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Ambidentate H-bonding of NO and O₂ in Heme Proteins

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Abstract

The affinity and reactivity of the gaseous molecules CO, NO and O₂ (XO) in heme protein adducts are controlled by secondary interactions, especially by H-bonds donated from distal protein residues. Vibrational spectroscopy, supported by DFT modeling, has revealed that for NO and O₂, but not for CO, a critical issue is whether the H-bond is donated to the outer or inner atom of the bound diatomic ligand. DFT modeling shows that bound NO and O₂ are ambidentate, both atoms separately acting as H-bond acceptors. This is not the case for CO, whose π^* orbital acts as a delocalized H-bond acceptor. Vibrational spectra of heme-XO adducts reveal a general pattern of backbonding variations, marked by families of negative correlations between frequencies associated with Fe-X and X-O bond stretches. For heme-CO adducts, H-bonding increases backbonding, the $\nu\text{FeX}/\nu\text{XO}$ points moving up the backbonding correlation established with model compounds. For NO and O₂ adducts, however, increased backbonding is only observed when the outer atom is the H-bond acceptor. H-bonding to the inner (X) atom instead produces a positive $\nu\text{FeX}/\nu\text{XO}$ correlation. This effect can be reproduced by DFT modeling. Its mechanism is polarization of the sp^2 orbital on the X atom, on the back side of the bent FeXO unit, drawing electrons from both the Fe-X and X-O bonds and weakening them together. Thus, the positioning of H-bond donors in the protein differentially affects bonding and reactivity in heme adducts of NO and O₂.

Keywords

Heme; Nitric oxide; Dioxygen; H-bonding; Backbonding; DFT; Raman

1. Introduction

Heme proteins are nature's receptors for the gaseous molecules CO, NO and O₂ (XO), which play key roles in metabolism and in signaling. Different heme proteins respond very differently to the presence of these molecules, despite having a common prosthetic group, a Fe-porphyrin, generally protoporphyrin IX. The protein responses differ because of differences in axial ligation by a protein proximal residue, generally histidine or cysteine, and in a variety of secondary interactions, especially H-bonding, with residues in the heme pocket.

H-bonding to the bound XO molecules affects their stability and reactivity. For example, the CO/O₂ affinity ratio is markedly reduced in myoglobin or hemoglobin, relative to protein-

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free heme, because the H-bond donated by a distal histidine residue is much stronger for bound O_2 than for bound CO.[1,2] The reactivity of bound NO or O_2 is also strongly affected by H-bonding. Thus, in cytochrome P450, which activates O_2 for substrate oxygenation, the reaction path is critically dependent on H-bonding from a distal threonine residue.[3,4]

In this article, we focus on the issue of which atom, X or O, gets the H-bond, and what the consequences are. For NO and O_2 , but not for CO, the outer and inner atoms can *both* accept H-bonds, with different effects on the electronic structure of the FeXO adduct. These effects have been explored through vibrational spectroscopy and supported by DFT computations[5–6], (A.V. Soldatova, T.G. Spiro, manuscript in preparation).

2. Backbonding Pattern of Vibrational Frequencies

The vibrational spectra of heme XO adducts contain modes which have dominant contributions from Fe-X and X-O bond stretching coordinates, and are labeled ν_{FeX} and ν_{XO} . When their frequencies are plotted against one another for a series of 5-coordinate Fe(II) porphyrin XO adducts (Figure 1), the result is a set of negative linear correlations. This is the expected result for variations in backbonding. The filled Fe(II) d_{π} orbitals are well matched to the empty XO π^* orbitals, and the resulting back-donation of electrons strengthens the Fe-X bond while weakening the X-O bonds (Figure 2), although to differing extents in the three FeXO adducts, due to their differing electronic and geometric structures (see below). The backbonding correlations were established with TPP-Y porphyrins (Figure 3) bearing variably electron-donating or -withdrawing substituents, which modulated back-donation through their inductive effect.[7–10] More electron-donating substituents increased back-donation, and shifted the ν_{FeX}/ν_{XO} point higher on the backbonding line. For different XO, the lines are shifted from one another because the Fe-X and X-O bonds have different intrinsic strengths for the different XO molecules. DFT computations, using a Fe(II)P-Y (porphine, P, bearing electron-donating and -withdrawing substituents, Y) (Figure 3) confirm that Fe-X and X-O bond distances and frequencies are negatively correlated for all the FeXO adducts[7, 8], (A.V. Soldatova, M. Ibrahim, T.G. Spiro, manuscript in preparation).

