Solid-State NMR Studies of Molecular Dynamics and Protein Hydration

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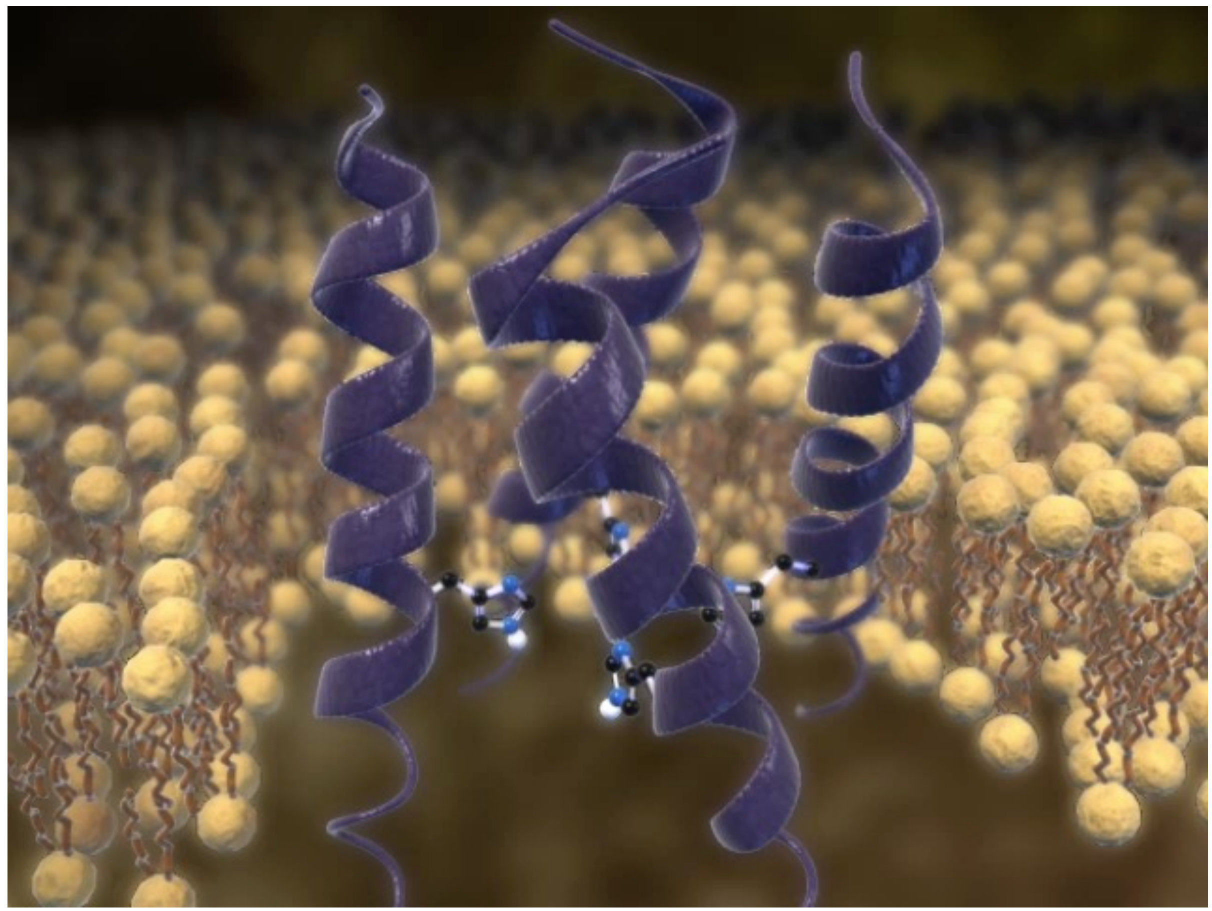
Motions are Abundant in Biomolecules

Histidine ring motion in the influenza M2 proton channel



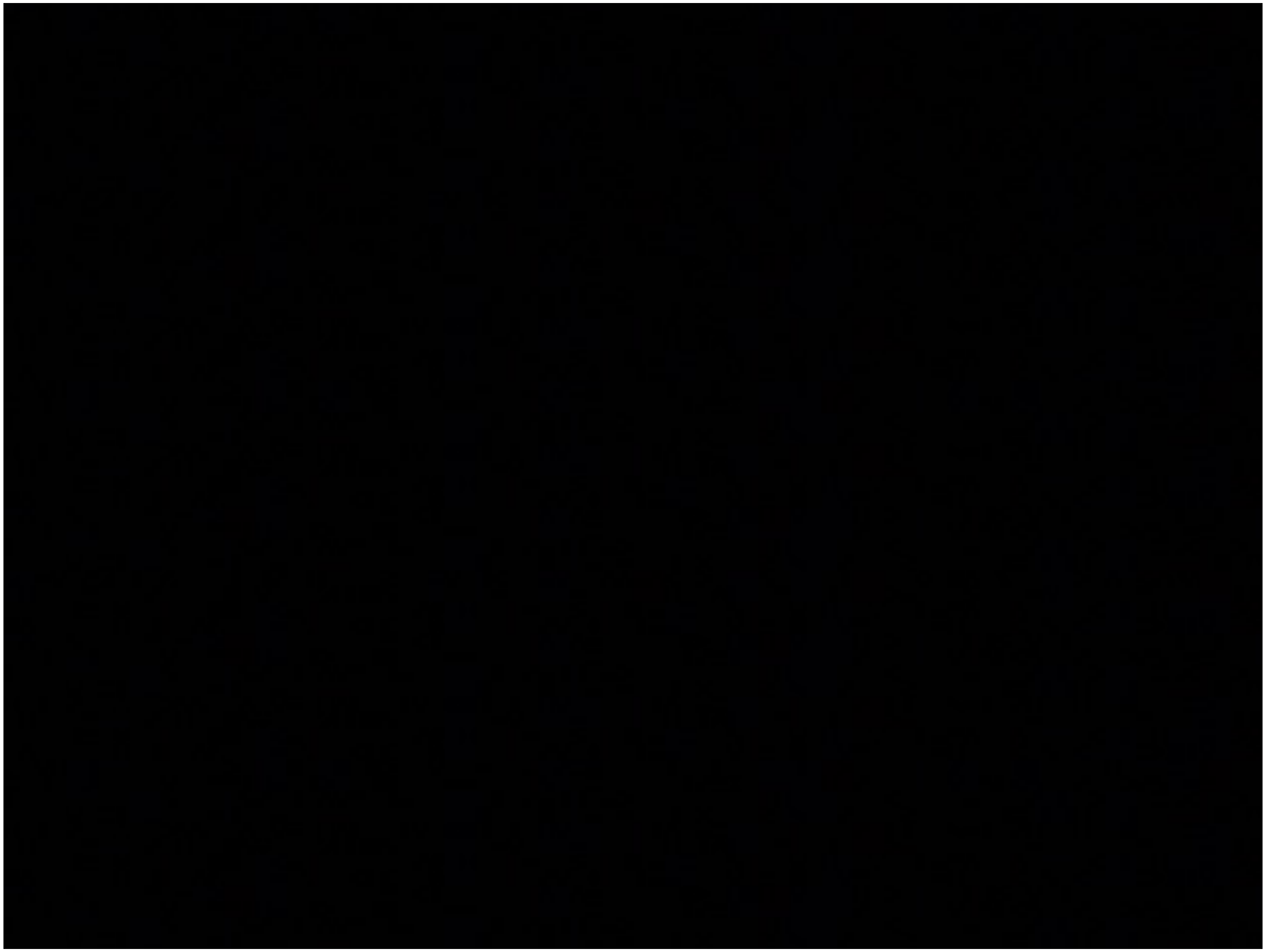
Protein motions enable:

- *Ion conduction*
- *Substrate transport*
- *Ligand binding*
- *Catalysis*

….

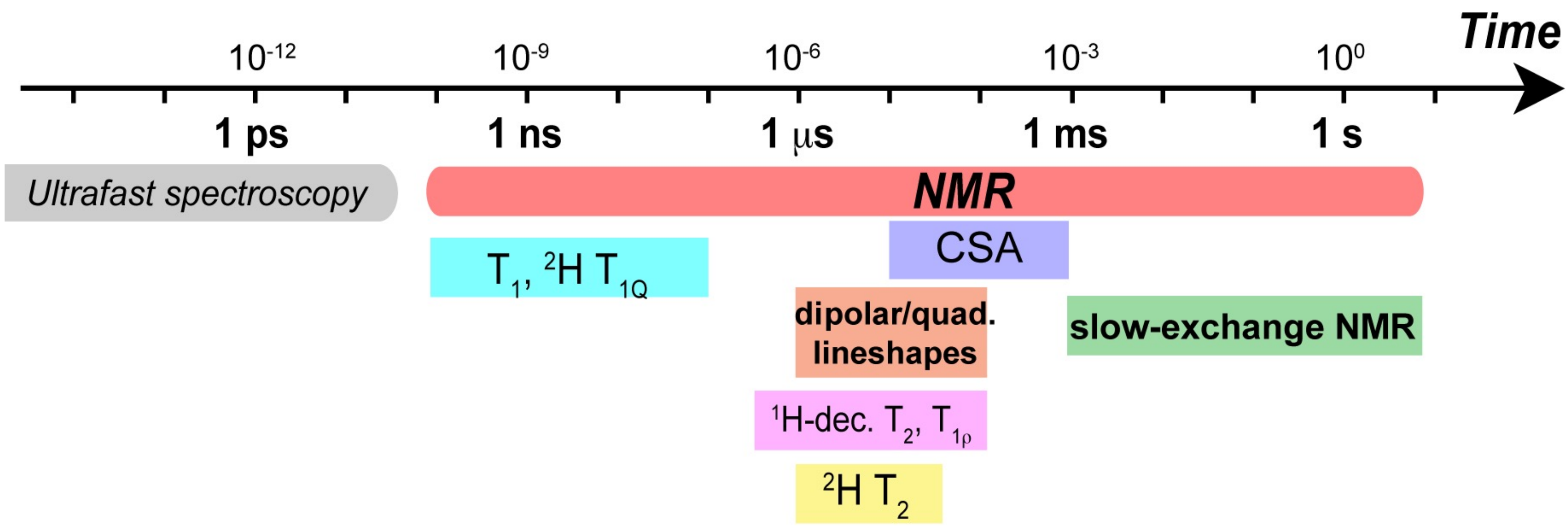
Hu et al, ***Science***, 2010.

Amantadine motion in the binding pocket of the M2 proton channel



Cady et al, ***Nature***, 2010.

Motional Timescales that are Accessible to NMR



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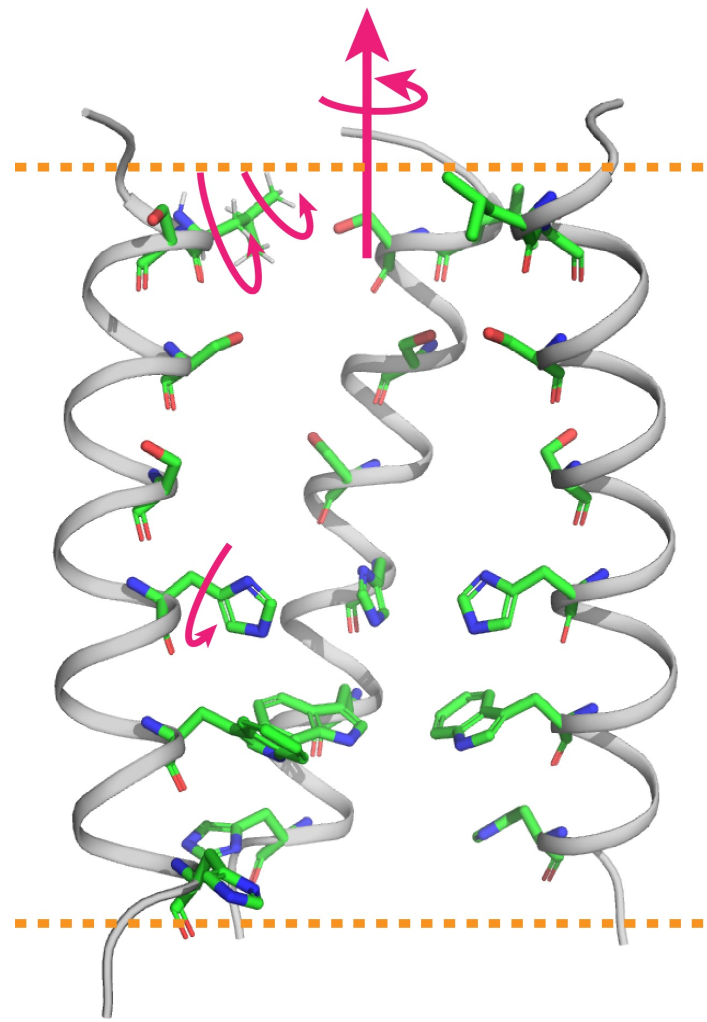
Common Protein Motions

Internal motions

- Methyl & amine three-site jumps
- Sidechain rotameric jumps (e.g. Leu mt - tp)
- *trans-gauche* isomerization (e.g. Lys, Arg)
- Aromatic ring flip
- Torsional fluctuation
- Loop & termini motions



Global motions

- Uniaxial diffusion of membrane proteins in lipid bilayers
- Correlated motions of protein domains

Effects of Molecular Motion on NMR Spectra

Molecular motions can:

- average NMR lineshapes;
- enhance or reduce peak intensities;
- speed up relaxation;
- complicate spectral quantification;
- allow spectral editing

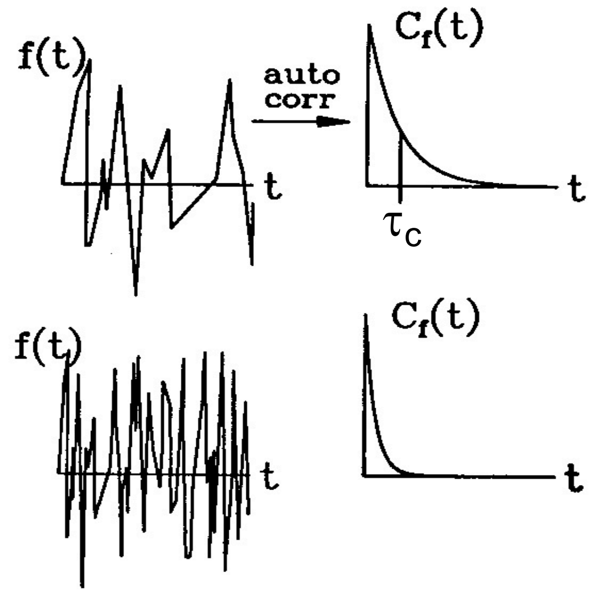
Outline

- *Timescales & amplitudes of motion from NMR*
- *Fast motion: average (sum) tensor*
- *Experiments for measuring fast motion*
- *Slow motion: difference tensor*
- *Experiments for measuring slow motion*

Rates & Amplitudes of Reorientations

- 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. C(t) decays with a characteristic time τ_c;

- ***Rates***: k (s^{-1}) is inversely related to correlation time τ_c.



- ***Amplitudes***: describes the reorientational angle β_R & the number of sites n_R.
- We do not consider translational motion, which can be studied by pulsed-field-gradient NMR.
- Diffusive motion: infinitesimal β_R, infinite n_R. e.g. isotropic tumbling, uniaxial diffusion, torsional fluctuations.
- Discrete motion: finite β_R, finite n_R;
  - Methyl 3-site jump: $\beta_R = 109.5^\circ$, $n_R = 3$ for C-H bonds
  - Phenylene ring flip: $\beta_R = 120^\circ$, $n_R = 2$ for ortho and meta C-H bonds

Motional Regimes in NMR

- ***Fast motion***: ***$k >> \Delta\omega$*** or **δ**, typically $\tau_c < 1$ µs
  - *Amplitudes*: obtained from spectral line narrowing.
    - e.g. ^{2}H spectra, DIPSHIFT, LG-CP, WISE, CSA narrowing.
  - *Rates*: > 10 x δ; Exact rates measured by relaxation NMR.

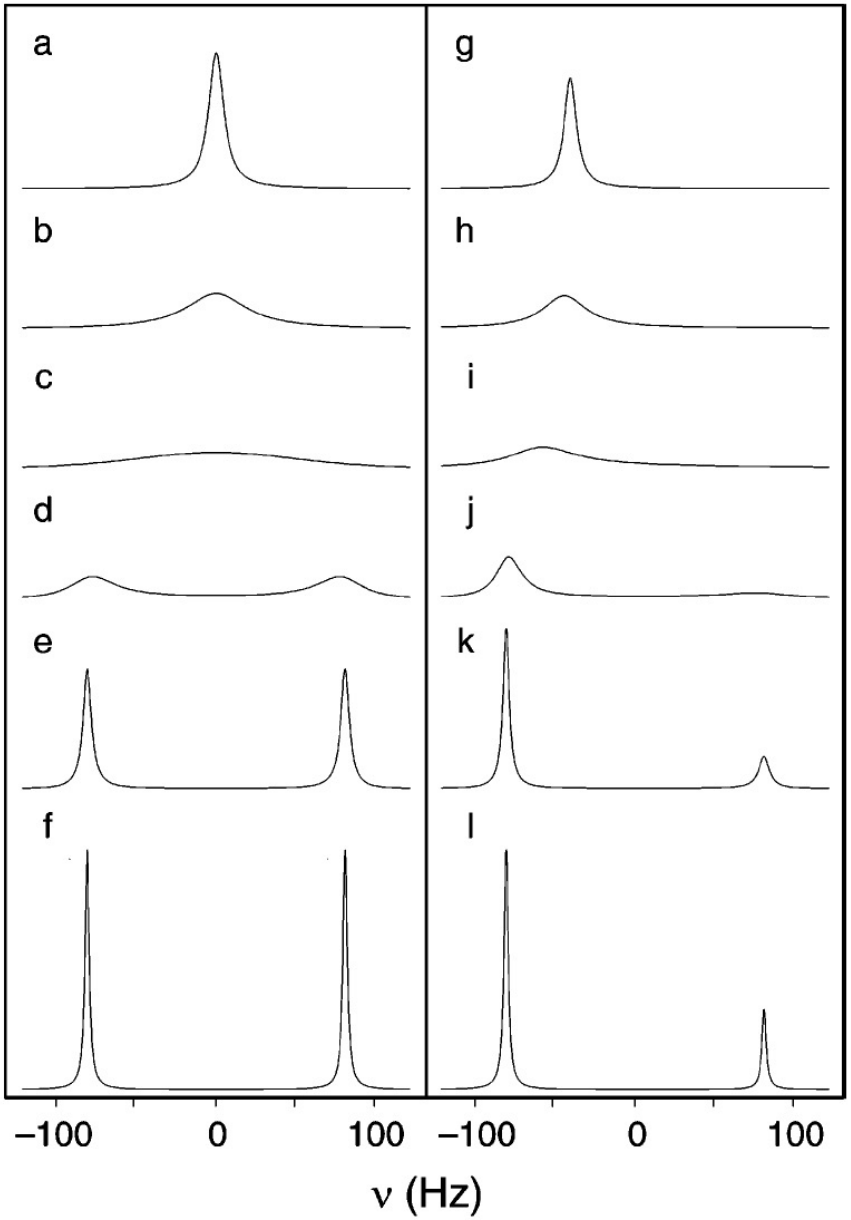
- ***Slow motion*: $k << \Delta\omega$,** typically $\tau_c > 10$ ms
  - *Amplitudes*: from cross peaks in 2D exchange spectra or from Nt_r-dependent CODEX intensities.
  - *Rates*: decay constant in mixing-time dependent intensities.
  - n_R: from the final value of the CODEX mixing-time curve.

- ***Intermediate motion***: $k \sim \Delta\omega$.
  - Causes loss of spectral intensity due to interference with ^{1}H decoupling & MAS.
  - *Rates*: from T_2 and $T_{1\rho}$ minima in $\log(T_{2,1\rho})$ plots vs 1/T.
  - *Amplitudes*: from asymmetric DIPSHIFT intensity decays

Effects of Motion on NMR Spectra

equal population
($p_A = p_B = 0.5$)

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



Palmer, Chem. Rev. 2004.

Fast motion: $k >> |\omega_A - \omega_B|$

Average frequencies $\overline{\omega}$

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

Slow motion: $k << |\omega_A - \omega_B|$

Measured during a mixing time.

