EPR spectroscopy for Bio-Inorganic Chemistry
Principles and Applications

FrenchBIC summer school
Carry-Le-Rouet / Marseille – 17-21 September 2017

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Summary

1- Basic principles
2- Improving EPR sensitivity
3- Transition metal ions: magnetic and EPR properties
4- Low spin Fe$^{3+}$ systems: hemes
5- High spin Fe$^{3+}$ systems
6- Spin transitions
7- Electron spin relaxation
8- Fe-S clusters and exchange interaction
9- Hyperfine coupling
10- HYSCORE spectroscopy on Mo(V) cofactor
11- Detection of intercenter magnetic couplings
Magnetism is related to motion of electric charges

In matter: moving charges are electrons and protons

- Electron magnetism: 
  $L$, orbital momentum  
  $S$, spin momentum  
  
  \[
  \vec{\mu}_e = - \beta_e (\vec{L} + g_e \vec{S}) = - g \beta_e \vec{S}
  \]

- Nuclear magnetism 
  $I$, nuclear spin  
  
  \[
  \vec{\mu}_N = g_N \beta_N \vec{I} = \gamma_N \hbar \vec{I}
  \]

$\beta_e = e \hbar / 2 m_e = 9.274 \cdot 10^{-24} \text{ A} \cdot \text{m}^2$  
$\beta_N = e \hbar / 2 m_p = 5.05 \cdot 10^{-27} \text{ A} \cdot \text{m}^2$

Bohr’s magneton $\gg$ Nuclear magneton
Electron Paramagnetic Resonance (EPR): A spectroscopy specific of single electron systems

For a system with spin \( S = \frac{1}{2} \) in a magnetic field \( B \):

\[
\vec{\mu} = - g \beta_e \vec{S}
\]

\[
E = - \vec{\mu} \cdot \vec{B} \quad \Rightarrow \quad H = - \vec{\mu} \cdot \vec{B} = g \beta_e \vec{S} \cdot \vec{B}
\]

Taking \( \vec{Z} \parallel \vec{B} \Rightarrow H = g \beta B S_Z \)

\( S_Z \) is quantified: only two values \( M_S = \pm \frac{1}{2} \)

Energies: \( E = g \beta_e B M_S = \pm \frac{1}{2} g \beta B \) (Zeeman effect)

\[
\Delta E = g \beta_e B
\]
Electron Paramagnetic Resonance (EPR): A spectroscopy specific of single electron systems

For a system with spin $S = \frac{1}{2}$ in a magnetic field $B$

$$E = - \mu \cdot B \quad \Rightarrow \quad H = - \mu \cdot B = g \beta_e S \cdot B$$

Taking $Z \parallel B \Rightarrow H = g \beta_e B S_Z$

$S_Z$ is quantified: only two values $M_S = \pm \frac{1}{2}$

Energies: $E = g \beta B M_S = \pm \frac{1}{2} g \beta_e B$ (Zeeman effect)

Resonance condition

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

$g = 2.00, B_0 = 0.3$ T

$\nu = 10$ GHz, $\lambda = 3$ cm

Microwaves (X-band)
Basic principles: the magnetic resonance phenomenon

2nd World War: development of RAdio Detecting And Ranging (RADAR)
- Microwave sources: klystron (Bernard Rollin 1940)
- Highly sensitive detection crystals
- Antenna, Magic-T, …
- Lock-in amplifiers
Basic principles:

EPR detectable systems: \( \mu_e \neq 0 \Rightarrow S \neq 0 \)

- **Odd electron number:**
  - Free radicals (organics, OH\(^{•}\), NO\(^{•}\), NO\(_2^{•}\), HCO\(_3^{•}\),…)
  - 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/inorganic molecular conductors, ferromagnets,….
Basic principles: the sensitivity of EPR

Thermal equilibrium and spin state populations

\[ E \]

\[ S = 1/2 \]

\[ M_S = +1/2 \]

\[ B \neq 0 \]

\[ M_S = -1/2 \]

\[ \Delta E = g \beta B \]

Weak value of \( \Delta E = g \beta B \)

\( B = 0.3 \, \text{T} \quad \Delta E \sim 0.3 \, \text{cm}^{-1} \)

Thermal equilibrium (Boltzmann’s law)

\[ \frac{N_+}{N_-} = \exp\left(- \frac{\Delta E}{k_B T}\right) \]

\[ \frac{N_+}{N_-} = \exp\left(- \frac{g \beta B}{k_B T}\right) \]

