Fundamentals of Electron Paramagnetic Resonance

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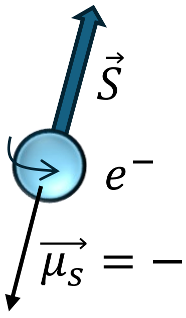
Bioinorganic Workshop 2024

EPR in 5 sec

Electron Paramagnetic Resonance



$\vec{\mu_s} = -g_e\beta_e\vec{\boldsymbol{S}}$

$\mu_s = g\beta\sqrt{s(s+1)}$

Electrons have spin angular momentum $\vec{S}$

The generated magnetic dipole moment μ_s interacts with external magnetic field

For $s = \frac{1}{2}$ two spin states: $m_s = +1/2$ and $m_s = -1/2$

With magnetic field - degeneracy is lifted.

$\beta_e = 9.27 \times 10^{-24}\, J/T$ - Bohr magneton

g - electron g-value

Free electron: $g = g_e = 2.0023$

s - spin quantum number

External magnetic oscillation @ microwave frequencies with energy matching that of the gap between m_S=+1/2 and m_S=-1/2 , will cause excitation of the system

** ESR = Electron Spin Resonance (mostly used interchangeably with EPR)*

Early history

- 1896. P. Zeeman and H. Lorentz:

Splitting of sodium spectral lines in magnetic field. (Zeeman effect)

- 1922. Stern, Gerlach:

Electron magnetic moment in an atom can take only discrete orientations.

- 1925. G.E. Uhlenbeck, S. Goudsmit:

Link between magnetic moment and electron spin angular momentum.

- 1938. I.I. Rabi.

Transition between levels induced by an oscillating magnetic field.

- 1944. E. Zavoisky (USSR):

First experimental observation of an EPR (in $CuCl_2 \bullet 2H_2O$)

- 1946. Cummerow, Halliday (USA).

EPR in manganous salts.

- 1947. Bagguley, Griffiths (UK)

EPR in chrome alum.

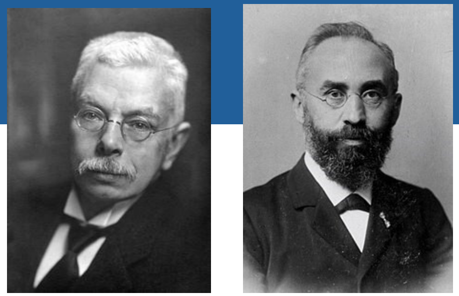
EPR spectroscopy

- Uses:
  - Organic free-radicals
  - Transition metal ions with an odd number of electrons or high spins
  - Excited states with S>0, e.g., triplet states
  - Conduction electron spin resonance in nanoparticles

- Molecules with all electrons paired are *EPR-silent*
- Most integer spin systems are EPR-silent too (but not all)

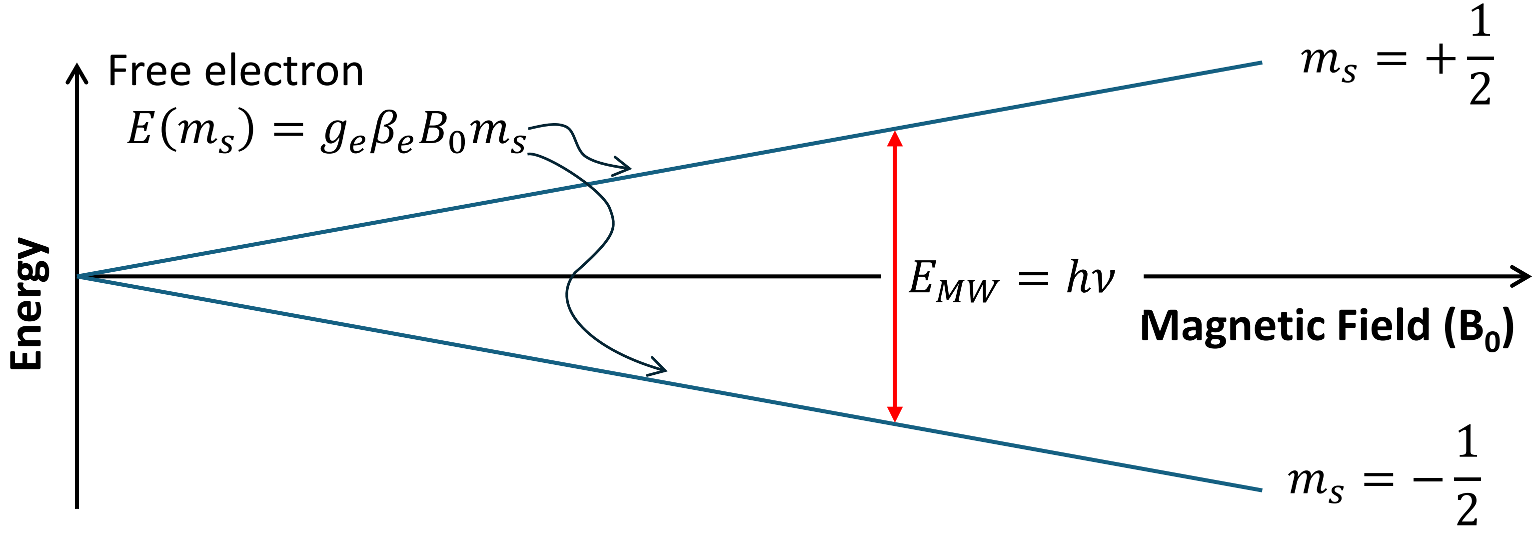
- Zeeman effect and g-anisotropy
- Hyperfine coupling
- Zero-field splitting
- Practical aspects

Zeeman effect

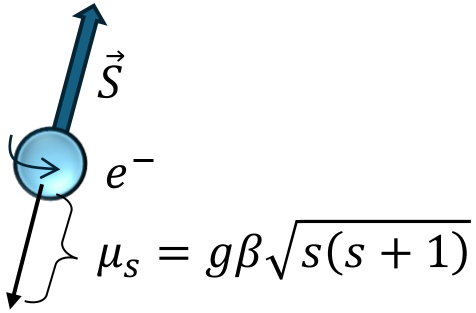


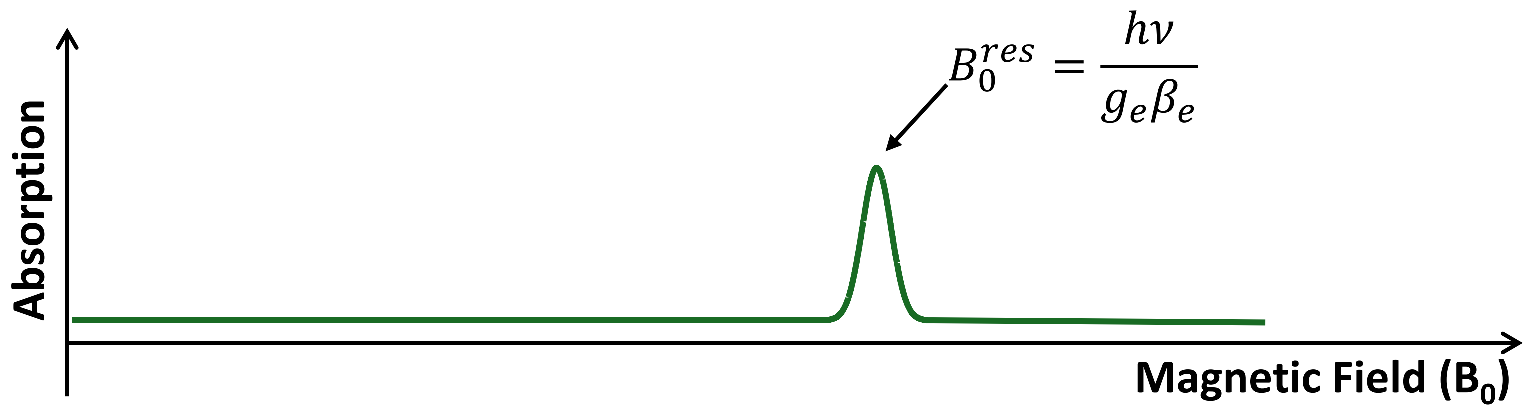
Pieter Zeeman reported spectral line splitting of sodium in magnetic field in 1896

Nobel prize in 1902, jointly with Hendrik Lorentz



$$\vec{\mu_s} = -g_e \beta_e \vec{\boldsymbol{S}}$$





μ_s – magnetic dipole moment
$\beta_e = 9.27 \times 10^{-24}\ J/T$ – Bohr magneton
g – electron g-value
Free electron: $g = g_e = 2.0023$
s – spin quantum number
$h = 6.626 \times 10^{-34}\ J \cdot sec$ – Plank constant
ν – microwave frequency

* It is useful to keep in mind that the g-factor is a slope of $E_{m_s}(B_0)$

Where it gets interesting

In virtually any “real” case, the g-factor is not isotropic

- Movement of e^- on an orbit induces additional magnetic dipole moment

$$\overrightarrow{\mu_L} = -\beta_e \vec{\boldsymbol{L}} \text{ and } \overrightarrow{\mu_S} = -\beta_e g_e \vec{\boldsymbol{S}}$$

- Spin orbit coupling mixes ground and excited states. So, interaction of the system with an external magnetic field is described by a Hamiltonian:

$$\mathcal{H}_{Zeeman} = \beta_e \overrightarrow{B_0}\left(\vec{L} + g_e \vec{S}\right) + \lambda \vec{L}\vec{S}$$

Because orbital momentum-derived magnetic moment is “locked” to the molecular frame (symmetry)

Zeeman interaction will depend on how the molecule is oriented w.r.t. the external field.

- the system is best described with a total angular momentum $\vec{J} = \vec{L} + \vec{S}$:

$$\mu = g\beta_e\sqrt{J(J+1)} \qquad \text{(recall Russell-Saunders terms)}$$

- Due to the SO-coupling (mixing) neither L or S are “good quantum numbers” (but J is)

Spin Hamiltonian Formalism

- If SOC contribution is relatively small, we can describe system purely in term of “spin” (spin Hamiltonian formalism)
- BUT, we need to account for SOC and anisotropy of the Zeeman effect.
- So, g-factor MUST encode anisotropy associated with the existence of the spin-orbit coupling → **g-tensor**.

$$\mathcal{H}_{eff} = \beta_e \overrightarrow{B_0}\boldsymbol{g}\vec{\boldsymbol{S}} = \beta_e [B_x \quad B_y \quad B_z] \begin{bmatrix} g_{xx} & g_{xy} & g_{xz} \\ g_{yx} & g_{yy} & g_{yz} \\ g_{zx} & g_{zy} & g_{zz} \end{bmatrix} \begin{bmatrix} S_x \\ S_y \\ S_z \end{bmatrix}$$

when SO coupling sufficiently small, we can use first-order perturbation to get:

$$g_{ij} = g_e + 2\lambda \sum_n \frac{\langle \phi_0 | L_i | \phi_n \rangle \langle \phi_n | L_j | \phi_0 \rangle}{E_n - E_0}$$

← mixing MOs

← gap between electronic states

We can choose the coordinate system so that g-tensor is diagonal:

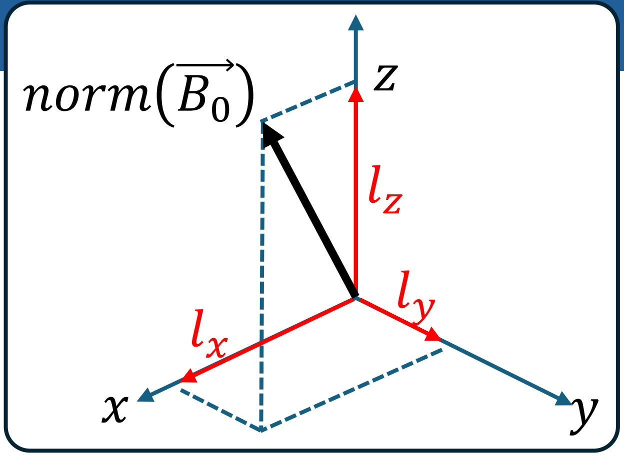
$$\mathcal{H}_{eff} = \beta_e \overrightarrow{B_0}\boldsymbol{g}\vec{\boldsymbol{S}} = \beta_e [B_x \quad B_y \quad B_z] \begin{bmatrix} g_x & 0 & 0 \\ 0 & g_y & 0 \\ 0 & 0 & g_z \end{bmatrix} \begin{bmatrix} S_x \\ S_y \\ S_z \end{bmatrix}$$

$g_{x,y,z}$ are principal components of the g-tensor.

This coordinate system is related to the molecular symmetry!

Because g-tensor parameterizes SOC, it is a “fingerprint” of the system

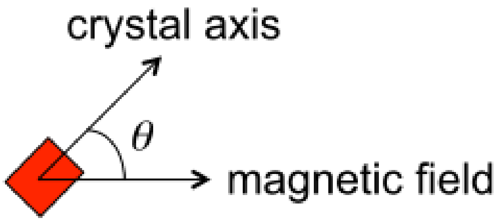
g-anisotropy

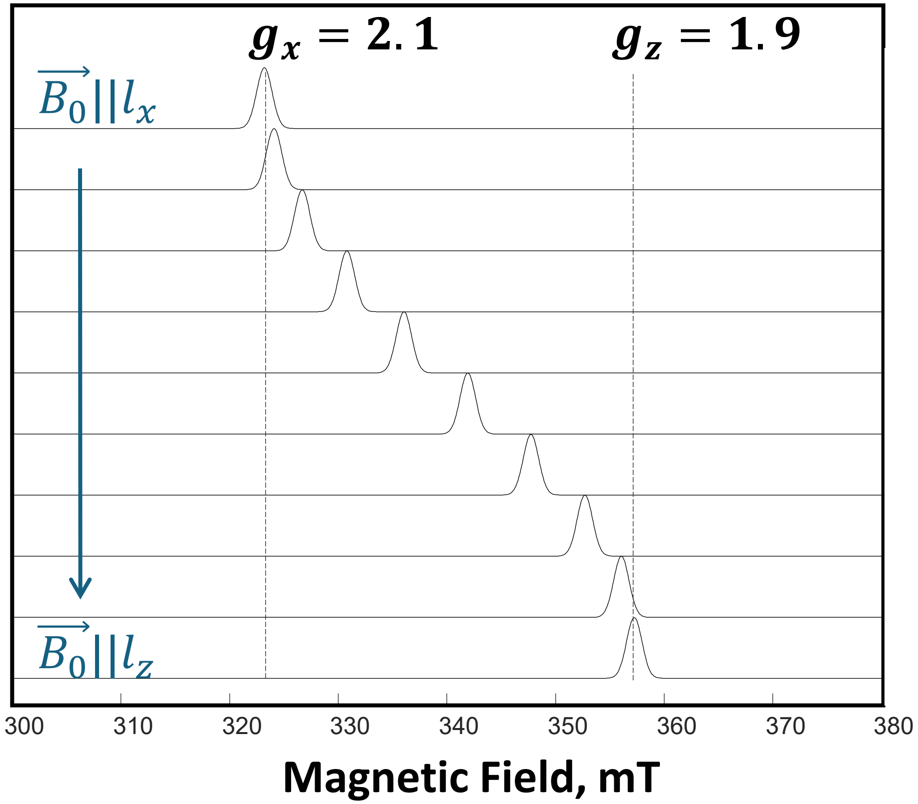


In the absence of any other magnetic interactions (yes, there is more …) resonance position on an EPR spectrum is dictated by

$$g_{eff} = \sqrt{g_x^2 l_x^2 + g_y^2 l_y^2 + g_z^2 l_z^2} \text{ and } B_0 = \frac{h\nu}{\beta_e g_{eff}}$$

