

A Fully Delocalized Mixed-Valence Bis- μ (Thiolato) Dicopper Complex: A Structural and Functional Model of the Biological Cu_A Center**

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The biological 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 (N₂OR),^[2] where it acts as a very efficient electron-transfer agent,^[3] cycling between the Cu^{1.5}Cu^{1.5} and Cu^ICu^I states.^[4] In the Cu_A center, the two copper ions bridged by two thiolate sulfur atoms of two cysteine residues form an almost-planar {Cu₂S₂} diamond core with a short Cu...Cu distance of 2.4–2.6 Å.^[5–8] Each copper is also bound to one histidine (N_δ) and a weakly coordinated axial ligand, which is either a methionine or a backbone carbonyl group (Figure 1 a). In its oxidized state, Cu_A is a completely delocalized mixed-valence class III Cu^{1.5}Cu^{1.5} system,^[9,10] with a direct Cu–Cu bond,

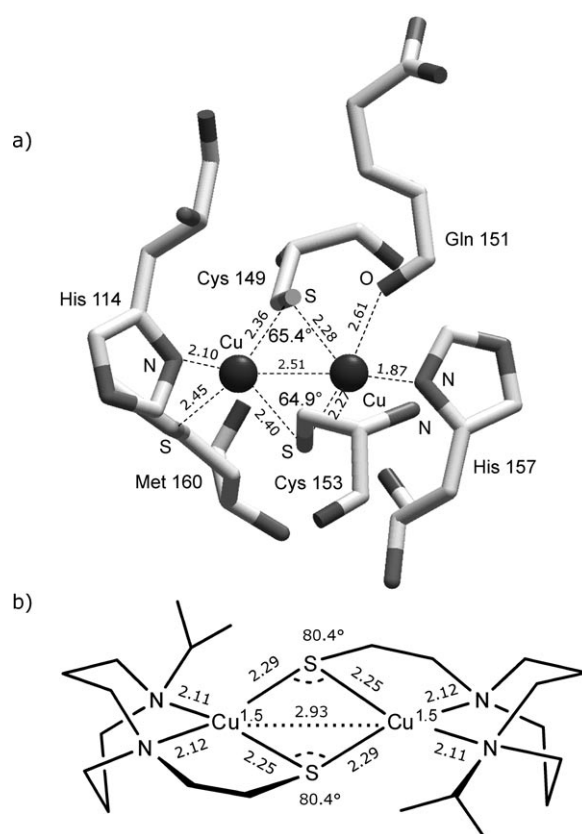


Figure 1. a) Structure of the Cu_A center of COX issued from *Thermus thermophilus* in the mixed-valence state;^[5] b) the model complex [Cu₂(iPrDacoS)₂]⁺.^[13]

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 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

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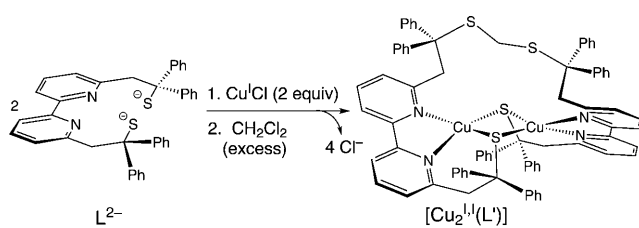
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delocalized mixed valence bis(μ -thiolato) dicopper complex, $[\text{Cu}_2(\text{iPrdacoS})_2]^+$ (iPrdacoS = 1-isopropyl-5-ethylthiolato-1,5-diazacyclooctane), has been isolated to date by Tolman and co-workers (Figure 1 b).^[13] However, this complex cannot be reduced to the $\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}$ 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 $\{\text{Cu}_2\text{S}_2\}$ core is $[\text{Cu}(\text{SC}_6\text{H}_4\text{CH}_3\text{-}o)(\text{phen})]_2$ (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, $[\text{Cu}_2^{1.5,1.5}\text{L}']^+$ and $[\text{Cu}_2^{1,1}\text{L}']$, thanks to the dinucleating character of the ligand L' (Scheme 1). The



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

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 $\text{Cu}^{1.5}\text{Cu}^{1.5}/\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}$ redox couple, thus making this system a functional model of the Cu_A site.

