Chapter 3

Resonance Raman and Electrocatalytic Behavior of Thiolate and Imidazole Bound Iron Porphyrin Complexes on Self Assembled Monolayers: Functional Modeling of Cytochrome P450
3.1. Introduction

Metalloporphyrins are versatile catalysts for O$_2$ activation and reduction. In Hemoglobin (Hb) and Myoglobin (Mb) the heme active sites bind O$_2$ reversibly;\textsuperscript{1-3} in Cytochrome c Oxidases (CcO) O$_2$ is reduced to H$_2$O and in Cytochrome P450 the O$_2$ reduction is coupled to substrate oxidation.\textsuperscript{4-8} In fact, Cytochrome P450 efficiently activates very strong C-H bonds using molecular O$_2$ in nature.\textsuperscript{9} All these active sites have the same heme cofactor but these vary in coordinating axial ligand and different distal environments. In Hb, Mb and peroxidases the heme is ligated to the protein by an imidazole head group of a histidine residue (Figure 3.1A) while in Cytochrome P450 the heme is ligated by a thiolate head group of a cysteine residue (Figure 3.1B). The different proximal ligands are proposed to have different “push-effects” and this has been a matter of great interest for some time now.\textsuperscript{10} In addition, different distal environments of some of these enzyme active sites are proposed to exert different “pull-effects”.\textsuperscript{10} Understanding the individual contributions of the different proximal ligands and distal environments to the diverse reactivities of these proteins have proved to be quite challenging. One of the key problems associated with investigating these effects in a protein active site by single point mutations of active site residues is the possible change of protein conformation associated with it.

![Figure 3.1. Active site structure of Myoglobin (A) (pdb id: 1A6M) and Cytochrome P450 (B) (pdb id: 1AKD).](image)
There is a growing demand for efficient man made catalysts that can catalyze the oxidation of inert C-H bonds using molecular O$_2$; a process that the Cytochrome P450 active site can catalyze efficiently under ambient conditions.\textsuperscript{11-13} In spite of great deal of research in this area, developing functional models of these active sites that reproduce these differences in axial and distal environments have been difficult using conventional synthetic techniques. This is mainly due to complications associated with the synthesis of stable heme complexes with a thiolate axial ligand. Only a few are reported so far and the ones reported are too unstable in presence of O$_2$ or in aqueous solvent to perform any elaborate experiments to investigate these properties.\textsuperscript{14-16} There have been several reports of iron and manganese porphyrin complexes and some non-heme Fe complexes that can catalyze substrate oxidation using peroxides, peracids and iodosyl benzene.\textsuperscript{17-30} While there are several recent reports of stoichiometric hydroxylations of organic substrates using oxygen there are no reported functional models or any catalyst that can catalytically oxidize C-H bonds using molecular oxygen like the native enzymes.\textsuperscript{31-36} In all known cases of C-H bond activation, a high valent metal-oxo species is produced using oxygen atom transfer agents (peracids, peroxides) and this high valent species activates inert C-H bonds. The challenge is to produce these species catalytically from molecular O$_2$.\textsuperscript{37} For iron porphyrins this process generally requires both protons and electrons, e.g. PFe$^{\text{II}}$ + O$_2$ + 2H$^+$ + e$^-$ $\rightarrow$ P$^+$Fe$^{\text{IV}}$=O (compound I) + H$_2$O (where P represents a porphyrinato ligand). Homogeneous catalysis, however, has a very significant inherent limitation in this regard i.e. controlling simultaneous addition of both O$_2$ and electrons to the catalyst from the same homogenous reaction medium. Rather the oxidation of the reducing agent (E$^0$ = -200 to -400 mV to reduce Fe-porphyrin) by either O$_2$ or H$^+$ is facile. In nature such a situation does not arise because the source of electron (i.e. the reductase component) stays protected in a protein active site and only transfers the electron to the active site. Further the electron transfer pathways and the H$^+$/O$_2$ transfer pathways leading into the enzyme active site are almost always orthogonal.

Self-Assembled Monolayer (SAM) of thiols on Au and Ag electrodes has been widely used in chemistry and biology. These thiols can be further functionalized in several different ways to attach different types of molecules to these surfaces. These molecules range from small
catalysts, DNA, proteins, antibodies, viruses.\textsuperscript{38-45} There have been several reports where electrocatalysts have been covalently attached to SAM. In several reports “click chemistry” has been used as a method for these covalent attachments.\textsuperscript{46,47,48} Collman group reported the attachment of Ru porphyrin complexes on Au surfaces by simple covalent coordination.\textsuperscript{49,50} Similar method has recently been used by the Nemykin group where they have successfully attached Zinc Tetraferrocenyl porphyrins on alkyl thiol SAM bearing an imidazole head group.\textsuperscript{51} This strategy can be used to construct a surface where the catalysts, bound to terminal functional headgroups, can be thinly dispersed between aliphatic thiols (referred to as diluents) thus allowing formation of site isolated active sites. The thiols insulate the electrode, source of electrons, from the bulk solution, source of H\textsuperscript{+} and O\textsubscript{2}. Electrons are transferred to the catalytic centre from the electrode via covalent bonds (C-C bonds). This mimics the inherent heterogeneity of protein active sites discussed above. Further the rate of electron transfer from the electrode to the catalyst can be controlled by using thiols of varying chain lengths.

In this chapter we report the formation of Bio-inspired catalytic surfaces which have been constructed, characterized and used for catalyzing vital multi-electron and multi-proton reactions, in particular, O\textsubscript{2} activation. Linkers, having a thiol group at one end, and a coordinating residue (imidazole/thiol) on the other terminal were synthesized. SAM functionalized Au electrodes having these different linkers which behave as different axial ligands were constructed. Various site isolated iron porphyrin based active sites differing in distal environment have been assembled on these surfaces by simple reversible covalent attachment. The surfaces were characterized by electrochemical and spectroscopic methods. These modified electrodes can catalytically oxidize cyclohexane and toluene using molecular O\textsubscript{2}.

### 3.2. Materials and Methods

#### 3.2.1. Materials

All reagents were of the highest grade commercially available and were used without further purification. 11-Bromoundecan-1-ol (BrC\textsubscript{11}OH), Methanesulfonyl chloride (OMsCl), Potassium thioacetate (KSAc), Octanethiol (C\textsubscript{8}SH), potassium hexafluorophosphate (KPF\textsubscript{6}), cyclohexanone, benzylalcohol and all buffers were purchased from Sigma-Aldrich. Di-sodium
hydrogen phosphate dihydrate (Na$_2$HPO$_4$. 2H$_2$O), potassium chloride (KCl), imidazole (Imd), conc. Hydrochloric acid (HCl), cyclohexane and cyclohexanol were purchased from Merck. Triethylamine (Et$_3$N), p-cresol and toluene were purchased from Spectrochem India Ltd. Au wafers were purchased from Platypus Technologies (1000 Å of Au on 50 Å of Ti adhesion layer on top of a Si(III) surface). Transparent Au wafers (100 Å of Au on 10 Å of Ti) were purchased from Phasis, Switzerland. Au discs for the Rotating Ring Disc Electrochemistry (RRDE) experiments and Ag discs for Surface Enhanced Resonance Raman Spectroscopy (SERRS) experiments were purchased from Pine Instruments, USA.

3.2.2. Instrumentation

UV-Vis absorption data were taken in an Agilent technologies spectrophotometer model 8453 fitted with a diode-array detector. All electrochemical experiments were performed using a CH Instruments (model CHI710D Electrochemical Analyzer). Bipotentiostat, reference electrodes, Teflon® plate material evaluating cell (ALS Japan) were purchased from CH Instruments. The RRDE set up from Pine Research Instrumentation (E6 series ChangeDisk tips with AFE6M rotor) was used to obtain the RRDE data. Surface Enhanced Resonance Raman data were collected using a Trivista 555 spectrograph (Princeton Instruments) and using 413.1 nm excitation from a Kr$^+$ laser (Coherent, Sabre Innova SBRC-DBW-K). FT-IR data and NMR data were measured on instruments as described in chapter 2 at room temperature. The mass spectra were recorded by QTOF Micro YA263 instrument. Gas Chromatography (GC) measurements were carried in an Agilent 6890N Network GC system.

3.2.3. Synthesis

3.2.3.1. 11-Imidazolyl undecan-1-thiol (ImdC$_{11}$SH)

The desired product was obtained starting from 11-Bromo undecan-1-ol through a series of steps as shown in Scheme 3.1. 11-Bromo undecan-1-ol was refluxed with excess imidazole in DMF followed by stirring with OMsCl and Et$_3$N which substituted –OH by mesyl group. The mesyl group was substituted by thioacetate group followed by refluxing in 0.6 (N) HCl to obtain the thiol group.
3.2.3.2. Undecan-1, 11-dithiol (SHC$_{11}$SH)

The desired product was obtained starting from 11-Bromo undecan-1-ol through a series of steps as shown in Scheme 3.2. 11-Bromo undecan-1-ol was stirred with OMsCl and Et$_3$N in dry THF to substitute –OH by mesyl group. The mesyl and bromo groups were substituted by thioacetate group by refluxing with 4 eq. KSAc in dry MeOH followed by refluxing in 1 (N) HCl to obtain the dithiol product.

