Spin Diffusion NMR For Distance Determination

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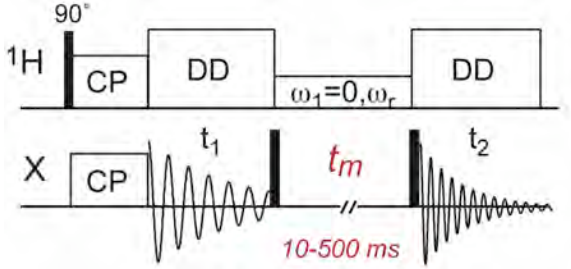
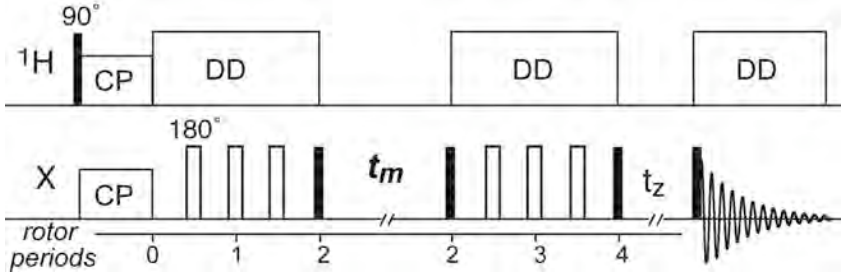
Spin Diffusion Methods in SSNMR

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

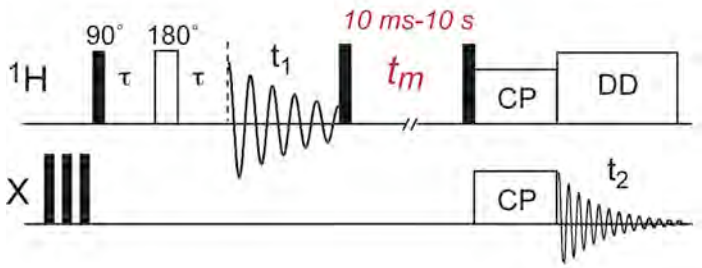


- ^{1}H-driven X-spin **anisotropic** spin diffusion: CODEX

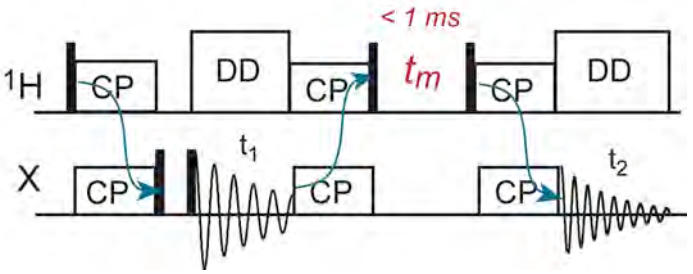


–> distances between chemically equivalent but orientationally inequivalent spins.

- Direct ^{1}H spin diffusion:
  - With ^{1}H evolution and X-spin detection

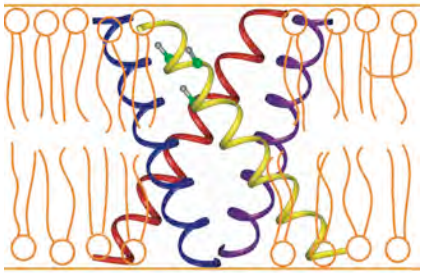


–> lipid-protein distances ~<20 Å.

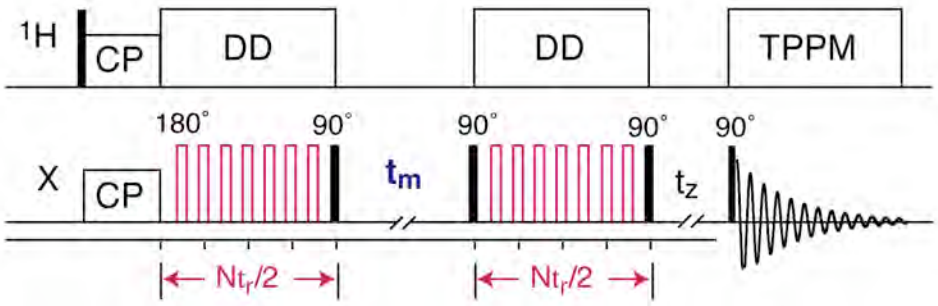


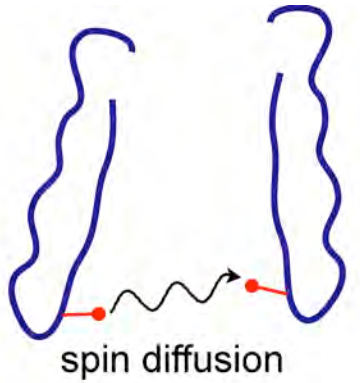
  - With X-spin evolution and X/Y detection (XHHY)

Oligomeric Structure From Anisotropic Spin Diffusion



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

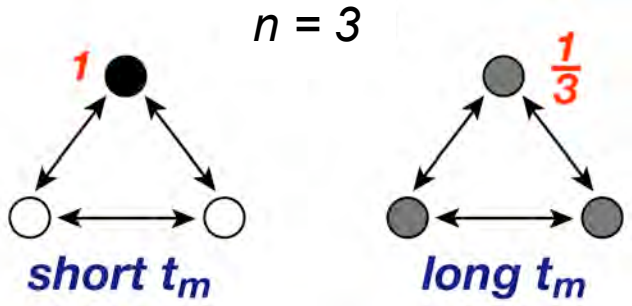




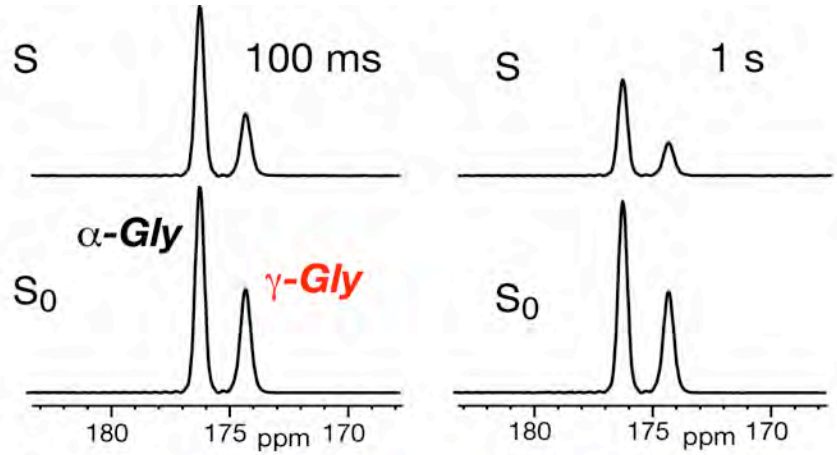
- The sequence detects reorientations due to either slow motion or spin diffusion. Can distinguish the two by:
  - varying temperature to affect motion, or
  - varying ^{1}H decoupling during t_m to affect spin diffusion.

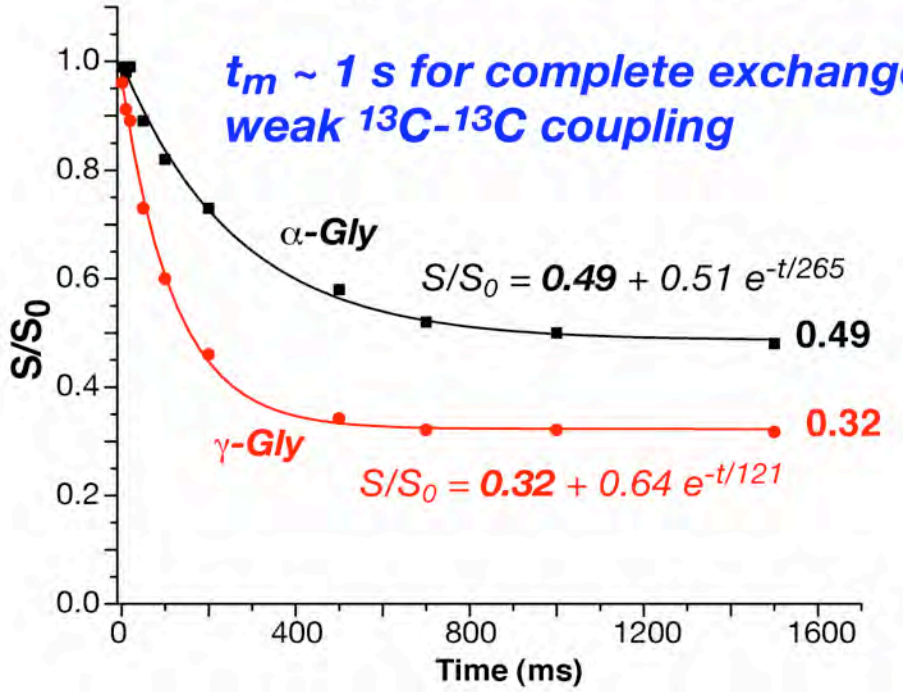
- Mechanism of spin diffusion: dipolar coupling –> distance determination.

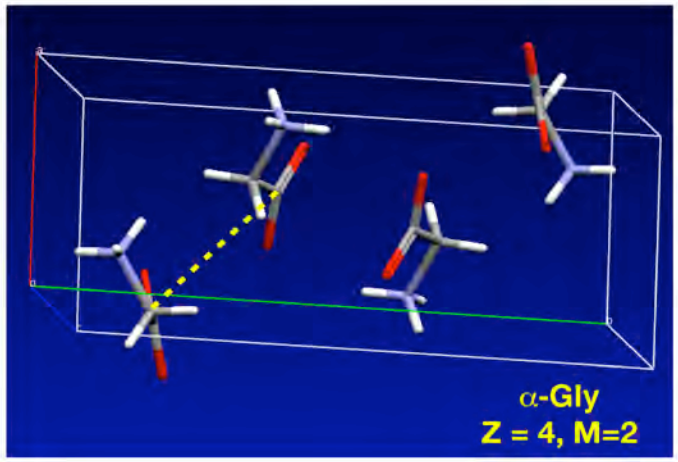
As $t_m \rightarrow 0$, $S/S_0 \rightarrow 1/n$, where n is the number of orientationally inequivalent sites.
→ spin counting



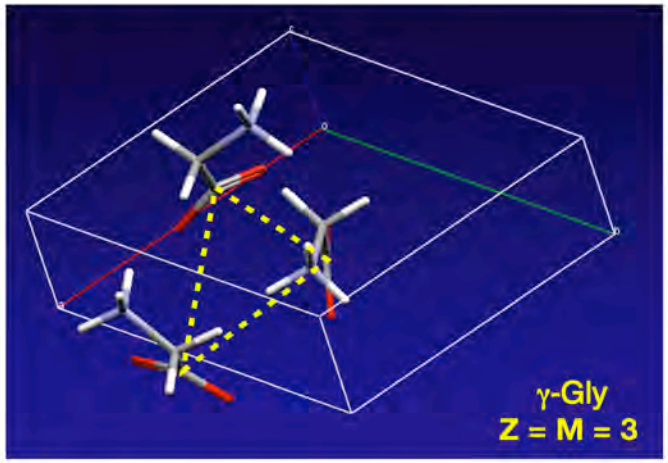
Spin Counting: ^{13}C CODEX







shortest C-C distance: 4.22 Å



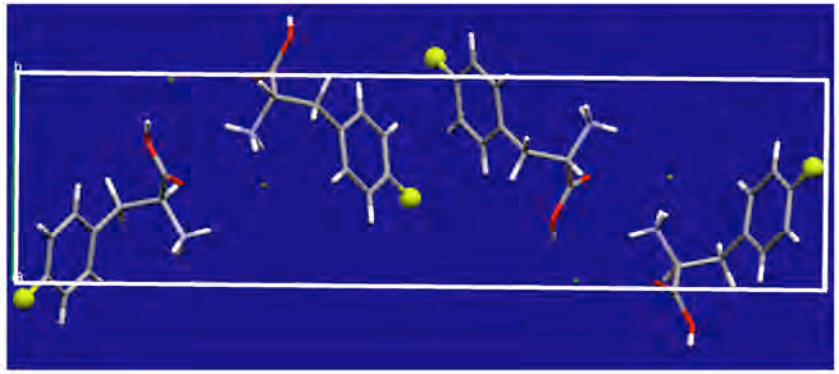
C-C distances: 4.17 Å, 5.23 Å

Buffy et al, **JACS**, 2004, 127, 4477 (2005).

