

Heme/Copper Assembly Mediated Nitrite and Nitric Oxide Interconversion

Shabnam Hematian, Maxime A. Siegler, and Kenneth D. Karlin*

Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218, United States

S Supporting Information

ABSTRACT: The heme_{a3}/Cu_B active site of cytochrome *c* oxidase is responsible for cellular nitrite reduction to nitric oxide; the same center can return NO to the nitrite pool via oxidative chemistry. Here, we show that a partially reduced heme/Cu assembly reduces NO₂[−] ion, producing nitric oxide. The heme serves as the reductant, but the Cu^{II} ion is also required. In turn, a μ-oxo heme-Fe^{III}–O–Cu^{II} complex facilitates NO oxidation to nitrite; the final products are the reduced heme and Cu^{II}–nitrito complexes.

Nitrogen oxides (NO_x) are components of great interest in both biological and environmental sciences. Nitric oxide (NO) is an important cellular signaling molecule and a powerful vasodilator involved in many physiological and pathological processes.¹ Nitrite (NO₂[−]) is the one-electron-oxidized product of endogenous NO metabolism. Recent studies indicate that nitrite plays a critical biological role by serving as a biochemical circulating reservoir for NO, in particular under conditions of physiologic hypoxia (low O₂ tensions; see also below) and ischemia. The nitrite-to-NO conversion represents an important alternative source of NO to the classical oxygen-dependent L-arginine-derived generation of NO catalyzed by nitric oxide synthase (NOS).² Subsequently, suggested conserved roles for the NO₂[−]/NO pool in cellular processes include oxygen sensing and oxygen-dependent modulation of intermediary metabolism.³ It is now considered that in order to stimulate NO signaling, nitrite reductase activity occurs widely in differing cellular environments and is effected by a variety of proteins/enzymes, including hemes, those with molybdenum,⁴ and what draws our current interest, cytochrome *c* oxidases (CcO's).^{3,5}

The link between nitrite/NO redox interconversion and O₂ sensing is thought to occur in mitochondria at the CcO binuclear heme_{a3}/Cu_B center; CcO is the terminal enzyme of the mitochondrial respiratory chain. Here, molecular oxygen consumption (i.e., O₂ reduction to water) is down-regulated in hypoxia by increased NO generation via CcO nitrite reductase activity, as reduced heme/Cu centers dominate when the O₂ concentration is low.^{3a,4,6} The NO thus generated inhibits CcO activity by reversibly binding to heme_{a3} in place of O₂, resulting in cellular O₂ accumulation (Figure 1). Some of the NO produced also participates in hypoxic signaling, the up-regulation of nuclear genes needed in response to the inherent dangers of low cellular O₂ concentrations.³

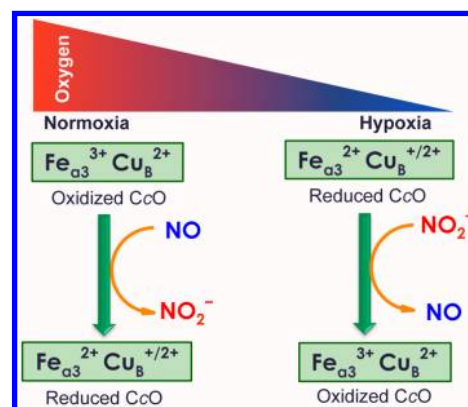
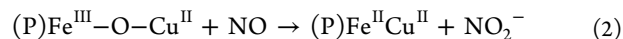
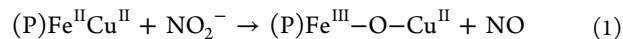


Figure 1. Cytochrome *c* oxidase (CcO) functioning in nitrite (NO₂[−])/nitric oxide (NO) interconversion as part of its role in regulation of O₂ balance. The availability of O₂ influences the redox state of the CcO containing the heme_{a3}/Cu_B binuclear center.

