EPR spectroscopy for Bio-Inorganic Chemistry
Principles and Applications

FrenchBIC summer school

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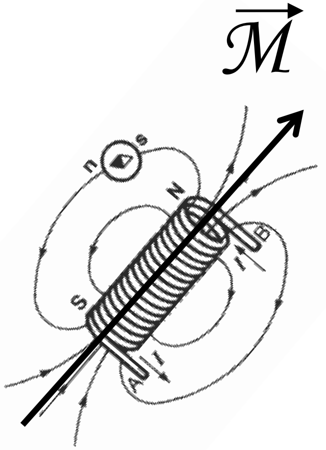
Bruno GUIGLIARELLI

Unité de Bioénergétique et Ingénierie des Protéines – UMR 7281

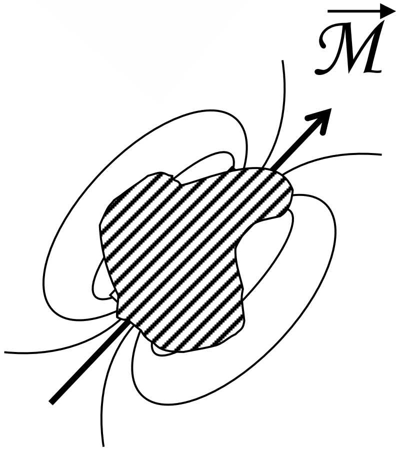
CNRS et Aix-Marseille Université

Summary

Basic principles



Magnetism is related to motion of electric charges



In matter : moving charges are electrons and protons

- Electron magnetism :
  - ***L***, orbital momentum
  - ***S***, spin momentum

$$\vec{\mu}_e = -\,\beta_e\,(\vec{L} + g_e\,\vec{S})$$
$$= -\,g\,\beta_e\,\vec{S}$$

EPR

- Nuclear magnetism
  - ***I***, nuclear spin

$$\vec{\mu}_N = g_N\,\beta_N\,\vec{I} = \gamma_N\,\hbar\,\vec{I}$$

NMR

$\beta_e = e\,\hbar\,/\,2\,m_e = 9.274\cdot10^{-24}\,\mathrm{A\cdot m^2}$ **>>** $\beta_N = e\,\hbar\,/\,2\,m_P = 5.05\cdot10^{-27}\,\mathrm{A\cdot m^2}$

Bohr's magneton >> Nuclear magneton

Basic principles : the magnetic resonance phenomenon

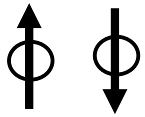
Electron Paramagnetic Resonance (EPR): A spectroscopy specific of single electron systems

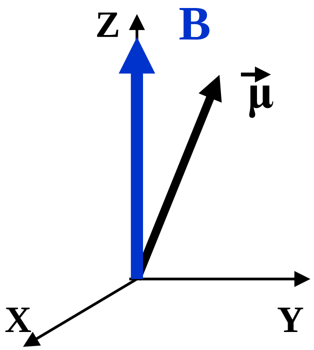
For a system with spin **S** = ½ in a magnetic field ***B***: $\vec{\mu} = - g\, \beta_e \vec{S}$

$E = - \vec{\mu} \bullet \vec{B} \longrightarrow H = - \vec{\mu} \bullet \vec{B} = g\, \beta_e \vec{S} \bullet \vec{B}$

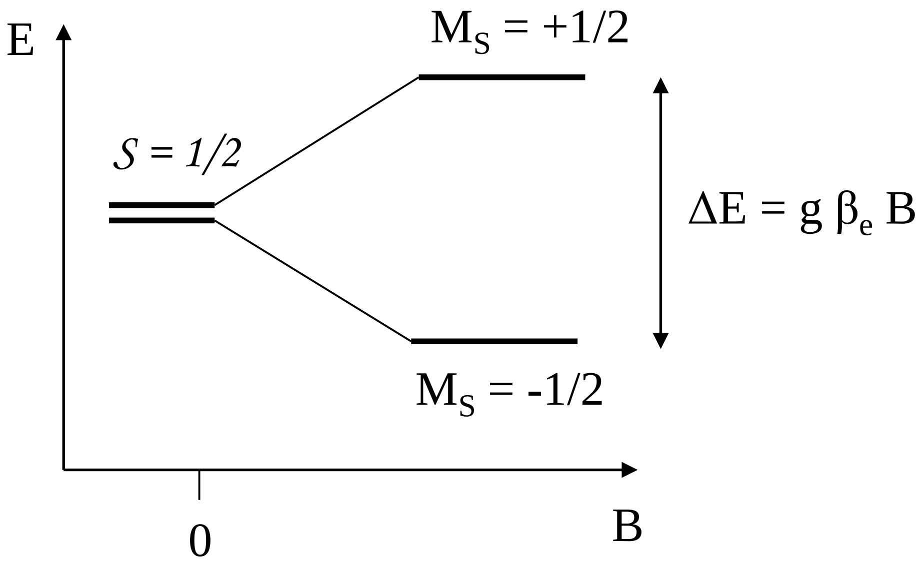
Taking $\vec{Z} \,//\, \vec{B} \Rightarrow H = g\, \beta\, B\, S_Z$

S_Z is quantified: only two values $M_S = \pm ½$





Energies : $E = g\, \beta_e\, B\, M_S = \pm ½\, g\, \beta\, B$ *(Zeeman effect)*



Basic principles : the magnetic resonance phenomenon

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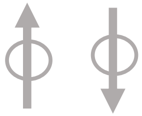
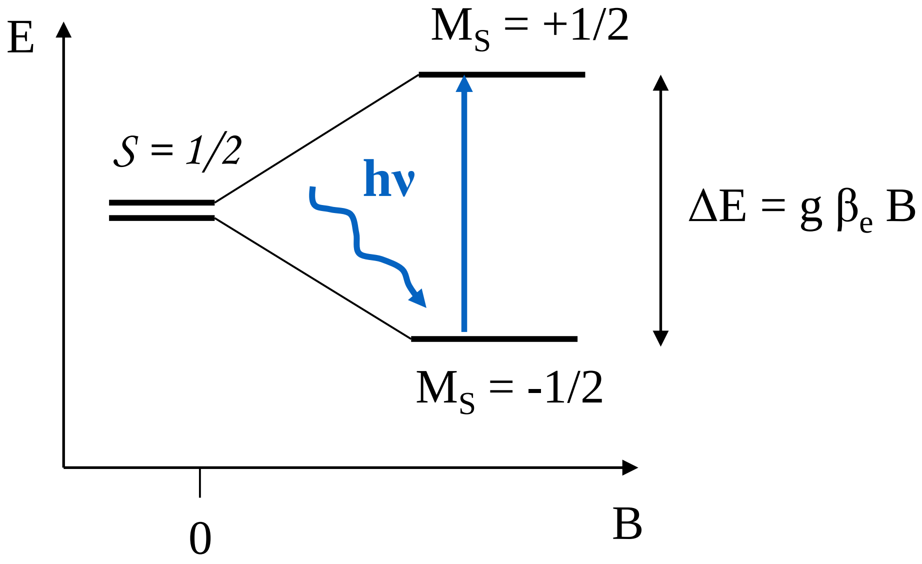
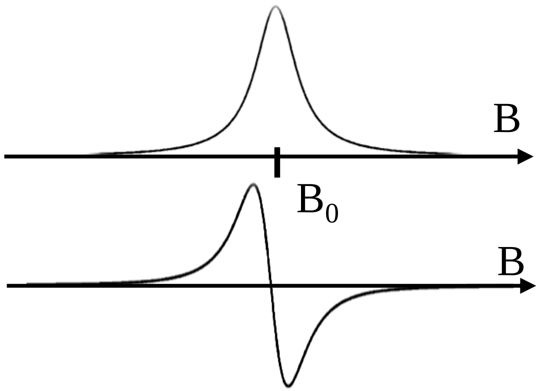


Energies : $E = g\,\beta\,B\,M_S = \pm ½\,g\,\beta_e\,B$ *(Zeeman effect)*

Resonance condition

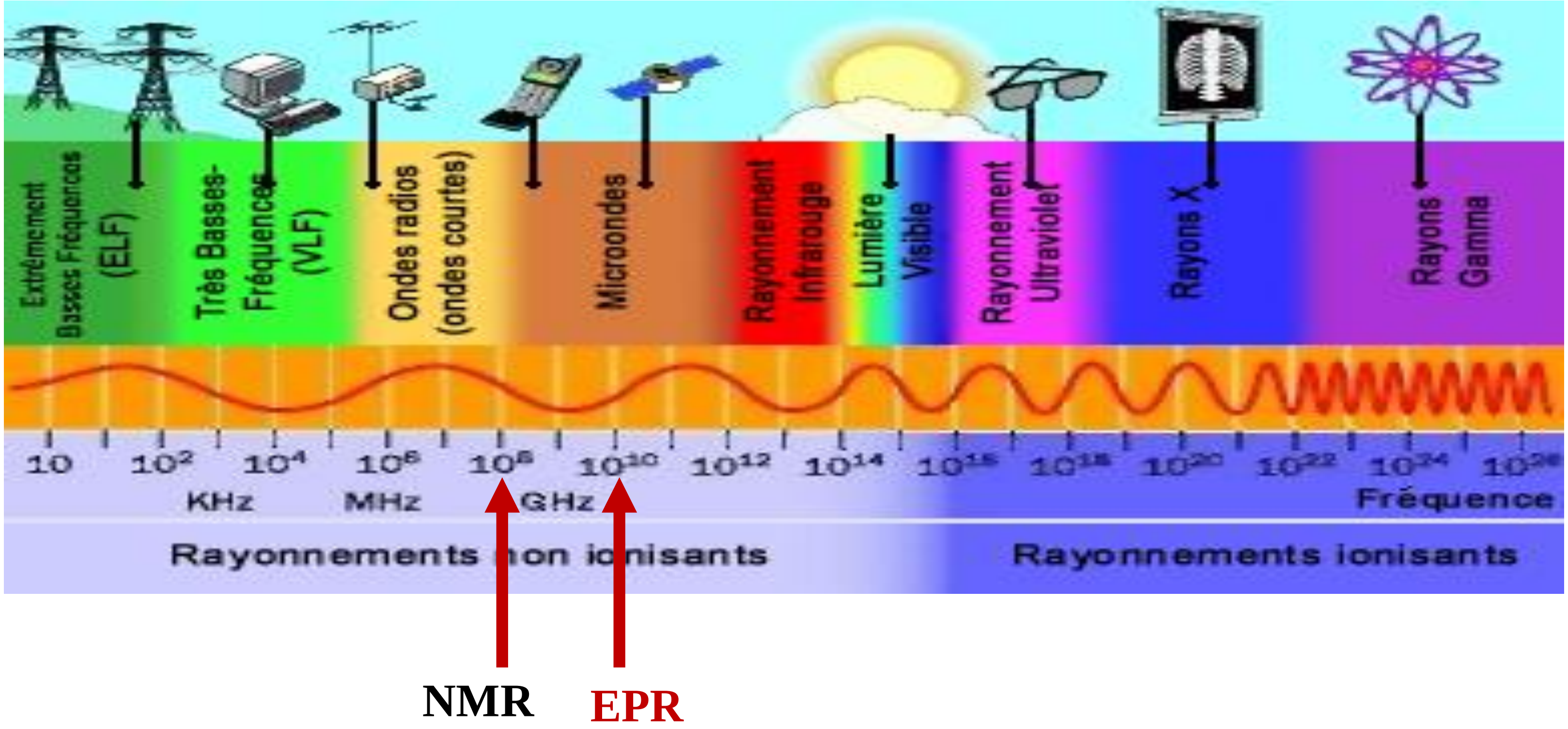
$$h\nu = g\,\beta_e\,B_0$$





g = 2.00, B_0 = 0.3 T
ν = 10 GHz, λ = 3 cm
Microwaves (X-band)

Basic principles : the magnetic resonance phenomenon

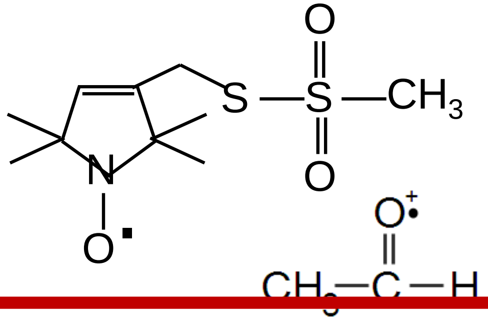


2nd World War: development of **RA**dio **D**etecting **A**nd **R**anging (RADAR)

- Microwave sources: klystron (Bernard Rollin 1940)
- Highly sensitive detection crystals
- Antenna, Magic-T, …
- Lock-in amplifiers

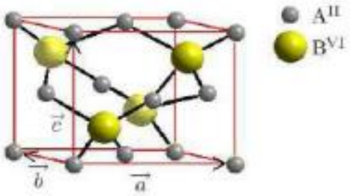
Basic principles:

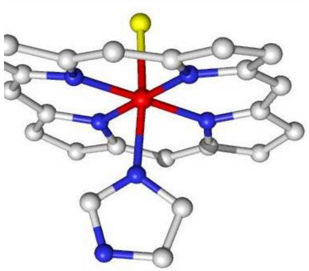
EPR detectable systems: $\boldsymbol{\mu_e \neq 0 \Rightarrow S \neq 0}$



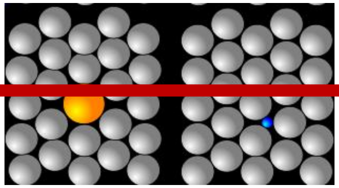
$CH_3-\overset{O^{+\bullet}}{\overset{\|}{C}}-H$

- **Odd electron number**:
  - Free radicals (organics, $OH^\bullet$, $NO^\bullet$, $NO_2^\bullet$, $HCO_3^\bullet$,…)
  - Transition metal ion compounds (Cu^{2+} , Fe^{3+} , Ni^{3+}, Mo^{5+}, V^{3+}, Ti^{3+},…)

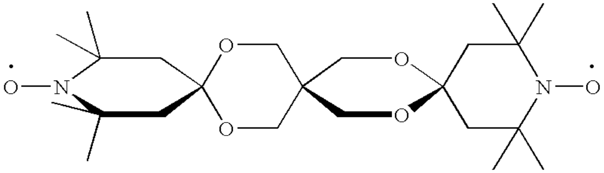




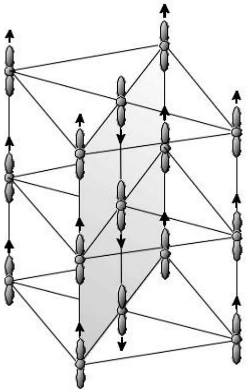
  - Impurities (doping) and defects in solids



- **Even electron number:**
  - Triplet states (excited or not), biradicals, O_2

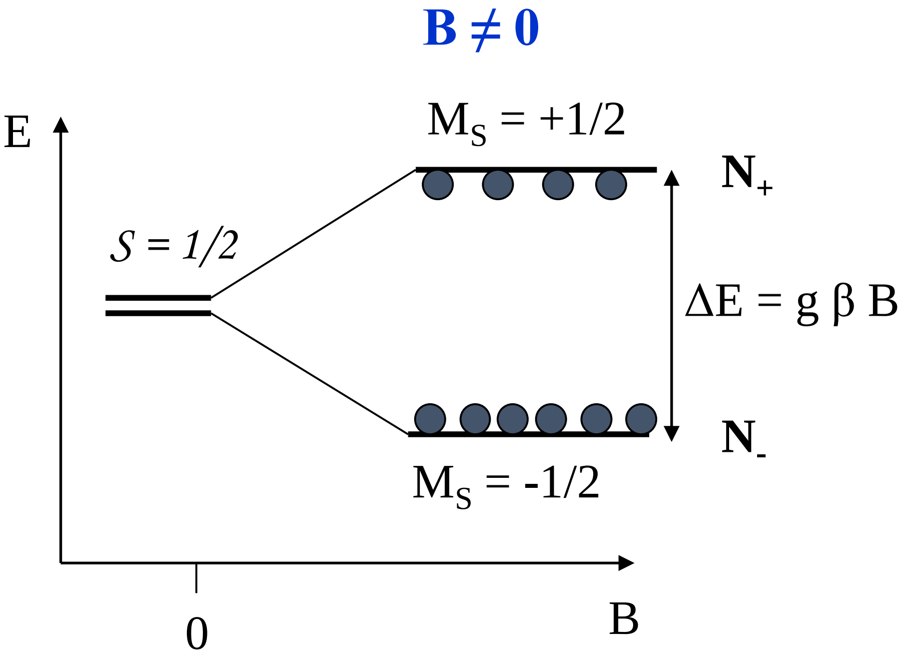


  - Conduction electrons, organic/inorganis molecular conductors, ferromagnets,….



Basic principles : the sensitivity of EPR

Thermal equilibrium and spin state populations



Weak value of $\Delta E = g\,\beta\,B$

$B = 0.3$ T $\Delta E \sim 0.3$ cm^{-1}

Thermal equilibrium (Boltzmann's law)

$$N_+ / N_- = \exp(-\Delta E / k_B T)$$

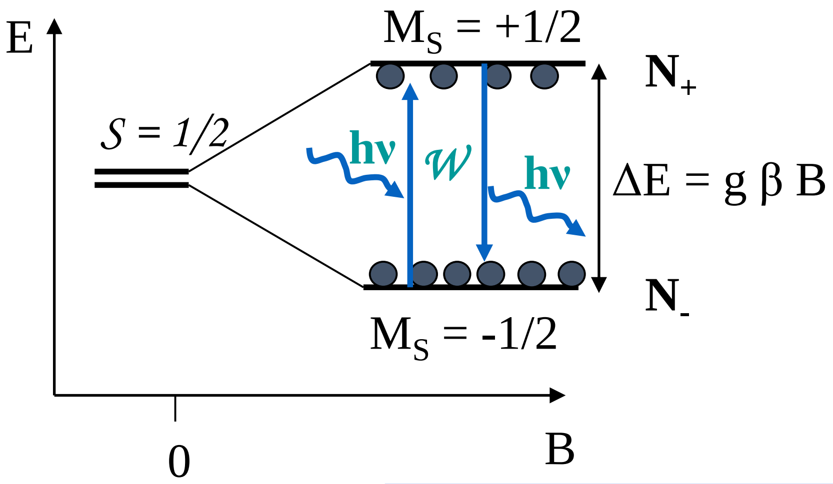
$$N_+ / N_- = \exp(-g\,\beta\,B / k_B T)$$

$T = 298$ K, $N_+/N_- = 0.9986$

Very weak spin polarization

$$p = (N_- - N_+)/(N_- + N_+) = 7{\cdot}10^{-4}$$

Basic principles : the sensitivity of EPR



Microwave induced transitions

$$B_1\,(t) = B_1 \cos(\omega t)$$

Same transition probability for absorption and emission

$$\mathcal{W} \propto B_1^2 \propto P_1 \ \text{(mW)}$$

EPR signal : net absorbed power

$P_{abs} = h\nu\,(\mathcal{W}N_- - \mathcal{W}N_+) = h\nu\,\mathcal{W}\,n$ with $n = N_- - N_+$

EPR signal intensity is directly related to n

Important consequence : Curie's law

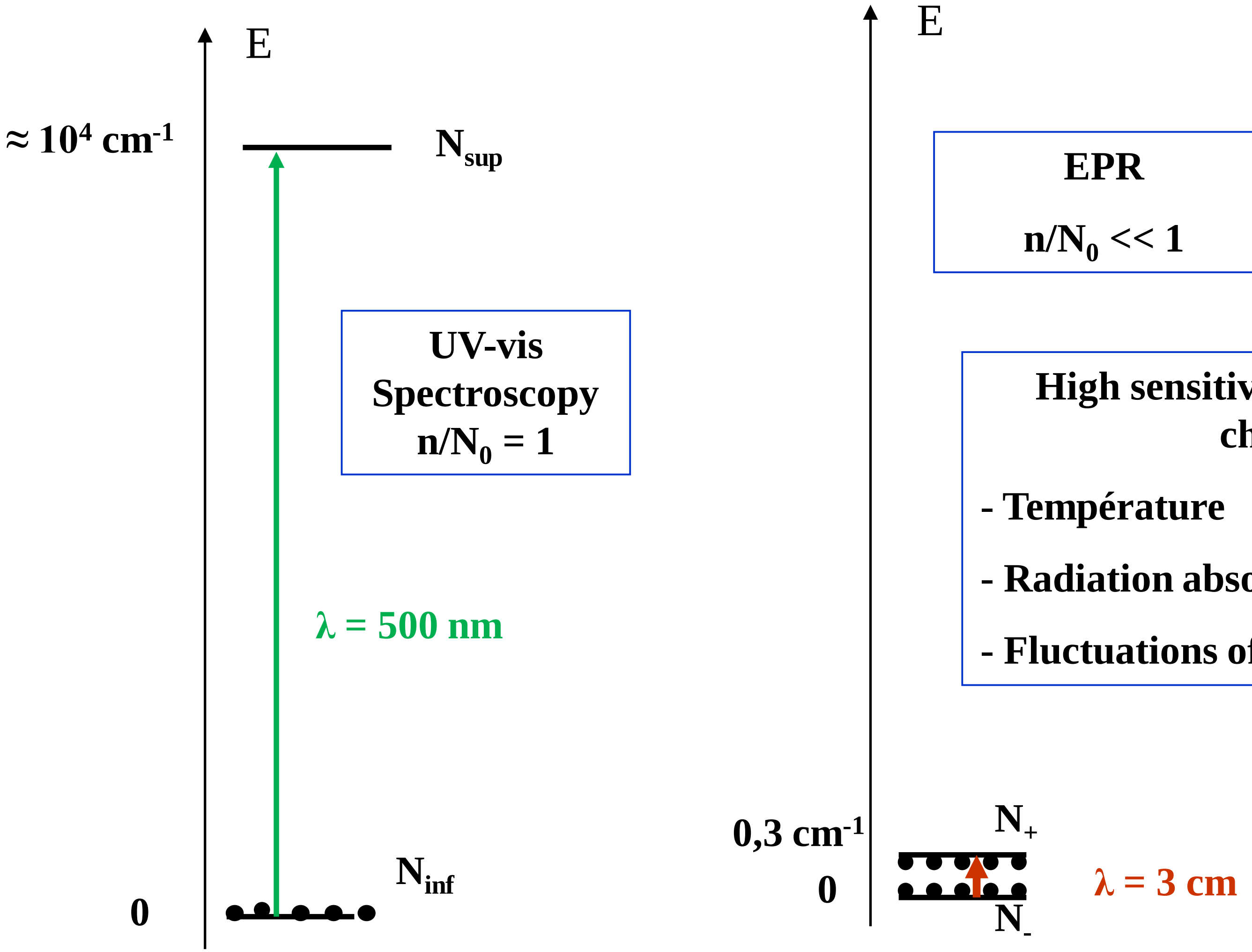
$I \propto n/N_0 = th(g\beta B_0\,/2k_BT) \approx g\beta B_0\,/2k_BT$

EPR signal intensity obeys the Curie's law **$I{\cdot}T = Cte$**

Pierre Curie

Basic principles : the sensitivity of EPR

$n = N_{inf} - N_{sup}$ population difference **Signal $\propto$ n**



UV-vis Spectroscopy
$n/N_0 = 1$

EPR

$n/N_0 << 1$

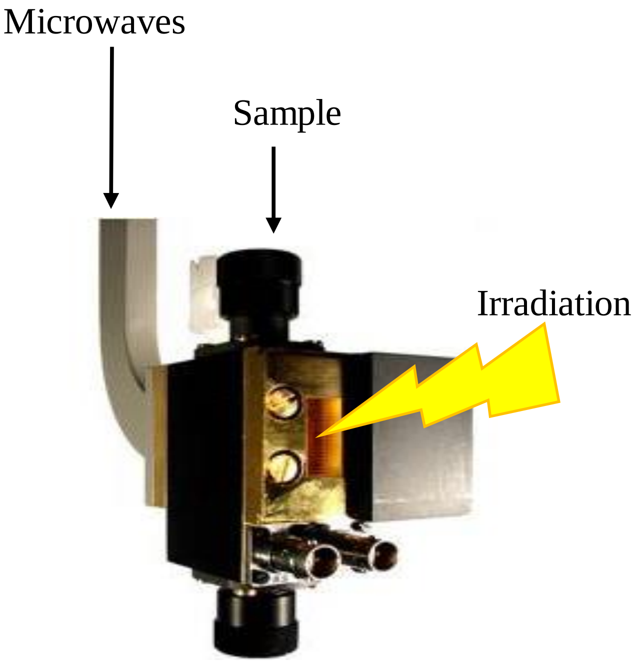
High sensitivity to population changes

- **Température**
- **Radiation absorption**
- **Fluctuations of the environment**

Basic principles: improving EPR sensitivity

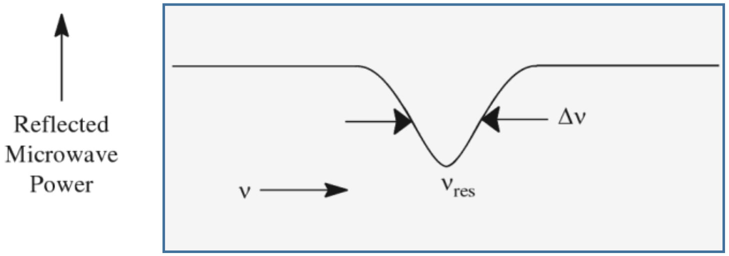
EPR signal intensity: $I \propto N_0\, g\beta B / 2k_B T$

- Sample concentration (N_0)
- Low temperatures (cryogeny: liquid N_2, He)
- High magnetic field / high frequency: Q-band 35GHz, W-band 95 GHz, …. 300 GHz
- Resonant cavity: Quality factor Q ~5-6000



Rectangular cavity TE102

Q = 2π énergie stockée / énergie dissipée = $\nu_{res} / \Delta\nu$



→ Sensitivity ∝ Q factor

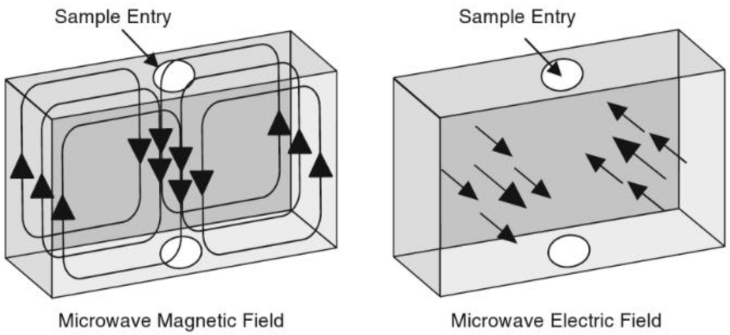
Basic principles: improving EPR sensitivity

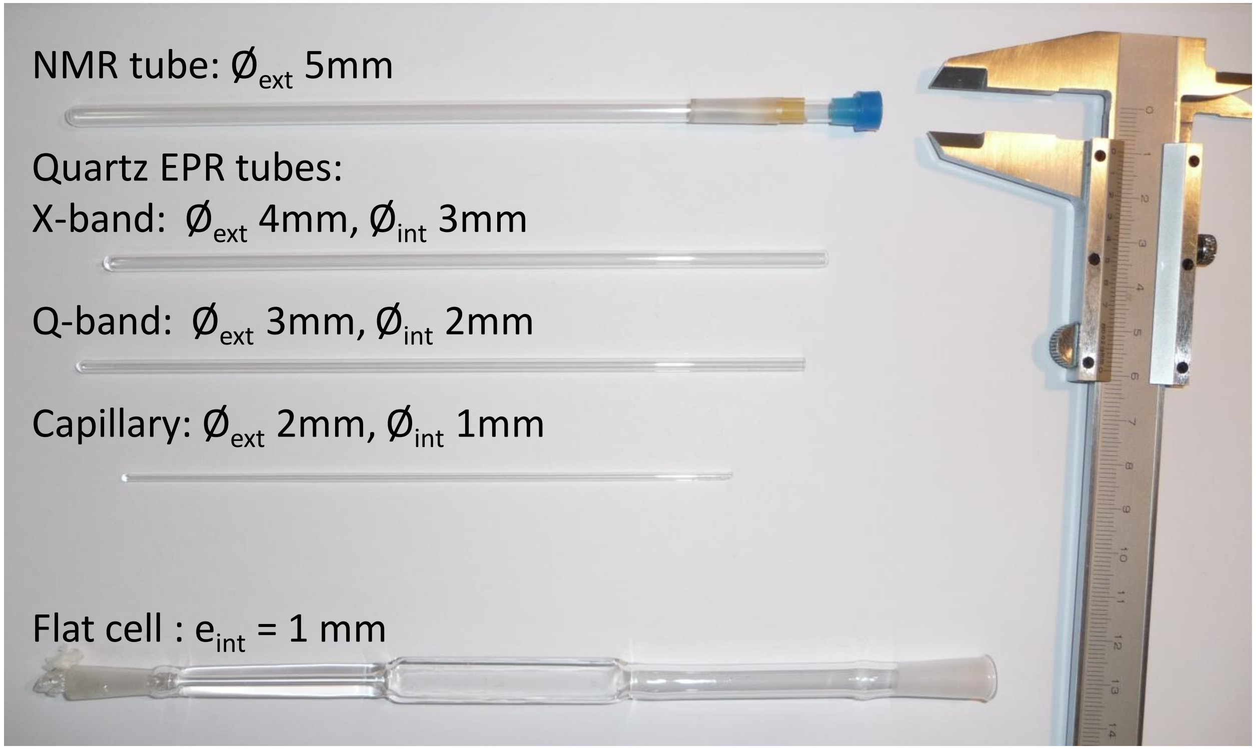
EPR does not like polar solvent: H2O, CH3OH, …

Dielectric absorption (ε_r)

↓

decrease of Q-factor





NMR tube: Ø$_{ext}$ 5mm

Quartz EPR tubes:

X-band: Ø$_{ext}$ 4mm, Ø$_{int}$ 3mm

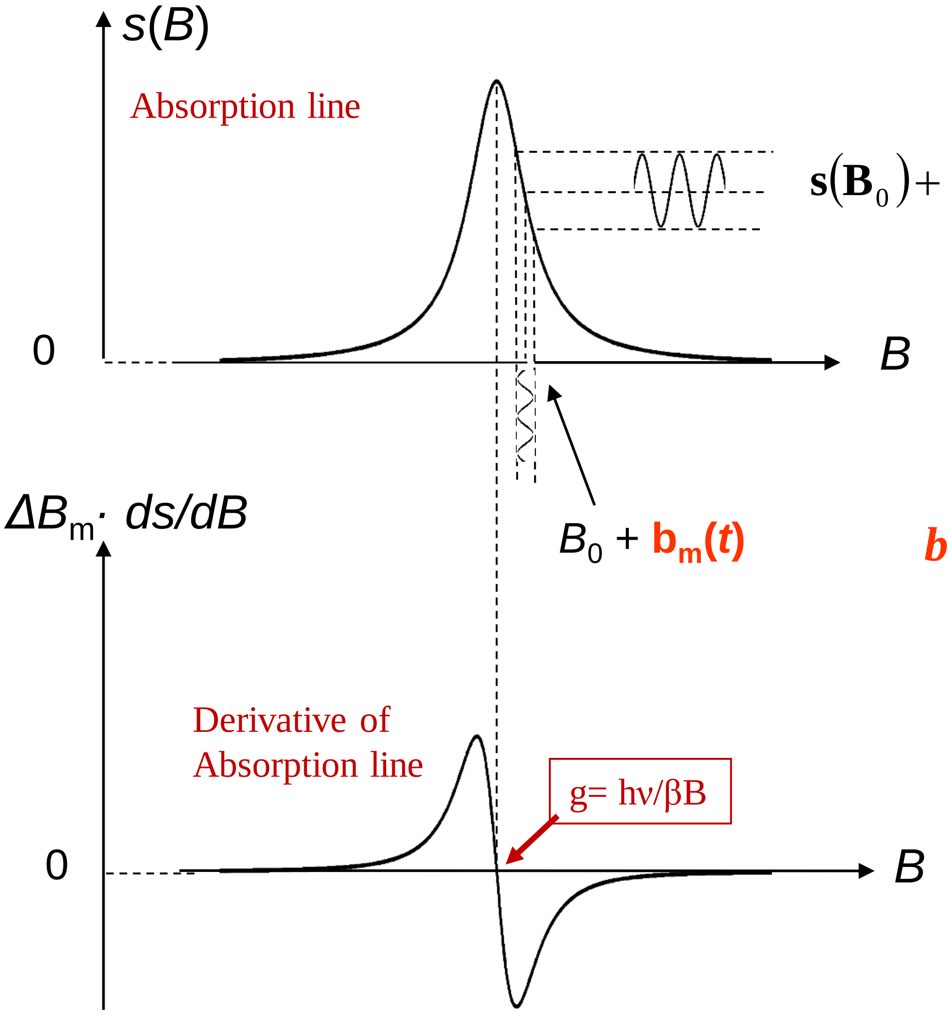
Q-band: Ø$_{ext}$ 3mm, Ø$_{int}$ 2mm

Capillary: Ø$_{ext}$ 2mm, Ø$_{int}$ 1mm

Flat cell : e_{int} = 1 mm

Basic principles: improving EPR sensitivity

Decrease the noise: Magnetic field amplitude modulation



ΔB_m << linewidth δB

if ΔB_m is « small »

$$s(B_0)+1/2\Delta B_m\left(\frac{ds}{dB}\right)_0\cos(2\pi\nu_m t)$$

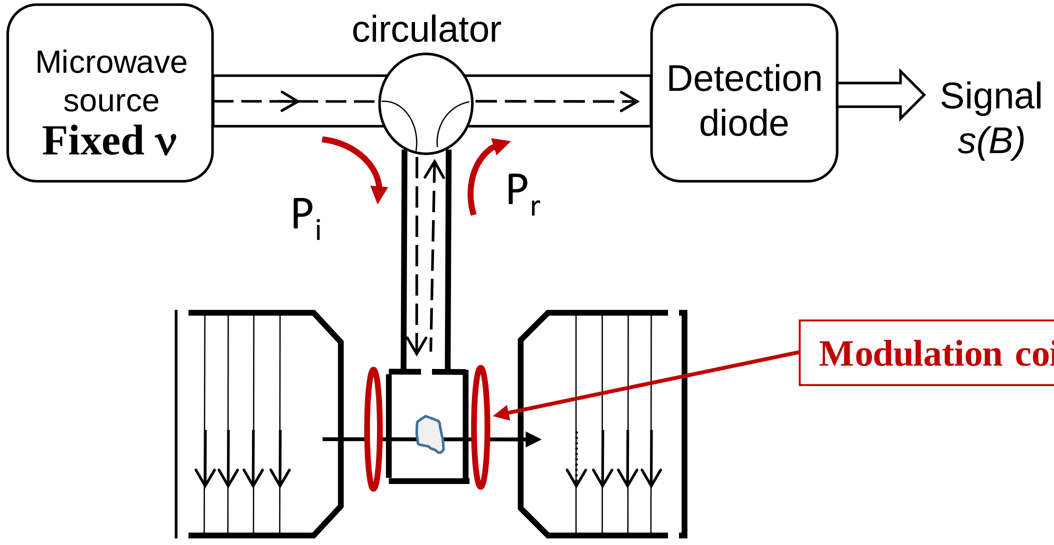
Magnetic field modulation

$$b_m(t) = 1/2\ \Delta B_m \cos(2\pi\nu_m t + \varphi)$$

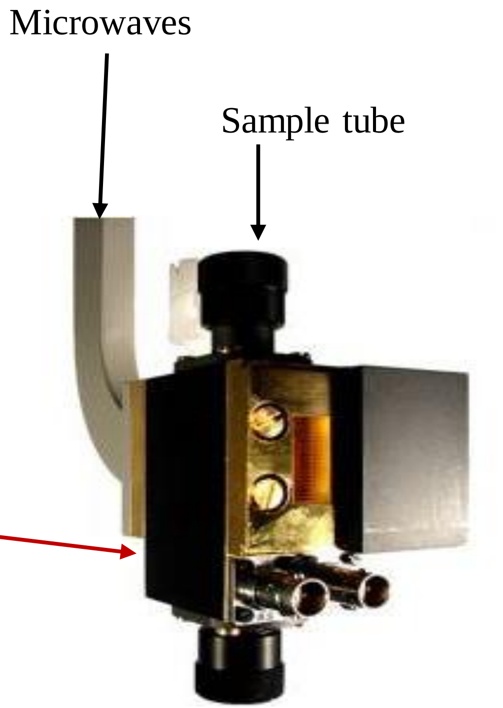
$\nu_m = 100$ **kHz**

Strong improvement of
the Signal /Noise ratio

Basic principles: improving EPR sensitivity



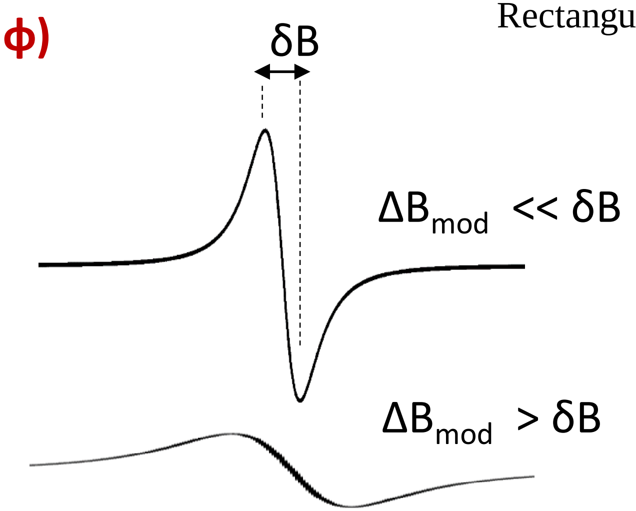
Modulation coils



Rectangular cavity TE102

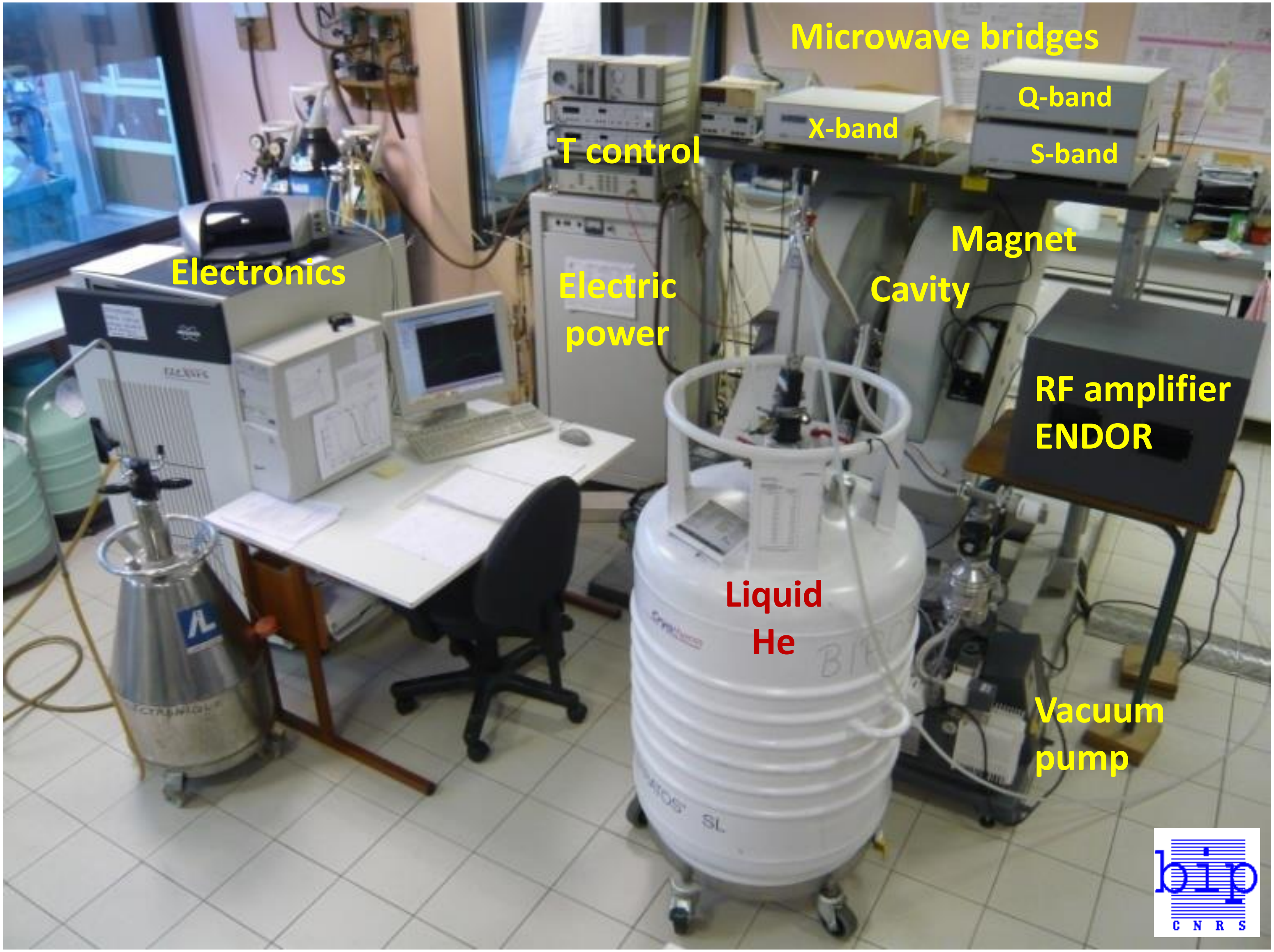
Resonant cavity

$\vec{B} + \Delta B_m \cos(2\pi\nu_m t + \phi)$



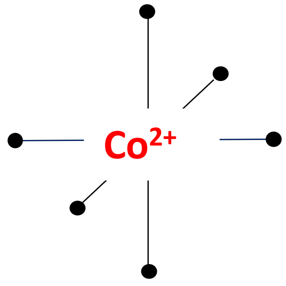
$\Delta B_m \ll$ linewidth δB
To avoid line broadening
by overmodulation

Multifrequency CW-EPR equipment

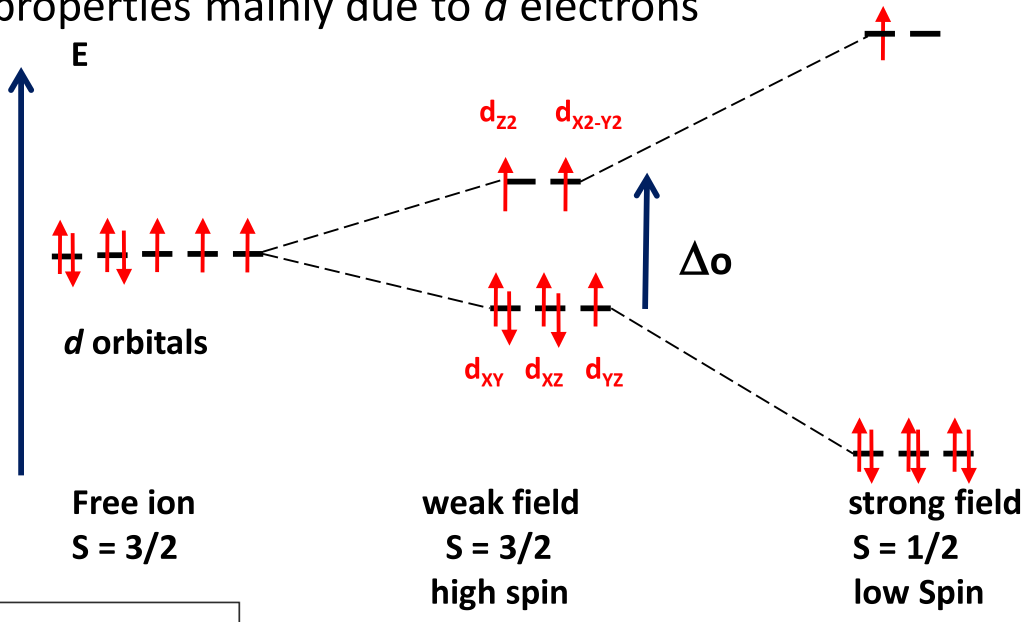


Transition metal compounds: Magnetic properties

Ligand field approach: Magnetic properties mainly due to *d* electrons



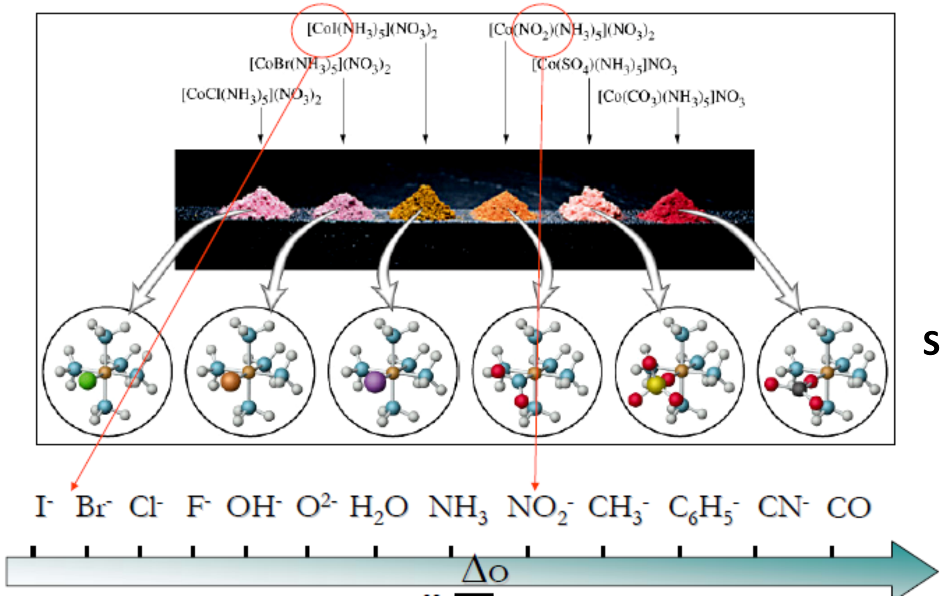
Octaedral complex (Oh) of Co^{2+} : $3d^7$



Free ion
S = 3/2

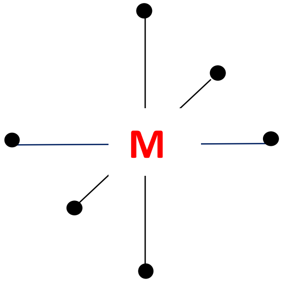
weak field
S = 3/2
high spin

strong field
S = 1/2
low Spin



Spectrochemical series $[Co\ X\ (NH_3)_5]^{2+}$

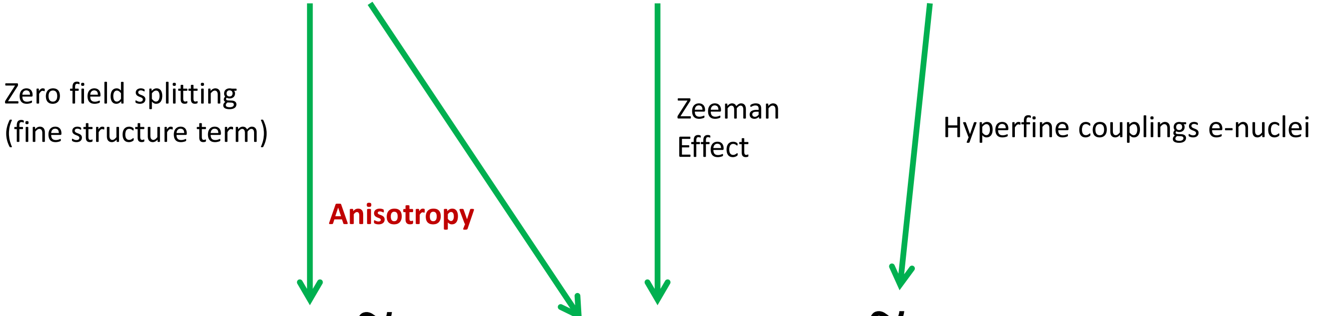
Transition metal compounds: Magnetic properties



Hamiltonian $$H = T_e + V_{en} + V_{ee} + V_L + \underbrace{H_{SO} + \text{magnetic terms}}_{\text{Requires the orbital part of the wavefunction to be calculated}}$$

Phenomenological approach: The spin hamiltonian H_S

$$H = \lambda\,\vec{L}\cdot\vec{S} + \beta\,(\vec{L} + g_e\vec{S})\cdot\vec{B} + \ couplages\,(\mu_e, \mu_n)\ldots$$



$$H_S = \vec{S}\,\tilde{D}\,\vec{S} + \beta\,\vec{S}\,\tilde{g}\,\vec{B} + \vec{S}\,\tilde{A}\,\vec{I}$$

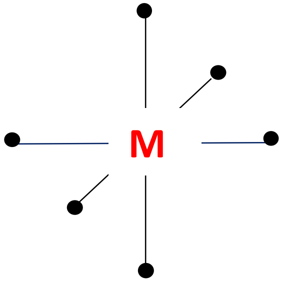
Terms : Fine structure Zeeman Hyperfine

$\tilde{D}, \tilde{g}, \tilde{A}$ Rank 2 tensors $\Rightarrow$ 3 x 3 matrix

$$\tilde{g} = \begin{bmatrix} g_{xx} & g_{xy} & g_{xz} \\ g_{yx} & g_{yy} & g_{yz} \\ g_{zx} & g_{zy} & g_{zz} \end{bmatrix}$$

Transition metal compounds: Magnetic properties

$$H_S = \vec{S}\,\tilde{D}\,\vec{S} + \beta\,\vec{S}\,\tilde{g}\,\vec{B} + \vec{S}\,\tilde{A}\,\vec{I}$$



Zeeman term: Zeeman effect + 2nd order effect of spin-orbit coupling

$$H_{Zeeman} = \beta\,\vec{S}\,\tilde{g}\,\vec{B}$$

$$H_{Zeeman} = \beta\,(S_X\ g_X\ B_X + S_Y\ g_Y\ B_Y + S_Z\ g_Z\ B_Z)$$

- Departure of *g* values from $g_e = 2.0023$
- Anisotropy

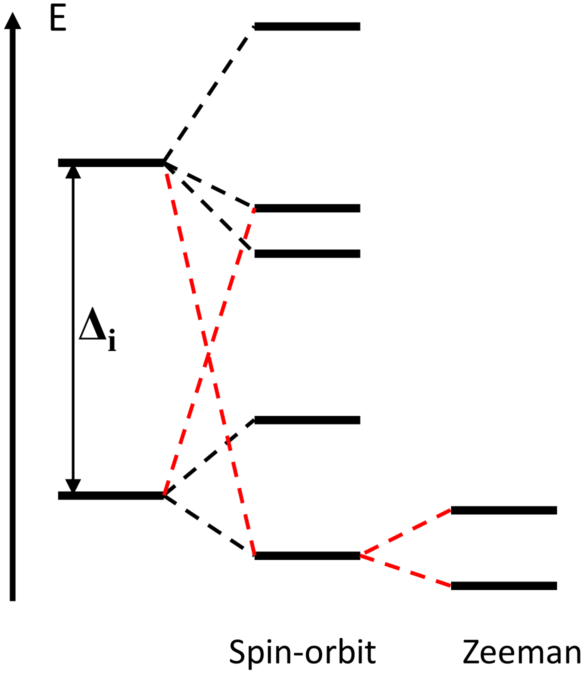
$$\mathrm{g_i} = \mathrm{g_e} - \alpha_\mathrm{i} \cdot \frac{\lambda}{\Delta_\imath} \quad \text{(i = x, y, z)}$$

λ = spin-orbit coupling constant

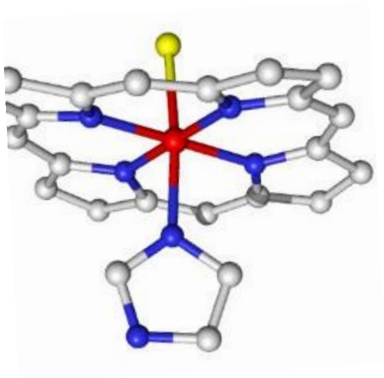
$\mathrm{d^n}$ configuration

$n < 5\ ,\ \lambda > 0 \Rightarrow \mathrm{g_i} < \mathrm{g_e} = 2.00$

$n > 5\ ,\ \lambda < 0 \Rightarrow \mathrm{g_i} > \mathrm{g_e} = 2.00$



Transition metal centers: Magnetic properties



Anisotropic $\tilde{g}$ tensor

(X, Y, Z) principal axes of g:

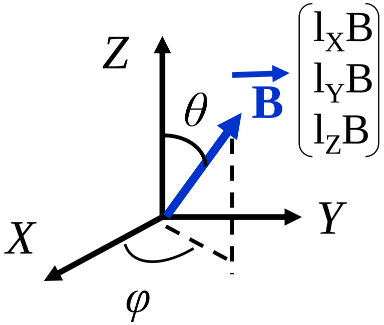
(g_X, g_Y, g_Z) principal g-values

Magnetic axes are related to symmetry axes and atomic positions

$$H_{Zeeman} = \beta\,\vec{S}\,\tilde{g}\,\vec{B}$$

$$H_{Zeeman} = \beta\,(S_X\,g_X\,B_X + S_Y\,g_Y\,B_Y + S_Z\,g_Z\,B_Z\,)$$

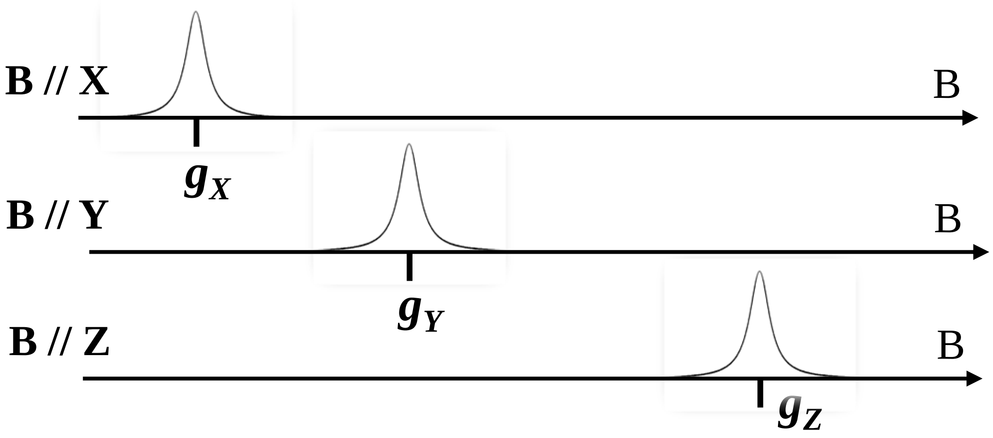
$$H_{Zeeman} = \beta\,g'\,\vec{S}\cdot\vec{B}'$$

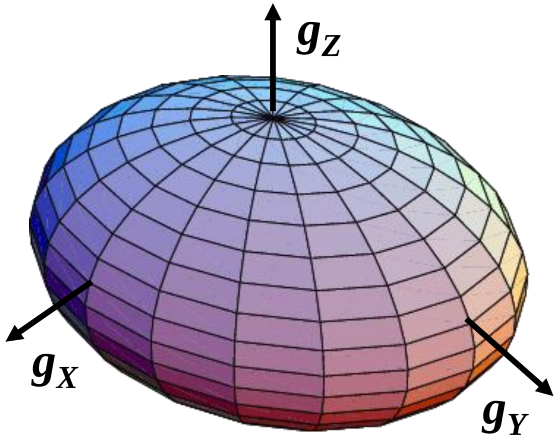


The line position ***g' depends on the B orientation***

$$g'^2 = l_X{}^2\,g_X{}^2 + l_Y{}^2\,g_Y{}^2 + l_Z{}^2\,g_Z{}^2$$

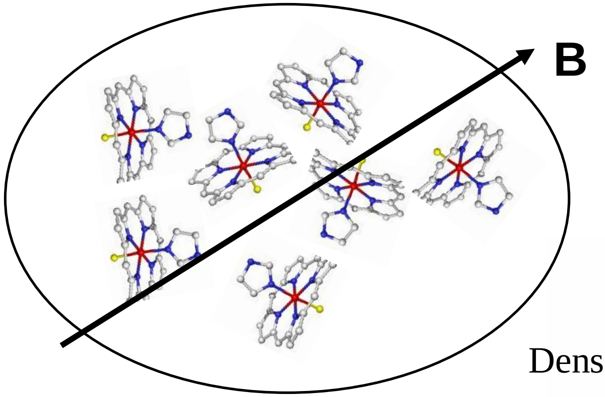
$$g'^2 = \cos^2\varphi\cdot\sin^2\theta\,g_X{}^2 + \sin^2\varphi\cdot\sin^2\theta\,g_Y{}^2 + \cos^2\theta\,g_Z{}^2$$



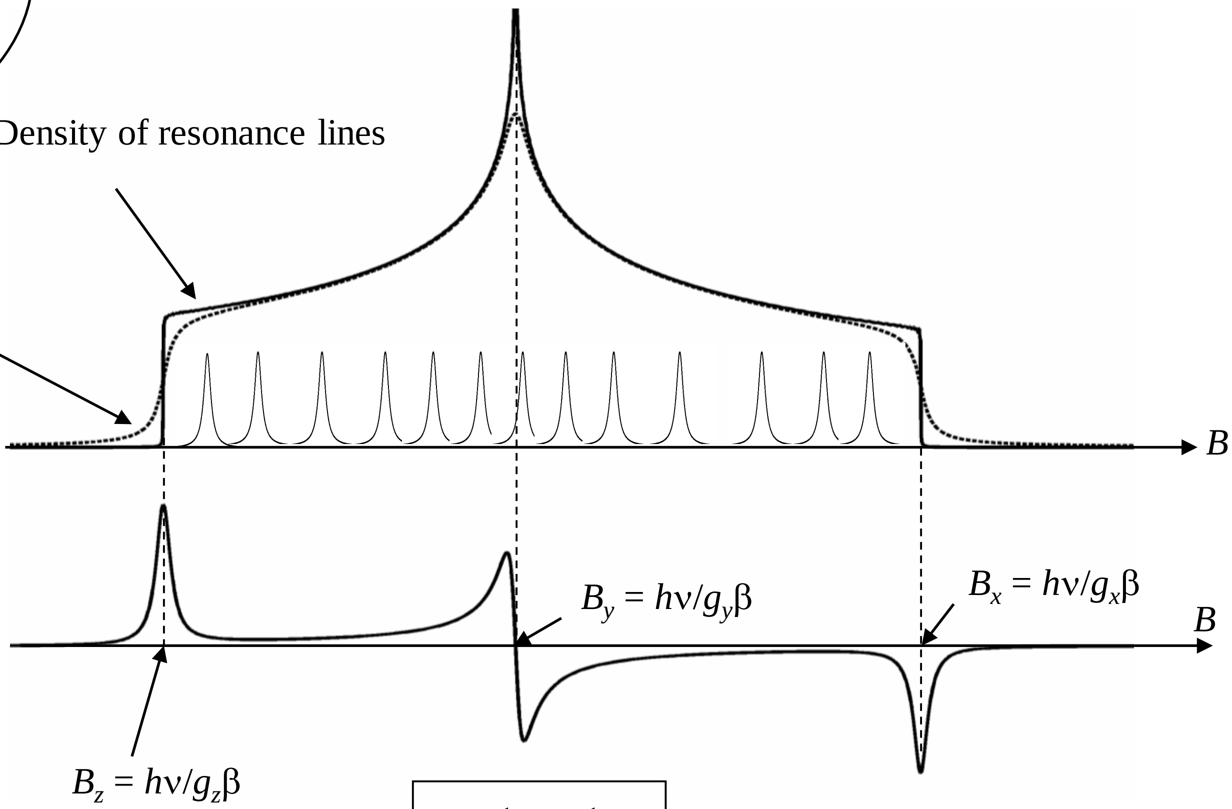


Transition metal compounds: Magnetic properties

Anisotropic *g* tensor– Powder or frozen solution spectrum



Disordered system: all the B orientations are present



Density of resonance lines

Absorption signal

Experimental spectrum = **derivative of absorption signal**

$B_z = h\nu/g_z\beta$

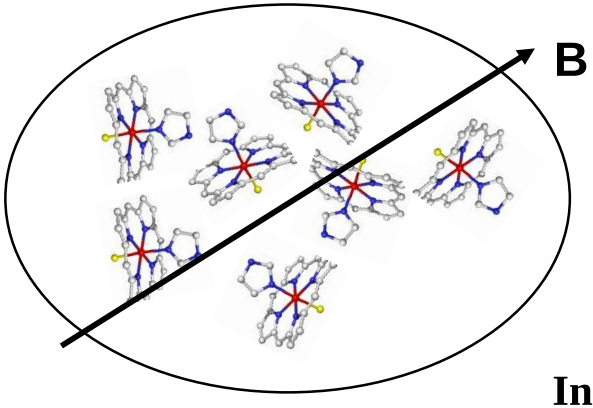
$B_y = h\nu/g_y\beta$

$B_x = h\nu/g_x\beta$

$g_X < g_Y < g_Z$

Transition metal compounds: Magnetic properties

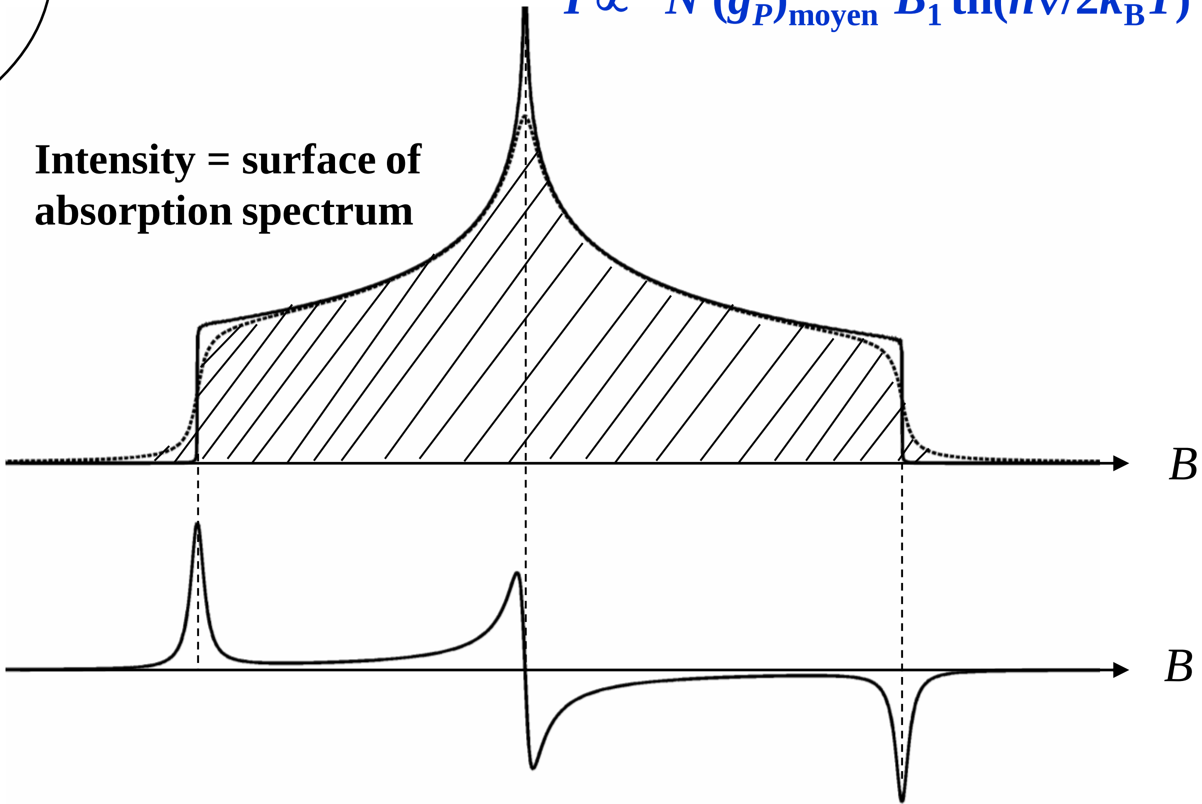
Anisotropic *g* tensor– Powder or frozen solution spectrum



Disordered system: all the B orientations are present

EPR signal intensity:

$$I \propto N\, (g_P)_{\text{moyen}}\, B_1\, \text{th}(h\nu/2k_BT)$$



Intensity = surface of absorption spectrum

double numerical integration

Experimental spectrum (absorption derivative)

Spin quantitation by comparison to a reference sample: $I/I_0 = N/N_0$

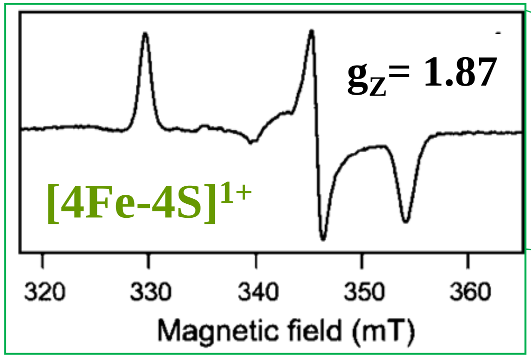
Transition metal compounds: Magnetic properties

g-tensor analysis

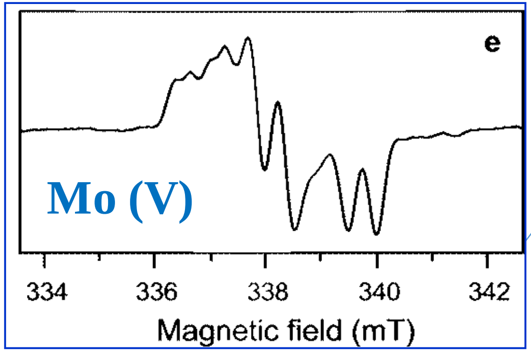
- Identification of magnetic centers
- Selective view of magnetic centers and of their environment (nuclei)
- No limit in size or physical state: solution, powder, crystals, membranes, cells…

g_X = 2.04 g_Y =1.94

15 K

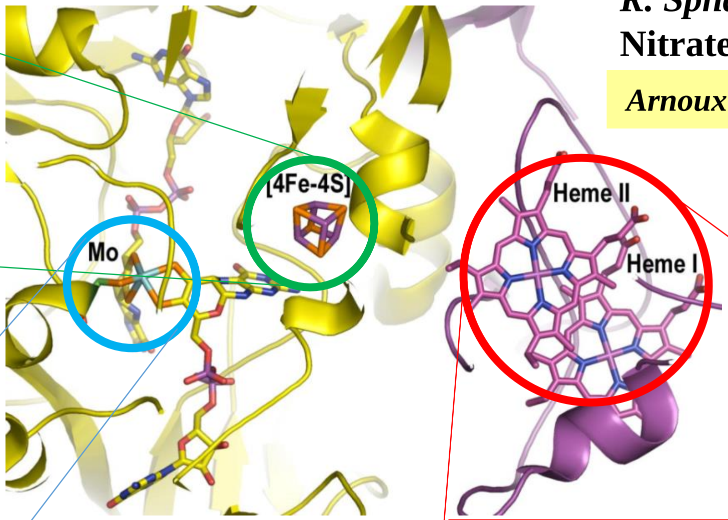


55 K



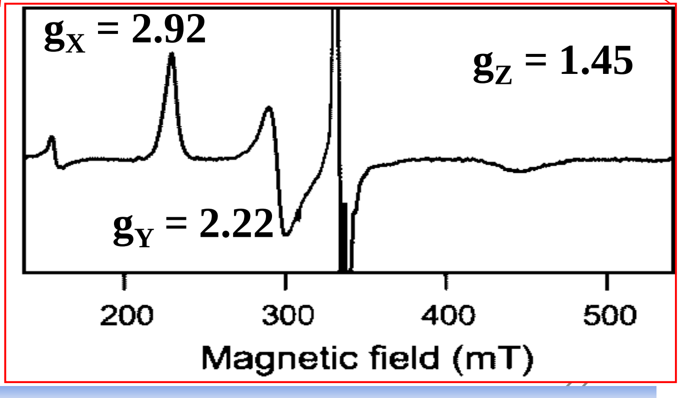
R. Sphaeroides periplasmic Nitrate reductase - NapAB

Arnoux et al., Nat. Struct. Biol. 2003

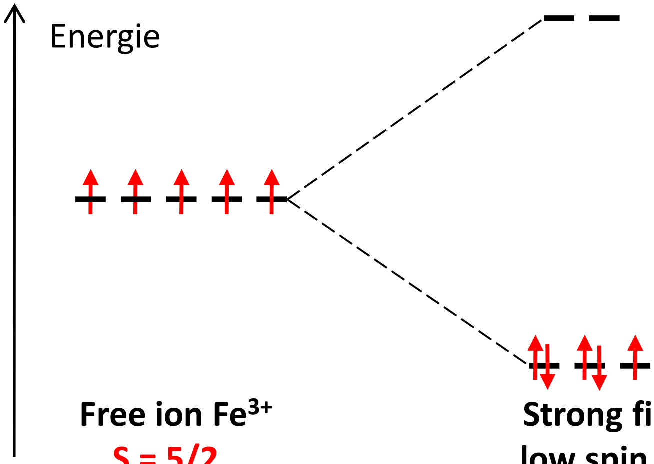


Hemes (Fe^{3+})

