Residual Dipolar Coupling Towards Understanding Structure and Dynamics of Biomolecules

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Indian Institute of Science Education and Research, Bhopal

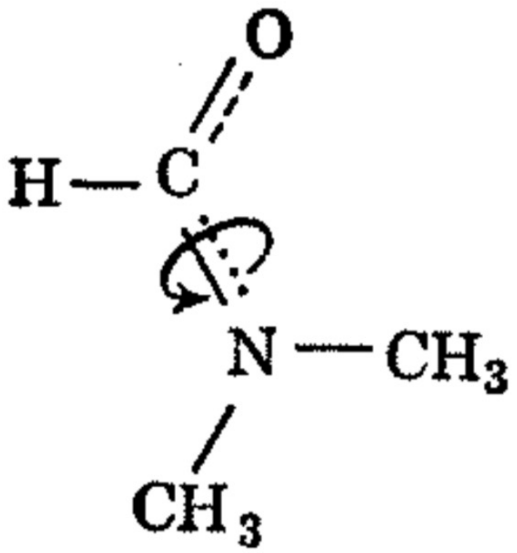
20th Dec 2018, Thursday

Teaching Session, 9:45 AM

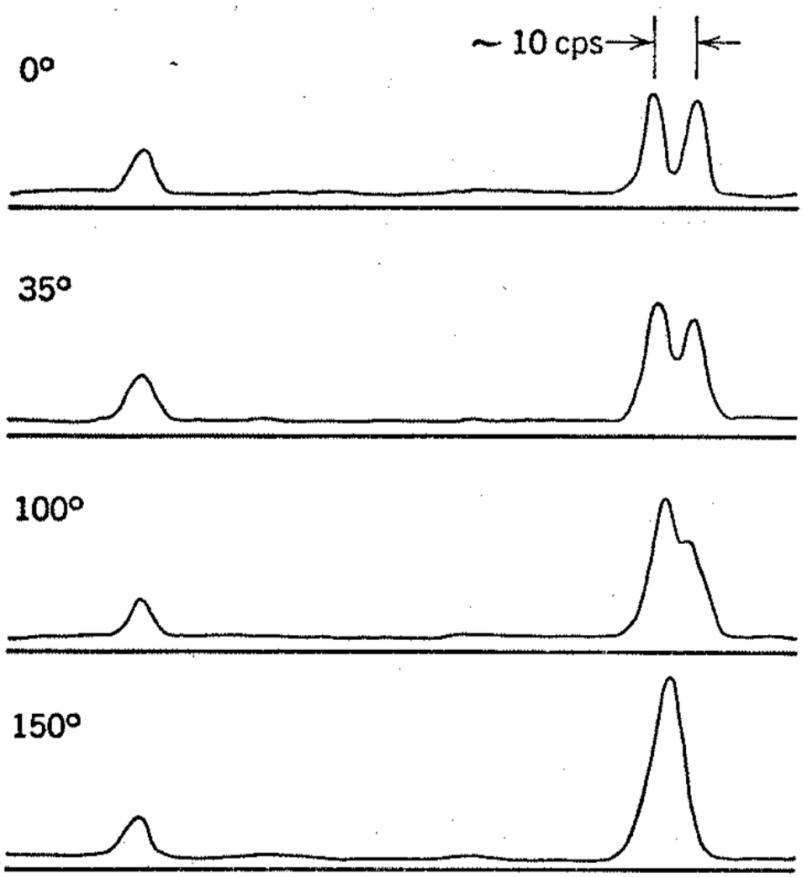
NMR Meets Biology 4th ed.

Khajuraho, Madhya Pradesh

Chemical Shifts



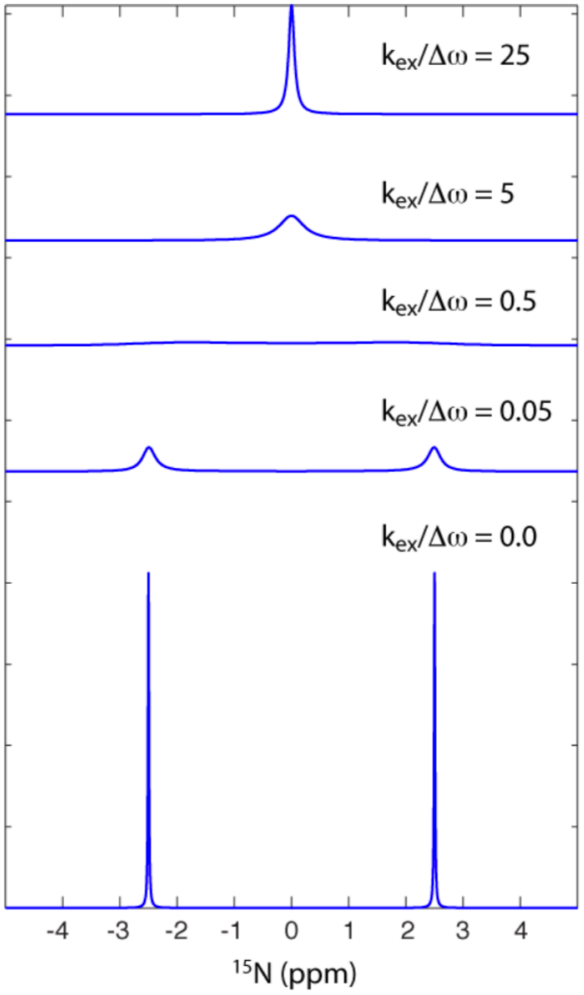
Dimethylformamide



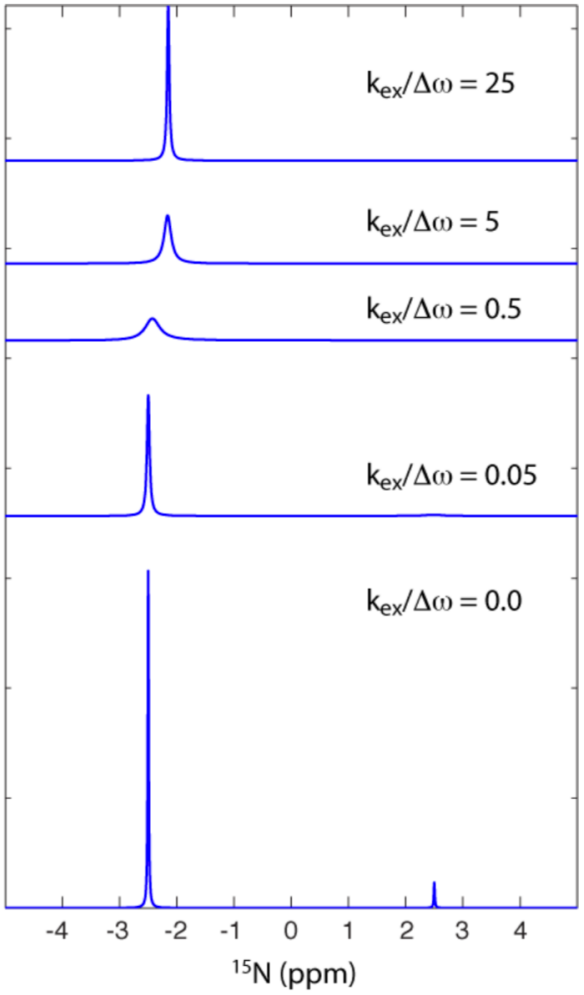
Phillips, W.D. *Journal of Chemical Physics,* **1955,** 23, 1363
Phillips, W.D. *Annals of New York Academy of Science,* **1958,** 70, 817
Picture: Roberts, J.D. Nuclear Magnetic Resonance – Applications to Organic Chemistry, **1959**, McGraw Hill Book Company (Figure 4-7, page 69).

Chemical Shifts upon Exchange

As we heard from Pramodh's talk, chemical shifts indicate dynamics based on exchange regimes…



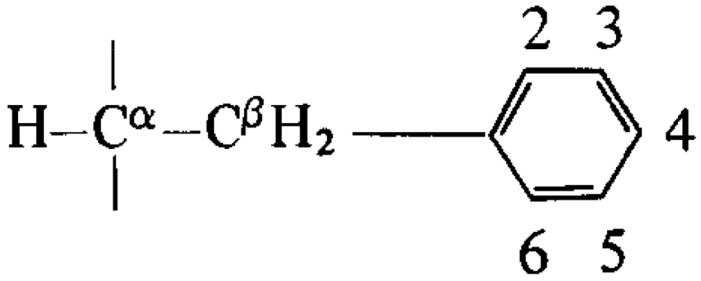
***Equal* Population**



***Unequal* Population**

NMR indicates molecular dynamics

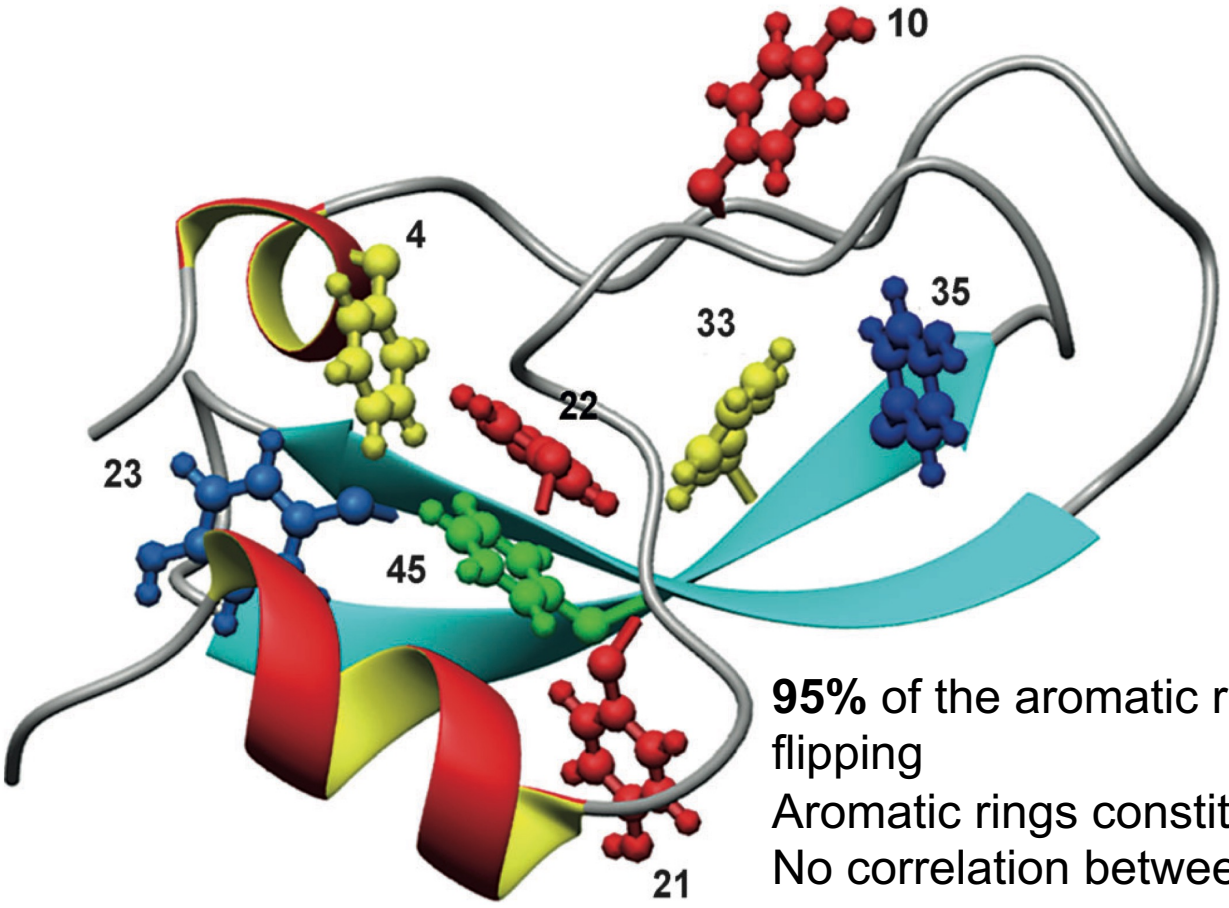
- Early bias towards **rigidity** of molecular structures from crystallographic studies
- First experimental evidence of molecular dynamics in the protein core were of reports of BPTI ^{1}H resonance assignment showed aromatic protons are “dynamic”
- Rotation about the X-angle of aromatic rings



Wagner, G., Demarco, A., Wuthrich, K. *Biophysics of Structure and Mechanism,* **1976,** 2, 139-158

Aromatic ring flips in BPTI

Bovine Pancreatic Trypsin Inhibitor



Slow:
Y35: k_{flip} ~4 s^{-1}
Y23: k_{flip} ~30 s^{-1}

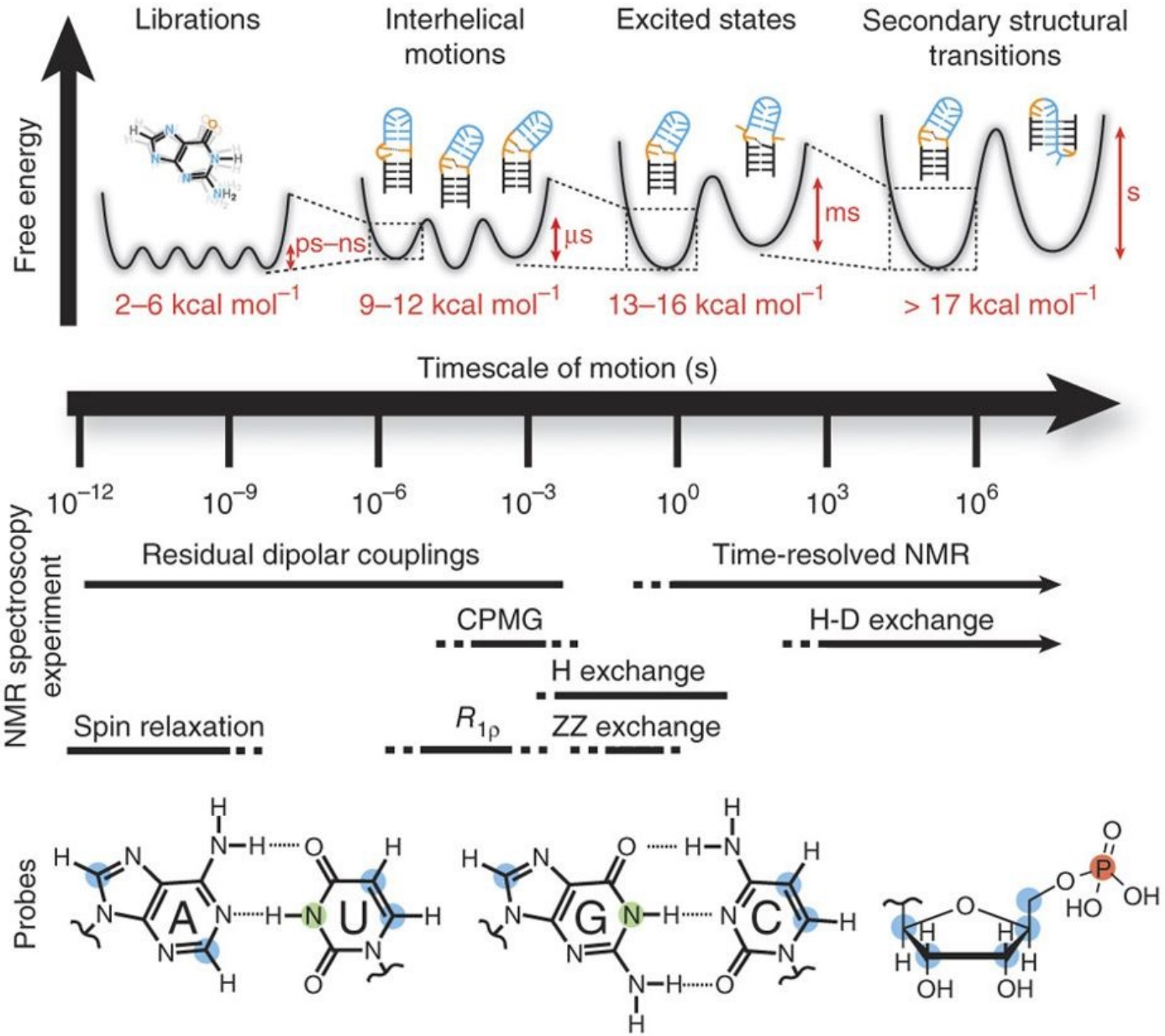
Intermediate:
F45: k_{flip} ~300 s^{-1}

Fast:
F4,Y10, Y21, F22*,
F33: $k_{flip} > 10^6$ s^{-1}

95% of the aromatic rings in proteins are fast flipping
Aromatic rings constitute **25%** of protein core
No correlation between ring position and flip rate

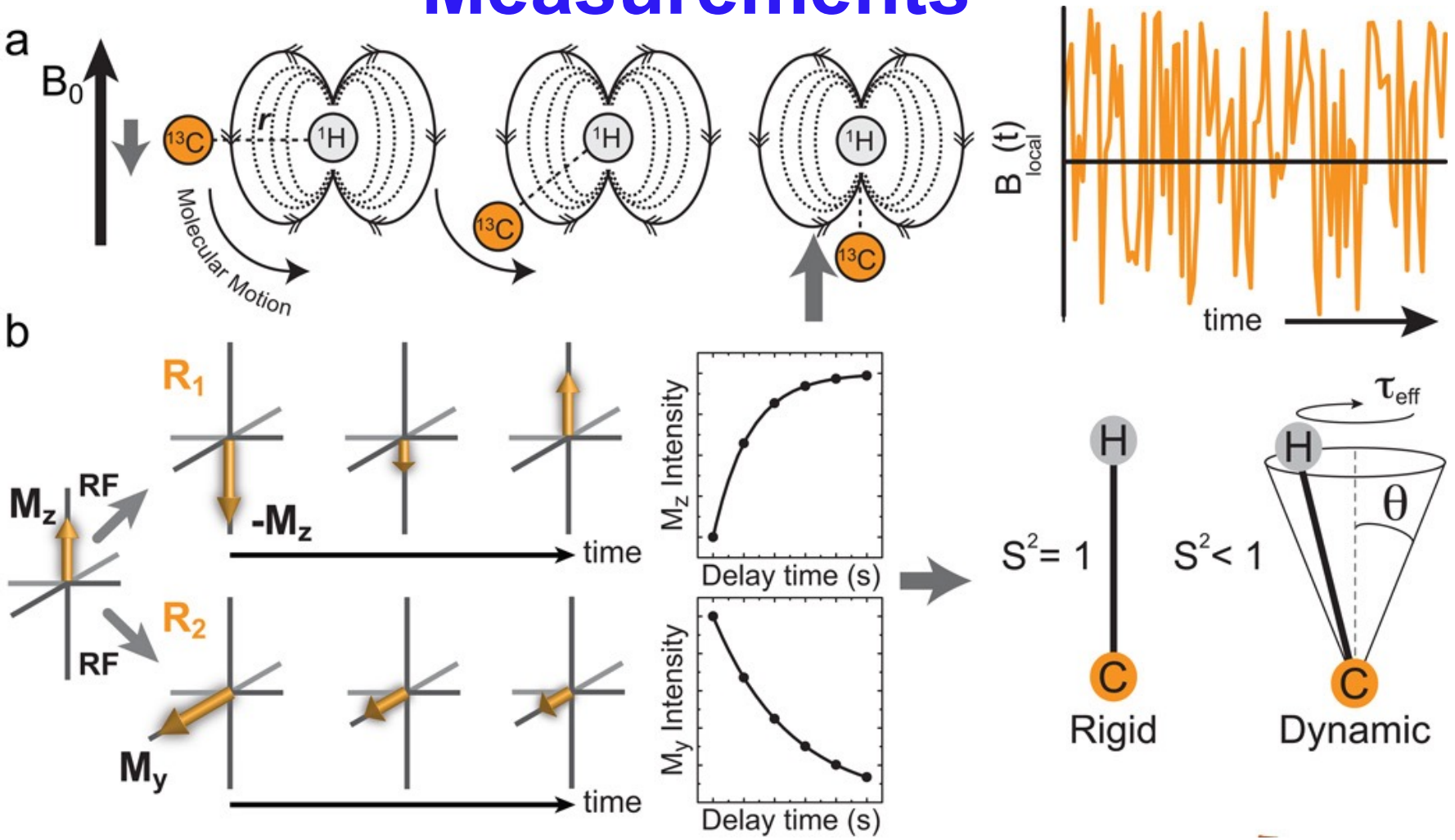
Wagner, G., Demarco, A., Wuthrich, K. *Biophysics of Structure and Mechanism,* **1976,** 2, 139-158
Wagner G, *FEBS Letters*, **1980**, 112(2), 280-284
Skalicky JJ, Mills JL, Sharma S, Szyperski T, *Journal of American Chemical Society*, **2001**, 24, 388-397