The universality of the backbonding pattern is remarkable because the FeXO adducts have different electronic and geometric structures.[11–14] In CO, both π^* orbitals are empty, and FeCO is a linear adduct, with $d_{\pi} - \pi^*$ overlaps in both perpendicular directions (x and y, with z as the FeCO axis, Figure 2). However, NO and O_2 have one and two π^* electrons, and their adducts are forced to bend ($\sim 145^\circ$ for FeNO, $\sim 120^\circ$ for FeO $_2$) in order to accommodate a π -antibonding interaction. The bonding can be thought of in terms of sp^2 -hybridization at the X atom, with one or two electrons in the lobe pointing away from the direction of bending (Figure 2). (NO can also form Fe(III)NO adducts, which are isoelectronic with Fe(II)CO). However, the π_{y^*} orbital remains available for backbonding from the Fe d_{yz} orbital in FeNO and FeO $_2$. Moreover, the π^* orbital energies decrease from CO to NO to O_2 . Thus, backdonation to π_{y^*} increases for FeNO and FeO $_2$, somewhat compensating the loss of π bonding in the x direction.

In FeO $_2$, backdonation results in nearly complete transfer of an electron, and the electronic structure is effectively Fe(III)(O $_2^-$); computations yield an open shell singlet ground state, with antiferromagnetic coupling between the spins on Fe(III) and O $_2^-$. [13–17] A weak π -bond between singly occupied orbitals, Fe- d_{yz} and O $_2$ - π_{y^*} , limits backdonation in Fe(III)(O $_2^-$);[14] as a result, ν_{XO} is less responsive to changes in ν_{FeX} for FeO $_2$ than for the other XO adducts, as can be seen in the much larger slope of the FeO $_2$ backbonding line (Figure 1).

That FeNO and FeO₂ vibrational frequencies follow the backbonding pattern is also remarkable in that ' νFeX ' is not a simple Fe-X stretching vibration. Because these adducts are bent, the Fe-X stretching and Fe-X-O bending coordinates mix in the vibrational modes, something that is symmetry-forbidden for the linear FeCO adduct. The mode chosen as ' νFeX ' is the one with the largest shift upon isotopic substitution at X.[8] While this mode has a major contribution from Fe-X stretching, Fe-X-O bending also contributes.[8,18] Despite this ambiguity, the backbonding lines are similar for all the adducts, albeit with different slopes, indicating that the coordinate mixing is constant, or else changes in proportion to the extent of backbonding.

In 6-coordinate FeXO adducts, the backbonding pattern is altered by the axial ligand, *trans* to the XO.[19] The axial ligand increases the electron density on the Fe, increasing backdonation and weakening the X-O bond. However, the expected Fe-X strengthening due to backbonding is offset by the σ competition between XO and the *trans* ligand for the Fe d_z^2 acceptor orbital, and the overall effect is Fe-X weakening. This effect is demonstrated by vibrational data for the Fe(II)(CO)TPP-Y adducts when 4-methylimidazole (4-MeImH – a surrogate for the histidine sidechain) is added as an axial ligand (Figure 4).[10,20] A new backbonding correlation is obtained, with νFeX shifted to lower values, as expected for a weaker Fe-X bond.

A shifted backbonding line is also obtained from vibrational data on 6-coordinate (4-MeImH)Fe(II)(NO)TPP-Y adducts (Figure 4) but in this case, the νFeX values are *higher*, not lower, than for the 5-coordinate adducts, despite the weakening of the Fe-X bond which is observed and calculated [8]. This anomaly is a manifestation of altered coordinate mixing in the ' νFeX ' mode, the issue mentioned above. In the 6-coordinate adducts, the contribution of Fe-X-O bending to this mode is increased [8], pushing the frequency higher. A similar effect is evident for 6- vs 5-coordinate Fe(II)O₂ adducts [21], although the data are too limited to determine a 6-coordinate backbonding line [9].