In turn, in normoxia, high local O₂ concentrations do not allow nitrite to compete as an oxidant at the CcO binuclear center; thus, NO/O₂ binding is noncompetitive,⁷ and heme_{a3}/Cu_B oxidizes NO back to nitrite (Figure 1) to rejoin the storage pool. NO is thought to first attack oxidized Cu_B, formally giving Cu^I–NO⁺; the latter hydrolyzes to nitrite.^{8,9}

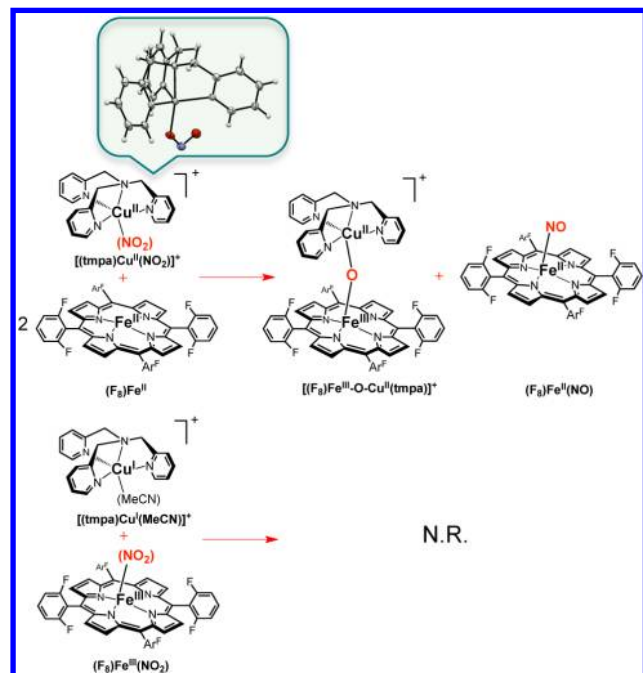
In this report, we describe a chemical system involving a heme/Cu assembly-mediated interconversion of these important nitrogen oxides. A partially reduced/oxidized state, with reduced heme and oxidized copper ion (i.e., Fe^{III}...Cu^{II}) efficiently converts nitrite to NO. When a fully oxidized Fe^{III}...Cu^{II} heme/Cu complex is employed, NO is readily oxidized to nitrite. The overall reactions are represented by eqs 1 and 2:



The nitrite reductase chemistry,¹⁰ here however in a heme/Cu chemical system, consisted of the iron(II) complex (F₈)Fe^{II} [F₈ ≡ tetrakis(2,6-difluorophenyl)porphyrinate(2−)]¹¹ and a preformed copper(II)–nitrito complex [(tmpa)Cu^{II}(NO₂)] [B-(C₆F₅)₄] [tmpa ≡ tris(2-pyridylmethyl)amine]; the latter was synthesized by adding AgNO₂ to a chloride precursor [(tmpa)Cu^{II}(Cl)] [B-(C₆F₅)₄], and its X-ray structure revealed an O-bound nitrito ligated Cu(II) ion (Scheme 1).¹²

Received: August 23, 2012

Scheme 1. Heme/Copper Assembly-Mediated Reduction of Nitrite to Nitric Oxide



When 2 equiv of $(F_8)Fe^{II}$ were mixed with 1 equiv of $[(tmpa)Cu^{II}(NO_2)]^+$ under a N_2 atmosphere in acetone at room temperature (RT), a reaction ensued, and on the basis of UV-vis, electron paramagnetic resonance (EPR), and IR spectroscopies, a 1:1 mixture of the heme-nitrosyl species $(F_8)Fe^{II}(NO)$ and the μ -oxo complex $[(F_8)Fe^{III}-O-Cu^{II}(tmpa)]^+$ were produced.¹² These products were readily identified, having been previously thoroughly characterized.¹³ To determine whether the heme or the copper ion is the reductant in this one-electron process ($NO_2^- \rightarrow NO$), we also carried out the reaction in which nitrite was added to the oxidized heme complex $[(F_8)Fe^{III}]SbF_6^{13b}$ (binding of nitrite to the ferric heme¹⁴ was indicated by a large UV-vis change)¹² and then the reduced complex $[(tmpa)Cu^I(MeCN)]^+$ ^{12,15} was added. In this case there was no reaction (Scheme 1), even over a period of days.¹² Control experiments showed that nitrite reacts only very slowly with $(F_8)Fe^{II}$ and not at all with $[(tmpa)Cu^I(MeCN)]^+$. Moreover, no nitrite reductase activity was observed for the fully reduced metal combination, nitrite plus $(F_8)Fe^{II}$ and $[(tmpa)Cu^I(MeCN)]^+$.¹²