\( T = 298 \, \text{K}, \quad \frac{N_+}{N_-} = 0.9986 \)

Very weak spin polarization

\[ p = \frac{N_+ - N_-}{N_+ + N_-} = 7 \times 10^{-4} \]
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

\[ P_{\text{abs}} = h\nu (\mathcal{W}N_+ - \mathcal{W}N_-) = h\nu \mathcal{W} n \quad \text{with} \quad n = N_- - N_+ \]

EPR signal intensity is directly related to \( n \)

Important consequence: Curie’s law

\[ I \propto n/N_0 = \text{th}(g\beta B_0 / 2k_B T) \approx g\beta B_0 / 2k_B T \]

EPR signal intensity obeys the Curie’s law \( I \cdot T = \text{Cte} \)
Basic principles: the sensitivity of EPR

\[ n = N_{\text{inf}} - N_{\text{sup}} \text{ population difference} \]

\[ \text{Signal} \propto n \]

\[ \approx 10^4 \text{ cm}^{-1} \]

\[ \lambda = 500 \text{ nm} \]

EPR

\[ n/N_0 \ll 1 \]

High sensitivity to population changes
- Température
- Radiation absorption
- Fluctuations of the environment

\[ \frac{n}{N_0} = 1 \]
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 \(Q \sim 5-6000\)

Microwaves

Sample

Irradiation

Rectangular cavity TE102

\[
Q = 2\pi \frac{\text{énergie stockée}}{\text{énergie dissipée}} = \frac{v_{\text{res}}}{\Delta v}
\]

Sensitivity \(\propto Q\) factor
Basic principles: improving EPR sensitivity

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

Dielectric absorption ($\varepsilon_r$)

decrease of Q-factor

NMR tube: $\phi_{\text{ext}}$ 5mm

Quartz EPR tubes:
X-band: $\phi_{\text{ext}}$ 4mm, $\phi_{\text{int}}$ 3mm

Q-band: $\phi_{\text{ext}}$ 3mm, $\phi_{\text{int}}$ 2mm

Capillary: $\phi_{\text{ext}}$ 2mm, $\phi_{\text{int}}$ 1mm

Flat cell: $e_{\text{int}}$ = 1 mm
Basic principles: improving EPR sensitivity

Decrease the noise: Magnetic field amplitude modulation

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

if \( \Delta B_m \) is « small »

\( b_m(t) = \frac{1}{2} \Delta B_m \cos(2\pi \nu_m t + \phi) \)

\( \nu_m = 100 \text{ kHz} \)

Strong improvement of the Signal /Noise ratio

\( \Delta B_m \ll \text{linewidth } \delta B \)
Basic principles: improving EPR sensitivity

\[ B + \Delta B_m \cos(2\pi v_m t + \phi) \]

\[ \Delta B_m << \text{linewidth } \delta B \]

To avoid line broadening by overmodulation

Microwaves

Sample tube

Rectangular cavity TE102

Microwave source

Fixed \( v \)

Detection diode

Signal \( s(B) \)

Modulation coils

\( \Delta B_{\text{mod}} \ll \delta B \)

\( \Delta B_{\text{mod}} > \delta B \)
Multifrequency CW-EPR equipment
Transition metal compounds: Magnetic properties

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

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

Free ion

$S = 3/2$

weak field

$S = 3/2$

strong field

$S = 1/2$

low Spin

$d$ orbitals

$\Delta o$

$\Delta o$

Spectrochemical series $[\text{Co} \ X \ (\text{NH}_3)_5]^{2+}$

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Transition metal compounds: Magnetic properties

Hamiltonian \[ H = T_e + V_{en} + V_{ee} + V_L + H_{SO} + \text{magnetic terms} \]

Requires the orbital part of the wavefunction to be calculated

Phenomenological approach: The spin hamiltonian \( H_S \)

\[ H = \lambda \vec{L} \cdot \vec{S} + \beta (\vec{L} + g_e \vec{S}) \cdot \vec{B} + \text{couplings} \ (\mu_e, \mu_n) \ldots \]

Zero field splitting (fine structure term)

Anisotropy

Zeeman Effect

Hyperfine couplings e-nuclei

\[ H_S = \vec{S} \ D \ \vec{S} + \beta \ S \ g \ B \ A \ I \]