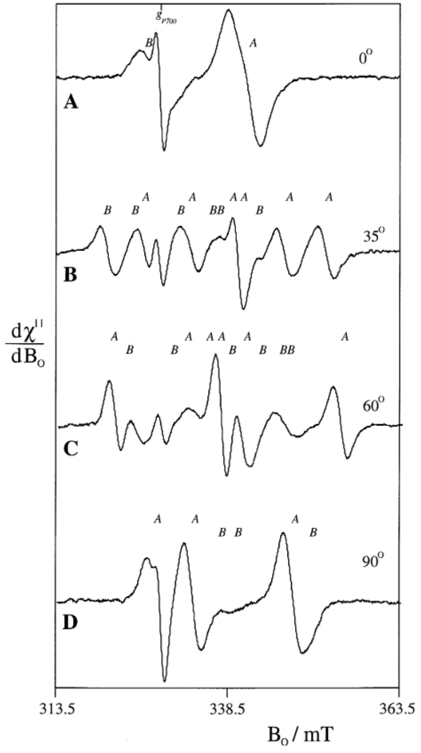
A single crystal "thought" experiment

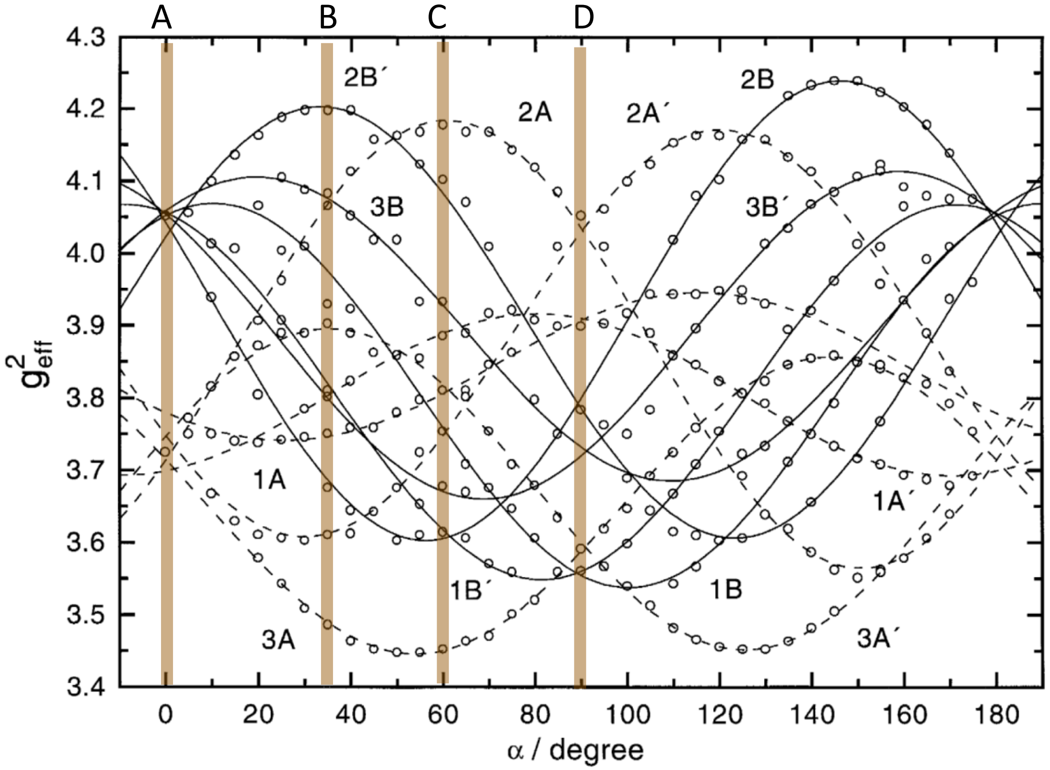


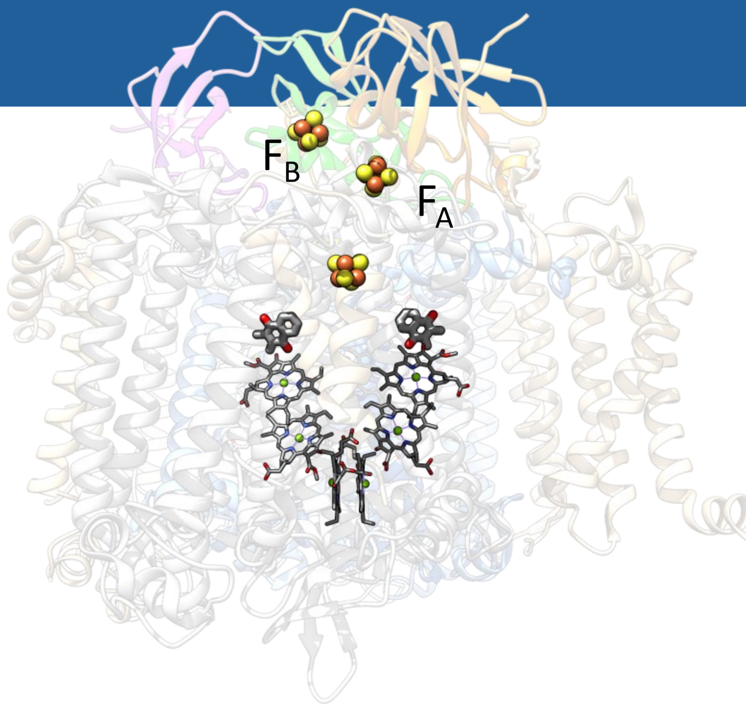


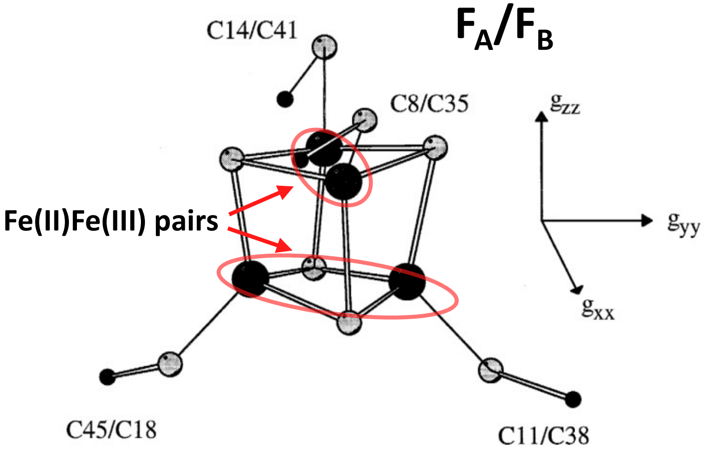
$* \frac{h}{\beta_e} = 71.448\ [mT]/[GHz]$

Single crystal study









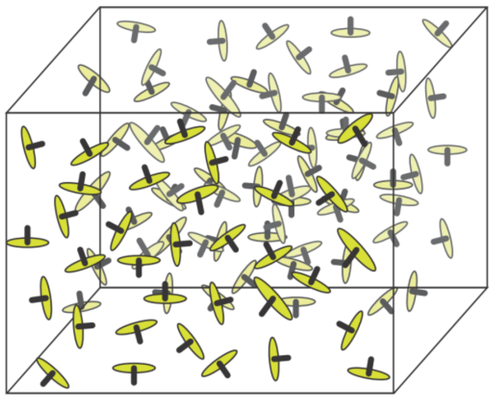
	g-Values of the FeS center			Angles with *c*-axis [a]		
	g_{xx}	g_{yy}	g_{zz}	β_X	β_Y	β_Z
F_A^-	1.856	1.941	2.051	53°	48°	64°
F_B^-	1.880	1.916	2.056	77°	60°	34°

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

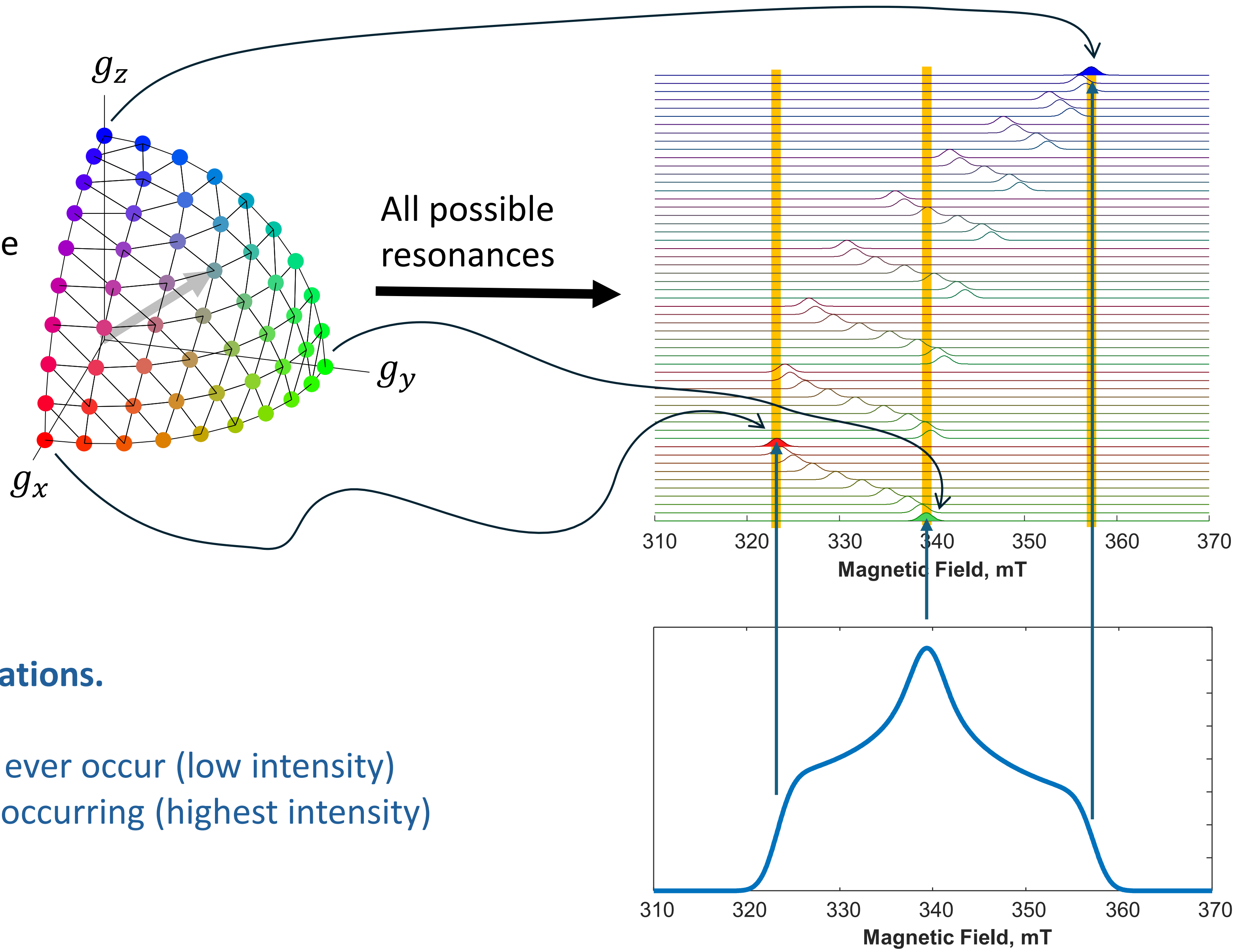
Powder averaging

$g_x = 2.1, g_y = 2.0, g_z = 1.9$

What if we have all possible orientations:



Orientaions of $\overrightarrow{B_0}$
w.r.t. molecular frame



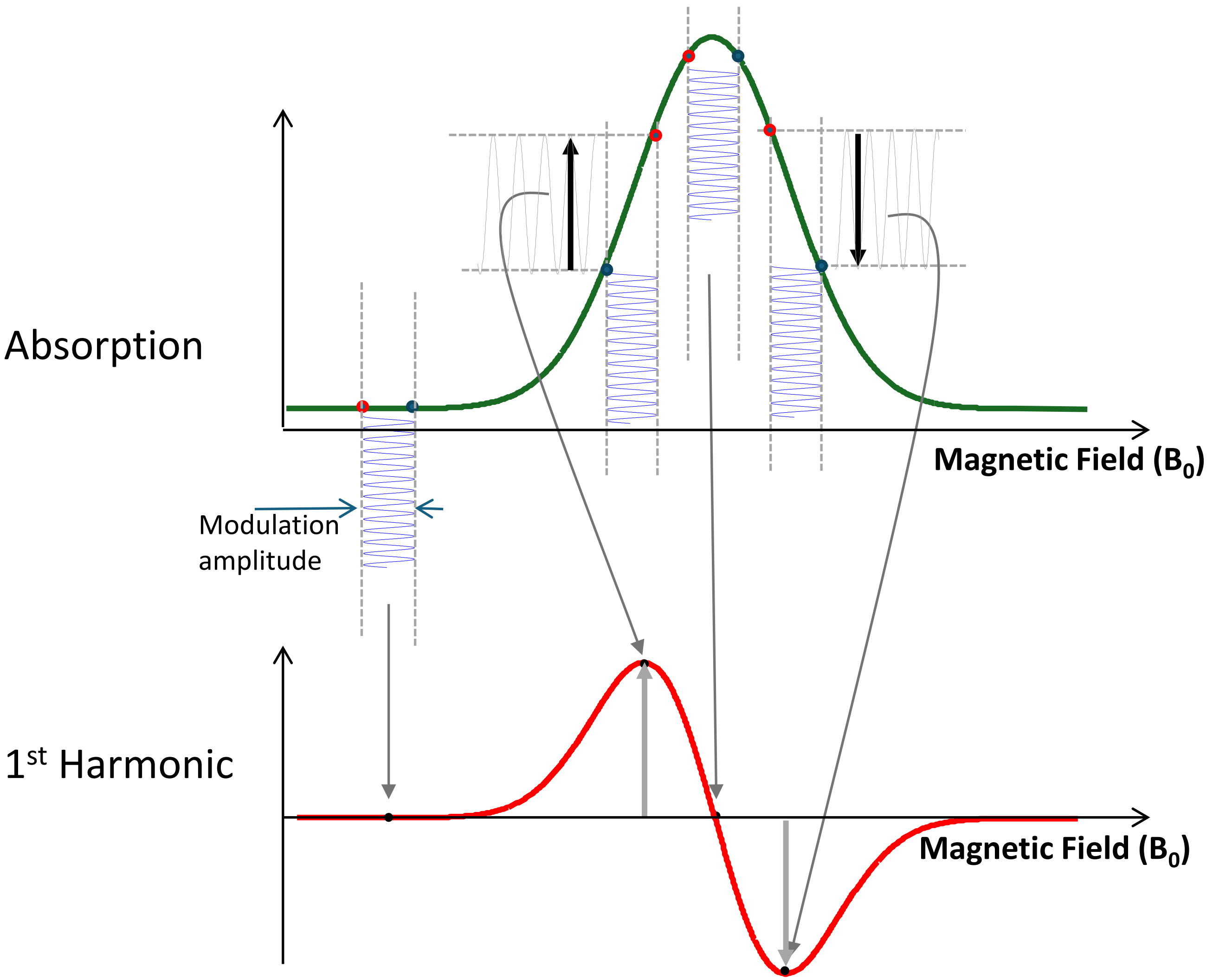
All possible
resonances

$$g_{eff} = \sqrt{g_x^2 l_x^2 + g_y^2 l_y^2 + g_z^2 l_z^2}$$

Powder averaging – sum of all possible orientations.

Lowest (g_z) and highest (g_x) g-values – hardly ever occur (low intensity)
Resonances at the middle g-value (g_y) – most occurring (highest intensity)

Field modulation, lock-in amplification



The amplitude and phase of the modulation is compared to a reference signal

We effectively record the 1^{st} derivative of the absorption line.

Types of shapes (g-anisotropy only)

Principal g-values can be obtained from the features of the spectra

Isotropic case

$g_x = g_y = g_z = \boldsymbol{g_{iso}}$

Axial case

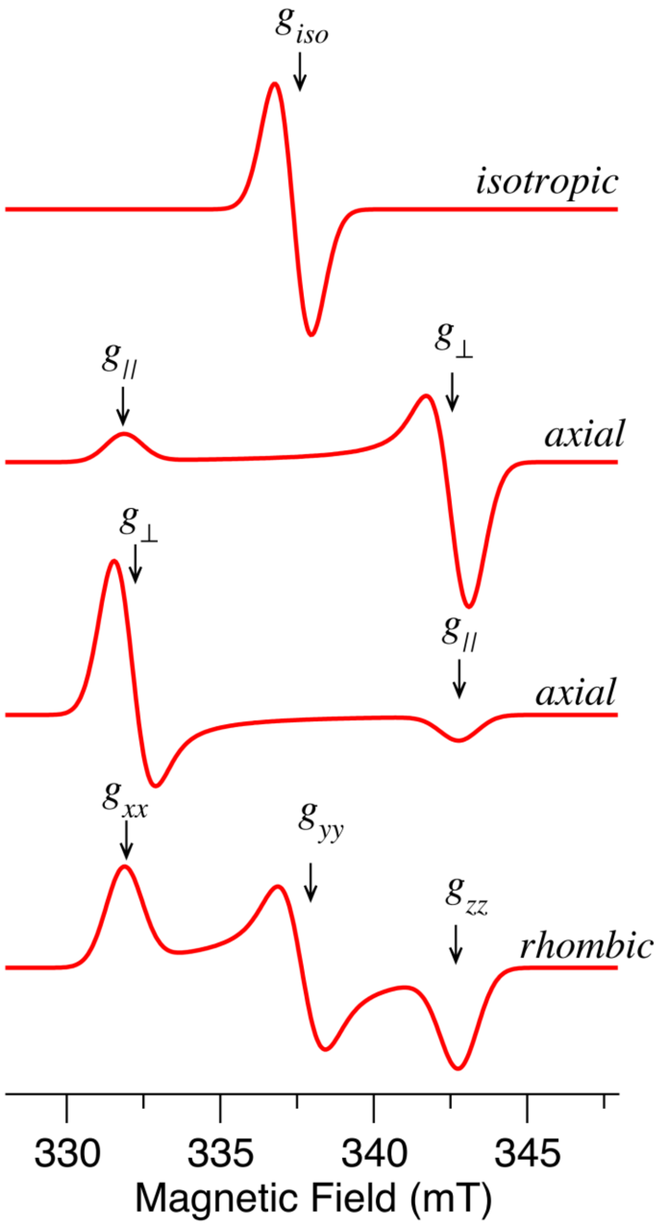
$g_x = g_y = \boldsymbol{g_\perp}, g_z = \boldsymbol{g_\parallel}$

it is common practice to assign $g_\parallel$ to g_z

Rhombic case

$\boldsymbol{g_x \neq g_y \neq g_z}$

exact assignment to the molecular frame depends on how much is known about the system (sometimes not much at all)



g-anisotropy

$$g_{ij} = g_e + 2\lambda \sum_n \frac{\langle \phi_0 | L_i | \phi_n \rangle \langle \phi_n | L_j | \phi_0 \rangle}{E_n - E_0}$$

