

## Paramagnetic metalloproteins

In paramagnetic systems:  $\mu_S = 658.2 \mu_I$

Dipole-dipole interaction:

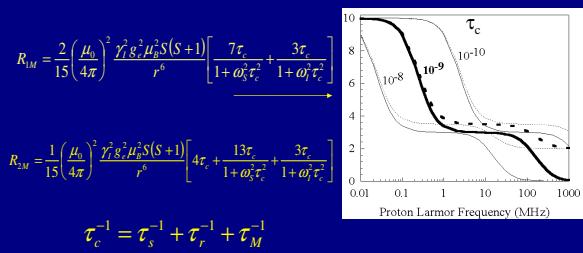
$$E^{\text{dip}} = -\frac{\mu_0}{4\pi} \left[ \frac{3(\boldsymbol{\mu}_1 \cdot \mathbf{r})(\boldsymbol{\mu}_2 \cdot \mathbf{r})}{r^5} - \frac{\boldsymbol{\mu}_1 \cdot \boldsymbol{\mu}_2}{r^3} \right]$$

$E^{\text{cont}} \propto A_c \langle S_z \rangle$

Relaxation rates:

$$R_{1,2} \propto \langle E^2 \rangle f(\tau_c, \omega)$$

## Nuclear relaxation due to the electron-nucleus dipolar coupling Solomon equations



## Paramagnetic metal ions

✓ They increase nuclear relaxation

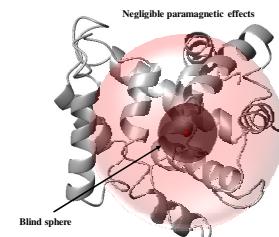
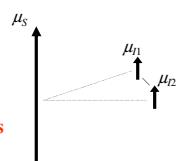
⇒ linewidth  $\Delta\nu = R_{2M} / \pi$

✓ They decrease the intensity of NOEs  
(if observed at all!)

$$\eta_{I1(I2)} = \frac{\sigma_{I1(I2)}}{\rho_{I1(I2)} + \rho_{I1(\text{other})} + R_{IM}}$$

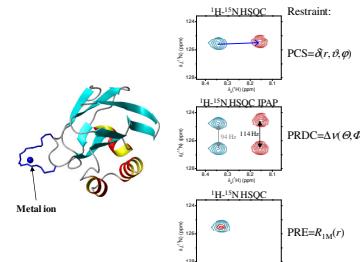
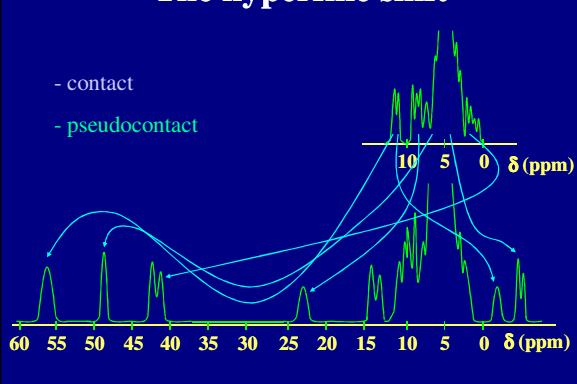
longitudinal relaxation of nucleus  $I$   
due to the coupling with other nuclei

cross relaxation rate,  
i.e. the magnetization transfer from the nucleus  $I2$   
to the nucleus  $I1$  when  $I2$  is saturated



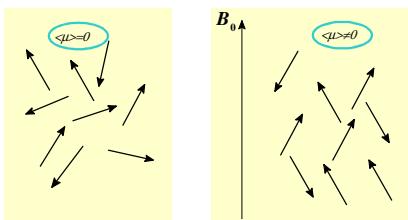
## The hyperfine shift

- contact
- pseudocontact



## Magnetic moments and magnetic fields

$$\mu_I = \hbar \gamma_I I \quad \mu = -\mu_B g_e S$$



## Average magnetic moment

$$\Delta E = g_N \mu_N B_0 = \hbar \gamma_N B_0 \quad \Delta E = g_e \mu_B B_0$$

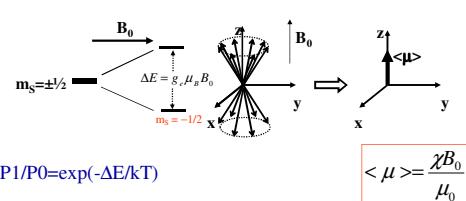
	$g_N$	$\hbar \gamma_N$	
<sup>1</sup> H	5.59	$2.81 \times 10^{-26} \text{ JT}^{-1}$	$g_e \mu_B = 1.86 \times 10^{-23} \text{ JT}^{-1}$
<sup>2</sup> H	0.86	0.43	
<sup>3</sup> H	5.96	2.99	
<sup>13</sup> C	1.40	0.71	$\omega_s = \frac{g_e \mu_B B_0}{\hbar} \gamma_N B_0 = \frac{g_e \mu_B}{\hbar \gamma_N} \omega_l$
<sup>14</sup> N	0.40	0.20	<b>400 MHz <sup>1</sup>H Larmor frequency</b>
<sup>15</sup> N	-0.57	-0.28	=
<sup>31</sup> P	2.26	1.14	<b>263 GHz of electron Larmor frequency</b>

$$\mu \text{ is 658 times larger than } \mu_I$$

$$\begin{aligned} \text{m}_S = \pm \frac{1}{2} &\xrightarrow{\Delta E = g_e \mu_B B_0} \mu = -\mu_B g_e S \\ &\downarrow \\ &< \mu > = -\mu_B g_e < S_z > \end{aligned}$$

## Paramagnetic susceptibility

Paramagnetic systems:



$\chi$  = paramagnetic susceptibility per molecule, independent on  $B_0$  and positive

## Curie law

$$\begin{aligned} < \mu > &= -\mu_B g_e < S_z > \\ &= \frac{\sum_{S,M_S} < S, M_S | S_z | S, M_S > \exp(-E_{S,M_S}/kT)}{\sum_{S,M_S} \exp(-E_{S,M_S}/kT)} \\ &= \frac{E_{Zeeman}}{E_{Zeeman} + E_{S,M_S}} \\ &= \frac{E_{Zeeman}}{E_{Zeeman} + g_e \mu_B M_S B_0} \end{aligned}$$

Zeeman energy of the level  
if no contribution from the orbital magnetic moment

$$\begin{aligned} \text{If } g_e \mu_B B_0 M_S \ll kT & \quad < S_z > = -\frac{g_e \mu_B B_0}{3kT} S(S+1) \\ & \quad < \mu > = \frac{\chi B_0}{\mu_0} \\ \text{Curie law: } \chi &= \frac{\mu_0 < \mu >}{B_0} = \mu_B^2 g_e^2 \frac{\mu_0}{3kT} S(S+1) \end{aligned}$$

## Magnetic susceptibility anisotropy

In systems that are orbitally non-degenerate, the anisotropy can be represented by an **anisotropy of the g-factor**

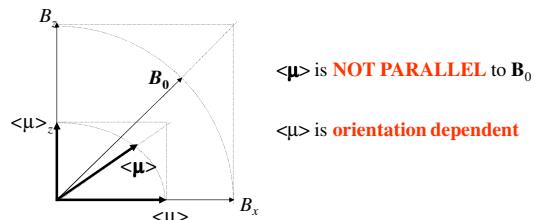
$$\begin{aligned} < \mu_{kk} > &= -\mu_B g_{kk} < S_{kk} > = -\mu_B \frac{\sum_i < \phi_i | L_{kk} + g_e S_{kk} | \phi_i > (1 - E_{i,kk}/kT)}{\sum_i (1 - E_{i,kk}/kT)} \\ & \quad < \phi | L_{kk} + g_e S_{kk} | \phi > = g_{kk} M_S \\ & \quad E_{i,kk} = g_{kk} \mu_B M_S B_0 \\ & \quad \text{Zeeman energy} \\ & \chi_{kk} = \mu_0 \mu_B^2 g_{kk}^2 \frac{S(S+1)}{3kT} \end{aligned}$$

Valid with **only one thermally populated multiplet** of spin number  $S$   
**NO zero field splitting**

## Average magnetic moment

If the orbital magnetic moment is considered,  $\chi$  is anisotropic

$$< \mu > = \frac{\chi \cdot \mathbf{B}_0}{\mu_0}$$



## Van Vleck equation

if the total energy of the system  $E^0 \gg$  Zeeman energy

$E^0$  = zero field splitting and ligand field energy

$$\chi_{kk} = \mu_0 \mu_B^2 \frac{\sum_i \left[ \frac{|\langle \phi_i | L_{kk} + g_e S_{kk} | \phi_i \rangle|^2}{kT} - 2 \sum_{j \neq i} \frac{|\langle \phi_i | L_{kj} + g_e S_{kj} | \phi_j \rangle|^2}{E_i^0 - E_j^0} \right] \exp(-E_i^0/kT)}{\sum_i \exp(-E_i^0/kT)}$$

Energies of ground and excited states

→  $\langle S_z \rangle \propto \chi$  are anisotropic

## The origin of contact shifts

Contact shift : contribution to the chemical shift due to the unpaired electron spin density on the resonating nucleus

The coupling constant between nucleus and e<sup>-</sup> I<sub>1</sub>...e<sup>-</sup> is called A  
⇒ the interaction is expressed by

$$H = AI \cdot S$$

A: contact hyperfine coupling proportional to the spin density of the resonating nucleus

## Contact shifts

$$H = AI \cdot S \quad \langle S_z \rangle = -\mu_B g_e \frac{S(S+1)B_0}{3kT}$$

H.M. McConnell, D.B. Chesnut, *J. Chem. Phys.* **1958**, 28, 107-117

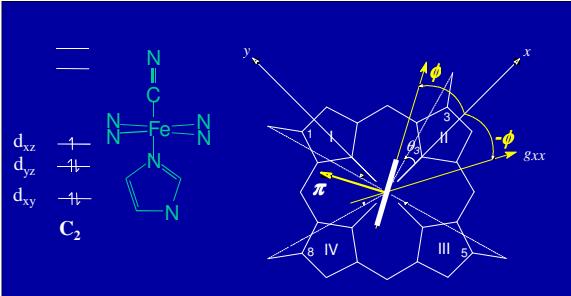
$$E^{\text{con}} = AI_z \langle S_z \rangle = -AI_z \frac{g_e \mu_B S(S+1)B_0}{3kT}$$

$$\delta^{\text{con}} = \frac{E_{\text{con}}}{\hbar \gamma_I I_z B_0} = \frac{A}{\hbar} \frac{g_e \mu_B S(S+1)}{3 \gamma_I k T}$$

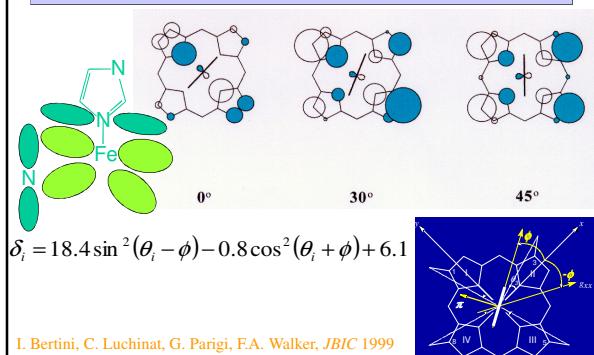
Kurland and McGarvey (1970) predict that  $\langle S_z \rangle$  and hence contact shift may be orientation dependent due to spin-orbit coupling

