

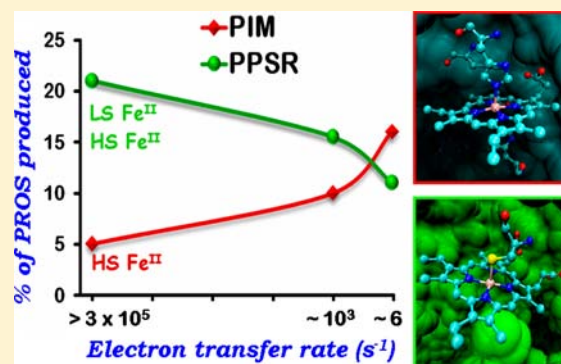
Electrocatalytic O₂ Reduction Reaction by Synthetic Analogues of Cytochrome P450 and Myoglobin: In-Situ Resonance Raman and Dynamic Electrochemistry Investigations

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Supporting Information

ABSTRACT: Bioinspired electrodes have been constructed by physisorption of two air stable iron porphyrin complexes, one bearing an imidazole coordination and the other bearing a thiolate coordination. To control the electron transfer (ET) rate to these O₂ reducing electrocatalysts, the complexes were immobilized on edge plane graphite electrode and alkyl thiol self-assembled monolayer (SAM) modified Au electrodes with varying chain lengths of the thiols. Catalyst immobilized SAM modified surfaces were characterized using surface enhanced resonance Raman spectroscopy (SERRS), and their electrocatalytic O₂ reduction properties were investigated using rotating ring disc electrochemistry (RRDE). While the imidazole bound complex showed increase in partially reduced oxygen species (PROS) on decreasing ET rate, the thiolate bound complex showed the opposite trend, that is, the value of PROS reduced on decreasing the ET rate. SERRS coupled to rotating disc electrochemistry (SERRS-RDE) technique helps gain insight into the O₂ reduction mechanism. The results obtained indicate that while the imidazole bound iron porphyrin complex reduces O₂ through an inner sphere mechanism using a high-spin (HS) Fe^{II} species, the thiolate ligated complex shows an inner sphere as well as outer sphere mechanism using a HS Fe^{II} and low-spin (LS) Fe^{II} species, respectively. The PROS formation by a HS Fe^{II} species of this thiolate bound complex increases with decreasing ET rates while that of a LS Fe^{II} species decreases with decreasing ET rates.



1. INTRODUCTION

Metallo-enzymes containing an iron porphyrin as the prosthetic group are ubiquitous in nature.^{1,2} The nature of axial ligands that are directly coordinated to the iron center of heme protein active sites play an integral role in modulating the properties and reactivities of these heme proteins.^{3–5} Myoglobin and hemoglobin which act as O₂ storage and transport proteins have imidazole coordination from histidine residues from the protein backbone.^{6–8} Cytochrome P450, which hydroxylates inert C–H bonds using molecular O₂, uses thiolate ligation originating from a cysteine residue.^{9–13} On the other hand catalase, which catalyzes the decomposition of hydrogen peroxide into H₂O and O₂, is coordinated to a phenolate group in the axial position originating from a tyrosine residue.^{3,14,15} Changes in the proximal ligation along with differences in the distal environment of these enzyme active sites exert different extents of “push” and “pull” effects.¹⁶ Thus investigating the roles of these axial ligands on the electronic structure and reactivity is an area of general interest. Efforts have been focused on developing functional mimics of active sites that reproduce the structural and/or functional properties of these enzyme active sites. However, a direct comparison between an imidazole bound and a thiolate bound heme and/or iron porphyrin centers is often complicated by changes in the

enzyme active site because of point mutation and/or lack of appropriately designed synthetic model complexes.

Recently the electronic structure of two structurally analogous imidazole coordinated (PIM) and thiolate coordinated (PSR) iron porphyrin complexes were compared using several spectroscopic methods and density functional theory (DFT) calculations.¹⁷ A thiolate coordination shifted the $E_{1/2}$ of the Fe^{III}/Fe^{II} couple ~500 mV more negative compared to imidazole coordination in solution phase because of covalent charge donation from thiolate to Fe. Notably, while most of the synthetic mimics of P450 are found to be unstable in air,^{18–21} the thiolate bound iron porphyrin complex bearing a “picket-fence” architecture (PPSR) is one of the rare examples^{22,23} which are stable in air. The stability derives from protecting the thiol group sterically using a “picket-fence” architecture as originally done by Higuchi and Hirobe.²⁴ These complexes reproduced the spin states and spectroscopic features associated with thiolate and imidazole bound active sites.

Oxygen reduction reaction (ORR) is an important reaction not only in life processes, such as biological respiration,^{25,26} but also in energy conversion relevant to fuel cell applications.²⁷ Although Fe porphyrins are known to be ORR catalysts, the

Received: April 24, 2013

Published: August 20, 2013

mechanism of electrochemical ORR and how it is affected by different axial ligands is not well understood. This multistep O_2 reduction involves many intermediates producing superoxide ($1e^-$ reduction), peroxide ($2e^-$ reduction), and water ($4e^-$ reduction) in aqueous medium depending on the nature of the electrode and catalysts used. Various such intermediates are trapped and characterized in solution using biological enzymes and their synthetic models such as metal-superoxides, metal-hydroperoxides, and metal-oxo species, and so on upon O_2 binding and during its activation and/or reduction under homogeneous conditions.^{9,12,21,28–41} While synthetic models of active sites of myoglobin and cytochrome c oxidase render it easier to detect the intermediates found during O_2 reduction using spectroscopic techniques, cytochrome P450 mimics hardly allow the detection and investigation of intermediates as most of these are highly unstable in O_2 even in their oxidized states.^{18–21} Although significant literature is available on C–H bond hydroxylation reactions in solution,^{22,42–47} little is known about the O_2 reduction mechanism of synthetic thiolate bound porphyrin complexes.⁴⁸ Such investigations are complicated by the inherent instability of Fe^{III} - RS^- complexes. But the stability of PPSR in air can remedy that. Homogeneous catalysis has a very significant inherent limitation which makes catalyzing multielectron multiproton processes such as O_2 reduction challenging; that is, simultaneous addition of both protons and electrons from the same homogeneous reaction medium to the catalyst. The electrons can, and in most cases will, have a tendency of reducing H^+ and/or O_2 present in the medium.

Electrocatalytic O_2 reduction on edge plane graphite (EPG) electrode by several iron porphyrin catalysts are reported in the literature where the catalysts are directly attached on the electrode surface.^{49–53} This results in facile electron transfer (ET) rate from the electrode to the catalyst. To control the ET rate under heterogeneous conditions, self-assembled monolayer (SAM) modified electrodes have been widely used.^{52,54–60} SAM formation enables one to tune the ET rate to the redox active site by varying the chain lengths of the linkers.^{54,52,61–63} Immobilization of the catalysts on SAM covered electrodes provide a good platform to fabricate bioinspired electrodes where the electrode serves the purpose of the reductase component of the enzyme by supplying electrons at a tunable rate to the active site. SAM acts as ET pathway insulating the substrates (O_2 , H^+) from the electrode which simulates the same mutually exclusive pathways for electrons and protons observed in the enzymatic active site. Recently, using a combination of synthesis and self-assembly, bioinspired electrodes mimicking the active sites of imidazole bound and thiolate bound iron porphyrins were reported.⁶⁴ In this study the axial ligands were grafted on a Au electrode and different Fe porphyrins were loaded on these to create a dilute monolayer of catalytic sites. The thiolate bound P450 inspired electrode was found to generate a high-valent intermediate during O_2 reduction which could catalytically hydroxylate inert C–H bonds using molecular oxygen in pH 7 buffer at room temperature, and >200 turnovers were observed.⁶⁴ Recently, a new experimental setup has been developed where dynamic electrochemistry, rotating disc electrochemistry (RDE) is coupled to a powerful spectroscopic tool, surface enhanced resonance Raman spectroscopy (SERRS) which allows direct in situ investigations of the reaction mechanism of electrocatalytic ORR using iron porphyrin catalysts.⁶⁵ Using this SERRS-RDE setup and utilizing a combination of spin and oxidation state marker ν_4 and ν_2 bands and $^{18}/^{16}O_2$ isotope effects a low-spin (LS) Fe^{III} –

OOH species and a $Fe^{IV}=\text{O}$ species were identified as intermediates of O_2 reduction for some synthetic iron porphyrin complexes involved in steady state electrocatalytic ORR.

In this paper we report the formation of bioinspired electrodes by simple physioadsorption of iron(III) porphyrin complexes bearing covalently attached imidazole (PIM) and thiolate (PPSR) ligands (Figure 1) which can catalyze the

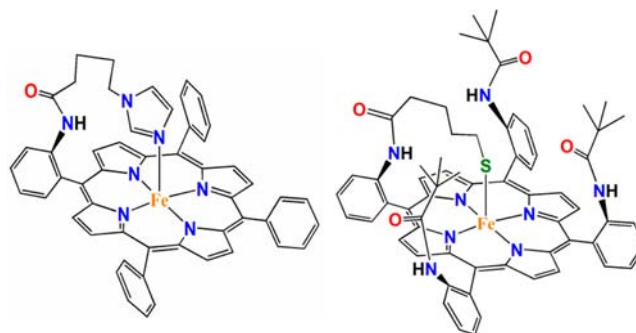


Figure 1. Pictorial representation of the catalysts used: PIM (left) and PPSR (right).

multielectron and multiproton O_2 reduction.⁶⁶ Heterogeneous electrocatalysis under physiological condition is investigated, and the results provide further insight into a detailed understanding of O_2 activation/reduction processes by the imidazole and thiolate coordinated porphyrin complexes. Remarkably, while the PROS production by the imidazole bound PIM increase with decrease in ET rate, the thiolate bound PPSR complex shows an opposite trend, that is, PROS decrease with decrease in ET rate.

