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Chemical Plausibility of Cu(III) with Biological Ligation in pMMO

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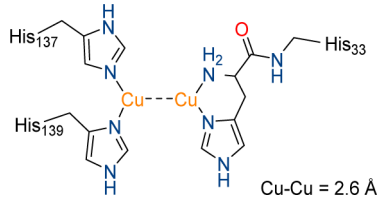
Supporting Information

ABSTRACT: The mechanisms of dioxygen activation and methane C−H oxidation in particulate methane monooxygenase (pMMO) are currently unknown. Recent studies support a binuclear copper site as the catalytic center. We report the low-temperature assembly of a high-valent dicopper(III) bis(μ-oxide) complex bearing marked structural fidelity to the proposed active site of pMMO. This unprecedented dioxygen-bonded Cu(III) species with exclusive biological ligation directly informs on the chemical plausibility and thermodynamic stability of the bis(μ-oxide) structure in such dicopper sites and foretells unusual optical signatures of an oxygenation product in pMMO. Though the ultimate pMMO active oxidant is still debated, C−H oxidation of exogenous substrates is observed with the reported Cu(III) complexes. The assembly of a high valent species both narrows the search for relevant pMMO intermediates and provides evidence to substantiate the role of Cu(III) in biological redox processes.

Methanotrophic bacteria incorporate methane, the principal component of natural gas, as a sole source of carbon and chemical energy. The first oxidative transformation in the metabolism of methane is catalyzed primarily by particulate methane monooxygenases (pMMOs), copper-dependent enzymes responsible for the selective conversion of methane to methanol in the presence of atmospheric dioxygen.[1−4] Mechanistic insight into pMMO has heretofore been limited. In copper starvation conditions, methane is oxidized in some organisms by action of well-studied, iron-dependent, soluble methane monooxygenases (sMMOs), which a recent time-resolved rRaman report has shown to operate presumably through a diiron(IV) bis(μ-oxide) "diamond core" as the active oxidant.[5] The ecological and economic significance of these reactions hold particular importance to chemists and the commodity carbon industry in that the kinetics of oxygen atom incorporation to a strong C−H bond (104 kcal mol^{-1}) are compatible with ambient conditions.[6]

Crystallographic and enzymological investigations have established the reactive center of pMMO to be a nonsymmetric binuclear copper site with a 2.60 Å separation in the pmoB subunit in which one copper is coordinated by two histidine imidazoles and another is chelated by the imidazole and primary amine of an N-terminal histidine (Chart 1).[3,7,8] To date, no study has shown definitive characterization of the activated, dioxygen-bonded adducts nor subsequent intermediates potentially responsible for methane oxygenation.[9] Conservation of the three critical active-site histidine residues may implicate similar oxidants in other important members of the copper membrane monooxygenase superfamily, such as ammonia monooxygenases (AMOs), central to terrestrial and marine nitrogen cycling by ammonia oxidizing archaea and bacteria.[10,11]

Chart 1. Binuclear Active Site of pMMO in *M. capsulatus*



In 2012, Rosenzweig and co-workers detected a putative oxygenated pMMO by optical spectroscopy[12]—a low intensity absorption feature at 345 nm; however, this data alone was insufficient to propose the nature of dioxygen binding to the binuclear site nor the structure of the oxygenated intermediate, as the observed energy and absorption intensity do not differentiate between a potentially relevant copper-dioxygen species or a resting state. While several modes of dioxygen coordination in binuclear systems are known, the short 2.60 Å Cu−Cu separation and protein-derived, bidentate ligation of each copper center in pMMO is consistent with a high-valent dicopper(III) bis(μ-oxide) species: minimal structural reorganization would be required to achieve a ca. 2.80 Å Cu−Cu distance with a nearly planar $N_2CuO_2CuN_2$ core and bidentate nitrogenous ligation of each copper center.[13−15] Indeed this might parallel the newly identified bis(μ-oxide) diamond core of intermediate Q in sMMO, which exhibits exceptionally short Fe−Fe distances.[5] However, the reported optical spectrum of pMMO is also inconsistent with known synthetic bis(μ-oxide) compounds, and the Cu(III) oxidation state is yet to be assigned definitively to any redox mechanism in biology.[9] Looming issues in current bioinorganic chemistry are the identity of pMMO oxygenated intermediates and the mechanism by which strong C−H bonds are activated.[16,17]

