Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Heterogeneous Bio-Inspired Peroxide Shunt for Catalytic Oxidation of Organic Molecules

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S.1. Experimental Section:

S.1.1. Materials:

All reagents were of the highest grade commercially available and were used without further purification. Octanethiol (C_8SH), Potassium hexafluorophosphate (KPF_6), benzylalcohol, benzaldehyde, styrene, styrene oxide, cinnamaldehyde and cinnamic acid were purchased from Sigma-Aldrich. Disodium hydrogen phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$), cyclohexane, cyclohexanol and cyclohexanone were purchased from Merck. Triethylamine (Et_3N), toluene, acetophenone and 1-phenyl ethanol were purchased from Spectrochem India Ltd. Au wafers were purchased from Platypus Technologies (1000 Å of Au on 50 Å of Ti adhesion layer on top of a Si (III) surface). Toluene-d8 was purchased from Sigma-Aldrich.

S.1.2. Instrumentation:

All electrochemical experiments were done using CH Instruments (model CHI700E Electrochemical Analyzer). Bi-potentiostat, reference electrodes (Ag/AgCl (satd. KCl)), Teflon plate material evaluating cell (ALS Japan) were purchased from CH Instruments. All qualitative and quantitative experiments were done using Gas Chromatography Mass Spectroscopy (GC-MS) technic. For this Agilent 7890B GC system with 5977A MS detector was used.

S.1.3. Experimental Procedure: Construction of the Electrode:

Cleaning of the Au wafers was performed electrochemically by sweeping several times between 1.7 V to -300 mV vs Ag/AgCl (satd. KCl) in 0.5 M H_2SO_4. SAM solutions were prepared using the concentration ratio of the linkers ($SHC_{11}SH$, $OPhC_{11}SH$ and $ImdC_{11}SH$) and diluent as shown in Table S.1. All these linkers were prepared using the reported procedure. In each cases the diluent used was C_8SH. Freshly cleaned Au wafers were rinsed with triple distilled water, ethanol, purged with N_2 gas, and immersed in the depositing solution for 48 h.

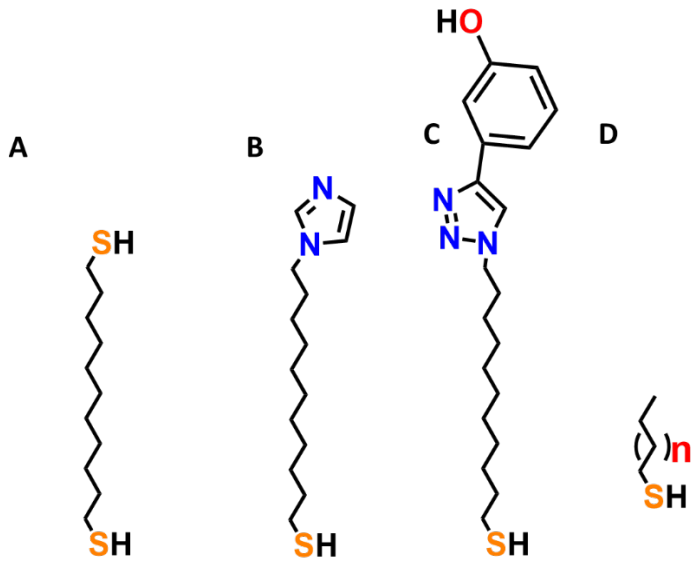


Figure S.1: A, B, C are the linker molecules; $SHC_{11}SH$, $ImdC_{11}SH$ and $OPhC_{11}SH$ respectively. D represents the diluent molecule C_8SH where n=5.

Table S.1: SAM solution preparation ratio		
Linkers	Molefraction	Total Concentration (mM)
$ImdC_{11}SH$	0.2	0.4
$OPhC_{11}SH$	0.2	0.4
$SHC_{11}SH$	0.2	3

S.1.4. Attachment of the Catalyst to the Electrode:

Au wafers immersed in the deposition solution were taken out before 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 the attachment of the Fe "picket-fence" (FePf) to the $ImdC_{11}SH$ linker the electrode surface was immersed in $CHCl_3$ solution of the catalysts for about 1.5 h. For the $SHC_{11}SH$ and $OPhC_{11}SH$ linkers the electrode surfaces were immersed first in Et_3N for 10 min and then in the FePf solutions for 2.5 h (Scheme S.1). To attach and α_4-Tetra-2-(4-carboxymethyl-1,2,3-triazolyl)-phenylporphyrinato Iron (FeEs4) to the $SHC_{11}SH$ same procedure was followed as mentioned earlier with FePf. After the respective times the surfaces were thoroughly rinsed with chloroform, ethanol, and triple distilled water before the catalysis. Thiolate ligation was characterised by Cyclic voltammograms, X-ray photoelectron spectroscopy and Resonance Raman spectroscopy.[1,2] Phenolate and imidazole ligations were characterised using cyclic voltammetry and Resonance Raman spectroscopy. [2,3]

