Protocol for Site-directed spin labeling EPR analysis of protein structure and dynamics.

Biochemical preparation

•Remove all native cysteines by mutating to alanine, serine or valine.

• Introduce single and double cysteines

• Express, and label. Amount of protein required per EPR data point is 10 μl of 50 μM (i.e 500 pmoles)

EPR spectroscopy

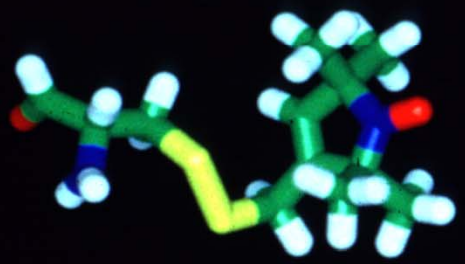
• Collect EPR spectrum to determine mobility

• Measure solvent accessibility.

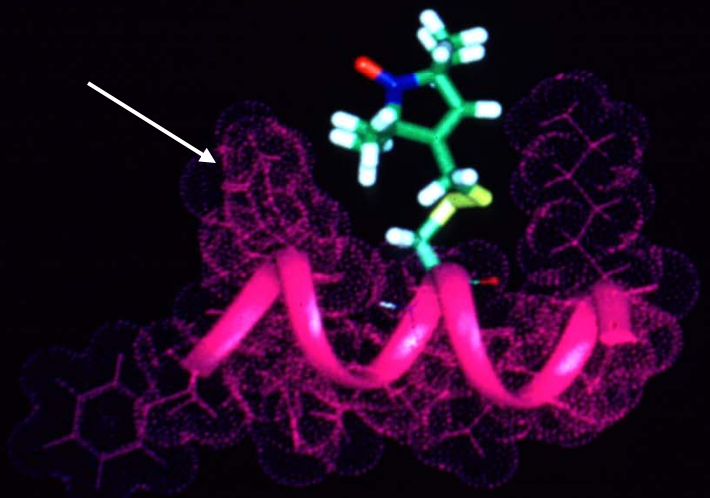
• Measure distances between pairs of spin labels.

The Mobility Parameter

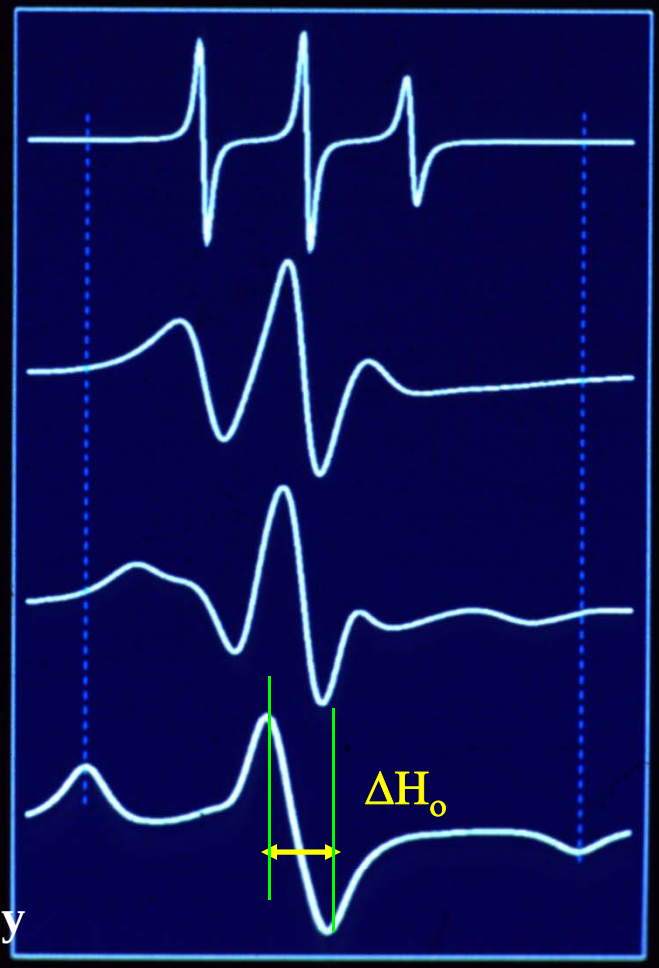
Protein Structure ⟶ Side –Chain Dynamics ⟶ Spectral Lineshape



Amino Acid R1



Motion of the label is restricted by surrounding atomic density



Motion of the label

fast ($\tau = 0.5ns$)

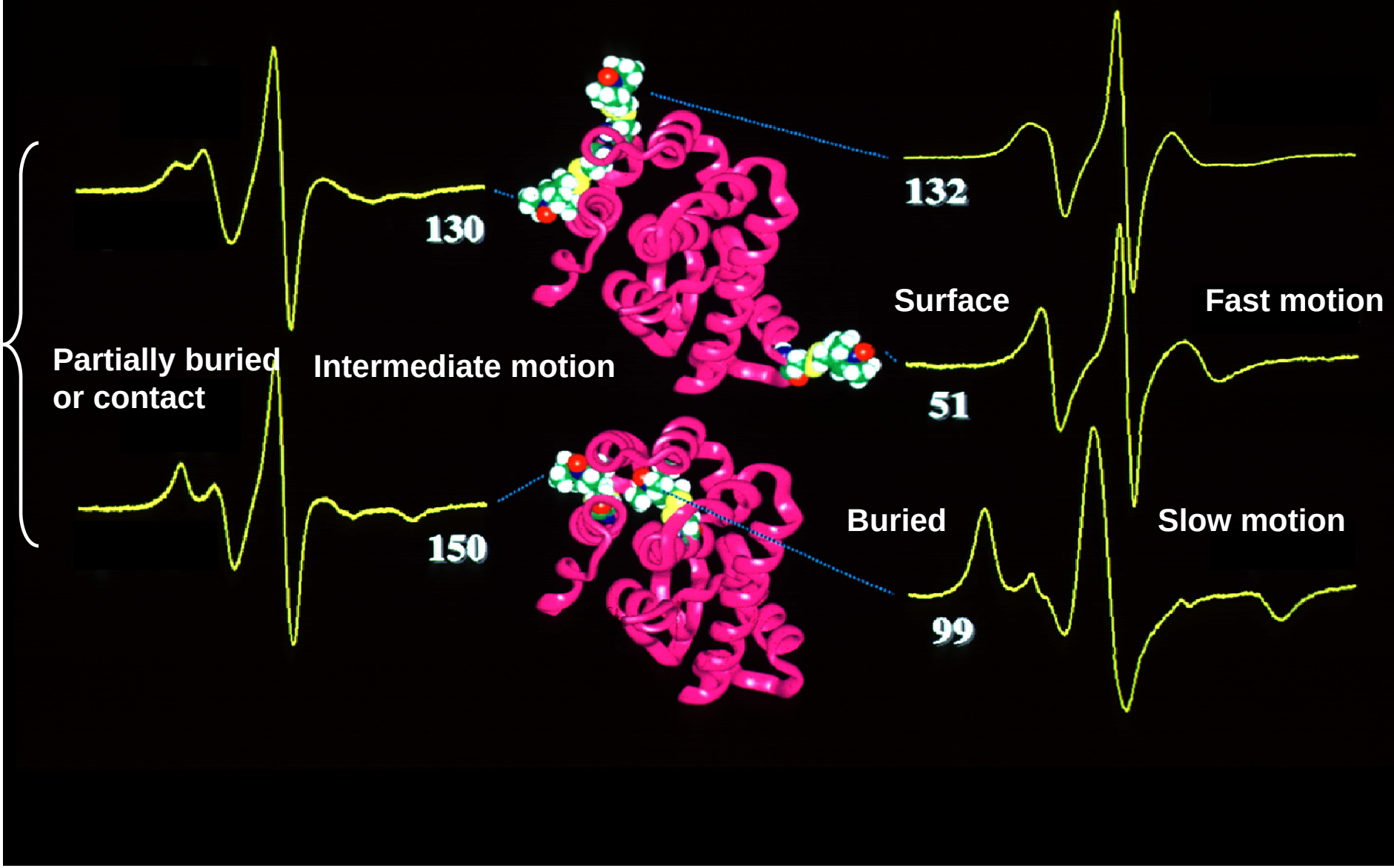
intermediate ($\tau = 3ns$)

