Insights into the Observed trans-Bond Length Variations upon NO Binding to Ferric and Ferrous Porphyrins with Neutral Axial Ligands

Rahul L. Khade, Erwin G. Abucayon, Douglas R. Powell, George B. Richter-Addo,* and Yong Zhang*

ABSTRACT: NO is well-known for its trans effect. NO binding to ferrous hemes of the form (por)Fe(L) (L = neutral N-based ligand) to give the {FeNO}^7 (por)Fe(NO)(L) product results in a lengthening of the axial trans Fe–L bond. In contrast, NO binding to the ferric center in [(por)Fe(L)]^+ to give the {FeNO}^6 [(por)Fe(NO)(L)]^+ product results in a shortening of the trans Fe–L bond. NO binding to both ferrous and ferric centers involves the lowering of their spin states. Density functional theory (DFT) calculations were used to probe the experimentally observed trans-bond shortening in some NO adducts of ferric porphyrins. We show that the strong σ antibonding interaction of d_x^2 and the axial (L) ligand p orbitals present in the Fe(II) systems is absent in the Fe(III) systems, as it is now in an unoccupied orbital. This feature, combined with a lowering of spin state upon NO binding, provides a rationale for the observed net trans-bond shortening in the {FeNO}^6 but not the {FeNO}^7 derivatives.

INTRODUCTION

In terms of its productive functions, the small molecule nitric oxide (NO) is perhaps most well-known for its activation of the mammalian enzyme soluble guanylyl cyclase (sGC) to trigger the eventual cyclization of 3',5'-cyclic guanosine monophosphate (cGMP). The first step in this activation process is the binding of NO to the Fe center of the ferrous (por)Fe^II(His) (por = porphyrin dianion) active site in the enzyme to form a {FeNO}^7 (por)Fe(NO)(His) derivative that converts to the 5-coordinate (por)Fe(NO)(N-base) form. This NO binding event correlates with activation of the enzyme, although there is some discussion about the possible involvement of a second NO binding site during this activation. Nevertheless, binding of NO to the ferrous porphyrin results in the weakening and/or disruption of the trans Fe–His linkage (Figure 1), a process that is believed to be integral to the subsequent protein conformation change that sets off the sequence of biochemical reactions leading to eventual blood pressure control. The structure of a related full-length sGC from Manduca sexta has recently been solved by cryo-electron microscopy. Interestingly, the mammalian enzyme that biosynthesizes NO, namely NO synthase, is also a heme-containing enzyme, and the product NO is a regulator of this enzyme. In this case, it binds to the ferric center to form a self-regulatory {FeNO}^6 derivative. Other {FeNO}^6 (sometimes referred to as ferric-NO or ferrous-NO+) hemes and heme biomolecules have been reported.

Data from high-resolution X-ray crystal structures of {FeNO}^7 heme models substantiate the trans-bond lengthening in 6-coordinate low-spin (por)Fe(NO)(N-base) derivatives. For example, excellent work by Scheidt and co-workers have demonstrated an increase in the axial trans-bond lengths of ~0.2 Å in some NO-bound (por)Fe(NO)(N-base) compounds when compared with their non-NO precursors. Numerous spectroscopic and computational investigations on relevant iron porphyrins have been reported that probe such trans-bond lengthening in {FeNO}^7 (por)Fe(NO)(N-base) derivatives.

Available data from {FeNO}^6 heme models have lagged behind the ferrous systems, in large part due to the earlier difficulty in obtaining tractable examples for crystal structural determinations. We reported the X-ray crystal structure of the 5-coordinate and 6-coordinate [(TPP)Fe(H_2O)]OTf/[(TPP)-Fe(NO)(H_2O)]OTf (TPP = tetraphenylporphyrin dianion) structural pair that was present in the same crystal. This allowed us to confidently ascertain the trans-bond shortening of the axial Fe–H_2O distance in the {FeNO}^6 derivative. The previously reported and related ferric N-ligated

Received: July 9, 2021
Published: September 15, 2021
pair [(OEP)Fe(2-MeIm)]+/[(OEP)Fe(NO)(2-MeIm)]+ (OEP = octaethylporphyrin dianion) also displayed a trans-bond shortening in the {FeNO}6 derivative.29,30 It is well-known that changes in oxidation and spin states are associated with concomitant changes in effective ionic radii of transition metals (e.g., low-spin Fe2+ and Fe3+ display smaller effective ionic radii than their high-spin forms).31,32 Thus, a transition from a higher-spin complex to its low-spin form will normally be expected to result in a shortening of M−L distances. For example, FeII−L bond lengths in homoleptic complexes may contract by ∼0.2 Å upon moving from high-spin to low-spin.33−35 However, as noted earlier in Figure 1, NO binding to the five-coordinate ferrous higher-spin (por)Fe(L) (L = neutral ligand)36,37 results in a lengthening of the trans-axial bond in the six-coordinate low-spin {FeNO}7 (por)Fe(NO)(L) derivative. In contrast, we note that NO binding to the high-spin ferric thiolate precursor (OEP)Fe(S-2,6-(CF3CONH)2C6H4)− (i.e., anionic axial ligand)38 generates the nitrosyl {FeNO}6 product with no significant change in the axial Fe−S bond distance.39 In the examples we show, NO binding to the higher-spin [(por)Fe(L)]+ precursors (L = neutral ligand) generates the low-spin {FeNO}6 [(por)Fe(NO)(L)]+ derivatives with shortened trans-axial bond lengths. Thus, although spin state lowering is present in all three examples described above, other factors likely contribute to the observed net variations in M−L distances.

In this article, we probe the contributions of NO binding and spin state changes on the trans-axial bond length in the {FeNO}6 [(por)Fe(NO)(L)]+ (L = neutral N- and O- donor ligand) derivatives. We utilize density functional theory (DFT) calculations to further elucidate the reasons behind the experimental observation of trans-bond shortening in these porphyrins.

■ RESULTS AND DISCUSSION

Computational Methodology and Geometry Optimizations. It is well-known that DFT has some difficulty in reproducing experimental spin states of porphyrins and nitrosyl porphyrins, as they often possess low lying spin states that are close in energy.40

To find an appropriate method to investigate the variations in trans-bond lengths upon NO binding to iron porphyrins, we examined several DFT methods using the experimentally investigated systems of this effect with the spin states from the respective experimental studies. We first studied the experi-

Figure 2. Molecular structures of the [(TPP)Fe(H2O)]OTf (1) and [(TPP)Fe(NO)(H2O)]OTf (2) pair.28 Thermal ellipsoids are drawn at 35% probability. The dashed lines represent H-bonding interactions of a water molecule and NO, and the aqua ligand with shared triflate anions (between 2 porphyrins).

