Introduction to Electron Paramagnetic Resonance Spectroscopy

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EPR Spectroscopy

EPR is magnetic resonance on unpaired electrons

Species that can be studied by EPR:

- free radicals
- transition metals with odd numbers of electrons or high spin
- excited states with $S \neq 0$ *e.g.* triplet states

Molecules with all electrons paired have no electron magnetic moment → no EPR spectrum.

Bioinorganic EPR

- The metals in metalloproteins usually do redox chemistry and are the active sites of the protein.
- The redox states are often paramagnetic.

  (two general classes: $S = ½$, $S > ½$)
- These states can be studied by EPR
- No background signals from the rest of the protein or sample.

Examples: Iron-sulfur proteins, heme and non-heme iron proteins, iron-nickel proteins, copper proteins

Outline

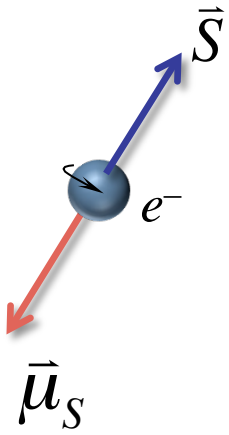
- Basics of the EPR experiment
- EPR in proteins at low temperature (S = ½)
  - g-anisotropy, single crystals, powder patterns
  - The hyperfine interaction
- Couplings between electrons, Zero Field Splitting (S > ½)
- High spin systems and Rhombograms

References

- Hagen (2009) “Biomolecular EPR Spectroscopy”, CRC Press
- Brustolon and Giamello (2008) “Electron Paramagnetic Resonance: A Practitioner's Toolkit” Wiley
- Weil and Bolton (2007) “Electron Paramagnetic Resonance: Elementary Theory and Practical Applications” Wiley
- Golbeck and van der Est (2013) in “Molecular Biophysics for the Life Sciences” Allewell, Narhi and Rayment Eds.

Basics of EPR

Electrons have spin angular momentum $\vec{S}$ which generates a magnetic dipole moment $\vec{\mu}_S$.



$$\mu_S = g_e \beta_e \sqrt{s(s+1)}$$

β_e = Bohr magneton
g_e = free electron g-value
s = spin angular momentum quantum number

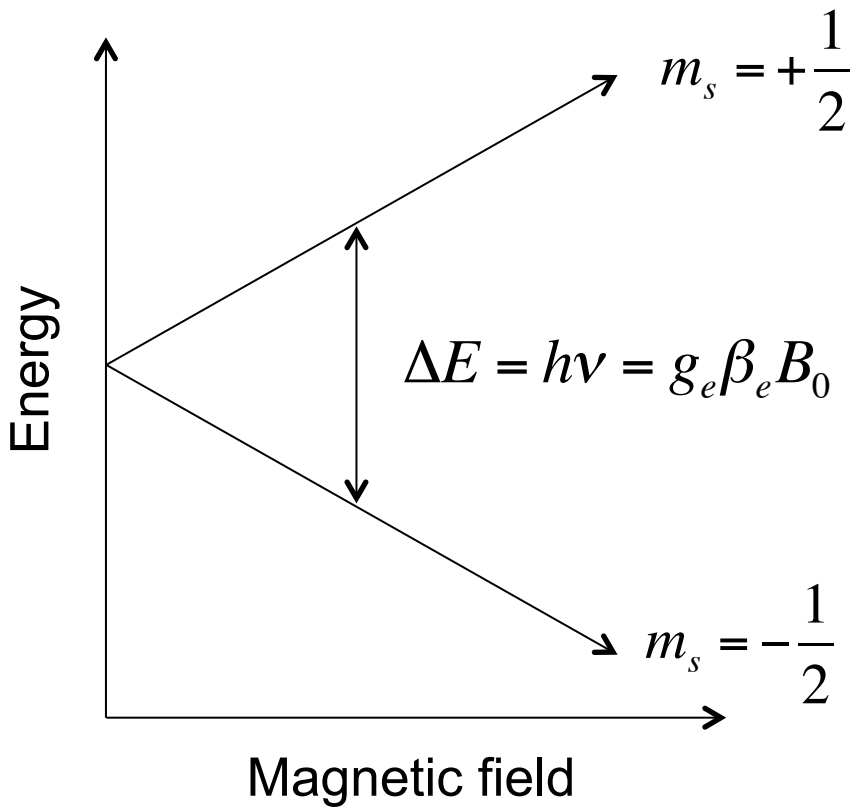
$$g_e = 2.002319 \qquad \beta_e = 9.27 \times 10^{-24}\, J/T$$

EPR Experiment

In a magnetic field the spin states are split by the Zeeman interaction.

Transitions with $\Delta m_s = \pm 1$ are allowed in an EPR experiment.

$$\frac{g_e \beta_e}{h} = 28.02 GHz / T$$



Comparison with NMR spectroscopy

The resonance frequency for a free electron is about 600 times larger than for a proton in the same magnetic field:

300 MHz ^{1}H NMR → 180 GHz EPR

180 GHz = 6 cm^{-1} microwave/far infrared

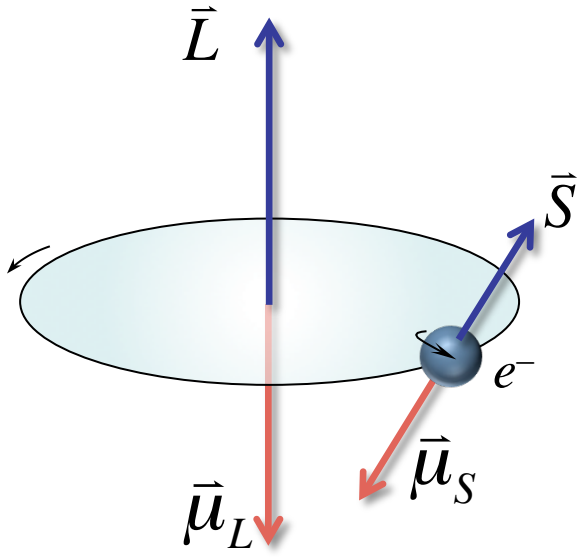
Couplings involving electrons are generally much stronger this leads to much broader spectra:

NMR: 1 Hz – 100 kHz

EPR: 1 MHz – several GHz

Basics of EPR

In atoms and molecules the electrons have both orbital and spin angular momentum. Each of these generates a magnetic dipole moment.



$$\mu_L = \beta_e \sqrt{l(l+1)}$$

$$\mu_S = g_e \beta_e \sqrt{s(s+1)}$$

Basics of EPR

The magnetic moment of a bound electron is determined by its total angular momentum $\vec{J}$

$$\mu = g\beta_e\sqrt{J(J+1)}$$

In molecules, the orbital angular momentum is generally quenched.

$\rightarrow \vec{J} \approx \vec{S}, \quad g \approx g_e$

Some residual orbital angular momentum remains

→ Exact g-value depends on the spin-orbit coupling:

Examples.

Cu(II) in $Cu(acac)_2$	g=2.13
Ti(III) ions in solid TiO_2	g=1.96

Choice of Field and Frequency

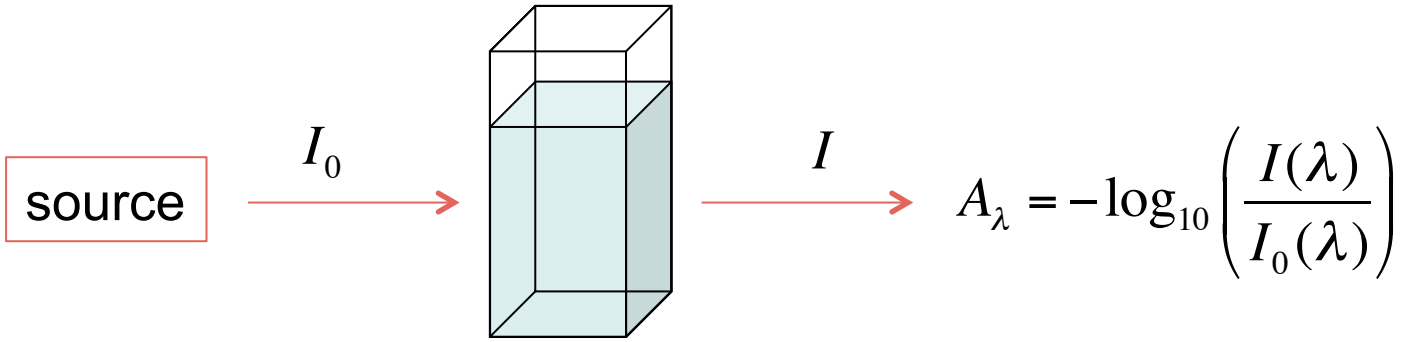
Commercially available spectrometers:

Frequency (GHz)	Frequency Band	Field for g=2.0023 (T)
1.2	L	0.043
2.4	S	0.086
9.5	X	0.34
34	Q	1.2
95	W	3.4
263	mm-band	9.4

X-band spectrometers are by far the most common.

The EPR Experiment

In most spectroscopic experiments the absorbance is measured as a function of frequency.

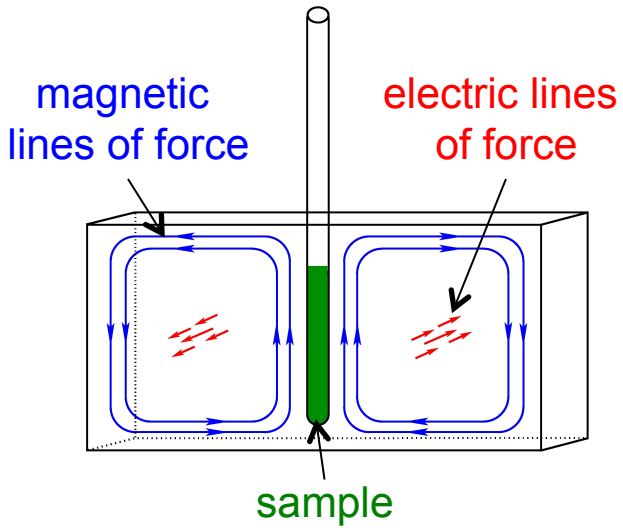


In an EPR experiment the absorbance is very weak and this method is only feasible at very high magnetic fields.

The EPR Experiment

To overcome the problem of weak signals a resonator is used:

- The sample is placed in a resonant cavity such that it sits in the magnetic component of the resonant microwave field



Many other resonator designs are possible. Each has its advantages

The EPR Experiment

The microwaves are usually brought to the resonator using a waveguide

An “iris” is placed at the entrance to the resonator to couple it.

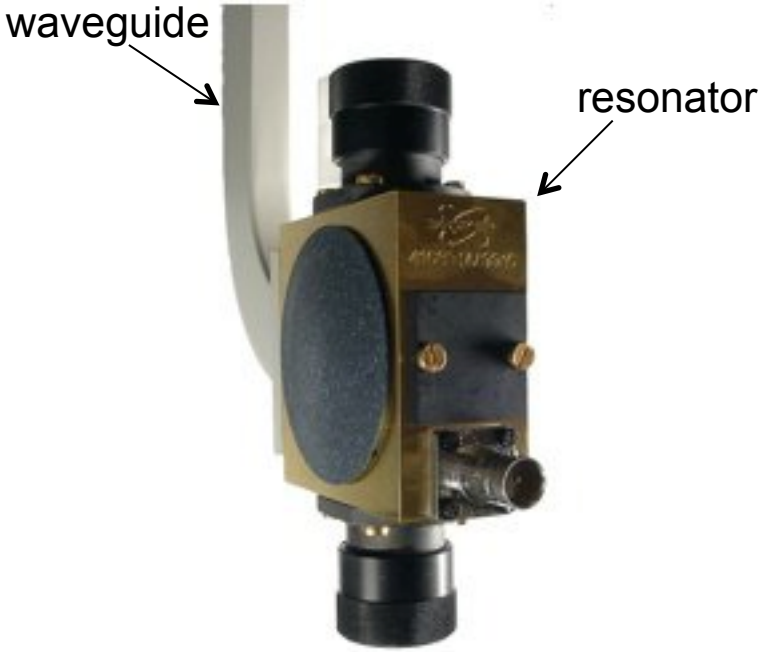
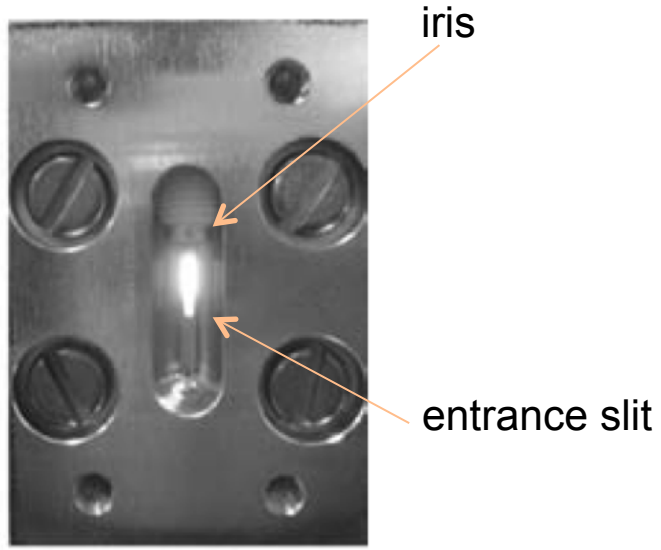


Image: Buker ER 4103TM cylindrical mode resonator
http://www.bruker.com/typo3temp/pics/e_75d2de1d39.jpg

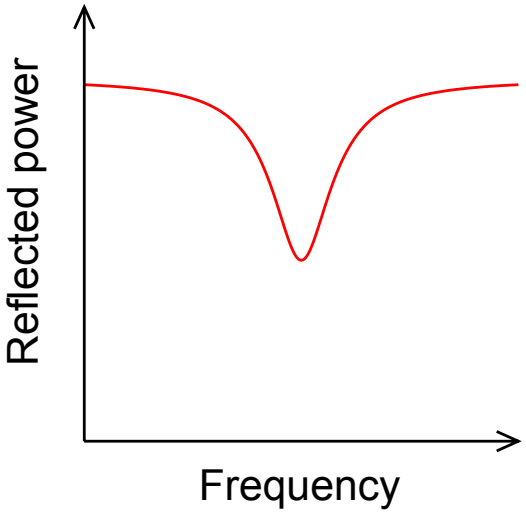


Hagen “Biomolecular EPR Spectroscopy” Fig. 2.6

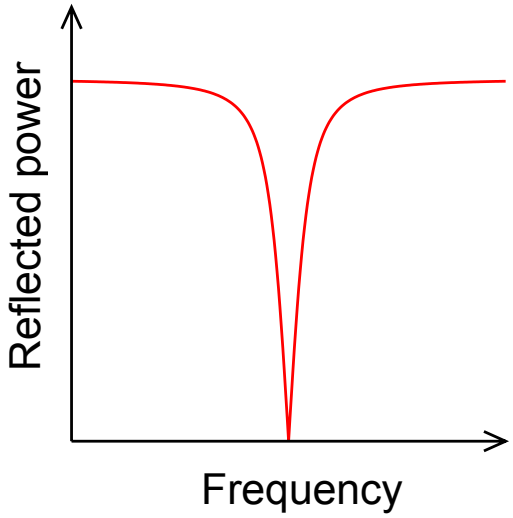
EPR Cavity Coupling

By adjusting the shape of the iris, the source is critically coupled to the cavity so no power is reflected.

