# *NMR Theory and Techniques for Studying Molecular Dynamics*

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Motivations:

- Molecular dynamics cause structural changes and heterogeneity.
- Molecular motion can average spectral lineshapes, reduce intensities, and affect NMR relaxation properties.
- Molecular motions are abundant in proteins and integral to their function.

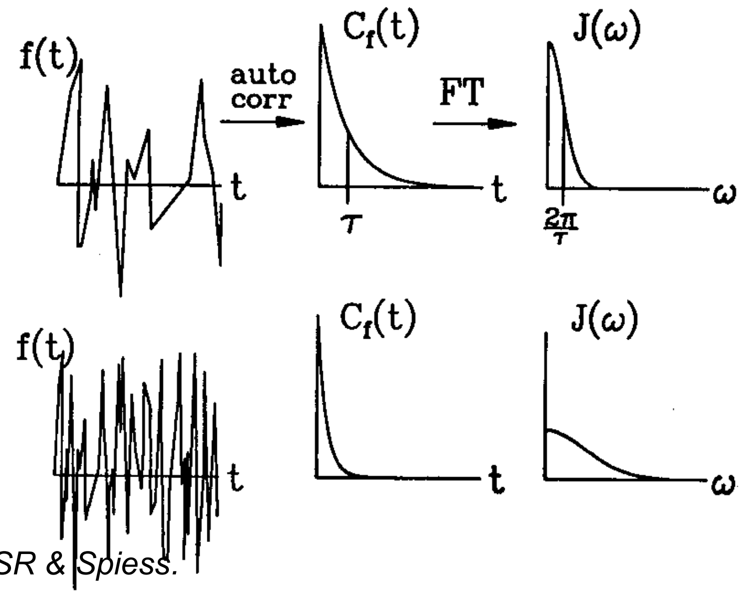
1. Timescale and amplitude of motion from NMR
2. Fast motion: average tensor & symmetry
3. Experiments for measuring amplitudes of fast motion
4. Order tensor and order parameter
5. Experiments for measuring slow motions
6. $^2$H quadrupolar NMR
7. Determining motional rates from relaxation NMR
8. Practical aspects of protein dynamics study by SSNMR

# Timescales and Amplitudes of Motion from NMR

• **Timescale**: rate $k$, correlation time $\tau_c \sim 1/k$, unit $s^{-1}$ reflects the stochastic nature of motion. Rate ≠ *frequency*.

• Correlation function C(t): provides a smooth curve for the random motion.

$$C(t) \sim \langle f(0) \cdot f(t) \rangle$$




*KSR & Spiess.*

• **Amplitude**: motional geometry, number of sites, and their relative orientation.

• Rotation vs. translation: here we only discuss rotation, or reorientation.

• Diffusive motion: infinite number of sites, infinitesimal step size. e.g. isotropic diffusion, on a cone and in a cone.

• Discrete motion: e.g. methyl 3-site jump, phenylene ring 2-site jump.

# Motional Regimes in NMR

- **Fast motion**: *k* >> $\delta$, which is the NMR interaction strength.
  - Amplitude and geometry information obtainable from spectral line narrowing (e.g WISE, DIPSHIFT, LG-CP, CSA recoupling, $^2$H quad echo);
  - Timescale from relaxation times ($T_1$, $T_{1\rho}$);
  - More geometry information from spectrally resolved $T_1$ and $T_2$ in $^2$H spectra.

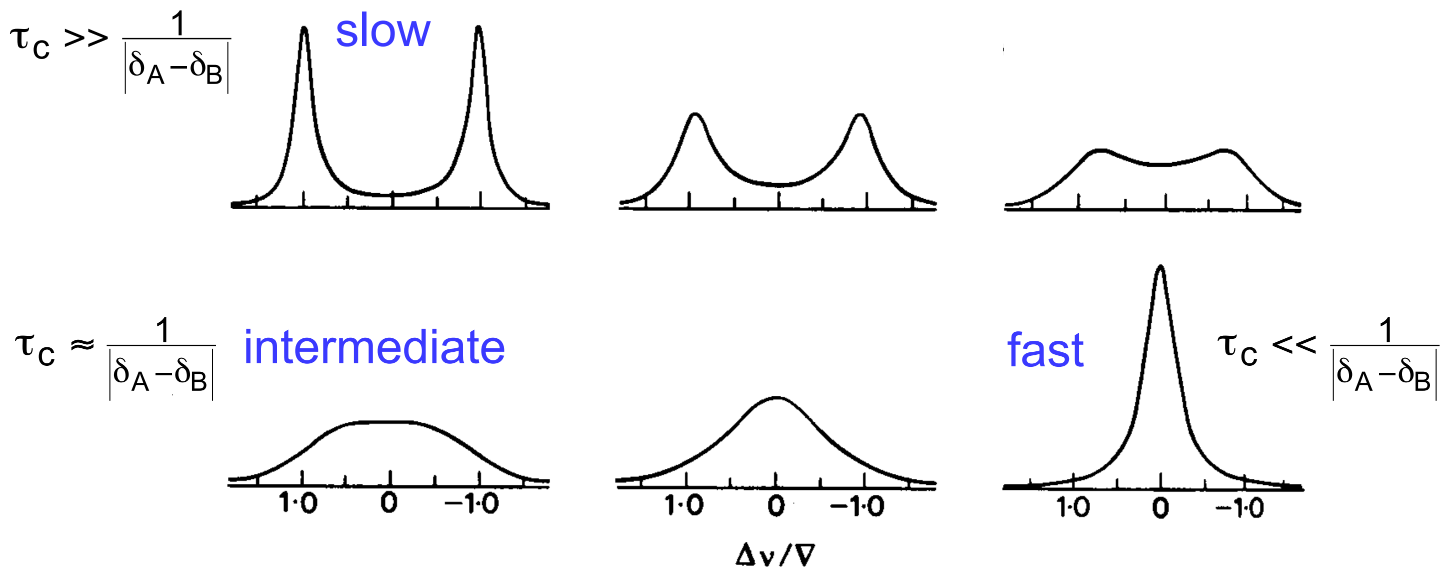
- **Slow motion**: k << $\delta$.
  - Exchange NMR: 2D exchange, stimulated echo, CODEX
  - Amplitude from the distance from diagonal in the 2D spectra, or $Nt_r$ dependence in CODEX.
  - Timescale from the $t_m$ dependence of the exchange intensity
  - Geometry from the final value in CODEX or the off-diagonal pattern.

- **Slow to intermediate motion**: k < $\delta$.
  - Echo experiments (Hahn echo or quadrupolar echo)
  - $T_2$ minimum, $T_{1\rho}$ minimum.

- **Intermediate motion**: k ~ $\delta$.
  - Amplitude and timescale from $^2$H lineshape and $T_{1\rho}$ relaxation times.
  - Interference with $^1$H decoupling.

# Motional Regimes in NMR

Example: equal-population 2-site exchange:




*Harris.*

- **Fast motion**: frequency view, order tensor **S** and order parameter.

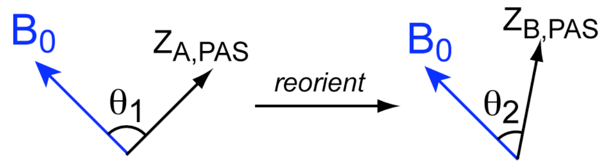
- **All other regimes:** time domain view essential.
  - Slow motion: occurs during the mixing time, is directly monitored.
  - Intermediate motion: explicit time-domain calculation.

# (Fast) Motional Averaging of NMR Frequencies

- NMR frequency is orientation-dependent: $\omega(\theta,\phi) = \delta\,\frac{1}{2}\left(3\cos^2\theta - 1 - \eta\sin^2\theta\cos 2\phi\right)$

where $(\phi, \theta)$ are the powder angles of $B_0$ in the tensor PAS.

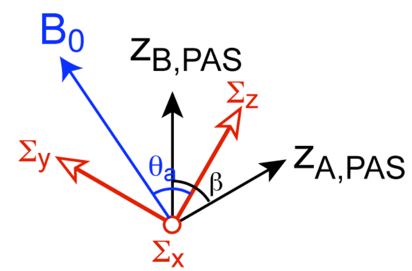
e.g: uniaxial interaction ($\eta$=0):




- Assuming fast motion among N sites with occupation probability $p_j$, then $\overline{\omega} = \sum_{j=1}^{N} p_j \omega_j$

- The average of a second rank tensor is still a second-rank tensor. So the average tensor $(\Sigma_1, \Sigma_2, \Sigma_3)$ has $\overline{\delta}$, $\overline{\eta}$ and powder angles $(\theta_a, \phi_a)$ w.r.t. $B_0$. The averaged NMR frequency is:

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




$\overline{\delta}, \overline{\eta}$ depend on motional geometry and symmetry.

$\overline{\delta}, \overline{\eta}$ of the averaged tensor usually differ from the $\delta$, $\eta$ of the original PAS.

$\overline{\delta}$ of an averaged dipolar coupling tensor can contain sign information.

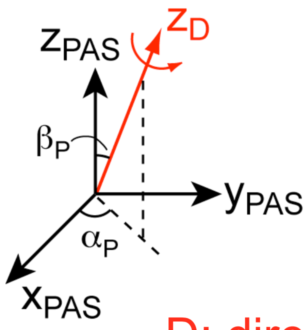
$\overline{\eta}$ of an averaged dipolar coupling tensor is generally not uniaxial.

# Motional Geometry: Symmetry Considerations

$\bar{\delta}$, $\bar{\eta}$ of the averaged tensor may be obtained from the symmetry of the motion:

- Tetrahedral jumps: $\bar{\delta} = 0$
- Isotropic diffusion: $\bar{\delta} = 0$
- Uniaxial rotation: $\bar{\eta} = 0$
- Discrete jumps over N≥3 sites with $C_N$ symmetry: $\bar{\eta} = 0$

For uniaxial rotation or jumps with $C_N$ symmetry (N≥3), the unique axis of the average tensor is the symmetry axis:




$$\sigma_{PAS} \rightarrow \sigma_D = \sum_{j=1}^{N} R^{-1}\left(\alpha_P, \beta_P, j\frac{360°}{N}\right) \cdot \sigma_{PAS} \cdot R\left(\alpha_P, \beta_P, j\frac{360°}{N}\right) \xrightarrow{\text{average}} \bar{\sigma}_D$$

How to calculate $\bar{\delta}$?

- $\bar{\delta}$ is the frequency obtained when $B_0$ is along the $Z_D$ axis. At this orientation, the frequency can be calculated by the equation without motion:

$$\bar{\delta} = \frac{1}{2}\delta\left(3\cos^2\beta_P - 1 - \eta\sin^2\beta_P\cos 2\alpha_P\right)$$

*KSR and Spiess.*

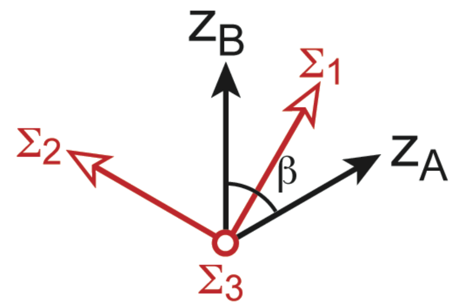
# Symmetry of the Average (Sum) Tensor

Consider equal-occupancy 2-site jumps between two uniaxial tensors (e.g. $^{13}C$-$^{1}H$ dipolar or $^{2}H$ quadrupolar couplings).

Average (or sum) tensor: $\Sigma = (\sigma_A + \sigma_B)/2 = (\sigma_B + \sigma_A)/2$
- must be invariant under the rotation.

For two uniaxial tensors, the three principal axes are:

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




| | $\sigma_A$ | $\sigma_B$ |
|---|---|---|
| $\Sigma_3$ axis: | $90^\circ$, | $90^\circ$ |
| $\Sigma_1$ axis: | $\beta/2$, | $\beta/2$ |
| $\Sigma_2$ axis: | $90^\circ+\beta/2$, | $90^\circ-\beta/2$ |

1, 2, 3 convention: left to right, or large to small frequencies: $\overline{\omega}_1 > \overline{\omega}_2 > \overline{\omega}_3$

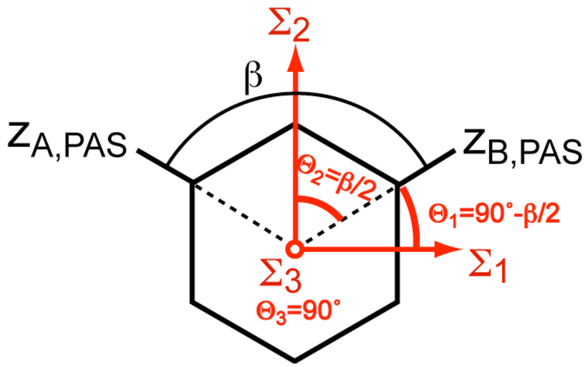
$\beta < 90^\circ$ and $\beta > 90^\circ$ have different axes labels.

Once the three principal axes directions are fixed, the three principal values are:

$$\overline{\omega}_n = \frac{1}{2}\delta\left(3\cos^2\Theta_n - 1\right),\ \Theta_n \text{ is the direction angle between } z_{PAS} \text{ and } \Sigma_1, \Sigma_2, \Sigma_3$$

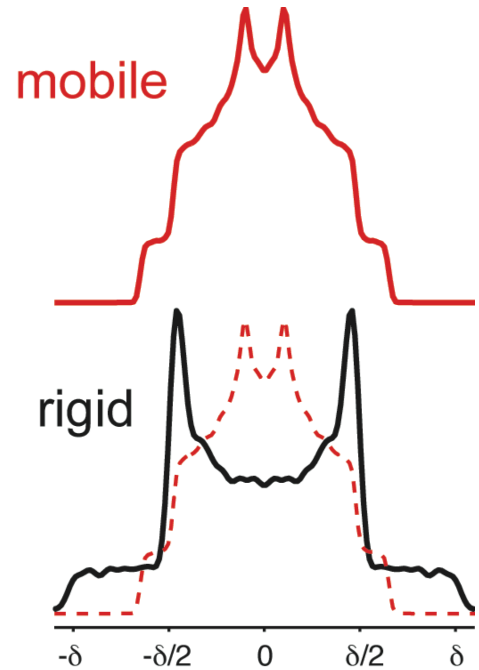
# Phenylene Ring Flip: Motionally Averaged Lineshape

Consider $^{2}H$ or C-H dipolar spectra ($\eta$=0 PAS):
Reorientation angle $\beta$ = 120˚




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

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




*KSR & Spiess.*

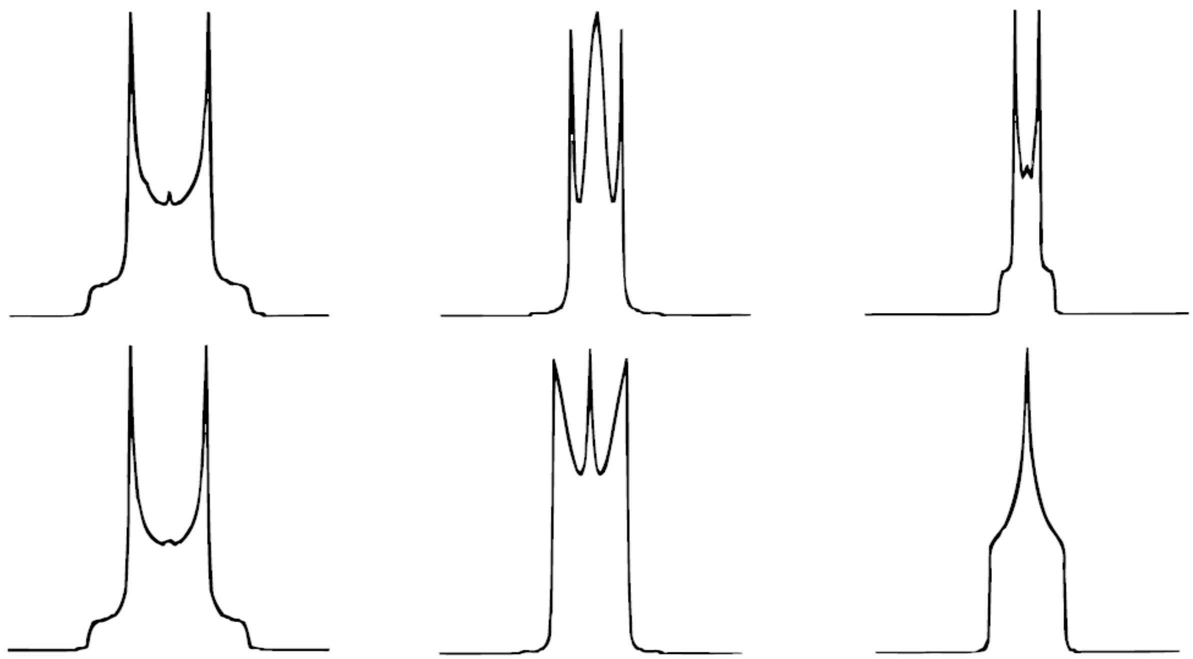
# Lineshapes of Two Other Common Motions

- Methyl 3-site jumps: $\beta$ = 109.5˚: $\bar{\delta} = -\delta/3$
- Equal-population trans-gauche isomerization: $\beta$ = 109.5˚.

slow | intermediate | fast

CD$_3$ jump

trans-gauche isomerization



$k$ | $10^4$ s$^{-1}$ | $10^6$ s$^{-1}$ | $10^8$ s$^{-1}$

*Palmer et al, JPC (1996), 100, 13293.*

# Orientation and Magnitude of the Difference Tensor

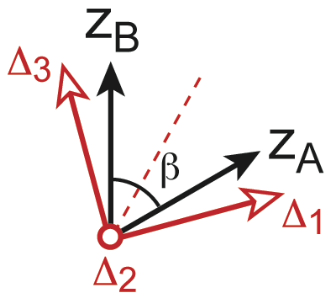
Difference tensor: $\Delta = \sigma_A - \sigma_B = -(\sigma_B - \sigma_A)$

- must have sign inversion under switch of the two individual tensors.
- relevant in exchange experiments.

