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A Fully Delocalized Mixed-Valence Bis-μ(Thiolato) Dicopper Complex: A Structural and Functional Model of the Biological $\mathrm{Cu_A}$ Center[**]

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The biological $\mathrm{Cu_A}$ site, a fully delocalized mixed-valence bis(μ-thiolato) dicopper complex, is implicated in the respiratory chain of all eukaryotes and some prokaryotes. It is found in cytochrome c oxidase (COX)[1] and nitrous oxidase reductase ($\mathrm{N_2OR}$),[2] where it acts as a very efficient electron-transfer agent,[3] cycling between the $\mathrm{Cu^{1.5}Cu^{1.5}}$ and $\mathrm{Cu^{I}Cu^{I}}$ states.[4] In the $\mathrm{Cu_A}$ center, the two copper ions bridged by two thiolate sulfur atoms of two cysteine residues form an almost-planar $\{\mathrm{Cu_2S_2}\}$ diamond core with a short Cu⋅⋅⋅Cu distance of 2.4–2.6 Å.[5–8] Each copper is also bound to one histidine ($\mathrm{N_\delta}$) and a weakly coordinated axial ligand, which is either a methionine or a backbone carbonyl group (Figure 1 a). In its oxidized state, $\mathrm{Cu_A}$ is a completely delocalized mixed-valence class III $\mathrm{Cu^{1.5}Cu^{1.5}}$ system,[9,10] with a direct Cu–Cu bond, which was the first metal–metal bond observed in nature.[11] Apart a slight elongation of the ligand bonds at copper and the Cu⋅⋅⋅Cu distance, any change in the copper ligand arrangement has been observed in the reduced state.[7] The exceptional structure and properties of the $\mathrm{Cu_A}$ site have motivated considerable efforts to understand the structure–function relationship in this highly unusual bimetallic center. With peptide ligands, an important number of synthetic models have been investigated.[11,12] In contrast, synthesizing a model with conventional organic ligands is far from trivial and remains a real challenge. A major synthetic problem is that such metal–thiolate complexes are generally prone to metal-reduction processes with concomitant formation of disulfides or, in the presence of dioxygen, to oxygenation at sulfur. To the best of our knowledge, a single example of a fully

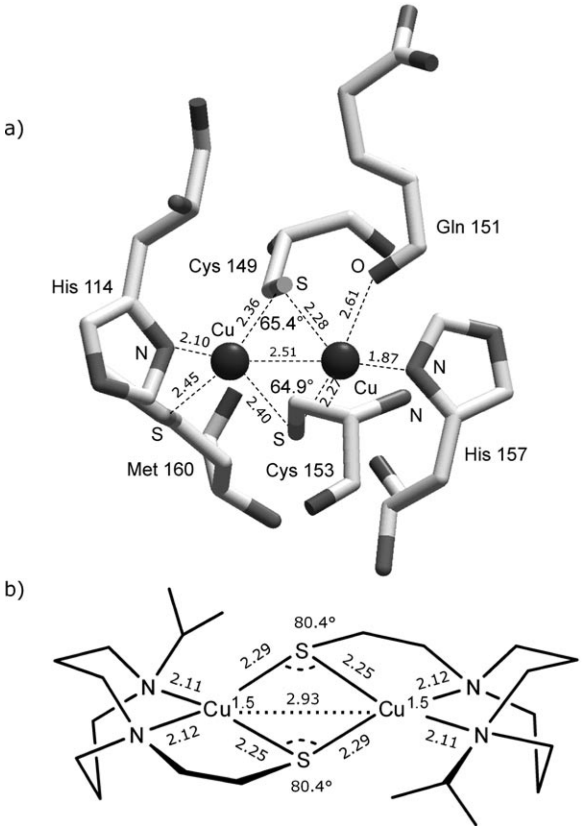

Figure 1. a) Structure of the $\mathrm{Cu_A}$ center of COX issued from *Thermus thermophilus* in the mixed-valence state;[5] b) the model complex $[\mathrm{Cu_2}(i\mathrm{PrdacoS})_2]^+$.[13]

