The vibrations originating from the iron phenolate bound chromophores reproduced those reported for heme tyrosine active sites in nature. The EPR and Fe-N$_{pyr}$ vibrations of phenolate and thiolate iron porphyrin complexes indicate that the phenolate ligand acts as a π-anisotropic ligand which is less covalent than a thiolate ligand. While the Fe$^{III/II}$ potential of the phenolate compound in a non-coordinating solvent is 110 mV more negative than that of the thiolate complex. DFT calculations reproduce the experimental results. The higher covalency of the thiolate ligand is responsible for the lower Fe-N$_{pyr}$ vibration whereas the greater electrostatic stabilization of the Fe$^{III}$-OPh bond is responsible for lowering the Fe$^{III/II}$ $E^0$ of the phenolate complex relative to the thiolate complex in a medium having a reasonable dielectric constant.
2.1. Introduction

The ability of axial ligands to modulate the chemistry of iron-porphyrins has been an area of considerable interest for many years.\textsuperscript{1-5} Most of the chemistry has been, however, focused on thiolate and imidazole bound iron porphyrin complexes because of the ubiquity of heme containing proteins bearing a cysteine (which bears a thiolate side chain) and histidine (which bears an imidazole side chain) axial ligands.\textsuperscript{6-12} In the case of heme catalases and some variants of hemoglobin, the heme cofactor is bound to phenolate oxygen from a tyrosine residue (Figure 2.1).\textsuperscript{13-15} The sixth coordination is less clear and a water molecule is often proposed to be ligated in the resting form. It appears to be vacant in the resting bovine catalase, as judged from the absence of electron density above the iron in the x-ray diffraction data, whereas electron density near the distal histidine in P. vitale catalase might be due to water molecule.\textsuperscript{15-17} This enzyme is highly efficient catalysing the dismutation of \( \text{H}_2\text{O}_2 \) and is only able to oxidize low molecular weight alcohols (e.g. methanol, ethanol) using \( \text{H}_2\text{O}_2 \).\textsuperscript{18-20} In the course of the enzymatic cycle, the active site of catalase gets oxidized to produce a highly reactive species known as compound 1.\textsuperscript{21-24} This species is best described as a high valent \( \text{Fe}^{IV}=\text{O} \) species with a radical cation localized on the porphyrin ring.\textsuperscript{25-29} Similar species have been generated in other heme proteins like cytochrome P450\textsuperscript{30-35} and even in \( \text{O}_2 \) carrier proteins like hemoglobin (Hb)\textsuperscript{36, 37} and myoglobin (Mb).\textsuperscript{38-40} The Fe center in the active site of the catalase in its resting form is best described as a five-coordinate (5C) high spin (HS) \( \text{Fe}^{III} \). Catalases are difficult to reduce under physiological conditions as their \( \text{Fe}^{III/II} \text{E}_0 \) are quite low (\(<-500 \text{ mV}\)) in solution and can only be stabilized via the formation of a CO adduct after photochemical reduction.\textsuperscript{41-43} This is a very important attribute of the catalase active site as the reaction of \( \text{H}_2\text{O}_2 \) with a ferrous center will lead to Fenton type reaction and generate reactive oxygen species.

Heme bound to tyrosine ligands were known previously only in hemoglobin (Hb) mutants.\textsuperscript{43, 44} The crystal structure of Hb M Boston shows that the distal histidine is replaced by a tyrosine that coordinates to the \( \text{Fe}^{III} \) and displaces the usual proximal His, yielding a five-coordinated iron that is incapable of \( \text{O}_2 \) binding.\textsuperscript{44} In Hb M Iwate, the
proximal histidine is replaced by tyrosine, low resolution x-ray data indicate that the iron is hexa-coordinated, binding both the abnormal tyrosine and the distal His.\textsuperscript{43} Resonance Raman (rR) spectra of these mutant hemoglobin proteins were investigated by Nagai et al. and these data revealed several vibrational bands between 1300-1320 cm\textsuperscript{-1} characteristic of a tyrosinate coordination.\textsuperscript{44, 45} So far few synthetic models of catalase active site are reported that mimic the axial phenolate coordination to a Fe\textsuperscript{III} porphyrin.\textsuperscript{14, 27, 29, 46, 47} In these complexes, where an external phenolate ligand was used to bind Fe\textsuperscript{III} porphyrins, the effects of hydrogen bonding interactions on the redox properties of these complexes were investigated.\textsuperscript{46}

Figure 2.1: (left) Active site structure of bovine liver catalase enzyme (pdb id: 8CAT) and (right) the chemical representation of the active site of catalase and the reaction it catalyzes.

Recently, a series of iron porphyrin complexes with axial imidazole and thiolate ligands were reported.\textsuperscript{7} The anionic thiolate ligand lowered the Fe\textsuperscript{III}/II \textit{E}\textsubscript{0} by 400 mV relative to a neutral imidazole ligand.\textsuperscript{7} The Fe-N\textsubscript{pyr} vibration varies 370-405 cm\textsuperscript{-1} and is found to be sensitive to the oxidation and spin state of the iron and even to the extent of covalent donation of the axial ligand.\textsuperscript{48-50} It was observed at 400 cm\textsuperscript{-1} for a neutral imidazole ligand and at 390 cm\textsuperscript{-1} for an anionic thiolate ligand.\textsuperscript{7} Previous investigations using spectroscopic and computational techniques have suggested that Fe-O bonding has greater electrostatic contribution than Fe-S bonding.\textsuperscript{51, 52} How these differences in bonding affect the electronic structure and spectroscopic properties of thiolate and phenolate bound iron porphyrin complexes remain to be explored.
In this study, the spectroscopic (EPR, rR) and redox properties of a phenolate bound iron porphyrin complex (POR, Figure 2.2) are compared to those of heme catalases and analogous thiolate (PPSR-yne, Figure 2.2), imidazole (PIM, Figure 2.2) bound complexes. The results suggested that while there are certain similarities between the ground state (GS) wave functions of thiolate and phenolate bound iron porphyrin complexes, differences in the extent of electrostatic and covalent interaction between the two lead to distinct spectroscopic and redox properties.

