Basic NMR theory

Lecture 1
Atom

◆ Has an electron cloud consisting of negatively charged electrons surrounding a dense nucleus.

◆ The nucleus contains positively charged protons and electrically neutral neutrons.

◆ When the number of protons in the nucleus equals the number of electrons, the atom is electrically neutral.

◆ Otherwise, it is an ion and has a net positive or negative charge.

◆ An atom is classified according to its number of protons and neutrons: the number of protons determines the “chemical element” and the number of neutrons determines the “isotope” of that element.

◆ Electrons determine the chemical properties of an element, and strongly influence an atom's magnetic properties.
Applications

**Structural Biology:** Proteins, DNA/RNA, Protein-DNA/RNA complexes, Protein-lipid complexes, and Polysaccharides

**Medicine:** Magnetic resonance imaging (MRI)

An example on medical use:

A distinctive chemical which is generally only seen in meningiomas
Chemistry: synthesis, pharmaceutical, quality control, structure, conformation, dynamics, kinetics, chemical exchange, equilibrium, molecular tumbling, etc...
Drug design: Structure Activity Relationships (SAR)

Petroleum industry: test water, oil, organic, inorganic composition.

Process control: mining, polymer production, cool analysis, cosmetics..

Food industry: drinks, solid foods, quality, contents, contamination..

Environmental: fertilizers, pesticides, pollution, heavy metals..

Natural product analysis: extracts, potential drug leads, etc..
History of NMR

1946 Bloch, Purcell  
First nuclear magnetic resonance (30 MHz)

1953 Overhauser  
NOE (nuclear Overhauser effect)

1966 Ernst, Anderson  
Fourier transform NMR

1975 Jeener, Ernst  
Two-dimensional NMR

1980 Kurt Wüthrich  
First solution structure of a small protein from NOE-derived distance restraints

1987/8  
3D NMR + $^{13}$C, $^{15}$N isotope labeling

1996/7  
New long-range structural parameters:
- residual dipolar couplings (also: anisotropic diffusion)
- cross-correlated relaxation
  TROSY (molecular weight > 100 kDa)

2003  
First solid-state NMR structure of a small protein

Nobel prizes

1944 Physics: Rabi (Columbia) [resonance method for recording the magnetic properties of atomic nuclei]

1952 Physics: Bloch (Stanford), Purcell (Harvard) [First NMR]

1991 Chemistry: Ernst (ETH) [development of NMR methodology]

2002 Chemistry: Wüthrich (ETH) [first 3D structures by NMR]

2003 Medicine: Lauterbur (Urbana), Mansfield (Nottingham) [MRI]
40 MHz NMR (1960)
First proton spectrum of ethanol @ 30 MHz

Ethanol proton spectrum @ 700 MHz
Details of the 20S proteasome structure: Quantification of dynamics and structure (670 kilodalton)

Why biomolecular NMR??

- Biological molecules such as proteins and nucleic acids can be large and complex. Knowing their structure is critical for understanding the relationship between structure and function.
- native-like conditions: solution, \textit{(in cell) NMR}
- many biological samples can be difficult to crystallize. e.g., nucleic acids structures are affected by crystal packing

- Limitation: molecular size. However, this is changing.
  
  Molecular weight: X-ray: $> 200 \text{ kDa}$,
  
  \begin{align*}
  \text{NMR} &< 50-100 \text{ kDa}, \\
  &\quad 900 \text{ kDa}!
  \end{align*}

- Ligand binding and molecular interactions in solution

- Characterization of dynamics and mobility (ps - days)
  - conformational dynamics, enzyme turnover, kinetics, folding
Structure determination by NMR

4D
A conceptual block diagram of the FT NMR
What is Spectroscopy?

• Spectroscopy is the study of the interactions between light and matter as a function of wavelength ($\lambda$).

• Light refers to any sort of electromagnetic radiation, such as visible light, UV, IR, and radiowaves.

• Depending on the frequency or wavelength of the radiation involved we can have different types of interactions with the matter (molecules).
Energy and frequency:

\[ \Delta E = h\nu \]

- \( h \) = Plank’s constant
- \( \nu \) = frequency

\( \lambda \)
NMR

• A physical phenomenon based upon the quantum mechanical magnetic properties of an atom's nucleus.

• Detects the absorption of radiofrequencies (electromagnetic radiation) by certain nuclei in a molecule.

• The nuclei of all atoms are characterized by:
  a nuclear spin quantum number (I)

• Only nuclei with spin number \( I \neq 0 \) can absorb/emit electromagnetic radiation. These should have an odd mass number.

