NMR: What are the Potentials?

Understanding the nuclear spin system
Spin choreography

Characterisation of various materials

Structure of compounds, biomolecules
Proteins, nucleic acids

Magnetic resonance imaging, MRI
NMR: What are the Potentials?

- **Structure elucidation**
  - Natural product chemistry
  - Synthetic organic chemistry

- **Study of dynamic processes**
  - Reaction kinetics
  - Study of equilibrium (chemical or structural)

- **Structure determination of macromolecules**
  - Proteins
  - Nucleotides, protein/DNA complexes
  - Polysaccharides

- **Drug Design**
  - Structure-Activity Relationship (SAR) by NMR

- **Medicine**
  - Magnetic Resonance Imaging
  - **Metabonomics**: combined use of spectroscopy & multivariate statistical approaches to studies of biofluids, cells & tissues. Gives a unique metabolic fingerprint for each complex biological mixture, sensitive to change
Nuclear spins are our spies to probe structure

Superconducting magnets give us the medium and mechanism to manipulate the spins

Talking to the spins in a language that they understand, which means we have to resonate with them
Nuclear spins are a fundamental property just like charge and mass.

Deuterium atom:
1 electron, 1 proton, and 1 neutron

Electronic spin = 1/2
Nuclear spin = 1

NMR uses the nuclear properties and nuclear spins to eavesdrop on others.

Paired nuclear spins are of no use to work as nuclear spies.

NMR needs unpaired, socially non-committed, nuclear spins to act as spies.

Helium atom
Net nuclear spin = 0
NMR non-observabe
<table>
<thead>
<tr>
<th>Element</th>
<th>Probed Nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td>N</td>
<td>$^{15}$N</td>
</tr>
<tr>
<td>Si</td>
<td>$^{29}$Si</td>
</tr>
<tr>
<td>P</td>
<td>$^{31}$P</td>
</tr>
</tbody>
</table>

**Common nuclei probed are $^{13}$C, $^{15}$N, $^{29}$Si, and $^{31}$P**
Many of the nuclei of industrial importance are quadrupolar spin nuclei manifesting in ceramics, glasses, zeolites and catalysts
Nuclear Spins

Charge

Nuclear spin

Spin

\[ \mu = \gamma \hbar I \]

- Magnetic moment
- Gyromagnetic ratio, depends on the nucleus
- Spin quantum number
A spinning gyroscope in a gravity field

A spinning charge in a magnetic field
The magnetic dipole moment $\mu$ and therefore the precession frequency are characteristic for each nucleus and scale with the gyromagnetic ratio $\gamma$.

Atomic nuclei are composed of protons and neutrons which have a spin.

<table>
<thead>
<tr>
<th>protons spin</th>
<th>neutrons spin</th>
<th>nuclear spin</th>
<th>$\mu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Even $\uparrow \downarrow$</td>
<td>even $\uparrow \downarrow$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Even $\uparrow \downarrow$</td>
<td>odd $\uparrow$</td>
<td>$\frac{1}{2}$</td>
<td>$&lt;0$</td>
</tr>
<tr>
<td>Odd $\uparrow$</td>
<td>even $\uparrow \downarrow$</td>
<td>$\frac{1}{2}$</td>
<td>$&gt;0$</td>
</tr>
<tr>
<td>Odd $\uparrow$</td>
<td>odd $\uparrow$</td>
<td>$n$</td>
<td>$&gt;0$</td>
</tr>
</tbody>
</table>

(Cavanagh, et al. "Protein NMR spectroscopy")
NMR Spies – Inside a Magnetic Field
NMR Spies – In Action
How do the spins probe the medium?

- Chemical shift anisotropy
- Dipole-dipole couplings
- Through-bond couplings
- Quadrupolar couplings

The magnetic field generated by one spin influences its neighbour.
The isotropic parts (manifest in solution-state) are time independent
Anisotropic parts cause line broadening
Internal Nuclear Spin Interactions

Spin > 1/2, $^{23}\text{Na}$, $^{17}\text{O}$…..

Spin 1/2, $^1\text{H}$, $^{13}\text{C}$…..

Electric

Quadrupolar

Isotropic quad. shift

1st, 2nd order quad. Interaction, anisotropic

Isotropic chemical shift

Chemical shift anisotropy, CSA

Chemical shift

Magnetic

Spin-spin couplings

Scalar, J-couplings

Dipolar

Heteronuclear

Homonuclear

Isotropic

Quadrupolar
NMR: Nuclear Spins, Magnetic Moments, and Resonance

Nuclear spin

NMR magnet

NMR spectrum

A nuclear spin of \( I > 0 \) is associated with a magnetic dipole moment \( \mu = \gamma I \).

Magnetic field

\[ \omega = \gamma B_0 \]

Apply radio frequency \( h\omega \) to measure the nuclear precession frequencies \( h\gamma B_0 \).

