ACCOUNTS of chemical research

Article

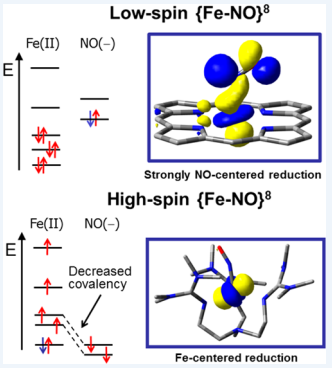
pubs.acs.org/accounts

Heme versus Non-Heme Iron-Nitroxyl {FeN(H)O}^{8} Complexes: Electronic Structure and Biologically Relevant Reactivity

Amy L. Speelman and Nicolai Lehnert*

Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States

CONSPECTUS: Researchers have completed extensive studies on heme and non-heme iron-nitrosyl complexes, which are labeled {FeNO}^{7} in the Enemark-Feltham notation, but they have had very limited success in producing corresponding, one-electron reduced, {FeNO}^{8} complexes where a nitroxyl anion (NO^-) is formally bound to an iron(II) center. These complexes, and their protonated iron(II)-NHO analogues, are proposed key intermediates in nitrite (NO_2^-) and nitric oxide (NO) reducing enzymes in bacteria and fungi. In addition, HNO is known to have a variety of physiological effects, most notably in the cardiovascular system. HNO may also serve as a signaling molecule in mammals. For these functions, iron-containing proteins may mediate the production of HNO and serve as receptors for HNO in vivo. In this Account, we highlight recent key advances in the preparation, spectroscopic characterization, and reactivity of ferrous heme and non-heme nitroxyl (NO^-/HNO) complexes that have greatly enhanced our understanding of the potential biological roles of these species.



Low-spin (ls) heme {FeNO}^{7} complexes (S = 1/2) can be reversibly reduced to the corresponding {FeNO}^{8} species, which are stable, diamagnetic compounds. Because the reduction is ligand (NO) centered in these cases, it occurs at extremely negative redox potentials that are at the edge of the biologically feasible range. Interestingly, the electronic structures of ls-{FeNO}^{7} and ls-{FeNO}^{8} species are strongly correlated with very similar frontier molecular orbitals (FMOs) and thermodynamically strong Fe-NO bonds. In contrast, high-spin (hs) non-heme {FeNO}^{7} complexes (S = 3/2) can be reduced at relatively mild redox potentials. Here, the reduction is metal-centered and leads to a paramagnetic (S = 1) {FeNO}^{8} complex. The increased electron density at the iron center in these species significantly decreases the covalency of the Fe-NO bond, making the reduced complexes highly reactive. In the absence of steric bulk, monomeric high-spin {FeNO}^{8} complexes decompose rapidly. Notably, in a recently prepared, dimeric [{FeNO}^{7}]$_2$ species, we observed that reduction leads to rapid N−N bond formation and N_2O generation, which directly models the reactivity of flavodiiron NO reductases (FNORs).

We have also made key progress in the preparation and stabilization of corresponding HNO complexes, {FeNHO}^{8}, using both heme and non-heme ligand sets. In both cases, we have taken advantage of sterically bulky coligands to stabilize these species. ls-{FeNO}^{8} complexes are basic and easily form corresponding ls-{FeNHO}^{8} species, which, however, decompose rapidly via disproportionation and H_2 release. Importantly, we recently showed that we can suppress this reaction via steric protection of the bound HNO ligand. As a result, we have demonstrated that ls-{FeNHO}^{8} model complexes are stable and amenable to spectroscopic characterization. Neither ls-{FeNO}^{8} nor ls-{FeNHO}^{8} model complexes are active for N−N coupling, and hence, seem unsuitable as reactive intermediates in nitric oxide reductases (NORs). Hs-{FeNO}^{8} complexes are more basic than their hs-{FeNO}^{7} precursors, but their electronic structure and reactivity is not as well characterized.

1. INTRODUCTION

The role of nitric oxide (NO) as a signaling molecule and a critical immune defense agent in mammals is now established,[1,2] and key proteins responsible for the production, detection, and detoxification of NO in mammals have been identified.[3] However, much less is known about the potential biological role of other NO_x derivatives and the enzymes involved in their generation and metabolism. In particular, nitrite (NO_2^-) and nitroxyl (NO^-/HNO) have been implicated as important signaling molecules, NO precursors and/or products of NO metabolism.[4,5] The currently accepted aqueous pK_a for free HNO is 11.6, which indicates that at physiological pH, the nitroxyl anion exists exclusively in its protonated form.[6] The physiological effects of HNO have been reviewed extensively in the literature and will only be discussed briefly here.[5,7,8] The most well-characterized effects of HNO stem from its interaction with thiols, as exemplified by inhibition of the enzyme aldehyde dehydrogenase by cyanamide (which is metabolized to HNO). In the cardiovascular system, HNO acts as a vasorelaxant, increases cardiac muscle contractility, and protects against ischemia reperfusion injuries. HNO also binds to a variety of globin proteins to form stable HNO complexes,[9] which suggests that iron centers could act as HNO sensors. Ferrous nitroxyl complexes are designated as {FeNO}^{8} or {FeNHO}^{8} (depending on the pH) in the Enemark-Feltham notation,[10] where the superscript denotes the number of Fe d plus NO π^* electrons.

In addition to the potential role of free HNO in biology, iron-nitroxyl complexes are important intermediates in NO-

Received: October 25, 2013
Published: February 20, 2014

 © 2014 American Chemical Society dx.doi.org/10.1021/ar400256u | Acc. Chem. Res. 2014, 47, 1106−1116

and nitrite-reducing enzymes in bacteria and fungi,[3] which perform important functions in the global nitrogen cycle. Here, nitric oxide reductases (NORs) catalyze the two-electron reduction of two molecules of NO to N_2O:

$$2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O \quad (1)$$

Respiratory NORs (rNORs) are found in denitrifying organisms that use nitrate as the terminal electron acceptor for respiration. Fungal (respiratory) NO reductase (Cyt. P450nor) is a cytochrome P450-type enzyme that binds NO to a ferric heme in the catalytically active state of the enzyme (Figure 1).[11] This is followed by direct hydride transfer from NAD(P)H to the ferric NO complex, generating an iron(II)-NHO intermediate. Either this HNO-bound intermediate or the corresponding, protonated species, best described as a Fe(IV)-NHOH(−) or Fe(III)-NHOH(radical) complex, reacts with NO to form the N−N bond, ultimately leading to N_2O production as shown in Scheme 1.[12−15]

In bacterial rNORs, including NorBC and qNOR, the active site corresponds to a very unusual heme/non-heme iron motif (Figure 2).[16] Of the many possible mechanisms that have been proposed for this enzyme, the ones summarized in Scheme 2 are considered most likely based on recent reports. Here, NO is activated by the non-heme (Fe_B) center via an Fe(II)-NO^- type intermediate,[17] or via a potential bridging coordination mode.[18] As there are currently no experimental data that provide direct insight into the key N−N bond forming step of this enzyme, elucidation of the reaction of free NO with Fe(II)-NO^- type complexes is of key importance to further validate the chemical basis for these proposed mechanisms.

Many pathogenic bacteria contain flavodiiron NO reductases (FNORs) that are specifically expressed to protect these organisms from exogenous NO.[19] These enzymes contain a non-heme diiron active site with an additional flavin cofactor in close proximity as shown in Figure 3. Reaction of the catalytically active, diferrous form of the active site with NO generates mono- and dinitrosyl intermediates that have been characterized spectroscopically.[20,21] Production of N_2O from non-heme iron nitrosyl monomers and dimers is either slow or nonexistent,[17,21,22] suggesting the enzyme must activate the

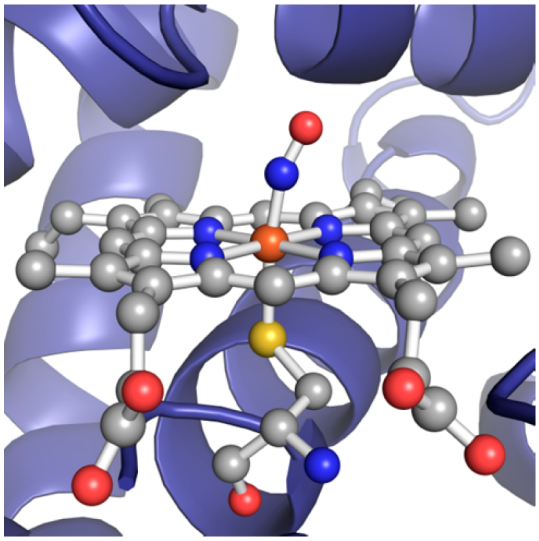


Figure 1. Ferric NO complex in the active site of P450nor (PDB 1CL6).