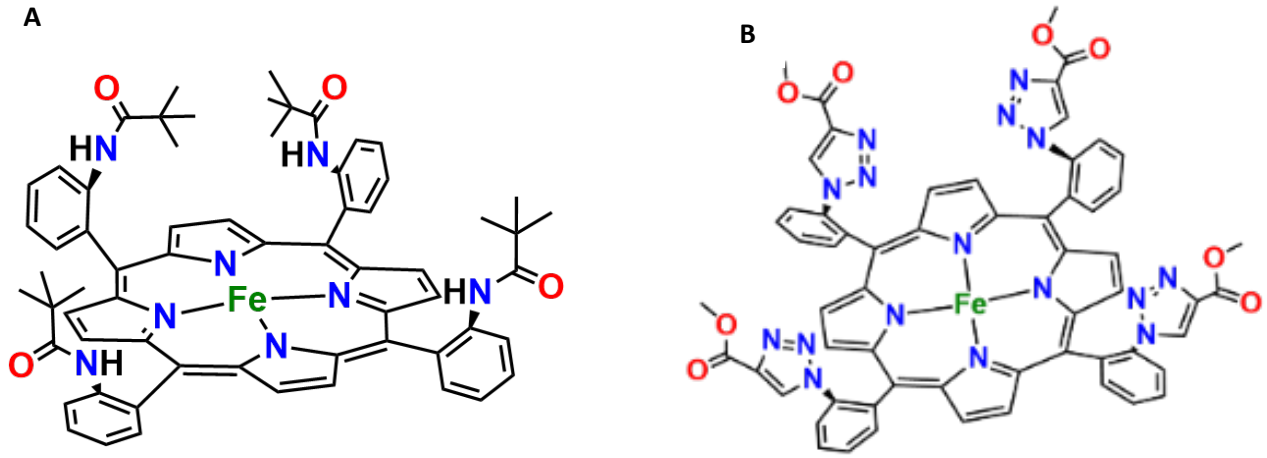
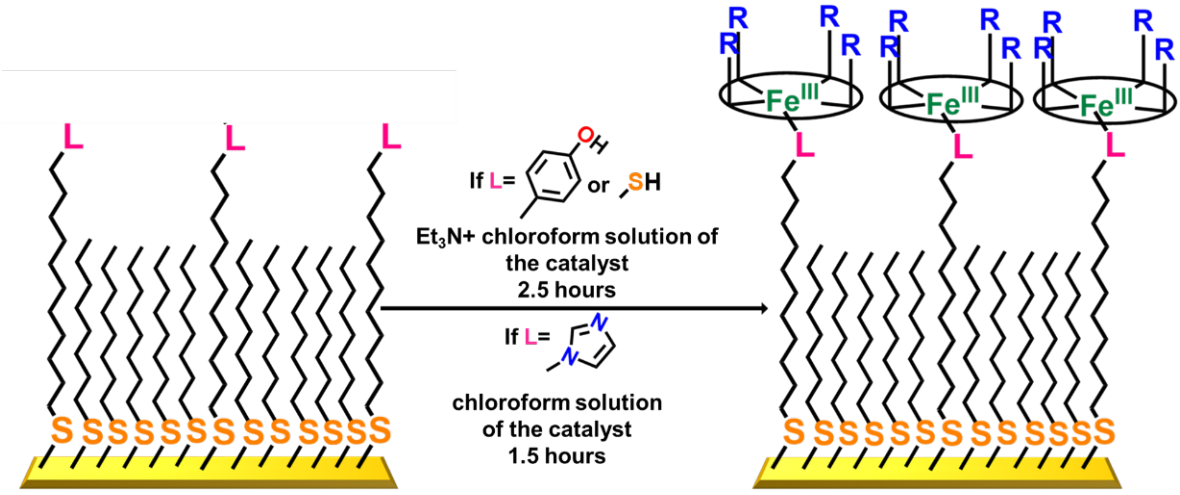


Figure S.2: Catalysts used in the work A) Fe "picket-fence" (FePf) and B) α_4-Tetra-2-(4-carboxymethyl-1,2,3-triazolyl)-phenylporphyrinato Iron (FeEs4).



Scheme S.1: Schematic representation of the construction of bio-inspired electrodes. Catalyst, iron-porphyrin, was attached on top of the SAM coated gold electrode.