<table>
<thead>
<tr>
<th>Mass number</th>
<th>Atomic #</th>
<th>( I )</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odd</td>
<td>Even or odd</td>
<td>1/2, 3/2, 5/2,…</td>
<td>( ^1\text{H}, , ^{13}\text{C}, , ^{15}\text{N}, , ^{31}\text{P} )</td>
</tr>
<tr>
<td>Even</td>
<td>Even</td>
<td>0</td>
<td>( ^{12}\text{C}, , ^{16}\text{O} )</td>
</tr>
<tr>
<td>Even</td>
<td>Odd</td>
<td>1, 2, 3</td>
<td>( ^{14}\text{N}, , ^2\text{H} )</td>
</tr>
</tbody>
</table>
The spinning nuclei possess angular momentum ($P$). Charge and motion of these nuclei gives rise to magnetic moment ($\mu$)

$$\vec{\mu} = \gamma \vec{P}$$

Vector quantities: They both have magnitude and direction

$\gamma = \text{gyromagnetic ratio}$: A measure of how “strongly magnetic” the atom is. It depends on the nature of each nuclei (constant for any given nuclei).

- Different nuclei have different magnetic moments.

- **Spin states** of the nucleus ($m$) are **quantized**:
  
  *magnetic quantum number* ($m$) = $2I+1$

  
  \[I, (I - 1), (I - 2), \ldots, -I\]

  
  For $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$, $^{31}\text{P}$ (biologically relevant nuclei with $I = \frac{1}{2}$):

  \[m = \frac{1}{2}, -\frac{1}{2}\]

  This means that these nuclei can have only two states (energy levels).
Atoms heavily Used in NMR?

Table 1-1.
Properties of some nuclides of importance in NMR spectroscopy.

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Spin</th>
<th>Electric quadrupole moment$^{(a)}$ [eQ] [10^-28 m^2]</th>
<th>Natural abundance$^{(b)}$ [%]</th>
<th>Relative sensitivity$^{(b)}$</th>
<th>Gyromagnetic ratio$^{(b)}$ γ [10^7 rad T^-1 s^-1]</th>
<th>NMR frequency [MHz]$^{(b)}$ ($B_0 = 2.3488$ T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>1/2</td>
<td>–</td>
<td>99.985</td>
<td>1.00</td>
<td>26.7519</td>
<td>100.00</td>
</tr>
<tr>
<td>$^2$H</td>
<td>1</td>
<td>$2.87 \times 10^{-3}$</td>
<td>0.015</td>
<td>$9.65 \times 10^{-3}$</td>
<td>4.1066</td>
<td>15.351</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>–</td>
<td>–</td>
<td>1.21</td>
<td>28.5350</td>
<td>106.664</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>$-6.4 \times 10^{-4}$</td>
<td>7.42</td>
<td>$8.5 \times 10^{-3}$</td>
<td>3.9371</td>
<td>14.716</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>$8.5 \times 10^{-2}$</td>
<td>19.58</td>
<td>$1.99 \times 10^{-2}$</td>
<td>2.8747</td>
<td>10.746</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>$4.1 \times 10^{-2}$</td>
<td>80.42</td>
<td>0.17</td>
<td>8.5847</td>
<td>32.084</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>1/2</td>
<td>–</td>
<td>98.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1/2</td>
<td>–</td>
<td>1.108</td>
<td>$1.59 \times 10^{-2}$</td>
<td>6.7283</td>
<td>25.144</td>
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<tr>
<td>$^{14}$N</td>
<td>1</td>
<td>$1.67 \times 10^{-2}$</td>
<td>99.63</td>
<td>$1.01 \times 10^{-3}$</td>
<td>1.9338</td>
<td>7.224</td>
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<tr>
<td>$^{15}$N</td>
<td>1/2</td>
<td>–</td>
<td>0.37</td>
<td>$1.04 \times 10^{-3}$</td>
<td>$-2.7126$</td>
<td>10.133</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>0</td>
<td>–</td>
<td>99.96</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$^{17}$O</td>
<td>5/2</td>
<td>$-2.6 \times 10^{-2}$</td>
<td>0.037</td>
<td>$2.91 \times 10^{-2}$</td>
<td>$-3.6280$</td>
<td>13.557</td>
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<tr>
<td>$^{19}$F</td>
<td>1/2</td>
<td>–</td>
<td>100</td>
<td>0.83</td>
<td>25.1815</td>
<td>94.077</td>
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<tr>
<td>$^{23}$Na</td>
<td>3/2</td>
<td>0.1</td>
<td>100</td>
<td>$9.25 \times 10^{-2}$</td>
<td>7.0704</td>
<td>26.451</td>
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<tr>
<td>$^{25}$Mg</td>
<td>5/2</td>
<td>0.22</td>
<td>10.13</td>
<td>$2.67 \times 10^{-3}$</td>
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<tr>
<td></td>
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<td>–</td>
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<td>19.865</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>–</td>
<td>100</td>
<td>$6.63 \times 10^{-2}$</td>
<td>10.8934</td>
<td>40.481</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>$5.5 \times 10^{-2}$</td>
<td>93.1</td>
<td>$5.08 \times 10^{-4}$</td>
<td>1.2499</td>
<td>4.667</td>
</tr>
<tr>
<td></td>
<td>7/2</td>
<td>$-5.0 \times 10^{-2}$</td>
<td>0.145</td>
<td>$6.40 \times 10^{-3}$</td>
<td>$-1.8028$</td>
<td>6.728</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>–</td>
<td>2.19</td>
<td>$3.37 \times 10^{-6}$</td>
<td>0.8687</td>
<td>3.231</td>
</tr>
<tr>
<td></td>
<td>7/2</td>
<td>0.42</td>
<td>100</td>
<td>0.28</td>
<td>6.3015</td>
<td>23.614</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>–</td>
<td>8.58</td>
<td>$5.18 \times 10^{-2}$</td>
<td>$-10.0318$</td>
<td>37.272</td>
</tr>
<tr>
<td></td>
<td>7/2</td>
<td>$-3.0 \times 10^{-3}$</td>
<td>100</td>
<td>$4.74 \times 10^{-2}$</td>
<td>3.5339</td>
<td>13.117</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>–</td>
<td>33.8</td>
<td>$9.94 \times 10^{-3}$</td>
<td>5.8383</td>
<td>21.499</td>
</tr>
</tbody>
</table>