**Scheme 3.2.** Schematic presentation of the synthesis of SHC$_{11}$SH.
3.2.3.3. Picketfence Iron(II) porphyrin (Fe picket-fence/ FePf), \( \alpha_4 \)-tetra-2-(4-ferrocenyl-1,2,3-triazolyl)-phenylporphyrinato iron (FeFc4) and \( \alpha_4 \)-tetra-2-(4-carboxymethyl-1,2,3-triazolyl)-phenylporphyrinato iron (FeEs4)

The catalysts have been prepared in our laboratory. Fe picket-fence has been synthesized as reported.\(^5\) The detailed synthetic scheme of the other two clickable porphyrins has been reported elsewhere.\(^5\),\(^6\) The catalysts have been pictorially presented in Figure 3.2.

![Figure 3.2. Pictorial representation of the catalysts used. I) Fe picket-fence, II) FeFc4 and III) FeEs4.](image)

3.2.4. Construction of the Electrodes

3.2.4.1. Formation of mixed Self Assembled Monolayer (SAM)

Au wafers and discs were cleaned electrochemically by sweeping several times between 1.5 V to -0.3 V in 0.5 M \( \text{H}_2\text{SO}_4 \). Ag discs were cleaned in alumina (size: 1 \( \mu \)) m, 0.3 \( \mu \) m and 0.05 \( \mu \) m) and then roughened in 0.1 M KCl solution as described in literature. SAM solutions were prepared using the concentration ratio of the linkers as shown on Table 3.1. In each case the diluent used was \( \text{C}_8\text{SH} \). Freshly cleaned Au wafers and discs and freshly roughened Ag discs were rinsed with triple distilled water, ethanol, purged with \( \text{N}_2 \) gas and immersed in the depositing solution for 48 hrs. Freshly cut transparent Au wafers, used in absorption spectroscopy were washed with ethanol, dried with \( \text{N}_2 \) gas and immersed in the SAM solution for the same period of time.
3.2.4.2. Attachment of the catalysts on to the SAM

Au wafers and discs and roughened Ag discs immersed in the deposition solution were taken out before experiments and rinsed with ethanol followed by triple distilled deionised water and then dried with N₂ gas. The wafers were then inserted into a Plate Material Evaluating Cell (ALS Japan) and the discs were mounted on a platinum ring disc assembly (Pine Instruments, USA). Solutions of the catalysts were prepared in chloroform. For the attachment of Fe picket-fence to the ImdC₁₁SH linker the electrode surface was immersed in CHCl₃ solution of the catalyst for about 1.5 hrs. For the SHC₁₁SH linker the electrode surfaces were immersed first in Et₃N for 10 mins and then in the Fe picket-fence porphyrin solution for 2.5 hrs (Scheme 3.3). For the attachment of FeFc₄ and FeEs₄ catalysts to the ImdC₁₁SH linker the electrode surface was immersed in the catalyst solution for about 1 hr and for the SHC₁₁SH linker the surfaces were immersed in a solution of Et₃N and catalyst for 1.5 hrs. After respective time the surfaces were thoroughly rinsed with chloroform, ethanol and triple distilled water before the electrochemical or SERRS experiments.

Table 3.1. SAM solution preparation ratios (C₈SH was used as diluent)

<table>
<thead>
<tr>
<th>Linkers</th>
<th>Mole-fraction</th>
<th>Total concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImdC₁₁SH</td>
<td>0.2</td>
<td>0.4 mM</td>
</tr>
<tr>
<td>SHC₁₁SH</td>
<td>0.2</td>
<td>3 mM</td>
</tr>
</tbody>
</table>

![Scheme 3.3. Schematic representation of construction of the Bio-inspired Electrodes.](image-url)
3.2.4.3 Physiabsorption vs Covalent Attachment

Iron porphyrins are known to physiadsorb on thiol surfaces. However, the work of Collman and Chidsey has shown that thorough rinsing of the surface with an organic solvent like CH₂Cl₂ or CHCl₃ removes any physiadsorbed material.⁴⁷ Control experiments were performed where the electrocatalytic behaviour of these surfaces were monitored before and after washing with CHCl₃. The data clearly show that the physiadsorbed complexes, which remain on the surface before washing with CHCl₃, show O₂ reduction at very different potentials (Figure 3S1 and 3S2, SI). However these species are easily removed on washing with CHCl₃ (Figure 3S3, SI).

3.2.5. Absorption Spectroscopy

For the heterogenous absorption experiment covalently attached catalysts on SAM modified transparent Au electrodes were used.

3.2.6. Cyclic Voltammetry (CV) Experiments

All CV experiments were done in pH 7 buffer (unless otherwise mentioned) containing 100 mM Na₂HPO₄.2H₂O and 100 mM KPF₆ (supporting electrolyte) using Pt wire as the counter electrode and Ag/AgCl as the reference electrode.

3.2.7. Coverage Calculation

The coverage for a particular species was estimated by integrating the oxidation and reduction currents of the respective species.⁴⁷,⁵⁶

3.2.8. Partially Reduced Oxygen Species (PROS)

The platinum ring and the Au disk were both polished by alumina powder (grit sizes: 1 µ, 0.3 µ and 0.05 µ) and electrochemically cleaned and inserted into the RRDE tip (Figure 3.3A) which was then mounted on the rotor and immersed into a cylindrical glass cell equipped with Ag/AgCl reference and Pt counter electrodes. The collection efficiency (CE) of the RRDE set-up was measured as described in Chapter 2. A 20±2% CE was generally recorded during these experiments (Figure 3.3B). The potential at which the ring was held during the collection experiments at pH 7 for detecting H₂O₂ was obtained from literature.⁵⁷
3.2.9. Surface Enhanced Resonance Raman Spectroscopy (SERRS)

Ag discs were cleaned using Alumina powder (grit sizes 1, 0.3 and 0.05 microns) and then roughened in 0.1 M KCl solution using reported procedures\textsuperscript{58,59} and immersed in SAM solutions. The roughened modified Ag discs were then inserted into the RRDE set-up for the collection of SERRS data.\textsuperscript{60,61} Catalysts were attached in a similar manner as described in section 3.2.4.2. Experiments were done using an excitation wavelength 413.1 nm and the power used at the electrode surface was around 10-12 mW.

3.2.10. Substrate oxidation

Substrates used here were toluene and cyclohexane. Buffer solutions (pH 7) were saturated with the substrate by vigorously shaking a suspension of these organic molecules in water. The mixture was allowed to settle and separated using a separating flask. The aqueous layer was extracted and O\textsubscript{2} reduction CV experiments were performed on the surfaces (3 Linear Sweep Voltammetry between 0.5 V to -0.5 V). The resultant aqueous solutions were extracted with Chloroform (CHCl\textsubscript{3}). The CHCl\textsubscript{3} layer was evaporated and the product left behind was subjected to ESI-MS and GC analysis.

3.2.11. Estimation of Turnover number (TON)

The amount of product was quantified using GC against known concentrations of pure compounds. As a control the same process, outlined above, was followed but without performing the electrochemical O\textsubscript{2} reductions in these solution. In such cases no new products

Figure 3.3. (A) RRDE assembly showing the Au disc and Pt ring. (B) A Collection efficiency plot.
were detected in the GC. The CV experiments were used to calculate the number of catalysts on the surface (Section 3.2.7). The ratio of the number of moles of product formed and the number of moles of catalyst present yields the turnover number. Further the integration of the \( \text{O}_2 \) reduction current provides the moles of \( \text{O}_2 \) reduced. The ratio of the moles of substrate oxidized (obtained from GC) and the moles of \( \text{O}_2 \) reduced is the Faradaic yield (FY) of the process. Typically FY of 9-10% was recorded i.e. only 9-10% of the reactive intermediates produced oxidized the substrate.

