Solid-State NMR Studies of Molecular Motion

and

Phase Cycling

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Molecular Motion is Abundant in Biomolecules & is Important for Function

- Ion conduction
- Protein folding
- Enzyme catalysis
- Ligand binding

![Time Scale Diagram]

- **Ultrafast spectroscopy**
- **NMR**
  - $T_1$, $^2H \ T_{1Q}$
  - CSA
  - dipolar/quad. lineshapes
- **slow-exchange NMR**
  - $^1H$-dec. $T_2$, $T_{1p}$
  - $^2H \ T_2$
Common Molecular Motions

Local motions

- Methyl & amine 3-site jumps
- Aromatic ring flip
- Small-amplitude torsional fluctuation
- Sidechain rotameric jumps (e.g. Leu mt - tp)
- trans-gauche isomerization
- Large-amplitude motions of flexible loops & termini

Global motions

- Rigid-body uniaxial diffusion of membrane proteins in lipid bilayers
- Correlated motions of protein domains
Effects of Motion on Solid-State NMR Spectra

Molecular motions can:
- average NMR lineshapes;
- reduce or enhance signal intensities;
- affect relaxation properties;
- complicate quantification;
- allow spectral editing

- Timescales & amplitudes of motion from NMR
- Fast motion: average (sum) tensor
- Experiments for measuring fast motion
- Order tensors & order parameters
- Slow motion: difference tensor
- Experiments for measuring slow motion
Rates & Amplitudes of Reorientational Motions

- For stochastic motions, correlation function \( C(t) \sim \langle f(0) \cdot f(t) \rangle \) describes how long it takes to randomize the molecular orientation. The characteristic decay time is \( \tau_c \).

- **Rates**: \( k \) (s\(^{-1}\)) is inversely related to correlation time \( \tau_c \). Rate (s\(^{-1}\)) \( \neq \) frequency (Hz).

- **Amplitudes**: denotes reorientational angle \( \beta_R \) and the number of sites \( n_R \).
- We do not consider translational motion here, which is typically studied by pulsed-field-gradient NMR.
- **Diffusive motion**: infinitesimal \( \beta_R \), infinite \( n_R \). e.g. isotropic tumbling, uniaxial diffusion, torsional fluctuations.
- **Discrete motion**: finite \( \beta_R \), finite \( n_R \):
  - Methyl 3-site jump: \( \beta_R = 109.5^\circ \), \( n_R = 3 \) for the C-H bonds
  - Phenylene ring flip: \( \beta_R = 120^\circ \), \( n_R = 2 \) for ortho and meta C-H bonds
Motional Regimes Accessible From NMR

• Fast motion: $k >> \Delta \omega$ or $\delta$, typically $\tau_c < 1 \mu s$
  • Amplitudes: obtained from spectral line narrowing.
    • e.g. $^2$H spectra, DIPSHIFT, LG-CP, WISE, CSA narrowing.
  • Rates: $> 10 \times \delta$; Exact value obtained from relaxation NMR.

• Slow motion: $k << \Delta \omega$, typically $\tau_c > 1 \text{ ms}$
  • Measured by exchange NMR;
  • Amplitudes: from 2D cross peaks & $N t_r$-dependent CODEX intensities.
  • Rates: measured as the decay constant in $t_m$-dependent intensities.
  • # of sites: from the final value of the CODEX mixing-time curve.

• Intermediate motion: $k \sim \Delta \omega$.
  • Manifests as intensity losses due to interference with $^1$H decoupling & MAS.
  • Rates: from $T_2$ and $T_1 r$ minima in log($T_{2,1 r}$) plots vs $1/T$.
  • Amplitudes: from asymmetric DIPSHIFT intensity decays
Effects of Motion on NMR Lineshapes

Equal population ($p_A = p_B = 0.5$)

Skewed population ($p_A = 0.75; p_B = 0.25$)

**Fast motion:** $k \gg |\omega_A - \omega_B|$

Average frequencies, $\bar{\omega}$, can be calculated for different motional models.

**Intermediate motion:** $k \approx |\omega_A - \omega_B|$

**Slow motion:** $k \ll |\omega_A - \omega_B|$

Measured during a mixing time.