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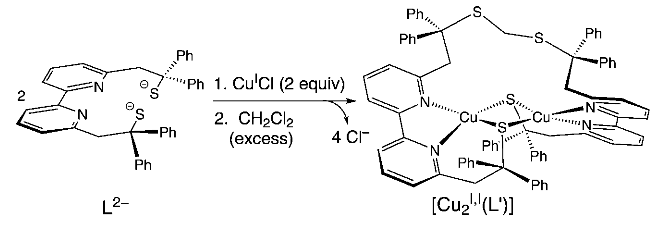
delocalized mixed valence bis(μ-thiolato) dicopper complex, $[Cu_2(i\text{PrdacoS})_2]^+$ (iPrdacoS = 1-isopropyl-5-ethylthiolato-1,5-diazacyclooctane), has been isolated to date by Tolman and co-workers (Figure 1b).[13] However, this complex cannot be reduced to the Cu^ICu^I redox state and it does not reproduce the complete set of structural and spectroscopic features of Cu_A. The other class III mixed-valence dicopper species are still rare and have been obtained by using either octaazacryptands[14,15] or different kinds of bridges between the two copper ions.[16–19] However, the lack of thiolate bridges leads to drastically different electronic properties than those of Cu_A. With regard to the reduced state, to date, the only described synthetic dinuclear Cu^I complex with a $\{Cu_2S_2\}$ core is $[Cu(SC_6H_4CH_3\text{-}o)(phen)]_2$ (phen = 1,10-phenanthroline).[20] Nevertheless, the aromatic thiolate ligands poorly mimic the cysteine-bridged ligands in Cu_A and its electrochemical properties have not been reported.

We report herein the synthesis and characterization of a new bis(μ-thiolato) dicopper complex that mimics most of the essential structural, spectroscopic, and functional properties of the Cu_A center. This complex has been obtained in the two formal oxidation states, $[Cu_2^{1.5,1.5}L']^+$ and $[Cu_2^{I,I}L']$, thanks to the dinucleating character of the ligand L′ (Scheme 1). The structures of both complexes were resolved by X-ray diffraction and investigated by EXAFS. The spectroscopic properties of the oxidized state were further studied by Cu K-edge XAS, CW X-band EPR optical and absorption spectroscopy, and DFT calculations. More importantly, an electrochemical investigation demonstrated the reversibility of the $Cu^{1.5}Cu^{1.5}/Cu^ICu^I$ redox couple, thus making this system a functional model of the Cu_A site.



Scheme 1. Synthesis of complex **1**.

The L^{2-} ligand (2,2′-(2,2′-bipyridine-6,6′-diyl)bis(1,1-diphenylethanethiolate)), in the presence of CuCl, reacts with CH_2Cl_2 under an inert atmosphere to form the green $[Cu_2^{I,I}L']$ (**1**) complex (Scheme 1). The in situ synthesis of L′ results from the formation of a methylene linker between two $[CuL]^-$ units by the reaction of one thiolate from each L ligand with CH_2Cl_2 (see the Supporting Information). Such reactivity has been reported with other thiolate ligands in nickel and ruthenium complexes,[21,22] but not with L in the cases of the mononuclear Fe^{III}[23] and Ni^{II}[24] complexes. The chemical oxidation of **1** by a mild oxidizing agent, such as 4-bromobenzenediazonium tetrafluoroborate (1 equiv), leads to the formation of a purple-colored one-electron oxidized form, which crystallizes in the presence of tetrakis(pentafluorophenyl)borate as $[Cu_2^{1.5,1.5}L'](B(C_6F_5)_4)$ (**2**).

The X-ray structures of both **1** and **2** are very similar (Figure 2) and contain a quasi planar $\{Cu_2S_2\}$ diamond core, which is slightly more bent in **1** than in **2** (153.1 and 173.0°). The presence of a unique counteranion per complex in **2** confirms that it is the one-electron oxidized form of **1**, and the similarity of the structural properties of both copper ions in **2** is in agreement with a delocalized mixed valence $Cu^{1.5}Cu^{1.5}$ form. The most remarkable difference between **1** and **2** is the Cu···Cu distance, which unexpectedly increases in the oxidized form, from 2.6378(14) to 2.9349(11) Å (as also verified by EXAFS; see below). A twist of the ligand around the methylene linker occurs in **2**, accompanied by an increase of the angle between the two CuN_2 planes (66 vs. 56° in **2** and **1**, respectively; Figure 2b). Similarly, the expansion of the Cu···Cu distance leads to an increase of the Cu-S-Cu angles in **2** (70 vs. 81°) and a decrease of the S-Cu-S angles (108 and 99°). In both structures, each copper ion is in the center of a distorted trigonal pyramid with the trigonal plane defined with the equatorial N2(4) atom (N_{eq}) and the two bridging

