Some Fundamentals of Mossbauer Spectroscopy and Relations to EPR. Examples Illustrating the Power of Combining the Two Techniques

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Penn State Bioinorganic Workshop 2014

Fundamentals (30 min)

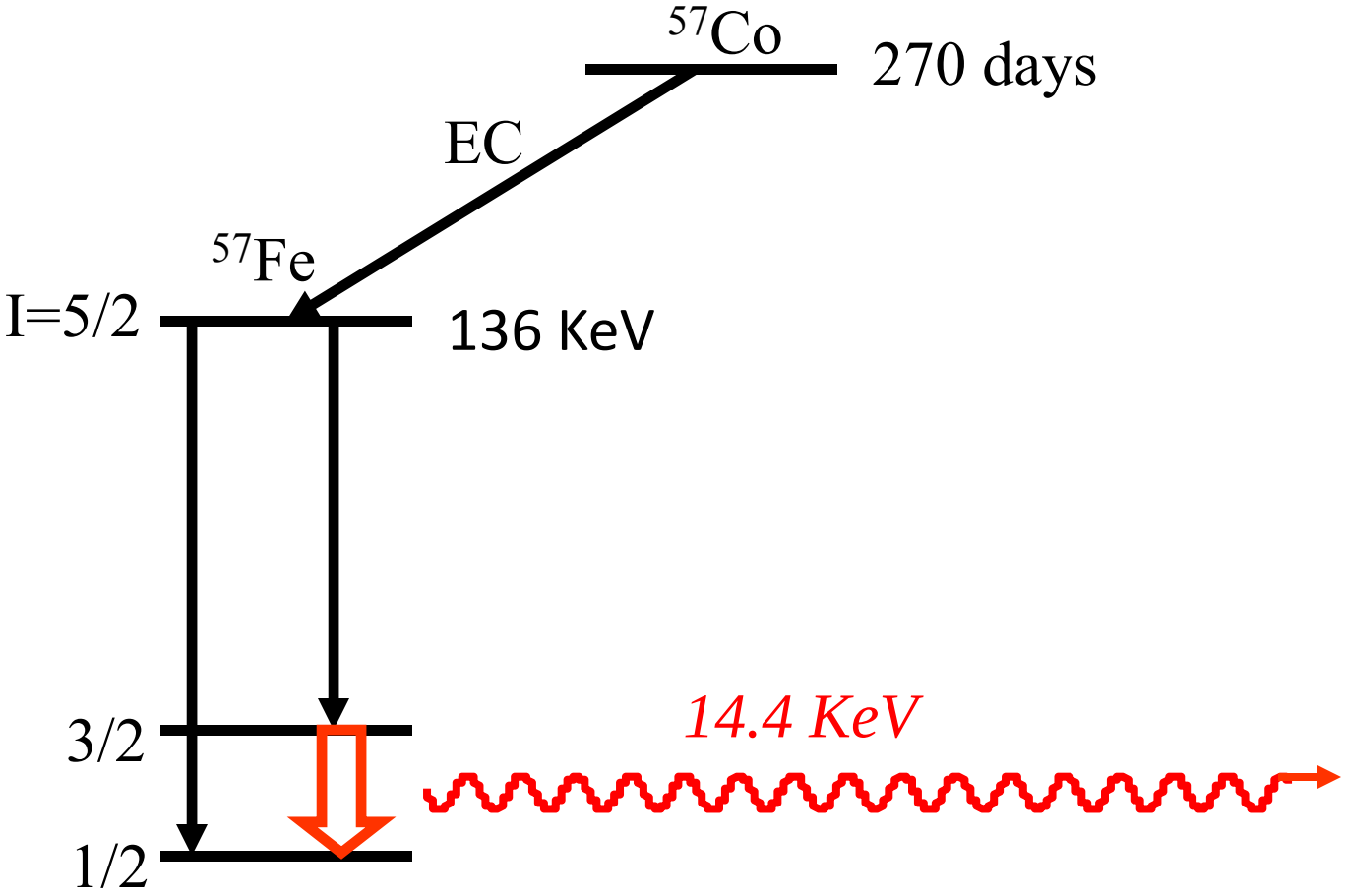
Applications

Coupled Chromophores of Sulfite Reductase

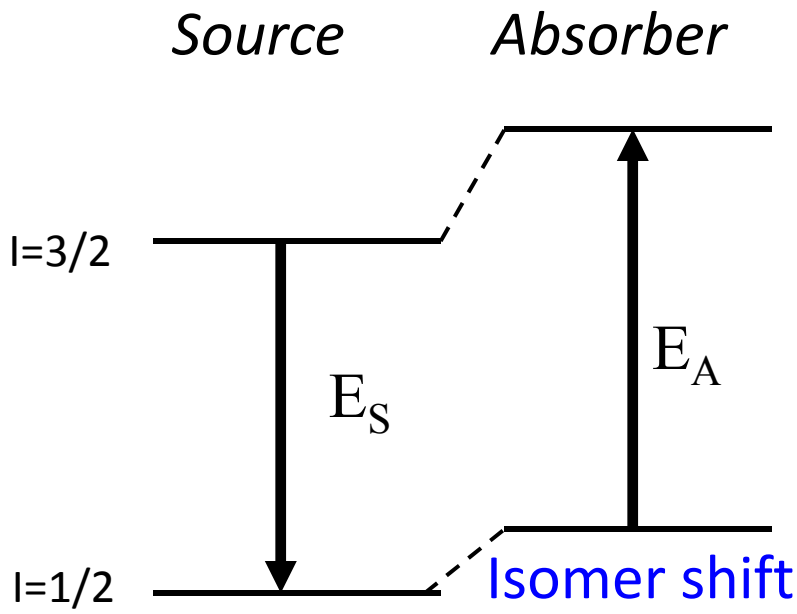
Fe(III) - radical intermediates in a dioxygenase

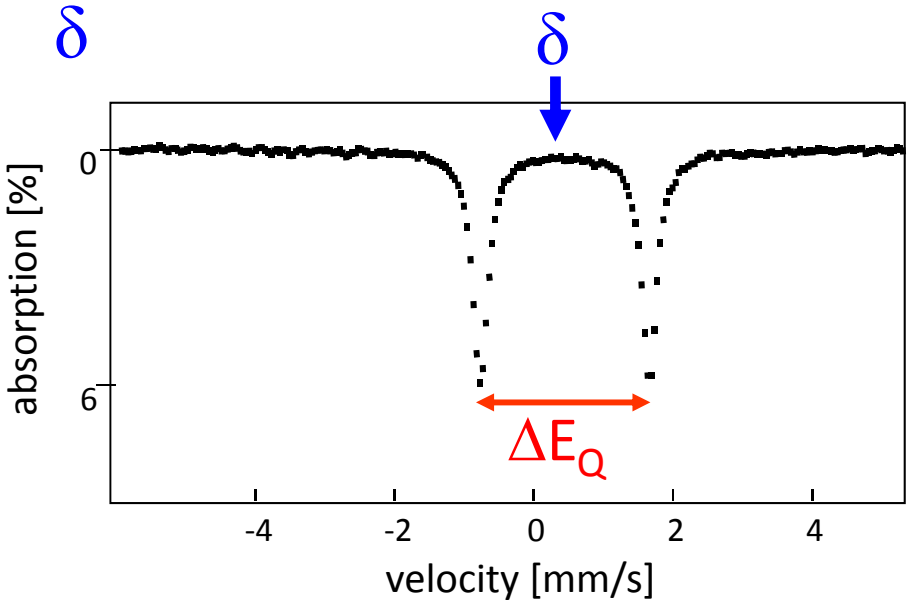
Discovery of 3Fe-4S clusters

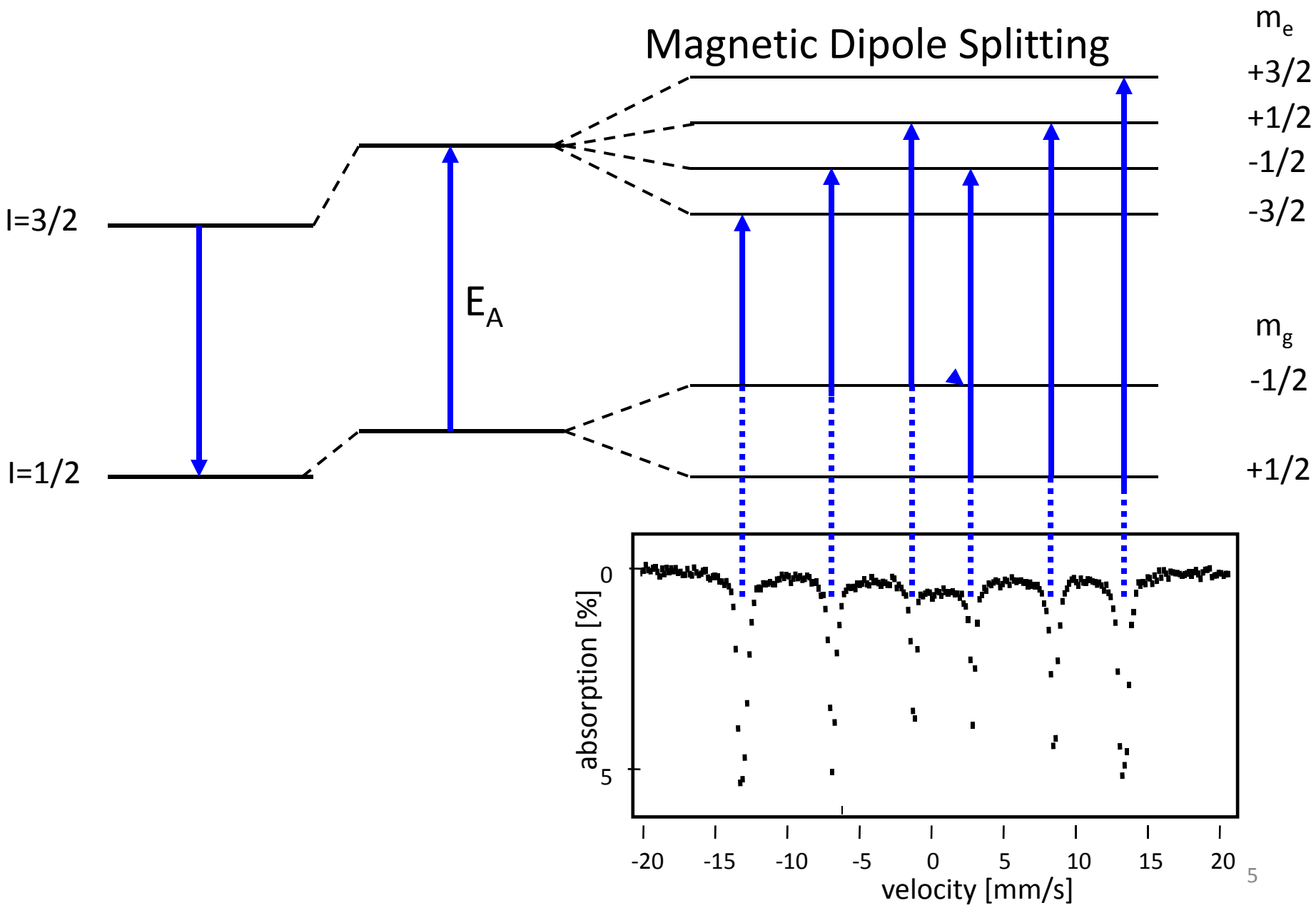
The Radiation Source

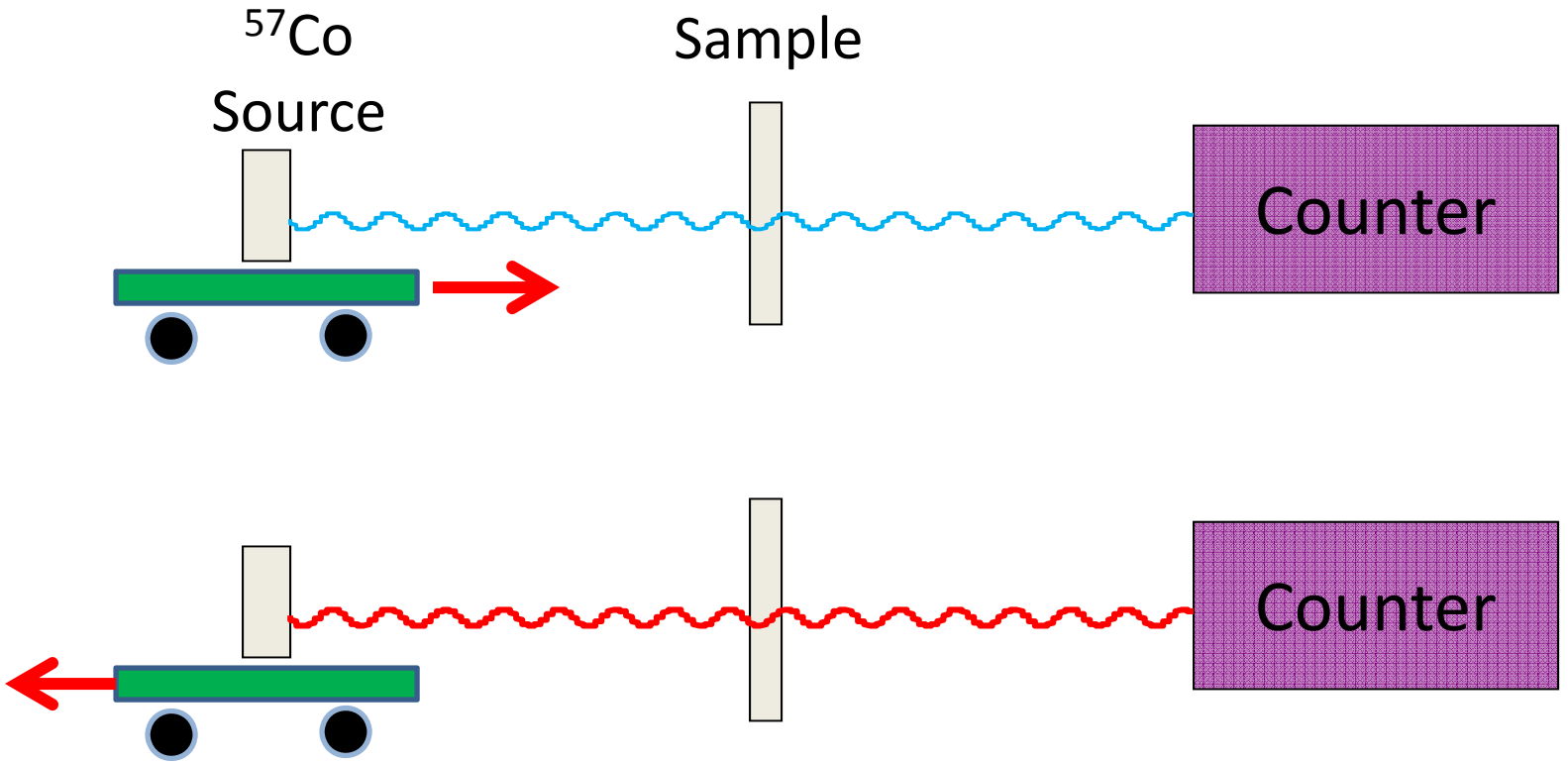


Zero field Mossbauer spectra






Magnetic Dipole Splitting
m_e
+3/2
+1/2
-1/2
-3/2
m_g
-1/2
+1/2
I=3/2
I=1/2
E_A
absorption [%]
0
5
-20
-15
-10
-5
0
5
10
15
20
velocity [mm/s]

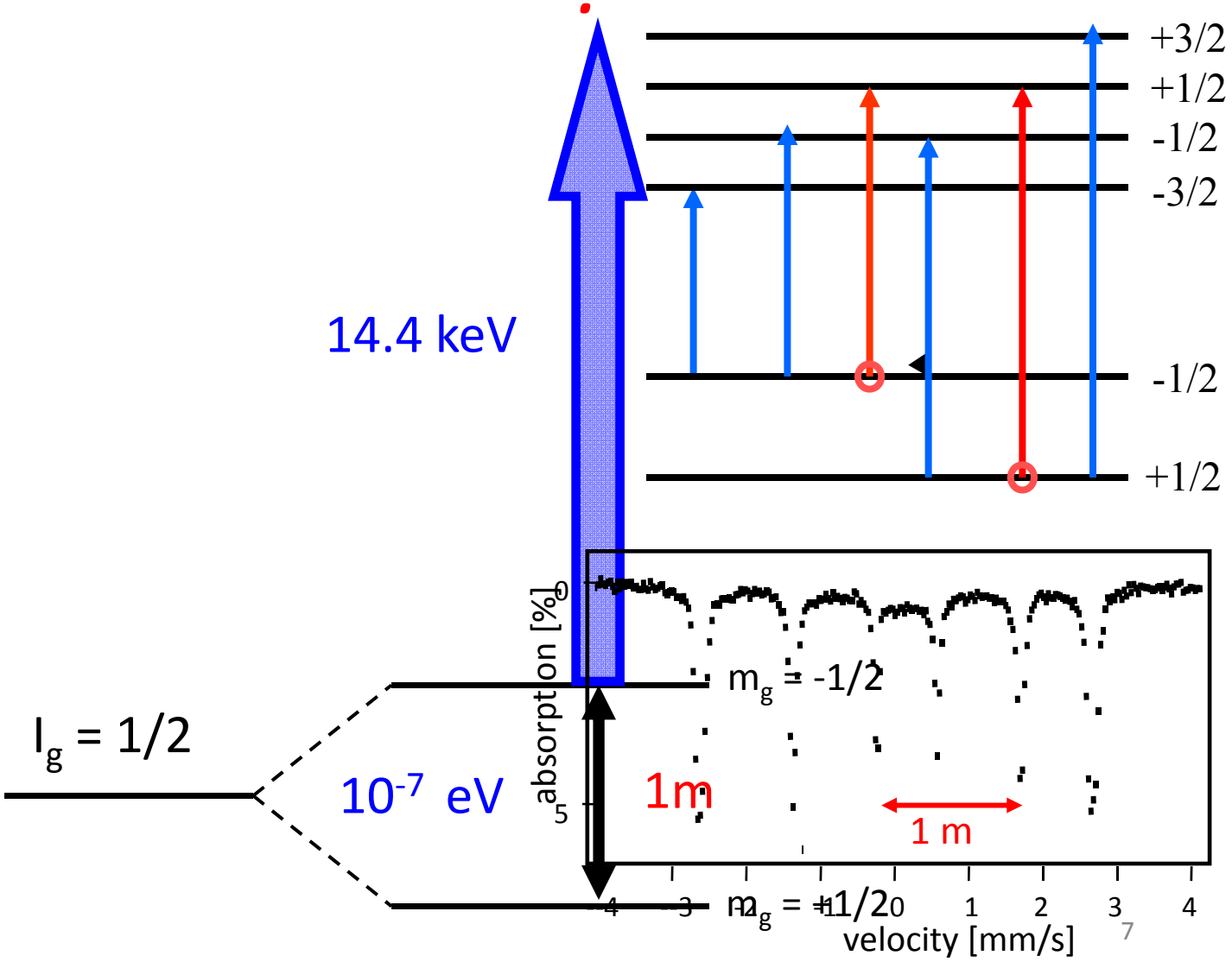


Doppler shift $\Delta E = v/c \; E_\gamma$

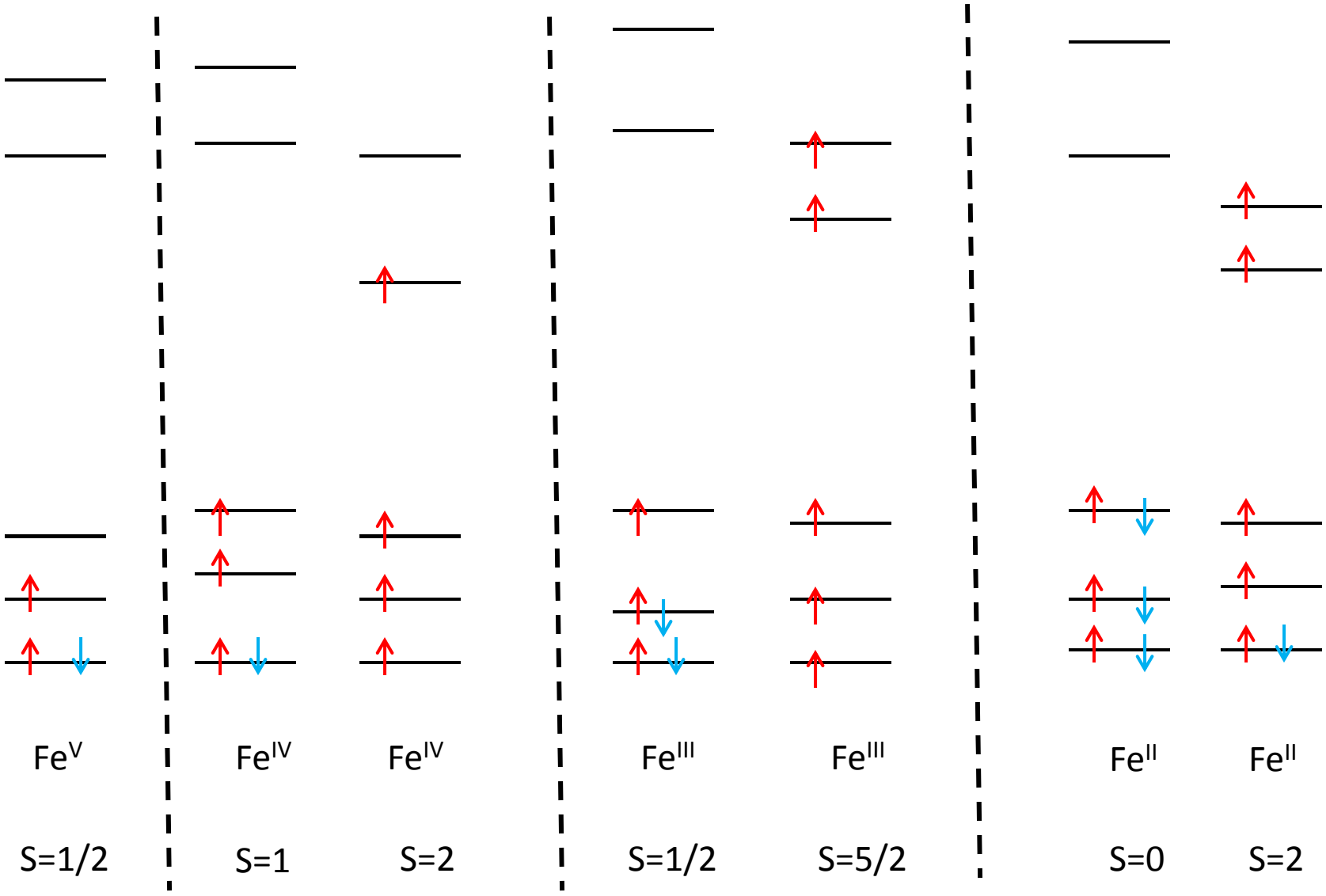
$$\frac{1 \text{ mm/s}}{3 \times 10^{11} \text{ mm/s}} \; 14.4 \times 10^{3} \text{ eV} = 4.8 \times 10^{-8} \text{ eV}$$

Where Is the Nuclear Excited State?

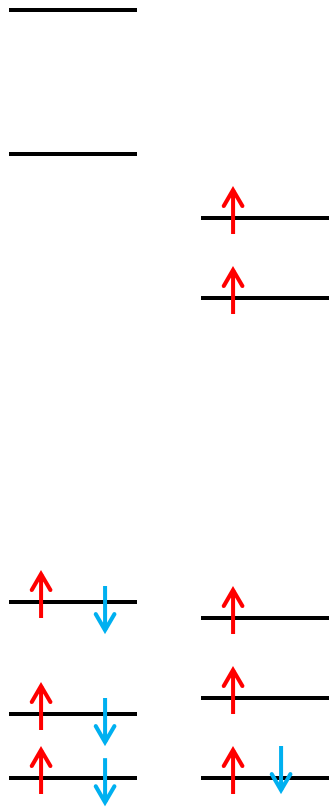
The excited state is at the Sun, 150 million km away.



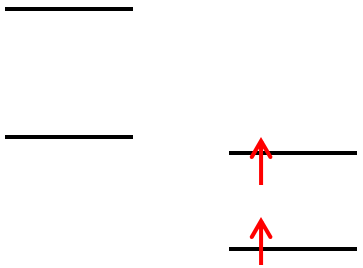
Common spin states of mononuclear Fe complexes

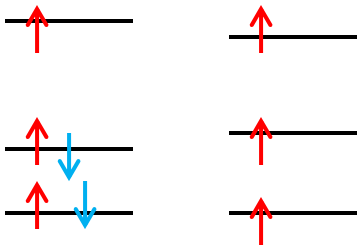


Common spin states of mononuclear Fe complexes



Common spin states of mononuclear Fe complexes

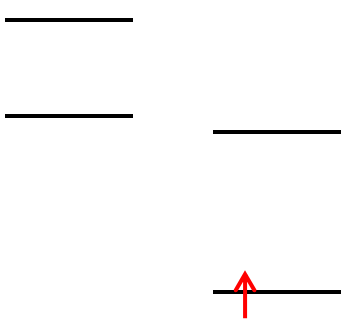


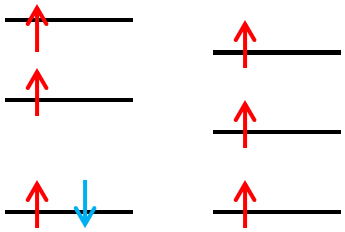


Fe^{III} Fe^{III}

S=1/2 S=5/2

Common spin states of mononuclear Fe complexes





Fe^{IV} Fe^{IV}

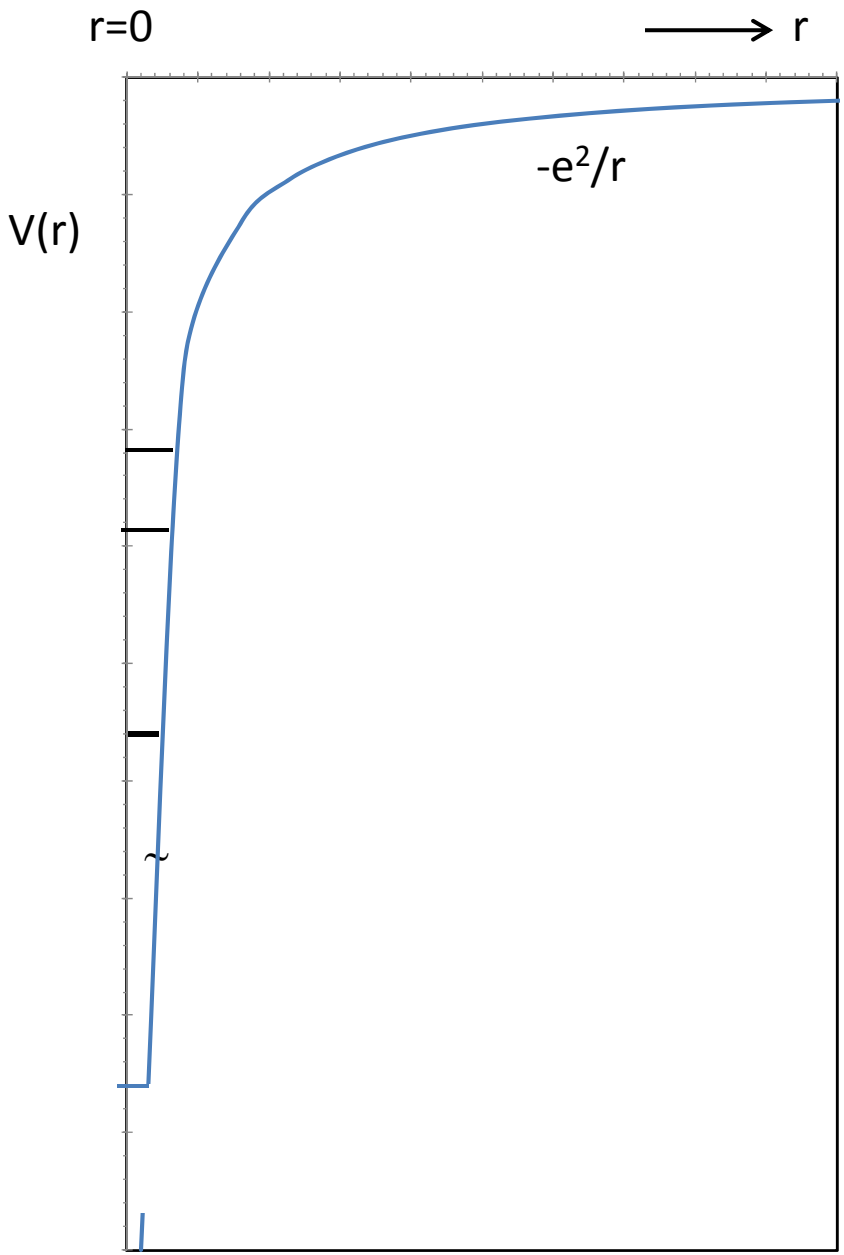
S=1 S=2

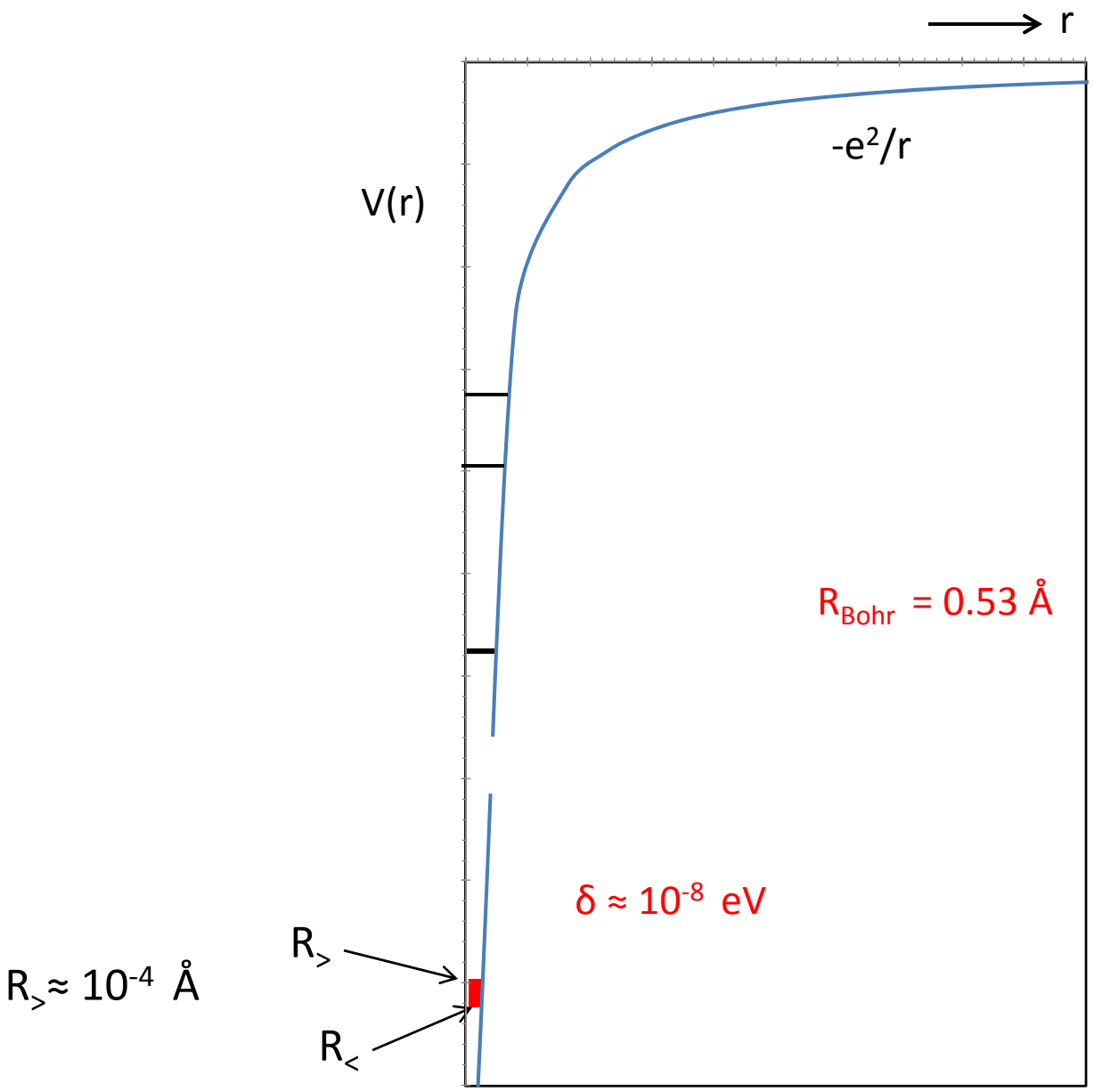
Common spin states of mononuclear Fe complexes

Fe^{V}

S=1/2

Isomer shift


r=0
r
V(r)
-e²/r
~

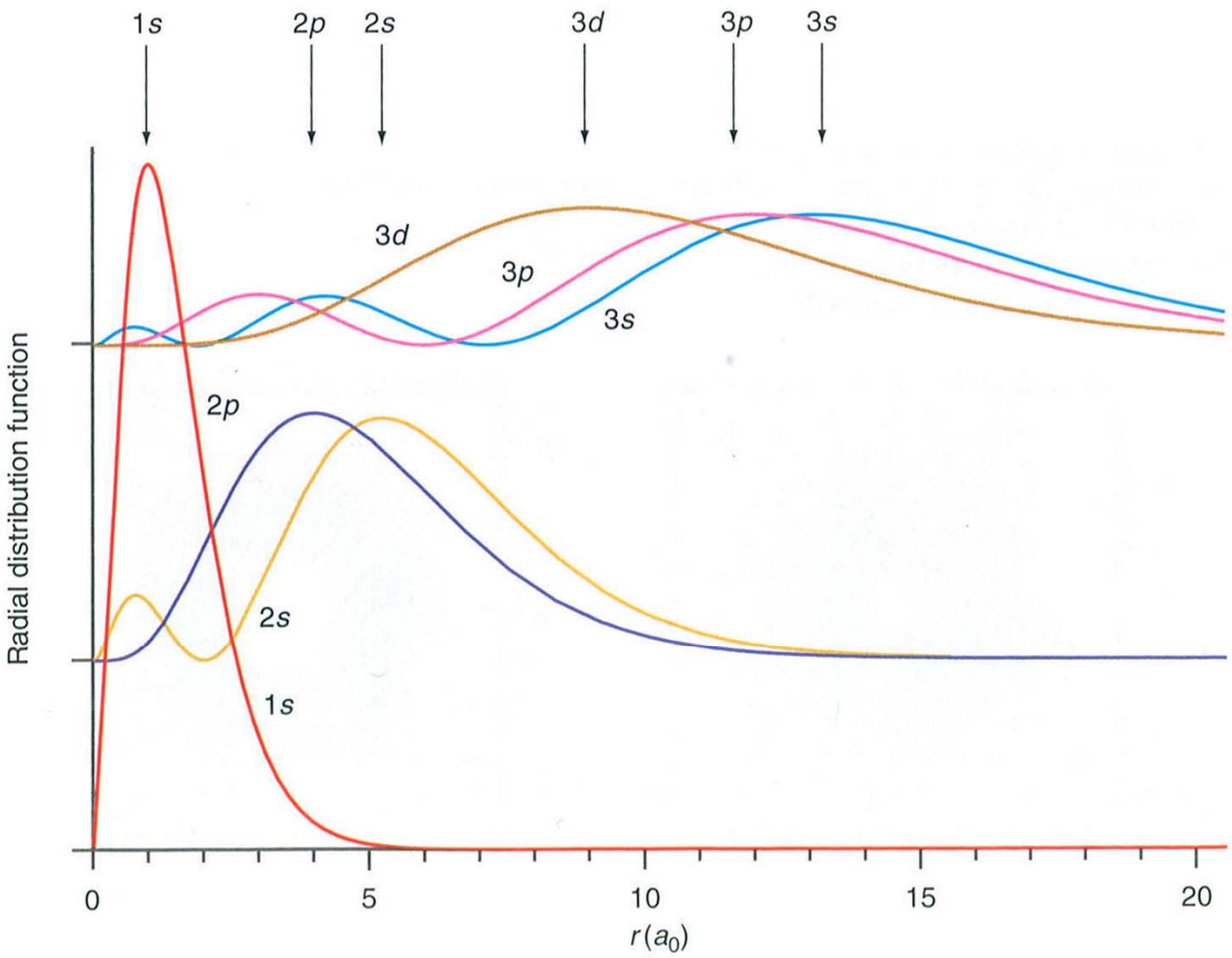

r
V(r)
$-e^2/r$
R_{Bohr} = 0.53 Å
δ ≈ 10^{-8} eV
$R_>$
$R_<$
$R_>$≈ 10^{-4} Å

$$\delta = 2/3\pi\, Z\, e^2 \{|\Psi(0)|^2_{Abs} - |\Psi(0)|^2_{Source}]\} \{<r^2>_{exc} - <r^2>_{grd}\}$$

Electronic property Property of ^{57}Fe nucleus

Ψ depends mainly on the s electron density at nucleus which in turn is influenced by the d electron density through shielding

Book: Gutlich, Link and Trautwein, Mossbauer spectroscopy and Transition Metal Chemistry


1s
2p
2s
3d
3p
3s
3d
3p
3s
2p
2s
1s
Radial distribution function
0
5
10
15
20
r(a0)

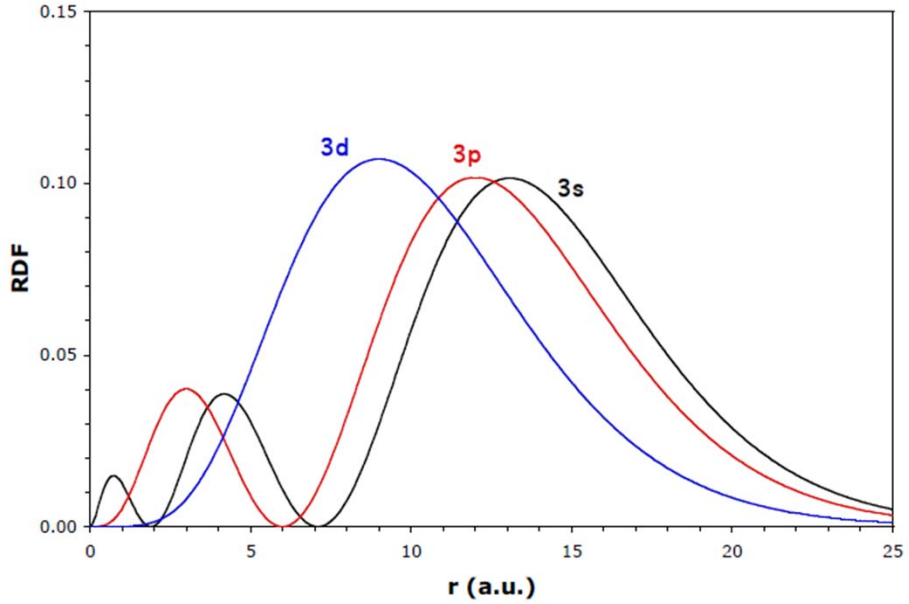
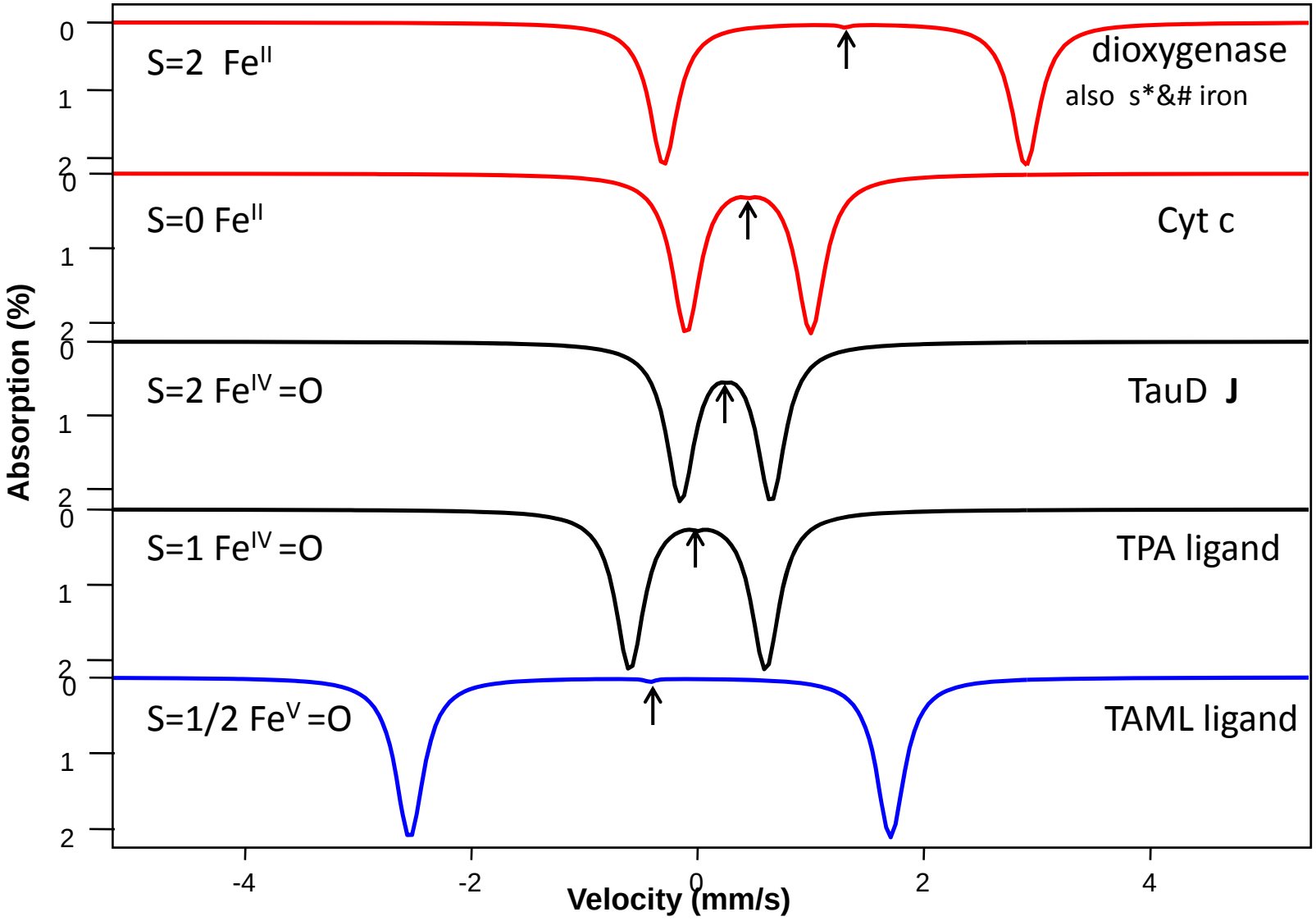


Figure 3. RDF plots for 3s, 3p, and 3d orbitals of the H atom.

