EPR Course

January 2, 2002

Lecture 11

NTU

Fall 2001

Chan

Transition Metals

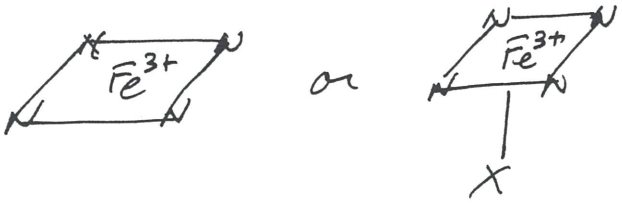
Free ions of the Transition Metals

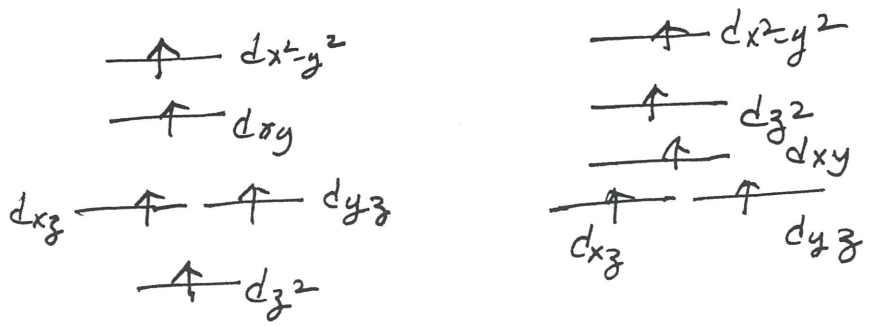
		Spin Arrangement in Free Ion	Total Spin S
$3d^1$	Ti^{3+}	↑ — — — —	1/2
$3d^2$	V^{3+}	↑↑ — — —	1
$3d^3$	V^{2+} Cr^{3+}	↑↑↑ — —	3/2
$3d^4$	Cr^{2+} Mn^{3+}	↑↑ ↑ ↑ —	2
$3d^5$	Mn^{2+} Fe^{3+}	↑↑ ↑ ↑ ↑	5/2
$3d^6$	Fe^{2+} Co^{3+}	↑↓ ↑ ↑ ↑ ↑	2
$3d^7$	Co^{2+}	↑↓ ↑↓ ↑↑↑	3/2
$3d^8$	Ni^{2+}	↑↓ ↑↓ ↑↓ ↑ ↑	1
$3d^9$	Cu^{2+}	↑↓ ↑↓ ↑↓ ↑↓ ↑	1/2
$3d^{10}$	Zn^{2+} Cu^{1+}	↑↓ ↑↓ ↑↓ ↑↓ ↑↓	0

Ligand field or Crystal field will lift the degeneracy of d-orbitals, and affect the distribution of the spins among the d-orbitals

For example,

(1) for Fe^{3+}-porphyrin that is four-coordinate (uncapped) or five coordinate

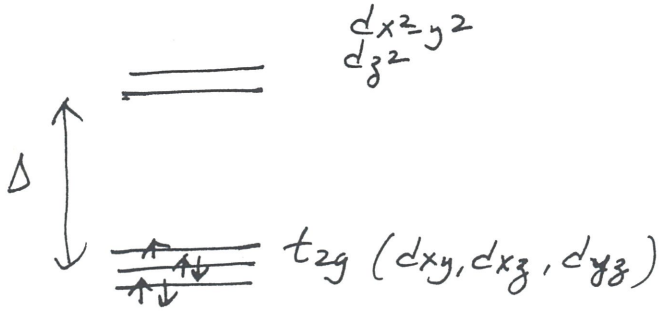


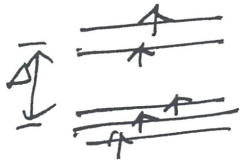


High-spin
$S = \frac{5}{2}$

High-spin
$S = 5/2$

(2) for Fe^{3+}-porphyrin that is six-coordinate





Strong crystal or ligand field

Δ large

Low-Spin $S = 1/2$

Weak crystal field

Δ small compared with electron-electron repulsion

High-Spin $S = 5/2$

Zero-field Splitting

If there is more than 1 unpaired electron spin, there will be magnetic interaction among the spins, and there will be a zero-field splitting.

The dipolar spin Hamiltonian takes the form

$$\mathcal{H}_D = \vec{S} \cdot \underset{\sim}{D} \cdot \vec{S}$$

where S is the total spin operator for the unpaired e's and $\underset{\sim}{D}$ is the zero-field splitting tensor!

For 2 unpaired electron spins,

$$D_{xx} = \tfrac{1}{2} g^2 \beta^2 \left\langle \frac{r_{12}^2 - 3x_{12}^2}{r_{12}^5} \right\rangle$$

$$D_{xy} = \tfrac{1}{2} g^2 \beta^2 \left\langle \frac{-3 x_{12} y_{12}}{r_{12}^5} \right\rangle$$

In the principal axes system of $\underset{\sim}{D}$,

$$\begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \longrightarrow \begin{pmatrix} -X & 0 & 0 \\ 0 & -Y & 0 \\ 0 & 0 & -Z \end{pmatrix}$$

so that

$$\mathcal{H}_D = -X S_{x'}^2 - Y S_{y'}^2 - Z S_{z'}^2$$

Now $X + Y + Z = 0$, so that only 2 of these quantities are independent!

