NMR (CHEM8028)

Solid-state NMR: Anisotropic interactions and how we use them

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NMR: From Molecular to Cellular Level

Solid State NMR

- Cell
- Mitochondrion
- Membrane
- Solution

Increasing complexity

Tissues
Organelles
Lipid/protein assemblies
Key proteins

Liquid NMR
Solid-state NMR spectra
Solid-state NMR

- Anisotropic Interactions
  - What are they, what do they do (to our spectra)
- How can we manipulate them
  - Oriented samples
  - Magic angle spinning
- How can we exploit them
  - Cross polarization
  - Dipolar recoupling
- How can we use them to probe structure/dynamics (2nd series of lectures)
Outline (1)

- What is anisotropy
- How does it effect NMR spectra
- What interactions give rise to anisotropic properties?
- Describing interactions: tensors
- Chemical Shielding Anisotropy
  - Orientational dependence of resonance frequency
  - Powder spectra
- Dipolar interactions
- Quadrupolar interactions
What is anisotropy

- Something whose properties depend on its orientation
  e.g. stress
How does it effect the NMR spectrum

- Each molecular orientation gives rise to a difference resonance frequency

- In powder we have the sum of all distributions

- In the liquid state these anisotropic properties are averaged on the NMR timescale
Which interactions in NMR

Isotropic

\[ H = H_{CS} \]

Anisotropic

\[ H = H_{CS} + H_J + H_{CSA} \]

\[ H = H_{CS} + H_J \]

\[ H = H_{CS} + H_J + H_{CSA} + H_{Dipolar} \]

\[ H = H_{CS} + H_J + H_{CSA} + H_{Dipolar} + H_Q \]
Describing interactions: tensors (1)

We are concerned with 3 flavours

- Zero rank tensors
  - Physical property independent of coordinate system in which it is described (scalar, distance)

- First rank tensors
  - Coordinate, depends on frame of reference (vector, has size and direction)

- Second rank tensors
  - Multiple first rank tensors e.g. stress (matrix)

- Higher rank exist – but we will not be considering
Describing interactions: tensors (2)

Rank zero tensor

Isotropic chemical shift, J-coupling

Rank one tensor

(0,0,B_z)

B_0
Describing interactions: tensors (3)

- Second rank tensors

\[
\sigma = \begin{pmatrix}
\sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz}
\end{pmatrix}
\]

\[
\sigma_{PAS} = \begin{pmatrix}
\sigma_{xx} & 0 & 0 \\
0 & \sigma_{yy} & 0 \\
0 & 0 & \sigma_{zz}
\end{pmatrix}
\]
Parameterizing 2\textsuperscript{nd} rank tensors

• In cartesian notation tensors defined by principle components, $A_{xx}, A_{yy}$ and $A_{zz}$

• Frequently parameterized with

$$a = Tr\{A\} = A_{xx} + A_{yy} + A_{zz}$$

$$\delta = A_{zz} - \frac{a}{3}$$

$$\eta = \frac{A_{yy} - A_{xx}}{\delta}$$

• This assumes

$$\left| A_{zz} - \frac{a}{3} \right| \geq \left| A_{xx} - \frac{a}{3} \right| \geq \left| A_{yy} - \frac{a}{3} \right|$$

• Thus the asymmetry $0.0 < \eta < 1.0$ and anisotropy can be both positive and negative
Chemical Shielding Anisotropy (1)

- Perturbation of the magnetic field due to interaction with surrounding electrons

- Inherently asymmetric (e.g. electron distribution surrounding carbonyl group)
Chemical Shielding Anisotropy (2)

• We can describe the perturbation of the main field ($B_0$), by the second rank tensor, $\sigma$.

$$B_S = \begin{bmatrix}
\sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz}
\end{bmatrix} 0
$$

• The Hamiltonian which describes the interaction with the modified field is:

$$H_{CSA} = \sum_k \gamma_k \begin{bmatrix}
\hat{I}_{kx} & \hat{I}_{ky} & \hat{I}_{kz} \\
\sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz}
\end{bmatrix} \begin{bmatrix} 0 \\ 0 \\ B_0 \end{bmatrix}$$

Which can be written in a simplified form as: $H_{CSA} = \sum_k \gamma_k \hat{I}_k \sigma_k \tilde{B}_0$
Chemical Shielding Anisotropy (3)

Thus the chemical shielding Hamiltonian simplifies to:

\[ H_{CSA} = \sum_k \gamma_k \hat{I}_{kz} \sigma^{(k)}_{zz} \mathbf{B}_0 \]

and the resonance frequency of the line is:

\[ \omega_{12} = -\sigma^{(k)}_{zz} \omega_0 \]

Thus the resonance frequency is proportional to \( \sigma_{zz} \) in the laboratory frame.