Resonance

Intensity vs. frequency

NMR spectrum
The Electromagnetic Spectrum

NMR resonance condition: $\omega = \gamma B_0$

- **Wavelength**: 1 nm, 1 µm, 1 mm, 1 m, 1 km
- **Frequency**: $10^{18}$, $10^{15}$, $10^{12}$, $10^9$, $10^6$ GHz, MHz

**Energy**
- **γ-rays**
  - nucleus transitions
- **X-rays**
  - inner electrons transitions
- **UV**
  - outer electrons vibrations
- **IR**
  - rotations
- **FIR**
  - $e^-$ magnetic transitions
- **Microwaves**
  - nuclear magnetic transitions
- **Radiowaves**
  - optical spectroscopy

**Spectroscopies**
- Moessbauer
- Optical spectroscopy
- ESR
- NMR
RF waves in

\[ B_0, \text{External magnetic field} \]

RF waves in

Nuclear magnetic moments in the sample

The frequency of emitted RF waves reveals information about the magnetic environment of atomic nuclei

RF waves out
Spins in the $\alpha$ (up) and $\beta$ (down) states populate the energy levels according to a Boltzmann distribution.

This leads to a small macroscopically observable magnetization along the $z$-axis $M_z$ (parallel to $B_0$).

No $x$- or $y$-magnetization is observed since the spin vectors are not *phase coherent*, i.e. they precess independently from each other around $B_0$, the $x,y$ components average to zero.
Radio frequency pulses:
Induce resonance by applying an external magnetic field that oscillates with the precession frequency of the spins (radio frequencies: MHz)

\[ \hbar \omega_0 = \Delta E = \gamma \hbar B_0 \]

\[ \omega_0 = \gamma B_0 \]
Sensitivity of NMR Spectroscopy

$S/N \sim N \gamma_{exc} \gamma_{det}^{3/2} B_0^{3/2} NS T_2^{1/2}$

- **S/N**: signal-to-noise
- **N**: number of spins
- **$\gamma_{exc}$**: gyromagnetic ratio of excited spins
- **$\gamma_{det}$**: gyromagnetic ratio of detected spins
- **$B_0$**: static magnetic field (e.g. 14.1 Tesla or 600 MHz for $^1$H)
- **NS**: number of scans
- **$T_2$**: transverse relaxation time

- Sample concentration
- Isotope labeling
- Magnet "size"
- Measurement time
- Molecular weight

Line width:

$\Delta \nu \sim 1/(\pi T_2)$
### Sensitivity of NMR Spectroscopy

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Net Spin</th>
<th>$\gamma$ / MHz T$^{-1}$</th>
<th>Abundance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>1/2</td>
<td>42.58</td>
<td>99.98</td>
</tr>
<tr>
<td>$^2$H</td>
<td>1</td>
<td>6.54</td>
<td>0.015</td>
</tr>
<tr>
<td>$^3$H</td>
<td>1/2</td>
<td>45.41</td>
<td>0.0</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>1/2</td>
<td>17.25</td>
<td>100.0</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>3/2</td>
<td>11.27</td>
<td>100.0</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>1</td>
<td>3.08</td>
<td>99.63</td>
</tr>
<tr>
<td>$^{15}$N</td>
<td>1/2</td>
<td>4.31</td>
<td>0.37</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>1/2</td>
<td>10.71</td>
<td>1.108</td>
</tr>
<tr>
<td>$^{19}$F</td>
<td>1/2</td>
<td>40.08</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Relative sensitivity of nuclei depends on:

- **Gyromagnetic ratio** ($\gamma$)
- **Natural abundance of the isotope**
Sensitivity of NMR Spectroscopy

$^1$H NMR spectra of organic compound
8 scans ~12 secs

$^{13}$C NMR spectra
8 scans ~12 secs

$^{13}$C NMR spectra
10,000 scans ~4.2 hours
Energy Levels: Why Big Magnets Are Needed?

Energy of a spin in a magnetic field:
\[ E = \mu B_0 = \gamma I B_0 \]
- \( I = \pm \frac{1}{2} \hbar \) nuclear spin
- \( \mu \) = magnetic moment
- \( \gamma \) = gyromagnetic ratio

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

The energy difference between the two states scales with the strength of the external magnetic field \( B_0 \).
Do We Need Bigger/Higher Magnets?

Highest superconducting magnet currently available is 23 T yielding proton Larmor frequency of 1000 MHz.
NMR used for structural characterisation
Should be able to distinguish functional groups unambiguously
Chemical Shift- Usefulness of NMR

$H^b$  $C$  $H^a$

$H^c$  $Cl$

$\nu^a$  $\nu^b$  $\nu^c$

frequency
Prof. Dharmatti was a student of Felix Bloch in Stanford and discovered chemical-shift phenomenon
Fourier-Transform NMR

How to efficiently detect a range of NMR frequencies (in a spectrum)

$f(t) \xrightarrow{\text{FT}} F(\omega)$

cw

Continuous Wave (CW) NMR

Fourier transform (FT) NMR

K. Zanier

(Ernst, et al. "Principles of Nuclear Magnetic Resonance")
Typical Experiment in NMR: RF Pulse

Effect of a $90^\circ$ $x$ pulse
After the Pulse: Nuclear Spin Evolution

RF receivers pick up the signals

The spins precess in the $xy$ plane and relax to the equilibrium value, free induction decay
Fourier Transformation

Fourier transformation

Relaxation - the nuclear spins return to equilibrium: $M_z = M_0$, $M_{xy} = 0$
There are two primary causes of spin relaxation:

Spin - lattice relaxation, $T_1$, longitudinal relaxation

Spin - spin relaxation, $T_2$, transverse relaxation
Spin Relaxation, $T_1$

$T_1$ determines the repetition rate of an experiment

For an optimum signal, there is a need to wait for a few $T_1$ times before which an experiment can be repeated for signal averaging.

