Objectives

- **Introduction to EPR theory**
  - *a) The resonance phenomenon*
  - *b) Anisotropy*
  - *c) Saturation*

- **Detailed protocol on using spin labels to determine protein structure and dynamics**
  - *a) Nitroxide scanning*
  - *b) Local environment and secondary structure*
  - *c) Tertiary and quaternary structure*
  - *d) Conformational dynamics*

Tools of the Trade

1- Atomic resolution: X-ray crystallography

2- NMR spectroscopy

3- de novo Modeling and structure determination, Homology modeling

4- CryoEM

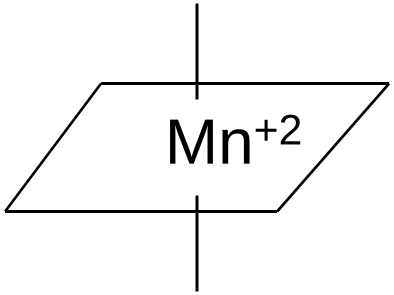
Why do we needs probes ?????

Issues: 1- sample requirement
2- nature of the environment
3- Dynamics and time scales

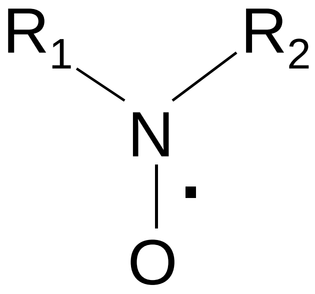
Electron *Paramagnetic* Resonance
Electron Spin Resonance

$OH^{\cdot}$

Free Radicals
Ascorbate
Hydroxyl
Tocopherol
etc.

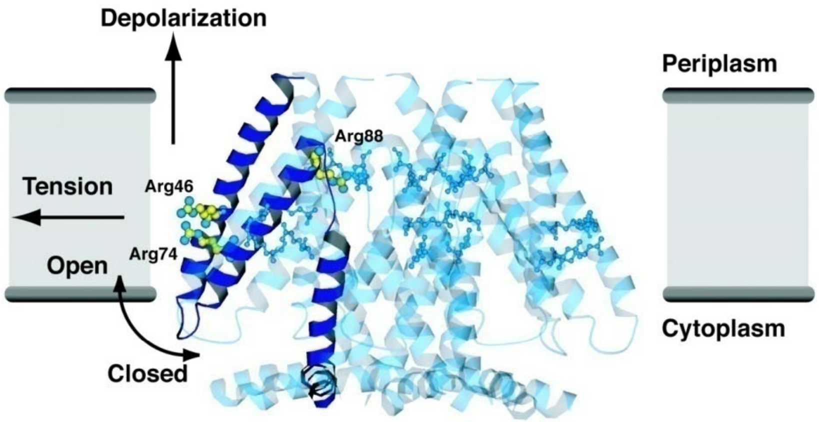


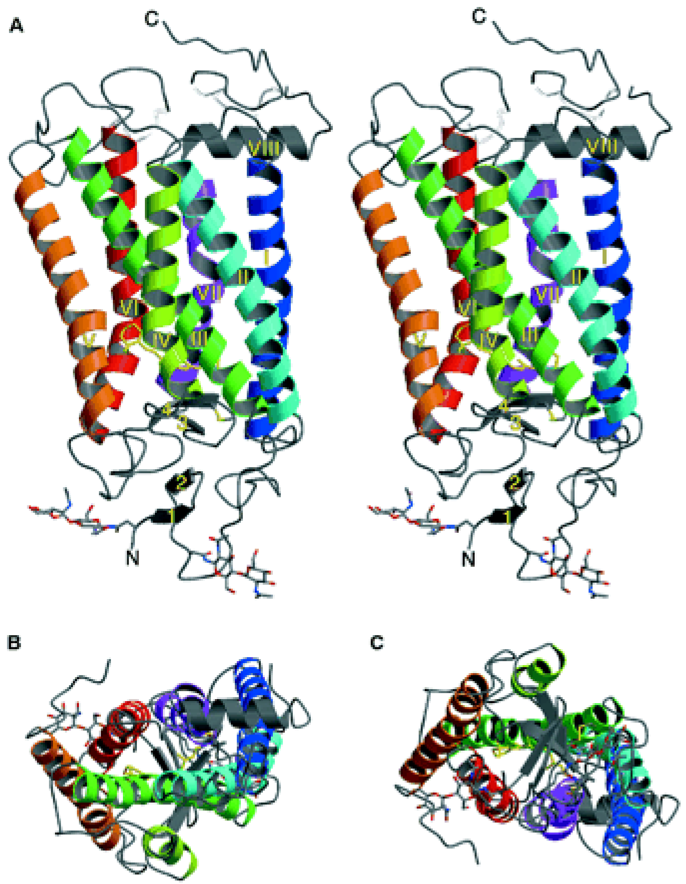
Transition Metals
All that have
unpaired electrons