Fast Motion: Averaging of NMR Frequencies

For a nuclear spin interaction tensor σ :

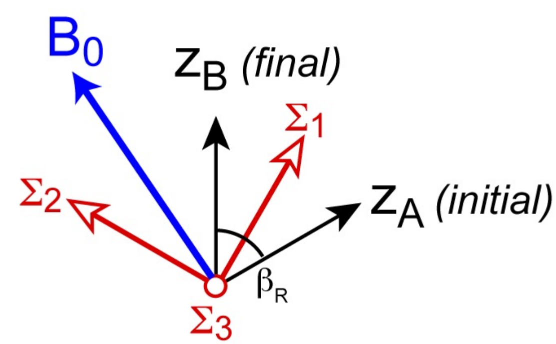
$$\omega(\theta,\phi) = \tfrac{1}{2}\delta\left(3\cos^2\theta - 1 - \eta\sin^2\theta\cos 2\phi\right)$$

Reorientation among N sites with probability p_j yields an average tensor:

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

- Σ has 3 principal axes (Σ_1, Σ_2, Σ_3).
- Σ is characterized by $\overline{\delta}$, $\overline{\eta}$, which reflect the geometry of motion.
- The orientation of B_0 in the Σ frame: (θ_a, ϕ_a).

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



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 $\overline{\eta}$?

Averaged Anisotropy & Asymmetry for Some Motional Geometries

- Isotropic motion ⇒ $\bar{\delta} = 0$

- Uniaxial rotation
- N ≥ 3 C_N jumps

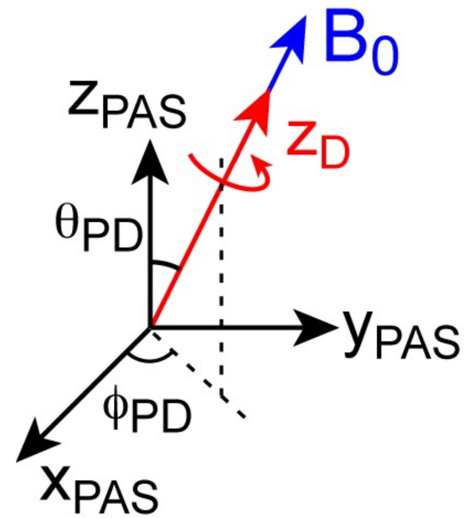
} ⇒ $\bar{\eta} = 0$

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

- The principal values δ_{ii} are the frequencies when B_0 is parallel to the principal axes.

- $\bar{\delta}$ is the frequency when B_0 is parallel to the z-axis of the $\mathbf{\Sigma}$ tensor.

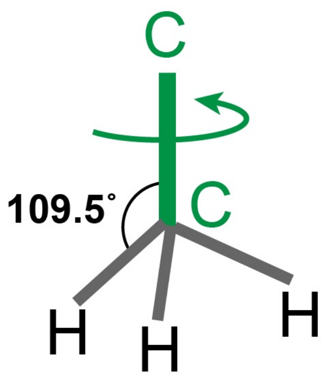
- Under this condition, motion does not change the PAS orientation relative to B_0, so the frequency depends on the fixed angles (θ_{PD}, ϕ_{PD}):



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

Fast Methyl Three-Site Jumps

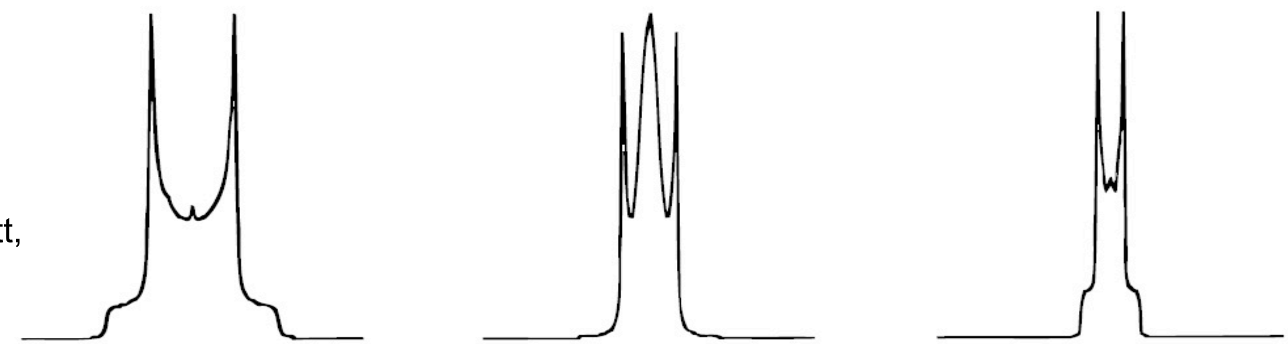
$$\bar{\delta} = \omega(\theta_{PD}, \phi_{PD}) = \tfrac{1}{2}\delta\left(3\cos^2\theta_{PD} - 1 - \eta\sin^2\theta_{PD}\cos 2\phi_{PD}\right)$$



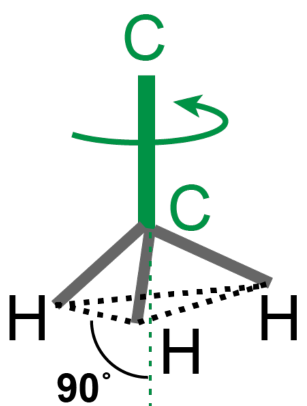
- **^{13}C-^{1}H dipolar coupling or ^{2}H quadrupolar coupling**

$$\eta = 0,\ \theta_{PD} = 109.5^\circ \Rightarrow \bar{\delta} = \tfrac{1}{2}\delta\left(3\cos^2 109.5^\circ - 1\right) = -\boldsymbol{\delta}/3, \quad \bar{\eta} = 0$$

Palmer, Williams, and McDermott, ***J. Phys. Chem.***, 100, 13293 (1996).



k	$10^4\ s^{-1}$	$10^6\ s^{-1}$	$10^8\ s^{-1}$
	slow	intermediate	fast



- **^{1}H-^{1}H dipolar coupling**

$$\eta = 0,\ \theta_{PD} = 90^\circ \Rightarrow \bar{\delta} = \tfrac{1}{2}\delta\left(3\cos^2 90^\circ - 1\right) = -\boldsymbol{\delta}/2, \quad \bar{\eta} = 0$$

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

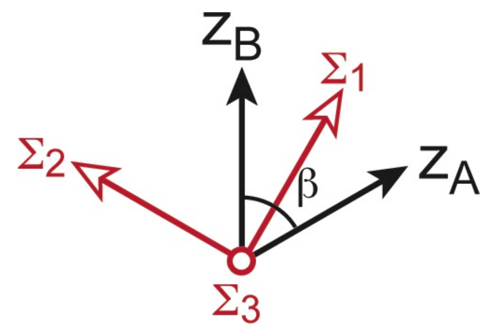
Average Tensor for Two-Site Jumps

2-site jumps averaging a uniaxial (η = 0) interaction: **calculate the frequency when B_0 is parallel to the 3 principal axes of the Σ tensor.**

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

By symmetry, the 3 principal axes should be:

Σ_1: Bisector of the AOB angle
Σ_2: Normal to the bisector in the AOB plane
Σ_3: Normal to the AOB plane



	σ_A	σ_B
Σ_1 axis:	$\beta/2$,	$\beta/2$
Σ_2 axis:	$90^\circ+\beta/2$,	$90^\circ-\beta/2$
Σ_3 axis:	90°,	90°

- 1, 2, 3 convention: left to right, i.e. $\overline{\omega}_1 > \overline{\omega}_2 > \overline{\omega}_3$
- $\beta < 90^\circ$ and $\beta > 90^\circ$ switch Σ_1 & Σ_2 axes.

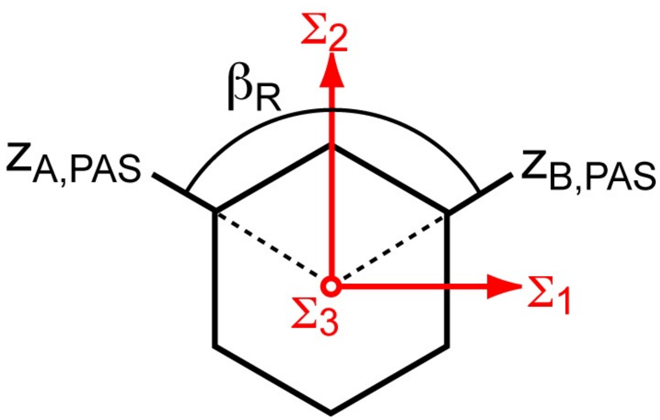
The principal values of the average tensor:

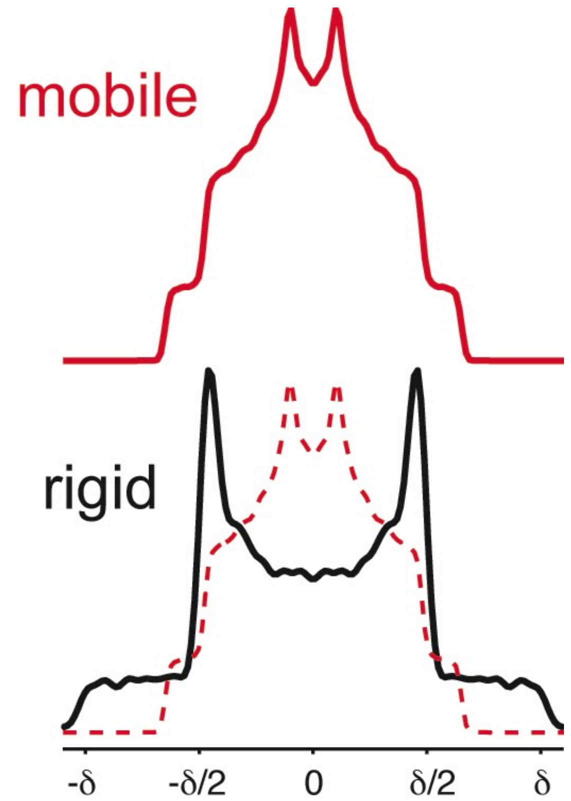
$$\overline{\omega}_n = \frac{1}{2}\delta\left(3\cos^2\Theta_n - 1\right)$$

Θ_n: angle between z_{PAS} and Σ_n

Two-Site Jumps: Phenylene Ring Flip

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





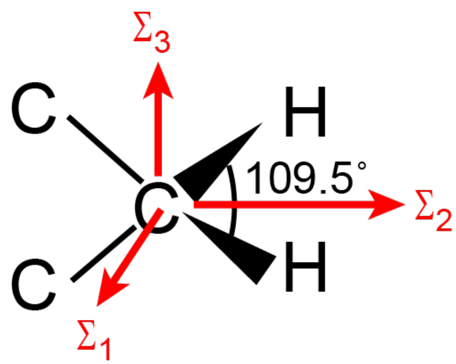
$$\overline{\omega}_n = \frac{1}{2}\delta\left(3\cos^2\Theta_n - 1\right)$$

$$\begin{cases} \Theta_1 = 30^\circ \\ \Theta_2 = 60^\circ \\ \Theta_3 = 90^\circ \end{cases} \Rightarrow \begin{cases} \overline{\omega}_1 = \frac{5}{8}\delta \\ \overline{\omega}_2 = -\frac{1}{8}\delta \\ \overline{\omega}_3 = -\frac{1}{2}\delta \end{cases} \Rightarrow \begin{cases} \overline{\delta} = \frac{5}{8}\delta \\ \overline{\eta} = 0.6 \end{cases}$$

$\overline{\eta} \neq 0$ for the average dipolar tensor.

Two-Site Jumps: *trans-gauche* Isomerization

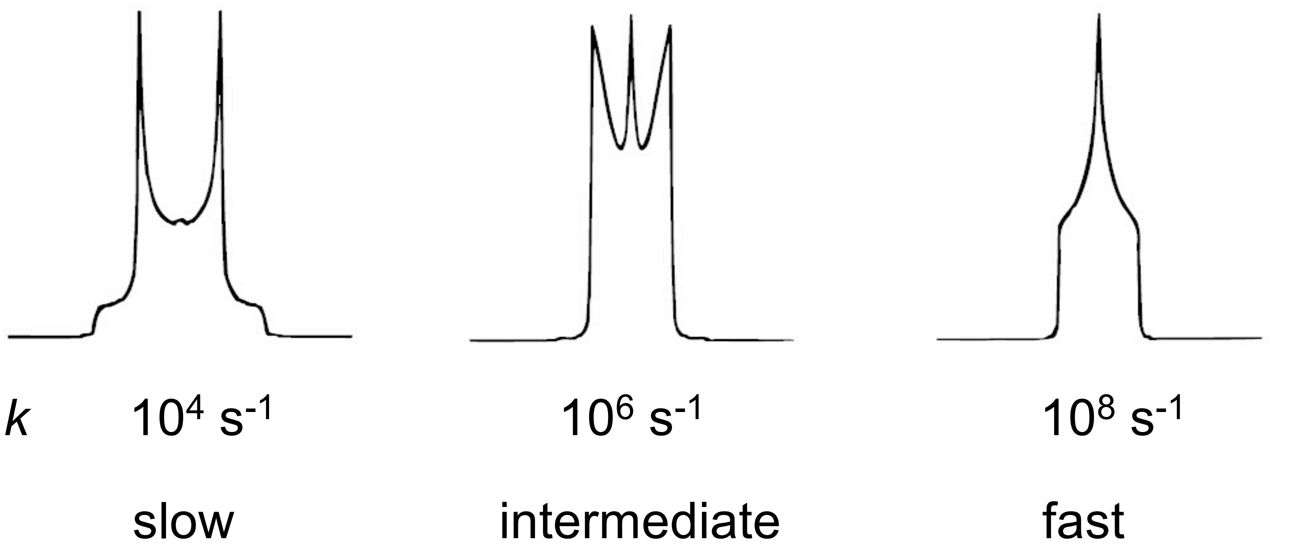
$$\overline{\omega}_n = \frac{1}{2}\delta\left(3\cos^2\Theta_n - 1\right)$$



- For C-H dipolar coupling or ^{2}H quadrupolar coupling:

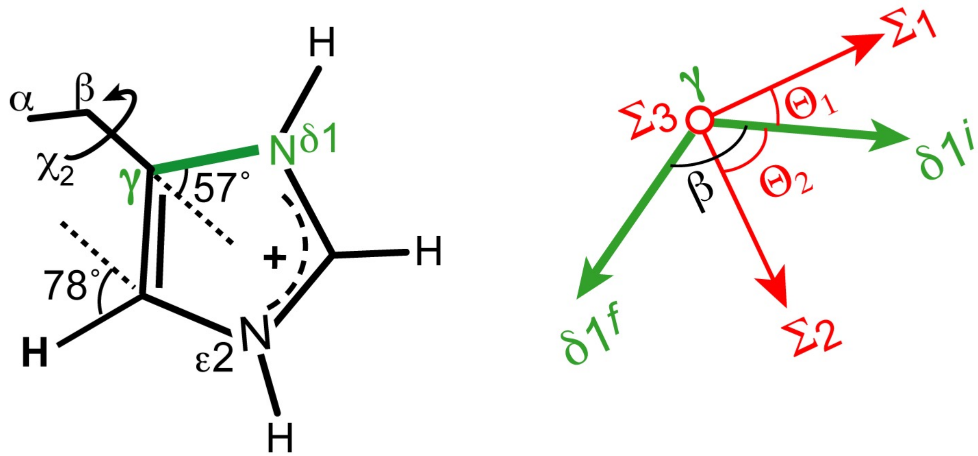
$\beta_R = 109.5^\circ$: $\theta_n = 35.3^\circ, 54.7^\circ, 90^\circ$: $\Rightarrow \overline{\omega}_n = \frac{\delta}{2},\ 0,\ -\frac{\delta}{2}.$

Palmer, Williams, and McDermott, ***J. Phys. Chem.***, 100, 13293 (1996).



$$\overline{\delta} = \frac{1}{2}\delta,\ \overline{\eta} = 1$$

Two-Site Jumps: Histidine Ring Flip



180° jump around the Cβ-Cγ bond:

For the Cγ-Nδ1 bond: $\beta_R = 2 \cdot 57° = 114°$

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

$$\begin{cases} \Theta_1 = 33° \\ \Theta_2 = 57° \\ \Theta_3 = 90° \end{cases} \Rightarrow \begin{cases} \overline{\omega}_1 = 0.56\delta \\ \overline{\omega}_2 = -0.06\delta \\ \overline{\omega}_3 = -0.5\delta \end{cases} \Rightarrow \begin{cases} \overline{\delta} = 0.56\delta \\ \overline{\eta} = 0.79 \end{cases} \Rightarrow S_{C\gamma-N\delta1} = 0.56$$

For the Cδ2-Hδ2 bond: $\beta_R = 156° \Rightarrow \overline{\delta} = 0.94\delta \Rightarrow S_{C\delta2-H\delta2} = 0.94$

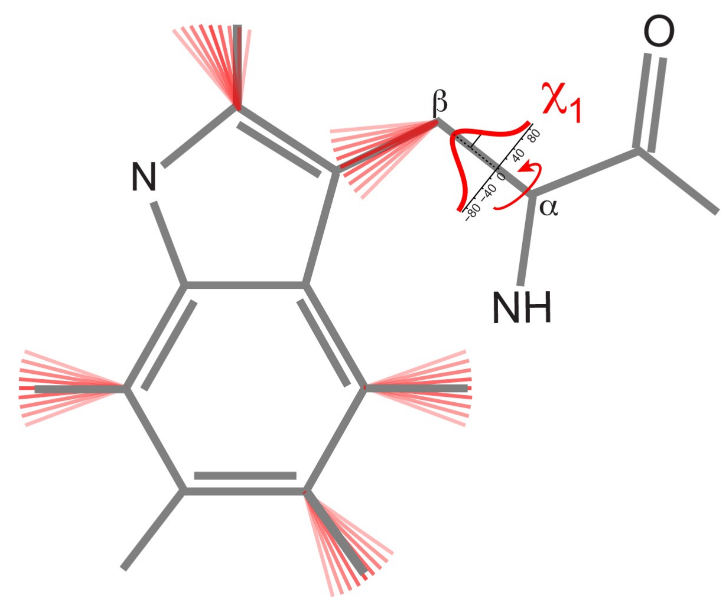
Multi-Site Jump: Gaussian Axial Fluctuation

• For motions involving ***multiple sites***, the sum tensor is the weighted average of individual tensors: $\bar{\omega} = \sum_j p_j \omega_j \rightarrow \Sigma = \sum_j p_j \sigma_j$

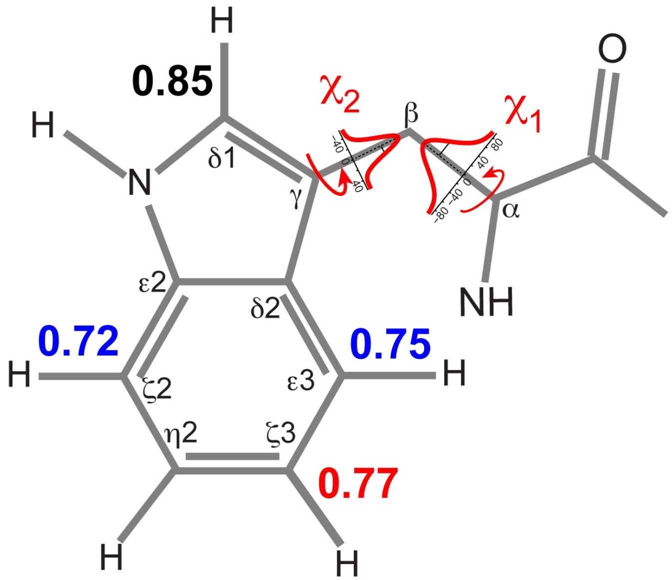
• The sum tensor can be diagonalized to give $\bar{\delta}$ and $\bar{\eta}$.