Terms: Fine structure Zeeman Hyperfine

\( \vec{D}, \vec{g}, \vec{A} \) Rank 2 tensors \( \Rightarrow 3 \times 3 \) matrix

\[ \vec{g} = \begin{bmatrix} g_{xx} & g_{xy} & g_{xz} \\
                        g_{yx} & g_{yy} & g_{yz} \\
                        g_{zx} & g_{zy} & g_{zz} \end{bmatrix} \]
Transition metal compounds: Magnetic properties

\[ H_S = \vec{S} \cdot \vec{D} \cdot \vec{S} + \beta \vec{S} \cdot \vec{g} \cdot \vec{B} + \vec{S} \cdot \vec{A} \cdot \vec{I} \]

**Zeeman term:** Zeeman effect + 2\textsuperscript{nd} order effect of spin-orbit coupling

\[ H_{\text{Zeeman}} = \beta \vec{S} \cdot \vec{g} \cdot \vec{B} \]

\[ H_{\text{Zeeman}} = \beta (S_X g_X B_X + S_Y g_Y B_Y + S_Z g_Z B_Z) \]

- Departure of \( g \) values from \( g_e = 2.0023 \)
- Anisotropy

\[ g_i = g_e - \alpha_i \cdot \frac{\lambda}{\Delta_i} \quad (i = x, y, z) \]

\( \lambda \) = spin-orbit coupling constant

\( d^n \) configuration

\( n < 5, \lambda > 0 \Rightarrow g_i < g_e = 2.00 \)

\( n > 5, \lambda < 0 \Rightarrow g_i > g_e = 2.00 \)
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

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

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

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

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

$B_z = h\nu/g_z\beta$

$B_y = h\nu/g_y\beta$

$B_x = h\nu/g_x\beta$

$g_X < g_Y < g_Z$
Disordered system: all the B orientations are present

Anisotropic $g$ tensor— Powder or frozen solution spectrum

EPR signal intensity:
$I \propto N \langle g_P \rangle_{moyen} B_1 \text{th}(h\nu/2k_B T)$

Intensity = surface of absorption spectrum

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…

\[
g_X = 2.04 \quad g_Y = 1.94 \quad g_Z = 1.87
\]

15 K

[4Fe-4S]^{1+}

\[
\begin{align*}
g_X &= 2.92 \\
g_Y &= 2.22 \\
g_Z &= 1.45
\end{align*}
\]

55 K

Mo (V)

R. Sphaeroides periplasmic Nitrate reductase - NapAB

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

Hemes (Fe^{3+})
Low spin Fe$^{3+}$ systems: hemes

Ground state $^2T_{2g}$
Orbital triplet state, $S = \frac{1}{2}$
No fine structure term, but strong influence of Spin-Orbit coupling on g-tensor anisotropy.

$$H_S = \beta \mathbf{\hat{S}} \mathbf{\hat{g}} \mathbf{\hat{B}}$$
Low spin Fe³⁺ systems: hemes

Magneto-structural correlations:

$t_{2g}$ hole model (Griffith, 1971)

Fe³⁺ ion in strong distorted octaedral ligand field

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

\[ g_i = f(\lambda, \mu, R) : \quad g_X^2 + g_Y^2 + g_Z^2 = 16 \]

Also accounts for g-strain broadening

(Cytochromes)

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

$\phi \approx 10\text{–}20^\circ$

$g_z = 3.0$

$\phi \approx 80\text{–}90^\circ$

$g_z = 3.8$

HALS hemes

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

EPR of *E. coli* membrane fractions

Control

WT

H187Y

H66Y

H56Y
Low spin Fe\textsuperscript{3+} systems: hemes

\textit{R. Sphaeroides} periplasmic Nitrate reductase - NapAB

\[ \text{Fe}^{3+} + \text{e}^- = \text{Fe}^{2+} \]

\[ S = \frac{1}{2} \quad S = 0 \]

\[ E^\circ_1 = 0 \text{ mV} \]
\[ E^\circ_2 = -120 \text{ mV} \]
High spin Fe$^{3+}$ systems

- Free Fe$^{3+}$ ion: $S = \frac{5}{2}$
  - Ground state: $^6S$
- Weak field: $S = \frac{5}{2}$
  - High spin: $^6A_1$
- Strong field: $S = \frac{1}{2}$
  - Low spin: $^2T_2$

**Energie**

- $d$ orbitals

**Myoglobin**
- $g_{\perp} = 6.0$, $g_{//} = 2.0$

**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 $\{|S, M_S>\}$

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

$D$ axial, $g$ isotropic

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

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

Case $D >> g \beta B$ (0.3 cm$^{-1}$ at 0.3 T)