Figure 3. Structures of the five-coordinate (left) and their respective NO-bound six-coordinate (middle) complexes with axial H2O. Sketches of the NO-bound derivatives are shown on the right; the thick horizontal lines represent the porphine (P) macrocycle except for 1/2, where it is for TPP. Atom color scheme: Fe-black, C-cyan, N-blue, O-red, S-yellow, F-green, and H-gray.
**Table 1. Changes in Geometrical Data Upon NO Binding to Some Iron Porphyrins (in Å and Deg)**

| Porphyrin            | S         | ΔR_{Fe-NO} | R_{Fe-L} | ΔR_{Fe-L} | R_{Fe-NO} | ΔFeNO | Err% | avErr% |
|----------------------|-----------|------------|----------|-----------|-----------|-------|------|--------|
| **Expt**             |           |            |          |           |           |       |      |        |
| [(TPP)Fe(NO)(H₂O)]SO₂CF₃ | 2.003     | 1.961      | 0.050    | 1.630     | 1.150     | 173.0 |      |        |
| [(TPP)Fe(H₂O)]SO₂CF₃  | 3/2       | 1.982      | 2.011    |           |           |       |      |        |
| [(TPP)Fe(NO)(H₂O)]SO₂CF₃ | 2.020     | 1.944      | 0.074    | 1.641     | 1.155     | 171.2 | 0.8  | 0.8    |
| [(TPP)Fe(H₂O)]SO₂CF₃  | 3/2       | 2.006      | 2.018    |           |           |       |      |        |
| [(P)Fe(NO)(H₂O)]SO₂CF₃ | 3/2       | 2.023      | 2.105    | 0.090     | 1.620     | 1.178 | 0.3  | 2.5    |
| [(P)Fe(NO)(H₂O)]²⁺      | 3/2       | 1.997      | 2.195    |           |           |       |      |        |
| **Calc**             |           |            |          |           |           |       |      |        |
| [(OEP)Fe(NO)(5-MeIm)]²⁺ | 0.009(3)  | 1.969(3)   | 0.082    | 1.637(3)  | 1.142(4)  | 177.9(3) |      |        |
| [(OEP)Fe(5-MeIm)]²⁺    | 2.003(9)  | 2.051(10)  |          |           |           |       |      |        |
| [(P)Fe(NO)(5-MeIm)]²⁺   | 3/2       | 2.026      | 2.022    | 0.128     | 1.640     | 1.148 | 1.8  | 1.8    |
| [(P)Fe(5-MeIm)]²⁺      | 2.006     | 2.150      |          |           |           |       |      |        |
| **Expt**             |           |            |          |           |           |       |      |        |
| [(OEP)Fe(2-MeIm)]²⁺    | 2.073     | 2.127      |          |           |           |       |      |        |
| [(P)Fe(2-MeIm)]²⁺      | 2.020     | 2.325      | 0.134    | 1.738     | 1.184     | 139.9 |      |        |
| [(P)Fe(2-MeIm)]²⁺      | 2.094     | 2.191      |          |           |           |       |      |        |

*a*NPor is the porphyrin nitrogen, *L* is the axial ligand other than NO, *Err%* is the average of percentage errors of all listed geometric parameters for one complex, and *avErr%* is the average of *Err%* of the NO bound and unbound complexes. *Ref 28 A*H₂O molecule of low occupancy was located near the axial NO ligand. *Ref 29 [Fe(OEP)(2-MeIm)(NO)]²⁺* (ruffled) was used for comparison, as the optimized structure is ruffled. *Ref 30 Average of all three [Fe(OEP)(2-MeIm)]²⁺ structures.* *This work.* *No available experimental structure.* *Ref 47.*

The results show that the best models, considering both accuracy and time, for the DFT geometry optimizations are 3/4 for this system (i.e., with the unsubstituted porphine but retaining the H-bonding partners). As seen in Table 1, the calculated trans-bond contraction upon NO binding was present for all of these structural pairs, suggesting that this feature exists for the ferric porphyrins regardless of porphyrin substitution or H-bonding partners. We compared these results with those using the hybrid HF-DFT mPW1PW91 method,⁴⁸ which has been used for other metal complexes (Table S1).⁴⁹,⁵⁰

We determined that the same conclusion can be made using this latter method, namely the 3/4 pair gave a similar excellent accuracy in geometry predictions compared to the full structural pair 1/2 (1.1 vs 0.9%), while models 5/6 missing the H-bonding pairs performed worse with an average error of 2.7%.

We then probed the previously reported experimental case of the N-ligated ferric pair [(OEP)Fe(2-MeIm)]²⁺ (high-spin) vs the [FeNO]⁶⁻ low-spin derivative [(OEP)Fe(NO)(2-MeIm)]⁴⁺ reported by Scheidt and co-workers,²⁹,³⁰ using the unsubstituted porphine models 9/10 (Figure 4) to examine more DFT methods (B3LYP,¹¹ M06,¹² ωB97XD,¹³ Table S1) as these molecules did not display additional H-bonding interactions. We also examined a larger 6-311++G(2d,2p) basis for the first coordination shell atoms (referred to as BS2) for the 9/10 pair. As shown in Table S1 with the results of mPW1PW91 and B3LYP, the use of the larger BS2 basis set has the best improvement of only 0.1%, and thus is not favorable, considering the additional time cost.

Using BPW91/BS1, the predicted trans-bond contraction of −0.040 Å upon NO binding to high-spin [(P)Fe(2-MeIm)]⁴⁺ (9) is in excellent agreement with the experimental value of −0.054 Å. As seen in Tables 1 and S1, all of these DFT methods can give excellent geometric predictions for the ferric/[FeNO]⁶⁻ pairs, with average errors of 0.7−1.4%. A slightly larger range of errors was determined for the ferrous complex (TPP)Fe(2-MeIm) (0.9% for M06 to 2.9% for B3LYP). However, except for BPW91, the other DFT methods have difficulty in reproducing the expected trans-bond lengthening in the ferrous porphyrins (ΔR_{Fe-NO} values of 11/12 in Table S1); mPW1PW91 and ωB97XD produced the wrong sign of the trans-bond length changes, while B3LYP and M06 yielded a negligible trans-bond length change (<0.01 Å) that is well within the error margin. As the main purpose of this work is to further understand the net
trans-bond contraction in the \{FeNO\}_6 porphyrins and based on our results described above, we used the BPW91/BS1-optimized geometries to further elucidate this net effect; the overall error of the optimized geometric parameters of all of the experimental ferric/(FeNO)_6 and ferrous/(FeNO)_7 pairs investigated here using BPW91/BS1 is 1.6%. The optimized geometries of all studied systems at the experimental spin states of the respective or similar systems are provided in the SI.

To further evaluate the accuracy of the BPW91/BS1-optimized geometries for our work, we investigated the calculated vs experimental Mössbauer properties of the relevant complexes 5, 9–11 and 14.24,29,30,47,54 As shown in Table 2 and Figure S1, the calculated Mössbauer quadrupole splittings ($\Delta E_Q$) are in excellent agreement with the experiment, with an average error of 3.5% in the whole experimental range. The calculated isomer shifts ($\delta_{iso}$) also agree very well with the experiment, with an average error of only 0.04 mm/s. In addition, the computational results of asymmetry parameters ($\eta$) are again very close to the experiment. These results indicate the general accuracy of the chosen quantum chemical method and the optimized structures.