Radial Distribution Function = $r^2 [R_{nl}(r)]^2$

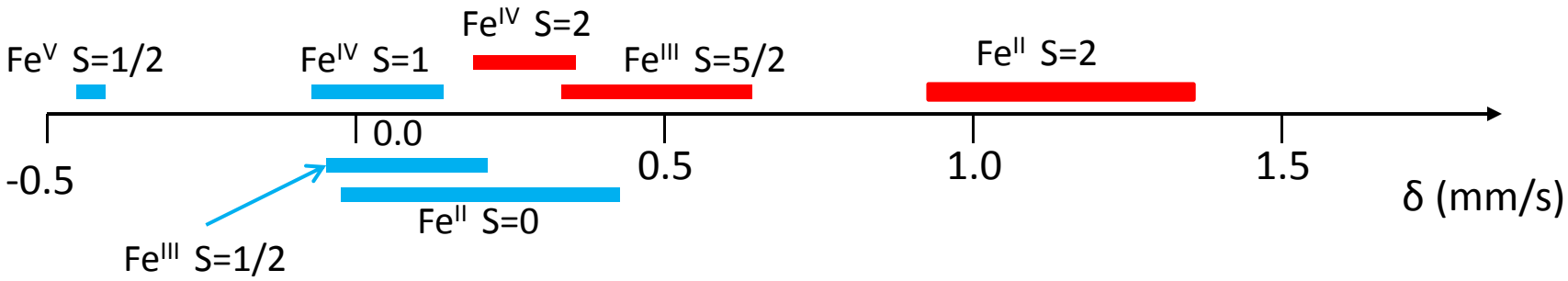
Plot from J. M. Standard handout

Typical quadrupole doublets



Isomer shift ranges for mononuclear octahedral complexes

(vs Fe metal) Diagram is very approximate and incomplete)



Note: Overlapping ranges can be sorted out by magnetic properties (spin)

Experimental errors are typically ± 0.01 mm/s

Isomer shift depends on

Oxidation state

Spin State

Nature of Ligands

Coordination Number

Geometry

Covalency

Quadrupole Splitting

Nuclear quadrupole interactions

$$\mathcal{H}_Q = (eQV_{zz}/12)\ \{\ 3\mathbf{I}_z^2\ -\ 15/4\ +\ \eta\ (\mathbf{I}_x^{\ 2}\ -\ \mathbf{I}_y^{\ 2}\ )\ \}$$

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

V_{xx} , V_{yy} , V_{zz} : principal components of the Electric Field Gradient (EFG) tensor

$$\Delta E_Q = (eQV_{zz}/2)\sqrt{(1 + \eta^2/3)}$$

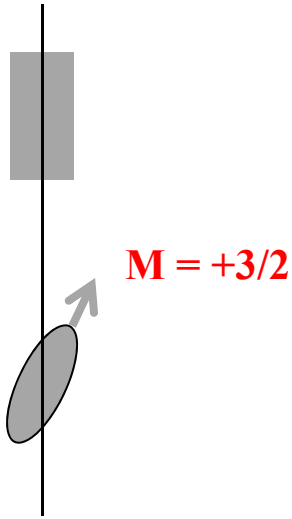
V(x,y,z) is the potential generated by the electronic environment at the ^{57}Fe nucleus

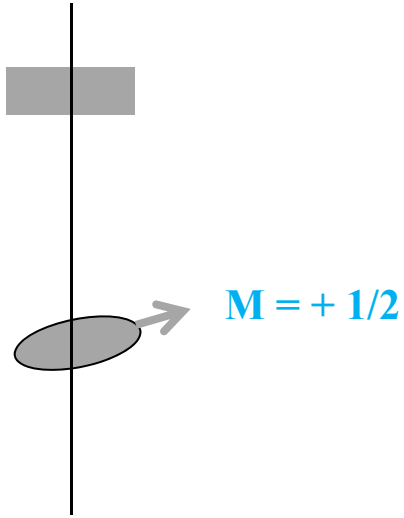
$$V_{zz} = \{\partial^2 V/\partial z^2\ \}_0$$

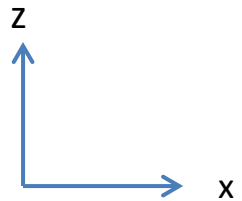
V_{zz} and η (and often more) can be determined experimentally

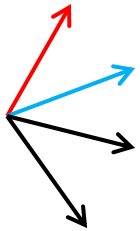
Spin around the vertical

Q > 0 for ^{57}Fe
cigar shaped
not pancake

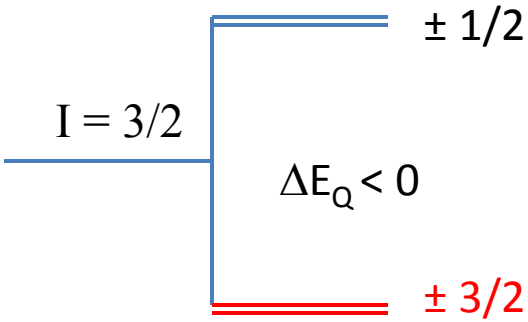


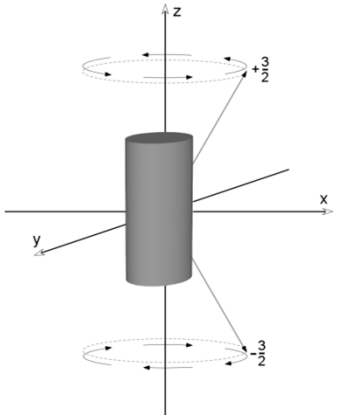


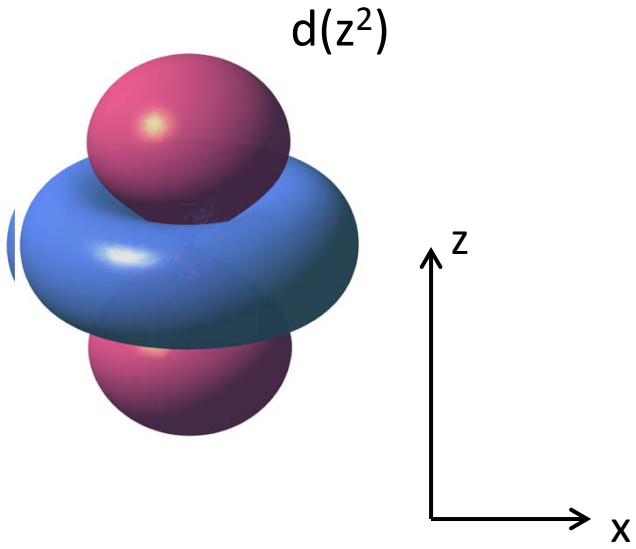


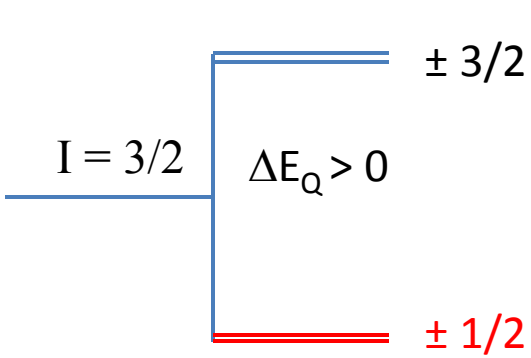


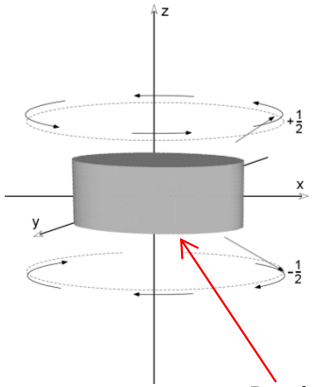
Sign of ΔE_Q



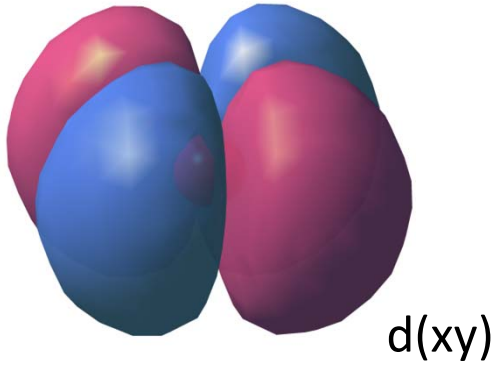




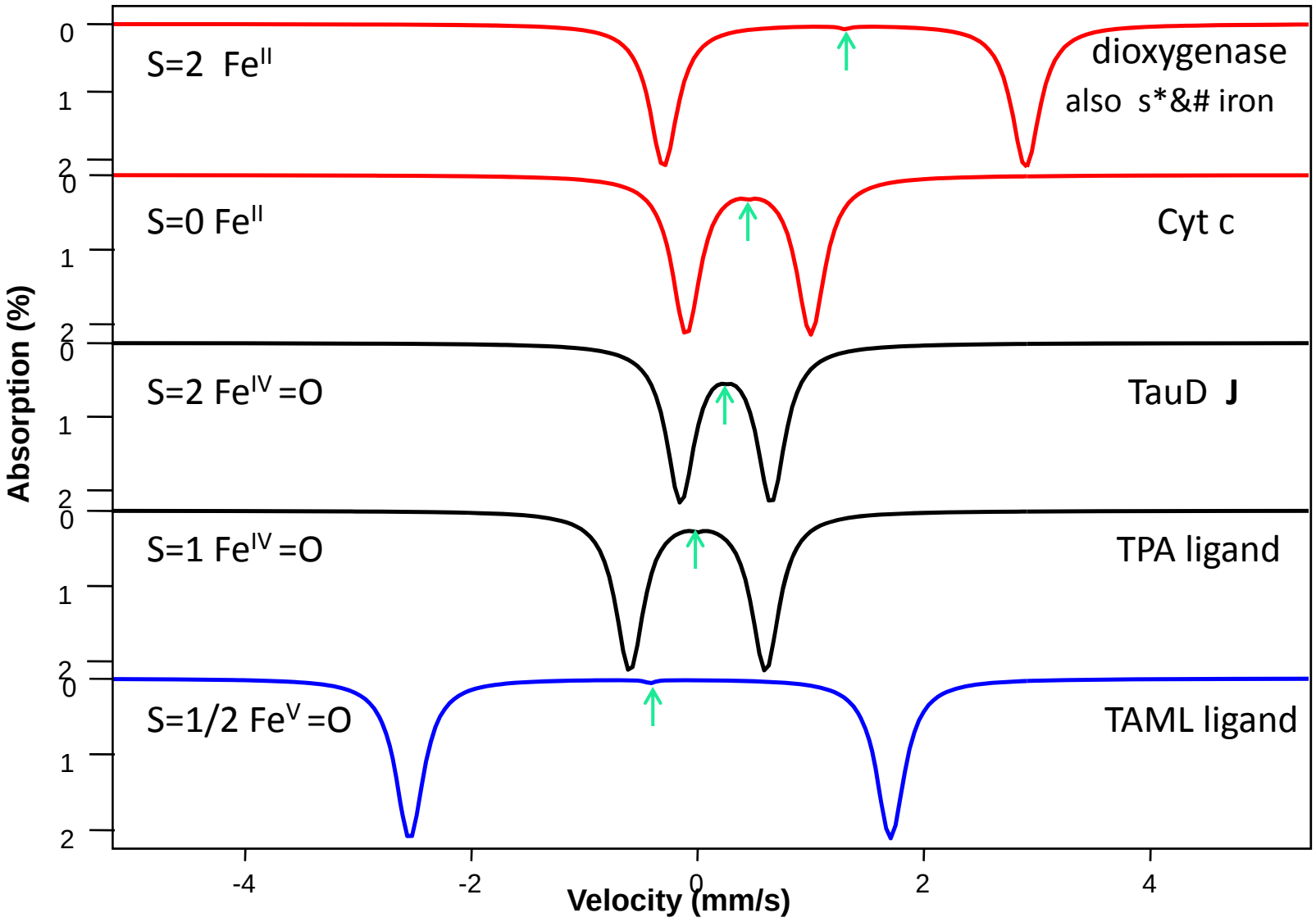




Positive charge



Typical quadrupole doublets



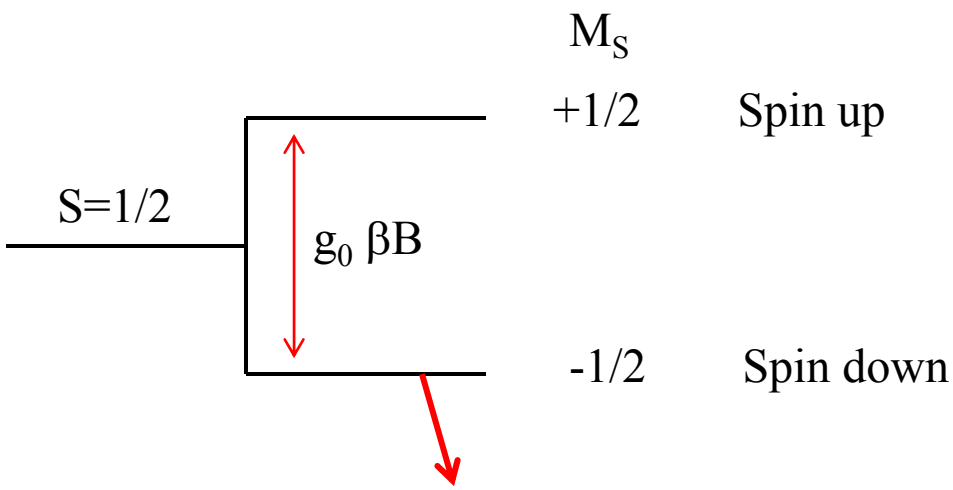
Comments on Electronic Properties

Electronic Zeeman Term

S= ½ and isotropic g-tensor: $g_x = g_y = g_z = g_0$

$\mathcal{H} = g_0 \beta B \mathbf{S}_z$

Call direction of **B** the z axis



$\langle \mathbf{S}_z \rangle$

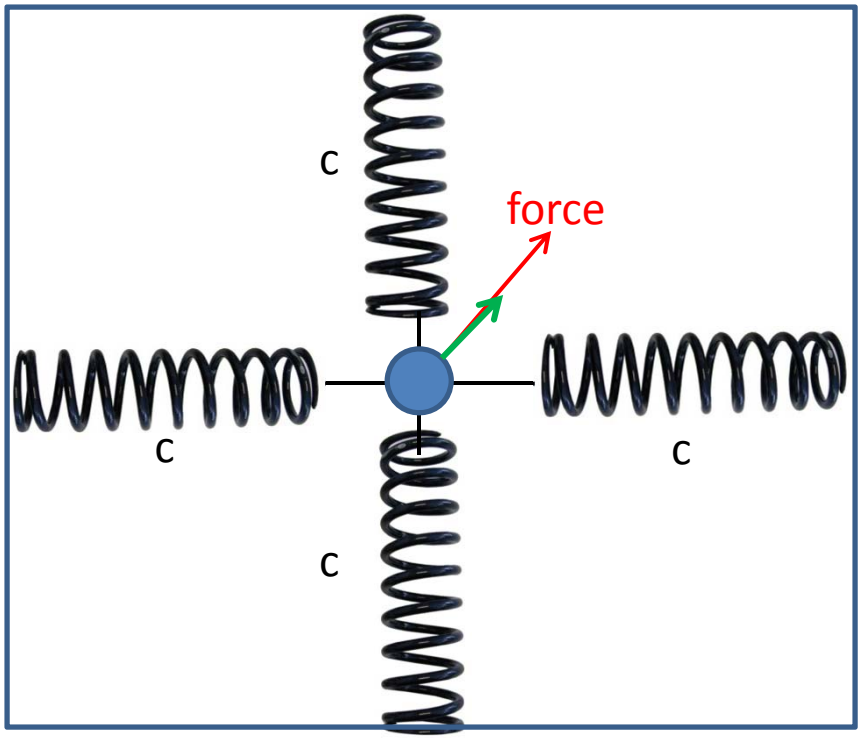
Expectation value of $\mathbf{S}_z$

$\langle \Psi_{\text{spin-down}} | S_z | \Psi_{\text{spin-down}} \rangle = \langle S_z \rangle = -1/2$

$\langle S_x \rangle = \langle S_y \rangle = 0$

Isotropic System

ACME

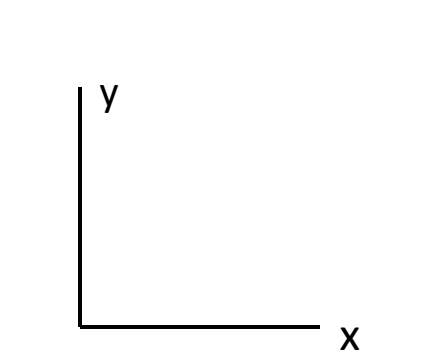


↗ = displacement

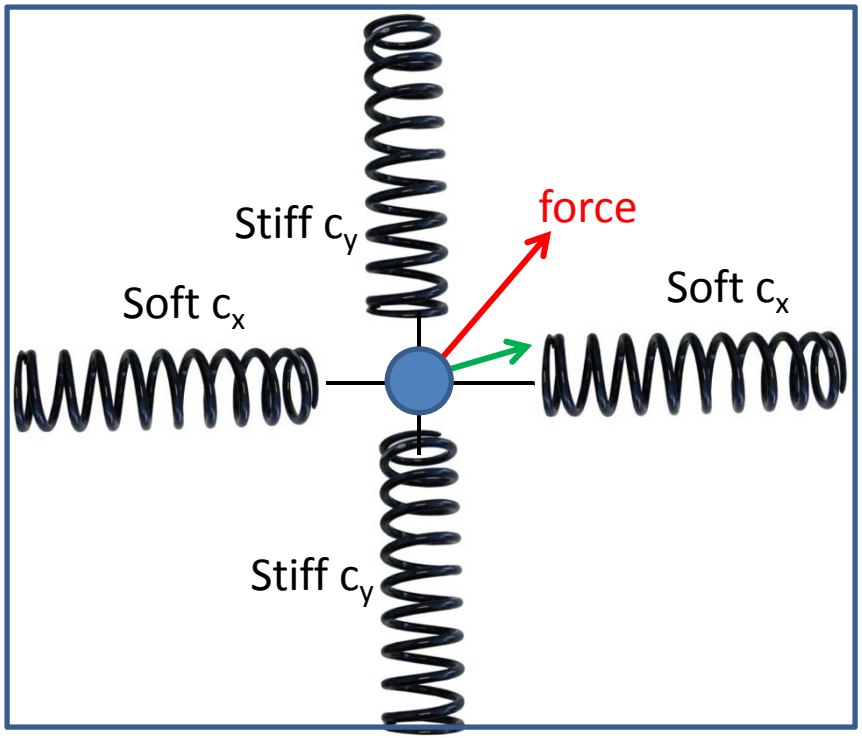
$$\mathbf{d} = c\,\mathbf{F}$$

displacement = c × force

Anisotropic System



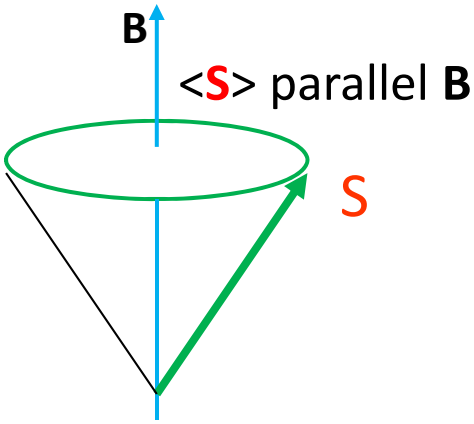
Principal axes of **c** tensor



ACME

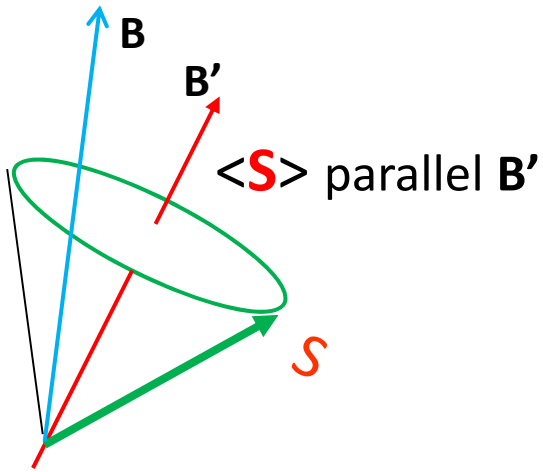
$$\mathbf{d} = \tilde{\mathbf{c}}\,\mathbf{F}$$

$\tilde{\mathbf{c}}$ is a 2 x 2 tensor



isotropic g: $g_x = g_y = g_z = g_0$

$\mathcal{H} = g_0 \beta \, \mathbf{S \bullet B}$

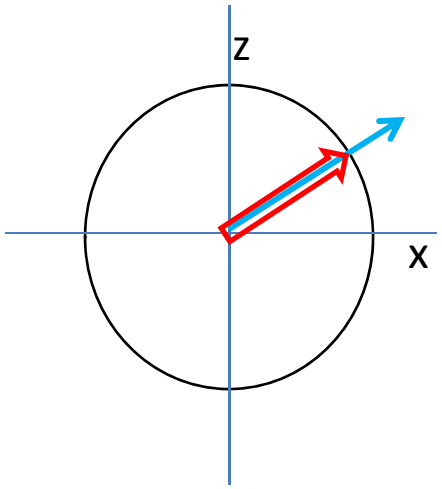


anisotropic g: $g_x \neq g_y \neq g_z$

$\mathcal{H} = \beta \, \mathbf{S \bullet g \bullet B} = \beta \, \mathbf{S \bullet B'}$

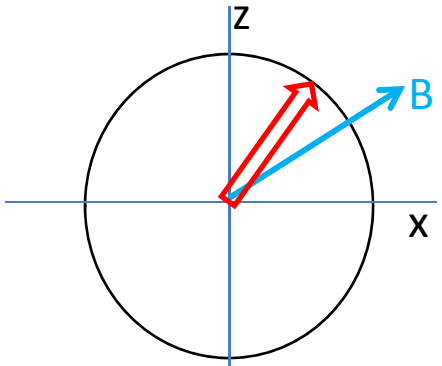
$\mathbf{B' = g \bullet B}$

For spin S = ½ we always have $\langle S_{B'} \rangle = \pm 1/2$



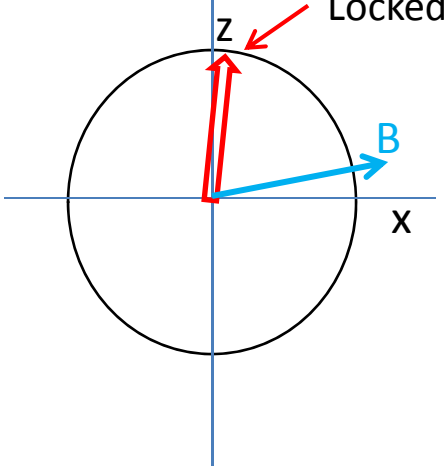
<S> is parallel to **B'**

Isotropic $g_z = g_x$



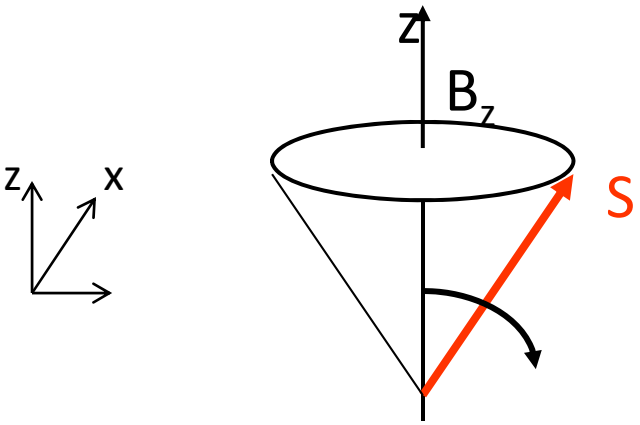
$g_z > g_x$

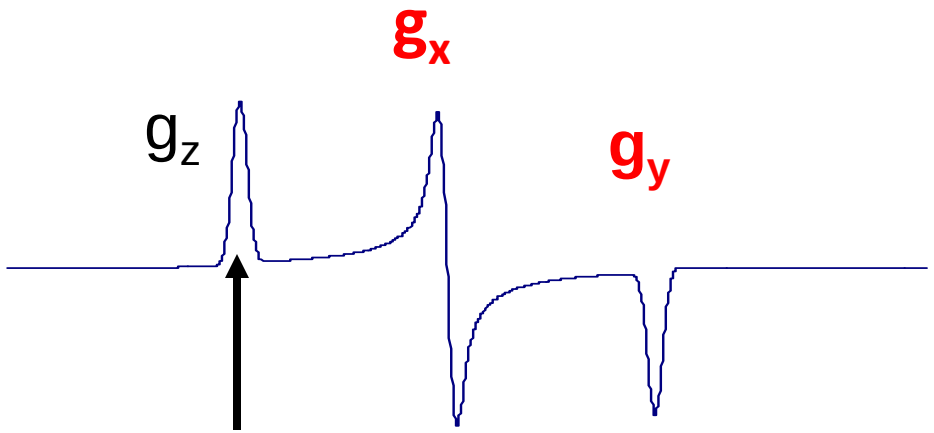
Locked near molecular z axis



$g_z >> g_x \approx 0$

Consider only x-y plane





Intensity at g_z

$\sim g_x^2 + g_y^2$ Aasa, Vanguard 1976

$$2S_x = (S_+ + S_-)$$

$$\mathcal{H} = g_z \beta B_z S_z + g_x \beta S_x B_{1x} \text{ (microwaves)}$$

$$\mathcal{H} = g_z \beta B_z S_z + (1/2) g_x \beta (S_+ + S_-) B_{1x} \text{ (microwaves)}$$

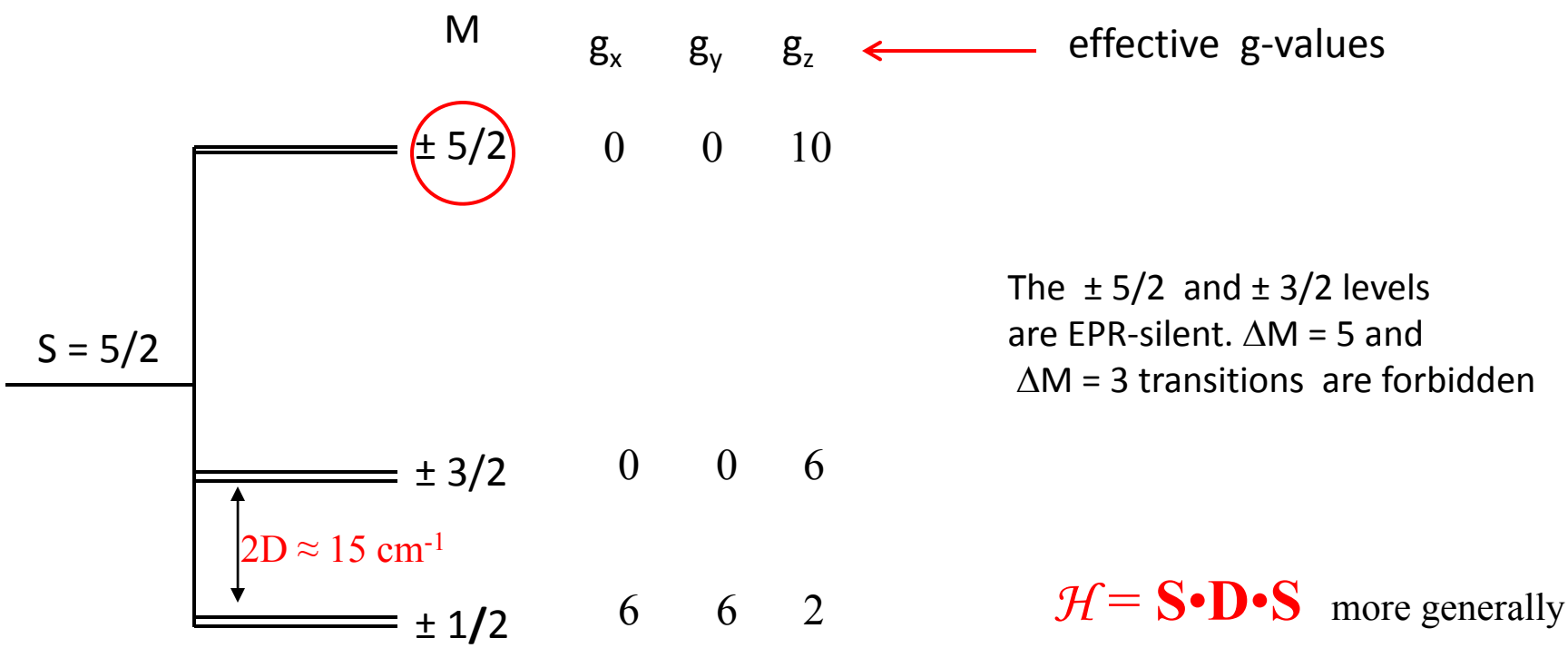
$$\text{EPR intensity} \sim |\langle \Psi_{\text{spin-up}} | g_x S_- B_{1x} | \Psi_{\text{spin-down}} \rangle|^2$$

when $g_x = 0$ and $g_y = 0$ there is no EPR and **<S>** is locked along z

Zero-field splitting in High-spin Fe^{III}

$$\mathcal{H} = D\ \{\mathbf{S}_z^{\,2} - 35/4 + (E/D)\,(\mathbf{S}_x^{\,2} - \mathbf{S}_y^{\,2})\} + g_0\,\beta\,\mathbf{S}\bullet\mathbf{B}$$

E/D = 0 = axial symmetry; often the case for hemes



The ± 5/2 and ± 3/2 levels are EPR-silent. ΔM = 5 and ΔM = 3 transitions are forbidden

$\mathcal{H} = \mathbf{S}\bullet\mathbf{D}\bullet\mathbf{S}$ more generally

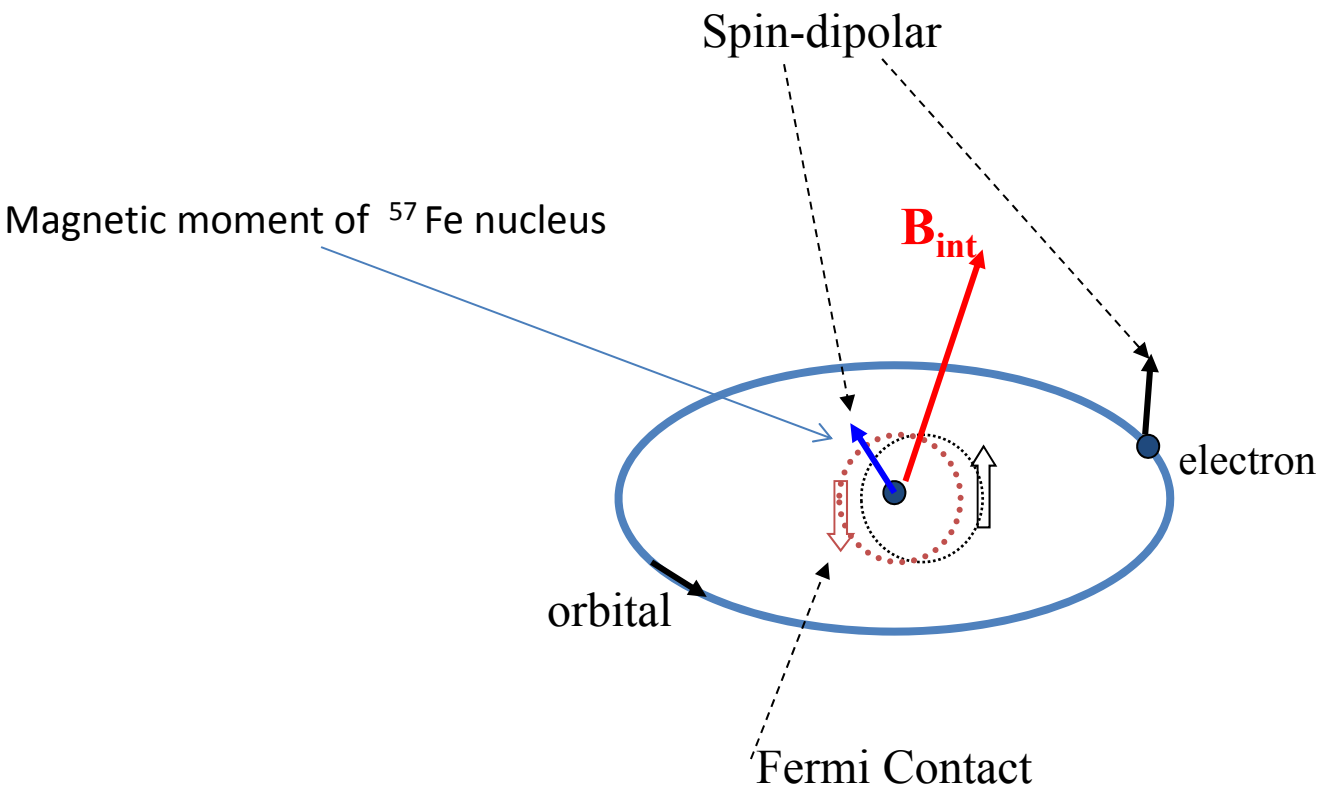
For simple isotropic S = ½ system

Expectation value of **S :** $\langle \Psi | S_z | \Psi \rangle = \langle S_z \rangle$

For Ψ = spin down

$\langle S_z \rangle = -1/2$ and $\langle S_x \rangle = \langle S_y \rangle = 0$

Comments on Magnetic Hyperfine interactions



$$B_{int} = B_{contact} + B_{spin\text{-}dipolar} + B_{orbital}$$

All lumped together in $\mathbf{S \cdot A \cdot I}$

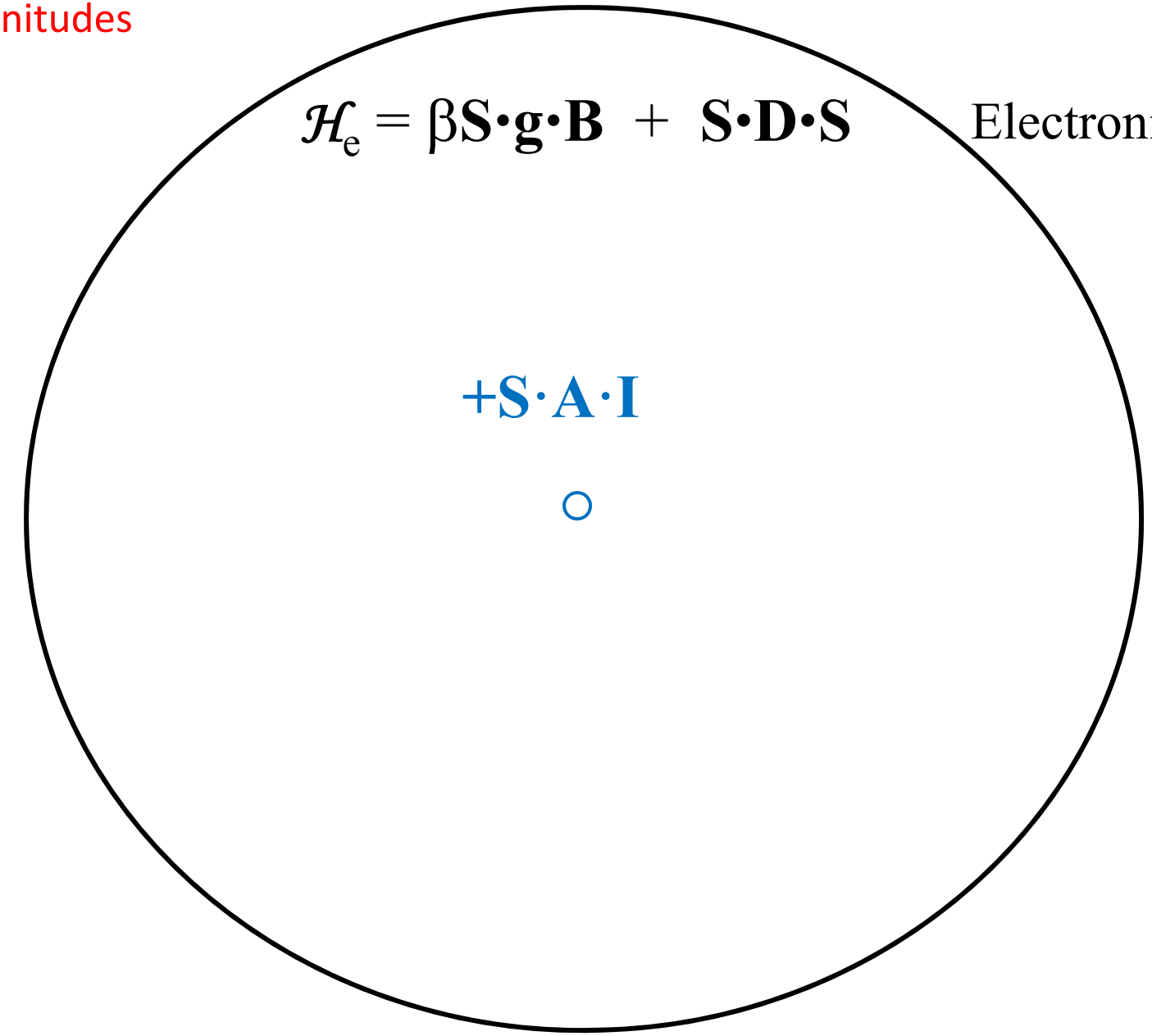
$$B_{int} = -\langle S \rangle \cdot A/g_N\beta_N$$

