Part 1
How can computational NMR contribute to structure determination of proteins with paramagnetic center?

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2014

Start

17.1.2013

" . . . we are sending the structure and the experimental pcs of a cobalt(II)-protein. The idea is for you to try to calculate the pcs from the present structure, and possibly increase the agreement with the experimental ones through changes in the coordination geometry of the metal ion. Here attached please find the structure 1RMZ (1.3 A resolution) of MMP12. The ZN ion with residue number 264 was replaced by cobalt(II). Pcs were measured, reported in the attached PNAS paper (in Table S2, labeled as PCS internal, Obs). The coordination sphere of the metal is composed of three imidazole groups of three histidine residues and of a bidentate ligand (hydroxamic acid). Best regards also on behalf of Claudio, Giacomo"

Paramagnetic shifts in solid-state NMR of proteins to elicit structural information

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Edited by Ann E. McDermott, Columbia University, New York, NY, and approved September 12, 2008 (received for review September 6, 2007)

The recent observation of pseudocontact shifts (pcs) in ^{13}C high-resolution solid-state NMR of paramagnetic proteins opens the way to their application as structural restraints. Here, by investigating a microcrystalline sample of cobalt(II)-substituted matrix metalloproteinase 12 [CoMMP-12 (159 AA, 17.5 kDa)], it is shown that a combined strategy of protein labeling and dilution of the paramagnetic species (i.e., ^{13}C-,^{15}N-labeled CoMMP-12 diluted in unlabeled ZnMMP-12, and ^{13}C-,^{15}N-labeled ZnMMP-12 diluted in unlabeled CoMMP-12) allows one to easily separate the pcs contributions originated from the protein internal metal (intramolecular pcs) from those due to the metals in neighboring proteins in the crystal lattice (intermolecular pcs) and that both can be used for structural purposes. It is demonstrated that intramolecular pcs are significant structural restraints helpful in increasing both precision and accuracy of the structure, which is a need in solid-state structural biology nowadays. Furthermore, intermolecular pcs provide unique information on positions and orientations of neighboring protein molecules in the solid phase.

matrix metalloproteinase | pseudocontact shift | microcrystal | cobalt(II)

Paramagnetic NMR restraints as relaxation times, pcs, and residual dipolar couplings (RDC) (21)—the latter two originating from anisotropy in the magnetic susceptibility tensor—are routinely used in solution NMR to refine structures (22), to investigate protein–protein interactions (23, 24), or to monitor dynamics (25, 26). Small paramagnetic molecules have been studied through magic angle spinning (MAS) SSNMR for decades (27–33). Paramagnetism in the solid state causes problems connected with the large shift anisotropy, inhomogeneous broadening (34), and the difficulties in obtaining efficient proton decoupling (30, 32). Pioneering works have shown that paramagnetic proteins are also affordable by SSNMR by using either perdeuterated substrates (35) or selective labeling (36). More recently, uniformly labeled paramagnetic proteins have also been studied (37–39), taking advantage of the absence of paramagnetic relaxation mechanisms related to the molecular tumbling (as Curie relaxation terms) (40). The prospective availability of fast and ultrafast MAS probes should allow one to reduce the limits posed by the presence of metals inducing large shift anisotropies (33, 40, 41).

Protein structure determination using ssNMR

- NOE (can be insufficient especially from ssNMR)

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Protein structure determination using ssNMR

- NOE (can be insufficient especially from ssNMR)
- Empirical angular restraints (TALOS)
- Pseudocontact shifts

Impact of a paramagnetic center in a protein

- Enhanced relaxation (blind zones . . .)
- Contact shift due to spin-density distribution
- **Pseudocontact shift due to dipolar coupling**
- RDCs in solution NMR

Pseudocontact shift

"experimentalists' view"

- A difference between chemical shift in paramagnetic and corresponding diamagnetic compound

Pseudocontact shift

"experimentalists' view"

- A difference between chemical shift in paramagnetic and corresponding diamagnetic compound
- . . . sufficiently far from paramagnetic center, such that:
  -contact shift is negligible
  -magnetic moment of the unpaired electrons can be approximated as a point dipole
  -(difference in orbital shielding is negligible)
- in present case: $Zn^{2+} \rightarrow Co^{2+}$ substitution does not have impact on the structure

Use of pseudocontact shifts

in study of macromolecules

- Iteratively obtain the χ tensor, utilizing also some low-resolution structure
- Impose long-range structure restraints
- Refine position of the magnetic moment / metal ion
- Study intermolecular interactions; crystal packing

$$\boldsymbol{\sigma}_{\mathbf{Dip}} = -\boldsymbol{\chi} \cdot \mathbf{D} \frac{1}{4\pi r_{k,s}^3} (\times 10^6 \mathrm{ppm}) \tag{1}$$

where

$$\mathbf{D} = 3\mathbf{n}_{k,s}\mathbf{n}_{k,s} - \mathbf{1}, \tag{2}$$

is the dimensionless dipolar coupling tensor where $\mathbf{n}_{k,s} = \mathbf{r}_{k,s}/r_{k,s}$ [1] then

$$\sigma_{\mathrm{PC}} = \frac{Tr(\boldsymbol{\sigma}_{\mathbf{Dip}})}{3} \tag{3}$$

[1] k, s label nuclear and electronic magnetic dipoles

Paramagnetic shielding

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}_{\mathrm{orb}} - \frac{\mu_{\mathrm{B}}}{\gamma k T}\, \boldsymbol{g} \cdot \langle \boldsymbol{S}\boldsymbol{S} \rangle_0 \cdot \boldsymbol{A} \tag{4}$$

[2]

Term name	Term in $\sigma_{\epsilon\tau}$	Number
σ_{orb}	σ_{orb}	0
σ_{con}	$g_e A_{\mathrm{con}} \langle S_\epsilon S_\tau \rangle_0$	1
σ_{dip}	$g_e \sum_b A^{\mathrm{dip}}_{b\tau} \langle S_\epsilon S_b \rangle_0$	2
$\sigma_{\mathrm{con},2}$	$g_e A_{\mathrm{PC}} \langle S_\epsilon S_\tau \rangle_0$	3
$\sigma_{\mathrm{dip},2}$	$g_e \sum_b A^{\mathrm{dip},2}_{b\tau} \langle S_\epsilon S_b \rangle_0$	4
σ_{ac}	$g_e \sum_b A^{\mathrm{as}}_{b\tau} \langle S_\epsilon S_b \rangle_0$	5
$\sigma_{\mathrm{con},3}$	$\Delta g_{\mathrm{iso}} A_{\mathrm{con}} \langle S_\epsilon S_\tau \rangle_0$	6
$\sigma_{\mathrm{dip},3}$	$\Delta g_{\mathrm{iso}} \sum_b A^{\mathrm{dip}}_{b\tau} \langle S_\epsilon S_b \rangle_0$	7
$\sigma_{\mathrm{c,aniso}}$	$A_{\mathrm{con}} \sum_a \Delta \tilde{g}_{\epsilon a} \langle S_a S_\tau \rangle_0$	8
σ_{pc}	$\sum_{ab} \Delta \tilde{g}_{\epsilon a} A^{\mathrm{dip}}_{b\tau} \langle S_a S_b \rangle_0$	9

Long-range terms in red

[2] PRL **100**, 2008, Pennanen T. O. & Vaara J.

χ in the modern shielding theory

$$E_{dip} = \mathbf{m}_k \cdot \mathbf{T} \cdot (-\boldsymbol{\chi} \cdot \mathbf{B}_0) / \mu_0 \tag{5}$$
$$= \hbar \gamma_k \mathbf{I}_k \cdot \boldsymbol{\sigma}_{\mathrm{Dip}} \cdot \mathbf{B}_0 \tag{6}$$

(here $\boldsymbol{\sigma}_{\mathrm{Dip}}$ is a sum of three (long range) terms of the breakdown of pNMR shielding)

$$-\mathbf{T} \cdot \boldsymbol{\chi} / \mu_0 = \boldsymbol{\sigma}_{\mathrm{Dip}} \tag{7}$$

see (Eq.1)
where $\mathbf{T}$ is the dipole-dipole interaction tensor for two dipoles also written like $\mathbf{T} = \mathbf{D}\frac{\mu_0}{4\pi r^3}$ where $\mathbf{D} = 3\mathbf{n}_{ks}\mathbf{n}_{ks} - \mathbf{1}$

$$\frac{\mu_0}{4\pi r^3 \mu_0}\mathbf{D} \cdot \boldsymbol{\chi} = \frac{\mu_B}{\gamma_k k T}\mathbf{g} \cdot \langle SS \rangle \cdot \mathbf{A}_{\mathrm{dip}}$$