### Table 2. Mössbauer Properties for Some Iron Porphyrins (Unit: mm/s)

| molecule | $S$ | $\Delta E_Q$ | $\delta_{iso}$ | $\eta$ |
|----------|-----|--------------|---------------|--------|
| Ferric/(FeNO)$_6^a$ | Expt 3/2 | 3.29 | 0.39 |
| ([P(Fe(NO)]$_6^a$ | Calc 3/2 | 3.59 | 0.40 | 0.00 |
| (OEP)Fe(NO)(2-Melm)$_5^a$ | Expt 0 | 1.88 | 0.05 | 0.86 |
| ([P(Fe(NO)](2-Melm)$_4^a$ | Calc 0 | 1.95 | 0.07 | 0.68 |
| (OEP)(Fe(2-Melm))$_4^a$ | Expt 5/2 | 1.39 | 0.40 |
| ([P(Fe(2-Melm))]$_4^a$ | Calc 5/2 | 1.25 | 0.31 | 0.38 |
| Ferrous/(FeNO)$_7^a$ | Calc 0 | 0.73 | 0.34 | 0.89 |
| TTPPFe(2-Melm)$_9^d$ | Expt 2 | -2.43 | 0.94 | 0.9 |
| (P)Fe(2-Melm)$_9^e$ | Calc 2 | -2.86 | 0.96 | 0.92 |
| TTPPFe(NO)(1-Melm)$_9^a$ | Expt 1/2 | 0.73 | 0.34 | 0.89 |
| (P)Fe(NO)(1-Melm)$_9^a$ | Calc 1/2 | 0.68 | 0.41 | 0.81 |

*Ref 54. *Ref 29. *Ref 30. *Ref 47 The asymmetry parameter $\eta$ is close to 1, so the sign is not certain. *Ref 24 average of all experimental data.

**Predictive Model.** With the BPW91/BS1 computational tool selected based on its accuracy in reproducing the experimentally determined structures in this work, we proceeded to examine other N-ligated ferric/(FeNO)$_6^b$ pairs not yet experimentally explored (L = 1-Melm (13/14), 5-Melm (15/16), and NH$_3$ (17/18)), using experimental spin states of similar systems. The relevant data of these complexes are collected in Table 3. To begin, and not unexpectedly, we find that the calculated low-spin ferric ([P(Fe(L)]$_9^a$ ($S = 1/2$) complexes show shorter axial Fe–L bonds than their high-spin ($S = 3/2$ or 5/2) forms.

Comparisons of the five-coordinate ferric ([P(Fe(L)]$_9^a$ precursors and the six-coordinate FeNO$_6^b$ ([P(Fe(NO)(L)]$_9^a$ products in their low-spin states were made. As can be seen in Table 3, when NO forms an adduct with low-spin ([P(Fe(L)]$_9^a$ ($S = 1/2$) to form the FeNO$_6^b$ ([P(Fe(NO)(L)]$_9^a$ ($S = 0$) product, a trans-bond lengthening of Fe–L is observed with the magnitude depending on the identity of L; with 2-Melm (+0.127 Å; 10) > 1-Melm/5-Melm (+0.096 Å; 14/16) > NH$_3$ (+0.076 Å; 18) and in order of steric bulk of the ligand ($\Delta R_{bac}$ in Table 3). Thus, when both the calculated ferric ([P(Fe(L)]$_9^a$ and FeNO$_6^b$ ([P(Fe(NO)(L)]$_9^a$ complexes are in the low-spin states, NO exhibits its “normal trans effect”.

**NO Binding to Higher-Spin Five-Coordinate ([P(Fe(L)]$_9^a$** to **Give Low-Spin Six-Coordinate FeNO$_6^b$ ([P(Fe(NO)(L)]$_9^a$** **Products.** Experimental determinations of the spin states of ([P(Fe(L)]$_9^a$’ precursors show that they are not low-spin species. Consistent with the experimental findings of Scheidt (P = OEP; L = 2-Melm), we also find from the calculations that the formation of the FeNO$_6^b$ ([P(Fe(NO)(L)]$_9^a$’ products from their higher-spin ferric ([P(Fe(L)]$_9^a$’ precursors results in a trans-bond shortening of the axial Fe–L bonds. In this case, however, the magnitude of the calculated shortening is in the reverse order of steric hindrance of the axial ligand L with NH$_3$ (18) > 1-Melm/5-Melm (14/16) > 2-Melm (10), when starting from both the high-spin ($S = 3/2$) or intermediate ($S = 3/2$) spin state precursors ($\Delta R_{bac}$ in Table 3). This suggests that a ligand with higher steric hindrance (e.g., 2-Melm) imposes a higher restraint of moving this ligand closer to Fe upon NO binding, which consequently reduces this effect.

---

*Figure 4. Structures of the five-coordinate (left) and their respective NO-bound six-coordinate (middle) porphine complexes with axial N-bound ligands. Sketches of the NO-bound derivatives are shown on the right; the thick horizontal lines represent the porphine (P) macrocycle. Atom color scheme: Fe-black, C-cyan, N-blue, O-red, and H-gray.*
Table 3. Calculated Geometrical Data for NO Binding in Various Iron Porphyrins (in Å)

| axial L(s) | S | $R_{Fe-N_p}$ | $R_{Fe-L}$ | $\Delta R_{Fe-L}$ | $R_{Fe-NO}$ |
|-----------|---|-------------|-------------|----------------|-------------|
| NO/H₂O    | 6 | 0.023       | 2.105       | 1.620           |             |
| H₂O       | 5 | 0.026       | 2.154       | -0.051          |             |
|           |   | 0.007       | 2.195       | -0.090          |             |
|           |   | 0.006       | 2.065       | +0.040          |             |
| NO/2-MeIm | 10| 0.021       | 2.076       | 1.635           |             |
| 2-MeIm    | 9 | 0.038       | 2.116       | -0.040          |             |
|           |   | 0.003       | 2.167       | -0.091          |             |
|           |   | 0.000       | 1.990       | +0.127          |             |
| NO/1-MeIm | 14| 0.026       | 2.021       | 1.640           |             |
| 1-MeIm    | 13| 0.015       | 2.107       | -0.086          |             |
|           |   | 0.007       | 2.149       | -0.128          |             |
|           |   | 0.006       | 1.925       | +0.096          |             |
| NO/5-MeIm | 15| 0.026       | 2.022       | 1.640           |             |
| 5-MeIm    | 15| 0.038       | 2.108       | -0.086          |             |
|           |   | 0.006       | 2.150       | -0.128          |             |
|           |   | 0.003       | 1.926       | +0.096          |             |
| NO/NH₃    | 18| 0.030       | 2.043       | 1.638           |             |
| NH₃       | 17| 0.069       | 2.183       | -0.140          |             |
|           |   | 0.001       | 2.209       | -0.166          |             |
|           |   | 0.000       | 1.967       | +0.076          |             |

$\Delta R_{Fe-L}$ is the difference in axial Fe−L bond lengths, namely the distance in the{Fe(NO)}⁺ product minus that in the {[(P)Fe(L)]⁺ reagent. The values are placed next to the specific spin state considered. The experimental spin state for this 5-coordinate FeIII/{FeNO}⁶ species from the reaction of NO with in situ prepared [(OEP)Fe(NO)(5-MeIm)]SbF₆ in CH₂Cl₂.

