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MINIREVIEW

William B. Tolman

Using synthetic chemistry to understand copper protein active sites: a personal perspective

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Abstract The results of studies performed in the author's laboratory are surveyed, with particular emphasis on demonstrating the value of a multidisciplinary synthetic modeling approach for discovering new and unusual chemistry helpful for understanding the properties of the active sites of copper proteins or assessing the feasibility of mechanistic pathways they might follow during catalysis. The discussion focuses on the progress made to date toward comprehending the nitrite reductase catalytic site and mechanism, the electronic structures of copper thiolate electron transfer centers, the sulfido-bridged "Cu_Z" site in nitrous oxide reductase, and the processes of dioxygen binding and activation by mono- and dicopper centers in oxidases and oxygenases.

Keywords Copper proteins · Thiolate · Sulfide · Nitric oxide · Dioxygen

Introduction

Copper proteins are involved in numerous processes that impact life and the environment [1]. The copper sites within this large class of metallobiomolecules function in a variety of ways, including transferring electrons over variable distances at divergent rates and potentials, binding and activating dioxygen for respiration and for oxidations of substrates in metabolically important reactions, and reducing nitrogen oxides in processes that are critical components of the global nitrogen cycle. Significant differences among the geometric, electronic structural, and spectroscopic features of copper protein active sites reflect a structural diversity that accompanies their functional variability. Despite extensive research, the understanding of structure–function relationships at the molecular level is incomplete, however, and many questions remain unanswered concerning the detailed mechanisms and fundamental chemistry underlying copper-mediated processes in biology. Such specific knowledge of bioinorganic copper chemistry also informs studies of other metal-containing biosites and ultimately will enable intelligent control of vital enzymatic and other catalytic reactions.

We are interested in obtaining fundamental chemical insights into copper protein active-site structure and mechanisms of action through the synthetic modeling approach. As described in more detail elsewhere [2–4], this approach entails using ligand design principles and appropriate control of reaction conditions to prepare low molecular weight complexes that resemble the targeted metalloprotein active site, either in a resting state or as a reactive intermediate. Detailed characterization of the new molecules by structural, spectroscopic, and theoretical techniques can then provide deep electronic structural insights potentially applicable to the biological systems. In addition, studies using synergistic experiments and theoretical calculations to examine the reactivity of the synthetic compounds and the mechanisms of their reactions can reveal new types of reaction pathways relevant to metalloenzyme catalysis. Finally, in the course of following the synthetic modeling approach, novel molecules may be synthesized that may be of intrinsic interest from a fundamental chemistry standpoint, irrespective of their biological relevance.

In this minireview, I present an overview of the results from my laboratory of research we have performed involving the use of synthetic methods to understand copper protein active-site chemistry. As a result, the discussion will be somewhat narrowly focused, yet hopefully it will nonetheless serve to illustrate what one may learn about copper protein active-site structure and function through the synthetic modeling approach. Broader perspectives more inclusive of the work of others in this dynamic research area are available in

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numerous comprehensive reviews, a limited selection of which is listed here [5–11].

Copper nitrite and copper nitrosyl complexes: models of the nitrite reductase catalytic site

Copper nitrite reductase (Cu NiR) plays a key role in biological denitrification [9, 12–14], an environmentally significant process whereby bacteria use nitrogen oxides as terminal electron acceptors in respiration and produce gaseous products (Eq. 1). The initially reported X-ray crystal structure [15] revealed that Cu NiR contains a type 1 electron transfer site connected via a His-Cys bridge to a copper ion ligated by three His imidazolyls and a water molecule (Fig. 1). Substrate (NO_2^-) is reduced at the His_3Cu center, with electrons transferred from the type 1 Cu via the His-Cys bridge [16–18]. Our modeling efforts were inspired by a report by Hulse et al. [19] in which it was stated that Cu NiR "produces N_2O via a bound nitrosyl intermediate, E-NO^+; this is presumably a cuprous nitrosyl, Cu^+–NO^+, of which there are no structurally characterized synthetic examples."

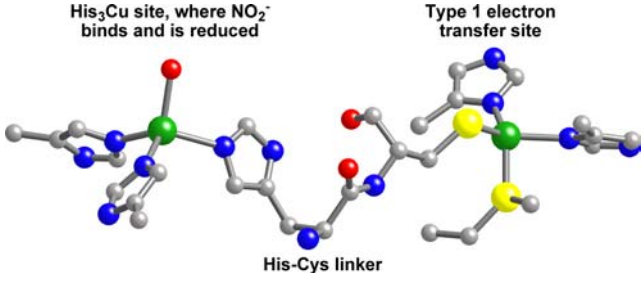

Fig. 1 The copper sites in nitrite reductase (PDB 1NIA). Atom colors are as follows: Cu *green*, N *blue*, O *red*, S *yellow*, C *gray*

We therefore decided to target a copper nitrosyl complex for synthesis and characterization, and found success by treating Cu(I) complexes of sterically hindered, facially coordinating tris(pyrazolyl)hydroborate ligands with NO (Fig. 2) [20–22]. End-on coordination of NO was confirmed by X-ray crystallography, and through detailed spectroscopic studies informed by theoretical calculations, insights into the electronic structure of the novel $[CuNO]^{11}$ unit were obtained (electron counting formalism described in Ref. [23]). Notably, the results indicated that this unit is best described as Cu(I)–$NO^{\bullet}$ rather than Cu(II)–NO^-. The binding of NO to the Cu(I) center is relatively weak, and when supporting ligands with substituents smaller than *t*-butyl were used, the nitrosyl complex reacted further with NO to yield a Cu(II)–NO_2^- complex and N_2O (Eq. 2) [24]. Mechanistic studies of this disproportionation reaction [22, 24] showed that attack of NO onto the copper nitrosyl complex is rate-determining. Identification of this facile process in the synthetic system suggested that a similar reaction may occur in the enzyme, providing one possible rationale for N_2O formation by Cu NiR under conditions of high concentrations of NO.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (1)$$

$$TpCuNO + 2NO \rightarrow TpCuNO_2 + N_2O \quad (2)$$

It is generally recognized that nitrite reduction by NiR involves coordination of NO_2^- at the His_3Cu site followed by protonation events that trigger loss of H_2O to yield a labile copper nitrosyl, which then releases NO [12–14]. The detailed molecular motions involved have been the subject of debate, however. X-ray crystallographic [25] and spectroscopic [26] studies showed that NO_2^- binds to the Cu(II) form of the active site via its O