For two uniaxial tensors, the three orthogonal axes of the difference tensor are:

- Normal of the AOB plane, $\Delta_2$
- In the AOB plane, 45˚ angles from the bisector, $\Delta_3$ and $\Delta_1$

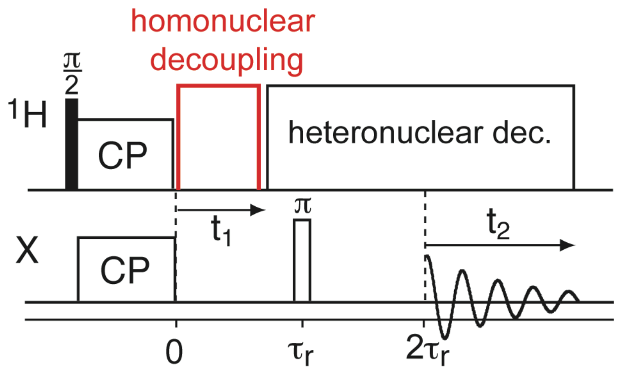
| | $\sigma_A$ | $\sigma_B$ |
|---|---|---|
| $\Delta_1$ axis: | 45˚-β/2, | 45˚+β/2 |
| $\Delta_2$ axis: | 90˚, | 90˚ |
| $\Delta_3$ axis: | 45˚+β/2, | 45˚-β/2 |




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

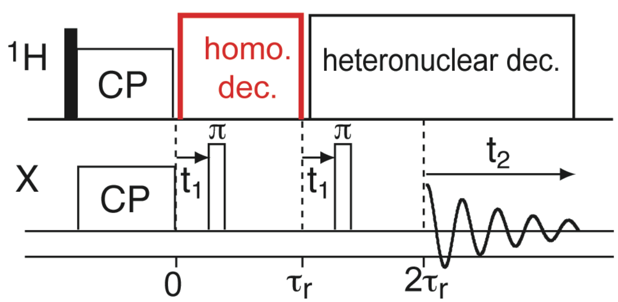
# Fast Motion Experiments: 1.DIPSHIFT

## Original DIPSHIFT




$$\omega_{exp} = \delta_{XH} \cdot k_{homo}$$

## Doubled DIPSHIFT




$$\omega_{exp} = 2 \cdot \delta_{XH} \cdot k_{homo}$$

- a separated-local field (SLF) technique: X magnetization evolves under X-H dipolar coupling.
- constant time $t_1$: sampling occurs within one rotor period.
- $^{1}$H homonuclear decoupling: e.g. MREV-8 for $\nu_r$<5 kHz, FSLG for $\nu_r$~ 5-15 kHz, DUBMO, etc.
- X isotropic shift in $\omega_2$ gives site resolution.

$$\Phi(t_1) = \int_0^{t_1} dt\,\omega(t) = \delta \int_0^{t_1} dt\left[C_1 \cos(\omega_r t + \gamma) + C_2 \cos(2\omega_r t + 2\gamma)\right],$$

$\delta \equiv -\mu_0 / 4\pi \cdot \gamma_H \gamma_X \hbar^2 / r_{HX}^3$, and $C_1, C_2$ are functions of powder angles $(\alpha, \beta, \gamma)$ and the asymmetry parameter $\eta$

$$\Phi_{CH}^{2x}(t_1) = \int_0^{t_1} dt\,\omega(t) - \int_{t_1}^{\tau_r} dt\,\omega(t)$$
$$= \int_0^{t_1} dt\,\omega(t) - \left[\int_0^{\tau_r} dt\,\omega(t) - \int_0^{t_1} dt\,\omega(t)\right]$$
$$= 2\int_0^{t_1} dt\,\omega(t) = 2\Phi_{CH}^{1x}(t_1)$$

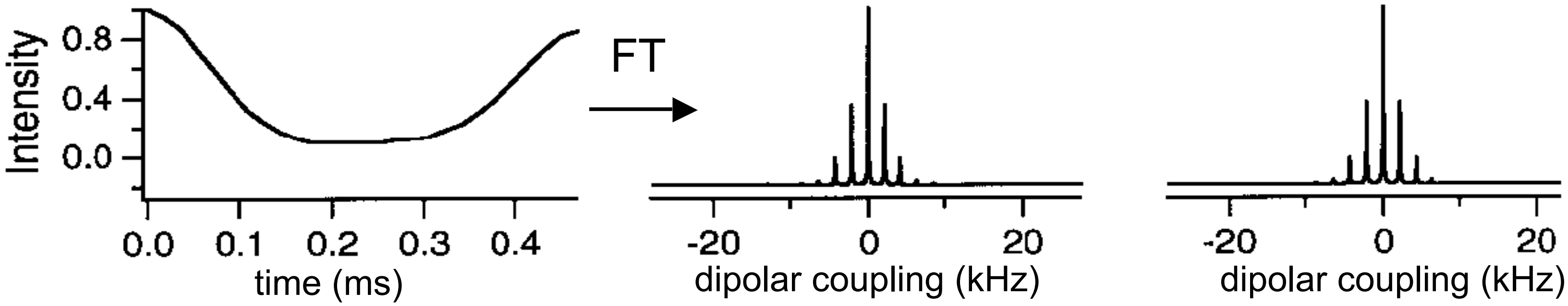
- doubling the phase allows higher $\nu_r$ to be used.

*Munowitz et al, JACS, 103, 2529 (1981).*
*Hong et al, JMR, 129,85 (1997).*

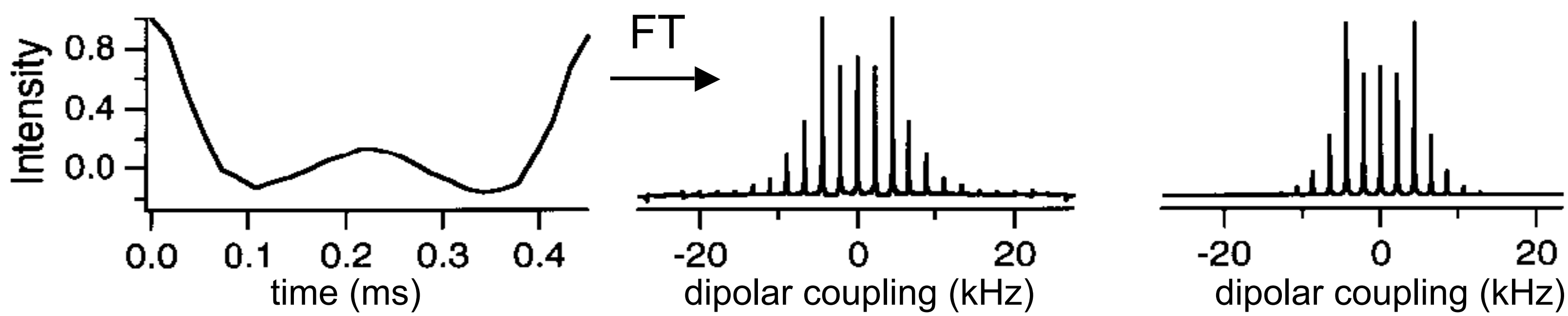
# DIPSHIFT Time and Frequency Signals

Original DIPSHIFT

$t_1$ modulation | expt. spectrum | simulated spectrum




Doubled DIPSHIFT



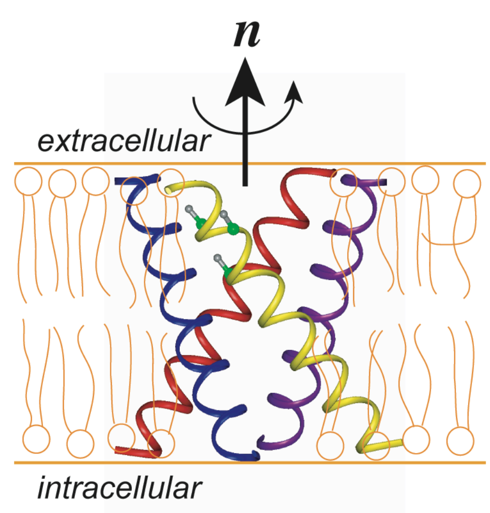

- Stick spectrum obtained by concatenating $f(t_1,\omega_r)$ over $n\tau_r$, followed by FT.
- Simulation can be in either frequency or time domain.
- Couplings can in principle be amplified by 2, 4…2n times by more $\pi$ pulses.

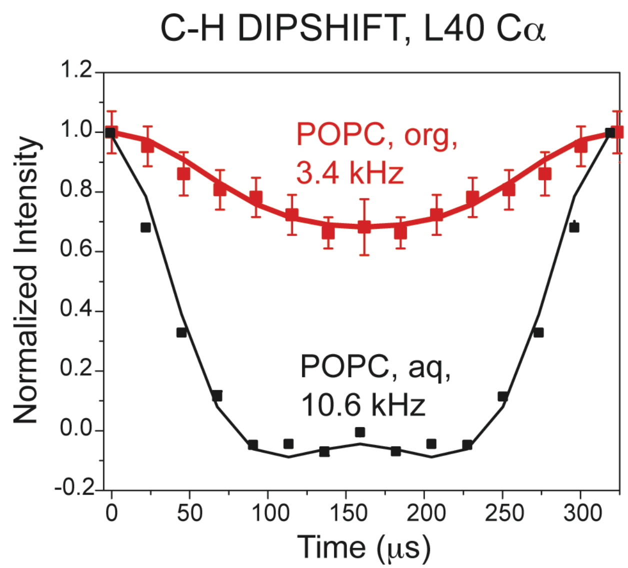
*Hong et al, JMR, 129,85 (1997).*

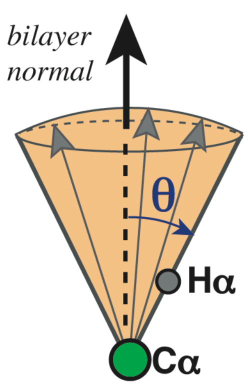
# Manifestation of Motion from DIPSHIFT Data

M2 transmembrane peptide of influenza A virus

22 27 34 41
**SSDPL VVAASII GILHLIL WILDRL**



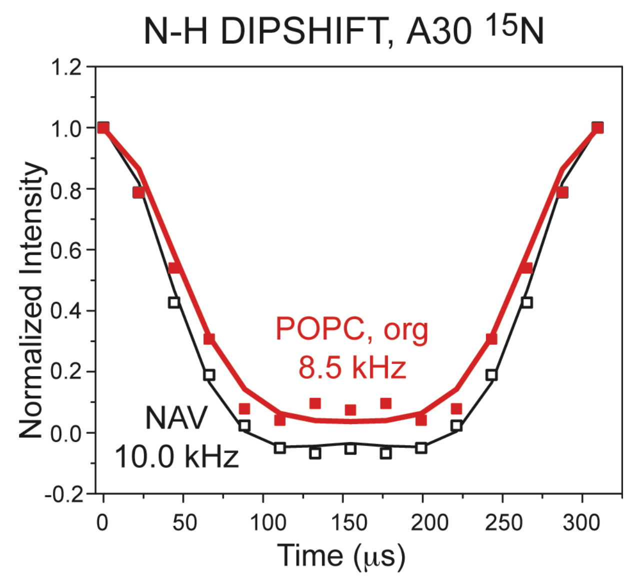




$$S = \frac{\overline{\Delta\nu}}{\Delta\nu} = \frac{1}{2}(3\cos^2\theta - 1)$$

rigid limit: 10.7 kHz

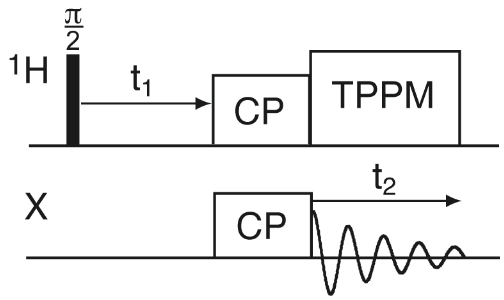
POPC (aq): S = 1, no motion.

POPC (org): S = ±0.32 → θ = 42°, 70°



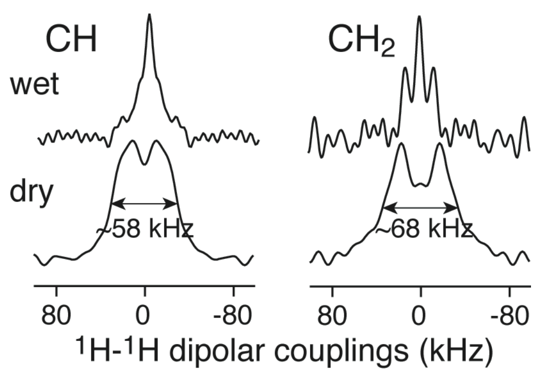

*Cady et al, JACS, 129, 5719 (2007).*

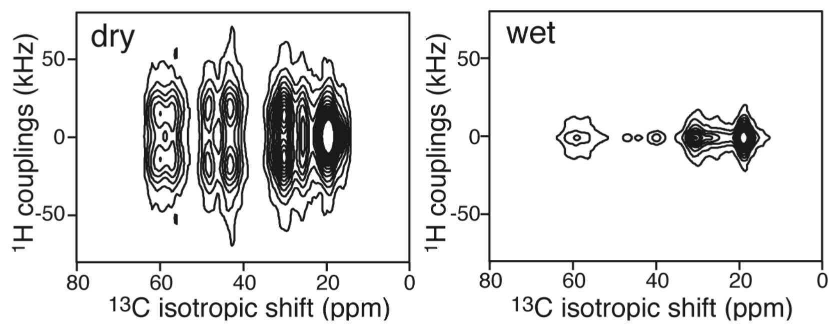
# Fast Motion Experiments: 2.WISE



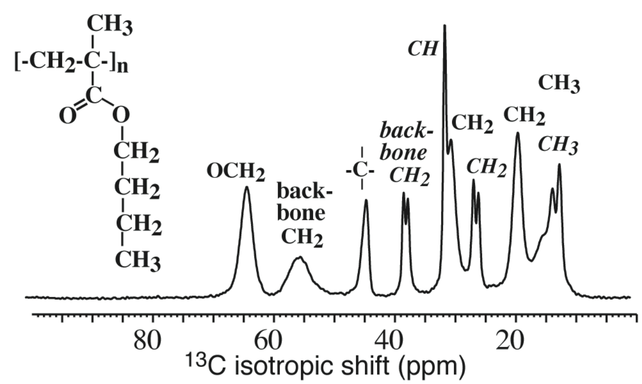

- $t_1$ dimension: $^1H$-$^1H$ dipolar coupling > $^1H$-X dipolar coupling >> $^1H$ CSA.
- Suitable for $\nu_r$ < 10 kHz.
- Qualitative and quick assessment of mobility.
- In proteins, rigid-limit FWHM are 55-60 kHz for CH groups, 65-70 kHz for $CH_2$ groups.