We have recently described low-temperature ligand exchange as a method of assembling high-valent dicopper(III) bis(μ-oxide) compounds with previously unknown, exclusive primary-amine ligation.[18] Here we present the first example of such species supported by biologically relevant histamine ligands, directly modeling the coordination sphere in pMMO. These complexes bear definite structural resemblance to the proposed binuclear

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Scheme 1. Sequential Ligand "Core Capture" with the Rank Ordered Thermodynamic Stability of 1, 2, 3, and 5

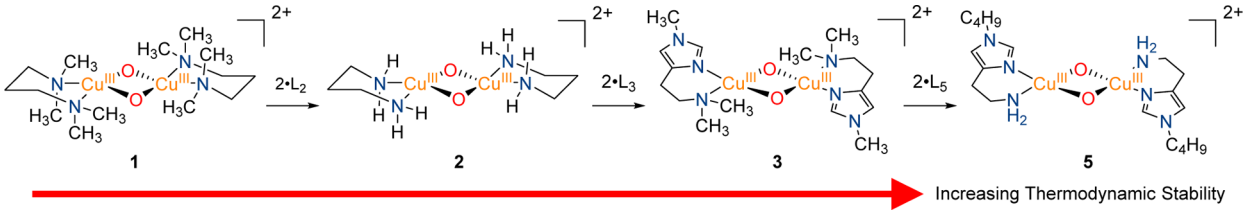
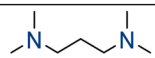
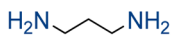
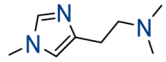
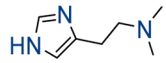
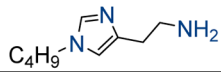


Table 1. Physical Properties of Compounds 1−5

Complex	Ligand		UV-Vis λ, nm (ε, mM^{-1} cm^{-1})[a,f]	Structural Metrics EXAFS[b,e,f] (DFT)[c,f] Cu-Cu (Å)	Cu-O (Å)	Cu-N (Å)[d,e,f]
1	L_1		403 (29.4) 302 (18.7)	2.85 (2.83)	1.84 (1.81)	2.02 (2.00)
2	L_2		375 (22.6) 278 (21.0)	2.77 (2.71)	1.86 (1.80)	2.00 (1.94)
3	L_3		380 (30.2) 280 (24.5)	2.80 (2.77)	1.82 (1.81)	1.97 (1.98 / 1.91)
4	L_4		380 (25.9) 283 (23.2)	2.81 (2.78)	1.82 (1.81)	1.96 (1.99 / 1.91)
5	L_5		363 (27.8) 262 (30.2)	2.78 (2.73)	1.82 (1.81)	1.96 (1.95 / 1.91)

[a] SbF_6^-, MeTHF, −125 °C, corrected ext. coeff. [b] Compounds **1**−**5** show Cu K pre-edge transitions at 8980.2, 8980.7, 8980.7, 8980.5, and 8980.6, respectively, consistent with a Cu(III) oxidation state. [c] Optimized at m06/tzvp/smd(THF) level of theory. [d] DFT gives asymmetric Cu−N coordination for **3**−**5** ($Cu-N_{amino}/Cu-N_{imd}$). [e] Resolution ≈ ±0.01 Å. [f] See ref 18 for **1** and **2**.

enzyme active site, and their unambiguous characterization as Cu(III) compounds suggests the accessibility of the Cu(III) oxidation state in biological systems in which histidine imidazole has been postulated to be insufficiently stabilizing.[19,20] Not only are these compounds potentially revelatory of the nature of O_2 activation in pMMO, they oxidize C−H bonds, a critical transformation in methane metabolism.[21,22] As the experimental tractability of these models currently outpaces enzymatic investigation, the unusual optical profiles described here may foretell key spectroscopic signatures by which pMMO active-site intermediates will be ultimately characterized.