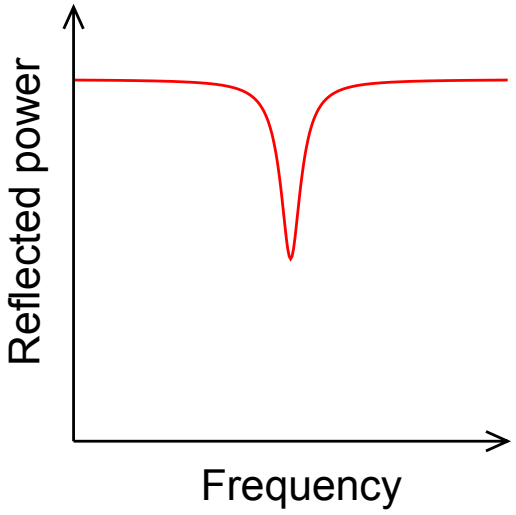
Over coupled



Critically coupled



Under coupled



The EPR Experiment

- When an EPR transition occurs in the sample, the resonance is disturbed and power is reflected
- The reflected power gives a stronger signal than directly measuring the absorbance of the sample

EPR Spectrometer

Typical resonator bandwidth: ~1-10 MHz

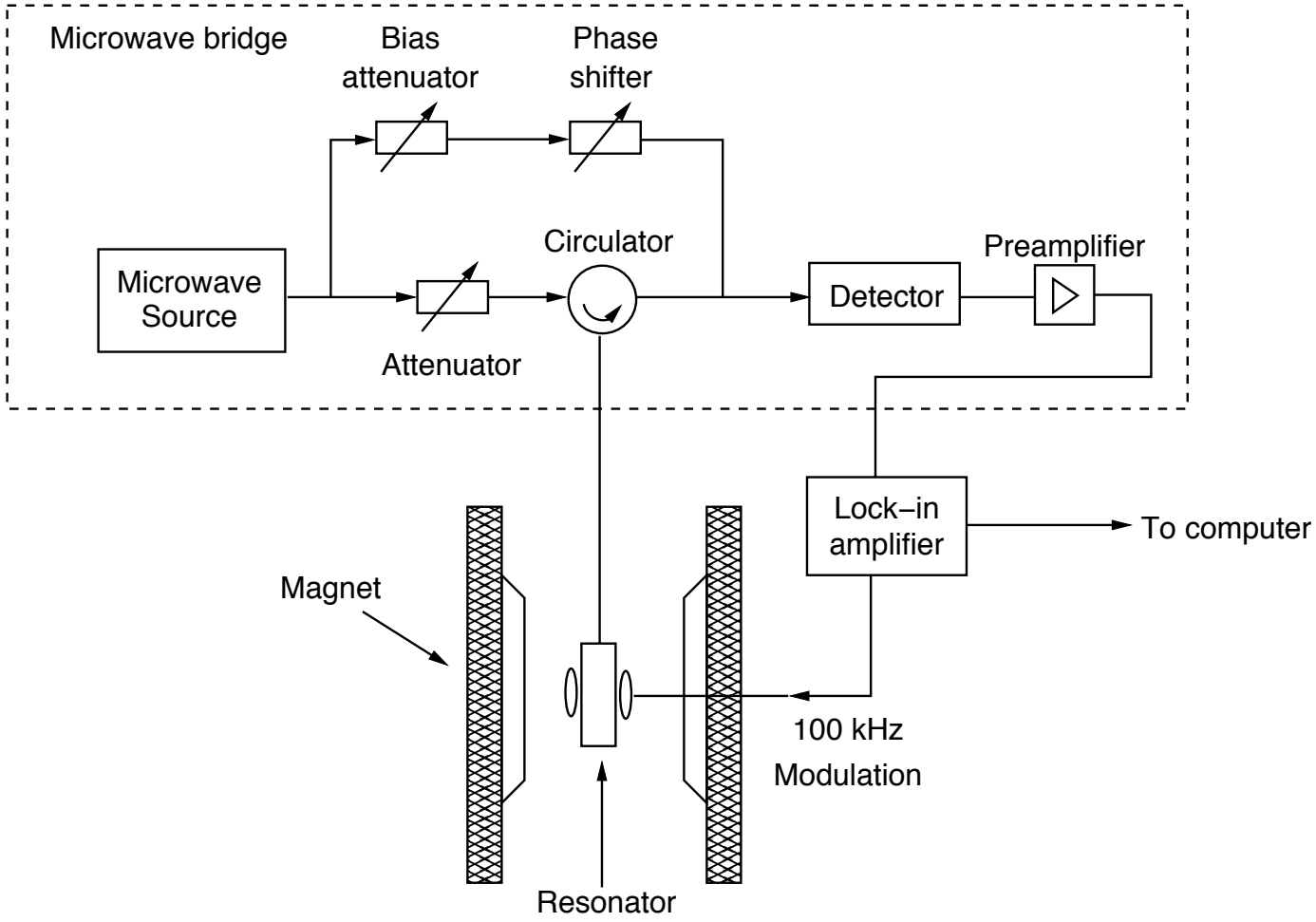
Spectral width: up to several GHz

Net result:
Cannot sweep the frequency.

Therefore EPR spectrometers typically use electromagnets and the microwave absorption is monitored as the field is varied.

Image: Bruker EMX EPR Spectrometer from Physikalische Technische Bundesanstalt http://www.ptb.de/de/org/6/62/624/bilder/apparat03.jpg

Schematic Diagram of an EPR Spectrometer



Field modulation technique:

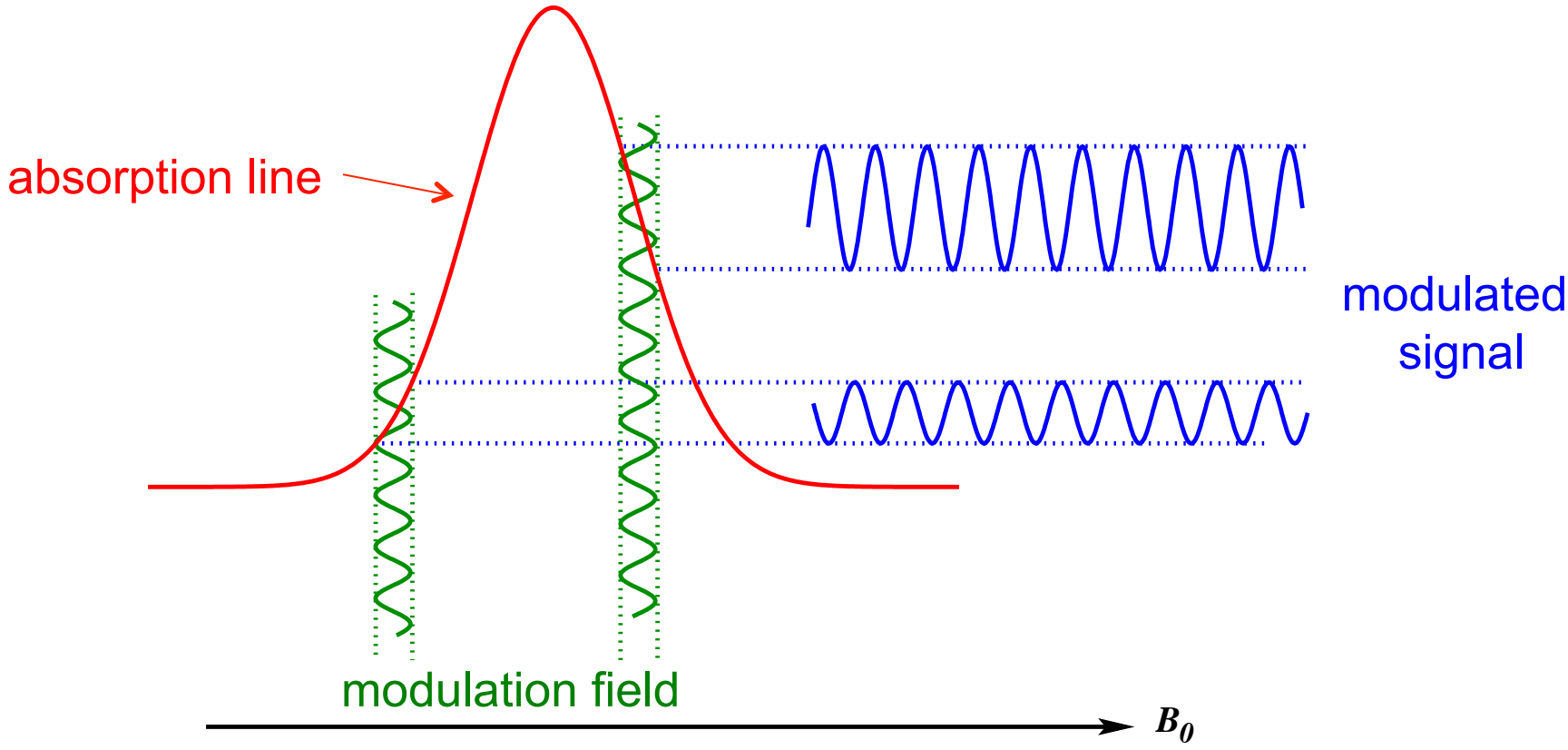
Even with a resonator the signals are still very noisy. So a different detection scheme is used.

To improve signal to noise, a small modulation field is added to the main magnetic field

The modulation coils are placed on the sides of the resonator

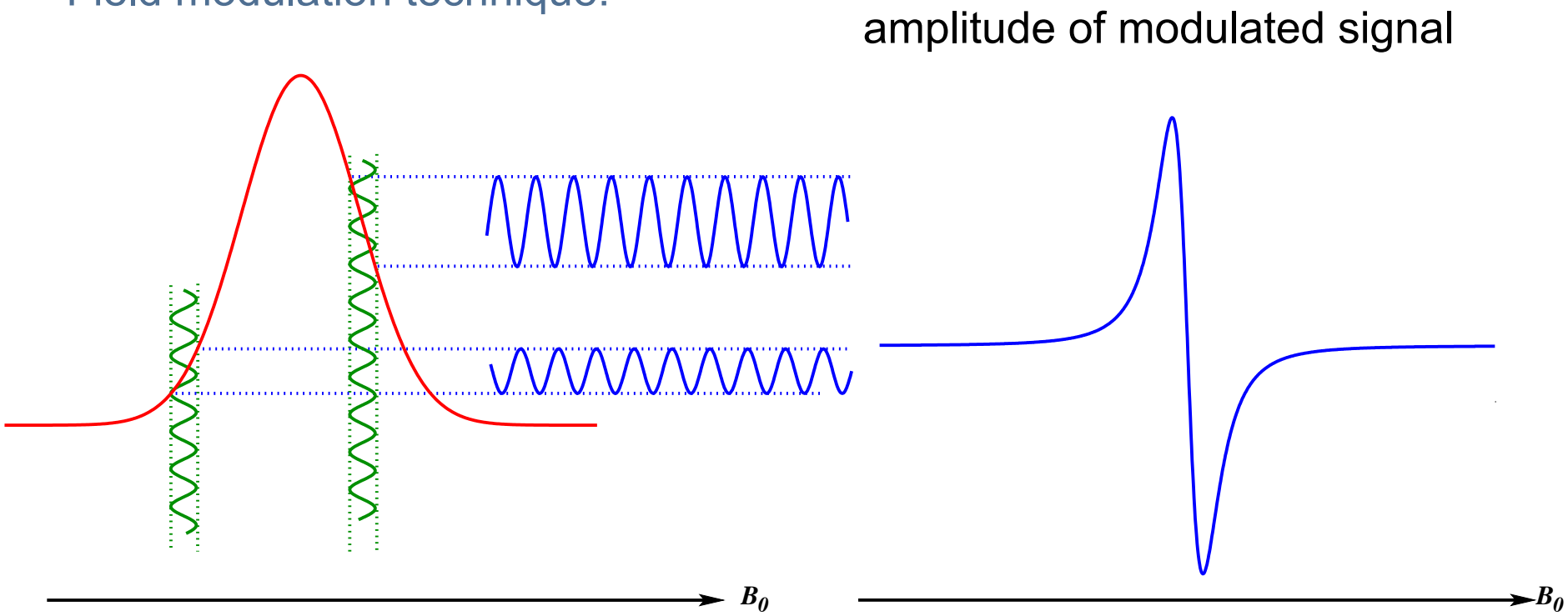
Image: Buker ER 4103TM cylindrical mode resonator
http://www.bruker.com/typo3temp/pics/e_75d2de1d39.jpg

Field modulation technique:



The amplitude of the modulated signal is measured and its phase is compared to a reference signal

Field modulation technique:



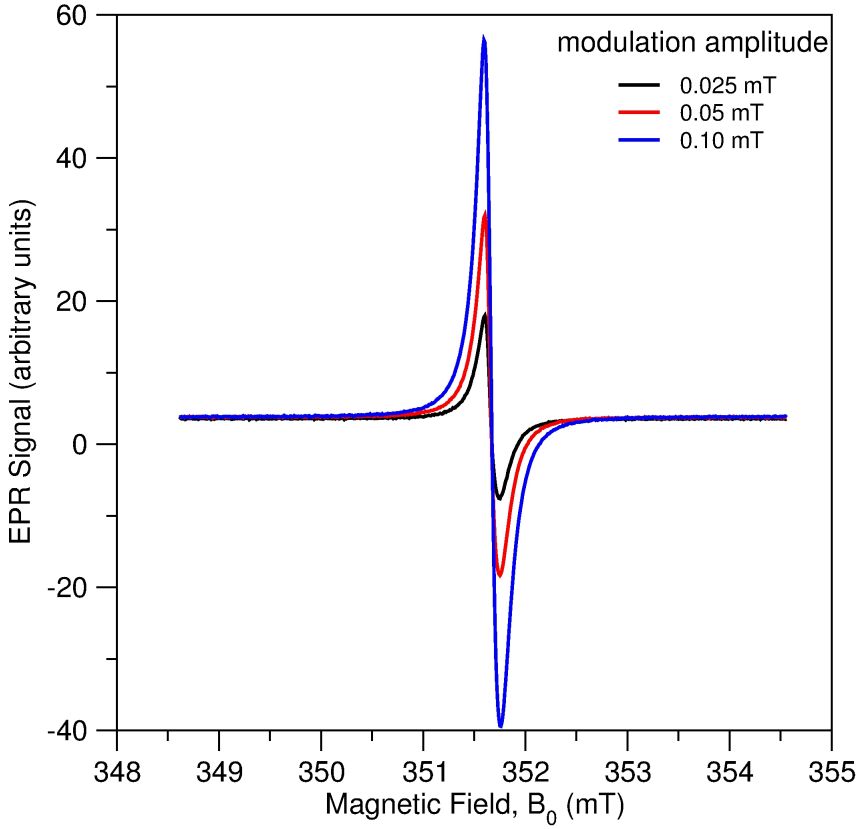
The amplitude of the modulated signal plotted as the EPR spectrum.