Figure 2.2: Schematic representation of the complexes.

2.2. EXPERIMENTAL SECTION

2.2.1. Materials and Instrumentation

Required materials are already discussed in the 1\textsuperscript{st} chapter. 3-hydroxy phenyl acetylene and cupper iodide were purchased from Aldrich chemical company. The solvents are used after purification by standard procedures. Unless otherwise mentioned all reactions were performed at room temperature and the column chromatography were performed on SiO\textsubscript{2} (60-120 mesh). The absorption spectra are measured in the Agilent technologies spectrophotometer model 8453 fitted with diode array detector. The FT-IR data are measured on the Parkin Elmer spectrometer instrument. All the NMR spectra were recorded on the Bruker DPX-300 or DPX-500
spectrometer at room temperature. The EPR spectrum is recorded on a JEOL instrument. The mass spectra are recorded by QTOF Micro YA263 instrument. Resonance Raman instrument discussed earlier.

### 2.2.2. Synthesis and Characterization

The synthetic strategy is described in detail in Scheme 2.1.

**meso-Mono(o-5-bromovaleramidophenyl)triphenylporphyrin(1)**

This step was already discussed in the chapter 1.

**meso-Mono(o-5-azidovaleramidophenyl)triphenylporphyrin(2)**

Sodium azide (10 mg, 1.2 eq.) was added to the solution of 1 (100 mg, 0.126 mmol) taken in 5 mL dry and degassed DMF, and the reaction was refluxed for 4 h in the dark. The formation of the desired product was monitored by TLC. After quenching the reaction mixture with H₂O, diethyl ether was added to collect the product in the organic layer. After the removal of the solvent, the product was purified by column chromatography using a 1% methanol/DCM mixture as the eluent. The IR data confirmed that Br group is replaced by N₃. Yield: 90 mg (~94.5%).

**Anal.** Calcd for C₄₉H₃₈N₈O: C, 77.96; H, 5.07; N, 14.84. Found: C, 76.85; H, 5.72; N, 13.40. (¹H NMR, CDCl₃) : δ -2.72 (s, 2H, NH ring), 0.84-0.9 (m, 4H, -CH₂), 0.98 (m, 2H, -CH₂), 2.52 (t, 2H, -CH₂), 6.74 (s, 1H, Aromatic), 7.5 (t, 1H, aromatic), 7.77-7.85 (m, 10H, Aromatic), 8.0(d, 1H, Aromatic), 8.2-8.25 (m, 6H, Aromatic), 8.69 (d, 1H, -CONH), 8.77 (d, 2H, pyrrolic), 8.88 (s, 6H, pyrrolic). SI-MS (+ve ion mode, methanol) m/z = 755.51 (100%, [MH]⁺). IR spectra (cm⁻¹) - 2093.4 (for N₃⁻ stretch).

**meso-Mono(o-5-azidovaleramidophenyl)triphenylporphyrinzinc(II)(3)**

Azide-functionalized porphyrin 2 (100 mg, 0.132 mmol) was dissolved in 15 mL of THF. Zn(OAc)₂.2H₂O (41 mg, 1.4 equiv) was added to the solution, and the reaction mixture was stirred for 3 h at RT. The reaction was quenched by H₂O followed by the addition of DCM. The organic layer was washed with a brine solution and was collected. The solvent was removed on a rotary evaporator, and the residue was separated by chromatography on a column packed with 60-120 mesh silica gels in 2% CH₂Cl₂/MeOH. The insertion of zinc metal into the porphyrin is
confirmed by absorption spectroscopy. The absorption data is given in the supporting information (S2.13). Yield: 100 mg (92.5%)

**Anal.** Calcd for C_{49}H_{36}N_{8}OZn: C, 71.92; H, 4.43; N, 13.69. Found: C, 70.85; H, 5.02; N, 12.80. (^{1}H NMR, CDCl_{3}): δ -0.94 (m, 2H, -CH_{2}), -0.14 (m, 2H, -CH_{2}), 0.87 (m, 4H, -CH_{2}), 6.85 (s, 1H, Aromatic), 7.58 (t, 1H, Aromatic), 7.76-7.85 (m, 10H, Aromatic), 8.17-8.36 (m, 8H, Aromatic), 8.82 (d, 2H, pyrrolic), 8.95 (s, 6H, pyrrolic H). IR spectra (cm^{-1}) - 2087.8 (for N_{3}^\text{-} stretch). ESI-MS - (+ve ion mode, methanol) m/z = 816.67.

**meso-Mono[o-5(3-hydroxyphenyl-1,2,3-triazolyl)]triphenylporphyrin zinc(4)**

To a solution of 3 (100 mg, 0.12 mmol) in 20 ml of dry THF, 3-hydroxyphenylacetylene (21 μL, 1.5 eq.) was added followed by the addition of CuSO_{4}/sodium ascorbate. The reaction was stirred overnight. When the reaction was completed, the reaction mixture was quenched by water followed by the addition of DCM. The organic layer was collected, dried over anhydrous Na_{2}SO_{4} and was purified by column chromatography using 3% DCM/methanol mixture as the eluent. The disappearance of N_{3} stretching is confirmed in the IR data. Yield: 95 mg (81%).

**Anal.** Calcd for C_{57}H_{42}N_{8}O_{2}Zn: C, 73.11; H, 4.52; N, 11.97. Found: C, 69.94; H, 4.90; N, 10.08. (^{1}H NMR, CDCl_{3}): δ -0.29 (m, 2H, -CH_{2}), 0.36 (m, 4H, -CH_{2}), 1.11 (m, 2H, -CH_{2}), 4.88 (s, 1H), 5.22 (s, 1H, ), 5.55 (s, 1H), 6.01 (m, 3H, Aromatic), 6.59 (d, 1H, Aromatic), 6.79 (s, 1H, Aromatic), 7.17 (d, 1H, Aromatic), 7.37-7.59 (m, 10H, Aromatic), 7.85-8.0 (m, 6H, Aromatic), 8.46 (d, 2H, pyrrolic H), 8.71 (s, 6H, pyrrolic H). ESI-MS (+ve ion mode, methanol) m/z = 957.7 (100%, [M+Na]^+.)