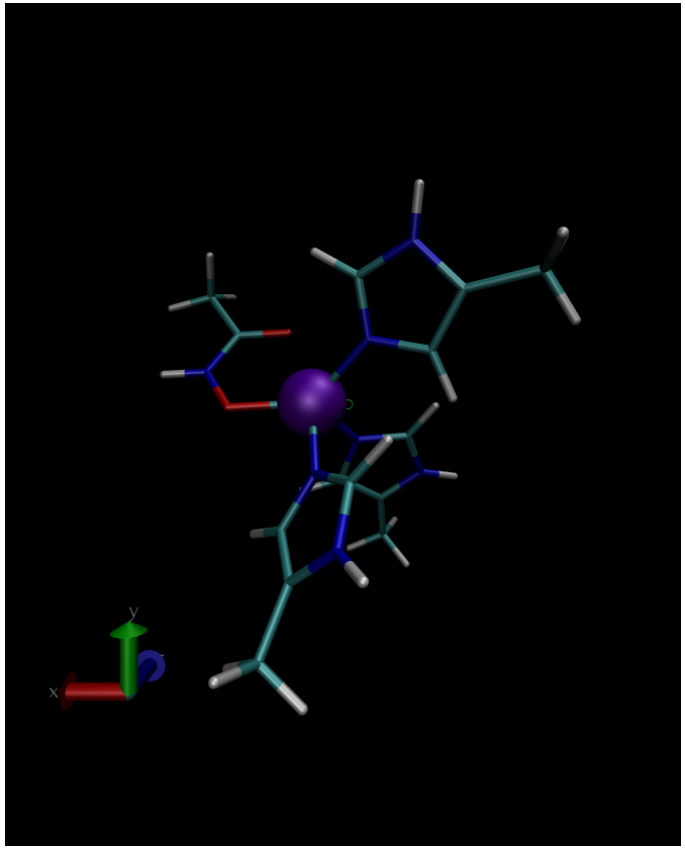
$$\mathbf{D} \cdot \boldsymbol{\chi} = \frac{\mu_B \mu_0}{kT}\mathbf{g} \cdot \langle SS \rangle \cdot \hbar \gamma_s \mathbf{D} \tag{8}$$

since $\hbar \gamma_s = g_e \mu_B$ the final expression for molecular susceptibility/magnetizability

$$\boldsymbol{\chi} = \frac{\mu_B^2 \mu_0}{kT}\mathbf{g} \cdot \langle SS \rangle g_e \tag{9}$$

Model of the paramagnetic center

This geometry was optimized (with alpha-Carbon atoms fixed) using the BP86 functional, def2-SVP (H,C,N,O,S) + def2-TZVP (Co) basis, and COSMO of water solvent.



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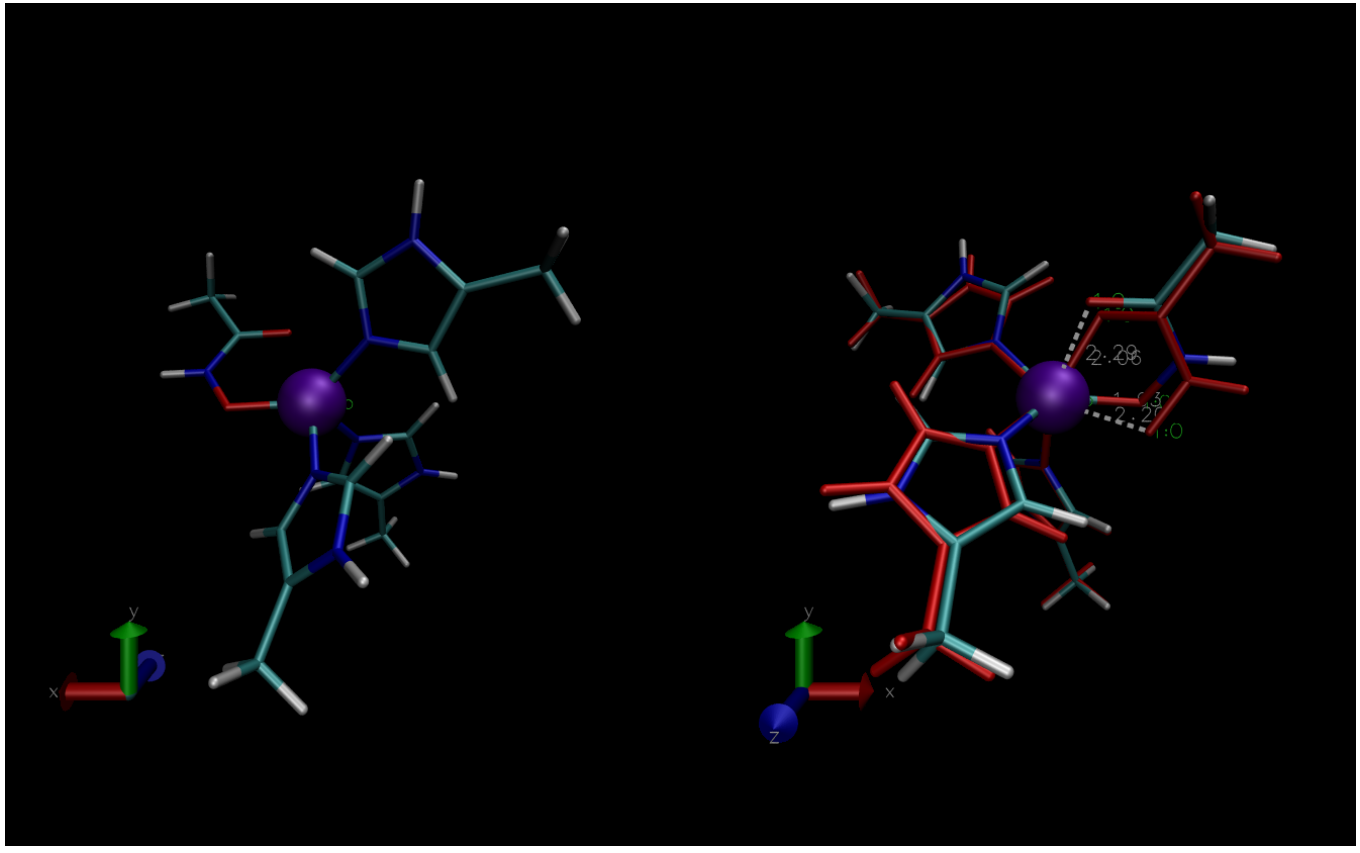
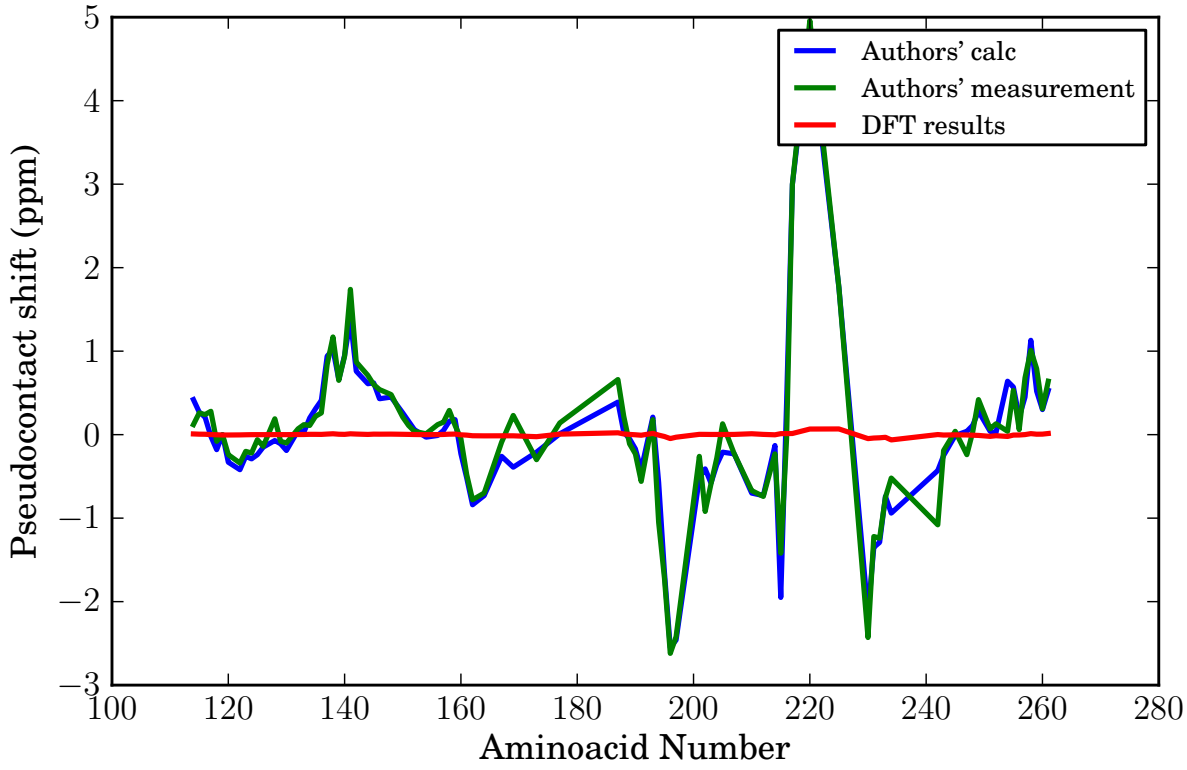