These observations indicate that the heme is the reductant in this heme/Cu nitrite reductase chemistry. The need for 2 equiv of $(F_8)Fe^{II}$ is due to the well-known high affinity of NO to bind ferrous hemes.¹⁶ The initially formed NO reacts very rapidly with $(F_8)Fe^{II}$; thus, if the reaction were carried out with equimolar quantities of $(F_8)Fe^{II}$ and $[(tmpa)Cu^{II}(NO_2)]^+$, only half of the iron would be available to reduce nitrite, and the rest would trap the NO as $(F_8)Fe^{II}(NO)$. The role of the Cu^{II} ion appears to be to provide a Lewis acid interaction with nitrite, facilitating NO_2^- (N–O) bond cleavage and stabilization of the resulting oxo anion via eventual formation of $[(F_8)Fe^{III}-O-Cu^{II}(tmpa)]^+$.

To demonstrate that heme/copper assemblies can mediate NO oxidation to nitrite, as occurs biologically in order to remove excess NO when it is not needed and return it to the nitrite pool (see above), we employed $[(F_8)Fe^{III}-O-Cu^{II}(tmpa)]^+$. Addition of NO to this fully oxidized

Scheme 2. Heme/Copper Assembly-Mediated Oxidation of Nitric Oxide to Nitrite

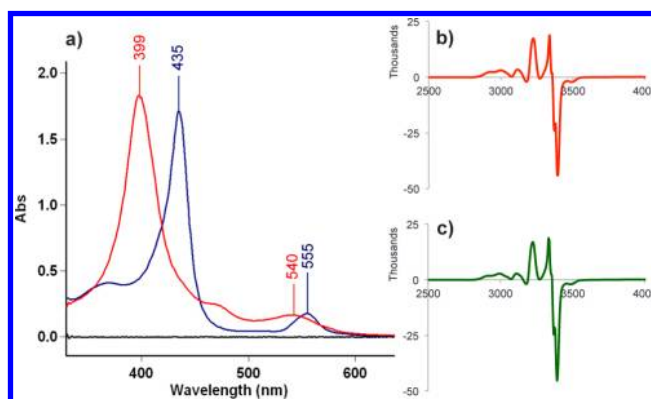
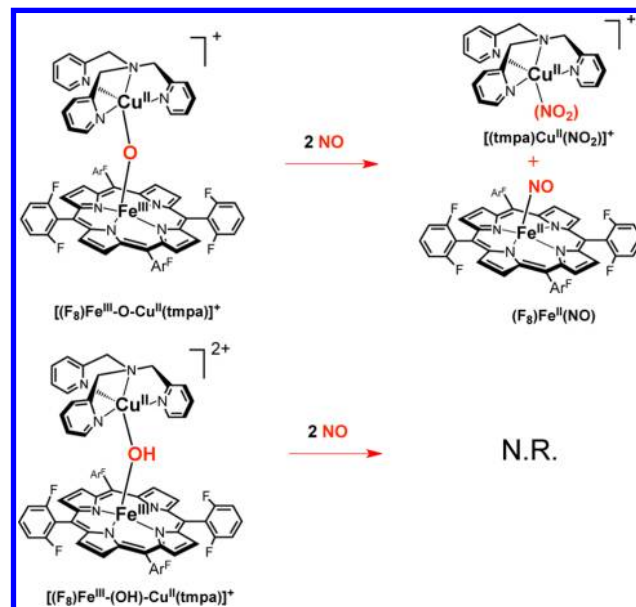