SSNMR Studies of Molecular Motion

- Timescales & amplitudes of motion from NMR
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Motional Averaging of NMR Frequencies

For a nuclear spin interaction tensor $\sigma$:

$$\omega(\theta, \phi) = \frac{1}{2} \delta \left( 3 \cos^2 \theta - 1 - \eta \sin^2 \theta \cos 2\phi \right)$$

Reorientation among N sites with probability $p_j$ gives an average tensor:

$$\overline{\omega} = \sum_j p_j \omega_j \quad \Rightarrow \quad \text{average tensor} \quad \Sigma = \sum_j p_j \sigma_j$$

- $\Sigma$ has 3 principal axes ($\Sigma_1, \Sigma_2, \Sigma_3$).
- $\Sigma$ is characterized by $\overline{\delta}, \overline{\eta}$, which reflect the geometry of motion.
- $B_0$ orientation with $\Sigma$: $(\theta_a, \phi_a)$.

$$\overline{\omega}(\theta_a, \phi_a) = \overline{\delta} \frac{1}{2} \left( 3 \cos^2 \theta_a - 1 - \overline{\eta} \sin^2 \theta_a \cos 2\phi_a \right)$$

Once the average tensor is known, we can predict the motionally averaged spectrum.

- In general, $\overline{\delta} \neq \delta$, $\overline{\eta} \neq \eta$.
- For dipolar couplings, $\overline{\delta}$ can be sign-sensitive, and $\overline{\eta} \neq 0$.

How do we determine $\overline{\delta}$ and $\overline{\eta}$?
Motionally Averaged $\delta$ and $\eta$

From symmetry:
- Isotropic motion $\Rightarrow \bar{\delta} = 0$
- Uniaxial rotation $\Rightarrow \bar{\eta} = 0$
- $N \geq 3$ $C_N$ jumps

For uniaxial rotation and $N \geq 3$ $C_N$ jumps, the z-axis of the average tensor is the symmetry axis, $z_D$.

$\bar{\delta}$ is the frequency when $B_0$ is parallel to the z-axis of the $\Sigma$ tensor. Under this condition, motion does not change $B_0$ orientation with the original PAS, thus the frequency is $\omega (\theta_{PD}, \phi_{PD})$.

\[
\bar{\delta} = \omega (\theta_{PD}, \phi_{PD}) = \frac{1}{2} \delta \left(3 \cos^2 \theta_{PD} - 1 - \eta \sin^2 \theta_{PD} \cos 2\phi_{PD}\right)
\]
Methyl 3-Site Jumps

\[
\bar{\delta} = \omega(\theta_{PD}, \phi_{PD}) = \frac{1}{2} \delta \left( 3 \cos^2 \theta_{PD} - 1 - \eta \sin^2 \theta_{PD} \cos 2 \phi_{PD} \right)
\]

- C-H ($\eta = 0$, $\theta_{PD} = 109.5^\circ$): $\bar{\delta} = \frac{1}{2} \delta \left( 3 \cos^2 109.5^\circ - 1 \right) = -\delta/3$, $\bar{\eta} = 0$

\[2^\text{H} \text{ spectra of C-H bond}\]

\begin{align*}
\text{k} & \quad 10^4 \text{ s}^{-1} \\
\text{slow} & \\
\text{k} & \quad 10^6 \text{ s}^{-1} \\
\text{intermediate} & \\
\text{k} & \quad 10^8 \text{ s}^{-1} \\
\text{fast} & \\
\end{align*}

- H-H ($\eta = 0$, $\theta_{PD} = 90^\circ$): $\bar{\delta} = \frac{1}{2} \delta \left( 3 \cos^2 90^\circ - 1 \right) = -\delta/2$, $\bar{\eta} = 0$

Order parameter: $S \equiv \bar{\delta}/\delta \begin{cases} S_{CH, \text{methyl}} = -1/3 \\ S_{HH, \text{methyl}} = -1/2 \end{cases}$

Average Tensor for Two-Site Jumps

For 2-site jumps averaging a uniaxial ($\eta = 0$) tensor, we can determine the $\Sigma$ tensor principal values using the same approach: calculate the frequency when $B_0$ is parallel to each of the 3 principal axes of the $\Sigma$ tensor.

The $\Sigma$ tensor is invariant under A-B switching: $\Sigma = (\sigma_A + \sigma_B)/2 = (\sigma_B + \sigma_A)/2$

So the 3 principal axes should be:

- $\Sigma_1$: Bisector of the AOB angle
- $\Sigma_2$: Normal to the bisector in the AOB plane
- $\Sigma_3$: Normal to the AOB plane

<table>
<thead>
<tr>
<th>$\Sigma_1$ axis</th>
<th>$\Sigma_2$ axis</th>
<th>$\Sigma_3$ axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta/2$,</td>
<td>$90^\circ + \beta/2$,</td>
<td>$90^\circ$,</td>
</tr>
<tr>
<td>$\beta/2$,</td>
<td>$90^\circ - \beta/2$,</td>
<td>$90^\circ$,</td>
</tr>
</tbody>
</table>

- 1, 2, 3 convention: left to right, i.e. $\omega_1 > \omega_2 > \omega_3$

$\beta < 90^\circ$ and $\beta > 90^\circ$ switch $\Sigma_1$ & $\Sigma_2$ axes.

