Presymposium Workshop

EPR and Mössbauer Spectroscopies

Carsten Krebs
Department of Chemistry
Department of Biochemistry and Molecular Biology
The Pennsylvania State University

Absorption Spectroscopy

- Does generally not provide information about the chemical identity of the absorbing species

- Is not quantitative for an unknown species
  (i.e. a small amount of a strongly absorbing species
  vs a large amount of a weakly absorbing species)

- Therefore, other spectroscopic methods for more detailed characterization of detectable species are required

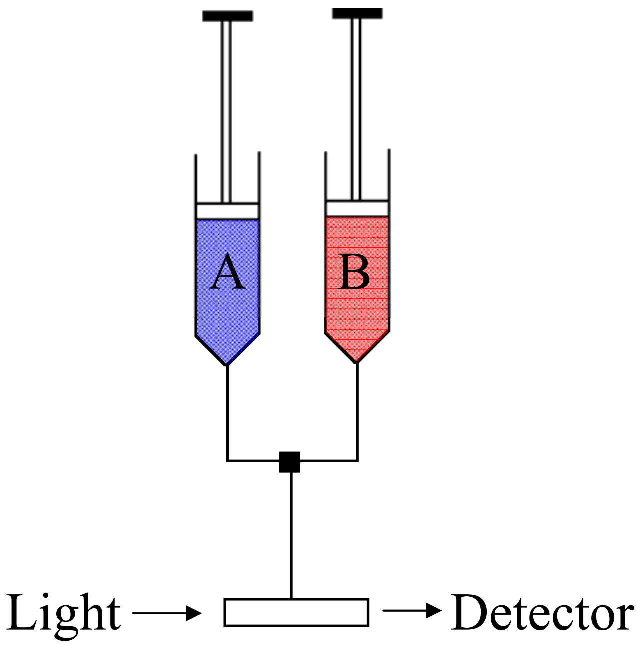
- Stopped-flow absorption spectroscopy often (but not always) provides detailed insight into the kinetics of the reaction studied, i.e. the time-scale for freeze-quench experiments.

Important Spectroscopic Methods for Characterization of Metalloenzymes

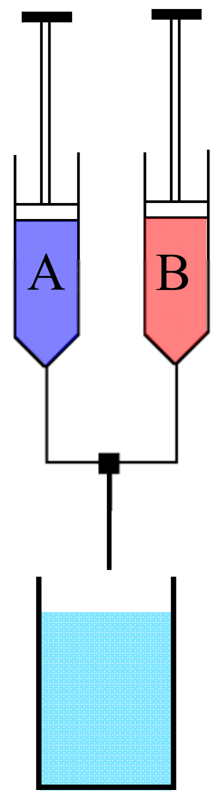
Electron Paramagnetic: Resonance (EPR)	Properties of the electron spin ground state of species with an odd number of unpaired electrons (half-integer spin)
Mössbauer:	Properties of Fe-species (spin state, oxidation state, type of ligands, relative amounts of all different Fe species in a sample)
ENDOR/ESEEM:	Hyperfine interactions between an EPR-active center and nearby magnetic nuclei
X-ray absorption:	Oxidation state of metal, distance to nearby atoms
Resonance Raman:	Detection of vibrational features associated with an UV/vis-absorbing species
MCD:	Number, position, and assignment of absorption bands; properties of the electron spin ground state

Time-Dependent Spectroscopic Methods

Stopped-flow Absorption

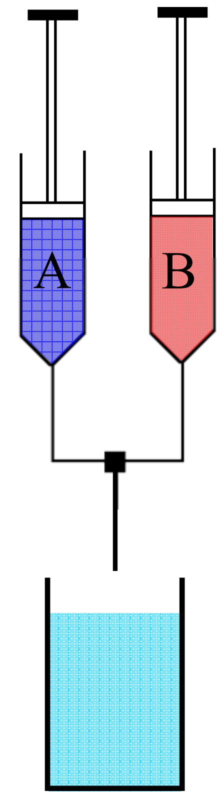


Freeze-quench



cryosolvent -150 °C
(isopentane or liquefied ethane)

Chemical-quench



Base
Organic solvent

UV/vis absorption, fluorescence

Mössbauer, EPR, X-ray absorption, resonance Raman, MCD

LC-MS

Important Spectroscopic Methods for Metalloenzymes

Electron Paramagnetic: Resonance (EPR)	Properties of the electron spin ground state of species with an odd number of unpaired electrons (half-integer spin)
Mössbauer:	Properties of Fe-species (spin state, oxidation state, type of ligands, relative amounts of all different Fe species in a sample)

- Provide information about the electron spin ground state (EPR probes spin states directly; Mössbauer probes spin ground state indirectly via hyperfine interaction)

- Both methods are quantitative

- Both methods are complementary and together allow ***all*** Fe-species in a sample

EPR and Mössbauer spectroscopy are complementary

Electron Spin / Method	Integer Spin $S = 0, 1, 2, 3, \ldots$	Half-Integer Spin $S = 1/2, 3/2, 5/2, \ldots$
EPR	EPR-silent (*in most cases*)	EPR-active
Mössbauer	Quadrupole doublet (*in most cases*) (analysis straightforward)	Magnetically Split Spectrum (analysis complex, but is facilitated using results from EPR)

Electron Zeeman effect

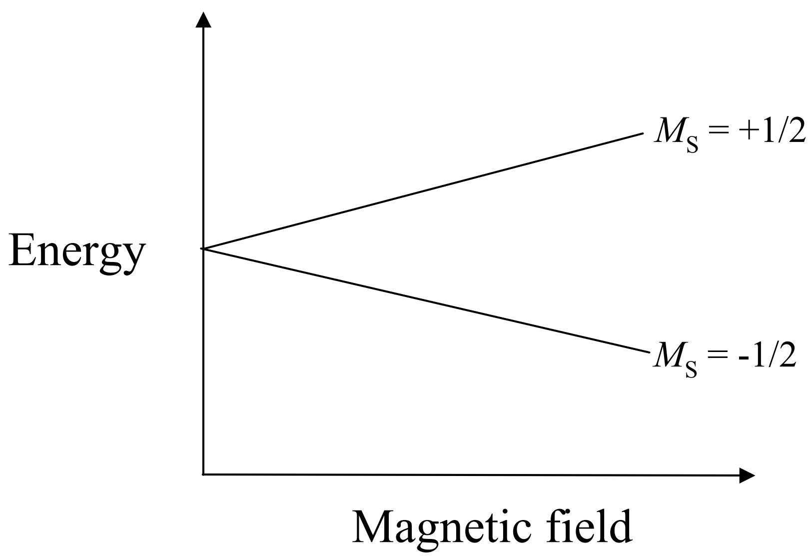
• The electron has an intrinsic angular momentum, called spin **S**, which has a value of S = 1/2 is quantized and can have 2 [= (2S + 1)] different M_S (range is –S, …,+S) values.

• In the absence of a magnetic field (**B**), the energies of the two states with M_S = -1/2 and M_S=+1/2 have the same energy (are degenerate).

• In the presence of a magnetic field, the energies differ (electron Zeeman effect)

$$E(S,M_S) = \mu_B g_e M_S \mathbf{B}$$

μ_B is the Bohr magneton
g_e is the electron g-factor (g_e = 2.0023…)
B is the magnetic field (variable).

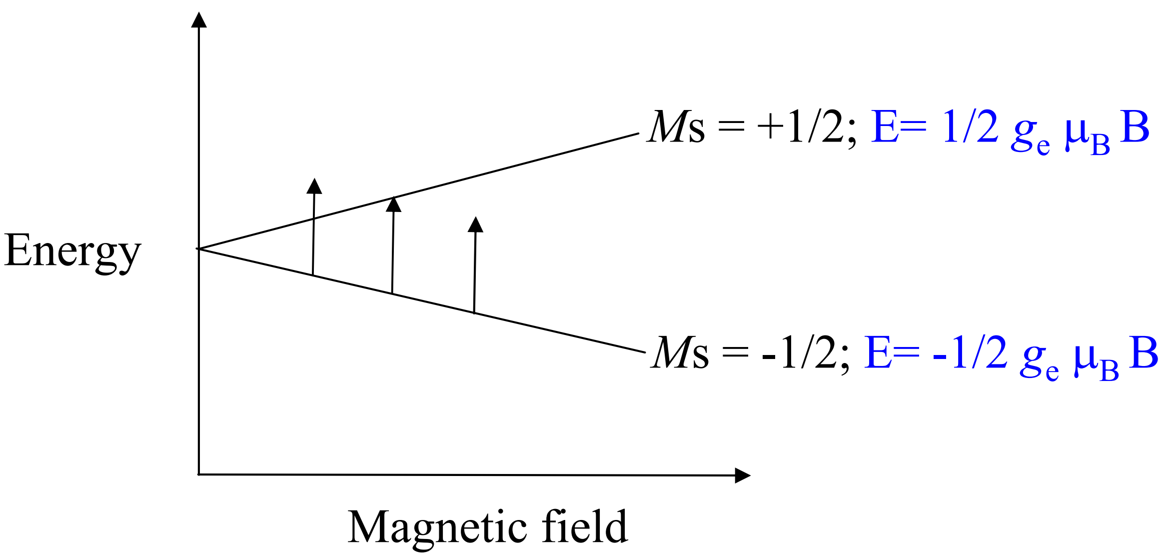


$$\hat{H} = \mu_B g_e \mathbf{S} \bullet \mathbf{B}$$

electron Zeeman

Continuous Wave (CW) EPR Spectroscopy

In CW-EPR the magnetic field is varied continuously. A microwave photon with a frequency ν field can be absorbed, when the following two requirements are met: (1) the separation of two states equals the energy of the microwave field and (2) the two states obey the selection rule $\Delta M_S = \pm 1$ (or $\Delta M_S = 0$ for parallel mode EPR).

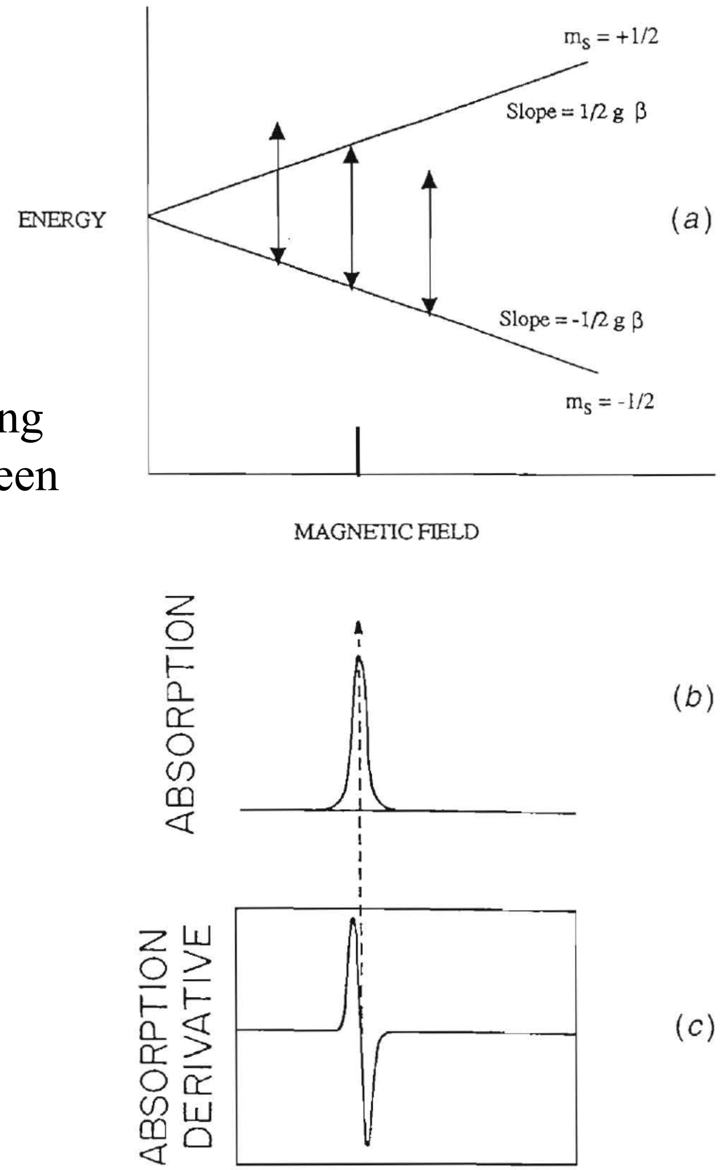


$\Delta E = g_e\, \mu_B\, B = h\, \nu$

Resonance condition

General shape of an EPR spectrum

• CW EPR spectra are recorded by modulating the magnetic field a little bit (typically between 1 and 10 G)



Taken from G. Palmer, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) 2000

Typical Microwave Frequencies used in EPR

Band	Frequency (GHz)	resonance field for g = 2 (G)
L	1	~360
S	3	~1,070
X	**9**	**~3,220**
P	15	~5,360
K	18	~6,440
Q	35	~12,500
W	94	~33,600
	139	~49,700
	245	~87,600

$$\boxed{1\ \mathrm{T} = 1{,}000\ \mathrm{mT} = 10{,}000\ \mathrm{G}}$$

EPR Spectra of “real molecules” with unpaired electrons

Overview

EPR Spectroscopy is a useful method, because the properties of the spin S in molecules are different from those of the free electron.

• Unpaired electron(s) is(are) in an orbital(s), which in most cases has angular momentum (e.g. unpaired electrons of transition metals are in d-orbitals with l = 2). Coupling of spin and orbital angular momenta (**spin orbit coupling**) leads to perturbations of the energy levels of the spin and therefore gives rise to perturbations in the spectrum.

• Coupling between electron spin and nuclear spin(s) leads to **hyperfine coupling**, which occurs when there is unpaired electron spin density at a nucleus with a magnetic moment $I \neq 0$.

• Interaction between two species with unpaired electrons (e.g. a dinuclear metal cluster composed of a high-spin Fe(III) center with $S_A = 5/2$ and a high-spin Fe(II) center with $S_B = 2$) leads to coupling of the two (or more) spins **(spin coupling)**. States are described by the total spin $\mathbf{S}_{total} = \mathbf{S}_1 + \mathbf{S}_2$.

Effect of orbital angular momentum

$$\hat{H} = \mu_B g_e \mathbf{S} \bullet \mathbf{B} \quad + \quad \mu_B \mathbf{L} \bullet \mathbf{B} \quad + \quad \lambda \mathbf{L} \bullet \mathbf{S}$$

electron Zeeman

spin-orbit coupling

$$= \mu_B [g_e \mathbf{S} + \mathbf{L}] \bullet \mathbf{B}$$

$$+ \quad \mathbf{S} \bullet \mathbf{D} \bullet \mathbf{S}$$

zero-field splitting

Only important for S > 1/2

will be covered later

$$= \boxed{\mu_B \mathbf{S} \bullet \mathbf{g} \bullet \mathbf{B}}$$

$$+ \quad \mathbf{S} \bullet \mathbf{D} \bullet \mathbf{S}$$

S and **B** are vectors (three components are $\mathbf{S}_x$, $\mathbf{S}_y$, $\mathbf{S}_z$ and $\mathbf{B}_x$, $\mathbf{B}_y$, $\mathbf{B}_z$).

The interaction between **S** and **B** is expressed by the **g**-tensor, which has $3 \times 3 = 9$ components.

$$\begin{matrix} g_{xx} & g_{xy} & g_{xz} \\ g_{yx} & g_{yy} & g_{yz} \\ g_{zx} & g_{zy} & g_{zz} \end{matrix} \quad \xrightarrow{\textit{DIAGONALIZATION}} \quad \begin{matrix} g_X & 0 & 0 \\ 0 & g_Y & 0 \\ 0 & 0 & g_Z \end{matrix}$$

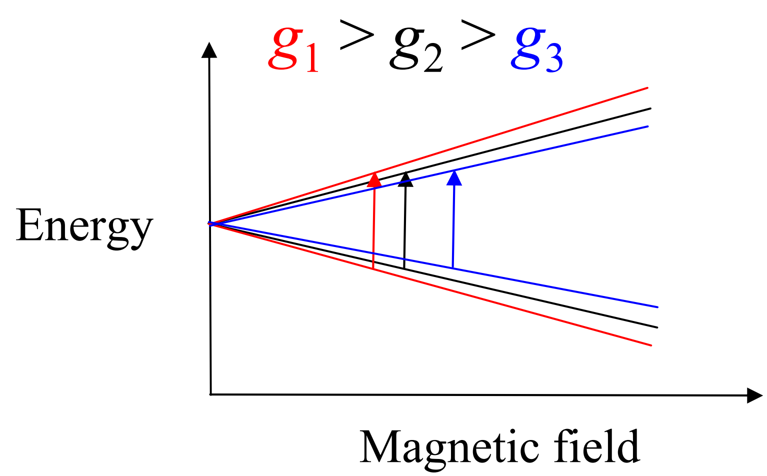
Deviations of g_X, g_Y, and g_Z from g_e are due to molecular structure and provide information about the paramagnetic species. g_X, g_Y, and g_Z may be different (**anisotropy**).

g-values

- *g*-values are inversely proportional to B

$$\Delta E = g\,\mu_B\,B = h\,\nu = \text{constant}$$

$$\boxed{g = 714.484\ \nu[\text{GHz}]\ /\ B\ [\text{G}]}$$



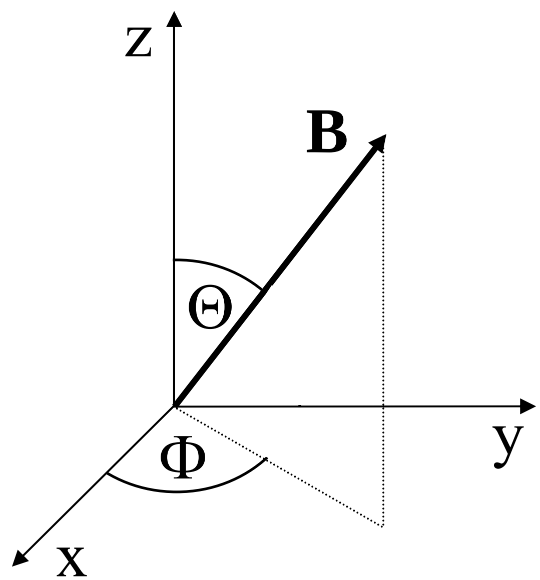
EPR of a randomly oriented sample (frozen solution)

• most metalloenzyme EPR studies are carried out on frozen solutions

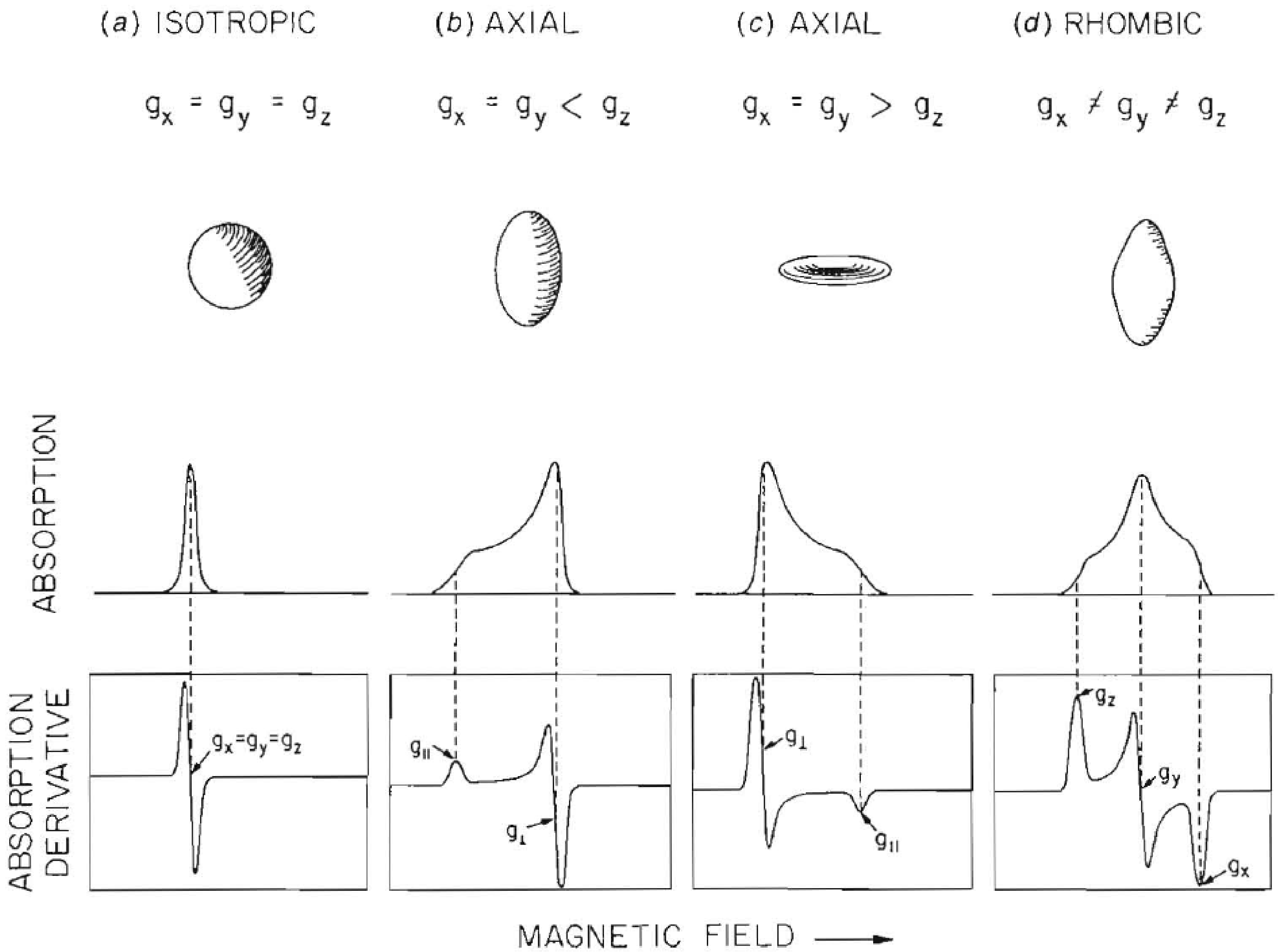
• molecules are randomly oriented relative to the external magnetic field

• *g*-value for an orientation specified by polar angles Θ and Φ is given by

$$\boxed{g_{\Theta\Phi} = [\, g^2_X \cos^2\Phi \sin^2\Theta \quad + \quad g^2_Y \sin^2\Phi \sin^2\Theta \quad + \quad g^2_Z \cos^2\Theta \,]^{1/2}}$$



Powder EPR spectra of species with anisotropic *g*-values



$$\boxed{g = 714.484\ \nu[\mathrm{GHz}] / B\ [\mathrm{G}]}$$

Taken from G. Palmer, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) 2000

g-values

The g-value of a free electron is $g_e = 2.0023\ldots.$

For organic radicals, the g-values are very close to the free-electron g_e-value.