- Organic free-radicals

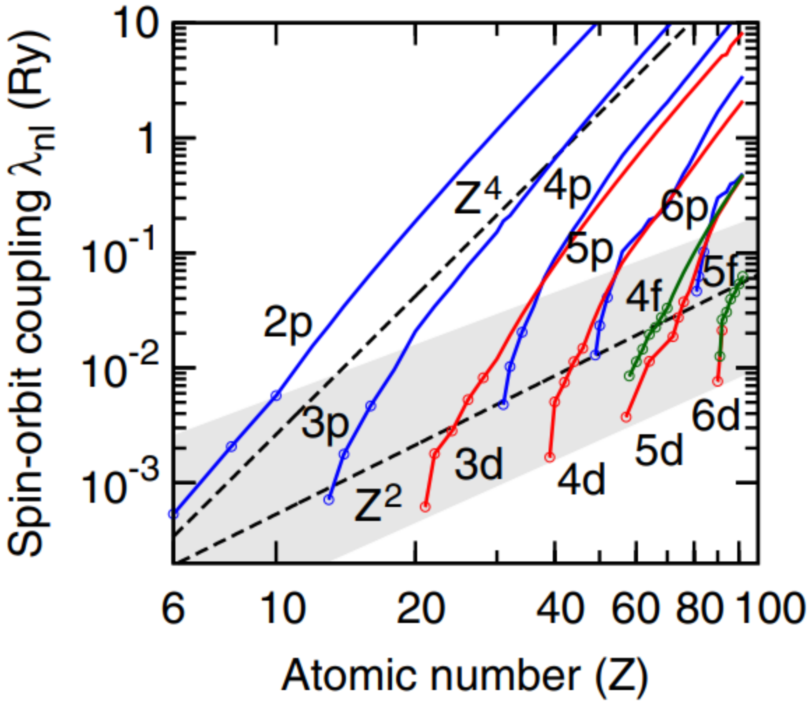
  large ΔE, small λ
  - hydrocarbon radicals: $g \approx 2.002 - 2.003$
  - N/O-based radicals: $g \approx 2.003 - 2.006$
  - S-based radicals : $g \approx 2.007 - 2.010$

- Transition metal ions

  (relatively) small ΔE, large λ
  - varies dramatically from system to system.
  - can be below or above $g_e = 2.0023$

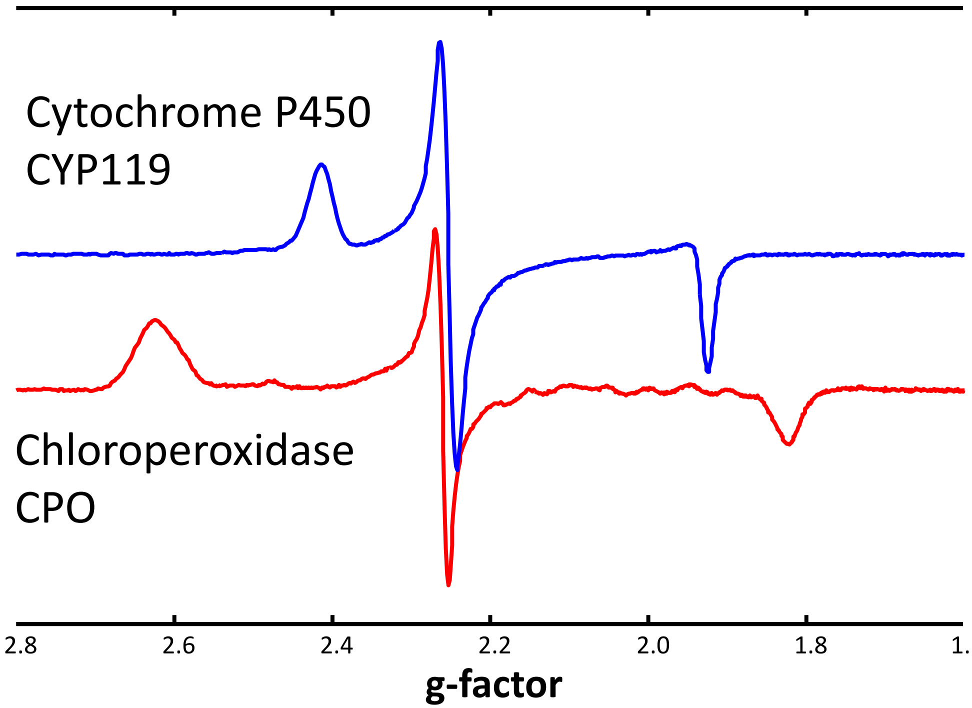
    V(IV)O, d^1,$g \approx 1.9 - 1.99$

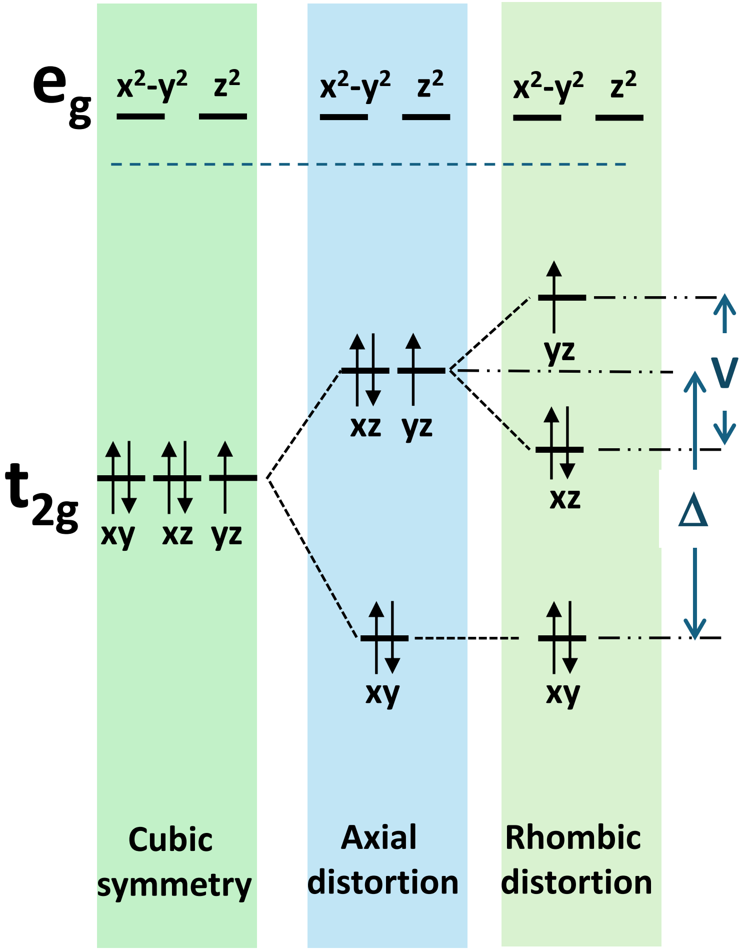
    Cu(II), d^9,$g \approx 2.1 - 2.2$

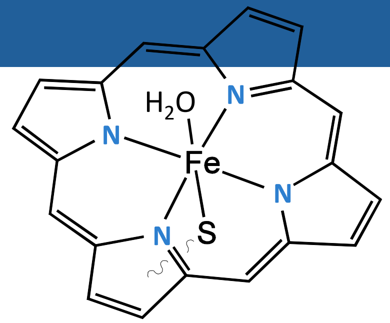


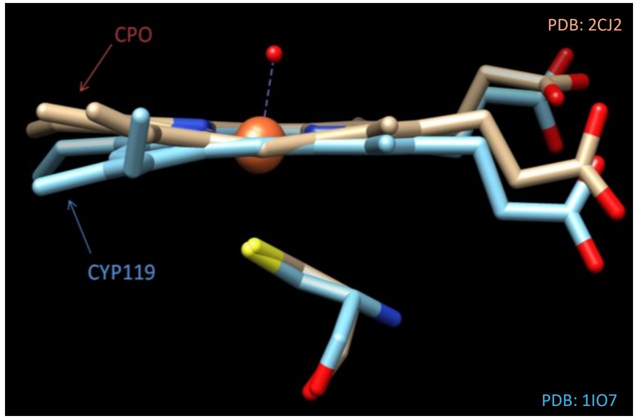
https://journals.aps.org/prb/pdf/10.1103/PhysRevB.92.035135

Example: low spin Ferric heme









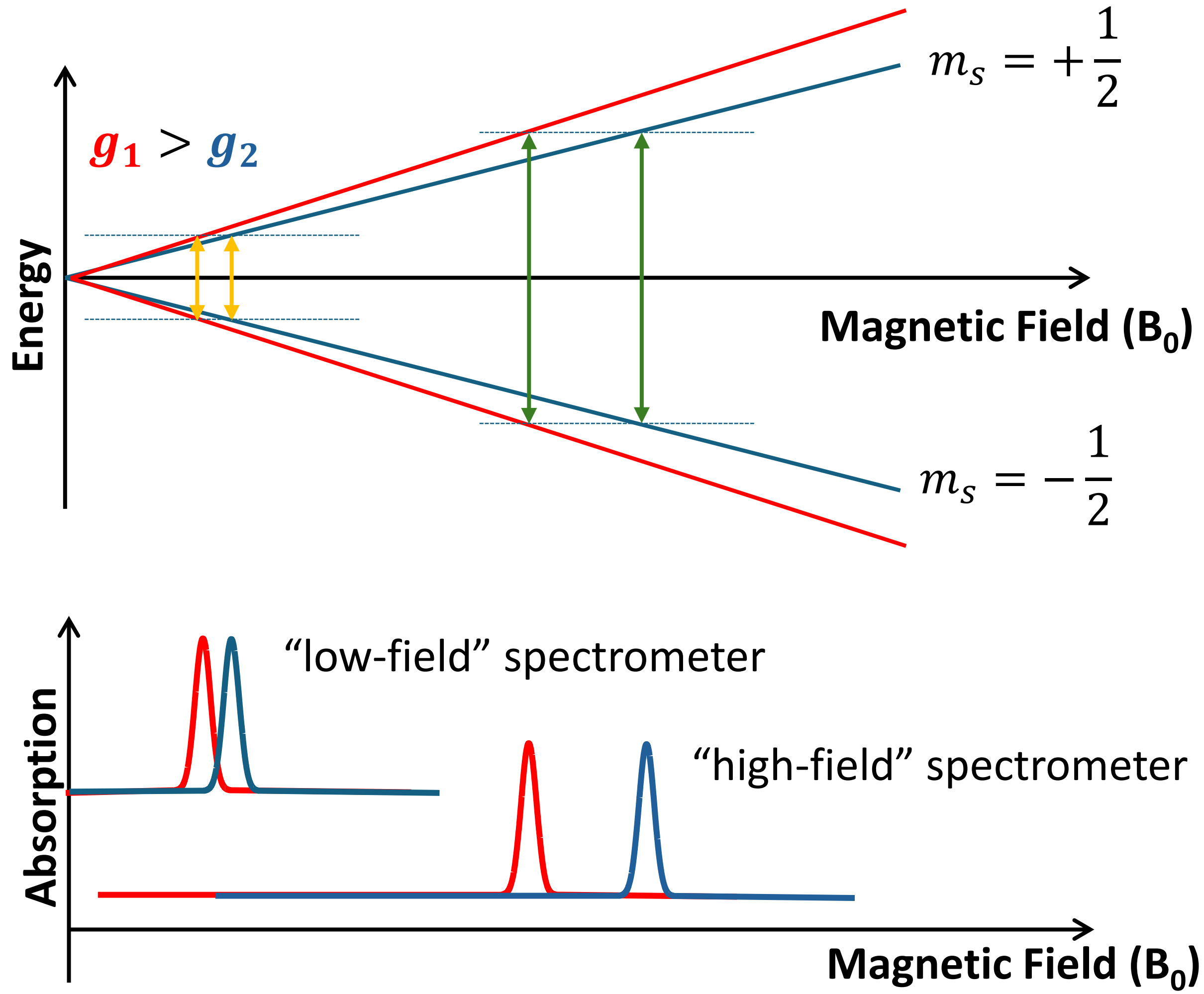
$$\frac{V}{\lambda} = \frac{g_x}{g_z+g_y} + \frac{g_y}{g_z-g_x}$$

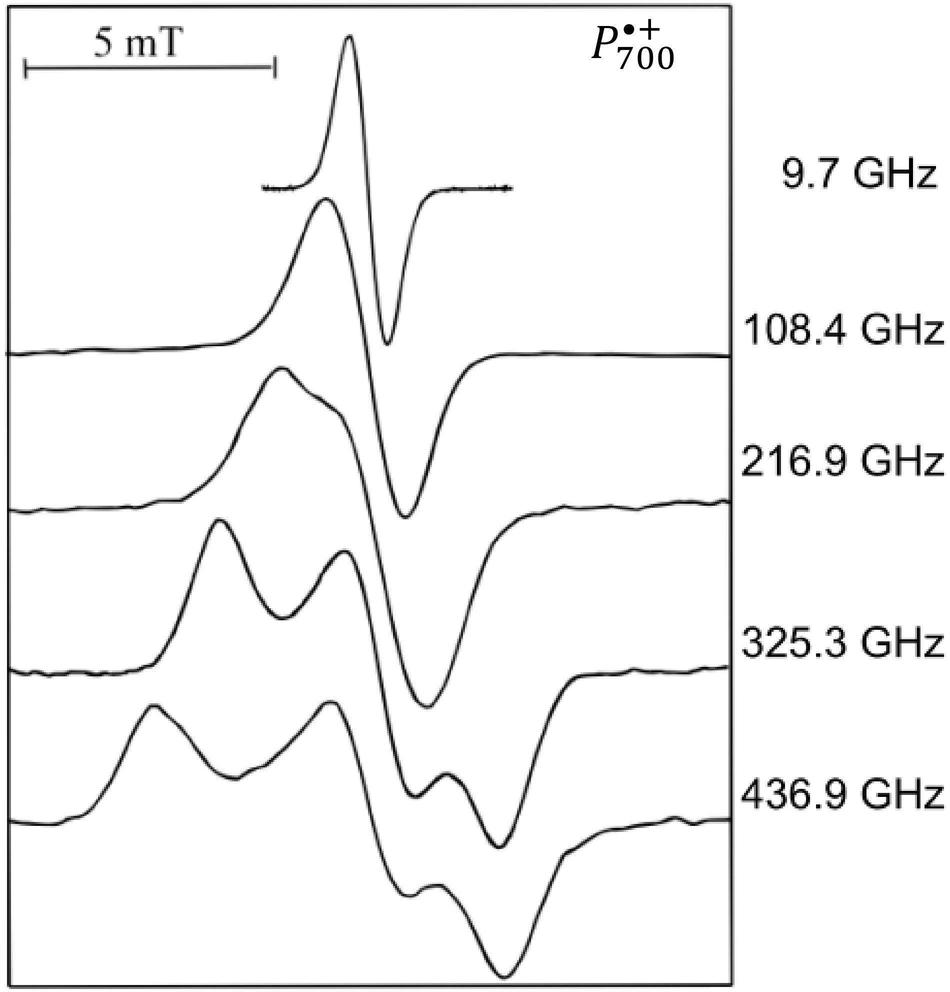
$$\frac{\Delta}{\lambda} = \frac{g_x}{g_z+g_y} + \frac{g_z}{g_z-g_x} - \frac{V}{\lambda}$$

Taylor CPS, Biochim. Biophys. Acta, 491 (1), 28: Pages 137–148 (1977)
https://doi.org/10.1016/0005-2795(77)90049-6