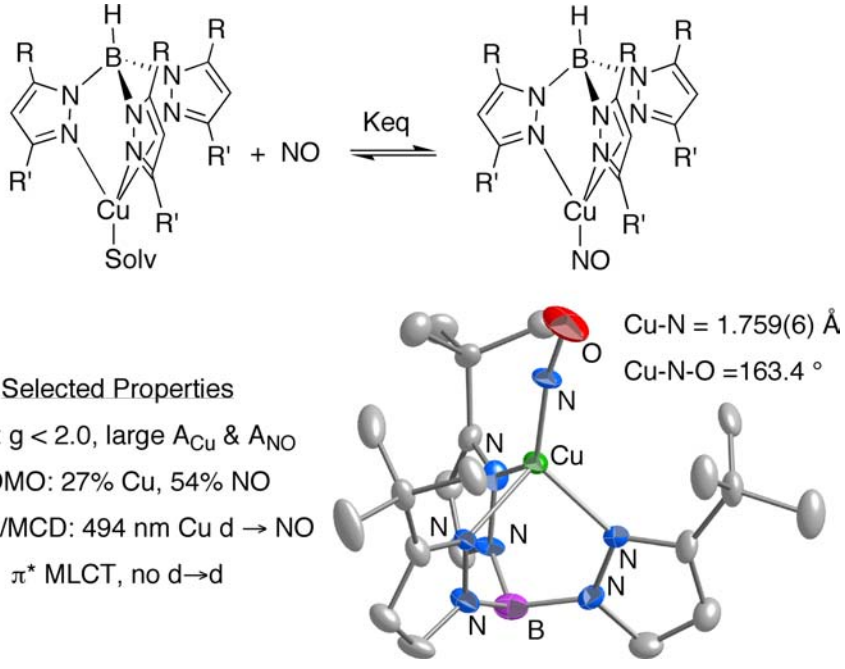

Fig. 2 Reversible binding of NO to a Cu(I) compound to yield a copper nitrosyl complex. The X-ray crystal structure of the product for R is H and R′ is *t*-Bu is shown (non-hydrogen atoms as 50% *thermal ellipsoids*), as are key selected properties determined through spectroscopic studies and theoretical calculations [21]

atoms, which raises the question of how an N-bound nitrosyl can subsequently be generated. One possibility is that reduction of the Cu center results in isomerization of the nitrite to an N-bound form. Precedent for this idea came from synthetic work from our laboratory [27, 28], wherein a mononuclear Cu(I)–NO_2^- complex supported by a biomimetic tridentate N-donor ligand (1,4,7-triisopropyltriazacylononane [29]) was shown to exhibit η^1-N coordination (Fig. 3) and to release NO upon treatment with acids (HOAc) or acid equivalents (trimethylsilyl trifluoromethanesulfonate, TMSOTf). This sequence has since been identified with other supporting ligand systems [30]. Another possible least-motion pathway for an O-bound nitrite to generate NO is for protonation and water loss to yield a side-on "η^2" nitrosyl adduct, which in transition metal chemistry is typically only a metastable species [31]. Such an adduct has recently been identified in Cu NiR by X-ray crystallography [32, 33], however, and has been examined by theoretical calculations at both the $[CuNO]^{10}$ and $[CuNO]^{11}$ redox levels [34]. Still, it remains unknown in synthetic copper chemistry, and thus is a tempting target for future research.

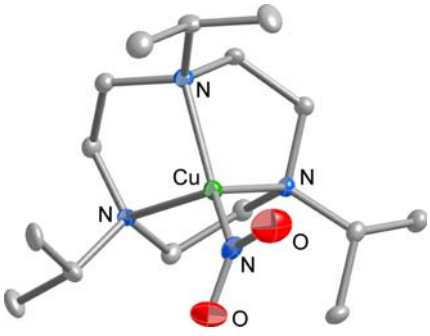

Fig. 3 Representation of the X-ray crystal structure of a Cu(I) complex with an η^1-nitrite ligand, shown as 50% thermal ellipsoids (hydrogen atoms omitted for clarity) [27, 28]

Copper thiolate complexes: models of the type 1 and "Cu_A" electron transfer sites

The type 1 site in Cu NiR is a member of a large class of copper centers in biology that function as electron transfer reagents [35]. Thiolate ligation is a common feature of such centers, which may be subdivided into two types according to their nuclearity. The "Cu_A" site is dinuclear, as determined originally from electron paramagnetic resonance (EPR) studies that were interpreted to indicate a mixed-valent fully delocalized ground state [36, 37], and later from X-ray crystallography (Fig. 4a) (the first reports of the structure of Cu_A in cytochrome *c* oxidase are in Refs. [38, 39]. Type 1 sites are mononuclear and have in common a short, covalent copper thiolate bond, yet their particular coordination geometries differ significantly. This may be illustrated by comparing the distorted tetrahedral Cu NiR type 1 moiety (Fig. 1) with the three-coordinate and trigonal bipyramidal sites of fungal laccase and azurin, respectively (Fig. 4b, c). A longstanding topic of research has been to understand how these structural variations contribute to the spectroscopic properties and electron transfer reactivity of these sites (e.g., rates and potentials) [35, 40–44]. Efforts to address this goal via the synthetic modeling approach have consumed inorganic chemists for decades [11, 45], a key success being the isolation of the compounds TpCuSR [Tp is tris(3,5-diisopropylpyrazolyl)hydroborate, R is CPh_3 or C_6F_5] that were found to exhibit the appropriately short Cu–SR bond and biomimetic EPR and UV–vis spectroscopic properties [46–48]. We sought structural variants for attaining closer structural fidelity to the natural sites and for uncovering structure–function relationships, in particular synthetic analogs of the three-coordinate fungal laccase site (notable owing to the presence of Cu(II) in an unprecedented low coordination number) and molecules that contain the typical thiolate/thioether ligation (cf. Cu NiR).