**meso-Mono[o-5(3-hydroxyphenyl-1,2,3-triazolyl)]triphenylporphyrin(5)**

1 ml of 6 M HCl was added to the solution of 4 (100 mg) in 15 ml of THF, and the reaction was stirred for 1 h in the dark. Then the reaction was neutralized by aq. NH_{3} solution followed by the addition of DCM and H_{2}O. The organic layer was collected, dried over anhydrous Na_{2}SO_{4} and was purified by flash column chromatography using 2% DCM/MeOH solvent mixture. The demetallation of Zn was confirmed by Absorption spectroscopy and Mass Spectroscopy. Yield: 90 mg (95%)

**Anal.** Calcd for C_{57}H_{44}N_{8}O_{2}: C, 78.42; H, 5.08; N, 12.84. Found: C, 77.34; H, 5.90; N, 11.08. (^{1}H NMR, CDCl_{3}): δ -2.85 (s, 2H, NH ring), 0.76 (m, 2H, -CH_{2}), 0.78 (m, 2H, -CH_{2}), 0.89 (m, 2H, -CH_{2}), 3.2 (t, 2H, -CH_{2}), 6.41 (d, 1H, Aromatic), 6.82 (s, 1H, triazole H), 6.85 (d, 1H, Aromatic), 6.87 (d, 1H, Aromatic), 7.41 (t, 1H, Aromatic), 7.51-7.89 (m, 11H, Aromatic), 7.93-
8.11 (m, 7H, Aromatic), 8.41 (d, 1H, Aromatic), 8.59 (d, 2H, pyrrolic), 8.63 (s, 6H, pyrrolic). ESI-MS (+ve ion mode, acetonitrile) m/z = 873.19 (100%, [M]+).

**meso-Mono[0-5(3-hydroxyphenyl-1,2,3triazolyl)]triphenylporphyriniron(II)bromide(6)**

Phenol-functionalized porphyrin (5) (100 mg, 0.11 mmol) was dissolved in 15 mL of dry and degassed THF. 2,4,6-Collidine (40 μL, 2 eq.) was added to this solution followed by the addition of FeBr2 (98 mg, 4 eq.). The solution was stirred overnight in a glove box in the dark. The reaction mixture was quenched by H2O followed by the addition of DCM. The organic layer was washed with a brine solution and was collected and dried over anhydrous Na2SO4. The solvent was removed on a rotary evaporator, and the residue was separated by chromatography on a column packed with 60-120 mesh silica gels in CH2Cl2. The column was eluted with a 5% MeOH/CH2Cl2 mixture. Yield: 90 mg (85%).

**Anal.** Calcd for C57H42FeN8O2Br: C, 68.00; H, 4.20; N, 11.13. Found: C, 67.04; H, 4.90; N, 10.03. ESI-MS (+ve ion mode, acetonitrile) m/z = 926.62 (100%, [M]+).

**meso-Mono[0-5(3-phenolate-1,2,3-triazolyl)]triphenylporphyriniron(III)(Fe^{III}(OPhP)(7)**

Iron phenol-functionalized porphyrin (6) (90 mg, 0.132 mmol) was dissolved in 15 mL of dry and degassed methanol in the presence of activated K2CO3. The solution was stirred overnight in a glove box. The reaction mixture was then filtered using Whatmann 40, and the filtrate was evaporated and dried using a vacuum pump inside the glove box. Yield: 85 mg (~94%).

**Anal.** Calcd for C57H41FeN8O2: C, 73.95; H, 4.46; N, 12.10. Found: C, 72.80; H, 4.92; N, 11.68. ESI-MS (+ve ion mode, acetonitrile) m/z = 925.91 (100%, [M]+).

**Bulky Benzylic Thiolate (PPSR-yne)**

This molecule is synthesized by our lab.⁵³ Synthesis of an air stable thiolate bound iron porphyrin complex has been previously reported.⁵⁴ The same basic design (i.e., sterically protected thiolate ligand) has been maintained, and terminal alkyne group has been introduced to allow covalent in situ attachment of this complex on to electrodes functionalized with azide
linkers. This required significant modification of the previously reported synthetic approach. Characterization of the complexes are in the supporting information (S2.1-S2.12).

![Scheme 2.1: Synthetic Procedure of the Desired Complex.](image)

### 2.3. Results

#### 2.3.1. EPR

The X-band EPR data of the POR complex in weakly coordinating solvents like THF shows an axial high-spin (HS) signal at g=6 (Figure 2.3, green), indicative of an S=5/2 GS. This is consistent with the S=5/2 GS observed in the active site of catalases. The EPR data of the precursor phenol complex POHR also shows a HS signal under the same condition. In a coordinating solvent like MeOH, POHR shows an S=5/2 axial EPR signal with slight rhombic distortion (g = 6.2, 5.8) suggesting formation of a MeOH bound complex [POHR-MeOH] but retaining its HS GS (Figure 2.3, red). On the contrary, the phenolate bound iron complex, POR shows an S=1/2 GS with g-values at 2.49, 2.16, 1.89 at 77 K in a coordinating solvent like
MeOH (Figure 2.3, violet). Thus, POR form S=5/2 species in weakly coordinating solvent like THF and S=1/2 in a coordinating solvent like MeOH. The rhombic parameter (V/λ) of the LS S=1/2 signal, calculated using the Taylor analysis,\textsuperscript{56} is consistent with the values obtained for LS analogues of catalase active site and indicates the presence of a strong π-anisotropic ligand like phenolate (Table 2.1).\textsuperscript{57} Note than the V/λ for the thiolate bound LS PPSR-yne complex is 5.68 which is much greater than that of the POR-MeOH complex. This is later addressed using DFT calculations (\textit{vide infra}).