Figure 2. (a) UV-vis spectra of $(F_8)Fe^{III}-O-Cu^{II}(tmpa)[B(C_6F_5)_4]$ (**1**) (blue) and $(F_8)Fe^{II}(NO)$ generated from **1** + $NO(g)$ ($12 \mu M$ in acetone at RT) (red). (b, c) EPR spectra of (b) the products of the reaction of $(F_8)Fe^{III}-O-Cu^{II}(tmpa)[B(C_6F_5)_4]$ and NO (red) and (c) an authentic sample of a 1:1 mixture of $(F_8)Fe^{II}(NO)$ and $[(tmpa)Cu^{II}(NO_2)][B(C_6F_5)_4]$ (green). The EPR spectra were recorded at 20 K (1 mM in MeTHF).

heterobinuclear complex led to rapid reaction (Scheme 2) and formation of nitrite, which bound to $Cu(II)$; the $(F_8)Fe^{II}$ formed¹⁷ in this redox reaction was trapped by a second equivalent of NO to give $(F_8)Fe^{II}(NO)$. UV-vis (Figure 2) and IR ($\nu_{NO} = 1688 \text{ cm}^{-1}$)¹² spectroscopies directly indicated nitrosyl complex formation. Nitrite analysis employing capillary electrophoresis revealed that this ion was produced in 95% yield.¹² EPR spectroscopy confirmed that a copper(II)–nitrito complex was produced (Figure 2); a sample taken from the reaction mixture was identical in all regards to that of an authentic sample of a 1:1 mixture of $(F_8)Fe^{II}(NO)$ and $[(tmpa)Cu^{II}(NO_2)]^+$.

It is important to explain why the reaction required 2 molar equiv of NO (Scheme 2). The second equivalent was not involved in the redox chemistry but was needed to trap the free $(F_8)Fe^{II}$ produced by the NO oxidase chemistry. When $(F_8)Fe^{II}$ is present, it effects the reverse reaction, namely, reduction of $Cu(II)$ -bound nitrite to give NO (Scheme 1). This was

demonstrated as follows: When 25 mL of a 10 μM solution containing a mixture of $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$ and $[(\text{tmpa})\text{Cu}^{\text{II}}(\text{NO}_2)]^+$ produced by the reaction of $[(\text{F}_8)\text{Fe}^{\text{III}}\text{O}-\text{Cu}^{\text{II}}(\text{tmpa})]^+$ with excess NO (green spectrum in Figure 3) was

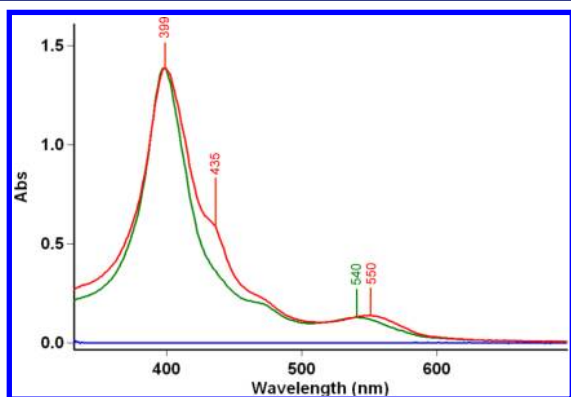


Figure 3. UV-vis spectra of a mixture of $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$ ($\lambda_{\text{max}} = 399$ nm) and $[(\text{tmpa})\text{Cu}^{\text{II}}(\text{NO}_2)]^+$ derived from the NO oxidase chemistry before (green) and after (red) the addition of 2 equiv of $(\text{F}_8)\text{Fe}^{\text{II}}$. The red spectrum shows the presence of a 2:1 mixture of $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$ and $[(\text{F}_8)\text{Fe}^{\text{III}}\text{O}-\text{Cu}^{\text{II}}(\text{tmpa})]^+$ (435 nm, sh). The new second equivalent of $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$ was derived from the nitrite reductase chemistry described by Scheme 1. See the text for further explanation.

