CHAPTER 9

SIX-COORDINATE HEME NITROSYLS: TEMPERATURE DEPENDENCE OF BONDING PARAMETERS AND LIGAND ORIENTATION

9.1 Introduction

In many of the physiological processes involving NO, the coordination of NO to a heme protein and the labilization of the ligand trans to NO or another rearrangement is the significant signaling event. Proposed mechanisms for the heme proteins cytochrome c′ and sGC have presented a scenario in which the coordination to heme changes during the physiological cycle. Understanding how these coordination events impact already coordinated ligands (and vice versa) is an important step in understanding the heme-NO interaction. At the literal center of these studies is the heme iron, whose motion along the coordinate axes is associated with reactive modes such as the $\nu_{\text{Fe-Im}}$, $\nu_{\text{Fe-NO}}/\delta_{\text{Fe-N-O}}$, and heme doming.

It is difficult to study the response that NO imparts on the Fe–N$_\text{Im}$ bond in heme protein models by studying a single 100 K crystal structure. Observation of the changes in bonding parameters as a result of temperature variation, however, has provided a range of Fe–N$_\text{Im}$ values allowing for a detailed study of peripheral substituent effects and ligand orientation effects on Fe–N$_\text{Im}$.

The multiple structures of [Fe(Porph)(NO)(1-MeIm)] complexes have also provide an opportunity to study the effects of temperature on ligand orientation and
concomitantly the effects of ligand orientation on other structural parameters. In small molecular models of the protein active site, nearly all reported structures contained multiple orientations of NO. However, in [Fe(TpXPP)(NO)(1-MeIm)], where X = F (triclinic and monoclinic forms, denoted tri and mono) and OCH₃, the NO ligand is totally ordered at 100 K. As described below, each of these structures have multiple orientations above 170 K. The factors that govern NO orientation, intermolecular interactions, will be investigated. Intermolecular interactions in the solid-state provide barriers to the rotation of NO about the Fe–N axis. These interactions are defined by a set of nonbonded contacts that provide an energy penalty for the rotation of NO. In many heme protein systems, such as MbNO, these nonbonded contacts are dominated by the interaction of the NO oxygen with a single amino acid residue (histidine-64). In Mb, this residue has been suggested to provide stabilization for the MbO₂ adduct. [224]

Mapping of the NO rotation in the solid state would provide information about potential barriers and path of rotation. An earlier study by Schappacher et al. [225] mapped the rotation O₂ in two cytochrome P450 model compounds using a potential model developed by Shmueli and Goldberg. [226] This study investigated the apparent rotational disorder of O₂ in two [Fe(TₚivPP)(X)(O₂)] complexes, X = C₆HF₄S⁻. The mapping method of Shmueli and Goldberg was used to aid in the interpretation of temperature dependent Mössbauer by defining relative rotation barriers in the solid state. In this study the picket fence porphyrin provides all substantial nonbonded contacts (intramolecular) involved in O₂ rotation.

Similar methodology is used herein to study the rotation of NO in several [Fe(TpXPP)(NO)(1-MeIm)] complexes. These complexes do not contain the protecting “pickets” of the picket fence porphyrin; all substantial barriers to NO
rotation are intermolecular. This allows an investigation into the intermolecular interactions, who by spacial arrangement in the crystal lattice, affect the orientation of axial ligands. This technique has been used on several complexes to develop strategies to explore the orientation of ligands in the solid state.

9.2 Experimental

DFT calculations provided the optimized structures and detailed vibrational predictions for the six-coordinate porphyrins \( \text{tri-[Fe(TpFPP)(1-MeIm)(NO)]} \) and \( \text{mono-[Fe(TpFPP)(1-MeIm)(NO)]} \). DFT calculations were performed with Gaussian 03,\(^{178}\) employing the B3LYP\(^{80}\) hybrid functional. We used Ahlrich’s VTZ\(^{179}\) basis set for Fe atom and 6-31G* basis set for all other atoms.

The synthesis and analysis of all \( [\text{Fe(TpXPP})(L)(NO)] \) complexes is described in detail in Chapter 7.

9.3 Results

The crystal and molecular structures of six six-coordinate NO iron(II) porphyrinates have been obtained at multiple temperatures between 100 K and 350 K\(^{1}\) and with several crystalline specimens. \( [\text{Fe(TpFPP})(NO)(1-MeIm)] \) was found to exist as two crystalline polymorphs: a monoclinic form denoted \( \text{mono-[Fe(TpFPP)(NO)(1-MeIm)]} \) and a triclinic form denoted \( \text{tri-[Fe(TpFPP)(NO)-(1-MeIm)]} \). The \( [\text{Fe(TpOCH\textsubscript{3}PP})(NO)(1-MeIm)], \text{Fe(TpCF\textsubscript{3}PP)(NO)(1-MeIm)]}, \text{and [Fe(TpNO\textsubscript{2}PP)(NO)(1-MeIm)]} \) derivatives were also studied. Cell parameters and crystallographic data for 23 distinct and completed measurements are

\(^{1}\)During the 350 K data collection for \( \text{tri-[Fe(TpFPP)(NO)(1- MeIm)]} \) sample decay became evident. The reported structure is based on a subset of the total reflections that represent 100% completeness to a resolution of 0.90 Å.
Data sets were acquired at multiple temperatures for each of the six-coordinate NO complexes. \textit{mono}-[Fe(TpFPP)(NO)(1-MeIm)], at 150 and 293 K, contains a second orientation of NO oxygen which has been refined with thermal parameters equal to the first orientation. Although the thermal parameters of the second orientation have been refined as equal to the first orientation, there is evidence (especially in structures above 100 K) of electron density between the first and second orientations that cannot be properly modeled as two or even three atoms. \textit{tri}-[Fe(TpFPP)(NO)(1-MeIm)] contains a second orientation of NO oxygen in data acquired at 200, 224, 293, and 350 K. Data was acquired on a second triclinic crystal of [Fe(TpFPP)(NO)(1-MeIm)] which contained two twinned components related by a $180^\circ$ rotation (minor component approximately 20%). This second example of the triclinic form also contained a second orientation of NO oxygen in data acquired at 224 and 293 K. [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] contains a second orientation of NO oxygen in data acquired at 293 and 330 K. Each of the [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] structures contain a disordered para-methoxy group modeled as two positions and modest rotational disorder of the 1-methylimidazole ligand that was left unmodeled. Attempts were made to refine the 1-methylimidazole with two rigid bodies or a disorder model with isotropic thermal parameters with no improvement to the structure. [Fe(TpNO$_2$PP)(NO)(1-MeIm)] contains three NO orientations at 100 K, while [Fe(TpCF$_3$PP)(NO)(1-MeIm)] contains two NO orientations at 100 and 290 K.

Populations for each of the NO oxygen orientations were established during the structure refinement routine using \textsc{xl}. The populations for \textit{tri}-[Fe(TpFPP)-(NO)(1-MeIm)] at 200, 224, 293, and 350 K (along with the second structural
determinations at 224 and 293 K) were analyzed to determine van’t Hoff parameters. The data was fit to an exponential with a correlation coefficient > 0.99. The populations of NO oxygen for mono-[Fe(TpFPP)(NO)(1-MeIm)] at 150 and 293 K and [Fe(TpOCH₃PP)(NO)(1-MeIm)] at 293 and 330 K were analyzed to estimate van’t Hoff parameters. Van’t Hoff parameters of [Fe(TPP)(NO)(1-MeIm)] were not estimated due to complicated NO orientation pattern.

Temperature-dependent infrared spectra were taken between 105 K and 350 K for [Fe(TpOCH₃PP)(NO)(1-MeIm)], tri-[Fe(TpFPP)(NO)(1-MeIm)], mono-[Fe-(TpFPP)(NO)(1-MeIm)], [Fe(TpCF₃PP)(NO)(1-MeIm)]. Experiments were performed in both ascending and descending temperature order with no indication of hysteresis.

\[ R = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}} \]

\(^2\)A linear correlation coefficient, Pearson’s R, was used to judge the fit to the data.
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**a** P1. **b** Z = 2. **c** second crystal with a twinned component. **d** P2₁/n. **e** Z = 4. **f** second crystal. **g** P2₁2₁2₁.
9.4 Discussion

9.4.1 \([\text{Fe(TpFPP)(1-MeIm)(NO)}]\) Polymorphs: The Vibrational Distinction

The investigation of multiple temperature-dependent crystal structure determinations was facilitated by the initial isolation of two crystalline polymorphs of \([\text{Fe(TpFPP)(1-MeIm)(NO)}]\) that display differing room temperature solid-state values of \(\nu_{\text{N-O}}\) (1631 cm\(^{-1}\) and 1640 cm\(^{-1}\)). Additional vibrational data for the polymorphic forms is available from NRVS. Spectra were obtained on powder samples of the two species and are displayed in Figure 9.1.

The powder data clearly shows differences in the overall iron vibrational modes. Oriented single-crystal NRVS data has also been obtained on both species that provides detailed information on the character of the modes, which allows for a detailed examination of the differences between the two. An analysis of their molecular structures and vibrational data yields a detailed view of how different molecular structure features affect the dynamics of the iron atom.

The crystalline polymorphs are in the triclinic and monoclinic crystal systems; both are isolated from the same crystallization experiments. Figure 9.2 illustrates the two molecular structures. The FeNO and imidazole planes are within 1\(^\circ\) of coplanarity (monoclinic form) and within 25\(^\circ\) (triclinic form). The relative orientation of the imidazole N–CH\(_3\) bond and the bent FeNO group are of the opposite sense in the two species; the monoclinic form has a “cisoid” arrangement and the triclinic a “transoid” one. The two NO ligands make angles of 38.5\(^\circ\) and 43.2\(^\circ\) with the closest Fe–N\(_p\) vector, so that when the four porphyrin nitrogen atoms are superimposed, the NO ligands are almost superimposed (see Figure 9.3). Major differences include relative rotations of the two imidazole ligands, the relative sense of NO and imidazole directions, small differences in the trans Fe–N\(_{\text{Im}}\)
bond distance, core conformations, and positions of the peripheral $p$-fluorophenyl groups. The changing peripheral group directions reflect and contribute to the differing crystal packing of the two complexes.

What is the basis for the differing vibrational characteristics of the two polymorphic forms? Although the differing relative orientations of the bent NO group with respect to the imidazole is the most obvious difference in the two structures, it plays at most a minor role in vibrational variation (see below); other structural distinctions are dominant. First, variations in $\nu_{N-O}$ are considered. At 293 K, the triclinic crystalline form has a $\nu_{N-O}$ 9 cm$^{-1}$ greater than the monoclinic form. Temperature-dependent IR has demonstrated that the frequency of $\nu_{N-O}$ decreases for each compound as the temperature is lowered. At 107 K, the frequency difference between the two is 11 cm$^{-1}$. At 100 K, the Fe–N$_{Im}$ distance in the triclinic form is 0.038 Å longer than in the monoclinic form. Structures for both forms have also been obtained at 293 K. The Fe–N$_{Im}$ bond distances in both increase by approximately 0.03 Å, and the difference between the two polymorphs
Figure 9.2. Thermal ellipsoid plot (50% probability) of
$\textit{tri}$-[Fe(TpFPP)(NO)(1-MeIm)] (left) and $\textit{mono}$-[Fe(TpFPP)(NO)(1-MeIm)] (right). Metrical information for $\textit{tri}$-[Fe(TpFPP)(NO)(1-MeIm)] and $\textit{mono}$-[Fe(TpFPP)(NO)(1-MeIm)] [in square brackets]. $\nu_{N-O} = 1641\,[1632]\,\text{cm}^{-1}$, Fe–N$_{\text{NO}}$ = 1.7521(9)
[1.7481(11)] $\text{Å}$, Fe–N$_{\text{Im}}$ = 2.1689(9) [2.1312(11)] $\text{Å}$, $<\text{Fe–N}_p>$ = 2.011(8)
[2.002(14)] $\text{Å}$, $<\text{Fe–N}_p\,\text{(adj)}>$ = 2.004(3) [1.992(4)] $\text{Å}$, $<\text{Fe–N}_p\,\text{(opp)}>$
= 2.018(3) [2.012(12)] $\text{Å}$, N–O = 1.1819(12) [1.1808(14)] $\text{Å}$. Fe–N$_{\text{NO}}$ tilt
= 5.1[7.2]$^\circ$, $\angle\text{Fe–N–O} = 138.64(8)\,[137.27(9)]^\circ$, N$_{\text{Im}}$–Fe–N$_{\text{NO}}$ = 176.08(4)
[175.08(4)]$^\circ$, N–Fe–N–O (dihedral) = 43.2 [38.5]$^\circ$.

Figure 9.3. Diagram showing overlap of molecules of the two
polymorphs. The two molecules are centered on the four porphyrin
nitrogen atoms. The triclinic species is drawn with thermal ellipsoids
contoured at the 50% probability level as solid surfaces, whereas atoms
of the monoclinic form are drawn with translucent spheres of arbitrary
radius. Atoms of the NO and the porphyrin core are seen to be close to
overlap, whereas the imidiazole and peripheral $p$-fluorophenyl groups are
not.
remains nearly constant at 0.033 Å. The change in the Fe–N_{Im} distance as a function of temperature is an unusual structural change in the absence of significant differences in the electronic structure (such as a spin-state transition). This is consistent with observed and calculated softness of this bond. However, it has been previously observed that changes in the Fe–N_{Im} bond trans to NO lead to variations in the observed value of ν_{N–O}.\[142\] Thus the shifts in ν_{N–O} are wholly consistent with the temperature dependent changes in the Fe–N_{Im} distances. Will the effects of trans Fe–N_{Im} distance differences also be propagated in the iron out-of-plane (OOP) modes?

DFT calculations were carried out to further explore the effect of ligand orientation and the character of the vibrational modes. Calculations carried out on the two polymorphic structures of [Fe(TpFPP)(NO)(1-MeIm)], where initial molecular configurations are based on X-ray crystal structures, and which resulted in energy-minimized structures that retained their initial cisoid and transoid configurations. However, other features of the structures became more similar such as the axial and equatorial bond distances. Variation in equatorial bonds with a tilted Fe–N_{NO} vector are also features of both calculations. The predicted vibrational spectra for the cisoid and transoid species, displayed in Figure 9.4, show no distinct differences.

NRVS data obtained from single crystals oriented to enhance (and identify) the in-plane and out-of-plane modes are shown in Figure 9.5. We observe particularly striking vibrational differences between the two polymorphs in the low frequency region for iron motion perpendicular to the porphyrin plane. Modes having frequencies (\(e_{Fe}^2\) values) of 33 cm\(^{-1}\) (0.06), 140 cm\(^{-1}\) (0.12), and 167 cm\(^{-1}\) (0.11) for \(tr\)-[Fe(TpFPP)(NO)(1-MeIm)] shift to 40 cm\(^{-1}\) (0.045), 153 cm\(^{-1}\) (0.07), and 177
Figure 9.4. Comparison of the calculated VDOS for powder samples of *mono-* and *tri-* [Fe(TpFPP)(1-MeIm)(NO)].

cmp^{-1} (0.11) in *mono-* [Fe(TpFPP)(NO)(1-MeIm)]. DFT calculations suggest that the highest two modes involve iron out-of-plane motion along with rotational and translational motion of the imidazole ligand and have 64% and 44% of the mode kinetic energy (KE) localized on the Fe(1-MeIm)(NO) fragment, but have little resemblance to a two-body Fe–Im oscillator. Figure 9.6 illustrates the character of the predicted modes, with frequencies ($e_{Fe}^2$ values) of 146 cm$^{-1}$ (0.10) and 165 cm$^{-1}$ (0.06). The significant frequency increases for low frequency modes of the Fe(1-MeIm)(NO) fragment in the cisoid species appear to reflect the 0.04 Å decrease in Fe–N$_{Im}$. However, the bands associated with the $\nu_{Fe-NO} = 432$ cm$^{-1}$ ($e_{Fe}^2 = 0.11$, cisoid) and 433 cm$^{-1}$ ($e_{Fe}^2 = 0.13$, transoid) and with $\nu_{Fe-NO}/\delta_{Fe-N-O} = 559$ cm$^{-1}$ (0.05) and 560 cm$^{-1}$ (0.06) show very small frequency differences consistent with the much smaller (∼0.004 Å) change in the Fe–N$_{NO}$ bond distance.