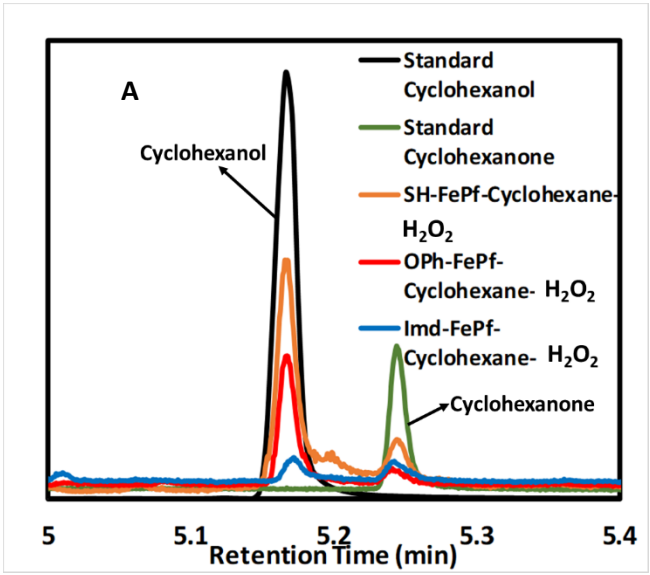
Table S.2. Characterisation of iron picket-fence porphyrin attached to the SAM coated gold electrode

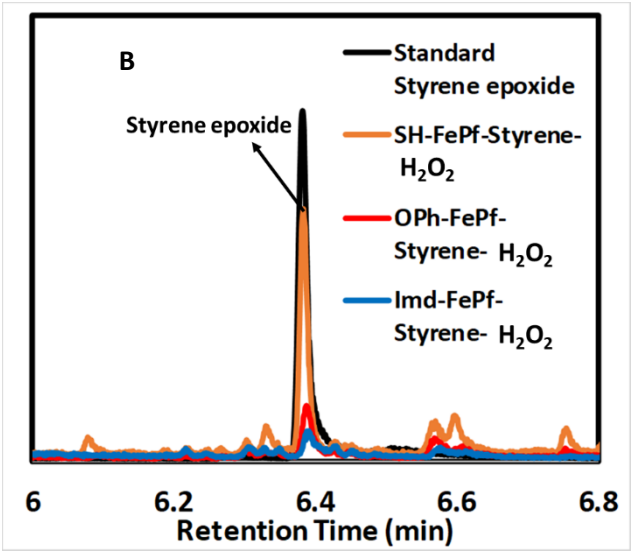
Electrode	Characterisation technics			Ref
	Cyclic voltammetry ($E_{1/2}$ vs Ag/AgCl (satd.KCl))	Resonance Raman Spectroscopy	X-ray Photoelectron Spectroscopy	
Thiolate-FePf	-250mV	ν_4=1362 cm^{-1} ν_3=1438 cm^{-1} ν_2=1554 cm^{-1} ν_8=392 cm^{-1} ν_{Fe-S}= 341 cm^{-1}	Fe $3p_{3/2}$= 56.8eV S 2p(1/2)=163.4 eV S 2p(3/2)=164.6 eV	1, 2
Phenolate-FePf	-202mV	ν_4=1362 cm^{-1} ν_3=1441 cm^{-1} ν_2=1555 cm^{-1} ν_{Fe-OPh}= 590 cm^{-1} ν_{C-O} = 1296 cm^{-1}	N.D	3
Imidazole-FePf	-217mV	ν_4=1364 cm^{-1} ν_3=1437 cm^{-1} ν_2=1554 cm^{-1} ν_8=389 cm^{-1}	N.D	2

S.1.5. Oxidation of the Substrate:

Substrates used here were cyclohexane, styrene, toluene, ethylbenzene and cinnamaldhyde. Phosphate buffer solutions (pH7) 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. Use of ethanol increases the solubility of the organic substrates into aqueous buffer. The mixture was allowed to settle and separated using a separating flask. The aqueous layer was extracted and 1ml of the extracted solution was taken onto the electrode surface and 30ul of 10M H_2O_2 was added to the solution. The resultant aqueous solution containing reactants and products were kept for 20 minutes and then extracted with Chloroform ($CHCl_3$). The $CHCl_3$ layer was dried, evaporated, and the product left behind was subjected to GC-MS analysis (Figure S. 3). Note that, ethanol, used here to make the substrates more soluble in water, acts as competing substrate in this reaction as acetaldehyde was detected in GC. However, the amount is low owing to its higher solubility in water and lesser affinity for the hydrophobic electrode surface. Concentration of H_2O_2 was varied to understand the role of H_2O_2 in overoxidation.

