Inorganic Chemistry
Forum Article

Inorg. Chem. **2010**, *49*, 6267–6282 **6267**
DOI: 10.1021/ic100033y

Bioinspired Heme, Heme/Nonheme Diiron, Heme/Copper, and Inorganic NOx Chemistry: $\bullet NO_{(g)}$ Oxidation, Peroxynitrite−Metal Chemistry, and $\bullet NO_{(g)}$ Reductive Coupling

Mark P. Schopfer,[†] Jun Wang,[†] and Kenneth D. Karlin*[,†,‡]

[†]*Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218, and* [‡]*Department of Bioinspired Science, Ewha Woman's University, Seoul 120-750, Korea*

Received January 6, 2010

The focus of this Forum Article highlights work from our own laboratories and those of others in the area of biochemical and biologically inspired inorganic chemistry dealing with nitric oxide [nitrogen monoxide, $\bullet NO_{(g)}$] and its biological roles and reactions. The latter focus is on (i) oxidation of $\bullet NO_{(g)}$ to nitrate by nitric oxide dioxygenases (NODs) and (ii) reductive coupling of two molecules of $\bullet NO_{(g)}$ to give $N_2O(g)$. In the former case, NODs are described, and the highlighting of possible peroxynitrite/heme intermediates and the consequences of this are given by a discussion of recent works with myoglobin and a synthetic heme model system for NOD action. Summaries of recent copper complex chemistries with $\bullet NO_{(g)}$ and $O_2(g)$, leading to peroxynitrite species, are given. The coverage of biological reductive coupling of $\bullet NO_{(g)}$ deals with bacterial nitric oxide reductases (NORs) with heme/nonheme diiron active sites and on heme/copper oxidases such as cytochrome *c* oxidase, which can mediate the same chemistry. Recently designed protein and synthetic model compounds (heme/nonheme/diiron or heme/copper) as functional mimics are discussed in some detail. We also highlight examples from the chemical literature, not necessarily involving biologically relevant metal ions, that describe the oxidation of $\bullet NO_{(g)}$ to nitrate (or nitrite) and possible peroxynitrite intermediates or reductive coupling of $\bullet NO_{(g)}$ to give nitrous oxide.

Introduction

In this Forum Article, we overview certain aspects of the biological inorganic chemistry and associated synthetic "biomimetic" chemistries relevant to transformations carried out on nitrogen monoxide (nitric oxide; •NO, indicating that it is a radical odd-electron species). These are, in order of coverage in this article, (i) oxidation of $\bullet NO_{(g)}$ to a nitrate anion (NO_3^-; eq 1), (ii) oxidation of $\bullet NO_{(g)}$ to a peroxynitrite anion [$O{=}NOO^-$; oxoperoxonitrate(1−) eq 2], or (iii) reductive coupling of two molecules of $\bullet NO_{(g)}$ to give nitrous oxide ("laughing gas", dinitrogen oxide, N_2O; eq 3). Thus, $\bullet NO_{(g)}$, the reductive derivative N_2O, or the gaseous aerobic surroundings of oxidation products, such as nitrogen dioxide ($\bullet NO_2$) and nitrous anhydride (dinitrogen trioxide, N_2O_3), occur naturally but are also important pollutants that have deleterious environmental and atmospheric chemistries. In nature, the reactions described by eqs 1 and 3 occur enzymatically and are part of the biochemistry of nitric oxide, which involves heme proteins. As we will point out, we speculate that the process described by eq 2 might as well take place as a metal ion (iron, copper, or manganese) center mediated reaction.

$$\bullet NO_{(g)} + M^{n+}(O_2) \rightarrow M^{n+1} + NO_3^- \quad (1)$$

$$\bullet NO_{(g)} + M^{n+}(O_2) \rightarrow M^{n+1}(O{=}NOO^-) \quad (2)$$

$$2\bullet NO_{(g)} + 2e^- \text{ (from metal ions)} + 2H^+ \rightarrow N_2O + H_2O \quad (3)$$

Bioinorganic chemists from a variety of subdisciplines have taken recent interest in such chemical reactivity, given their biological importance as well as the fact that these processes involve very interesting and important metal-ion-mediated electron-transfer and/or atom-transfer conversions. We will describe here a number of recently described chemical systems that are structural and/or functional models for these transformations, while placing our discussions into the context of the biochemistry that is known. However, first we need to provide some background on $\bullet NO_{(g)}$.

Nitric oxide can be produced in vivo from a variety of sources (Figure 1) including (i) nitric oxide synthase (NOS) [as members of a class of heme-containing monooxygenases, NOSs specifically catalyze the oxidation of L-arginine to L-citrulline, producing $\bullet NO_{(g)}$],[1−3] (ii) nitrite reductase (NiR)

*To whom correspondence should be addressed. E-mail: karlin@jhu.edu.

(1) Marletta, M. A. *J. Biol. Chem.* **1993**, *268*, 12231–12234.

Published on Web 07/12/2010

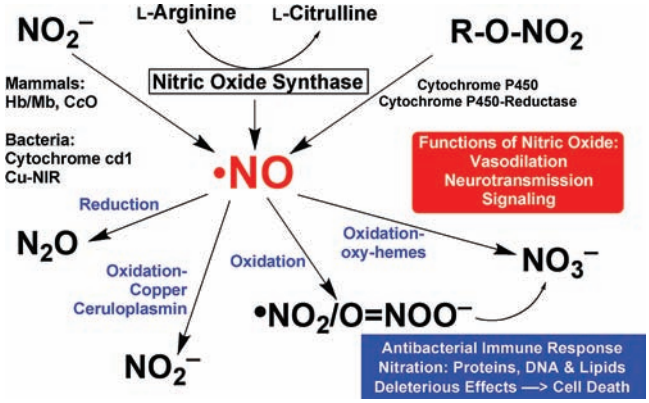


Figure 1. Chemistry of •$NO_{(g)}$: sources of production in biological systems, its functions and roles, and multiple oxidation and reduction pathways, leading to the formation of a variety of species that may effect a diverse range of biomolecules and functions. See also the text.

[in bacteria, nitrite reduction to form nitric oxide is the second step during anaerobic bacterial denitrification, which is catalyzed by two genetically unrelated classes of NiRs: heme NiRs (cytochrome cd_1 nitrite reductase, cd_1-NiR) or copper nitrite reductase (Cu-NiR)],[4,5] (iii) reductions of nitrite (NO_2^-) with various metalloproteins,[6] all of whose main functions are not nitrite reduction, such as hemoglobin/myoglobin (Hb/Mb) (oxygen transport/storage),[7−9] xanthine oxidoreductase (XOR),[10,11] cytochrome *c* oxidase (C*c*O) (O_2 reduction: proton translocation),[12,13] and possibly soluble guanylate cyclase (sGC)],[14] (iv) reductions of organic nitrates [R−O−NO_2; e.g., nitroglycerin has been widely employed for cardiovascular therapy accredited to its ability to generate •$NO_{(g)}$, and although the mechanism of reduction to •$NO_{(g)}$ is ill-defined, it is thought to be mediated by cytochrome P450 (P450) and cytochrome P450 reductase in mammals],[15] and (v) nonenzymatic nitrite reduction as well as acidic disproportionation.

The production of •$NO_{(g)}$ is critical to living systems in a number of biochemical processes that include blood pressure regulation, neurotransmission, and immune response to protect against pathogens.[16−18] Perhaps the most well-known function of nitric oxide is the signaling agent role it plays in the critical process of smooth muscle relaxation involved in vasodilation (blood pressure regulation). •$NO_{(g)}$ is biosynthesized from NOS and is able to act on the enzyme sGC, converting guanosine triphosphate to cyclic guanosine monophosphate, which is a secondary messenger in smooth muscle relaxation and similarly in neurotransmission. Another important role of •$NO_{(g)}$, or other oxides of it, is assisting in the destruction of invading microorganisms; although production of •$NO_{(g)}$ and its oxidation products is used to protect against these pathogens/diseases, local environments of high concentrations can lead to deleterious or inhibitory effects even on the host cells themselves.[19,20] Nitric oxide also plays a role in regulating (P450) enzymatic activities during inflammation by inhibiting its catalytic activity.[21,22] Inhibition of other enzymes such as terminal oxidases and catalase has also been suggested. At high enough concentrations in vivo or through the use of •$NO_{(g)}$ releasing agents, it is suggested that nitric oxide can reversibly inhibit the catalytic functions of these enzymes by binding to the heme, or it can irreversibly inhibit the enzyme if nitrogen oxides derived from •$NO_{(g)}$ oxidation; e.g., peroxynitrite anion ($^-$OON=O; PN) or the nitrogen dioxide radical (•NO_2) react with and alter the overall structure of the protein, e.g., via protein tyrosine nitration.[17,23,24] A 2009 symposium was to discuss advances in the understanding of the roles that nitrite and nitrate may play in therapeutics, nutrition, and, more generally, biology.[6] Because the reduction of nitrate to nitrite and nitrite to •$NO_{(g)}$ are now known to be catalyzed by numerous enzymatic and nonenzymatic pathways, as discussed above, these species are now quickly becoming increasingly interesting to nutritional researchers as well as possessing possible therapeutic applications regarding various cardiovascular diseases including ischemia reperfusion and hypertension.[25]

Nature has developed ways to maintain •$NO_{(g)}$ homeostasis in vivo by both oxidative and reductive pathways of removing •$NO_{(g)}$. Certain bacterial and mammalian enzymes possess nitric oxide dioxygenase (NOD) activity, the ability to catalyze the reaction of •$NO_{(g)}$ and O_2 to yield the biologically benign nitrate (NO_3^-). The copper enzyme

(2) Crane, B. R. *Biochem. Soc. Trans.* **2008**, *36*, 1149–1154.
(3) Zhu, Y.; Silverman, R. B. *Biochemistry* **2008**, *47*, 2231–2243.
(4) Wasser, I. M.; de Vries, S.; Moënne-Loccoz, P.; Schröder, I.; Karlin, K. D. *Chem. Rev.* **2002**, *102*, 1201–1234.
(5) Brenner, S.; Heyes, D. J.; Hay, S.; Hough, M. A.; Eady, R. R.; Hasnain, S. S.; Scrutton, N. S. *J. Biol. Chem.* **2009**, *284*, 25973–25983.
(6) Lundberg, J. O.; Gladwin, M. T.; Ahluwalia, A.; Benjamin, N.; Bryan, N. S.; Butler, A.; Cabrales, P.; Fago, A.; Feelisch, M.; Ford, P. C.; Freeman, B. A.; Frenneaux, M.; Friedman, J.; Kelm, M.; Kevil, C. G.; Kim-Shapiro, D. B.; Kozlov, A. V.; Lancaster, J. R.; Lefer, D. J.; McColl, K.; McCurry, K.; Patel, R. P.; Petersson, J.; Rassaf, T.; Reutov, V. P.; Richter-Addo, G. B.; Schechter, A.; Shiva, S.; Tsuchiya, K.; van Faassen, E. E.; Webb, A. J.; Zuckerbraun, B. S.; Zweier, J. L.; Weitzberg, E. *Nat. Chem. Biol.* **2009**, *5*, 865–869.
(7) Moller, J. K. S.; Skibsted, L. H. *Chem. Rev.* **2002**, *102*, 1167–1178.
(8) Gladwin, M. T.; Grubina, R.; Doyle, M. P. *Acc. Chem. Res.* **2009**, *42*, 157–167.
(9) Kim-Shapiro, D. B.; Gladwin, M. T.; Patel, R. P.; Hogg, N. The Reaction between Nitrite and Hemoglobin: The Role of Nitrite in Hemoglobin-mediated Hypoxic Vasodilation. In *The Smallest Biomolecules: Diatomics and their Interactions with Heme Proteins*; Ghosh, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2008; pp 269−289.
(10) Li, H.; Cui, H.; Kundu, T. K.; Alzawahra, W.; Zweier, J. L. *J. Biol. Chem.* **2008**, *283*, 17855–17863.
(11) Duranski, M. R.; Greer, J. J. M.; Dejam, A.; Jaganmohan, S.; Hogg, N.; Langston, W.; Patel, R. P.; Yet, S. F.; Wang, X. D.; Kevil, C. G.; Gladwin, M. T.; Lefer, D. J. *J. Clin. Invest.* **2005**, *115*, 1232–1240.
(12) Castello, P. R.; Woo, D. K.; Ball, K.; Wojcik, J.; Liu, L.; Poyton, R. O. *Proc. Natl. Acad. Sci.U.S.A.* **2008**, *105*, 8203–8208.
(13) Benamar, A.; Rolletschek, H.; Borisjuk, L.; Avelange-Macherel, M.-H.; Curien, G.; Mostefai, H. A.; Andriantsitohaina, R.; Macherel, D. *Biochim. Biophys. Acta* **2008**, *1777*, 1268–1275.
(14) Alzawahra, W. F.; Talukder, M. A. H.; Liu, X. P.; Samouilov, A.; Zweier, J. L. *Am. J. Phys.* **2008**, *295*, H499–H508.
(15) Li, H. T.; Liu, X. P.; Cui, H. M.; Chen, Y. R.; Chen, Y. R.; Cardounel, A. J.; Zweier, J. L. *J. Biol. Chem.* **2006**, *281*, 12546–12554.
(16) *Nitric Oxide, Biology and Pathobiology*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2000.
(17) Ischiropoulos, H. *Arch. Biochem. Biophys.* **2009**, *484*, 117–121.
(18) Rose, M. J.; Mascharak, P. K. *Curr. Opin. Chem. Biol.* **2008**, *12*, 238–244.
(19) Lim, C. H.; Dedon, P. C.; Deen, W. M. *Chem. Res. Toxicol.* **2008**, *21*, 2134–2147.
(20) Cape, J. L.; Hurst, J. K. *Arch. Biochem. Biophys.* **2009**, *484*, 190–196.
(21) Miranda, K. M.; Nims, R. W.; Thomasa, D. D.; Espey, M. G.; Citrin, D.; Bartberger, M. D.; Paolocci, N.; Fukuto, J. M.; Feelisch, M.; Wink, D. A. *J. Inorg. Biochem.* **2003**, *93*, 52–60.
(22) Ouellet, H.; Lang, J.; Couture, M.; Ortiz de Montellano, P. R. *Biochemistry* **2009**, *48*, 863–872.
(23) Ulrich, V.; Kissner, R. *J. Inorg. Biochem.* **2006**, *100*, 2079–2086.
(24) Gunaydin, H.; Houk, K. N. *Chem. Res. Toxicol.* **2009**, *22*, 894–898.
(25) Lundberg, J. O.; Weitzberg, E.; Gladwin, M. T. *Nat. Rev. Drug Discovery* **2008**, *7*, 156–167.

