Spin Diffusion NMR For Distance Determination

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Spin Diffusion Methods in SSNMR

- $^1$H-driven X-spin isotropic spin diffusion:
  - no $^1$H decoupling (PDSD)
  - with $^1$H decoupling, $\omega_1=\omega_r$ (DARR/RAD)

- $^1$H-driven X-spin anisotropic spin diffusion: CODEX
  -> distances between chemically equivalent but orientationally inequivalent spins.

- Direct $^1$H spin diffusion:
  - With $^1$H evolution and X-spin detection
    -> lipid-protein distances $\sim<20$ Å.
  - With X-spin evolution and X/Y detection (XHHY)
Oligomeric Structure From Anisotropic Spin Diffusion

Goal: determine the intermolecular packing and distances of oligomeric protein assemblies.

- The sequence detects reorientations due to either slow motion or spin diffusion. Can distinguish the two by:
  - varying temperature to affect motion, or
  - varying $^1$H decoupling during $t_m$ to affect spin diffusion.

- Mechanism of spin diffusion: dipolar coupling $\rightarrow$ distance determination.

As $t_m \rightarrow 0$, $S/S_0 \rightarrow 1/n$, where $n$ is the number of orientationally inequivalent sites. $\rightarrow$ spin counting
Spin Counting: $^{13}$C CODEX

$S_0$ \( \text{100 ms} \) \( S \) \( 1 \text{ s} \)

\( \alpha\)-Gly \( \gamma\)-Gly

$t_m \sim 1 \text{ s for complete exchange: weak }^{13}$C-$^{13}$C coupling

$S/S_0 = 0.49 + 0.51 \ e^{-t/265}$

$S/S_0 = 0.32 + 0.64 \ e^{-t/121}$

shortest C-C distance: 4.22 Å

C-C distances: 4.17 Å, 5.23 Å

$^{19}$F Spin Diffusion: Faster than $^{13}$C

F-F coupling is 14-fold stronger than C-C coupling for the same distance.

F-F coupling is 14-fold stronger than C-C coupling for the same distance.

$\text{[1-}^{13}\text{C]} \text{ Phe}$

$\text{[4-}^{19}\text{F]} \text{ Phe}$
CODEX Decay Trajectory: Rate Matrix Approach

• For spin diffusion among \( n \) X spins, the time-evolution of the \( n \)-dimensional vector of the z magnetization, \( M(t) \), is given by the differential equation:

\[
\frac{d\tilde{M}(t)}{dt} = -K\tilde{M}(t)
\]

\[
\begin{pmatrix}
\frac{dM_1(t)}{dt} \\
\vdots \\
\frac{dM_n(t)}{dt}
\end{pmatrix} =
\begin{pmatrix}
k_{11} & \cdots & k_{1n} \\
\vdots & \ddots & \vdots \\
k_{n1} & \cdots & k_{nn}
\end{pmatrix}
\begin{pmatrix}
M_1(t) \\
\vdots \\
M_n(t)
\end{pmatrix}
\]

\( K \)

• \( M(t) = M_z(t) - M(0) \).
• \( K \): \( n \)-D exchange matrix of rate constants \( k_{ij} \).
• \( T_1 \) relaxation not included since it’s removed by the CODEX control \( S_0 \).

• Detailed balance of equilibrium \( M \) requires:
  • the sum of each column of the \( K \) matrix is zero, \( k_{ii} = -\sum_{j \neq i} k_{ji} \)

\[
\frac{dM_1}{dt} + \cdots + \frac{dM_n}{dt} = 0 \quad \Rightarrow \quad (k_{11}M_1 + \cdots + k_{1n}M_n) + (\cdots) + (k_{n1}M_1 + \cdots + k_{nn}M_n) = 0 \quad \Rightarrow \quad k_{11} + k_{21} + \cdots + k_{n1} = 0, \quad \ldots \quad k_{n1} + k_{n2} + \cdots + k_{nn} = 0
\]

\[
-\sum_{j \neq i} k_{ji} = k_{ii}
\]

\( k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0) \)

\[
K = \begin{pmatrix}
k_{11} & \ldots & k_{1n} \\
\ldots & \ldots & \ldots \\
k_{n1} & \ldots & k_{nn}
\end{pmatrix}
\]

\[k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0)\]

• \(k_{ij} = k_{ji}\) for equal populations of equilibrium M.
• Thus sum of each row is also zero.