![Figure 2.3: X-band EPR data of the POR and POHR complexes in THF and MeOH at 77 K, 10 mW power, and gain 1 x 10⁴.](image)

<table>
<thead>
<tr>
<th>Table 2.1: EPR Parameters for the Heme Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spin</td>
</tr>
<tr>
<td>Catalase resting\textsuperscript{58}</td>
</tr>
<tr>
<td>Catalase-N\textsubscript{3}\textsuperscript{58, 59}</td>
</tr>
<tr>
<td>Cyt P450\textsuperscript{57}</td>
</tr>
<tr>
<td>POR</td>
</tr>
<tr>
<td>POR-MeOH</td>
</tr>
<tr>
<td>PPSR-yne-MeOH</td>
</tr>
</tbody>
</table>
2.3.2. Resonance Raman (rR)

Resonance Raman spectra of the complex POR are obtained at 77 K with 413.1 nm excitation. The complex POR in THF shows that the oxidation and spin state marker $v_4$ and $v_2$ bands are at 1364 cm$^{-1}$ and 1555 cm$^{-1}$ (Figure 2.4A, pink), respectively. These values indicate that Fe center in the POR complex in THF is HS Fe(III), consistent with the EPR data. Note that there is some reduced HS Fe(II) component in the spectrum with $v_4$ and $v_2$ vibration at 1347 and 1535 cm$^{-1}$, respectively. This is due to photo-reduction of POR in the laser (S2.14). The rR data of the precursor complex POHRin THF shows that the $v_4$ and $v_2$ bands are at 1363 cm$^{-1}$ and 1552 cm$^{-1}$ (S2.15), respectively. However, the rR spectrum of the POR in MeOH (Figure 2.4A, deep green) shows that the $v_4$ and $v_2$ bands are at 1368 cm$^{-1}$ and 1567 cm$^{-1}$, respectively, indicating that it exists as a six coordinated LS species, consistent with the EPR data. The rR data along with the EPR data indicate that the Fe center in complex POR and POHR in THF are HS Fe$^{III}$ and while the Fe center in complex POHR retain its HS ground state in MeOH, complex POR in MeOH exists in a LS state.

![Figure 2.4](image.png)

Figure 2.4. rR data in the A. high-energy region (1300–1600 cm$^{-1}$). B. low frequency region (700 cm$^{-1}$). Laser excitation wavelength= 413.1 nm; power = 10 mW.

Proteins and synthetic models bearing iron phenolate bonds (or iron tyrosinate in a protein active site) exhibit highly characteristic resonance-enhanced vibrational modes of the coordinated phenolate ligand. In general, Fe$^{III}$-O vibration of Fe$^{III}$-phenolate species lies between 530-600 cm$^{-1}$ (Table 2.2). The rR, in the low frequency region of the complex POR, show peaks at 573 and 589 cm$^{-1}$ corresponding to Fe-OPh vibrations (Fe–O stretch and $\nu_{6b}$) (Figure 2.4B, blue), consistent with the values reported for the active site of tyrosine bound heme.
sites (Table 2.2).\textsuperscript{61,62} These vibrations are not observed for the precursor complex POHR (which bears a protonated phenol). This supports the assignments of these vibrations as the Fe\textsuperscript{III}-O stretch resulting from the phenolate coordination to Fe\textsuperscript{III}-porphyrin. Note that two Fe-O vibrations are observed instead of one. This is analysed using DFT calculations. Multiple features are observed between 1100-1600 cm\textsuperscript{-1} which is typical of metal phenolate complexes, consistent with the values reported for catalase enzyme (S2.16) and other Fe\textsuperscript{III}-phenolate complexes.\textsuperscript{15} Unfortunately many of these vibrations overlap with the porphyrin ring modes. However, the C-O stretch at 1320 cm\textsuperscript{-1} is clearly observed in complex POR and not in the complexes POHR, PIM and PPSR-yne, further supporting phenolate coordination in complex POR (Figures 2.5B and S2.17). Note that while the vibrations observed for POR are consistent with those observed for mutants of Hb bearing an axial tyrosine ligand and another synthetic model complex, these vibrations are not resolved for catalase active sites.\textsuperscript{15,45,62} Generally Fe–O vibrations have been selectively enhanced with excitation near 500 nm.\textsuperscript{15,61} However, here the rR data suggest that the Fe–O vibrations are observed by exciting into the soret band. This implies mixing of the porphyrin and the Fe–OPh bonding orbitals in these complexes (vide infra).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|l|}
\hline
Active Site & \textit{v}(Fe-O) & \textit{v}(C-O) & Ref. \\
\hline
Hemoglobin M Boston & 603 & 1279 & \textsuperscript{45} \\
Hemoglobin M Iwate & 588 & 1308 & \textsuperscript{62} \\
Bovine liver catalase & 1244 (?) & & \textsuperscript{15} \\
[Fe(TTOP)]\textsubscript{2} & 623 & 1293 & \textsuperscript{15} \\
POR & 573, 589 & 1320 & This work \\
\hline
\end{tabular}
\caption{Characteristic Resonance Raman Frequencies (cm\textsuperscript{-1}) for Fe(III)-Phenolates}
\end{table}

The \textit{v}_8 vibration, which represents the Fe-N\textsuperscript{pyr} (Fe-pyrrole nitrogen) symmetric stretch, is observed at 395 cm\textsuperscript{-1} for the S = 5/2 POR in THF (Figure 2.5A, red).\textsuperscript{63} The Fe-N\textsuperscript{pyr} vibration reflects the relative donor strengths of the axial ligands between complexes having the same spin states. The data suggest that the S = 5/2 PIM complex, which has an axial imidazole ligand, has a higher Fe-N\textsuperscript{pyr} vibration (400 cm\textsuperscript{-1}) relative to the S = 5/2 PPSR-yne and POR complexes, which
have a thiolate and phenolate axial ligand, respectively. This indicates that the thiolate and phenolate axial ligands are much better donors than imidazole. Further the Fe-N$_{\text{pyr}}$ of the thiolate bound PPSR-yne is at 390 cm$^{-1}$ which is 5 cm$^{-1}$ weaker than the Fe-N$_{\text{pyr}}$ vibration of the phenolate bound POR complex (395 cm$^{-1}$). Similarly the Fe-N$_{\text{pyr}}$ vibration of the LS PIM-MeOH, POR-MeOH and PPSR-yne-MeOH complexes are at 395 cm$^{-1}$, 391 cm$^{-1}$ and 389 cm$^{-1}$, respectively, i.e. showing the same trend observed for the 5C HS complexes (Figure S2.18). DFT calculations have been utilized to understand this effect in detail (*vide infra*).

