Electron Paramagnetic Resonance (EPR)
SDSL – Site Directed Spin Labeling

1. **Discovery of EPR** – E. Zavoisky, USSR, 1945
   (http://kfti.knc.ru/eng/about/index.html)

2. **Development as a Biophysical Tool.** - Synthesis of a stable free radical (the nitroxide), and labeled biomolecules (Spin labels) (Humphries & McConnell *in* Methods of Experimental Physics, vol. **20**, G. Ehrenstein and H. Lecar, eds., 1982, Academic Press, p. 53-122.)

3. **Applications** – dynamics of molecular motions in macromolecules and supramolecular assemblies. Examples taken from Hubbell, Cafiso and Altenbach. 2000. Identifying conformational changes with site-directed spin labeling. *Nat. Struct. Biol.* **7**:735-739. & Columbus and Hubbell. 2002. A new spin on protein dynamics. *Trends in Biochemical Sciences.* 27:288-295. (Review)

2. Basic Properties of ESR

1. **Electrons have 'spin'.** A circulating electric charge has a magnetic moment.

$$\mu = -g\beta \mathbf{S}$$

2. **Energy Levels are Nondegenerate in a Magnetic Field**. Electrons (and some nuclei) with spin = ½, have two energy levels, which results in one transition.

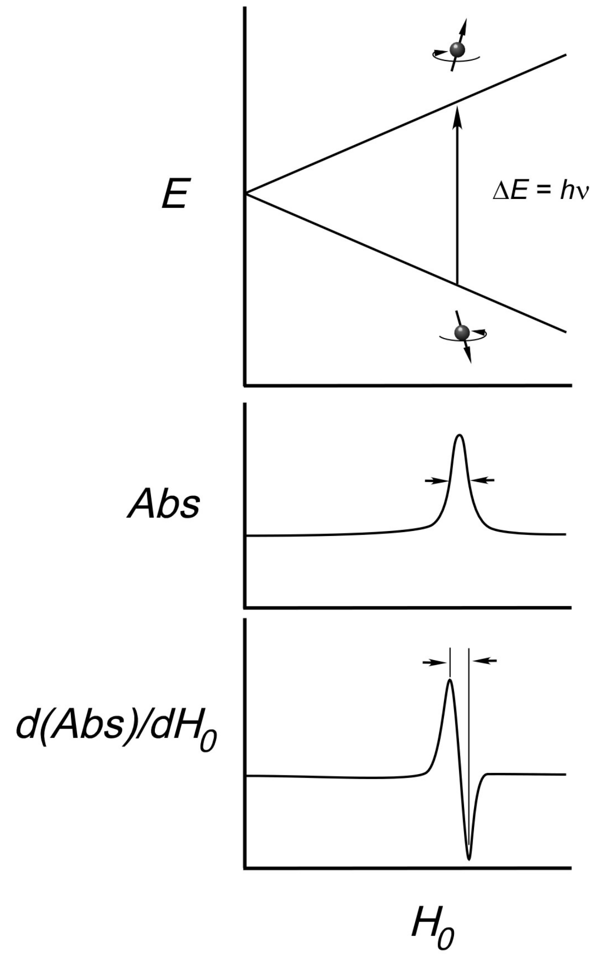
$$E = -\mu \cdot \mathbf{H}_0 = g\beta \mathbf{S} \cdot \mathbf{H}_0$$

In an external magnetic field applied along the z-axis, the energies can be on the z-component of the spin, +/- ½

3. **The Transition Energy** is given as the difference in the low and high energy levels:

$$h\nu = E(S_z = +1/2) - E(S_z = -1/2)$$

$$h\nu = g\beta H_0$$



(Humphries & McConnell, 1982)

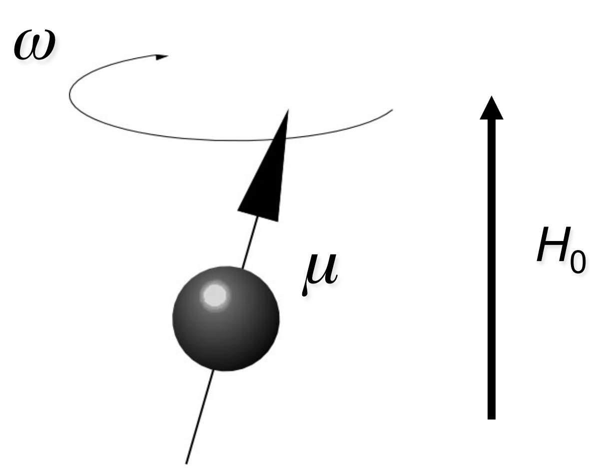
2. Basic Properties of ESR

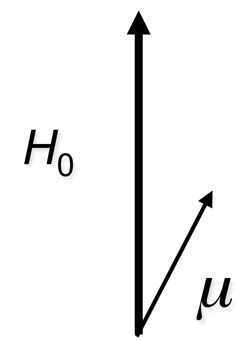
The Transition Energy is also expressed as:

$$h\nu = g\beta H_0 \quad \rightarrow \quad 2\pi\nu = g\beta H_0 / \hbar$$

$$\omega = \gamma H_0$$

γ is the magnetogyric (or gyromagnetic) ratio

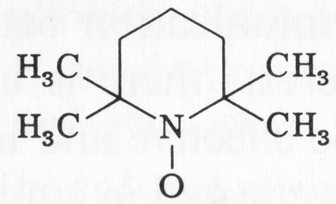




$$\frac{d}{dt}\boldsymbol{\mu} = \gamma\boldsymbol{\mu} \times \mathbf{H}$$

(Humphries & McConnell, 1982)

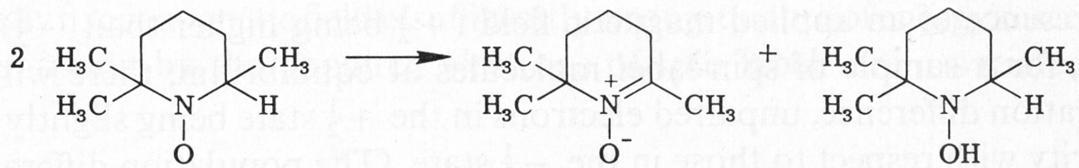
2. Nitroxide Spin Labels



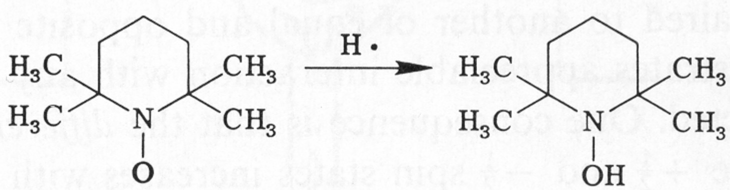
2,2,6,6-Tetramethyl-piperidine-1-oxyl (TEMPO)

Nitroxide Spin Labels are Stable between pH 3 and 10

They must be tetra-substituted for stability

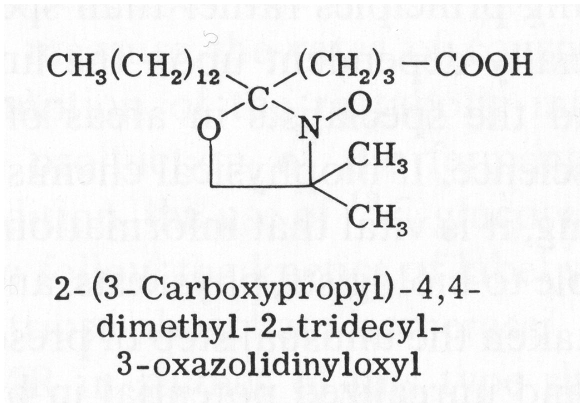


They are sensitive (quenched by) reductants

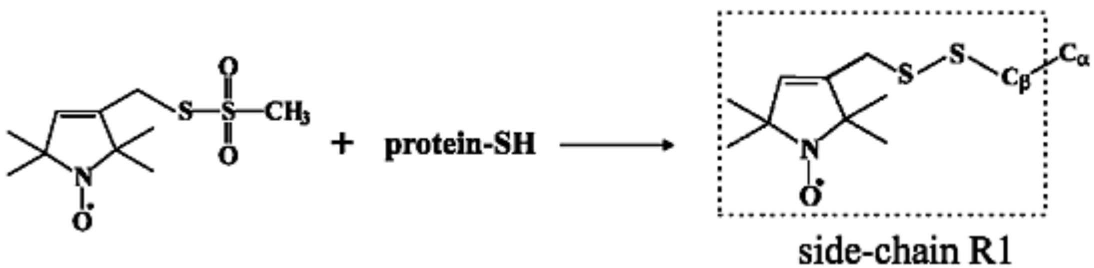


(Humphries & McConnell, 1982)

