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Metalloporphyrin–NO Bonding: Building Bridges with Organometallic Chemistry

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Metalloporphyrin−NO Bonding: Building Bridges with Organometallic Chemistry

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ABSTRACT

DFT calculations in our laboratory and elsewhere have elucidated fundamental aspects of the structure and bonding of a variety of metalloporphyrin−diatomic complexes, including the biologically important heme CO, NO, and O_2 complexes. We have also studied some more exotic species such as metalloporphyrin−dinitrosyl, −dialkyl, and −diaryl complexes. In the course of this research, we discovered a number of unexpected similarities (isolobal analogies) between the bonding in metalloporphyrin−NO and organometallic compounds. Equally important, DFT calculations have played a significant role in advancing our understanding of *selective* diatomic ligand binding by heme proteins.

1. Introduction

Heme−diatomic interactions are at the heart of many key biological processes such as dioxygen storage and transport by the respiratory proteins myoglobin and hemoglobin, NO sensing by soluble guanylate cyclase, and nitrite and NO reduction, key processes in the global nitrogen cycle. An important question in this area is how a heme protein manages to discriminate among the small, essentially isosteric diatomics CO, NO, and O_2.[1,2] It is fair to say that our understanding of this problem has undergone a major transformation in the last 10−15 years, with quantum chemical studies playing not a small part.[3,4]

The subject of heme−diatomic interactions captured the imagination of inorganic chemists early on. Myoglobin and hemoglobin played a central role in the early development of crystallography in the 1940s and 1950s. Moreover, small-molecule X-ray crystallographic instrumentation became widely available in the 1960s. These two factors led to the synthesis and spectroscopic and structural characterization of a plethora of metalloporphyrin−diatomic model complexes.[5] From the point of view of this Account, particular mention may be made of some of the more recent crystallographic studies on FeNO porphyrins by Scheidt and co-workers.[5] In combination with a theoretical picture[6] of the bonding involved, these model complexes have placed our knowledge of heme protein−diatomic interactions in a much broader context.

Born 1964 in India, Abhik Ghosh obtained his Ph.D. from the University of Minnesota in 1992. In 1996, he took up a faculty position at the University of Tromsø, Norway, where he is now a professor of chemistry. From 1997 to 2004, he has also been associated with the San Diego Supercomputer Center as a Senior Fellow. His current research focuses on electronic structural aspects of transition metal complexes and involves both experimental and theoretical approaches.

Some of the essentials of metalloporphyrin−diatomic electronic structure are as follows. The metal centers in all known metalloporphyrin CO, NO, and O_2 complexes are low-spin as a result of the strong π-accepting ability of these ligands. The coordination geometries of metalloporphyrin−NO complexes are generally closely related to the so-called Enemark−Feltham electron count (n), which is the sum of the number of metal d and NO π^* electrons.[7] Thus, for $\{MNO\}^n$ complexes, where n is the Enemark−Feltham count, the MNO angles are about 180°, 145°, and 120° for $n = 6$, 7, and 8, respectively.

Recently, we have investigated the electronic structures of a number of less known metalloporphyrin species such as metalloporphyrin−dinitrosyl and −dialkyl/diaryl complexes. Although we undertook these studies simply as isolated intellectual puzzles, we soon discovered a common thread, in the form of similar metal−ligand orbital interactions, across many of these molecules. A key goal of this Account is to convey a sense of this commonality. Gratifyingly, our calculated results not only deepen our understanding of metalloporphyrin−diatomic bonding, but they also seem to provide a broader chemical context for biological heme−diatomic interactions.

2. Carbonmonoxyhemes: A Unique Tilting and Bending Potential Energy Surface

The question of how myoglobin (as well as hemoglobin) apparently discriminates against CO in favor of life-giving O_2 provides a good starting point for our discussion. The textbook explanation, based on inaccurate, early myoglobin−CO crystal structures, is that the protein forces the heme-bound CO into a high-energy bent conformation, while such a conformation is natural for a bound O_2. Based on high-resolution myoglobin−CO crystal structures[1] and IR absorption[8] and photoselection[9] studies, this picture changed dramatically in the 1990s. These studies seemed to indicate not only a relatively linear FeCO unit but also one that was rigidly so. The high FeCO bending frequency of around 550 cm^{-1} of carbonmonoxyhemes (which is higher than the FeC stretching frequency of about 450 cm^{-1})[8] suggested an essentially unbendable FeCO unit. This picture too turned out to be partially incorrect.

In 1996, using DFT calculations, Ghosh and Bocian[3] showed that FeCO units and indeed $\{MXO\}^6$ units in general are extremely flexible with respect to *cooperative* tilting and bending of the MXO unit but remarkably stiff when tilting and bending occur in opposite directions, the tilting and bending angles being defined in Figure 1. Subsequently, this picture was confirmed by Spiro and Kozlowski,[10] while Nakamoto and co-workers[11] provided an explanation for this effect. As shown in Figure 1, while in-phase tilting and bending hardly disrupts M−X−O π bonding, out-of-phase tilting and bending does. In light of the Ghosh−Bocian potential,[3] the 550 cm^{-1} FeCO

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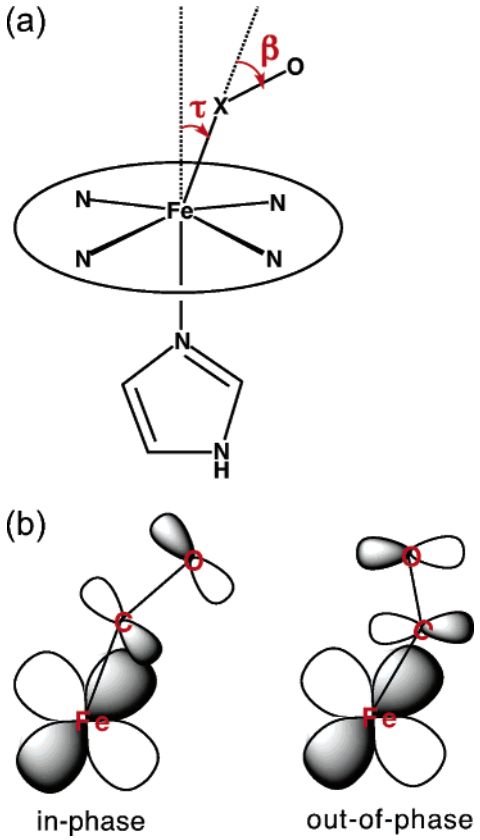

FIGURE 1. (a) Definition of the tilt and bend angles. (b) In- and out-of-phase tilting-and-bending deformations.

vibration could be assigned to the high-energy out-of-phase tilting and bending mode.[3]

Given the softness of the FeCO unit toward cooperative tilting and bending, how then can we explain the relatively linear FeCO geometries observed for myoglobin−CO and other heme−CO protein derivatives? The protein active sites are apparently simply too open to force the CO to bend over significantly. The softness of the FeCO unit also means that any reasonable amount of deformation cannot deliver a sizable chunk of the 4 kcal/mol by which myoglobin discriminates against CO in favor of O_2, relative to protein-free heme.[1] We now know that the discrimination energy largely reflects differential electrostatic stabilization of heme-bound O_2 by a distal pocket hydrogen bond, rather than a specific destabilization of the bound CO.[1−4]

3. {FeNO}^{7} Porphyrins: Intrinsic Tilting

Long considered as simply a pollutant and poison, NO has emerged as a ubiquitous signaling molecule in biology.[12] In biological systems, it is produced by the heme protein NO synthase (NOS) by the oxidative decomposition of arginine. The mammalian NO-sensing enzyme soluble guanylate cyclase (sGC) catalyzes the conversion of GTP to cyclic GMP (cGMP), a second messenger that mediates a variety of signaling pathways, notably the process of vasodilation.[12] Understanding heme−NO bonding is therefore of significant biological interest.

