Di-heme Proteins: Effect of Heme-Heme Interactions

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](image)

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

Figure. A schematic diagram comparing the diheme cytochrome c (DHC2) with the model ethane-bridged diheme 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

- 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

\[ X = \text{ClO}_4^-, \text{SO}_3\text{CF}_3^- \]

**Selected geometrical parameters**

<table>
<thead>
<tr>
<th></th>
<th>Fe\textsuperscript{III}(bisporphyrin)(X)\textsubscript{2}</th>
<th>Fe-\textsuperscript{I}O[A] ( \times ) 10(^{-2} )</th>
<th>Fe-N\textsubscript{h}[Å] ( \times ) 10(^{-1} )</th>
<th>( \Delta A_{24} ) [Å]</th>
<th>( \Delta A_{4n} ) [Å]</th>
<th>( A_{24} ) [Å]</th>
<th>( S )</th>
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<tbody>
<tr>
<td>Fe-\textsuperscript{I}O [Å]</td>
<td>1.906(4) \times 10(^{-2} )</td>
<td>2.067(4) \times 10(^{-1} )</td>
<td></td>
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<tr>
<td>Fe-N\textsubscript{h} [Å]</td>
<td>2.059(9) \times 10(^{-1} )</td>
<td>1.978(3) \times 10(^{-1} )</td>
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<tr>
<td>( \Delta A_{24} ) [Å]</td>
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<td>0.30</td>
<td></td>
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<tr>
<td>( \Delta A_{4n} ) [Å]</td>
<td>0.44</td>
<td>0.25</td>
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<tr>
<td>( A_{24} ) [Å]</td>
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<td>0.21</td>
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<tr>
<td>( S )</td>
<td>5/2</td>
<td>3/2</td>
<td></td>
<td></td>
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</tr>
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</table>

**Reversal of Ligand-field Strength**

**Magnetocochemical Series:**

\[ \text{Ag(CB}_{11}\text{H}_{12})^3^- < \text{CH}_{11}\text{H}_{12}^- < \text{SbF}_6^- < \text{ClO}_4^- < \text{C(CN)}_3^- < \text{CF}_3\text{SO}_3^- < \text{BF}_4^- < \text{ReO}_4^- < \text{OTrF}_5^- \]

**1H NMR**

**Ligand-field strength observed:**

\[ \text{ClO}_4^- > \text{SO}_3\text{CF}_3^- \]

\( X = \text{OTf} \)

Intermediate spin

\( X = \text{ClO}_4^- \)

High spin

\[ \text{Reversal of ligand-field strength in di-hemes} \]

*Chem. Commun. 2011, 47, 4790*
Axial Phenoxide Coordination: Effect of Heme-Heme Interactions

Mössbauer Spectra at 298K

X-ray Structural Parameters at 100 K

<table>
<thead>
<tr>
<th>X, Y</th>
<th>H</th>
<th>Br</th>
<th>Fe-Fe(A)</th>
<th>Fe-C-Fe</th>
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<tbody>
<tr>
<td>Fe-Cl(Å)</td>
<td>1.916(2)</td>
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<td>1.804(4)</td>
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<td>Cl-M(Å)</td>
<td>2.056(3)</td>
<td>2.076(3)</td>
<td>2.068(5)</td>
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<td>A24-Fe(Å)</td>
<td>1.34(3)</td>
<td>1.52(3)</td>
<td>1.65(4)</td>
<td>1.62(4)</td>
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</table>

X=Br, S=3/2

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

Out-of-plane displacement plots

(A)  
(B)
Change of Fe Spin: Effect of Heme-Heme Interactions

\[ ^1H \text{ NMR} \]

(A)

\[ m^t-H \quad -CH_2^- \quad m \quad m' \quad Me \]

(B)

\[ m^t-H \quad -CH_2^- \quad m \quad m' \quad Me \]

(C)

\[ S=3/2 \]

\[ S=5/2 \]


Effect of Metal-Spins on the Redox Potential

\[ 1^{st} \text{ oxidation} \]

\[ 1^{st} \text{ reduction} \]

Deviation from linearity due to change in spin state

Axial Thiophenolate Coordination

EPR in DCM at 77 K
- Experimental
- Simulated

Mulliken spin densities

$^1$H NMR in CDCl$_3$ at 298K

High Spin
(S = 5/2)

Mössbauer spectra

Inorg. Chem. 2014, 52, 11925
UV-visible-NIR Spectral Change

Electronic Distribution: Spin Coupling Model

Diamagnetic

Strong intramolecular radical-radical coupling makes the overall molecule diamagnetic
Changing Metal from Fe to Zn: Step-wise Oxidations

\[
\text{(di-cation di-radical complex)}
\]

Neutral Complex  \hspace{1cm} 1e-oxidized Complex  \hspace{1cm} 2e-oxidized Complex

\text{‘U’ Conformation}

\begin{align*}
\text{Absorbance} & \quad 1.5 \\
\text{Wavelength [nm]} & \quad 300 \quad 600 \quad 900 \quad 1200 \quad 1500 \quad 1800
\end{align*}

\text{Inorg. Chem. 2016, 55, 3229}
Silver(III)···Silver(III) Interaction Stabilizes the Syn-form Upon Oxidation

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, ASAP.*

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

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


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-FeIV MauG: Isolation of Highly Reactive Radical Intermediates**

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


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

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


6. **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)*
7. **Axial Thiophenolate Coordination on Diiron(III)bisporephyrin: 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*


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

   S. Dey and S. P. Rath*


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


10. **Control of Spins by Ring Deformation in a Diiron(III)bisporephyrin: Reversal of ClO_4^- and CF_3SO_3^- Ligand Field Strength on the Magnetochemical Series**

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


11. **Synthesis and Characterization of Anti-bisFe(III) Porphyrins, Syn-bisFe(III)-μ-oxo Porphyrin and Syn-bisFe(III)-μ-oxo Porphyrin Cation Radical**

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


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