2. EXPERIMENTAL DETAILS

2.1. Materials. All reagents were of the highest grade commercially available and were used without further purification. Octanethiol (C_8SH), Hexadecanethiol ($C_{16}SH$), potassium hexafluorophosphate (KPF_6), and all buffers were purchased from Sigma-Aldrich. Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and potassium chloride (KCl) were purchased from Merck. 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). Edge plane pyrolytic graphite (EPG) electrodes and Au discs for the Rotating Ring Disc Electrochemistry (RRDE) experiments and Ag discs for SERRS and SERRS coupled with Rotating Disc Electrochemistry (SERRS-RDE) experiments were purchased from Pine Instruments, U.S.A. The catalysts *meso*-Mono[*o*-5-(*N*-imidazolyl) valeramidophenyl]-triphenylporphyrinatoiron(III)-bromide (PIM) and *meso*-Tris (α,α,α -*o*-pivalamidophenyl- α -*o*-5-thiolatevaleramidophenyl)porphyrinatoiron(III) (PPSR) (Figure 1) have been prepared following literature reports.¹⁷

2.2. Instrumentation. All electrochemical experiments were performed using a CH Instruments (model CHI710D Electrochemical Analyzer). Biopotentiostat, 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). X-ray photoelectron spectroscopy (XPS) data were collected using an instrument from Omicron Nanotechnology GmbH, Germany (serial number-0571).

2.3. Construction of the Electrodes. **2.3.1. Physioadsorption of the Catalysts on EPG.** A 60 μL portion of catalyst from a 1 mM solution of the respective catalysts in chloroform ($CHCl_3$) is deposited

on a freshly cleaned EPG electrode mounted on a RRDE setup. After the evaporation of the solvent, the surface is thoroughly dried with N_2 gas and sonicated in ethanol. Finally before using it for electrochemical experiments, the modified electrodes are washed with triple distilled water.

2.3.2. Formation of SAM. Au wafers and discs are cleaned electrochemically by sweeping several times between 1.5 V to -0.3 V in 0.5 M H_2SO_4 . Ag discs are cleaned in alumina (size: 1 μ , 0.3 μ , and 0.05 μ) and then roughened in 0.1 M KCl solution as described in the literature.⁶⁷ SAM solutions are prepared using the concentration of the thiols as shown in Table 1. Freshly cleaned Au wafers and discs and

Table 1. SAM Solution Preparation Concentrations

thiols used	concentration (mM)
C_8SH	0.4
$C_{16}SH$	0.4

freshly roughened Ag discs are rinsed with triple distilled water, ethanol, purged with N_2 gas, and immersed in the depositing solution for around 8 h.

2.3.3. Physiaabsorption of the Catalysts on to the SAM. Au wafers and discs and roughened Ag discs immersed in the deposition solution are taken out before experiments and rinsed with ethanol followed by triple distilled deionized water and then dried with N_2 gas. The wafers are then inserted into a Plate Material Evaluating Cell (ALS Japan) and the discs are mounted on a platinum ring disc assembly (Pine Instruments, U.S.A.). These SAM modified surfaces are immersed in the $CHCl_3$ solution of the catalyst for around 30 min and are then rinsed with chloroform, ethanol, and triple distilled water followed by drying with N_2 gas before the XPS, electrochemical or SERRS experiments.

2.4. Cyclic Voltammetry (CV) Experiments. All CV experiments are done in pH 7 buffer (unless otherwise mentioned) containing 100 mM $Na_2HPO_4 \cdot 2H_2O$ and 100 mM KPF_6 (supporting electrolyte) using Pt wire as the counter electrode and Ag/AgCl as the reference electrode.

2.5. Coverage Calculation. The coverage for a particular species is estimated by integrating the oxidation and reduction currents of the respective species.^{62,68}

2.6. Partially Reduced Oxygen Species (PROS). The platinum ring and the Au disc are 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 which is 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 setup is measured in a 2 mM $K_3Fe(CN)_6$ and 0.1 M KNO_3 solution at 10 mV/S scan rate and 300 rpm rotation speed. A $20 \pm 2\%$ CE is generally recorded during these experiments. The potential at which the ring is held during the collection experiments at pH 7 for detecting H_2O_2 has been obtained from the literature.⁶⁹

2.7. SERRS. Ag discs are cleaned using Alumina powder (grit sizes 1, 0.3, and 0.05 μ) and then roughened in 0.1 M KCl solution using reported procedures^{67,70} and immersed in SAM solutions. The roughened modified Ag discs are then inserted into the RRDE setup for the collection of SERRS data.^{71,72} Catalysts are physiaabsorbed in a similar manner as described in section 2.3.3. Experiments are done using an excitation wavelength of 413.1 nm, and the power used at the electrode surface was around 10–12 mW. While collecting the spectra at the resting/oxidized state the disc is held at 0 V and at -0.5 V to obtain a reduced spectrum.

2.8. SERRS-RDE. Preparation of the roughened modified Ag surfaces and immobilization of catalysts on these surfaces are done likewise as described in section 2.7. Experiments have been done using an excitation wavelength of 413.1 nm, and the power used at the electrode surface is around 10–12 mW. The electrode is rotated at constant speed of about 200 rpm.⁶⁵ Similarly, while collecting the spectra at resting/oxidized state the disc was held at 0 V and at -0.5 V to obtain a spectrum during steady state O_2 reduction.

2.9. XPS. XPS is performed on the C_8SH modified Au surface bearing catalyst (physiaabsorbed), using Mg $K\alpha$ radiation (1253.6 eV) for excitation. High resolution scans, with a total energy resolution of about 1.0 eV, are recorded with pass energy of 20 eV, step size of 0.2 eV. Base pressure of the chamber initially was 1×10^{-10} mbar and during the experiment was $\sim 3 \times 10^{-10}$ mbar. Binding energy spectra are calibrated by the Ag $3d_{5/2}$ peak at 368.2 eV. An error of ± 0.1 eV is estimated for all the measured values. The surfaces for XPS are prepared following the same procedure as described in section 2.3.3 and then subjected to XPS experiments.

3. RESULTS

3.1. CV. The CV of the PIM and PPSR complex functionalized electrodes show well developed Fe^{III}/Fe^{II} CV both when physiaabsorbed on EPG and on C_8SH SAM modified Au in the absence of oxygen (Figure 2). The $E_{1/2}$ of the

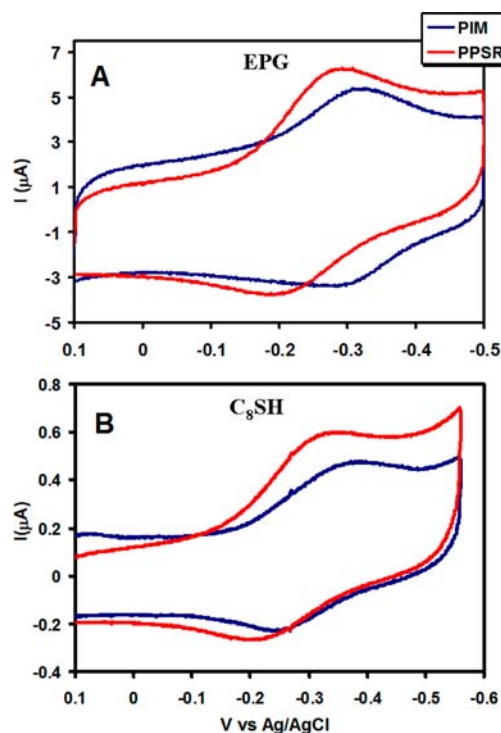


Figure 2. CV data of PIM (blue) and PPSR (red) in deoxygenated pH 7 buffer when physiaabsorbed on EPG (A) and C_8SH SAM modified Au (B) using Ag/AgCl as reference and Pt wire as counter electrodes respectively under Ar atmosphere.

porphyrin Fe^{III}/Fe^{II} process of PIM and PPSR, physiaabsorbed on EPG, appear at -300 mV and -230 mV respectively (Figure 2A). When physiaabsorbed on C_8SH modified Au electrode the $E_{1/2}$ for the PIM and PPSR complexes appears at -308 mV and -263 mV, respectively (Figure 2B). In the case of PIM the redox potential appears at more negative value in both cases compared to that for PPSR. This is different from the trend observed in noncoordinating organic solvents where the $E_{1/2}$ of thiolate bound iron porphyrin complex (PSR) was found to be ~ 500 mV more negative compared to PIM.¹⁷ In an aqueous medium PIM, which has a neutral imidazole axial ligand and bears an overall positive charge in its resting Fe^{III} state, binds a OH^- ligand (to charge neutralize), which will lower its $E_{1/2}$ significantly relative to the value observed in a noncoordinating organic solvents. Furthermore, because of the OH^- coordination, PIM is six coordinate while PPSR is five

coordinate under experimental conditions. CV experiments at different pHs are performed to verify the nature of this hydroxide ligand. A plot of $E_{1/2}$ vs pH shows a proton coupled ET (PCET) process (Supporting Information, Figure S1) and is consistent with an $\text{Imd-Fe}^{\text{III}}-\text{OH}^- + e^- + \text{H}^+ = \text{Imd-Fe}^{\text{II}}-\text{OH}_2$ redox equilibrium for PIM. The presence of an axial OH^- ligand is further confirmed by the characteristic Fe–OH vibration (*vide infra*). Note that both the oxidation and the reduction waves are quite broad. This may be due to the presence of both high-spin (HS) and low-spin (LS) species on the surface. SERRS is used to understand the nature of the species (section 3.2).

