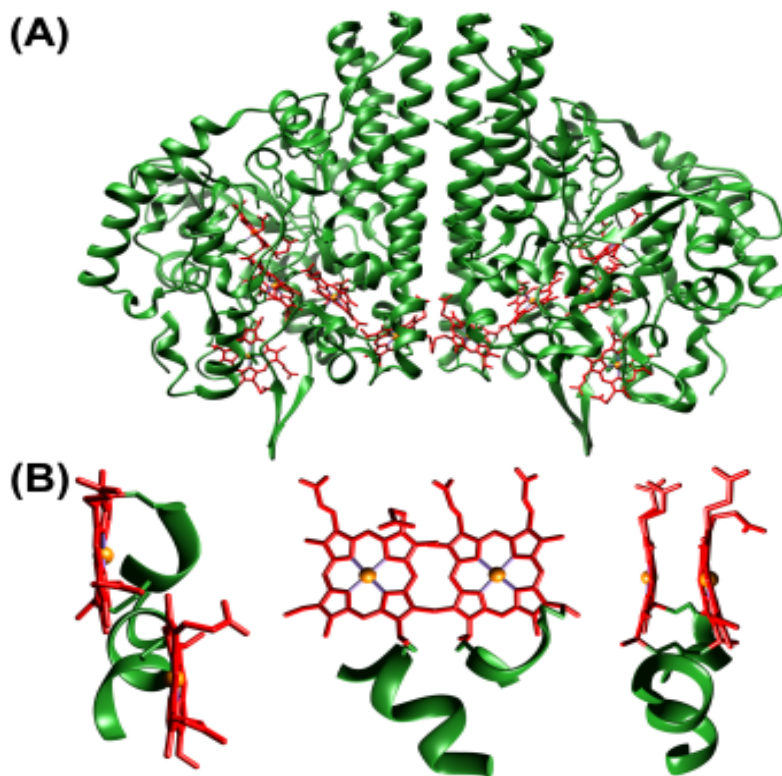
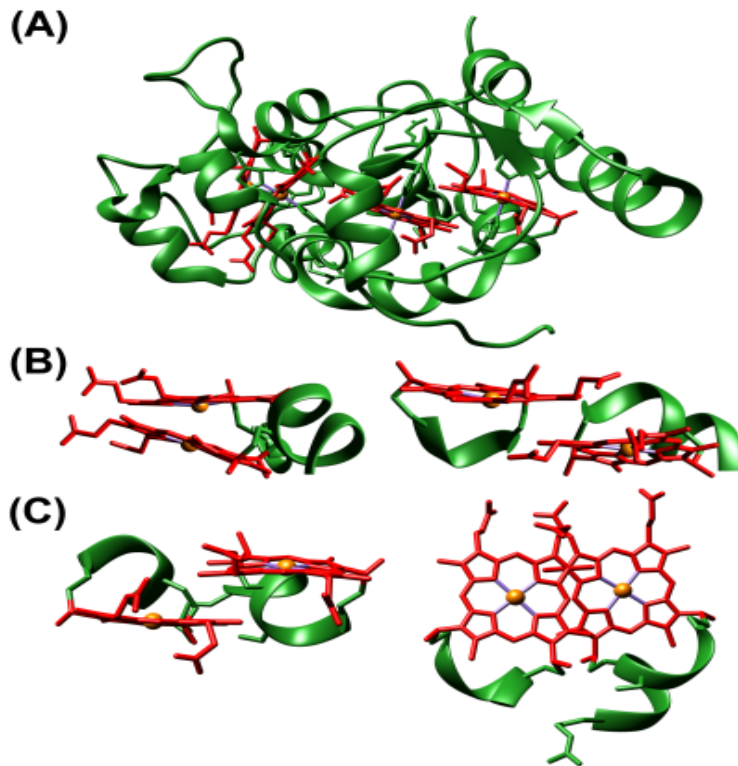


## Di-heme Enzymes: A Family with Superior Biological Activity

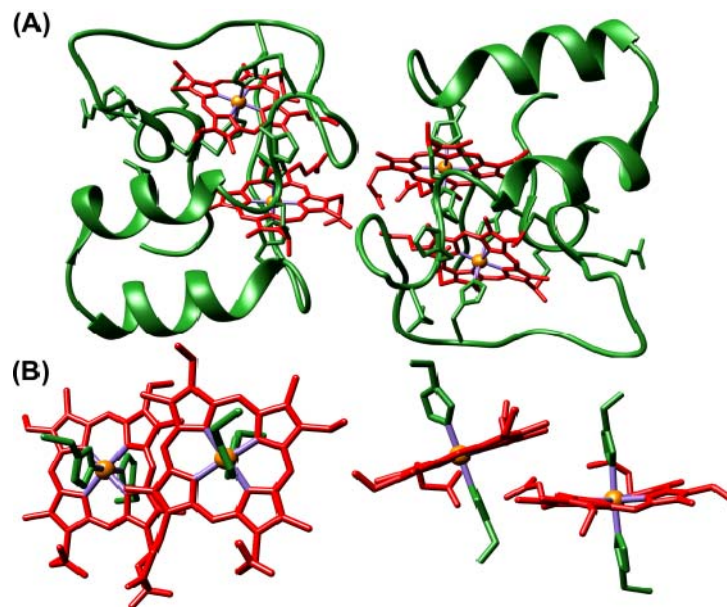
Multiheme cytochrome constitutes a widespread class of proteins with essential functions in electron transfer and enzymatic catalysis. Their functional properties are in part determined by the relative arrangement of multiple heme cofactors. Understanding the significance of these motifs is crucial for the elucidation of the highly optimized properties of multiheme cytochromes *c*, but their spectroscopic investigation is often restricted by the presence of a large number and efficient coupling of the individual centers. The diheme cytochrome *c* (DHC2) from *G. sulfurreducens* is, however, the simplest member of such family, with two heme groups attached through a single polypeptide chain that are found to be different. A large number of diheme enzymes such as *MauG* and bacterial diheme cytochrome *c* peroxidases (*bCcP*) are also known which catalyze various important chemical transformations in biology. These attractive features have prompted us to investigate on the biomimetic model of multi-heme cytochromes as a part of our ongoing research.



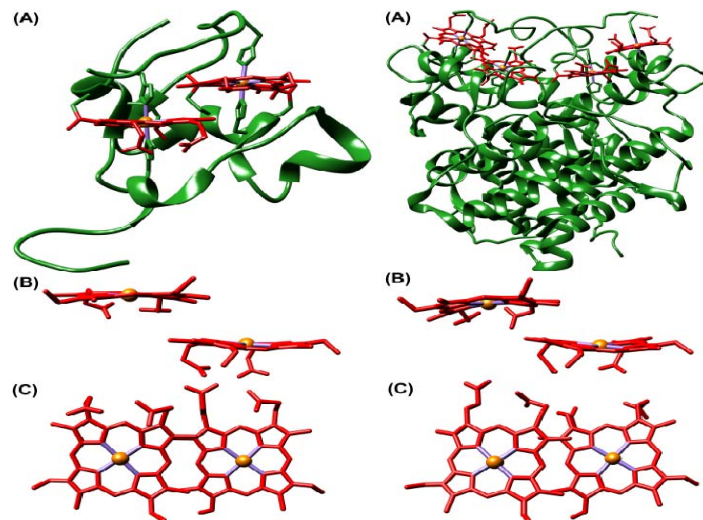
**Figure.** (A) Structure of cytochrome *c* nitrite reductase (PDB code 1QDB) in which the protein chain and the heme groups have been colored green and red, respectively. (B) Different views of the diheme motif formed by heme 3 and heme 4 in the nitrite reductase.