Radicals

Some radicals as so isotropic, they require a high field to resolve g-anisotropy





g: [2.00317, 2.00264, 2.00226]
Bratt et al. J.Phys.Chem.B (1997) 101 9689-9689

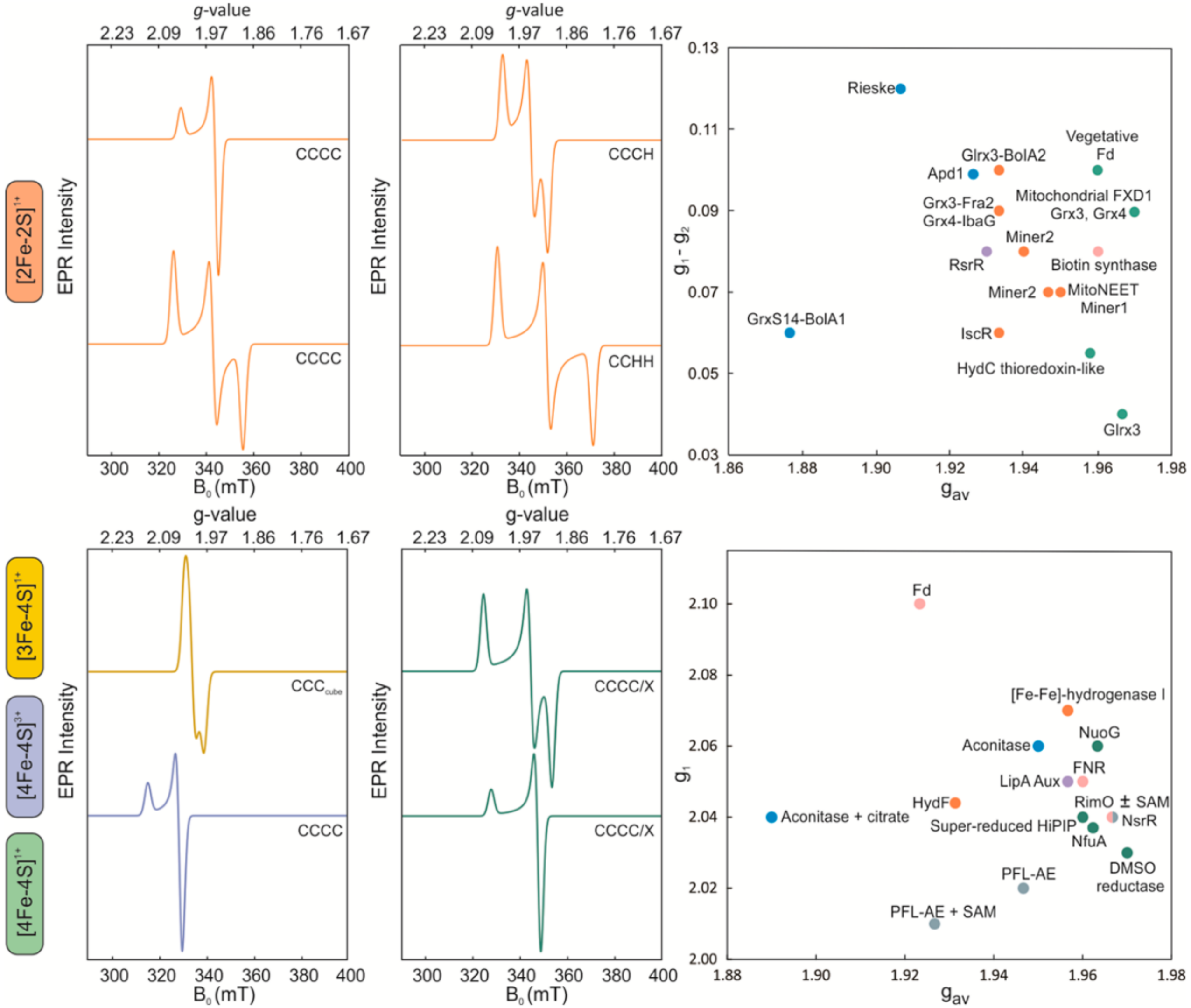
FeS clusters etc.

Coupled clusters are a more complicated case.

While the EPR spectrum may be relatively simple…
Note that we (typically) only observe the ground state multiplet.

Apparent g-anisotropy:

- + SOC parameters of individual ions (intrinsic g,D)
- + valence delocalization (“double exchange”)
- + spin-spin interactions (exchange)



Quist et al. Inorganics 2023, 11(12), 475

Hyperfine interaction

Interaction between unpaired electron and a magnetic nucleus leads to splitting of energy levels

Isotropic contribution:

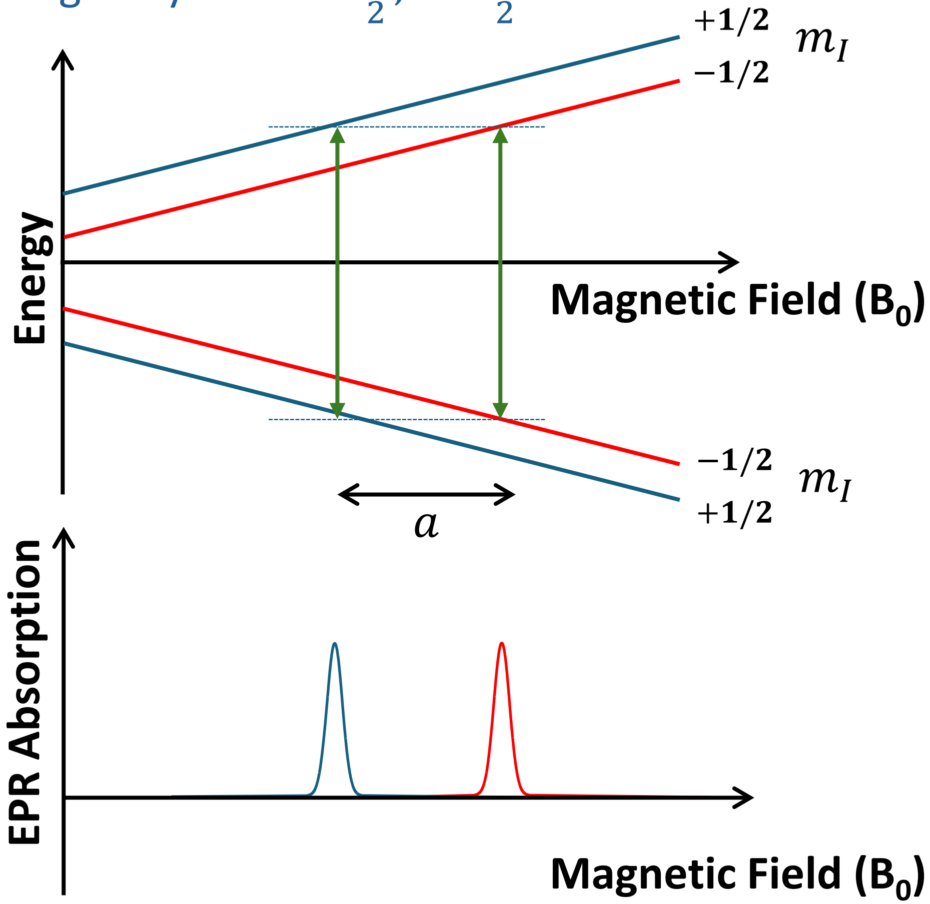
Direct spin density at the nucleus

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

Anisotropic contribution (dipolar):

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

single crystal. $S = \frac{1}{2}, I = \frac{1}{2}$

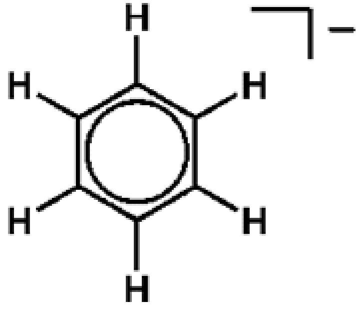


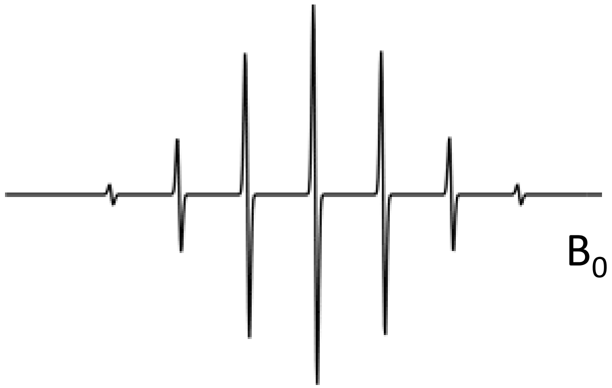
Isotropic HF coupling

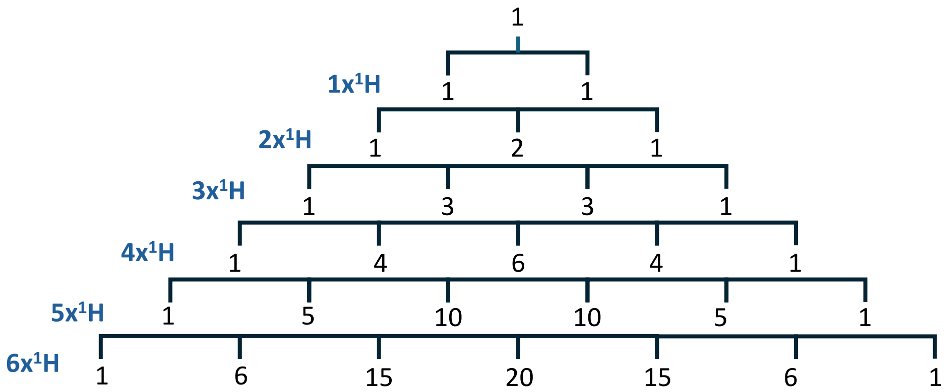
Each nucleus adds to the splitting

Extreme case of high symmetry:

Benzene Radical Anion





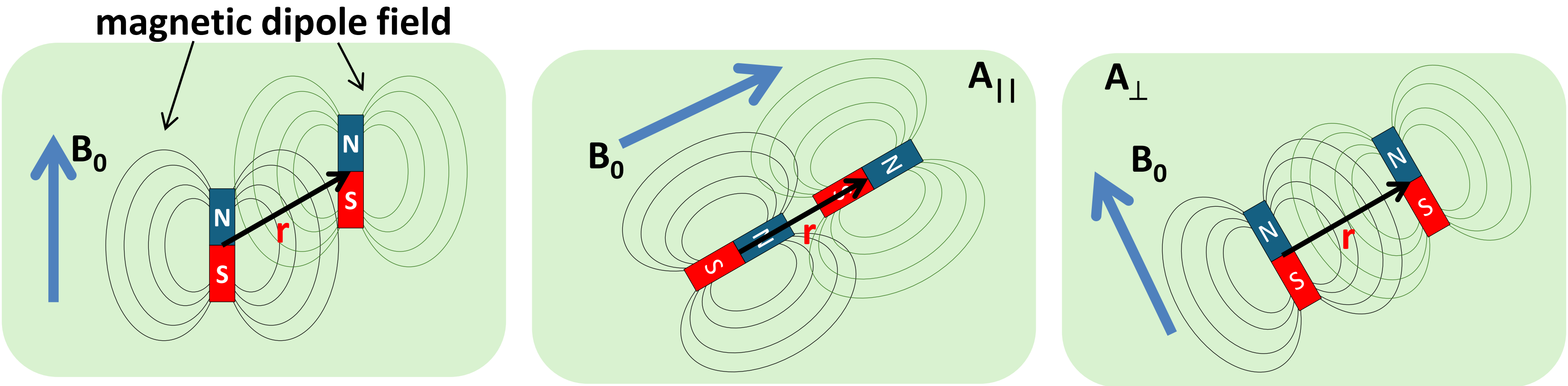


McConnel rule for ^{1}H HF couplings on C_α:

$A^{\alpha}_{iso} = \rho^{\alpha}_{\pi} Q_\alpha$, ρ^{α}_{π}-spin density on C_α, Q_α-proportionality constant

Dipolar HF coupling

“classical” interpretation: interaction of two spins (nuclear and/or electron) through space:



attraction *repulsion*

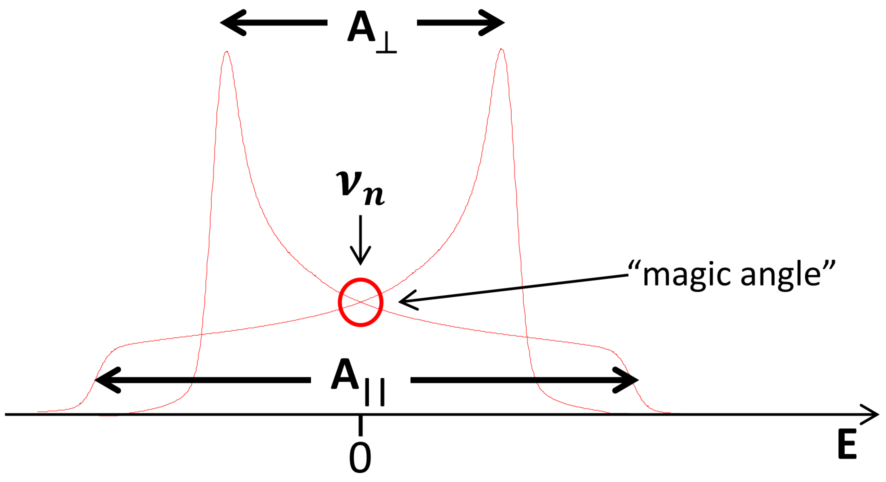
Energy of the interaction change sign as we rotate the sample in external magnetic field

Pure Hyperfine dipole-dipole interaction:

$$A_{x,y} = A_\perp = T$$

$$A_z = A_{||} = -2T$$

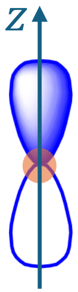
$$T = \underbrace{\frac{\mu_0}{4\pi h}\beta_e\beta_n}_{\text{just constants}} \cdot \frac{g_e g_n}{r^3}$$



Anisotropic HF coupling

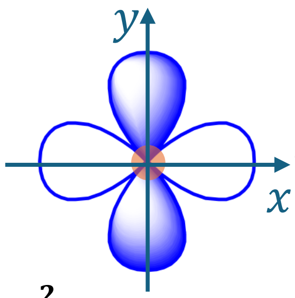
Nucleus at the center of anisotropic electron spin density (e.g. metal centers ^{57}Fe, $^{61/63}$Cu etc.) will exhibit anisotropic HF interaction

For example:



$$\left\{\begin{array}{l} a_z = a_{iso} + \frac{4}{5}a^2 P_p \\ a_{x,y} = a_{iso} - \frac{2}{5}a^2 P_p \end{array}\right.$$

$\boldsymbol{p_z}$



$$\left\{\begin{array}{l} a_z = a_{iso} + P_p\left[-\frac{4}{7}a^2 + \delta g_{SOC}^{z}\right] \\ a_{x,y} = a_{iso} + P_p\left[+\frac{2}{7}a^2 + \delta g_{SOC}^{xy}\right] \end{array}\right.$$

$\boldsymbol{d_{x^2-y^2}}$

a - covalency, e.g., amount of p (or d) character in MO

P_p, P_d- proportionality constants

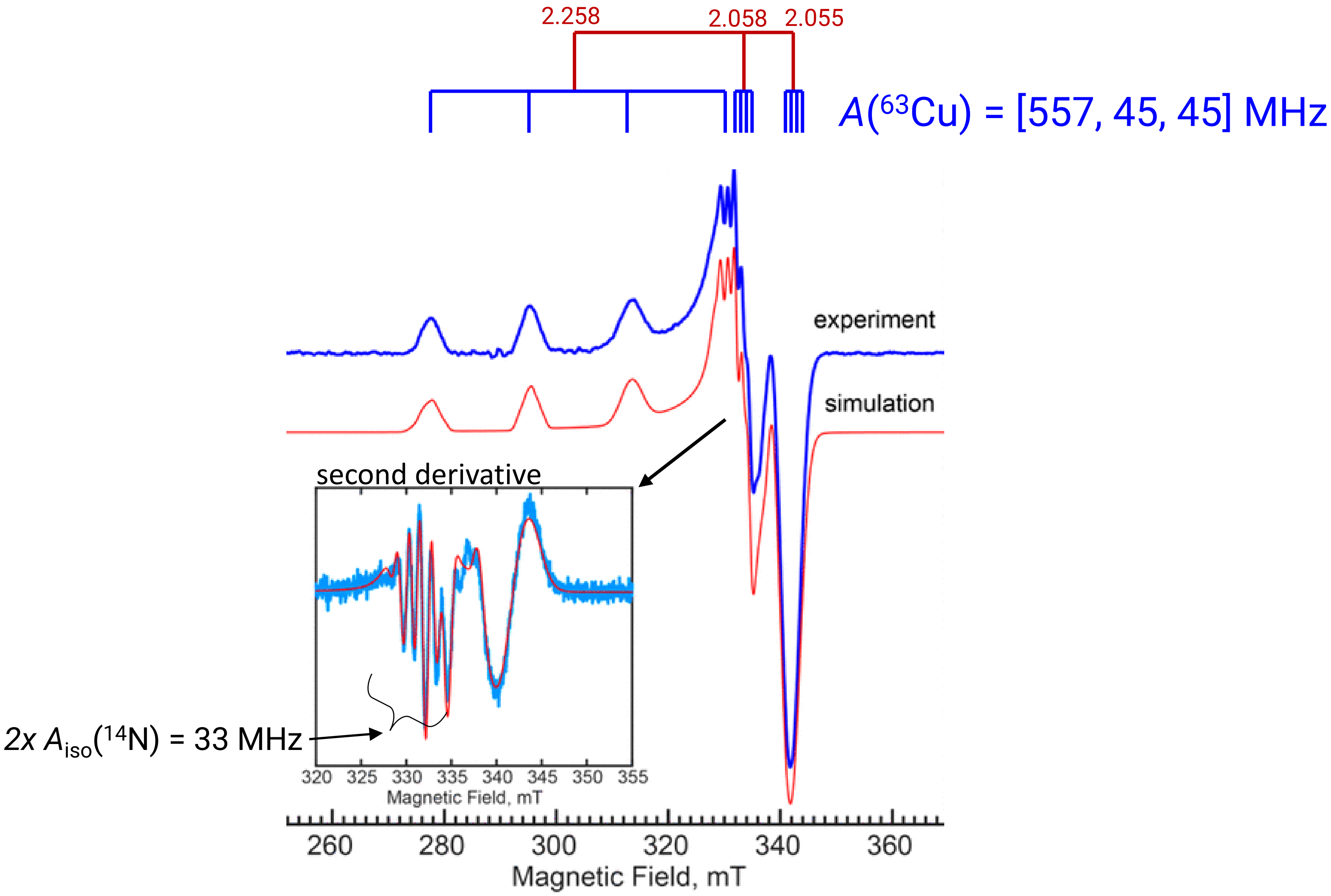
δg_{SOC}-contributions from spin-orbit coupling (SOC)