The integration of the current under the $\text{Fe}^{\text{III/II}}$ process yields the surface coverage of the functionalized electrodes, that is, the number of the electroactive species present (Table 2). The PIM

Table 2. Calculated Surface Coverages (mol/cm^2)

catalyst	EPG	C_8SH
PIM	$(1.08 \pm 0.08) \times 10^{-11}$	$(2.69 \pm 0.03) \times 10^{-12}$
PPSR	$(1.2 \pm 0.02) \times 10^{-11}$	$(3.52 \pm 0.05) \times 10^{-12}$

complex shows coverage of around $1.08 \times 10^{-11} \text{ mol}/\text{cm}^2$ and $2.69 \times 10^{-12} \text{ mol}/\text{cm}^2$ on EPG and C_8SH surfaces, respectively. For the PPSR complex the coverages on EPG and C_8SH are found to be $1.2 \times 10^{-11} \text{ mol}/\text{cm}^2$ and $3.52 \times 10^{-12} \text{ mol}/\text{cm}^2$, respectively. These values indicate a very dilute layer of catalysts are present on the electrodes and not a multilayer which yields coverages that are 2 orders of magnitude higher.⁷³ Similar results were obtained in previous reports which utilized this approach.^{64,74}

3.2. SERRS. SERRS data were collected by physisorbing the catalysts on C_8SH modified roughened Ag electrodes. The SERRS data of PIM in the resting oxidized state show the oxidation and spin state marker ν_4 and ν_2 bands at 1360 cm^{-1} and 1553 cm^{-1} , respectively, indicating the presence of a HS Fe^{III} species (Figure 3A, red; Table 3).^{75,76} A Lorentzian fit of these data indicates the presence of a minor LS Fe^{III} component (Supporting Information, Figure S2). The ν_8 band appears at 396 cm^{-1} (Supporting Information, Figure S3). When reduced, a HS Fe^{II} species is generated with the ν_4 and ν_2 bands appearing at 1347 cm^{-1} and 1543 cm^{-1} , respectively (Figure 3A, black).^{75,77} Complete conversion of the Fe^{III} species does not occur as is reflected in these bands (Supporting Information, Figure S4). The LS Fe^{III} component (ν_2 at 1565 cm^{-1}) remains at this potential. This may be expected as the LS Fe^{III} complex at the potential at which the SERRS is performed (-0.5 V), which will have a lower potential than a HS Fe^{III} complex, is not completely reduced. Additionally, the $E_{1/2}$ is slightly lower in the roughened Ag disc relative to that on Au discs (Supporting Information, Figure S5).

The SERRS data of the oxidized PPSR show the ν_4 band appears at 1362 cm^{-1} and ν_2 appears at 1552 cm^{-1} (Figure 3B, red and Supporting Information, Figure S6). The values correspond to a HS Fe^{III} species. Lorentzian fit of the data indicate the presence of weak peaks corresponding to LS Fe^{III} species (Supporting Information, Figure S6). The ν_8 band appears at 391 cm^{-1} (Supporting Information, Figure S3) which is lower than that observed for PIM. Upon reduction the ν_4 and ν_2 bands shift to 1352 cm^{-1} and 1558 cm^{-1} , respectively (Figure 3B). These bands are very broad which clearly indicate the presence of a mixture of species. Lorentzian fit of the data

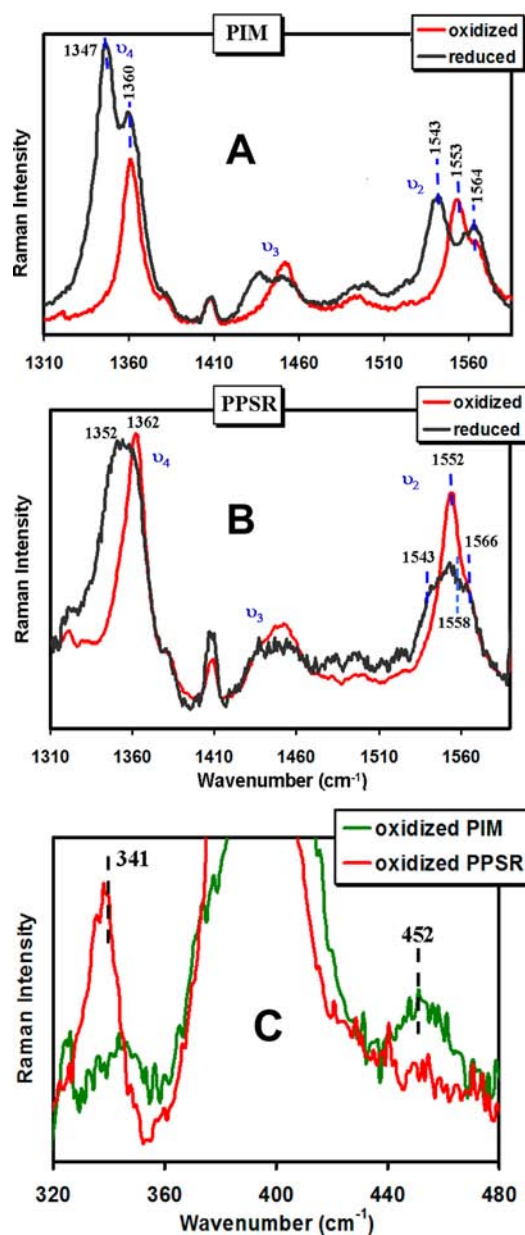


Figure 3. SERRS data of PIM (A) and PPSR (B), in the high frequency region, when physisorbed on C_8SH modified roughened Ag electrodes under oxidizing (red) and reducing (black) conditions in pH 7 buffer under anaerobic atmosphere. (C) SERRS data of PIM (green) and PPSR (red) in the low frequency region under resting (oxidized) condition.

Table 3. SERRS Data of the Complexes Physisorbed on C_8SH SAM^a

complexes		marker bands (in cm^{-1})	
		ν_2	ν_4
PIM	<i>oxidized</i>	1553, 1566	1360, 1365
	<i>reduced</i>	1543, 1564	1347, 1362
PPSR	<i>oxidized</i>	1552, 1565	1362, 1365
	<i>reduced</i>	1558, 1552, 1566	1352, 1362, 1367

^aThe values in bold denote the most intense components. The components are obtained from the best fits of the data.

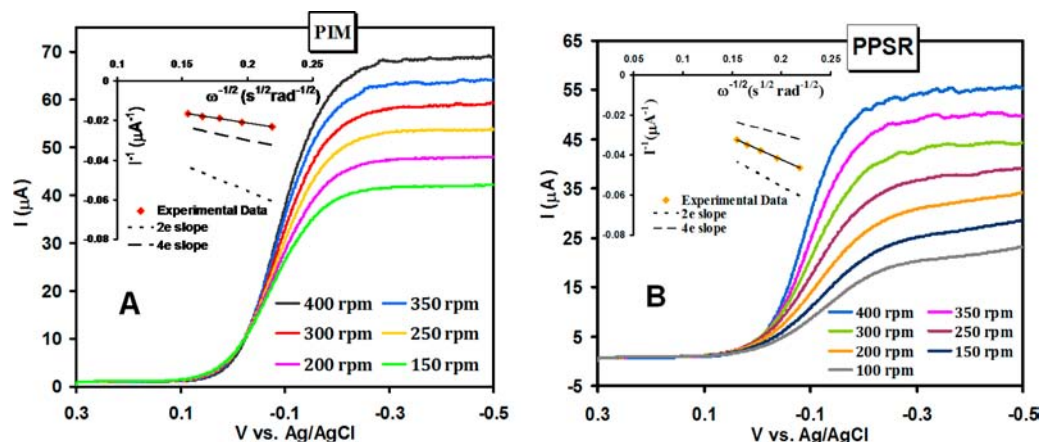


Figure 4. LSV of PIM (A) and PPSR (B) physisorbed on EPG in air saturated pH 7 buffer at a scan rate of 50 mV/s at multiple rotations using Ag/AgCl as reference and Pt wire as counter electrodes. Koutecky–Levich plots of the respective catalysts are given in black bold line in the inset of the figures. The theoretical plots for 2e⁻ and 4e⁻ processes are indicated by the dotted and dashed lines respectively.

indicates the presence of HS Fe^{II}, LS Fe^{II}, some unreduced HS Fe^{III} species and LS Fe^{III} species (Figure 3B, black and Supporting Information, Figure S7).^{75,77}

The SERRS data in low frequency region of PIM and PPSR, in their resting state under aqueous condition, clearly show features unique to these complexes (Figure 3C). A peak at 452 cm⁻¹ in the case of PIM represents a characteristic band of the Fe^{III}–OH stretching frequency where the Fe center is 6C and axially ligated to a imidazole group and is HS in nature.⁷⁸ This is consistent with the PCET behavior of PIM and eliminates the possibility of any oxo-bridged complex.⁷⁹ PPSR shows distinct peaks at 341 cm⁻¹ and 639 cm⁻¹ (Figure 3C and Supporting Information, Figure S8) which probably correspond to Fe–SR and C–S stretching frequencies, respectively, of a HS thiolate bound iron porphyrin complex as observed for other thiolate bound HS synthetic mimics and native cytochrome P450 enzyme active site.^{17,80} These Fe–S and C–S vibrations disappear upon reduction and reappear on reoxidation eliminating the possibility of dissociation of the thiolate ligand on reduction (Supporting Information, Figure S8). Note that a band around 345 cm⁻¹ is also seen in the low frequency region for both PIM and PPSR (for PPSR, the band overlaps with the Fe–thiolate stretching vibration) corresponding to out-of-plane deformation (γ_{por}) of any porphyrin macrocycle.^{81,82}