Nuclear Zeeman term

$$-\mathbf{B} \cdot \boldsymbol{\mu} = -\mathbf{B} \cdot (g_N \beta_N \mathbf{I})$$

nuclear
magnetic moment

magnitudes


$\mathcal{H}_e = \beta \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{B} + \mathbf{S}\cdot\mathbf{D}\cdot\mathbf{S}$
Electronic terms
$+\mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}$

$$\mathcal{H}_e = \beta \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{B} + \mathbf{S}\cdot\mathbf{D}\cdot\mathbf{S} + \mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I} - g_N\beta_N\mathbf{B}\cdot\mathbf{I}$$

Nuclear Zeeman term

$$\mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I} = \frac{-\langle\mathbf{S}\rangle\cdot\mathbf{A}\cdot(-g_N\beta_N\mathbf{I})}{g_N\beta_N}$$

$$\mathbf{B}_{int} = -\langle\mathbf{S}\rangle\cdot\mathbf{A}/g_N\beta_N$$

$$\mathcal{H}_N = -g_N\beta_N\mathbf{I}\cdot(\mathbf{B}_{int} + \mathbf{B})$$

$$\mathcal{H}_N = -g_N\beta_N\mathbf{I}\cdot\mathbf{B}_{eff}$$

The ^{57}Fe nucleus sees B_{eff}

There is more to $\mathcal{H}_e$ **

Antisymmetric exchange

$$\mathcal{H}_e = \sum_{n=1,2} \{\beta S_n \cdot g_n \cdot B + S_n \cdot D \cdot S_n\} + J\, S_1 \cdot S_2 + d \cdot S_1 x S_2$$

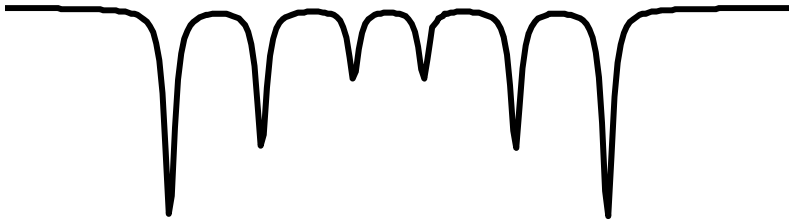
Isotropic exchange

All these terms influence **<S>** and thus $\mathbf{B}_{int}$

** But we have enough of it, haven't we?

4.2 K Mössbauer Spectra for B = 0

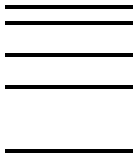
Half-Integral Spin



S=3/2

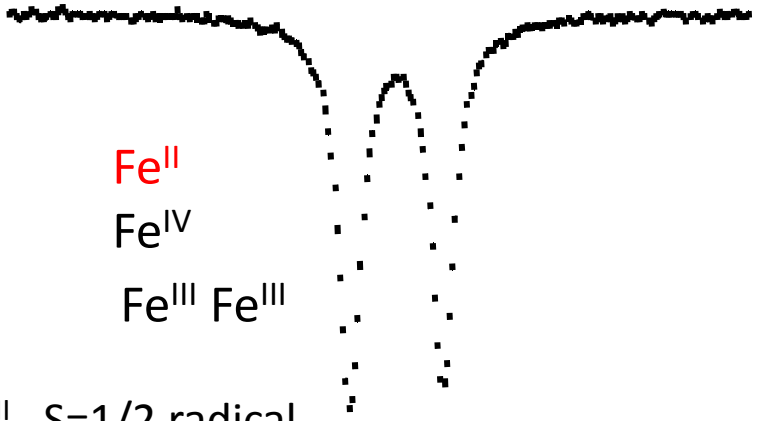
Kramers doublets

S = 2



Generally singlets have
<S> = 0 in zero field

Integer or Zero Spin



Fe^{II}

Fe^{IV}

Fe^{III} Fe^{III}

or Fe^{III} - S=1/2 radical

e. g. Fe^{III} Fe^{I} Fe^{V}

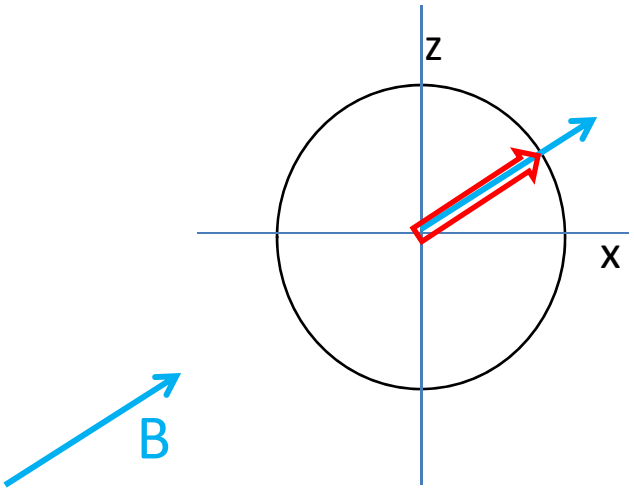
Fe^{III} Fe^{II} and $Fe^{IV}Fe^{III}$

Isotropic system

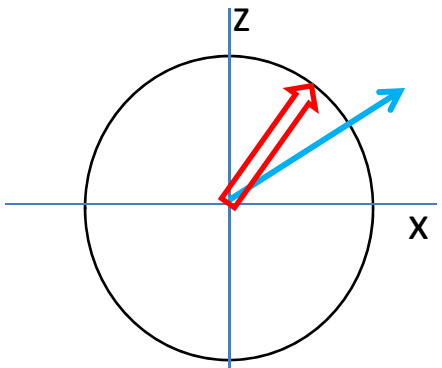
$$\mathbf{B}_{int} = - \langle \mathbf{S} \rangle \bullet A_0 / g_N \beta_N$$

For isotropic system: $\mathbf{B}_{int}$ is parallel to $\langle \mathbf{S} \rangle$, which is parallel to **B**

$\mathbf{B}_{int}$ is parallel to **B**

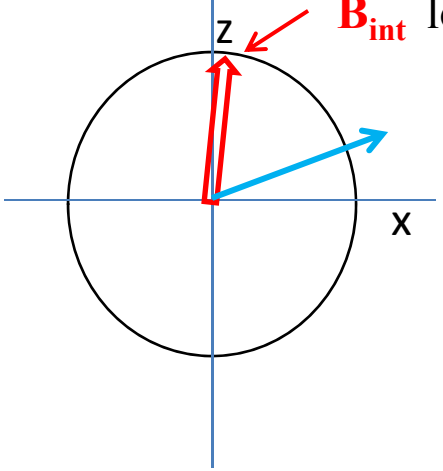


Isotropic $\mathbf{B}_{int}$ is parallel to **B**



somewhat anisotropic

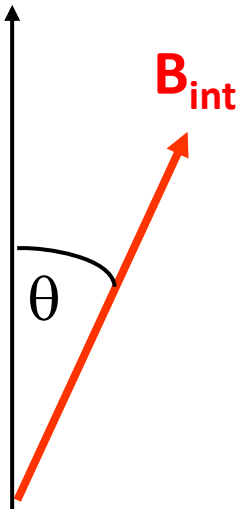
$\mathbf{B}_{int}$ not parallel to **B**



$\mathbf{B}_{int}$ locked along molecular z axis

Very anisotropic favoring z

γ-rays

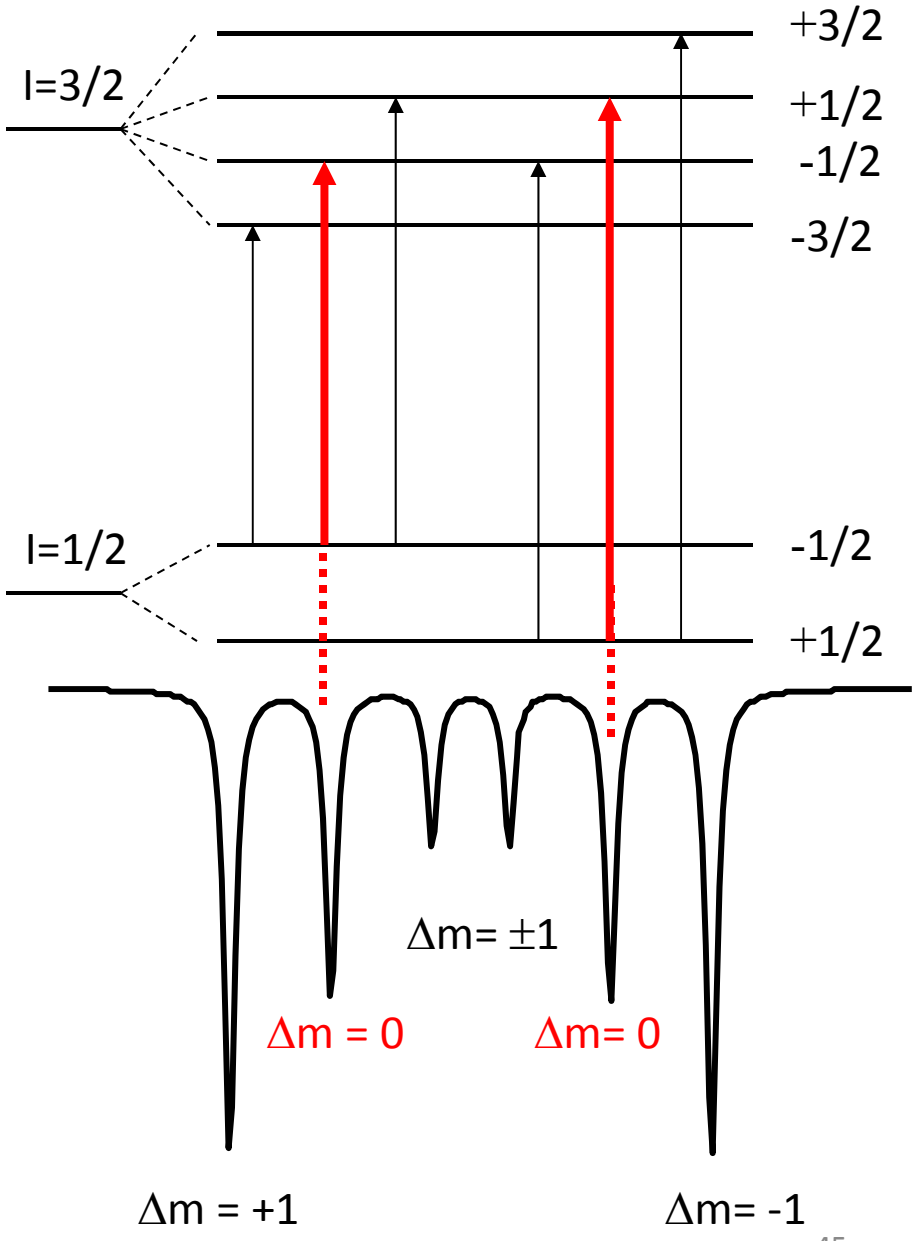


Intensities

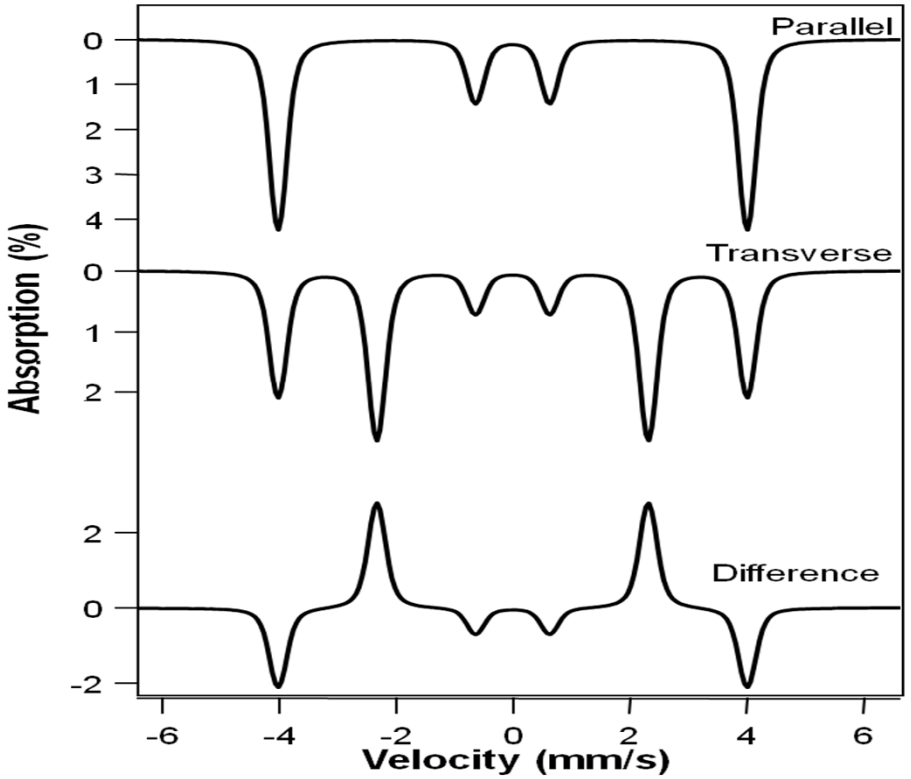
$\Delta m = \pm 1 \quad \sim \mathbf{1 + \cos^2\theta}$

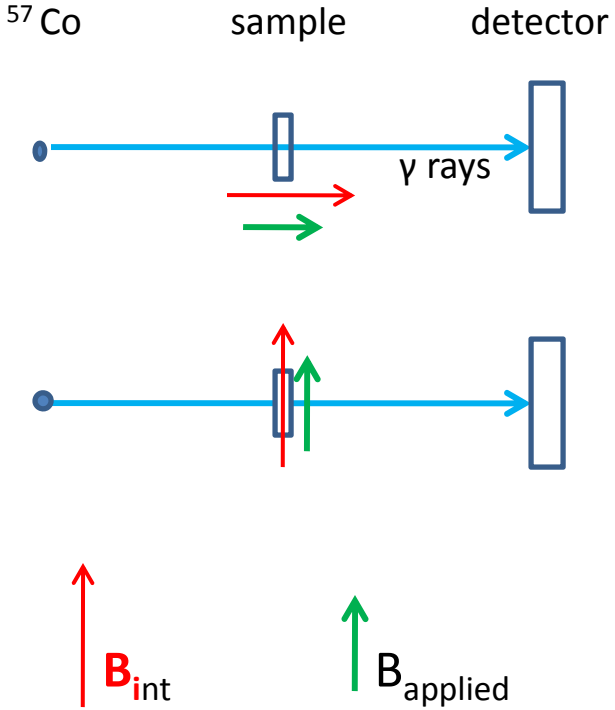
$\Delta m = 0 \quad \sim \mathbf{\sin^2\theta}$

Magnetic dipole transition (property of ^{57}Fe)



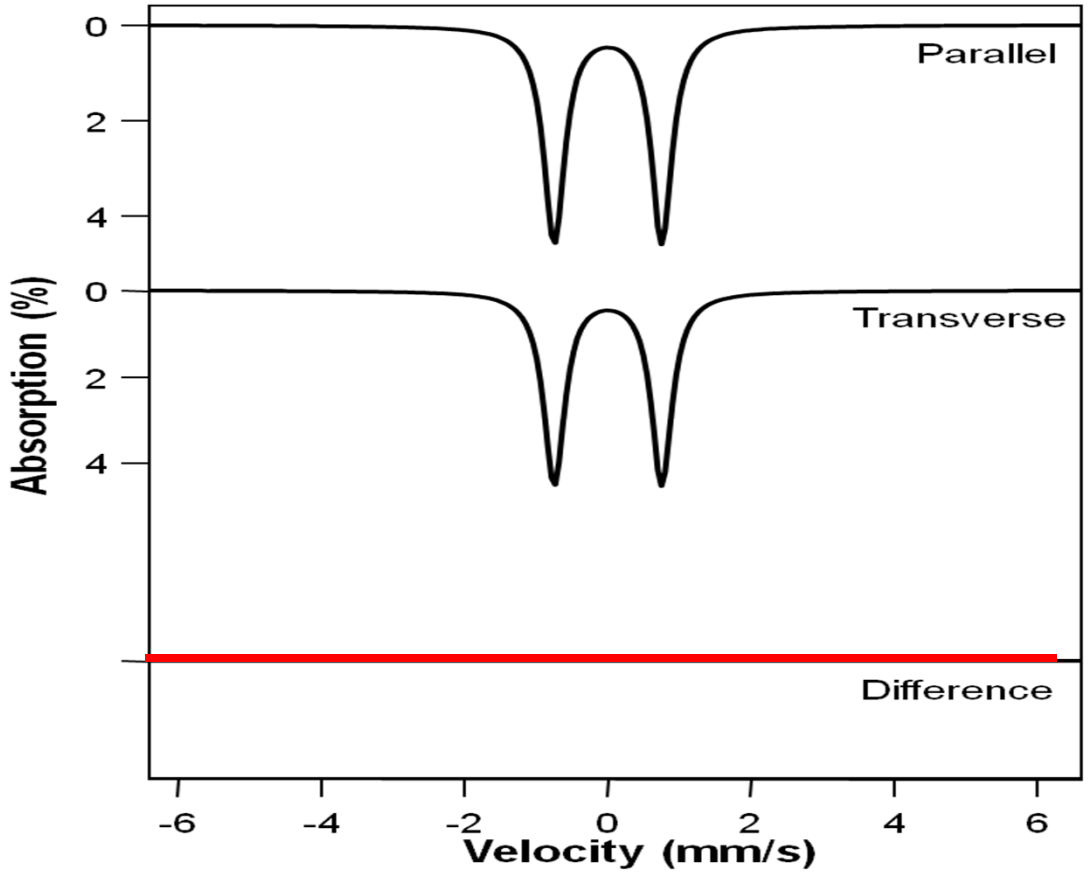
For an isotropic system





$$\mathbf{B}_{int} = -\langle \mathbf{S} \rangle \bullet A_0 / g_n \beta_n$$

Quadrupole doublets cancel for “parallel minus transverse”

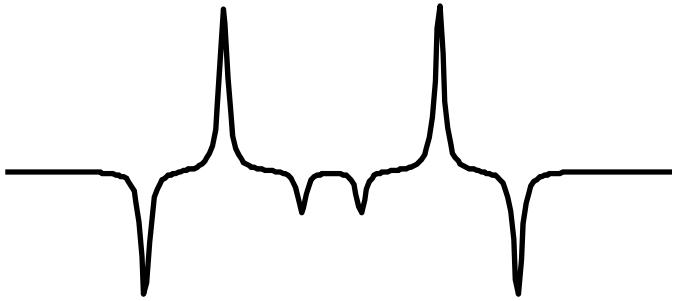


500 gauss applied field

When you observe

this

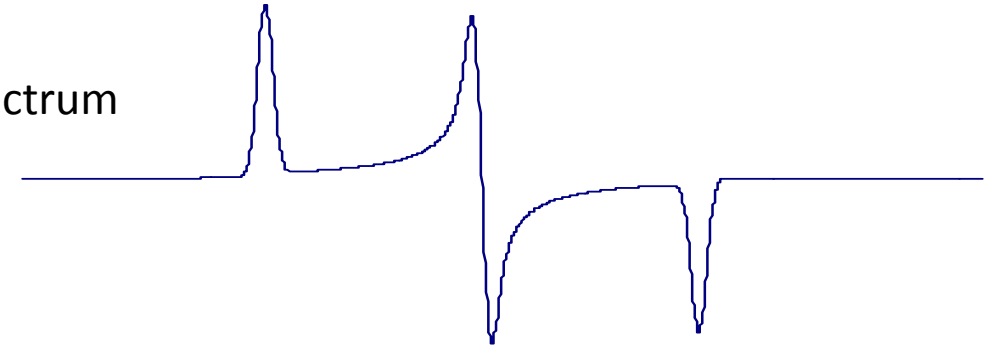
Difference spectrum



You MUST observe

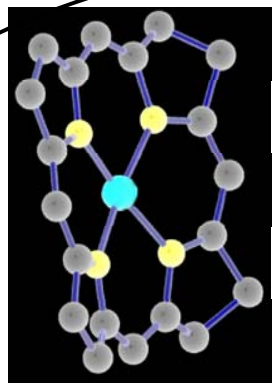
that

EPR spectrum

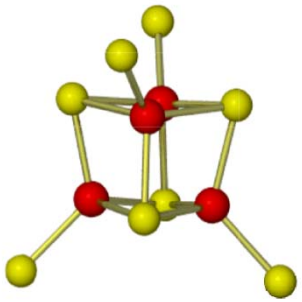


What can we do with that?

E. coli Sulfite Reductase

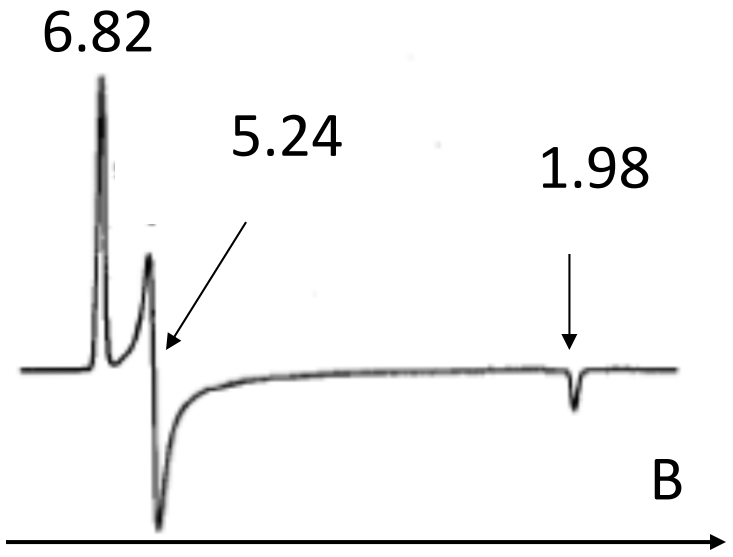


Siroheme



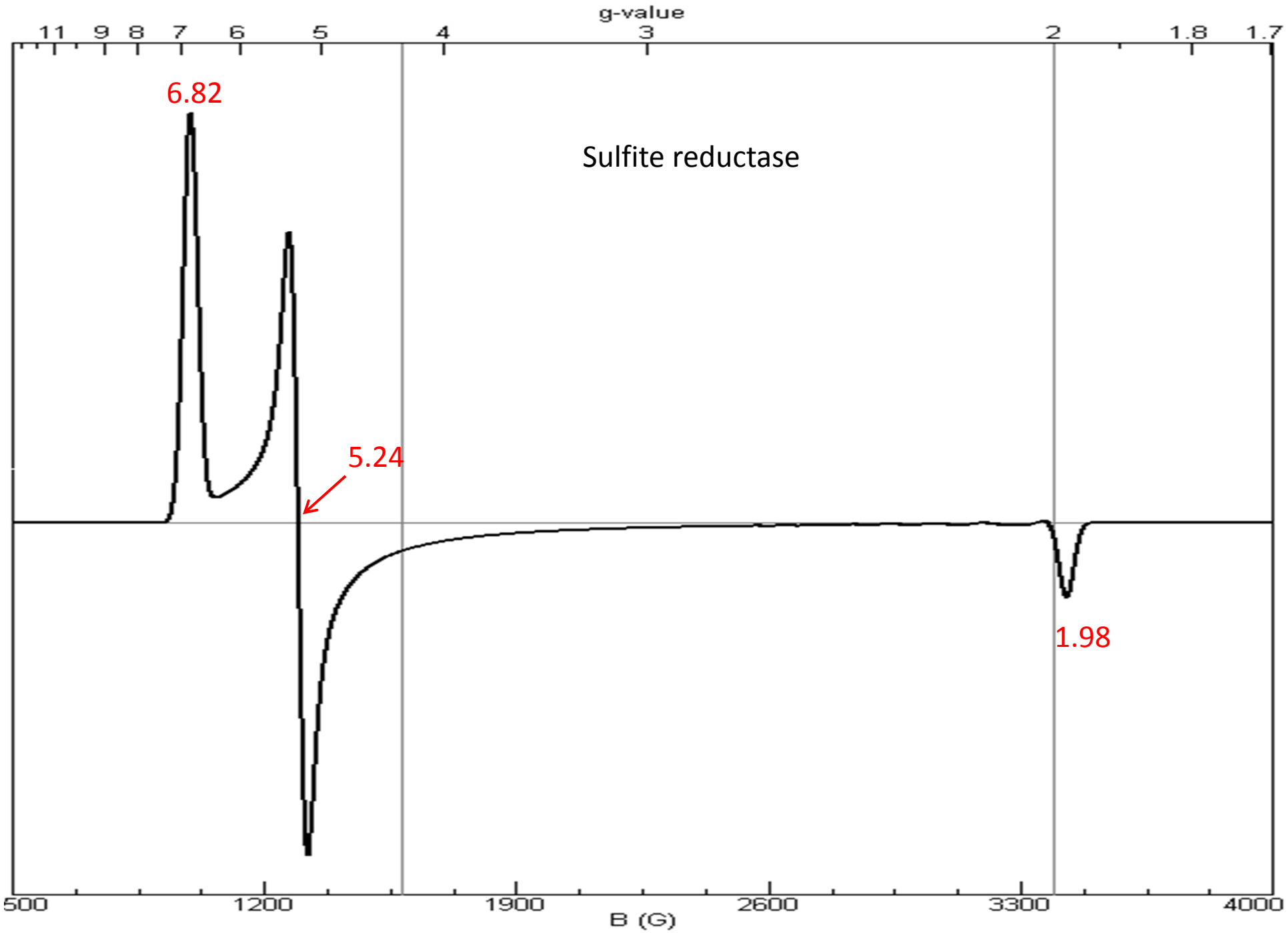
4Fe-4S

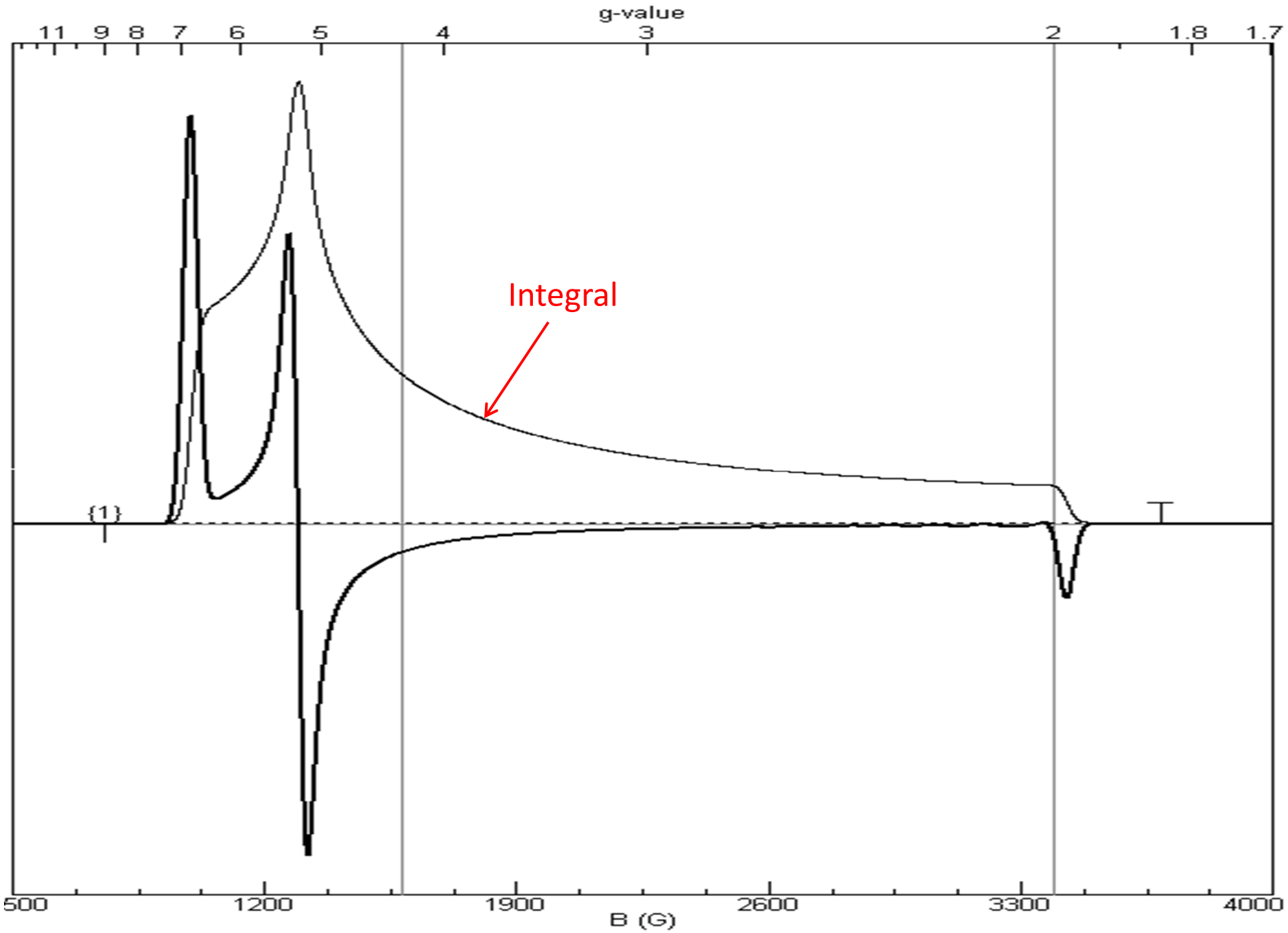
10 K X-band EPR

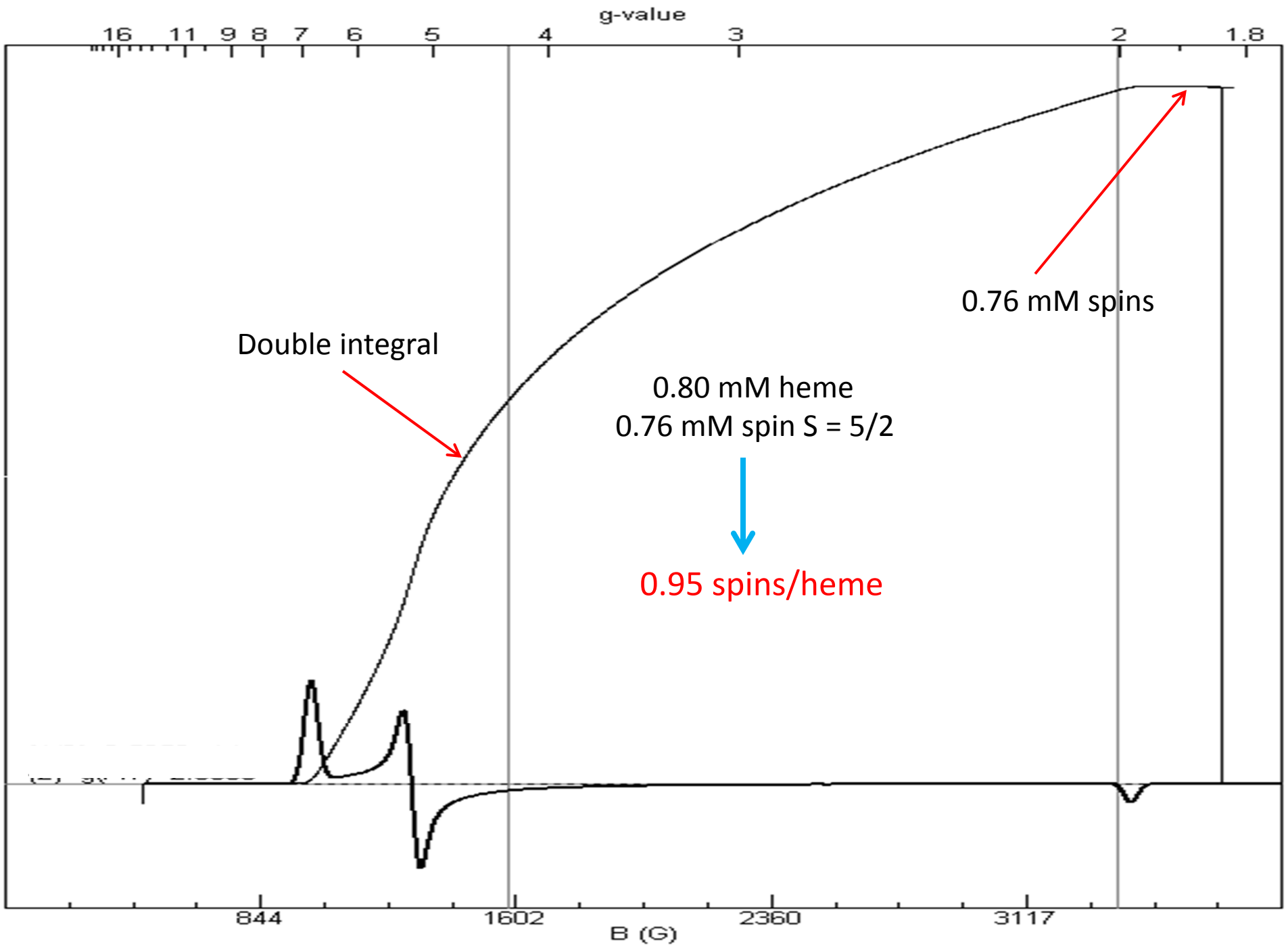


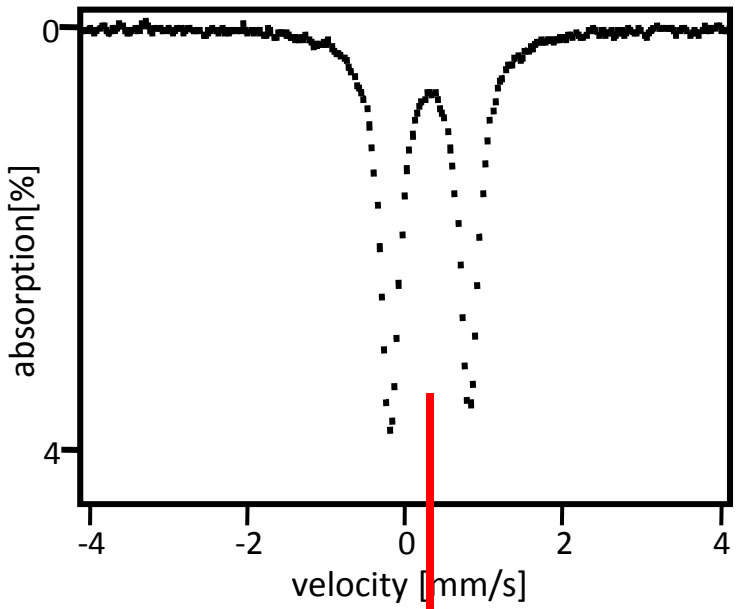
0.95 spins/heme

L. M. Siegel, P. Janick, J. Christner, E. Munck


g-value
11
9
8
7
6
5
4
3
2
1.8
1.7
6.82
Sulfite reductase
5.24
1.98
500
1200
1900
2600
3300
4000
B (G)

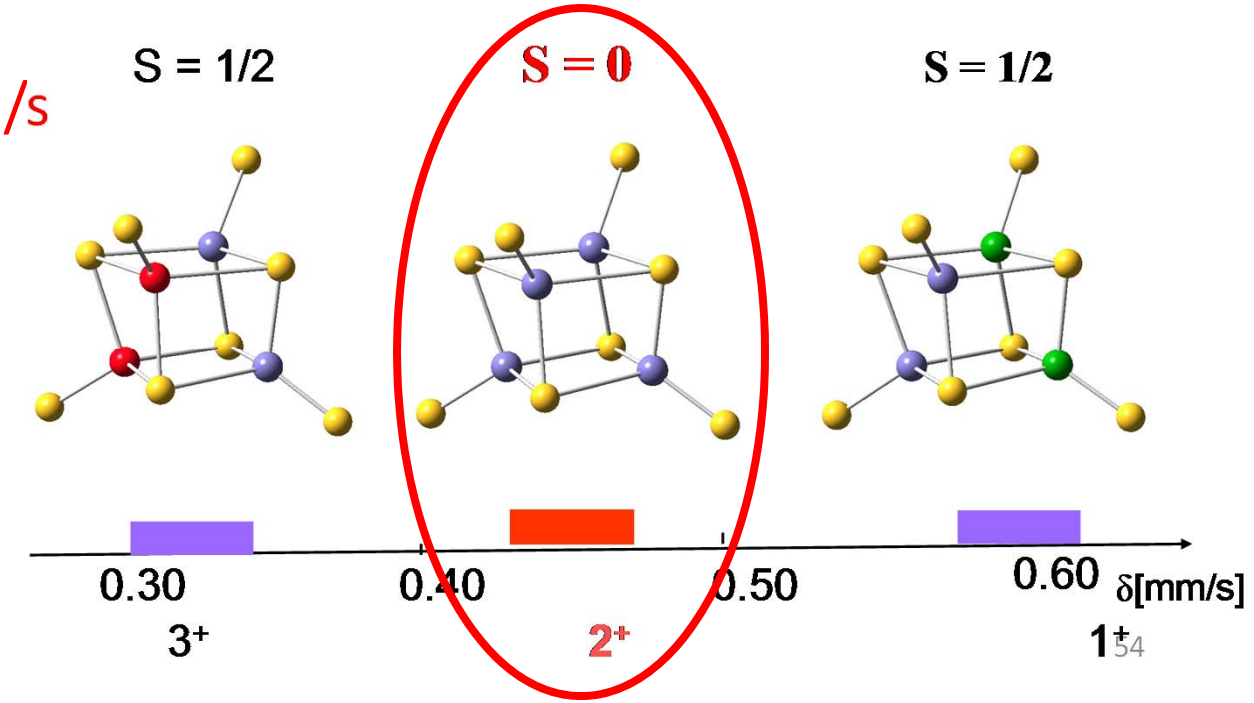

g-value
11
9
8
7
6
5
4
3
2
1.8
1.7
Integral
(1)
500
1200
1900
2600
3300
4000
B (G)


g-value
16
11
9
8
7
6
5
4
3
2
1.8
Double integral
0.76 mM spins
0.80 mM heme
0.76 mM spin S = 5/2
0.95 spins/heme
844
1602
2360
3117
B (G)