### 3.3. Results and Analysis

#### 3.3.1. Absorption Spectroscopy

Iron porphyrins have intense Soret band which helps in monitoring these complexes after attachment on the modified surfaces. Absorption data obtained on \( \text{FeFc}_4 \) attached to imidazole linker show a Soret at 422 nm (Figure 3.4A, red). When the sample is reduced by dithionite solution the Soret shifts to 432 nm (Figure 3.4A, green), which upon binding CO sharpens and moves to 426 nm (Figure 3.4A, blue).\(^{62}\) These values are consistent with previous reports of imidazole coordinated Fe-porphyrin.\(^{63}\) Absorption data obtained on electrode bearing the \( \text{FeFc}_4 \) attached to dithiol linker show a Soret at 420 nm (Figure 3.4B, green) which upon reduction shifts to 438 nm. Upon reduction a 565 nm band at the Q-band region is also seen. Binding CO to the reduced complexes results in a Soret at 422 nm and not at 450 nm as may be expected for a P450 like active site (Figure 3S4, SI). These results reproduce previous reports on several thiolate bound heme active sites and is likely due to protonation of the thiolate ligand on CO binding.\(^{64}\) When the catalyst is bound to imidazole the Soret shifts from 420 nm to 426 nm indicating the formation of a low spin complex upon binding strong donor like imidazole (Figure 3.4B, yellow).\(^{65, 66}\) A band at 482 nm gains intensity when bound to external imidazole ligand. This band is not Soret.\(^{67}\) The observed values and their changes with changes in oxidation state of iron and the trans axial ligand (e.g. \( \text{H}_2\text{O}, \text{CO}, \) imidazole) are in general consistent with previous reports on P450 and thiolate bound heme complexes. These data are collected on a monolayer modified transparent electrode surface and thus appear noisy if compared to solution and in many cases the value of the Soret transition can only be tentatively assigned. However, in spite
of such dilution, the very high absorption coefficient of the porphyrin results in resonable absorption features. Considering the molar extinction coefficient of native P450 (~$10^5$ M$^{-1}$ cm$^{-1}$), the beam area (0.385 cm$^2$) and the path-length (0.1 cm) of the cell the number of molecules (i.e. the coverage) has been estimated to be around $1.5\pm0.5 \times 10^{-11}$ moles/cm$^2$.

**Figure 3.4.** (A) Absorption spectra of FeFc$_4$ attached to a monolayer of ImdC$_{11}$SH when oxidized (red), reduced (green) and when reduced and CO bound (blue) in pH 7. (B) Similar spectra of FeFc$_4$ attached to SHC$_{11}$SH when oxidized (green), reduced (red) in pH 7 and in 100 mM imidazole containing pH 7 (yellow).

### 3.3.2. Surface Enhanced Resonance Raman Spectroscopy (SERRS)

The SERRS spectra of the FeEs$_4$ complex attached to the imidazole linker show the oxidation and spin state marker $v_4$ and $v_2$ bands at 1367 cm$^{-1}$ and 1566 cm$^{-1}$, respectively (Figure 3.5A, orange). This reflects a low-spin ferric state of the Iron. The corresponding $v_4$ vibrations for the thiolate linker is at 1365 cm$^{-1}$ and there are two $v_2$ vibrations at 1551 cm$^{-1}$ and 1565 cm$^{-1}$ indicating that there is a mixture of high spin ($v_2$ at 1551 cm$^{-1}$) and low spin ($v_2$ at 1565 cm$^{-1}$) species (Figure 3.5A, blue). The $v_8$ vibration which represents the symmetric Fe-N$_{pyrrole}$ stretch, is at 391 cm$^{-1}$ for the imidazole linker and it shifts to 388 cm$^{-1}$ for the thiolate linker (Figure 3.5A, inset) consistent with previous results. This may reflect the stronger donation by the anionic covalent thiolate donor which weakens the Fe-N$_{pyrrole}$ bond. However this
comparison is only speculative as the $v_8$ of the thiolate bound site is a mixture of high spin (HS) and low spin (LS) species.

![Figure 3.5. SERRS data of FeEs$_4$ when attached to ImdC$_{11}$SH (orange) and SHC$_{11}$SH (blue) in the (A) high frequency region and in the (B) low frequency region.](image)

In the low frequency region, additional vibrations are observed for the thiolate linker at 341 cm$^{-1}$ and 675 cm$^{-1}$ (Figure 3.5B). Further there is an increase in intensity of the 770 cm$^{-1}$ vibration. It is tempting to assign the 341 cm$^{-1}$ vibration as a Fe-S stretching mode for the high-spin species and the 675 cm$^{-1}$ vibration and the 770 cm$^{-1}$ vibration as C-S stretching modes. However, such assignment needs appropriate isotopic substitution to be confirmed which is beyond the scope of this study.

In the presence of 100 mM imidazole, the SERRS data of the FeEs$_4$ complex attached to the thiolate linker show the $v_4$ and $v_2$ vibrations at 1366 cm$^{-1}$ and at 1565 cm$^{-1}$ indicating that a LS species exists under these conditions (Figure 3S5, grey). Thus binding of an external axial imidazole ligand increases the population of low spin species on these surfaces, consistent with the data obtained with imidazole linker.

The SERRS data of FeFc$_4$ bound to imidazole SAM (Figure 3.6A, orange) show the $v_4$ and the $v_2$ bands at 1368 cm$^{-1}$ and 1566 cm$^{-1}$, respectively, indicating that the Fe in the complex is in a LS Fe(III) state. Alternatively, the SERRS data of the FeFc$_4$ complex bound to the dithiol SAM (Figure 3.6A, blue) show a major species with the $v_4$, $v_3$ and $v_2$ bands at 1363 cm$^{-1}$, 1448 cm$^{-1}$
and 1556 cm$^{-1}$, respectively. This originates from a HS Fe(III) centre. The $v_2$ region shows a shoulder at 1567 cm$^{-1}$ indicating the presence of a minor LS Fe(III) species. Overall these results are consistent with those observed for the FeEs$_4$ complex i.e. the imidazole bound complex is mainly LS while the thiolate bound complex is mixture of high and low spin species. In the low frequency region, unique vibrations are observed between 520 cm$^{-1}$ and 741 cm$^{-1}$ (Figure 3.6B, blue). These vibrations may originate from Fe-S-C motif (C-S stretching vibration) and can only be assigned with appropriate isotopic substitution.

Figure 3.6. SERRS data of FeFc$_4$ when attached to ImdC$_{11}$SH (blue) and SHC$_{11}$SH (red) in the (A) high frequency region and in the (B) low frequency region.

In the presence of 100 mM imidazole, the SERRS data of the FeFc$_4$ complex attached to the thiolate linker is similar to that of the FeEs$_4$ complex. The $v_4$ and $v_2$ vibrations appear at 1369 cm$^{-1}$ and at 1566 cm$^{-1}$, respectively, indicating that the iron porphyrin complex exists as a purely LS species (Figure 3.6, grey) on binding an axial imidazole ligand.

SERRS data of Fe picket-fence complex attached to the imidazole SAM (Figure 3.7, orange) show the $v_4$ and the $v_2$ bands at 1364 cm$^{-1}$ and 1554 cm$^{-1}$, respectively, indicating that the Fe in the complex is in a HS Fe(III) state. For the thiolate bound Fe picket-fence catalyst the $v_4$ band appear at 1362 cm$^{-1}$ and the $v_2$ band appear at 1554 cm$^{-1}$ and 1566 cm$^{-1}$ indicating the presence of the Fe in the complex as a mixture of high and low-spin Fe(III) states (Figure
In the presence of 100 mM imidazole, the SERRS data of the Fe picket-fence complex attached to the thiolate linker show the increase in LS Fe(III) state (Figure 3.7, green).

![SERRS data of Fe picket-fence attached to ImdC11SH (orange) and SHC11SH in pH 7 (blue) and 100 mM imidazole containing pH 7 (green).](image)

**Figure 3.7.** SERRS data of Fe picket-fence attached to ImdC11SH (orange) and SHC11SH in pH 7 (blue) and 100 mM imidazole containing pH 7 (green).

The SERRS data obtained for different catalyst are summarized in Table 3.2. The data suggest clear differences in the SERRS data of these catalysts bound to linkers and the data obtained by physiadsorbing these catalysts on octane thiol surfaces. In particular for the physiadsorbed surfaces the $v_3$ vibration is $>$1450 cm$^{-1}$, the $v_4$ and the $v_2$ vibrations and the distribution of the high spin and low spin species are quite distinct from those obtained for the coordinated surfaces (Figure 3S7, SI and Table 3.2). Further the spin states of the iron centre depend on the nature of the coordinating ligand. The triazole bearing porphyrins stabilize a low spin ground state with an imidazole ligand but result in a dominantly HS state (minor low spin species present as well) with a thiolate ligand. Note that this distribution of HS and LS is also seen in case for P450 enzyme and its mutant.\(^{74,75}\) In addition to these there are several unique vibrations in the low energy region of the SERRS spectra which agree with known values of Fe-S and C-S vibrations supporting the formation of thiolate coordinated heme sites on the surface. The Fe picket-fence complex bound to imidazole is high spin. However, all of these complexes become low spin when incubated in a 100 mM imidazole buffer due to an exogenous imidazole binding. Unfortunately SERRS data could not be obtained on the FeFc$_4$ and the FeEs$_4$ bound to...
the imidazole linker in the presence of 100 mM imidazole as it led to dissociation of the iron porphyrin from the surface (*vide infra*).