• e.g. 4-spin \(K\) matrix:
\[
K = \begin{pmatrix}
k_{AB} + k_{AC} + k_{AD} & -k_{BA} & -k_{CA} & -k_{DA} \\
-k_{AB} & k_{BA} + k_{BC} + k_{BD} & -k_{CB} & -k_{DB} \\
-k_{AC} & -k_{BC} & k_{CA} + k_{CB} + k_{CD} & -k_{DC} \\
-k_{AD} & -k_{BD} & -k_{CD} & k_{DA} + k_{DB} + k_{DC}
\end{pmatrix}
\]

• The rate matrix includes both direct and relayed transfer effects. e.g. magn. transfer from A to C: \(-k_{AC}, -k_{AB}\) and \(-k_{BC}\).

• CODEX is a natural method to measure distances in inherently multi-spin environments, among spins of the same identity but in different molecules → intermolecular distance constraints in oligomeric assemblies.
CODEX Decay to Equilibrium Value

• The solution to the differential equation of $M(t)$ is:

$$\tilde{M}(t) = e^{-Kt}\tilde{M}(0)$$

• The exponential operator can be treated by diagonalization of $K$ or calculated in a matrix-based software. Expressed in terms of the diagonalized exchange matrix $\Lambda = UKU^{-1}$ ($K=U^{-1}\Lambda U$) where $U$ is the eigenvector matrix of $K$,

$$\tilde{M}(t) = e^{-Kt} \cdot \tilde{M}(0) = e\left(\tilde{U}\Lambda U^{-1}\right)t \cdot \tilde{M}(0) = \left(Ue^{-\Lambda t}U^{-1}\right)\tilde{M}(0) = U\begin{pmatrix} e^{-\lambda_1 t} & 0 & 0 \\ 0 & \ldots & 0 \\ 0 & 0 & e^{-\lambda_n t} \end{pmatrix}U^{-1} \cdot \tilde{M}(0)$$

• For an $n$-D matrix (for $n$ spins) with zero-sum columns, one eigenvalue is always zero with the eigenvector of $\left(1/\sqrt{n} \quad \ldots \quad 1/\sqrt{n}\right)^T$, while all other eigenvalues are positive.

Proof:

$$K \cdot \begin{pmatrix} 1/\sqrt{n} \\ \vdots \\ 1/\sqrt{n} \end{pmatrix} = \sum_{n} K_{mn} \cdot \frac{1}{\sqrt{n}} = \frac{1}{\sqrt{n}} \sum_{n} K_{mn}$$

sum over row

$K_{mn}=K_{nm}$,

$\sum_{m} K_{mn}=0$, $m$

$$\rightarrow e^{-\lambda_1 t} = \frac{1}{\sqrt{n}} \cdot 0 = 0 \cdot \begin{pmatrix} 1/\sqrt{n} \\ \vdots \\ 1/\sqrt{n} \end{pmatrix}$$
• Thus, at long mixing times $t_m$,

$$\tilde{\mathbf{M}}(t) = \left( \mathbf{U} e^{-\Lambda t} \mathbf{U}^{-1} \right) \cdot \tilde{\mathbf{M}}(0) \Rightarrow$$

$$\tilde{\mathbf{M}}(t >> \frac{1}{\lambda_i}) = \sum_{i=1}^{\infty} \tilde{\mathbf{M}}(0) \cdot \left( \tilde{\mathbf{u}}_i \cdot e^{-\lambda_i t} \cdot \tilde{\mathbf{u}}_i^{-1} \right) = \sum_{i=1}^{\infty} \tilde{\mathbf{M}}(0) \cdot \left( \tilde{\mathbf{u}}_i \cdot e^{-\infty} \cdot \tilde{\mathbf{u}}_i^{-1} \right) + \tilde{\mathbf{M}}(0) \cdot \left( \begin{array}{c} 1/\sqrt{n} \\ 1/\sqrt{n} \\ \vdots \\ 1/\sqrt{n} \end{array} \right) e^{0 \cdot t} \left( \begin{array}{c} 1/\sqrt{n} \\ 1/\sqrt{n} \\ \vdots \\ 1/\sqrt{n} \end{array} \right)$$