R.J. Kurland, B.R. McGarvey, *J. Magn. Reson.* **1970**, 2, 286-301

## Electronic configuration of LS Fe<sup>III</sup> Heme

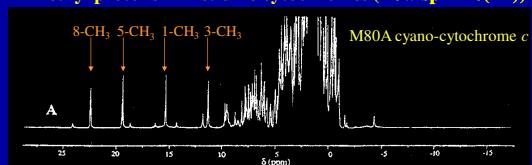


## Calculated vs. observed shifts of methyl protons in histidine-cyanide cytochromes



## Contact shift restraints: unpaired electron spin density on heme nuclei is a function of axial ligand orientation

### Methyl protons in histidine cytochromes (Low spin Fe(III))



From the fit of the methyl shifts:  $\phi = 57^\circ \pm 9^\circ$

Bren, Gray, Banci, Bertini, Turano *J. Am. Chem. Soc.* (1995)  
 $\phi_{\text{structure}} = 49^\circ$  Banci, Bertini et al. *JBIC* (1996)

### Contact shift restraints: iron-sulfur proteins

$$\left(\frac{\Delta\nu}{\nu_0}\right)^{con} = -\left(\frac{A_c}{\hbar\gamma_l B_0}\right) \langle S_z \rangle = \left(\frac{A_c}{\hbar}\right) \frac{\overline{g}^2 \mu_B^2 S(S+1)}{3\gamma_N kT}$$

**H $\beta$  of iron-coordinated cysteines**

$\delta \propto \sin^2 \theta$

$\delta_{H\beta} = a \sin^2 \theta + b \cos \theta + c$

Bertini, Capozzi, Luchinat, Piccioli, Vila,  
*J. Am. Chem. Soc.*, 1994

### Electron-nucleus dipole-dipole interaction

$\mathbf{B}_0$

$\mu_1 = \hbar\gamma_l \mathbf{I}$

$\langle \mu \rangle = \chi \cdot \mathbf{B}_0 / \mu_0$

Dipolar shift tensor

$$H = -\frac{\hbar\gamma_l}{4\pi} \left[ \frac{3(\mathbf{I} \cdot \mathbf{r})(\mathbf{r} \cdot \chi \cdot \mathbf{B}_0)}{r^5} - \frac{\mathbf{I} \cdot \chi \cdot \mathbf{B}_0}{r^3} \right] = \hbar\gamma_l \mathbf{I} \cdot \sigma \cdot \mathbf{B}_0$$

### Pseudocontact shifts

in the principal frame of the  $\chi$  tensor

$$\sigma = -\frac{1}{4\pi r^5} \begin{pmatrix} (3x^2 - r^2)\chi_{xx} & 3xy\chi_{yy} & 3xz\chi_{zz} \\ 3xy\chi_{xx} & (3y^2 - r^2)\chi_{yy} & 3yz\chi_{zz} \\ 3xz\chi_{xx} & 3yz\chi_{yy} & (3z^2 - r^2)\chi_{zz} \end{pmatrix}$$

The **rotational average** of the dipolar interaction causes a shift of the NMR signals, called pseudocontact shift:

$$\delta^{pes} = \langle \text{Dipolar energy} \rangle / \text{Zeeman energy} (I_z \hbar \gamma_l B_0)$$

$$\delta^{pes} = -\frac{1}{3} \text{Tr}(\sigma)$$

Note: if  $\chi$  is isotropic,  $\delta^{pes} = 0$

### Pseudocontact shifts

$$\delta^{pes} = \frac{1}{12\pi r^3} \frac{(3x^2 - r^2)\chi_{xx} + (3y^2 - r^2)\chi_{yy} + (3z^2 - r^2)\chi_{zz}}{r^2}$$

$$\begin{pmatrix} \chi_{xx} & 0 & 0 \\ 0 & \chi_{yy} & 0 \\ 0 & 0 & \chi_{zz} \end{pmatrix} \rightarrow \chi_{iso} I + \begin{pmatrix} \chi_{xx} - \chi_{iso} & 0 & 0 \\ 0 & \chi_{yy} - \chi_{iso} & 0 \\ 0 & 0 & \chi_{zz} - \chi_{iso} \end{pmatrix}$$

In polar coordinates:

$$\delta^{pes} = \frac{1}{12\pi r^3} \left[ 4\chi_{ax} (3\cos^2 \vartheta - 1) + \frac{3}{2} 4\chi_{rh} \sin^2 \vartheta \cos 2\phi \right]$$

$$\Delta\chi_{ax} = \chi_{zz} - \frac{\chi_{xx} + \chi_{yy}}{2}$$

$$\Delta\chi_{rh} = \chi_{xx} - \chi_{yy}$$

written in the frame of the  $\chi$  tensor

### Pseudocontact shifts

Surfaces with constant  $\delta^{pes}$  values:

Axial                          Totally Rhombic

$\Delta\chi_{rh} = 0$                $\square$  positive       $\Delta\chi_{rh} = (2/3)\Delta\chi_{ax}$        $\blacksquare$  negative

### Pseudocontact shifts

- **proportional to  $r^{-3}$**

$$\delta^{pes} = \frac{1}{12\pi r^3} \left[ 4\chi_{ax} (3\cos^2 \vartheta - 1) + \frac{3}{2} 4\chi_{rh} \sin^2 \vartheta \cos 2\phi \right]$$

⇒ the effect is propagated to atoms far from the paramagnetic center ( $R_1$  propagates as  $r^{-6}$ )

- **depend on angular parameters**

⇒ provide information on the spatial position of the atom with respect to the metal ion

**Problems:**

- \*strong **covariance** between  $\theta$ ,  $\phi$  and  $r$
- \*angular dependence provided by **quadratic** trigonometric functions:

⇒ they are difficult to be used efficiently as restraints in torsion angle dynamics programs like DYANA/CYANA or Xplor-NIH

## Residual dipolar couplings

Dipolar interaction between nuclear magnetic moments:

$$H = -\frac{\mu_0}{4\pi} \left[ \frac{3(\mu_1 \cdot r)(\mu_2 \cdot r)}{r^5} - \frac{\mu_1 \cdot \mu_2}{r^3} \right]$$

$$H = -\frac{\mu_0}{4\pi} \frac{\hbar^2 \gamma_A \gamma_B}{r_{AB}^3} I_z^A I_z^B (3 \cos^2 \gamma - 1)$$

Rotational average:  $\frac{1}{2} \int_{-1}^1 (3 \cos^2 \gamma - 1) d(\cos \gamma) = 0$

**different orientations not all equally populated**

$$\Delta V^{rdc} = \frac{\Delta E}{h} = -\frac{\mu_0 \hbar \gamma_A \gamma_B}{4\pi^2 r_{AB}^3} < \frac{3 \cos^2 \gamma - 1}{2} > \text{Orientation tensor}$$

$$\Delta V^{rdc} = -\frac{\mu_0 \hbar \gamma_A \gamma_B}{8\pi^2 r_{AB}^3} [S_{zz} (3 \cos^2 \Theta - 1) + (S_{xx} - S_{yy}) \sin^2 \Theta \cos 2\Phi]$$

## Partial orientation

bilayer    micelle    bicelle

Orientation induced by restriction in space

$$rdc(Hz) = -\frac{\mu_0}{4\pi^2} \frac{\gamma_A \gamma_B \hbar}{2m_{AB}} \left[ D_{ax} (3 \cos^2 \theta - 1) + \frac{3}{2} D_{rh} (\sin^2 \theta \cos 2\phi) \right]$$

## Self-orientation rdc

Anisotropic electron average magnetic moment

$E \propto \int_0^{2\pi} \mu_i(\gamma) (3 \cos^2 \gamma - 1) \sin \gamma d\gamma \neq 0$

**Consequences:**  
The molecular orientations are not equally probable  
The N-H dipole-dipole interaction does not average zero upon rotation

## Self orientation versus orientation induced by external agents

- ❖ Advantages of paramagnetic rdc
- ❖ No perturbations due to the **interactions** with the orienting material
- ❖ Disadvantages of paramagnetic rdc
- ❖ **Line broadening** due to the presence of the paramagnetic ion. However, line broadening is  $\propto r^{-6}$ 
  - ⇒ no disadvantages far from the paramagnetic ion
- ❖ Common disadvantages
- Simulated annealing protocols not optimized for multiple minima constraints

## Self-orientation tensor

The orientation tensor  $S_{ii}$ :

$$S_{ii} = \frac{\int \frac{3 \cos^2 \alpha - 1}{2} \exp(-E^{\text{aniso}}(\alpha, \beta) / kT) d \cos \alpha d \beta}{\int \exp(-E^{\text{aniso}}(\alpha, \beta) / kT) d \cos \alpha d \beta}$$

In paramagnetic systems:

$$E^{\text{aniso}} = - \int_0^{<\mu>} \mathbf{B}_0 \cdot \mathbf{d} <\mu> = - \frac{\mathbf{B}_0 \cdot \chi \cdot \mathbf{B}_0}{2\mu_0}$$

$$S_{ii} = \frac{3}{2} \frac{B_0^2}{15\mu_0 kT} (\chi_{ii} - \bar{\chi})$$

## Self-orientation rdc

$$rdc(Hz) = -\frac{1}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_H \gamma_N \hbar}{4\pi^2 r_{HN}^3} \left[ \Delta \chi_{ax}^{\text{mol}} (3 \cos^2 \theta - 1) + \frac{3}{2} \Delta \chi_{rh}^{\text{mol}} (\sin^2 \theta \cos 2\phi) \right]$$
5

## Residual dipolar couplings

$$rdc(Hz) = -\frac{1}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_H \gamma_N h}{4\pi^2 r_{HN}^3} \left[ \Delta\chi_{ax}^{mol} (3\cos^2 \theta - 1) + \frac{3}{2} \Delta\chi_{rh}^{mol} (\sin^2 \theta \cos 2\phi) \right]$$

- depend on the NH / C<sup>α</sup>H<sup>α</sup> / C'C<sup>α</sup> / ... vector orientation

- do not depend on any distance with respect to the metal ion

Therefore, values of the same order of magnitude can be obtained for all the coupled nuclei of the protein

- they all refer to the same reference system, and thus relate all the internuclear vectors to the same frame (and not the internuclear vectors to one another).

