

# Catalytic C–H Bond Oxidation Using Dioxygen by Analogues of Heme Superoxide

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**ABSTRACT:** Heme active sites are capable of oxidizing organic substrates by four electrons using molecular oxygen (heme dioxygenases), where a dioxygen (O<sub>2</sub>) adduct of heme (Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup>) acts as the primary oxidant, in contrast to monooxygenases, where high-valent species are involved. This chemistry, although lucrative, is difficult to access using homogeneous synthetic systems. Over the past few years using a combination of selfassembly and in situ resonance Raman spectroscopy, the distribution of different reactive intermediates formed during the electrochemical reduction of oxygen has been elucidated. An Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> species, which is the reactive species of dioxygenase, is an intermediate in heterogeneous electrochemical O<sub>2</sub> reduction by iron porphyrins and its population, under electrochemical conditions, may be controlled by controlling the applied potential. Iron porphyrins having different axial ligands are constructed on a self-assembled



monolayer of thiols on an electrode, and these constructs can activate  $O_2$  and efficiently catalyze the dioxygenation of 3-methylindole and oxidation of a series of organic compounds having C–H bond energies between 80 and 90 kcal mol<sup>-1</sup> at potentials where Fe<sup>III</sup>- $O_2^{\bullet-}$  species are formed on the electrode. Isotope effects suggest that hydrogen-atom transfer from the substrate is likely to be the rate-determining step. Axial thiolate ligands are found to be more efficient than axial imidazoles or phenolates with turnover numbers above 60000 and turnover frequencies over 60 s<sup>-1</sup>. These results highlight a new reaction engineering approach to harness  $O_2$  as a green oxidant for efficient chemical oxidation.

### **1. INTRODUCTION**

Utilization of dioxygen  $(O_2)$  as a green oxidant for useful chemical transformations has been a long-time pursuit of the scientific community.<sup>1-6</sup> In nature, metalloenzymes like heme dioxygenases (HDOs; Figure 1A)<sup>7,8</sup> and cytochrome P450 (cytP450; Figure 1B)<sup>9–11</sup> have evolved to utilize  $O_2$  to oxidize a wide variety of organic substrates playing key roles in several biochemical processes. These metalloenzyme active sites can activate oxygen controllably to generate reactive intermediates, which act as the oxidants<sup>9,11,12</sup> (Figure 2). In HDOs, the oxygen-bound heme iron(III) superoxide ( $P[Fe^{III}-O_2^{\bullet-}]$ , where P represents the porphyrin ring) species reacts with the substrate<sup>7</sup> (Figure 1A). In cytP450, a resting ferrous active site binds oxygen, and utilizing two electrons and two protons generates a highly reactive species known as compound I, which has a ferryl center bound to a porphyrin radical-cation species  $P^+[Fe^{IV}=O]$ . In cytP450, a Fe<sup>III</sup>-OOH species, which precedes compound I, is also proposed to be an active oxidant involved in alkene epoxidation (Figure 1C).<sup>13–15</sup>

The chemistry community has been pursuing emulating this elegant chemistry for several decades.<sup>16–18</sup> Activation of oxygen by small-molecule ferrous complexes outside the protein environment to oxidize organic substrates has proven to be challenging.<sup>19–22</sup> This is primarily due to two reasons. First, the generation of high-valent intermediates like



Figure 1. Schematic representation of the active intermediates of (A) indole 2,3-dioxygenase, (B) cytP450 monooxygenase, (C) intradiol catechol dioxygenase, and (D) a synthetic model for HAT from a pendant quinol system by iron superoxide species.

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Figure 2. Schematic representation of the intermediates involved in oxygen reduction and their reactivity in both enzyme and synthetic systems.

P+FelV(

P<sup>+</sup>Fe<sup>IV</sup>O

compound I from oxygen requires controlled delivery of electrons and protons; while deftly engineered in metalloenzymes, it is challenging to control in most synthetic constructs.  $^{3,23,24}$  Second, the reaction of O<sub>2</sub> with ferrous complexes generates reactive oxygen species like superoxide and hydroxyl radicals and the corresponding ferric complexes.<sup>25,26</sup> While these radicals are unselective oxidants,<sup>27</sup> the end product ferric species do not react with  $O_2$ , thereby jeopardizing catalysis.<sup>3</sup> Early efforts to chemically regenerate the active reduced metal form by an external reducing agent to obtain catalysis resulted in the nonspecific oxidation of both styrene and cyclohexane with very limited turnovers.<sup>28</sup> Recently, photocatalysis has been utilized to regenerate the reactive species, and it has been proven that photogeneration of the reactive intermediate occurs via a free-radical pathway.<sup>29,30</sup> In very rare cases,  $Fe^{III}-O_2^{\bullet-}$  has been found to be capable of oxidizing organic substrates in a controllable manner.<sup>31-33</sup> A recent example is the two-electron oxidation of a quinol group appended to the porphyrin ring by  $Fe^{III}-O_2^{\bullet-}$ species (Figure 1D) via two consecutive hydrogen-atomtransfer (HAT) steps.<sup>34</sup> The above few cases represent twoelectron oxidation of the substrate and not four-electron oxidation typical of dioxygenases. In dioxygenases, all four electrons required to reduce O<sub>2</sub> are obtained from an organic substrate, which, in turn, gets oxidized by four electrons and incorporates the oxygen atoms. Such reactivity utilizing O<sub>2</sub> as a four-electron oxidant is yet to be demonstrated in synthetic heme systems.