15 K



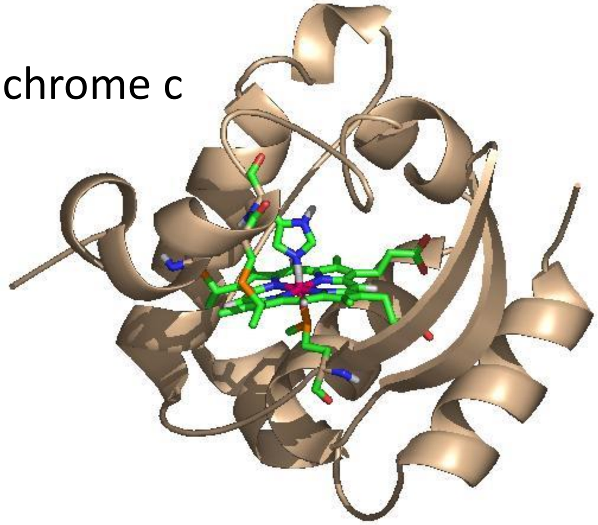
Low spin Fe^{3+} systems: hemes

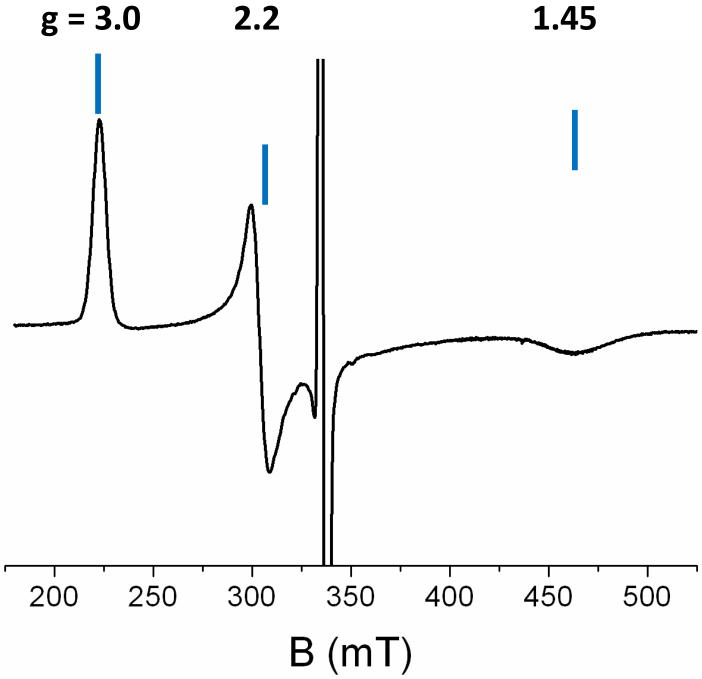


Free ion Fe^{3+}
S = 5/2
Ground states ^6S

Strong field
low spin S = 1/2
$^2T_2$

Cytochrome c





Ground state $^2T_{2g}$

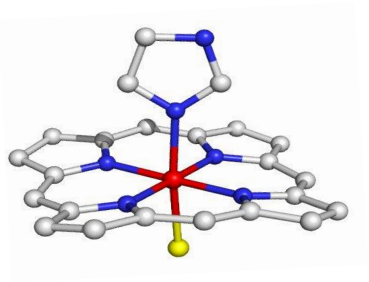
Orbital triplet state, S = ½

No fine structure term, but strong influence of Spin-Orbit coupling on g-tensor anisotropy.

$$H_S = \beta \vec{S} \tilde{g} \vec{B}$$

Low spin Fe^{3+} systems: hemes

Cytochromes



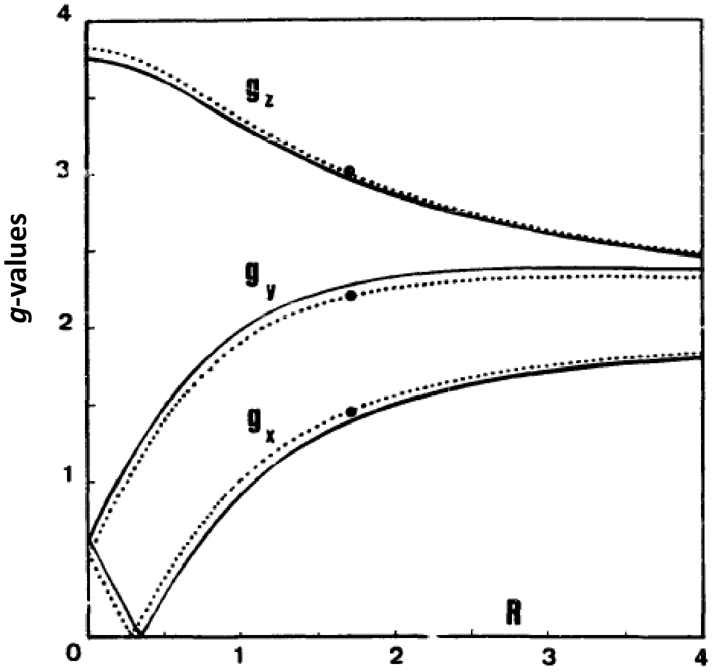
Magneto-structural correlations:

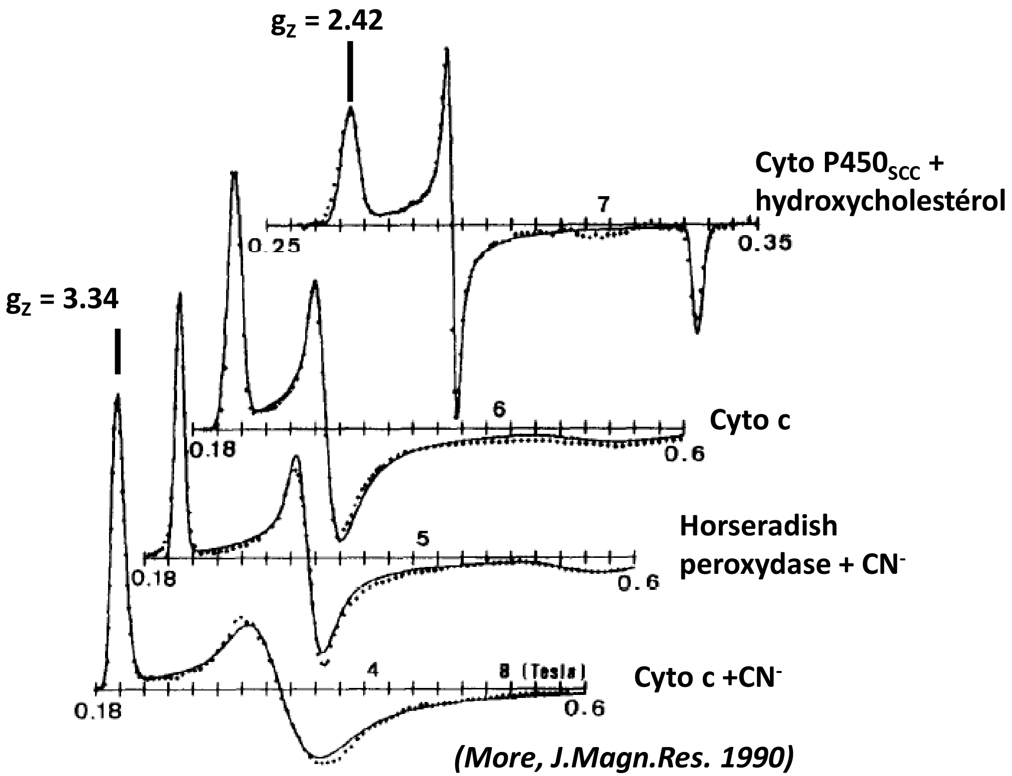
t$_{2g}$ hole model (Griffith, 1971)
Fe^{3+} ion in strong distorted octaedral ligand field

$$\mathscr{H}(\mu, R, \lambda) = -\lambda \mathbf{L}\cdot\mathbf{S} + \frac{\mu}{9}(3L_z^2 - L(L+1)) + \frac{R}{12}(L_+^2 + L_-^2)$$

μ and R, axial and rhombic components of the ligand field
$g_i = f(\lambda, \mu, R)$: $g_X^2 + g_Y^2 + g_Z^2 = 16$

Also accounts for g-strain broadening

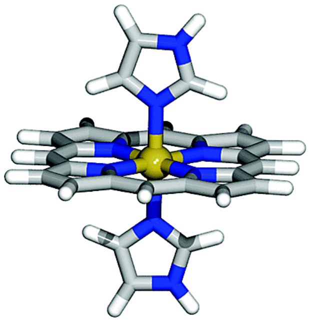




(More, J.Magn.Res. 1990)

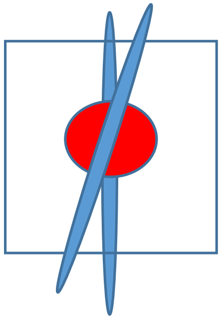
Low spin Fe^{3+} systems: hemes

Magneto-structural correlations: Hemes with bis-Histidine axial coordination

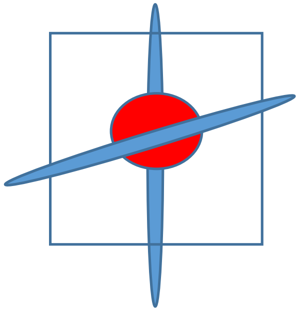


The rhombicity depends on the ϕ angle between imidazole planes

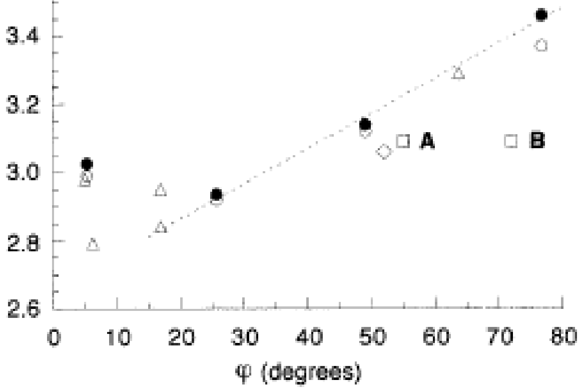
g_z increases when ϕ increases



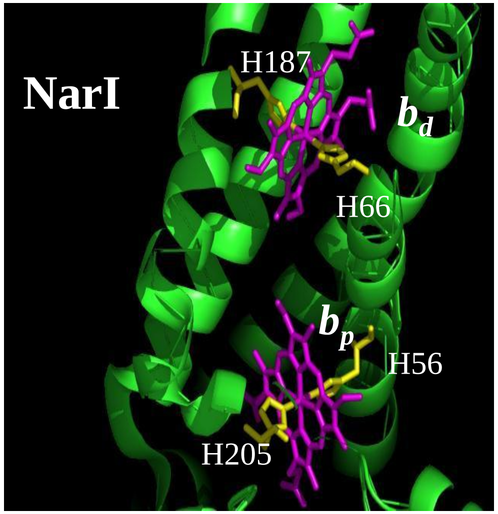
ϕ ≈ 10- 20°
g_z = 3.0

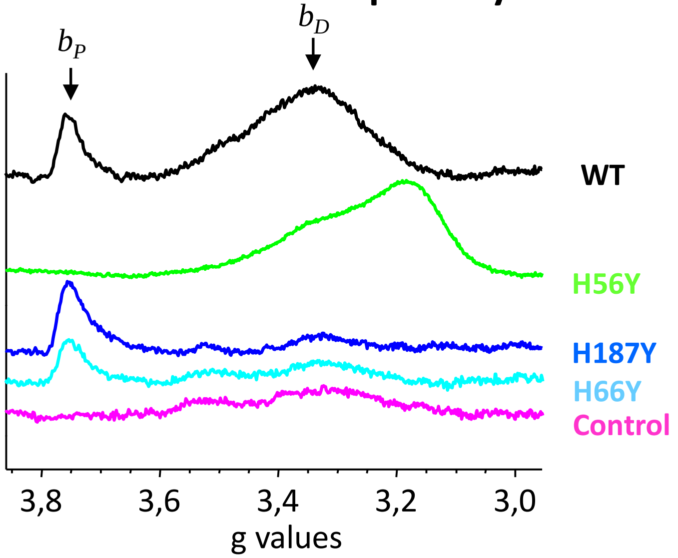


ϕ ≈ 80-90°
g_z = 3.8
HALS hemes



b-type hemes of the membrane-bound subunit of the respiratory nitrate reductase NarGHI





EPR of *E. coli* membrane fractions

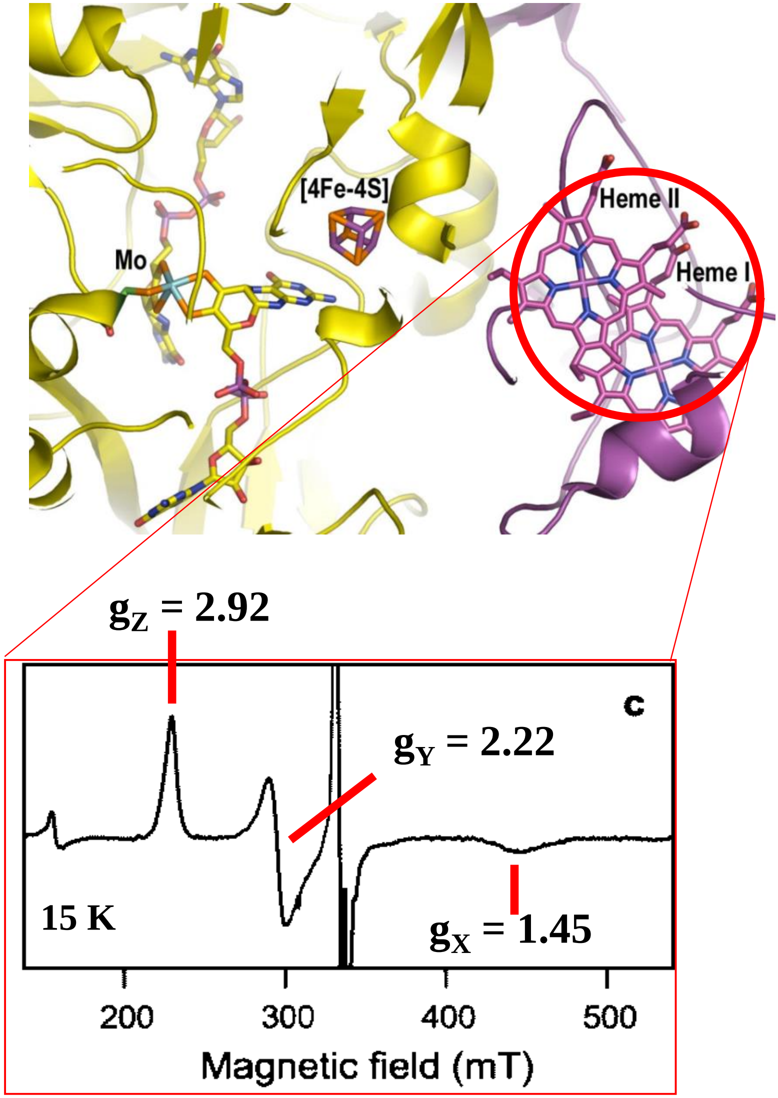
Low spin Fe^{3+} systems: hemes

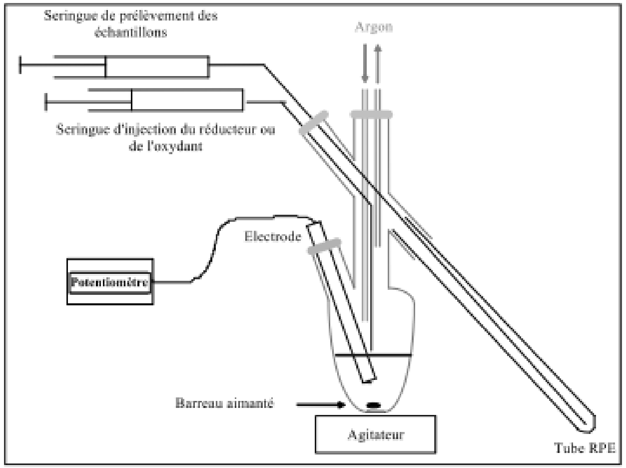
Redox titrations: E° measurements

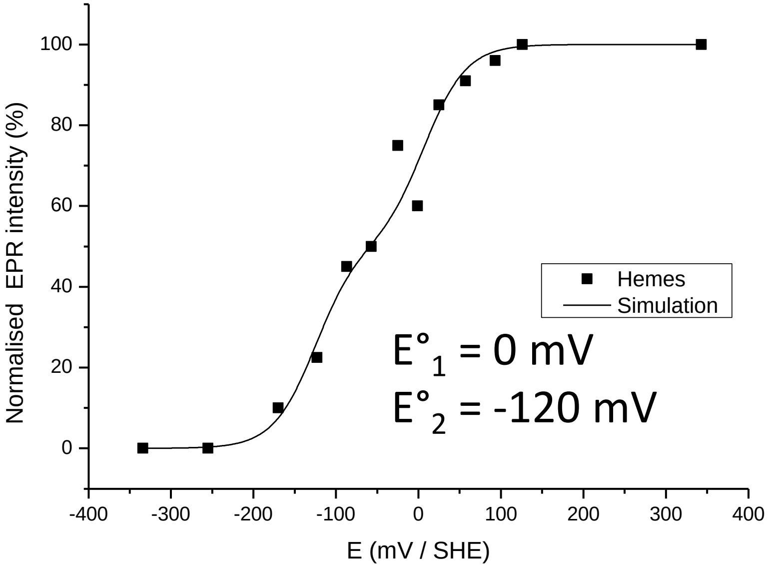
$Fe^{3+} + e^{-} = Fe^{2+}$

S=1/2 S=0

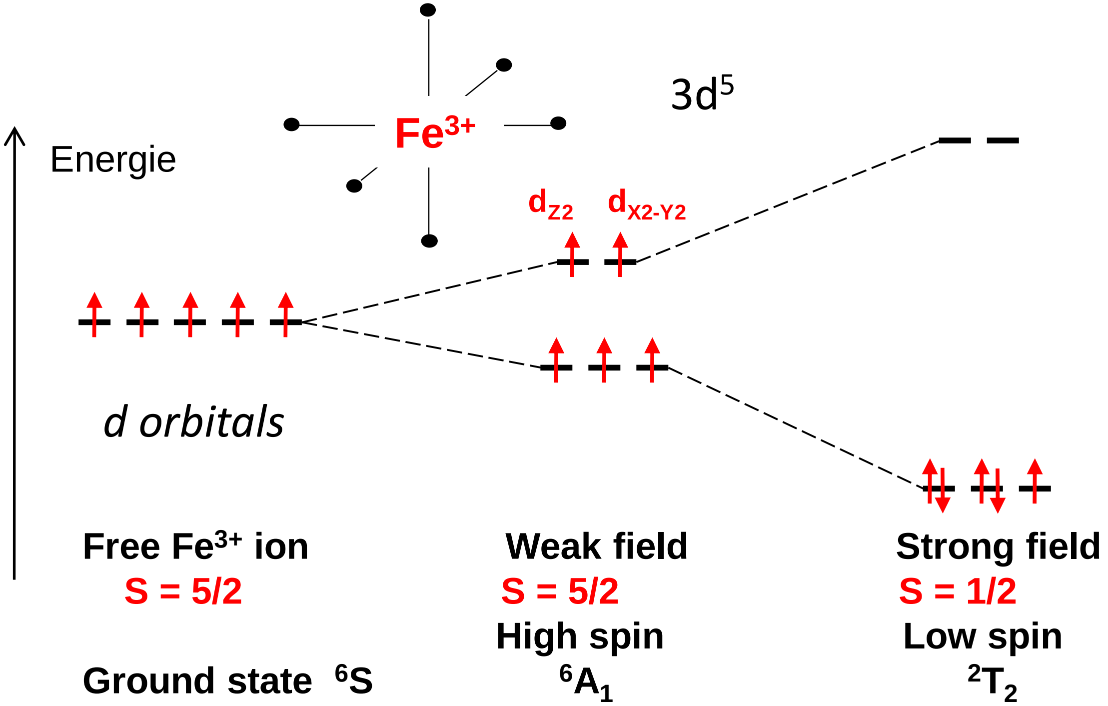
***R. Sphaeroides* periplasmic Nitrate reductase - NapAB**







High spin Fe^{3+} systems

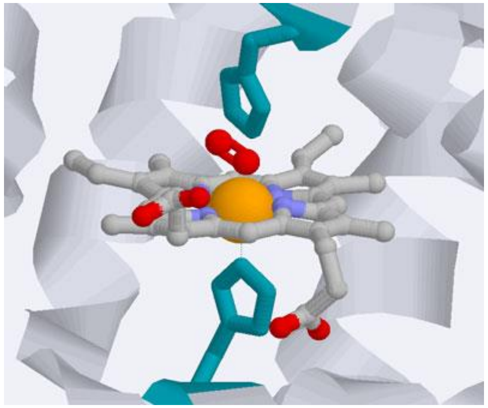


Free Fe^{3+} ion
$S = 5/2$

Ground state ^6S

Weak field
$S = 5/2$
High spin
$^6A_1$

Strong field
$S = 1/2$
Low spin
$^2T_2$



Myoglobine
$g_{\perp} = 6.0$, $g_{//} = 2.0$

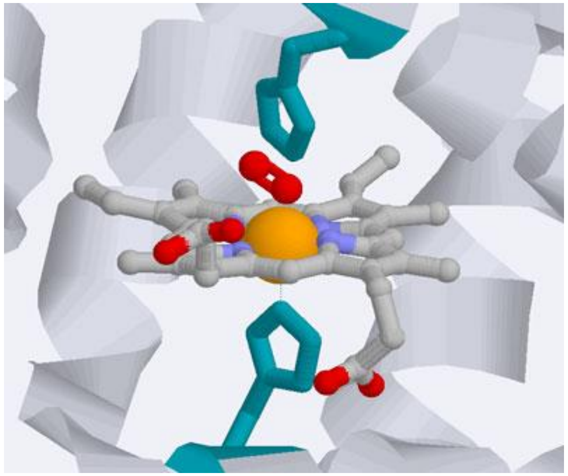
Cytochrome b_2
g= 2.92, 2.27, 1.5

High spin Fe^{3+} systems

S = 5/2 , M_S = -5/2, -3/2, -1/2, +1/2, +3/2, +5/2

6 states {**|S, M_S>}**

Heme in Myoglobine



Axial symmetry– Influence of fine structure (Zero field splitting)

D axial, ***g*** isotropic

$$H_S = \vec{S}\ \tilde{D}\ \vec{S} + \beta\ \vec{S}\ \tilde{g}\ \vec{B}$$

$$H_{SF} = D\,(S_Z^2 - \frac{1}{3}S(S+1)) + g\ \beta\ \vec{S} \cdot \vec{B}$$

Case **D >> g β B** **(0.3 cm^{-1} at 0.3 T)**

- B = 0 : Zero field splitting

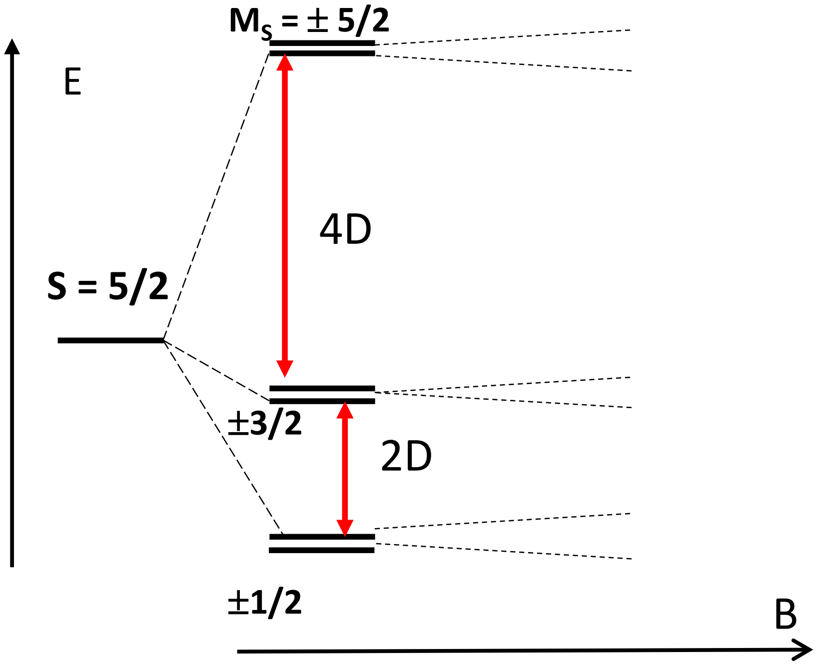
$S_Z\ |S,M_S> = M_S\ |S,M_S>$

$\Delta M_S = 0$

$S_Z^2\ |S,M_S> = M_S^2\ |S,M_S>$

E (M_S) = D (M_S^2 – 1/3 S(S+1))

E (M_S) = D (M_S^2 – 35/12)



High spin Fe^{3+} systems

– Axial symmetry – Influence of fine structure

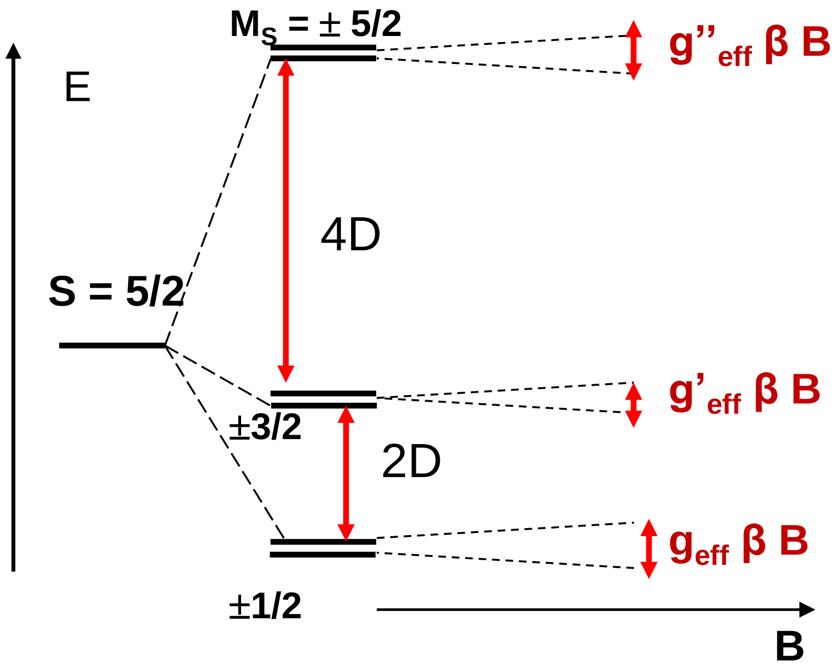
Case **D >> g β B** **($0.3\ cm^{-1}$ at X-band)**

- B ≠ 0 :

$$H_{Zeeman} = g\,\beta\,(S_X B_X + S_Y B_Y + S_Z B_Z)$$

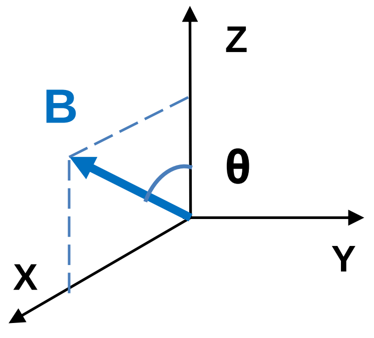
$$= g\beta\,B\,(S_Z \cos\theta + 1/2(S_+ + S_-)\sin\theta)$$

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



Perturbation approach:

$$H_{Zeeman} = \tfrac{1}{2}\, g\, \beta\, B \quad \begin{array}{c|cc|cc|cc|c}
|M_S> & |-5/2> & |+5/2> & |-3/2> & |+3/2> & |-1/2> & |+1/2> & \\
 & -5\cos\theta & 0 & X & 0 & 0 & 0 & |-5/2> \\
 & 0 & +5\cos\theta & 0 & X & 0 & 0 & |+5/2> \\
\hline
 & X & 0 & -3\cos\theta & 0 & X & 0 & |-3/2> \\
 & 0 & X & 0 & +3\cos\theta & 0 & X & |+3/2> \\
\hline
 & 0 & 0 & X & 0 & -\cos\theta & 3\sin\theta & |-1/2> \\
 & 0 & 0 & 0 & X & 3\sin\theta & +\cos\theta & |+1/2>
\end{array}$$



$$S_X = ½\,(S_+ + S_-)$$

$S_+ |S,M_S> = [(S(S+1) - M_S (M_S + 1)]^{1/2} |S,M_S +1>$

$S_- |S,M_S> = [(S(S+1) - M_S (M_S - 1)]^{1/2} |S,M_S -1>$

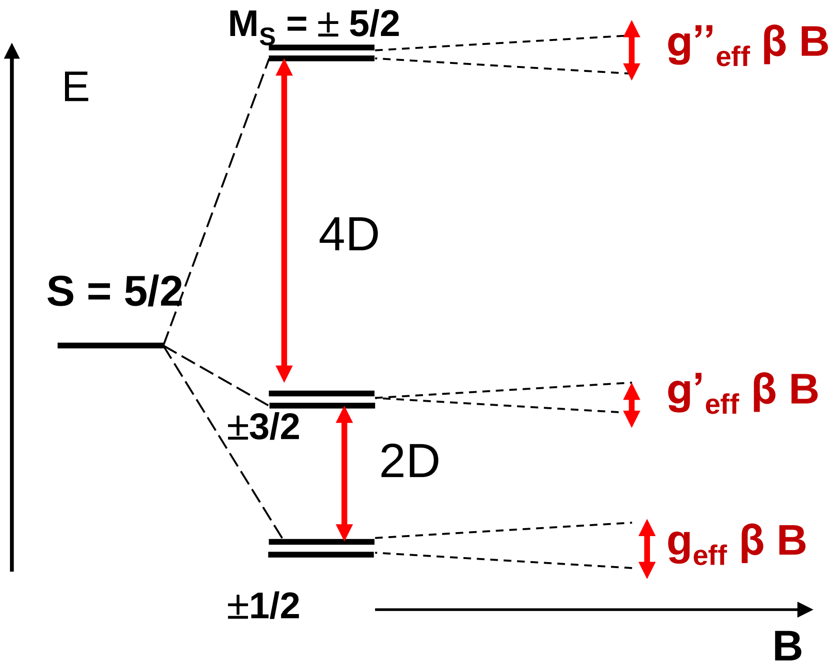
High spin Fe^{3+} systems

Axial symmetry - **$D >> g \beta B$**

$M_S = \pm 5/2$ g''_{eff} axial : $g''_{eff\,//} = 5g = 10$, $g''_{eff\perp} = 0$

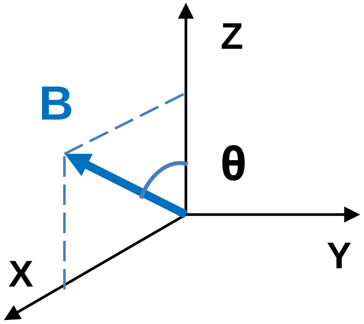
$M_S = \pm 3/2$ g'_{eff} axial : $g'_{eff\,//} = 3g = 6$, $g'_{eff\perp} = 0$

$M_S = \pm 1/2$ g_{eff} axial : $g_{eff\,//} = g = 2$, $g_{eff\perp} = 3g = 6$



1st order perturbation calculation

$$H_{Zeeman} = \frac{1}{2} g \beta B \quad \begin{array}{c|cc|cc|cc} |M_S> & |-5/2> & |+5/2> & |-3/2> & |+3/2> & |-1/2> & |+1/2> \\ \hline & -5\cos\theta & 0 & X & 0 & 0 & 0 \\ & 0 & +5\cos\theta & 0 & X & 0 & 0 \\ \hline & X & 0 & -3\cos\theta & 0 & X & 0 \\ & 0 & X & 0 & +3\cos\theta & 0 & X \\ \hline & 0 & 0 & X & 0 & -\cos\theta & 3\sin\theta \\ & 0 & 0 & 0 & X & 3\sin\theta & +\cos\theta \end{array}$$



High spin Fe^{3+} systems

Axial symmetry- **D >> g β B**

$M_S = \pm 5/2$ g''_{eff} axial : $g''_{eff\,//} = 5g = 10$, $g''_{eff\perp} = 0$

$M_S = \pm 3/2$ g'_{eff} axial : $g'_{eff\,//} = 3g = 6$, $g'_{eff\perp} = 0$

$M_S = \pm 1/2$ g_{eff} axial : $g_{eff\,//} = g = 2$, $g_{eff\perp} = 3g = 6$

Allowed EPR transitions: $\Delta M_S = \pm 1$

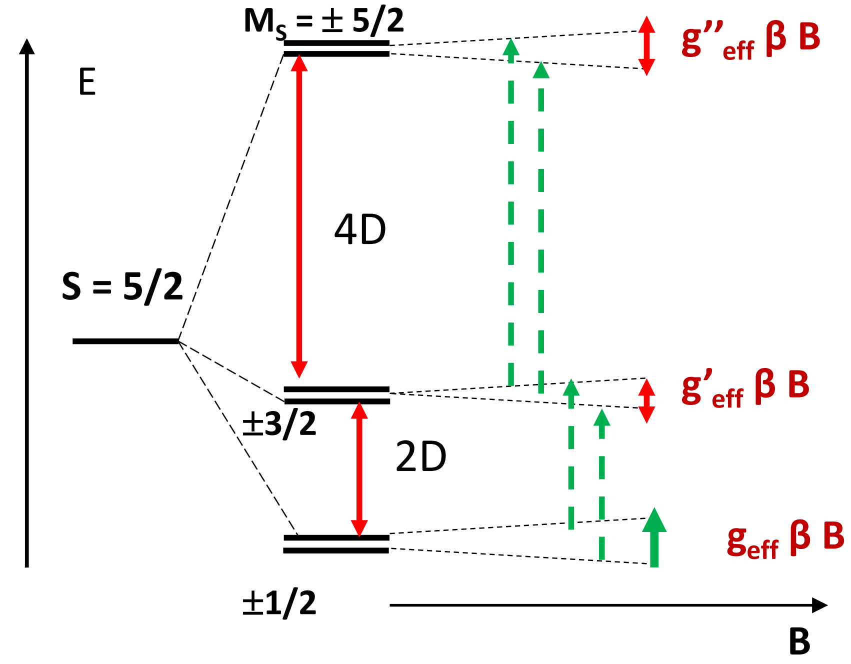
5 allowed transitions 🟩

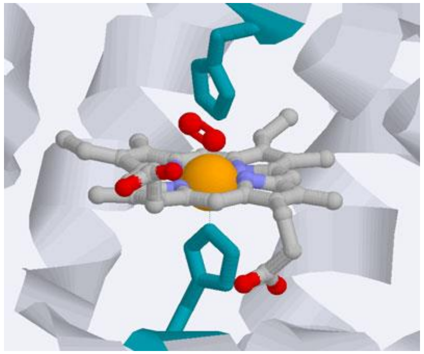
Only one is energetically accessible :