$$= 0 + (0 \ldots 1 \ldots 0) \left( \begin{array}{c} 1/\sqrt{n} \\ \sqrt{n} \\ \vdots \\ 1/\sqrt{n} \end{array} \right) \cdot \left( \begin{array}{c} 1/\sqrt{n} \\ \sqrt{n} \end{array} \right) = 1/\sqrt{n} \cdot \left( \begin{array}{c} 1/\sqrt{n} \\ 1/\sqrt{n} \end{array} \right) = \left( \begin{array}{c} 1/n \\ 0 \ldots 0 \end{array} \right)$$

$$\mathbf{M}(t >> \frac{1}{\lambda_i}) = \left( \begin{array}{c} 1/n \\ 1/n \ldots 1/n \end{array} \right)$$

Complete equilibration of CODEX magnetization.

Luo & Hong, JACS, 128, 7242 (2006)
Rate Constant and Overlap Integral

- In the rate constant expression: \( k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0) \)

\[ \omega_{ij} = \frac{\mu_0}{4\pi} \frac{\gamma^2 \hbar}{r_{ij}^3} \frac{(1 - 3\cos^2 \theta_{ij})}{2} \]

- The angular term, \((1-3\cos^2\theta_{ij})\) depends on the powder angles of the molecules in the \(B_0\) field. The square of \(\omega_{ij}\) can be simplified by its powder-averaged value, 0.8.

Main adjustable parameter in the \(\omega_{ij}\) extraction: \(F_{ij}(0)\)

- **Overlap integral**: probability that SQ transitions occur at the same frequency for spins i and j:

\[ F_{ij}(0) = \int_{-\infty}^{+\infty} f_i(\omega - \omega_i)f_j(\omega - \omega_j)d\omega \]

- \(f_i(\omega-\omega_i)\): normalized SQ lineshape of spin i without \(^1H\) decoupling.
- \(\omega_i\): center of the lineshape.
- \(F_{ij}(0)\): reflects the overlap area of two \(^1H\) undecoupled SQ lines, and is related to the normalized ZQ lineshape at 0 frequency.
- The larger the \(F_{ij}(0)\), the faster the decay, the larger the spin diffusion rate \(k_{ij}\), and the smaller the decay constant \(\tau_{SD}\).
- \(F_{ij}(0)\) has the unit of time (s).
Overlap Integral

\[ F_{ij}(0) = \int_{-\infty}^{+\infty} f_i(\omega - \omega_i)f_j(\omega - \omega_j) d\omega \]

- \( F_{ij}(0) \) depends on the
  - isotropic shift difference
  - anisotropic chemical shift
  - \( X-H \) dipolar coupling
  - \( ^1H-^1H \) dipolar coupling
  - Spinning speed

- For singly labeled systems, can approximate \( F_{ij}(0) \) as the same for all intermolecular spin pairs \( ij \).

- The rate constant \( k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0) \) was developed for \(^1H\)-driven \( X \)-spin diffusion. But it has been used to analyze direct \(^1H\) spin diffusion as well, and on small molecule compounds it gives good agreement with the crystal-structure distances.

Determining F(0) from Model Compounds

For small-molecule compounds, need to consider distances over a number of unit cells.

\[ \omega_{ij}^2 \rightarrow \sum \omega_{ij}^2 \]

second moment coupling

\[ \sum \omega_{ij}^2 \text{ converges within } 15 - 20 \, \text{Å} \]

\[ \gamma\text{-Gly: } \sum \omega_{ij}^2 \approx 2 \omega_{ij}^2 \]

shortest distance between spin pair
dipolar coupling square \( \omega_{ij}^2 \)

read another distance

Add the dipolar coupling square to \( \omega_{ij}^2 \)

The sum converge?

Assume F(0) value

Exchange probability \( k_{ij} \)

Exchange matrix \( k \)

\[ M(t) = \exp(-Kt) M(t = 0) \]

Use another F(0) value

RMSD

\[ \text{RMSD} = \sqrt{\frac{\sum (M_{\text{sim}} - M_{\text{exp}})^2}{N}} \]

Smallest RMSD?