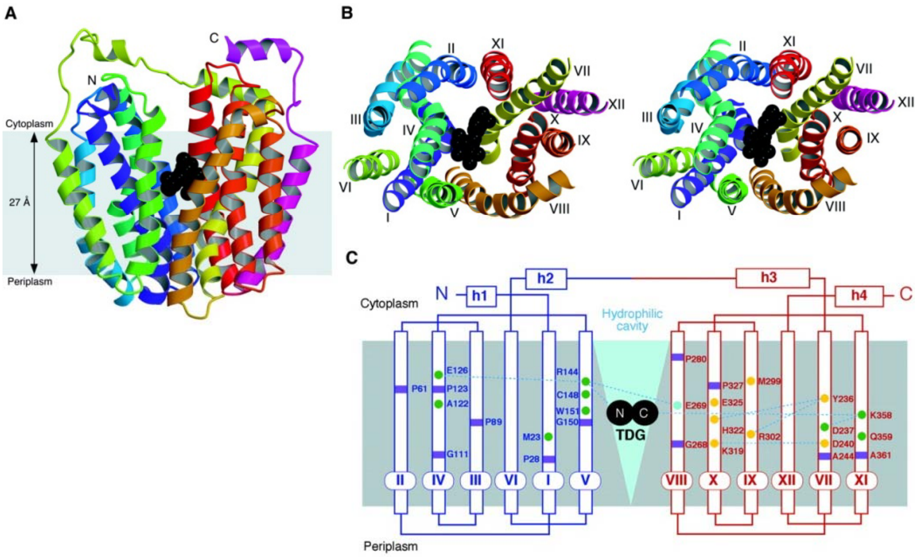


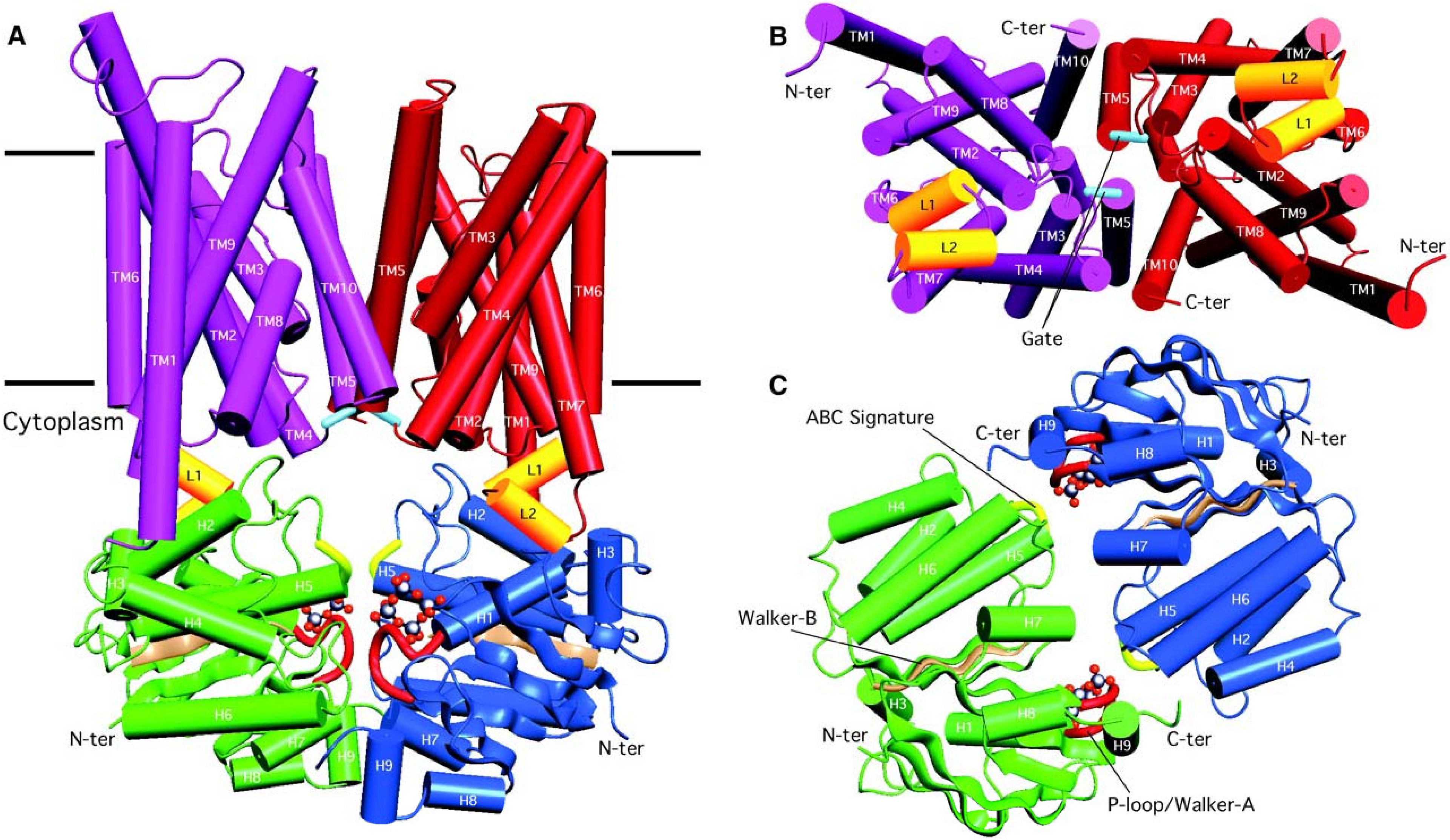
Spin Labels
R_1 & R_2 chosen
to provide
specificity

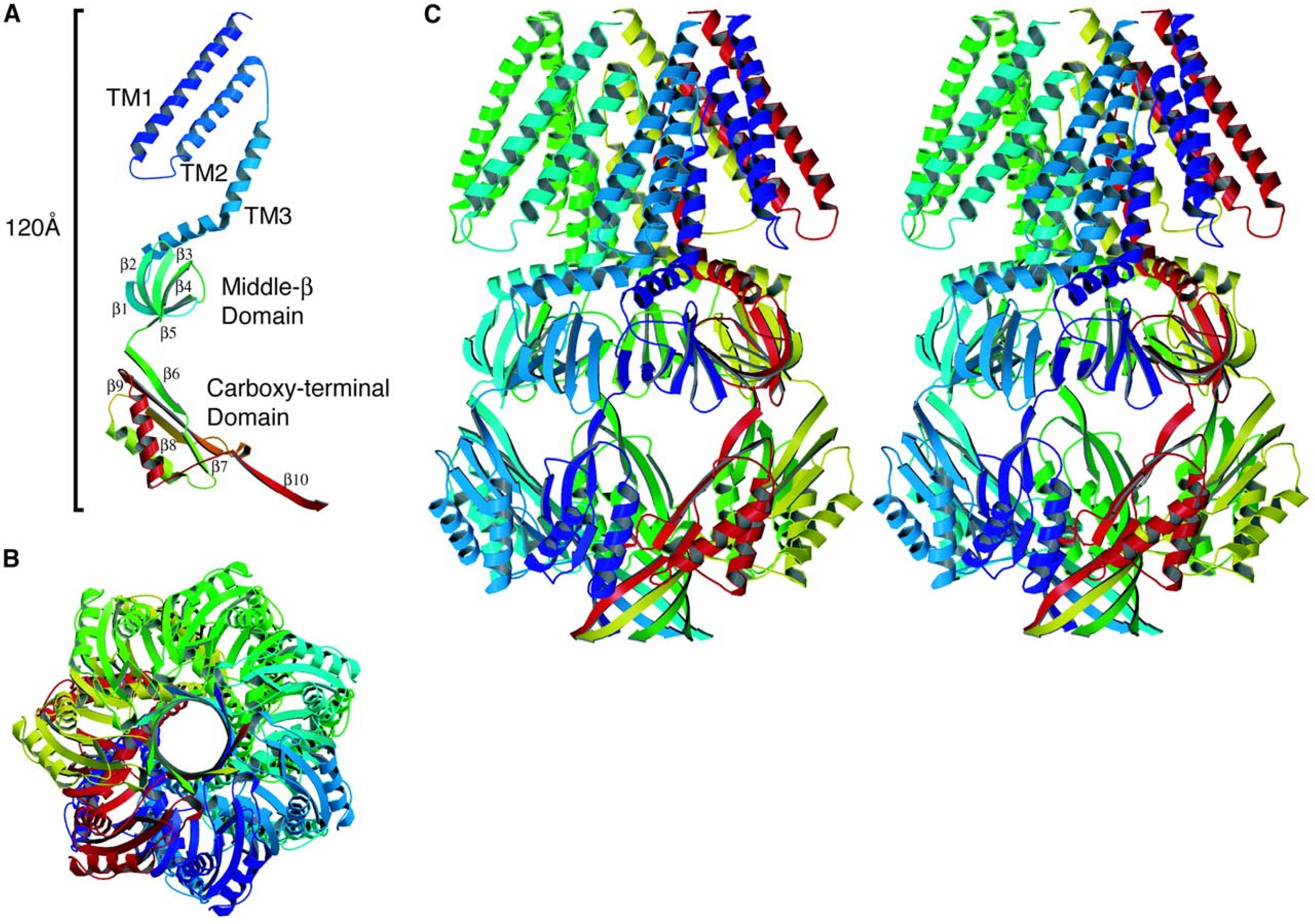
Functional Dynamics of Proteins



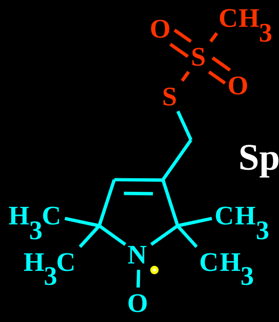




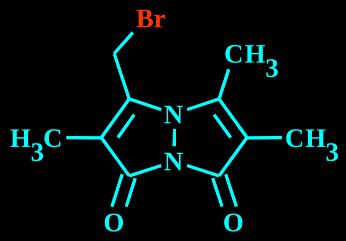

A
Cytoplasm
TM1
TM2
TM3
TM4
TM5
TM6
TM7
TM8
TM9
TM10
L1
L2
H1
H2
H3
H4
H5
H6
H7
H8
H9
N-ter
N-ter
B
C-ter
N-ter
Gate
C-ter
N-ter
C
ABC Signature
Walker-B
P-loop/Walker-A
C-ter
N-ter
N-ter
C-ter