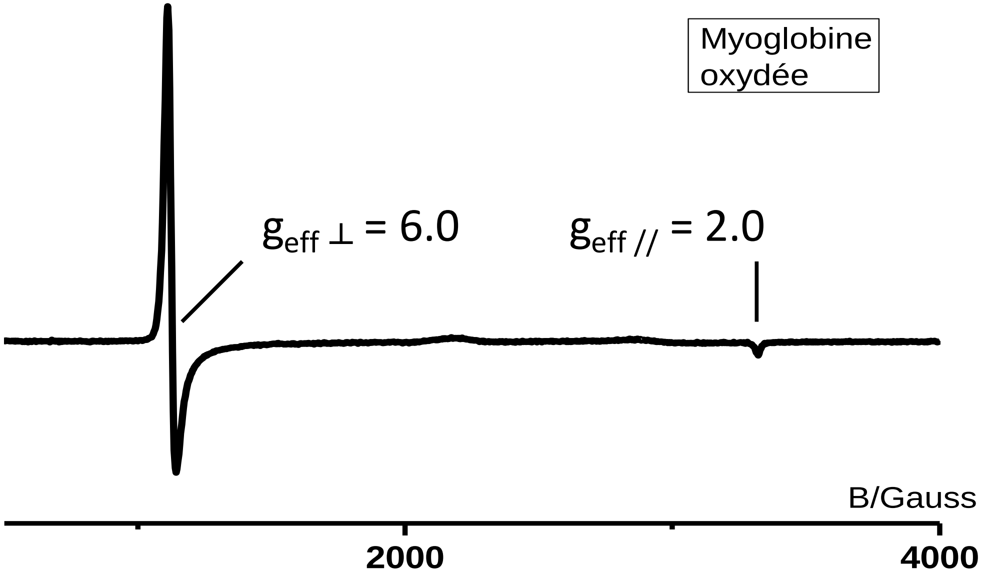
$h\nu \approx g\beta B \ll D$

Forbidden transitions 🟥

$\Delta M_S = \pm 3$; $\Delta M_S = \pm 5$







High spin Fe^{3+} systems

Axial Symmetry - **$D \gg g\,\beta\,B$**

Measurement of *D*: Temperature study

$M_S = \pm 1/2$ EPR spectrum intensity

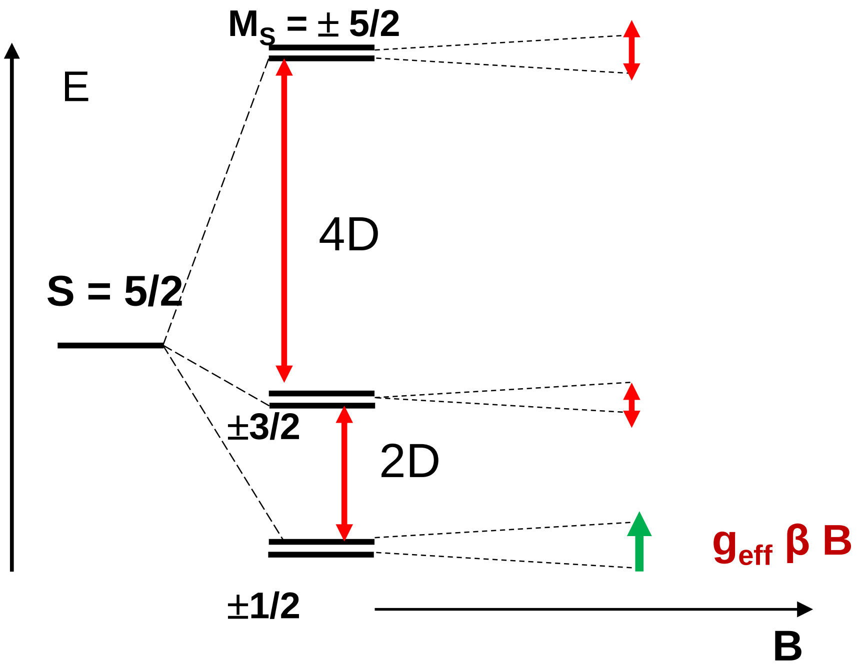
$$I \propto N_{(\pm 1/2)} / T$$

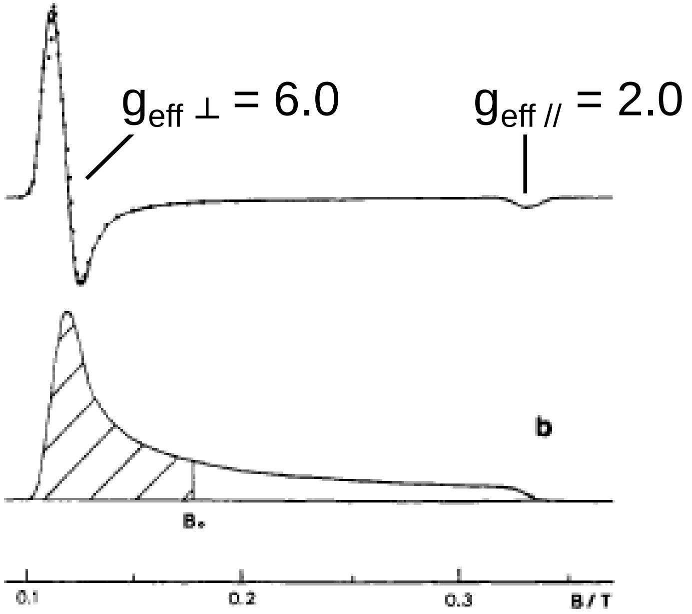
$$N_0 = N_{(\pm 1/2)} + N_{(\pm 3/2)} + N_{(\pm 5/2)}$$

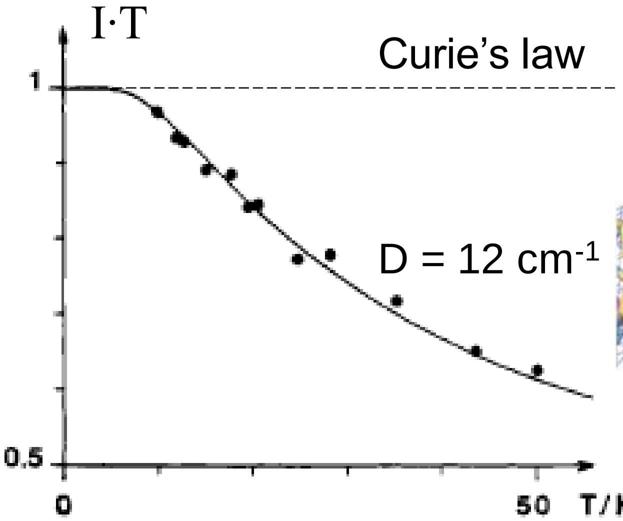
$$N_{(\pm 3/2)} / N_{(\pm 1/2)} = \exp(-2D/kT) \quad \text{(Boltzmann)}$$

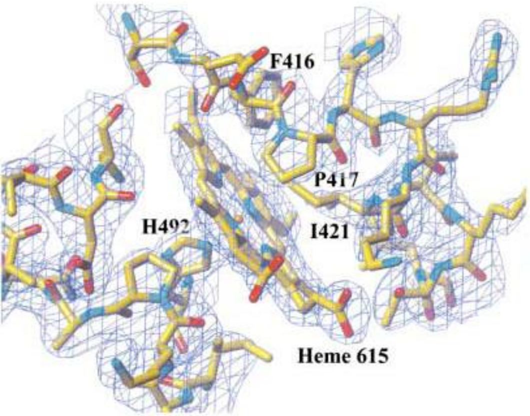
$$N_{(\pm 5/2)} / N_{(\pm 1/2)} = \exp(-6D/kT)$$

$$I \cdot T \propto 1 / [1 + \exp(-2D/kT) + \exp(-6D/kT)]$$









High spin Fe^{3+} systems

Rhombic fine structure: (D, E) >> g β B

$$\mathbf{H_{SF}} = \mathbf{D}\,(\mathbf{S}_Z^2 - \frac{1}{3}\mathbf{S}(\mathbf{S}+1)) + \mathbf{E}(\mathbf{S}_X^2 - \mathbf{S}_Y^2)$$

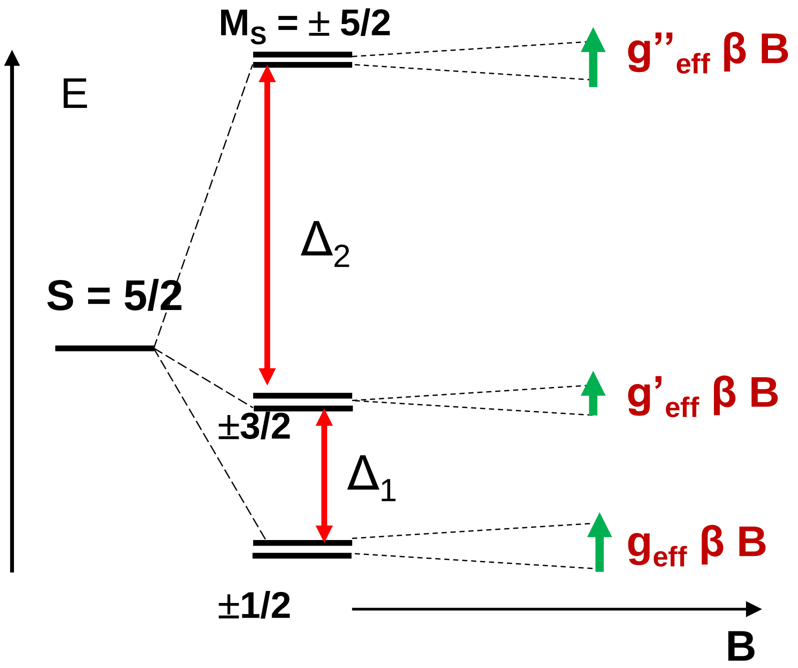
$$\mathbf{H_{SF}} = \mathbf{D}\,(\mathbf{S}_Z^2 - \frac{1}{3}\mathbf{S}(\mathbf{S}+1)) + \frac{\mathbf{E}}{2}(\mathbf{S}_+^2 + \mathbf{S}_-^2)$$

Mixing of states $\Delta M_S = \pm 2$

Forbidden transitions become allowed !

$\Delta M_S = \pm 3$; $\Delta M_S = \pm 5$

But the g_{eff} calculations are more complex…



Weak rhombicity: E/D < 0.1

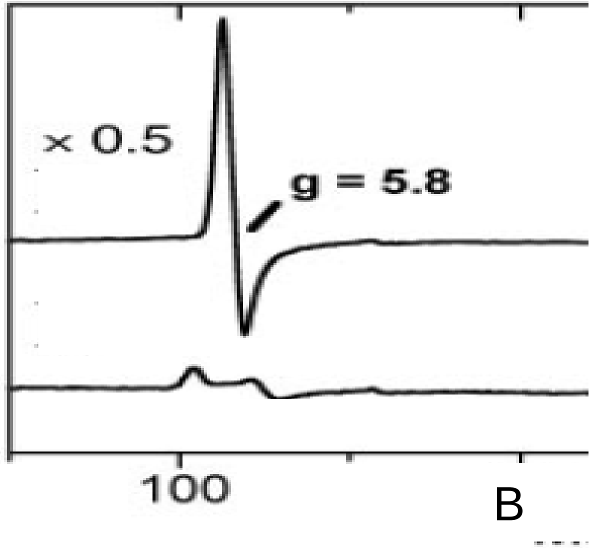
In manifold $M_S = \pm 1/2$

$g_{eff\ X} = 6 + 24$ E/D

$g_{eff\ Y} = 6 - 24$ E/D

$g_{eff\ Z} = 2.0$

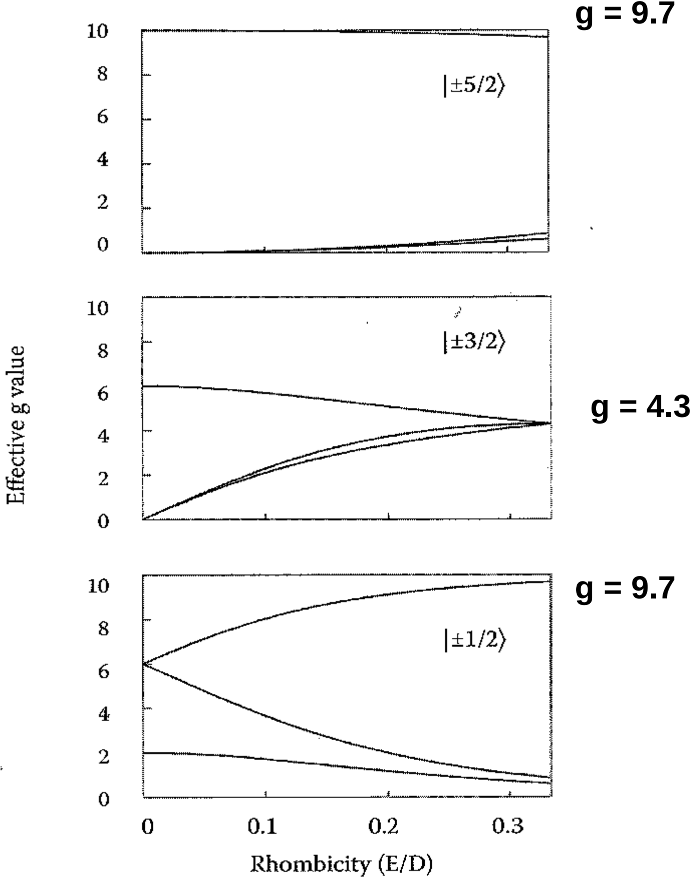
=> Determination of E/D



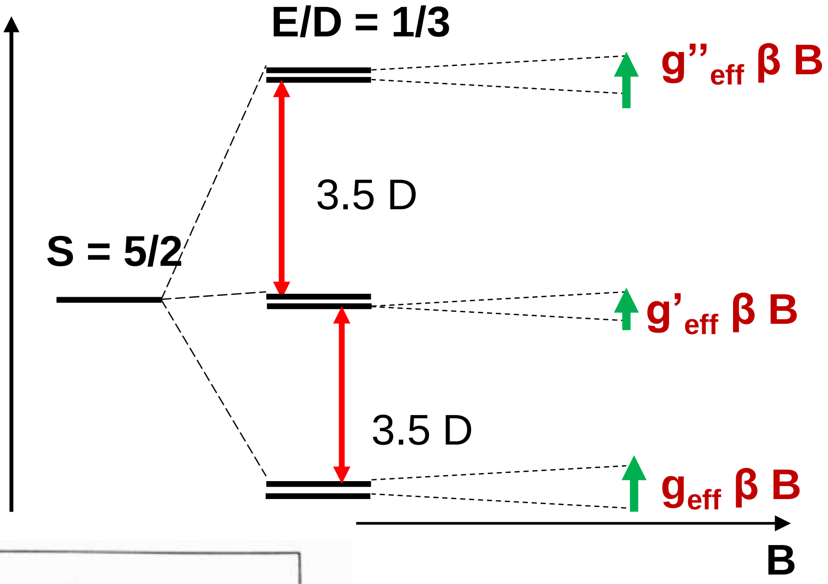
High spin Fe^{3+} systems

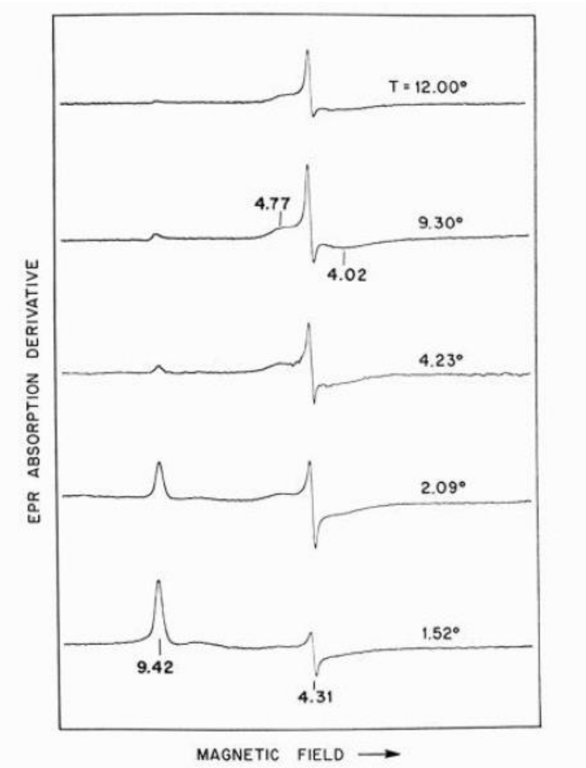
Rhombic fine structure: (D, E) >> g β B

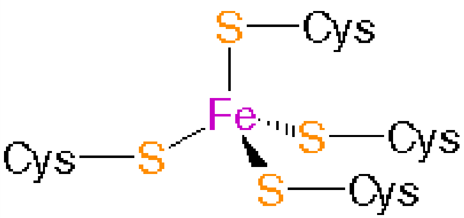
Rhombogram for S=5/2



Isotropic line at g = 4.3 for adventitious Fe^{3+}







Oxidized Rubredoxin
g = 9.4 , 1.2 , 0.9
g = 4.77, 4.3 , 4.0
D = 1.76 cm^{-1} E = 0.495 cm^{-1}
(*Peisach* 1971)

B

High spin / Low spin Fe^{3+} systems

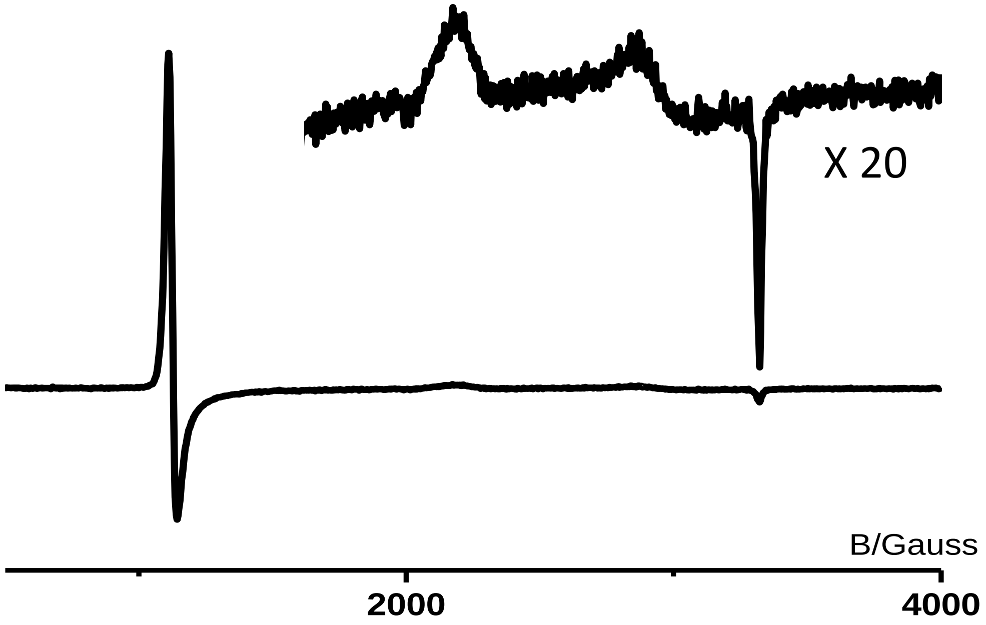
EPR Signal intensity: $I \propto N\,(g_P)_{av}\,B_1\,\mathrm{th}(h\nu/2k_BT)$

$(g_P)_{av} \approx \{2/3\,[(g_x^2 + g_y^2 + g_z^2)/3]^{1/2} + 1/3\,[(g_x + g_y + g_z)/3]\}$

Influence of the transition probability

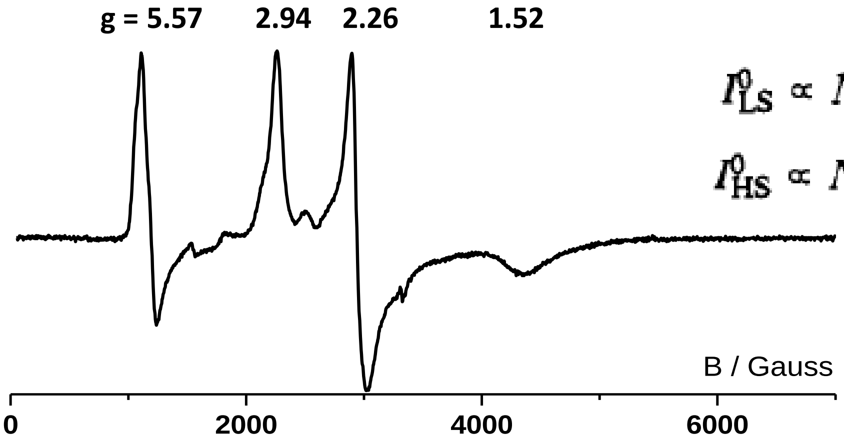
Higher sensitivity for signals with high g-values (low magnetic field)

Equimolar solution of myoglobin (HS) and cytochrome c (LS)



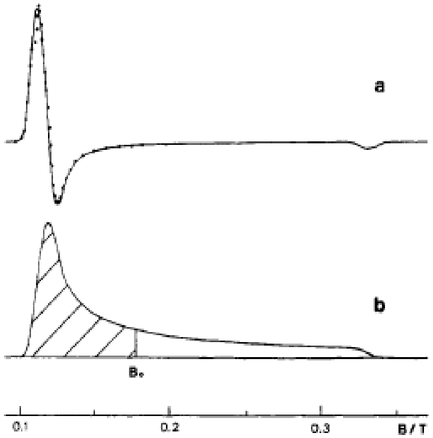
High spin / Low spin Fe^{3+} systems

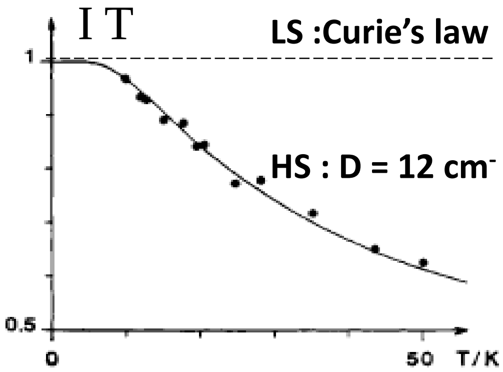
High Molecular weight Cytochrome (HMC) : 16 hemes
High Spin + Low Spin hemes ?



$$I^0_{LS} \propto N_{LS}\,(g_p^{av})_{LS}$$

$$I^0_{HS} \propto N_{HS}\,(g_p^{av})_{HS}$$

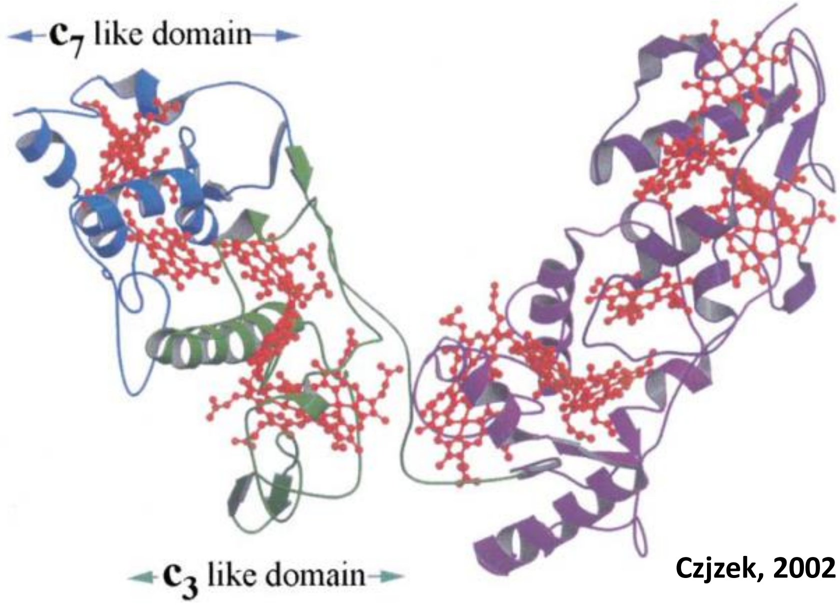




Determination of HS heme spin intensity by spectral simulation and comparison with a standard

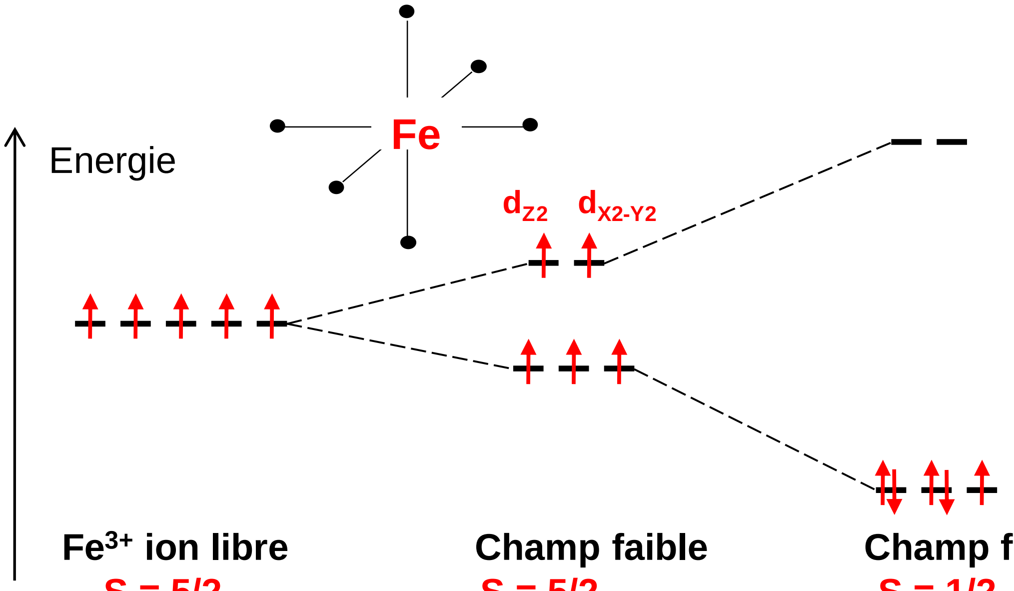
$$(g_p^{av})_{LS}\, n_{LS} + (g_p^{av})_{HS}\, n_{HS}\, f(16\ K) = 38$$

16 hemes : 1 HS + 15 LS



Czjzek, 2002

Spin transitions in Fe^{3+} systems



Fe^{3+} ion libre
S = 5/2

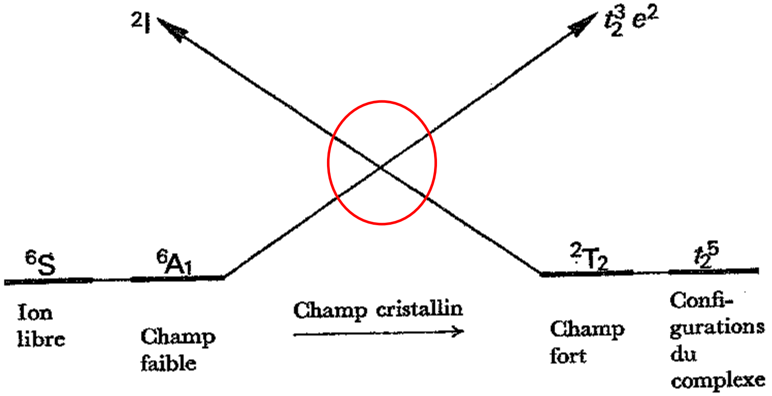
Terme ^6S

Champ faible
S = 5/2
Spin fort
$^6A_1$

Champ fort
S = 1/2
Spin faible
$^2T_2$

Valence ion Fe	1+	2+	3+	4+	5+
$3d^n$	7	6	5	4	3
HS	3/2	2	5/2	2	3/2
LS	1/2	0	1/2	0	1/2

Spin Transitions

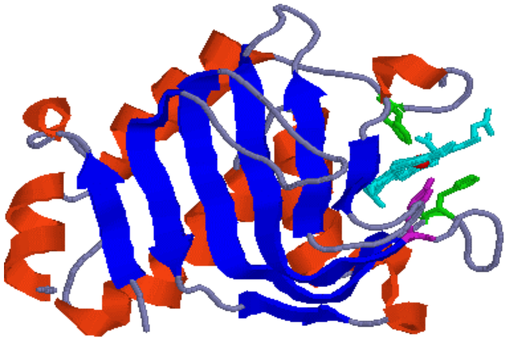


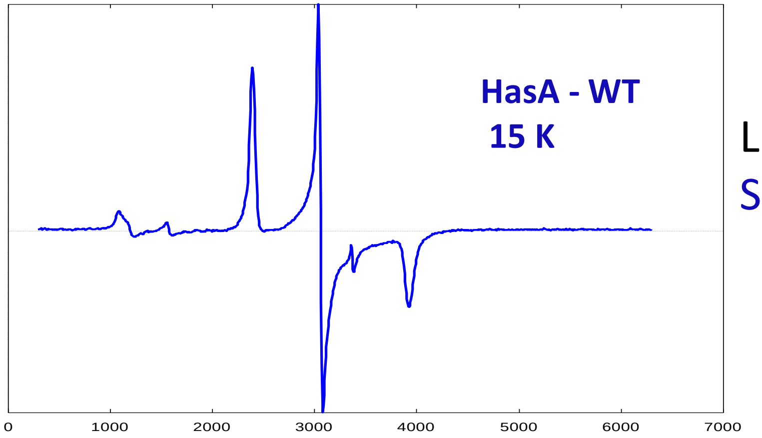
Spin transitions are induced by:
- Change of ligand field strength: change of ligand, compression
- T variations

Spin transitions in Fe^{3+} systems: HasA protein

HasA protein : Heme acquisition system
Enable pathogenic bacteria (*Serratia marcescens Yersinia pestis)*
to take heme group from hemoglobin in human

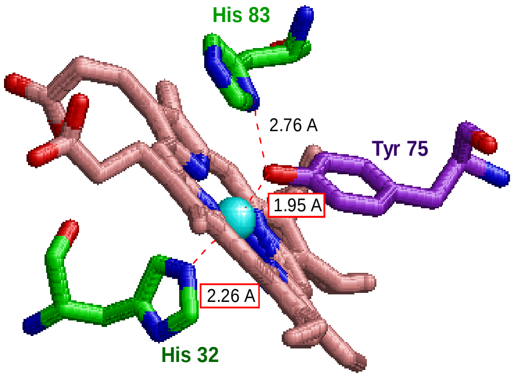
Very strong affinity for heme: $K_D = 10^{-11}$ M



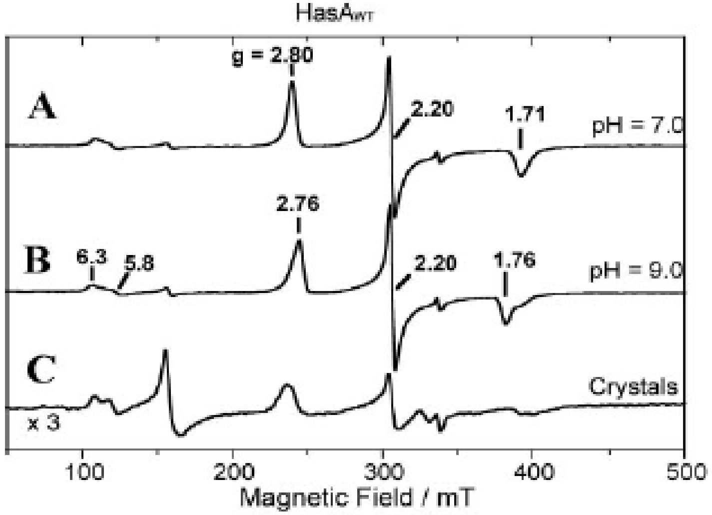


Low spin Fe^{3+}
$S = 1/2$

Axial coordination of Fe^{3+}
by His32-Tyr78



(Arnoux, NSB 1999)



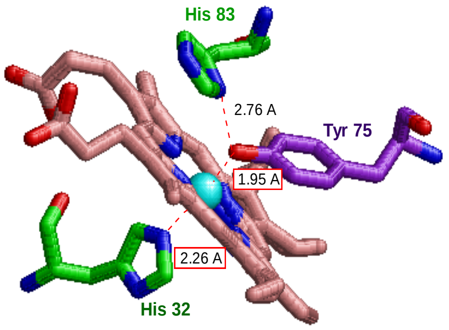
Same coordination of Fe^{3+} in solution and in the cristal state

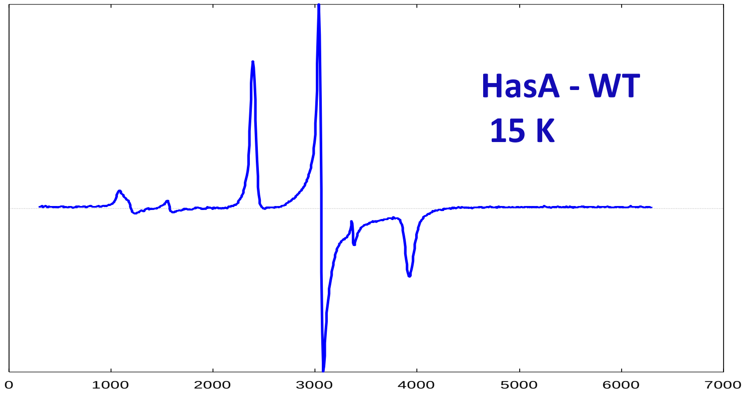
Polycrystals

Spin transitions in Fe^{3+} systems: HasA protein

HasA protein : Heme acquisition system
Enable pathogenic bacteria (*Serratia marcescens Yersinia pestis)*
to take heme group from hemoglobin in human

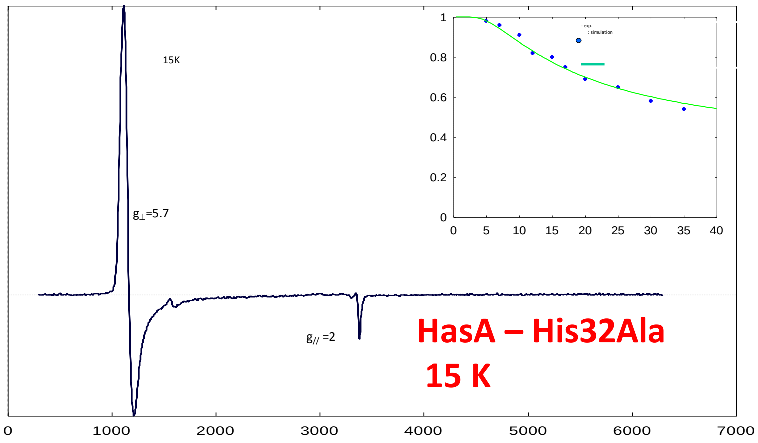
Very strong affinity for heme: $K_D = 10^{-11}$ M