![Figure 2.5](image)

Figure 2.5. rR data in the A. low frequency region (300-700 cm$^{-1}$). B. high-energy region A. (1250–1350 cm$^{-1}$).

### 2.3.3. Cyclic Voltammetry (CV)

Cyclic Voltammetry of the POR complex shows one oxidation reduction processes (Figure 2.6, red). The quasi-reversible wave with E$_{1/2}$ of -1.14 V represents the Fe$^{\text{III/II}}$ couple; consistent with the values reported for other phenolate bound iron porphyrin complexes. The thiolate complex shows a reversible wave with E$_{1/2}$ of -1.03 V represents the Fe$^{\text{III/II}}$ couple (Figure 2.6, blue). For an analogous imidazole ligated complex PIM E$_{1/2}$ is -0.58 V, reported previously. Thus the presence of the axial anionic π-donor ligand lowers the Fe$^{\text{III/II}}$ potential by 560 mV relative to the neutral imidazole ligand. The reduction potential of the POR complex is thus 110 mV more negative relative to the thiolate complex. The lower E$^0$ of the phenolate complex relative to the thiolate complex reproduces the lower E$^0$ of the 5C HS catalase site relative to P450 in solution. Note that based on the relative magnitude of the Fe-N$_{\text{pyr}}$ vibration with an axial thiolate ligand and having the lowest Fe-N$_{\text{pyr}}$ vibration (i.e. weakest Fe-N$_{\text{pyr}}$ bond) may have been expected to have the lower Fe$^{\text{III/II}}$ E$^0$ of the three. Contrary to
expectations, the phenolate complex is found to have 110 mV lower Fe\textsuperscript{III/II} $E^0$ than the thiolate complex. This difference likely originates from a larger electrostatic interaction between Fe and O in phenolate relative to Fe and S in thiolate and is analysed using DFT calculations (\textit{vide infra}).

![Figure 2.6](image)

Figure 2.6. CV of the above complexes in a CH\textsubscript{2}Cl\textsubscript{2} solvent having 100 mM TBAP as supporting electrolyte, glassy carbon as the working electrode, scan rate = 100 mV/s, and Fe/Fc\textsuperscript{+} is used as a internal reference electrode.

### 2.4. DFT Calculations

#### 2.4.1. Geometry

Geometry optimized DFT calculations are performed to obtain a possible structure of the complex PORin its HS and LS forms (Figure 2.7).\textsuperscript{64, 65} The optimized geometries obtained using BP86 functional indicate the Fe-O bond is 1.84 Å and 1.81 Å (Table 2.3, values obtained with B3LYP provided in Table S2.19) in the 5C HS and the 6C LS states, respectively. These values are in good agreement with the reported structures of catalase active sites as well as those of synthetic model complexes.\textsuperscript{46, 47} The Fe-OH\textsubscript{2} bond, present in the 6C LS model, is 2.10 Å which is in good agreement with previous reports on LS Fe\textsuperscript{III} complexes bearing a bound H\textsubscript{2}O.\textsuperscript{7} The Fe-N\textsubscript{pyr} bonds are longer by 0.01 Å in the HS thiolate-bound PSR complex relative to the tyrosine bound HS POR complex, indicating a weaker Fe-N\textsubscript{pyr} bond in the former. Similarly the Fe-N\textsubscript{pyr} bonds of HS imidazole bound PIM complex are 2.05 Å which is shorter relative to PSR (a smaller model for PPSR-yne) and POR complexes respectively.
Figure 2.7: DFT-optimized structures of complexes POR and POR-H₂O.

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Mulliken Charges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-N\text{pyr}</td>
<td>Fe-L\text{a}</td>
</tr>
<tr>
<td>S=5/2</td>
<td>BLC\text{b}</td>
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<td></td>
<td>POR</td>
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<tr>
<td></td>
<td>POR</td>
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<tr>
<td></td>
<td>PSR</td>
</tr>
</tbody>
</table>

a. L= axial ligand coordinating atom S for PPSR-yne, N for PIM and O for POR b. resting bovine liver catalase (8CAT) b. N₃⁻ bound bovine liver catalase (1TH2)

The Fe-O-Ph angle in BLC (X-tal) and POR (optimized) is ~140° in the high spin state. The larger Fe-O-Ph angle in POR likely leads to kinematic coupling of the Fe-O and the ν₆b mode resulting in enhancement of both at the same time.

2.4.2. Vibrational Frequencies

The calculated vibrational frequencies (using both BP86 and B3LYP) are listed in Table 2.4. The calculations using the BP86 functional reproduce the experimentally observed symmetric ν₄ and ν₂ intra-ligand modes for the phenolate bound complex with good accuracy (within ±20 cm⁻¹ ~1% error). These calculations further reproduce the experimentally observed
relative magnitudes in the ν₈ vibrations (i.e. the Fe-Npyrr stretch) of the HS complexes i.e. PIM > POR > PSR. Thus the BP86 functional is suitable for calculated these set of molecule and is used for further calculations. Note that B3LYP underestimates the intra-ligand modes in general and, in particular, for PIM.

The calculated Fe-O-C angle of the complex is 145° which is much wider than 109° that may be expected for a sp³ hybridized O center. As a consequence of this wide Fe-O-C angle (also observed in the crystal structure of BLC i.e 143°) the Fe-O vibration couples (calculated to be at 613 cm⁻¹) with the ν₆b bending mode of the phenolate (calculated to be at 590 cm⁻¹) also observed. Note that the Fe-L vibrations are overestimated by 2.8% at this level of theory. Enhancement of both the Fe-O and ν₆b of phenolate in the rR spectra is also observed for a bovine liver catalases which has a Fe-O-C angle of 143°. This may explain the observation of two vibrations (Fe-O at 573 cm⁻¹ and phenolate ν₆b at 589 cm⁻¹) instead of one Fe-O vibration in this region.

