Nuclear Magnetic Resonance (NMR) Spectroscopy

Felix Bloch & Edward Purcell
Physics - 1952

Richard Ernst
Chemistry - 1991

Kurt Wuthrich
Chemistry - 2002

Paul Lauterbur & Peter Mansfield
Medicine - 2003

Brian Sykes

Lewis Kay
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What does NMR tell us ?

1) Primary structure characterization
2) Dynamics - psec-sec timescales
3) Equilibrium binding
4) Folding/Unfolding
5) Three dimensional structure

Some Advantages

1) Solution based
2) Non-destructive
3) Residue specific information
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<table>
<thead>
<tr>
<th>Nucleus</th>
<th>I</th>
<th># Protons</th>
<th># Neutrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1\text{H}$</td>
<td>$1/2$</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$^{12}\text{C}$</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>$^{13}\text{C}$</td>
<td>$1/2$</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>$^{14}\text{N}$</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>$^{15}\text{N}$</td>
<td>$1/2$</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Absorption of energy by nucleus - depends on nuclear spin (I)  
= sum of \textit{unpaired} protons + neutrons (spin 1/2)

$I \neq 0$ - NMR observed - spin will have magnetic moment $\mu = \gamma I$
For proteins $^1\text{H}, \; ^{13}\text{C}, \; ^{15}\text{N}$ ($^{31}\text{P}$)
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\[
\begin{align*}
I &= -\frac{1}{2} \\
S &= \alpha \\
\beta &= \beta \\
m &= (-I, -I+1, \ldots, I-1, I)
\end{align*}
\]
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Normal

\[ \mu = -\gamma I_z \]

Magnetic Field \( (B_0) \)

[Diagram showing normal and magnetic field]
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So how does this give us an “NMR signal”? 

\[
m_I = +1/2 \quad m_I = -1/2
\]
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This occurs for all $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$ in magnetic field $B_0$

- $^1\text{H}$: 26.75 $\gamma$ ($\times 10^7$ rad / T sec)
- $^{13}\text{C}$: 6.73 $\gamma$
- $^{15}\text{N}$: -2.72 $\gamma$
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\[
\Delta E = \gamma \hbar B_0 = h\nu \\
\nu = \frac{\gamma B_0}{2\pi} \text{ (Hz)} \\
\omega = \gamma B_0 \text{ (rad)}
\]

**Larmor Precession**

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>(\gamma \times 10^7 \text{ rad/T sec} )</th>
<th>(\nu) at 14.09 T</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H})</td>
<td>26.75</td>
<td>600.00</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>6.73</td>
<td>150.87</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>-2.71</td>
<td>60.82</td>
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</table>
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NMR is an insensitive method

<table>
<thead>
<tr>
<th></th>
<th>$^1$H</th>
<th>$N=10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.75T</td>
<td>18.79T</td>
</tr>
<tr>
<td></td>
<td>499,980</td>
<td>499,968</td>
</tr>
<tr>
<td></td>
<td>500,020</td>
<td>500,032</td>
</tr>
</tbody>
</table>
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Sensitivity

\[ n_\alpha - n_\beta = \Delta n = \frac{N\gamma\hbar B_o}{2kT} \]

\[ M_o = n_\alpha u_{z\alpha} + n_\beta u_{z\beta} = \Delta n u_{z\alpha} = \frac{1\gamma\hbar\Delta n}{2} = \frac{1N(\gamma\hbar)^2B_o}{4kT} \]
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Net Magnetization

Rotating Frame
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How is the NMR Signal Obtained?
- employ a rf pulse at $\nu$ of desired nucleus

![Diagram showing magnetic field orientations and rf pulse](image)
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\[ \theta = \frac{\pi}{2} \]

\[ \gamma B_1 = 41 \text{ kHz} \]

\[ \text{pw} = 6 \mu\text{sec} \]

rf on \hspace{1cm} \text{off}
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\[ M_z(t) = M_o(1 - e^{-t/T_1}) \]

\[ M_y(t) = M_y(0) e^{-t/T_2} \]
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<table>
<thead>
<tr>
<th>Molecular Weight</th>
<th>T&lt;sub&gt;1&lt;/sub&gt; (sec)</th>
<th>T&lt;sub&gt;2&lt;/sub&gt; (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW 100</td>
<td>0.5 Hz</td>
<td>10 Hz</td>
</tr>
<tr>
<td>MW 20,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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NMR Instrumentation

Magnet - $B_0$ 18.79 T

22.31 T (2006)
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Magnet Technology

![Graph showing cycles and peaks labeled I, II, III, IV.](image)
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Magnet Technology
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Probe Technology
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Cold “Cryogenic” Probe

\[
S/N \sim 1/\left\{ R_s \cdot (T_s + T_{pa}) + (R_c \cdot (T_c + T_{pa})) \right\}^{1/2}
\]

- \( T_{pa} \) and \( T_c \) - lowered to 298°C \( \rightarrow \) 20°C
- \( R_s \), \( T_s \) - near 298°C

3-4 times more sensitive

500 MHz + cold probe = 1.6x S/N 800 MHz
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Block Diagram of NMR Spectrometer

- Probe
- Transmitter
- Preamplifier
- Duplexer
- CPU
- Computer
- Receiver

Arrows indicate the direction of signal flow:
- obs, dec, lk from Transmitter to Duplexer
- obs, lk from Duplexer to Preamplifier
- obs, lk from Preamplifier to Receiver
- obs, lk from Receiver to CPU