### 3.3.3. Electrochemistry

The CV of the Fe picket-fence complex functionalized electrodes show well developed Fe$^{III}$/Fe$^{II}$ CV for both imidazole and thiolate linkers in the absence of O$_2$ (Figure 3.8). For the imidazole linker the $E_{1/2}$ of the porphyrin Fe$^{III}$/Fe$^{II}$ process is at -217 mV (Figure 3.8, blue) and for the thiolate linker this value is obtained at -250 mV (Figure 3.8, red, Table 3.3). Note that the reduction of the iron could lead to ligand dissociation. However that would lead to loss of catalyst from the surface and there will be loss of CV intensity between successive scans. However that is not the case indicating that both the oxidized and reduced iron porphyrin complexes are bound to the surface.

### Table 3.2. Marker bands (in cm$^{-1}$) of different Fe-porphyrins attached to SHC$_{11}$SH or ImdC$_{11}$SH linker in pH 7 buffer

<table>
<thead>
<tr>
<th>Linker</th>
<th>$\nu_4$</th>
<th>$\nu_3$</th>
<th>$\nu_2$</th>
<th>$\nu_8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeEs$_4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_8$SH</td>
<td>1363</td>
<td>1450</td>
<td>1557/1564</td>
<td>388</td>
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<tr>
<td>SHC$_{11}$SH</td>
<td>1364</td>
<td>1439</td>
<td>1551/1565</td>
<td>388</td>
</tr>
<tr>
<td>SHC$_{11}$SH (with 100 mM imidazole)</td>
<td>1366</td>
<td>-</td>
<td>1565</td>
<td>389</td>
</tr>
<tr>
<td>ImdC$_{11}$SH</td>
<td>1367</td>
<td>-</td>
<td>1566</td>
<td>391</td>
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<tr>
<td>FeFc$_4$</td>
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<tr>
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<td>1454</td>
<td>1555/1565</td>
<td>390</td>
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<td>SHC$_{11}$SH</td>
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<td>1556/1567</td>
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<tr>
<td>SHC$_{11}$SH (with 100 mM imidazole)</td>
<td>1369</td>
<td>-</td>
<td>1566</td>
<td>391</td>
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<td>389</td>
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<td>Fe picket-fence</td>
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<td>1362</td>
<td>1438/1456</td>
<td>1554/1566</td>
<td>392</td>
</tr>
<tr>
<td>SHC$_{11}$SH (with 100 mM imidazole)</td>
<td>1357/1368</td>
<td>-</td>
<td>1554/1565</td>
<td>393</td>
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<tr>
<td>ImdC$_{11}$SH</td>
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<td>1437/1454</td>
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</tbody>
</table>
Similarly, the CV of the FeFc₄ catalyst functionalized electrodes show well developed Fc⁺/Fc and Fe³⁺/Fe²⁺ CVs for both imidazole and thiolate linkers in the absence of O₂ (Figure 3.9). For the imidazole linker the E₁/₂ of the Fc⁺/Fc and the porphyrin Fe³⁺/Fe²⁺ processes are at 365 mV and -210 mV, respectively (Figure 3.9, blue). For the thiolate linker these values are obtained at 362 mV and -244 mV, respectively (Figure 3.9, orange, Table 3.3). In the presence of CO when FeFc₄ is bound to thiolate the Fc/Fc⁺ CV appears at same potential but the Fe³⁺/Fe²⁺ CV is not observed consistent with thiolate protonation indicated by the UV-Vis data (Figure 3S8, SI). The electroactive ferrocene groups acts as an internal redox marker and it does not shift depending on the linker. Thus, for both the Fe picket-fence and FeFc₄ catalysts, the Fe³⁺/Fe²⁺ process shifts to negative potentials for the thiolate linker relative to the imidazole linker. The CV data for the Fe³⁺/Fe²⁺ couple is much broader than that of the Fc⁺/Fc couple. This may be because of the presence of two different spin states of these complexes (see SERRS data above) resulting in two different redox processes under the same wave.⁷⁶
Figure 3.9. CV data of FeFc$_4$ in deoxygenated pH 7 buffer when attached to ImdC$_{11}$SH (blue) and SHC$_{11}$SH (orange) using Ag/AgCl as reference and Pt wire as counter electrodes under Ar atmosphere.

<table>
<thead>
<tr>
<th>Table 3.3. Observed $E_{1/2}$ values (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst</td>
</tr>
<tr>
<td>Fe picket-fence</td>
</tr>
<tr>
<td>FeFc$_4$</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

The integration of the current under the Fc$^+/Fc$ and/or Fe$^{III}/Fe^{II}$ processes yields the total number of electroactive species immobilized on the surface i.e. surface coverage (Table 3.4).$^{47}$ The Fe “picket fence” shows coverage of 0.7% and 0.9% on thiol and imidazole functionalized SAM, respectively. For the FeFc$_4$ complex the coverages for the Fc$^+/Fc$ and Fe$^{III}/Fe^{II}$ processes are found to be 6.6% and 1.4% and 6.6% and 1.6% on thiol and imidazole functionalized SAM, respectively. Thus the Fc$^+/Fc$ current is about four times that of Fe$^{III}/Fe^{II}$, consistent with the stoichiometry of the molecule (i.e. four Fc groups per Fe heme center). Note that $\sim$1% coverage of the total electroactive surface indicates that a very dilute monolayer of catalyst is present on the electrode.$^{77}$
CV experiments are performed at different pHs to gain insight into the nature of trans axial ligand. For the imidazole linker, both Fe picket-fence and FeFc4 complexes show a pH dependent $E_{1/2}$ process (Figure 3.10A and 3.10B). [Note that Fc+/Fc process in the FeFc4 complex is pH independent]. The $E_{1/2}$ for the porphyrin bound Fe$^{III/II}$ shifts to more negative values at higher pHs. Approximately 60 mV shift in $E_{1/2}$ per unit shift in pH is observed between pH 6-8 characteristic of a single proton coupled electron transfer (PCET) process and is consistent with an $\text{Imd-Fe}^{III}-\text{OH} + e^- + H^+ = \text{Imd-Fe}^{II}-\text{OH}_2$ redox equilibrium.\textsuperscript{78, 79} The data also suggest that the pKa’s involved are higher for the FeFc4 complex relative to the Fe picket-fence complex.\textsuperscript{80} In the presence of 100 mM imidazole in the buffer, the pH dependence of the Fe$^{III/II}$ process for the picket-fence complex is abolished (Figure 3.10A, green).\textsuperscript{81} This is consistent with the replacement of the ionizable H$_2$O ligand by non-ionizable imidazole i.e. $\text{Imd-Fe}^{III}$-Imd + e$^-$ = $\text{Imd-Fe}^{II}$-Imd. The original electrochemistry behaviour is reinstated once the imidazole containing buffer is replaced with normal pH 7 buffer.

**Table 3.4. Calculated surface coverages (%)**

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>ImdC$_{11}$SH</th>
<th>SHC$_{11}$SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe “picket fence”</td>
<td>0.9 ± 0.02</td>
<td>0.7 ± 0.05</td>
</tr>
<tr>
<td>FeFc4</td>
<td>Fc+/Fc</td>
<td>6.6 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Fe$^{III/II}$</td>
<td>1.6 ± 0.05</td>
</tr>
</tbody>
</table>

**Figure 3.10.** Plot of $E_{1/2}$ values at various pHs of Fe picket-fence (A) and FeFc4 (B) attached to ImdC$_{11}$SH SAM vs pH with and without 100 mM imidazole.
In the case of thiolate linker, the CV data of the FeFc\textsubscript{4} and the Fe picket-fence complexes indicate that the $E_{1/2}$ shows little pH dependence between pHs 6-9 (Figure 3.11A and 3.11B) relative to the imidazole linker. This is consistent with a RS-Fe\textsuperscript{III}-OH\textsubscript{2} + e\textsuperscript{-} = RS-Fe\textsuperscript{II}-OH\textsubscript{2} redox equilibrium. SERRS data indicates that the thiolate linker results in a mixture of high-spin and low-spin species (vide supra). However the pH dependence of $E_{1/2}$ indicates that in both of these cases (i.e. high-spin and low-spin) a PCET is not involved.

![Figure 3.11](image_url)

**Figure 3.11.** Plot of $E_{1/2}$ values at various pHs of Fe picket-fence (A) and FeFc\textsubscript{4} (B) attached to SHC\textsubscript{11}SH SAM vs pH with (red) and without (green) 100 mM imidazole.