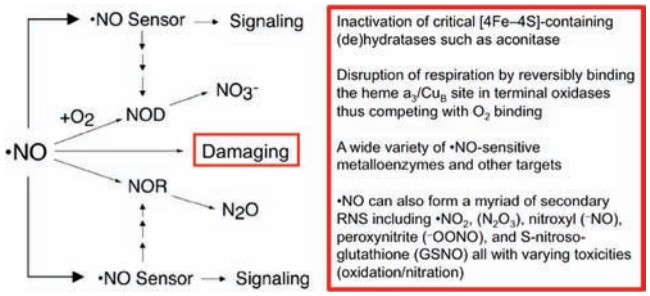


Figure 2. Various deleterious effects of $\bullet NO_{(g)}$ and formation of reactive nitrogen species (RNS) in vivo. Adapted with permission from Gardner.[28]

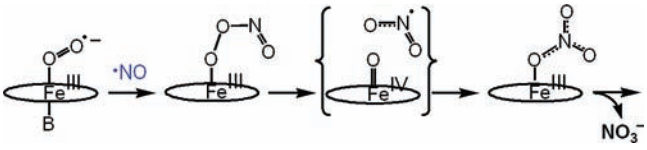
ceruloplasmin, normally oxidizing the Fe^{II} ion, has recently been shown to oxidize $\bullet NO_{(g)}$ to nitrite, perhaps to remove excess $\bullet NO_{(g)}$ and "store" it in the nitrite pool.[26,27] Other enzymes possess nitric oxide reductase (NOR) activity, the ability to reductively couple two molecules of $\bullet NO_{(g)}$, giving N_2O and water. Both NOD and NOR pathways will be discussed in more detail below, highlighting some recent work in the field done in our laboratory as well as others.

Nitric Oxide, PN, $\bullet NO_2$, and Hemes

NOD. Given the variety of sources of $\bullet NO_{(g)}$ production in vivo, it is necessary that living systems possess a defense against excess concentrations of $\bullet NO_{(g)}$ (Figure 2), possibly analogous to an antioxidant defense system protecting against hydrogen peroxide and other reactive oxygen species (ROS). Indeed, Hb and Mb appear to possess this type of reactivity, and it has been suggested that $\bullet NO_{(g)}$ detoxification may have been a more ancient function of the two heme proteins.[28] Hb and Mb are major sinks of $\bullet NO_{(g)}$ in mammals, and it has been shown that truncated Hbs (trHbs) and microbial flavohemoglobins (flavoHbs) exhibit NOD activity.[28,29]

Gardner and co-workers used electrospray ionization mass spectrometry (ESI-MS) to analyze the stoichiometric product nitrate in the reaction of doubly ^{18}O-labeled red blood cell oxyHb [$Hb(Fe^{III}\text{-}^{18}O_2^{\bullet-})$], sperm whale oxyMb [$Mb(Fe^{III}\text{-}^{18}O_2^{\bullet-})$], and *Escherichia coli* oxyflavoHb [$flavoHb(Fe^{III}\text{-}^{18}O_2^{\bullet-})$] with $\bullet NO_{(g)}$, seeing greater than 99% incorporation of the labeled O_2.[30] These data support a mechanism that involves a rapid reaction of the oxyheme [$Fe^{III}O_2^{\bullet-}$] with $\bullet NO_{(g)}$ to form a transient PN species [$Fe^{III}OON{=}O^-$], which then rapidly forms the NO_3^- anion. A more detailed understanding of this final step is being investigated by a number of groups, and the most commonly accepted mechanism (Scheme 1) is thought to involve (after PN formation) isomerization via homolytic O−O bond cleavage, producing an oxoferryl

Scheme 1



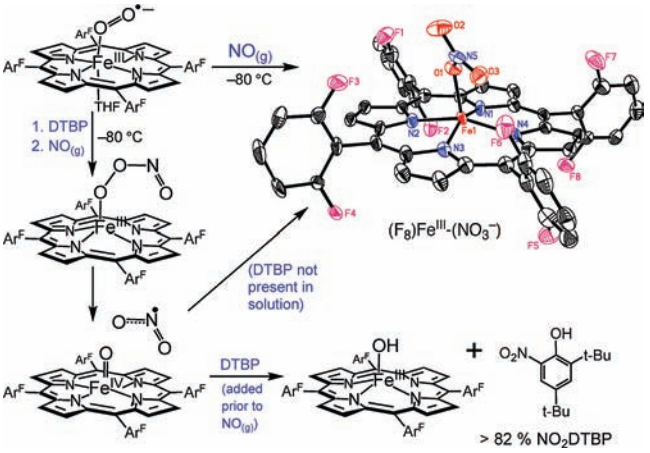
($Fe^{IV}{=}O$) species and $\bullet NO_2$, which within the cage attacks the ferryl O atom to produce NO_3^-.[24,31−37] However, recent reactive molecular dynamics simulations on a trHb structure suggest O−O bond cleavage is likely too slow to be involved in the dioxygenation mechanism.[38] An intermediate species detected by UV−vis and electron paramagnetic resonance (EPR) spectroscopy has been assigned to a PN complex, but a recent resonance Raman study clearly supports its reassignment to a heme/nitrate (product) complex.[34]

NOD Model Compound with Implication for PN Formation. Recent work from our group using a synthetic oxyheme that exhibits NOD reactivity has been explored with the hope of adding to the understanding of the above mechanism.[39] A reduced heme $(F_8)Fe^{II}$ [F_8 = tetrakis-(2,6-difluorophenyl)porphyrinate(2−)] reacts reversibly with O_2 to give a diamagnetic iron(III) superoxo species $(S)(F_8)Fe^{III}(O_2^{\bullet-})$, which is stable in solution below −40 °C in coordinating but nonaqueous solvents (S).[40,41] The addition of 1 equiv of $\bullet NO_{(g)}$ [at −80 °C in tetrahdydrofuran (THF)] produces the five-coordinate nitratoheme complex $(F_8)Fe^{III}(NO_3^-)$ in near-quantitative yield, as is seen in the NOD reactions. To further investigate the possible proposed intermediates in this reaction, we sought chemical evidence that might suggest the formation of a PN species. In fact, we observed effective nitration chemistry when 2,4-di-*tert*-butylphenol (DTBP; 1 equiv) was added *prior* to the addition of 1 equiv of $\bullet NO_{(g)}$ to the superoxo species (Scheme 2). Workup of the reaction solution revealed that the ferric hydroxo product $(F_8)Fe^{III}OH$ formed (∼85% yield) along with high yields (> 82%) of 2,4-di-*tert*-butyl-6-nitrophenol (NO_2DTBP). These findings, along with appropriate control experiments, indicated that an as yet unobserved heme/NO_x intermediate must have formed, and it was able to effect a phenol nitration reaction faster than its own isomerization to the nitrate complex.[39]

Peroxynitrite Myoglobin Chemistry. Recent work from Groves' laboratory indicates that a PN/heme, generated by the reaction of PN with oxidized (met) horse heart Mb,

(26) 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*, 486–493.
(27) Samuel, T. K.; Gitlin, J. D. *Nat. Chem. Biol.* **2006**, *2*, 452–453.
(28) Gardner, P. R. *J. Inorg. Biochem.* **2005**, *99*, 247–266.
(29) Ouellet, H.; Ouellet, Y.; Richard, C.; Labarre, M.; Wittenberg, B.; Wittenberg, J.; Guertin, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5902–5907.
(30) Gardner, P. R.; Gardner, A. M.; Brashear, W. T.; Suzuki, T.; Hvitved, A. N.; Setchell, K. D. R.; Olson, J. S. *J. Inorg. Biochem.* **2006**, *100*, 542.
(31) Lee, J.; Hunt, J. A.; Groves, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 7493–7501.
(32) Groves, J. T. *Curr. Opin. Chem. Biol.* **1999**, *3*, 226–235.
(33) Pacher, P.; Beckman, J. S.; Liaudet, L. *Physiol. Rev.* **2007**, *87*, 315–424.
(34) Yukl, E. T.; de Vries, S.; Moënne-Loccoz, P. *J. Am. Chem. Soc.* **2009**, *131*, 7234–7235.
(35) Herold, S.; Koppenol, W. H. *Coord. Chem. Rev.* **2005**, *249*, 499–506.
(36) Blomberg, L. M.; Blomberg, M. R. A.; Siegbahn, P. E. M. *J. Biol. Inorg. Chem.* **2004**, *9*, 923–935.
(37) Ford, P. C.; Lorkovic, I. M. *Chem. Rev.* **2002**, *102*, 993–1017.
(38) Mishra, S.; Meuwly, M. *J. Am. Chem. Soc.* **2010**, *132*, 2968–2982.
(39) Schopfer, M. P.; Mondal, B.; Lee, D.-H.; Sarjeant, A. A. N.; Karlin, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 11304–11305.
(40) 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*, 5754–5767.
(41) Kim, E.; Helton, M. E.; Wasser, I. M.; Karlin, K. D.; Lu, S.; Huang, H.-w.; Moënne-Loccoz, P.; Incarvito, C. D.; Rheingold, A. L.; Honecker, M.; Kaderli, S.; Zuberbühler, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3623–3628.

Scheme 2



produces nitrate along with other RNS.[42] Stopped-flow spectrophotometry evidence was presented to show that ferrylMb (i.e., a high-valent compound II like the Fe^{IV}=O species) and •NO_2 are formed in this reaction. In these experiments, a large excess of PN relative to oxidized Mb allowed for multiple reaction cycles between metMb and PN such that the ferrylMb species could accumulate to high concentrations and produce unambiguous results. Fluorescein (Fl) was used to trap •NO_2 escaping from the protein active-site "cage"; Fl was nitrated by •NO_2 to form NO_2Fl, the formation of which was followed by its spectral changes. The ratio of the rates of in-cage rebound to cage escape (k_r/k_e) was approximately 10 (Figure 3). Groves' results are thus also consistent with the primary mechanism mentioned above, that is, by the typically described NOD process where oxyMb reacts with •$NO_{(g)}$ to give a ferryl and •NO_2; however, the latter may escape and effect nitration of a nearby tyrosine (phenol) moiety.

PN, discussed above with respect to NOD chemistry and/or heme/PN interactions, is known to form from the near-diffusion-controlled reaction of superoxide anion ($O_2^{\bullet-}$) with •$NO_{(g)}$ (Figure 4). This RNS can form other free radicals such as nitrogen dioxide, a hydroxyl radical, and, in the presence of CO_2, a carbonate radical anion. All of these are strong oxidants and, in the case of •NO_2, also a nitrating agent. A variety of biomolecules are oxidized/nitrated by PN-derived radicals including, tyrosine, thiols, unsaturated-fatty-acid-containing lipids, and DNA. Such oxidations/nitrations have been associated with a number of disease states; PN- and/or •NO_2-derived nitration of protein tyrosine residues may interfere with critical cell-signaling functions.[43−45] Metal ions react with PN, can form complexes,[35,46] and, as described above, carry out chemistry with hemes. PN interactions and nitration chemistry have also

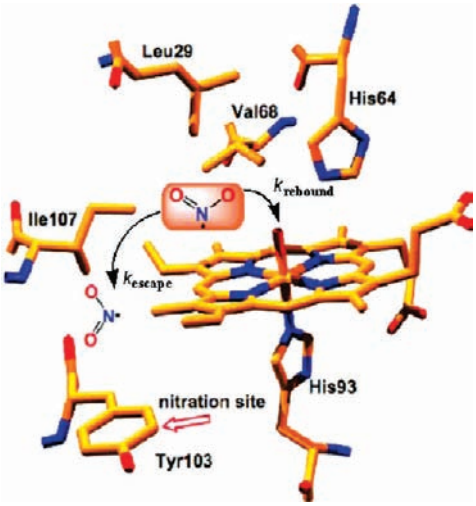


Figure 3. Depiction of the Mb active site following reaction of the oxidized heme with PN [or which would form from an oxyheme plus •$NO_{(g)}$ reaction], which generates a caged radical pair, [Fe^{IV}=O •NO_2]. Nitrogen dioxide can either attack the oxo group, leading to nitrate anion, or escape, which may result in tyrosine nitration. Adpated with permission from Su and Groves.[42]

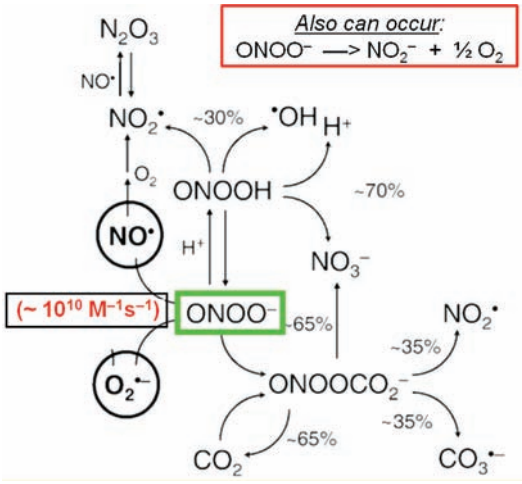


Figure 4. PN chemistry showing formation of a nitrogen dioxide radical, a hydroxyl radical, and a carbonate radical anion. These species can effect protein tyrosine nitration, protein oxidation, DNA, lipid and thiol oxidation/nitration, and reactions with metalloproteins including Fe/Mn/Cu. As discussed, metal ions may also react with PN, for example, methemes, to give [Fe^{IV}=O •NO_2] caged radicals. Adapted from ref 56.

been described with respect to manganese[47,48] and copper/zinc superoxide dismutases.[49−51] Other heme enzymes include cytochrome P450 monooxygenases.[52] For example, in separate experiments, reactions of PN with various ferric P450 enzymes led to generation of an appreciable amount of compound I [i.e., ($P^{\bullet+}$)Fe^{IV}=O; P = porphyrinate],