However, \( \sigma \) is usually defined in the principle axis system (PAS) not in the lab frame (LF). Therefore, we need to transform \( \sigma \) from the PAS to LF.
Transformations

Principle Axis System

Lab Frame

- Rotation characterized by the three Euler angles ($\alpha, \beta, \gamma$)
- Multiple $\mathcal{J}$ by rotation matrix $R$
Transformation matrix

Can derive a rotation matrix which bring about the rotation described above:

\[
R(\alpha, \beta, \gamma) = \begin{pmatrix}
    \cos \alpha \cos \beta \cos \gamma - \sin \alpha \sin \gamma & \sin \alpha \cos \beta \cos \gamma + \cos \alpha \sin \gamma & -\sin \beta \cos \gamma \\
    -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma & -\sin \alpha \cos \beta \sin \gamma + \cos \alpha \cos \gamma & \sin \beta \sin \gamma \\
    \cos \alpha \sin \beta & -\sin \alpha \sin \beta & \cos \beta
\end{pmatrix}
\]

To determine \( \sigma \) in the laboratory frame, need to apply to the chemical shielding tensor \( \sigma \) in the principle axis system:

\[
\sigma_{LAB} = R(\alpha, \beta, \gamma) \sigma_{PAS} R^{-1}(\alpha, \beta, \gamma)
\]

This can be simplified to give general Hamiltonian for CSA in lab frame of:

\[
H_{CS} = \left( -\gamma_k B_0 \left( \sigma_{iso} + \frac{\delta}{2} \left[ 3 \cos^2 \beta - 1 \right] - \eta \sin^2 \beta \cos 2\alpha \right) \right) \hat{I}_{kz}
\]

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Effect on resonance position

\( \sigma_{zz} = 3000 \text{Hz} \)
\( \sigma_{xx} = -1500 \text{Hz} \)
\( \sigma_{yy} = -1500 \text{Hz} \)

\[ \sigma_{iso} = \frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz}) = 0 \text{Hz} \]

\[ \delta = \sigma_{zz} - \sigma_{iso} = 3000 \text{ Hz} \]

\[ \left| \frac{\sigma_{yy} - \sigma_{xx}}{\delta} \right| = 0.0 \]

\[ H_{CS} = -\gamma k B_0 \left( \sigma_{iso} + \frac{\delta}{2} \left[ (3 \cos^2 \beta - 1) - \eta \sin^2 \beta \cos 2\alpha \right] \right) \hat{i}_k \]
Powder Patterns

• In powders we have a random distribution of molecular orientations.

• Thus the lineshape is the weighted superposition of all the different orientations:

\[
s(t) = \frac{1}{8\pi^2} \int_0^{2\pi} \int_0^{2\pi} \int_0^{2\pi} s(\alpha, \beta, \gamma, t) \delta\alpha \sin \beta \cdot \delta\beta \cdot \delta\gamma
\]
Empirical relation between PAS and MF

1) Methyl carbons → axially symmetric, axis along threefold symmetry axis
2) Ring carbons → three distinct tensor elements, most shielded perpendicular to plane, least shielded bisecting C-C-C angle of ring
3) Most shielded direction:
   1) Perpendicular to ring in aromatic carbons
   2) Along C3 axis for methyl carbons
   3) Perpendicular to the sp2 plane for carbonyl/carboxylic acids
4) Least shielded direction:
   1) In the ring plane, bisecting C-C-C angle
   2) Perpendicular to C3 axis for methyl groups
   3) In the sp2 place for carbonyl/carboxylic acids
5) Intermediate shielding
   1) Tangential to ring for aromatic systems
   2) In the sp2 plane and perpendicular to the C-C bond for COOH
**Dipolar Interaction**

**Classical interpretation**

Classical interaction energy between two magnetic (dipole) moments when both are aligned with the magnetic field:

\[ E = \frac{\mu_0}{4\pi} \frac{1}{r_{12}^3} \mu_1 \mu_2 (1 - 3 \cos^2 \theta) \]

**Quantum mechanical**

\[ H_D = \frac{\mu_0 \gamma_1 \gamma_2}{4\pi} \frac{\hbar}{r_{12}^3} \left( \tilde{I}_1 \tilde{I}_2 - \frac{3}{r_{12}^2} \left( \tilde{I}_1 \cdot \tilde{r}_{12} \right) \left( \tilde{I}_2 \cdot \tilde{r}_{12} \right) \right) \]

\[ \left. \quad = \tilde{I}_1 D \tilde{I}_2 \right. \]

where:

\[ D = \frac{\mu_0 \gamma_1 \gamma_2}{4\pi} \frac{\hbar}{r_{12}^3} \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -2 \end{pmatrix} \]