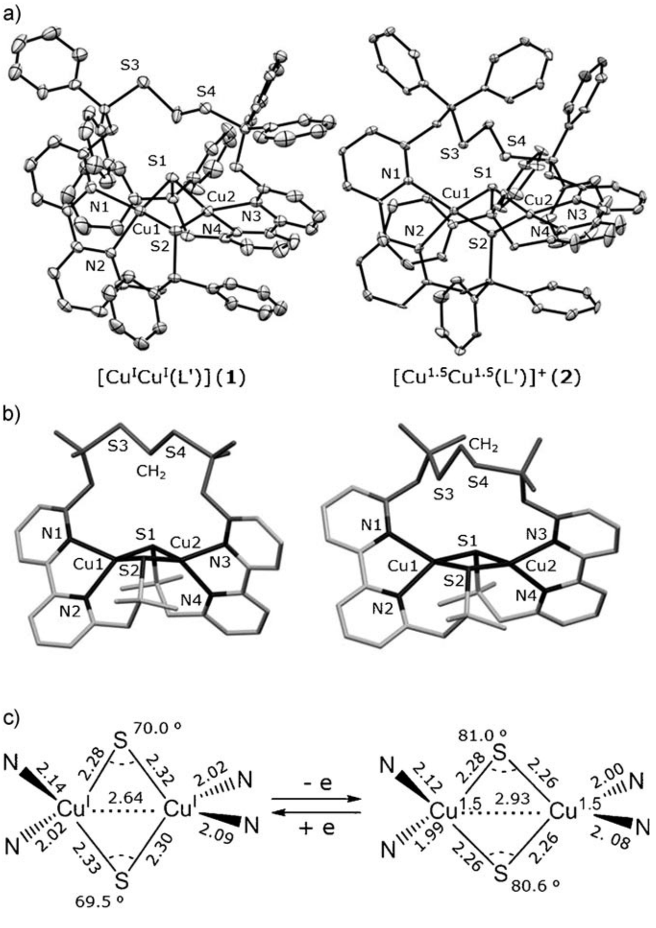


Figure 2. Different representations of complexes **1** and **2**: a) ORTEP views with ellipsoids set at 30% probability (hydrogen atoms and anion omitted for clarity). The same enantiomer of both complexes is depicted, and only one crystallographically independent complex of $[Cu_2L']^+$ is shown. b) Molecular views (hydrogen atoms, phenyl groups, and anion omitted for clarity) to enlighten the major conformational modifications between the two redox states involving the methylene linker (opposite orientations) and the $\{Cu_2S_2\}$ core (more flattened in the case of $[Cu_2L']^+$). c) Representation with principal distances and angles of the $\{Cu_2S_2\}$ cores.

aliphatic thiolate sulfur atoms of L′, from which Cu1(2) is only slightly displaced (less than 0.49 Å). The N1(3) atom (N_{ax}) acts as axial ligand. The $\{Cu_2S_2\}$ core of **2** presents Cu–S/N_{eq} bond lengths that are slightly shorter than in **1**, which is consistent with an increase of the oxidation level of the copper ion (4 and 2 pm, respectively). This is in excellent agreement with EXAFS data reported on the native Cu_A center, which indicate an average decrease of the Cu–S_{cys} and Cu–N_{his} distances between the mixed valence and the reduced states of 4 and 1 pm, respectively.[7]

The normalized Cu K-edge data for **1** and **2** are compared in Figure 3 a. Upon oxidation, the edge shifts up by about 1 eV in energy. A weak 1s to 3d pre-edge feature is present in **2**, which is absent in **1**. This is consistent with the presence of d-hole character in the mixed valent species, and confirmed by the X-band EPR spectrum of **2**, which exhibits an axial signal with discernible seven-line copper hyperfine lines in both the *g* components (Figure 3 b). The seven hyperfine lines are associated with the coupling of the unpaired electron ($S = \frac{1}{2}$) to the two equivalent copper ions ($I = 3/2$).[9] The observation that $g_{\parallel} > g_{\perp} > 2.0023$ together with a seven-line hyperfine pattern shows that the unpaired electron is delocalized over both copper centers and that the singly occupied molecular orbital (SOMO) contains contributions from the local Cu $d_{x^2-y^2}$ orbitals. The temperature-independent behavior of the EPR spectrum between 5 and 100 K confirms the fully delocalized class III nature of **2**, and the extremely small hyperfine splitting of 118 MHz on the $g_{\parallel}$ signal indicates a high degree of delocalization of the electron onto the ligands, as previously discussed for Cu_A ($117 A_{\parallel} < 123$ MHz).[9] As shown in Figure 3 b, the EPR properties of **2** closely resemble those obtained for the Cu_A center.