3 Additional changes appear in the 200-220 cm$^{-1}$ region, but are more difficult to interpret because of overlapping contributions from modes with Fe displacement parallel and perpendicular to the porphyrin plane. The most prominent out-of-plane mode predicted in this region has some FeIm stretching character, but only 16% of the mode KE is localized on the Fe(1-MeIm)(NO) fragment and may be less sensitive to the Fe coordination sphere.
Figure 9.5. Comparison of the measured VDOS for mono- and tri-[Fe(TpFPP)(NO)(1-MeIm)] with crystals oriented with porphyrin planes aligned either perpendicular to or parallel to the excitation beam.
between these derivatives. Nonetheless, the Fe–N$_{\text{Im}}$ bond distance variation must change the electron density at iron, which in turn modulates the electron density that iron donates to the NO ligand, without significant effects on the stiffness of the Fe–N$_{\text{NO}}$ bond.

Earlier work showed the extreme sensitivity of in-plane modes to peripheral substituents.\textsuperscript{[76]} The two polymorphs also provide a unique opportunity to explore the systematics of in-plane mode variation. The calculated character of the four prominent modes are shown in Figure 9.7. The KE of the 350 cm$^{-1}$ mode is predominantly located on iron ($e^2_{Fe} \sim 0.40$), while the modes near 200 and 460 cm$^{-1}$ are predominately located on the porphyrin ($e^2_{Fe} \sim 0.06$). DFT calculations suggest that the direction of iron motion in the 350 cm$^{-1}$ mode is perpendicular to the FeNO plane, not along the Fe–N$_p$ directions. This is distinctly different from that of the analogous carbonyl adducts where the iron motion is governed by the porphyrin Fe–N$_p$ bonds.\textsuperscript{[78]} These two systems suggest that the orientation of
imidazole has little effect on the in-plane iron motion. We can thus conclude that the cisoid and transoid configurations are vibrationally indistinguishable since the dihedral angle between the FeNO plane and the closest N\textsubscript{p}–Fe–N\textsubscript{p} plane are almost identical. The feature that does lead to the higher frequencies in the monoclinic phase is the systematically shorter Fe–N\textsubscript{p} bonds (all approximately 0.01 Å, despite the equatorial asymmetry).

The [Fe(TpFPP)(1-MeIm)(NO)] data presented gives further evidence of the extremely soft nature of the Fe–N\textsubscript{Im} bond; changes in the length of this bond are correlated with variation in \(\nu_{N-O}\). Studies on the [Fe(TpFPP)(1-MeIm)(NO)] polymorphs initiated a broader investigation into the temperature-dependent effects on structural and vibrational parameters of [Fe(TpXPP)(NO)(1-MeIm)] complexes.

9.4.2 Temperature-Dependent Structural Features of [Fe(TpXPP)(NO)(1-MeIm)]

A “fortuitous” failure of the crystal cooling system led to the initial collection of a data set for \textit{tri}-[Fe(TpFPP)(NO)(1-MeIm)] at room temperature. The structure revealed two orientations of the NO ligand; a disordered NO is common with many prior examples.\cite{64, 66,142} Upon repair of the cryocooler, the structure was re-determined at 100 K. The same crystalline sample now showed a \textit{single} orientation of the NO ligand at the lower temperature. This suggests that the second orientation of NO in \textit{tri}-[Fe(TpFPP)(NO)(1-MeIm)] is a temperature-dependent phenomenon. Accordingly, we collected a number of additional data sets at different temperatures and on two crystal samples for \textit{tri}-[Fe(TpFPP)(NO)(1-MeIm)]. ORTEP diagrams of \textit{tri}-[Fe(TpFPP)(NO)(1-MeIm)], at all temperatures, are given in Figure 9.8 and Figure 9.9. A total of ten distinct data sets were collected; the order of
Figure 9.7. Predicted vibrational modes for the in-plane motion of the FeNO group. The imidazole group is omitted for clarity. The experimentally observed values that correspond to the calculated values in parentheses for mono-[Fe(TpFPP)(1-MeIm)(NO)] are 219 (219), 343 (339), and 456/471 (463/466) cm$^{-1}$ and for tri-[Fe(TpFPP)(1-MeIm)(NO)] are 218 (219), 338 (339), and 457 (463/466) cm$^{-1}$. 
data collections are indicated in Table 9.2. The graphical depiction in Figure 9.12 shows experiment order, experiment temperature, NO orientations and the population of the major NO orientation. Several generalizations are apparent from the figure for tri-[Fe(TpFPP)(NO)(1-MeIm)]. First, at low temperature the NO ligand is ordered for two different crystal samples. Second, similar populations of the NO orientation are observed at the same temperature. Third, the population of the second orientation increases with increasing temperature. Fourth, the order/disorder of NO is a thermally driven, reversible process. Finally, the results appear to be sample independent, although in this experiment and the others following have been necessarily limited to two samples each.

A smaller set of temperature-dependent structure determinations were subsequently made for mono-[Fe(TpFPP)(NO)(1-MeIm)] and [Fe(TpOCH<sub>3</sub>PP)(NO)(1-MeIm)]. ORTEP diagrams of mono-[Fe(TpFPP)(NO)(1-MeIm)], at all temperatures, are given in Figure 9.10, while ORTEP diagrams of [Fe(TpOCH<sub>3</sub>PP)(NO)-(1-MeIm)] are given in Figure 9.11. Temperature-dependent NO orientations were also observed. The experimental order is given in Table 9.2 and depicted graphically in Figure 9.13 and Figure 9.14. Both representations show the apparent reversibility of the NO orientation. At room temperature, two orientations of NO were found in both complexes. However, at lower temperatures, each of the two samples contains only a single orientation of the NO ligand.

Finally, three additional structures, [Fe(TpCF<sub>3</sub>PP)(NO)(1-MeIm)], [Fe(TpNO<sub>2</sub>-PP)(NO)(1-MeIm)], and [Fe(TPP)(NO)(1-MeIm)], were determined at one or two temperatures for comparison with the previous data sets.

These studies allow for the observation of possible temperature-dependent variations or trends in the coordination group geometry of {FeNO}<sup>7</sup> porphyrinates.
Figure 9.8. ORTEP diagrams (50% probability ellipsoids) of $tri$-[Fe(TpFPP)(NO)(1-MeIm)] at 100, 150, 175, and 200 K. Hydrogen atoms are omitted for clarity.
Figure 9.9. ORTEP diagrams (50% probability ellipsoids) of $trr$-[Fe(TpFPP)(NO)(1-MeIm)] at 224, 293, and 350 K. Hydrogen atoms are omitted for clarity.
Figure 9.10. ORTEP diagrams (50% probability ellipsoids) of \( \text{mono-[Fe(TpFPP)(NO)(1-MeIm)]} \) at 100, 125, 150, and 293 K. Hydrogen atoms are omitted for clarity.
Figure 9.11. ORTEP diagrams (50% probability ellipsoids) of [Fe(TpOCH₃PP)(NO)(1-MeIm)] at 100, 293, and 330 K. Hydrogen atoms are omitted for clarity.

Figure 9.12. Diagram displaying the order of data acquisition for tri-[Fe(TpFPP)(NO)(1-MeIm)]. The temperature of data acquisition and occupancy factors for the major orientations are also displayed.
Figure 9.13. Diagram displaying the order of data acquisition for *mono*-[Fe(TpFPP)(NO)(1-MeIm)]. The temperature of data acquisition and occupancy factors for the major orientations are also displayed.

Figure 9.14. Diagram displaying the order of data acquisition for [Fe(TpOCH₃PP)(NO)(1-MeIm)]. The temperature of data acquisition and occupancy factors for the major orientations are also displayed.
Bond lengths and other geometrical parameters for the coordination group are given in Table 9.2. Most of the metrical parameters show little or no temperature dependence. Although a second orientation of the NO becomes evident at higher temperatures, the absolute and relative orientations of the imidazole and the major NO orientation do not show any temperature dependence. The Fe–N\text{Im} distance and the Fe–N\text{NO} tilt angle show the largest temperature-dependent effect.

Tilting of the Fe–N\text{NO} vector, as described above, is an inherent characteristic of \{FeNO\}^7 porphyrinates. The Fe–N\text{NO} tilt shows an apparent temperature dependence; the tilt is smaller with increased temperature. However, the true degree of temperature variation is difficult to measure due to uncertainties associated with the NO nitrogen position. The two orientations of the NO ligand lead to two positions of the nitrogen that should lead to a small change in the apparent refined position which is the average of the population-weighted positions. This, along with possible effects of increased thermal motion, lead to apparent shifts in the nitrogen position and concomitant decreases in the tilt angle that overstate the temperature-dependent change.
TABLE 9.2

SELECTED STRUCTURAL FEATURES AND ν N–O FOR

[Fe(TpXPP)(NO)(L)] COMPLEXES

<table>
<thead>
<tr>
<th>Complex</th>
<th>Temp., a</th>
<th>Fe–N N O b</th>
<th>Fe–N Im b</th>
<th>Fe–N p b</th>
<th>Fe–N p long</th>
<th>Fe–N NO, ν N–O e</th>
<th>rotation, change f</th>
<th>% maj. orien.</th>
</tr>
</thead>
<tbody>
<tr>
<td>tri-[Fe(TpFPP)- (NO)(1-MeIm)] g</td>
<td>100 [4]</td>
<td>1.7521(9)</td>
<td>2.1689(12)</td>
<td>2.004(3)</td>
<td>2.018(3)</td>
<td>5.1, 3.3</td>
<td>1635.74</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>150 [7]</td>
<td>1.7480(12)</td>
<td>2.1725(12)</td>
<td>2.0021(12)</td>
<td>2.015(5)</td>
<td>4.6, 2.8</td>
<td>1636.73</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>200 [9]</td>
<td>1.7477(13)</td>
<td>2.1774(13)</td>
<td>2.002(2)</td>
<td>2.016(4)</td>
<td>4.6, 3.0</td>
<td>1637.72</td>
<td>207, 27</td>
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<tr>
<td></td>
<td>224 [6]</td>
<td>1.7473(12)</td>
<td>2.1814(12)</td>
<td>2.0030(6)</td>
<td>2.012(2)</td>
<td>4.3, 2.8</td>
<td>1638.20</td>
<td>207, 28</td>
</tr>
<tr>
<td></td>
<td>224 [2]</td>
<td>1.7579(14)</td>
<td>2.1880(14)</td>
<td>2.0038(4)</td>
<td>2.015(2)</td>
<td>3.9, 5.5</td>
<td>1638.20</td>
<td>207, 28</td>
</tr>
<tr>
<td></td>
<td>293 [5]</td>
<td>1.7427(14)</td>
<td>2.1906(14)</td>
<td>2.004(2)</td>
<td>2.012(3)</td>
<td>3.4, 2.5</td>
<td>1639.56</td>
<td>209, 30</td>
</tr>
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<td></td>
<td>350 [10]</td>
<td>1.724(3)</td>
<td>2.193(3)</td>
<td>2.004(3)</td>
<td>2.007(3)</td>
<td>3.2, 2.7</td>
<td>1640.69</td>
<td>210, 30</td>
</tr>
<tr>
<td>mono-[Fe(TpFPP)- (NO)(1-MeIm)] i</td>
<td>100 [1]</td>
<td>1.7481(11)</td>
<td>2.1312(11)</td>
<td>1.992(4)</td>
<td>2.011(11)</td>
<td>7.2, 2.6</td>
<td>1624.39</td>
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<td>125 [4]</td>
<td>1.748(3)</td>
<td>2.128(3)</td>
<td>1.99(2)</td>
<td>2.014(11)</td>
<td>6.1, 2.5</td>
<td>1625.18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>150 [3]</td>
<td>1.7558(15)</td>
<td>2.1325(14)</td>
<td>1.991(6)</td>
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<td>6.3, 1.9</td>
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<td>293 [2]</td>
<td>1.753(3)</td>
<td>2.158(2)</td>
<td>1.995(1)</td>
<td>2.011(13)</td>
<td>5.4, 1.7</td>
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<td>1, 79</td>
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<tr>
<td>[Fe(TpOCH3PP)- (NO)(1-MeIm)] k</td>
<td>100 [1]</td>
<td>1.7486(8)</td>
<td>2.1659(8)</td>
<td>1.993(11)</td>
<td>2.014(4)</td>
<td>4.9, 5.7</td>
<td>1616.14</td>
<td>203</td>
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<td></td>
<td>100 [4]</td>
<td>1.7532(10)</td>
<td>2.1690(11)</td>
<td>2.000(11)</td>
<td>2.020(3)</td>
<td>5.0, 6.0</td>
<td>1616.14</td>
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<tr>
<td></td>
<td>293 [2]</td>
<td>1.7464(12)</td>
<td>2.1902(12)</td>
<td>2.000(8)</td>
<td>2.015(5)</td>
<td>4.6, 6.0</td>
<td>1619.53</td>
<td>201, 286</td>
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<td></td>
<td>330 [3]</td>
<td>1.7438(14)</td>
<td>2.2030(14)</td>
<td>1.999(7)</td>
<td>2.016(4)</td>
<td>4.1, 5.7</td>
<td>1620.18</td>
<td>202, 287</td>
</tr>
<tr>
<td>[Fe(TpCF3PP)- (NO)(1-MeIm)] j</td>
<td>100 [1]</td>
<td>1.7530(15)</td>
<td>2.1464(14)</td>
<td>2.001(7)</td>
<td>2.017(7)</td>
<td>3.4, 0.9</td>
<td>1631.57</td>
<td>183, 9</td>
</tr>
<tr>
<td></td>
<td>290 [2]</td>
<td>1.7499(17)</td>
<td>2.1943(15)</td>
<td>2.008(2)</td>
<td>2.013(2)</td>
<td>1.2, 1.6</td>
<td>1636.84</td>
<td>183, 6</td>
</tr>
<tr>
<td>[Fe(TpNO2PP)- (NO)(1-MeIm)]</td>
<td>100 [1]</td>
<td>1.755(2)</td>
<td>2.164(3)</td>
<td>2.000(7)</td>
<td>2.012(2)</td>
<td>3.6, 0.5</td>
<td>1641</td>
<td>161, 279, 339</td>
</tr>
<tr>
<td>[Fe(TPP)- (NO)(1-MeIm)]</td>
<td>100 [1]</td>
<td>1.7491(10)</td>
<td>2.1698(10)</td>
<td>1.9973(11)</td>
<td>2.0166(3)</td>
<td>1.9, 3.7</td>
<td>1631.74</td>
<td>336, 249</td>
</tr>
<tr>
<td></td>
<td>293 [1]</td>
<td>1.745(2)</td>
<td>2.186(2)</td>
<td>2.0019(3)</td>
<td>2.0105(5)</td>
<td>1.7, 3.8</td>
<td>1629.54</td>
<td>358, 261, 194</td>
</tr>
</tbody>
</table>

a degrees K. b Å. c degrees. d uncertainties at 100 K are about 0.1 ° and are somewhat larger at higher temperatures where disordered NO is found, see text. e cm⁻¹. Frequencies for a particular temperature are interpolated from the best fit line in Figure 9.17. f ° is the 1-methyl group position of 1-MeIm, rotations are clockwise. g experiments 1–3 used a crystal with a twin component. Experiments 4–10 used a second crystal with no twinning. h experiments 2–4 used a second crystal. i experiment 2 used a second crystal. j experiment 2 used a second crystal. k IR at 293 K.
As described in Chapter 7, the tilting of the Fe–N\textsubscript{NO} vector in \{FeNO\}\textsuperscript{7} porphyrinates induces asymmetry in the Fe–N\textsubscript{p} bonds. All of the complexes presented, at every temperature, have a similar pattern of a pair of shortened Fe–N\textsubscript{p} bonds and a pair of elongated Fe–N\textsubscript{p} bonds. Average values of short and long Fe–N\textsubscript{p} for tri-[Fe(TpFPP)(NO)(1-MeIm)], mono-[Fe(TpFPP)(NO)(1-MeIm)], [Fe-(TpOCH\textsubscript{3}PP)(NO)(1-MeIm)], which contain a single ordered NO at 100 K, have been calculated and are displayed in Table 9.2. Additionally, average values of short and long Fe–N\textsubscript{p} bond distance for [Fe(TpCF\textsubscript{3}PP)(NO)(1-MeIm)], [Fe-(TpNO\textsubscript{2}PP)(NO)(1-MeIm)], and [Fe(TPP)(NO)(1-MeIm)], which contain a multiple NO orientations at 100 K, have been calculated and are also displayed in Table 9.2. Although variation of the Fe–N\textsubscript{p} bond lengths attributed to multiple orientations should be observed, the measured differences are smaller than the uncertainties; bond asymmetry is observed in all cases.

