Chemical Shift and Dipolar Tensors as Probes of Dynamics: Integration of MAS NMR, MD Simulations, and Density Functional Theory

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Outline

Introduction

RN-symmetry based CSA and dipolar tensor recoupling

Quantifying motions in dynamic systems: integration of MAS NMR, MD, and Density Functional Theory (HIV-1 capsid)
Protein Dynamics by NMR

NMR Methods
- Averaged anisotropic couplings
- Spin-lattice relaxation
- Quadrupolar tensors
- CSA tensors
- Dipolar tensors
- Rotating frame relaxation
- Relaxation dispersion
- Exchange NMR

Protein Motions
- Bond vibration
- Local flexibility
- Domain motions
- Methyl group rotation
- Brownian tumbling
- Protein folding
- H-transfer

Adapted from Krushelnitsky and Reichert, Prog. NMR Spectr. (2005)
Tensorial Restraints in MAS NMR: Structure and Dynamics Probes

**Dipolar tensors**

- H-N$^H$ – dynamics on ns-us timescales, hydrogen bonding
- H-C$^\alpha$ – dynamics on ns-us timescales, less sensitive to local structure
- C$^\alpha$-N$^H$ – dynamics on ms timescales

**CSA tensors**

- C$^\alpha$, C$'$ – local structure, dynamics
- C$^\beta$, C$^\gamma$, C$^\delta$, C$^\varepsilon$, C$^\zeta$ - local structure, dynamics
- N$^H$ – local structure (H bonding), dynamics
- H$^N$– local structure (H bonding), dynamics

Molecular Motion: From NMR Parameters to Motional Model

SIMPLE SYSTEMS, SIMPLE SYMMETRIES

Average tensor values can be used to derive the motional geometry

PROTEINS AND PROTEIN ASSEMBLIES

Multiple motional modes, complex trajectories, simple analysis impossible

HOW TO RELATE NMR PARAMETERS TO A MOTIONAL MODEL?

Collect as many experimental data sets as possible:
For $^1$H, $^2$H, $^{15}$N, $C'$, $C^\alpha$, $C^\beta$, sidechains
$T_1$, $T_2$, $T_{1\rho}$, $T_Q$
Lineshapes- dipolar, CSA, quadrupolar

Perform MD simulations to extract the conformational space and rates
Site-Specific Measurement of Protein Dynamics by MAS NMR: Atomic-Resolution Picture

HIV-1 CA protein (231 aa), tubular assembly

Gly peaks:
✓ Very well resolved
✓ Shifts reflect 2° structure and dynamics
Site-Specific Measurement of Protein Dynamics by MAS NMR: Atomic-Resolution Picture

HIV-1 CA protein (231 aa), tubular assembly

Sensitive to motions on timescales of
ns to μs (N/C-H) and μs to ms (N-C)
Site-Specific Measurement of Protein Dynamics by MAS NMR: Atomic-Resolution Picture

$^{15}\text{N}$, $^{13}\text{C}$, $^1\text{H}$ Chemical Shift Tensors

HIV-1 CA protein (231 aa), tubular assembly

Sensitive to motions on timescales of $\text{ns to } \mu\text{s}$
Dipolar and CSA Recoupling Experiments by MAS NMR

**Dipolar tensors** – see Mei’s lecture and references therein

- DIPSHIFT- LGCP, FSLG, PMLG, eDUMBO REDOR
- RN-symmetry recoupling
- PARS and wPARS
- CPVC

**Heteronuclear CSA tensors**

- ROCSA (Chan and Tycko)
- RNCSA

\(^1\)H CSA tensors

- Combination of RNCSA and RN-dipolar experiments

Rotations: Irreducible Spherical Tensors and Reference Frames in MAS NMR

Irreducible Spherical Tensors;
Wigner Rotation Matrices (see Rob’s l & PS)

Rank \( l \) spherical tensor: \( 2l+1 \) components;
\( m = -l, -l+1, \ldots, l-1, l \)

\[
R(\Omega) T_{\lambda m} R(\Omega)^\dagger = \sum_{m'=-l}^{+l} T_{\lambda m'} D_{m' m}^l (\Omega)
\]

- Rotation operator
- Wigner rotation matrix

\( \Omega = \{\alpha, \beta, \gamma\} \)