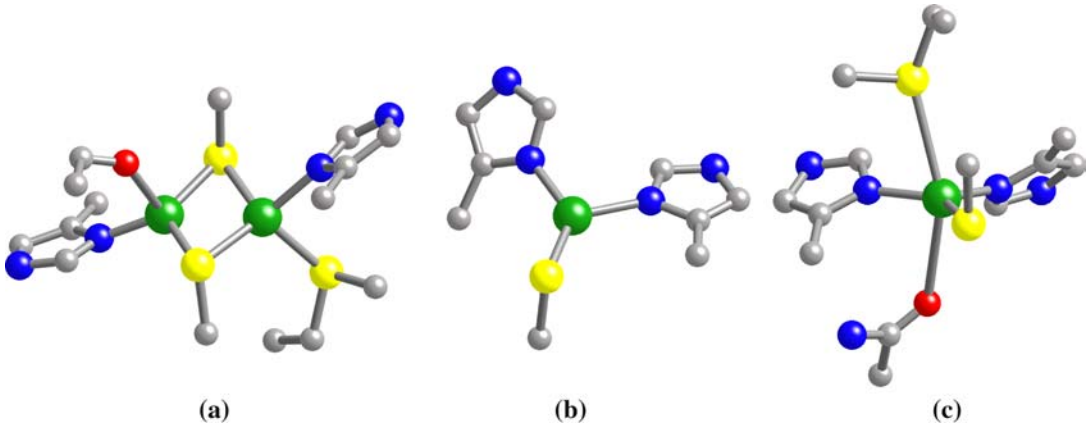

Fig. 4 Depictions from X-ray crystal structures of **a** the Cu_A site in cytochrome *c* oxidase (PDB 2CUA), **b** the type 1 site in fungal laccase (PDB 1HFU), and **c** the type 1 site in azurin (PDB 2AZA). Atom colors are as follows: Cu *green*, N *blue*, O *red*, S *yellow*, C *gray*

The synthesis of the first example of a three-coordinate Cu(II) complex, LCuCl (L is β-diketiminate ligand), laid the groundwork for the preparations of the targeted Cu(II) thiolate complexes (Fig. 5a, b) [49, 50]. The key to the syntheses was the use of the sterically hindered isopropyl-substituted version of L; less hindered variants yielded dimeric precursors $[LCuCl]_2$ that failed to provide stable thiolate derivatives [51]. The compounds LCuSR (R is *t*-Bu or *o*-dimethylphenyl) and $LCu(SC(Ph)_2CH_2SMe)$ are accurate structural biomimics insofar as their coordination geometries agree closely with those of the fungal laccase site or the Cu NiR site, respectively. Most illuminating, however, were detailed spectroscopic studies of LCuSR that focused on drawing comparisons with TpCuSR and the fungal laccase site [52, 53]. Notable among the many conclusions drawn from these studies was the finding that the key redox orbital in LCuSR (Fig. 5c) closely resembles that determined for the Tp complex and the protein, but with subtle differences due to the especially strong electron donating character of the β-diketiminate ligand. In short, through an enhanced *trans* influence the β-diketiminate increases the *d*-orbital splitting and weakens the Cu d + S $p\pi$ interaction relative to the splitting and interaction of the other centers. This property of the β-diketiminate underlies a reduction potential of -0.18 V versus the normal hydrogen electrode that is drastically lower than that of the fungal laccase site (more than $+0.7$ V) and also has repercussions in Cu/O_2 chemistry (see later).

Our efforts to build a model of the dinuclear Cu_A site were initially stimulated by a lively debate over the nature of this center at a conference attended in 1992 (the proceedings are published in Ref. [54]), but after its bis(μ-thiolato)dicopper structure had been confirmed [38, 39], further impetus was provided by the need to understand the basis for its unusual mixed-valence delocalized "Cu(1.5)Cu(1.5)" electronic structure [55]. Significant synthetic efforts culminated in the successful preparation of a complex that mimicked key aspects of Cu_A, in particular a $[Cu_2(SR)_2]^+$ core with full valence delocalization by EPR spectroscopy (Fig. 6) [56]. Nonetheless, structural differences between the model and the biosite resulted in important and informative disparities in spectroscopic properties [41, 57–59]. For example, the Cu–Cu distance in the model is 2.9306(9) Å, significantly longer than in Cu_A (approximately 2.4 Å), and the model contains two strongly bound terminal ligand N donors, whereas Cu_A has only one (in addition to a much more weakly coordinating group). While similar Cu–S bond covalencies in the model complex and Cu_A were indicated by S K-edge and Cu L-edge X-ray absorption spectroscopy (XAS) [59], the mixed-valent $\psi \rightarrow \psi^*$ transition energies differed by approximately 7,800 cm^{-1} ($Cu_A >$ model). These results were interpreted to provide quantitative support for the previously suggested and unique Cu–Cu bonding interaction in Cu_A [60] that is absent in the model complex. The calculated highest occupied molecular orbitals (HOMOs) for the model complex and Cu_A (Fig. 6) illustrate the different metal–metal interactions, which were argued to arise in large part from differences in the terminal ligand geometries [58]. Importantly, in this work the most significant insights into the electronic structure of the biosite came from analysis of key *differences* between it and the model complex. Thus, comparative studies on *inexact* models of a metalloprotein active site may sometimes be especially informative.

