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Heme/Copper Assembly Mediated Nitrite and Nitric Oxide Interconversion

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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 NO_2^- ion producing nitric oxide. The heme serves as the reductant while the Cu^{II} ion is also required. In turn, a μ-oxo hemeFe^{III}-O-Cu^{II} complex facilitates NO oxidation to nitrite; the final products are the reduced heme and Cu^{II}-nitrite 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 powerful vasodilator involved in many physiological and pathological processes.[1] Nitrite (NO_2^-), 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 O_2-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 NO generation catalyzed by nitric oxide synthase (NOS).[2] Subsequently, suggested conserved roles for the NO_2^-/NO pool in cellular processes are such as 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 it is effected by a variety of proteins/enzymes, including hemes, those with molybdenum,[4] and what draws our current interest, cytochrome *c* oxidases (C*c*O). [3,5]

The link between nitrite/NO redox interconversion and O_2-sensing is thought to occur in mitochondria at the C*c*O binuclear heme$_{a3}$/Cu$_B$ center; C*c*O is the terminal enzyme of the mitochondrial respiratory chain. Here, molecular oxygen consumption (i.e., O_2 reduction to water) is down-regulated in hypoxia by increased NO generation via C*c*O nitrite reductase activity, as reduced heme/Cu centers dominate when the O_2 concentration is low.[3a,4,6] The NO thus generated inhibits C*c*O activity by reversibly binding to heme$_{a3}$, in place of O_2, resulting in cellular O_2 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 O_2 cellular concentrations.[3]

In turn, in normoxia, high local O_2 concentrations do not allow nitrite to compete as an oxidant at the C*c*O binuclear center; NO/O_2 binding is non-competitive[7] and heme$_{a3}$/Cu$_B$

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ASSOCIATED CONTENT

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

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., $Fe^{II}..Cu^{II}$, efficiently converts nitrite to NO. When we employ a fully oxidized $Fe^{III}…Cu^{II}$ heme/Cu complex, NO is readily oxidized to nitrite. The overall reactions are represented by equations 1 and 2.

$$(P)Fe^{II}Cu^{II}+NO_2^- \rightarrow (P)Fe^{III}-O-Cu^{II}+NO \quad (1)$$

$$(P)Fe^{III}-O-Cu^{II}+NO \rightarrow (P)Fe^{II}Cu^{II}+NO_2^- \quad (2)$$

Nitrite reductase chemistry,[10] here however in a heme/Cu chemical system, consists of the iron(II) complex $(F_8)Fe^{II}$ (F_8 ≡ tetrakis(2,6-difluorophenyl)porphyrinate(2−))[11] and a preformed copper(II)-nitrite complex $[(tmpa)Cu^{II}(NO_2)]$-$[B(C_6F_5)_4]$ (tmpa ≡ tris(2-pyridyl-methylamine); the latter was synthesized by adding $AgNO_2$ to a chloride precursor $[(tmpa)Cu^{II}(Cl)][B(C_6F_5)_4]$ and its X-ray structure reveals an O-bound nitrito ligated Cu(II) ion (Scheme 1).[12]