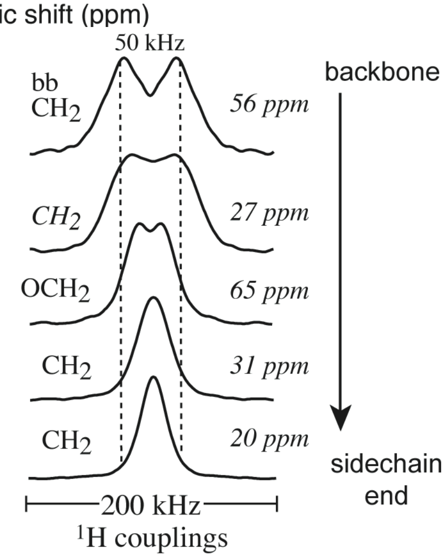
e.g: hydration dynamics of elastin $(VPGVG)_n$




e.g: mobility gradient in poly(n-butyl methacrylate).

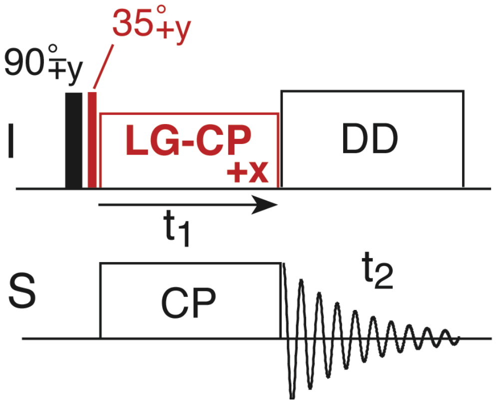


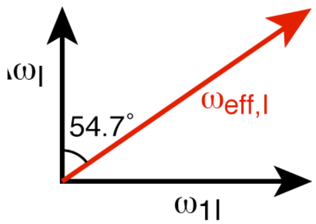





*Clauss et al, Macromolecules, 25, 5208 (1992).*
*Yao et al, MRC, 42, 267 (2004).*
*KSR and Mao, JACS, 124, 13938 (2002).*

# Fast Motion Experiments: 3.LG-CP



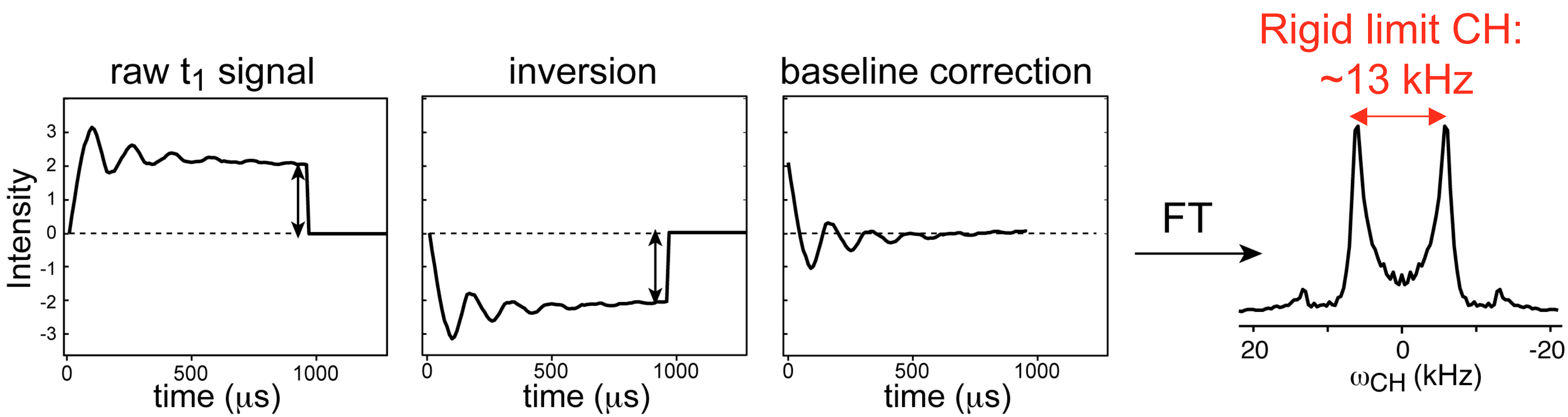

- simple to use: increment contact time to obtain $t_1$ dipolar modulation.
- 35° pulse prepares $\rho_0$ along the tilted $^1$H effective field.
- $^1$H homonuclear decoupling by magic-angle spin lock, removes H-H coupling, reveals X-H dipolar oscillation.
- suitable under fast MAS ($\nu_r$ >~10 kHz).
- symmetrized $\omega_1$ and site resolution in $\omega_2$.

**LG-CP condition:**

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

$$\omega_{IS,LG} = \delta \cdot \cos\theta_m = 0.577 \cdot \delta$$

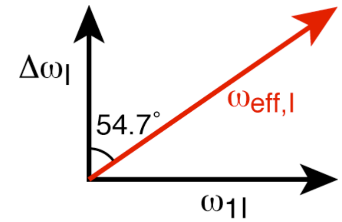
*Van Rossum et al, JACS, 122, 3465 (2000).*
*Hong et al, JPC, 106, 7355 (2002).*



# LG-CP Average Hamiltonian

- in the doubly rotating frame:

$$H = \underbrace{\omega_{1I} I_x + \omega_{1S} S_x}_{\text{rf part}} + \Delta\omega_I I_z + \underbrace{\omega_{IS}(t) I_z S_z}_{\text{I–S hetero. dipolar}} + \underbrace{\omega_{II}(t)\left(3 I_z^i I_z^j - I^i \cdot I^j\right)}_{\text{I-I homo. dipolar}}$$




where $\omega_{IS}(t) = 2\delta\left[C_1 \cos(\omega_r t + \gamma) + C_2 \cos(2\omega_r t + 2\gamma)\right]$, and $\delta = -\frac{\mu_0}{4\pi}\frac{\gamma_I \gamma_S \hbar^2}{r_{IS}^3}$

- In the tilted frame defined by the pulses, $R = e^{-i\theta_m I_y} e^{-i\frac{\pi}{2} S_y}$,

$$H^T = \underbrace{\omega_{\text{eff},I} I_z + \omega_{1S} S_z}_{\text{rf part}} + \omega_{IS}(t)\left(\sin\theta_m I_x S_x - \cos\theta_m I_z S_x\right),$$

- In the interaction frame defined by the rf pulses, $H_{IS}^T = e^{iH_{rf}t} H_{IS} e^{-iH_{rf}t}$

under the n=±1 matching condition: $\omega_{\text{eff},I} - \omega_{1S} = \pm\omega_r$

the time-independent average I-S dipolar coupling is:

$$\overline{H_{IS}^T} = \frac{1}{2}\delta \sin\theta_m \left[I_x^{(23)}(C_1 \cos\gamma) - I_y^{(23)}(C_1 \sin\gamma)\right] = \frac{1}{2}\delta \sin\theta_m C_1 \left[I_x^{(23)} \cos\gamma - I_y^{(23)} \sin\gamma\right]$$

while $\overline{H_{II}^T} = 0$.

where the 0-quantum 2-spin operators are:

$$I_x^{(23)} \equiv I_x S_x + I_y S_y, \quad I_y^{(23)} \equiv I_y S_x - I_x S_y, \quad I_z^{(23)} \equiv \frac{1}{2}(I_z - S_z)$$

# LG-CP: Evolution of $\rho$ Under the ZQ Hamiltonian

- The averaged I-S dipolar Hamiltonian can be written as a scalar product between the ZQ spin operator and an effective tilted LG field:

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

- In the tilted frame,

$$\rho_0 \propto I_z = I_z^{(14)} + I_z^{(23)}$$

$$\Rightarrow \rho(t) \propto I_z^{(14)} + I_z^{(23)}\cos(\omega_{IS,LG}t) = \frac{I_z}{2}\left(1+\cos\omega_{IS,LG}t\right) + \frac{S_z}{2}\left(1-\cos\omega_{IS,LG}t\right)$$

where $\omega_{IS,LG} = \frac{\delta}{2}\sin\theta_m C_1$

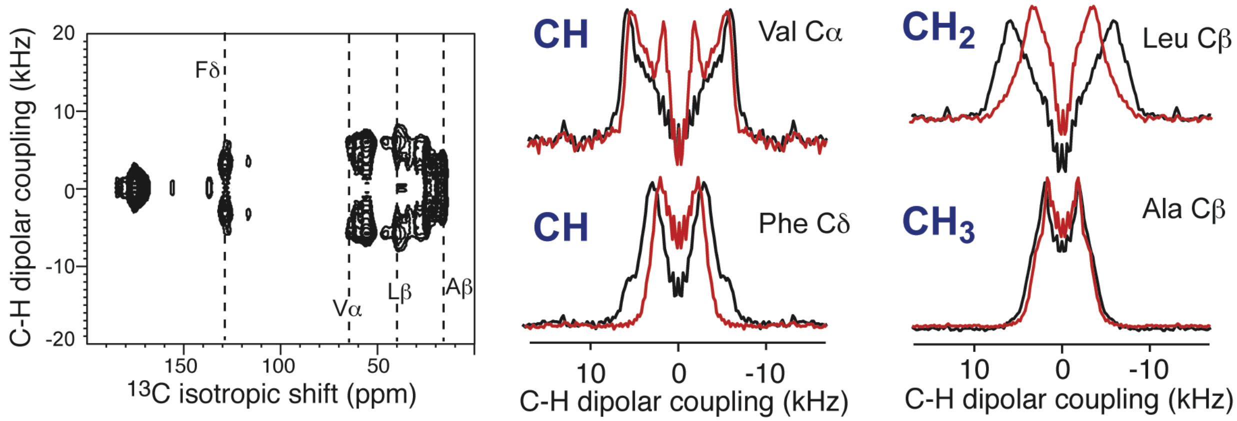
$$\text{Powder average: } C_1 = \frac{3}{4}\sin 2\theta_m \sin 2\beta_{ij},$$

$$\omega_{IS,LG}(\beta_{ij}) = \frac{\delta}{2}\sin\theta_m \frac{3}{4}\sin 2\theta_m \sin 2\beta_{ij} = \frac{1}{2}\underline{\delta\cos\theta_m}\sin 2\beta_{ij}$$

$$\Rightarrow \text{LG}-\text{CP splitting} = \delta\cos\theta_m = 0.577\cdot\delta$$

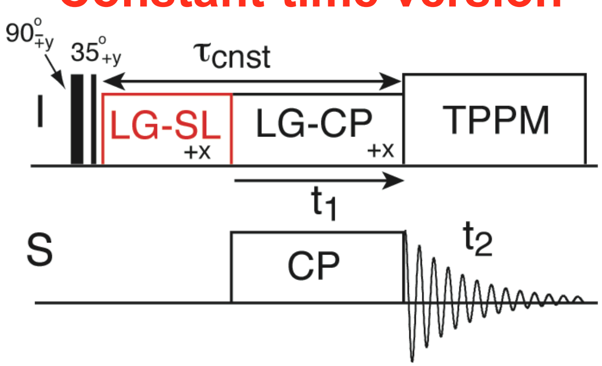
# LG-CP for Studying Membrane Protein Motion

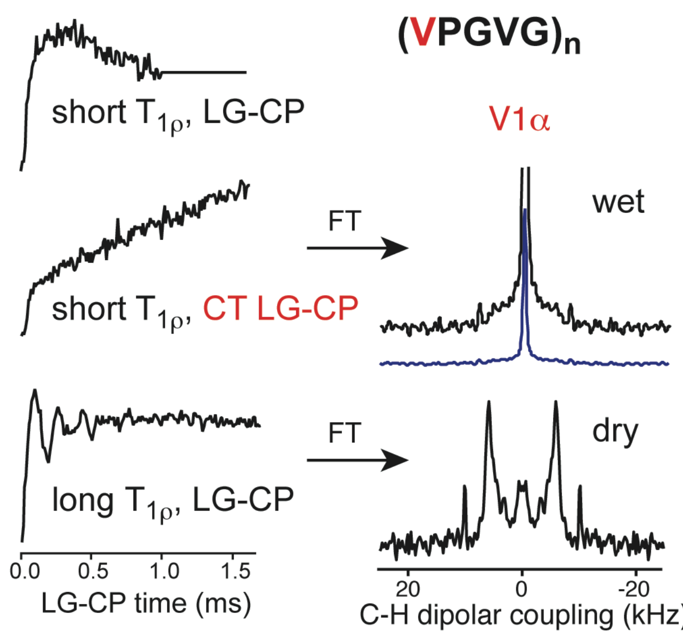
e.g. colicin Ia channel domain, soluble –> membrane-bound state




Improvements of LG-CP **#1**:

**Constant-time version**



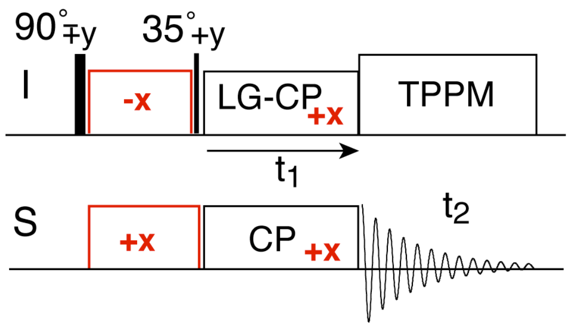




- removes $T_{1\rho}$ effect in $\omega_1$.
- lower sensitivity.