- $B = 0$: Zero field splitting

$S_Z \ |S,M_S> = M_S \ |S,M_S> \quad \Delta M_S = 0$

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

$$E (M_S) = D (M_S^2 - 1/3 S(S+1))$$

$$E (M_S) = D (M_S^2 - 35/12)$$

Heme in Myoglobin
High spin Fe$^{3+}$ systems

- Axial symmetry – Influence of fine structure

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

- $B \neq 0$

\[
H_{\text{Zeeman}} = g \beta (S_X B_X + S_Y B_Y + S_Z B_Z)
\]

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

$\Delta M_S = 0$ \hspace{1cm} $\Delta M_S = \pm 1$

Perturbation approach:

\[
\begin{pmatrix}
-5 \cos \theta & 0 & X & 0 & 0 & 0 \\
0 & +5 \cos \theta & 0 & X & 0 & 0 \\
X & 0 & -3 \cos \theta & 0 & X & 0 \\
0 & X & 0 & +3 \cos \theta & 0 & X \\
0 & 0 & X & 0 & - \cos \theta & 3 \sin \theta \\
0 & 0 & 0 & X & 3 \sin \theta & + \cos \theta \\
\end{pmatrix}
\]

$H_{\text{Zeeman}} = \frac{1}{2} g \beta B$

\[
S_X = \frac{1}{2} (S_+ + S_-)
\]

$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 - $D >> g \beta B$

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

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

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

1$^{\text{st}}$ order perturbation calculation

$H_{\text{Zeeman}} = \frac{1}{2} g \beta B$

\[
\begin{pmatrix}
-5 \cos \theta & 0 & X & 0 & 0 & 0 \\
0 & +5 \cos \theta & 0 & X & 0 & 0 \\
X & 0 & -3 \cos \theta & 0 & X & 0 \\
0 & X & 0 & +3 \cos \theta & 0 & X \\
0 & 0 & X & 0 & -\cos \theta & 3 \sin \theta \\
0 & 0 & 0 & X & 3 \sin \theta & + \cos \theta \\
\end{pmatrix}
\]
**High spin Fe$^{3+}$ systems**

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

- $M_S = \pm 5/2$  $g''_{\text{eff}}$ axial: $g''_{\text{eff} //} = 5g = 10, g''_{\text{eff} \perp} = 0$
- $M_S = \pm 3/2$  $g'_{\text{eff}}$ axial: $g'_{\text{eff} //} = 3g = 6, g'_{\text{eff} \perp} = 0$
- $M_S = \pm 1/2$  $g_{\text{eff}}$ axial: $g_{\text{eff} //} = g = 2, g_{\text{eff} \perp} = 3g = 6$

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

5 allowed transitions

Only one is energetically accessible: $hv \approx g \beta B << D$

**Forbidden transitions** $\Delta M_S = \pm 3; \Delta M_S = \pm 5$
High spin Fe$^{3+}$ systems

Axial Symmetry - $D >> g \beta B$

Measurement of $D$: Temperature study

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

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

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

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

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

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

$g_{eff \perp} = 6.0 \quad g_{eff \parallel} = 2.0$

Curie’s law

$D = 12 \text{ cm}^{-1}$

$M_S = \pm 5/2$

$S = 5/2$

$\pm 3/2$

$\pm 1/2$

$E$

$4D$

$2D$

$g_{eff \perp} \beta B$

$g_{eff \parallel} \beta B$

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High spin Fe$^{3+}$ systems

Rhombic fine structure: \((D, E) \gg g \beta B\)

\[
H_{SF} = D (S_Z^2 - \frac{1}{3} S(S+1)) + E(S^2_X - S^2_Y)
\]

\[
H_{SF} = D (S_Z^2 - \frac{1}{3} S(S+1)) + \frac{E}{2} (S^2_+ + S^2_-)
\]

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 \(M_S = \pm 1/2\)
\(g_{eff \, X} = 6 + 24 E/D\)
\(g_{eff \, Y} = 6 - 24 E/D\)
\(g_{eff \, Z} = 2.0\)

\(\Rightarrow\) 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

$g = 9.4, 1.2, 0.9$
$g = 4.77, 4.3, 4.0$

$D = 1.76\text{ cm}^{-1}$ $E = 0.495\text{ cm}^{-1}$

(Peisach 1971)
High spin / Low spin Fe$^{3+}$ systems

EPR Signal intensity: $I \propto N (g_P)_{av} B_1 \text{th}(hv/2k_B T)$

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

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 ?