(42) Su, J.; Groves, J. T. *J. Am. Chem. Soc.* **2009**, *131*, 12979–12988.
(43) Ullrich, V.; Kissner, R. *J. Inorg. Biochem.* **2006**, *100*, 2079–2086.
(44) Schopfer, F. J.; Baker, P. R. S.; Freeman, B. A. *Trends Biochem. Sci.* **2003**, *28*, 646–654.
(45) Monteiro, H. P.; Arai, R. J.; Travassos, L. R. *Antioxid. Redox Signaling* **2008**, *10*, 843.
(46) Roncaroli, F.; Videla, M.; Slep, L. D.; Olabe, J. A. *Coord. Chem. Rev.* **2007**, *251*, 1903–1930.
(47) Quint, P.; Reutzel, R.; Mikulski, R.; McKenna, R.; Silverman, D. N. *Free Radical Biol. Med.* **2006**, *40*, 453.
(48) MacMillan-Crow, L. A.; Crow, J. P.; Kerby, J. D.; Beckman, J. S.; Thompson, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11853–11858.
(49) Alvarez, B.; Demicheli, V.; Duran, R.; Trujillo, M.; Cervenansky, C.; Freeman, B. A.; Radi, R. *Free Radical Biol. Med.* **2004**, *37*, 813–822.
(50) Ischiropoulos, H.; Zhu, L.; Chen, J.; Tsai, M.; Martin, J. C.; Smith, C. D.; Beckman, J. S. *Arch. Biochem. Biphys.* **1992**, *298*, 431–437.
(51) Macfadyen, A. J.; Reiter, C.; Zhuang, Y. X.; Beckman, J. S. *Chem. Res. Toxicol.* **1999**, *12*, 223–229.
(52) Ortiz de Montellano, P. R. *Chem. Rev.* **2010**, *110*, 932–948.
(53) Behan, R. K.; Hoffart, L. M.; Stone, K. L.; Krebs, C.; Green, M. T. *J. Am. Chem. Soc.* **2007**, *129*, 5855–5859.
(54) Sheng, X.; Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **2008**, *130*, 13310–13320.

and compound II [(P)Fe^{IV}=O] derivatives along with the more stable Fe^{III}NO species, as determined by UV−vis, Mössbauer, and X-ray absorption spectroscopies.[52−55] These reactions also led to nitration of approximately three tyrosine residues within the enzyme.[52]

While $\bullet NO_{(g)}$ oxidations by oxyheme [$Fe^{III}(O_2^{\bullet-})$] are discussed with respect to heme NOD reactivity, there exists an inorganic chemistry wherein metal nitrosyl complexes [derived from metal ion (M^n) complexes plus $\bullet NO_{(g)}$] react with O_2 to generate either metal nitro/nitrito species ($M^{n+1}NO_2$), metal nitrates ($M^{n+1}NO_3$), or both.[37] While not detected previously, these reactions are thought to proceed through PN [$M^{n+1}(^-OONO)$] intermediates. The first example of this comes from Clarkson and Basolo, where they described the kinetics of the O_2 reaction with a cobalt nitrosyl complex in the presence of coordinating bases to give the corresponding nitro complex (eq 4); the reaction mechanism has been suggested to involve an N-coordinated PN species as an intermediate, before transfer of one of its O atoms to a second 1 equiv of cobalt nitrosyl.[57] A similar O_2 reaction was carried out with an iridium nitrosyl complex, and formation of an N-coordinated PN was also suggested as an intermediate before its isomerization to the iridium nitrate complex final product (eq 5).[58] Additionally and more recently, when a reduced nitroprusside was reacted with O_2, the authors suggest that its reaction proceeds through an N-coordinated PN complex, as suggested from their density functional theory (DFT) calculations, before decomposition (eq 6).[46,59]

$$2Co(salen)(NO) + 2py + O_2 \rightarrow 2Co(salen)(NO_2) \quad (4)$$

$$Ir(PPh_3)_2(CO)(Cl)(X)(NO) + O_2 \rightarrow Ir(PPh_3)_2(CO)(Cl)(X)(NO_3) \quad (5)$$

$$Fe^{II}(CN)_5(NO)^{3-} + O_2 \rightarrow Fe^{III}(CN)_5(N(O)O_2{}^-)^{3-} \rightarrow \rightarrow \quad (6)$$

Given the known inorganic chemistry of metal nitrosyl complexes and O_2 (vide supra) and the abundance of metalloproteins in biological systems, it would seem possible that biological PN generation could occur via other (e.g., copper, manganese) metal-ion-mediated pathways, rather than only from $O_2^{\bullet-} + \bullet NO_{(g)}$ (Figure 4) or heme reactivity. For example, does a $M(O_2)$ complex (M = Mn or Cu) react with $\bullet NO_{(g)}$ to give PN-like chemistry; conversely, does a M(NO) complex react with O_2, giving similar results? This led us to initiate investigations of (ligand)$Cu^I/\bullet NO/O_2$ chemistry.

(55) Newcomb, M.; Halgrimson, J. A.; Horner, J. H.; Wasinger, E. C.; Chen, L. X.; Sligar, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 8179–8184.
(56) Bauer, G.; Chatgillaloglu, C.; Gebicki, J. L.; Gebicka, L.; Gescheidt, G.; Golding, B. T.; Goldstein, S.; Kaizer, J.; Merenyi, G.; Speier, G.; Wardman, P. *Chimia* **2008**, *62*, 704–712.
(57) Clarkson, S. G.; Basolo, F. *Inorg. Chem.* **1973**, *12*, 1528–1534.
(58) Kubota, M.; Phillips, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 5637–5638.
(59) Videla, M.; Roncaroli, F.; Slep, L. D.; Olabe, J. A. *J. Am. Chem. Soc.* **2007**, *129*, 278–279.

Copper Peroxynitrite Generation, Characterization, and Transformation

Copper Peroxynitrite Complexes and Chemistry. Discrete metal peroxynitrite complexes are rare,[35] but as discussed above, they are suggested to form as transients from metal−NO + O_2(g) or metal−O_2 + $\bullet NO_{(g)}$ reactions.[35,37,46] However, we have recently been able to generate discrete copper(II) peroxynitrite complexes via two approaches:[60,61] the reaction of $\bullet NO_{(g)}$ with the copper superoxide species $[(TMG_3tren)Cu^{II}(O_2^{\bullet-})]^+$ (eq 7)[62,63] or the complementary reaction of O_2 with a copper nitrosyl species $[Cu^I(AN)(NO)]^+$ (eq 8).

$$[(TMG_3tren)Cu^{II}(O_2^{\bullet-})]^+ + \bullet NO \rightarrow [(TMG_3tren)Cu^{II}(^-OON{=}O)]^+ \rightarrow [(TMG_3tren)Cu^{II}(^-ONO)]^+ + 0.5O_2 \quad (7)$$

$$[(AN)Cu^I(NO)]^+ + O_2 \rightarrow [(AN)Cu^{II}(O{=}NOO^-)]^+ \rightarrow [(AN)Cu^{II}(NO_2{}^-)]^+ + 0.5O_2 \quad (8)$$

Starting with the copper/dioxygen species $[(TMG_3tren)Cu^{II}(O_2^{\bullet-})]^+$, bubbling $\bullet NO_{(g)}$ at −80 °C results in the formation of a yellowish-green complex, formulated as the PN species $[(TMG_3tren)Cu^{II}(^-OON{=}O)]^+$, the first example of such a species. The formulation was, in part, confirmed by the ESI-MS evidence.[60] This species' EPR spectrum is distinctly tetragonal, which is consistent with the square-pyramidal (SP) geometry predicted by DFT calculations (B3LYP), which also led to the lowest-energy structure possessing a cyclic bidentate κ^2-*O*,*O*′-OONO moiety (Figure 5).

Upon warming to room temperature or just prolonged storage at −80 °C, $[(TMG_3tren)Cu^{II}(^-OON{=}O)]^+$ transforms to the nitrite complex $[(TMG_3tren)Cu^{II}(^-ONO)]^+$ in high yield, along with O_2 in 30−35% yield (50% theoretical; see eq 7). The X-ray crystal structure of $[(TMG_3tren)Cu^{II}(^-ONO)]^+$ shows an η^1-*O*-nitrito bound to the Cu^{II} ion in an overall trigonal-bipyramidal (TBP) environment, which is in accordance with the reverse axial EPR spectrum. The dramatic spectroscopic differences between the $^-$OON=O (peroxynitrito) and ONO^- (nitrito) complexes highlight the distinctly different nature of these species (Figure 5).

The complementary reaction to that just described involves the complex $[Cu^I(AN)(NO)]^+$, formed at −80 °C by reaction of the precursor copper(I) compound with $\bullet NO_{(g)}$ (Scheme 3).[61] As is now well appreciated,[64−68] this

(60) Maiti, D.; Lee, D.-H.; Narducci Sarjeant, A. A.; Pau, M. Y. M.; Solomon, E. I.; Gaoutchenova, K.; Sundermeyer, J.; Karlin, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 6700–6701.
(61) Park, G. Y.; Deepalatha, S.; Puiu, S. C.; Lee, D.-H.; Mondal, B.; Narducci Sarjeant, A. A.; del Rio, D.; Pau, M. Y. M.; Solomon, E. I.; Karlin, K. D. *J. Biol. Inorg. Chem.* **2009**, *14*, 1301–1311.
(62) Würtele, C.; Gaoutchenova, E.; Harms, K.; Holthausen, M. C.; Sundermeyer, J.; Schindler, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3867–3869.
(63) Maiti, D.; Lee, D.-H.; Gaoutchenova, K.; Würtele, C.; Holthausen, M. C.; Sarjeant, A. A. N.; Sundermeyer, J.; Schindler, S.; Karlin, K. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 82–85.
(64) Ghosh, S.; Dey, A.; Usov, O. M.; Sun, Y.; Grigoryants, V. M.; Scholes, C. P.; Solomon, E. I. *J. Am. Chem. Soc.* **2007**, *129*, 10310–10311.
(65) Usov, O. M.; Sun, Y.; Grigoryants, V. M.; Shapleigh, J. P.; Scholes, C. P. *J. Am. Chem. Soc.* **2006**, *128*, 13102–13111.

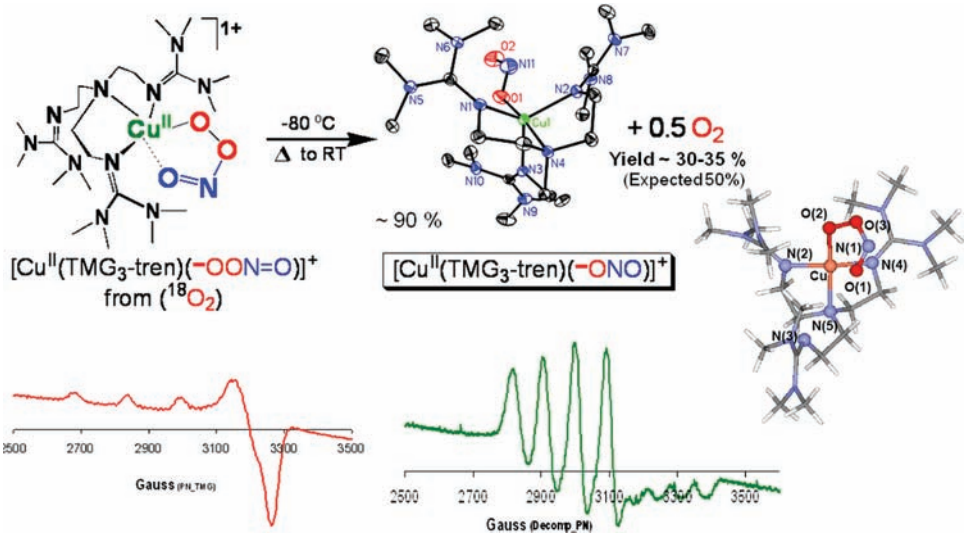
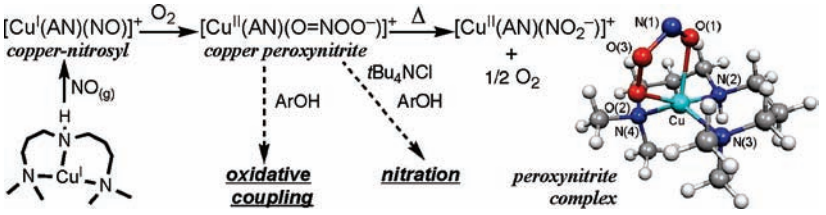


Figure 5. Peroxynitrito complex $[(TMG_3tren)Cu^{II}(^{-}OON{=}O)]^+$ formed from $Cu/O_2/{\bullet}NO_{(g)}$ chemistry and its formulation confirmed by ESI-MS experiments including ^{18}O labeling (**O** is ^{18}O). An X-ray crystal structure of the nitrite product $[(TMG_3tren)Cu^{II}(^{-}ONO)]^+$ was obtained. Dramatic differences in the EPR spectra of the $^{-}OON{=}O$ and ^{-}ONO species (SP vs TBP) are observed, and the calculated lowest-energy structure for the peroxynitritocopper(II) complex is shown.[60]

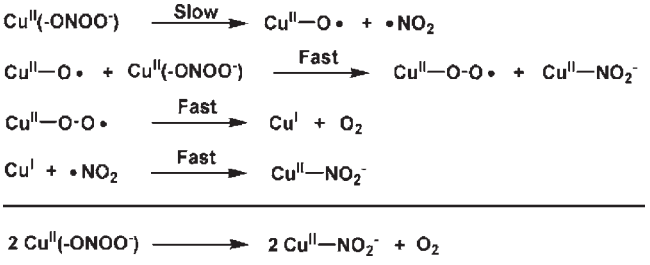
Scheme 3



is best described as a $Cu^I(\bullet NO$ radical) species. Bubbling it with $O_2(g)$ at −80 °C results in a light- to deep-blue-colored species formulated as the mononuclear PN complex $[Cu^{II}(AN)(O{=}NOO^-)]^+$, which possesses a typical tetragonal Cu^{II} EPR spectrum. DFT calculations suggest that the lowest-energy structure for $[Cu^{II}(AN)(O{=}NOO^-)]^+$ favors a cyclic bidentate κ^2-*O*,*O*′-O=NOO moiety (Scheme 3). These computations also indicate that the overall chemistry proceeds via $\bullet NO_{(g)}$ dissociation from $[Cu^I(AN)(NO)]^+$, O_2 interaction to give a transient $[Cu^{II}(AN)(O_2^{\bullet-})]^+$ species, followed by reattack by $\bullet NO_{(g)}$ to give $[Cu^{II}(AN)(O{=}NOO^-)]^+$.[61] The same sort of chemistry is thought to occur in a protein (heme)Fe(NO) + $O_2(g)$ reaction to give nitrate.[35,37]

While $[Cu^{II}(AN)(O{=}NOO^-)]^+$ is moderately stable in solution at −80 °C, it also thermally transforms to the copper nitrite complex $[Cu^{II}(AN)(NO_2^-)]^+$, as identified by EPR and UV−vis spectroscopies along with X-ray crystallography. O_2 is also released as per eq 8. Because an initial homolytic O−O cleavage is most often associated with PN chemistry, a plausible mechanism of reaction, as adapted from that proposed by Babich and Gould[69] in a copper(II) plus peroxynitrite aqueous system is shown in Scheme 4.