S.2. GC-MS traces:





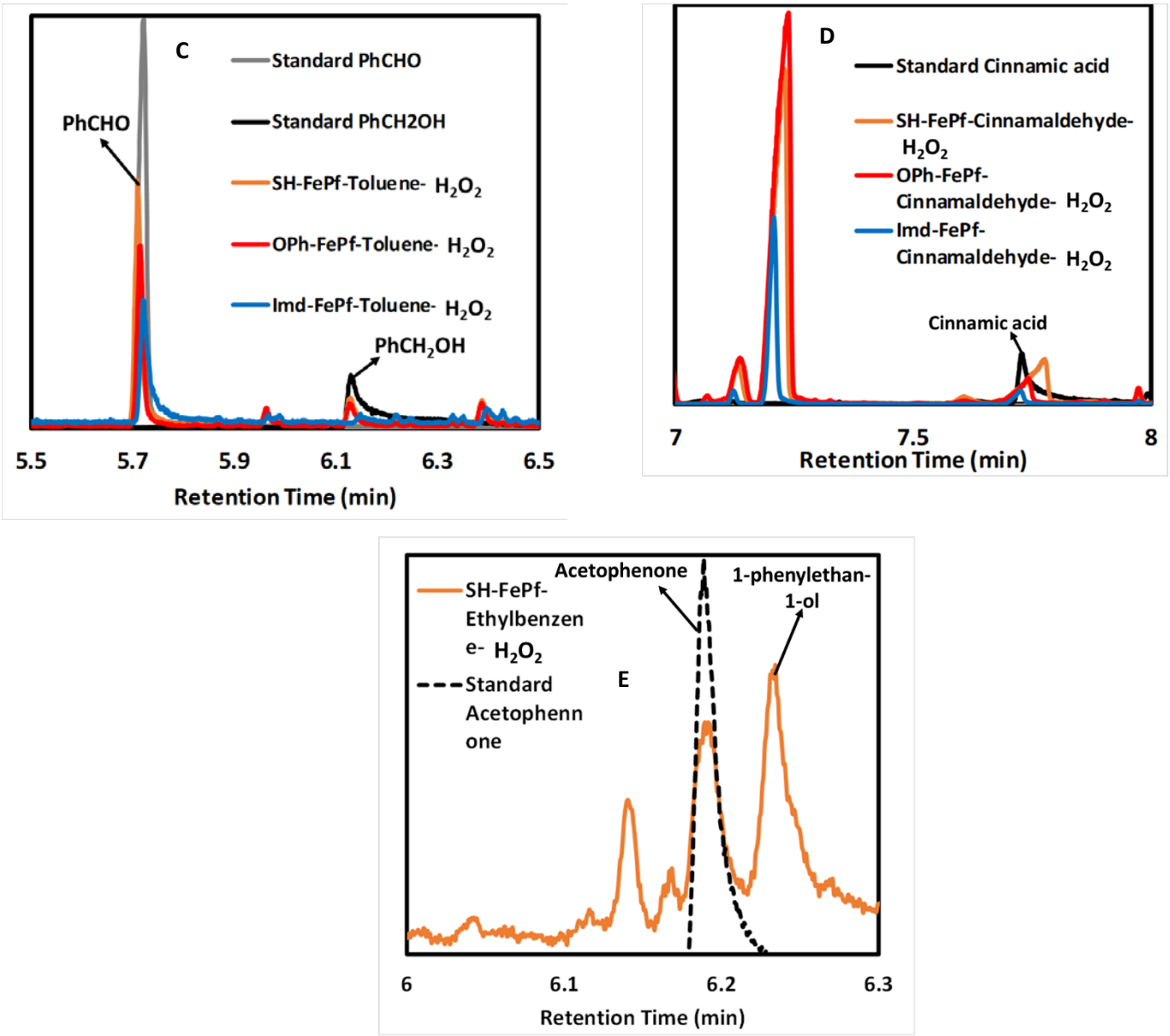


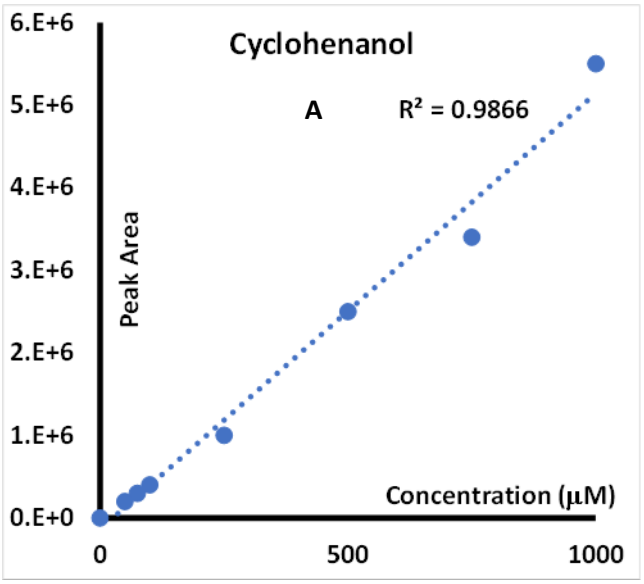
Figure S.3: GC traces of A. Cyclohexane, B. Styrene, C. Toluene, D. Cinnamaldehyde and E. Ethylbenzene oxidation