In summary, the pH dependent CV data suggest that these complexes bear a trans OH\textsuperscript{-} ligand when bound to imidazole and a trans H\textsubscript{2}O ligand when bound to thiolate at pH 7. In the case of the imidazole linker, the trans OH\textsuperscript{-} ligand is protonated upon reduction and hence its $E_{1/2}$ follows a pH dependence befitting a PCET mechanism\textsuperscript{79}. Alternatively, no protonation is involved in the case of the thiolate linker and hence its $E_{1/2}$ is pH independent.

### 3.3.4. O\textsubscript{2} Reactivity

#### 3.3.4.1. O\textsubscript{2} reduction

In the presence of O\textsubscript{2} in the buffer a linear sweep voltammetry (LSV) experiment shows large electrocatalytic O\textsubscript{2} reduction currents at negative potentials for all the three catalysts for both imidazole and thiolate linkers (Figure 3.12). Note that at these potentials the iron
porphyrin catalyst is reduced to Fe$^{II}$ (Figure 3.8 and 3.9). The data indicate that for the thiolate linker the O$_2$ reduction occurs at more negative potentials relative to an imidazole linker (Figure 3.12).$^{82,83,84}$ This is consistent with lower reduction potentials of the thiolate ligated complexes and reflects that when bound to an anionic thiolate linker more driving force is needed to reduce O$_2$ relative to a neutral imidazole linker. The potentials for O$_2$ reduction where no linker is present (i.e. catalyst physiadsorbed on thiol surfaces) are distinct from these potentials (Section 3.2.4.3). This, in addition to the SERRS and UV-Vis data, clearly indicates the formation of thiolate and imidazole coordinated active sites on the electrode.

![Figure 3.12. LSV data of Fe picket-fence (A), FeEs$_4$ (B) and FeFc$_4$ (C) in air saturated pH 7 buffer when attached to ImdC$_{11}$SH (green) and SHC$_{11}$SH (red) using Ag/AgCl as reference and Pt wire as counter electrodes.](image)

### 3.3.4.2. Partially Reduced Oxygen Species

Rotating ring disc electrochemistry (RRDE) is used to estimate the amount of partially reduced oxygen species (PROS) produced due to incomplete O$_2$ reduction. In this technique any O$_2^-$ or O$_2^{2-}$ i.e. a 1e$^-$ or 2e$^-$ reduction produced in the modified Au working electrode is radially diffused, due to the hydrodynamic current created by the rotation, to the ring, which is held at 0.7 V, where these are oxidised back to O$_2$ (Scheme 3.4).$^{82,85}$ This results in an oxidation current in the ring and the ratio of the catalytic current of the disc ($i_c$) and the ring ($i_v$) yields the % of PROS produced. Note that if O$_2$ is reduced to H$_2$O, no current is detected in the ring.
The Fe picket-fence catalyst when bound to the imidazole linker produces 5% PROS i.e. 95% of the O₂ is reduced to H₂O. Alternatively, when the same catalyst is bound to thiolate almost twice the amount of PROS (10%) is produced (Table 3.5). This reflects the trans effect of the thiolate ligand which leads to greater hydrolysis of the Fe^{III}-O₂⁻ and/or Fe^{III}-OOH species produced during O₂ reduction (Scheme 3.5) generating more PROS. The FeEs₄ catalyst produces 10% PROS when bound to the imidazole linker and 17% PROS when bound to the thiolate linker. The increase in PROS in the FeEs₄ catalyst relative to the Fe picket-fence catalyst (either imidazole or thiolate coordinated) reflects the greater hydrophilicity of the distal pocket in the FeEs₄ catalyst which increases the hydrolysis of the Fe^{III}-O₂⁻/Fe^{III}-OOH species produced on the electrode generating greater PROS. Even for the FeEs₄ catalyst almost twice the amount of PROS is produced when bound by thiolate consistent with the result obtained with Fe picket-fence. Note that the “push-effect” of thiolate is also reflected in the auto-oxidation rate of P450 enzyme when compared to Myoglobin (vide infra). The FeFc₄ catalyst which bears the hydrophilic distal pocket as well as the electron transfer site produces ~10% PROS for both imidazole and thiolate linkers. The presence of additional electron donors counter the trans effect of the thiolate ligand.

Scheme 3.4. Schematic representation of PROS detection mechanism by a RRDE set-up.

\[
\text{disk: } O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \\
O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \\
\text{ring: } H_2O_2 \rightarrow O_2 + 2e^- + 2H^+
\]
Scheme 3.5. Generic oxygen reduction mechanism for Fe-porphyrins.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>ImdC\textsubscript{11}SH</th>
<th>SHC\textsubscript{11}SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe picket-fence</td>
<td>5 ± 1</td>
<td>9.8 ± 1</td>
</tr>
<tr>
<td>FeEs\textsubscript{4}</td>
<td>10 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>FeFc\textsubscript{4}</td>
<td>9 ± 1</td>
<td>10.5 ± 1</td>
</tr>
</tbody>
</table>

3.3.5. Substrate oxidation

The CV of Fe picket-fence bound to the dithiol linker in pH 7 buffer containing 100 mM imidazole shows a well resolved Fe\textsuperscript{III}/Fe\textsuperscript{II} CV (Figure 3.13A, green). After one oxygen reduction cycle (i.e. a CV run between +0.5 V to -0.5 V in air saturated pH 7 buffer) 60% decay in the Fe\textsuperscript{III}/Fe\textsuperscript{II} current is observed (Figure 3.13A, orange). This implies that 60% of the catalyst has decayed possibly due to the production of highly oxidizing species during O\textsubscript{2} reduction. No such decay is observed when the same catalyst is bound to the imidazole linker (Figure 3S10). Alternatively, when the FeFc\textsubscript{4} catalyst bound to the dithiol linker is used, i.e. the catalyst bearing electron donor Fc groups, only 20% decay is observed (Figure 3.13B). This further supports the formation of a highly oxidizing intermediate during the oxygen reduction cycle, as the ferrocene
groups present reduces the reactive intermediate thus slowing down the decay of the catalyst leading to greater stability of the catalyst. It is likely PROS produced during O$_2$ reduction may lead to catalytic decay. However both the FeFc$_4$ catalyst and the Fe “picket fence” catalyst produce 10% PROS when bound to thiolate but the former shows 20% decay whereas the later shows 60% decay. Thus the decay is likely to be caused by O$_2$ derived reactive species and not by PROS.

Figure 3.13. CV of Fe picket-fence (A) and FeFc$_4$ (B) attached to SHC$_{11}$SH linker in pH 7 buffer containing 100 mM imidazole before (light green) and after (orange) one O$_2$ reduction cycle in pH 7 buffer using Ag/AgCl as reference and Pt wire as counter electrodes.

To chemically evaluate the possibility of formation of high valent intermediates during O$_2$ reduction by these iron porphyrin active sites on the electrodes, the O$_2$ reduction reactions are performed in the presence of K$_4$[Fe(CN)$_6$] using a RRDE set-up. In these experiments the Pt ring is held at 0 V where it will reduce any [Fe(CN)$_6$]$^{3-}$ to [Fe(CN)$_6$]$^{4-}$. As the potential of the working electrode bearing the catalysts is gradually lowered and it starts to reduce O$_2$ (Figure 3.14, red and blue lines), the ring current simultaneously increases (Figure 3.14, dashed red and blue) suggesting concomitant oxidation of [Fe(CN)$_6$]$^{4-}$ present in the solution to [Fe(CN)$_6$]$^{3-}$. The formal potential of [Fe(CN)$_6$]$^{3-/4-}$ is 0.24 V vs Ag/AgCl at pH 7 is much higher than the potentials applied to the working of the Pt ring electrode during these experiments. Thus neither the working electrode or the Pt ring electrode can oxidize [Fe(CN)$_6$]$^{4-}$ to [Fe(CN)$_6$]$^{3-}$. Hence the iron porphyrin catalysts must be producing species with $E^o > 0.24$ V which is capable
of oxidizing the $[\text{Fe(CN)}_6]^{4-}$ present in the solution to $[\text{Fe(CN)}_6]^{3-}$ which is then detected in the ring (Figure 3.14, dashed blue and red). Since the $E_{1/2}$ of Fe$^{III/II}$ for iron porphyrin complexes are well below this value (Figure 3.9) which indicates that species having oxidation states higher than +III (Fe$^{IV}$=O of compound I type) are being generated on the electrode during O$_2$ reduction by iron porphyrin catalysts bound to both imidazole and thiol linkers. Note that a SAM covered Au electrode, i.e. without any catalyst, does not show any $[\text{Fe(CN)}_6]^{3-}$ formation when swept over the same potential range. The amount of PROS (O$_2$, H$_2$O$_2$) produced on these bio-inspired electrodes (5-10%) is much less than the amount of $[\text{Fe(CN)}_6]^{4-}$ oxidation observed (~15%). Thus these species cannot account for the oxidation of $[\text{Fe(CN)}_6]^{4-}$ to $[\text{Fe(CN)}_6]^{3-}$ at these low potentials. In a control experiment, the oxidation of ferrocyanide to ferricyanide was monitored using a bare Au electrode as the working electrode. Bare Au disc (i.e. not covered by SAM) produces 55% PROS in pH 7. However 55% PROS produced oxidizes only about 17% ferrocyanide i.e. ~1/3rd. We think this is due to slow kinetics of oxidation of ferrocyanide to ferricyanide by H$_2$O$_2$ ~ 10$^{-4}$ min$^{-1}$. Thus, 5-10% PROS in these catalysts can only produce 1/3rd i.e. 1-3% ferricyanide. Hence we thought the formation of 15% ferricyanide was likely due to oxidation of ferrocyanide by high-valent iron species formed during O$_2$ reduction.