Customary to define

$$D = \frac{1}{2}(X+Y) - Z$$

$$E = -\frac{1}{2}(X-Y)$$

Then

$$\mathcal{H}_D = -X S_{x'}^2 - Y S_{y'}^2 - Z S_{z'}^2$$

$$= D\left(S_z^2 - \frac{1}{3}S^2\right) + E\left(S_x^2 - S_y^2\right)$$

Will drop "prime" henceforth, remembering that D and E are always referred to principal axes of the zero-field splitting tensor (X, Y, Z)

Second-order Spin-orbit Energy

Aside from magnetic interactions among unpaired spins, spin-orbit coupling can lead to an indirect electron spin-spin coupling.

Spin-orbit energy to second order

$$= -\lambda^2 \sum_n \frac{\langle 0 | \vec{L}\cdot\vec{S} | n \rangle \langle n | \vec{L}\cdot\vec{S} | 0 \rangle}{E_n - E_0}$$

which could be rewritten as

$$\mathcal{H}_{SO} = \hat{S} \cdot \underline{D} \cdot \hat{S}$$

with $D_{ik} = -\lambda^2 \sum_n \frac{\langle \psi_0 | L_i | \psi_n \rangle \langle \psi_n | L_k | \psi_0 \rangle}{E_n - E_0}$

where $i, k = x, y, z$

For transition metals, spin-orbit contribution to $\underset{\sim}{D}$ usually dominates!

Nevertheless, still the same spin Hamiltonian

$$\boxed{\mathcal{H}_{ZS} = D\left(S_z^2 - \tfrac{1}{3}S^2\right) + E\left(S_x^2 - S_y^2\right)}$$

where we lump $\mathcal{H}_D + \mathcal{H}_{SO}$ together, so that D and E now include contributions from magnetic dipolar and spin-orbit interactions.

Some concrete examples

(a) $\boxed{S = 1/2}$ $\quad S = 1/2$

$S_z = \pm 1/2$

so that $\langle S_z^2 - \frac{1}{3}S^2 \rangle$

$= \langle \frac{1}{4} - \frac{1}{3} S(S+1) \rangle$

$= \frac{1}{4} - \left[\frac{1}{3} \cdot \frac{1}{2}\left(\frac{3}{2}\right)\right] = 0$

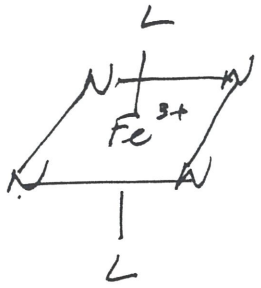
also $\langle S_x^2 \rangle = \langle S_y^2 \rangle = \frac{1}{4}$

So $\mathcal{H}_{ZS} = 0$ $\quad$ **No ZS splitting for $S = 1/2$**

and $\mathcal{H}_S = \mathcal{H}_Z = \beta \vec{S} \cdot \underset{\sim}{g} \cdot \vec{H}$

g-tensor anisotropic due to spin-orbit coupling.

A good example would be low spin d^5, as for a six-coordinate Fe(III) porphyrin with strong-field axial ligands, schemically shown as



where

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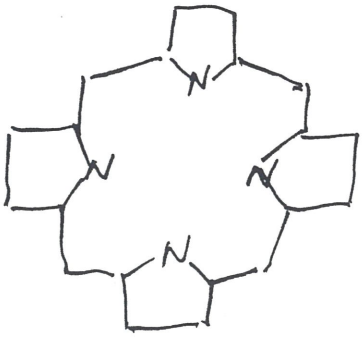
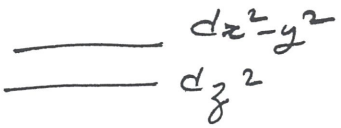
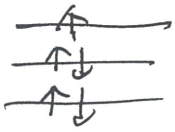


or its derivatives





or 1 unpaired electron in t_{2g} orbitals (O_h) octahedral

or 1 unpaired electron in d_{xz}, d_{yz} (degenerate) for square bipyramid

Effective g-values : 3, 2, 1.5

(b) In contrast $\boxed{S = \frac{5}{2}}$

e.g. high spin Fe^{3+}-porphyrin

5 unpaired electrons : $(d_{xy}^1\ d_{xz}^1\ d_{yz}^1\ d_{z^2}^1\ d_{x^2-y^2}^1)$

- Exchange interaction among five unpaired spins

$$\Longrightarrow S = 5/2 \text{ ground state}$$

- But the unpaired electrons also interact magnetically via magnetic dipole-dipole interaction and via second-order spin-orbit coupling

Therefore $\mathcal{H}_S = \mathcal{H}_{ZS} + \mathcal{H}_Z$

$$= D\left(S_z^2 - \tfrac{1}{3}S(S+1)\right) + E\left(S_x^2 - S_y^2\right)$$

$$+ \beta \vec{H} \cdot \underset{\sim}{g} \cdot \vec{S}$$

For Fe-porphyrin

$z \perp$ porphyrin plane; x, y in porphyrin plane.

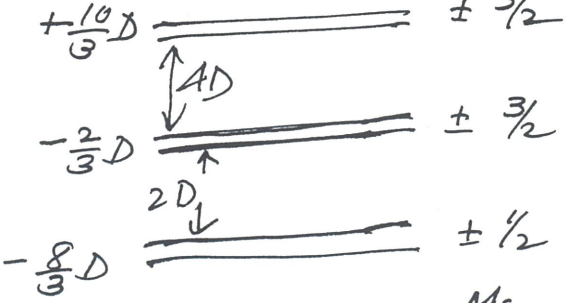
For D_{4h} porphyrin, $E = 0$, so $\mathcal{H}_{ZS} = D\left(S_z^2 - \frac{1}{3}S(S+1)\right)$

Since $\langle S_z^2 \rangle = M_S^2$ where $M_S = \pm 5/2, \pm 3/2, \pm 1/2$

(projection of spin along porphyrin normal)