With only one structural [(P)Fe(N-base)]¹⁺/[(P)Fe(NO)(N-base)]¹⁺ pair available experimentally, namely the OEP/2-MeIm pair,²⁹,₃⁰ we sought to prepare and crystallize the ferric derivatives containing axial 1-MeIm and/or 5-MeIm and their NO adducts. Gratifyingly, we were able, after a multi-year effort, to obtain and crystallize the ferric 5-coordinate [(OEP)Fe(N-(5-MeIm))]⁺ and 6-coordinate [(OEP)Fe(NO)(5-MeIm)]⁺ pair (c.f., the porphine models 15/16). Their crystal structures are shown in Figure 5.

The 5-coordinate ferric complex [(OEP)Fe(S-MeIm)]SbF₆ was prepared as an S = 3/2 species from the reaction of (OEP)FeFSbF₅ with 1.0 equiv of S-MeIm in CH₂Cl₂. The strict 1:1 reagent stoichiometry was important, as the bis-imidazole derivatives generally form in the presence of >1 equiv reactant. The values are placed next to the specific spin state considered. The experimental spin state for this 5-coordinate FeIII/{FeNO}⁶ species from the reaction of NO with in situ prepared [(OEP)Fe(NO)(5-MeIm)]SbF₆ in CH₂Cl₂.

The 5-coordinate ferric complex [(OEP)Fe(S-MeIm)]SbF₆ was prepared as an S = 3/2 species from the reaction of (OEP)FeFSbF₅ with 1.0 equiv of S-MeIm in CH₂Cl₂. The strict 1:1 reagent stoichiometry was important, as the bis-imidazole derivatives generally form in the presence of >1 equiv reactant. The porphyrin plane in [(OEP)Fe(NO)(5-MeIm)]⁺ displays a slight wave conformation with a apical displacement of +0.28 Å of the Fe atom toward the axial S-MeIm ligand. The compound has an average Fe−N(por) bond length of 2.023(10) Å and an Fe−N(axial) bond distance of 2.051(10). The π−π interaction (Figure 5B) between pairs of [(OEP)Fe(S-MeIm)]⁺ ions in the crystal is characterized by a mean plane separation (M.P.S.) of 3.436 Å and a lateral shift (L.S.) of 3.537 Å. The distances between Fe⋅⋅⋅Fe centers and between centroids Ct−...Ct are 3.141 and 4.736 Å, respectively. The π−π interaction in this compound is weaker than that observed in [(OEP)Fe(2-MeIm)]ClO₄ displaying a M.P.S. and L.S. of 3.312 and 1.49 Å, respectively.

The NO adduct [(OEP)Fe(NO)(5-MeIm)]SbF₆ (S = 0) was successfully synthesized from the reaction of NO with in situ prepared [(OEP)Fe(S-MeIm)]SbF₆ in CH₂Cl₂. The molecular structure (Figure 5C and S1C) exhibits a near-linear Fe−N−O linkage (∠177.9(3)°), with an average Fe−N(por) bond length of 2.009(3) Å. Its IR spectrum reveals a $\nu_{NO}$ at 1890 cm⁻¹, which is similar to those of previously reported 6-coordinate [(por)Fe(NO)(L)]⁺ complexes.³ The porphyrin plane exhibits minor deviations from planarity, with only a slight Fe-apical displacement of 0.028 Å toward the NO ligand.

Importantly, and consistent with the data in Table 3 for the model porphine derivatives 15/16 (Table 3), the trans-axial Fe−N(S-MeIm) bond distance (1.970 Å) in this low-spin NO adduct [(OEP)Fe(NO)(5-MeIm)]⁺ (S = 0) is significantly

Figure 5. (A) Molecular structure of the cation of 5-coordinate [(OEP)Fe(S-MeIm)]SbF₆. (B) Edge-on view of the π−π interaction between adjacent cations. (C) Molecular structure of the cation of 6-coordinate [(OEP)Fe(NO)(S-MeIm)]SbF₆. Thermal ellipsoids are drawn at 35% probability. The H atoms (except for the imidazole N6 protons) and the anions have been omitted for clarity.
shorter than that in the S-coordinate higher-spin \((S = 3/2)\) non-NO adduct \([(OEP)Fe(5-MeIm)]_2SbF_6\) (experimental \(\Delta R_{Fe-L} = -0.082\) Å versus calculated \(-0.128\) Å). For comparison, we also prepared the six-coordinate low-spin \([(OEP)Fe(5-MeIm)]_2SbF_6\) \((S = 1/2)\) complex and determined its crystal structure (Figure 6); the experimentally observed axial Fe–N bond length decreases, as expected, when the low-spin 6-coordinate derivative \((1.969(1)\) Å; \(\Delta\)) forms from its higher-spin S-coordinate analogue \((2.051(10)\) Å). Such a decrease is also observed in the limited number of ferric and ferrous \([(por)Fe(N-base)]^{5+/6+}/[(por)Fe(N-base)]^{6+/7+}\) pairs reported that show a spin state decrease. Conversely, axial Fe–N bond lengths can increase when going from 5-coordinate to 6-coordinate when spin states do not change significantly (Table S3).

**NO Binding to Ferric and Ferrous Porphyrins.** It is well-known that NO binding to iron porphyrins results in the generation of low-spin derivatives. In the case of six-coordinate low-spin \({\text{FeNO}}^6\) \([(\text{por})\text{Fe(NO)}(\text{L})]^+\) products with neutral ligands, we proceeded to investigate the electronic structure differences between ferric and ferrous porphyrins upon NO binding (Figure 7) at the experimental spin states. We selected the well-known ferric \([(OEP)\text{Fe}(2-MeIm)]^+:\text{Fe}^9\) \((S = 5/2)\) and the \([(OEP)\text{Fe}(2-MeIm)]^+(S = 0)\) pair. We examined both their \{\text{FeNO}\}^6 \((\text{por})\text{Fe(NO)}(\text{L})\) \((\text{L} = \text{neutral ligands})\) compounds and inspected the frontier MOs containing Fe d, f orbitals that could interact with the axial ligands. In the ferric precursor \((\text{P})\text{Fe(NO)}(2-MeIm))^+:\text{Fe}^9\) \((\text{L} = \text{neutral ligands})\), the high-spin d ferric center has an electronic configuration of \((d_{xz})^2(d_{yz})^2(d_{xy})^2(d_{x^2-y^2})^2(d_{z^2})^3\). As shown in the first column of MOs in Figure 8, the highest occupied molecular orbital (HOMO) has a strong π antibonding interaction between the Fe d\(z^2\) and ligand π orbital, with HOMO–3 having a weak π antibonding interaction between the Fe d\(z^2\) and ligand π* orbitals. The Fe d\(z^2\) orbital has basically a non-bonding interaction with 2-MeIm, as shown by HOMO–4. NO binding induces a large change in the electronic configuration of the resulting complex \(10\) \([(\text{P})\text{Fe(NO)}(2-MeIm)]^+:\text{Fe}^9\) \((\text{L} = \text{neutral ligands})\). The Fe center now is basically low-spin \(d^6\) due to the electron transfer from NO in the lowest energy state: \(\Delta\). Consequently, the strong π antibonding interaction between Fe d\(z^2\) and ligand π orbitals is now moved to an unoccupied MO, LUMO+2, while interactions of the Fe d\(x^2-y^2\) and ligand orbitals are basically the same; see the second column of MOs in Figure 8.