Most of the vibrational data come from resonance Raman spectroscopy, because resonance with the porphyrin $\pi\text{-}\pi^*$ transition (the intense Soret band near 400 nm is generally utilized) enhances electronically coupled vibrations, including the Fe-X-O vibrations, without interference from background vibrations (solvent, substituents or protein.) These background vibrations obscure most regions of the infrared spectrum. However, νCO vibrations occur in an uncluttered region of the spectrum, and can be monitored via infrared spectroscopy.[22–27]

3. H-bonding Effects

Heme protein vibrational spectroscopy, supported by DFT modeling, has led to key insights regarding distal H-bonding to bound XO molecules. For Fe(II)CO adducts, DFT modeling of distal H-bond donors indicates that the preferred orientation of the H-bond is towards the middle of the CO bond [28]. This is indeed the orientation of the distal imidazole in the CO adduct of myoglobin (Mb - Figure 5).[29] The CO π^* orbitals, partially occupied by backbonding, act as delocalized acceptors for the H-bond. The effect of the H-bond is to polarize the Fe(II)CO unit, enhancing back-bonding. Accordingly, νFeC shifts up, while νCO shifts down. Indeed, for MbCO variants having distal residue replacements (Figure 6) that produce variable distal H-bonds, the $\nu\text{FeC}/\nu\text{CO}$ points track the same backbonding correlation as do model compounds with variably electron donating substituents (Figure 7) [8,10]. The points shift higher on the backbonding line for stronger H-bonding, or other positively polar interactions, and lower for weaker H-bonding and for negatively polar interactions.

However, for both Fe(II)NO and Fe(II)O₂, modeling of a distal H-bond donor demonstrates XO ambidenticity. A stable energy structure is obtained for H-bonding to *either* O or to X. Figure 8 shows the computed structures for (ImH)Fe(II)NO porphine having the H-bond through either N or O.[5] Similar structures are also computed for the O₂ analog (A.V. Soldatova, T.G. Spiro, manuscript in preparation). The $\nu\text{FeX}/\nu\text{XO}$ frequencies behave quite differently in these adduct, depending on whether H-bond is directed toward X or O, as computation reveals (*vide infra*).

3.1 Fe(II)NO

When $\nu\text{FeX}/\nu\text{XO}$ data are plotted for heme protein Fe(II)NO adducts, the results are very different than for the Fe(II)CO adducts (Figure 7). The points can be seen to describe a fan. The top of the fan is the backbonding correlation obtained for the model compounds (4-MeImH)Fe(II)(NO)TPP-Y (Figure 4).[8] Some of the heme proteins fall on this backbonding line, but many do not.

DFT modeling provides the key to understanding the experimental fan. When H-bond donors of differing strength are allowed to interact with the bound NO, the effect on bonding depends on whether the interaction is with O_{NO} or N_{NO} (Figure 8).[5] H-bonding to O_{NO} increases backbonding; the Fe-N distance decreases while the N-O distance increases and the associated frequencies shift in the opposite directions (Figure 9). A negative frequency correlation is obtained, much like the top of the experimental fan. However, H-bonds directed at N_{NO} produce *positive* correlations between Fe-N and N-O distances and frequencies (Figure 9). For both sets of correlations, frequencies and distances obeyed Badger's rule [30], despite the coordinate mixing in the ' νFeN ' mode, because H-bonding has little effect on the mode composition.[5]

The physical mechanism of the positive distance and frequency correlations is that H-bonding to N_{NO} draws electrons from *both* the Fe-N and N-O bonds into the singly occupied non-bonding sp² orbital on N_{NO} (Figure 2); the Fe-N and N-O bonds therefore weaken together. The increased occupancy of the sp² orbital should also decrease the Fe-N-O angle, and the computed angle does indeed decrease [5]. Although the decrease was only modest, 3°, between the weakest and strongest H-bonds (NH₃ and NH₄⁺), H-bonding to N_{NO} was also found to soften the Fe-N-O bending potential significantly.

A similarly modest increase in the Fe-N-O angle was computed when the H-bond was directed at O_{NO}. [5]. In this case, the H-bond draws electron density out of the sp² orbital into the NO π^* orbital, increasing both the Fe-N-O angle and the extent of backbonding, thus, accounting for the negative distance and frequency correlations.

The heme protein Fe(II)NO adducts that fall on the backbonding correlation, at the top of the fan in Figure 7, likely have O_{NO}-directed H-bonds, if any. In the case of the heme cd₁ nitrite reductase (NiR), whose point falls well up on the backbonding line, a strong H-bond from a distal histidine residue to O_{NO} is evident in the crystal structure.[31]

The bottom of the fan is a line defined by the set of Mb variants having residue substitution distal to the heme (Figure 6).[9,32] The points along the line represent variants with different degrees of H-bonding to the bound NO. Wild-type Mb, having a distal histidine (H64), is in the middle of the line, but when it is replaced by hydrophobic residues (H64I, H64V), the point is close to the TPP-Y backbonding line. In between is H64Q, since the glutamine replacement forms a weaker H-bond than histidine. The point moves down the line from the wild-type position in the L29F variant, because the leucine at this position buttresses H64, and its replacement with the bulkier phenylalanine strengthens the H-bond.