Dynamics using NMR Spectroscopy



Bothe R., Nikolova E.N., Eichhorn C.D., Chugh J., Hansen A.L., Al-Hashimi H.M., *Nature Methods,* **2011,** 8, 919-931

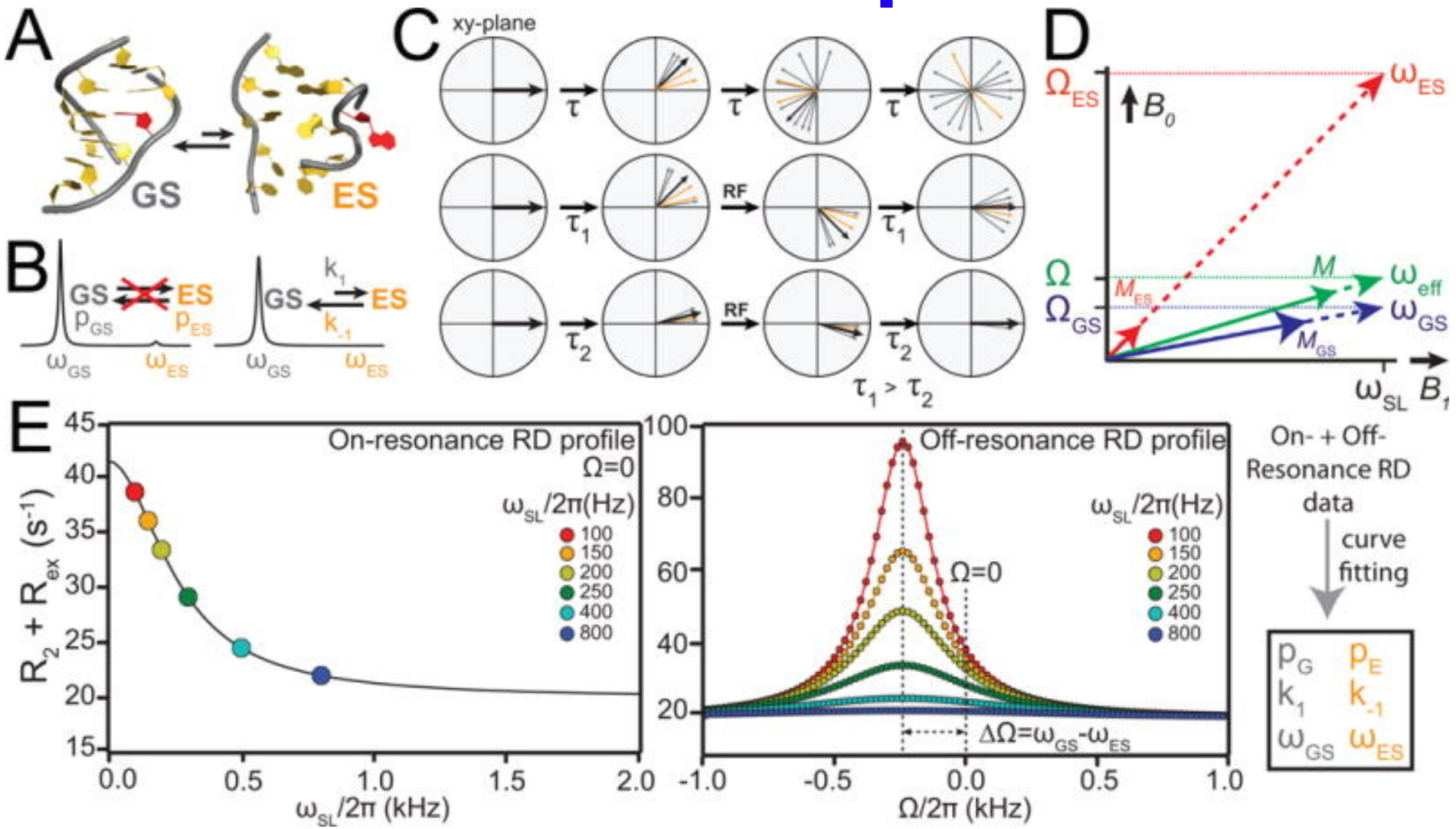
Nuclear Spin Relaxation Measurements



- Time-scale limited by overall tumbling of the molecule
- Provides relative motions across bonds within a molecule

Bothe R., Nikolova E.N., Eichhorn C.D., Chugh J., Hansen A.L., Al-Hashimi H.M., *Nature Methods,* **2011,** 8, 919-931

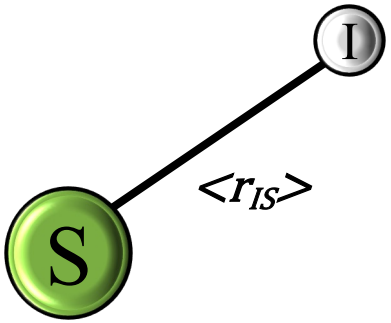
Relaxation Dispersion



- Microseconds to milliseconds
- Provides rates, population and chemical shift of minor state(s)

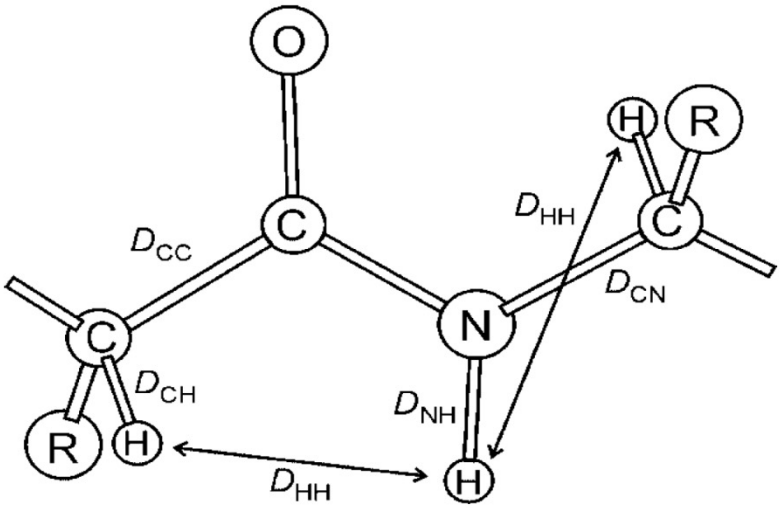
Xue Y, Kellogg D, Kimsey IJ, Sathyamoorthy B, Stein ZW, McBrairty M, Al-Hashimi H.M., *Methods Enzymology,* **2015,** 558, 39-74

Dipolar Coupling



I, S are the two spins coupled to each other.
$\mathbf{r_{IS}}$ is the inter-nuclear vector between them.
$\boldsymbol{\mu_0}$ is the magnetic permeability of vacuum.
$\boldsymbol{\gamma_I}$ and $\boldsymbol{\gamma_s}$ are the magnetogyric ratio of the two spins.

$$\mathcal{H}^{IS}_{dioplar} = \left(\frac{\mu_0 \hbar}{4\pi}\right)\left(\frac{\gamma_I \gamma_S}{\langle r_{IS} \rangle^3}\right)\left[I \cdot S - \frac{3(I \cdot r_{IS})(S \cdot r_{IS})}{\langle r_{IS} \rangle^2}\right]$$



$D_{C\alpha H\alpha}$	= 48 kHz		
D_{NH}	= -24 kHz		
$D_{H.H\alpha}$	= 22 kHz	(i, i)	
$D_{H.H\alpha}$	= 10 kHz	(i, i-1)	
$D_{C\alpha C'}$	= 5 kHz		
$D_{NC\alpha}$	= -2 kHz		

Residual Dipolar Coupling

$$\mathcal{H}_{dioplar}^{IS} = \left(\frac{\mu_0 \hbar}{4\pi}\right)\left(\frac{\gamma_I \gamma_S}{\langle r_{IS}\rangle^3}\right)\left[I \cdot S - \frac{3(I \cdot r_{IS})(S \cdot r_{IS})}{\langle r_{IS}\rangle^2}\right]$$

$$\mathcal{H}_{dioplar}^{IS} = \left(\frac{\mu_0 \hbar}{4\pi}\right)\left(\frac{\gamma_I \gamma_S}{\langle r_{IS}\rangle^3}\right)\left[\hat{A} + \hat{B}^{ZQ} + \hat{C}^{SQ} + \hat{D}^{DQ}\right]$$

$$\hat{A} \quad = \frac{1}{2}(3\cos^2\theta - 1).\,[2I_z S_z]$$

$$\hat{B}^{ZQ} = \frac{1}{8}(3\cos^2\theta - 1).\,[2I^+S^- + 2I^-S^+]$$

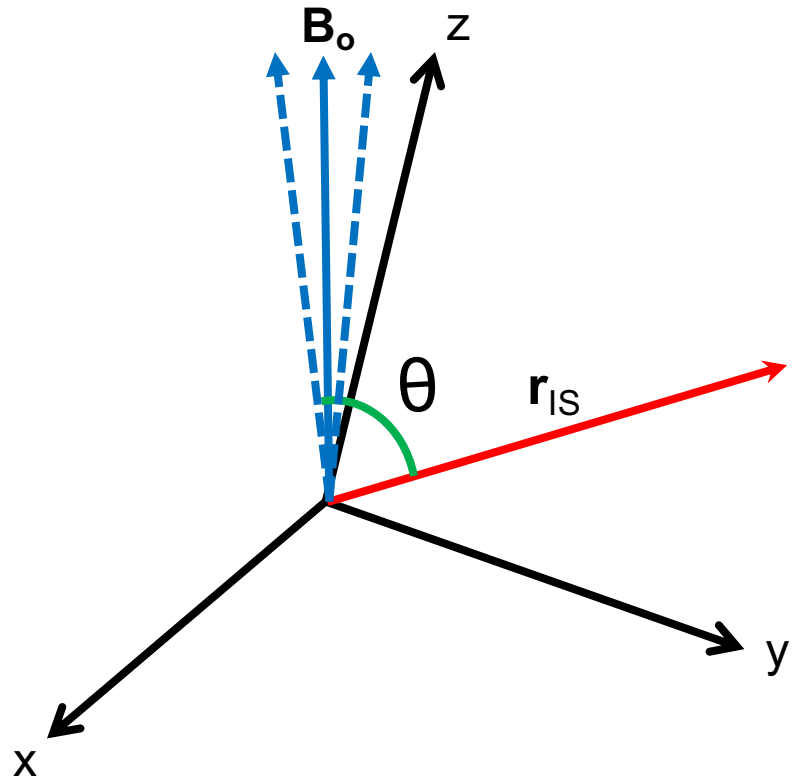
$$\hat{C}^{SQ} = \frac{3}{8}\sin 2\theta\left[e^{-i\phi}(2I^+S_z + 2I^-S_z) + e^{+i\phi}(2I_zS^- + 2I_zS^+)\right]$$

$$\hat{D}^{DQ} = \frac{3}{8}\sin^2\theta\left[e^{-i2\phi}I^+S^+ + e^{+i2\phi}I^-S^-\right]$$

Theory

$$\hat{H}_{rdc} = -\left(\frac{\mu_0}{4\pi}\right)\hbar\left(\frac{\gamma_I\gamma_S}{\langle \mathbf{r}_{IS}\rangle^3}\right)\left\langle\overline{\frac{3\cos^2\theta - 1}{2}}\right\rangle(2I_zS_z)$$

$$= D_{IS}^{\max}\overline{\langle P_2(\cos\theta)\rangle}(2I_zS_z)$$



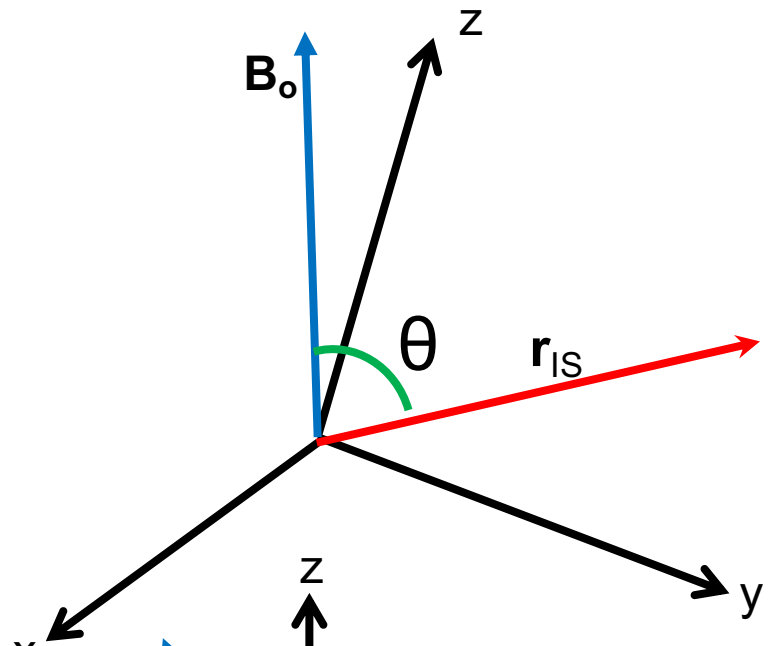
$$D = D_{IS}^{\max}\overline{\langle P_2(\cos\theta)\rangle}$$

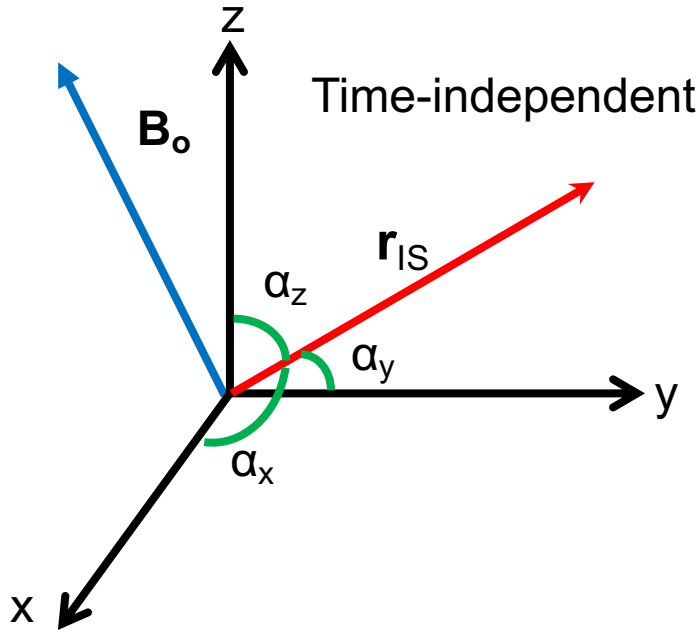
$$P_2(x) = \frac{1}{2}(3x^2 - 1)$$

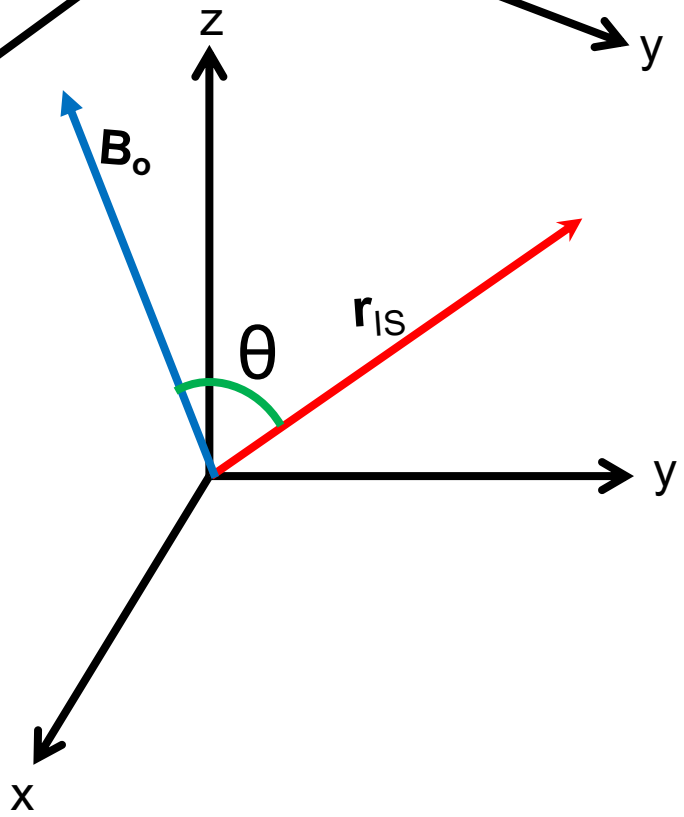
$$D_{IS}^{\max} = -\left(\frac{\mu_0}{4\pi}\right)\hbar\left(\frac{\gamma_I\gamma_S}{\langle \mathbf{r}_{IS}\rangle^3}\right)$$

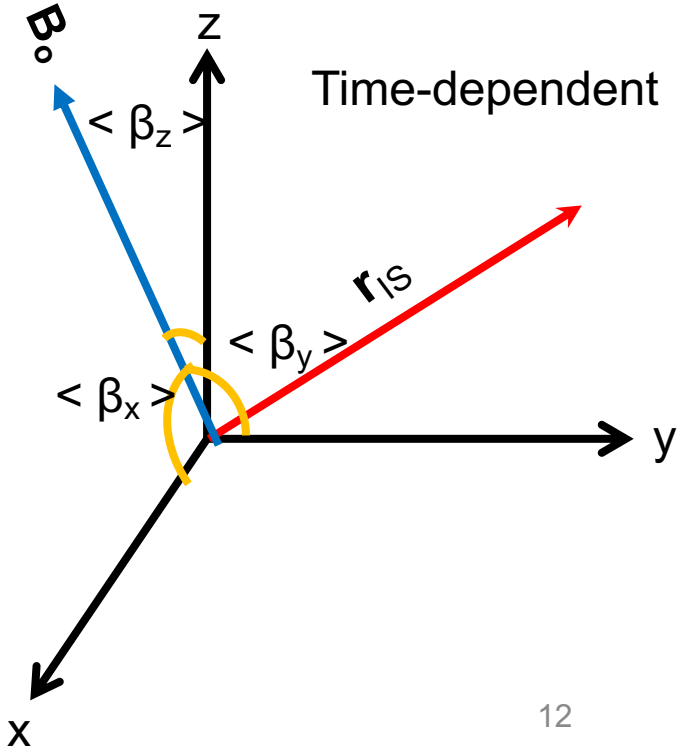
x, y, z represents the molecular axis frame.