$\{FeNO\}^7$ units. This has been proposed to occur via formation of a bridging mononitrosyl complex (Scheme 3).[20,21] Alternatively, a recent model complex study has shown that reduction of a diferrous dinitrosyl, $[\{FeNO\}^7]_2$, to the corresponding dinitroxyl complex, $[\{FeNO\}^8]_2$, leads to fast and efficient N_2O formation, providing strong evidence that the enzyme might utilize a similar pathway (the "super-reduced" mechanism according to ref 19) for catalysis (Scheme 3).[22]

Finally, multiheme cytochrome *c* nitrite reductases (C*c*NIRs) are found in denitrifying bacteria and catalyze the direct six-electron reduction of nitrite to ammonia.[23] Due to the large number of hemes present in these systems, experimental insight into the mechanism of these enzymes is quite limited, but it is thought that a ferrous heme-nitrosyl, $\{FeNO\}^7$, complex is formed as a central intermediate of catalysis. Since these species have very negative reduction potentials and are not basic, it is likely that the next step in the mechanism corresponds to a coupled proton and electron transfer, creating a ferrous HNO intermediate $\{FeNHO\}^8$.[24] Further protonation and reduction of this species then yields ammonia as the final product. Correspondingly, electrochemical reduction of ferrous heme-nitrosyl model complexes in the presence of acid generates ammonia in high yield (but using quite negative reduction potentials).[25−27] Further studies are necessary to fully elucidate the mechanism of multiheme C*c*NIRs.

Based on these findings, heme and non-heme nitroxyl complexes are intricately involved in NO_x reduction processes in biology, but surprisingly, the fundamental properties of these species are not well understood. In this Account, we highlight recent key advances in our understanding of the electronic structure and reactivity of iron-nitroxyl complexes, which sheds new light on the potential biological roles of these species.

2. HEME-NITROXYL COMPLEXES

In the past, many mechanistic proposals have been put forth for respiratory NORs where heme-bound nitroxyl intermediates of type $\{FeN(H_n)O\}^8$ (n = 0−2) react with NO (see Introduction). Importantly, the systematic evaluation of this proposed reactivity as a function of the protonation state of the heme $\{FeNO\}^8$ intermediate can only be based on model complexes, which allow for a precise control of the availability of protons in the reaction medium.

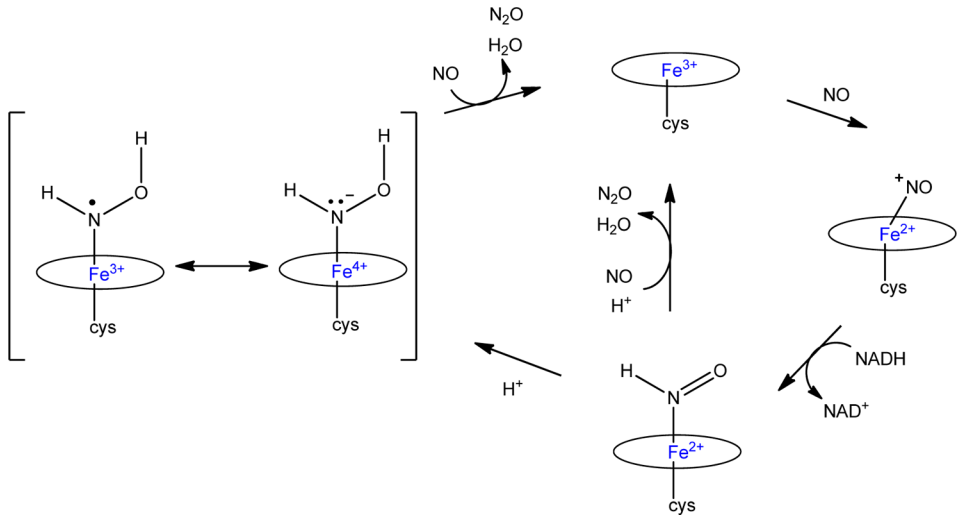
Two approaches can be used to generate the nonprotonated $\{FeNO\}^8$ complexes. First, ferrous heme-nitrosyls, $\{FeNO\}^7$, can be reduced by one electron using electrochemical methods:

$$[Fe(Porph)(NO)] + e^- \rightarrow [Fe(Porph)(NO)]^- \quad (2)$$

Pioneering studies by Kadish and co-workers demonstrated reversible one-electron reduction of five-coordinate (5C) ferrous heme-nitrosyls to generate the corresponding Fe(II)-NO^- species in spectroelectrochemical experiments.[28] Ryan and co-workers later obtained vibrational data for [Fe(TPP)(NO)]$^-$ and [Fe(OEP)(NO)]$^-$ (TPP^{2-} = tetraphenylporphyrin dianion; OEP^{2-} = octaethylporphyrin dianion), and also performed reactivity studies on these (and related) compounds.[29] Doctorovich and co-workers reported in 2010 the first *isolation* of a 5C Fe(II)-NO^- complex (generated by chemical reduction of the Fe(II)-NO precursor), using the extremely electron-poor porphyrin $H_2TFPPBr_8$.[30] Using a similar approach, Harrop and co-workers reported the isolation of an $\{FeNO\}^8$ heme analog.[31,32] Most recently, our group reported a series of heme-nitroxyl complexes and demonstrated a strong correlation of the electronic structures of analogous 5C

dx.doi.org/10.1021/ar400256u | *Acc. Chem. Res.* 2014, 47, 1106−1116

Scheme 1



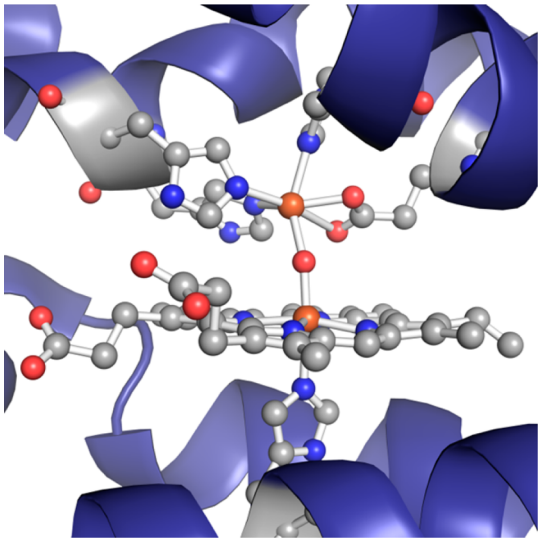


Figure 2. Oxo-bridged, diferric resting state of the NorBC active site (PDB 3O0R).

{FeNO}^{7} and {FeNO}^{8} complexes, and new reactivity data using a bis-picket fence porphyrin model system.[27] In addition, we introduced a new method for the preparation of bulk material of {FeNO}^{8} complexes that starts from a ferrous porphyrin complex, which is first reduced and then reacted with NO gas (Figure 4):

$$[\mathrm{Fe(Porph)}] + e^- \rightarrow [\mathrm{Fe(Porph)}]^- \quad (3)$$

$$[\mathrm{Fe(Porph)}]^- + \mathrm{NO} \rightarrow [\mathrm{Fe(Porph)(NO)}]^- \quad (4)$$

This method is more robust for the preparation of bulk {FeNO}^{8} complexes compared to that in eq 2, which often generates material that is quite impure.

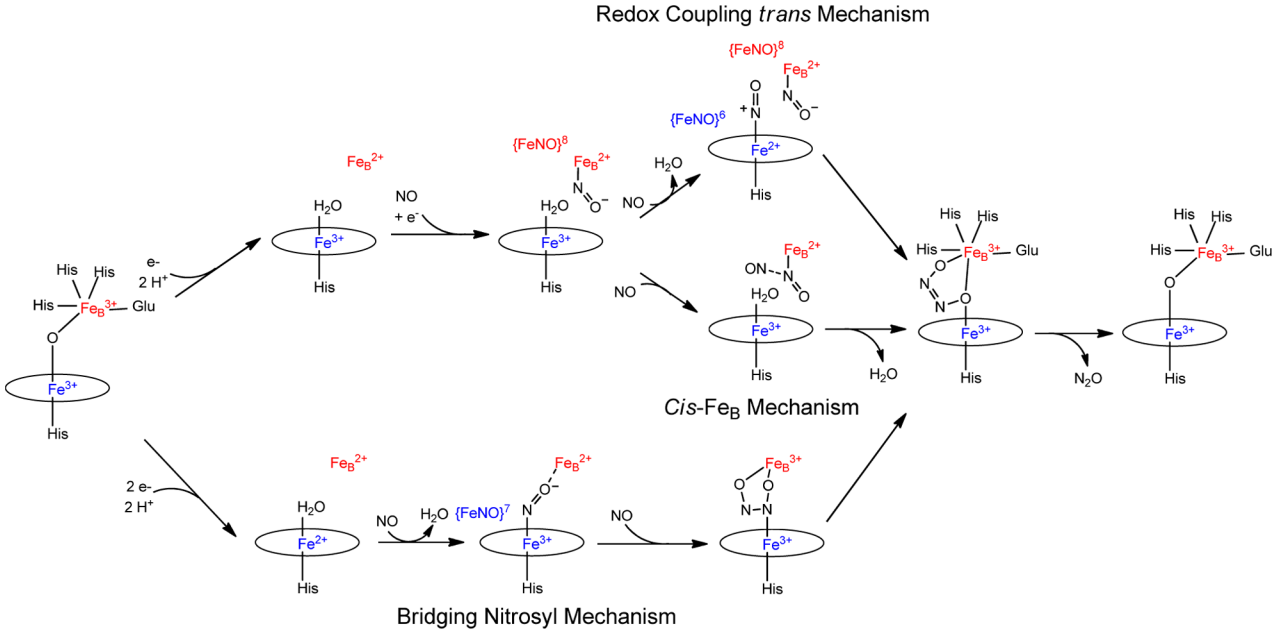
The spectroscopic properties and electronic structures of heme {FeNO}^{8} complexes are best understood in comparison to their ferrous heme-nitrosyl, {FeNO}^{7}, precursors, which have been studied extensively (see ref 33 for a detailed discussion). In short, ferrous heme-nitrosyls exhibit S = 1/2 ground states where the iron center is in the low-spin (ls) state, and are therefore also designated as "ls-{FeNO}^{7}". Their Fe-NO units show short Fe-NO distances of about 1.71−1.76 Å with a bent Fe−N−O unit. In 5C complexes, the Fe−NO and N−O stretching frequencies are observed in the 520−530 and 1675−1700 cm^{-1} range. The Fe−NO bond in these complexes is characterized by strong σ donation from the singly occupied π^* orbital of NO into d_{z^2} of iron (with the Fe−NO bond along the z axis). The SOMO constitutes the bonding combination of these orbitals.[34] In addition, significant π backbonding is observed between the empty π^* orbital of NO and the occupied $t_{2(g)}$ orbitals of iron. Due to the pronounced thermodynamic *trans* effect of NO (also called the *trans* "interaction" or *trans* "influence"),[35] caused by the strong Fe-NO σ bond, N-donor ligands like pyridine (Py) and imidazole (Im) in model systems and histidine (His) in proteins show only small binding constants trans to NO in the 10−100 M^{-1} range. In corresponding six-coordinate (6C) complexes with axially coordinated imidazole derivatives, a simultaneous weakening of the Fe-NO and N−O bonds is observed as a consequence of the *trans* effect,[36] leading to reduced Fe−NO and N−O stretching frequencies at ∼440 cm^{-1} and in the 1610−1630 cm^{-1} range.[37]