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 $[\text{Cu}_2^{1,1}\text{L}']$ (**1**) complex (Scheme 1). The in situ synthesis of L' results from the formation of a methylene linker between two $[\text{CuL}]^-$ 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 $[\text{Cu}_2^{1.5,1.5}\text{L}'](\text{B}(\text{C}_6\text{F}_5)_4)$ (**2**).

The X-ray structures of both **1** and **2** are very similar (Figure 2) and contain a quasi planar $\{\text{Cu}_2\text{S}_2\}$ diamond core,

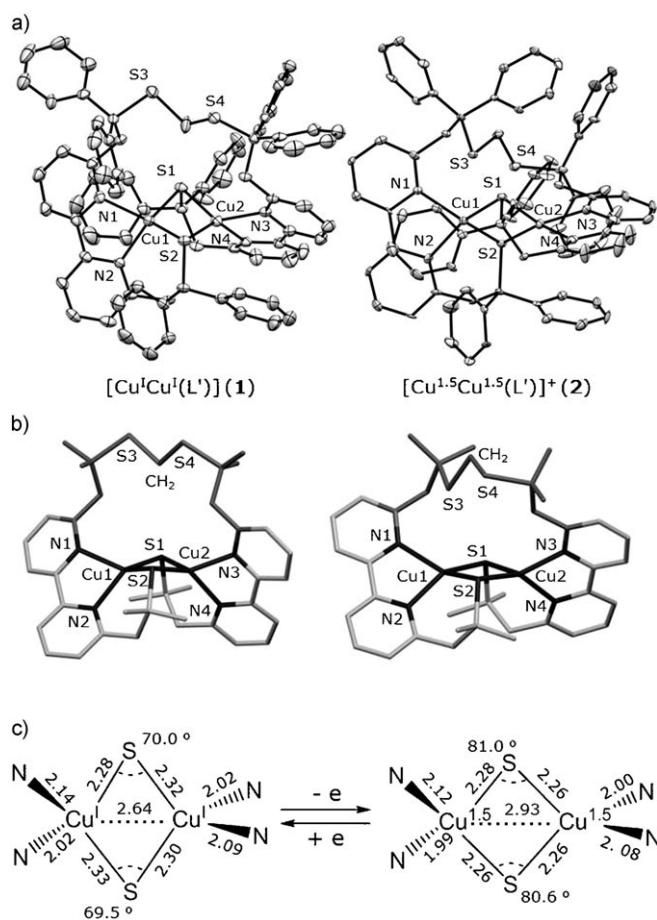


Figure 2. Different representations of complexes **1** and **2**: a) ORTEP views with ellipsoids set at 30% probability (hydrogen atoms and anion omitted for clarity). The same enantiomer of both complexes is depicted, and only one crystallographically independent complex of $[\text{Cu}_2\text{L}']^+$ 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 $\{\text{Cu}_2\text{S}_2\}$ core (more flattened in the case of $[\text{Cu}_2\text{L}']^+$). c) Representation with principal distances and angles of the $\{\text{Cu}_2\text{S}_2\}$ cores.

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 $\text{Cu}^{1.5}\text{Cu}^{1.5}$ form. The most remarkable difference between **1** and **2** is the $\text{Cu}\cdots\text{Cu}$ 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 $\text{Cu}\cdots\text{Cu}$ distance leads to an increase of the $\text{Cu}\cdots\text{S}\cdots\text{Cu}$ angles in **2** (70 vs. 81°) and a decrease of the $\text{S}\cdots\text{Cu}$ 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 $\text{N}_2(4)$ atom (N_{eq}) and the two bridging

aliphatic thiolate sulfur atoms of **1**, 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 3a. 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 3b). The seven hyperfine lines are associated with the coupling of the unpaired electron ($S = 1/2$) to the two equivalent copper ions ($I = 3/2$).^[9] The observation that $g_{||} > g_{\perp} > 2.0023$ 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 $g_{||}$ signal indicates a high degree of delocalization of the electron onto the ligands, as previously discussed for Cu_A (117 $A_{||} < 123$ MHz).^[9] As shown in Figure 3b, the EPR properties of **2** closely resemble those obtained for the Cu_A center.