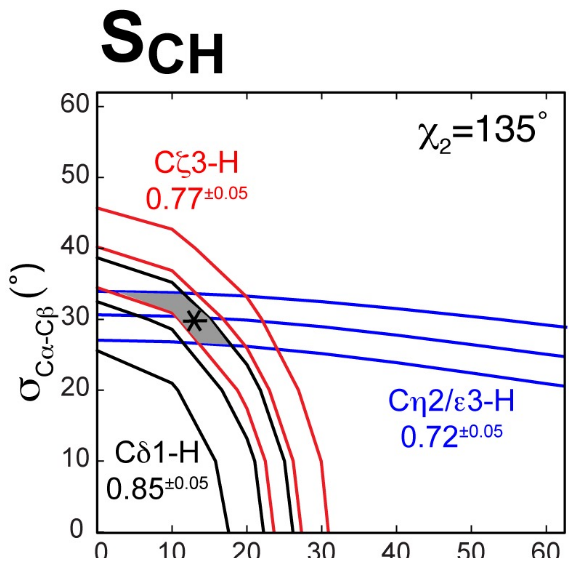
Example

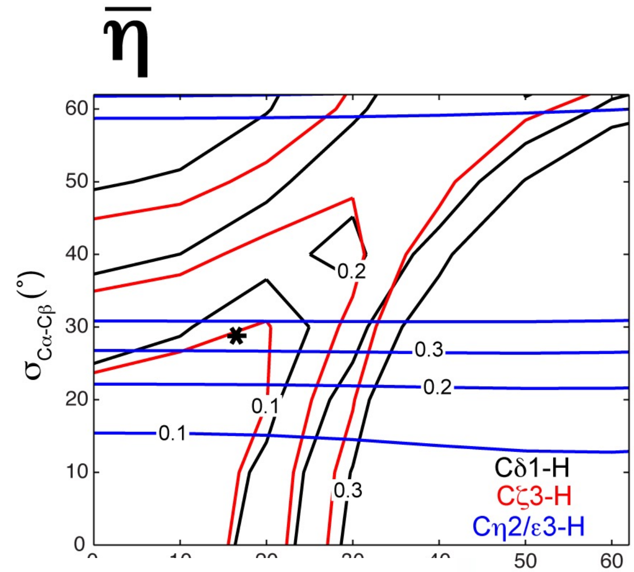
- Motion of a Trp sidechain in influenza M2.
- The measured order parameters rule out a simple 2-site jump motion around a single axis.
- Use a ***Gaussian biaxial fluctuation model*** with widths $\sigma_{\alpha\beta}$ and $\sigma_{\beta\gamma}$ to calculate the average couplings.



Motion of Trp41 in the M2 Proton Channel

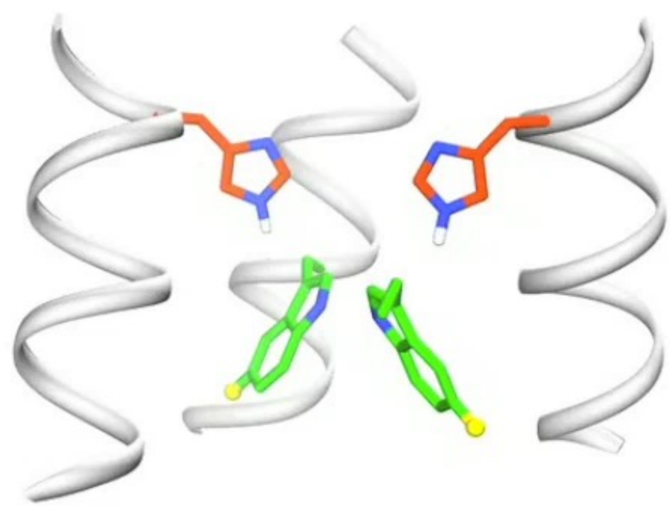




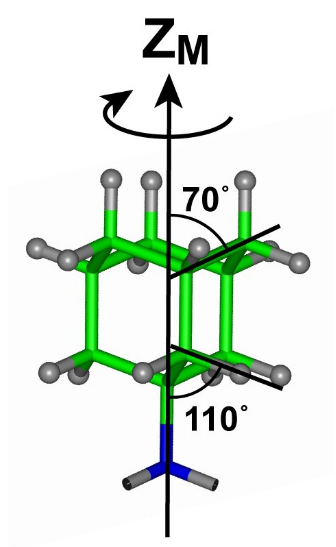


$\sigma_{\alpha\beta} \approx 30^{\circ}$

$\sigma_{\beta\gamma} \approx 15^{\circ}$



Uniaxial Rotation of a Rigid Molecule



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

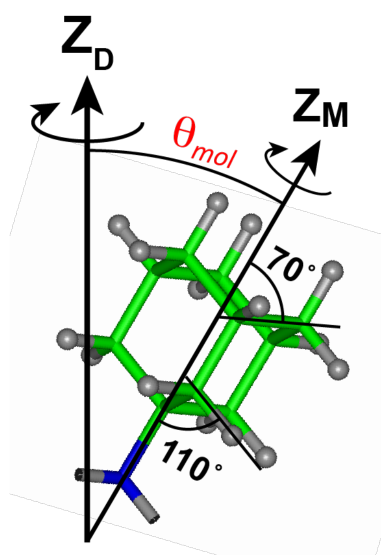
Relative to Z_M:

- 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:

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

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



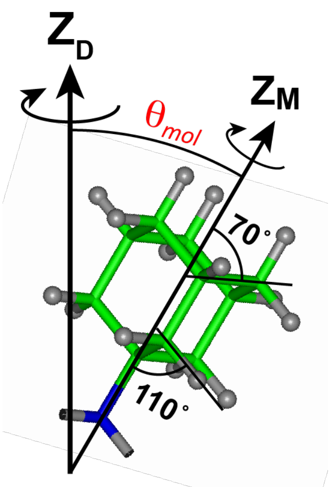
- If amantadine also rotates around an external axis, the bilayer normal Z_D:

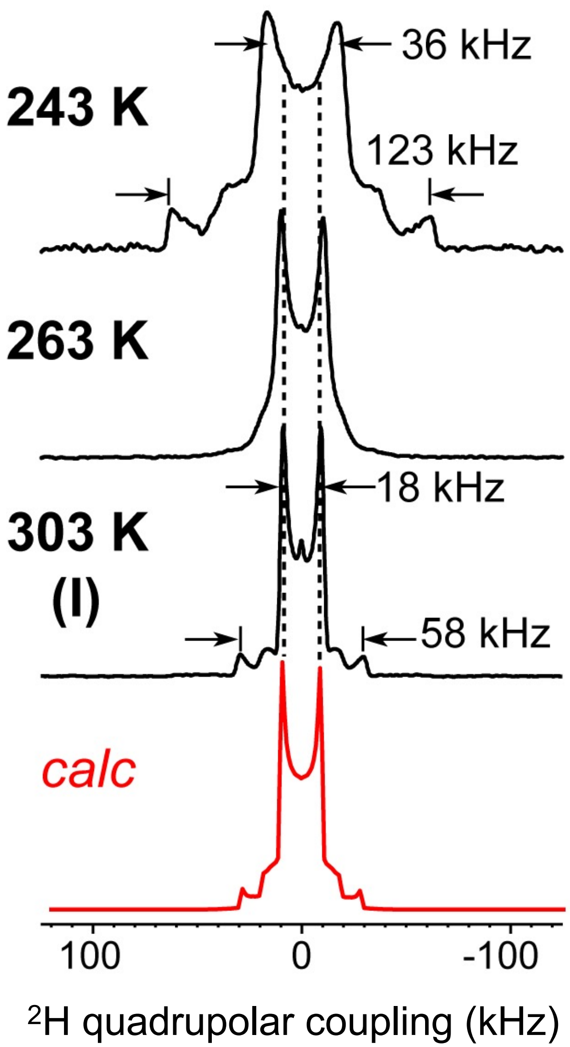
$$\bar{\bar{\delta}} = \tfrac{1}{2}\delta\left(3\cos^2\theta_{PM} - 1\right)\cdot\tfrac{1}{2}\left(3\cos^2\theta_{MD} - 1\right)$$

$$= \tfrac{1}{2}\delta\left(3\cos^2\theta_{PM} - 1\right)\cdot S_{mol}$$

Cady et al, ***Nature***, 2010.

Amantadine Dynamics in Lipid Bilayers



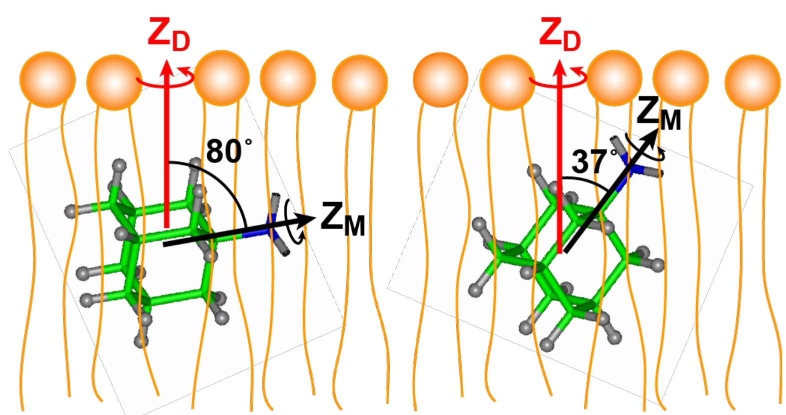


- 12 CD bonds: $0.33 \cdot \delta = 40\ kHz$
- 3 CD bonds : $1.0 \cdot \delta = 125\ kHz$

Gel phase: $S_{mol} \approx 1 \Rightarrow \theta_{MD} = 0°$

Liquid-crystalline phase:

$S_{mol} = \pm 0.46 \Rightarrow \theta_{MD} = 37°,\ 80°$



In the lipid bilayer

Cady et al, ***Nature***, 2010.

SSNMR Studies of Molecular Motion

- *Timescales & amplitudes of motion from NMR*
- *Fast motion: average (sum) tensors*
- *Experiments for measuring fast motion*
- *Slow motion: difference tensors*
- *Experiments for measuring slow motion*

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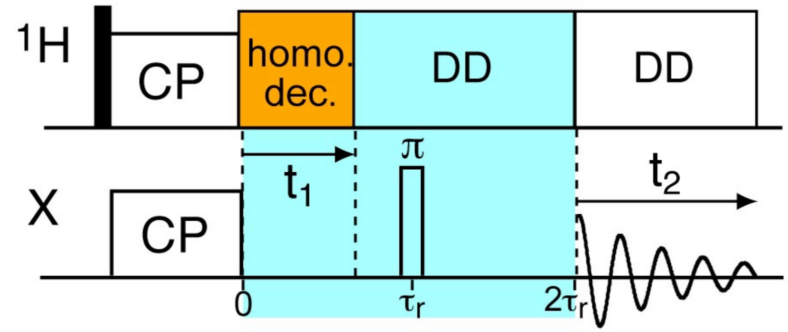
The X-^{1}H DIPSHIFT Experiment

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

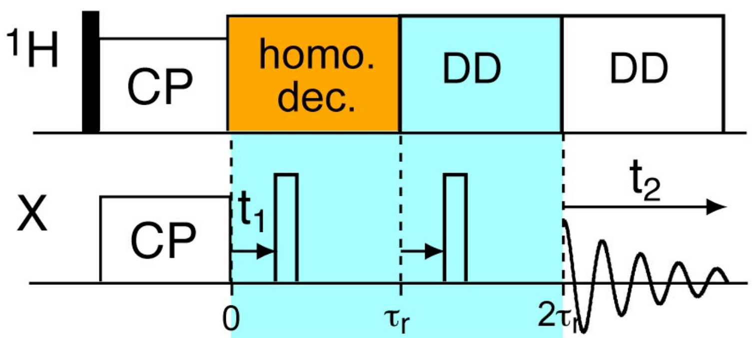
$$\Psi(t_1) = \int_0^{t_1} \omega(t)\,dt, \text{ where } \omega \propto \delta \cdot S \cdot k$$

Coupling constant *Scaling factor*

DIPSHIFT



Doubled DIPSHIFT



$$\Psi^{2\times}(t_1) = \int_0^{t_1} \omega(t)\,dt - \int_{t_1}^{\tau_r} \omega(t)\,dt = \int_0^{t_1} \ldots - \left(\underbrace{\int_0^{\tau_r} \ldots}_{0} - \int_0^{t_1} \ldots \right)$$

$$= 2\int_0^{t_1} \omega(t)\,dt = 2\Psi(t_1)$$

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

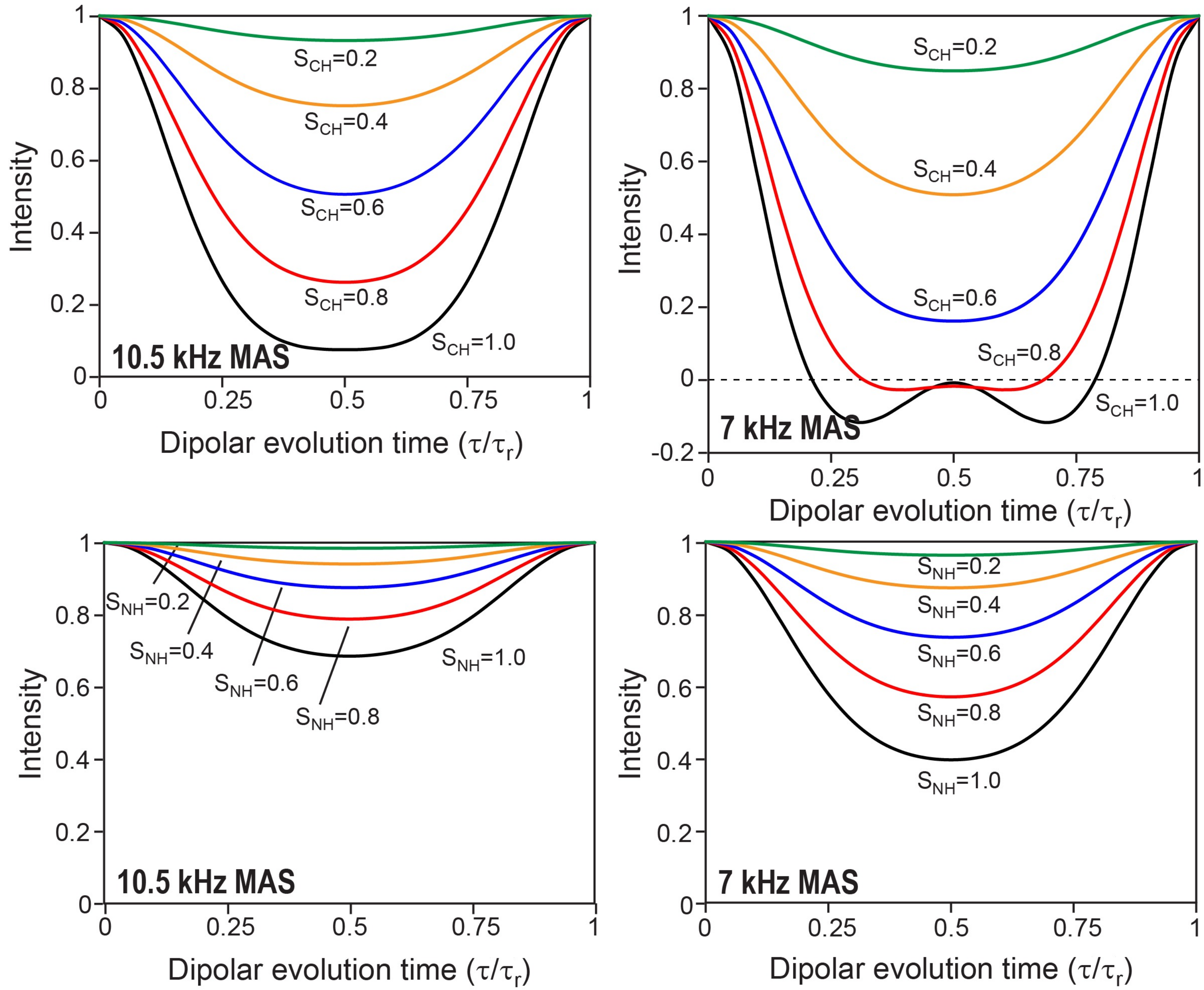
Munowitz et al, ***J. Am. Chem. Soc.***, 103, 2529 (1981);
Hong et al, **J. Magn. Reson.** 129, 85 (1997).

Simulated C-H & N-H DIPSHIFT Time Signals

FSLG for ^{1}H homonuclear decoupling (k = 0.577)

2 x C-H

2 x N-H



Python Code for Simulating DIPSHIFT Curves

http://meihonglab.com/

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RESEARCH PUBLICATIONS LAB PEOPLE MEI HONG SOFTWARE CONTACT

SOFTWARE: **FITTING PROGRAMS FOR DIPOLAR-CHEMICAL SHIFT CORRELATION (DIPSHIFT) EXPERIMENT**

Python Script

DOWNLOAD

This Python script calculates dipolar dephasing curves for the DIPSHIFT experiment. Input parameters that the user can vary are found at the top of the script.

Jupyter Python Script

DOWNLOAD

This script accomplishes the same things as the Python script above but is written to be used in the Jupyter Notebook web application.

Fortran Program

DOWNLOAD

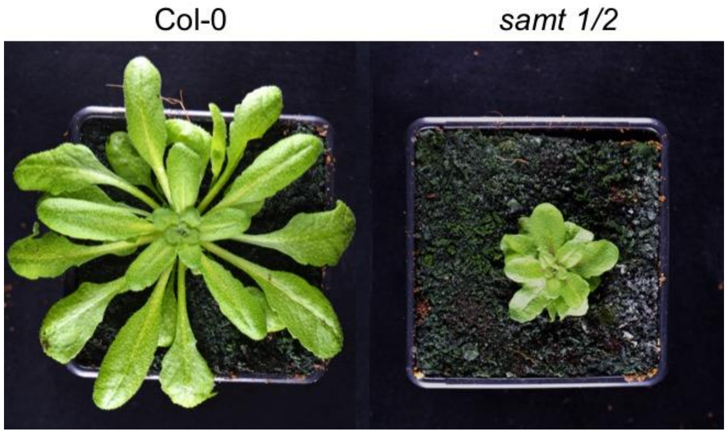
Fortran Input File

DOWNLOAD

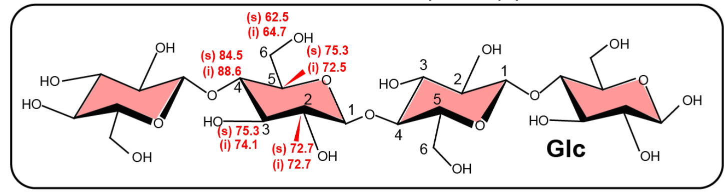
This file allows you to input the dipolar coupling value and experimental conditions such as the spinning speed for the Fortran program

Install Anaconda Navigator or Jupyter

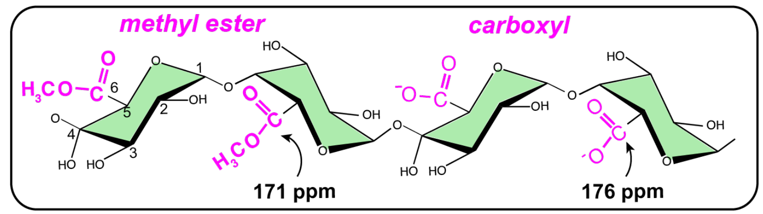
Polysaccharide Dynamics in Plant Cell Walls