The absorption spectrum of **2** is shown in Figure 3 c together with those of the Cu_A mixed-valence state and of Tolman's model complex $[Cu_2(i\text{PrdacoS}_2)_2]^+$. In contrast to Cu_A, both synthetic models display the same intense transition in the near-infrared region ($\lambda_{max} = 1622$ and 1466 nm in **2** and $[Cu_2(i\text{PrdacoS}_2)_2]^+$, respectively).[13] This feature has been assigned to the mixed-valence $\psi \rightarrow \psi^*$ transition associated with spin delocalization between the two copper ions.[25] In fact, in both complexes, the distance between the two copper ions is too large for significant direct copper–copper orbital overlap to occur and leads to a drastic decrease of the $\psi \rightarrow \psi^*$ transition energy relative to Cu_A ($\lambda_{max} = 780$–810 nm).[11]

As expected, the SOMO of **2** is completely delocalized over the two copper ions and the bridging sulfur atoms of the $\{Cu_2S_2\}$ diamond core (Figure 4). The combined copper spin population (Mulliken analysis) amounts to 48 %, while the sulfur ligands carry as much as 39 % of the spin. These numbers, while not representing physical observables, are close to what has been interpreted for the native Cu_A site (48 % on Cu vs. 44 % on S).[26] Noticeable spin density is also found on the N atoms (5 % in **2** and Cu_A). The SOMO analysis shows that the π_u ground state of **2** is of a similar nature as in $[Cu_2(i\text{PrdacoS}_2)_2]^+$, which is consistent with a long Cu···Cu distance. By contrast, the shorter Cu···Cu distance in Cu_A leads to a ground state with the σ_u^* orbital that is singly occupied.[27]