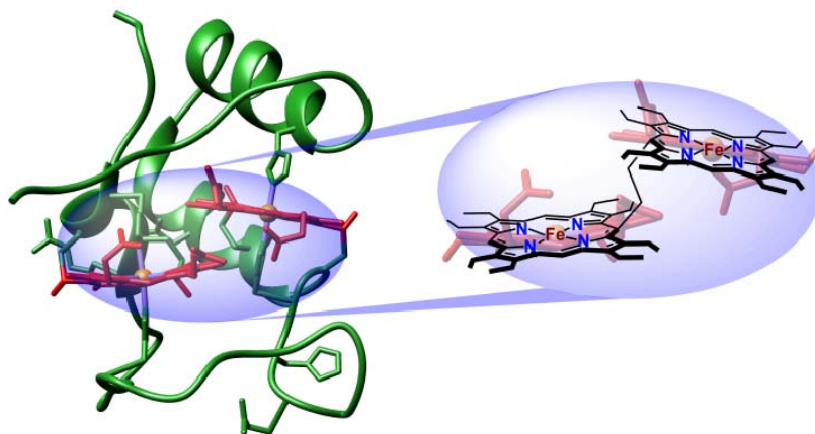
**Figure.** (A) Structure of the oxidized state of tetraheme cytochrome c554 from *Nitrosomonas europaea* (PDB code 1FT5). The protein chain and the heme groups have been colored green and red, respectively. Different views of the diheme motifs formed by (B) hemes I and III and (C) hemes II and IV.



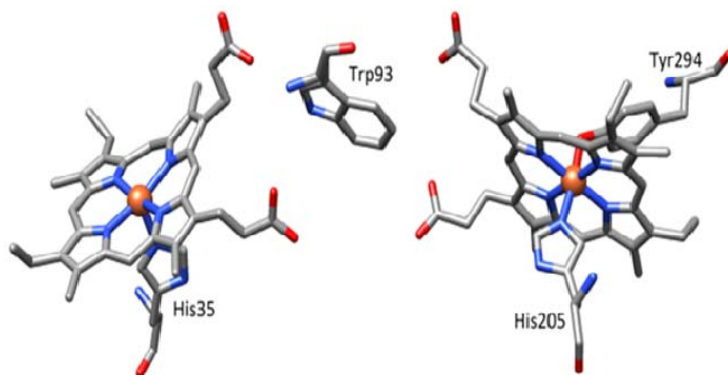
**Figure.** (A) Structure of the diheme cytochrome c, DHC2, from *Geobacter sulfurreducens* (PDB code 2CZS) containing 2 monomer per asymmetric unit. Each monomer has two heme groups covalently attached to the protein chain. The protein chain and the heme groups have been colored green and red, respectively. (B) Different views of structural arrangements of the heme groups.



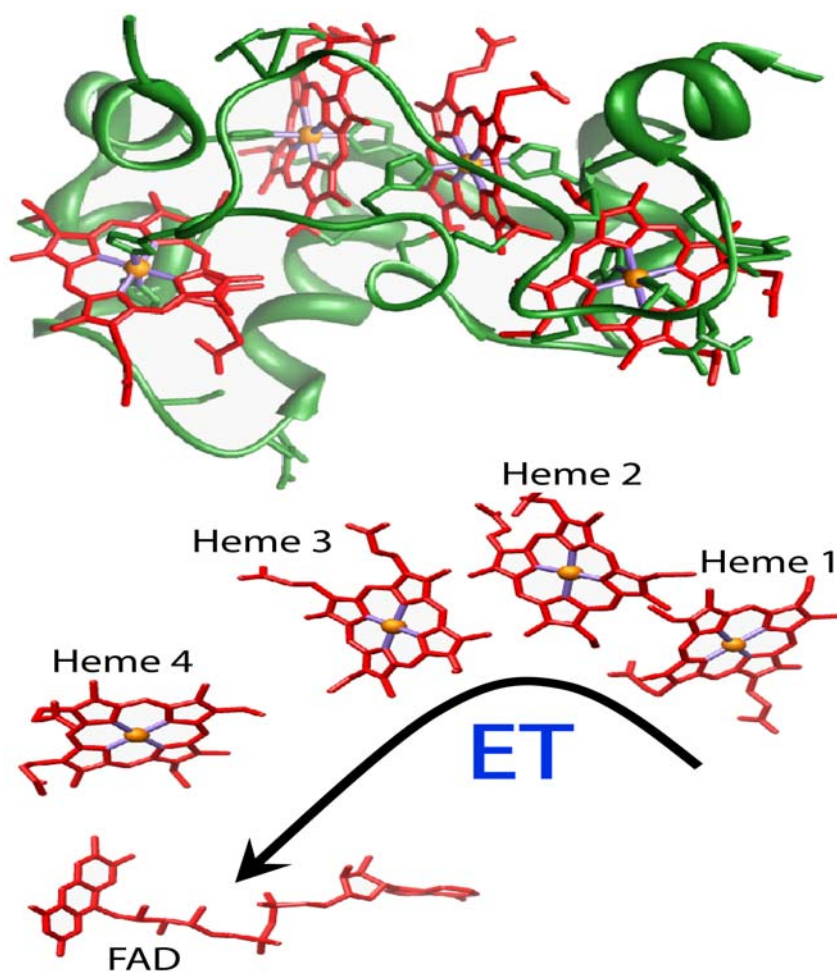
**Figure.** *Left*, (A) structure of the di-heme cytochrome *c*, NapB, from *Haemophilus influenzae* (PDB code 1JNI); (B) side and (C) top views of di-heme motifs therein. *Right*, (A) structure of the aerobic form of the split-Soret di-heme cytochrome *c* from *Desulfovibrio desulfuricans* ATCC 27774 (PDB code 1H21); (B) side and (C) top views of di-heme motifs therein. The protein chain and the heme groups have been colored green and red, respectively.



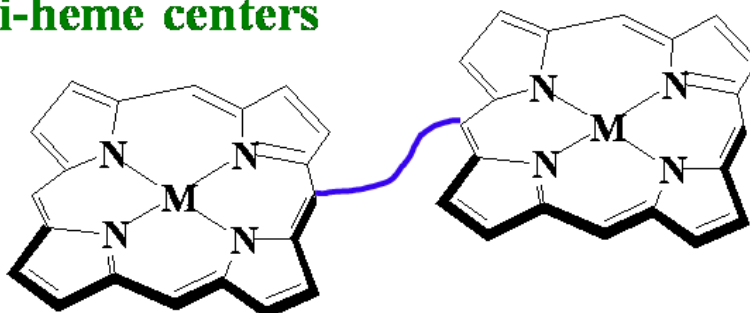
**Figure.** A schematic diagram comparing the di-heme cytochrome *c* (DHC2) with the model ethane-bridged di-heme architecture.



MauG is a terminal enzyme involved in the biosynthesis of the catalytic tryptophan tryptophenylquinone (TTQ) cofactor of methylamine dehydrogenase (MADH)



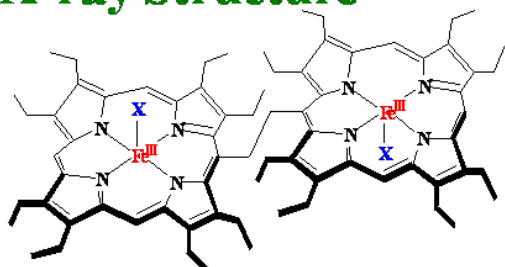
## Model di-heme centers



**M:** Fe, Mn, Co, Ni, Cu, Zn etc

- ❖ Judicious choice of the spacer will allow precise control in the spatial arrangement for inter-macrocycle interactions and possible electronic communications.
- ❖ Focus will be on how the nature and extent of heme-heme interactions influence the spectral and electrochemical properties of the individual heme centers.