\[ g = 5.57 \quad 2.94 \quad 2.26 \quad 1.52 \]

\[ I_{LS} \propto N_{LS} (g_{p}^{av})_{LS} \]
\[ I_{HS} \propto N_{HS} (g_{p}^{av})_{HS} \]

\[ B \text{ / Gauss} \]

\[ \text{IT} \]
\[ \text{LS : Curie's law} \]
\[ \text{HS : } D = 12 \text{ cm}^{-1} \]

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

\[ (g_{p}^{av})_{LS} n_{LS} + (g_{p}^{av})_{HS} n_{HS} f(16 \text{ K}) = 38 \]

16 hemes : 1 HS + 15 LS

Czjzek, 2002

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Spin transitions in Fe\(^{3+}\) systems

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

<table>
<thead>
<tr>
<th>Valence ion Fe</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>3d(^n)</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>HS</td>
<td>3/2</td>
<td>2</td>
<td>5/2</td>
<td>2</td>
<td>3/2</td>
</tr>
<tr>
<td>LS</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Spin Transitions

- 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

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

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

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

HasA - WT

Polycrystals

(Arnoux, NSB 1999)
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

Mutant of the axial coordination:
His32Ala
Lack of sixth ligand or H$_2$O molecule
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: \(K_D = 10^{-11} \text{ M}\)

![Image](image)

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

\[
\text{Low spin Fe}^{3+} \quad S = 1/2
\]

\[
\text{pH = 5.5} \quad \text{High spin Fe}^{3+} \quad S = 5/2
\]

Bruno GUIGLIARELLI
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

![Diagram of HasA protein structure]

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

\[ \Delta E = g \beta B \]

\[ \frac{N_+}{N_-} = \exp\left(- \frac{g \beta B}{k T}\right) = \frac{W_+}{W_-} \]

\[ N_+ W_- = N_- W_+ \]

**Thermal equilibrium (Boltzmann)**

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

⇒ 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

\[
dn/dt = -2 \mathcal{W} n + (n_0 - n) / T_1 \quad (\mathcal{W} \propto B_1^2)
\]

\[\Rightarrow\] Steady state in continuous wave EPR

Net absorbed microwave power at steady state

\[
P_{\text{abs}} = h \nu (\mathcal{W} N_+ - \mathcal{W} N_-) = h \nu \mathcal{W} n_{\text{Stat}}
\]

High power: \(n_{\text{stat}} \rightarrow 0\) : **Power saturation**

\[\Rightarrow T_1 \text{ and } T_2 \text{ measurements}\]
Spin-lattice relaxation:
- Coupling between spins and vibrations (phonons)
- Strong dependence on spin-orbit coupling
  \[ H_{SO} = \lambda L \cdot S \]
- If \( T \) increases, \( T_1 \) decreases.

When \( T_1 \approx T_2 \) broadening of the resonance line:
\[ \delta B = \frac{\hbar}{g\beta} \cdot \frac{1}{T_1} \]

Electron spin relaxation: Temperature dependence

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
- Increasing temperature to suppress a signal by relaxation broadening
- Separation of signals from species with different relaxation properties
Fe-S clusters

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

Typical Fe-S EPR signals

[Pandelia, BBA 2015]
Fe-S clusters: fast electron spin relaxation

Relaxation broadening of a [3Fe-4S]^{1+} signal (S = \frac{1}{2}) upon T increase

![Graph showing the relaxation broadening of a [3Fe-4S]^{1+} signal upon T increase.](image)
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)

Mo-bisPGD cofactor
Selective EPR view of metal cofactors in respiratory nitrate reductase

Fe-S clusters: fast electron spin relaxation

Relaxation properties (oxidized state)

Magnetic Field (mT)

12.5K
1mW
+210 mV

Å

5.4
8.9
9.4
9.6
9.7
11.2

FS4
FS3
FS2
FS1
FS0
Moco

Bruno GUIGLIARELLI
French-BIC School – Carry Le Rouet, 17th September 2017
Fe-S clusters: fast electron spin relaxation

Selective EPR view of metal cofactors in respiratory nitrate reductase

Relaxation properties (oxidized state)

- [3Fe-4S]
- 12.5K 100mW +210 mV
- 12.5K 1mW +210 mV

Magnetic Field (mT)

- 320
- 340
- 360

Å
- b_D
- 5.4
- b_P
- 8.9
- FS4
- 9.4
- FS3
- 9.6
- FS2
- 9.7
- FS1
- 11.2
- FS0
- Moco

Bruno GUIGLIARELLI
Fe-S clusters: fast electron spin relaxation

Selective EPR view of metal cofactors in respiratory nitrate reductase

Relaxation properties (oxidized state)