*Values from [1, 2].
Values from the Bruker Almanac, 1992; sensitivity is expressed relative to $^1$H (= 1) for constant field and equal numbers of nuclei.
$^3$H is radioactive.
• For a particular nuclei, the **magnetic moment** \((\mu)\):

\[
\mu = \gamma I \hbar / 2\pi
\]

• When in a magnet, the energy of a spin \((E)\) depends on a static magnetic field \((B_o)\) and \(\mu\).

\[
E = -\mu \cdot B_o
\]

Tesla (T)
Magnetic energy and populations

- When $B_o$ is applied, spins have two possible energies.

\[ E = - \mu \cdot B_o \]

\[ E_\alpha = - \frac{\gamma h B_o}{4\pi} \]

\[ E_\beta = \frac{\gamma h B_o}{4\pi} \]

\[ \Delta E = E_\beta - E_\alpha = \frac{\gamma h B_o}{2\pi} \]

- The larger the $B_o$, the larger the energy difference.

- $\Delta E$ for $^1H$ at $B_o = 9.4$ T is $4 \times 10^{-5}$ Kcal/mol.
At equilibrium, there is excess of nuclei in the $\alpha$ state:

![Diagram showing Boltzmann distribution](image)

The **population ratio** between the two levels depends on $\Delta E$.

We apply Boltzmann distribution: a certain distribution function or probability measure for the distribution of the states of a system.

\[
\frac{N_\alpha}{N_\beta} = e^{\frac{\Delta E}{K_B T}} \quad \text{and} \quad \frac{N_\beta}{N_\alpha} = e^{-\frac{\Delta E}{K_B T}}
\]

$K_B =$ Boltzmann constant $= 1.3805 \times 10^{-23}$ JK$^{-1}$

Since $\Delta E$ is very small compared to the average energy of $K_B T$ thermal motion:

\[
\frac{N_\beta}{N_\alpha} = 1 - \Delta E / K_B T = 1 - \left( \frac{\gamma \hbar B_o}{2\pi K_B T} \right)
\]
Nuclei with larger \( \gamma \) will absorb/emit more energy, and will therefore be more sensitive. Sensitivity is proportional to \( \mu \) and \( \frac{N_\alpha}{N_\beta} \)

\[
\gamma(^{13}\text{C}) = 6,728 \text{ rad/G} \\
\gamma(^{1}\text{H}) = 26,753 \text{ rad/G}
\]

- \(^1\text{H}\) is \( \sim 64 \) times more sensitive than \(^{13}\text{C}\) only due to \( \gamma \).