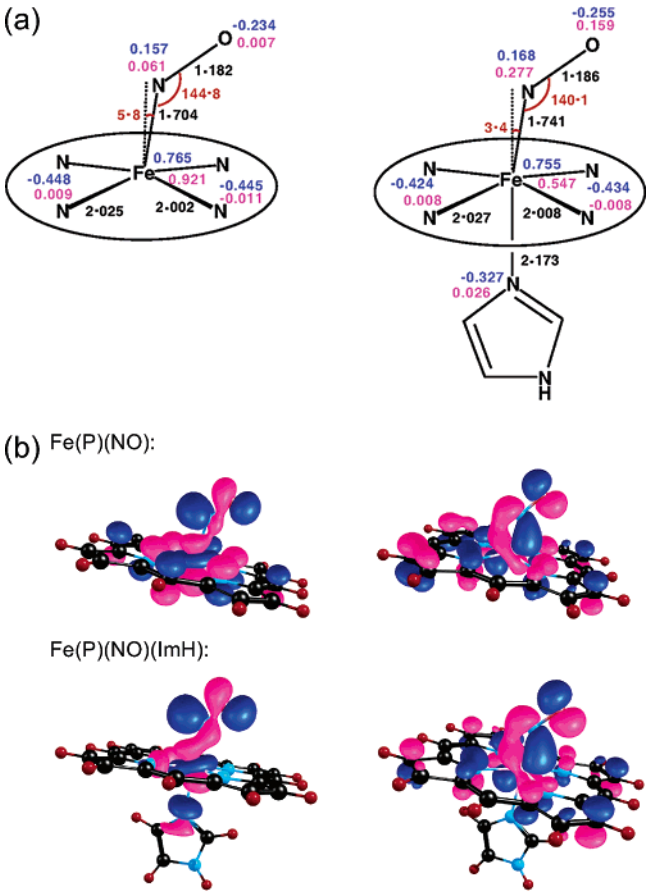

FIGURE 2. Calculated results on Fe(P)(NO) and Fe(P)(ImH)(NO). (a) Optimized distances (Å, in black), angles (deg, in red), Mulliken charges (in blue), and spin populations (in magenta). (b) Selected MOs involving Fe(d)-NO(π^*) bonding. The MOs to the left are the SOMOs, while those to the right are doubly occupied.

Without exception, {FeNO}^{7} heme−NO derivatives exhibit a strongly bent FeNO unit.[13] To a first approximation, this bent geometry reflects the shape of the singly occupied molecular orbital (SOMO), which involves a σ-type interaction between the Fe d_{z^2} orbital and an NO π^* orbital. EPR spectra of these $S = {}^1/_2$ complexes show hyperfine coupling to the NO nitrogen, indicating that the NO carries a significant amount of the spin density.[7] Recent DFT studies in our laboratory[14,15] and elsewhere[16,17] have added a number of fascinating details to this basic picture, which may be enumerated as follows. Figure 2 presents some key DFT[18] results on Fe(P)(NO), a five-coordinate model complex, and on Fe(P)(ImH)(NO), a six-coordinate complex (P = porphine, ImH = imidazole).

Both the five- and six-coordinate complexes carry a significant amount of spin density on the NO nitrogens, qualitatively consistent with EPR spectroscopy. However, the sixth ligand, imidazole in this case, does have a significant effect on the spin density profile; as shown in Figure 2b, it pushes a considerable amount of the SOMO density away from the Fe and on to the NO. This has also long been appreciated from EPR studies.[7]

Second, note that the Fe−N_{ImH} bond is unusually long, which reflects the trans-labilizing effect of NO or, more specifically, the antibonding metal(d_{z^2})−ImH interaction

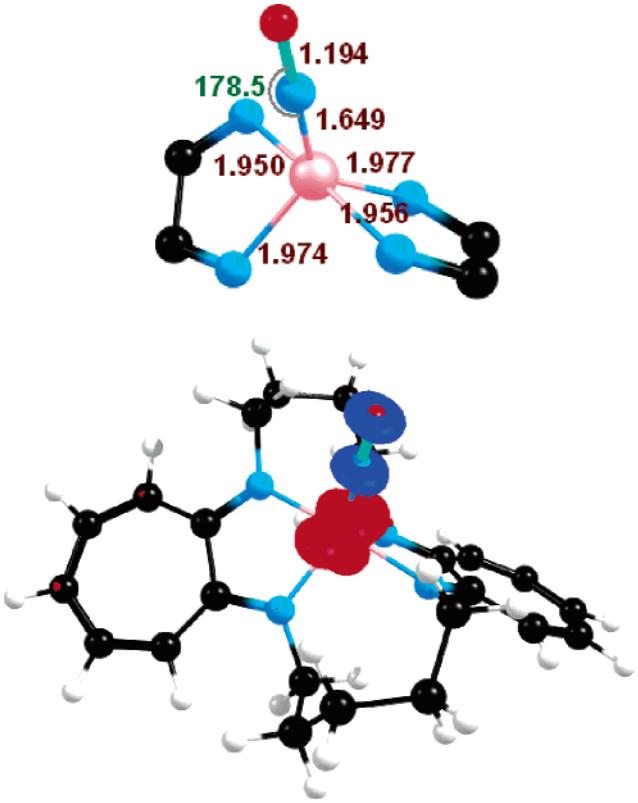

FIGURE 3. Highlights of the coordination geometry around the iron (Å in brown, deg in green) and spin density profile (majority spin in red, minority spin in blue) for Fe(5,5-tropocoronand)(NO).

present in the SOMO of Fe(P)(ImH)(NO) (Figure 2b). It has been proposed that the such a labilization of the proximal histidine in sGC by NO is of great functional importance to the enzyme and plays a direct role in the hydrolysis of GTP.[19]

Third, note that the Fe−N_{NO} vector is tilted relative to the heme normal,[14] which has also been observed experimentally;[13] this tilting may seem paradoxical, given that in Figure 2 the Fe d_{z^2} orbital component of the HOMO seems to tilt in the *opposite* direction relative to the heme normal. Why then does the Fe−N_{NO} vector tilt the way it does? The answer lies in another lower energy orbital interaction, namely an a′ Fe(d_π)−NO(π^*) π interaction (also shown in Figure 2), which apparently wins out.[15]

Finally, like the FeCO units, {FeNO}^{7} units are also substantially flexible,[15−17] which may be viewed as another manifestation of the Ghosh−Bocian potential.[3] Indeed, an artificial C_{4v} Fe(P)(NO) structure, with a linear FeNO group, is barely 0.2 eV (or 5 kcal/mol) higher in energy than the bent global minimum. The unpaired electron in the C_{4v} structure occupies a pure Fe d_{z^2} orbital that by symmetry cannot overlap with the NO π^* orbitals. The dramatic flexibility of the low-spin {FeNO}^{7} unit is perhaps best illustrated by the nonheme Fe(5,5-tropocoronand)(NO) complex (see Figure 3) reported by Franz and Lippard,[20] which we have recently studied with DFT calculations.[21] (The 5,5-tropocoronand ligand consists of a pair of aminotropiminates, i.e., nitrogen versions of tropolonates, linked by a pair of pentamethylene tethers.)