Euler angles

\[
D_{m' m}^l (\Omega) = \exp\{-im'\alpha\} d_{m' m}^l (\beta) \exp\{-im\gamma\}
\]

- reduced Wigner rotation matrix element

Reference Frames in MAS NMR

P- principal tensor frame
M – molecular reference
R – rotor; L - laboratory

**Rotational Signatures of Homonuclear Spin Interactions**  
*(Diamagnetic Systems, I=1/2)*

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Space rank, $l$</th>
<th>Spin rank, $\lambda$</th>
<th>Field rank</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
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<tr>
<td>Chemical shift anisotropy</td>
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<tr>
<td>$J$-coupling</td>
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<tr>
<td>Dipole–dipole coupling</td>
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<td>0</td>
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</table>

**Components of Homonuclear Spin Interactions in the Interaction Frame of an Applied RF Field, for Exact Magic Angle Spinning**

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Space rank, $l$</th>
<th>Space components, $m$</th>
<th>Spin rank, $\lambda$</th>
<th>Spin components, $\mu$</th>
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<td>$J$-coupling</td>
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<td>Dipole–dipole coupling</td>
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# Rotational Signatures of Interactions in Heteronuclear Spin Systems

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<tr>
<th>Interaction</th>
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<th>$I$-Spin rank, $\lambda_I$</th>
<th>$S$-Spin rank, $\lambda_S$</th>
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<td>$I$-Spin DD-coupling</td>
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<td>$S$-Spin $J$-coupling</td>
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<td>0</td>
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<td>$S$-Spin DD-coupling</td>
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<tr>
<td>IS $J$-coupling</td>
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# Components of $I$ and $S$ Spin Interactions in the Interaction Frame of Two Resonant RF Fields, for Exact Magic-Angle Spinning

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Space rank, $l$</th>
<th>Space components, $m$</th>
<th>$I$-Spin rank, $\lambda_I$</th>
<th>$I$-Spin components, $\mu_I$</th>
<th>$S$-Spin rank, $\lambda_S$</th>
<th>$S$-Spin components, $\mu_S$</th>
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<td>${-1, 0, 1}$</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
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<td>2</td>
<td>${-2, -1, 0, 1, 2}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$S$-Spin isotropic shift</td>
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<td>${-1, 0, 1}$</td>
</tr>
<tr>
<td>$S$-Spin CSA</td>
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<td>0</td>
<td>0</td>
<td>${-1, 0, 1}$</td>
</tr>
<tr>
<td>$S$-Spin $J$-coupling</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>$S$-Spin DD-coupling</td>
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<td>${-2, -1, 1, 2}$</td>
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<td>0</td>
<td>2</td>
<td>${-2, -1, 0, 1, 2}$</td>
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<tr>
<td>IS $J$-coupling</td>
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<td>0</td>
<td>1</td>
<td>${-1, 0, 1}$</td>
<td>1</td>
<td>${-1, 0, 1}$</td>
</tr>
<tr>
<td>IS DD-coupling</td>
<td>2</td>
<td>${-2, -1, 1, 2}$</td>
<td>1</td>
<td>${-1, 0, 1}$</td>
<td>1</td>
<td>${-1, 0, 1}$</td>
</tr>
</tbody>
</table>

\( \text{CN}_n^\nu - \text{Symmetry Sequences} \)

Figure 3: Visualization of the symmetry numbers for (a) \( C7_{1/2} \) sequences, and (b) \( C14_{1/2} \) sequences. The left-hand helices represent the spatial rotation of the sample as a function of time. The right-hand helices depict the modulation of the rf phase as a function of time. \( n \) spatial rotations are completed in the same time as \( \nu \) spin rotations. The rf phase is modulated in \( N \) discrete steps, depicted by the planes in the right-hand diagrams. (Adapted from Ref. 38)

$R N_n^\nu$ – Symmetry Sequences

$n$ complete sample revolutions

\[ \begin{array}{cccc}
0 & 1 & 2 & 3 \\
\hline
\end{array} \quad \begin{array}{c}
N - 1 \\
\end{array} \]

\[ \pi \text{ Pulse} \]