titrated with 25 mL of a 20 μM solution (i.e., 2 equiv) of $(\text{F}_8)\text{Fe}^{\text{II}}$, the product solution (red spectrum in Figure 3) contained $\sim 5 \mu\text{M}$ $[(\text{F}_8)\text{Fe}^{\text{III}}\text{O}-\text{Cu}^{\text{II}}(\text{tmpa})]^+$ along with 10 μM $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$; the spectral intensity was equivalent to that observed in the starting mixture because of dilution. This proves that the backward reaction can and does occur, that is, that $[(\text{tmpa})\text{Cu}^{\text{II}}(\text{NO}_2)]^+$ reacts first with $(\text{F}_8)\text{Fe}^{\text{II}}$ in a 1:1 stoichiometry to give NO, which is then trapped by the second equivalent of $(\text{F}_8)\text{Fe}^{\text{II}}$.

We also tested the μ -hydroxo complex $[(\text{F}_8)\text{Fe}^{\text{III}}(\text{OH})-\text{Cu}^{\text{II}}(\text{tmpa})]^{2+}$ for “NO oxidase” chemistry, but upon addition of NO, there was no nitrite production (Scheme 2).¹² Instead, very slow (hours) reductive nitrosylation¹⁸ occurred, and all of the heme present was converted to $(\text{F}_8)\text{Fe}^{\text{II}}(\text{NO})$. It is thus clear that the μ -oxo complex $[(\text{F}_8)\text{Fe}^{\text{III}}\text{O}-\text{Cu}^{\text{II}}(\text{tmpa})]^+$ is efficient or at least special in its ability to effect a redox reaction (formally $\text{Fe}^{\text{III}} \rightarrow \text{Fe}^{\text{II}}$) that includes oxo transfer.¹⁹

In summary, this report has described new chemistry with heme/Cu assemblies and nitrogen oxide interconversion: nitrite reduction to nitric oxide can readily be effected with our heme/copper chemistry. The reduced heme is the source of the one electron required. The presence of Cu^{II} ion as a Lewis acid is crucial. While nitrite reduction to NO is well-known to occur via heme proteins such as hemoglobin and myoglobin,²⁰ bacteria/fungal heme cd_1 ²¹ or copper nitrite reductases,²² and certain copper(I) complexes,^{22a,23} it appears that the transformation with heme/Cu synthetic complexes has not been examined to date. We have shown here that both heme and Cu are required, at least in our system. It is notable that the heme is the reductant on the basis of the observed products; however, cyclic voltammetric determination of the redox potentials for the separate complexes $(\text{F}_8)\text{Fe}$ (-0.20 V vs Fc^+/Fc)¹² and $\text{Cu}(\text{tmpa})$ (-0.42 V vs Fc^+/Fc)¹² indicate the latter is a better reductant. For CcO, the opposite appears to be the case, as the heme_{a3} has a lower redox potential than does Cu_B .²⁴

On the other hand, the heme is also the redox entity, as $[(\text{F}_8)\text{Fe}^{\text{III}}\text{O}-\text{Cu}^{\text{II}}(\text{tmpa})]$ effects oxidation of NO to nitrite

by oxo transfer (see above)¹⁹ with reduction of $(\text{F}_8)\text{Fe}^{\text{III}}$. The closely related species $[(\text{F}_8)\text{Fe}^{\text{III}}(\text{OH})-\text{Cu}^{\text{II}}(\text{tmpa})]^+$ and/or heme-only complexes do not enable this reaction. However, as already mentioned, it is the Cu_B in CcO that is thought to oxidize NO,⁹ and it is well-known that NO can react with Cu^{II} complexes,²⁵ affording nitrite. This may imply that in CcO it is a particular coordination environment, a specific structural and/or redox state, that is required for mediation of the NO oxidase chemistry.