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

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

Phe crystal structure



Z = 4, distances: 4 - 17 Å

~ 10 s for complete exchange

[1-^{13}C] Phe

~ 50 ms for complete exchange

[4-^{19}F] 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:

$$\frac{d\vec{M}(t)}{dt} = -\mathbf{K}\vec{M}(t) \qquad \begin{pmatrix} dM_1(t)/dt \\ \dots \\ dM_n(t)/dt \end{pmatrix} = \underbrace{\begin{pmatrix} k_{11} & \dots & k_{1n} \\ \dots & \dots & \dots \\ k_{n1} & \dots & k_{nn} \end{pmatrix}}_{\mathbf{K}} \begin{pmatrix} M_1(t) \\ \dots \\ M_n(t) \end{pmatrix}$$

- 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.

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

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

$$\frac{dM_1}{dt} + \dots + \frac{dM_n}{dt} = 0 \quad \rightarrow \quad \left(k_{11}M_1 + \dots + k_{1n}M_n\right) + \left(\dots\right) + \left(k_{n1}M_1 + \dots + k_{nn}M_n\right) = 0 \rightarrow$$

$$\left(k_{11} + k_{21} \dots + k_{n1}\right)M_1 + \left(\dots\right) + \left(k_{n1} + k_{n2} \dots + k_{nn}\right)M_n \equiv 0$$

$$\Rightarrow k_{11} + k_{21} \dots + k_{n1} = 0, \quad \dots k_{n1} + k_{n2} \dots + k_{nn} = 0 \quad \rightarrow \quad -\sum_{j \neq i} k_{ji} = k_{ii}$$

Luo & Hong, **JACS**, 128, 7242 (2006)

$$\mathbf{K} = \begin{pmatrix} k_{11} & \dots & k_{1n} \\ \dots & \dots & \dots \\ k_{n1} & \dots & k_{nn} \end{pmatrix}$$

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

- $k_{ij} = k_{ji}$ for equal populations of equilibrium M.
- Thus sum of each row is also zero.

- e.g. 4-spin **K** matrix:

$$\mathbf{K} = \begin{pmatrix} k_{AB}+k_{AC}+k_{AD} & -k_{BA} & -k_{CA} & -k_{DA} \\ -k_{AB} & k_{BA}+k_{BC}+k_{BD} & -k_{CB} & -k_{DB} \\ -k_{AC} & -k_{BC} & k_{CA}+k_{CB}+k_{CD} & -k_{DC} \\ -k_{AD} & -k_{BD} & -k_{CD} & k_{DA}+k_{DB}+k_{DC} \end{pmatrix}$$

- 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:

$$\vec{M}(t) = e^{-\mathbf{K}t}\vec{M}(0)$$

- 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 $\Lambda=\mathbf{UKU}^{-1}$ ($\mathbf{K}=\mathbf{U}^{-1}\Lambda\mathbf{U}$) where **U** is the eigenvector matrix of **K**,

$$\vec{M}(t) = e^{-\mathbf{K}t}\cdot\vec{M}(0) = e^{-\left(\mathbf{U}\Lambda\mathbf{U}^{-1}\right)t}\vec{M}(0) = \left(\mathbf{U}e^{-\Lambda t}\mathbf{U}^{-1}\right)\vec{M}(0) = \mathbf{U}\begin{pmatrix} e^{-\lambda_1 t} & 0 & 0 \\ 0 & \dots & 0 \\ 0 & 0 & e^{-\lambda_n t} \end{pmatrix}\mathbf{U}^{-1}\cdot\vec{M}(0)$$

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

Proof:

$$\mathbf{K}\cdot\begin{pmatrix} 1/\sqrt{n} \\ \dots \\ 1/\sqrt{n} \end{pmatrix} = \sum_n K_{mn}\cdot\frac{1}{\sqrt{n}} = \frac{1}{\sqrt{n}}\underbrace{\sum_n K_{mn}}_{\text{sum over row}} \xrightarrow{\substack{K_{mn}=K_{nm},\\ \sum_m K_{mn}=0}} = \frac{1}{\sqrt{n}}\cdot 0 = 0\cdot\begin{pmatrix} 1/\sqrt{n} \\ \dots \\ 1/\sqrt{n} \end{pmatrix}$$

- Thus, at long mixing times t_m,

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

$$\vec{M}(t >> \frac{1}{\lambda_i}) = \sum_{i=1}^{n}\vec{M}(0)\cdot\left(\vec{u}_i\cdot e^{-\lambda_i t}\cdot\vec{u}_i^{-1}\right) = \sum_{i=1}^{n-1}\vec{M}(0)\cdot\left(\vec{u}_i\cdot e^{-\infty}\cdot\vec{u}_i^{-1}\right) + \vec{M}(0)\cdot\begin{pmatrix}1/\sqrt{n}\\ \dots \\ 1/\sqrt{n}\end{pmatrix}e^{-0\cdot t}\begin{pmatrix}1/\sqrt{n}\\ \dots \\ 1/\sqrt{n}\end{pmatrix}$$

$$= 0 + \begin{pmatrix}0 & \dots & 1 & \dots 0\end{pmatrix}\begin{pmatrix}1/\sqrt{n}\\ \dots \\ 1/\sqrt{n}\end{pmatrix}\cdot 1\cdot\begin{pmatrix}1/\sqrt{n}\\ \dots \\ 1/\sqrt{n}\end{pmatrix} = 1/\sqrt{n}\cdot\begin{pmatrix}1/\sqrt{n}\\ \dots \\ 1/\sqrt{n}\end{pmatrix} = \begin{pmatrix}1/n\\ \dots \\ 1/n\end{pmatrix}$$

$$M\left(t >> \frac{1}{\lambda_i}\right) = \left(1/n, 1/n, \dots 1/n\right)$$

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

Main adjustable parameter in the ω_{ij} extraction: $F_{ij}(0)$

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

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

- $f_i(\omega\text{-}\omega_i)$: normalized SQ lineshape of spin i without ^{1}H decoupling.
- ω_i: center of the lineshape.
- $F_{ij}(0)$: reflects the overlap area of two ^{1}H 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 τ_{SD}.
- $F_{ij}(0)$ has the unit of time (s).

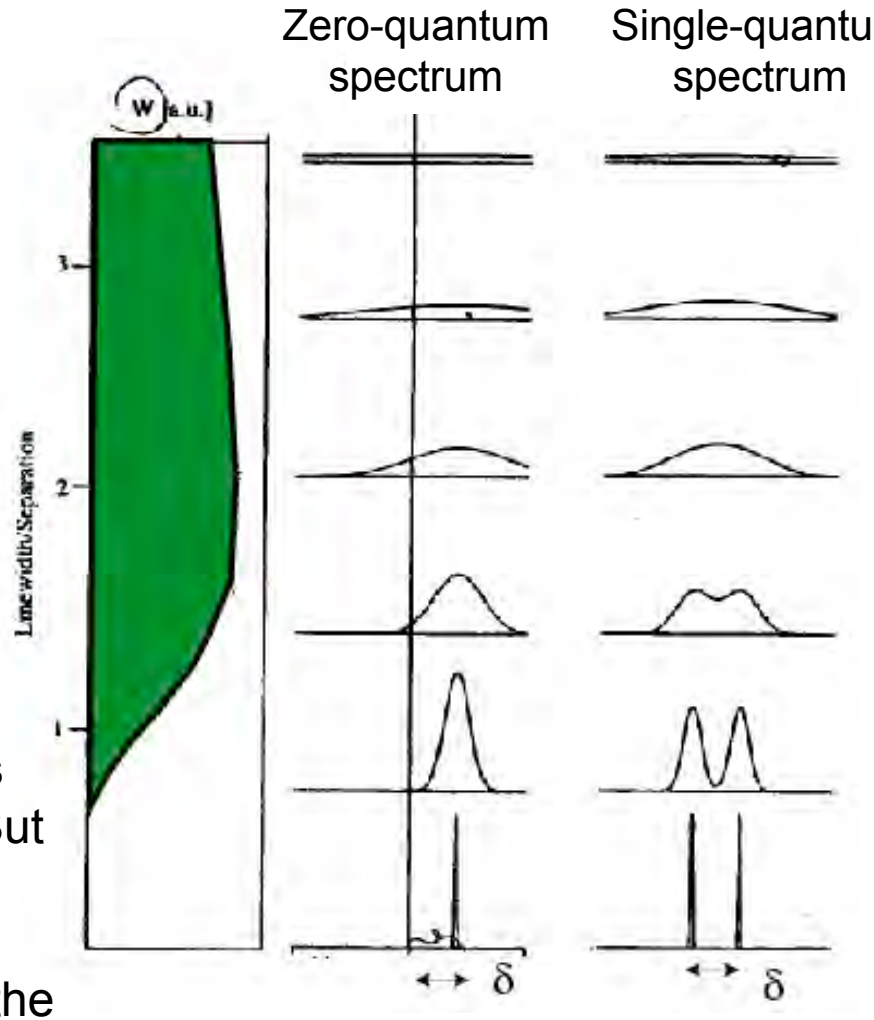
Overlap Integral

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

- $F_{ij}(0)$ depends on the
  - isotropic shift difference
  - anisotropic chemical shift
  - X-^{1}H dipolar coupling
  - ^{1}H-^{1}H 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 ^{1}H-driven X-spin diffusion. But it has been used to analyze direct ^{1}H spin diffusion as well, and on small molecule compounds it gives good agreement with the crystal-structure distances.



Meier, **Adv. Magn. Reson.** 18, 1 (1994).

