

## Supporting Information

# Electron Transfer Control of Reductase vs Monooxygenase: Catalytic C-H Bond Hydroxylation and Alkene Epoxidation by Molecular Oxygen

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**S.1. Oxygen Reduction Reaction (ORR) current of Iron Picketfence (FePf) attached SAM coated gold electrode:**

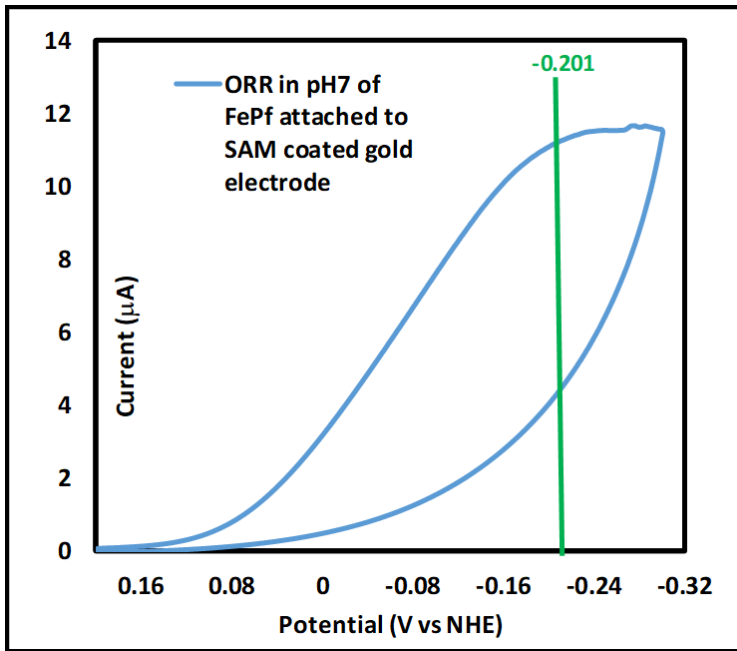
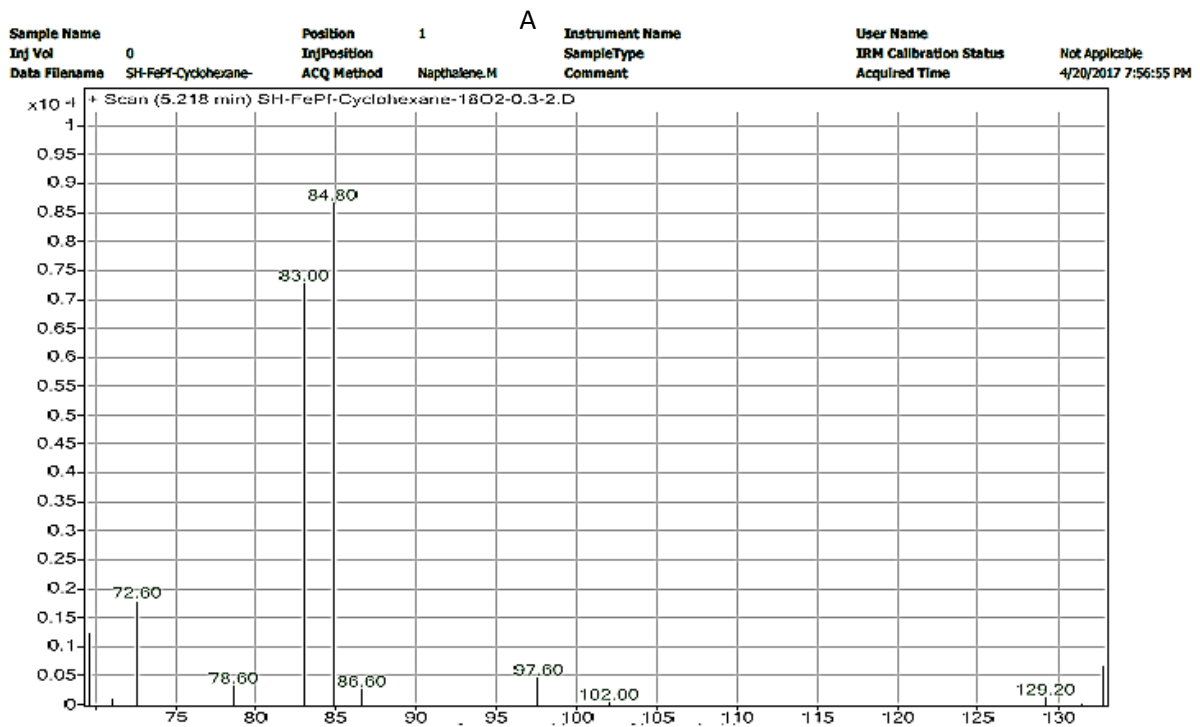