12.5K
100mW

12.5K
1mW

50K
4mW

Magnetic Field (mT)

[3Fe-4S]

+210 mV

Mo^V

+210 mV

b_D
5.4

b_P
8.9

FS4
9.4

FS3
9.6

FS2
9.7

FS1
11.2

FS0

Moco

Bruno GUIGLIARELLI
Unusual FeS cluster in NarGHI nitrate reductase

Unusual coordination of FeS0 cluster:
Cys motive HxxxCxxxC...C

FS0

g ~ 5

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

g ~ 2

NarG subunit

FeSO

Mo

17 Å

10 Å

wt

H50S

FeS0 : a S=3/2 [4Fe-4S]^{+1} cluster coordinated by His
(Lanciano, J.Phys.Chem. 2007)
Unusual FeS cluster in NarGHI nitrate reductase

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

$$\Delta$$

$$\Delta = [D^2 (1 + 3(E/D)^2)]^{1/2}$$

$$\Delta = 4.35 \text{ cm}^{-1} \gg g\beta B$$

(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)

![Diagram of NarJ and NarGHI complex]

**Step 1**: NarJ helps in the insertion of FeS and Mo into NarGHI complex.

**Step 2**: NarJ facilitates the insertion of Mo into the Moco.

Fe-S clusters: Exchange interaction determination from relaxation broadening

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

\[ H_{ex} = J \vec{S}_1 \cdot \vec{S}_2 \]

\[ S_T = \vec{S}_1 + \vec{S}_2 \]

\[ |S_1 - S_2| \leq S_T \leq S_1 + S_2 \]

\[ S_T = 1/2, 3/2, 5/2, 7/2, 9/2 \]

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

\[ E = J S(S+1) + \text{Cte} \]

Antiferromagnetic coupling: J > 0

Ground state: \( S_T = 1/2 \)

\[ \Delta = 3/2 \text{ J} \]

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 \( \text{C-X}_4\text{-C-X}_{35}\text{-C-} \text{X}_3\text{-C} \)
Signal broadening for \( T > 160 \text{ K} \)

Cys motive \( \text{C-X}_{15}\text{-C-X}_2\text{-C-} \text{X}_{13}\text{-C} \)
Signal broadening for \( T > 50 \text{ K} \)
Disappearance at \( T = 100 \text{ K} \)

For each S manifold:
\[
\tilde{g} = K_1 g_1 + K_2 g_2
\]
\[
K_1 = \frac{S(S+1)+S_1(S_1+1)-S_2(S_2+1)}{2S(S+1)}
\]
\[
K_2 = \frac{S(S+1)+S_2(S_2+1)-S_1(S_1+1)}{2S(S+1)}
\]
\[
K_1 + K_2 = 1
\]

For the ground state \( S = 1/2 \):
\[ g = \frac{7}{3} g_1 - \frac{4}{3} g_2 \]

\( \text{Fe}^{3+}, S_1 = 5/2 \), state \( ^6A_1 \), only weak variations of \( g_1 \)

\( \text{Fe}^{2+}, S_2 = 5/2 \), state \( ^2T_2 \), \( g_2 \) very sensitive to structure variations

\( \Rightarrow \) Correlations between g-values variations reflect structural changes of the \( \text{Fe}^{2+} \) site

Fe-S clusters: Exchange interaction determination from relaxation broadening

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

Cys motive **C-X**$_4$**-C-**X$_{35}$**-C-**X$_3$-**C

Signal broadening for $T > 160$ K

Cys motive **C-X**$_{15}$**-C-**X$_2$-**C-**X$_{13}$-**C

Signal broadening for $T > 50$ K

Disappearance at $T = 100$ K

Broadening by Orbach relaxation process

\[
1 / T_1 \propto \exp \left( - \frac{\Delta}{kT} \right)
\]

\[
\Delta = 3/2 \, J
\]

Hnd$_D$ : $J = 180$ cm$^{-1}$

Hnd$_A$ : $J = 560$ cm$^{-1}$
**Hyperfine coupling**

\[ H_S = \vec{S} \cdot \vec{D} \cdot \vec{S} + \beta \vec{S} \cdot \vec{g} \cdot \vec{B} + \vec{S} \cdot \vec{A} \cdot \vec{I} \]

Magnetic coupling between electron and nuclear spins

**Two physical contributions:**
- **Dipolar magnetic coupling**

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

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

\[
\hat{H} = \hat{S} \hat{\vec{T}} \hat{\vec{I}} \quad \text{Anisotropic term, } Tr(T) = 0
\]