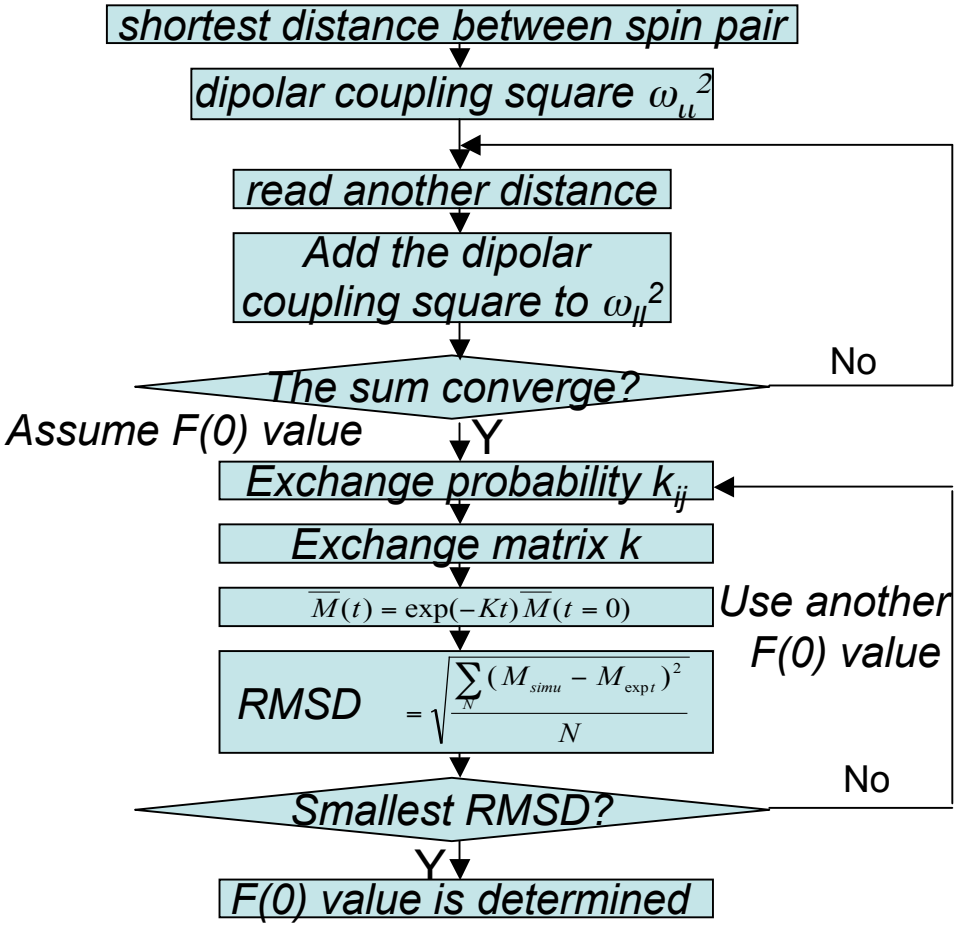
Determining F(0) from Model Compounds

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

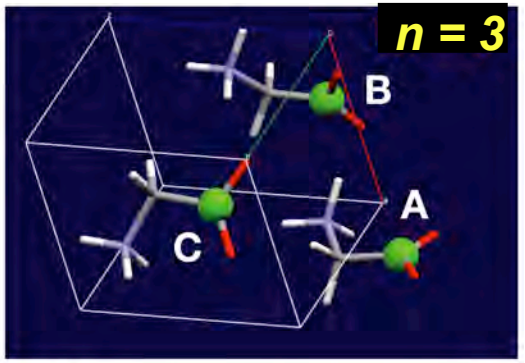
$\omega_{ij}^2 \longrightarrow \sum_{m,n} \omega_{i_m j_n}^2$ second moment coupling

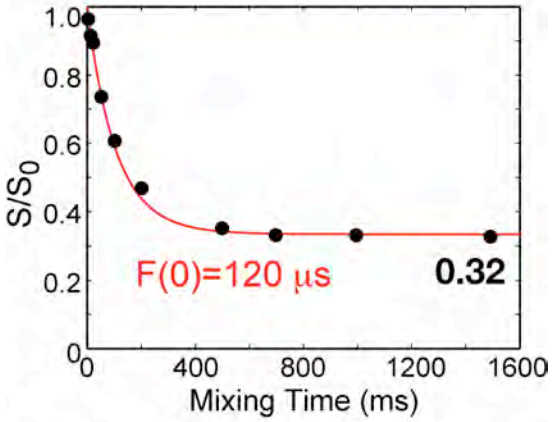
$\sum_{m,n} \omega_{i_m j_n}^2$ converges within 15 - 20 Å.

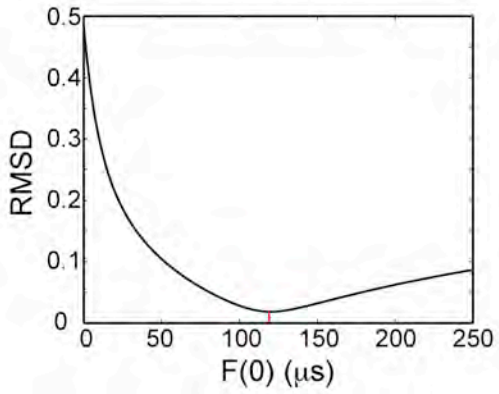
γ-Gly: $\sum_{m,n} \omega_{i_m j_n}^2 \approx 2\omega_{i_n j_n}^2$



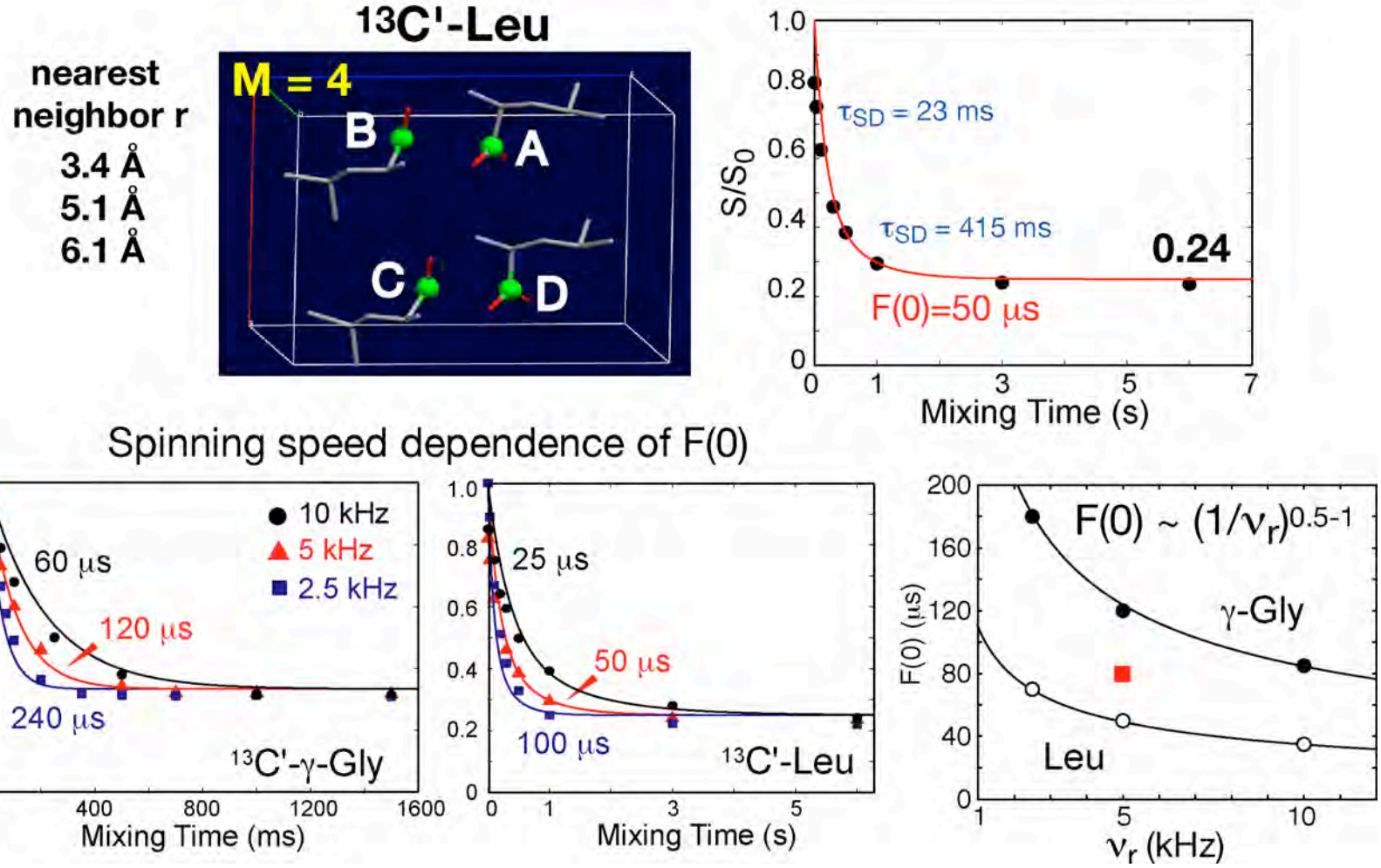
γ-Gly, 4.17 Å







F(0) of ^{13}C CODEX



- *At 5 kHz MAS, F(0) ≈ 80 μs.*
- *Faster spinning reduces F(0) –> slower spin diffusion.*
- *F(0) ~ $(1/\nu_r)^{0.5\text{-}1}$.*

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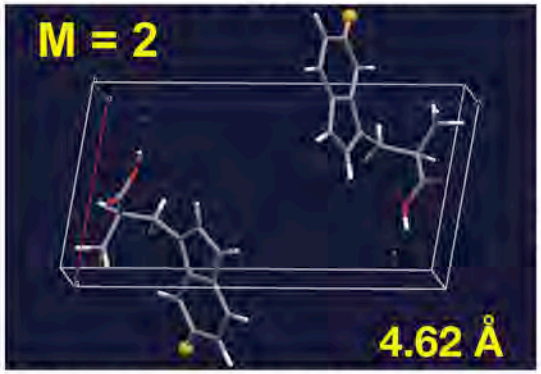
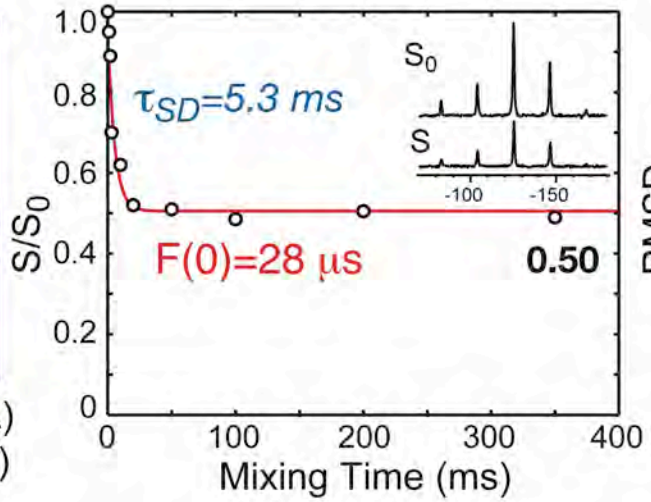
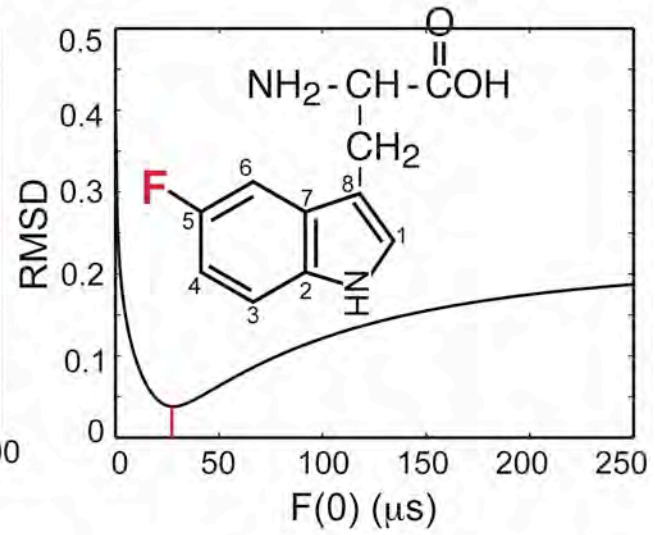
F(0) of ^{19}F CODEX