In our previous report, a dicopper(III) bis(μ-oxide) compound **2**, ligated by propylenediamine ($\mathbf{L_2}$), was shown to be accessible through the intermediacy of stable, preformed **1**, by ligand displacement or "core capture" (Scheme 1).[18,23] While **1** is formed by oxygenation of its ligand-Cu(I) precursor, the direct oxygenation of Cu(I) complexes with any NH_2-bearing ligand yields complex mixtures of copper-dioxygen species, even at −125 °C, presumably due to highly reactive dioxygen adducts initially formed. Core capture can be applied to the synthesis of histamine-ligated dicopper(III) bis(μ-oxide) complexes: injection of two molar equivalents of ligands $\mathbf{L_3}$−$\mathbf{L_5}$ into solutions of **1** equilibrated at −125 °C in 2-MeTHF cleanly yields dicopper(III) bis(μ-oxide) complexes **3**−**5**, respectively, unprecedented Cu(III)-dioxygen compounds ligated by imidazole ligands (Scheme 1). Compounds **3**−**5** each exhibit two characteristic, intense UV ligand-to-metal charge transfer (LMCT) transitions[15] (380−363 and 280−262 nm), attributable to dicopper(III) bis(μ-oxide) cores. However, these features are significantly blue-shifted with respect to the parent **1** with exclusive tertiary amine ligation (403 and 302 nm, vide infra) (Table 1). Cu K-edge X-ray absorption spectra (XAS) of frozen solutions of **3**−**5** show clean pre-edge transitions near 8981 eV, indicative of the Cu(III) oxidation state.[24] Modeling of the extended X-ray absorption fine structure (EXAFS) data is consistent with a short Cu−Cu scattering interaction in each case (**3**−**5**: 2.80, 2.81, 2.78 Å; Table 1), typical of the compact geometry of a dicopper(III) bis(μ-oxide) structure. Cu-imidazole multiple scattering contributions significantly improve the fit of the EXAFS data,[23] indicating histamine chelation. DFT optimized structures and time-dependent DFT calculated spectra support identification of **3**−**5** as the products of ligand substitutions by successfully reproducing trends in the experimental XAS metrical parameters and faithfully predicting the systematic blue-shifting of the LMCT optical bands with respect to **1** (Figure S12).

The strength of ligand−metal interactions of imidazole and NH_2 with copper is immediately discernible in the systematically blue-shifted optical spectra of **2**−**5** with respect to **1** (Figure S1). Careful inspection of TD-DFT optical transitions indicates that energetic perturbation of the accepting orbitals tunes the LMCT transitions characteristic of the dicopper(III) bis(μ-oxide) structure.[15,18] Compound **2** expectedly absorbs at higher energies than does **1** (λ_{max} = 375 vs 403 nm) because the primary amines of **2**, lacking the methyl substituents of **1**, associate more closely, forming stronger σ-interactions to the copper centers (2.00 vs 2.02 Å by EXAFS). Tighter associations raise the energy of the accepting orbitals and blue-shift the transitions. This effect is confirmed in the comparison of the LMCT transitions of **5** (λ_{max} = 363 nm) with **3** or **4** (λ_{max} = 380 nm), differing only in the degree of amine alkylation. Intriguingly, we find that imidazole binding leads to greater blue-shifting than does primary amine ligation, evidenced in the comparison of **5** with **2** (λ_{max} = 363 vs 375 nm); imidazole induces increased orbital perturbation by stronger σ-associations

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with Cu (**5**, Cu-N_{avg} (EXAFS) = 1.96 Å, Cu-N_{Imd} (DFT) = 1.91 Å; **2**, Cu−N (EXAFS) = 2.00 Å, Cu−N (DFT) = 1.94 Å). In fact, inspection of crystallographic databases reveals the vast majority of characterized histamine or histidine chelated Cu(II) structures exhibit significantly shorter Cu−N_{Imd} distances than Cu−N_{amino} distances, a trend common to other transition metal complexes as well.[25]