The regeneration of a reactive ferrous form can also be, in principle, achieved electrochemically. Such attempts are complicated by the fact that the application of a cathodic potential to iron porphyrins (to reduce them) in the presence of  $O_2$  leads to the electrochemical reduction of oxygen. While intermediates such as  $Fe^{III}-O_2^{\bullet-}$  and  $P^+Fe^{IV}=O$  can be envisaged to be involved in the process, it has not been clear as to how to access these species controllably using  $O_2$ . Very recently, the electrochemical reduction of  $O_2$  using iron porphyrin complexes in conjunction with in situ resonance Raman spectroscopy has led to the development of a general understanding of the mechanism for the oxygen reduction

process (Figure 2).<sup>35</sup> A ferric porphyrin is first reduced to a ferrous porphyrin as the applied potential is lowered below the thermodynamic reduction potential of the iron porphyrin  $[E^{\circ}(Fe^{III/II})$  in Figure 2]. The ferrous porphyrin binds O<sub>2</sub>, generating a  $Fe^{III} - O_2^{\bullet-}$  species, which is the reactive species in HDOs and has been detected and characterized in solution in spectroelectrochemical experiments.<sup>36</sup> The  $Fe^{III}$ - $O_2^{\bullet-}$  species is then further reduced as the applied potential if further lowered to a Fe<sup>III</sup>-OOH species (Figure 2), which is known as compound 0 in heme enzymes. This reduction step is either a proton-coupled electron-transfer (PCET) or a electron-transfer proton-transfer (ETPT) pathway depending on the nature of the axial ligand, solvent, and distal environment.<sup>37,38</sup> Again, depending on the nature of the axial ligand and distal structure of the porphyrin, the Fe<sup>III</sup>-OOH species can lead to either a  $Fe^{IV} = O$  (compound II) or a  $P^+Fe^{IV} = O$  (compound I) species, which are accumulated on the electrode during steadystate  $O_2$  reduction.<sup>39-42</sup> The oxygen-derived species that accumulate on the electrode are chemically the same as the reactive oxidants in different heme enzymes (Figure 2). The electrochemical approach of generating these reactive species is advantageous because these different species are separated by the thermodynamic potential, which can be controlled by externally applying the requisite potential.

Using this approach, thiolate-bound iron porphyrins, immobilized on self-assembled monolayers (SAMs) of thiols on gold electrodes, demonstrated monooxygenase activity, where the two electrons required for it were supplied from the electrode.43 Catalytic hydroxylation of aliphatic C-H bonds having a bond dissociation energy of  $\geq 100$  kcal mol<sup>-1</sup> using O<sub>2</sub> as an oxidant was demonstrated with a turnover number (TON) of >20000, and a 100% <sup>18</sup>O<sub>2</sub>-incorporated product indicated that molecular oxygen was the source of the oxygen atom. High-valent intermediates such as compound I  $(P^+Fe^{IV} = O in Figure 2)$  generated during oxygen reduction could be utilized to oxidize organic substrates in these electrochemical analogues of the cytP450 system. Normally, such high-valent intermediates formed during electrochemical O<sub>2</sub> reduction are reduced by rapid electron transfer (ET) from the electrode, which in these cases is generally held at cathodic potentials. When the rate of ET was controlled by controlling the chain length of the thiols used to form the SAM, these intermediates could be used to oxidize inert C-H bonds catalytically. These recent developments raise the possibility of harnessing the reactivity of  $Fe^{III}-O_2^{\bullet-}$  species at potentials where the  $Fe^{III}-O_2^{\bullet-}$  species may exist. In this manuscript, catalytic chemical oxidation of organic substrates by four electrons is achieved using molecular oxygen as the oxidant. The oxidant, a  $Fe^{III}-O_2^{\bullet-}$  species, is generated electrochemically on SAM-covered gold electrodes by the reaction of  $O_2$ with iron(II) porphyrins having different axial ligands (e.g., thiolate, phenolate, and imidazole). Apart from indoles, a series of substrates having C-H bond dissociation free energies  $(BDFE_{CH})$  of less than 90 kcal mol<sup>-1</sup> could be oxidized with turnover number (TON) and turnover frequency (TOF) greater than 60000 and 60  $s^{-1}$ , respectively. Isotope effects, <sup>18</sup>O<sub>2</sub> labeling, and the effect of the applied potential suggest that HAT by a Fe<sup>III</sup>- $O_2^{\bullet-}$  species is likely to be the key step in catalysis.