- Symmetric second rank axially symmetric tensor.
- Again we need to rotate from the PAS to LF to obtain resonance frequency.
Orientation dependence of dipolar interaction

Homo-nuclear Dipolar Hamiltonian:

\[
H_{D,II} = \frac{\mu_0}{4\pi} \frac{\gamma_i \gamma_j \hbar}{r_{12}^3} \frac{(1 - 3 \cos^2 \theta)}{2} \left( 3\hat{I}_{1z}\hat{I}_{2z} - \left( \hat{I}_1 \cdot \hat{I}_2 \right) \right)
\]

Hetero-nuclear Dipolar Hamiltonian:

\[
H_{D,JS} = \frac{\mu_0}{4\pi} \frac{\gamma_i \gamma_j \hbar}{r_{12}^3} \frac{(1 - 3 \cos^2 \theta)}{2} \left( 2\hat{I}_{1z}\hat{I}_{2z} \right)
\]

\[\delta_{\text{dip}} = 20 \text{ kHz}\]
Quadrupolar Interaction (1)

If spin > 1/2, nucleus contains an electronic quadrupole moment \( Q \).

Electronic quadrupole moment interacts with surrounding electron cloud (electric field gradient (EFG), \( V \)).

\[
H_Q = \vec{I}_k \vec{Q} \vec{I}_k
\]

where:

\[
Q = \frac{eQ}{2I(2I-1)} \begin{bmatrix}
V_{xx} & 0 & 0 \\
0 & V_{yy} & 0 \\
0 & 0 & V_{zz}
\end{bmatrix}
\]

Again we can define the anisotropy and asymmetry:

\[
\delta_Q = Q_{zz} = \frac{e^2 qQ}{2I(2I-1)\hbar}
\]

\[
\eta_Q = \frac{V_{yy} - V_{xx}}{V_{zz}}
\]
Quadrupolar Interaction (2)

To calculate the resonance frequency, we must transform from the PAS of the EFG to the laboratory frame.

Retaining only the “secular terms” gives the following Hamiltonian in the LF:

\[ H_Q = \frac{\delta_Q}{2} \left[ (3\cos^2 \beta - 1) - \eta_Q \sin^2 \beta \cos 2\alpha \right] (3I_z^2 - I(I + 1)) \]
Exploitation of anisotropic interaction

- Oriented samples
  - Single Crystal studies
  - Oriented Biological Membranes
- Dynamics
  - Averaging of anisotropic interaction
- Local electronic environment
  - Perturbation in chemical shielding anisotropy
Dynamics: averaging of anisotropy

Rotational diffusion: Scaling of interaction by $\frac{1}{2}(3\cos^2 \theta - 1)$ where $\theta$ is the angle between axis of motional averaging and the PAS of the interaction.
Oriented samples

Necessary to introduce macroscopic alignment:
1) Crystallization
2) Oriented membranes
3) Fibres (Silk/DNA)
Oriented samples – ligand orientations

**Graphical Representation**

- **Orientation ± 5°**
  - Graph showing frequency distribution with B₀ axis.
  - Peak at 0° orientation.

- **Mosaic Spread ± 5°**
  - Graph showing frequency distribution with B₀ axis.
  - Spread indicated at ±5°.

**Additional Visuals**

- 3D models of molecular structures indicating orientation and mosaic spread.

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Protein Backbone Orientation

$^{15}\text{N}$ chemical shielding anisotropy

$^{15}\text{N}$-$^1\text{H}$ hetero-nuclear dipolar coupling

Opella et al. 1998
Local electronic environment

As we shall see next week, typically these parameters are obtained under conditions of magic-angle spinning to enhance signal to noise.

FIGURE 1: Proton-decoupled $^{15}$N powder spectra of all-trans-retinyldenebutyl$^{15}$N)imine: (a) unprotonated; (b) the HCl salt. In (a) the powder pattern is $\sim$600 ppm (18 kHz) wide with an isotropic shift of 315 ppm. Protonation narrows the spectrum to a breadth of 270 ppm and moves the isotropic shift to 172 ppm.
An aside: spherical tensors

- Make the calculations a lot easier to handle
- Frequently used in papers
Sensitivity and resolution enhancement in solid-state NMR
Resume

Isotropic

\[ H = H_{CS} \]

H = H_{CS} + H_J

H = H_{CS} + H_J + H_{CSA}

Anisotropic

\[ H = H_{CS} + H_J + H_{CSA} + H_{Dipolar} \]

\[ H = H_{CS} + H_J + H_{CSA} + H_{Dipolar} + H_Q \]
Oriented samples

• Increase resolution by orienting interactions, therefore all spins resonate at the same frequency

• As all spins resonate with the same frequency the sensitivity of the measurements is higher
Magic-angle spinning
Magic Angle Spinning