The strong trans effect of NO causes the rupture of the Fe–His bond in a number of protein systems to give five-coordinate \{FeNO\}\textsuperscript{7} systems,[15–17] while in others, upon NO binding the heme remains six-coordinate \{FeNO\}\textsuperscript{7}.[16, 18] Clearly understanding the nature of this bond and its effects are essential to understanding heme \{FeNO\}\textsuperscript{7} NO-sensing systems. The activation “switch” in sGC is an apparent rupture of the Fe–His bond.[62] Structural changes induced by the elongation and eventual scission of this bond are believed to be essential in signal transduction, ultimately leading to vasodilatation.[15, 19]

The known trans directing effect of NO on the Fe–N\textsubscript{Im} bond suggests that the bond might be described as “soft” and be especially susceptible to temperature-dependent environmental effects.\textsuperscript{4} This is indeed the case. The length of the biologically important Fe–N\textsubscript{Im} bond in each of the six-coordinate \{FeNO\}\textsuperscript{7} porphyrinates

\textsuperscript{4}The binding constants for the addition of an imidazole to a five-coordinate nitrosyl are known to be small and are summarized in Reference [65]. Neither the trans effect nor the binding constant absolutely predict that the Fe–N\textsubscript{Im} bond will show a strong temperature dependence.
Figure 9.15. Plot of cell volume per molecule (Å³) or Fe–N_{Im} bond distance (Å) vs temperature (K). Data illustrated are: (red circles) \textit{tri-}[Fe(TpFPP)(NO)(1-MeIm)], (blue squares) \textit{mono-}[Fe(TpFPP)(NO)(1-MeIm)], and (green diamonds) \textit{[Fe(TpOCH_{3}PP)(NO)(1-MeIm)]}. Data points in each of the plots are fit linearly. Correlation coefficients for the top[bottom] plots are: \textit{tri-}[Fe(TpFPP)(NO)(1-MeIm)] 0.99[0.96], \textit{mono-}[Fe(TpFPP)(NO)(1-MeIm)] 0.99[0.97], and \textit{[Fe(TpOCH_{3}PP)(NO)(1-MeIm)]} 0.97[0.99].
nates shows a linear dependence on temperature. Plotted in the lower panel of Figure 9.15 is the temperature dependence of the Fe–N_{Im} bond for *tri*-[Fe(TpFPP)-(NO)(1-MeIm)], *mono*-[Fe(TpFPP)(NO)(1-MeIm)], and [Fe(TpOCH_{3}PP)(NO)(1-MeIm)]. The change in cell volume as a function of temperature is shown in the top panel of Figure 9.15 (complexes with < 3 data sets are not illustrated); the correlation between changes in cell volume and the Fe–N_{Im} bond length is evident. The change in cell volumes for the three complexes between 100 and 293 K is about 3.6 to 3.8%. The changes in cell volume are not unusual for porphyrin complexes in our experience, although perhaps at the upper end of ranges observed.\(^5\) The change of the Fe–N_{Im} bond distance in [Fe(TpOCH_{3}PP)(NO)-(1-MeIm)] (2.1659(8)–2.2030(14) Å, 100–330 K), *tri*-[Fe(TpFPP)(NO)(1-MeIm)] (2.1669(12)–2.1934(28) Å, 100–350 K), *mono*-[Fe(TpFPP)(NO)(1-MeIm)] (2.1312(11)–2.158(2) Å, 100–293 K), [Fe(TpCF_{3}PP)(NO)(1-MeIm)] (2.1464(14)–2.1694(15) Å, 100–290 K), and [Fe(TPP)(NO)(1-MeIm)] (2.1698(10)–2.186(2) Å, 100–293 K) is small but unidirectional.

The effects of the temperature-dependent crystal packing on the Fe–N_{Im} bond is best seen in *tri*-[Fe(TpFPP)(NO)(1-MeIm)]. Figure 9.16 displays the relative shift of adjacent molecules in the unit cell for *tri*-[Fe(TpFPP)(NO)(1-MeIm)] at 100 K. In this figure, coordinates of the porphyrin nitrogen atoms of the 100 and 350 K structures have been overlaid for the two molecules shown at the bottom right. The relative differences in the intermolecular spacing of \(~0.5\) Å can now be seen between the 100 K structure (shown in blue) and the 350 K structure (shown in red). The contraction of the cell and the tighter intermolecular contacts in the 100 K structure can be clearly seen. This cell contraction is manifested in a

\(^{5}\text{For example, a six-coordinate CO complex, [Fe(TPP)(CO)(1-MeIm)], showed a 3.2% change in cell volume.}\)
shortening of Fe–N$_{\text{Im}}$ by about 0.02 Å. Clearly the weakness of the Fe–N$_{\text{Im}}$ bond has contributed to the temperature dependence of the Fe–N$_{\text{Im}}$ bond distance. The red vector shown in Figure 9.16 has approximately equal components in the $a$ and $b$ directions that is also seen in the relative changes in the cell constants. Although the directions are not as clear in [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] and mono-[Fe-(TpFPP)(NO)(1-MeIm)], they similarly display a shortening of the Fe–N$_{\text{Im}}$ bond due to contraction of the unit cell and decreased intermolecular interactions with reduced temperature.

9.4.3 Variable-Temperature Infrared Studies.

Temperature-dependent infrared data were collected for [Fe(TpOCH$_3$PP)(NO)-(1-MeIm)], tri-[Fe(TpFPP)(NO)(1-MeIm)], [Fe(TpCF$_3$PP)(NO)(1-MeIm)], and mono-[Fe(TpFPP)(NO)(1-MeIm)] at temperatures ranging from 107 to 348 K. In-
Figure 9.17. Plot of $\nu_{\text{N-O}}$ (cm$^{-1}$) vs temperature (K). Data illustrated are: tri-$[\text{Fe(TpFPP})(\text{NO})(1\text{-MeIm})]$ (red circles), [Fe(TpCF$_3$PP)(NO)(1-MeIm)] (grey triangles), mono-$[\text{Fe(TpFPP})(\text{NO})(1\text{-MeIm})]$ (blue squares) and [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] (green diamonds). Each set of data points is fit linearly with correlation coefficients of $R > 0.99$. 
Infrared measurements were made to determine if the distinct NO orientations observed in the X-ray diffraction experiments would be vibrationally observable. In all four complexes, a single $\nu_{\text{N} - \text{O}}$ band was observed with no discernible broadening that might suggest a second $\nu_{\text{N} - \text{O}}$ peak. The most significant change observed was an increasing value of $\nu_{\text{N} - \text{O}}$ with increasing temperature. The temperature dependence (20 degree intervals) of $\nu_{\text{N} - \text{O}}$ versus T is given in Figure 9.17. In all cases the change in $\nu_{\text{N} - \text{O}}$ versus temperature demonstrates a linear dependence with high correlation.

What effect leads to the T-dependence? The theoretical study above, of the two polymorphic forms of $[\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]$, suggests equivalent energy-minimized structures and vibrational data despite the differences in the relative orientation of the NO and imidazole planes (i.e., cisoid and transoid).[223] Thus, relative differing orientations appear not to have an effect on $\nu_{\text{N} - \text{O}}$. Rather, we believe that it is the temperature-dependent changes in the Fe–N$_{\text{Im}}$ bond length, not any environmental differences of the NO ligand, that causes the variation of $\nu_{\text{N} - \text{O}}$ with temperature. These values are strongly correlated for each complex. Figure 9.18 displays a plot of the Fe–N$_{\text{Im}}$ bond length versus the N–O stretching frequency for $\text{tri-[Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]$ (red circles), $\text{mono-[Fe(TpFPP)}(\text{NO})-(1-\text{MeIm})]$ (blue squares), and $[\text{Fe(TpOCH$_3$PP)}(\text{NO})(1-\text{MeIm})]$ (green diamonds). The two polymorphic forms of $[\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]$ fall on the same line (best fit line in black) and demonstrate that the correlation is independent of crystalline form. Plots of Fe–N$_{\text{Im}}$ versus $\nu_{\text{N} - \text{O}}$ for $[\text{Fe(TpOCH$_3$PP)}(\text{NO})(1-\text{MeIm})]$ (green diamonds), however, do not fall onto the same line as $\text{tri-[Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]$ and $\text{mono-[Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]$. These differences can be attributed to the cis effect of different para-substituted porphyrinates.[136] The
Figure 9.18. Plot of $\nu_{N-O}$ (cm$^{-1}$) vs Fe–N$_{Im}$ bond distance (Å). Data illustrated are triclinic (red circles) and monoclinic (blue squares) forms of [Fe(TpFPP)(NO)(1-MeIm)], [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] (green diamonds), [Fe(TpCF$_3$PP)(NO)(1-MeIm)] (yellow square), and [Fe(TPP)(NO)(1-MeIm)]$^{66}$ (grey triangle). Frequencies for a particular temperature are interpolated from the best fit line in Figure 9.17. Data points are fit with a correlation coefficient of $R = 0.98$ for [Fe(TpFPP)(NO)(1-MeIm)] (black line) and $R = 0.98$ for [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] (green line).
electron-donating character of the methoxy-substituent in \([\text{Fe(TpOCH}_3\text{PP})(\text{NO})-(1-\text{MeIm})]\) increases the electron density on iron thereby pushing more electron density into the SOMO and increasing the \(\pi^*\) antibonding character of NO. This is seen experimentally in the lower \(\nu_{N-O}\) frequencies observed for \([\text{Fe(TpOCH}_3\text{PP})(\text{NO})-(1-\text{MeIm})]\). The electron-withdrawing fluoro-substituted analog reduces electron density at the iron center thereby causing the higher observed value for \(\nu_{N-O}\) in the \([\text{Fe(TpFPP})(\text{NO})(1-\text{MeIm})]\) derivatives.

Although a complete temperature profile for \([\text{Fe(TPP)}(1-\text{MeIm})(\text{NO})]\) and \([\text{Fe(TpCF}_3\text{PP})(\text{NO})(1-\text{MeIm})]\) are not available, the room temperature value for \([\text{Fe(TPP)}(1-\text{MeIm})(\text{NO})]\) (grey triangle) is in fact at an intermediate value \((\nu_{N-O} = 1625 \text{ cm}^{-1})\[^{[66]}\] and the \([\text{Fe(TpCF}_3\text{PP})(\text{NO})(1-\text{MeIm})]\) (yellow squares) value is close to that of \(\text{para}\)-fluoro substituted porphyrinate \((\nu_{N-O} \approx 1631–1636 \text{ cm}^{-1})\), both consistent with their expected cis effects.

Additionally, the effect of varying the length of the Fe–N\(_{\text{Im}}\) bond is different for the two porphyrinates. As observed in Figure\[^{[9.18]}\] the slope of \([\text{Fe(TpFPP})(\text{NO})-(1-\text{MeIm})]\) is more than \(2\times\) larger than in \([\text{Fe(TpOCH}_3\text{PP})(\text{NO})(1-\text{MeIm})]\); the effect of changing the Fe–N\(_{\text{Im}}\) bond distance on \(\nu_{N-O}\) is twice as large. This further reflects differences in the electronic characteristics imparted by the differing porphyrinates.

The trans effect of the heme axial ligands are thought to be of great utility to diatomic sensing heme-proteins.\[^{[19]}\] Correlations of bonding parameters across the porphyrin plane have been previously noted.\[^{[142, 223]}\] The interaction between the axial ligands in heme nitrosyls are made through the SOMO. The SOMO of \([\text{Fe(Porph)}(\text{NO})(1-\text{MeIm})]\) has been described as a combination of the \(\pi^*\) orbital on NO and the \(d_{z^2}\) orbital, which is an antibonding interaction with respect to
the imidazole ligand. A shortening in the Fe–N\textsubscript{Im} bond distance must lead to (small) increases in electron donation to Fe, which decreases the donation from the $\pi^*$ orbital of NO with a concomitant decrease in the $\nu\textsubscript{NO}$ value. The converse is also possible. There has been suggestions that distal pocket interactions, steric, electronic, or both, directly influence the proximal coordination and by association signal transduction.\cite{15} The observation of spin-spin coupling between the imidazole $^{14}$N and NO observable in the EPR spectrum of [Fe(Porph)(NO)(1-MeIm)] suggests at least modest interaction between the two axial ligands.\cite{220}

9.4.4 Mapping NO Movement in the Crystalline State

The crystal and molecular structure of tri-[Fe(TpFPP)(NO)(1-MeIm)] was initially investigated at 293 K. At this temperature, the NO ligand was found in two positions separated by a 180° rotation about the heme normal. Although disordered NO groups are a common feature in coordination chemistry, the large separation between the two orientations was unusual. Moreover, a subsequent structure determination at 100 K, on the same crystal specimen, displayed a completely ordered NO. A second crystal specimen was studied to validate the temperature-dependent phenomenon. The structure was acquired first at 100 K, then at 293 K. The same temperature-dependent position and atomic populations were found in the duplicate experiments. Clearly, substantial motion of the NO ligand was required for this solid-state transition.

These results suggested that the NO orientation is controlled by temperature-dependent interactions in the solid state, i.e., crystal packing effects. Crystal packing effects are often cited as the explanation of stereochemical features, however this system appears to provide an opportunity for a quantitative study of
“crystal packing.” Knowledge of the specific interactions, and the degree of interaction, would be useful to predict NO position and occupancy in the solid state. Thus, an extensive exploration into the nature of the temperature-dependent NO motion in \( \text{tri-[Fe(TpFPP)(NO)(1-MeIm)]} \) was initiated.

Figure 9.12 shows experiment order, experiment temperature, NO orientations and the population for the major NO orientation of ten crystal structure determinations. At temperatures \( \leq 175 \text{ K} \), only a single NO orientation is observed. However, at a temperature above 175 K, the onset of a second NO ligand orientation occurs. Two NO orientations, and their respective temperature-dependent populations, are reproducibly observed in multiple crystalline samples of \( \text{tri-[Fe(TpFPP)(NO)(1-MeIm)]} \). These results suggest that there is a pathway in the solid state between the two molecular orientations. Presented below is a detailed investigation of the influence of crystal packing effects in crystalline \( \text{[Fe(TpFPP)-(NO)(1-MeIm)]} \) on NO motion in the solid state.

The effect of rotating a coordinated (bent) NO ligand in solid-state \( \text{[Fe(TpXPP)-(NO)(1-MeIm)]} \) is investigated by moving the oxygen atom of the nitrosyl group to simulate the progression from the major orientation to the minor orientation. The \text{CART} \(^6\) program, was used to calculate the positions of 35 additional oxygen atom positions using the crystallographically determined oxygen position as an initial point and placing each additional oxygen atom at 10° intervals around a circle parallel to the porphyrin plane at a fixed distance from the nitrosyl nitrogen. A depiction of the 35 additional positions of nitrosyl oxygen is illustrated in Figure 9.19. The program \text{MERCURY}[227] was then used to determine the overlap of the van der Waals radius of oxygen with the van der Waals

\(^6\)Local program used to generate atom positions in a cartesian coordinate system and translate back to fractional coordinates.
Figure 9.19. Schematic displaying the rotation of nitrosyl oxygen parallel to the porphyrin plane. Rotational direction and labeling scheme are also displayed. Selected positions of oxygen (gold) are labeled.

Radii of atoms from adjacent molecules in the crystal lattice. Values of van der Waals radii are those given on the Cambridge Crystallographic Data Centre website (http://www.ccdc.cam.ac.uk/products/csd/radii/). Plots were constructed by summing all van der Waals overlaps for each of the 35 calculated positions and the crystallographically determined positions of the nitrosyl oxygen, then plotting them against their rotation clockwise from a position defined by the projection of the methyl group of the coordinated 1-methylimidazole onto the porphyrin (with the NO ligand on top). These plots, which may estimate a pseudopotential of rotation based solely on the sum of van der Waals overlaps, display interactions of the rotating nitrosyl ligand in the solid state.

The tri-[Fe(TpFPP)(NO)(1-MeIm)] system is studied extensively to understand the structural parameters that cause the observed temperature dependence of NO orientation. The barriers that prevent NO rotation are provided by in-
Figure 9.20. Sum of the van der Waals radii overlaps (Å) vs the rotation of NO oxygen from the found position (deg.) indicated by a point for tri-[Fe(TpFPP)(NO)(1-MeIm)] at 100 K. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation.
termolecular interactions; no substantial intramolecular interactions are observed. Barriers to rotation of NO are provided by adjacent molecules in the crystal lattice. Areas of reduced interaction provide either local or global energy minima. The motion of NO rotation in the solid state is mapped by a summation of van der Waals interactions of NO oxygen with intermolecular atomic interactions.