Pseudocontact shifts, DFT results

PCS plotted for C_α of every observed aminoacid residue



Authors ← Balayssac, Bertini, Bhaumik, Luchinat
calc ← (Eq.1), from X-ray structure and fitted χ from the measured PCSs

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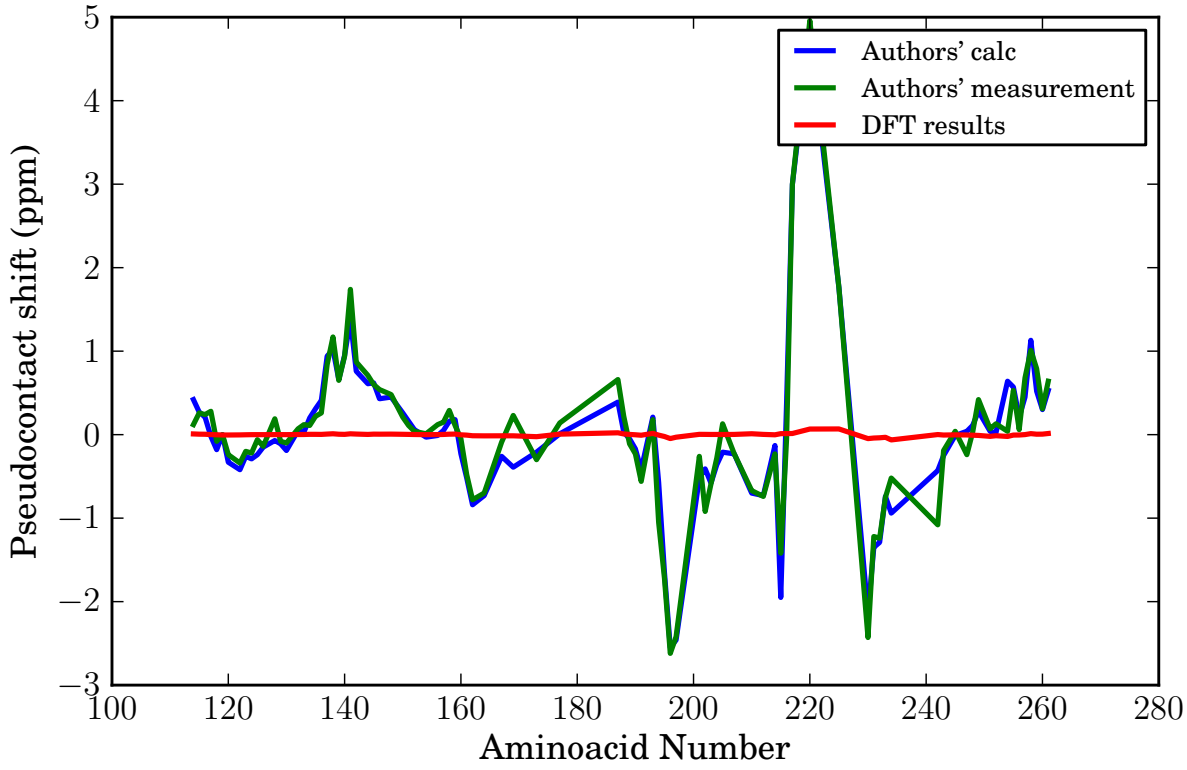

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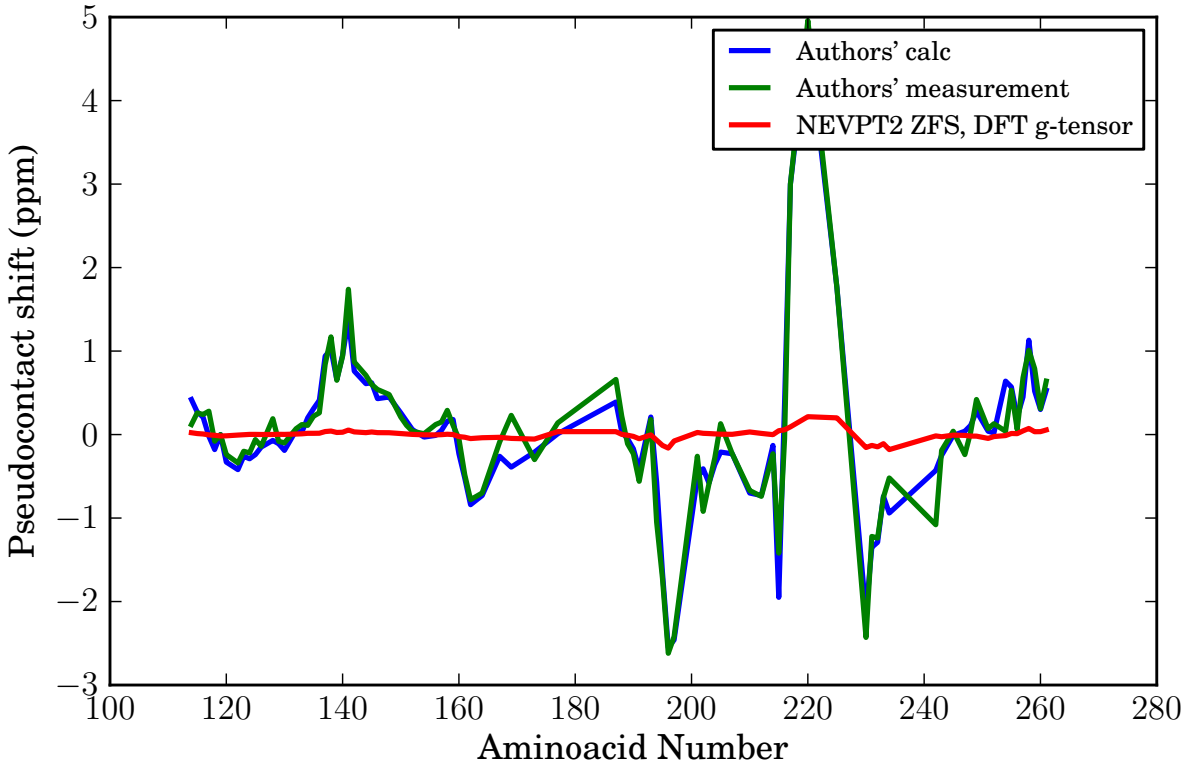
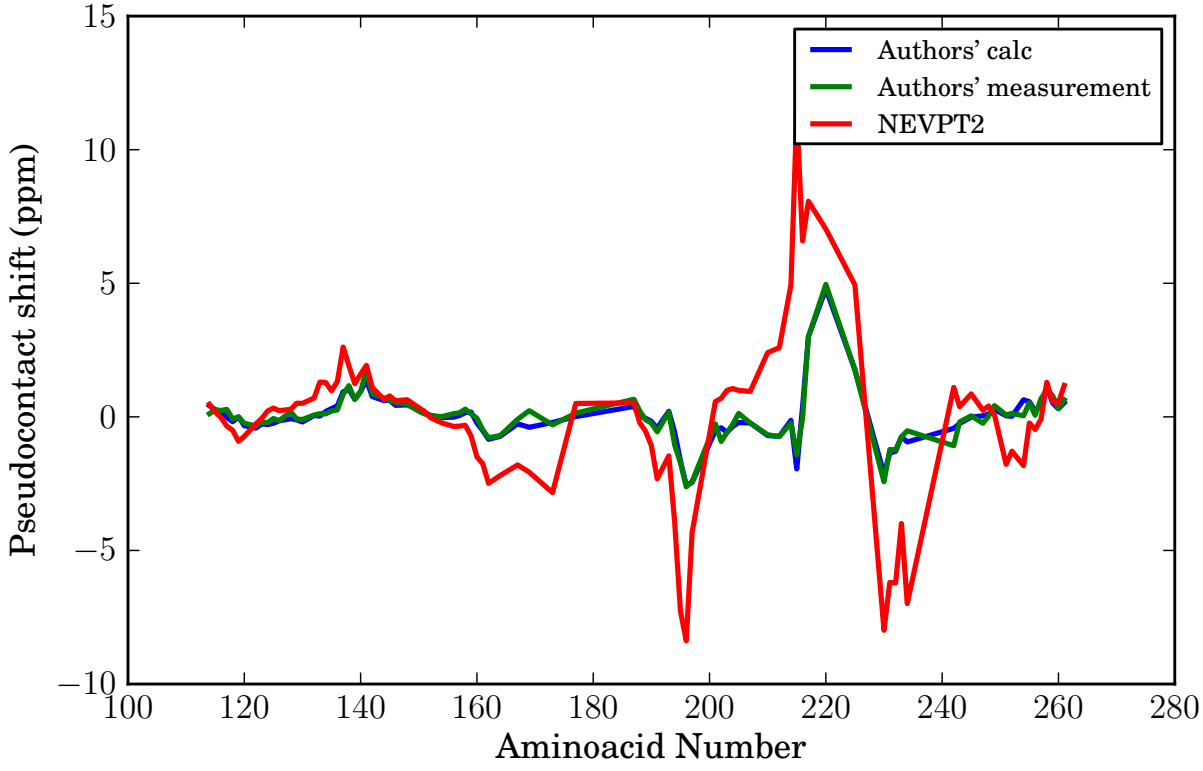
$D = 4.35 cm^{-1}$, $E/D = 0.279$ $g_{\mathrm{iso}} = 2.0657$