## Pseudocontact shift

$$\delta^{pc} = \frac{1}{12\pi r^3} \left[ \Delta\chi_{ax} (3\cos^2 \theta - 1) + \frac{3}{2} \Delta\chi_{rh} \sin^2 \theta \cos 2\phi \right]$$

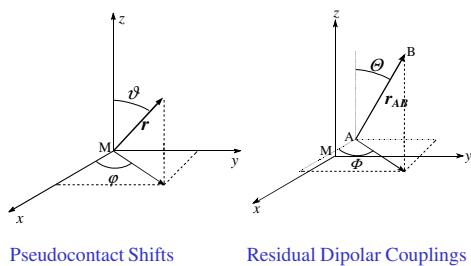
$r$ ,  $\theta$  and  $\phi$  are the polar coordinates of the resonating nucleus in the metal  $\chi$  frame

## Residual Dipolar Coupling

$$\Delta\nu(Hz) = -\frac{1}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_H \gamma_N h}{4\pi^2 r_{HN}^3} \left[ \Delta\chi_{ax}^{mol} (3\cos^2 \Theta - 1) + \frac{3}{2} \Delta\chi_{rh}^{mol} (\sin^2 \Theta \cos 2\Phi) \right]$$

$\Theta$  and  $\Phi$  are the angles defining the orientation of the NH vector

in the molecular  $\chi$  frame



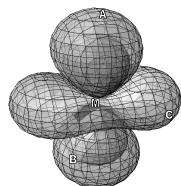
## PCS + RDC

RDC obtained by difference between splittings measured on the paramagnetic and the diamagnetic sample at the same field depend on  $\Delta\chi^{para}$

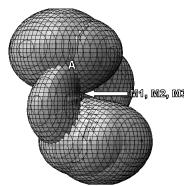
$$\begin{aligned} {}^1J(B_0) &= {}^1J + \Delta\nu_{RDC}^{\text{dia}} + \Delta\nu_{RDC}^{\text{para}} \\ \Delta\nu^{mol} - \Delta\nu^{\text{dia}} &= \Delta\nu_{ndc}^{\text{para}} \quad \text{shift}^{\text{para}} - \text{shift}^{\text{dia}} = pcs \quad \Delta\chi^{para} \end{aligned}$$

$$\Delta rdc(Hz) = -\frac{1}{4\pi} \frac{B_0^2}{15kT} \frac{\gamma_H \gamma_N h}{4\pi^2 r_{HN}^3} \left[ \Delta\chi_{ax}^{para} (3\cos^2 \theta - 1) + \frac{3}{2} \Delta\chi_{rh}^{para} (\sin^2 \theta \cos 2\phi) \right]$$

## Pseudocontact shifts

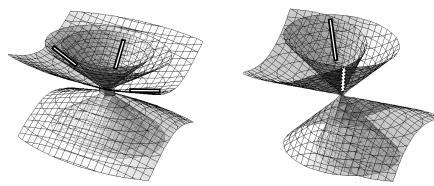


Three atoms,  
the same metal ion

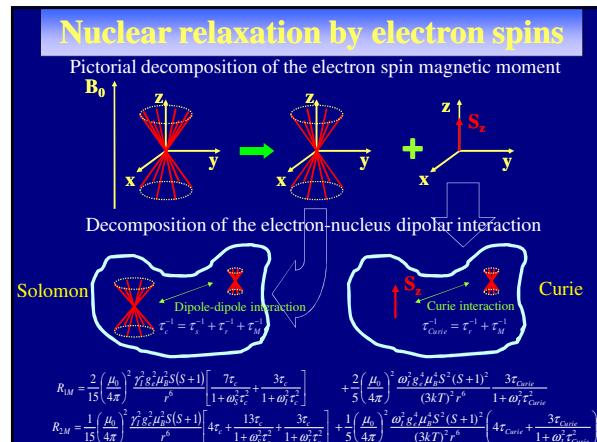
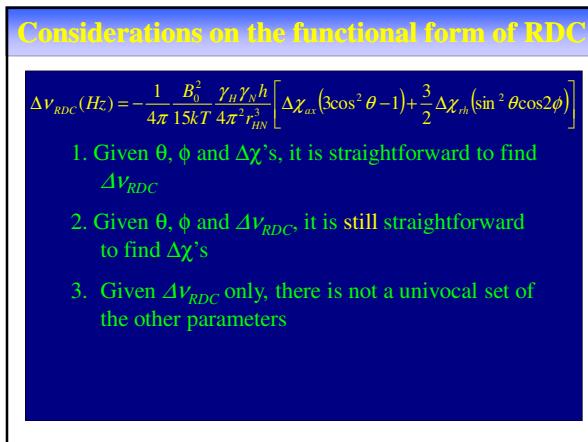
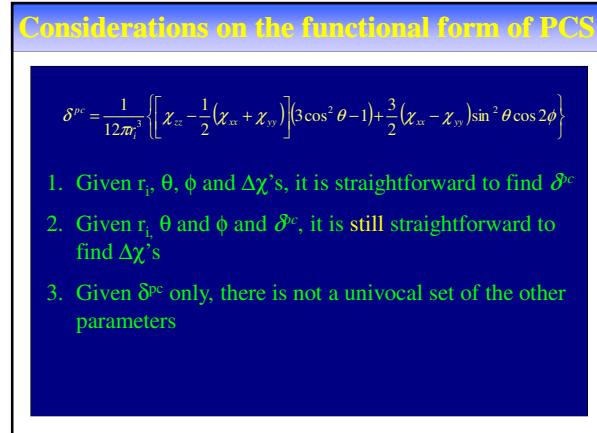
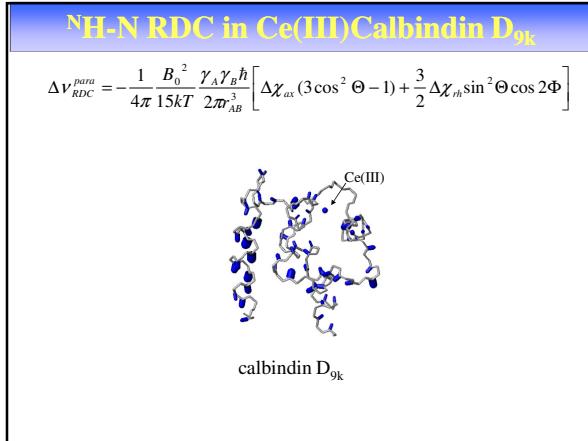
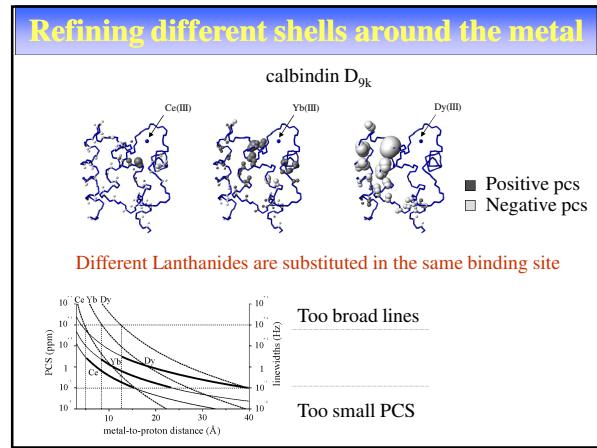
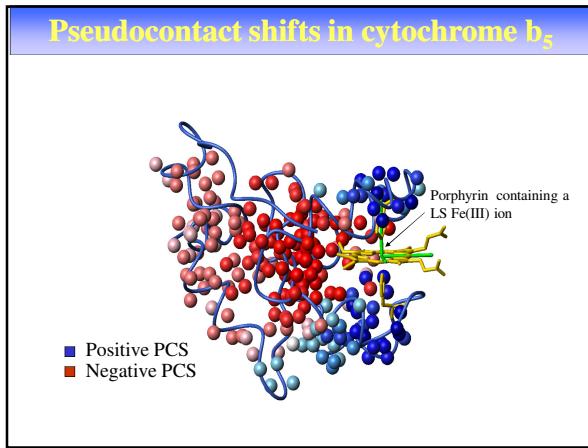


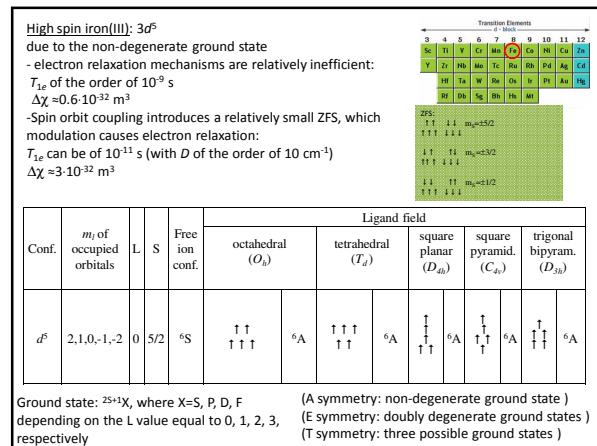
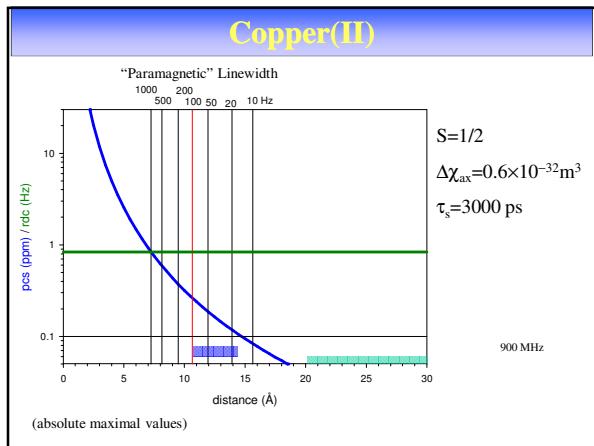
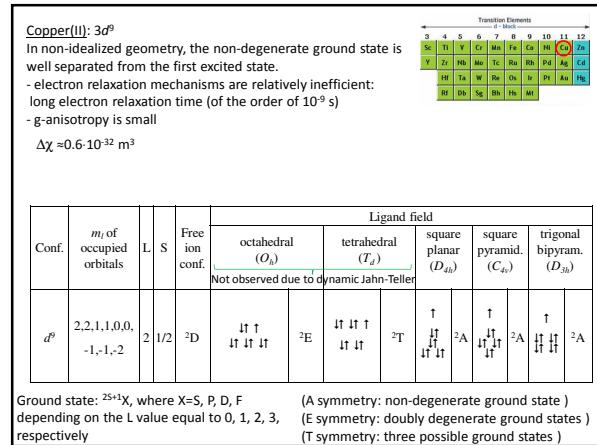
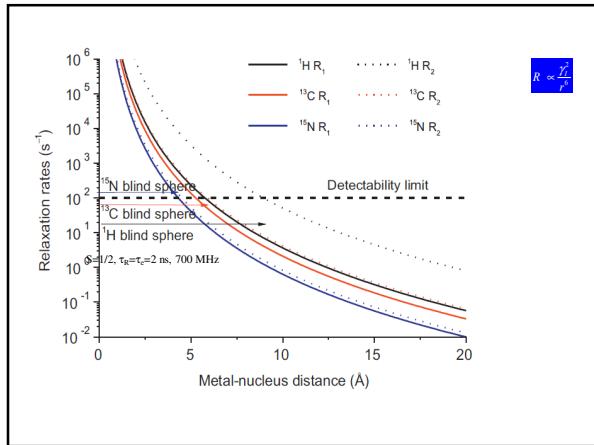
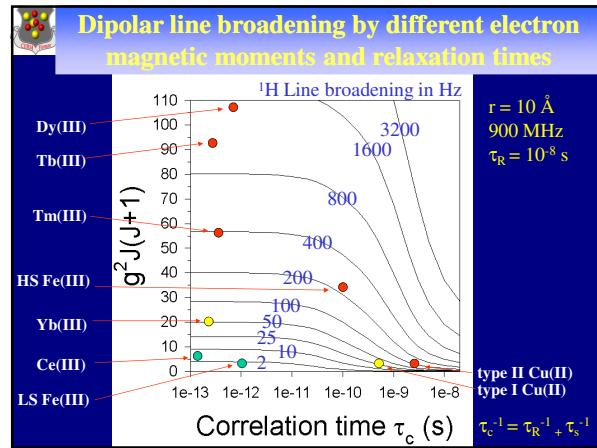
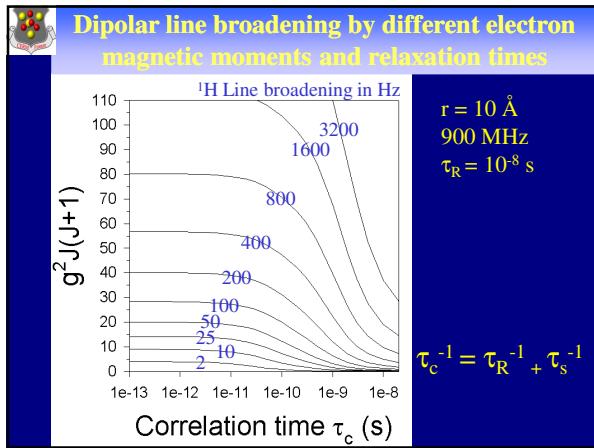
Three metal ions,  
the same atom