*Hong et al, JPC, 106, 7355 (2002);*
*Yao et al, MRC,42, 267 (2004).*

# LG-CP Variants

Improvements of LG-CP **#2**:
PILGRIM




If at the end of the first CP period the S-spin has the same magnetization as the I-spin, then

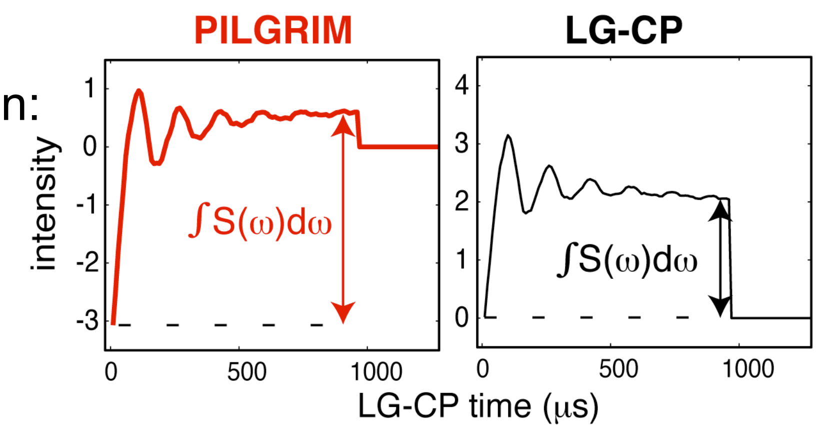
$$\rho(0) = I_z - S_z = 2I_z^{(23)},$$

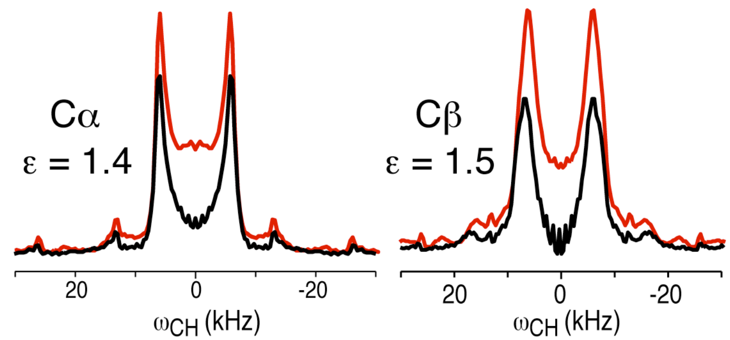
$$\text{compared to original LG-CP } \rho(0) = I_z^{(14)} + I_z^{(23)},$$

2 – fold sensitivity enhancement

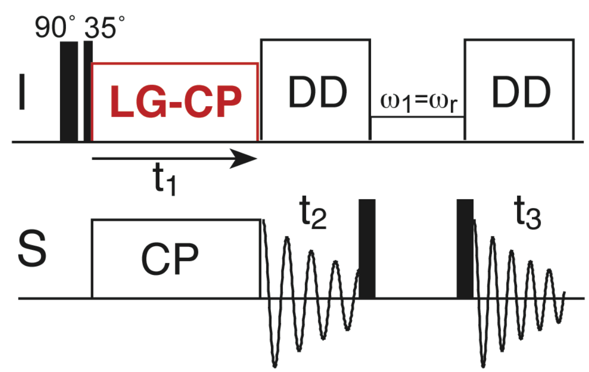
$$\rho(t) = I_z \cos\omega_{IS,LG}t - S_z \cos\omega_{IS,LG}t$$

e.g. Gln:




Improvements of LG-CP **#3**: 3D LG-CP

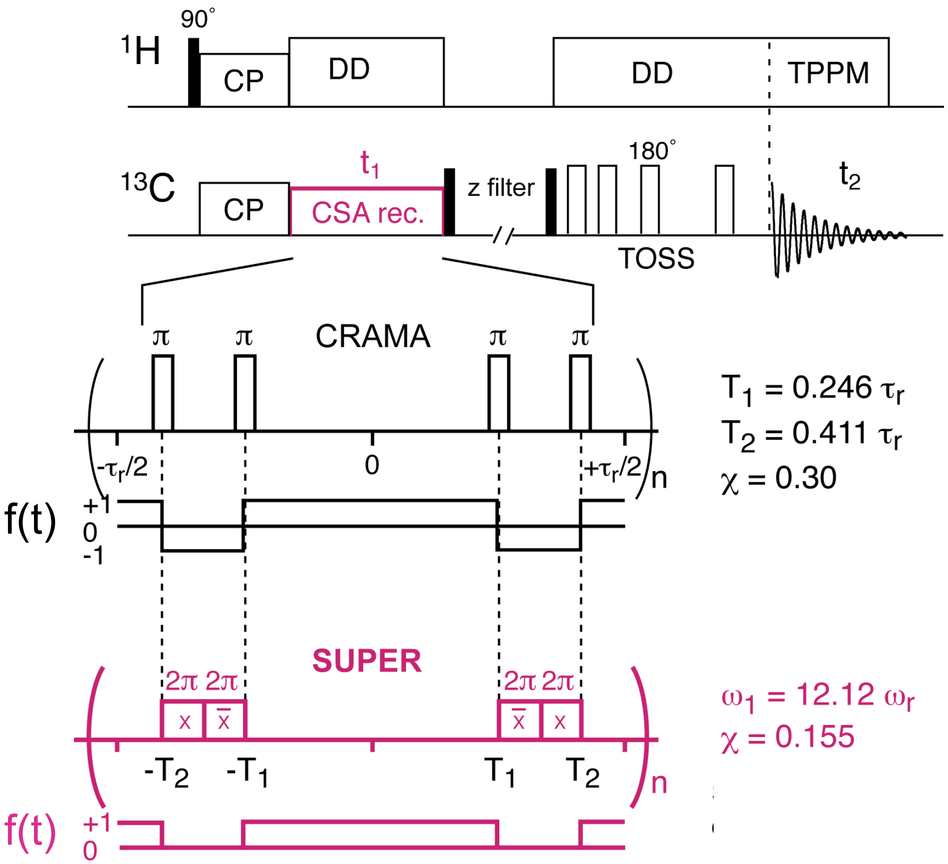



*Hong et al, JPC, 106, 7355 (2002).*
*Lorieau and McDermott, JACS,128, 11505 (2006).*

# Motionally Averaged CSA Lineshape

Main advantage over dipolar coupling:

- Spectra give asymmetry of the motion: e.g. uniaxial motion –> $\overline{\eta} = 0$
- Can be recoupled by SUPER for isolated labels.




2π pulses: better compensation

$$\omega(t) = C_1 \cos\omega_r t + C_2 \cos 2\omega_r t + S_1 \sin\omega_r t + S_2 \sin 2\omega_r t,$$

**static lineshape** : $\omega \propto \mathbf{C_1 + C_2}$

MAS phase under $\pi$ pulses :

$$\Phi = \int_{-\tau_r/2}^{\tau_r/2} \omega(t) f(t) dt$$

choice of pulse positions :

to make $\Phi \propto C_1 + C_2$

$\Rightarrow$ quasi – static lineshape

- $f(\tau_r/2 - t) = f(\tau_r/2 + t)$, even function;
- $\int_0^{\tau_r/2} f(t) \cos\omega_r t \cdot dt = \int_0^{\tau_r/2} f(t) \cos 2\omega_r t \cdot dt$

## Under 2π pulses:

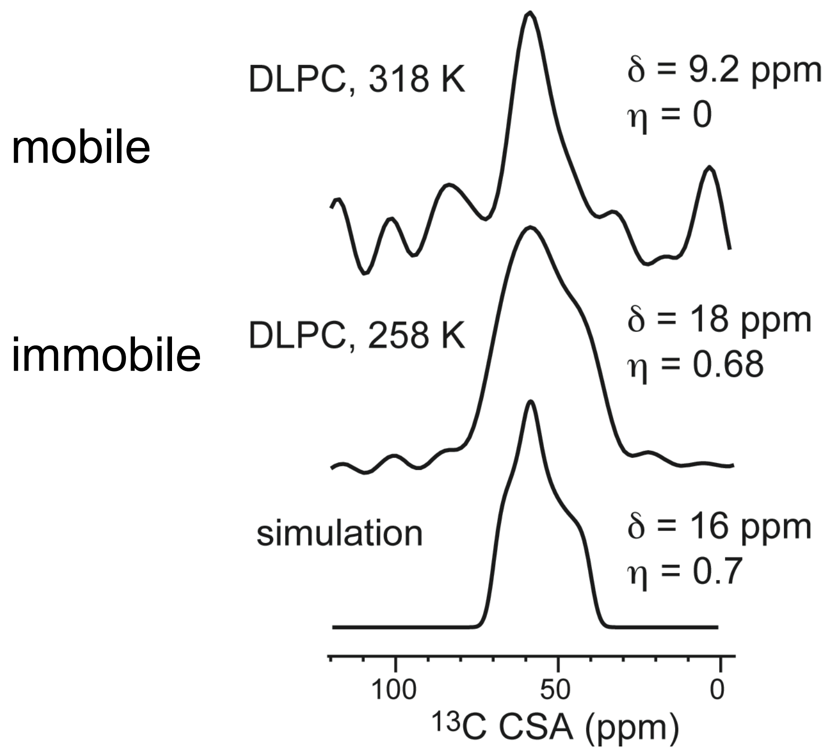
$$\int_0^{\tau_r} f(t)^{SUPER} \cdot dt = \frac{1}{2} \int_0^{\tau_r} f(t)^{CRAMA} \cdot dt$$

$\Rightarrow$ scaling factor $\chi$ is half that of the original expt.

*Tycko et al, JMR, 85, 265 (1989).*
*Liu et al, JMR, 155, 15 (2002).*

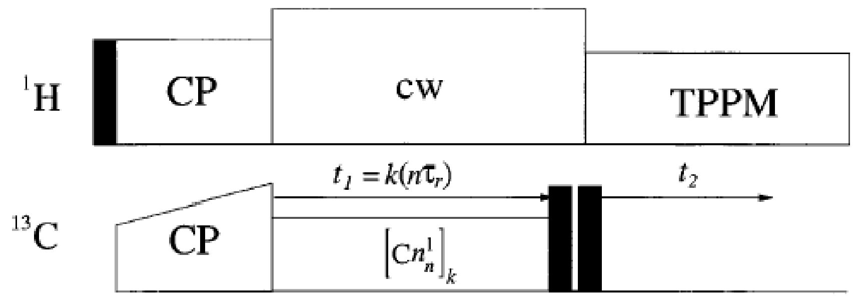
# SUPER Lineshape of a Membrane Peptide

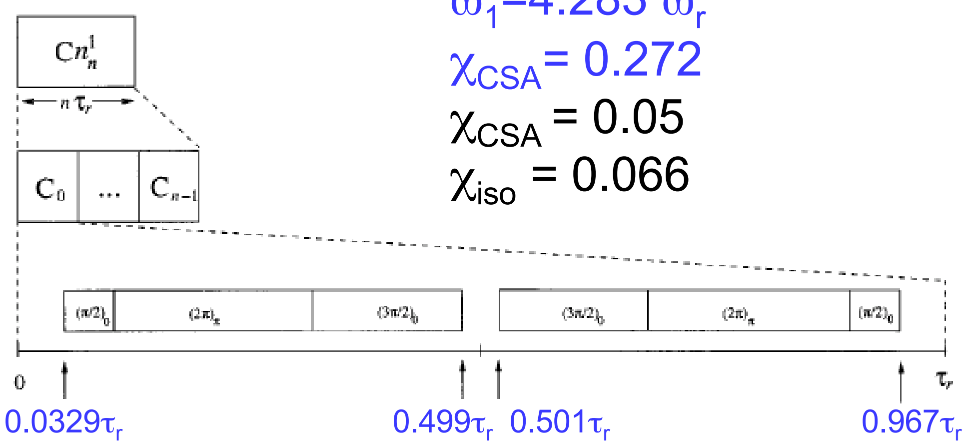
e.g. M2 peptide of influenza A virus, L40 Cα




*Cady et al, JACS, 129, 5719 (2007)*

# Motionally Averaged CSA: U-$^{13}$C Labeled Proteins



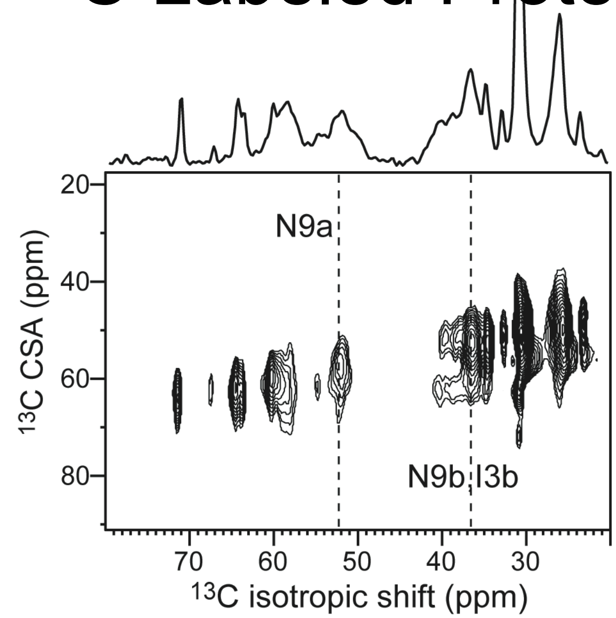



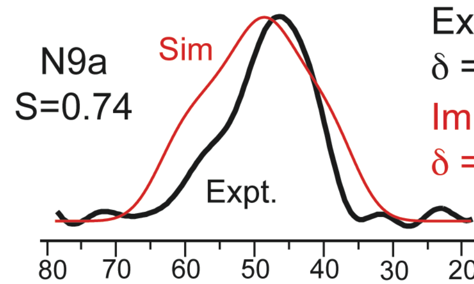

$\omega_1$=4.283 $\omega_r$

$\chi_{CSA}$= 0.272

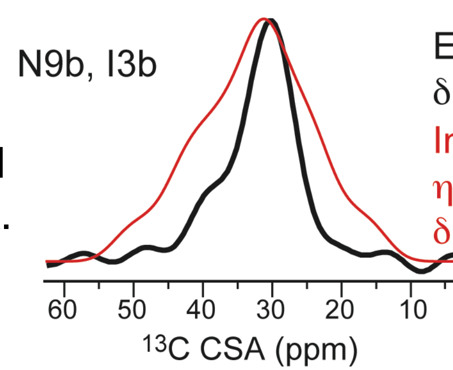
$\chi_{CSA}$ = 0.05

$\chi_{iso}$ = 0.066




Expt: η = 0
δ = 10.6 ppm
Immobile: η = 0.94
δ = 14.4 ppm




Expt: η = 0
δ = 9.0 ppm
Immobile:
η = 0.92, 0.82
δ = 20.5, 11.8 ppm

Example: U-$^{13}$C, $^{15}$N-I3,N9-labeled penetratin in DMPC/DMPG, 303 K.

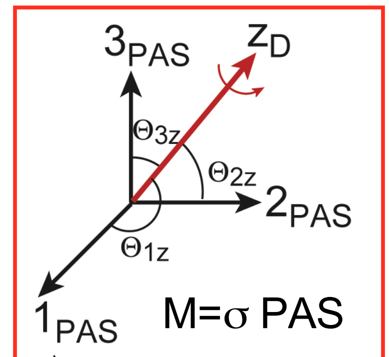
*Chan & Tycko, JCP, 118, 8378, (2003).*
*Su et al, unpublished data.*

# *NMR Theory and Techniques for Studying Molecular Dynamics*

4. Order tensor and order parameter
5. Experiments for measuring slow motions
6. $^{2}H$ quadrupolar NMR
7. Determining motional rates from relaxation NMR
8. Practical aspects of protein dynamics study by SSNMR

# Derivation of Order Tensor - Uniaxial Motion

- Info from motionally averaged spectra usually expressed as order parameter S, which is the simplified version of order tensor **S**.
- Consider a molecule undergoing uniaxial rotation around a director $Z_D$.
- The measured NMR frequency is the z-component of the interaction $\sigma$ in the lab frame. It can be obtained from two coordinate transformations:




$$\sigma^M \xrightarrow{\Theta_{ij}} \sigma^D \xrightarrow{\text{uniaxial motion}} \left\langle \sigma^D \right\rangle \xrightarrow{\Phi_{ij}} \sigma^L$$

- Since $<\sigma^D>$ is axially symmetric, the only relevant frequency in the $\sigma^M$ –> $\sigma^D$ transformation is the frequency along $Z_D$:

$$\sigma^D_{zz} \equiv Z^M_D \cdot \sigma^M \cdot Z^M_D = \begin{pmatrix} \cos\Theta_{1z} & \cos\Theta_{2z} & \cos\Theta_{3z} \end{pmatrix} \begin{pmatrix} \sigma^M_{11} & \sigma^M_{12} & \sigma^M_{13} \\ \sigma^M_{21} & \sigma^M_{22} & \sigma^M_{23} \\ \sigma^M_{31} & \sigma^M_{32} & \sigma^M_{33} \end{pmatrix} \begin{pmatrix} \cos\Theta_{1z} \\ \cos\Theta_{2z} \\ \cos\Theta_{3z} \end{pmatrix}$$

$$= \sum_{i,j} \cos\Theta_{iz} \cdot \sigma^M_{ij} \cdot \cos\Theta_{jz}, \quad \xrightarrow{\text{averaging}}$$

$$\left\langle \sigma^D_{zz} \right\rangle = \sum_{i,j} \left\langle \cos\Theta_{iz} \cos\Theta_{jz} \right\rangle \cdot \sigma^M_{ij} = \frac{2}{3} \sum_{i,j} \underbrace{\left\langle \mathbf{\frac{3}{2} \cos\Theta_{iz} \cos\Theta_{jz} - \frac{1}{2} \delta_{ij}} \right\rangle}_{\mathbf{S_{ij}}} \cdot \sigma^M_{ij} + \frac{1}{3} \sum_{i,j} \delta_{ij} \sigma^M_{ij}$$

$$\Rightarrow \bar{\delta} \equiv \left\langle \sigma^D_{zz} \right\rangle - \sigma_{iso} = \frac{2}{3} \sum_{i,j} S^M_{i,j} \cdot \sigma^M_{ij}$$

# Definition of Order Tensor

- The general Saupe matrix (**order tensor**) **S** is defined as:

$$\mathbf{S}_{ij} \equiv \frac{1}{2}\left\langle 3\cos\Theta_i\cos\Theta_j - \delta_{ij}\right\rangle$$

- For uniaxial motion, the diagonalized $\left\langle \sigma^D \right\rangle$ is: $\left\langle \sigma^D \right\rangle = \begin{pmatrix} \sigma_\perp & 0 & 0 \\ 0 & \sigma_\perp & 0 \\ 0 & 0 & \sigma_{//} \end{pmatrix}$,

where $\sigma_{//} = \left\langle \sigma^D_{zz} \right\rangle$, $\sigma_\perp = \frac{3}{2}\sigma_{iso} - \frac{1}{2}\sigma_{//}$

$\bar{\delta} = \left\langle \sigma^D_{zz} \right\rangle - \sigma_{iso} = \boxed{\frac{2}{3}\sum_{i,j} S_{i,j} \cdot \sigma_{ij}}$ is true in any common frame of S and $\sigma$.