Low spin Fe^{3+}
$S = 1/2$

Mutant of the axial coordination:
His32Ala
Lack of sixth ligand or H_2O molecule

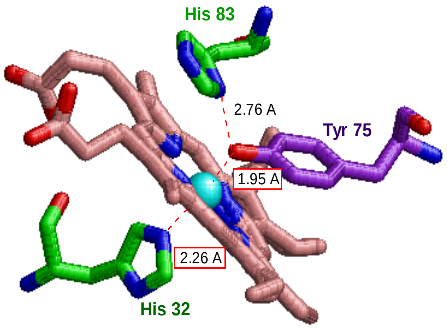


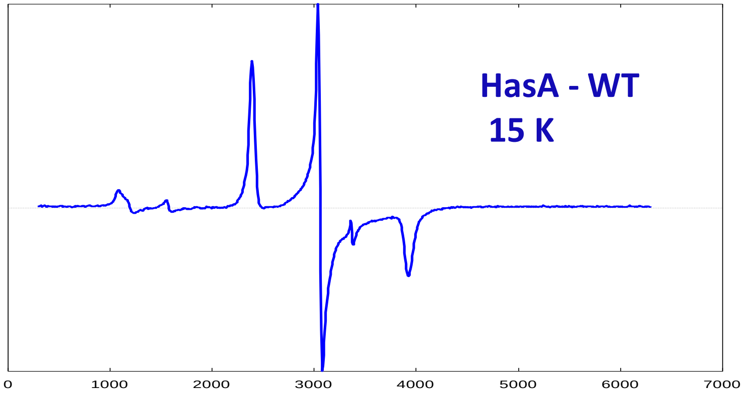
High spin Fe^{3+}
$S = 5/2$

Spin transitions in Fe^{3+} systems: HasA protein

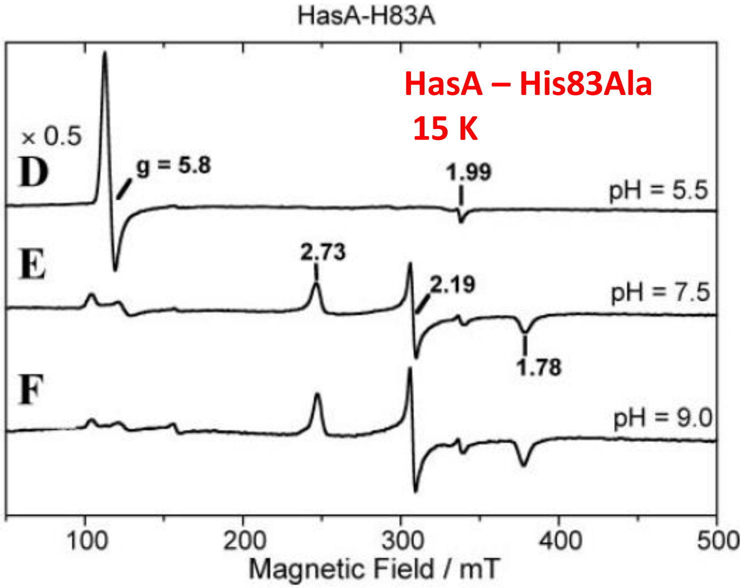
HasA protein : Heme acquisition system
Enable pathogenic bacteria (*Serratia marcescens Yersinia pestis)*
to take heme group from hemoglobin in human

Very strong affinity for heme: $K_D = 10^{-11}$ M





Low spin Fe^{3+}
$S = 1/2$



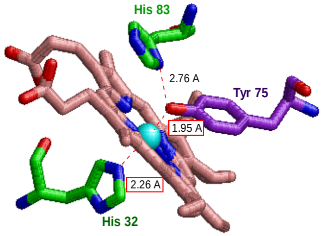
pH =5.5
High spin
Fe^{3+} $S = 5/2$

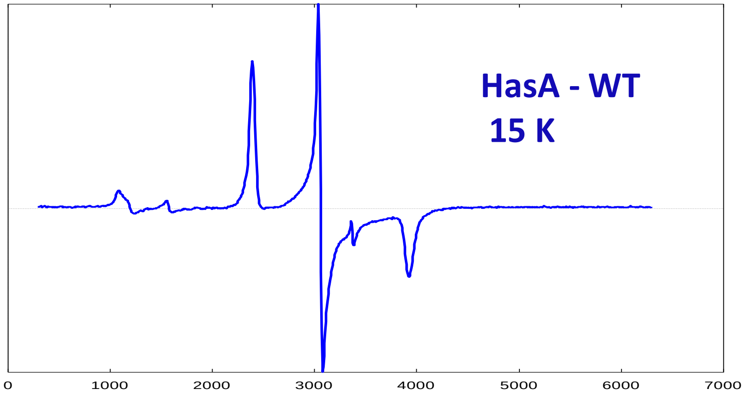
Mutant of the axial coordination:
His83Ala
Decoordination of Tyr75 at acidic pH

Spin transitions in Fe^{3+} systems: HasA protein

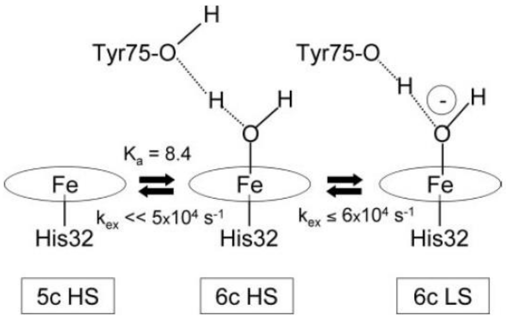
HasA protein : Heme acquisition system
Enable pathogenic bacteria (*Serratia marcescens Yersinia pestis)*
to take heme group from hemoglobin in human

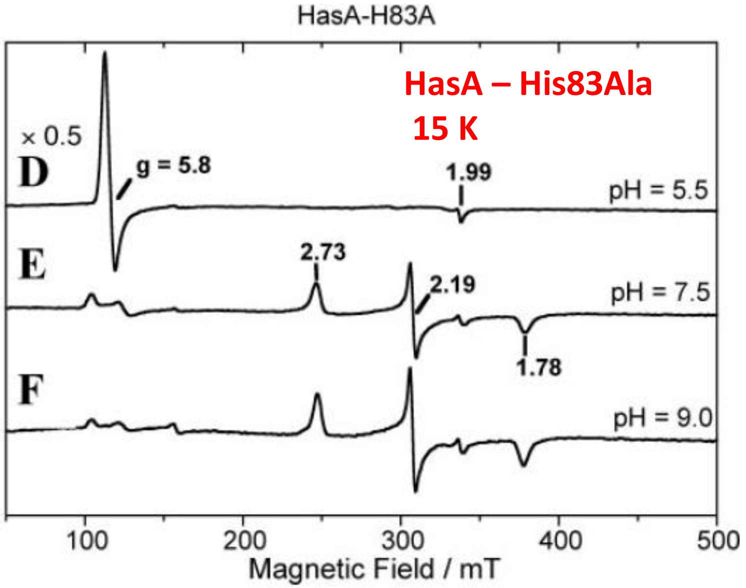
Very strong affinity for heme: $K_D = 10^{-11}$ M





Low spin Fe^{3+}
$S = 1/2$



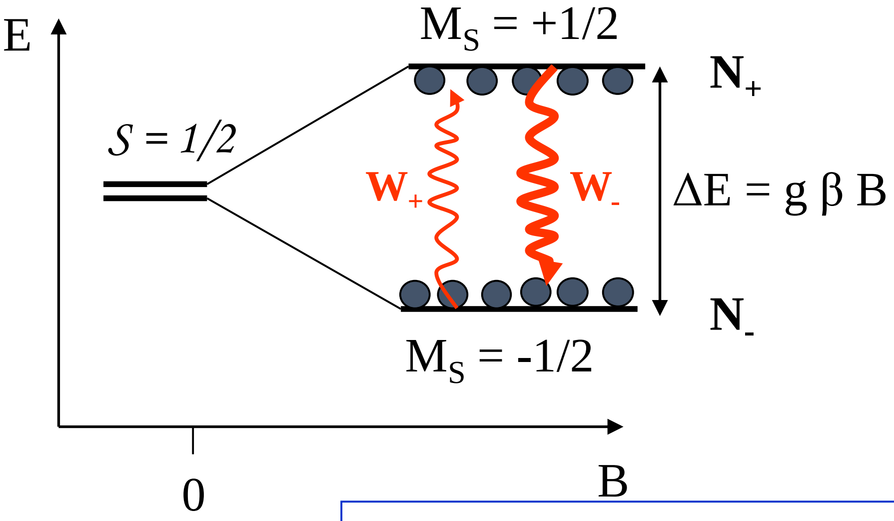


pH =5.5
High spin
Fe^{3+} $S = 5/2$

Model : Breaking the His83-Tyr75 H-bond decrease heme affinity and enable the transfer to the membrane bound receptor HasR

(*Caillet, J.Biol.Chem., 2008*)

Electron spin relaxation



Thermal equilibrium (Boltzmann)

$$N_+ / N_- = \exp\,(-\,g\,\beta\,B\,/\,k\,T\,) = W_+ / W_-$$

$$N_+\,W_- = N_-\,W_+$$

Spontaneous transitions (W_+, W_-) maintain thermal equilibrium

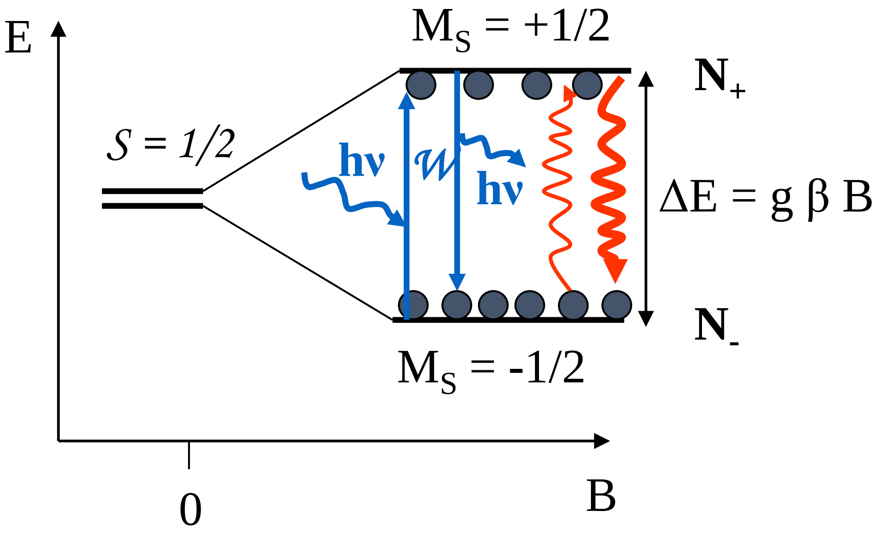
$\Rightarrow$ Induced by fluctuations of magnetic field: $B = B_0 + B(r, t)$

Fluctuations of the environment:

- Thermal motions: translations, rotations, vibrations, collisions
- Magnetic neighbours: nuclei, other paramagnetic centers
- Thermal radiation

- Energy exchange between spins : spin-spin relaxation (T_2)
- Energy exchange between spins and surrounding : spin-lattice relaxation (T_1)

Electron spin relaxation



Upon microwave irradiation, competition between Absorption/Relaxation

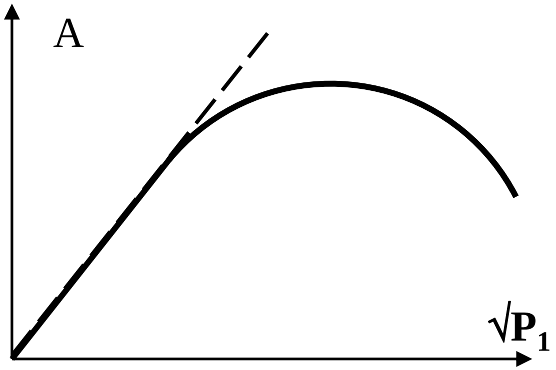
$$\mathbf{dn/dt = -2\,\mathcal{W}n + (n_0 - n) / T_1 \qquad (\mathcal{W} \propto B_1^2)}$$

=> Steady state in continuous wave EPR

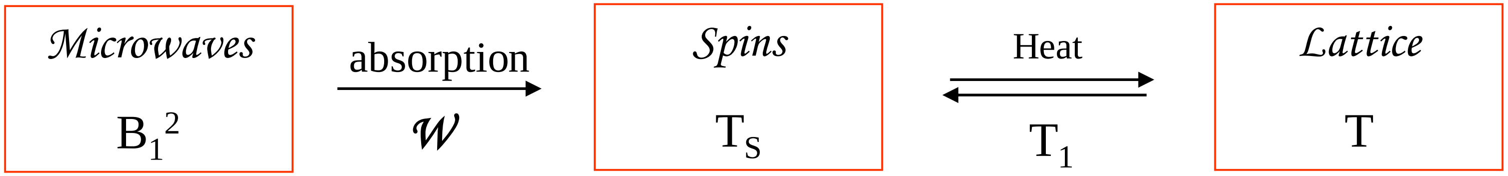
Net absorbed microwave power at steady state

$$P_{abs} = h\nu\,(\mathcal{W}N_- - \mathcal{W}N_+) = h\nu\,\mathcal{W}\,\mathbf{n_{Stat}}$$

High power: $n_{stat} \rightarrow 0$: **Power saturation**



=> **T_1 and T_2 measurements**



Microwaves

B_1^2

absorption

$\mathcal{W}$

Spins

T_S

Heat

T_1

Lattice

T

Electron spin relaxation: Temperature dependence

Spin-lattice relaxation :

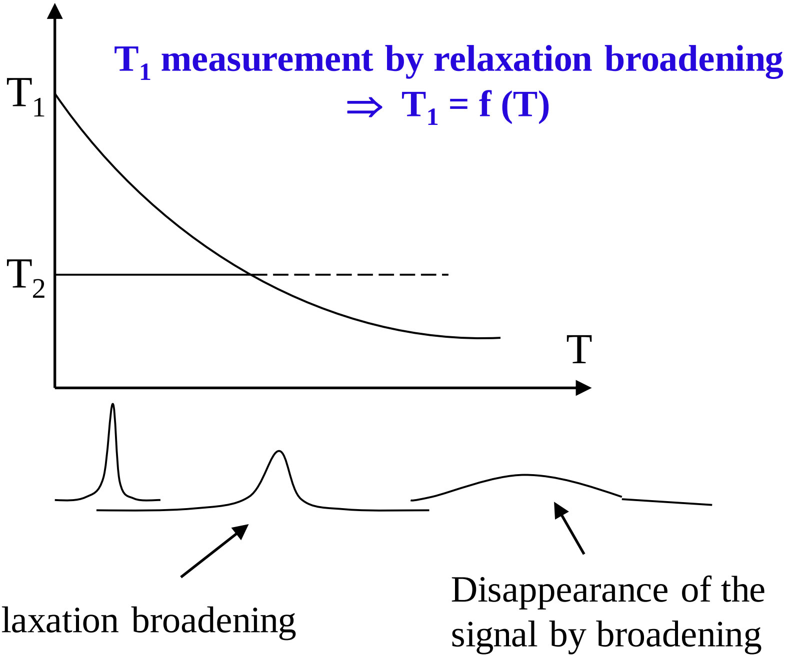
- Coupling between spins and vibrations (phonons)
- Strong dependence on spin-orbit coupling

$$\mathbf{H_{SO} = \lambda\, L\cdot S}$$

- If T increases, T_1 decreases.

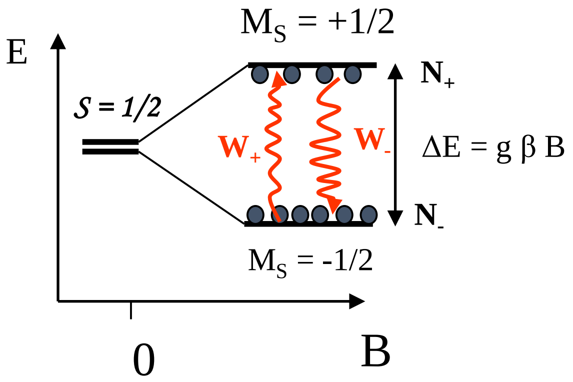
When $T_1 \approx T_2$ broadening of the resonance line:

$$\mathbf{\delta B = \hbar/g\beta \cdot 1/T_1}$$



T_1 measurement by relaxation broadening

$\Rightarrow$ T_1 = f (T)

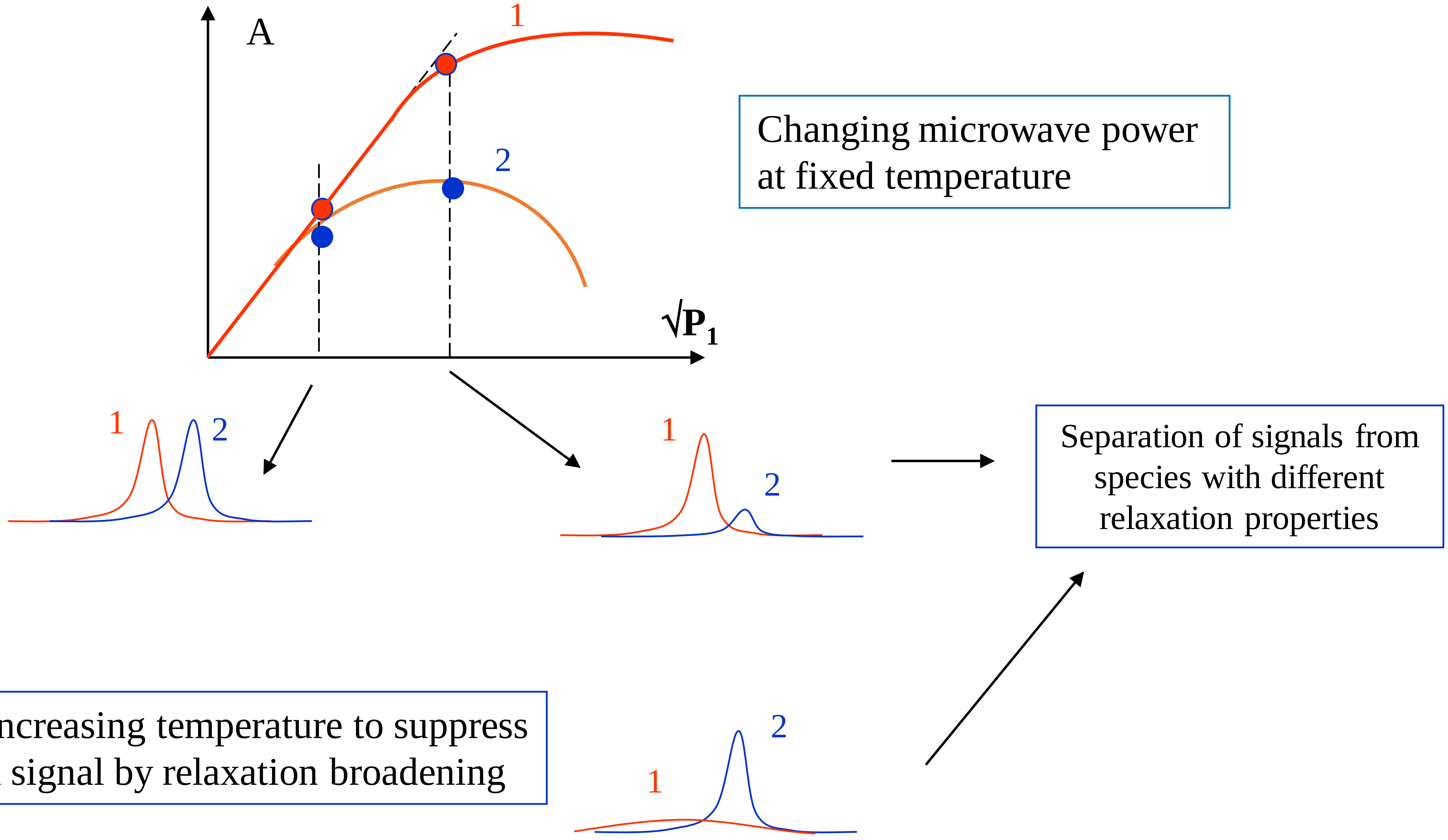


For transition metal ions
Strong spin-orbit coupling

- ***g*-tensor anisotropy**
- **Fast relaxation**
- **EPR study at low T**

Electron spin relaxation: Temperature dependence

Strategies for separating signals from different species



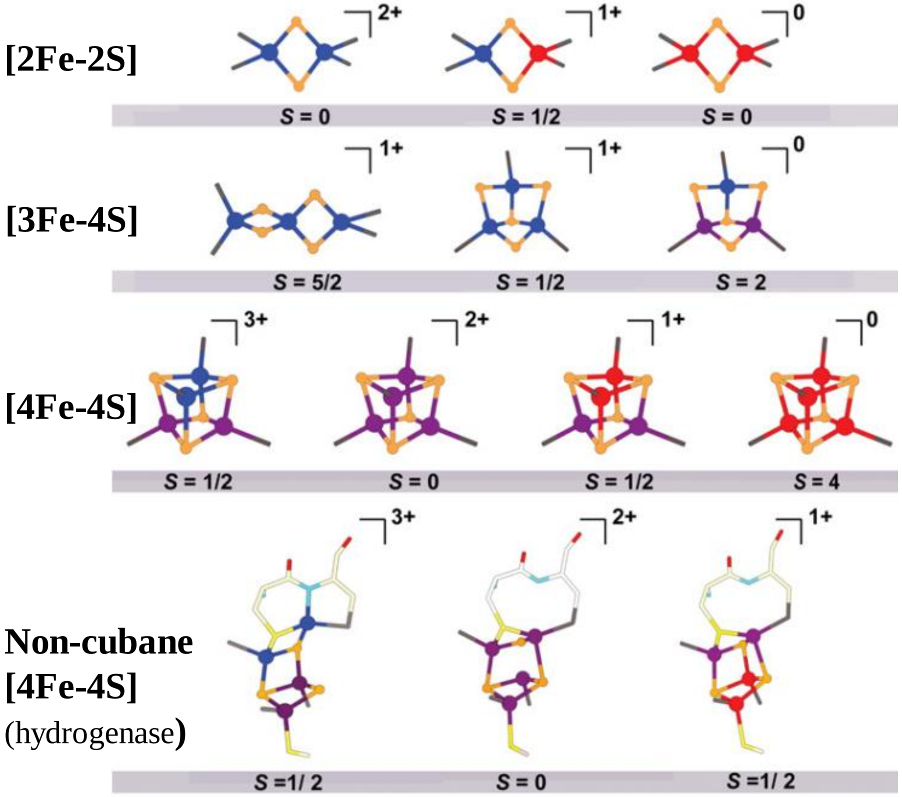
Changing microwave power at fixed temperature

Separation of signals from species with different relaxation properties

Increasing temperature to suppress a signal by relaxation broadening

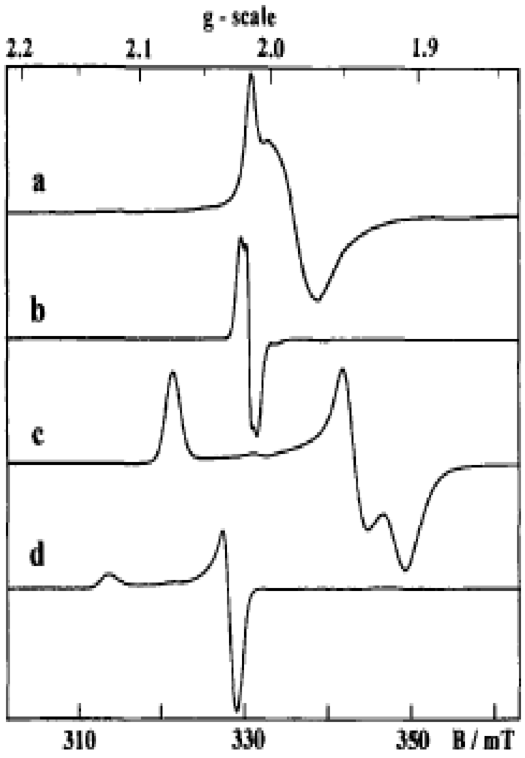
Fe-S clusters

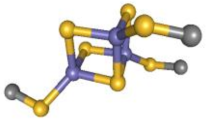
Magnetic properties arise from exchange coupling between Fe^{3+} (S=5/2) and Fe^{2+} (S=2) ions



[Pandelia, BBA 2015]

Typical Fe-S EPR signals

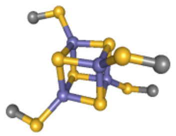




$[3Fe\text{-}4S]^{1+}$
S = 1/2

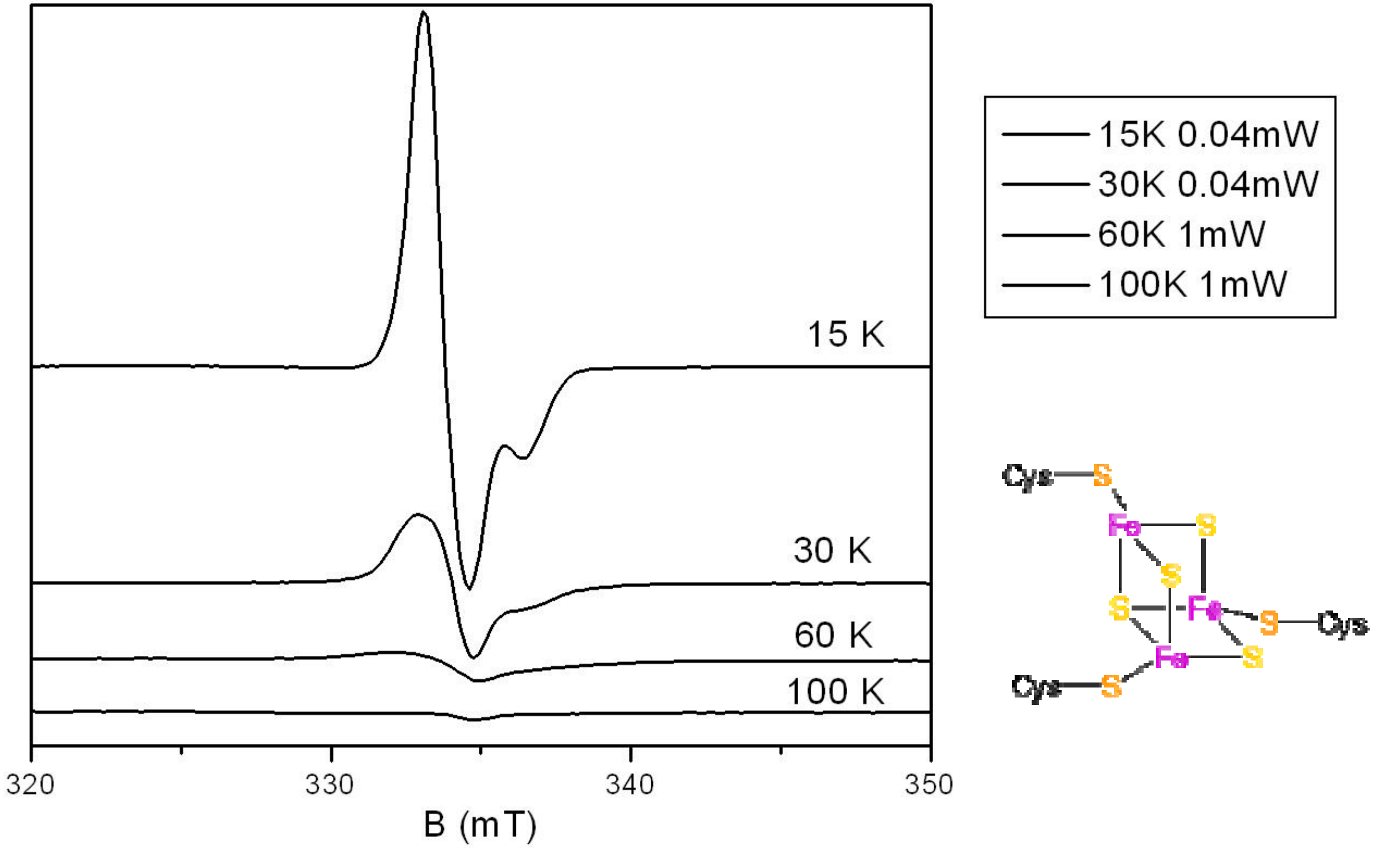
$[4Fe\text{-}4S]^{1+}$
S = 1/2

$[4Fe\text{-}4S]^{3+}$
S = 1/2



Fe-S clusters: fast electron spin relaxation

Relaxation broadening of a $[3Fe\text{-}4S]^{1+}$ signal ($S = ½$) upon T increase

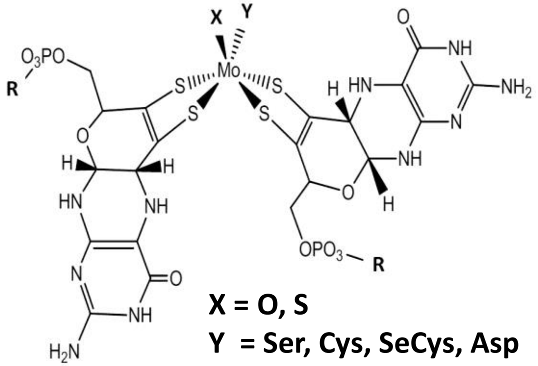


Fe-S clusters: fast electron spin relaxation

Selective EPR view of metal cofactors in *E. coli* respiratory nitrate reductase

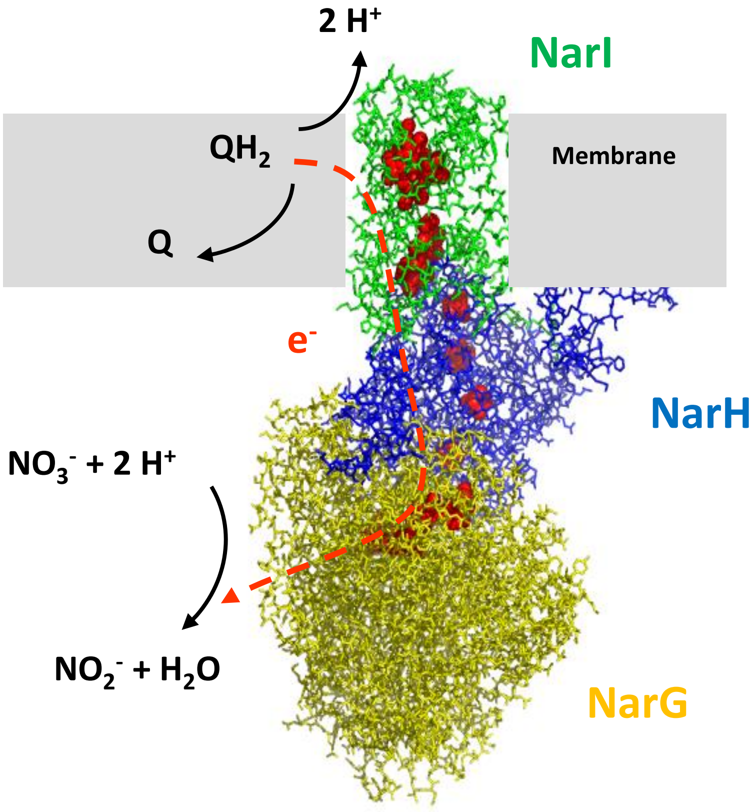
NarGHI
- Structure
- Mechanism
- Interaction with quinones
- Reactivity of Molybdenum cofactor
- Substrate specificity
- Biogenesis

(Coll. A. Magalon, CNRS Marseille)



X = O, S
Y = Ser, Cys, SeCys, Asp

Mo-bisPGD cofactor

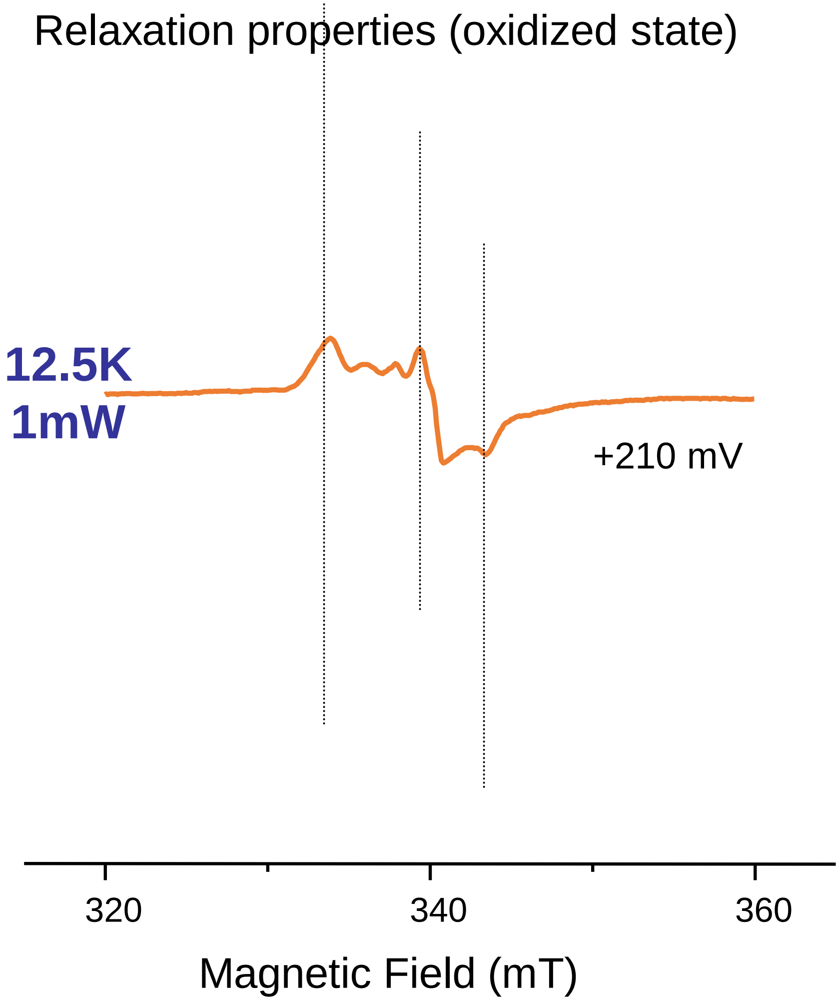


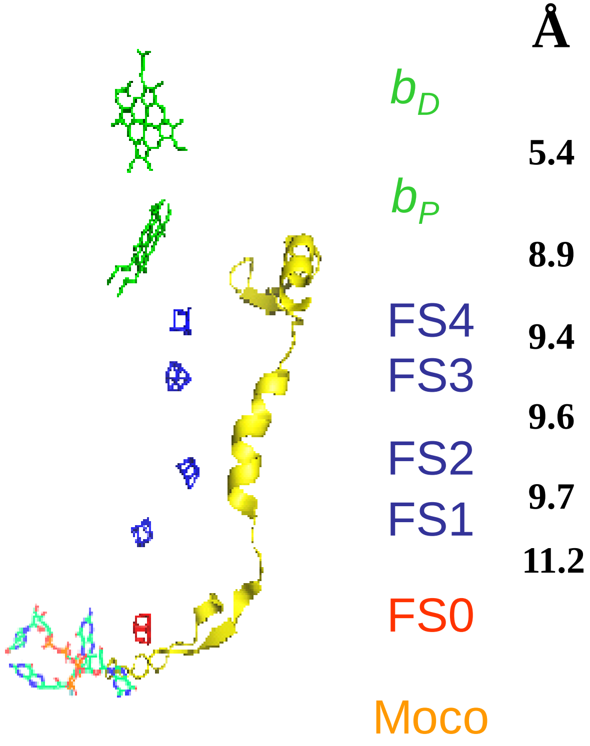
Membrane-bound Nitrate reductase from *E. coli* (NarGHI)