Energy levels

quofield splitting for $E \sim 0$



Typically
$D \sim 2\text{-}10\ cm^{-1}$
$E \sim 0\text{-}0.04\ cm^{-1}$

Metmyoglobin
$D = 9\ cm^{-1}$

Metmyoglobin fluoride $D \cong 6\ cm^{-1}$

- Low Temperature (4.2 K) EPR is the easiest and the most unequivalent way to determine the spin state of an iron heme in the ferric state.
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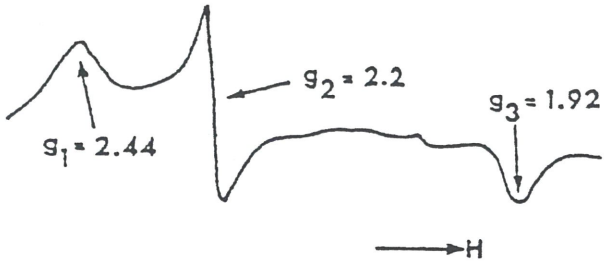
(1) Low-spin d^5



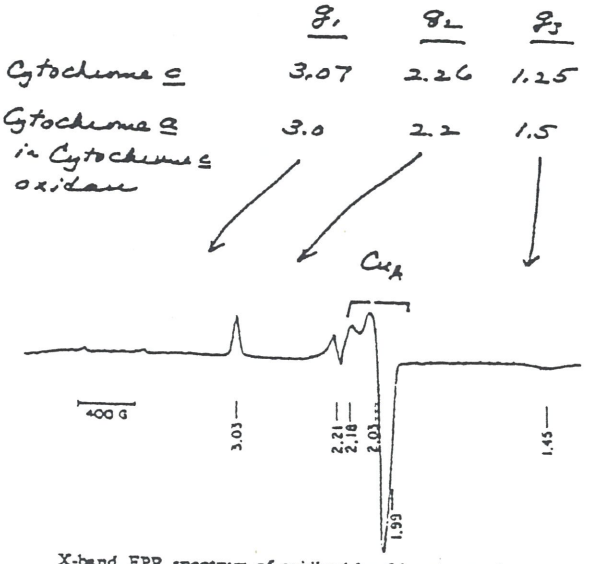
EPR of resting (oxidized) cychrome P-450 before substrate is bound.

(2) Other low-spin hemes in hemeproteins

g-values of low-spin hemoproteins

	g_1	g_2	g_3
Mb.OH	2.61	2.19	1.82
Mb.N_3	2.8	2.25	1.75
Hb.OH	2.6	2.3	1.7
Hb.N_3	2.82	2.2	1.70
Peroxidase.OH	2.36	2.12	1.67
Cytochrome *c* peroxidase	2.7	2.2	1.33
Cytochrome b_5	3.03	2.23	1.93
Catalase.N_3	2.80	2.18	1.74
	(2.53	2.2	1.85)
Catalase.CN	2.84	2.25	1.66
	2.56	2.31	1.81
	(2.43	2.17	1.895)

Mb is myoglobin and Hb is hemoglobin.



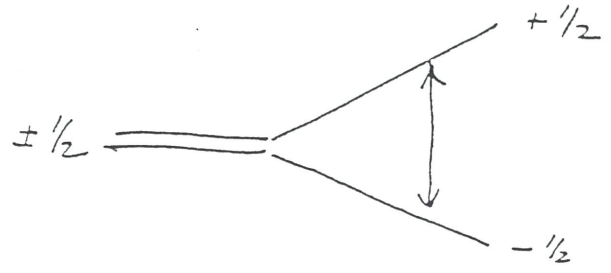
X-band EPR spectrum of oxidized beef heart cytochrome *c* oxidase at 10 K.

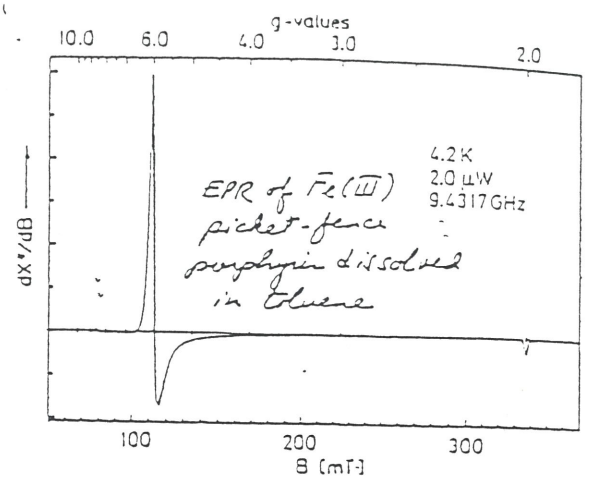
(3) High-spin heme centers

Hemoprotein	g_1	g_2	g_3
Met Mb	5.9		2.0
Catalase	6.6	5.4	2.0
$+F^-$	6.5	5.5	2.0
$+N_3^-$	6.1	5.2	2.0
P-450	6.6	5.3	2.0

Conventional EPR only observed for $\pm 1/2$ level, the so-called

Kramer doublet





$g_{eff} \cong 2$ when $\vec{H} \parallel z$ (along porphyrin normal)

6 when $\vec{H} \perp z$ (in porphyrin plane)

$g_{xy}^{eff} = 6 \pm 24\left(\frac{E}{D}\right)$ for deviation from axial symmetry

Note that Fe^{3+}-porphyrins are usually observed near liquid helium temperatures, where essentially the lowest level is populated ($S_z = \pm 1/2$ Kramer doublet if $D > 0$)

Of course, for $D < 0$, $S_z = \pm 5/2$ lowest occupied and no EPR is expected (!) at low temperatures.