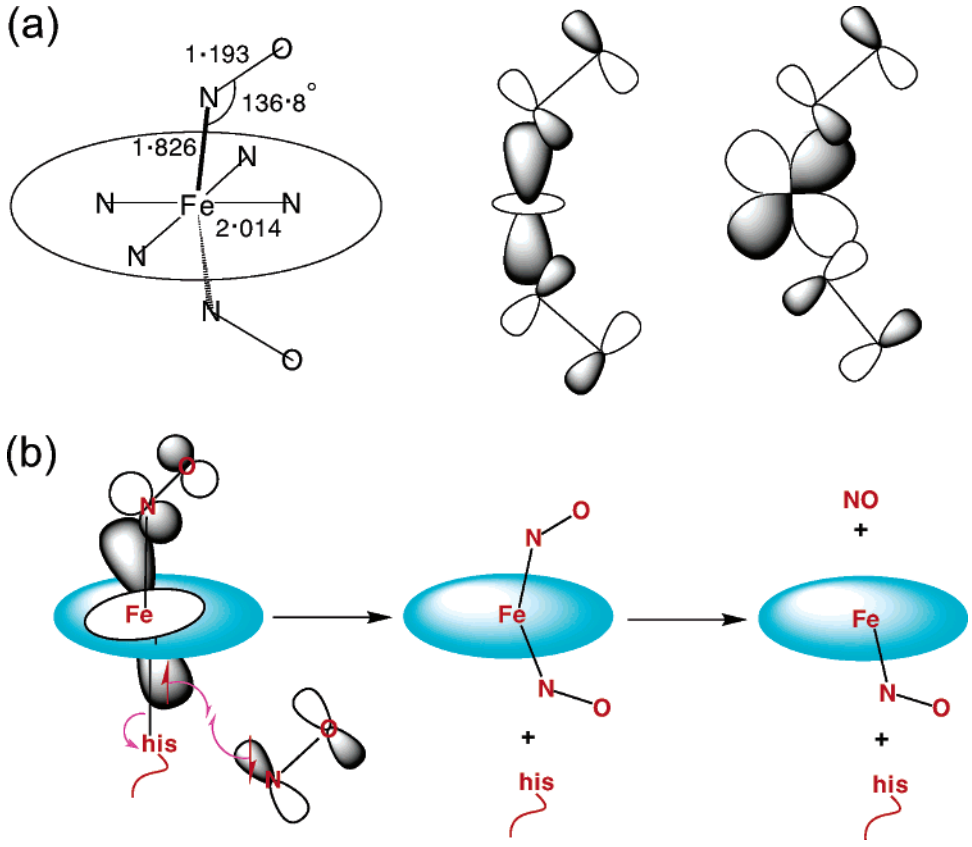

FIGURE 4. (a) Optimized structure of dinitrosylheme and schematics of key shape-determining orbital interactions. (b) Schematic representation of the putative formation of a proximal heme−NO complex via a dinitrosylheme intermediate.

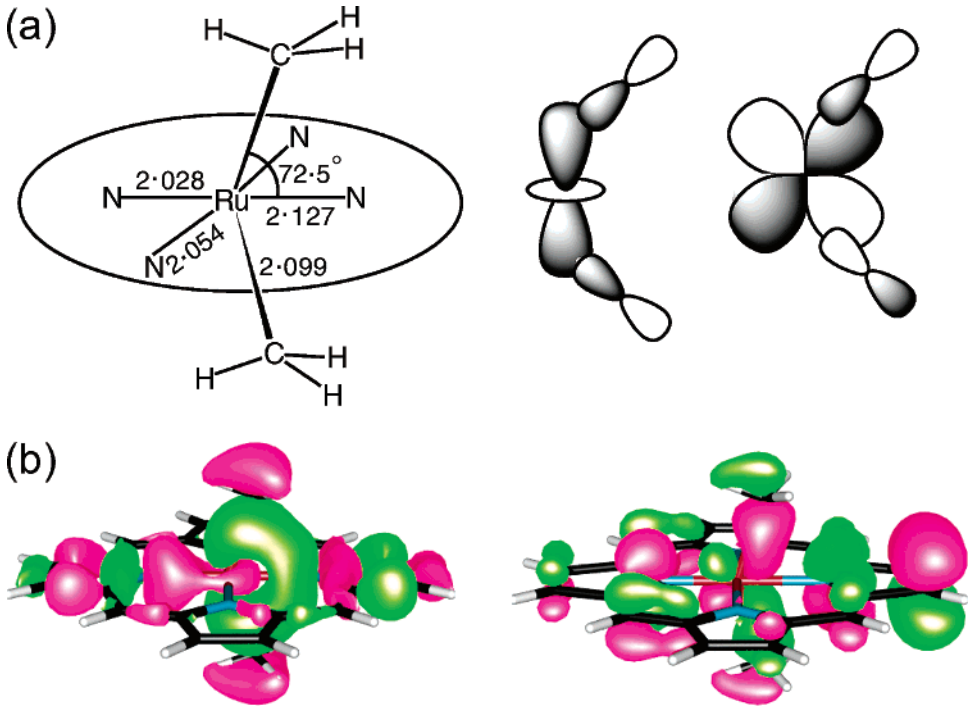

FIGURE 5. $Ru(P)(CH_3)_2$. (a) Highlights of the optimized structure and schematics of the "shape-determining" MOs. (b) Computer graphics of the corresponding canonical MOs.

Although this complex features a nearly *linear* $\{FeNO\}^7$ unit, the bonding turns out to be surprisingly similar to that in a heme−NO complex. If we regard the $Fe-N_{NO}$ direction as the z direction, the SOMO may be described as roughly $d_{x^2-z^2}$ (rather than d_{z^2}). This difference in d orbital hybridization stems from the trigonal bipyramidal geometry enforced by the tropocoronand ligand. In turn, the $d_{x^2-z^2}$ orbital seems to be less able to σ-bond with an NO π^* orbital, which results in a linear FeNO unit with maximum Fe−NO π bonding.

4. Dinitrosylheme: A Unique Trans−Syn Stereochemistry

Lorkovic and Ford have reported unstable, diamagnetic *trans*-dinitrosyl adducts of Fe^{II} and Ru^{II} porphyrins,[22] which have thus far not been characterized by X-ray crystallography. Given the trans effect of NO, the electronic structure of *trans*-dinitrosylheme presented an obvious conundrum, which we set about exploring with DFT calculations.[23] The results proved surprising: the $\{Fe(NO)_2\}^8$ porphyrins exhibit a clear preference for a peculiar trans-syn conformation. In other words, as shown in Figure 4, the two NOs on opposite sides of the porphyrin bend over in a syn or cisoid manner, relative to the porphyrin normal. A clear preference for such a unique structure seemed to implicate a primal, very definite, effect, most likely something involving orbital symmetry. Ultimately, we figured out that the two metal(d)−NO(π^*) interactions, shown in Figure 4a, which we might call the "shape-determining" orbital interactions, specifically favor the trans-syn conformation. If the NOs were upright or bent in opposite directions, one of these orbital interactions would become disallowed on symmetry grounds.[23]

It turns out that a dinitrosylheme intermediate may actually occur in nature, as an intermediate for the bacterial heme protein cytochrome *c*′.[24,25] The exact function of this protein is unclear, but it has been suggested that it suppresses potentially toxic levels of NO. Like sGC, cytochrome *c*′ forms five-coordinate heme−NO complexes. Amazingly, in the case of the *Alcaligenes xylosoxidans* protein, a crystal structure of the five-coordinate NO adduct shows the NO to be on the *proximal* side, where it has replaced the proximal histidine ligand.[24] As shown in Figure 4b, the intermediacy of a dinitrosylheme intermediate provides an elegant explanation of this unique phenomenon.