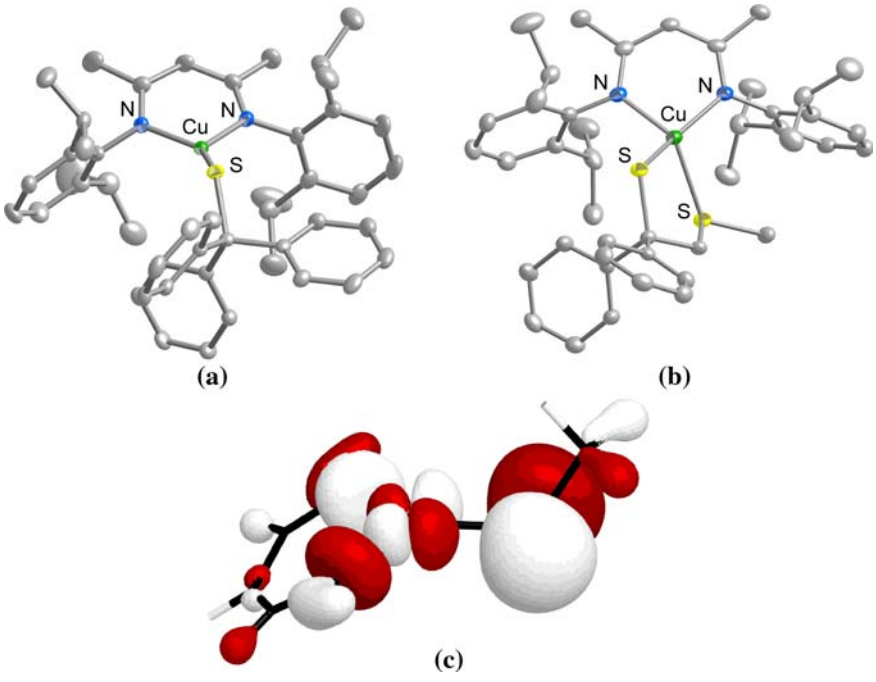

Fig. 5 X-ray crystal structures of **a** $LCuSC(Ph)_3$ and **b** $LCuSC(Ph)_2CH_2SMe$, as 50% thermal ellipsoids (hydrogen atoms omitted for clarity) [49, 50]. The calculated singly occupied molecular orbital for $LCuSC(Ph)_3$ is shown in **c** [52]

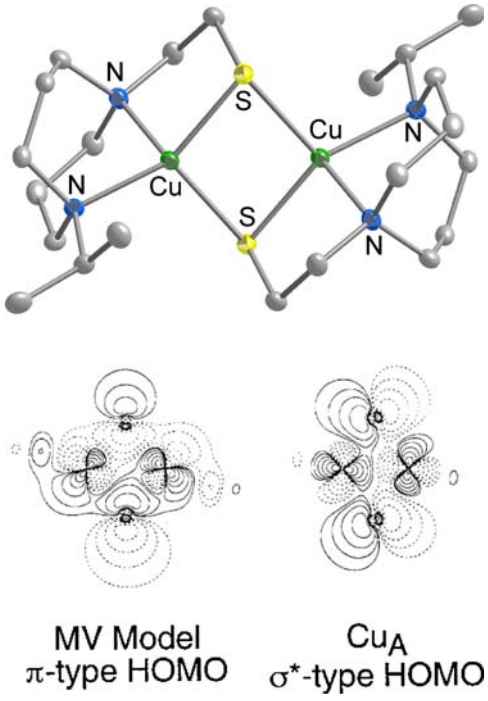

Fig. 6 X-ray crystal structure of a model complex of Cu_A (*top*, shown as 50% thermal ellipsoids) [56] and the highest occupied molecular orbitals (*HOMOs*) calculated for the model and Cu_A (*bottom*, reproduced with permission from Ref. 41)

Copper sulfide chemistry: toward models of the nitrous oxide reductase catalytic center ("Cu_Z")

The reduction of N_2O to N_2 during denitrification is catalyzed by nitrous oxide reductase (N_2OR) [61, 62], which contains two types of copper sites, a Cu_A-type center proposed to perform an electron transfer function, and a catalytic cluster ("Cu_Z") recently shown by X-ray crystallography [63, 64] and resonance Raman spectroscopy [65, 66] to contain four Cu ions bridged by a μ_4-sulfido moiety (Fig. 7). The fully reduced $Cu(I)_4$ form of the cluster has been proposed to bind and reduce N_2O via pathways supported by theoretical calculations, although more oxidized forms have been identified and may be catalytically relevant [62, 67–72]. Impetus for synthetic modeling of Cu_Z is provided by its structural novelty and the provocative nature of the mechanisms proposed for its activity. While Cu(I) sulfido species have been reported with supporting soft, abiological donor ligands (e.g., phosphines) [73], little is known about copper–sulfur chemistry with N-donor ligands akin to the histidine imidazolyl donors present in Cu_Z. Thus, we are actively exploring fundamental aspects of copper–sulfur chemistry using such supporting ligands, with the ultimate goal of constructing accurate models of Cu_Z with which to perform detailed comparative spectroscopic and reactivity studies.

So far, these explorations have led to the isolation of copper complexes of varying nuclearity that feature sulfur in multiple redox forms, including S_2^{2-}, $S_2^{\bullet-}$, and S^{2-} [74–76]. Using copper-containing starting materials supported by anionic β-diketiminate or anilido-imine ligands, a class of (μ-η^2:η^2-S_2^{2-})dicopper(II) complexes was prepared, one member of which is shown in Fig. 8a. Related (μ-S_2^{2-})dicopper(II) compounds with tri- or tetradentate N-donor ligands have been reported [77–80].