When two equiv $(F_8)Fe^{II}$ are mixed with one equiv $[(tmpa)Cu^{II}(NO_2)]^+$ under a N_2 atmosphere in acetone at RT, a reaction ensues and based on UV-vis, EPR and IR spectroscopies, a one-to-one mixture of the heme-nitrosyl species $(F_8)Fe^{II}(NO)$ and the μ-oxo complex $[(F_8)Fe^{III}$-O-$Cu^{II}(tmpa)]^+$ are produced.[12] These products are readily identified, having been previously thoroughly characterized.[13] To determine whether it is the heme or the copper ion that is the reductant in this one-electron process (NO_2^- —> NO), we also carried out the reaction where nitrite was added to the oxidized heme complex $[(F_8)Fe^{III}]SbF_6$ [13b] (binding of nitrite to the ferric heme[14] is indicated by the large UV-vis change which occurs)[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 show that nitrite reacts only very slowly with $(F_8)Fe^{II}$ and not at all with $[(tmpa)Cu^{I}(MeCN)]^+$. Moreover, nitrite reductase activity is not 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 two equiv of complex $(F_8)Fe^{II}$ is due to the well-known high affinity of NO to bind ferrous hemes.[16] Nitric oxide formed first reacts very rapidly with $(F_8)Fe^{II}$; thus, if the reaction is carried out with equimolar quantities of $(F_8)Fe^{II}$ and $[(tmpa)Cu^{II-}(NO_2)]^+$, only ½ of the iron is available to reduce nitrite, and the rest traps the NO as $(F_8)Fe^{II}(NO)$. The role of the Cu^{II} ion appears to be that 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-$Cu^{II}(tmpa)]^+$.

In demonstrating 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 restore it into the nitrite pool (vide supra), we employed $[(F_8)Fe^{III}$-O-$Cu^{II}(tmpa)]^+$. Addition of NO to this fully oxidized hetero-binuclear complex leads to rapid reaction (Scheme 2) and formation of nitrite which binds to Cu(II); the $(F_8)Fe^{II}$ which forms[17] in this redox reaction is trapped by a 2nd equiv of NO to give $(F_8)Fe^{II}(NO)$. UV-vis (Figure 2) and IR (ν_{NO} = 1688 cm^{-1})[12] spectroscopies directly indicate nitrosyl complex formation. Nitrite analysis employing capillary electrophoresis reveals production of a 95% yield of this ion.[13] EPR spectroscopy confirms that a copper(II)-nitrito complex is produced (Figure 2); a sample taken from the

reaction mixture is 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 requires two mole-equiv of NO (Scheme 2). The 2nd equiv is not involved in redox chemistry, but is needed to trap free left-over $(F_8)Fe^{II}$ which is produced by the NO oxidase chemistry.

If $(F_8)Fe^{II}$ is present, it effects the reverse reaction, i.e., reduction of nitrite bound to Cu(II), giving NO, Scheme 1. This can be demonstrated as follows: When 25 mL of a 10 μM solution containing the reaction product mixture $(F_8)Fe^{II}(NO)$ and $[(tmpa)Cu^{II}(NO_2)]^+$, that derived from $[(F_8)Fe^{III}\text{-}O\text{-}Cu^{II}(tmpa)]^+$ + xs NO, (green spectrum, Figure 3), is titrated with 25 mL of a 20 μM (2 equiv) solution of $(F_8)Fe^{II}$, the product solution (red spectrum, Figure 3) shows that ~5 μM $[(F_8)Fe^{III}\text{-}O\text{-}Cu^{II}(tmpa)]^+$ is present, along with 10 μM $(F_8)Fe^{II}(NO)$, the spectral intensity now equivalent to that observed in the starting mixture, because of dilution. This proves that the backward reaction can and does occur, i.e., that $[(tmpa)Cu^{II}(NO_2)]^+$ reacts firstly with $(F_8)Fe^{II}$ in a 1:1 stoichiometry to give NO, then trapped by the second equiv $(F_8)Fe^{II}$.

We also tested the μ-hydroxo complex $[(F_8)Fe^{III}\text{-}(OH)\text{-}Cu^{II}(tmpa)]^{2+}$ for 'NO oxidase' chemistry, but upon addition of NO, there is no nitrite production (Scheme 2).[12] Instead, very slow (hours) reductive nitrosylation[18] occurs and all the heme present is converted to $(F_8)Fe^{II}(NO)$. It is thus clear that the μ-oxo complex $[(F_8)Fe^{III}\text{-}O\text{-}Cu^{II}(tmpa)]^+$ is efficient or at least special in its ability to effect a redox reaction (formally Fe^{III} —> Fe^{II}) which includes oxo-transfer.[19]