5. Dialkylruthenium(IV) Porphyrins: An Organometallic Connection

"I don't doubt it, Mr. Holmes, but that is no business of ours."

"Is it not? Is it not? Breadth of view, my dear Mr. Mac, is one of the essentials of our profession. The interplay of ideas and the oblique uses of knowledge are often of extraordinary interest. You will excuse these remarks from one who, though a mere connoisseur of crime, is still rather older and perhaps more experienced than yourself."

"... Surely our profession, Mr. Mac, would be a drab and sordid one if we sometimes did not set the scene so as to glorify our results. The blunt accusation, the brutal tap upon the shoulder − what can one make of such a denouement? But the quick inference, the subtle trap, the clever forecast of coming events, the triumphant vindication of bold theories − are these not the pride and justification of our life's work? ..."

The Valley of Fear by Sir Arthur Conan Doyle

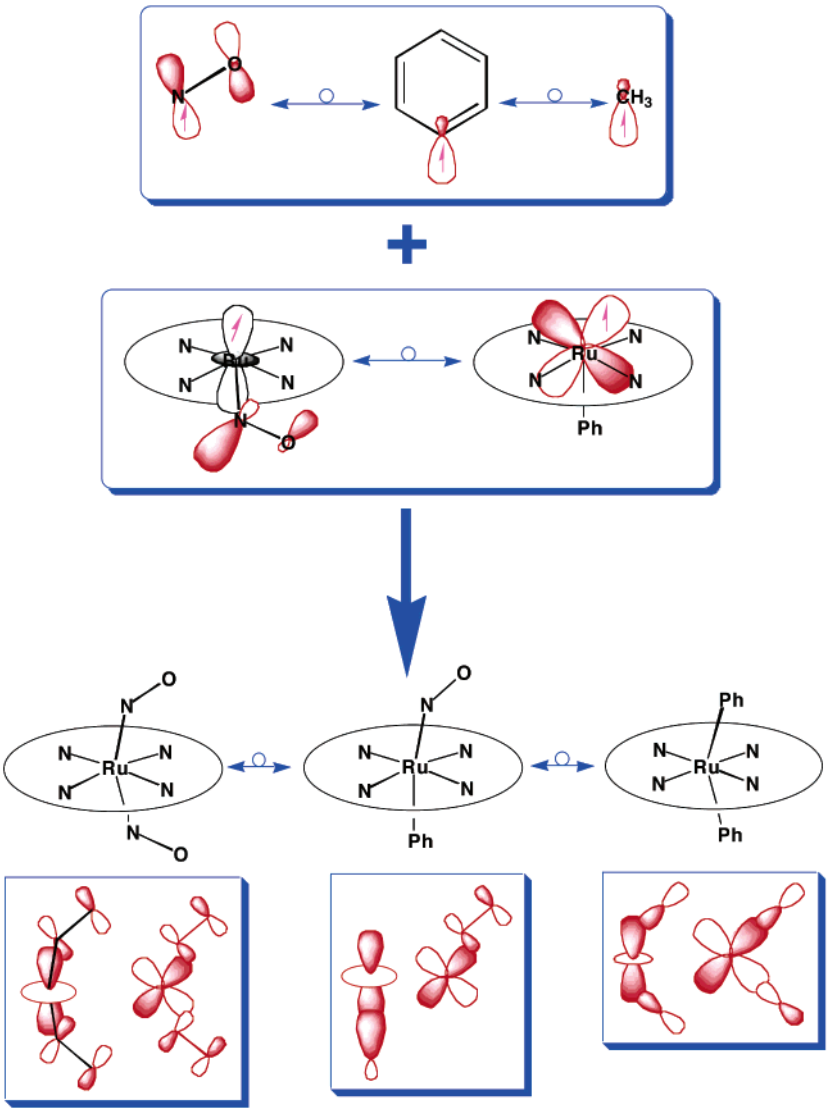

FIGURE 6. Toward an NO/alkyl/aryl isolobal analogy. Note the topological similarity of the shape-determining MOs of $Ru(P)(NO)_2$, Ru(P)-(NO)Ph, and $Ru(P)Ph_2$.

Around the time of our dinitrosylheme study,[23] we also wished to expand our horizons by examining electronic-structural problems involving second-row transition-metal porphyrins. We began this endeavor by trying to understand the diamagnetism of ruthenium(IV)−dialkyl and −diaryl complexes, first studied in depth by Collman and co-workers.[26,27] Geometry optimizations of $Ru(P)(CH_3)_2$ and $Ru(P)Ph_2$ ($S = 0$) led to remarkable structures with strong cisoid or syn tilting of the Ru−C vectors relative to the porphyrin normal.[28] As shown in Figure 5, the C−Ru−C angle in the optimized structure of $Ru(P)(CH_3)_2$ is only about 140°. Two specific Ru(d)−methyl orbital interactions account for the trans-syn geometry of $Ru(P)(CH_3)_2$.[28] Note that one of these interactions would be precluded on symmetry grounds for an upright orientation of the axial methyl groups.[28] The four electrons from the two methyl lone pairs might be imagined as occupying the two MOs shown in Figure 5, which might be described as based on the d_{z^2} and d_{xz} orbitals, while the four 4d electrons of the Ru^{IV} center may be thought of as occupying the metal d_{xy} and d_{yz} orbitals. Fortunately, this theoretical picture does seem to be supported by some concrete experimental evidence. Thus, like our calculated Ru structures, a crystal structure of $Os(TTP)(CH_2\text{-}TMS)_2$ (TTP = *meso*-tetra(*p*-tolyl)porphyrin, TMS = trimethylsilyl) also exhibits strongly cisoid-tilted trimethylsilylmethyl groups, with a C−Os−C angle of about 140°![29]

Initially,[23] our understanding of the unique conformational preference of dinitrosylheme was rudimentary. It was the MO analysis of $Ru(P)(CH_3)_2$ that gave us the hindsight we needed to really understand the electronic structure of dinitrosylheme. Chemists have a specific name for such "oblique uses of knowledge", namely isolobal analogies.[30] We are thus led to entertain the notion of methyl radicals and NO as isolobal fragments, at least with reference to Fe^{II}, Ru^{II}, and Os^{II} porphyrins as their bonding partners. We will try to extend this analogy to additional systems in the rest of this Account.