In the solid state, the spatial arrangement of molecules in the crystal lattice provides intermolecular interactions. These interactions are estimated using the sum of the van der Waals radii overlaps. For example: in a given orientation, the van der Waals radius of an oxygen atom is overlapped by 0.1 Å with the van der Waals radius of a carbon atom. This *single* interaction leads to a sum of the van der Waals radii overlap value of −0.1 Å. Summing *all* interactions gives the total overlap for the oxygen at any given position. Plotted in Figure 9.20 is the sum of the van der Waals radii overlaps for *tri*-[Fe(TpFPP)(NO)(1-MeIm)] at 100 K for a rotation of 360° from the crystallographically determined NO position about the Fe–NNO bond. The point represents the crystallographically determined oxygen position and the abscissa values represent the rotation (clockwise is positive) of the FeNO plane from the imidazole plane at 0°. In the sum of the van der Waals radii plots with a second crystallographically determined NO position, the minor orientation point may not be located on the line for the predicted rotation of the NO oxygen from the majority occupied NO position. This discrepancy is the result of not modeling the tilt of the Fe–NNO vector off of the heme normal. Therefore, the interactions at these positions may be slightly overestimated or underestimated.
Figure 9.21. Fourier maps of $\text{tri}$-$[\text{Fe(TpFPP)(NO)(1-MeIm)}]$ at 100 K (a) and 293 K (b) displaying observed electron density contoured in a plane at 2.64 Å above the four pyrrole nitrogen plane. The contoured distance above this plane is defined by the distance between the nitrosyl oxygen and the four atom pyrrole nitrogen mean plane. The contours are at 0.1 electron intervals from 0.8 to 1.5 $e^-/Å^3$. 

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Figure 9.22. Sum of the van der Waals radii overlaps (Å) vs rotation of NO oxygen from found positions (deg.) indicated by points for tr[-Fe(TpFPP)(NO)(1-MeIm)] at all temperatures where data was acquired. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. The major orientation is rotated ∼180° from the minor orientation.

Population values are given in percent.
In the 100 K sum of the van der Waals radii plot of \textit{tri-}[Fe(TpFPP)(NO)(1-MeIm)], the peaks near $-55^\circ$ and $105^\circ$ represent the orientations of NO oxygen with largest intermolecular interactions. The valleys at $-150^\circ$ and $25^\circ$ represent orientations of NO oxygen with smallest intermolecular interactions. The valleys at $-150^\circ$ and $210^\circ$ are identical, related by a $360^\circ$ rotation. The height of the peaks (value of the overlap in Å) is determined by the degree of interaction and is related to specific intermolecular interactions. Figure 9.21 shows the intermolecular interactions represented by a Fourier map of \textit{tri-}[Fe(TpFPP)(NO)(1-MeIm)] at 100 K displaying observed electron density contoured at 2.64 Å above the mean plane of the porphyrin. As the NO oxygen is rotated clockwise about the Fe–N\textsubscript{NO} bond, starting with the FeNO plane parallel to the imidazole plane, it interacts with the phenyl ring of an adjacent molecule near $90^\circ$ and a phenyl ring from a second adjacent molecule near $210^\circ$ shown by the electron density. Due to the spatial arrangement in crystalline \textit{tri-}[Fe(TpFPP)(NO)(1-MeIm)] the most likely orientation would put the FeNO plane nearly parallel to the imidazole plane.

At 100 K, the barriers to rotation at $-55^\circ$ and $105^\circ$ are nearly equal. The valley located at $-150^\circ$ contains 100% of the NO orientation, while the broad valley at $25^\circ$ is unoccupied. As the temperature of the crystal was increased above 200 K a second orientation of the NO oxygen was populated at a $180^\circ$ rotation from the major orientation. The second, distinct, orientation is located in the valley at $25^\circ$. Plots of the sum of the van der Waals radii overlaps for structures measured at all temperatures are given in Figure 9.22. At 200 K, the second orientation is occupied 3% of the time. At this temperature, the barrier at $-55^\circ$ is reduced to a greater extent than the barrier at $105^\circ$. All structures measured above 200 K contain a second orientation of NO at the $25^\circ$ position. As the temperature is
TABLE 9.3

TEMPERATURE AND NO SITE POPULATIONS FOR A NUMBER OF [Fe(TpXPP)(NO)(1-MeIm)] COMPLEXES

<table>
<thead>
<tr>
<th>compound</th>
<th>$T^a$[exp.]</th>
<th>orien. $1^b$</th>
<th>orien. $2^b$</th>
<th>orien. $3^b$</th>
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</thead>
<tbody>
<tr>
<td>trr-[Fe(TpFPP)(NO)(1-MeIm)]$^c$</td>
<td>100 [4]</td>
<td>1.00</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>100 [3]</td>
<td>1.00</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>150 [7]</td>
<td>1.00</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>175 [8]</td>
<td>1.00</td>
<td>0.03</td>
<td>–</td>
</tr>
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<td></td>
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<td>0.07</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>224 [6]</td>
<td>0.93</td>
<td>0.05</td>
<td>–</td>
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<td></td>
<td>224 [2]</td>
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<td>293 [5]</td>
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</tr>
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<td></td>
<td>350 [10]</td>
<td>0.73</td>
<td>0.27</td>
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</tr>
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<td>293 [2]</td>
<td>0.74</td>
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<tr>
<td>[Fe(TpOCH$_3$PP)(NO)(1-MeIm)]$^d$</td>
<td>100 [1]</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>100 [4]</td>
<td>1.00</td>
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<td>–</td>
</tr>
<tr>
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<td>330 [3]</td>
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</tr>
<tr>
<td>[Fe(TpCF$_3$PP)(NO)(1-MeIm)]$^e$</td>
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<td>–</td>
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<tr>
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<td>0.31</td>
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</tr>
<tr>
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<td>100 [1]</td>
<td>0.63</td>
<td>0.28</td>
<td>0.09</td>
</tr>
</tbody>
</table>

$a$ degrees K.  $^b$ Percent site occupation/100.  $^c$ experiments 1–3 used a crystal with a twin component. Experiments 4–10 used a second crystal with no twinning.  $^d$ experiments 2–4 used a second crystal.  $^e$ experiment 2 used a second crystal.
increased, the barrier at $-55^\circ$ decreases to a much larger extent than the peak at $105^\circ$. Additionally, as the temperature is increased to 350 K the valley at $25^\circ$ becomes less broad. The population of the NO orientations for all temperatures are given in Table 9.3. The population and depopulation of these positions is temperature dependent. Structural data for tri-$[\text{Fe(TpFPP)(NO)(1-MeIm)}]$ was collected in ascending and descending order. The populations were reproducible at a given temperature indicating the reversibility of the solid-state motion. In the solid state, the motion of the NO ligand could progress over either the $-55^\circ$ peak or the $105^\circ$ peak. However, the energy barrier for the $-55^\circ$ peak is clearly lower as the temperature is increased. It is apparent from these and subsequent measurements that when the barrier is smaller than $\sim -2.3$ Å, rotation occurs as judged by a newly populated position.

$\text{tri-[Fe(TpFPP)(NO)(1-MeIm)]}$ fits into the first class of solid-state interactions where intermolecular interactions lead to discrete NO orientations. An ORTEP diagram showing 50% probability ellipsoids for the two distinct orientations of oxygen at 293 K and the single orientation of oxygen at 100 K for $\text{tri-[Fe(TpFPP)(NO)(1-MeIm)]}$ is given in Figure 9.23. No rotational disorder (elongation of the ellipsoid along the rotation axis) is observed in the thermal ellipsoid of the minor orientation of oxygen (O1b). This observation agrees well with the sum of the van der Waals radii overlaps plot (Figure 9.22) for $\text{tri-[Fe(TpFPP)(NO)(1-MeIm)]}$ where there are well-defined barriers between oxygen orientations.

Using the sum of the van der Waals radii plots, several additional NO heme complexes, where the ligand orientation might be controlled by intermolecular interactions, are investigated. Structural data were originally collected at 100 K for $[\text{Fe(TpOCH}_3\text{PP})(\text{NO})(1-\text{MeIm})]$ and the van der Waals radii plot contained two
Figure 9.23. ORTEP diagrams (50% probability ellipsoids) of
$\text{tri-}[\text{Fe(TpFPP)(NO)(1-MeIm)}]$ at (a) 100 K and (b) 293 K. The
molecules are oriented with the plane of the porphyrins in the page to
emphasize the orientations of NO oxygens. Hydrogen atoms and phenyl
rings are omitted for clarity.
Figure 9.24. Sum of the van der Waals radii overlaps (Å) vs the rotation of NO oxygen from found positions (deg.) indicated by points for [Fe(TpOCH$_3$)PP](NO)(1-MeIm)]. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. The major orientation is rotated $\sim$90° from the minor orientation. Population values are given in percent.
well defined valleys at 205° and 305° (see Figure 9.24). Like the trī-[Fe(TpFPP)-(NO)(1-MeIm)] complex, the two valleys are distinct with a large barrier between them. Unlike the trī-[Fe(TpFPP)(NO)(1-MeIm)] complex, the peak heights are disproportionate. The large barrier at 25° is the result of interactions with the 1-MeIm ligand of an adjacent molecule, while the small barrier at 260° is the result of interactions with the methoxy group of an adjacent molecule. At 100 K, the barrier at 25° has a sum of the van der Waal radii of $\sim -3.0 \, \text{Å}$ and the barrier at 260° has a sum of the van der Waals radii of $\sim -2.1 \, \text{Å}$. Using the 100 K data, we predict that a second orientation of NO, 100° rotated from the major NO orientation, will become populated at higher temperatures as a result of changes in the height of the barrier at 260°. Structures were measured at 293 and 330 K, each containing a second orientation of NO (see Figure 9.24). The populations of the second position is temperature dependent (see Table 9.3). The small barrier at 260° is reduced to a sum of the van der Waals radii value of $> -1.5 \, \text{Å}$, which leads to the population of a second discrete position. Rotation of NO most likely proceeds over this barrier. The larger barrier at 25° also decreases with increased temperature, however, the sum of the van der Waals radii value is still $< -2.6 \, \text{Å}$, even at 330 K. The study of the [Fe(TpOCH₃PP)(NO)(1-MeIm)] complex demonstrates the predictive nature of this method of solid-state mapping. trī-[Fe-(TpFPP)(NO)(1-MeIm)] and [Fe(TpOCH₃PP)(NO)(1-MeIm)] represent the first class of solid-state interactions observed in the sum of the van der Waals radii plots. These complexes have two distinct barriers and two distinct valleys. Additionally, the minor NO orientation is isolated from the major NO orientation by intermolecular interactions at all measured temperatures where multiple orientations are present.
Figure 9.25. Sum of the van der Waals radii overlaps (Å) vs rotation of NO oxygen from found positions (deg.) indicated by points for mono-[Fe(TpFPP)(NO)(1-MeIm)]. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. The major orientation is rotated ∼80° from the minor orientation. Population values are given in percent.
\textit{mono-[Fe(TpFPP)(NO)(1-MeIm)]} represents a second class of solid-state interactions where the NO orientations are not distinct at all measured temperatures. At 100 K, \textit{mono-[Fe(TpFPP)(NO)(1-MeIm)]} has a single orientation of NO. The sum of the van der Waals radii plot for \textit{mono-[Fe(TpFPP)(NO)(1-MeIm)]} is displayed in Figure 9.25. The large peak at $-150^\circ$ is the result of interactions of NO with the 1-MeIm ligand of an adjacent molecule. At 100 K, a small peak near the major NO position is observed. The peak, located at $55^\circ$, is caused by the interaction of NO with the fluoro group from an adjacent molecule. At 100 K there are also two valleys. The first valley is located at $0^\circ$ and a second, less well defined valley, is located at $70^\circ$. As the temperature of the crystal is increased to $\geq 150$ K, a second orientation of NO is populated in the valley $70^\circ$ rotated from the major NO orientation (see Table 9.3). In Figure 9.25 two low potential regions are observed, however, at temperatures $< 150$ K only the valley near $0^\circ$ is populated. At temperatures $\geq 150$ K the barrier near $55^\circ$ is overcome by NO rotation and the small valley near $70^\circ$ becomes populated. Even at 125 K, the barrier at $55^\circ$ is very small, but only a single orientation of NO is observed. However, determining the population of any position of oxygen is limited by the detection limits of the X-ray diffraction experiment. This is especially problematic when determining that a site is unpopulated. In these studies, a 3\% ($\sim 0.25$ electron) occupied oxygen was successfully modeled. More typically, 0.5–1.0 electron (6–12\% occupancy) may be confidently found and properly refined.

Finally, this second class of complexes does not have well-defined positions of NO oxygen at temperatures $\geq 150$ K. The 293 K structure of \textit{mono-[Fe(TpFPP)-(NO)(1-MeIm)]}, reported earlier in this Chapter, was refined with the two oxygen positions having equal thermal parameters to better calculate the populations of
each of the two sites. The complex was also refined with independent thermal parameters for the two oxygen atoms to better observe the unconstrained shape of the thermal ellipsoids. The statistical parameters are similar for both the constrained and unconstrained models. $R_1 (wR_2)$ for the constrained and unconstrained models are 0.0518 (0.1288) and 0.0514 (0.1282), respectively. An ORTEP diagram of $\text{mono}$$-[\text{Fe(TpFPP)(NO)(1-MeIm)}]$ at 293 K with independent thermal parameters for the two oxygen atoms is given in Figure 9.26. It is apparent from the elongated thermal ellipsoid of the minor orientation of oxygen (located at 70°, O1b) that there are unresolved NO positions. This is most likely the result of the loss of the barrier at 55° and the formation of a broad valley from −40° to 100°.

This new broad valley includes the positions of the two valleys observed at 100 K. The elongated oxygen thermal ellipsoids at 293 K in Figure 9.26b and the broad ill-defined valley from −40° to 100° both suggest that the NO oxygen is not two discrete NO oxygen positions at 293 K, rather the NO oxygen is oriented over several unresolved positions. At temperatures $\leq$ 150 K, however, the barrier at 55° allows for only a single, discrete, NO orientation (see Figure 9.26a for the 100 K ORTEP of $\text{mono}$$-[\text{Fe(TpFPP)(NO)(1-MeIm)}]$).

[Fe(TPP)(NO)(1-MeIm)] is a second example in the class of complexes where the positions of the NO oxygen are not isolated by the intermolecular contacts. The 100 and 298 K structures of [Fe(TPP)(NO)(1-MeIm)] were previously reported, each containing two orientations of NO.[64, 66] The 100 K structure had NO populations of 85/15% and the 293 K structure had NO populations of 67/32%. As described above and in Chapter 7, the structural data at 100 and 293 K were reacquired to study the temperature dependence of NO site population in the solid state. At 100 K the populations of the NO orientations were similar,
Figure 9.26. ORTEP diagrams (50% probability ellipsoids) of \emph{mono-}[Fe(TpFPP)(NO)(1-MeIm)] at (a) 100 K and (b) 293 K. The molecules are oriented with the plane of the porphyrins in the page to emphasize the elongated thermal ellipsoid of the minor oxygen (O1b) orientation in the (b) 293 K structure. The major oxygen orientation (O1a) (a and b) is also shown. Hydrogen atoms and phenyl rings are omitted for clarity.
78/22% (see Table 9.3), however at 293 K the structure was better modeled with three NO orientations. The populations of the three orientations, 38/31/31%, agree well with the 67/32% populations observed in the original structure, however the 67% position is now modeled as two positions, 38% and 31% occupied.

At 100 K, there are two well-defined valleys at 241° and 350° in the sum of the van der Waals radii plot of [Fe(TPP)(NO)(1-MeIm)]. There are also two peaks. The major barrier, located at 120°, results from the interaction of the NO oxygen with the phenyl group of an adjacent molecule. The minor barrier, located at 300°, results from the interaction of the NO oxygen with the 1-MeIm ligand of an adjacent molecule. As the temperature is increased to 293 K, the major barrier decreases and the minor barrier increases modestly, although, the minor barrier changes in an atypical manner possible reflecting anisotropic crystal expansion. The largest variation occurs in the broadness of the valley near 225°. At 293 K, the broadness of the valley allows for additional rotational motion which is modeled as two NO sites. The statistical parameters are similar for both the three NO position model and two NO position model. $R_1 (wR_2)$ for the three NO position and two NO position models are 0.0564 (0.1702) and 0.0569 (0.1723), respectively. An ORTEP diagram of [Fe(TPP)(NO)(1-MeIm)] at 100 and 293 K is given in Figure 9.28. It is apparent from the thermal ellipsoids of the oxygen atoms (O1a and O1b) at 293 K that there is electron density between the two modeled positions resulting from unresolved NO positions. The broadness of the valley at 225° in the sum of the van der Waals radii plots and the elongated thermal ellipsoids of the ORTEP diagram both suggest that the NO oxygen is not two discrete NO oxygen positions at 293 K near 225°, rather the NO oxygen is located at several unresolved positions. As the temperature is decreased to 100 K,
Figure 9.27. Sum of the van der Waals radii overlaps (Å) vs the rotation of NO oxygen from found positions (deg.) indicated by points for [Fe(TPP)(NO)(1-MeIm)]. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. Population values are given in percent.
the valley at 241° narrows and the NO oxygen can be best described as a single orientation.