In summary, this report describes new chemistry with heme/Cu assemblies and nitrogen oxide interconversion: Nitrite reduction to nitric oxide can be readily effected with our heme/copper chemistry. The reduced heme is the source of the one-electron required. The presence of Cu^{II} ion, as Lewis Acid, is crucial. While nitrite reduction to NO is well known to occur via heme proteins such as Hb and Mb,[20] bacteria/fungal heme cd_1 [21] or copper nitrite reductases[22] and certain copper(I) complexes,[22a,23] the transformation with heme/Cu synthetic complexes until now seems to not have been examined. 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, based on the observed products; however cyclic voltammetric determination of redox potentials for separate $(F_8)Fe$ (−0.20 V vs Fc^+/Fc)[12] and Cu(tmpa) (−0.42 V vs Fc^+/Fc)[12] complexes indicate the latter is a better reductant. For C*c*O, 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 $[(F_8)Fe^{III}\text{-}O\text{-}Cu^{II}(tmpa)]$ effects NO-oxidation to nitrite by oxo-transfer (vide supra);[19] $(F_8)Fe^{III}$ becomes reduced. The closely related species $[(F_8)Fe^{III}\text{-}(OH)\text{-}Cu^{II}(tmpa)]^+$ and/or heme-only complexes do not enable this reaction. Yet, as already mentioned, it is the Cu_B in C*c*O which 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 C*c*O it is a particular coordination environment, a specific structural and/or redox state, that is required to mediate 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 heme reductive capability and the nitrite-Cu binding mode, e.g., O-, vs O,O'- vs N-bound. As our heme and Cu centers have switched redox capabilities compared to C*c*O, we will wish to change the heme or the Cu-ligand so as 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] Further investigations are required. "The devil is in the details."