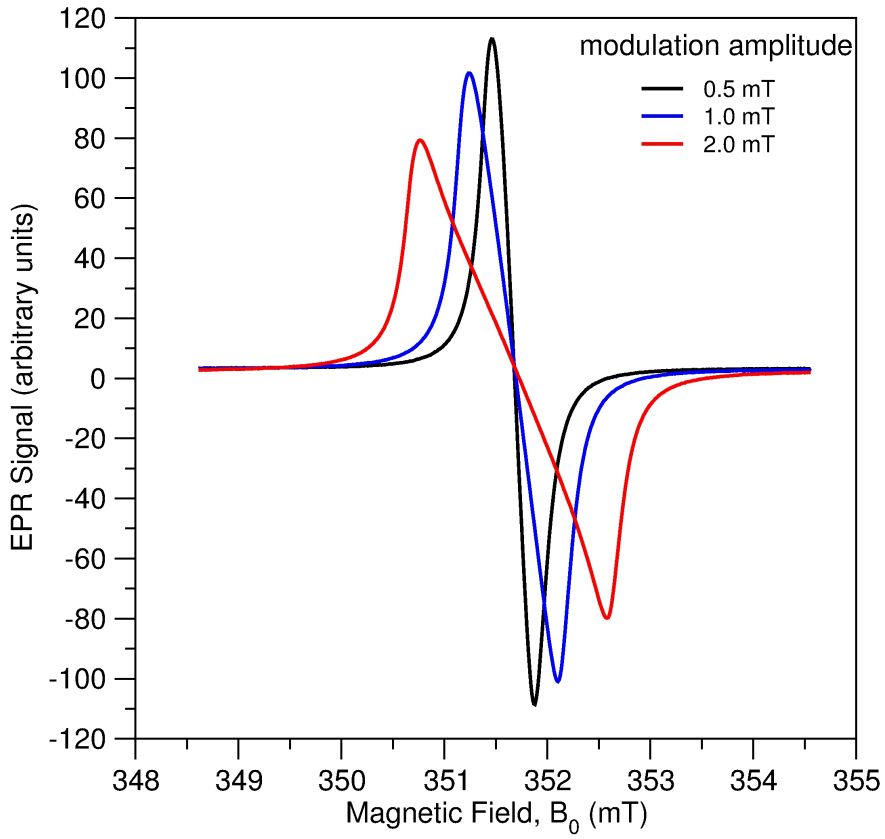
Field modulation technique

Two drawbacks:

The first derivative of the spectrum is obtained

The signal amplitude and shape depends on the modulation amplitude

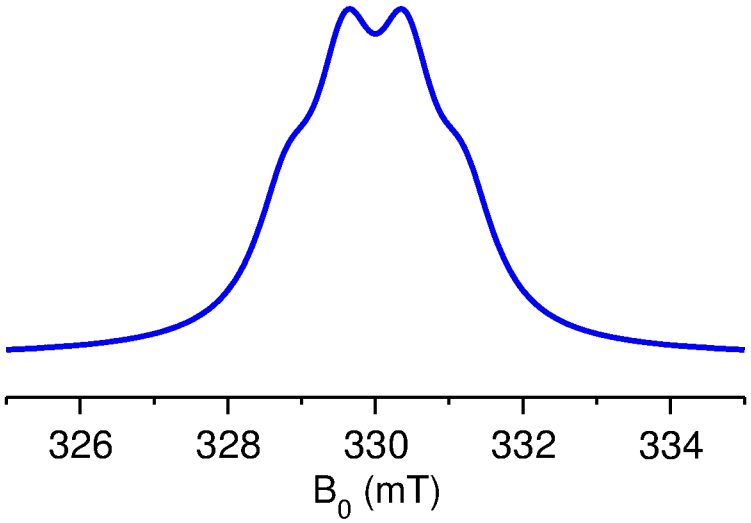




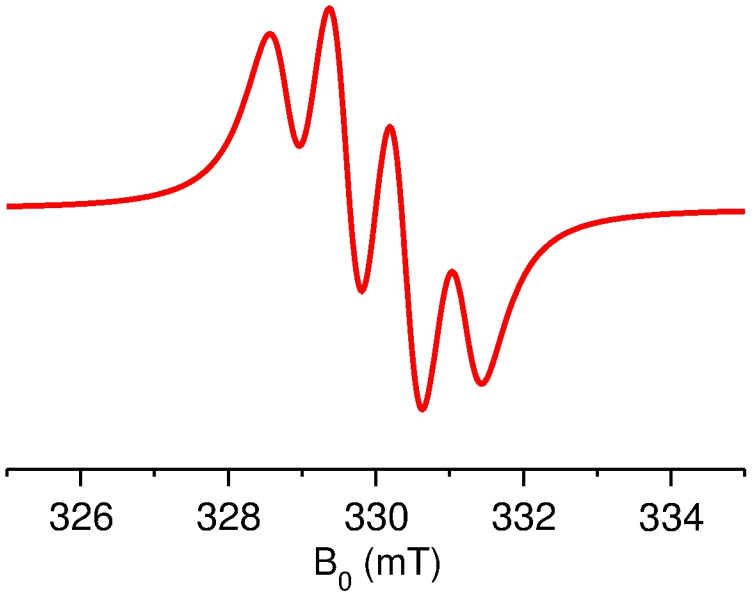
Field modulation technique

Main advantages:
Much better signal to noise
Structure of spectrum is emphasized in first derivative

absorption spectrum



first derivative



Saturation and Relaxation

The population difference between the spin states is small:

$$N_\alpha / N_\beta = \exp(-g_e \beta_e B_0 / kT)$$

$\Delta N / N = 10^{-3}$ for $B_0 = 330$ mT at 298 K

The EPR signal strength depends on the population difference.

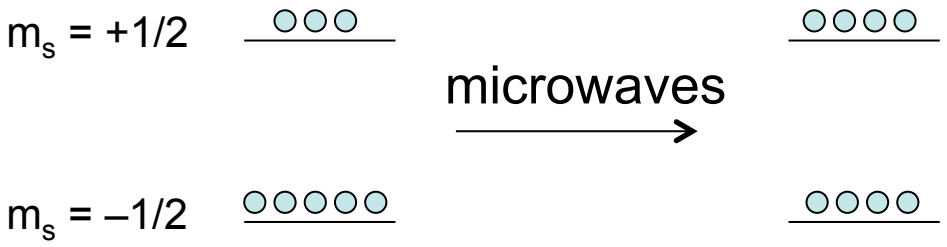
$m_s = +1/2$ ○○○

$m_s = -1/2$ ○○○○○

At low temperature the difference is larger.

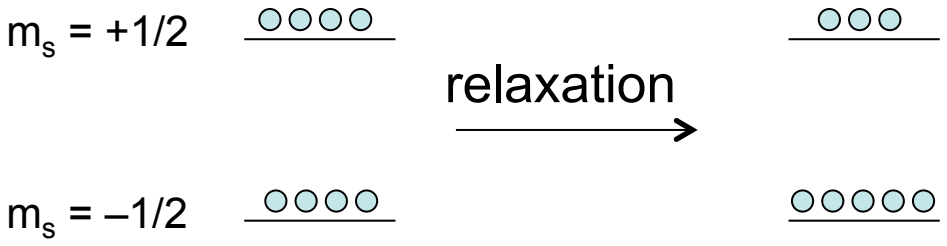
Saturation and Relaxation

EPR transitions tend to equalize the populations:



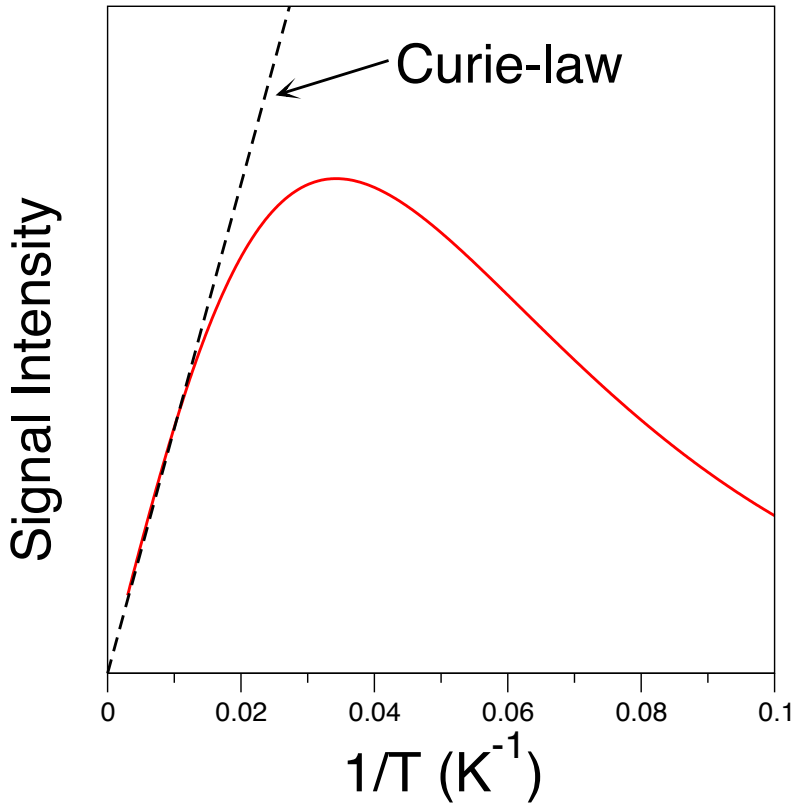
no EPR signal!

The population difference is restored by spin relaxation:

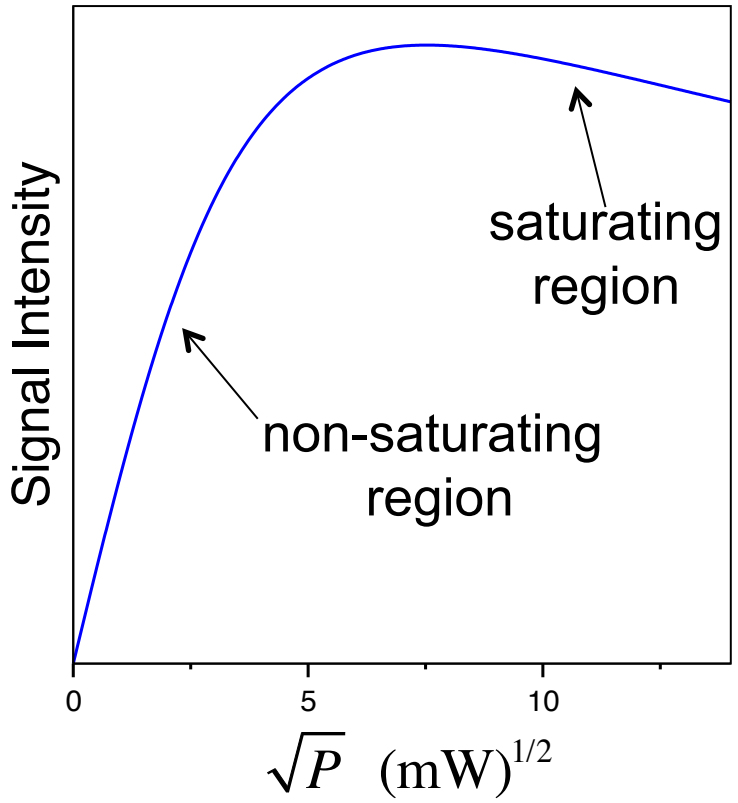


Factors influencing signal intensity

Temperature



Microwave power



Factors influencing signal intensity

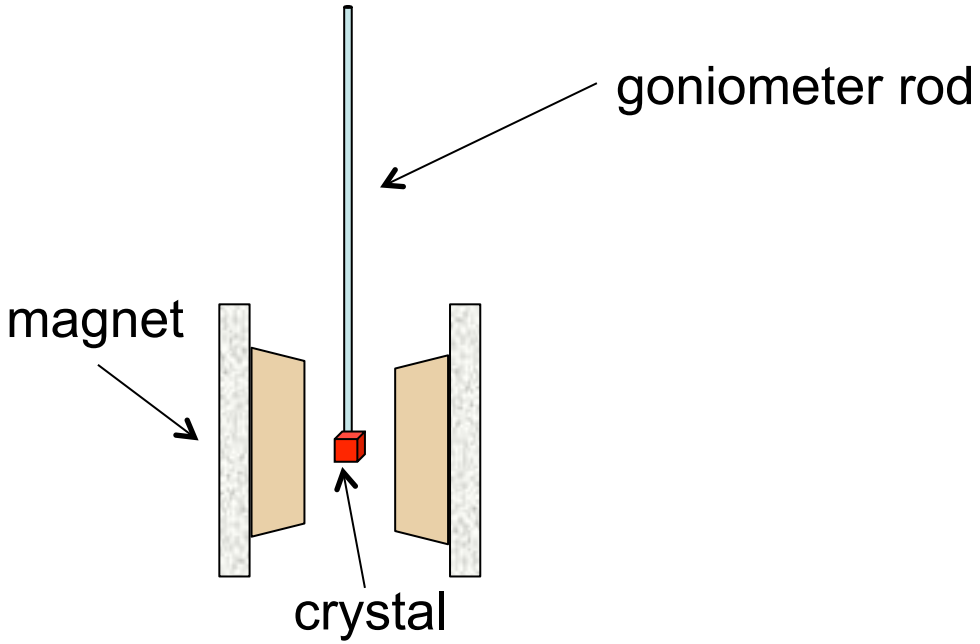
For each sample there is an optimal microwave power and temperature.

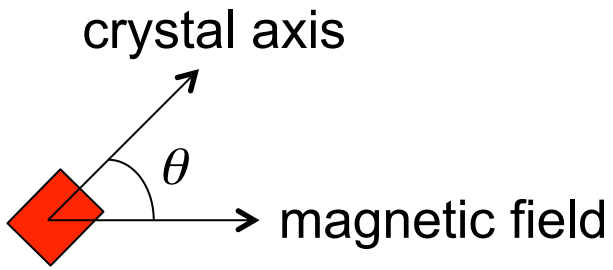
Metalloproteins are usually measured at low temperature.

This means the orientation dependence of the g-values is important.

Single crystal EPR

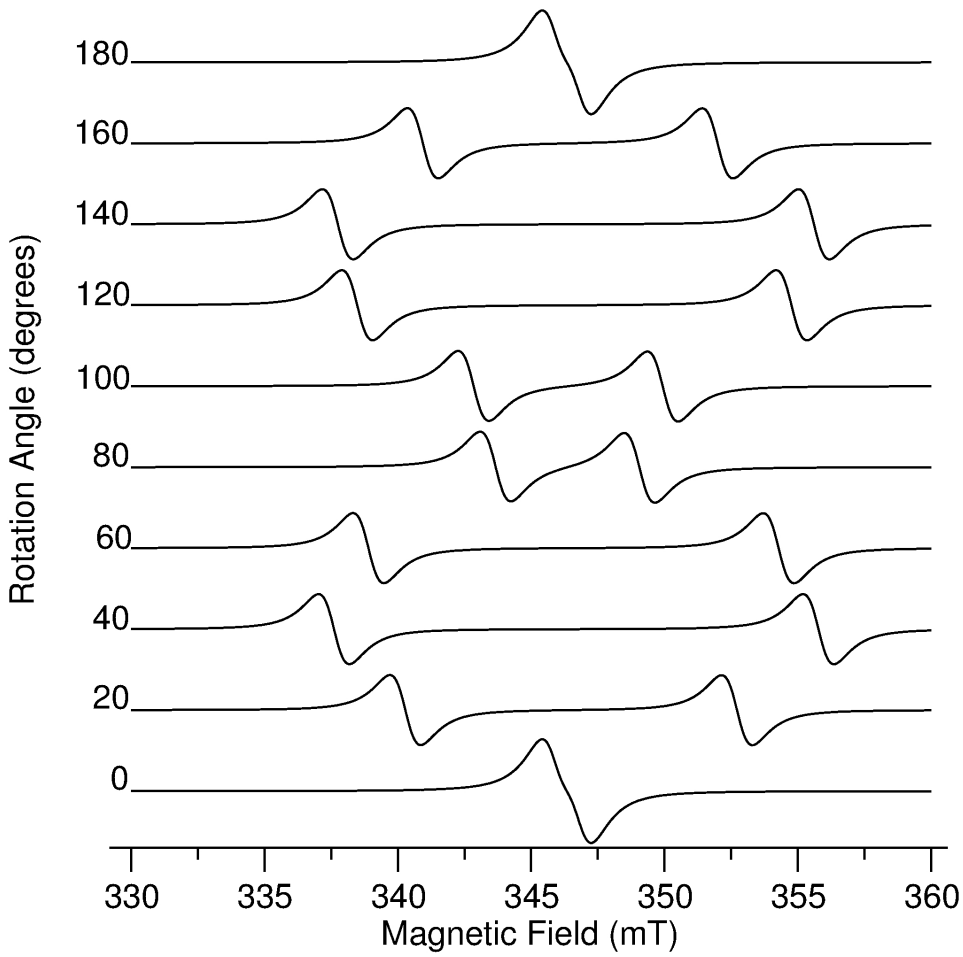
The orientation dependence of the spectra can be studied in single crystals





Single crystal EPR

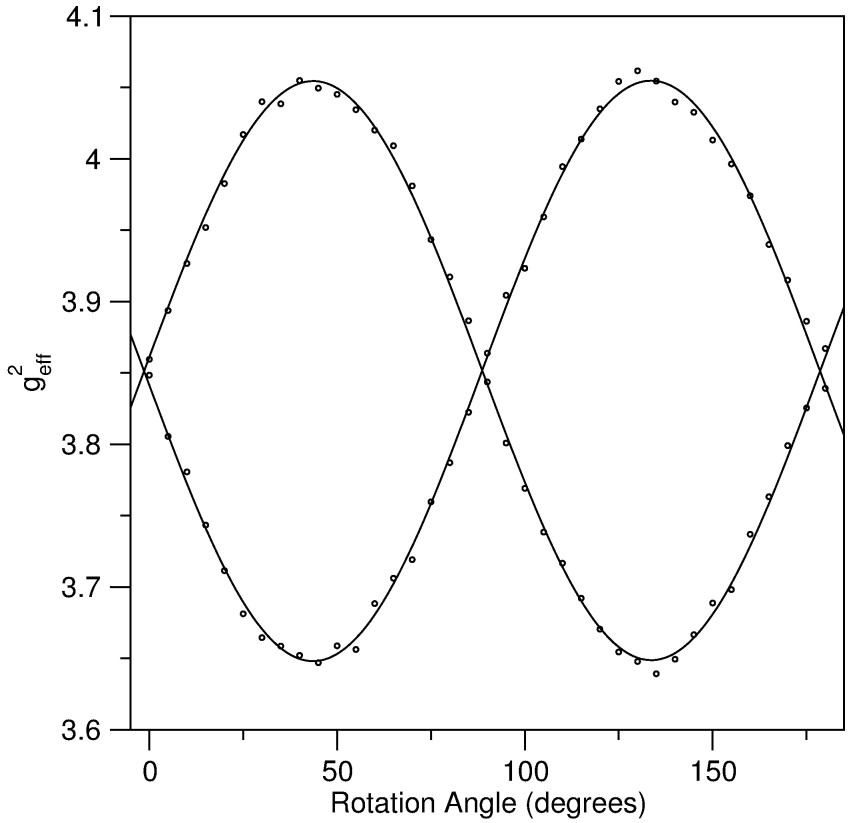
A series of spectra are collected at different orientations …



