In Vivo NMR Spectroscopy & Imaging

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Basic Principles of In Vivo NMR Spectroscopy
Slide #1: Atomic particles with spin angular momentum behave like little magnetic tops that rotate around an axis within the particle. The tops behave like little bar magnets oriented along the axis of rotation. Particles with spin include electrons, neutrons, protons, quarks and nuclei with an odd number of protons and/or an odd number of neutrons. These particles are said to possess a magnetic moment, which is a vector quantity oriented along the axis of rotation. In vector terminology the magnetic moment $\mu = \gamma I$, where $\mu$ and $I$ are vectors pointing along the rotation axis, and $\gamma$ is a scalar called the magnetogyric ratio, a constant for any particle that equals the ratio of its magnetic moment to spin angular momentum. The magnitude of $I$ is an odd or even multiples of 1/2. The faster the particle spins, the larger the value of $\gamma$ and the larger is its magnetic moment.
Slide #2: In the absence of an external magnetic field (e.g. in outer space far away from the sun or any planet or in a specially shielded chamber) the nuclear magnetic moments are randomly oriented. The sum of their magnetic moments is zero because there is equal probability for each orientation, and hence the moments cancel each other out.
Slide #3: However in the presence of a magnetic field the nuclei exhibit a form of motion which no longer makes them randomly oriented. They spin around their intranuclear axis, but the axis itself rotates around the direction of the applied magnetic field. The locus of the head of the magnetic moment vector is a circle in a plane perpendicular to the external magnetic field. This motion is called precession.

If we designate the direction of the applied field (Ho) as Z, then the x- and y- components of the nuclear magnetization cancel because the various spins are rotating with random phase relative to each other (i.e., $M_x = \Sigma \mu_x = M_y = \Sigma \mu_y = 0$). However there is a slight “preference” for spins rotating in the direction of the applied field (i.e., +z direction; the energy in the +z direction is slightly less than the energy in the −z direction). Hence there is a net magnetization in the z direction (i.e., $\mu_z \neq 0$).

If all the nuclei are of the same type (e.g., protons) and we disregard the effects of the electrons and internuclear interactions for the present moment, then all the nuclei precess at the same frequency ($\omega_0$), which is proportional to the applied magnetic field, i.e., $\omega_0 = \gamma H_0$, where $H_0$ is the magnitude of the applied external field and $\gamma$ is the magnetogyrict ratio, a constant for a given nucleus. The precessional frequency is also called the Larmor frequency of the nucleus.
Slide #4: This motion is completely analogous to the motion of a spinning top or gyroscope in a gravitational field. The top spins around its axis, and the axis precesses around the direction of the gravitational field. The key difference in this analogy is that there is no “friction” for nuclear precession. The top gradually increases the angle between its axis and the gravitational field as it dissipates energy through friction and eventually collapses. The nuclear spin never loses energy (unless another field is applied) and keeps spinning at the same rate and same direction. (Actually Brownian motion will change the orientation of individual spins, but on average there is no change in relative distribution of spins in the ensemble.)
Slide #5: If all nuclei of a given type (\(^1\)H, \(^{13}\)C, \(^{15}\)N, \(^{31}\)P, etc.) precessed at a frequency characteristic only of that nucleus, NMR would be profoundly uninteresting to the chemist. It would only be a method for identifying specific isotopes, and would be a very inefficient method at that. Mass spectrometry is much more sensitive for this purpose.

Fortunately, electrons exert a very strong influence on the precessional (or Larmor) frequency. This is because electrons have much larger magnetic moments than nuclei (e.g. \(\mu_{\text{electron}} \sim 630 \times \mu_{\text{proton}}\)). Moreover, the magnetic field generated by electrons is extremely sensitive to the chemical environment: The total field around the nucleus (\(H_{\text{tot}}\)) is the sum of the applied field and \(H_{\text{loc}}\), the field generated by the electrons (of the nucleus or of a neighboring nucleus) i.e. \(H_{\text{tot}} = H_0 + H_{\text{loc}}\). Hence the Larmor frequency strongly depends on the chemical environment since:

\[ \omega = \gamma H_{\text{tot}} = \gamma (H_0 + H_{\text{loc}}) \]  