Rotation of NO in solid-state [Fe(TPP)(NO)(1-MeIm)] occurs in the 180°–260° valley and only modestly over the barriers at 120° and near 300° as evidenced from the small change in oxygen populations (22 to 31% from 100 to 293 K). However, the found NO oxygen position (at 350°) in the 293 K structure is 40° rotated from the lowest point in the valley, possibly reflecting the error associated with not modeling the tilt of the Fe–N_{NO} vector. The barrier at 120° is reduced with increased temperature, although, the sum of the van der radii value is still approximately −2.5 Å at 293 K, making NO rotation less likely. The NO rotation most likely occurs over the smaller barrier (sum of the van der Waals radii = −1.0 Å at 100 K), although, the barrier gets modestly larger at 293 K.

The sum of the van der Waals interactions for [Fe(TpCF_{3}PP)(NO)(1-MeIm)] represents the only compound in the third class and is more complicated than the other two classes. [Fe(TpCF_{3}PP)(NO)(1-MeIm)] contains two orientations of NO oxygen at 100 K. The two positions are occupied 94% and 6% of the time (see Table 9.3). A sum of the van der Waals radii plot of [Fe(TpCF_{3}PP)(NO)-(1-MeIm)] at 100 K is given in Figure 9.29. The plot contains two valleys, one at 177° and one at 9°, which have small van der Waals overlap with adjacent molecules. This plot also contains two peaks located at −80° and 95° which are similar in height (≈−2.5 Å). Both of these peaks result from the interactions of NO with the phenyl group of an adjacent molecule. When the temperature of the crystal structure is increased to 290 K, the barriers at −80° and 95° show no apparent change, however the valley at 9° shows a reduction in the intermolecular interactions. [Fe(TpCF_{3}PP)(NO)(1-MeIm)] is unique because the populations of
Figure 9.28. ORTEP diagrams (50% probability ellipsoids) of [Fe(TPP)(NO)(1-MeIm)] at (a) 100 K and (b) 293 K. The molecules are oriented with the plane of the porphyrins in the page to emphasize the orientations of NO oxygens. At 100 K, the populations of the O1a and O1c positions are 78 and 22 %, respectively. At 293, the populations of the O1a, O1b, and O1c positions are 38, 31, and 31%, respectively. Hydrogen atoms and phenyl rings are omitted for clarity.
the NO oxygen positions are greatly affected by the depth of the valleys. The populations of these two positions change with respect to temperature. Table 9.3 displays the population values for each of the two orientations. Although the sum of the van der Waals radii difference between the 100 and 290 K structures is small, subtle differences in the depth of the valley at 9° influences the population and depopulation of the two positions resulting in large changes in site population. The population of the minor NO orientation increases from 6% at 100 K to 40% at 290 K. The similarity in barrier heights at 100 and 290 K suggests that valley depth controls the population and depopulation of the NO positions. As the temperature is increased to 290 K, the valley at 9° becomes more energetically favorable than at 100 K. At 100 K the valley at 180° is lower, resulting in a higher population. At 100 and 290 K, both of the barriers are nearly equal making the determination of rotation pathway somewhat ambiguous.

Finally, the sum of the van der Waals interactions of $[\text{Fe(TpNO}_2\text{PP})(\text{NO})(1-\text{MeIm})]$ is investigated. Only one data set was collected for $[\text{Fe(TpNO}_2\text{PP})(\text{NO})(1-\text{MeIm})]$, however, the observed interactions are different from other six-coordinate nitrosyl complexes. The interactions causing the large barrier located from $-60^\circ$ to $140^\circ$ or $300^\circ$ to $500^\circ$ are complicated due to the site disorder of an adjacent solvent molecule. The adjacent site is populated by 1-methylimidazole 72% of the time, chloroform 20% of the time, and a second chloroform that could not be modeled. Figure 9.30 depicts the interaction of the NO oxygen with either 1-MeIm, chloroform, or a sum of the two scaled by their crystallographically defined occupancies. The NO position at 339° is populated 9% of the time. As Figure 9.30 depicts, the interactions causing the 339° barrier changes greatly based on the solvent molecule present, while differences in the sum the interactions at 261° and 161° caused by the varied solvate, are much smaller. When the solvate is 1-
Figure 9.29. Sum of the van der Waals radii overlaps (Å) vs the rotation of NO oxygen from found positions (deg.) indicated by points for [Fe(TpCF₃PP)(NO)(1-MeIm)]. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. The major orientation is rotated ~180° from the minor orientation. Population values are given in percent.
methylimidazole, the intermolecular interactions are greatly increased at the 339° position at least suggesting that when there is a 1-MeIm present, the NO position cannot be occupied. This site is therefore occupied only 9% of the time, and only when the solvate is a chloroform.

9.4.5 Energetics of NO Movement

The least-squares-determined populations for the NO oxygen sites in samples with two orientations (see Table 9.3) can be used to determine thermodynamic parameters of the NO disorder. The van’t Hoff equation (Equation 9.1) is used to calculate the \( \Delta H^\circ \) and \( \Delta S^\circ \) of the process that rotates the NO ligand from the major orientation to the minor orientation. The equilibrium constant, \( K \), is calculated from the found populations of the NO orientations. We make the assumption that all crystallographic measurements represent thermal equilibrium positions. Plots of the equilibrium constant versus 1/T are given in Figures 9.31 and 9.32. Data are fit using the van’t Hoff equation (Equation 9.1) to determine \( \Delta H^\circ \) and \( \Delta S^\circ \). The Gibbs free energy equation (Equation 9.2) is then used to calculate \( \Delta G^\circ \).

\[
\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{9.1}
\]

\[
\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{9.2}
\]

The van’t Hoff parameters for \textit{tri}=[Fe(TpFPP)(NO)(1-MeIm)], are \( \Delta H^\circ = 8.9 \) kJ/mol, \( \Delta S^\circ = 17 \) J/mol-K and \( \Delta G^\circ = 3.8 \) kJ/mol. The expectation that pop-
Figure 9.30. Sum of the van der Waals radii overlaps (Å) vs the rotation of NO oxygen from found positions (deg.) indicated by points for [Fe(TpNO₂PP)(NO)(1-MeIm)]. This “pseudopotential” plot estimates the strength of intermolecular interactions felt by the NO oxygen during rotation. The green curve represents interactions when the solvate molecule is CHCl₃, the red curve represents interactions when the solvate molecule is 1-methylimidazole, and the black curve is a sum of both sets of interactions. The green and red curves are weighted, based on the refined populations of the solvent molecules. Population values are given in percent.
ulating the second, higher energy, orientation would be nonspontaneous is found to be true as evidenced by the positive values of $\Delta G^\circ$. The value of $\Delta G^\circ$ is small reflecting the subtle free energy differences between the minor and major orientations caused by intermolecular interactions. Van’t Hoff plots were also used to estimate thermodynamic properties of mono-[Fe(TpFPP)(NO)(1-MeIm)] ($\Delta H^\circ = 4.8$ kJ/mol, $\Delta S^\circ = 8$ J/mol-K and $\Delta G^\circ = 2.3$ kJ/mol) and [Fe(TpOCH$_3$PP)(NO)-(1-MeIm)] ($\Delta H^\circ = 5.2$ kJ/mol, $\Delta S^\circ = 6$ J/mol-K and $\Delta G^\circ = 3.4$ kJ/mol). The relative depth of the two valleys ultimately governs the thermodynamic parameters calculated by the van’t Hoff equation. The difference in the sum of the van der Waals radii values at 293 K for the two found positions in tri-[Fe(TpFPP)(NO)(1-MeIm)], mono-[Fe(TpFPP)(NO)(1-MeIm)], and [Fe(TpOCH$_3$PP)(NO)(1-MeIm)] are $-0.804$, $-0.229$, and $-0.676$ Å, respectively. The $\Delta G^\circ$ values and sum of the van der Waals radii values for the minor and major orientations suggest that the larger the difference in the sum of the van der Waals radii values at the two found positions, the larger the free energy difference between the two found positions. This agrees well, at least qualitatively, with the description of the major to minor orientation transition in the solid state being a nonspontaneous process. It is difficult, however, to directly correlate the sum of van der Waals radii values with energy. Using van der Waals radii overlap as an estimate of the rotational energetics makes a number of assumptions. This method assumes that atoms are completely static in the crystal lattice. This method also assumes the systems energy increases linearly when you bring two atoms in close proximity. Finally, this method treats all atoms as non-interacting spheres. Even with these assumptions, this method is useful to describe specific interactions in the solid state. It gives a degree of predictably to ligand orientation and allows for a description of rotational barriers in the solid state.
9.4.6 Ligand Orientation in Nitrosyl Coordinated Myoglobin

The relevance of these rotational barriers to biological systems is not well understood. In MbNO there is a single dominating peak in the sum of the van der Waals radii plot. The sum of the van der Waals radii plot for MbNO is illustrated in Figure 9.33. Coordinates for the MbNO structures are from the Protein Data Bank, 1NPF and 1HJT. All non-hydrogen interactions are considered. The two X-ray structures of MbNO show very similar sum of the van der Waals radii plots. A single strong interaction at 190° is observed in each of the two crystal structures of MbNO. The strong interaction attributed to this peak is with the HIS-64 residue. It has been suggested that this strong interaction provides stabilization in the MbO₂ adduct, although the biological significance of the His-64/NO interaction in MbNO is not well understood.

9.5 Summary

New six-coordinate nitrosyl-ligated iron(II) porphyrinates have been prepared and characterized at several temperatures. Three of the new complexes contain an ordered nitrosyl group at 100 K and are the first examples of completely ordered nitrosyls in [Fe(Porph)(NO)(1-MeIm)] complexes. At temperatures > 200 K all complexes have multiple nitrosyl orientations. Temperature-dependent variations or trends in the coordination group geometry may be observed by studying this reversible process. These trends indicate a strong correlation between the physiologically relevant Fe–N_im bond and the stretching frequency of NO on the opposite side of the porphyrin. Additionally, the relative orientation of the axial ligands has no appreciable influence on the vibrational dynamics of these porphyrinate systems.
Figure 9.31. Plot of the K value vs 1/T for tri-[Fe(TpFPP)(NO)(1-MeIm)]. The equilibrium constants, K, are calculated from the found populations of NO orientations for the transition from highest populated position to the lowest populated position. Data are fit using the van’t Hoff equation to determine $\Delta H^\circ$ and $\Delta S^\circ$. The thermodynamic parameters for tri-[Fe(TpFPP)(NO)(1-MeIm)] are $\Delta H^\circ = 8.9\pm0.5$ kJ/mol and $\Delta S^\circ = 17\pm1.4$ J/mol-K.
Figure 9.32. Plot of the K value vs 1/T for 
mono-[Fe(TpFPP)(NO)(1-MeIm)] (blue) and 
[Fe(TpOCH$_3$PP)(NO)(1-MeIm)] (red). The equilibrium constants, K, are calculated from the found populations of NO orientations for the transition from highest populated position to the lowest populated position. Data are fit using the van’t Hoff equation to determine $\Delta H^\circ$ and $\Delta S^\circ$. The thermodynamic parameters for 
mono-[Fe(TpFPP)(NO)(1-MeIm)] are $\Delta H^\circ = 4.8 \pm 1.0$ kJ/mol and $\Delta S^\circ = 8 \pm 3.7$ J/mol-K. The thermodynamic parameters for 
[Fe(TpOCH$_3$PP)(NO)(1-MeIm)] are $\Delta H^\circ = 5.2 \pm 0.5$ kJ/mol and $\Delta S^\circ = 6 \pm 1.4$ J/mol-K.
Figure 9.33. Sum of the van der Waals radii overlaps (Å) vs rotation of NO oxygen from imidazole N2 position (deg.). The found NO oxygen position is indicated by a circle. These “pseudopotential” plots estimate the strength of intermolecular interactions felt by the NO oxygen during rotation. Coordinates from Protein Data Bank, 1NPF (red) and 1HJT (blue). \[228\] [229]
The orientation of the NO ligand in solid-state \([\text{Fe(TpXPP)(NO)(1-MeIm)}]\) systems has also been investigated. These studies have displayed two major themes. The rotation about the Fe–N\(_{\text{NO}}\) bond occurs in the solid state by populating energy-accessible orientations. Finally, the energy barriers to populating energy-accessible orientations are provided by intermolecular interactions; populations are controlled by crystal packing effects. These effects are an inherent property of the crystal, not the isolated molecules.
10.1 Introduction

The importance of the Fe–His bond in the NO and CO sensing proteins has been commented on at length in previous chapters, especially in the sGC system. The Fe–His bond, which is typically the only covalent bond between the heme and the protein, transfers the signal of coordination state from the heme to the protein, thereby eliciting a physiological response.

Prior to ligand coordination, diatomic sensing heme proteins are typically high-spin iron(II) with a coordinated histidine. The strength of the Fe–His bond in the five-coordinate systems has been used as a gauge of protein ligation and activation states, and as a possible predictor of protein activity. The $\nu_{\text{Fe–His(Im)}}$ is observed by rR in a number of deoxy (5-coordinate iron(II)) heme proteins and model compounds at $\sim 220 \text{ cm}^{-1}$. Unfortunately, much less information is available about the Fe–His(Im) bond in six-coordinate iron(II) hemes with diatomic ligands that would provide details of, for example, the control of ligand binding. Some insight into the nature of the Fe–His(Im) bond post ligand ejection has been attained through flash photolysis experiments with MbXO and HbXO.
Rai et al. were the first investigators to directly observe the $\nu_{\text{Fe-His(Im)}}$ in a six-coordinate heme diatomic system. This study used NRVS data and normal coordinate analysis to identify the $\nu_{\text{Fe-His(Im)}}$ mode in [Fe(TPP)(CO)(1-MeIm)]. This initial investigation demonstrated that the $\nu_{\text{Fe-His(Im)}}$ in six-coordinate heme diatomic systems could be studied using NRVS.

This chapter contains a detailed investigation of the $\nu_{\text{Fe-His(Im)}}$ in [Fe(Porph)-(XO)(L)] complexes where XO = CO, NO, and O$_2$. Modes with significant Fe–Im motion will be described in detail. Additionally, this study will compare the effects of the diatomic ligands on the vibrational dynamics of the Fe–Im bond.

10.2 Experimental

The synthesis and characterization of [Fe(TpXPP)(NO)(L)] complexes were described in Chapter 7. The synthesis and characterization of [Fe(Porph)(CO)-(XIm)] complexes were described in Chapters 4 and 5.

NRVS was performed on powder and single-crystal samples. A description of the NRVS technique is given in Chapter 1. The NRVS experiment results in the observation of all vibrational modes with iron character. The single-crystal alignment and other NRVS experimental details are described in Chapter 3. Single-crystal samples are aligned with the X-ray beam parallel or perpendicular to the porphyrin plane. In the parallel orientation the in-plane vibrations are enhanced and the out-of-plane vibrations are suppressed. In the perpendicular orientation the out-of-plane vibrations are enhanced and the in-plane vibrations are suppressed.

NRVS data were collected on powder samples of [Fe(OEP)(CO)(1-MeIm)], [Fe(TPP)(1-PhIm)(CO)], and [Fe(TP$_{piv}$P)(1-MeIm)(O$_2$)] and single-crystal samples of [Fe(OEP)(2-MeHIm)], [Fe(TPP)(1-MeIm)(CO)], [Fe(TPP)(1,2-DiMeIm)(CO)]-
\[ \text{C}_7\text{H}_8, [\text{Fe(TPP)(4-MePip)(NO)}] \cdot \text{CHCl}_3, [\text{Fe(TPP)(4-MePip)(NO)}], [\text{Fe(TPP)(1-MeIm)(NO)}], \text{tri-[Fe(TpFPP)(NO)(1-MeIm)]}, \text{mono-[Fe(TpFPP)(NO)(1-MeIm)]}, \text{and [Fe(TpOCH}_3\text{PP)(1-MeIm)(NO)]} \] resulting in the observation of all vibrational modes with iron character.