Fe-S clusters: fast electron spin relaxation

Selective EPR view of metal cofactors in respiratory nitrate reductase

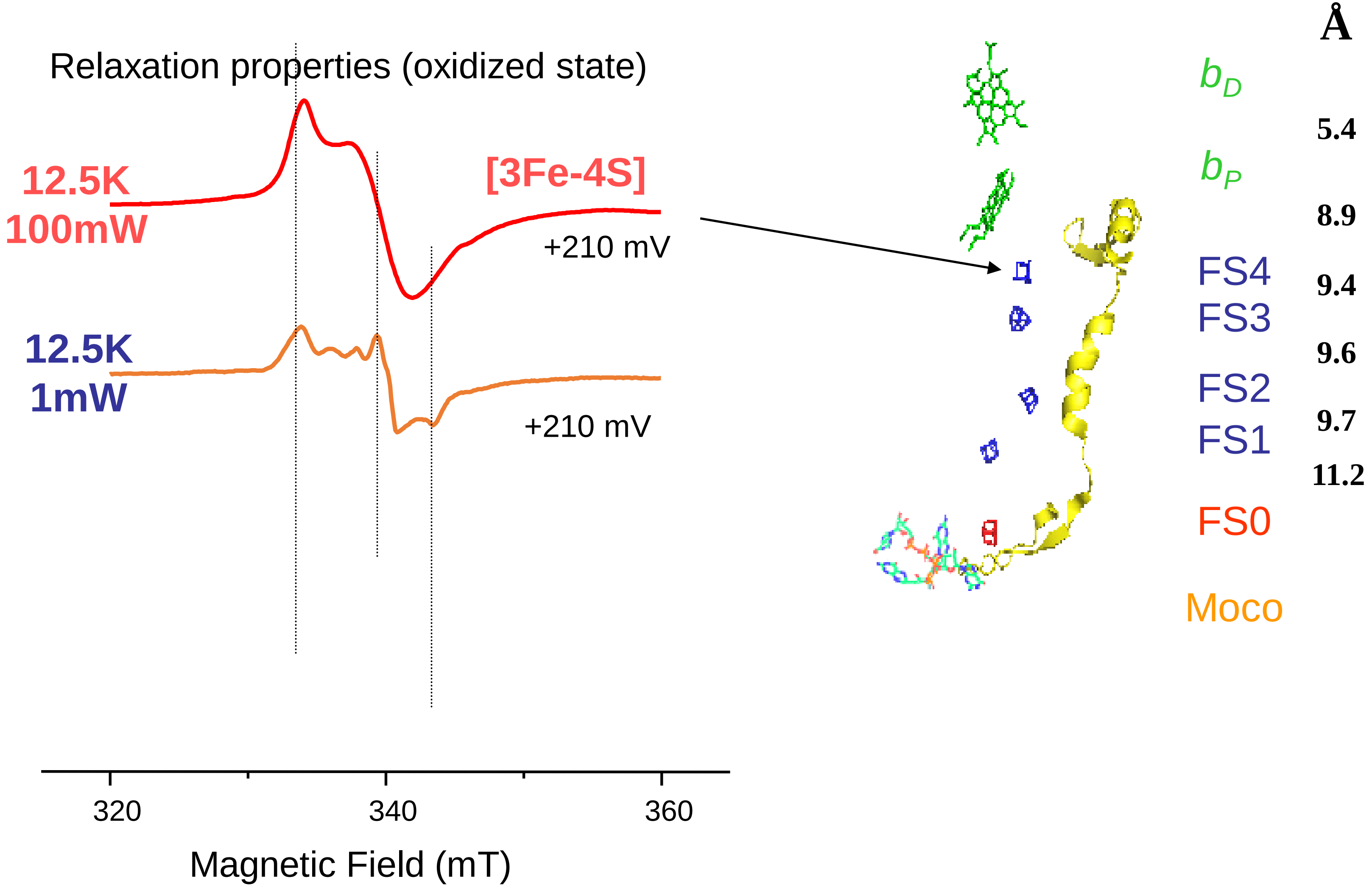
Relaxation properties (oxidized state)





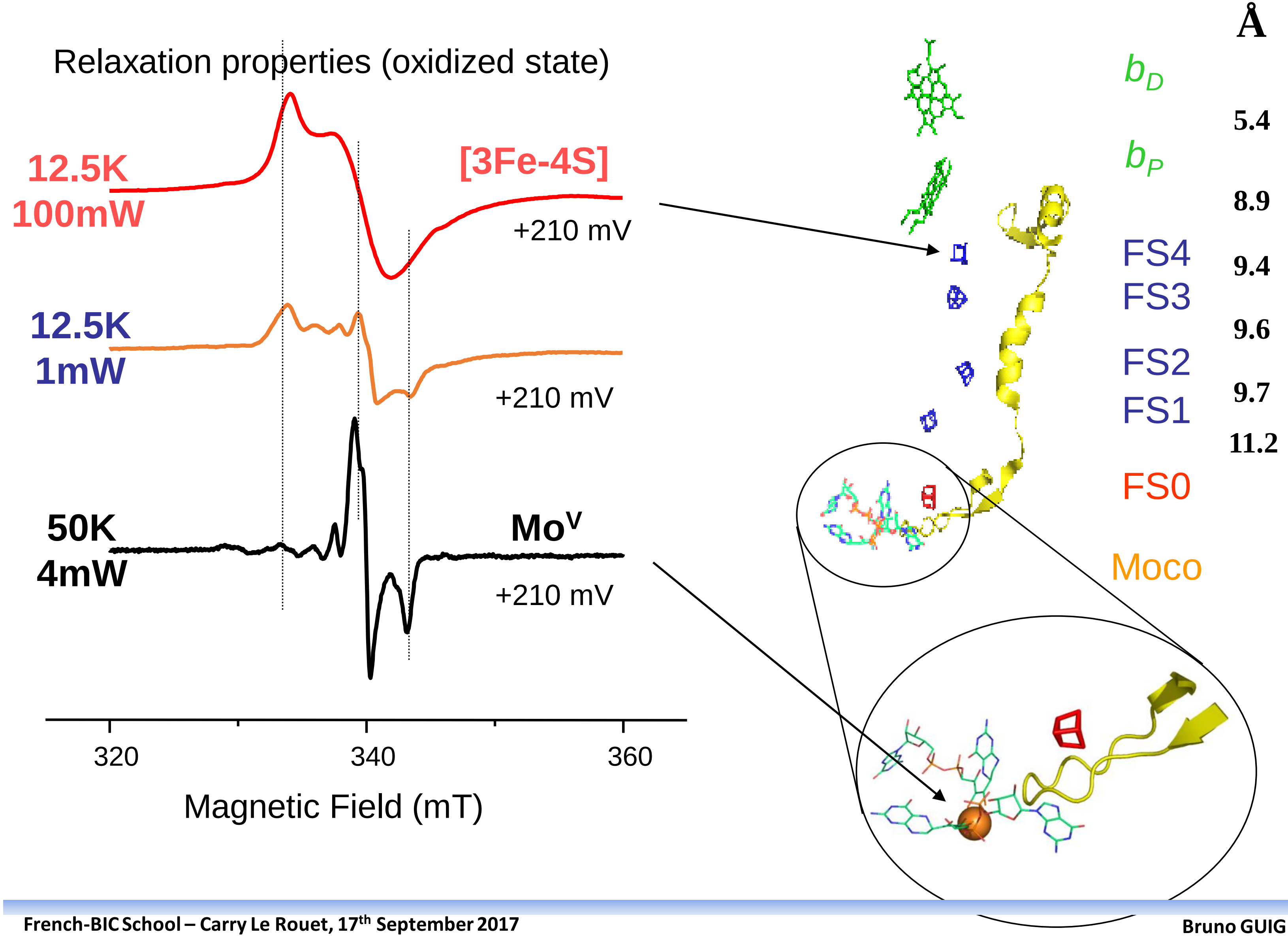
Fe-S clusters: fast electron spin relaxation

Selective EPR view of metal cofactors in respiratory nitrate reductase

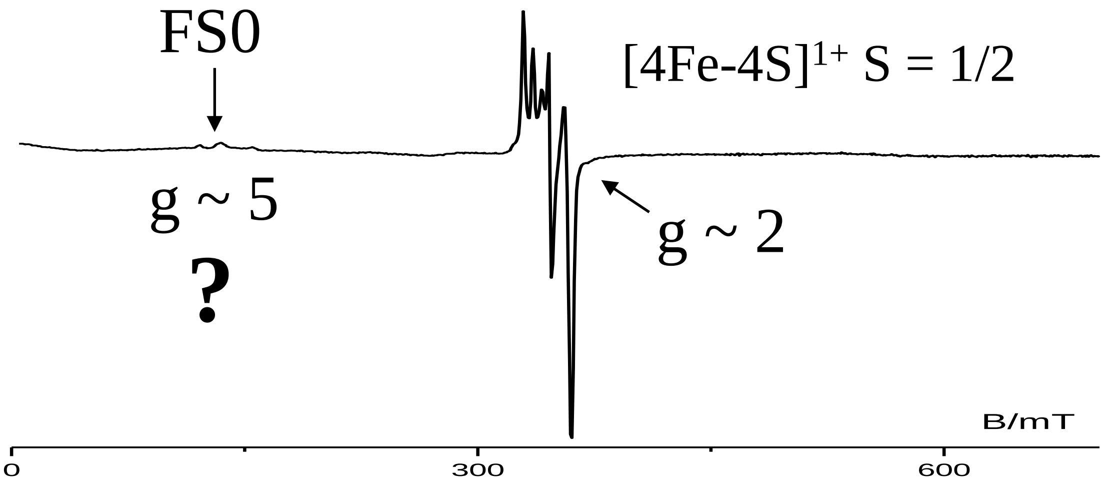


Fe-S clusters: fast electron spin relaxation

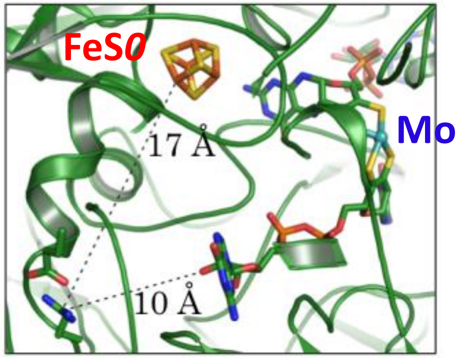
Selective EPR view of metal cofactors in respiratory nitrate reductase



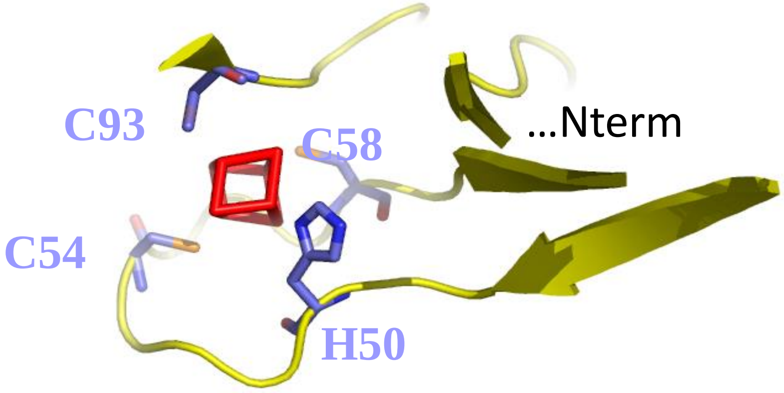
Unusual FeS cluster in NarGHI nitrate reductase

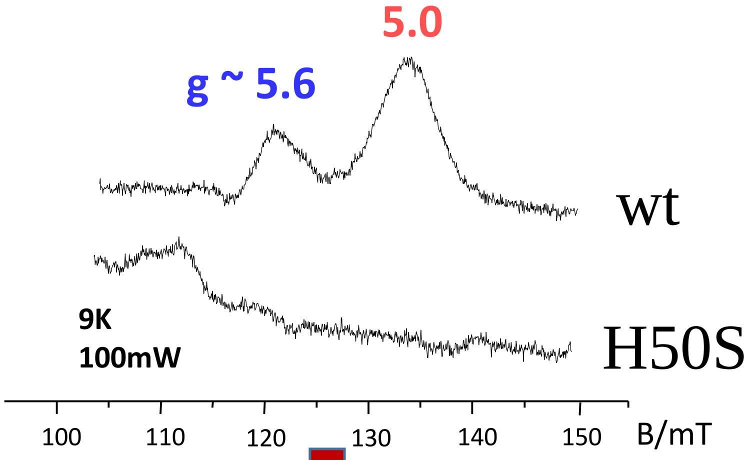


NarG subunit



Unusual coordination of FeS0 cluster:
Cys motive HxxxCxxxC…C

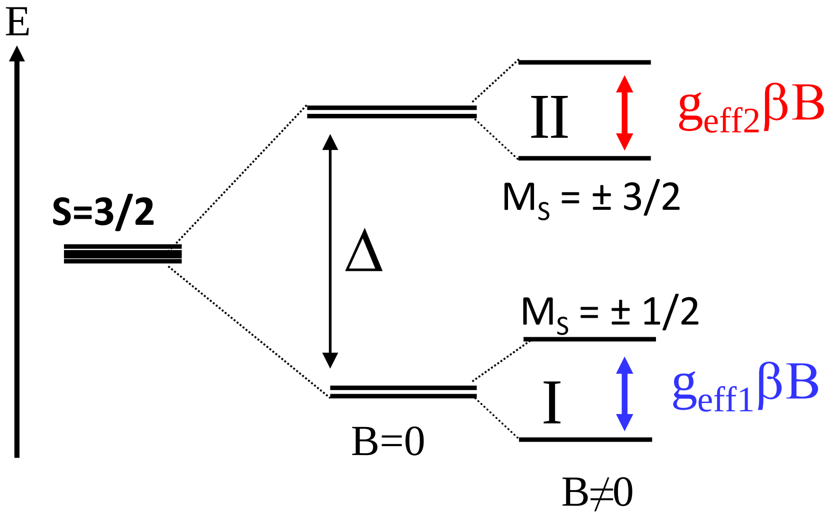




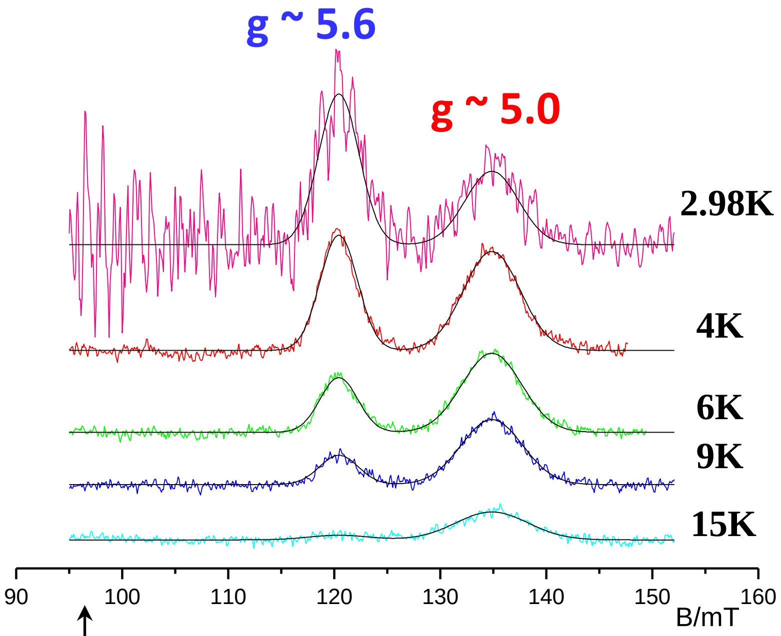
FeS0 : a S=3/2 $[4Fe\text{-}4S]^{+1}$ cluster coordinated by His
(*Lanciano, J.Phys.Chem. 2007*)

Unusual FeS cluster in NarGHI nitrate reductase

$[4Fe\text{-}4S]^{+1}$ **$3Fe^{2+}$ S = 2** **$1Fe^{3+}$ S = 5/2** } S = 3/2



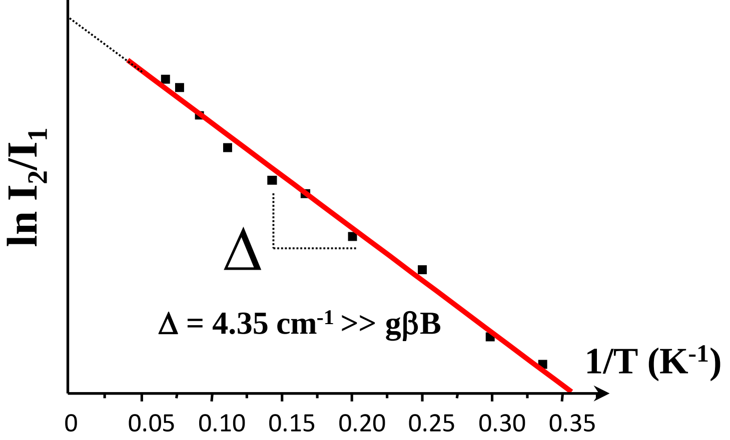
T dependence EPR study



$$\tilde{H}_S = \tilde{\mathbf{H}}_{\mathbf{ZFS}} + \tilde{H}_{Zeeman}$$

$$\tilde{H}_S = D\left[S_z^2 - \frac{1}{3}S(S+1) + {}^{E}\!/_{D}\left(S_x^2 - S_y^2\right)\right] + \beta\vec{B}\tilde{g}\vec{S}$$

$$\Delta = [D^2 (1 + 3(E/D)^2)]^{½}$$

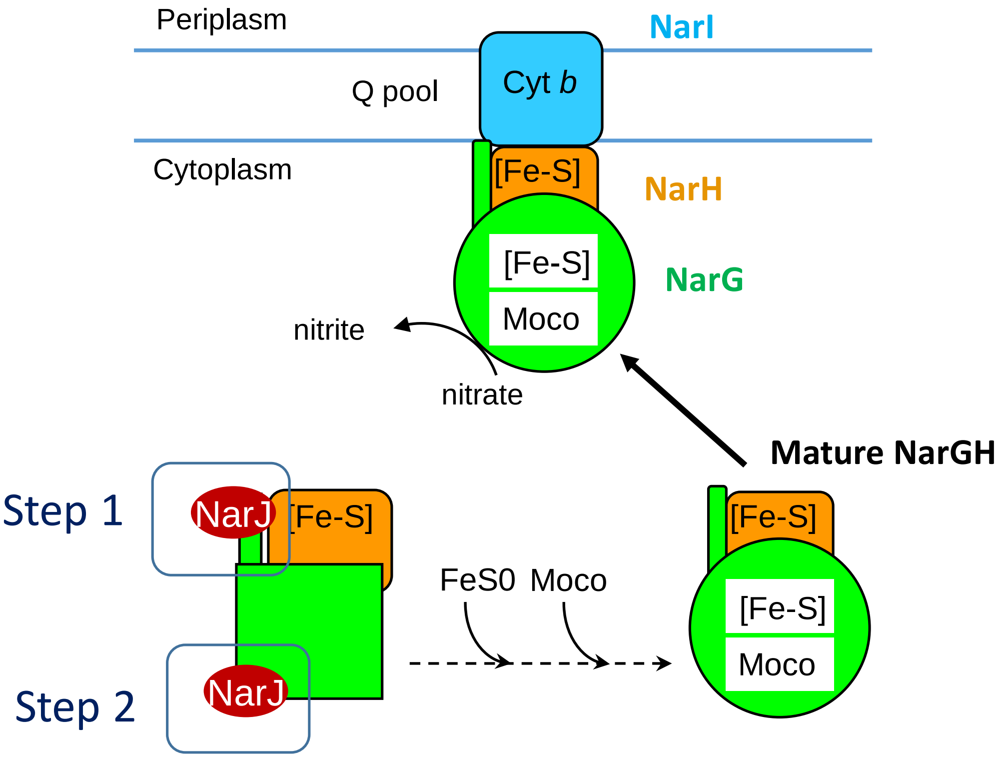


(*Lanciano, J.Phys.Chem. 2007*)

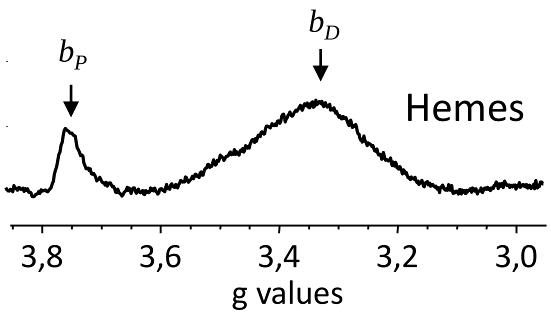
Insertion of the Mo cofactor in NarGHI nitrate reductase – EPR view

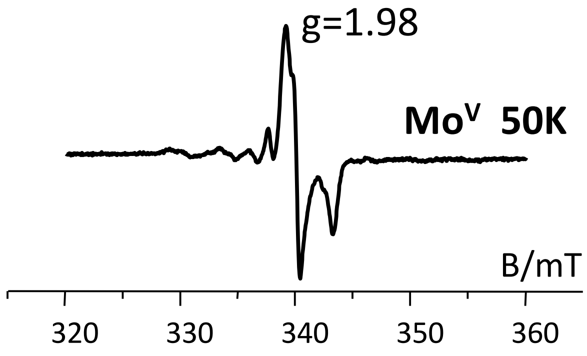
NarJ : a specific chaperone of NarGH complex
a **multifunctional protein**

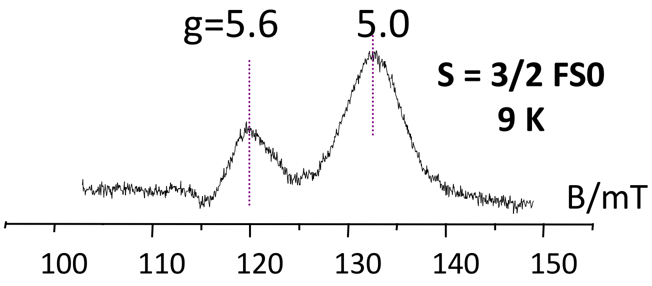
✓ Association to the N-ter of NarG prevent premature membrane anchoring
✓ Sequential Insertion of metal centers (FeS et Moco)



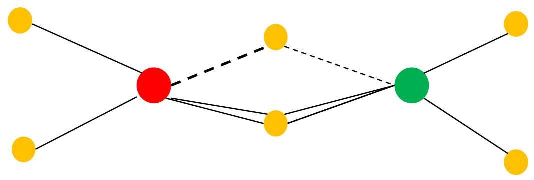
Vergnes A. *et al*, **2006**, *J. Biol. Chem.*
Lanciano P. *et al*, **2007**, *J. Biol. Chem.*

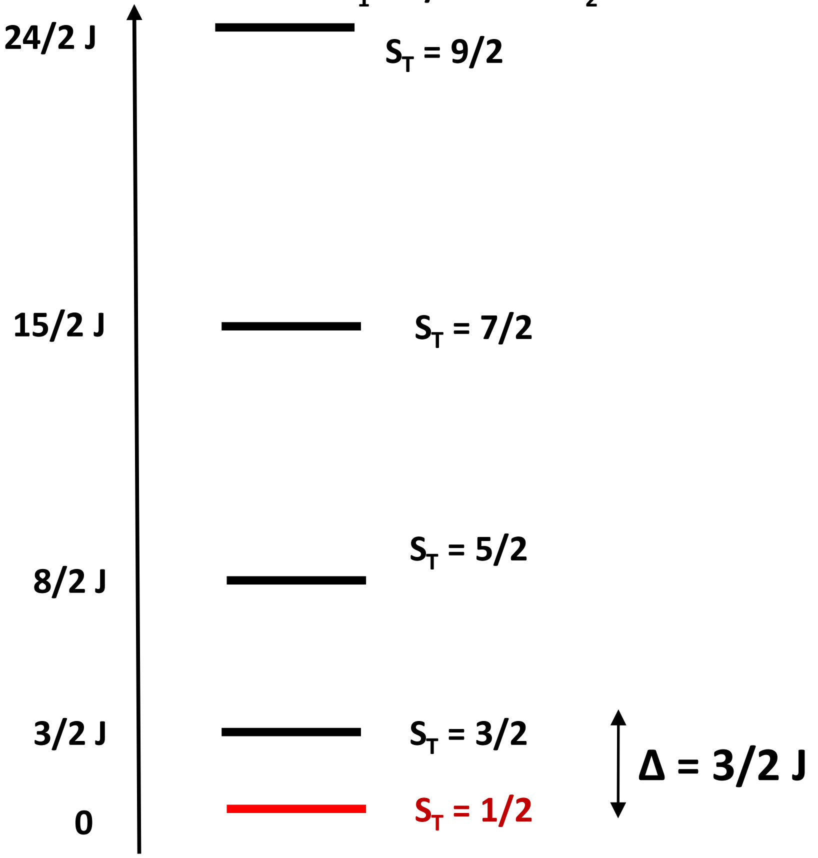






Fe-S clusters: Exchange interaction determination from relaxation broadening





Δ = 3/2 J

Exchange interaction between Fe ions in $[2Fe\text{-}2S]^{+1}$

$$H_{ex} = J\,\vec{S}_1 \cdot \vec{S}_2$$

$$S_T = \vec{S}_1 + \vec{S}_2$$

$$|S_1 - S_2| \leq S_T \leq S_1 + S_2$$

$$S_T = 1/2, 3/2, 5/2, 7/2, 9/2$$

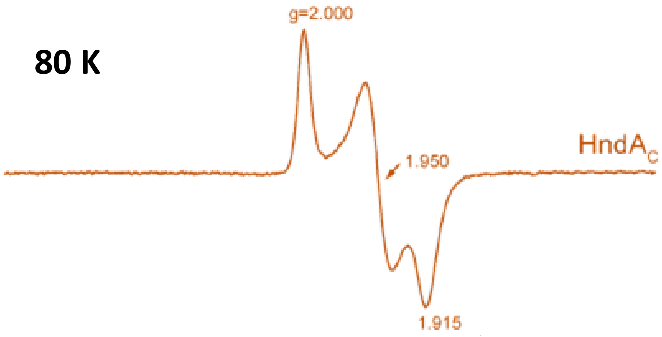
$$H_{ex} = \frac{J}{2}(S^2 - S_1^2 - S_2^2)$$

$$E = J\,S(S+1) + Cte$$

Antiferromagnetic coupling: J > 0

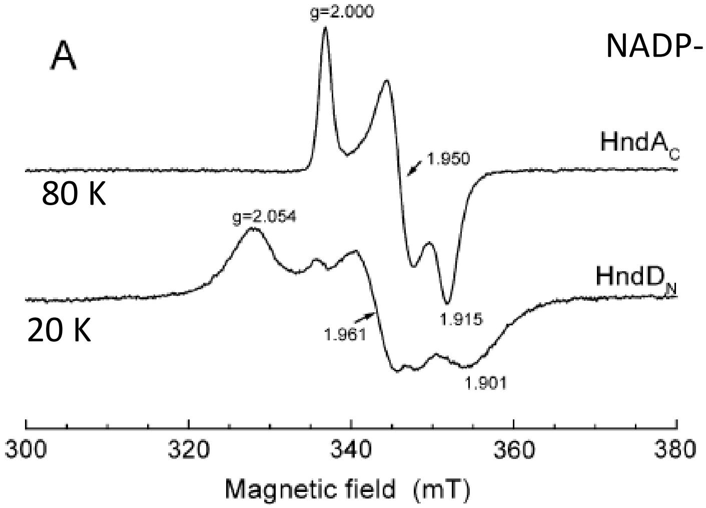
Ground state: $S_T = 1/2$

80 K



Fast relaxation of the EPR signal: Orbach process

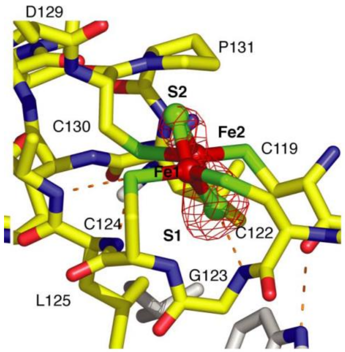
Fe-S clusters: Exchange interaction determination from relaxation broadening



NADP-dependent Fe-Fe hydrogenase from *D. fructosovorans*

Cys motive **$C\text{-}X_4\text{-}C\text{-}X_{35}\text{-}C\text{–}X_3\text{-}C$**
Signal broadening for T > 160 K

Cys motive **$C\text{-}X_{15}\text{-}C\text{-}X_2\text{-}C\text{–}X_{13}\text{-}C$**
Signal broadening for T > 50 K
Disappearance at T = 100 K

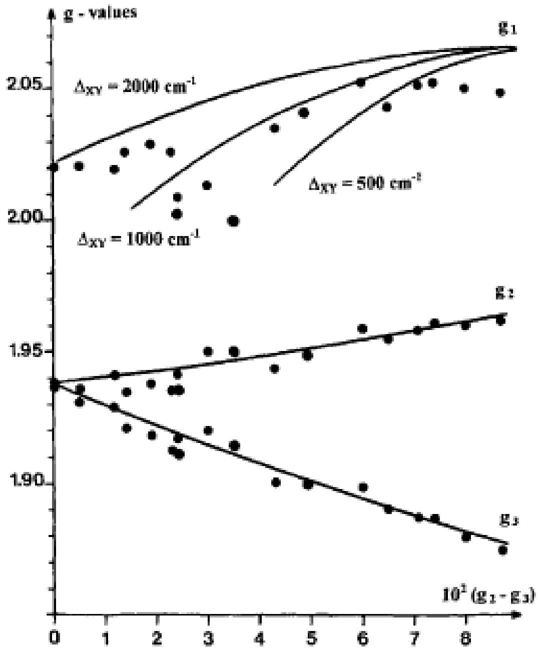


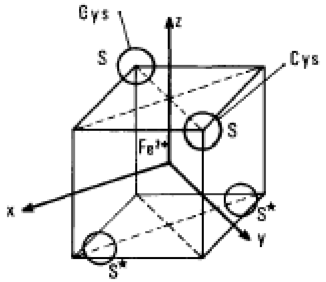
For each S manifold: $\tilde{g} = K_1\tilde{g}_1 + K_2\tilde{g}_2$

$$K_1 = \frac{S(S+1)+S_1(S_1+1)-S_2(S_2+1)}{2S(S+1)}$$

$$K_2 = \frac{S(S+1)+S_2(S_2+1)-S_1(S_1+1)}{2S(S+1)}$$

$$K_1 + K_2 = 1$$



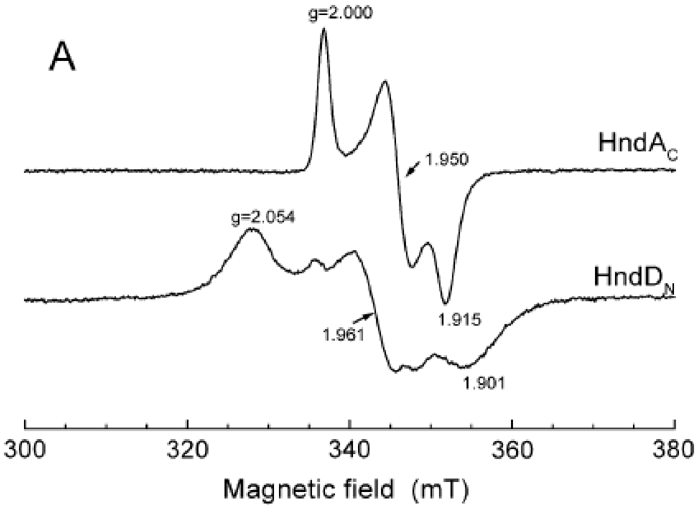


For the ground state S = 1/2: $g = 7/3\ g_1 - 4/3\ g_2$
Fe^{3+} , $S_1 = 5/2$, state $^6A_1$, only weak variations of g_1
Fe^{2+} , $S_2 = 5/2$, state $^2T_2$, g_2 very sensitive to structure variations
⇒ Correlations between g-values variations reflect structural changes of the Fe^{2+} site

(Bertrand & Guigliarelli, Adv.Inorg.Chem1999)

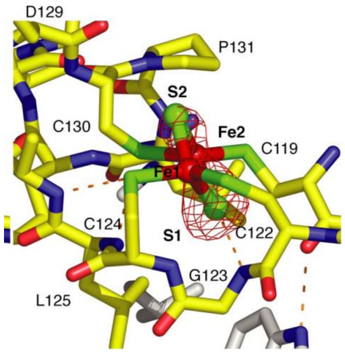
Fe-S clusters: Exchange interaction determination from relaxation broadening

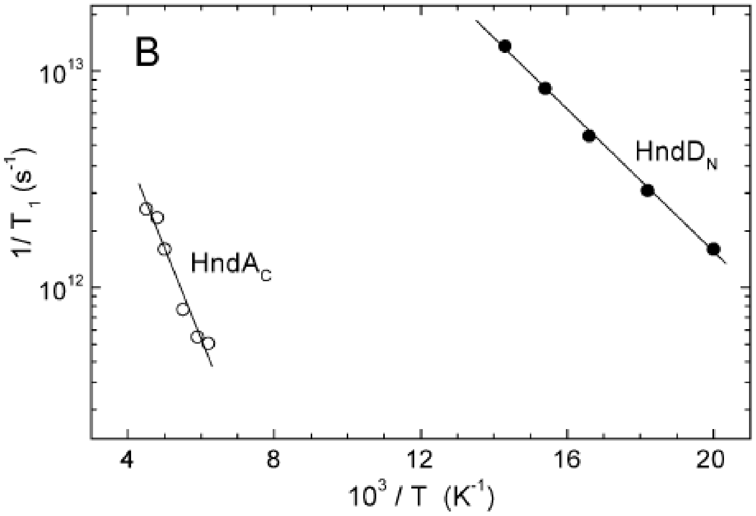
NADP-dependent Fe-Fe hydrogenase from *D. fructosovorans*



Cys motive **$C\text{-}X_4\text{-}C\text{-}X_{35}\text{-}C\text{–}X_3\text{-}C$**
Signal broadening for T > 160 K

Cys motive **$C\text{-}X_{15}\text{-}C\text{-}X_2\text{-}C\text{–}X_{13}\text{-}C$**
Signal broadening for T > 50 K
Disappearance at T = 100 K





Broadening by Orbach relaxation process
$1 / T_1 \propto \exp(-\Delta / kT)$

$\Delta = 3/2\ J$

HndD$_N$: J = 180 cm^{-1}

HndA$_C$: J = 560 cm^{-1}

Hyperfine coupling

$$H_S = \vec{S}\,\tilde{D}\,\vec{S} + \beta\,\vec{S}\,\tilde{g}\,\vec{B} + \vec{S}\,\tilde{\mathrm{A}}\,\vec{\mathrm{I}}$$