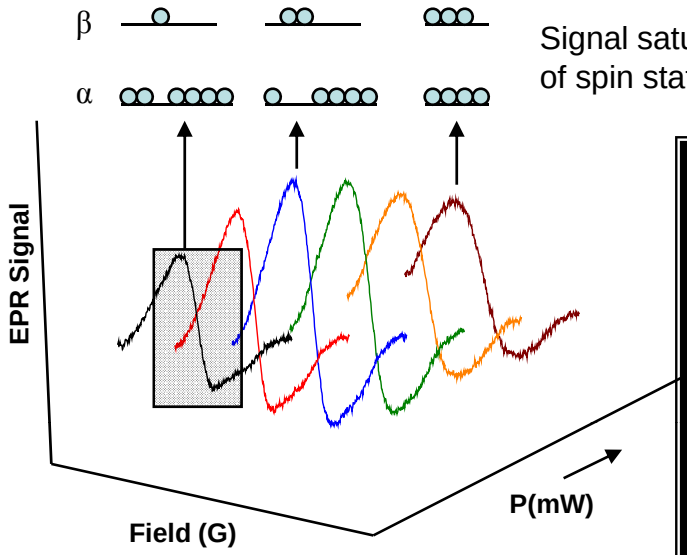
slow ($\tau = 10ns$)

rigid ($\tau > 100ns$)

τ is the characteristic time of the motion

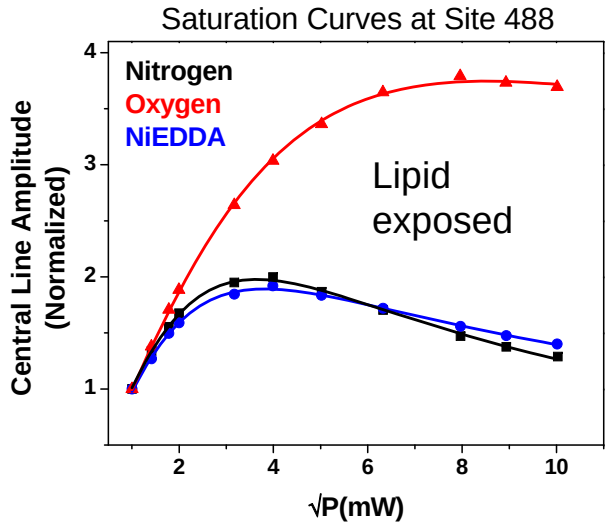
Correlation between Spin Label Mobility and Structural Environment





Signal saturation = equalization of spin states

$$A = IP^{1/2}\,[1+(2^{1/\varepsilon}-1)P/P_{1/2}]^{-\varepsilon}$$



Theoretical

$W_{ex} = \Delta[1/T_{1e}] = k_{ex}C_R$:

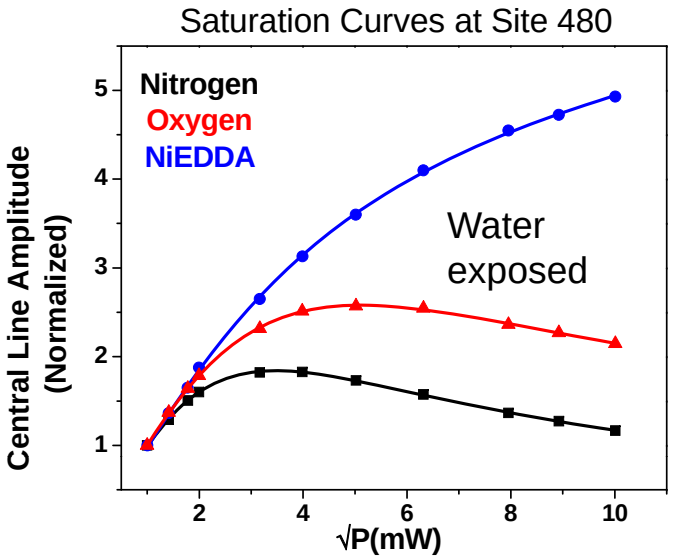
$$P_{1/2} \alpha\ 1/(T_1{*}T_2)$$

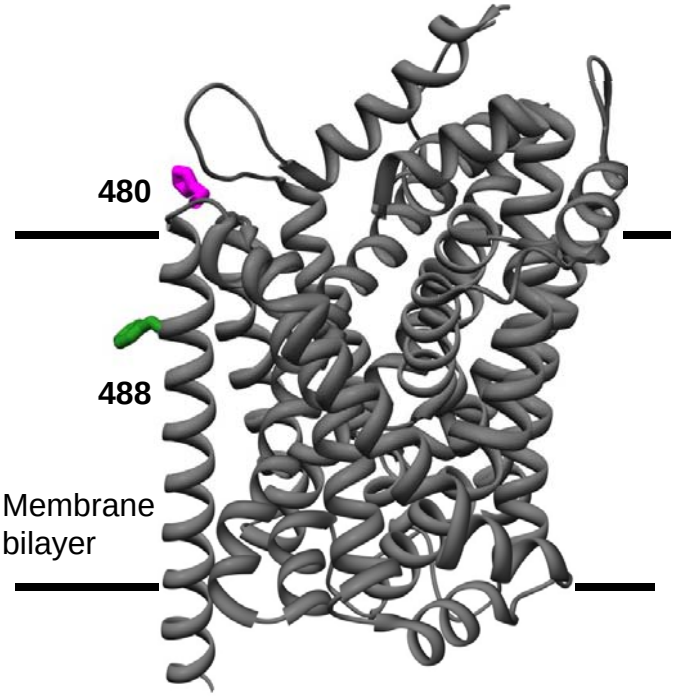
$$\Delta P_{1/2} \alpha\ 1/(T_1{*}T_2)_a - 1/(T_1{*}T_2)_b$$

Experimental

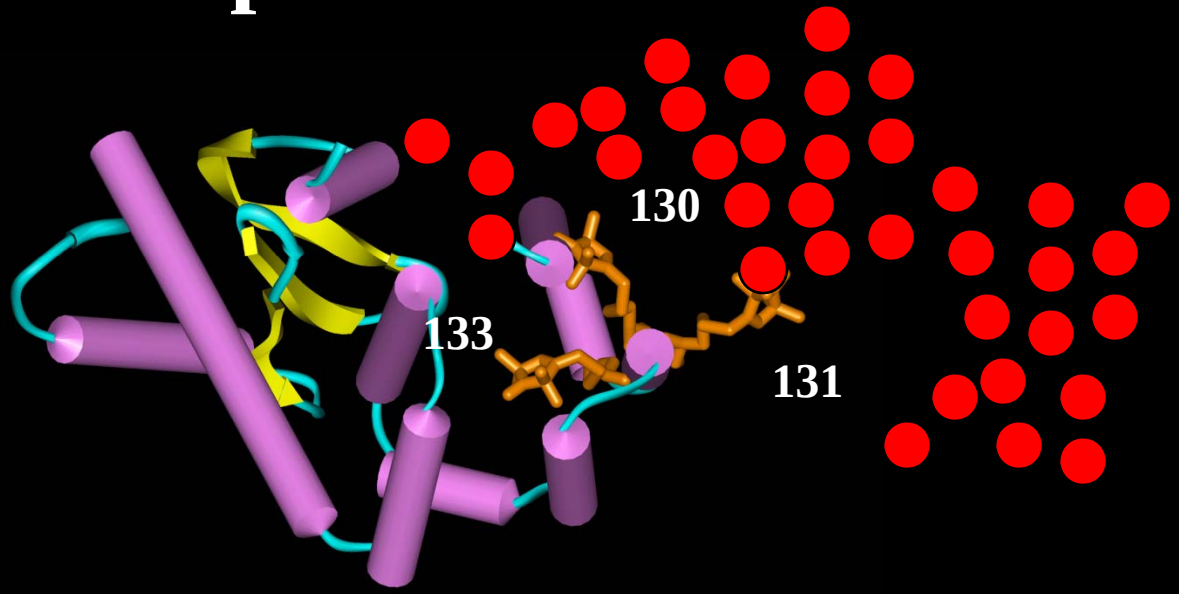
$$\Delta P_{1/2} = P_{1/2\,(Ni/O2)} - P_{1/2(N2)}$$