The time scale with which the $z$-component of the magnetisation has relaxed back to the equilibrium magnetisation, $M_0$.
Behaviour of $T_1$ and $T_2$ Relaxation Times

$T_1$ or $T_2$ relaxation time

- Long
- Short

$T_1$ and $T_2$ at short correlation times

- Gases
- Small molecules
- Medium sized molecules
- Large molecules

$T_1$ minimum

- Fast motion
  - Short $\tau_c$
- Slow motion
  - Long $\tau_c$

Optimal frequency for $T_1$ relaxation (MHz frequencies)

Correlation time:

- 0
- 0.2
- 0.4
- 0.6
- 0.8
- 1.0 ns
$T_1$ Measurement- Inversion Recovery Experiment

$\tau=0$

- Initial state: Spin oriented along the $z$ axis.
- 180° pulse: Spin flip along the $x$ axis.
- 90° pulse: Spin rotates into the $y$ plane.

Some $\tau$

- Spin precesses for a certain time $\tau$.
- 90° pulse: Spin rotates into the $y$ plane.

Large $\tau$

- Spin precesses for a large time $\tau$.
- 90° pulse: Spin rotates into the $y$ plane.

The figure illustrates the change in the spin orientation with respect to time $\tau$. The use of pulses to manipulate the spin orientation is a key aspect of NMR spectra and other magnetic resonance experiments.
Measure the NMR signal as a function of $\tau$.

Bloch equation for longitudinal magnetisation:

$$\frac{dM_z}{dt} = - \frac{M_z - M_0}{T_1}$$

$$M_z = M_0(1 - 2e^{-\frac{\tau}{T_1}})$$
Inversion Recovery - Measure NMR Intensity as a function of the delay time $\tau$ and fit to an exponential function

\[ M_z = M_o \left(1 - 2e^{-\tau/T_1}\right) \]
The faster the dephasing, the faster the decay of the time domain signal, the broader the line.

Line widths are related to $T_2$ relaxation. $LW \sim 1/ T_2$ (not always true due to inhomogeneous broadening).

$T_2$ is always faster (shorter) than or equal to $T_1$. 
$T_2$ Measurement - Spin-Echo Experiment

Bloch equation for transverse magnetisation:

$$\frac{dM_{x,y}}{dt} = -\frac{M_{x,y}}{T_1}$$

$$M_{x,y} = M_0 e^{-\frac{\tau}{T_2}}$$

Plot of the peak amplitude as a function of $\tau$ in the spin-echo experiment.
Physics Today, 1953

E. Hahn, Physical Review, 80, 1950
Spin-Echo Experiment

- Magnetisation is refocused
- Formation of an echo
- Decay of echo only due to $T_2$
T₂ Relaxation, Line Width and Correlation Times

\[ \tau_c = \frac{4\eta_w r^3_H}{3k_B T} \]

\[ \eta_w = \text{viscosity of the solvent} \]

\[ r^3_H = \text{hydrated radius} \]

mobile, flexible chain has narrower line widths than globular protein

$^{15}\text{N}$

![Diagram showing $^{1H}$ and $^{15}\text{N}$ with line widths indicating T$_2$ Relaxation, Line Width and Correlation Times]
Mobility is also expressed in $T_1$ relaxation times.

$t = 10\, \text{us}$

$t = 100\, \text{us}$

$t = 1000\, \text{us}$

$t = 5000\, \text{us}$
To summarise:

The first point of the FID determines the intensity of the resonance signal.

The duration of the FID determines the resolution of the resonance signal.
**Information in a NMR Spectra**

1) Energy $E = h\nu$

$h$ is Planck constant
$\nu$ is NMR resonance frequency

### Observable

<table>
<thead>
<tr>
<th>Observable</th>
<th>Name</th>
<th>Quantitative</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak position</td>
<td>Chemical shifts ($\delta$)</td>
<td>$\delta$(ppm) = $u_{\text{obs}} - u_{\text{ref}}/u_{\text{ref}}$ (Hz)</td>
<td>chemical (electronic) environment of nucleus</td>
</tr>
<tr>
<td>Peak Splitting</td>
<td>Coupling Constant (J) Hz</td>
<td>peak separation (intensity ratios)</td>
<td>neighboring nuclei (torsion angles)</td>
</tr>
<tr>
<td>Peak Intensity (ratio)</td>
<td>Integral</td>
<td>unit less (ratio)</td>
<td>nuclear count</td>
</tr>
<tr>
<td>Peak Shape</td>
<td>Line width</td>
<td>relative height of integral curve</td>
<td>quantifying</td>
</tr>
</tbody>
</table>