The principal values of the average tensor:

$$\frac{1}{2} \delta \left( 3 \cos^2 \Theta_n - 1 \right)$$

$\theta_n$: angle between $z_{\text{PAS}}$ and $\Sigma_n$
Two-Site Jumps: Phenylene Ring Flip

Consider $^2$H or C-H dipolar spectra ($\eta = 0$):
Reorientation angle $\beta_R = 120^\circ$.

\[
-\omega_n = \frac{1}{2} \delta \left( 3 \cos^2 \Theta_n - 1 \right)
\]

\[
\begin{align*}
\Theta_1 &= 30^\circ \\
\Theta_2 &= 60^\circ \\
\Theta_3 &= 90^\circ
\end{align*}
\]

\[
\begin{align*}
-\omega_1 &= \frac{5}{8} \delta \\
-\omega_2 &= -\frac{1}{8} \delta \\
-\omega_3 &= -\frac{1}{2} \delta
\end{align*}
\]

\[
\bar{\delta} = \frac{5}{8} \delta \\
\bar{\eta} = 0.6
\]

$\bar{\eta} \neq 0$ for the average dipolar tensor.
Two-Site Jumps: *trans-gauche* Isomerization

For C-H dipolar coupling or $^2$H quadrupolar coupling:

$$\beta_R = 109.5^\circ: \theta_n = 35.3^\circ, 54.7^\circ, 90^\circ: \quad \Rightarrow \quad \ddot{\omega}_n = \frac{\delta}{2}, 0, -\frac{\delta}{2}. $$

$^2$H spectra

\[ k \quad 10^4 \text{ s}^{-1} \quad 10^6 \text{ s}^{-1} \quad 10^8 \text{ s}^{-1} \]
slow \quad intermediate \quad fast

\[ \bar{\delta} = \frac{1}{2} \delta, \quad \bar{\eta} = 1 \]

Two-Site Jumps: Histidine 180° Ring Flip

180° jump around the Cβ-Cγ bond (χ2 change):

For the Cγ-Nδ1 bond: \( β_R = 2 \cdot 57° = 114° \)

\[
\bar{ω}_n = \frac{1}{2} \delta \left( 3 \cos^2 Θ_n - 1 \right)
\]

\[
\begin{align*}
Θ_1 &= 33° \\
Θ_2 &= 57° \\
Θ_3 &= 90°
\end{align*}
\]

\[
\begin{align*}
\bar{ω}_1 &= 0.56δ \\
\bar{ω}_2 &= -0.06δ \\
\bar{ω}_3 &= -0.5δ
\end{align*}
\]

For the Cδ2-Hδ2 bond: \( β_R = 156° \)

\( \bar{δ} = 0.94δ \) \( S_{Cδ2-Hδ2} = 0.94 \)
SSNMR Studies of Molecular Motion

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X-\textsuperscript{1}H Dipolar-Shift Correlation (DIPSHIFT)

- Passive sampling of X-\textsuperscript{1}H dipolar coupling in a rotor period combined with active \textsuperscript{1}H-\textsuperscript{1}H decoupling
  - MREV-8: \leq 7 kHz
  - LG-CP: < 40 kHz
  - FSLG, PMLG, eDUMBO: \sim < 40 kHz

- Active recoupling of X-\textsuperscript{1}H dipolar coupling
  - e.g. \textsuperscript{15}N-\textsuperscript{1}H REDOR in deuterated proteins with back exchanged \textsuperscript{1}H

- Symmetry-based R sequences, \sim < 40 kHz
  - e.g. \textsuperscript{1}R14\textsubscript{3}, \textsuperscript{1}R16\textsubscript{3}, \textsuperscript{1}R18\textsubscript{4}, \textsuperscript{1}R26\textsubscript{5}, \textsuperscript{1}R28\textsubscript{5}

\begin{align*}
\omega_1 / \omega_r & \quad 2.33 \quad 2.67 \quad 2.25 \quad 2.6 \quad 2.8 \\
k & \quad 0.27 \quad 0.28 \quad 0.26 \quad 0.27 \quad 0.28
\end{align*}

Schanda, Meier, Ernst et al.