Labels have been incorporated into biomolecules



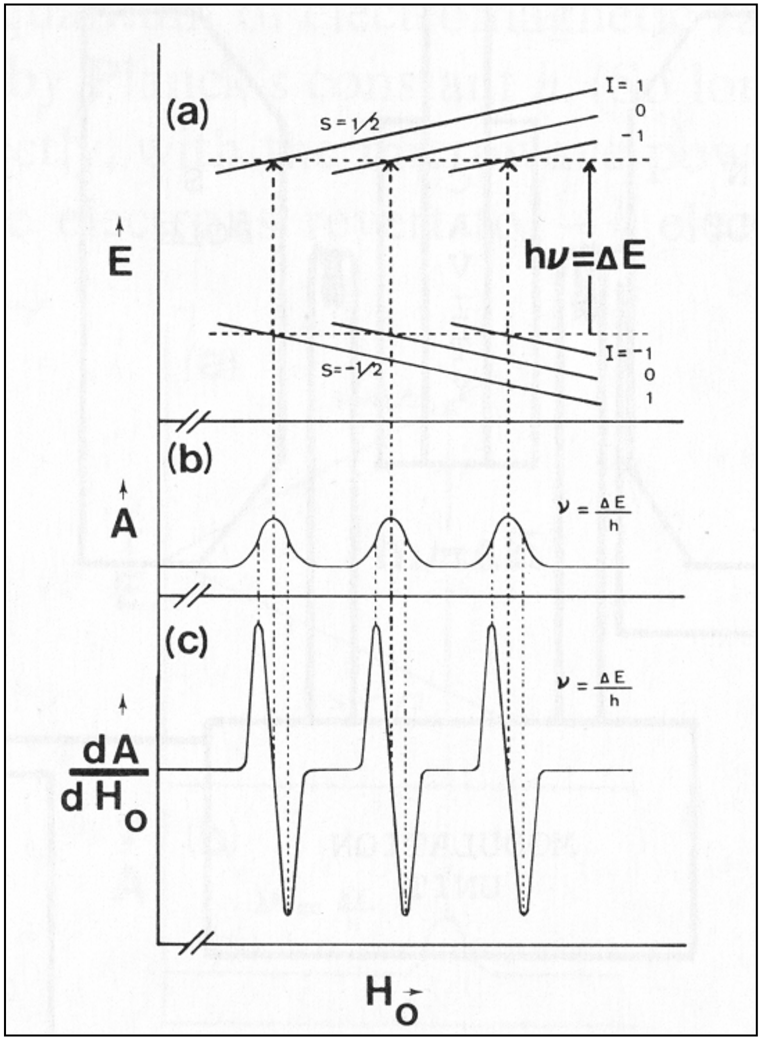
Lipids

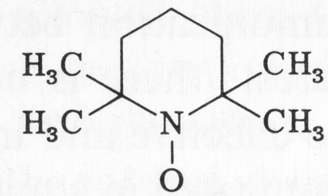


Proteins

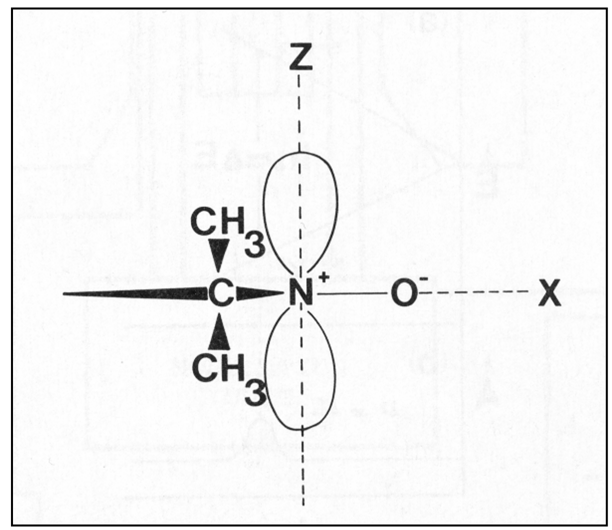
(Humphries & McConnell, 1982)

The Local Field Produced by the Magnetic Moment of ^{14}N Results in Hyperfine Splitting