5-^{19}F-Tryptophan

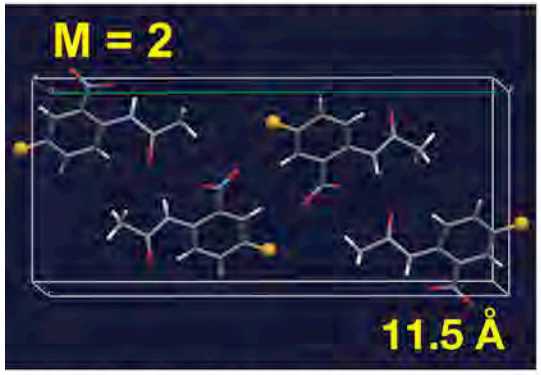


nearest neighbor: 1.1 kHz (4.6 Å)
second moment: 1.6 kHz (4.0 Å)

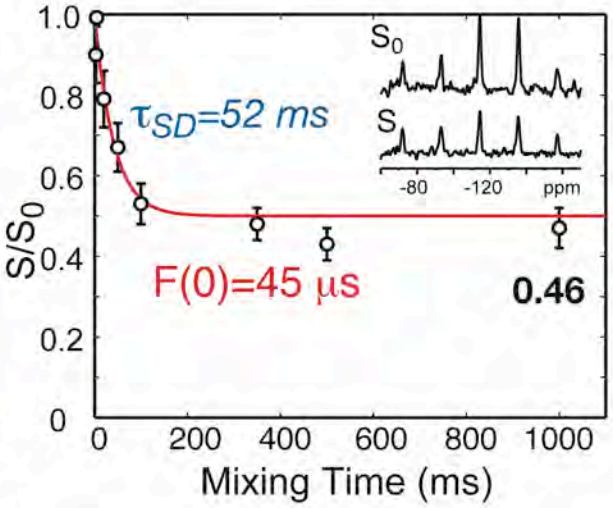


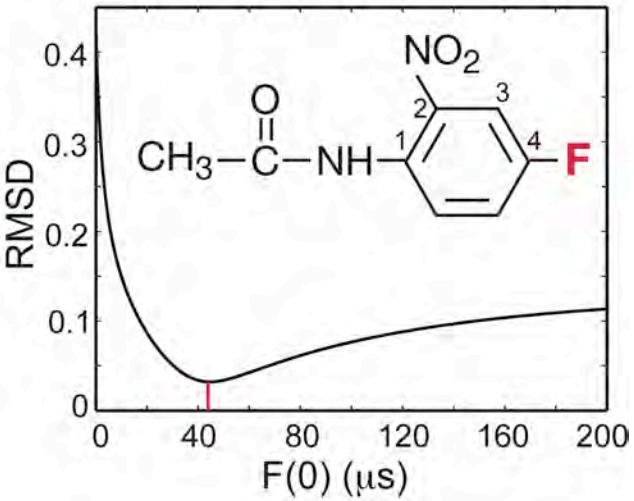


4-^{19}F-2'-nitroacetanilide



nearest neighbor: 70 Hz (11.5 Å)
second moment: 470 Hz (6.1 Å)

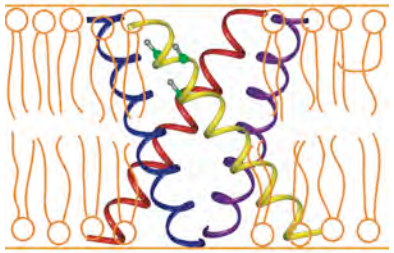


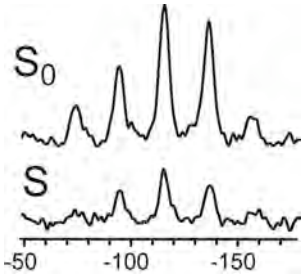


Consensus ^{19}F F(0) at 8 kHz MAS is 37 μs.

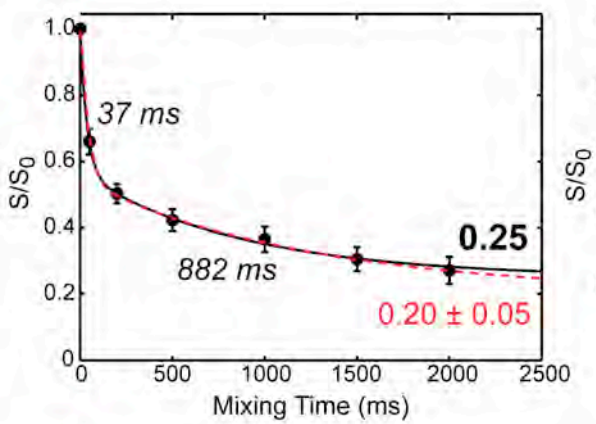
$k_{ij} = 0.5\pi \cdot \omega_{ij}^2 \cdot F_{ij}(0) \propto F_{ij}(0)/r^6$ ⇒ k is much less sensitive to F(0) than r.

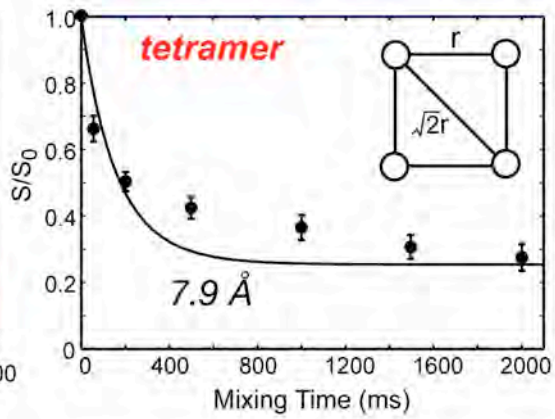
M2-TMP: a Tetrameric H^+ Channel in the Membrane

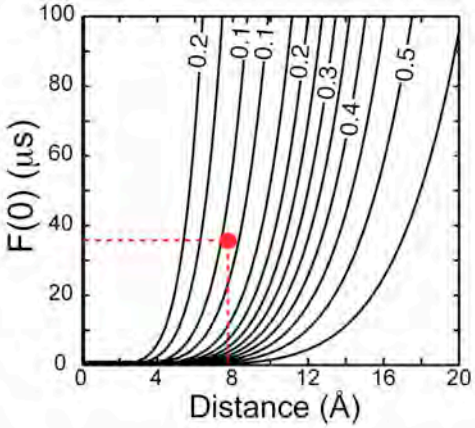


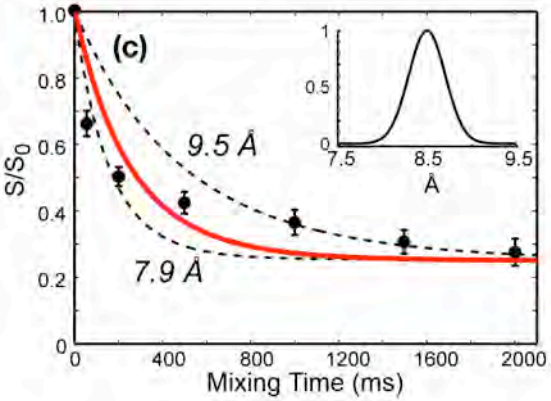


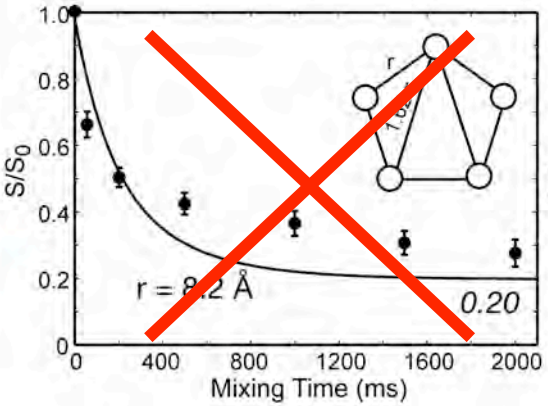
Ala30 –> [4-^{19}F] Phe30,
P:L = 1:15, DMPC bilayers,
240 K, 8 kHz MAS

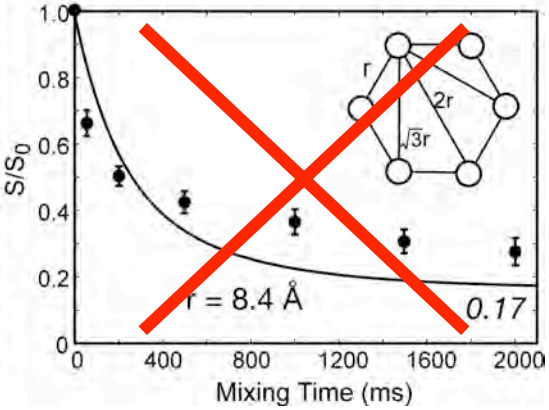












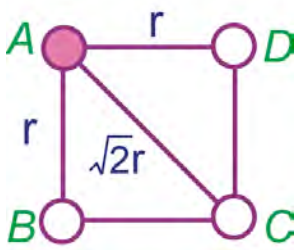
Luo & Hong, **JACS**, 128, 7242 (2006)

Other Practical Aspects of CODEX for Oligomeric Structure Determination

- Symmetric oligomers: only one unknown distance in the **K** matrix.

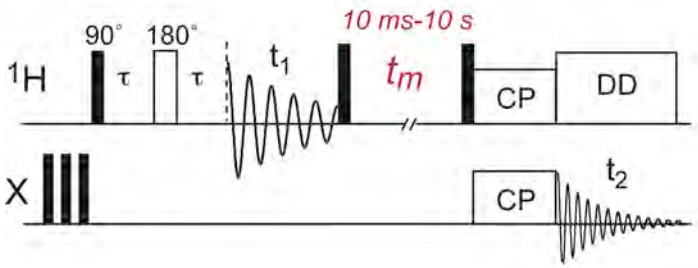
$$\text{e.g. } k_{AB} = k_{AD} = 0.5\pi F(0)\cdot\omega\left(r\right)^2,$$

$$k_{AC} = 0.5\pi F(0)\cdot\omega\left(\sqrt{2}r\right)^2 = \frac{1}{2^{3/2}}0.5\pi F(0)\cdot\omega\left(r\right)^2$$



- 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 ~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˚ orientation differences between molecules.
- Need to ensure no slow motion is present at the desired temperature.

^{1}H Spin Diffusion of Membrane Proteins

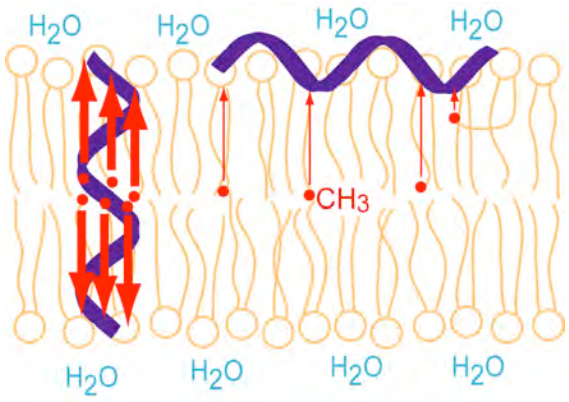


Purposes:
- Protein distance to the membrane center.
- Protein distance to the membrane surface.

- 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 –> rigid** transfer.
  - X spin detection can be ^{13}C, ^{15}N, ^{31}P, etc.