Single crystal EPR

The g-values of the lines are fitted to the equation:

$$g_{eff}^2 = g_{aa}^2 \cos^2\theta + 2g_{ab}^2 \cos\theta \sin\theta + g_{bb}^2 \sin^2\theta$$



Rotation in 3 independent planes gives values of

$$g_{aa}^2, g_{bb}^2, g_{cc}^2, g_{ab}^2, g_{ac}^2, g_{bc}^2$$

Single crystal EPR

The g-tensor is then diagonalized numerically

$$\begin{bmatrix} g_{aa}^2 & g_{ab}^2 & g_{ac}^2 \\ g_{ab}^2 & g_{bb}^2 & g_{bc}^2 \\ g_{ac}^2 & g_{bc}^2 & g_{cc}^2 \end{bmatrix} \longrightarrow \begin{bmatrix} g_{xx}^2 & 0 & 0 \\ 0 & g_{yy}^2 & 0 \\ 0 & 0 & g_{zz}^2 \end{bmatrix}$$

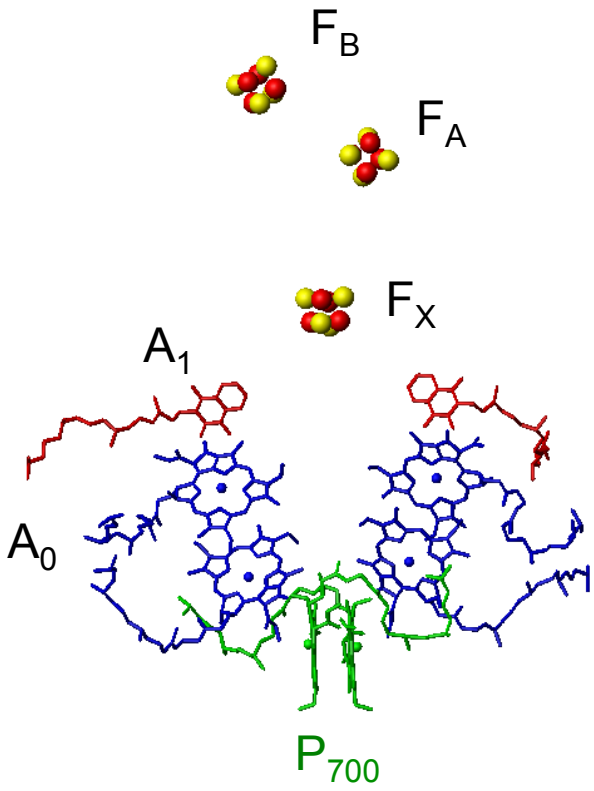
this gives the principal g-values g_{xx}, g_{yy} and g_{zz}.

The diagonalization is achieved by the transformation:

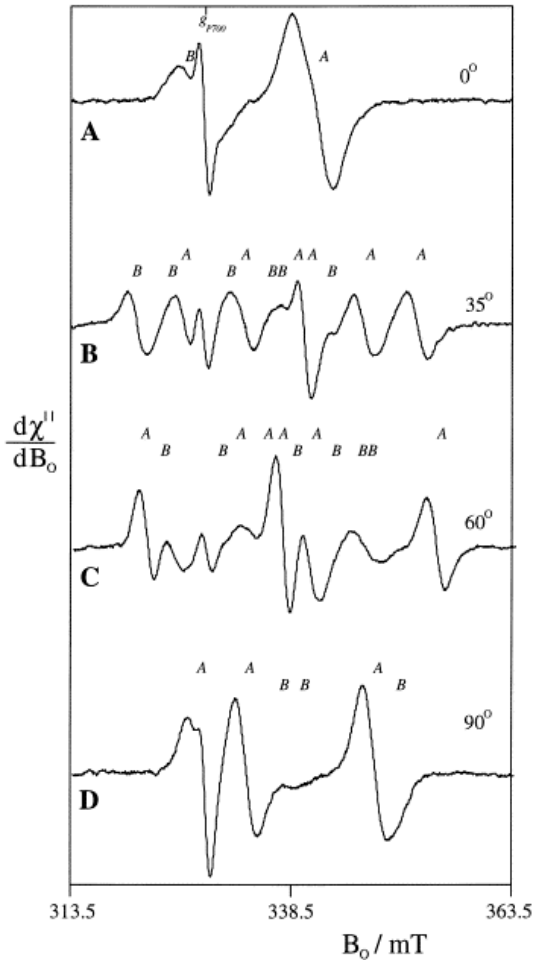
$$\boldsymbol{U}\boldsymbol{g}^2\boldsymbol{U}^{-1} = \boldsymbol{g}_{diagonal}^2$$

The transformation matrix $\boldsymbol{U}$ gives the orientation of the principal axes x,y,z in the crystal axis system a,b,c

Example Iron Sulfur Clusters in Photosystem I:

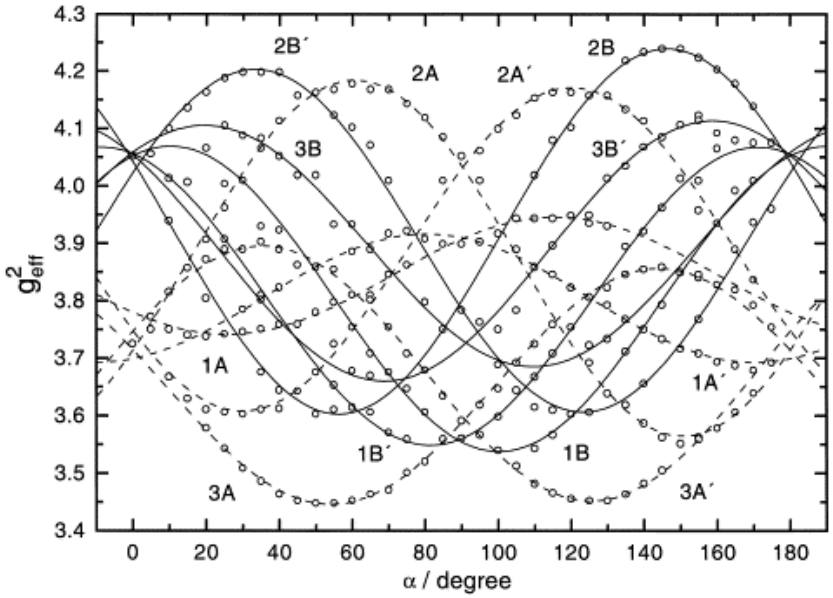


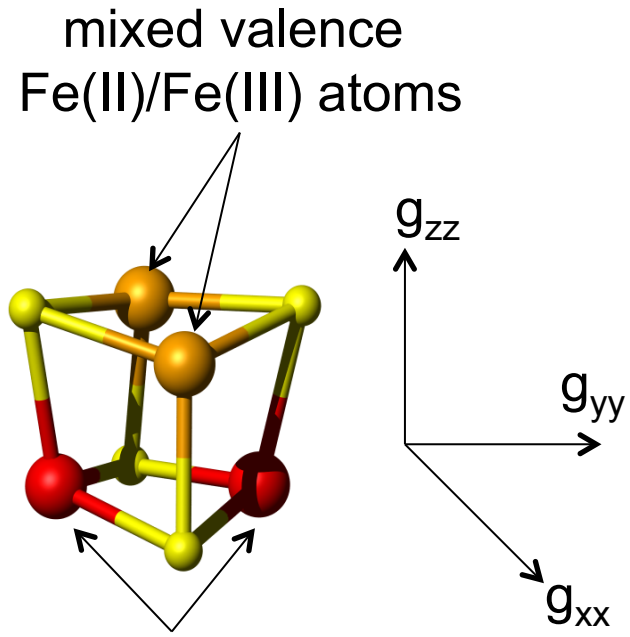
Rotation about *c*-axis



Kamlowski *et al* Biochim. Biophys. Acta 1319 (1997) 185–198

Example Iron Sulfur Clusters in Photosystem I:





	g_{xx}	g_{yy}	g_{zz}
F_A^-	1.856	1.941	2.051
F_B^-	1.880	1.916	2.056

Kamlowski *et al* Biochim. Biophys. Acta 1319 (1997) 185–198

Symmetry Terminology

For any tensorial property, T

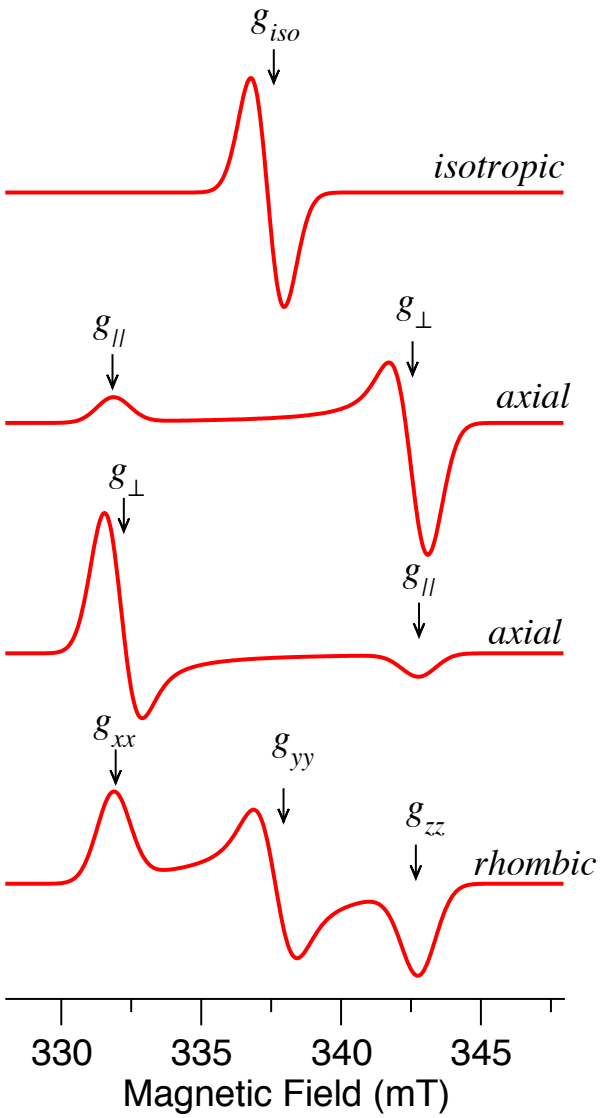
Tensor Elements	Term	Symmetry
$T_{xx}=T_{yy}=T_{zz}$	*isotropic*	Tetrahedral or higher
$T_{xx}=T_{yy}\neq T_{zz}$ *or* $T_{xx}\neq T_{yy}=T_{zz}$	*axial*	3-fold or higher rotation axis
$T_{xx}\neq T_{yy}\neq T_{zz}$	*rhombic*	2-fold rotation or lower

Powder Spectra

For randomly oriented samples the spectrum is a sum of all possible orientations.

The principal g-values can be obtained from features in the spectra.

The shape of the spectrum depends on the symmetry of the molecule



g-Anisotropy

The g-anisotropy depends on the spin orbit coupling.
Perturbation theory gives:

mixing of molecular orbitals

$$g_{ij} = g_e + 2\lambda \sum_n \frac{\langle \psi_0 | \hat{L}_i | \psi_n \rangle \langle \psi_n | \hat{L}_j | \psi_0 \rangle}{E_n - E_0}$$

spin-orbit coupling parameter

g-anisotropy

General trends:

- Radicals with light elements e.g. C, H, O, N .
  - Weak spin orbit coupling
  - Small g-anisotropy and signals near g=2.0023.
- Transition metals
  - Moderate to strong spin-orbit coupling
  - Larger g-anisotropy
  - g-anisotropy depends on the electronic configuration and the symmetry of the ligand field.

g-Anisotropy and Spin-Orbit Coupling

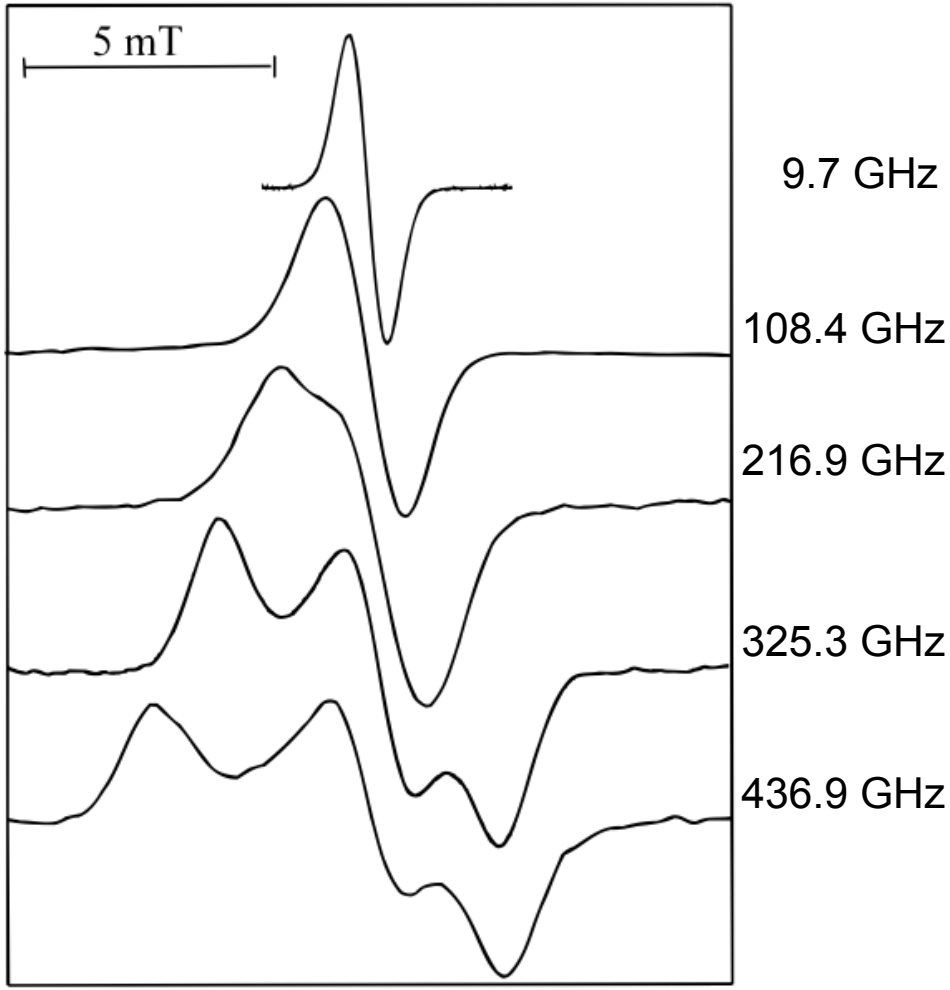
Chlorophyll cofactor $P_{700}^{\bullet+}$