Cytochrome *a*3 in Cytochrome *c* oxidase	6.0	2.0

(4) Recall for high-spin heme, conventional EPR arise from $m_s = \pm 1/2$ Kramer doublet:

- g~6 when $\bar{H}$ is in porphyrin plane
- g~2 when $\bar{H}$ is $\perp$ porphyrin plane

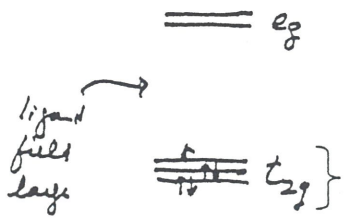
When porphyrin has D_{4h} symmetry (axial), E=0 and g=6.0. When zero-field interaction has a rhombic component, i.e., E≠0, then

$$g_{\perp} = 6.0 \pm 24(E/D)$$

e.g. for E~0.04cm^{-1} D~10cm^{-1} (Cytochrome a_3 in cytochrome *c* oxidase)

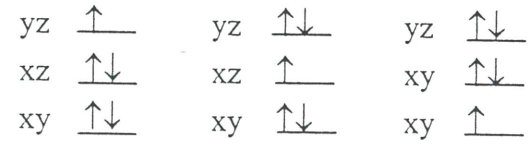
E~0.04cm^{-1} is a relatively small rhombic distortion.

(5) For low spin heme, deviation of g-values from 2.0023 arises from spin-orbital interaction.



$\therefore$ can focus attention at t_{2g} orbitals!

At this level of approximation, problem is orbitally degenerate $\Rightarrow$ John-Teller distortion to D_{2h} symmetry, which lifts degeneracy of 3 t_{2g} orbitals.



ground state | 1st excited state | 2nd excited state

$(d_{yz})^+$ | $(d_{xz})^+$ | $(d_{xy})^+$ ← spin"up"

Spin-orbit interaction mixes these states

For example,

$$\Psi_1^+ = A_1(d_{yz})^+ + B_1(i)(d_{xz})^+ + C_1(d_{xy})^-$$

and $\Psi_1^- = -A_1(d_{yz})^- + B_1(i)(d_{xz})^- + C_1(d_{xy})^+$

More explicitly

$$\Psi_1^+ = A_1(d_{yz})^+ + \frac{\left\langle d_{xz}^+ \middle| -\lambda \vec{L}\cdot\vec{S} \middle| d_{yz}^+ \right\rangle}{\varepsilon_{yz} - \varepsilon_{xz}}(d_{xz})^+ + \frac{\left\langle d_{xy}^- \middle| -\lambda \vec{L}\cdot\vec{S} \middle| d_{yz}^+ \right\rangle}{\varepsilon_{yz} - \varepsilon_{xy}}(d_{xy})^-$$

$$= A_1(d_{yz})^+ + \frac{i\lambda}{2(\varepsilon_{yz} - \varepsilon_{xz})}(d_{xz})^+ + \frac{\lambda}{2(\varepsilon_{yz} - \varepsilon_{xy})}(d_{xy})^-$$

where ε_{yz}, ε_{xz}, ε_{xy} are the energies of $(d_{yz})^{\pm}$, $(d_{xz})^{\pm}$, $(d_{xy})^{\pm}$ the so called "hole" states.

ε_{xy} _____ $(d_{xy})^{\pm}$
ε_{xz} _____ $(d_{xz})^{\pm}$
ε_{yz} _____ $(d_{yz})^{\pm}$

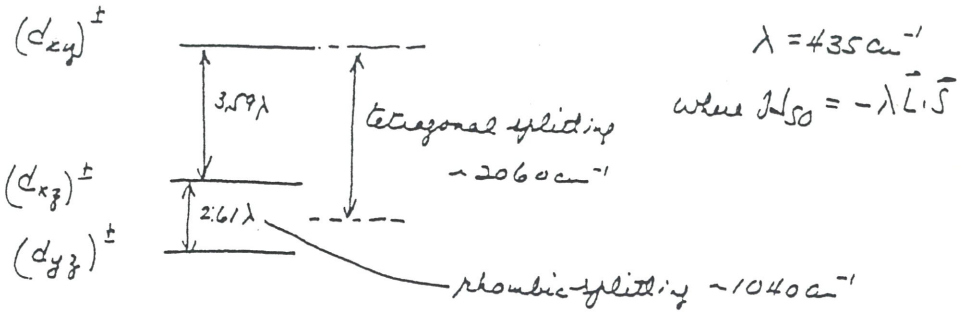
spin-orbit interaction mixes these "hole" states

Similar final wavefunctions can be written for remaining two Kramer doublets.

For azidoferrihemoglobin (Hb, N_3), where $g_1 = 2.80$, $g_2 = 2.22$ and $g_3 = 1.72$

	i	Ai	Bi	Ci	
Kramer	1	(0.973)	–0.207	–0.097	$\sim(d_{yz})^{\pm}$
doublet	2	0.219	(0.970)	0.108	$\sim(d_{xz})^{\pm}$
	3	0.017	–0.126	(0.990)	$\sim(d_{xy})^{\pm}$

To fit g-values for azidoferrihemoglobin, requies



at 4.2 - 20 K, only $(d_{yz})^{\pm}$ Kramer doublet is populated!

Ref. F.S.Griffith, "Theory of Transition –Metal Ions" Cambridge University Press.
M. Kotani, Advances in Chem. Physics 7, 159 (1964)

(6) Since different axial ligands affect the tetragonal and rhombic splittings differently, the g-values for a low-spin heme can be used to infer the axial ligands.