F(0) value is determined
• At 5 kHz MAS, $F(0) \approx 80 \mu s$.
• Faster spinning reduces $F(0) \rightarrow$ slower spin diffusion.
• $F(0) \sim (1/\nu_r)^{0.5-1}$.

F(0) of $^{19}$F CODEX

Consensus $^{19}$F F(0) at 8 kHz MAS is 37 µs.

$$k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0) \propto F_{ij}(0)/r^6 \Rightarrow k \text{ is much less sensitive to } F(0) \text{ than } r.$$
M2-TMP: a Tetrameric H\(^+\) Channel in the Membrane

\[ \text{Ala30} \rightarrow [4^{19}F] \text{Phe30}, \ P:L = 1:15, \ DMPC \text{ bilayers,} \ 240 \text{ K, 8 kHz MAS} \]

Luo & Hong, \textbf{JACS}, 128, 7242 (2006)
Other Practical Aspects of CODEX for Oligomeric Structure Determination

- **Symmetric oligomers**: only one unknown distance in the $K$ matrix.
  
  $k_{AB} = k_{AD} = 0.5\pi F(0) \cdot \omega(r)^2$,
  
  $k_{AC} = 0.5\pi F(0) \cdot \omega(\sqrt{2}r)^2 = \frac{1}{2^{3/2}} 0.5\pi F(0) \cdot \omega(r)^2$

- **Asymmetric oligomers**: multiple distances unknown. Unclear whether the CODEX curve can yield multiple distances. The rigorous approach: measure multiple distances to avoid under-determining the problem.

- With $^{19}$F-$^{19}$F dipolar coupling, the maximum distance detected in model compounds is $\sim 15$ Å.

- Phenylene ring $4^{19}$F position insensitive to ring flip: good for distance expts.

- **CF$_3$ labels** not recommended: fast $^{19}$F $T_1$ relaxation during $t_m$.

- Other aromatic $^{19}$F-labels for proteins: $5^{19}$F-Trp, $6^{19}$F-Trp.

- Large $^{19}$F CSA is sensitive to small-angle differences between two molecules. E.g. $\delta \approx 55$ ppm for $4^{19}$F-Phe; at 9.4 T, $\delta \approx 20$ kHz. With $Nt_r=250$ µs, $2\pi\delta Nt_r \approx 10\pi$, sensitive to $10^\circ$ orientation differences between molecules.

- Need to ensure no slow motion is present at the desired temperature.
$^1$H Spin Diffusion of Membrane Proteins

Purposes:
- Protein distance to the membrane center.
- Protein distance to the membrane surface.

Main features:
- Undecoupled $^1$H $T_2$ filter before $t_1$ selects mobile components.
- $^1$H undecoupled $t_1$ evolution further suppresses rigid components.
- Direct $^1$H spin diffusion, mobile $\rightarrow$ rigid transfer.
- X spin detection can be $^{13}$C, $^{15}$N, $^{31}$P, etc.

Application modes:
- Ambient temp. (LC phase): lipid (L) $\rightarrow$ protein (P) transfer,
  - $2\tau \sim 2$ ms
  - $t_m \sim [10$ ms, 10 s]
  - mainly 2D (can also be 1D), to resolve multiple mobile $^1$H signals.
- Low temp. (gel phase): water (W) $\rightarrow$ protein transfer,
  - $2\tau \sim 0.2$ ms
  - $t_m \sim [0.1$ ms, 25 ms]
  - 1D, no $^1$H evolution needed (only water remains).
LC Phase $^1$H Spin Diffusion: Intensity Buildup Reflects Minimum L-P Distance

- $^1$H spectrally resolved mobile components in a membrane sample:
  - $\text{H}_2\text{O}$: $S \sim 0.03$
  - lipid $\text{CH}_3$: $S \sim 0.02-0.04$
  - lipid $(\text{CH}_2)_n$: $S \sim 0.08 - 0.20$
  - lipid $\text{H}_\gamma$: $S$ very small

- If the protein is mostly immobile, $S >\sim 0.7$, then spin diffusion is slow within the soft lipid matrix and water, and rapid within the protein.

- A rate-limiting step in the L/W $\rightarrow$ P transfer is transfer across the intermolecular interface due to translational and rotational diffusion of L/W.