Bax A, Kontaxis G, Tjandra N. *Methods Enzymology,* **2001,** 339, 127-74









Bax A, Kontaxis G, Tjandra N. *Methods Enzymology,* **2001,** 339, 127-74

$$\left\langle P_2(\cos\theta)\right\rangle = \frac{1}{2}\left(3\cos^2\theta - 1\right)$$

$$\mathbf{B}_o = \cos\beta_x\hat{x} + \cos\beta_y\hat{y} + \cos\beta_z\hat{z}$$

$$\mathbf{r}_{IS} = \cos\alpha_x\hat{x} + \cos\alpha_y\hat{y} + \cos\alpha_z\hat{z}$$

$$\cos\theta = \frac{\mathbf{B}_o \cdot \mathbf{r}_{IS}}{\left|\mathbf{B}_o\right|\left|\mathbf{r}_{IS}\right|} = \frac{\left(\cos\beta_x\hat{x} + \cos\beta_y\hat{y} + \cos\beta_z\hat{z}\right)\cdot\left(\cos\alpha_x\hat{x} + \cos\alpha_y\hat{y} + \cos\alpha_z\hat{z}\right)}{\sqrt{\cos^2\beta_x + \cos^2\beta_y + \cos^2\beta_z}\sqrt{\cos^2\alpha_x + \cos^2\alpha_y + \cos^2\alpha_z}}$$

But $\beta_i = \left\langle\beta_i\right\rangle$

$$\cos\theta = \frac{\left(\cos\left\langle\beta_x\right\rangle\cos\alpha_x + \cos\left\langle\beta_y\right\rangle\cos\alpha_y + \cos\left\langle\beta_z\right\rangle\cos\alpha_z\right)}{\sqrt{\cos^2\left\langle\beta_x\right\rangle + \cos^2\left\langle\beta_y\right\rangle + \cos^2\left\langle\beta_z\right\rangle}\sqrt{\cos^2\alpha_x + \cos^2\alpha_y + \cos^2\alpha_z}}$$

$\left\langle C_i\right\rangle = \cos\left\langle\beta_i\right\rangle$ and $c_i = \cos\alpha_i$

$$\cos\theta = \frac{\left\langle C_x\right\rangle c_x + \left\langle C_y\right\rangle c_y + \left\langle C_z\right\rangle c_z}{\sqrt{\left\langle C_x\right\rangle^2 + \left\langle C_y\right\rangle^2 + \left\langle C_z\right\rangle^2}\sqrt{c_x{}^2 + c_y{}^2 + c_z{}^2}}$$

$$\because \sqrt{\left\langle C_x\right\rangle^2 + \left\langle C_y\right\rangle^2 + \left\langle C_z\right\rangle^2} = \sqrt{c_x{}^2 + c_y{}^2 + c_z{}^2} = 1$$

$$\cos\theta = \left\langle C_x\right\rangle c_x + \left\langle C_y\right\rangle c_y + \left\langle C_z\right\rangle c_z$$

$$\cos^2\theta = \left\langle C_x\right\rangle^2 c_x{}^2 + \left\langle C_y\right\rangle^2 c_y{}^2 + \left\langle C_z\right\rangle^2 c_z{}^2 + 2\left\langle C_xC_y\right\rangle c_xc_y + 2\left\langle C_yC_z\right\rangle c_yc_z + 2\left\langle C_zC_x\right\rangle c_zc_x$$

Bax A, Kontaxis G, Tjandra N. *Methods Enzymology,* **2001,** 339, 127-74

$$\cos^2\theta = \langle C_x\rangle^2 c_x^{\,2} + \langle C_y\rangle^2 c_y^{\,2} + \langle C_z\rangle^2 c_z^{\,2} + 2\langle C_x C_y\rangle c_x c_y + 2\langle C_y C_z\rangle c_y c_z + 2\langle C_z C_x\rangle c_z c_x$$

$$\langle P_2(\cos\theta)\rangle = \frac{1}{2}\left(3\cos^2\theta - 1\right)$$

$$= \frac{1}{2}\left[3\left(\langle C_x\rangle^2 c_x^{\,2} + \langle C_y\rangle^2 c_y^{\,2} + \langle C_z\rangle^2 c_z^{\,2} + 2\langle C_x C_y\rangle c_x c_y + 2\langle C_y C_z\rangle c_y c_z + 2\langle C_z C_x\rangle c_z c_x\right) - 1\right]$$

$$= \sum_{i,j=x,y,z} S_{ij} c_i c_j$$

where, S_{ij} is defined as,

$S_{ij} = \frac{1}{2}\left(3\langle C_i C_j\rangle - \delta_{ij}\right)$ δ_{ij} is the Kronecker delta.

$$S_{ji} = \frac{1}{2}\left(3\langle C_j C_i\rangle - \delta_{ij}\right) = S_{ij} \qquad \ldots(1)$$

$$S_{ii} = \frac{1}{2}\left(3\langle C_i\rangle^2 - 1\right) \Rightarrow \sum_{i=x,y,z} S_{ii} = \frac{1}{2}\left(3\langle C_x\rangle^2 + \langle C_y\rangle^2 + \langle C_z\rangle^2 - 3\right) = 0 \qquad \ldots(2)$$

The matrix formed by the elements S_{ij} is called the *Saupe Order Matrix*, ***S***.

Do not confuse with order parameter S_{LS}^2!

S is traceless and symmetric. These two conditions imply that out of the 9 elements that form the 3X3 matrix, it is enough if 5 are determined (3 off diagonal & 2 diagonal).

Bax A, Kontaxis G, Tjandra N. *Methods Enzymology,* **2001,** 339, 127-74

$$\mathbf{S'} = \begin{pmatrix} S_{11} & S_{12} & S_{13} \\ S_{21} & S_{22} & S_{23} \\ S_{31} & S_{32} & S_{33} \end{pmatrix}$$

$$\mathbf{S} = \begin{pmatrix} S_{xx} & 0 & 0 \\ 0 & S_{yy} & 0 \\ 0 & 0 & S_{zz} \end{pmatrix} \text{ in Principle Axis System.}$$

$$|S_{zz}| \geq |S_{yy}| \geq |S_{xx}|$$

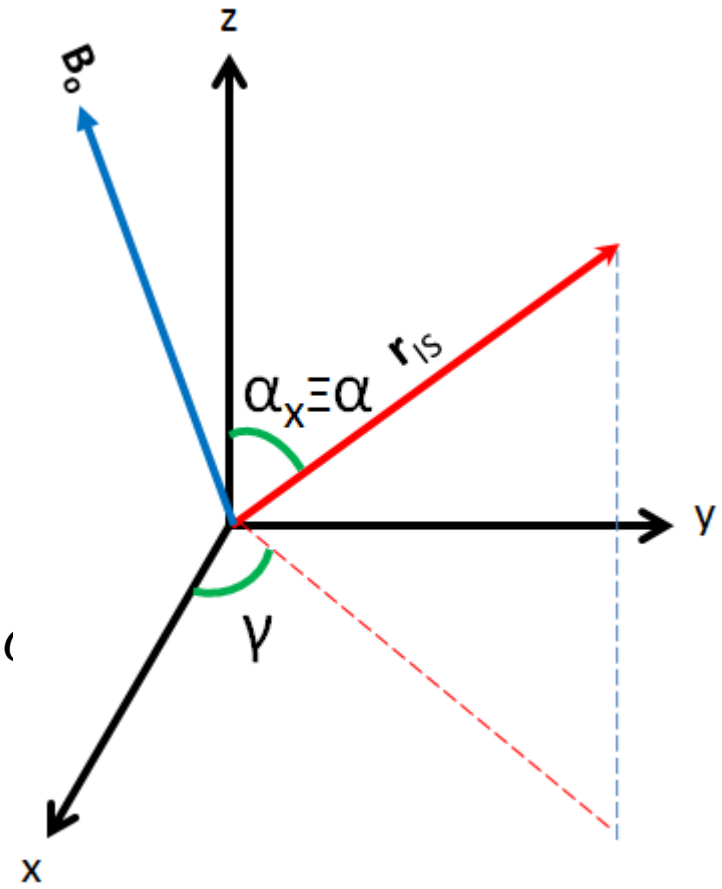
$$D = D_{IS}^{\max} \langle P_2(\cos\theta) \rangle = D_{IS}^{\max} \left[\frac{3}{2} \left(\langle C_x \rangle^2 c_x^{\,2} + \langle C_y \rangle^2 c_y^{\,2} + \langle ($$

$$D = D_{IS}^{\max} \left[\frac{3}{2} \left(\langle C_x \rangle^2 c_x^{\,2} + \langle C_y \rangle^2 c_y^{\,2} + \langle C_z \rangle^2 c_z^{\,2} \right) - \frac{1}{2} \right]$$

$$D = \frac{3}{2} D_{IS}^{\max} \left[\left(\langle C_x \rangle^2 c_x^{\,2} + \langle C_y \rangle^2 c_y^{\,2} + \langle C_z \rangle^2 c_z^{\,2} \right) - \frac{1}{3} \right]$$

Now going to the spherical polar co - ordinates

$$\langle C_i \rangle^2 = \frac{1}{3} + A_{ii} \text{ and } c_x = \sin\alpha\cos\gamma; c_y = \sin\alpha\sin\gamma; c_z = \cos\alpha;$$



CAUTION:
Notations followed!

Bax A, Kontaxis G, Tjandra N. *Methods Enzymology,* **2001,** 339, 127-74
Prestegard J.H., Al-Hashimi H.M., Tolman J.R. *Quarterly Reviews of Biophysics*, **2000**, 33(4), 371-424

$$D = \frac{3}{2} D_{IS}^{\max} \left[\left(\langle C_x \rangle^2 c_x^{\,2} + \langle C_y \rangle^2 c_y^{\,2} + \langle C_z \rangle^2 c_z^{\,2} \right) - \frac{1}{3} \right]$$

$$\langle C_i \rangle^2 = \frac{1}{3} + A_{ii} \text{ and } c_x = \sin\alpha\cos\gamma; c_y = \sin\alpha\sin\gamma; c_z = \cos\alpha;$$

$$D = \frac{3}{2} D_{IS}^{\max} \left[A_{zz}\cos^2\alpha + A_{xx}\sin^2\alpha\cos^2\gamma + A_{yy}\sin^2\alpha\sin^2\gamma \right]$$

$$D = \frac{3}{2} D_{IS}^{\max} \left[A_{zz}\left(\frac{3\cos^2\alpha - 1}{2} \right) + \left(\frac{A_{xx} - A_{yy}}{2} \right) \sin^2\alpha\cos 2\gamma \right]$$

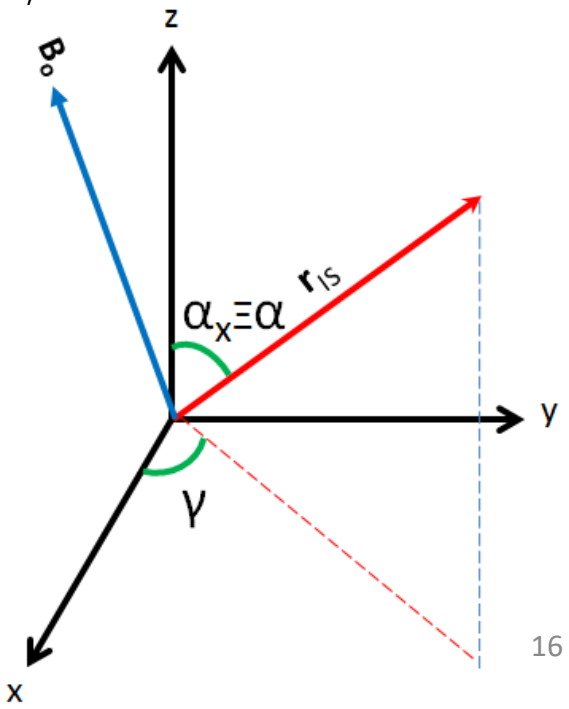
Defining an axial and rhombic alignment tensor A_a and A_r as

$$A_a = \frac{3}{2} A_{zz} \text{ and } A_r = \left(A_{xx} - A_{yy} \right)$$

$$D = D_{IS}^{\max} \left[\left(\frac{3\cos^2\alpha - 1}{2} \right) A_a + \frac{3}{4} A_r \sin^2\alpha\cos 2\gamma \right]$$

$$D = D_{IS}^{a} \left[\left(3\cos^2\alpha - 1 \right) + \frac{3}{2} R \sin^2\alpha\cos 2\gamma \right]$$

$$D_{IS}^{a} = \frac{1}{2} D_{IS}^{\max} A_a \text{ and } R = \left(\frac{A_{xx} - A_{yy}}{A_{zz}} \right)$$



Frequently seen expressions in literature:

I. Default expression

$$D = \frac{3}{2} D_{IS}^{\max} \left[A_{zz} \left(P_2(\cos\alpha) \right) + \left(\frac{A_{xx} - A_{yy}}{2} \right) \sin^2\alpha \cos 2\gamma \right]$$

II. Defining an axial and rhombic alignment tensor A_a and A_r as

$$D = D_{IS}^{\max} \left[\left(P_2(\cos\alpha) \right) A_a + \frac{3}{4} A_r \sin^2\alpha \cos 2\gamma \right]$$

$$A_a = \frac{3}{2} A_{zz} \quad \text{and} \quad A_r = \left(A_{xx} - A_{yy} \right)$$

III. Rhombicity R

$$D = D_{IS}^{a} \left[\left(3\cos^2\alpha - 1 \right) + \frac{3}{2} R \sin^2\alpha \cos 2\gamma \right]$$

$$D_{IS}^{a} = \frac{1}{2} D_{IS}^{\max} A_a \text{ and } R = \left(\frac{A_{xx} - A_{yy}}{A_{zz}} \right)$$

$$P_2(\cos\alpha) = \frac{3\cos^2\alpha - 1}{2}$$

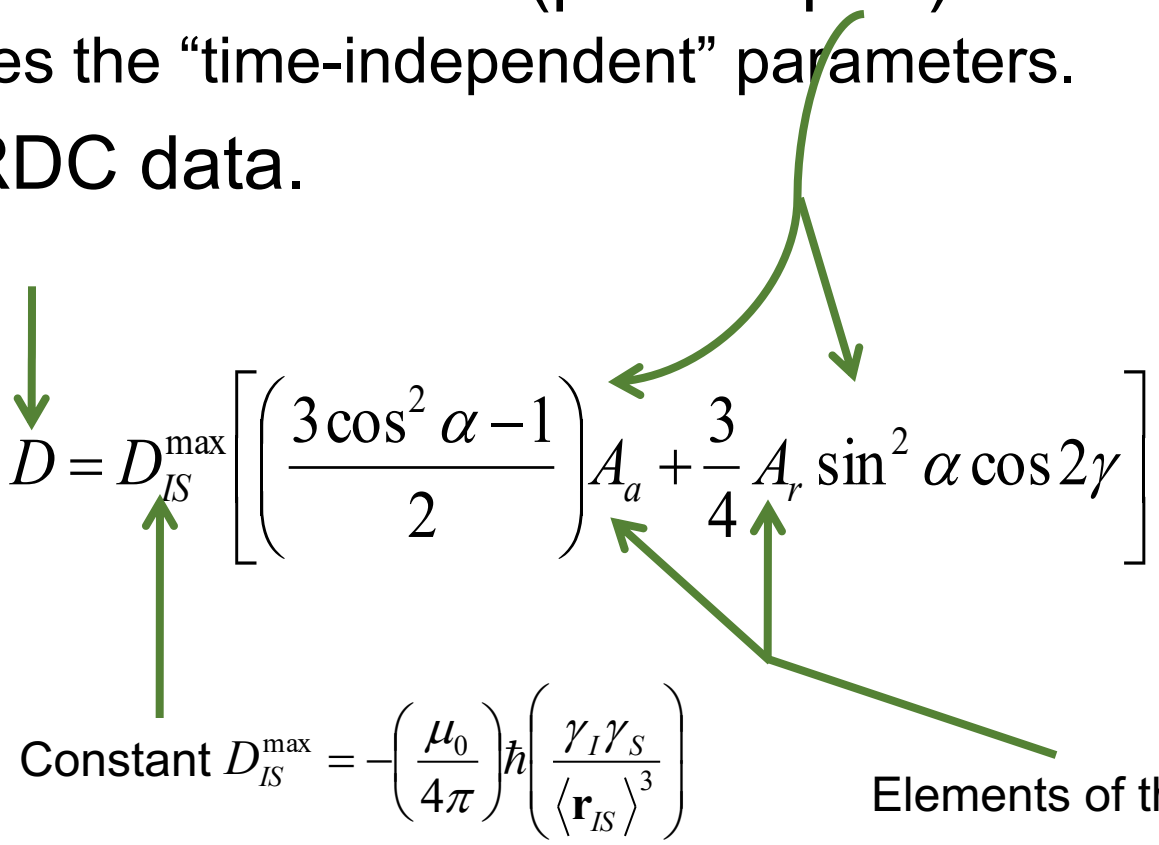
$$D_{IS}^{\max} = -\left(\frac{\mu_0}{4\pi} \right) \hbar \left(\frac{\gamma_I \gamma_S}{\left\langle \mathbf{r}_{IS} \right\rangle^3} \right)$$

Determination of alignment tensor

Protein with structure.

Input files:

1. The co-ordinates file. (protein.pdb)
   - Gives the “time-independent” parameters.
2. The RDC data.



Order matrix analysis by SVD

$$\langle P_2(\cos\theta)\rangle = \frac{1}{2}\left[3\left(\langle C_x\rangle^2 c_x^{\,2} + \langle C_y\rangle^2 c_y^{\,2} + \langle C_z\rangle^2 c_z^{\,2} + 2\langle C_x C_y\rangle c_x c_y + 2\langle C_y C_z\rangle c_y c_z + 2\langle C_z C_x\rangle c_z c_x\right) - 1\right]$$

$$\langle P_2(\cos\theta)\rangle = \frac{1}{2}\left[3\left(S_{xx} c_x^{\,2} + S_{yy} c_y^{\,2} + S_{zz} c_z^{\,2} + 2S_{xy} c_x c_y + 2S_{yz} c_y c_z + 2S_{zx} c_z c_x\right) - 1\right]$$

$$\langle P_2(\cos\theta)\rangle = \frac{1}{2}\left[3\left(\left(-S_{yy} - S_{zz}\right) c_x^{\,2} + S_{yy} c_y^{\,2} + S_{zz} c_z^{\,2} + 2S_{xy} c_x c_y + 2S_{yz} c_y c_z + 2S_{zx} c_z c_x\right) - 1\right]$$

$$\langle P_2(\cos\theta)\rangle = \frac{1}{2}\left[3\left(S_{yy}\left(c_y^{\,2} - c_x^{\,2}\right) + S_{zz}\left(c_z^{\,2} - c_x^{\,2}\right) + 2S_{xy} c_x c_y + 2S_{yz} c_y c_z + 2S_{zx} c_z c_x\right) - 1\right]$$

From PDB

RDC input.