Rf phase alternation $\pm \frac{\pi \nu}{N}$

$\text{RN}_n^\nu$ – Symmetry Sequences for Dipolar and CSA Tensor Recoupling

(a)  
\[
\begin{array}{cccccccc}
\phi & -\phi & \phi & -\phi & \phi & \phi & -\phi \\
\end{array}
\] ..........................  \[
\begin{array}{cccccccc}
\phi & -\phi & \phi & -\phi & \phi & \phi & -\phi \\
\end{array}
\]

number of the total $\pi$ pulses: $N$
phase shift $\phi = \nu \pi / N$

(b)  
\[
\begin{array}{cccccccccccccccc}
+\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} & +\frac{\pi}{14} & -\frac{\pi}{14} \\
\end{array}
\] 3$T_R$

$R_{143}^1$: 14 $\pi$ pulses per 3 rotor periods; $\phi = \pm \pi / 14$, $w_{rf} = 7/3 \omega_R$.

$H_{\lambda\mu}^\Lambda \neq 0$, if $mn - \mu \nu = Z_\lambda \cdot N/2$

First-order selection rules

**RN\textsubscript{n}^\nu – Symmetry Sequences:**

**Average Hamiltonian and Selection Rules**

**First-order average Hamiltonian (interaction frame):**

\[
\overline{\mathcal{H}}^{(1)}(t^0) = \sum_{lm\lambda\mu} \overline{\mathcal{H}}^{(1)}_{lm\lambda\mu}(t^0)
\]

\[
\overline{\mathcal{H}}^{(1)}_{lm\lambda\mu}(t^0) = T^{-1} \int_{t^0}^{t^0+T} dt \, \mathcal{H}_{lm\lambda\mu}(t)
\]

**Euler angle symmetry:**

- Individual components modulated by spatial rotations and rotations of spin polarizations by rf field

\[
\beta_{rf}\left(t + \frac{n\tau_r}{N}\right) = \beta_{rf}(t) \pm \pi
\]

\[
\gamma_{rf}\left(t + \frac{n\tau_r}{N}\right) = \gamma_{rf}(t) - \frac{2\pi \nu}{N}
\]

**First-order selection rules:**

\[
\overline{\mathcal{H}}^{(1)}_{lm\lambda\mu} = 0 \quad \text{if} \quad mn - \mu \nu \neq \frac{N}{2} Z_{\lambda}
\]

\[Z_{\lambda} - \text{any integer with the same parity as the spin rank } \lambda.\]

Even \(\lambda\): \(Z_{\lambda} = 0, \pm 2, \pm 4, \ldots\);
Odd \(\lambda\): \(Z_{\lambda} = \pm 1, \pm 3, \pm 5, \ldots\)

Rather restrictive for RN, very few higher-order terms

\( \text{RN}_n^\nu - \text{Symmetry Sequences for CSA Tensor Recoupling: Average Hamiltonian and Selection Rules} \)

<table>
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<th>Interaction</th>
<th>Space rank, ( l )</th>
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<th>Spin rank, ( \lambda )</th>
<th>Spin components, ( \mu )</th>
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<tr>
<td>Isotropic chemical shift</td>
<td>0</td>
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<td>{(-1, 0, 1)}</td>
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<tr>
<td>Chemical shift anisotropy</td>
<td>2</td>
<td>{-2, -1, 1, 2}</td>
<td>1</td>
<td>{-1, 0, 1}</td>
</tr>
</tbody>
</table>

**First-order average Hamiltonian (interaction frame):**

\[
H^\Lambda = K_{m\lambda\mu} A^\Lambda_{lm} (\Omega_{PR}) d_{m0}^l (\beta_{RL}) T_{\lambda\mu}^\Lambda,
\]

**First-order selection rules**

\[
H^\Lambda_{lm\lambda\mu} \neq 0, \quad \text{if} \quad mn - \mu v = Z_{\lambda} \cdot \frac{N}{2}
\]

**First-order average Hamiltonian according to the spatial rank \((m=0, \pm1, \pm2)\):**

\[
\tilde{H}^{\text{CSA}} = \tilde{H}^{\text{CSA}}_{(0)} + \tilde{H}^{\text{CSA}}_{(1)} + \tilde{H}^{\text{CSA}}_{(2)}
\]

\[
= \sum_{m=0,\lambda,\mu} K_{m\lambda\mu} A^\Lambda_{lm} (\Omega_{PR}) d_{m0}^l (\beta_{RL}) T_{\lambda\mu}^\Lambda
\]