Scheme 4



Another possibility is a disproportionation reaction involving PN because peroxynitrate is a known "decomposition" product of PN, however, producing singlet oxygen (eq 9).[70−72] Thus, we speculate that the peroxynitritocopper(II) to nitritocopper(II) conversion might proceed according to eq 10.

$$\begin{aligned} ONOO^- + ONOOH &\rightarrow O_2NOO^- + NO_2^- + H^+ \\ O_2NOO^- &\rightarrow NO_2^- + O_2(g) \\ \text{Effective Net Reaction}: \quad 2ONOO^- &\rightarrow 2NO_2^- \\ &\quad + O_2(\text{singlet}) \end{aligned} \tag{9}$$

(66) Wasbotten, I. H.; Ghosh, A. *J. Am. Chem. Soc.* **2005**, *127*, 15384–15385.
(67) Fujisawa, K.; Tateda, A.; Miyashita, Y.; Okamoto, K.; Paulat, F.; Praneeth, V. K. K.; Merkle, A.; Lehnert, N. *J. Am. Chem. Soc.* **2008**, *130*, 1205–1213.
(68) Merkle, A. C.; Lehnert, N. *Inorg. Chem.* **2009**, *48*, 11504–11506.
(69) Babich, O. A.; Gould, E. S. *Res. Chem. Intermed.* **2003**, *29*, 343–348.
(70) Miyamoto, S.; Ronsein, G. E.; Correa, T. C.; Martinez, G. R.; Medeiros, M. H. G.; Mascio, P. D. *Dalton Trans.* **2009**, 5720–5729.
(71) Gupta, D.; Harish, B.; Kissner, R.; Koppenol, W. H. *Dalton Trans.* **2009**, 5730–5736.
(72) Goldstein, S.; Lind, J.; Merenyi, G. *Chem. Rev.* **2005**, *105*, 2457–2470.

$$[(AN)Cu^{II}(O{=}NOO^-)]^+ + [(AN)Cu^{II}(O{=}NOO^-)]^+ \rightarrow [(AN)Cu^{II}(O_2NOO^-)]^+ + [(AN)Cu^{II}(ONO^-)]^+$$

$$[(AN)Cu^{II}(O_2NOO^-)]^+ \rightarrow [(AN)Cu^{II}(ONO^-)]^+ + O_{2(g)} \quad (10)$$

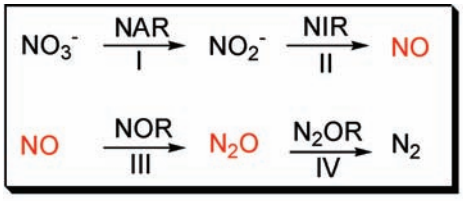
Possessing PN-like oxidative capability, $[Cu^{II}(AN)(O{=}NOO^-)]^+$ effects exogenous phenol substrate oxidative coupling chemistry, such as the oxidation of DTBP to form a bisphenol with a yield of 80%. A small amount (~5%) of o-nitrated phenol is also observed (Scheme 3). The main copper product is the nitrito complex $[Cu^{II}(AN)(NO_2^-)]^+$, as identified by comparison with authentic material. After the addition of chloride (tBu_4NCl) to replace the $O{=}NOO^-$ group on Cu^{II}, it is suggested that a free PN group or species derived from $[Cu^{II}(AN)(O{=}NOO^-)]^+$ is (are) present and this is supported by the observation that added DTBP is now primarily nitrated (Scheme 3). Thus, under the solvent and reaction conditions employed, peroxynitritocopper(II) complex $[Cu^{II}(AN)(O{=}NOO^-)]^+$ and free PN react differently, but both in a manner consistent with the PN formulation for $[Cu^{II}(AN)(O{=}NOO^-)]^+$.

With these works, we have accomplished the generation of PN via (ligand)Cu^I/•NO/O_2 reactivity studies. Clearly, mechanistic insight is lacking for the thermal transformation chemistry of peroxynitritocopper(II) compounds. It is notable that both systems lead to nitrite plus O_2 products and not the isomerization that would give nitrate. We suspect that a change in the ligand environment around the copper ion might alter the PN reactivity.[73] Further studies will focus on reaction mechanisms, (ligand)Cu^I/•NO/O_2, with differing ligands and examination of how copper binding affects the inherent reactivity of the PN anion or peroxynitrous acid. Given the presence of a copper ion and copper protein active sites present in biological fluids, we speculate that $Cu(O_2)$ or Cu(NO) species that form transiently may generate PN-type chemistry.

Bacterial NORs, Models, and Inorganic Chemistry

It has been long known that microorganisms transform nitrogen-containing oxyanions to gaseous products.[74,75] Anaerobic denitrifying bacteria use nitrogen oxides to dump metabolically derived reducing equivalents, starting with nitrate, finally releasing N_2. All of the enzymes in this pathway are metalloenzymes, including molybdenum, iron, or copper (Scheme 5). NORs effect the third reaction, mediating the reductive coupling of 2 mol equiv of $•NO_{(g)}$ to $N_2O(g)$.[4,76−78] Via such chemistry, NORs also serve to reduce toxic levels of $•NO_{(g)}$. We should note that in fungi

Scheme 5



I. NAR: Nitrate Reductase (Mo-oxotransferase)
II. NIR: Nitrite Reductase (heme cd_1 or multicopper)
III. NOR: Nitric Oxide Reductase (heme/non-heme diiron)
IV. N_2OR: Nitrous Oxide Reductase (multicopper sulfide)

NOR enzymes are a mononuclear heme/iron of the P450 type, that is, with a cysteinate sulfur axial ligand.[4,79,80] Another type of NOR is shown to exist in strict or facultative anaerobic bacteria, Archaea, and also in some eukaryotes such as microaerobic protozoan parasites.[77,81] Here, the active sites are nonheme/diiron (i.e., no heme); they are flavodiiron proteins (one is a so-called flavorubredoxin)[77,81−85] which contributes resistance to the potential toxic effects of $•NO_{(g)}$.[75]

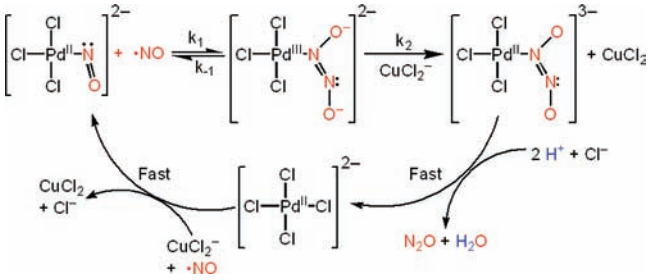
Inorganic/Organometallic $•NO_{(g)}$ Reductive Coupling. For the purposes of this bioinorganic-oriented article, our focus will be on chemical mimics of heme/nonheme/diiron or heme/copper enzymes. However, we note some examples from the inorganic/organometallic literature, studies carried out to make fundamental advances concerning $•NO_{(g)}$ interactions with metal ions, and for potential environmental chemistry applications.[86] In the 1970s, a stoichiometric iridium-bound NO reduction to form N_2O was reported, accompanied with the oxidation of CO to CO_2, during which dinitrogen dioxide (N_2O_2) (or perhaps more accurately a $N_2O_2^{2-}$ hyponitrite dianion)[87−89] was detected as an intermediate that transferred an O atom to CO (eqs 11 and 12).[86,90−93]

$$[Ir(NO)_2(PPh_3)_2]^+ + 4CO \rightarrow [Ir(CO)_3(PPh_3)_2]^+ + CO_2 + N_2O \quad (11)$$

$$2•NO_{(g)} + CO \rightarrow N_2O + CO_2 \quad (12)$$

(73) We have observed that if the ligand AN is changed to MeAN, with a methyl group input to the 2° central N atom, analogous chemistry leads to substantial amounts of nitrate; unpublished observations.
(74) Zumft, W. G. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533–616.
(75) Poole, R. K. *Biochem. Soc. Trans.* **2005**, *33*, 176–180.
(76) Zumft, W. G. Respiratory Nitric Oxide Reductases, NorB and NorZ, of the Heme−Copper Oxidase Type. In *The Smallest Biomolecules: Diatomics and their Interactions with Heme Proteins*; Ghosh, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2008; pp 327−353.
(77) Moënne-Loccoz, P. *Nat. Prod. Rep.* **2007**, *24*, 610–620.
(78) Zumft, W. G. *J. Inorg. Biochem.* **2005**, *99*, 194–215.
(79) Daiber, A.; Shoun, H.; Ulrich, V. Nitric Oxide Reductase (P450nor) from *Fusarium oxysporum*. In *The Smallest Biomolecules: Diatomics and their Interactions with Heme Proteins*; Ghosh, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2008; pp 354−377.
(80) Daiber, A.; Shoun, H.; Ullrich, V. *J. Inorg. Biochem.* **2005**, *99*, 185–193.
(81) Di Matteo, A.; Scandurra, F. M.; Testa, F.; Forte, E.; Sarti, P.; Brunori, M.; Giuffre, A. *J. Biol. Chem.* **2008**, *283*, 4061–4068.
(82) Silaghi-Dumitrescu, R.; Kurtz, D. M.; Ljungdahl, L. G.; Lanzilotta, W. N. *Biochemistry* **2005**, *44*, 6492–6501.
(83) Silaghi-Dumitrescu, R.; Ng, K. Y.; Viswanathan, R.; Kurtz, D. M. *Biochemistry* **2005**, *44*, 3572–3579.
(84) Kurtz, D. M., Jr. *Dalton Trans.* **2007**, 4115–4121.
(85) Gardner, A. M.; Helmick, R. A.; Gardner, P. R. *J. Biol. Chem.* **2002**, *277*, 8172–8177.
(86) Lee, D.-H.; Mondal, B.; Karlin, K. D. NO and N_2O Binding and Reduction. In *Activation of Small Molecules: Organometallic and Bioinorganic Perspectives*; Tolman, W. B., Ed.; Wiley-VCH: New York, 2006; pp 43−79.
(87) Arulsamy, N.; Bohle, D. S.; Imonigie, J. A.; Moore, R. C. *Polyhedron* **2007**, *26*, 4737–4745.
(88) Arulsamy, N.; Bohle, D. S.; Imonigie, J. A.; Levine, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 2371–2373.
(89) Xu, N.; Campbell, A. L. O.; Powell, D. R.; Khandogin, J.; Richter-Addo, G. B. *J. Am. Chem. Soc.* **2009**, *131*, 2460–2461.

Scheme 6

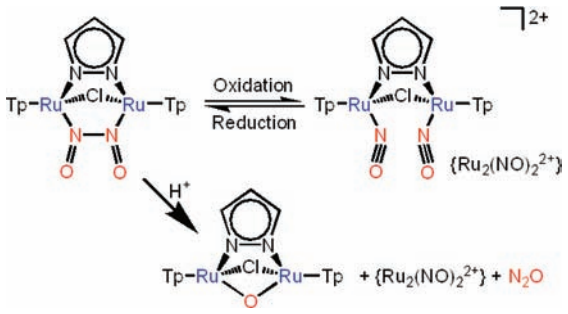


Eisenberg and co-workers showed that, in an aqueous solution of HCl and ethanol, the anionic complex $[RhCl_2(CO)_2]^-$ catalytically mediated the CO reduction of NO. There it was shown using isotope labeling studies that water played the role of an O-atom transfer agent.[91,94,95] It has also been shown by others that chloride salts of palladium, copper, or platinum could catalyze NO reductive coupling to N_2O, using either CO or olefins as reducing agents.[86]

In a significant work, Trogler and co-workers[96] carried out detailed kinetic mechanistic studies of a palladium/copper-catalyzed reduction of $\bullet NO_{(g)}$ in an aqueous solution, where, in a rate-limiting step, a cuprous salt reduces the dinitrogen dioxide species $PdCl_3(N_2O_2)^{2-}$ to give $PdCl^{4-}$, N_2O, and water. The proposed mechanism is outlined in Scheme 6 (adapted from ref 96). The remarkable resemblance here to biological $\bullet NO_{(g)}$ reductive coupling from either diiron bacterial NORs or heme/copper-containing C*c*O's (see detailed discussions below) is that the second metal ion, here copper, does not bind $\bullet NO_{(g)}$ very strongly and seems primarily to serve as a key reductant; the first metal ion, here palladium (but heme in biological systems), binds the first $\bullet NO_{(g)}$ ligand.

A very exciting chemical system illustrating N−N coupling from metal nitrosyl fragments and crystallographic characterization of the $\{N_2O_2\}$ intermediate was recently described for a pyrazolylborate-bridged diruthenium complex.[97] A "curious" N−N bond distance observed for the coupled product was 1.861(3) Å, which is shorter than the *cis*-NO dimer (2.18 Å), but longer than the typical N−N single bond (1.42 Å). Remarkably, reversible oxidation gave the dinitrosyl complex $[\{TpRu(NO)\}_2(\mu\text{-Cl})(\mu\text{-pz})]^{2+}$ (Scheme 7, adapted from ref 97). Preliminary results showed that acidification produced a μ-oxo compound (21% yield) with release of nitrous oxide (5% yield). As will be seen from the further discussion (vide infra), this chemistry bears a remarkable resemblance to what is known for the enzymatic diiron NOR chemistry.