$$\Pi = [\Delta P_{1/2}/\Delta Hpp]\,[P_{1/2}/\Delta Hpp]_{ref}$$



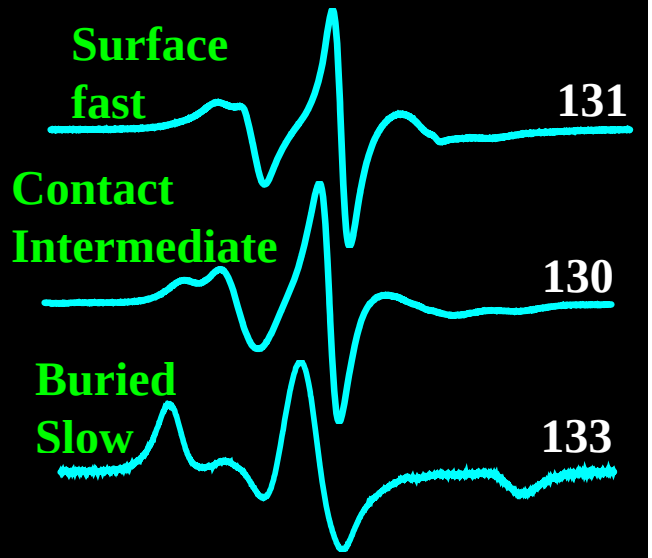


Spin Label SolventAccessibility



Note the correlation between accessibility and mobility

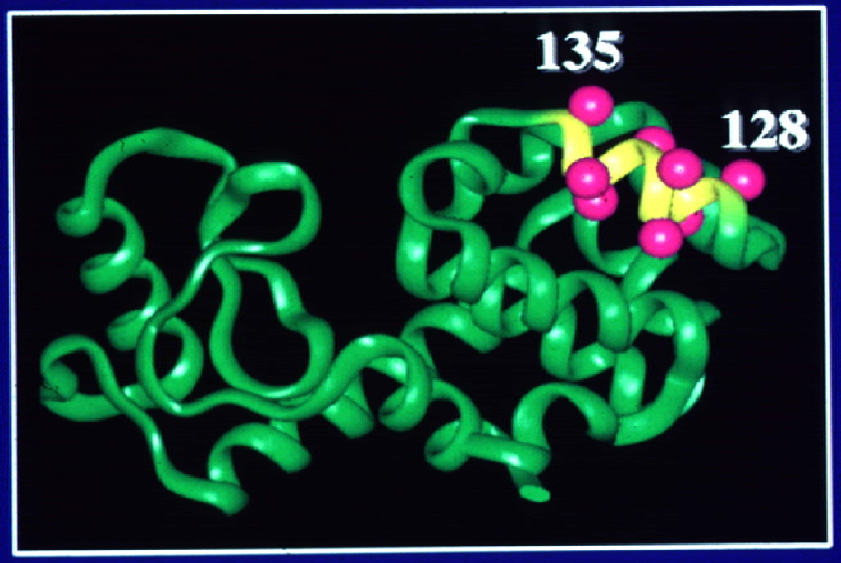
Spin label Mobility

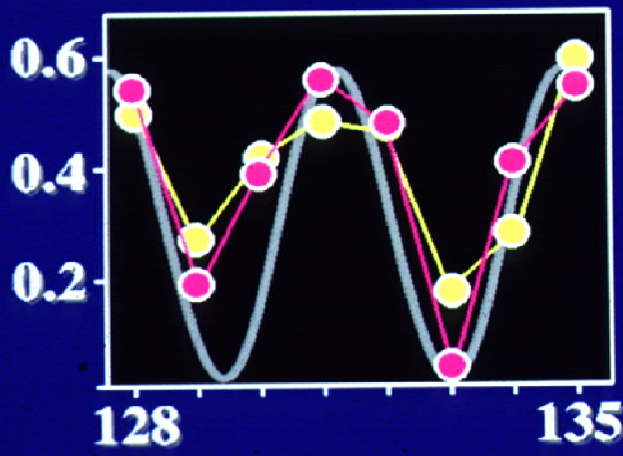


- **Deduced from measurement of the collision frequency with small paramagnetic molecules such as NiEDDA and Oxygen.**
- **Reagents are differentially soluble :assignment of the topographical location.**
- **Quantitative measurement: high resolution structural assignment.**