A
120Å
TM1
TM2
TM3
β3
β2
β4
β1
β5
Middle-β
Domain
β6
β9
Carboxy-terminal
Domain
β8
β7
β10
B
C

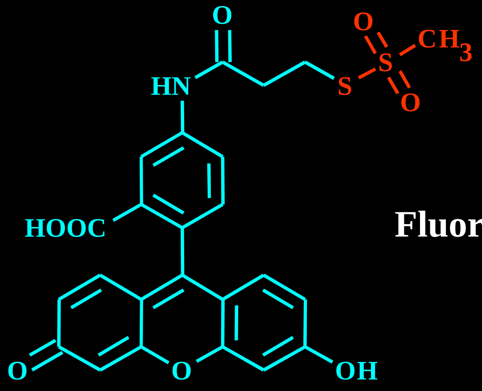
Site-Directed Labeling



Spin label



Bimane



Fluorescein

Probe spectroscopy

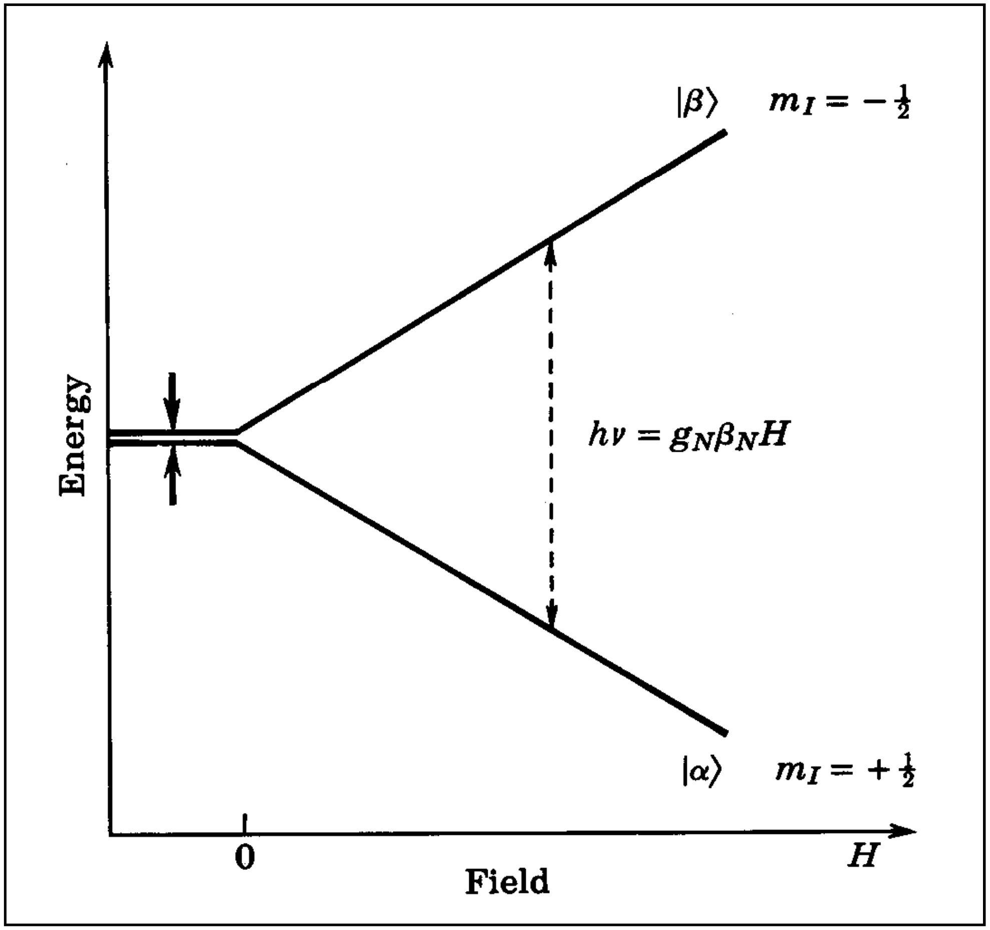
Spectral parameter → Protein structure

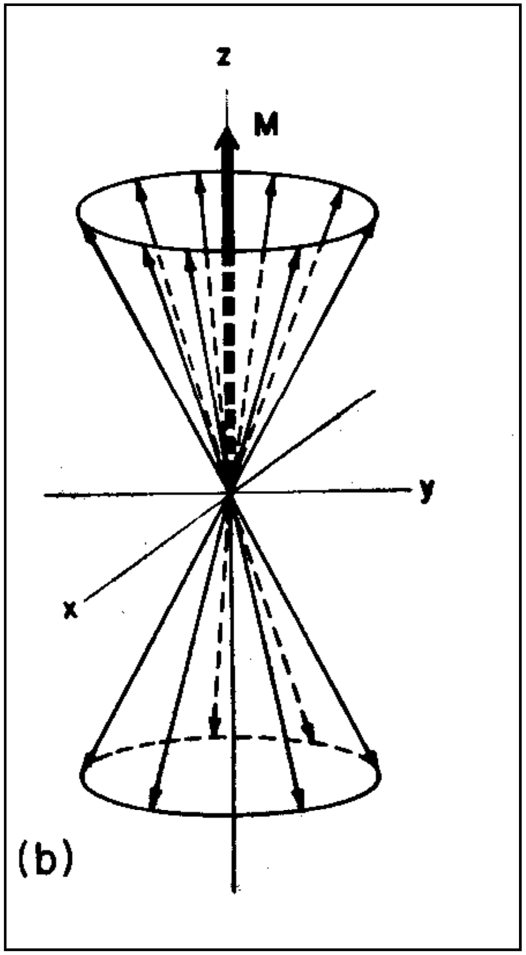
Biggest challenge: What is the transfer function?
Examples :

Uncertainty principle of chemical modification:
Implications: limited resolution both on the static and dynamic levels