Pseudocontact shifts, DFT g-tensor, NEVPT2 ZFS



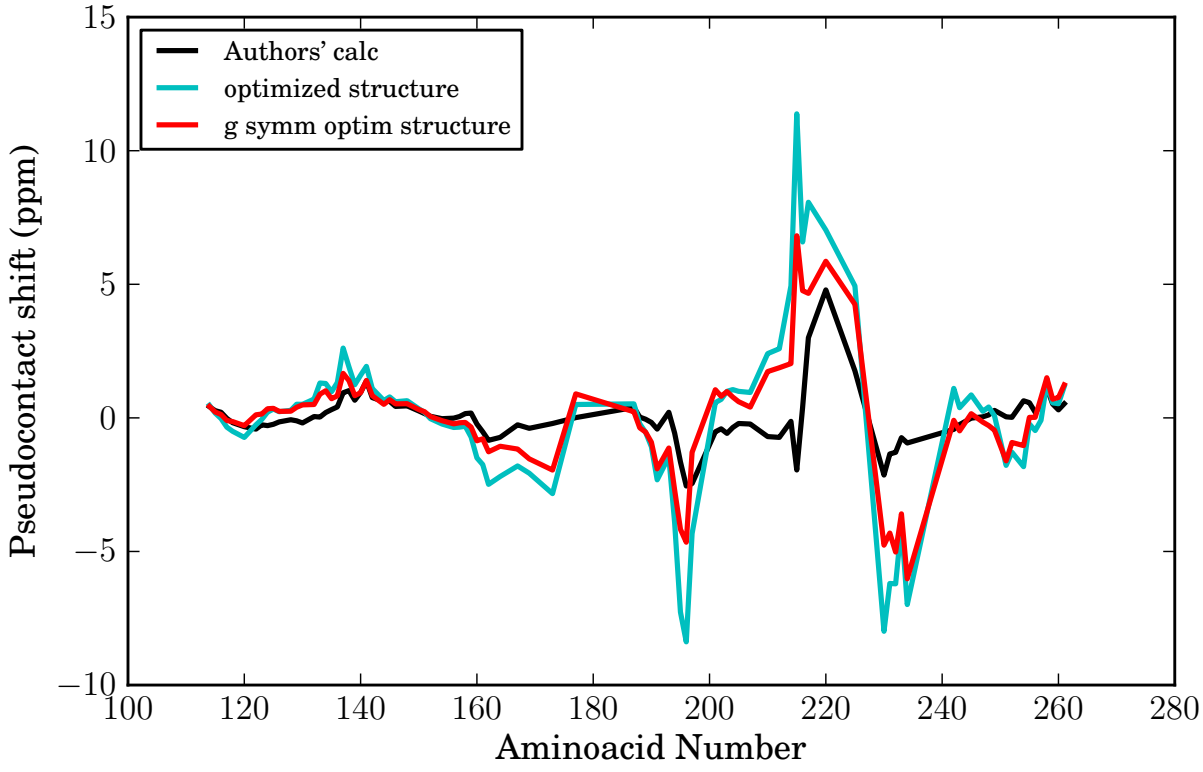
$D = -27.44 cm^{-1}$, $E/D = 0.267$

Pseudocontact shifts, NEVPT2 results

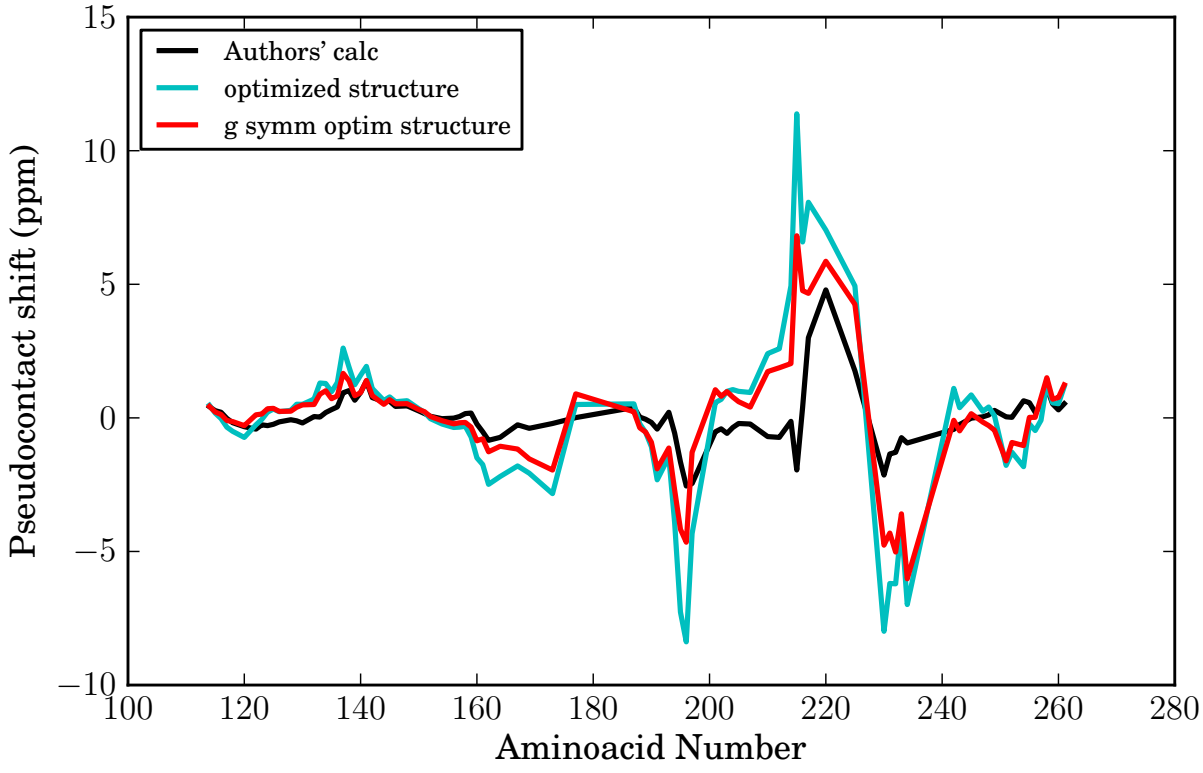


$D = -27.44 cm^{-1}$, $E/D = 0.267$ $g_{\mathrm{iso}} = 3.33$

About symmetrization of the g-tensor