$\Delta\nu = 1/\pi T_2$
peak half-height

$wavelength (\text{cm})$

$10^{-10}$ $10^{-8}$ $10^{-6}$ $10^{-4}$ $10^{-2}$ $10^0$ $10^2$

**Radiations**

- $\gamma$-rays
- x-rays
- UV VIS
- IR
- $\mu$-wave
- radio
a) Small local magnetic fields ($B_{loc}$) are generated by electrons as they circulate around nuclei.
b) These local magnetic fields can either oppose or augment the external magnetic field
1) Typically oppose external magnetic field
2) Nuclei “see” an effective magnetic field ($B_{eff}$) smaller than the external field
3) $\sigma$ – magnetic shielding or screening constant
   i. depends on electron density
   ii. depends on the structure of the compound

\[ B_{eff} = B_o - B_{loc} \rightarrow B_{eff} = B_o(1 - \sigma) \]

HO-CH$_2$-CH$_3$

$\sigma$ – reason why we observe three distinct NMR peaks instead of one based on strength of $B_o$

$\nu = \gamma B_o/2\pi$

Shielding – local field opposes $B_o$
Rather than measure the exact resonance position of a peak, we measure how far downfield it is shifted from TMS.
The shift observed for a given proton in Hz also depends on the frequency of the instrument used. Higher frequencies = larger shifts in Hz.
### ppm and Hz

<table>
<thead>
<tr>
<th>$^1$H operating frequency</th>
<th>Hz equivalent of ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 MHz</td>
<td>500 Hz</td>
</tr>
<tr>
<td>600 MHz</td>
<td>600 Hz</td>
</tr>
<tr>
<td>800 MHz</td>
<td>800 Hz</td>
</tr>
</tbody>
</table>
Chemical shift \( \delta = \frac{\text{shift in Hz}}{\text{spectrometer frequency in MHz}} = \text{ppm} \)

Higher frequency leads to more dispersion

- 500 MHz
- 600 MHz
- 800 MHz

Parts per million
• For protons, ~ 15 ppm:
  - Alcohols, protons adjacent to ketones
  - Acids, Aldehydes
  - Amides
  - Olefins
  - Aliphatic

For carbon, ~ 220 ppm:
  - C=O in ketones
  - Aromatics, conjugated alkenes
  - Olefins
  - Aliphatic CH₃, CH₂, CH
  - C=O of Acids, aldehydes, esters
  - Carbons adjacent to alcohols, ketones

Chemical-Shift Scales

ppm

TMS

H₃C—Si—CH₃

CH₃

CH₃
Chemical-Shift Scales

<table>
<thead>
<tr>
<th></th>
<th>acid COOH</th>
<th>aldehyde CHO</th>
<th>benzene CH</th>
<th>alkene =C-H</th>
<th>C-H where C is attached to an electronegative atom X-C-H</th>
<th>CH on C next to pi bonds X=C-C-H</th>
<th>aliphatic C-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Interpretation of most 1D spectra is possible with this information
Factors Determining Resonance Positions of $^1\text{H}$

- Deshielding by electronegative elements
- Anisotropic fields usually due to pi-bonded electrons in the molecule
- Deshielding due to hydrogen bonding
Deshielding by an Electronegative Element

Chlorine “deshields” the proton, that is, it takes valence electron density away from carbon, which in turn takes more density from hydrogen deshielding the proton.

“deshielded“ protons appear at low field

highly shielded protons appear at high field

Deshielding moves proton resonance to lower field
Electronegativity Dependence of Chemical Shift

<table>
<thead>
<tr>
<th>Compound CH₃X</th>
<th>CH₃F</th>
<th>CH₃OH</th>
<th>CH₃Cl</th>
<th>CH₃Br</th>
<th>CH₃I</th>
<th>CH₄</th>
<th>(CH₃)₄Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element X</td>
<td>F</td>
<td>O</td>
<td>Cl</td>
<td>Br</td>
<td>I</td>
<td>H</td>
<td>Si</td>
</tr>
<tr>
<td>Electronegativity of X</td>
<td>4.0</td>
<td>3.5</td>
<td>3.1</td>
<td>2.8</td>
<td>2.5</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Chemical shift δ</td>
<td>4.26</td>
<td>3.40</td>
<td>3.05</td>
<td>2.68</td>
<td>2.16</td>
<td>0.23</td>
<td>0</td>
</tr>
</tbody>
</table>

Dependence of the Chemical Shift of CH₃X on the element X

- Deshielding increases with the electronegativity of atom X
- Most deshielded
- TMS
Substitution Effects on Chemical Shift

The effect decreases with increasing distance.

The effect increases with greater numbers of electronegative atoms.

most deshielded

<table>
<thead>
<tr>
<th>Compound</th>
<th>CHCl₃</th>
<th>CH₂Cl₂</th>
<th>CH₃Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp. PPM</td>
<td>7.27</td>
<td>5.30</td>
<td>3.05</td>
</tr>
</tbody>
</table>

most deshielded

<table>
<thead>
<tr>
<th>Compound</th>
<th>-CH₂-Br</th>
<th>-CH₂-CH₂Br</th>
<th>-CH₂-CH₂CH₂Br</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp. PPM</td>
<td>3.30</td>
<td>1.69</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Aromatic Ring Current Effects

The presence of pi-bonds leads to anisotropic fields and affect chemical shift

Aromatic ring currents are observed in molecules like benzene and naphthalene

The applied magnetic field gives rise to a ring current in the delocalised pi-electrons of the aromatic ring

Benzene rings have the greatest effect

Ring protons can get deshielded (induced and static fields add) while inner protons get shielded (induced and static fields oppose)
Aromatic Ring Current Effects

**Ring Current in Benzene**

- Ring protons come at 7.3 ppm instead of the 5.6 ppm of the vinylic protons in cyclohexane.
- Secondary magnetic field generated by circulating $\pi$ electrons deshields aromatic protons.
- Circulating $\pi$ electrons.
- Deshielded fields add together.