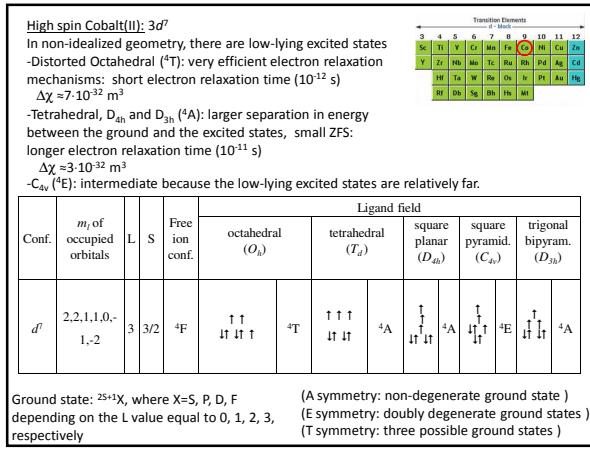
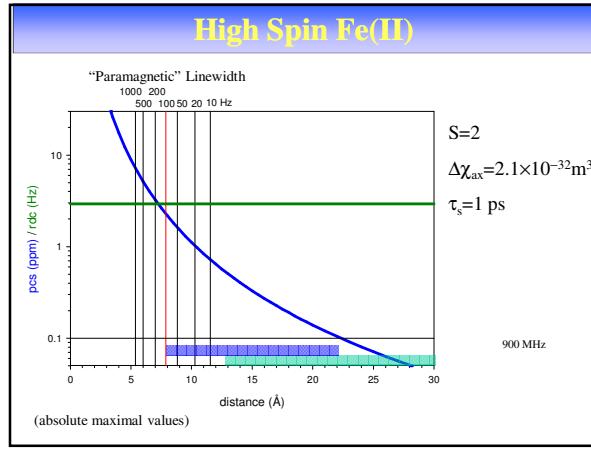
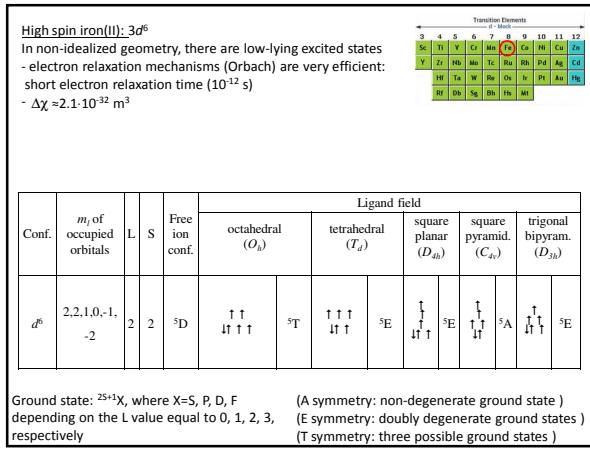
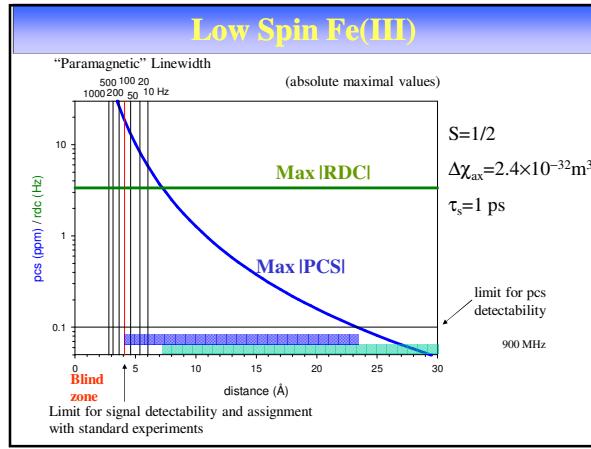
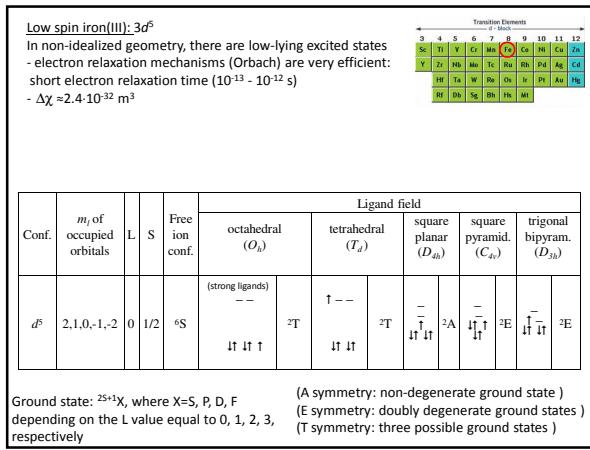
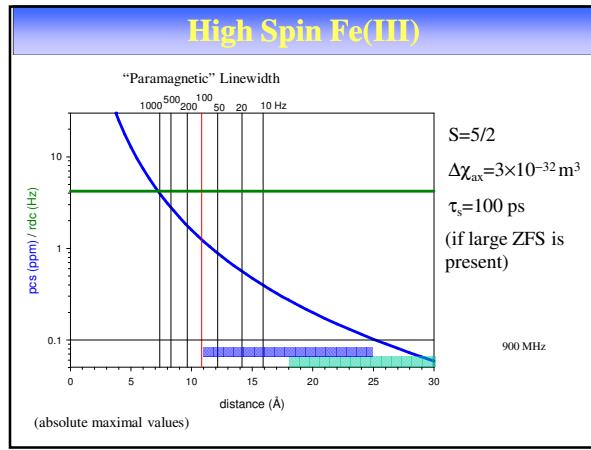
## Residual dipolar couplings

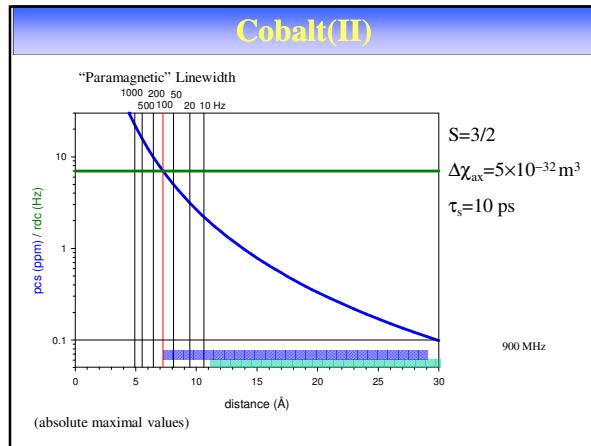


Three NH, the same metal ion      Two metal ions, the same NH









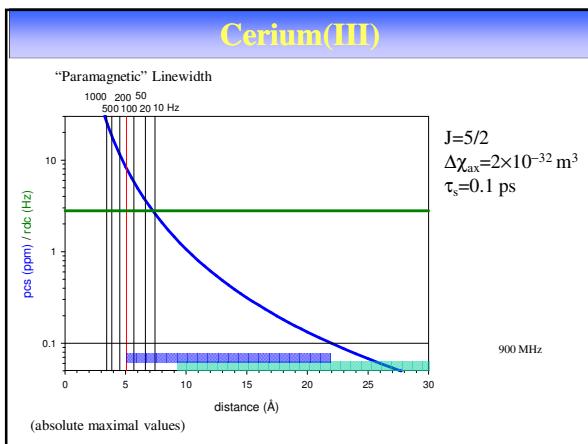
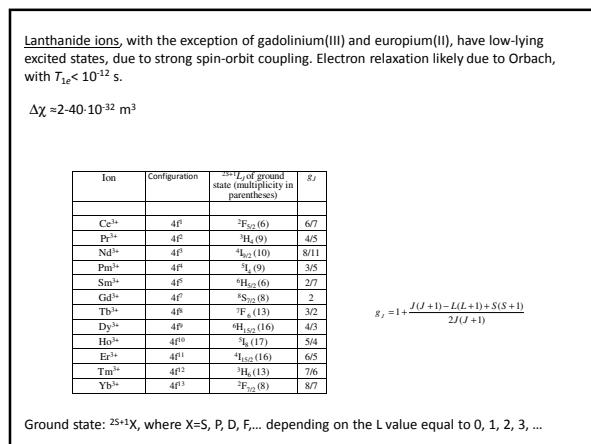
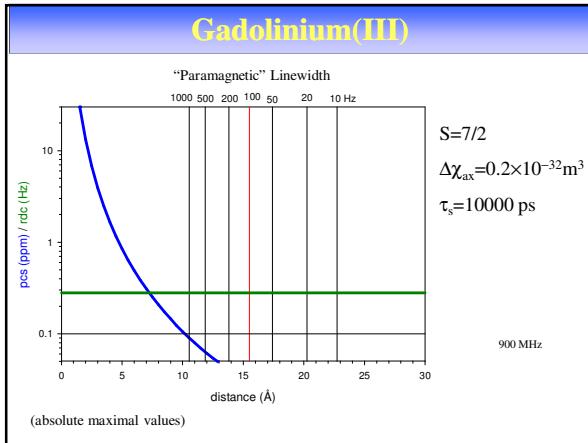
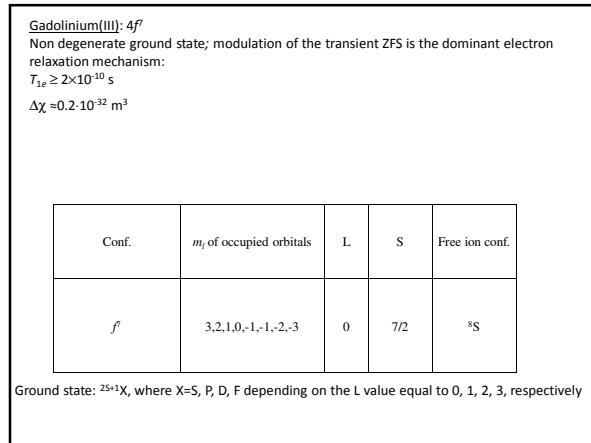
**Manganese(II):**  $3d^5$  (like high spin iron(III))  
due to the non-degenerate ground state electron relaxation mechanisms are relatively inefficient.