6. Some Unusual $\{MNO\}^6$ Complexes

Richter-Addo, Scheidt, and co-workers[31] have reported a remarkable set of $\{MNO\}^6$ M(Por)(NO)Ar (M = Fe, Ru, and

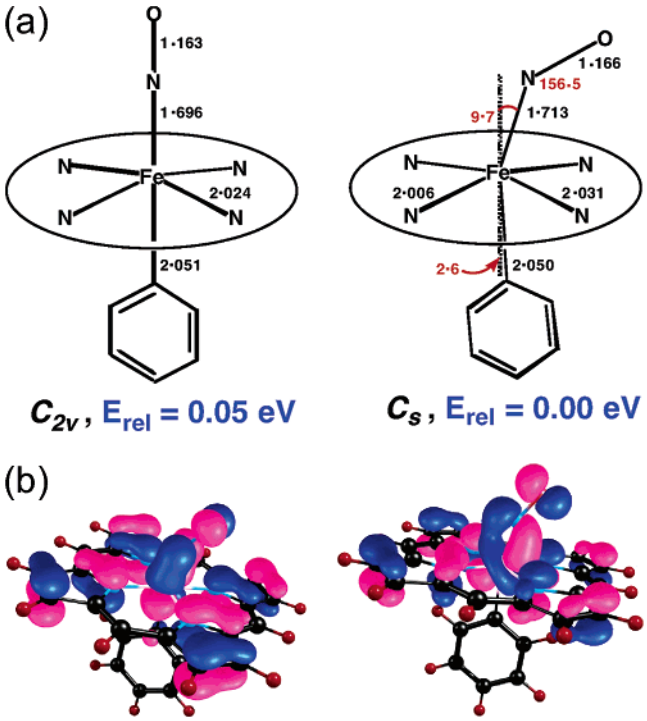

FIGURE 7. Fe(P)(NO)Ph ($S = 0$). (a) Highlights of optimized structures (Å, deg). (b) Selected occupied MOs involving Fe(d_π)-NO(π^*) bonding.

Os) complexes with distinctly bent MNO groups in which the M−N_{NO} and M−C_{Ar} vectors both tilt away in the same direction from the porphyrin normal, albeit to different degrees. Indeed, pursuing the NO/alkyl/aryl isolobal analogy proposed above, it is useful to view these complexes as hybrids of the dinitrosyl and dialkyl/aryl complexes discussed above. I have attempted to illustrate these isolobal relationships in Figure 6. However, what exactly accounts for the tilted and bent NO units in these complexes, which are essentially unique for {MXO}^{6} porphyrin derivatives?

Figure 7 shows selected calculated results on Fe(P)(NO)Ph. In essence, the metal d_{z^2} orbital may be viewed as tied up in a bonding interaction with the aryl group and essentially unable to σ bond with a lone pair on the NO nitrogen. Instead, the metal d_{xz} orbital engages in a favorable three-center bent π bond with an NO π^* orbital. However, as might be expected on the basis of the Ghosh−Bocian potential,[3] the energy associated with linearizing the NO is relatively small in these complexes, being only about 1 kcal/mol. Interestingly, a strongly bent {FeNO}^{6} unit with a *trans*-thiolate ligand has also been noted in a high-resolution crystal structure of nitrophorin 4, a salivary protein of the blood-sucking insect *Rhodnius prolixus*.[32]

7. Side-On NO Coordination

It turns out that {MNO}^{6} units are actually even more flexible than implied by the Ghosh−Bocian potential energy function. Thus, using IR spectroscopy at low-temperature, Richter-Addo, Coppens, and co-workers detected a side-on photoisomer for a {RuNO}^{6} porphyrin.[33] Figure 8a depicts a DFT optimized structure for Ru(P)(η^2-NO)Cl (C_s, $S = 0$),[34] chosen as a model of the experimentally observed compound, and its three primarily Ru 4d-based MOs. The MOs provide a nice explanation for the rather unsymmetrical structure of the Ru(η^2-NO) core. Thus, if we define the Ru(η^2-NO) plane as the *xz* plane, then in the HOMO-4, the Ru d_{xz} orbital can be seen to π-bond nicely with the NO π^* orbital in the *xz* plane. However, the HOMO-3 shows that the Ru d_{yz} orbital can only π-bond with one end of the other NO π^* orbital, and it does so with the nitrogen end, which explains the considerable inequality of the Ru−N_{NO} and Ru−O distances.

Digressing momentarily to nonheme metalloproteins, I should mention that a side-on CuNO intermediate has recently been observed in high-resolution crystal structures[35,36] of copper nitrite reductase (CuNIR), an environmentally significant bacterial enzyme that reduces nitrite to NO. To understand the electronic structure of this intermediate, we studied both $S = 0$ {CuNO}^{10} and $S = {}^1/_2$ {CuNO}^{11} model complexes with the hydrotris(4-imidazolyl)borate ($HBim_3^-$) supporting ligand.[37] In each case, a side-on structure was obtained as a local minimum, with the optimized geometries being shown in Figure 8b. Compared with the end-on forms, the energies of the side-on structure are only 0.47 and 0.23 eV for {CuNO}^{10} and {CuNO}^{11}, respectively. Moreover, compared to {CuNO}^{10}, the Cu−ligand distances in the side-on {CuNO}^{11} structure (2.0 ± 0.1 Å) are in distinctly better agreement with the experiment. As shown in Figure 8b, the unpaired electron in the latter structure is localized in one of the NO π^* orbitals, indicating a Cu^I−$NO^\cdot$ electronic description.

8. A Unique Linear *Trans*-{Mn(NO)$_2$}^{8} Unit

Unlike the *trans-syn* {Fe(NO)$_2$}^{8} and {Ru(NO)$_2$}^{8} porphyrins, the crystal structure of an apparently isoelectronic *trans*-{Mn(NO)$_2$}^{8} phthalocyanine complex, [Mn(Pc)(NO)$_2$]$^-$, exhibits upright and linear NO units,[38] which is unexpected for Enemark−Feltham electron counts exceeding 6. It turns out that the linearity of the *trans*-{Mn(NO)$_2$}^{8} unit reflects a different, $S = 1$ ground state for the Mn complex, relative to the Fe and Ru analogues. DFT calculations showed that the metal center in the Mn complex is best described as low-spin d^6 Mn^I, while the NO(π^*) orbitals overlap with the Mn(d_π) orbitals in an "allyl-like manner" to generate a pair of doubly degenerate MOs that accommodate the two unpaired electrons.[39] Highlights of the 4-fold-symmetric optimized structure, atomic spin populations, a plot of the spin density, and a plot of one of the two doubly degenerate singly occupied MOs (SOMOs) are shown in Figure 9.

9. Cis, Eclipsed, and Attracto: A {Mo(NO)$_2$}^{6} Porphyrin

The isolobal analogy is a model. It is the duty of our scientific craft to push it to extremes, and being only a model it is certain to fail somewhere, for any model, as ingenious a construction as it might be, is bound to abstract only a piece of reality. ...