XPS data obtained on PPSR physisorbed on C₈SH SAM modified Au surface after being immersed into an aqueous medium (Supporting Information, Figure S9) show the presence of two types of S 2p ionizations at 163.5 and 162.7 eV.^{83,84} Note that thiol S 2p binding energy appears at 170 eV.⁸⁵ The S 2p peak at 162.7 eV is characteristic of thiolate S atom bound to Au at the SAM interface,⁸³ and the S 2p ionization at 163.5 eV is characteristic of Fe^{III}–SR species.⁸⁴ The Fe 3p ionization at 58.9 eV is characteristic of an Fe^{III} complex.⁸⁶ XPS also shows the presence of three different types of carbon (aliphatic C at 284.5 eV, aromatic C at 285.3 eV and carbonyl C at 286.2 eV) and oxygen (originating from carbonyl oxygen) present in the complex (Supporting Information, Figure S9). Thus both the SERRS and XPS data confirm the presence of an Fe^{III}–SR bond in the PPSR complex immobilized on SAM and exposed to aqueous environment. Note that the XPS data on the electrochemically reduced surfaces could not be obtained using the currently available experimental setup.

The SERRS data for the two catalysts under resting and reduced state are summarized in Table 3. The data suggest clear differences in the spectra of these two catalysts reflecting the difference in the nature of the iron center. The oxidation and spin states of the metal center depend on the axial coordination and also on the coordination number. Note that the ν_8 vibration represents the symmetric Fe–N_{pyrrole} stretch and appears at 396 cm⁻¹ in PIM and shifts to 391 cm⁻¹ in PPSR. While the absolute values are different, this trend of lowering ν_8 vibration in the case of thiolate coordination is consistent with previous data obtained on PIM and PSR complexes in homogeneous organic solutions (Supporting Information, Figure S3).¹⁷ This reflects strong covalent donation from the anionic thiolate ligand weakening the Fe–N_{pyrrole} bond. The data indicate that while the PIM complex is mostly HS in both oxidized and reduced state, the PPSR complex has a significant population of the LS state in both oxidation states. It may be expected that a thiolate bound Fe^{III} porphyrin complex will show a LS component at room temperature. However observation of a significant LS Fe^{II} component is rather rare in the P450 literature.^{87,88}

3.3. Electrochemical O₂ Reactivity. **3.3.1. EPG.** O₂ reactivity of these catalysts under physiological conditions was studied using a heterogeneous approach. In the presence of O₂ in pH 7 buffer, in a linear sweep voltammetry (LSV) experiment, a large electrocatalytic O₂ reduction current is observed at negative potentials for both the catalysts when physisorbed on EPG (Figure 4 and 5A). At these potentials the iron porphyrin catalysts are reduced to Fe^{II} (Figure 2A). The data indicate that for PPSR, the O₂ reduction occurs at more negative potentials, although the reduction potential of Fe^{III/II} couple is positive, compared to PIM. This implies that the potential defining step of ORR for PIM and PPSR catalysts are different and it is not the Fe^{III} to Fe^{II} reduction step.

3.3.1.1. RDE. The electrocatalytic O₂ reduction at different rotation rates (RDE) have been performed for both PIM and PPSR, which not only helps in determining the number of electrons involved in ORR but also provides a direct proof of the stability as well as durability of these catalysts on the electrode surfaces (Figure 4). The O₂ reduction current increases with increasing rotation rates following the Koutecky–Levich equation, $I^{-1} = i_{\text{K}}^{-1} + i_{\text{L}}^{-1}$, where i_{K} is the potential dependent kinetic current and i_{L} is the Levich

current.⁸⁹ i_L is expressed as $0.62nFA[O_2](D_{O_2})^{2/3}\omega^{1/2}\nu^{-1/6}$, where n is the number of electrons transferred to the substrate, A is the macroscopic area of the disc (0.125 cm^2), $[O_2]$ is the concentration of O_2 in an air saturated buffer (0.26 mM) at $25\text{ }^\circ\text{C}$, D_{O_2} is the diffusion coefficient of O_2 ($1.8 \times 10^{-5}\text{ cm}^2\text{ s}^{-1}$) at $25\text{ }^\circ\text{C}$, ω is the angular velocity of the disc, and ν is the kinematic viscosity of the solution ($0.009\text{ cm}^2\text{ s}^{-1}$) at $25\text{ }^\circ\text{C}$.⁹⁰ The plot of I^{-1} at multiple rotation rates vs the inverse square root of the angular rotation rate ($\omega^{-1/2}$) is linear. The slopes obtained from the experimental data for PIM closely matches with the theoretical slope predicted for a $4e^-$ process (Figure 4A, inset). However, for PPSR the slope suggests the value of n to be 3.25 ± 0.25 (Figure 4B, inset and Supporting Information, Figure S10). The values of n obtained indicate that under very fast ET O_2 undergoes almost complete $4e^-$ reduction to H_2O by PIM but not by PPSR (vide infra). Note that the SERRS data clearly show that there are two species on the electrodes in both the oxidized and the reduced states. Thus the RDE data, where the rotation rates are varied (i.e., the O_2 flux is varied), may be complicated by differential O_2 binding rates of the two species present. To gain further insight into the ORR mechanism electrocatalytic O_2 reduction by PIM and PPSR at fast (EPG), slow (C_8SH modified Au), and very slow ($C_{16}SH$ modified Au) ET rates were investigated.

3.3.1.2. RRDE. RRDE has been used to calculate the amount of PROS produced because of incomplete reduction of O_2 . In this technique any O_2^- or O_2^{2-} produced in the modified Au working electrode due to $1e^-$ or $2e^-$ reduction of O_2 is radially diffused, because of the hydrodynamic current created by the rotation, to the ring, which is held at 0.7 V , where these are oxidized back to O_2 .^{89,91} This results in an oxidation current in the ring, and the ratio of the catalytic current of the ring (i_r) and the disc (i_c) yields the % of PROS produced. In a generally accepted mechanism generation of PROS entails hydrolysis of a $Fe^{III}-O_2^-$ species produced during O_2 reduction in the aqueous environments. Note that no current is detected in the ring when O_2 is reduced to H_2O by the catalyst. PIM produces about $3.5 \pm 1\%$ PROS when physisorbed on EPG, whereas PPSR produces $19 \pm 1.5\%$ PROS (Figure 5 and 6). Note that these results are independent of rotation rates of the electrode (Supporting Information, Figure S11).⁹² This may reflect the higher trans effect of the thiolate ligand, compared to the neutral imidazole ligand, which leads to facile hydrolysis of Fe^{III} bound O_2^- (oxy adduct) or OOH (peroxy adduct) species.⁶⁴ A value of $n = 3.25$ O_2 reduction by PPSR on EPG relative to $n = 4$ reduction by PIM is consistent with the higher amount of PROS generated by PPSR relative to PIM (Figure 4). Interestingly, for PIM, the inflection point of the i_c is at the same potential as the inflection point of i_r . Alternatively, for PPSR, the inflection point of the i_c is offset from the inflection point of i_r by $\sim 70\text{ mV}$ (Figure 5B). This is mechanistically significant (vide infra). It should be noted that the increase of ring current after the inflection potential does not arise from the O_2 reduction of the bare electrode surfaces, that is, background (Supporting Information, Figure S12).

3.3.2. SAM. To probe the O_2 reduction mechanism, the selectivity of O_2 reduction was studied under slow ET by physisorbing on C_8SH and $C_{16}SH$ SAM modified Au electrodes. In the presence of O_2 in pH 7 buffer both the catalysts, physisorbed on C_8SH SAM, show large electrocatalytic current at negative potentials similar to those observed when physisorbed on EPG (Figure 7). When physisorbed on $C_{16}SH$ SAM, neither of the catalysts show saturation in the

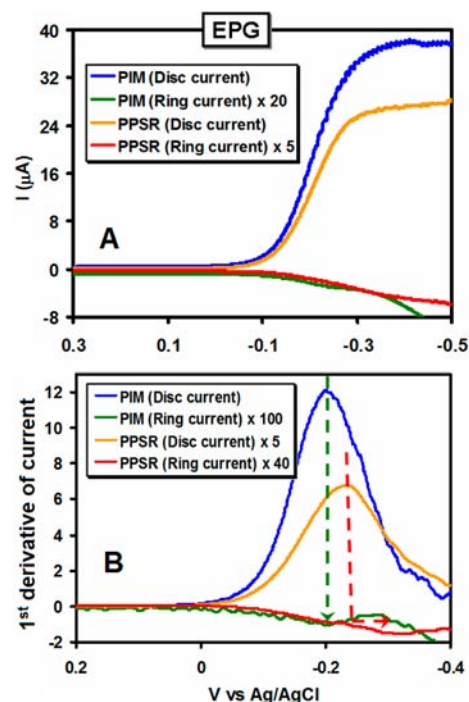


Figure 5. (A) RRDE data of PIM and PPSR, showing the disc and Pt ring currents, physisorbed on EPG in air saturated pH 7 buffer at a scan rate of 10 mV/s and rotation speed of 300 rpm , using Ag/AgCl reference and Pt wire counter electrodes. (B) The corresponding first derivative plots of the disc and ring currents of PIM and PPSR as shown in A.