- Further transforming $<\sigma^D>$ to the lab frame gives the standard expression for the measured NMR frequency with orientation dependence:

$$\sigma^L_{zz} - \sigma_{iso} = \sum_i \cos\Phi_{iz} \cdot \left\langle \sigma^D_{ii} - \sigma_{iso} \right\rangle \cdot \cos\Phi_{iz} = -\frac{1}{2}\bar{\delta}\left(\cos^2\Phi_{xz} + \cos^2\Phi_{yz}\right) + \bar{\delta}\cos^2\Phi_{zz}$$

$$= -\frac{1}{2}\bar{\delta}\left(1 - \cos^2\Phi_{zz}\right) + \bar{\delta}\cos^2\Phi_{zz} = \boxed{\bar{\delta} \cdot \frac{1}{2}\left(3\cos^2\Phi_{zz} - 1\right)}$$

$\Phi_{zz}$: polar angle of the director in the lab frame.

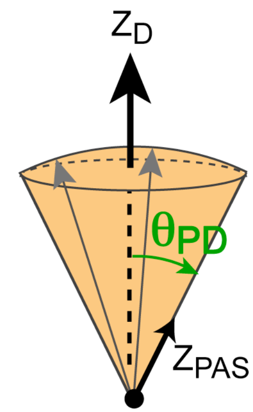
# Properties of the Order Tensor

- The order tensor is traceless: $\sum_i S_{ii} = \frac{3}{2}\sum_i \cos^2\Theta_i - \frac{3}{2} = 0$
- The order tensor is symmetric: $S_{ij} = S_{ji}\ (\cos\Theta_i \cos\Theta_j = \cos\Theta_j \cos\Theta_i)$
- Thus, **S** tensor has 5 independent elements, and can always be diagonalized to give its own principal axis system.

- The order parameter along a bond is the "projection" of the order tensor onto that vector.

$$\bar{\delta} = \frac{2}{3}\sum_i S_{ii}^{\sigma,PAS} \cdot \sigma_{ii}^{PAS} = \frac{2}{3}\left[\delta \cdot S_{zz}^{\sigma,PAS} - \frac{\delta}{2}\left(S_{xx}^{\sigma,PAS} + S_{yy}^{\sigma,PAS}\right)\right]$$

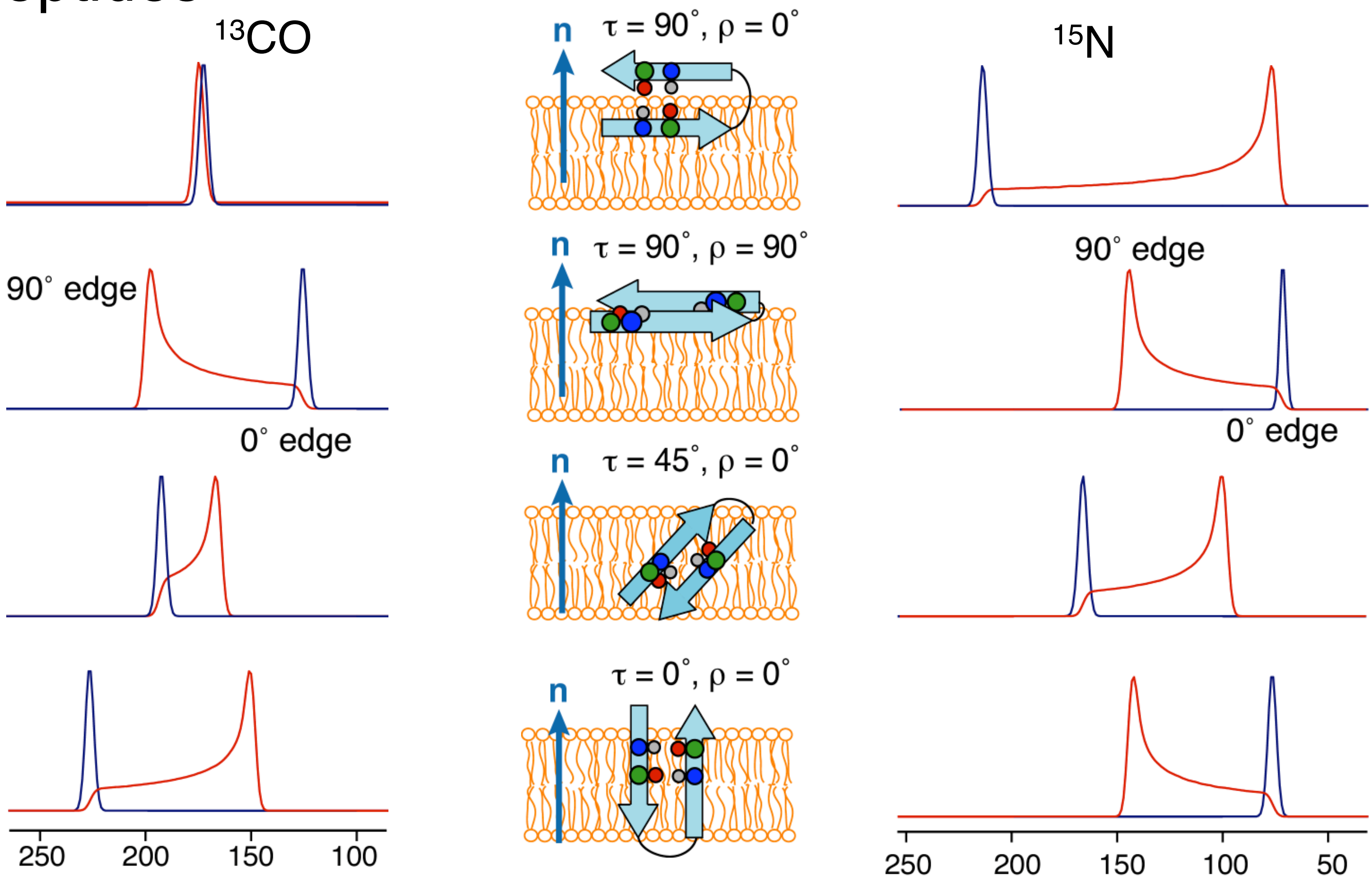
$$= \delta \cdot S_{zz}^{\sigma,PAS} = \delta\left\langle \frac{1}{2}\left(3\cos^2\theta_{PD} - 1\right)\right\rangle \quad \text{where } \theta_{PD} = \Theta_{3Z}$$




- Thus, the order parameter along a tensor's principal axis, $S_{zz}^{\sigma,PAS}$, is directly measurable as the ratio $\bar{\delta}/\delta$.

- Since $\omega_{0^\circ\ oriented} = \frac{1}{2}\delta\left(3\cos^2\theta_{PD} - 1\right) + \omega_{iso}$, bond order parameters contain the same orientation information that is sought after in aligned-membrane experiments.

# Uniaxially Averaged CSA Lineshapes of β-Sheet Peptides




ap. β-sheet: $\phi$ = -139°,$\psi$ = +135°; rigid-limit $^{13}$CO CSA: 248, 170, 100 ppm; rigid-limit $^{15}$N CSA: 217, 77, 64 ppm.



*Hong and Doherty, Chem. Phys. Lett, 432, 296 (2006).*

# Order Tensor and Order Parameter of Limiting Cases

• Determining a traceless and symmetric **S** tensor requires 5 independent NMR couplings; Symmetry considerations may reduce the number of unknowns.

• If the molecule is rigid (i.e. no segmental motion), then there is a single **S** tensor for the whole molecule (all segments have the same order).

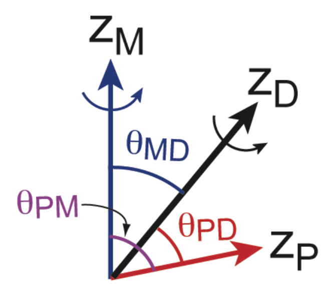
• If the rigid molecule rotates about a single director axis, then the **S** tensor is uniaxial, i.e. $\eta_S = 0$, with the unique axis along the director, and $S_{zz}^{PAS} = 1$ (complete order).

• If the rigid molecule rotates about its own molecular axis and an external director axis, then the **S** tensor is uniaxial along the molecular axis,

$$S_{zz}^{PAS} = S_{mol} = \frac{1}{2}\left\langle 3\cos^2\theta_{MD} - 1\right\rangle$$

• In this case, S along a bond, $S_{bond}$ is related to $S_{mol}$ by:

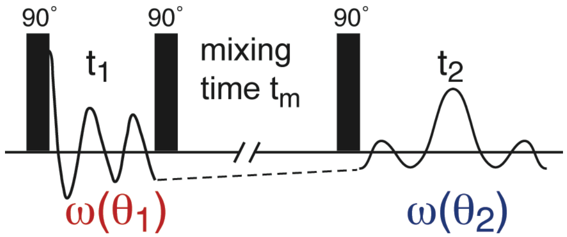
$$S_{bond}(\theta_{PD}) \equiv \frac{\bar{\delta}}{\delta} = \frac{1}{2}\left(3\cos^2\theta_{PM} - 1\right)\cdot S_{mol}$$

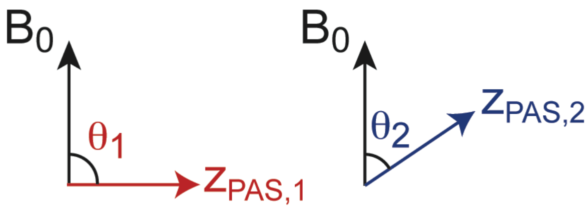


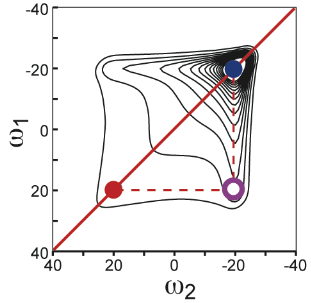

• $S_{mol}$ can be smaller than 1 due to tilt of the molecular axis from $Z_D$ or wobbling of the molecular axis.

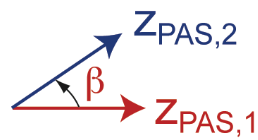
• Example: - Cholesterol rings in the lipid membrane;
- Lipid chains have additional internal segmental motions.

# Slow Motion: 2D Exchange NMR



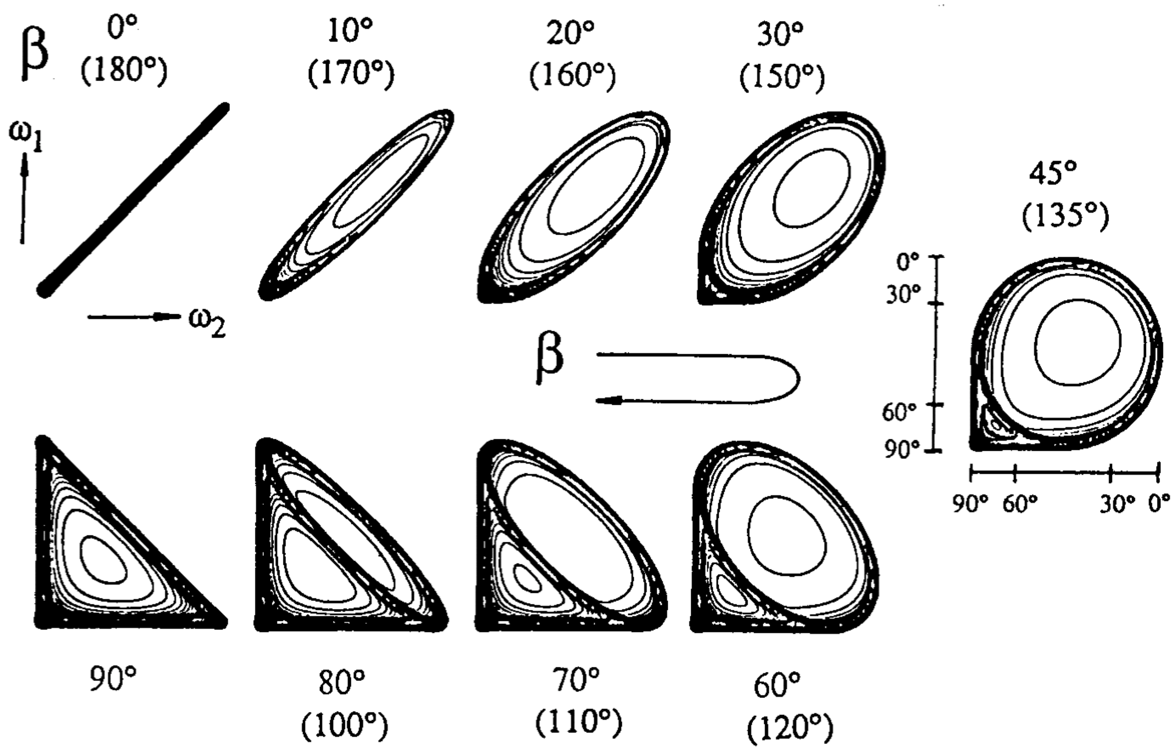




2D spectrum $S(\omega_1, \omega_2)$ is a joint probability:

- intensity distribution: geometry of motion
- geometry encoded in a single angle $\beta$ for a uniaxial interaction
- $t_m$ dependence –> correlation time.