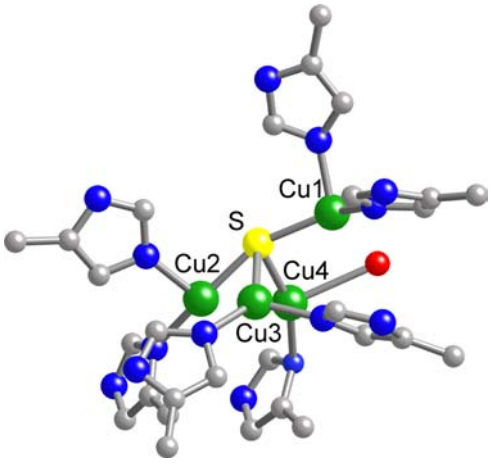

Fig. 7 The Cu_Z site of nitrous oxide reductase (PDB 1FWX)

A different reaction course was followed, however, when Cu(I) complexes of the neutral bidentate N donors *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) or *N*,*N*,*N*′,*N*′-tetramethylcyclohexane-1,2-diamine (TMCHD) were treated with S_8 [75]. In this case, we isolated novel clusters comprising $[Cu_3(\mu\text{-}S)_2]^{3+}$ cores with SbF_6^- or PF_6^- counterions (Fig. 8b). These clusters model attributes of Cu_Z, insofar as the (μ-sulfido)tricopper subunit resembles the portion of Cu_Z comprising Cu2, Cu3, Cu4, and S (Fig. 7). Interestingly, while a previously reported analog with a $[Cu_3(\mu\text{-}O)_2]^{3+}$ core is valence localized [81, 82], with one Cu(III) and two ferromagnetically coupled Cu(II) ions that exhibit divergent metal–ligand bond distances, the $[Cu_3(\mu\text{-}S)_2]^{3+}$ core exhibits complete delocalization, as indicated by EPR spectroscopy (10-line Cu-hyperfine in $g = 4$ signal at X-band at 10 K) and X-ray diffraction (local D_{3h} cluster symmetry with equivalent metal–ligand bond distances). Theoretical calculations rationalize these differences in electronic delocalization by invoking σ-type antibonding interactions between the S *p* orbitals that underlie an inversion in the frontier molecular orbital energy ordering relative to that of the oxo-bridged cluster [75].

More recently, we found that when S_8 was added to the Cu(I) complex of TMEDA having a $CF_3SO_3^-$ counterion, a compound with a novel $[Cu_2(S_2)_2]^{2+}$ core and axially coordinated $CF_3SO_3^-$ groups is generated (Fig. 8c) [76]. On the basis of structural data (cf. S–S = 1.95 Å) and resonance Raman spectroscopy [ν(S–S) = 613 cm^{-1}], the core is best formulated as having Cu(II) ions linked by $S_2^{\bullet-}$ bridges rather than Cu(III)

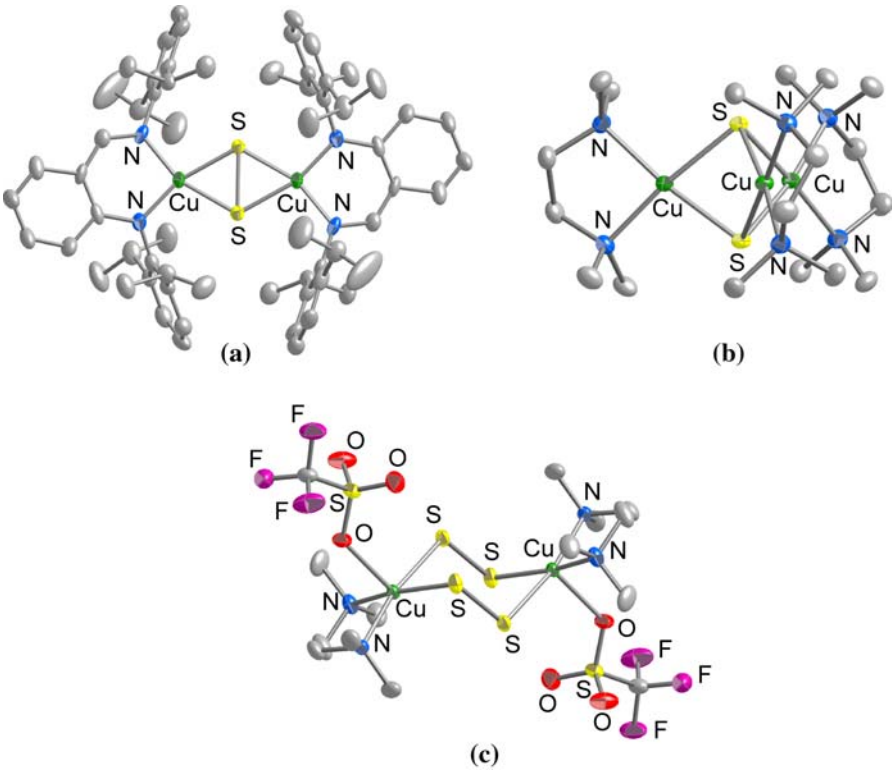

Fig. 8 X-ray crystal structures of **a** a (μ-η^2:η^2-S_2^{2-})dicopper(II) complex supported by an anilido-imine ligand [74], **b** the tricationic fragment of $[(TMEDA)_3Cu_3(\mu\text{-}S)_2](SbF_6)_3$ [75], and **c** $[(TMEDA)_2Cu_2(S_2)_2(O_3SCF_3)_2]$ [76]. Each structure is shown as 50% thermal ellipsoids, with heteroatoms labeled and hydrogen atoms omitted for clarity. *TMEDA N,N′,N′*-tetramethylethylenediamine

ions with S_2^{2-} units. Apparently, widely varying copper–sulfur core topologies and oxidation levels can result from rather subtle changes in N-donor ligands and/or counterions, and we are therefore inspired to further explore Cu–S chemistry with the ultimate goal of modeling the unique Cu_Z site.

