess together and their bulk magnetization in the xy plane decays more rapidly than it intrinsically should - the signal for that proton is broadened and if we were to measure the simple decay to get T_2 we would get the wrong value.

In the pulse sequence above, after the first 90° pulse, these spins separate from each other in the plane. However, after the 180° pulse, they refocus exactly! Only true dephasing will keep them from refocusing and producing our spin echo. Thus measuring T_2 by the spin echo method gives us the true T_2.

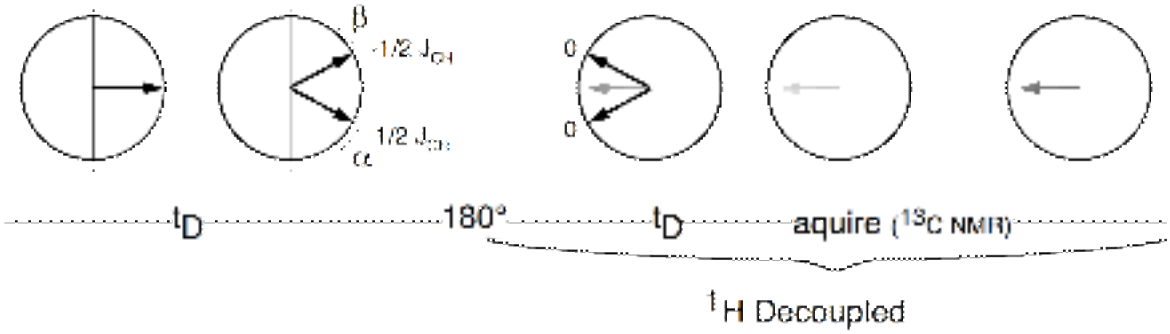
J-Modulated Spin Echo (Heteronuclear)

We have discussed how spin-spin interactions occur because one nucleus "feels" the magnetic field produced by a neighboring nucleus. Let's consider not just two different kinds (chemical shifts) of spins, but two different kinds of nucleus (eg. ^1H and ^{13}C) - heteronuclear coupling. As an example, let's look at the ^{13}C NMR spectrum of chloroform: $CHCl_3$. We know that the ^{13}C resonance will be split into two by interaction with the ^1H nucleus (which is either spin up or down). The ^{13}C resonances precess at □$_o$ ± J_{CH}/2, or in the rotation frame, they precess at +J_{CH}/2 and -J_{CH}/2.