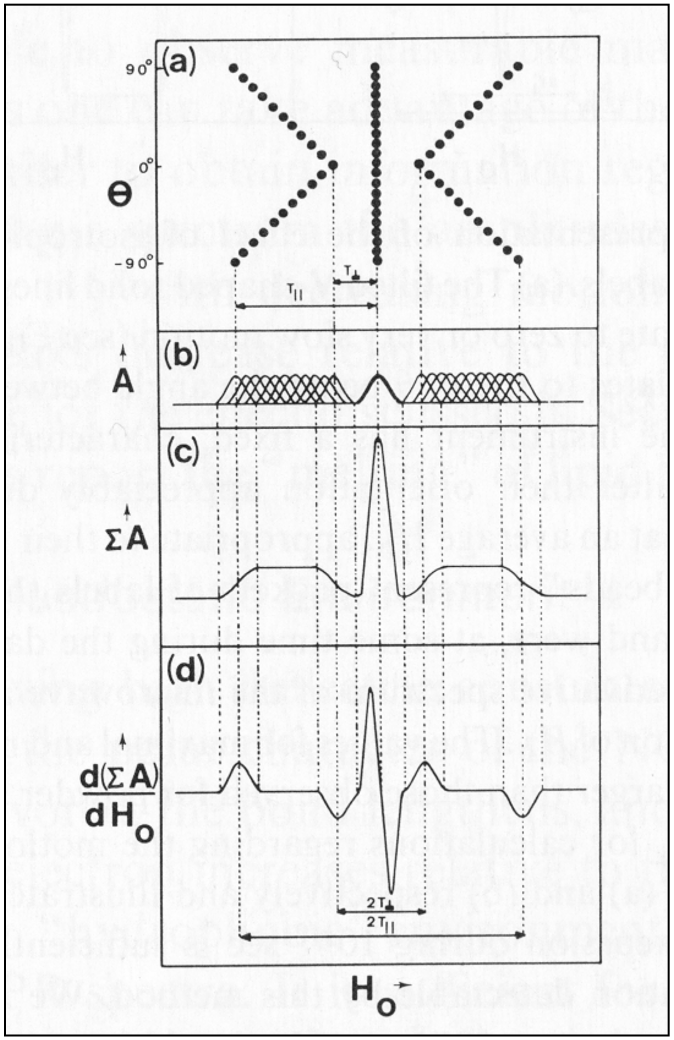


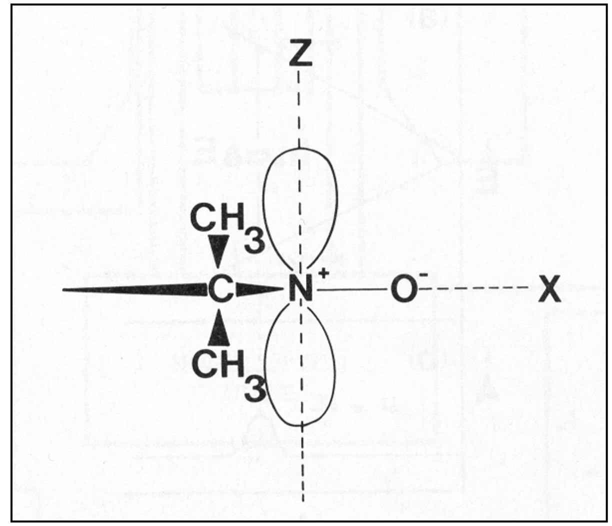
2,2,6,6-Tetramethyl-piperidine-1-oxyl (TEMPO)



(Humphries & McConnell, 1982)

Hyperfine Splitting Depends on Orientation of Molecule with Respect to the Magnetic Field



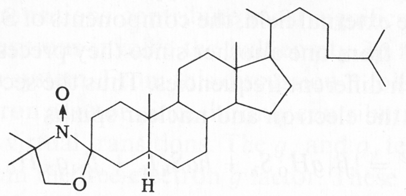


← 'Powder Pattern' of an Isotropic (Unoriented) Immobilized Label

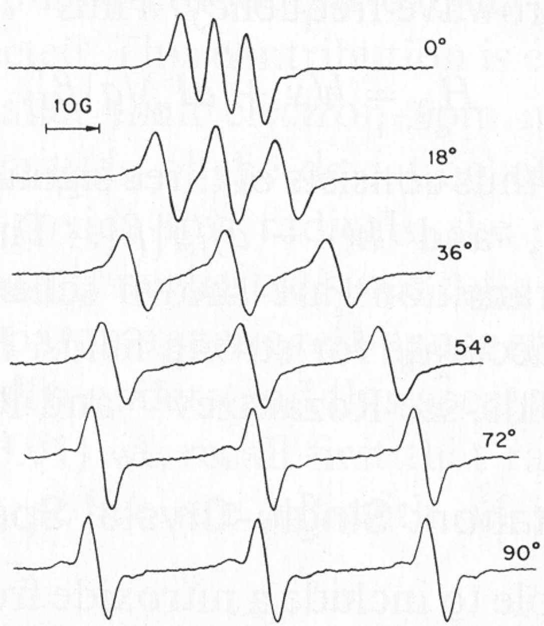
(Humphries & McConnell, 1982)