### 2. RESULTS

**2.1. Oxidation of Substrates.** Iron porphyrin having a hydrophobic distal site like iron "picketfence" porphyrin,<sup>17</sup>



Figure 3. (A) Schematic representation of the heterogeneous electrode system used, where L stands for different axial ligands such as imidazole (md), phenolate (OPh<sup>-</sup>), and thiolate (RS<sup>-</sup>). Fe<sup>III/II</sup> cyclic voltammograms in the absence of O<sub>2</sub> (solid line; scan rate 1 V s<sup>-1</sup>) and the electrocatalytic O<sub>2</sub> reduction current (dashed line; scan rate 50 mV s<sup>-1</sup>) for (B) imidazole-, (C) phenolate-, and (D) thiolate-ligated FePf in a 100 mM pH 7 phosphate buffer.

FePf, is attached to imidazole, phenolate, and thiolate terminal headgroups of thiols thinly dispersed in a SAM on gold electrodes (Figure 3A, where R = o-pivolylacetamidophenyl) as described earlier.<sup>44</sup> In the absence of oxygen, these electrodes show reversible  $Fe^{III/II}$  processes at -202, -217, and -250 mV versus Ag/AgCl (saturated KCl) for phenolate-, imidazole-, and thiolate-ligated FePf, respectively (parts C, B, and D of Figure 3, respectively). In the presence of oxygen in a pH 7 buffer solution, these show electrochemical oxygen reduction, which has been investigated in detail.<sup>35,40,44,45</sup> When 3methylindole, a modified indole dioxygenase substrate, is taken in the buffer solution used for oxygen reduction reaction and a constant potential of -300 mV versus Ag/AgCl (saturated KCl) is applied in a bulk electrolysis experiment, N-(2-acetylphenyl)formamide (Figure 4A and Figures S.3 and S.4) is found during product analysis, which is the product of a dioxygenase reaction, suggesting that the electrochemical activation of O<sub>2</sub> provides access to dioxygenase reactivity in this construct. The same reactivity is observed irrespective of the axial ligand used (i.e., thiolate, phenolate, and imidazole). Having observed the oxidation of 3-methylindole, a convenient substrate has to be chosen to understand the reactivity of the oxidant produced during electrochemical oxygen reduction in detail [rate, kinetic isotope effect (KIE),  ${}^{18}$ O incorporation, etc.]. Recently, an FeTPP-based Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> species has been demonstrated to perform HAT from a covalently attached pendant quinol having a BDFE<sub>OH</sub> of 80 kcal mol-1 (Figure 1D).<sup>34</sup> Accompanying density functional theory calculations



**Figure 4.** Oxidation of (A) 3-methylindole and (B) toluene by  $Fe^{III}$ - $O_2^{\bullet-}$  with different axial ligands.

suggested that the BDFE<sub>OH</sub> value of Fe<sup>III</sup>-O<sub>2</sub>H bond is 83.9 kcal mol<sup>-1</sup>. Accordingly, toluene (BDFE<sub>CH</sub> of 89 kcal mol<sup>-1</sup>) is chosen as a substrate to investigate the reactivity of these systems. In this case, a four-electron-oxidized product of toluene, benzaldehyde, is detected as the main product with TONs as high as  $6.1 \times 10^4$  and TOFs as high as  $67.93 \text{ s}^{-1}$  for the thiolate-bound FePf complex (Table 1). Thus, the construct developed here can catalyze the reaction PhCH<sub>3</sub> +  $O_2 \rightarrow$  PhCHO + H<sub>2</sub>O efficiently at room temperature and in an aqueous medium (Figure 4B). Along with benzaldehyde, minor amounts of benzyl alcohol are also observed, suggesting that the reaction proceeds via benzyl alcohol as an intermediate.

Table 1. TONs, TOFs, the Maximum TON Possible for Monooxygenase Reaction  $(TON_M)$ , and the Ratio of TON and  $TON_M$  ( $\mu$ ) for the Products Obtained after the Oxidation of Substrates by Differently Ligated FePf Complexes

Substrate	Product	La	TON (10 <sup>3</sup> ) <sup>b</sup>	TOF (s <sup>-1</sup> ) <sup>c</sup>	TON <sub>M</sub> (10 <sup>3</sup> ) <sup>d</sup>	$\boldsymbol{\mu}^{e}$
$\frown$		Imd	35	39.5	4.1	9.1
$\frown$		PhO <sup>-</sup>	29	32.6	3.4	9.5
$\frown$		RS <sup>-</sup>	61	67.9	3.1	22.8
	С		01	0.9	6.1	0.1
С		RS <sup>-</sup>	45	49.1	5.9	7.7
			48	54	2.95	16.9
	OH		42	47.5	5.4	0.5
		RS	43	47.5	5.1	8.5
ССНО	ССООН	RS	32	36.1	3.2	10.1
		RS <sup>-</sup>	Observed			
		PhO <sup>-</sup>	Observed			
H		Imd	Observed			
	ОН	RS <sup>-</sup>	21	23	2.98	0.70
		PhO <sup>-</sup>	NA			
		Imz	NA			
$\bigcirc$		RS⁻	5.7	6	1.27	0.45
		PhO <sup>-</sup>	NA			
		Imz		NA		

<sup>*a*</sup>L is the axial ligand. <sup>*b*</sup>TON is the turnover number. <sup>*c*</sup>Turnover frequency (TOF) averaged over the entire duration of the experiment. <sup>*d*</sup>Maximum turnover expected from monooxygenase reactivity. <sup>*e*</sup>The ratio of TON and TON<sub>M</sub>.