One-electron reduction of these ls-{FeNO}^{7} heme complexes is generally reversible, but occurs at very negative reduction potentials of −0.6 to −1.0 V vs NHE (except for porphyrins with very electron poor hemes),[27,28] which, even in the best cases, is at the edge of biologically feasible redox potentials.[38] This reduction leads to a distinct shift of ν(N−O) to lower energy by about 180−230 cm^{-1}, as conveniently observed by spectroelectrochemistry (see Figure 5). This drop in the N−O stretch reflects a significant amount of NO-centered reduction in ls-{FeNO}^{8} complexes. In addition, there is a strong correlation between the N−O stretching frequencies in the {FeNO}$^{7/8}$ couples as shown in Figure 6,[27] which indicates that the electronic structures of these species are strongly correlated. Indeed, DFT calculations emphasize that reduction of ls-{FeNO}^{7} complexes simply leads to a double-occupation of their SOMOs, but that the nature of this MO remains essentially unchanged.[27] The resulting ls-{FeNO}^{8} complexes are therefore diamagnetic with S = 0 ground states, and their electronic structure is somewhat intermediate between Fe(II)-NO$^-$ and Fe(I)-NO(radical) with strong NO reduction.[14]

This prediction has a number of consequences that are experimentally testable. First, double occupation of the

dx.doi.org/10.1021/ar400256u | *Acc. Chem. Res.* 2014, 47, 1106−1116

Scheme 2



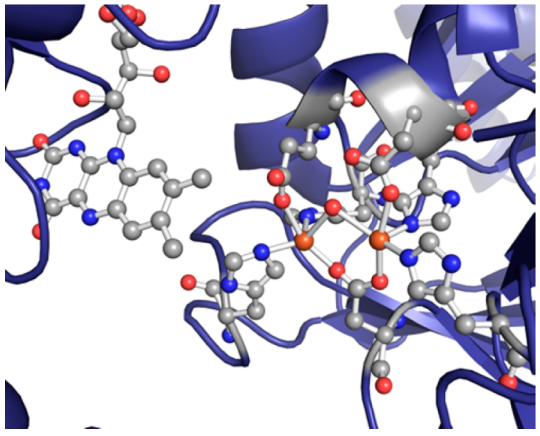


Figure 3. Diferrous acitve site of *Moorella thermacetica* FNOR (PDB 1YCG) shown with the proximal flavin (FMN) cofactor.

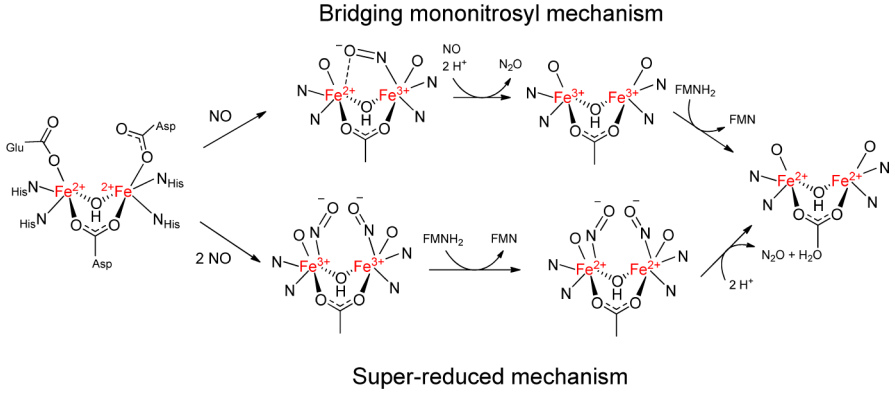
(mostly) Fe-NO σ bonding SOMO of the ls-{FeNO}^{7} complexes should lead to a further increase of the *trans* effect. Indeed, the binding constant of N-donor ligands trans to NO drops by several orders of magnitude in ls-{FeNO}^{8} complexes; for example, from 2055 M^{-1} in [Fe(T*o*-F_2PP)(NO)] to <0.2 M^{-1} in [Fe(T*o*-F_2PP)(NO)]$^-$.[27] Hence, the formally NO^- ligand in ls-{FeNO}^{8} complexes has a stronger *trans* effect than NO! Second, double occupation of the SOMO in ls-{FeNO}^{7} complexes should lead to an overall strengthening of the Fe-NO bond (although some of this is compensated by loss in π backbonding and increased Coulomb repulsion; see ref 33) and, hence, a noticeable increase in ν(Fe-NO). This is indeed observed; for example, the Fe-NO stretch in [Fe(TPP)(NO)] shifts from 525 cm^{-1} (in THF) to 549 cm^{-1} upon reduction to [Fe(TPP)(NO)]$^-$.[29]

Based on this electronic structure picture, one would expect that ls-{FeNO}^{8} complexes are quite basic, and indeed, these complexes are easily protonated by weak acids.[29] However, the formed Fe(II)-NHO model complexes, {FeNHO}^{8}, are not stable and disproportionate with H_2 release to regenerate the ferrous heme-nitrosyl starting materials:[27,29,30]

$$[Fe(Porph)(NO)]^- + H^+ \rightarrow [Fe(Porph)(NHO)] \quad (5)$$

$$2[Fe(Porph)(NHO)] \rightarrow 2[Fe(Porph)(NO)] + H_2 \quad (6)$$

Scheme 3



dx.doi.org/10.1021/ar400256u | *Acc. Chem. Res.* 2014, 47, 1106−1116

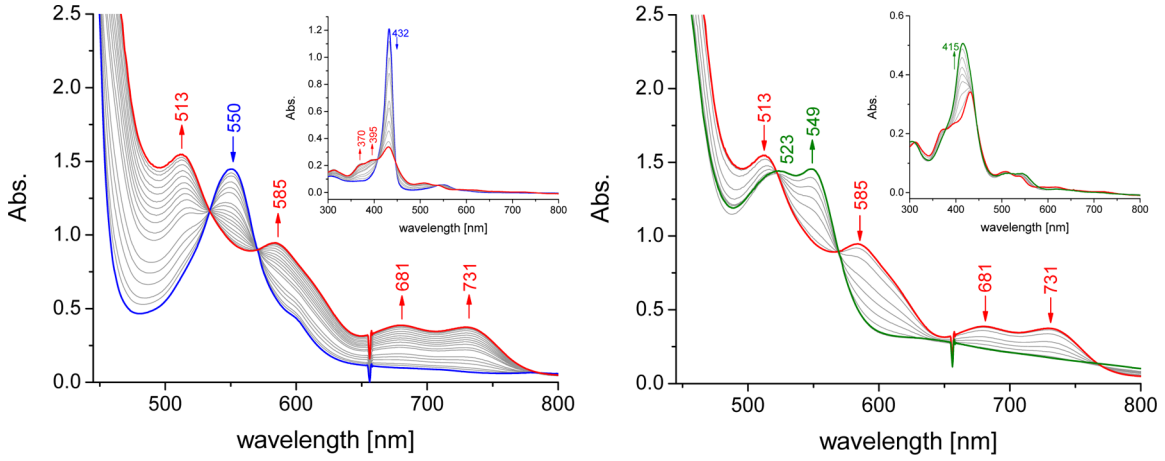


Figure 4. Left: UV−vis spectra for the one-electron reduction of [Fe(3,5-Me-BAFP)] (blue) to [Fe(3,5-Me-BAFP)]$^-$ (red). Right: Subsequent reaction with NO(g) in THF at room temperature resulting in [Fe(3,5-Me-BAFP)(NO)]$^-$ formation (green). Reprinted with permission from ref 27. Copyright 2013 American Chemical Society.