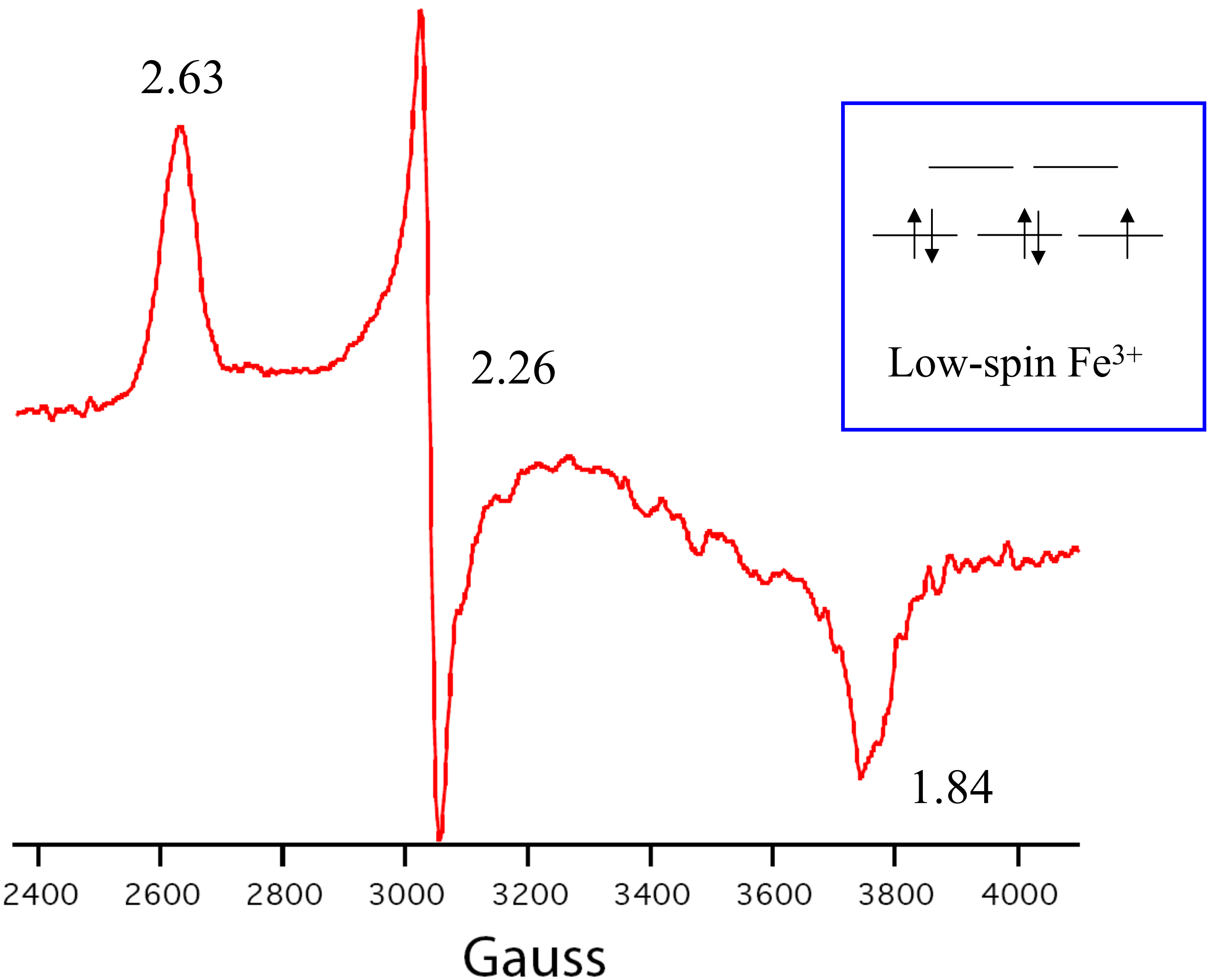
For transition metals, the g-values may differ significantly from g_e.

Examples: (1) Cu^{2+} has very often two g-values ($g_{||}$ and $g_{\perp}$); for most cases $g_{||}>2.0$ and $g_{\perp} \approx 2.0$

(2) High-spin Fe^{3+} has usually almost isotropic g-values close to 2.0.

(3) Low-spin Fe^{3+} has anisotropic g-values.

EPR spectrum of the low-spin ferric center in the heme-dependent enzyme chloroperoxidase



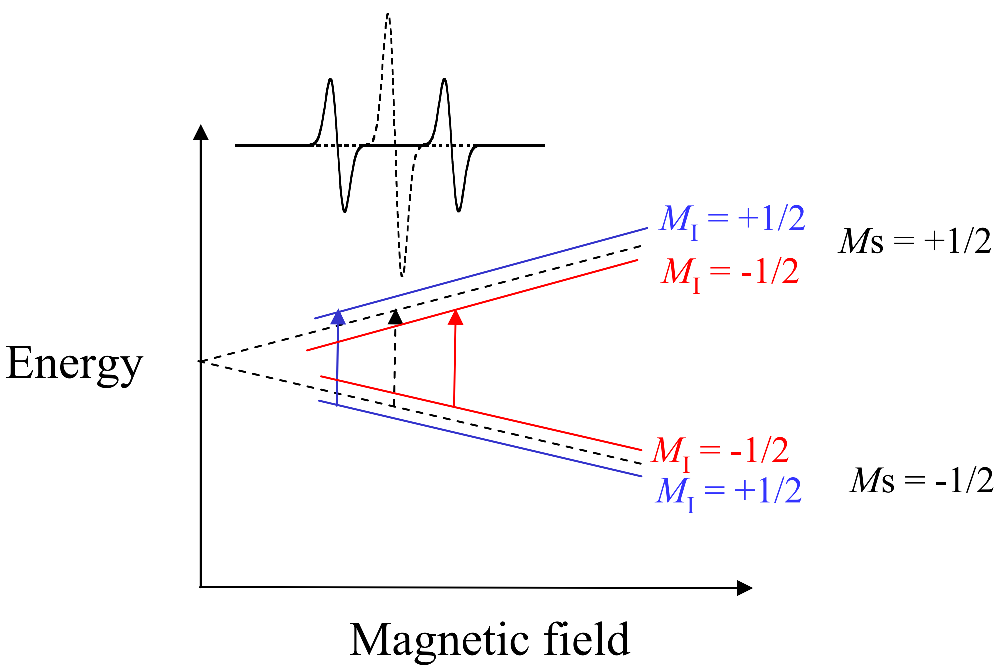
Hyperfine coupling

Interaction between the nuclear spin **I** and the electron spin **S** is described by the hyperfine coupling tensor **A** and results in splitting into $(2I + 1)$ lines.

Two main contributions:

(1) Fermi contact term (requires spin density at the nucleus; caused by spin polarization); the Fermi contact term is isotropic.

(2) Dipolar interaction of the nuclear magnetic moment with the magnetic moment of the electron (both spin and orbital part). This interaction is anisotropic, i.e. it has an orientation dependence.



$$\boxed{\hat{H} = \underbrace{\mu_B \mathbf{S} \bullet \mathbf{g} \bullet \mathbf{B}}_{\text{electron Zeeman}} + \underbrace{\mathbf{S} \bullet \mathbf{A} \bullet \mathbf{I}}_{\text{hyperfine}}}$$

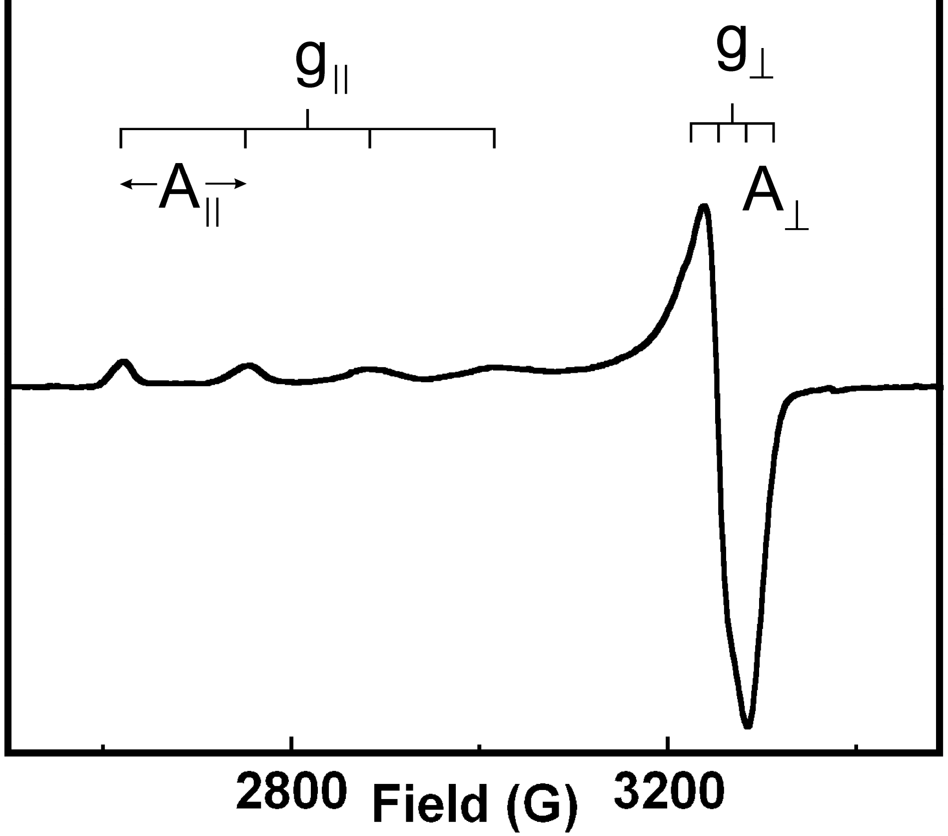
Selection rules:

$$\Delta M_S = \pm 1$$
$$\Delta M_I = 0$$

Important nuclear spins

Isotope	Nuclear spin (*I*)	nat. abundance (%)
^{1}H	1/2	99.9
^{2}H	1	0.02
^{12}C	0	98.9
^{13}C	1/2	1.1
^{14}N	1	99.6
^{15}N	1/2	0.4
^{16}O	0	99.8
^{17}O	5/2	0.04
^{31}P	1/2	100.0
^{32}S	0	95.0
^{33}S	3/2	0.8
^{55}Mn	5/2	100.0
^{56}Fe	0	91.7
^{57}Fe	1/2	2.2
^{63}Cu	3/2	69.0
^{65}Cu	3/2	31.0

EPR spectrum of $Cu^{II}(ClO_4)_2$



Spin orbit coupling / Zero field splitting

Arises from the interaction of the spin **S** with the orbital angular momentum **L** (λ**L•S**).

For example, in high spin Fe^{3+}, the interaction is caused by the interaction of the $^6A_1$ ground term with the excited $^4T_1$ term.

2nd order perturbation treatment of this interaction can be described as follows:

$$\hat{H} = \mathbf{S} \bullet \mathbf{D} \bullet \mathbf{S}$$

$$= D\,[\,S_z^2 - 1/3\,S\,(S + 1) + E/D\,(S_x^2 - S_y^2)]$$

Spin-orbit coupling lifts the (2*S*+1)-fold degeneracy of the energies of the spin multiplet, i.e. it causes the splitting of the states without application of a magnetic field, hence zero field splitting.

D and *E* are the *axial and rhombic zero-field-splitting parameters*, respectively.

$\boxed{\text{$E/D$ is called rhombicity and can take values ranging from 0 to 1/3.}}$

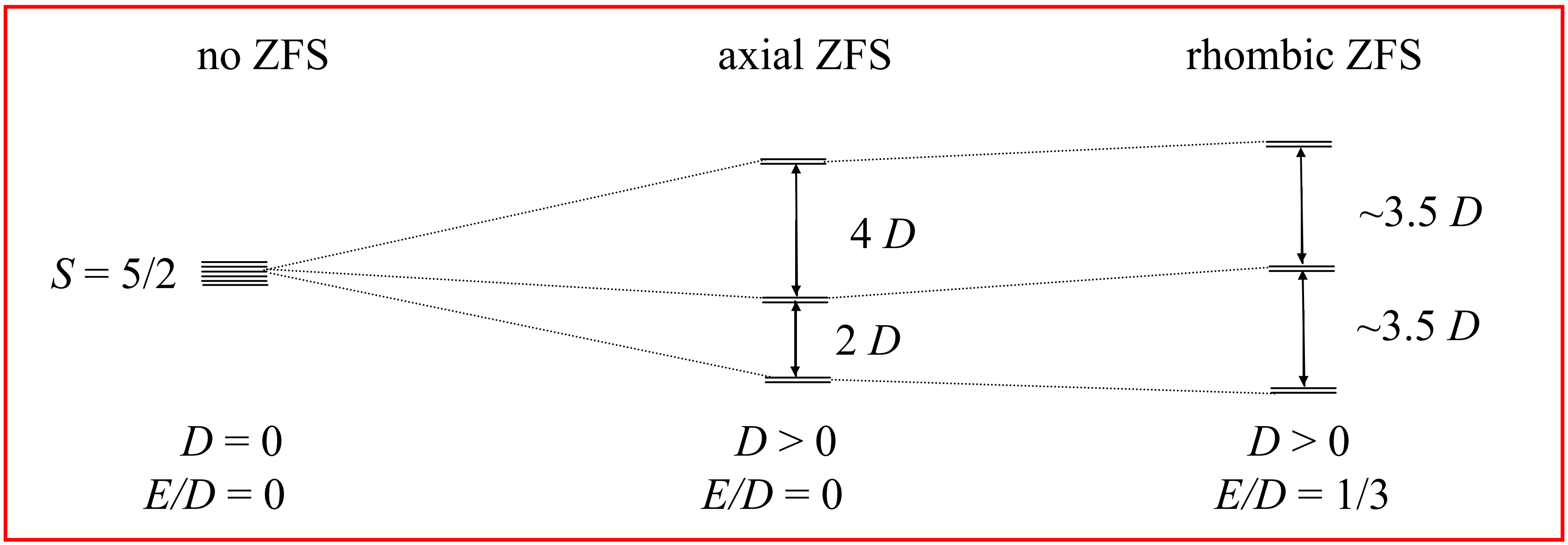
Zero field splitting of half-integer spin systems

Half-integer spin systems are those with $S = 1/2, 3/2, 5/2, 7/2, \ldots$.

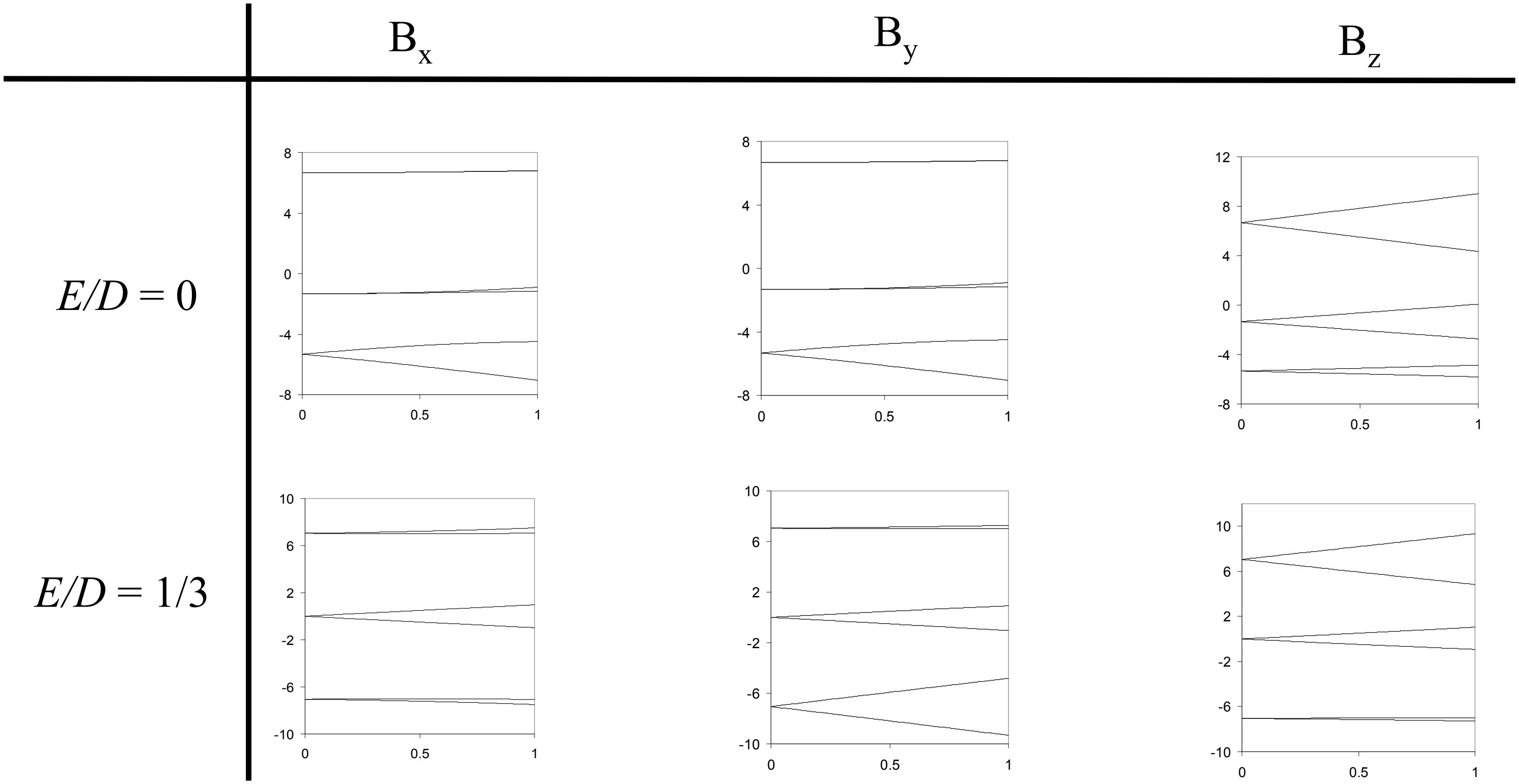
ZFS splits the $(2S + 1)$ states into $(S + 1/2)$ doublets, which are called Kramers doublets.

Example: $S = 5/2$ has $(2 \times 5/2 + 1) = 6$ states. ZFS splits it into $(5/2 + 1/2) = 3$ Kramers doublets.

Important: The two states of the Kramers doublet have always the same energy without a magnetic field, irrespective of the values of D and E/D.



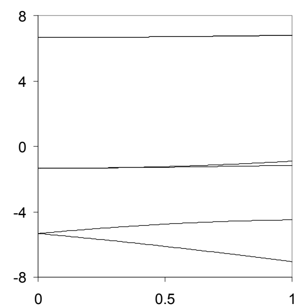
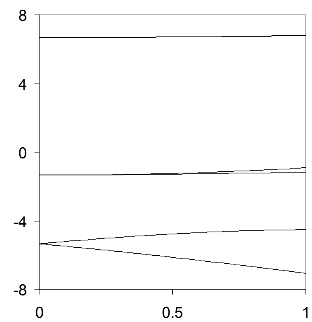
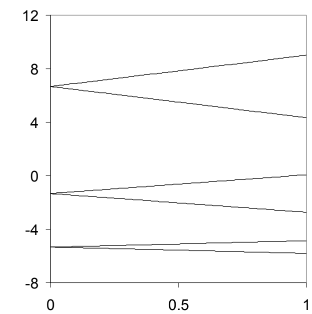
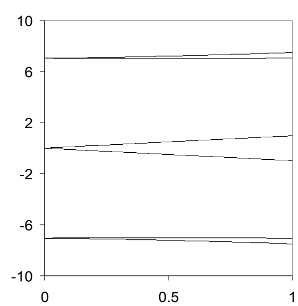
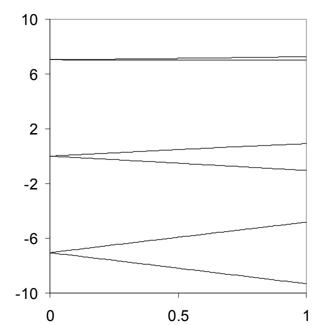
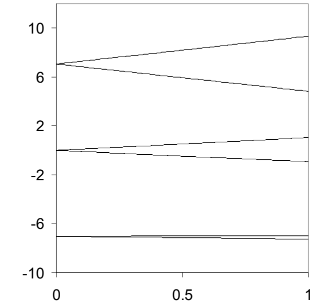
Electron Zeeman effect for an S = 5/2 spin system with ZFS (D = + 2 cm^{-1})



Plotted are energies of the states (y-axis) vs B (x-axis).