\[
+ \sum_{m=\pm1,\lambda,\mu} K_{m\lambda\mu} A^\Lambda_{lm} (\Omega_{PR}) d_{m0}^l (\beta_{RL}) T_{\lambda\mu}^\Lambda
\]

\[
+ \sum_{m=\pm2,\lambda,\mu} K_{m\lambda\mu} A^\Lambda_{lm} (\Omega_{PR}) d_{m0}^l (\beta_{RL}) T_{\lambda\mu}^\Lambda
\]

\[
= \gamma B_0 \sigma_0 I_z + \gamma B_0 \sigma_1 \cos(\gamma_{PR} + \omega_r t + \delta_1) I_z
\]

\[
+ \gamma B_0 \sigma_2 \cos(2(\gamma_{PR} + \omega_r t + \delta_2)) I_z,
\]

\[
\sigma_0 = \sigma^{120} + P_3 (\cos \beta_{RL}) \left[ P_2 (\cos \beta_{PR}) (\sigma^{\text{PAS}}_{zz} - \sigma^{120}) + \frac{1}{2} \sin \beta_{PR} \cos 2\alpha_{PR} (\sigma^{\text{PAS}}_{xx} - \sigma^{\text{PAS}}_{yy}) \right],
\]

\[
\sigma_1 = \sqrt{\sigma_{\text{cos1}}^2 + \sigma_{\text{sin1}}^2}
\]

\[
= \sin \beta_{RL} \cos \beta_{RL} \sin \beta_{PR} \left[ \cos \beta_{PR} (-3(\sigma^{\text{PAS}}_{zz} - \sigma^{120})
\]

\[
+ \cos 2\alpha_{PR} (\sigma^{\text{PAS}}_{xx} - \sigma^{\text{PAS}}_{yy}) \right)^2
\]

\[
+ \left[ \sin 2\alpha_{PR} (\sigma^{\text{PAS}}_{xx} - \sigma^{\text{PAS}}_{yy}) \right]^2 \right)^{1/2},
\]

\[
\sigma_2 = \sqrt{\sigma_{\text{cos2}}^2 + \sigma_{\text{sin2}}^2}
\]

\[
= \frac{1}{2} \sin^2 \beta_{RL} \left\{ \frac{3}{2} \sin^2 \beta_{PR} (\sigma^{\text{PAS}}_{zz} - \sigma^{120})
\]

\[
+ \frac{1}{2} (1 + \cos 2\beta_{PR}) \cos 2\alpha_{PR} (\sigma^{\text{PAS}}_{xx} - \sigma^{\text{PAS}}_{yy}) \right)^2
\]

\[
+ \left[ \cos \beta_{PR} \sin 2\alpha_{PR} (\sigma^{\text{PAS}}_{xx} - \sigma^{\text{PAS}}_{yy}) \right]^2 \right)^{1/2}.
\]

**RN_n**<sup>υ</sup> – Symmetry for CSA Tensor Recoupling: Suitable Sequences

<table>
<thead>
<tr>
<th>Symmetry numbers</th>
<th>Scaling factors for $\sum F_{\text{CSA}}(l, m, \lambda, \mu)$, $m = \pm 1$</th>
<th>Scaling factors for $\sum F_{\text{CON}}(l, m, \lambda, \mu)$</th>
<th>$\omega_{l} / \omega_{0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>$n$</td>
<td>$V$</td>
<td>$0.2250$</td>
</tr>
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</table>

Table S1. Summary of $\gamma$-Encoded R-Symmetry Sequences Suitable for Recoupling of the CSA Interaction. $N$ is from 10 to 20, $N/2n$ is from 1.0 to 8.0.
**RNₙⁿ⁻ Symmetry Sequences for CSA Tensor Recoupling: Scaling Factors for σ₁⁻ and σ₂⁻ CSA Sequences**

(a) First-order CSA recoupling \((m = ±1)\)

\[
\overline{H}_{imλμ}^\Lambda(t^0) = κ_{imλμ}[A_{im}^\Lambda]^R \exp\{-im(α_{RL}^0 - ω_r t^0)\}T_{λμ}^\Lambda
\]

\[
k_{imλμ} = τ^{-1}d_{m0}^l(β_{RL}) \times \int_0^τ dt d_{μ0}^l(-β_{rf}(t)) \exp\{i(μγ_{rf}(t) - μ \frac{πν}{N} + mω_r t)\}
\]