At the low end of the H-bonding line is the V68N variant; the valine in this position abuts the bound NO, and its replacement by asparagine introduces a strong H-bond.

For Fe(II)CO adducts, these same variably H-bonding Mb variants simply move the $\nu\text{FeX}/\nu\text{XO}$ up and down the backbonding line defined by the TPP-Y models,[9] but for the Fe(II)NO they define an entirely different line, with a *positive* slope. The reason is that the distal histidine in Mb is oriented toward the side of the bound NO (Figure 5), with the NH group closer to N_{NO} than to O_{NO} . [33–35] For the CO adduct, this orientation is of little consequence. The H-bond acceptor is the delocalized CO π^* orbital, and H-bonding enhances backdonation, regardless of orientation. But for NO, the sideways interaction with N_{NO} is critical.

There are four reported Mb(II)NO crystal structures, differing in the method of preparation, and in the source of the Mb.[33–35] They all show distinctly shorter distances from the nearest N atom of the distal histidine to N_{NO} than to O_{NO} (Table 1). Interestingly, two of the reported Fe-N-O angles, 121° [35] and 112° [34] are much smaller than the other two, 147° [33] and 144° [35], and they are associated with significantly shorter ($\text{His64})\text{N}^{\cdots}\text{N}_{\text{NO}}$ distances. This trend is as expected from the DFT computations. The extent of the extra Fe-N-O bending is surprisingly large, but may reflect the expected softening of the bending potential and the moderate resolution of the crystal structures ($1.3 - 1.9 \text{ \AA}$). The reason for the stronger H-bonds in the two highly bent structures is unclear, but perhaps reflects different distributions of solvent molecules or salt ions, stemming from different crystallization conditions.

H-bonding to N_{NO} can also have biochemical consequences, by modulating the reactivity of the bound NO. For example, it can facilitate reduction to HNO, a species of considerable biological interest.[36–38] By pulling electron density into the sp^2 orbital on N_{NO} , and weakening the Fe-N and N-O bonds, the H-bond raises the Fe(II/I) reduction potential, and is itself poised to protonate the developing NO^- . HNO intermediates have been implicated in the enzymatic mechanisms of NO synthase [39], and of nitrite [40] and NO [41] reductases.

It is notable that reduction of Mb(II)NO is facile, and results in a stable HNO adduct,[42,43] although HNO itself is highly unstable. As expected, the Fe-N and N-O bonds are longer in the HNO adduct than in the NO adduct.[44,45] The reduction mechanism can be readily envisioned as complete transfer of the distal histidine NH proton to N_{NO} , concerted with electron transfer from the reductant to the NO adduct (proton coupled electron transfer - Figure 10). Subsequent histidine re-protonation from the solvent, and reorientation of the HNO adduct would produce a new H-bond, now to the O atom of HNO, which NMR analysis [46] indicates to be a key aspect of Mb(HNO) stability.

3.2 Fe(II)O₂

The study of FeO_2 vibrations poses special problems, because the adducts are prone to oxidation. The few 5-coordinate data, plotted in Figure 1 are from low-temperature and matrix isolation experiments, or from superstructured porphyrins that afford steric protection. Most data are from heme proteins, which sequester the FeO_2 adducts, protecting them from rapid oxidation.

Another serious difficulty is that the O-O stretching mode, νOO , is normally not detectable in RR (resonance Raman) spectra, due to weak enhancement. And while the νOO mode is active in IR spectra, its frequency, $\sim 1200 \text{ cm}^{-1}$, places it in a spectral region that is crowded with porphyrin and protein vibrations, making detection and assignment difficult [47]. However, there are circumstances under which νOO does become detectable in RR spectra,

presumably reflecting altered electronic coupling, although the mechanisms are currently uncertain.[48] RR enhancement of νOO is found when the axial ligand is thiolate; data are available for a number of cysteinyl heme proteins and model complexes.[49–53]