For *n* RDCs measured.

$$\begin{pmatrix} D_1 \\ D_2 \\ D_3 \\ D_4 \\ D_5 \\ . \\ . \\ D_n \end{pmatrix} = D_{IS}^{\max} \begin{pmatrix} c_{1y}^{\,2} - c_{1x}^{\,2} & c_{1z}^{\,2} - c_{1x}^{\,2} & 2c_{1x}c_{1y} & 2c_{1y}c_{1z} & 2c_{1z}c_{1x} \\ c_{2y}^{\,2} - c_{2x}^{\,2} & c_{2z}^{\,2} - c_{2x}^{\,2} & 2c_{2x}c_{2y} & 2c_{2y}c_{2z} & 2c_{2z}c_{2x} \\ c_{3y}^{\,2} - c_{3x}^{\,2} & c_{3z}^{\,2} - c_{3x}^{\,2} & 2c_{3x}c_{3y} & 2c_{3y}c_{3z} & 2c_{3z}c_{3x} \\ c_{4y}^{\,2} - c_{4x}^{\,2} & c_{4z}^{\,2} - c_{4x}^{\,2} & 2c_{4x}c_{4y} & 2c_{4y}c_{4z} & 2c_{4z}c_{4x} \\ c_{5y}^{\,2} - c_{5x}^{\,2} & c_{5z}^{\,2} - c_{5x}^{\,2} & 2c_{5x}c_{5y} & 2c_{5y}c_{5z} & 2c_{5z}c_{5x} \\ . & . & . & . & . \\ . & . & . & . & . \\ c_{ny}^{\,2} - c_{nx}^{\,2} & c_{nz}^{\,2} - c_{nx}^{\,2} & 2c_{nx}c_{ny} & 2c_{ny}c_{nz} & 2c_{nz}c_{nx} \end{pmatrix} \begin{pmatrix} S_{yy} \\ S_{zz} \\ S_{xy} \\ S_{yz} \\ S_{zx} \end{pmatrix}$$

S matrix: **TBD**.

$$D_{n\times1} = C_{n\times5}.S_{5\times1}$$

$$\left(C_{n\times5}\right)^{-1}.D_{n\times1} = S_{5\times1}$$

$$C_{n\times5} = U_{n\times5}.A_{5\times5}.\left(V_{5\times5}\right)^{T} \qquad \text{where } A_{5\times5} \text{ is a diagonal matrix}$$

$$\left(U_{n\times5}\right).\left(U_{n\times5}\right)^{T} = I_{n\times n}$$

$$\left(V_{5\times5}\right).\left(V_{5\times5}\right)^{T} = I_{5\times5}$$

$$\left(C_{n\times5}\right)^{-1} = V_{5\times5}.A_{5\times5}^{-1}.\left(U_{n\times5}\right)^{T}$$

$$\therefore S_{5\times1} = \left(V_{5\times5}.A_{5\times5}^{-1}.\left(U_{n\times5}\right)^{T}\right).D_{n\times1}$$

$$\left(A_{5\times5}\right)^{-1}$$

$$\begin{pmatrix} a & 0 & 0 & 0 & 0 \\ 0 & b & 0 & 0 & 0 \\ 0 & 0 & c & 0 & 0 \\ 0 & 0 & 0 & d & 0 \\ 0 & 0 & 0 & 0 & e \end{pmatrix}^{-1} = \begin{pmatrix} 1/a & 0 & 0 & 0 & 0 \\ 0 & 1/b & 0 & 0 & 0 \\ 0 & 0 & 1/c & 0 & 0 \\ 0 & 0 & 0 & 1/d & 0 \\ 0 & 0 & 0 & 0 & 1/e \end{pmatrix}$$

$$\mathbf{S'} = \begin{pmatrix} S_{11} & S_{12} & S_{13} \\ S_{21} & S_{22} & S_{23} \\ S_{31} & S_{32} & S_{33} \end{pmatrix} \text{ in Molecular Axis System.}$$

$$\mathbf{S} = \begin{pmatrix} S_{xx} & 0 & 0 \\ 0 & S_{yy} & 0 \\ 0 & 0 & S_{zz} \end{pmatrix} \text{ in Principle Axis System.}$$

Thus, the alignment tensor is determined for the given protein (for that conformer) in that particular alignment media under a specific set of conditions. (temperature, pH, ionic strength, magnetic field, etc.)

Residual Dipolar Coupling

$$\hat{\mathcal{H}}_{rdc} = \left(\frac{\mu_0 \hbar}{\pi}\right)\left(\frac{\gamma_I \gamma_S}{\langle r_{IS}\rangle^3}\right)\left(\frac{3\cos^2\theta - 1}{2}\right).[2\boldsymbol{I_z S_z}]$$

$$\hat{\mathcal{H}}_{scalar} = (\pi J_{IS}).[2\boldsymbol{I_z S_z}]$$

- One-bonded RDCs are therefore commonly measured as they are easy to measure and r_{IS} is well defined
- r^{-3} dependence renders RDCs to be more global parameters than NOEs (extending them to 20-30 Å)
- Over-determination of the AT provides more reliable prediction of RDCs

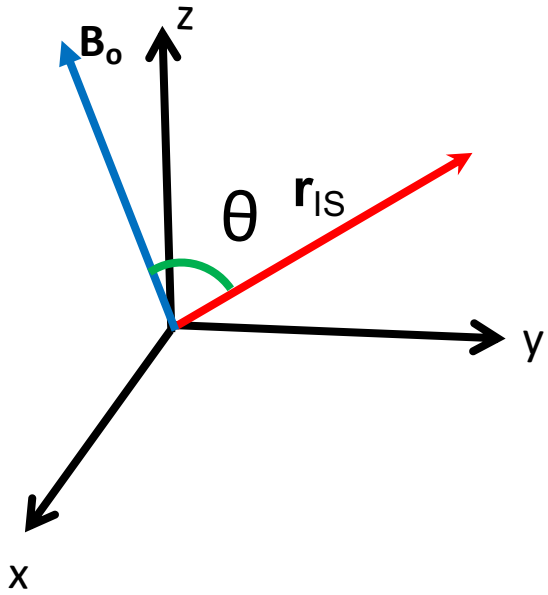
$$Q = \sqrt{\frac{D_{calc}^2 - D_{obs}^2}{D_{obs}^2}}$$

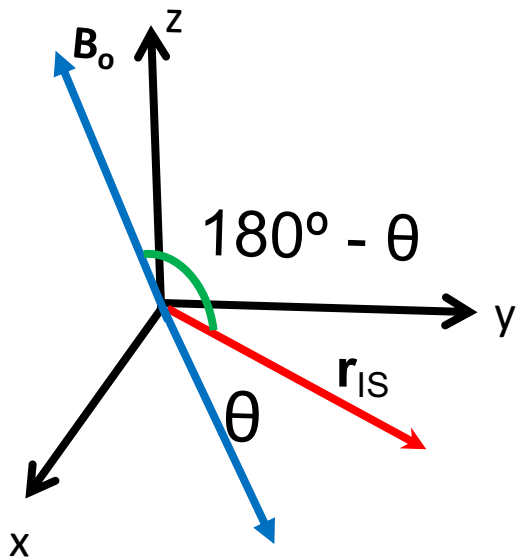
Which way is the bond aligned? θ or – θ?

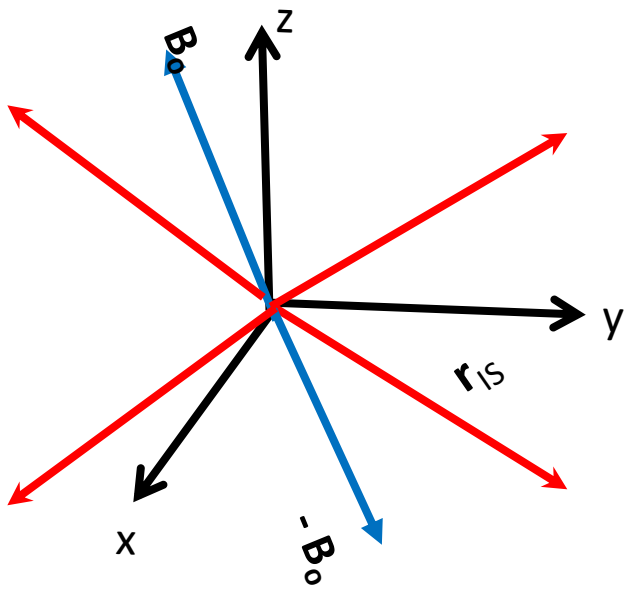
$$\cos(\pi-\theta)=-\cos\theta$$

$$\text{but } \cos^2(\pi-\theta)=\cos^2\theta$$

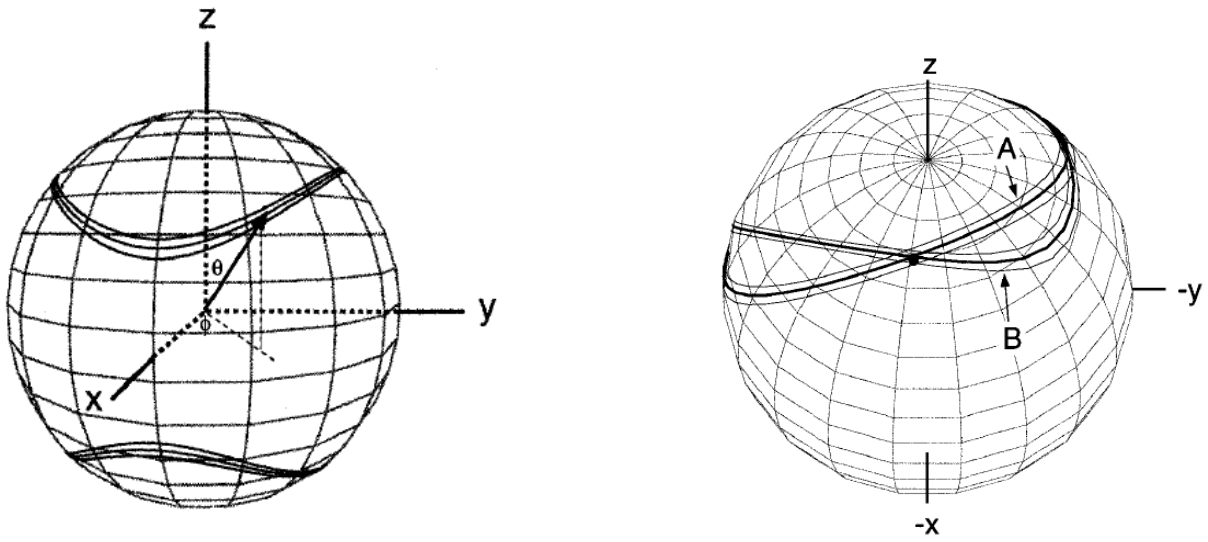
$$\text{Similarly, } \cos^2\theta=\cos^2(\pi-\theta)=\cos^2(\pi+\theta)=\cos^2(2\pi-\theta)$$







Measurement with one media gives a range of polar angles. Thus another alignment media would help in more precise determination of the angles.



Including the other constraints helps reduce possible orientations.

Alignment Tensor Absence of Structure

Assuming different, fixed distance inter-nuclear vector types are isotropically distributed *relative* to the alignment tensor , then…

A histogram of the *normalized* RDC for several vectors approximates a powder pattern, from which the axial and rhombic components are readily extracted.

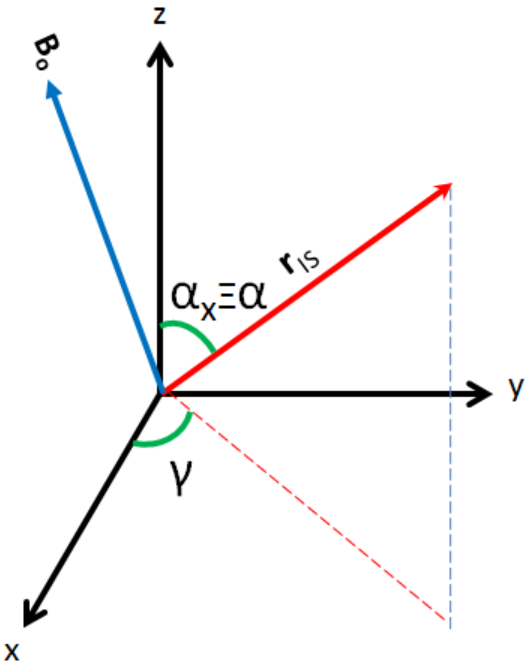
- Extreme values when:

α=0º (closest to z of PAS)and α=γ=90º (to y)

$$D = D_{IS}^{a}\left[\left(3\cos^2\alpha - 1\right) + \frac{3}{2}R\sin^2\alpha\cos 2\gamma\right]$$

$$D_{\max} = 2D_{IS}^{a} \qquad (\alpha = 0)$$

$$D_{\min} = -D_{IS}^{a}\left(1 + 1.5R\right) \qquad (\alpha = \gamma = \pi/2)$$

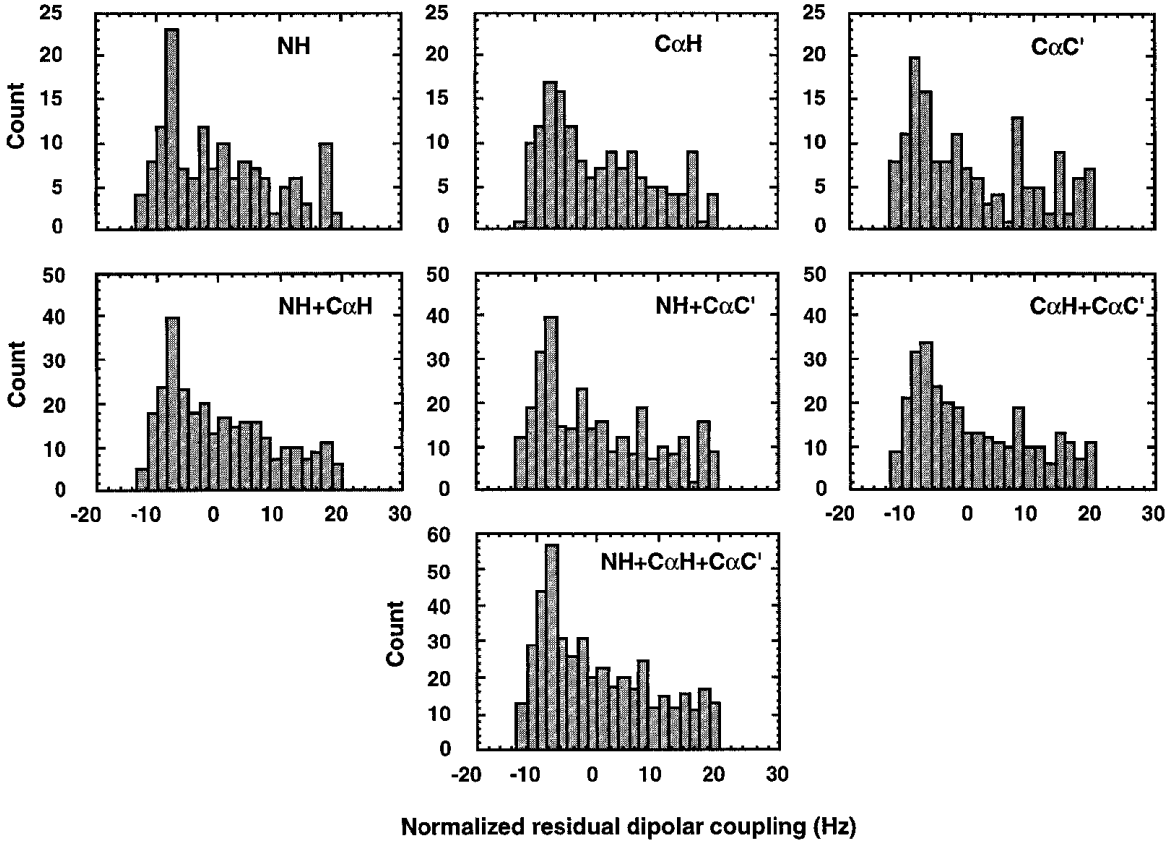


If the internuclear vectors are distributed uniformly and isotropically, a histogram describing the probability of finding D values between these two values will have the same shape as a CSA powder pattern (?).

- The highest probability when:

α=90,γ=0 (to x) $D = D_{IS}^{a}\left[\left(3\cos^2\alpha - 1\right) + \frac{3}{2}R\sin^2\alpha\cos 2\gamma\right]$

$$D_{\min} = -D_{IS}^{a}\left(1 - 1.5R\right) \qquad (\alpha = \pi/2, \gamma = 0)$$



interleukin-1β (153 residues, 17.5 kDa)

D_a = 10.0 and R=0.2

Some simulations…

- Over to Kaustubh…
- Let us take an example of 10 RDCs measured from experiment
- Best fit it against structural coordinate
  - Determine D_{IS}^{max}
  - Assume Cartesian coordinate as molecular frame and obtain direction cosines
- Perform singular value decomposition to obtain alignment tensor
- Back-predict RDCs, check Q-factor and RMSD with input

$$\begin{pmatrix} D_1 \\ D_2 \\ D_3 \\ D_4 \\ D_5 \\ . \\ . \\ D_n \end{pmatrix} = D_{IS}^{\max} \begin{pmatrix} c_{1y}^2 - c_{1x}^2 & c_{1z}^2 - c_{1x}^2 & 2c_{1x}c_{1y} & 2c_{1y}c_{1z} & 2c_{1z}c_{1x} \\ c_{2y}^2 - c_{2x}^2 & c_{2z}^2 - c_{2x}^2 & 2c_{2x}c_{2y} & 2c_{2y}c_{2z} & 2c_{2z}c_{2x} \\ c_{3y}^2 - c_{3x}^2 & c_{3z}^2 - c_{3x}^2 & 2c_{3x}c_{3y} & 2c_{3y}c_{3z} & 2c_{3z}c_{3x} \\ c_{4y}^2 - c_{4x}^2 & c_{4z}^2 - c_{4x}^2 & 2c_{4x}c_{4y} & 2c_{4y}c_{4z} & 2c_{4z}c_{4x} \\ c_{5y}^2 - c_{5x}^2 & c_{5z}^2 - c_{5x}^2 & 2c_{5x}c_{5y} & 2c_{5y}c_{5z} & 2c_{5z}c_{5x} \\ . & . & . & . & . \\ . & . & . & . & . \\ c_{ny}^2 - c_{nx}^2 & c_{nz}^2 - c_{nx}^2 & 2c_{nx}c_{ny} & 2c_{ny}c_{nz} & 2c_{nz}c_{nx} \end{pmatrix} \begin{pmatrix} S_{yy} \\ S_{zz} \\ S_{xy} \\ S_{yz} \\ S_{zx} \end{pmatrix}$$

Alignment media

Alignment Mechanisms:

1. Steric
2. Electrostatics –
    a) Attractive
    b) Repulsive

Alignment media:

- Magnetic field alignment
- Poly Acrylamide Gel
- Liquid Crystals
    - Bicelles
    - Phages
    - Non-ionic LC media

Magnetic field alignment

- Macromolecules with large anisotropic magnetic susceptibility tend to align at high magnetic field strength.

- Generally,
  - Metalloproteins (cyanometglobulin)
  - Regularly structured diamagnetic structures (nucleic acids)

- Number of metal binding sites matters.
- Metalloproteins: Replacement of the already present Fe^{+2} by Lanthanides (Dy^{+3} & Tb^{+3}).