Seeks to reintroduce averaging process through mechanical rotation
Averaging of anisotropic interactions
The Hamiltonian becomes time dependent:

$$\hat{H}_{CS}(t) = \omega(\alpha, \beta, \gamma, t)\hat{I}_z$$

We can deconvolute this into the iso- and anisotropic contributions:

$$\omega(\alpha, \beta, \gamma, t) = \omega_{iso} + \omega_{CSA}(\alpha, \beta, \gamma)$$

where

$$\omega_{iso} = \omega_0 \bar{\sigma}$$

and

$$\omega_{CSA}(\alpha, \beta, \gamma) = C_1 \cos(\omega t - \gamma) + S_1 \sin(\omega t - \gamma)$$

$$+ C_2 \cos(2\omega t - 2\gamma) + S_2 \sin(2\omega t - 2\gamma)$$

Where $C_1$, $C_2$, $S_1$ and $S_2$ relate the anisotropic interaction to magnetic field (Appendix 1).
Analysis of MAS spectra

- All anisotropic interactions become time dependent
- To analyze spectra need to treat these time dependencies
- Several mathematical descriptions that allow us to do this
  - Average Hamiltonian Treatment
  - Floquet Theory
  - Piece wise integration
Slow speed spinning

- Rotational echoes apparent in fid which characterise the anisotropy of the interaction

- At lower spinning speed the intensity of the sidebands characterises the anisotropic interaction ($\delta$ and $\eta$)
Herzfeld-Berger Analysis

Expression exist to calculate the intensity of sidebands for a given anisotropic interaction:

\[ I(N\omega_r) = \frac{1}{4\pi} \int_0^{2\pi} \int_0^{\pi} \delta\alpha \delta\beta \sin\beta |F(\alpha, \beta, N\omega_r)|^2 \]

where

\[ F(\alpha, \beta, N\omega_r) = \exp(i\Phi^{CSA}) \]

and

\[ \Phi^{CSA}(\alpha, \beta, \gamma, t) = \Phi^{iso} + \Phi^{CSA} \]

\[ \Phi^{iso}(t) = \omega^{iso} t \]

\[ \Phi^{CSA}(\alpha, \beta, \gamma, t) = \frac{C_1}{\omega_r} \sin(\omega_r t - \gamma) - \frac{S_1}{\omega_r} \cos(\omega_r t - \gamma) \]

\[ + \frac{C_2}{2\omega_r} \sin(2\omega_r t - \gamma) - \frac{S_2}{2\omega_r} \sin(2\omega_r t - \gamma) \]

CSA analysis in reality

Several programs now available that now facilitate this task:

1) Tables – Paper by Herzfeld and Berger

2) matNMR (routines for analysis of both CSA and quadrupolar interactions in both static and MAS spectra)
   http://matnmr.sourceforge.net/ (requires matlab)

3) MAS sideband analysis (Levitt group homepage)
   http://www.mhl.soton.ac.uk/public/Main/index.html (requires mathematica)
Effect of off-angle MAS

- Anisotropic interaction scaled by $\frac{1}{2}(3\cos^2\theta - 1)$
- Useful for characterizing anisotropy whilst gaining some sensitivity
- Indicates why magic angle should be carefully set!
When does MAS not work?

• Homogeneous interactions
  – e.g. Homonuclear dipolar interactions
• Heterogeneous line-broadening
  – e.g. Samples with conformational heterogeneity (lyophilized solids)
• Nuclei with large quadrupolar interactions
• When samples are not ‘solid’
Applications of MAS

- Resolution/Sensitivity Enhancement ✓
- Low speed spinning – characterisation of anisotropy

Isotropic chemical shifts in the protein backbone are sensitive to secondary structure.

Analysis of the principle components of the chemical shielding tensor reveals that larger changes are seen in $\sigma_{22}$ making it a sensitive probe of protein secondary structure.

Wei et al. 2001 JACS 123: 6118-26
Applications of MAS

- Low speed spinning
  - anisotropy → mobility

Amyloid precursor protein in differing lipid environments has different propensity to oligomerise. Sideband analysis reveals changes in peptide mobility

Magic-angle spinning and metabolomics
Tale of a hungry worm

4kHz MAS spectrum of *C. elegans* (400MHz)
Carbohydrate metabolism
Fatty acid metabolism
MAS-NMR and metabolism

Spectroscopically:
• Simple markers for metabolites
• Observe changes in metabolite levels
• Labelling possible to aid in assignment

Biologically:
• Genetics of *C. elegans* well characterized
• Large library of mutations
• Development well understood
• Genes linked to phenotype
• Behavioral differences
A molecular view of biological systems

Structure of high affinity ligands

Membrane protein complexes

Regulation of intra-cellular trafficking

Whole organisms