Hyperfine Splitting in Oriented Spin Label Crystals (Spin Label doped in Cholesteryl Chloride)

As one example of this type of experiment, Fig. 12 shows paramagnetic resonance spectra of the *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-α-cholestan-3-one (**I**) when this substance is incorporated as a substitutional impurity (at a concentration of the order of one percent) in single crystals of cholesteryl chloride.[12]

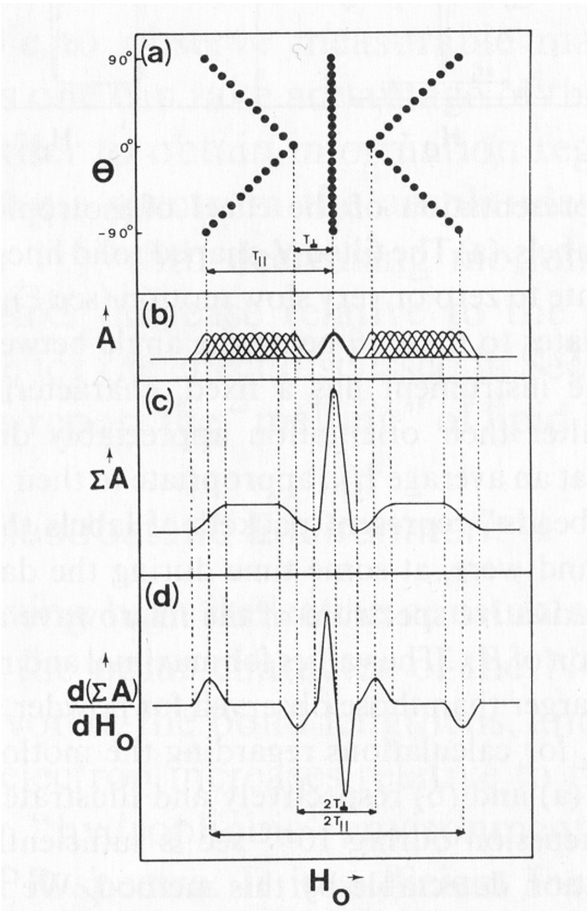


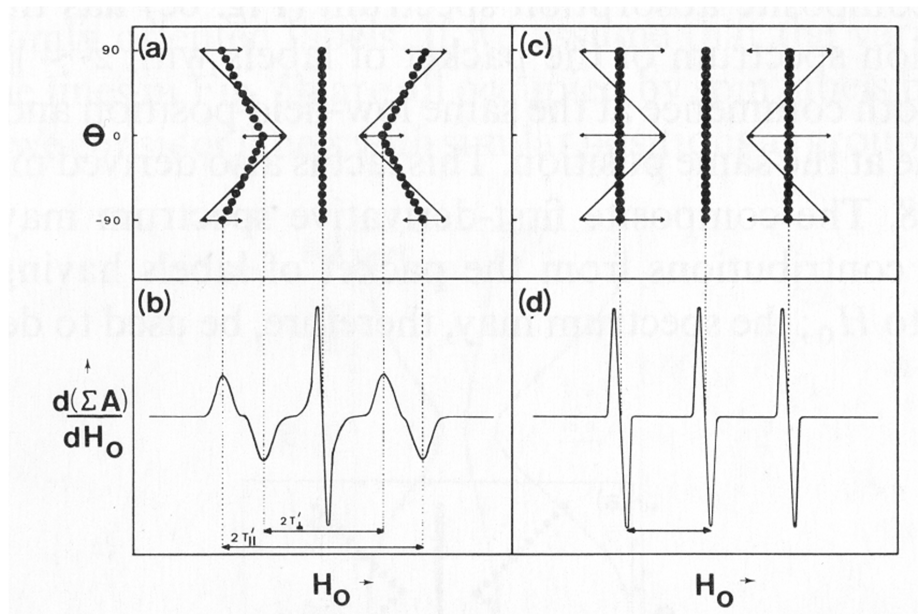
I



(Humphries & McConnell, 1982)

Effects of Mobility





Restricted Mobility

Mobile

Immobilized

(Humphries & McConnell, 1982)