Strong donation of imidazole and NH_2 to Cu is well illustrated by the dynamics of core capture and the energetic stabilization of Cu(III) by imidazole. Core capture by ligands $\mathbf{L_3}$−$\mathbf{L_5}$ proceeds rapidly and leaves no detectable concentration of the parent **1** in equilibrium with **3**−**5**, indicating favorable kinetics and thermodynamics of ligand exchange at these low solutions temperatures. Stepwise titration of two equivalents of $\mathbf{L_3}$−$\mathbf{L_5}$ per **1**, monitored by *in situ* UV−vis spectroscopy, shows tight isosbestic conversion, without observation of an intermediate mixed-ligand species. Such behavior signifies an increase in the successive free energies of ligand exchange, with substantial stabilization of the imidazole-bonded species with respect to **1**. As previously stated, $\mathbf{L_2}$ effects core capture of **1** to form **2** with exclusive primary amine ligation. Compound **2** in turn is transformed to **3** when mixed with $\mathbf{L_3}$. The energetic preference for imidazole coordination to Cu(III) outcompetes even the favorable primary-amine binding over peralkylated-amine ligation; i.e., imidazole for $-NH_2$ substitution is favorable despite the energetic penalty of tertiary amine for $-NH_2$ substitution. The most thermodynamically stabilized complex under these conditions is **5**. $\mathbf{L_5}$ is capable of displacing $\mathbf{L_3}$, a reaffirmation of the affinity of copper for primary over peralkylated amines. DFT computed isodesmic exchange reactions fully corroborate these observations and predict the rank ordered stability of the complexes enumerated in Scheme 1 (cf. Figure S23).

Compounds **2**, **3**, and **5** are capable of oxidizing C−H bonds of exogenous substrates by a mechanism best described as H-atom abstraction (HAT), even at the extreme solution temperatures of −125 °C.[18] Under identical conditions, **1** exhibits no identifiable reaction rate with exogenous C−H substrates. First-order decay of **5** is observed in the presence of excess 9,10-dihydro-methylacridine (C−H BDE ≈ 74 kcal mol^{-1}, k = 4.6 M^{-1} s^{-1}, $KIE_{H/D}$ = 20), 9,10-dihydroanthracene (C−H BDE ≈ 76 kcal mol^{-1}, k = 2.5 × 10^{-1} M^{-1} s^{-1}, $KIE_{H/D}$ = 31, ca. 50% yield anthracene), 1,4-cyclohexadiene (C−H BDE ≈ 76 kcal mol^{-1}, k = 0.7 × 10^{-1} M^{-1} s^{-1}), and xanthene (BDE ≈ 76 kcal mol^{-1}, k = 2.0 × 10^{-1} M^{-1} s^{-1}, $KIE_{H/D}$ = 22).[26,27] The background thermal decay of **5** at −125 °C is not enhanced by addition of approximately 1700 equiv of toluene (C−H BDE ≈ 89 kcal mol^{-1}), potentially providing an upper-bound reactivity limit.