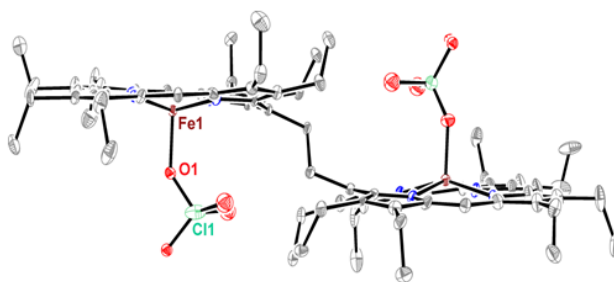
# X-ray Structure



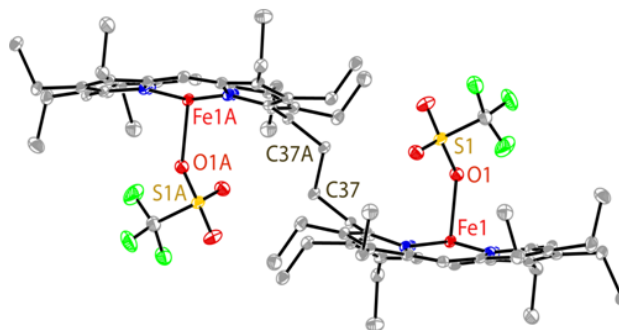
X = ClO<sub>4</sub>, SO<sub>3</sub>CF<sub>3</sub>

Selected geometrical parameters

	Fe <sub>2</sub> <sup>III</sup> (bisporphyrin)(X) <sub>2</sub>	
	X = ClO <sub>4</sub>	X = SO <sub>3</sub> CF <sub>3</sub>
Fe-O [Å]	1.906(4)	2.067(4)
Fe-N <sub>p</sub> [Å]	2.059(9)	1.978(3)
Δ <sup>Fe<sub>24</sub></sup> [Å]	0.50	0.30
Δ <sup>Fe<sub>4N</sub></sup> [Å]	0.44	0.25
Δ <sub>24</sub> [Å]	0.11	0.21
S	5/2	3/2



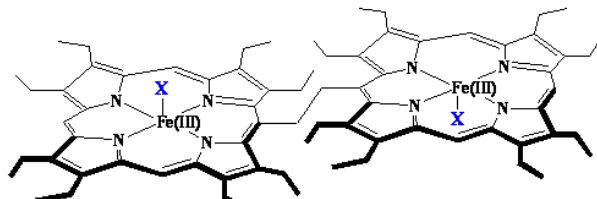
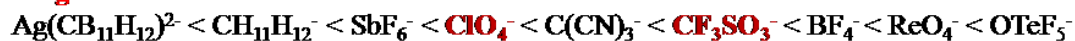
Pure high-spin (S=5/2)



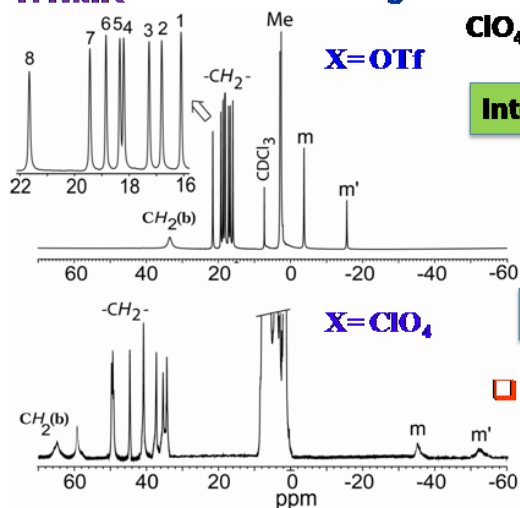
Pure Intermediate-spin (S=3/2)

## ❖ Reversal of Ligand-field Strength

Magnetochemical Series:



<sup>1</sup>H NMR



❖ Ligand-field strength observed:

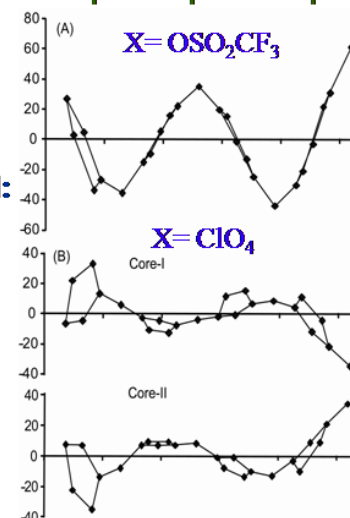
ClO<sub>4</sub><sup>-</sup> > SO<sub>3</sub>CF<sub>3</sub><sup>-</sup>

Intermediate spin

High spin

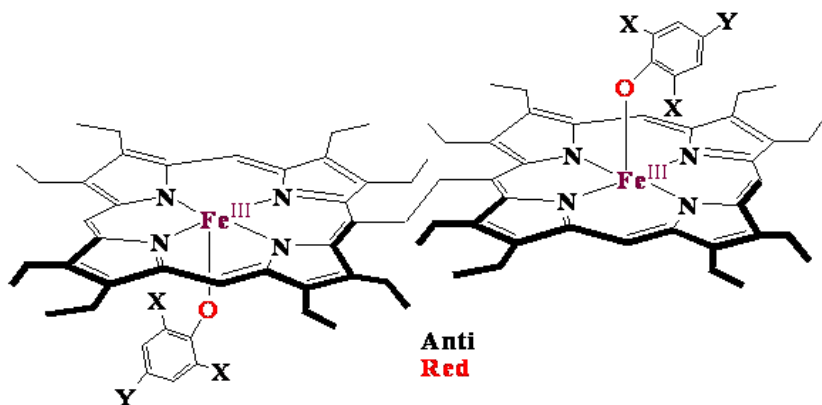
❑ Reversal of ligand-field strength in di-hemes