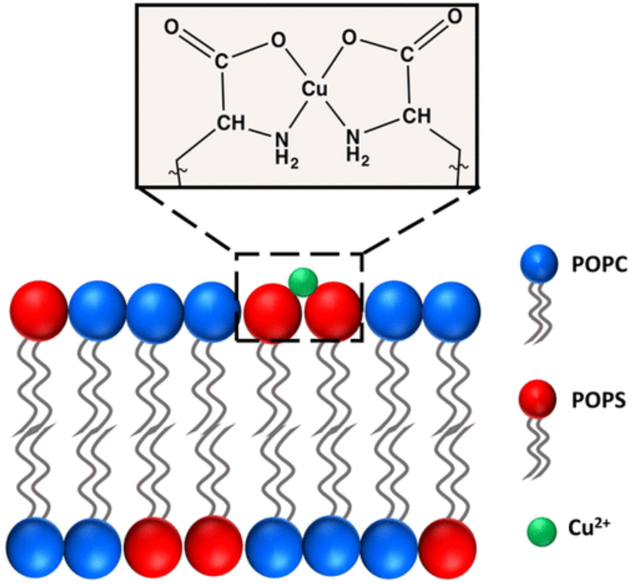
a_{iso}- originating from a pseudo-fermi contact (think of it as *s-p*, *s-d* MO mixing)

J.R. Morton and K.F. Preston, *J.Mag.Res.* 1978, 30, 577

P.H. Rieger, *J.Mag.Res.* 1997, 124, 140

Example: Cu^{2+} EPR



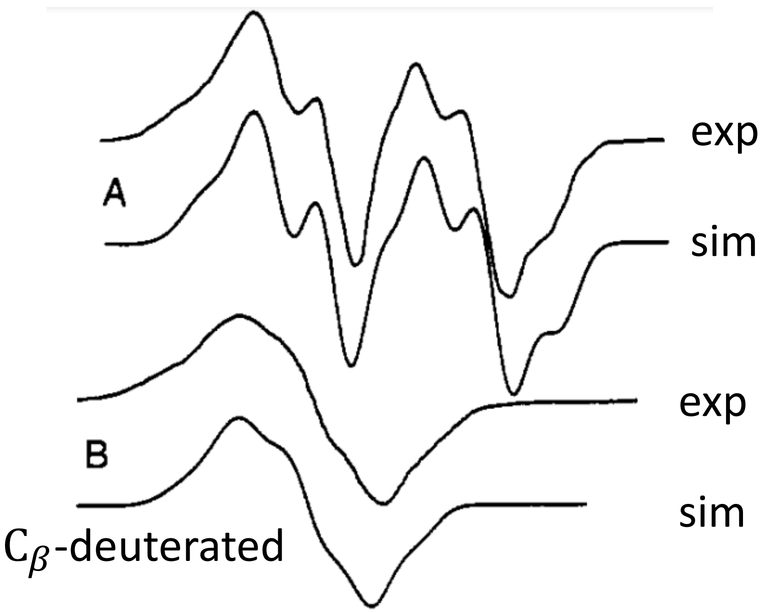


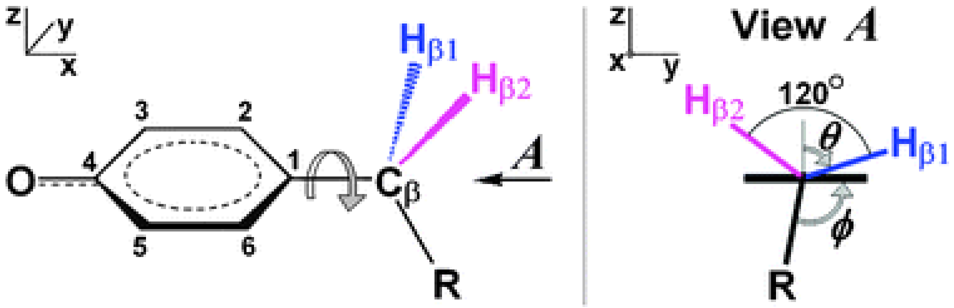
*note that ^{63}Cu and ^{61}Cu isotopes have similar abundance.
Second simulation using g_n-scaled HFCs for the latter added into total simulation.

Kusler et al. J. Phys. Chem. B 2016, 120, 50, 12883−12889

Example: Tyrosyl radical

E.coli RNR





$$A_{iso}^{\beta_1} = \rho_{C1}(B' + B'' \cos^2 \Theta)$$

$$A_{iso}^{\beta_2} = \rho_{C1}(B' + B'' \cos^2\{\Theta - 120°\})$$

g_{xyz} = [2.0077, 2.0054, 2.0023]

$A_{xyz}^{3,5}$= [-9.6, -2.8, -7] MHz

$A_{xyz}^{\beta_1}$= [19.6, 19.6, 21.2] MHz

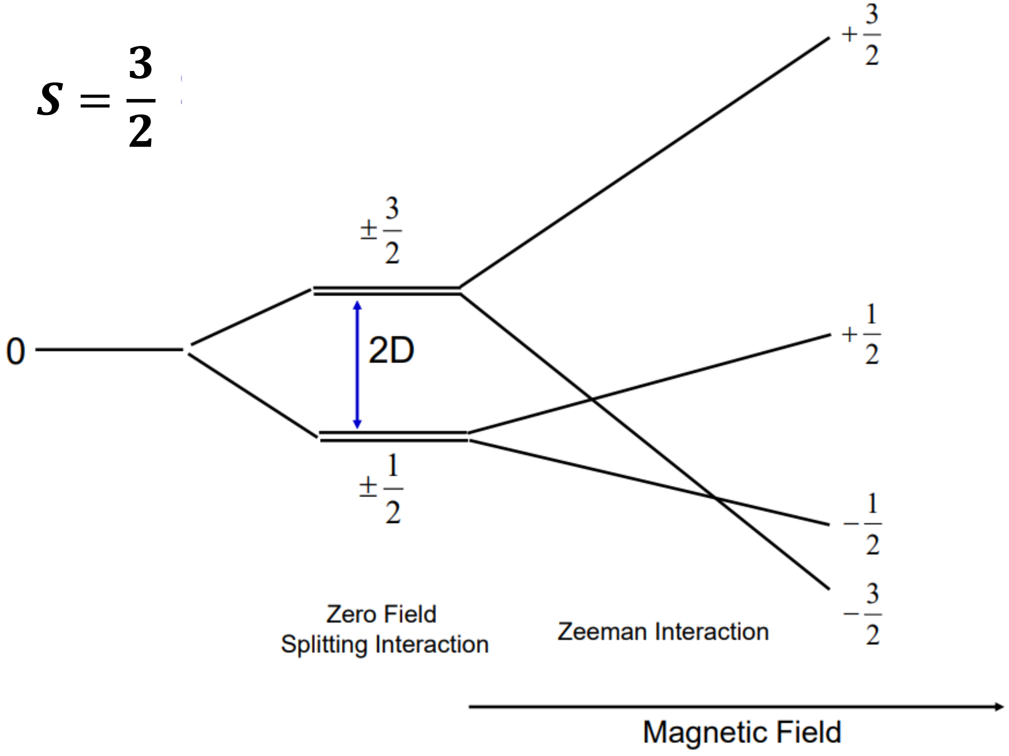
$A_{xyz}^{\beta_2}$= [-0.17, -0.17, 1.75] MHz

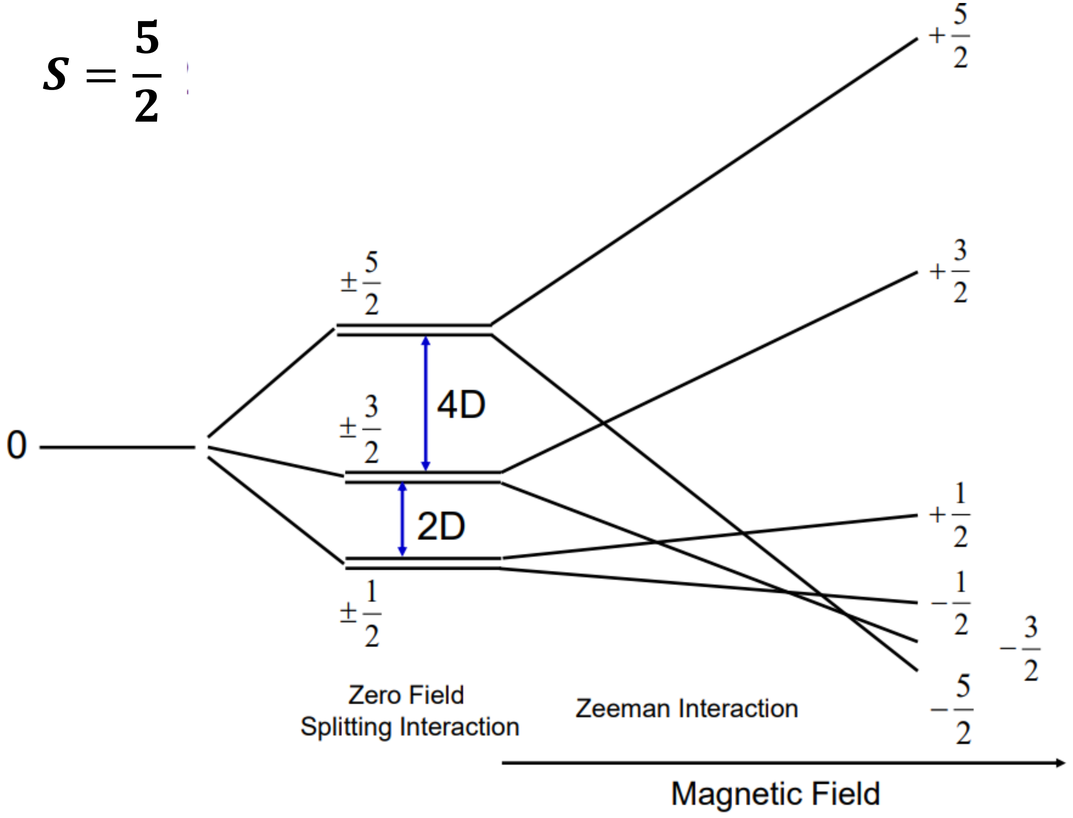
Hoganson, Babcock, Biochemistry, 1992, 31, 11874-11880

Zero Field Splitting

S>1/2: SOC and spin-spin coupling splits the spin states according to $|m_s|$

This effect has a profound effect on the EPR spectra





Zero Field Splitting

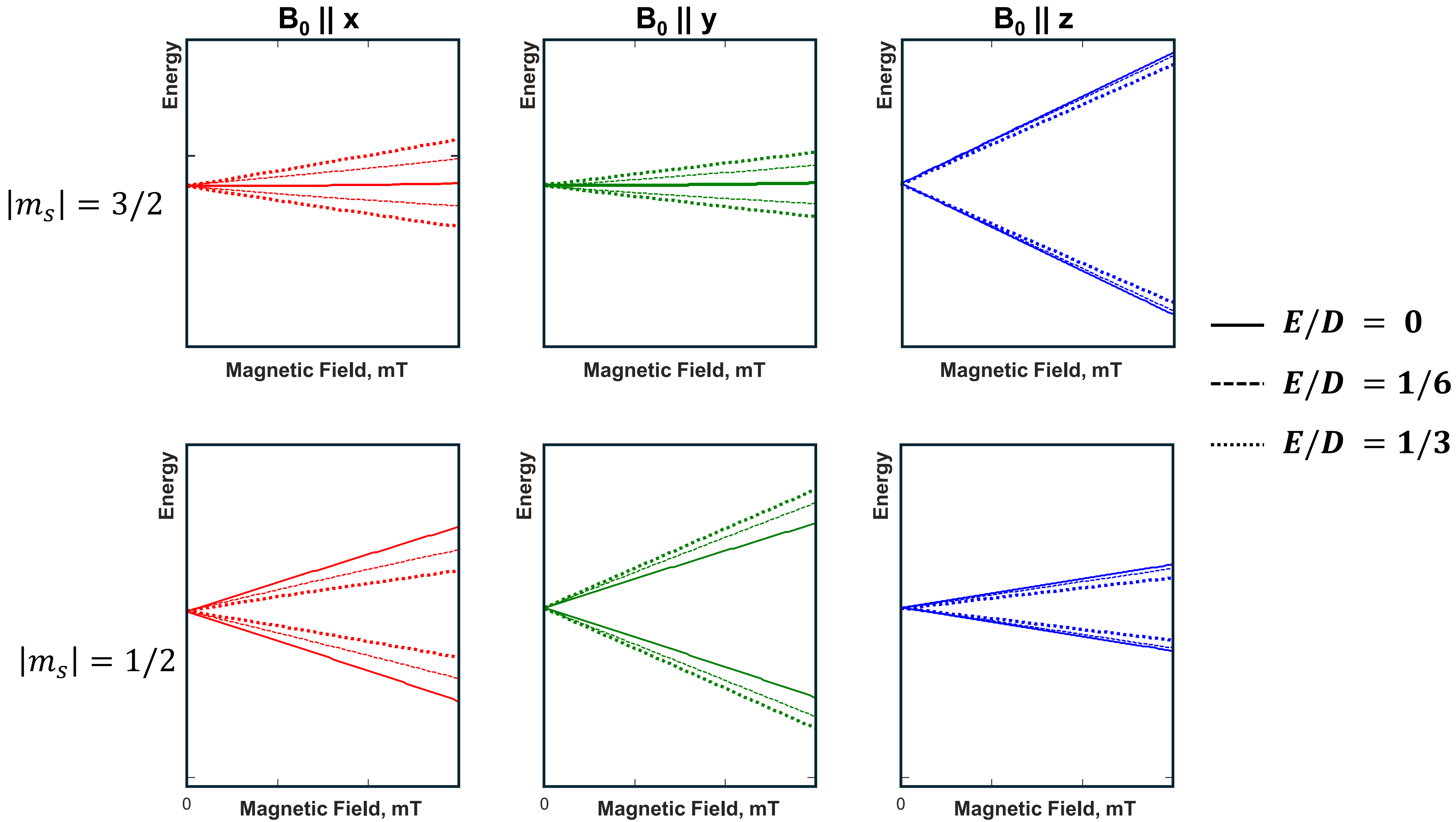
Addition to spin Hamiltonian: $\mathcal{H}_{ZFS} = \vec{\boldsymbol{S}}\boldsymbol{D}\vec{\boldsymbol{S}}$

In its principal axes: $\boldsymbol{D} = \begin{bmatrix} -\frac{1}{3}D + E & 0 & 0 \\ 0 & -\frac{1}{3}D - E & 0 \\ 0 & 0 & \frac{2}{3}D \end{bmatrix}$

High symmetry systems with little-to-no SOC, $D, E \cong 0$
Systems with an axial symmetry, $E \cong 0$

Note that “rhombicity” E takes values from 0 to $\frac{1}{3}D$

Zero Field Splitting (S=3/2)

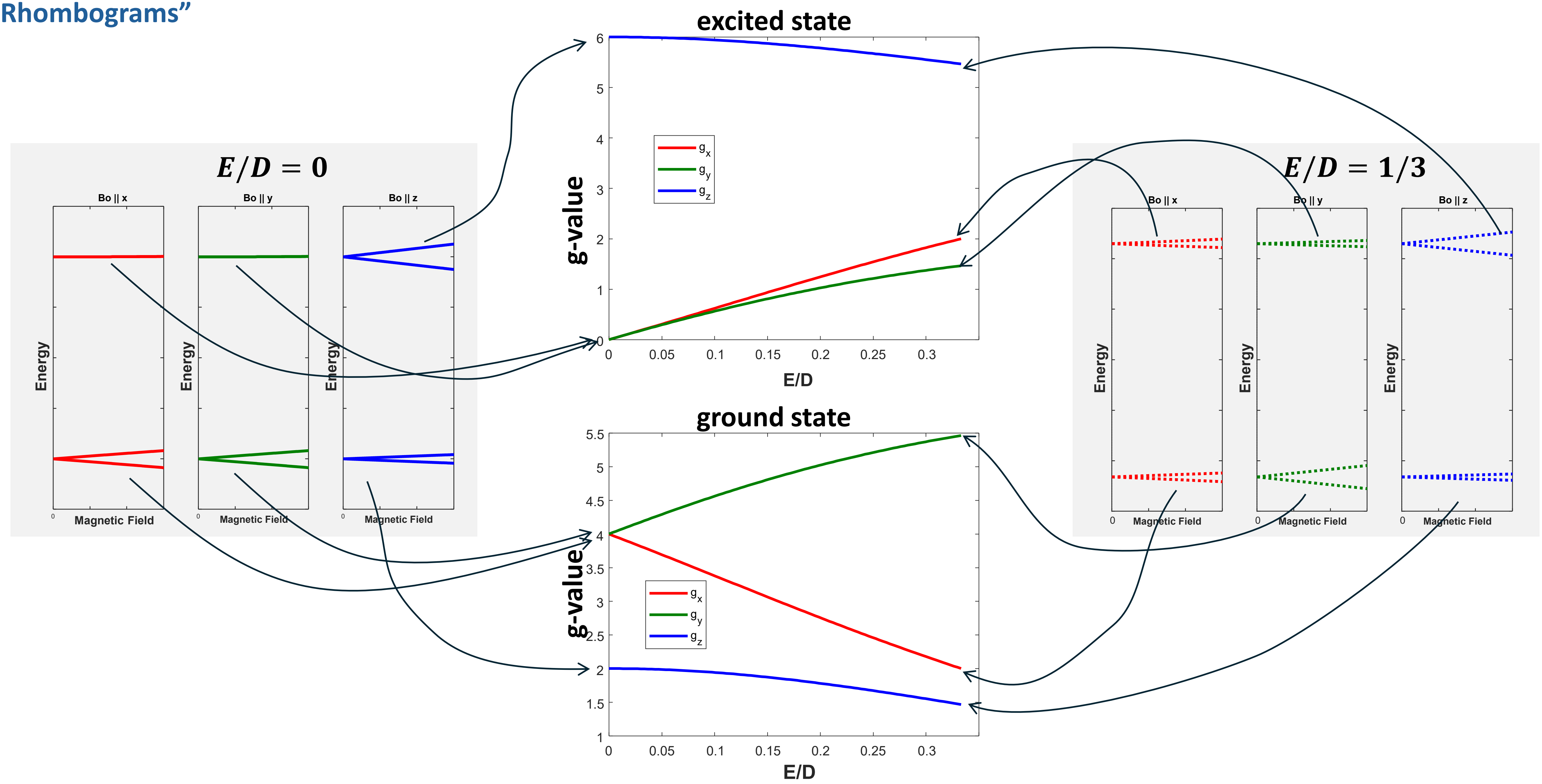


Wouldn't it be nice to streamline prediction of EPR spectra in terms of effective g-values?