The first-order substrate kinetics and the large H/D kinetic isotope effects for each reaction strongly support C−H bond homolysis by HAT. However, the C−H bond strengths of the substrates explored here are not comparable to that in methane (104 kcal mol^{-1}). While C−H activation nearly 150 °C below room temperature is extremely rare, the kinetics and inability to oxidize more difficult substrates could potentially rule out the dicopper(III) bis(μ-oxide) as the active oxidant in pMMO, in favor of further activated intermediates succeeding the initial oxygenation product. Nevertheless, it is interesting to note that compound **3** also oxidizes 9,10-dihydro-methylacridine by a similar mechanism ($KIE_{H/D}$ = 14), albeit at a rate more than ten times slower than that measured for **5** ($k_5/k_3 \approx 12$). Compound **2**, with exclusive primary-amine ligation, reacts comparably to **5** (k_2 = 9.2 M^{-1} s^{-1}, $k_2/k_5 \approx 2$). Space-filling models show substrate access to the electrophilic oxides of the Cu_2O_2 core is impaired by the presence of methyl groups of **3**, in contrast to **2** and **5** (Figure S22). DFT transition state optimizations of the reaction of **5** with cyclohexadiene clearly show a linear approach of the substrate C−H bond, to the O−O vector in the lowest energy pathway; this association is hindered by amine alkylation (Figure S20).[18] Primary amine ligation in pMMO could allow fast kinetics with a strong substrate C−H bond by providing substrate accessibility to oxide ligands of the active oxidant intermediate.

Compounds **3**−**5** are significant to the discussion of the Cu(III) oxidation state in biology in which histidine imidazole is a common ligand.[9] In no study of metalloenzymes has Cu(III) been observed, even transiently, even though examples of Cu(III)−O_2 compounds now far outnumber oxygenated Cu(II) complexes in synthetic chemistry.[15] This disparity is often rationalized by the use of electronically stabilizing donors such as tertiary amines, guanidines, and anionic nitrogen in synthetic Cu_2O_2 chemistry, while biology employs the considerably less basic imidazole group. It is hypothesized by others that stabilization of a Cu(III) complex with biological ligation is energetically inaccessible.[19,20] Compounds **3**−**5** undermine the generality of this hypothesis and suggest preferential stabilization, with respect to other binuclear intermediates, of a dicopper(III) bis(μ-oxide) oxygenated species in the active site environment of pMMO.

The spectroscopic analysis above is relevant to the characterization of oxygenated pMMO:[12] a Cu(III) intermediate bonded by three imidazoles and a primary amine is predicted to exhibit optical transitions at even higher energies than those of **5** at 363 nm. This feature approaches the high energy absorbances of isomeric dicopper(II) structures, e.g., μ−η^2:η^2-side-on peroxides of oxy-tyrosinase and oxy-hemocyanin, a hypothesized origin of the 345 nm feature observed by Rosenzweig in oxygenated pMMO samples.[9,12] Future optical spectroscopy of a potential dicopper(III) oxygenated state of pMMO may not be congruent with typical synthetic dicopper(III) bis(μ-oxide) complexes known in synthetic literature nor definitive of an active-site structure.

The identification of the binuclear reactive center of pMMO and other homologous copper membrane monooxygenases introduces a new and distinct class of active-site environments of dioxygen-activating copper enzymes. The tris(imidazole) coordination of Cu(I) in tyrosinase, hemocyanin, and NspF and their ca. 4.10 Å Cu−Cu separation[9] predispose the active-sites toward stabilization of oxygenated dicopper(II) μ−η^2:η^2-peroxide intermediates (Cu−Cu ca. 3.50 Å). Synthetic model complexes reproducing this type of imidazole coordination either self-assemble or preferentially form Cu(II)−peroxide species.[23,28] The coordination environment in pMMO is unlike that in other well-characterized copper enzymes. Primary amine-to-metal ligation is in itself uncommon in biology, though this class of ligation is emerging in the literature, mostly from the dioxygen-dependent mononuclear copper polysaccharide monooxygenases.[29−32]

Given the postulate of a binuclear copper active site of pMMO,[7] we hypothesize initial formation of a dicopper(III) bis(μ-oxide) oxygenated intermediate, in preference to other dicopper(II) isomers, before C−H oxidation. Homolytic O−O bond cleavage and oxidation to high-valency would unify dioxygen activation by methane monooxygenases.[5] In pMMO, limited ligation of imidazole and primary amine groups, along with the short 2.60 Å Cu−Cu separation, are more conducive to the formation of a Cu(III)−Cu(III) state. The chief consid-