The relation between EPR parameters and structure of low spin ferric heme compounds (taken from [11])

Compound	Axial ligands		g values		
Glycera Hb $MeNH_2$	imid	RNH_2	3.30	1.98	1.20
Leg Hb pyridine	imid	pyr	3.26	2.10	0.82
Cytochrome c	imid•	met	3.07	2.26	1.25
Bis imid heme	imid•	imid•	3.02	2.24	1.51
Bis $imid^-$ heme	$imid^-$	$imid^-$	2.80	2.26	1.72
MbOH	$imid^-$	OH^-	2.55	2.17	1.85
Cyt. $P\text{-}450_{cam}$	imid•	RS^-	2.45	2.26	1.92
Cyt. c oxidase	imid•	imid•	3.0	2.2	1.5

this approach was used to argue that cytochrome a in cytochrome c oxidase is six-coordinate with N from neutral imdazoles as axial ligands

A better approach to infer the axial ligands of hemes in hemoproteins is EXAFS.

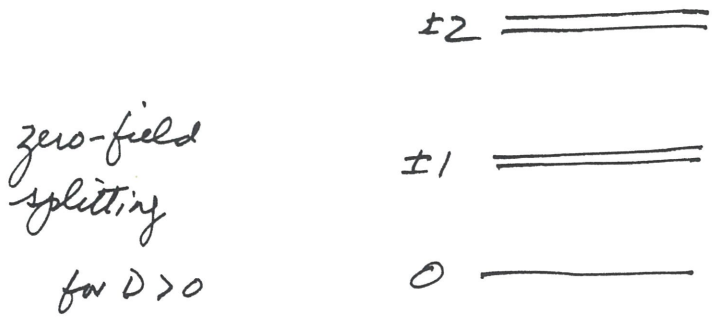
EXAFS spectroscopy has provided the first direct, compelling evidence demonstrating that the proximal ligand to the heme in P-450 (in states 1-5) and chloroperoxidase (state 2 and 4) is a thiolate sulfur.
Ref. S.P. Cramer, J. H. Dawson, K.O. Hodgson and L. P. Hager, *J. Am. Chem. Soc.* 100, 7282 (1978).

Other spin systems

(c) High-spin d^6 and high-spin d^4

e.g. high-spin Fe^{2+} e.g. Mn^{3+}

$\boxed{S = 2}$ (4 unpaired electrons)



No Kramer doublet → no conventional EPR!

(d) Low-spin d^6

e.g. Fe^{2+}-porphyrin in oxy Mb or reduced cytochrome a in Cytochrome c oxidase

$\boxed{S = 0}$

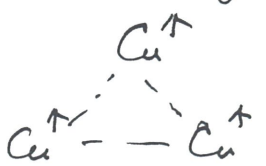
no EPR!

(e) $S = 3/2$, e.g. Co^{2+} (d^7);

Mo^{3+} in $MoFe_3S_3$ cluster

$(4d^3)$

or ground state of ferromagnetically coupled oxidized trinuclear Cu^{2+} cluster

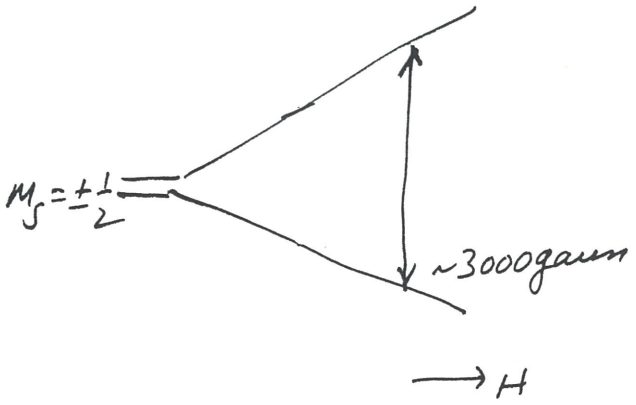


(in C-cluster of PMMO)

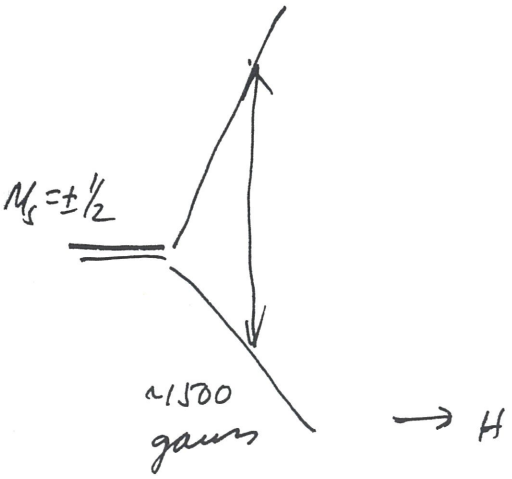
$\pm 3/2$

zero-field splitting →

$\pm 1/2$ Kramer Doublet

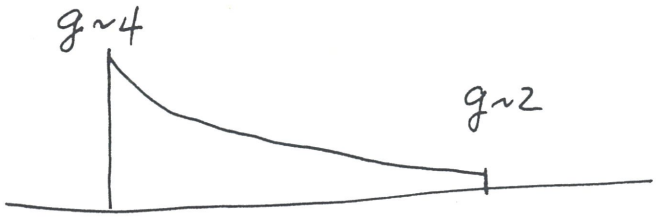


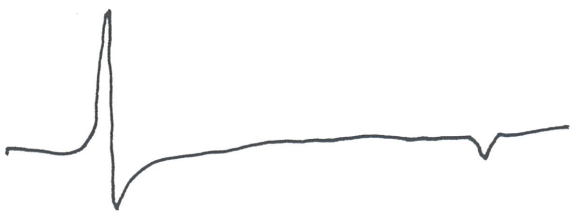
$g \simeq 2$ when $\vec{H}$ along z-axis or ⊥ triangular plane of trinuclear cluster