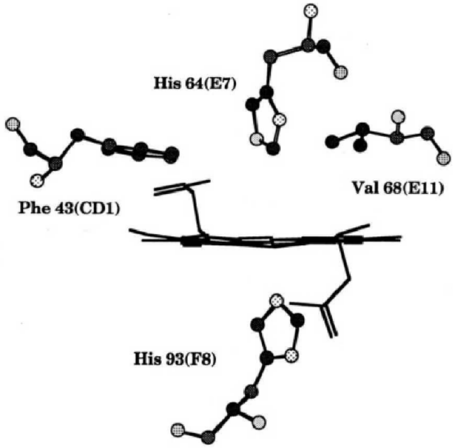
Zero Field Splitting (S=3/2)

Let’s treat every doublet as an effective $S = 1/2$
The slope of $E(B_0)$ represents effective g value

“Rhombograms”

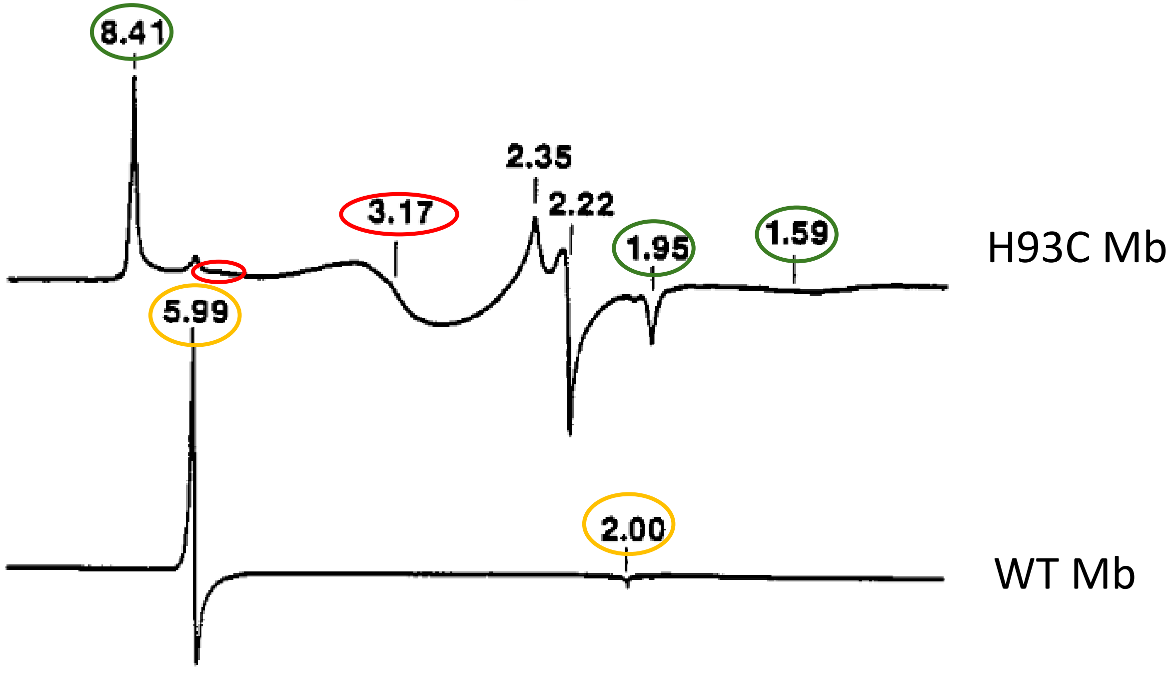


Example: High spin Human Myoglobin (S=5/2)

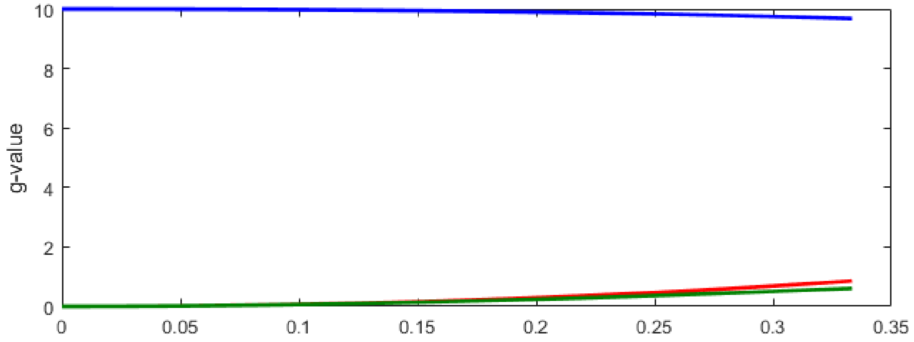


$|m_s| = 3/2$

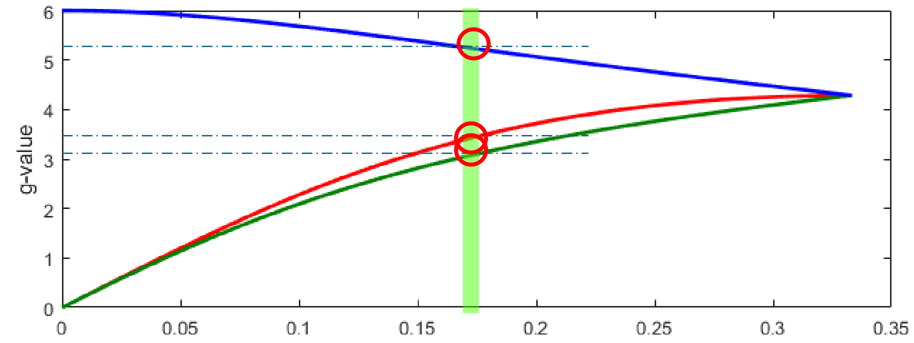
At 4.2 K



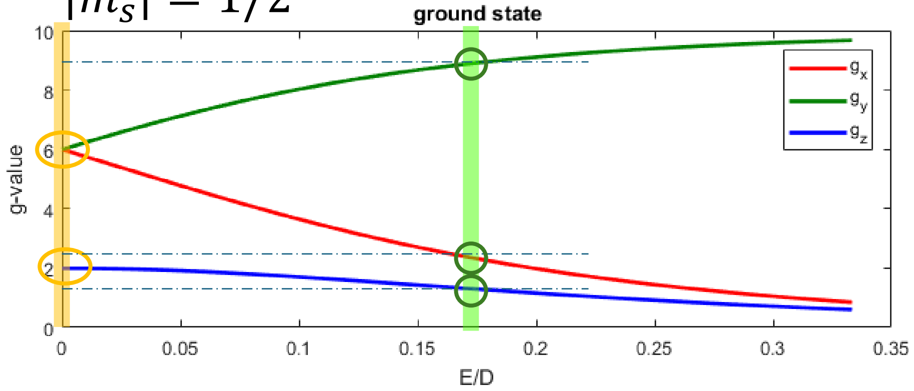
$|m_s| = 5/2$



$|m_s| = 3/2$

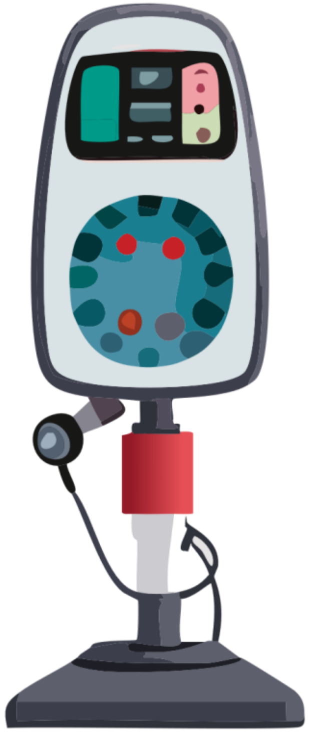


$|m_s| = 1/2$



Adachi et al. Biochemistry 1993, 32, 1, 241-252

Let’s talk about instrumentation



* “EPR spectrometer” according to AI

Spectrometer design

It is much easier to change magnetic field than do a broad-band sweep @ MW frequency

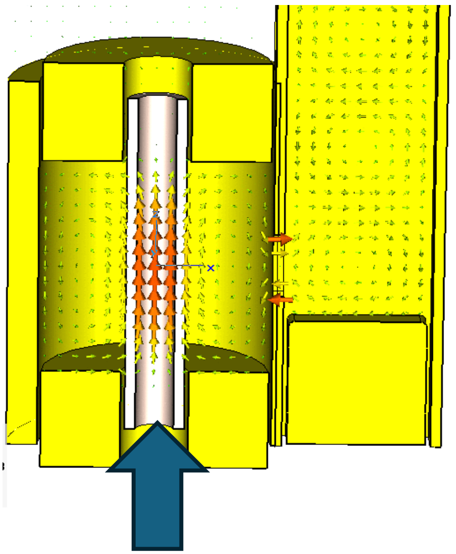
- Frequency is fixed within a range
- Magnetic field is adjusted

Resonators

- Really narrows the band, but substantially improves sensitivity

Cylindrical cavity

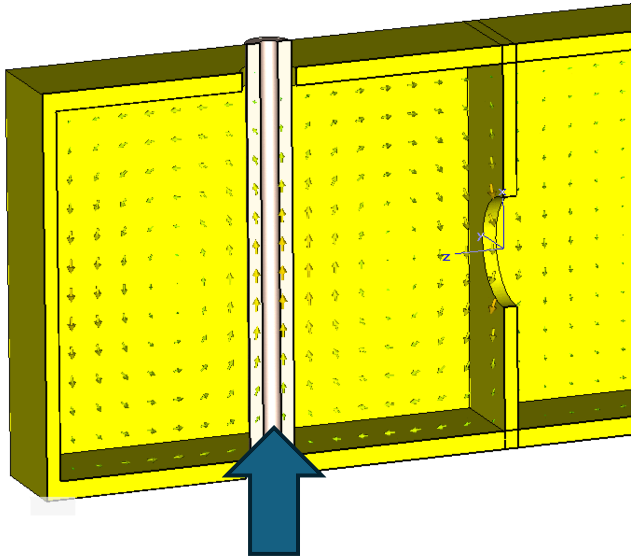
Silakov lab, Q-band pulse EPR



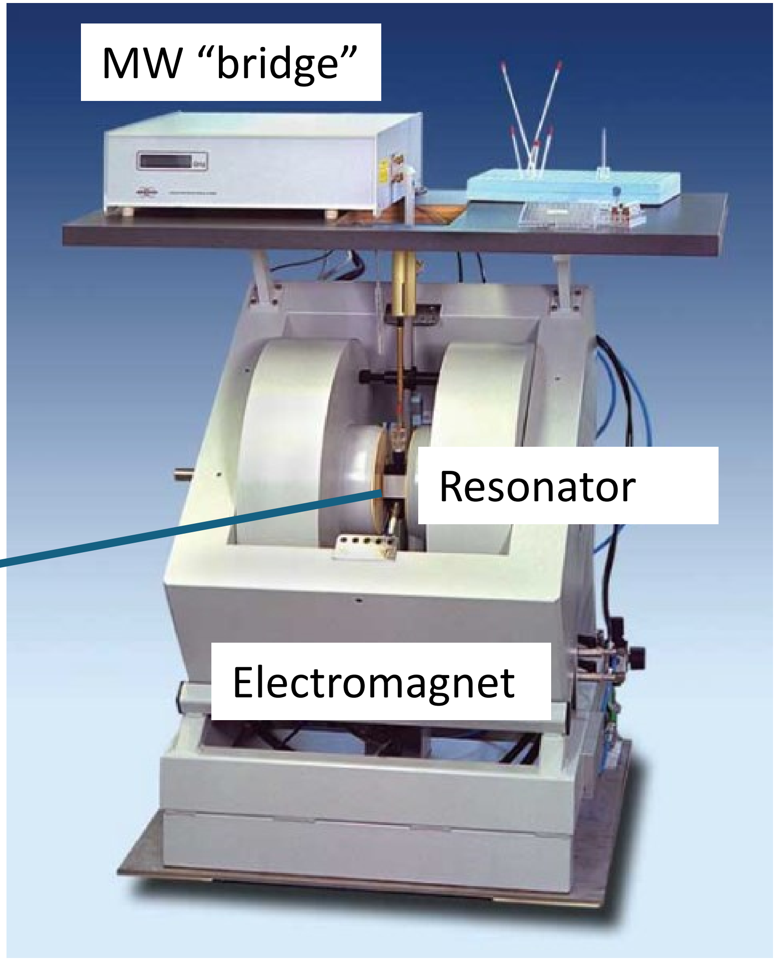
Sample

Rectangular cavity

Silakov lab, X-band CW EPR



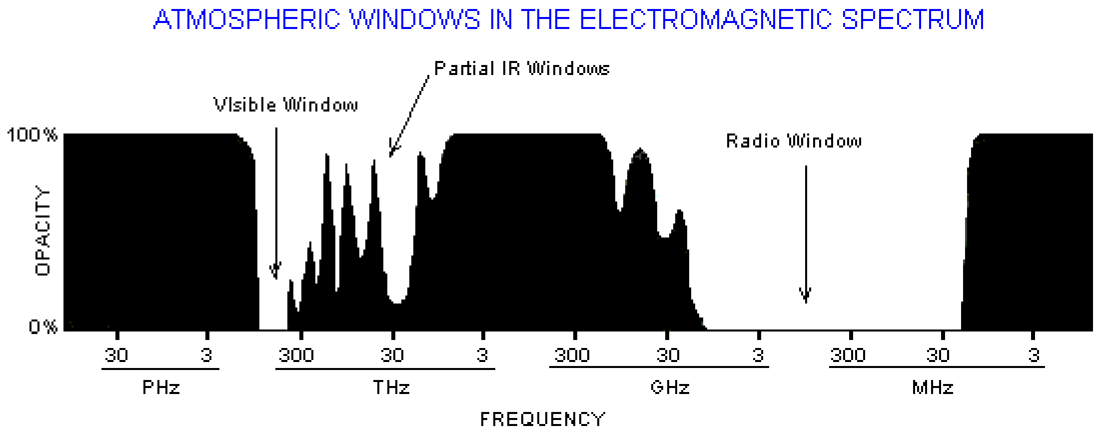
Sample



Spectrometer design

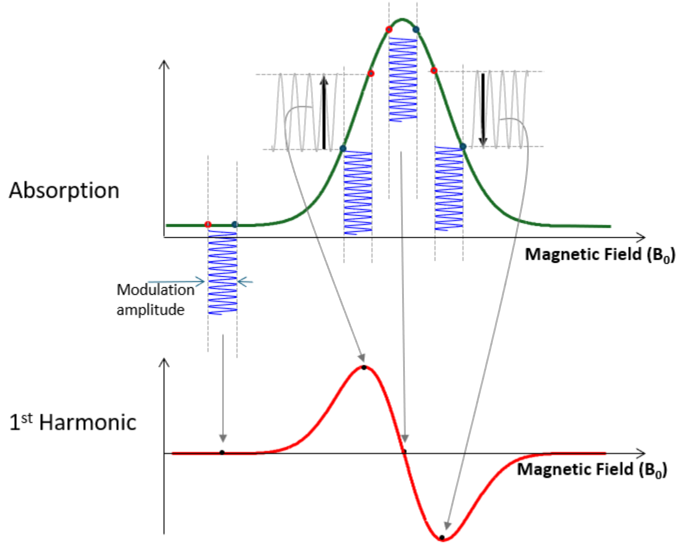
Historically, only microwave components at specific MW “bands” were available due to gaps in atmospheric EM absorption.