Figure S.1: Oxygen reduction reaction current of the thiolate ligated FePf on top of the SAM coated gold electrode.

**S.2. Amount of <sup>18</sup>O inclusion:**



B

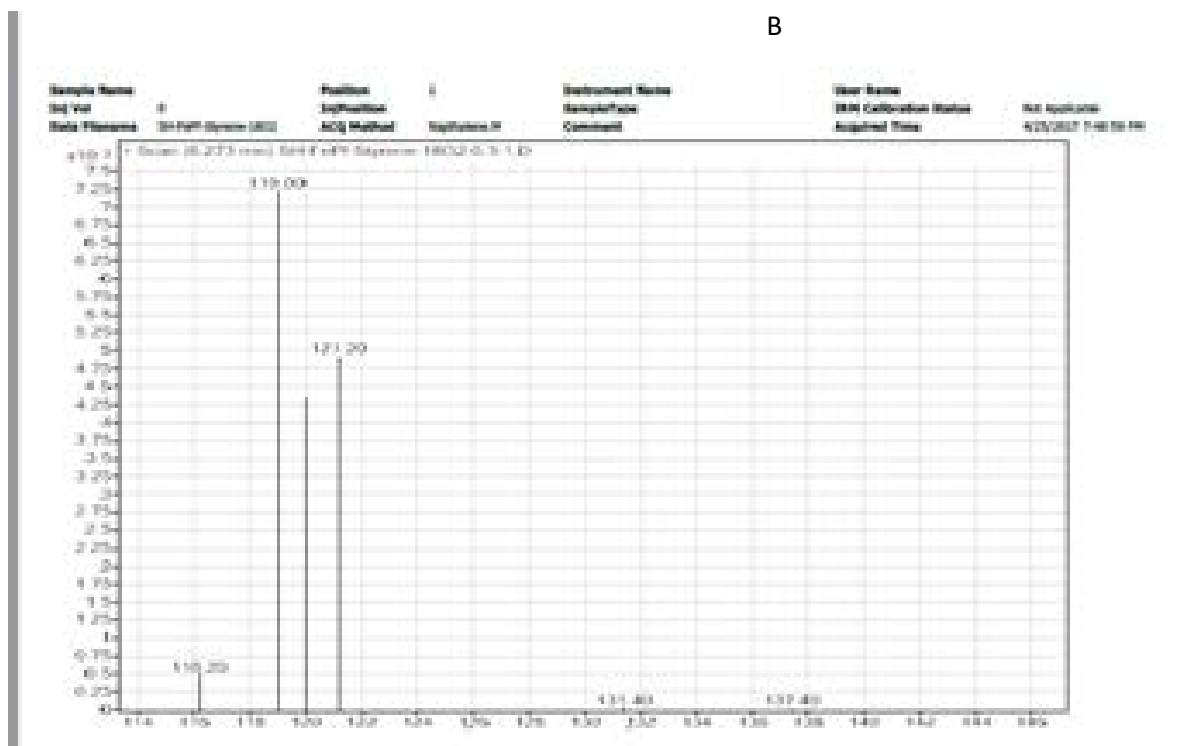


Figure S.2: Mass spectrum of the oxidized product of A) Cyclohexane and B) Styrene when the catalysis was performed in presence of  $^{18}\text{O}_2$  instead of  $\text{O}_2$ .