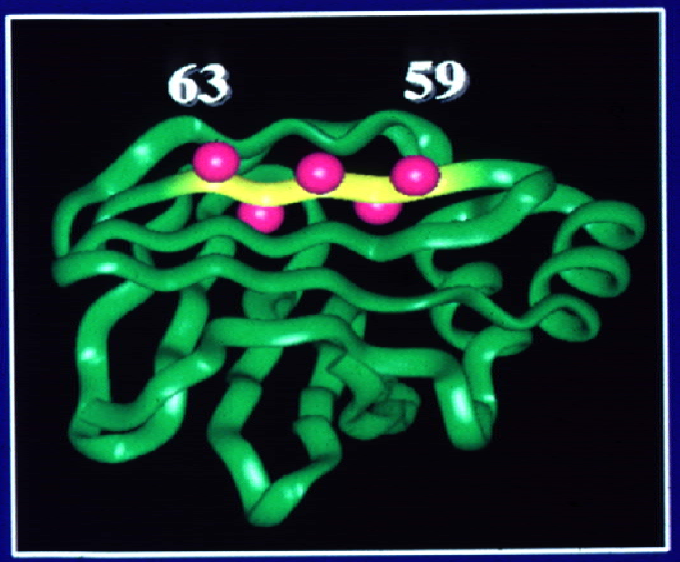
Nitroxide Scanning

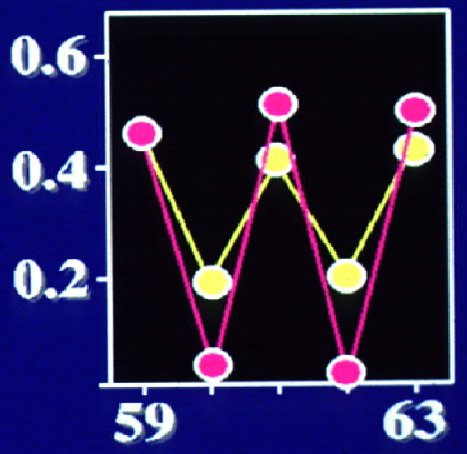
T4 Lysozyme





CRBP

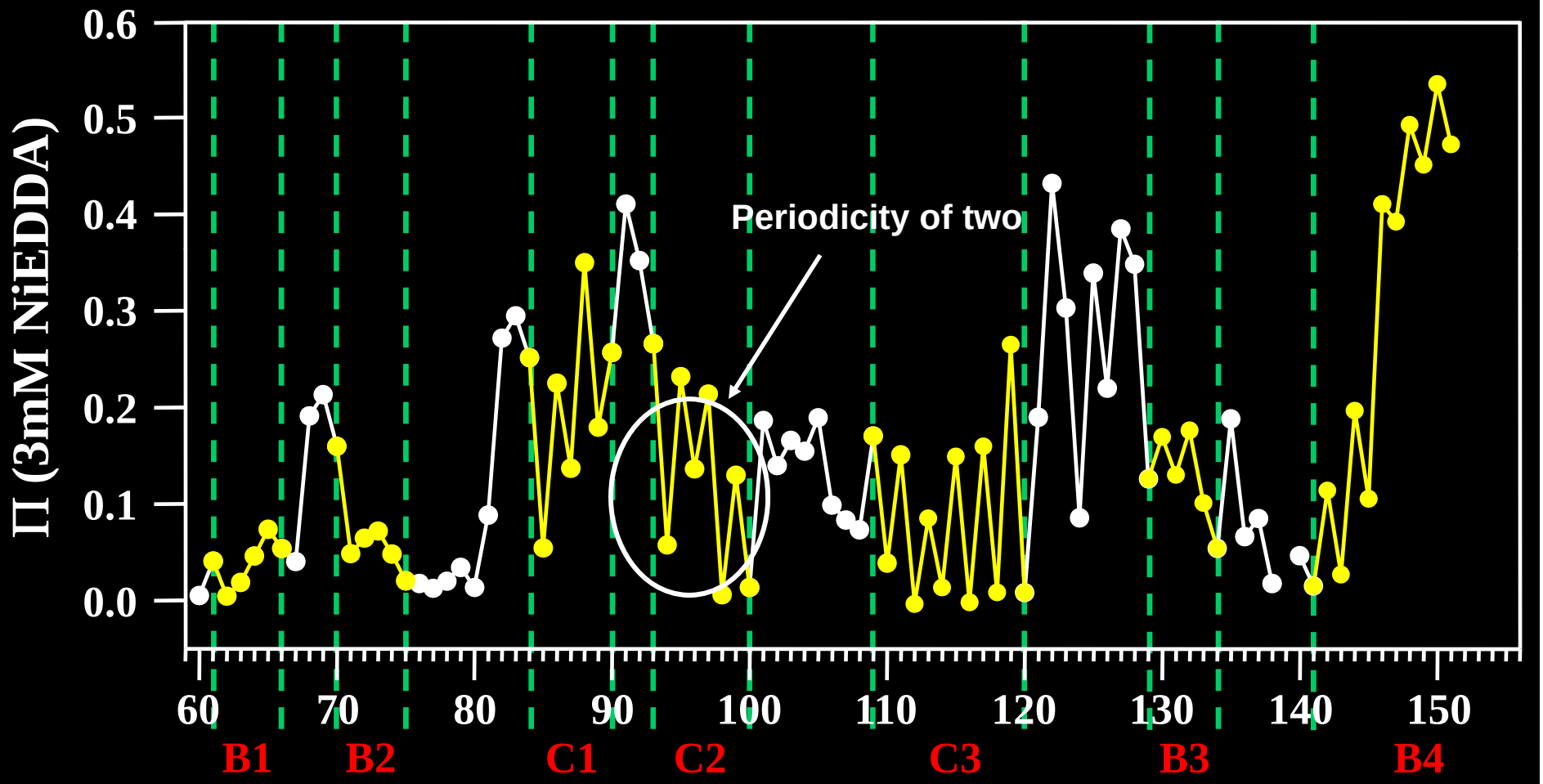




ΔH_0^{-1}

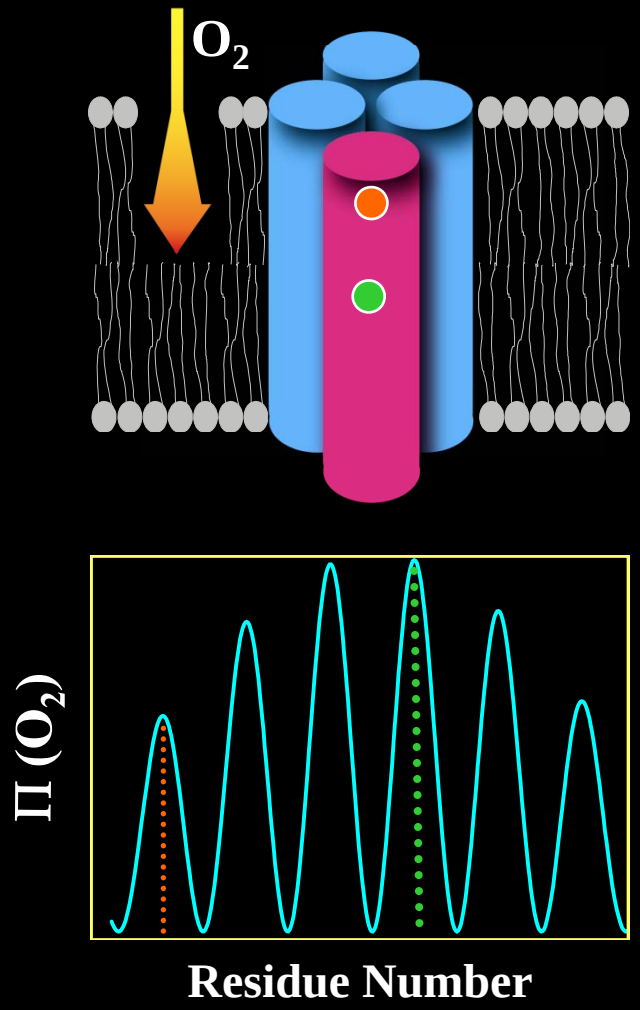
$\Pi(O_2)$

Secondary Structure Assignment of the α-Crystallin Domain

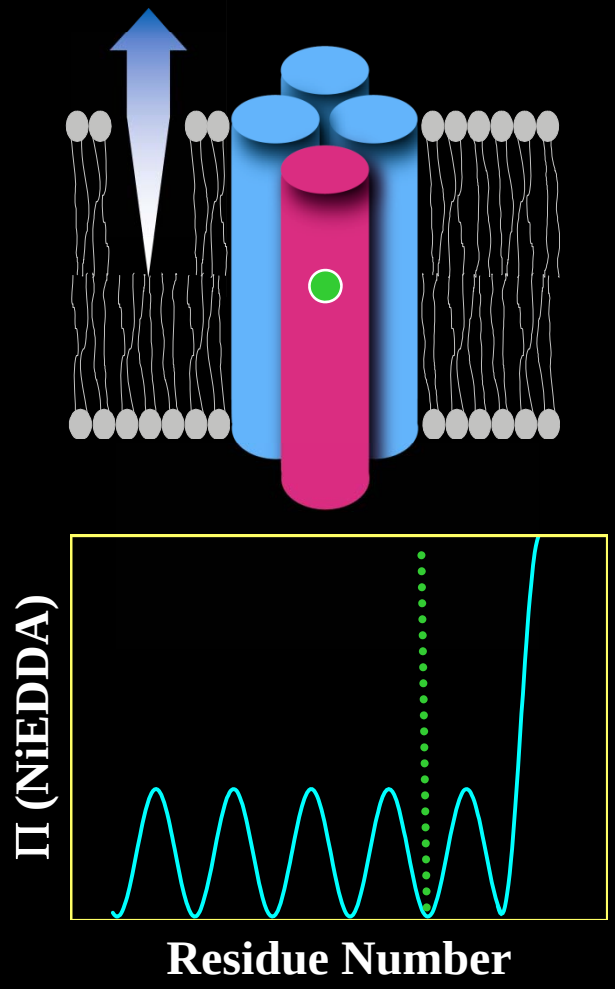


Accessibility Pattern of Anisotropically Solvated Transmembrane Helix

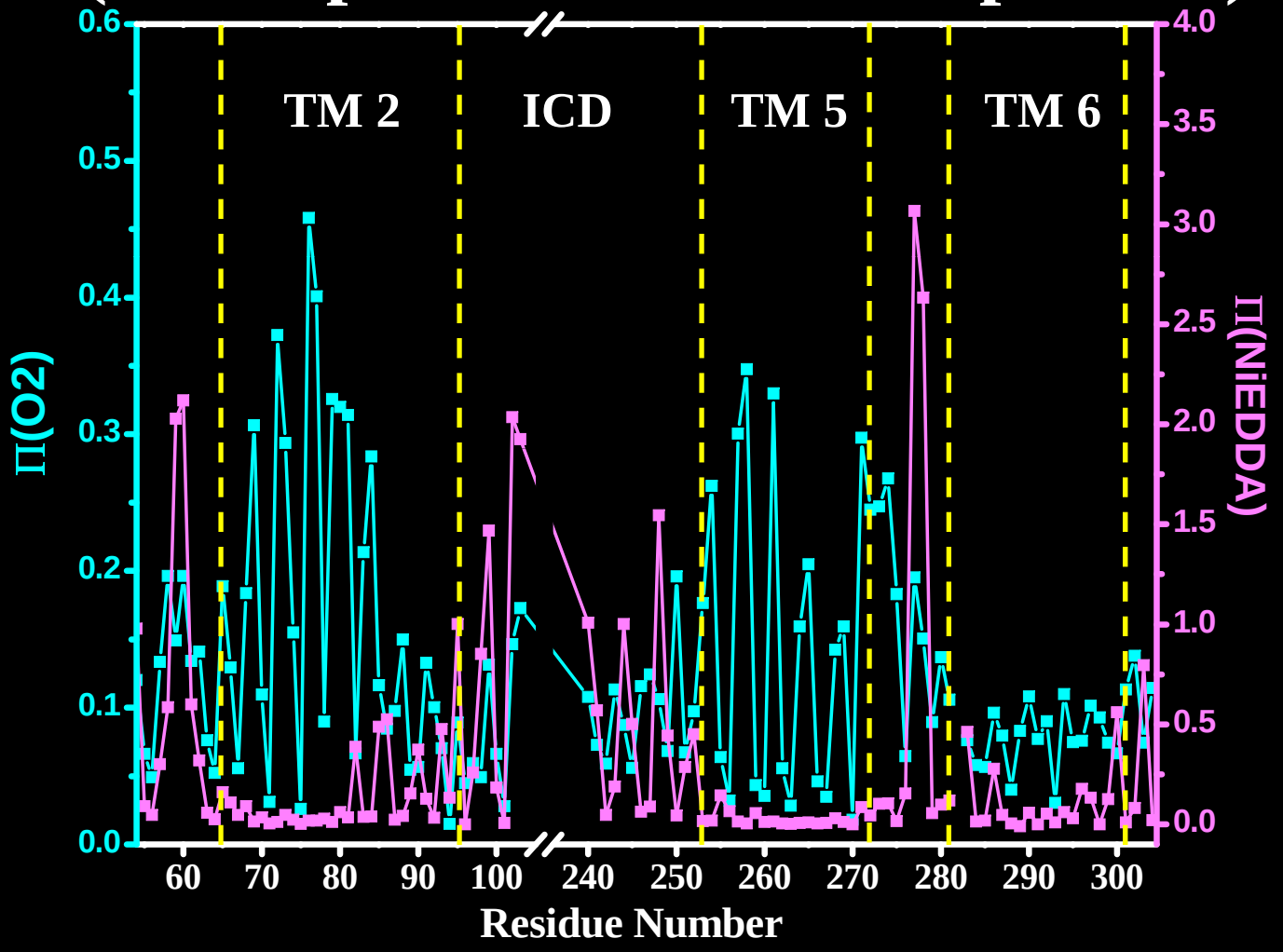
O_2 Gradient in the Bilayer



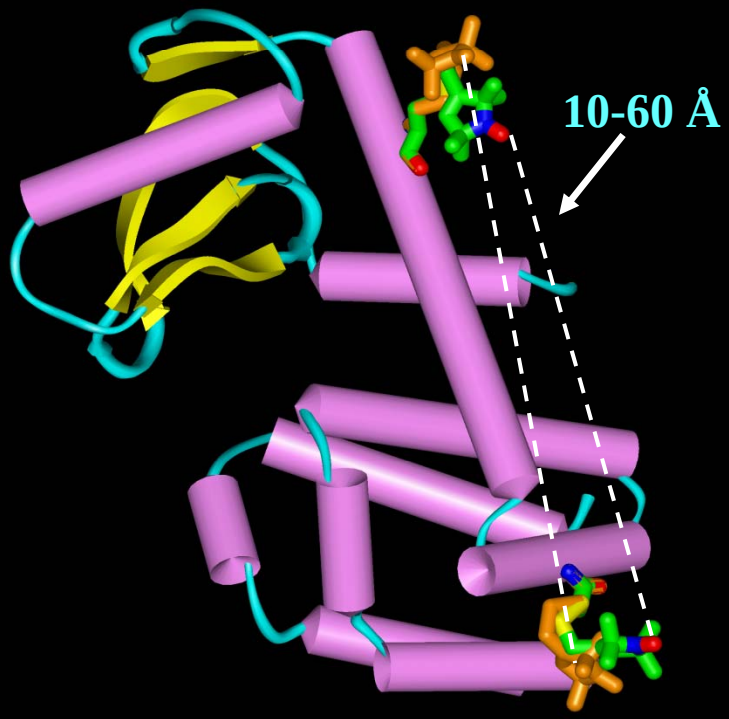
NiEDDA Concentration in the Bilayer

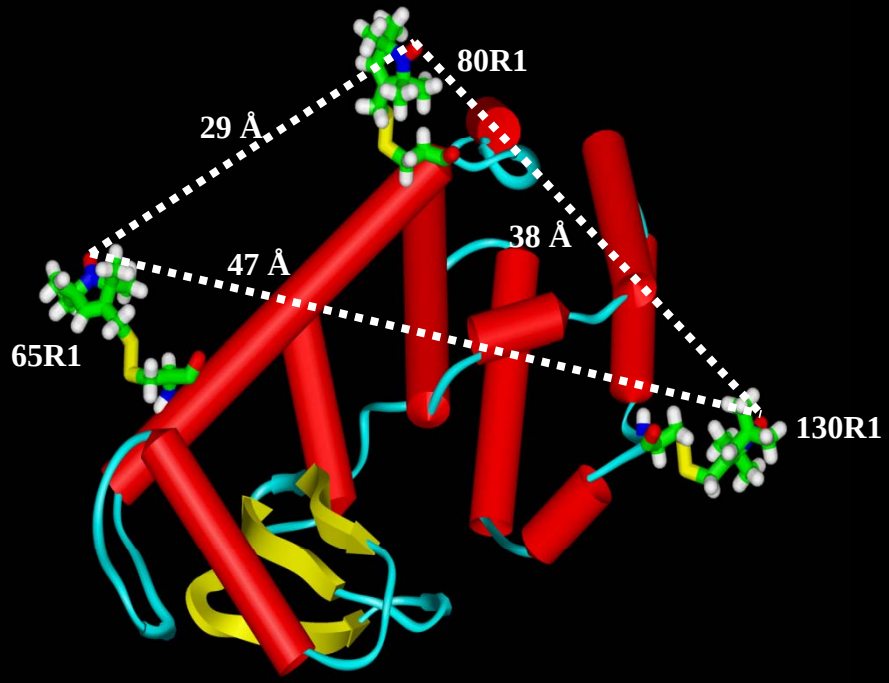


Accessibilities of Spin label Side Chains Along Selected Segments of Apo-MsbA (Example of a membrane protein)



Distance Determination in Proteins





Pair	*Ca- Ca*	*R1-R1*
65/80	23 Å	28 Å