Peroxodicopper and bis(μ-oxo)dicopper complexes: models of oxygenase intermediates

Understanding how dioxygen is bound and activated at multicopper sites in proteins such as hemocyanin [83], the multicopper oxidases [84–86], particulate methane monooxygenase [85, 87], and tyrosinase [84, 85, 88] has been a longstanding objective of synthetic modeling studies [5–10, 89–91]. Inspired by the pioneering work of Karlin [92] and Kitajima et al. [93], who were the first to structurally define *trans*-1,2-peroxodicopper and μ-η^2:η^2-peroxodicopper complexes, respectively, we examined the low-temperature oxygenations of Cu(I) complexes of substituted 1,4,7-triazacyclononane ligands [94]. In the course of this work, we isolated and characterized bis(μ-oxo)dicopper(III) complexes and showed that in one case (using a supporting ligand with *i*-Pr substituents) this species can rapidly equilibrate with a μ-η^2:η^2-peroxodicopper isomer [95], thus illustrating how the O–O bond in dioxygen may be reversibly broken and formed (Fig. 9). These results, as well as those of others that have demonstrated the ubiquity of the bis(μ-oxo)dicopper core and the generality of this reversible isomerization, have been reviewed recently elsewhere [6, 7, 90]. Here, I focus on a key mechanistic question that consideration of these results raised [96]: Which is the active oxidant in tyrosinase, the observed μ-η^2:η^2-peroxodicopper intermediate or a derived bis(μ-oxo) isomer? While only studies of the enzyme itself can directly address this question, through the synthetic modeling approach one can determine what is chemically possible; that is, whether or not either isomer is capable of performing the critical tyrosinase oxidation step, hydroxylation of an aromatic ring (e.g., phenol).

A number of studies have shown that the μ-η^2:η^2-peroxodicopper unit can hydroxylate aromatic rings through an electrophilic mechanism [7, 90, 91, 97, 98]. Of these studies, perhaps the most definitive is the extensive examination of the hydroxylation of the *meta*-xylyl bridge of a binucleating pyridyl-amine ligand upon decay of a μ-η^2:η^2-peroxodicopper complex [97]. On the other hand, when bis(μ-oxo)dicopper complexes are allowed to decompose, supporting N-donor ligand substituent C–H bonds positioned near the bridging oxygen

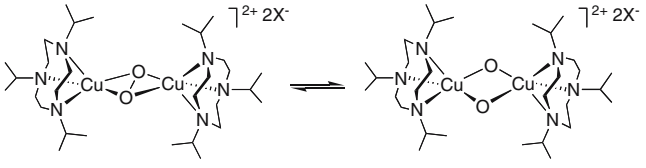

Fig. 9 Equilibrium between μ-η^2:η^2-peroxodicopper and bis(μ-oxo)dicopper complexes

atoms are attacked, leading to N-dealkylation via a hydrogen atom abstraction pathway that is typical for such bis(μ-oxo) cores [7]. In order to avoid this type of process and to assess the capability of the bis(μ-oxo)dicopper unit to attack an arene instead, we designed a bidentate ligand which would favor bis(μ-oxo)dicopper complex formation and position an arene near the bridging oxygen atoms, with other substituent C–H bonds oriented such that they would not be as susceptible to attack [99, 100]. Upon low-temperature oxygenation of the Cu(I) complex of this new ligand, only spectroscopic features of a bis(μ-oxo)dicopper complex were observed, and upon warming and removal of the copper ions, products corresponding to hydroxylation of the arene were identified (Fig. 10). The hydroxylation product yields and rates of formation increased with the electron donating capabilities of the substituents *X* and no H/D kinetic isotope effect was observed, consistent with an electrophilic aromatic substitution pathway.

While these results seem to demonstrate that the bis(μ-oxo)dicopper core is indeed capable of hydroxylating an appropriately positioned aromatic ring, they do not constitute proof for this idea, for one cannot rule out arene attack by a small amount of a reactive μ-η^2:η^2-peroxodicopper isomer formed in a rapid pre-equilibrium step. Arguments against the viability of a bis(μ-oxo)dicopper intermediate in tyrosinase have been made on the basis of theoretical calculations [101], but methodological issues that complicate attempts to accurately determine the relative energies of μ-η^2:η^2-peroxodicopper and bis(μ-oxo)dicopper isomers have been raised recently that render these conclusions suspect [102, 103]. In a particularly relevant report [104], a phenolate adduct to a bis(μ-oxo)dicopper complex was identified at low temperature and found to yield phenol hydroxylation products (catechol and *o*-quinone) upon warming. Theoretical calculations support an intramolecular process involving attack of the bis(μ-oxo)dicopper core lowest unoccupied molecular orbital on the phenolate ligand HOMO and, thus, the feasibility of oxidation of phenol by the bis(μ-oxo)dicopper core. Still, the possible involvement of a μ-η^2:η^2-peroxodicopper species cannot be ruled out definitively by experiment in this case either, so from a formal standpoint at least the question of which species is the active oxidant in tyrosinase remains unresolved.

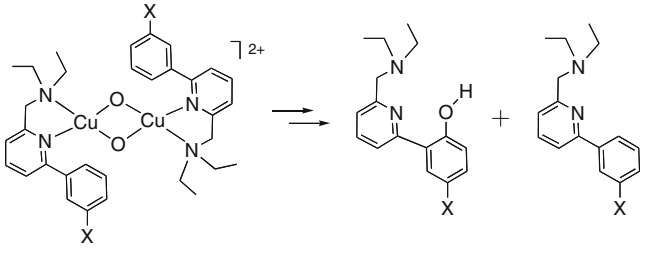

Fig. 10 Hydroxylation of an arene substituent upon decomposition of a bis(μ-oxo)dicopper complex