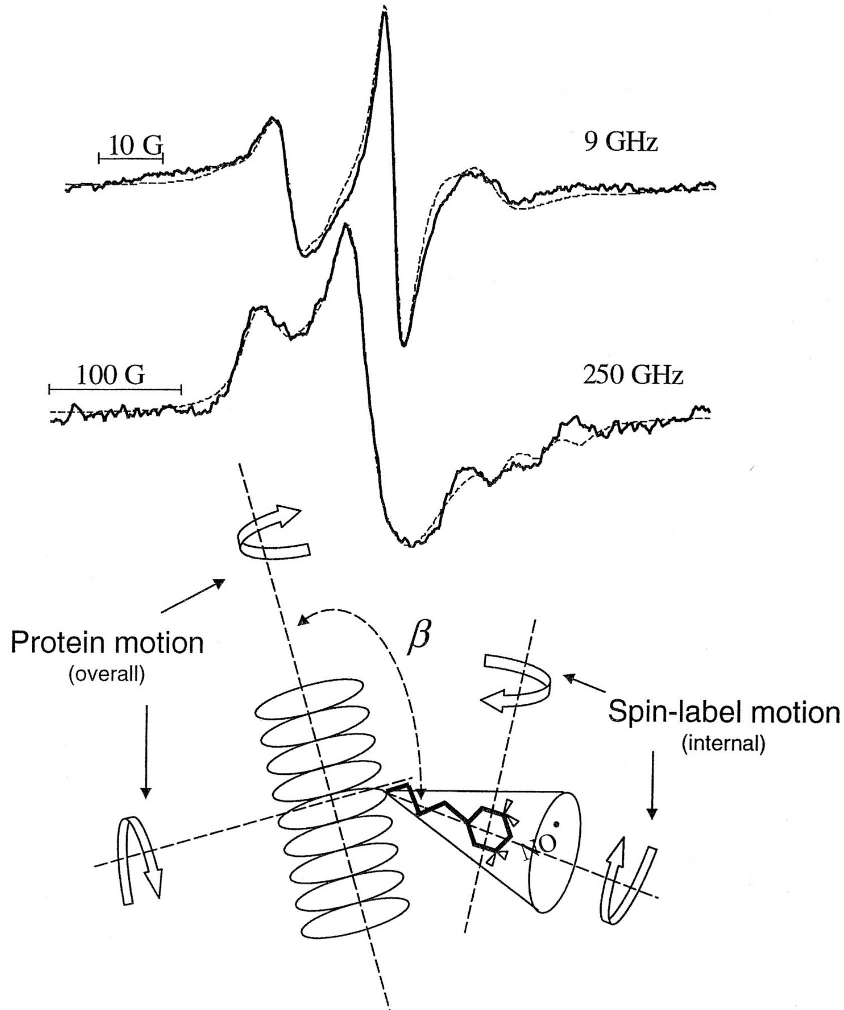
Magnetic Field Dependence of EPR Line Shapes

Increase Magnetic Field

→ Increase energy level separation

→ Shorten lifetime of excited state by a change in the spontaneous decay rate

→ Shift the observation window of molecular dynamics from longer to shorter time scales