Cellulose (1–>4) β-D-Glc

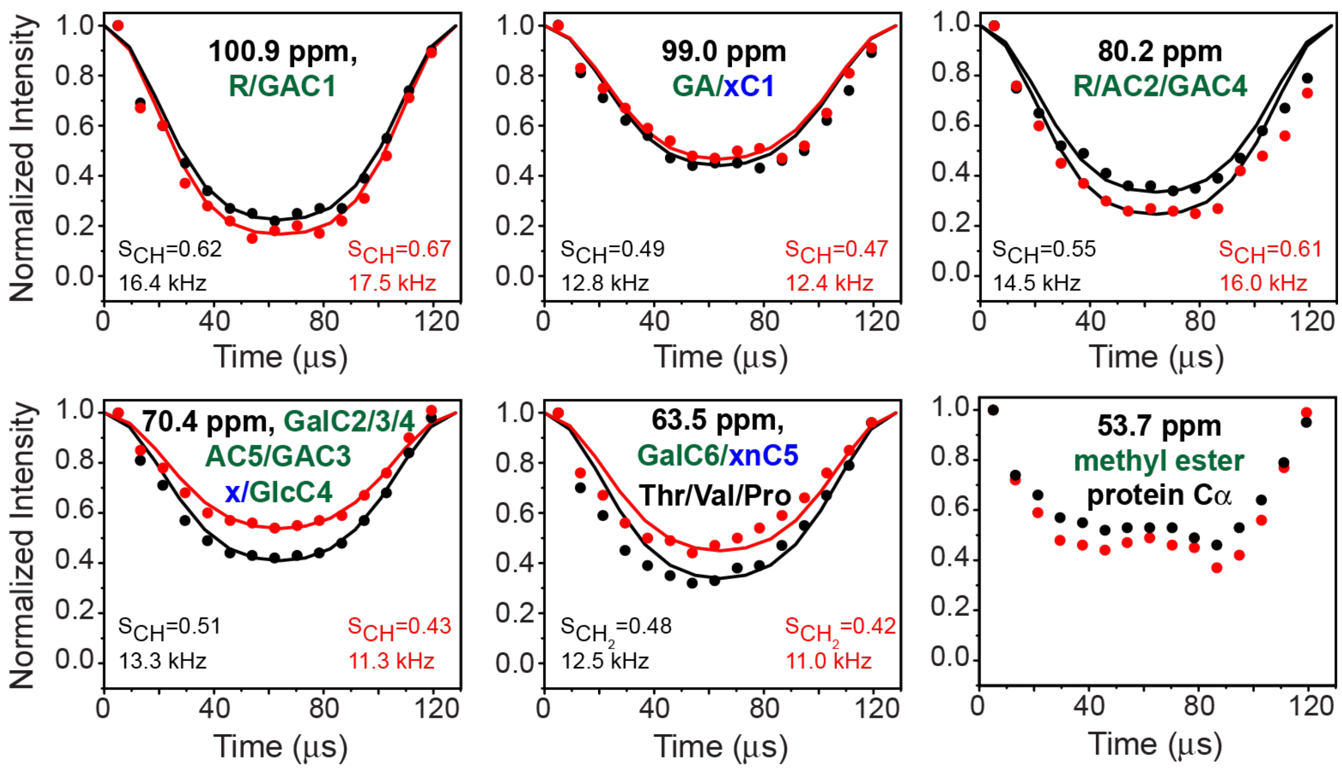


Homogalacturonan (1-4) α-D-GalA



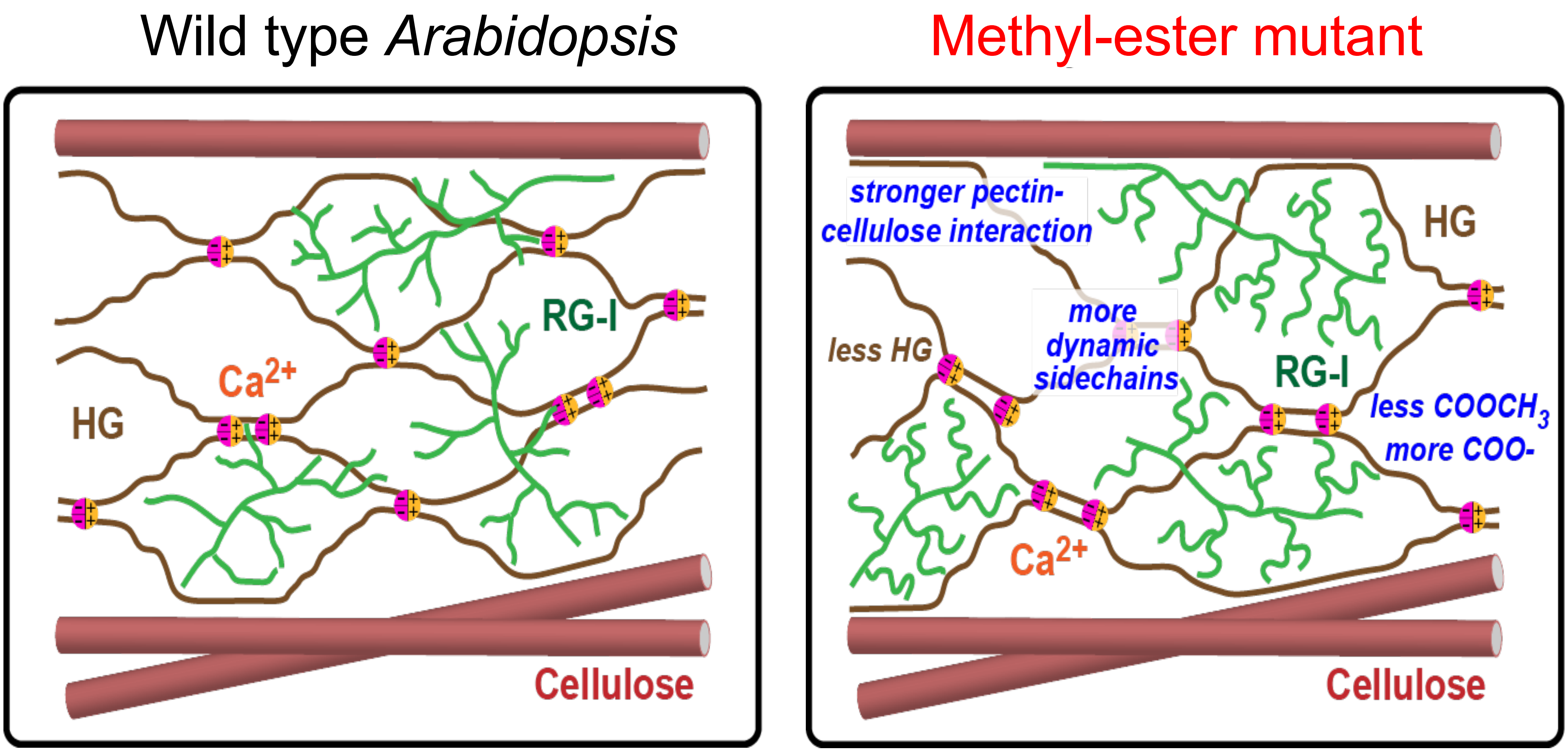
Wild type *Arabidopsis*

low-methyl-ester mutant



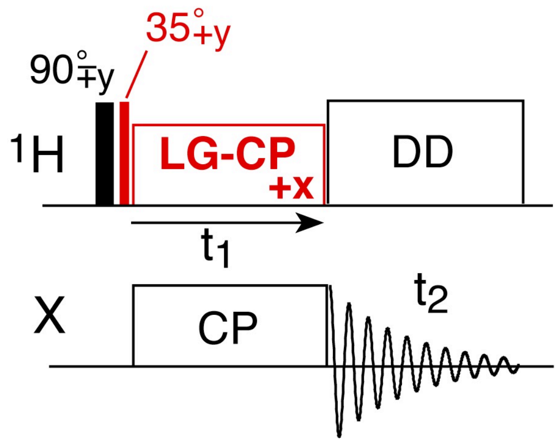
Pyae Phyo

Pectin Tethering of Cellulose Slows Cell Wall Loosening

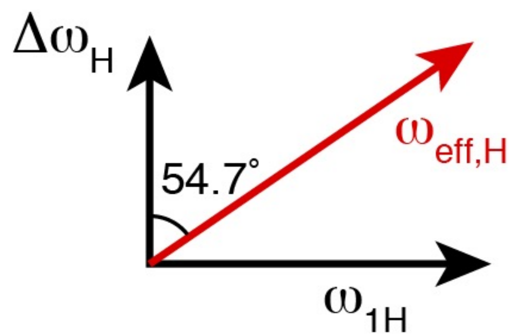


HONG LAB

2D Lee-Goldberg CP for Measuring ^{13}C-^{1}H Dipolar Couplings



Magic-angle tilted spin lock on ^{1}H:



LG-CP condition:

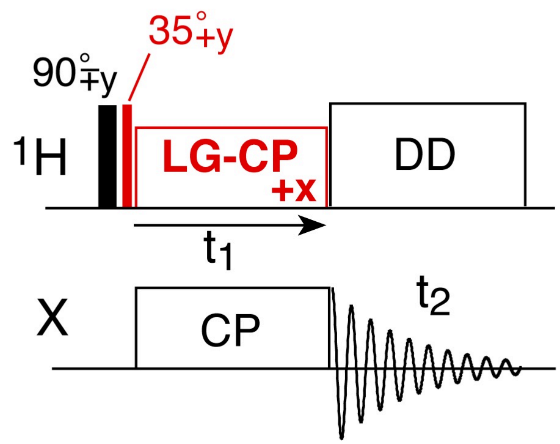
$\omega_{1,X} = \omega_{eff,H} \pm \omega_r$

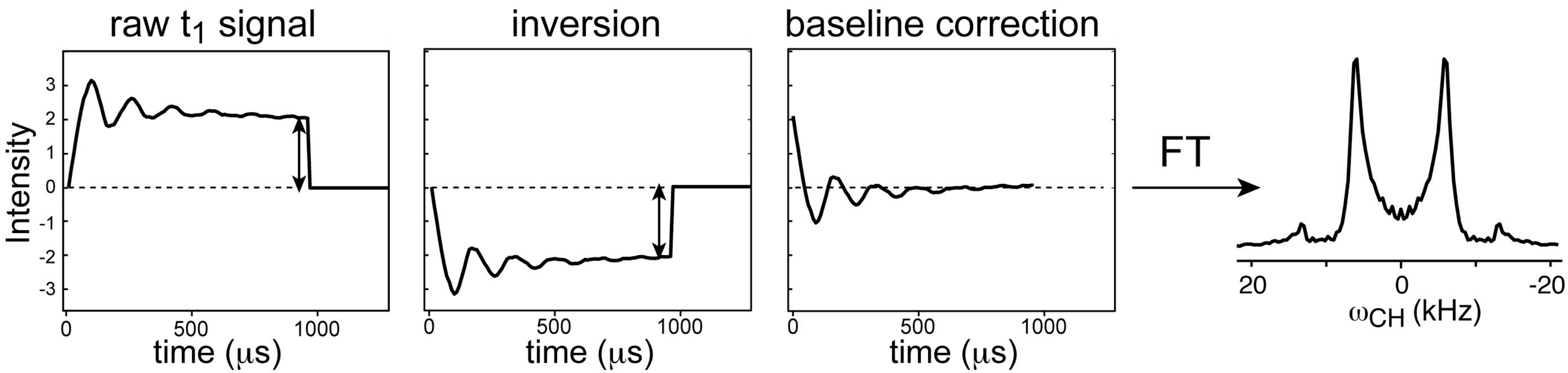
- ☺ Simple: increment CP contact time as t_1.
- ☺ ^{1}H-^{1}H dipolar coupling is removed by LG spin lock.
- ☺ Can be done under fast MAS (10 - 40 kHz)
- ☺ Frequency-domain dipolar spectrum resolves multiple splittings.
- ☺ Scaling factor: $k = \cos(54.7^\circ) = 0.577$.

- ☹ CP matching may be unstable under fast MAS.

Van Rossum et al ***JACS***, 122, 3465 (2000). Hong et al ***JPC***, 106, 7355 (2002).

LG-CP Time Signals

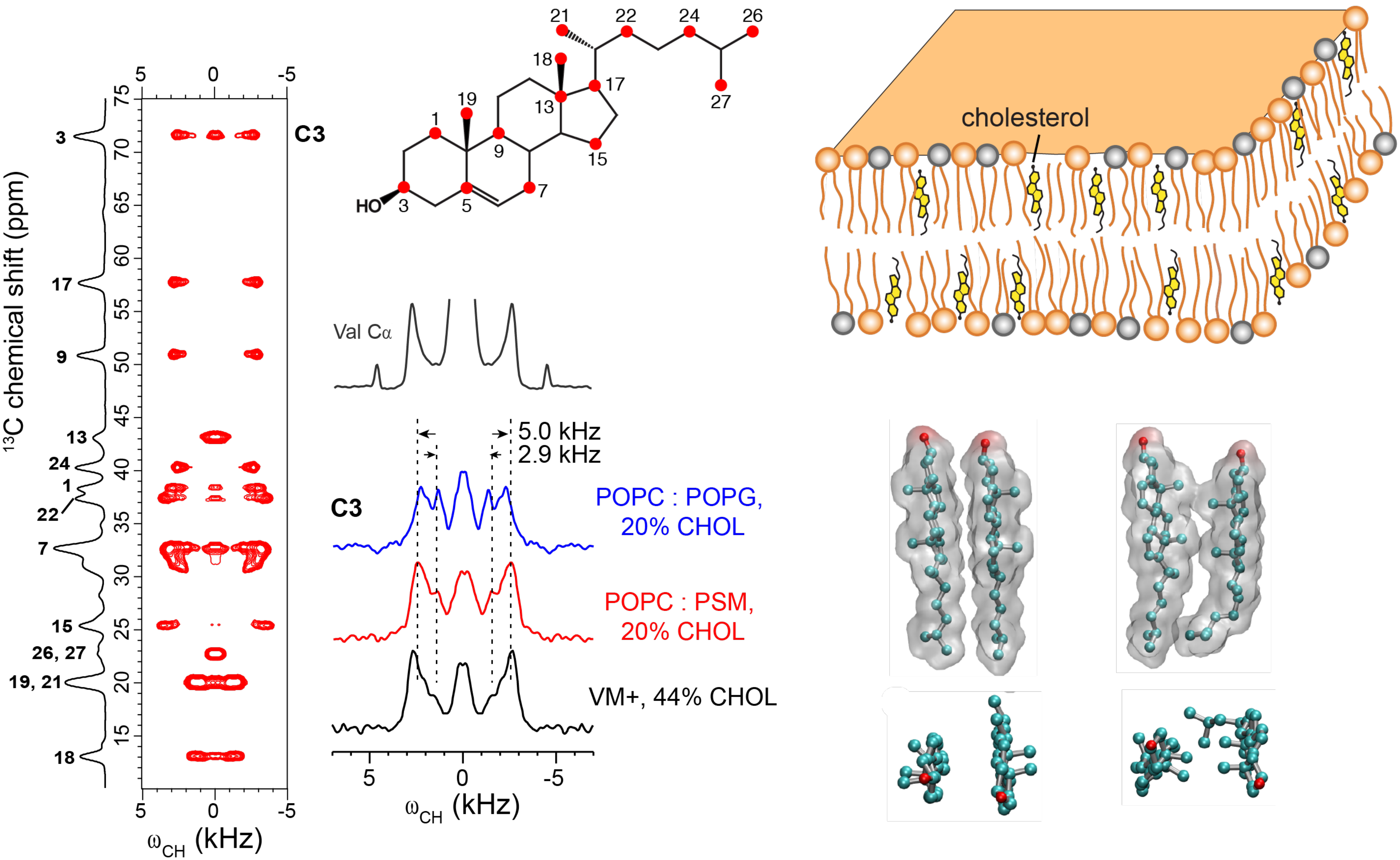




Hartman-Hahn CP does not show distinct dipolar oscillations due to the presence of multi-spin ^1H-^1H dipolar couplings under slow MAS.

Van Rossum et al ***JACS***, 122, 3465 (2000). Hong et al ***JPC***, 106, 7355 (2002).

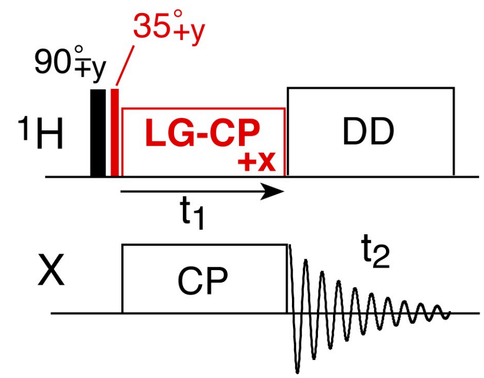
Cholesterol Dynamics in Lipid Membranes



Elkins, Bandara, Pantelopulos, Straub and Hong, submitted.

LG-CP Average Hamiltonian Analysis

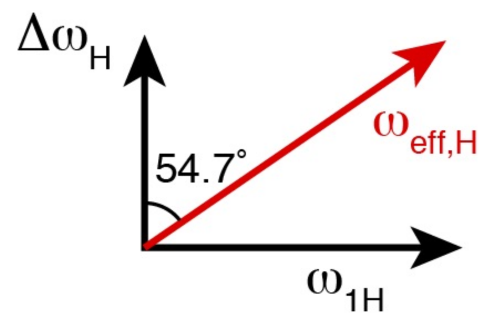
$$H(t) = \underbrace{\omega_{1I} I_x + \omega_{1S} S_x}_{\text{rf pulses}} + \Delta\omega_I I_z + \underbrace{\omega_{IS}(t) I_z S_z}_{\text{to transfer pol.}} + \underbrace{\omega_{II}(t)\left(3 I_z I_z - I \cdot I\right)}_{\text{to remove}}$$



$$\omega_{IS}(t) = 2\delta\left[C_1 \cos\left(\omega_r t + \gamma\right) + C_2 \cos\left(2\omega_r t + 2\gamma\right)\right]$$

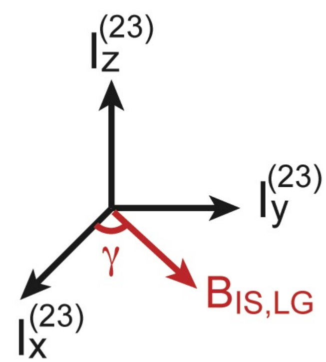
Transform into a tilted frame and the interaction frame of the rf pulses, under the sideband matching condition

$$\omega_{eff,H} - \omega_{1S} = \pm\omega_r$$



It can be shown that the average I-S dipolar coupling is the scalar product between a ZQ spin operator and a tilted effective field:

$$\overline{\tilde{H}}_{IS}^{T(0)} = \underbrace{\tfrac{1}{2}\delta \sin\theta_m C_1}_{\omega_{IS,LG}} \bullet \underbrace{\left(I_x^{(23)},\ I_y^{(23)},\ I_z^{(23)}\right)}_{\overrightarrow{I^{(23)}}} \underbrace{\begin{pmatrix} \cos\gamma \\ \sin\gamma \\ 0 \end{pmatrix}}_{B_{IS,LG}}$$

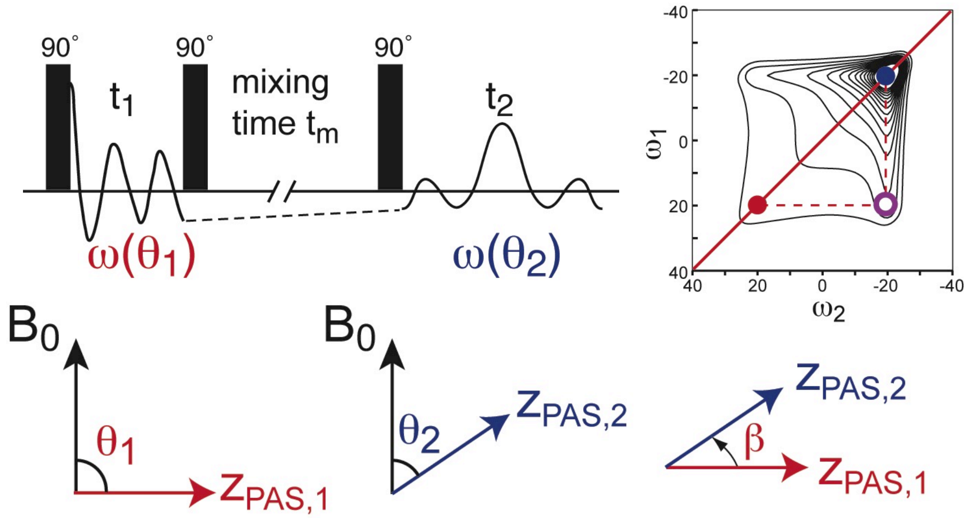


$$\overline{\tilde{H}}_{II}^{T,(0)} = 0$$

SSNMR Studies of Molecular Motion

- *Timescales & amplitudes of motion from NMR*
- *Fast motion: average tensors*
- *Experiments for measuring fast motion*
- *Slow motion: difference tensors*
- *Experiments for measuring slow motion*

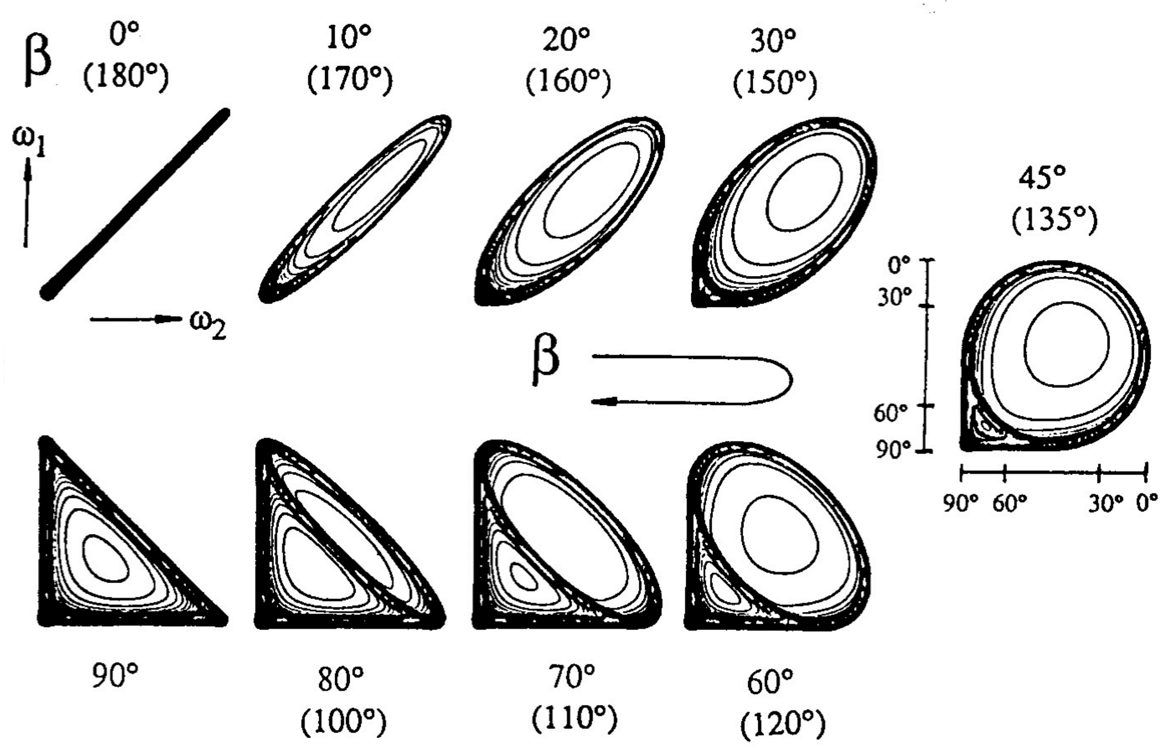
Slow Motion: 2D Exchange NMR



2D spectrum $S(\omega_1, \omega_2)$: a joint probability:

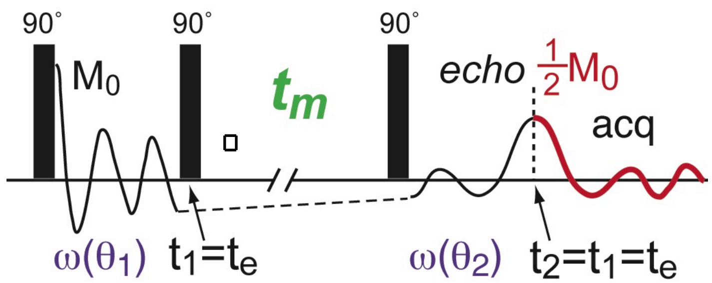
- intensity distribution: motion geometry.
- t_m dependence: correlation time.