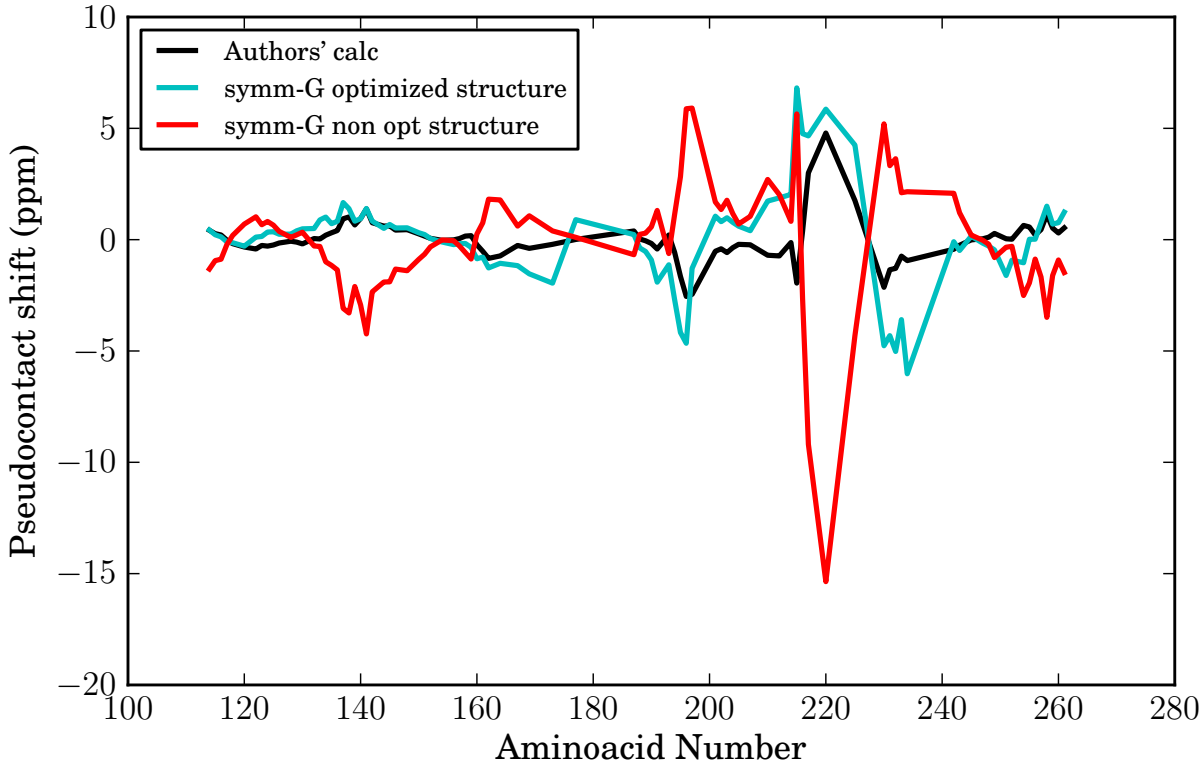


About symmetrization of the g-tensor



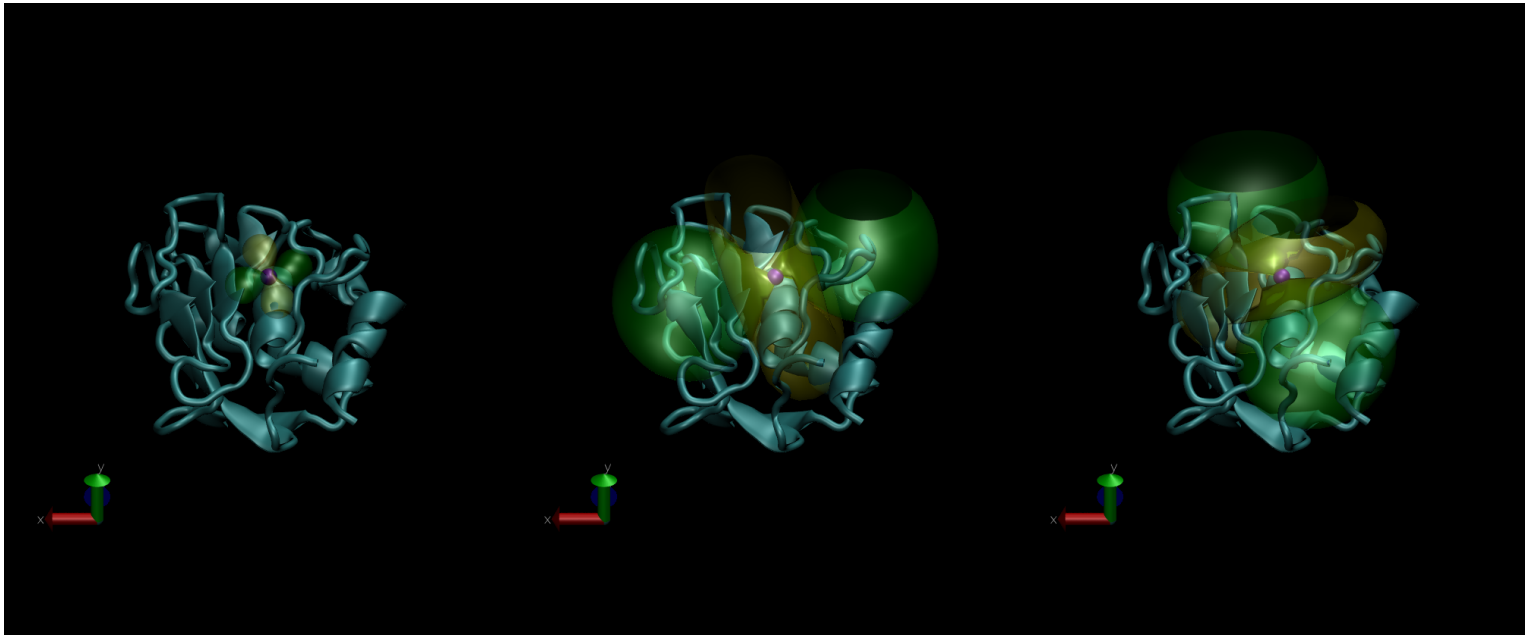
final results?

Optimized vs experimental structure

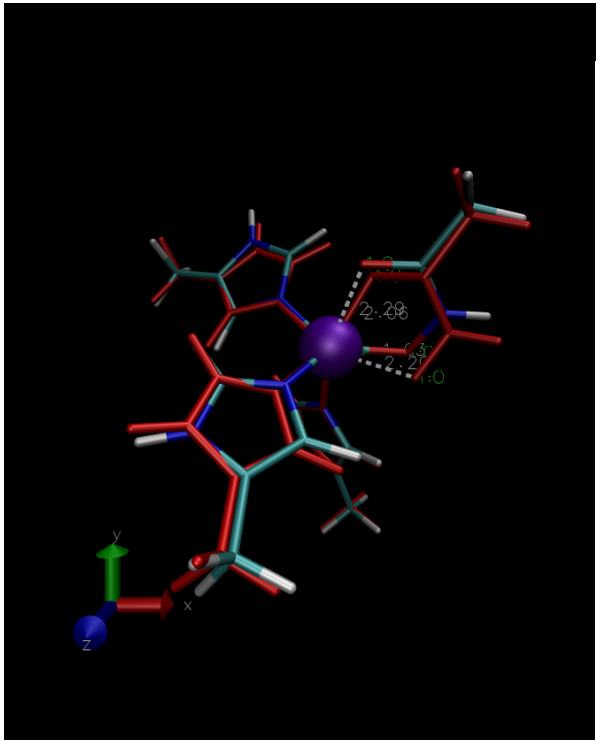


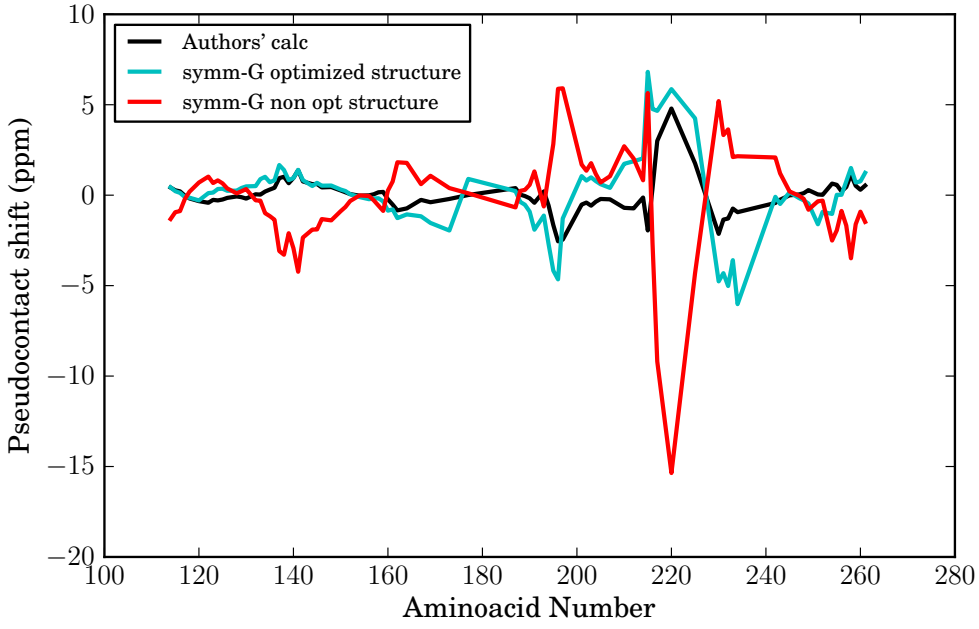
Pseudocontact shift isosurfaces of ± 1.5 ppm