$B_0$
Carboxylic acids have strong hydrogen bonding - they form dimers.

With carboxylic acids the O-H absorptions are found between 10 and 12 ppm very far downfield.

In methyl salicylate, which has strong internal hydrogen bonding, the NMR absorption for O-H is at about 14 ppm, way, way downfield.
The chemical shift depends on how much hydrogen bonding is taking place.

Alcohols vary in chemical shift from 0.5 ppm (free OH) to about 5.0 ppm (lots of H bonding).

Hydrogen bonding lengthens the O-H bond and reduces the valence electron density around the proton - it is deshielded and shifted downfield in the NMR spectrum.
Influence of Hydrogen Bonding

OH upfield shifted, No H-bonding

OH downfield shifted, H-bonding
SPIN-SPIN SPLITTING
Splitting of the resonances, multiplets, are due to spin-spin coupling and can be predicted by the n+1 rule.
Scalar Coupling

J-coupling is facilitated by the electrons in the bonds that connect the nuclei

Scalar coupling, through-bond coupling

The coupling constants can be related to a number of physical properties: Hybridisation, dihedral bond angles, and electronegativity of substituents

Geminal and vicinal couplings may be used to determine the bond angles and torsion angles
Geminal Scalar Coupling: $^2J$ Coupling

Smaller the $J$ value, larger the bond angle

Example:

- $\alpha = 109^\circ$
  - $^2J = 12 \text{ - } 18 \text{ Hz}$

- $\alpha = 118^\circ$
  - $^2J = 3 \text{ - } 7 \text{ Hz}$

- $\alpha = 120^\circ$
  - $^2J = 0 \text{ - } 3 \text{ Hz}$
The magnitude of the J coupling is dictated by the torsion angle between the two coupling nuclei according to the Karplus equation.

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

right-handed alpha helix $^3J_{N\alpha} = 3.9$

antiparallel beta sheet $^3J_{N\alpha} = 8.9$

parallel beta sheet $^3J_{N\alpha} = 9.7$

The Ramachandran Plot.

- Labeled regions indicate the types of secondary structures:
  - **Beta-sheet.**
  - **Left handed alpha-helix.**
  - **Right handed alpha-helix.**
Spin System Classification

Chemical equivalence among the spins:

• If the spins of the same isotopic species
• There exists a molecular symmetry operation that exchanges the two spins

Magnetic equivalence among the spins:

• This is a stronger form of the chemical equivalence
• The spins should have the same chemical shifts
• Either the spins have identical couplings to all other spins in the molecule or there are no other spins in the molecule
Spin System Classification

Not magnetically equivalent

\[ \delta_{Ha} = \delta_{Hb}, \text{ and } \delta_{Fa} = \delta_{Fb} \text{ but } \]
\[ J_{HaFb} \neq J_{HaFa} \text{ and } J_{HbFb} \neq J_{HbFa} \]

Also here \( J_{Ha} \neq J_{Hb} \neq 0 \)

It is an AA’XX’ spin system

Magnetically equivalent

It is an \( A_2X_3 \) spin system

The most shielded spin is notated as \( A \)

In this case, difluoromethane, \(^1\text{Hs} \) and \(^{19}\text{Fs} \) are magnetically equivalent not due to rotation, but to symmetry around the carbon. It is an \( A_2X_2 \) system
this hydrogen peak is split by its two neighbours

two neighbours
$n+1 = 3$
triplet

these hydrogens are split by their single neighbour

one neighbour
$n+1 = 2$
doublet

$n+1$ rule
1) Protons that are equivalent by symmetry usually do not split one another

\[
\text{protons equivalent by symmetry}
\]

\[
X-\text{CH-CH-Y} \quad X-\text{CH}_2-\text{CH}_2-\text{Y}
\]

no splitting if \(x=y\)

no splitting if \(x=y\)

2) Protons in the same group usually do not split one another

\[
\text{protons in the same group}
\]

[diagram]

\[
\text{or}
\]

[diagram]
Some Splitting Patterns

\[ \text{X-CH-CH-Y} \quad (x \neq y) \]

\[ \text{CH}_3\text{-CH-} \]

\[ \text{CH}_2\text{-CH-} \]

\[ \text{CH}_3\text{-CH}_2\text{-} \]

\[ \text{X-CH}_2\text{-CH}_2\text{-Y} \quad (x \neq y) \]

\[ \text{CH}_3\text{-CH-} \]

\[ \text{CH}_3\text{-} \]

\[ \text{CH}_3 \]
The coupling constant is the distance \( J \) (measured in Hz) between the peaks in a multiplet.