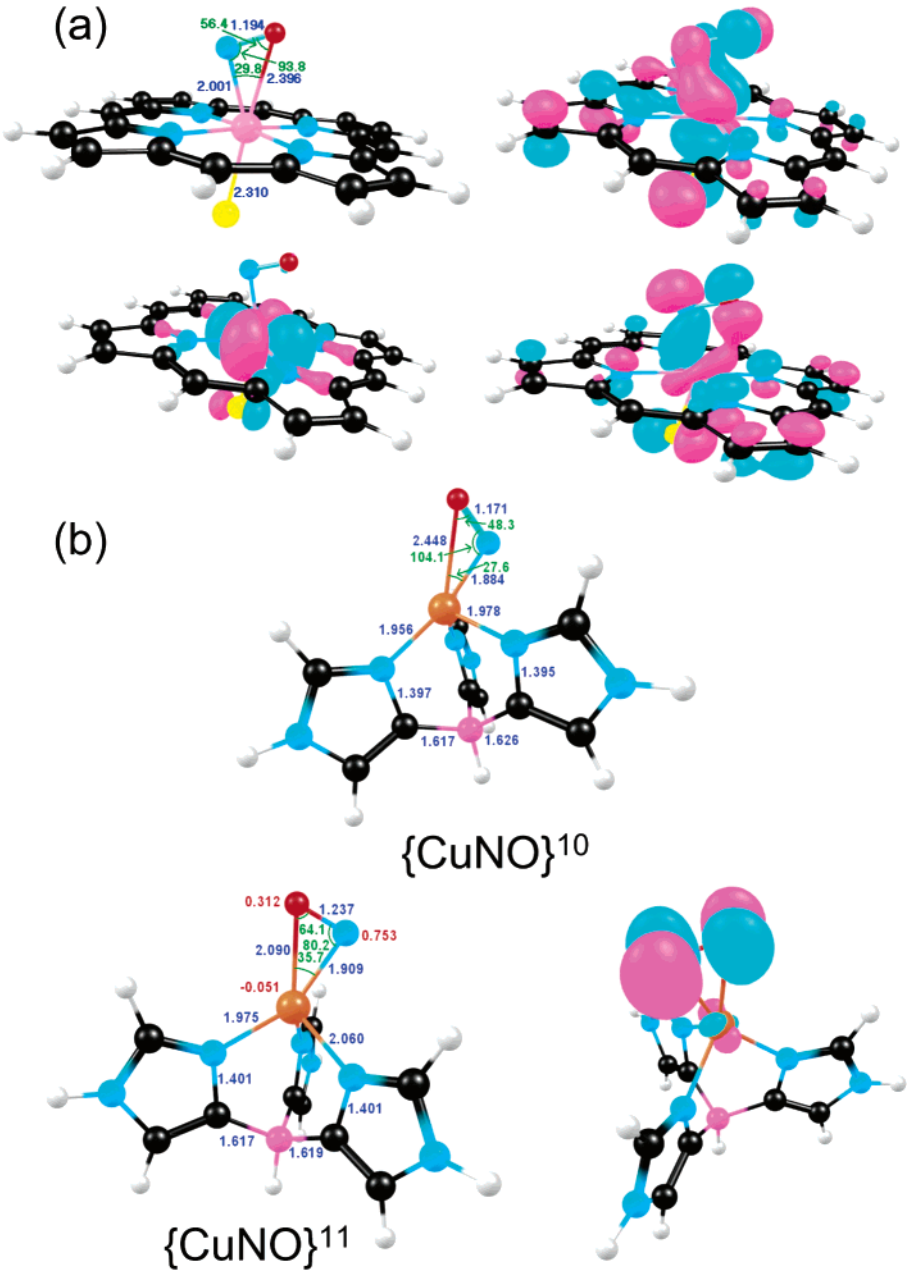

FIGURE 8. Side-on NO. (a) Optimized structure (Å, deg) and the three primarily Ru 4d-based MOs of Ru(P)(η^2-NO)Cl ($S = 0$). (b) Optimized structures (Å, deg) for side-on $\{CuNO\}^{10}$ (top, $S = 0$) and $\{CuNO\}^{11}$ (bottom, $S = {}^1/_2$) $HBim_3^-$ model complexes. Selected spin populations (in red) and the "open-shell" MO are also shown for the latter molecule.

The pleasing aspect of this particular model is that it brings together different subfields of our central science. We are separated, split asunder − organic, inorganic, physical, biological, analytical chemists − by the very largesse of our creation. The variety of molecules we create, and the methods we use to study them breed jargon and specialization. Yet underneath the seeming complexity there must be a deep unity.

Roald Hoffmann, Nobel lecture, 1982

Good mathematicians see analogies between theorems and theories, the very best ones see analogies between analogies.

Stefan Banach, quoted by Stanislaw L. Ulam in "*Adventures of a Mathematician*"

The crystal structure of *cis*-Mo(P)$(NO)_2$, an $\{M(NO)_2\}^6$ system, exhibits a number of interesting features.[40] Thus, the $Mo(NO)_2$ moiety eclipses a pair of opposite Mo−N bonds; moreover, the two NO units are peculiarly bent in a so-called attracto conformation, for which no explanation has been offered. Pursuing the NO/alkyl isolobal analogy, we were able to ascribe the attracto conformation to a bonding interaction between the Mo(d_{z^2}) orbital and a pair of NO(π^*) orbitals;[41] note that this MO is analogous to one shown in Figure 10a for $Zr(P)(CH_3)_2$.

Parts b and c of Figure 10 depict highlights of the optimized geometry of *cis*-Mo(P)$(NO)_2$ as well as the three doubly occupied MOs with substantial Mo(4d) character, which may be described as follows. While the d_{z^2} orbital

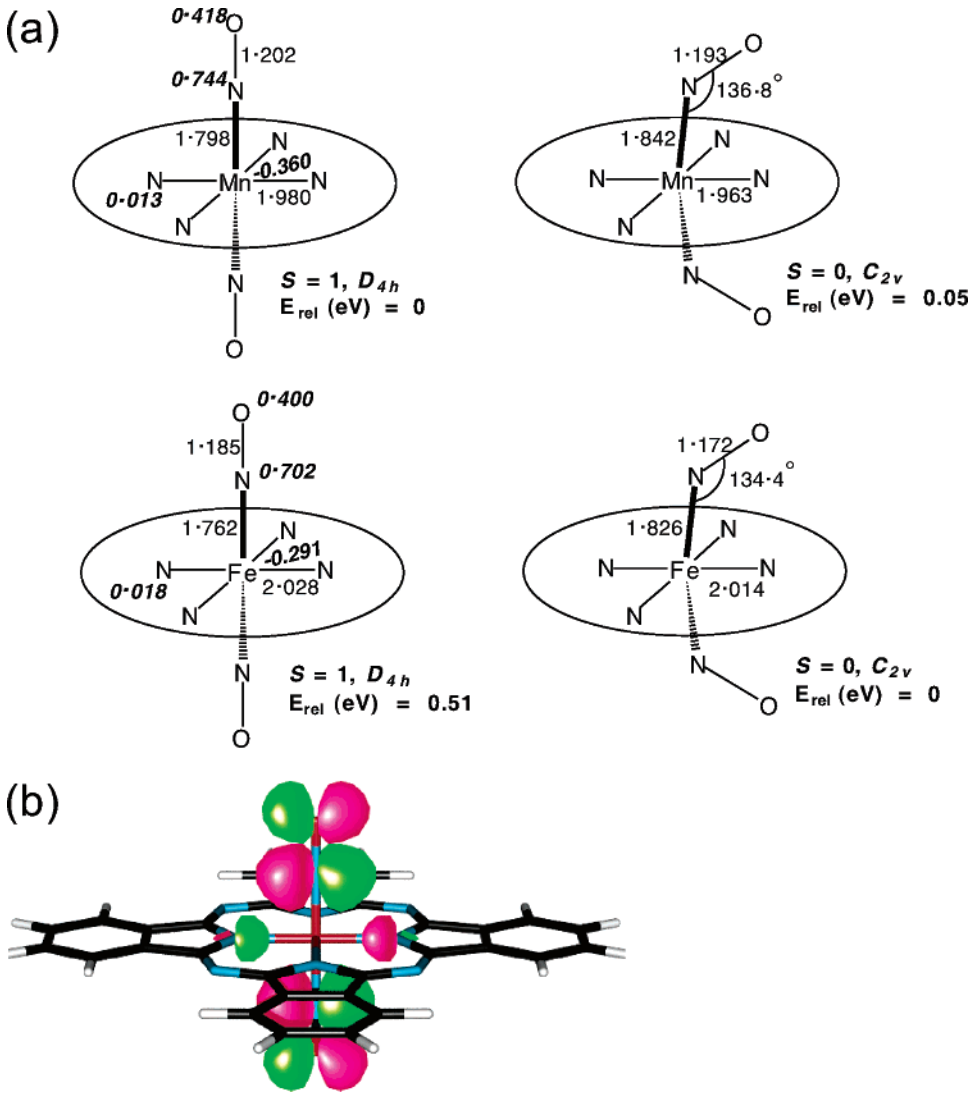

FIGURE 9. $[Mn(Pc)(NO)_2]^-$. (a) Key geometry parameters (Å, deg) and Mulliken spin populations (bold). (b) One of the *e* SOMOs. For comparison, the analogous iron structures are also shown in a.