1.0 < \(N/2n\) ≤ 8.0

Compatible with modern hardware (attainable rf powers)

(b) Second-order CSA recoupling \((m = ±2)\)

Advantages of σ₁⁻ RNCSA sequences:
- Larger scaling factors
- Less sensitive to the required rf field strength

In practice: RNₙⁿ⁻ sequences with higher \(N/2n\) ratio are advantageous, at a given MAS frequency
RN-Symmetry Based $^{15}\text{N}$ CSA Recoupling: Experimental Results

RN-Symmetry Based $^{13}$C CSA Recoupling: Experimental Results

[U-$^{13}$C,$^{15}$N-Histidine]

$[{^{15}}N]$-N-acetyl-valine (natural abundance $^{13}$C)

RN-Symmetry Dipolar Recoupling for Protein Dynamics:
3D Sequences

RN-Symmetry Dipolar Recoupling for Protein Dynamics: Suitable Symmetries

Summary of R-symmetry sequences suitable for heteronuclear dipolar recoupling under fast MAS conditions, $10 \leq N \leq 30$, and $1 < N/2n \leq 5.0^*$. 

<table>
<thead>
<tr>
<th>$N$</th>
<th>$n$</th>
<th>$\nu$</th>
<th>$\omega_I/\omega_N$</th>
<th>$K_{ef}$</th>
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<td>14</td>
<td>13</td>
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<td>0.1254</td>
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| 30   | 14  | 13    | 1.071               | 0.1254  |

| 4    | 1   | 4     | 2               | 0.5     |

* $N$, $n$, and $\nu$ are the symmetry numbers associated with each sequence. $\omega_I$ and $\omega_N$ denote the RF field strength and the MAS frequency, respectively. $K_{ef}$ is the effective scaling factor of the dipolar lineshape recoupled by a specific symmetry sequence.

RN-Symmetry Dipolar Recoupling for Protein Dynamics: Scaling Factors

**U-^{13}C,^{15}N-Alanine**

**U-^{15}N-N-Acetylamide**

<table>
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<tr>
<td>(a)</td>
<td>R22_1</td>
<td>8.9 ± 0.2 kHz</td>
</tr>
<tr>
<td>(b)</td>
<td>R18_1</td>
<td>9.3 ± 0.2 kHz</td>
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<tr>
<td>(c)</td>
<td>R14_1</td>
<td>10.1 ± 0.3 kHz</td>
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<td>(d)</td>
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<tr>
<td>(e)</td>
<td>R16_1</td>
<td>10.0 ± 0.2 kHz</td>
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RN-Symmetry Dipolar Recoupling for Protein Dynamics: Compatible with Fast-MAS Conditions

R16\textsuperscript{3} C-H Dipolar Lineshapes

Dynactin’s CAP-Gly domain

Phase-Alternating $\text{RN}_n^\nu$ – Symmetry (PARS) Dipolar Recoupling

Advantages (particularly at high fields):

✓ Suppression of $^1\text{H}$ CSA contribution
✓ Weak sensitivity to RF field mismatch

Dipolar Recoupling at Fast MAS (60 – 110 kHz): CPVC

Advantages:
✓ Lack of sensitivity to RF inhomogeneity
✓ No contribution of $^1$H CSA to lineshapes

RN-Symmetry Based $^1$H CSA Recoupling: Requires 3 Experiments

$^1$H($^{15}$N$_{und}$): $^1$H CSA + H-N DIP

$^1$H($^{15}$N$_{dec}$): $^1$H CSA

$^{15}$N: H-N DIP

<table>
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<tr>
<th>Compound</th>
<th>$\delta_\sigma$(ppm)</th>
<th>$\eta$</th>
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<td>NAV</td>
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<td>MLF/Leu</td>
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<td>MLF/Phe</td>
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Incomplete averaging of heteronuclear dipolar term during $^1$H CSA recoupling non-trivial contribution due to small $^1$H CSA

RN-Symmetry Based $^1$H CSA Recoupling: Histidine

(a) RN-$^1$H($^{15}$N$_{cat}$) RN-$^1$H($^{15}$N$_{neu}$) RN-$^{15}$N
H-Nr1 (cat) H-Nr2 (cat) H-Nr2 (neu)