Disadvantages

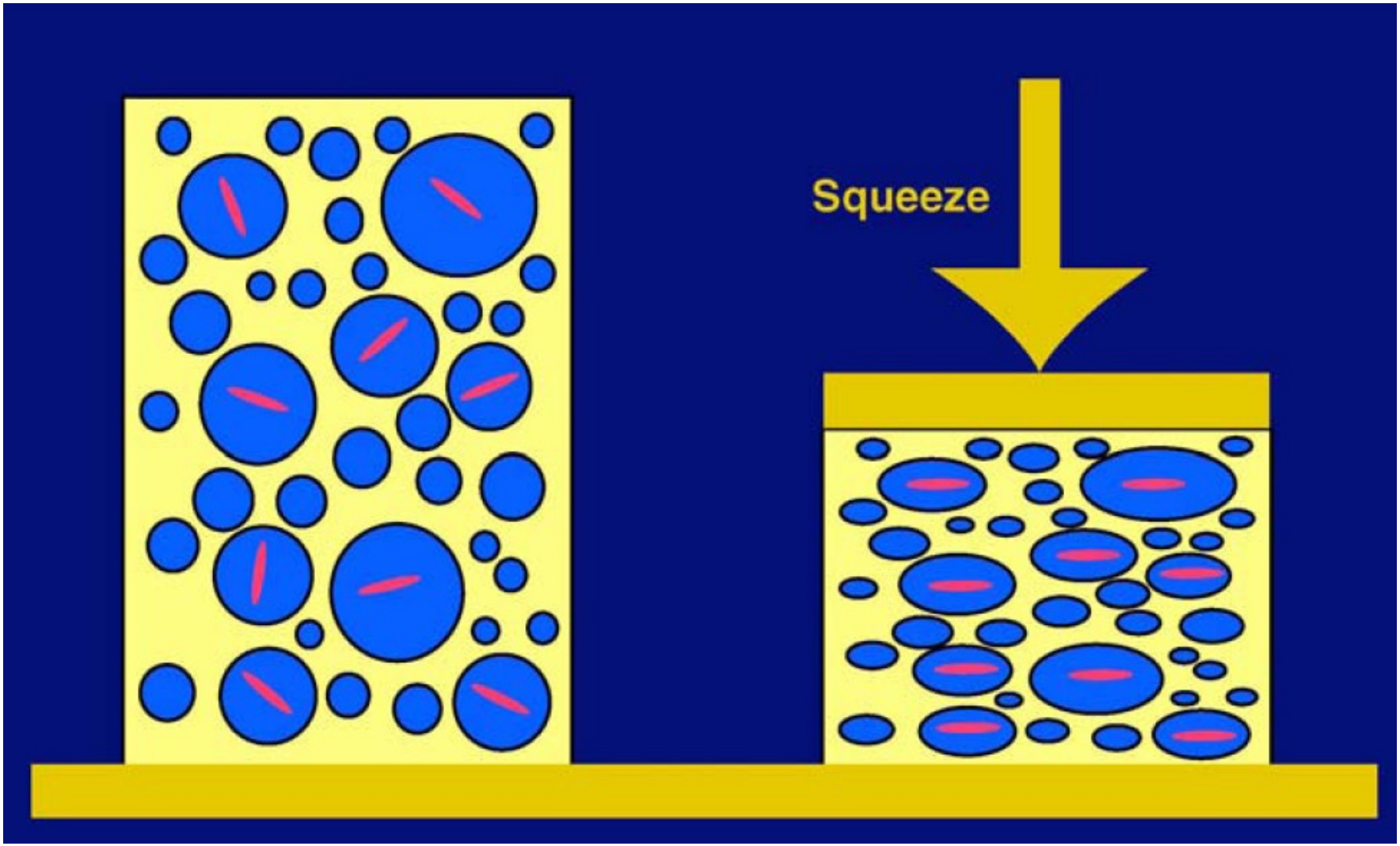
- Small contributions to splitting such as dynamic frequency shifts and cross-correlation effects can become significant.
- RDC to be measured at least in 2 field strengths to determine J & D.
- RDC values an order less than that of obtained using LC even under high magnetic fields.
- Line broadening effects (decreases sensitivity) are observed when Lanthanides are used (Eu^{+3}, Yb^{+3}, Ce^{+3}).
- Alignment $\alpha\ \chi B^2$.

Poly Acrylamide Gels (PAG)

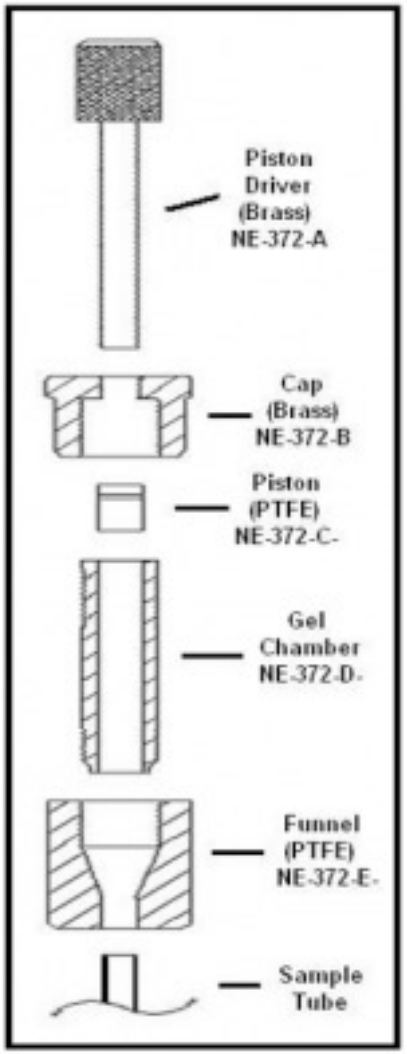
- Strain induced alignment in gels.
- Protein diffuses into “casted” PAG, transferred to NMR tube and compressed.
- The “cavities” in the gel now have a oblate character. When placed vertically in a magnet diffusing proteins into the aqueous phase will, on average, have their longer axis orthogonal to magnetic field.
- Advantages: Inertness and solute recovery
- Disadvantages: Inhibits rotational diffusion rate leads to line broadening.

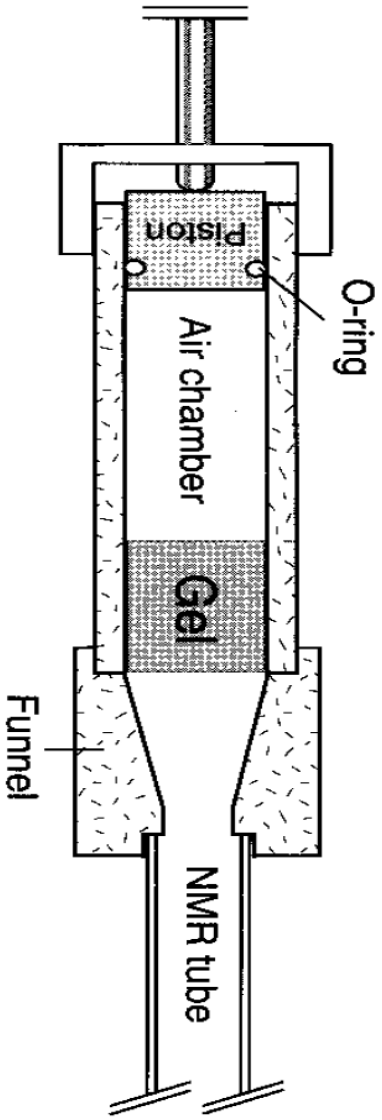
Tycko R, Blanco F.J., Ishi Y. *Journal of the American Chemical Society,* **2000**, 122(33), 9340-41

Partial Alignment in PAG



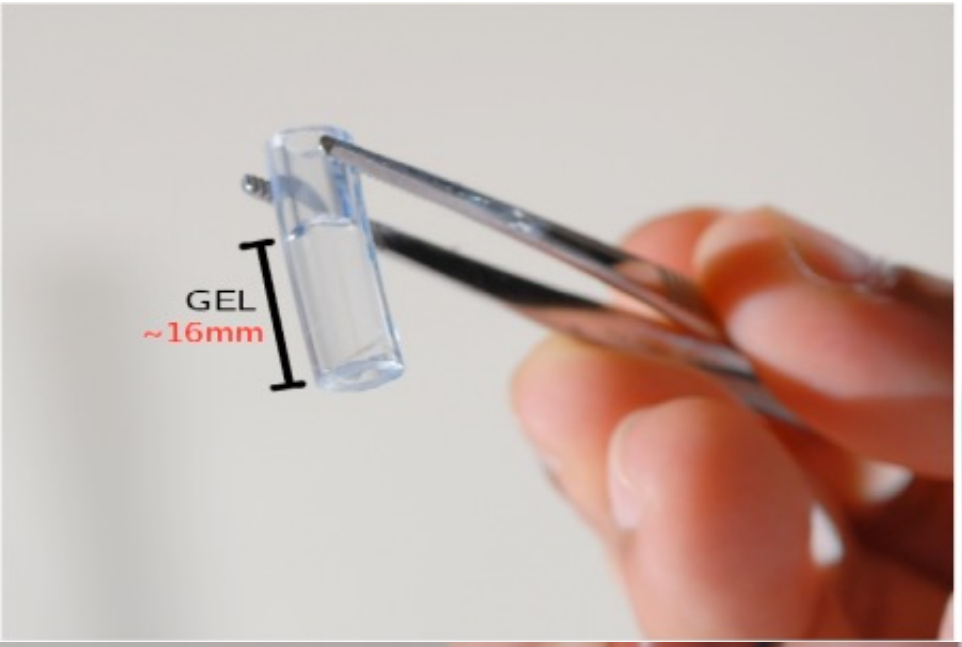
Stretched Gel

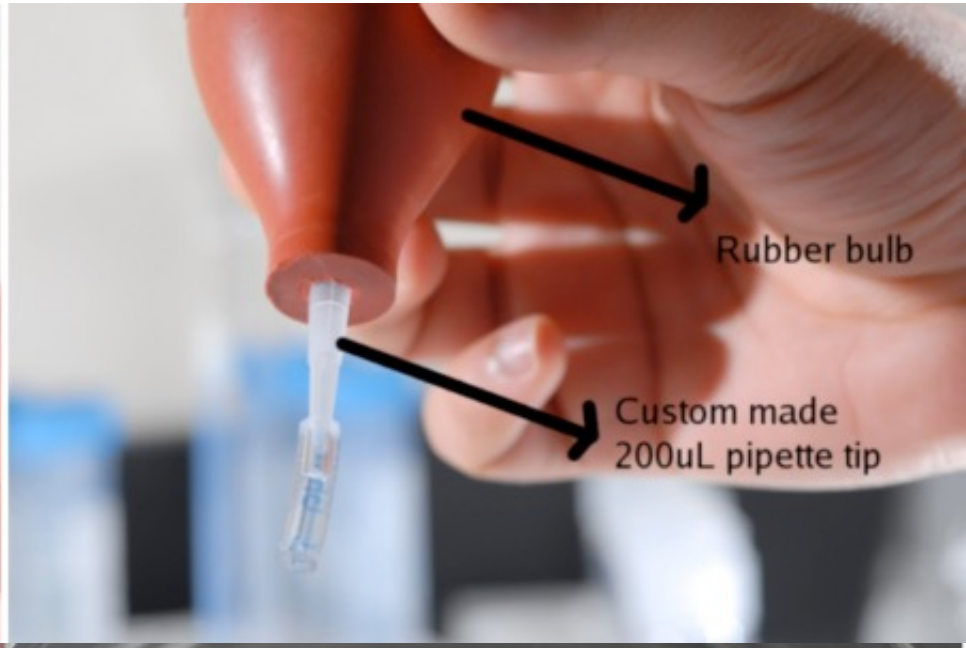


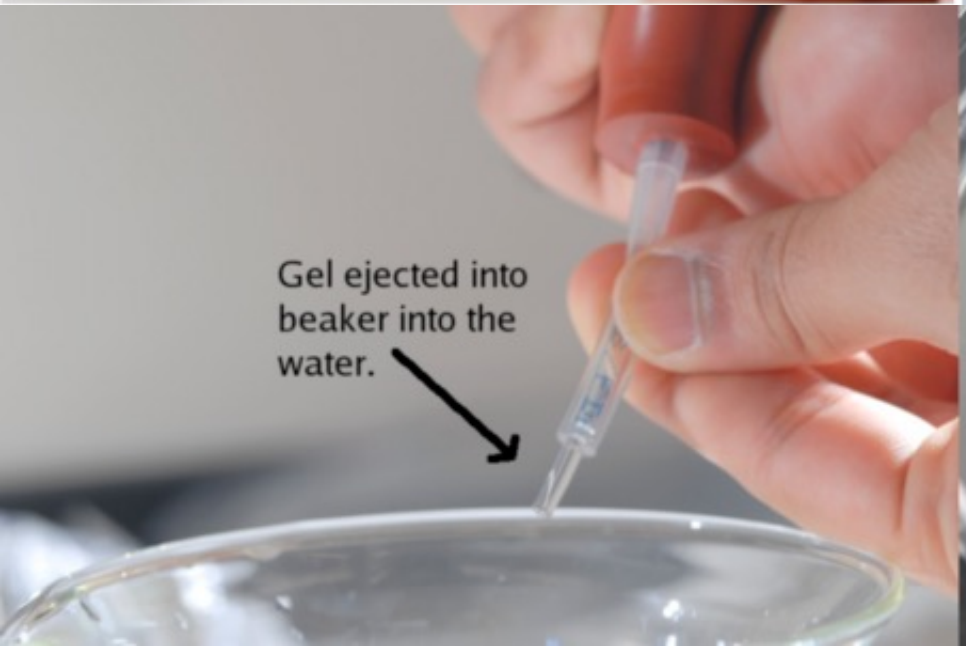


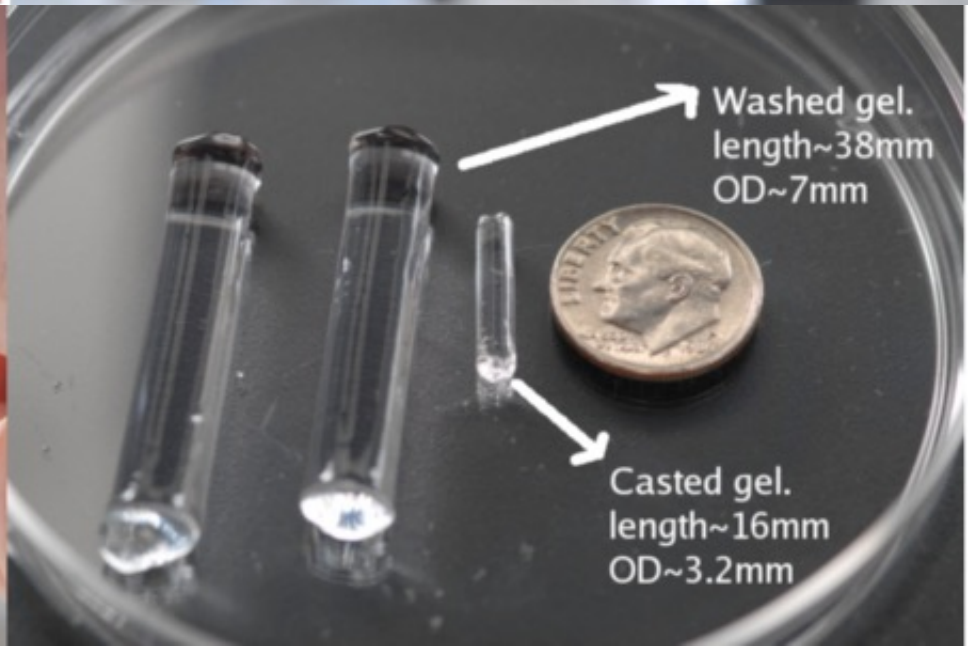
Chou J.J., Gaemers S, Howder B, Louis JM, Bax A. *Journal of Biomolecular NMR,* **2001**, 21, 377-82

Compressed gel

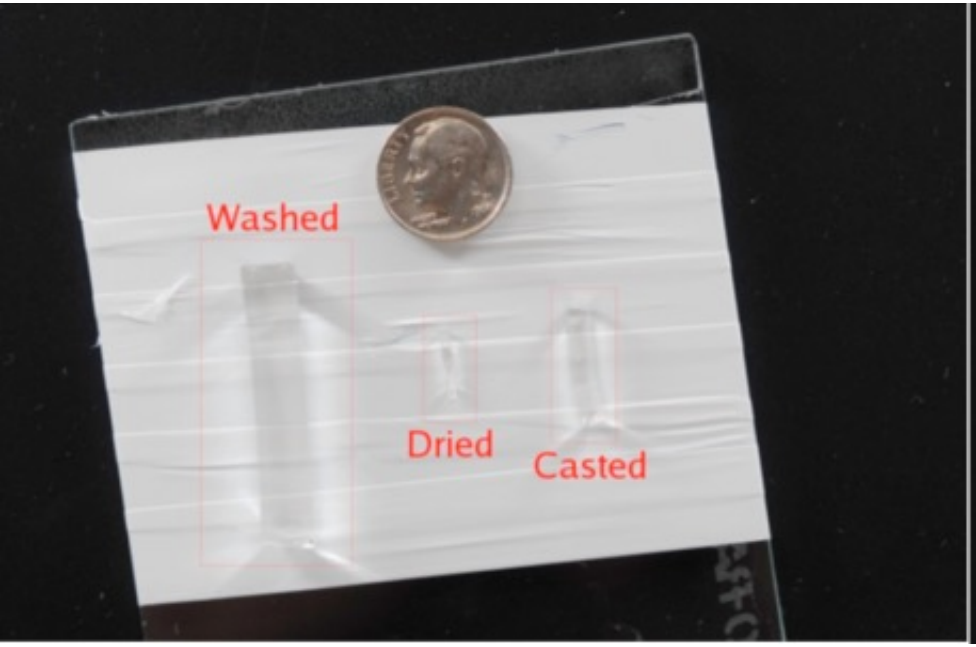


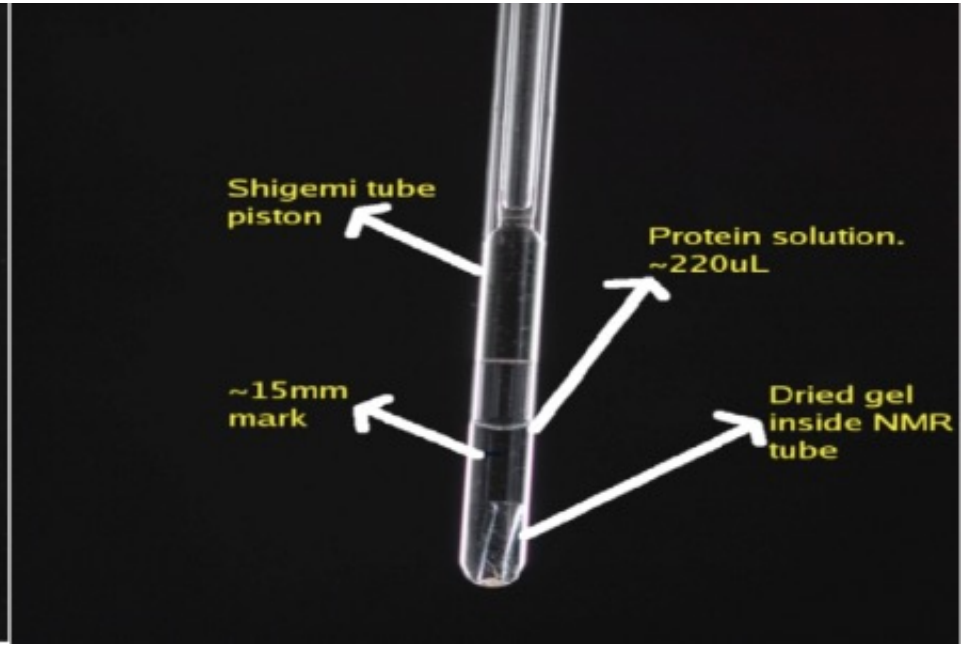






Compressed gel





Cierpicki T, Liang B, Tamm LK, Bushweller JH. *Journal of the American Chemical Society,* **2006**, 128(21), 6947-51

Liquid crystals – Bicelles

Various mixtures available.

- DMPC:DHPC::3~3.5:1

DMPC – dimyristoyl phosphatidylcholine.

DHPC – dihexanoyl phosphatidylcholine

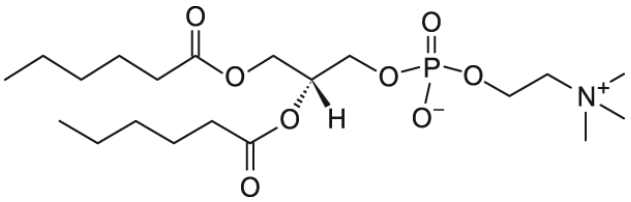
– DIODPC:CHAPSO::4.3:1

DIODPC – 1,2-di-O-dodecyl-sn-glycero-3-phosphocholine.