engages in σ-type interactions with the NO(π^*) orbitals, the d_{yz} orbital may be described as engaged in π interactions with the NO(π^*) interaction, where we have identified the $Mo(NO)_2$ plane as the *xz* plane. The third doubly occupied MO may be described as the d_{xy} orbital (pointing between the porphyrin nitrogens) and engaging in a δ-like interaction with a pair of π^* orbitals on the two NOs; note that this orbital interaction explains why the $Mo(NO)_2$ moiety is eclipsed (rather than staggered) relative to a pair of opposite $Mo-N_{porphyrin}$ bonds.

Why do the Zr and Mo complexes exhibit cis stereochemistries, unlike the iron and ruthenium derivatives discussed earlier (in sections 4−6)? We believe this has to do with the inherently larger ionic radii of the early transition metals. Note the long Zr−N and Mo−N lengths in Figure 10, compared with analogous distances for Fe and Ru in Figures 4a and 5a.

Overall, the above analysis appears to have resulted in an interesting jigsaw of analogies: an NO/alkyl/aryl isolobal analogy plus an early/late transition-metal analogy. Although superficial by mathematicians' standards of "analogies between analogies", these insights do capture the essence of the bonding in many intriguing molecules, including metalloporphyrin−NO complexes.

10. Diatomic Ligand Discrimination by Heme Proteins

Let us now return to our discussion on how heme proteins discriminate among the different diatomic ligands.[42] As mentioned above, the "bent CO" hypothesis can no longer account for the observed ligand discrimination. Instead, hydrogen bonding involving a distal pocket residue appears to be the key factor governing the selective binding of either O_2 or CO/NO for many (but not all) diatomic-binding heme proteins.[1−4,43] The following are a few thumbnail sketches.

Myoglobin.[1] For a five-coordinate, substituted imidazole-ligated protoheme in benzene at room temperature, $K_{CO}/K_{O_2} = 22\,000$, an unacceptable ratio for a respiratory heme protein. This ratio is reduced to about 25 for sperm whale myoglobin, which corresponds to a discrimination

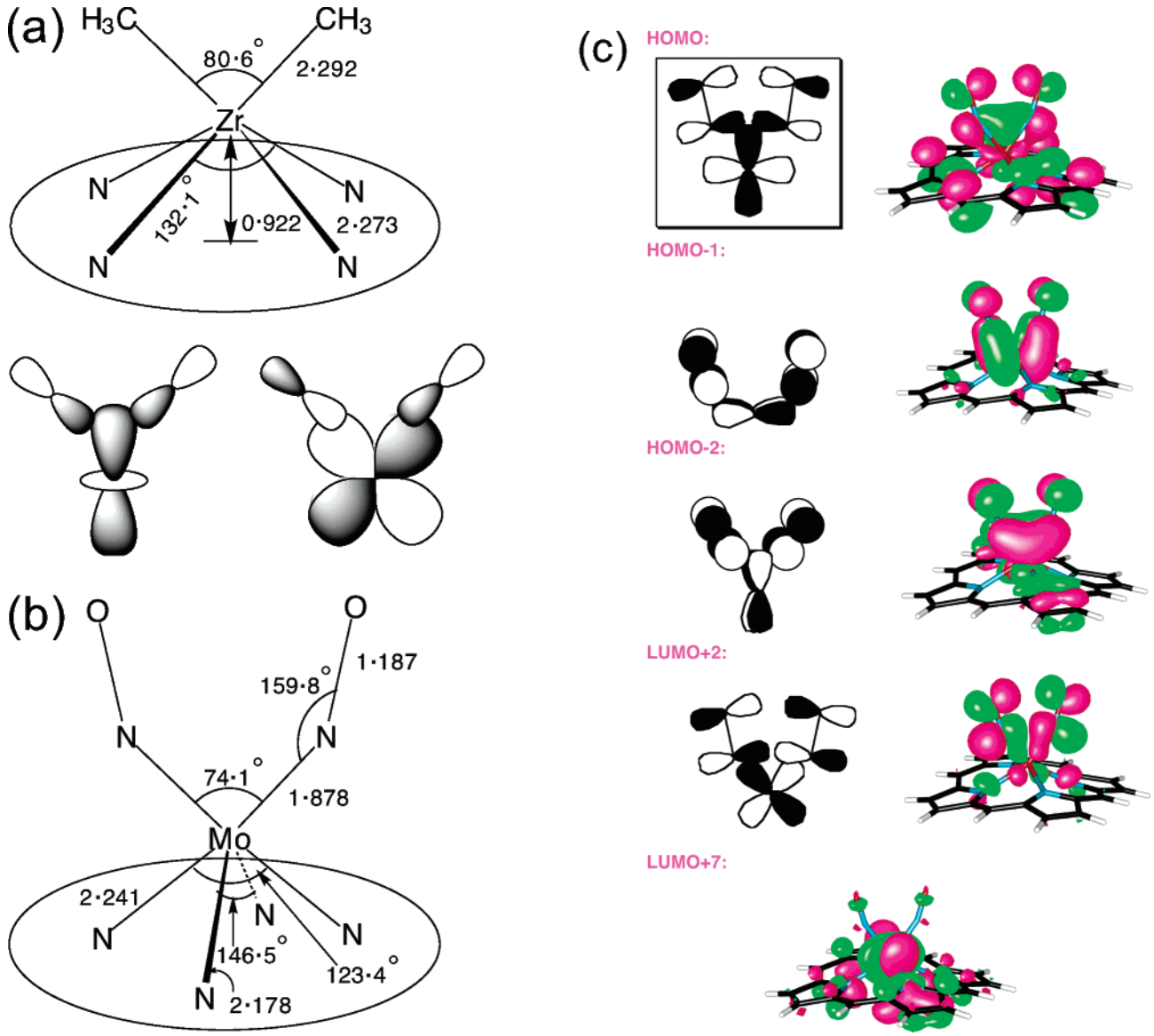

FIGURE 10. (a) Optimized geometry and key orbital interactions in $Zr(P)(CH_3)_2$. (b) Optimized geometry and (c) selected MOs of $Mo(P)(NO)_2$. Note the topological similarity of the "boxed" HOMO in c with the first orbital interaction shown in a.