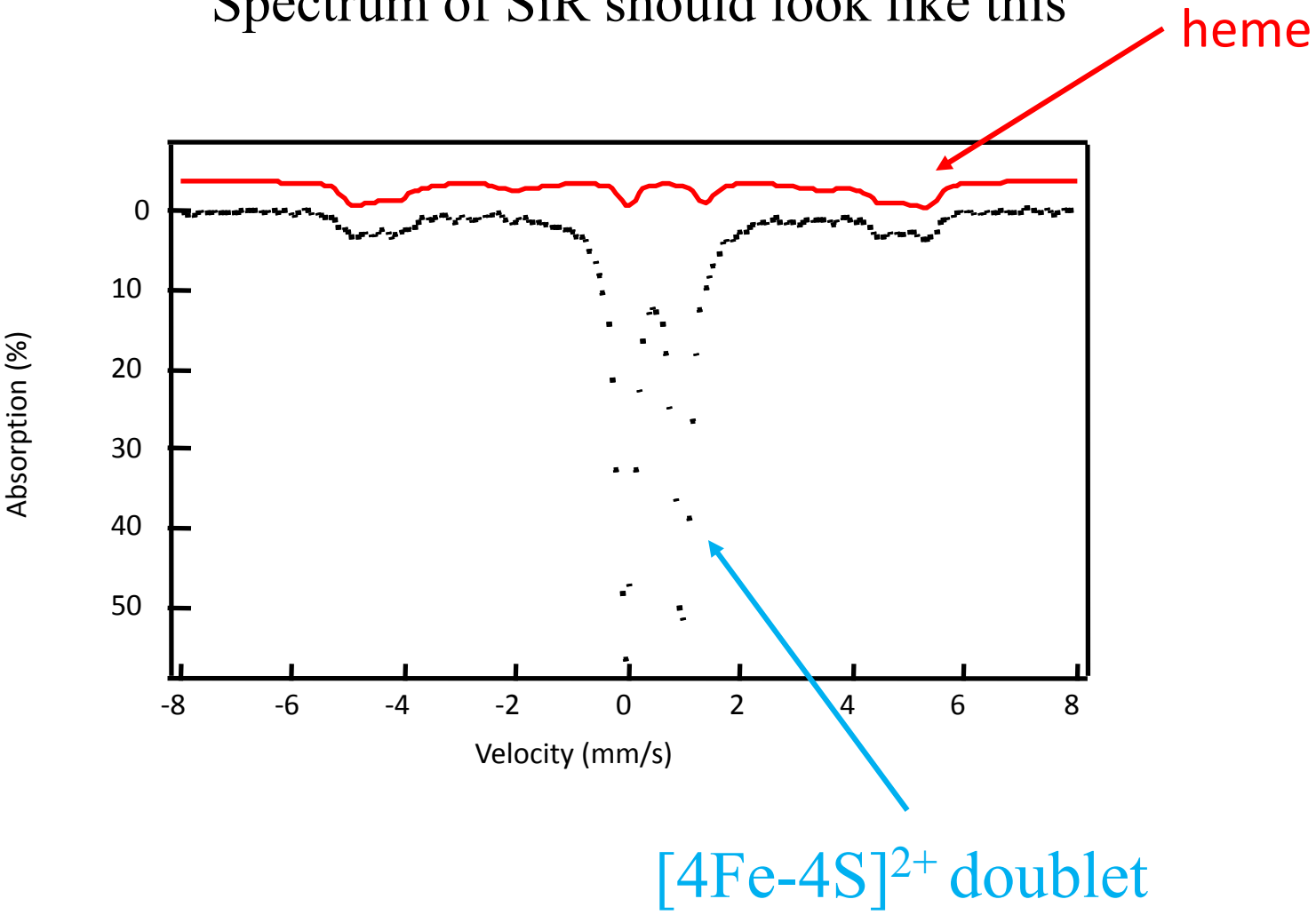


190-K Mössbauer Spectrum of the [4Fe-4S] Cluster.
By chance, the four Fe of the cluster and the Fe of the Siroheme have the same δ and ΔE_Q values

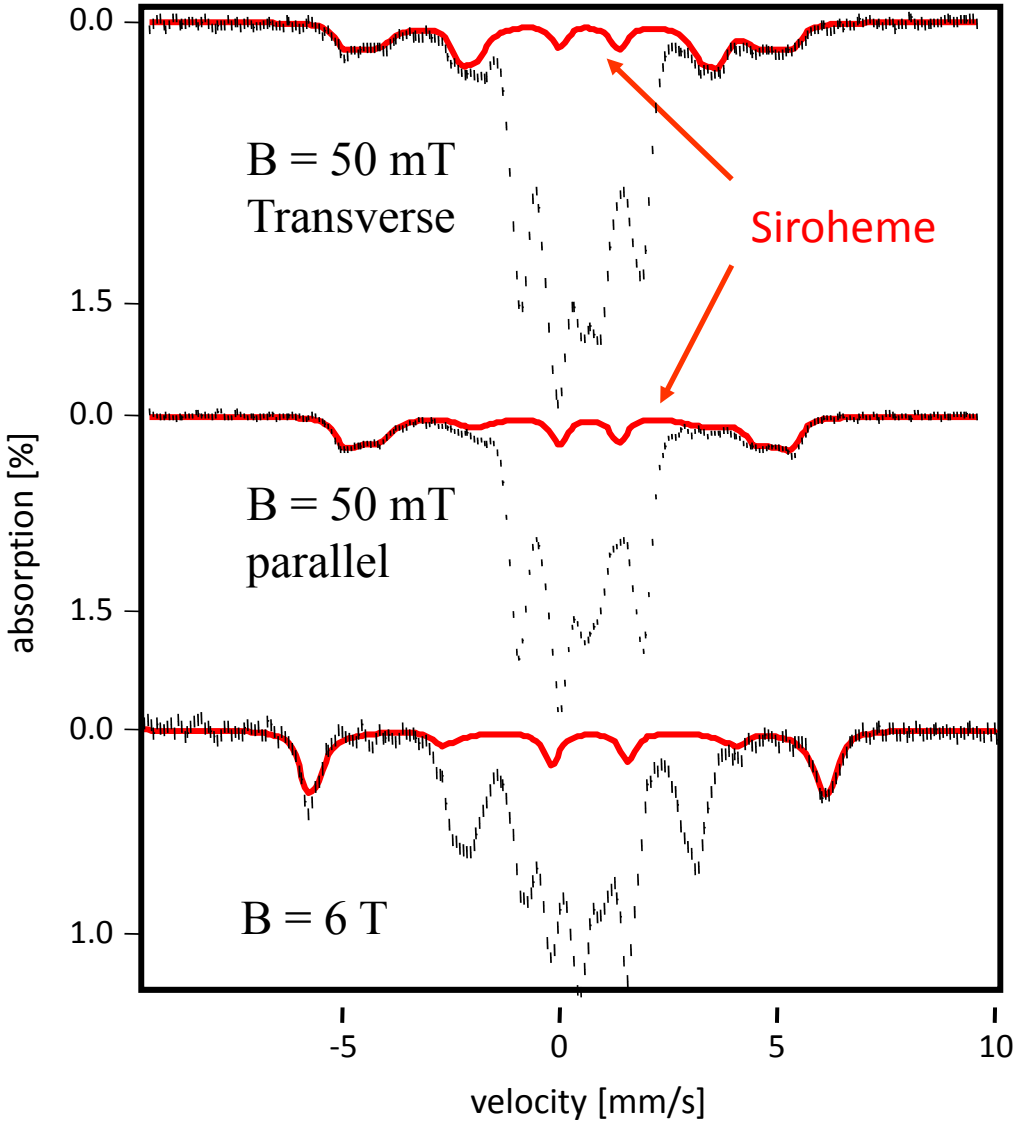
$\delta = 0.45 \pm 0.01$ mm/s



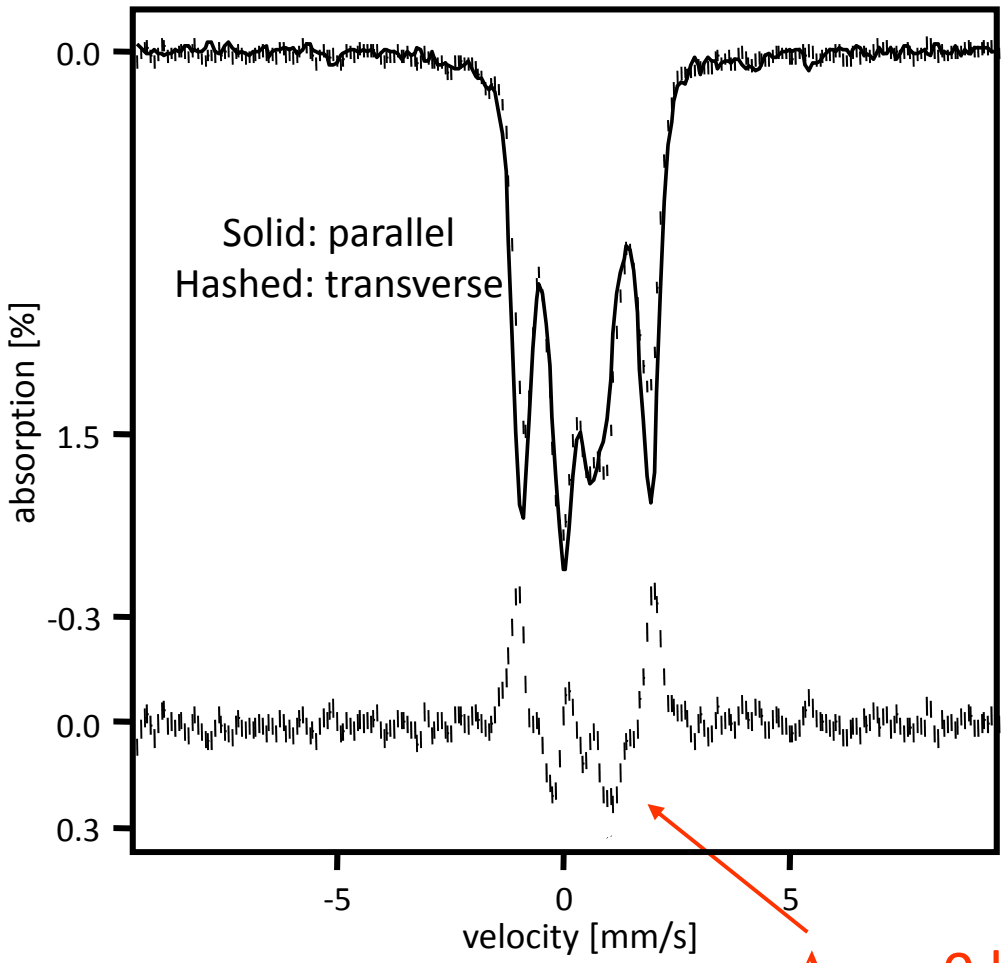
Spectrum of SiR should look like this



But doesn't


0.0
1.5
0.0
1.5
0.0
1.0
absorption [%]
B = 50 mT
Transverse
Siroheme
B = 50 mT
parallel
B = 6 T
-5
0
5
10
velocity [mm/s]

$[4Fe\text{-}4S]^{2+}$ Cluster of Sulfite Reductase



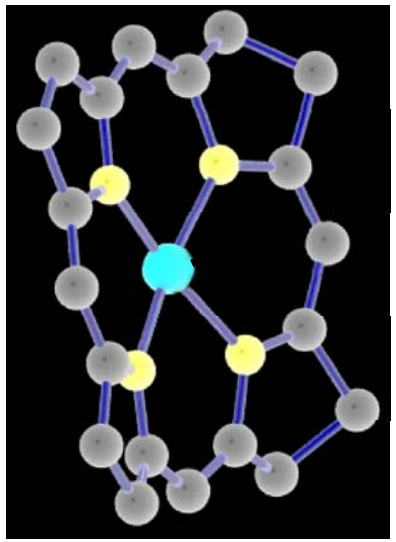
After removal of heme spectrum

Conclusion

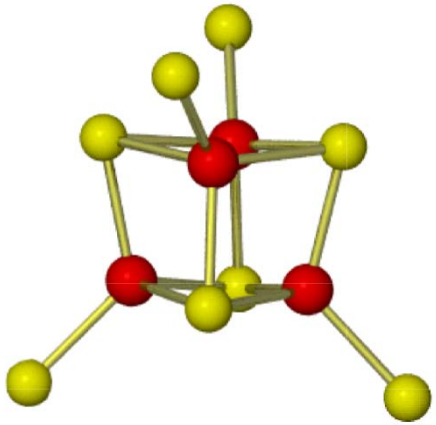
[4Fe-4S] cluster must be associated with the EPR signal.

Christner,J.;P.;Münck, E.;Janick,P.A.;Siegel,L.M. *J. Biol. Chem*. 1982, *356*, 2089

Coupled Chromophores !!



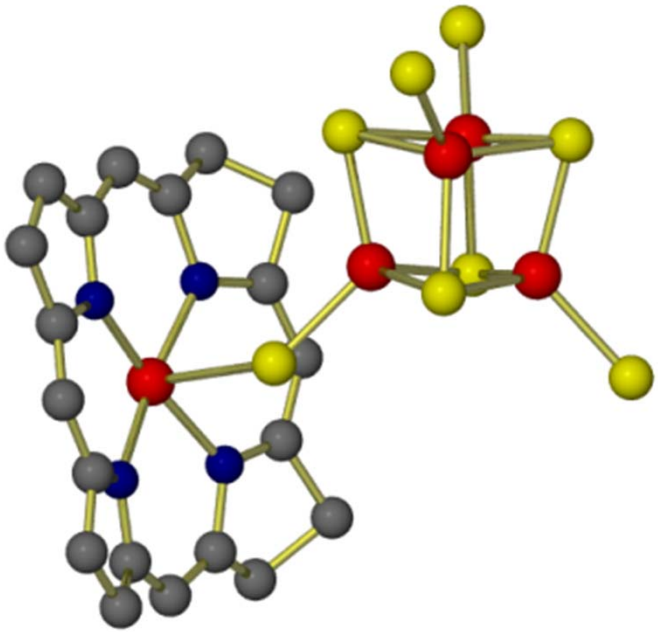
Siroheme



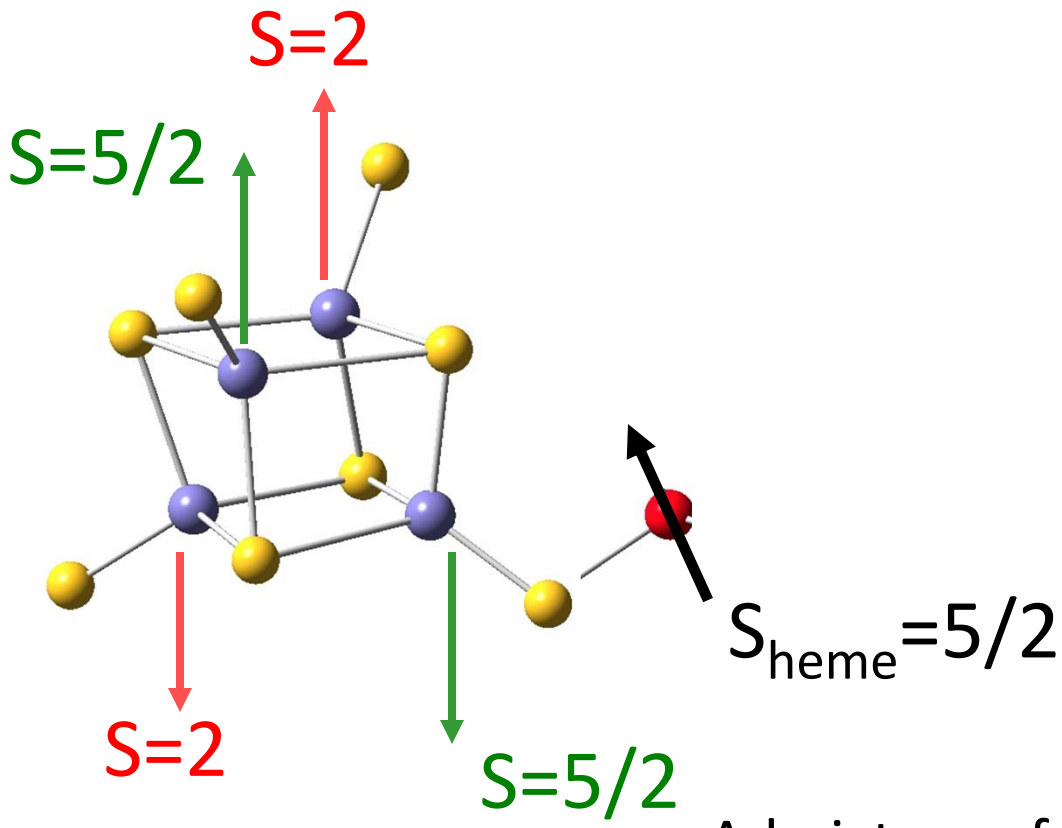
4Fe-4S

Mössbauer Study 1982

X-ray structure: D. E. McRee et al. *J. Biol. Chem.* 1986, *261,*10277

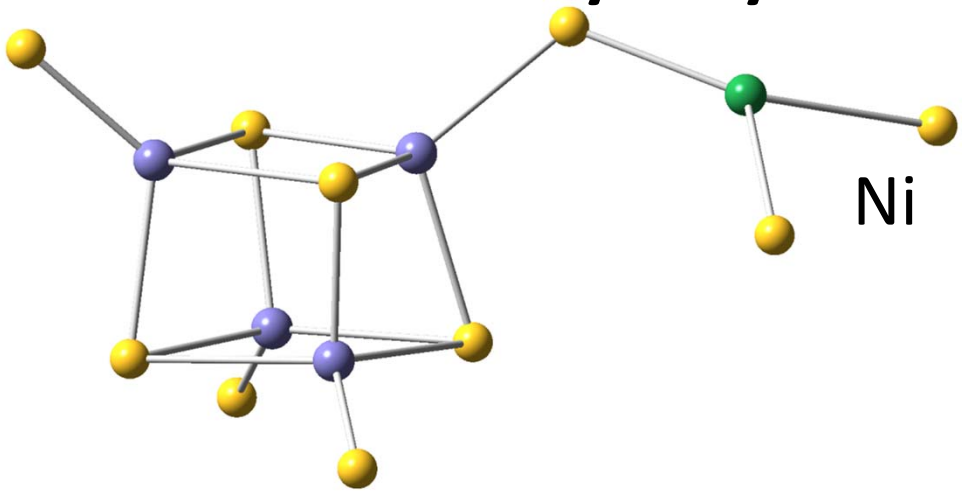


Paramagnetism of $[4Fe\text{-}4S]^{2+}$ Cluster



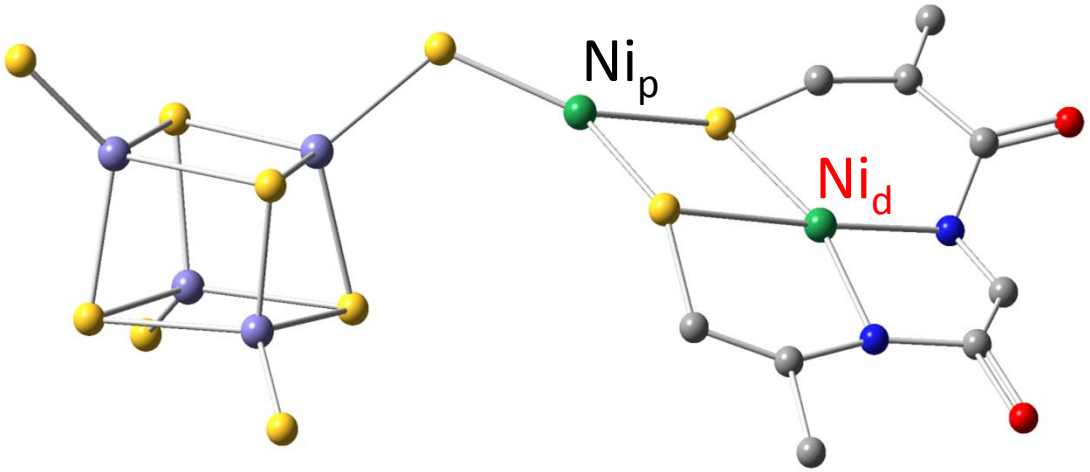
S=0 ⟶ Admixture of S=1 excited state in the S=0 ground state.

Acetyl Synthase A-cluster



by Mössbauer Analysis 1997

Xia, J., Hu, X., Popescu, C., Lindahl, P.A., Münck, E. *JACS,* 1997,*119*, 8301



by X-ray Crystallography 2003

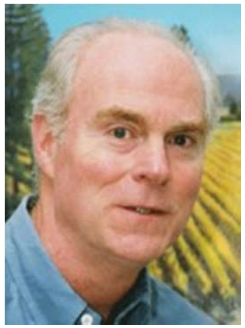
C. Darnault et al. *Nat. Struct. Biol*. 2003, *10*, 271

2,3 homoprotocatechuate dioxygenase

2,3 HPCD

M. M. Mbughuni,[a] M. Chakrabarti,[b] J. A.Hayden,[b] E. L. Bominaar,[b] M. P. Hendrich,[b] E. Münck,[b] and J. D. Lipscomb[a1] *PNAS* 2010 ; Mbughuni et al. *Biochemistry* 2011; Mbughuni et al *Biochemistry* 2012;

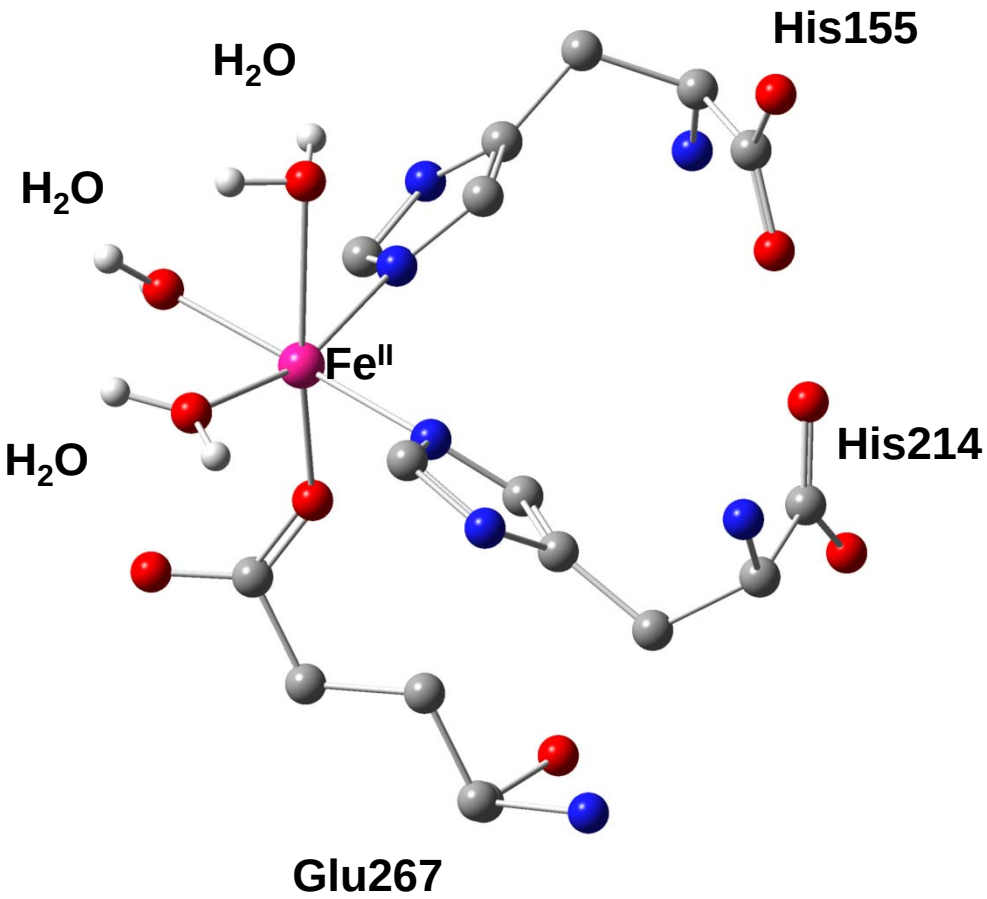
Homoprotocatechuate 2,3-Dioxygenase (HPCD)



Catalyze ring cleave of aromatic substrates.

Incorporate both atoms of O2 into product.

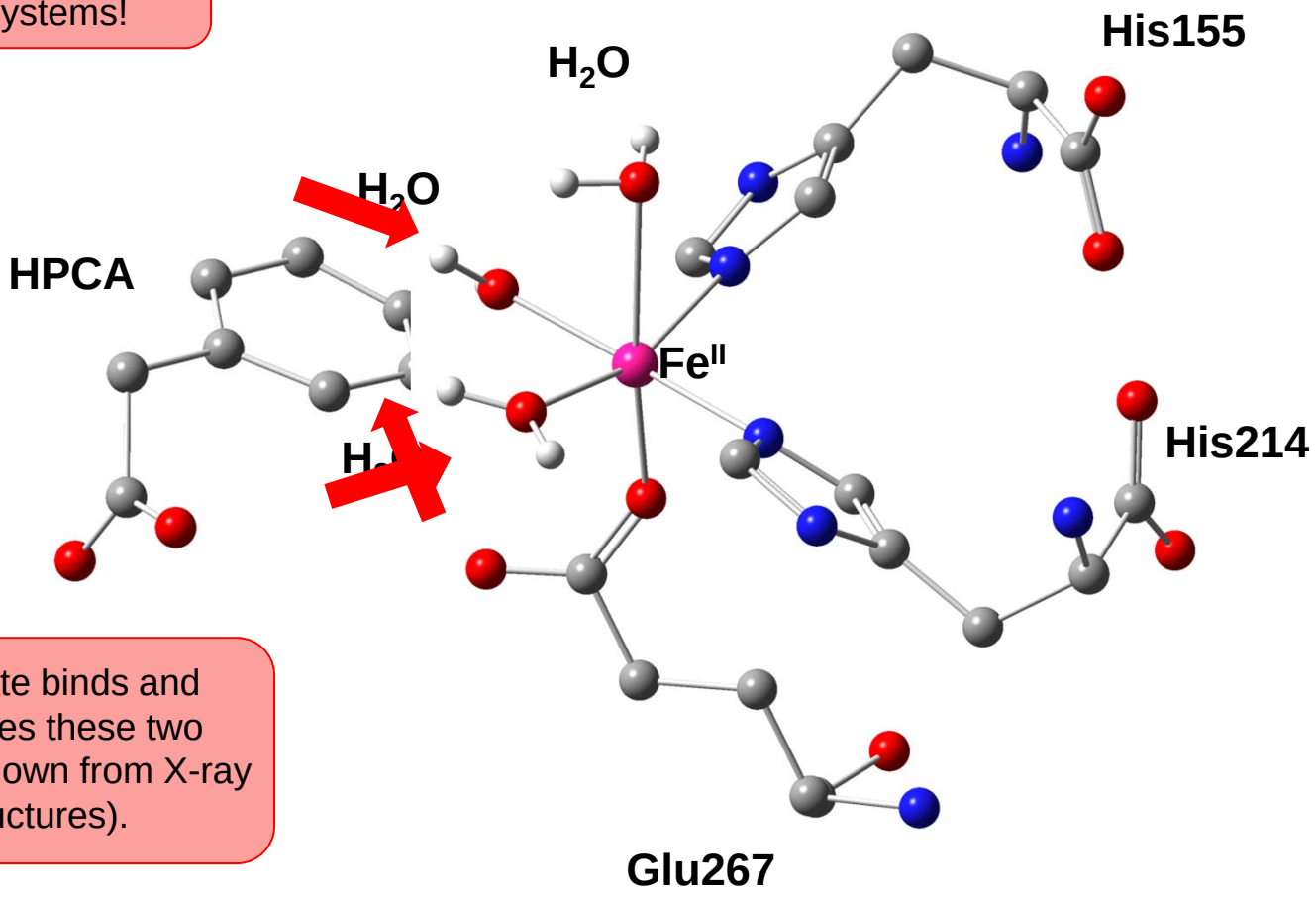
Roles in human health and maintenance of global carbon cycle



Katie Meier

HPCD Active Site

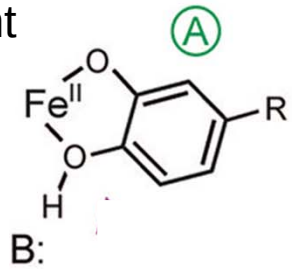
2-His, 1-Carboxylate facial triad motif – in many systems!



Substrate binds and eliminates these two waters (known from X-ray structures).

This is a simplified picture:please ignore (wrong) carboxylates at the tail of His 214, 155 and Glu 267

(A) multistep substrate binding and displacement of solvents



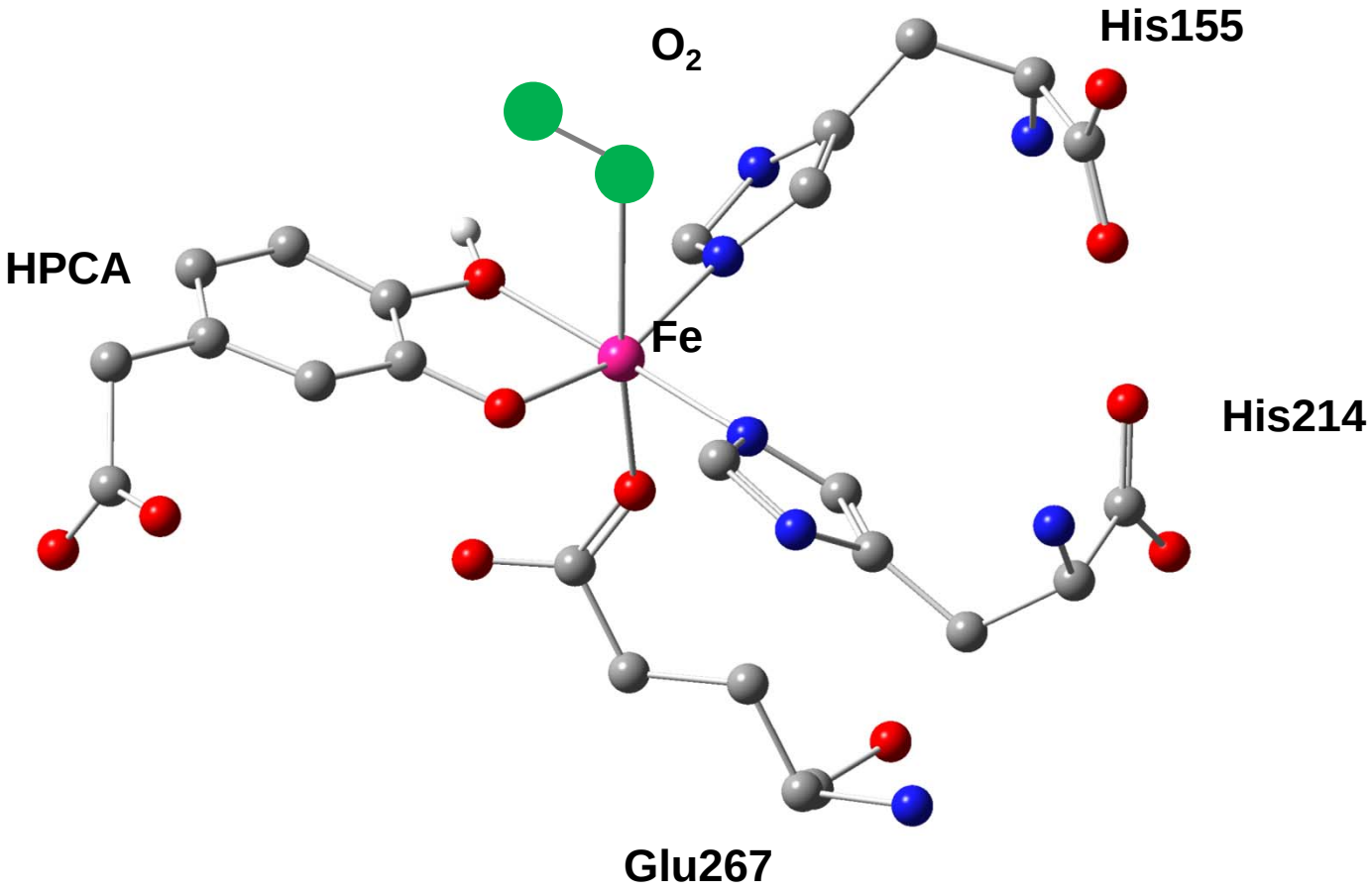
(E) formation of an alkyl-peroxo intermediate

(G) hydrolysis of the lactone to yield the bound product ready for release

(F) O-O bond cleavage yielding a substrate lactone intermediate and a Fe-OH moiety,

Mbughuni, M. M., Chakrabarti, M., Hayden, J. A., Meier, K. K., Dalluge, J. J., Hendrich, M. P., Munck, E., Lipscomb, J.D. “Oxy Intermediates of Homoprotocatechuate 2,3-Dioxygenase: Facile Electron Transfer between Substrates.” *Biochem.,* **2011**, *50*, pp 10262-10274.

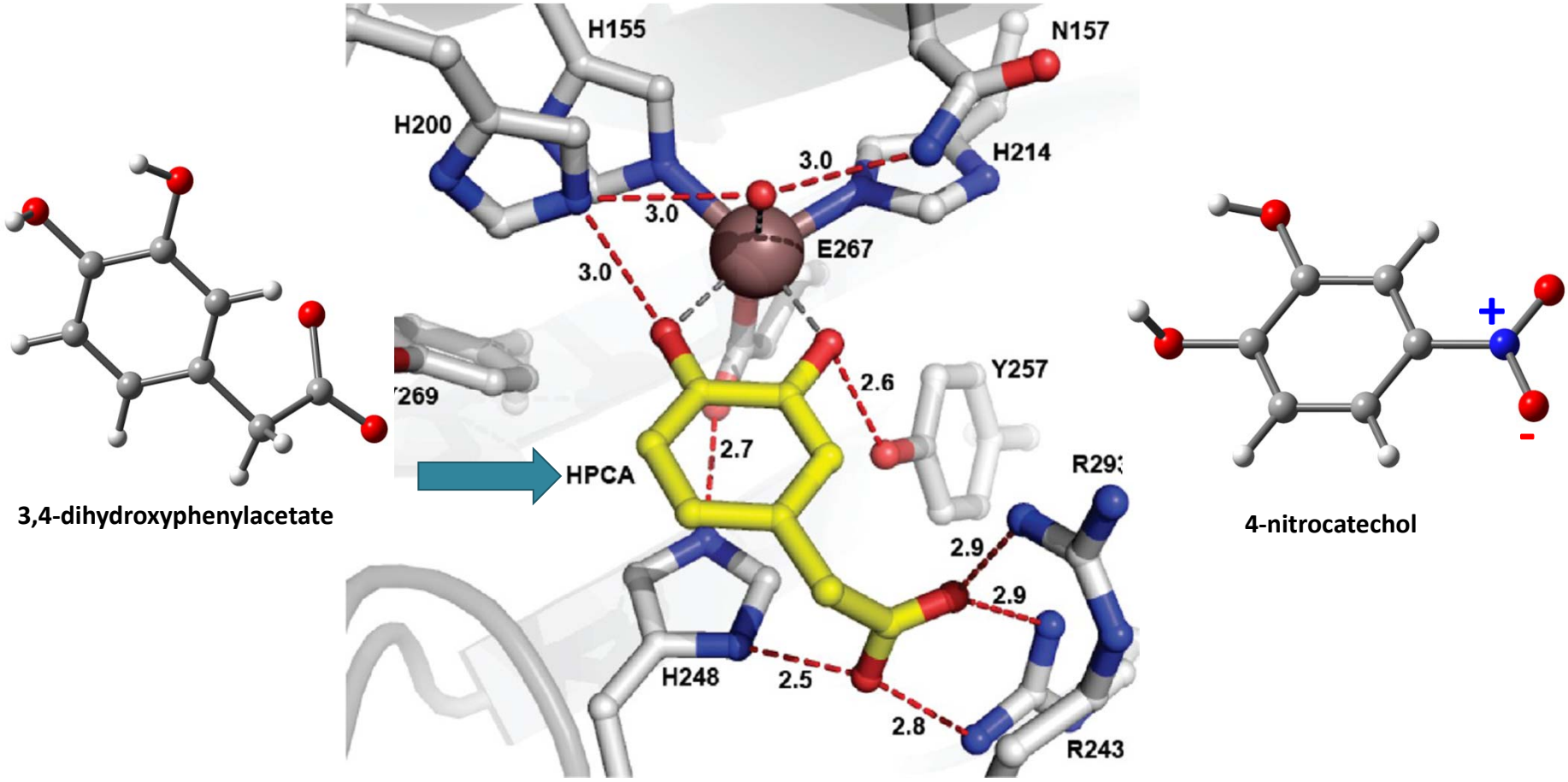
HPCD-Substrate-O_2 Adduct



Two types of modifications are useful to slow down intermediates or to give insight into reaction pathway

Next two pics also show secondary coordination sphere

1: Alternative Substrate



3,4-dihydroxyphenylacetate

4-nitrocatechol

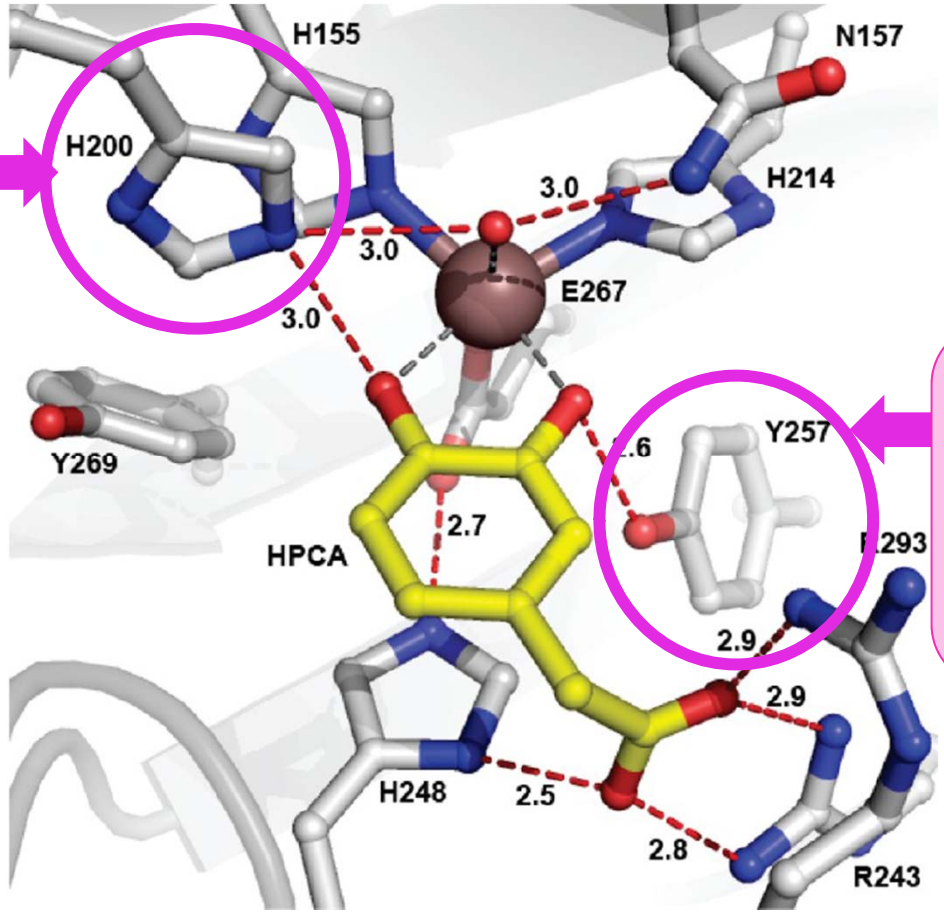
Kovaleva, E.G., Lipscomb, J.D. “Structural Basis for the Role of Tyrosine 257 of Homoprotocatechuate 2,3-Dioxygenase in Substrate and Oxygen Activation.” *Biochemistry,* **2012**, *51* (44), pp 8755-8763. (PDB ID: 4GHG)

2: Mutagenesis

Strategies for changing electron density

H200N: HPCA

(1) Mutation removes the potential for efficient hydrogen bonding.
(2) Cannot catalyze acid-base chemistry.