P. P. Borbat et al., Science 291, 266 -269 (2001)

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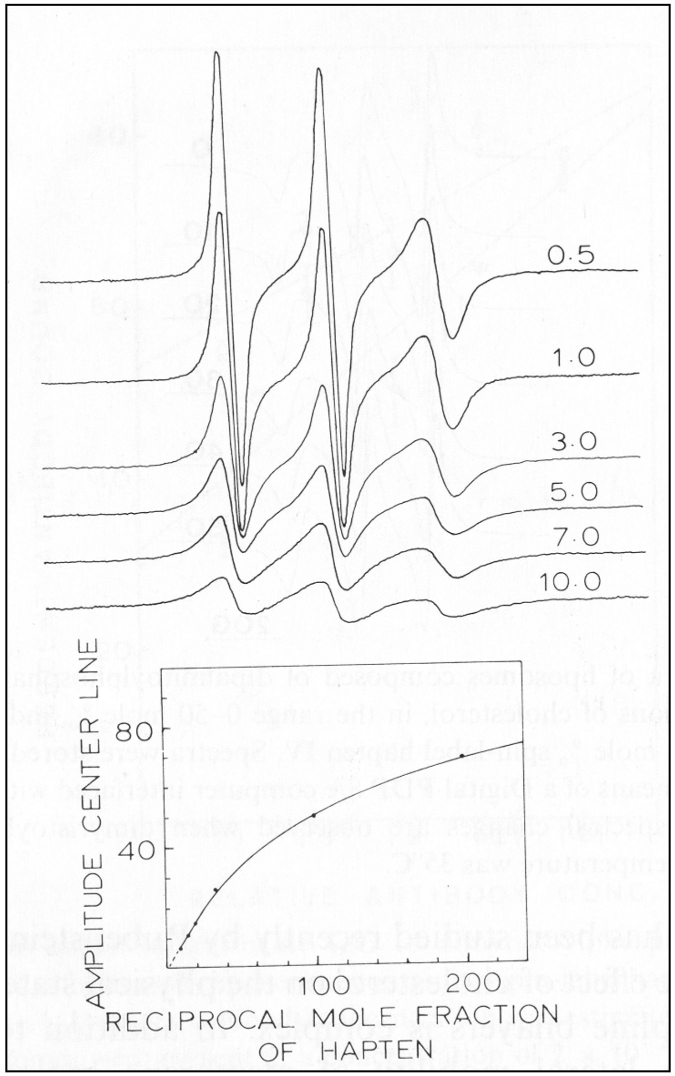
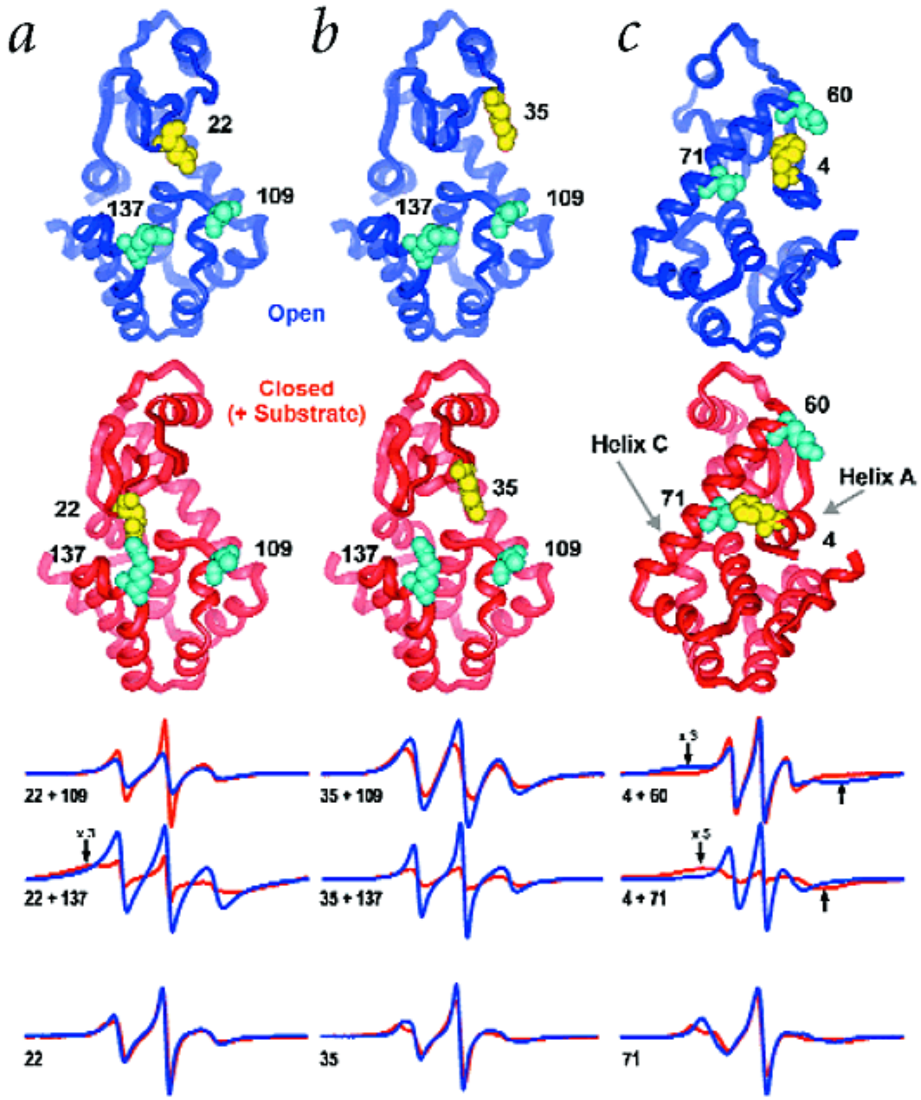
Dipole-Dipole Exchange Interactions



(Humphries & McConnell, 1982)

Exchange Interactions in Double-Labeled T4 Lysozyme Samples Reflect Domain Motion