**Figure 6.** Molecular structure of the cation of six-coordinate \([(OEP)\text{Fe}(5-MeIm)]_2SbF_6\). Thermal ellipsoids are drawn at 35% probability. The H atoms (except for the imidazole N4 protons) and the anions have been omitted for clarity.

**Figure 7.** Sketch of the varied effects of NO binding on trans-axial bond lengths in ferrous and ferric porphyrins with neutral N-based and O-based ligands \((\text{L})\) in this work.
The MO results of the ferrous \((P)Fe(2-MeIm)\) (11) and \((FeNO)^7\) \((P)Fe(NO)(2-MeIm)\) (12) are shown in the third and fourth columns of MOs in Figure 8, respectively. The frontier MOs of \((P)Fe(2-MeIm)\) are similar to those of the deoxyMb model \((P)Fe(5-MeIm)\), with a high-spin d^6 configuration of \((d_{x^2})^2(d_{y^2})^2(d_{z^2})^1(d_{x^2}y^2)^1(d_{xy})^1\).

The strong σ antibonding interaction between the Fe d^z_2 and ligand p orbitals is evident in HOMO−1, while the Fe d_{x^2}/d_{y^2} and ligand orbitals are basically of non-bonding interactions (HOMO−3 and HOMO−5 in the third column of MOs in Figure 8). After NO binds to the precursor 11, the low-spin Fe center in the product 12 is of the electronic structure of \((d_{x^2})^3(d_{y^2})^3(d_{z^2})^1(d_{x^2}y^2)^1(d_{xy})^1\), as reported previously in similar ferrous NO porphyrins.57,58 The Fe d^z_2 orbital now moves to a higher energy orbital, HOMO, in the NO-bound product that enhances the trans effect, while the Fe d_{x^2}/d_{y^2} and ligand orbitals’ non-bonding interactions retain the same feature.

This kind of bonding analysis was also done for the O-based ligand water for which the ferric precursor to-\((FeNO)^6\) reaction also exhibits M–L bond contraction, while the ferrous- to- \((FeNO)^7\) system again shows M–L bond elongation (Table 3), as found above, for N-based ligands. For the ferric NO-free system 5, the strong σ antibonding interaction between the Fe d^z_2 and ligand p orbitals is evident in HOMO−5, which is again moved to the unoccupied orbital (LUMO) when NO is bound; see Figure 9, top two MOs on the left.

In contrast, for the ferrous NO-free system 7, the strong σ antibonding interaction between the Fe d^z_2 and ligand p orbitals evident in HOMO−1, is now moved to a higher orbital, HOMO, as in the case for the N-based ligand discussed above, when NO is bound; see Figure 9, top two MOs on the right. This enhances the trans effect. For all of these systems (5–8), the Fe d_{x^2}/d_{y^2} and ligand orbitals are basically of non-bonding interactions (see Figure 9, bottom two rows of MOs), which may not be important for the axial M−L trans effect. These MO features are qualitatively the same as found above for the N-based ligands.

In summary, these MO results show that the largest difference between Fe^II and Fe^III porphyrins upon NO binding at the experimental spin states is that, in contrast with the normal trans effect for \((FeNO)^7\) systems due to the strong antibonding interaction of d^z_2 and the axial ligand orbitals, this antibonding interaction is absent in the \((FeNO)^6\) systems (with neutral axial ligands) because it is now in an unoccupied orbital. The absence of this strong trans interaction, together with the distance shortening from the relatively higher-spin state for the NO-free ferric porphyrins to the low-spin state for the NO bound complexes, results in the observed color effect. Our results contribute toward an understanding of the versatility of NO in inducing the experimentally observed effect in iron porphyrins, where the trans Fe−L bond length may lengthen, or remain unchanged upon NO binding in different situations.

**EXPERIMENTAL SECTION**

**Computational.** All quantum chemical calculations were performed using Gaussian 09.60 All models investigated in this work were subjected to full geometry optimizations and subsequent frequency calculations to verify that they were minimum energy states of their potential energy surfaces. Descriptions of the calculations of Mössbauer properties are presented in the Supporting Information.

**General.** All reactions were performed anaerobically using standard Schlenkware and under nitrogen, unless otherwise noted. Air-sensitive samples and reagents were handled inside the glovebox. Solvents were collected under nitrogen from a PureSolv 400−5-MD solvent purification system (Innovative Technology) or distilled from appropriate drying agents under an atmosphere of nitrogen. Nitric oxide (NO) gas was passed through a double KOH column and then through a cold trap (dry ice/acetone) to minimize the introduction of NOx impurities. FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer. UV−vis spectra were collected using a Hewlett Packard 8453 diode array instrument. ^1H NMR experiments were performed on a 400 MHz Varian NMR spectrometer.

**Preparation of [(OEP)Fe(5-MeIm)]SbF_6.** To a CH_2Cl_2 (6 mL) solution of (OEP)FeSbF_6 (13.6 mg, 0.016 mmol)55 was added 4/5-methylimidazole (1.4 mg, 0.017 mmol), and the resulting solution was stirred overnight, during which time, the color of the reaction mixture changed from purple-brown to purple-red. The volume of the solution was reduced to ~1 mL.
under reduced pressure. The solution was then transferred to a vial (10 mL) and carefully layered with n-hexane (~3 mL) inside a glovebox. Slow evaporation of this solution gave crystals of \([(OEP)Fe(5-MeIm)]\)SbF6 (69% isolated yield) that were suitable for characterization by X-ray crystallography. IR (KBr): 662 and 640 cm\(^{-1}\) (\(\nu\) (SbF\(_6^−\)) anion). Evans NMR method (400 MHz, CDCl\(_3\), −50 °C)\(^{61−63}\) revealed a spin-only magnetic moment (\(\mu\)) of 3.67 BM, which suggests a spin system of \(S=3/2\).

Preparation of \([(OEP)Fe(NO)(5-MeIm)]\)SbF6. Following the general procedure above, 4/5-methylimidazole (1.5 mg, 0.018 mmol) was added to a stirred solution of \([(OEP)FeFSbF6\)] (13.8 mg, 0.017 mmol) in CH\(_2\)Cl\(_2\) (5 mL), and the reaction mixture was left to stir overnight. The volume of the solution was reduced to ~1.5 mL under reduced pressure. The Schlenk tube containing the reaction mixture was taken into a glovebox, and NO gas was bubbled (via needle) containing the reaction mixture was taken into a glovebox, and NO gas was bubbled (via needle) to give the product, which was isolated by filtration and dried in vacuo.