- The two states of a Kramers doublet split symmetrically
- The magnitude of the splitting depends on the orientation of **B** to ZFS tensor

Effective *g*-values for an *S* = 5/2 spin system with ZFS

	B_x	$g_{eff,x}$	B_y	$g_{eff,y}$	B_z	$g_{eff,z}$
$E/D = 0$		0 0 6		0 0 6		10 6 2
$E/D = 1/3$		0.9 4.3 0.9		0.6 4.3 9.6		9.6 4.3 0.6

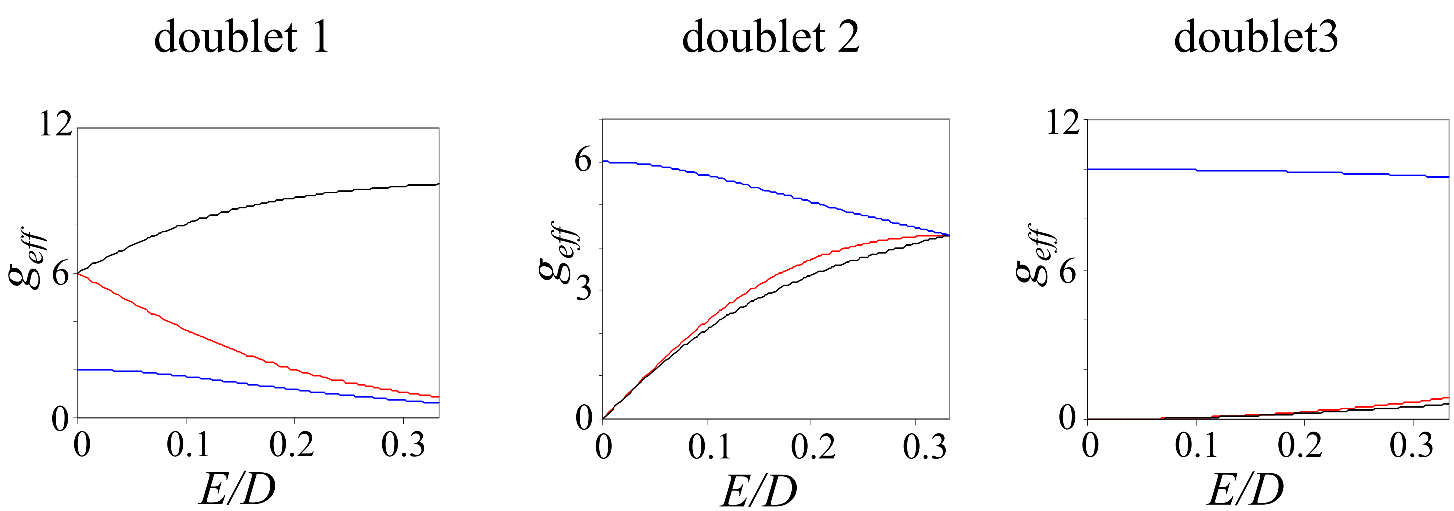
- From the splitting, the effective *g*-values can be calculated

$$\boxed{\Delta E = g_{eff}\, \mu_B\, B = h\, \nu}$$

$$\boxed{g_{eff} = 714.484\ \nu[\mathrm{GHz}]\ /\ B\ [\mathrm{G}]}$$

The three rhombograms for S = 5/2

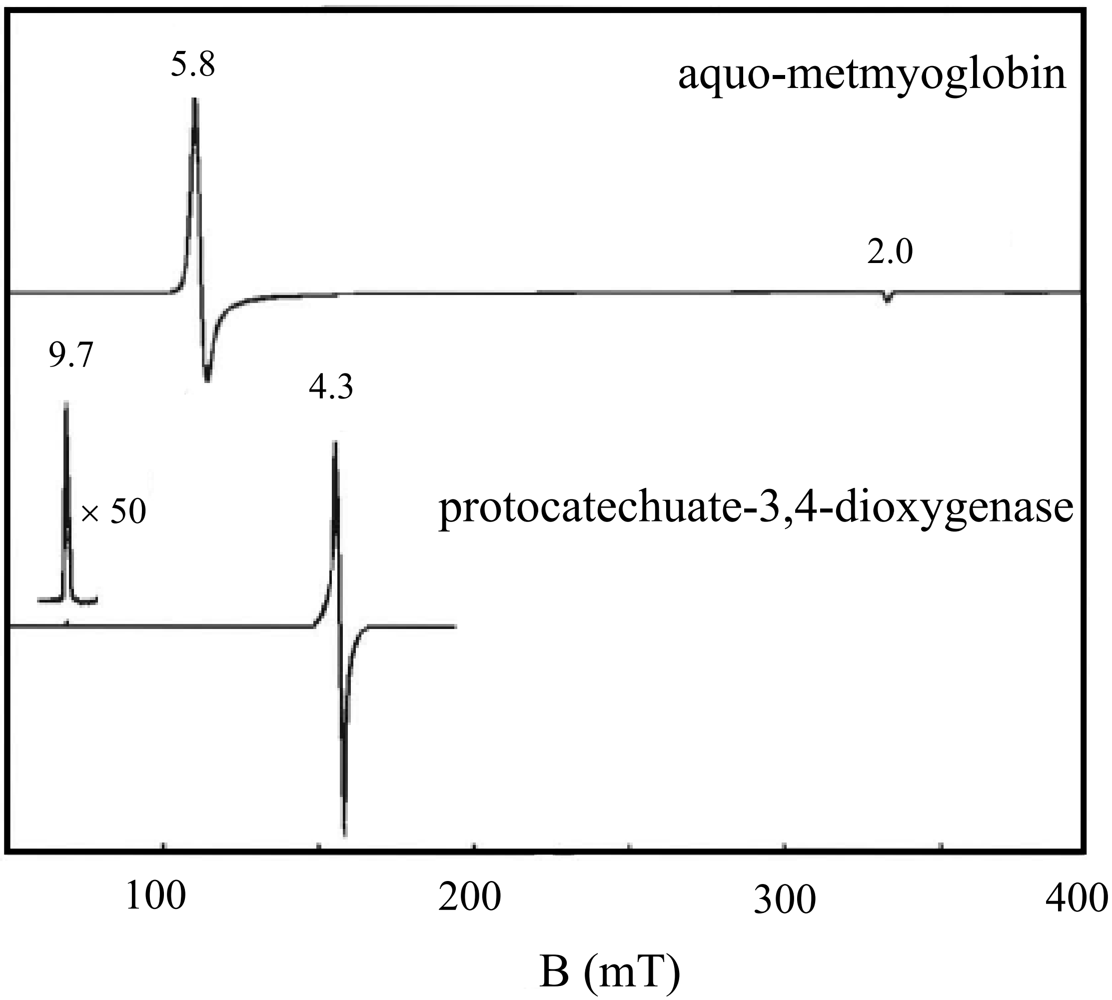
In a rhombogram the effective g-values (g_{eff}) are plotted as a function of the rhombicity E/D (varied from 0 to 1/3 in order to cover all possible cases).



Each rhombogram contains three curves corresponding to the orientation of the magnetic field **B** along the molecular x (red), y (black), and z (blue) axes defined by the ZFS interaction.

E/D is a value that is specific to the species under investigation.

Examples for axial and rhombic high-spin Fe(III) sites



axial “6,6,2”-signal

rhombic 4.3-signal

Adapted from G. Palmer, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) 2000

Rhombograms for other half-integer spin states

Rhombograms for other half-integer spin systems can be generated in an analogous fashion.

Those for $S = 3/2$, $5/2$, $7/2$, and $9/2$ are in your materials.

Further reading

W. R. Hagen, *EPR Spectroscopy of Iron-Sulfur Proteins*, Adv. Inorg. Chem. 38, 164-222 (1992)

G. Palmer, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) (2000)

W. R. Hagen, *Biomolecular EPR Spectroscopy*, CRC Press (2009)

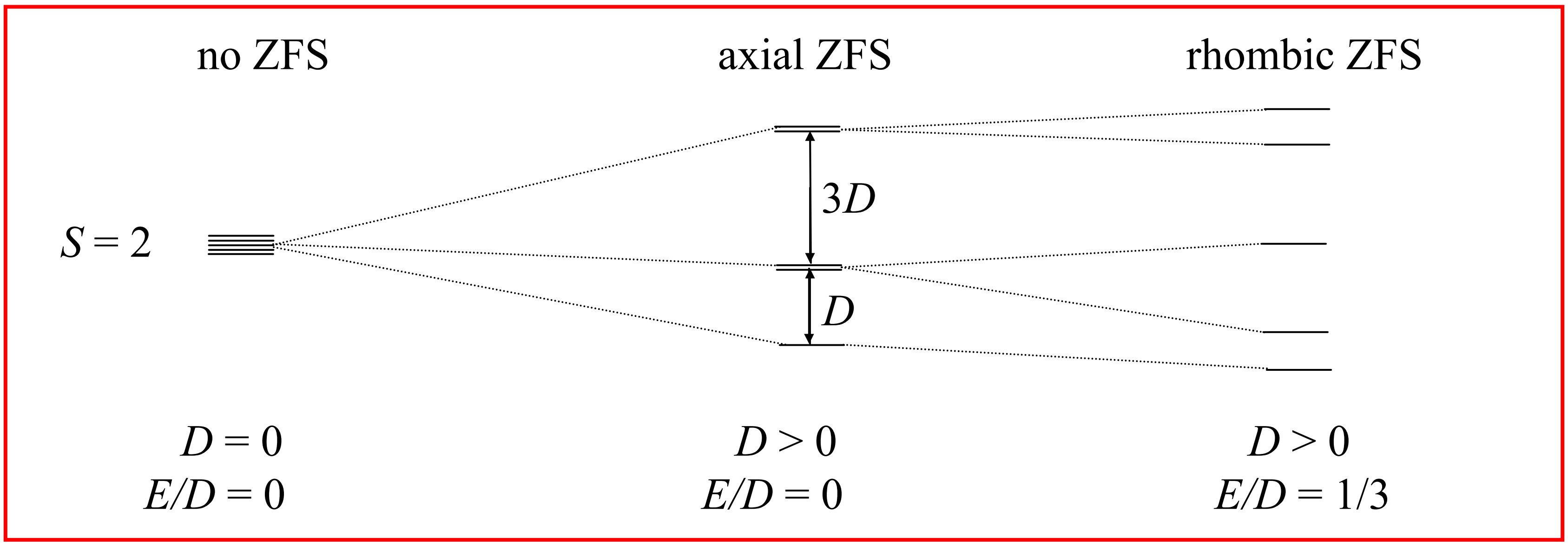
Zero field splitting of integer spin systems

Integer spin systems are those with $S = 0, 1, 2, 3, 4, \ldots$.

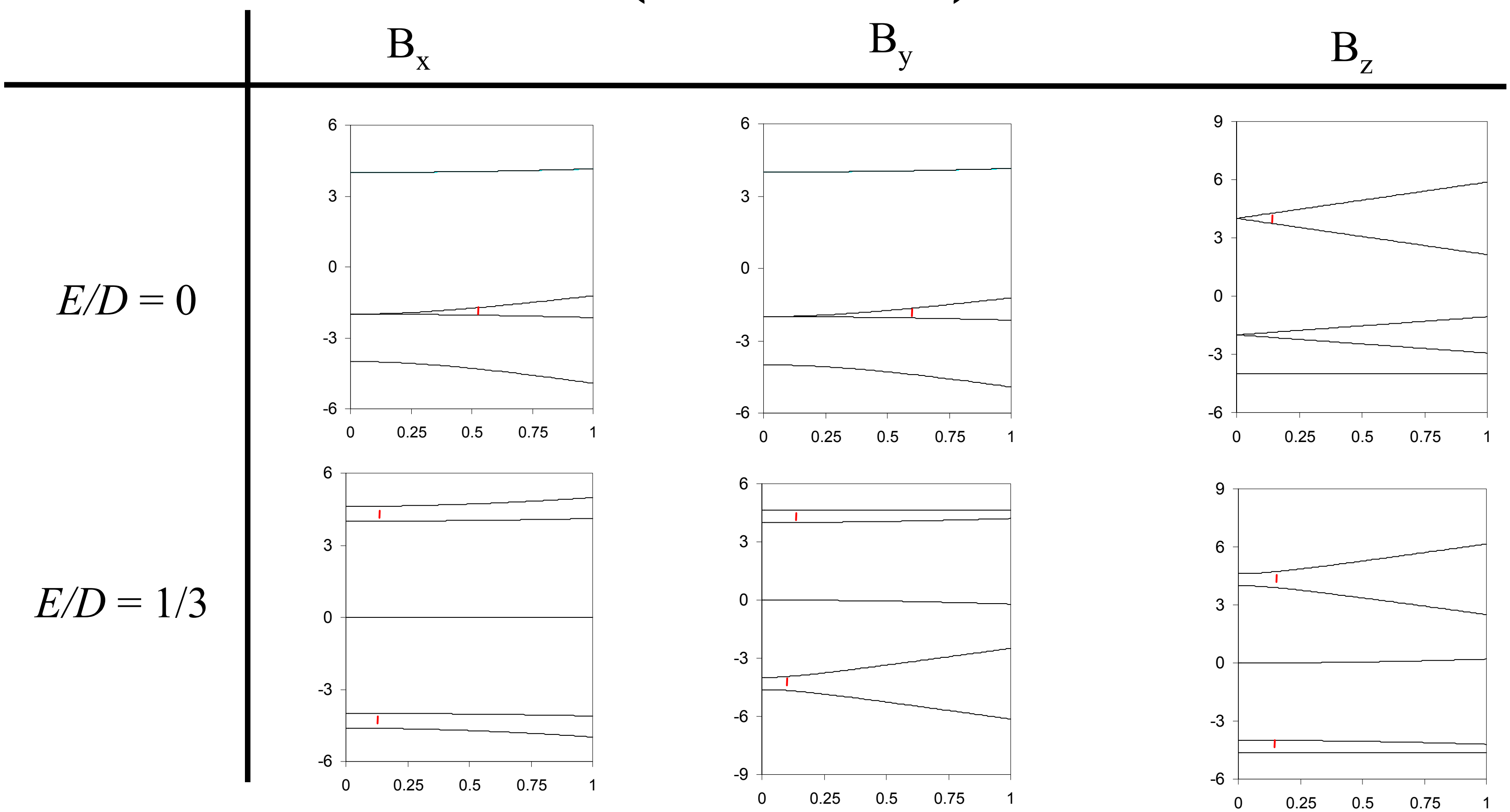
ZFS splits the $(2S + 1)$ states. For axial systems (i.e. $E/D = 0$) they are split into S doublets and one singly degenerate state.

$E/D \neq 0$ removes the degeneracy of the S doubly degenerate states. In this case all $(2S + 1)$ states have different energies.

Example: $S = 2$ has $(2 \times 2 + 1) = 5$ states. Axial ZFS splits it into 2 doublets and one additional state, and $E/D \neq 0$ removes the twofold degeneracy of the doublets.



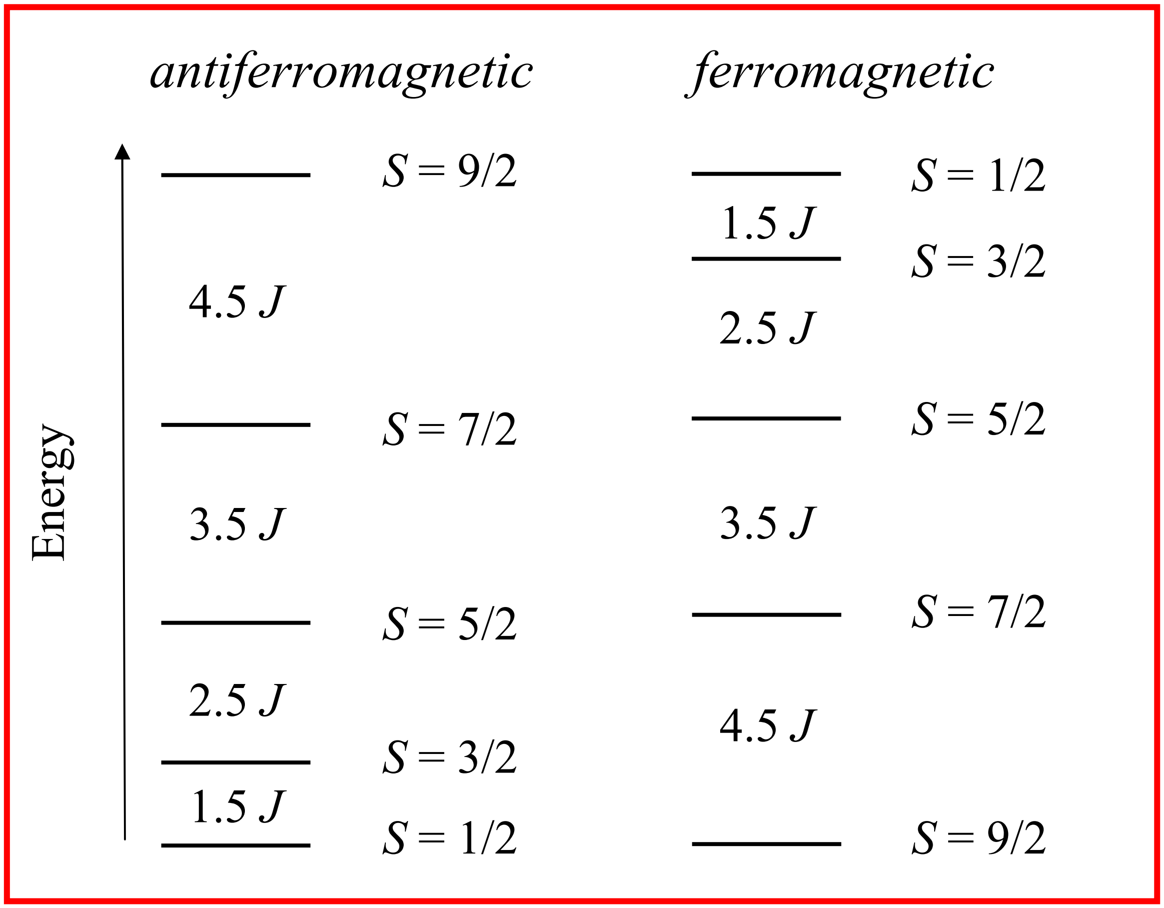
Electron Zeeman effect for an *S* = 2 spin system with ZFS (D = + 2 cm^{-1})



- Energy separation between states is in most (but not all) cases greater than X-band microwave photon (0.3 cm^{-1}), shown in red.
- Further reading on integer spin EPR
  G. Palmer, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) 2000

Spin coupling

- Let's assume a dinuclear cluster with a high-spin Fe^{3+} ($S_1 = 5/2$) and a high-spin Fe^{2+} ($S_2 = 2$).
- The two local spins S_1 and S_2 couple to give total spin states S.
- Allowed values for S are 1/2, 3/2, 5/2, 7/2, and 9/2 (range is $|S_1 - S_2| = 1/2$ and $S_1 + S_2 = 9/2$).



$\hat{H} = J\ \mathbf{S}_1 \bullet \mathbf{S}_2$

$E(S) = J/2\ S\ (S + 1)$

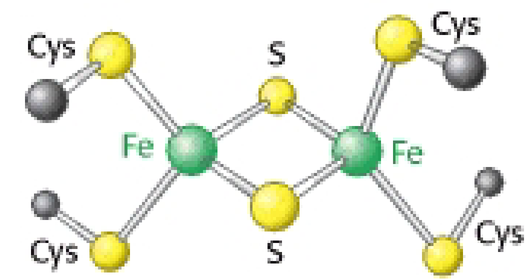
$J > 0$ antiferromagnetic

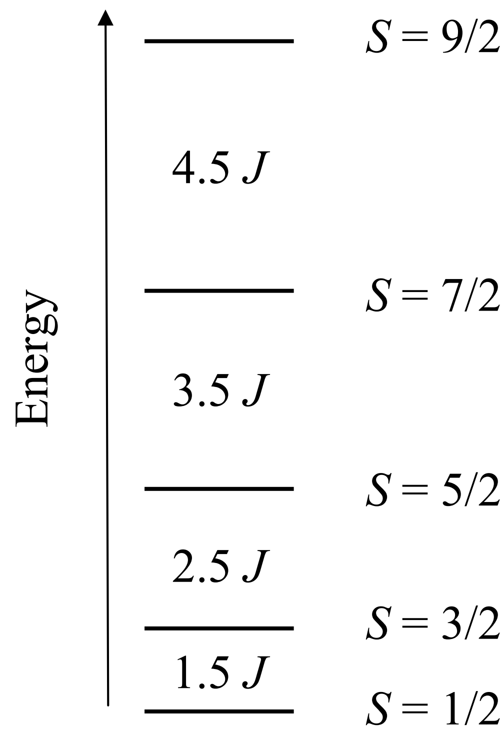
$J < 0$ ferromagnetic

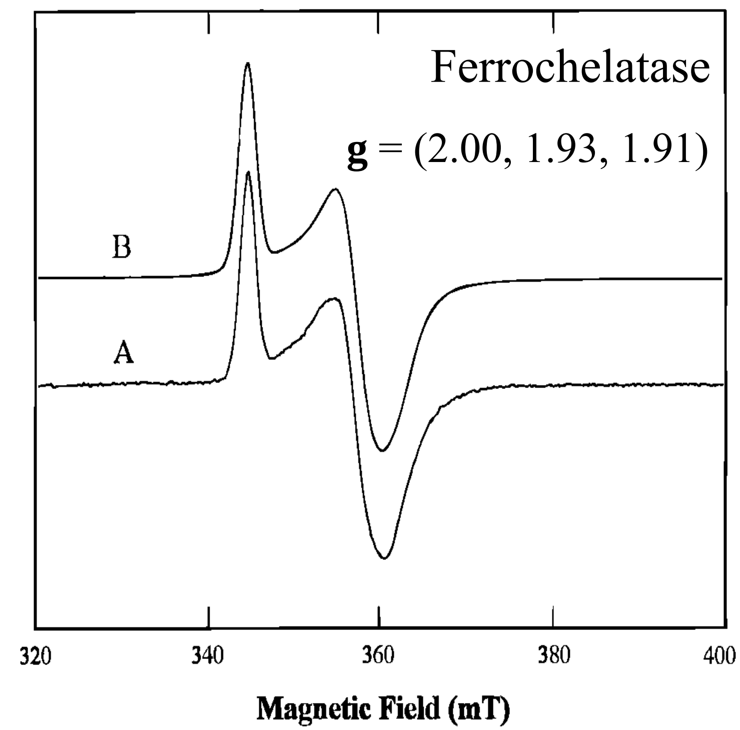
Spin-coupled clusters: Example 1

- A [2Fe-2S]$^{+}$ consists of a high-spin Fe^{3+} ion ($S_1 = 5/2$) and a high-spin Fe^{2+} ion ($S_2 = 2$), which are antiferromagnetically coupled.