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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17. By analogy to what has in the past been suggested for the enzyme reaction,[8b] nitric oxide attacks at the cupric center, formally leading to Cu^{I}-NO^{+}, with oxo transfer to the latter, along with electron transfer from Cu^{I} to Fe^{III}, giving the Cu^{II}-nitrito complex, and Fe^{II} (which is then trapped by a second NO molecule). Separately, we can demonstrate that with the present ligand-complexes, $[(tmpa)Cu^{I}(MeCN)]^{+}$ and $[(F_8)Fe^{III}]SbF_6$, the Cu^{I}-to-Fe^{III} electron-transfer readily occurs in acetone, see Supporting Information.
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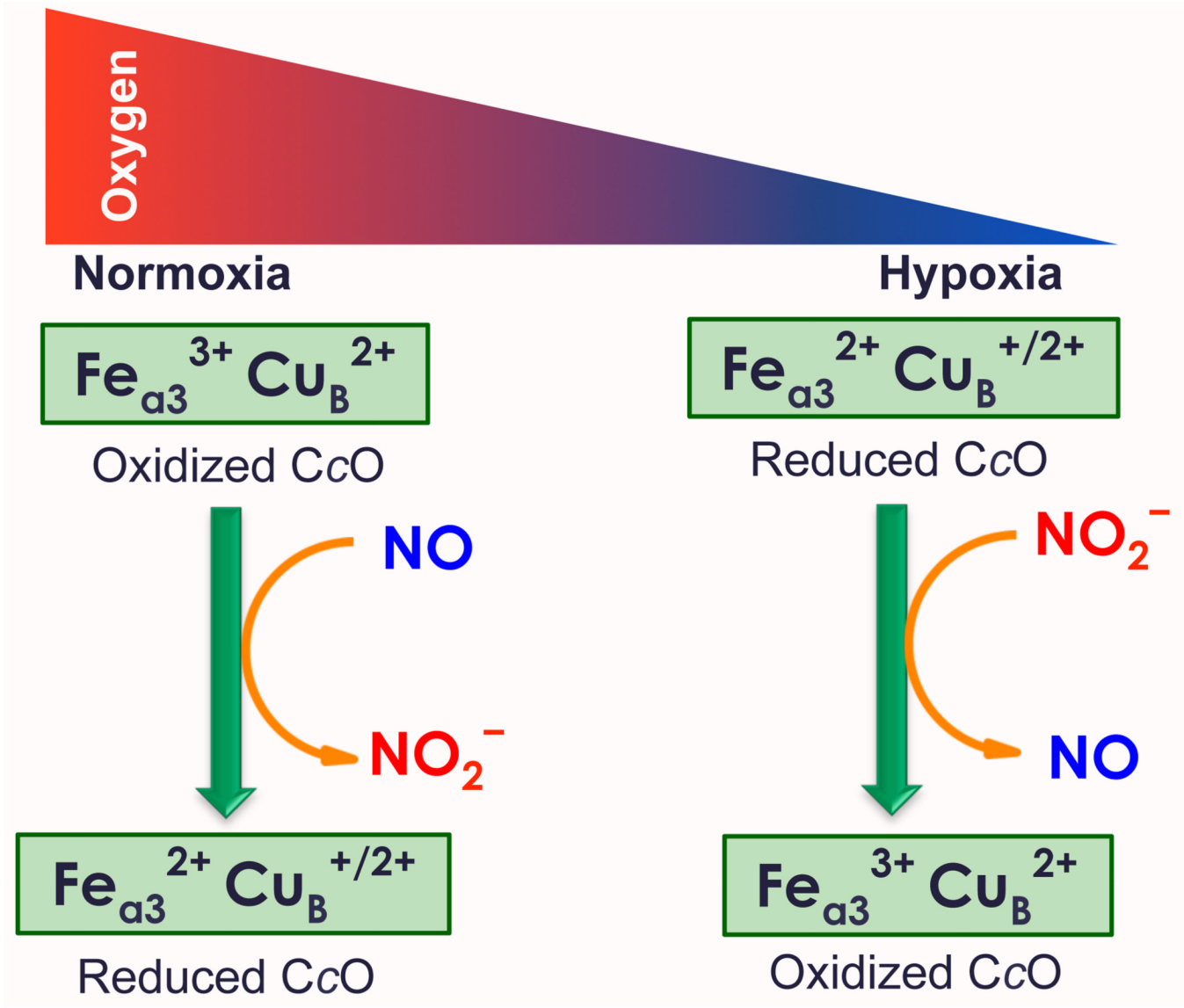


Figure 1.
Cytochrome *c* oxidase (C*c*O) functioning in nitrite (NO_2^-) and nitric oxide (NO) interconversion, as part of its role in regulation of dioxygen balance. Molecular oxygen availability influences the redox state of the C*c*O containing (heme$_{a3}$/Cu_B) binuclear center.