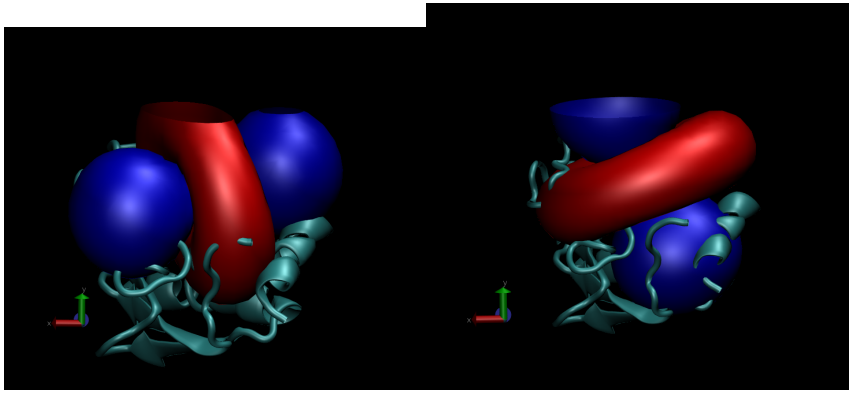
DFT g-tensor NEVPT2 NEVPT2 exp. str.



PCS optimized vs crystal structure model



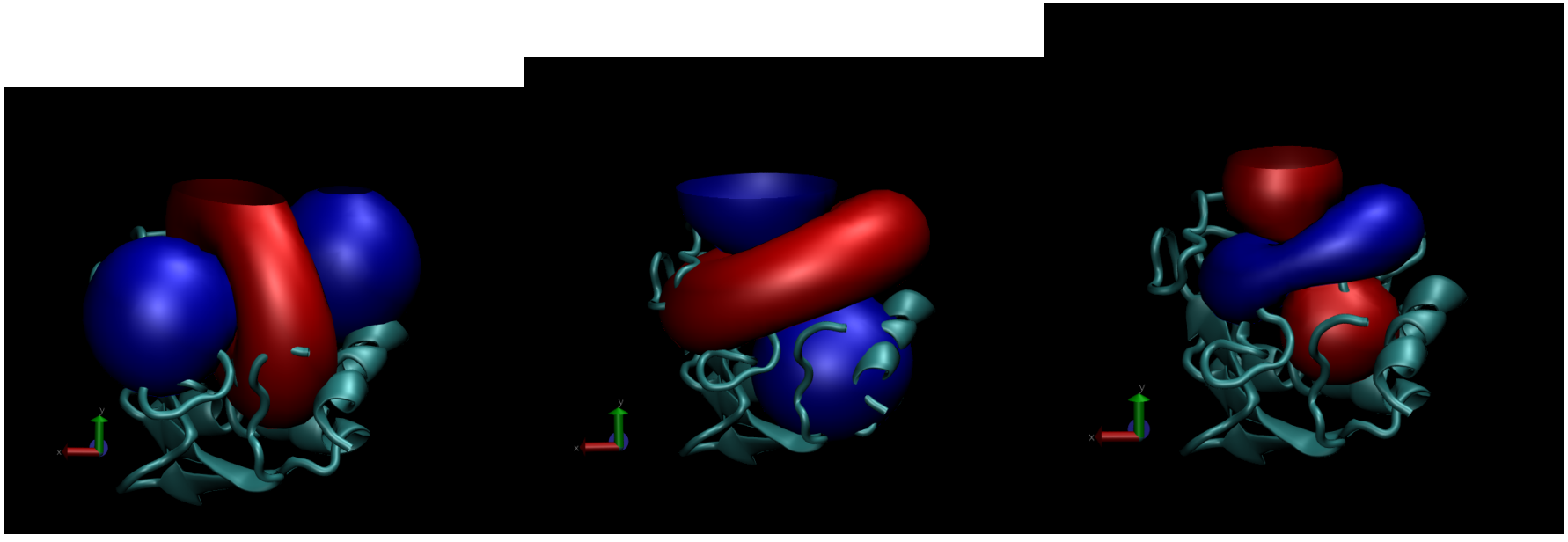




NEVPT2 optimized str

NEVPT2 exp. str.