- Application modes:
  - ambient temp. (LC phase): lipid (L) –> protein (P) transfer,
    - 2τ ~ 2 ms
    - t_m ~ [10 ms, 10 s]
    - mainly 2D (can also be 1D), to resolve multiple mobile ^{1}H signals.
  - low temp. (gel phase): water (W) –> protein transfer,
    - 2τ ~ 0.2 ms
    - t_m ~ [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

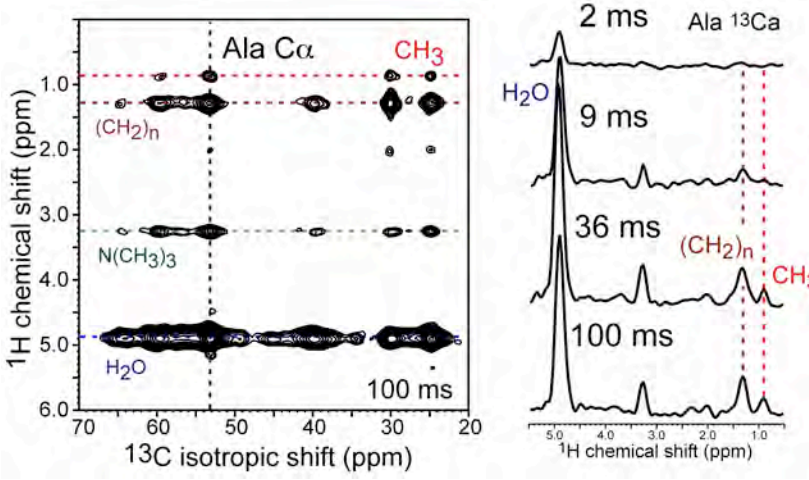


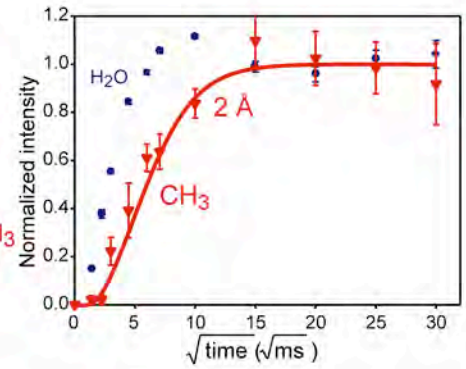
- ^{1}H spectrally resolved mobile components in a membrane sample:
  - **H**$_2$O: S ~ 0.03
  - lipid C**H**$_3$: S ~ 0.02-0.04
  - lipid (C**H**$_2$)$_n$: S ~ 0.08 - 0.20
  - lipid Hγ: S very small

- If the protein is mostly immobile, S >~ 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 –> 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 ≤1 ms (~CHHC), obliterating distance resolution for typical t_m values of ~100 ms and higher.
- Buildup curve (Intensity vs $\sqrt{t_m}$) reflects the shortest distance from the source spin to the protein –> qualitative information of protein topology.
- It doesn't matter where the ^{13}C/^{15}N label is in the protein.

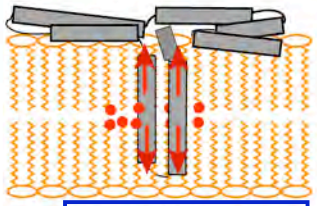
2D Data and Buildup Curves

Colicin Ia channel domain in POPC/POPG membrane





Transmembrane model



X-ray diffraction

monolayer: 23 Å

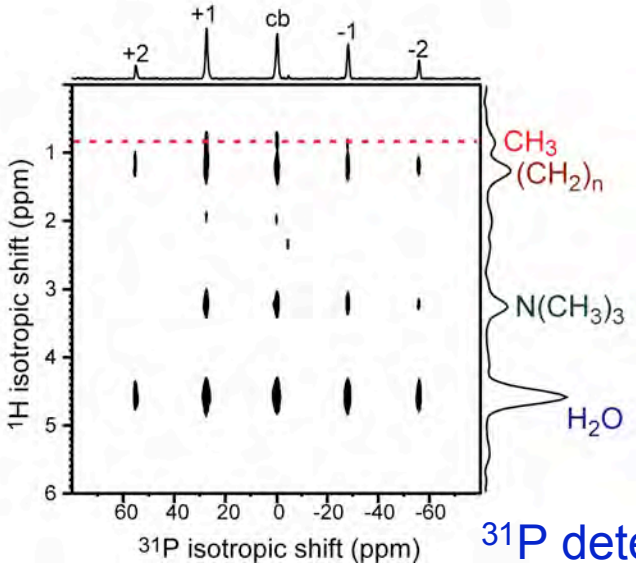
protein: an array of ~ 150 Å.

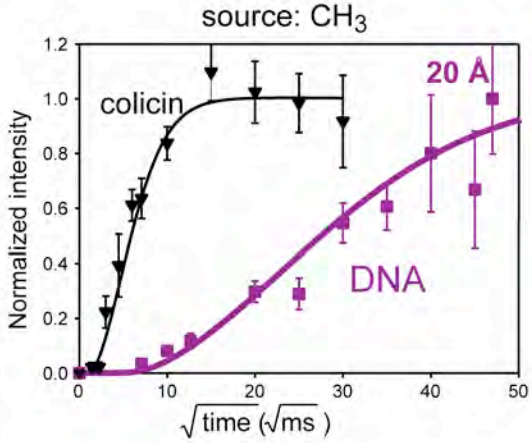
D (nm²/ms)
source: 0.012
interface: 0.0025
protein: 0.3

^{13}C detection

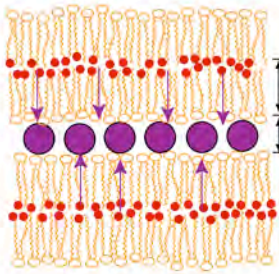
Huster et al, JACS, 124, 874 (2002)

DNA - cationic membrane mixture





Surface model



monolayer: 23 Å

DNA: 20 Å

D (nm²/ms)
source: 0.012
interface: 0.00025
DNA: 0.3

^{31}P detection

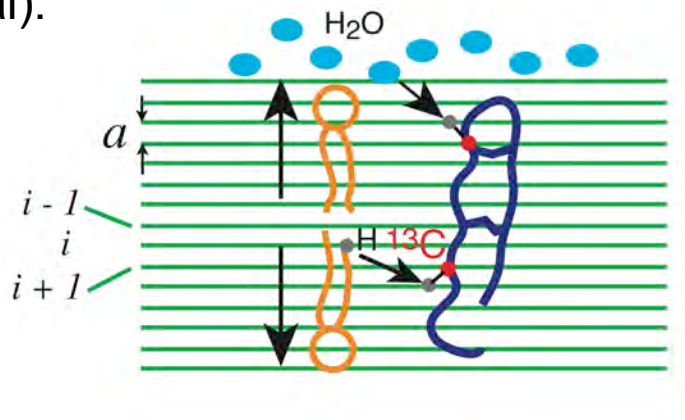
Distances from Linear-Chain Spin Diffusion Calculation

- 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)
Ω: transfer rate = D/a^2
a: lattice spacing, 2 Å or 1 Å

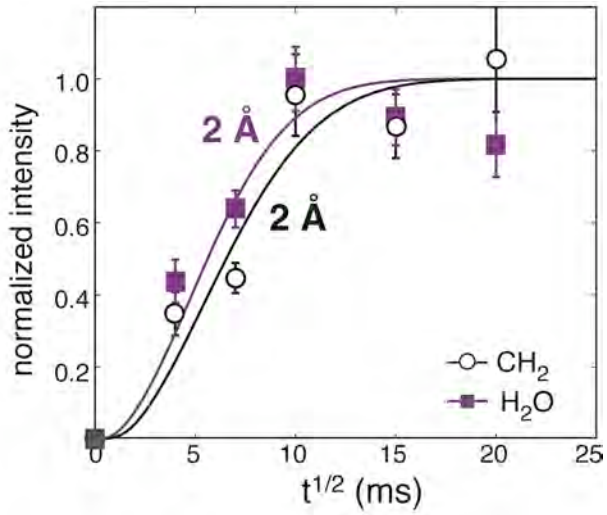


- Ω or D is related to the ^{1}H-^{1}H dipolar couplings. In rigid polymers, D≈0.8 nm^2/ms has been measured, equivalent to Ω ≈20 kHz for two protons 2.0 Å apart.
- Two lipid vicinal protons are ~2.4 Å apart (rigid-limit δ=8.8 kHz).
- Using a S ≈ 0.04 for protons close to the acyl chain termini, the motionally averaged ^{1}H-^{1}H coupling is Ω ≈ 350 Hz.
- With a spacing a of 2 Å, the resulting $D_L = \Omega\, a^2$ is ~0.014 nm^2/ms.

- For proteins with S ≈ 0.7, the ^{1}H-^{1}H coupling is Ω (2.4 Å) ~ 6.0 kHz, => D_P ≈ 0.25 nm^2/ms (a = 2 Å).
- For interfacial transfer, typical D_{int} ~ 0.002 nm^2/ms (order of magnitude).

Sample simulation:

	D (nm²/ms)	*r (Å)*
Source - lipid CH_3:	*0.012*	*4 Å*
Source - H_2O:	*0.03*	*2 Å*
Gap:	*0.012*	*x Å*
Interface:	*0.00125*	*2 Å*
Sink - peptide	*0.3*	*30 Å*



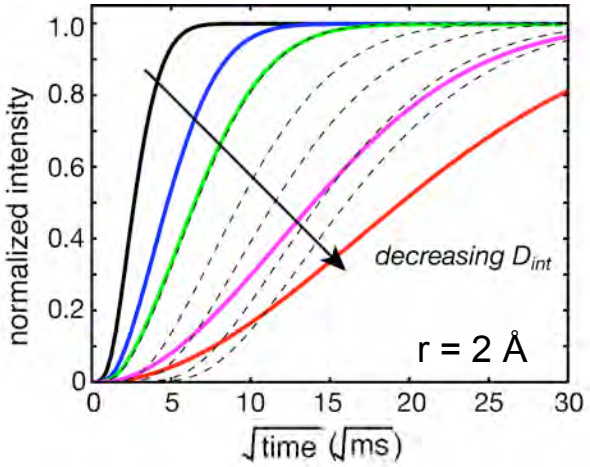
Effects of D_{int} and Distance on the Buildup Curves

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

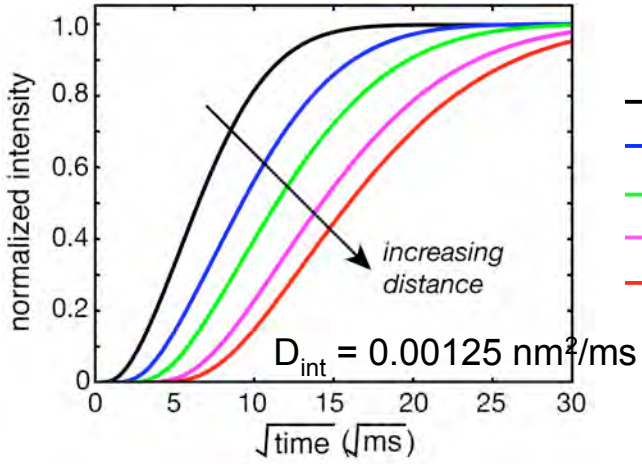
D_L = 0.0125 nm²/ms
D_p = 0.3 nm²/ms

D_{int} (nm²/ms)
- 0.0125
- 0.0025
- 0.00125
- 0.00025
- 0.000125

Varying D_{int}



Varying r



r
- 2 Å
- 6 Å
- 10 Å
- 16 Å
- 20 Å

- D_{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_{int}~0.0025 nm²/ms, lipid-DNA have D_{int}~0.00025 nm²/ms (low ^{1}H density in DNA), and cholesterol-containing membranes also give D_{int} ~ 0.00025 nm²/ms.