(b) RN-$^1$H($^{13}$C$_{cat}$) RN-$^1$H($^{13}$C$_{neu}$) RN-$^{13}$C
H-Cr1 (cat) H-Cr1 (neu) H-Cr2 (cat) H-Cr2 (neu) H-Cr (cat) H-Cr (neu)

pH 6.0

RN-Symmetry Based $^1$H CSA Recoupling: Histidine

- $\delta_{zz} - \delta_{iso}$
- $\delta_{yy} - \delta_{iso}$
- $\delta_{xx} - \delta_{iso}$

Chemical Shift (ppm)

DFT: b3lyp/6-311G
MAS NMR

RN-Symmetry Based $^1$H CSA Recoupling: Dynactin’s CAP-Gly

3D R-$^1$H($^{13}$N$_{dec}$): H CSA
3D R-$^1$H($^{13}$N$_{und}$): H CSA + H-N DIP

Hou, Paramasivam, Yan, Polenova, Vega, (2013) JACS
$^1$H CSA Tensors in CAP-Gly: Principal Components

Linear regression (solution NMR data):

Hou, Paramasivam, Yan, Polenova, Vega, (2013) JACS
\(^1\)H CSA Tensors and Hydrogen Bond Length: CAP-Gly

Data fit to the electrostatic model: \(\text{CSA} = a + b/(R - c)^2\) Tjandra and Bax (1997) J. Am. Chem. Soc.

\(^1\)H CSA tensors are sensitive to H bonding:

Hou, Paramasivam, Yan, Polenova, Vega, (2013) JACS
CA Assemblies: ‘Ultra’Fast (100-110 kHz) MAS, $^1$H Detection

With CRMN-ENS Lyon and Bruker:

- 100-111 kHz MAS; ~0.1-0.3 mg U-$^{13}$C,$^{15}$N-CA tubes
- Unprecedented resolution and sensitivity: data collection takes hours vs. days/weeks for a single 3D
- Streamlined 3D structure determination, using H-based distance restraints

<table>
<thead>
<tr>
<th>Sample Amount</th>
<th>Moderate MAS</th>
<th>Fast MAS</th>
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<tbody>
<tr>
<td>Sample Amount</td>
<td>20-30 mg</td>
<td>0.2-0.3 mg</td>
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<td>8-9 days</td>
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<td>CH HECTCOR</td>
<td>7-8 h</td>
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<td>NH HECTCOR</td>
<td>4-5 h</td>
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<td>NCACX</td>
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<tr>
<td>RNCSA</td>
<td>1-2 days</td>
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</table>

'H CSA Tensors by 'H-Detected MAS NMR, 110 kHz- Is One Experiment Sufficient?

'\(^1\)H CSA Lineshapes \(R_{18}^8\)^\(^7\)

A word of caution:

'\(^1\)H CSA lineshapes may contain a contribution of '\(^1\)H-'\(^1\)\(^5\)N dipolar term, requiring triple fit of ('\(^1\)H CSA, '\(^1\)H-'\(^1\)\(^5\)N dipolar, and '\(^1\)H CSA+'\(^1\)H-'\(^1\)\(^5\)N dipolar) spectra at slower MAS (see Hou et al., JACS (2013), 135, 1358-1368)

Validate '\(^1\)H CSA values by DFT calculations (see Vega et al. Isr. J. Chem. (2014), DOI: 10.1002/ijch.201300099)

Integrating MAS NMR and MD Simulations to Study Functional Dynamics

(HIV-1 Virus Capsid)
Integrated Structural Biology of Protein Assemblies: Challenges

HIV-1 (and other viral) assemblies

Complex architecture
- viral capsids and maturation intermediates
- pleomorphic, sequence variants

Functional interactions with (human) host factor proteins
- promote or inhibit viral infection
- large multicomponent complexes with capsid assemblies

Highly dynamic systems
- dynamics occur on many decades of motional timescales
- regulate viral lifecycle and infectivity
- essential to characterize the motions, in the context of function
Integrated Structural Biology of (HIV-1) Assemblies: NMR Methods

Samples
- tailored isotopic labeling
- control of morphology
- design of ‘model’ assemblies via mutagenesis

Integration of NMR, DNP, MD, DFT, cryo-EM
Essential for comprehensive understanding of biology

Computation
- MD simulations – characterization of dynamics
- QM/MM and QM/MM/MD – chemical shifts
HIV-1 Capsid Assembly