The incorporation of molecular O2 in the product is confirmed by labeling experiments using <sup>18</sup>O<sub>2</sub>. While the benzyl alcohol produced is 100% labeled with <sup>18</sup>O, the benzaldehyde produced is not because the rate of exchange of a <sup>18</sup>O-labeled aldehyde oxygen with unlabeled water is  $\sim 10^5$ M<sup>-1</sup> s<sup>-1</sup> in buffered solutions.<sup>46</sup> Recently, <sup>18</sup>O<sub>2</sub> incorporation in aldehyde in unlabeled water was established by reducing it in situ to the corresponding alcohol using NaBH4.47 In the presence of NaBH4, the <sup>18</sup>O-labeled benzaldehyde produced immediately gets reduced to labeled benzyl alcohol and the alcoholic oxygen atom thus produced does not get further exchanged.47 Thus, the electrochemical oxygen reduction is performed in a <sup>18</sup>O<sub>2</sub>-saturated buffer in the presence of toluene and excess NaBH<sub>4</sub>. This resulted in a substantially greater amount of <sup>18</sup>O-labeled benzyl alcohol (Figure S.8) than that produced without labeled oxygen. The excess <sup>18</sup>O-labeled benzyl alcohol is obtained from the reduction of <sup>18</sup>O-labeled benzaldehyde produced via oxidation of toluene with <sup>18</sup>Olabeled molecular oxygen. The total yields of benzaldehyde are lower for axial imidazole and phenolate ligands relative to that for an axial thiolate ligand (Table 1, rows 1-3). A series of substrates, including benzyl alcohol, benzaldehyde, and cinnamaldehyde, could be oxidized by molecular oxygen using the same approach. These substrates all have BDFE<sub>C-H</sub> < 89 kcal mol<sup>-1</sup> (Table 1, rows 4–6). However, stronger C–H bonds of cyclohexane or epoxidation of styrene by a  $\tilde{Fe}^{III}$ - $O_2^{\bullet-}$ 

species could not be achieved with axial imidazole and phenolate ligations (Table 1, rows 8 and 9) using this approach in the same potential range of -100 to -500 mV versus Ag/AgCl (saturated KCl).

2.2. Quantification of the Faradaic Efficiency. Monooxygenase activity  $(R-H + O_2 + 2H^+ + 2e^- \rightarrow R-OH + H_2O)$ requires two electrons from the electrode per substrate oxidized. Thus, if an electrolysis experiment consumes a total charge Q, the moles of the product obtained can be at most Q/*nF*, where F is a Faraday constant of 96500 C mol<sup>-1</sup>. For a monooxygenase-type reaction, the maximum possible TON  $(TON_M in Table 1, column 6)$  can be estimated from the total charge consumed using this formula, where n = 2. For example, as demonstrated recently, a compound I type species is formed during oxygen reduction by iron porphyrins with axial thiolate ligands at cathodic potentials that can hydroxylate and epoxidize organic substrates via a cytP450-like monooxygenase activity.<sup>43</sup> It was observed that the ratio of the actual TON and TON<sub>M</sub>(defined henceforth as  $\mu$ ; section S.8) was always <1 because some of the electrons are consumed as a result of a competing oxygen reduction reaction, which does not lead to product formation (Table 1, column 7). For a dioxygenasetype reaction or a four-electron oxidation of an organic substrate, the reactive ferrous form is regenerated at the end of the cycle and  $\mu$  can be theoretically infinite.<sup>48,49</sup> However, when attached to an electrode and dipped in an aqueous solution, there is always a competing oxygen reduction reaction and hydrolysis of the intermediates involved (e.g., Fe<sup>III</sup>-OOH), which consumed electrons.<sup>43</sup> In such a case, the experimentally observed  $\mu$  can be expected to be more than 1 (n = 2) but not infinite. Note that while the competing oxygen reduction is expected to reduce the efficiency of the dioxygenase activity, the active ferrous species can be regenerated at the electrode at the applied cathodic potential and help to reinstate catalysis.

Quantification of the oxidation product of toluene, benzaldehyde, reveals that the yields in these cases, where the applied potential is held in the kinetically controlled region of the oxygen reduction reaction, are substantially higher than what monooxygenase activity would allow ( $TON_M$  in Table 1), necessarily implying an oxidation mechanism that does not require regeneration of the active ferrous form at the end of the catalytic cycle, i.e., a four-electron oxidation by O<sub>2</sub>. The ratio of actual TON and TON<sub>M</sub>,  $\mu$ , is  $\gg 1$  for almost all substrates where  $BDFE_{CH}$  is <89 kcal mol<sup>-1</sup>. For stronger C–H bonds as well as the epoxidation of styrene, oxidation is only observed with an axial thiolate ligand, akin to cytP450, having  $\mu < 1$  for reasons explained before. A  $\mu$  of 22 in the case of thiolatebound FePf implies that there are 22 turnovers for the fourelectron oxidation of toluene to benzaldehyde before the iron center needs to be reduced by the electrode. Radical-chain reactions like Russel termination and Gif reactions can also lead to excess substrate oxidation but are rescinded based on product distribution and isotope effects that are discussed below. The TON and TOF are higher for the thiolate-ligated porphyrin relative to the imidazole- and phenolate-ligated porphyrins. Moreover, the amount of oxidized product increases  $\sim$ 5 times when the aqueous electrolyte, containing 0.2 mM dissolved  $O_2$ , is saturated with  $O_2$  (~1 mM), consistent with a dioxygenation-type four-electron oxidation of the substrate (Figure S.7 and Table S.3). Thus, <sup>18</sup>O incorporation, very high  $\mu$  values, and dependence of the rate on the concentration of oxygen in the solution imply that a four-electron oxidation of the substrate is catalyzed by iron