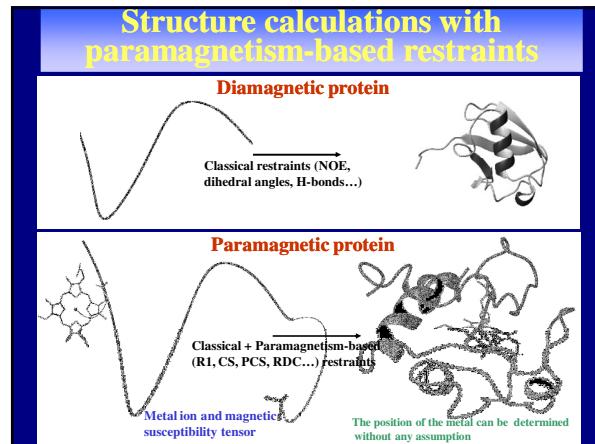
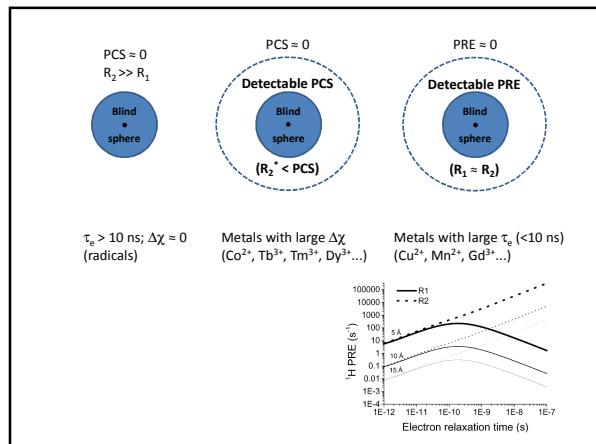
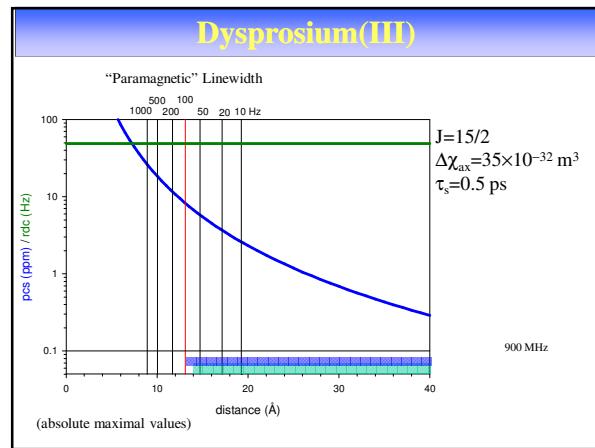
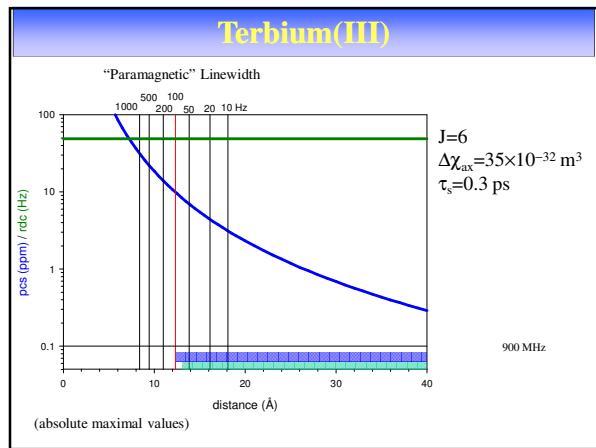
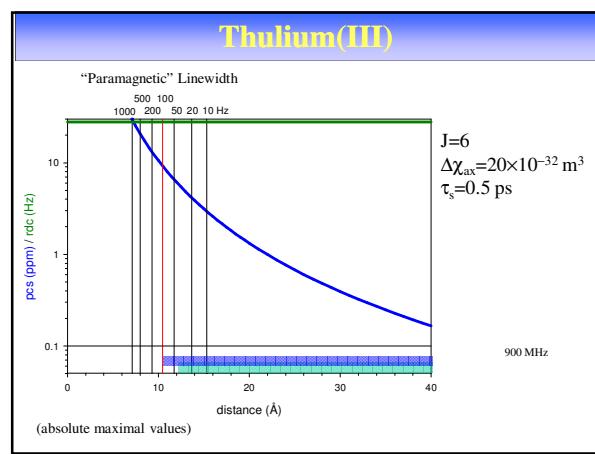
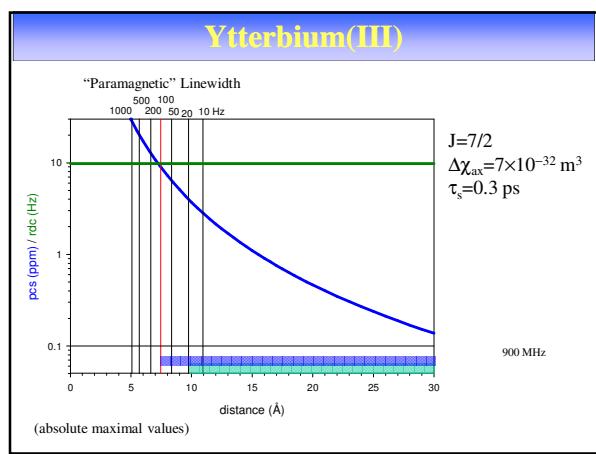
Spin orbit coupling introduces a relatively small ZFS, generally smaller than that in high spin iron(III) - because the spin orbit coupling constant is smaller due to the smaller charge of the manganese(II) ion, and because excited states are closer in manganese(II) than in iron(III) :-  
 $T_{1e}$  of the order of  $10^{-9}\text{--}10^{-10} \text{ s}$  ( $D$  around  $1 \text{ cm}^{-1}$ )

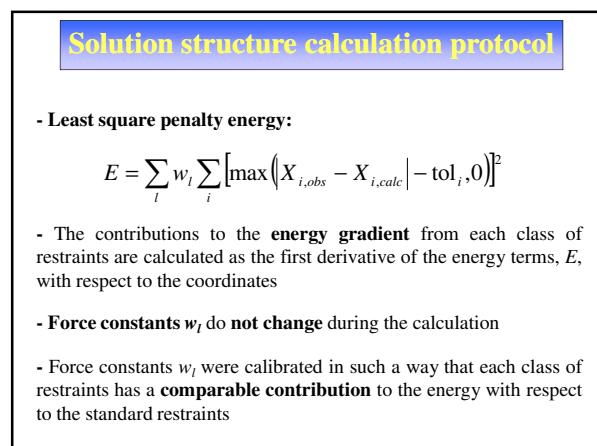
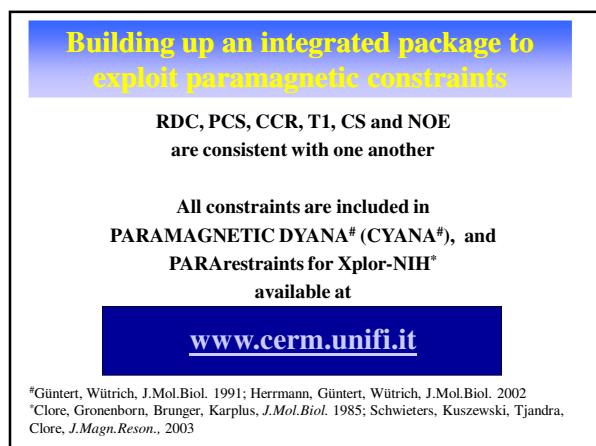
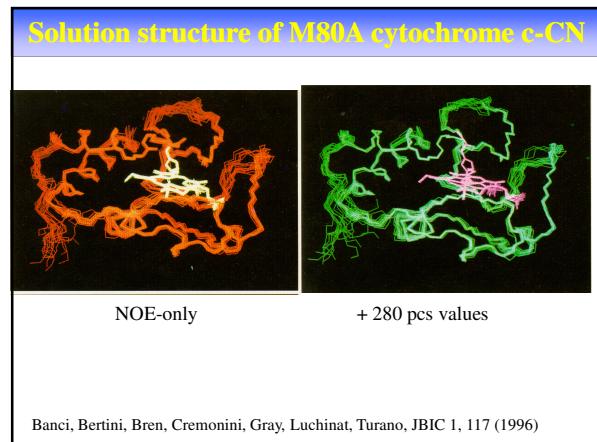
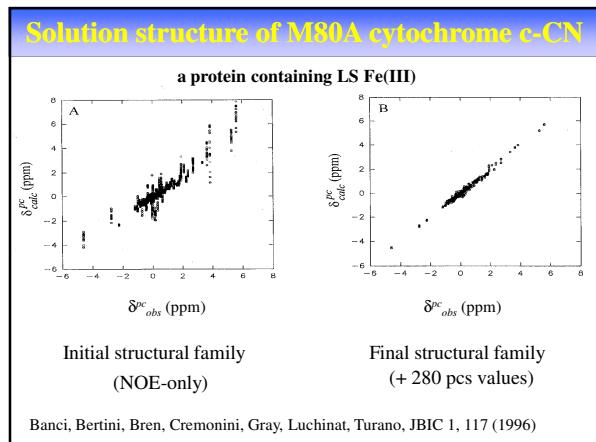
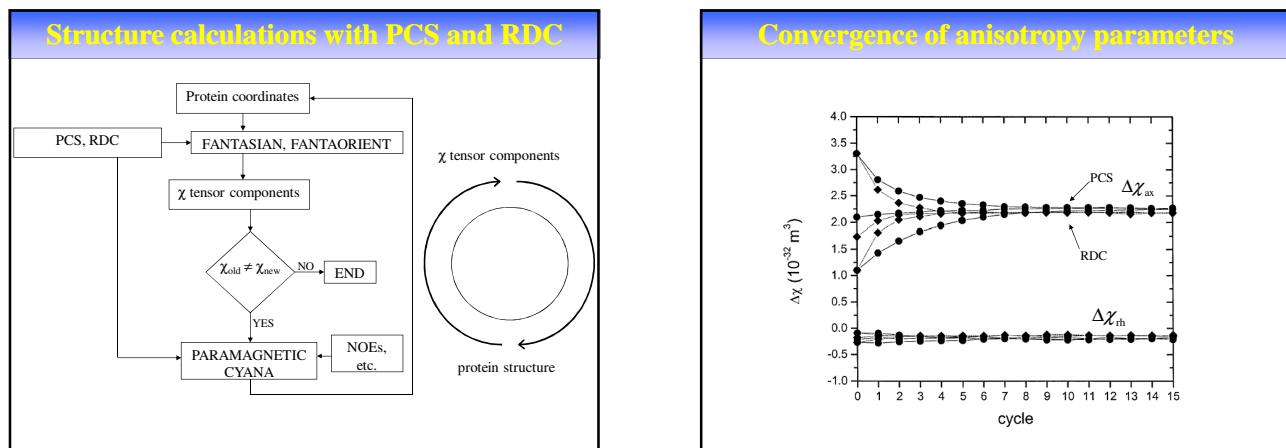
Transition Elements I - Block											
Sc	Ti	V	Cr	Fe	Co	Ni	Cu	Zn	Y	Zr	Hf
Y	Zr	Hf	Mo	Tc	Ru	Rh	Pt	Ag	Cd		
La	Ta	W	Os	Ir	Pd	Os	Pt	Ag	Cd		
Lu	W	Os	Os	Ir	Pd	Os	Pt	Ag	Cd		