Very high frequency EPR is needed to resolve the g-anisotropy

g_{xx} = 2.00317

g_{yy} = 2.00264

g_{zz} = 2.00226



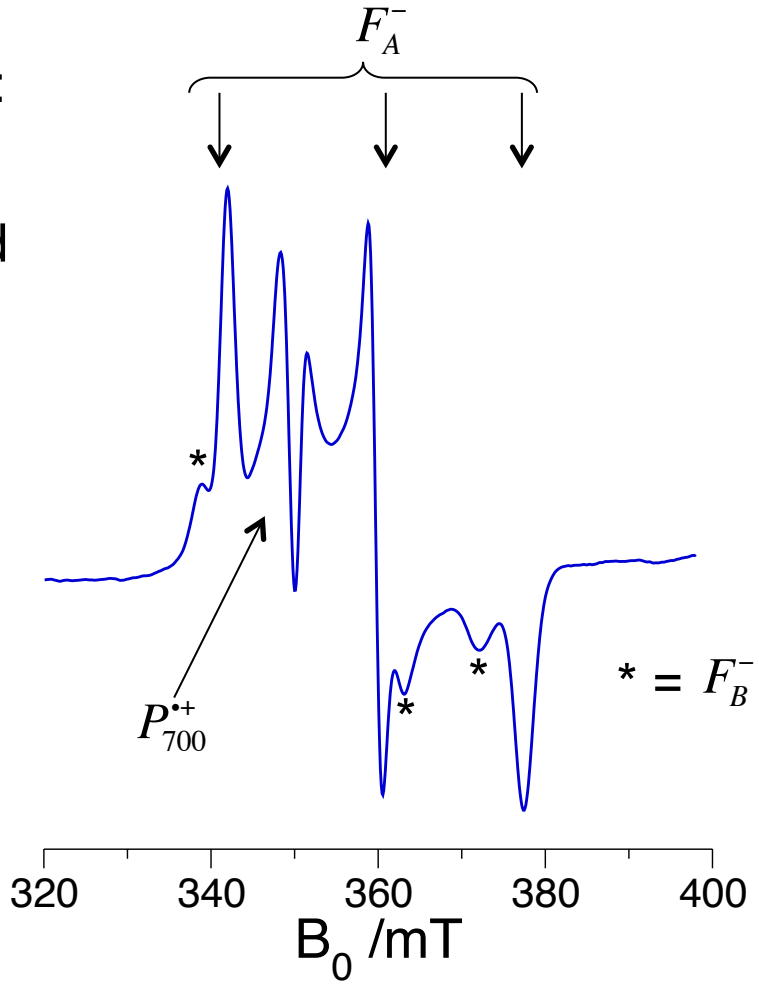
Bratt *et al*J. Phys. Chem. B, (1997),101,9686–9689

g-Anisotropy and Spin-Orbit Coupling

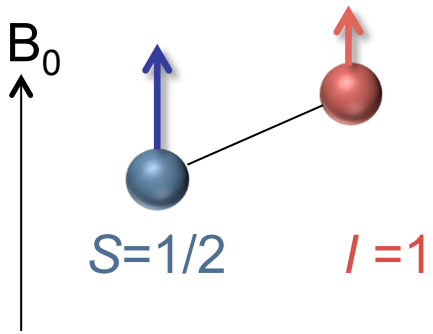
FeS clusters in Photosystem I:

Spectra well resolved at X-band (9.5 GHz).

Spin-orbit coupling is much stronger because of the metal atoms

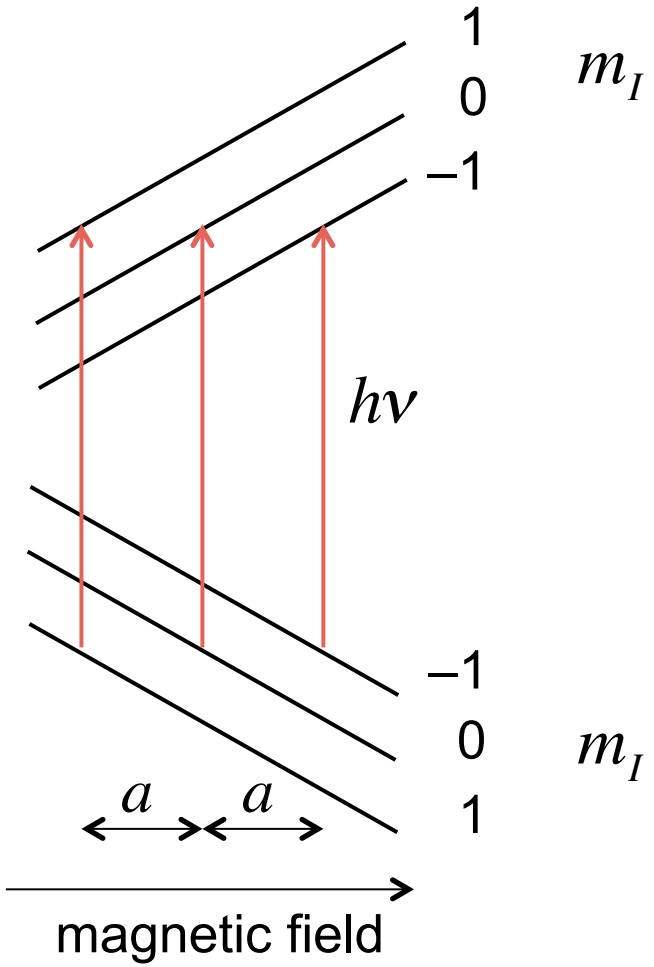


Hyperfine coupling



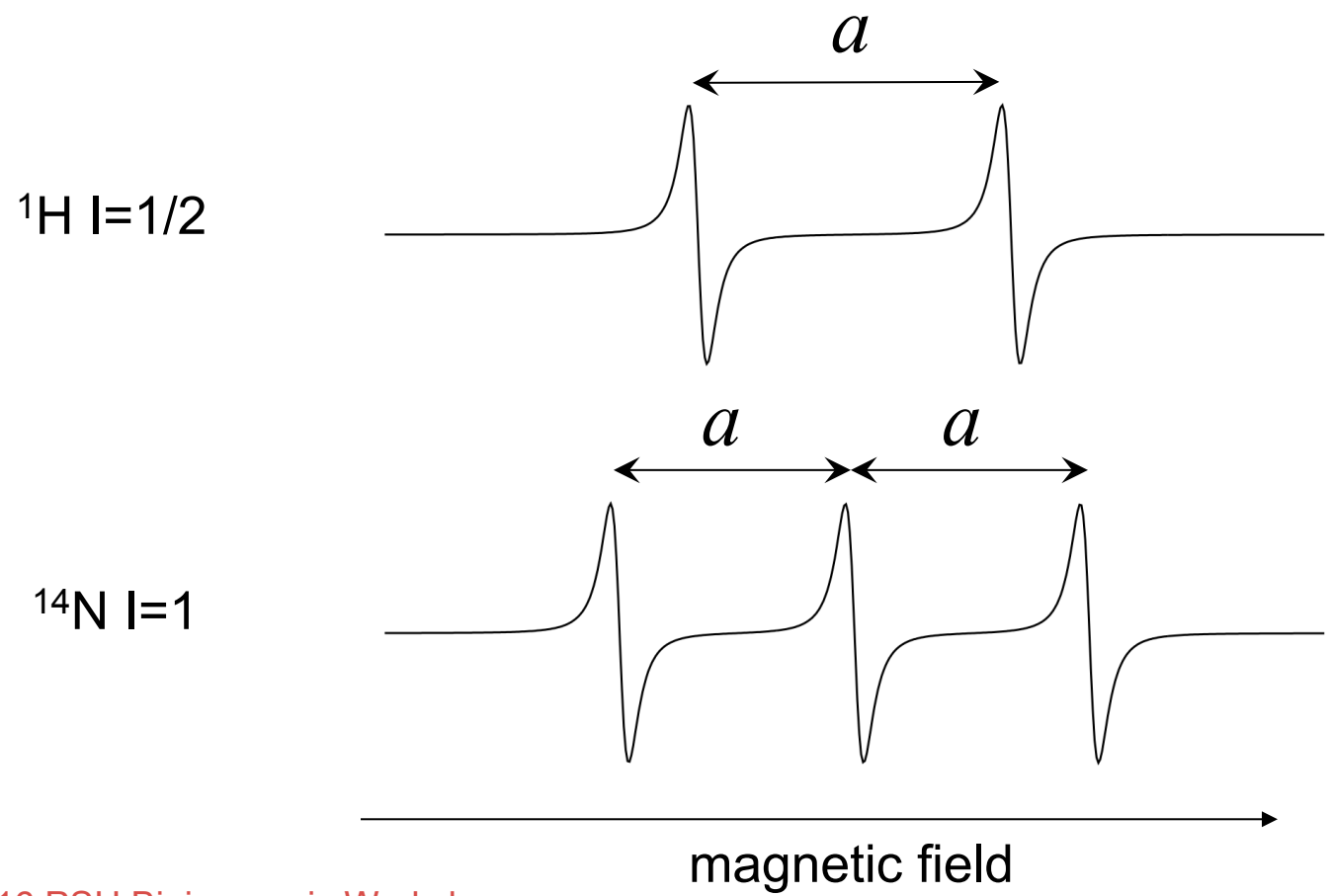
The interaction between the unpaired electron and neighbouring nuclei leads to splitting of the energy levels and the spectrum.

Energy level diagram for coupling to a nitrogen nucleus with *I* = 1



Hyperfine coupling (Isotropic case)

Each nucleus with $I \neq 0$ that couples to the unpaired electron gives $2I + 1$ lines of equal intensity.



Hyperfine Coupling to Multiple Nuclei

Groups of equivalent nuclei give characteristic patterns of lines.

The number of hyperfine lines, n_{hfs}, from a group of, n, equivalent nuclei of spin I is:

$$n_{hfs} = (2nI + 1)$$

The total number of hyperfine lines, n, from several groups of equivalent nuclei:

$$n_{total} = \prod_i (2n_i I_i + 1) = (2n_1 I_1 + 1)(2n_2 I_2 + 1)...$$

This number can become very large

Interpretation of the hyperfine coupling:

Hyperfine coupling constants have two main contributions:

Fermi contact $$a_{iso} = \frac{2}{3}\frac{\mu_0 \beta_e \beta_n}{h} g_e g_n \left|\psi(0)\right|^2$$

electron spin density at the nucleus

Dipolar coupling $$a_{dipolar} = \frac{\mu_0}{4\pi}\frac{\beta_e \beta_n}{h} g_e g_n \left\langle \frac{3\cos^2\theta - 1}{r^3} \right\rangle$$

Notice that this term is orientation dependent

S=1/2 Metal Centres in Proteins:

- Small hyperfine couplings are not resolved (too many lines)
- Strong couplings may be (partially) resolved from nuclei with high electron spin density
- Couplings are orientation dependent

$$a = a_{iso} + a_{dipolar}$$

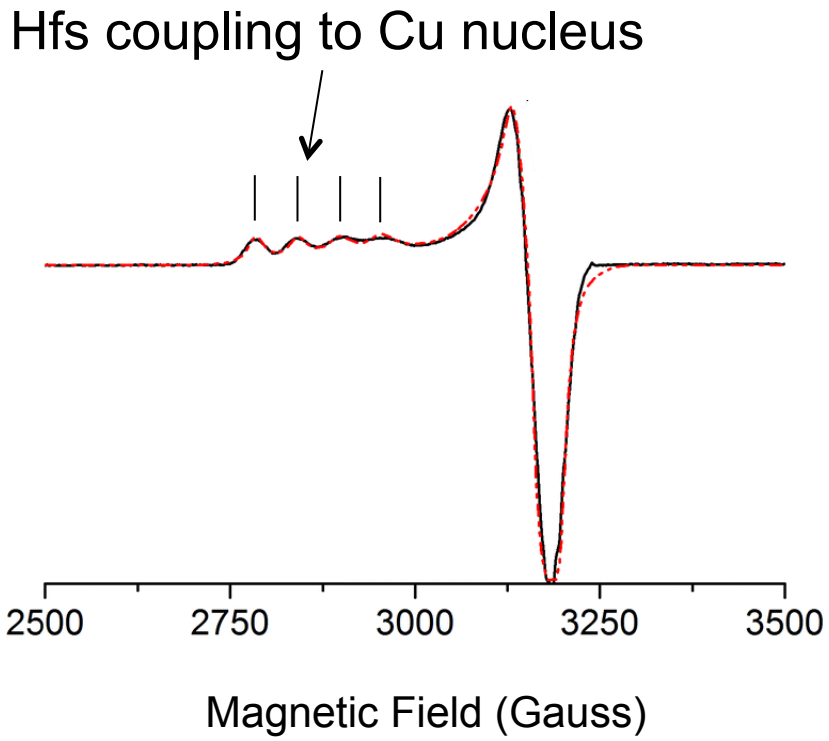
The sign of this term depends on orientation

Resolved Hyperfine Coupling

Azurin:

In proteins containing Cu(II) hyperfine coupling to the I=3/2 copper nucleus is observed.

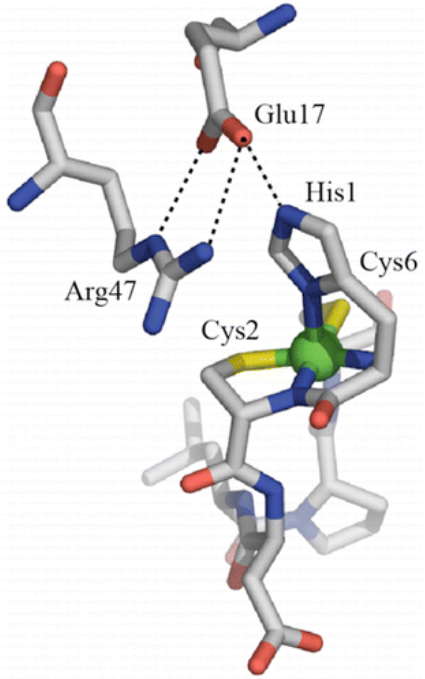
Usually it is only resolved on the $g_{\perp}$ component



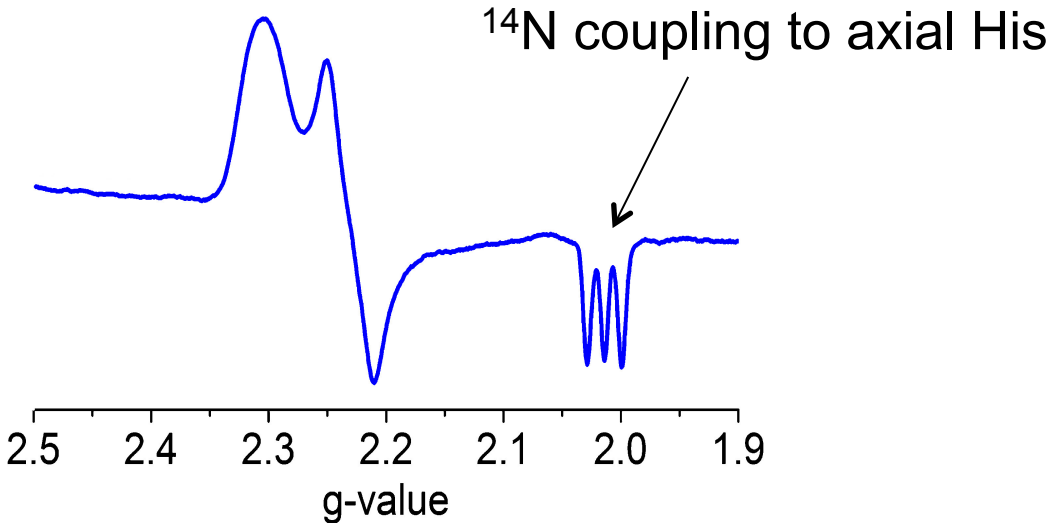
K.M. Clark, *et al.* Inorg. Chem. Frontiers (2014) **1**, 153-158

Resolved Hyperfine Coupling

Ni Superoxide dismutase:



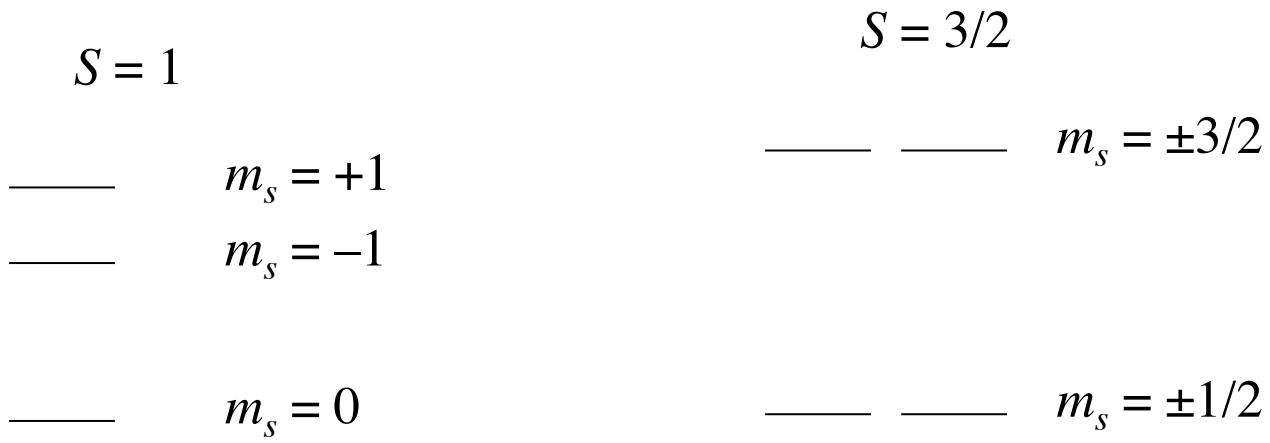
Hyperfine coupling to ligands is also sometimes resolved on one or more of the g-tensor components



Ryan *et al* Biochemistry (2015) **54**, 1016–1027

Zero-Field Splitting

For systems with S > 1/2, spin-orbit coupling and spin-spin coupling, split the spin states :



This splitting has a large impact on the EPR spectra

Zero-Field Splitting

Because the splitting occurs even when there is no magnetic field present is referred to as Zero-Field-Splitting:

The term in the spin Hamiltonian describing this interaction has the form:

$$H_{ZFS} = \boldsymbol{S} \bullet \boldsymbol{D} \bullet \boldsymbol{S}$$

In its principal axes the matrix $\boldsymbol{D}$ can be written:

$$\boldsymbol{D} = \begin{pmatrix} -\frac{1}{3}D + E & 0 & 0 \\ 0 & -\frac{1}{3}D - E & 0 \\ 0 & 0 & \frac{2}{3}D \end{pmatrix}$$

Zero-Field Splitting and Symmetry

For an *isotropic* system $D, E = 0$

For an *axial* system $D \neq 0, E = 0$

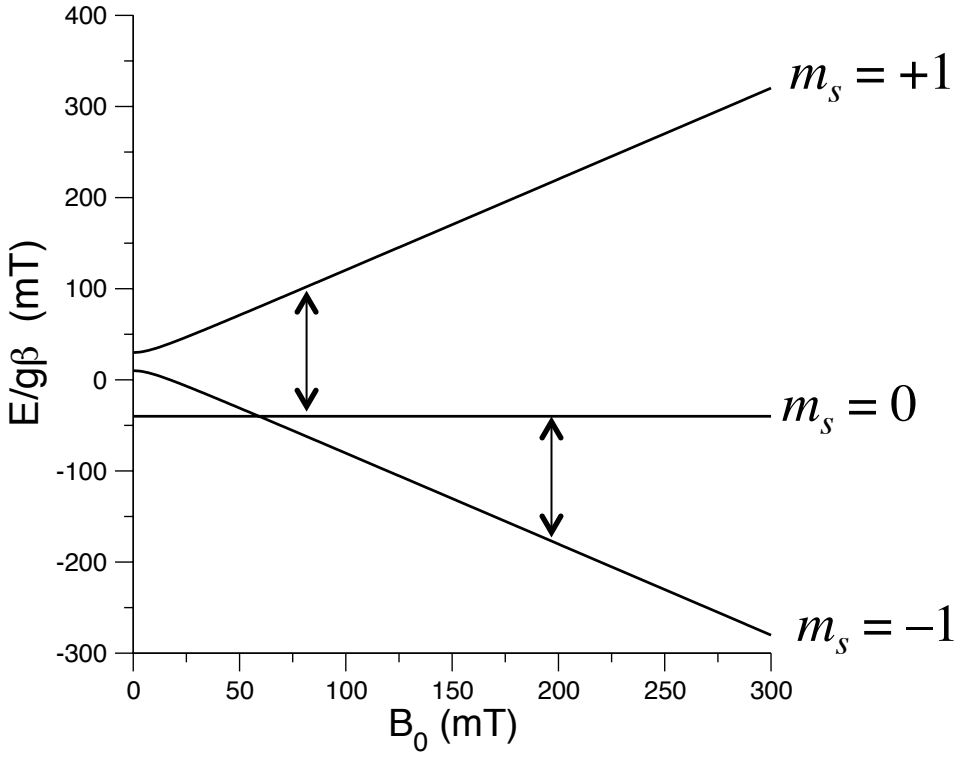
For a *rhombic* system $D \neq 0, E \neq 0$

The ratio E/D is a measure of the *rhombicity* of the zero-field splitting

The rhombicity lies between 0 and 1/3

Zero-Field Splitting

Organic triplet sates: The parameters *D* and *E* are generally smaller than the Zeeman energy at X-band

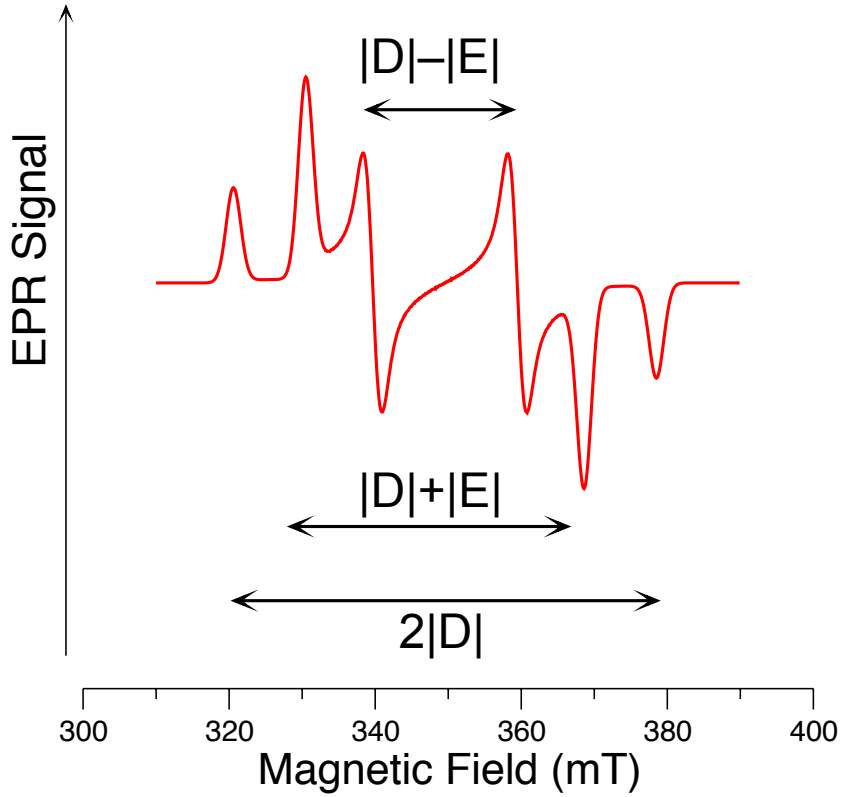


There are two allowed transitions in the EPR.

They occur at different field values because of the ZFS

Zero-Field Splitting

For a powder, the spectrum is a so-called “Pake pattern”

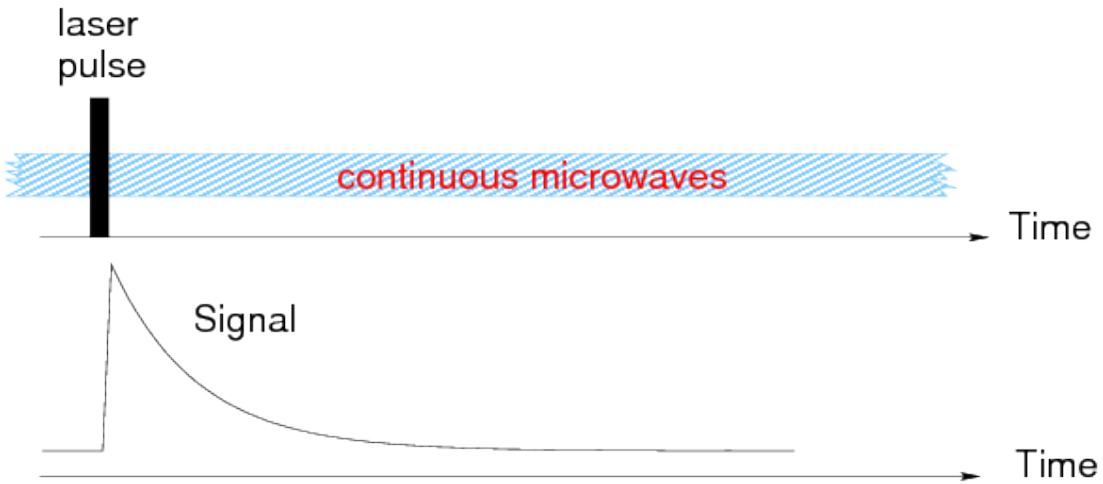


The parameters D and E can be determined from the positions of the features in the spectrum.

Light-induced Triplet States

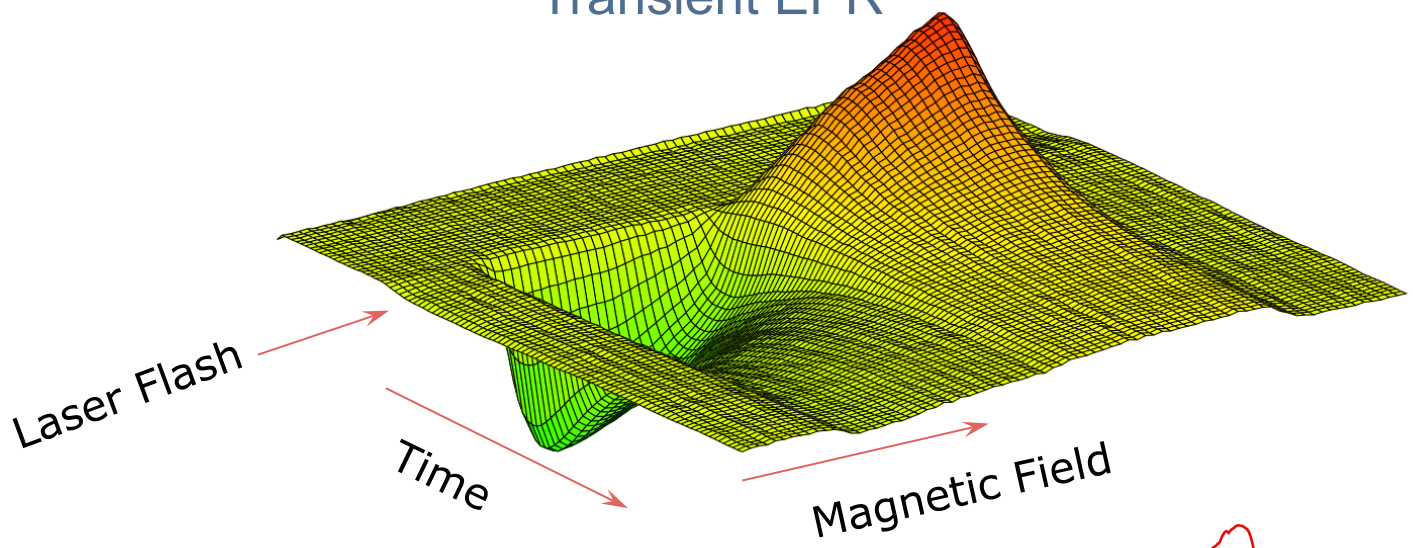
Few organic molecules have triplet ground states but excited triplet states are often long-lived enough to be observed by EPR.

Such measurements require transient EPR

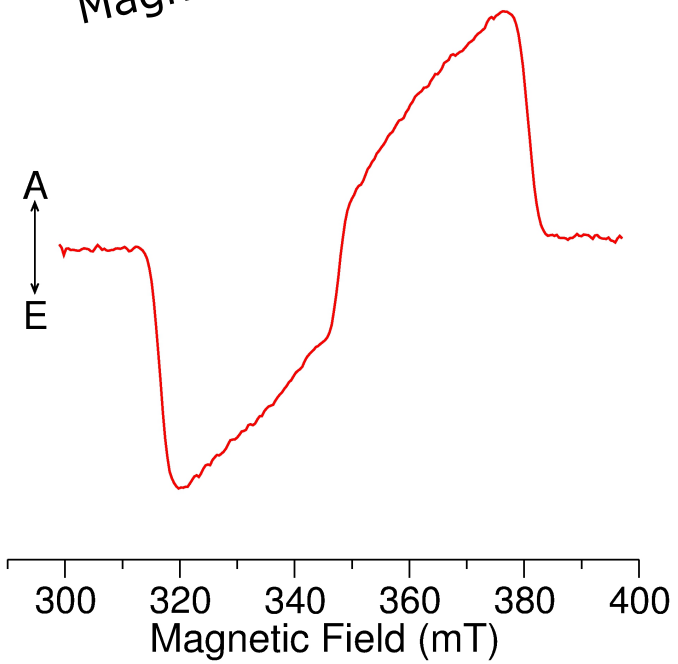


Direct detection is usually used. (No field modulation)

Transient EPR

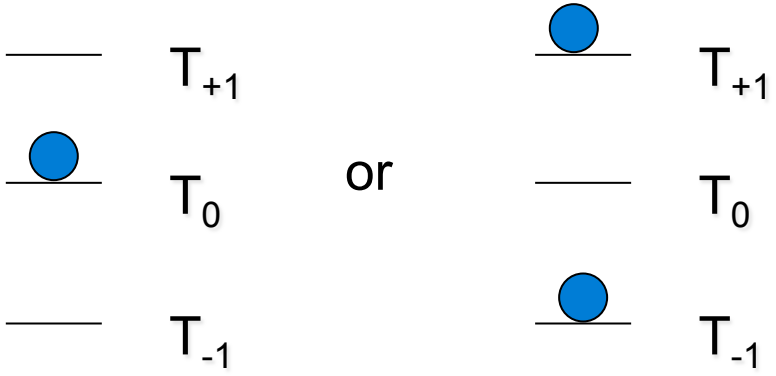


Spectrum extracted from dataset



Spin Polarization of Triplet States

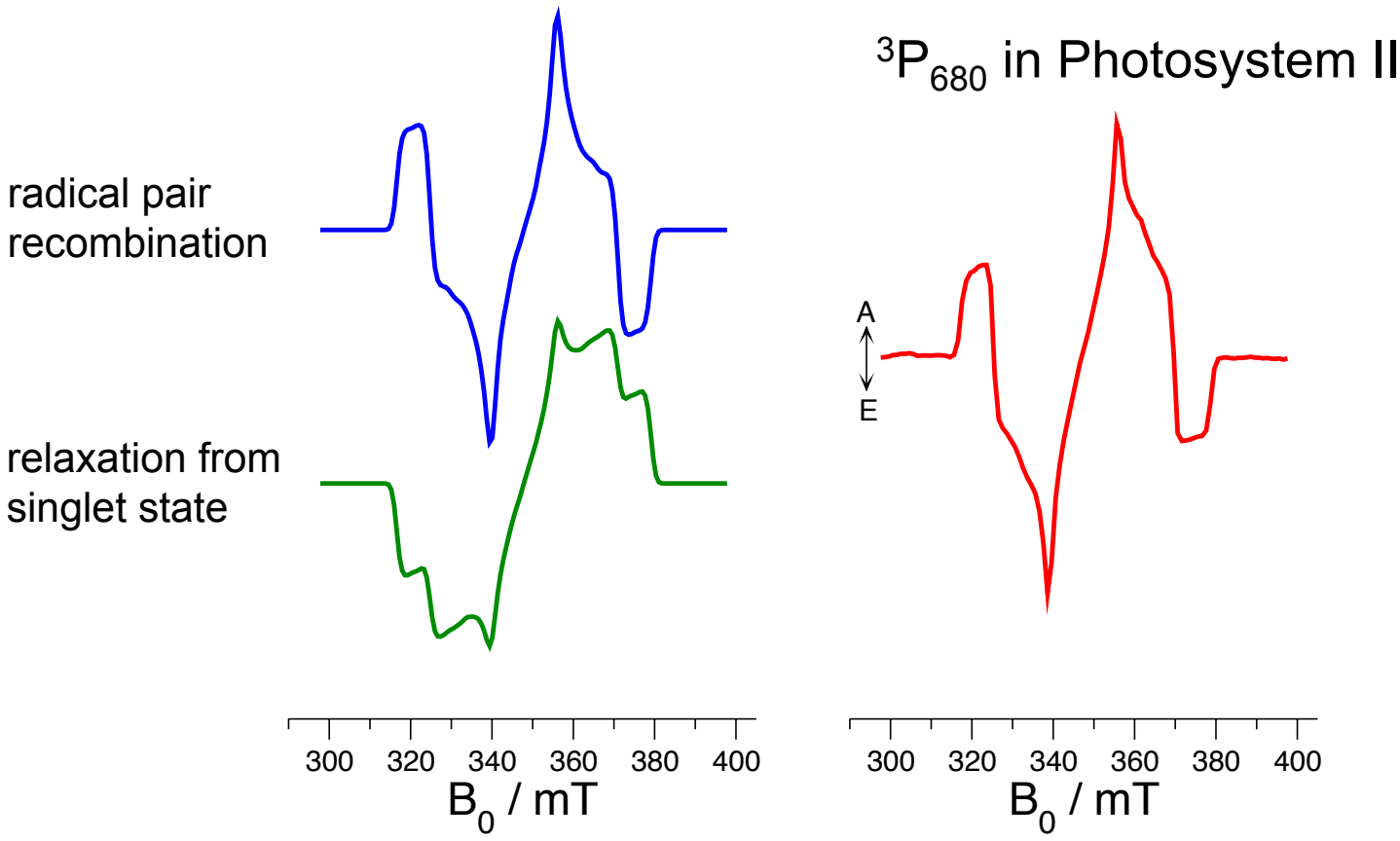
The sublevels of a light-induced triplet state are selectively populated



The selectivity is determined by the pathway by which the triplet state is populated.