- ➢ EPR Spectrometers are operated at specific “bands”



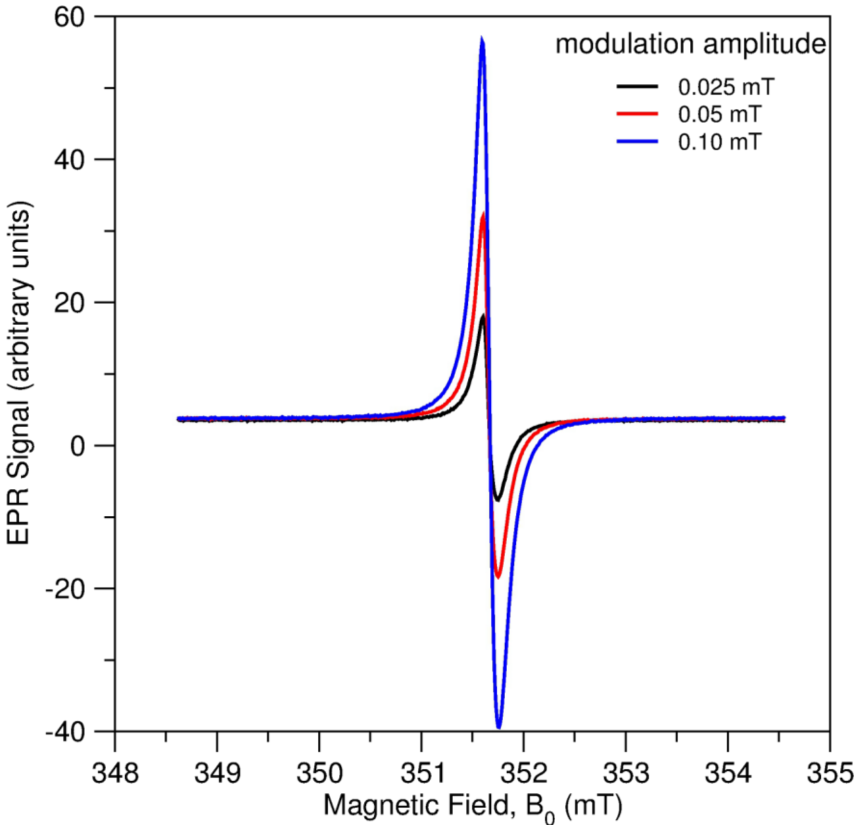
Frequency (GHz)	Frequency Band	Field for g=2.0023 (T)
1.2	L	0.043
2.4	S	0.086
9.5	X	0.34
34	Q	1.2
95	W	3.4
263	mm-band	9.4

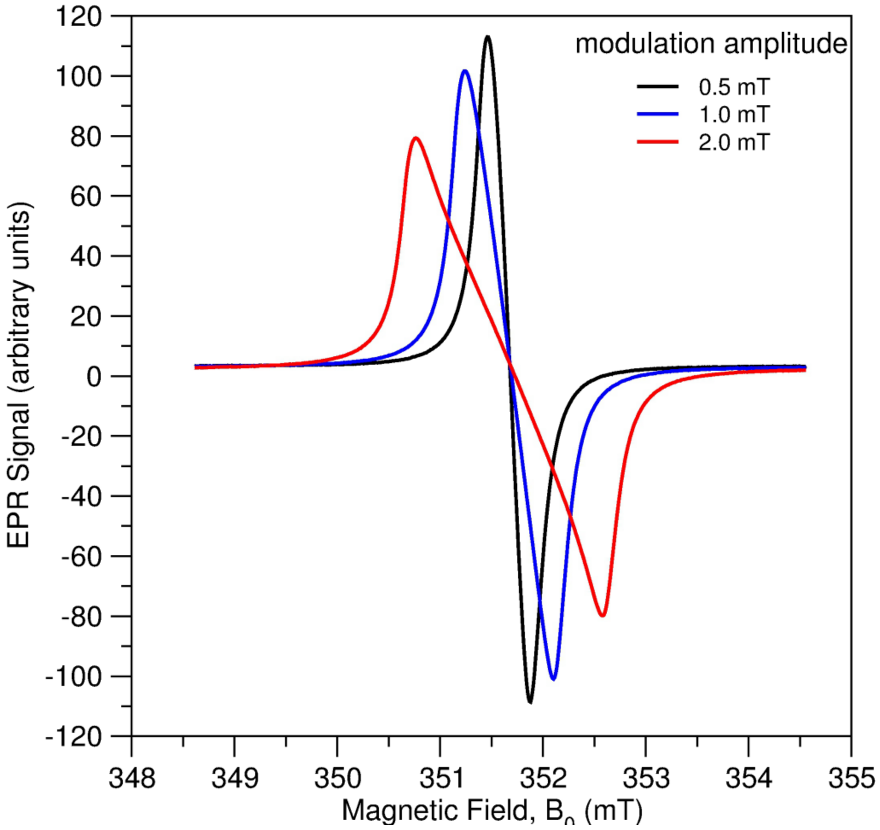
Field Modulation



Direct detection of low-power microwave frequencies is hard due to noise.

- ➢ Virtually all commercial continuous wave EPR instruments use field modulation





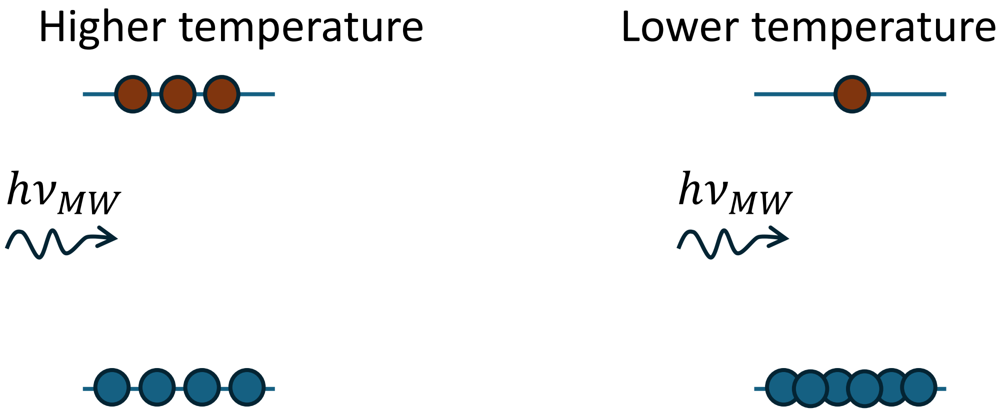
more ≠ better

Why cryogenic temperatures?

1. Combat Boltzmann population distribution

EPR signal depends on population difference between spin states:

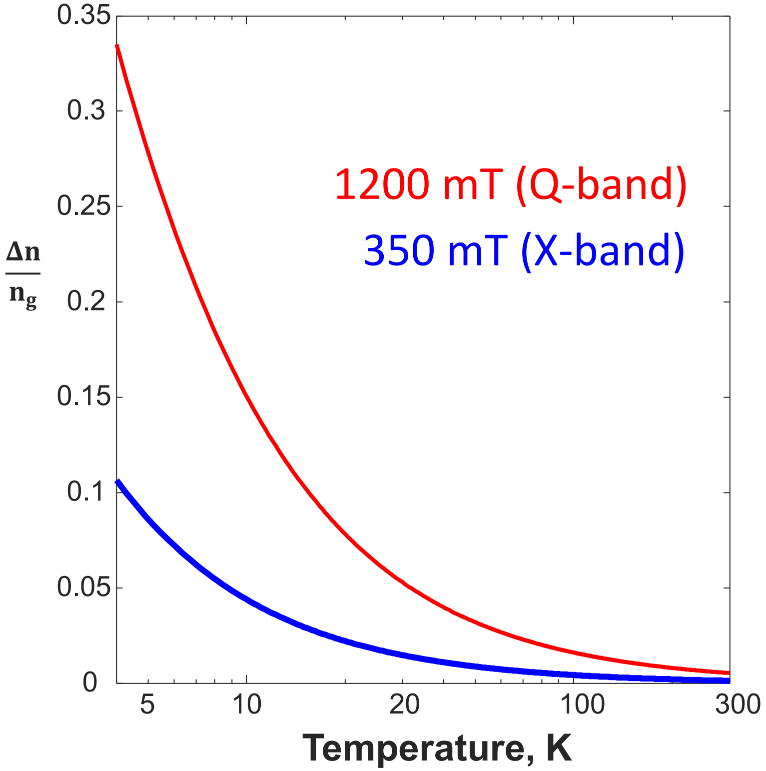
$$\frac{n_{excited}}{n_{ground}} = \exp\left(-\frac{g\beta_e B_0}{kT}\right)$$



Higher temperature

Lower temperature

Lower temperature = more signal (not the end of the story, though)

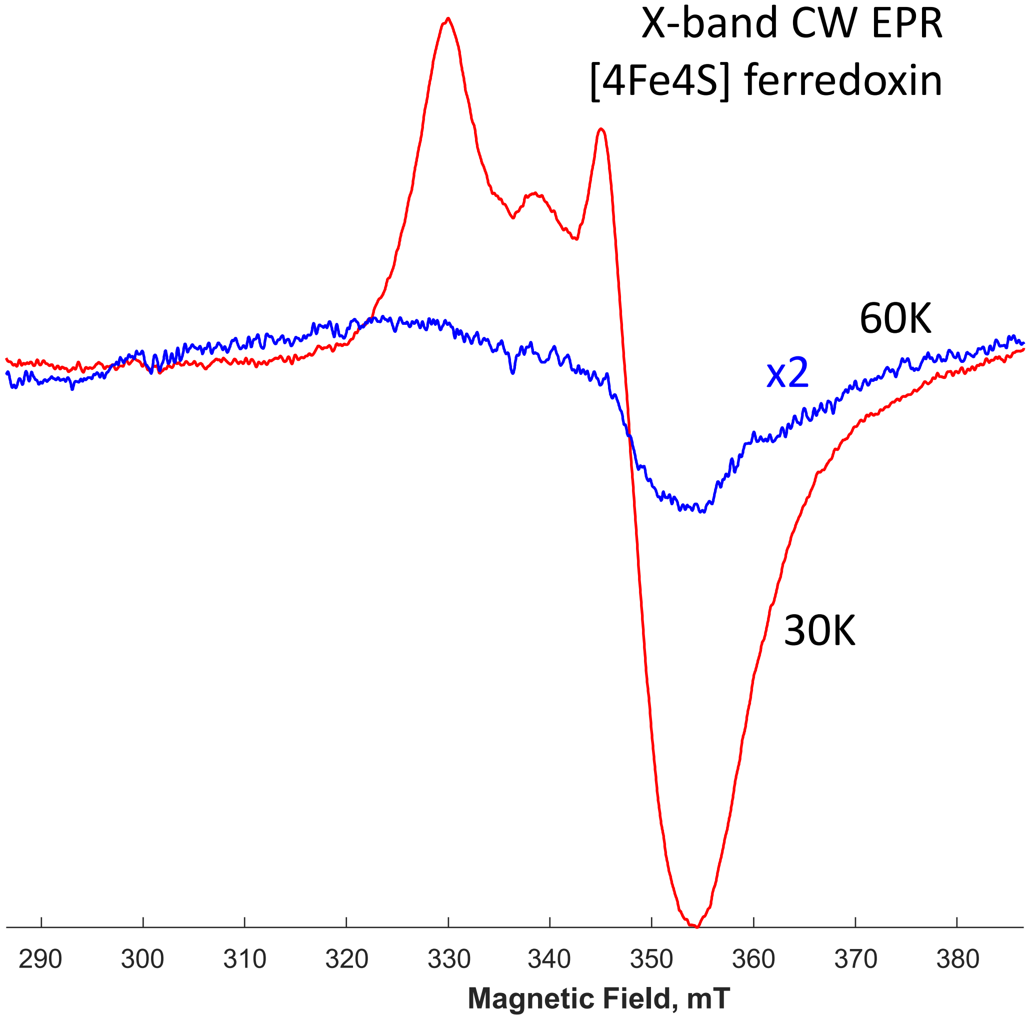


Why cryogenic temperatures?

2. Combat fast relaxation (line broadening)

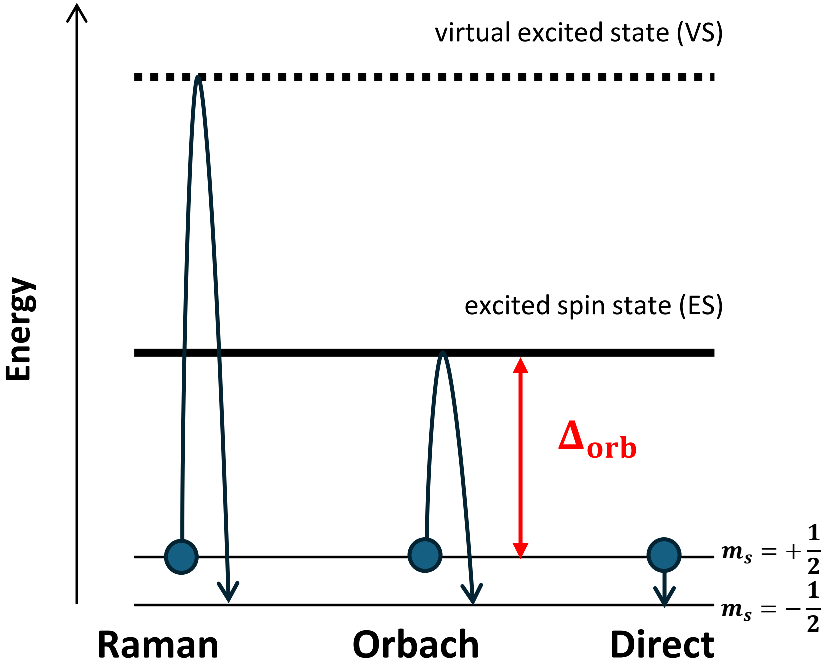
$$\Delta B \propto \frac{1}{T_1} + \frac{1}{T_2}$$

T_2 normally has a moderate temperature behavior
T_1 has strong temperature behavior



Relaxation of coupled systems

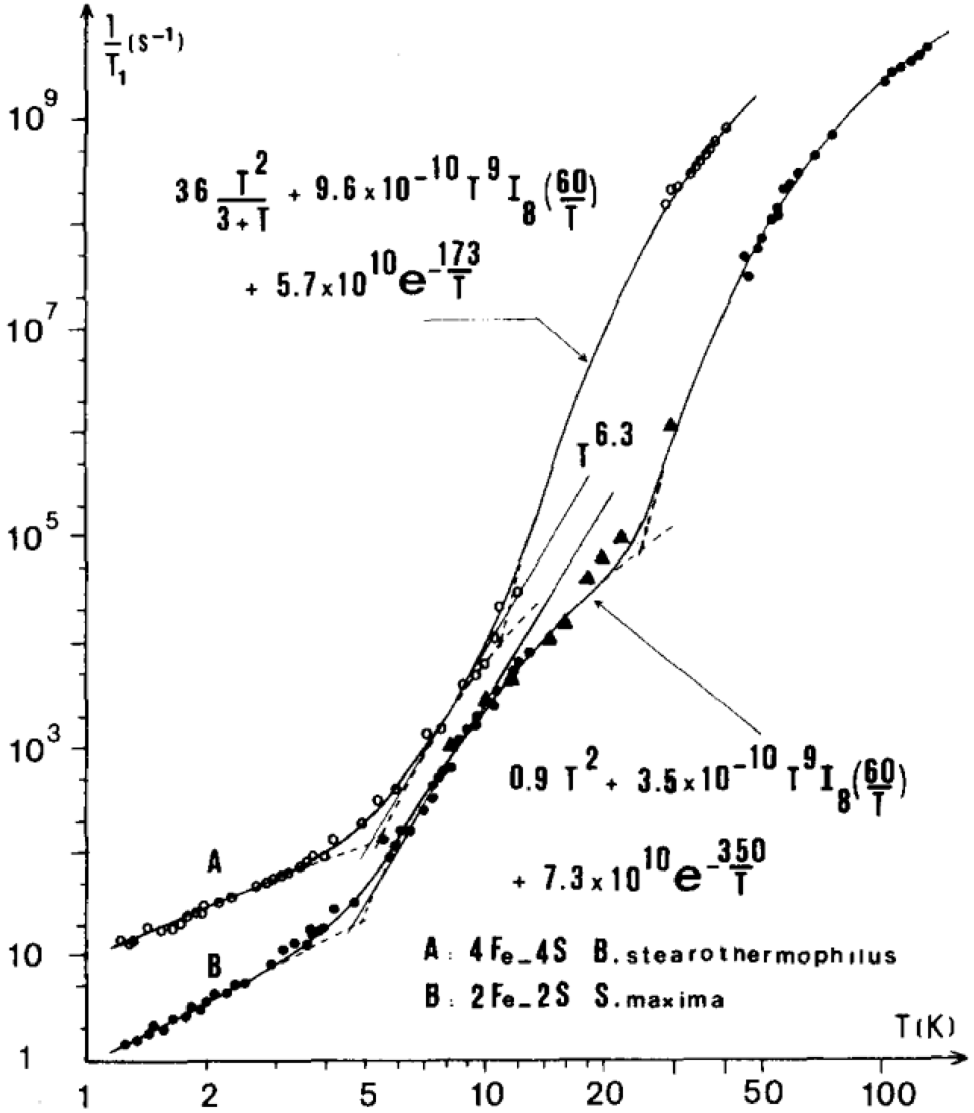
Multiple pathways for spin-lattice relaxation (T_1)



$$\left(\frac{1}{T_1}\right)_{raman} \propto T^9$$

$$\left(\frac{1}{T_1}\right)_{orbach} \propto \frac{1}{exp\left(\frac{\Delta_{orb}}{T}\right)-1}$$