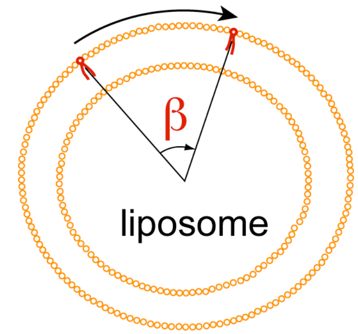
$S(\omega_1,\omega_2;\beta)$ for an $\eta=0$ tensor

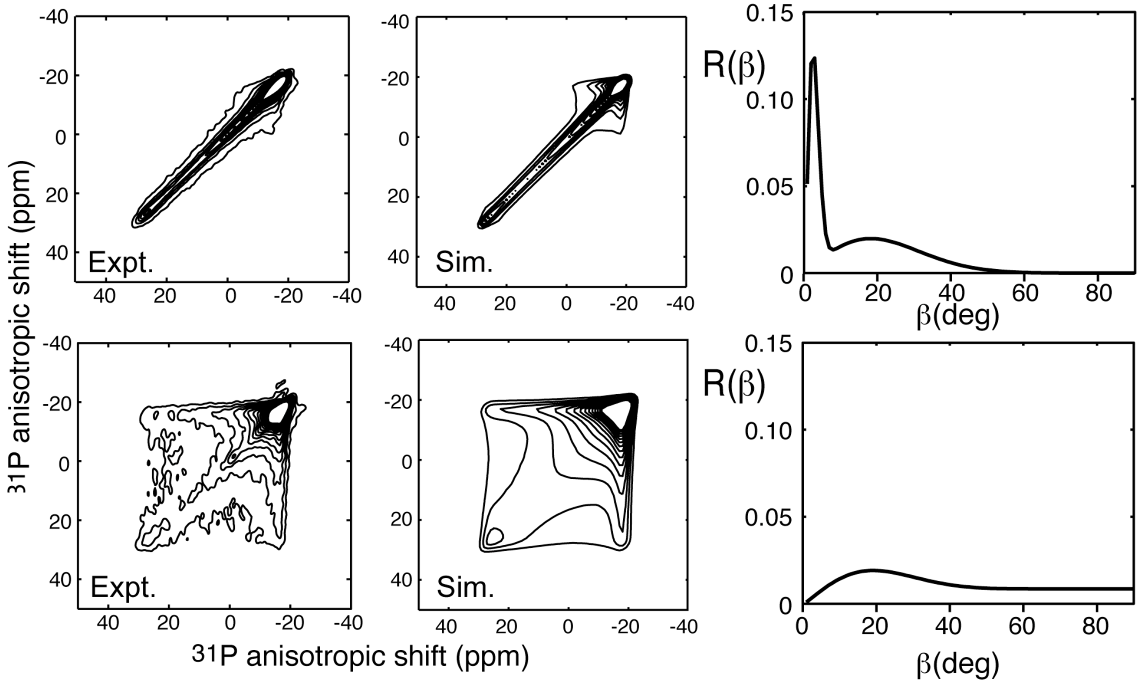



*KSR and Spiess.*

# 2D Exchange of Lipid Membranes

e.g. lateral diffusion of lipids over the curved surface of liposomes.




• Tensor PAS: uniaxial due to lipid axial rotation;

• Reorientation-angle distribution $R(\beta;\ t_m)$.

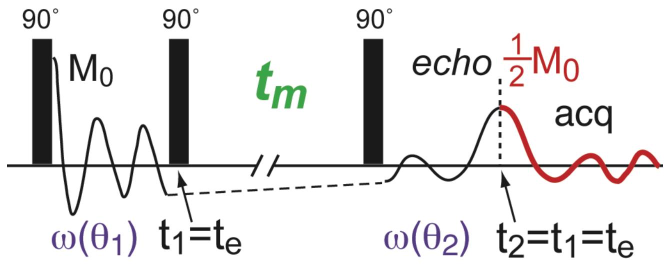
$$S(\omega_1, \omega_2; t_m) = \int_{0^\circ}^{90^\circ} d\beta R(\beta; t_m) S(\omega_1, \omega_2; \beta)$$

• $t_m$ =0, no exchange.

• $t_m$ dependence gives info on the lateral diffusion $\tau_c = r^2/6D_L$, which reveals the vesicle size r.

*Marasinghe et al, JPC B, 109, 22036 (2005).*

# 1D Stimulated Echo: Time-Domain Exchange




- 1D analog of 2D exchange: $t_1 = \tau$.
- Allows fast determination of $\tau_c$ by varying $t_m$, avoids multiple 2D.
- similar to 2D exchange, most often applied to static samples.

2D time signal:

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

$\langle \ldots \rangle$ denotes powder averaging.

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

- Segments without frequency change: $\omega(\theta_1)=\omega(\theta_2)=\omega$ (diagonal), or $t_m=0$

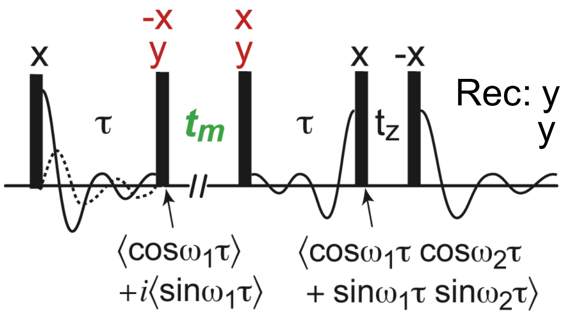
$$M_{SE}(t_e) = \left\langle e^{-i\omega t_e} \cdot e^{i\omega t_e} \right\rangle = \underbrace{\langle \mathbf{1} \rangle}_{\text{2 scans}}$$

- Segments with frequency change,

$$M_{SE}(t_e) = \left\langle e^{-i\omega(\theta_1)t_e} \cdot e^{i\omega(\theta_2)t_e} \right\rangle = \left\langle e^{i\omega\left[(\theta_2)-\omega(\theta_1)\right]t_e} \right\rangle \rightarrow 0$$ due to destructive interference.

1D simulated echo intensity = 2D spectrum's diagonal intensity.

# Stimulated Echo: An Improved Sequence




- Built-in z-filter eliminates $T_1$ effects.
  - $S_0$: first $t_z$ = 1 ms, then $t_m$
  - S: first $t_m$, then $t_z$ = 1 ms
- Second and third 90˚ pulses phase-cycled together to create $\cos(\omega_1 t)\cos(\omega_2 t)$ in one scan and $\sin(\omega_1 t)\sin(\omega_2 t)$ in another.

Every 2 scans:

$$M_{SE}(\tau;t_m) = M_0\left\langle \cos(\omega_1\tau)\cos(\omega_2\tau) + \sin(\omega_1\tau)\sin(\omega_2\tau)\right\rangle$$

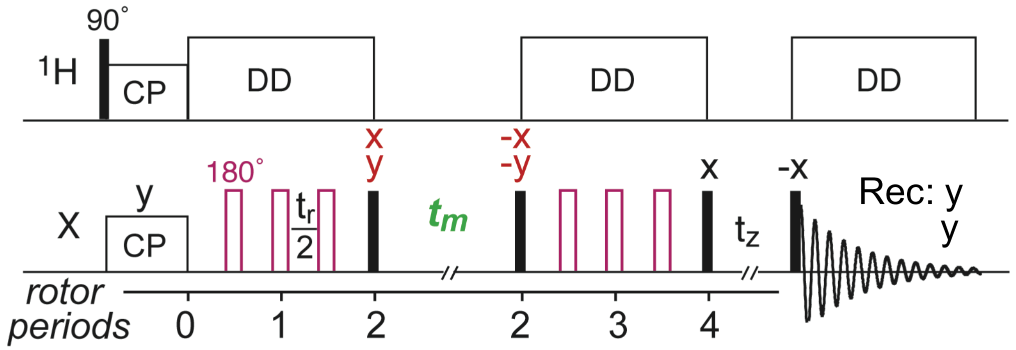
$$= M_0\underline{\left\langle \cos(\omega_1-\omega_2)\tau\right\rangle} = \int \cos\Big((\omega_1-\omega_2)\tau\Big)\cdot S\Big(\omega_1,\omega_2;t_m\Big)d\omega_1 d\omega_2$$

- Powder averaging: 2D spectrum $S(\omega_1,\omega_2;\, t_m)$ is the probability of finding molecules with freq $\omega_1$ before $t_m$ and freq $\omega_2$ after $t_m$ .
- At $t_m$=0, $\omega_1 = \omega_2, \;\Rightarrow\; \cos\Big(\omega_1-\omega_2\Big)\tau = 1, \;\Rightarrow\; M_{SE}\Big(\tau;0\Big) = \int S\Big(\omega_1,\omega_2;t_m\Big)d\omega_1 d\omega_2$
- With increasing $t_m$, $\omega_2 \neq \omega_1$, echo intensity decreases.
- For symmetric *n*-site jumps, the stimulated echo intensity decays exponentially with a time constant $\tau_c$:

$$M_{SE}\left(\tau >> \frac{1}{\delta};t_m\right) = \frac{1}{n} + (1-\frac{1}{n})\cdot M_0\cdot e^{-t_m/\tau_c}$$

*Marasinghe et al, JPC B, 109, 22036 (2005).*

# Stimulated Echo Under MAS: CODEX




- 180° pulse train recouples X-spin CSA.
- 90° storage and read-out pulses are phase-cycled together; after the second recoupling period, the accumulated MAS phase for 2 scans is:

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

where

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

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

- No reorientation: $\omega_1 = \omega_2$, –> $\Phi_1+\Phi_2 = 0$, $\cos(\Phi_1+\Phi_2)=1$, a stimulated echo.
- With reorientation, $\omega_1 \neq \omega_2$, –> $\cos(\Phi_1+\Phi_2) < 1$, echo decay.
- Same $T_1$ correction by $t_m/t_z$ switch between $S_0$ and S.



*deAzevedo et al, JCP, 112, 8988 (2000).*

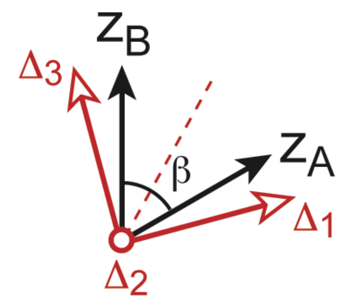
# CODEX Sensitive to Small-Angle Reorientation

- The normalized CODEX signal is parameterized by the product of CSA and recoupling time, $\delta N t_r$ (analogous to REDOR).
- Normalized signal can be considered in terms of the difference phase:

$$S(t_m, \delta N t_r) / S_0(t_m, \delta N t_r) = \cos(|\Phi_2| - |\Phi_1|) = \cos(\Phi^{\Delta})$$

$$\text{where } \Phi^{\Delta} = N \int_2^{t_r/2} (\omega_2 - \omega_1) dt = N \int_2^{t_r/2} \omega^{\Delta}(t) dt$$

- The difference tensor of a uniaxial interaction ($\eta$=0) has the symmetry-determined orientation on the right –>
- It can be shown that:




$$\omega_{22}^{\Delta} = 0$$

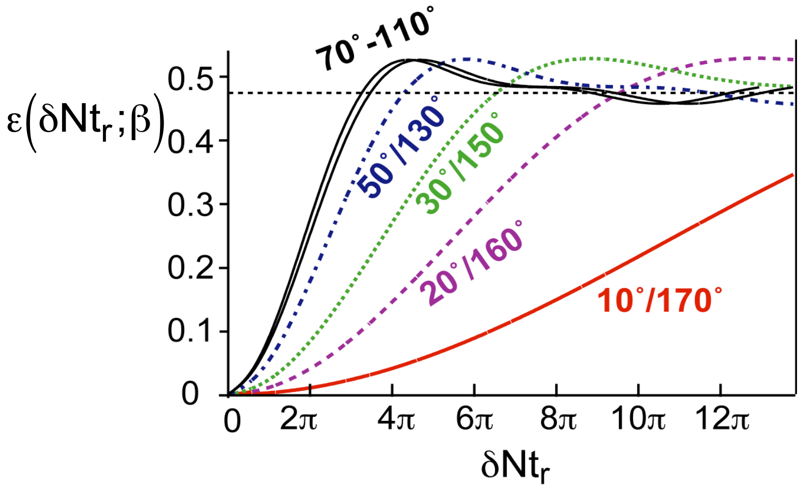
$$\omega_{33}^{\Delta} = -\omega_{11}^{\Delta} = \frac{3}{2}\delta \cdot \sin\beta$$

$$\text{i.e. } \eta^{\Delta} = 1$$

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

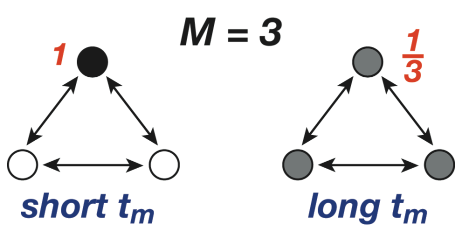
- Thus, the CODEX signal scales with $\sin\beta$ or $\beta$ for small angles.
- Usual angle dependence is $(3\cos^2\beta-1)2$, which scales as $\beta^2$ .

# CODEX: Reorientation Angles and Number of Sites



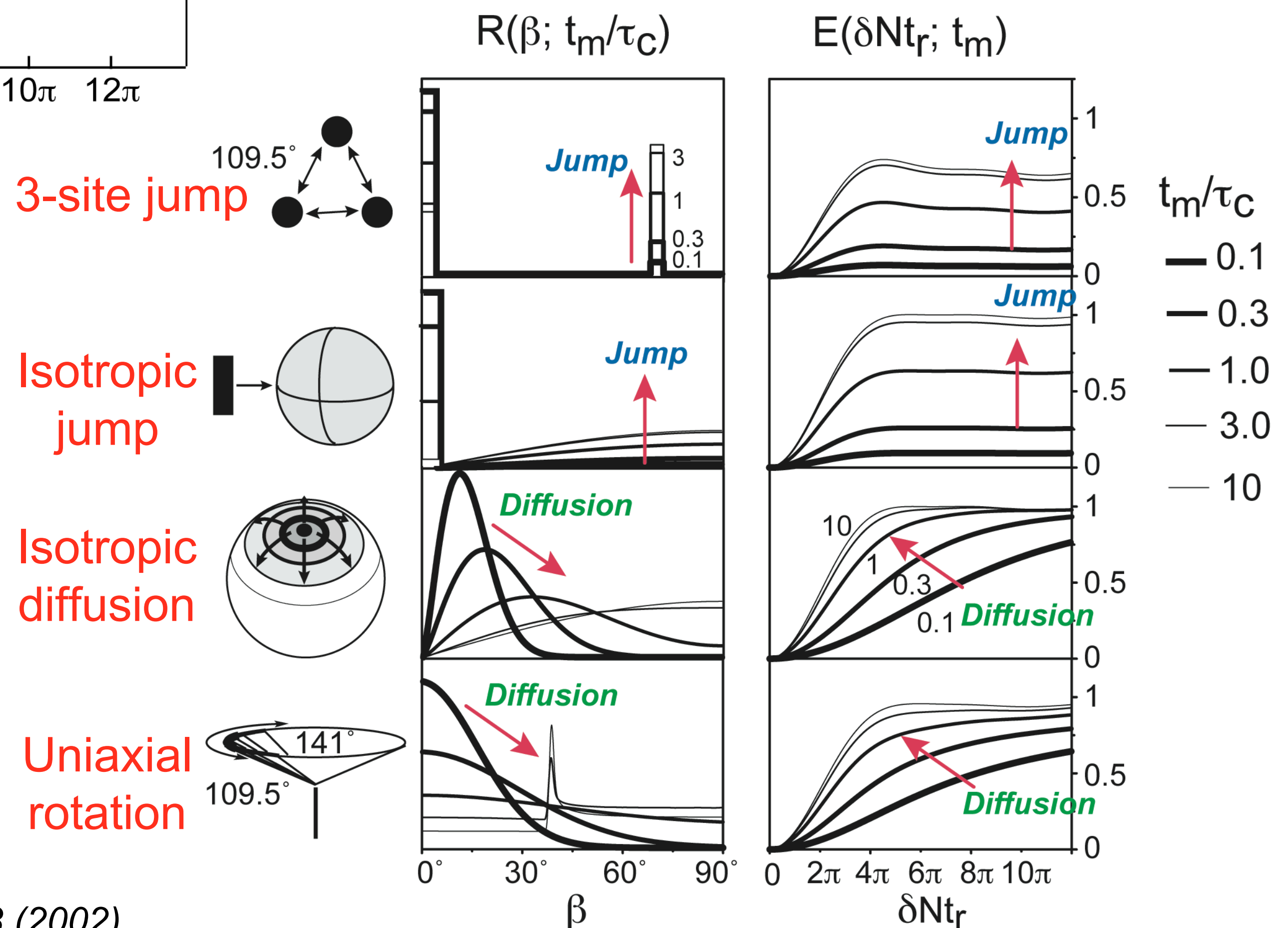

Total signal is a weighted sum of β-dependent curves:

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




Jump motions:

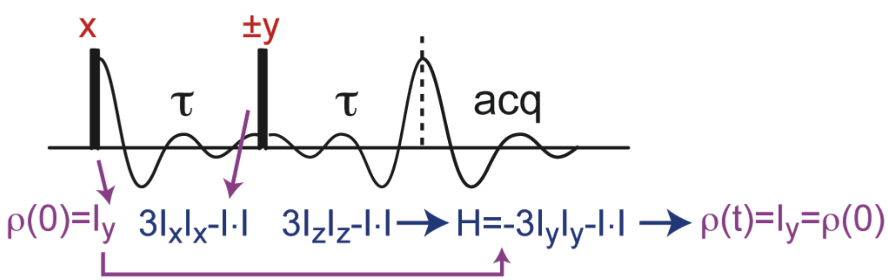
$$\frac{S}{S_0}\left(t_m \gg \tau_c, \delta N t_r \gg 1\right) = \frac{1}{M}$$




*KSR et al, Encyclop NMR, 9, 633 (2002).*

# $^2$H NMR for Studying Protein Motion

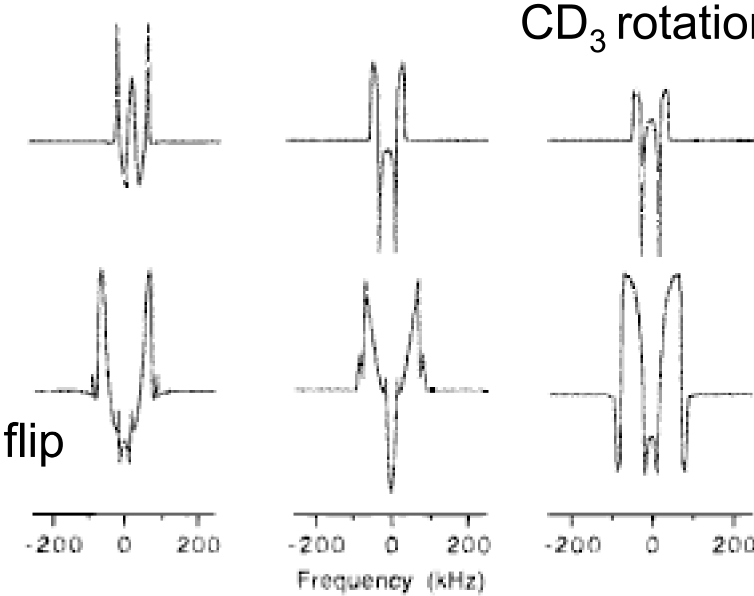
- large interaction strength, 160-200 kHz, sensitive to motional geometry.
- anisotropic relaxation experiments probe ns - ms motions.
- requires site-specific labeling.
- no multiplex advantage.
- fast motion amplitude information replaced by C-H dipolar experiments under MAS, e.g. LG-CP.




- Quadrupolar echo
- Anisotropic $T_2$
- Anisotropic $T_1$ : different relaxation rates across motionally averaged lineshape; large frequencies give faster relaxation.
- 2D exchange: $\omega_1$ symmetrized version of the $\eta$=0 CSA pattern.

CD$_3$ rotation

Phe ring flip



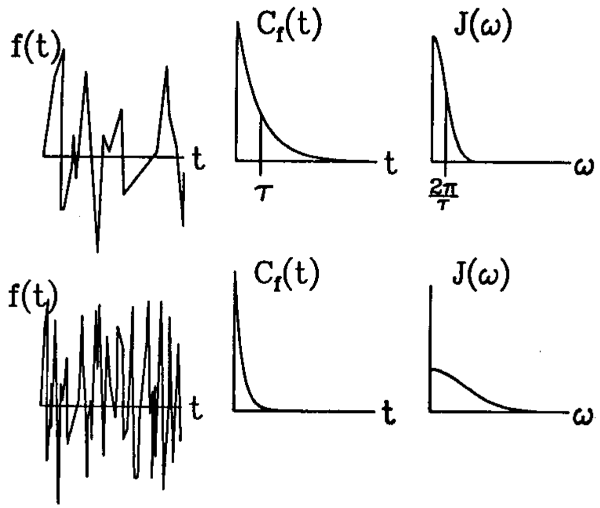

*Palmer et al, JPC, 100, 13293 (1996).*

# *NMR Theory and Techniques for Studying Molecular Dynamics*

7. Determining motional rates from relaxation NMR

8. Practical aspects of protein dynamics study by SSNMR

# NMR Relaxation - A Primer




- Requirements for relaxation:
  - a magnetic interaction.
  - random motion at the appropriate frequency.

- Consider an isolated spin subject to an isotropic random field $B_L(t)=\langle B_L\rangle f(t)$.

$$\left\langle \frac{d\rho(t)}{dt} \right\rangle = \underbrace{\left\langle -\frac{i}{\hbar}\left[H(t),\rho(0)\right] \right\rangle}_{0} - \frac{1}{\hbar^2}\left\langle \int_0^t \left[H(t),\left[H(t-\tau),\rho\right]\right]d\tau \right\rangle = -\frac{1}{\hbar^2}\left\langle \int_0^t \underbrace{\left[H(t),\left[H(t-\tau),\rho\right]\right]}_{C(\tau)}d\tau \right\rangle$$

- The spatial part of H(t) is $\langle \gamma B_L \rangle \cdot f(t)$

so double commutation means $\langle f(t)\cdot f(t-\tau)\rangle \equiv C(\tau)$

- Assume $C(\tau) = e^{-\tau/\tau_c}$, i.e. decays to 0, isotropic motion.
- In the rotating frame, H(t) is modulated by $e^{-in\omega_0 t}$
- So Fourier transform $C(\tau)$: $\int_0^\infty C(\tau)e^{-i\omega_0\tau}d\tau \equiv J(\omega_0) \quad \Rightarrow \quad J(\omega_0) = \frac{\tau_c}{1+\omega_0^2\tau_c^2}$
- $J(\omega_0) \equiv$ spectral density, power available from the fluctuations at frequency $\omega_0$

*Abragam*

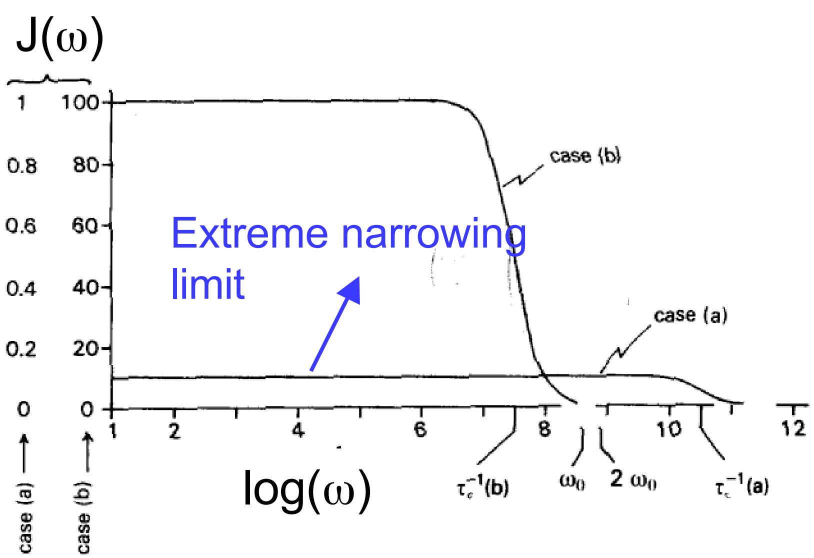
# Relaxation Rate, J(ω), & Correlation Time

- The above calculations lead to relaxation rates including terms like:

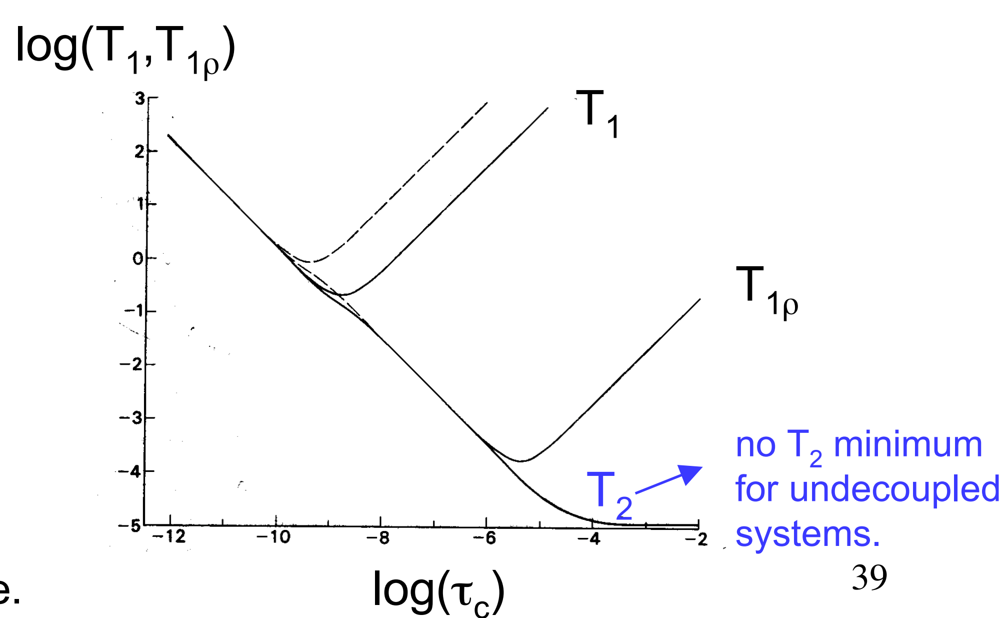
$$T_1^{-1} \propto \langle \gamma B_L \rangle^2 \frac{\tau_c}{1+\omega_0^2\tau_c^2}$$

a Lorentzian function centered at $\omega_0 = 0$

- $<\gamma B_L>$: strength of the local field driving relaxation.
- For heteronuclear dipolar relaxation, $\gamma B_L = \omega_{IS}$.
- $<\gamma B_L>^2$ dependence: relaxation is second order.
- $\omega_0\tau_c << 1,\ T_1^{-1} \propto \tau_c$; $\omega_0\tau_c \sim 1,\ T_{1,min}^{-1} \propto \omega^2/\omega_0$




Logarithmic plot stretches small ω regime.



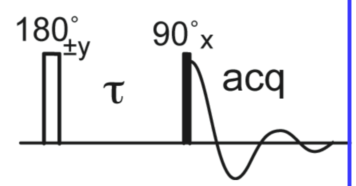



*Harris.*

# $T_1$ and $T_{1\rho}$ Relaxation

- Main local fields driving relaxation:
  - dipolar couplings
  - quadrupolar couplings
  - CSA

Inversion recovery

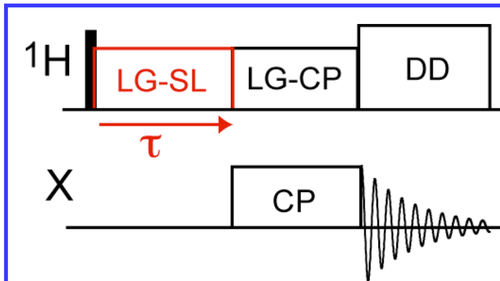



- For X-spin $T_1$ relaxation due to H-X dipolar coupling and X-spin CSA,

$$R_{1,X} = \frac{\omega_{HX}^2}{4}\left[\underline{J(\omega_H-\omega_X)}+3\underline{J(\omega_X)}+6\underline{J(\omega_H+\omega_X)}\right]+c^2\underline{J(\omega_X)},\quad \text{where } c=\Delta\sigma\cdot\omega_X/\sqrt{3}$$

- $T_1$ relaxation is sensitive to motion near the Larmor frequencies, ~$10^{-9}$ $s^{-1}$

- For $^1H$ $T_{1\rho}$ relaxation under LG spin lock at $\omega_e$, driven by H-H and H-X dipolar couplings,

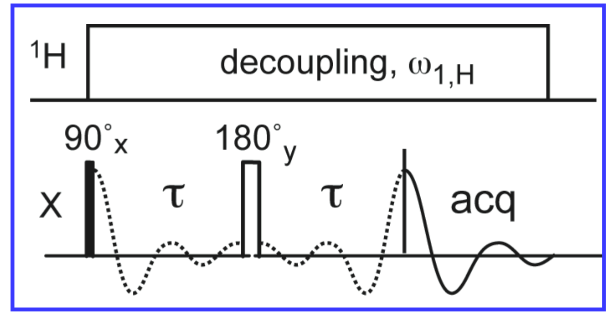



$$R_{1\rho,H} = \frac{1}{10}\delta_{HH}^2\left\{\underline{J(\omega_e)}+2\underline{J(2\omega_e)}+6J(\omega_H)+6J(2\omega_H)\right\}$$
$$+\frac{1}{30}\delta_{XH}^2\left\{2\underline{J(\omega_e)}+3J(\omega_X)+J(\omega_X-\omega_H)+3J(\omega_H)+6J(\omega_X+\omega_H)\right\}$$

- $T_{1\rho}$ is sensitive to motion near ~$10^5$ $s^{-1}$, which is common in membrane proteins.
- X-spin $T_{1\rho}$ is usually not measured due to the need for $^1H$ decoupling, which causes undesirable reverse CP.

*Mehring.*
*Huster et al, Biochemistry, 40, 7662 (2001).*

# $^1$H Decoupled X-Spin $T_2$




- For X-spin $T_2$ under a $^1$H decoupling field of $\omega_{1,H}$,

$$R_{2,X} = \frac{\gamma_H^2 \gamma_X^2 \hbar^2}{5 \cdot r^6} \underline{J(\omega_{1,H})} = \frac{\gamma_H^2 \gamma_X^2 \hbar^2}{5 \cdot r^6} \frac{\tau_c}{1 + \omega_{1,H}^2 \tau_c^2}$$

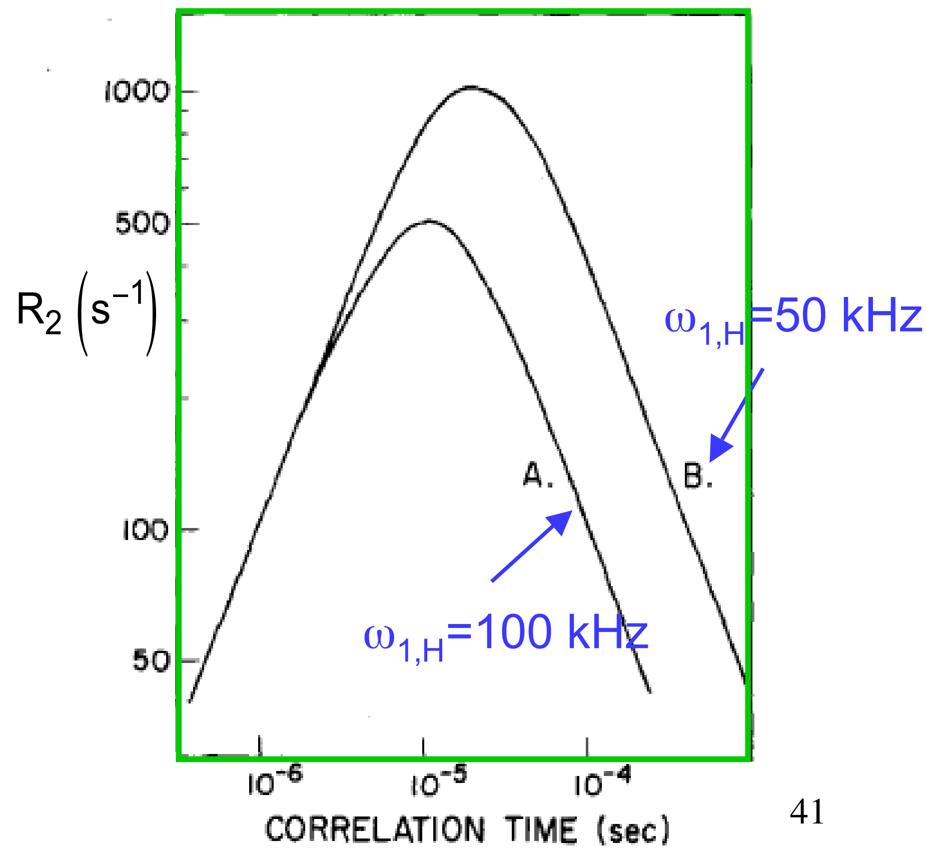
$$\omega_{1,H}\tau_c << 1, \quad R_{2,X} \propto \tau_c$$

$$\omega_{1,H}\tau_c >> 1, \quad R_{2,X} \propto \frac{\tau_c}{\omega_{1,H}^2}$$

- Solids X-spin $T_2$ has a minimum.