The absorption spectrum of **2** is shown in Figure 3c together with those of the Cu_A mixed-valence state and of Tolman's model complex $[Cu_2(iPrdacoS_2)_2]^+$. 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 $[Cu_2(iPrdacoS_2)_2]^+$, 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 $[Cu_2(iPrdacoS_2)_2]^+$, 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]

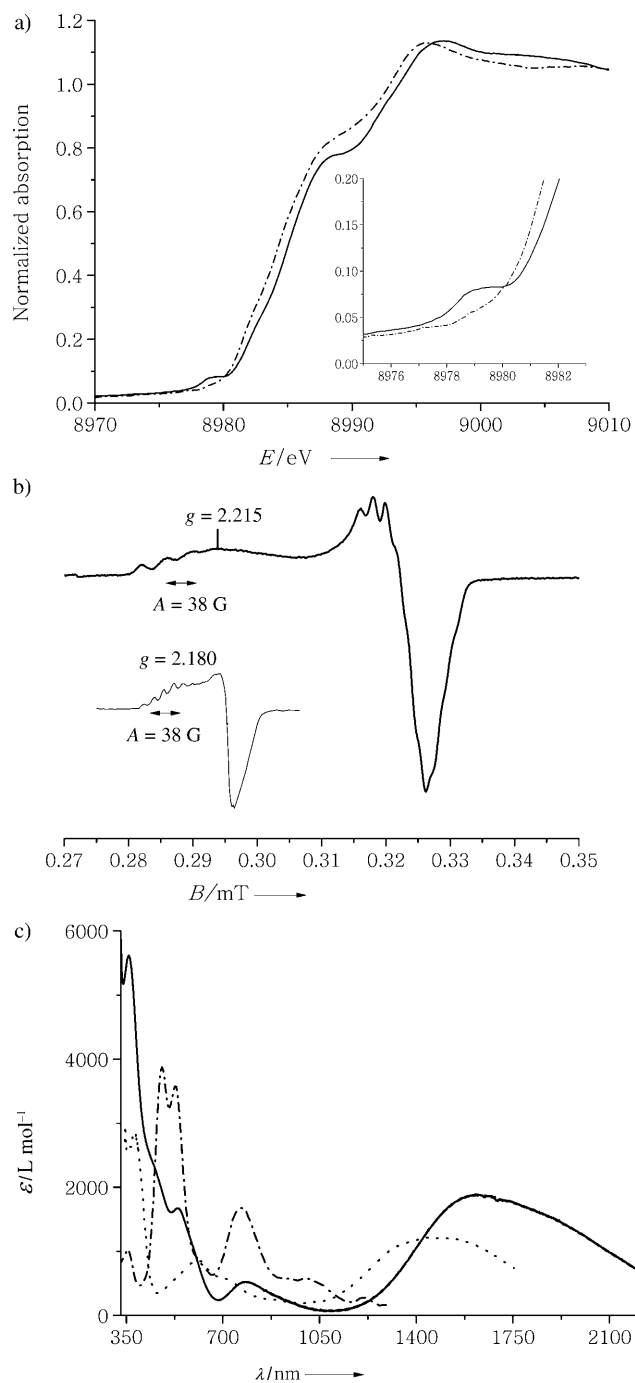


Figure 3. a) Comparison of the normalized Cu K-edge data for **1** (---) and **2** (—); b) X-band EPR spectrum of **2** recorded in CH_2Cl_2 at 5 K. Parameters used for the simulation: $g_{||} = 2.215$, $g_{\perp} = 2.002$, $A_{||} = 118$ MHz, $A_{\perp} = 48$ MHz. Inset: EPR spectrum of Cu_A of N_2OR from *P. stutzeri*;^[34] c) absorption spectra of **2** (—), $[Cu_2(iPrdacoS_2)_2]^+$ (••••),^[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 5a): a reversible $Cu^{1.5}Cu^{1.5}/Cu^I Cu^I$ 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.