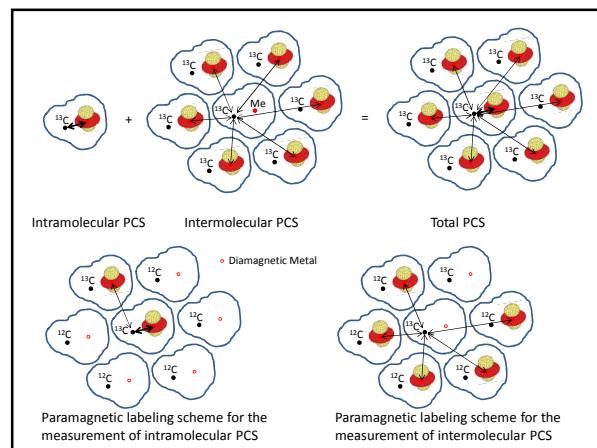
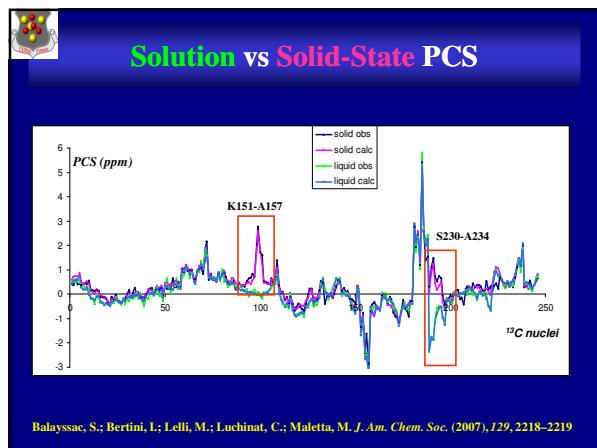
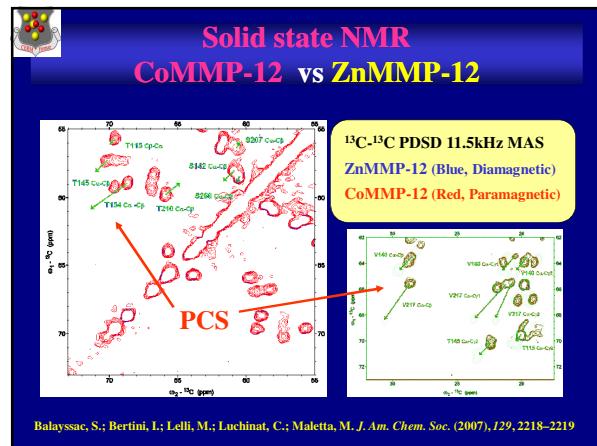
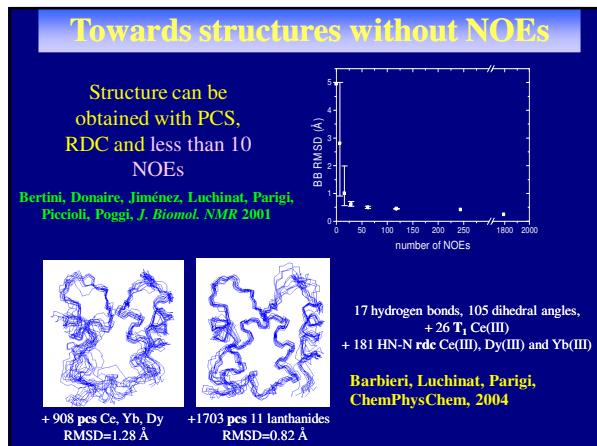
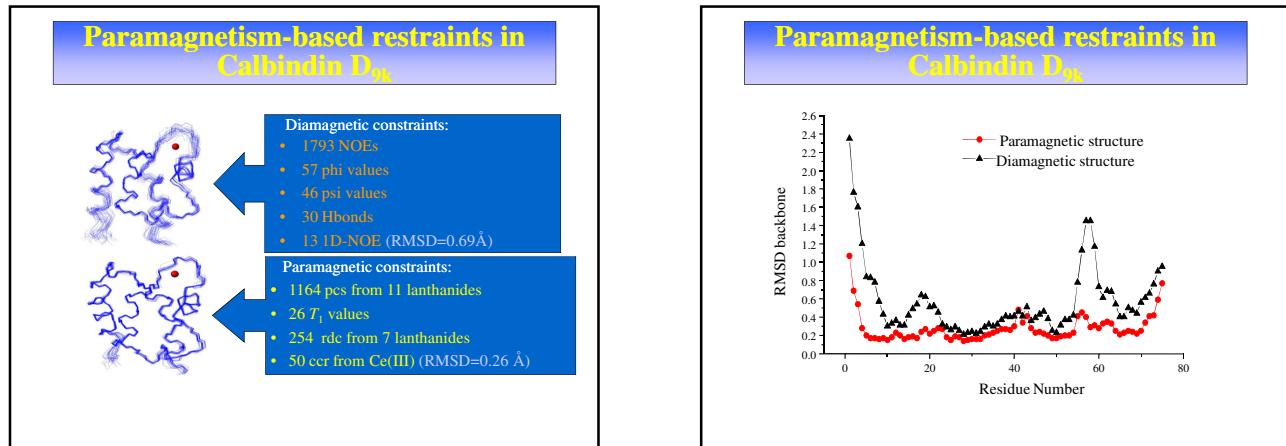
Conf.	$m_l$ of occupied orbitals	L	S	Free ion conf.	Ligand field									
					octahedral ( $O_h$ )		tetrahedral ( $T_d$ )		square planar ( $D_{4h}$ )		square pyramid. ( $C_{4v}$ )		trigonal bipyram. ( $D_{3h}$ )	
$d^5$	2,1,0,-1,-2	0	5/2	$^6S$	$\uparrow\uparrow$ $\uparrow\uparrow\uparrow$	$^6A$	$\uparrow\uparrow\uparrow$ $\uparrow\uparrow$	$^6A$	$\uparrow\uparrow$ $\uparrow\uparrow$	$^6A$	$\uparrow\uparrow$ $\uparrow\uparrow$	$^6A$	$\uparrow\uparrow$ $\uparrow\uparrow$	$^6A$

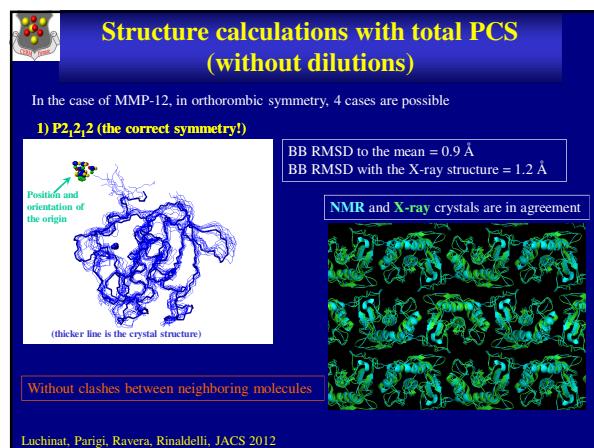
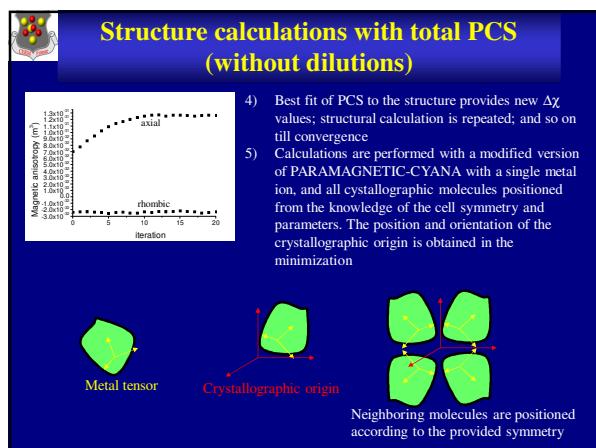
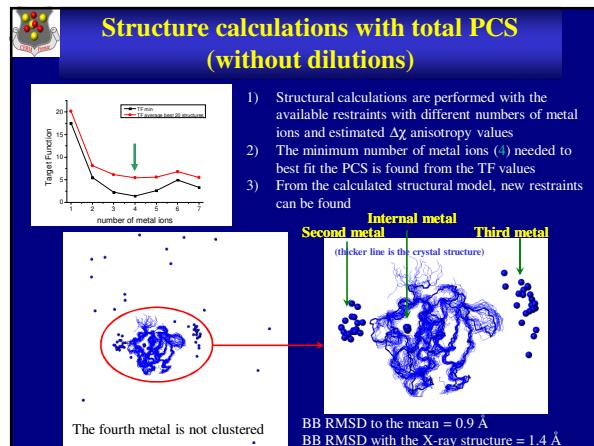
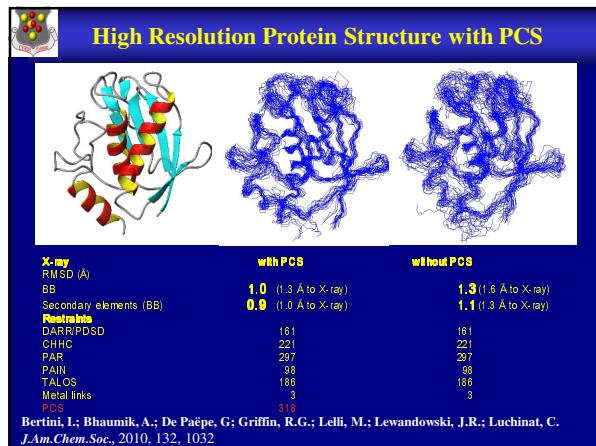
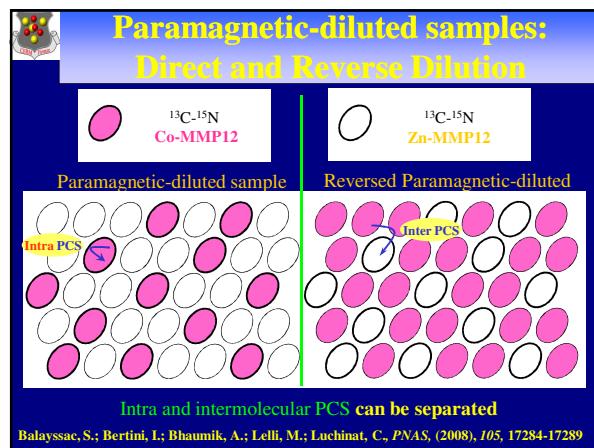
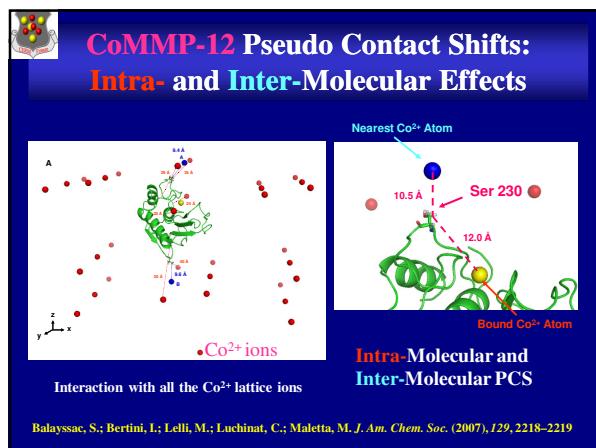
Ground state:  $^{2s+1}X$ , where X=S, P, D, F depending on the L value equal to 0, 1, 2, 3, respectively  
(A symmetry: non-degenerate ground state )  
(E symmetry: doubly degenerate ground states )  
(T symmetry: three possible ground states )