Further investigations will include our probing of the mechanisms of the reactions described in this report. For nitrite reduction, critical mechanistic components will certainly include the heme reductive capability and the nitrite-Cu binding mode (e.g., O- vs O'- vs N-bound). As our heme and Cu centers have switched redox capabilities compared with CcO, we also wish to change the heme or the Cu ligand to make the Cu center a better oxidant than the heme. For our heme/Cu NO oxidation chemistry, we are uncertain about which metal is the real oxidant,¹⁸ so further investigations are required; as is often the case, “the devil is in the details”.

■ ASSOCIATED CONTENT

📄 Supporting Information

Synthetic and analytical details; UV-vis, IR, and EPR spectra; cyclic voltammetry and capillary electrophoresis results; and X-ray structural details and CIF files. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

karlin@jhu.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (GM060353 to K.D.K.). We also thank Dr. Richard C. Himes for help in synthesizing $[(\text{tmpa})\text{Cu}^{\text{II}}(\text{Cl})][\text{B}(\text{C}_6\text{F}_5)_4]$.

■ REFERENCES

- (1) (a) Ignarro, L. J. Nitric Oxide as a Communication Signal in Vascular and Neuronal Cells. In *Nitric Oxide: Principles and Actions*; Lancaster, J., Ed.; Academic Press: New York, 1996; p 111. (b) Schopfer, M. P.; Wang, J.; Karlin, K. D. *Inorg. Chem.* **2010**, *49*, 6267.
- (2) (a) Samouilov, A.; Kuppusamy, P.; Zweier, J. L. *Arch. Biochem. Biophys.* **1998**, *357*, 1. (b) Dezfulian, C.; Raat, N.; Shiva, S.; Gladwin, M. T. *Cardiovasc. Res.* **2007**, *75*, 327.
- (3) (a) Castello, P. R.; David, P. S.; McClure, T.; Crook, Z.; Poyton, R. O. *Cell Metab.* **2006**, *3*, 277. (b) Feelisch, M.; Fernandez, B. O.; Bryan, N. S.; Garcia-Saura, M. F.; Bauer, S.; Whitlock, D. R.; Ford, P. C.; Janero, D. R.; Rodriguez, J.; Ashrafian, H. *J. Biol. Chem.* **2008**, *283*, 33927.
- (4) van Faassen, E. E.; Bahrami, S.; Feelisch, M.; Hogg, N.; Kelm, M.; Kim-Shapiro, D. B.; Kozlov, A. V.; Li, H.; Lundberg, J. O.; Mason, R.; Nohl, H.; Rassaf, T.; Samouilov, A.; Slama-Schwok, A.; Shiva, S.; Vanin, A. F.; Weitzberg, E.; Zweier, J.; Gladwin, M. T. *Med. Res. Rev.* **2009**, *29*, 683.
- (5) (a) Sarti, P.; Giuffrè, A.; Barone, M. C.; Forte, E.; Mastronicola, D.; Brunori, M. *Free Radic. Biol. Med.* **2003**, *34*, 509. (b) Gupta, K. J.; Igamberdiev, A. U. *Mitochondrion* **2011**, *11*, 537. (c) Sarti, P.; Forte, E.; Mastronicola, D.; Giuffrè, A.; Arese, M. *Biochim. Biophys. Acta* **2012**, *1817*, 610. (d) Curtis, E.; Hsu, L. L.; Noguchi, A. C.; Geary, L.