Out-of-plane displacement plots

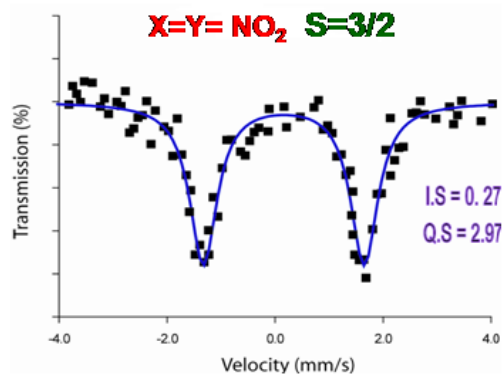


Chem. Commun. 2011, 47, 4790

# Axial Phenoxide Coordination: Effect of Heme-Heme Interactions

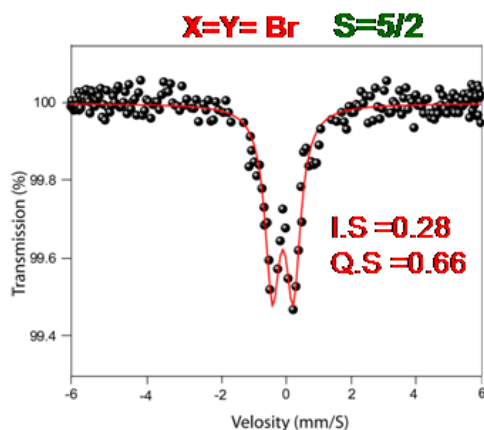


## Mössbauer Spectra at 298K

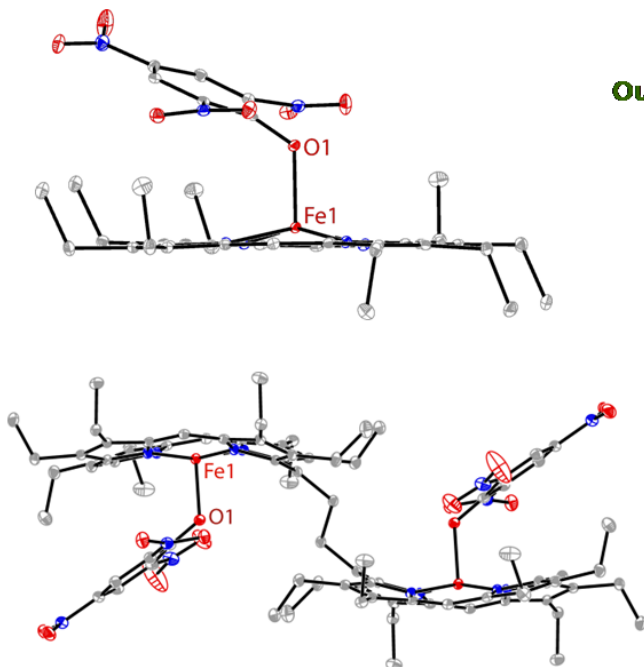


## X-ray Structural Parameters at 100 K

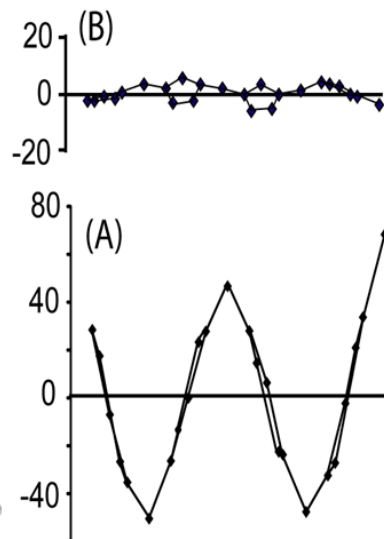
X, Y	H	Br	iPr		NO <sub>2</sub>
Fe-O[A]	1.916 (2)	1.864 (3)	1.894(4)	1.813(4)	2.000 (2)
Fe-N <sub>1</sub> [Å]	2.056 (3)	2.076 (3)	2.060(5)	2.069(5)	1.972 (3)
Fe-O-C [°]	134.4(2)	152.0(3)	165.7(4)	162.7(4)	127.2(2)
Δ24 <sup>Fe</sup> [Å]	0.51	0.42	0.55	0.56	0.27
Δ24 [Å]	0.12	0.08	0.20	0.13	0.26
S	5/2	5/2	5/2		3/2



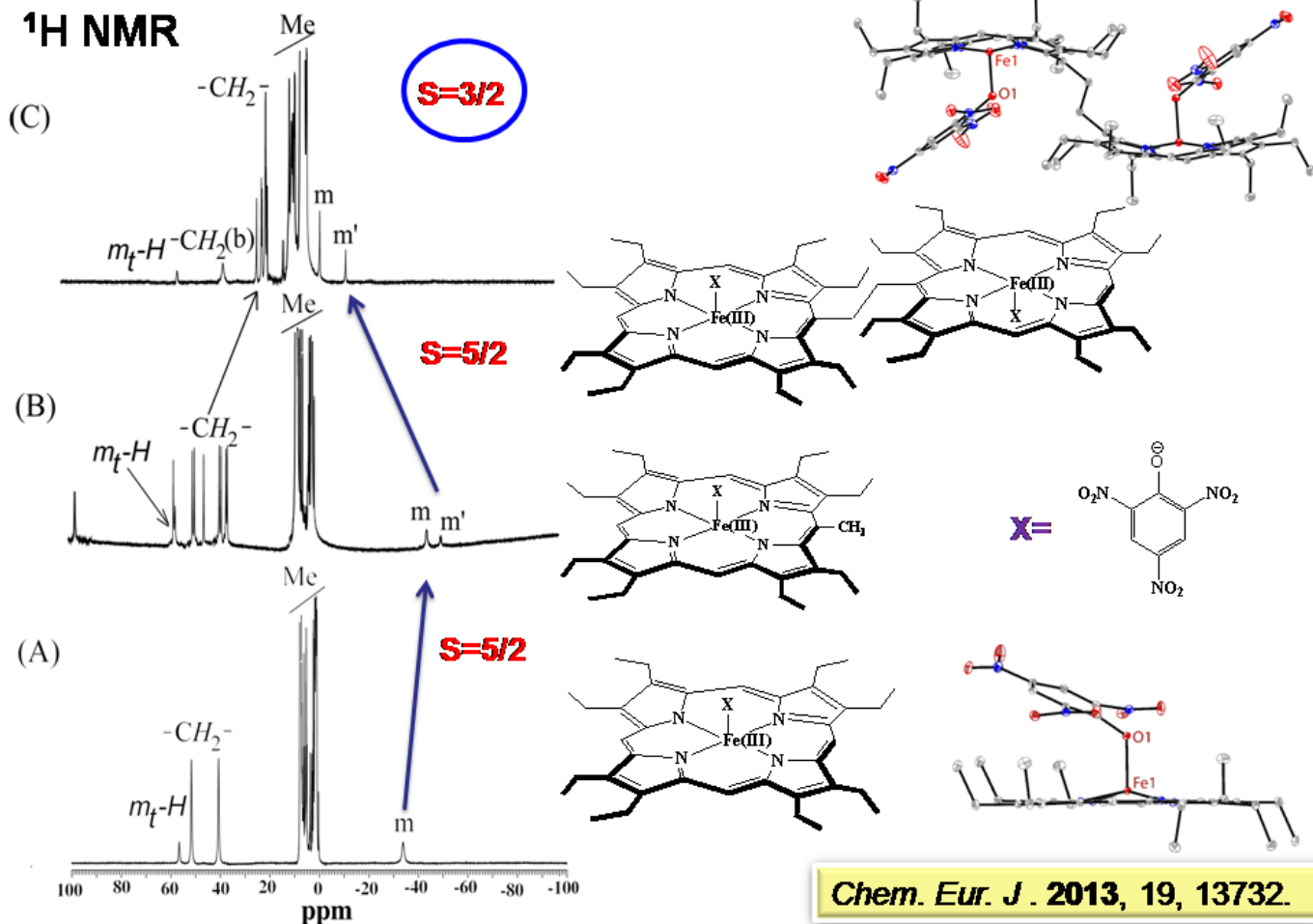
## ❖ Unusual stabilization of intermediate spin of Fe, for the first time



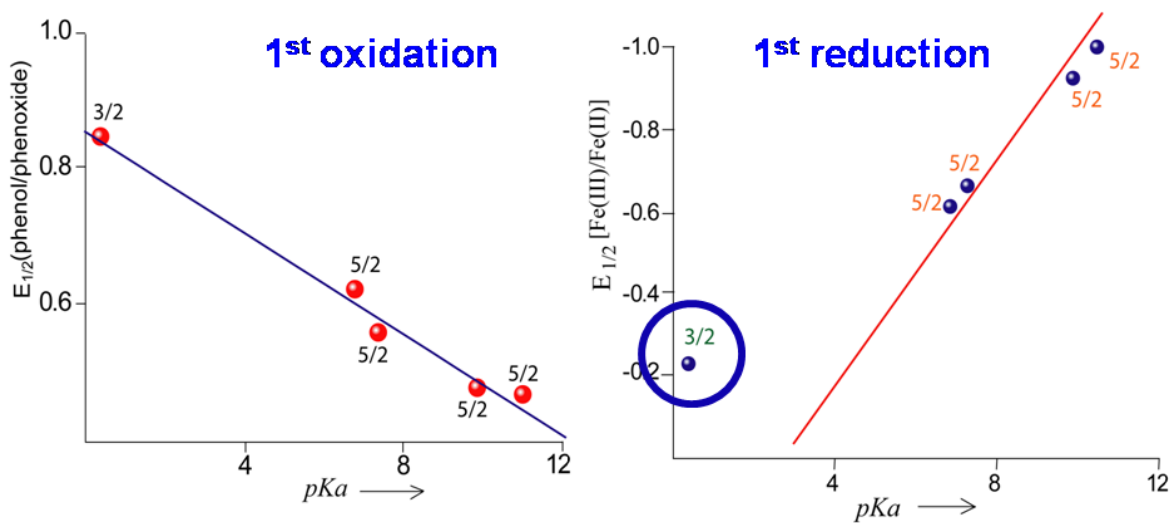
## Out-of-plane displacement plots



## Change of Fe Spin: Effect of Heme-Heme Interactions



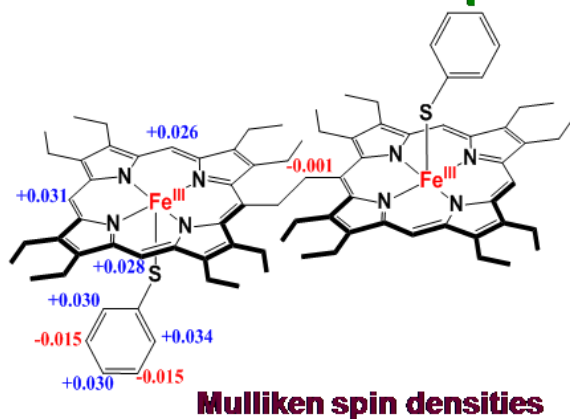
## Effect of Metal-Spins on the Redox Potential