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



Schmidt-Rohr and Spiess, ***Multidimensional Solid-State NMR and Polymers***, 1994.

1D Stimulated Echo: Time-Domain Exchange



- 1D analog of 2D exchange spectra.
- Allows rapid measurement of τ_c without 2D.

2D time signal:

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

1D time signal: $t_2 = t_1 = t_e$.

- Segments without frequency change: $\omega(\theta_1) = \omega(\theta_2) = \omega(\text{diagonal})$.

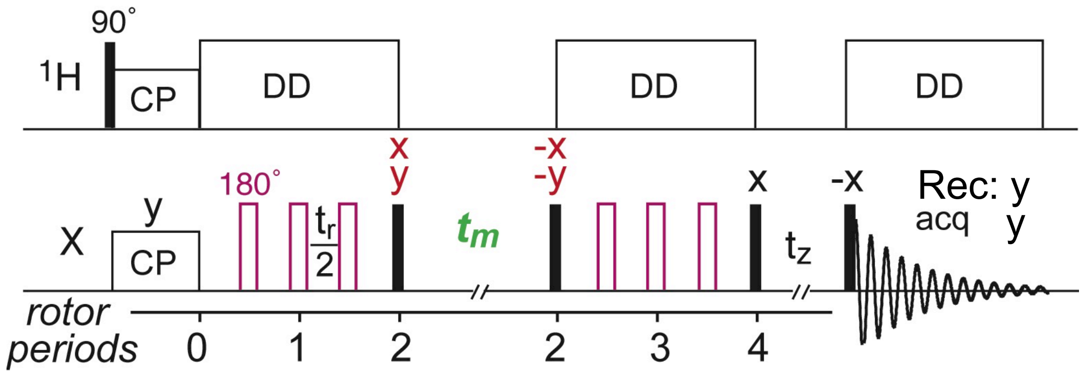
$$M(t_e)=\left\langle e^{-i\omega t_e}\cdot e^{i\omega t_e}\right\rangle = \underbrace{\langle \mathbf{1}\rangle}_{2\ \text{scans}}$$

- Segments with frequency change:

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

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 2nd recoupling period, the MAS phase for 2 scans is:

$$\cos\Phi_1\cos\Phi_2-\sin\Phi_1\sin\Phi_2=\cos\left(\Phi_1+\Phi_2\right)=\cos\left(\left|\Phi_2\right|-\left|\Phi_1\right|\right)$$

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

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

- No motion: $\omega_1=\omega_2$, –> $\cos(\Phi_1+\Phi_2)=1$, full echo.
- With motion: $\omega_1\neq\omega_2$, –> $\cos(\Phi_1+\Phi_2)<1$, reduced echo.

deAzevedo…Schmidt-Rohr, ***J. Chem. Phys.***, 112, 8988 (2000).

Exchange NMR Involves Difference Tensor

CODEX signal:

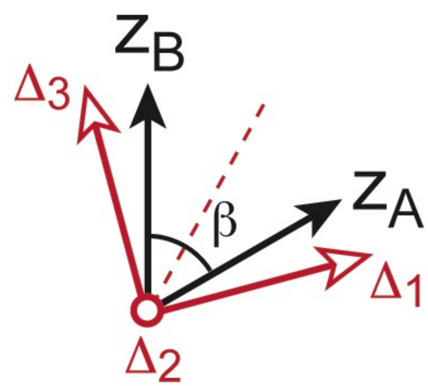
$$\frac{S(t_m,\delta N t_r)}{S_0(t_m,\delta N t_r)} = \cos(|\Phi_2|-|\Phi_1|) = \cos(\Phi^\Delta), \text{ where } \Phi^\Delta = N\int_2^{t_r/2} \omega^\Delta(t)\,dt$$

Difference tensor: $\Delta \equiv \sigma_A - \sigma_B$

Reflection of the Z_A and Z_B axes with the bisector plane gives the opposite of the original difference tensor.

For η = 0, the Δ tensor's principal axis directions are:

- Δ_2: Normal of the AOB plane;
- Δ_3 and Δ_1: in the AOB plane, 45° from the bisector.



	σ_A	σ_B
Δ_1 axis:	45°-β/2,	45°+β/2
Δ_2 axis:	90°,	90°
Δ_3 axis:	45°+β/2,	45°-β/2

$$\omega_n^\Delta = \tfrac{1}{2}\delta\left(3\cos^2\Theta_{A,n} - 1\right) - \tfrac{1}{2}\delta\left(3\cos^2\Theta_{B,n} - 1\right)$$

CODEX is Sensitive to Small-Angle Reorientations

For Z_A :

$$\omega_{A,2} = \tfrac{1}{2}\delta\left(3\cos^2 90° - 1\right) = -\tfrac{1}{2}\delta$$

$$\omega_{A,1} = \tfrac{1}{2}\delta\left(3\cos^2\left(45° - \beta/2\right) - 1\right)$$

$$\omega_{A,3} = \tfrac{1}{2}\delta\left(3\cos^2\left(45° + \beta/2\right) - 1\right)$$

For Z_B

$$\omega_{B,2} = \tfrac{1}{2}\delta\left(3\cos^2 90° - 1\right) = -\tfrac{1}{2}\delta$$

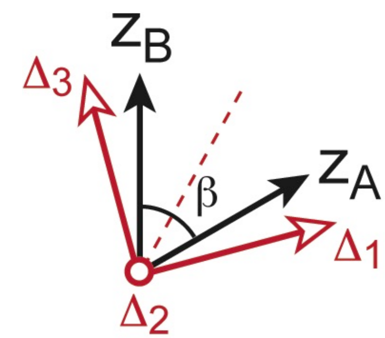
$$\omega_{B,1} = \tfrac{1}{2}\delta\left(3\cos^2\left(45° + \beta/2\right) - 1\right)$$

$$\omega_{B,3} = \tfrac{1}{2}\delta\left(3\cos^2\left(45° - \beta/2\right) - 1\right)$$

$$\omega_2^\Delta = \omega_{A,2} - \omega_{B,2} = 0$$

$$\omega_1^\Delta = \omega_{A,1} - \omega_{B,1} = \tfrac{3}{2}\delta \cdot \tfrac{1}{2}\left[\cos\left(90° - \beta\right) - \cos\left(90° + \beta\right)\right] = \tfrac{3}{2}\delta\sin\beta$$

$$\omega_3^\Delta = \omega_{A,3} - \omega_{B,3} = \tfrac{3}{2}\delta \cdot \tfrac{1}{2}\left[\cos\left(90° + \beta\right) - \cos\left(90° - \beta\right)\right] = -\tfrac{3}{2}\delta\sin\beta$$

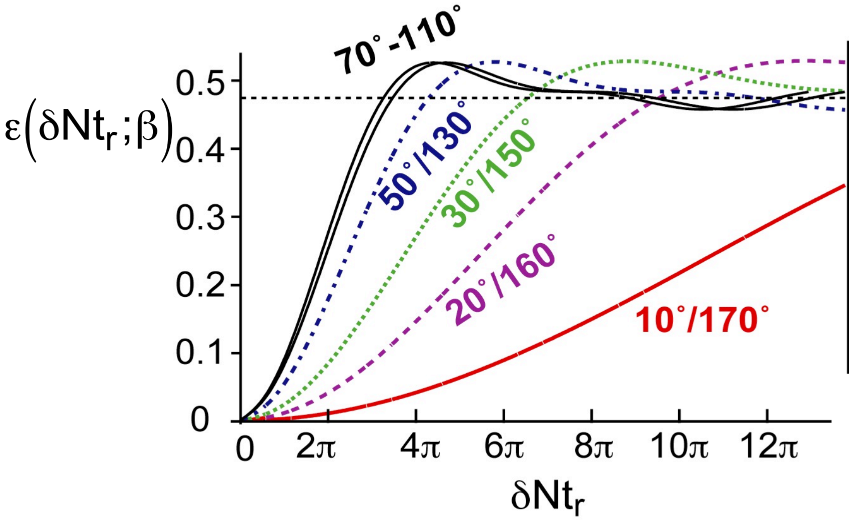


$$\Rightarrow \quad \eta^\Delta = 1$$

$$\left|\omega_{33}^\Delta - \omega_{11}^\Delta\right| = 3|\delta| \cdot \sin\beta = \left|\omega_{33} - \omega_{11}\right| \cdot 2\sin\beta$$

- CODEX signal scales ~ $sin\beta$, which is ~β for small angles.
- Usual angular dependence is $(3\cos^2\beta-1)/2$, which scales ~β^2 .

CODEX: Reorientation Angles & Number of Sites



$$E\left(t_m, \delta N t_r\right) = \int_0^{90^\circ} R(\beta)\,\varepsilon\left(\delta N t_r;\beta\right) dt \cdot d\beta$$

Jump motions:

$$\frac{\Delta S}{S_0}\left(t_m >> \tau_c, \delta N t_r >> 1\right)$$

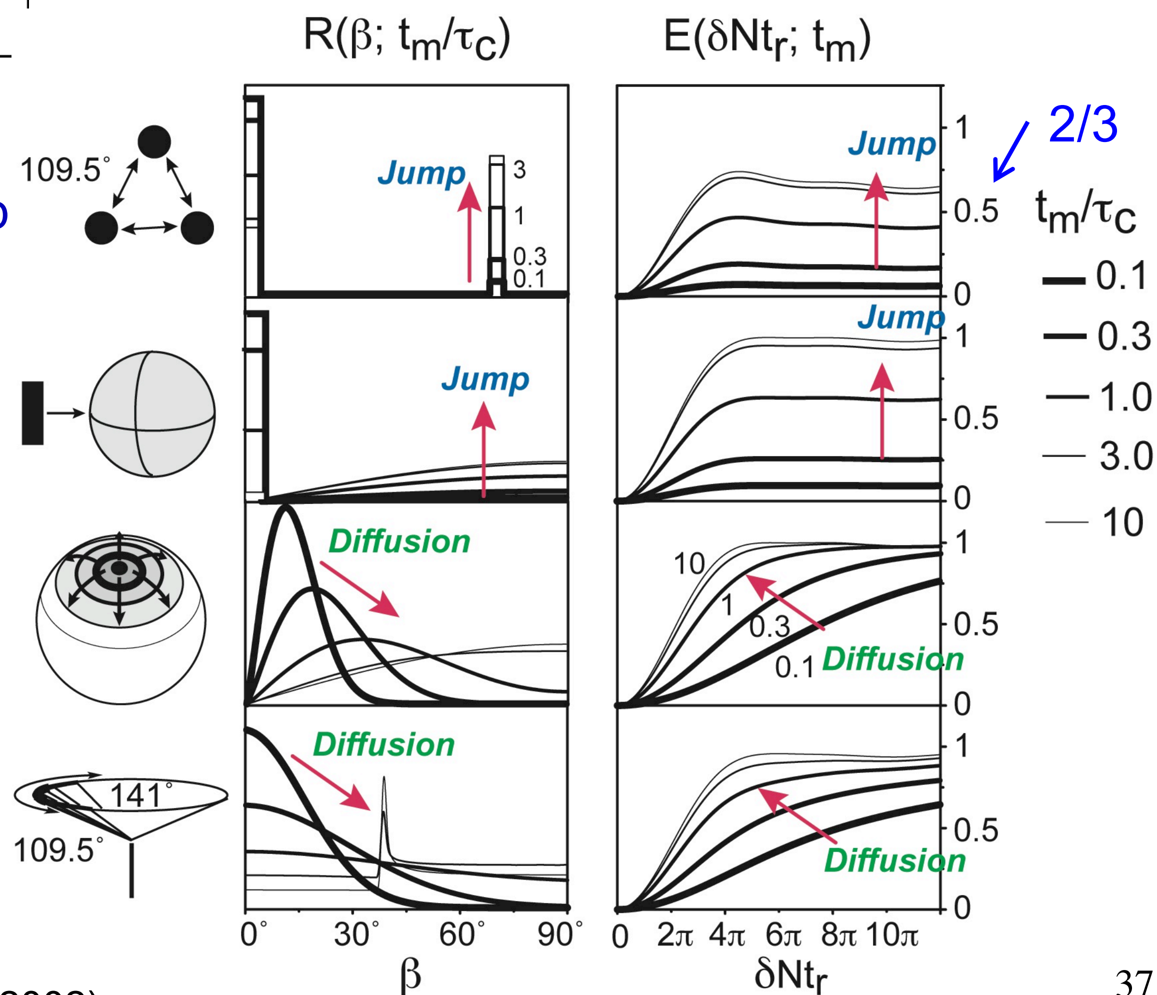
$$= 1 - \frac{1}{M}$$

3-site jump

Isotropic jump

Isotropic diffusion

Uniaxial rotation



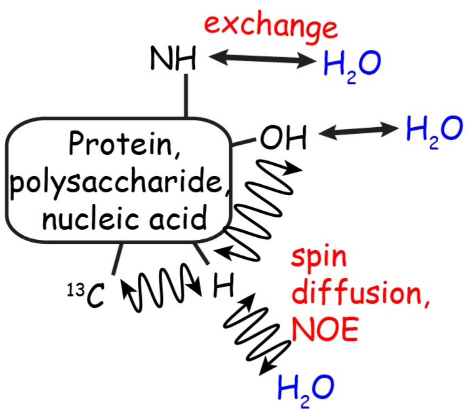
Schmidt-Rohr et al, ***Encyclop NMR***, 9, 633 (2002).

Summary

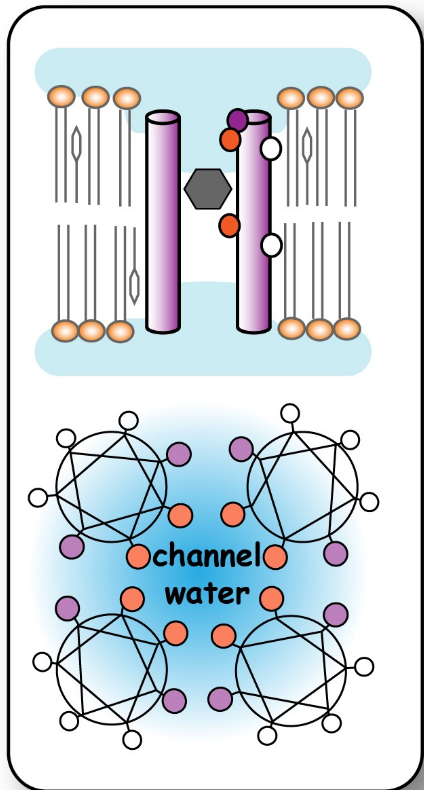
Motions are ubiquitous in biological molecules.

- Fast motions *average* the interaction tensors and *narrow the spectra*.
- The *average tensors* and spectral lineshapes of several common motions can be analytically derived.
- Fast motions can be measured using 2D SLF experiments that resolve dipolar couplings by chemical shifts.
- *Order parameters & order tensors* give information on rigid-body motions as well as internal motions.
- Slow motions can be measured as *2D exchange cross peaks* or 1D *CODEX* stimulated echo intensities.
- The geometry of slow motion is described by *difference tensors*.

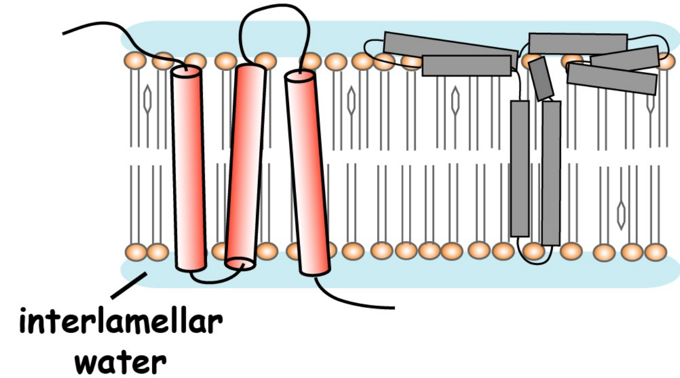
Hydration of Biomolecular Systems



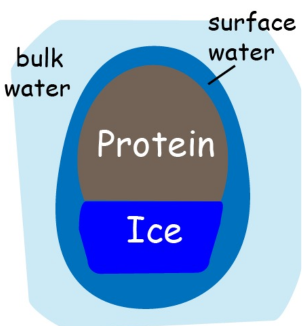
Ion channels



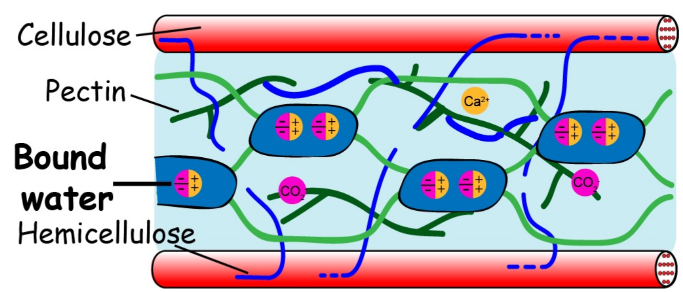
Polytopic membrane proteins



Ice binding proteins



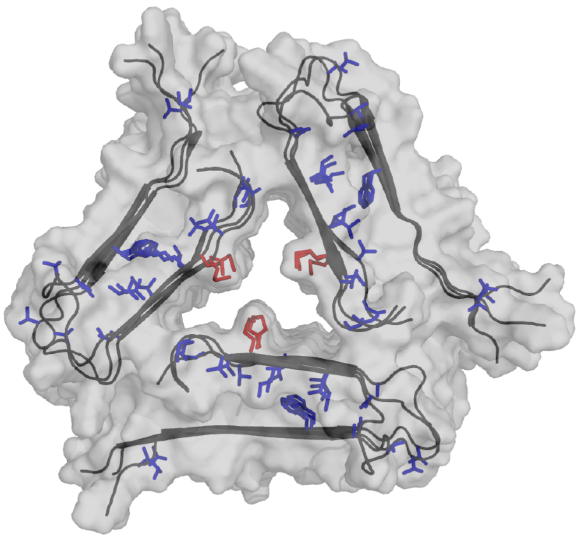
Plant cell walls and other complex biomaterials



Williams & Hong, ***JMR***, 2014.

