Structure Determination by NMR

* Introduction to NMR
* 2D NMR, resonance assignments

\underline{J–Correlated Based Experiments}

* COSY - Correlated Spectroscopy
* NOESY - Nuclear Overhauser Effect Spectroscopy
* HETCOR - Heteronuclear Correlated Spectroscopy/HMQC
* COLOC/HMBC
* J-Resolved
Structure Determination by NMR

Biological molecules such as proteins and nucleic acids can be large and complex. They can easily exceed 2000 atoms. Knowing their structure is critical in understanding the relationship between structure and function.

X-ray crystallography is an excellent method to determine detailed 3D structures of even some of the largest biological molecules. However, it has some significant difficulties. Getting crystals and is the structure biologically relevant.

NMR can be used to determine 3D structure and dynamics in solution! It’s limitation is molecular size. However, this is changing.
TATA Box Binding Protein Bound to DNA Duplex

2071 atoms
2175 bonds
NMR Structure Determination

- What is NMR?
- How does NMR work?
- How is a three dimensional structure elucidated?
Nuclear Magnetic Resonance

Nuclear spin
\[ \mu = \gamma I \hbar \]
\(\mu\) - magnetic moment
\(\gamma\) - gyromagnetic ratio
\(I\) - spin quantum number
\(\hbar\) - Planck's constant

\(I\) is a property of the nucleus

<table>
<thead>
<tr>
<th>Mass #</th>
<th>Atomic #</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odd</td>
<td>Even or odd</td>
<td>1/2, 3/2, 5/2,…</td>
</tr>
<tr>
<td>Even</td>
<td>Even</td>
<td>0</td>
</tr>
<tr>
<td>Even</td>
<td>Odd</td>
<td>1, 2, 3</td>
</tr>
</tbody>
</table>

As an exercise determine \(I\) for each of the following \(^{12}\text{C}, ^{13}\text{C}, ^{1}\text{H}, ^{2}\text{H}, ^{15}\text{N}\).
Apply an external magnetic field
(i.e., put your sample in the magnet)

Spin 1/2 nuclei will have two orientations in a magnetic field +1/2 and -1/2.

\[ \omega = \gamma B_0 = \nu/2\pi \]

- \( \omega \) - resonance frequency in radians per second, also called Larmor frequency
- \( \nu \) - resonance frequency in cycles per second, Hz
- \( \gamma \) - gyromagnetic ratio
- \( B_0 \) - external magnetic field (the magnet)
Net magnetic moment

\[ \vec{B}_0 \]

\[ \mu \]

\[ \omega \]

\[ +1/2 \]

\[ -1/2 \]

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2D NMR
Ensemble of Nuclear Spins

\[ \vec{B}_o = 0 \]
Randomly oriented

\[ \vec{B}_o > 0 \]
Highly oriented

Each nucleus behaves like a bar magnet.

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The net magnetization vector allows us to look at the system as a whole.

Many nuclei

\[ \mathbf{M}_0 \] - net magnetization vector allows us to look at the system as a whole.

One nucleus
Allowed Energy States for a Spin 1/2 System

\[ \Delta E = \gamma \hbar B_o = \hbar \nu \]

Therefore, the nuclei will absorb light with energy \( \Delta E \) resulting in a change of the spin states.
Energy of Interaction

\[ \Delta E = \gamma \hbar B_0 = h \nu \]

The frequency, \( \nu \), corresponds to light in the radiofrequency range when \( B_0 \) is in the Teslas.

This means that the nuclei should be able to absorb light with frequencies in the range of 10’s to 100’s of megaherz.

Note: FM radio frequency range is from ~88MHz to 108MHz. \(^{77}\text{Se}, \gamma = 5.12 \times 10^7 \text{ rad sec}^{-1} \text{ T}^{-1}\)

\[ \nu = \gamma B_0 / 2\pi \]
Nuclear Spin Dynamics

Effect of a 90° x pulse
Nuclear Spin Evolution

RF receivers pick up the signals
Free Induction Decay

The signals decay away due to interactions with the surroundings.

A free induction decay, FID, is the result.

Fourier transformation, FT, of this time domain signal produces a frequency domain signal.
Spin Relaxation

There are two primary causes of spin relaxation:

Spin - lattice relaxation, $T_1$, longitudinal relaxation.