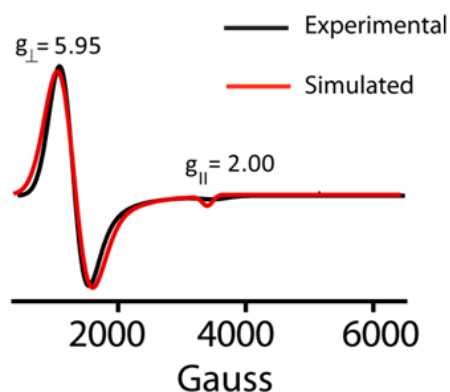
Deviation from linearity due to change in spin state

Chem. Eur. J. 2013, 19, 13732.

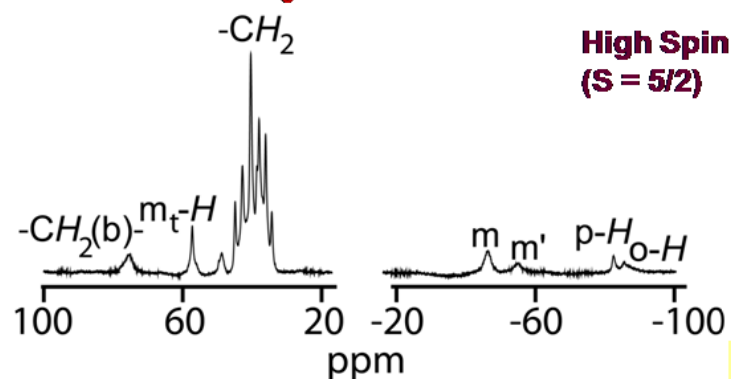
## Axial Thiophenolate Coordination



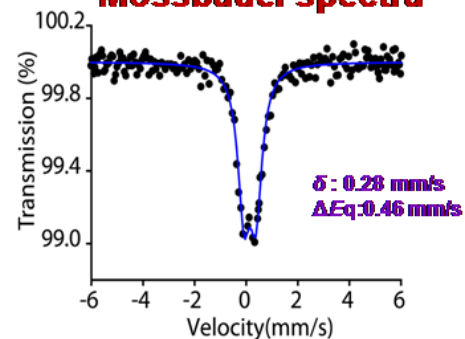
### EPR in DCM at 77 K



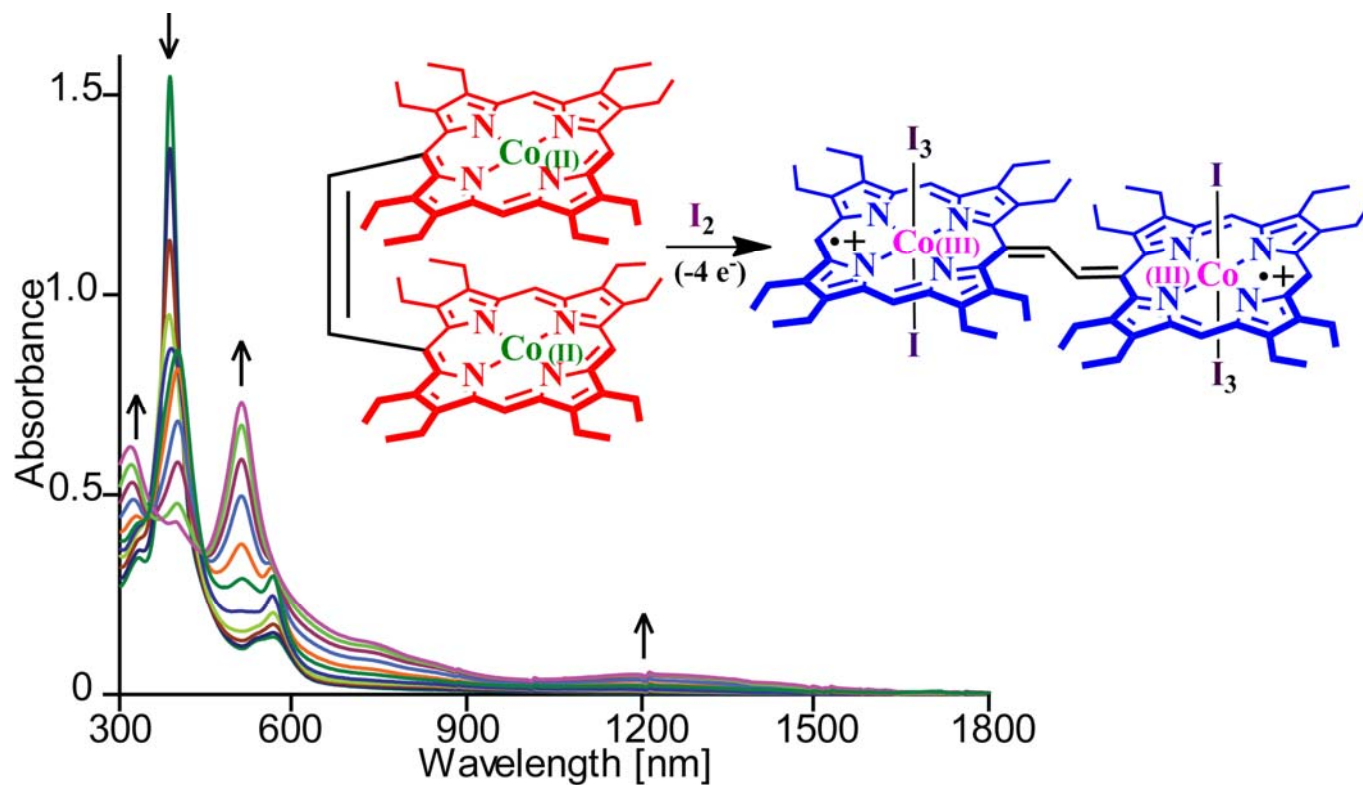
### <sup>1</sup>H NMR in CDCl<sub>3</sub> at 298K