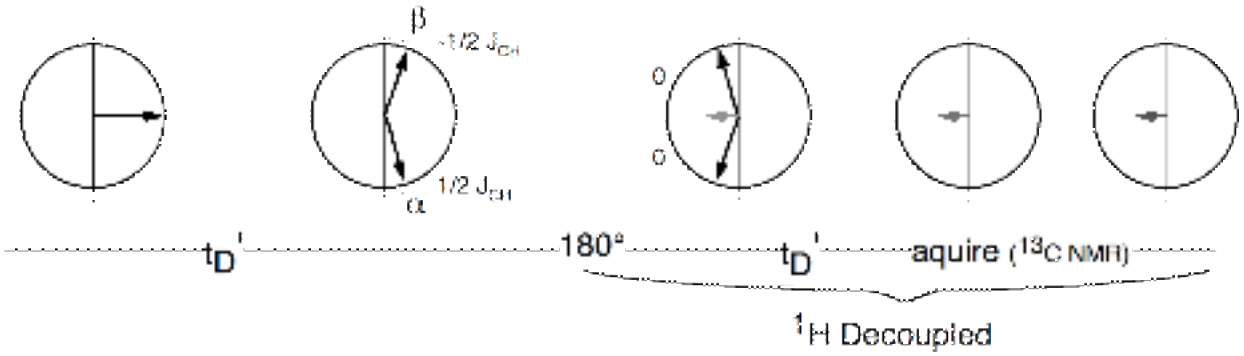




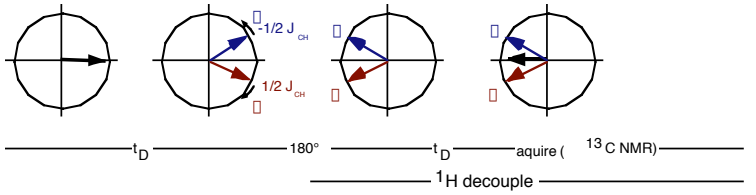
But now, let's repeat the experiment but applying ^1H decoupling throught the second delay (t_D) and the aquisition (but *not* during the initial delay. When decoupling is on, the two ^{13}C spins now precess at the original (chemical shift) □$_o$ (in other words they *do not* precess in our rotating frame). We get an FID from them, corresponding to a single peak centered at the chemical shift. But notice that the intensity of this peak is less than what it would have been had there been decoupling throughout the entire process.



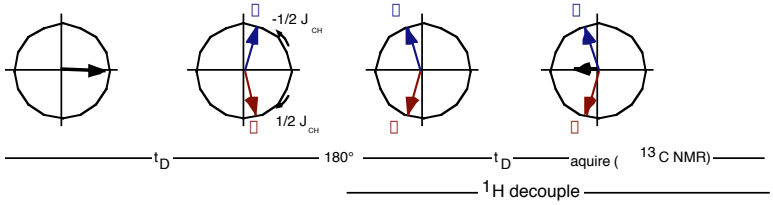
Now let's lenghten the delay time t_D:

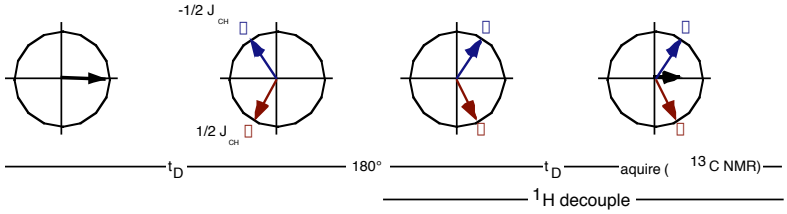


In fact, the extent to which this signal is decreased is a function of the delay time t_D and the J-coupling precession frequency ($J_{CH}/2$). Depending on their relationship, the resulting "static" vector can be large (1/2 J t_D = 0,2π), zero (1/2 J t_D = π/2,3π/2), or negative (1/2 J t_D = π).



We have talked before how the FID is simply a collection of sine waves and that when we Fourier transform this "time domain" we get a "frequency domain" spectrum. We can think of t_D as a new time variable. By convention, since t_D comes first it is often called t_1 (not to be confused with T_1 and the FID time domain is called t_2. When we FT time domain t_2 in this case each resonance will vary sinusoidally with t_1, with the frequency of that oscillation determined by the J/2 coupling (modulated by J coupling). So if we FT the entire set of spectra obtained obtained in the aquistion, we will get a frequency domain in this second dimension. 2□dimensional NMR!

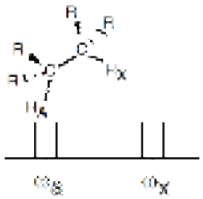




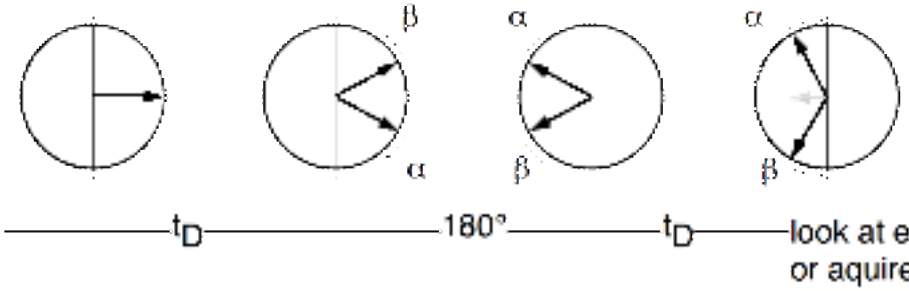
Note that the extent to which the spins have precessed during the delay is a function of their spin-spin (in this case) coupling strength and the delay time. This coupling will be different for different spins with different couplings.