- It has an EPR-active $S = 1/2$ ground state.





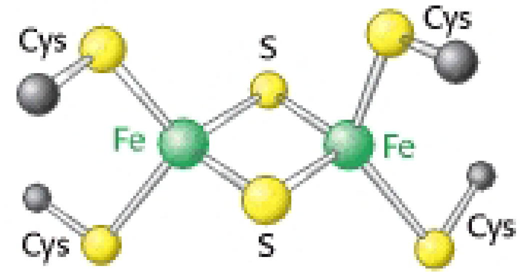


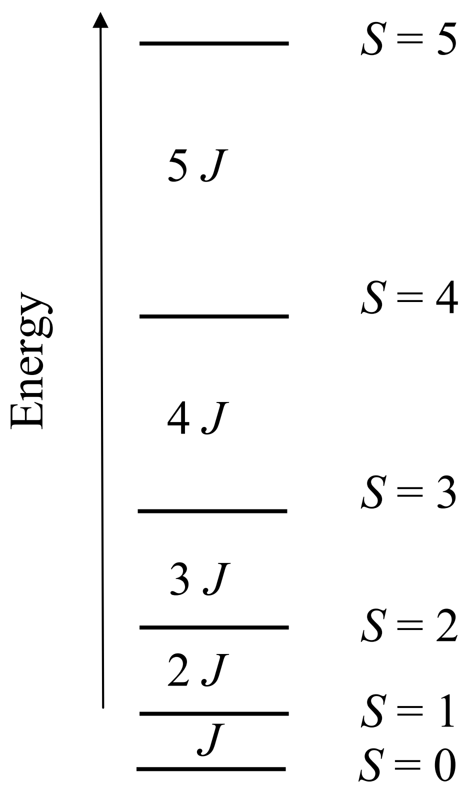
- EPR-spectroscopy probes the **total ground spin state** of a coupled cluster!

Spin-coupled clusters: Example 2

- $[2Fe\text{-}2S]^+$ clusters can be oxidized to the $[2Fe\text{-}2S]^{2+}$ form.

- $[2Fe\text{-}2S]^{2+}$ clusters have two high-spin Fe^{3+} ions, which are antiferromagnetically coupled to yield a $S = 0$ ground state.





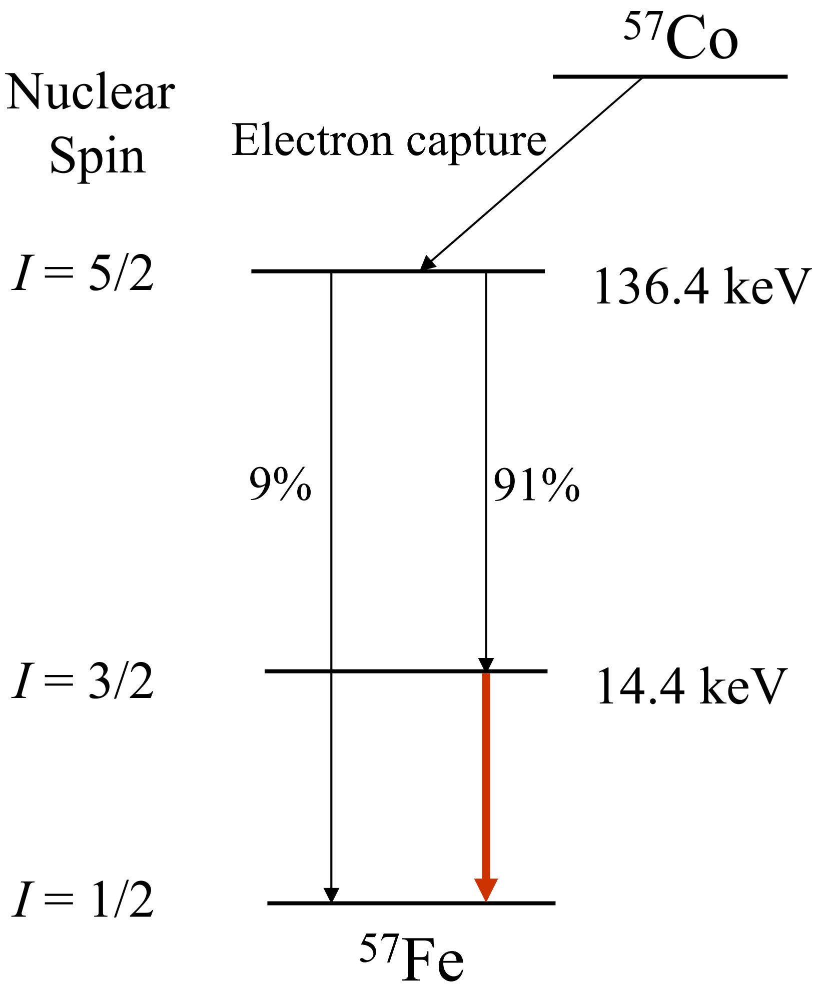
The $S = 0$ ground state is diamagnetic and therefore ***not*** EPR-active.

Other physical methods are required for characterization of EPR-silent species …

Mössbauer spectroscopy

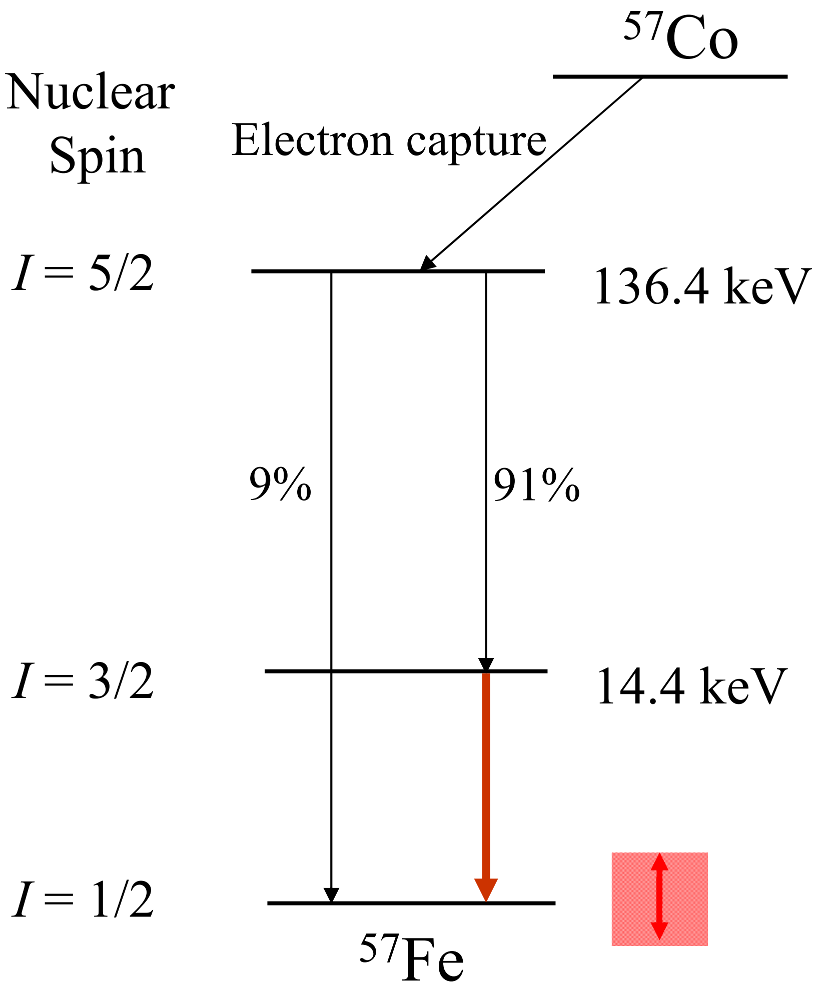
Energy source:	14.4 keV γ-photon from ^{57}Co nucleus.
Physical process:	Nuclear resonance transition between the ground and excited states of ^{57}Fe nucleus.
Physical quantities detected:	Hyperfine interactions between the ^{57}Fe nucleus and its surrounding electrons.
Information gained:	Detailed electronic properties of Fe atoms in the samples; e.g. spin and oxidation state, cluster nuclearity.
Sample constraints:	~0.4 mL frozen solution with > 0.5 mM 57Fe for each distinct Fe site; avoid high concentrations of relatively heavy atoms (e.g. Cl, S, P) sample must be a solid (otherwise no recoilless absorption and emission of γ-photon)

Mössbauer spectroscopy
The "light source": Decay scheme of ^{57}Co



- 3,300 times the energy of a 285-nm UV photon
- recoil imparts significant change of energy of the photon
- emitting and absorbing nuclei must be embedded in solid lattice
- there is recoil-less emission and absorption of γ-photons (f-factor)
- at low temperatures, all Fe species have same f-factor
- **fraction of Fe species in sample is proportional to area of Mössbauer subspectrum**

Mössbauer spectroscopy
The "light source": Decay scheme of ^{57}Co



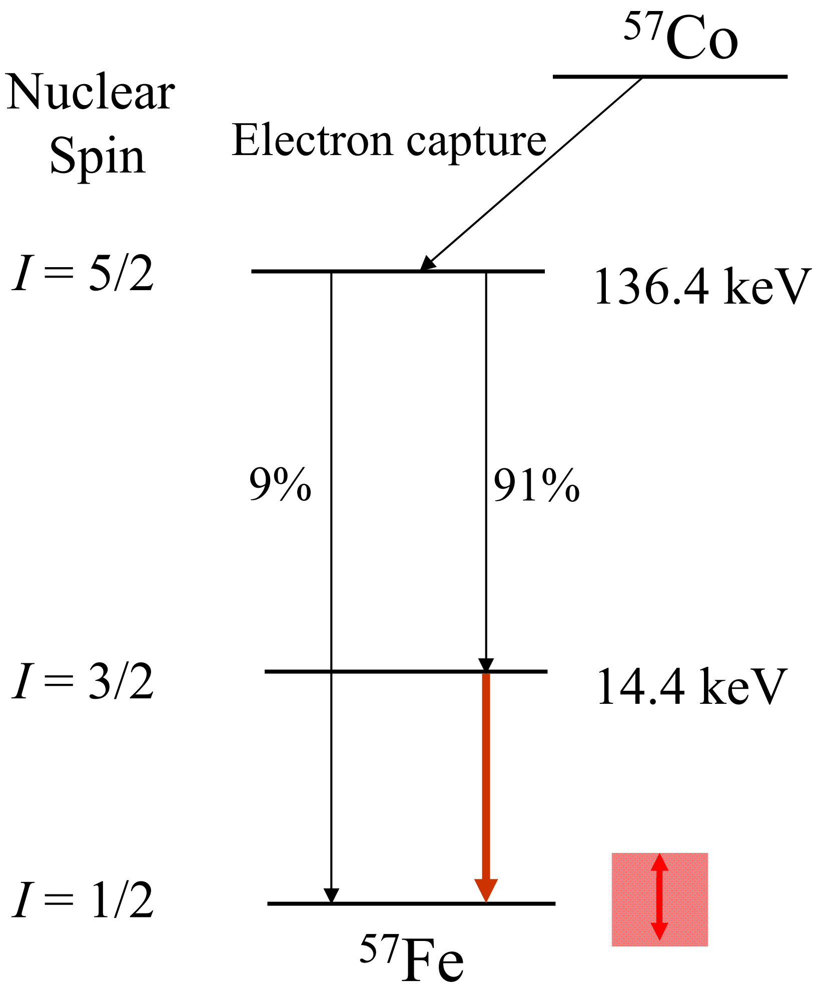
$$\Delta E = \frac{v}{c} E_\gamma$$

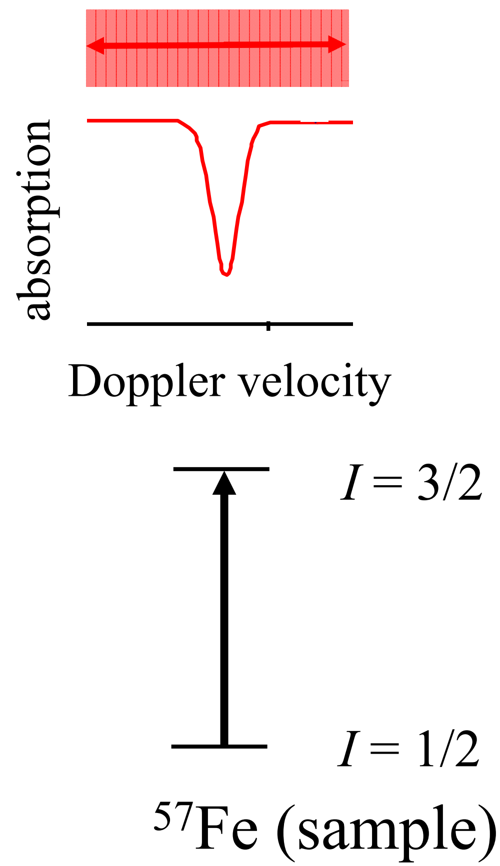
v = source velocity

c = speed of light

- Doppler effect allows the energy of the photon to be varied slightly

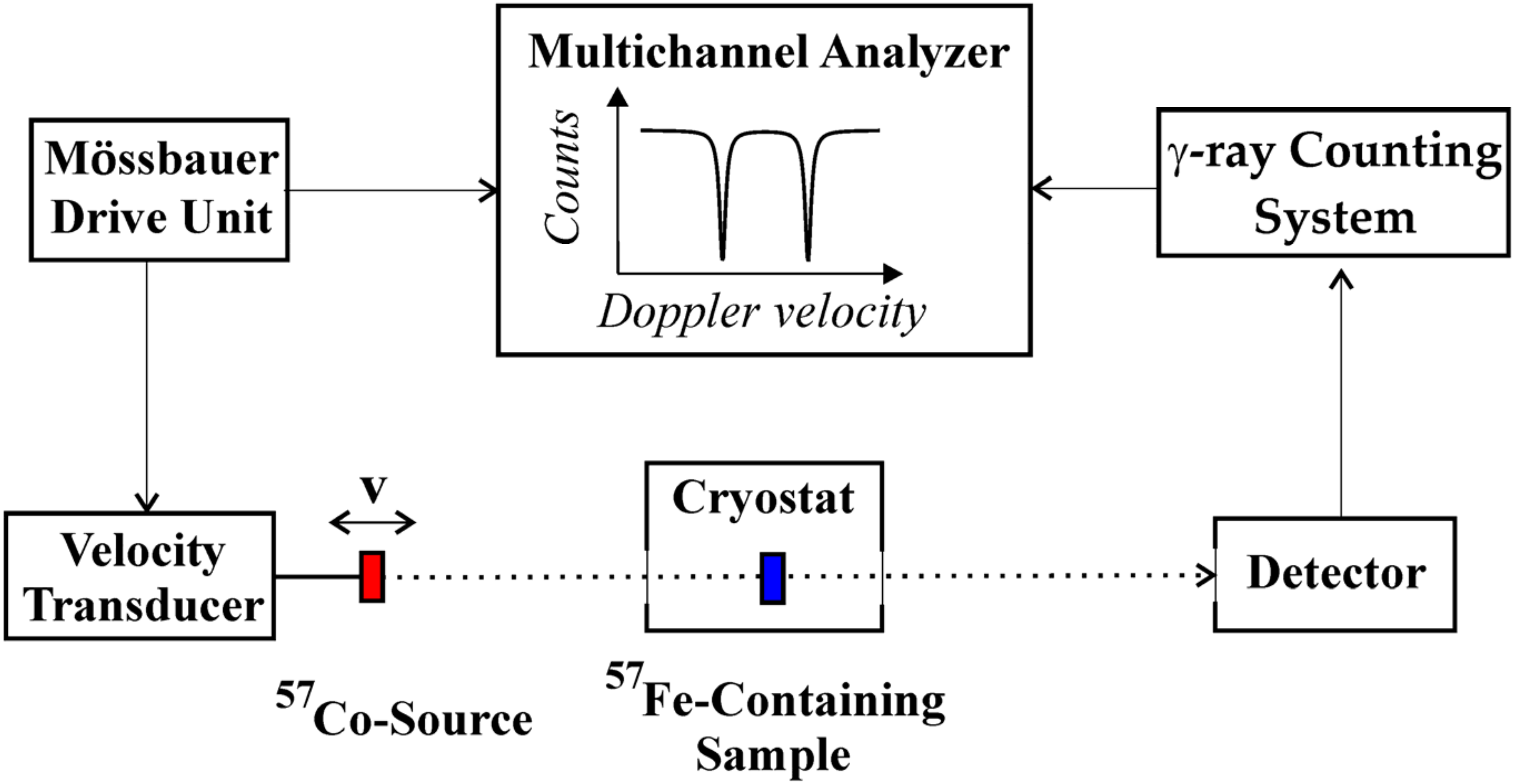
Mössbauer spectroscopy
The "light source": Decay scheme of ^{57}Co





- Photon can be absorbed by a ^{57}Fe nucleus in the sample

Experimental setup (transmission geometry)



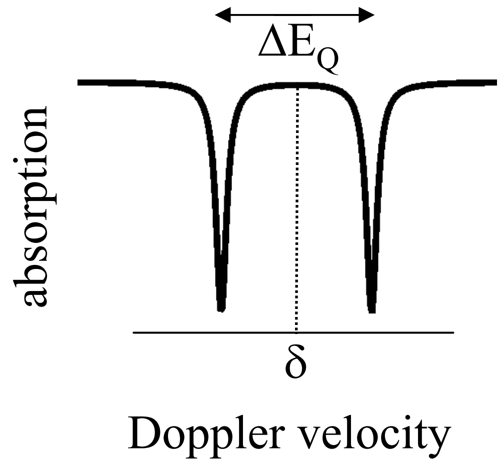
$$v = 1 \text{ mm/s} \Rightarrow \Delta E = 4.8 \times 10^{-8} \text{ eV}$$

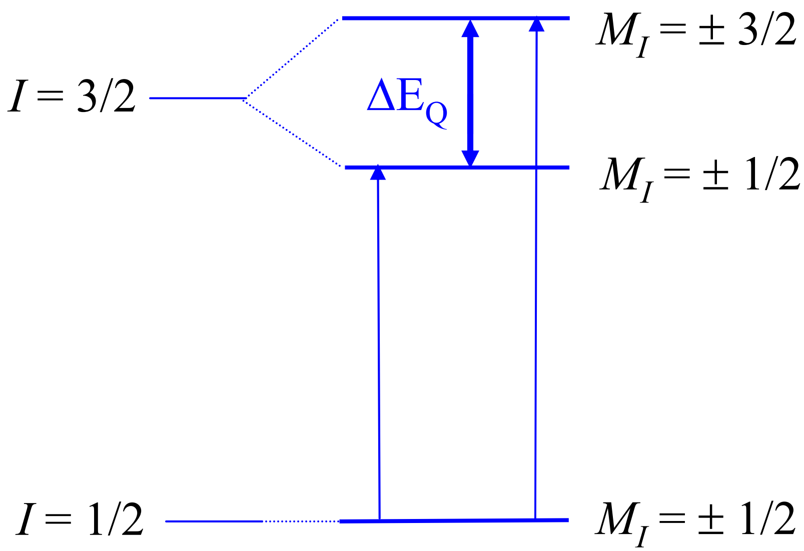
$$= 11.6 \text{ MHz}$$

$$= 3.9 \times 10^{-4} \text{ cm}^{-1}$$

$$= 5.6 \text{ K}$$

Types of Mössbauer spectra: 1) Quadrupole Doublet





Parameters

Isomer shift (δ):

s-electron density (oxidation state)

Quadrupole splitting (ΔE_Q):

caused by interaction of the quadrupole moment **Q** of the $I = 3/2$ excited state with the electric field gradient **V** generated by charges (electrons) surrounding the Fe site

Isomer shift

$$\delta = \underbrace{\left(|\psi_{sample}(0)|^2 - |\psi_{source}(0)|^2 \right)}_{\textit{electron density at nucleus}} \times \underbrace{4/5\, \pi\, Ze^2\, R^2 \times (\Delta R/R)}_{\textit{properties of } ^{57}\textit{Fe nucleus}}$$

$\Delta R/R$ is the change of radius in ground and excited state (negative for ^{57}Fe).
$|\psi(0)|^2$ is the probability to find an electron at the ^{57}Fe nucleus (only s-electrons have non-zero probability to be at nucleus, other electrons affect s-electron density by shielding).