- Similar to $^1$H $T_{1\rho}$, decoupled X-spin $T_2$ is sensitive to ~$10^5$ $s^{-1}$ motions.

- Many membrane peptides and proteins exhibit $R_2$-enhanced line broadening due to μs motion.




*Rothwell and Waugh, JCP, 74, 2721 (1981)*

# More about Time Correlation Function

- Definition: $C(\tau) = \langle f(t) \cdot f(t-\tau) \rangle$
- For example, $f(t) = \frac{1}{2}\left[3\cos^2\theta(t) - 1\right]$

  which leads to the $2^{nd}$-order correlation function:

$$C_2(\tau) \equiv 5\langle P_2(\cos\theta(t)) \cdot P_2(\cos\theta(t-\tau)) \rangle$$

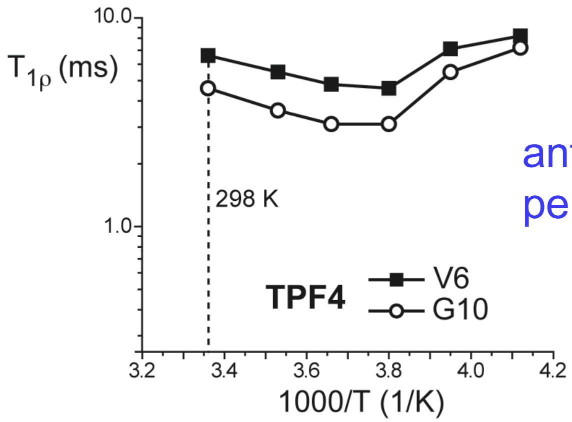
- Higher order correlation functions also exist. In general,

$$C_L(\tau) \propto \langle P_L(\cos\theta(t)) \cdot P_L(\cos\theta(t-\tau)) \rangle$$

- For discrete jumps, $\tau_c(C_2) = \tau_c(C_4) = \tau_c(C_6)\ldots$
- For diffusive motion, $\tau_c$ from higher order $C_L$ functions differ from $\tau_c(C_2)$.
- Determining $\tau_c(C_L)$ for different order can distinguish jump motion and diffusive motion.

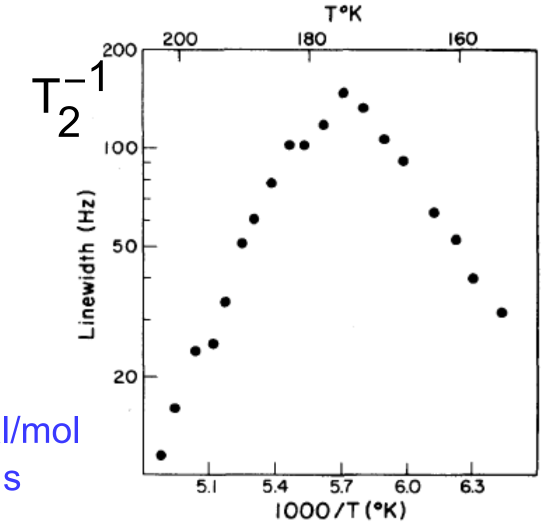
# Determining $\tau_c$ in Anisotropically Mobile Systems

- For activated processes, $\tau = \tau_0 e^{E_a/RT}$
- Varying temperature to map out $\tau_0$ and $E_a$.



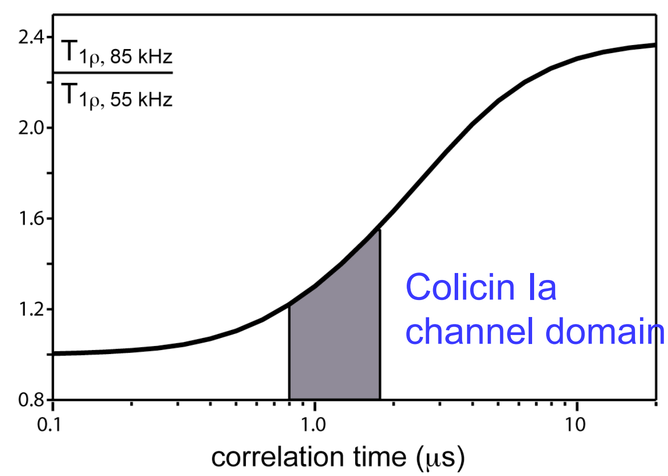

antimicrobial peptide TPF4

$E_a$ = 6.4 kcal/mol
$\tau_0$=2 x $10^{-14}$ s




e.g. hexamethylbezene, 3 phases:
I: $CH_3$ $C_3$ motion (< 116 K),
II: $C_6$ + $C_3$ motions (116-383 K)

- For anisotropic motion: $C(\tau)$ decays to a finite value, $S^2 > 0$.
- $J(\omega)$ depends on S: $J(\omega) = (1 - S^2)\tau_c / (1 + \omega^2 \tau_c^2)$
- Varying the field: $\omega_0$ for $T_1$, $\omega_1$ for $T_{1\rho}$.
- Relaxation time ratios at different fields depend only on $\tau_c$.




*Rothwell and Waugh, JCP, 74, 2721 (1981)*
*Huster et al, Biochemistry, 40, 7662 (2001).*
*Doherty et al, Biochemistry, Epub (2008)*

# Practical Aspects of Protein Dynamics From SSNMR

Types of Motions in Proteins

## Segmental

- methyl group rotation ($CH_3$)
- amine rotation ($NH_3$)
- phenylene ring flip (Phe, Tyr)
- no ring flip (Trp, His)
- torsional libration
  - sidechains: mobility gradient
  - backbone amides (NH-CO)
- trans-gauche isomerization
- large-amplitude diffusion of loops and termini

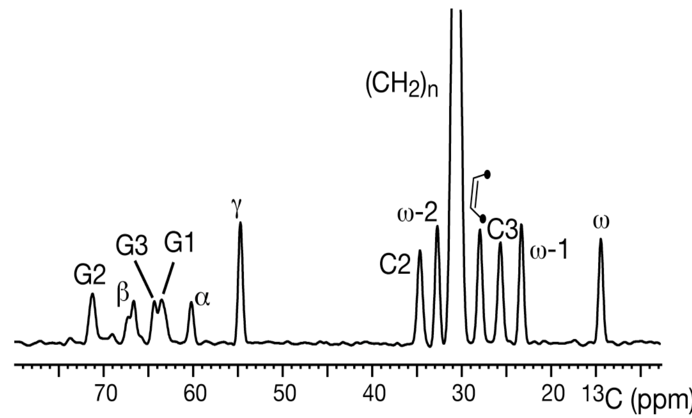
## Global

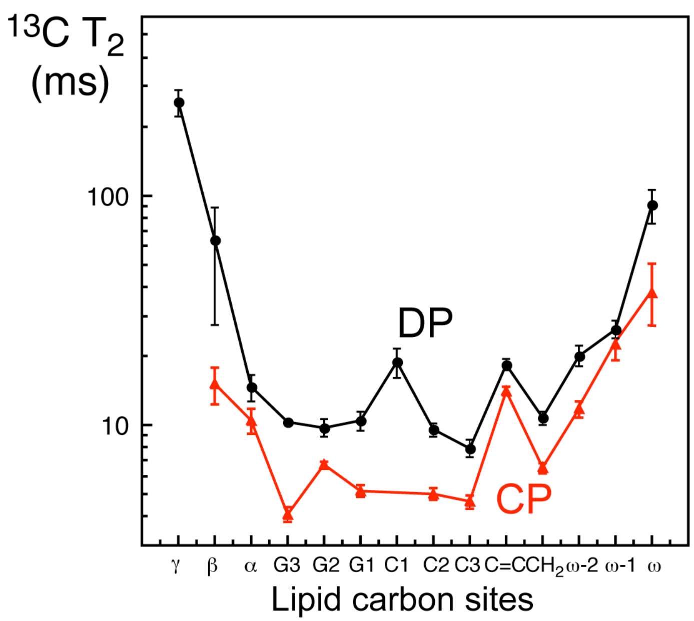
- whole-body uniaxial rotation (membrane peptides)
- near isotropic diffusion (e.g. elastin)

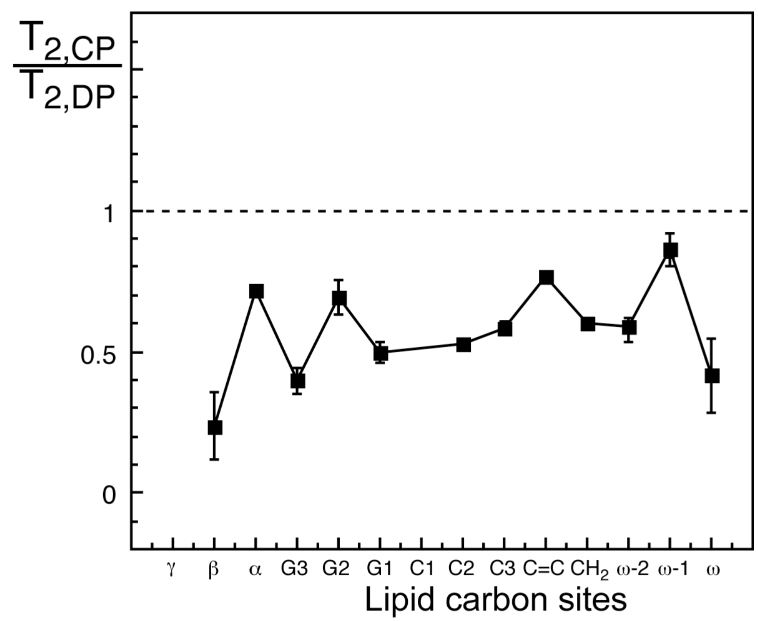
# Diagnostic Methods to Identify Motion - 1

1. CP versus DP (direct polarization)
   - CP: selects immobile species.
   - DP: selects mobile species due to narrow lines, high intensity, and short $^{13}C$ $T_1$.
   - heterogeneously mobile systems give different $T_2$'s under CP and DP.

e.g. POPC/POPG membrane in the presence of an antimicrobial peptide



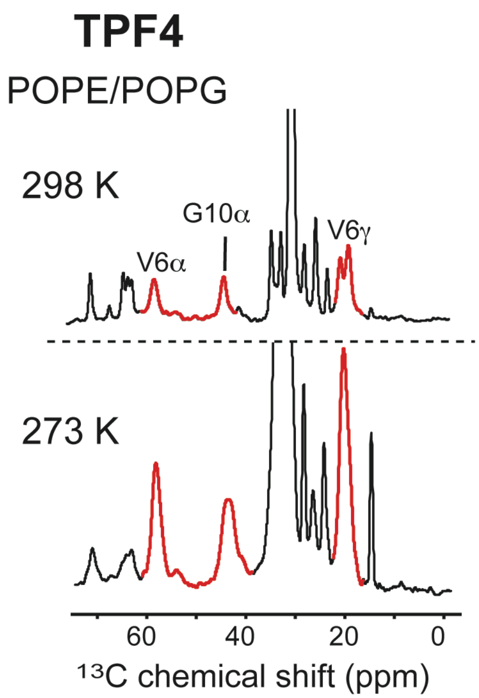

*Mani et al, Biochemistry, 43, 13839 (2004).*

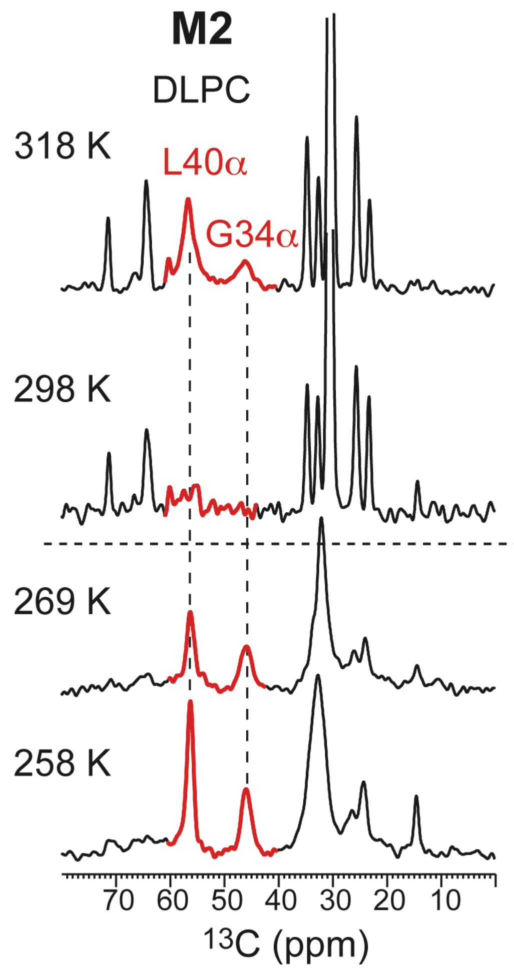
# Diagnostic Methods to Identify Motion - 2

2. CP intensity loss: intermediate timescale motion interferes with:

- $^{1}$H-X CP
- $^{1}$H decoupling
- Intensity can be retrieved by varying T.

$^{13}$C CP

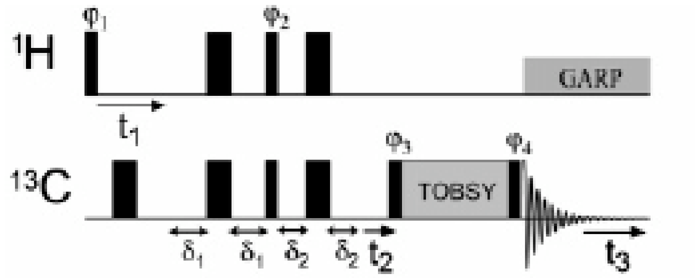



*Doherty et al, Biochemistry, 2007 (Epub ahead of print)*
*Cady et al, JACS, 129, 5719 (2007).*

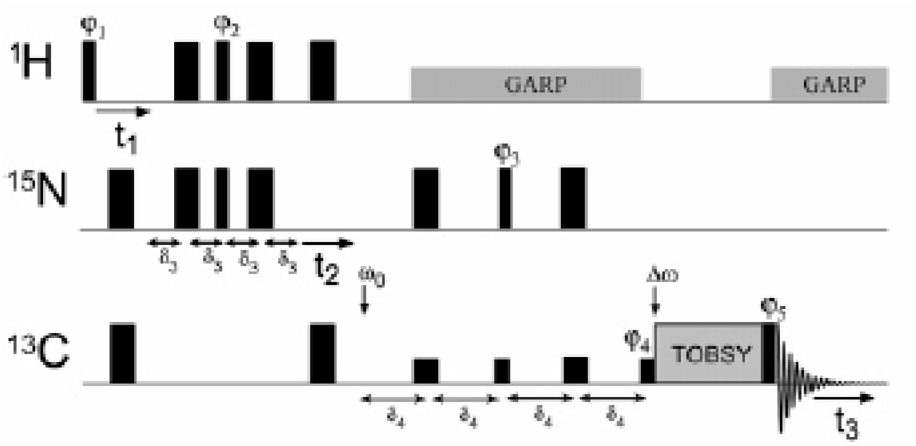
# Other Mobility-Tailored Techniques

3. Very large-amplitude motion: J-coupling based techniques can be used to selectively detect mobile segments.




J-INEPT HCC

*Andronesi, JACS, 127, 12965 (2005).*




J-INEPT HNCACB

4. Rigid-body uniaxial rotation of membrane peptides around the bilayer normal allows *orientation determination*.