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

\[
\hat{H}_{\text{Fermi}} = \frac{2\mu_0}{3} g_e \beta_e g_n \beta_n |\psi(0)|^2 \vec{S} \cdot \vec{I} = \vec{a}_{\text{iso}} \vec{S} \cdot \vec{I} \quad \text{isotropic, reflects spin density on the nucleus}
\]

\[
\hat{H}_{\text{Hyp}} = \vec{S} \cdot \vec{A} \cdot \vec{I} \quad \text{Anisotropic term, with } Tr(A) = a_{\text{iso}}
\]
Hyperfine coupling: spectral effects

\[ H_S = \hat{S} \hat{D} \hat{S} + \beta \hat{S} \vec{g} \vec{B} + \hat{S} \vec{A} \vec{I} \]

Spin states: |S, M_S>, |I, M_I>: (2S+1)(2I+1) states
- For isotropic \( g \) and isotropic \( A \) tensor:
  \[ H_S = g\beta \hat{S} \cdot \vec{B} + A\hat{S} \cdot \vec{I} = g\beta S_Z B + A(S_Z I_Z) \]
  \[ E_{(M_S,M_I)} = g\beta B M_S + A M_S M_I \] (1st order)

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

B_0 = h\nu/g\beta
Hyperfine coupling: spectral effects

\[ H_S = \vec{S} \cdot \vec{D} \cdot \vec{S} + \beta \vec{S} \cdot \vec{g} \cdot \vec{B} + \vec{S} \cdot \vec{A} \cdot \vec{I} \]

General case: anisotropic \( g \) and \( A \) tensor

\( \Rightarrow (2I+1) \) hyperfine components for each principal direction of \( g \)

\[ A_X / g_X \beta \quad \text{and} \quad A_Y / g_Y \beta \quad \text{and} \quad A_Z / g_Z \beta \]

\( I = 1 \)

\( A \) and \( g \) with parallel axes

\[ g^2 = l_x^2 g_x^2 + l_y^2 g_y^2 + l_z^2 g_z^2 \]

\[ A^2 g^2 = l_x^2 A_x^2 g_x^2 + l_y^2 A_y^2 g_y^2 + l_z^2 A_z^2 g_z^2 \]
Hyperfine coupling: spectral effects

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

Exemple of copper enzymes

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

$^{63}\text{Cu}$, $^{65}\text{Cu}$ : $I = 3/2$ \hspace{1cm} $2I+1 = 4$

Lytic Polysaccharides Monooxygénases (LPMO), *Pseudospora ancerina*

Partial resolution of hyperfine lines / linewdhts
Hyperfine coupling: spectral effects

General case: anisotropic \( g \) and \( A \) tensor

Mo(V) cofactor (4d\(^1\)) of periplasmic nitrate reductase (\textit{Rhodobacter sphaeroides})

2 protons \( I = 1/2 \)
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

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

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

Hyperfine Quadrupolar $(K, \eta)$

HYSCORE pulse sequence
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

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

- **NarG**
- **NarH**
- **NarI**

**Monodentate**
- Asp222
- His546

**Closed pyrane**

Structure of the different Mo(V) species?

- $\text{NO}_3^- + 2 \text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$
- $\text{NarG}$
- **Membrane**
- $2 \text{H}^+$
- $\text{QH}_2$
- **Q**
- $\text{e}^-$

*(Jormakka, 2004)*

- **pH 8.1 as prep**
- **pH 5.9 (E=+345 mV)**
- **$^{95, 97}\text{Mo I} = 5/2$**

High-pH

Low-pH
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Evidence for two $^{14}$N nuclei, $N_1$ and $N_2$, 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

- **N$_i$ parameter determination**

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

  - Hyperfine and quadrupolar parameters:
    
    $A_{iso} = 1.0$ MHz \hspace{1cm} $\kappa = 0.6-0.7$ MHz \hspace{1cm} $A < 2\nu_1(N)$
    
    $T = 0.25$ MHz \hspace{1cm} $\eta = [0-1]$

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

- **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 $^{15}\text{N}$ ($I=1/2$) - $^{98}\text{Mo}$ of NarGH