Monocopper–dioxygen adducts: modeling monocopper oxidases and oxygenases

The binding and activation of dioxygen by a single copper site are central steps in the mechanisms of several biologically significant metalloenzymes [105, 106], including the amine oxidases, dopamine β-monooxygenase (DβM) [107], and peptidylglycine α-hydroxylating monooxygenase (PHM) [108]. Extensive studies of DβM and PHM in particular have led to the proposal that a 1:1 Cu/O_2 adduct is responsible for attacking the target C–H bond of their respective substrates [109, 110]. This adduct has been suggested to feature O_2 coordinated in η^1 "end-on" fashion to "Cu_M," the copper site ligated by two His imidazoles and a Met thioether, on the basis of an X-ray crystal structure of PHM determined by using a slow-reacting substrate (Fig. 11) [111]. Many questions about the structural aspects and functional role(s) of this and other postulated 1:1 Cu/O_2 adducts in proteins have stimulated extensive efforts to isolate and characterize such species synthetically [7, 106]. Such efforts have been complicated by the tendency for 1:1 Cu/O_2 adducts to react rapidly with a second Cu(I) ion to yield relatively stable peroxodicopper or bis(μ-oxo)dicopper compounds. At the outset of our work, this reaction had been inhibited successfully in only one instance to yield an η^2 "side-on" complex supported by a sterically hindered tris(pyrazolyl)hydroborate ligand (Fig. 12a) [112]. Structural, spectroscopic, and theoretical studies showed that this adduct is best formulated as a Cu(II) superoxide [113], notwithstanding an apparently underestimated O–O bond distance (1.22 Å) in the reported X-ray structure [114].

We found that bis(μ-oxo)dicopper complexes were generated upon reaction of Cu(I) complexes of sterically undemanding β-diketiminates with O_2 at low temperature [115, 116]. On the other hand, when more sterically hindered β-diketiminates or related anilido-imine ligands were used, the reactions yielded isolable 1:1 Cu/O_2 adducts that have been extensively characterized

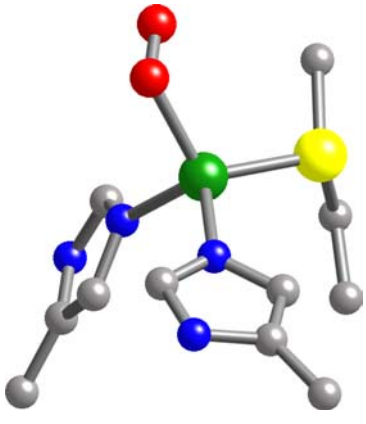

Fig. 11 The O_2 adduct at the Cu_M site of peptidylglycine α-hydroxylating monooxygenase (PDB 1SDW)

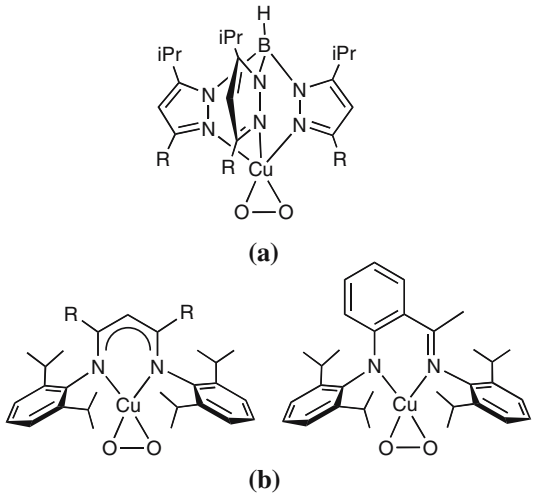

Fig. 12 Structurally defined 1:1 Cu/O_2 adducts, where in **a** R is *t*-Bu or adamantyl and in **b** R is Me or *t*-Bu

(Fig. 12b) [115, 117–119]. The combined results support significant Cu(III) peroxide character for these adducts. Key data include (1) an O–O bond distance determined to be 1.392(2) Å from a high-resolution X-ray structure that falls between distances characteristic of coordinated superoxide and peroxide ligands [119], (2) a Cu K-edge XAS spectrum with a $1s \rightarrow 3d$ pre-edge transition energy (approximately 3,981 eV) essentially identical to those of bis(μ-oxo)dicopper compounds [118], and (3) corroborating theoretical results that show, for example, orbital occupation numbers from multiconfigurational CASPT2 calculations for the Cu/O_2 moiety that are closer to d^8/O_2^{2-} than to d^9/O_2^- [119, 120]. These findings make sense in view of the powerful electron-donating capabilities of the β-diketiminate and anilido-imine ligands, which stabilize the Cu(III) state and effectively "push" electron density onto the coordinated dioxygen unit.

Considerable insight into the mechanism of formation of the 1:1 Cu/O_2 adducts from the reaction of the Cu(I) precursor LCu(NCR) (L is β-diketiminate, R is alkyl or aryl) with O_2 was obtained through synergistic low-temperature stopped-flow kinetics studies and theoretical calculations [118]. In brief, the kinetics data support a dual pathway mechanism involving a second-order associative path wherein O_2 binds and nitrile then dissociates (pathway A; Fig. 13), as well as a competing path with a zero-order dependence on O_2 concentration that involves prior displacement of nitrile by tetrahydofuran (THF) solvent (solvolysis) followed by a rapid associative substitution by O_2 (pathway B). The transition states for each pathway were calculated by density functional theory, revealing interesting differences in the O_2 binding geometries to the ligand being displaced that depend on the nature of the solvent (end-on for THF vs. asymmetric side-on for CH_3CN, shown in Fig. 13). Overall, the reaction is calculated to be highly exergonic, in good agreement with the experimental finding that the binding of O_2 is irreversible, such that argon purging removes excess O_2 without perturbing the spectroscopic features of the 1:1 Cu/O_2 adduct. We have taken advantage of this irreversible binding behavior by using the adduct as a synthon for the stepwise construction of bis(μ-oxo)dimetal complexes that feature different metals (cf. Cu–Ni [121]) or different ligands on each metal