energy ($\Delta\Delta G°$) of about 4 kcal/mol.[1] Thus, experimentally, hydrogen bonding with the distal histidine and other electrostatic effects favor O_2 binding over CO binding by about 4 kcal/mol. In contrast, for a distal ImH residue, DFT calculations indicate a hydrogen-bond energy of 3−4 kcal/mol for Fe(P)(CO)(ImH),[44] as well as for Fe(P)(ImH)(NO),[15] but an energy of about 9−10 kcal/mol for Fe(P)(ImH)O_2.[44] Thus, based on these model complexes, the DFT estimate of the O_2 versus CO discrimination energy is about 6 kcal/mol. Combined quantum and molecular mechanics simulations of myoglobin further improve this value to 5 kcal/mol, which is pretty much in agreement with experiment.[45]

H−NOX Domains.[2] Hydrogen bonding to a suitable distal residue seems to be critical for the formation of a stable O_2 complex. Thus, sGC, which does not have a distal hydrogen-bond donor, exhibits a remarkable reverse discrimination, relative to myoglobin and hemoglobin: it does not bind O_2 in a normal aerobic atmosphere but selectively binds CO and NO. The recent discovery and structural characterization[46−48] of a number of bacterial proteins (named H−NOX for heme−nitric oxide/oxygen binding) with sequence homology to sGC has shed significant light on selective ligand binding by not only sGC but also heme proteins in general. Like sGC (which has not yet been structurally characterized), *certain* bacterial orthologs also exclude O_2 under aerobic conditions, while binding NO selectively; unlike the respiratory heme proteins, these do not have a hydrogen-bond donor in the distal pocket, which would stabilize a heme-bound O_2. However, certain other H−NOX proteins found, curiously enough, in obligate anerobic bacteria *do* have a strategically positioned distal pocket tyrosine (or other hydrogen-bond donor), and these reversibly bind O_2 as well as NO and CO. Marletta and co-workers have suggested that the O_2 binding regulates downstream chemical events, which in turn lead to taxis (locomotion) toward regions of lower O_2 concentration.[2]

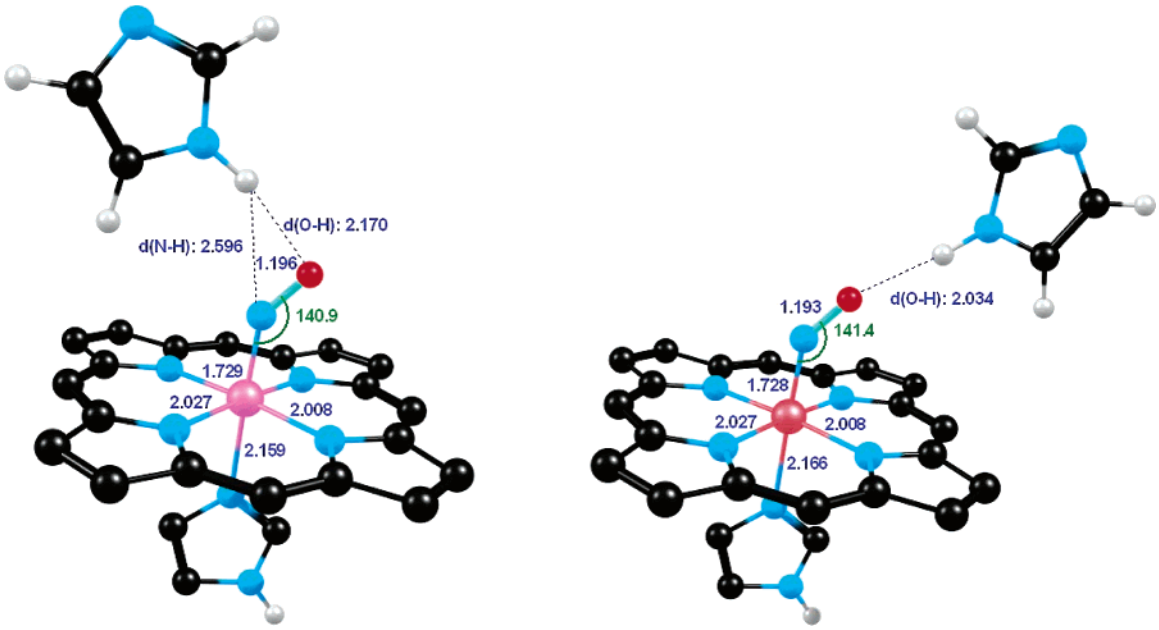

FIGURE 11. Alternative hydrogen-bond geometries involving Fe(P)(NO)(ImH) and a distal imidazole. Porphyrin and proximal hydrogens are omitted for clarity.

FIGURE 12. Views, pro and con, on MO arguments. Drawn by artist Mr. Odd Klaudiussen based on directions from the author.

In view of the exciting new discoveries on H−NOX domains, we undertook a DFT study of heme−NO groups as hydrogen-bond acceptors. Thus, we calculated hydrogen-bond energies of five- and six-coordinate heme−NO complexes with phenol, with the latter chosen as a model for the distal pocket tyrosine mentioned above. The calculated energies are approximately 2 kcal/mol, which is about 4 times smaller than the energies calculated for heme−O_2 complexes hydrogen bonding with a distal histidine. The interaction energies are essentially the same for five- and six-coordinate complexes, which may not be surprising but are nonetheless contrary to a recent suggestion![48]

We also investigated two alternative geometries of hydrogen bonding between a heme-bound NO and a "distal" ImH ligand. As shown in Figure 11, the results show that the NO can indeed accept hydrogen bonds in two ways, via the oxygen alone or sideways, via both the nitrogen and oxygen. While both geometries are equally favorable in energy terms, the latter geometry has actually been observed in a recent 1.9 Å crystal structure of the NO complex of horse heart myoglobin.[49]

CooA: CO versus NO Discrimination.[50] As mentioned above, heme-bound CO and NO are nearly equally effective (or ineffective) as hydrogen-bond accepors.[15] Thus, distal pocket hydrogen bonding cannot lead to effective CO versus NO discrimination; nature has devised other strategies for that. While little computational work has been done on this problem, our discussion would be incomplete unless we touch on at least some of the strategies involved.

Although sGC can bind both CO and NO, only NO results in a large acceleration of the enzymatic reaction, GTP hydrolysis. In the same spirit, in the case of the CO-sensing transcriptional regulator CooA from *Rhodospirillum rubrum*, only CO binding leads to effective signal

transduction and DNA binding. The CO-free Fe^{II} form of CooA features unique six-coordinate hemes with proline and histidine residues as axial ligands. While CO binding displaces the proline and initiates DNA binding, NO binding displaces both axial ligands, presumably as a result of NOs strong trans-labilizing effect, and does not result in any bioactivity.[50]

Finally, unlike CO and O_2, NO has the unique ability to bind to both Fe^{II} and Fe^{III} centers. Not surprisingly, certain heme proteins such as the nitrophorins and cytochrome P_{450} NO reductase exploit Fe^{III} centers for selectively binding NO.[32]

11. Concluding Remarks

Modern computational chemistry is increasingly about brute force computing, with decreasing emphasis on qualitative reasoning. In part, this state of affairs reflects the maturing frontier of inorganic chemistry. The qualitative aspects of bonding in simple transition-metal complexes are generally well-understood. In part, this also has to do with the reigning fads of the moment. Under these circumstances, the research described here has given me and my co-workers an opportunity to think in qualitative terms, to marvel at the force and beauty of orbital symmetry, and to revel in those "oblique uses of knowledge" called isolobal analogies, and all this in an important biochemical context![51] As I have tried to illustrate with a touch of humor in Figure 12, hardcore theoreticians might deride these pursuits as worthless, but they give us chemists what we crave: insight, understanding, and the ability to think creatively about new molecules.

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