Homonuclear J-Modulated Spin Echo

Now let's look at the same situation, but one in which protons split protons (homonuclear). Consider two spins A and X.

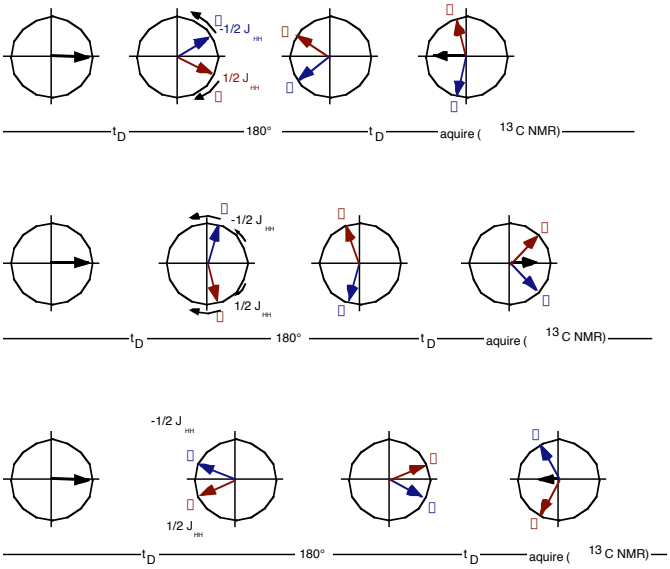


Let's look at our spin echo pulse sequence for the spin H_A. Use the rotating frame Ω_a.

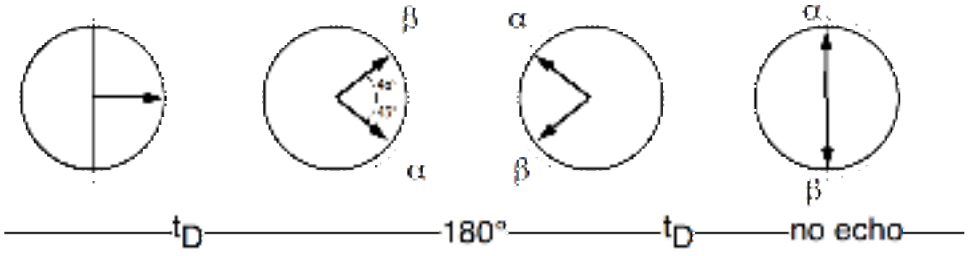


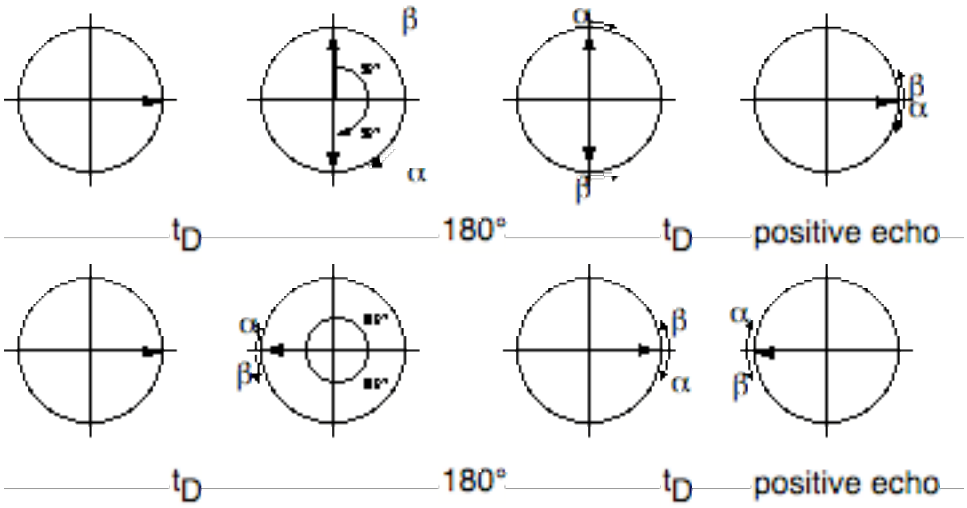
As before, we do a 90° pulse, followed by a delay t_D. During this time, the spins precess in opposite directions as before.