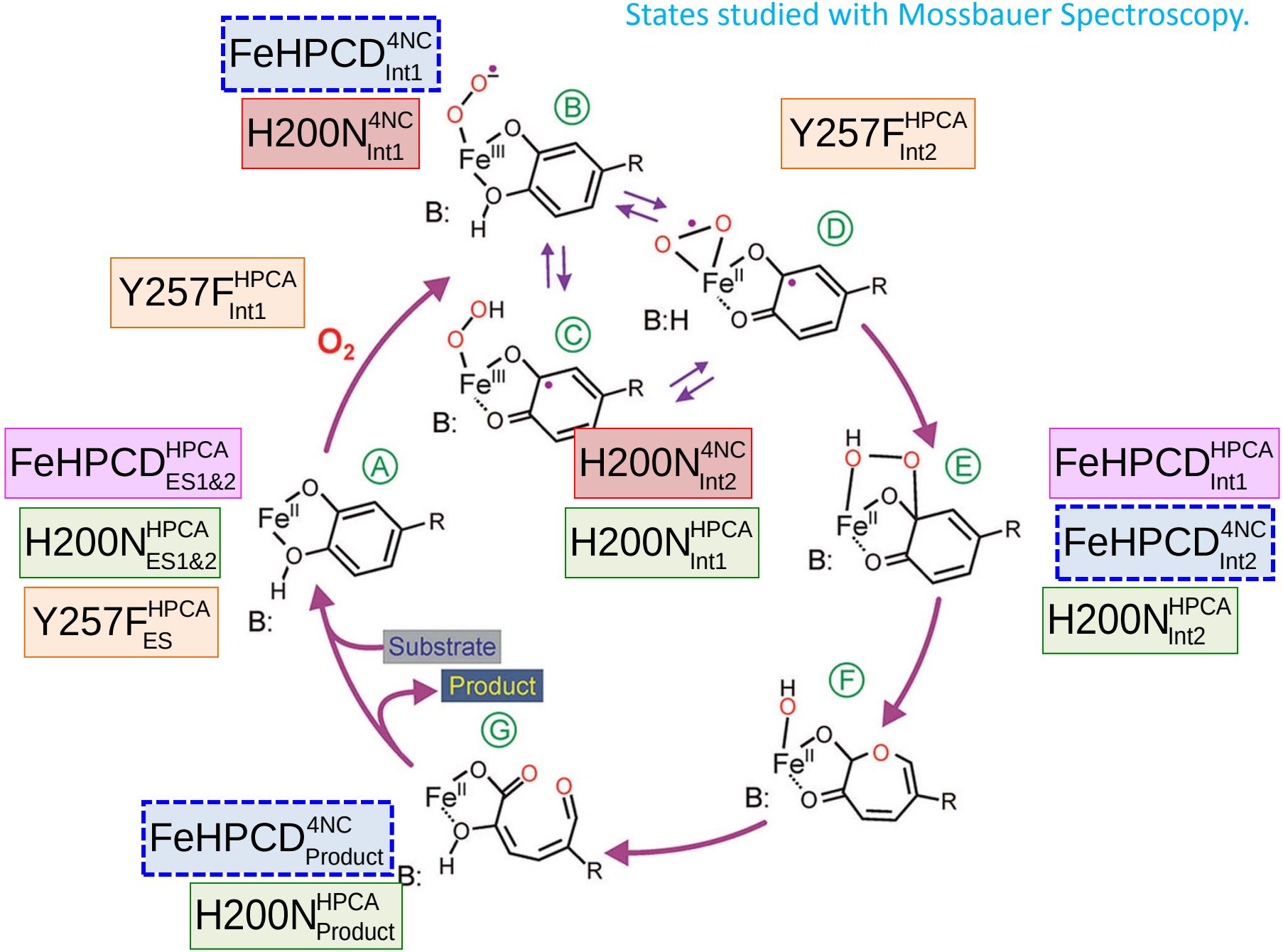


Y257F: HPCA

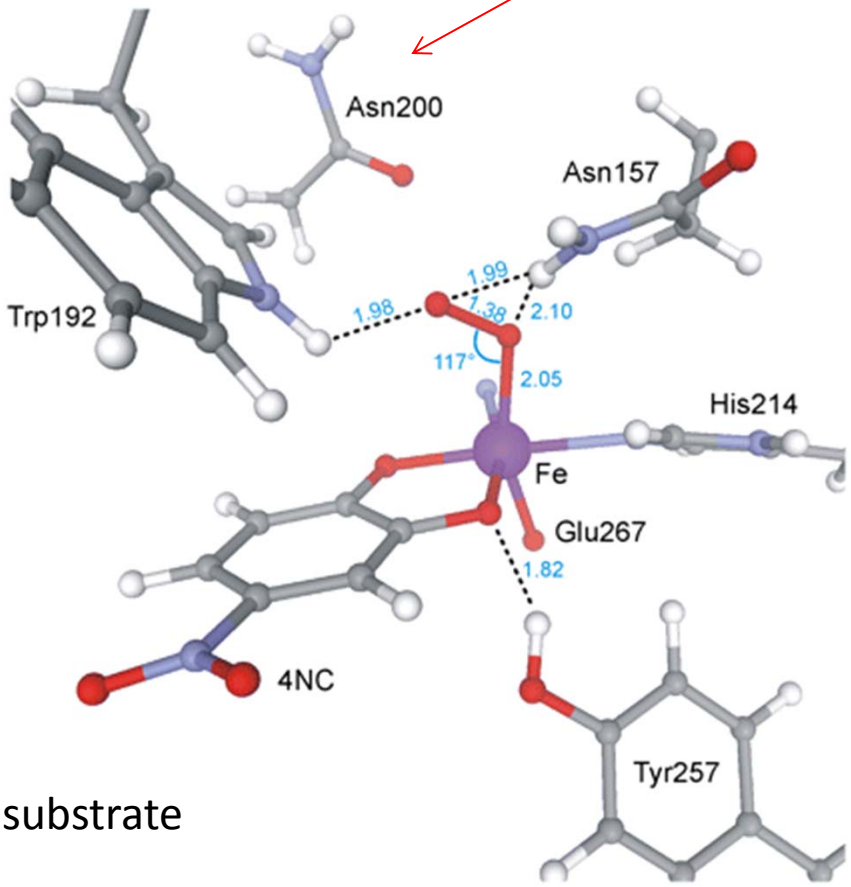
(1) Phenylalanine preserves spatial characteristics of Tyr.
(2) Removes ability to H-bond or sterically interact with substrate.

Kovaleva, E.G., Lipscomb, J.D. “Structural Basis for the Role of Tyrosine 257 of Homoprotocatechuate 2,3-Dioxygenase in Substrate and Oxygen Activation.” *Biochemistry,* **2012**, *51* (44), pp 8755-8763. (PDB ID: 4GHG)

States studied with Mossbauer Spectroscopy.



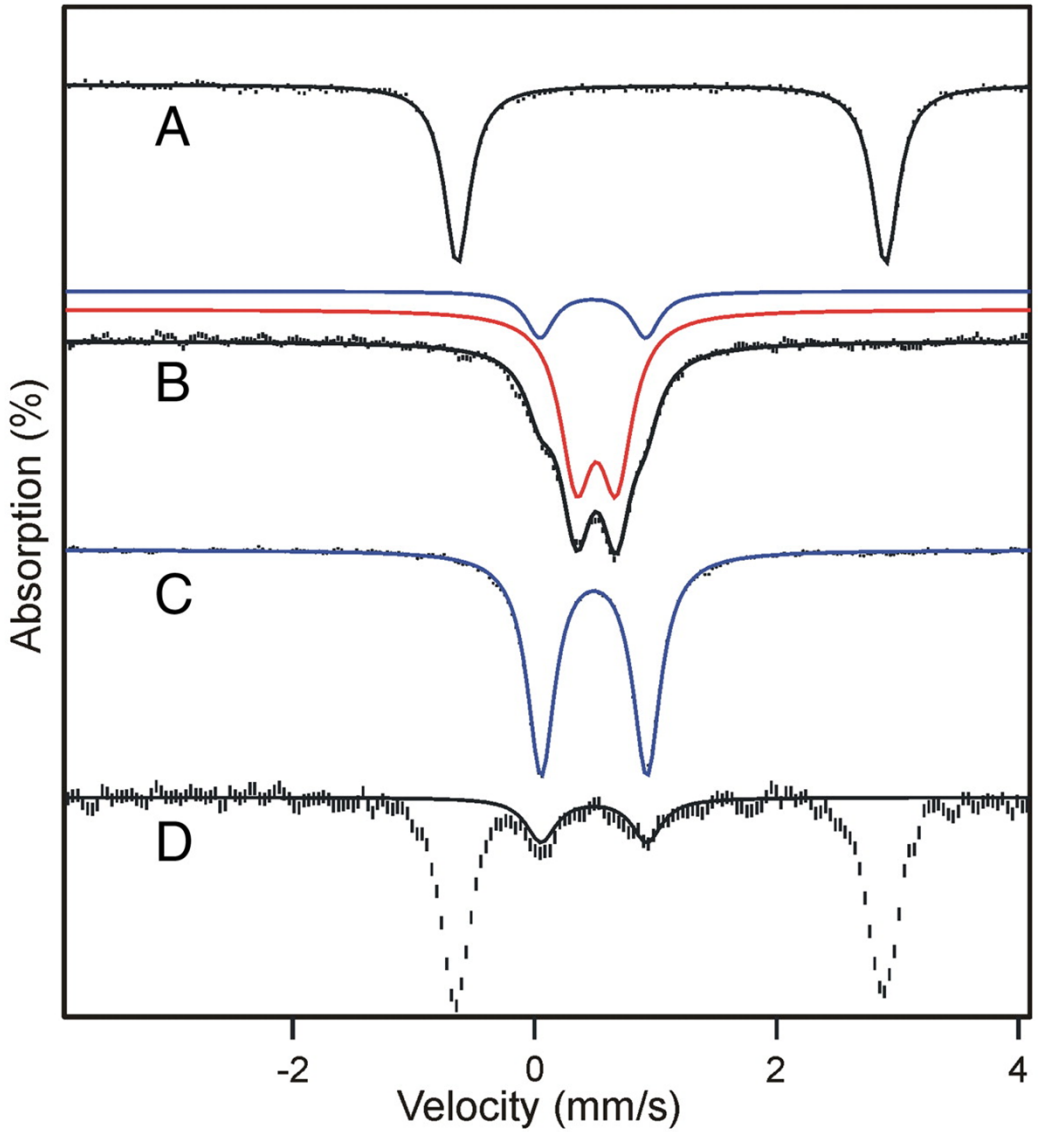
H200N mutant allows to trap Int-1



4NC is a slow substrate

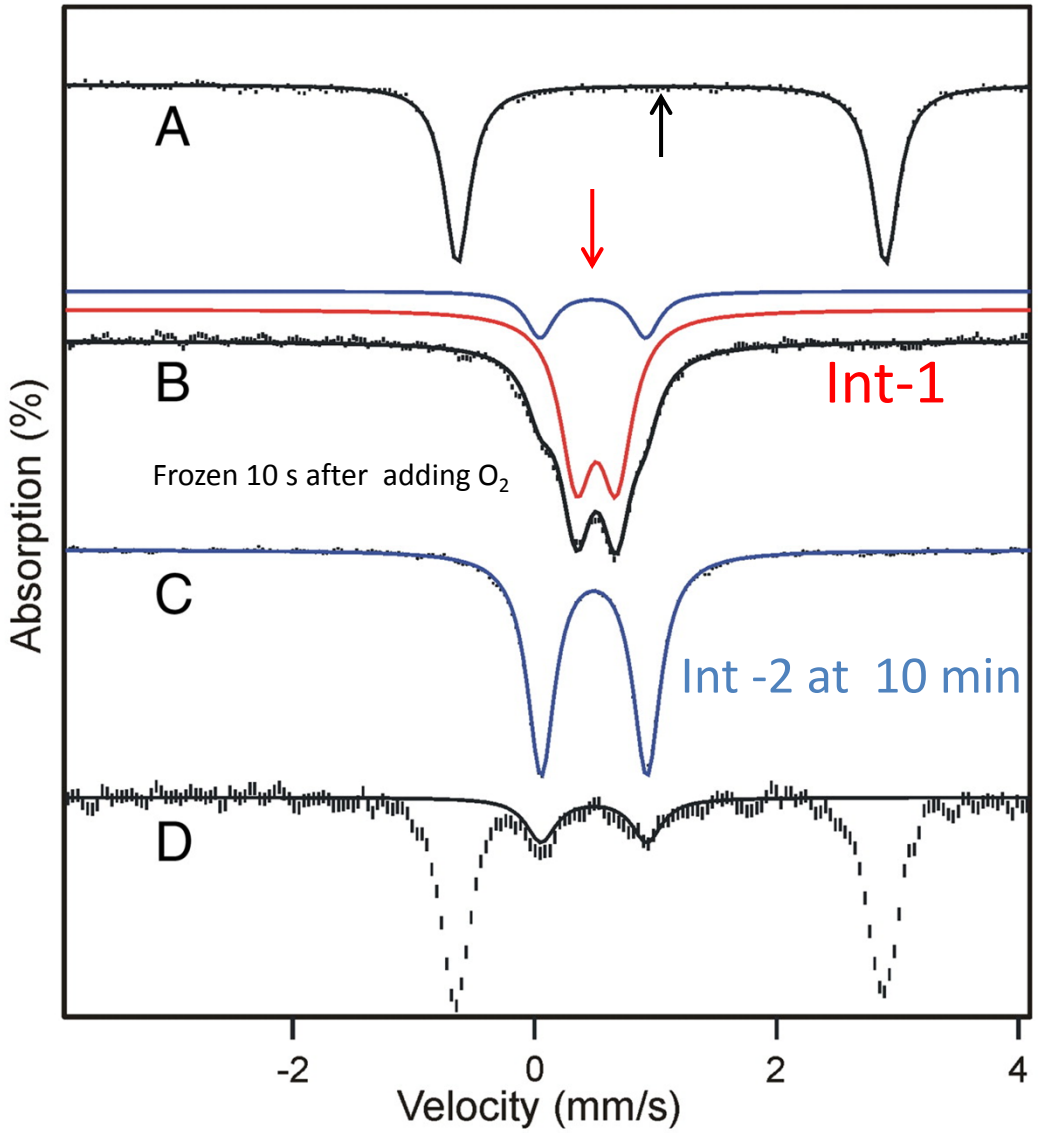
Histidine 200 → asparagine

Zero field Mossbauer Spectra of H200N mutant



2,3 HPCD + 4-nitrocatechol

Zero field Mossbauer Spectra at 4.2 K: H200N mutant



2,3 HPCD + 4-nitrocatechol

δ = 1.12 mm/s

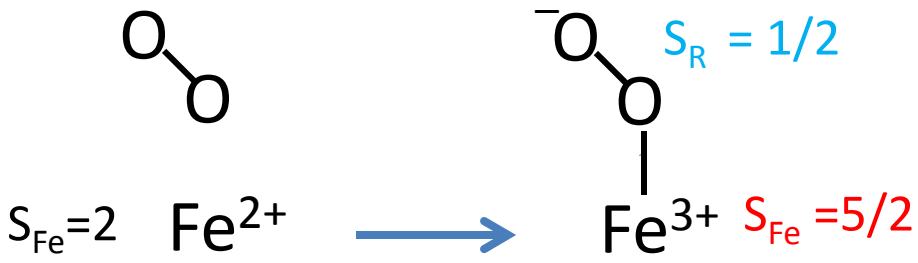
→ Fe^{II}

$\delta = 0.50$ mm/s suggests S= 5/2 Fe^{III} but Int-1 behaves like an integer spin species

Int-2 is also high-spin Fe^{III}

2,3 HPCD + product and remaining Int-2

Formation of a Superoxo Complex

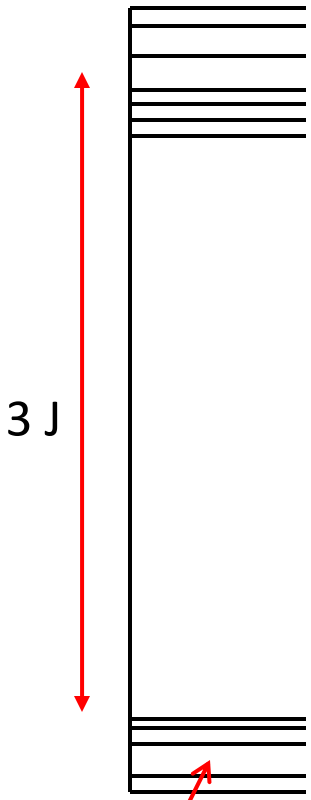


Described by exchange coupling

$$\mathcal{H} = J\, \mathbf{S}_{Fe} \bullet \mathbf{S}_R$$

$\mathcal{H}$ produces a spin ladder with energies

$$E = J\,\{S(S+1) - 35/4 - \tfrac{3}{4}\}/2$$



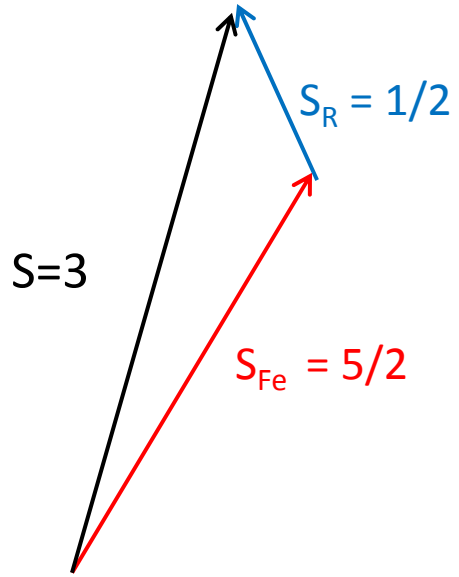
S = 3 multiplet

$\mathbf{S} = \mathbf{S}_{Fe} + \mathbf{S}_{R}$

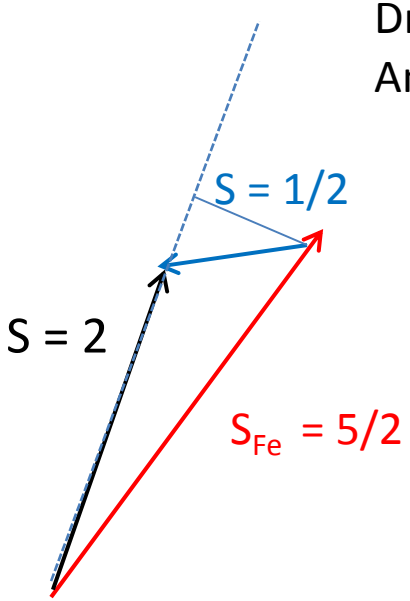
$\mathcal{H} = J\, \mathbf{S}_{Fe} \bullet \mathbf{S}_{R}$

S = 2 multiplet
(with zero-field splitting)

Zero field splitting



Drawn for J > 0,
Antiferromagnetic

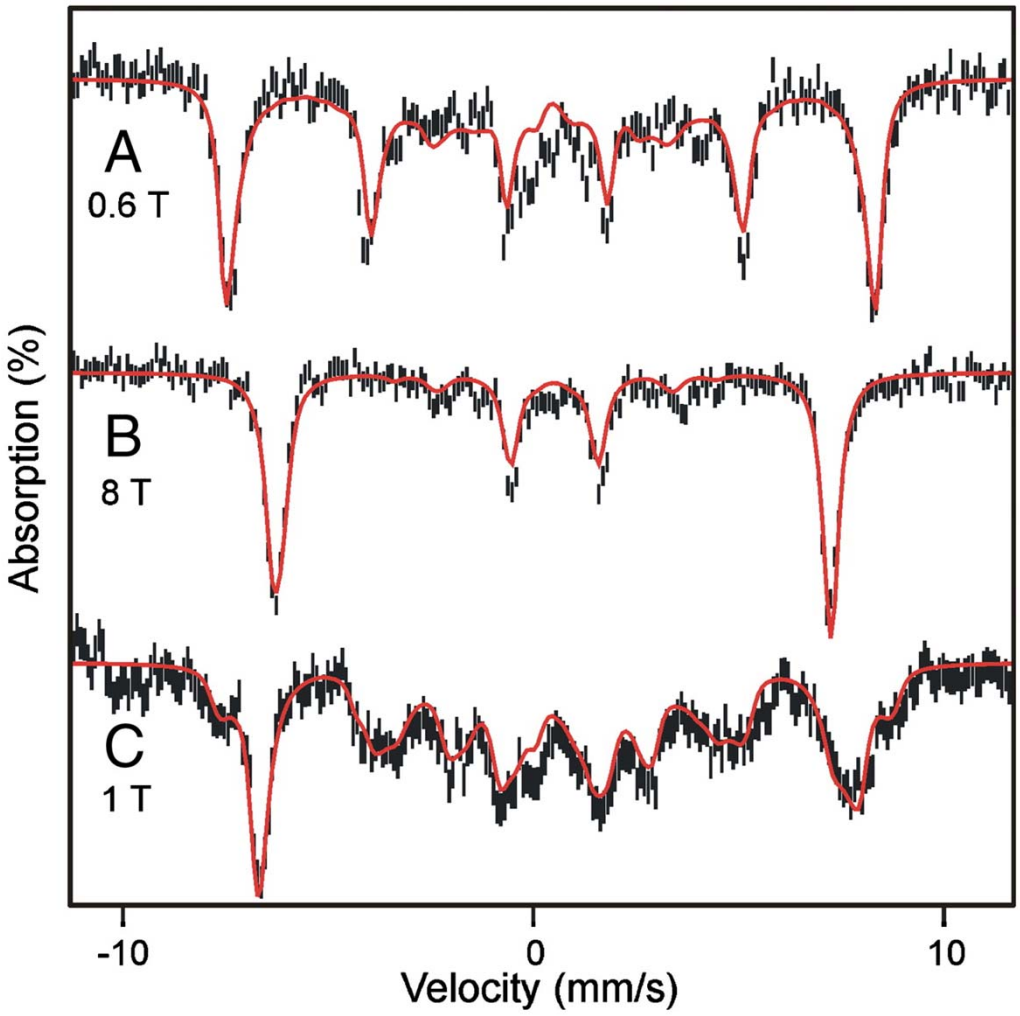


Mossbauer: The iron of Int-1 is high-spin (S_1=5/2) ferric

Full splitting for B = 0.6 T suggests two closely spaced (< 0.3 cm^{-1}) electronic levels. That is less than hν at X-band

Do EPR

Explained below



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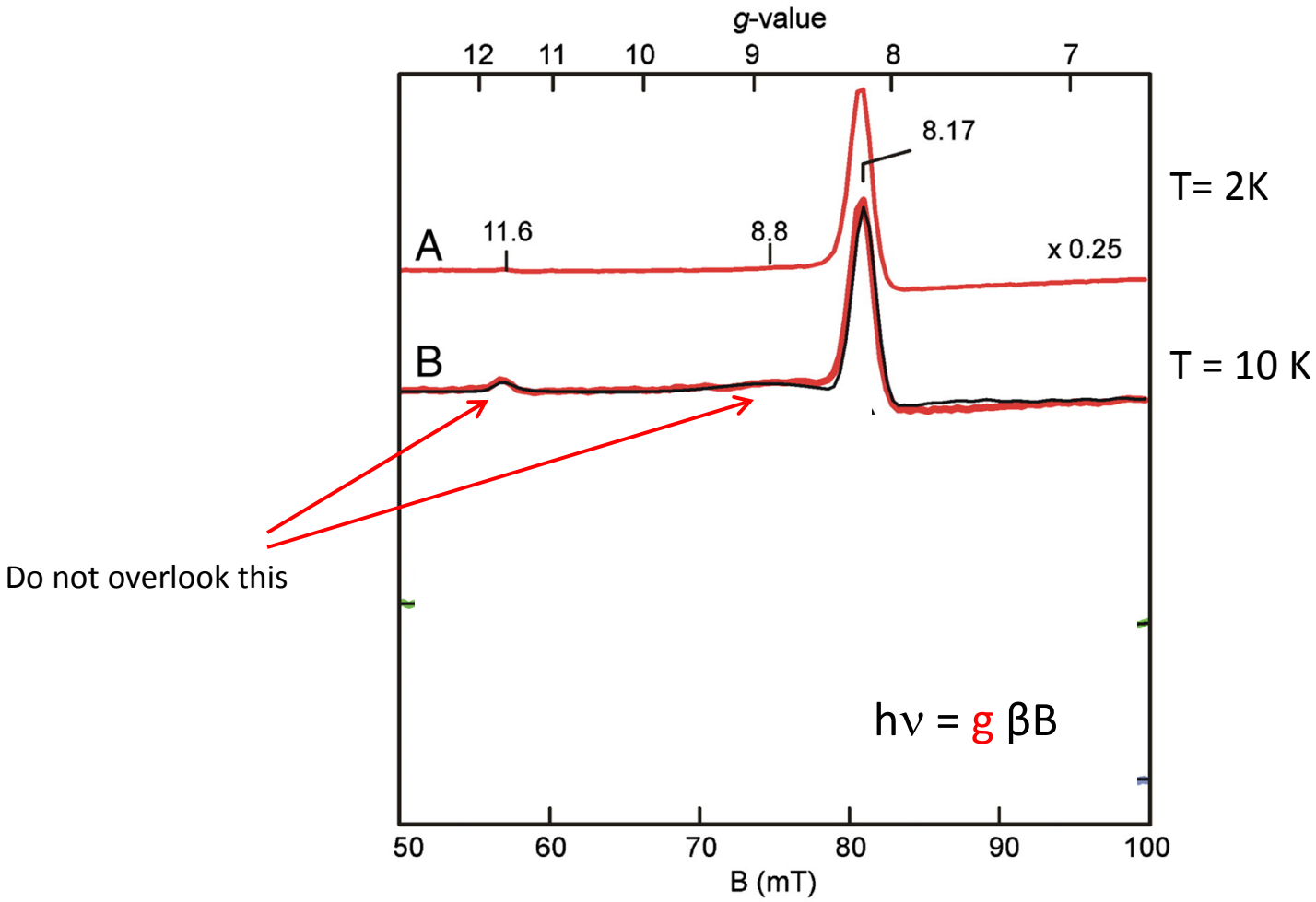
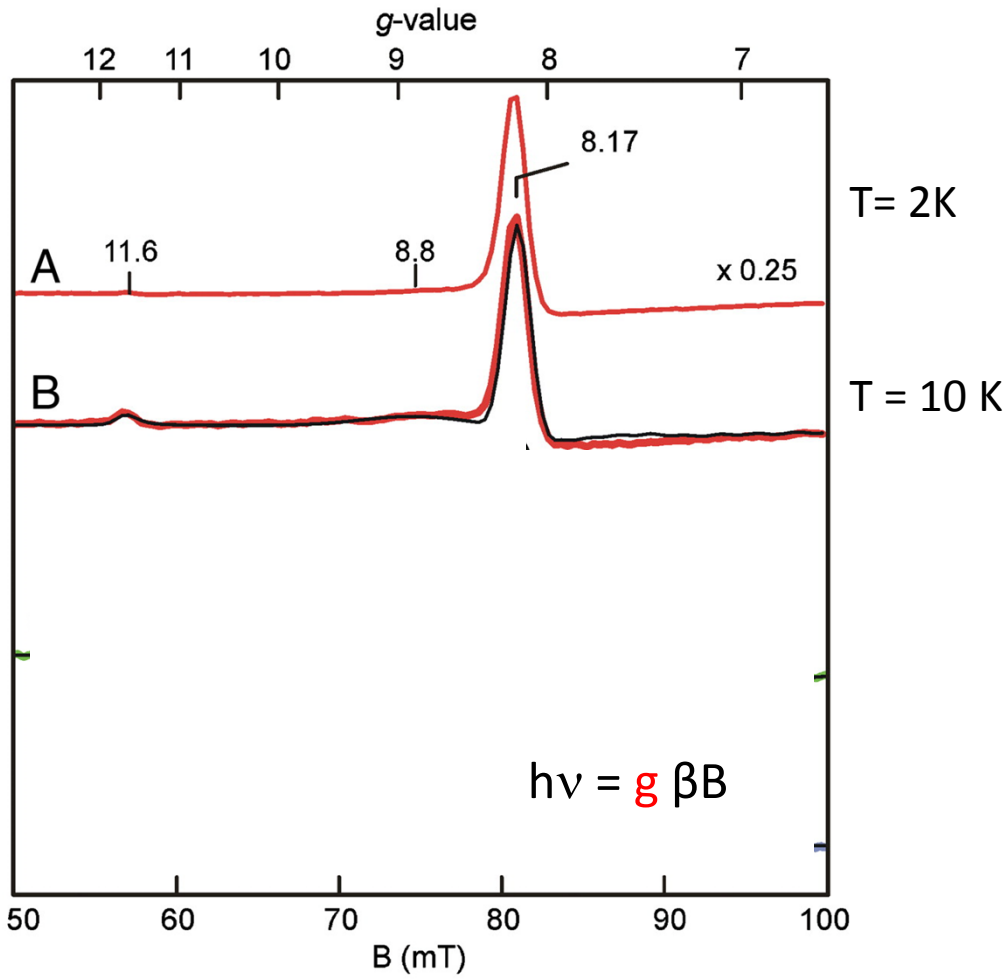
Parallel mode EPR of Int-1

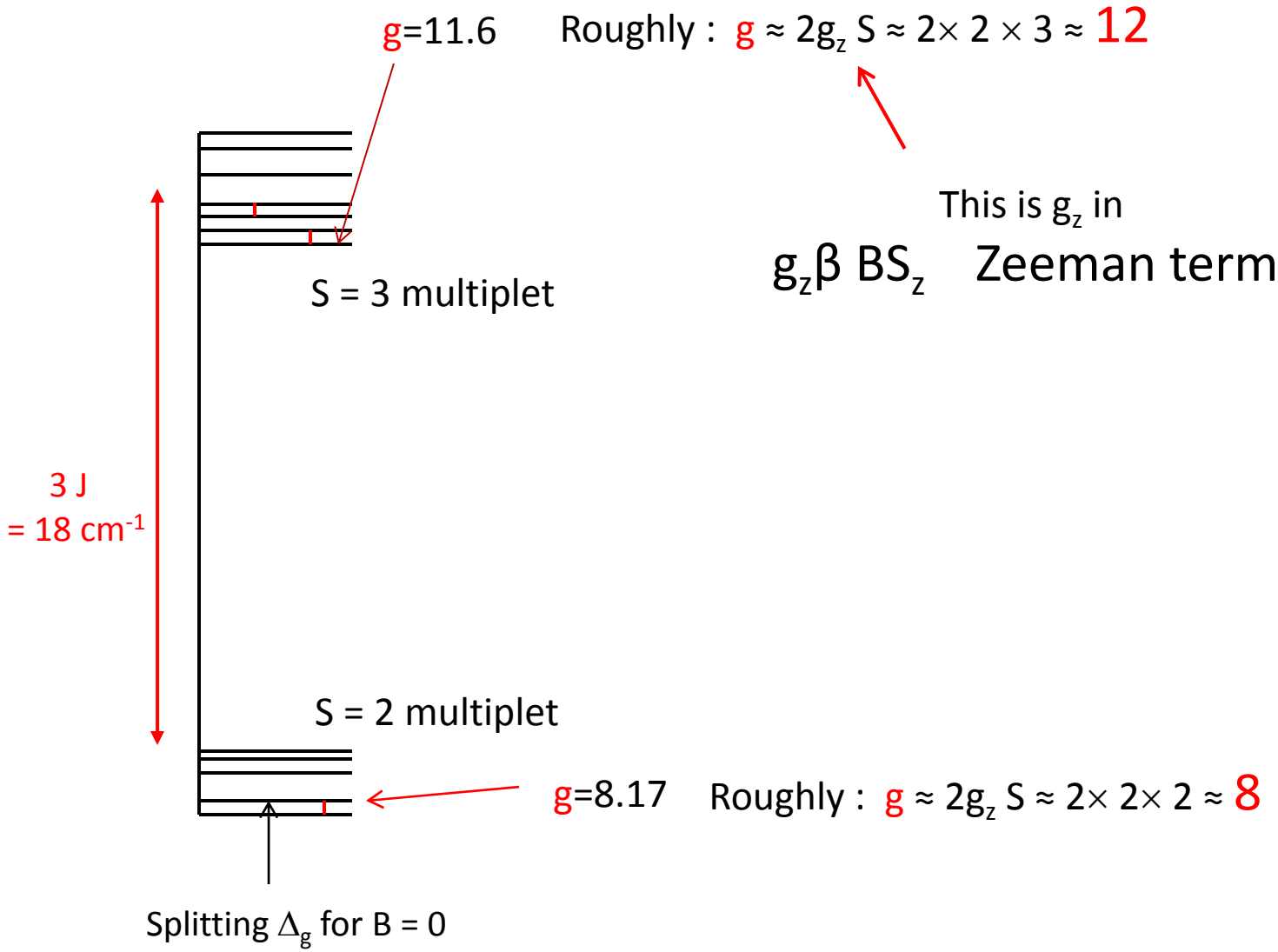


The g = 8.17 feature is characteristic of an S = 2 system

Parallel mode EPR of Int-1



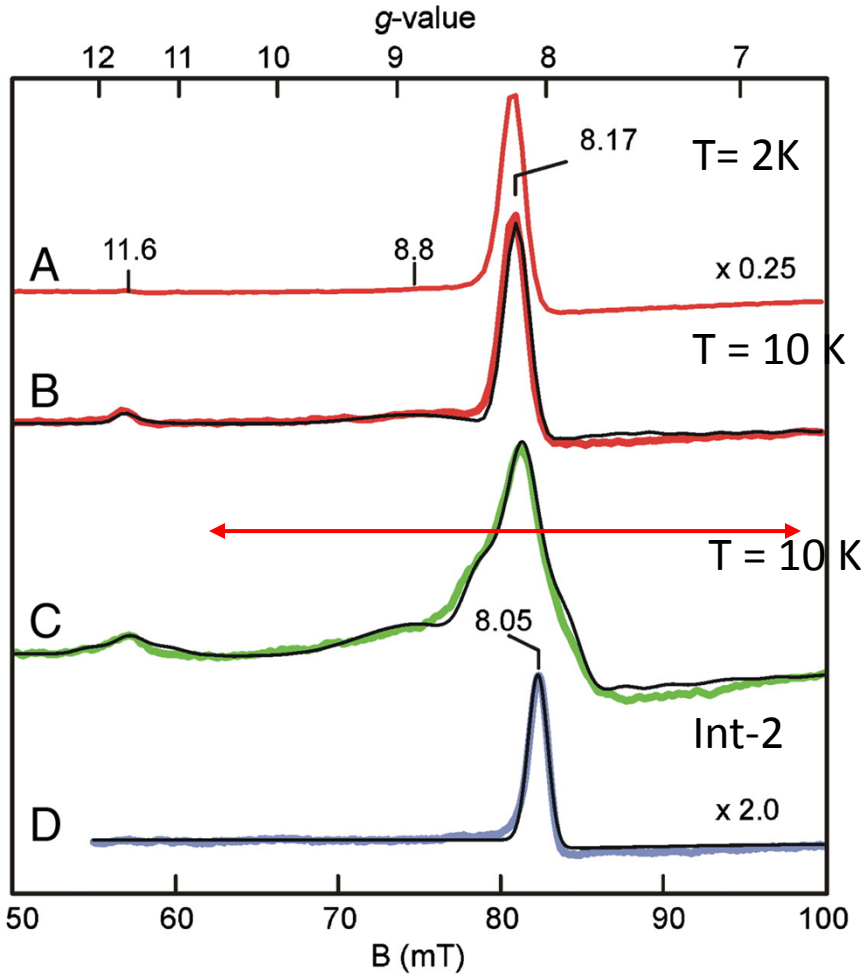
The g = 8.17 feature is characteristic of an S = 2 system



Comment: For a "doublet" with $M_s = \pm S$ one observes a resonance near $g = 2g_zS$ provided Δ_g is sufficiently small

MP Hendrich did the EPR

Parallel mode X-band EPR of Int-1



Nearly 400 gauss
^{17}O splitting (70% enriched)

$A_z(^{17}O)$ = 180 MHz

The **g = 8.17** feature is characteristic of an **S = 2** system

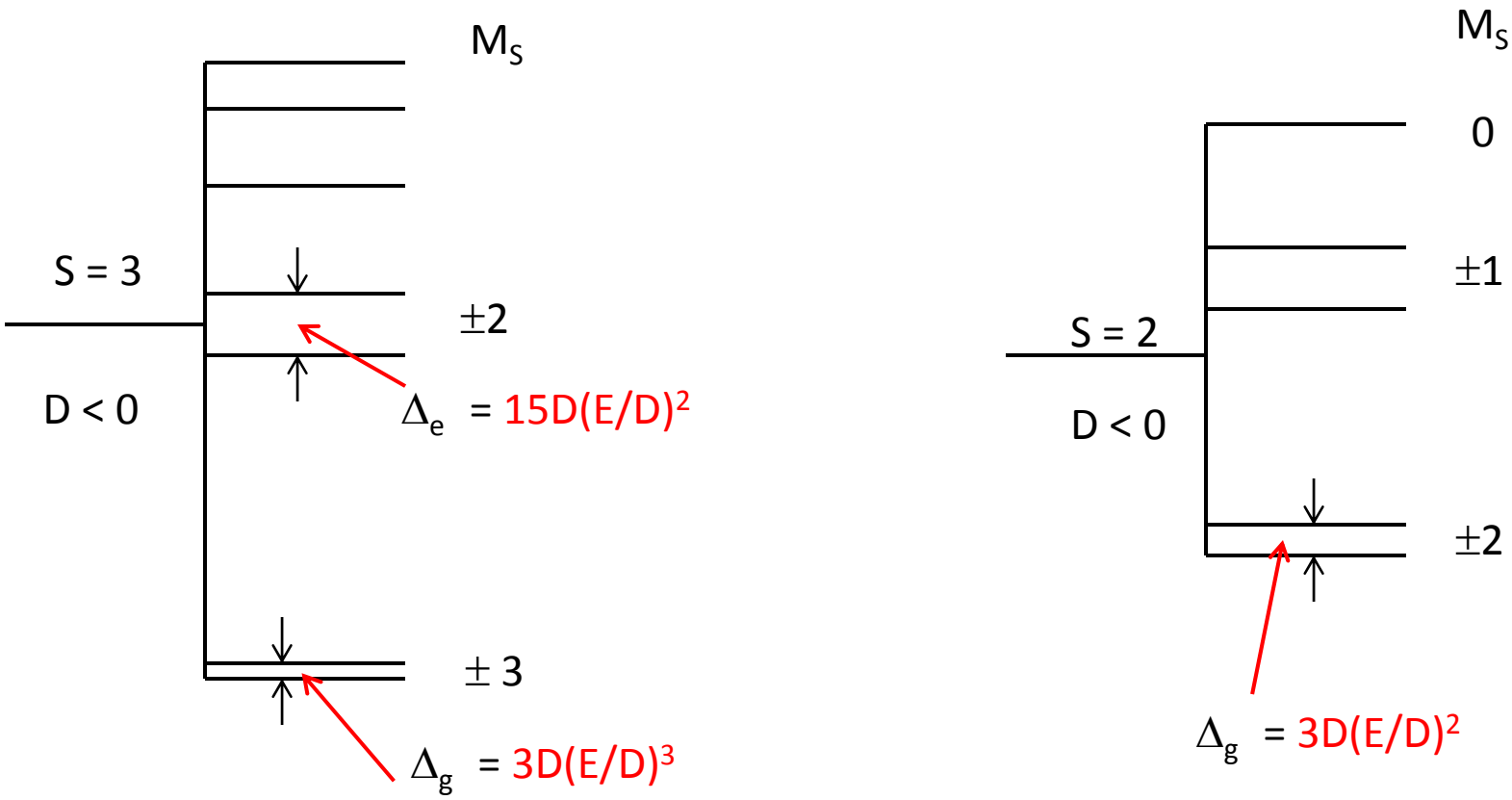
Back to Mossbauer Spectra

Zero-field Splitting Hamiltonian

$$\mathcal{H} = D \{S_z^2 - S(S+1)/3 + (E/D)(S_x^2 - S_y^2)\}$$

We get D and E/D from EPR as well as Mossbauer Spectroscopy

Zero-field splitting parameters D and E



E/D can be restricted to $0 \leq E/D \leq 1/3$

For B = 0 the $|M_S>$ nomenclature is inappropriate. Better: e.g. $|\phi^{3a}>= (|+3> - |-3>)/\sqrt{2}$

Pay attention to the Δ's

If the Δ's are $>$ 0.005 cm^{-1} there will be a quadrupole doublet

for B = 0 !