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65/135	34 Å	46 Å

Borbat, P.P., Mchaourab, H.S., and Freed, J.H. (2002).
***Journal of the American Chemical Society.* 124, 5304-5314.**

Dipolar Interaction

The energy of interaction of a magnetic dipole μ_1 with magnetic dipole μ_2 at distance r is

$$E = U = \frac{\mu_1 \mu_2}{r^3}$$

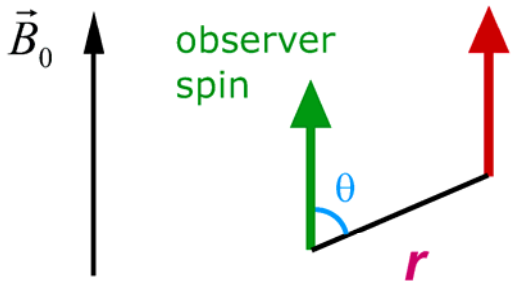
More generally, considering the vector properties of the magnetic dipoles

$$U_{dipolar} = \frac{\mu_0}{4\pi}\left[\frac{\vec{\mu}_1 \cdot \vec{\mu}_2}{r^3} - \frac{3(\vec{\mu}_1 \cdot \vec{r})(\vec{\mu}_2 \cdot \vec{r})}{r^5}\right]$$

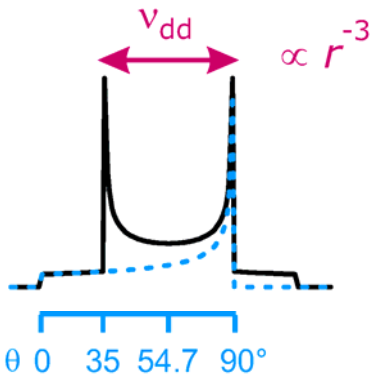
Which is proportional to $(1\text{-}3\cos^2\theta)$ where θ is the angle between the interspin vector and the external magnetic field.