### Mössbauer spectra

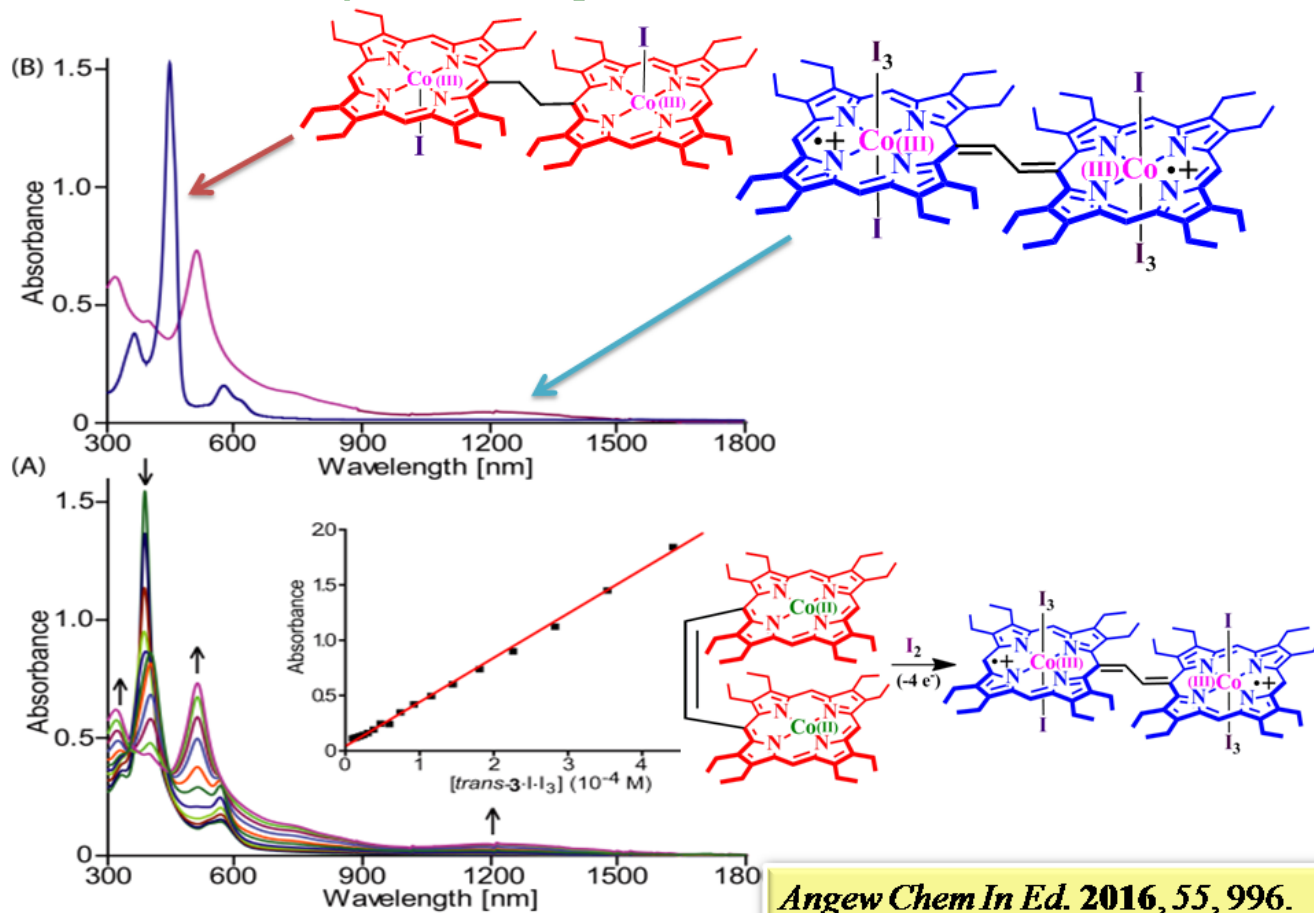


*Inorg. Chem.* **2014**, *52*, 11925

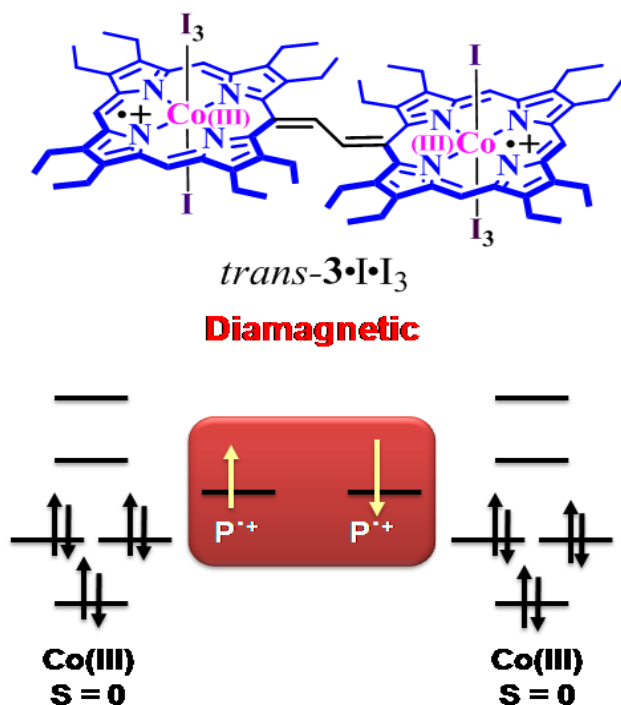




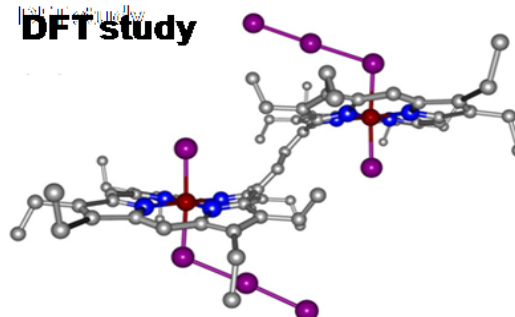
## UV-visible-NIR Spectral Change



## Electronic Distribution: Spin Coupling Model



### DFT study

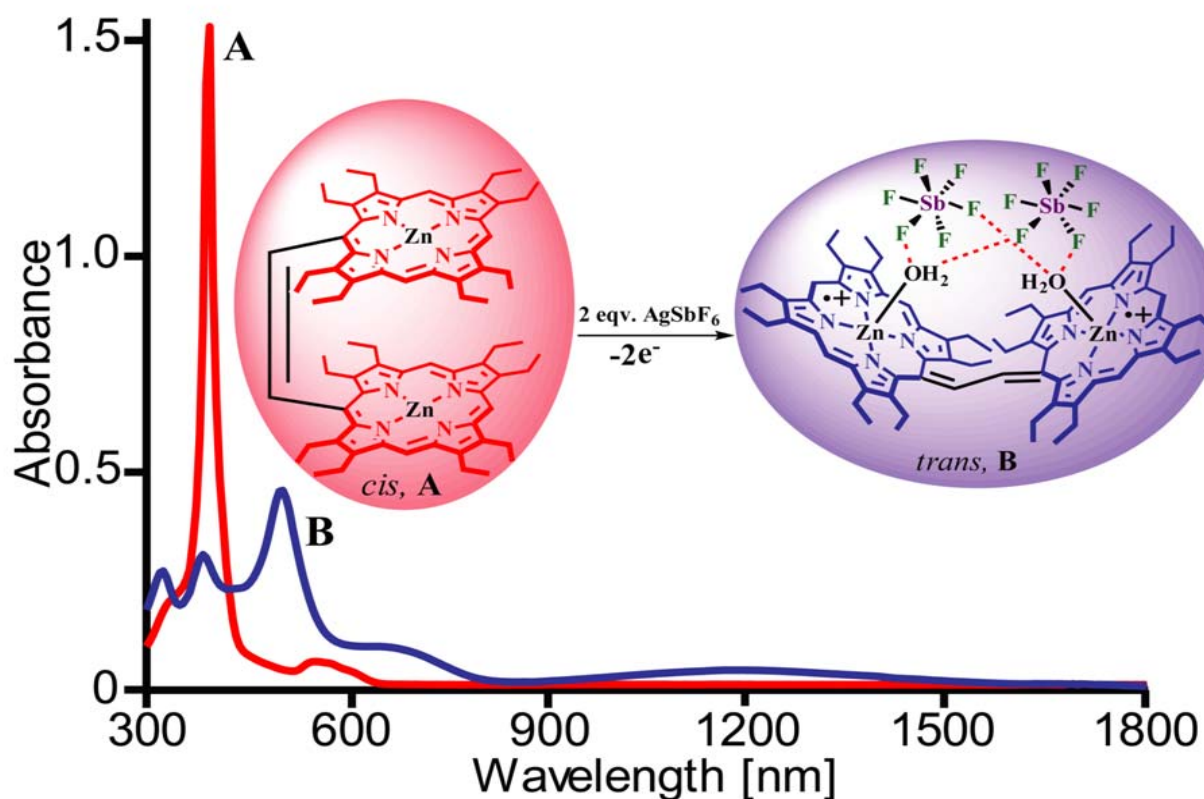
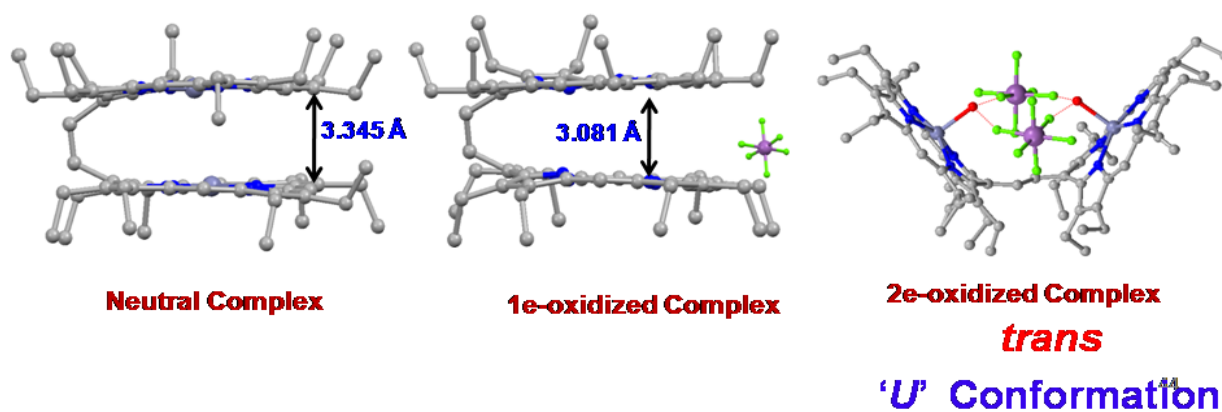
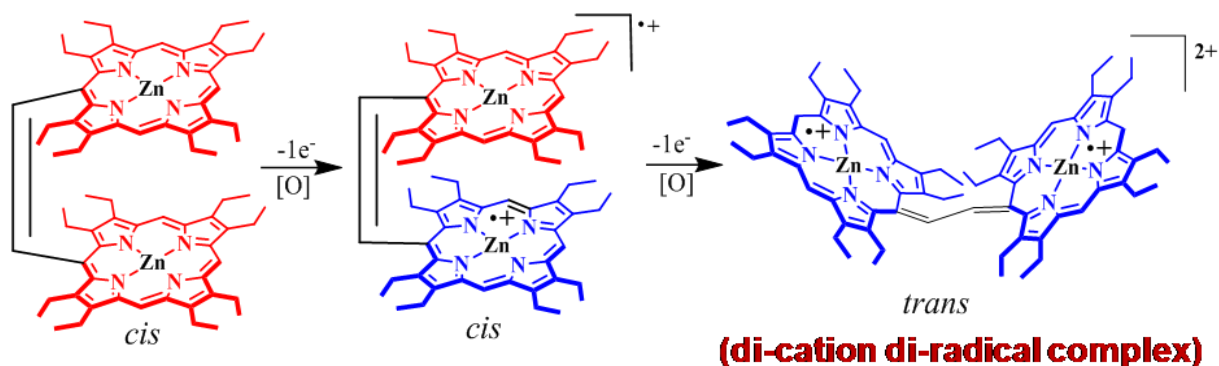


$Co-N_p = 1.974 [1.967]$   