$g \simeq 4$ when $\vec{H}$ in xy plane (triangular plane)

Powder spectrum



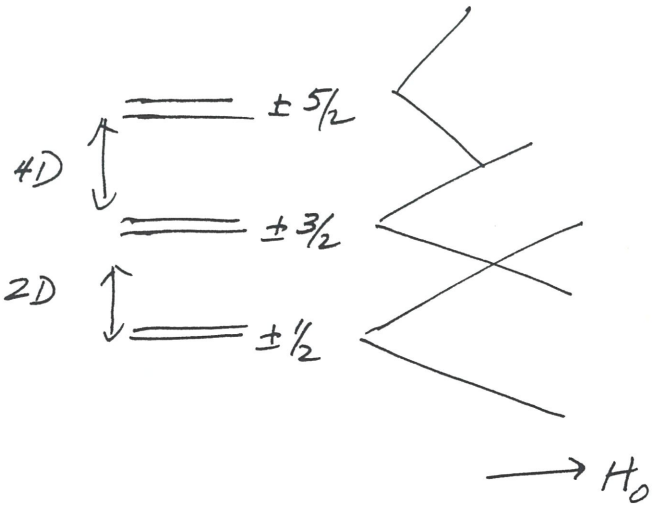


derivative spectrum

(f) Mn^{2+}

high-spin d^5

D usually small $\sim 0.1\,cm^{-1} - 1\,cm^{-1}$

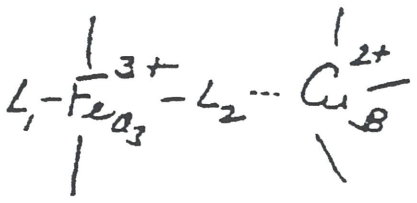


$D \sim g\beta H_0/hc$

- Ligand Superhyperfine Interactions in EPR and ENDOR spectra
  provide more direct evidence of identity of ligands

(1) When ligand superhyperfine structure is large enough to be resolved in conventional EPR spectrum.
Illustrate this by way of the cytochrome a_3, Cu_B cluster of cytochrome c oxidase.

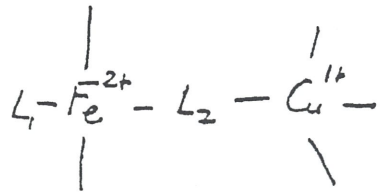
(a) Oxidized Cluster



high-spin
S=5/2 S=1/2 } Antiferromagnetic
Coupling ⇒ S=2
if L_2= H_2O, F^-, formate
No conventional EPR!

low-spin
S=1/2 S=1/2 } S=0 antiferromagnetic
or 1 ferromagnetic
if L_2= OH^-, CN^-

(b) Reduced Cluster



high-spin
S=2
if L_2= H_2O

No conventional EPR!

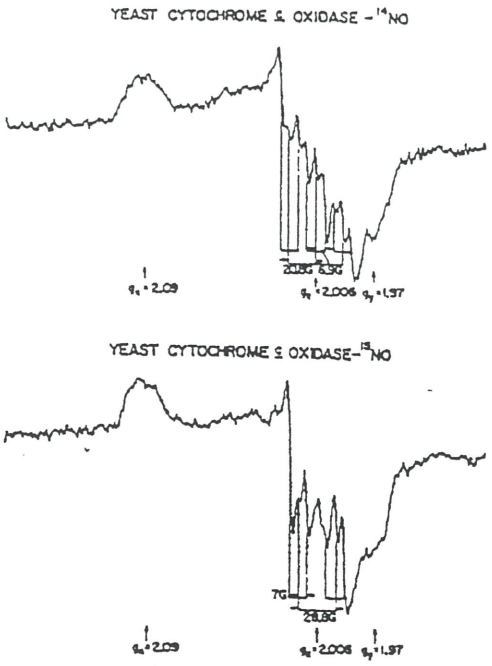
low-spin
S=0
if L_2=CO,NO

No EPR for CO complex ; but NO is paramagnetic, so although NO-binding to iron heme convert Fe to low spin (S=0), the nitrosyl heme is an odd electron system with S=1/2

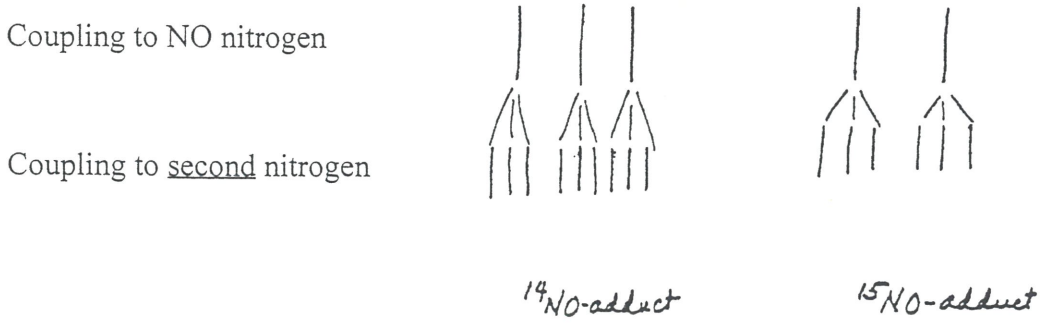
(c) EPR spectrum of ^{14}NO- and ^{15}NO adducts of reduced cytochrome *c* oxidase.