DFT calculations provide the optimized structures and detailed vibrational predictions for the porphyrin complexes listed above. Calculations were performed with Gaussian 03,[178] using the 6-31G* basis set for N, O, C, F, and H atoms, Ahlrich’s VTZ basis set for the Fe atom,[179] and the Becke-Lee-Yang-Parr composite exchange correlation functional (B3LYP).[80] The initial atom coordinates were acquired from the X-ray crystal structures. The KED is determined for each vibrational mode from the relative Cartesian displacements of individual atoms, as described previously.[76] The calculations describe the atomic vibrations using a set of relative atomic displacements for which the mode composition factors \( (e^2_{j\alpha}) \) for each atom \( j \) and mode \( \alpha \), are directionally dependent.[78]

10.3 Discussion

This chapter details the start of a broader investigation into the Fe–His(Im) vibrational dynamics in six-coordinate hemes with bound diatomic molecules. The novelty of these initial measurements lies in the difficulty of measuring the \( \nu_{\text{Fe–His(Im)}} \) mode for six-coordinate heme diatomic complexes using traditional vibrational spectroscopy. In five-coordinate heme complexes bound with histidine (imidazole), however, the bond is sufficiently enhanced to observe by rR. The \( \nu_{\text{Fe–His(Im)}} \) is observed by rR in a number of deoxy (5-coordinate iron(II)) heme proteins and model compounds at \( \sim 220 \text{ cm}^{-1} \).[16] [58] [68] [78] Some insight into the nature of the Fe–His(Im) bond in six-coordinate heme diatomics has been
acquired indirectly by studying the 5-coordinate Fe–His(Im) bond post diatomic ligand ejection in MbXO and HbXO. The NRVS technique is used to investigate the vibrational dynamics of the biologically significant Fe–His(Im) bond in six-coordinate heme diatomics. Single-crystal NRVS is especially well-suited for the measurement of the Fe–His(Im) vibrational modes. Single crystals are oriented so that the modes with motion along the heme normal are enhanced, such as the Fe–His(Im) vibrational modes, and the vibrational modes in the porphyrin plane are suppressed.

Initial investigations into the Fe–His(Im) bond were performed on 5-coordinate heme imidazole complexes. Single-crystal measurements on [Fe(TPP)(2-MeHIm)] identified out-of-plane modes at 216, 228, and 246 cm$^{-1}$, with $e_{\text{Fe}}^2 = 0.20, 0.06,$ and 0.19, respectively. Empirical normal mode calculations identified Fe–Im contributions to each of these three modes.

Measurements on single crystals of [Fe(OEP)(2-MeHIm)] reveal modes with significant out-of-plane character at 24, 71, 111, 140, and 222 cm$^{-1}$. The out-of-plane and powder spectra of [Fe(OEP)(2-MeHIm)] are given in Figure 10.1. The feature near 224 cm$^{-1}$ in the out-of-plane spectrum of [Fe(OEP)(2-MeHIm)] can be fit with two peaks, with frequencies 217 and 233 cm$^{-1}$ and areas $e_{\text{Fe}}^2 = 0.34$ and 0.20, respectively. The total $\sum e_{\text{Fe}}^2 = 0.54$ approaches the value $e_{\text{Fe}}^2 = m_{\text{Im}}/(m_{\text{Fe}} + m_{\text{Im}}) = 0.59$ expected for a two-body Fe–Im oscillator.

10.3.1 The Fe–Im Bond in [Fe(Porph)(XIm)(CO)] Compounds

This chapter is dedicated to characterizing the Fe–Im vibrational modes, which have not been clearly identified for low-spin heme complexes with diatomic ligands bound trans to the imidazole (although symmetric Im-Fe-Im stretching modes...
Figure 10.1. NRVS spectra displaying the powder (black) and out-of-plane (red) data for $\text{[Fe(OEP)(2-MeHIm)]}$. The mode assigned as the $\nu_{\text{Fe-His(Im)}}$, at 224 cm$^{-1}$, is fit with two components with frequencies 217 and 233 cm$^{-1}$ and areas $e^2_{\text{Fe}} = 0.34$ and 0.20, respectively.

were reported between 182 and 226 cm$^{-1}$ in the Raman spectra of several 6-coordinate bis-imidazole Fe(II) porphyrins and bis-histidine ligated heme proteins.\textsuperscript{233, 236} Raman frequencies sensitive to Fe isotopic substitution are occasionally reported and tentatively assigned to Fe–Im vibrational modes for diatomic ligand complexes with heme proteins, including $\text{Chironomus HbCO}$ (317 cm$^{-1}$),\textsuperscript{237} and $\text{MbO}_2$ (263 cm$^{-1}$).\textsuperscript{238} as well as ferric cyanide complexes with $\text{Chironomus Hb}$ (309 cm$^{-1}$)\textsuperscript{239} and $\text{Chlamydomonas Hb}$ (315 cm$^{-1}$).\textsuperscript{240} But the Fe isotope shift does not distinguish Fe–Im modes from the in-plane Fe vibrations observed by NRVS in the same frequency region. No modes sensitive to isotope labeling of the imidazole were identified in the Raman spectra of metMb or MbCO.\textsuperscript{241}

A previous analysis of the $\text{[Fe(TPP)(1-MeIm)(CO)]}$ powder data, based on an empirical calculation,\textsuperscript{82} located the $\nu_{\text{Fe-Im}}$ mode at 226 cm$^{-1}$ and the Fe–Im rotation (bending) mode at 172 cm$^{-1}$. NRVS data on an oriented single crystal
Figure 10.2. Comparison between the measured single-crystal NRVS spectrum of [Fe(TPP)(CO)(1-MeIm)] (top panel), and the DFT calculated single-crystal spectrum of [Fe(TPP)(CO)(1-MeIm)] (bottom panel). DFT predicted mode values are convolved with an 8 cm$^{-1}$ Lorentzian function.
of [Fe(TPP)(1-MeIm)(CO)] reveals several modes with out-of-plane character at 324 cm\(^{-1}\) and below, in addition to the FeCO modes discussed in Chapter 6. The single-crystal NRVS spectrum of [Fe(TPP)(CO)(1-MeIm)] has features at 39, 64, 127, 172, 225, 331, and 507 cm\(^{-1}\) with strong out-of-plane character. A comparison of the powder NRVS data and the out-of-plane single-crystal data is given in Figure 10.2. Density Functional Theory was used to calculate energy minimized structures and predict vibrational mode frequencies and character.

Density Functional Theory calculations for [Fe(TPP)(1-MeIm)(CO)] predict six out-of-plane Fe modes below 400 cm\(^{-1}\). The calculated mode at 320 cm\(^{-1}\) consists of motion of the entire FeCO group translating along the heme normal with consequent stretching of the Fe–Im bond, but insignificant imidazole motion. The character of the 320 cm\(^{-1}\) mode is given in Figures 10.3 and 10.4. It was difficult to observe this mode in the original investigation of powder [Fe(TPP)-(1-MeIm)(CO)] because it is obscured by stronger in-plane modes. However, this mode is clearly revealed in the oriented single-crystal data at 331 cm\(^{-1}\) (see Figure 10.2). The modes at 157, 182, and 214 cm\(^{-1}\), which contain motion of both iron and imidazole, are given in Figures 10.3 and 10.4. The mode composition factors for each of the modes are given in Table 10.1. However, none of the aforementioned modes is a pure \(\nu_{\text{Fe–His(Im)}}\) mode, and each has an \(e_{\text{Fe}}^2\) value significantly lower than predicted for a two-body Fe–Im oscillator (\(e_{\text{Fe}}^2 = 0.59\)).

A similar distribution of Fe–Im mode character among multiple vibrational modes appeared in previous calculations on truncated model porphyrins.\(^{[194]}\) Calculations on these unsubstituted iron porphyrins also predict significant mixing between Fe–Im stretching and pyrrole tilting modes,\(^{[242]}\) consistent with an analysis of all available isotope data for deoxyMb, which suggested that the Fe–His
Figure 10.3. Predicted vibrational modes of [Fe(TPP)(1-MeIm)(CO)] with frequencies (a) 157, (b) 182, (c) 214, and (d) 320 cm$^{-1}$ involve Fe–Im motion.
Figure 10.4. Experimentally observed (black) and DFT predicted (red and blue) vibrational modes of [Fe(TPP)(1-MeIm)(CO)] and [Fe(TPP)(1,2-DiMeIm)(CO)]. Measured (black) and calculated $\epsilon_{Fe}^2$ (red) and $\epsilon_{Fe}^2 + \epsilon_{Im}^2$ (blue) values are given in parentheses.
vibration could not be described as a simple two-body oscillator \[241\].

Calculations of the energy-minimized structures of [Fe(TPP)(1-MeIm)(CO)] and [Fe(TPP)(CO)(1,2-Me$_2$Im)] predict an elongation of the Fe–Im bond by 0.08 Å in the porphyrinates coordinated by 1,2-Me$_2$Im. This observation agrees well with the experimentally determined structures in which the Fe–Im bond is 0.03–0.09 Å longer in [Fe(TPP)(CO)(XIm)] when XIm = 2-MeHIm or 1,2-Me$_2$Im.\[107\] The 0.08 Å increase of the Fe–Im bond length is correlated with modest decreases of the 182, 214, and 320 cm$^{-1}$ frequencies predicted for [Fe(TPP)(1-MeIm)(CO)] compared to 166, 212, and 320 cm$^{-1}$ for [Fe(TPP)(CO)(1,2-Me$_2$Im)]. Values of all modes with significant Fe–Im motion are given in Table 10.2.
TABLE 10.1

DFT PREDICTED ImFeCO KINETIC ENERGY DISTRIBUTION
FOR [Fe(TPP)(CO)(L)]

<table>
<thead>
<tr>
<th>freq. cm(^{-1})</th>
<th>(e^2_{Fe})</th>
<th>(e^2_{C})</th>
<th>(e^2_{O})</th>
<th>(\sum e^2_{Im})</th>
<th>(\sum e^2_j)</th>
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<tr>
<td>26</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.09</td>
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</tr>
<tr>
<td>50</td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
<td>0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>52</td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>55</td>
<td>0.01</td>
<td>0.01</td>
<td>0.08</td>
<td>0.24</td>
<td>0.34</td>
</tr>
<tr>
<td>64</td>
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<td>0.01</td>
<td>0.14</td>
<td>0.28</td>
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</tr>
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<td>96</td>
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<td>0.39</td>
</tr>
<tr>
<td>157</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.68</td>
<td>0.74</td>
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<tr>
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<td>0.07</td>
<td>0.01</td>
<td>0.01</td>
<td>0.52</td>
<td>0.61</td>
</tr>
<tr>
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<td>0.09</td>
<td>0.02</td>
<td>0.03</td>
<td>0.08</td>
<td>0.22</td>
</tr>
<tr>
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<td>0.02</td>
<td>0.00</td>
<td>0.20</td>
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<tr>
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<td>0.05</td>
<td>0.02</td>
<td>0.00</td>
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</tr>
<tr>
<td>318</td>
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<td>0.04</td>
<td>0.01</td>
<td>0.05</td>
<td>0.37</td>
</tr>
<tr>
<td>320</td>
<td>0.11</td>
<td>0.10</td>
<td>0.15</td>
<td>0.00</td>
<td>0.36</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>323</td>
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<td>0.02</td>
<td>0.00</td>
<td>0.04</td>
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<td>379</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>0.90</td>
<td>0.94</td>
</tr>
</tbody>
</table>

\(\sum e^2_{Im}\) is equal to the sum of the mode composition factors for all the 12 atoms in the imidazole group. All modes < 400 cm\(^{-1}\) with \(e^2_{Fe} \geq 0.01\) are included.

The DFT calculations predict frequency changes for several modes involving rotation of the Im ligand, from 157, 263, and 283 cm\(^{-1}\) in [Fe(TPP)(1-MeIm)(CO)] to 152, 267, and 281 cm\(^{-1}\) in [Fe(TPP)(CO)(1,2-Me\(_2\)Im)], perhaps reflecting the increased steric interaction of the methyl group with the porphyrin. The Fe amplitudes predicted for some of these modes also change. For [Fe(TPP)(1-MeIm)(CO)], the predicted Fe amplitude is slightly smaller for the 157 cm\(^{-1}\) mode than for the 182 cm\(^{-1}\) mode, while for [Fe(TPP)(1,2-Me\(_2\)Im)(CO)] the mean-squared amplitude predicted for the 152 cm\(^{-1}\) mode (\(e^2_{Fe} = 0.04\)) is about half as large as the 166 cm\(^{-1}\) mode (\(e^2_{Fe} = 0.09\)).
None of the identified out-of-plane Fe vibrations involving the imidazole ligand can be described as a simple two-body vibration. Nevertheless, measurements and calculations suggest that the frequencies of the features observed experimentally at 151, 172, 225, and 331 cm\(^{-1}\) in [Fe(TPP)(1-MeIm)(CO)] have modest sensitivity to the Fe–Im bond length.

The experimentally observed vibrational modes corresponding to Fe–Im motion are influenced by the 0.03–0.09 Å Fe–Im bond length increase in [Fe(TPP)(CO)(1,2-Me\(_2\)Im)] compared to [Fe(TPP)(CO)(1-MeIm)]. Out-of-plane features attributed to motion of the Fe–Im group found in [Fe(TPP)(CO)(1,2-Me\(_2\)Im)] at 139, 168, and 221 cm\(^{-1}\) are shifted to 151, 172, and 225 cm\(^{-1}\) in [Fe(TPP)(1-MeIm)(CO)]. The highest frequency Fe–Im mode observed in [Fe(TPP)(CO)(1,2-Me\(_2\)Im)], 320 cm\(^{-1}\), is insensitive to this bond length change. DFT predicted values agree well with the experimental data. Replacement of the 1-MeIm ligand with 1,2-Me\(_2\)Im results in a 0.03–0.09 Å lengthening of the Fe–Im and a corresponding downshift in the 151, 172, 225, and 331 cm\(^{-1}\) modes to 139, 168, 221, and 315/327 cm\(^{-1}\). However, the mode compositions, displayed in Figure 10.4, are similar for the carbonyl complexes coordinated with 1-MeIm and 1,2-Me\(_2\)Im.
Only small perturbations are observed as a result of additional methyl group of 1,2-Me$_2$Im.

Three additional six-coordinate heme carbonyls systems have been measured. Assigning the Fe–Im vibrational modes in proteins and powders of small molecules has proven to be more difficult than in the single-crystal measurements. The features in the 150–300 cm$^{-1}$ region of MbCO are not sufficiently resolved to confidently assign. Powder data acquired on [Fe(TPP)(CO)(1-PhIm)] has features at 169 and 219 cm$^{-1}$ in the same region as the 151, 172, and 225 cm$^{-1}$ modes are observed in [Fe(TPP)(CO)(1-MeIm)]; however, without single-crystal measurements, definitive assignments are difficult. Powder data was also acquired on [Fe(OEP)(CO)(1-MeIm)] which has features at 151, 168, and 222 cm$^{-1}$ suggesting weak perturbation caused by peripheral effects. Definitive assignments of these modes are again difficult without single-crystal measurements.

10.3.2 The Fe–Im Bond in Six-Coordinate Heme Nitrosyls

The Fe–His(Im) bond in six-coordinate heme nitrosyls is of great interest due to its biological importance. Proposed mechanisms for the heme proteins cytochrome $c'$ and sGC have presented a scenario in which the coordination to heme changes during the physiological cycle.[63] Unfortunately, little information is available about the Fe–His(Im) bond in six-coordinate nitrosyl iron(II) hemes. Knowledge of the vibrational dynamics of these systems would provide, for example, insight into control of ligand binding and ejection.