**Figure 5.**  $\mu$  of benzadehyde obtained as a function of the applied potential for (A) imidazole-, (B) phenolate-, and C) thiolate-bound FePf in a pH 7 buffer solution at room temperature.

porphyrins using a molecular oxygen by four electrons, where  $Fe^{III}$ - $O_2^{\bullet-}$  is a reactive intermediate formed upon binding of  $O_2$  to the ferrous porphyrin produced on the electrode. The population of a  $Fe^{III}$ - $O_2^{\bullet-}$  species on an electrode is potential-dependent because this species is further reduced to a  $Fe^{III}$ -OOH species in the process of  $O_2$  reduction and that automatically requires the product distribution to be potential-dependent as well.

2.3. Effect of the Applied Potential. The extent of fourelectron-oxidized product produced during constant potential electrolysis reactions, expressed in  $\mu$  (section S.8), is quantified at different applied potentials for FePf bound to axial imidazole, phenolate, and thiolate ligands. In all of these cases, the maximum yield is obtained at potentials where the  $Fe^{III}-O_2^{\bullet-}$  species is likely most abundant on the electrode (Figure 5), i.e., at the potential-dependent kinetic region of the electrocatalytic current where ET is the rate-determining step (rds). This is the potential region where the rate of  $O_2$ reduction is limited by the reduction of Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> via ET from the electrode.<sup>38</sup> At more negative applied potentials [-500 mV vs Ag/AgCl (saturated KCl)], surface-enhanced resonance Raman spectroscopy coupled to rotating disk electrochemistry experiments have indicated the presence of Fe<sup>III</sup>-OOH (major) and Fe<sup>IV</sup>=O species (minor); i.e., the Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> species has been further reduced.<sup>35</sup> The extent of substrate oxidation is reduced substantially at this potential, with  $\mu$  approaching the monooxygenation limit of <1. At these potentials, O<sub>2</sub> is already reduced by two electrons to generate peroxide species or Fe<sup>IV</sup>=O/P<sup>+</sup>Fe<sup>IV</sup>=O species (produced after O–O bond cleavage) and, hence,  $\mu$  approaching a value <1 is expected.

**2.4. ET Rate.** In case a high-valent intermediate like compound I or II generated during  $O_2$  reduction is the oxidant, increasing the rate of ET from the electrode to the catalyst by altering the chain length of the thiol reduces the yield of the oxidized product.<sup>43</sup> For example, when compound I generated during  $O_2$  reduction is used to oxidize cyclohexane to cyclohexanol in thiolate-bound FePf,  $\mu$  decreases rapidly as the rate of ET from the electrode to catalyst is enhanced by decreasing the chain length of thiols used to construct the SAM. In contrast, the yield of benzaldehyde from toluene oxidation is determined to be almost independent of the ET rate from the electrode, suggesting that the majority of oxidation occurs via a dioxygenase-type reactivity that does not require ET from the electrode during catalytic turnover (Table 2).

Apart from four-electron oxidation of these substrates by oxygen, it is also possible that oxidation occurs via a radicalchain reaction where a substrate radical reacts with oxygen in the medium. There are two possible pathways: (a) Russel

Table 2. Changes in  $\mu$  with Changes in the ET Rate

Substrate	Product	Axial ligand	Diluent	k <sub>ET</sub> (sec <sup>-1</sup> ) <sup>51-54</sup>	μ
$\frown$		RS <sup>-</sup>	C <sub>8</sub> SH	~ 10 <sup>3</sup>	22.8
$\frown$		RS	C₄SH	~ 105	20.8
$\frown$		RS⁻	C <sub>10</sub> SH	~ 5x10 <sup>2</sup>	22.6
	•				

termination;<sup>50</sup> (b) Gif reaction. Russel termination would result in a 1:1 ratio of the aldehyde and alcohol, which is not the case here. Gif reaction would require the formation of a Fe–C bond and has a minimal KIE. The formation of a Fe–C bond would inhibit oxygen binding to the iron, which would, in turn, inhibit O<sub>2</sub> reduction, contrary to the experimental results. Thus, neither of these radical mechanisms fits the product profile and KIE (see below) obtained here.