$$\delta_{Fe(II)} > \delta_{Fe(III)} > \delta_{Fe(IV)}$$

$$\delta_{high\text{-}spin} > \delta_{low\text{-}spin}$$

$$\delta_{octahedral} > \delta_{tetrahedral}$$

Quadrupole splitting

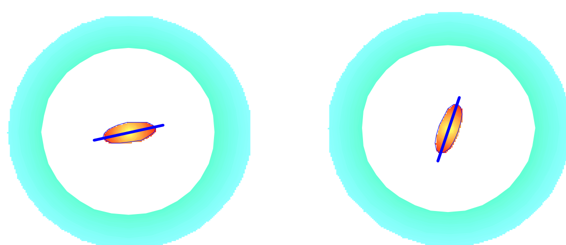
$$\hat{H}_q = \mathbf{I} \cdot \mathbf{Q} \cdot \mathbf{I}$$

$$= eQV_{zz}/12\ [\ 3\ I_z^2 - I\,(I + 1) + \eta\,(I_x^2 - I_y^2)]$$

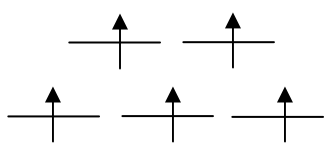
$$\Delta E_Q = eQV_{zz}/2\ [\ 1 + \eta^2/3]^{1/2}$$

$$\eta = (V_{xx} - V_{yy})\ /\ V_{zz} \text{ (asymmetry parameter)}$$

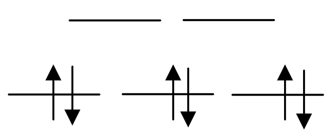
Spherical distribution



ΔE_Q typically small

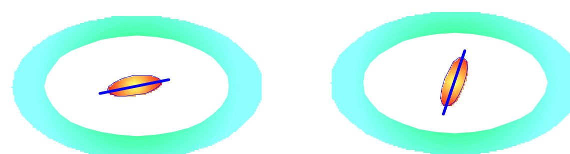


High-spin Fe^{3+}

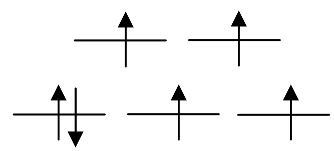


Low-spin Fe^{2+}

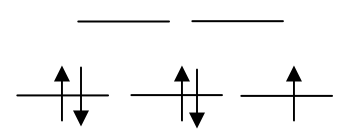
Asymmetric distribution



ΔE_Q typically large



High-spin Fe^{2+}



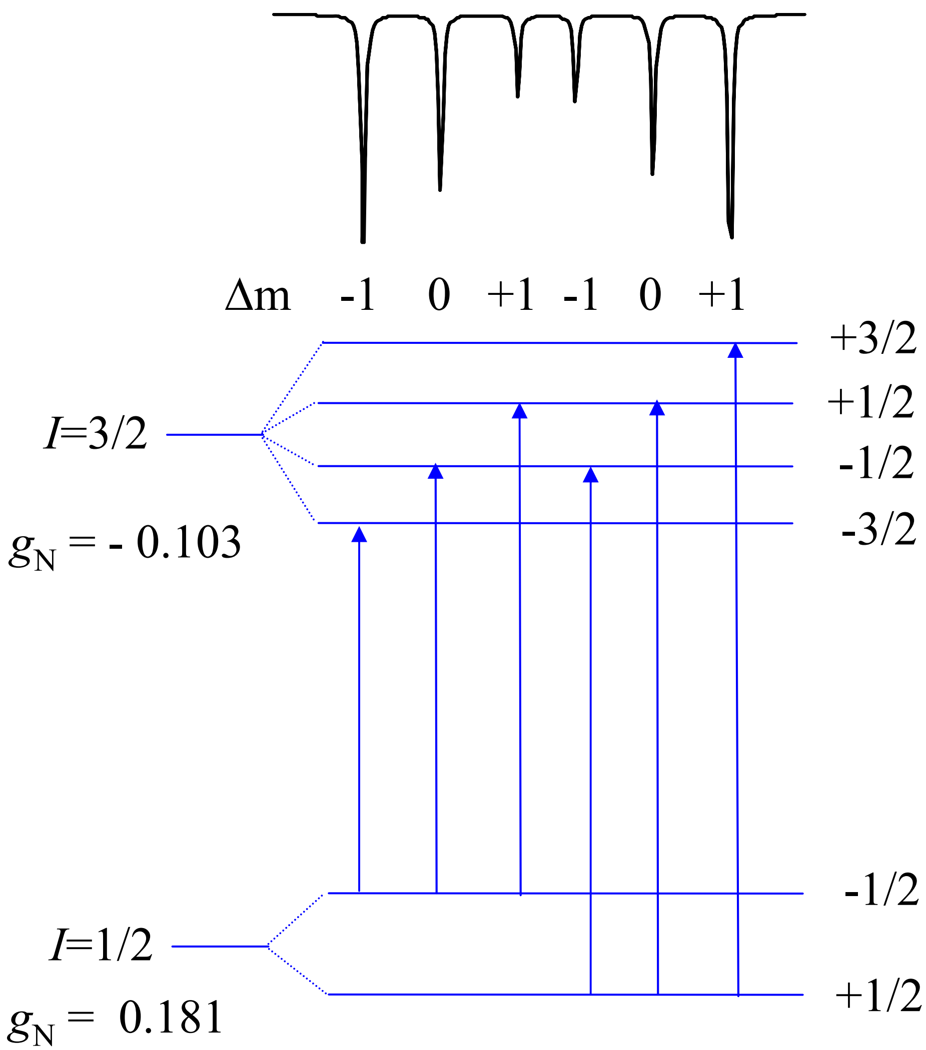
Low-spin Fe^{3+}

Typical values of δ and ΔE_Q for biological samples

Oxidation state	Spin state	Ligands	δ (mm/s)	ΔE_Q (mm/s)
Fe(II)	$S = 2$	heme	0.85 - 1.0	1.5 - 3.0
		Fe-(O/N)	1.1 - 1.3	2.0 - 3.2
		Fe/S	0.60 - 0.70	2.0 - 3.0
	$S = 0$	heme	0.30 - 0.45	< 1.5
Fe(III)	$S = 5/2$	heme	0.35 – 0.45	0.5 – 1.5
		Fe-(O/N)	0.40 – 0.60	0.5 – 1.5
		Fe/S	0.20 – 0.35	< 1.0
	$S = 3/2$	heme	0.30 – 0.40	3.0 – 3.6
	$S = 1/2$	heme	0.15 – 0.25	1.5 – 2.5
		Fe-(O/N)	0.10 – 0.25	2.0 – 3.0
Fe(IV)	$S = 2$	Fe-(O/N)	0.0 – 0.35	0.5 – 1.5
	$S = 1$	heme	0.0 – 0.10	1.0 – 2.0
		Fe-(O/N)	-0.20 – 0.10	0.5 – 4.3

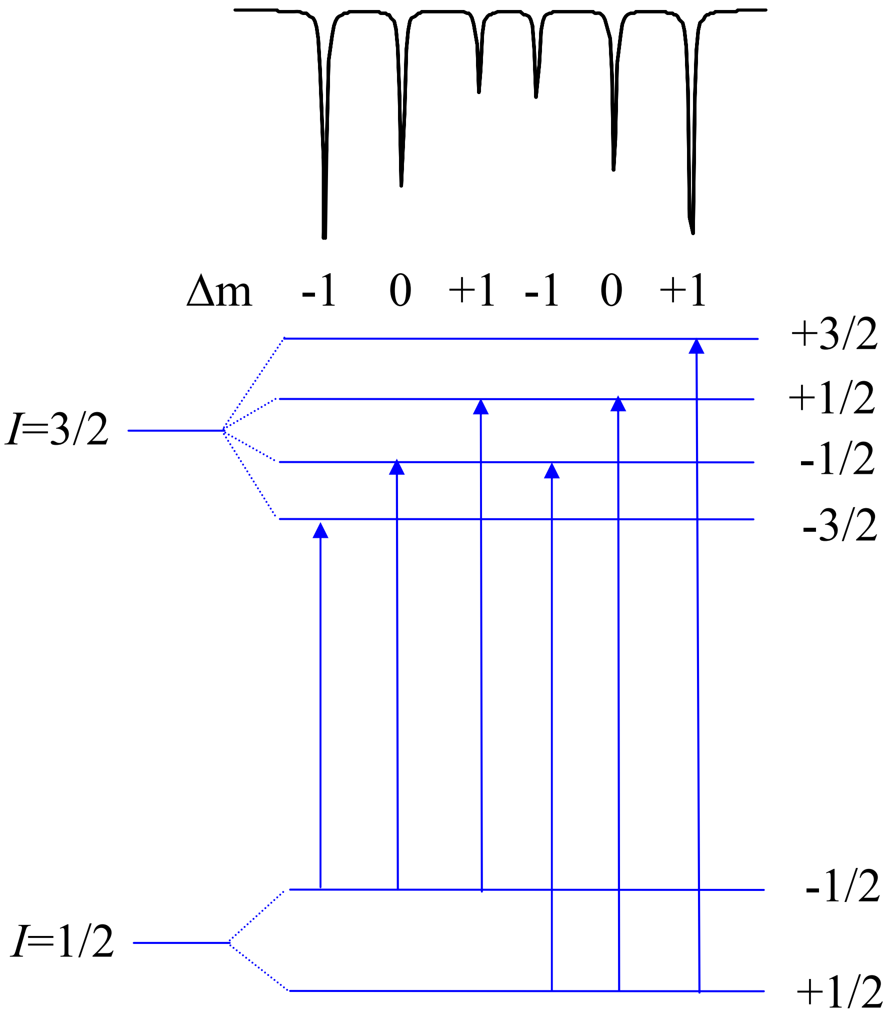
Adapted from E. Münck, *Physical Methods in Bioinorganic Chemistry*, L. Que (ed) 2000

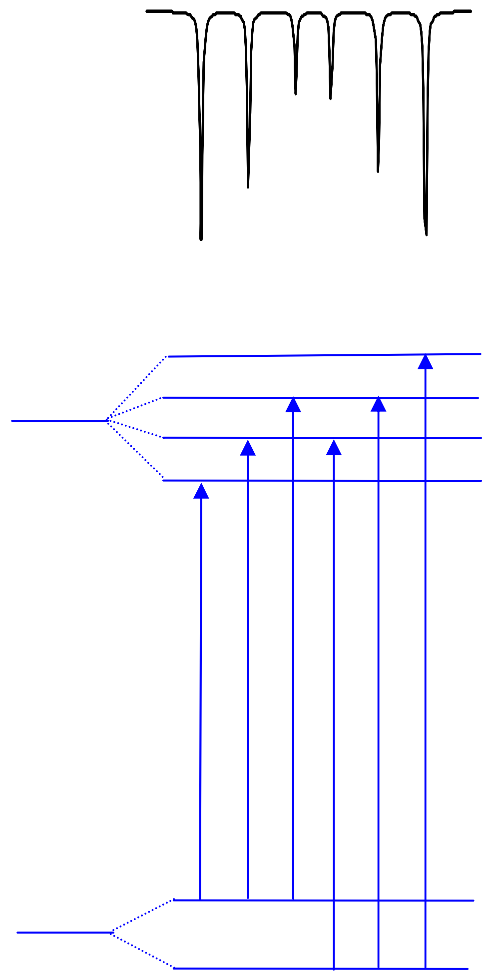
Types of Mössbauer spectra: 2) Magnetic Spectra



- Ground and excited state of ^{57}Fe are split due to nuclear Zeeman effect

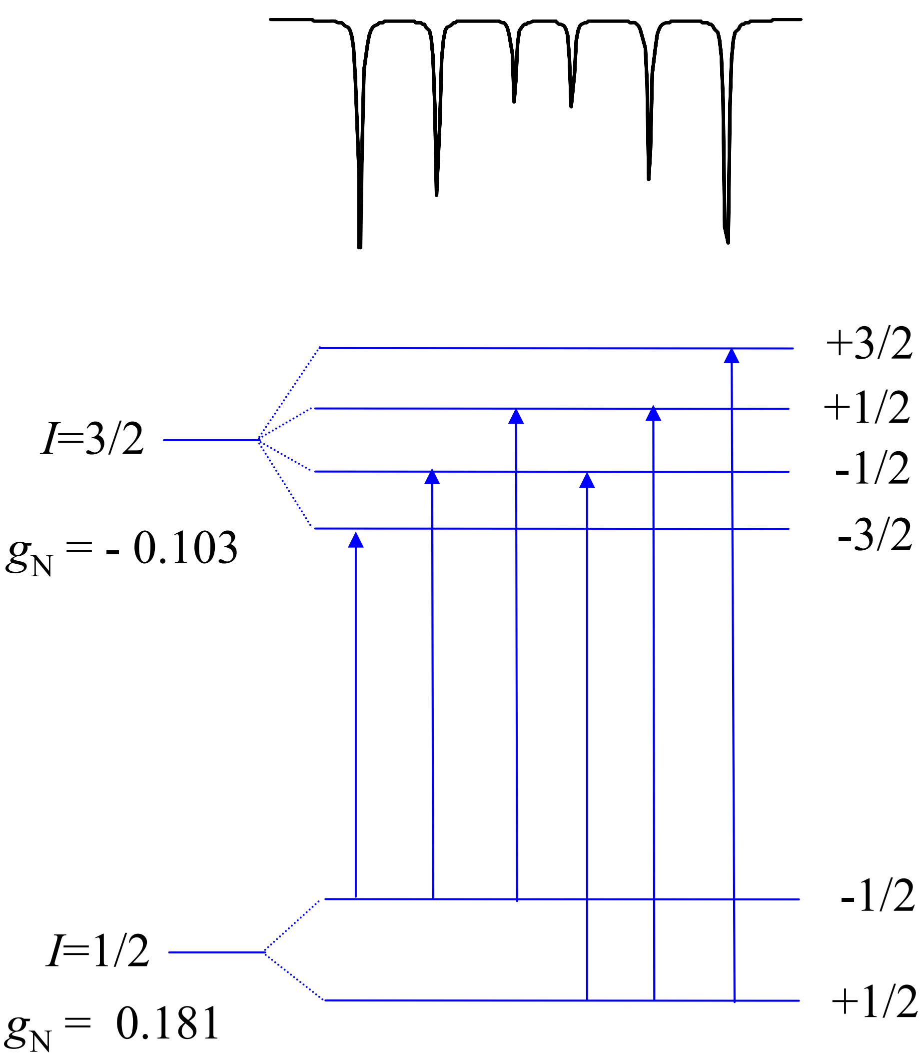
Types of Mössbauer spectra: 2) Magnetic Spectra

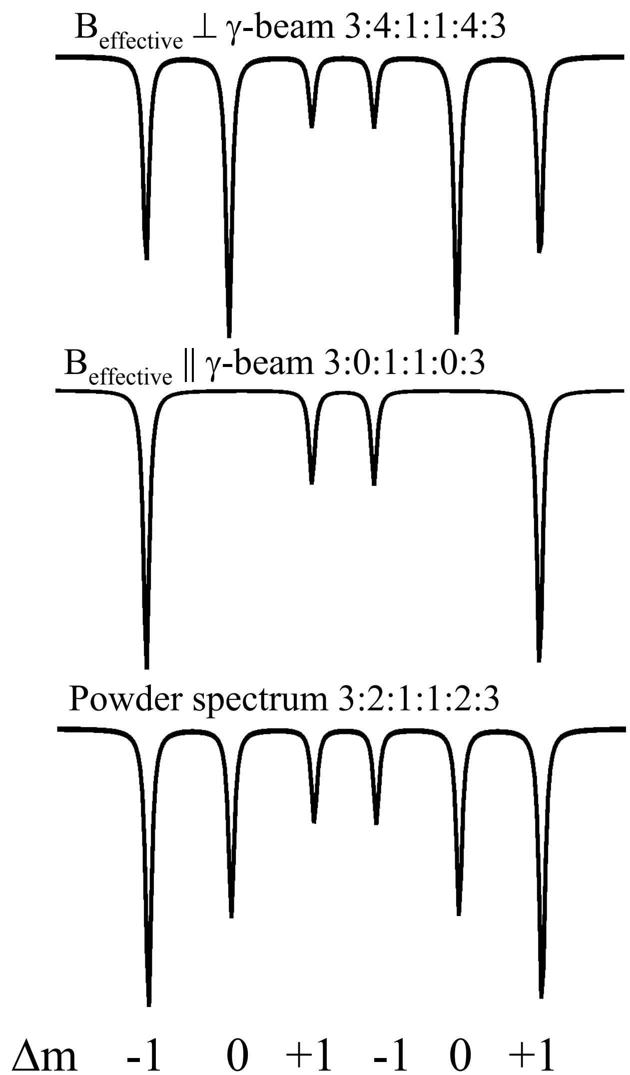




- Splitting of the six lines increases as the magnetic field experienced by the ^{57}Fe nucleus (the effective magnetic field) increases

Types of Mössbauer spectra: 2) Magnetic Spectra





- Intensity ratio of the six lines depends on the orientation of the effective magnetic field to the propagation direction of the γ beam.

Remaining topics about magnetic Mössbauer

- What is the internal field and how can it be calculated?
- How is it correlated with EPR spectroscopy?
- What is the orientation of the internal field?
- How does the relaxation rate of the electronic states affect the spectrum?
- Magnetic Mössbauer spectra of polynuclear clusters

Spin Hamiltonian for Mössbauer Spectroscopy

electron spin *hyperfine coupling* *nuclear spin*

$$\hat{H} = \boxed{\mu_B \mathbf{S} \bullet \mathbf{g} \bullet \mathbf{B} \quad + \quad \mathbf{S} \bullet \mathbf{D} \bullet \mathbf{S}} \quad + \quad \mathbf{S} \bullet \mathbf{A} \bullet \mathbf{I} \quad - \quad g_N \mu_N \mathbf{B} \bullet \mathbf{I} \quad + \quad \mathbf{I} \bullet \mathbf{Q} \bullet \mathbf{I}$$

electron Zeeman — zero field splitting — hyperfine — ^{57}Fe nuclear Zeeman — quadrupole splitting

$$= \langle \mathbf{S} \rangle \bullet \mathbf{A} \bullet \mathbf{I} \quad - \quad g_N \mu_N \mathbf{B} \bullet \mathbf{I} \quad + \quad \mathbf{I} \bullet \mathbf{Q} \bullet \mathbf{I}$$

$\langle \mathbf{S} \rangle$ is spin expectation value; it contains information of electronic structure

$$= - g_N \mu_N [- \langle \mathbf{S} \rangle \bullet \mathbf{A} / g_N \mu_N + \mathbf{B}] \bullet \mathbf{I} \quad + \quad \mathbf{I} \bullet \mathbf{Q} \bullet \mathbf{I}$$

internal field — external field

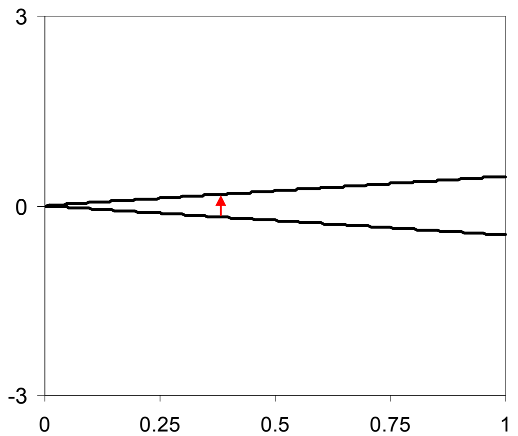
effective field

- $\langle \mathbf{S} \rangle$ is proportional to dE/dB

Spin expectation value

⟨S⟩ ~ dE/dB; therefore, Mössbauer senses how steep the slope is, i.e. the larger dE/dB, the larger is ⟨S⟩, the larger is the splitting in the Mössbauer spectrum.

Half-integer spin systems



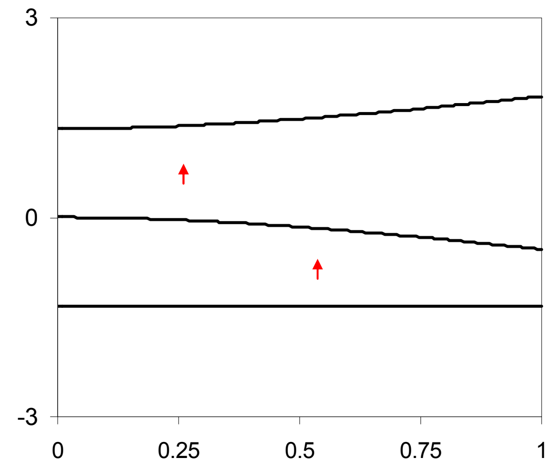
Have large ⟨S⟩, even with ~50mT B_{ext}

For small $B_{external}$: $\langle S \rangle \approx g_{eff}/4$

Magnetically split spectra

Correlation between EPR and Mössbauer !