Spin Polarization

Example: Photosystem II



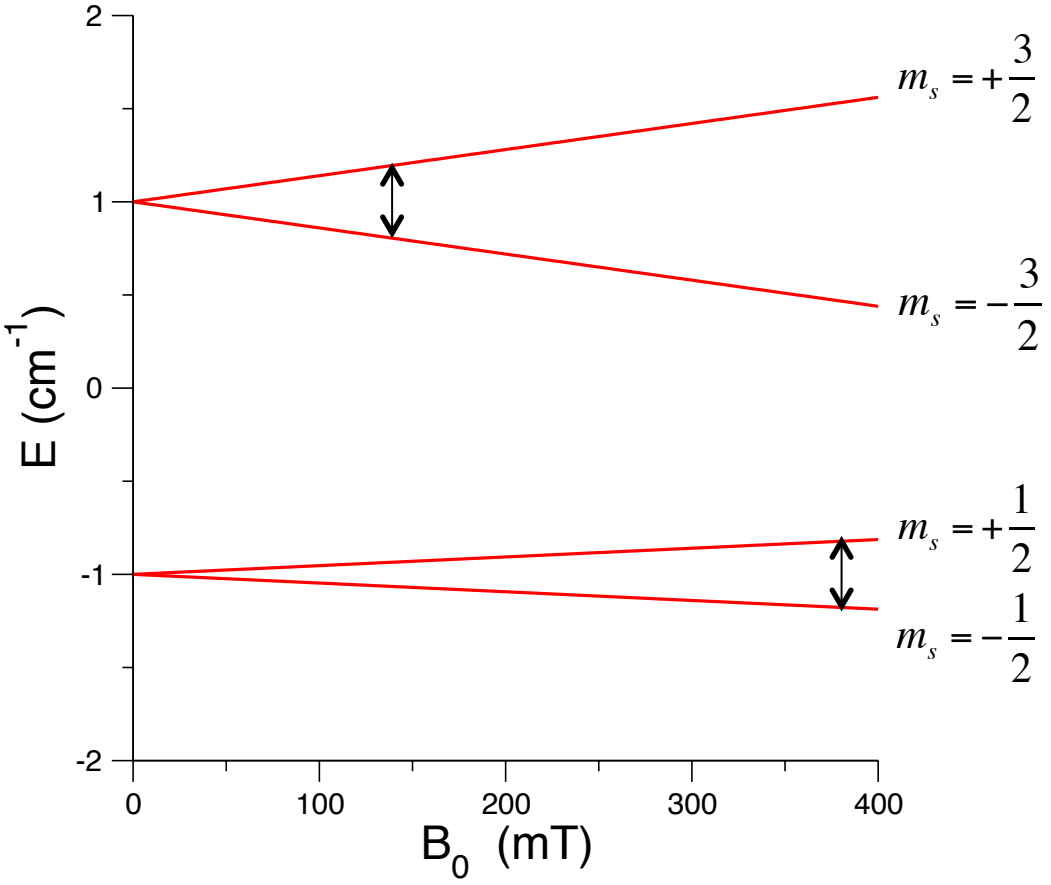
Kamlowski et al , Ber. der Bunsenges. Phys. Chem. 100, 2045–2051 , (1996)

High Spin Systems

For metals with S > 1/2, the zero field splitting is often much larger than the Zeeman interaction

e.g. S = 3/2

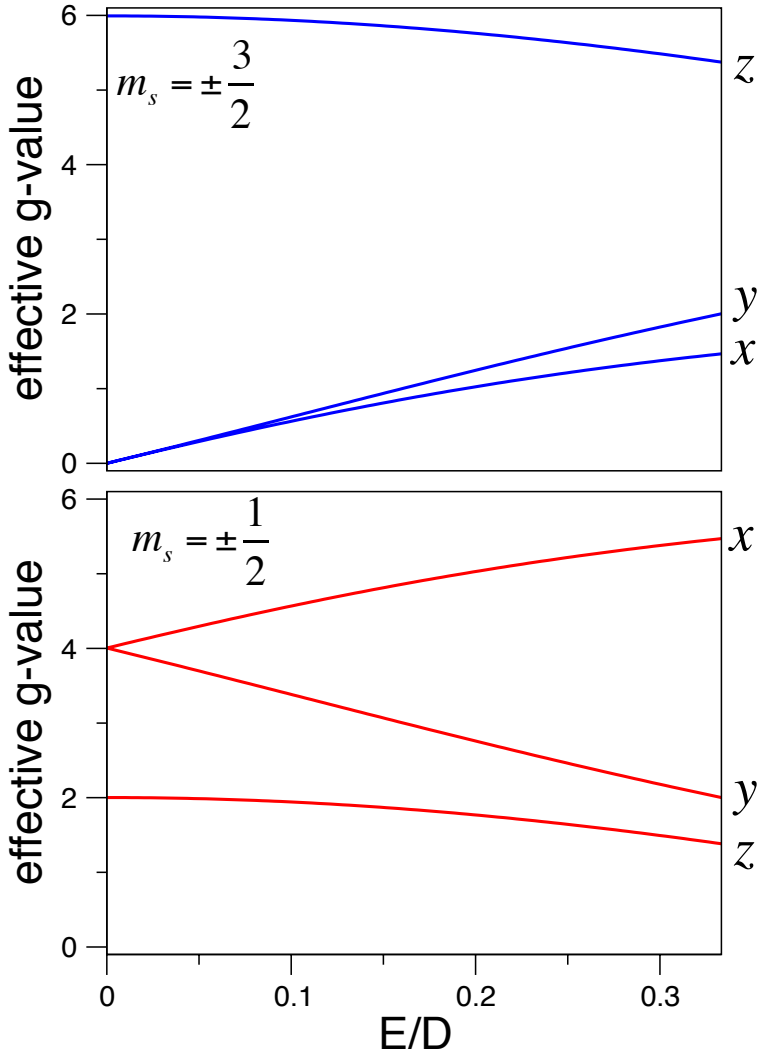
For half-integer spins, transitions are often observed at low field.



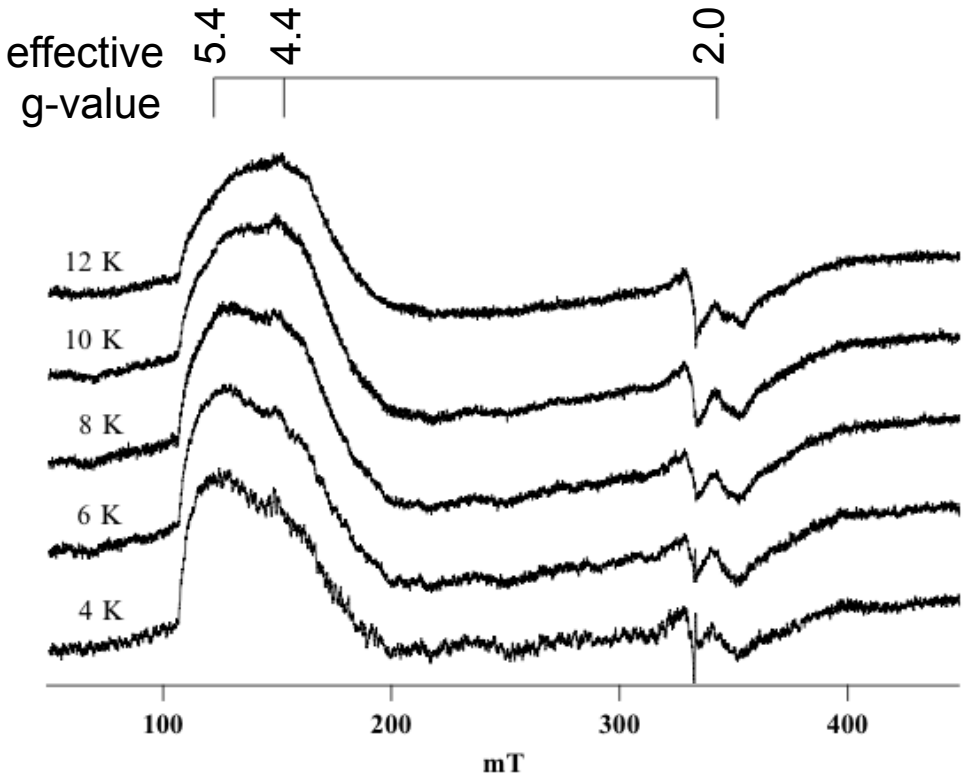
Rhombograms

The positions of the features in the spectrum depend on the ratio of the zero field splitting parameters D and E.

The expected peak positions can be calculated as a function of E/D in a so-called *Rhombogram*



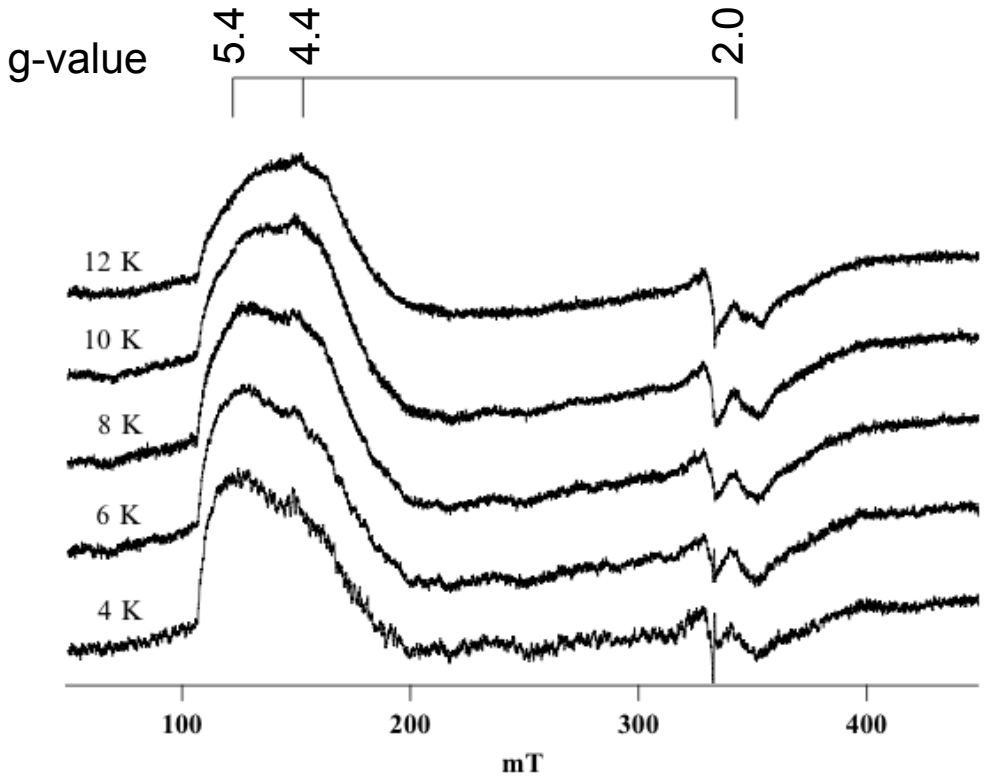
Rhombograms

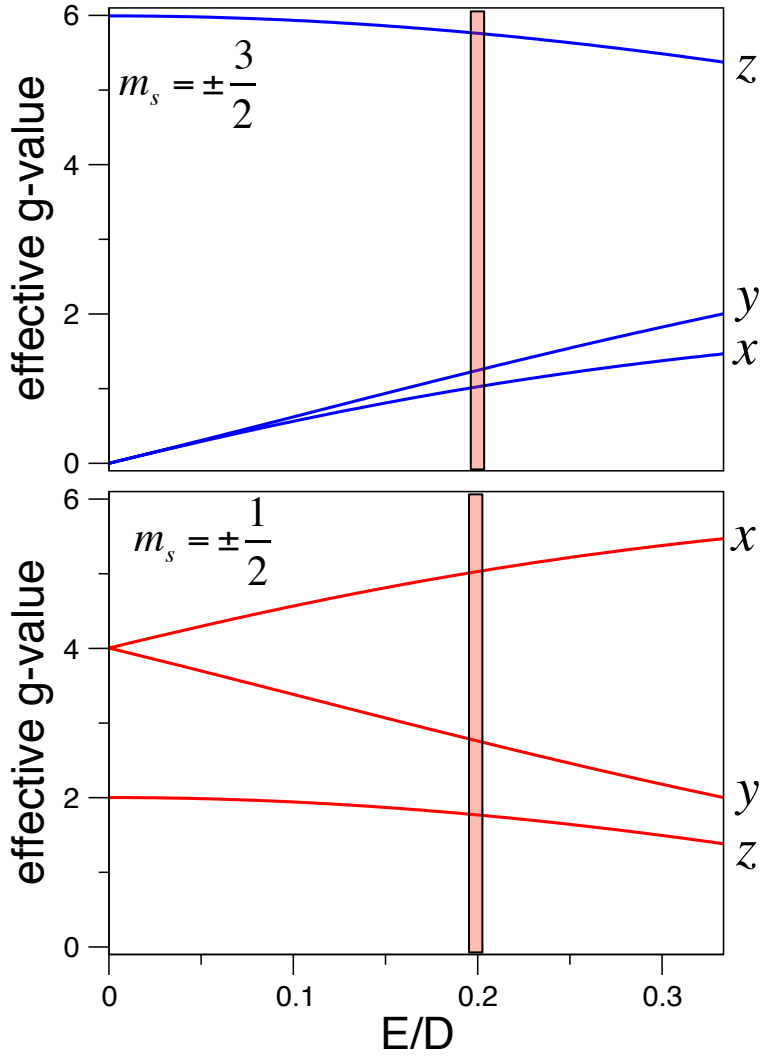


EPR spectrum of the reduced iron-sulfur cluster F_X in the reaction centre of heliobacteria

Golbeck and van der Est (2013) in "Molecular Biophysics for the Life Sciences", Allewell, Nahri & Rayment, Eds.