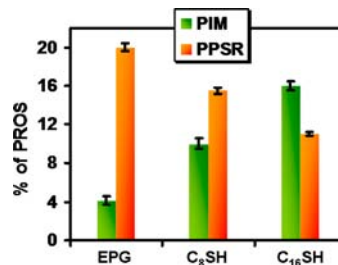


Figure 6. Percentage of PROS formed by PIM (green) and PPSR (orange) in air saturated pH 7 buffer under fast (EPG), slow (C_8SH SAM on Au), and very slow ($C_{16}SH$ SAM on Au) electron fluxes using Ag/AgCl reference and Pt wire counter electrodes. Rotation speed = 300 rpm , scan rate = 10 mV/s . Error bars indicate standard deviation from the mean.

O_2 reduction current because the slow ET rate shifts the saturation to a more negative potential (Supporting Information, Figure S13). The Tafel slopes of the ORR current for both PIM and PPSR are $\sim 400\text{ mV/decade}$ increase in current (Supporting Information, Figure S14) indicating that the electron flux to these physisorbed catalysts are comparable. RRDE is used to estimate the amount of PROS formed under these moderate ($\sim 10^3\text{ s}^{-1}$ in C_8SH)^{93,94} and slow ($\sim 4\text{--}6\text{ s}^{-1}$ $C_{16}SH$)^{52,95} ET rates.⁹⁶ PIM produces $10 \pm 0.5\%$ and $16 \pm 1\%$ PROS when physisorbed on C_8SH and $C_{16}SH$ SAM, respectively. Thus, with a decrease in the ET rate an increase in the PROS formation is observed for PIM (Figure 6). This is in good agreement with previous reports of synthetic imidazole ligated iron porphyrins where, under slow ET, PROS production increases.⁵² In the case of PPSR, a decrease in

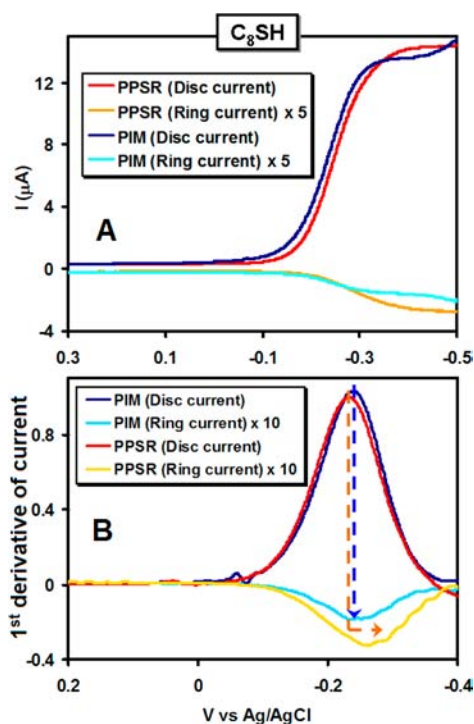


Figure 7. (A) RRDE data of PIM and PPSR, showing the Au and Pt currents, physisorbed on C_8SH SAM in air saturated pH 7 buffer at a scan rate of 10 mV/s and rotation speed of 300 rpm, using Ag/AgCl reference and Pt wire counter electrodes. (B) The corresponding first derivative plots of the disc and ring currents of PIM and PPSR as shown in A.

PROS formation is observed with decrease in the ET rate. When physisorbed on C_8SH SAM PPSR produces about $15.5 \pm 0.5\%$ PROS, while in $C_{16}SH$ it shows about $11 \pm 1\%$ PROS (Figure 6). Such decrease in PROS with decrease in ET flux has not been observed before, that is, this is an opposite trend compared to PIM or other reported O_2 reducing iron porphyrin complexes (Figure 6).⁹⁷ This opposite trend in PROS production of PIM and PPSR suggests that these two complexes, differing in the axial ligation, reduce molecular O_2 in different mechanistic pathways. In situ SERRS experiments were performed to understand this effect (vide infra).

The inflection point and saturation of the disc current matches closely the onset of the ring current in the case of PIM (Figure 7). This implies that the PROS (i_r) are a side product of the catalytic cycle operative during O_2 reduction (i_c). This, however, is not the case for PPSR. There is a distinct lag between the catalytic current and the ring current. This is better represented in the first derivatives of i_c and i_r . A maximum/minimum in the first derivative represents an inflection point in the currents. The inflection points of i_c and i_r are the same for PIM (Figure 7B, blue and light blue) when, for PPSR, the inflection point of i_r is at ~ 70 mV lower potential than i_c (Figure 7B, orange and yellow). This implies that (a) the PROS production is not solely the part of the catalytic cycle of O_2 reduction for PPSR which occurs at the onset of the electrocatalytic current and (b) another cathodic process ~ 70 mV lower than the O_2 reduction process is responsible for PROS production. Since, by definition, PROS production must be associated to O_2 reduction, there must be another electrocatalytic O_2 reduction step at ~ 70 mV lower potential.

Judging by the greater PROS production at this potential this step is less selective toward a $4e^- O_2$ reduction.

3.4. SERRS-RDE. SERRS-RDE data of the catalysts were obtained by rotating the electrode at constant rotation speed (200 rpm) and applying a constant potential of -0.5 V at the electrode, that is, under steady state condition.⁶⁵ SERRS-RDE data of PIM in the oxidized condition is same as discussed in section 3.2 (Supporting Information, Figure S2). In the presence of O_2 in pH 7 buffer, when the electrode is held at reducing potential (i.e., at -0.5 V), the ν_2 band at 1565 cm^{-1} corresponding to a LS Fe^{III} species is found to increase in intensity (Figure 8).⁷⁵ Along with the Fe^{III} species some HS

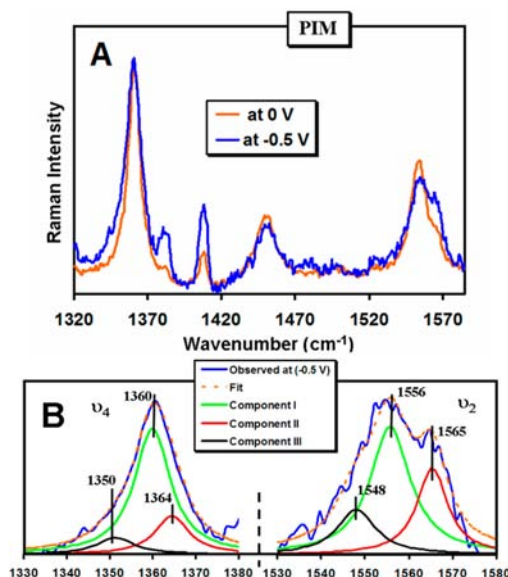


Figure 8. (A) SERRS-RDE data of PIM, physisorbed on C_8SH modified Ag electrode, in the high frequency region under oxidized (orange) and steady state (blue) conditions in air saturated pH 7 buffer using Ag/AgCl reference and Pt wire counter electrodes, rotating the electrode at 200 rpm. (B) The ν_4 and ν_2 bands of the steady state spectra along with the fits showing different components.

Fe^{II} species, corresponding to ν_4 and ν_2 bands at 1350 cm^{-1} and 1548 cm^{-1} , respectively, can also be observed (Figure 8). Thus during steady state O_2 reduction a LS Fe^{III} species and a HS Fe^{II} species are accumulated on the electrode.⁷⁷

SERRS-RDE data of PPSR were collected using similar experimental conditions like those for PIM. Under oxidizing potential similar distribution of products were observed as discussed in section 3.2. During steady state electrocatalytic O_2 reduction conditions the ν_4 bands at 1344 cm^{-1} , 1354 cm^{-1} , and 1367 cm^{-1} and ν_2 bands at 1543 cm^{-1} , 1556 cm^{-1} , and 1566 cm^{-1} increase in intensity (Figure 9). These bands correspond to HS Fe^{II} , LS Fe^{II} , and LS Fe^{III} species, respectively.⁷⁵ Alternatively, the ν_4 band at 1361 cm^{-1} and ν_2 band at 1551 cm^{-1} (HS Fe^{III}) remain which implies that either the initial HS Fe^{III} species is not reduced fully under the applied potential or a HS Fe^{III} species accumulates on the electrode surface after catalytic ORR cycle during steady-state turnover. Note that among these species LS Fe^{II} has higher intensity than others (Figure 9B). This could indicate higher resonance enhancement and/or a greater population, the latter being more likely. Thus in addition to the HS Fe^{II} and LS Fe^{III} species produced in PIM during steady state O_2 reduction, a LS Fe^{II} species is produced in PPSR.