Dipolar coupling and distance

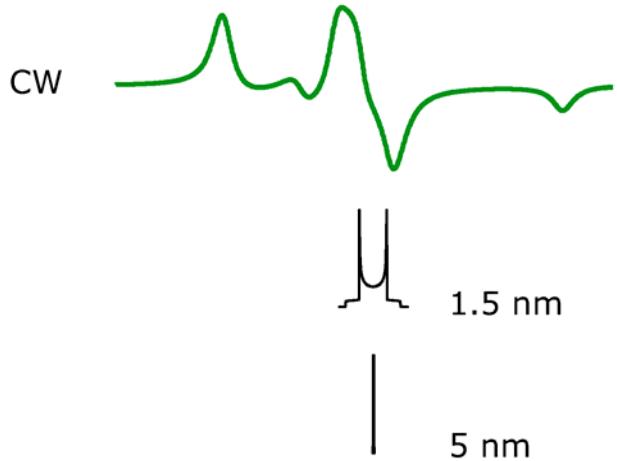
Dipolar interaction of aligned spins



Pake pattern



Electron-electron coupling



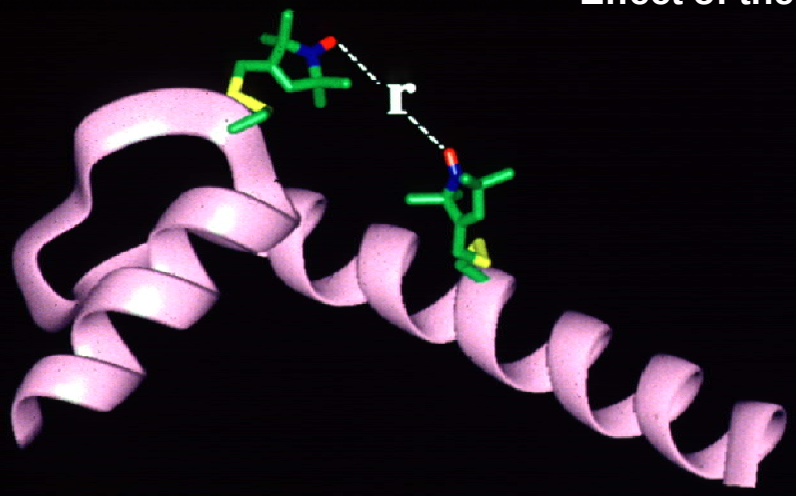
Electron-nuclear coupling at *r* = 1 nm

^{1}H: 79 kHz

^{31}P: 32 kHz

^{14}N: 5.7 kHz

Distance Determination in Proteins



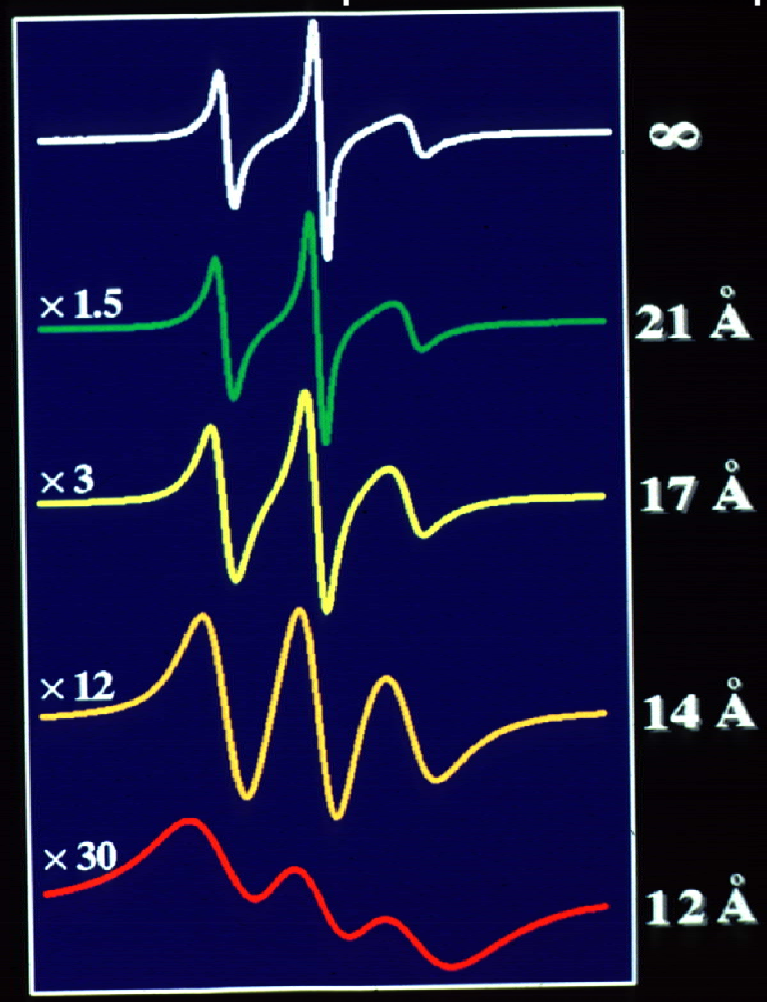
Nitroxide–Nitroxide

Static Limit ($\propto r^{-3}$)

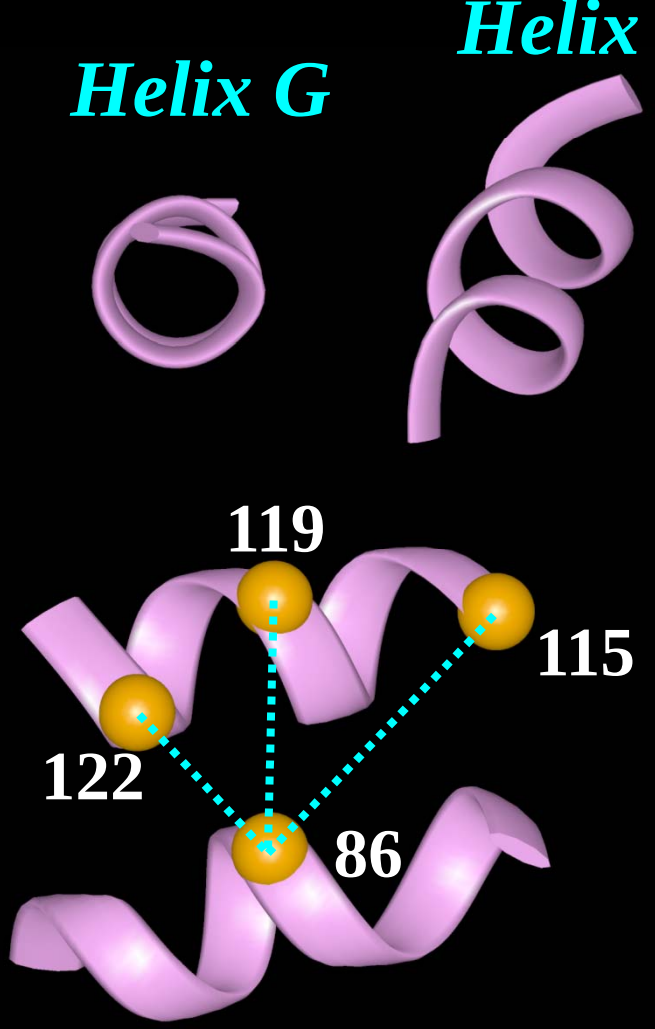
$\tau_r \gg (3\pi g^2\beta^2/(hr^3))^{-1}$