### S.3. Comparison between the Catalytic Activity of iron-picketfence porphyrin (FePf) and iron-half picketfence (FehPf) porphyrin:

Comparison between the catalytic activity of FePf and Fehpf was done by performing oxidation of cyclohexane, adamantane, styrene and ethyl benzene with both the porphyrins. The observable amount of cyclohexanone and adamantanone during the oxidation of cyclohexane and adamantane respectively with Fehpf supports hypothesis of the steric demand of the picketfence architecture. Moreover, the higher amount of styrene oxide and benzaldehyde (side product due to over oxidation) in styrene oxidation emphasizes on better approach of the substrates in to the active site cavity. With ethyl benzene there is no trace of primary aliphatic C-H hydroxylation and only benzylic oxidation is observed as is known to be the case for most systems reported till date.

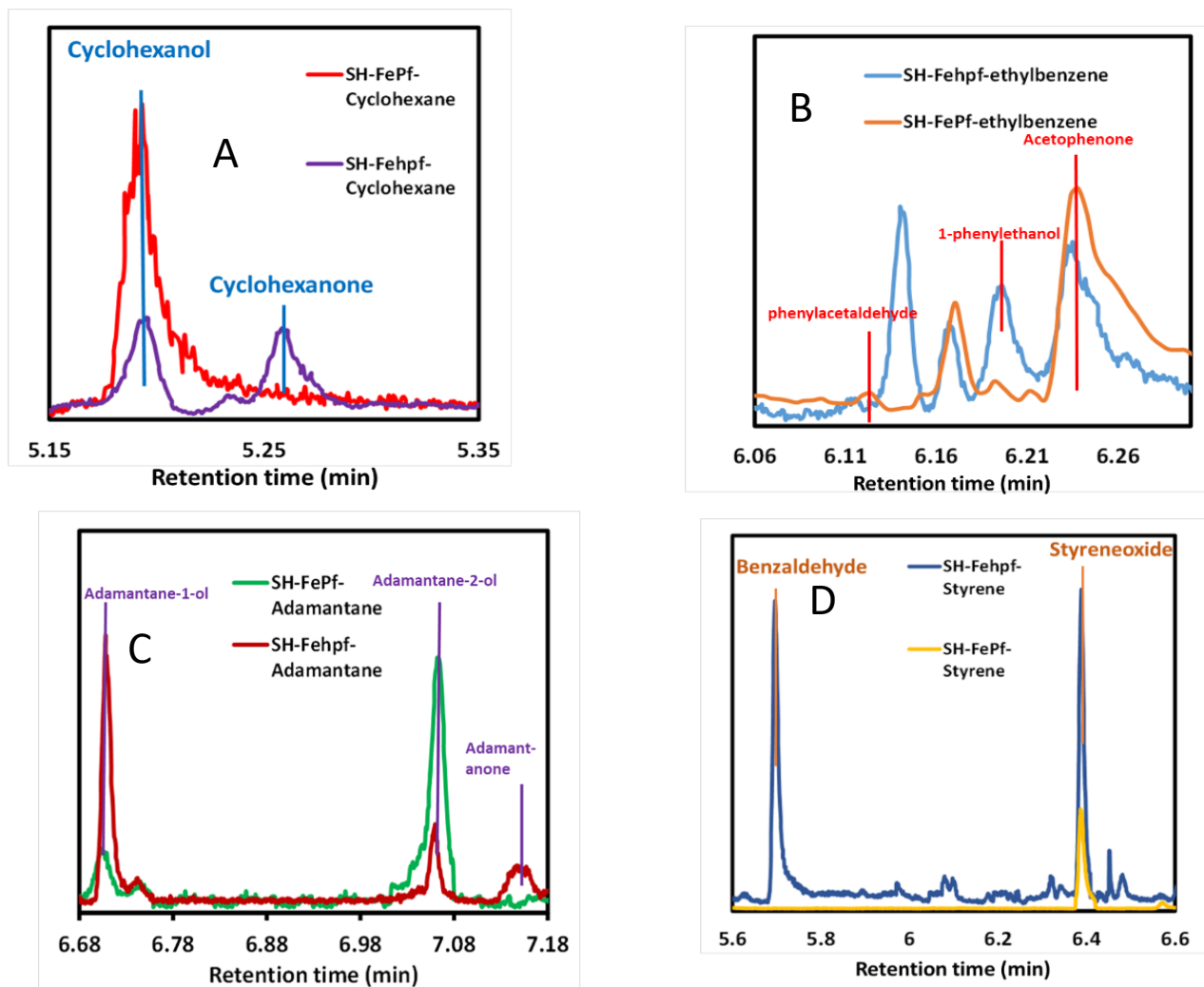


Figure S.3: Comparison between the Catalytic Activity of FePf and Fehpf during A. Cyclohexane, B. Ethylbenzene, C. Adamantane and D. Styrene oxidation

#### S.4. Faradic Yield (FY) at different applied potential:

As the potential of the electrode made more negative, FY and Turnover frequency for Toluene oxidation decreases dramatically. This is again consistent with the proposed in-situ generated high valent oxo species as the catalyst. At low applied potentials, the rate of electron transfer from the electrode increases, resulting in rapid reduction of this species.