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eration is the viability and energetic accessibility of Cu(III) in a protein medium. Generally, Cu(III) is reasonably excluded in most mononuclear systems due to very positive reduction potentials, which lie outside the biological window. However, this presumption may not be appropriate in the context of strong donation from two oxide ligands within a planar dimeric copper site, fully congruent with the structural preference of Cu(III), a d^8 metal. In fact, experimentally calibrated, DFT isodesmic redox calculations reasonably estimate the standard reduction potential of **5** at 750 mV (Figure S26), equivalent to or below the functional (II/I) potential of the blue copper site of fungal laccase (780 mV vs NHE[33]) and well within a conservative estimate of the biological window. Additionally the characterization of compounds **3**−**5** positively demonstrates that the dicopper(III) bis(μ-oxide) core is indeed thermodynamically stable with respect to Cu(I) and dioxygen in the presence of histamine ligands. It follows from the enhanced core stability of successive imidazole for primary amine substitutions, observed in ligand competition experiments, that a dicopper(III) bis(μ-oxide) is a thermodynamically plausible intermediate with ligation by three imidazoles and one primary amine and with the compact 2.60 Å Cu−Cu separation within the active site. In a recent computational study of C−H activation in pMMO by a proposed reactive Cu(II)−(O)(OH)−Cu(III) active oxidant, Yoshizawa and Shiota favor exclusion of a bis(μ-oxide) in preference to dioxygen activation to a dicopper(II) μ−η^2:η^2-peroxide adduct.[22] However, this result might be attributable to axial coordination of a distant glutamate residue (Cu−O(Glu35) = 4.38 Å), a disputable active site model.[16]