<table>
<thead>
<tr>
<th>Mode</th>
<th>PSR</th>
<th>POR</th>
<th>PIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ν₂</td>
<td>1554</td>
<td>1551</td>
<td>1477</td>
</tr>
<tr>
<td>ν₃</td>
<td>1451</td>
<td>1447</td>
<td>1421</td>
</tr>
<tr>
<td>ν₄</td>
<td>1361</td>
<td>1351</td>
<td>1326</td>
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<tr>
<td>ν₈</td>
<td>391</td>
<td>378</td>
<td>373</td>
</tr>
<tr>
<td>C-O</td>
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<td>Fe-S</td>
<td>336</td>
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<td>290</td>
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<tr>
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<td>369</td>
<td>369</td>
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<td></td>
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<td>410</td>
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</tr>
<tr>
<td>Fe-O</td>
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</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>573</td>
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<td></td>
<td>-</td>
<td></td>
<td>614</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td>600</td>
</tr>
</tbody>
</table>

Table 2.4: Calculated Vibrational Frequencies (cm⁻¹)

2.4.3. Electronic Structure

5C

The GS wave function of the HS 5C complex (POR) shows a t₂³e² configuration with a square pyramidal ligand field. The dₓ₂₋₇ᵧ₂ orbital is highest in energy due to its σ antibonding interaction with the in-plane pyrrole orbitals. There are two interactions between the Fe and the
phenolate ligand; a σ interaction between the out-of-plane phenolate π* orbital and the d_{xz} orbital (Figure 2.8, middle) and a π interaction with the in-plane phenolate π* orbital and the d_{yz} orbital (S2.20). While the interactions between the phenolate and the Fe are similar to that its other well studied analogue; the alkyl thiolate, there are certain nuances worth mentioning. Note that the d_{x2-y2} orbital of the PIM complex are at a higher energy than that of the POR and PSR complexes respectively. The higher Z_{eff} on the Fe in the POR complex will result in stronger σ-bonding and π interaction with the occupied anionic porphyrin donor ligand orbitals (in-plane pyrrole orbitals, in-plane-phenolate π* orbital). This reflects stronger of the Fe-N_{pyr} bonds in the POR complex relative to the PSR complex. The molecular orbital (MO) contributions (Table 2.5) reveal that the d_{yz} and d_{xz} orbital in the POR model have 15% S_{3p} mixed into it, while the d_{x2} orbital has 4% S_{3p} mixed into it; i.e., there is significant covalent interaction between Fe and O (both σ and π). The t_2 orbitals contain contributions from both the phenolate ligand as well as the porphyrin. Similarly the porphyrin π* shows significant mixing of the phenolate ligand. This mixing between these two centers may provide a mechanism how the Fe-O and C-O modes of POR are observed in the rR data when the Soret band (π→π*) is excited.

<table>
<thead>
<tr>
<th>Table 2.5: MO Compositions of the β Unoccupied Orbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbital Contribution</td>
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<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>d_{x2-y2}</td>
</tr>
<tr>
<td>π*</td>
</tr>
<tr>
<td>d_{yz}</td>
</tr>
<tr>
<td>d_{xz}</td>
</tr>
<tr>
<td>d_{xy}</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Notably the spin density on the thiolate sulfur is significantly more than the phenolate ligand in the HS state. This implies that, in spite of the fact that the Fe-S bond is significantly longer than that of the Fe-O bond; the Fe-S bond is much more covalent relative to the Fe-O bond. The lower charge transfer from the phenolate to Fe holds true for both the π and σ interactions.
Figure 2.8. Calculated (BP86) GS MO diagram of 5C PIM, POR and PSR complexes are shown. Only unoccupied β orbitals are shown.

**6C**

The GS wave function of the LS 6C POR-H$_2$O complex is very similar to that of the PSR-MeOH complex. It has a distorted octahedral ligand field. The GS wave function of the 6C LS POR-H$_2$O complex shows a normal $t_2^5e^0$ electronic structure. The singly occupied $t_2$ orbital forms a π bond (Figure 2.9) while the unoccupied $e$ orbital forms an σ bond (Figure 2.9, left) with the in-plane and out-of-plane orbital of the phenolate ligand, respectively. In the POR-H$_2$O complex ~6% of occupied Fe $t_2$ character is mixed into this porphyrin π* orbital while for the PSR-MeOH complex ~8.2 % of occupied Fe $t_2$ character is mixed into this porphyrin π* orbital. This is due to higher energies of the Fe$_{3d}$ orbital of the PSR-MeOH complex relative to the POR-H$_2$O complex as greater covalent charge donation from the anionic thiolate ligand to the iron in
the PSR-MeOH complex (28% π + 2x26% σ = 0.8 e) lowers the \( Z_{\text{eff}} \) on the Fe and increases the energy of the 3d manifold. This allows better overlap with the \( t_2 \) orbitals and \( \pi^* \) orbital. Note that the \( d_{yz} \) orbital, which is raised up in energy by strong \( \pi \)-bonding interaction with the thiolate carries the unpaired electron. Thus while both the phenolate and the thiolate ligands exhibit considerable \( \pi \) and \( \sigma \) interaction with the \( d_{yz/xz} \) and \( d_{z^2} \) orbitals, the delocalization is significantly more for the thiolate ligand i.e. the Fe-S bond is considerably more covalent that the Fe-OPh bond.

Figure 2.9: MO diagram of the 6C POR-H\(_2\)O (left) and PSR-MeOH (right) complexes.

### 2.4.4. Reduction potentials

DFT calculations were also used to calculate the \( E^0 \) of these complexes. While the calculated values can not be directly compared to the experimental value as these use different
reference point, the relative magnitudes can be. The PSR complex is calculated to have an $E_{1/2}$ which is 34 mV more negative than the POR complex in the gas phase (Table 2.7). But when solvation is included (DCM) the POR complex is calculated to have a more negative potential (7 mV) relative to the PSR complex reproducing the experimentally observed trend. This likely reflects differences in the nature of bonding in the PSR and POR complex (*vide infra*).