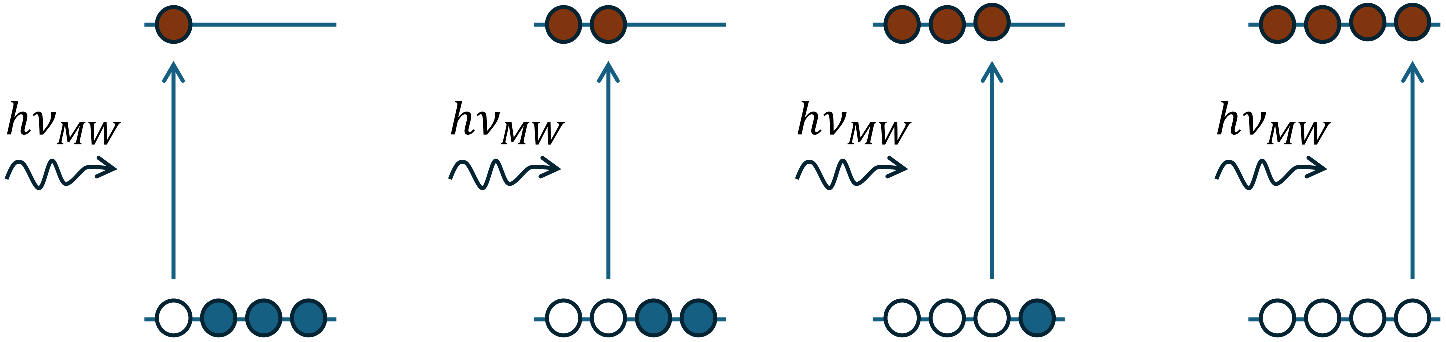
$$\left(\frac{1}{T_1}\right)_{direct} \propto T^1$$



Bertrand et al. J.Chem.Phys. 1982, 76(10), 4715-4719

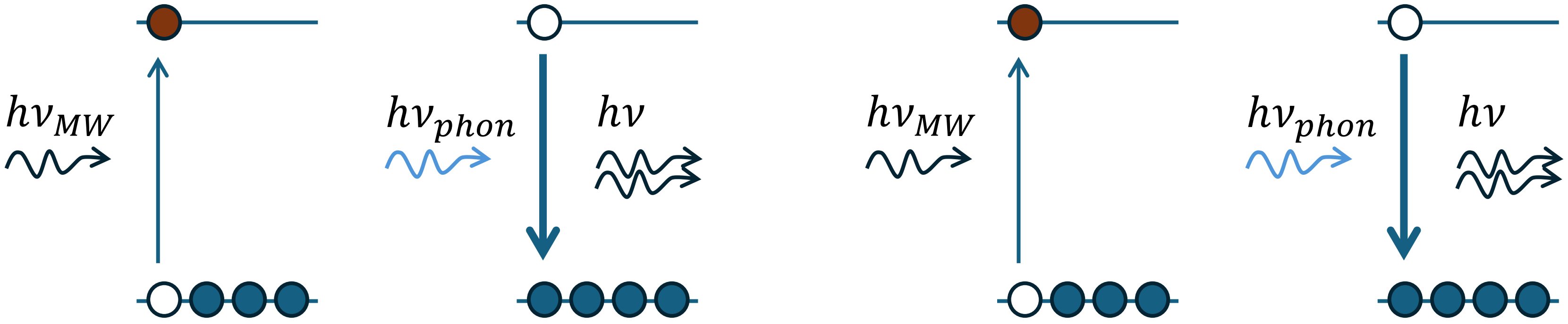
Power saturation

Extreme case #1. Insignificantly slow relaxation



Nothing left to do
No more quants of energy can be absorbed
SATURATION

Extreme case #2. FAST (direct) relaxation



We will never run out of things to do

Power Saturation

MW amplitude ($\sqrt{P_{MW}}$) ≈ # of quants of energy, i.e., how quickly we can “pump” energy into the system

If relaxation is **very fast**

more power → more absorption events → **more** EPR signal

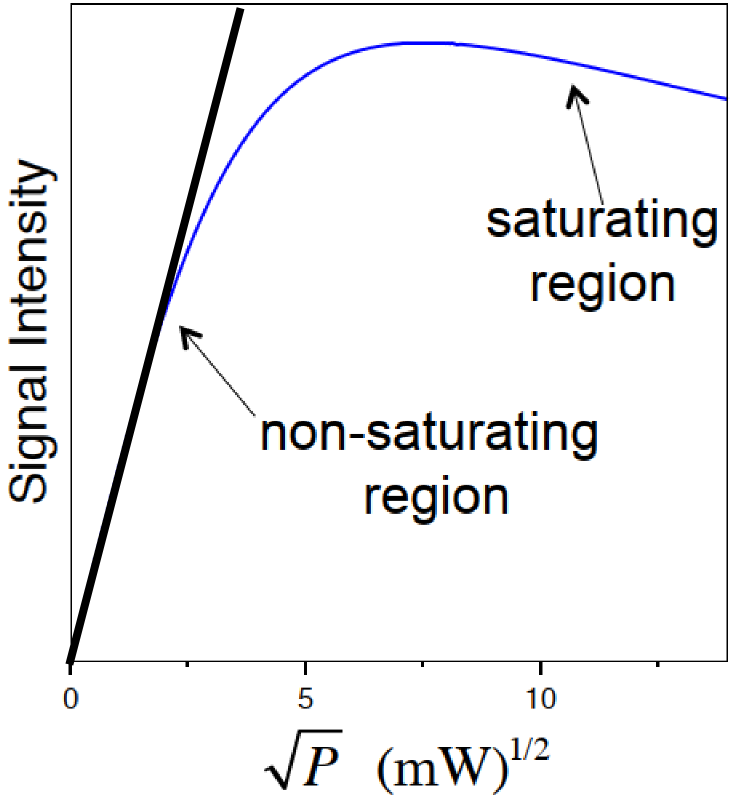
If relaxation is **very slow**

more power → faster transition saturates → **less** EPR signal

$$A = a\frac{\sqrt{P}T_2^2}{\left(1+\frac{P}{P_{0.5}}\right)^{1/2}}$$

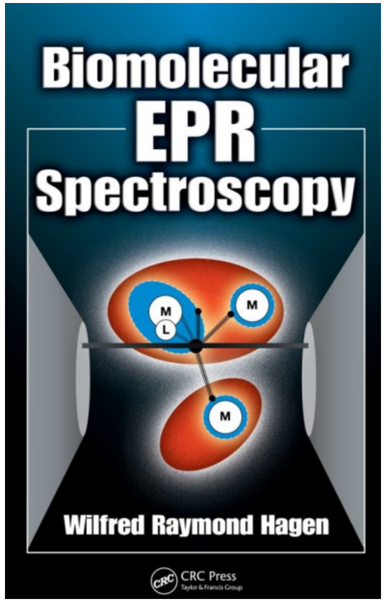
a – instrument dependent constant

$$P_{0.5} = (\gamma_e^2 T_1 T_2)^{-1}$$



Lower temperature → Slower relaxation → Need less power to saturate

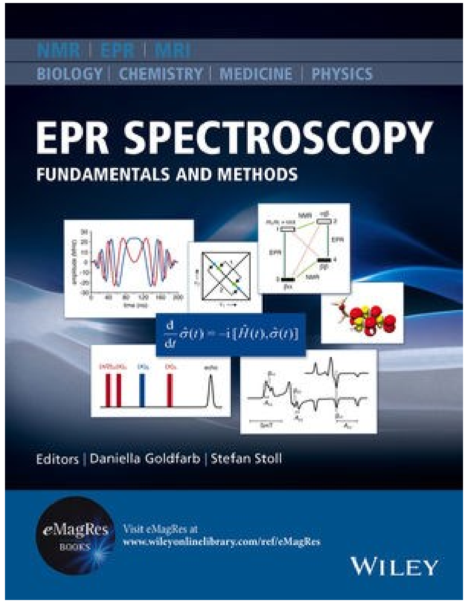
Literature



Biomolecular EPR Spectroscopy

Wilfred Raymond Hagen

CRC Press
Taylor & Francis Group



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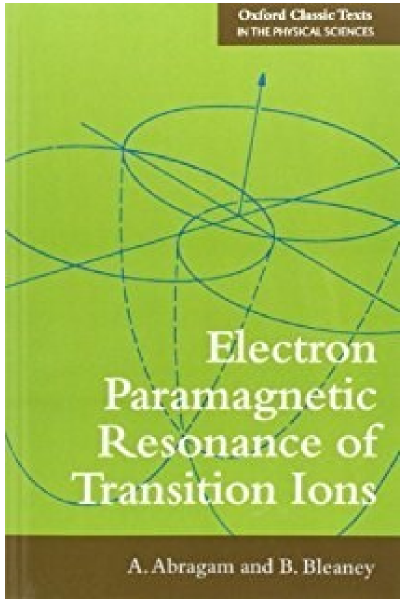

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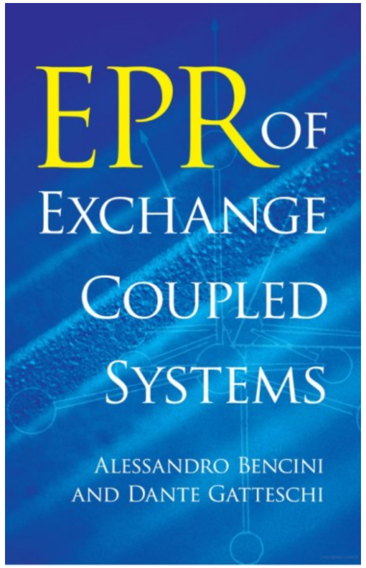
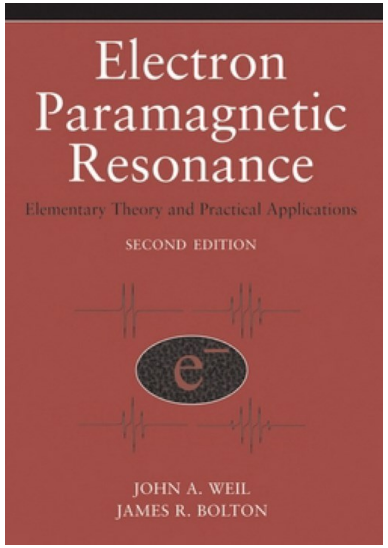
Electron Paramagnetic Resonance of Transition Ions

A. Abragam and B. Bleaney



EPR OF EXCHANGE COUPLED SYSTEMS

ALESSANDRO BENCINI
AND DANTE GATTESCHI



Electron Paramagnetic Resonance
Elementary Theory and Practical Applications
SECOND EDITION

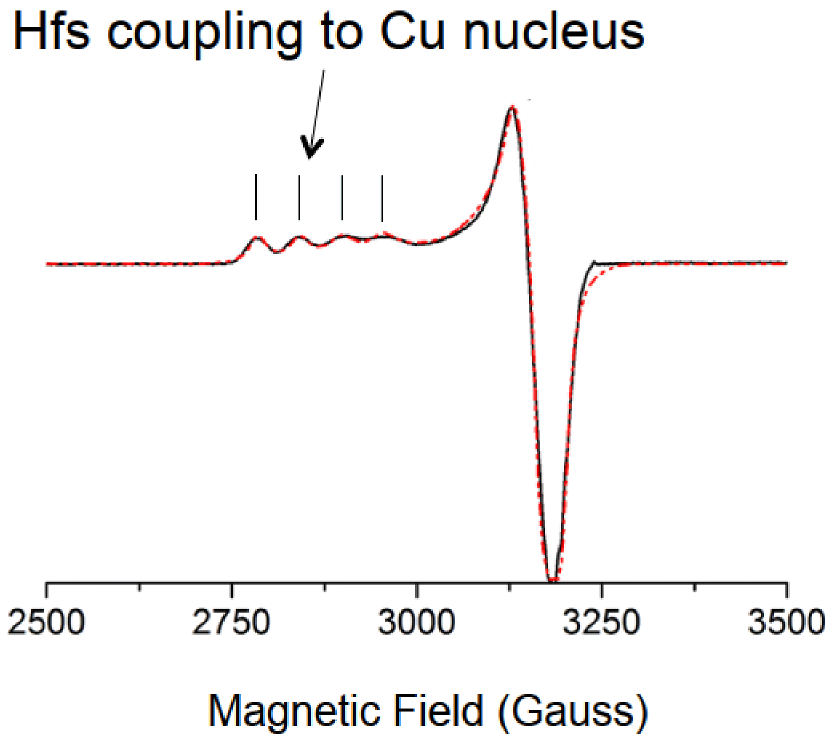
JOHN A. WEIL
JAMES R. BOLTON

Resolved Hyperfine Coupling

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

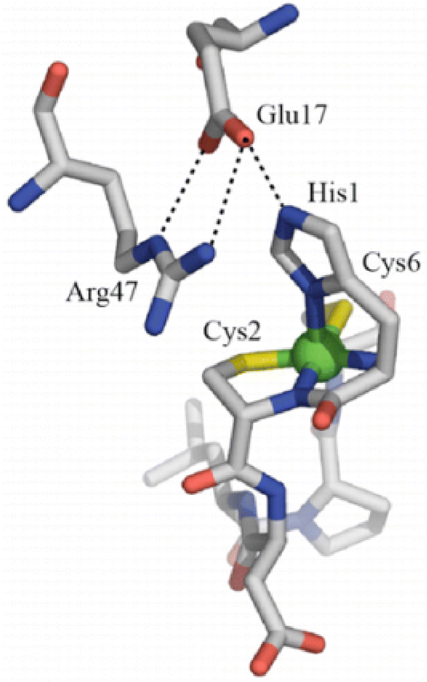
Azurin:



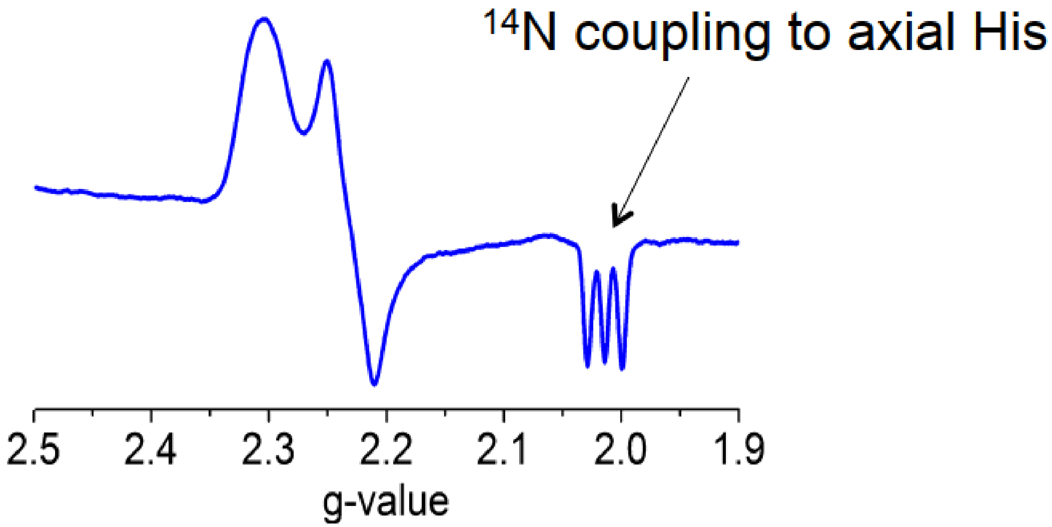
K.M. Clark, *et al.* Inorg. Chem. Frontiers (2014) **1**, 153-158

Resolved Hyperfine Coupling

Ni Superoxide dismutase:

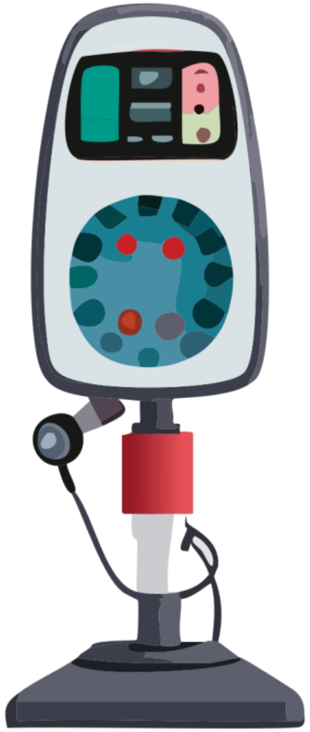


Hyperfine coupling to ligands is also sometimes resolved on one or more of the g-tensor components



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

Let’s talk about instrumentation



“EPR spectrometer” according to AI