Polenova et al.
Constant-Time $^{1}{H}$ DIPSHIFT

- A separated-local-field (SLF) technique.
- $^{1}{H}-^{1}{H}$ homonuclear decoupling

$$\Psi(t_1) = \int_0^{t_1} \omega(t) \, dt, \text{ where } \omega \propto \delta \cdot S \cdot k$$

- Allows higher $v_r$ to be used to measure small couplings.
- Constant time removes $^{1}{H} \, T_2$ decay during $t_1$.

DIPSHIFT Time Signals

7 kHz MAS, FSLG decoupling ($k = 0.577$),
Typical rigid-limit values: C-H bond: 22.7 kHz, covalent N-H: 10.5 kHz

1x, C-H

1x, N-H

2x, C-H

2x, N-H

Insensitive.
$^1$H-X Dipolar Coupling from LG-CP Oscillations

Magic-angle tilted spin lock on $^1$H:

Simple: increment CP contact time as $t_1$.

$^1$H-$^1$H dipolar coupling is removed by LG spin lock.

Can be conducted under relatively fast MAS ($\sim 40$ kHz)

Detection in the frequency domain resolves multiple splittings.

Large scaling factor: $k = \cos(54.7^\circ) = 0.577$.

CP matching may be unstable under fast MAS.

Hong et al *JPC*, 106, 7355 (2002).
Lee-Goldburg CP Time Signals

HH-CP does not have as distinct C-H oscillations due to the presence of multi-spin $^1\text{H}-^1\text{H}$ dipolar couplings at regular MAS frequencies.
Example: a Bacterial Toxin Increases Motional Amplitudes Upon Membrane Binding

Colicin Ia channel domain

$^2$H Sideband Patterns Reflect Fast Motion

15 kHz MAS
- $C_Q = 50$ kHz
- $C_Q = 100$ kHz
- $C_Q = 150$ kHz (C-D)
- $C_Q = 200$ kHz
- $C_Q = 250$ kHz (O-D)

20 kHz MAS

15 kHz MAS
- $C_Q = 170$ kHz (C-D)
- $S_{CD} = 0.20$
- $S_{CD} = 0.30$
- $S_{CD} = 0.40$
- $S_{CD} = 0.50$
$^{13}$C-$^2$H Correlation: Polarization Transfer by RESPIRATION-CP

Jain...Nielsen, PCCP, 2014;
Wei...Nielsen, JPC Letters, 2011.

CD-labeled Bacterial Cellulose
CDN-labeled Valine
HD exchanged $^{13}$C-labeled D-Glucose


$$\eta_{D\rightarrow C,C} = \frac{\gamma_C I_{D\rightarrow C}}{\gamma_D I_C}$$
2D $^2$H-$^{13}$C Correlation Spectra of Cellulose

Bacterial Cellulose, Crystalline

Plant Cell Wall Cellulose, Disordered

$^{13}$C DP

$^2$H-$^{13}$C $^{\text{resp}}$ CP

interior cellulose C4, 89 ppm

Experimental

$C_\text{g} = 167^{+7}_{-5}$ kHz

$\eta = 0$

surface cellulose C6, 61 ppm

Experimental

$C_\text{g} = 80^{+20}_{-10}$ kHz

$\bar{\eta} = 1$

$\bar{\eta} = 0$

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A Common Motion of Membrane Peptides: Rigid-Body Uniaxial Diffusion

Ovispirin, antimicrobial peptide

Protegrin-1, antimicrobial peptide

Tachyplesin-1, antimicrobial peptide

PMX30016, a planar antimicrobial arylamide

Influenza M2 transmembrane domain

Order Parameters in a Rigid Uniaxial Molecule

- For a uniaxially diffusive system with director $Z_D$, the bond order parameter is:

$$S_{bond} \equiv \bar{\delta} = \frac{1}{2} \langle \left( 3 \cos^2 \theta_{PD} - 1 \right) \rangle$$

- $\theta_{PD}$: angle of the bond with the director.
- $\langle \rangle$ denote averaging over different $\theta_{PD}$.

- For a rigid molecule undergoing uniaxial rotation, $\theta_{PD}$ is fixed; thus,

$$S_{bond} = \frac{1}{2} \left( 3 \cos^2 \theta_{PD} - 1 \right)$$

- If the rigid molecule rotates around a molecular axis and the molecular axis rotates or wobbles around $Z_D$ (e.g. cholesterol) then:

$$S_{bond} = \frac{1}{2} \left( 3 \cos^2 \theta_{PM} - 1 \right) \cdot \frac{1}{2} \left( 3 \cos^2 \theta_{MD} - 1 \right) = \frac{1}{2} \left( 3 \cos^2 \theta_{PM} - 1 \right) \cdot S_{mol}$$
Uniaxial Rotation of a Rigid Small Molecule

Amantadine is rigid, and all bonds lie on a diamond lattice with tetrahedral angles relative to the molecular axis, $Z_M$.