Preparation of \([(OEP)Fe(5-MeIm)2]SbF6\). To a CH\(_2\)Cl\(_2\) (5 mL) solution of \([(OEP)FeFSbF6\)] (9.2 mg, 0.011 mmol) was added excess 4/5-methylimidazole (6.3 mg, 0.077 mmol), and the resulting solution was stirred overnight. The volume of the solution was reduced to ~1.5 mL under reduced pressure, and the product was precipitated by the addition of n-hexane (10 mL). The supernate was decanted, and the solid was washed with n-hexane (3 × 10 mL) and subsequently dried in vacuo. The solid was redisolved in CH\(_2\)Cl\(_2\) (~1.5 mL), and the solution was carefully layered with n-heptane (~1.5 mL) in an open vial inside a glovebox. Slow evaporation of this solution afforded X-ray quality crystals of the product, which was identified as \([(OEP)Fe(5-MeIm)2]SbF6\] (70% isolated yield) by X-ray crystallography. IR (KBr): 667, 654, 631 cm\(^{-1}\) (\(\nu\) (SbF\(_6^−\)) anion). Evans NMR method (400 MHz, CDCl\(_3\), −20 °C)\(^{61−63}\) revealed a spin-only magnetic moment (\(\mu\)) of 1.84 BM, which suggests a spin system of \(S=1/2\).

X-ray Crystallography. Details of crystal data and refinement are given in Table 1. Single-crystal X-ray diffraction data were collected using an APEX II diffractometer with a CCD area detector\(^{64,65}\) and a sealed-tube Mo K\(_{\alpha}\) radiation source (\(\lambda = 0.71073\) Å). Diffraction data were collected from the samples at 100(2) K. The structures were solved by direct methods and using the SHELXTL system and refined by full-matrix least-squares methods on \(F^2\)\(^{66,67}\). Details of crystal data and structure refinement parameters are collected in Table S2 in the SI.

\([(OEP)Fe(5-MeIm)]\)SbF6. A purple block-shaped crystal of dimensions 0.110 × 0.160 × 0.180 mm\(^3\) was selected for structural analysis. Cell parameters were determined from a least-squares fit of 1002 peaks in the range 2.24 < \(2\theta\) < 17.01°. A total of 9547 data were measured in the range 1.595 < \(2\theta\) < 22.208° using \(\phi\) and \(\omega\) oscillation frames. The data were corrected for absorption by the semi-empirical method\(^{68}\) giving minimum and maximum transmission factors of 0.625 and 0.745, respectively. The data were merged to form a set of 9547 independent data with \(R\) (int) = 0.0467 and a coverage of 96.5%. The triclinic space group \(P\) was determined by statistical tests and verified by subsequent refinement. The positions of hydrogens bonded to carbons were initially determined by geometry and were refined using a riding model. The hydrogen bonded to N\(_6\) was located on the difference map, and its position was refined independently with restraints. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atom displacement parameters were set to 1.2 times (1.5 for methyl) the isotropic equivalent displacement parameters of the bonded atoms.

The selected crystal was twinned by non-merohedry, with a refined twin ratio of 0.3125(13). The intensity data were truncated to 0.94 Å because the data in higher resolution shells all had \(\langle F^2 - F^2 \rangle / \sigma > 2\). The anion was disordered with occupancies refining to 0.75(6) and 0.250(6) for the unprimed and primed atoms, respectively; restraints on the positional and displacement parameters of the disordered atoms were required. A total of 549 parameters were refined against 632 restraints and 9547 data to give \(wR(F^2) = 0.1872\) and \(S = 1.012\) for weights of \(w = 1/\sigma^2(F^2) + (0.0650P)^2 + 5.6000P\), where \(P = [F^2 + 2F^2] / 3\). The final \(R(F)\) was 0.0732 for the 5321 observed, \([F > 4\sigma(F)]\), data. The largest shift/s.u. was 0.000 in the final refinement cycle.

\([(OEP)Fe(NO)(5-MeIm)]\)SbF6. A red block-shaped crystal of dimensions 0.110 × 0.180 × 0.180 mm\(^3\) was selected for structural analysis. Cell parameters were determined from a least-squares fit of 4020 peaks in the range 2.48 < \(2\theta\) < 29.88°. A total of 22523 data were measured in the range 1.478 < \(2\theta\) < 29.911° using \(\phi\) and \(\omega\) oscillation frames. The data were corrected for absorption by the empirical method\(^{68}\) giving minimum and maximum transmission factors of 0.377 and 0.564. The data were merged to form a set of 22 523 independent data with \(R\) (int) = 0.0486 and a coverage of 100.0%. The triclinic space group \(P\) was determined by statistical tests and verified by subsequent refinement. The positions of hydrogens bonded to carbons were initially determined by geometry and were refined using a riding model. The hydrogen bonded to N\(_6\) was located on the difference map, and its position was refined independently. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atom displacement parameters were set to 1.2 times (1.5 for methyl) the isotropic equivalent displacement parameters of the bonded atoms.

The selected crystal was twinned by non-merohedry. The ratio of the twin components was refined to 0.2020 (7). The anion was disordered and was best modeled in three orientations, with occupancies refining to 0.9595(19), 0.0230(19), and 0.0175(19) for the unprimed, primed, and double-primed atoms, respectively; restraints on the positional and displacement parameters of the disordered atoms were required. A total of 638 parameters were refined against 1240 restraints and 22523 data to give \(wR(F^2) = 0.1473\) and \(S = 1.008\) for weights of \(w = 1/\sigma^2(F^2) + (0.0840P)^2 + P\), where \(P = [F^2 + 2F^2] / 3\). The final \(R(F)\) was 0.0489 for the 15 942 observed, \([F > 4\sigma(F)]\), data. The largest shift/s.u. was 0.002 in the final refinement cycle.

\([(OEP)Fe(5-MeIm)]\)SbF6. A black block-shaped crystal of dimensions 0.160 × 0.300 × 0.560 mm\(^3\) was selected for structural analysis. Cell parameters were determined from a
least-squares fit of 9325 peaks in the range 2.41 < θ < 30.89°. A
total of 27 350 data were measured in the range 1.858 < θ <
31.043° using φ and ω oscillation frames. The data were
corrected for absorption by the empirical method68 giving
minimum and maximum transmission factors of 0.6376 and
0.7462. The data were merged to form a set of 6507 independent
data with R(int) = 0.0232 and a coverage of 100.0%. The triclinic
space group P1 was determined by statistical tests and verified
by subsequent refinement. The positions of hydrogens bonded to
carbons were initially determined by geometry and were refined
using a riding model. The hydrogen bonded to N4 was located
on the difference map, and its position was refined independently. Non-hydrogen atoms were refined with
anisotropic displacement parameters. Hydrogen atom displace-
ment parameters were set to 1.2 times (1.5 for methyl) the
isotropic equivalent displacement parameters of the bonded
atoms.

Both the metal complex and the SbF₆ anion were located on a
center of symmetry. Two ethyl side groups of the porphyrin
were disordered. The occupancies of atoms C11 and C12 were
refined to 0.708(S) and 0.292(S) for the unprimed and primed atoms,
respectively. The occupancies of atoms C17 and C18
refined to 0.818(S) and 0.182(S) for the unprimed and primed atoms,
respectively. Restraints on the positional and displace-
ment parameters of the disordered atoms were required. A total
of 316 parameters were refined against 60 restraints and 6507
data to give wR(F²) = 0.0713 and S = 1.012 for weights of w = 1/
[σ²(F²) + (0.0420P)² +0.6400P²], where P = [F² + 2F₂]/3. The
final R(F) was 0.0257 for the 5940 observed, [F > 4σ(F)], data. The
largest shift/s.u. was 0.000 in the final refinement cycle.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c03610.