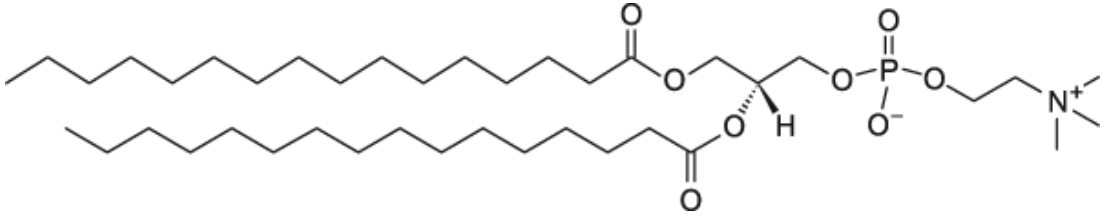
CHAPSO – 3-(cholamidopropyl) dimethylammonio-2-hyrdroxyl-1-propane sulfate.

0.1 equivalents of CTAB is added.

CTAB – hexadecyl (cetyl) trimethyl ammonium bromide.

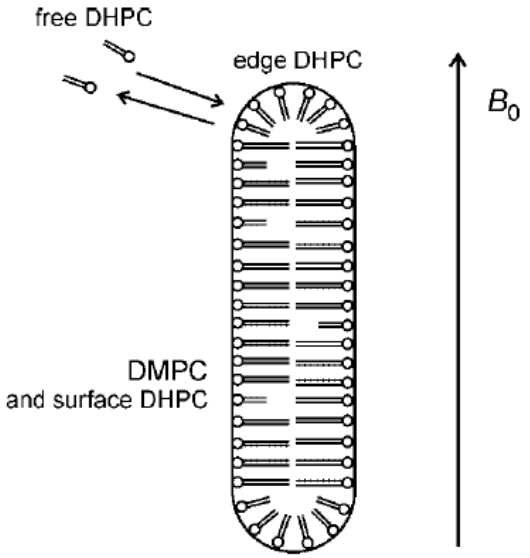


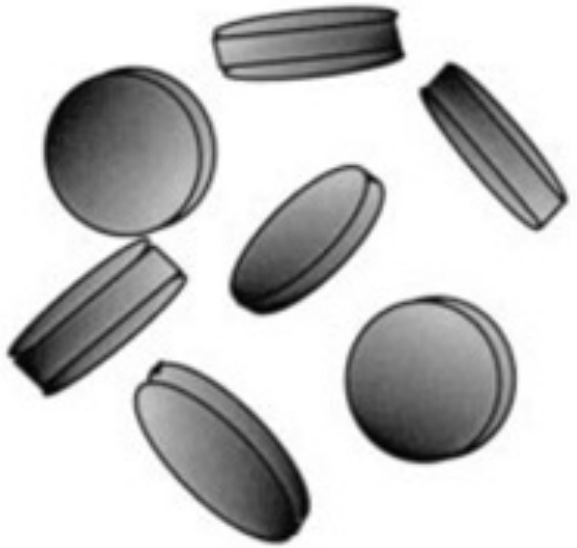
DHPC

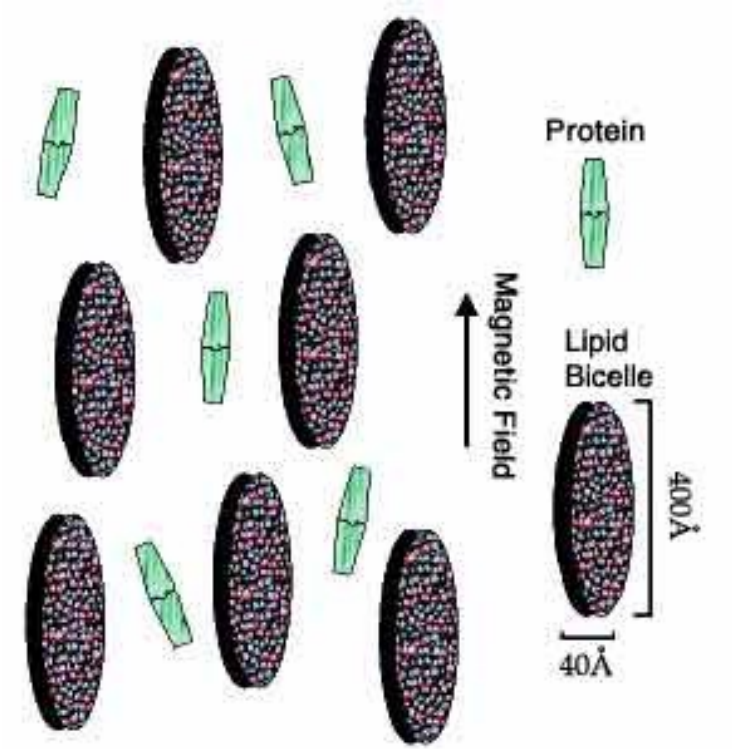


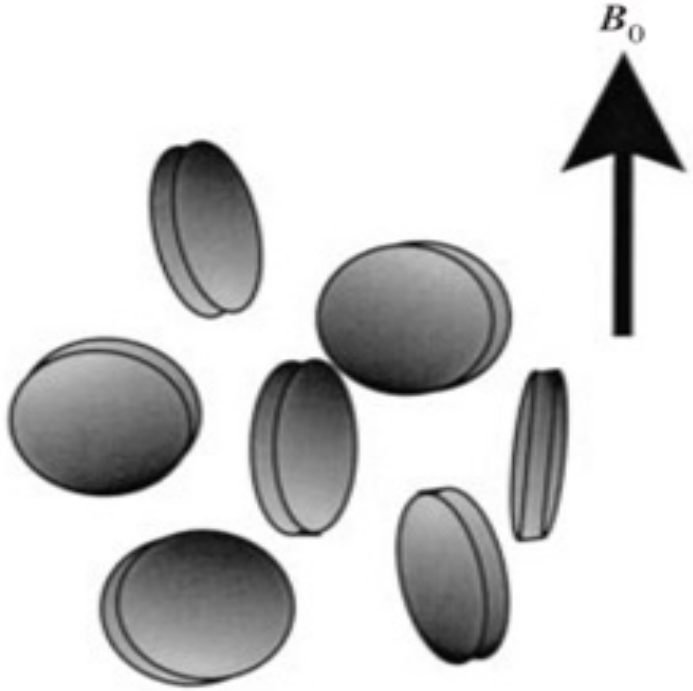
DMPC

Tjandra N, Bax A. *Journal of Biomolecular NMR,* **1997**, 10, 289-292









Prestegard J.H., Al-Hashimi H.M., Tolman J.R. *Quarterly Reviews of Biophysics*, **2000**, 33(4), 371-424

Liquid crystals – Bicelles

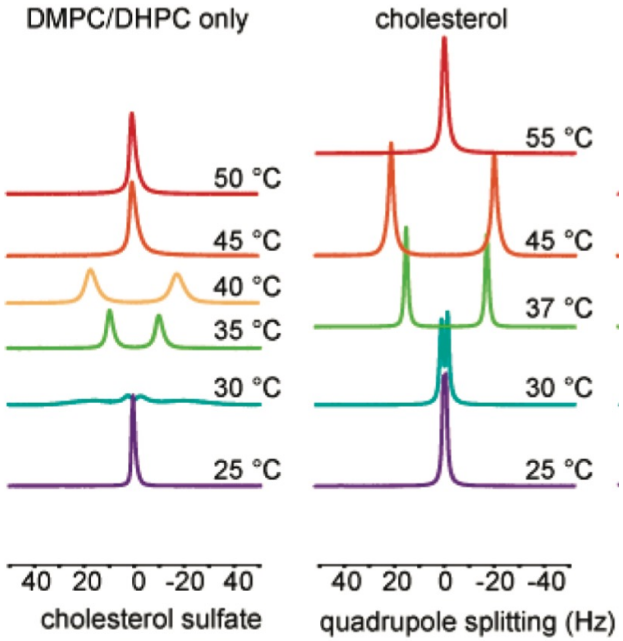
Advantages:

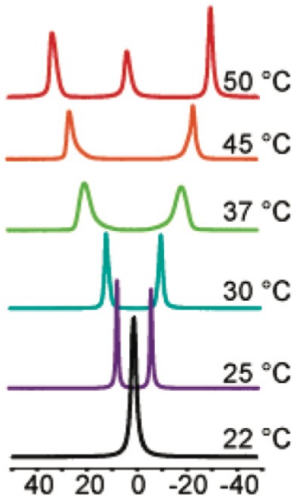
- Both sterically (uncharged) and electronic alignment (charged)
- Macromolecule orientation α lipid concentration
- Temperature dependent formation of bicelles

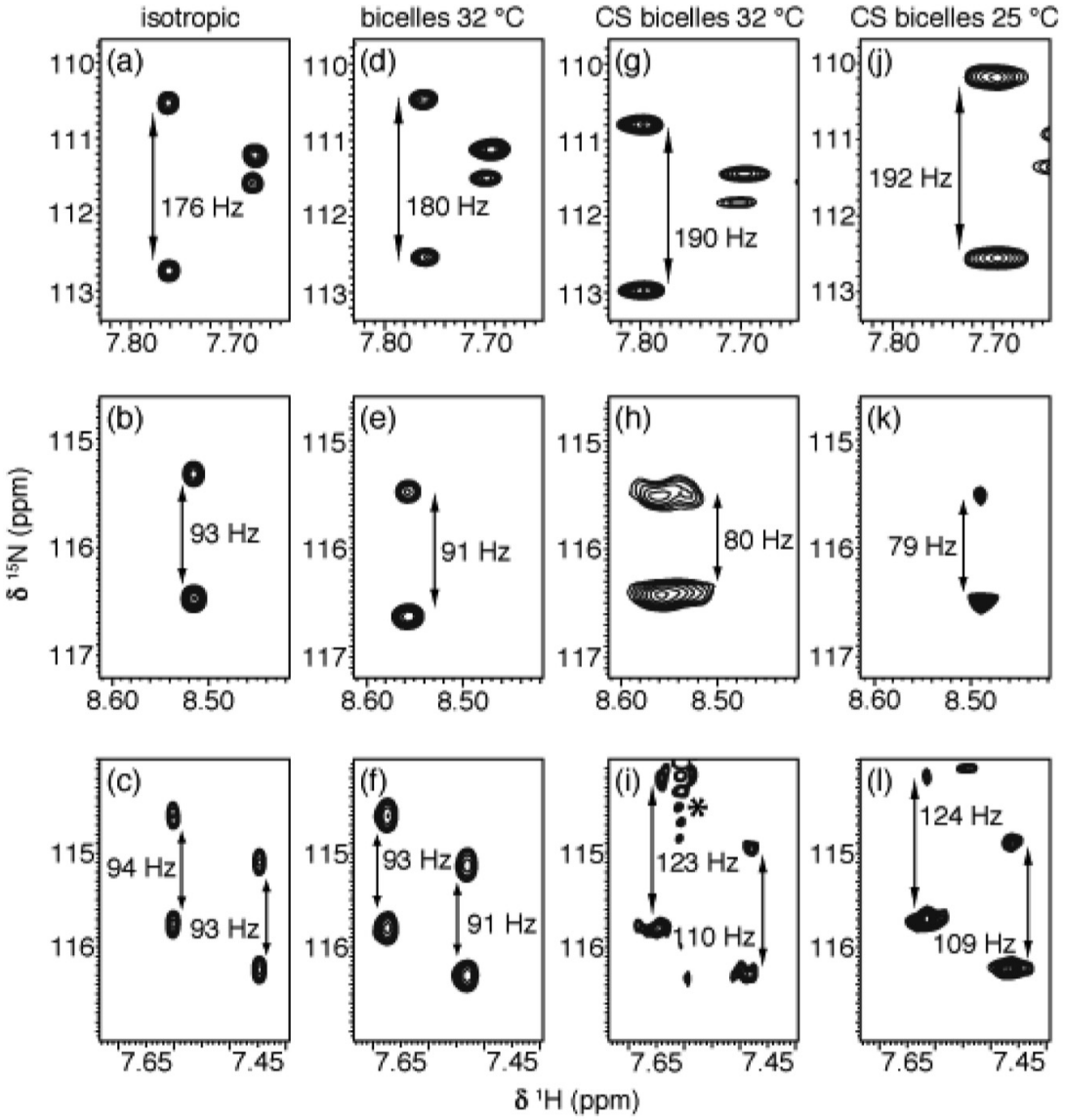
Disadvantages:

- DMPC/DHPC – Hydrolyses at low pH
- DIODPC/CHAPSO – Unstable in neutral pH
- Cetylpyridinium chloride/n-hexanol - require high salt concentration and high protein concentration destabilizes LC
- Cetylpyridinium bromide/n-hexanol – works well only in low salt concentrations

Cholesterol doped bicelles



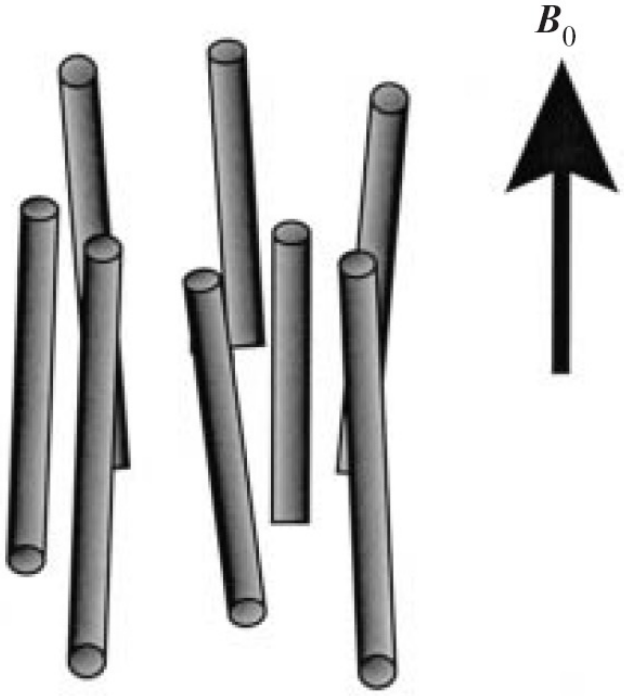




Shapiro R, Brindley A, Martin R.W.. *Journal of the American Chemical Society,* **2010**, 132, 11406-07

Liquid Crystals: Phages & Membranes

- Rod shaped viruses (TMV), phages (Pf1 and fd)
- Ease of sample preparation, extraordinary stability under extreme conditions.



- Remain ordered over a wide range of temperature.(Diameter of 20 Å, length of 2 μm).
- Tends to aggregate < pH 6
- Tendency to phase separation (CTAB helps to an extent).

Hansen MR, Mueller L, Pardi A. *Nature Structural and Molecular Biology*, **1998**, 5(12), 1065-74
Clore, G.M., Starich, M.R., Gronenborn, A.M. *Journal of the American Chemical Society,* **1998**, 120, 10571-72

Combination of co-solvents

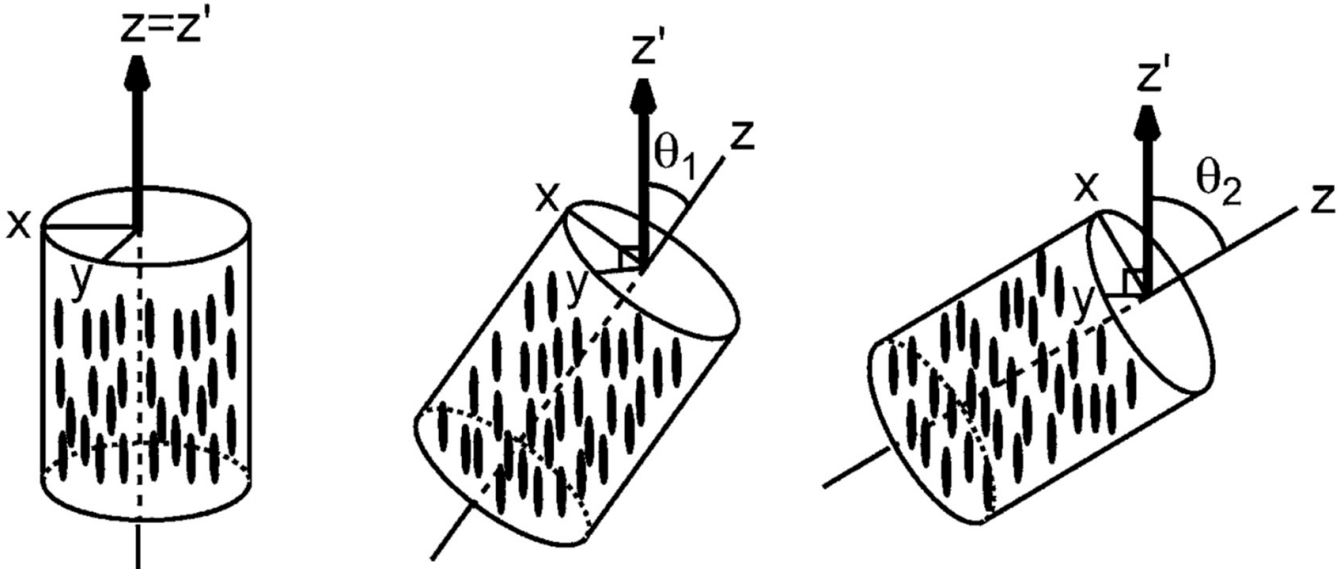


Table 1. Experimental Ubiquitin Alignment Tensors Using Various Composite Pf1 and SAG Media (pH 6.6, 25 °C)

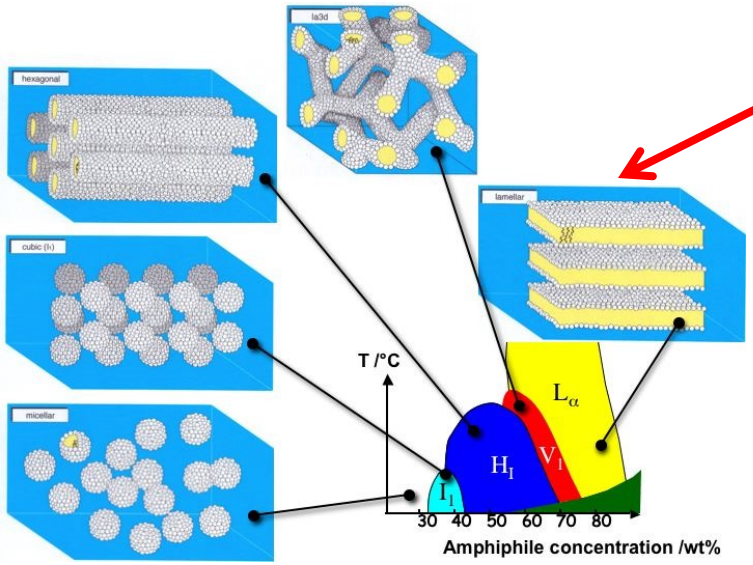
medium	Pf1[a]	acrylamide[b]	Mg^{2+} [c]	θ[d]	A_{zz} ($\times 10^{-4}$)	η	α	β	γ
A	4.0	5%	10	55°	−12.7	0.28	−27.9	123.0	121.8
B	4.0	5%	10	0°	−18.5	0.40	−28.8	125.8	45.1
C	3.0	5%	10	30°	−11.5	0.28	−26.8	121.7	104.5
D	3.0	5%	20	30°	−8.9	0.24	−26.3	122.5	94.8
E	3.0	5%	10	55°	−9.2	0.13	−28.3	119.1	95.0
F		5%[e]		NA	6.1	0.78	−158.4	147.3	−131.9
G[f]	3.5			NA	−3.0	0.54	−39.3	125.9	45.9

Ruan K., Tolman J.R. *Journal of the American Chemical Society,* **2005**, 127(43), 15032-33

Liquid crystals – Nonionic media

1. n-alkyl polyethylene glycol in n-alkyl alcohols.
2. Glucopone in n-hexanol.

Alcohols tend to reduce the temperature of formation of lyotropic phase.



Lyotropic phase (L_α).

Bilayer formation.

Thickness dictated by surfactant concentration.