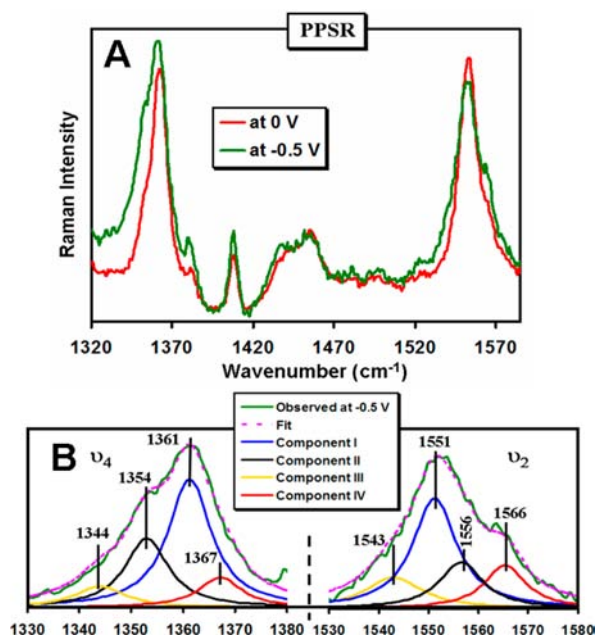


Figure 9. (A) SERRS-RDE data of PPSR, physisorbed on C_8SH modified roughened Ag electrode, in the high frequency region under oxidized (red) and steady state (green) conditions in air saturated pH 7 buffer using Ag/AgCl reference and Pt wire counter electrodes, rotating the electrode at 200 rpm. (B) The ν_4 and ν_2 bands of the steady state spectra along with the fits showing different components.

4. DISCUSSIONS

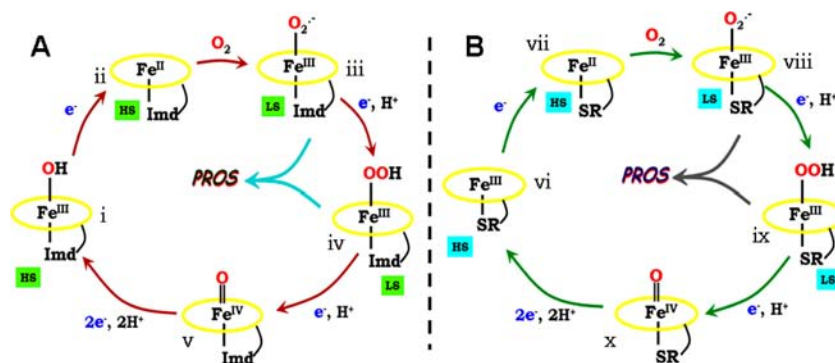
A combination of electrochemical and spectroscopic techniques has been used to investigate the reactivity of imidazole ligated and thiolate ligated iron porphyrin complexes physisorbed on electrode surfaces. The only difference in their structural pattern is the “picket-fence” type architecture present in PPSR which stabilizes this complex in an oxic environment.⁹⁸ The spectroscopic results obtained in this study indicate that in an aqueous environment both these species mostly exist as HS Fe^{III} in their resting state. When reduced electrochemically in an anaerobic aqueous environment a unique LS Fe^{II} species is observed for PPSR in addition to a HS Fe^{II} species, whereas, under same conditions a HS Fe^{II} species is observed for PIM. Occurrence of a thiolate bound LS Fe^{II} species is rare and has been reported in cytochrome P450 enzymes CYP119 and CYP102.⁸⁷ A previous report with PIM and PPSR in a homogeneous nonaqueous solution showed that, in the resting oxidized ferric state, PPSR exists as a six coordinate LS Fe^{III}

species in a coordinating solvent while PIM remains HS under the same conditions.¹⁷ The tendency of an anion, that is, thiolate ligand, to stabilize a 6C LS Fe^{III} species was rationalized by a unique iron t_{2g} to porphyrin π^* back bonding interaction which was operative only in the presence of a π donor thiolate ligand. The same argument may be presented in case of the thiolate bound LS Fe^{II} species encountered in this study. Fe^{II} having lesser Z_{eff} than Fe^{III} will have higher 3d orbital energies which will result in better back bonding interaction in the former. In fact, while all 5C Fe^{II} heme enzymes bearing axial thiolate ligand are HS, several 6C Fe^{II} heme enzymes bound to an axial thiolate ligand were found to be LS.⁹⁹

During electrocatalytic O_2 reduction at steady state, SERRS-RDE data of PIM shows build up of HS Fe^{II} and LS Fe^{III} species on the electrode surface (Figure 8). This LS Fe^{III} species may suggest the formation of either $Fe^{III}-O_2^-$ or $Fe^{III}-OOH$ species (iii, iv; Scheme 1A), though conclusive assignments cannot be made without isotope labeling with $^{18}O_2$ saturated buffer. No Raman bands could be observed at $1371-1373\text{ cm}^{-1}$ and $1570-1575\text{ cm}^{-1}$ region, characteristic of $Fe^{IV}=O$ species.^{100,101} Thus O_2 binding to the HS Fe^{II} species and decay of the LS Fe^{III} species are the slowest steps in the ORR catalyzed by imidazole bound Fe-porphyrin.

Generally on an EPG electrode, where the catalyst is directly immobilized on the conducting surface, the O–O bond cleavage reaction, which requires transfer of electron(s) to the initial $Fe^{III}-O_2^-$ species formed, is facile and thus minimum amount of PROS is produced. Slowing down the ET rate by immobilizing the catalyst on octanethiol SAM (k_{ET} for C_8SH SAM is $\sim 10^3\text{ s}^{-1}$)^{93,63,94} allows enough time for the competing hydrolysis reaction. This leads to formation of PROS (Scheme 1A). If the ET rate is further slowed down by immobilizing the catalyst on hexadecanethiol SAM (k_{ET} for $C_{16}SH$ SAM is $\sim 4-6\text{ s}^{-1}$)^{52,95} the probability of the hydrolysis reaction increases further increasing the amount of PROS production. This trend has been observed for all O_2 reducing iron porphyrin based electrocatalysts reported to date.^{60,102} The fact that slowing down ET leads to increased PROS suggests that the species responsible for PROS production is not produced by ET. If that were the case, then slowing down ET would automatically lower PROS production. Rather, in this case, PROS production is a competing reaction of the species responsible for PROS production (iii, Scheme 1) which dominates when the ET rate is slowed down. The hydrolysis of the $Fe^{III}-O_2^-$ species fits the description of the competing reaction. As the ET rate is reduced the hydrolysis rate, which remains constant, dominates and produces greater PROS.

Scheme 1. Oxygen Reduction Scheme of PIM (A) and PPSR (B) Immobilized on a Surface



During O_2 reduction by PPSR under steady state condition, in addition to the HS Fe^{II} , HS Fe^{III} , and LS Fe^{III} species present on the surface, a significant amount of LS Fe^{II} species is observed (Figure 9). This species is also observed when the PPSR complex is reduced in the absence of O_2 . Thus, this LS Fe^{II} species is likely to be $OH_2-Fe^{II}-SR$ (the sixth ligand is invoked to be H_2O as it is the solvent). There are only few reports of native cytochrome P450 enzymes where a LS Fe^{II} state has been observed. In those rapid auto-oxidation rates are observed as well, that is, $Fe^{II} + O_2 \rightarrow Fe^{III} + O_2^-$. In the RRDE setup the O_2^- species produced because of auto-oxidation is detected in situ as PROS. The data indicate that in PPSR the PROS decrease as the ET rate decreases. Note that in case of $C_{16}SH$ SAM the ET rate is very slow irrespective of the catalyst used, as reflected from the similar Tafel slopes obtained for PIM and PPSR during O_2 reduction (Supporting Information, Figure S14), and is thus the rate determining step in the catalytic ORR. This is contrary to all observations made to date where PROS production increases with decrease in ET rate.¹⁰² One likely scenario is in addition to a normal $4e^-/4H^+$ O_2 reduction process catalyzed by a HS Fe^{II} species at the onset of O_2 reduction, there is an additional O_2 reduction by a LS Fe^{II} species at ~ 70 mV lower potential. It is logical that a LS $Fe^{III/II}$ process will have a lower potential than a HS $Fe^{III/II}$ process. Note that, the presence of HS and LS species were indicated by the SERRS data obtained in both oxidized and reduced states. Binding of O_2 to this LS Fe^{II} species, in the case of inner sphere mechanism, entails the breaking of a $Fe-OH_2$ bond of a 6C LS Fe^{II} species with a $t_{2g}^6 e_g^0$ configuration which can be expected to be thermodynamically uphill (Scheme 2) making O_2 binding

Scheme 2. Alternate O_2 Reduction Pathway of PPSR in Case of Formation of a LS Fe^{II} Species



slow.¹⁰³ Rather, outer sphere O_2 reduction generating O_2^- (detected by RRDE) where the rate of PROS production increases with increase in ET rate and vice versa (as more LS Fe^{II} species will accumulate on the surface) is consistent with SERRS-RDE and RRDE data. There are several reports on both heme and nonheme Fe^{II} complexes showing outer sphere $1e^-$ O_2 reduction.^{104–106} More importantly the active sites of cyt P450 CYP 101, CYP 102, and CYP 119 show some population of the LS Fe^{II} state, and these indeed exhibit high outer sphere rates of auto oxidation by O_2 .⁸⁷ Fe^{II} LS after reducing O_2 through an outer sphere mechanism gets oxidized to Fe^{III} LS, which immediately takes up e^- and gets reduced to Fe^{II} LS species (Scheme 2). Thus both LS Fe^{III} and LS Fe^{II} species will be present on the electrode during steady state as observed in the SERRS-RDE data.