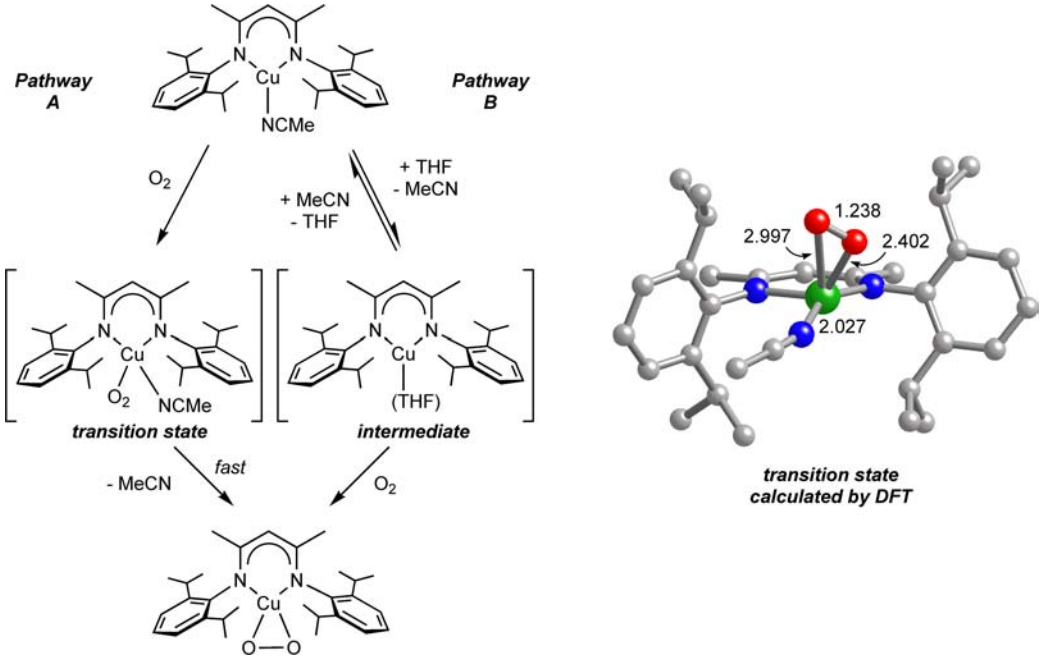

Fig. 13 Proposed dual pathway mechanism for the formation of the 1:1 Cu/O_2 adduct and the calculated structure of the transition state for the associative pathway A

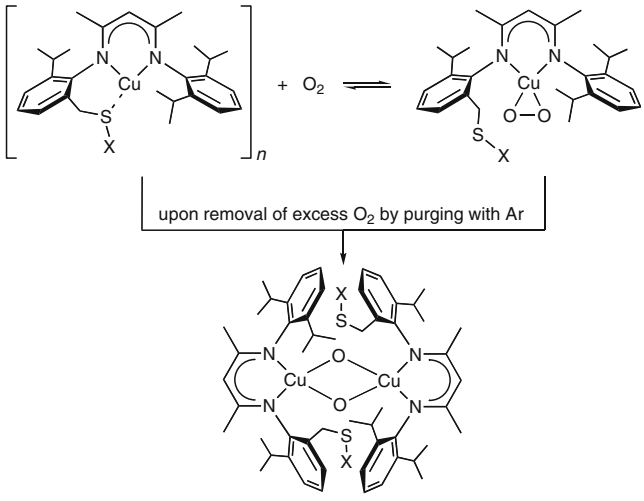

Fig. 14 $Cu(I)/O_2$ reactivity of complexes with thioether-modified β-diketiminate ligands (*X* is Me or Ph)

[117]. Further applications of this methodology for the synthesis of novel heterobimetallic compounds or higher nuclearity models of multicopper protein active sites are currently under investigation.

More recently, we have probed the role of the Met thioether ligand in PHM by incorporating a thioether substituent into the β-diketiminate framework (Fig. 14) [122]. Copper(I) complexes of the new ligands react with O_2 at low temperature to yield 1:1 Cu/O_2 adducts that we postulate on the basis of spectroscopic data and theoretical calculations have similar side-on Cu(III) peroxo structures to those of the parent ligand system lacking the thioether. Yet, while the structure of the 1:1 Cu/O_2 adduct is not perturbed by the thioether, the O_2 binding equilibrium is affected, as evidenced by conversion of the adduct to a bis(μ-oxo)dicopper complex upon purging with argon. The bis(μ-oxo)dicopper species forms via loss of O_2 from the 1:1 Cu/O_2 adduct to yield a Cu(I) species that is then trapped by remaining adduct. Thus, the thioether appears to decrease the equilibrium constant for O_2 binding to the copper ion by decreasing the rate of O_2 coordination, increasing the rate of O_2 dissociation, or both. These results provide precedence for the hypothesis of similar influences of the Met ligand on the binding of O_2 to the Cu_M site in DβM and PHM [109, 110].

Conclusions and perspective

A deeper understanding of the structures, properties, and reactivity of a range of copper protein active-site species has been obtained through studies of model complexes synthesized using relatively simple supporting ligands. Central to these studies has been the characterization of new copper complexes using multiple complementary and synergistic techniques, often in collaboration with experts in the fields of advanced spectroscopy, rapid kinetics, and theory. In the end, the value of this approach lies in the ability to discover new and unusual chemistry that can rationalize observed features of the metalloproteins themselves or, in special cases, indicate a new way of thinking about how a metalloenzyme might work. Perhaps most importantly, discoveries of new synthetic model chemistry raise new questions that stimulate further research. In this regard, many targets remain for future synthetic studies. These include a (μ-sulfido)tetracopper cluster model of Cu_Z in N_2OR, tricopper/O_2 species with which to test the feasibility of intermediates postulated for the multicopper oxidases, and a low-coordinate monocopper complex featuring end-on O_2 coordination like that postulated as the reactive species in oxygenases like PHM. The exciting prospects for gaining new knowledge of copper protein properties and for discovering novel chemistry in pursuit of these and other related objectives provide a basis for continued confidence in the utility of the synthetic modeling approach in bioinorganic chemistry.

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