Relative to $Z_M$:
- 12 CD bonds: $\theta_{PM} = 70.5^\circ, 109.5^\circ$
- 3 CD bonds: $\theta_{PM} = 0^\circ$

- If amantadine rotates only around the molecular axis, then the average $^2$H quadrupolar coupling is:

$$\bar{\delta} = \frac{1}{2} \delta \left(3 \cos^2 \theta_{PM} - 1\right)$$

- 12 CD bonds: $0.33 \cdot \delta = 40 \text{ kHz}$
- 3 CD bonds: $1.0 \cdot \delta = 125 \text{ kHz}$

- If amantadine also rotates around the bilayer normal $Z_D$:

$$\bar{\delta} = \frac{1}{2} \delta \left(3 \cos^2 \theta_{PM} - 1\right) \cdot \frac{1}{2} \left(3 \cos^2 \theta_{MD} - 1\right)$$

$$= \frac{1}{2} \delta \left(3 \cos^2 \theta_{PM} - 1\right) \cdot S_{mol}$$
**Amantadine Dynamics in Lipid Bilayers**

- 12 CD bonds: \(0.33 \cdot \delta = 40 \ \text{kHz}\)
- 3 CD bonds: \(1.0 \cdot \delta = 125 \ \text{kHz}\)

**Gel phase:** \(S_{mol} \approx 1 \Rightarrow \theta_{MD} = 0^\circ\)

**Liquid-crystalline phase:**
\(S_{mol} = \pm 0.46 \Rightarrow \theta_{MD} = 37^\circ, 80^\circ\)

Order Tensor: Flexible Molecules

There are many internally flexible biomolecules, e.g. multiple-domain proteins, RNA helices with hinge motions, sugars with flexible glycosidic linkages, and lipids.

Saupe matrix:

\[ S_{ij} = \frac{1}{2} \left( 3 \cos \theta_i^D \cos \theta_j^D - \delta_{ij} \right) \]

\( \theta_i^D \) : angle of the director with the i-th axis of the molecule

- Each rigid segment in the molecule has one S tensor, with its own principal axis system (where S is diagonal).

- Each S matrix is traceless & symmetric, thus it has 5 independent elements, which requires 5 NMR couplings to determine.

Average NMR couplings:

\[ \overline{\delta} = \frac{2}{3} \sum_{i,j} S_{ij}^M \sigma_{ij}^M \]

\( \sigma_{ij}^M \) : interaction tensor elements in the molecule-fixed frame

- Once the order tensor is known, one can calculate the motionally averaged NMR couplings for any direction in the rigid segment.
Origin of the Order Tensor

The order tensor derivation is mathematically involved, but is useful when a uniaxially diffusive system is no longer rigid but has *flexible internal parts*.

- The NMR frequency is the z-component of an interaction tensor in the lab frame:

\[ \sigma^M \xrightarrow{\theta^D} \sigma^D \xrightarrow{\text{uniaxial motion}} \langle \sigma^D \rangle \xrightarrow{\beta} \sigma^L \]

- Since \( \langle \sigma^D \rangle \) is axially symmetric, the only relevant frequency in the \( \sigma^M \rightarrow \sigma^D \) transformation is the frequency along \( Z_D \):

\[
\sigma^{D}_{33} = Z^M_D \cdot \sigma^M \cdot Z^M_D = \begin{pmatrix}
\cos \theta^D_1 & \cos \theta^D_2 & \cos \theta^D_3 \\
\sigma^M_{11} & \sigma^M_{12} & \sigma^M_{13} \\
\sigma^M_{21} & \sigma^M_{22} & \sigma^M_{23} \\
\sigma^M_{31} & \sigma^M_{32} & \sigma^M_{33}
\end{pmatrix}
\]

\[
= \sum_{i,j} \cos \theta^D_i \cdot \sigma^M_{ij} \cdot \cos \theta^D_j \xrightarrow{\text{averaging}} \langle \sigma^D_{33} \rangle = \sum_{i,j} \langle \cos \theta^D_i \cos \theta^D_j \rangle \cdot \sigma^M_{ij}
\]
Origin of the Order Tensor

\[
\left\langle \sigma^{D}_{33} \right\rangle = \sum_{i,j} \left\langle \cos \theta_i^D \cos \theta_j^D \right\rangle \cdot \sigma_{ij}^M = \frac{2}{3} \sum_{i,j} \left( \frac{3}{2} \cos \theta_i^D \cos \theta_j^D - \frac{1}{2} \delta_{ij} \right) \cdot \sigma_{ij}^M + \frac{1}{3} \sum_{i,j} \delta_{ij} \sigma_{ij}^M
\]