\( J \) is a measure of the amount of interaction between the two sets of hydrogens creating the multiplet.
Representative Coupling Constants

- **vicinal**
  - \[
  \begin{array}{c}
    & H & H \\
    C - C & & \\
  \end{array}
  \]
  - 6 to 8 Hz
  - three bond
  - \(^3\)J

- **trans**
  - \[
  \begin{array}{c}
    H & C = C & H \\
    & & \\
  \end{array}
  \]
  - 11 to 18 Hz
  - three bond
  - \(^3\)J

- **cis**
  - \[
  \begin{array}{c}
    H & C = C & H \\
    & & \\
  \end{array}
  \]
  - 6 to 15 Hz
  - three bond
  - \(^3\)J

- **geminal**
  - \[
  \begin{array}{c}
    & C = C & H \\
    & & \\
  \end{array}
  \]
  - 0 to 5 Hz
  - two bond
  - \(^2\)J
Scalar Coupling- Splitting of Resonances

A splitting of a signal means that we have more energies involved in the transition of a certain nuclei. So why do we have more energies?

Coupling constants do not depend on the applied magnetic field, unlike the CSA.
The nuclear magnetic moment of $^{19}$F polarises the F bonding electron (up), which, since we are following quantum mechanics rules, makes the other electron point down (the electron spins have to be antiparallel).

Now, since we have different states for the $^1$H electrons depending on the state of the $^{19}$F nucleus, we will have slightly different energies for the $^1$H nuclear magnetic moment (remember that the $1s$ electron of the $^1$H generates an induced field...).

This difference in energies for the $^1$H result in a splitting of the $^1$H resonance line.
Scalar Coupling - Splitting of Resonances

Similar analysis for a CH$_2$ system as well.

The state of one of the $^1$H spins gets transmitted to the other $^1$H spins via the electrons in the bond (things get a bit complicated here due to the sp$^3$ hybridization etc. in general).
At any given time about half of the molecules in solution will have spin $+1/2$ and the other half will have spin $-1/2$. Shift of $H_A$ is affected by the spin of its neighbour.
The resonance positions (splitting) of a given hydrogen is affected by the possible spins of its neighbor.
Spin Arrangements

two neighbors
n+1 = 3
triplet

one neighbor
n+1 = 2
doublet

methylene spins

methine spins
Scalar Coupling- Energy Level Picture

$J = 0$
$\nu A_1 = \nu A_2$

$J > 0$

$J < 0$
In weakly coupled spin systems: Consider ethyl acetate, CH$_2$ at 4.5 ppm and CH$_3$ at 1.5 ppm

J is typically 7 Hz, hence weakly coupled, also called first-order spin system

Each $^1$H in CH$_3$ will see three possible states of $^1$H in CH$_2$
Each $^1$H in CH$_2$ will see four possible states of $^1$H in CH$_3$
Note the $^1$H in CH$_3$ and that in CH$_2$ are equivalent
1\textsuperscript{st} order systems (continued)

• A coupled to \( n \) identical nuclei \( X \) (of spin \( \frac{1}{2} \)) yields \( n + 1 \) lines in the spectrum of \( A \).

• Therefore, the \( \text{CH}_2 \) in \( \text{EtOAc} \) will show up as four lines, or a \textit{quartet}.
• Analogously, the \( \text{CH}_3 \) in \( \text{EtOAc} \) will show up as three lines, or a \textit{triplet}.

• The separation of the lines will be equal to the coupling constant between the two types of nuclei (\( \text{CH}_2 \)’s and \( \text{CH}_3 \)’s in \( \text{EtOAc} \), approximately 7 Hz).

• Intensities can also be derived from the diagram of the possible states:

• Since we have the same probability of finding the system in any of the states, and states in the same rows have equal energy, the intensity will have a ratio 1:2:1 for the \( \text{CH}_3 \), and a ratio of 1:3:3:1 for the \( \text{CH}_2 \).
The splitting of the resonance of a nuclei A by a nuclei X with spin number I will be $2I + 1$.

Start with one $^1\text{H}$

Coupling to the first $^1\text{H}$
$(2 \times \frac{1}{2} + 1 = 2)$

Coupling to the second $^1\text{H}$

Coupling to the third $^1\text{H}$

In general, the number of lines in these cases will be a binomial expansion, known as the Pascal Triangle:

$1 : n / 1 : n(n - 1) / 2 : n(n - 1)(n - 2) / 6 : ...$
Here $n$ is the number of equivalent spins 1/2 we are coupled to: The results for several $n$’s is

\[
\begin{array}{ccccccc}
1 \\
1 & 1 \\
1 & 2 & 1 \\
1 & 3 & 3 & 1 \\
1 & 4 & 6 & 4 & 1 \\
\end{array}
\]

- In a spin system in which we have a certain nuclei coupled to more than one nuclei, all first order, the splitting will be basically an extension of what we saw before.