Liquid crystals – Nonionic media

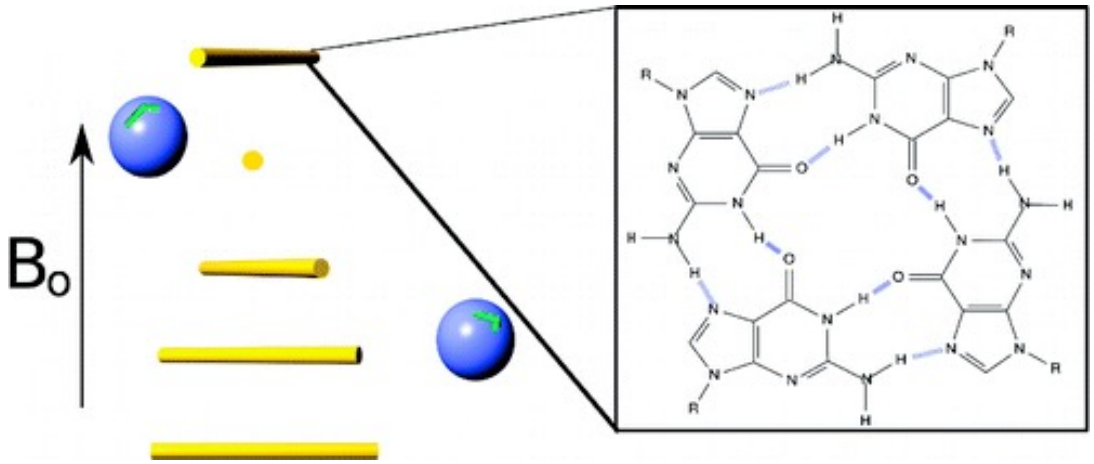
Advantages:

- Fairly insensitive to pH and ionic strength.
- Little or no binding affinity to proteins.
- Stable over a long temperature range (0-40°C)

Disadvantages:

- Glucopone – negatively charged.
- Recovery of proteins.

G-tetrad liquid crystals



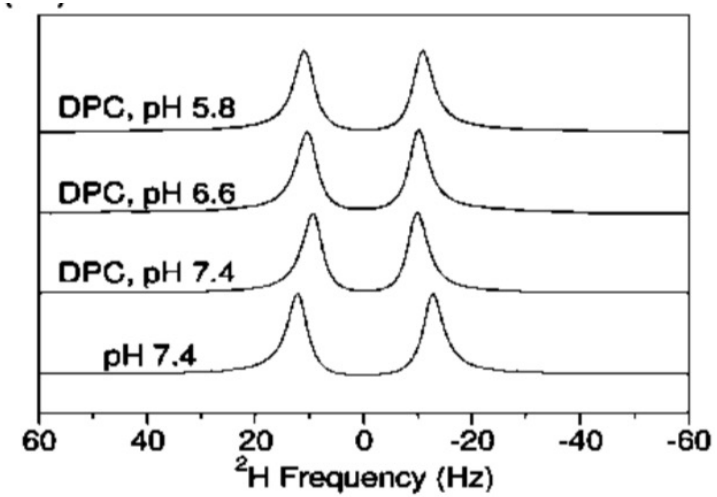
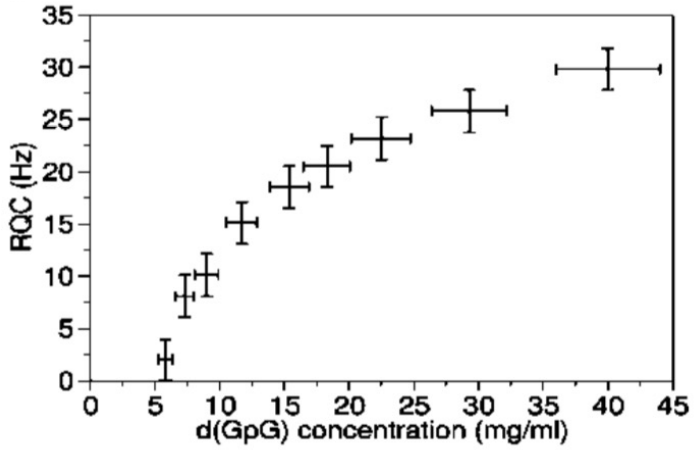


Table 1. Alignment Tensor Parameters in Liquid Crystalline Media for U-{^{13}C,^{15}N}-[K19E,D40N,V42E]-GB3[a] [15,22]

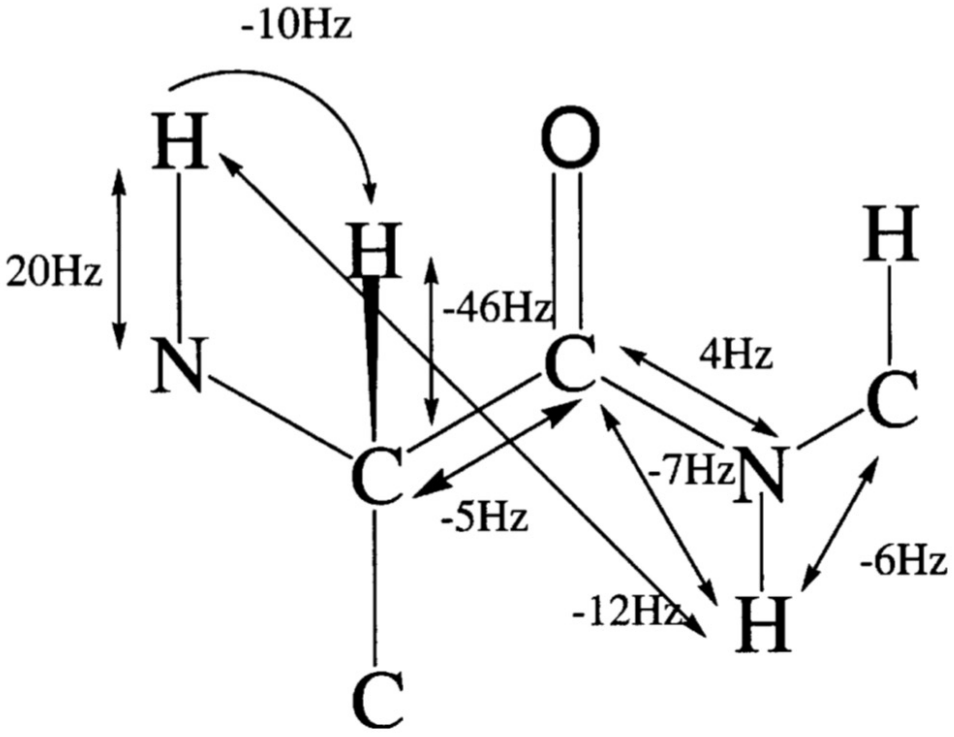
LC medium	D_a (Hz)	R	tensor orientation[b]	Q-factor
Pf1	−7.12	0.321	(154°, 90°, 220°)	0.083
K-dGpG	8.30	0.434	(156°, 90°, 232°)	0.096



Lorieau J, Yao L, Bax A. *Journal of the American Chemical Society,* **2008**, 130(24), 7536-37

Measurement

- HSQC (F1/F2 coupled)
- TROSY (CE-TROSY)
- J-modulation experiment (ARTSY)



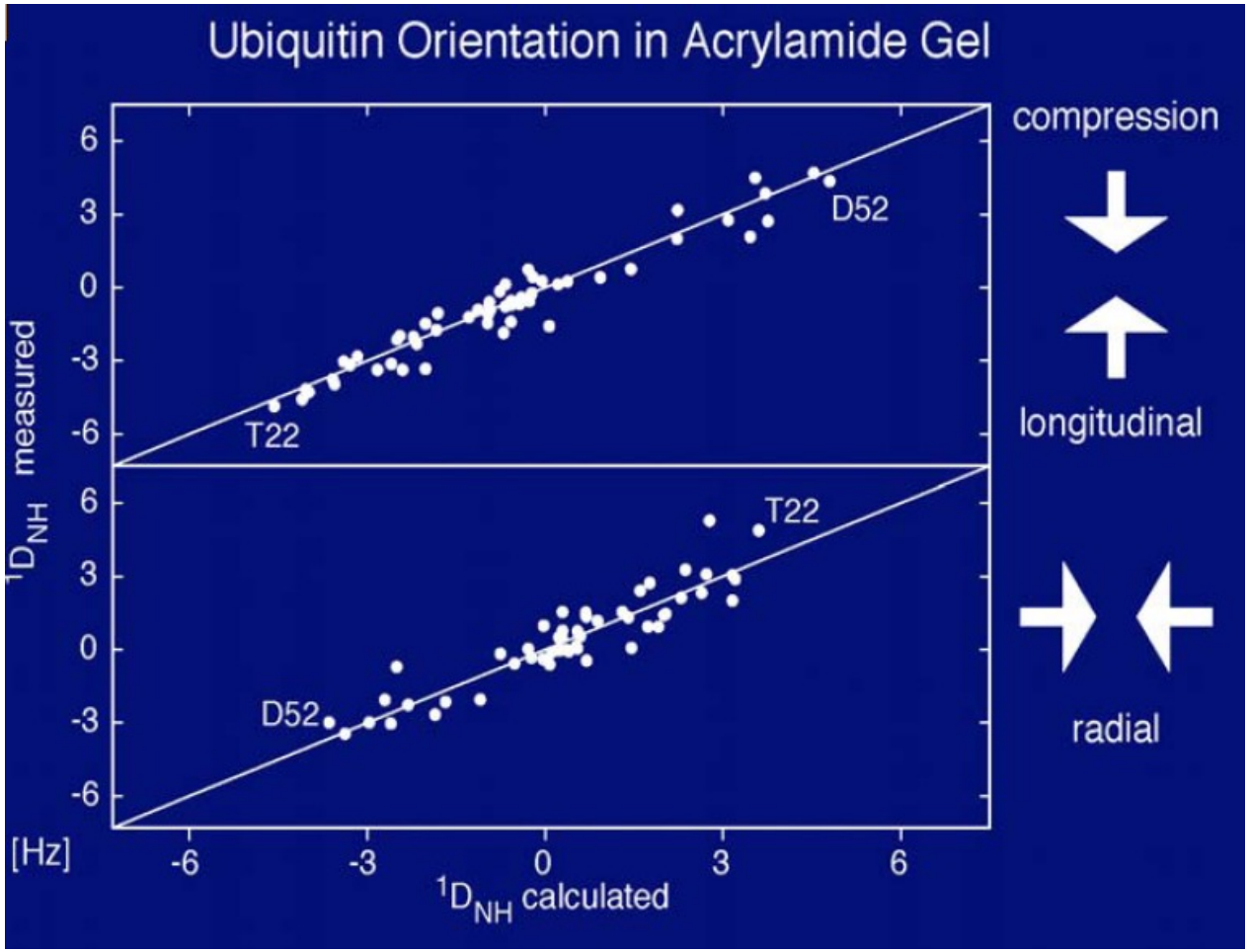
Prestegard J.H., Al-Hashimi H.M., Tolman J.R. *Quarterly Reviews of Biophysics*, **2000**, 33(4), 371-424

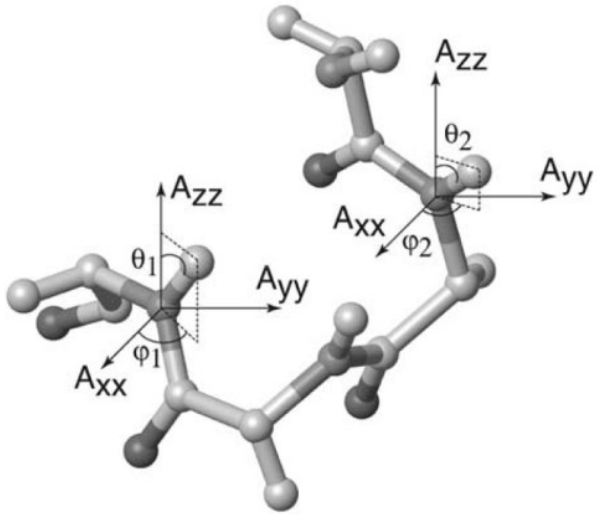
Software

- RAMAH (SVD_ORDERTEN)
- Prediction of molecular ALignmEnt from Structure (PALES)
- REsidual Dipolar Coupling Analysis Tool (REDCAT)
- Module
- Dipocoup

- XPLOR-NIH
- CYANA (3 and above)
- REDCRAFT

Structure Validation

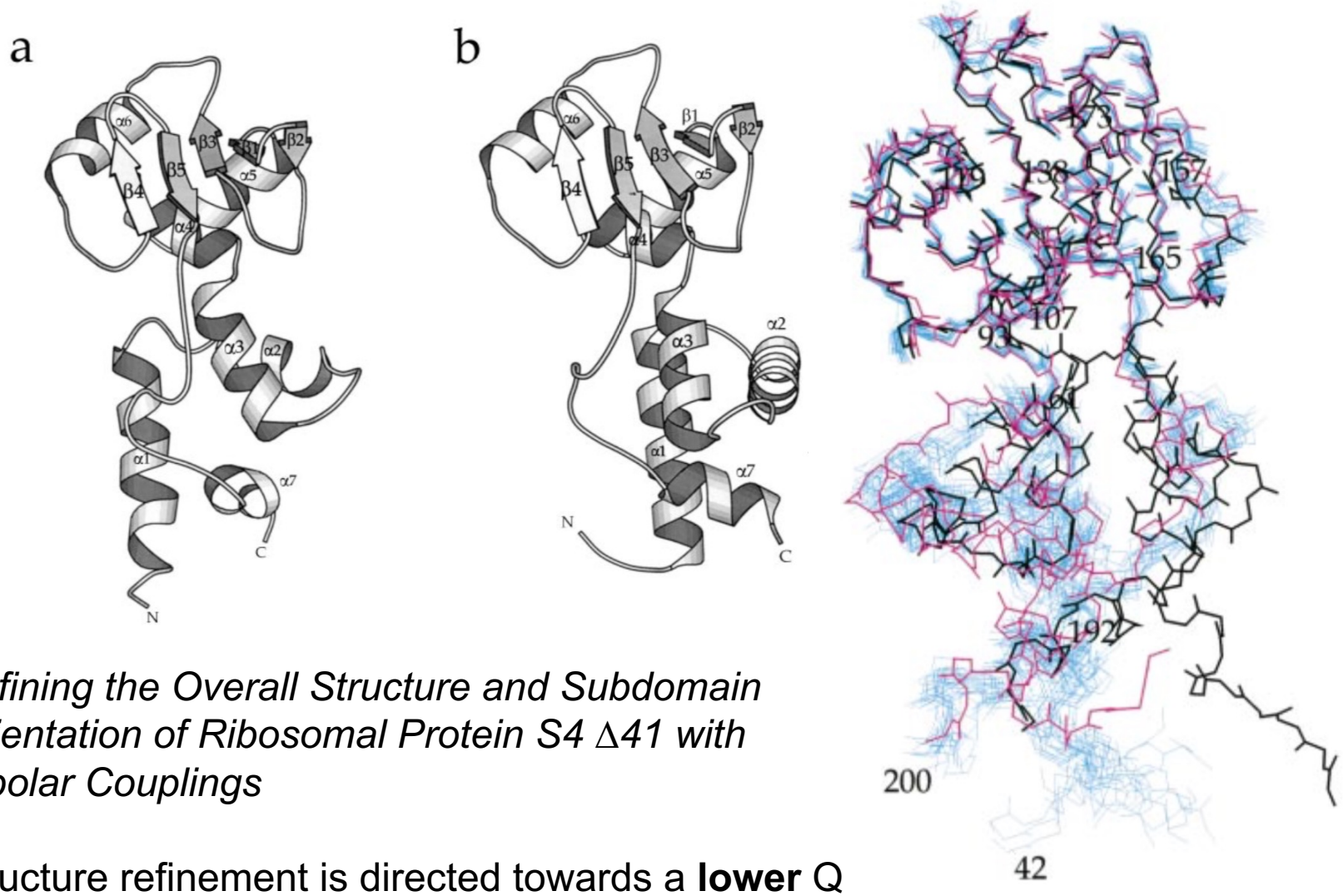




$$Q = \sqrt{\frac{D_{calc}^2 - D_{obs}^2}{D_{obs}^2}}$$

Q-factor < 0.2 implies well refined structure
0.2 ≤ Q ≤ 0.4 requires refinement
Q > 0.4 poor quality structure

Structure Determination

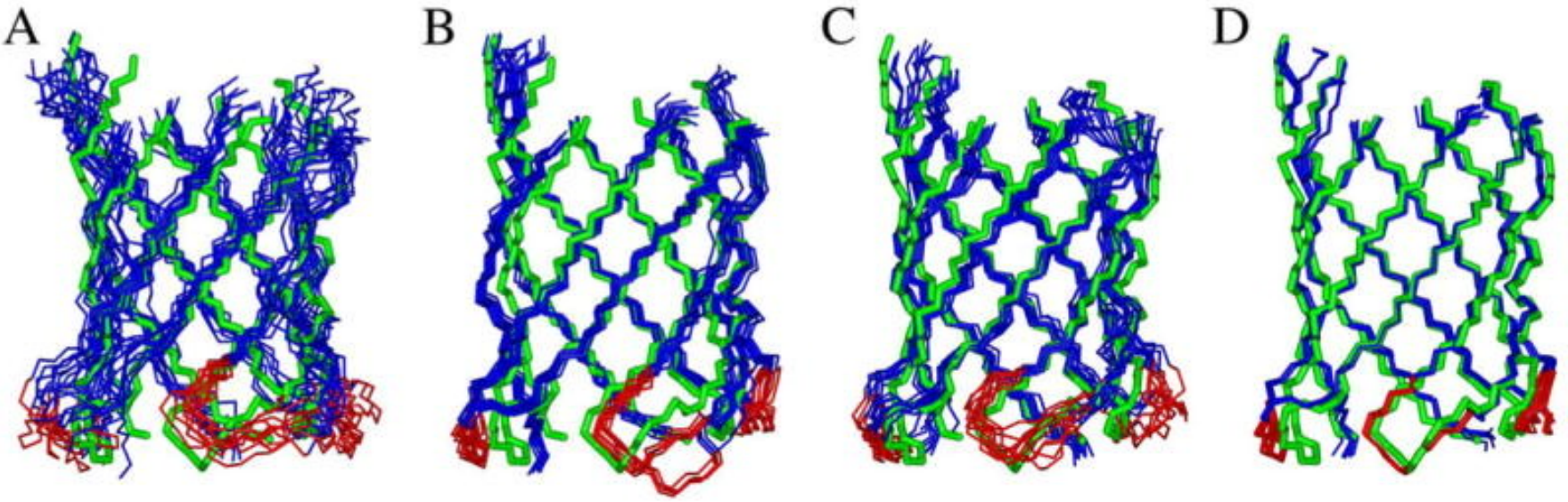


Refining the Overall Structure and Subdomain Orientation of Ribosomal Protein S4 Δ41 with Dipolar Couplings

Structure refinement is directed towards a **lower** Q factor value.