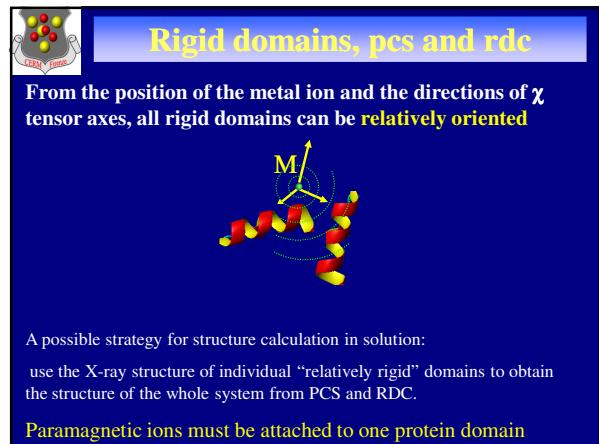
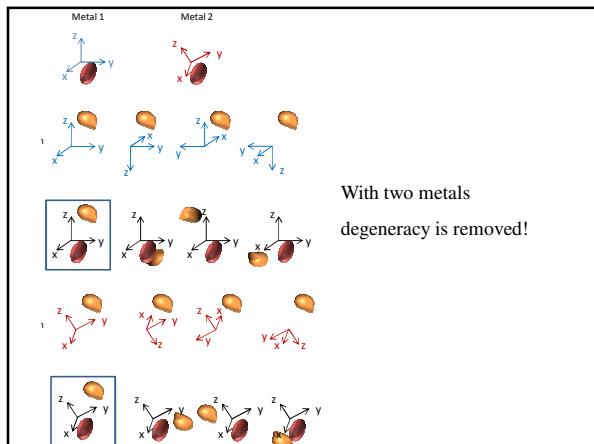
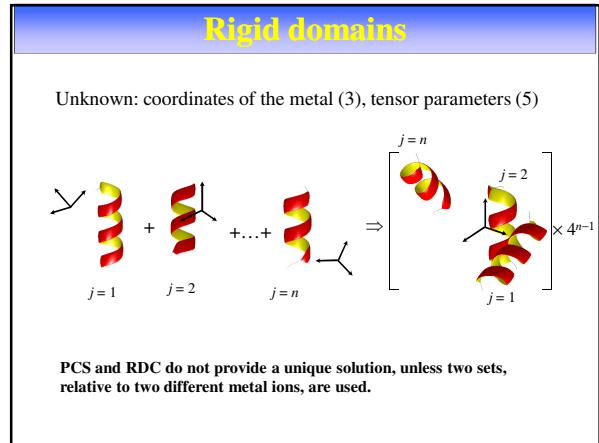
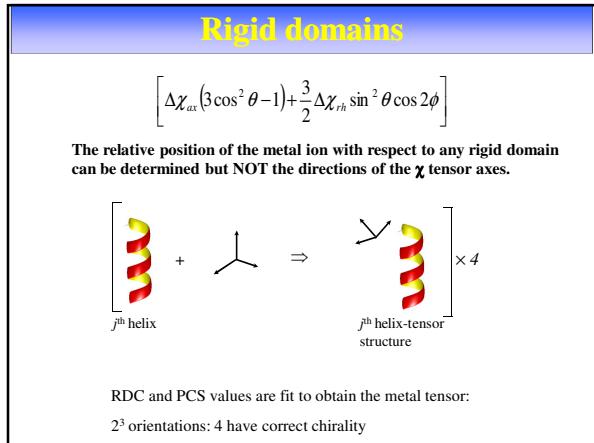
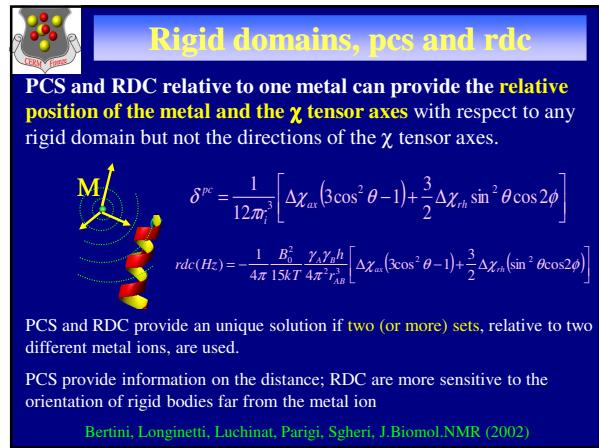
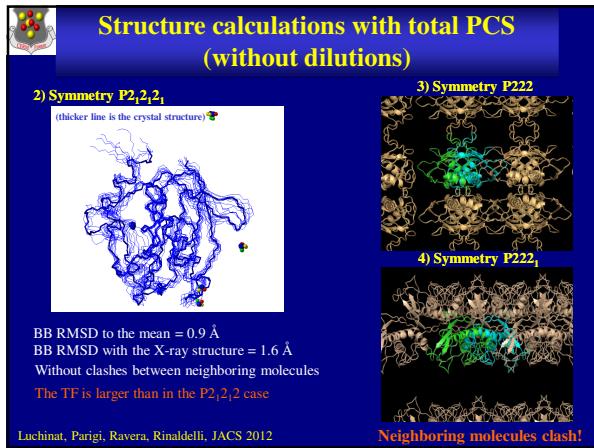


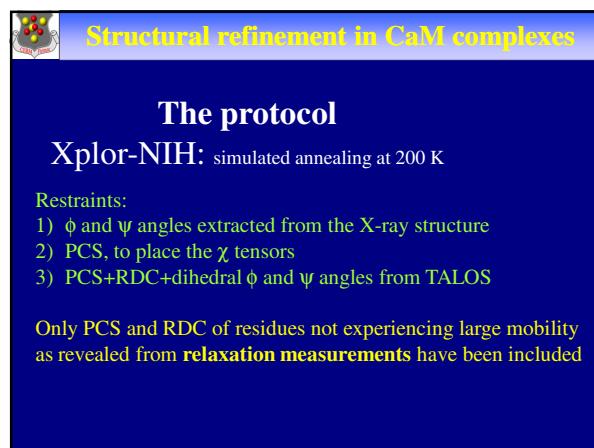
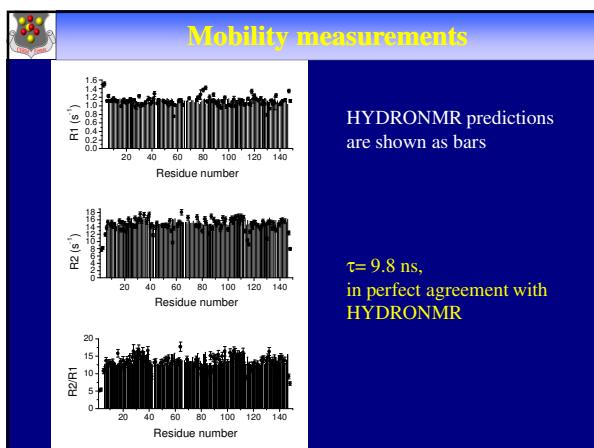
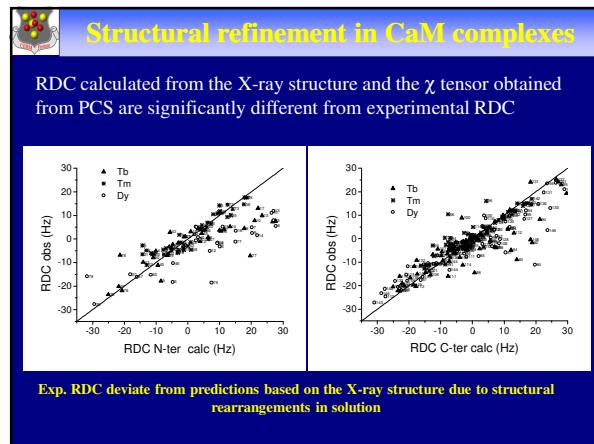
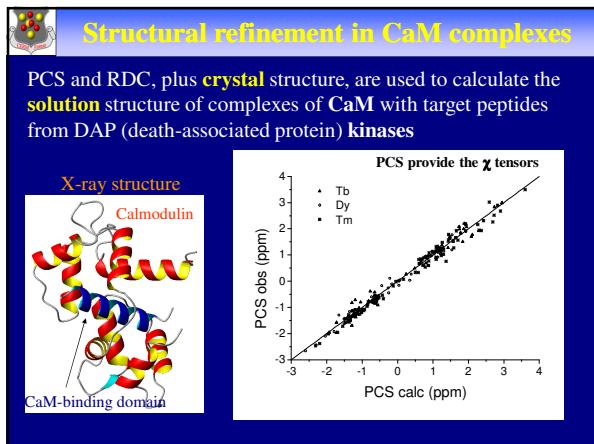
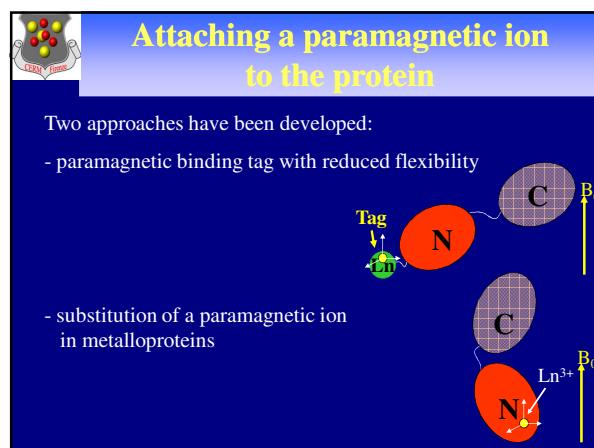
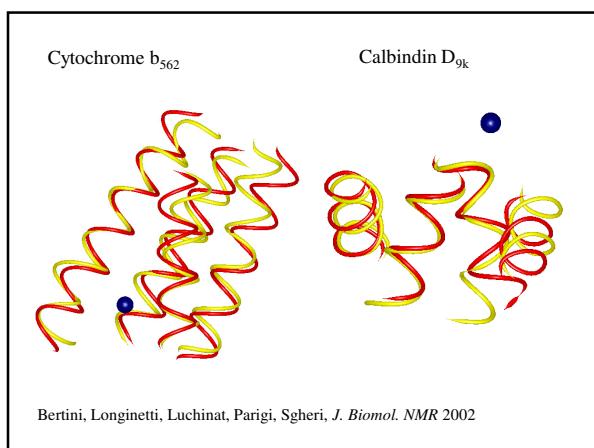