- Say that we have a CH (A) coupled to a CH$_3$ (M) with a $J_{AM}$ of 7 Hz, and to a CH$_2$ (X) with a $J_{AX}$ of 5 Hz. We basically go in steps. First the big coupling, which will give a quartet:

- Then the small coupling, which will split each line in the quartet into a triplet:

- This is called a *triplets of quartet* (big effect is the last...).
2-Nitropropane

1:6:15:20:16:6:1 in higher multiplets the outer peaks are often nearly lost in the baseline
Scalar Coupling: Strong Coupling

\[ |\omega_{0A} - \omega_{0X}| \sim \pi J_{AX} \]

Second-order spectra, AB spin system

\[ J > 0 \]

\[ \alpha \beta \cos \theta + \beta \alpha \sin \theta \]

\[ -\alpha \beta \sin \theta + \beta \alpha \cos \theta \]

\[ \omega_{0A} - \omega_{0X} \]

\[ \omega_{0X} \]

\[ D = \left[ (\omega_{0A} - \omega_{0B})^2 + \pi J^2 \right]^{1/2} \]

\[ \sin 2\theta = J/D \]

\[ \text{sos} 2\theta = (\omega_{0A} - \frac{1}{2} \omega_{0BX})/D \]

\[ E_4 = \frac{1}{2} \omega_{0A} + \frac{1}{2} \omega_{0BX} + \frac{1}{4} \pi J_{AB} \]

\[ E_3 = \frac{1}{2} D - \frac{1}{4} \pi J_{AB} \]

\[ E_2 = -\frac{1}{2} D - \frac{1}{4} \pi J_{AB} \]

\[ E_1 = -\frac{1}{2} \omega_{0A} - \frac{1}{2} \omega_{0B} + \frac{1}{4} \pi J_{AB} \]
• As $\Delta \nu$ approaches $J$, more and more transitions of similar energy occur.

• Our system is now a second-order system. We have effects that are not predicted by the simple multiplicity rules that were described earlier.
Scalar Coupling: Second-Order Spectra

• **AB** system has the *roofing effect*: coupled pairs will lean towards each other, making a little roof:

• The chemical shifts of nuclei A and B are not at the center of the doublets. They will be at the center of mass of both lines. $\Delta v$ the $\nu_A - \nu_B$ chemical shift difference

\[
\Delta v^2 = |(f_1 - f_4)(f_2 - f_3)|
\]

\[
\nu_A = \nu_Z - \Delta v / 2
\]

\[
\nu_B = \nu_Z + \Delta v / 2
\]

• Peak intensities can be computed similarly:

\[
\frac{I_2}{I_1} = \frac{I_3}{I_4} = \frac{|f_1 - f_4|}{|f_2 - f_3|}
\]

\[
|J_{AB}| = |f_1 - f_2| = |f_3 - f_4|
\]
Scalar Coupling: Second-Order Spectra

Transition from $A_2X$ to $A_2B$
J and Magnetic Field: Comparison

Coupling constants are **constant** - they do not change at different field strengths.

100 MHz

J = 7.5 Hz

200 Hz

7.5 Hz

100 Hz

200 MHz

Separation is larger

J = 7.5 Hz

400 Hz

200 Hz

The **shift** is dependant on the field.
Note the compression of multiplets in the 200 MHz spectrum when it is plotted on the same scale as the 100 MHz spectrum instead of on a chart which is twice as wide.
Why buy a higher field instrument?

Spectra are simplified!

Overlapping multiplets are separated.

Second-order effects are minimized.

J and Magnetic Field: Comparison

50 MHz
J = 7.5 Hz

100 MHz
J = 7.5 Hz

200 MHz
J = 7.5 Hz
Besides identifying the type of hydrogen, we can also obtain the relative Numbers of each type of hydrogen by integration.

Integration is determining the area under a peak.

The area under a peak is proportional to the number of hydrogens that generate the peak.
Integration of a Peak

The integral line rises an amount proportional to the number of H in each peak

Benzyl Acetate

\[
55: 22: 33 = 5: 2: 3 \text{ simplest ratio of the heights}
\]
• Each different type of hydrogen gives a peak or group of peaks (multiplet)

• The chemical shift, in ppm, is suggestive of the type of hydrogen generating the peak

• The integral gives the relative numbers of each type of hydrogen

• Spin-spin splitting gives the number of hydrogens on adjacent carbons

• The coupling constant $J$ also gives information about the arrangement of the atoms involved, dihedral angles for instance
$^{12}\text{C}$ is not NMR active, $I=0$

$^{13}\text{C}$ is NMR active, $I=1/2$

However, $^{13}\text{C}$ is only 1.08% abundant:

- Low gyromagnetic ratio
- Signals about 6000 times weaker than $^1\text{H}$

The chemical-shift range of $^{13}\text{C}$ is larger than that of $^1\text{H}$, about 0-200 ppm
For a given field strength $^{13}\text{C}$ has its resonance at a different (lower) frequency than $^1\text{H}$.

Divide the hydrogen frequency by 4 (approximately) for carbon-13.
Due to the low natural abundance of $^{13}$C spins, the probability of finding two $^{13}$C atoms next to each other in a single molecule is very small.

$^{13}$C-$^{13}$C coupling NO! Not probable

$^{13}$C spectra are determined by many molecules contributing to the spectrum, each having only one $^{13}$C atom.