**2.5. Isotope Effects.** These reactions were performed with toluene- $d_8$  to estimate the KIE. The axial thiolate, phenolate, and imidazole ligands produce KIEs of 5, 9, and 12, respectively (Table 3 and Figure S.9). These formidable

Table 3. KIE and KSIE for Toluene Oxidation with Differently Ligated FePf

Substrate	Product	Axial	KIE	KSIE
		Ligand		
$\frown$	<u>_</u>	RS-	5.05±0.1	4.26±0.18
$\frown$	<del>ر</del>	OPh-	8.8±0.7	1.12±0.05
$\bigtriangledown$	<u>ر</u>	Imd	11.7±0.3	1.03±0.02

KIEs for the axial imidazole and phenolate ligands suggest that HAT is the rds in these reactions. This rds is likely to be HAT from toluene to the Fe<sup>III</sup>-O<sub>2</sub><sup>--</sup> intermediate formed during O<sub>2</sub> reduction on the electrode. <sup>51-54</sup> Because these reactions are performed in aqueous solvents, the solvent kinetic isotope effects (KSIEs) are measured (Figure S.10). Consistent with a HAT mechanism, neither axial phenolate nor imidazole ligands have any KSIEs. The axial thiolate ligand exhibits a KSIE of 4, which along with a weak KIE may imply a different rds. Note that a monooxygenase reaction by the thiolate-bound FePf complex exhibited a KSIE of 13 because the proton-assisted heterolytic O–O bond cleavage of Fe<sup>III</sup>-OOH was involved.<sup>43</sup>

### 3. MECHANISM OF ACTION

On the basis of the experimental observations, a mechanism of action for the observed reactivity can be proposed. A Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> species performs the first HAT from the substrate, forming a Fe<sup>III</sup>-OOH species and a substrate radical. The Fe<sup>III</sup>-OOH rebounds via homolytic cleavage of the O–O bond to form a hydroxylated substrate and Fe<sup>IV</sup>=O. The Fe<sup>IV</sup>=O, being a better oxidant than Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup>, performs another HAT from the two-electron-oxidized substrate, and via a final rebound, it forms the four-electron-oxidized substrate, regenerating the Fe<sup>II</sup> reactive state, which can enter another catalytic cycle. The ability of Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> to perform two consecutive HATs from a substrate, as proposed here, was recently established using an iron porphyrin bearing a pendant quinol group.<sup>34</sup> The

proposed Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup>, Fe<sup>III</sup>-OOH, and Fe<sup>IV</sup>=O intermediates were all characterized using resonance Raman and electron paramagnetic resonance spectroscopy.<sup>34</sup> The KIEs are consistent with the HAT being the rds as proposed.

The proposed mechanism requires the Fe<sup>IV</sup>=O species to complete the second two-electron oxidation. While imidazoleand phenolate-bound Fe<sup>IV</sup>=O species are known to be stable at neutral pH,<sup>55</sup> thiolate-bound Fe<sup>IV</sup>=O species have  $pK_a >$ 9<sup>56</sup> and are protonated at pH 7. Similarly, a thiolate-bound  $Fe^{III}$ -OOH is prone to heterolysis. While protonation of  $Fe^{IV}$ = O would compete with HAT from the monooxygenated substrate, protonation of Fe<sup>III</sup>-OOH would compete with its rebound (Figure 6) to the substrate radical. However, the bulky pivolyl groups in FePf create a hydrophobic pocket that disfavors water binding to the iron. This is evident from the fact that the resonance Raman data of thiolate-bound FePf show that it is five-coordinate even in an aqueous environment, unlike thiolate-bound iron porphyrins without the bulky pivolyl groups, which, as the resonance Raman data show, form wate-bound six-coordinate low-spin species.<sup>57</sup> Similarly, protonation of Fe<sup>III</sup>-OOH by bulk water during O<sub>2</sub> reduction or monooxygenation, for example, has a KSIE of 13.43 Accordingly, when an iron porphyrin with two pivolyl groups is used, FehPf (Figure S.11), the benzadehyde produced is reduced substantially and  $\mu$  values are reduced to 12.4 relative to 22.8 in FePf because of the greater access of water to the active site, causing more O2 reduction and monooxygenase activity, consistent with the proposed mechanism.

The low KIE and high KSIE suggest that, for thiolate-bound FePf, the rds is not likely to be HAT by a  $Fe^{III}-O_2^{\bullet-}$  species because a HAT step does not have a water involved. Alternatively, considering the high  $pK_a$  of a thiolate-bound  $Fe^{IV}=O$ , it is possible that the basic  $Fe^{IV}=O$  abstracts the proton from the benzyl alcohol produced from toluene, followed by hydride abstraction from the resultant alkoxide (Figure 7A). A similar mechanism for benzyl alcohol oxidation to benzaldehyde has been proposed for basic  $Fe^V {=\!\!\!\!=} O$ supported by a tetraamidate ligand.<sup>58</sup> Because the proton of benzyl alcohol, which sits atop of the active site cavity, is rapidly exchangeable with the solvent (faster than the time scale of these reactions) but the benzylic C-H is not, such a rds will show both KIE and KSIE as observed here. Consistently, KSIE is not observed when benzyl alcohol is used as the substrate for thiolate-bound FePf, where benzoic acid is the major product (forms benzylbenzoate in situ). This is because the first two-electron oxidation of benzyl alcohol will lead to benzaldehyde (Figure 7B), which does not have an exchangeable proton and hence no KSIE.