Functional analysis of labeled mutants is a REQUIREMENT

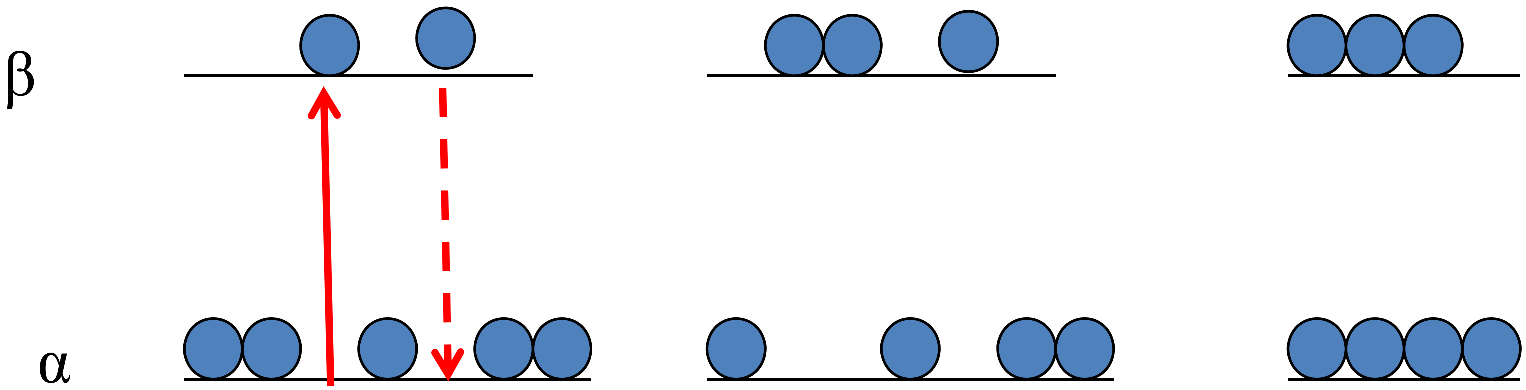
Spin ½ Electron in an External Field





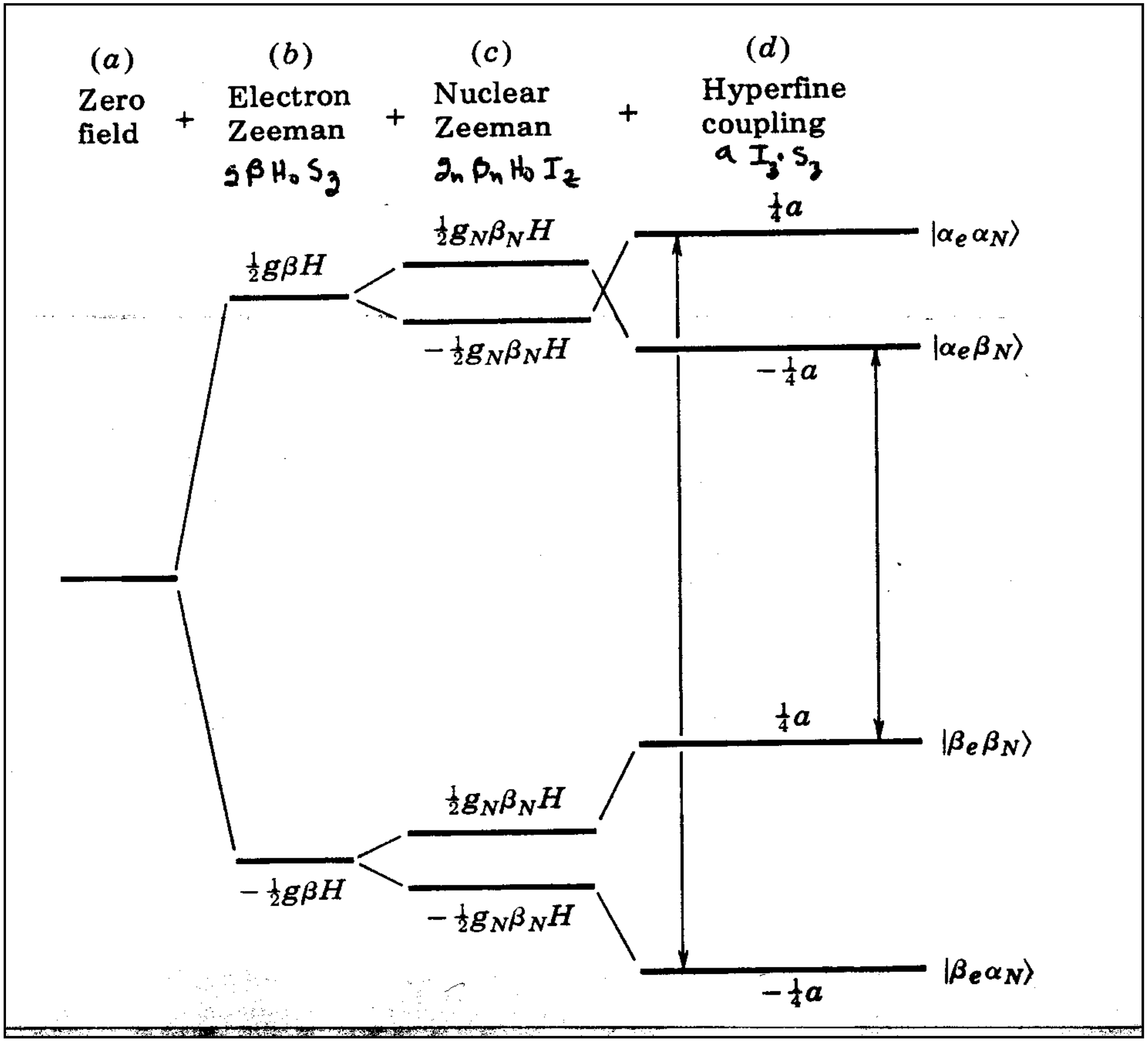
Electron Spins Precess
@ the Larmor Frequency
about the External Field
γ_e = 1.76e^7 rad/sec/G