![Image of RRDE experiment](image_url)

**Figure 3.14.** RRDE experiment of Fe picket-fence when bound to ImdC$_{11}$SH (blue) and SHC$_{11}$SH (red) linkers in pH 7 buffer containing 10 mM $K_4[\text{Fe(CN)}_6]$ and ring was held at 0 V using Ag/AgCl reference and Pt wire counter electrodes. The disc current is shown in bold line and ring current in dashed line.
Enthused by the possibility of formation of high valent intermediates formed during O$_2$ reduction by the thiolate and imidazole ligated Fe picket-fence catalyst, the reactivity of these intermediates towards inert C-H bonds were investigated. Indeed, when substrates like cyclohexane and toluene are present in the aqueous buffer (saturated solutions) hydroxylations of very inert C-H bonds are observed (Table 3.6 and SI) on the thiolate bound Fe “picket fence” surfaces. Gas chromatography (Figure 3S11 and 3S12, SI) and GC-MS of the products indicated that cyclohexane was oxidized to cyclohexanol and cyclohexanone with turnover numbers of 241 and 27, respectively.\(^8\) Alternatively toluene, that bears both benzylic and aromatic C-H bonds, was oxidized to produce benzyl alcohol and p-cresol i.e. both benzylic and aromatic C-H bond hydroxylations were observed.\(^9\) Interestingly, further oxidation of the hydroxylated products to ketones was not observed. This is possibly due to higher solubility of the hydroxylated products in water. This reactivity is unique to Fe picket-fence porphyrin bound to thiolate. No detectable reaction was observed when thiolate is replaced by imidazole or when the hydrophobic Fe picket-fence is replaced by hydrophilic FeEs$_4$. These surfaces produce however significant amount of PROS (thiolate bound ester produces 17% PROS). This suggests that the observed hydroxylations are catalysed by metal centred high valent species and not by PROS. Attempts to label the oxidized products by $^{18}$O, by using $^{18}$O$_2$ instead of $^{16}$O$_2$ during the formation of the high valent species, was not successful because of the very fast exchange rate between Fe$^{IV}=^{18}$O and H$_2^{16}$O.\(^22,90,91\)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product (TON)</th>
</tr>
</thead>
</table>
| Cyclohexane | Cyclohexanol (241)  
Cyclohexanone (27) |
| Toluene | Benzyl alcohol (13)  
p-cresol (27) |

One of the advantages of attaching catalysts to ligands immobilized on the surface is that these electrodes are recyclable. This is demonstrated by functionalizing a thiol linker bearing electrode with the Fe picket-fence catalyst (Figure 3S15, green) and then, after utilizing
this electrode to catalytically reduce O\textsubscript{2}, washing off the electrode with dil. HCl (or any dilute acid). This protonated the axial thiolate ligand and the Fe-S bond was cleaved. The free catalyst was then washed off and an electrode with no catalyst bound is generated which does not reduce O\textsubscript{2} (Figure 3S15, cyan). This electrode is again successfully functionalized with Fe picket-fence and the catalytic O\textsubscript{2} reduction activity is gained back (Figure 3S15, red). This allowed recycling of electrodes bearing thiolate and imidazole linkers even after catalyst decay.

### 3.4. Discussions

A combination of spectroscopic and electrochemical techniques indicates that dilute site-isolated active sites can be created where the axial ligand, thiolate or imidazole, is provided from the electrode. This approach has been previously demonstrated for imidazole and pyridine linkers and reduces the synthetic onus involved in making synthetic models.\textsuperscript{49,51} Conventionally synthesizing a Fe\textsuperscript{III}-SR porphyrin complex is complicated because of the \(2\text{Fe}^\text{III} + 2\text{RS}^- \rightarrow 2\text{Fe}^\text{II} + \text{RSSR} \) process. However, this possibility is eliminated on very dilute surfaces such as the ones used here as the thiolates are spatially separated and cannot form disulfide dimers. Similarly, synthesis of iron porphyrins with covalently bound imidazole ligands are complicated by the formation of bridged dimers. Such a possibility is again reduced on a dilute surface employed here. SERRS and CV data allow determination of the nature of the active sites and the trans axial ligands. In spite of the fact that thiolate is a much stronger donor compared to imidazole, SERRS data show that the thiolate bound active sites are a mixture of five coordinated HS and six coordinated LS species while the imidazole bound active sites are all LS.\textsuperscript{71} In the low frequency region of SERRS data (Figure 3.5B and 3.6B) few additional bands are observed for thiolate axial ligands which may arise from the C-S stretching vibration, but conclusive assignments cannot be made without isotope (S\textsuperscript{32}) labelling. The Fe\textsuperscript{III/II} \(E_{1/2}\) potential is determined to be more negative for the thiolate bound active site at pH 7. However, the \(E_{1/2}\) of the imidazole and the thiolate bound active sites are difficult to compare at this stage as these sites vary in their spin states and also in the nature of the trans axial ligands (i.e. H\textsubscript{2}O vs OH\textsuperscript{-}) which may significantly affect the \(E_{1/2}\).\textsuperscript{92} The variation of the \(E_{1/2}\) for the imidazole linker between pH 5-9 (~60 mV per pH unit) is suggestive of the presence of a proton coupled electron transfer process.\textsuperscript{79} The
oxidized ferric state is possibly bound to a hydroxide ligand and upon reduction accepts a neutral H₂O ligand thus the reduction of the imidazole coordinated site requires a proton and hence its E₁/₂ is pH dependent. Note that the protonation of the imidazole ligand does not occur in this pH range. The E₁/₂ value for the thiolate linker is pH dependent below pH 6 and above pH 9 but remains independent in the range 6-9. These data are consistent with the presence of two pH equilibriums for a thiolate bound iron porphyrin complex under aqueous environments. In the pH range 6-9 the axial ligands are thiolate and H₂O for both the oxidized and reduced forms and thus the E₁/₂ remains pH independent. Below pH 6 an RS⁻/RSH equilibrium and above pH 9 an OH⁻/H₂O equilibrium exists, respectively. Both the processes require protonation and are thus pH dependent. However, like some P450 active sites, in the presence of a strong trans axial ligand like CO, the pKa of the thiolate ligand is raised and it gets protonated at pH 7. Notably the E₁/₂ values obtained at various pHs has a contribution from both the HS and LS components as observed in SERRS with the HS species being the major one. Similar pH dependence of Fe₃/II E₁/₂ is reported for some P450 family of enzymes although the analysis there is complicated by a synchronous change in spin state of the iron center.

The pH dependence of the Fe₃/II E₁/₂ suggest that the thiolate axial ligand raises the pKa of the trans aquo ligand such that it remains H₂O for the oxidized ferric site even at pHs as high as 9. This is very similar to the very high pKa observed for the trans H₂O ligand present in the Cytochrome P450 active site. Alternatively, pKa of H₂O ligand bound to iron porphyrin complexes and active sites bearing coordinated imidazole ligand vary from 6-8 depending on the environment. In iron porphyrin complexes, bearing no additional redox active site, the amount of PROS detected with a thiolate linker is twice of that detected with an imidazole linker irrespective of the porphyrin architecture. PROS result from the hydrolysis of Fe²/II-O₂/Fe³/II-OOH species and thus may reflect the higher “push-effect” of the trans thiolate ligand. In the presence of triazole groups in the distal pocket (in case of FeEs₄) greater amount of PROS is produced for both the thiolate and the imidazole linkers. This could be due to hydrophobicity of the picket fence architecture and/or stabilization of the Fe-O₂ adduct due to hydrogen bonding from the amide groups. However the thiolate still produces ~2 times more PROS relative to the imidazole linker. The auto-oxidation rate of P450 enzyme is 20 s⁻¹ whereas that
of myoglobin is $10^{-5}$ s$^{-1}$. Thus the higher PROS generation of the thiolate ligand is in general agreement with the higher rate of auto-oxidation in cytochrome P450 relative to myoglobin. However, in these systems, the PROS vary by a factor of two when in the enzymes the 1st order auto-oxidation rates vary by $10^6$. Thus during steady state the push effect of the thiolate possibly significantly enhances the rate of O-O bond cleavage to compete with auto-oxidation. In case of FeFc$_4$ the presence of extra reducing centre on the porphyrin distal structure minimizes this effect.