The proposed mechanism suggests that the same reactivity, in principle, should be attainable under homogeneous conditions under both single turnover conditions (starting with ferrous porphyrins) and homogeneous electrocatalytic conditions. Accordingly, cyclic voltammetry (CV) of FePf with *N*-methylimidazole in the presence of O<sub>2</sub> and toluene shows a large electrocatalytic current at potentials where the iron is reduced to its ferrous state (section S.14 and Figure S.12). This current, which is absent in the presence of O<sub>2</sub> alone, corresponds to the oxidation of toluene by the Fe<sup>III</sup>-O<sub>2</sub><sup>•-</sup> species formed, and benzaldehyde and benzoic acid are indeed detected by gas chromatography/mass spectrometry (GC/MS).

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Figure 6. Proposed mechanism of oxidation of toluene to benzaldehyde by  $O_2$  catalyzed by FePf covalently attached to the electrode via axial ligands.



#### Hydride transfer

Figure 7. Proposed mechanism for the second oxidation of (A) benzyl alcohol, produced from toluene, and (B) benzaldehyde, produced from benzyl alcohol with thiolate-bound FePf.

### 4. CONCLUSION

In summary, the catalytic oxidation of benzylic C–H bonds by iron porphyrins using  $O_2$  could be demonstrated with TONs and TOFs as high as ~60000 and ~60 s<sup>-1</sup>, respectively, at room temperatures and in water solvent. The combination of the self-assembly of an active site atop an electrode and knowledge of the detailed mechanism of  $O_2$  reduction by iron porphyrins immobilized on the electrode allowed control over the species present on the electrode using an applied potential,

thereby harnessing a dioxygenase-type reactivity likely via a proposed  $Fe^{III}$ - $O_2^{\bullet-}$  species. The rds for this reaction, as indicated by KIE and KSIE, is HAT by  $Fe^{III}$ - $O_2^{\bullet-}$  for axial imidazole and phenolate ligands, while for an axial thiolate ligand, it is likely to be hydride transfer to a thiolate-bound  $Fe^{IV}$ =O from a benzyl alcohol formed in situ. These results not only open up new reaction engineering approaches for harnessing a green oxidant like  $O_2$  for useful chemical oxidations but also offer direct insight into the role of axial ligands in tuning the reactivity of  $Fe^{III}$ - $O_2^{\bullet-}$  species, which are proposed to be formed during electrochemical oxygen reduction and are ubiquitous in nature in all heme oxidases and oxygenases.

### 5. EXPERIMENTAL SECTION

**5.1. Materials.** All reagents were of the highest grade commercially available and were used without further purification. Octanethiol ( $C_8SH$ ), butanethiol ( $C_4SH$ ), decanethiol ( $C_{10}SH$ ), potassium hexafluorophosphate (KPF<sub>6</sub>), benzyl alcohol, benzaldehyde, cinnamaldehyde and 3-methylindole were purchased from Sigma-Aldrich. Disodium hydrogen phosphate dihydrate (Na<sub>2</sub>HPO<sub>4</sub>: 2H<sub>2</sub>O) was purchased from Merck. Triethylamine (Et<sub>3</sub>N) and toluene were purchased from Spectrochem India Ltd. Isotopic labeled O<sub>2</sub> was purchased from Platypus Technologies [1000 Å of gold on 50 Å of a titanium adhesion layer on top of a silicon(III) surface].

**5.2.** Instrumentation. All electrochemical experiments were done using CH Instruments (model CHI700E electrochemical analyzer). The bipotentiostat, reference electrodes [Ag/AgCl (saturated KCl)], and Teflon plate material evaluating cell (ALS Japan) were purchased from CH Instruments. All qualitative and quantitative experiments were done using GC/MS. For this, an Agilent 7890B GC system with a 5977A MS detector was used.

**5.3. Construction of the Electrode.** Cleaning of the gold wafers was performed electrochemically by sweeping several times between 1.7 V and -300 mV versus Ag/AgCl (saturated KCl) in 0.5 M H<sub>2</sub>SO<sub>4</sub>. SAM solutions were prepared using the concentration ratio of the linkers (SHC<sub>11</sub>SH, OPhC<sub>11</sub>SH, and ImdC<sub>11</sub>SH; Figure 8) and



**Figure 8.** (A–C) Linker molecules  $SHC_{11}SH$ ,  $ImdC_{11}SH$ , and  $OPhC_{11}SH$ , respectively. (D) Diluent molecule (n = 1, 5, and 7 respectively for C<sub>4</sub>SH, C<sub>8</sub>SH, and C<sub>10</sub>SH).

Table 4. SAN	A Solution	Preparation	Ratio
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linker	mole fraction of the linker	total concentration (mM)
ImdC <sub>11</sub> SH	0.2	0.4
OPhC <sub>11</sub> SH	0.2	0.4
SHC11SH	0.2	3

diluent shown in Table 4. All of these linkers were prepared using the reported procedure.<sup>44</sup> In each case, the diluent used was C<sub>8</sub>SH, except for the cases where the ET control reactivity was measured. For both C<sub>4</sub>SH and C<sub>10</sub>SH diluents, the SAM solutions were prepared using the same diluent and linker (SHC<sub>11</sub>SH) ratio that was used for SAM solutions containing a C<sub>8</sub>SH diluent. Freshly cleaned gold wafers were rinsed with triple-distilled water and ethanol, purged with N<sub>2</sub> gas, and immersed in the depositing solution for 48 h.