- Conical capsid
- CA hexamer (216)
- CA pentamer (12)

Cyclophilin A (CypA)
- 17.9 kDa; 164 AA
- Peptidyl-prolyl isomerase
- Directly interacts with capsid
- CA/CypA interactions are essential for viral infectivity

Host cell

References:
HIV-1 Capsid Assembly

- Assembled capsid is a dynamic structure
- Motions of individual residues and structural units span timescales from nanoseconds to milliseconds and slower
- Dynamics are functionally important for capsid assembly, capsid maturation, and interactions with host factors
- Static structures provide only partial information, knowledge of dynamics is necessary to understand capsid’s functions
- Atomic-level, quantitative picture—by integrated MAS NMR, MD simulations, and Density Functional Theory

Host cell

MAS NMR Studies of HIV-1 Capsid

Yun Han, Chris Suiter
Manman Lu, Maple Wang, Cait Quinn
HIV-1 Capsid is Remarkably Dynamic: Visualizing the Motions

- Assembled capsid is a dynamic structure
- Motions occur on multiple timescales
- Motions include: backbone and sidechain reorientations; loop motions; domain motions; motions of entire CA molecules in the capsid

Understanding Dynamics of HIV-1 Protein Assemblies at Atomic Resolution by Integrated MAS NMR, MD, and DFT Approach

1. Residue/atom-specific NMR parameters:
   - chemical shift tensors/order parameters
   - dipolar tensors/order parameters
   - spectral intensities and frequencies

2. MD simulations
   - conformational space sampled by each residue/atom
   - angular probability distributions, dipolar tensors
   - PCA analysis, coupled motions, etc.

3. Density Functional Theory calculations
   - Chemical shift tensors for substructures in MD trajectory
   - Critical for NMR structure calculations for dynamic systems

NMR: Guangjin Hou, Manman Lu
DFT calculations: Huilan Zhang

MD trajectory from Zhao et al., Nature 497:643-646 (2013)
MD calculations by Juan Perilla and Klaus Schulten
Site-Specific Measurement of Protein Dynamics by MAS NMR: Atomic-Resolution Picture

HIV-1 CA protein (231 aa), tubular assembly

N(C)-H/N-C Dipolar Tensors

G61 Rigid
G89 Dynamic
G101 Rigid
G116
G220

Sensitive to motions on timescales of
ns to μs (N/C-H)
and
μs to ms (N-C)
HIV-1 Capsid is Remarkably Dynamic: Integrating NMR & MD

- $^1$H-$^{15}$N dipolar lineshape experiments
- 3D RN-symmetry based experiments
- Nano- to microsecond timescales
- **HXB2 wild type**: unusually dynamic CypA loop
- **NL4-3 A92E**: loop motions are significantly reduced
- Experimental MAS NMR data are in quantitative agreement with the MD simulations
- Reproducible order parameters from MD trajectories of individual CA hexamers in the assembly

Lu, Hou, Zhang, Suiter, Ahn, Byeon, Perilla, Langmead, Hung, Gor’kov, Gan, Brey, Aiken, Zhang, Schulten, Gronenborn, Polenova (2015) PNAS
Dynamics of Protein Assemblies by MAS NMR: 
From MD Trajectory to Dipolar Order Parameter

In the presence of fast-limit motions (ns-us), dipolar coupling constant is a discrete average of values for different angular orientations (from MD):

\[
\langle \omega_{HN} \rangle = \frac{1}{N} \sum_{i=1}^{N} \frac{\hbar \gamma_i \gamma_{15N}}{(r_{HN}^3)_{i}} (1-3\cos^2 \theta_i)
\]

Dipolar order parameter is then

\[
S_{HN} = \frac{\langle \omega_{HN} \rangle}{\langle \omega_{HN} \rangle_{\text{rigid}}}
\]
Dynamics of CA: Angular Distributions

Rigid regions

G8

TS4

N121

HXB2

NL4-3

A92E

CypA loop

V86

A88

A92/E92

G94

Probability

Polar angle $\theta$

Lu, Hou, Zhang, Suiter, Ahn, Byeon, Perilla, Langmead, Hung, Gor’kov, Gan, Brey, Aiken, Zhang, Schulten, Gronenborn, Polenova (2015) PNAS
Site-Specific Measurement of Protein Dynamics by MAS NMR: Atomic-Resolution Picture