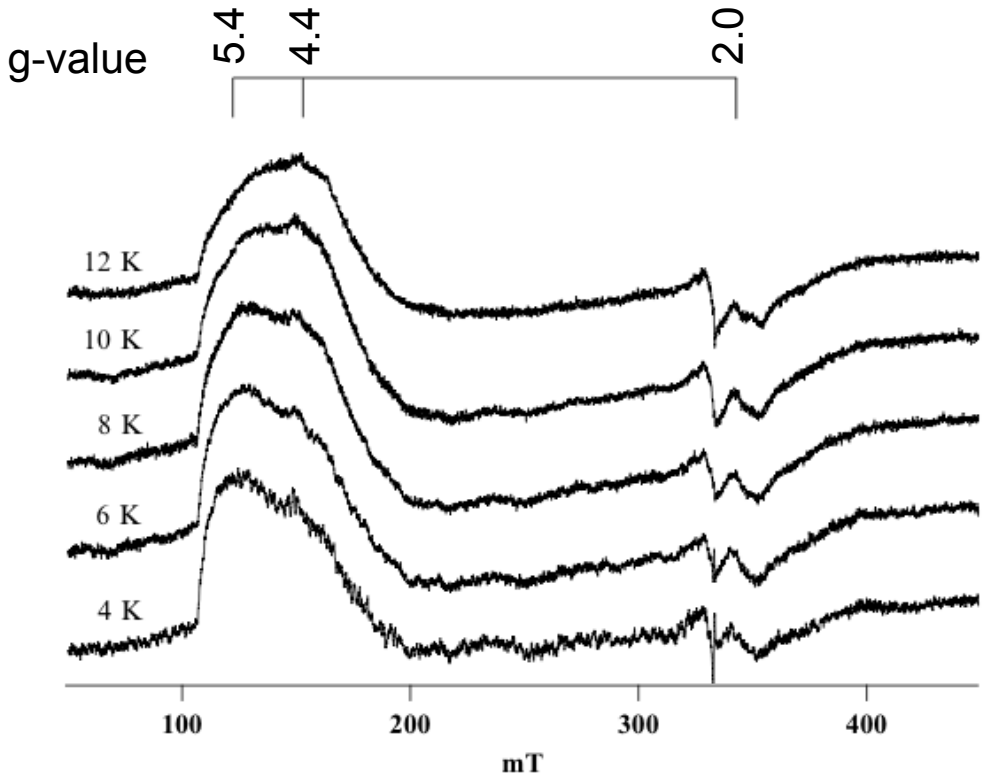
Rhombograms





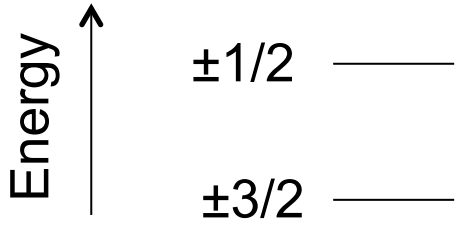
The main features in the spectrum correspond to E/D = ~0.2 for a spin 3/2 system.

Rhombograms

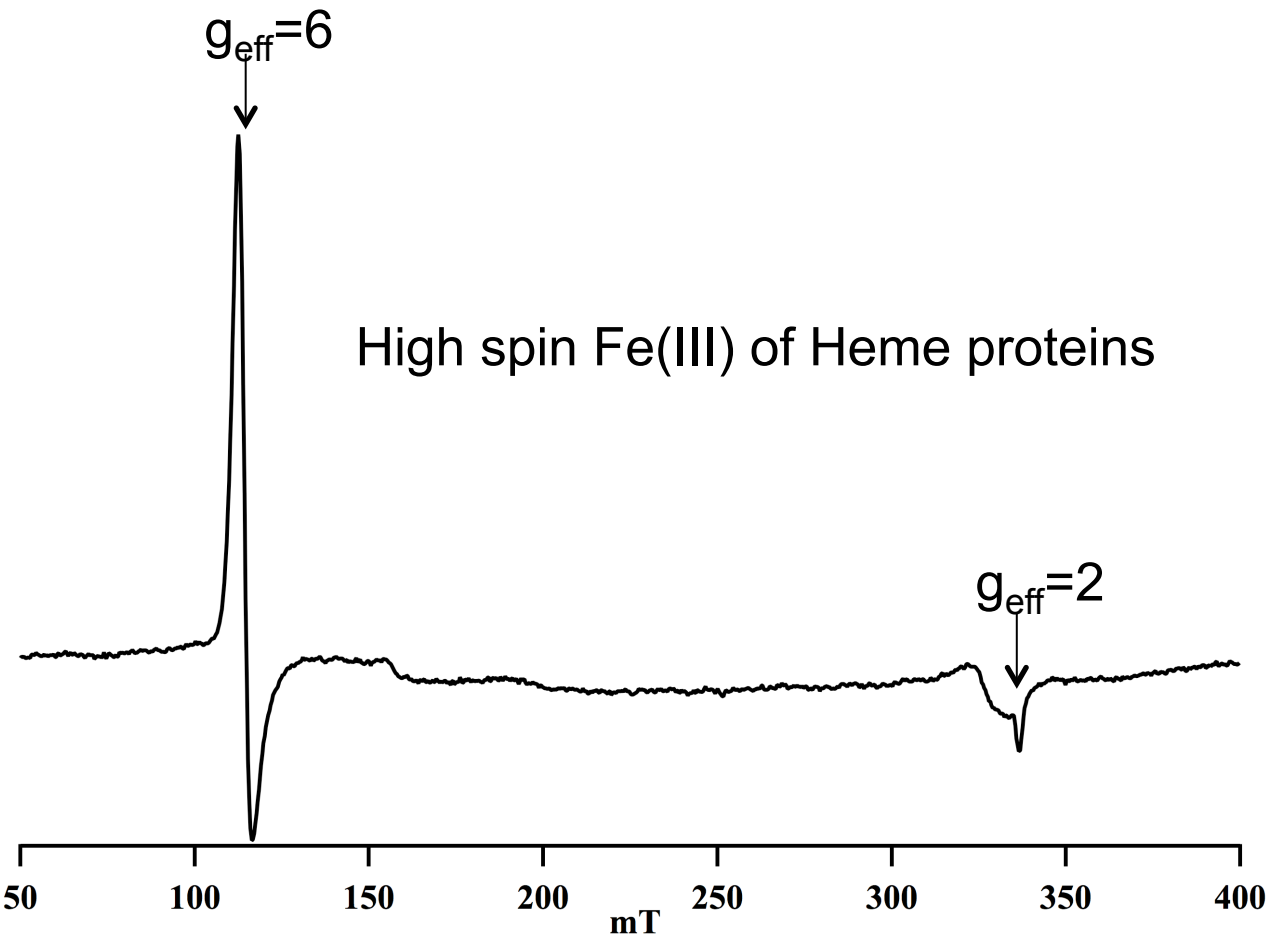


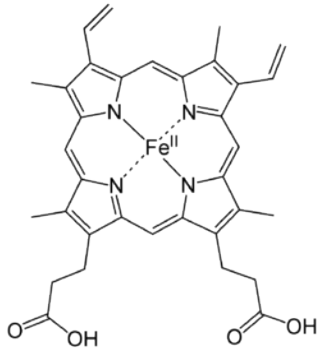
The feature at g=5.4 from the m_s= ±3/2 levels increases with temperature.

So we have:



Example Myoglobin

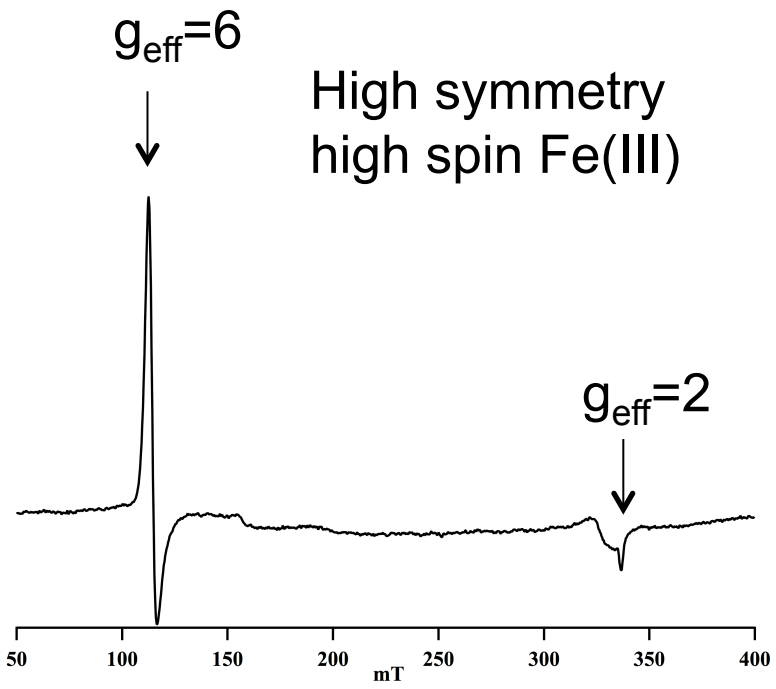


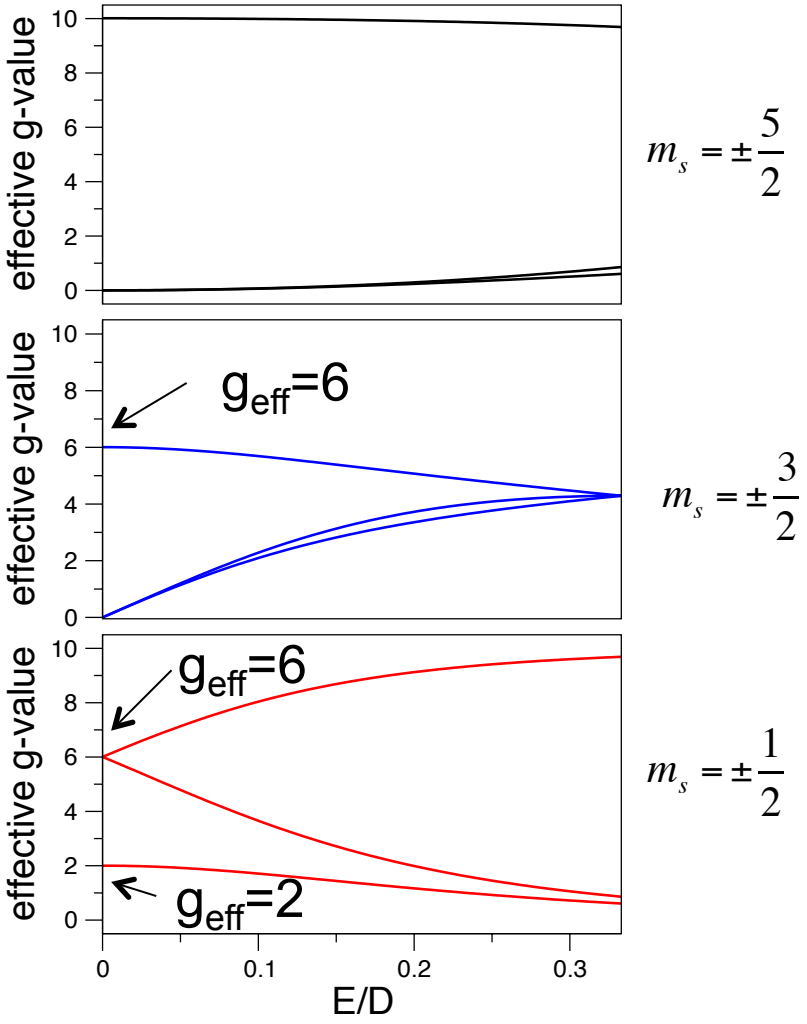


Heme

Golbeck and van der Est (2013) in "Molecular Biophysics for the Life Sciences", Allewell, Nahri & Rayment, Eds.

Example Myoglobin

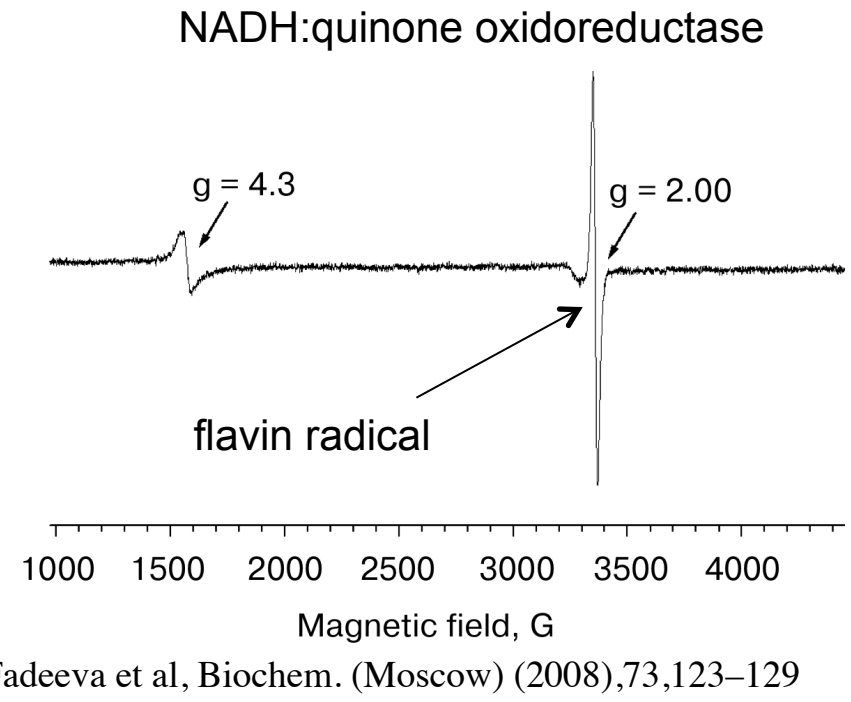




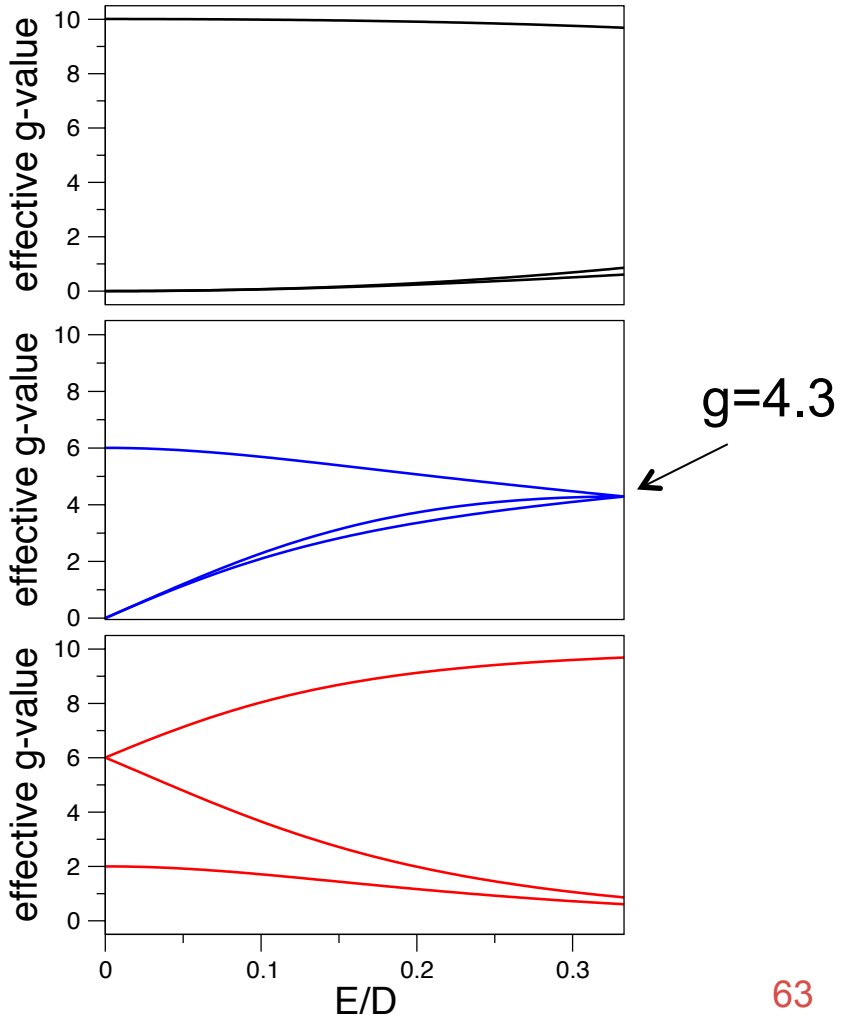
Golbeck and van der Est (2013) in "Molecular Biophysics for the Life Sciences", Allewell, Nahri & Raymond, Eds.

"Junk Iron"

Many biological samples show a signal at g=4.3 from non-specifically bound "junk" Fe(III).



Fadeeva et al, Biochem. (Moscow) (2008),73,123–129



Summary

- Basics of the EPR experiment
- Orientation dependence g-anisotropy, single crystals
- Hyperfine coupling
- Couplings between electrons, Zero Field Splitting
- High spin systems and Rhombograms