Spin - spin relaxation, $T_2$, transverse relaxation.
Nuclear Overhauser Effect

Caused by dipolar coupling between nuclei.

The local field at one nucleus is affected by the presence of another nucleus. The result is a mutual modulation of resonance frequencies.
Nuclear Overhauser Effect

The intensity of the interaction is a function of the distance between the nuclei according to the following equation.

\[ I = A \left( \frac{1}{r^6} \right) \]

- \( I \) - intensity
- \( A \) - scaling constant
- \( r \) - internuclear distance

Arrows denote cross relaxation pathways:
- \( r_{1,2} \) - distance between protons 1 and 2
- \( r_{2,3} \) - distance between protons 2 and 3

The NOE provides a link between an experimentally measurable quantity, I, and internuclear distance. NOE is only observed up to ~5Å.
Scalar J Coupling

Electrons have a magnetic moment and are spin 1/2 particles.

J coupling is facilitated by the electrons in the bonds separating the two nuclei. This through-bond interaction results in splitting of the nuclei into $2I + 1$ states. Thus, for a spin 1/2 nucleus the NMR lines are split into $2(1/2) + 1 = 2$ states.

$$\text{Multiplet} = 2nI + 1$$

$n$ - number of identical adjacent nuclei
$I$ - spin quantum number
Scalar J Coupling

The magnitude of the J coupling is dictated by the torsion angle between the two coupling nuclei according to the Karplus equation.

\[ J = A + B \cos(\theta) + C \cos^2(\theta) \]

\[ A = 1.9, \quad B = -1.4, \quad C = 6.4 \]

A, B and C on the substituent electronegativity.
Coupling constants can be measured from NMR data.

Therefore, from this experimental data we can use the Karplus relation to determine the torsion angles, $\theta$.

Coupling constants can be measured between most spin 1/2 nuclei of biological importance,

$$^1\text{H}, ^{13}\text{C}, ^{15}\text{N}, ^{31}\text{P}$$

The most significant limitation is usually sensitivity, S/N.
The chemical shift is the most basic of measurements in NMR. The Larmor frequency of a nucleus is a direct result of the nucleus, applied magnetic field and the local environment.

If a nucleus is shielded from the applied field there is a net reduction if the magnetic field experienced by the nucleus which results in a lower Larmor frequency.

δ is defined in parts per million, ppm.

\[ \delta = (\omega - \omega_o)/\omega_o \times 10^6 \]
Biomolecular NMR Experiments

J Correlated Based Experiments
- COSY - Correlated Spectroscopy
- 2QF-COSY - Double Quantum Filtered Spectroscopy
- HETCOR - Heteronuclear Correlated Spectroscopy
- E.COSY - Exclusive COSY
- HOHAHA - Homonuclear Hartmann Hahn (TOCSY)

Nuclear Overhauser Based Experiments
- NOESY - Nuclear Overhauser Effect Spectroscopy
- ROESY - Rotating Frame Overhauser Effect Spectroscopy

Three Dimensional Experiments Use a Combination
- NOESY - TOCSY
- NOESY - NOESY
Summary

There are three primary NMR tools used to obtain structural information:

- **Nuclear Overhauser effect** - internuclear distances
- **$J$ Coupling** - torsion angles
- **Chemical shift** - local nuclear environment

(Chemical exchange can also be monitored by NMR.)
Homonuclear 2D correlation techniques

• **Through Bond:** $^{n}J_{HH}$ (scalar coupling)
  
  **COSY** : Correlated Spectroscopy
  Directly coupled neighbors

  **Relay-COSY** : RELAY-Correlated Spectroscopy
  Directly coupled neighbors and protons coupled to the coupled neighbors (relay transfer)

  **TOCSY** : Total Correlation Spectroscopy
  Directly coupled neighbors and protons coupled to the coupled neighbors (More efficient than Relay)

• **Through Space:** Distance

  **NOESY** : NOE Spectroscopy
  **ROESY** : ROE Spectroscopy
  
  *NOE in Rotating frame*
$\text{C}_4\text{H}_8\text{O}$

$\text{CH}_3 – \text{CH}_2 – \text{CH}_2 – \text{OH}$

$^1\text{H}$ NMR Spectrum
(300 MHz, CDCl$_3$ solution)