- Shiva, S. *Antioxid. Redox Signal.* **2012**, *17*, 951. (e) Toledo, J. C.; Augusto, O. *Chem. Res. Toxicol.* **2012**, *25*, 975.
- (6) Poyton, R. O.; Castello, P. R.; Ball, K. A.; Woo, D. K.; Pan, N. *Ann. N.Y. Acad. Sci.* **2009**, *1177*, 48.
- (7) Mason, M. G.; Nicholls, P.; Wilson, M. T.; Cooper, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 708.
- (8) (a) Antunes, F.; Boveris, A.; Cadenas, E. *Antioxid. Redox Signal.* **2007**, *9*, 1569. (b) Torres, J.; Sharpe, M. A.; Rosquist, A.; Cooper, C. E.; Wilson, M. T. *FEBS Lett.* **2000**, *475*, 263.
- (9) (a) The multicopper enzyme ceruloplasmin has also been shown to effect NO oxidase ($\text{NO} \rightarrow \text{NO}_2^-$) chemistry. (b) Shiva, S.; Wang, X.; Ringwood, L. A.; Xu, X.; Yuditskaya, S.; Annavajjhala, V.; Miyajima, H.; Hogg, N.; Harris, Z. L.; Gladwin, M. T. *Nat. Chem. Biol.* **2006**, *2*, 486. (c) Paradis, M.; Gagné, J.; Mateescu, M.-A.; Paquin, J. *Free Radic. Biol. Med.* **2010**, *49*, 2019.
- (10) (a) Castiglione, N.; Rinaldo, S.; Giardina, G.; Stelitano, V.; Cutruzzola, F. *Antioxid. Redox Signal.* **2012**, *17*, 684. (b) Merkle, A. C.; Lehnert, N. *Dalton Trans.* **2012**, *41*, 3355.
- (11) (a) Kopf, M.-A.; Neuhold, Y.-M.; Zuberbühler, A. D.; Karlin, K. D. *Inorg. Chem.* **1999**, *38*, 3093. (b) Ghiladi, R. A.; Kretzer, R. M.; Guzei, I.; Rheingold, A. L.; Neuhold, Y.-M.; Hatwell, K. R.; Zuberbühler, A. D.; Karlin, K. D. *Inorg. Chem.* **2001**, *40*, 5754.
- (12) See the Supporting Information.
- (13) (a) Wang, J.; Schopfer, M. P.; Sarjeant, A. A. N.; Karlin, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 450. (b) Wang, J.; Schopfer, M. P.; Puiu, S. C.; Sarjeant, A. A. N.; Karlin, K. D. *Inorg. Chem.* **2010**, *49*, 1404. (c) Karlin, K. D.; Nanthakumar, A.; Fox, S.; Murthy, N. N.; Ravi, N.; Huynh, B. H.; Orosz, R. D.; Day, E. P. *J. Am. Chem. Soc.* **1994**, *116*, 4753. (d) Fox, S.; Nanthakumar, A.; Wikström, M.; Karlin, K. D.; Blackburn, N. J. *J. Am. Chem. Soc.* **1996**, *118*, 24.
- (14) (a) Finnegan, M. G.; Lappin, A. G.; Scheidt, W. R. *Inorg. Chem.* **1990**, *29*, 181. (b) Wyllie, G. R. A.; Scheidt, W. R. *Chem. Rev.* **2002**, *102*, 1067.
- (15) Lucas, H. R.; Meyer, G. J.; Karlin, K. D. *J. Am. Chem. Soc.* **2010**, *132*, 12927.
- (16) Cooper, C. E. *Biochim. Biophys. Acta* **1999**, *1411*, 290.
- (17) By analogy to what has in the past been suggested for the enzyme reaction,^{8b} nitric oxide attacks at the cupric center. Formally, this affords $\text{Cu}^{\text{I}}\text{-NO}^+$, to which oxo transfer occurs, along with electron transfer from Cu^{I} to Fe^{III} to give the $\text{Cu}^{\text{II}}\text{-nitrito}$ complex and Fe^{II} , which is then trapped by a second NO molecule. Separately, we can demonstrate that with the present complexes $[(\text{tmpa})\text{Cu}^{\text{I}}(\text{MeCN})]^+$ and $[(\text{F}_8)\text{Fe}^{\text{III}}]\text{SbF}_6$, the Cu^{I} -to- Fe^{III} electron transfer readily occurs in acetone (see the Supporting Information).