Integer spin systems



Have in most cases $\langle S \rangle \approx 0$ for $B_{ext} = 0$

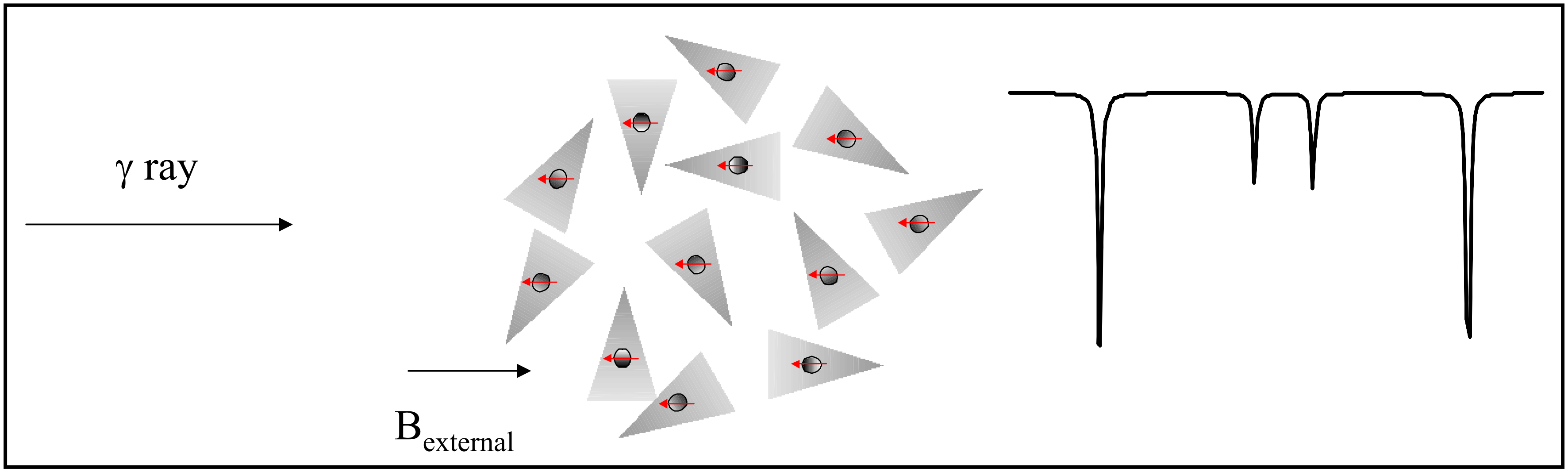
=> Quadrupole doublets

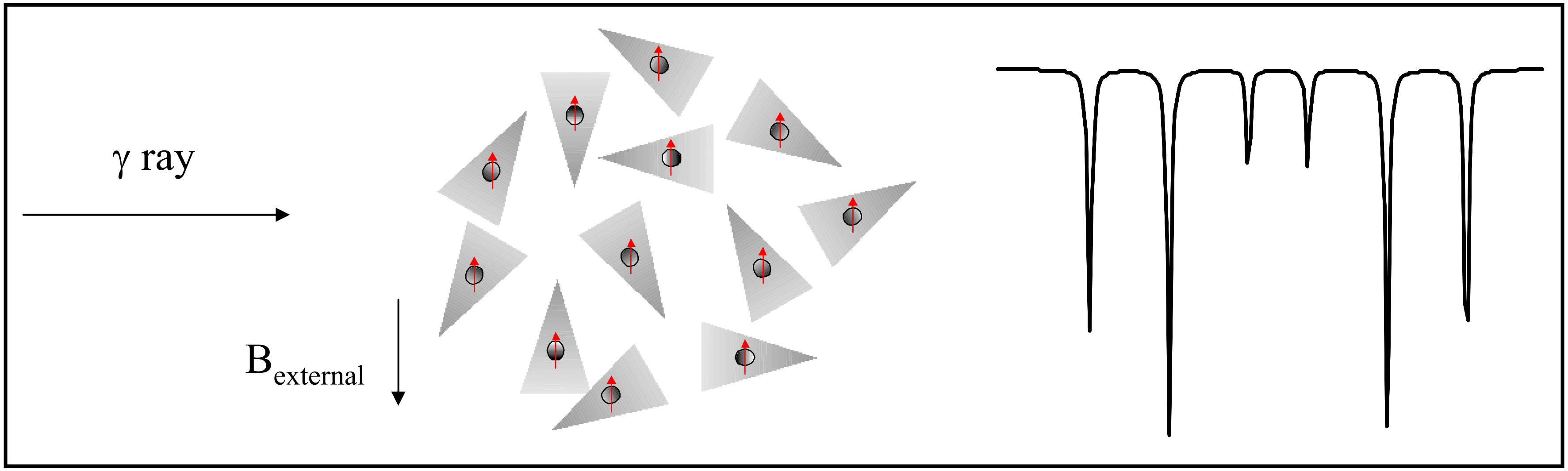
Small B_{ext} (50 mT) may result in small ⟨S⟩

=> broadened quadrupole doublets

Typically EPR-silent

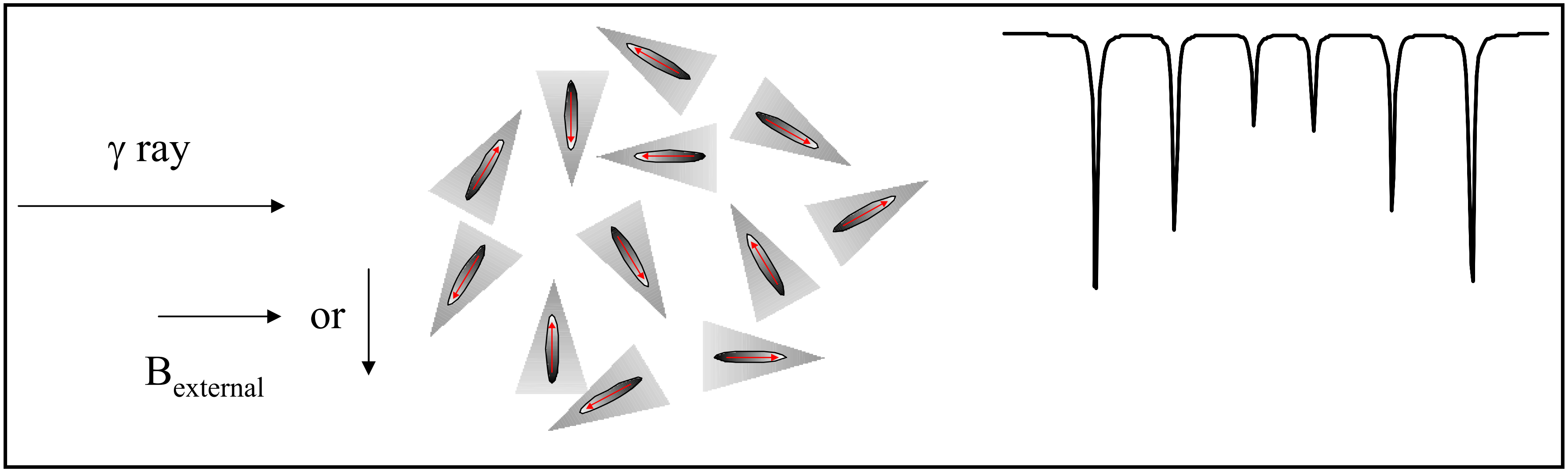
Internal Field of an isotropic electronic spin system





• The internal field (large, ~30-60T) is aligned antiparallel to the external field (~50mT).

Internal Field of an uniaxial electronic spin system



• Consider a spin system with highly anisotropic, uniaxial ⟨S⟩
(e.g. ground doublet of rhombic *S* = 5/2, which has values of 2.4, 0.23, and 0.15)

• The internal field is oriented along the axis with the greatest component of ⟨S⟩
[i.e. the component of ⟨S⟩ aligned antiparallel to the external field is maximized].

• The orientation of ⟨S⟩ depends on molecular frame; thus, because molecules are frozen randomly, the internal fields are oriented randomly (powder averaged spectrum)

Relaxation of the electronic states and their effect on the Mössbauer spectrum

Paramagnetic Fe-sites have more than one electronic state; relaxation between electronic states needs to be considered for such systems.

Three cases are possible:

- The relaxation between electronic states is slow compared to the Larmor frequency of the ^{57}Fe nucleus (time scale of Mössbauer spectroscopy).
(typically encountered for metalloproteins at 4.2K)

- The relaxation between electronic states is fast compared to the Larmor frequency of the ^{57}Fe nucleus.
(encountered at "high" temperatures; depends on system under consideration)

- The relaxation between electronic states is comparable to the Larmor frequency of the ^{57}Fe nucleus. This case is difficult to treat and we try to avoid it by choosing different experimental conditions (temperature, external field).

Slow and fast relaxation limit

Slow relaxation

Calculate $\langle \mathbf{S} \rangle$ for each electronic state.

Calculate Mössbauer spectrum for each electronic state.

Add the subspectra of all electronic states according to their Boltzmann factors [~exp(-E/kT)].

The resulting spectrum contains multiple subspectra (one for every electronic state).

The subspectra are split by the hyperfine interaction, i.e. magnetic spectra.

Fast relaxation

Calculate $\langle \mathbf{S} \rangle$ for each electronic state.

Calculate the average $\langle \mathbf{S}_{av} \rangle$ from the individual $\langle \mathbf{S} \rangle$ values according to their Boltzmann factors.

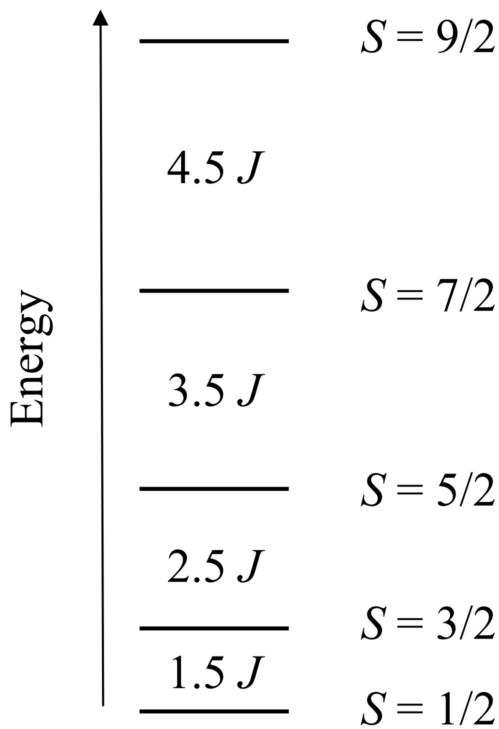
Calculate Mössbauer spectrum using $\langle \mathbf{S}_{av} \rangle$. The resulting spectrum contains only one subspectrum.

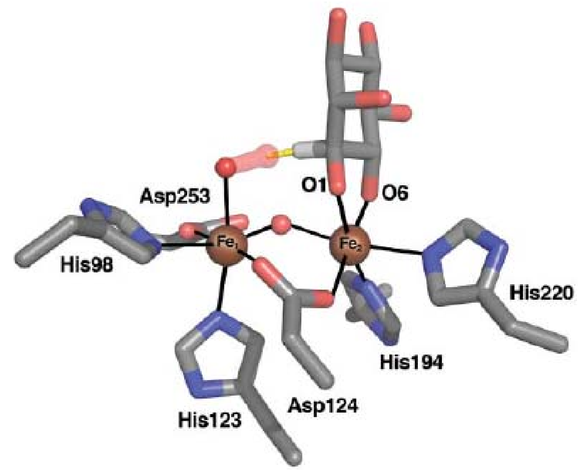
In small magnetic fields $\langle \mathbf{S}_{av} \rangle \approx 0$, therefore no hyperfine interactions, i.e. spectrum is a quadrupole doublet.

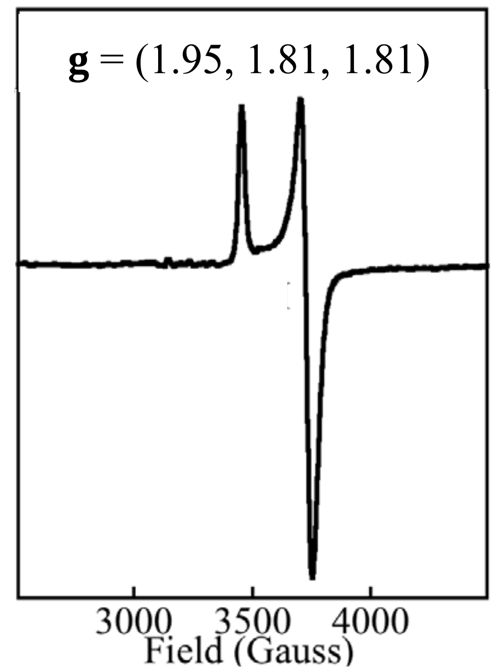
The spin-coupled $Fe_2^{II/III}$ cluster in *myo*-inositol oxygenase

- The active form of *myo*-inositol oxygenase harbors a dinuclear site with a high-spin Fe^{3+} ion (S_1 = 5/2) and a high-spin Fe^{2+} ion (S_2 = 2), which are antiferromagnetically coupled.

- It has an EPR-active S = 1/2 ground state.

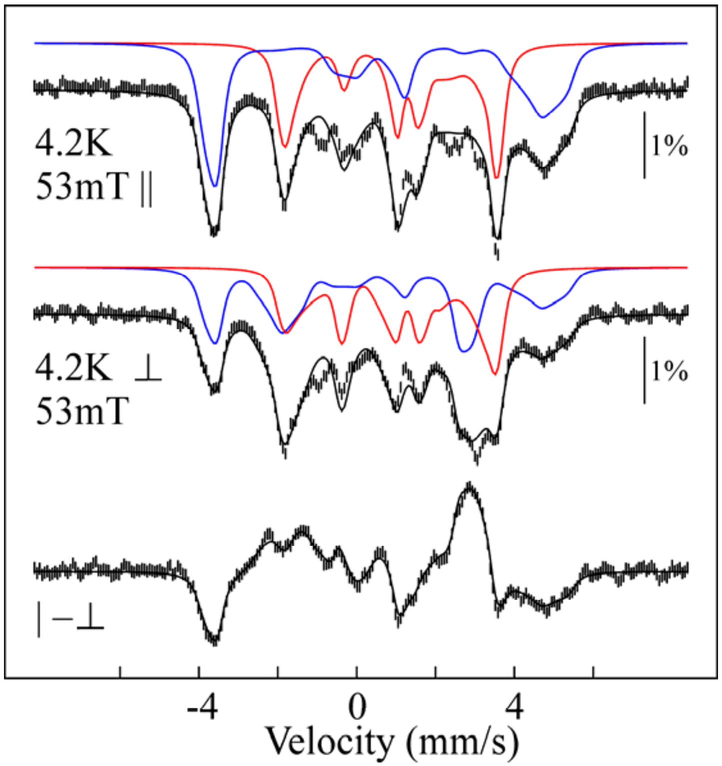


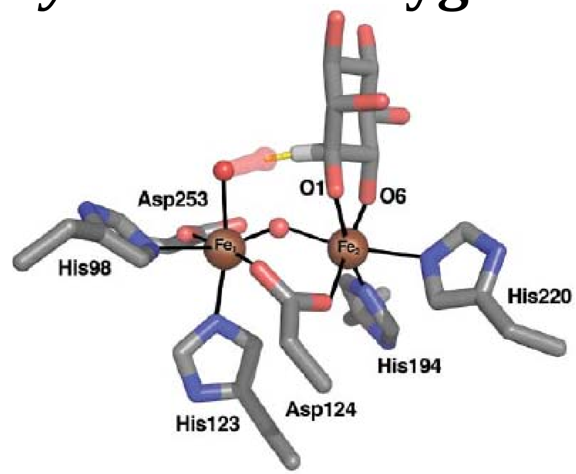


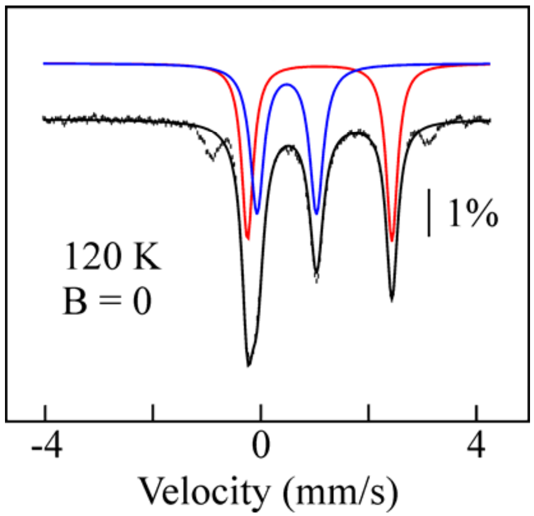


- EPR-spectroscopy probes the **total ground spin state** of a coupled cluster.

The spin-coupled $Fe_2^{II/III}$ cluster in *myo*-inositol oxygenase





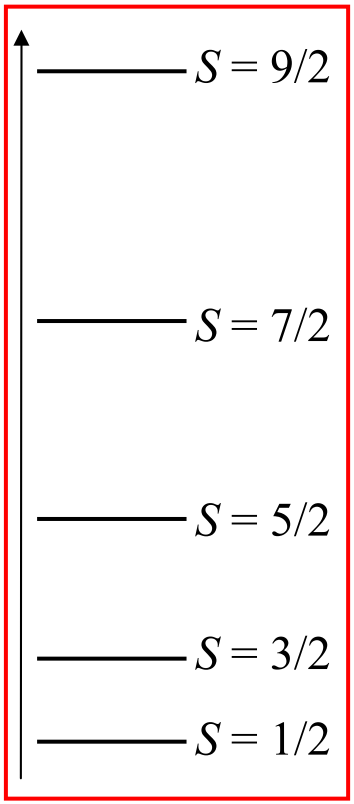


- Mössbauer-spectroscopy probes the **local spin state** of each ^{57}Fe-labeled site of a coupled cluster.
- At 4.2 K: slow-relaxation limit => magnetically split spectra
- At 120 K: fast-relaxation limit => quadrupole doublets

Spin Hamiltonian of an exchange-coupled cluster

$$\hat{\mathrm{H}}_{\mathrm{total}} = \hat{\mathrm{H}}_{\mathrm{el}} + \hat{\mathrm{H}}_{\mathrm{hf}} + \hat{\mathrm{H}}_{\mathrm{nuc}}$$

$$\boxed{\hat{\mathrm{H}}_{\mathrm{el}} = \underbrace{\mu_{\mathrm{B}}\,\mathbf{S_1}\bullet\mathbf{g_1}\bullet\mathbf{B}}_{\text{el. Zeeman 1}} + \underbrace{\mu_{\mathrm{B}}\,\mathbf{S_2}\bullet\mathbf{g_2}\bullet\mathbf{B}}_{\text{el. Zeeman 2}} + \underbrace{\mathbf{S_1}\bullet\mathbf{D_1}\bullet\mathbf{S_1}}_{\text{ZFS 1}} + \underbrace{\mathbf{S_2}\bullet\mathbf{D_2}\bullet\mathbf{S_2}}_{\text{ZFS 2}} + \underbrace{J_{12}\,\mathbf{S_1}\bullet\mathbf{S_2}}_{\text{exchange coupling}}}$$



- Solving this electronic Hamiltonian provides detailed insight into ALL electronic states (i.e. also the excited $S = 3/2$, $5/2$, $7/2$, and $9/2$ states)
- Experimentally, only the $S = 1/2$ ground state is probed.

The following (much simpler) Hamiltonian is often used:

$$\boxed{\hat{\mathrm{H}}_{\mathrm{el}} = \mu_{\mathrm{B}}\,\mathbf{S_{tot}}\bullet\mathbf{g_{tot}}\bullet\mathbf{B}}$$

Spin Hamiltonian of an exchange-coupled cluster

$$\hat{H}_{hf} = \underbrace{\mathbf{S_1 \bullet A_1 \bullet I_1}}_{\text{hyperfine 1}} + \underbrace{\mathbf{S_2 \bullet A_2 \bullet I_2}}_{\text{hyperfine 2}}$$

If we want to do the simpler calculation only involving the ground state, we need to use modified hyperfine coupling tensors, which are multiplied by the appropriate **spin projection coefficients**

$$= \mathbf{S_{tot}} \bullet \{\mathbf{S_1}/\mathbf{S_{tot}} \times \mathbf{A_1}\} \bullet \mathbf{I_1} + \mathbf{S_{tot}} \bullet \{\mathbf{S_2}/\mathbf{S_{tot}} \times \mathbf{A_2}\} \bullet \mathbf{I_2}$$

$$= \mathbf{S_{tot}} \bullet \{c_1 \times \mathbf{A_1}\} \bullet \mathbf{I_1} + \mathbf{S_{tot}} \bullet \{c_2 \times \mathbf{A_2}\} \bullet \mathbf{I_2}$$

Spin projection factors

$$c_i = [S(S+1) + S_i(S_i+1) - S_j(S_j+1)] \,/\, [2S(S+1)]$$

For $S = 1/2$ ground state, $c_1 = +7/3$ and $c_2 = -4/3$

See A. Bencini and D. Gatteschi, *EPR of Exchange Coupled Systems*, Springer, 1989 for derivation of spin coupling coeff.

Spin Hamiltonian of an exchange-coupled cluster

$$\hat{H}_{hf} = \underbrace{\mathbf{S_1 \cdot A_1 \cdot I_1}}_{\text{hyperfine 1}} + \underbrace{\mathbf{S_2 \cdot A_2 \cdot I_2}}_{\text{hyperfine 2}}$$

$$= \mathbf{S_{tot}} \cdot \{c_1 \times \mathbf{A_1}\} \cdot \mathbf{I_1} + \mathbf{S_{tot}} \cdot \{c_2 \times \mathbf{A_2}\} \cdot \mathbf{I_2}$$

- $\mathbf{A_1}$ and $\mathbf{A_2}$ (the intrinsic **A**-tensors given with respect to the local spin) are dominated by the Fermi contact term, which is ~20 to -22 T.

- Analysis of field-dependent Mössbauer spectra allows $c_1 \times \mathbf{A_1}$ and $c_2 \times \mathbf{A_2}$ to be determined.

- by estimating $\mathbf{A_1}$ and $\mathbf{A_2}$, one can determine $c_1$and c_2 and therefore determine the nature of the spin coupling of the cluster.

- if hyperfine coupling is resolved in EPR, then $|c_1 \times \mathbf{A_1}|$ and $|c_1 \times \mathbf{A_1}|$ can be determined, but not the sign of c_1 and c_2.