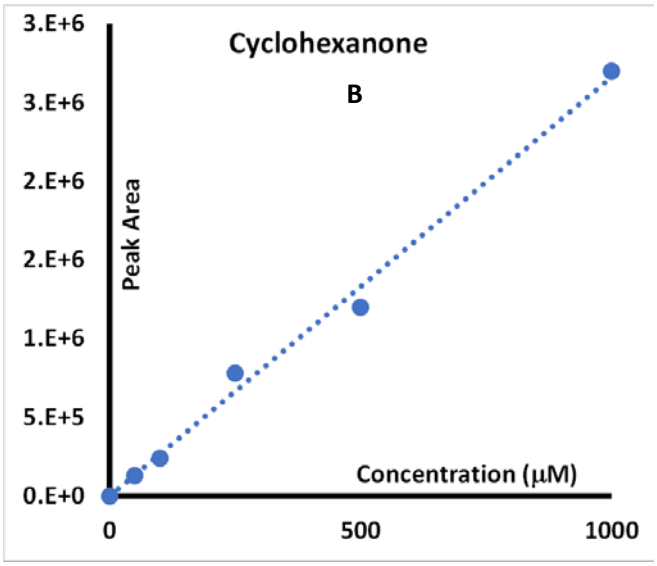
S.3. Estimation of the Turnover Number (TON) and Turnover Frequency (TOF):

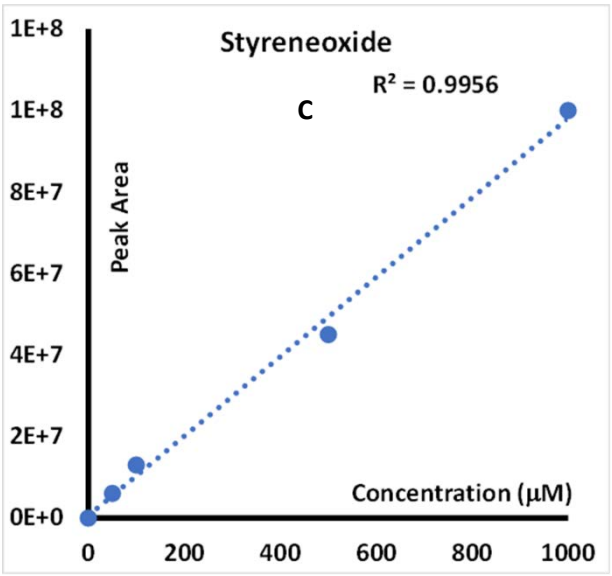
The amount of oxidized product was determined by GC. In order to do that area of the peaks of pure samples of different known concentrations were determined and plotted against the concentration of the samples. This plot represents the calibration plot for that particular. The amount of the oxidized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

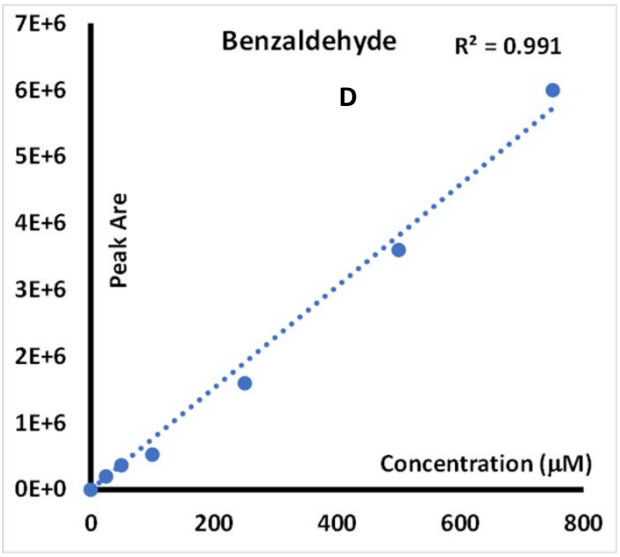
Table S.2: $E_{1/2}$ and amount of the catalyst with different axial ligands on the SAM modified Gold electrode			
Catalyst	**Axial ligand**	**$E_{1/2}$ (mV vs Ag/AgCl (satd. KCl))**	**Surface Coverage (moles/cm^2)**
FePf	RS^-	-250	$5.23x10^{-13}$
FePf	PhO^-	-202	$5.22x10^{-13}$
FePf	Imd	-217	$6.71x10^{-13}$

S.4. Calibration curve:









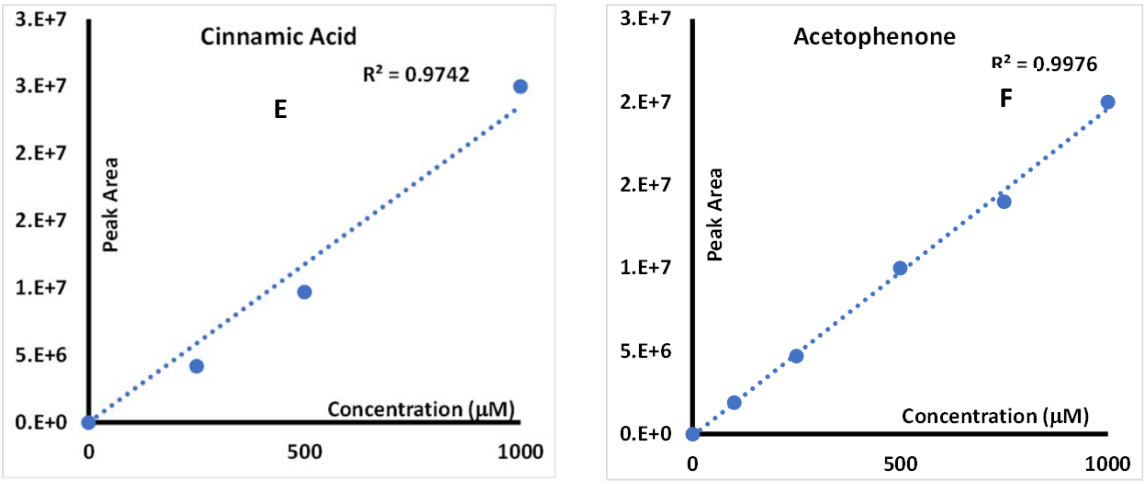


Figure S.4: Calibration curves of A. Cyclohexanol, B. Cyclohexanone, C. Styreneoxide, D. benzaldehyde, E. cinnamic acid and F. acetophenone.

S.5. Estimation of the Kinetic Isotope Effect (KIE) and Solvent Kinetic Isotope Effect (SKIE)

To measure the KIE for Toluene oxidation, Toluene-d_8 was used as the substrate. The deuterated substrate was dissolved in buffer and catalysis was performed as mentioned above with the catalyst attached electrode. The products were quantified by GCMS and then the amount was compared with the product obtained for substrates stated above.

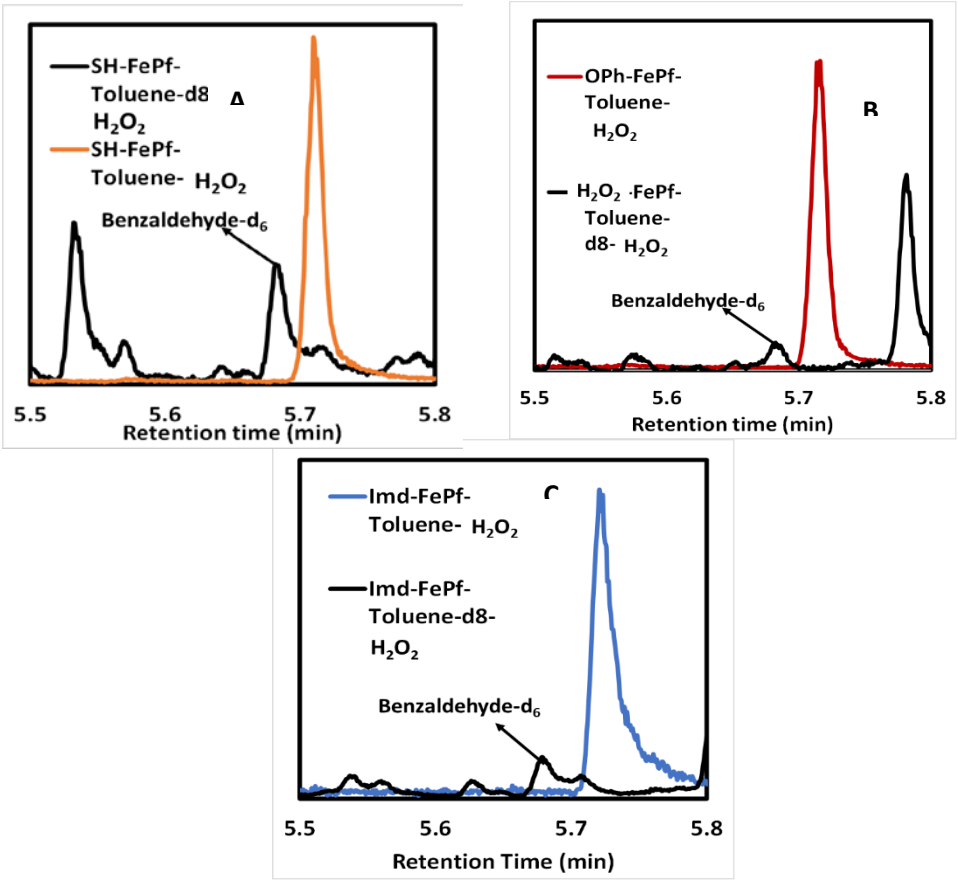


Figure S.5: Kinetic Isotope effect during the Toluene oxidation with A) thiolate, B) Phenolate and C) Imidazole ligated FePf with H_2O_2. Peak at 6.78 min is the peak of benzaldehyde-d_6.