Scheme 7



An interesting example of another type is where a terminal osmium nitride complex [Tp = hydrotris(pyrazolyl)borate] reacts with 2 equiv of $\bullet NO_{(g)}$, first transferring an "N atom" to generate N_2O and finally $(Tp)Os(NO)Cl_2$ (eq 13).[98] See a recent review for more inorganic examples of metal complex $\bullet NO_{(g)}$ reduction, including cases where the N−O bond is cleaved.[86]

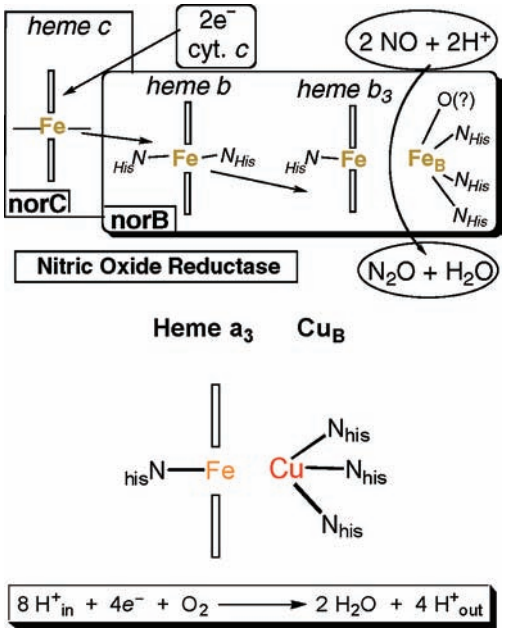
$$(Tp)Os(N)Cl_2 + \bullet NO_{(g)} \rightarrow \{(Tp)Os(N_2O)Cl_2\} \rightarrow N_2O + \{(Tp)OsCl_2\} \rightarrow \bullet NO_{(g)} \rightarrow (Tp)Os(NO)Cl_2 \quad (13)$$

Bacterial NORs.[4,76−78,99] In fact, bacterial NORs possess a binuclear active site with a high-spin heme b_3 and adjacent nonheme/Fe_B that catalyzes the reduction of nitric oxide to nitrous oxide (Scheme 8). The electron source derives from soluble cytochrome *c*. Fe_B is coordinated by N atoms from three histidine ligands. This NorB subunit, which includes the heme/nonheme/diiron active site, exhibits weak homology to subunit I of C*c*O. C*c*O and other members of the heme/copper oxidase (HCO) superfamily serve in the mitochondrial electron-transport chain of aerobic organisms to couple the reduction of O_2 to water with membrane proton translocation; the latter is utilized for ATP synthesis, the cell's energy carrier.[100,101] In fact, the four histidine residues that ligate heme a_3 and Cu_B in C*c*O (Scheme 8) are conserved in NORs. In essence, the Cu_B site of C*c*O has been replaced by a nonheme/iron (Fe_B) in NOR. HCOs and NORs are structural and functional homologues, and it is found that some HCOs and NORs reduce both O_2(g) and $\bullet NO_{(g)}$ (also see below) with different selectivities.

There are as yet no published bacterial NOR X-ray structures. The three conserved histidine residues clearly serve as Fe_B ligands, while an additional O-atom donor from a glutamate residue carboxylate has been proposed or discussed. Recent investigations, including a computer model alignment of a NorB catalytic subunit from *Pseudomonas denitrificans* with evolutionarily related HCOs, suggest that while a series of conserved glutamic acid residues act as a proton entry point and pathway proceeding

(90) Bhaduri, S.; Johnson, B. F. G.; Savory, C. J.; Segal, J. A.; Walter, R. H. *J. Chem. Soc., Chem. Commun.* **1974**, 809–810.
(91) Meyer, C. D.; Eisenberg, R. *J. Am. Chem. Soc.* **1976**, *98*, 1364–1371.
(92) Bhaduri, S.; Johnson, B. F. G.; Pickard, A.; Raithby, P. R.; Sheldrick, G. M.; Zuccaro, C. I. *J. Chem. Soc., Chem. Commun.* **1977**, 354–355.
(93) Haymore, B. L.; Ibers, J. A. *J. Am. Chem. Soc.* **1974**, *96*, 3325–3327.
(94) Eisenberg, R.; Meyer, C. D. *Acc. Chem. Res.* **1975**, *8*, 26–34.
(95) Hendriksen, D. E.; Eisenberg, R. *J. Am. Chem. Soc.* **1976**, *98*, 4662–4664.
(96) MacNeil, J. H.; Berseth, P. A.; Bruner, E. L.; Perkins, T. L.; Wadia, Y.; Westwood, G.; Trogler, W. C. *J. Am. Chem. Soc.* **1997**, *119*, 1668–1675.
(97) Arikawa, Y.; Asayama, T.; Moriguchi, Y.; Agari, S.; Onishi, M. *J. Am. Chem. Soc.* **2007**, *129*, 14160–14161.
(98) McCarthy, M. R.; Crevier, T. J.; Bennett, B.; Dehestani, A.; Mayer, J. M. *J. Am. Chem. Soc.* **2000**, *122*, 12391–12392.
(99) Watmough, N. J.; Field, S. J.; Hughes, R. J. L.; Richardson, D. J. *Biochem. Soc. Trans.* **2009**, *037*, 392–399.
(100) Kim, E.; Chufán, E. E.; Kamaraj, K.; Karlin, K. D. *Chem. Rev.* **2004**, *104*, 1077–1133.
(101) Collman, J. P.; Decréau, R. A. *Chem. Commun.* **2008**, 5065–5076.

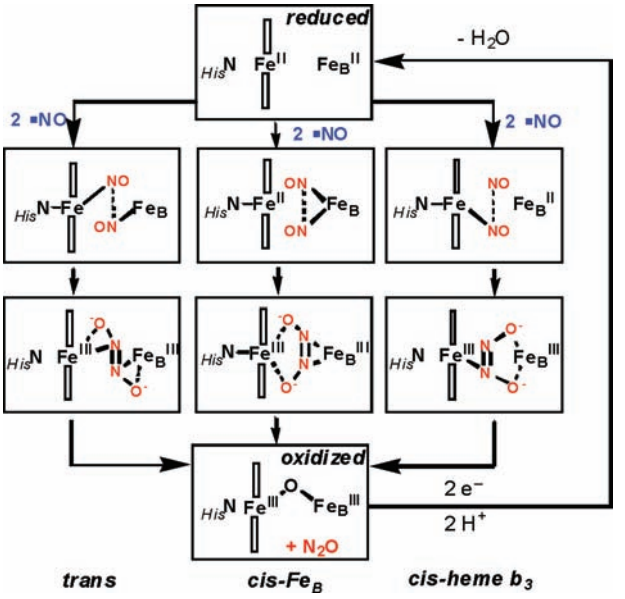
Scheme 8



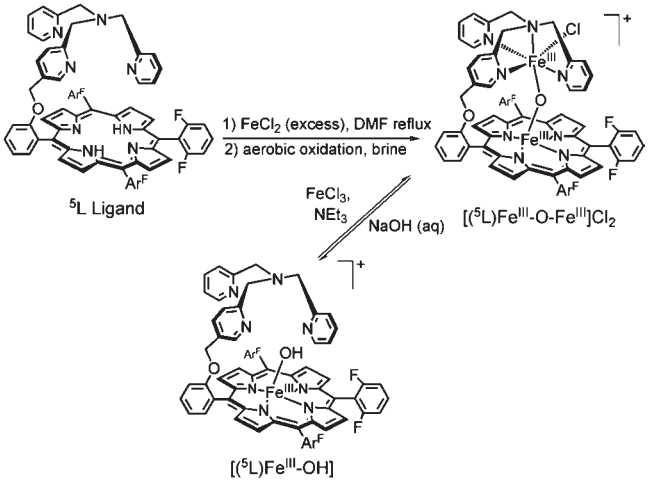
to the active site, the nearest glutamate is still too far away from Fe_B to be ligated.[102,103]

NOR Reaction Mechanism. Scheme 9 depicts three possible mechanisms that have discussed likely steps in NOR action.[77,104] In a trans mechanism, the binding of two NO's to separate Fe atoms leads to N−N coupling and a hyponitrite $N_2O_2^{2-}$ intermediate. Supporting this mechanism are the known strong affinity of $\bullet NO_{(g)}$ for reduced hemes,[105] and observations that *both* Fe atoms can bind CO [ν_{C-O} = 1972 and 2068 (Fe_B) cm^{-1}] or NO.[106,107] For *cis*-Fe_B, two Fe_B-bound NO's couple to give a hyponitrite, which eliminates N_2O and H_2O following protonation/N−O cleavage; the heme serves in electron transfer and perhaps stabilizes the $N_2O_2^{2-}$ ligand. This mechanism can be seen as reasonable in that it would not involve a generally highly stable "dead-end" heme/Fe^{II}-NO species.[77,108] For *cis*-heme b_3, an initial heme b_3−NO reacts directly with NO to give a hyponitrite radical and then (or simultaneously) is reduced and coordinated by Fe_B. Siegbahn's calculations[104] favor this scenario; N−O bond cleavage (→ N_2O) is aided by $Fe^{IV}{}_B$=O formation. The "timing" of N−O scission, further electron transfer, protonation (by a nearby Glu?), and possible $N_2O_2^{2-}$ isomerization to an O-bound form are events that remain to be defined.[77] Other mechanisms can be envisioned, for example, one that is initiated by a "mixed-valent"

Scheme 9



Scheme 10

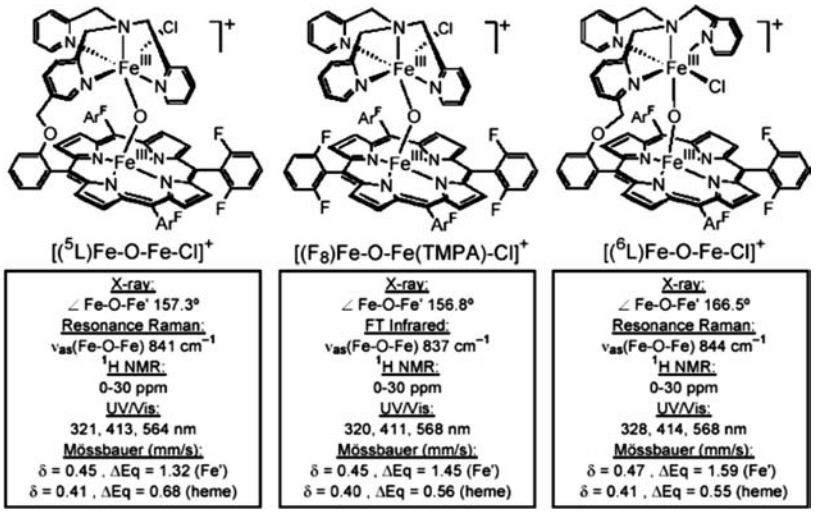


heme$^{III}\cdots Fe^{II}{}_B$ species.[77,108] Also, note that there is general agreement that the oxidized form of the enzyme, which might be involved in actual turnover,[77] is a high-spin heme coupled to Fe_B via a μ-oxo (O^{2-}) ligand; here, the heme's proximal histidine may be dissociated (Scheme 9).

C*c*O to μ-Oxo Diferric NOR Models. Our own entry into heme/metal NO chemistry derived from our early efforts in HCO modeling and the design and synthesis of heterobinucleating ligands. For example, the synthesis of ^{5}L and the introduction of excess $FeCl_2$ led to our generation and structural characterization of [(^{5}L)Fe^{III}-OFe^{III}Cl]$^+$.[109] The nonheme/iron in the tetradentate tris-(2-pyridyl)methyl coordination environment can be (reversibly) removed by the addition of base and "rusting", giving [(^{5}L)Fe^{III}OH] (Scheme 10). This high-spin complex could be "reconstituted" with iron or copper, in

(102) Flock, U.; Lachmann, P.; Reimann, J.; Watmough, N. J.; Ädelroth, P. *J. Inorg. Biochem.* **2009**, *103*, 845–850.
(103) Reimann, J.; Flock, U.; Lepp, H.; Honigmann, A.; Adelroth, P. *Biochim. Biophys. Acta* **2007**, *1767*, 362–373.
(104) Blomberg, L. M.; Blomberg, M. R. A.; Siegbahn, P. E. M. *Biochim. Biophys. Acta* **2006**, *1757*, 240.
(105) Praneeth, V. K. K.; Nather, C.; Peters, G.; Lehnert, N. *Inorg. Chem.* **2006**, *45*, 2795–2811.
(106) Lu, S.; Suharti; de Vries, S.; Moenne-Loccoz, P. *J. Am. Chem. Soc.* **2004**, *126*, 15332–15333.
(107) Kumita, H.; Matsuura, K.; Hino, T.; Takahashi, S.; Hori, H.; Fukumori, Y.; Morishima, I.; Shiro, Y. *J. Biol. Chem.* **2004**, *279*, 55247–55254.
(108) Grönberg, K. L. C.; Watmough, N. J.; Thomson, A. J.; Richardson, D. J.; Field, S. J. *J. Biol. Chem.* **2004**, *279*, 17120–17125.
(109) Martens, C. F.; Murthy, N. N.; Obias, H. V.; Karlin, K. D. *Chem. Commun.* **1996**, 629–630.

Chart 1



the latter case, to give an interesting heterobinuclear complex with a $Fe^{III}OCu^{II}$ core structure.[110]

In fact, we were able to generate two other μ-oxo heme/nonheme/diiron(III) complexes to compare and contrast (Chart 1).

Both heme and nonheme irons are in their high-spin states but are magnetically strongly antiferromagnetically coupled, as shown from Mössbauer, ^1H or ^2H NMR spectroscopies, and SQUID susceptometry for $[(^6L)FeOFeCl]^+$ ($J = -115\ cm^{-1}$ and $S = 0$),[111,112] and the structural parameters are consistent with these findings.[113] This includes the fact that the heme Fe atoms are well above (~0.5 Å) the heme plane.[111] These complexes are very stable and do not react with pyridine or imidazole bases, in line with the conclusion from enzyme studies that the heme's proximal histidine in the oxidized NOR active site is dissociated.

Thus, these synthetic diiron complexes have been good first-generation models for the presumed μ-oxodiiron(III) enzymes (Chart 1). Such μ-oxo complexes may be important in the enzyme mechanism and turnover. We speculate that, once the diiron active site becomes fully reduced and is able to react with the $\bullet NO_{(g)}$ substrate, the timing of active site protonation is such that the enzyme can first employ a driving force associated with a reaction to form the μ-oxo complex, i.e., $2Fe^{II} + 2\bullet NO_{(g)} \rightarrow$ (heme)-$Fe^{III}OFe^{III} + N_2O(g)$. Subsequent protonation and reduction would give fully reduced enzyme plus water as byproducts.