Order tensor

Saupe matrix:

\[
S_{ij} = \frac{1}{2} \left\langle 3 \cos \theta_i^D \cos \theta_j^D - \delta_{ij} \right\rangle
\]

\[
\overline{\delta} \equiv \left\langle \sigma^{D}_{33} \right\rangle - \sigma_{iso} = \frac{2}{3} \sum_{i,j} S_{ij}^M \sigma_{ij}^M \Rightarrow \overline{\delta} = \frac{2}{3} Tr\{S \cdot \sigma\}
\]

Once the S tensor is known, then one can calculate motionally averaged couplings along any direction.
Relating Order Tensor to Order Parameter

Bond order parameter is the “projection” of the order tensor onto the bond.

\[ S_{bond} = S_{33}^{\sigma\ PAS} = \left\langle \frac{1}{2} \left( 3 \cos^2 \theta_{PD} - 1 \right) \right\rangle = \bar{\delta}/\delta \]

\[ \bar{\delta} = \frac{2}{3} \sum_{i} S_{ii}^{\sigma\ PAS} \cdot \sigma_{ii}^{\sigma\ PAS} = \frac{2}{3} \left[ \delta \cdot S_{33}^{\sigma\ PAS} - \frac{\delta}{2} \left( S_{11}^{\sigma\ PAS} + S_{22}^{\sigma\ PAS} \right) \right] = \frac{2}{3} \cdot \frac{3}{2} \cdot \delta \cdot S_{33}^{\sigma\ PAS} \]

\[ \Rightarrow \quad \bar{\delta} = \delta \cdot S_{33}^{\sigma\ PAS} = \delta \left\langle \frac{1}{2} \left( 3 \cos^2 \theta_{3, \sigma\ PAS} - 1 \right) \right\rangle \]

For uniaxially mobile proteins that are oriented along \( B_0 \):

\[ \omega(0^\circ) - \omega_{iso} = \frac{1}{2} \left( 3 \cos^2 0 - 1 \right) \]

\[ = \bar{\delta} = S_{bond} \cdot \delta \]

Thus, \( S_{bond} \) contains orientation information.
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Slow Motion: 2D Exchange NMR

2D spectrum $S(\omega_1, \omega_2)$ is a joint probability:
- intensity distribution: motion geometry.
- $t_m$ dependence $\rightarrow$ correlation time.

$S(\omega_1, \omega_2; \beta)$ (for $\eta = 0$)

Schmidt-Rohr and Spiess, 1994.
1D Stimulated Echo: Time-Domain Exchange

- 1D analog of 2D exchange.
- Allows measurement of $\tau_c$ without many 2D spectra.

2D time signal:

$$f(t_1, t_2) = \left\langle \left[ \cos \omega(\theta_1)t_1 - i \sin \omega(\theta_1)t_1 \right] \cdot e^{i\omega(\theta_2)t_2} \right\rangle = \frac{1}{\text{powder averaging}} \left\langle e^{-i\omega(\theta_1)t_1} \cdot e^{i\omega(\theta_2)t_2} \right\rangle$$

1D time signal: $t_2 = t_1 = t_e$.
- Segments without frequency change: $\omega(\theta_1) = \omega(\theta_2) = \omega(\text{diagonal})$.

$$M(t_e) = \left\langle e^{-i\omega t_e} \cdot e^{i\omega t_e} \right\rangle = \left\langle 1 \right\rangle$$

- 2 scans

- Segments with frequency change:

$$M(t_e) = \left\langle e^{-i\omega(\theta_1)t_e} \cdot e^{i\omega(\theta_2)t_e} \right\rangle = \left\langle e^{i\omega[(\theta_2)-\omega(\theta_1)]t_e} \right\rangle \xrightarrow{\text{long } t_m} 0$$

**1D stimulated echo intensity = 2D diagonal intensity**
1D Stimulated Echo Under MAS: CODEX


- 180° pulse train recouples X-spin CSA.
- 90° storage and read-out pulses are phase-cycled together.
- After the 2\textsuperscript{nd} recoupling period, the MAS phase for 2 scans is:

\[
\cos \Phi_1 \cos \Phi_2 - \sin \Phi_1 \sin \Phi_2 = \cos(\Phi_1 + \Phi_2) = \cos(|\Phi_2| - |\Phi_1|)
\]

\[
\Phi_1 = \frac{N}{2} \left( \int_0^{t_r/2} \omega_1(t) \, dt - \int_{t_r/2}^{t_r} \omega_1(t) \, dt \right) = N \int_0^{t_r/2} \omega_1(t) \, dt
\]

\[
\Phi_2 = \frac{N}{2} \left( -\int_0^{t_r/2} \omega_2(t) \, dt + \int_{t_r/2}^{t_r} \omega_2(t) \, dt \right) = -N \int_0^{t_r/2} \omega_2(t) \, dt
\]