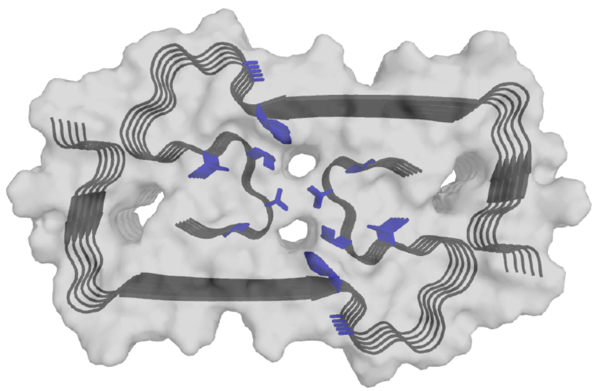
Water in Amyloid Fibrils

Wild-type Aβ40



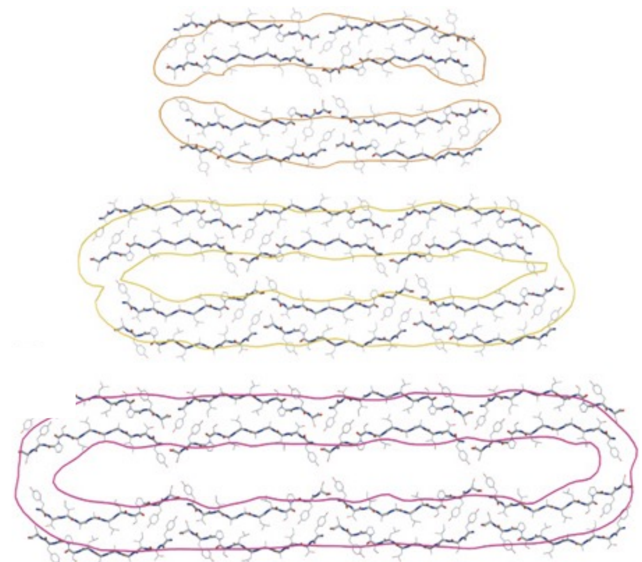
Paravastu et al, ***PNAS***, 2008.

(E22Δ) Osaka Aβ40



Schultz et al, ***Angew. Chemie,*** 2015.

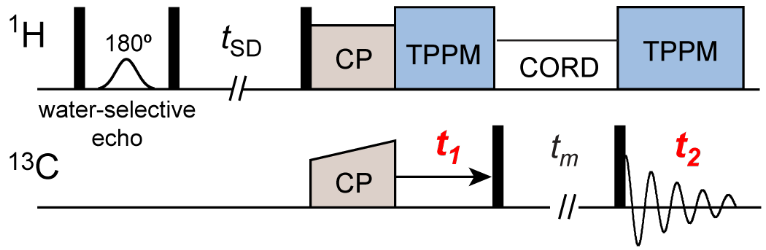
Transthyretin TTR(105-115)



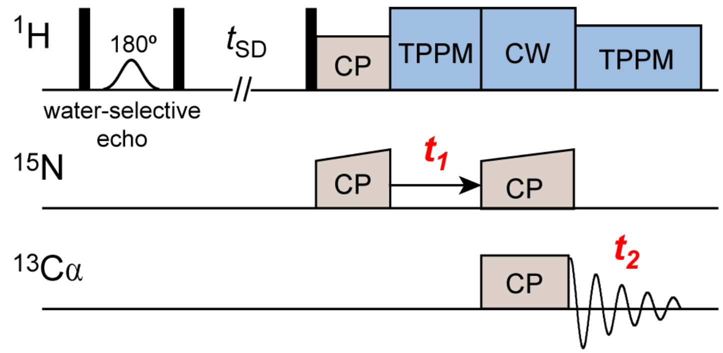
Fitzpatrick et al, ***PNAS***, 2013.

Water-Edited 2D Solid-State NMR Experiments

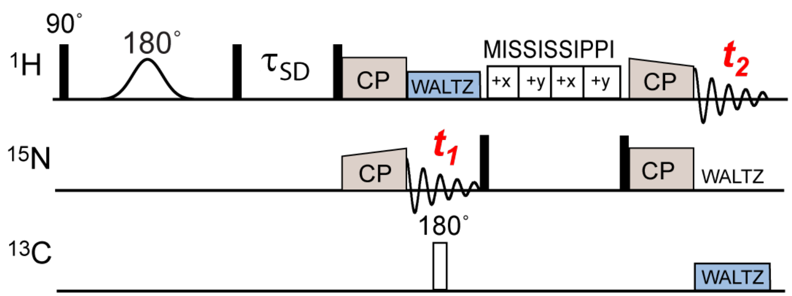
Water-edited 2D CC



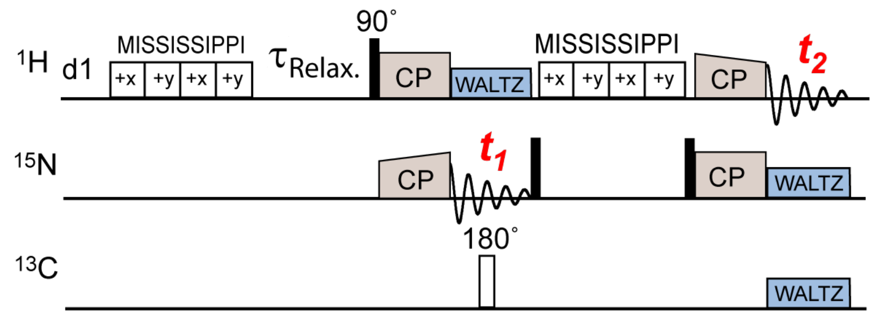
Water-edited 2D NCα



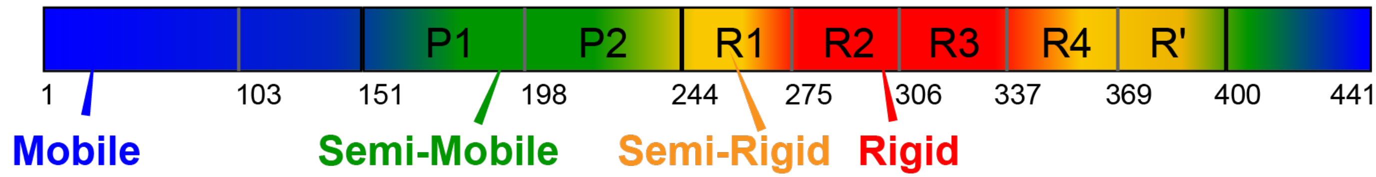
Water-Edited 2D hNH

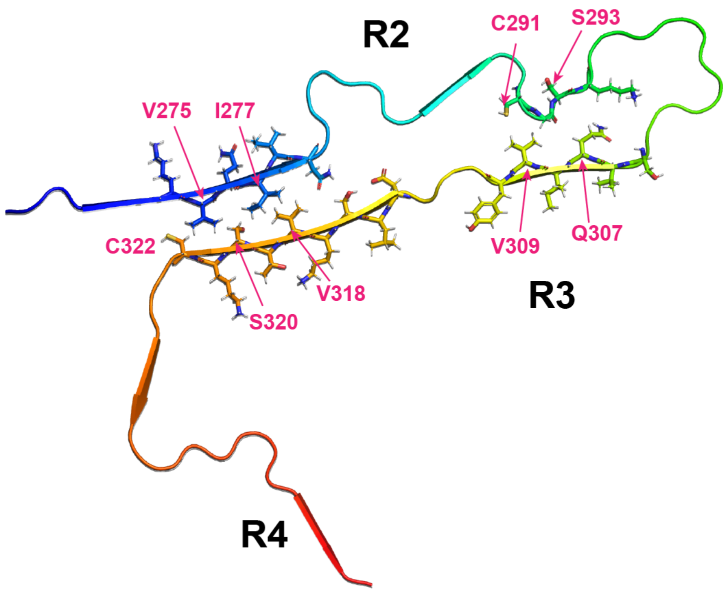


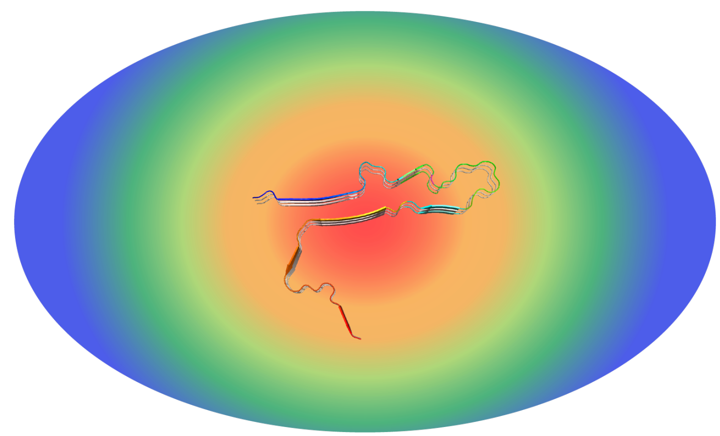
^{1}H T_1 Saturation-Recovery 2D hNH



Structure and Dynamics of Tau Fibrils

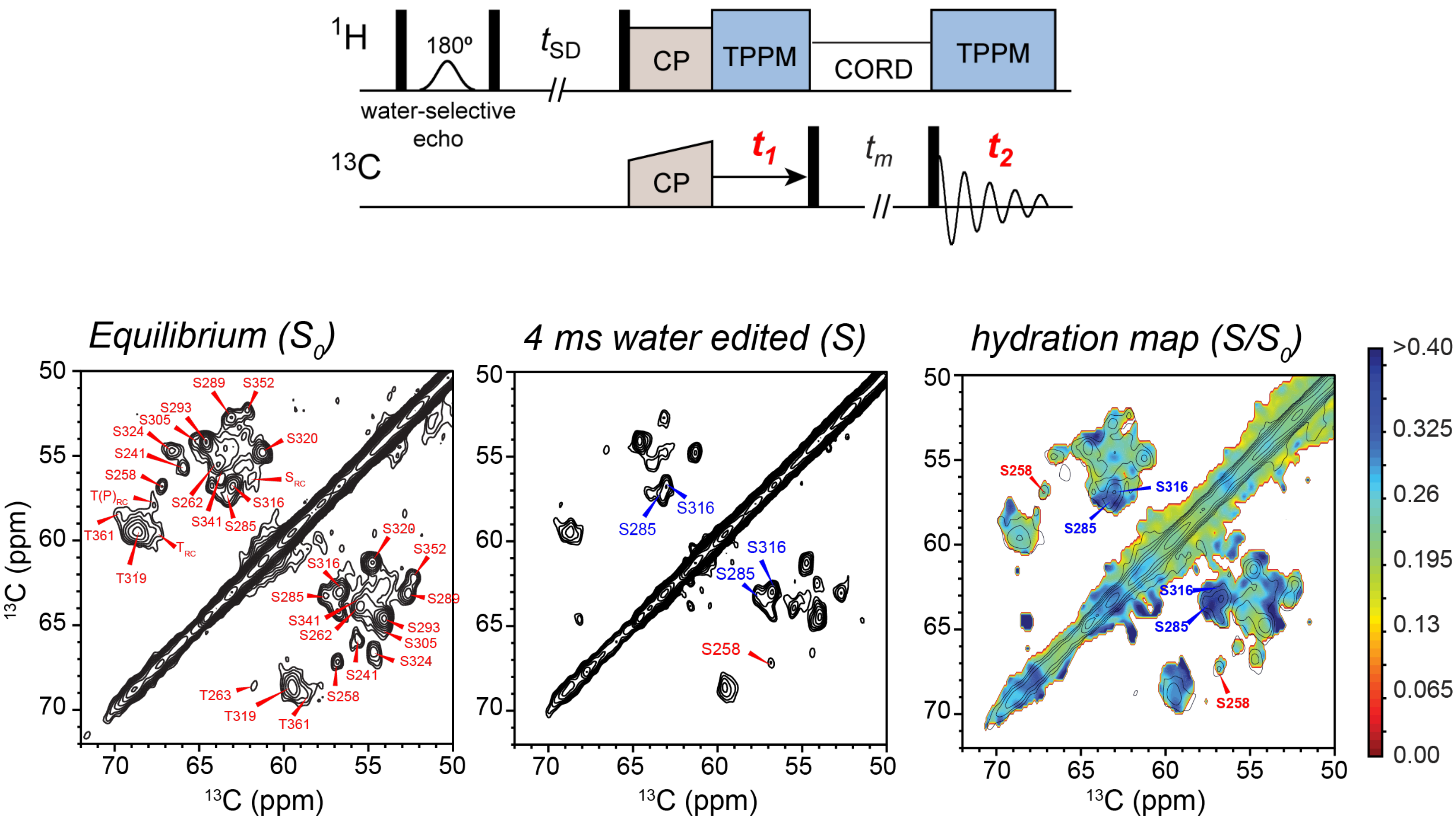






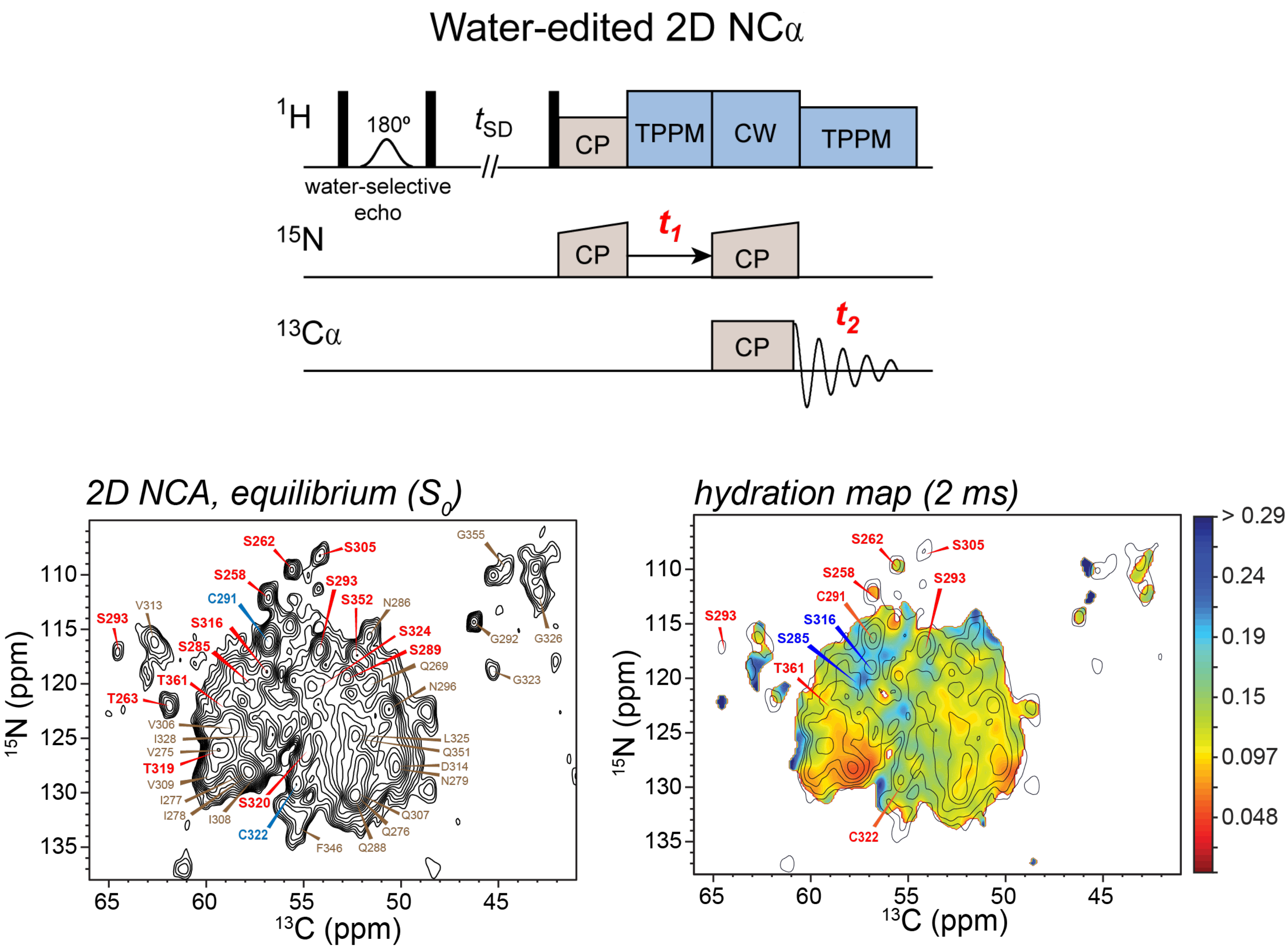
Water-Edited 2D CC Spectra of Tau

Water-edited 2D CC

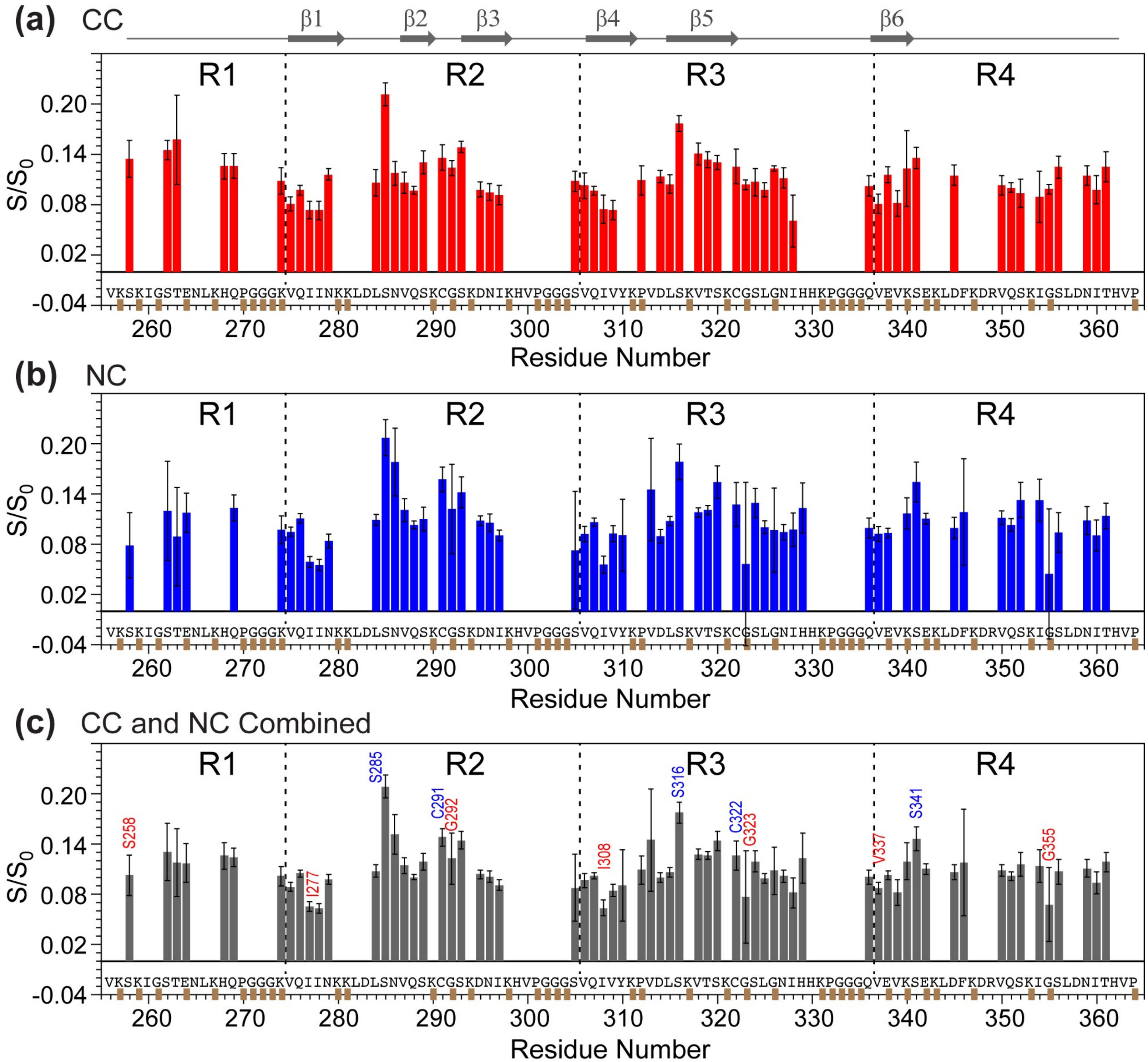


Dregni, Duan, and Hong, ***Biochemistry***, 2020.