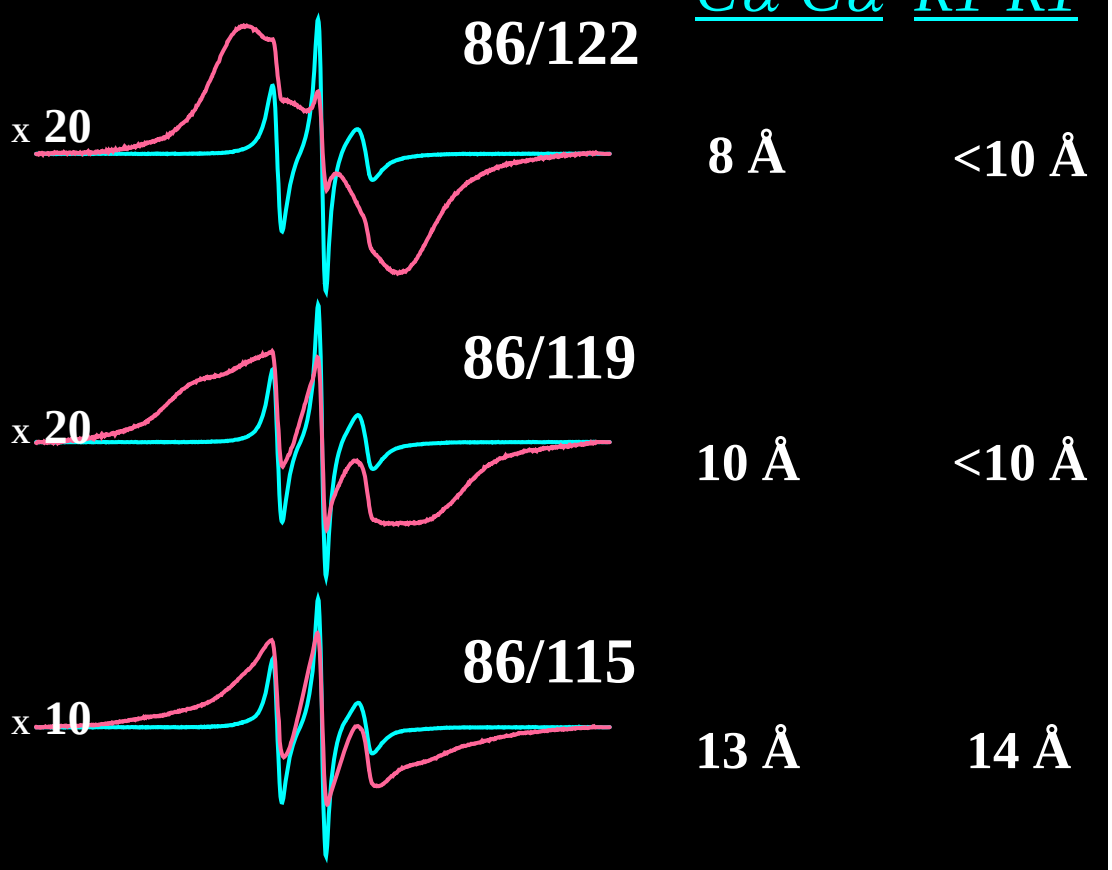
Range: 10–25 A

Effect of the distance between spin labels on the EPR spectrum



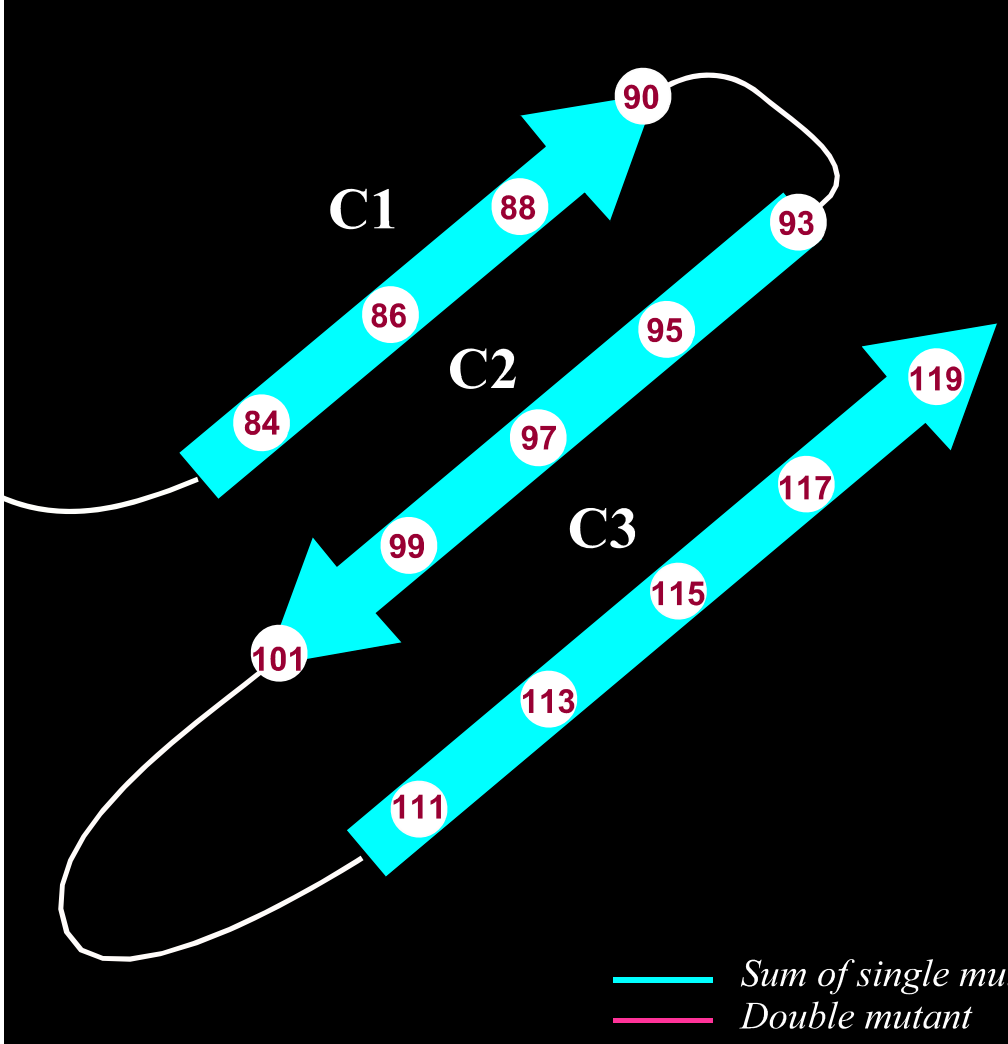
Packing of Helices D and G In T4 Lysozyme

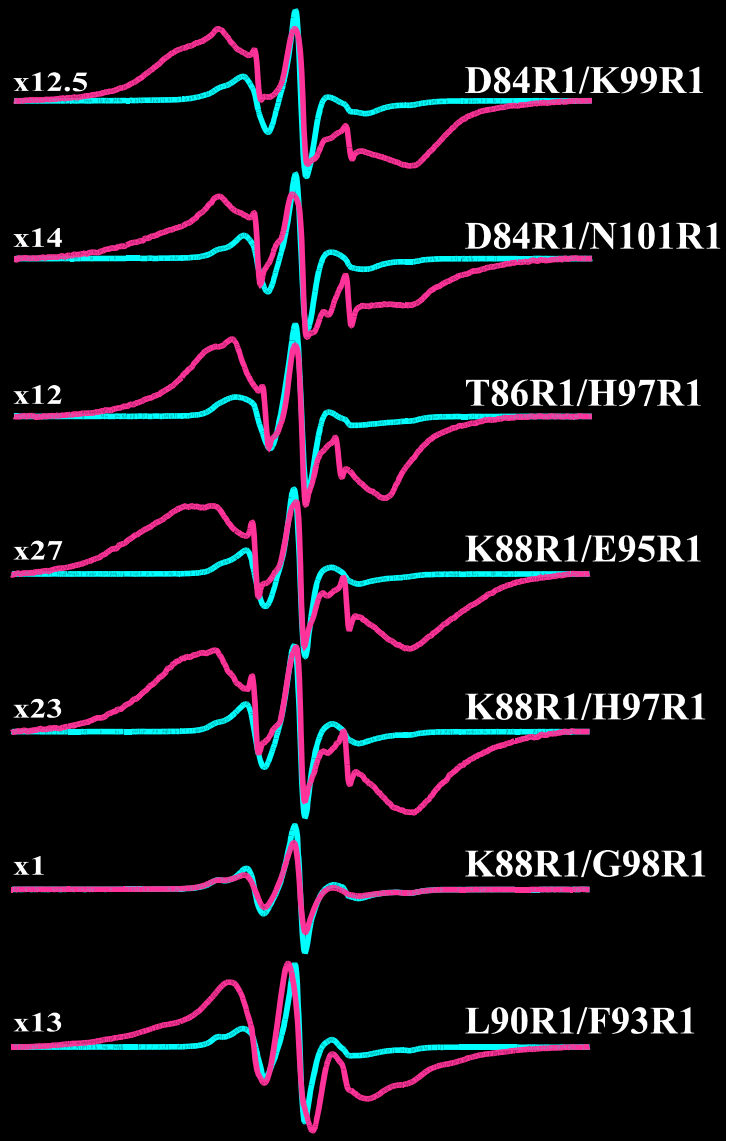




Conclusion: Distance between labels reflects the packing of secondary structure.