Several mimics of Cytochrome P450 are available in literature but almost all are unstable in air.$^{100,52,101,102}$ There are only very few reports of these thiolate bound active sites which are stable in air but neither has explored O$_2$ activation.$^{103,104}$ Reports do exist where thiolate bound systems have activated C-H bonds using H$_2$O$_2$, m-CPBA.$^{27}$ These systems are not equivalent to Cyt P450 which uses molecular O$_2$. Here we report, for the first time, C-H hydroxylation by a thiolate bound porphyrin using molecular O$_2$, a function so far attributed only to Cytochrome P450. The thiolate ligated Fe picket-fence is very reactive and decays fast during O$_2$ reduction. This suggests formation of highly reactive, possibly high-valent, intermediates during O$_2$ reduction. These highly reactive intermediates have been used to oxidize inert C-H bonds. Cyclohexane (BDE = 99.3 Kcal/mol)$^{23}$ is oxidized to cyclohexanol and cyclohexanone and Toluene (BDE, benzylic = 89.9 Kcal/mol, aromatic = 85 Kcal/mol)$^{105}$ is oxidized to cresol and benzyl alcohol. These oxidations occur in pH 7 buffer (saturated with the substrate) at room temperature and using molecular O$_2$. Turnover numbers as high as 200 are estimated. The results indicate that while diffusion of the hydrophobic substrate to the hydrophobic distal environment is favoured, further oxidation of the hydroxylated products is not observed possibly due to higher solubility of these complexes in aqueous medium which diffuses them out into the bulk solvent and away from the reaction center localized on the hydrophobic monolayer (Figure 3.516, SI). When coordinated to the imidazole linker, the iron porphyrin site does produce Fe(IV) species (likely to be Fe$^{IV}$=O) during O$_2$ reduction which can oxidize [Fe(CN)$_6$]$^{4-}$ to [Fe(CN)$_6$]$^{3-}$. However this species is not catalytically competent to hydroxylate C-H bonds. Further, no C-H bond hydroxylations were observed when a porphyrin catalyst with a hydrophilic distal cavity was used. This is likely due to fact that the hydrophobic substrates used
here do not dock in these hydrophilic distal cavity which is possibly essential for reactivity. The hydrophobic distal structure of “picket fence” porphyrin is ideal for this purpose. Note that the C-H bond hydroxylation may be catalyzed by high valent reactive species formed via a peroxide shunt (from the PROS formed) or during reduction of molecular oxygen. RRDE data indicate that the FeEs$_4$ complex bound to a thiolate ligand produce maximum PROS (17%) but it shows no C-H hydroxylation. Similarly, neither of the imidazole bound iron porphyrin complexes shows any detectable activity in spite of producing detectable PROS. Thus O$_2$ derived high-valent species i.e. Fe$^{IV}$=O and not PROS is likely to catalyze the C-H bond hydroxylation observed here.

Catalytic C-H bond hydroxylation using molecular O$_2$ has been a long term goal for chemists. C-H bond hydroxylation is a 2e$^-$ oxidation while the reduction of molecular O$_2$ requires 4e$^-$. In Cytochrome P450 the two additional electrons needed during substrate hydroxylation using O$_2$ are provided by a reductase component (flavin or ferredoxin). These reduced active sites, in spite of having very low E$^0$, does not reduce O$_2$ as they are protected inside a protein environment. A formidable challenge involved in making synthetic catalysts that can catalyze the same reaction is to provide electrons to the active site but not directly to molecular O$_2$ (a reducing agent capable of reducing P450 type complexes, i.e. E$^0$ < -0.5 V, may, and often will, reduce O$_2$ directly). In these systems the electrode acts as the reductase component by providing electron to the active site during turnover. Here electrons do not directly reduce O$_2$ as it is insulated by the SAM i.e. SAM provides the insulation analogous to the environment in a protein.

Previous attempts of electrochemically oxidizing organic alcohols used Rh porphyrins to generate high valent species in aqueous solutions by applying high potentials (> 1 V) on the electrodes.$^{106, 107, 108}$ In these attempts the oxidizing equivalents are provided by the electrode and not by molecular O$_2$. There are some inspiring early reports where high valent oxo species of Mn or Fe porphyrins were generated electrochemically in organic solvents or immobilized in electrodes and were used to activate C-H bonds using molecular O$_2$.$^{109, 110, 111, 112}$ These works report high Turnover numbers (TON) and very high Faradaic yields (FY). In this chapter, by mimicking the thiolate ligation and hydrophobic substrate binding pocket of “picket fence” porphyrins, we are able to oxidize C-H bonds using molecular O$_2$ in aqueous solvents using
similar approach. In our case though TON is almost similar to these reports, FY is about ~10%. In the current approach there is always a competition between reaction of the high-valent intermediates with the substrate (catalytic process, Scheme 3.6, blue arrow) and reduction of these by electron transfer from the electrodes (Scheme 3.6, green arrow) leading to $4e^-/4H^+ O_2$ reduction. This compromises the FY of this process.\textsuperscript{113} The electron transfer rate can be retarded by increasing the chain length of the thiols used. This should, in principle, increase the life-time of the reactive species on the electrode and hence increase the probability of the catalytic process.

![Scheme 3.6. Schematic representation of the competing reactions on a Bio-inspired electrode.](image_url)

Finally a key advantage of these electrodes is that they can be reversibly constructed which minimizes the effort and time of reloading. An attachment via an irreversible covalent bond formation or through thin layer deposition is also well known. However, in those cases the electrode and the monolayer become dysfunctional as the catalyst decays. Alternatively, the metal ligand bonds are easily cleaved via protonation. This allows the metal catalyst to be easily removed after washing with dilute acid without affecting the monolayer.
3.5. Conclusion

In summary thiolate and imidazole bound site-isolated active sites are created on self-assembled monolayers. These easy to construct recyclable electrodes can activate molecular O₂ in pH 7 and generate high-valent species in the process which can catalyze C-H bond hydroxylations at room temperatures. Turnover number as high as 200 is estimated for oxidation of cyclohexane to cyclohexanol using molecular O₂.

3.6. References

(55) Lower concentrations than this did not result in good SAM.
(62) The λ of the Soret were determined by taking a 1st derivative of the spectrum (derivative=0 at maximum). However, the data on the reduced imidazole bound complex did not offer a clean maximum using the 1st derivative. In that case the maximum was approximated.
(73) The Fe-S vibration for the S=1/2 species is reported to be ~390 cm⁻¹ which overlaps with the strong υ₈ band.
The CV waves retain their nature and the $E_{1/2}$ values remain unchanged even after background correction (Figure S8, SI).

This is also indicated from the UV-Vis data. Note that the coverages obtained from both the methods are in agreement.

FeFc complex dissociates in the presence of 100 mM imidazole in the buffer. So no CV of imidazole bound FeFc attached to the Imidazole linker could be recorded.

The reaction of decomposed porphyrin with oxygen cannot be ruled out and may also be a reason for PROS generation.

In page 36 where the equilibrium has been mentioned the trans ligand is H$_2$O so it remains as S- at pH 7. But when the trans ligand is CO (as in UV-vis) the pKa shifts higher and so the linker remains protonated i.e. as SH.
(113) It is surprising that the previous reports show high FY in the presence of O$_2$ and H$^+$ in the medium as all of these porphyrins are known to electrocatalytically reduce O$_2$ in organic solvents.
3.7. Supporting Information (SI)

Synthesis of 11-Imidazole undecan-1-thiol (ImdC11SH)

3.7.1.1. Synthesis of 11-Imidazole undecan-1-ol (a)

11-Bromo undecan-1-ol was refluxed with 2 eqv. of imidazole in about 7 mL DMF for overnight. Excess imidazole was used which acted itself as a base for the reaction (Use of other bases like Et₃N sometimes resulted in a dimer product of imidazole). The reaction mixture was worked up with Diethyl ether and double distilled water. The ether layer was evaporated and pure compound was collected and characterized.

¹H NMR (300 MHz, CDCl₃): δ 1.18 (m, 18H), 3.53 (t, 2H, J=6.5 Hz, 7.0 Hz), 3.84 (t, 2H, J= 7.0 Hz, 7.5 Hz), 6.82 (s, 1H), 6.94 (s, 1H), 7.37 ppm (s, 1H).