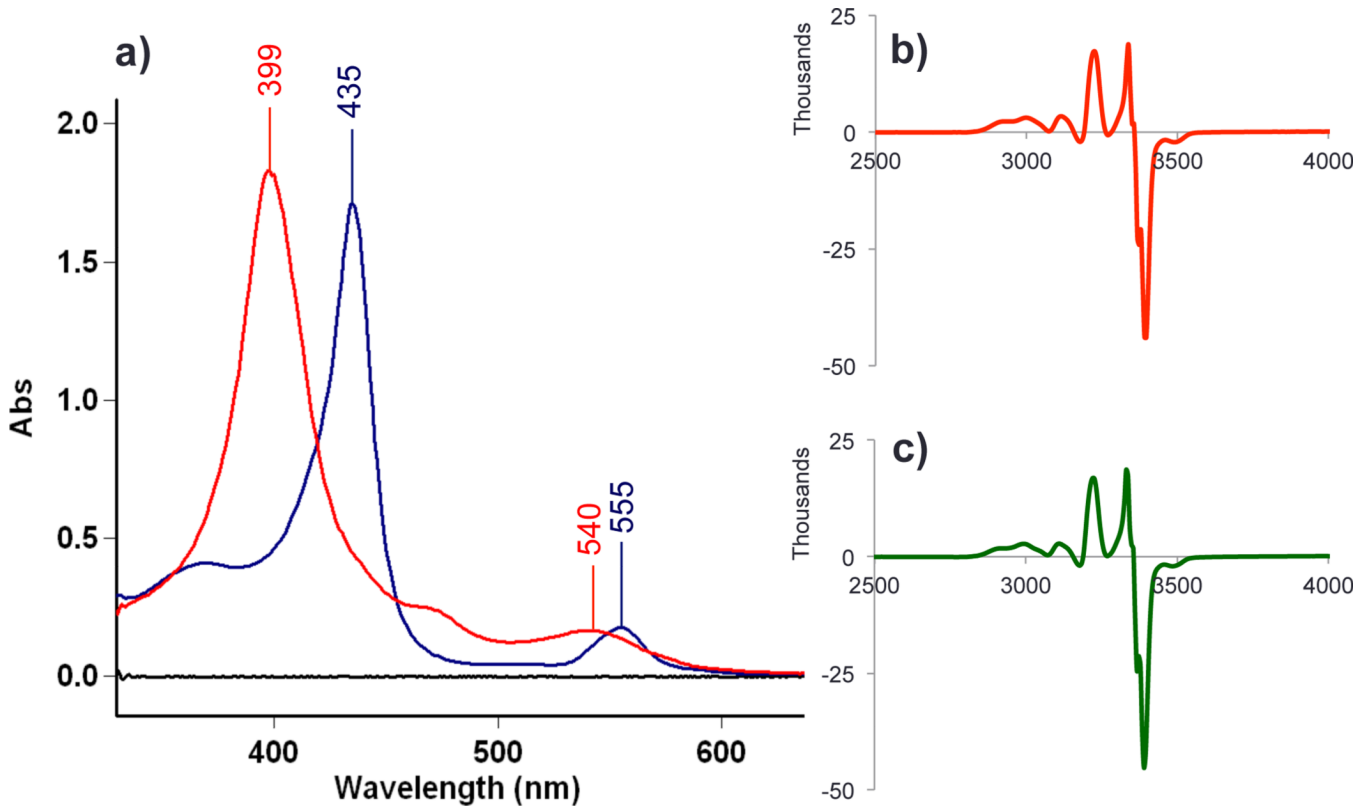


Figure 2.

a) UV-vis spectra of $(F_8)Fe^{III}$-O-Cu^{II}(tmpa)][$B(C_6F_5)_4$] (**1**) (blue), $(F_8)Fe^{II}(NO)$ (red) generated from **1** + $NO_{(g)}$ (12 μM in acetone at RT); b) EPR spectrum of the reaction products of $(F_8)Fe^{III}$-O-Cu^{II}(tmpa)][$B(C_6F_5)_4$] and NO (red) and; c) EPR spectrum of an authentic sample of a 1:1 $F_8Fe^{II}(NO)$ and [(tmpa)$Cu(NO_2)$][$B(C_6F_5)_4$] mixture (green). EPR spectra were recorded at 20 K (1 mM in MeTHF).