→All 3 g-components are resolved
(g_1=2.09;g_2=2.006;g_3=1.97)

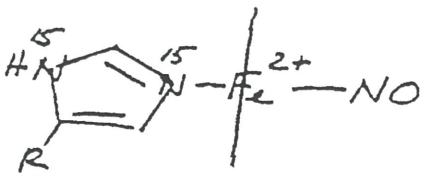
→The g=2.006 component exhibits a (^{14}NO-adduct) nine-line hyperfine patterns, which can be interpreted in terms of the superposition of these sets of three lines arising from two nonequivalent nitrogens (I=1) interacting with the unpaired electron. The larger of the two hyperfine coupling constants is 20.8 Gauss and the smaller 6.9 Gauss.



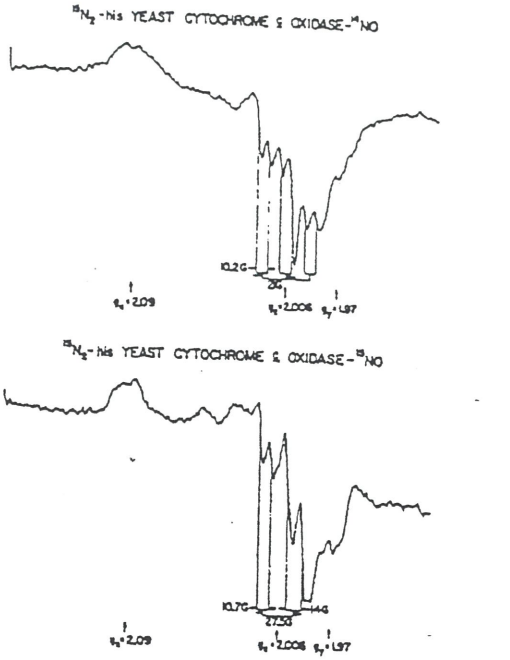
<u>^{15}NO-adducts</u>: When ^{15}NO is used in the experiment, the ^{15}NO-bound protein exhibits an EPR spectrum with g-value identical to those of the ^{14}NO-bound species, but the g=2.006 component shows a hyperfine pattern consisting of two sets of three lines. This pattern is consistent with the presence of one ^{14}N and one ^{15}N nitrogen bound axially to cytochrome a_3 with a 28.2 Gauss splitting for the ^{15}N and a 7.0 G splitting for the ^{14}N ligand.



→When experiment were repeated on a yeast cytochrome c oxidase preparation wherein all the histidines substituted with ^{15}N at both imidazole ring positions, the EPR spectra of the ^{14}NO- and ^{15}NO-aducts are altered.



The EPR spectra of the ^{15}NO- adduct of the (^{15}N) His protein consists of two sets of doublets, with a ^{15}NO nitrogen splitting of 27.5 Gauss and a splitting of 12 Gauss for the ^{15}N nitrogen of the histidine. The ^{14}NO-adduct exhibits a pattern consisting of 3 sets of doublets, with splitting of 21 Gauss to 10.2 G for the ^{14}NO and histidine ^{15}N nitrogen respectively.



EPR spectra of the ^{14}NO and ^{15}NO complexes of reduced native and (^{15}N)His yeast cytochrome c oxidase. Frequency, 9.2 GHz; temperature, 30 K.

→These experiments provide unequivocal identification of histidine as the endogenous fifth ligand to cytochrome a_3 (in the reduced state).

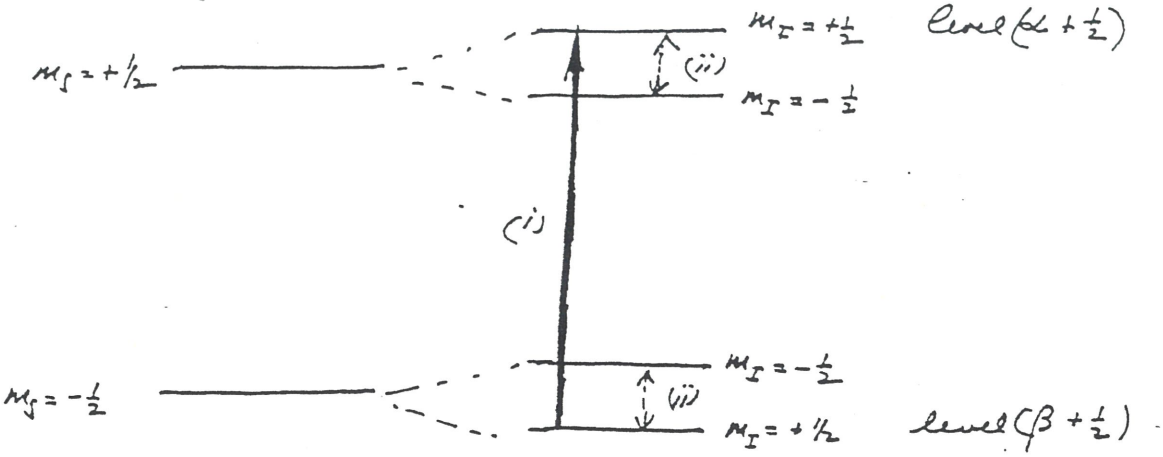
(2) When ligand superhyperfine interaction is too small to be resolved in EPR spectrum

Appeal to ENDOR spectroscopy
≡Electron Nuclear Double Resonance spectroscopy

Excite nuclear spins coupled to unpaired electron spin and use change in intensity of EPR spectrum to indicate resonance.

a) $\underline{I = \frac{1}{2}}$ nucleus coupled to electron spin

Energy level diagram



coupling to nuclear

(i) Saturate one of EPR transitions (EPR intensity = 0)

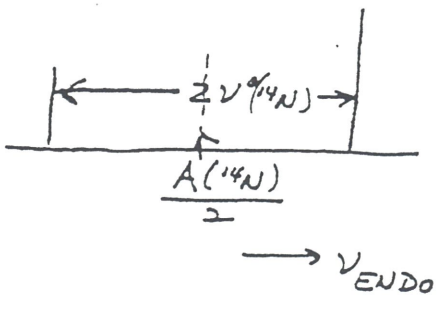
(ii) Slowly vary NMR frequency. At resonance, the population of level $(\beta + \frac{1}{2})$ will decrease, and population of level $(\alpha + \frac{1}{2})$ will increase. EPR will no longer be saturated and EPR intensity $\neq 0$.