Now apply the 180° pulse. As before, the spins are flipped, but note that the spins that these spins are coupled to are also flipped (in other words the the H_A spin above labeled Ω originally saw the neighboring X spin as "up" - hence the term Ω). Now that same spins sees its neighbor "down" and so we now call that H_A spin Ω. The direction of the subsequent precession depends on this and do the spins precess in the direction opposite to what we saw before. They will in general ***not*** produce an intense echo after a delay t_D. You can also view this by noting that the spins are now **out of phase** with respect to before (by exactly 4π t_D J_{ax} radians).



Look at specific delay times:





Concept: Population transfer

Measuring Through-Bond Couplings - COSY

We saw before that spin-spin couplings (J-couplings) measure the interaction between two nuclei. In particular, these interactions occur *through-bond* - they require that the nuclei be connected by a small number of covalent bonds. Therefore, J-coupling information is valuable in assigning the covalent structure of a molecule.

A *two-dimensional* NMR technique called COrrelated SpectroscopY (COSY) measures J-couplings in a single set of pulse experiments. We do not have time to go into the mechanics of how this is done, but the end result is a two-dimensional spectrum in which chemical shift appears on both in-plane axes. The vertical axis contains a "spectrum" in which peaks occur only in the two one-dimensional spectra at at the intersections of two J-coupled transitions.

Measuring Through-Space Couplings - NOESY

We have seen before that two dipoles can interact in a purely *through-space* manner (for example, in Förster energy transfer). A similar interaction occurs in NMR to produce (mainly) relaxational effects. Another two-dimensional approach exploits this effect, such that cross-peaks in the two-dimensional spectrum reflect nuclei coupled via a dipole-dipole mechanism. This effect is called the Nuclear Overhauser effect, the spectrum is called NOESY.

ESR

Electronic Zeeman Interaction

$E_{Zeeman} = +m_s \beta H = +m_s g \beta H$

A spin with $m_s = +1/2$ is said to be □, while a spin with $m_s = -1/2$ is said to be □.

$m_s = +1/2$ (□) refers to a spin aligned *with* the field (as in NMR). Since the charge of an electron is negative, all interactions are opposite that which we described previously for interaction with an applied magnetic field. Energy is increased by this unfavorable interaction. Conversely for spin with $m_s = -1/2$ (□).

Spin-Spin Interactions - Hyperfine

For the interaction between an electron and a nucleus

$E_{hyperfine} = +m_s m_I A$ A = hyperfine coupling constant (compare with NMR's J)

If the nuclear spin is aligned with the field, then the total field that the electron feels is larger. This results in a relative destabilization of an electron which is also aligned with the field. In other words, if the electron and nuclear spins are both □ (or both □), then the energy of the system *increases* (opposite to NMR). However, if the spins are aligned opposite to each other, the energy of the system is *decreased* relative to the energy for no interaction.

This yields the following for the total energy of the system

$$E_{Zeeman} = -m_{I_A} g_{N_A} \beta_N H - m_{I_B} g_{N_B} \beta_N H - m_{I_A} m_{I_B} J_{AB}$$

Environmental Differences - g value

When we talk about local fields effecting the total field that a local electron feels, we refer to variations in g value. This is effectively like chemical shift in NMR. The difference is that variations in g value usually arise from orbital effects, rather than environment. Organic radicals are simple systems and the g value is very near that of the free electron (g_e). For metals, however, interactions between the magnetic moment of the electron and the orbital angular momentum of the electron can lead to large variations in g value.

g-anisotropy

Since g values in metals arises from interactions with the orbital angular momentum, it is not surprising that this interaction varies with the angle between the applied field and the electronic orbital of interest (for electronic orbitals above the fully symmetric s orbital). This is refered to g-anisotropy.

applications

radicals and metal centers in proteins
nitroxide radicals monitor motion

Do we want to cover chemical kinetics anywhere???