3.7.1.2. Synthesis of 11-Imidazole undecan-1-mesyl (b)

11-Imidazole undecan-1-ol was stirred in dry THF on an ice bath containing 2 eqv. of triethylamine (Et₃N). To it 2 eqv. of Mesyl chloride dissolved in dry THF was added dropwise (Care must be taken in the dropwise addition because addition of mesyl chloride all at once lead to the elimination of the imidazole group). The reaction mixture was allowed to stir for 4 hrs. The resulting solution was evaporated and the compound was extracted in DCM. Evaporating the DCM layer gave the pure product.

¹H NMR ( 300 MHz, CDCl₃): δ 1.14 (m, 14H), 1.67 (m, 2H), 1.82 (m, 2H), 2.94 (s, 3H), 4.15 (t, 2H, J=6.6 Hz), 4.22 (t, 2H, J=7.2 Hz), 7.07 (s, 1H), 7.33 (s, 1H), 9.33 ppm (s, 1H).

1.3. Synthesis of 11-Imidazole undecan-1-thioacetate (c)

11-Imidazole undecan-1-mesyl was refluxed with excess potassium thioacetate in dry methanol for about 8 hrs. Methanol was evaporated and the resulting mixture was extracted in DCM and water. The DCM layer was evaporated to obtain the desired compound.

¹H NMR (300 MHz, CDCl₃): δ 1.18 (m, 16H), 1.46 (m, 2H), 2.24 (s, 3H), 2.78 (t, 2H, J=7.5 Hz, 7.2 Hz), 3.85(t, 2H, J=7.2 Hz, 6.9 Hz), 6.83 (s, 1H), 6.98 (s, 1H), 7.41 ppm (s, 1H).

1.4. Synthesis of 11-Imidazole undecan-1-thiol (d)

The thioacetate derivative was refluxed in dry MeOH with about 2 eqv. 0.6 N HCl for about 8 hrs. Methanol was evaporated and the resulting mixture was extracted in DCM and water (It
had to be worked up with water several times to remove excess acid). The DCM layer was evaporated to obtain the desired compound.

$^1$H NMR (500 MHz, CDCl$_3$): 1.19 (m, 16H), 1.31 (m, 2H), 2.60 (t, 1H, $J=7.5$ Hz), 2.87 (t, 1H, $J=7.5$ Hz, 7.0 Hz), 4.03 (t, 2H, $J=7.5$ Hz, 7.0 Hz), 6.95 (s, 1H), 7.13 (s, 1H), 8.31 ppm (s, 1H).

ESI-MS (+ve ion mode, methanol): m/z = 254.03 (93%), [M]$^+$; m/z = 285.98 (100%), [M+CH$_3$OH]$^+$.

2. Synthesis of Undecan-1, 11-dithiol (SHC$_{11}$SH)

2.1. Synthesis of 11-Bromo undecan-1-mesy (e)

11-Bromo undecan-1-ol along with 2 eqv. of Et$_3$N was stirred in dry THF on a ice bath. 2 eqv. of Mesyl chloride was added dropwise to the reaction mixture. The reaction was continued for about 4 hrs. THF was evaporated and the compound was extracted in DCM. The DCM layer was evaporated to obtain the product.

2.2. Synthesis of Undecan-1, 11-dithioacetate (f)

11-Bromo undecan-1-mesy derivative was refluxed in dry MeOH with 4 eqv. of Potassium thioacetate for about 8 hrs. The reaction mixture was worked up with DCM and water. The DCM layer was evaporated and a TLC in 5% DCM-MeOH mixture suggested the formation of two compounds. The desired product was obtained by separating it from the other product by column chromatography in 5% DCM-MeOH mixture.

FT-IR data: 1691 cm$^{-1}$ for thioacetate (SAC$^-$)

2.3. Synthesis of Undecan-1, 11-dithiol (g)

The dithioacetate derivative was refluxed in dry MeOH with about 2 mL of 1 N HCl for overnight. MeOH was evaporated and the reaction mixture was worked up with DCM and double distilled water. (Care has to be taken to remove excess acid). The DCM layer was evaporated to obtain the pure product.

CHN Anal. Calcd. for C$_{11}$H$_{24}$S$_2$: C, 59.9; H, 10.9; S, 29.09. Found: C, 58.55; H, 9.80; S, 29.05.

FT-IR data: no 1691 cm$^{-1}$ of thioacetate.

ESI-MS (+ve ion mode, acetonitrile): m/z = 263.09 (100%), [M + CH$_3$CN]$^+$.
$^1$H NMR (300 MHz, CDCl$_3$) of (a)

$^1$H NMR (300 MHz, CDCl$_3$) of (b)
$^1$H NMR (300 MHz, CDCl$_3$) of (c)

$^1$H NMR (300 MHz, CDCl$_3$) of (d)
ESI-MS (in CH$_3$OH) of (d)

FT IR of (f)
FT IR of (g)

ESI-MS (in CH$_3$CN) of (g)
Figure 3S1. CV data of Fe “picket-fence” in air saturated pH 7 buffer when attached to SHC\textsubscript{11}SH (green) and ImdC\textsubscript{11}SH (blue) and when physiadsorbed in C\textsubscript{8}SH (orange) monolayers at 50 mV/s using Ag/AgCl reference and Pt wire counter electrodes. Note that they show O\textsubscript{2} reduction at clearly different potentials.

Figure 3S2. CV data of FeFc\textsubscript{4} in air saturated pH 7 buffer when attached to SHC\textsubscript{11}SH (green) and ImdC\textsubscript{11}SH (blue) and when physiadsorbed in C\textsubscript{8}SH (red) monolayers at 50 mV/s using Ag/AgCl reference and Pt wire counter electrodes. Note that they show O\textsubscript{2} reduction at clearly different potentials.
**Figure 3S3.** CV data of FeFc₄ in air saturated pH 7 buffer when attached ImdC₁₁SH monolayer before (red) and after (blue) CHCl₃ wash at 50 mV/s using Ag/AgCl reference and Pt wire counter electrodes. Note that the O₂ reduction current seen due to physiabsorption is gone after CHCl₃ wash.

**Figure 3S4.** Absorption spectra of FeFc₄ attached to a monolayer of SHC₁₁SH when oxidized (green), reduced (red) and when reduced and CO bound (blue) in pH 7.
Figure 3S5. SERRS data of FeEs$_4$ attached to SHC$_{11}$SH in pH 7 (red) and 100 mM imidazole containing pH 7 (grey).

Figure 3S6. SERRS data of FeFc$_4$ attached to SHC$_{11}$SH in pH 7 (red) and 100 mM imidazole containing pH 7 (grey).
Figure 3S7. SERRS data of FeEs₄ (A) and FeFc₄ (B) when attached to ImdC₁₁SH (blue) and SHC₁₁SH (green) and when physiadsorbed in C₈SH (red) in pH 7 in the high frequency region. The $\nu_2$, $\nu_4$, $\nu_8$, and $\nu_3$ bands obtained during attachments are different when compared to physiadsorbed case.

Figure 3S8. CV data of FeFc₄ bound to SHC₁₁SH in deoxygenated pH 7 buffer (green) and CO containing pH 7 buffer (orange) using Ag/AgCl as reference and Pt wire as counter electrodes under Ar atmosphere.
**Figure 3S9.** (A) CV data of Fe “picket-fence” in deoxygenated pH 7 buffer when attached to ImdC₁₁SH before and after background correction and (B) CV data of FeFc₄ in deoxygenated pH 7 buffer when attached to SHC₁₁SH before and after background correction using Ag/AgCl as reference and Pt wire as counter electrodes under Ar atmosphere.

**Figure 3S10.** CV of Fe “picket-fence” attached to ImdC₁₁SH linker in pH 7 buffer containing 100 mM imidazole before (green) and after (red) one O₂ reduction cycle in pH 7 buffer using Ag/AgCl as reference and Pt wire as counter electrodes.
Figure 3S11. GC plot of the oxidized sample of cyclohexane along with standards.

Figure 3S12. GC plot of the oxidized sample of toluene along with standards.
Figure 3S13. Calibration curve obtained from peak area (of GC) vs known concentration of cyclohexanol. The concentration of the oxidized sample is obtained from this curve.

Figure 3S14. Calibration curve obtained from peak area (of GC) vs known concentration of cyclohexanone. The concentration of the oxidized sample is obtained from this curve.
Figure 3S15. CV of Fe “picket-fence” attached on SHC₁₁SH linker in pH 7 after initial load (green), after HCl wash (blue) and after reload (red) using Ag/AgCl as reference and Pt wire as counter electrodes.

Figure 3S16. Schematic presentation of the substrate oxidation steps occurring with Fe picket-fence attached to SHC₁₁SH SAM.