**pH = 8.5**

- $N_{ll}$ parameter determination

**pH 8.5 preparation of $^{14}\text{N}$/ $^{98}\text{Mo}$ NarGH

- Hyperfine and quadrupolar parameters:
  - $A_{iso} = 2.7$ MHz
  - $\kappa = 0.7$ MHz
  - $T = 0.56$ MHz
  - $\eta = 0.4$ MHz
  - Cancelation Condition: $A \sim 2\nu_i(N)$

\[
^{15}\text{N} : I = 1/2 ; \quad \frac{A(14N)}{A(15N)} = \frac{g_n(14N)}{g_n(15N)} = 0.712
\]

**pH10 preparation of $^{15}\text{N}$/ $^{98}\text{Mo}$ NarGH

- Hyperfine coupling:
  - $A_{iso} = 3.4$ MHz
  - $T = 0.7$ MHz
Detection of unresolved hyperfine coupling: HYSCORE spectroscopy

Experimental

pH = 6.0

\[ {^{14}N_I} \]

\[ A = 2.7 \text{ MHz} \]

\[ \kappa = 0.66 \text{ MHz} \]

\[ \eta = 0.4 \]

High pH form

pH = 8.5

\[ {^{14}N_{II}} \]

\[ A = 1.1 \text{ MHz} \]

\[ \kappa = 0.69 \text{ MHz} \]

\[ \eta = 0.44 \]

Low pH form

Simulations

80% \[ {^{14}N_I} \] + 20% \[ {^{14}N_{II}} \]

15% \[ {^{14}N_I} \] + 85% \[ {^{14}N_{II}} \]

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

| Nucleus | Frequency (MHz) | |     |
|---------|-----------------|---------|
| $^{14}N_i$ | 0.69            | 0.44    | Low pH |
| $^{14}N_{II}$ | 0.66          | 0.4     | High pH |

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

$pH = 6$

Assignment to Asn52-N$_6$
- 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

HYSCORE Study of Nar

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

Excess reductant → Rapid freezing (<1 min)

"as prepared" enzyme → Rapidly reduced enzyme

80 K

15 K

T dependence of the spectral shape
Detection of intercenter magnetic coupling

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

$\text{Mo(V)} \leftrightarrow [4\text{Fe-4S}]^{+1}$

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

$J \vec{S}_1 \cdot \vec{S}_2 + S_1 \cdot D_{\text{dip}} \vec{S}_2$

Point dipole model

Resting enzyme

$r = 15\ \text{Å}$

$\Theta = 35^\circ$, $\varphi = 45^\circ$

$J = 0$

$J = 0$

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

4Fe\textsubscript{ox} (Mo(V) resting) → Excess reductant → Rapid freezing (<1 min) → 4Fe\textsubscript{red} (Mo(V) resting) → Activation → 4Fe\textsubscript{red} (Mo(V) active)

Detection of intercenter magnetic coupling
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-4S]⁺¹

\[ \hat{H}_{\text{int}} = \hat{H}_{\text{exch}} + \hat{H}_{\text{dip}} \]

\[ J \tilde{S}_1 \cdot \tilde{S}_2 + \tilde{S}_1 \cdot D_{\text{dip}} \tilde{S}_2 \]

Mo(V) EPR signal saturation properties

30 K

Activated enzyme

\[ r = 15 \text{Å} \]
\[ \Theta = 35°, \varphi = 45° \]
\[ J = 0.5 \text{ mT} \]

Exp.

Simul.

B / mT

1/T₁ = 1/T°₁ + k₁dip + k₁ex
1/T₂ = 1/T°₂ + k₂dip + k₂ex

Active, 4Fe reduced
Resting, 4Fe reduced
Resting, 4Fe oxidized
Detection of intercenter magnetic coupling

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

\[
\hat{H}_{\text{int}} = \hat{H}_{\text{exch}} + \hat{H}_{\text{dip}}
\]

\[
J \vec{S}_1 \cdot \vec{S}_2 + S_1 D_{\text{dip}} \vec{S}_2
\]

- 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.

Mo(V) ↔ [4Fe-4S]**+1**
Detection of intercenter magnetic coupling

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)
Acknowledgements

LBC, CEA, CNRS, AMU
David PIGNOL
Pascal ARNOUX
Monique SABATY

BIP, CNRS & AMU
Stéphane GRIMALDI
Julia RENDON
Frédéric BIASO
Sinan AL ATTAR
Elisabetta MILEO
Alessio BONUCCI
Valérie BELLE
Bénédicte BURLAT
Kamal ZEAMARI

LCB, CNRS & AMU
Axel MAGALON
Sinan AL ATTAR
Anne WALBURGER
Thank you for your attention

International EPR school
Carry-Le-Rouet / Marseille
3-7 June 2018