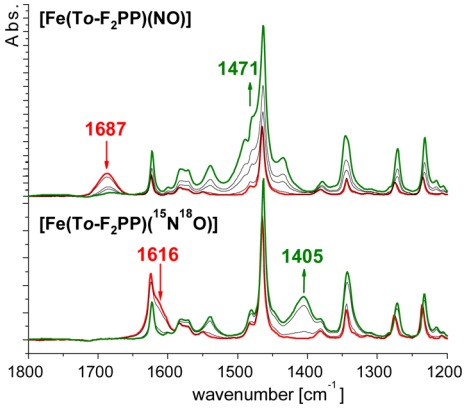


Figure 5. Infrared spectra for the spectroelectrochemical reduction of [Fe(T*o*-F$_2$PP)(NO)] (top) and [Fe(T*o*-F$_2$PP)(^{15}N^{18}O)] (bottom). Reprinted with permission from ref 27. Copyright 2013 American Chemical Society.

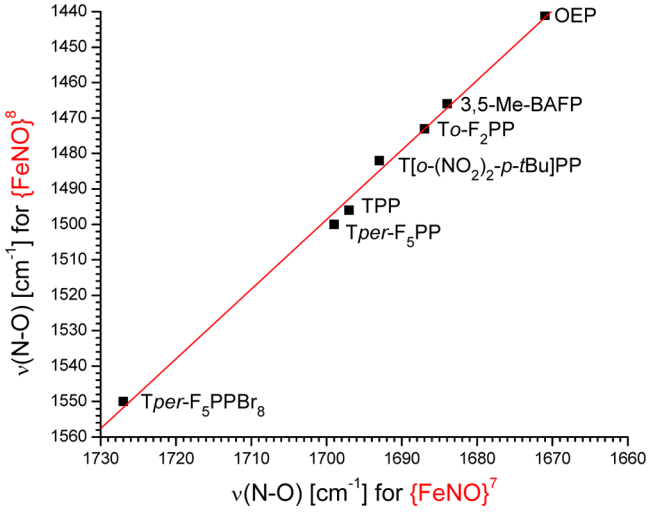


Figure 6. Comparison of N−O stretching frequencies in {FeNO}^{7} and {FeNO}^{8} porphyrin complexes. Adapted from ref 27.

Our group has recently shown that this disproportionation can be suppressed by sterically shielding the bound HNO ligand. The model complex [Fe(3,5-Me-BAFP)(NO)]$^-$ that contains a bis-picket fence porphyrin (Figure 7) allows for the generation of a stable Fe(II)-NHO complex via reversible protonation with acetic acid in THF (Figure 8).[27] Hence, with this ligand system, analogous {FeNO}^{8} and {FeNHO}^{8} complexes are now available for the first time that enable investigations into the biologically relevant reactivity of these species in direct comparison (see below).

The basicity of ls-{FeNO}^{8} complexes was further demonstrated by Farmer and co-workers, who studied the reduction of the ferrous myoglobin-NO adduct, Mb(II)-NO. Their seminal studies demonstrated that Mb(II)-NO can be reduced directly to the corresponding nitroxyl complex, forming Mb(II)-NHO in an aqueous environment. However, this required very negative redox potentials of about −650 mV (vs NHE).[39] The resulting Mb(II)-NHO complex turned out to be surprisingly stable with an HNO binding constant of about 4×10^9 M^{-1},[40] and was therefore amendable to spectroscopic studies.[7] Based on these results, the complex is clearly N-protonated with a diamagnetic ground state (S = 0). The bound HNO ligand shows a diagnostic ^{1}H NMR signal for the H-NO proton around 15 ppm. The Fe(II)-NHO unit is further characterized by Fe−N and N−O stretching frequencies of 651 and 1385 cm^{-1}, respectively.[41] The HNO ligand itself is only a weak σ donor, and acts mostly as a π acceptor when bound to ferrous heme,[42,43] which is distinctly different from the unprotonated Fe(II)-NO$^-$ heme complexes described above. The pK_a of the bound HNO ligand is higher than 10, likely close to 11,[7] again emphasizing the basicity of ls-{FeNO}^{8} heme complexes.

Ferrous heme-nitrosyls are very stable and the bound NO ligand is generally unreactive toward NO and other nitrosyl complexes, which renders these species less suitable as catalytically competent intermediates in NORs.[33] Recent computational work emphasizes that ferrous heme-nitrosyls only become reactive toward NO for N−N bond formation after reduction and in the presence of at least one equivalent of protons,[44] which corresponds to the reaction:

$$[Fe^{II}(Porph)(NHO)] + NO \rightarrow [Fe^{III}(Porph)(N(OH)-NO)] \quad (7)$$

that would then generate a ferric hyponitrite complex. Importantly, the bis-picket fence porphyrin complex [Fe(3,5-

dx.doi.org/10.1021/ar400256u | Acc. Chem. Res. 2014, 47, 1106−1116

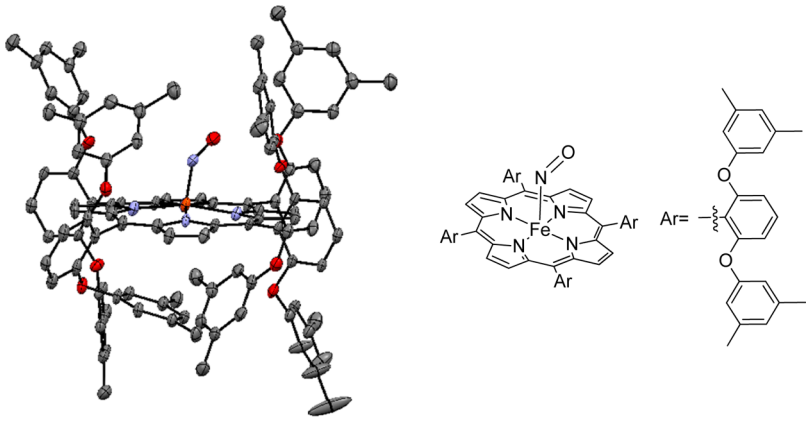


Figure 7. Crystal structure of the bis-picket fence porphyrin model complex [Fe(3,5-MeBAFP)(NO)].[27] Thermal ellipsoids shown at 30% probability.

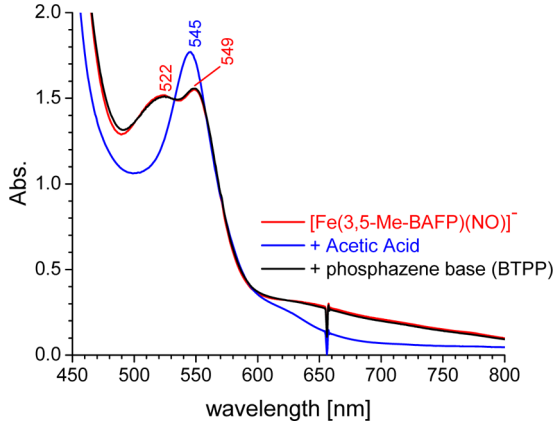


Figure 8. UV−visible spectrum for the reaction of [Fe(3,5-Me-BAFP)(NO)]$^-$ (red) with acetic acid to generate [Fe(3,5-Me-BAFP)(NHO)] (blue), followed by deprotonation with phosphazene base P$_1$-*t*Bu-tris(tetramethylene) (BTPP) to regenerate [Fe(3,5-Me-BAFP)(NO)]$^-$ (black). Adaped from ref 27.

Me-BAFP)(NO)] now provides a first look into these mechanistic possibilities. First, unprotonated ls-{FeNO}^{8} model complexes react with free NO gas exclusively by simple outer sphere electron transfer, regenerating the {FeNO}^{7} starting material and likely producing free NO$^-$, the decomposition of which was not further investigated.[27] Since the resting potential of [Fe(Porph)(NO)]$^-$ complexes is more negative than that of free NO gas (except for very electron-poor hemes), this is not a surprise, but nevertheless indicates that the reaction

$$[Fe(Porph)(NO)]^- + NO \rightarrow [Fe^{III}(Porph)(N(O^-)-NO)]^- \quad (8)$$

is unfavorable. More importantly, the protonated complex [Fe(3,5-Me-BAFP)(NHO)] does not react with NO under N−N bond formation either, and no ferric hyponitrite complex (or any other ferric product) is observed in this reaction. Instead, the observed product of this reaction is again the parent {FeNO}^{7} complex.[27] This provides a first indication that a Fe(II)−NHO complex is not catalytically competent for N−N bond formation in respiratory NORs, and supports the idea that the critical intermediate in Cyt. P450nor is actually the doubly protonated complex:

$$[Fe^{II}(Porph)(NHO)] + H^+ \rightarrow \text{“}[Fe^{IV}(Porph)(NHOH)]^+\text{”} \quad (9)$$

$$\text{“}[Fe^{IV}(Porph)(NHOH)]^+\text{”} + NO \rightarrow [Fe^{III}(Porph)(N(OH)-NOH)]^+ \quad (10)$$

Nevertheless, further studies on Fe(II)-NHO model complexes are required to solidify these mechanistic conclusions for P450nor. Furthermore, these results disagree with the earlier proposed “*cis*-heme b_3” mechanism for bacterial rNORs where a ls-{FeN(H)O}^{8} heme complex would be formed as the critical intermediate prior to N−N bond formation (see ref 17). In addition, the redox potential of the Fe$_B$ center is quite positive,[16] which further rules out electron transfer from the ferrous Fe$_B$ to the putative heme {FeNO}^{7} complex to generate such a ls-{FeN(H)O}^{8} heme intermediate. This emphasizes that the Fe$_B$ center must be intricately involved in the N−N bond forming step in NorBC.[17,18]

3. NON-HEME NITROXYL COMPLEXES

As noted in the Introduction, non-heme {FeN(H)O}^{8} complexes have been implicated in the catalytic cycles of both bacterial rNORs and FNORs. In contrast to heme {FeNO}^{7} complexes, which are low-spin (S = 1/2) and have an Fe(II)−NO(radical) electronic structure, non-heme {FeNO}^{7} complexes are generally high-spin (S = 3/2) and are best described as Fe(III)−NO$^-$ systems (see ref 17 for details). Thus, a different electronic structure and reactivity can be anticipated for non-heme {FeNO}^{8} complexes as compared to their heme analogues, but experimental evidence for this assumption has been lacking.