S.6. Control Experiments:

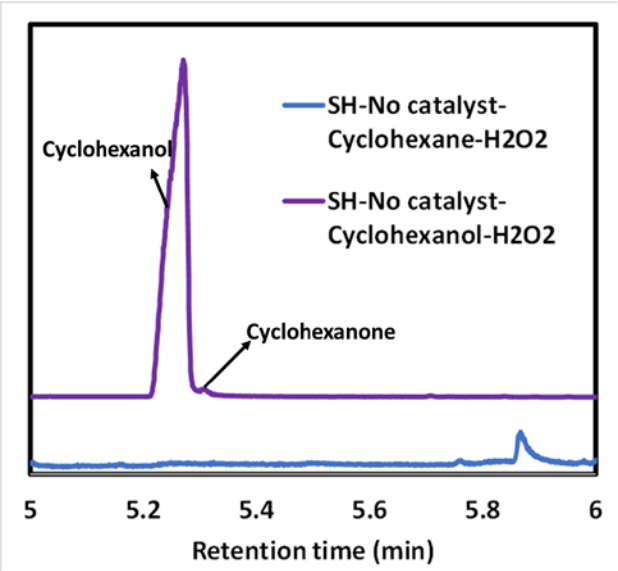


Figure S.6: GC traces of A) cyclohexane and B) Cyclohexanol oxidation without any catalyst using H_2O_2 as the oxidizing agent.

S.7. Structure of FehPf:

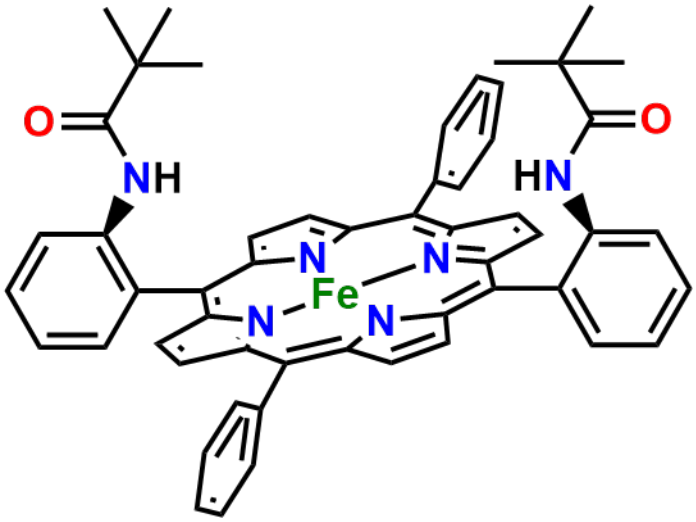


Figure S.7: Figure of FehPf

S.8. Surface Enhanced Resonance Raman Spectra of FePf and $FeEs_4$ with H_2O_2:

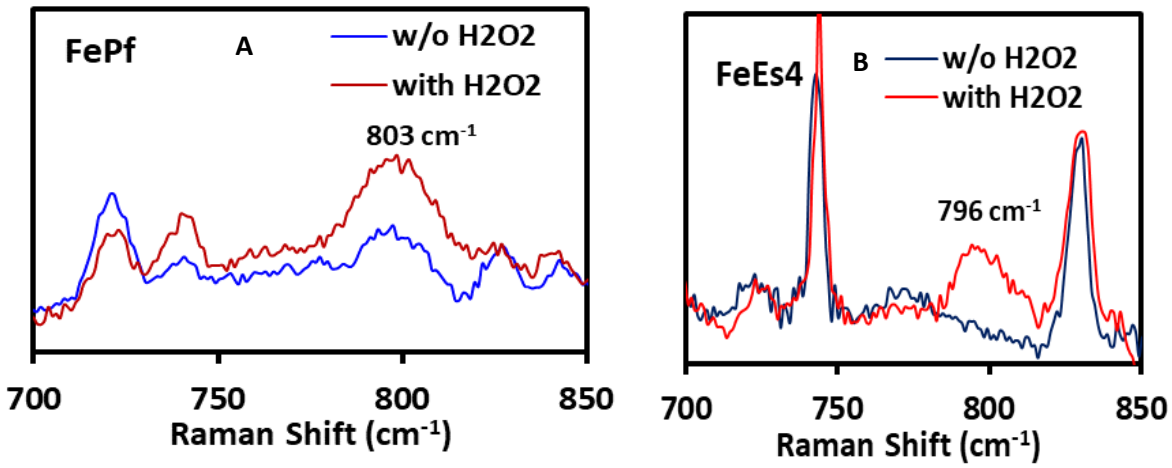


Figure S.8: SERRS data of A) FePf and B) FeEs4 in presence of H_2O_2. The peaks at803 cm^{-1} and 796 cm^{-1} depict the peaks of compound I for FePf and $FeEs_4$ respectively.