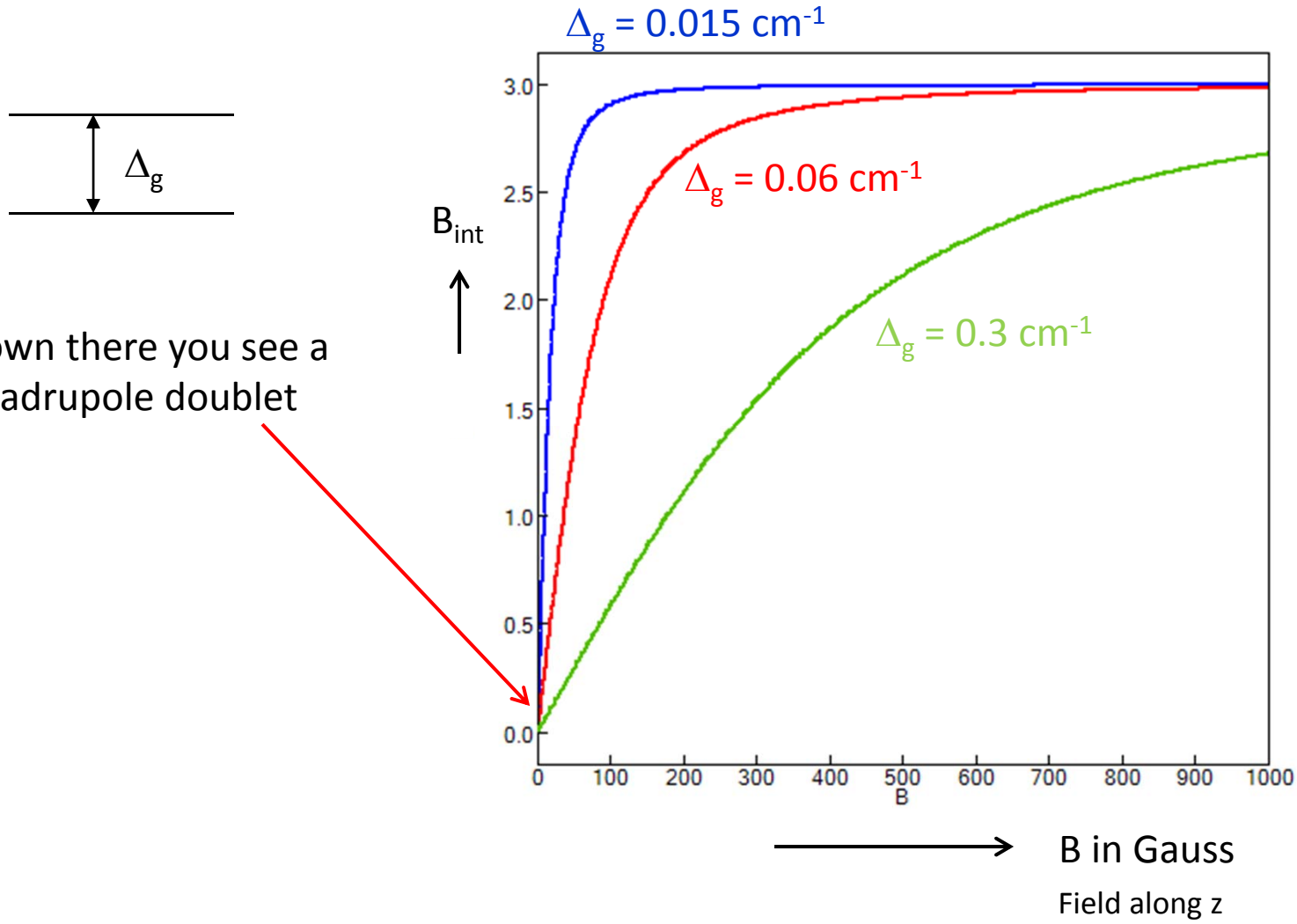
$$\phi = \{|S=2, +2\rangle + |S=2, -2\rangle\}/\sqrt{2}$$

M_S

$$\langle \phi | S_{x,y,z} | \phi \rangle = 0$$

Hence $B_{int} = 0$

B_{int} (z) versus B



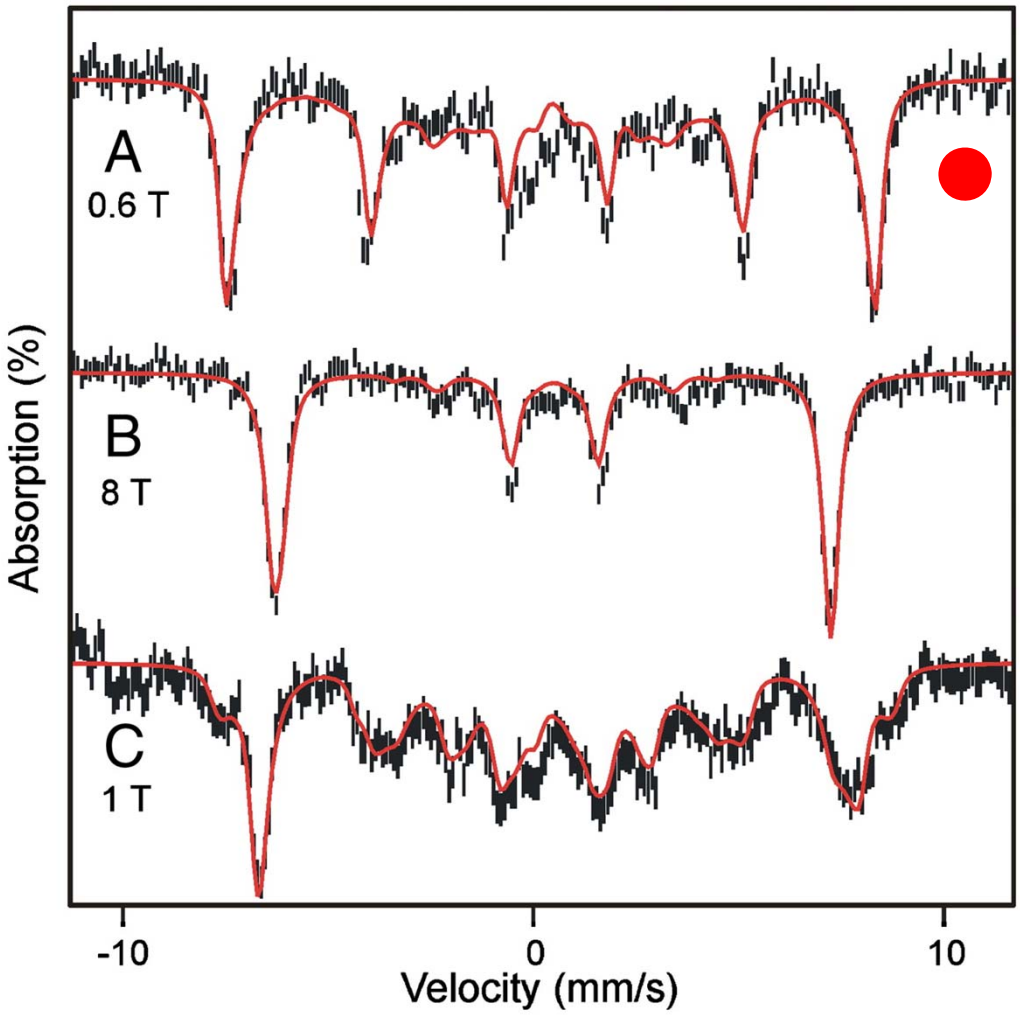
Down there you see a quadrupole doublet

All listed Δ_g are in range of X-band EPR, i.e. $h\nu > \Delta_g$

Mossbauer: The iron of Int-1 is high-spin (S_1=5/2) ferric

Full splitting for B = 0.6 T suggests two closely spaced (< 0.3 cm^{-1}) electronic levels.

Δ_g = 0.012 cm^{-1}



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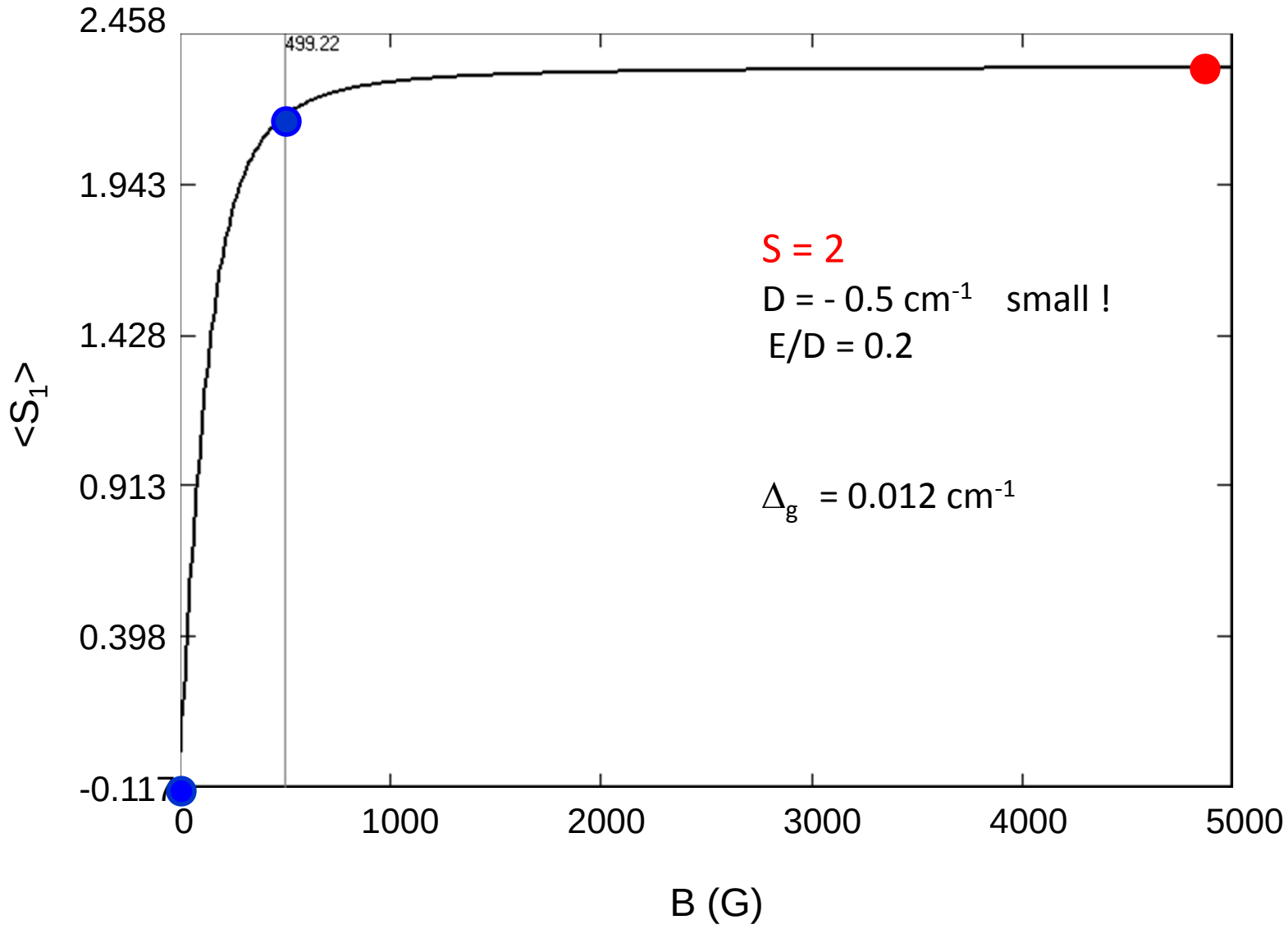
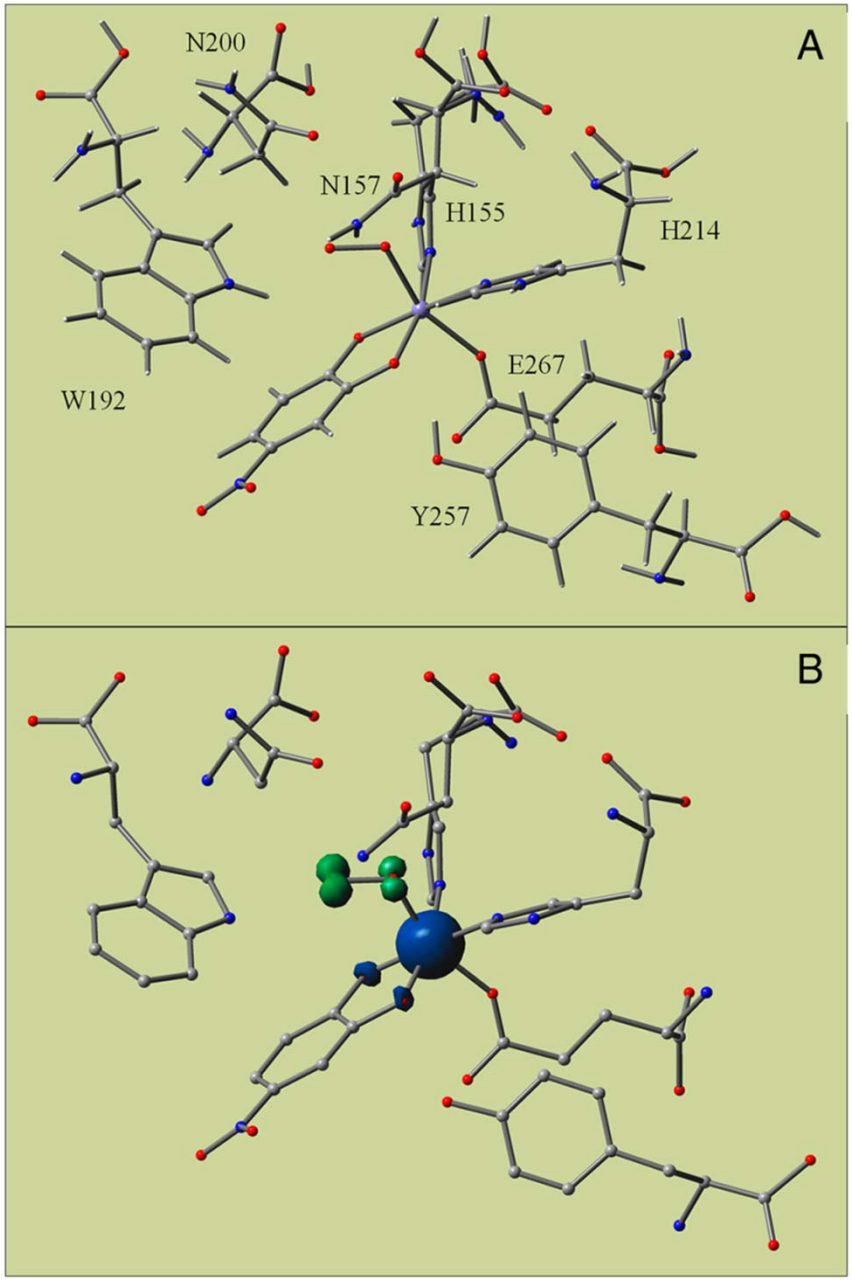

2.458
1.943
1.428
0.913
0.398
-0.117
499.22
<S1>
0
1000
2000
3000
4000
5000
B (G)
S = 2
D = - 0.5 cm-1 small !
E/D = 0.2
Δg = 0.012 cm-1



Blue: α spin density
Green: β spin density

Broken symmetry state of Int 1

These are the parameters you get

$J = 6\ cm^{-1}$

$D = -0.59\ cm^{-1}$

$E/D = 0.20$

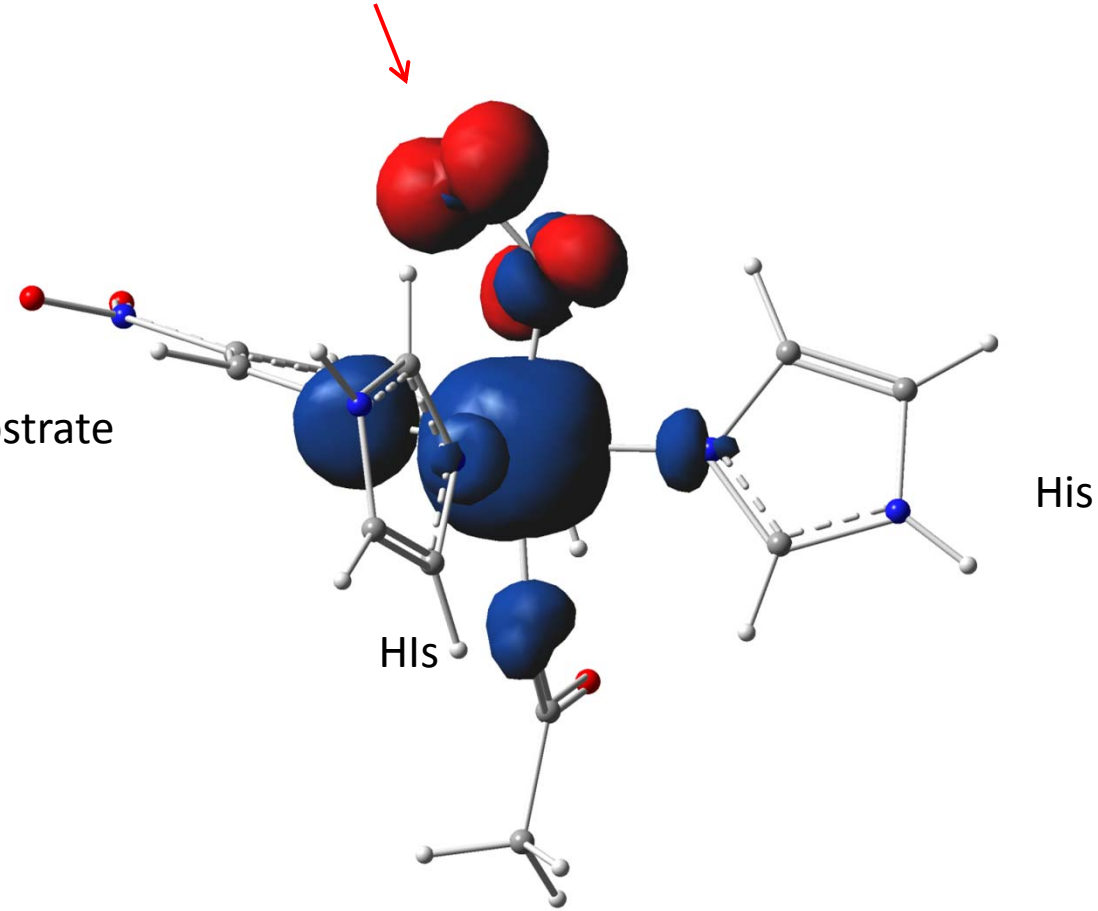
$A_{x,y,z}/g_n \beta_n = -(21.4, 21.4, -21.4)\ T$

$\Delta E_Q = 0.33\ mm/s \quad \eta = -3$

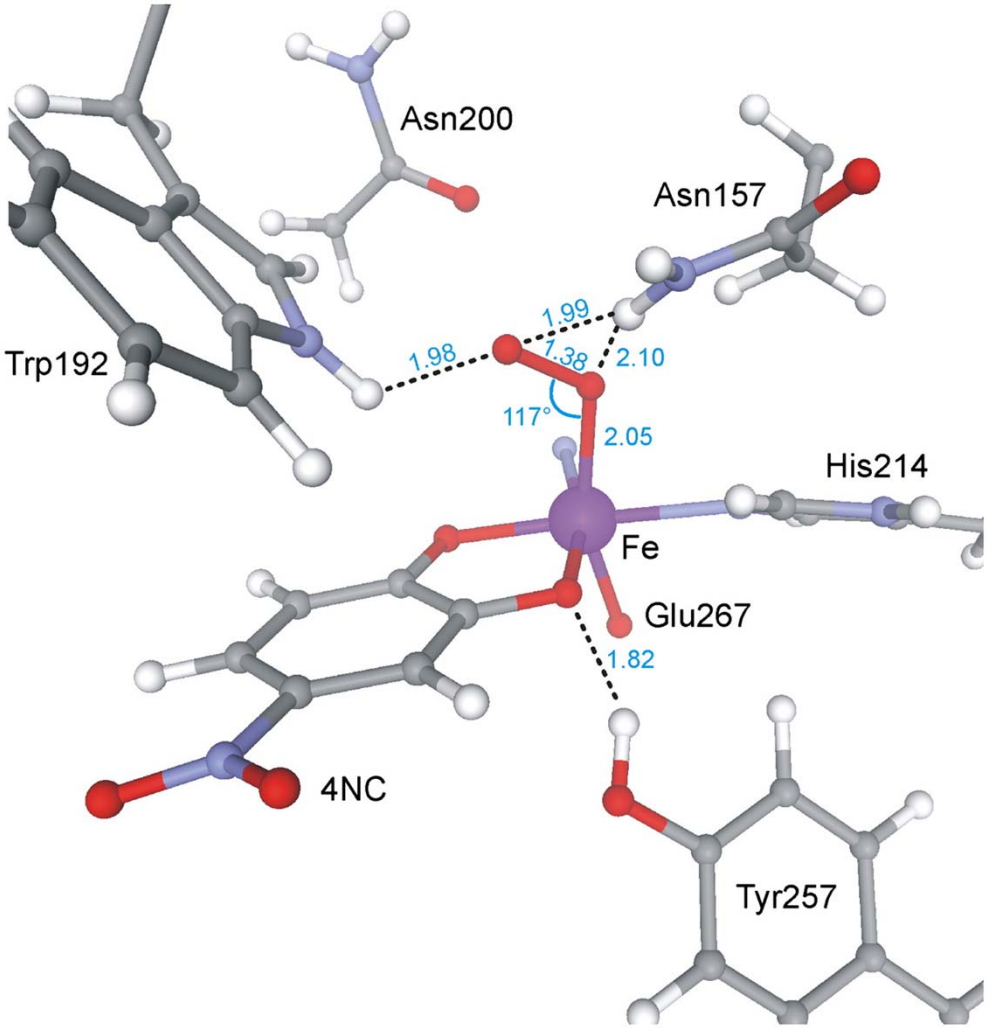
$\delta = 0.50\ mm/s$

$A_z\ (^{17}O) = 18\ MHz$

Superoxide radical is centered on distal O



DFT geometry optimization of Int-1.



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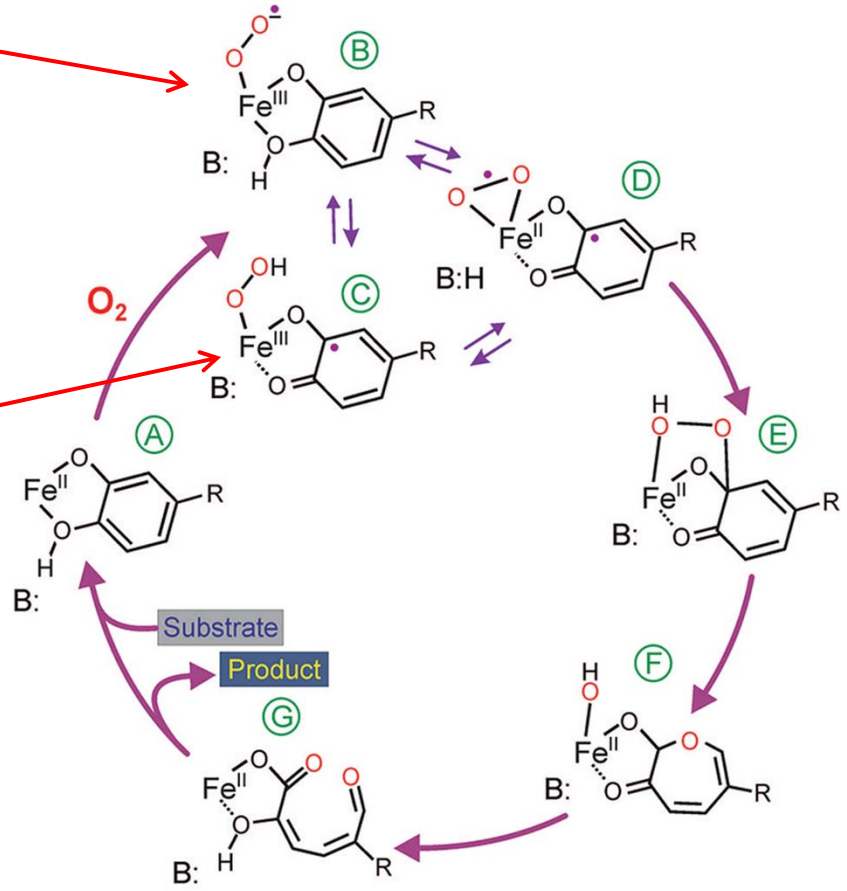
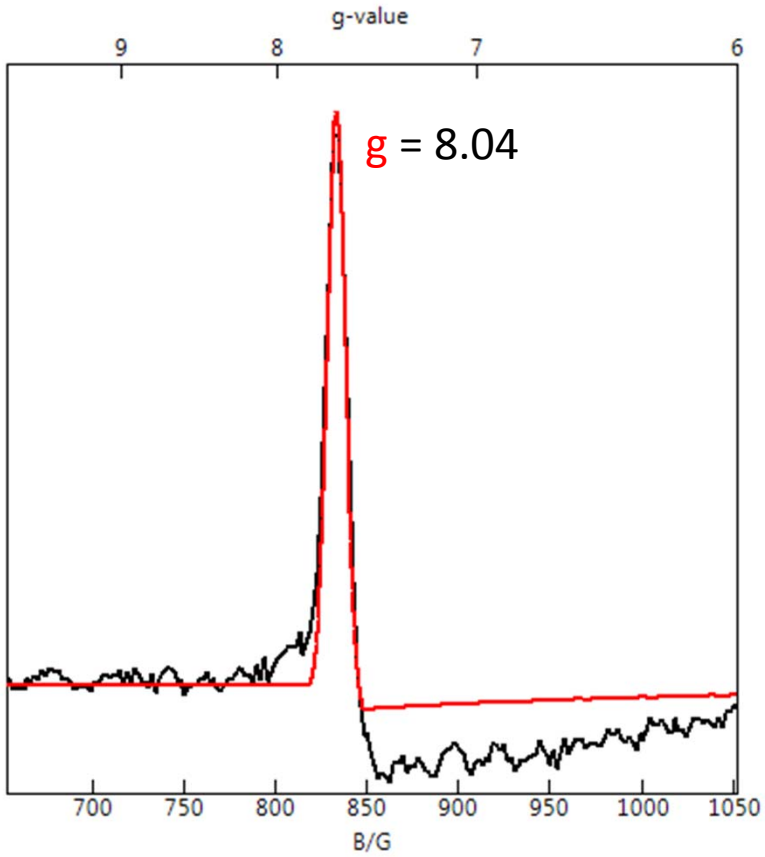
You were here

Let’s go there



Int-2 from another HPCD mutant

EPR of HPCD Int-2 (different mutant)



No ^{17}O-^{17}O broadening
i.e. radical is not O-O

Parallel mode EPR

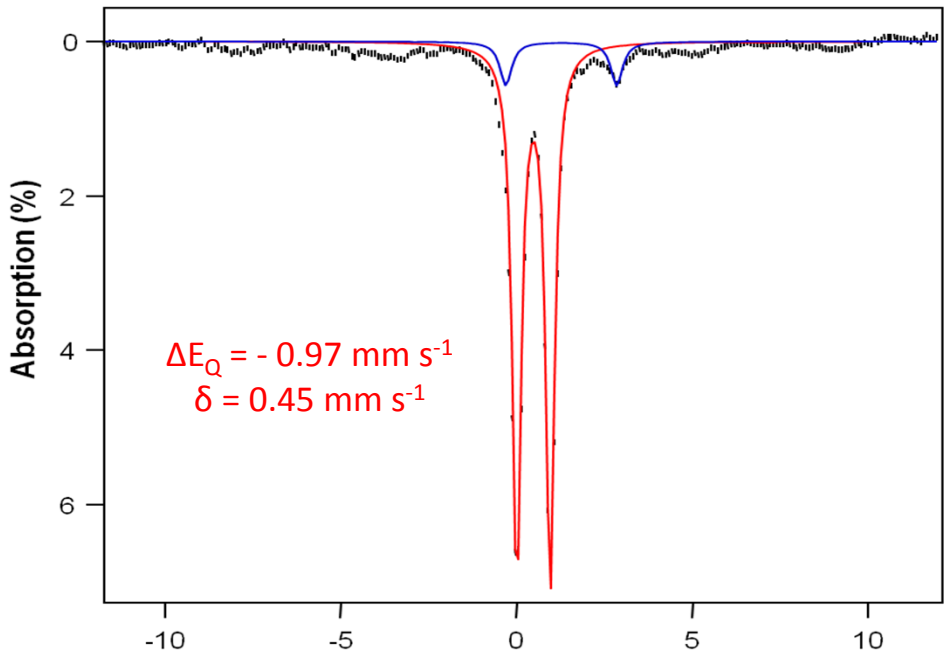
T = 10 K

This feature is 3 times sharper than that of Int-2 of H200N-nitrocatechol

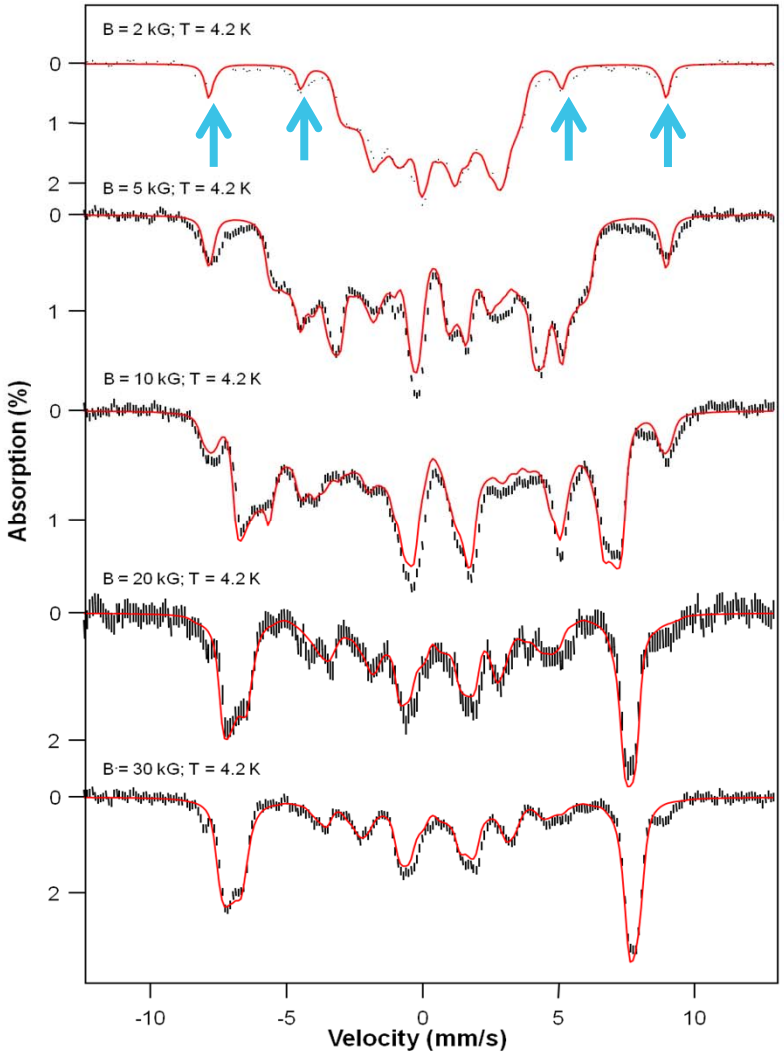
Katie Meier, Melanie Rogers, Emile Bominaar, John Lipscomb, EM, in progress

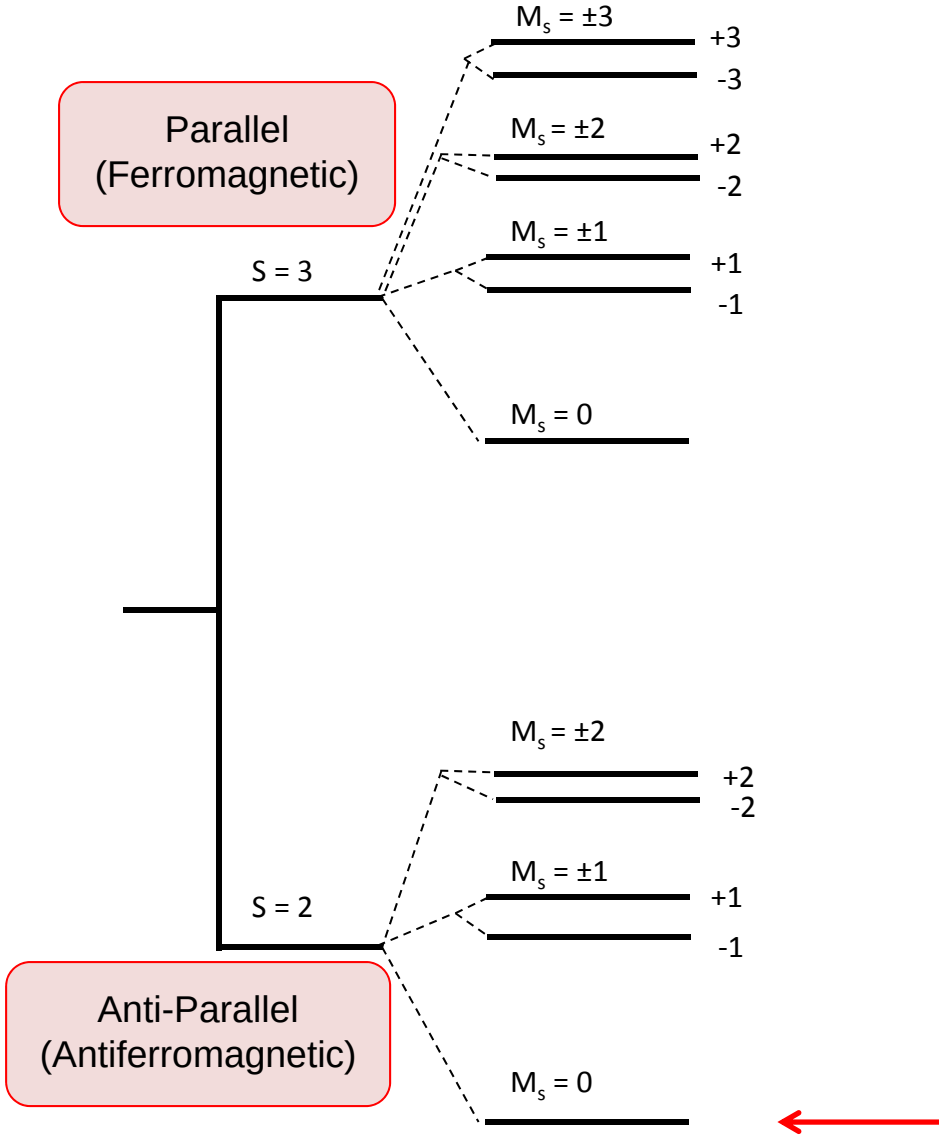
4.2 K Mossbauer spectrum of Int-2

Blue: high-spin Fe^{II}

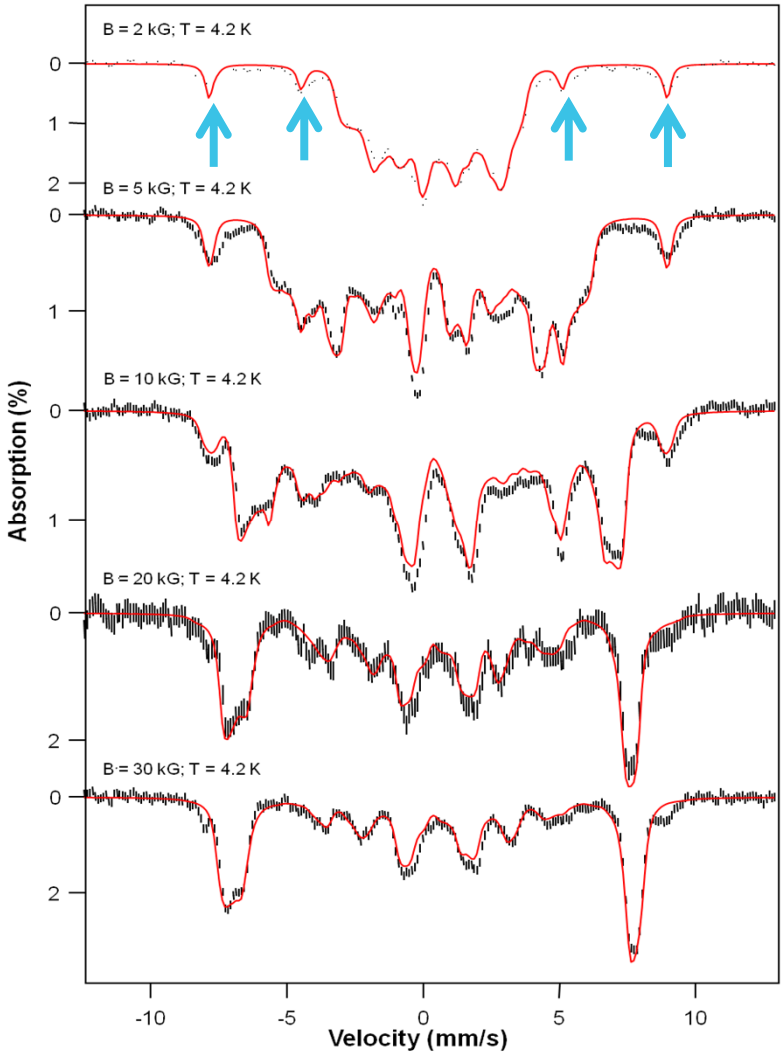


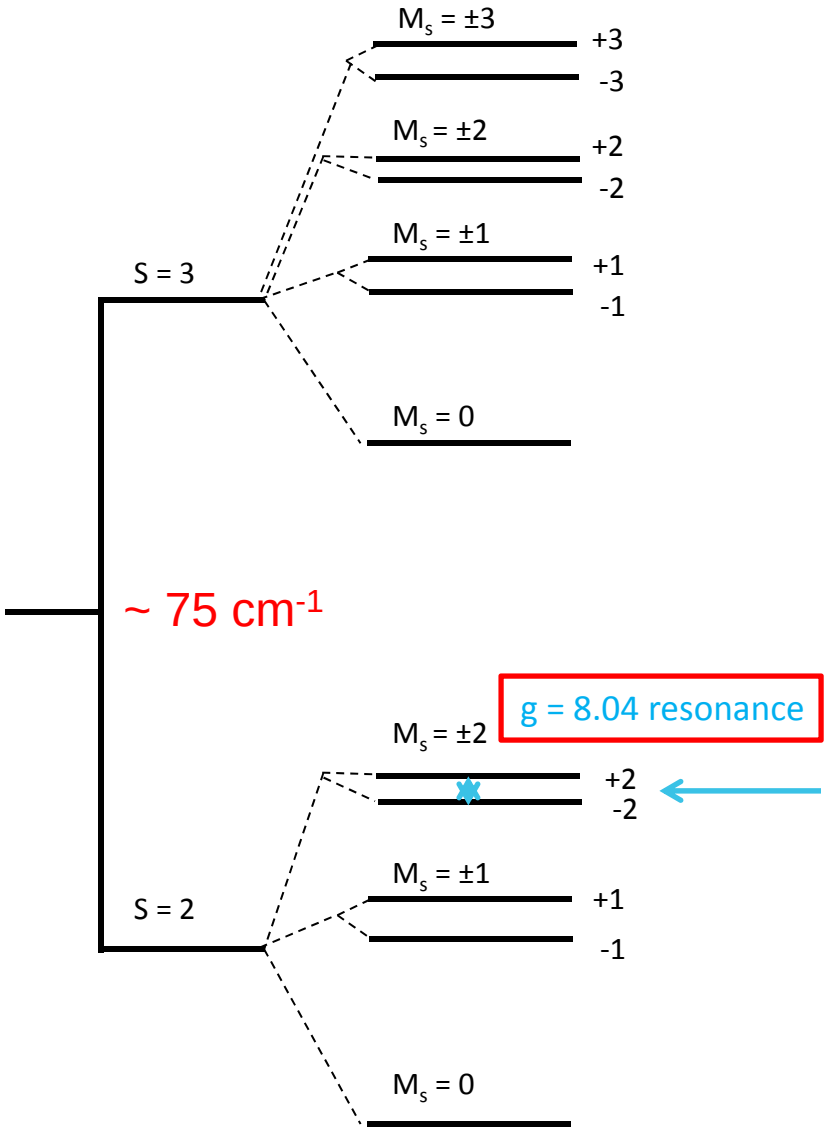
$Fe^{2+} + O_2$


B = 2 kG; T = 4.2 K
B = 5 kG; T = 4.2 K
B = 10 kG; T = 4.2 K
B = 20 kG; T = 4.2 K
B = 30 kG; T = 4.2 K
Absorption (%)
Velocity (mm/s)
-10
-5
0
5
10