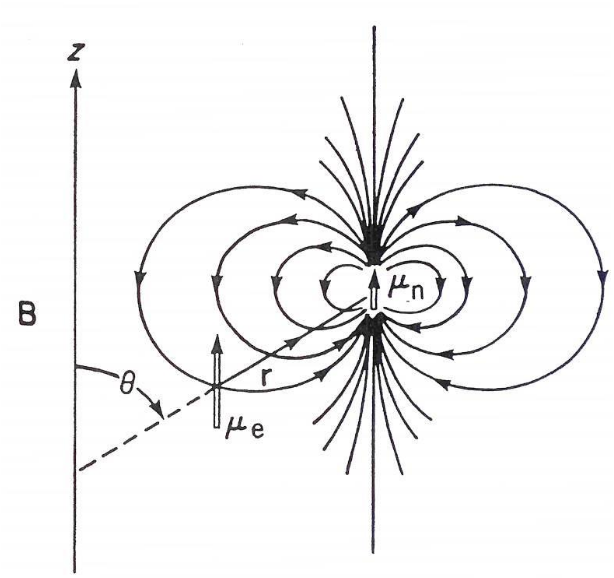
Magnetic coupling between electron and nuclear spins

Two physical contributions:
- Dipolar magnetic coupling

$$E_{dip} = -\vec{\mu}_e.\vec{B}_{induit} = \frac{\mu_0}{4\pi}\left(\frac{\vec{\mu}_e.\vec{\mu}_n}{r^3} - \frac{3(\vec{\mu}_n.\vec{r})(\vec{\mu}_e.\vec{r})}{r^5}\right)$$

$$\hat{H}(\mathbf{r}) = -\frac{\mu_0}{4\pi} g_e\beta_e g_n\beta_n\left(\frac{\hat{S}.\hat{I}}{r^3} - \frac{3(\hat{S}.\vec{r})(\hat{I}.\vec{r})}{r^5}\right)$$

$$\hat{H} = \hat{S}\tilde{T}\hat{I}$$ Anisotropic term, *Tr(T) = 0*



- Fermi contact term (non-zero probability of electron on nucleus)

$$\hat{H}_{Fermi} = \frac{2\mu_0}{3} g\beta_e\, g_n\beta_n |\psi(0)|^2 \vec{S}\cdot\vec{I} = a_{iso}\vec{S}\cdot\vec{I}$$

isotropic, reflects spin density on the nucleus

$$\boxed{H_{Hyp} = \vec{S}\,\tilde{A}\,\vec{I}}$$ Anisotropic term, with *Tr(A)* = a_{iso}

Hyperfine coupling: spectral effects

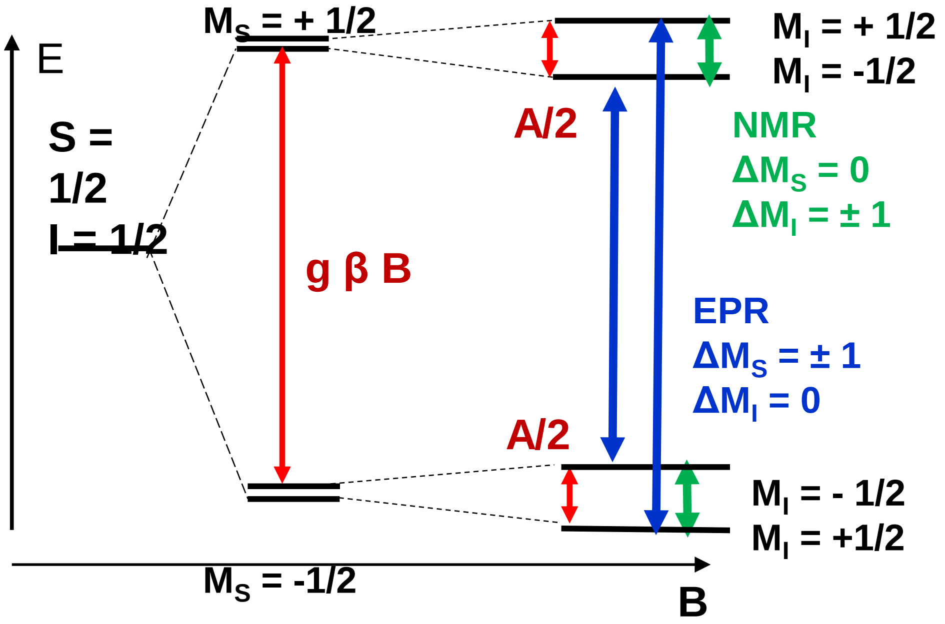
$$H_S = \vec{S}\,\tilde{D}\,\vec{S} + \beta\,\vec{S}\,\tilde{g}\,\vec{B} + \vec{S}\,\tilde{\mathrm{A}}\,\vec{\mathrm{I}}$$

Spin states: |S, M_S>, |I, M_I> : (2S+1)(2I+1) states

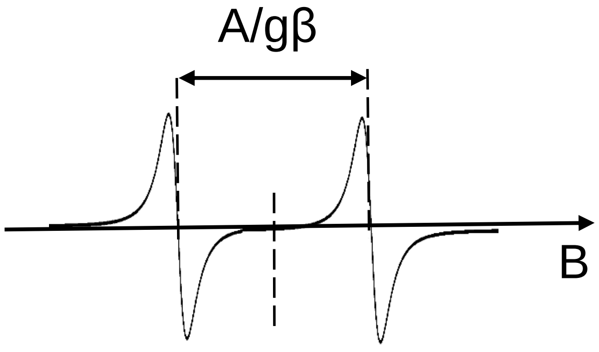
- For isotropic *g* and isotropic *A* tensor:

$$\mathbf{H_S = g\beta\,\vec{S}\cdot\vec{B} + A\vec{S}\cdot\vec{I} = g\beta\,S_Z B + A(S_Z I_Z)}$$

$$\boxed{\mathbf{E_{(M_S,M_I)} = g\beta\,B\,M_S + A\,M_S\,M_I}} \quad \text{(1}^{\text{st}}\text{ order)}$$



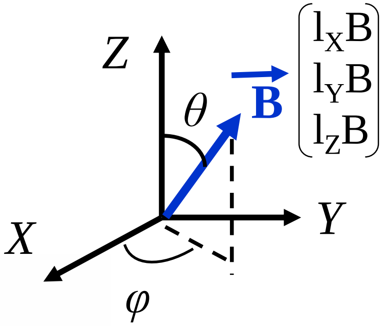
Splitting of the EPR line into (2 I +1) = 2 Hyperfine components



B_0 = hν/gβ

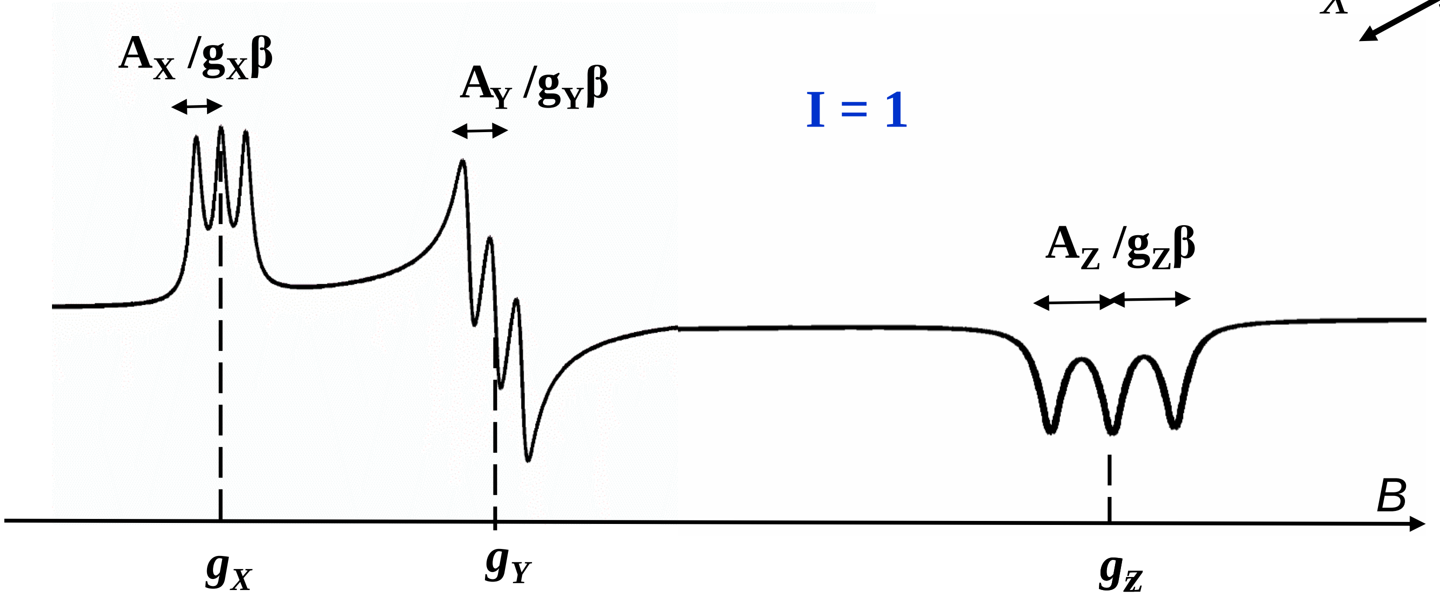
Hyperfine coupling: spectral effects

$$H_S = \vec{S}\,\tilde{D}\,\vec{S} + \beta\,\vec{S}\,\tilde{g}\,\vec{B} + \vec{S}\,\tilde{A}\,\vec{I}$$



General case: anisotropic *g* and *A* tensor

=> (2I+1) hyperfine components for each principal direction of *g*



***A* and *g* with parallel axes**

$$\boxed{\begin{aligned} g^2 &= l_X^2 g_X^2 + l_Y^2 g_Y^2 + l_Z^2 g_Z^2 \\ A^2 g^2 &= l_X^2 A_X^2 g_X^2 + l_Y^2 A_Y^2 g_Y^2 + l_Z^2 A_Z^2 g_Z^2 \end{aligned}}$$

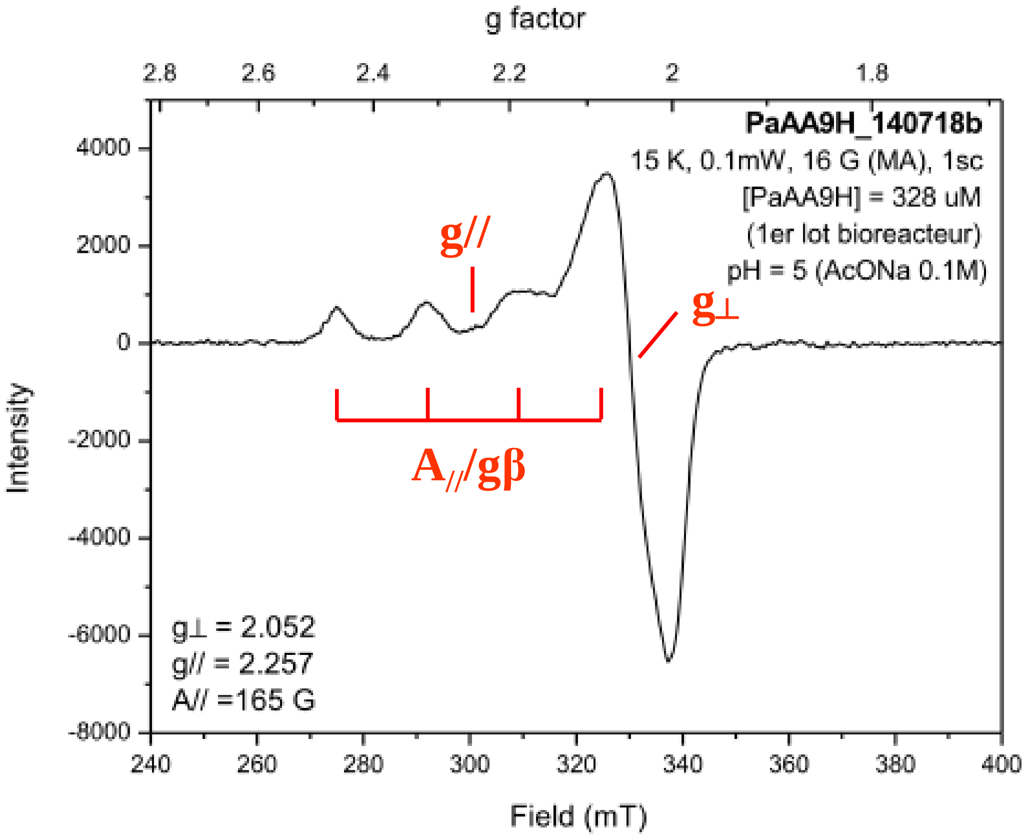
Hyperfine coupling: spectral effects

General case: anisotropic ***g*** and ***A*** tensor

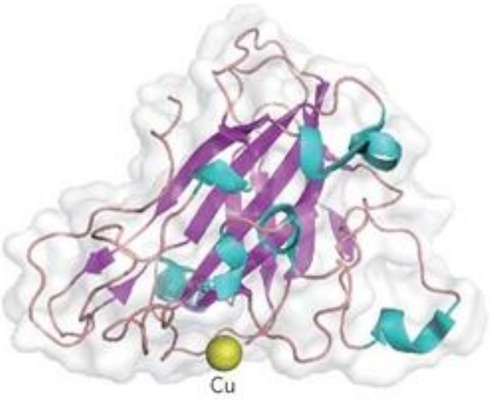
Exemple of copper enzymes

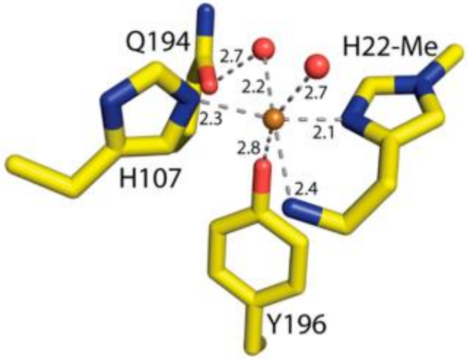
Cu^{2+} : $3d^9$ S=1/2

^{63}Cu, ^{65}Cu : I = 3/2   2I+1 = 4



Lytic Polysaccharides Monooxygénases (LPMO), *Pseudospora ancerina*



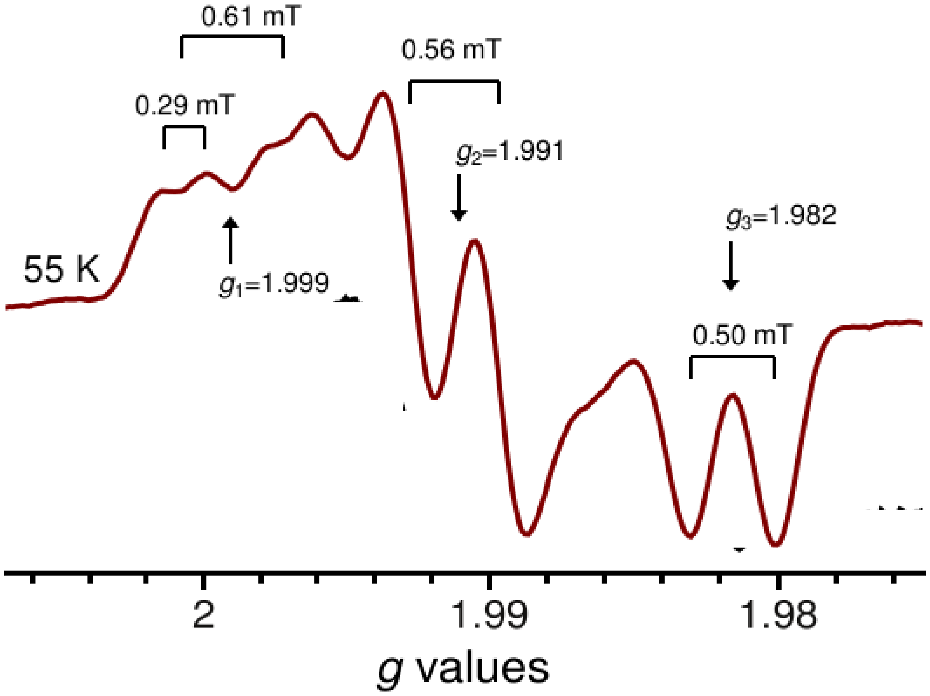


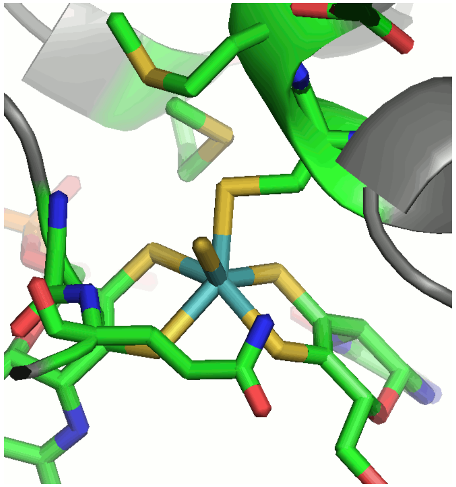
Partial resolution of hyperfine lines / linewidths

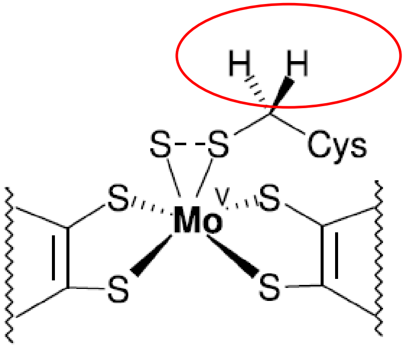
Hyperfine coupling: spectral effects

General case: anisotropic ***g*** and ***A*** tensor

Mo(V) cofactor ($4d^1$) of periplasmic nitrate reductase (*Rhodobacter sphaeroides)*



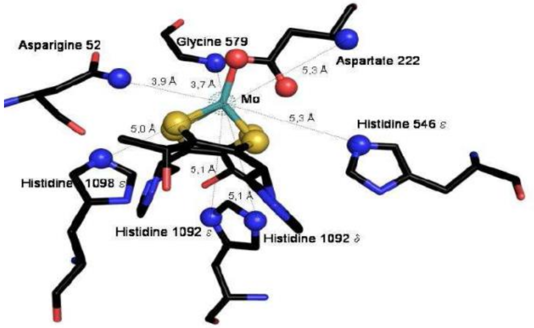


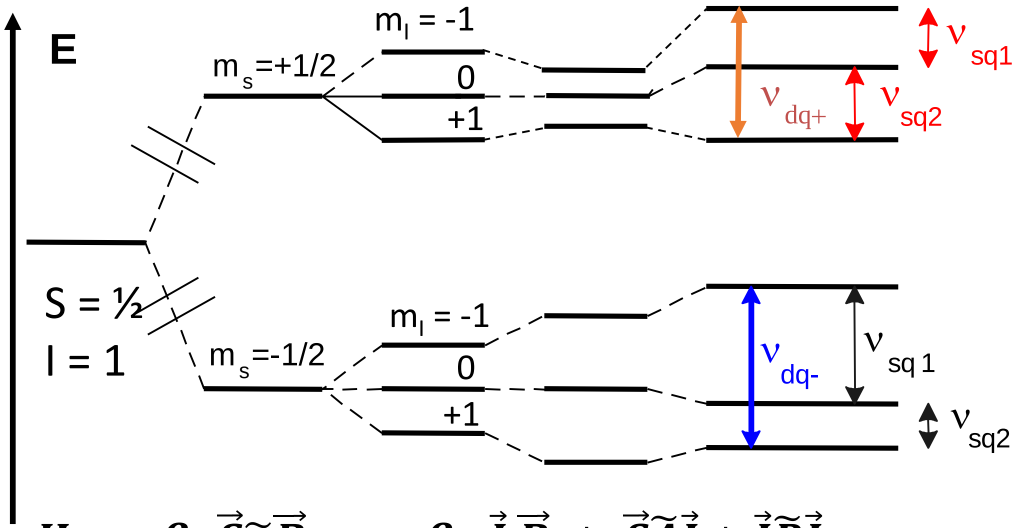


2 protons I = 1/2

Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Hyperfine sublevels correlation for ^{14}N (I = 1)

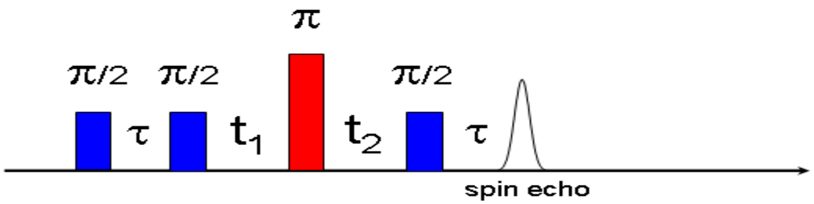




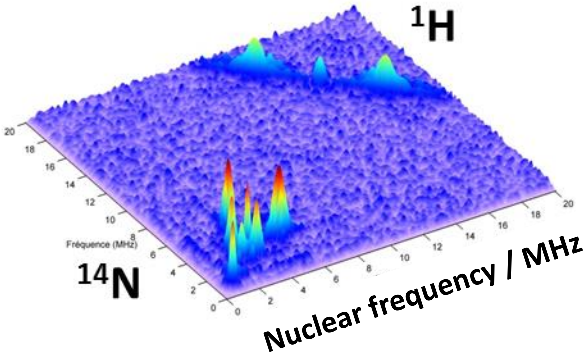
$$H_S = \beta_e \vec{S}\tilde{g}\vec{B} - g_n\,\beta_n\,\vec{I}\,\vec{B} + \vec{S}\tilde{A}\vec{I} + \vec{I}\tilde{P}\vec{I}$$

Hyperfine

Quadrupolar (K, η)

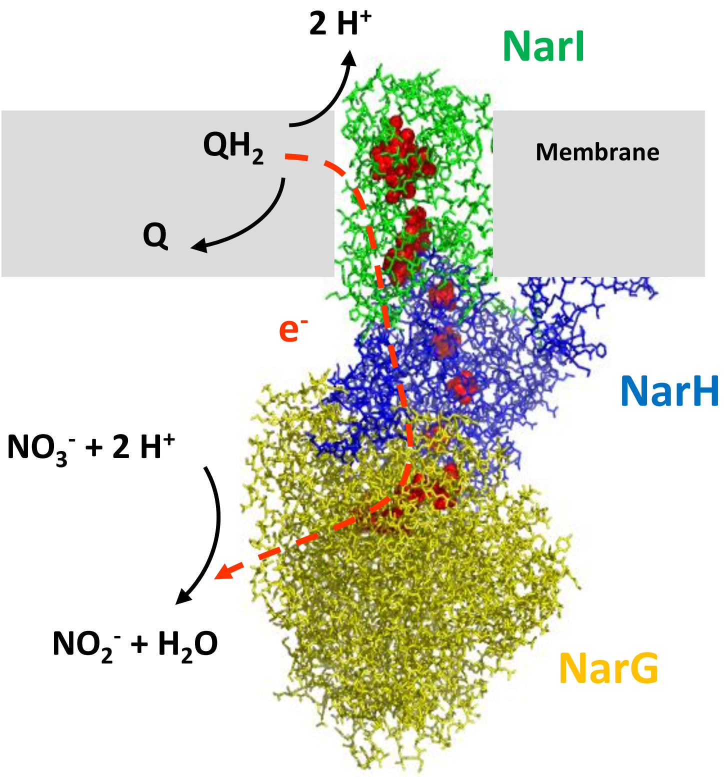


HYSCORE pulse sequence

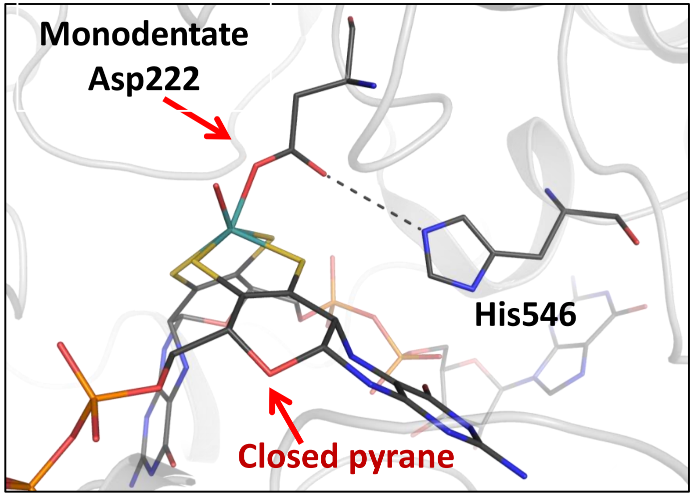


Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

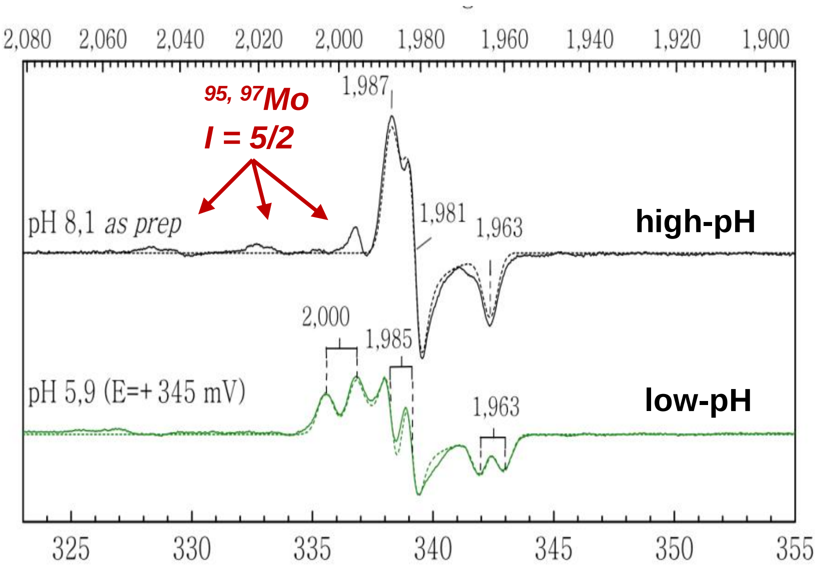
Membrane-bound Nitrate reductase from *E. coli* (NarGHI)



NarGH



(Jormakka, 2004)

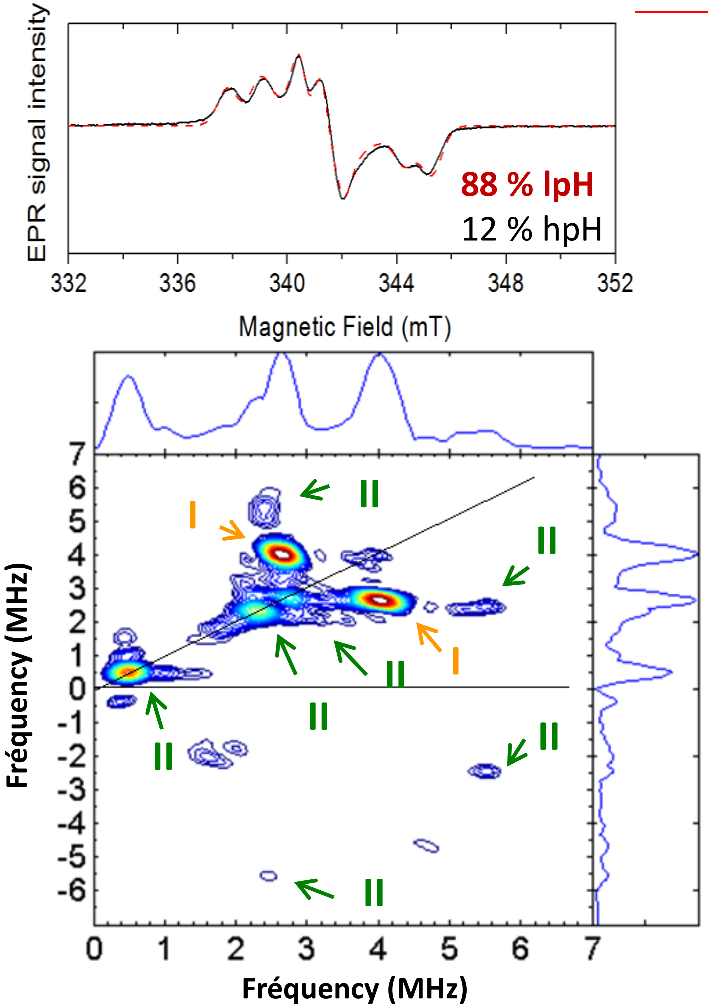


Structure of the different Mo(V) species ?

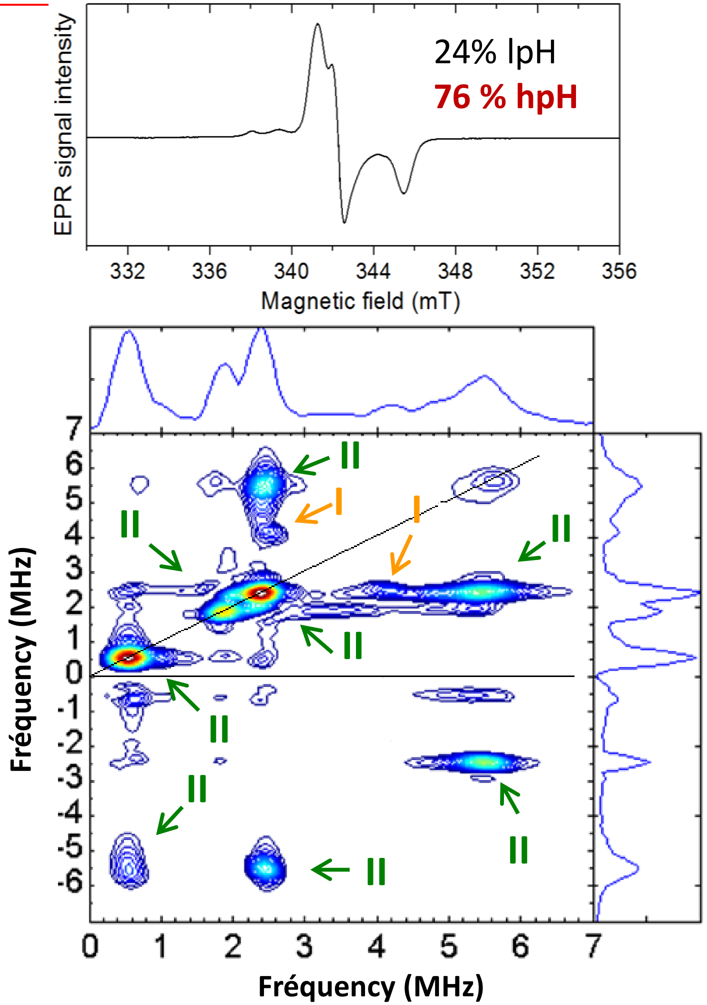
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

^{14}N / ^{98}Mo - NarGH

pH = 6



pH = 8.5

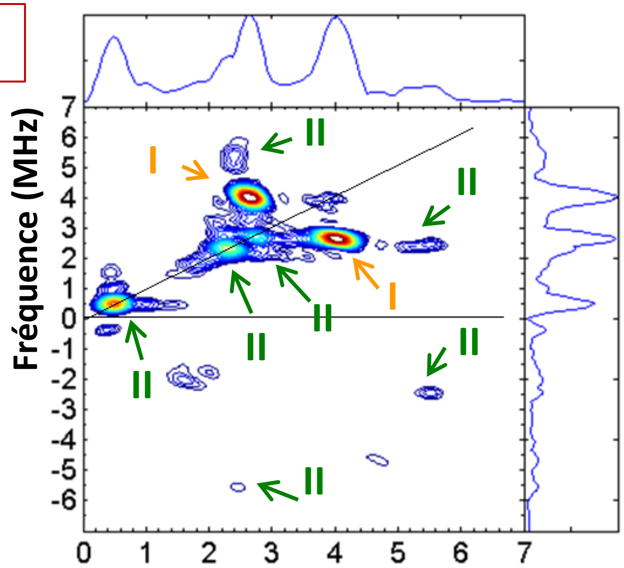


Evidence for two ^{14}N nuclei,
N_I and N_{II} associated to low pH and high pH Mo(V), respectively

Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Simplify HYSCORE spectrum with double isotope labeling ^{15}N (I=1/2) - ^{98}Mo of NarGH

pH = 6



- N_I parameter determination

pH = 6 preparation of ^{14}N / ^{98}Mo-NarGH

- Hyperfine and quadrupolar parameters:

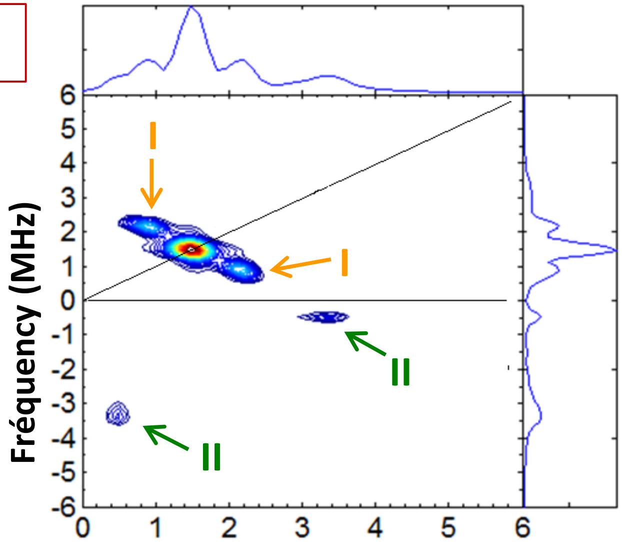
A_{iso} = 1.0 MHz
T = 0.25 MHz

κ = 0.6-0.7 MHz
η = [0-1]

A < $2\upsilon_I$(N)

^{15}N : *I = 1/2 ;* $\frac{A(14N)}{A(15N)} = \frac{g_n(^{14}N)}{g_n(^{15}N)} = 0.712$

pH = 6



Double isotope labelling:
pH = 6 preparation of ^{15}N / ^{98}Mo-NarGH

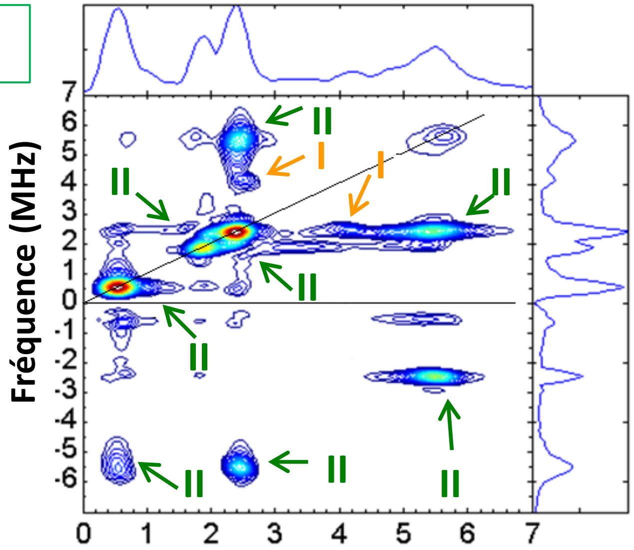
- Hyperfine parameters :

A_{iso} = 1.5 MHz
T = 0.4 MHz

Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Simplify HYSCORE spectrum with double isotope labeling ^{15}N (I=1/2) - ^{98}Mo of NarGH

pH = 8.5



- N_{II} parameter determination

pH 8.5 preparation of ^{14}N/ ^{98}Mo NarGH

- Hyperfine and quadrupolar parameters :