RR enhancement of the νOO band can also be observed for some imidazole-ligated heme proteins that have distal H-bonds to the bound O_2 . [48,54–58] A number of $\nu\text{FeO}/\nu\text{OO}$ data pairs have been reported for such proteins, and are plotted in Figure 11. They describe a fan of points similar to that found for Fe(II)NO adducts (Figure 6). A clear connection to distal H-bond strength has been demonstrated for the points at the bottom of the fan, which belong to variants of Ctb [54,57], a truncated hemoglobin from the bacterium *C. jejuni*. The distal pocket of Ctb contains tryptophan, tyrosine and histidine residues, all within H-bond distance of bound heme ligands, and with one another. The wild-type protein occupies the lowest point of the $\nu\text{FeO}/\nu\text{OO}$ fan, but when the H-bond donors are successively replaced by residues with non-polar sidechains, the Ctb points move steadily up a positive correlation, defining the bottom of the fan (Figure 11).

The similarity of the $\nu\text{FeX}/\nu\text{XO}$ fans for Fe(II)NO and Fe(II)O_2 suggests that, as in the former case, H-bonding to the terminal, or outer, atom, O^t , of Fe(II)O_2 , enhances backbonding, and leads to a negative $\nu\text{FeO}/\nu\text{OO}$ correlation (top of the fan), while H-bonding to the proximal, or inner, atom, O^p , weakens the Fe-O as well as the O-O bond, and produces a positive correlation (bottom of the fan). Unfortunately, structural data of high enough resolution to discern the dominant H-bond contacts are sparse. In Ctb, the positive correlation suggests O^p as the dominant receptor. A crystal structure is available only for the cyanide adduct.[59] However, the distal tryptophan, whose mutation produces the largest $\nu\text{FeO}/\nu\text{OO}$ shift, appears to be properly oriented to H-bond primarily with O^p . Also consistent with the O^p/O^t dichotomy is the position of hemoglobin, the highest point at the top of the fan [60–62], whose crystal structure reveals short H-bonds to O^t [63]. Points falling within the fan (Figure 11) likely reflect situations in which H-bonds to both O atoms are of comparable importance.

DFT modeling designed to test the O^p/O^t dichotomy (A.V. Soldatova, T.G. Spiro, manuscript in preparation), confirmed that H-bond donors of variable strength increase the Fe-O and O-O distances in concert when they are directed at O^p , but decrease the Fe-O distance while increasing the O-O distance (backbonding) when they are directed at O^t (Figure 12). The mechanism is the same as for Fe(II)NO adducts, although the positive slope is larger (2.0 vs 1.5), and the negative slope is smaller (-0.31 vs -1.0), for Fe(II)O_2 . Vibrational mode computations for the Fe(II)O_2 adducts are currently in progress (A.V. Soldatova, T.G. Spiro, manuscript in preparation).

Conclusions

Backbonding is the dominant motif in Fe(II)XO ($\text{X} = \text{C}, \text{N}, \text{O}$) porphyrin adducts, as revealed by families of $\nu\text{FeX}/\nu\text{XO}$ negative correlations, the lines being modulated by the nature of X and by effects of *trans* ligands. The degree of backbonding is controlled by inductive effects of porphyrin substituents and by polar residues near the bound XO, including H-bond donors. For NO and O_2 , a straightforward backbonding effect is seen only if the H-bond acceptor is the outer atom of XO. H-bonding to the inner atom, X, weakens the Fe-X and X-O bonds in concert, by polarizing electrons in the sp^2 orbital on the back side of X. The positioning of H-bond donors by the protein differentially controls bonding and reactivity in heme-NO and $-\text{O}_2$ adducts.

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Abbreviations

IR	infrared
RR	resonance Raman
NMR	nuclear magnetic resonance
DFT	density functional theory
H-bond	hydrogen bond
Mb	myoglobin
Hb	hemoglobin
HbN,HbO	hemoglobin I & II from <i>Mycobacterium tuberculosis</i>
Ch/Hb	<i>Chlamydomonas</i> hemoglobin
ScHb	<i>Synechocystis</i> hemoglobin
HemDGC	heme-containing diguanylate cyclase
Ctb	hemoglobin III from <i>Campylobacter jejuni</i>
NiR	nitrite reductase
AXCP	cytochrome <i>c'</i> from <i>Alcaligenes xylosoxidans</i>
BjFixLH	<i>Bradyrhizobium japonicum</i> FixL heme PAS
MtDosH	<i>Methanobacterium thermoautotrophicum</i> Dos heme-PAS
TtTar4H	<i>Thermoanaerobacter tengcongensis</i> Tar4 protein heme domain
TPP,TMP, OEP	tetraphenyl-, tetramethyl- & octaethyl- porphyrins
Pc	phthalocyanine
ImH	MeImH, imidazole, 4-methylimidazole

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Highlights

- DFT modeling together with available vibrational experimental data showed differential response of Fe(II)XO (XO = NO and O₂) heme to the position of H-bond donors.
- H-bonding to Fe(II)XO from heme pocket residues increases backbonding only if the H-bond acceptor is the outer atom of XO.
- H-bonding to the inner atom, X, of the Fe(II)XO adduct weakens the Fe-X and X-O bonds in concert due to enhanced orbital mixing.