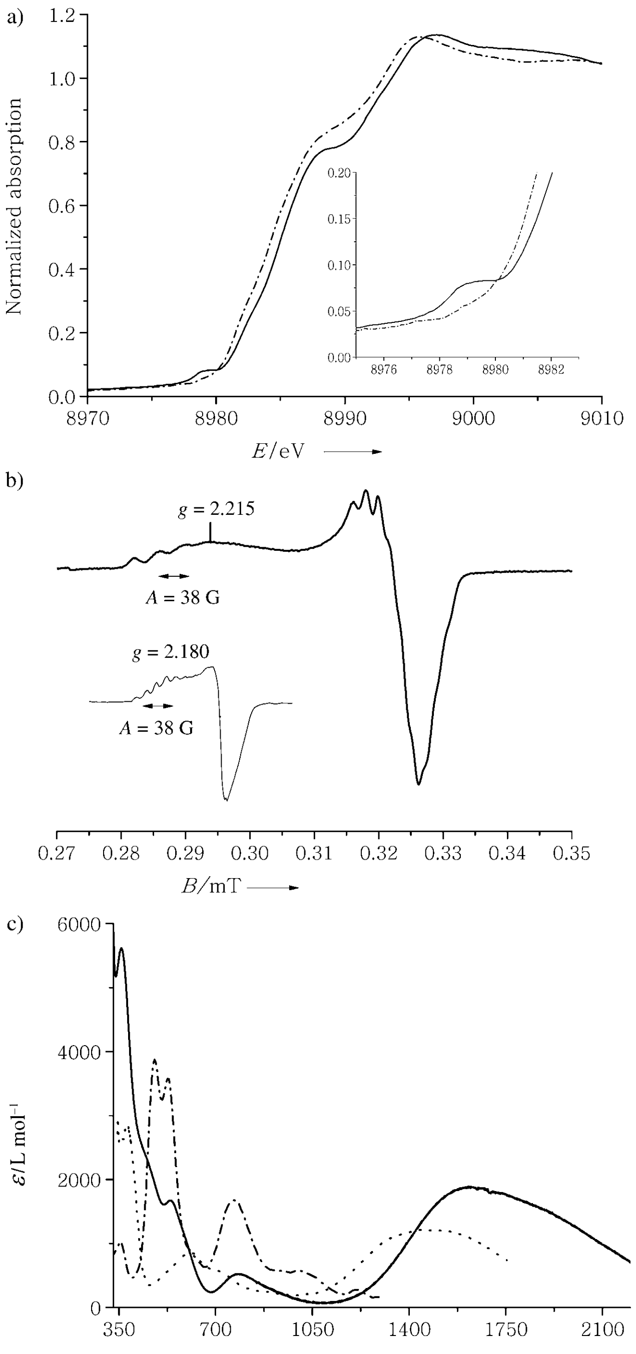


Figure 3. a) Comparison of the normalized Cu K-edge data for **1** (–•–•) and **2** (——); b) X-band EPR spectrum of **2** recorded in CH_2Cl_2 at 5 K. Parameters used for the simulation: $g_{\parallel} = 2.215$, $g_{\perp} = 2.002$, $A_{\parallel} = 118$ MHz, $A_{\perp} = 48$ MHz. Inset: EPR spectrum of Cu_A of N_2OR from *P. stutzeri*;[34] c) absorption spectra of **2** (——), $[Cu_2(i\text{PrdacoS}_2)_2]^+$ (•••••),[13] and Cu_A of COX from *B. subtilis* (–•–•).[35]

The electrochemical behavior of complexes **1** and **2** was investigated. The cyclic voltammogram (CV) of **1**, recorded in CH_2Cl_2 under argon, displays two successive one-electron oxidation processes (Figure 5 a): a reversible $Cu^{1.5}Cu^{1.5}$/Cu^ICu^I wave at $E_{1/2} = -0.79$ V vs. Fc^+/Fc ($\Delta E_p = 80$ mV) and a partially reversible wave at $E_{1/2} = +0.05$ V ($\Delta E_p = 100$ mV) that is assigned to the $Cu^{II}Cu^{II}$/$Cu^{1.5}Cu^{1.5}$ redox couple.

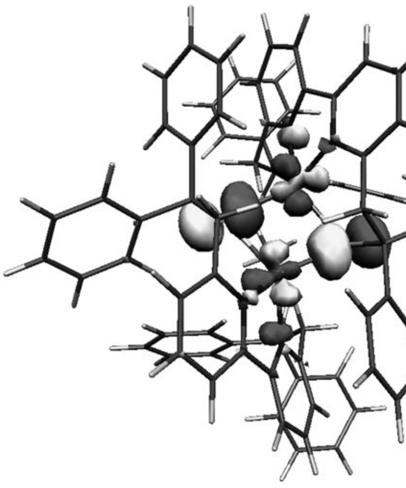


Figure 4. Contour plot of the redox-active, half-filled molecular orbital (SOMO) of complex **2**.

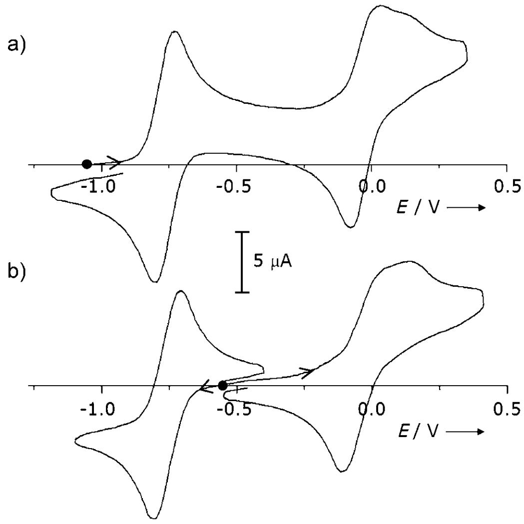


Figure 5. a) Cyclic voltammogram of **1** (0.9 mm) in CH_2Cl_2 with 0.1 m Bu_4NPF_6 at a carbon vitreous electrode and b) after electrolysis at −0.610 V (1 electron per molecule of **1** involved), scan rate of 100 mVs^{-1}. Potentials are given versus Fc^+/Fc.