Parallel
(Ferromagnetic)
S = 3
$M_s = \pm 3$
+3
-3
$M_s = \pm 2$
+2
-2
$M_s = \pm 1$
+1
-1
$M_s = 0$
Anti-Parallel
(Antiferromagnetic)
S = 2
$M_s = \pm 2$
+2
-2
$M_s = \pm 1$
+1
-1
$M_s = 0$

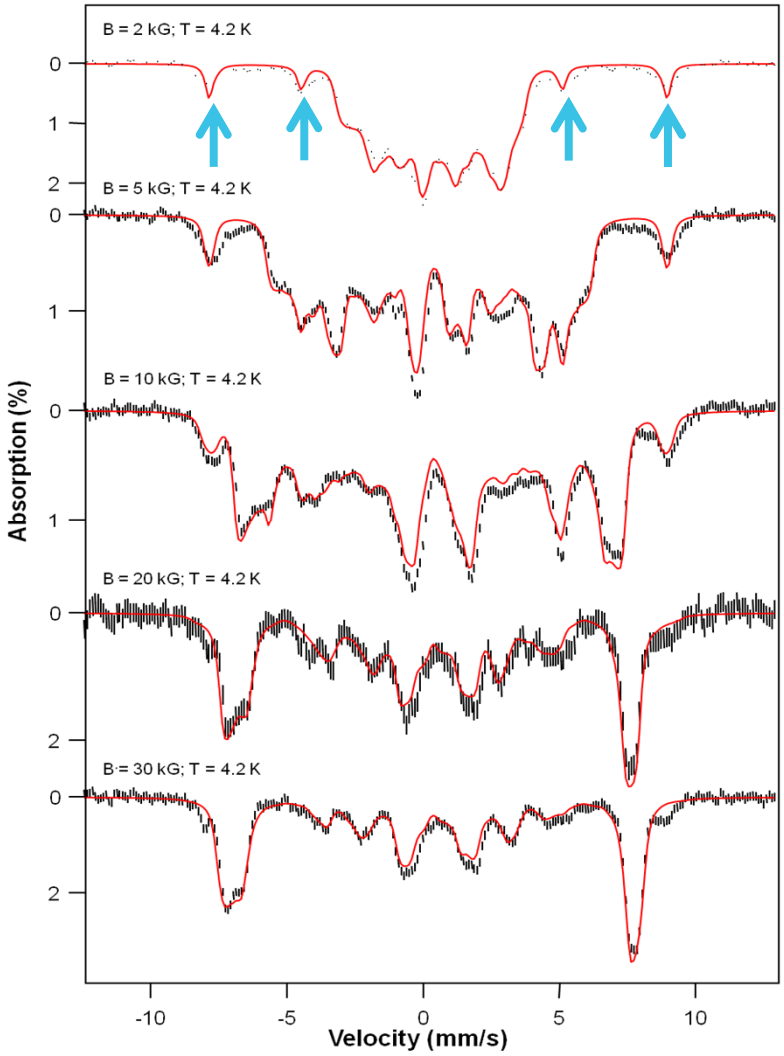
$Fe^{2+} + O_2$

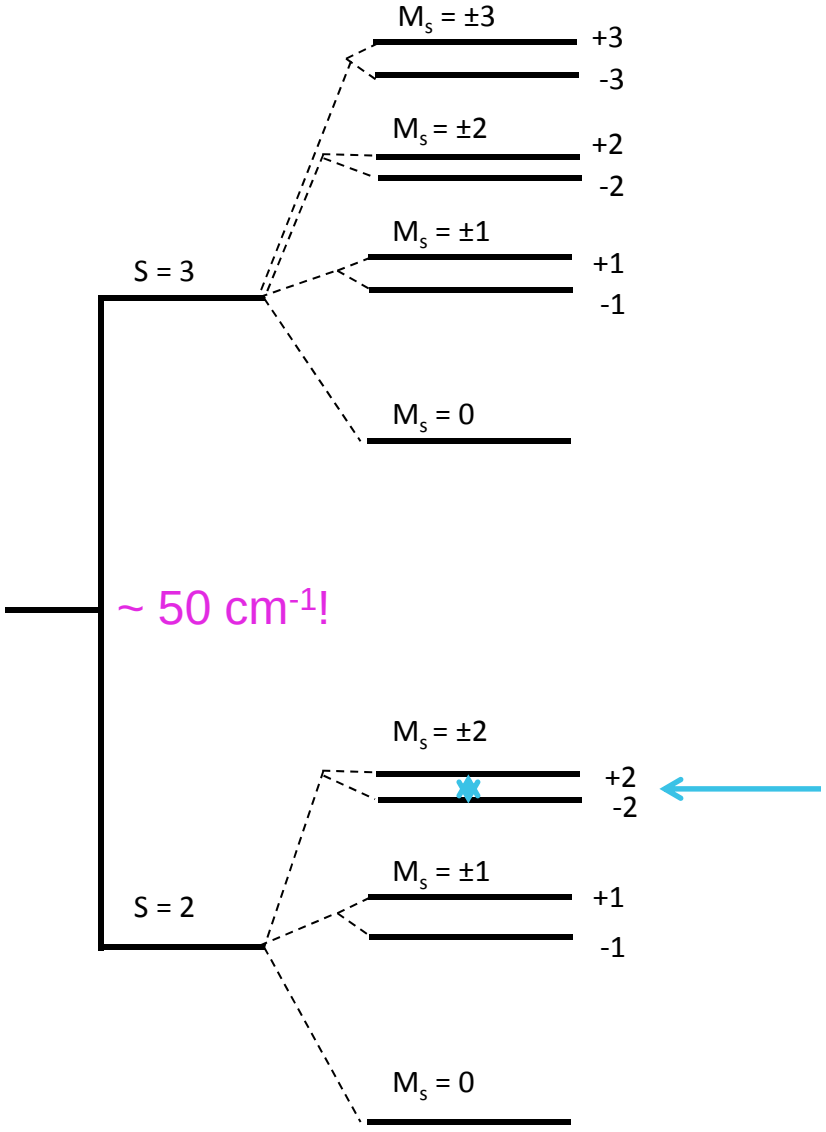




Check J

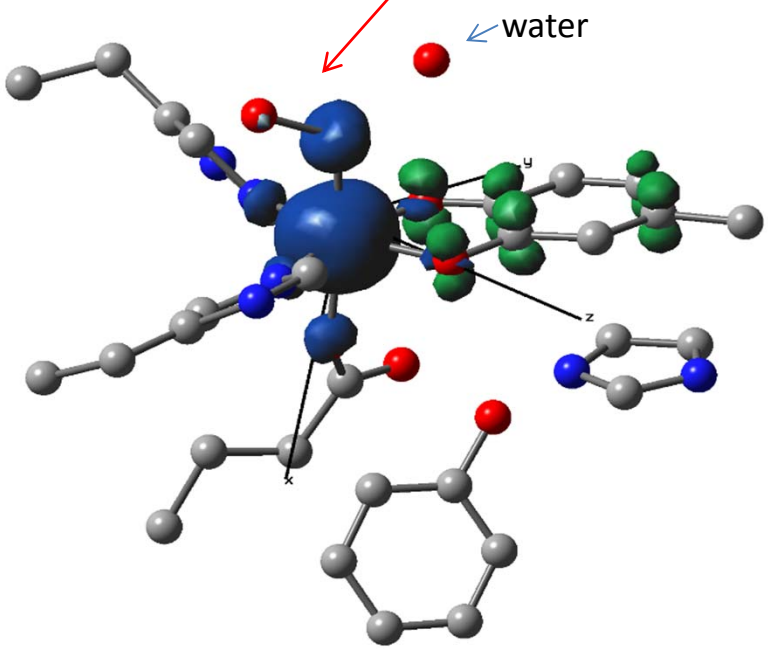
Fe^{2+}-Substrate + O_2





Preliminary DFT of Int-2

Hydroperoxo (should point towards substrate)



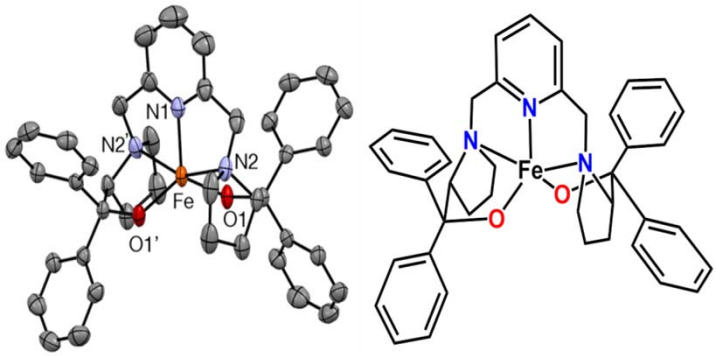
α density blue

β density green

This is work in progress

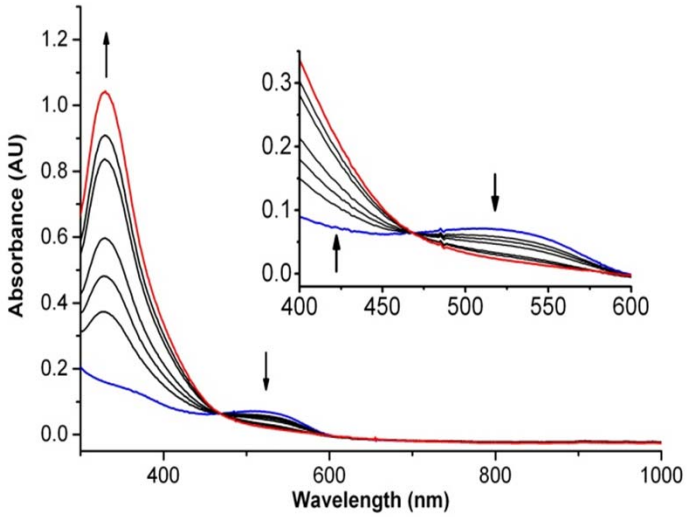
Spin density contour

A non-Heme Iron-Superoxo Complex



Fe^{II}(BDPP)

Add O_2 at -80 C to Fe^{II}(BDPP)



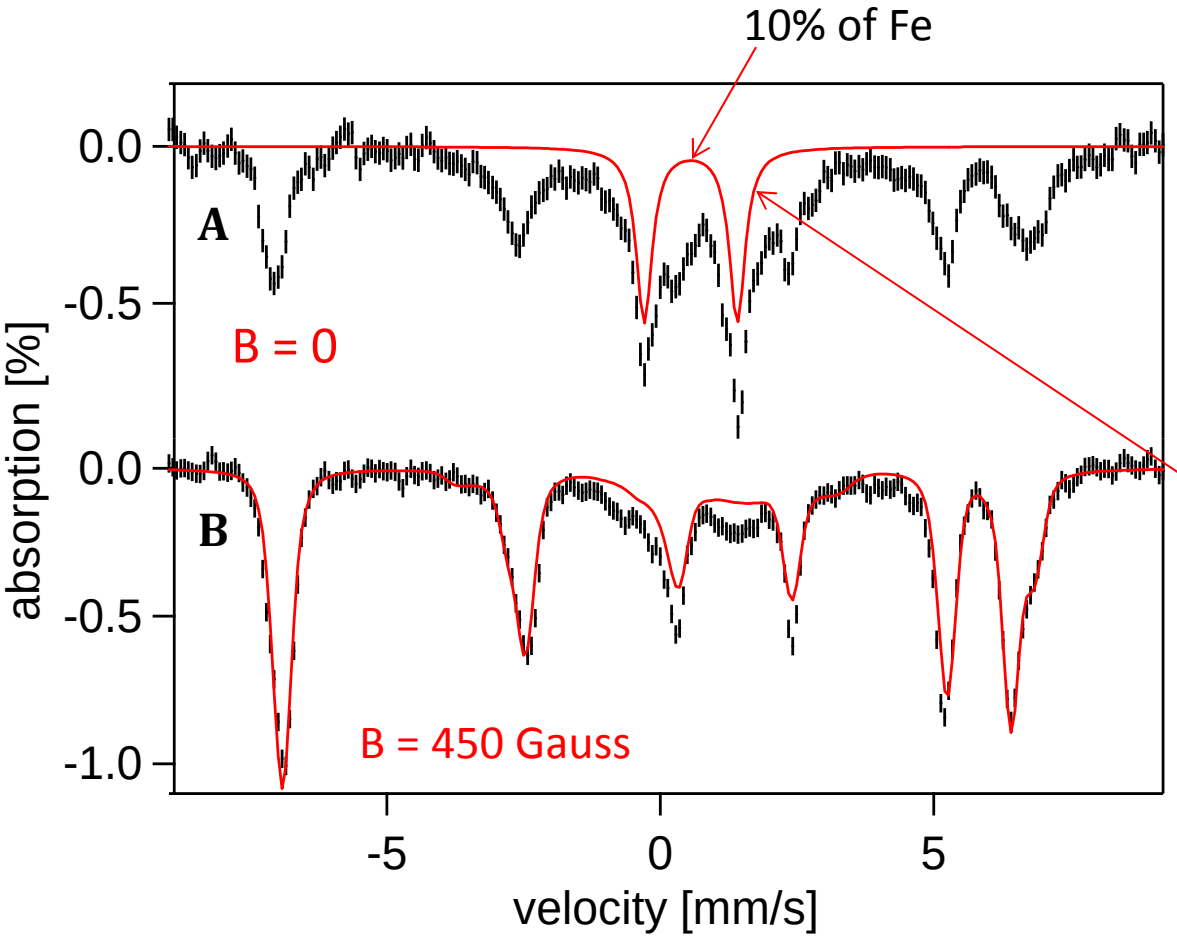
rRaman ^{16}O-^{16}O stretch at 1125 cm^{-1} and ^{18}O-^{18}O at 1062 cm^{-1} after 413 nm excitation: typical of superoxo species

Chiang, Scott Kleespies, Heather Stout, Meier, Li, Bominaar, Que, Munck, Lee, submitted

An iron(III) –superoxo complex has integer spin, so

we expect to observe a quadrupole doublet for B = 0

4.2 K Mossbauer spectra of Fe(BDPP) after adding O_2

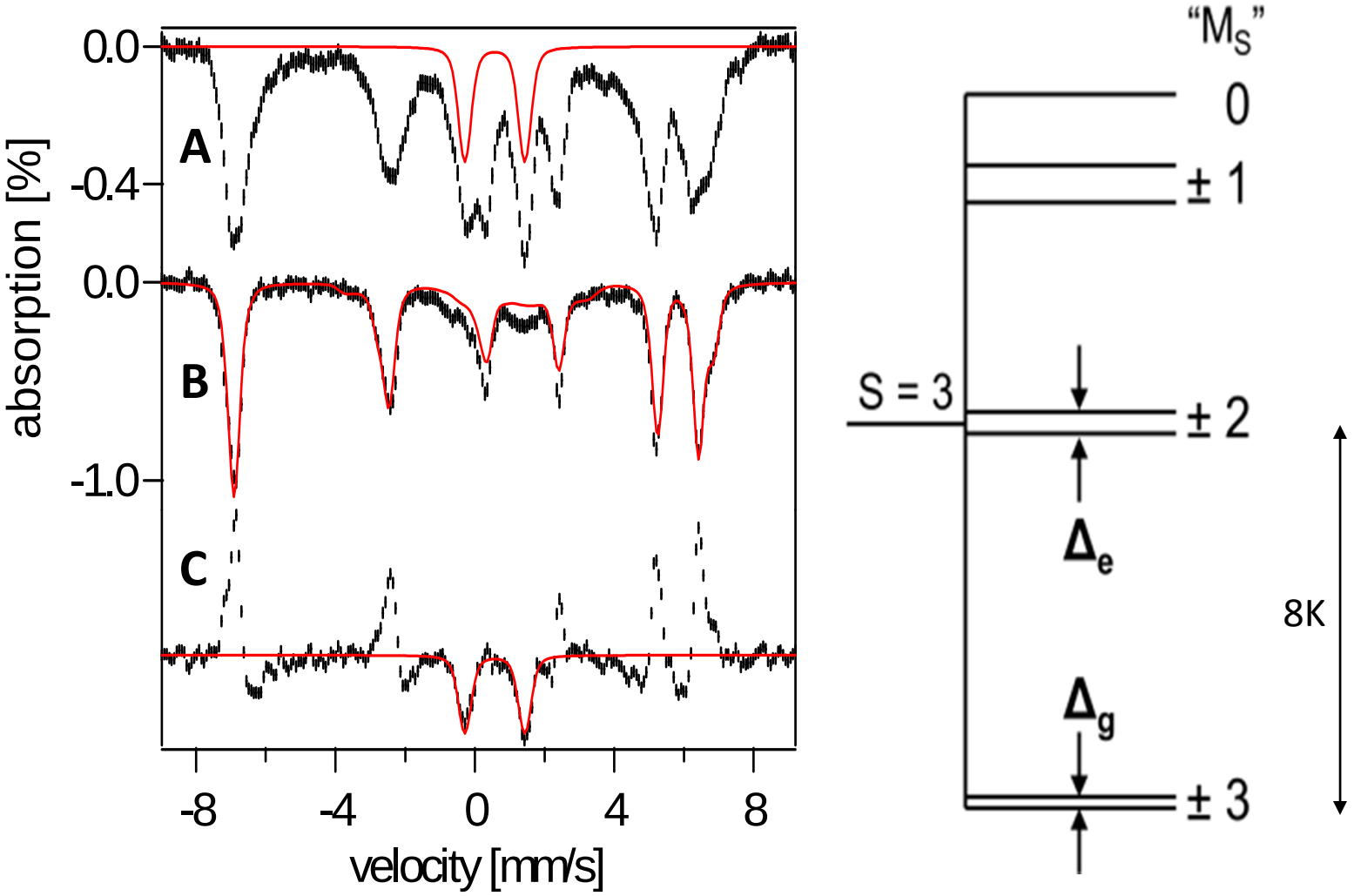


ΔE_Q = 1.7 mm/s

δ = 0.58 mm/s

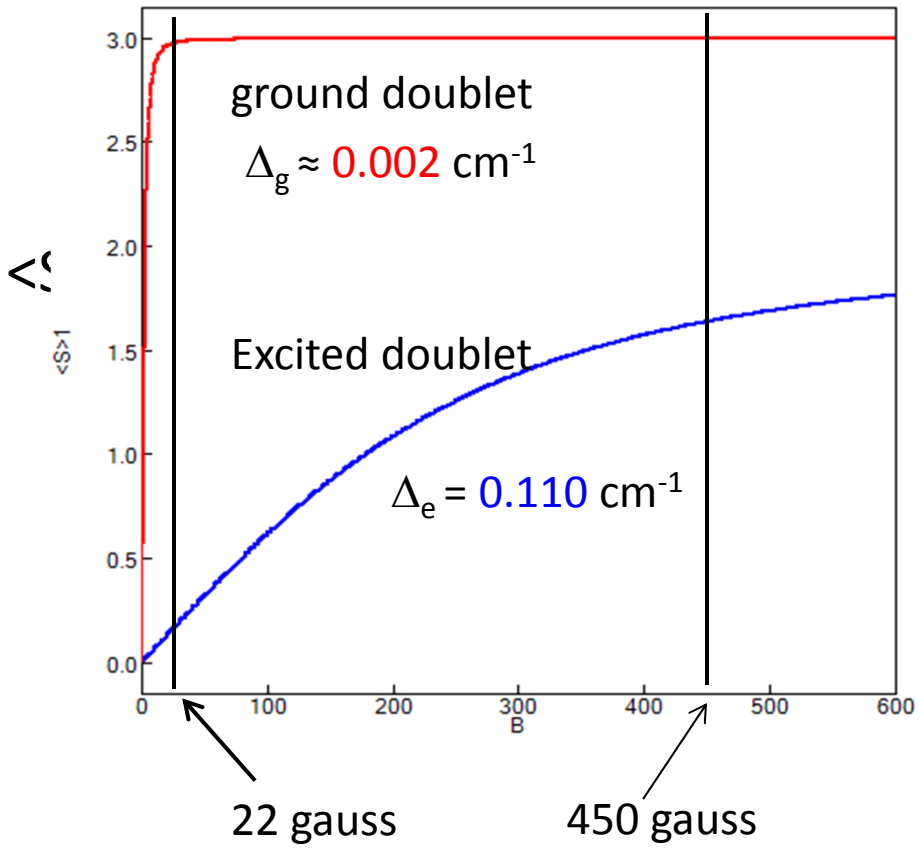
high-spin Fe^{III}

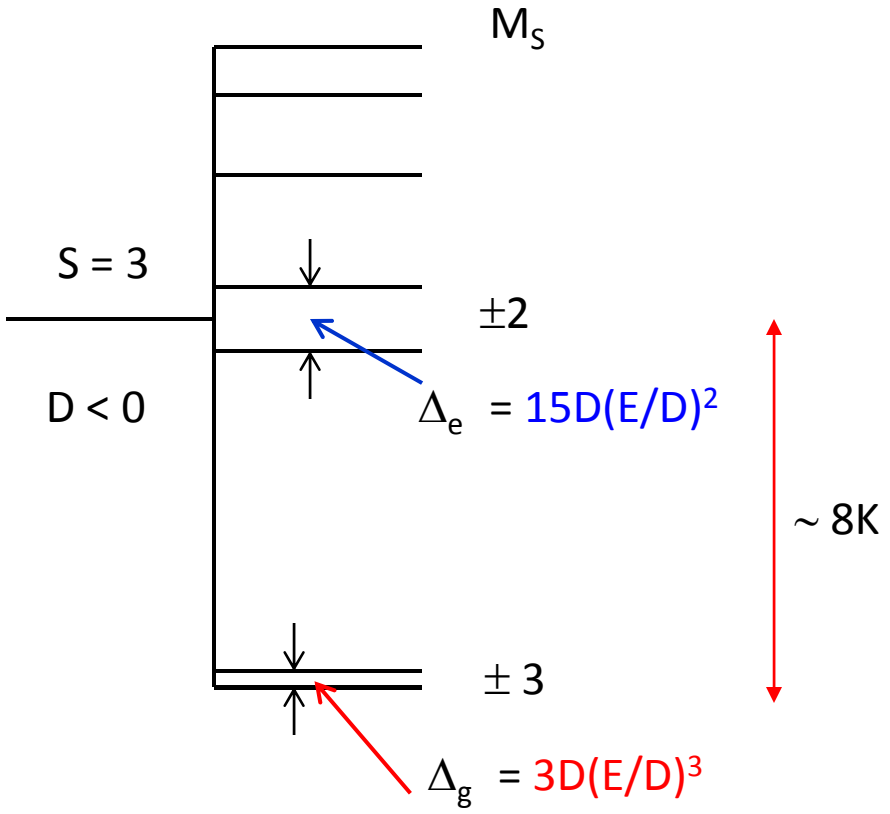
Scott did that to us



The splitting pattern in (B) yields exactly the same ΔE_Q and δ as the red doublet in (A)

$B_{int}(z) = -\langle S_z \rangle A_z / g_n \beta_n$

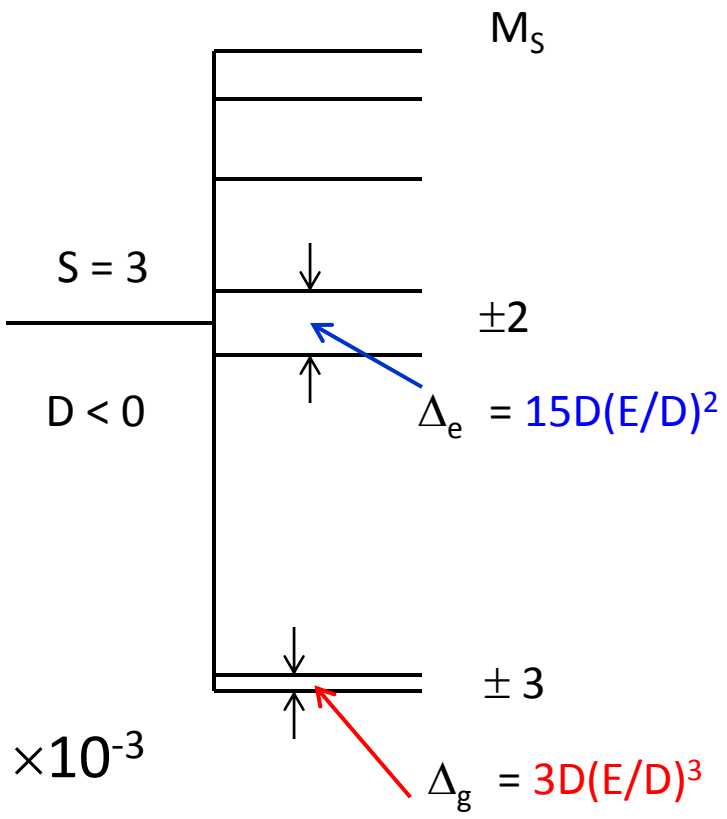




At 22 Gauss: Why does the Δ_e electronic doublet produce a quadrupole doublet and the Δ_g doublet a magnetically split spectrum ?

How about EPR?

EPR Intensity $\sim \Delta^2$



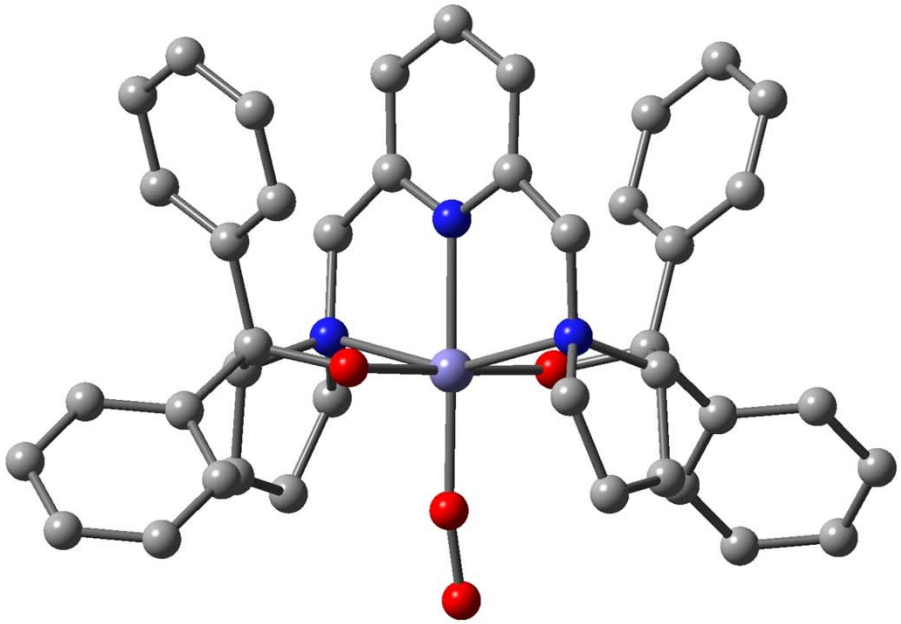
Fe^{III} HPCD --superoxo $(E/D)^4 = (0.2)^4 = 1.6 \times 10^{-3}$

Fe^{III} (BDPP)-superoxo $(E/D)^6 = (0.08)^6 \approx 2.5 \times 10^{-7}$ fat chance

Quadrupole doublet

$\|M_s = +3\rangle$	$\|M_s = -3\rangle$

$\phi^{3a} \approx \{|M_s = +3\rangle - |M_s = -3\rangle\}/\sqrt{2}$ dragon wins



A DFT picture of Fe(III)(BDPP)-superoxo

Dear Scott,

You are not as bad as we initially thought. Sample is about

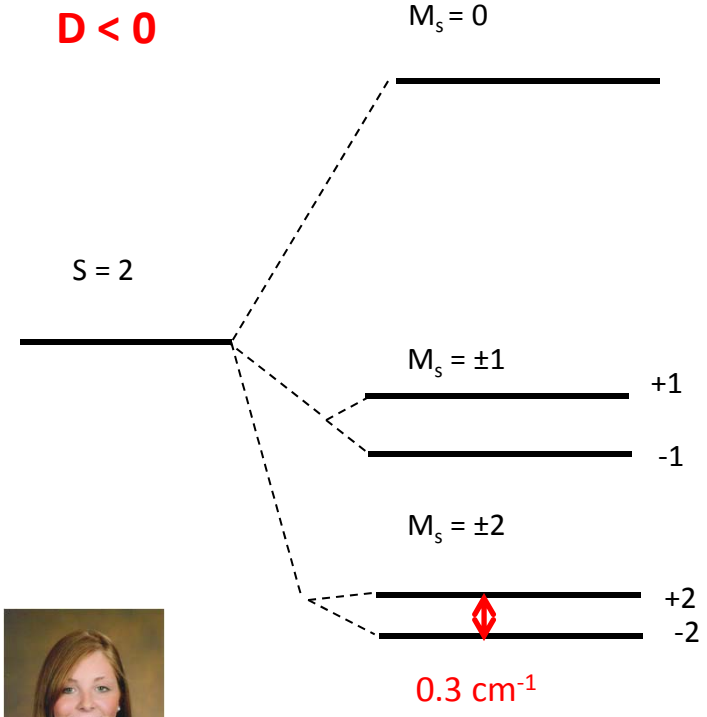
85% Fe(III) superoxo, or better. Keep enjoying the Minnesota mosquitos.

Best

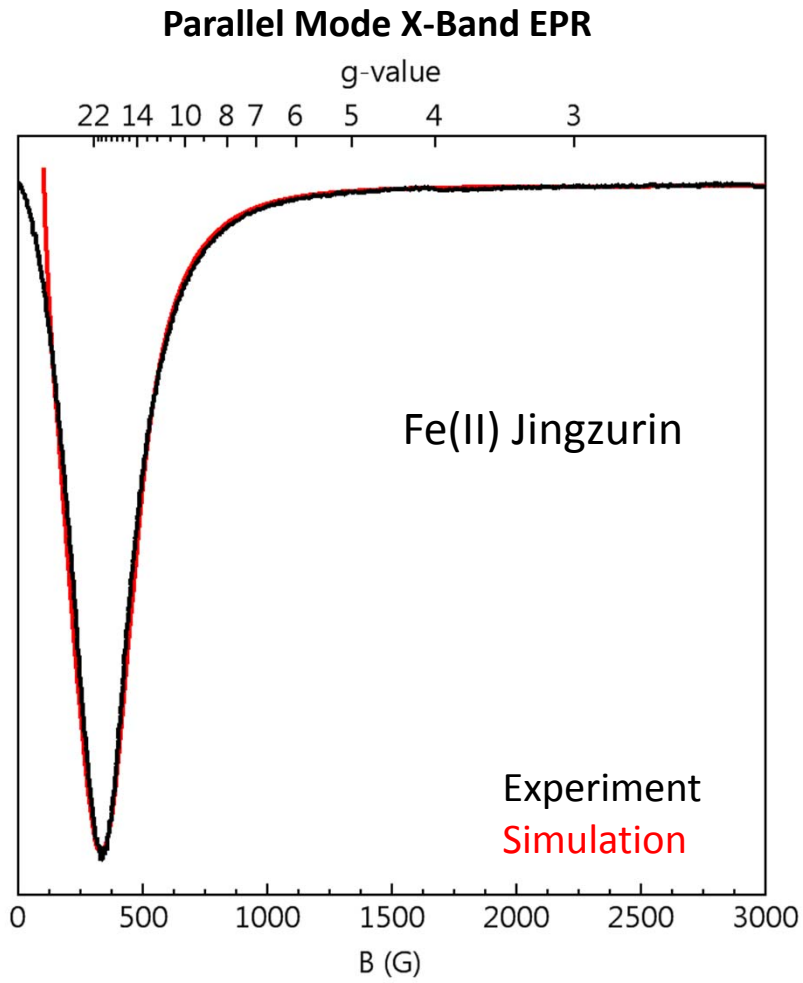
Heather, Katie and Eckard

PS: Please send a sample in a glassing solvent such as MeTHF

Splitting Diagram for S=2 (HS Fe^{2+})