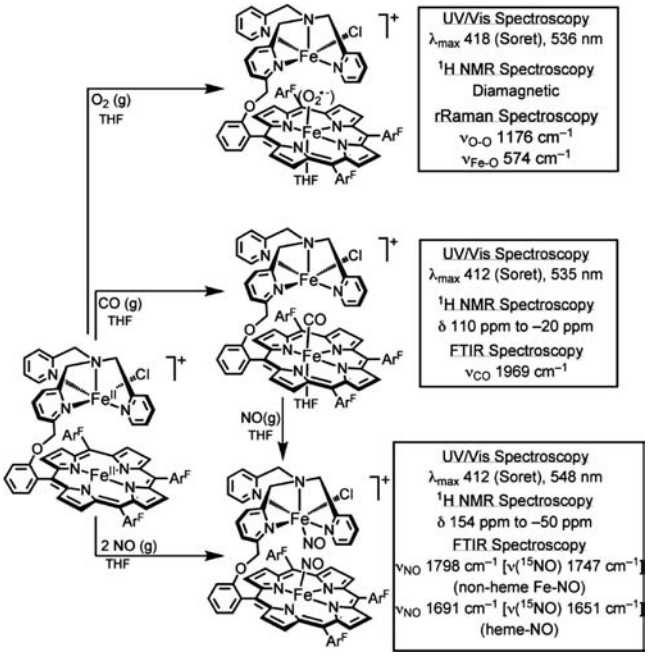
Reduced Diiron(II) Complexes and Reactivity. The heme/nonheme/diiron(II) complex $[(^6L)Fe^{II}\cdots Fe^{II}Cl]^+$ was generated by the reduction of $[(^6L)Fe^{III}OFe^{III}(Cl)]^+$.[114] Interestingly, its reaction with the enzyme substrate surrogate O_2 at −80 °C gives a product formulated as $[(^6L)Fe^{III}(O_2^-)(THF)\cdots Fe^{II}Cl]^+$ {$\lambda_{max} = 418$ (Soret), 536 nm; $\nu_{O-O} = 1176\ cm^{-1}$, $\nu_{Fe-O} = 574\ cm^{-1}$}, i.e., where the nonheme/iron does not react with O_2, nor does it participate in bridge bonding to the O_2-derived fragment. The heme O_2 adduct, formally an iron(III) superoxide species, models the spectroscopic and physical properties of oxyHbs or oxyMbs. In the reaction with CO (Scheme 11), a heme/carbonyl complex is formed, $[(^6L)Fe^{II}(CO)(THF)\cdots Fe^{II}-Cl]^+$. This observation suggests that future generations of model compounds need an altered coordination environment for the nonheme Fe atom, perhaps without a chloride ligand and with one less N donor, to enable this "Fe_B" center to bind CO as the enzyme does.[77,115]

However, the reaction of carefully purified $\bullet NO_{(g)}$ with $[(^6L)Fe^{II}\cdots Fe^{II}-Cl]^+$ leads to the dinitrosyl complex $[(^6L)Fe(NO)\cdots Fe(NO)\text{-}Cl]^+$ (Scheme 11). This complex is quite stable, and thus far we have not been able to induce it to couple the nitrosyl fragments to produce N_2O and the hoped for μ-oxo complex $[(^6L)Fe^{III}OFe^{III}(Cl)]^+$. The addition of protons also does not effect the formation of nitrous oxide, and with an excess of a base such as 1,5-dicyclohexylimidazole, $\bullet NO_{(g)}$ is liberated: $[(^6L)Fe(NO)\cdots Fe(NO)Cl]^+$ + excess "base" $\rightarrow Fe^{II}\cdots Fe^{II} + 2\bullet NO_{(g)}$.[114]

Functional Model for the Stoichiometric Reaction. On the other hand, Collman's group has succeeded in this regard. In recent exciting reports,[116,117] a fully reduced heme/$Fe_H^{II}\cdots Fe_B^{II}$ synthetic complex reacts with 2 equiv of $\bullet NO_{(g)}$ to yield 1 equiv of $N_2O(g)$ and a bisferric complex. A similar reduction of $\bullet NO_{(g)}$ was not observed upon reaction of a mixed-valent complex heme/$Fe_H^{III}\cdots Fe_B^{II}$. The product $N_2O(g)$ was quantified by use of the enzyme nitrous oxide reductase, and the bisferric species was identified by Fourier transform infrared (FT-IR) and EPR spectroscopies.[116] A follow-up investigation for possible intermediates during this $\bullet NO_{(g)}$

(110) Obias, H. V.; van Strijdonck, G. P. F.; Lee, D.-H.; Ralle, M.; Blackburn, N. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 9696–9697.
(111) Wasser, I. M.; Martens, C. F.; Verani, C. N.; Rentschler, E.; Huang, H. W.; Moenne-Loccoz, P.; Zakharov, L. N.; Rheingold, A. L.; Karlin, K. D. *Inorg. Chem.* **2004**, *43*, 651–662.
(112) 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*, 4753–4763.
(113) Scheidt, W. R.; Reed, C. A. *Chem. Rev.* **1981**, *81*, 543–555.
(114) Wasser, I. M.; Huang, H. W.; Moenne-Loccoz, P.; Karlin, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 3310–3320.
(115) Hayashi, T.; Lin, M. T.; Ganesan, K.; Chen, Y.; Fee, J. A.; Gennis, R. B.; Moënne-Loccoz, P. *Biochemistry* **2009**, *48*, 883–890.
(116) Collman, J. P.; Yang, Y.; Dey, A.; Decréau, R. A.; Ghosh, S.; Ohta, T.; Solomon, E. I. *Proc. Nat. Acad. Sci. U.S.A.* **2008**, *105*, 15660–15665.
(117) Collman, J. P.; Dey, A.; Yang, Y.; DecreAAau, R. A.; Ohta, T.; Solomon, E. I. *J. Am. Chem. Soc.* **2008**, *130*, 16498–16499.

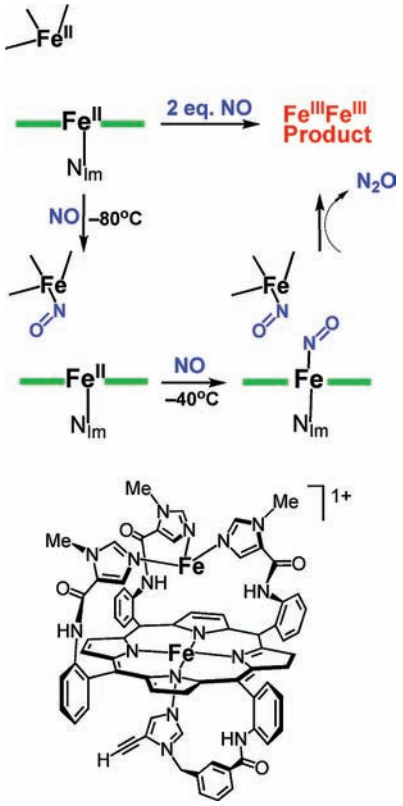
Scheme 11



reductive coupling reaction suggested the existence of two nitrosyl intermediates, in the form of heme/$Fe_H^{II}\cdots Fe_B^{II}$-NO at −80 °C and heme/$Fe_H^{II}NO\cdots Fe_B^{II}NO$ at −40 °C, as monitored by EPR, resonance Raman, and IR spectroscopies (Scheme 12). These authors suggest that their results support the trans mechanism (Scheme 9).[117]

Carboxylate-Containing Ligands for Fe_B in NOR Models. Collman and co-workers,[101,118] who have in recent years moved to emphasize the use of "faithful" or "accurate" mimics, i.e., use of imidazole ligand donors both proximal and distal (i.e., for a nonheme iron or copper ion), have also now generated binucleating ligands with a distal RCO_2^- group, which is speculated to be an Fe_B ligand (but see above). One should be reminded that it is by definition not possible to deduce enzyme chemistry from model system investigations. This can only be accomplished from studies on the enzyme itself, in part because no model can truly mimic an enzyme's local environment, i.e., with respect to the exact geometry and juxtaposition of groups, local polarity, or dielectric (and resulting redox potential) and because of second coordination sphere modulating effects. Models are not meant to duplicate data but to "sharpen the questions" being asked[119] or, if possible, to build/create something that works so the system can be truly understood[119] and to carry out systematic investigations where input synthetic modifications are tested for structure, spectroscopic properties,

Scheme 12



and/or function, so as to enhance our fundamental understanding.[120−122] It will be most interesting to learn of diiron/NO(g) reactivity studies, which may be carried out on the synthetically sophisticated ligands depicted in Chart 2.

Engineered Protein Bacterial NOR Model. Using protein design/engineering,[123] Lu and co-workers[124] used sperm whale Mb as a scaffold to engineer an Fe_B binding site into the distal pocket of the heme showing the resulting heme/nonheme/diiron protein to be a structural and *functional* mimic of the NOR active site. The nonheme "Fe_B" atom was inserted and from X-ray crystallography was found to be bound by three histidines and a glutatmate O atom, as may occur in NORs. Exposure of excess $\bullet NO_{(g)}$ to the deoxy ($Fe^{II}\cdots Fe^{II}$) Fe_BMb protein, formed by the addition of two reducing equivalents, yielded ∼30% N_2O(g), and the process could be repeated stepwise. Most recently,[125] this group's protein engineering has been further developed to include *two* glutamates, one new one (I107E; Figure 6) near the active site. A number of NOR protein glutamates are conserved and known to be critical for activity,[102,126] likely facilitating a hydrogen-bonding network and providing proton delivery for NO reduction. For Lu's engineered protein, introduction of the second glutamate (nonbonding to Fe_B; Figure 6) increased the yield of N_2O by ∼100%,

(118) Collman, J. P.; Boulatov, R.; Sunderland, C. J.; Fu, L. *Chem. Rev.* **2004**, *104*, 561–588.

(119) These quotes or paraphrases come from physical scientists from other disciplines, commenting on "models" in scientific research: "sharpen the questions", see http://www.gap-system.org/∼history/Biographies/Karlin.html; "... so the system can be truly understood", see http://www.goodreads.com/quotes/show/8414.

(120) Karlin, K. D. *Science* **1993**, *261*, 701–708.

(121) Holm, R. H.; Solomon, E. I. *Chem. Rev.* **2004**, *104*, 347–348.

(122) Lee, Y.; Karlin, K. D. Highlights of Copper Protein Active-Site Structure/Reactivity and Synthetic Model Studies. In *Concepts and Models in Bioinorganic Chemistry*; Metzler-Nolte, N., Kraatz, H.-B., Eds.; Wiley-VCH: New York, 2006; pp 363−395.

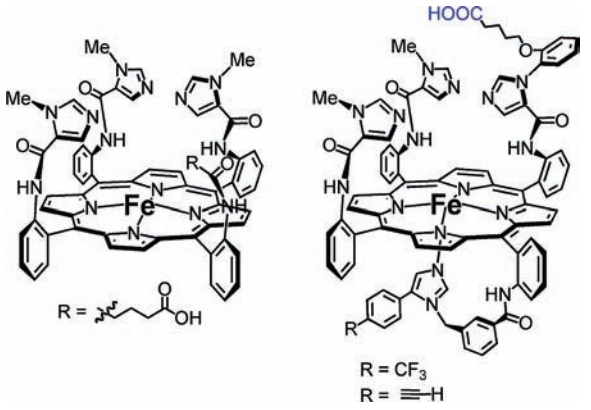
(123) Lu, Y.; Yeung, N.; Sieracki, N.; Marshall, N. M. *Nature* **2009**, *460*, 855–862.

(124) Yeung, N.; Lin, Y.-W.; Gao, Y.-G.; Zhao, X.; Russell, B. S.; Lei, L.; Miner, K. D.; Robinson, H.; Lu, Y. *Nature* **2009**, *462*, 1079–1082.

(125) Lin, Y.-W.; Yeung, N.; Gao, Y.-G.; Miner, K. D.; Tian, S.; Robinson, H.; Lu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 8581−8586.

(126) Butland, G.; Spiro, S.; Watmough, N. J.; Richardson, D. J. *J. Bacteriol.* **2001**, *183*, 189–199.

Chart 2



suggesting the importance of the second coordination sphere as well as the dual roles of the two glutamates. Their finding[125] that redox-active Fe^{2+} or Cu^{+} (vs Zn^{2+}) in the Fe_B site is necessary for NO reduction also suggests the importance of electron donation and mediation of the heme/NO intermediate.

Thus, there now exists heme/nonheme/diiron synthetic and engineered protein NOR functional models that signify considerable accomplishments and advances, providing insights and promise for more in the near future. Notwithstanding all of these research efforts from the perspective of microbiology, biophysics, biochemistry, and model compound synthetic coordination chemistry, there is still much to learn about bacterial NOR chemistry and their chemical mechanisms. In particular, aspects concern the coordination chemistry of •NO, CO, and even O_2 at this binuclear but unsymmetrical diiron center, the nature of the intermediates and their redox states, protonation events and their timing, all of which are related to the important N−N bond-forming and N−O bond-breaking steps. Fundamental characteristics of such NO chemistries apply to many other subareas, e.g., PN formation and reactivity,[127,128] biological nitrite reduction to $\bullet NO_{(g)}$ (a research area that has undergone explosive growth),[6,128] N_2O reduction to N_2,[129] and even to fundamental aspects of O_2 reduction to water or O_2 utilization for oxidation−oxygenation reactivity.

$\bullet NO_{(g)}$ Interactions with Heme/Copper-Containing C*c*O, Including Reductive Coupling. As mentioned, NORs and C*c*O's are evolutionarily related, both considered as part of a heme/M genetic superfamily. Specifically, certain types of C*c*O's such as ba_3 and caa_3 oxidases from *Thermus thermophilus*, cbb_3 oxidase from *Pseudomonas stutzeri*, and bo_3 from *E. coli* also carry out $\bullet NO_{(g)}$ reductive coupling, that is, NOR chemistry (Scheme 13), although at reaction rates much lower than for the diiron NORs.

However, the study of C*c*O-mediated $\bullet NO_{(g)}$ reductive coupling has recently received considerable attention from the biophysical−biochemical and computational research communities.[34,115,130−132] This interest certainly, in part, derives from the fact that X-ray structures are available for a good number of HCOs, and the study of these systems can/should provide valuable fundamental insights.