- Once intermolecular transfer occurs, $^1$H magnetization equilibrates in the protein in $\leq 1$ ms ($\sim\text{CHHC}$), obliterating distance resolution for typical $t_m$ values of $\sim 100$ ms and higher.

- Buildup curve (Intensity vs $\sqrt{t_m}$) reflects the shortest distance from the source spin to the protein $\rightarrow$ qualitative information of protein topology.

- It doesn’t matter where the $^{13}\text{C}/^{15}\text{N}$ label is in the protein.
2D Data and Buildup Curves

Colicin Ia channel domain in POPC/POPG membrane

13C detection

DNA - cationic membrane mixture

31P detection

Huster et al, JACS, 124, 874 (2002)
Distances from Linear-Chain Spin Diffusion Calculation

• General 1D diffusion equation (Fick’s 2nd Law):

\[ \frac{\partial M}{\partial t} = D \cdot \frac{\partial^2 M}{\partial x^2} \]

(Nature abhors a wrinkle.)

• On a discrete 1D lattice (along the bilayer normal):

\[ \frac{\Delta M_i}{\Delta t} = D \cdot \frac{1}{a^2} \left[ \left( M_{i+1} - M_i \right) - \left( M_i - M_{i-1} \right) \right] = \Omega \left( -2M_i + M_{i+1} + M_{i-1} \right) \]

D: diffusion coefficient (nm\(^2\)/ms)
\( \Omega \): transfer rate = D/\(a^2 \)
\( a \): lattice spacing, 2 Å or 1 Å

• \( \Omega \) or D is related to the \(^1\)H-\(^1\)H dipolar couplings. In rigid polymers, D\( \approx \)0.8 nm\(^2\)/ms has been measured, equivalent to \( \Omega \approx 20 \) kHz for two protons 2.0 Å apart.

• Two lipid vicinal protons are \( \sim 2.4 \) Å apart (rigid-limit \( \delta = 8.8 \) kHz).

• Using a \( S \approx 0.04 \) for protons close to the acyl chain termini, the motionally averaged \(^1\)H-\(^1\)H coupling is \( \Omega \approx 350 \) Hz.

• With a spacing \( a \) of 2 Å, the resulting \( D_L = \Omega a^2 \) is \( \sim 0.014 \) nm\(^2\)/ms.
• For proteins with $S \approx 0.7$, the $^1$H-$^1$H coupling is $\Omega (2.4 \, \text{Å}) \sim 6.0 \, \text{kHz}$, $\Rightarrow D_p \approx 0.25 \, \text{nm}^2/\text{ms}$ ($a = 2 \, \text{Å}$).

• For interfacial transfer, typical $D_{\text{int}} \sim 0.002 \, \text{nm}^2/\text{ms}$ (order of magnitude).

Sample simulation:

<table>
<thead>
<tr>
<th>Source - lipid CH$_3$:</th>
<th>0.012</th>
<th>4 Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source - H$_2$O:</td>
<td>0.03</td>
<td>2 Å</td>
</tr>
<tr>
<td>Gap:</td>
<td>0.012</td>
<td>$x$ Å</td>
</tr>
<tr>
<td>Interface:</td>
<td>0.00125</td>
<td>2 Å</td>
</tr>
<tr>
<td>Sink - peptide</td>
<td>0.3</td>
<td>30 Å</td>
</tr>
</tbody>
</table>
Effects of $D_{\text{int}}$ and Distance on the Buildup Curves

$D_{\text{int}}$: the adjustable parameter in the SD simulation. Estimate by reproducing the slope of the experimental buildup curve.

$D_L = 0.0125 \text{ nm}^2/\text{ms}$
$D_p = 0.3 \text{ nm}^2/\text{ms}$

- $D_{\text{int}}$ mainly changes the slope of the buildup curve.
- $r$, lipid-protein distance, mainly changes the initial lag of the buildup curve.
- Empirically, phospholipid-protein mixtures have $D_{\text{int}} \sim 0.0025 \text{ nm}^2/\text{ms}$, lipid-DNA have $D_{\text{int}} \sim 0.00025 \text{ nm}^2/\text{ms}$ (low $^1\text{H}$ density in DNA), and cholesterol-containing membranes also give $D_{\text{int}} \sim 0.00025 \text{ nm}^2/\text{ms}$.