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, $M(x,t) = e^{-(x-x_0)^2/4Dt} \Big/ \sqrt{\pi Dt}$.

- A domain source $M_{dom}(x,t)$ is a superposition of many point sources:

$$M_{dom}(x,0) = \int_{-\infty}^{0} \delta(x - x_0)\, dx_0$$

- $M_{dom}(x,t)$ evolves as an error function centered at the source-sink interface:

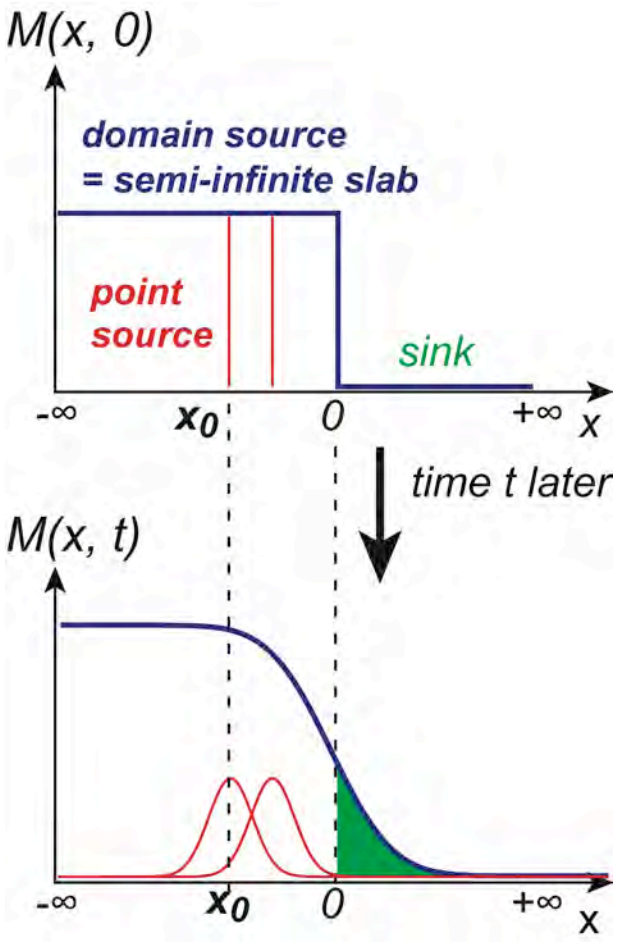
$$M_{dom}(x,t) = \int_{-\infty}^{0} \frac{e^{-(x-x_0)^2/4Dt}}{\sqrt{\pi Dt}} dx_0 \xrightarrow{x' = \frac{x-x_0}{\sqrt{4Dt}}} \begin{pmatrix} x_0 = -\infty, x' = +\infty \\ x_0 = 0, x' = x/\sqrt{4Dt} \\ dx' = -dx_0/\sqrt{4Dt} \end{pmatrix}$$

$$= \frac{-\sqrt{4Dt}}{\sqrt{\pi Dt}} \cdot \int_{+\infty}^{x/\sqrt{4Dt}} e^{-x'^2} dx' = \frac{2}{\sqrt{\pi}} \cdot \int_{x/\sqrt{4Dt}}^{+\infty} e^{-x'^2} dx' = \boxed{\operatorname{erfc}\left(\frac{x}{\sqrt{4Dt}}\right)}$$

- The total magn $I_{sink}(t)$ of the sink increases as $t^{1/2}$:

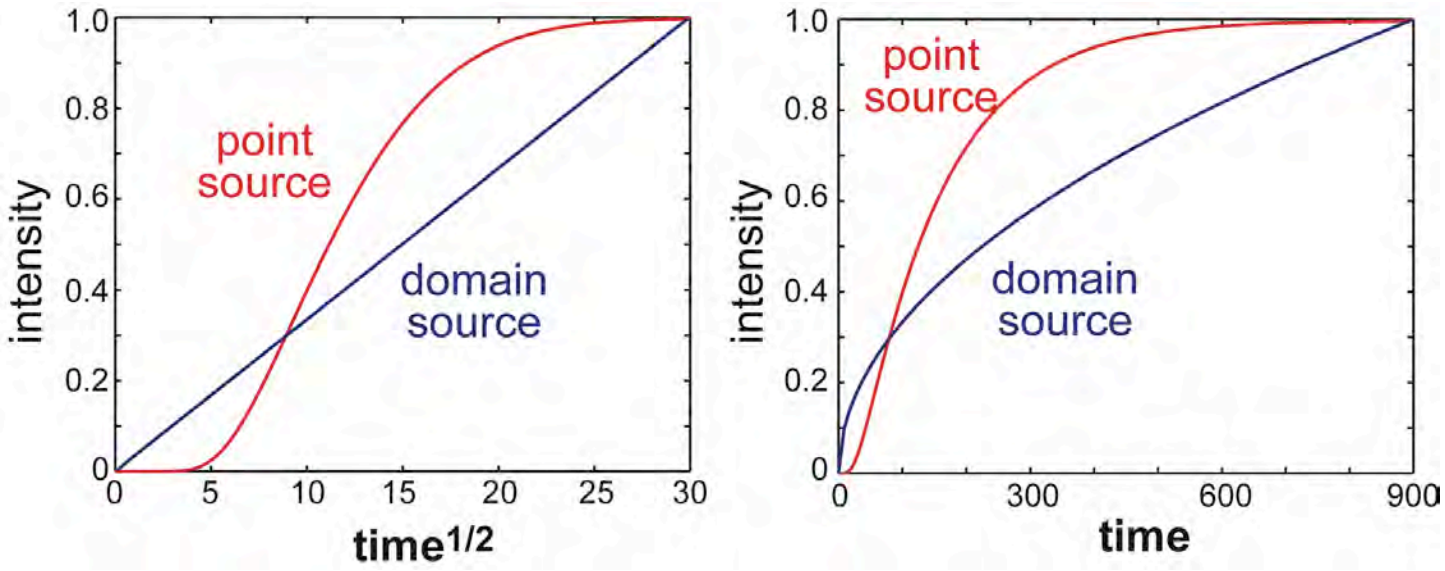
$$I_{sink}(t) \propto \int_{0}^{+\infty} M_{dom}(x,t)dx = \int_{0}^{+\infty} \operatorname{erfc}\left(\frac{x}{\sqrt{4Dt}}\right) dx \xrightarrow{x'' = \frac{x}{\sqrt{4Dt}}}$$

$$\begin{pmatrix} x = 0, x'' = 0 \\ x = +\infty, x'' = +\infty \\ dx'' = dx/\sqrt{4Dt} \end{pmatrix} = \sqrt{4Dt} \int_{0}^{+\infty} \operatorname{erfc}(x'')dx'' = \sqrt{4Dt}\, \frac{1}{\sqrt{\pi}} = \sqrt{\frac{4D}{\pi}} \cdot \boxed{\sqrt{t}}$$



Buildup Curves Plotted with Time$^{1/2}$ vs Time

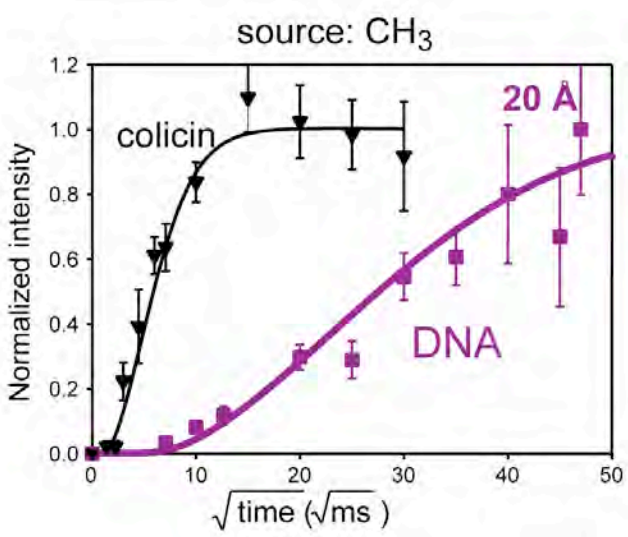
- Thus, for domain spin diffusion, the I(t$^{1/2}$) plot is linear.
- For point-source spin diffusion, there is a latency period (M≈0) whose duration depends on the distance from the point source.
- Plotting I(t$^{1/2}$) stretches out the initial period compared to I(t), thus better distinguishing different distances.

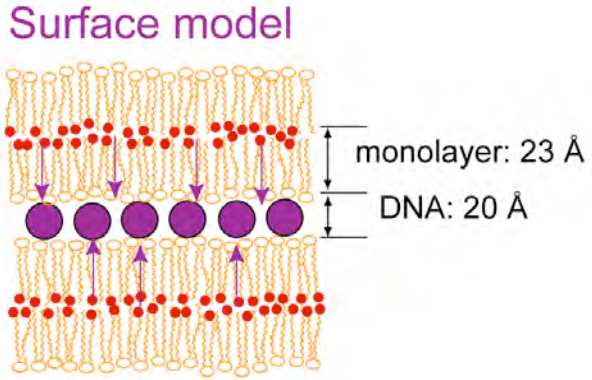


Buildup Curves of Non-Transmembrane Systems

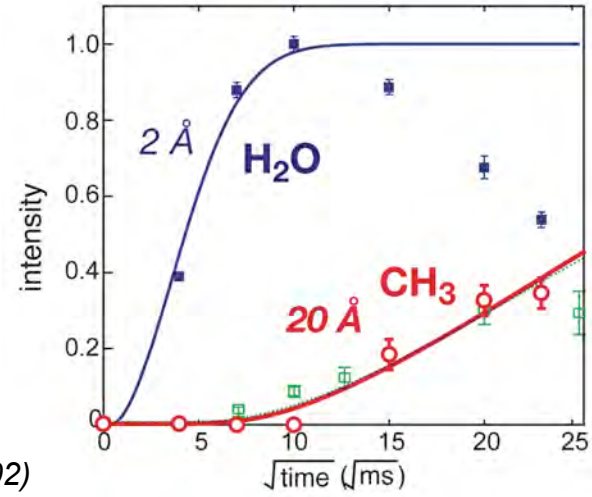
• In membrane systems, spin diffusion is usually from point sources, giving a lag period in the $I_{sink}(t^{1/2})$ plot. This is especially clear in non-TM macromolecules.