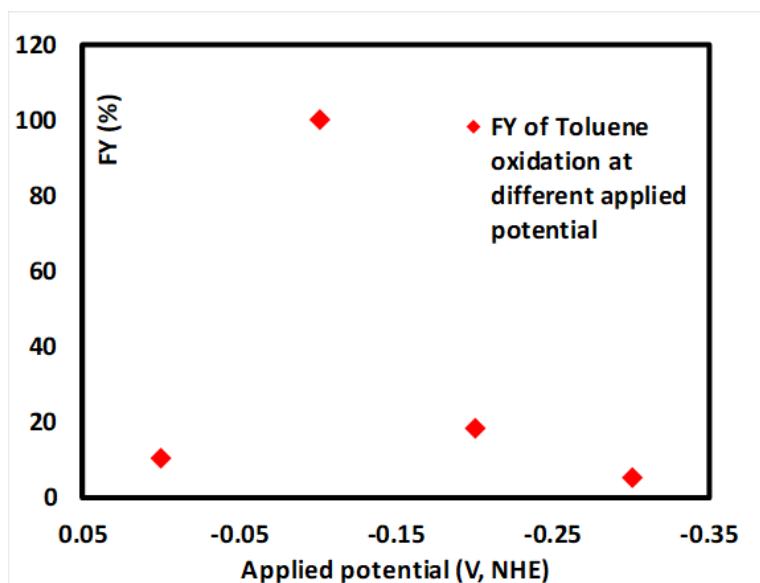


Figure S.4.: FY of Toluene oxidation at different applied potential.

### S.5. Characterisation of iron picketfence porphyrin attached to the SAM coated gold electrode:

#### S.5.1. Background current of SAM coated gold electrode vs Oxygen Reduction Reaction current of Iron Picketfence (FePf) attached electrode:

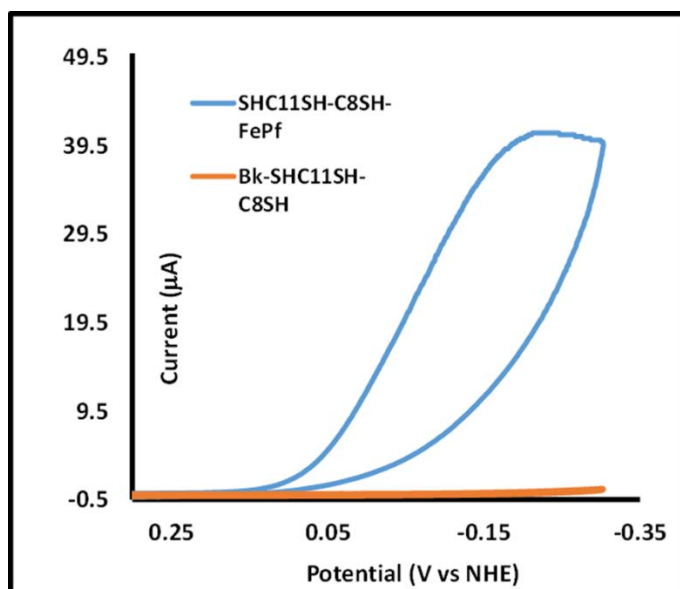


Figure S.5.1: Overlay of background current of SAM coated gold electrode (without catalyst, orange) and Oxygen reduction reaction current of the same electrode after the catalyst (FePf, blue) attachment

### S.5.2 X-ray Photoelectron Spectroscopy:

X-ray photoelectron spectroscopic (XPS) data also indicates the presence of Fe, C, N, S and O on a FePf bound protein bearing surface (Fig. S2). The presence of  $3p_{3/2}$  binding energy peak at 56.8 eV indicate the presence of Fe in the porphyrin, present on the surface.