Water-Edited 2D NC Spectra of Tau

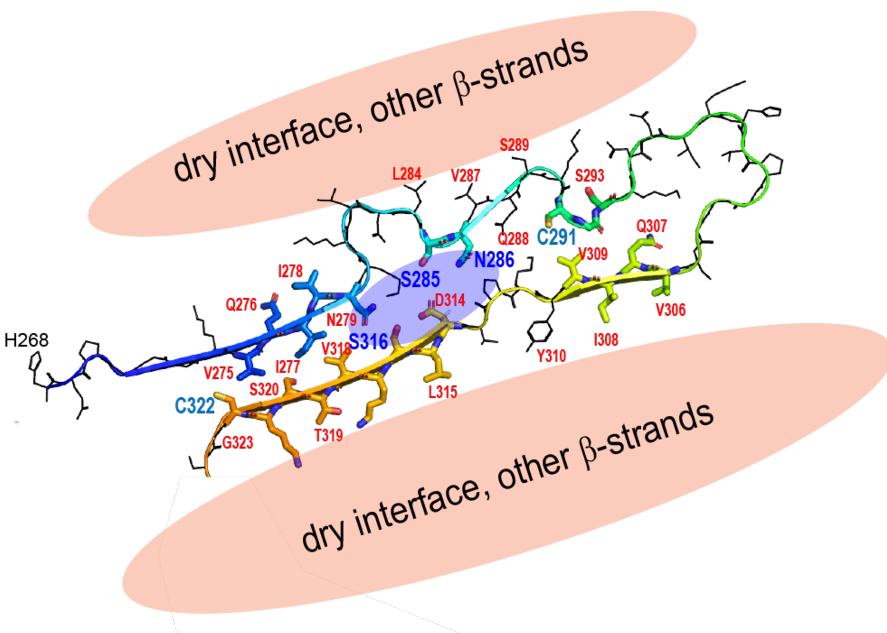


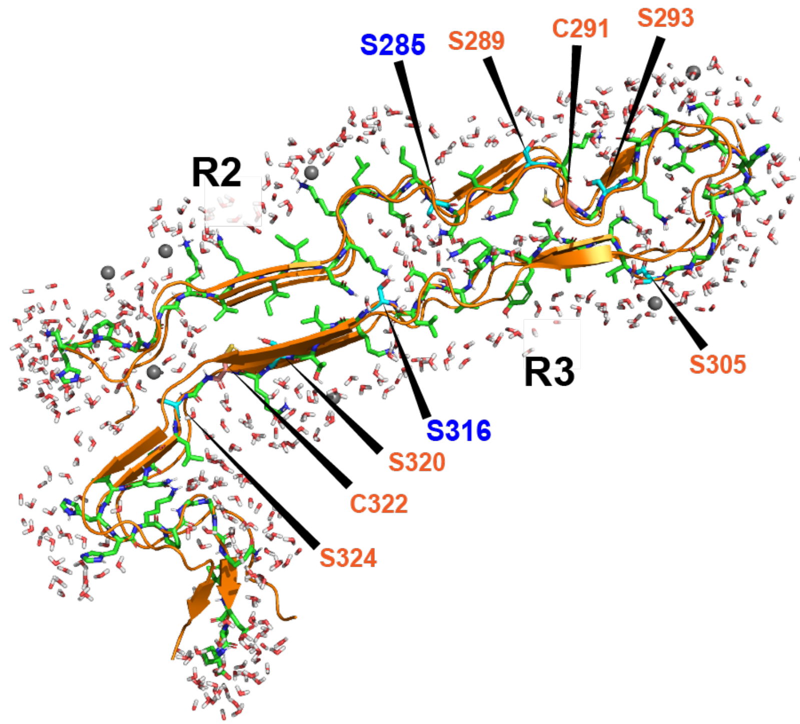
Dregni, Duan, and Hong, ***Biochemistry***, 2020.

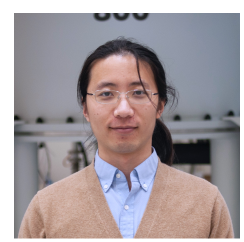


Dregni, Duan, and Hong, ***Biochemistry***, 2020.

Hydrated versus Dry Residues in Tau Fibrils



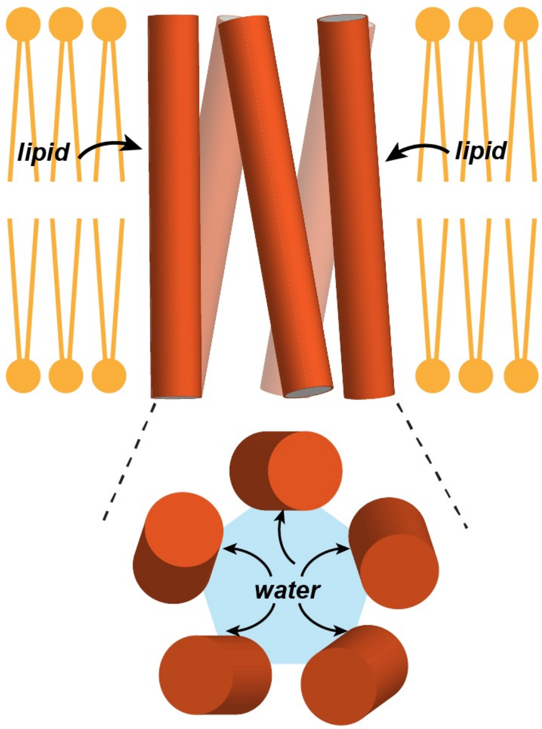


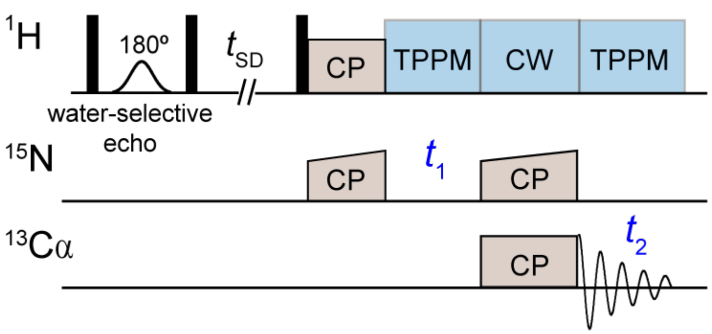


Pu Duan

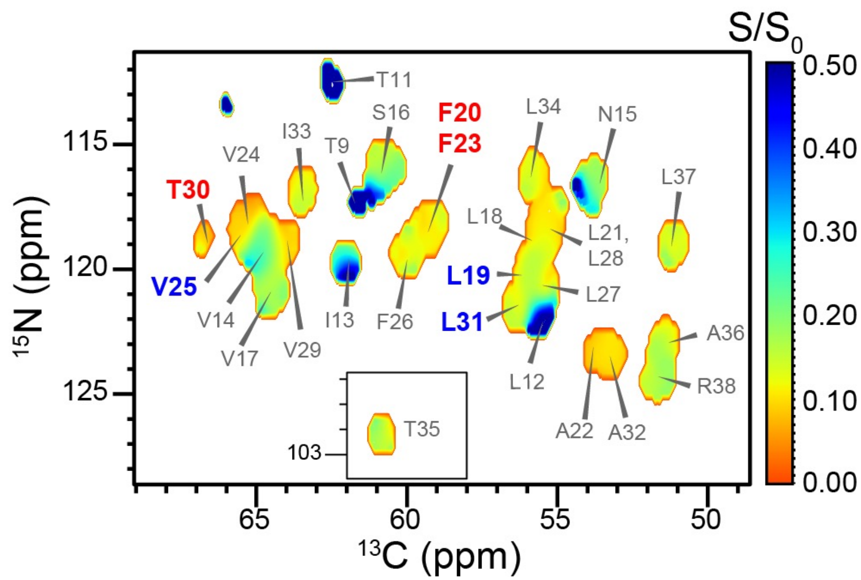
Dregni, Duan, and Hong, ***Biochemistry***, 2020.

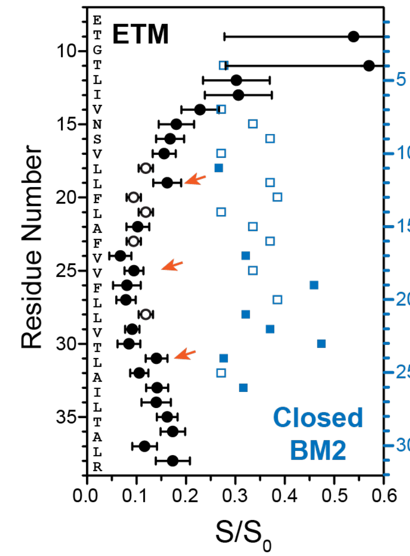
Hydration of a Membrane Ion Channel



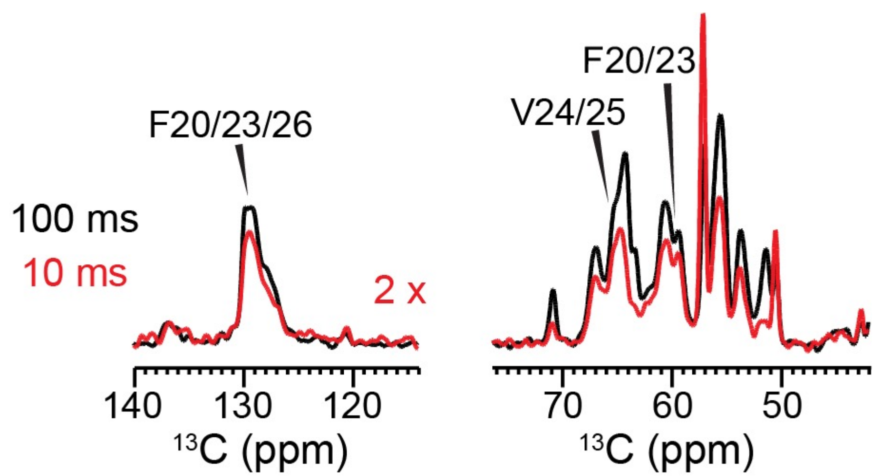


Water-transferred intensities



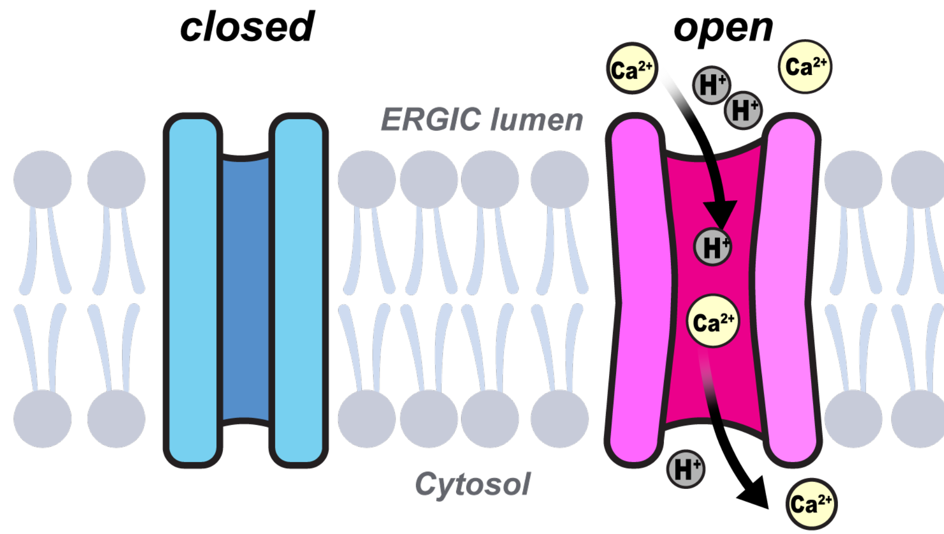


Lipid-transferred intensities



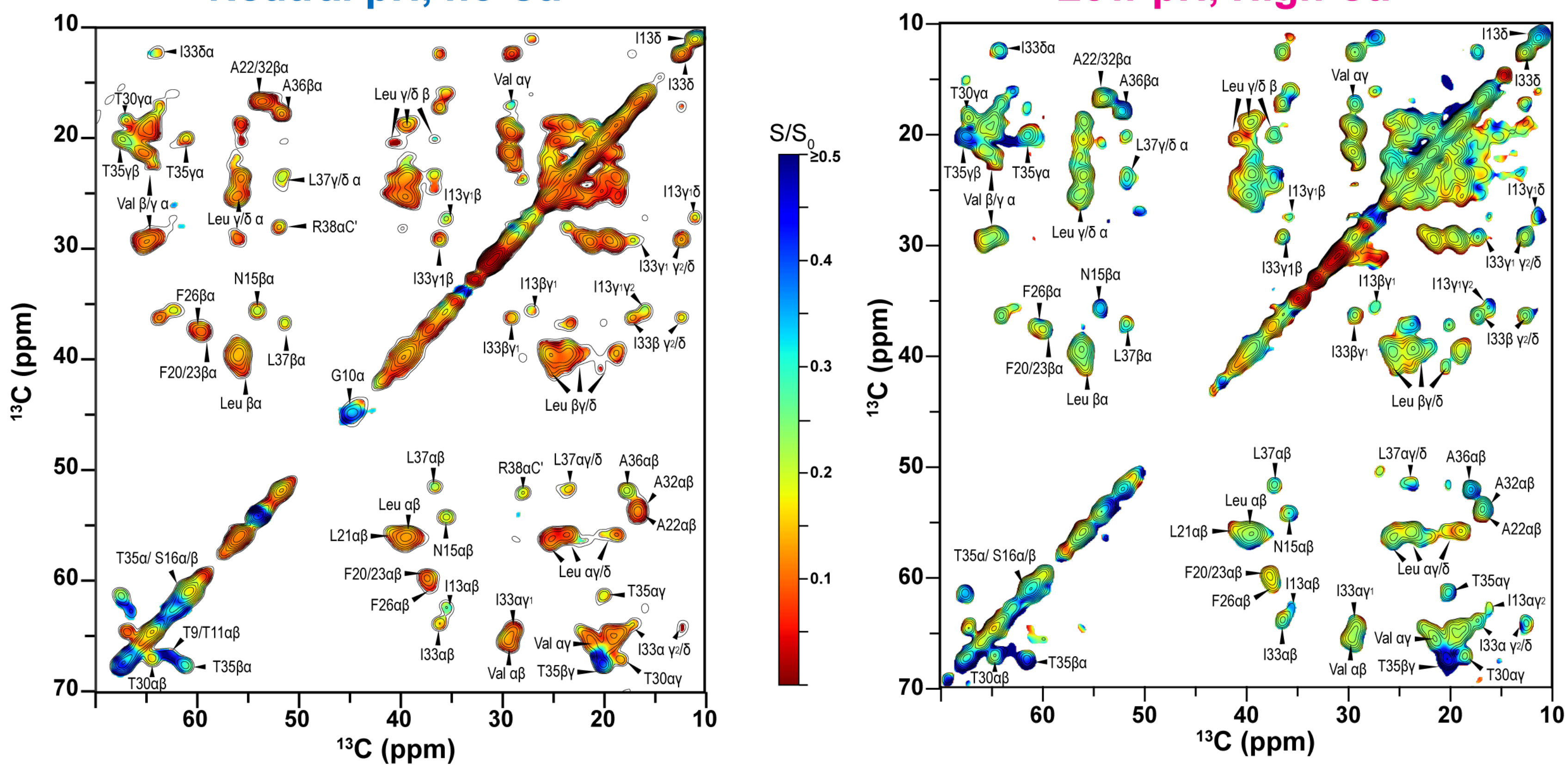
Mandala, McKay, Shcherbakov, Dregni, Kolocouris & Hong, ***Nat. Struc. Mol. Biol***, 2020.

Open State of the E Channel: Higher Water Accessibility



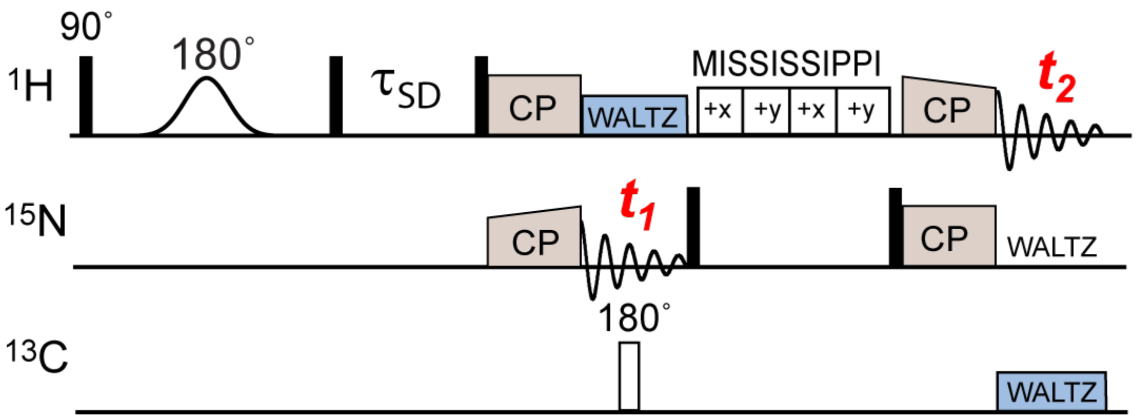
Water-edited 2D CC: 9 ms & 100 ms ^{1}H mixing