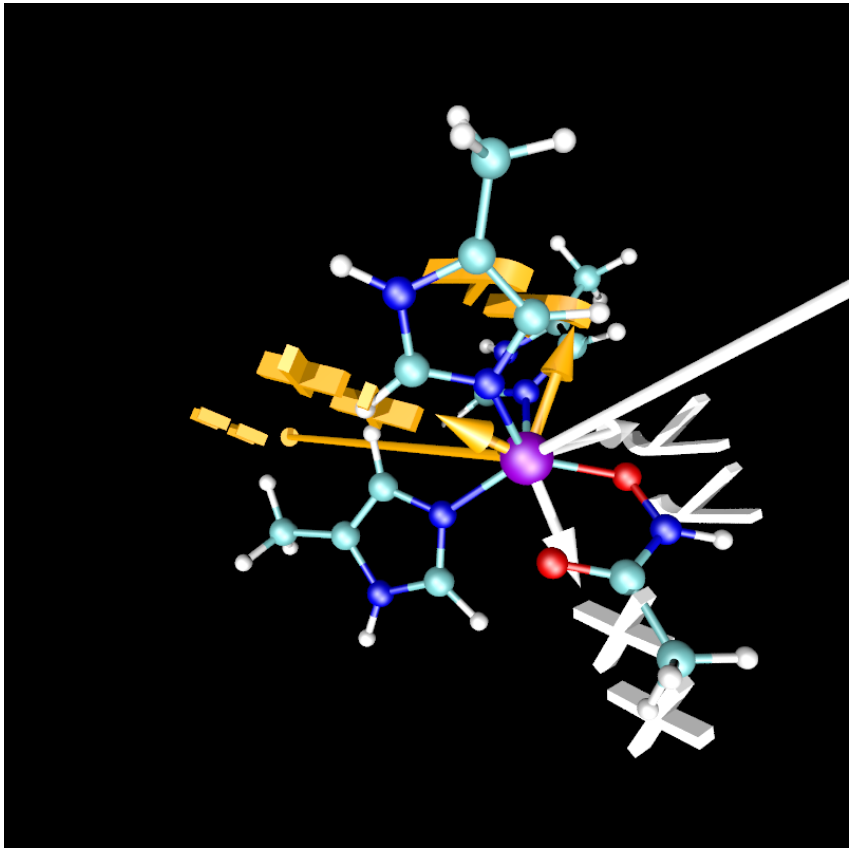
experimental PCS



g and ZFS in optimized/ nonoptimized structure

G tensor

ZFS tensor



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G tensor

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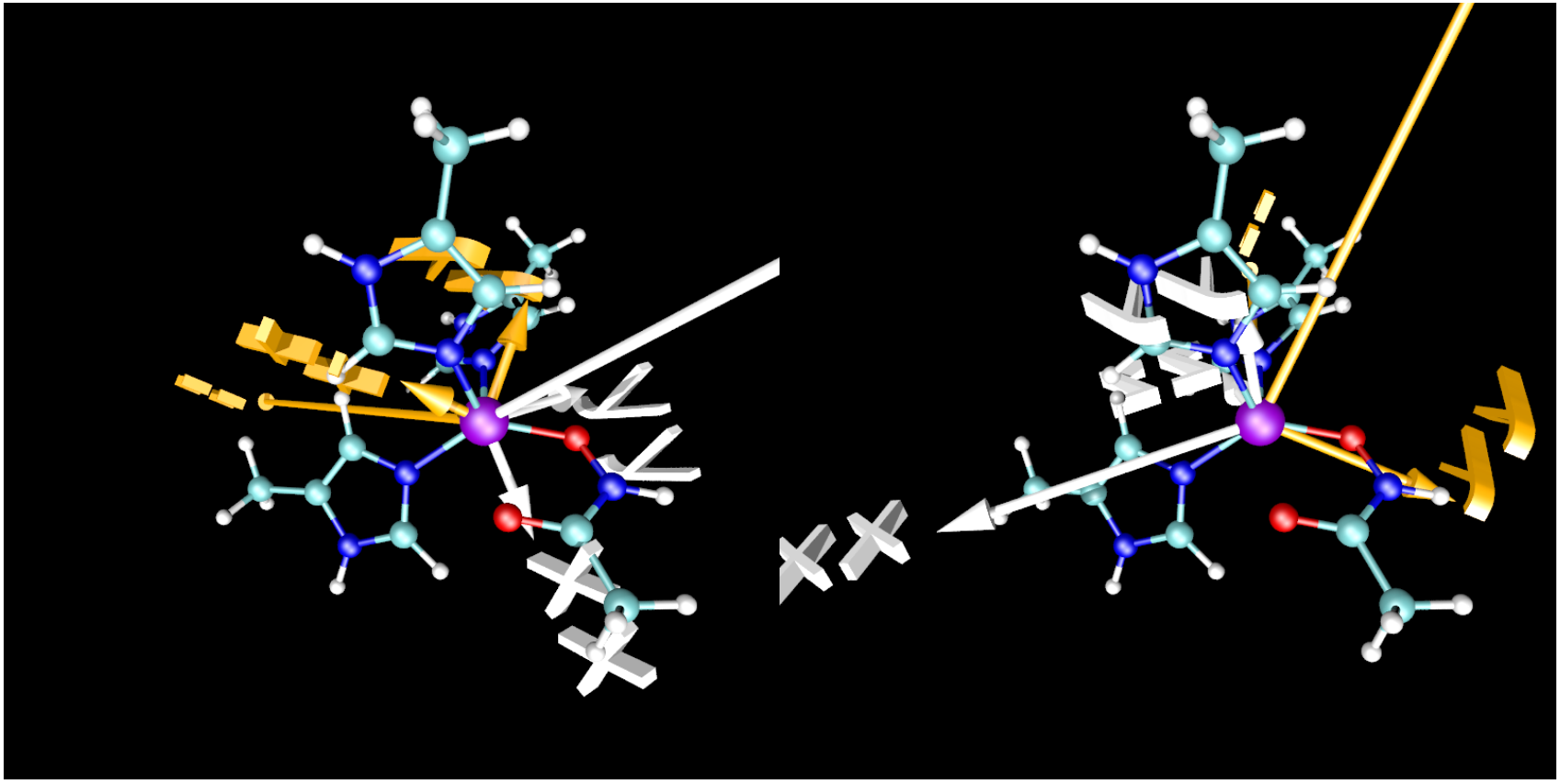
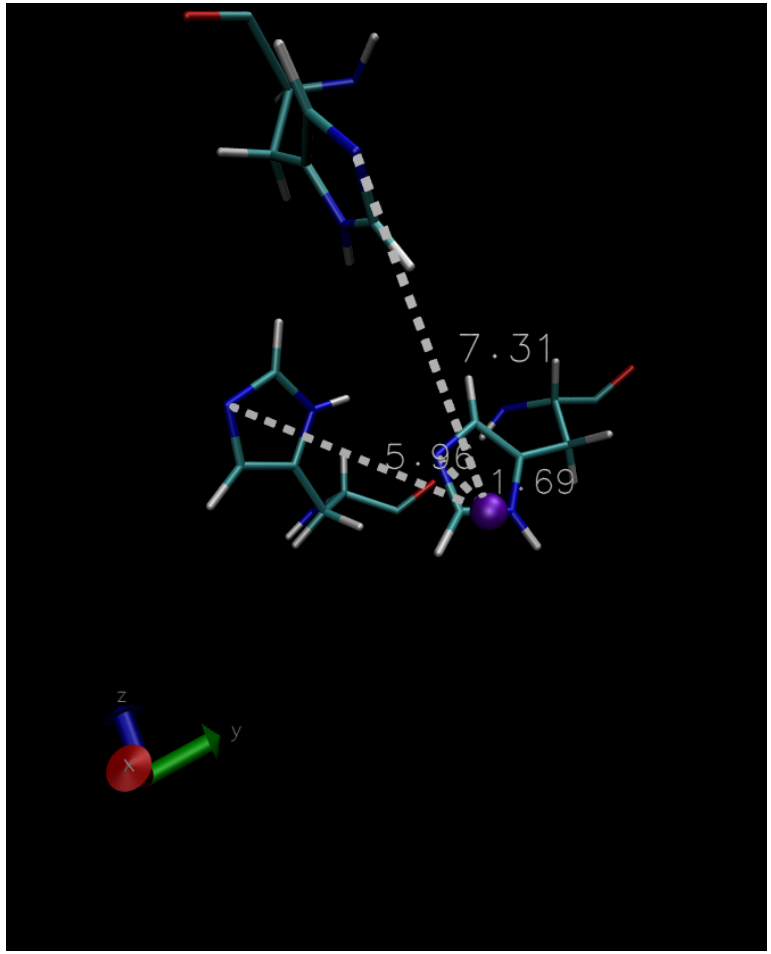


How can computational NMR contribute to structure determination of proteins with paramagnetic center?

- Knowing PCSs
- (Capable to accurately calculate χ)
- Not knowing structure:
  1. Of/near the paramagnetic center
  2. More distant from the paramagnetic center:
  3. *Intermediate (blind zone of H)*

Simple case of point 1. shown in this work.

More difficult case . . .



PDB: 2K9C [3]

[3] PNAS **105**, 2008, Balayssac, Bertini, Bhaumik, Luchinat

More distant from the paramagnetic center

Can we help with ?

Common case of protein structure elucidation, have to optimize:

- axiality, rhombicity and orientation of χ
- position of protein atoms (with a help of other information such as NOE)

or

- Know paramagnetic center center (spin-label, porphyrin, FeS?), or able to model the center well.
- Can reduce number of optimized parameters when doing the structure optimization. (axiality, rhombicity of χ are known) Is it significant?

Conclussions 1

1. PCSs (of distant regions of a protein) calculated using QC methods **on the model of the paramagnetic center** are in qualitative agreement with the measured PCSs.

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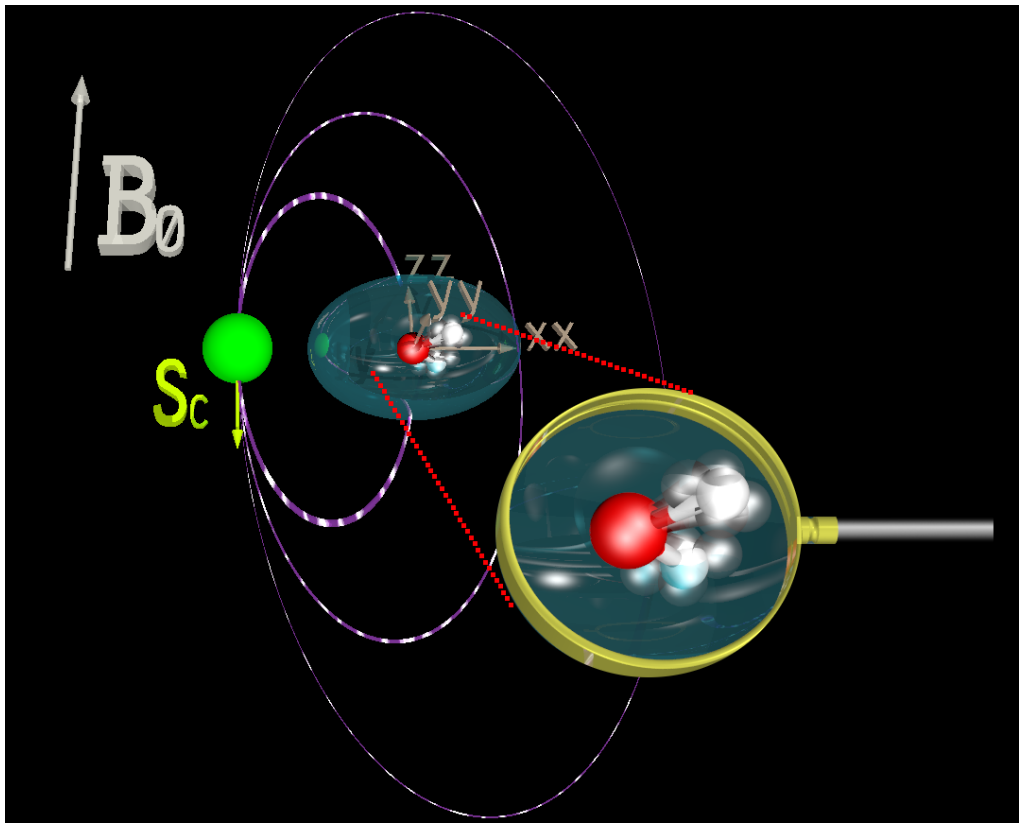
Conclussions 1

1. PCSs (of distant regions of a protein) calculated using QC methods **on the model of the paramagnetic center** are in qualitative agreement with the measured PCSs.
2. $\rightarrow$ serve for indirect proof that the geometry optimization of the paramagnetic center has improved the model
3. χ expressed consistently with the paramagnetic nuclear shielding theory of Pennanen and Vaara 2008
4. remaining questions

Part 2

Curie-type paramagnetic NMR relaxation in the aqueous solution of Ni(II)



Magnetic field of the Curie spin manifests itself as both the pNMR shielding tensor and Curie relaxation, in analogy with CSA relaxation theory.[4]

[4] Mareš, Hanni, Lantto, Lounila, Vaara PCCP 2014, in press.