$^{15}\text{N}, ^{13}\text{C}, ^{1}\text{H}$
Chemical Shift Tensors

HIV-1 CA protein (231 aa), tubular assembly

Sensitive to motions on timescales of ns to μs
Implications of Dynamics for Capsid Structure Determination: Effect of Motional Averaging on Chemical Shifts

CypA loop

\[ \text{Isotropic shifts-no pronounced effect} \]

CS anisotropy-clear dynamic averaging

Quantum DFT calculations from X-ray structure—not in any agreement with NMR experiment

MD/SHIFTX-better agreement, isotropic shifts only

MD/DFT-strong agreement with experiment

Dynamically Averaged $^{15}N^H$ CSA Tensors: Calculation by MD – QM/MM

Frame 1 of MD:
$$\delta^1 = R_1^{-1} \cdot \Lambda^1 \cdot R_1$$
$$\Lambda^1 = \begin{bmatrix} \delta_{11}^1 & 0 & 0 \\ 0 & \delta_{22}^1 & 0 \\ 0 & 0 & \delta_{33}^1 \end{bmatrix}$$

Frame i of MD:
$$\delta^i = M_i^{-1} \cdot (R_1^{-1} \cdot \Lambda^1 \cdot R_1) \cdot M_i$$

$$R = \begin{bmatrix} \cos \alpha \cos \beta \cos \gamma - \sin \alpha \sin \gamma & \sin \alpha \cos \beta \cos \gamma + \cos \alpha \sin \gamma & - \sin \beta \cos \gamma \\ - \cos \alpha \cos \beta \sin \gamma - \sin \alpha \cos \gamma & - \sin \alpha \cos \beta \sin \gamma + \cos \alpha \cos \gamma & \sin \beta \sin \gamma \\ \cos \alpha \sin \beta & \sin \alpha \sin \beta & \cos \beta \end{bmatrix}$$

Average CSA tensor
$$\langle \delta \rangle = \frac{1}{N} \cdot \begin{bmatrix} \sum_{i=1}^N \delta_{xx}^i & \sum_{i=1}^N \delta_{xy}^i & \sum_{i=1}^N \delta_{xz}^i \\ \sum_{i=1}^N \delta_{yx}^i & \sum_{i=1}^N \delta_{yy}^i & \sum_{i=1}^N \delta_{yz}^i \\ \sum_{i=1}^N \delta_{zx}^i & \sum_{i=1}^N \delta_{zy}^i & \sum_{i=1}^N \delta_{zz}^i \end{bmatrix}$$
Effect of Dynamic Averaging on Principal Components of \(^{15}N\)H CSA Tensors in HIV-1 CA Assemblies

![Graphs showing dynamic and static components of CSA tensors](image)

Effect of Dynamic Averaging on Euler Angles of $^{15}N^H$ CSA Tensors in HIV-1 CA Assemblies

Effect of Dynamic Averaging on $^{15}\text{N}^H$ CSA Tensors: HIV-1 CA Assemblies

G89 (dynamic)

E98 (static)

$t = 1\ \text{ns}$

G89 (dynamic)

E98 (static)

After averaging for 100 ns

$\langle \delta_{\text{MD/DFD}} \rangle = 23.28\ \text{ppm}$  $\langle \delta_{\text{NMR/exp}} \rangle = 40.35\ \text{ppm}$

$\langle \delta_{\text{MD/DFD}} \rangle = 92.94\ \text{ppm}$  $\langle \delta_{\text{NMR/exp}} \rangle = 101.43\ \text{ppm}$

Implications of Dynamics for Capsid Structure Determination: Effect of Motional Averaging on Chemical Shifts

- Excellent correlation between experiment and theory for CS anisotropy
- Considerable deviations for isotropic CS
- Quantitative understanding of capsid’s dynamics is essential for atomic-level structure determination by NMR, using chemical shifts and dipolar (distance) information

Motional Averaging of Chemical Shifts is the Result of CSA Tensor Reorientation

- Reorientation of molecular frame during the course of motion is the main contributor to dynamic averaging
- Principal components of CSA tensor affected to a negligible degree
- Simplifies the structure calculation: QM calculation needs to be done on a single frame, and rotated during motion using classical MD trajectories

... when no change in secondary structure (folding-unfolding)
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U of Illinois
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