NRVS is used to study the Fe–Im bond in [Fe(TpXPP)(NO)(L)] complexes where L = 1-MeIm and 4-MePip. The frequency and character of the Fe–L vibrational modes are assigned using single-crystal NRVS and DFT calculations.
TABLE 10.3

SELECTED STRUCTURAL PARAMETERS FOR [Fe(TPP)(NO)(4-MePip)]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fe–N&lt;sub&gt;pyr&lt;/sub&gt; short, Å</th>
<th>Fe–N&lt;sub&gt;pyr&lt;/sub&gt; long, Å</th>
<th>Fe–N&lt;sub&gt;Pip&lt;/sub&gt;, Å</th>
<th>Fe–N&lt;sub&gt;NO&lt;/sub&gt;, Å</th>
<th>∠FeNO, deg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe(TPP)(NO)(4-MePip)]&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.990(15)</td>
<td>2.015(7)</td>
<td>2.3998(14)</td>
<td>1.7481(13)</td>
<td>140.30(12)</td>
</tr>
<tr>
<td>[Fe(TPP)(NO)(4-MePip)]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0103(4)</td>
<td>2.0331(4)</td>
<td>2.3998</td>
<td>1.75474</td>
<td>139.58</td>
</tr>
<tr>
<td>[Fe(TPP)(NO)(4-MePip)]·CHCl&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.003(11)</td>
<td>2.015(4)</td>
<td>2.2834(17)</td>
<td>1.7512(18)</td>
<td>138.04(15)</td>
</tr>
<tr>
<td>[Fe(TPP)(NO)(4-MePip)]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.014(7)</td>
<td>2.0256(16)</td>
<td>2.2834</td>
<td>1.76715</td>
<td>139.17</td>
</tr>
<tr>
<td>[Fe(TPP)(NO)(4-MePip)]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.014(6)</td>
<td>2.027(2)</td>
<td>2.2305</td>
<td>1.77255</td>
<td>139.32</td>
</tr>
</tbody>
</table>

<sup>a</sup> experimentally determined crystal structure. <sup>b</sup> DFT predicted values. The refinement was made holding the Fe–N<sub>4</sub>–MePip bond constant at 2.3998 Å. <sup>c</sup> DFT predicted values. The refinement was made holding the Fe–N<sub>4</sub>–MePip bond constant at 2.2834 Å. <sup>d</sup> DFT predicted values, all parameters were refined.

Thirty years ago, before knowledge of the role of NO in biology, two crystalline forms of [Fe(TPP)(NO)(4-MePip)] were isolated and characterized by Scheidt et al. The ν<sub>NO</sub> of the unsolvated form was found to be shifted 15 cm<sup>−1</sup> toward higher frequency when compared to the unsolvated form. The shift in ν<sub>NO</sub> value was attributed to the 0.14 Å elongated Fe–N<sub>4</sub>–MePip bond in the unsolvated form. [Fe(TPP)(NO)(4-MePip)] and [Fe(TPP)(NO)(4-MePip)]·CHCl<sub>3</sub> were prepared to investigate the effect of the Fe–N<sub>4</sub>–MePip bond length on the overall vibrational dynamics. Synthetic and structural information are presented in Chapter 7.

DFT was used to calculate the energy minimized structure and iron vibrational modes of [Fe(TPP)(NO)(4-MePip)] using the unsolvated form as an starting structure. Two additional calculations were conducted refining all parameters with the exception of the Fe–N<sub>4</sub>–MePip bond for the long (2.3998(14) Å Fe–N<sub>4</sub>–MePip bond) and short (2.2834(17) Å Fe–N<sub>4</sub>–MePip bond) forms of [Fe(TPP)(NO)(4-MePip)]. Notable predicted and experimentally determined structural characteristics of the [Fe(TPP)(NO)(4-MePip)] calculations are given in Table 10.3. The calculated
structural trends agree well with the trends observed in the experimentally determined structures.

Predicted Fe vibrational modes also agree well with the expected trends resulting from Fe–N$_{4-\text{MePip}}$ elongation. The predicted out-of-plane NRVS spectra for the long (2.3998(14) Å Fe–N$_{4-\text{MePip}}$ bond), short (2.2834(17) Å Fe–N$_{4-\text{MePip}}$ bond), energy minimized forms of [Fe(TPP)(NO)(4-MePip)] are given in Figure 10.5. The elongation of the Fe–N$_{4-\text{MePip}}$ bond causes the shift of several modes with Fe–N$_{4-\text{MePip}}$ character to lower frequencies. Modes with significant Fe–N$_{4-\text{MePip}}$ motion, and mode composition factors are given in Figure 10.6, Figure 10.7, and Table 10.4. The energy minimized structure contains the shortest Fe–N$_{4-\text{MePip}}$ bond (2.2305 Å), and subsequently the highest frequency modes with significant Fe–N$_{4-\text{MePip}}$ character. Modes at ~95, 123, 138, ~145, 206, and 330 cm$^{-1}$, shift to 75, 100, 106, 125, 204, and 315 in the prediction with the longest Fe–N$_{4-\text{MePip}}$ bond (2.3998 Å) (see Table 10.4 and Figure 10.5). The minor Fe–N$_{4-\text{MePip}}$ stretching modes at ~95 and 138 cm$^{-1}$, the ligand rotational (or bending) mode at ~145 cm$^{-1}$, the major $\nu_{\text{Fe–4MePip}}$, and FeNO translation show a large dependence on the Fe–N$_{4-\text{MePip}}$ bond length. The mode near 205 cm$^{-1}$, however, shows little dependence on the Fe–N$_{4-\text{MePip}}$ bond length. The total predicted kinetic energy contribution to the 205 cm$^{-1}$ mode from the NO–Fe–4-MePip unit is 13%, the rest of KED is involved in motion of the porphyrin and periphery. This mode is contrasted with the lowest frequency minor $\nu_{\text{Fe–4MePip}}$ value where the kinetic energy contribution from the NO–Fe–4-MePip unit is 80% (Fe–N$_{4-\text{MePip}}$ = 2.3998 Å) and 59% (Fe–N$_{4-\text{MePip}}$ = 2.2305 Å). Additional modes in the 400–600 cm$^{-1}$ region shift slightly (< 2 cm$^{-1}$) to higher frequency as the Fe–N$_{4-\text{MePip}}$ distance is increased. The 400–600 cm$^{-1}$ region has previously been assigned to FeNO modes.
As described in Chapter 8, the FeNO vibrational modes strongly influenced by the trans ligand.

The experimental out-of-plane spectra of [Fe(TPP)(NO)(4-MePip)] and [Fe(TPP)(4-MePip)(NO)]·CHCl₃ are given in Figure 10.8. Vibrational modes with strong out-of-plane character are observed at 33, 119, 201, 248, 309, 427, 467, and 530 cm⁻¹ for [Fe(TPP)(4-MePip)(NO)] and 30, 127, 143, 206, 248, 313, 406, 427, 469, and 534 for [Fe(TPP)(4-MePip)(NO)]·CHCl₃ (the relatively large intensity of the mode at 119 cm⁻¹ in [Fe(TPP)(4-MePip)(NO)] compared to the pair of modes near 135 cm⁻¹ in [Fe(TPP)(4-MePip)(NO)]·CHCl₃ suggests that the 119 cm⁻¹ mode may be a pair of unresolved peaks). Mode assignments are made by comparing the experimental data with the DFT predicted data. Vibrational modes assigned to Fe–N₄–MePip motion are given in Table 10.5. All four vibrational modes are dependent on the length of the Fe–N₄–MePip bond. The modes shift 7,
Figure 10.6. DFT predicted modes of [Fe(TPP)(NO)(4-MePip)] with significant Fe–Im motion. The modes are predicted from data with the Fe–N$_{4\text{-MePip}}$ bond restrained to 2.3998 Å(left) or 2.2834 Å(right).
Figure 10.7. DFT predicted modes of [Fe(TPP)(NO)(4-MePip)] with significant Fe–Im motion. The modes are predicted from data with the Fe–N_{4-MePip} bond restrained to 2.3998 Å(left) or 2.2834 Å(right).
23, 4, \sim 15 \text{ cm}^{-1} \text{ toward higher frequency when the Fe–N}_{4-MePip} \text{ bond is shortened by} 0.12 \text{ Å. The modes near 120 and 205 cm}^{-1} \text{ are assigned to the } \nu_{Fe-4MePip} \text{ and the modes near 310 cm}^{-1} \text{ are assigned to the FeNO translation. In } [Fe(TPP)(4-MePip)(NO)]\cdot\text{CHCl}_3 \text{ the 313 cm}^{-1} \text{ contribution maybe the result of a large in-plane mode being partially visible in the out-of-plane spectrum. The porphyrin packing of } [Fe(TPP)(NO)(4-MePip)]\cdot\text{CHCl}_3 \text{ in the } P2_12_12_1 \text{ space group causes the single-crystal measurement to be acquired with the X-ray beam } 90 \pm 13.8^\circ \text{ from the porphyrin plane, rather than the typical } 90^\circ \text{ orientation which should more fully suppress the in-plane vibrational modes. Finally, the modes in the } 400–600 \text{ cm}^{-1} \text{ region shift to higher frequency as the Fe–N}_{4-MePip} \text{ bond length is increased. These modes, which have previously been assigned to FeNO, do not follow the same pattern observed in the predicted spectra. The mode near 425 cm}^{-1} \text{ for } [Fe(TPP)(NO)(4-MePip)] \text{ is predicted to be the Fe–NO stretch, however it is difficult to make a one-to-one correlation between the mode near 427 cm}^{-1} \text{ in } [Fe(TPP)(NO)(4-MePip)] \text{ and a split set of modes at 409 and 437 cm}^{-1} \text{ in the solvated form. The modes at 467 and 531 cm}^{-1} \text{ shift to higher frequency (469 and 534 cm}^{-1} \text{) when the Fe–N}_{4-MePip} \text{ bond is shortened. These shifts are of the same magnitude observed in the predicted spectra, but the opposite sense. This observation is also contrary to the 0.003 Å shorter Fe–N}_{4-MePip} \text{ bond in } [Fe(TPP)(4-MePip)(NO)]\cdot\text{CHCl}_3. \text{ It is clear from the split nature of the peak near 420 cm}^{-1} \text{ in the NRVS data of } [Fe(TPP)(4-MePip)(NO)]\cdot\text{CHCl}_3 \text{ that there are additional influences to the positioning of this mode that are not properly modeled. Perhaps the variation in the O–N···N–C}_1 \text{ dihedral angle } (–21^\circ \text{ for } [Fe(TPP)(4-MePip)(NO)]\cdot\text{CHCl}_3 \text{ and } 61^\circ \text{ for } [Fe(TPP)(4-MePip)(NO)]) \text{ influence the mode compositions in a manner not observed in the } [Fe(Porph)(NO)(1-MeIm)] \text{ complexes.}
TABLE 10.4

PREDICTED VIBRATIONAL MODES IN [Fe(TPP)(NO)(4-MePip)]
WITH SIGNIFICANT Fe–N₄–MePip MOTION

<table>
<thead>
<tr>
<th>Mode</th>
<th>[Fe(TPP)(NO) (4-MePip)]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>[Fe(TPP)(NO) (4-MePip)]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>[Fe(TPP)(NO) (4-MePip)]&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>minor stretch</td>
<td>75&lt;sup&gt;c&lt;/sup&gt; (0.04)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>92/97 (0.01 total)</td>
<td>93/97 (0.04 total)</td>
</tr>
<tr>
<td>minor stretch</td>
<td>100 (0.03)</td>
<td>120 (0.15)</td>
<td>123 (0.01)</td>
</tr>
<tr>
<td>major stretch</td>
<td>106 (0.24)</td>
<td>128 (0.15)</td>
<td>138 (0.23)</td>
</tr>
<tr>
<td>bend</td>
<td>125/125 (0.03 total)</td>
<td>138 (0.01)</td>
<td>143/143/147 (0.01 total)</td>
</tr>
<tr>
<td>minor stretch</td>
<td>204 (0.05)</td>
<td>206 (0.05)</td>
<td>206 (0.06)</td>
</tr>
<tr>
<td>FeNO translation</td>
<td>315 (0.31)</td>
<td>330 (0.33)</td>
<td>333 (0.33)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DFT predicted values. The refinement was made holding the Fe–N₄–MePip bond constant at 2.3998 Å.<br />
<sup>b</sup> DFT predicted values. The refinement was made holding the Fe–N₄–MePip bond constant at 2.2834 Å.<br />
<sup>c</sup> DFT predicted values, all parameters were refined freely.<br />
<sup>d</sup> ε<sub>Fe</sub>².

Single-crystal NRVS data acquired on [Fe(TPP)(NO)(1-MeIm)] contains modes with significant out-of-plane character at 40, 148, 174, 211, and 311 cm⁻¹. The DFT predicted out-of-plane spectrum of [Fe(TPP)(NO)(1-MeIm)] is compared to the experimentally acquired spectrum in Figure [10.9]. The calculated data predict four modes with significant Fe–Im motion and having frequencies of 145, 166, 209, and 326 cm⁻¹. DFT calculations suggest that the two lowest frequency modes involve iron out-of-plane motion along with rotational and translational motion of the imidazole ligand and have 71% and 78% of the mode kinetic energy localized on the Fe(1-MeIm)(NO) fragment. The mode predicted at 209 cm⁻¹ has only 11% of the mode kinetic energy localized on the Fe(1-MeIm)(NO) fragment. Finally, the mode predicted at 322 cm⁻¹ has 37% of the mode kinetic energy localized on the Fe(1-MeIm)(NO) fragment. These modes all have substantial Fe–Im motion, however they have little resemblance to a two-body Fe–Im oscillator. The predicted modes and there mode composition factors are given in Figure [10.10].
TABLE 10.5

EXPERIMENTAL VIBRATIONAL MODES IN
[Fe(TPP)(4-MePip)(NO)] AND [Fe(TPP)(4-MePip)(NO)]·CHCl₃ WITH
SIGNIFICANT Fe–N₄–MePip MOTION

<table>
<thead>
<tr>
<th>[Fe(TPP)(NO)(4-MePip)]</th>
<th>[Fe(TPP)(NO)(4-MePip)]·CHCl₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>119⁴</td>
<td>127</td>
</tr>
<tr>
<td>119⁵</td>
<td>143</td>
</tr>
<tr>
<td>201</td>
<td>206</td>
</tr>
<tr>
<td>309</td>
<td>313/323⁶</td>
</tr>
</tbody>
</table>

⁴ cm⁻¹, ⁵ the relatively large intensity of the mode at 119 cm⁻¹ in [Fe(TPP)(4-MePip)(NO)] compared to the pair of modes near 135 cm⁻¹ in [Fe(TPP)(4-MePip)(NO)]·CHCl₃ suggests that the 119 cm⁻¹ mode may be a pair of unresolved peaks. ⁶ the 313 cm⁻¹ contribution maybe the result of a large in-plane mode being partially visible in the out-of-plane spectrum due to porphyrin packing of [Fe(TPP)(NO)(4-MePip)]·CHCl₃ in the P2₁2₁2₁ space group causing the measurement to be acquired with the X-ray 90±13.8° from the porphyrin plane rather that the typical 90° orientation.
predicted modes with motions of all atoms in [Fe(TpFPP)(1-MeIm)(NO)] is given in Figure 10.11.

The first two vibrational modes in [Fe(TPP)(NO)(1-MeIm)] at 148 and 174 cm\(^{-1}\) involve iron out-of-plane motion along with rotational and translational motion of the imidazole. The mode at observed at 211 cm\(^{-1}\) is assigned to the \(\nu_{\text{Fe--Im}}\), although only a small portion of the kinetic energy of this mode is predicted to involve NO-Fe-Im motion. Finally, the mode observed experimentally at 322 cm\(^{-1}\) is assigned to the FeNO translation. All four of these modes are similar to those observed in six-coordinated heme carbonyls, see Figures 10.3 and 10.4. However, the Fe–L bond trans to the diatomic ligand is >0.1 Å longer in the six-coordinate nitrosyl complexes than the six-coordinate carbonyl complexes of [Fe(Porph)(1-MeIm)(XO)]; three of the four modes with significant Fe–Im motion shift to lower frequency when XO is nitric oxide. The vibrational modes assigned to the Fe–
Figure 10.9. Comparison of experimental out-of-plane NRVS spectra of [Fe(TPP)(NO)(1-MeIm)] (black) and DFT predicted out-of-plane spectra of [Fe(TPP)(NO)(1-MeIm)] (grey). Experimental spectra have been smoothed (five point). DFT predicted mode values are convolved with 16 cm\(^{-1}\) Lorentzian functions.

Im motion in [Fe(TPP)(1-MeIm)(CO)] are at 147, 174, 211, and 322 cm\(^{-1}\) (see Figure 10.10), while Fe–Im modes for [Fe(TPP)(1-MeIm)(CO)] are observed at 151, 172, 225, and 331 cm\(^{-1}\) (see Figure 10.3). The large change in Fe–Im bond length causes several small changes in vibrational modes with Fe–Im character. This experimental observation supports the idea of distributed Fe–Im vibrational modes, thereby making a singular \(\nu_{\text{Fe-Im}}\) mode assignment incomplete.