 $C20-C37 = 1.374 [1.430]$   
 $C37-C37A = 1.430 [1.377]$   
 $\Delta_{24}^{Co} = 0.10 [0.10]$   
 $\Delta G_{TS} = 15.6$   
 $trans\text{-}13\text{-}I\text{-}I_3 [trans\text{-}3\text{-}I\text{-}I_3]$

Basis set combination: LANL2DZ/6-31G\*\*

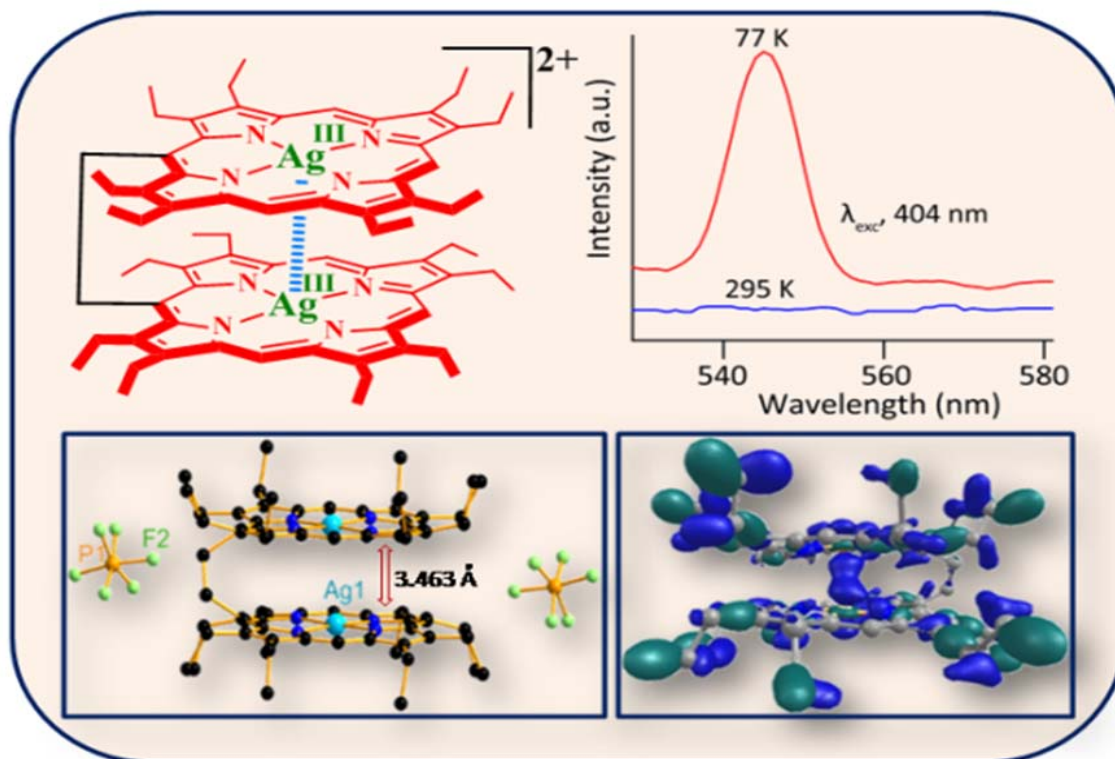
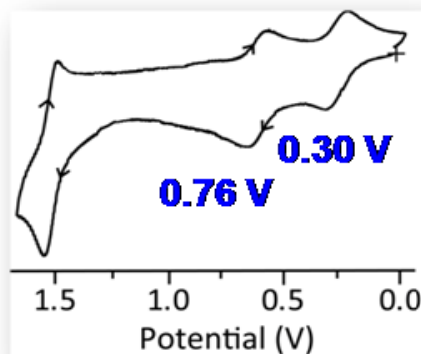
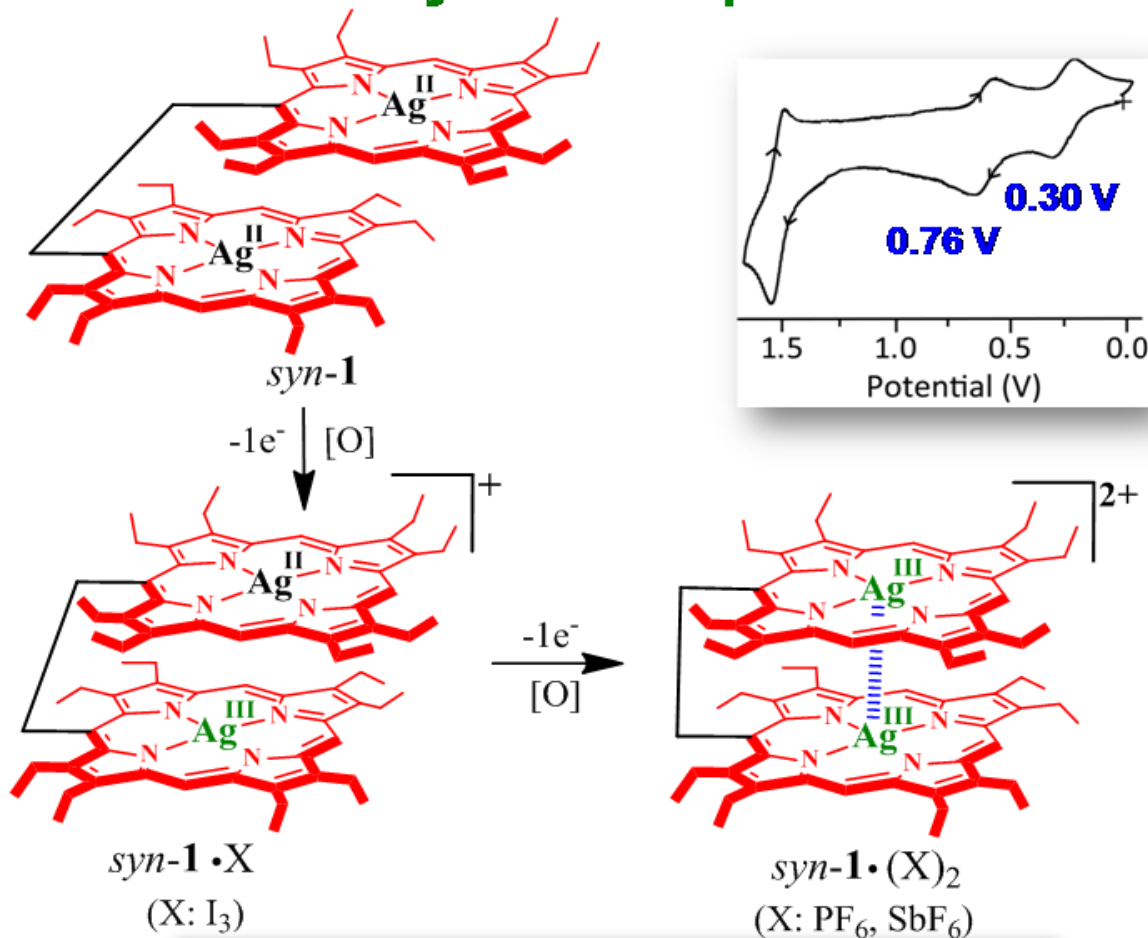
Strong intramolecular radical-radical coupling makes the overall molecule diamagnetic