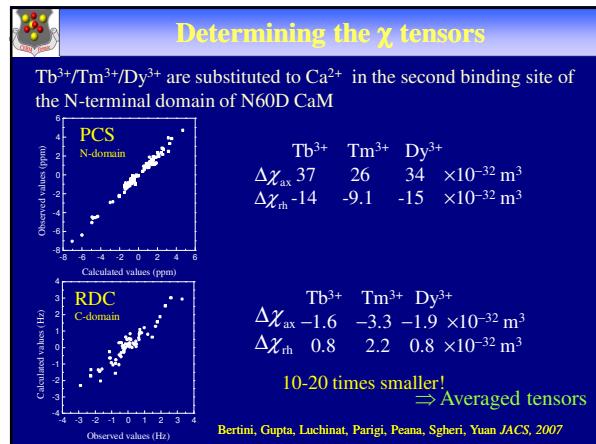
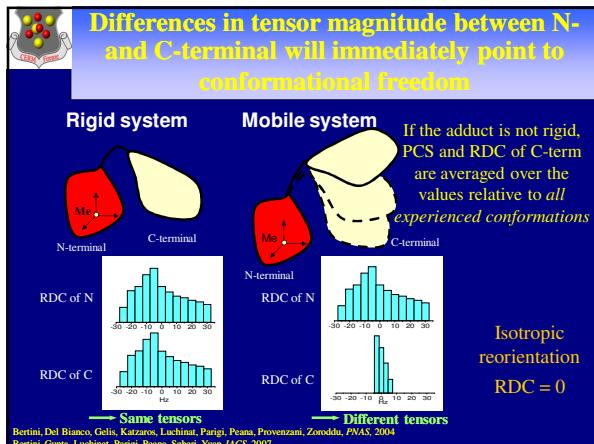
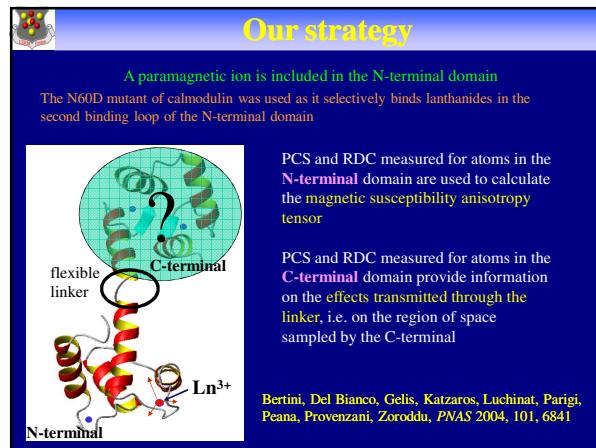
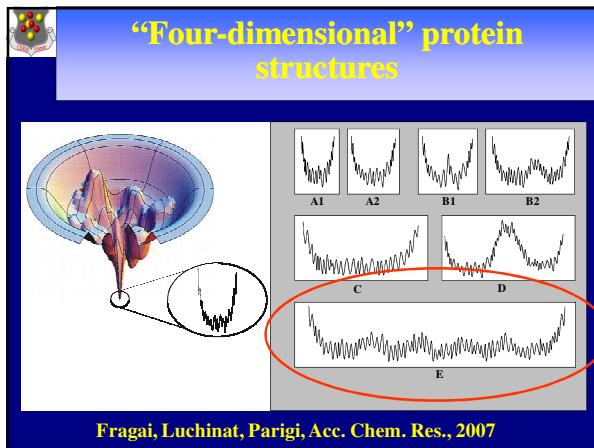
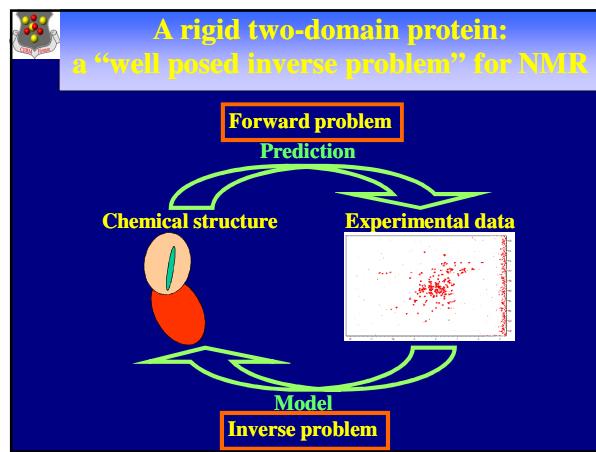
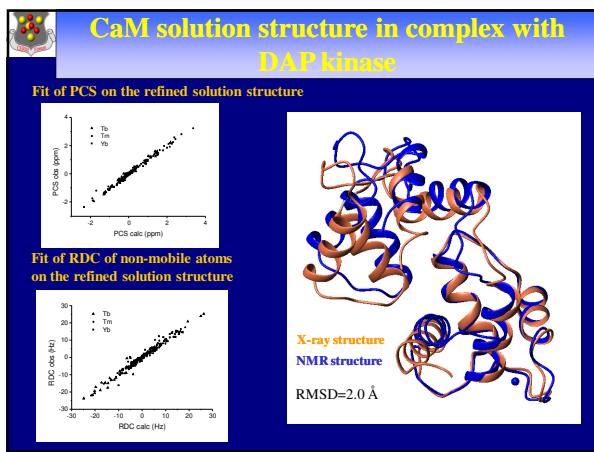


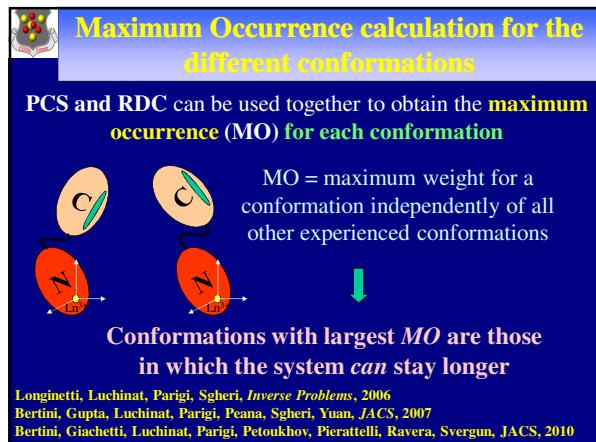
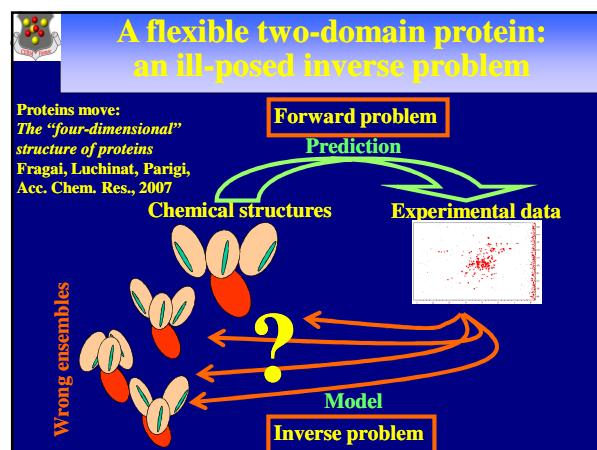
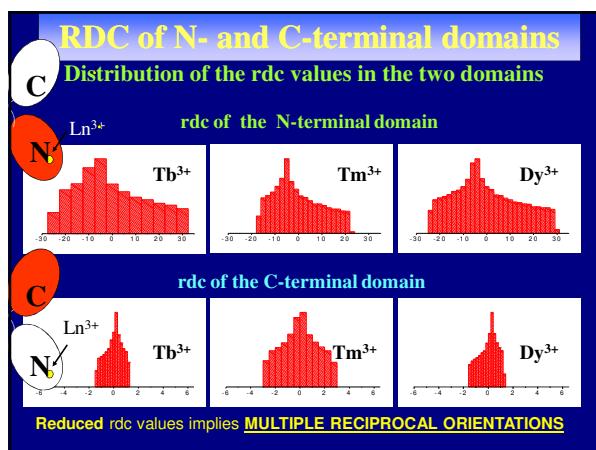












- References:**
- I. Bertini, C. Luchinat, G. Parigi, "Paramagnetic constraints: an aid for quick solution structure determination of paramagnetic metalloproteins", *Concepts in Magn. Reson.* (2002) 14, 259-286.
  - I. Bertini, C. Luchinat, G. Parigi, "Magnetic susceptibility in paramagnetic NMR", *Prog. in NMR Spectr.* (2002) 40, 249-273.
  - I. Bertini, C. Luchinat, G. Parigi, "<sup>1</sup>H NMRD profiles of paramagnetic aquo-complexes and metalloproteins", *Adv. Inorg. Chem.* (2005) 57, 105-172. In R. van Eldik and I. Bertini, "Relaxometry of water-metal ion interactions".
  - I. Bertini, C. Luchinat, G. Parigi, R. Pierattelli, "NMR Spectroscopy of Paramagnetic Metalloproteins", *ChemBioChem* (2005) 6, 1536-1549.
  - M. Fragai, C. Luchinat, G. Parigi, "Four dimensional protein structures: examples from metalloproteins", *Acc. Chem. Res.* (2006) 39, 909-917.
  - I. Bertini, C. Luchinat, G. Parigi, R. Pierattelli, "Perspectives in paramagnetic NMR of metalloproteins", *Dalton Trans.* (2008) 3782-3790.
  - I. Bertini, C. Luchinat, G. Parigi, "Moving the frontiers in solution and solid state bioNMR", *Coord. Chem. Rev.* (2011) 255, 649-663.
  - I. Bertini, C. Luchinat, G. Parigi, "Paramagnetic molecules" in "NMR of Biomolecules: Towards Mechanistic Systems Biology", Bertini I, McGreevy KS, Parigi G (Eds.), Wiley-VCH, Weinheim, Germany, 2012, pag. 155-171.
  - M. Fragai, C. Luchinat, G. Parigi, E. Ravera, "Conformational freedom of metalloproteins revealed by paramagnetism-assisted NMR", *Coord. Chem. Rev.* (2013) 257, 2652-2667.
  - A. Bhaumik, C. Luchinat, G. Parigi, E. Ravera, M. Rinaldelli, "NMR crystallography on paramagnetic systems: solved and open issues", *CrystEngComm* (2013) 15, 8639-8656.