- (18) (a) Ford, P. C. *Inorg. Chem.* **2010**, *49*, 6226. (b) Fernandez, B. O.; Lorkovic, I. M.; Ford, P. C. *Inorg. Chem.* **2003**, *42*, 2.
- (19) Man, W.-L.; Lam, W. W. Y.; Ng, S.-M.; Tsang, W. Y. K.; Lau, T.-C. *Chem.—Eur. J.* **2012**, *18*, 138.
- (20) (a) Doyle, R. P.; Pickering, R. A.; Deweert, T. M.; Hoekstra, J. W.; Pater, D. J. *Biol. Chem.* **1981**, *256*, 12393. (b) Gladwin, M. T.; Grubina, R.; Doyle, M. P. *Acc. Chem. Res.* **2009**, *42*, 157. (c) Shiva, S.; Huang, Z.; Grubina, R.; Sun, J. H.; Ringwood, L. A.; MacArthur, P. H.; Xu, X. L.; Murphy, E.; Darley-Usmar, V. M.; Gladwin, M. T. *Circ. Res.* **2007**, *100*, 654. (d) Yi, J.; Heinecke, J.; Tan, H.; Ford, P. C.; Richter-Addo, G. B. *J. Am. Chem. Soc.* **2009**, *131*, 18119.
- (21) (a) Averill, B. A. *Chem. Rev.* **1996**, *96*, 2951. (b) Zumft, W. G. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533. (c) Lehnert, N.; Berto, T. C.; Galinato, M. G. I.; Goodrich, L. E. The Role of Heme–Nitrosyls in the Biosynthesis, Transport, Sensing, and Detoxification of Nitric Oxide (NO) in Biological Systems: Enzymes and Model Complexes. In *The Handbook of Porphyrin Science*; Kadish, K. M., Smith, K., Guillard, R., Eds.; World Scientific: Singapore, 2011; Vol. 14, pp 1–247.
- (22) (a) Wasser, I. M.; de Vries, S.; Moënné-Loccoz, P.; Schröder, L.; Karlin, K. D. *Chem. Rev.* **2002**, *102*, 1201. (b) Merkle, A. C.; McQuarters, A. B.; Lehnert, N. *Dalton Trans.* **2012**, *41*, 8047.
- (23) (a) Hsu, S. C. N.; Chang, Y.-L.; Chuang, W.-J.; Chen, H.-Y.; Lin, I. J.; Chiang, M. Y.; Kao, C.-L.; Chen, H.-Y. *Inorg. Chem.* **2012**, *51*, 9297. (b) Kumar, M.; Dixon, N. A.; Merkle, A. C.; Zeller, M.; Lehnert, N.; Papish, E. T. *Inorg. Chem.* **2012**, *51*, 7004.
- (24) (a) Nicholls, P.; Wrigglesworth, J. M. *Ann. N.Y. Acad. Sci.* **1988**, *550*, 59. (b) Moskovitz, J. *Biochim. Biophys. Acta* **2005**, *1703*, 213. (c) Gorbikova, E. A.; Vuorilehto, K.; Wikstrom, M.; Verkhovsky, M. I. *Biochemistry* **2006**, *45*, 5641. (d) Belevich, I.; Bloch, D. A.; Belevich, N.; Wikstrom, M.; Verkhovsky, M. I. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2685. (e) CcO metal center redox potentials are highly dependent on the redox states of the other metal cofactors as well as the pH/protonation state.
- (25) (a) Tran, D.; Skelton, B. W.; White, A. H.; Laverman, L. E.; Ford, P. C. *Inorg. Chem.* **1998**, *37*, 2505. (b) Kalita, A.; Kumar, P.; Deka, R. C.; Mondal, B. *Chem. Commun.* **2012**, *48*, 1251.