In mammalian systems, the study of C*c*O interaction with $\bullet NO_{(g)}$ interactions has a long history;[133−136] NO is thought to be a (reversible) cellular C*c*O respiration inhibitor, and C*c*O/$\bullet NO_{(g)}$ interactions have physiological and pathological consequences, including influencing the NO signaling and cell death.[135,136] $\bullet NO_{(g)}$ can react at the heme and/or Cu_B,[131,135] and NO conversions to iron nitrosyl and/or nitrite (via Cu_B redox chemistry) are known.[135−137] Recent literature suggests that $\bullet NO_{(g)}$ reversible inhibition of C*c*O's and $\bullet NO_{(g)}$ oxidation to nitrite at C_cO active sites are natural functions and occur as a consequence of the available O_2(g) concentrations (i.e., organ or tissue hypoxia vs hyperoxia), all to lead to proper $\bullet NO_{(g)}$ homeostasis via production of an appropriate nitrite [as source of $\bullet NO_{(g)}$] pool.[12,13] Recent papers even suggest that $\bullet NO_{(g)}$ oxidation to nitrite and not C*c*O inhibition is a primary C*c*O/$\bullet NO_{(g)}$ interaction.[138,139] For NO_2^- formation, PN (O=NOO$^-$) may form as an intermediate,[140] but this has been partially disputed.[141] Nitrite-dependent $\bullet NO_{(g)}$ production appears to occur under hypoxic conditions, and this $\bullet NO_{(g)}$ may be involved in cell signaling via tyrosine nitration.[12]

C*c*O NOR Activity. Concerning heme/Cu $\bullet NO_{(g)}$ reductive coupling (bio)chemistry, recent biophysical studies and DFT calculations from Varotsis, Kitagawa, Ohta, and coworkers[130−132,142] have led to the suggestion of what is referred to as a trans mechanism (Figure 7). This was adopted on the basis of their biophysical and DFT studies, with reference to the literature on the diiron NORs (see Scheme 9). Rate-limiting addition of $\bullet NO_{(g)}$ and a proton to a heme/nitrosyl complex leads to a "N_2O_2H" intermediate, one-electron-oxidized from a hyponitrite dianion ($N_2O_2^{2-}$) and protonated, that possesses a N−N bond. Note that reduced Cu^{I}_B is proposed to be present, and a DFT-calculated structure for this intermediate that is N-bound to both the heme iron and copper ions has been presented.[132] These researchers also assert direct detection of the a_3 nitrosyl and hyponitrite species via resonance

(127) Goldstein, S.; Merenyi, G.; Robert, K. P. *Methods Enzymol.* **2008**, *436*, 49–61.
(128) Heinecke, J.; Ford, P. C. *Coord. Chem. Rev.* **2010**, *254*, 235–247.
(129) Chen, P.; Gorelsky, S. I.; Ghosh, S.; Solomon, E. I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4132–4140.
(130) Ohta, T.; Kitagawa, T.; Varotsis, C. *Inorg. Chem.* **2006**, *45*, 3187–3190.
(131) Pinakoulaki, E.; Ohta, T.; Soulimane, T.; Kitagawa, T.; Varotsis, C. *J. Am. Chem. Soc.* **2005**, *127*, 15161–15167.
(132) Varotsis, C.; Ohta, T.; Kitagawa, T.; Soulimane, T.; Pinakoulaki, E. *Angew. Chem., Int. Ed.* **2007**, *46*, 2210–2214.
(133) Brudvig, G. W.; Stevens, T. H.; Chan, S. I. *Biochemistry* **1980**, *19*, 5275–5285.
(134) Pilet, E.; Nitschke, W.; Liebl, U.; Vos, M. H. *Biochim. Biophys. Acta* **2007**, *1767*, 387–392.
(135) Mason, M. G.; Nicholls, P.; Wilson, M. T.; Cooper, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 708–713.
(136) Brunori, M.; Forte, E.; Arese, M.; Mastronicola, D.; Giuffre, A.; Sarti, P. *Biochim. Biophys. Acta* **2006**, *1757*, 1144–1154.
(137) Pearce, L. L.; Bominaar, E. L.; Hill, B. C.; Peterson, J. *J. Biol. Chem.* **2003**, *278*, 52139–52145.
(138) Cooper, C. E.; Mason, M. G.; Nicholls, P. *Biochim. Biophys. Acta* **2008**, *1777*, 867–876.
(139) Antunes, F.; Boveris, A.; Cadenas, E. *Antioxid. Redox Signaling* **2007**, *9*, 1569–1580.
(140) Pearce, L. L.; Kanai, A. J.; Birder, L. A.; Pitt, B. R.; Peterson, J. *J. Biol. Chem.* **2002**, *277*, 13556–13562.
(141) Giuffre, A.; Forte, E.; Brunori, M.; Sarti, P. *FEBS Lett.* **2005**, *579*, 2528–2532.
(142) Pinakoulaki, E.; Ohta, T.; Daskalakis, V.; Gialou, I.; Kitagawa, T.; Soulimane, T.; Varotsis, C. A. *Biophys. J.* **2005**, *88*, 391A–391A.

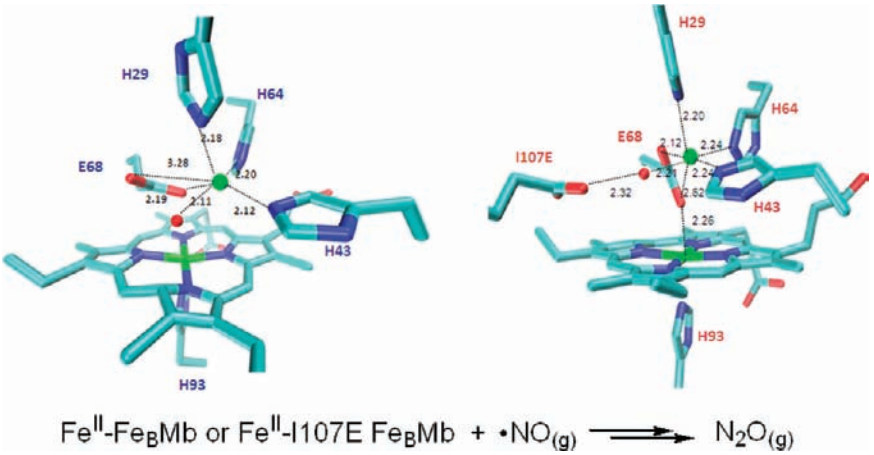
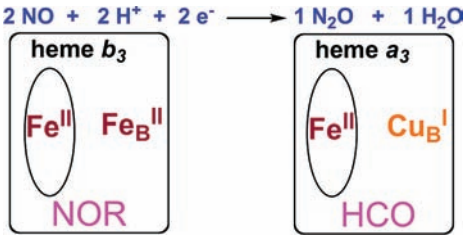


Figure 6. Depiction of two X-ray crystallographically determined forms of the engineered protein Fe^{II}−Fe_BMb (left) and Fe^{II}−I107E Fe_BMb (right), both exhibiting NOR activity.[124,125] Both structures possess the "distal" histidine (H64) from wild-type Mb plus the designed (i.e., mutated) new histidines (H29 and H43) and glutamate residues (E68) that serve as nonheme/iron ligands. The right structure reveals the new glutamate, which is not bound to the Fe_B (E107) but which leads to dramatically enhanced protein activity. See the text for further discussions.

Scheme 13



Raman spectroscopic interrogation; we note that the latter hyponitrite complex was generated by adding excess $\bullet NO_{(g)}$ to an oxidized ($Fe^{III}\cdots Cu^{II}$) form of the enzyme. Finally, it is suggested that further protonation leads to $N_2O(g)$ generation.

The computational studies carried out by Blomberg, Blomberg, and Siegbahn[143] favor a NOR *cis*-heme-like mechanism (Figure 7 and Scheme 9). The heme/nitrosyl complex would undergo direct attack by $\bullet NO_{(g)}$ because the latter is expected to bind the cuprous ion only weakly at best. The hyponitrite complex formed is suggested to be O-bound to $Cu^{II}{}_B$. These latter two suggestions are also in line with recent studies by Moënne-Loccoz and co-workers.[77,115] From studies using FT-IR spectroscopy, following photolysis of the heme/nitrosyl moiety, it was demonstrated that NO binds to Cu_B weakly and in an η^2 side-on or η^1 O-bound (end-on) form for C*c*O from *T. thermophilus*, but no Cu_B interaction is observed at all for the C*c*O bo_3 from *E. coli*. Such observations suggest how a second $\bullet NO_{(g)}$ molecule might slip in to form an N−N bond with the nitrosyl/heme already N-bound to iron but leave a hyponitrite product O-bound to the Cu_B ion.

One of many foci for future studies on the proteins or models (designed protein or synthetic, vide infra) will be on the nature of the putative hyponitrite complex intermediate, its structure, and redox or protonation state. There are very few hyponitrite metal complexes known from inorganic chemistry, but they may coordinate or bridge metals in many different ways.[87,89] A very recent

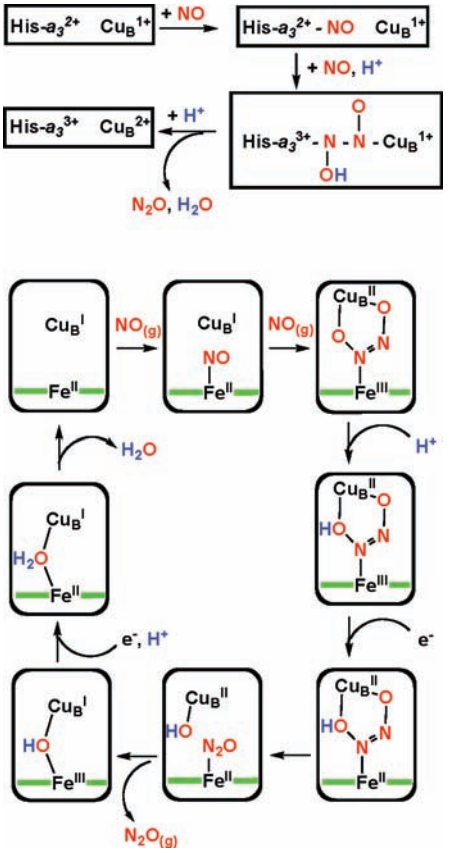
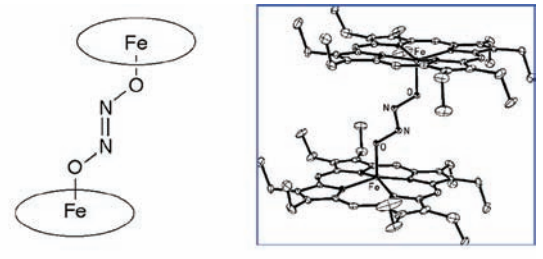


Figure 7. Proposed mechanisms for $\bullet NO_{(g)}$ reductive coupling in heme/Cu C*c*O's: (top) proposal from Varotsis, Kitagawa, Ohta, and co-workers, based on biophysical studies and DFT calculations; (bottom) computationally derived mechanism from Blomberg, Blomberg, and Siegbahn. See the text for further discussions and references.

complex from Richter-Addo and co-workers[89] may be relevant (Scheme 14). They reported on the first structurally characterized heme/hyponitrite complex, a stable bimetallic species from the μ-oxo species $[(OEP)Fe]_2(\mu\text{-}O)$ (OEP = octaethylporphyrinate) complex and hyponitrous acid. It is notable that the $N_2O_2^{2-}$ anion is O-bound to the Fe^{III} atoms, suggesting that this is a stable bonding arrangement.

(143) Blomberg, L. M.; Blomberg, M. R. A.; Siegbahn, P. E. M. *Biochim. Biophys. Acta* **2006**, *1757*, 31–46.

Scheme 14



The addition of acid to their complex releases $N_2O(g)$, and so it will be interesting to eventually know if the structure here is relevant to the NOR and/or heme/copper oxidase (C*c*O) mechanisms and intermediate structures proposed (vide supra).

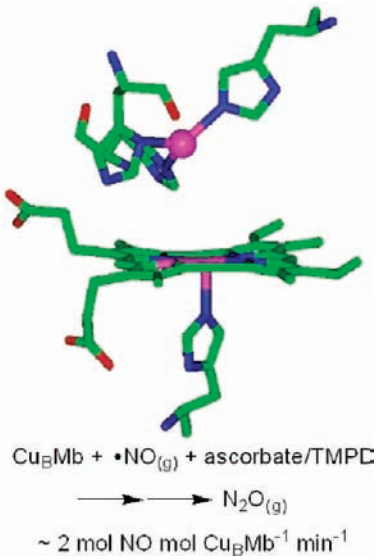
Model Systems, Protein and Synthetic. Lu and coworkers,[144] in a protein-based model system, demonstrated CcO type NOR chemistry coming (again) from a modified/engineered Mb protein from sperm whale. They designed in histidine residues which serve as copper ion ligands (i.e., the Cu_B), leading to a binuclear heme/Cu center. With an added external reductant (ascorbate with tetramethylphenylenediamine (TMPD), they demonstrated $\bullet NO_{(g)}$ to $N_2O_{(g)}$ conversion (Scheme 15). From their studies, the presence of Cu was seen to weaken the proximal His interaction with the heme-iron, allowing for the ability to form a 5-coordinate heme-NO species, which was seen as being critical for the chemistry.

In the last two years, efforts have been made toward the study of $\bullet NO_{(g)}$ interactions with synthetic HCO (e.g., C*c*O) model compounds. Collman et al.[100,101,118] have over the years generated numerous such constructs, many as functional models which electrocatalytically reduce dioxygen to water. In a recent study by that group[145] however, a reduced heme-Cu complex was shown to bind $\bullet NO_{(g)}$, but it did not give reductive coupling chemistry; further O_2-reaction with the NO-adduct gave an oxidized heme product, and it was suggested that the reaction involved formation of nitrate anion (although this was not directly detected).[145]

In the last year, our research group has in fact been able to demonstrate NOR functional modeling, i.e, employment of heme-copper synthetic complexes to effect the coupling of two $\bullet NO_{(g)}$ molecules to give N_2O. In our initial efforts, we employed a binucleating ligand ^{6}L, whose iron and heme-copper derivatives have been previously used in our investigations involving O_2^- chemistry.[146] In the presence of protons, two equivalents $\bullet$NO which bind to the heme are reductively coupled to yield one equivalent N_2O (Figure 8).[147]

The starting point was the reductive nitrosylation of $(^6L)Fe^{III}(OH)$ to straightforwardly give the iron(II) nitrosyl compound $(^6L)Fe(NO)$ (Figure 8, red EPR spectrum), possessing the expected classic three-line hyperfine split EPR spectrum. The affiliated bis-nitrosyl species, $(^6L)Fe(NO)_2$, was generated via the addition of $\bullet NO_{(g)}$ to $(^6L)Fe(NO)$. $(^6L)Fe(NO)_2$ is EPR silent, as expected, and only stable at low-temperature; warming releases one mol-equiv $\bullet NO_{(g)}$ and $(^6L)Fe(NO)$ is reformed (Figure 8). $(^6L)Fe(NO)_2$ was deemed an ideal starting point, as addition of a copper(I) source would result in this ion's binding to the uncoordinated tris(pyridylmethyl)amine "tether" portion of ^{6}L and facilitate further chemistry. This indeed was the case and with the addition of $[Cu^I(MeCN)_4]^+$ and two mole-equivalents of acid, $N_2O_{(g)}$ is indeed produced in high yield (80%), as determined from gas-chromatographic analysis. Consistent with this stoichiometry expected and that each metal ion is a source of one reducing equivalent, the oxidized metal complex product is $[(^6L)Fe^{III}\cdots Cu^{II}(D)]^{3+}$ (D = water or solvent), as demonstrated by UV−vis and EPR spectroscopies (Figure 8).[147]