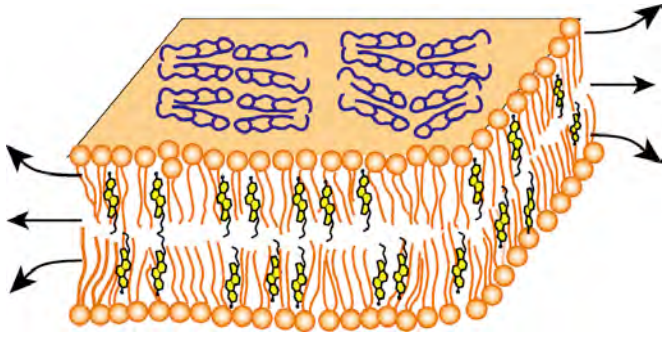
DNA - cationic membrane





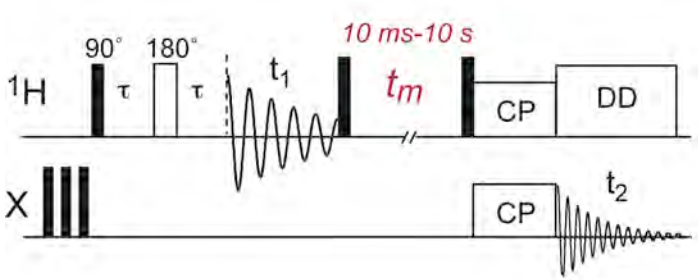
POPC/cholesterol membrane with PG-1





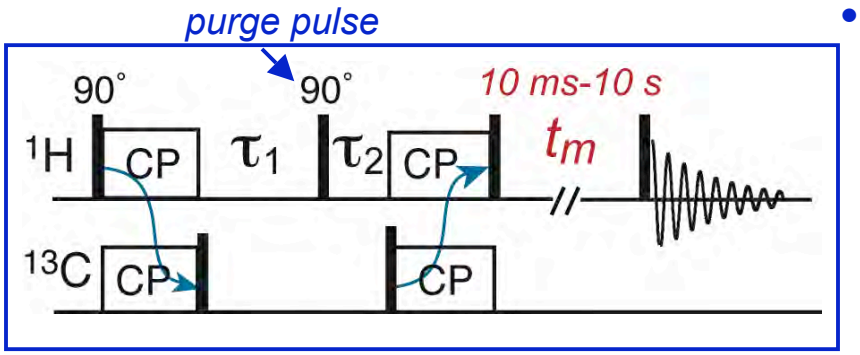
Huster et al, JACS, 124, 874 (2002)
Mani et al, PNAS, 103, 16242 (2006)

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.



τ_1~10 ms, τ_2 ~ 5 ms.

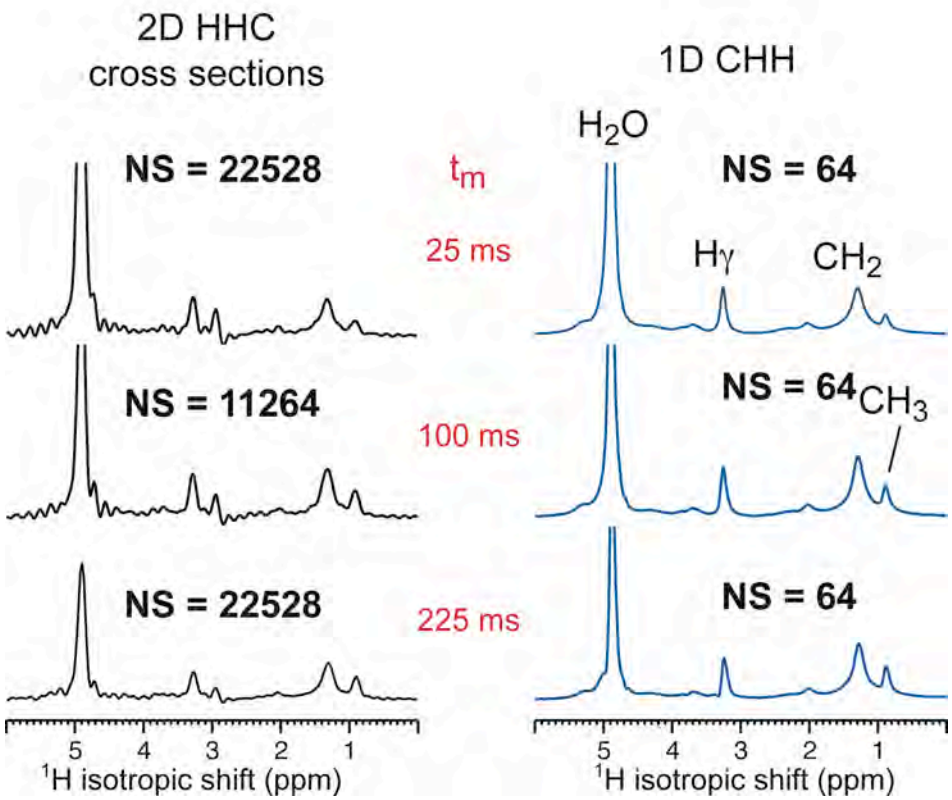
- Alternative, 1D CHH:
  - Invert the ^{1}H and ^{13}C dimensions.
  - Remove ^{13}C t_1 altogether since no distance resolution!
  - Sensitivity gain due to ^{1}H detection.
  - ^{1}H detection requires no homonuclear decoupling.

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

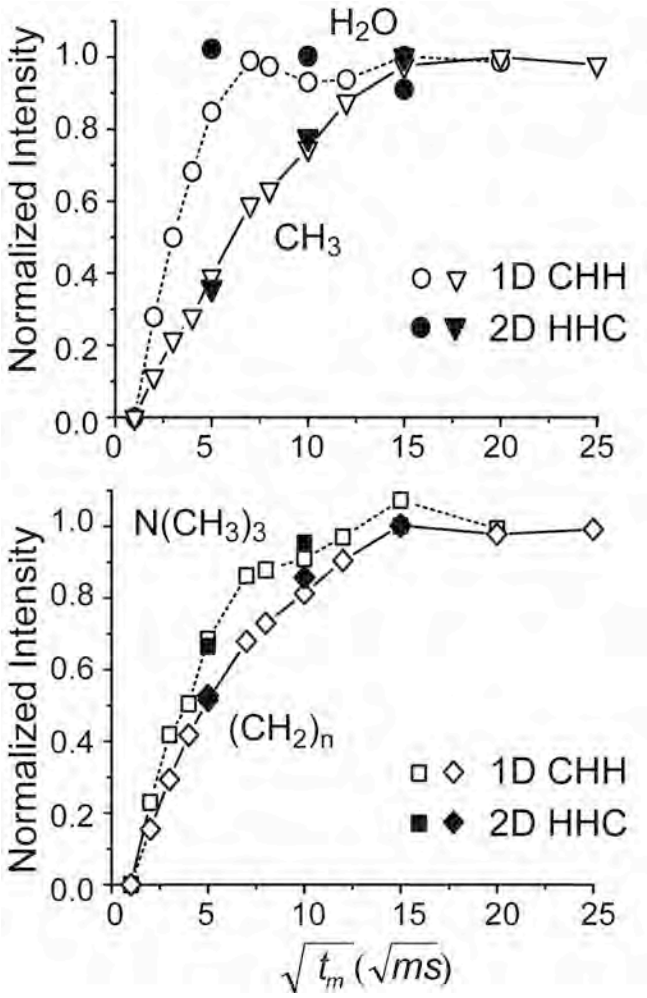
- Two obstacles of 1D CHH:
  - Suppressing large equilibrium ^{1}H magnetization of lipids & water.
  - Sensitivity gain limited by the fraction of labeled ^{13}C sites versus natural abundance lipid ^{13}C.

1D CHH Protein-Lipid Spin Diffusion

TEASE U-^{13}C, ^{15}N-labeled colicin Ia 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≈1:3:2 and a ^{13}C labeling level of ~50%, the calculated fraction of detected protons is ~2.5%. This gave reproducible and correct CHH buildup curves.
- The experiment needs to suppress ~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 >~15% for CHH to work.

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Prof. Wonhwa Cho (UIC)
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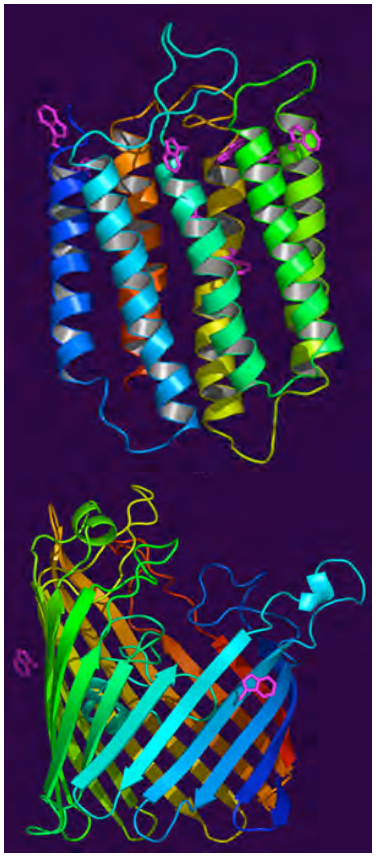
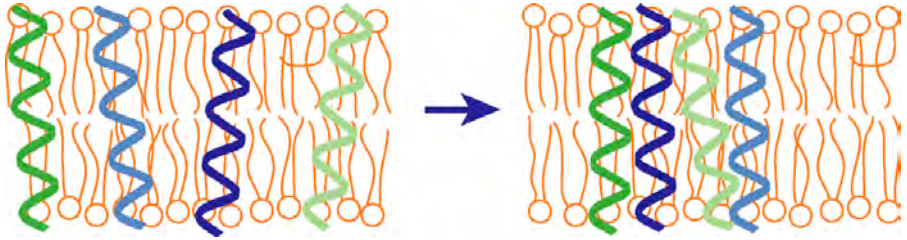
^{19}F Spin Diffusion for Determining Intermolecular Distances in Oligomeric Membrane Proteins

Mei Hong, Iowa State University



Membrane protein structural features:

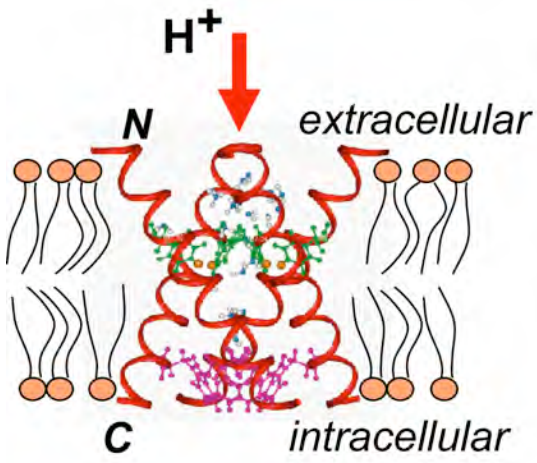
- Orientation.
- Depth of Insertion.
- Sidechain conformation.
- Assembly of polypeptide chains: quaternary structure.