Origin of the resonance signal

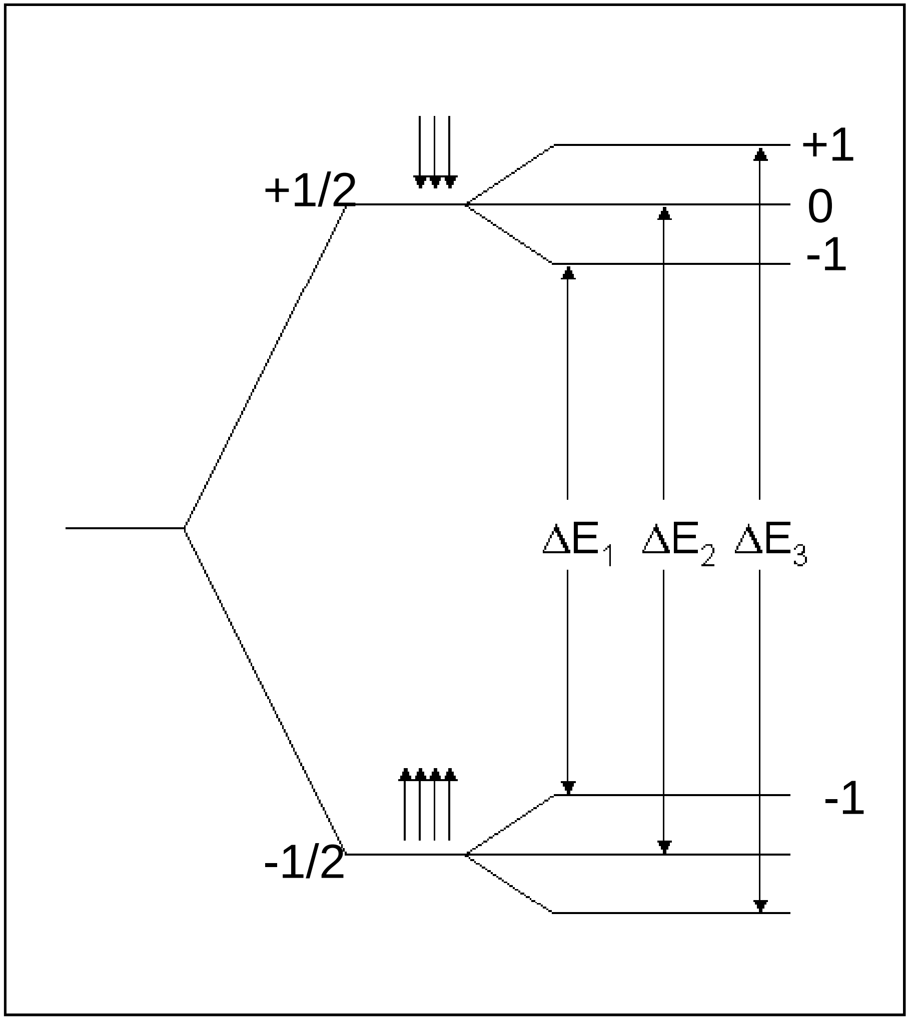


- Difference in population defined by Boltzman Distribution
- The concept of thermal equilibrium: Relaxation
- Saturation

Energy level Diagram for a ^{15}N Nitroxide
S=½; I=½



Approximate Energy Levels for a ^{14}N Nitroxide Spin Label; S =½, I=1



In general, EPR gives information on the # and nuclear spin state
of nearby nuclei –coordination sphere of metal centers in metalloproteins

Block Diagram of EPR Spectrometer

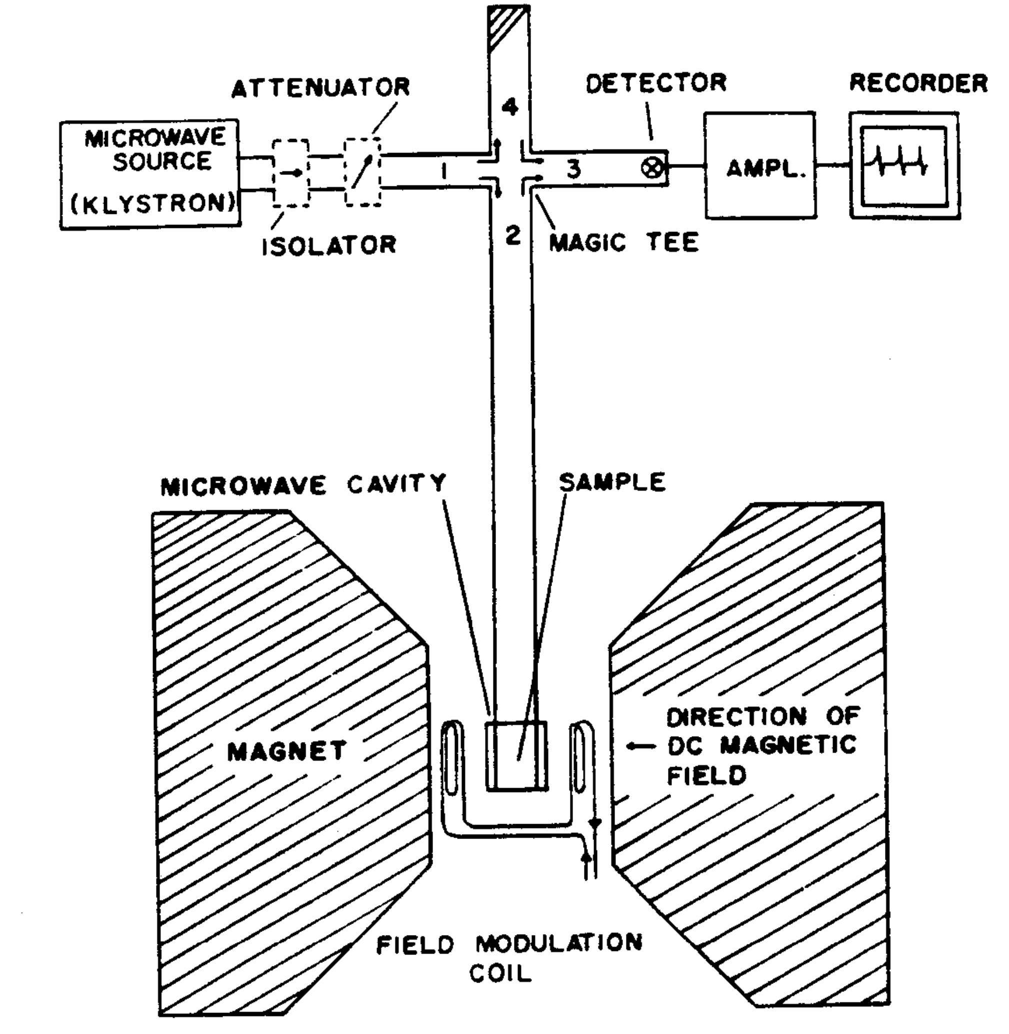
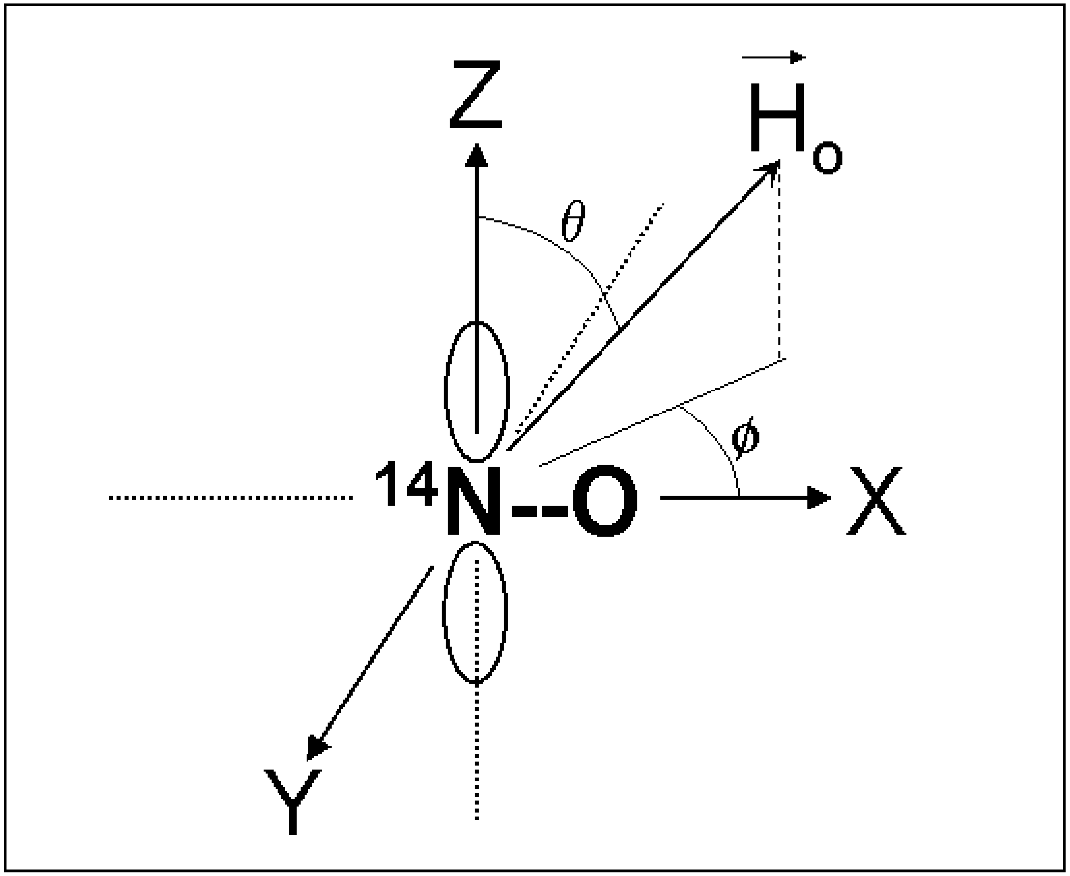