Markus M.A, Gerstner R.B., Draper D.E, Torchia D.A. *Journal Molecular Biology,* **1999**, 292, 375-387

Membrane Protein Structure Determination



Distances + Dihedrals
Accuracy ~ 3 Å

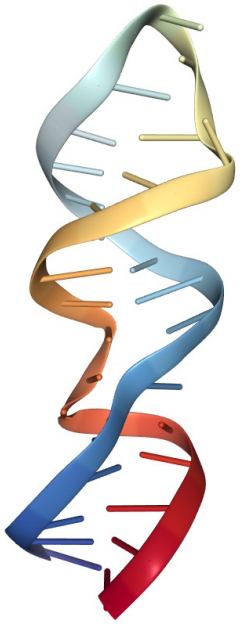
Distances + **RDCs** + Dihedrals
Accuracy ~ 2 Å

Distances + H-bonds + Dihedrals
Accuracy ~ 1.5 Å

Distances + H-bonds + Dihedrals + **RDCs**
Accuracy ~ 1 Å

Cierpicki T, Liang B, Tamm LK, Bushweller JH. *Journal of the American Chemical Society,* **2006**, 128(21), 6947-51

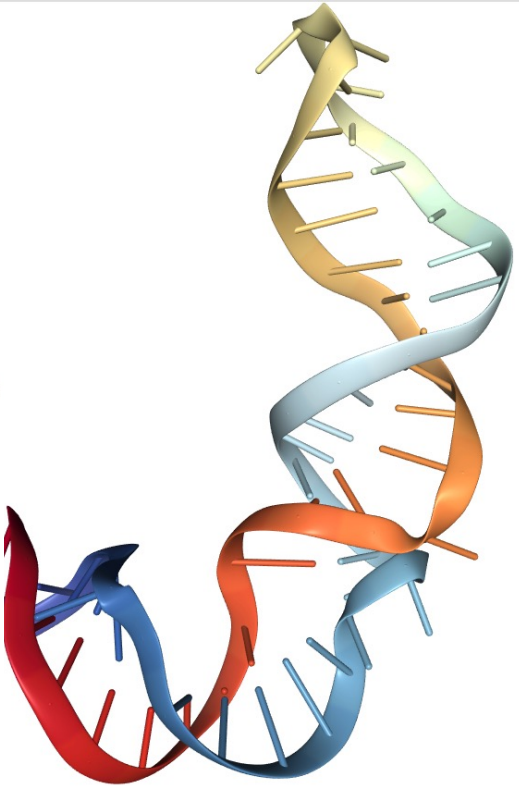
RNA Structure Determination



34-nt
HCV IRES
Domain IIa
PDB: 1P5M

55-nt
HCV IRES
Domain IIb
PDB: 1P5N

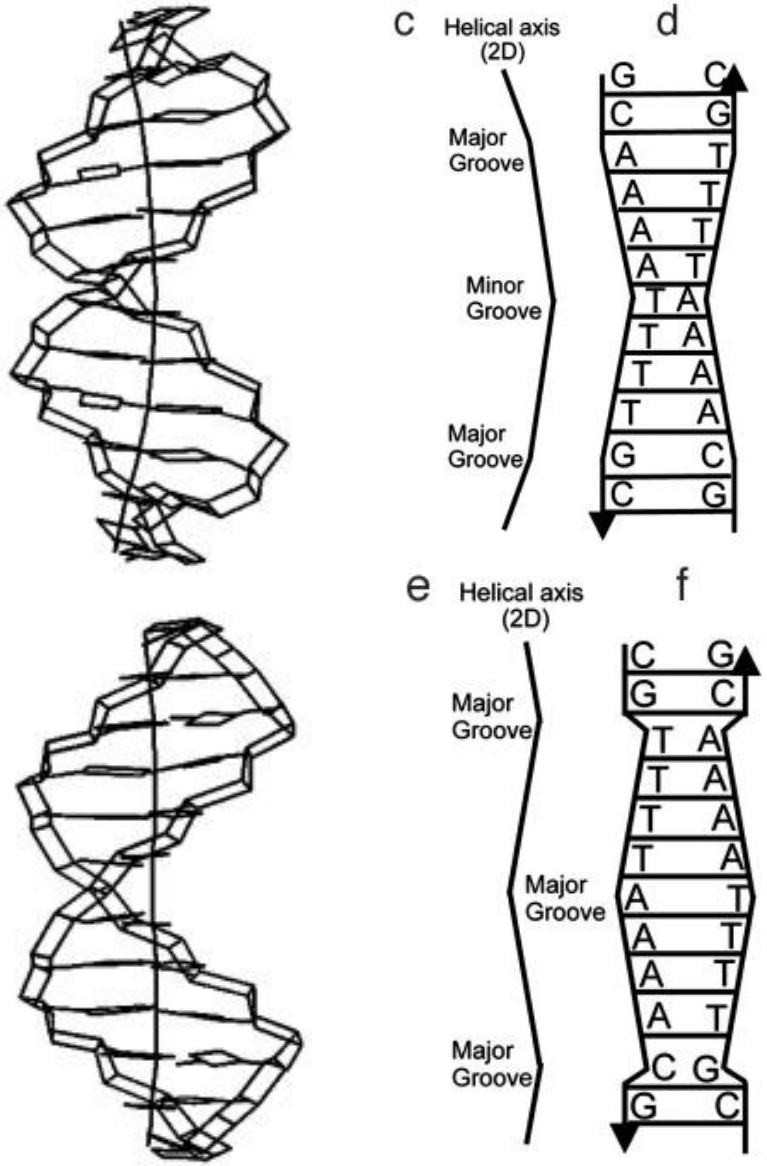
77-nt
HCV IRES
Domain II
PDB: 1P5O



77-nt
HCV IRES
Domain II
PDB: 1P5P

Lukavsky, P.J., Kim, I., Otto, G.A., Puglisi, J.D. *Nature Structure and Molecular Biology,* **2003**, 10, 1033-38

DNA Structure Determination



DNA A-tract bending in 3D: solving the dA_4T_4 vs. dT_4A_4 conundrum.

Stefl R, Wu H, Ravindranathan S, Sklenár V, Feigon J. *Proc. Natl. Acad. Sci. USA*, **2004**, 101(5), 1177-82

A bit of history…

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9279–9283, September 1995
Biophysics

Nuclear magnetic dipole interactions in field-oriented proteins: Information for structure determination in solution

(myoglobin/NMR/paramagnetic proteins)

J. R. TOLMAN*, J. M. FLANAGAN†, M. A. KENNEDY‡, AND J. H. PRESTEGARD*

NMR evidence for slow collective motions in cyanometmyoglobin

J. R. Tolman[1], J. M. Flanagan[2], M. A. Kennedy[3] and J. H. Prestegard[1]

articles

Bax, A., Tjandra, N. *Nature Structure and Molecular Biology,* **1997**, 4(4), 292-297

But are proteins floppier?

Are proteins even floppier than we thought?

Ad Bax[1] and Nico Tjandra[2]

In our opinion, therefore, because dipolar couplings are exquisitely sensitive to structure but only weakly to dynamics, they hold most promise for structure determination[10], not for the study of dynamics.

Bax, A., Tjandra, N. *Nature Structure and Molecular Biology,* **1997**, 4(4), 254-256c

Dynamics from RDCs

J. Am. Chem. Soc. **2001,** *123,* 1416−1424

Structural and Dynamic Analysis of Residual Dipolar Coupling Data for Proteins

Joel R. Tolman,*,†,∥ Hashim M. Al-Hashimi,‡,§ Lewis E. Kay,† and James H. Prestegard§

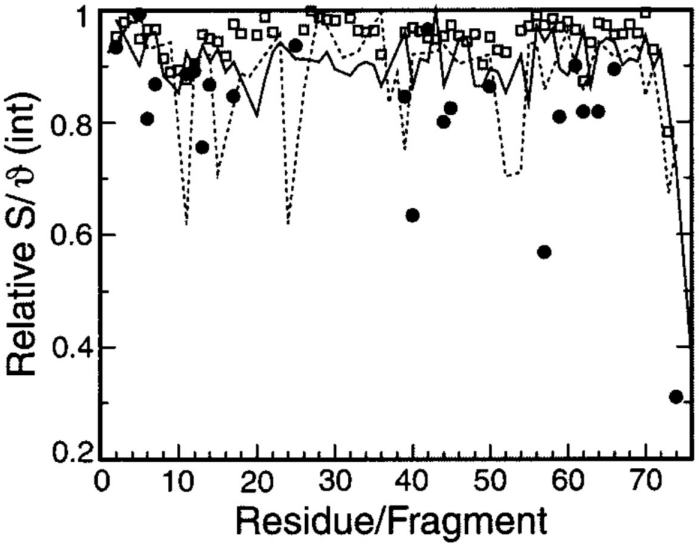


Figure 8. Comparison of ϑ(int) (filled circles) with spin relaxation derived order parameters. Shown are S_{NH} order parameters reported by Tjandra et al.[43] (open squares) and Schneider et al.[15] (solid line), as well as $S_{C\alpha H\alpha}$ order parameters reported by Wand, et al.[48] (dotted line). Values of ϑ(int) were those corresponding to solutions of minimum orientational deviation from the 1UBQ X-ray structure. Within each set, all values were scaled relative to the largest ϑ (or S) observed.

J. Am. Chem. Soc. **2001,** *123,* 6098−6107

Model-Free Approach to the Dynamic Interpretation of Residual Dipolar Couplings in Globular Proteins

Jens Meiler,[†] Jeanine J. Prompers,[‡] Wolfgang Peti,[†] Christian Griesinger,*[,†,§] and Rafael Brüschweiler*[,‡]

$$\frac{\langle D \rangle}{D_{zz}} = \sqrt{\frac{4\pi}{5}}\left(\langle Y_{20}(\theta,\varphi)\rangle + \sqrt{\frac{3}{8}}R(\langle Y_{22}(\theta,\varphi)\rangle + \langle Y^*_{22}(\theta,\varphi)\rangle)\right)$$

$$S^2_{LS} = \frac{4\pi}{5}\sum_{M=-2}^{2}\langle Y_{2M}(\theta,\phi)\rangle\langle Y^*_{2M}(\theta,\phi)\rangle\Big|_0^{\tau_c}$$

$$S^2_{rdc} = \frac{4\pi}{5}\sum_{M=-2}^{2}\langle Y_{2M}(\theta,\phi)\rangle\langle Y^*_{2M}(\theta,\phi)\rangle\Big|_0^{ms}$$

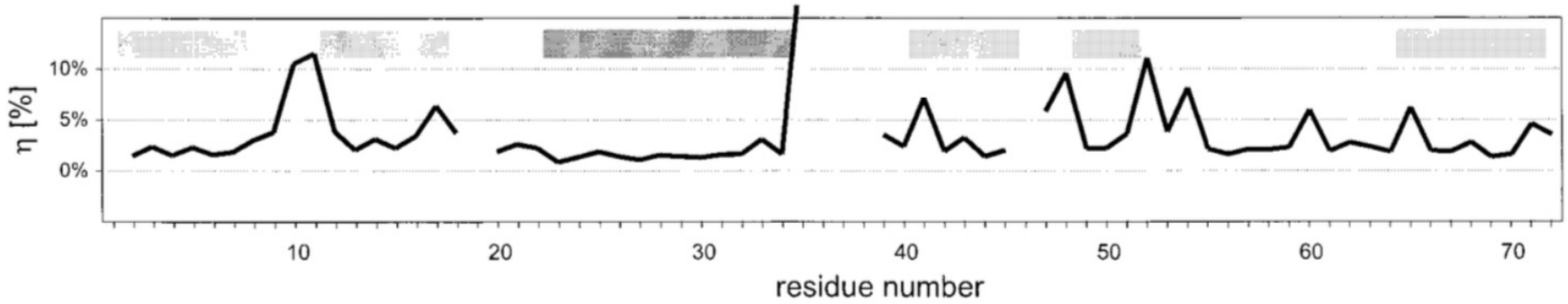
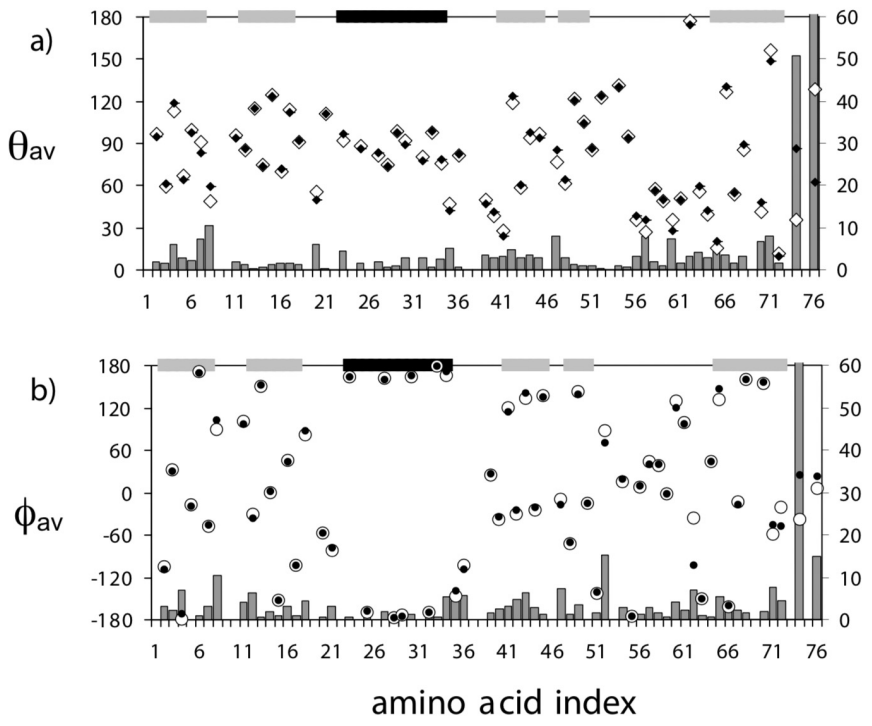
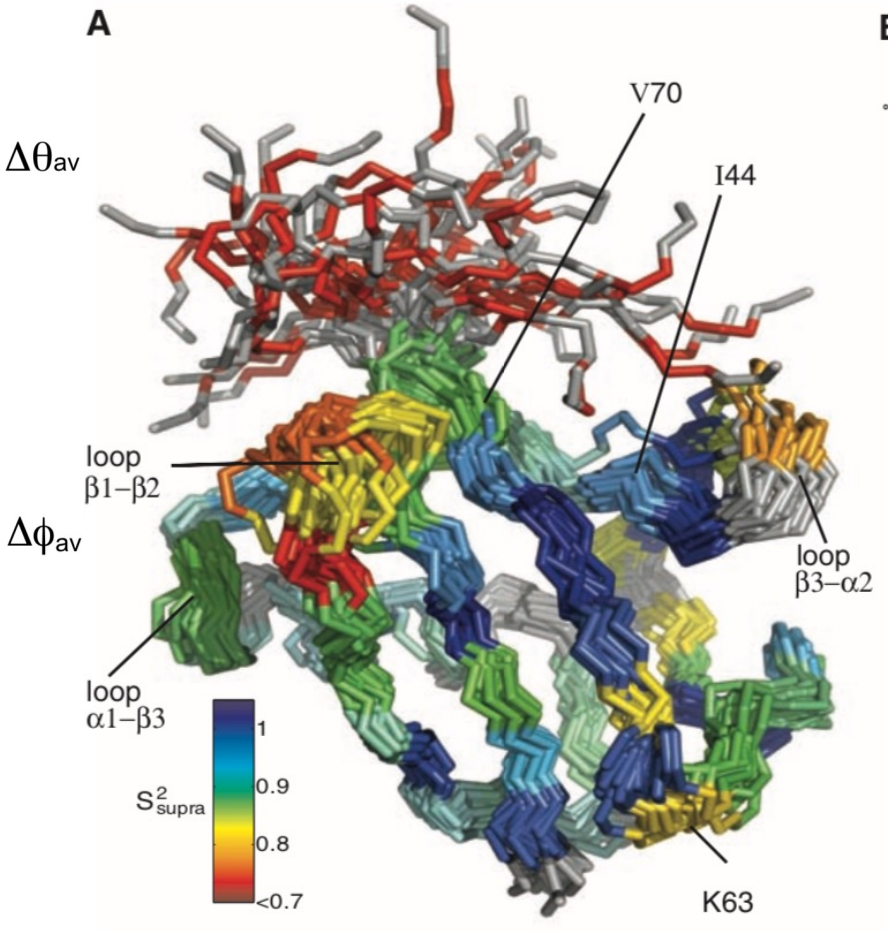


Figure 6. Motional asymmetry parameter η defined in eq 11 for $N-H^N$ vectors as a function of the residue number. In regular secondary structure, η varies between 1% and 6%, while in more mobile loop regions the asymmetry can exceed 10%.

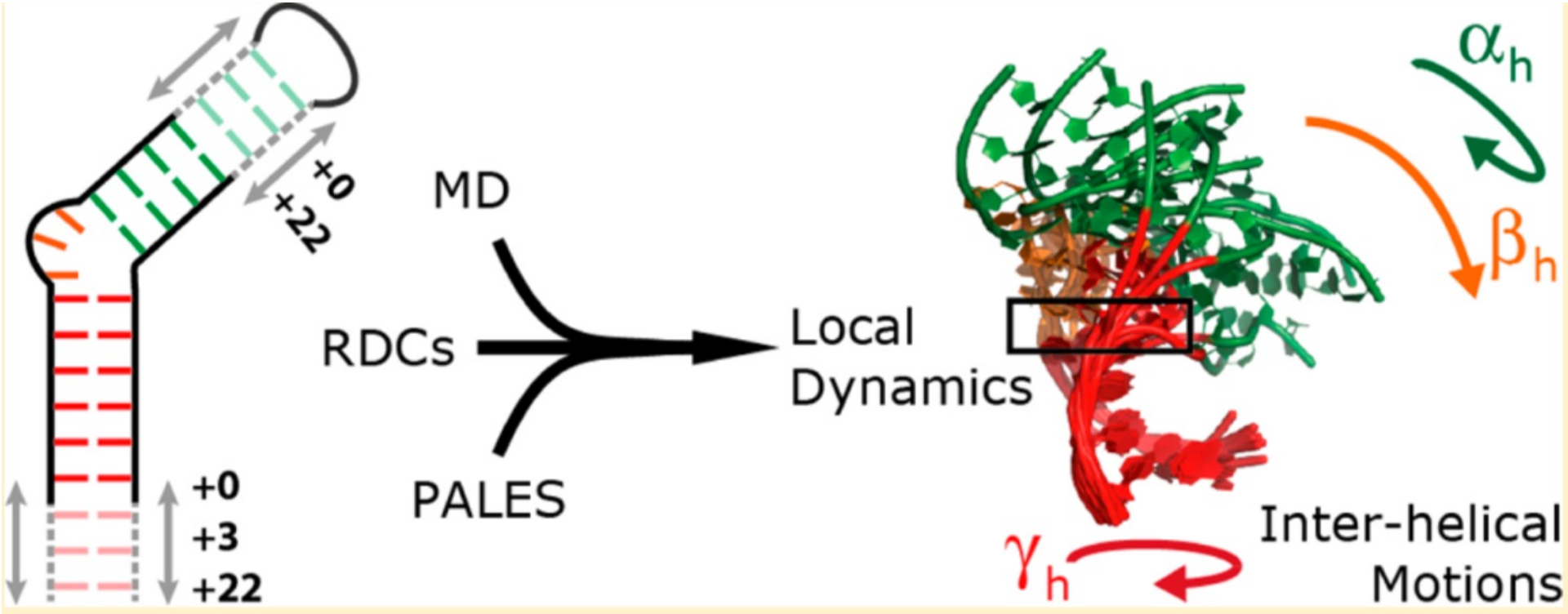
Ubiquitin Dynamics from 36 alignments!





Lange, O.F, Lamolek N-A, Fares, C., Schröder G.F, …, Greisinger, C., Groot B.L.de. *Science,* **2008**, 320, 1471-75
Lamolek, N-A. Thesis. Gottingen 2008: "New Methods in NMR Spectroscopy for the Study of Protein Dynamics "

Nucleic Acid Dynamics



Salmon, L, Bascom G, Andricioaei I, Al-Hashimi H.M. *Journal of the American Chemical Society,* **2013**, 135, 5457-5466

To conclude..

- RDCs offer complementary way of structure determination, validation and refinement
- Provides insights in dynamics at slower time scales than spin relaxation

Thanks

NMB for opportunity

Kaustubh for the quick numerical simulation

to all of for attention