Studies on reduced high-spin (hs) non-heme {FeNO}^{8} species are rare in the literature. In fact, the cyclic voltammograms of hs-{FeNO}^{7} complexes typically show quasi-reversible to irreversible {FeNO}$^{7/8}$ couples, suggesting the reduced species are unstable and cannot be isolated for further studies.[17] Lippard and co-workers showed that in the case of the *β*-diketiminate ligand Ar-acnac$^-$, reduction of its hs-{FeNO}^{7} complex results in rapid disproportionation yielding an {Fe(NO)$_2$}^{10} dinitrosyl iron complex (DNIC):[45]

dx.doi.org/10.1021/ar400256u | *Acc. Chem. Res.* 2014, 47, 1106−1116

$$2[Fe(NO)Br(Ar\text{-}nacnac)]^{-} \rightarrow [Fe(NO)_2(Ar\text{-}nacnac)]^{-} + [Fe^{II}(Ar\text{-}nacnac)Br_2]^{-} \quad (11)$$

In light of this, steric protection of the Fe-NO core may be crucial to synthesize a stable $\{FeNO\}^8$ complex. In support of this strategy, our group showed that a stable high-spin non-heme $\{FeNO\}^8$ complex can in fact be obtained by employing the sterically encumbering ligand TMG_3tren.[46] The $\{FeNO\}^8$ species $[Fe(TMG_3tren)(NO)]^+$ is obtained by chemical or electrochemical reduction of the corresponding $\{FeNO\}^7$ precursor, shown in Figure 9. Importantly, ^1H NMR measurements demonstrate that, in contrast to diamagnetic low-spin $\{FeNO\}^8$ complexes, $[Fe(TMG_3tren)(NO)]^+$ is paramagnetic with a total spin of S = 1. Reduction is accompanied by a decrease of $\nu(N{-}O)$ by 130 cm^{-1}.

DFT calculations provide insight into the electronic structure of the new hs-$\{FeNO\}^8$ complex compared to its hs-$\{FeNO\}^7$ precursor (Scheme 4). The electronic structure of the hs-$\{FeNO\}^7$ unit is best described as a high-spin Fe(III) antiferromagnetically (AF) coupled to a triplet NO^- in terms of limiting electronic structures.[17] Note that in this case, the Fe−NO bond is highly covalent and thus, the NO^- does *not* behave like a nitroxyl. The bonding is dominated by strong π-donation from NO^- into the unoccupied β-spin d_{xz} and d_{yz} orbitals of iron (where the Fe−N(O) bond is located on the *z*-axis) as shown in Scheme 4, left.[47] Importantly, reduction of the hs-$\{FeNO\}^7$ unit leads to population of the β-spin d_{xy} orbital, *which is nonbonding with respect to the Fe−NO unit* (see Scheme 4, right). Hence, this corresponds to a metal-centered reduction, leading to an Fe(II)−NO^- complex where a high-spin Fe(II) is AF coupled to triplet NO^-. The increased electron density at the iron center in the hs-$\{FeNO\}^8$ complex then leads to a moderate decrease in π-donation from NO^- to the Fe(II), which accounts for the relatively small downshift in $\nu(N{-}O)$ upon reduction. Due to the decreased π-donation from NO^- to Fe(II) in the hs-$\{FeNO\}^8$ case, the Fe-NO bond is significantly less covalent as compared to the $\{FeNO\}^7$ precursor, as indicated by the percentage of Fe and NO π^* character in the corresponding β MOs. This indicates that reduction serves as a potent method to activate stable non-heme $\{FeNO\}^7$ units toward further reactivity, for example, protonation or N−N coupling in NORs.

A metal-centered reduction has also been proposed for the ferrous NO adduct of the mononuclear non-heme iron enzyme taurine dioxygenase (TauD).[48] In this case, γ-irradiation of a frozen sample of the $\{FeNO\}^7$ adduct resulted in 31% formation of the $\{FeNO\}^8$ complex. The increase in the Mössbauer isomer shift from 0.71 mm/s in TauD-$\{FeNO\}^7$ to 1.07 mm/s in TauD-$\{FeNO\}^8$ indicates a metal-centered reduction; however, direct assignment of the spin state was challenging. Based on DFT calculations, an S = 1 spin state consisting of high-spin Fe(II) AF coupled to NO^- as described above for the TMG_3tren system was indirectly assigned for TauD-$\{FeNO\}^8$. Interestingly, despite the dissimilar ligand frameworks in the TMG_3tren and TauD systems, the proposed electronic structures of the two hs-$\{FeNO\}^8$ species are extremely similar, which strongly suggests that metal-centered reduction is a general feature of high-spin $\{FeNO\}^{7/8}$ units. Considering that hs-$\{FeNO\}^7$ complexes have an Fe(III)−NO^- electronic structure, a metal-centered reduction should in fact be favored over an NO-based reduction since anionic NO^- should have a very low electron affinity.

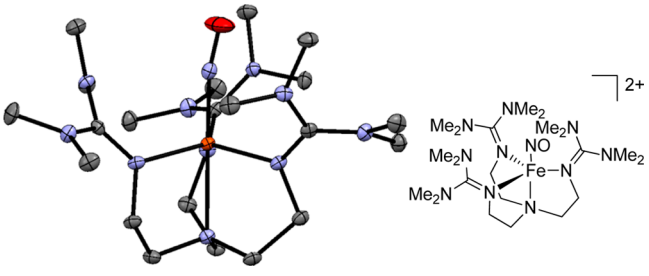


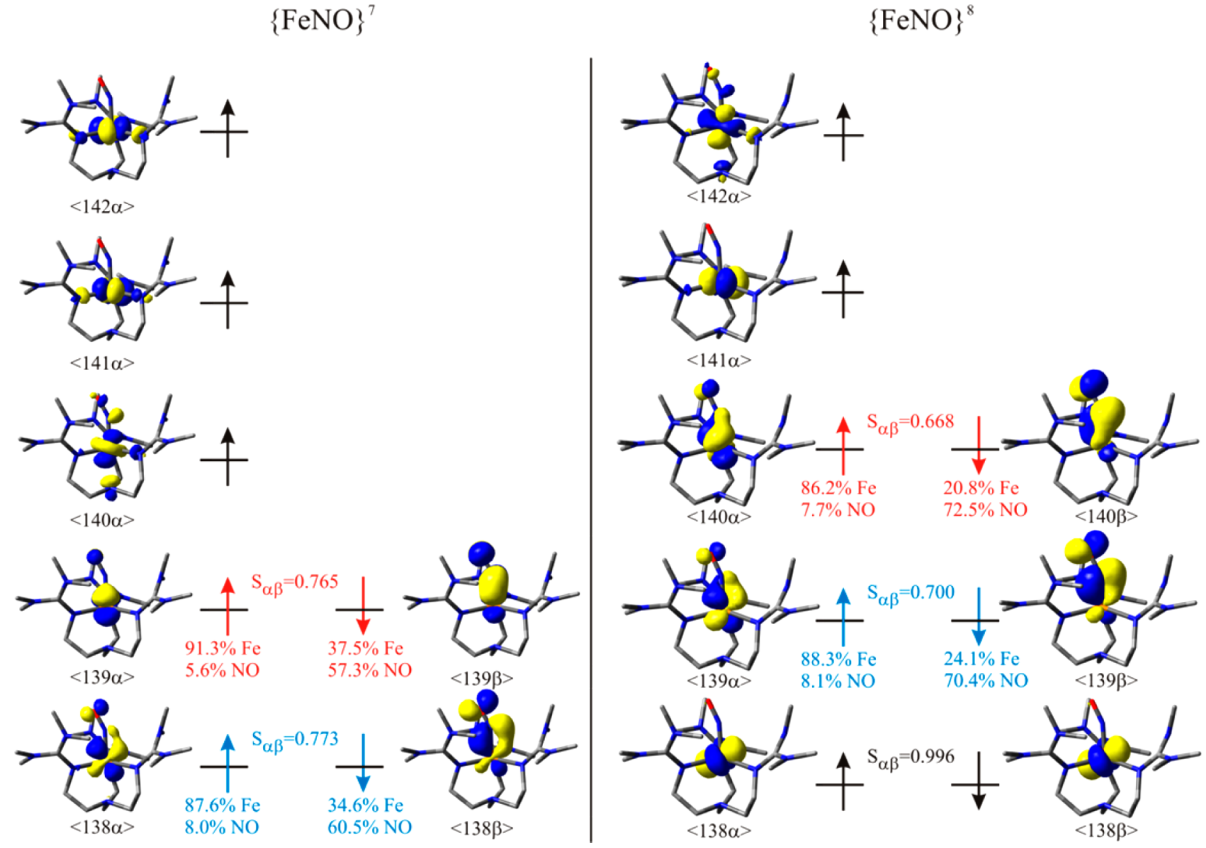
Figure 9. Crystal structure of the non-heme $\{FeNO\}^7$ complex $[Fe(TMG_3tren)(NO)](OTf)_2$ shown with thermal ellipsoids at 50% probability. Solvent, counterions, and hydrogen atoms have been omitted for clarity.[46]