Fig. 1. A block diagram of a simple ESR spectrometer showing the essential components.

Solids
Liquids
Gases
High S/N

Most EPR
done @
X-band
9-10 GHz
3 kG Field

Nitroxide Reference Frame



g_{xx} = 2.0086
g_{yy} = 2.0056
g_{zz} = 2.0022
A_{xx} = 7 G
A_{yy} = 6 G
A_{zz} = 34 G

$$H_{res} = [h\nu/\beta_e g_{eff}(\theta,\phi)] + m_i A_{eff}(\theta,\phi)$$

$$g_{eff} = g_{xx}\sin^2\theta\cos^2\phi + g_{yy}\sin^2\theta\sin^2\phi + g_{zz}\cos^2\theta$$

$$A_{eff} = [A_{xx}{}^2\sin^2\theta\cos^2\phi + A_{yy}{}^2\sin^2\theta\sin^2\phi + A_{zz}{}^2\cos^2\theta]^{½}$$

Variation in the EPR Spectrum for a Single Nitroxide

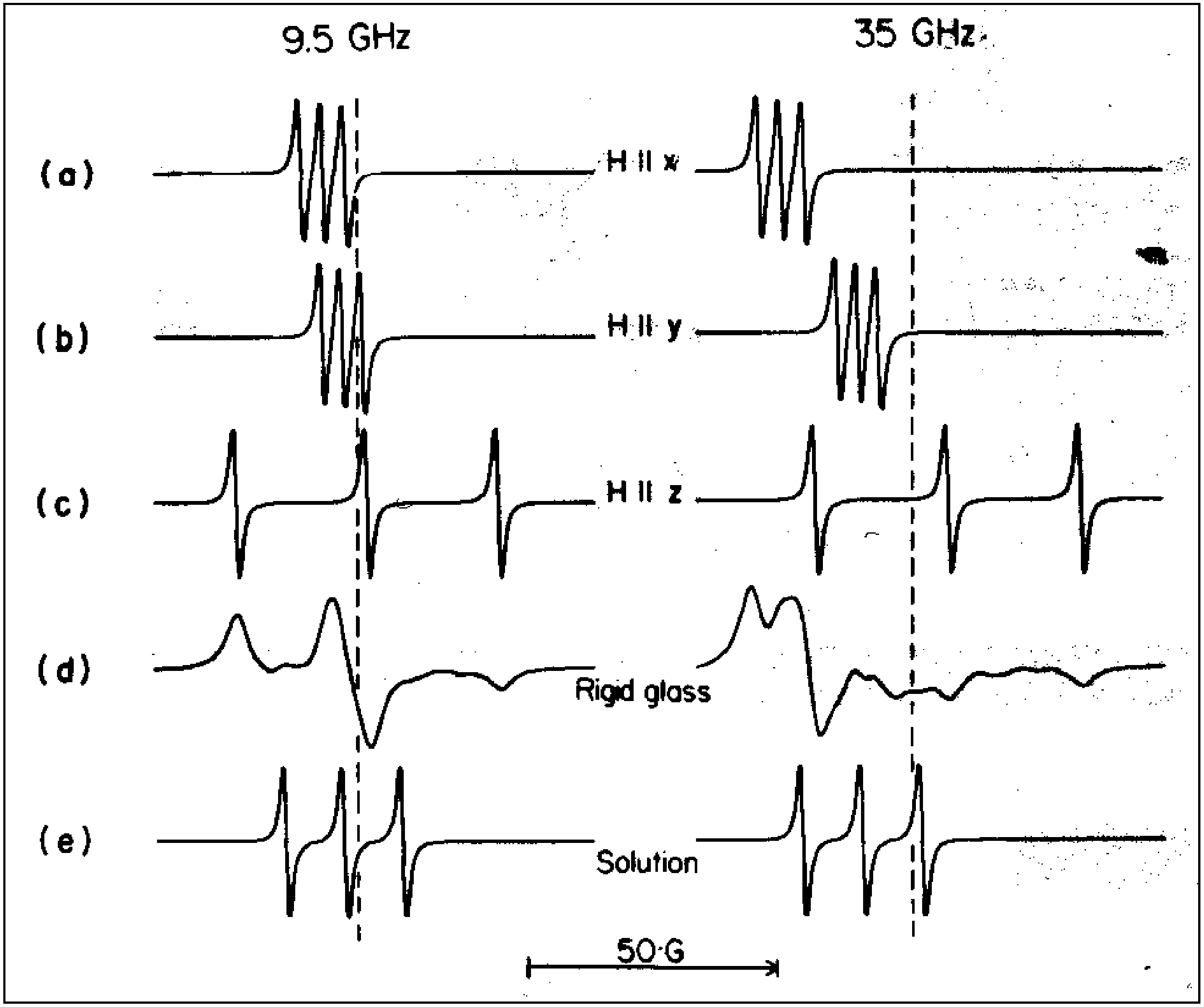
Single Crystal - x

Single Crystal - y

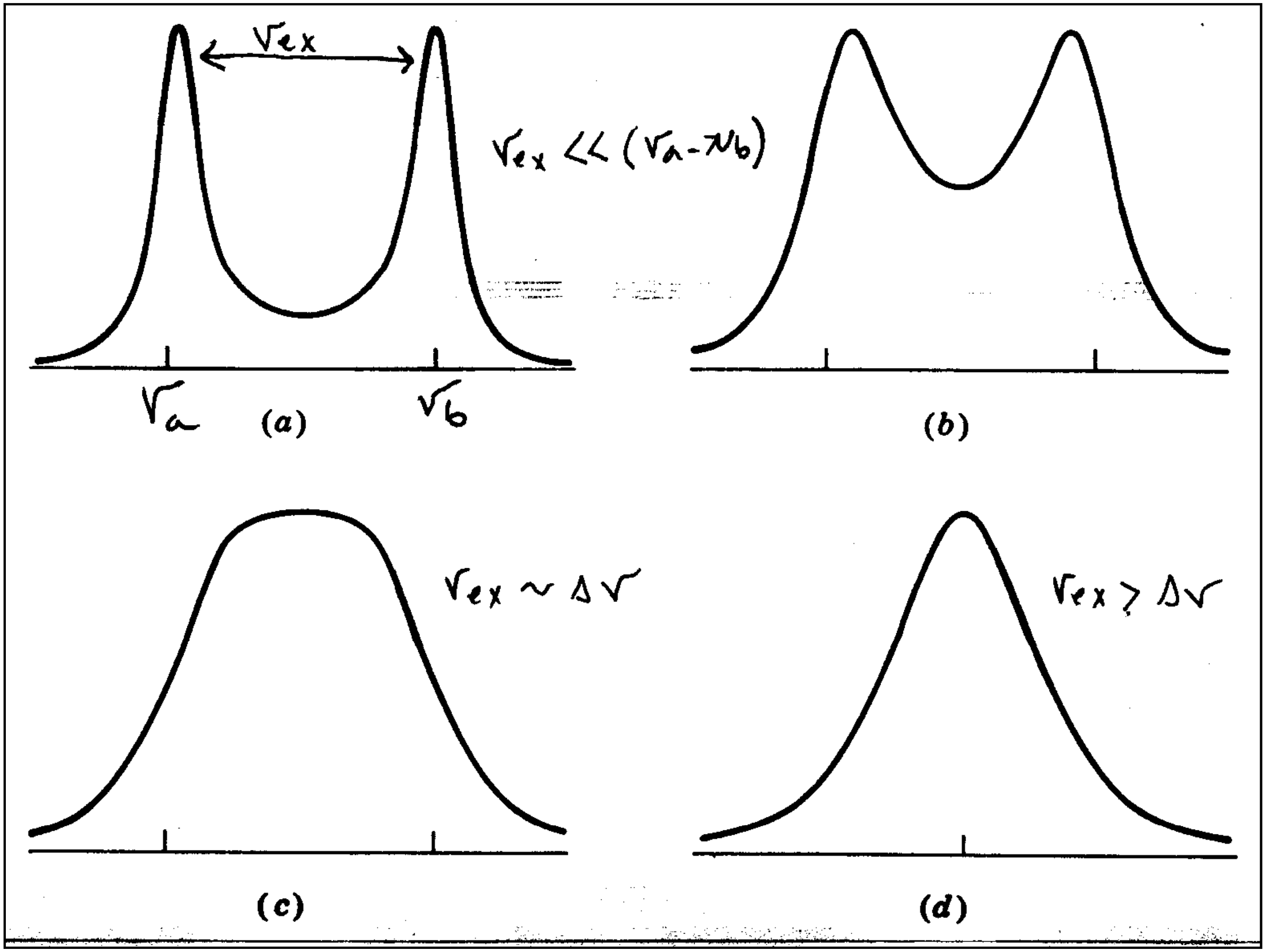
Single Crystal - z

Polycrystalline

Crystal Dissolved
in Water



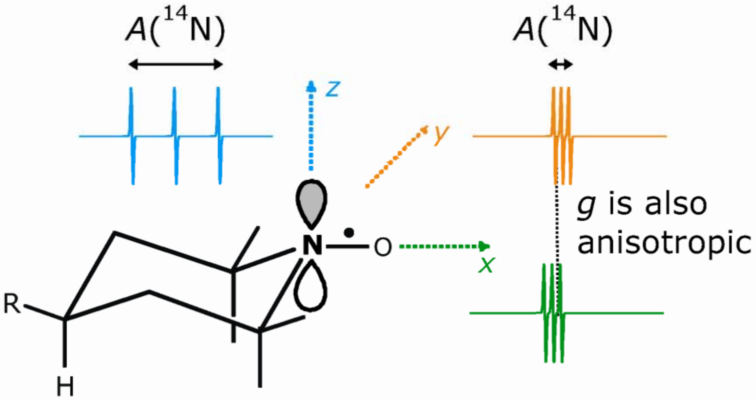
Two-Site Exchange Model
Effects of Motion on EPR Spectra

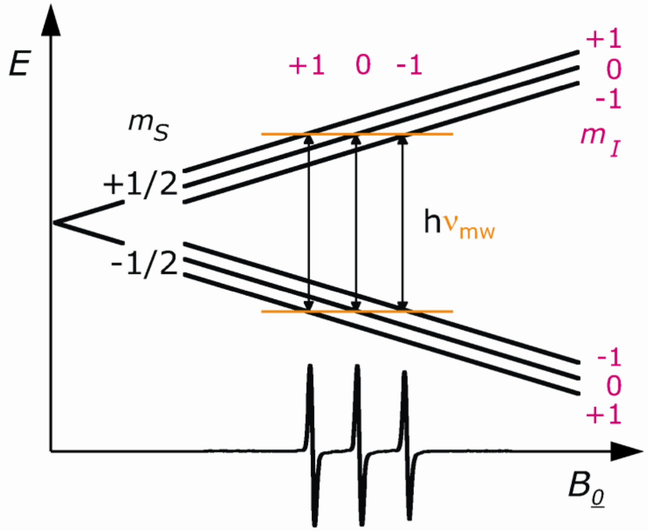


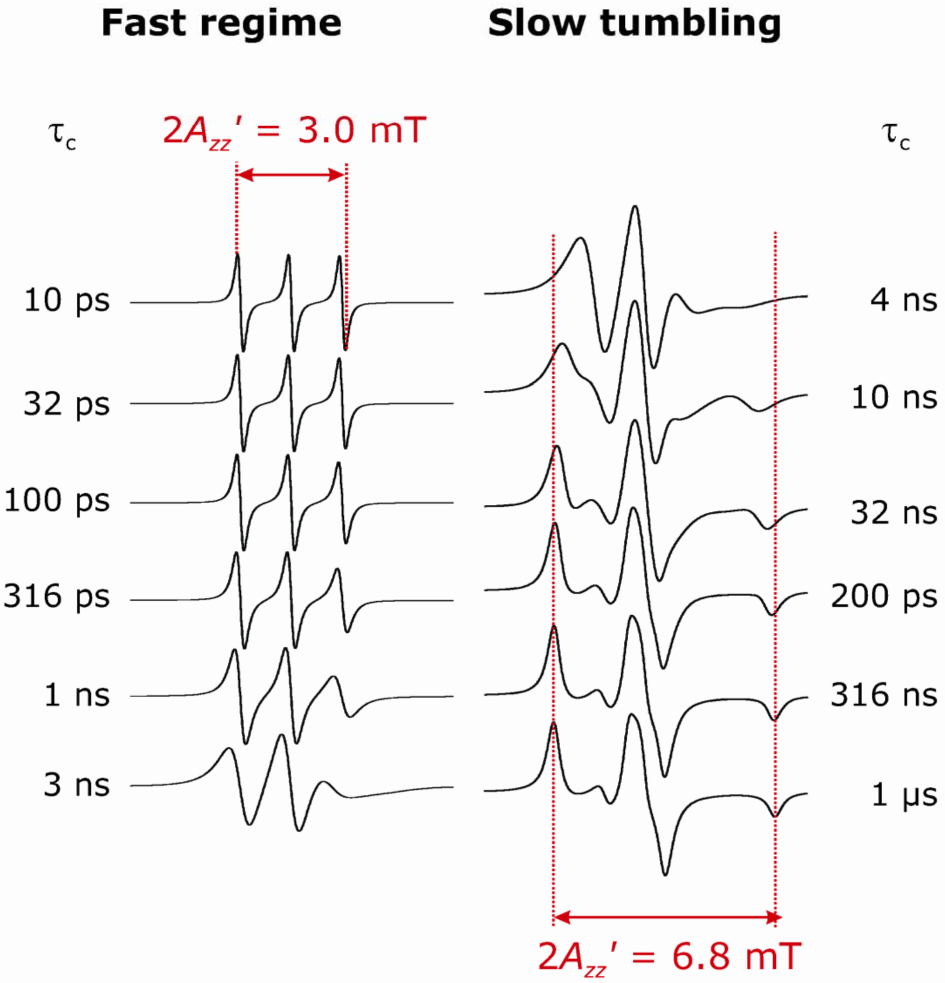
(a) (b)