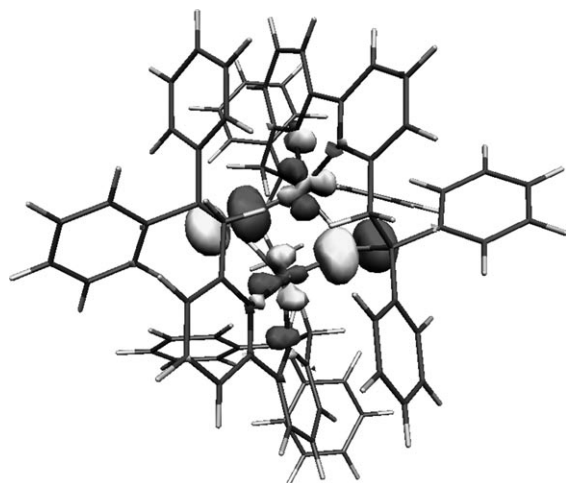


Figure 4. Contour plot of the redox-active, half-filled molecular orbital (SOMO) of complex **2**.

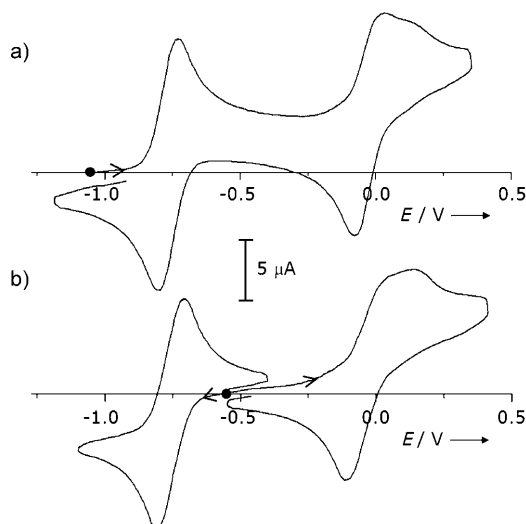


Figure 5. a) Cyclic voltammogram of **1** (0.9 mM) in CH_2Cl_2 with 0.1 M Bu_4NPF_6 at a carbon vitreous electrode and b) after electrolysis at -0.610 V (1 electron per molecule of **1** involved), scan rate of 100 mVs^{-1} . Potentials are given versus Fc^+/Fc .

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 $\text{Cu}^{1.5}\text{Cu}^{1.5}$ and Cu^1Cu^1 redox states most likely results from the preorganization of the system owing to the dinucleating character of L' that allows the stabilization of the $\{\text{Cu}_2\text{S}_2\}$ diamond core. The potential of the $\text{Cu}^{1.5}\text{Cu}^{1.5}/\text{Cu}^1\text{Cu}^1$ 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 , $\text{CH}_2\text{Cl}_2 < +0.025$ V),^[14,16,18] except for one with a $\{\text{Cu}_2\text{P}_2\}$

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 ($k_{\text{el}} = 7.7(1) \times 10^{-3} \text{ cm s}^{-1}$, or estimated as $k_{\text{hom}} = 1.37(2) \times 10^6 \text{ L mol}^{-1} \text{ s}^{-1}$).^[31] This k_{hom} value indicates that our model can achieve electron transfer rates faster than mononuclear Cu^{II} complexes, including plastocyanin ($k_{\text{hom}} = 1.5 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$),^[32] but more slowly than Cu_A ($k_{\text{hom}} = 2.2 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$),^[33] which is certainly due to the lack of the Cu–Cu bond in **2**.

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

In summary, we have isolated and characterized the first dicopper system with a $\{\text{Cu}_2\text{S}_2\}$ 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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