Calculation flow

1. Molecular dynamics
2. Snapshot calculations (ZFS, g, HFC)$\rightarrow$ pNMR
3. Correlation functions, spectral density functions of the pNMR shielding
4. Redfield theory (CSA) $\rightarrow R_1, R_2$ relaxation rates due to Curie relaxation

ZFS, g, HFC

	calc (experim)
ZFS Δ_5 (cm^{-1})	3.5 (2.6, 3.0)
g, iso	2.10 (2.25)
HFC, $A_{\mathrm{dip},33}$, (MHz)	8.22 (?)

pNMR shielding

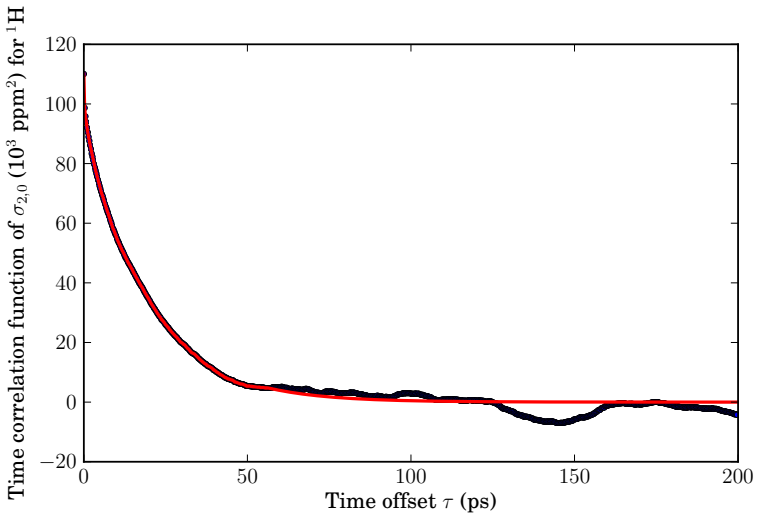
$\sigma_{2,0}$

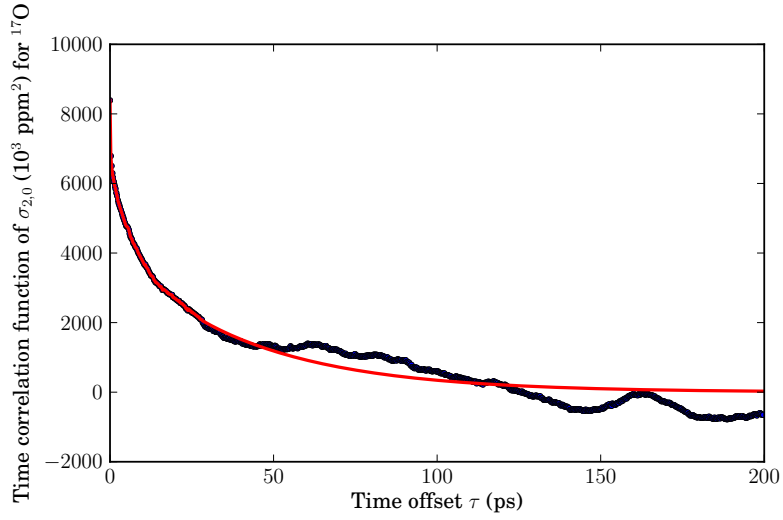
Term name	FSS/^{1}H	SSS/^{1}H	FSS/^{17}O	SSS/^{17}O
σ_{orb} [d]	-	-	-	-
σ_{con} [e]	1.50	0.0364	131	1.78
σ_{dip}	304	63.3	2673	93.2
$\sigma_{\mathrm{con},2}$	0.0182	0.000959	1.08	0.00694
$\sigma_{\mathrm{dip},2}$	14.1	3.00	109	3.14
σ_{ac}	0.0153	0.00176	0.139	0.00628
$\sigma_{\mathrm{con},3}$	0.0765	0.001835	6.68	0.0904
$\sigma_{\mathrm{dip},3}$	15.1	3.15	133	4.65
$\sigma_{\mathrm{c,aniso}}$	0.369	0.00908	33.2	0.441
σ_{pc}	0.518	0.100	4.89	0.146

FSS : First Solvation Shell

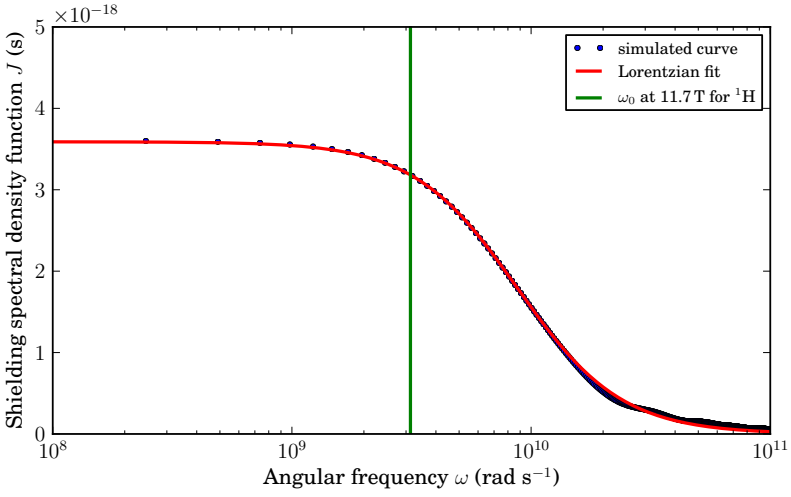
SSS : Second Solvation Shell

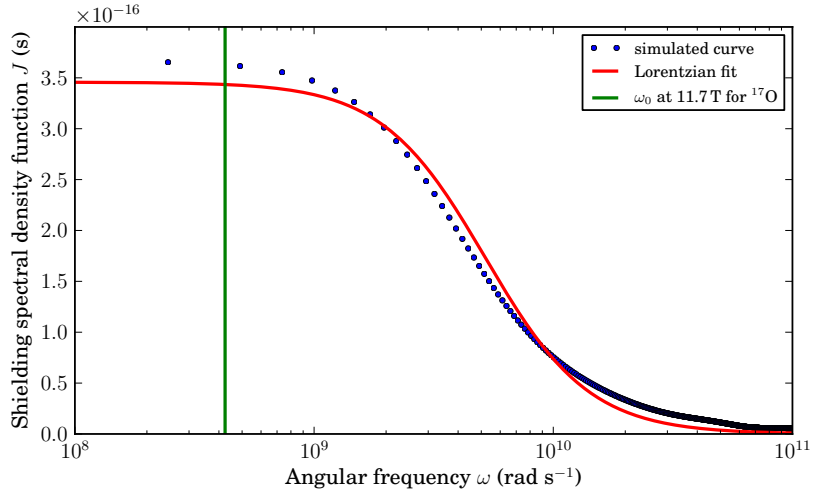
Simulated time correlation function of the spherical $\sigma_{2,0}$ component of the shielding tensor





The spectral density functions





Relaxation rates of Curie relaxation

11.7 T

$$R_1 = \frac{1}{2}\omega_0^2 J(\omega_0)$$

$$R_2 = \frac{1}{12}\omega_0^2 [4J(0) + 3J(\omega_0)]$$

Shielding term	^{1}H (FSS)	^{1}H (bulk, 0.12M)	^{1}H (1 M total)
σ_{dip}	13; 17	0.30; 0.41	1.7; 2.3
$\sigma_{\mathrm{dip},2}$	0.032; 0.042	3.2×10^{-4}; 3.9×10^{-4}	3.8×10^{-3}; 5.0×10^{-3}
$\sigma_{\mathrm{dip},3}$	0.032; 0.041	7.6×10^{-4}; 1.0×10^{-3}	4.3×10^{-3}; 5.5×10^{-3}
σ_{tot}	16; 20	0.45; 0.52	2.2; 2.7

Conclussions 2

1. For Ni(II) (*aqua*), the Curie relaxation mechanism is a very minor one, available only computationally.
2. Using the theory of pNMR shielding, Curie relaxation can be reliably calculated using the analogy with CSA relaxation in diamagnetic systems

People

Juha Vaara, Ladislav Benda, Giacomo Parigi, Martin Kaupp, Matti Hanni, Perttu Lantto, Juhani Lounila