<table>
<thead>
<tr>
<th>Complex</th>
<th>Potential (gas phase)</th>
<th>Potential (DCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSR</td>
<td>-1.917</td>
<td>-0.784</td>
</tr>
<tr>
<td>POR</td>
<td>-1.883</td>
<td>-0.791</td>
</tr>
</tbody>
</table>

The dependence of $E^0$ on Fe-L bond energy (BE) can be approximately expressed as:

$$E^0 = BE_{ox} - BE_{red}$$

BE on the other hand can have both covalent and electrostatic contribution i.e,

$$BE = BE_{cov} + BE_{elec}$$

As indicated by the calculated GS wave function for the 5C HS state, the Fe-S bond has more covalent character than the Fe-OPh bond. The contribution of $BE_{elec}$ can be approximately estimated by Coulomb’s law;

$$BE_{elec} = \frac{Z_{\text{eff}}^{Fe}Z_{\text{eff}}^{L}}{r_{Fe-L}} \times 14.1 \text{ kcal/mol.}$$

The calculated Mulliken charges on the Fe and S atoms for PSR and Fe and O atoms for POR in the gas phase and after solvation are listed in Table 2.8. The electrostatic interaction between the iron and the coordinating atom of the axial ligand is considered. For both PSR the electrostatic stabilization of the reduced state is more than that of the oxidized state. On the contrary the electrostatic stabilization of the oxidized state of POR is more than that for the reduced state. This is a direct result of the strong covalent bonding between the thiolate sulfur and iron in the oxidized state which reduces the partial charges on the individual centers reducing the electrostatic contribution to bonding. The electrostatic contribution to bonding stabilizes the oxidized state of POR by 0.79 Kcal/mol and destabilizes the oxidized state of the PSR complex by 0.74 Kcal/mol. Thus the electrostatic contribution favors the oxidation of POR complex by 1.53 Kcal/mol relative to the PSR complex. This may be expected to lower the
reduction potential of the POR complex relative to the PSR complex by 66 mV. The corresponding calculated $E^0$ of the PSR is 34 mV more negative in the gas phase. This, of course, represents the combined effect of the covalent and electrostatic contributions to bonding. In this case the large covalency of the Fe-S bond in the oxidized state, as indicated in the reduction of the $v_8$ vibration and the calculated GS wave function, results in a more negative $E^0$ for the PSR complex relative to the POR complex.

The electrostatic stabilization increases in when a PCM is used as the polarization of the environment favors charge separation in the GS wave function. The enhanced electrostatic stabilization of the POR complex, on including solvation, in its oxidized form relative to the reduced form (0.94 Kcal/mol) is now expected to lower its potential by 81 mV relative to the PSR complex where the oxidized state is now destabilized by 0.92 Kcal/mol. Thus the inclusion of the polarized medium is expected to lower the $E^0$ of the POR complex relative to the PSR complex 80 mV (1.86 Kcal/mol), 14 mV more relative to the gas phase. The calculated $E^0$ for the PSR is 34 mV more negative in the gas phase while the calculated $E^0$ of POR is 7 mV more negative in DCM. Thus the calculations show that inclusion of the PCM medium in the calculations shift the POR $E^0$ more negative by 41 mV relative to the PSR complex. This is likely due to the larger electrostatic stabilization of the oxidized POR complex.

### Table 2.8: Mulliken Charges and Electrostatic Interaction Energies

<table>
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<tr>
<th></th>
<th>Fe$^{III}$</th>
<th></th>
<th>Fe$^{II}$</th>
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<tr>
<td></td>
<td>$q_{Fe}$</td>
<td>$q_x$</td>
<td>$r_{Fe-L}$</td>
<td>$E_{elec}$</td>
<td>$q_{Fe}$</td>
<td>$q_x$</td>
<td>$r_{Fe-L}$</td>
<td>$E_{elec}$</td>
<td>$\Delta E_{elec}$</td>
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<td><strong>Gas Phase $\epsilon=1$</strong></td>
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<tr>
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<tr>
<td><strong>In DCM Solution ($\epsilon=8.9$)</strong></td>
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<td>PSR</td>
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<td>-0.64</td>
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<td>-6.90</td>
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</table>
2.5. DISCUSSION

The rR of the POR complex indicates that Fe-O and the ν₆b vibration of a 5C HS Fe-OPh unit are at 589 cm⁻¹ and 573 cm⁻¹. The C-O vibration of the phenolate ligand is identified at 1320 cm⁻¹. These values are consistent with those reported for the 5C HS resting site of heme proteins bearing a tyrosine axial ligand. The EPR, the oxidation and spin state marker bands of the POR complex shows that it becomes 6C LS in a coordination solvent. The g values are anisotropic and the V/λ ratio, obtained from Taylor analysis, indicates that the phenolate ligand is a strong π anisotropic ligand. The V/λ of the POR complex is 4.02 which is less than that of a corresponding axial thiolate ligand bound complex PPSR-yne which is 5.68. The ν₈ vibration, which is a reporter of the extent of charge donation from the axial ligand to the iron, of the POR complex is 395 cm⁻¹ which is less than that of a imidazole bound complex, PIM, but higher than that of the thiolate bound complex PPSR-yne (390 cm⁻¹). The higher ν₈ of PIM relative to the POR complex indicate that the axial phenolate is more covalent than the imidazole. Alternatively, both the V/λ and lower ν₈ indicate that the Fe-OPh is less covalent than Fe-SR. This is substantiated by DFT calculations which reproduce the experimentally observed vibrational parameters. The calculated wave functions show that the axial phenolate ligand has an σ and a π bonding interaction with the dₓ² and dₓ²ᵧᵧ orbital in both the 5C HS and 6C LS states. The 5C wave function shows 15% and 4% mixing of the phenolate ligand into the π bonding dₓᵧᵧ and σ bonding dₓ² orbital. These are much lower than the 29% and 16% mixing of the thiolate ligand into the π and σ bonding d orbitals, respectively. Similarly for the 6C LS GS the π and σ antibonding d orbitals have 11% and 20% phenolate mixed into them. While the contribution of the phenolate ligand into the metal 3d wave functions increase significantly relative to those of the 5C HS state, these are still much lower than those of an axial thiolate ligand which show 28% and 52% mixing into the π and σ 3d orbitals, respectively.