A detailed investigation of the effects of the Fe–Im bond length on the vibrational dynamics in [Fe(TpFPP)(NO)(1-MeIm)] was presented in the previous Chapter 9. The two polymorphic forms of [Fe(TpFPP)(NO)(1-MeIm)], \(\text{tri-}[\text{Fe(TpFPP)(NO)(1-MeIm)}]\) and \(\text{mono-}[\text{Fe(TpFPP)(NO)(1-MeIm)}]\), contained a number of vibrational modes that shifted to a lower frequency as a result of the longer Fe–Im bond in \(\text{tri-}[\text{Fe(TpFPP)(NO)(1-MeIm)}]\). This experiment suggested that the Fe–Im bond length, not the relative orientation of the axial ligands causes the observed differences in the vibrational dynamics of Fe. Presented below is the
<table>
<thead>
<tr>
<th>Structure</th>
<th>Measured (Units, Error)</th>
<th>Calculated (Units, Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe(TPP)-(1-MeIm)(NO)]</td>
<td>148 (0.06, 0.43)</td>
<td>140 (0.12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147 (0.08, 0.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>153 (0.07)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>147 (0.08, 0.70)</td>
</tr>
<tr>
<td>tri-[Fe(TpFPP)-(1-MeIm)(NO)]</td>
<td>164 (0.10, 0.68)</td>
<td>167 (0.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>163 (0.10, 0.62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>165 (0.06, 0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>164 (0.03, 0.27)</td>
</tr>
<tr>
<td>mono-[Fe(TpFPP)-(1-MeIm)(NO)]</td>
<td>208 (0.02, 0.09)</td>
<td>206 (0.08, 0.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 (0.06, 0.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>195 (0.06, 0.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>174 (0.09, 0.18)</td>
</tr>
<tr>
<td>[Fe(TpOCH3PP)-(1-MeIm)(NO)]</td>
<td>329 (0.33, 0.36)</td>
<td>313 (0.32, 0.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>316 (0.30, 0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>323 (0.14, 0.16)</td>
</tr>
</tbody>
</table>

Figure 10.10. Experimentally observed (black) and DFT predicted (red) vibrational modes of [Fe(TPP)(NO)(1-MeIm)], tri-[Fe(TpFPP)(NO)(1-MeIm)], mono-[Fe(TpFPP)(NO)(1-MeIm)], and [Fe(TpOCH3PP)(NO)(1-MeIm)]. Measured (black) and calculated $\epsilon_{Fe}^2$ (red) and $\epsilon_{Fe}^2 + \epsilon_{Im}^2$ (blue) values are given in parentheses.
Figure 10.11. Predicted vibrational modes of $[\text{Fe(TpFPP})(1-\text{MeIm})(\text{NO})]$ with frequencies (a) 147, (b) 163, (c) 200, and (d) 320 cm$^{-1}$ involve Fe–Im motion.
assignment of the Fe–Im vibrational modes in [Fe(TpOCH3PP)(NO)(1-MeIm)].

As described in Chapters 7–9, several of the vibrational and structural features are affected by the Fe–Im bond length. However, cis electronic effects and peripheral effects also influence these parameters. These three influences make it difficult to correlate the length of the Fe–Im length with the Fe–Im vibrational modes across a range of porphyrinates with varied peripheral substituents. Previous studies on [Fe(TpFPP)(NO)(1-MeIm)] and [Fe(TPP)(NO)(4-MePip)] are more appropriate to study the systematic influences of the Fe–Im bond length on the Fe vibrational dynamics.

The out-of-plane NRVS spectra for tri-[Fe(TpFPP)(NO)(1-MeIm)], [Fe(TPP)-(NO)(1-MeIm)], [Fe(TpOCH3PP)(NO)(1-MeIm)], and mono-[Fe(TpFPP)(NO)(1-MeIm)] are displayed in Figure 10.12. Assignments of the modes with large Fe–Im motion are made using DFT mode assignment predictions and assignments discussed previously. The values for all modes assigned to the Fe–Im motion are given in Figure [10.10] and Table [10.6]. The lowest and second lowest frequency modes in the two polymorphic forms of [Fe(TpFPP)(NO)(1-MeIm)] show an increase in the Fe–Im mode frequency as the Fe–Im bond length is shortened. Comparing these modes in [Fe(TPP)(NO)(1-MeIm)] and [Fe(TpOCH3PP)(NO)(1-MeIm)] with mono-[Fe(TpFPP)(NO)(1-MeIm)], where the length of the Fe–Im bond is similar, a > 7 cm\(^{-1}\) shift to higher frequency is observed. This discontinuity may be the result of peripheral or cis electronic effects, although parsing these influences would be difficult. The mode near 200 cm\(^{-1}\), in which the majority of the KED is located on the porphyrin and periphery, also shows no correlation with the length of the Fe–Im bond. Finally, the mode near 310 cm\(^{-1}\), the FeNO translation, seems to be more strongly correlated with the length of the Fe–Im bond.
Figure 10.12. Experimental out-of-plane NRVS spectra of (from top to bottom) tri-[Fe(TpFPP)(NO)(1-MeIm)], [Fe(TPP)(NO)(1-MeIm)], [Fe(TpOCH₃PP)(NO)(1-MeIm)], and mono-[Fe(TpFPP)(NO)(1-MeIm)]. Spectra have been smoothed (five point).
The Fe–Im bond length values in *tri*-\([\text{Fe(TpFPP})(\text{NO})(1-\text{MeIm})]\), [\text{Fe(TPP)}(\text{NO})(1-\text{MeIm})], [\text{Fe(TpOCH}_3\text{PP})(\text{NO})(1-\text{MeIm})], and *mono*-\([\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]\) do not correlate well with the four vibrational modes assigned to Fe–Im motion. The vibrational modes with Fe–Im motion are distributed over several frequencies in each of these complexes. The distribution of KE contributions to the each Fe–Im mode is most likely different for the varying porphyrinates. This in conjunction with the unmodeled peripheral substitution and cis-electronic effects, make the observation of correlations between the Fe–Im bond length and the Fe–Im vibrational modes difficult. However, in the systems with similar porphyrinates, [\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})] and [\text{Fe(TPP)}(\text{NO})(4-\text{MePip})], correlations of the Fe–Im bond length and vibrational modes are observed.

The Fe–Im vibrational modes, as predicted from density functional theory, are similar in *tri*-\([\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})], [\text{Fe(TPP)}(\text{NO})(1-\text{MeIm})], [\text{Fe(TpOCH}_3\text{PP)}(\text{NO})(1-\text{MeIm})], and *mono*-\([\text{Fe(TpFPP)}(\text{NO})(1-\text{MeIm})]\) (see Figure 10.10) despite the variation in the Fe–Im bond length and varied nature of the porphyrinates. Additionally it is interesting to note that the Fe–Im vibrational modes are very similar in the measured [\text{Fe(TPP)}(\text{CO})(\text{XIm})] and [\text{Fe(Porph)}(\text{NO})(1-\text{MeIm})] complexes.
Comparing the modes in Figure 10.4 and Figure 10.10 an increase in the frequency of each mode is observed for the carbonyl complexes. This agrees well with the >0.1 Å decrease in the length of the Fe–Im bond. However, the motions of the four vibrational modes associated with the Fe–Im stretch are very similar in all six complexes. Only small differences are observed as a result of the reduction in symmetry associated with the bent FeNO unit.

10.3.3 Fe–Im Vibrational Modes in [Fe(TpivPP)(1-MeIm)(O₂)]

The strength of the Fe–His bond in the five-coordinate systems has been used as a gauge of protein ligation and activation states, and as a possible predictor of protein activity.[58] Some insight into the nature of the Fe–His(Im) bond post ligand ejection has been attained through flash photolysis experiments with MbXO and HbXO.[68] [231] [232] However, directly studying the Fe–Im bond in [Fe(Porph)(1-MeIm)(O₂)] complexes has been difficult. NRVS has allowed for the
observation of Fe–Im in a [Fe(Porph)(1-MeIm)(O₂)] for the first time.

Powder NRVS and DFT predicted NRVS spectra are used to assign the Fe–Im modes in [Fe(TpivPP)(1-MeIm)(O₂)]. The predicted powder and out-of-plane spectra are given in Figure 10.13. The first two modes predicted at 150 and 152 cm⁻¹ involve iron out-of-plane motion along with rotational and translational motion of the imidazole. These modes have similar composition to the first two Fe–Im modes predicted for six-coordinate carbonyls and nitrosyls. The next three modes are located at 176, 193, and 200 cm⁻¹. The first mode, at 176 cm⁻¹, has 24% of its KED involved in the motion of the Im ligand while only 6% involved the motion of FeO₂. The modes at 193 and 200 cm⁻¹ each have ~ 20% of their KED involved in the motion of FeO₂ and 20% of their KED involved in the motion of Im. Finally, the mode at 330 cm⁻¹ is again assigned to the FeXO translation, although in [Fe(TpivPP)(1-MeIm)(O₂)] only 22% of the total KED involves the motion of the FeO₂ unit (~35% for [Fe(TPP)(1-MeIm)(CO)] and (~40% for [Fe(TPP)(1-MeIm)(NO)]).
TABLE 10.7

VIBRATIONAL MODES IN [Fe(Porph)(XO)(1-MeIm)] WITH SIGNIFICANT Fe–Im MOTION

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fe–Im, Å</th>
<th>151</th>
<th>172</th>
<th>225</th>
<th>331</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Fe(TPP)(CO)(1-MeIm)] 2.0503(14)</td>
<td>170</td>
<td>202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe(TpivPP)(1-MeIm)(O₂)] 2.07(2)</td>
<td>170</td>
<td>202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe(TPP)(NO)(1-MeIm)] 2.1698(10)</td>
<td>148</td>
<td>174</td>
<td>211</td>
<td>311</td>
<td></td>
</tr>
</tbody>
</table>

a cm⁻¹. b from reference [145]

The predicted out-of-plane and experimental powder spectra are given in Figure 10.14. The experimental powder data have two peaks in the region assigned to the Fe–Im rotation (bend) and the Fe–Im stretching modes. These modes are observed experimentally at 170 cm⁻¹ for the Fe–Im rotation (bend) and 202 cm⁻¹ for the Fe–Im stretch. Only two distinct modes are observed in this region, however, multiple unresolved modes could contribute to either of these peaks. Additionally, analyzing only the powder data makes a definitive assignment of Fe–Im vibrational modes difficult. A more definite assignment of the modes in this region, and an observation of the FeO₂ translational mode, might be possible if a suitable single-crystal measurement was available. At this time no single-crystal sample of [Fe(TpivPP)(1-MeIm)(O₂)] has been measured.

10.4 Summary

This chapter describes the Fe–Im vibrational modes in [Fe(Porph)(XO)(L)] complexes where XO = CO, NO, and O₂. Modes with significant Fe–Im motion in Fe(Porph)(XO)(1-MeIm)] are given in Table 10.7. The length of the Fe–Im bond is correlated with the Fe–Im vibrational modes in the nitrosyl and carbonyl
complexes. The shifts in frequencies, however, are small and distributed between at least four vibrational modes. Comparing the oxygen complex with the nitrosyl and carbonyl complexes is more difficult. The correlation between the Fe–Im bond length and the Fe–Im vibrational modes is no longer observed when the O₂ complex is included in the analysis. This may be the result of the influences from the picket fence porphyrinate or the modes may be assigned improperly due to the lack of single-crystal data.

Contributions to the Fe–Im motion are distributed over at many vibrational modes. Modes with Fe–Im rotation (bending), Fe–Im stretching, and FeXO translation are observed for all six-coordinate complexes in which single-crystal NRVS data has been measured. The nature of these modes are similar, yet their frequencies vary depending on the coordinated diatomic ligand and the porphyrinate used in the investigation. No single vibrational mode may be described as the Fe–Im stretch, the motion of this bond along the heme normal is a combination of many vibrational modes, none of which resemble a two-body oscillator. These investigations provide a detailed description of the Fe–Nᵢm vibrational modes which are believed to be involved in signaling processes of diatomic gas sensing heme proteins.
CHAPTER 11

CONCLUSION

The initial goal of this research project was to study the $\nu_{\text{Fe-His(Im)}}$ mode in six-coordinate heme diatomic complexes. It is apparent from the preceding chapters that the work done in this dissertation is not only a study of the Fe–Im bond in heme complexes, but also a collection of investigations studying the interaction of diatomic ligands with heme and the changes in structural and vibrational parameters that are caused by diatomic molecule ligation.

The interaction of diatomic molecules with heme has several consequences. For the biologically relevant, high-spin iron(II) hemes with histidine(imidazole) as a ligand, coordination results in a spin transition to low spin and movement of the iron atom into the plane of the porphyrin. Six-coordinate heme carbonyls have Fe–XO bonds ranging from 1.70–1.80 Å, although more typically these bonds are $\sim$1.75 Å. For the five-coordinate heme carbonyls this bond length decreases to $\sim$1.71 Å reflecting the loss of the competition of the imidazole for bonding electrons. The $\nu_{\text{C-O}}$ for both the five- and six-coordinate species are similar, suggesting that the Fe $\pi$-donation into the CO $\pi^*$ orbitals is not perturbed by the imidazole ligand. However, the $\pi$ system is greatly perturbed by the solid-state interactions between CO and adjacent molecules. The IR spectra of five [Fe(Porph)(CO)(1-MeIm)] species showed similar values of $\nu_{\text{C-O}}$ in toluene solution, however, large differences (55 cm$^{-1}$ range) in $\nu_{\text{C-O}}$ are observed in the solid
state. These frequency differences result from the differing crystalline environments of the CO. Finally, with data of sufficient accuracy a correlation of Fe–C vs C–O distances may be made akin to the spectroscopically based correlations of \( \pi \)-back-bonding observed in metal carbonyls.

The Fe–Im bond has been discussed at great length in this dissertation, including its biological significance. The coordination of diatomic molecules to heme has a large effect on the Fe–Im bond. The average value for a Fe–Im bond in [Fe(Porph)(XIm)] decreases from 2.147 Å to 2.04–2.10 Å in [Fe(TPP)(XIm)-(CO)] and increases to \( \sim 2.16 \) Å in [Fe(TPP)(XIm)(NO)] (100 K). The changes that incur as a result of CO coordination reflect the high-spin to low-spin transition. However, the large trans-deactivating effect of NO, leads to an elongation of the Fe–Im bond upon NO coordination. This weak bond (Fe–Im) is especially susceptible to changes in the cell volume. The length of this bond is correlated with the contraction and expansion of the unit cell in the solid state. Changes in the Fe–Im bond length have only a modest effect on the Fe–NO bond length, however, they influence the \( \nu_{N-O} \) though the NOFeIm \( \pi \)-system. The vibrational dynamics of Fe are strongly affected by coordination of a diatomic molecule. The vibrational consequence of changes in the Fe–Im bond are discussed in Chapter 10. The variation in the strength of the Fe–Im bond between unligated, CO ligated, and NO ligated is reflected in the Fe–Im vibrational modes. Overall shifts in modes attributed to Fe–Im motion are observed, however, the differences per mode are small. The Fe vibrational modes with strong FeXO character are also observed upon diatomic molecule coordination.

The nature of the Fe-ligand modes are addressed in Chapters 6, 8 and 10 detailing the assignment of modes that were difficult to definitively assign using
IR or rR. Modes with Fe–Im rotation (bending), Fe–Im stretching, and FeXO translation are observed for all six-coordinate complexes in which single-crystal NRVS data has been measured. These experiments describe four major modes with Fe–Im motion. The two lowest frequency modes had motion parallel to the heme normal, however, also contained rotational motion of the imidazole ligand. The third vibrational mode showed only a small amount of total kinetic energy attributed to motion of XOFeIm unit. Finally, the fourth mode, which may be observed only in single-crystal NRVS data, is assigned as a FeXO translational mode. These four modes have not been described previously for six-coordinated heme nitrosyls. Finally, the FeNO vibrations are strongly mixed with at least three modes having both $\nu_{\text{Fe-NO}}$ and $\delta_{\text{FeNO}}$ character, however, for simplicity a single (best) mode is assigned for each of these peaks (see Chapter 8). Neither the Fe-ligand modes of the NO complexes nor the CO complexes resemble two-body oscillators.

This dissertation investigates the effect of diatomic molecules on heme. The structural and vibrational consequences of these interactions are believed to be involved in biological processes of heme proteins. Small molecular models of these systems have aided in the understanding of specific interactions and small structural variations that may be difficult to observe in the biological systems. These investigations ultimately model changes in ligation state, and the sequential changes in bonding parameters, essential to heme protein function.
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