EPR and Mössbauer spectroscopy are complementary

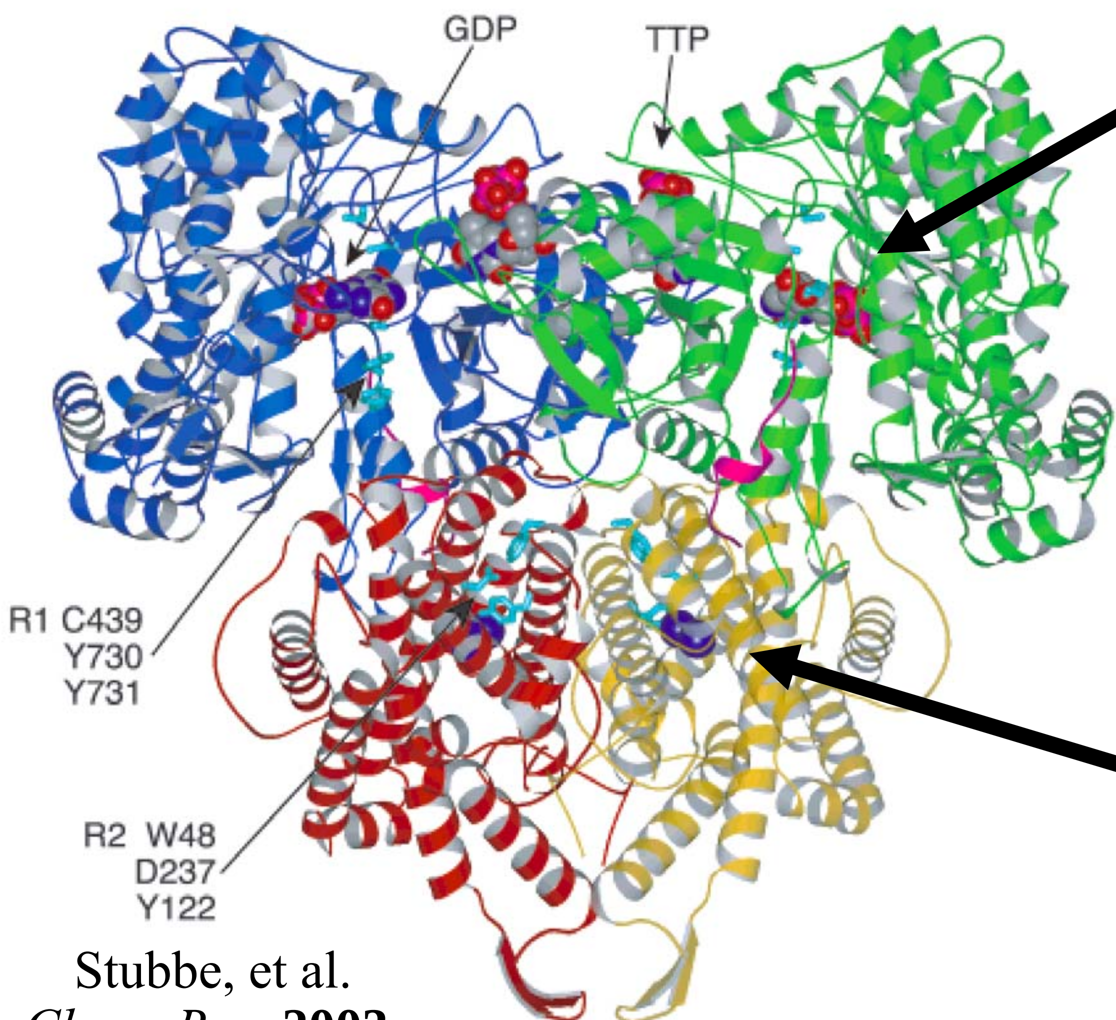
Electron Spin / Method	Integer Spin $S = 0, 1, 2, 3, \ldots$	Half-Integer Spin $S = 1/2, 3/2, 5/2, \ldots$
EPR	EPR-silent (*in most cases*)	EPR-active
Mössbauer	Quadrupole doublet (*in most cases*) (analysis straightforward)	Magnetically Split Spectrum (analysis complex, but is facilitated using results from EPR)

The two case studies for the practical part

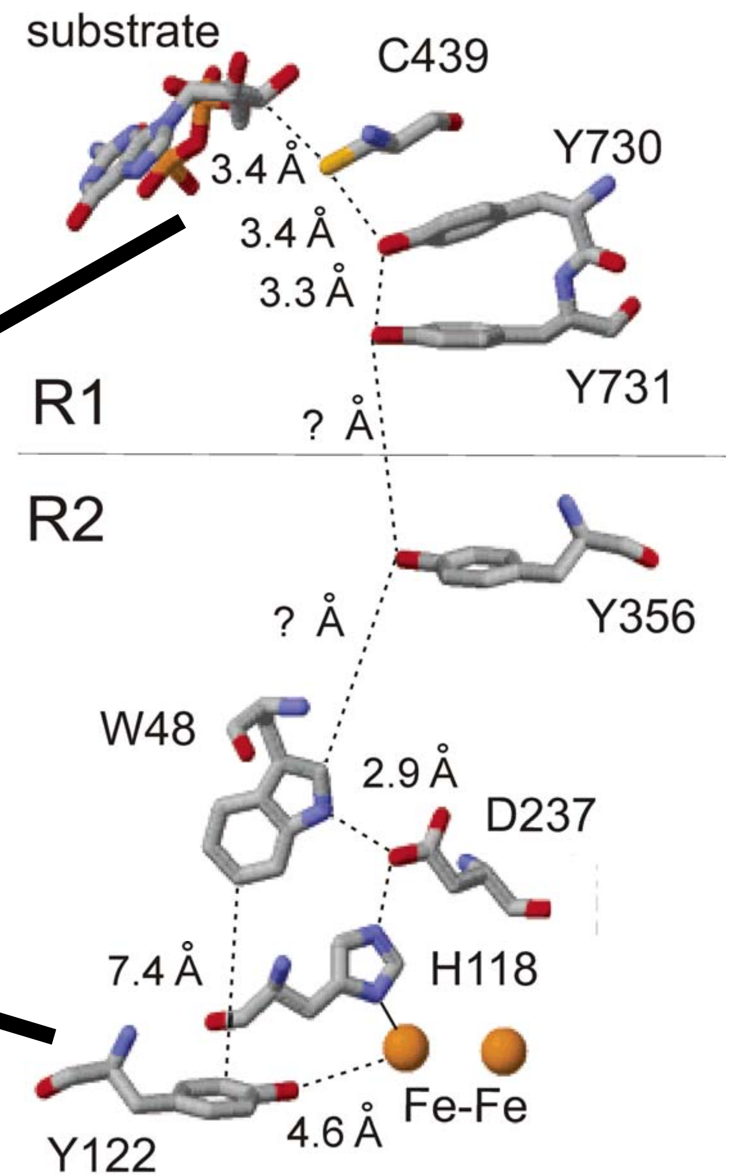
(1) The Mn/Fe-containing class I ribonucleotide reductase from *Chlamydia trachomatis*

(2) The reaction cycle of taurine:α-ketoglutarate dioxygenase

Class I Ribonucleotide Reductase from *E. coli*

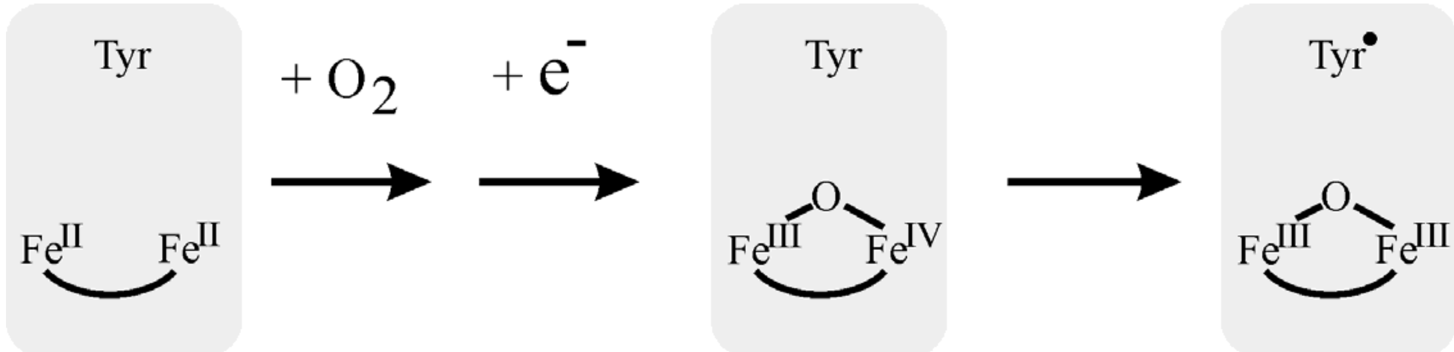


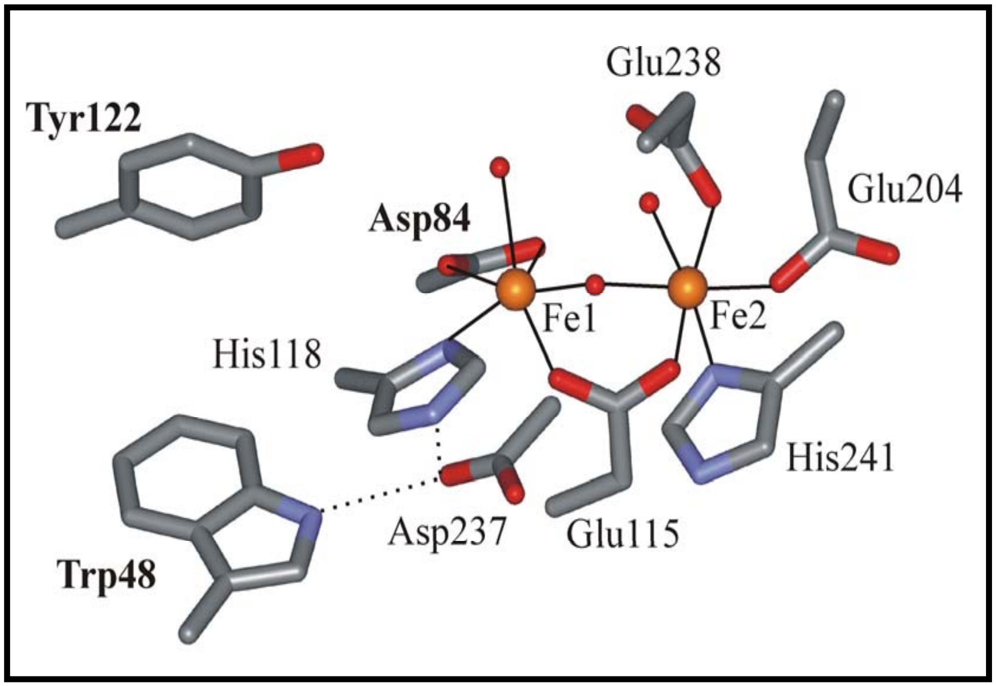
Stubbe, et al. *Chem. Rev.* **2003**, 2167-2202.



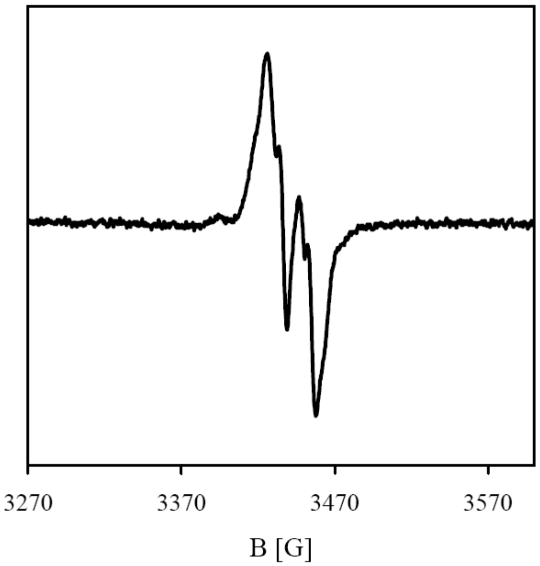
Proposed **PCET** (Proton Coupled Electron Transfer) Pathway

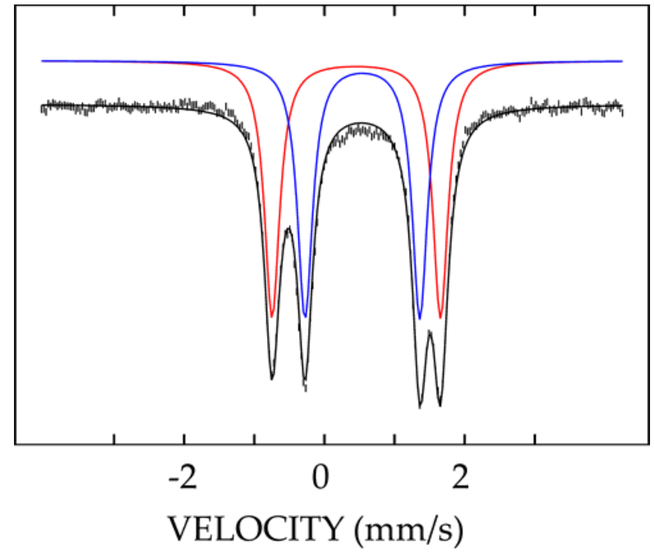
Example: Cofactor generation of *E. coli* ribonucleotide reductase

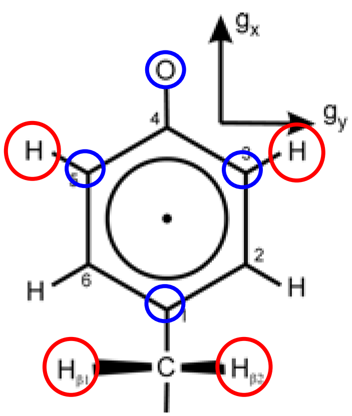


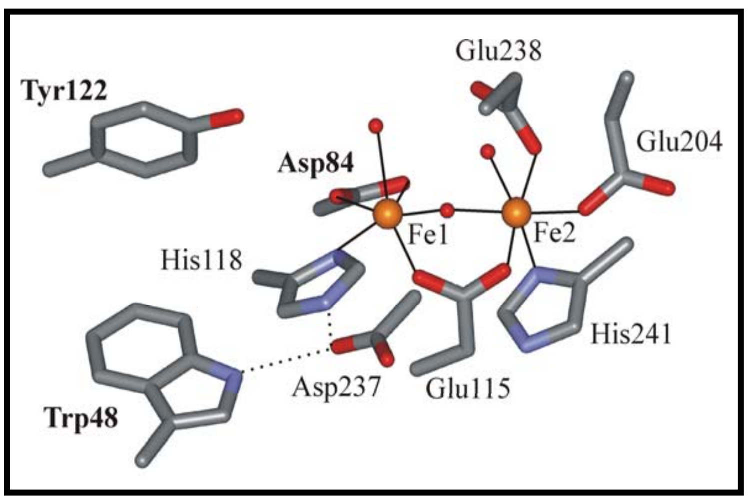


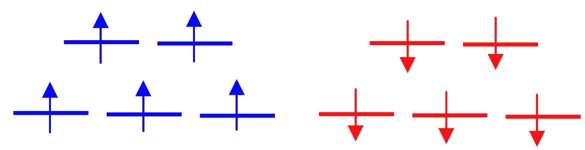
Spectroscopic signatures of the active $Fe_2^{III/III}$-Y122$^{\bullet}$ form









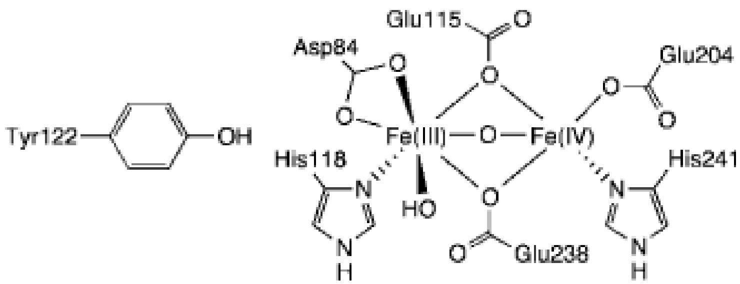


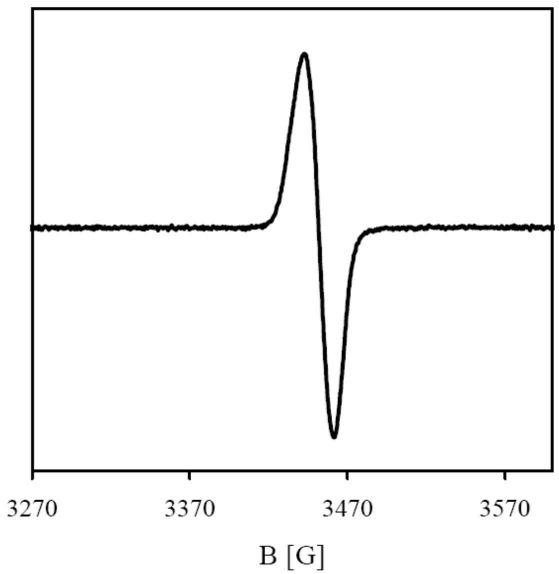
Fe(III)
$S = 5/2$

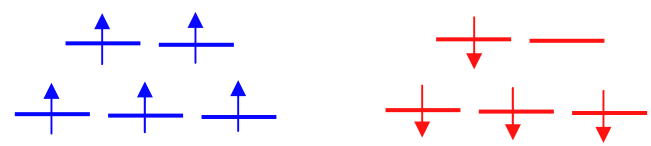
Fe(III)
$S = 5/2$

Ground state: $S_{total} = 0$

Spectroscopic signatures of the $Fe_2^{III/IV}$ intermediate "X"





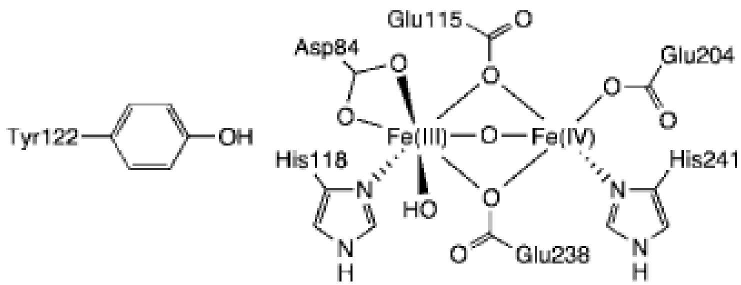


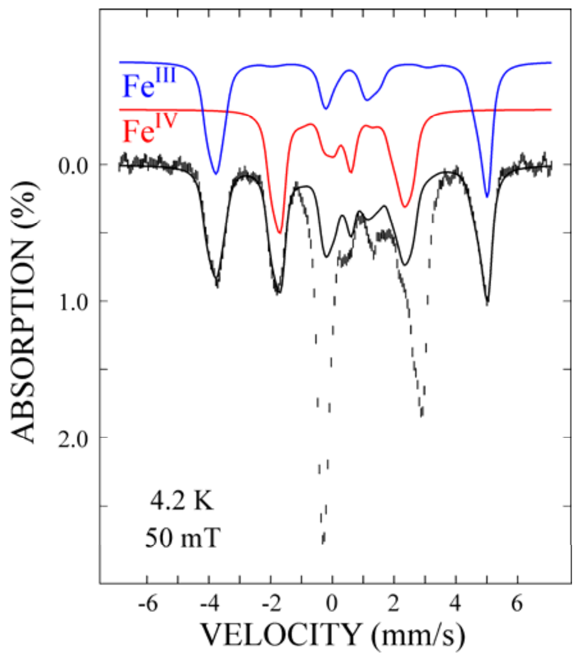
Fe(III)
$S = 5/2$

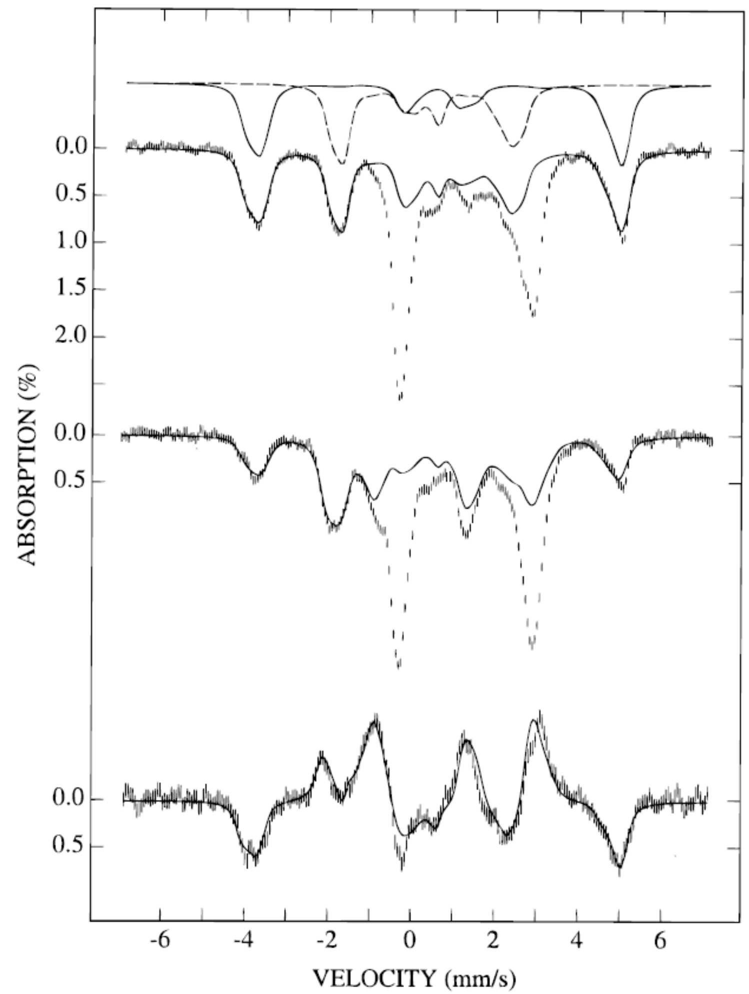
Fe(IV)
$S = 2$

Ground state: $S_{total} = 1/2$

Spectroscopic signatures of the $Fe_2^{III/IV}$ intermediate "X"

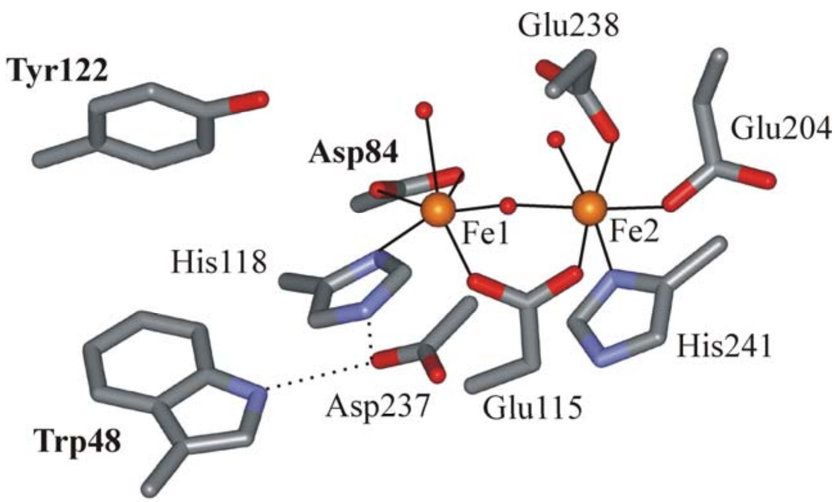




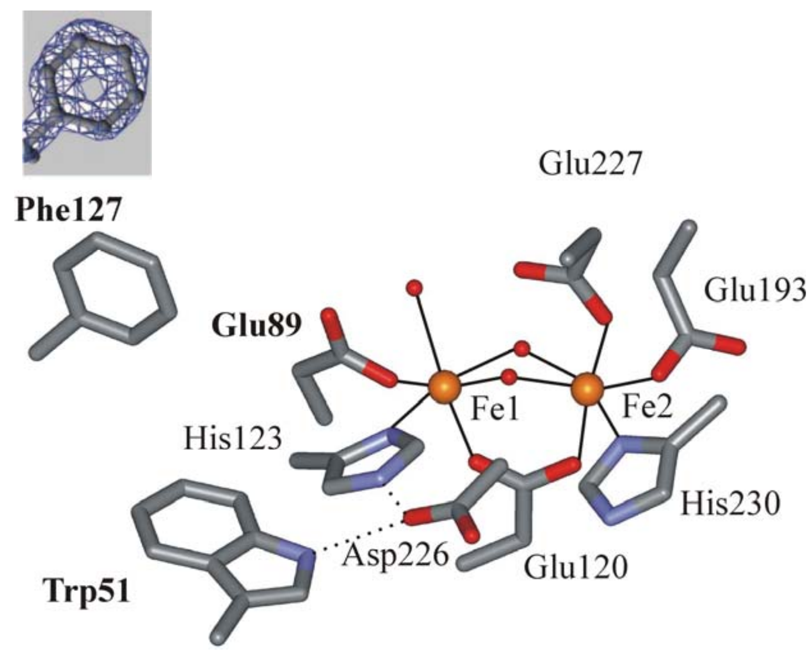


R2 Subunit from *Chlamydia trachomatis* Lacks Radical-Harboring Tyrosine but is Still Active!