S.9. Effect of Distal Pocket:

The enzymatic active site of CytP450 has a hydrophobic pocket consisting of Leu 244, Val 247, Val 295 and Phe87 amino acid residues[4,5]. This hydrophobic pocket is responsible for the binding of hydrophobic substrates like camphor. Apart from FePf, bearing hydrophobic secondary structure, oxidation of the same substrates with H_2O_2 was also tested with α_4-Tetra-2-(4-carboxymethyl-1,2,3-triazolyl)-phenylporphyrinato Iron ($FeEs_4$) [6](Figure 2C) which bears a hydrophilic cavity. This complex stabilizes water molecule in its distal site via H-bonding to the triazoles which has a remarkable effect on its O_2 binding and CO_2 reduction activity. The results show a significant decrease in both the TON and TOF of the oxidation hydrophobic substrates like cyclohexanol and styrene. The yields of hydrophilic substrates like aldehydes, on the other hand, are similar suggesting that hydrophobic pocket generally facilitates the access of the hydrophobic organic substrates to the reactive intermediate.

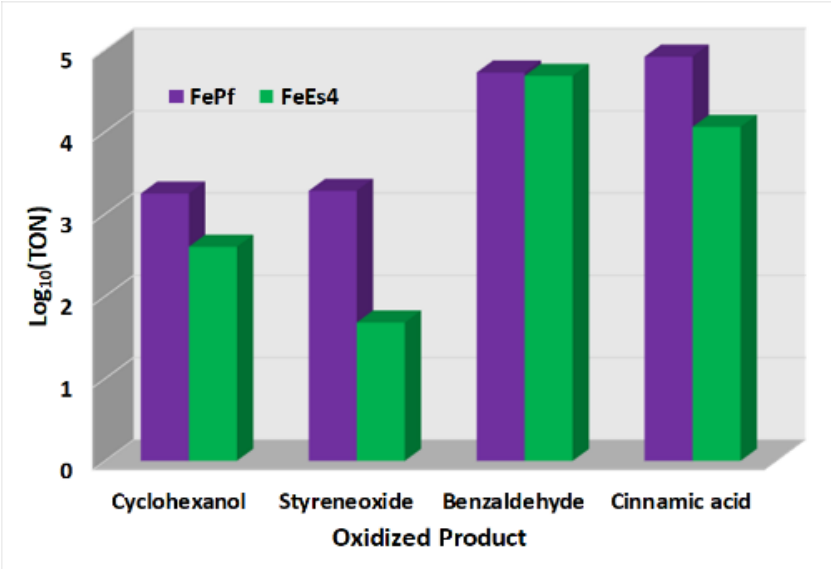


Figure S.9: Comparison between the Log_{10}(TON) of different oxidized product $FeEs_4$ (green) and FePf (purple).

References:

(1) Mukherjee, M.; Dey, A. Electron Transfer Control of Reductase versus Monooxygenase: Catalytic C–H Bond Hydroxylation and Alkene Epoxidation by Molecular Oxygen. *ACS Cent. Sci.* **2019**.

(2) Sengupta, K.; Chatterjee, S.; Samanta, S.; Bandyopadhyay, S.; Dey, A. Resonance Raman and Electrocatalytic Behavior of Thiolate and Imidazole Bound Iron Porphyrin Complexes on Self Assembled Monolayers: Functional Modeling of Cytochrome P450. *Inorg. Chem.* **2013**, *52*, 2000-2014.

(3) Sengupta, K.; Chatterjee, S.; Dey, A. Catalytic H2O2 Disproportionation and Electrocatalytic O2 Reduction by a Functional Mimic of Heme Catalase: Direct Observation of Compound 0 and Compound I in Situ. *ACS Catal.* **2016**, *6*, 1382-1388.

(4) Colthart, A. M.; Tietz, D. R.; Ni, Y.; Friedman, J. L.; Dang, M.; Pochapsky, T. C. Detection of substrate-dependent conformational changes in the P450 fold by nuclear magnetic resonance. *Scientific Reports* **2016**, *6*, 22035.

(5) Williams, P. A.; Cosme, J.; Ward, A.; Angove, H. C.; Matak Vinković, D.; Jhoti, H. Crystal structure of human cytochrome P450 2C9 with bound warfarin. *Nature* **2003**, *424*, 464-468.

(6) Mittra, K.; Chatterjee, S.; Samanta, S.; Sengupta, K.; Bhattacharjee, H.; Dey, A. A hydrogen bond scaffold supported synthetic heme FeIII–O2− adduct. *ChemComm* **2012**, *48*, 10535-10537.