(1)
Slide #5 continued: Since $H_{\text{loc}}$ usually is negative, it serves to shield the nucleus from the external field. One can therefore describe the effect of the local field in terms of a shielding constant ($\sigma$) defined by:

$$H_{\text{tot}} = H_0 (1-\sigma) \quad (3)$$

And the Larmor frequency is:

$$\omega = \gamma H_0 (1-\sigma) \quad (4)$$

$\sigma$ is basically the chemical shift. It is measured in ppm since rearranging equation 3 we obtain:

$$\sigma = (H_0 - H_{\text{tot}}) / H_0 = (V_0 - V_{\text{tot}}) / V_0 \quad \text{where } V = \omega/2\pi \quad (5)$$

Chemical shift reference e.g. TMS
Slide #6: The NMR experiment consists of changing the orientations of the nuclear moments in order to produce a detectable change in sample magnetization that can be related back to the Larmor frequencies or $\alpha$-values of the various nuclei. This, in turn, will give us chemical information about the various nuclei in the sample. We will also soon see that it could provide spatial information about the sample.

There are two ways to describe how this is accomplished. The first and simpler explanation is in terms of quantum mechanics (in a very simple form). The second involves classical mechanics. The classical explanation is necessary to understand key phenomena like relaxation times that are involved in NMR imaging and also to understand Fourier transform NMR (FT-NMR) techniques.

Let's begin with the simplified quantum mechanical explanation. We can consider two basic spin states or eigenstates as the physicists call them. One is called the spin $+1/2$ state and precesses around the direction of the applied magnetic field (i.e. the $+z$-axis). The other precesses around the $-z$-axis and is called the spin $-1/2$ state. Here $\pm 1/2$ refer to what are called quantum numbers for the $z$-component of the magnetic moment. We are restricting ourselves to nuclei with spin angular momentum quantum number $1/2$ ($^1H$, $^{13}C$, $^{15}N$, $^{31}P$, etc.). Other nuclei have higher quantum numbers and it's a little more difficult to describe them, but the same basic principles apply.
The z-component of the magnetic moment of any spin 1/2 nucleus can be written in the form:

\[ \mu_z = \alpha \mu_z^{1/2} + (1-\alpha)\mu_z^{-1/2}, \quad 0 \leq \alpha \leq 1 \]

where the superscripts refer to the two eigenstates we've described above. In other words, the entire ensemble (i.e. sample) of spins can be considered as the weighted sum of the +1/2 and -1/2 eigenstates. It is much simpler and exactly equivalent to think of the entire ensemble (i.e. sample) consisting of just the eigenstates themselves (i.e. we have mixtures of only the +1/2 states and -1/2 states). The net magnetization of the sample is just difference between the spin up (i.e.+1/2) and spin down (i.e.-1/2) states. For protons at room temperature this is an extremely small member (about 5-6 spins for 10^6 spins). The spin eigenstates precess around the z-axis at the Larmor frequency. Their x- and y-components cancel. To generate a spin transition (or flipping of the states from the spin up to the spin down state), we apply a rotating field perpendicular to \( H_0 \) which rotates around the z-axis at an angular frequency \( \omega_1 \). As long as \( \omega_1 \) is different from the Larmor frequency (\( \omega_B \)), nothing happens. However when the frequency of \( H_1 \) matches the Larmor frequency (\( \omega_1 \approx \omega_B \)) the sample absorbs energy from the rotating field (which is in the radio frequency range and, hence, is called the rf field) and some of the spin up spins flip over and become spin down spins.
There is a net change in the magnetization of the sample which can be detected electronically (as we shall soon see). This phenomenon is called resonance because the rf field \( H_1 \) exactly matches the precessional frequency of the nuclei. Once the rf field is turned off, the sample returns to its equilibrium magnetization by the appropriate proportion of spins flipping from the higher energy spin down states \((-1/2)\) to the lower energy spin up state \((+1/2)\) until Boltzmann equilibrium is reestablished, i.e.,

\[
N_+ / N_- = \exp(\Delta E / kT)
\]

Where \( N_+ \) and \( N_- \) refer to the number of spins in the \( 1/2 \) and \(-1/2 \) eigenstates, respectively, and \( \Delta E \) is their difference in energy.