Due to their highly covalent Fe−NO bonds, non-heme $\{FeNO\}^7$ species are typically unreactive and cannot be protonated.[17] As noted above, reduction of these complexes leads to a decrease in Fe-NO covalency and thus, $\{FeNO\}^8$ species should be more basic than their $\{FeNO\}^7$ counterparts. In the case of TMG_3tren, the $\{FeNO\}^8$ complex reacts rapidly with weak acid, although the putative HNO species is not stable at room temperature (Figure 10).[46] Upon annealing of TauD-$\{FeNO\}^8$ at 193 K, a new species with δ = 0.80 mm/s formed, which was tentatively assigned as a quintet $\{FeNHO\}^8$ complex based on DFT calculations.[48] This suggests a possible pathway for HNO synthesis in biological systems. As noted above, reduction of *heme* $\{FeNO\}^7$ complexes is NO-centered and therefore occurs at extremely negative potentials, so formation of heme-nitroxyl complexes from ferrous heme-nitrosyl precursors is unfavorable under physiological conditions. In contrast, because the high-spin state offers partially occupied (acceptor) d-orbitals at low energy, reduction of *non-heme* $\{FeNO\}^7$ complexes is expected to occur at more positive, biologically feasible potentials. Although reduction potentials for biological non-heme $\{FeNO\}^7$ sites have not been measured, model complex studies indicate that this is indeed the case. For example, the model complex [Fe(BMPA-Pr)]X (X = OTf, ClO_4) exhibits an $\{FeNO\}^{7/8}$ redox couple at −300 mV vs NHE, which is within the biologically feasible range.[47] Thus, non-heme iron centers could in principle act as HNO synthases in vivo, if the HNO ligand formed upon reduction is labile and could be released from the active site.

The decreased covalency in $\{FeNO\}^8$ species could also cause other reactivity, most notably N−N coupling in NO reductases. Recently, our group reported the first functional model for FNORs, $[Fe_2(BPMP)(OPr)(NO)_2](BPh_4)_2$ (Figure 11).[22] This $[\{FeNO\}^7]_2$ dimer is stable in solution and does not show any N−N coupling activity. However, upon reduction of the diiron core to the $[\{FeNO\}^8]_2$ dimer, near quantitative N_2O production was observed in approximately one minute. N_2O production is not observed upon reduction of a corresponding monomeric species, which indicates that a diiron motif is required for efficient N_2O production. These results support the proposed "super-reduced" mechanism for FNORs (Scheme 3), in which the flavin cofactor plays a central role in the catalytic cycle by reducing a diferrous dinitrosyl intermediate. For this mechanism to work efficiently, the observed weak electronic coupling between the two $\{FeNO\}^7$ units in the dimer is of key significance, as this allows two electrons to transfer to the $[\{FeNO\}^7]_2$ dimer at very similar, biologically feasible redox potentials.

dx.doi.org/10.1021/ar400256u | *Acc. Chem. Res.* 2014, 47, 1106−1116

Scheme 4. Schematic MO Diagram for the Non-Heme {FeNO}^{7} Complex [Fe(TMG$_3$tren)(NO)]$^{2+}$ (left) and Its {FeNO}^{8} Counterpart (right)[a]



[a] $S_{\alpha\beta}$ indicates the degree of overlap of the α- and β-spin orbitals. Adaped from ref 46.

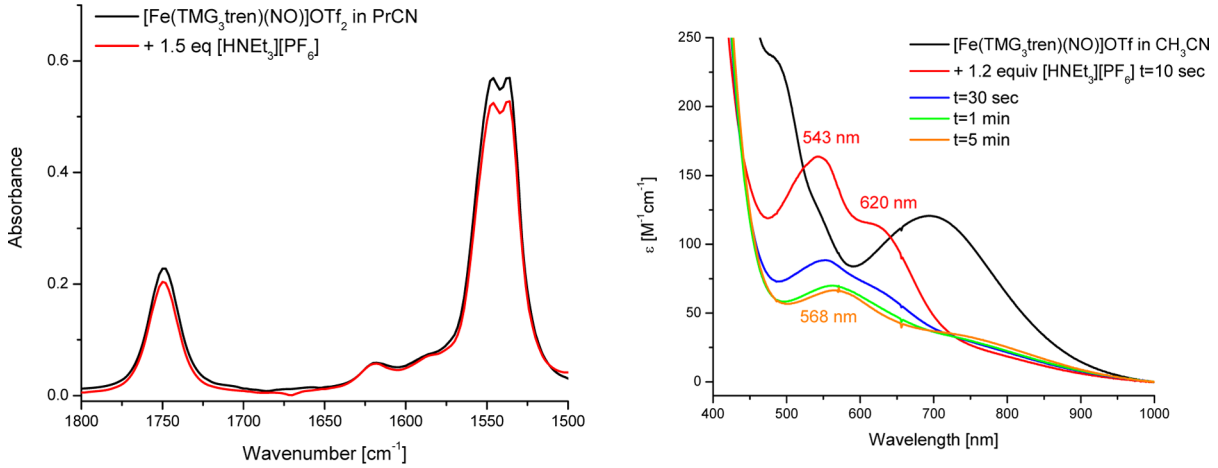


Figure 10. Acid stability of the {FeNO}^{7} complex (left, observed by IR) and protonation of the {FeNO}^{8} complex (right, monitored by UV−vis) of TMG$_3$tren using a weak acid. Adapted from ref 46.

A non-heme {FeN(H)O}^{8} species could also be envisioned as a central intermediate in bacterial rNORs. Such an intermediate would be primed with both electrons required for the reduction of NO to N_2O. A second equivalent of NO would then be provided either by diffusion of free NO gas into the active site in a *cis*-type mechanism or by a ferric heme nitrosyl in a redox-type *trans* coupling mechanism (Scheme 2).[17] In this way, the formation of a stable heme {FeNO}^{7} species in the catalytic cycle could be avoided. At present, the reactivity of high-spin non-heme nitroxyl complexes is largely undefined. Further studies are needed to explore the fundamental chemistry of these species with NO and other important biomolecules.

4. SUMMARY: COMPARISON OF HEME AND NON-HEME COMPLEXES

Based on all of these findings, it is clear that heme (ls) and non-heme (hs) nitroxyl complexes have radically different electronic structures and properties. Reduction of ls-{FeNO}^{7} complexes

dx.doi.org/10.1021/ar400256u | Acc. Chem. Res. 2014, 47, 1106−1116

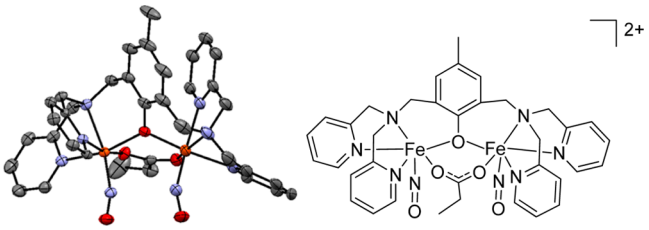


Figure 11. Crystal structure of the $[\{FeNO\}^7]_2$ dimer $[Fe_2(BPMP)(OPr)(NO)_2](BPh_4)_2$. The BPh_4^- counterions and all hydrogen atoms have been omitted for clarity.[22]

leads to double occupation of the Fe-NO bonding SOMO as indicated in Scheme 5 and, hence, a strengthening of the Fe−NO bond. This goes along with a significant degree of NO reduction. In the ls complex [Fe(cyclam-ac)(NO)], the reduction is almost entirely *NO-centered*, which causes a very large downshift in ν(N−O) of 336 cm^{-1}.[49] In heme systems, the electronic structure of the ls-$\{FeNO\}^8$ species is intermediate between Fe(II)−NO^- and Fe(I)-NO(radical), leading to smaller downshifts in ν(N−O) of ~200 cm^{-1}. The resulting ls-$\{FeNO\}^8$ complexes contain a bound singlet NO^- ligand and, hence, are diamagnetic. These species are stable and generally unreactive, except that the bound nitroxyl ligand is very basic and, hence, fully protonated at pH = 7.