The lower charge donation from the phenolate oxygen to the Fe relative to the thiolate sulfur results in a larger contribution of electrostatic interaction in Fe-L bond in the former and a more covalent contribution in the later. This produces an interesting anomaly between measured properties. The measured vibrational and EPR data clearly indicate that the Fe-SR bond is more covalent than the Fe-OPh bond. On the contrary the measured E⁰ of the POR complex is 110 mV more negative than that of the PPSR-yne complex in DCM. Normally, other ligands remaining
the same, a more covalent axial ligand would result in a lower $E^0$. For example the PIM complex with a neutral imidazole axial ligand has 400-500 mV more positive $E^0$ than PPSR-yne and POR. Thus considering the higher covalency of the Fe-SR bond in the PPSR-yne complex, it may be expected to have a lower $E^0$ than the POR complex. This is not the case here. DFT calculations indicate that indeed in the gas-phase the calculated $E^0$ for the PSR complex in lower than that of the POR complex. However, calculations which include solvation show that the POR complex has a lower $E^0$ than the PSR complex. This is due to greater electrostatic contribution to axial ligand Fe bonding in the less covalent POR complex relative to the PSR complex which has a very covalent Fe-S bond. The electrostatic interaction contributes more in a polarized medium relative to the gas phase. This leads to significant stabilization of the oxidize Fe$^{III}$ state in the POR complex which results in lowering its Fe$^{III}$/II $E^0$ relative to the PSR complex in DCM.

Of the three axial ligands prevalent in the heme active sites, phenolate and thiolate are both anionic. The additional negative charge in the P450 active site lowers the $pK_a$ of trans ligands such that the compound II species is protonated (Fe$^{IV}$-OH) whereas compound II in neutral imidazole bound sites are deprotonated (Fe$^{IV}$=O). Additionally the covalent donation from the anionic thiolate ligand helps stabilize high valent intermediates in the P450 type active sites and the basicity of the Fe$^{IV}$=O unit in the oxidant, compound I, help achieve strong C-H bond abstraction using a low potential oxidant. Catalases, on the other hand, are bound via an anionic ligand, giving it a distinct edge over neutral imidazole bound active sites in stabilizing high valent species are characterized by a lower Fe$^{III}$/Fe$^{II}$ $E^0$ (-226 on electrode to -500 mV in solution) relative to P450 (-175 mV in solution) in their 5C HS resting form. The results presented in this study suggests that differences in covalent and electrostatic contribution to the ground state electronic structures of these anionic phenolate and thiolate bound heme active sites play a major role in determining their biophysical properties. The thiolate axial ligand forms more covalent Fe-S bond which lowers the Lewis acidity of the iron center whereas the phenolate axial ligand forms less covalent bond and results in higher Lewis acidity at the metal site. As a result the water derived axial ligands at the P450 active site will have greater $pK_a$ relative to the catalase active site. The fact that compound II is protonated in P450 but deprotonated in compound II in catalase serves as an illustrative example.

The lower $E^0$ of a phenolate bound iron porphyrin complex relative to a thiolate or imidazole bound complex is suggestive of yet another role the tyrosine axial ligand may be
playing in the active site of catalase. Presumably it is absolutely essential to avoid the reduction of the heme site of catalase to its ferrous state under physiological conditions as reaction of $\text{H}_2\text{O}_2$, its substrate, with the reduced ferrous site will result in the generation of reactive oxygen species by Fenton’s reaction. The results obtained using POR suggests that the enhanced stabilization of the oxidized state in a medium with low dielectric (organic solvent for POR and protein environment for catalase) resulting from the electrostatic nature of the Fe-O bond is likely to be responsible for the very low Fe$^{III/II}$ potential observed in the catalase active site avoiding its reduction by most physiological reducing agents.

2.6. References

2.7. Supporting Information

S2.1: $^1$H NMR spectrum of complex 2.
S2.2. IR spectrum of complex 2.

S2.3. Mass spectrum of complex 2.
S2.4. Mass spectrum of complex 3.

S2.5. IR spectrum of complex 3.

S2.7. $^1$H NMR spectrum of complex 4.

S2.9. $^1$H NMR spectrum of complex 5.
S2.10. Mass spectrum of complex 5.

S2.11. Mass spectrum of complex 6.

2.1A. UV-Vis absorption spectroscopy of the synthesized porphyrin complexes:

The free ligand of azide-porphyrin has an intense soret band at 419 nm and weaker bands at 514, 549, 592, and 650 nm (S2.13, pink). The Zn\textsuperscript{II}-azide complex of has the soret band at 424 nm and Q bands at 557, 595 and 623 nm (S2.13, blue). In the Zn\textsuperscript{II}-phenol complex, the soret at 424 nm and new set of bands appear at 557 and 596 nm (S2.13, deep green). The free ligand phenol precursor (5) complex shows soret band at 419 nm and Q band at 515, 549, 592 and 647 nm (S2.13, violet).

S2.13. Absorption data of azide(2) in pink, metallated Zn–azide(3) in blue, Zn–phenol(4) in deep green, demetallated phenol(5) in violet, free ligand (5), complex POHR (6) and POR (7) in dry THF.
2.2A. Power dependence of resonance Raman (rR):

1 mM solution of POR complex is taken in the NMR tube. The sample is excited in different laser excitation power.

S2.14. rR spectra of the range 1300 – 1600 cm\(^{-1}\), showing oxidized complex POR in THF. Laser wavelength = 413.1 nm. All spectra were recorded at 77 K.

S2.15. rR spectra of the range 1300 – 1600 cm\(^{-1}\), showing oxidized complex POHR in THF. Laser wavelength = 413.1 nm, Laser power on sample = 10 mW. The spectrum was recorded at 77 K.
S2.16: rR spectra of the range 300 – 1600 cm\(^{-1}\), showing oxidized complex POR in THF. Laser wavelength= 413.1 nm, Laser power on sample = 10 mW. The spectrum was recorded at 77 K.

S2.17: The rR data of the complex POR, POHR and PIM in the C-O vibration region.

S2.18: The rR data of the above complexes.
**S2.20.** π interaction with the in-plane phenolate π* orbital and the d_{yz} orbital.

Table S2.19: Optimized Bond Lengths (Å) of the Models and Relevant Mulliken charges

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<thead>
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<th>Geometry</th>
<th>Mulliken Charges</th>
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