That's really all there is to NMR except for a lot of mathematical detail describing how \( H_1 \) causes these spin transitions and how the spins return back to equilibrium by two pathways, spin-lattice and spin-spin relaxation which are easier to visualize in the classical formulation (which will be described later).
Slide #7: This shows a schematic of a very simple NMR spectrometer. The sample solution is placed between the poles of the magnet in a cylindrical tube oriented perpendicular to the field of the magnet (H₀, which is in the horizontal direction). The probe consists of a simple solenoidal coil of copper wire wound around the sample. An rf oscillator is connected in series with the coil, producing a linear sinusoidal field around the sample.

\[ H_1 = A_0 \cos(\omega_1 t) \]

This field is just the sum of two rotating fields, one in the clockwise direction and the other in the counterclockwise direction since these fields can be as written in complex variables.

Clockwise:

\[ 1/2A_0 \exp(i\omega_1 t) = 1/2A_0 \cos \omega_1 t + 1/2A_0 \sin \omega_1 t \]

Counterclockwise:

\[ 1/2A_0 e^{i\omega_1 t} = 1/2A_0 \cos \omega_1 t - 1/2A_0 \sin \omega_1 t \]

sum = \( A_0 \cos \omega t \)
One of these fields will rotate in the same direction as the spins precess and the other will rotate in the opposite direction. Only the in phase component is relevant; the other has no effect. We place an ammeter in the circuit to monitor the current. If we vary $\omega_1$ while keeping $H_0$ constant, nothing happens until we are in resonance with one of the nuclei in the sample. Then the magnetization of the sample changes. We know from Lenz’s law that when a coil is placed in a magnetic field that varies, a current is induced that opposes the current in the coil. Hence the current in the ammeter decreases. This occurs every time our oscillator matches a Larmor frequency of a nucleus in the sample. If we plot current vs. frequency, we obtain a spectrum (see next slide). The same result can be obtained by keeping the rf constant at $\omega_1$ and varying the $H_0$ field until $\omega_0$ matches $\omega_1$. Either way we arrive at resonance, which triggers a change in current detected by the ammeter. The first experiment is called a frequency sweep experiment, whereas the second is called a field sweep experiment.
TYPICAL SPECTRUM

ENERGY ABSORBED

\[ \begin{align*}
H_0 \text{ or } I_0 & \rightarrow A \\
& \rightarrow \text{REFERENCE}
\end{align*} \]

\[ \leftarrow \nu (\text{Hz}) \text{ or } \delta (\text{ppm}) \]

CHEMICAL SHIFT

\[ \nu = \nu_0^{\text{ref}} - \nu_0^{A} \]

\[ \delta = \sigma_{\text{ref}} - \sigma_{A} = \nu / \text{oscillator frequency} \]

COMMON REFERENCES

\begin{align*}
\text{CH}_3 \\
\text{CH}_3 - \text{Si} - \text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_3 - \text{Si} - \text{CH}_3\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}^+ \\
\text{DSS (water)} \\
\text{(nonaqueous solvents)}
\end{align*}
Slide #8: Now let’s go back to the classical formalism. The spins are precessing relative to the z-direction. They have different magnitudes. Let’s move all their magnetic moment vectors to a common origin in a fixed coordinate system. The x- and y-magnetizations are random and therefore cancel out. There is, however, a finite z-magnetization denoted as $M_z = M_0 \kappa$

where $\kappa$ is a unit basis vector in the z-direction.
Slide #9: Next we apply a rotating field perpendicular to $H_0$. In a fixed coordinate system $H_1$ rotates at a frequency $\omega_1$ in the x-y plane. The laws of physics are very complicated in a fixed coordinate system if one is dealing with rotating fields (electrical, gravitational, etc.). The mathematics gets a lot simpler if we define a coordinate system rotating at the same rate as the applied rf field. In this system $H_1$ is fixed. Let’s define its direction as the $x'$-direction (where the prime indicates that we are in the rotating frame). The need for this transformation is apparent if we think about describing the effects of gravity on objects on earth. If we define our coordinates as fixed on the earth, gravity simply points down. To an observer in outer space using a fixed coordinate system, gravity at a specific position on earth is much more complicated to describe.
Slide #10: Now let’s consider the effect of $H_1$ on $M$. Classical physics implies that nothing happens so long as $\omega_1$ is significantly different than $\omega_0$. 
Slide #11: When $\omega_1 \sim \omega_0 = \gamma H_0$, resonance occurs and M rotates around the x-axis by an angle $\theta$ given by