- If we take into account the natural abundance, \(^{13}\text{C}\) (1.1\%) ends up being 6400 less sensitive than \(^1\text{H}\).

- \( \frac{N_\alpha}{N_\beta} \) ratio is only 1.000064. Calculate!

- In 1 million spins, difference is just 64
Precession

- In the presence of a magnetic field, two forces act on the spins: One that tries to align them with \( B_o \), while the other tries to maintain their angular momentum.
- The net result is that the nuclei spins trace a circular path about the applied field.
- This phenomenon is called **Larmor precession** (named after Joseph Larmor) or angular frequency, \( \omega_o \) (radians) or \( \nu \) (Hz):

\[
\omega_o = - \gamma B_o \text{ (rad s}^{-1})
\]

\[
\nu = - \frac{\gamma B_o}{2\pi} \text{ (Hz)} \quad \text{Larmor Frequency}
\]
The net magnetization vector

- There are several magnetic fields acting on the spins. One is $B_0$, which is constant and generates the precession at $\omega_0$. The others are fluctuating due to the molecular anisotropy and its environment, and make the spins ‘try’ all the possible orientations with respect to $B_0$ in a certain amount of time.

- Orientations in favor of $B_0$ will have lower magnetic energy, and will be slightly favored. After a certain time, a net magnetization ($M_0$) pointing in the direction of $B_0$ will develop.

One nucleus

Many nuclei

$M_0$ - net magnetization vector allows us to look at system as a whole
• Where does the net magnetization come from?

To figure it out, translate all the spins to the origin of the coordinate system.

• There is a slight excess of spins aligned with $B_o$, but at any angle with respect to $Z$. The distribution is proportional to $N_\alpha / N_\beta$.

• If you break down the $\mu$ vectors in $Z$ and $<xy>$:

- The net magnetization is aligned with $B_o$, and this is what we use in NMR.
Each nucleus behaves like a bar magnet.

\[ B_0 = 0 \]
Randomly oriented

\[ B_0 > 0 \]

- **Nuclear magnetic resonance occurs when the nucleus changes its spin state from \( \alpha \) to \( \beta \).**
- This is driven by the absorption of a quantum of energy.
- Coming from electromagnetic radiation, whose frequency must match that of Larmor precession:
NMR excitation

• To detect NMR signal, we have to move the system away from equilibrium. That is, we have to **perturb its populations**.

We need the system to absorb energy. That energy will induce transitions between energy levels that is to cause magnetic resonance to occur.

**How do you achieve that?**

The energy source is an oscillating electromagnetic radiation generated by an alternating current.

\[ B_1 = C \times \cos (\omega_0 t) \]
• The oscillating magnetic field (B1) is considered to have two counter-acting magnetic vectors, the resultant of which corresponds exactly to the applied oscillating field.

-only the vector that rotates at $+\omega_0$ interacts with $M_o$ to achieve a resonant condition (because it rotates in the same direction of the precession of $M_o$)

* After the oscillating magnetic field and all the $\mu$'s interact, a torque is generated, and they rotate.

Classical Larmor Precession
Since $\mathbf{B}_1$ and $\mathbf{M}_o$ rotate by the same amount, the macroscopic effect is that $\mathbf{M}_o$ rotates around the $x$ axis to generate *transverse magnetization* ($\mathbf{M}_{xy}$):

Since we altered the population ratio between energy levels (i.e., $N_\alpha/N_\beta$), the system absorbed energy. i.e., we disturbed the equilibrium.

The individual spins keep precessing under the effect of $\mathbf{B}_o$, the transverse magnetization $\mathbf{M}_{xy}$ will rotate around the $z$ axis at the precession frequency, $\omega_o$. 
Detection of $M_{xy}$ and return to equilibrium

- The oscillation of $M_{xy}$ generates a fluctuating magnetic field which can be used to generate a current in a coil

In the absence of the external $B_1$, $M_{xy}$ will go back to the $z$ axis ($M_o$, equilibrium) by restoring the original $N_\alpha/N_\beta$. This phenomenon is called *relaxation* (to be discussed later).
Detection of $M_{xy}$ and return to equilibrium

- The oscillation of $M_{xy}$ generates a fluctuating magnetic field which can be used to generate a current in a coil

In the absence of the external $B_1$, $M_{xy}$ flips back to the $z$ axis by restoring the original $N_\alpha/N_\beta$. This phenomenon is called relaxation (to be discussed later).