<u>The Diferric Cluster of *Ec* R2</u>

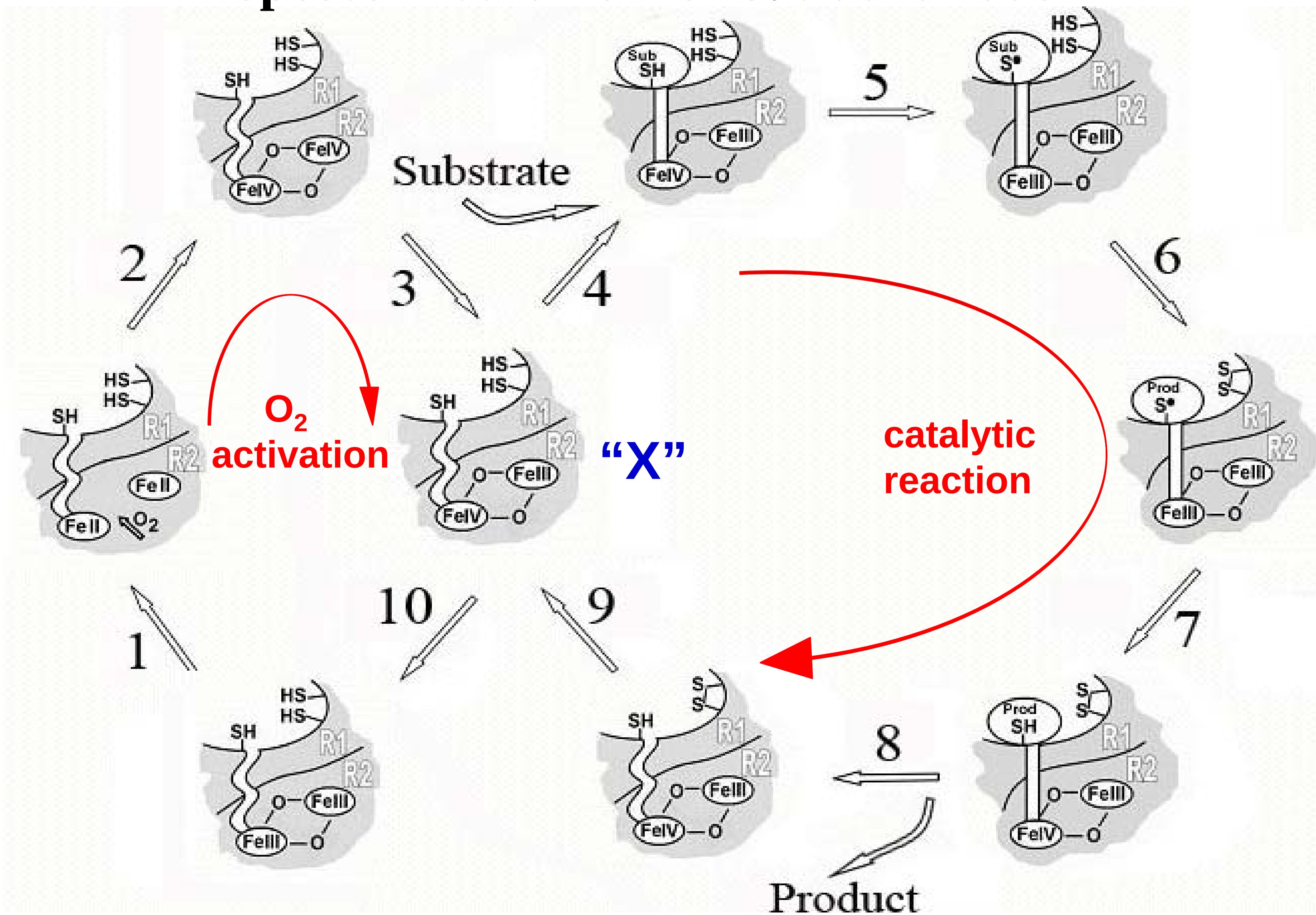


<u>The Diferric Cluster of *Ct* R2</u>



Högbom, et al. *<u>Science</u>* **2004**, 245-248.

Proposed mechanism of *C. trachomatis* RNR

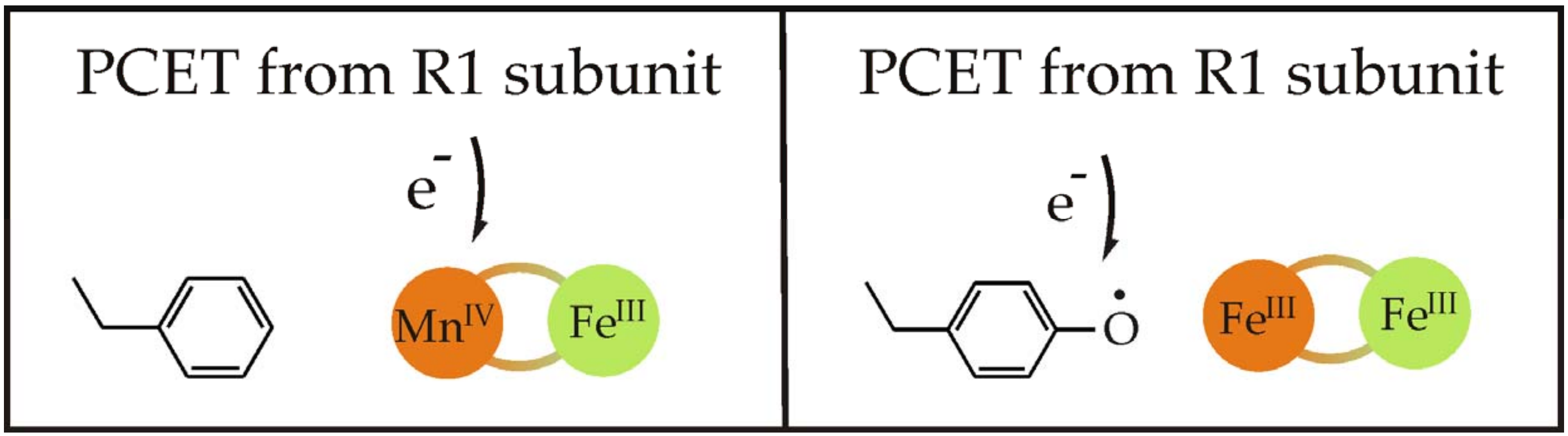


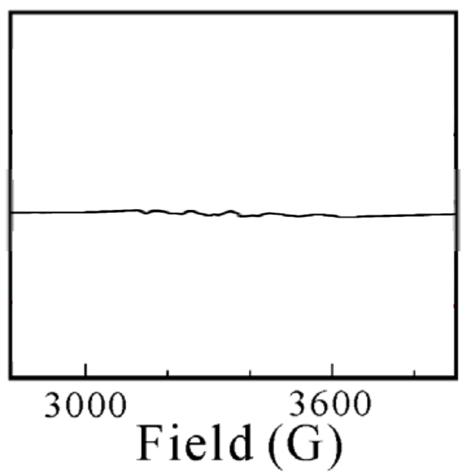
Voevodskaya, et al. *PNAS* **2006**, 9850-9854

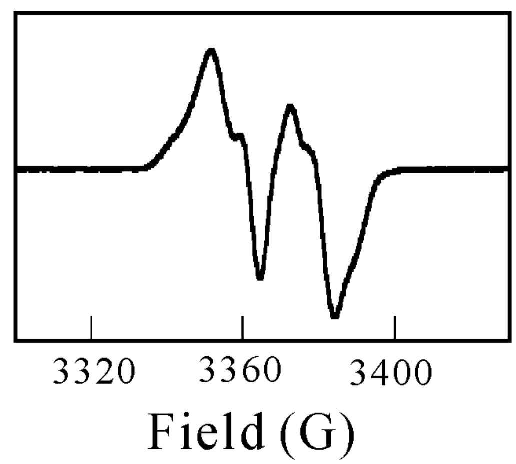
Oxidized PCET-initiating cofactors of class I RNRs

Class Ic

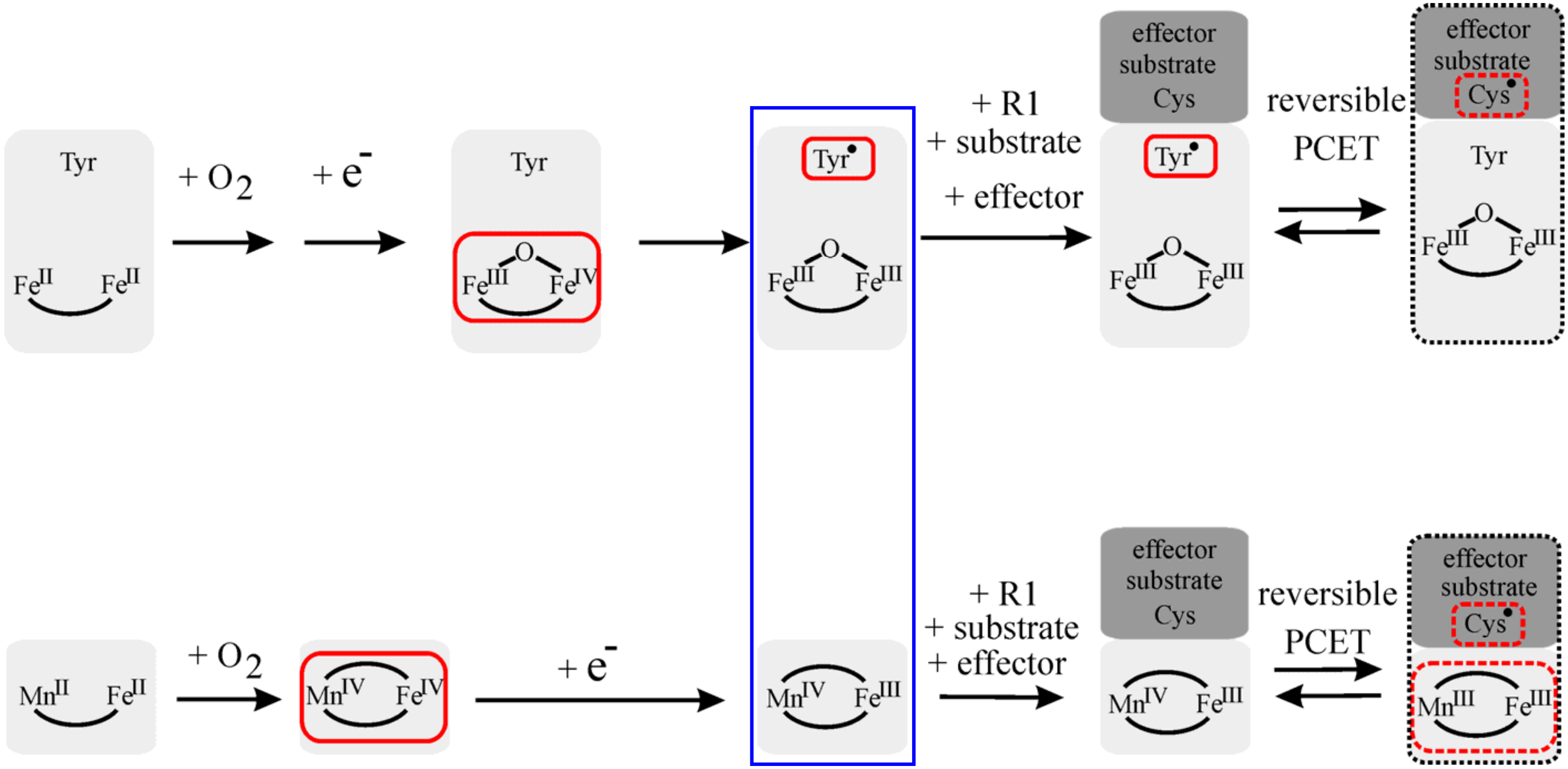
"Regular" Class I







Comparison of Class I and Class Ic RNRs

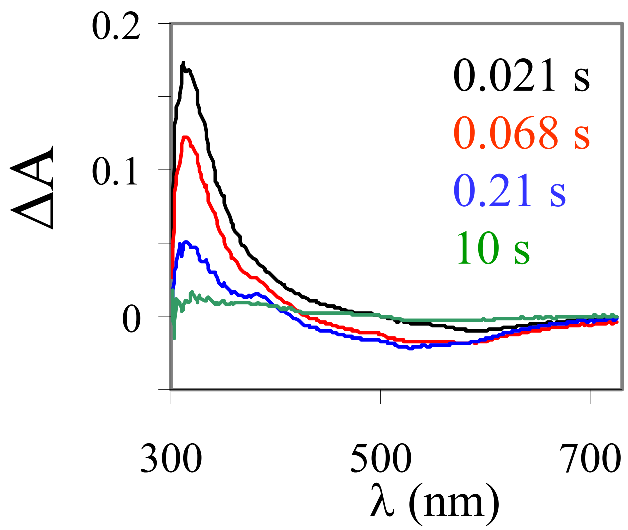


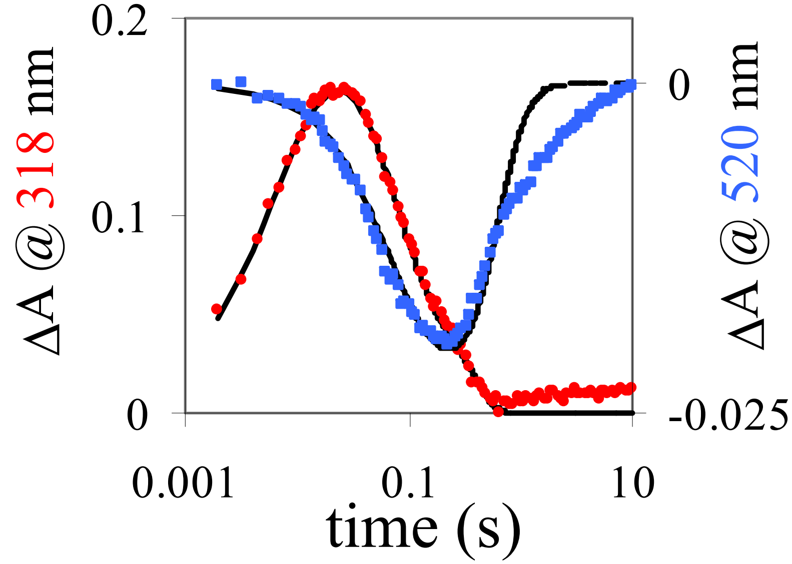
The two case studies for the practical part

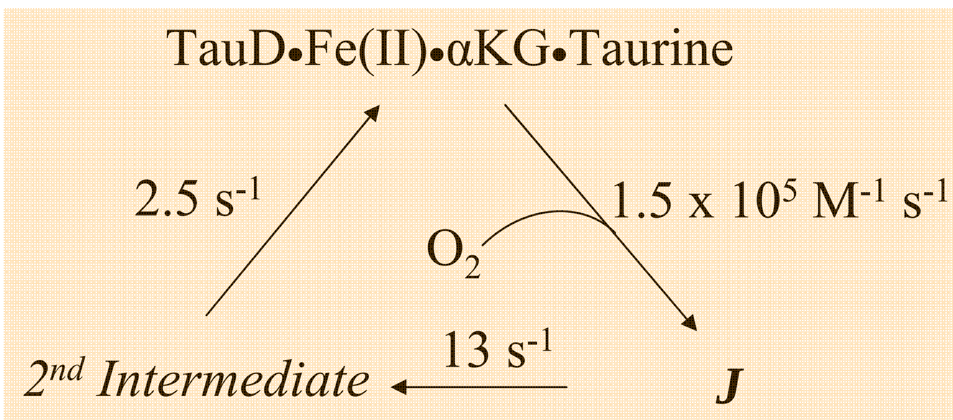
(1) The Mn/Fe-containing class I ribonucleotide reductase from *Chlamydia trachomatis*

(2) The reaction cycle of taurine:α-ketoglutarate dioxygenase

Stopped-Flow Absorption Spectroscopy of Taurine:α-Ketoglutarate Dioxygenase (TauD)

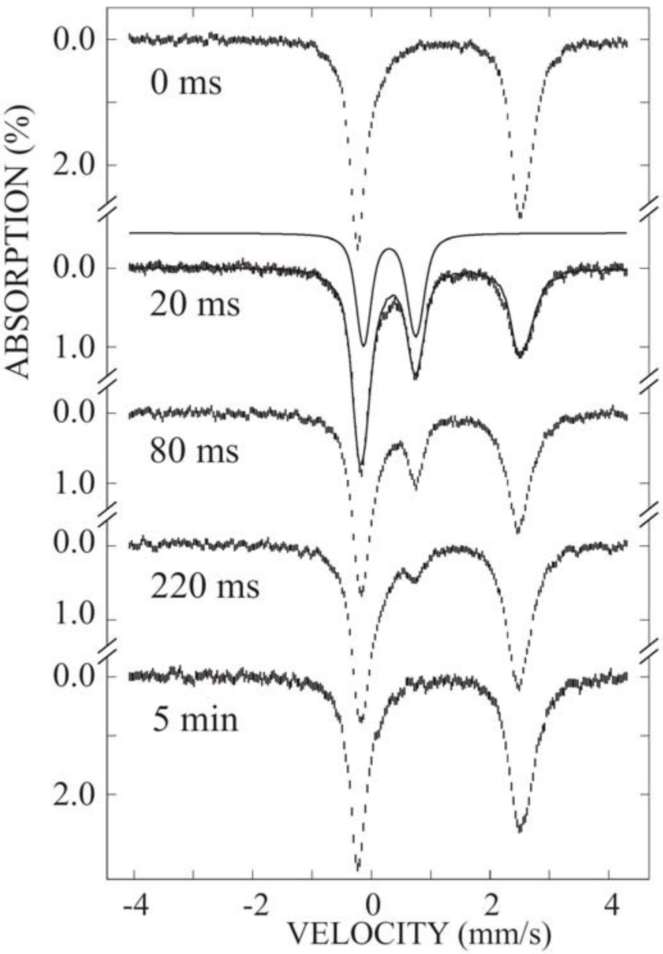






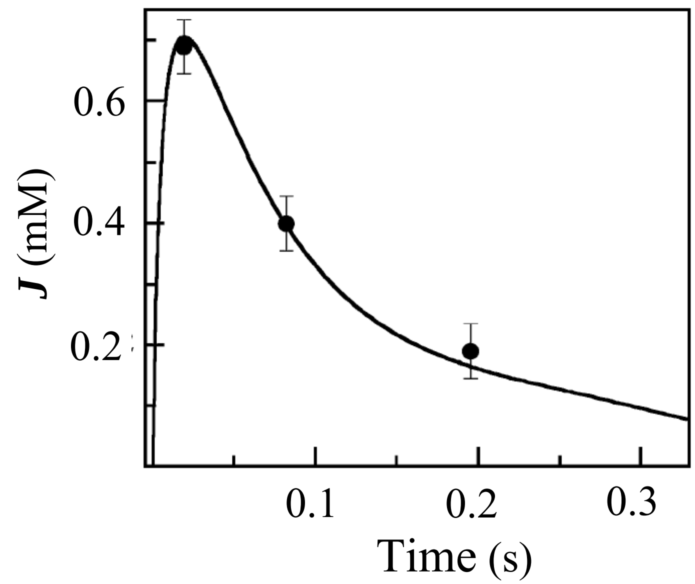
- Evidence for accumulation of two reaction intermediates

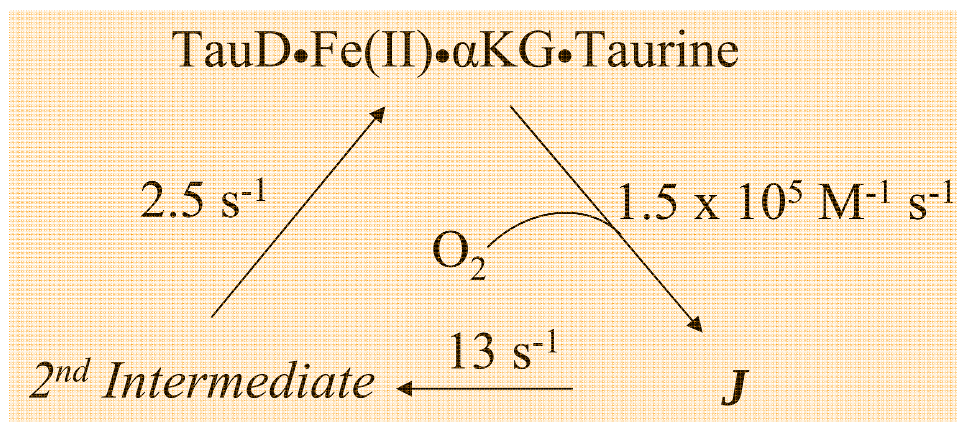
Evidence for an Fe(IV) Intermediate (*J*) by Mössbauer Spectroscopy



$\delta = 1.16$ mm/s
$|\Delta E_Q| = 2.76$ mm/s

$\delta = 0.30$ mm/s
$|\Delta E_Q| = 0.90$ mm/s





Fe(II) Fe(IV)

Mössbauer Evidence that *J* has an Integer Spin Ground State with *S* = 2