(c) (d)

Rotational correlation times

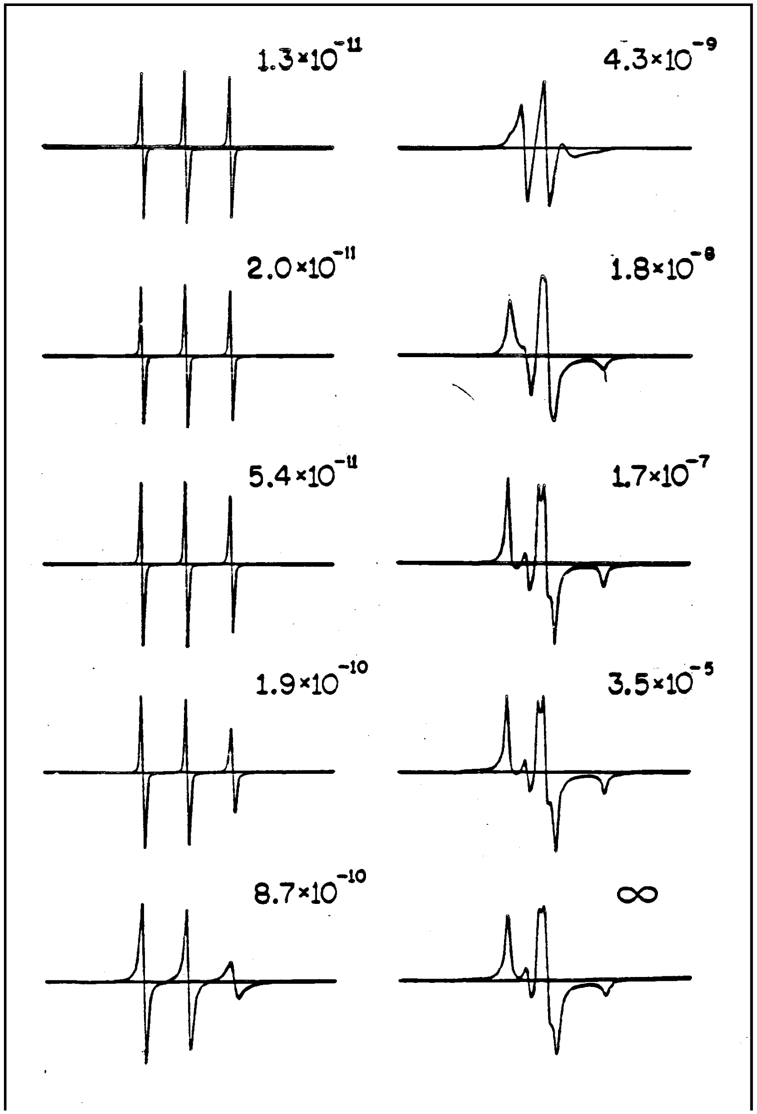






EPR Spectra as a Function of Rotational Correlation Time; $\tau_r = 1/6D_r$

$\tau_r = 4\pi\eta r_h^3 / 3kT$

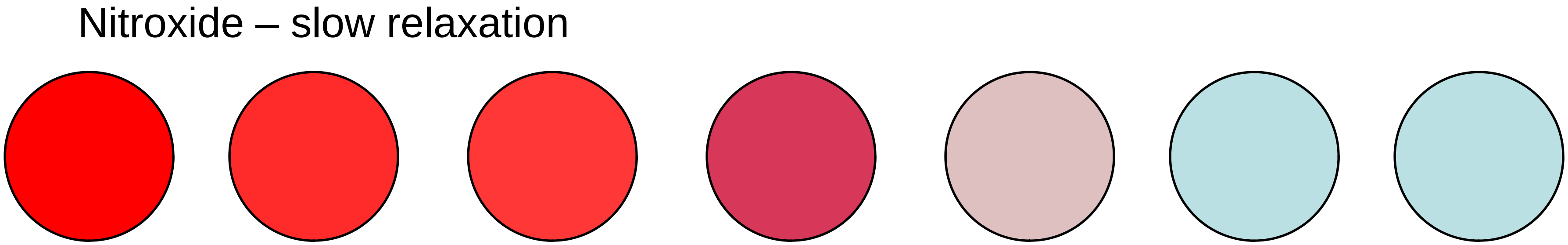


Small Protein - EGF

Nitroxide in $H_2$0

Large Protein – 1 MDa

Electron Spin-Spin Relaxation - Accessibility

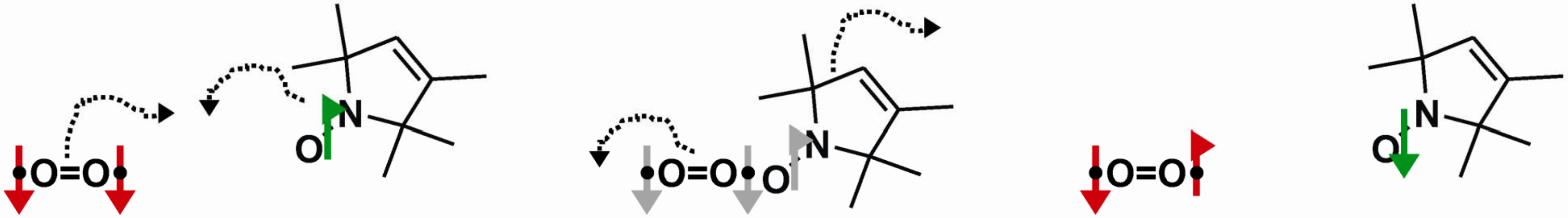


T_{1e}'s of isolated Nitroxide spin labels are on the order of a few μsec's at physiological temperatures

Relaxation via Collisional Exchange

Diffusing paramagnetic species

before **during** **and after**



observer spin up ↑

electrons in overlapping
orbitals are indistinguishable

observer spin down ↓

collision

⇒ relaxation time T_1 decreases with increasing exchange rate W_{ex}

- most easily detected via saturation curves (CW EPR)

$$\Delta P_{1/2} \propto W_{ex}/T_{2e}$$

ALTENBACH et al., *Proc. Natl. Acad. Sci. USA* **91**, 1667-1671 (1994)