In contrast, hs-$\{FeNO\}^7$ complexes show *metal-based* reductions that lead to a decrease in Fe-NO covalency (Scheme 5) and, hence, weaker Fe-NO bonds. The resulting hs-$\{FeNO\}^8$ species have S = 1 ground states with coordinated triplet NO^- ligands, and are generally reactive and susceptible to decomposition. Interestingly, corresponding $[\{FeNO\}^7]_2$ dimers produce N_2O upon reduction, indicating that hs-$\{FeNO\}^8$ complexes are generally competent intermediates for N−N bond formation in NORs. The degree of NO reduction is substantially smaller compared to ls-$\{FeNO\}^8$ species as reflected by the reduced drop in ν(N−O) upon reduction, but still significant enough to enable protonation and HNO generation under physiological conditions.

ls-$\{FeNO\}^7$ complexes have very negative reduction potentials, rendering their reduction to ls-$\{FeN(H)O\}^8$ species challenging. In contrast, stable hs-$\{FeNO\}^7$ complexes reduce at much milder potentials, which makes hs-$\{FeN(H)O\}^8$ species easily accessible, reactive intermediates with potentially widespread implications in biological systems.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

Biographies

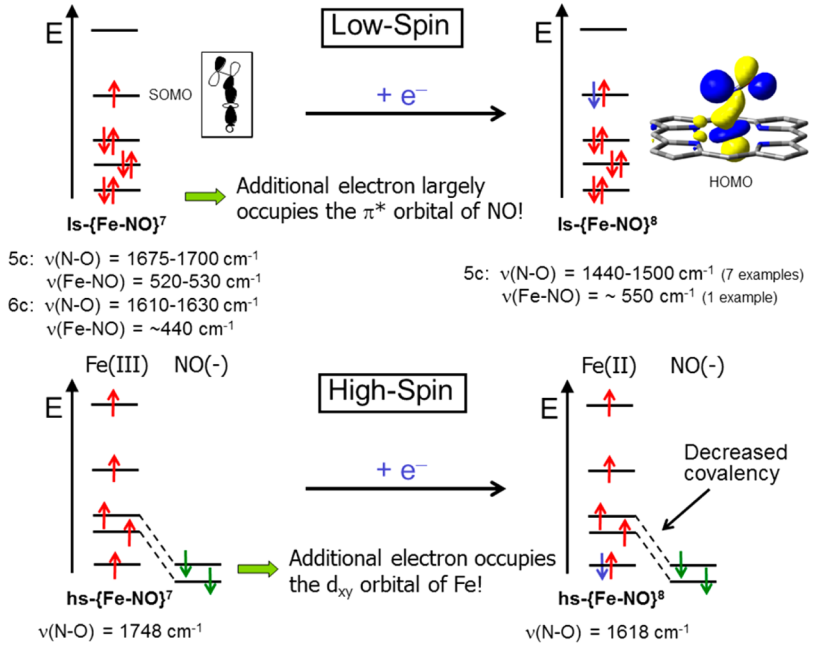
Amy L. Speelman received her B.S. in Chemistry from Hope College in 2010 and is currently a graduate student at the University of Michigan in Prof. Nicolai Lehnert's group. Her research focuses on the synthesis and characterization of non-heme $\{FeNO\}^7$ and $\{FeNO\}^8$ model complexes and spectroscopic studies on heme proteins.

Nicolai Lehnert obtained a Diploma in Chemistry in 1995 from the Heinrich-Heine-University Düsseldorf, Germany. In 1999, he received his Ph.D. from the Johannes Gutenberg-University Mainz, Germany, working on model systems for nitrogenase under supervision of Priv.-Doz. Dr. F. Tuczek and Prof. P. Gütlich. He carried out postdoctoral research, from 1999 to 2001, under Prof. E. I. Solomon at Stanford University. He received his Habilitation in 2006 from the Institute of Inorganic Chemistry, Christian-Albrechts-University Kiel, Germany, working with Prof. F. Tuczek. In 2006, he accepted a faculty position at the University of Michigan.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (CHE-0846235 and CHE-1305777 to N.L.). A.L.S. acknowledges support from an NSF Graduate Research Fellowship (DGE-0718132).

Scheme 5. Illustration of the Differences in Electronic Structure between High-Spin and Low-Spin Nitroxyl Complexes



REFERENCES

(1) Moncada, S.; Palmer, R. M.; Higgs, E. A. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **1991**, *43*, 109−142.

(2) Ignarro, L.: *Nitric Oxide: Biology and Pathobiology*; Academic Press: San Diego, 2000.

(3) Lehnert, N.; Berto, T. C.; Galinato, M. G. I.; Goodrich, L. E.: The Role of Heme-Nitrosyls in the Biosynthesis, Transport, Sensing, and Detoxification of Nitric Oxide (NO) in Biological Systems: Enzymes and Model Complexes. In *The Handbook of Porphyrin Science*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Hackensack, NJ, 2011; Vol. *14*; Chap. 63, pp 1−247.

(4) Gladwin, M. T.; Grubina, R.; Doyle, M. P. The New Chemical Biology of Nitrite Reactions with Hemoglobin: R-State Catalysis, Oxidative Denitrosylation, and Nitrite Reductase/Anhydrase. *Acc. Chem. Res.* **2009**, *42*, 157−167.

(5) Miranda, K. M. The chemistry of nitroxyl (HNO) and implications in biology. *Coord. Chem. Rev.* **2005**, *249*, 433−455.

(6) Bartberger, M. D.; Liu, W.; Ford, E.; Miranda, K. M.; Switzer, C.; Fukuto, J. M.; Farmer, P. J.; Wink, D. A.; Houk, K. N. The reduction potential of nitric oxide (NO) and its importance to NO biochemistry. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10958−10963.

(7) Kumar, M. R.; Fukuto, J. M.; Miranda, K. M.; Farmer, P. J. Reactions of HNO with Heme Proteins. *Inorg. Chem.* **2010**, *49*, 6283−6292.

(8) Fukuto, J. M.; Cisneros, C. J.; Kinkade, R. L. A comparison of the chemistry associated with the biological signaling and actions of nitroxyl (HNO) and nitric oxide (NO). *J. Inorg. Biochem.* **2013**, *118*, 201−208.

(9) Kumar, M. R.; Pervitsky, D.; Chen, L.; Poulos, T.; Kundu, S.; Hargrove, M. S.; Rivera, E. J.; Diaz, A.; Colón, J. L.; Farmer, P. J. Nitrosyl Hydride (HNO) as an O_2 Analogue: Long-Lived HNO Adducts of Ferrous Globins. *Biochemistry* **2009**, *48*, 5018−5025.

(10) Enemark, J. H.; Feltham, R. D. Principles of Structure, Bonding, and Reactivity for Metal Nitrosyl Complexes. *Coord. Chem. Rev.* **1974**, *13*, 339−406.

(11) Nakahara, K.; Tanimoto, T.; Hatano, K.; Usuda, K.; Shoun, H. Cytochrome P-450 55A1 (P-450dNIR) acts as nitric oxide reductase employing NADH as the direct electron donor. *J. Biol. Chem.* **1993**, *268*, 8350−8355.

(12) Obayashi, E.; Takahashi, S.; Shiro, Y. Electronic Structure of Reaction Intermediate of Cytochrome P450nor in Its Nitric Oxide Reduction. *J. Am. Chem. Soc.* **1998**, *120*, 12964−12965.

(13) Daiber, A.; Nauser, T.; Takaya, N.; Kudo, T.; Weber, P.; Hultschig, C.; Shoun, H.; Ullrich, V. Isotope effects and intermediates in the reduction of NO by $P450_{nor}$. *J. Inorg. Biochem.* **2002**, *88*, 343−352.

(14) Lehnert, N.; Praneeth, V. K. K.; Paulat, F. Electronic Structure of Iron(II)-Porphyrin Nitroxyl Complexes: Molecular Mechanism of Fungal Nitric Oxide Reductase (P450nor). *J. Comput. Chem.* **2006**, *27*, 1338−1351.

(15) Riplinger, C.; Neese, F. The Reaction Mechanism of Cytochrome P450 NO Reductase: A Detailed Quantum Mechanics/Molecular Mechanics Study. *ChemPhysChem* **2011**, *12*, 3192−3203.

(16) Hino, T.; Matsumoto, Y.; Nagano, S.; Sugimoto, H.; Fukumori, Y.; Murata, T.; Iwata, S.; Shiro, Y. Structural basis of biological N_2O generation by bacterial nitric oxide reductase. *Science* **2010**, *330*, 1666−1670.

(17) Berto, T. C.; Speelman, A.; Zheng, S.; Lehnert, N. Mono- and Dinuclear Non-Heme Iron-Nitrosyl Complexes: Models for Key Intermediates in Bacterial Nitric Oxide Reductases. *Coord. Chem. Rev.* **2013**, *257*, 244−259.

(18) Blomberg, M. R. A.; Siegbahn, P. E. M. Mechanism for N_2O Generation in Bacterial Nitric Oxide Reductase: A Quantum Chemical Study. *Biochemistry* **2012**, *51*, 5173−5186.

(19) Kurtz, D. M., Jr. Flavo-diiron enzymes: nitric oxide or dioxygen reductases? *J. Chem. Soc., Dalton Trans.* **2007**, 4115−4121.

(20) Hayashi, T.; Caranto, J. D.; Matsumura, H.; Kurtz, D. M.; Moënne-Loccoz, P. Vibrational Analysis of Mononitrosyl Complexes in Hemerythrin and Flavodiiron Proteins: Relevance to Detoxifying NO Reductase. *J. Am. Chem. Soc.* **2012**, *134*, 6878−6884.