Exchanges with D$_2$O
C₄H₈O: COSY

CH₃ – CH₂ – CH₂ – OH

CH₂ OH

CH₃

CH₂
\( ^1H \) NMR of glucose derivative
2D-COSY of glucose derivative
C$_5$H$_8$O$_2$  \( I = 5 - 8/2 + 1 = 2 \)

$\text{CH}_2 - O$

$^1$H NMR Spectrum
(600 MHz, C$_6$D$_6$ solution)

$^{13}$C NMR Spectrum
(150 MHz, C$_6$D$_6$ solution)

O-C=O

CH$_2$ - O

solvent

ppm
C₅H₈O₂

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C₈H₁₆O : I = 8 – 16/2 + 1 = 1

¹H NMR Spectrum
(600 MHz, Benzene-D₆ solution)

CH₂ – CO – CH₂

⁻⁴H⁻¹⁻²⁻¹⁻\(\text{ppm}\)

¹³C NMR Spectrum
(150 MHz, Benzene-D₆ solution)

Ketone
C=O
C8H16O

Me1 – CH22 – CH23 – CH24

CH25

C=O

CH27 — Me-8

Me8

1H-1H COSY Spectrum
(600 MHz, Benzene-D6 solution)
$\text{C}_{11}\text{H}_{20}\text{O}_4$

$^1H$ NMR Spectrum
(300 MHz, CDCl$_3$ solution)

$\text{O} - \text{CH}_2 - \text{CH}_3$

$^13\text{C}$ NMR Spectrum
(75 MHz, CDCl$_3$ solution)

$\text{O} - \text{C}=\text{O}$
C_{11}H_{20}O_4

\begin{align*}
O &= C - O - \text{CH}_2 - \text{CH}_3 \\
\text{CH}_3 - \text{CH}_2 - C - \text{CH}_2 - \text{CH}_3 \\
O &= C - O - \text{CH}_2 - \text{CH}_3 \\
\end{align*}

Only missing quaternary carbon

\text{\textsuperscript{1}H-\textsuperscript{1}H COSY Spectrum} \\
(300 MHz, CDCl_3 solution)
$J_{1,2} \Rightarrow \text{negative}$

$J_{1,3} J_{2,3} \Rightarrow \text{positive}$
If we consider cross peak 2/1 Passive couplings – involving passive nuclei 3 - ($J_{1,3}$ and $J_{2,3}$) have same Sign (positive slant)

If we consider cross peak 3/1 Passive couplings – involving passive nuclei 2 - ($J_{1,2}$ and $J_{2,3}$) have different Sign (negative slant)
Phase Sensitive COSY

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2D NMR Spectroscopy
COSY-90: disaccharide

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**COSY45**: H2 and H4 overlap at ~ 4.7 ppm. Cross-peak in COSY-45 allow to assign H3 and H5a/b unambiguously as H4 is coupled to a geminal pair => different sign in J.
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2D NMR Spectroscopy
Deshielded multiplet
<table>
<thead>
<tr>
<th>$^1H$</th>
<th>$^13C$</th>
<th>Assignment</th>
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<tbody>
<tr>
<td>6.6</td>
<td>113</td>
<td>8</td>
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<tr>
<td>6.5</td>
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<tr>
<td>4.8</td>
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<td>9</td>
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<td>4.2</td>
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<td>18</td>
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<tr>
<td>2.6</td>
<td>40</td>
<td>16</td>
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<tr>
<td>2.6 &amp; 2.4</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td>2.4</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>2.0 &amp; 1.8</td>
<td>36</td>
<td>17</td>
</tr>
</tbody>
</table>

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HMOC of menthol in CDC13 obtained on I400 with the inverse probe

Pulse Sequence: HMOC

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2D NMR Spectroscopy
HMBC Spectra
J-Resolved Spectrum
How we solve the structure of an unknown compound with the help of 2D-NMR Spectrum???
\(-\text{CHOH}\text{-CHOH}\text{-CH}_2\text{-OH} = \text{m/z : 91}\)

- \text{CH} - \text{OH}
- \text{CH}_2 - \text{OH}
- \text{CH}_2 - \text{OH}
$-\text{CHOH} -$ \text{CHOH} - \text{CH}_2 - \text{OH} = m/z : 91$

Broad band

DEPT Spectrum
\[- \text{CHOH– CHOH– CH}_2– \text{OH} = m/z : 91\] \times 2 = 182
COSY_45
HSQC
HMBC
J-resolved
$J$-resolved