At the potentials where the outer sphere O_2 reduction by the LS Fe^{II} state of the PPSR complex is operative, the inner sphere O_2 reduction by the HS Fe^{II} species, which starts at more positive potentials, is operative as well. Thus the PROS observed represent a summation of the PROS produced by the individual processes. Since resonance Raman is not quantitative

it is hard to estimate the relative rates of the outer sphere and the inner sphere O_2 reductions from the intensities of the ν_4 and ν_2 bands corresponding to the HS and LS Fe^{II} species observed on the electrode during steady state O_2 reduction. However if the outer sphere O_2 oxidation rate of the LS Fe^{II} species were fast then (a) a much higher amount of O_2^- would be detected in the RRDE experiments and (b) the SERRS-RDE data would not be dominated by the LS Fe^{II} species (i.e., it will not accumulate on the electrode during steady state turnover if its reaction with O_2 is fast); both these observations suggest that the rate of outer sphere reduction of O_2 by the LS Fe^{II} species is relatively smaller than the rate of inner sphere O_2 reduction by the HS Fe^{II} species.

5. CONCLUSION

In summary, the SERRS, RRDE, and SERRS-RDE data indicate that a neutral imidazole axial ligand bound PIM complex uses the HS $Fe^{II/III}$ couple to reduce O_2 whereas the anionic thiolate axial ligand bound PPSR complex uses both HS and LS $Fe^{II/III}$ couples, the LS $Fe^{III/II}$ potential being lower than the HS $Fe^{III/II}$ potential. A HS Fe^{II} species (with an open axial coordination) have a tendency toward inner sphere reduction of O_2 to H_2O where the O_2 binding and either a PCET to a $Fe^{III}-O_2^-$ species or the O–O bond cleavage of a $Fe^{III}-OOH$ species are the slowest steps in ORR. A LS Fe^{II} species (only observed in PPSR) likely reduces O_2 to O_2^- using an outer sphere mechanism. As the rate of ET from the electrode to the catalyst is reduced, the ORR by a HS Fe^{II} species produces more PROS while ORR by a LS Fe^{II} species produces less PROS.

■ ASSOCIATED CONTENT

Supporting Information

SERRS, XPS, CV, and RRDE data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the Department of Science and Technology, India, Grant # DST/SR/IC-35-2009 and by Council of Scientific and Industrial Research (CSIR), India, 01(2412)10/EMr-II. S.C., K.S., and P.K.D. acknowledge CSIR-JRF and CSIR-SRF fellowships, respectively. S.S. acknowledges the integrated Ph.D. program of IACS. The IACS XPS facility funded by the DST unit of Nano-Science is gratefully acknowledged.

■ REFERENCES

- (1) Bertini, I.; Gray, H. B.; Lippard, S. J.; Valentine, J. S. *Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.
- (2) Kadish, K. M.; Smith, K. M.; Guilard, R. Heme Proteins. In *Handbook of Porphyrin Science*; World Scientific Publishing Co.: Singapore, 2010; Vol. 5, p 465.
- (3) Dawson, J. H. *Science* **1988**, *240*, 433–439.
- (4) Wuttke, D. S.; Gray, H. B. *Curr. Opin. Struct. Biol.* **1993**, *3*, 555–563.
- (5) Poulos, T. L. *Nat. Struct. Mol. Biol.* **1996**, *3*, 401–403.
- (6) Riggs, A. F. *Curr. Opin. Struct. Biol.* **1991**, *1*, 915–921.

- (7) Møller, J. K. S.; Skibsted, L. H. *Chem. Rev.* **2002**, *102*, 1167–1178.
- (8) Flögel, U.; Merx, M. W.; Gödecke, A.; Decking, U. K. M.; Schrader, J. r. *Proc. Natl. Acad. Sci.* **2001**, *98*, 735–740.
- (9) Kim, E.; Chufan, E. E.; Kamaraj, K.; Karlin, K. D. *Chem. Rev.* **2004**, *104*, 1077–1134.
- (10) Ferguson-Miller, S.; Babcock, G. T. *Chem. Rev.* **1996**, *96*, 2889–2908.
- (11) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841–2888.
- (12) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. *Chem. Rev.* **2005**, *105*, 2253–2278.
- (13) Ortiz de Montellano, P. R., Ed.; *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 3rd ed.; Kluwer Academic/Plenum publishers: New York, 2005.
- (14) Dunford, H. B.; Stillman, J. S. *Coord. Chem. Rev.* **1976**, *19*, 187–251.
- (15) Frew, J. E.; Jones, P. *Advances in Inorganic and Bioinorganic Mechanisms*; Academic Press: Orlando, FL: 1984; Vol. 3, p 175.
- (16) Dawson, J. H.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255–1276.
- (17) Das, P. K.; Chatterjee, S.; Samanta, S.; Dey, A. *Inorg. Chem.* **2012**, *51*, 10704–10714.
- (18) Collman, J. P.; Sorrell, T. N.; Hoffman, B. M. *J. Am. Chem. Soc.* **1975**, *97*, 913–914.
- (19) Chang, C. K.; Dolphin, D. *J. Am. Chem. Soc.* **1976**, *98*, 1607–1609.
- (20) Ueno, T.; Nishikawa, N.; Moriyama, S.; Adachi, S.; Lee, K.; Okamura, T.-a.; Ueyama, N.; Nakamura, A. *Inorg. Chem.* **1999**, *38*, 1199–1210.
- (21) Tani, F.; Matsura, M.; Nakayama, S.; Naruta, Y. *Coord. Chem. Rev.* **2002**, *226*, 219–226.
- (22) Suzuki, N.; Higuchi, T.; Urano, Y.; Kikuchi, K.; Uekusa, H.; Ohashi, Y.; Uchida, T.; Kitagawa, T.; Nagano, T. *J. Am. Chem. Soc.* **1999**, *121*, 11571–11572.
- (23) Ueyama, N.; Nishikawa, N.; Yamada, Y.; Okamura, T.-a.; Nakamura, A. *J. Am. Chem. Soc.* **1996**, *118*, 12826–12827.
- (24) Higuchi, T.; Uzu, S.; Hirobe, M. *J. Am. Chem. Soc.* **1990**, *112*, 7051–7053.
- (25) Chance, B. *J. Gen. Physiol.* **1965**, *49*, 163–188.
- (26) Babcock, G. T.; Wikstrom, M. *Nature* **1992**, *356*, 301–309.
- (27) Gewirth, A. A.; Thorum, M. S. *Inorg. Chem.* **2010**, *49*, 3557–3566.
- (28) Kahlow, M. A.; Loehr, T. M.; Zuberi, T. M.; Gennis, R. B. *J. Am. Chem. Soc.* **1993**, *115*, 5845–5846.
- (29) Verkhovsky, M. I.; Morgan, J. E.; Wikstroem, M. *Biochemistry* **1994**, *33*, 3079–3086.
- (30) Solomon, E. I.; Chen, P.; Metz, M.; Lee, S.-K.; Palmer, A. E. *Angew. Chem., Int. Ed.* **2001**, *40*, 4570–4590.
- (31) Marchal, S.; Girvan, H. M.; Gorren, A. C. F.; Mayer, B.; Munro, A. W.; Balny, C.; Lange, R. *Biophys. J.* **2003**, *85*, 3303–3309.
- (32) Abu-Soud, H. M.; Raushel, F. M.; Hazen, S. L. *Biochemistry* **2004**, *43*, 11589–11595.
- (33) Han, W.-G.; Noodleman, L. *Inorg. Chem.* **2008**, *47*, 2975–2986.
- (34) D'Antonio, J.; Ghiladi, R. A. *Biochemistry* **2011**, *50*, 5999–6011.
- (35) Holm, R. H.; Kennepohl, P.; Solomon, E. I. *Chem. Rev.* **1996**, *96*, 2239–2314.
- (36) Groves, J. T. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 3569–3574.
- (37) Collman, J. P.; Boulatov, R.; Sunderland, C. J.; Fu, L. *Chem. Rev.* **2004**, *104*, 561–588.
- (38) Meunier, B.; de Visser, S. P.; Shaik, S. *Chem. Rev.* **2004**, *104*, 3947–3980.
- (39) Watanabe, Y.; Nakajima, H.; Ueno, T. *Acc. Chem. Res.* **2007**, *40*, 554–562.
- (40) Que, L.; Tolman, W. B. *Nature* **2008**, *455*, 333–340.
- (41) Ohta, T.; Liu, J.-G.; Naruta, Y. *Coord. Chem. Rev.* **2013**, *257*, 407–413.
- (42) Bell, S. R.; Groves, J. T. *J. Am. Chem. Soc.* **2009**, *131*, 9640–9641.
- (43) Rittle, J.; Green, M. T. *Science* **2010**, *330*, 933–937.
- (44) Ortiz de Montellano, P. R. *Chem. Rev.* **2010**, *110*, 932–948.
- (45) Shoji, O.; Wiese, C.; Fujishiro, T.; Shirataki, C.; Wünsch, B.; Watanabe, Y. *J. Biol. Inorg. Chem.* **2010**, *15*, 1109–1115.
- (46) Johnston, J. B.; Ouellet, H.; Podust, L. M.; Ortiz de Montellano, P. R. *Arch. Biochem. Biophys.* **2011**, *507*, 86–94.
- (47) Mak, L. H.; Sadeghi, S. J.; Fantuzzi, A.; Gilardi, G. *Anal. Chem.* **2010**, *82*, 5357–5362.
- (48) van der Felt, C.; Hindoyan, K.; Choi, K.; Javdan, N.; Goldman, P.; Bustos, R.; Star, A. G.; Hunter, B. M.; Hill, M. G.; Nersissian, A.; Udit, A. K. *J. Inorg. Biochem.* **2011**, *105*, 1350–1353.
- (49) Shigehara, K.; Anson, F. C. *J. Phys. Chem.* **1982**, *86*, 2776–2783.
- (50) Shi, C.; Anson, F. C. *Inorg. Chem.* **1990**, *29*, 4298–4305.
- (51) Ricard, D.; L'Her, M.; Richard, P.; Boitrel, B. *Chem.—Eur. J.* **2001**, *7*, 3291–3297.
- (52) Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y.-L.; Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D. *Science* **2007**, *315*, 1565–1568.
- (53) Samanta, S.; Mitra, K.; Sengupta, K.; Chatterjee, S.; Dey, A. *Inorg. Chem.* **2013**, *52*, 1443–1453.
- (54) Chidsey, C. E. D. *Science* **1991**, *251*, 919–922.
- (55) Alleman, K. S.; Weber, K.; Creager, S. E. *J. Phys. Chem.* **1996**, *100*, 17050–17058.
- (56) Zou, S.; Clegg, R. S.; Anson, F. C. *Langmuir* **2002**, *18*, 3241–3246.
- (57) Smalley, J. F.; Finklea, H. O.; Chidsey, C. E. D.; Linford, M. R.; Creager, S. E.; Ferraris, J. P.; Chalfant, K.; Zawodzinski, T.; Feldberg, S. W.; Newton, M. D. *J. Am. Chem. Soc.* **2003**, *125*, 2004–2013.
- (58) Devaraj, N. K.; Decreau, R. A.; Ebina, W.; Collman, J. P.; Chidsey, C. E. D. *J. Phys. Chem. B* **2006**, *110*, 15955–15962.
- (59) Hosseini, A.; Barile, C. J.; Devadoss, A.; Eberspacher, T. A.; Decréau, R. A.; Collman, J. P. *J. Am. Chem. Soc.* **2011**, *133*, 11100–11102.
- (60) Samanta, S.; Sengupta, K.; Mitra, K.; Bandyopadhyay, S.; Dey, A. *Chem. Commun.* **2012**, *48*, 7631–7633.
- (61) Creager, S.; Yu, C. J.; Bamdad, C.; O'Connor, S.; MacLean, T.; Lam, E.; Chong, Y.; Olsen, G. T.; Luo, J.; Gozin, M.; Kayyem, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 1059–1064.
- (62) Collman, J. P.; Devaraj, N. K.; Eberspacher, T. P. A.; Chidsey, C. E. D. *Langmuir* **2006**, *22*, 2457–2464.
- (63) Clark, R. A.; Bowden, E. F. *Langmuir* **1997**, *13*, 559–565.
- (64) Sengupta, K.; Chatterjee, S.; Samanta, S.; Bandyopadhyay, S.; Dey, A. *Inorg. Chem.* **2013**, *52*, 2000–2014.
- (65) Sengupta, K.; Chatterjee, S.; Samanta, S.; Dey, A. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 7051–7056.
- (66) Note that PSR though being structurally similar to PIM could not be used as it is unstable in air.
- (67) Bulovas, A.; Talaikyte, Z.; Niaura, G.; Kazemekaite, M.; Marcinkeviciene, L.; Bachmatova, I.; Meskys, R.; Razumas, V. *Chemija* **2007**, *18*, 9–15.
- (68) Pramanik, D.; Sengupta, K.; Mukherjee, S.; Dey, S. G.; Dey, A. *J. Am. Chem. Soc.* **2012**, *134*, 12180–12189.
- (69) Zhang, Y.; Wilson, G. S. *J. Electroanal. Chem.* **1993**, *345*, 253–271.
- (70) Hildebrandt, P.; Macor, K. A.; Czernuszewicz, R. S. *J. Raman Spectrosc.* **1988**, *19*, 65–69.
- (71) Murgida, D. H.; Hildebrandt, P. *Chem. Soc. Rev.* **2008**, *37*, 937–945.
- (72) Sharma, B.; Frontiera, R. R.; Henry, A.-I.; Ringe, E.; Van Duyne, R. P. *Mater. Today* **2012**, *15*, 16–25.
- (73) Bettelheim, A.; Chan, R. J. H.; Kuwana, T. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *110*, 93–102.
- (74) Mukherjee, S.; Sengupta, K.; Das, M.; Jana, S.; Dey, A. *J. Biol. Inorg. Chem.* **2012**, *17*, 1009–1023.
- (75) Burke, J. M.; Kincaid, J. R.; Peters, S.; Gagne, R. R.; Collman, J. P.; Spiro, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 6083–6088.
- (76) Abe, M.; Kitagawa, T.; Kyogoku, Y. *Chem. Lett.* **1976**, *5*, 249–252.