Figure 1.

Negative frequency correlations for vibrational modes associated with Fe-X and X-O bond stretching are seen for 5-coordinate Fe(II) porphyrin complexes of all three X-O molecules, X = C, N, O. See text for references to the data.

Figure 2. Schematic diagram of geometric and electronic structure of Fe(II) porphyrin XO adducts, showing the orbitals available for π backbonding.

Figure 3.

Structural diagram for porphyrins used to establish Fe(II)XO backbonding correlations via substituent variation, experimentally via RR spectroscopy (left: FeTPP-Y), and computationally via DFT (right: FeP-Y)

Figure 4.

Experimental correlations of νFeX and νXO for 5- and 6-coordinate CO and NO adducts of FeTPP-Y. Axial ligation shifts νFeX down for CO but up for NO, although the Fe-X bond is weakened in both cases. The anomalous νFeN upshift reflects an increased contribution of Fe-N-O bending to the ' νFeN ' vibrational mode (see text).

Figure 5. Overlay of MbXO structures (*gray*, MbCO, pdb #: 1BZR; *blue*, MbNO, pdb #: 2FRJ; *red*, MbO₂, pdb #: 1A6M), showing the sideways contact of the distal histidine with the bound XO.

Figure 6. MbCO structure (pdb #:1A6G) showing the distal residues which have been mutated to investigate the effects of distal electric fields and H-bonds on MbXO adducts.

Figure 7.

Contrasting response of FeCO and FeNO adducts to distal H-bonds in heme proteins. Varying the H-bond strength shifts the $\nu\text{FeX}/\nu\text{XO}$ point along the backbonding line established with model FeTPP-Y adducts with CO, and also with NO, provided that the H-bond is directed toward O_{NO}. A positive correlation is seen if the H-bond is directed toward N_{NO} (see text for references).

Figure 8. Ambidentate H-bonding by Fe(II)NO. Stable structures are obtained via DFT when a H-bond donor (HCl) is directed at either O_{NO} or N_{NO} , of NO bound to imidazole-ligated FeP [5].

Figure 9.

DFT modeling [5] confirms that Fe-N and N-O bond distances and frequencies correlate negatively or positively when H-bond donors of varying strength are directed, respectively, at O_{NO} or N_{NO} of $(\text{ImH})\text{FeP}(\text{NO})$.

Figure 10.
Suggested mechanism for the facile reduction of Mb(NO) to Mb(HNO) [5].

Figure 11.

RR data on O_2 adducts for a number of imidazole-ligated heme proteins are suggestive of ambidentate distal H-bonding, showing behavior similar to NO adducts (Figure 7). For the Ctb truncated Hb [54,57], a positive correlation with the strength of distal H-bonds is revealed by site mutants, consistent with H-bonding to O^{P} .

Figure 12.

DFT modeling establishes that Fe-O and O-O bond distances correlate negatively or positively when H-bond donors of varying strength are directed, respectively, at O^t or O^p of $(\text{ImH})\text{FeP}(\text{O}_2)$.

Table 1

Selected parameters for MbXO crystal structures.

Structure	FeXO (deg)	(His64)N ^{'''} X (Å)	(His64)N ^{'''} O (Å)	Reference
MbCO _{sw} ^a	171	3.59	3.21	[29]
MbNO _{hh} ^b	147	2.98	3.18	[33]
MbNO _{hh} ^c	144	3.05	3.34	[35]
MbNO _{hh} ^d	121	2.72	3.33	[35]
MbNO _{sw} ^e	112	2.78	3.38	[34]
MbOO _{sw} ^f	122	3.08	2.97	[64]

pdb #:

^a1BZR (at 1.15 Å);^b1NPF (at 1.90 Å);^c2FRJ (at 1.30 Å);^d2FRK (at 1.30 Å);^e1HJT (at 1.70 Å);^f1A6M (at 1.00 Å).