Exhaustive electrolysis of **1** at −0.61 V results in the transfer of one electron and quantitatively yields **2** (Figure 5b). Compound **1** is fully regenerated by back electrolysis at −0.91 V, demonstrating the perfect reversibility of this one-electron process. The accessibility of the two $Cu^{1.5}Cu^{1.5}$ and Cu^ICu^I redox states most likely results from the preorganization of the system owing to the dinucleating character of L′ that allows the stabilization of the $\{Cu_2S_2\}$ diamond core. The potential of the $Cu^{1.5}Cu^{1.5}/Cu^ICu^I$ redox system of **1** is lower than that of the Cu_A site (+0.24 V vs. NHE,[28] or −0.44 V vs. Fc^+/Fc[29]), in accordance to the weaker axial bonding interactions in the biological site.[30] The other fully delocalized mixed valence dicopper complexes present higher oxidation potentials than **1** and Cu_A ($-0.390 < E_{1/2}$ vs. Fc^+/Fc, $CH_2Cl_2 < +0.025$ V),[14,16,18] except for one with a $\{Cu_2P_2\}$ core (−0.920 V vs. Fc^+/Fc, CH_2Cl_2).[19] The electron self-exchange rate constant was measured for the **2**/**1** redox couple by means of electrochemical experiments ($k_{el} = 7.7(1) \times 10^{-3}$ cms^{-1}, or estimated as $k_{hom} = 1.37(2) \times 10^6$ L$mol^{-1}s^{-1}$).[31] This k_{hom} value indicates that our model can achieve electron transfer rates faster than mononuclear Cu^{II} complexes, including plastocyanin ($k_{hom} = 1.5 \times 10^5$ L $mol^{-1}s^{-1}$),[32] but more slowly than Cu_A ($k_{hom} = 2.2 \times 10^8$ L $mol^{-1}s^{-1}$),[33] which is certainly due to the lack of the Cu−Cu bond in **2**.

A bulk electrolysis experiment at +0.64 V of a electrogenerated solution of **2** demonstrates that the $Cu^{II}Cu^{II}$ form is not stable. This is in parallel with the redox behavior observed for the Cu_A center for which the fully oxidized $Cu^{II}Cu^{II}$ state has not been reported to date.[28]

In summary, we have isolated and characterized the first dicopper system with a $\{Cu_2S_2\}$ core that is stable in the two oxidation states (I,I) and (1.5,1.5) and that can be related to the biological Cu_A site. However, in contrast to Cu_A, the short Cu···Cu distance of the reduced complex **1** notably increases in the oxidized mixed valence form **2**, despite the geometric constraints imposed by L′. Investigations are underway in our laboratories to understand this unexpected elongation of the Cu···Cu distance upon the oxidation process.

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[1] B. G. Malmstroem, *Chem. Rev.* **1990**, *90*, 1247–1260.
[2] Suharti, M. J. F. Strampraad, I. Schröder, S. de Vries, *Biochemistry* **2001**, *40*, 2632–2639.
[3] G. T. Babcock, M. Wikstrom, *Nature* **1992**, *356*, 301–309.
[4] D. W. Randall, D. R. Gamelin, L. B. LaCroix, E. I. Solomon, *J. Biol. Inorg. Chem.* **2000**, *5*, 16–29.
[5] P. A. Williams, N. J. Blackburn, D. Sanders, H. Bellamy, E. A. Stura, J. A. Fee, D. E. McRee, *Nat. Struct. Biol.* **1999**, *6*, 509–516.
[6] K. Brown, M. Tegoni, M. Prudencio, A. S. Pereira, S. Besson, J. J. Moura, I. Moura, C. Cambillau, *Nat. Struct. Biol.* **2000**, *7*, 191–195.
[7] N. J. Blackburn, S. de Vries, M. E. Barr, R. P. Houser, W. B. Tolman, D. Sanders, J. A. Fee, *J. Am. Chem. Soc.* **1997**, *119*, 6135–6143.
[8] N. J. Blackburn, M. Ralle, E. Gomez, M. G. Hill, A. Pastuszyn, D. Sanders, J. A. Fee, *Biochemistry* **1999**, *38*, 7075–7084.
[9] F. Neese, W. G. Zumft, W. E. Antholine, P. M. H. Kroneck, *J. Am. Chem. Soc.* **1996**, *118*, 8692–8699.
[10] J. A. Farrar, F. Neese, P. Lappalainen, P. M. H. Kroneck, M. Saraste, W. G. Zumft, A. J. Thomson, *J. Am. Chem. Soc.* **1996**, *118*, 11501–11514.
[11] M. G. Savelieff, Y. Lu, *J. Biol. Inorg. Chem.* **2010**, *15*, 461–483.
[12] Y. Lu, *Angew. Chem.* **2006**, *118*, 5714–5728; *Angew. Chem. Int. Ed.* **2006**, *45*, 5588–5601.
[13] R. P. Houser, V. G. Young, W. B. Tolman, *J. Am. Chem. Soc.* **1996**, *118*, 2101–2102.
[14] C. Harding, J. Nelson, M. C. R. Symons, J. Wyatt, *J. Chem. Soc. Chem. Commun.* **1994**, 2499–2500.
[15] M. E. Barr, P. H. Smith, W. E. Antholine, B. Spencer, *J. Chem. Soc. Chem. Commun.* **1993**, 1649–1652.
[16] C. He, S. J. Lippard, *Inorg. Chem.* **2000**, *39*, 5225–5231.