$$\theta = \gamma H_1 t_p$$

where $t_p$ is the duration of application of the $H_1$ field. This field is usually applied as a pulse of short and precise duration.
Slide #12: Suppose we apply this pulse just long enough to flip M by 90° onto the y-axis. This is called a 90° pulse (typically on the order of 10 μsec). The y-magnetization is now $M_y$. 
Slide #13: Once $H_1$ is turned off, we again have a sample magnetization ($M=M_{xy}$) perpendicular to a constant field. This will again produce a rotation of $M_{xy}$ around the applied field ($H_0$, which is in the z-direction). $M_{xy}$ then rotates in the xy-plane at the Larmor frequency ($\omega_0$). The angle of rotation after some time $t$ is the net rotational frequency in the rotating frame ($\omega_0 - \omega_r$) times the time after the pulse was turned off ($t$).

However the magnitude of the magnetization vector ($M_{xy}$) does not remain constant but diminishes exponentially with time according to

$$M_{xy} = M_0 \, e^{-t/T_2}$$

where the time constant $T_2$ is defined as the spin-spin relaxation time or the transverse relaxation time. The reason why spin-spin relaxation occurs is depicted in the next slide.
Slide #14: Because magnets are never completely homogeneous, and because of interactions between spins of the same nuclear species, the various spins observe slightly different magnetic fields (even if they are chemically identical). They, therefore, precess at slightly different Larmor frequencies. The magnetization vectors tend to fan out in the XY plane and, hence, cancel each other out. This is obvious if you define an in-phase and out-of-phase coordinate for the wave packet. The in-phase coordinate corresponds to the center of the packet, and the out-of-phase is perpendicular to it. As time progresses, the in-phase coordinate gets smaller and the out-of-phase coordinate gets larger, but they have equal probability of their being positive or negative (hence they cancel out). When the spins totally dephase in the xy-plane, there is no net xy-magnetization. It is important to note that this process involves no energy transfer from the spins to the surroundings.
Slide #15: In NMR spectroscopy the signal is detected with a phase sensitive detector which measures magnetization 90° out of phase with the rf field (i.e. along the y'-direction in the rotating frame). This signal is proportional to $M_{xy} \cos \phi$ or using the values of these parameters defined in the previous figures the detected signal is proportional to $M_0 \exp(-t/T_2) \cos (\omega - \omega_x) t$. 
Slide #16: For a single spin this signal looks like an exponentially decaying sinusoid. We call it the free induction decay (FID). Note that time is the independent variable. To determine the frequency of this signal ($\omega - \omega_1$), we perform a Fourier transformation and obtain a single Lorenzian line.
Slide #17: This slide shows how Fourier transformation, a procedure that can readily be performed with a digital computer, determines the frequency of an FID. The top figure shows an FID with a low frequency. The second figure shows one with a higher frequency, and the third shows one with a still higher frequency. The fourth shows the composite FID obtained by combining all three of the above FIDs. Note that the FT of the composite FID produces all three peaks in the frequency domain that were obtained by FT of the individual FIDs.
Slide #18: This figure shows the FID obtained by adding together very many weak FIDs from the $^{13}$C signals of a sample of cholesterol acetate. The FT yields the complete spectrum of this compound. Note that for each chemically distinct nucleus there is a resolved peak (or resonance). The S/N increases with the square root of the number of scans. Since an FID can be measured in approximately one second, where a frequency or field sweep scan takes about 500 seconds, the FT methods permits very rapid averaging of many spectra and much more efficient improvement in S/N compared to field or frequency sweep NMR.
Table 1. Relative Sensitivities of NMR Nuclei