Packing of Strands C1 and C2

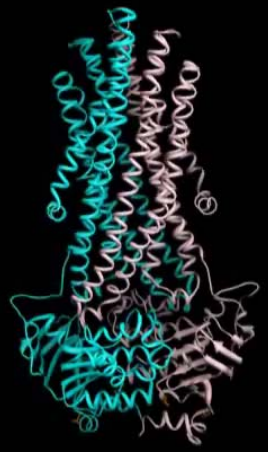




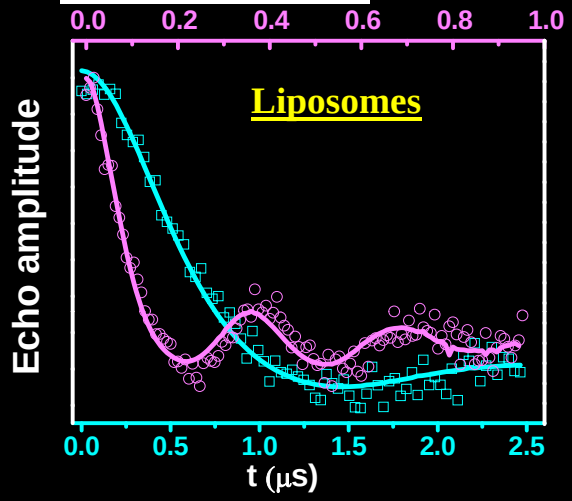
Distance Changes at the ABCs

Borbat et al. (2007). PLoS Biology.

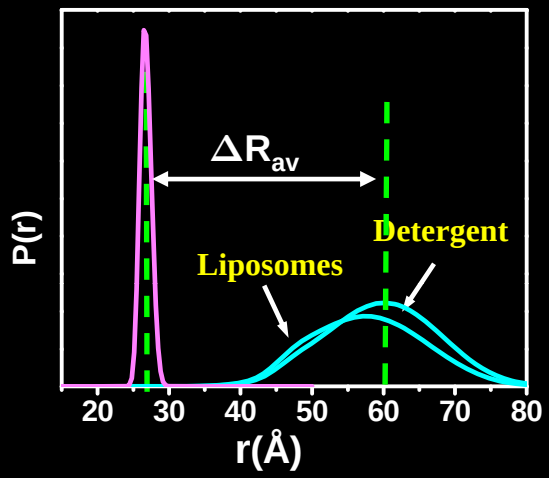
How far do the ABCs move?



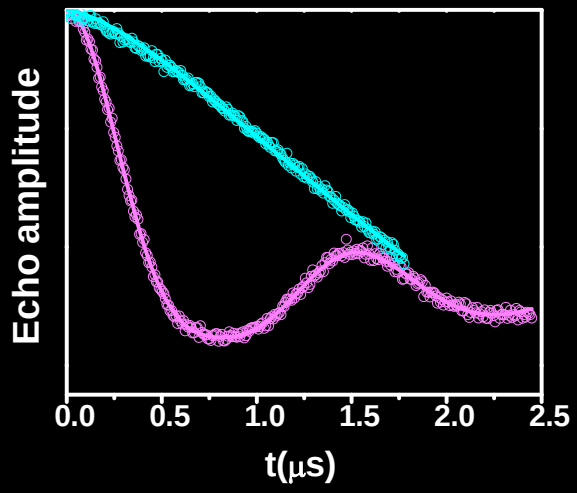
Raw DEER Data

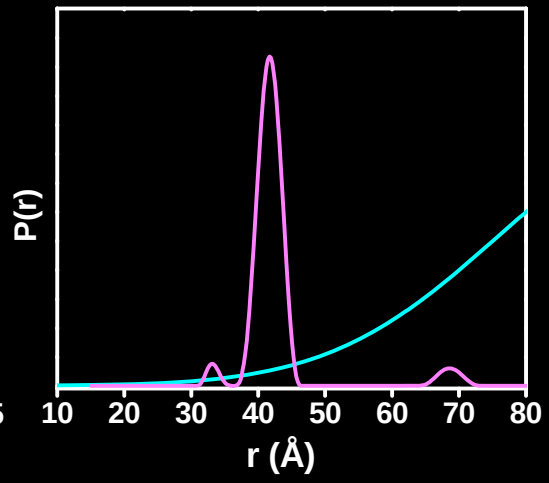


Distance Distribution

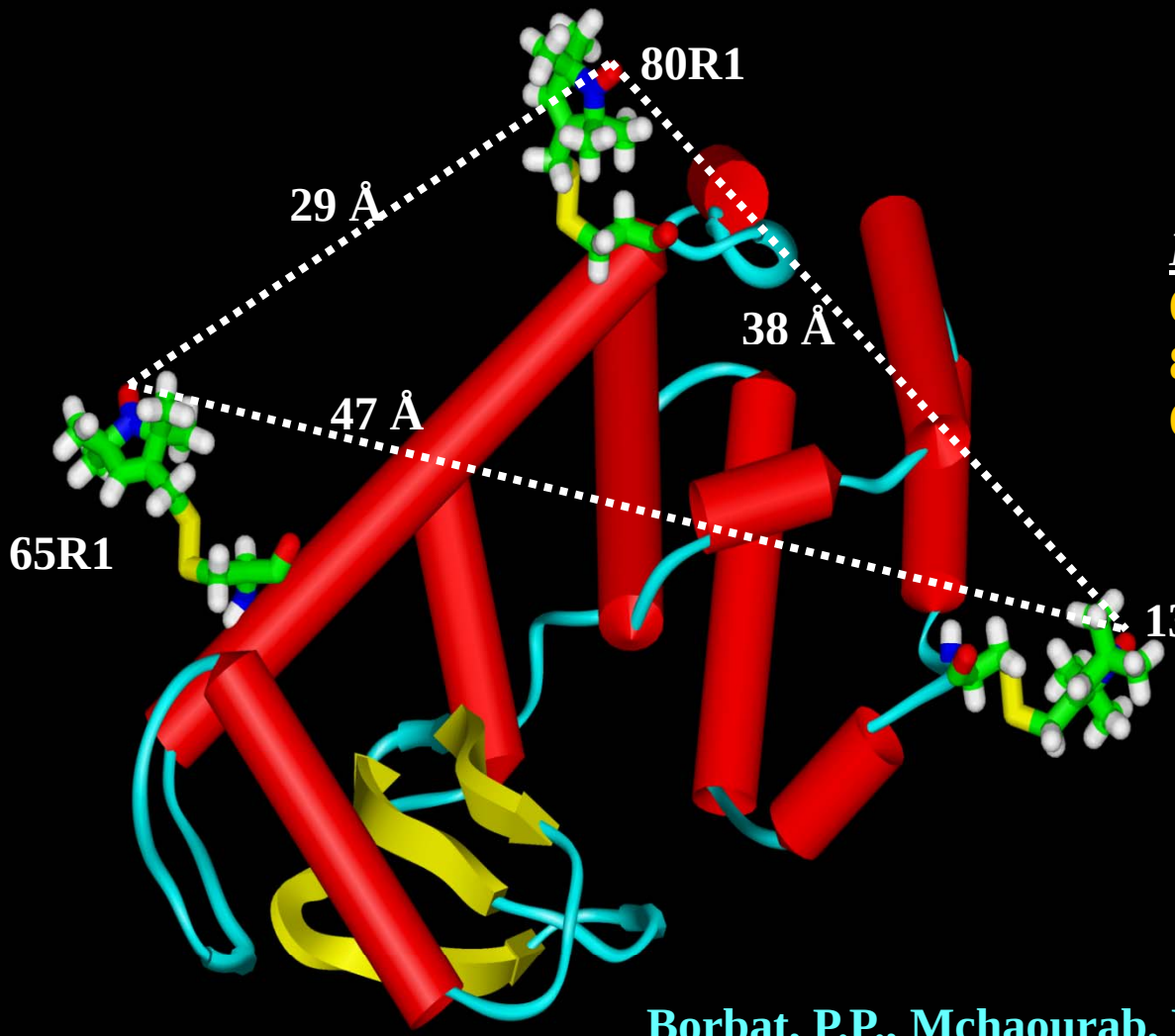


No change in spin label dynamics ……. a change in NBD separation and order





Long Range Distance Measurements in T4 Lysozyme by Double Electron-Electron Resonance



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