(iii) The first order ENDOR spectrum of a nucleus of type I consists of transitions at frequencies given by:

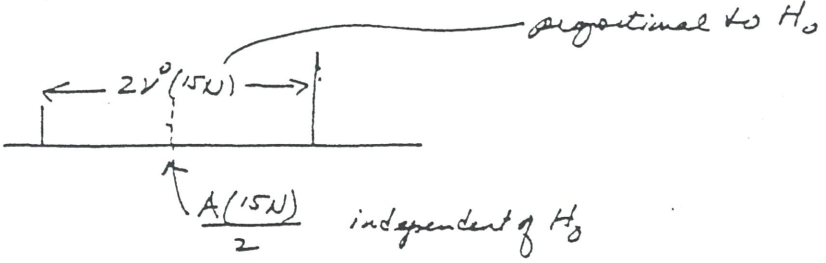
(a) $\underline{\text{When } \nu^0 = \text{NMR Larmor frequency} < A}$

$$\nu_{ENDOR} = \frac{A}{2} \pm \nu^0$$

e.g. $\underline{^{14}N}$: a Larmor-split doublet centered at $\frac{A(^{14}N)}{2}$ split by $2\nu^0(^{14}N)$ (further splitting by electric quadrupole interaction)



^{15}N: a Larmor-split doublet centered at $A(^{15}N)/2$ and split by $2\nu^0(^{15}N)$.

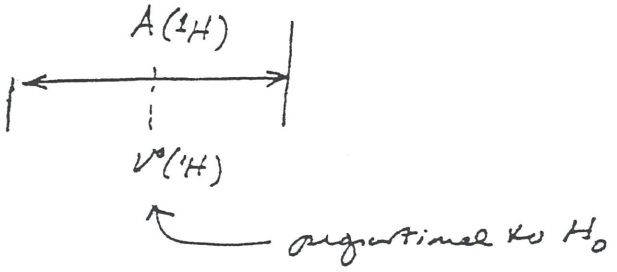


Note $\frac{\nu^0(^{15}N)}{\nu^0(^{14}N)} = \frac{A(^{15}N)}{A(^{14}N)} = 1.403$

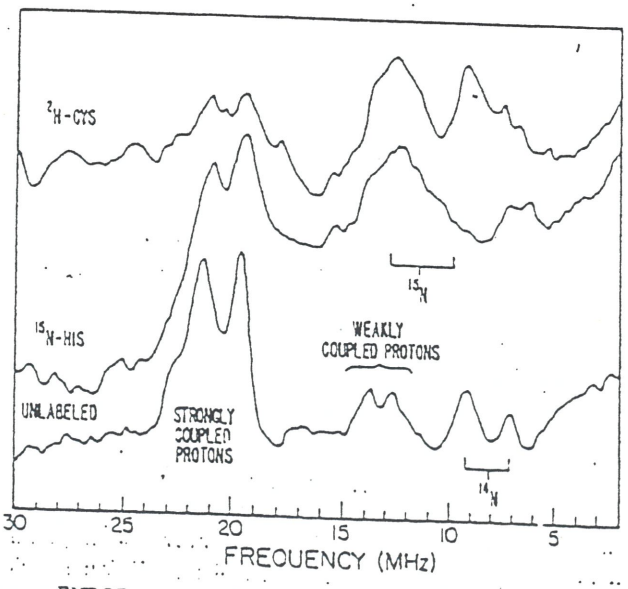
see page 14 for ENDOR at two field strengths

(b) When $\nu^0 > A$ (e.g. ^1H)

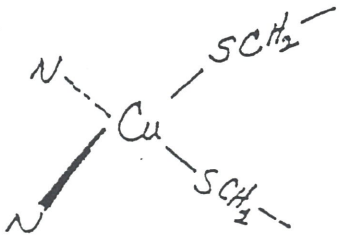
Then $\nu_{ENDOR} = \nu^0(^1H) \pm \frac{A(^1H)}{2}$



(c) ^1H, ^{14}N and ^{15}N ENDOR of Cu_A site of cytochrome c oxidase



ENDOR spectra of native, (^{15}N)His and (^{2}H)Cys yeast cytochrome c oxidase observed at $g = 2.04$, microwave frequency 9.12 GHz and temperature 2.1 K.



"Cu_A site"

In the ENDOR of the Cu_A site, there are NMR signals assignable to ^{14}N, weakly-coupled protons, and strongly coupled protons for native yeast cytochrome c oxidase.

→ ^{14}N signals is replaced by ^{15}N when the yeast is grown on ^{15}N-substituted (ring) histidine (99%)

→ Strongly coupled protons disappear or attenuated in intensity when the yeast is grown on deuterated cysteine with 2H (>90%) at the β-carbon (β-methylene)

→ These results provide unambiguous evidence for histidine and cysteine as ligands to Cu_A.

Ref: D. F. Blair et al Chemica Scripta 21, 43-53 (1983);
T. H. Stevens & S. I Chan, J. Biol. Chem 256, 1069 (1981);
T. H. Stevens et al, J. Biol. Chem. 257, 12106 (1982)
T. H. Stevens, D. F, Bocian & S. I Chan, FEBS 97, 314 (1979)