Computational and crystallographic details (PDF)

[(OEP)Fe(5-MeIm)]SbF₆ (CIF)

[(OEP)Fe(NO)(5-MeIm)]SbF₆ (CIF)

[(OEP)Fe(S-MeIm)]₂SbF₆ (CIF)

Accession Codes
CCDC 2080223–2080225 contain the supplementary crystallo-
graphic data for this paper. These data can be obtained free of
charge via www.ccdc.cam.ac.uk/data_request/cif or by emailing
data_request@ccdc.cam.ac.uk or by contacting the Cambridge
Crystallographic Data Centre, 12 Union Road, Cambridge CB2
1EZ, UK; fax: ++44 1223 336033.

AUTHOR INFORMATION

Corresponding Authors

George B. Richter-Addo — Price Foundation Institute of Structural Biology and Department of Chemistry and
Biochemistry, University of Oklahoma, Norman, Oklahoma
73019, United States; orcid.org/0000-0001-9400-0113;
Email: grichteraddo@ou.edu

Yong Zhang — Department of Chemistry and Chemical Biology,
Stevens Institute of Technology, Castle Point on Hudson,
Hoboken, New Jersey 07030, Unites States; orcid.org/
0000-0001-7207-6416; Email: yong.zhang@stevens.edu

Authors

Rahul L. Khade — Department of Chemistry and Chemical
Biology, Stevens Institute of Technology, Castle Point on
Hudson, Hoboken, New Jersey 07030, Unites States

Erwin G. Abucayon — Price Foundation Institute of Structural
Biology and Department of Chemistry and Biochemistry,
University of Oklahoma, Norman, Oklahoma 73019, United
States

Douglas R. Powell — Price Foundation Institute of Structural
Biology and Department of Chemistry and Biochemistry,
University of Oklahoma, Norman, Oklahoma 73019, United
States

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.1c03610

Author Contributions

The manuscript was written through contributions of all
authors. Computations were performed at SIT and the
experiments were performed at OU. All authors have given
approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health
(GM-085774 to Y.Z.) and by (while GBR-A was serving at) the
National Science Foundation (CHE-1900181 and
CHE-0130835). Any opinion, findings, and conclusions or recom-
mandations expressed in this material are those of the authors
and do not necessarily reflect the views of the NSF.

REFERENCES

(1) Iganno, L. J. Nitric oxide: A unique endogenous signaling
molecule in vascular biology (Nobel lecture). Angew. Chem., Int. Ed.
1999, 38, 1882–1892.

(2) Culotta, E.; Koshland, D. E. No news is good news. Science 1992,
258, 1862–1865.

(3) Herzik, M. A.; Jonnalagadda, R.; Kuriyan, J.; Marletta, M. A.
Structural insights into the role of iron-histidine bond cleavage in nitric
oxide-induced activation of H-NOX gas sensor proteins. Proc. Natl.
Acad. Sci. U.S.A. 2014, 111, E4156–E4164.

(4) Cheng, L.; Richter-Addo, G. B. Binding and Activation of Nitric
Oxide by Metalloporphyrins and Heme. In The Porphyrin Handbook;
Guillard, R.; Smith, K.; Kadish, K. M., Eds.; Biochemistry and Binding:
Activation of Small Molecules; Academic Press: New York, 2000; Vol.
4, pp 219–291.

(5) Under the {MNO}ⁿ Enemark-Feltham notation,² "n" represents
the d electron count of the metal center (minus the contribution of the
NO ligand) plus an added electron for each NO ligand. Thus, the NO
adduct of a ferrous d⁸ center is represented as [Fe(NO)]²⁻.

(6) Enemark, J. H.; Feltham, R. D. Principles of structure, bonding,
and reactivity for metal nitrosyl complexes. Coord. Chem. Rev. 1974, 13,
339–406.

(7) Ballou, D. P.; Zhao, Y.; Brandish, P. E.; Marletta, M. A. Revisiting
the kinetics of nitric oxide (NO) binding to soluble guanylate cyclase:
The simple no-binding model is incorrect. Proc. Natl. Acad. Sci. U.S.A.
2002, 99, 12097–12101. and references therein

(8) Diersk, E. A.; Hu, S.; Vogel, K. M.; Yu, A. E.; Spiro, T. G.; Burstin,
J. N. Demonstration of the role of scission of the proximal histidine-iron
bond in the activation of soluble guanylyl cyclase through metal-
loporphyrin substitution studies. J. Am. Chem. Soc. 1997, 119, 7316–
7323.

(9) Marti, M. A.; Capece, L.; Crespo, A.; Doctorovich, F.; Estrin, D. A.
Nitric oxide interaction with cytochrome c’ and its relevance to

24785 https://doi.org/10.1021/acsomega.1c03610
ACS Omega 2021, 6, 24777–24787
guanylate cyclase. Why does the iron histidine bond break? J. Am. Chem. Soc. 2005, 127, 7721–7728.

(10) Marti, M. A.; Scherlis, D. A.; Doctorovich, F. A.; Ordejon, P.; Estrin, D. A. Modulation of the NO trans effect in heme proteins: implications for the activation of soluble guanylate cyclase. J. Biol. Inorg. Chem. 2003, 8, 595–600.

(11) Dai, Z.; Farquhar, E. R.; Arora, D. P.; Boon, E. M. Is histidine dissociation a critical component of the NO/H-NOX signaling mechanism? Insights from X-ray absorption spectroscopy. Dalton Trans. 2012, 41, 7984–7993.

(12) Lehnhart, N.; Sage, J. T.; Silvein, N.; Scheidt, W. R.; Alp, E. E.; Sturhahn, W.; Zhao, J. Oriented single-crystal nuclear resonance vibrational spectroscopy of [Fe(TPP)(NO)]: quantitative assessment of the trans effect of NO. Inorg. Chem. 2010, 49, 7197–7215.

(13) Praneeth, V. K. K.; Nather, C.; Peters, G.; Lehnhart, N. Spectroscopic properties and electronic structure of five- and six-coordinate iron(II) porphyrin NO complexes: Effect of axial N-donor ligand. Inorg. Chem. 2006, 45, 2795–2811.

(14) Vestnes, V. K.; Paulat, F.; Berto, T. C.; George, S. D.; Nather, C.; Sulok, C. D.; Lehnert, N. Electronic structure of six-coordinate iron(III) complexes. Coord. Chem. Rev. 2018, 362, 44–63.

(15) Obayashi, E.; Tsukamoto, K.; Adachi, S.-I.; Takahashi, S.; Ishii, S.; Sakurai, H.; Nakamaki, A. Synthesis and properties of octaethylporphino(arenethiolato)iron(III) complexes with intra-molecular NH...S hydrogen bond: Chemical function of the hydrogen bond. Inorg. Chem. 1998, 37, 2415–2421.

(16) Xu, N.; Powell, D. R.; Cheng, L.; Richter-Addo, G. B. The first structurally characterized nitroso heme thiolate model complex. Chem. Commun. 2006, 2030–2032.