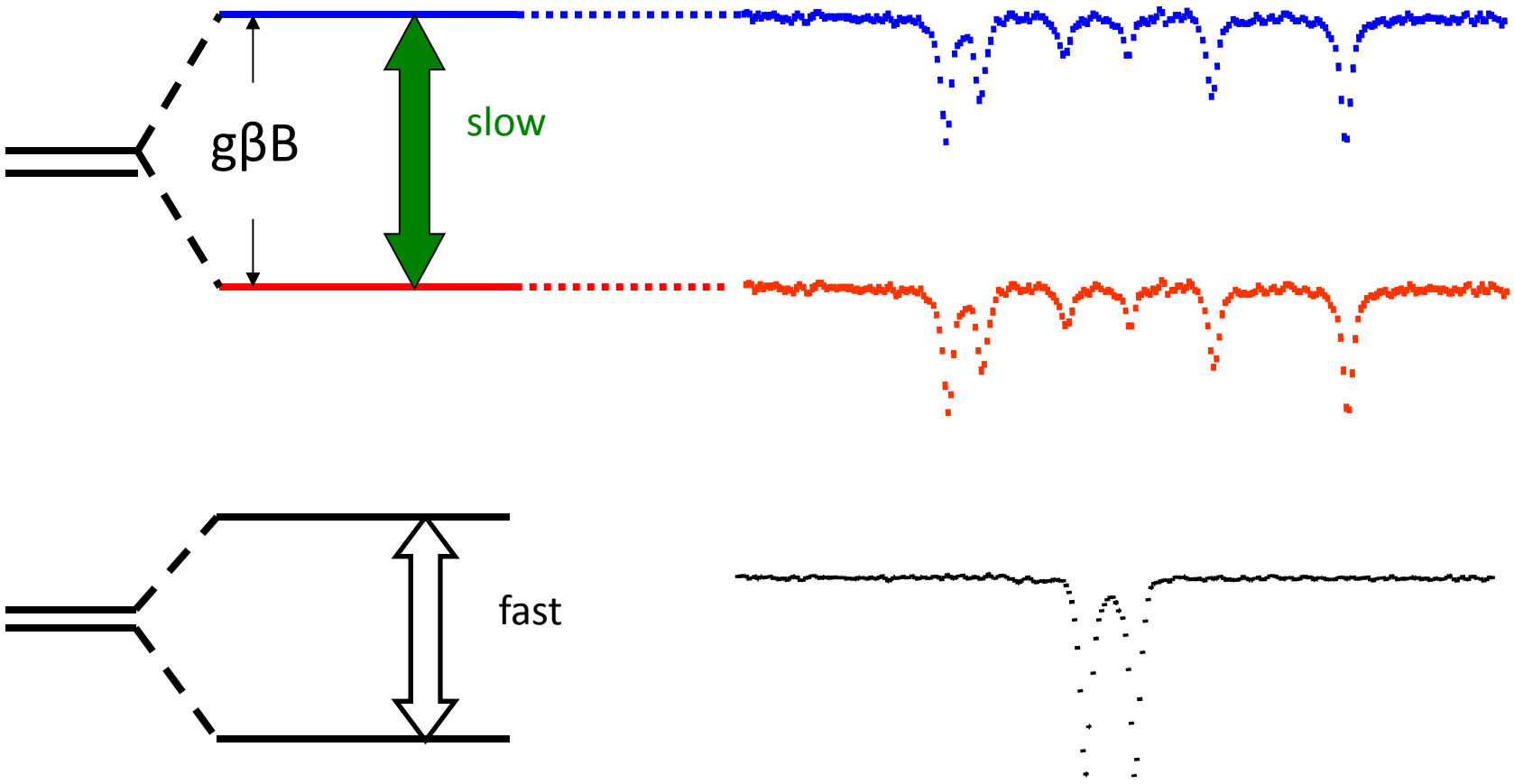
Sample from Jing Liu



Fe(II) M121E-Azurin

Discovery of 3Fe-4S clusters

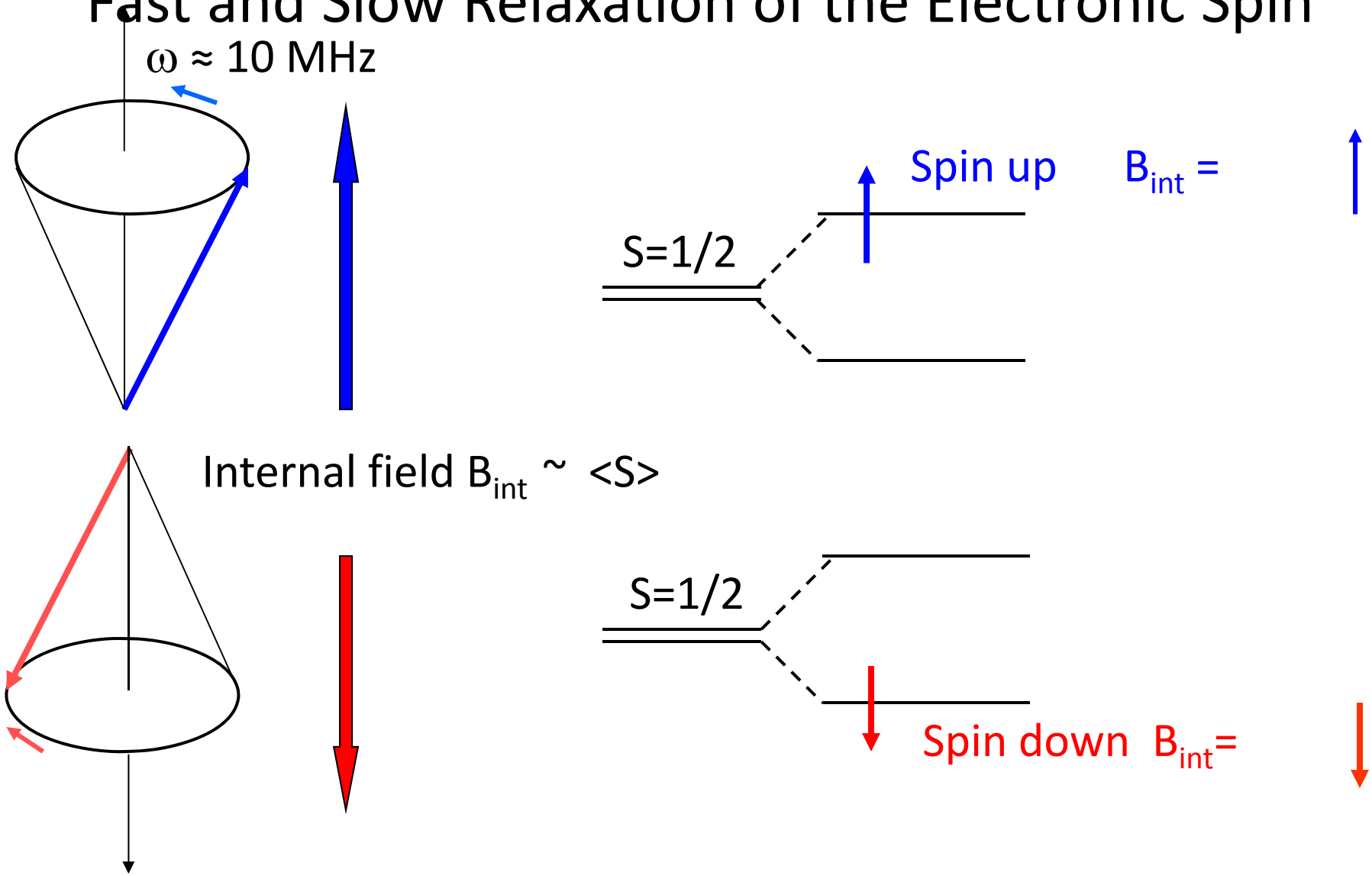
Slow and Fast Spin Relaxation



Assumption: βB/kT << 1 = 0.01 at 4.2 K and 500 gauss

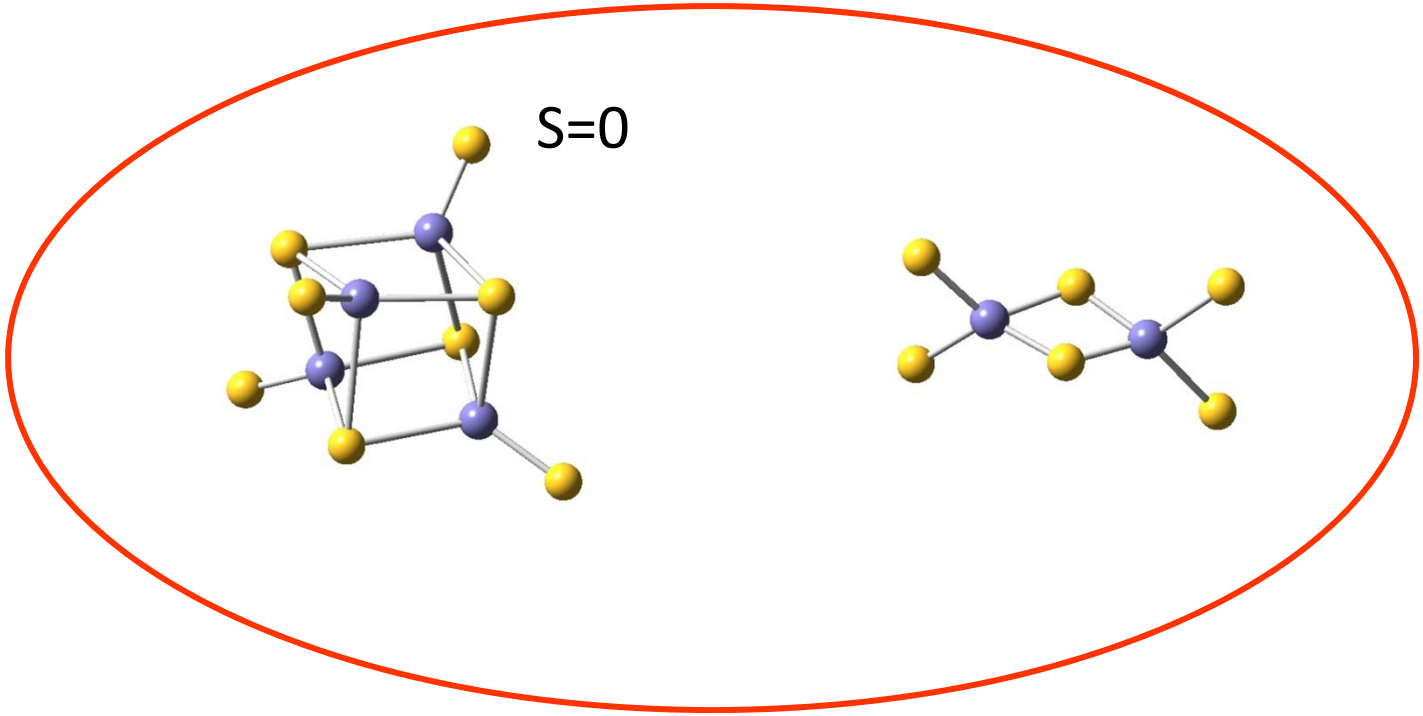
Tutorial

Fast and Slow Relaxation of the Electronic Spin



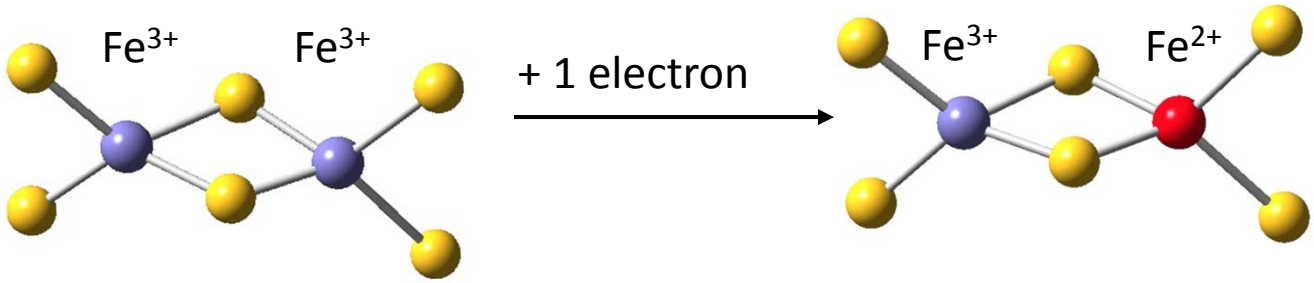
Tutorial

Azotobacter vinelandii Ferredoxin



X-ray Structure: *Nature* 1979

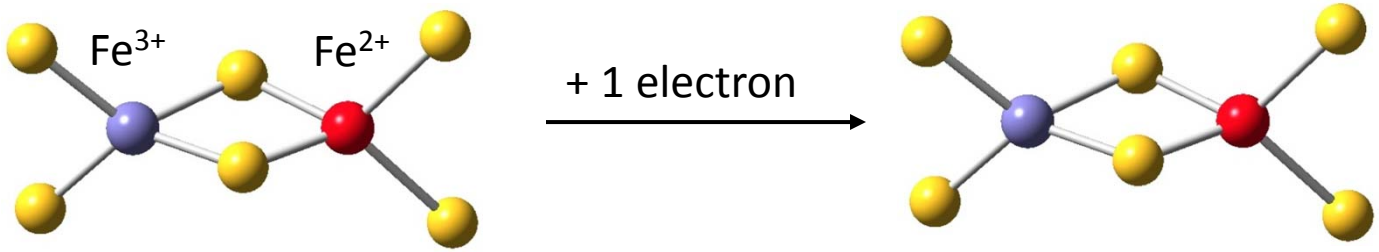
Typical [2Fe-2S] cluster



S = 0

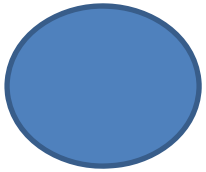
S = ½ g =1.94

Azotobacter cluster

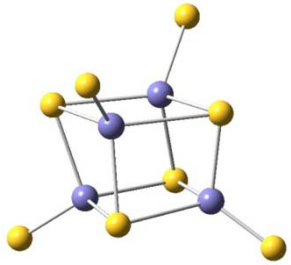


S = ½ g =2.01

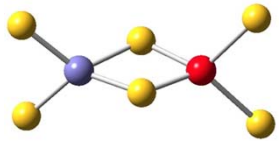
Integer Spin



Oxidized

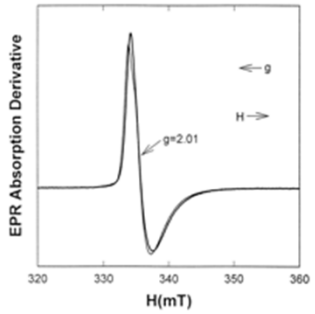


S=0

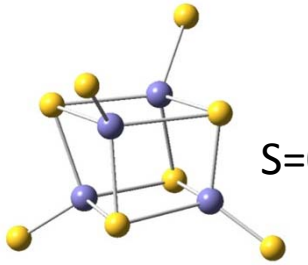


S=1/2

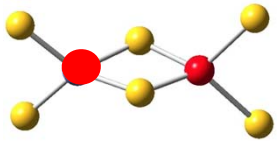
g=2.01



Reduced

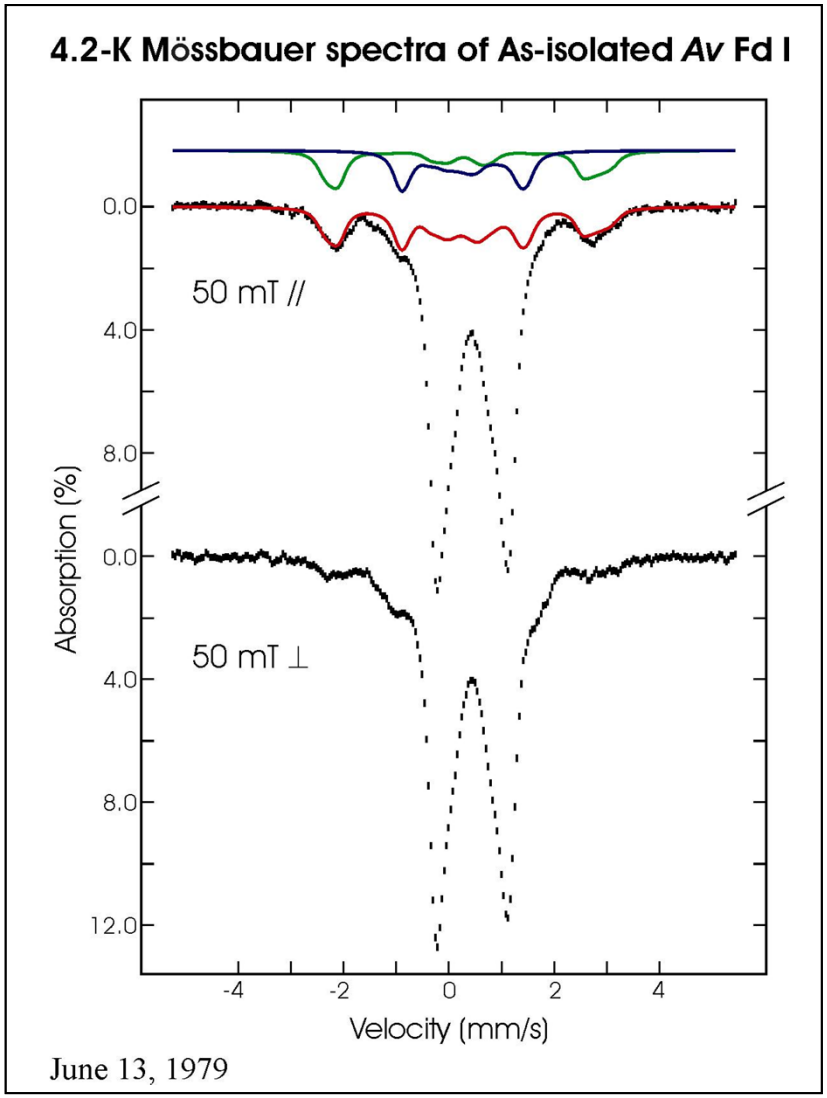


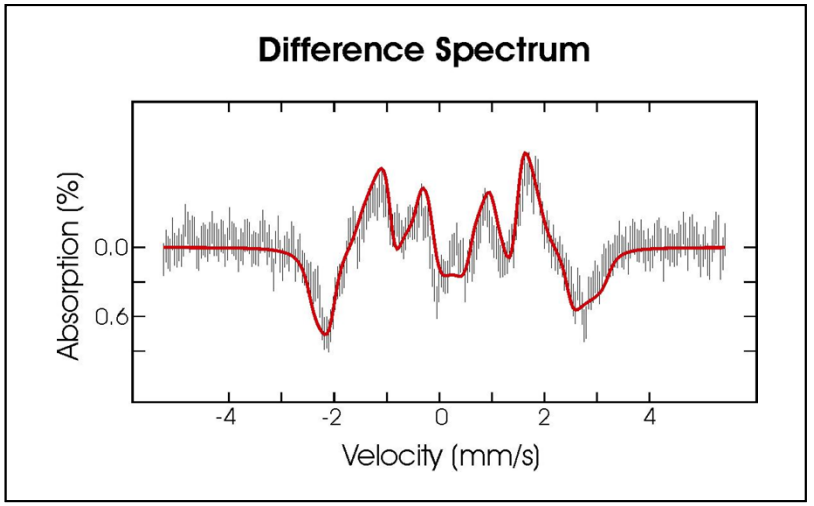
S=0



No EPR (yet)

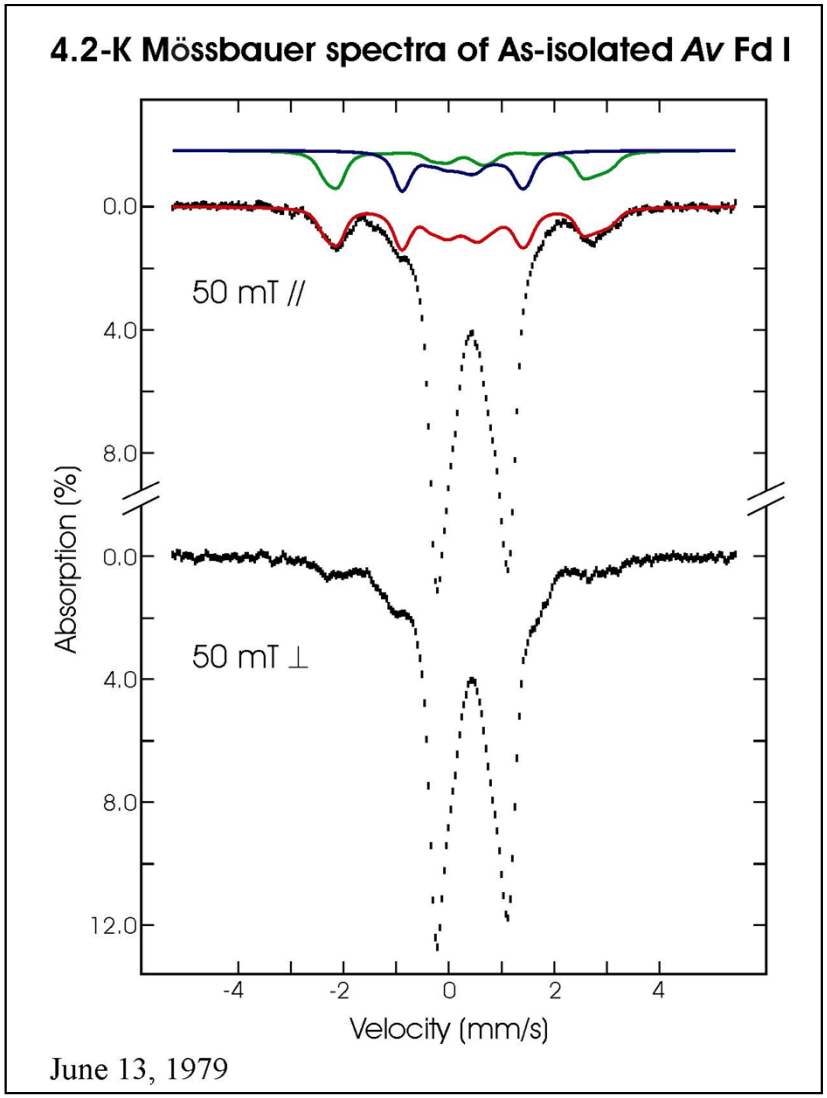
Mossbauer spectra of oxidized state

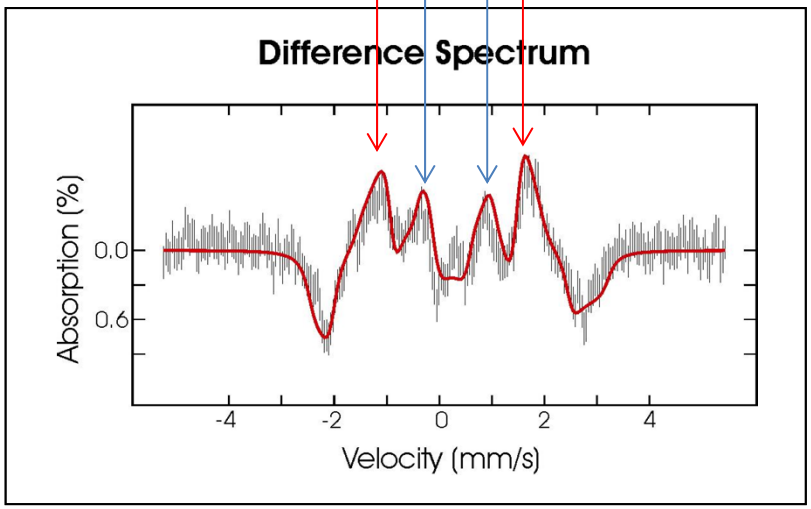




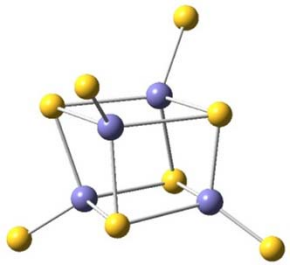
Mossbauer spectra of oxidized state

Obviously a 2Fe center

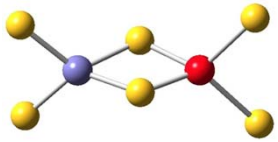




Oxidized



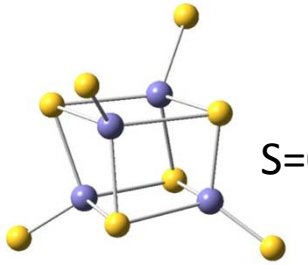
S=0



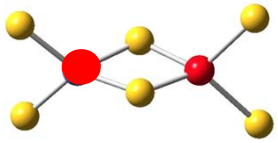
S=1/2

g = 2.01

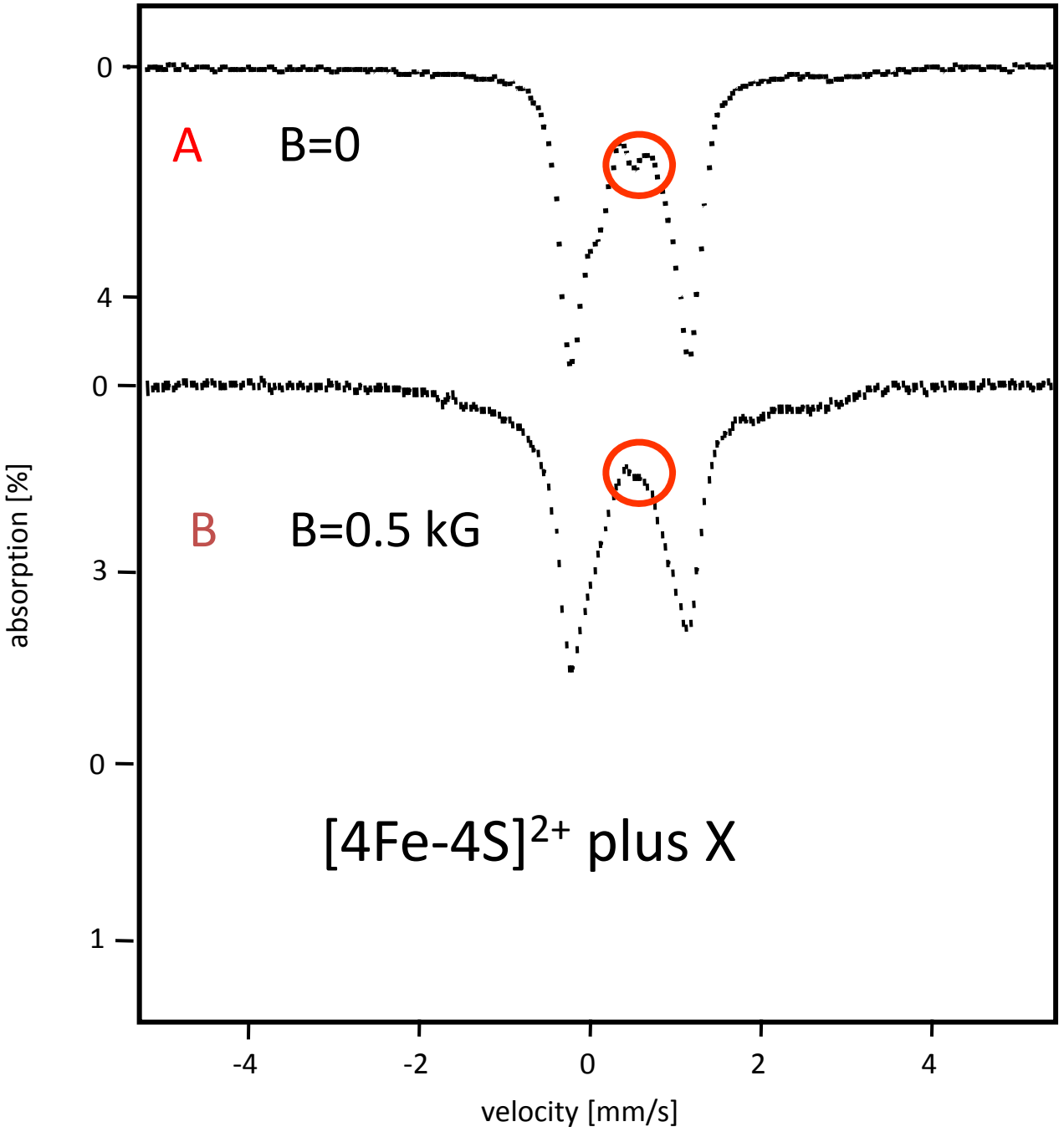
Reduced



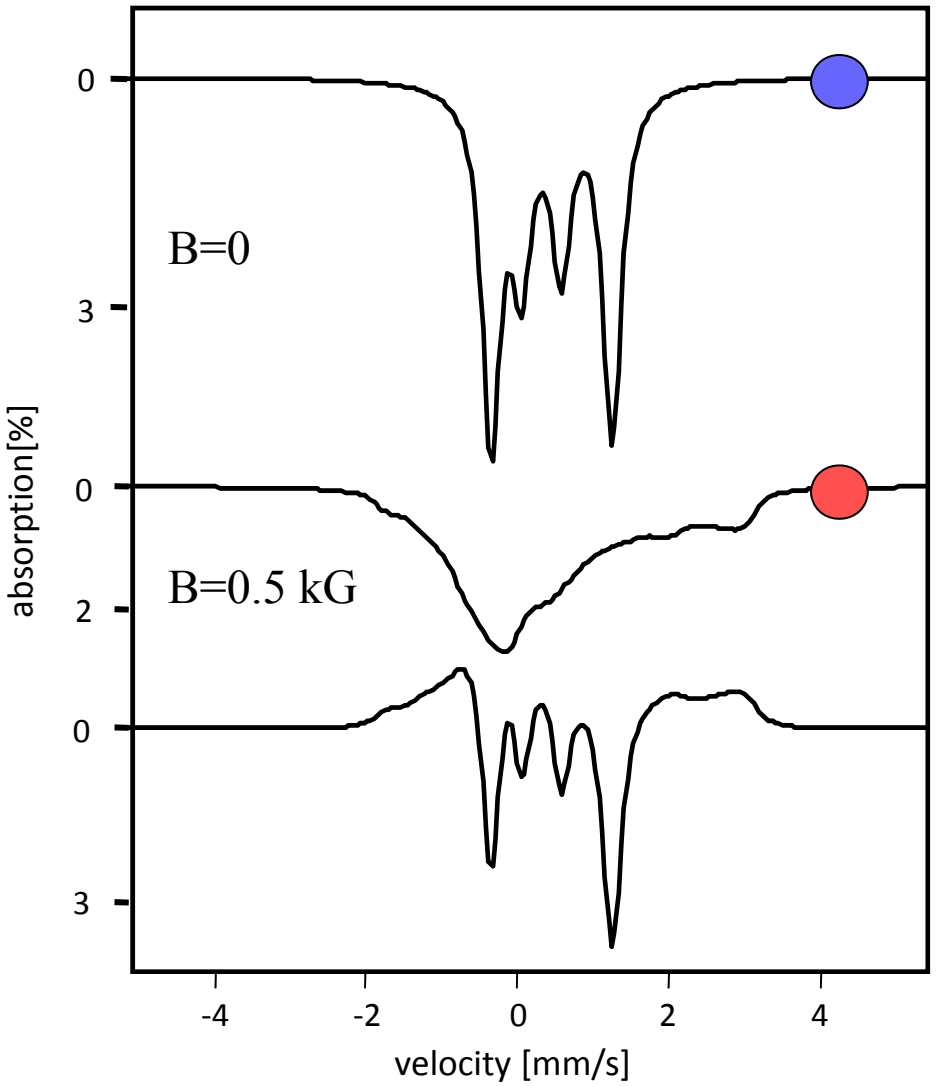
S=0

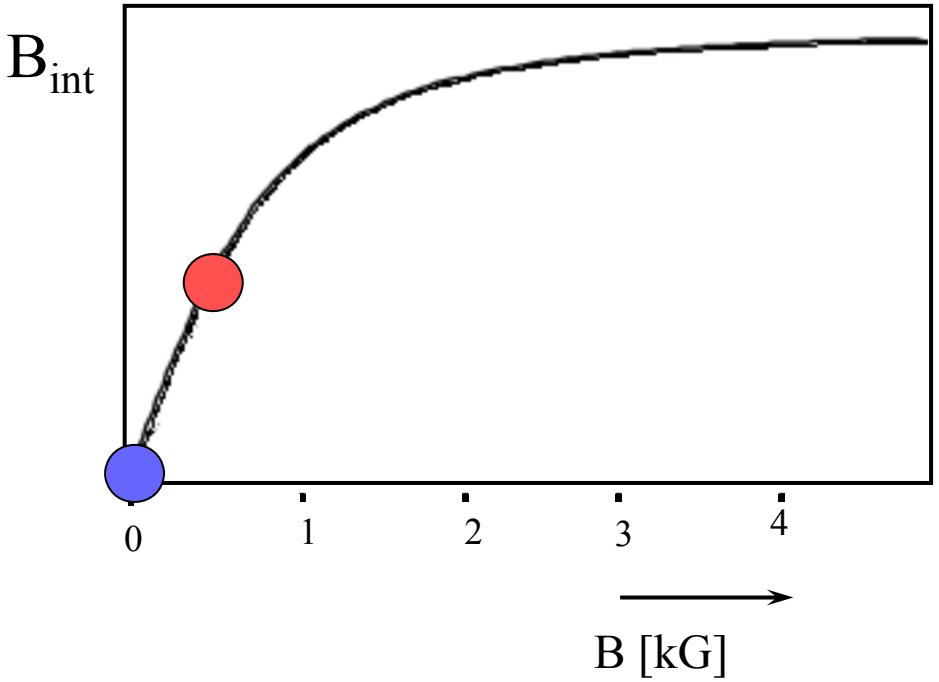


No EPR (yet)

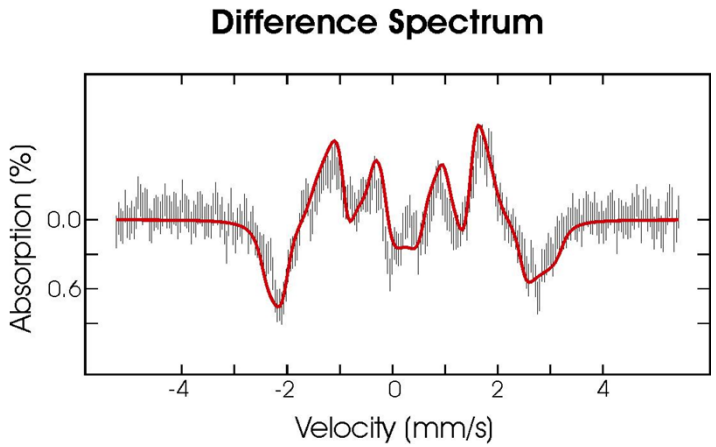

A B=0
B B=0.5 kG
[4Fe-4S]2+ plus X
absorption [%]
velocity [mm/s]
0
4
0
3
0
1
-4
-2
0
2
4

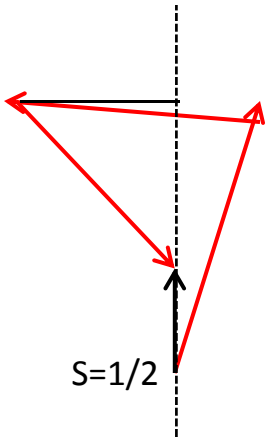
Simulation with S = 2 Spin Hamiltonian

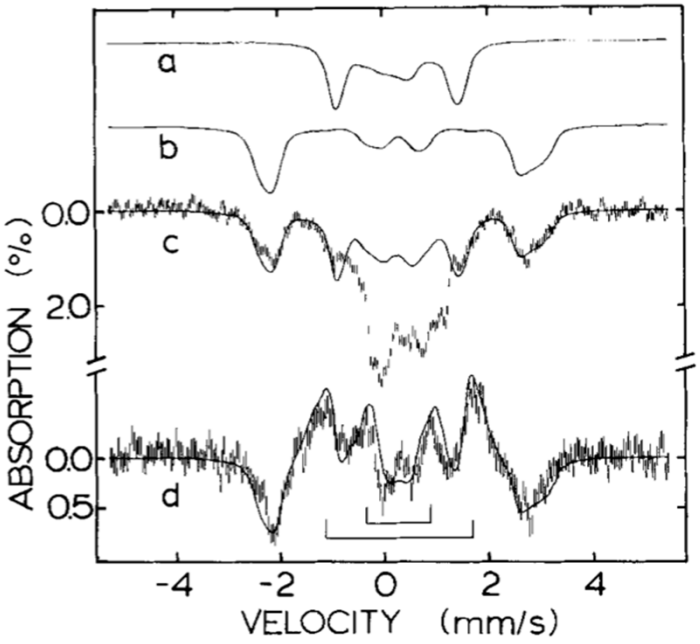


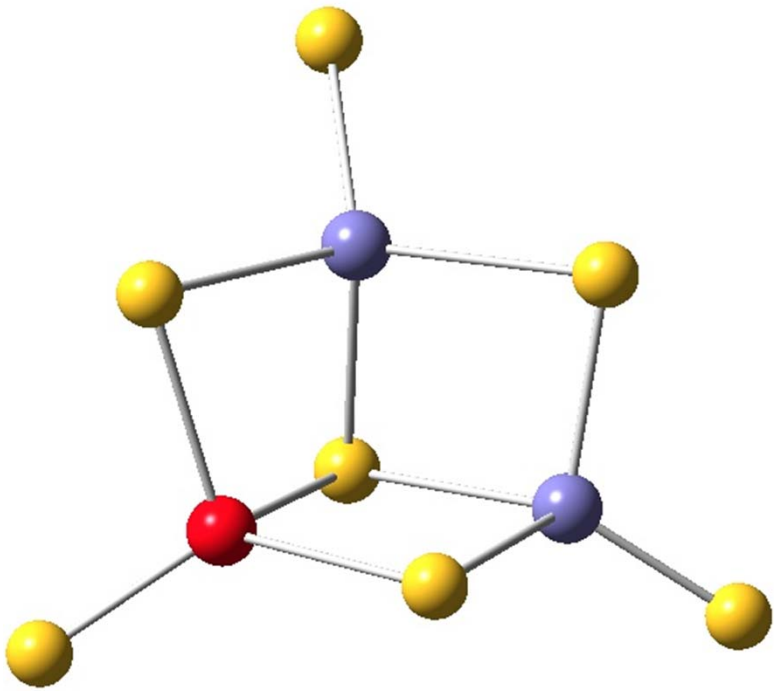


Why did we miss 3rd iron in oxidized state ?







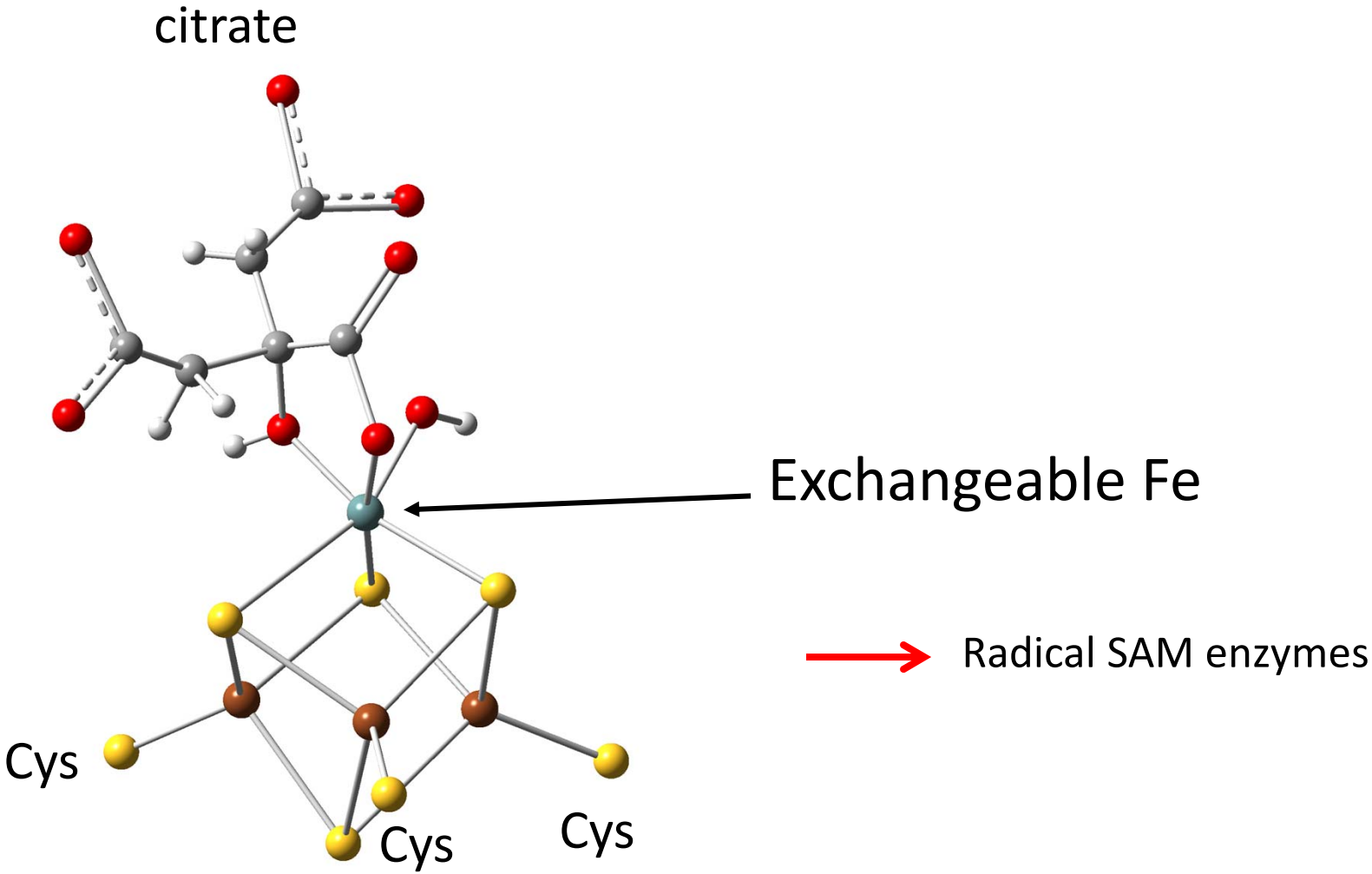


$[3Fe\text{-}4S]^{+}$ Cluster in reduced S=2 state

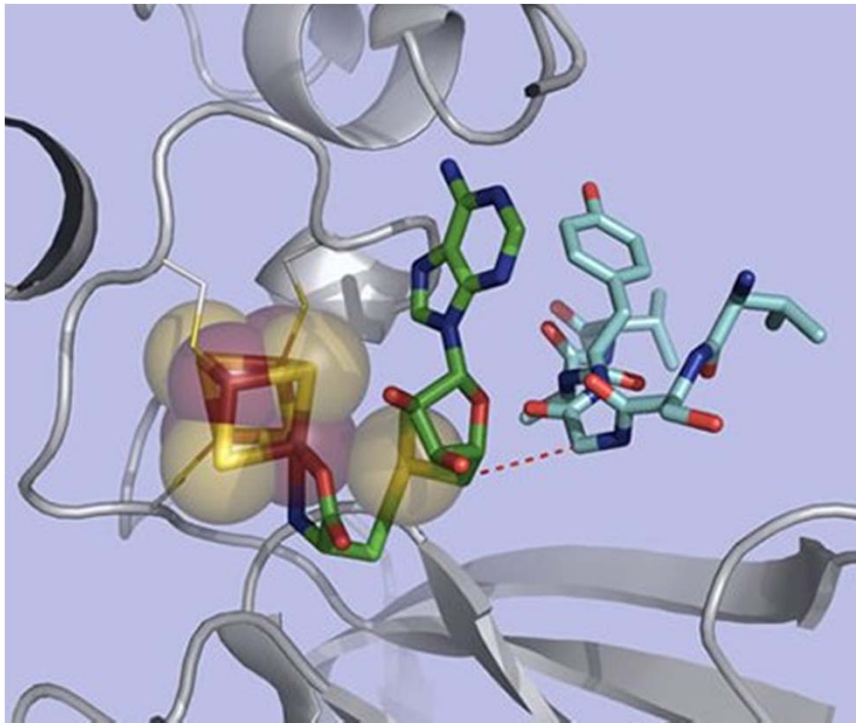
Fe^{3+}

$Fe^{2.5+}$

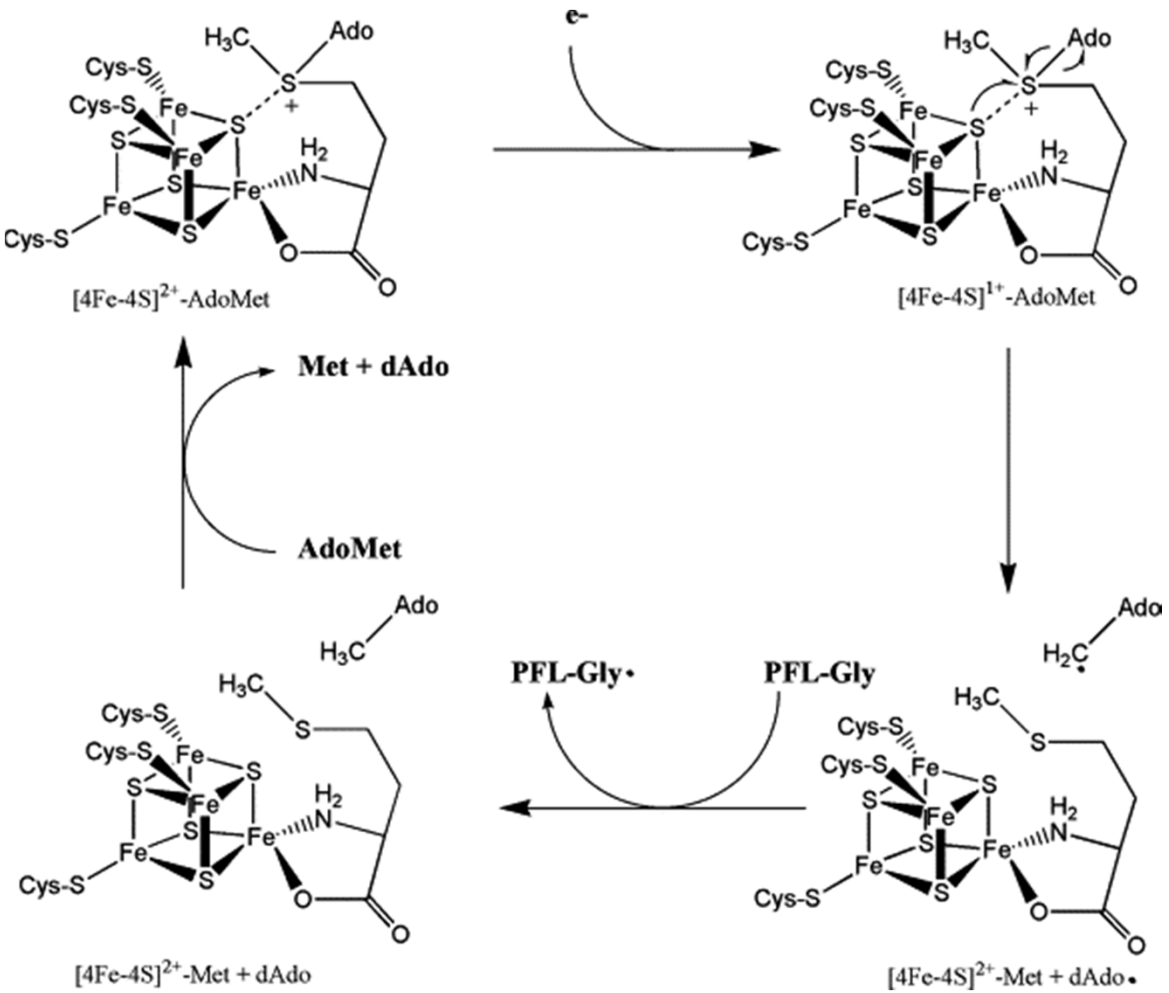
Aconitase with Substrates



Pyruvate Formate Lyase as seen on Joan Broderick's home page



Radical SAM Enzymes



A selfie with a Mossbauer spectroscopist, taken on May 29, 2014, will be worth at least $ 10, 000,000 in 20 years**

** probably even more