Scheme 15



By contrast, if acid is not added along with the copper(I) source, the chemistry occurring is that of the well-known[4,86] copper(I) complex mediated disproportionation of $\bullet NO_{(g)}$:

$$3\bullet NO_{(g)} + [(L)Cu^I]^+ \rightarrow N_2O(g) + [(L)Cu^{II}(NO_2^-)]^+ \quad (14)$$

Because the binding of one $\bullet NO_{(g)}$ to the heme is very strong, i.e., in $(^6L)Fe(NO)$, only the other 1 equiv of $\bullet NO_{(g)}$ is available, and the "upper" copper(I) chelate effects its disproportionation. The amount of copper(II) complex present should be at a level of one-third of the amount of heme/nitrosyl complex present (see eq 14 and Figure 8); thus, the g = 2.0−2.3 region of the EPR spectrum for this reaction mixture (Figure 8) is dominated by the heme/nitrosyl complex signal on top of a typical copper(II) tetragonal EPR signal. The yield of $N_2O(g)$ was 90%, based on this expected stoichiometry, and the presence of a nitrite anion was proven using ion chromatography. Further supporting evidence was that a close analogue of just the copper(I) chelate present in the

(144) Zhao, X.; Yeung, N.; Russell, B. S.; Garner, D. K.; Lu, Y. *J. Am. Chem. Soc.* **2006**, *128*, 6766–6767.
(145) Collman, J. P.; Dey, A.; Decreau, R. A.; Yang, Y.; Hosseini, A.; Solomon, E. I.; Eberspacher, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9892–9896.
(146) Chufán, E. E.; Mondal, B.; Gandhi, T.; Kim, E.; Rubie, N. D.; Moënne-Loccoz, P.; Karlin, K. D. *Inorg. Chem.* **2007**, 46, in press.
(147) Wang, J.; Schopfer, M. P.; Sarjeant, A. A. N.; Karlin, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 450–451.

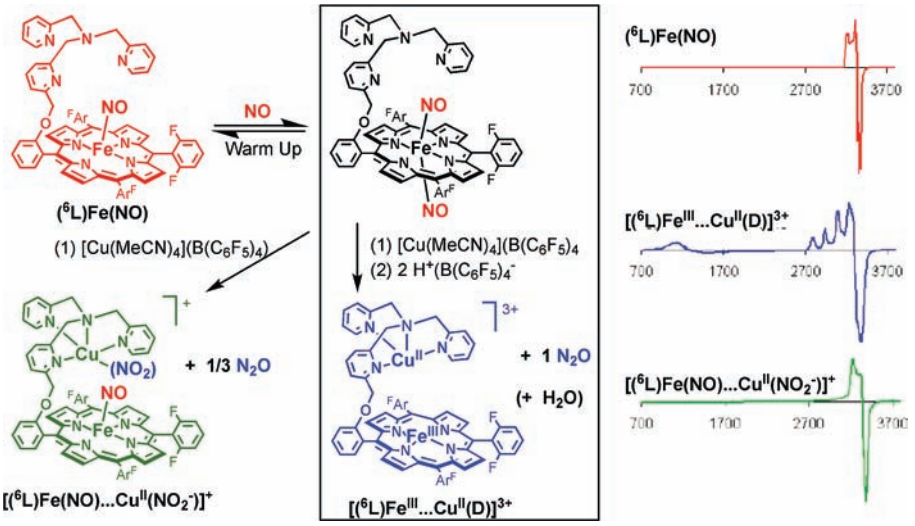
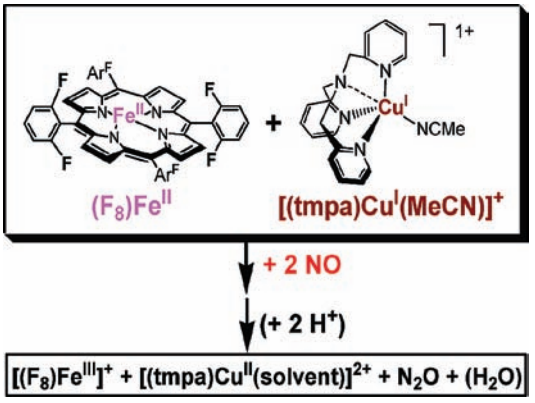


Figure 8. Reductive coupling chemistry mediated by the heme/copper complex with a binucleating ^{6}L ligand. The addition of a copper(I) source to the heme/dinitrosyl complex, with acid, leads to $\bullet NO_{(g)}$ reductive coupling to give N_2O(g). The EPR spectrum (blue) for the oxidized heme/Cu product $[(^6L)Fe^{III}\cdots Cu^{II}(D)]^{3+}$ shows the presence of a $g = 6$ signal for high-spin Fe^{III} and a typical Cu^{II} spectrum in the $g = 2.0-2.3$ region. See the text for further explanations.[147].

Scheme 16



^{6}L-containing heme/copper metal complex does, in fact, disproportionate $\bullet NO_{(g)}$ according to eq 14.[147]

More recently,[148] a similar $\bullet NO_{(g)}$ reductive coupling reaction was shown to occur when the component complexes are employed, $(F_8)Fe^{II}$ and $[(tmpa)Cu^I(MeCN)]^+$ [tmpa = tris(2-pyridylmethyl)amine] as metal complexes and sources of electrons, i.e., one from each. Formation of the heme/dinitrosyl complex $(F_8)Fe(NO)_2$ by the −80 °C addition of excess $\bullet NO_{(g)}$ to $(F_8)Fe^{II}$ [vacuum−purge removal of any excess $\bullet NO_{(g)}$] and the addition of the copper(I) complex and acid effect the reductive coupling and production of nitrous oxide (Scheme 16). Here, more detailed UV−vis, EPR (Figure 9), and NMR characterizations were described, such as those for the heme/nitrosyl (pentacoordinate, $\nu_{N-O} = 1691$ cm^{-1} in CH_2Cl_2; hexacoordinate with a THF axial base ligand, $\nu_{N-O} = 1670$ cm^{-1} in THF) and heme/dinitrosyl complexes (EPR-silent; Figure 9). The overall chemistry is essentially the same (Figure 10), as described by Figure 8. Again, without acid present, only disproportionation of

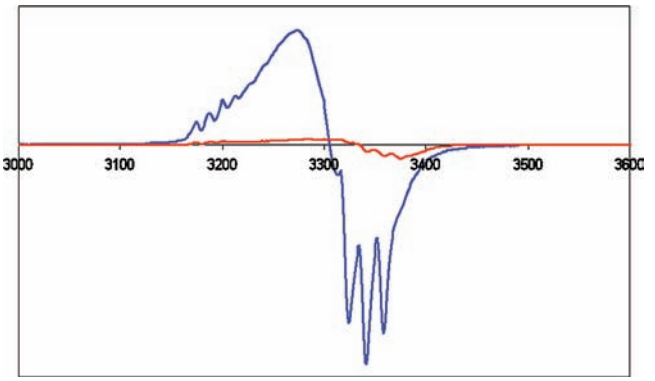


Figure 9. EPR spectra of $(F_8)Fe(NO)$ ligand ($g_1 = 2.113$, $g_2 = 2.078$, $g_3 = 2.025$; blue) and the dinitrosyl species $(F_8)Fe(NO)_2$ (EPR-silent; red) at the same concentration in acetone, recorded at 77 K.[148].

1 of the 2 mol equiv of $\bullet NO_{(g)}$ present at the beginning took place.

For both chemical systems described above (Figures 8 and 10), acid alone plus the heme/dinitrosyl complexes does not effect $\bullet NO_{(g)}$ reductive coupling. The copper ion has a critical role in this NOR mimetic chemistry. We suggest possible steps in the chemistry described here. A likely first one would be the attack of the copper(I) complex moiety on the $\bullet NO_{(g)}$ ligand in the heme/dinitrosyl fragment. This may release one $\bullet NO_{(g)}$ molecule, which now attacks a binuclear heme/$Fe^{II}(NO)\cdots Cu^I$ species to give a hyponitrite-type species. Then, protonation, electron transfer from the copper ion, and N−O cleavage could give N_2O(g), water, and the heme Fe^{III} and Cu^{II} complexes observed. As yet, we do not possess any direct mechanistic information, nor have we yet been able to detect any intermediates. However, we expect new insights to be made from further low-temperature monitoring and/or kinetic studies, and hopefully these will provide useful information on this fundamentally important nitrogen oxide chemistry and possibly provide further clues to the mechanistic details associated with the enzymatic reactions.

(148) Wang, J.; Schopfer, M. P.; Puiu, S. C.; Sarjeant, A. A. N.; Karlin, K. D. *Inorg. Chem.* **2010**, *49*, 1404–1419.

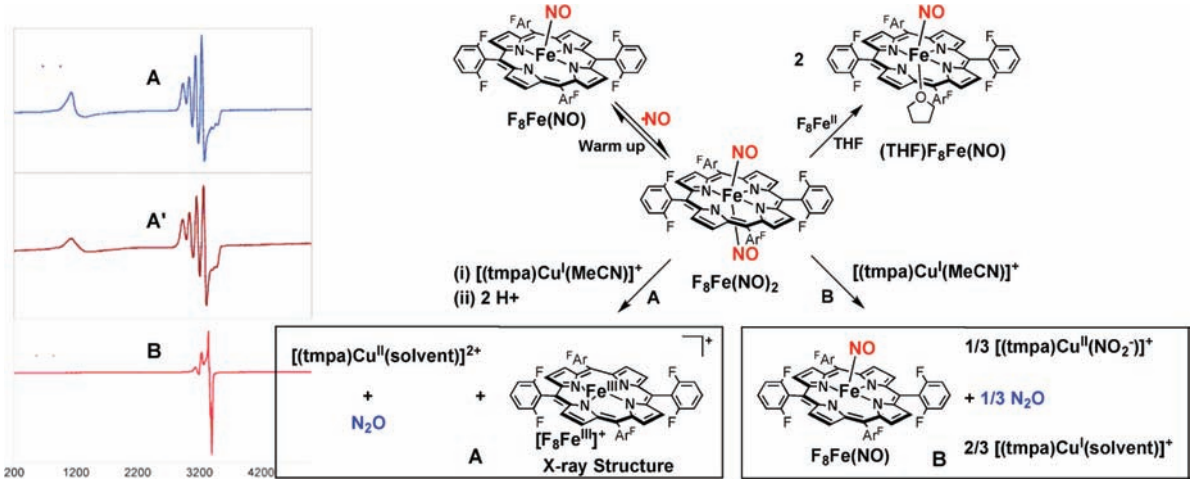


Figure 10. Outline of the $\bullet NO_{(g)}$ reductive coupling chemistry mediated by the component system, starting with 1:1 mixtures of heme and copper complexes $(F_8)Fe(NO)$ and $[(tmpa)Cu^{I}(MeCN)]^{+}$. When the dinitrosyl species $(F_8)Fe(NO)_2$ is reacted with $[(tmpa)Cu^{I}(MeCN)]^{+}$ and acid, the products obtained are shown in panel A, associated with EPR spectrum A (blue). The TBP nature of $[(tmpa)Cu^{I}(MeCN)]^{2+}$ leads to the expected "reverse" axial spectrum, while the $g = 6$ feature is associated with a high-spin $[(F_8)Fe^{III}]^{+}$. Specturm A′ is a made-up 1:1 mixture of authentic compounds showing a match with spectrum A. EPR spectrum B is a mixture ascribed to that product of the reaction without acid (panel B). See the text for further explanation.[148].

Conclusions

In this Forum Article, we have emphasized recent works, especially from our own laboratories, pertaining to iron- and/or copper-mediated chemistry with nitrogen oxides. At the center of the picture is $\bullet NO_{(g)}$, and although it was not emphasized here, its formation from sources other than NOSs, especially nitrite, is a particularly important and right now a "hot" subject. We have focused on bioinspired inorganic chemistry and biochemistry involved with either $\bullet NO_{(g)}$ oxidation or reduction. For the former, oxidation by heme-containing NODs, including Hb and Mb, which carry out this function, nitrate is the product. However, PN and/or $\bullet NO_{2(g)}$ may form as intermediates and may leak from the center of action, i.e., the enzyme active sites. Because these species have their own chemistry, other types of products might appear, such as nitrite, a hydroxyl radical, or O_2. Concerning the reduction of $\bullet NO_{(g)}$, we have emphasized its heme/nonheme/diiron- or heme/copper-mediated reductive coupling to give $N_2O_{(g)}$ and inorganic/model systems, which give rise to this biologically interesting and important reaction.

There is considerable current interest and activity in the subjects discussed here. From the synthetic bioinorganic perspective, we suggest that a considerable increase in activity will soon take place. There is already a strong history of nitrogen oxide research in the literature, but there are many fundamental aspects to still be uncovered, having chemical, biological, medicinal, and environmental implications. As the very exciting biology continues to be unraveled, inorganic chemists, certainly ourselves, will watch with great interest and be inspired to carry out new inorganic chemistry.

Note Added in Proof. Very recent reports bear on subjects discussed in this review. A detailed vibrational spectroscopic analysis and DFT modeling of $\bullet NO_{(g)}$ bonding to ferric hemes {*J. Am. Chem. Soc.* **2010**, *132*, 4614−4625} includes a discussion of the NOR N-N coupling mechanism and possible heme $Fe^{III}-N-O$ bending in the presence of a nucleophile such as that from an Fe_B-coordinated NO^{-}. Of broader interest and in the context of nitrogen oxide metabolism, another account {*Nature* **2010**, *464*, 543−548 and *Nature* **2010**, *464*, 500−501} provides an intriguing proposal for the existence of an as yet unknown "NO dismutase". This would explain the finding of an anaerobic methane oxidizing bacterium which is linked to nitrite utilization. The latter is reduced to $\bullet NO$, which dismutates, $2\ \bullet NO \rightarrow N_2 + O_2$, supplying the O_2 required for CH_4 oxidative metabolism. Note that elsewhere the term "NO dismutase" has a different meaning {*Angew. Chem., Int. Ed.* **2008**, *47*, 8735−8739}.

Acknowledgment. The authors acknowledge support of the National Institutes of Health (Grants GM28962 and GM60353) and Korea WCU Program R31-2008-000-10010-0.