The thermodynamics, reduction potentials, tight Cu−N associations, and strong ligand donation exhibited in the complexes explored here provide the first chemically plausible experimental evidence of the ability of pMMO-like coordination to stabilize a high-valent Cu(III) dioxygen intermediate and beg for the introduction of the Cu(III) state to the biological canon of copper redox chemistry. Enhancement of C−H oxidation kinetics by an unknown active oxidant may be an attendant benefit of minimal ligation in this active site.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and results, computational details, and spectroscopic data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b02157.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lieberman, R. L.; Rosenzweig, A. C. *Nature* **2005**, *434*, 177.
(2) Bollinger, J. M., Jr. *Nature* **2010**, *465*, 40.
(3) Lieberman, R. L.; Shrestha, D. B.; Doan, P. E.; Hoffman, B. M.; Stemmler, T. L.; Rosenzweig, A. C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3820.
(4) Smith, S. M.; Rawat, S.; Telser, J.; Hoffman, B. M.; Stemmler, T. L.; Rosenzweig, A. C. *Biochemistry* **2011**, *50*, 10231.
(5) Banerjee, R.; Proshlyakov, Y.; Lipscomb, J. D.; Proshlyakov, D. A. *Nature* **2015**, *518*, 431.
(6) Hermans, I.; Spier, E.; Neuenschwander, U.; Turrà, N.; Baiker, A. *Top. Catal.* **2009**, *52*, 1162.
(7) Balasubramanian, R.; Smith, S. M.; Rawat, S.; Yatsunyk, L. A.; Stemmler, T. L.; Rosenzweig, A. C. *Nature* **2010**, *465*, 115.
(8) Himes, R. A.; Barnese, K.; Karlin, K. D. *Angew. Chem., Int. Ed.* **2010**, *49*, 6714.
(9) Solomon, E. I.; Heppner, D. E.; Johnston, E. M.; Ginsbach, J. W.; Cirera, J.; Qayyum, M.; Kieber-Emmons, M. T.; Kjaergaard, C. H.; Hadt, R. G.; Tian, L. *Chem. Rev.* **2014**, *114*, 3659.
(10) Lawton, T. J.; Ham, J.; Sun, T.; Rosenzweig, A. C. *Proteins: Struct.. Funct.. Bioinf.* **2014**, *82*, 2263.
(11) Liew, E. F.; Tong, D.; Coleman, N. V.; Holmes, A. J. *Microbiology* **2014**, *160*, 1267.
(12) Culpepper, M. A.; Cutsail, G. E.; Hoffman, B. M.; Rosenzweig, A. C. *J. Am. Chem. Soc.* **2012**, *134*, 7640.
(13) Lieberman, R. L.; Kondapalli, K. C.; Shrestha, D. B.; Hakemian, A. S.; Smith, S. M.; Telser, J.; Kuzelka, J.; Gupta, R.; Borovik, A. S.; Lippard, S. J.; Hoffman, B. M.; Rosenzweig, A. C.; Stemmler, T. L. *Inorg. Chem.* **2006**, *45*, 8372.
(14) Rosenzweig, A. C.; Sazinsky, M. H. *Curr. Opin. Struct. Biol.* **2006**, *16*, 729.
(15) Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013.
(16) Balasubramanian, R.; Rosenzweig, A. C. *Acc. Chem. Res.* **2007**, *40*, 573.
(17) Culpepper, M. A.; Rosenzweig, A. C. *Crit. Rev. Biochem. Mol. Biol.* **2012**, *47*, 483.
(18) Citek, C.; Lin, B.-L.; Phelps, T. E.; Wasinger, E. C.; Stack, T. D. P. *J. Am. Chem. Soc.* **2014**, *136*, 14405.
(19) Kaim, W.; Rall, J. *Angew. Chem., Int. Ed.* **1996**, *35*, 43.
(20) Crichton, R. R.; Pierre, J. L. *BioMetals* **2001**, *14*, 99.
(21) Himes, R. A.; Karlin, K. D. *Curr. Opin. Chem. Biol.* **2009**, *13*, 119.
(22) Shiota, Y.; Juhász, G.; Yoshizawa, K. *Inorg. Chem.* **2013**, *52*, 7907.
(23) Citek, C.; Lyons, C. T.; Wasinger, E. C.; Stack, T. D. P. *Nat. Chem.* **2012**, *4*, 317.
(24) DuBois, J. L.; Mukherjee, P.; Collier, A. M.; Mayer, J. M.; Solomon, E. I.; Hedman, B.; Stack, T. D. P.; Hodgson, K. O. *J. Am. Chem. Soc.* **1997**, *119*, 8578.
(25) Allen, F. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *58*, 380.
(26) Larsen, A. S.; Wang, K.; Lockwood, M. A.; Rice, G. L.; Won, T. J.; Lovell, S.; Sadilek, M.; Turcek, F.; Mayer, J. M. *J. Am. Chem. Soc.* **2002**, *28*, 10112.
(27) Warren, J. J.; Tronic, T. A.; Mayer, J. M. *Chem. Rev.* **2010**, *110*, 6961.
(28) Lynch, W. E.; Kurtz, D. M.; Wang, S. K.; Scott, R. A. *J. Am. Chem. Soc.* **1994**, *116*, 11030.
(29) Quinlan, R. J.; Sweeney, M. D.; Lo Leggio, L.; Otten, H.; Poulsen, J.-C. N.; Johansen, K. S.; Krogh, K. B. R. M.; Jørgensen, C. I.; Tovborg, M.; Anthonsen, A.; Tryfona, T.; Walter, C. P.; Dupree, P.; Xu, F.; Davies, G. J.; Walton, P. H. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 15079.
(30) Hemsworth, G. R.; Henrissat, B.; Davies, G. J.; Walton, P. H. *Nat. Chem. Biol.* **2014**, *10*, 122.
(31) Hemsworth, G. R.; Taylor, E. J.; Kim, R. Q.; Gregory, R. C.; Lewis, S. J.; Turkenburg, J. P.; Parkin, A.; Davies, G. J.; Walton, P. H. *J. Am. Chem. Soc.* **2013**, *135*, 6069.
(32) Li, X.; Beeson, Iv; William, T.; Phillips; Christopher, M.; Marletta; Michael, A.; Cate; Jamie, H. D. *Structure* **2012**, *20*, 1051.
(33) Vernon, T. T.; Malmström, B. G.; Anson, F. C.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3387.

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