- No motion: $\omega_1 = \omega_2$, $\Rightarrow \cos(\Phi_1 + \Phi_2) = 1$, full echo.
- With motion: $\omega_1 \neq \omega_2$, $\Rightarrow \cos(\Phi_1 + \Phi_2) < 1$, reduced echo.
Exchange NMR Involves Difference Tensor

CODEX signal:
\[
\frac{S(t_m, \delta N t_r)}{S_0(t_m, \delta N t_r)} = \cos \left( |\Phi_2| - |\Phi_1| \right) = \cos (\Phi^\Delta), \quad \text{where} \quad \Phi^\Delta = N \int_2^{t_r/2} \omega^\Delta (t) \, dt
\]

Difference tensor: \( \Delta = \sigma_A - \sigma_B \)

Reflection of the \( Z_A \) and \( Z_B \) axes with the bisector plane gives the negative of the original difference tensor.

For \( \eta = 0 \), the \( \Delta \) principal axis directions are:
- \( \Delta_2 \): Normal of the AOB plane;
- \( \Delta_3 \) and \( \Delta_1 \): In the AOB plane, 45° from the bisector.

<table>
<thead>
<tr>
<th>( \Delta ) axis</th>
<th>( \sigma_A )</th>
<th>( \sigma_B )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta_1 )</td>
<td>45° - ( \beta/2 ), 45° + ( \beta/2 )</td>
<td>45° + ( \beta/2 ), 45° - ( \beta/2 )</td>
</tr>
<tr>
<td>( \Delta_2 )</td>
<td>90°</td>
<td>90°</td>
</tr>
<tr>
<td>( \Delta_3 )</td>
<td>45° + ( \beta/2 ), 45° - ( \beta/2 )</td>
<td>45° - ( \beta/2 ), 45° + ( \beta/2 )</td>
</tr>
</tbody>
</table>

\[
\omega^\Delta_n = \frac{1}{2} \delta \left( 3 \cos^2 \Theta_{A,n} - 1 \right) - \frac{1}{2} \delta \left( 3 \cos^2 \Theta_{B,n} - 1 \right)
\]
CODEX is Sensitive to Small-Angle Reorientations

For $Z_A$:
\[
\omega_{A,2} = \frac{1}{2} \delta \left( 3 \cos^2 90^\circ - 1 \right) = -\frac{1}{2} \delta \\
\omega_{A,1} = \frac{1}{2} \delta \left( 3 \cos^2 \left( 45^\circ - \beta / 2 \right) - 1 \right) \\
\omega_{A,3} = \frac{1}{2} \delta \left( 3 \cos^2 \left( 45^\circ + \beta / 2 \right) - 1 \right)
\]

For $Z_B$:
\[
\omega_{B,2} = \frac{1}{2} \delta \left( 3 \cos^2 90^\circ - 1 \right) = -\frac{1}{2} \delta \\
\omega_{B,1} = \frac{1}{2} \delta \left( 3 \cos^2 \left( 45^\circ + \beta / 2 \right) - 1 \right) \\
\omega_{B,3} = \frac{1}{2} \delta \left( 3 \cos^2 \left( 45^\circ - \beta / 2 \right) - 1 \right)
\]

\[
\omega_2^\Delta = \omega_{A,2} - \omega_{B,2} = 0 \\
\omega_1^\Delta = \omega_{A,1} - \omega_{B,1} = \frac{3}{2} \delta \cdot \frac{1}{2} \left[ \cos (90^\circ - \beta) - \cos (90^\circ + \beta) \right] = \frac{3}{2} \delta \sin \beta \\
\omega_3^\Delta = \omega_{A,3} - \omega_{B,3} = \frac{3}{2} \delta \cdot \frac{1}{2} \left[ \cos (90^\circ + \beta) - \cos (90^\circ - \beta) \right] = -\frac{3}{2} \delta \sin \beta
\]

\[
\Rightarrow \quad \eta^\Delta = 1 \\
\left| \omega_{33}^\Delta - \omega_{11}^\Delta \right| = 3|\delta| \cdot \sin \beta = \left| \omega_{33} - \omega_{11} \right| \cdot 2 \sin \beta
\]