[17] R. Gupta, Z. H. Zhang, D. Powell, M. P. Hendrich, A. S. Borovik, *Inorg. Chem.* **2002**, *41*, 5100 – 5106.

[18] S. B. Harkins, J. C. Peters, *J. Am. Chem. Soc.* **2004**, *126*, 2885 – 2893.

[19] N. P. Mankad, E. Rivard, S. B. Harkins, J. C. Peters, *J. Am. Chem. Soc.* **2005**, *127*, 16032 – 16033.

[20] R. K. Chadha, R. Kumar, D. G. Tuck, *Can. J. Chem.* **1987**, *65*, 1336 – 1342.

[21] Q. Wang, A. C. Marr, A. J. Blake, C. Wilson, M. Schroder, *Chem. Commun.* **2003**, 2776 – 2777.

[22] C. A. Grapperhaus, S. Poturovic, M. S. Mashuta, *Inorg. Chem.* **2002**, *41*, 4309 – 4311.

[23] M. A. Kopf, D. Varech, J. P. Tuchagues, D. Mansuy, I. Artaud, *J. Chem. Soc. Dalton Trans.* **1998**, 991 – 998.

[24] M. Gennari, M. Orio, J. Pécaut, F. Neese, M.-N. Collomb, C. Duboc, *Inorg. Chem.* **2010**, *49*, 6399 – 6401.

[25] K. R. Williams, D. R. Gamelin, L. B. LaCroix, R. P. Houser, W. B. Tolman, T. C. Mulder, S. deVries, B. Hedman, K. O. Hodgson, E. I. Solomon, *J. Am. Chem. Soc.* **1997**, *119*, 613 – 614.

[26] S. DeBeer George, M. Metz, R. K. Szilagyi, H. X. Wang, S. P. Cramer, Y. Lu, W. B. Tolman, B. Hedman, K. O. Hodgson, E. I. Solomon, *J. Am. Chem. Soc.* **2001**, *123*, 5757 – 5767.

[27] S. I. Gorelsky, X. J. Xie, Y. Chen, J. A. Fee, E. I. Solomon, *J. Am. Chem. Soc.* **2006**, *128*, 16452 – 16453.

[28] C. Immoos, M. G. Hill, D. Sanders, J. A. Fee, C. E. Slutter, J. H. Richards, H. B. Gray, *J. Biol. Inorg. Chem.* **1996**, *1*, 529 – 531.

[29] N. G. Connelly, W. E. Geiger, *Chem. Rev.* **1996**, *96*, 877 – 910.

[30] G. N. Ledesma, D. H. Murgida, H. K. Ly, H. Wackerbarth, J. Ulstrup, A. J. Costa-Filho, A. J. Vila, *J. Am. Chem. Soc.* **2007**, *129*, 11884 – 11885.

[31] M. J. Weaver, *J. Phys. Chem.* **1990**, *94*, 8608 – 8613.

[32] M. R. b. Jensen, D. F. Hansen, J. J. Led, *J. Am. Chem. Soc.* **2002**, *124*, 4093 – 4096.

[33] C. E. Slutter, R. Langen, D. Sanders, S. M. Lawrence, P. Wittung, A. J. Di Bilio, M. G. Hill, J. A. Fee, J. H. Richards, J. R. Winkler, B. G. Malmström, *Inorg. Chim. Acta* **1996**, *243*, 141 – 145.

[34] H. S. McHaourab, S. Pfenninger, W. E. Antholine, C. C. Felix, J. S. Hyde, P. M. H. Kroneck, *Biophys. J.* **1993**, *64*, 1576 – 1579.

[35] D. R. Gamelin, D. W. Randall, M. T. Hay, R. P. Houser, T. C. Mulder, G. W. Canters, S. de Vries, W. B. Tolman, Y. Lu, E. I. Solomon, *J. Am. Chem. Soc.* **1998**, *120*, 5246 – 5263.