(21) Hayashi, T.; Caranto, J. D.; Wampler, D. A.; Kurtz, D. M.; Moënne-Loccoz, P. Insights into the Nitric Oxide Reductase Mechanism of Flavodiiron Proteins from a Flavin-Free Enzyme. *Biochemistry* **2010**, *49*, 7040−7049.

(22) Zheng, S.; Berto, T. C.; Dahl, E. W.; Hoffman, M. B.; Speelman, A. L.; Lehnert, N. The Functional Model Complex $[Fe_2(BPMP)(OPr)(NO)_2](BPh_4)_2$ Provides Insight into the Mechanism of Flavodiiron NO Reductases. *J. Am. Chem. Soc.* **2013**, *135*, 4902−4905.

(23) Einsle, O.; Messerschmidt, A.; Huber, R.; Kroneck, P. M. H.; Neese, F. Mechanism of the Six-Electron Reduction of Nitrite to Ammonia by Cytochrome *c* Nitrite Reductase. *J. Am. Chem. Soc.* **2002**, *124*, 11737−11745.

(24) Bykov, D.; Neese, F. Reductive activation of the heme iron-nitrosyl intermediate in the reaction mechanism of cytochrome *c* nitrite reductase: A theoretical study. *J. Biol. Inorg. Chem.* **2012**, *17*, 741−760.

(25) Murphy, W. R., Jr.; Takeuchi, K. J.; Meyer, T. J. Interconversion of Nitrite and Ammonia: Progress toward a Model for Nitrite Reductase. *J. Am. Chem. Soc.* **1982**, *104*, 5817−5819.

(26) Liu, Y.; Ryan, M. D. The electrochemical reduction of iron porphyrin nitrosyls in the presence of weak acids. *J. Electroanal. Chem.* **1994**, *368*, 209−219.

(27) Goodrich, L. E.; Saikat, R.; Alp, E. E.; Zhao, J.; Hu, M. Y.; Lehnert, N. Electronic Structure and Biologically Relevant Reactivity of Low-Spin $\{FeNO\}^8$ Porphyrin Model Complexes: New Insight from a Bis-Picket Fence Porphyrin. *Inorg. Chem.* **2013**, *52*, 7766−7780.

(28) Lançon, D.; Kadish, K. M. Electrochemical and spectral characterization of iron mono- and dinitrosyl porphyrins. *J. Am. Chem. Soc.* **1983**, *105*, 5610−5617.

(29) Choi, I.-K.; Liu, Y.; Feng, D.; Paeng, K.-J.; Ryan, M. D. Electrochemical and Spectroscopic Studies of Iron Porphyrin Nitrosyls and Their Reduction Products. *Inorg. Chem.* **1991**, *30*, 1832−1839.

(30) Pellegrino, J.; Bari, S. E.; Bikiel, D. E.; Doctorovich, F. Successful Stabilization of the Elusive Species $\{FeNO\}^8$ in a Heme Model. *J. Am. Chem. Soc.* **2010**, *132*, 989−995.

(31) Patra, A. K.; Dube, K. S.; Sanders, B. C.; Papaefthymiou, G. C.; Conradie, J.; Ghosh, A.; Harrop, T. C. A thermally stable $\{FeNO\}^8$ complex: properties and biological reactivity of reduced MNO systems. *Chem. Sci.* **2012**, *3*, 364−369.

(32) Sanders, B. C.; Rhine, M. A.; Harrop, T. C. Properties of $\{FeNO\}^8$ and $\{CoNO\}^9$ Metal Nitrosyls in Relation to Nitroxyl Coordination Chemistry. *Struct. Bonding (Berlin, Ger.)* **2013**, DOI: 10.1007/430_2012_87.

(33) Lehnert, N.; Scheidt, W. R.; Wolf, M. W. Structure and Bonding in Heme−Nitrosyl Complexes and Implications for Biology. *Struct. Bonding (Berlin, Ger.)* **2013**, DOI: 10.1007/430_2013_92.

(34) Goodrich, L. E.; Paulat, F.; Praneeth, V. K. K.; Lehnert, N. Electronic Structure and Reactivity of Heme-Nitrosyls and Its Significance for Nitric Oxide Sensing, Transport, and Catalysis in Biological Systems. *Inorg. Chem.* **2010**, *49*, 6293−6316.

(35) Traylor, T. G.; Sharma, V. S. Why NO? *Biochemistry* **1992**, *31*, 2847−2849.

(36) Praneeth, V. K. K.; Näther, C.; Peters, G.; Lehnert, N. Spectroscopic Properties and Electronic Structure of Five- and Six-Coordinate Iron(II) Porphyrin NO Complexes: Effect of the Axial N-Donor Ligand. *Inorg. Chem.* **2006**, *45*, 2795−2811.

(37) Lehnert, N.; Sage, J. T.; Silvernail, N. J.; Scheidt, W. R.; Alp, E. E.; Sturhahn, W.; Zhao, J. Oriented Single-Crystal Nuclear Resonance Vibrational Spectroscopy of [Fe(TPP)(MI)(NO)]: Quantitative Assessment of the trans Effect of NO. *Inorg. Chem.* **2010**, *49*, 7197−7215.

(38) Guo, M.; Sulc, F.; Ribbe, M. W.; Immoos, C. E.; Farmer, P. J.; Burgess, B. K. Direct Assessment of the Reduction Potential of the $[4Fe\text{-}4S]^{1+/0}$ Couple of the Fe Protein from *Azotobacter vinelandii*. *J. Am. Chem. Soc.* **2002**, *124*, 12100−12101.

(39) Farmer, P. J.; Sulc, F. Coordination chemistry of the HNO ligand with hemes and synthetic coordination complexes. *J. Inorg. Biochem.* **2005**, *99*, 166−184.
(40) Zapata, A. L.; Kumar, M. R.; Pervitsky, D.; Farmer, P. J. A singular value decomposition approach for kinetic analysis of reactions of HNO with myoglobin. *J. Inorg. Biochem.* **2013**, *118*, 171−178.
(41) Immoos, C. E.; Sulc, F.; Farmer, P. J.; Czarnecki, K.; Bocian, D. F.; Levina, A.; Aitken, J. B.; Armstrong, R. S.; Lay, P. A. Bonding in HNO-Myoglobin as Characterized by X-ray Absorption and Resonance Raman Spectroscopies. *J. Am. Chem. Soc.* **2005**, *127*, 814−815.
(42) Ling, Y.; Mills, C.; Weber, R.; Yang, L.; Zhang, Y. NMR, IR/Raman, and Structural Properties in HNO and RNO (R = Alkyl and Aryl) Metalloporphyrins with Implication for the HNO-Myoglobin Complex. *J. Am. Chem. Soc.* **2010**, *132*, 1583−1591.
(43) Goodrich, L. E.; Lehnert, N. The *trans* effect of nitroxyl (HNO) in ferrous heme systems: Implications for soluble guanylate cyclase activation by HNO. *J. Inorg. Biochem.* **2013**, *118*, 179−186.
(44) Yi, J.; Morrow, B. H.; Campbell, A. L. O. C.; Shen, J. K.; Richter-Addo, G. B. Nitric oxide coupling mediated by iron porphyrins: the N-N bond formation step is facilitated by electrons and a proton. *Chem. Commun.* **2012**, *48*, 9041−9043.
(45) Tonzetich, Z. J.; Héroguel, F.; Do, L. H.; Lippard, S. J. Chemistry of Nitrosyliron Complexes Supported by a β-Diketiminate Ligand. *Inorg. Chem.* **2011**, *50*, 1570−1579.
(46) Speelman, A. L.; Lehnert, N. Characterization of the First High-Spin Non-Heme $\{FeNO\}^8$ Complex: Implications for the Reactivity of Iron Nitroxyl Species in Biology. *Angew. Chem., Int. Ed.* **2013**, *52*, 12283−12287.
(47) Berto, T. C.; Hoffman, M. B.; Murata, Y.; Landenberger, K. B.; Alp, E. E.; Zhao, J.; Lehnert, N. Structural and Electronic Characterization of Non-Heme Fe(II)-Nitrosyls as Biomimetic Models of the Fe_B Center of Bacterial Nitric Oxide Reductase (NorBC). *J. Am. Chem. Soc.* **2011**, *133*, 16714−16717.
(48) Ye, S.; Price, J. C.; Barr, E. W.; Green, M. T.; Bollinger, J. M.; Krebs, C.; Neese, F. Cryoreduction of the NO-Adduct of Taurine:α-Ketoglutarate Dioxygenase (TauD) Yields an Elusive $\{FeNO\}^8$ Species. *J. Am. Chem. Soc.* **2010**, *132*, 4739−4751.
(49) Serres, R. G.; Grapperhaus, C. A.; Bothe, E.; Bill, E.; Weyhermüller, T.; Neese, F.; Wieghardt, K. Structural, Spectroscopic, and Computational Study of an Octahedral, Non-Heme $\{Fe-NO\}^{6-8}$ Series: $[Fe(NO)(cyclam\text{-}ac)]^{2+/+/0}$. *J. Am. Chem. Soc.* **2004**, *126*, 5138−5153.