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Resonance Frequency @ 4T</th>
<th>Resonance Frequency @ 9.4T</th>
<th>Natural Abundance %</th>
<th>Relative Sensitivity per Nucleus</th>
<th>Relative Sensitivity at Natural Abundance</th>
<th>Spin Quantum Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>178.305</td>
<td>400.224</td>
<td>100</td>
<td>1.000</td>
<td>1.000</td>
<td>1/2</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>42.828</td>
<td>85.640</td>
<td>1.108</td>
<td>1.59×10⁻³</td>
<td>1.76×10⁻³</td>
<td>1/2</td>
</tr>
<tr>
<td>$^19$F</td>
<td>160.220</td>
<td>376.517</td>
<td>100</td>
<td>0.834</td>
<td>0.834</td>
<td>1/2</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>45.040</td>
<td>105.863</td>
<td>100</td>
<td>9.27×10⁻¹</td>
<td>9.27×10⁻¹</td>
<td>3/2</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>68.940</td>
<td>162.009</td>
<td>100</td>
<td>6.64×10⁻¹</td>
<td>6.64×10⁻¹</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Figure #19: This table summarizes the NMR properties of a number of nuclei.
Slide #20: (circa 1972)

This shows an old advertisement for a Bruker HX-90 spectrometer. On the left we have an electromagnet. The sample (out of sight) is between the pole faces. In the center is the console with the signal on the oscilloscope. This console generates the rf frequencies and records the spectrum. On the right is the computer that does the signal averaging and Fourier transformation. The teletype in the far rights is the input device.
Slide #21: This slide shows a more modern Bruker CXP-200/300 spectrometer equipped with a wide bore 4.7T (or 7.0T) magnet. It also shows Thian Ng, who was the manager of our NMR facility in Birmingham, Alabama. He’s showing off the tumor probe he built.
Slide #22: While all this decay of magnetization is occurring in the XY-plane, recovery of magnetization proceeds in the z-direction. This process is called spin-lattice relaxation because it involves energy transfer from the sample to the surroundings (in the lung or heart). It occurs at an exponential rate, i.e.

\[ M_z = M_0 \left( 1 - e^{-t/T_1} \right) \]

Where \( T_1 \) is the spin-lattice or longitudinal relaxation time.
Slide #23: Measurement of $T_1$ by an inversion recovery experiment: We apply a 180° pulse flipping the magnetization onto the - z-axis. This is followed by a delay during which time the magnetization decays from $-M_0$ to some smaller negative magnetization at $-M_z$ at $t=\tau$. We measure this magnetization with a 90° pulse. The experiment is repeated varying the delay between the 180° and 90° pulses. A plot of magnetization vs. time appears at the bottom of the figure. The exponential curve can be fit by non-linear regression analysis to measure $T_1$. 
Figure 2.4. The Hahn spin-echo experiment. (a) A 90° pulse applied along $x'$ at time 0 causes $\mathbf{M}$ to tip to the positive $y'$ axis. (b) The macroscopic magnetizations, $m_i$, of nuclei in different parts of the sample dephase as a result of the inhomogeneity in $H_0$. Those nuclei precessing faster than the average (the rotation rate of the frame) appear in the rotating frame to move toward the observer. Looking down from the positive $x'$ axis they appear to move clockwise, while those slower than average move counterclockwise. (c) A 180° pulse along $x'$ at time $\tau$ causes all $m_i$ to rotate 180° about the $x'$ axis. (d) The faster nuclei, still moving clockwise in the rotating frame, now go away from the observer, while the slower nuclei move counterclockwise toward the observer. (e) At time $2\tau$ the $m_i$ rephase along the $-y'$ axis. (f) At time $>2\tau$ the $m_i$ again dephase.

Slide #24: (see caption) This shows how a spectra experiment can be used to measure $T_2$ (more about this later).
Slide #25: This shows how $T_1$ and $T_2$ vary with the dipolar rotational correlation time. Small molecules rotate rapidly and exhibit short $T_{1,2}$. Note that in this limit $T_1 = T_2$. At longer rotational correlation times $T_1$ reaches a minimum and then increases. $T_2$ decreases monotonically. Note that $T'_2 \leq T_2$.

For proteins and other large molecules,

$$\tau_c \approx 10^{-8} \text{ sec} \Rightarrow T_2 \text{ very short} \Rightarrow \text{ very broad resonances}$$

This is why in in vivo NMR we see only small molecules.

END OF SPECTROSCOPY LECTURE