- (77) Grochol, J.; Dronov, R.; Lisdat, F.; Hildebrandt, P.; Murgida, D. *H. Langmuir* **2007**, *23*, 11289–11294.
- (78) Das, T. K.; Franzen, S.; Pond, A.; Dawson, J. H.; Rousseau, D. L. *Inorg. Chem.* **1999**, *38*, 1952–1953.
- (79) Collman, J. P.; Gagne, R. R.; Halbert, T. R.; Marchon, J. C.; Reed, C. A. *J. Am. Chem. Soc.* **1973**, *95*, 7868–7870.
- (80) Champion, P. M.; Stallard, B. R.; Wagner, G. C.; Gunsalus, I. C. *J. Am. Chem. Soc.* **1982**, *104*, 5469–5472.
- (81) Asher, S. A.; Schuster, T. M. *Biochemistry* **1979**, *18*, 5377–5387.
- (82) Crisanti, M. A.; Spiro, T. G.; English, D. R.; Hendrickson, D. N.; Suslick, K. S. *Inorg. Chem.* **1984**, *23*, 3897–3901.
- (83) Castner, D. G.; Hinds, K.; Grainger, D. W. *Langmuir* **1996**, *12*, 5083–5086.
- (84) Hasan, M. N.; Kwakernaak, C.; Sloof, W.; Hagen, W.; Heering, H. *J. Biol. Inorg. Chem.* **2006**, *11*, 651–662.
- (85) Sodhi, R. N. S.; Cavell, R. G. *J. Electron Spectrosc. Relat. Phenom.* **1986**, *41*, 1–24.
- (86) Brion, D. *Appl. Surf. Sci.* **1980**, *5*, 133–152.
- (87) Denisov, I. G.; Hung, S.-C.; Weiss, K. E.; McLean, M. A.; Shiro, Y.; Park, S.-Y.; Champion, P. M.; Sligar, S. G. *J. Inorg. Biochem.* **2001**, *87*, 215–226.
- (88) Segall, M. D.; Payne, M. C.; Ellis, W.; Tucker, G. T.; Boyes, N. *Chem. Res. Toxicol.* **1998**, *11*, 962–966.
- (89) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; p 300.
- (90) McCrory, C. C. L.; Ottenwaelde, X.; Stack, T. D. P.; Chidsey, C. E. D. *J. Phys. Chem. A* **2007**, *111*, 12641–12650.
- (91) Collman, J. P.; Fu, L.; Herrmann, P. C.; Zhang, X. *Science* **1997**, *275*, 949–951.
- (92) Different types of rotation rates during RRDE experiments yielding similar observations and results further support the durability and stability of both catalysts under aqueous conditions.
- (93) Hosseini, A.; Collman, J. P.; Devadoss, A.; Williams, G. Y.; Barile, C. J.; Eberspacher, T. A. *Langmuir* **2010**, *26*, 17674–17678.
- (94) Davis, K. L.; Drews, B. J.; Yue, H.; Waldeck, D. H.; Knorr, K.; Clark, R. A. *J. Phys. Chem. C* **2008**, *112*, 6571–6576.
- (95) Shein, J. B.; Lai, L. M. H.; Eggers, P. K.; Paddon-Row, M. N.; Gooding, J. J. *Langmuir* **2009**, *25*, 11121–11128.
- (96) Slow ET rate in C₁₆SH SAM is also reflected in CV experiments (Supporting Information, Figures S14 and S15). Catalysts physisorbed on C₆SH SAM yielded good CV both in normal pH 7 buffer and in presence of 100 mM pyridine. However, in case of C₁₆SH no CV was observed in the potential window suggesting splitting of the oxidation and reduction waves.
- (97) The amount of PROS formation by PIM and PPSR when physisorbed on C₁₆SH SAM is a property of the catalysts. Note that under both the case the ET rate is similar and is rate determining for the catalytic process as obtained from the Tafel slope analysis (Supporting Information, Figure S14).
- (98) Note that the other thiolate bound analogue (PSR) reported earlier is unstable in air and thus cannot be used in this study.
- (99) Dawson, J. H.; Andersson, L. A.; Sono, M. *J. Biol. Chem.* **1983**, *258*, 13637–13645.
- (100) These bands are generally assigned for the Fe^{IV}=O species of synthetic mimics.
- (101) Hashimoto, S.; Mizutani, Y.; Tatsuno, Y.; Kitagawa, T. *J. Am. Chem. Soc.* **1991**, *113*, 6542–6549.
- (102) Collman, J. P.; Decréau, R. A. *Chem. Commun.* **2008**, *0*, 5065–5076.
- (103) Collman, J. P.; Decréau, R. A.; Dey, A.; Yang, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 4101–4105.
- (104) Momenteau, M.; Reed, C. A. *Chem. Rev.* **1994**, *94*, 659–698.
- (105) Wallace, W. J.; Houtchens, R. A.; Maxwell, J. C.; Caughey, W. S. *J. Biol. Chem.* **1982**, *257*, 4966–4977.
- (106) Stanbury, D. M.; Haas, O.; Taube, H. *Inorg. Chem.* **1980**, *19*, 518–524.