A_{iso} = 2.7 MHz
T = 0.56 MHz

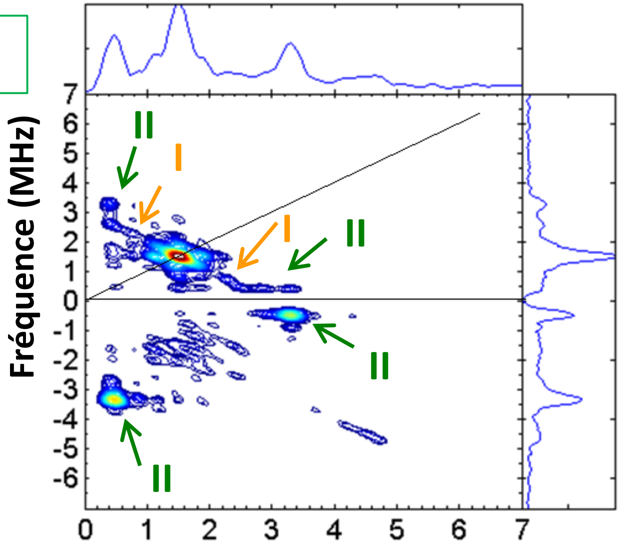
κ = 0.7 MHz
η = 0.4 MHz

Cancelation Condition
$A \sim 2\upsilon_I(N)$

^{15}N : I = 1/2 ;

$$\frac{A(14N)}{A(15N)} = \frac{g_n(^{14}N)}{g_n(^{15}N)} = \mathbf{0.712}$$

pH = 10



pH10 preparation of ^{15}N/ ^{98}Mo NarGH

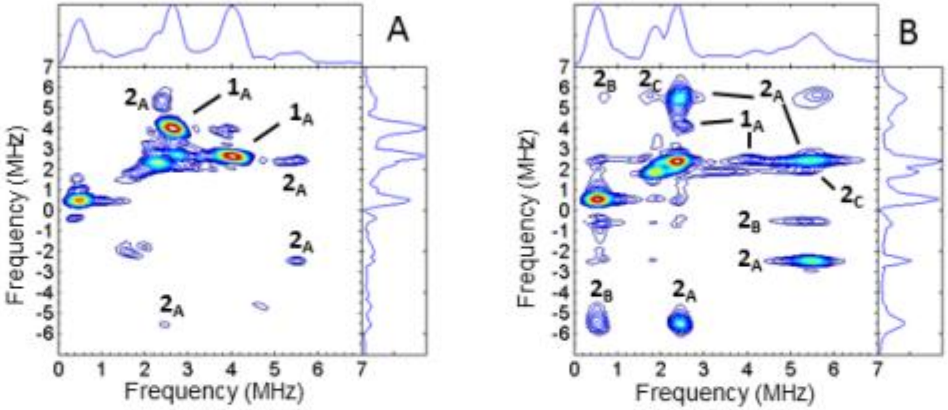
- Hyperfine coupling :

A_{iso} = 3.4 MHz
T = 0.7 MHz

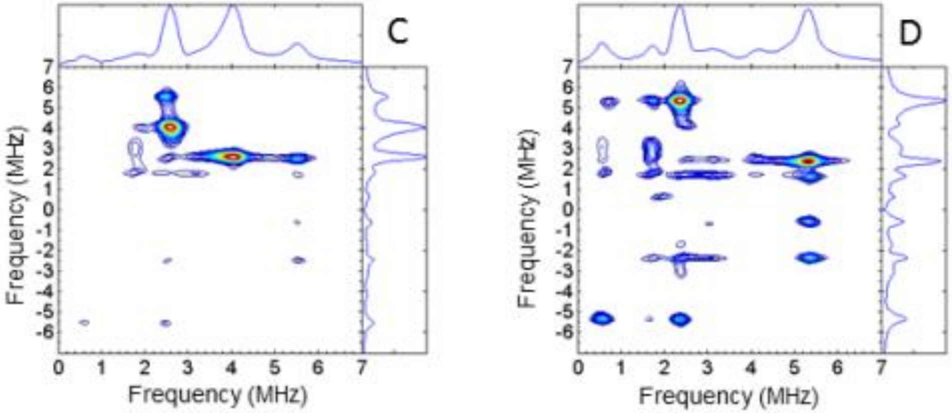
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

pH = 6.0 **pH = 8.5**

Experimental



Simulations

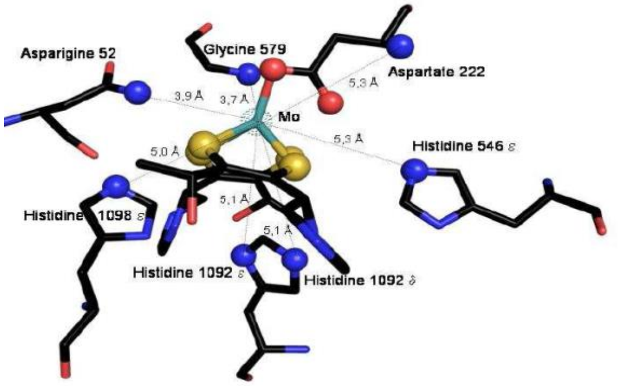


80% $^{14}N_I$ + 20% $^{14}N_{II}$

15% $^{14}N_I$ + 85% $^{14}N_{II}$

	^{14}N parameters	Assignement
$^{14}N_{II}$	A = 2.7 MHz κ = 0.66 MHz η = 0.4	High pH form
$^{14}N_I$	A = 1.1 MHz κ = 0.69 MHz η = 0.44	Low pH form

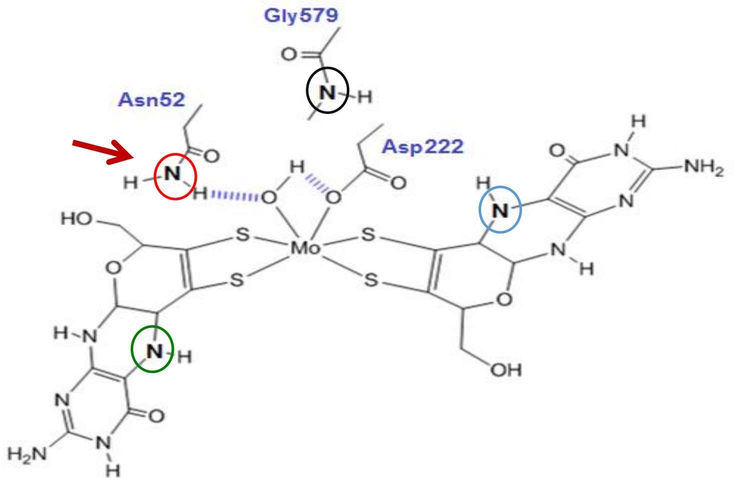
Similar quadrupole parameters for N_I et N_{II} :
Do they arise from the same chemical group ?



Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Structure model for Mo(V) low pH species

- **Entire tetrahydropyranopterin**
- **Amino-acids with closest N atoms : Asn52, Gly579**



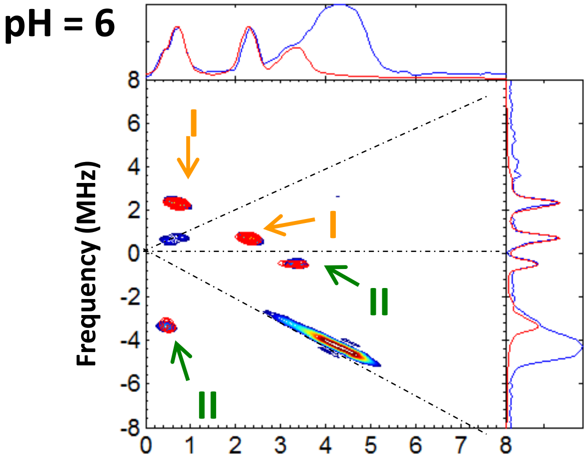
DFT

Nucleus	|κ| [MHz]	η
$^{14}N_Q$	1.433	0.162
$^{14}N_P$	1.422	0.168
$^{14}N_{N52}$	**0.786**	**0.418**
$^{14}N_{G579}$	0.916	0.205

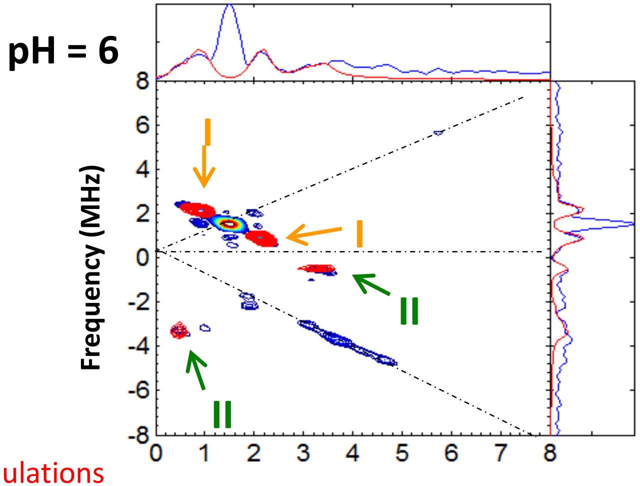
HYSCORE

$^{14}N_I$	**0.69**	**0.44**	**Low pH**
$^{14}N_{II}$	**0.66**	**0.4**	**High pH**

Selective ^{15}N-Asn labeling of ^{98}Mo-NarGH



^{15}N / ^{98}Mo-NarGH



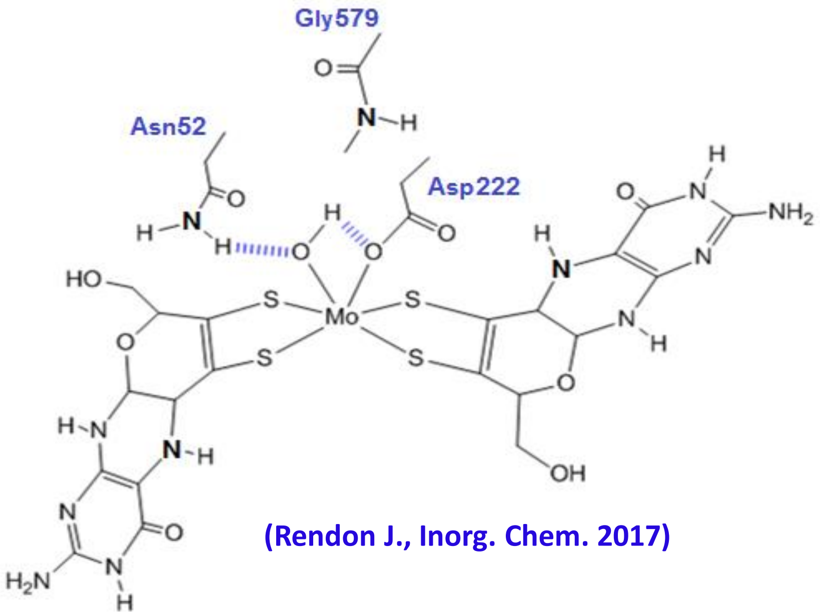
🟥 Simulations

Assignment to Asn52-N_δ
- N_I to low pH Mo(V)
- N_{II} to high pH Mo(V)

(Rendon, Inorg. Chem. 2017)

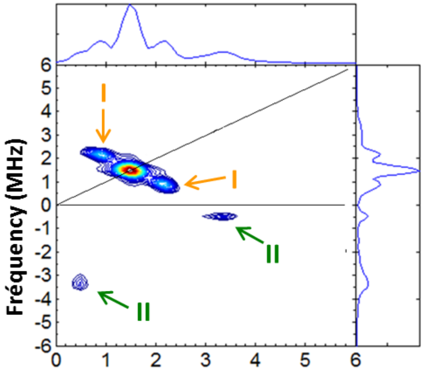
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

First structural model of the low pH Mo(V) species in NarGH



(Rendon J., Inorg. Chem. 2017)

HYSCORE Study of Nar



Soon: W-band EPR (95 GHz, 3.5 T)

In progress:

- ^{1}H, ^{2}H HYSCORE analysis in progress
- Structure of high pH Mo(V)
- Influence of distant amino-acids

Detection of intercenter magnetic coupling

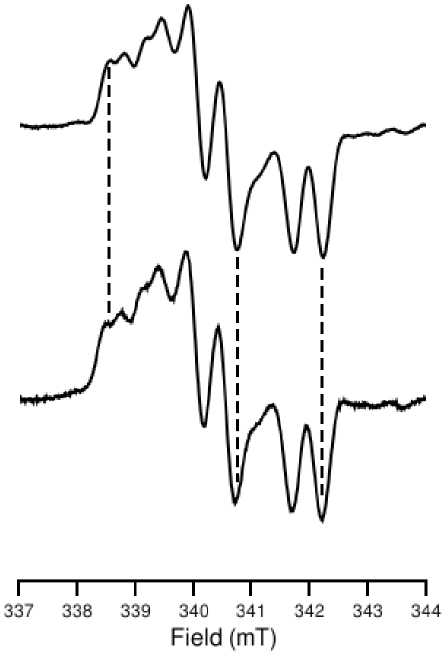
Analysis of spin-spin coupling between high g Mo(V) and reduced 4Fe-4S center in NapAB

$4Fe_{ox}$ Mo(V) resting → Excess reductant, Rapid freezing (<1 min) → $4Fe_{red}$ Mo(V) resting

"as prepared" enzyme

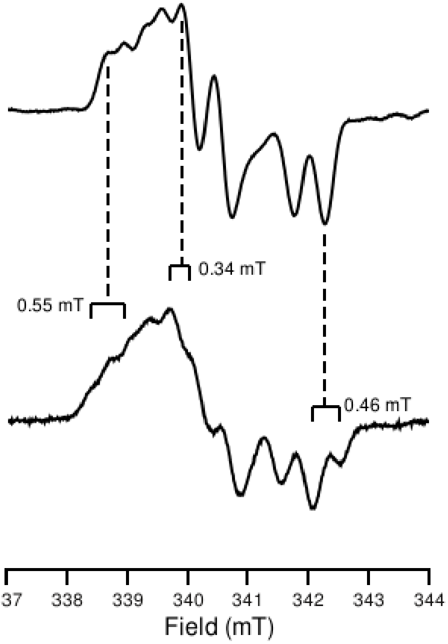
Rapidly reduced enzyme

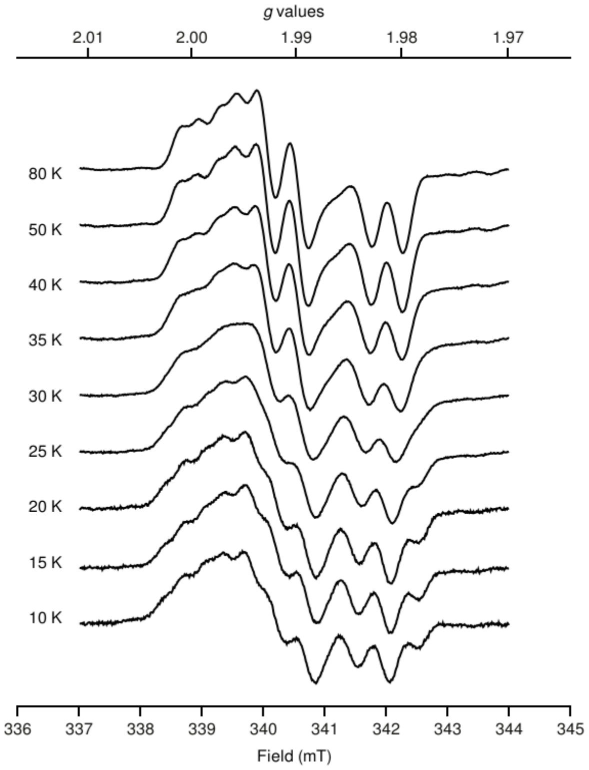
T dependence of the spectral shape



80 K

15 K

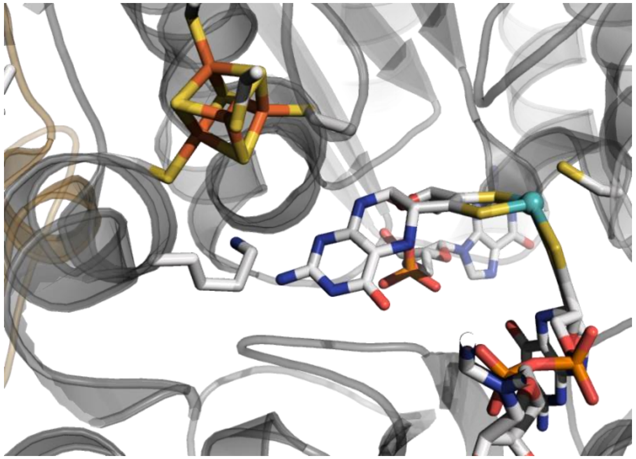




Detection of intercenter magnetic coupling

Analysis of spin-spin coupling between high g Mo(V) and reduced 4Fe-4S center in NapAB

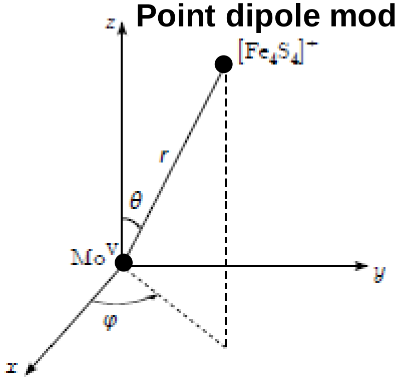
Mo(V) ↔ $[4Fe\text{-}4S]^{+1}$

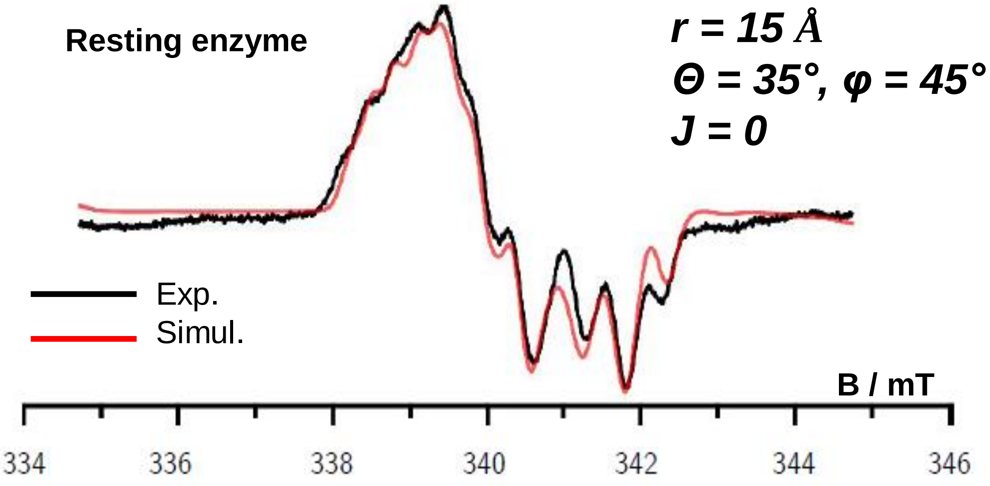


$$\hat{H}_{int} = \hat{H}_{exch} + \hat{H}_{dip}$$

$$J\,\vec{S}_1 \cdot \vec{S}_2 + \vec{S}_1\, D_{dip} \vec{S}_2$$

Point dipole model





Resting enzyme

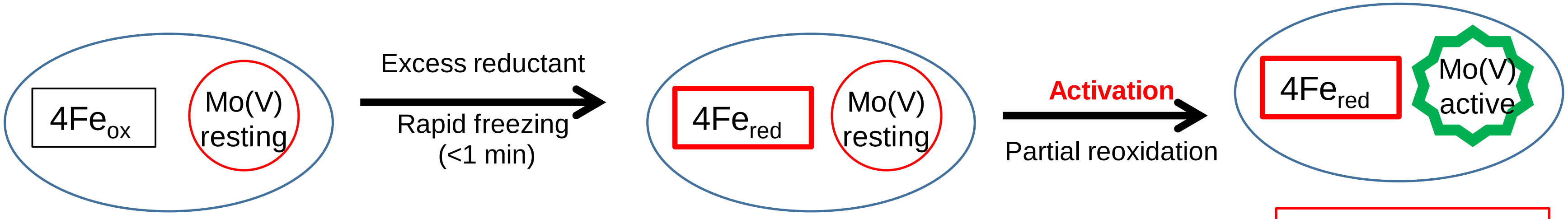
r = 15 Å
Θ = 35°, φ = 45°
J = 0

J = 0

No exchange coupling in inactive Nap between high g resting Mo(V) and FeS center

Detection of intercenter magnetic coupling

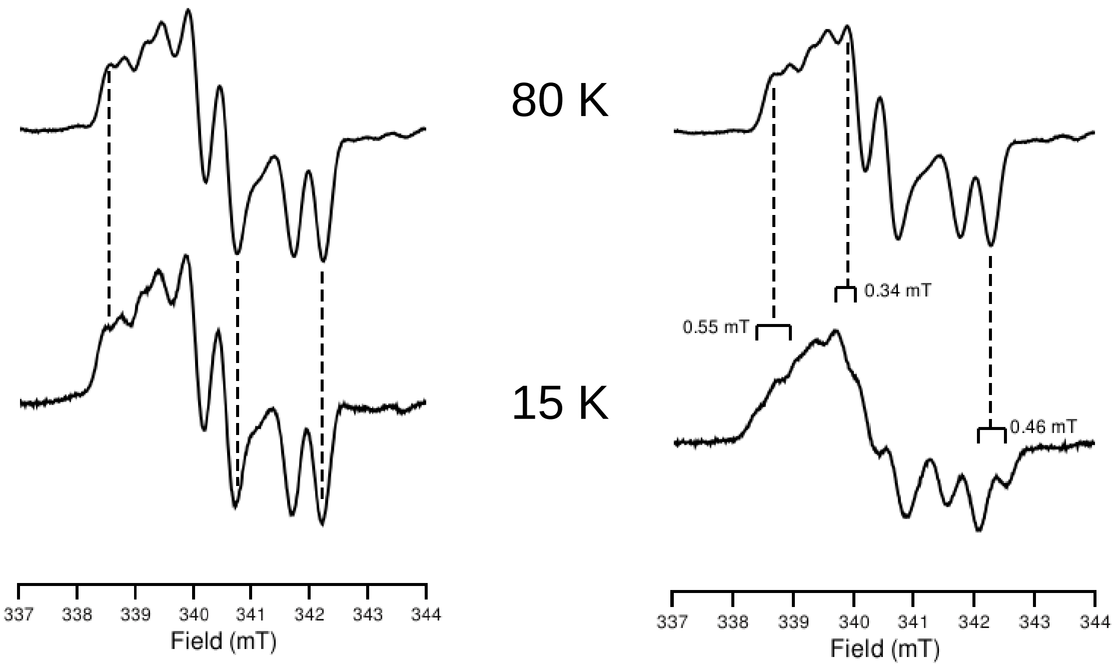
Influence of activation on the spin-spin coupling between high g Mo(V) and reduced Fe-S center

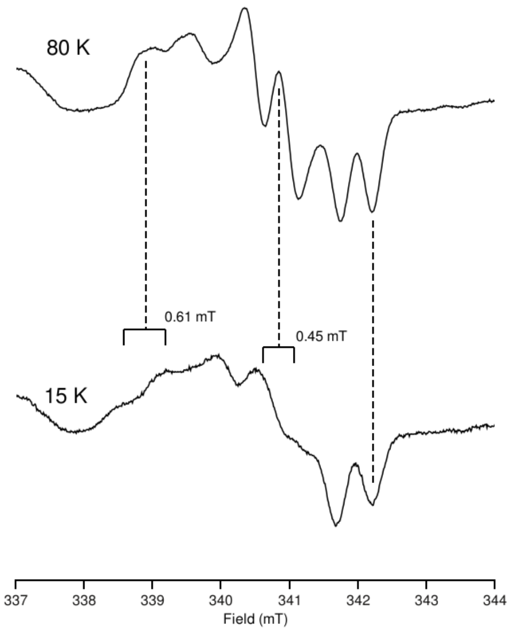


"as prepared" enzyme

Rapidly reduced enzyme

Activated enzyme



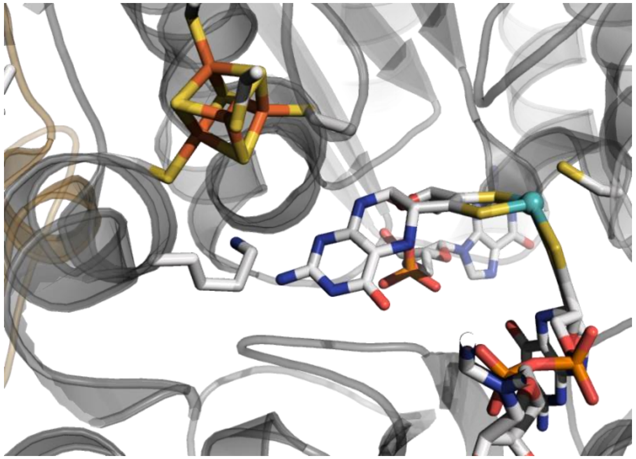


Change of the spin-spin coupling upon enzyme activation

Detection of intercenter magnetic coupling

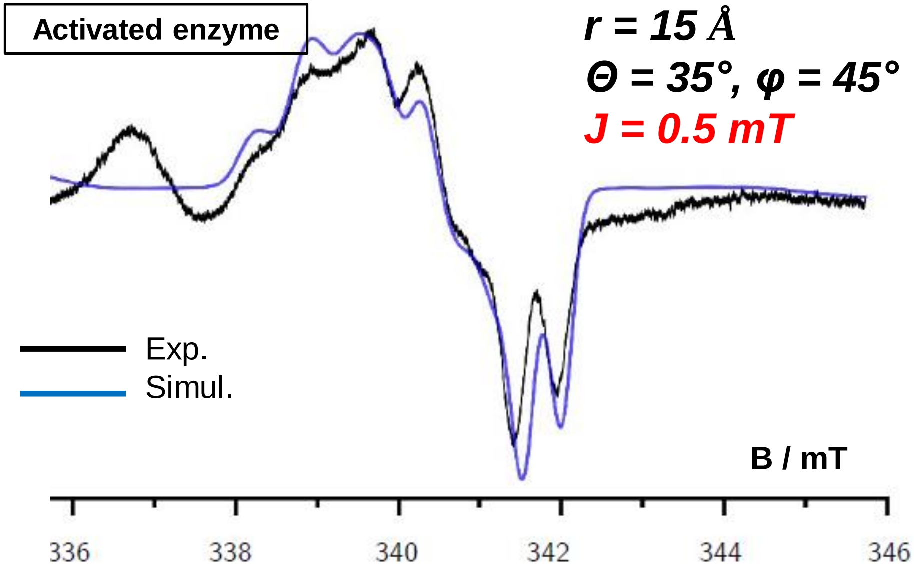
Analysis of spin-spin coupling between high g Mo(V) and reduced 4Fe-4S center in NapAB

Mo(V) ↔ $[4Fe\text{-}4S]^{+1}$



$$\hat{H}_{int} = \hat{H}_{exch} + \hat{H}_{dip}$$

$$J\,\vec{S}_1 \cdot \vec{S}_2 + \vec{S}_1\,D_{dip}\,\vec{S}_2$$

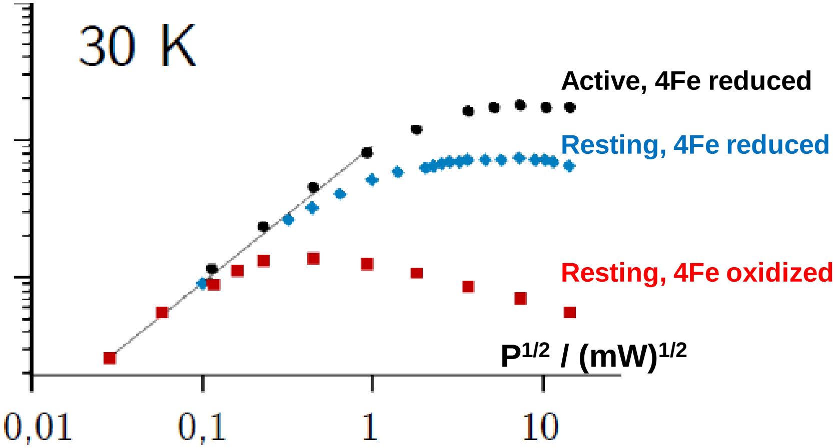


r = 15 Å

Θ = 35°, φ = 45°

J = 0.5 mT

Mo(V) EPR signal saturation properties



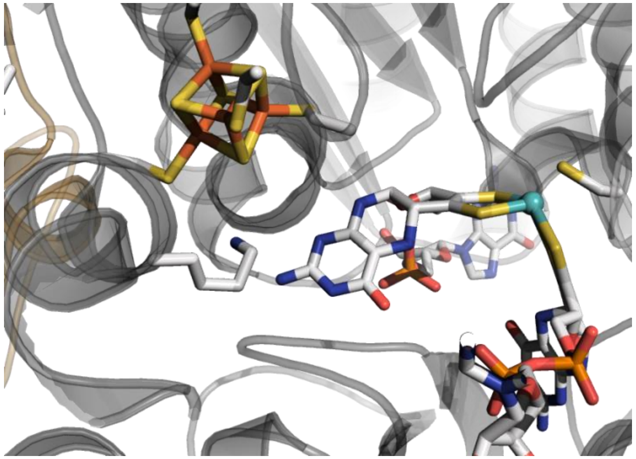
$$1/T_1 = 1/T°_1 + k_{1dip} + k_{1ex}$$

$$1/T_2 = 1/T°_2 + k_{2dip} + k_{2ex}$$

Detection of intercenter magnetic coupling

Analysis of spin-spin coupling between high g Mo(V) and reduced 4Fe-4S center in NapAB

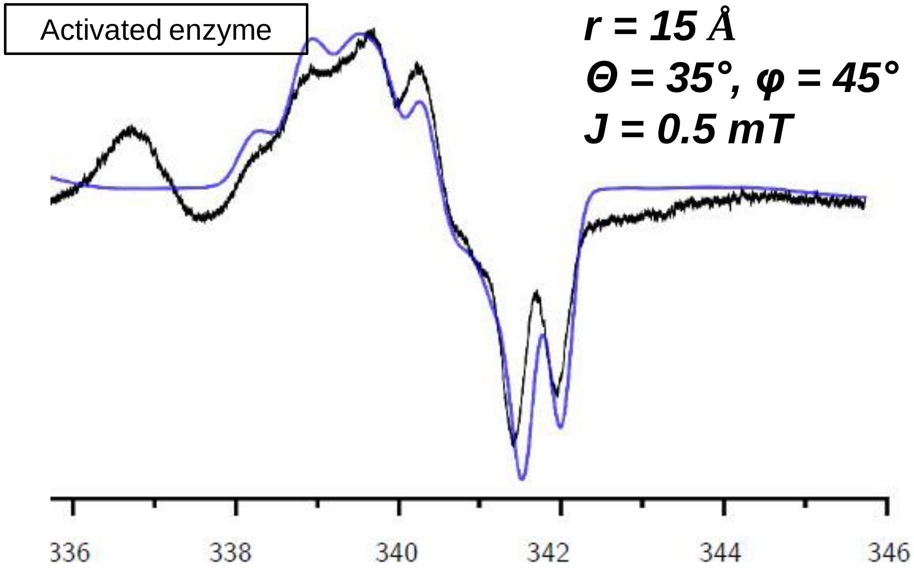
Mo(V) ↔ $[4Fe\text{-}4S]^{+1}$



$$\hat{H}_{\mathrm{int}} = \hat{H}_{\mathrm{exch}} + \hat{H}_{\mathrm{dip}}$$

$$\downarrow \qquad\qquad \downarrow$$

$$J\,\vec{S}_1 \cdot \vec{S}_2 + \vec{S}_1\, D_{dip} \vec{S}_2$$



r = 15 Å
Θ = 35°, φ = 45°
J = 0.5 mT

- In resting and activated enzymes, the high-g Mo(V) signals are very similar

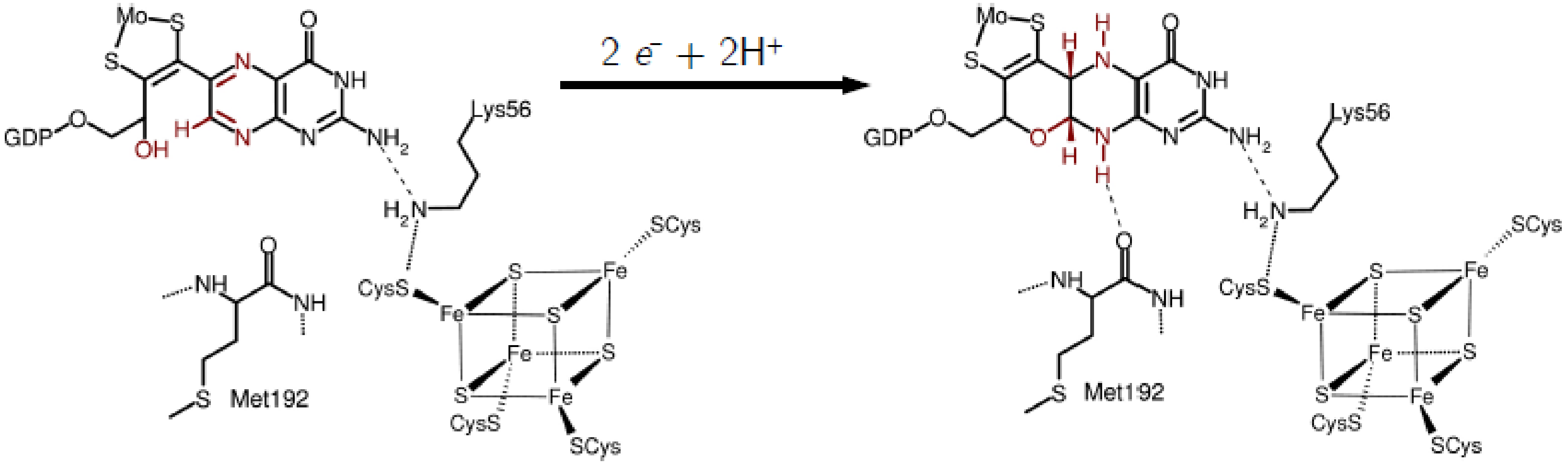
- No change of the first coordination sphere of the Mo ion in the activation process

- Change of the exchange coupling between Mo and Fe-S centers in the activation process.

Detection of intercenter magnetic coupling

Analysis of spin-spin coupling between high g Mo(V) and reduced 4Fe-4S center in NapAB

Model for NapAB activation : Pterin as a non-innocent ligand



Inactive enzyme

High g resting Mo(V), oxidized pterine

J = 0 , no electron transfer

Activated enzyme

High g « activated » Mo(V), reduced pterine

J = 0.5 mT, restored electron transfer

Change of hydrogen bond network around Mo ion

(J. Jacques, BBA 2014)

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Aix*Marseille université

A*Midex Initiative d'excellence Aix-Marseille

AGENCE NATIONALE DE LA RECHERCHE ANR
MOLYERE

Région Provence-Alpes-Côte d'Azur

TGE RENARD

Institut de Microbiologie de la Méditerranée imm

Thank you for your attention

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