Blue – Enzyme minus substrate

Red – Enzyme plus substrate

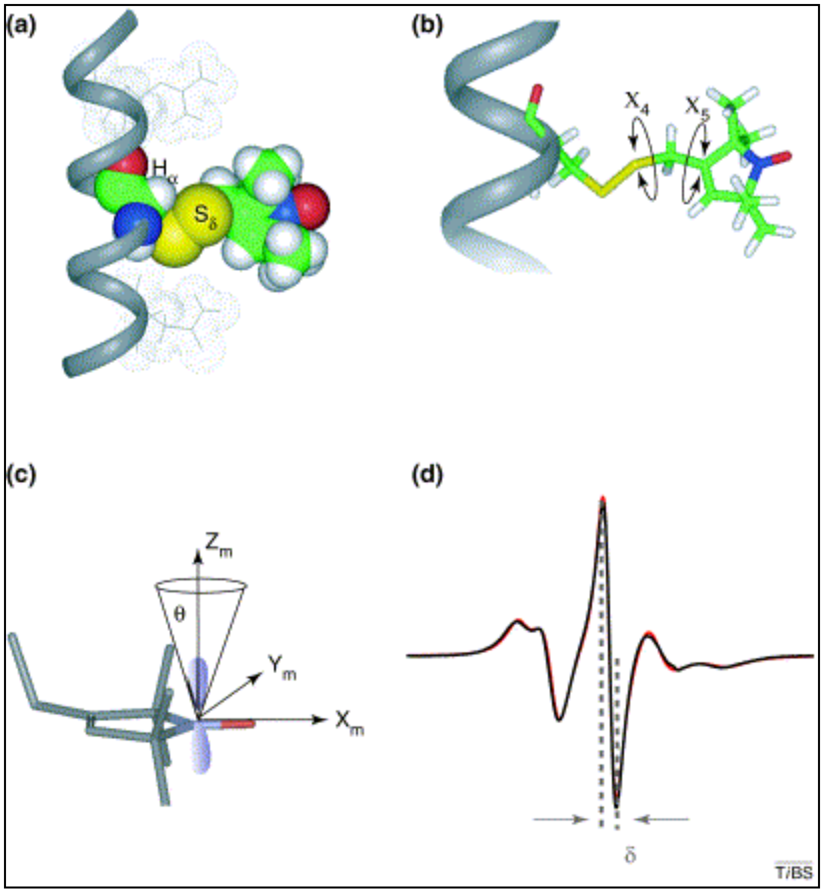
←Double Labeled Samples

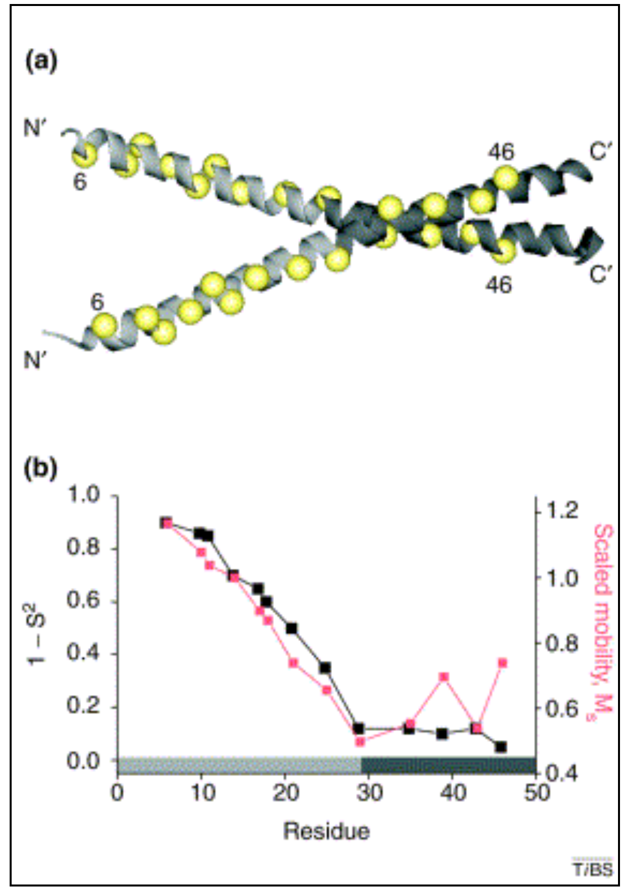
← Single Labeled Samples

(Hubbell, Cafiso & Altenbach, NSB, 2000)

Measurement of, and changes in, Restricted Mobility

Scaled mobility: $M_s = \frac{(\delta^{-1} - \delta_i^{-1})}{(\delta_m^{-1} - \delta_i^{-1})}$ where δ, δ_m, δ_i are the measured peak width, the most mobile label peak width and the least mobile label width, respectively. In these examples δ_m = 2.1 Gauss and δ_i = 8.4 Gauss.





(Columbus & Hubbell, 2002)