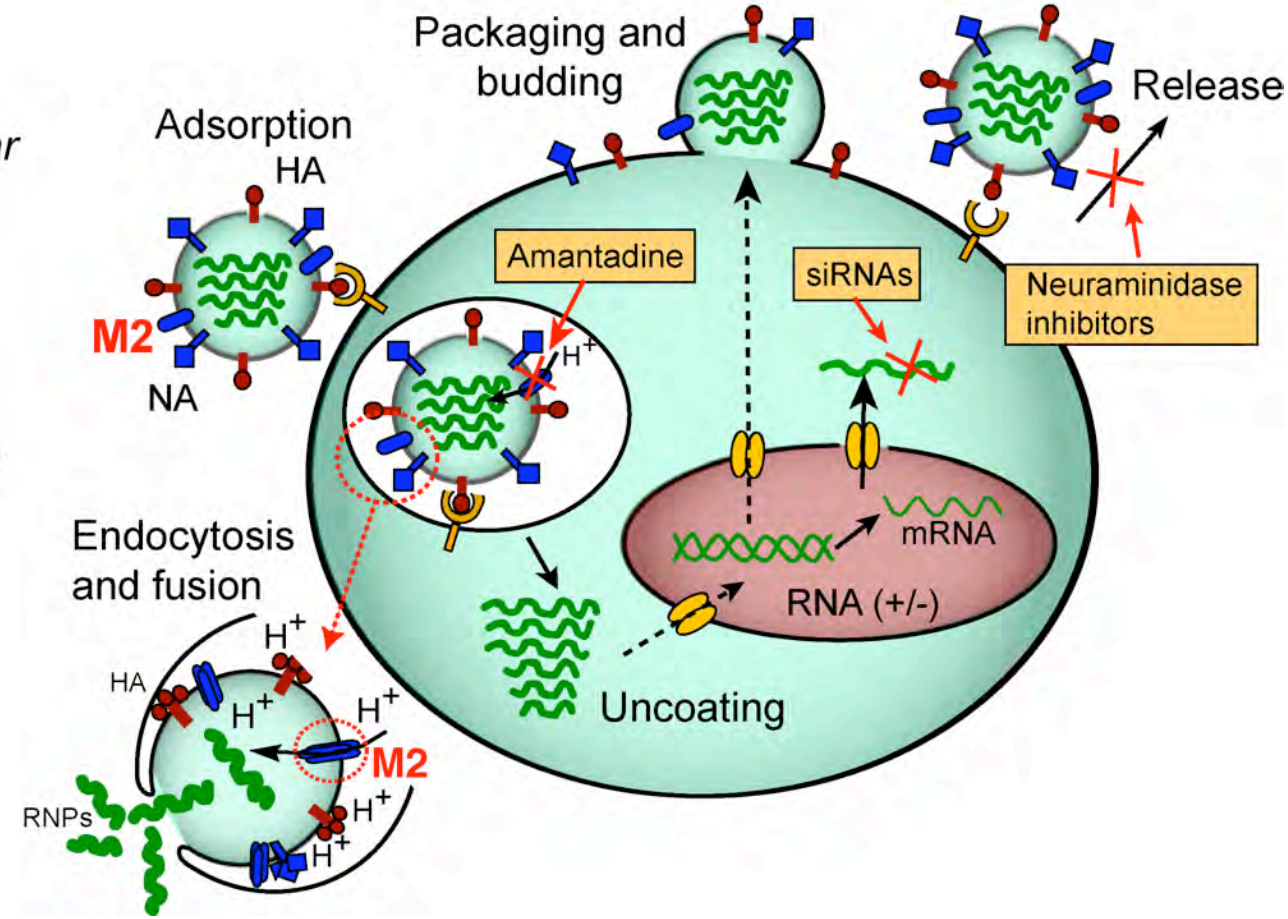


Oligomeric structure of membrane proteins:

- Oligomeric number
- Intermolecular distance constraints.

M2 Protein: a Proton Channel of Influenza A Virus

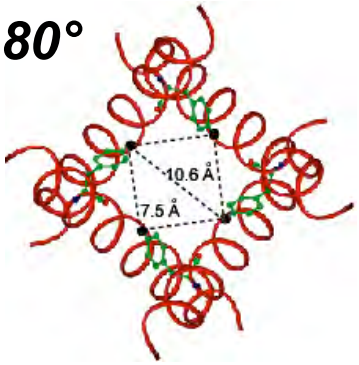




- ***A H^+ channel, open at pH < 7 and closed at pH > 7.***
- ***Forms tetrameric bundles in micelles.***
- ***Oligomeric state in the lipid bilayer unknown. Only one short interhelical distance reported (Cross et al.).***

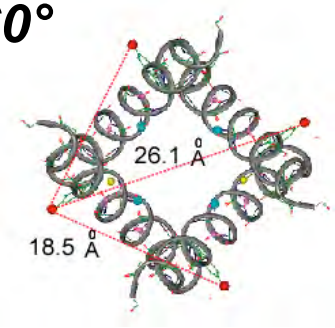
F-F Distance Confirms the Tetramer Model

χ_1= 180°



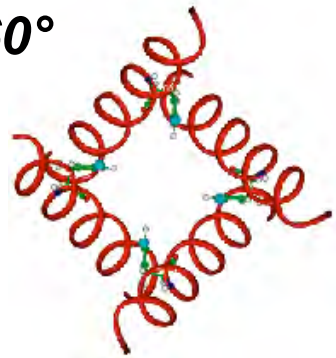
Most probable rotamer:
*r = 7.5 Å, **F(0)=28 μs***

χ_1= -60°



r = 18.5 Å,
F(0)=2000 μs

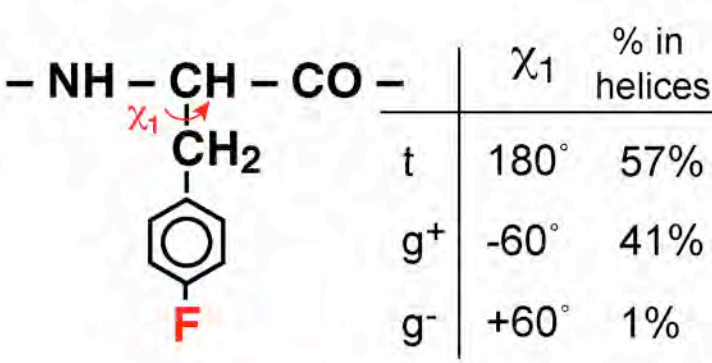
χ_1=60°



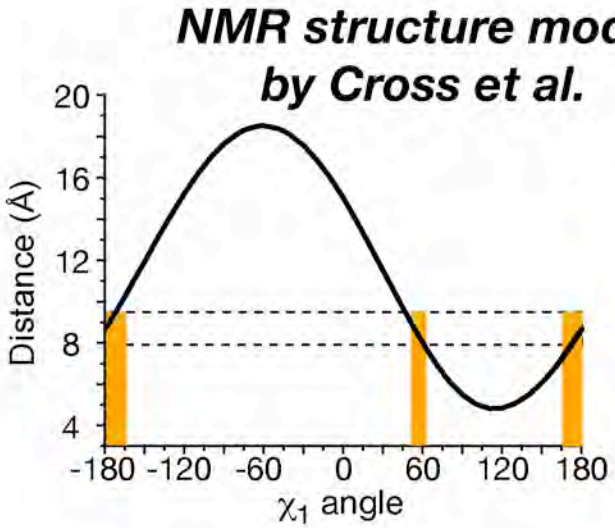
Least probable rotamer:
ring clashes with backbone

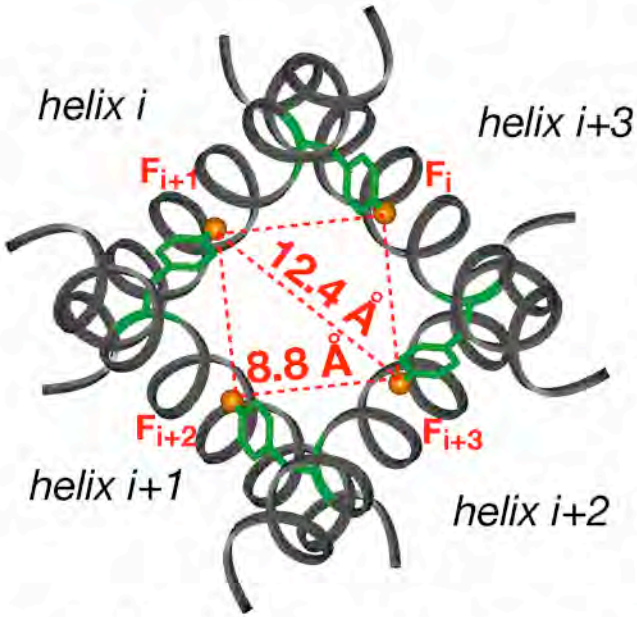
The interhelical distance of 7.9 - 9.5 Å for Phe30 agrees well with the M2 tetramer model obtained from ^{15}N orientation data (Cross et al).

F-F Distance Confirms Existing Tetramer Model



	χ_1	% in helices	r_{FF}
t	180°	57%	8.8 Å
g^+	-60°	41%	19 Å
g^-	+60°	1%	8.7 Å

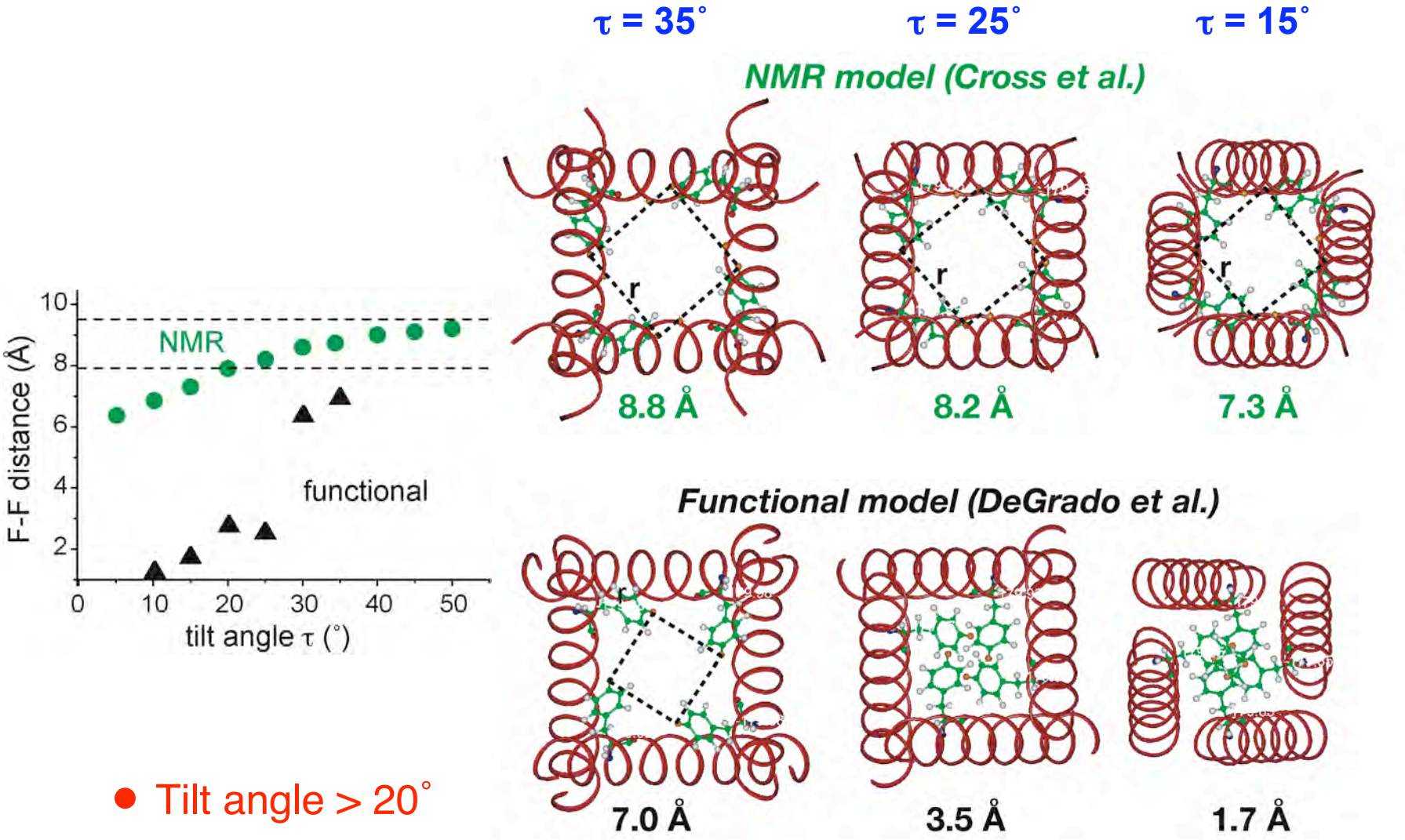




- +60° rotamer: forbidden by steric clash with the backbone
- Only the trans rotamer is possible.

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 > 20°
- Functional model may have un-optimized rotation angles.