- CODEX signal scales $\sim \sin \beta$, which is $\sim \beta$ for small angles.
- Usual angular dependence is $(3\cos^2 \beta - 1)/2$, which scales $\sim \beta^2$. 
CODEX: Reorientation Angles & Number of Sites

\[ E(t_m, \delta N_{tr}) = \int_0^{\frac{90^\circ}{t}} R(\beta) e(\delta N_{tr}; \beta) \, dt \cdot d\beta \]

Jump motions:
\[
\frac{\Delta S}{S_0}(t_m >> \tau_c, \delta N_{tr} >> 1) = 1 - \frac{1}{M}
\]

- 3-site jump
- Isotropic jump
- Isotropic diffusion
- Uniaxial rotation

Dipolar CODEX

Use dipolar coupling instead of CSA to probe orientational change:

- $^{15}$N-$^1$H: Use perdeuterated proteins to minimize $^1$H spin diffusion
- $^{13}$C-$^1$H and $^{15}$N-$^1$H: decouple $^1$H-$^1$H couplings to suppress $^1$H spin diffusion
- $^{15}$N-$^{13}$C: $\sim$1 kHz couplings, probes $t_c >> 1$ ms

McDermott et al;

Krushelnitsky, Saalwächter, Reichert, et al.
Phase Cycling:

Selecting Desired Signals and Removing Artifacts
Phase Cycling: 6 Rules of Thumb

**Purposes:**

- **Remove**
  - unwanted signals from the stator, coil, or compounds of a different nature from the sample of interest

- **Compensate for**
  - flip angle errors (e.g. 180° pulses)
  - quadrature imbalance
  - DC offset
  - Incomplete transverse dephasing

- **Select**
  - double-quantum or multiple-quantum coherences while removing single-quantum coherence.

**Phase cycles:**

- +/-, usually on the 1H 90° excitation pulse & constant-phase 1H spin lock

- Exorcycle of the 180° pulse
- CYCLOPS: +x, +y, -x, -y
- +/-, part of the CYCLOPS
- Invert the phase of the 1st z-filter pulse, keep Rec phase constant

- DQ coherence phase winds in the opposite direction from SQ coherence in response to phase increment of the DQ block
Phase Cycling for Cross Polarization

- The $^1$H spin lock phase should be perpendicular to the $90^\circ$ excitation phase.

- $^{13}$C spin lock phase is independent of the $^1$H spin lock phase.

- The $^1$H M vs $B_1$ relation is inverted every other scan to cause inversion of the $^{13}$C M and the receiver phase, so that M of $^{13}$C spins uncoupled or weakly coupled to $^1$H can be canceled (if desired).

- The relative orientation between M and $B_1$ must be the same between the two channels.
Echo 180° Pulses and z Mixing 90° Pulses

**Echo**

- Exorcycle of the 180° pulse compensates for flip angle (β) errors. Even when β ≠ 180°, M refocuses with the correct phase, except for an intensity scaling of (1-cosβ)/2.


**Z-filter**

- Z-filter: 1st 90° phase is inverted against M every other scan while the 2nd 90° phase remains constant;
  - The 2nd 90° phase does not need to be parallel to the 1st 90° phase.
  - The inversion of the desired M and the receiver removes undestroyed transverse M.
Complete Phase Cycling for 2D PDSD Expts

- CP: $2 \times 4 = 8$ (inversion & CYCLOPS)
- Z-filter: $x \times 2$ for 1st $90^\circ$ inversion
- (Optional) echo detection:
  - $180^\circ$ phase along the M; or
  - $180^\circ$ phase exorcycled against M ($x \times 4$)

- Total # of phase cycling steps:
  - $16 = 2 \times 4 \times 2$;
  - $64 = 2 \times 4 \times 2 \times 4$ (with exorcycle);

- Extension to 3D, 4D…
  - # of z-mixing periods increases; doubling of phase-cycle steps for every z-period is impractical.
  - Under ultrafast MAS, $T_2$ lengthens.
  - Better to actively dephase transverse M by dipolar recoupling.
Summary

*Motions are ubiquitous in biological molecules.*

- Fast motions average the interaction tensors and *narrow the spectra*.
- Based on symmetry, the *average tensors* and spectral lineshapes of several motions can be analytically derived.
- Fast motions can be measured using 2D experiments that resolve dipolar couplings by chemical shifts.
- *Order parameters & order tensors* give information on whole-body motions and internal motions.
- Slow motions can be measured as *2D exchange cross peaks* or 1D stimulated echo intensities using CODEX.
- The geometry of slow motion is described by *difference tensors*.
- 6 rules of thumb allow the construction of phase cycles in many NMR experiments.