Neutral pH, no Ca^{2+} | **Low pH, High Ca^{2+}**

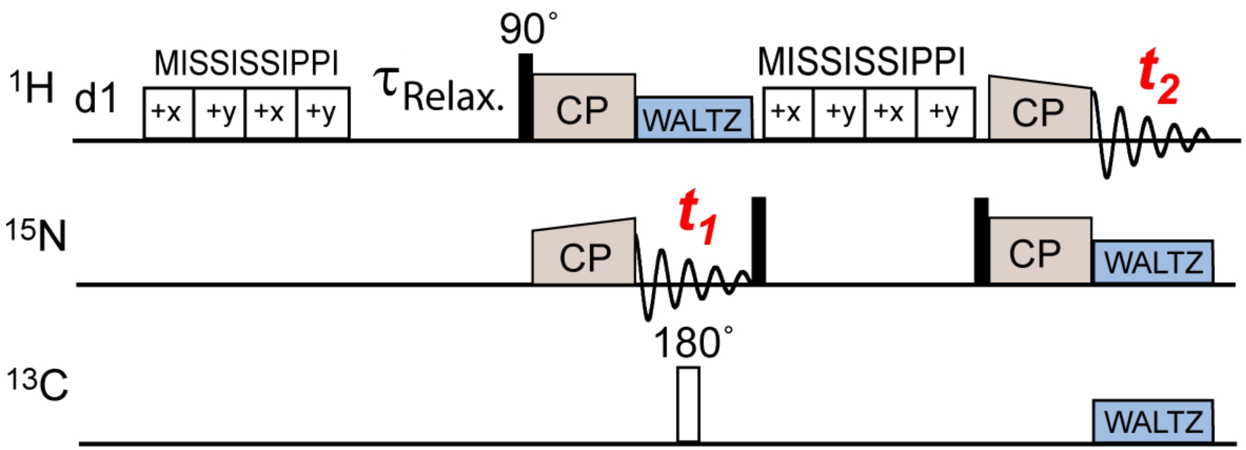


^{1}H-Detected Water-Edited 2D NMR Experiments

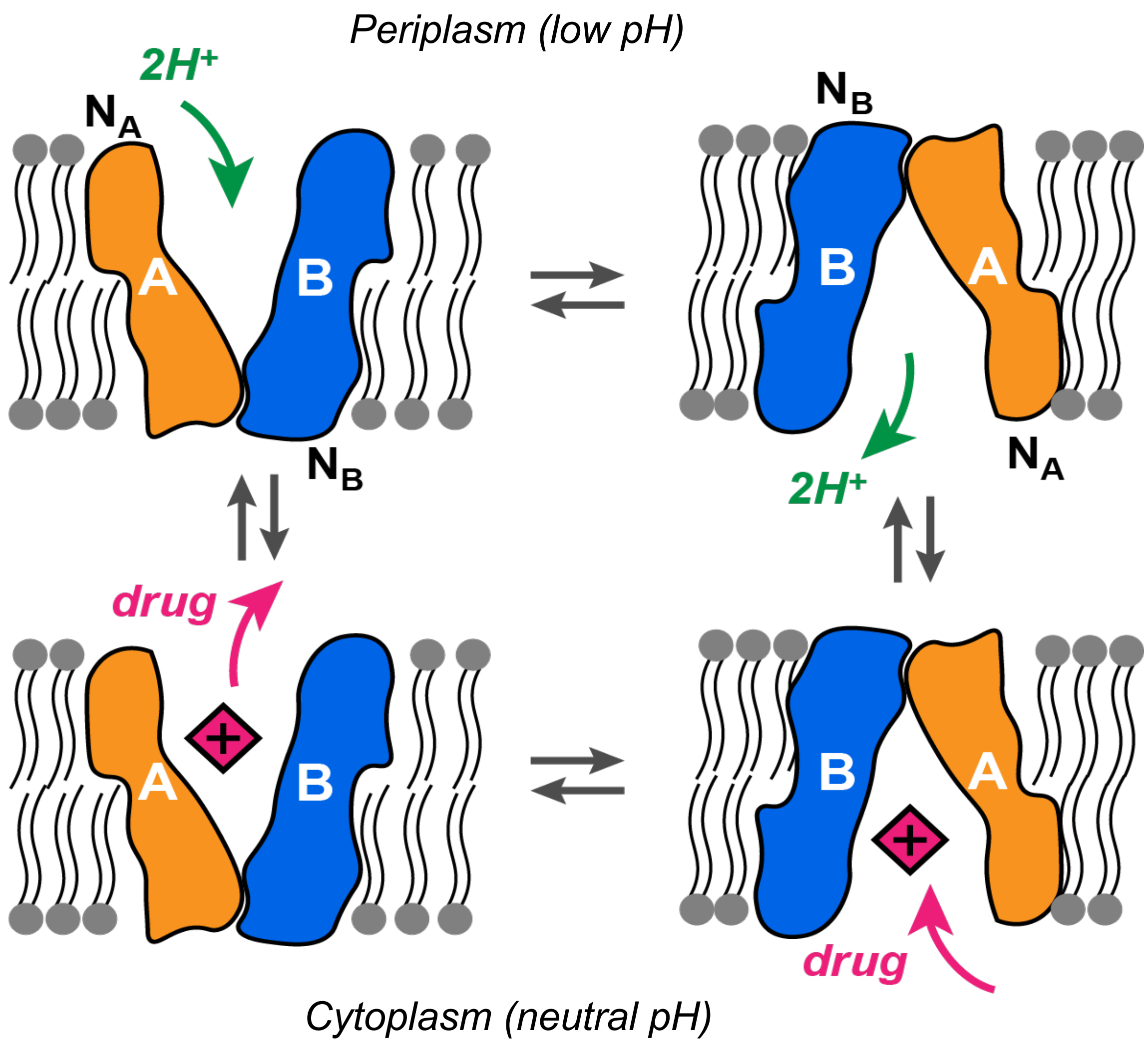
Water-Edited 2D hNH



^{1}H T_1 Saturation-Recovery 2D hNH

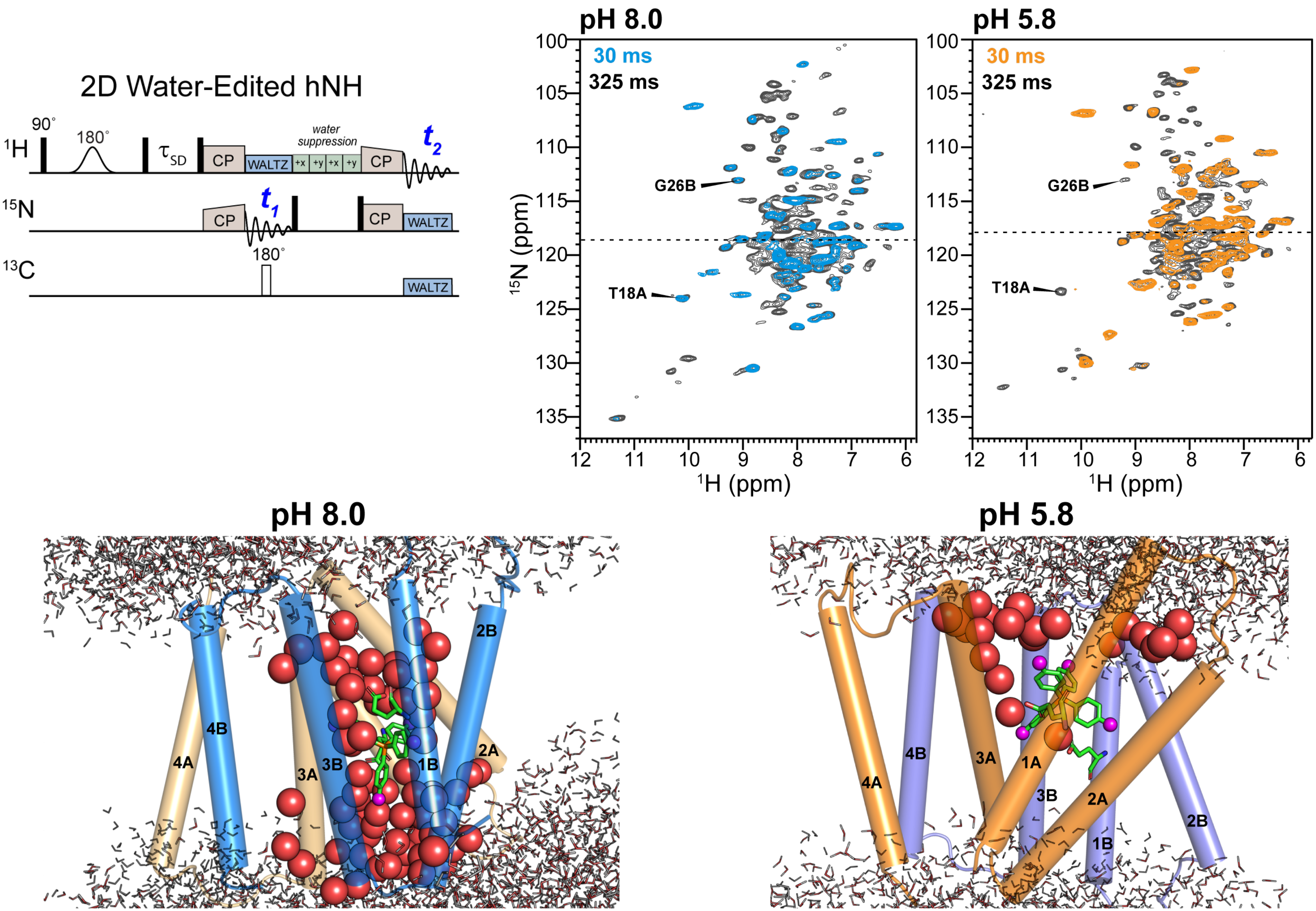


EmrE: A Proton-Coupled Multidrug-Resistance Transporter



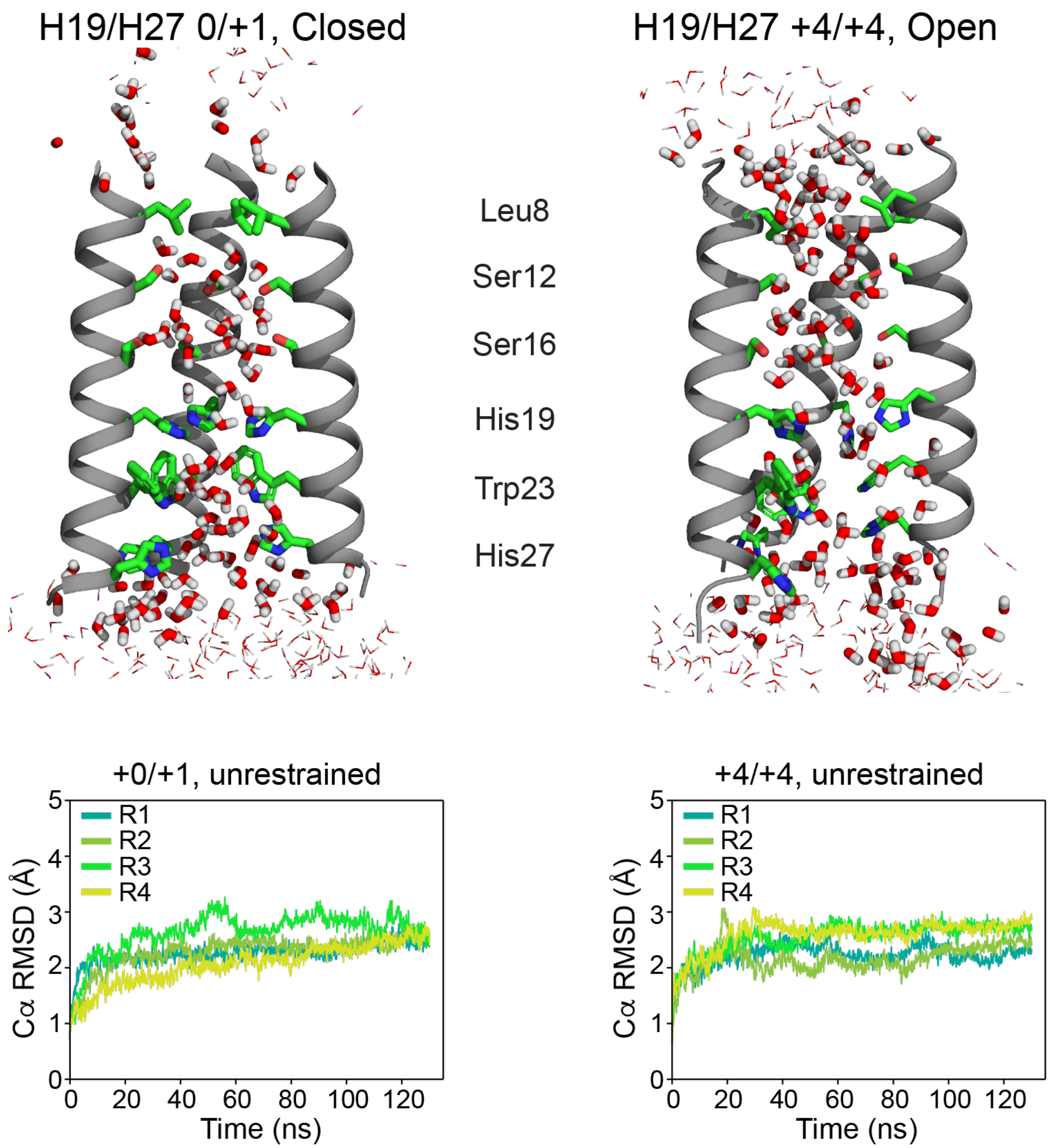
Prior NMR studies: **Katie Henzler-Wildman,** Nate Traaseth, Clemens Glaubitz

Hydration of the Substrate Binding Pocket of EmrE



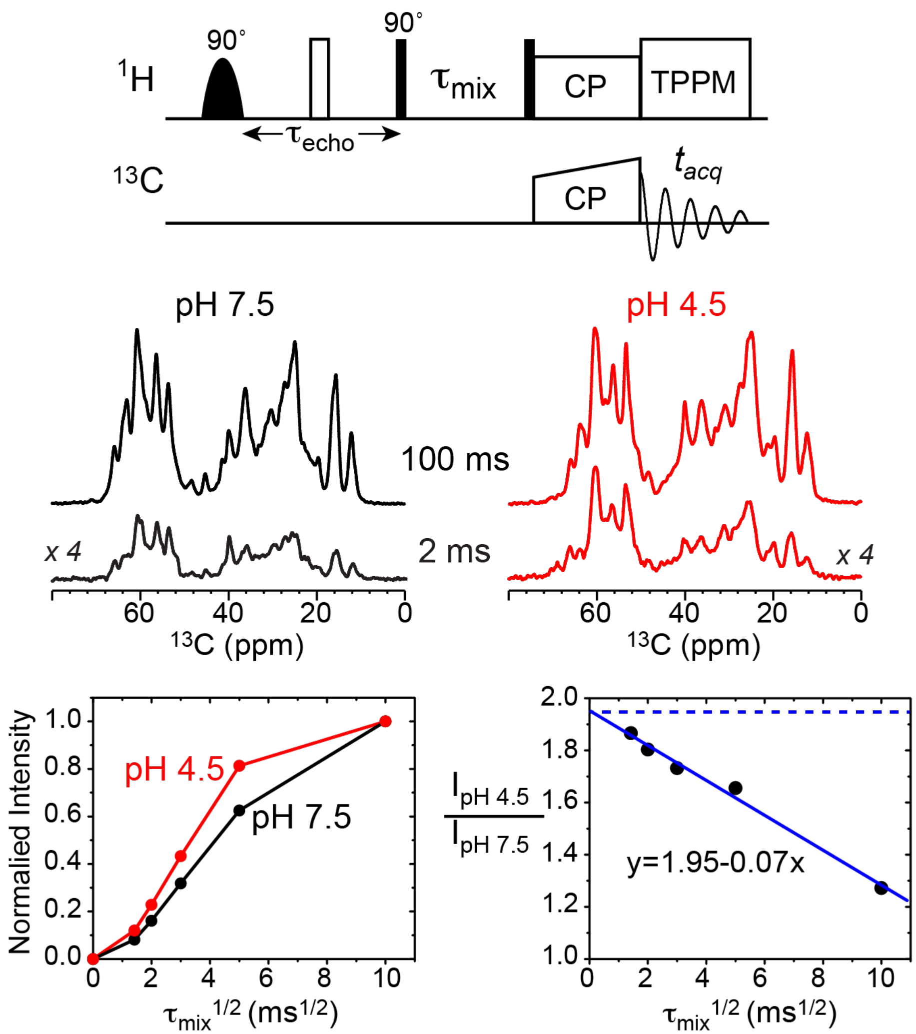
Shcherbakov, Spreacker, Dregni, Henzler-Wildman & Hong, ***Nature Commun***. *2022.*

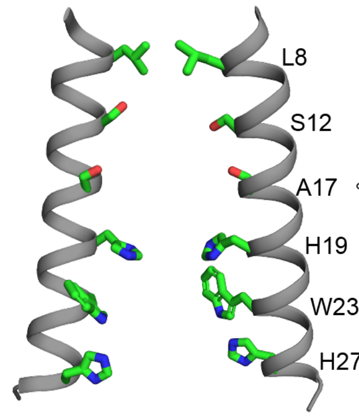
MD Simulations of Water in Influenza M2 Channels

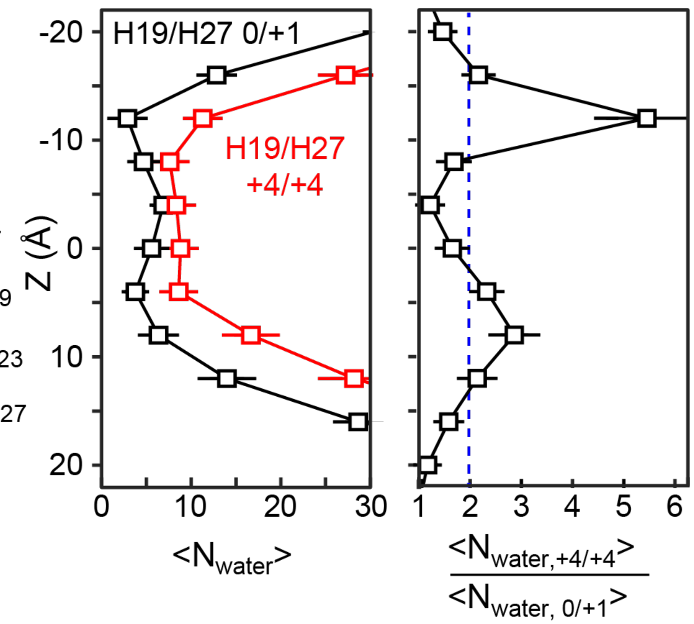


Michiel Niesen, Dina Sharon, Adam Willard

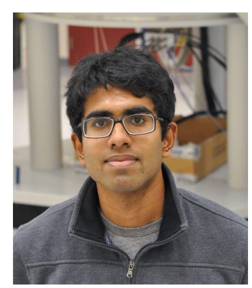
Water in BM2 Channels: More Water @ Low pH







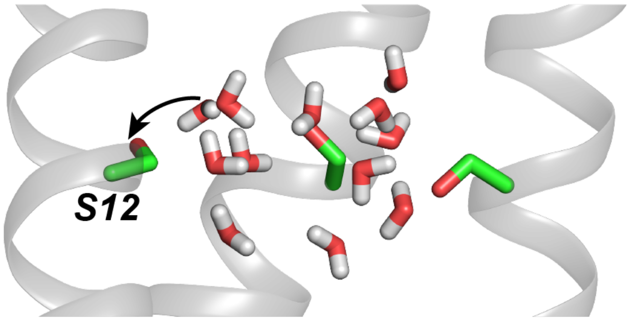
Marty Gelenter



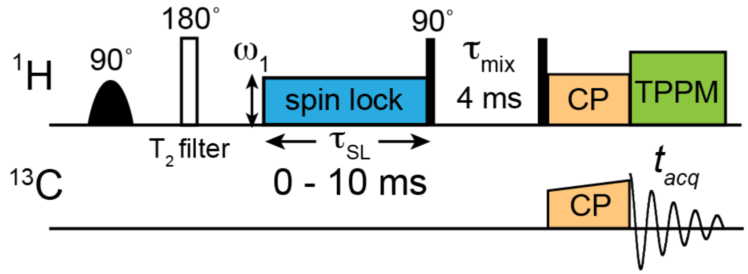
Shiva Mandala

Gelenter …Hong, ***Comm. Biol.*** 2021

Water in the BM2 Channel is Anisotropic

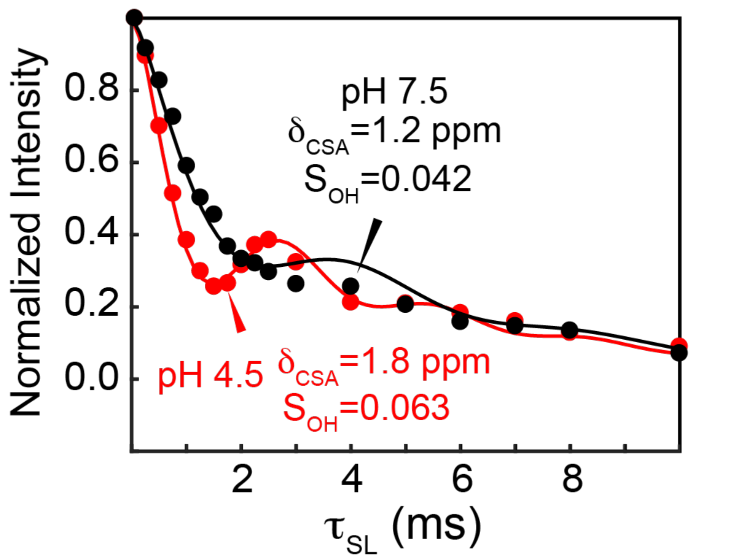


^{13}C-detected water ^{1}H anisotropy

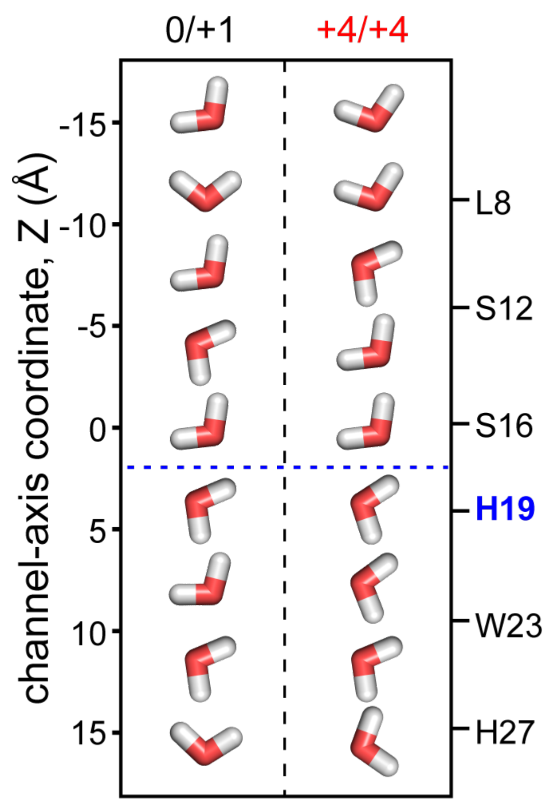


Rigid-limit water
^{1}H CSA: 28 ppm

$\omega_1 = 2\omega_r$
14 kHz MAS, spin lock @ 28 kHz



Most probable water orientations



Marty

Gelenter, Mandala, Niesen, Sharon, Dregni, Willard and Hong, ***Commun. Biol.*** 2021