(17) Smith, D. M. A.; Dupuis, M.; Vorpagel, E. R.; Stratsma, T. P. Characterization of electronic structure and properties of a bisthaldine heme model complex. J. Am. Chem. Soc. 2003, 125, 2711–2717. and references therein

(18) Becke, A. D. Density-functional energy exchange approximation with correct asymptotic behavior. Phys. Rev. A 1988, 38, 3098–3100.

(19) Perdew, J. P.; Burke, K.; Wang, Y. Generalized gradient approximation for the exchange-correlation hole of a many-electron system. Phys. Rev. B 1996, 54, 16533–16539.

(20) Wachtler, A. J. Gaussian basis set for molecular wavefunctions containing third-row atoms. J. Chem. Phys. 1970, 1033–1036.

(21) Liu, Y. L.; Guerra, F.; Wang, K.; Wang, W. X.; Li, J. K.; Huang, C. Z.; Zhou, Z.; Houllihan, K.; Li, Z.; Zhang, Y.; Nair, S. K.; Oldfield, E. Structure, function and inhibition of the two- and three-domain 4Fe-4S IspG proteins. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 8558–8563.

(22) Wang, K.; Wang, W. X.; No, J. H.; Zhang, Y. H.; Zhang, Y.; Oldfield, E. Inhibition of the Fe₄S₄-cluster-containing protein IspH (LytB): Electron paramagnetic resonance, metalloclaycs, and mechanisms. J. Am. Chem. Soc. 2010, 132, 6719–6727.

(23) Zhang, Y.; Oldfield, E. Cytoschrome P450: An investigation of the Mossbauer spectra of a reaction intermediate and an Fe(IV)=O model system. J. Am. Chem. Soc. 2004, 126, 4470–4471.

(24) Ghosh, A. Metalloporphyrin–NO bonding: Building bridges with organometallic chemistry. Acc. Chem. Res. 2005, 38, 943–954.

(25) Xu, N.; Powell, D. R.; Richter-Addo, G. B. Nitrosylation in a crystal: Remarkable movements of iron porphyrins upon binding of nitric oxide. Angew. Chem., Int. Ed. 2011, 50, 9694–9696.

(26) Ellison, M. K.; Schulz, C. E.; Scheidt, W. R. Nitrosyliron(III) porphyrinates: Porphyrin core conformation and FeNO geometry. Any correlation? J. Am. Chem. Soc. 2002, 124, 13833–13841.

(27) Scheidt, W. R.; Geiger, D. K.; Lee, Y. J.; Reed, C. A.; Lang, G. Characterization of 5-coordinate monomimidazole)porphino(iron(III) complexes. J. Am. Chem. Soc. 1985, 107, 5693–5699.

(28) Shannon, R. D. Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. Acta Crystallogr., Sect. A: Found. Adv. 1976, 32, 751–767.

(29) Shannon, R. D.; Prewitt, C. T. Effective ionic radii in oxides and fluorides. Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem. 1969, 25, 925–946.

(30) Scott, H. S.; Staniland, R. W.; Kruger, P. E. Spin crossover in homoleptic Fe(II) imidazolylidene complexes. Coord. Chem. Rev. 2018, 362, 44–63.
parameters: The mPW and mPW1PW models. *J. Chem. Phys.* 1998, 108, 664–675.

(49) Zhang, Y.; Guo, Z. J.; You, X. Z. Hydrolysis theory for cisplatin and its analogues based on density functional studies. *J. Am. Chem. Soc.* 2001, 123, 9378–9387.

(50) Stevenson, S.; Ling, Y.; Coumbe, C. E.; Mackey, M. A.; Confair, B. S.; Phillips, J. P.; Dorn, H. C.; Zhang, Y. Preferential encapsulation and stability of La3N cluster in 80 atom cages: Experimental synthesis and computational investigation of La3N@C79N. *J. Am. Chem. Soc.* 2009, 131, 17780–17782.

(51) Becke, A. D. Density-functional thermochemistry. 3. The role of exact exchange. *J. Chem. Phys.* 1993, 98, 5648–5652.

(52) Zhao, Y.; Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* 2008, 120, 215–241.

(53) Chai, J.-D.; Head-Gordon, M. Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections. *Phys. Chem. Chem. Phys.* 2008, 10, 6615–6620.

(54) Cheng, B.; Safo, M. K.; Orosz, R. D.; Reed, C. A.; Debrunner, P. G.; Scheidt, W. R. Synthesis, structure, and characterization of five-coordinate aquo(octaethylporphinato)iron(III) perchlorate. *Inorg. Chem.* 1994, 33, 1319–1324.

(55) Kintner, E. T.; Dawson, J. H. Spectroscopic studies of ferric porphyrins with quantum mechanically admixed intermediate-spin states: Models for cytochrome c’. *Inorg. Chem.* 1991, 30, 4892–4897.

(56) Abucayong, E. G.; Powell, D. R.; Richter-Addo, G. B. unpublished results.

(57) Zhang, Y.; Mao, J.; Godbout, N.; Oldfield, E. Mössbauer quadrupole splittings and electronic structure in heme proteins and model systems: A density functional theory investigation. *J. Am. Chem. Soc.* 2002, 124, 13921–13930.

(58) Zhang, Y.; Gossman, W.; Oldfield, E. A density functional theory investigation of Fe-N-O bonding in heme proteins and model systems. *J. Am. Chem. Soc.* 2003, 125, 16387–16396.

(59) Xu, N.; Goodrich, L. E.; Lehner, N.; Powell, D. R.; Richter-Addo, G. B. Preparation of the elusive (por)Fe(NO)(O-ligand) complex by diffusion of nitric oxide into a crystal of the precursor. *Angew. Chem., Int. Ed.* 2013, 52, 3896–3900.

(60) Frisch, M. J. et al. *Gaussian 09*, revision B.01; Gaussian, Inc.: Wallingford CT, 2010.

(61) Evans, D. F. The determination of the paramagnetic susceptibility of substances in solution by nuclear magnetic resonance. *J. Chem. Soc.* 1959, 2003–2005.

(62) Yatsunyk, L. A.; Walker, F. A. Structural, NMR, and EPR studies of $S = 1/2$ and $S=3/2$ Fe(III)bis(4-cyanopyridine) complexes of dodecasubstituted porphyrins. *Inorg. Chem.* 2004, 43, 757–777.

(63) Bain, G. A.; Berry, J. F. Diamagnetic corrections and Pascal’s constants. *J. Chem. Educ.* 2008, 85, 532–536.

(64) APEX2 Data Collection: APEX2 Software Reference Manual. Bruker-AXS: Madison, WI, 2007.

(65) SAINT: Data Reduction and Correction Program. Data Reduction: SAINT Software Reference Manual; Bruker-AXS: Madison, WI, 2007.

(66) Sheldrick, G. M. *SHELXT - Integrated space-group and crystal-structure determination*. *Acta Crystallogr., Sect. A: Found. Adv.* 2015, 71, 3–8.

(67) Sheldrick, G. M. Crystal Structure Refinement with SHELXL. *Acta Crystallogr., Sect. C: Struct. Chem.* 2015, 71, 3–8.

(68) Krause, L.; Herbst-Irmer, R.; Sheldrick, G. M.; Stalke, D. Comparison of silver and molybdenum microfocus X-ray sources for single-crystal structure determination. *J. Appl. Crystallogr.* 2015, 48, 3–10.