**5.4. Attachment of the Catalyst to the Electrode.** Gold wafers immersed in the deposition solution were taken out before the experiments and rinsed with ethanol followed by triple-distilled deionized water and then dried with  $N_2$  gas. The wafers were then inserted into a plate material evaluating cell (ALS Japan). Catalysts were dissolved in chloroform. For attachment of FePf (Figure 9) to



Figure 9. Catalysts used in the work with FePf.

the ImdC<sub>11</sub>SH linker, the electrode surface was immersed in a CHCl<sub>3</sub> solution of the catalyst for about 1.5 h. For the SHC<sub>11</sub>SH and OPhC<sub>11</sub>SH linkers, the electrode surfaces were immersed first in Et<sub>3</sub>N for 10 min and then in the FePf solution for 2.5 h (Scheme 1). After the respective times, the surfaces were thoroughly rinsed with chloroform, ethanol, and triple-distilled water before electrochemical catalysis. Thiolate ligation was characterized by CV, X-ray photoelectron spectroscopy,<sup>43</sup> and resonance Raman spectroscopy (section S.1). Phenolate and imidazole ligations were characterized using CV and resonance Raman spectroscopy.

**5.5. CV Experiments.** All CV experiments were done in pH 7 buffer (unless otherwise mentioned) containing 100 mM  $Na_2HPO_4$ .  $2H_2O$  and 100 mM KPF<sub>6</sub> (supporting electrolyte) using a platinum wire as the counter electrode and Ag/AgCl (saturated KCl) as the reference electrode.

5.6. Oxidation of the Substrates. The substrates used here were toluene, benzyl alcohol, benzaldehyde, cinnamaldhyde, and 3methylindole. Phosphate buffer solutions (pH 7) were saturated with these substrates by adding 2 drops of ethanol to the suspension of these organic molecules in water, followed by vigorous shaking of the previously mentioned suspension. The use of ethanol increases the solubility of the organic substrates in aqueous buffer. The mixture was allowed to settle and separated using a separating flask. The aqueous layer was extracted, and electrocatalysis was done as reported before.<sup>43</sup> Electrochemical analyses were performed with this aqueous solution (Figure S.1). Electrolysis was performed at a constant potential of -300 mV [vs Ag/AgCl (saturate KCl)] with this solution for 15 min. The potential dependence of the oxidation reaction was evaluated by performing electrolysis experiments at several potentials between -100 and -500 mV versus Ag/AgCl (saturated KCl) and analyzing the products. Product analysis represents the average of 3-5 individual experiments. The CV data collected after bulk electrolysis (Figure S.1.A) indicate the extent of decay of the catalysts during electrolysis. After the electrochemical experiments, the resultant aqueous electrolytes containing reactants and products were extracted with chloroform (CHCl<sub>3</sub>). The CHCl<sub>3</sub> layer was dried and evaporated, and the product left behind was subjected to GC/MS analysis (Figure S.2). Note that ethanol, used here to make the substrates more soluble in water, acts as a competing substrate in this reaction because acetaldehyde was detected by GC. However, the amount is low because of its higher solubility in water and lesser affinity for the hydrophobic electrode surface. For toluene oxidation with differently ligated FePf, electrolysis was done in this potential

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### Scheme 1. Schematic Representation of the Construction of Bioinspired Electrodes<sup>a</sup>



<sup>a</sup>The catalyst, iron porphyrin, was attached on top of the SAM-coated gold electrode.

range, and in Table 1, the maximum values of TON, TOF, and  $\mu$  were reported for each of the axial ligands. Control experiments without axial ligands were also done to confirm that the axially ligated iron porphyrin is responsible for oxidation of the substrates (section S.5 and Figure S.5).

**5.7. Estimation of TON and TOF.** The amount of oxidized product was determined by GC (Figure S.2). In order to do that, the areas of the peaks of pure samples of different known concentrations were determined and plotted against the known concentrations of the samples. This plot represents the calibration plot for that particular sample (Figure S.6). The amount of the oxidized product, outlined above, was actually quantified by using the respective calibration plot. CV experiments were used to calculate the amount of catalyst on the electrode surface (Table S.2). The ratio of the number of moles of product formed and number of moles of catalyst depicts the TON. TON divided by the reaction time gives the TOF of the overall reaction.

**5.8. Estimation of KIE and KSIE.** To measure KIE for toluene oxidation, toluene- $d_8$  was used as the substrate. The deuterated substrate was dissolved in aqueous buffer, and catalysis was performed as mentioned above with the catalyst attached to the electrode. For KSIE measurement, instead of pH 7 phosphate buffer, pD 7 buffer with the same concentration of electrolyte was used, and the substrates were saturated in this buffer to perform catalytic reactions with the modified electrode. In both cases, the products were quantified by GC/MS, and then the amounts were compared with the products obtained for the substrates stated above.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.9b03767.

Control experiments and additional CV data (PDF)

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### Notes

The authors declare no competing financial interest.

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