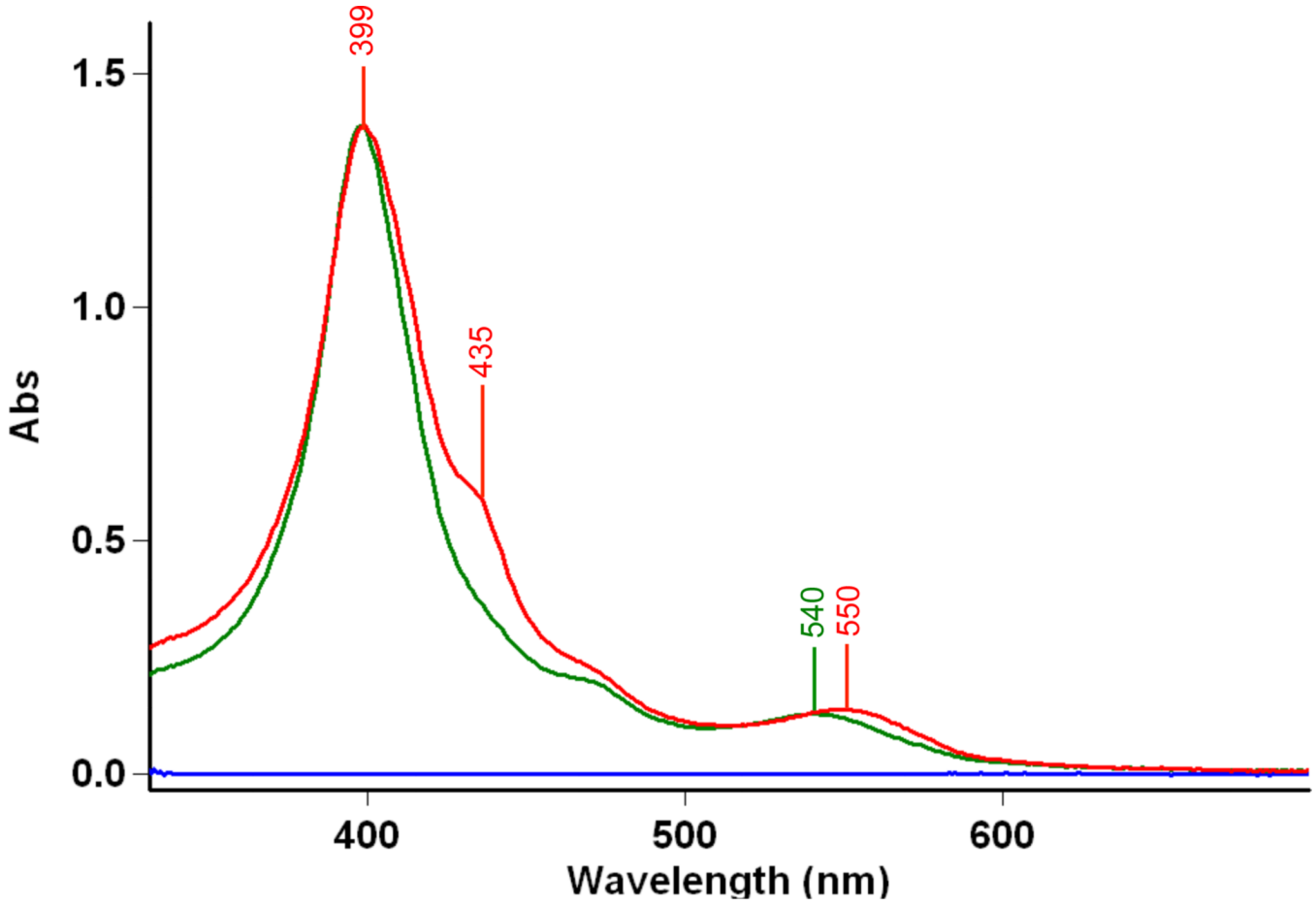
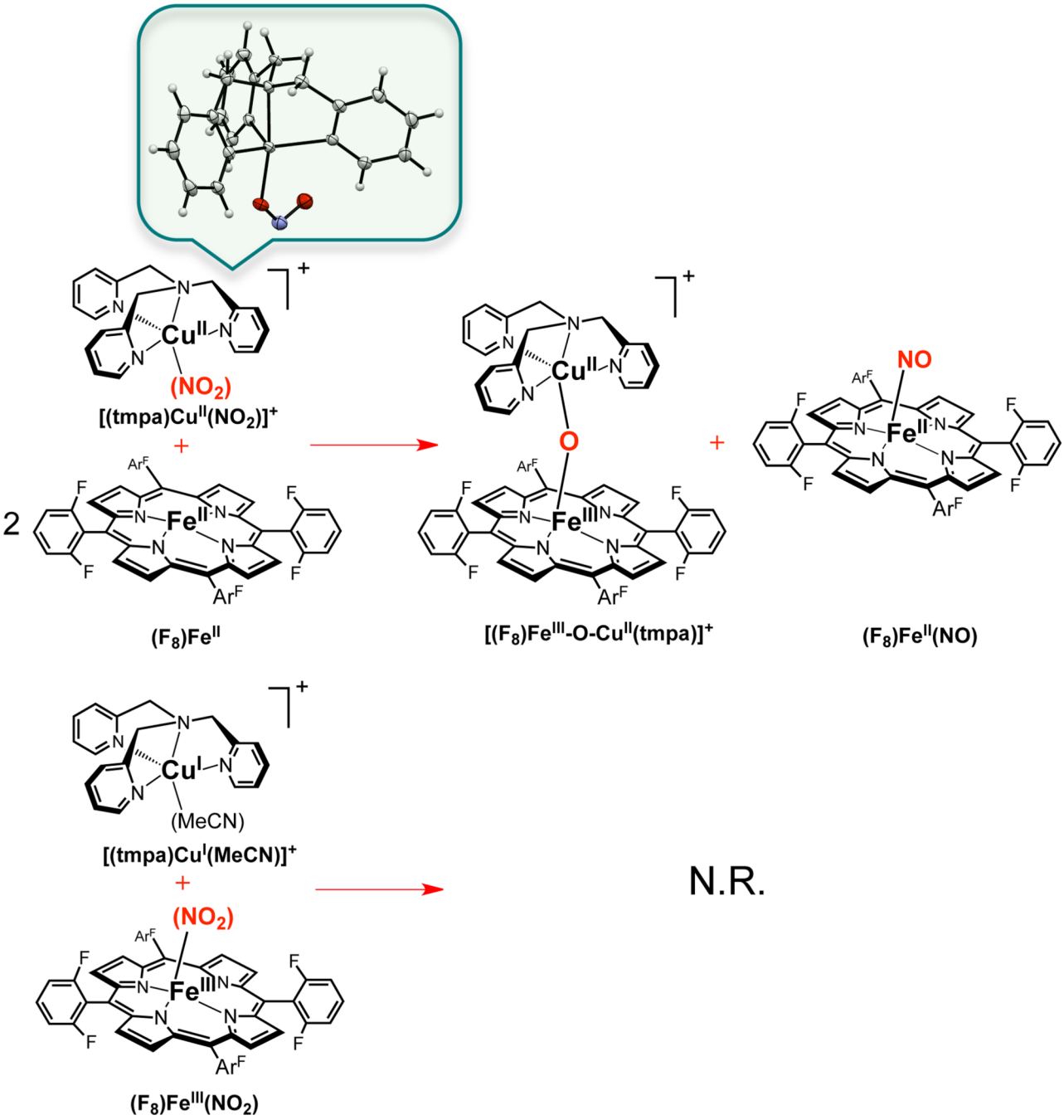
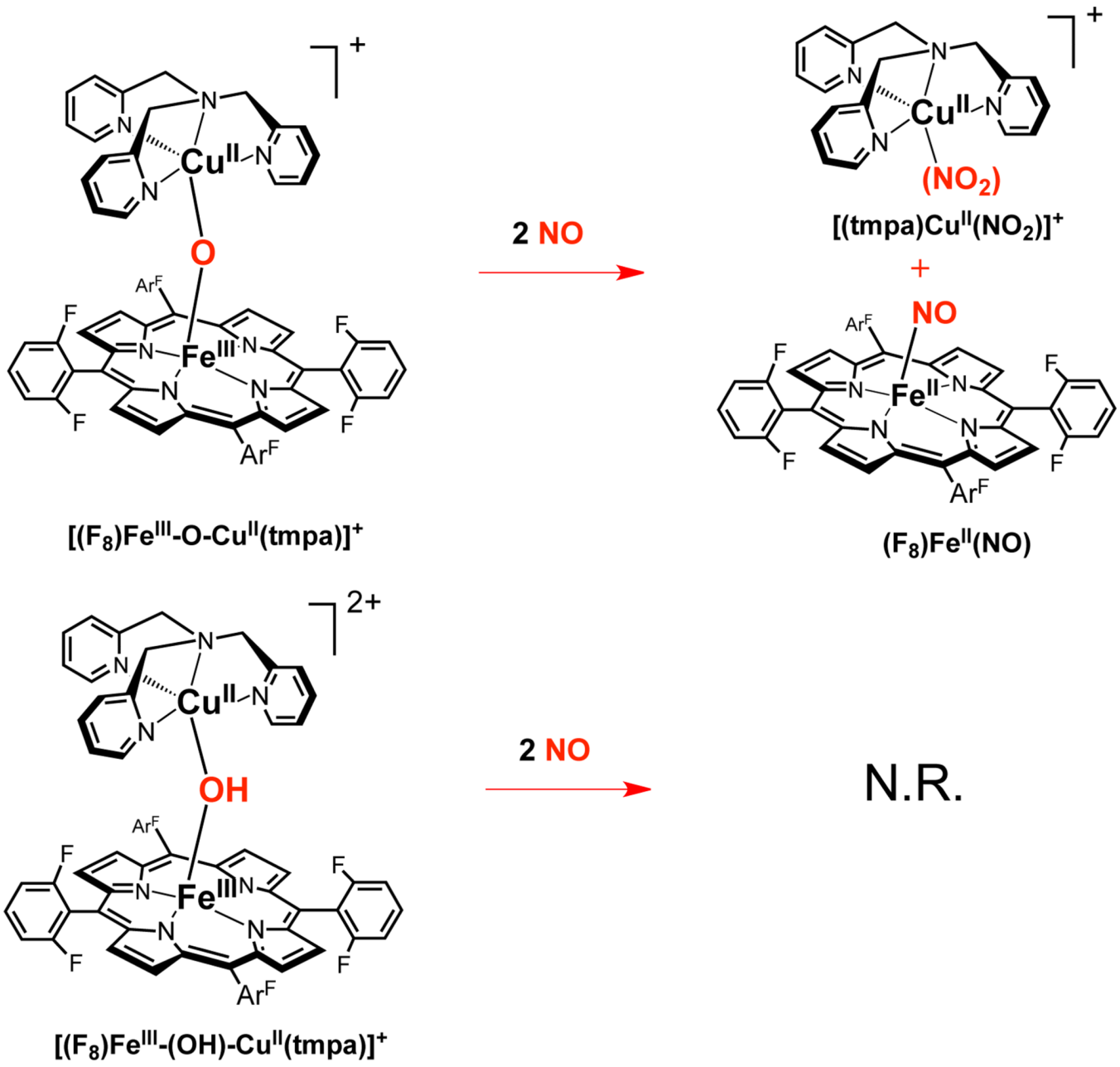


Figure 3.

To the product solution $(F_8)Fe^{II}(NO)$ (λ_{max} = 399 nm) plus $[(tmpa)Cu^{II}(NO_2)]^+$ (green), derived from the NO oxidase chemistry, is added 2 equiv $(F_8)Fe^{II}$. The resulting solution (red spectrum) reveals the presence of a 2:1 mixture of $(F_8)Fe^{II}(NO)$ (λ_{max} = 399 nm) and $[(F_8)Fe^{III}\text{-}O\text{-}Cu^{II}(tmpa)]^+$ (435 nm, sh). The new 2nd equiv $(F_8)Fe^{II}(NO)$ derives from nitrite reductase chemistry described by Scheme 1. See text for further explanation.



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



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