However, $^{13}$C does couple to $^1$H (spin $\frac{1}{2}$)

$^{13}$C-$^1$H coupling YES! Very common
$^{13}\text{C}$-$^1\text{H}$ J Coupling, Heteronuclear Coupling

The effect of attached protons on $^{13}\text{C}$ resonances

( $n+1$ rule applies )  
(J’s are large ~ 100 - 200 Hz)
$^{13}$C-$^1$H J Coupling, Heteronuclear Coupling

Ethyl phenylacetate

$^{13}$C coupled to the hydrogens
In cases of many nuclear spins, J couplings can reduce the sensitivity and crowd the spectra, hence, the need to remove them. Heteronuclear/homonuclear J decoupling.
Heteronuclear Decoupling in Solution-State NMR

$^{1}H$
$I$

$^{13}C$
$(\pi/2)_y$

S spin detection

Decoupling

RF

Decoupling
### Heteronuclear Decoupling in Solution-State NMR

<table>
<thead>
<tr>
<th>Situation</th>
<th>Decoupling Scheme</th>
<th>Rational</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decouple $^{15}\text{N}$, observe protons.</td>
<td>WALTZ-16.</td>
<td>Narrow bandwidth of $^{15}\text{N}$, typically 30 ppm ($\Delta F = 1.8$ kHz) with no $^{15}\text{N}^{15}\text{N}$ coupling, WALTZ-16 provides excellent line-narrowing.</td>
</tr>
<tr>
<td>Decouple $^{13}\text{C}$, observe protons.</td>
<td>GARP-1 for natural abundance.</td>
<td>Require high bandwidth to cover carbon spectrum. Typical bandwidth is 80 ppm ($\Delta F = 12$ kHz).</td>
</tr>
<tr>
<td></td>
<td>DIPSI-3 for uniformly labeled samples, provided sufficient bandwidth can be generated. This will depend on the hardware and the desired bandwidth.</td>
<td>$^{13}\text{C}^{13}\text{C}$ couplings can interfere with GARP-1 decoupling.</td>
</tr>
<tr>
<td>Decouple $^{1}\text{H}$, observe carbon or nitrogen.</td>
<td>DISPI-2 or DIPSI-3. Timing constraints may force the use of DIPSI-2 in triple resonance experiments.</td>
<td>Moderate proton bandwidth needed, 4 ppm ($\Delta F = 2.4$ kHz) for amides, 6 ppm for aliphatics ($\Delta F = 3.6$ kHz). $^{1}\text{H}^{1}\text{H}$ couplings will interfere with WALTZ-16 and GARP-1.</td>
</tr>
</tbody>
</table>
Heteronuclear decoupling- A double-resonance scheme

Others being Nuclear Overhauser Effect, Spin Tickling and other sophisticated schemes
Decoupling: Spectral Simplification

Ethyl phenylacetate
### $^{13}$C Chemical Shift Chart

<table>
<thead>
<tr>
<th>Type of Carbon</th>
<th>Electronwith Effects</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated carbon - sp$^3$</td>
<td>no electronegativity</td>
<td>8 - 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 - 55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 - 60</td>
</tr>
<tr>
<td>Saturated carbon - sp$^3$</td>
<td>electronegativity</td>
<td>40 - 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 - 80</td>
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<tr>
<td></td>
<td></td>
<td>25 - 65</td>
</tr>
<tr>
<td>Unsaturated carbon - sp$^2$</td>
<td></td>
<td>65 - 90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 - 150</td>
</tr>
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<td></td>
<td></td>
<td>110 - 175</td>
</tr>
<tr>
<td>Aldehyde and Ketones</td>
<td></td>
<td>155 - 185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>185 - 220</td>
</tr>
<tr>
<td>Aromatic ring carbons</td>
<td></td>
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</tbody>
</table>
13C PPM Chart for Carbonyl and Nitrile Functional Groups
Structural Determination with 1D NMR

• Structure determination of small molecules possible with 1D NMR

• Chemical shift, J coupling, geometry ...........

• Other sophisticated experiments
  
  • INEPT
  • DEPT
  • Nuclear Overhauser Effect, NOE
  • Relaxation measurements
  • Exchange experiments
  • Diffusion experiments
  • Variable temperature experiments
Homodecoupling

- Simple & quick means of determining if two spins are coupled
- Effective on molecules with simple, well-dispersed spectra
- Involves irradiation of selected resonance with low power, thus eliminating any coupling to this spin
- Comparison of homodecoupled spectrum with the normal one, coupling partners of the irradiated peak are determined

2D counterpart ➔ COSY  Homodec. spectrum of ethyl benzene
Conclusions

• NMR is a very powerful method to probe geometry, dynamics, and other structural information parameters

• Chemical shift, scalar coupling, relaxation rate constants, peak integrals are some of the methods used in one-dimensional NMR

• 1D NMR can yield a host of information regarding a wide range of molecules

• $^1$H and $^{13}$C are the normal probes in 1D NMR

• Other probes, such as, $^{31}$P, $^{29}$Si, $^{11}$B, and many other spin ½ or spin higher nuclei may be used to characterise materials

• NMR also is a thorough test bed for many quantum mechanics principles

• A versatile tool with far reaching implications in Physics, Chemistry, Biology, and Medicine