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Mani et al, PNAS, 103, 16242 (2006)
Origin of the $t^{1/2}$ Dependence of Intensity Buildup

- For a point source at $x_0$, $M(x,0) = \delta(x-x_0)$, the solution of the diffusion equation $\partial M/\partial t = D \cdot \partial^2 M/\partial x^2$ is a Gaussian function of $x$, $M(x,t) = e^{-\frac{(x-x_0)^2}{4Dt}}/\sqrt{\pi Dt}$.

- A domain source $M_{\text{dom}}(x,t)$ is a superposition of many point sources:
  $$M_{\text{dom}}(x,0) = \int_{-\infty}^{0} \delta(x-x_0) \, dx_0$$

- $M_{\text{dom}}(x,t)$ evolves as an error function centered at the source-sink interface:
  $$M_{\text{dom}}(x,t) = \int_{-\infty}^{0} e^{-\frac{(x-x_0)^2}{4Dt}} \sqrt{\frac{\pi}{4Dt}} \, dx_0 \int_{\frac{x-x_0}{\sqrt{4Dt}}}^{\frac{x-x_0}{\sqrt{4Dt}}+\infty} e^{-x'^2} \, dx' = \frac{-\sqrt{4Dt}}{\sqrt{\pi Dt}} \cdot \int_{\frac{-\infty}{\sqrt{4Dt}}}^{\frac{x-x_0}{\sqrt{4Dt}}} e^{-x'^2} \, dx' = \frac{2}{\sqrt{\pi}} \cdot \int_{\frac{x-x_0}{\sqrt{4Dt}}}^{\infty} e^{-x'^2} \, dx' = \text{erfc}\left(\frac{x}{\sqrt{4Dt}}\right)$$

- The total magn $I_{\text{sink}}(t)$ of the sink increases as $t^{1/2}$:
  $$I_{\text{sink}}(t) \propto \int_{0}^{+\infty} M_{\text{dom}}(x,t) \, dx = \int_{0}^{+\infty} \text{erfc}\left(\frac{x}{\sqrt{4Dt}}\right) \, dx = \frac{x}{\sqrt{4Dt}} \bigg|_{0}^{+\infty} = \frac{1}{\sqrt{\pi}} \cdot \sqrt{t}$$
Thus, for domain spin diffusion, the \( I(t^{1/2}) \) plot is linear.

For point-source spin diffusion, there is a latency period \((M \approx 0)\) whose duration depends on the distance from the point source.

Plotting \( I(t^{1/2}) \) stretches out the initial period compared to \( I(t) \), thus better distinguishing different distances.
Buildup Curves of Non-Transmembrane Systems

- In membrane systems, spin diffusion is usually from **point sources**, giving a lag period in the $I_{\text{sink}}(t^{1/2})$ plot. This is especially clear in non-TM macromolecules.

**DNA - cationic membrane**

**POPC/cholesterol membrane with PG-1**

*Huster et al, JACS, 124, 874 (2002)*  
*Mani et al, PNAS, 103, 16242 (2006)*
Higher-Sensitivity LC-Phase $^1$H Spin Diffusion

- **2D HHC:**
  - Indirect $^1$H dimension of lipids and water, high resolution, require long $t_1$.
  - Direct $^{13}$C dimension of protein, lower resolution and sensitivity.

- Buildup curves require multiple 2D, long expt time, need careful monitoring of CP stability, sample hydration etc, to obtain reliable curves.

- **Alternative, 1D CHH:**
  - Invert the $^1$H and $^{13}$C dimensions.
  - Remove $^{13}$C $t_1$ altogether since no distance resolution!
  - Sensitivity gain due to $^1$H detection.
  - $^1$H detection requires no homonuclear decoupling.

Detection sensitivity gain: $\left(\frac{\gamma_H}{\gamma_C}\right)^{3/2} = 8$

- **Two obstacles of 1D CHH:**
  - Suppressing large equilibrium $^1$H magnetization of lipids & water.
  - Sensitivity gain limited by the fraction of labeled $^{13}$C sites versus natural abundance lipid $^{13}$C.
1D CHH Protein-Lipid Spin Diffusion

TEASE U-$^{13}$C, $^{15}$N-labeled colicin la channel domain in POPC/POPG membrane. P/L = 1:100, ~50% $^{13}$C labeling.