## Changing Metal from Fe to Zn: Step-wise Oxidations



# Silver(III)---Silver(III) Stabilizes the *Syn*-form Upon Oxidation

## Interaction



## Representative Publications:

1. **Silver(III)•••Silver(III) Interaction Stabilizes the *Syn*-form in a Porphyrin Dimer Upon Oxidation**

A. K. Singh, F. S. T. Khan and S. P. Rath\*

*Angew. Chem. Int. Ed.* **2017**, *56*, 8849-8854.

2. **Highly Oxidized Cobalt Porphyrin Dimer: Spin Coupling and Stabilization of 4e-Oxidized Product**

S. Dey, D. Sil and S. P. Rath\*

*Angew. Chem. Int. Ed.* **2016**, *55*, 996-1000.

3. **Oxidation Triggers Extensive Conjugation and Unusual Stabilization of Two Di-heme Dication Diradical Intermediates: Role of Bridging Group for Electronic Communication**

D. Sil, S. Dey and S. P. Rath\*

*Chem. Sci.*, **2016**, *7*, 1212-1223. (*Edge Article*)

4. **Probing Bis-Fe<sup>IV</sup> MauG: Isolation of Highly Reactive Radical Intermediates**

T. Guchhait, S. Sarkar, Y. A. Pandit and S. P. Rath\*

*Chem. Eur. J. (Communication)* **2017**, *23*, 10270-10275.

5. **Intermacrocyclic Interaction Triggers Facile One-step Synthesis of a Chlorin-Porphyrin Heterodimer**

Y. A. Pandit and S. P. Rath\*

*Chem. Eur. J.* **2017**, *23*, ASAP. (DOI: 10.1002/chem.201701943)

6. **Oxo- and Hydroxo-bridged Diiron(III) Porphyrin Dimers: Inorganic and Bio-inorganic Perspectives and Effects of Intermacroscopic Interactions**

T. Guchhait, S. Sasmal, F. S. T. Khan, and S. P. Rath\*

*Coord. Chem. Rev.* **2017**, *337*, 112-144.

**7. Effect of Two Interacting Rings in Metalloporphyrin Dimers upon Stepwise Oxidations**

S. Dey, D. Sil, Y. A. Pandit and S. P. Rath\*

*Inorg. Chem.* **2016**, *55*, 3229-3238.

**8. Ethane-bridged Porphyrin Dimer as Model of Di-heme Proteins: Inorganic and Bioinorganic Perspectives and Consequences of Heme-Heme Interactions**

D. Sil and S. P. Rath\*

*Dalton Trans.* **2015**, *44*, 16195 - 16211. (*Invited Perspective article*)

**9. Axial Thiophenolate Coordination on Diiron(III)bisporphyrin: Influence of Heme-Heme Interactions on Structure, Function and Electrochemical Properties of the Individual Heme Center**

D. Sil, F. S. T. Khan and S. P. Rath\*

*Inorg. Chem.* **2014**, *53*, 11925 - 11936.

**10. Syn-Anti Conformational Switching in an Ethane-bridged Co(II)bisporphyrin Induced by External Stimuli: Effects of Inter-macrocyclic Interactions, Axial Ligation and Chemical and Electrochemical Oxidations**

S. Dey and S. P. Rath\*

*Dalton Trans.* **2014**, *43*, 2301 - 2314.

**11. Unusual Stabilization of an Intermediate Spin of Iron upon Axial Phenoxide Coordination on a Diiron(III)bisporphyrin: Effect of Heme-Heme Interactions**

S. Bhowmik, S. Dey, D. Sahoo and S. P. Rath\*

*Chem. Eur. J.* **2013**, *19*, 13732 - 13744.

**12. Control of Spins by Ring Deformation in a Diiron(III)bisporphyrin: Reversal of  $\text{ClO}_4^-$  and  $\text{CF}_3\text{SO}_3^-$  Ligand Field Strength on the Magnetochemical Series**

S. Bhowmik, S. K. Ghosh and S. P. Rath\*

*Chem. Commun.* **2011**, *47*, 4790 - 4792.

**13. Synthesis and Characterization of Anti-bisFe(III) Porphyrins, Syn-bisFe(III)- $\mu$ -oxo Porphyrin and Syn-bisFe(III)- $\mu$ -oxo Porphyrin Cation Radical**

S. K. Ghosh, R. Patra and S. P. Rath\*

*Inorg. Chem.* **2010**, *49*, 3449 - 3460.

**14. Remarkably Bent, Ethane-Linked, Diiron(III)  $\mu$ -Oxobisporphyrin: Synthesis, Structure, Conformational Switching, and Photocatalytic Oxidation**

S. K. Ghosh, R. Patra and S. P. Rath\*

*Inorg. Chem.* **2008**, *47*, 10196 - 10198.