Time-saving: 180-350 fold. 1D reproduces the 2D buildup curves.

Luo & Hong, SSNMR., 29, 163-169 (2006)
Sensitivity of the CHH Spin Diffusion Experiment

• All detected $^1$H magn originates from the labeled $^{13}$C sites ($C_p$) in the protein. So the sensitivity mainly depends on the $^{13}$C labeling level.

• Sensitivity also depends on the % of mobile protons ($H_L+H_W$) in the sample.

• Assuming complete equilibrium (CP + SD), the number of detected protons is:

$$H_{CHH} = C_p \times \frac{H_P}{H_P + C_p} \times \frac{H_L + H_W}{H_P + H_L + H_W}$$

• The % detected $^1$H’s among the total lipid and water protons is $H_{CHH}/(H_L + H_W)$

• For a membrane protein sample with mass ratio $P:L:W \approx 1:3:2$ and a $^{13}$C labeling level of $\sim 50\%$, the calculated fraction of detected protons is $\sim 2.5\%$. This gave reproducible and correct CHH buildup curves.

• The experiment needs to suppress $\sim 98\%$ undesired $^1$H signals. This is achieved by the $T_2$ filter, phase cycling, and a 90° purge pulse. Suppression of the rigid $^1$H magn is easy, but of the mobile $^1$H magn. of the natural abundance lipid $^{13}$C is more difficult.

• Empirically, $<0.8\%$ detected protons causes systematic errors in the buildup curves. Thus, $^{13}$C labeling level needs to be $>\sim 15\%$ for CHH to work.
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19F Spin Diffusion for Determining Intermolecular Distances in Oligomeric Membrane Proteins

Mei Hong, Iowa State University

Membrane protein structural features:
- Orientation.
- Depth of Insertion.
- Sidechain conformation.
- Assembly of polypeptide chains: quaternary structure.

Oligomeric structure of membrane proteins:
- Oligomeric number
- Intermolecular distance constraints.
M2 Protein: a Proton Channel of Influenza A Virus

- A $H^+$ channel, open at $pH < 7$ and closed at $pH > 7$.

- Forms tetrameric bundles in micelles.

- Oligomeric state in the lipid bilayer unknown. Only one short interhelical distance reported (Cross et al.).
F-F Distance Confirms the Tetramer Model

\[ \chi_1 = 180^\circ \]

Most probable rotamer:
\[ r = 7.5 \, \text{Å}, \quad F(0)=28 \, \mu s \]

\[ \chi_1 = -60^\circ \]

\[ r = 18.5 \, \text{Å}, \quad F(0)=2000 \, \mu s \]

\[ \chi_1 = 60^\circ \]

Least probable rotamer:
ring clashes with backbone

The interhelical distance of 7.9 - 9.5 Å for Phe30 agrees well with the M2 tetramer model obtained from \(^{15}\text{N}\) orientation data (Cross et al).
F-F Distance Confirms Existing Tetramer Model

- NH - CH - CO -

<table>
<thead>
<tr>
<th>$\chi_1$</th>
<th>% in helices</th>
<th>$r_{FF}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>180°</td>
<td>57%</td>
</tr>
<tr>
<td>g$^+$</td>
<td>-60°</td>
<td>41%</td>
</tr>
<tr>
<td>g$^-$</td>
<td>+60°</td>
<td>1%</td>
</tr>
</tbody>
</table>

NMR structure model by Cross et al.

- +60° rotamer: forbidden by steric clash with the backbone
- Only the trans rotamer is possible.

The inter-helical distance of 7.9 - 9.5 Å for F30 agrees well with the M2 tetramer model obtained from $^{15}$N orientational data.
Distance Restraint for Helix Orientation

- Tilt angle $\tau = 35^\circ$
- Tilt angle $\tau = 25^\circ$
- Tilt angle $\tau = 15^\circ$

NMR model (Cross et al.)

Functional model (DeGrado et al.)

- Tilt angle $> 20^\circ$
- Functional model may have un-optimized rotation angles.