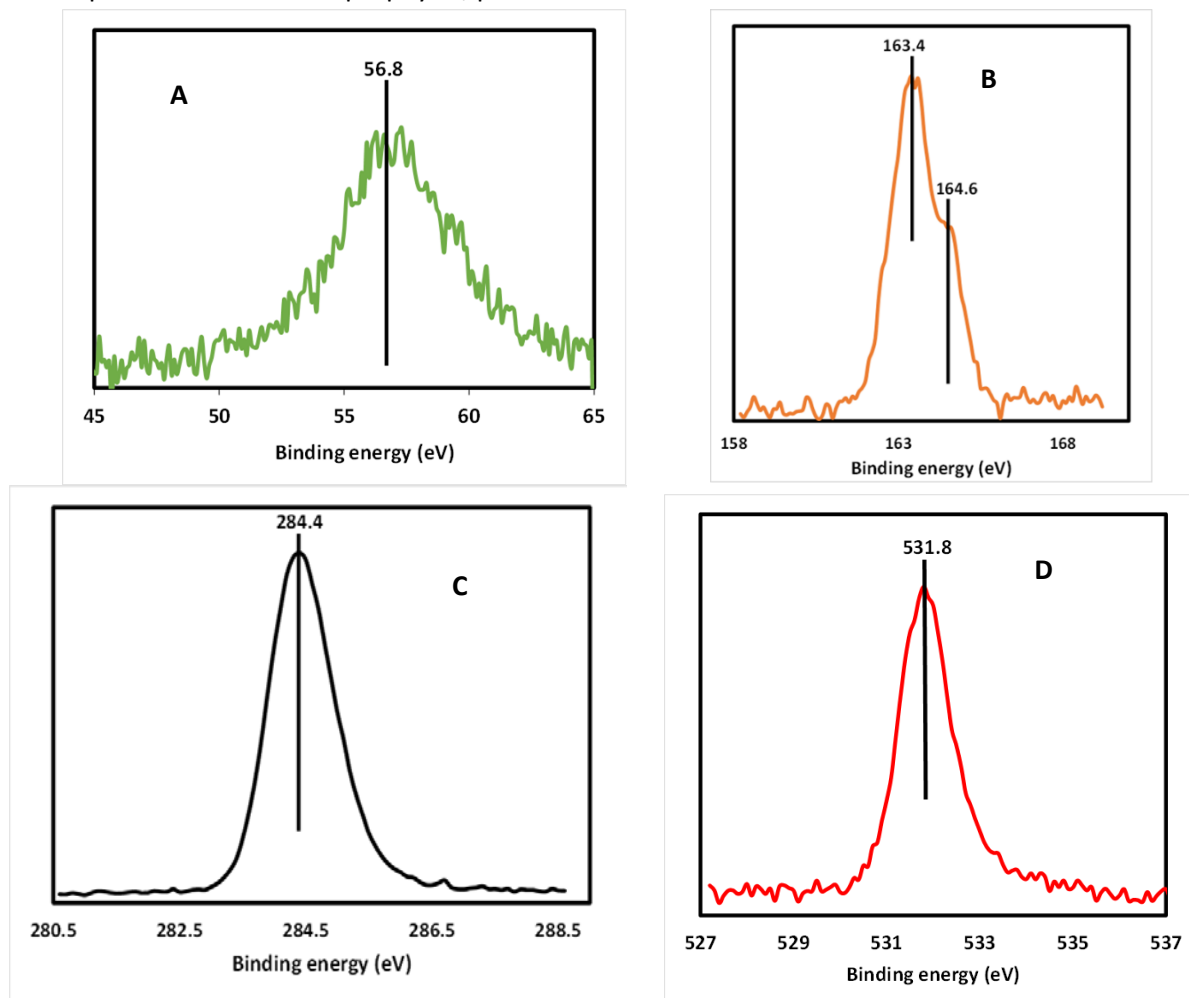
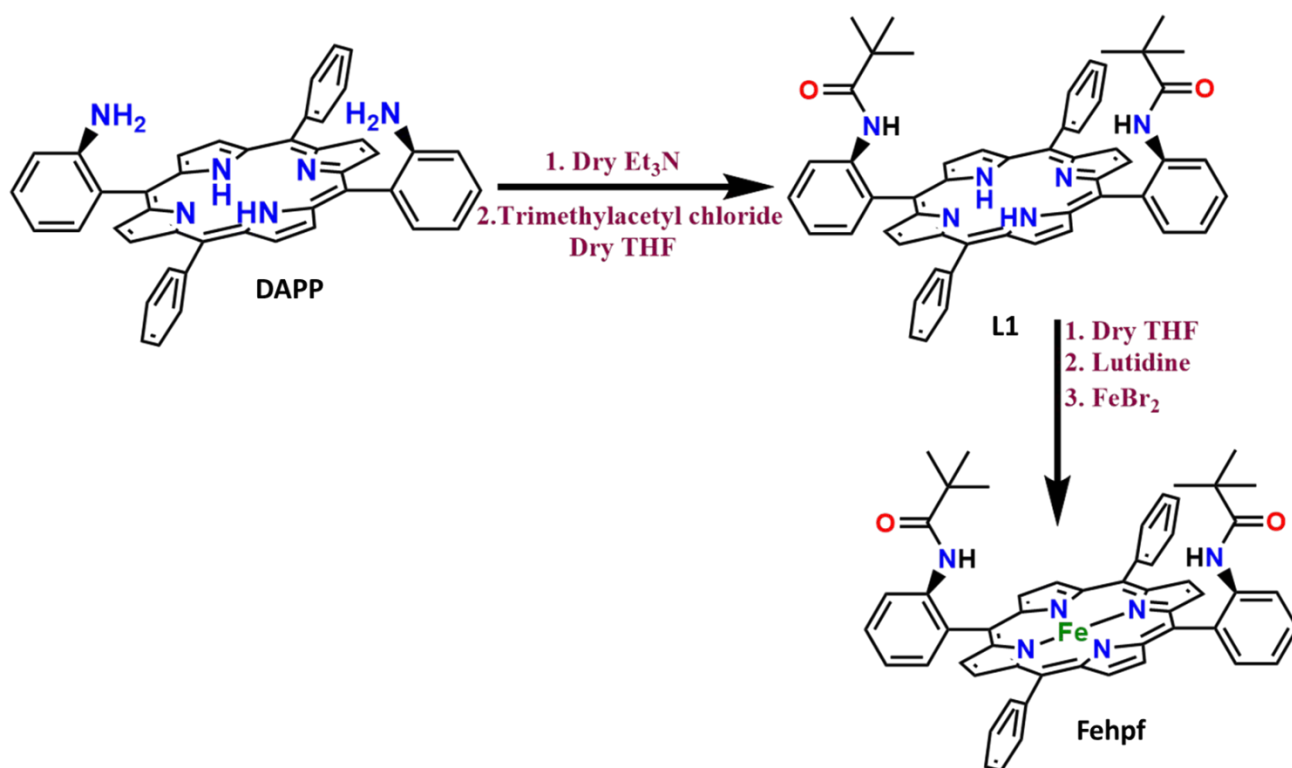


Figure S.5.2: High-resolution X-ray Photoelectron spectra for iron picketfence (FePf) on the SAM covered Au surface. (A) Fe  $3p_{3/2}$  (green) (B) S  $2P_{1/2}$  and  $2P_{3/2}$  (orange) (C) C  $1s_{1/2}$  (black) and (D) O  $1s_{1/2}$  (red).

### S.6. Synthesis of the Catalysts:

In order to prepare iron half picketfence porphyrin (Fehpf), 5,15-bis(ortho-aminophenyl)-10,20-bis(phenyl)porphyrin (DAPP)<sup>1</sup> was used as the precursor complex and synthesis was done as mentioned in scheme I. To prepare the ligand (L1) 40 mg of DAPP was dissolved in 20 ml of Dry THF in a round bottom flask. In it 4 eq of dry triethyl amine ( $\text{Et}_3\text{N}$ ) and 4 eq of trimethylacetyl chloride were added and this whole mixture was stirred overnight under Argon Atmosphere. Ligand was characterised using Mass spectrometry and NMR spectroscopy.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  -2.64 (s, 2H), 0.18

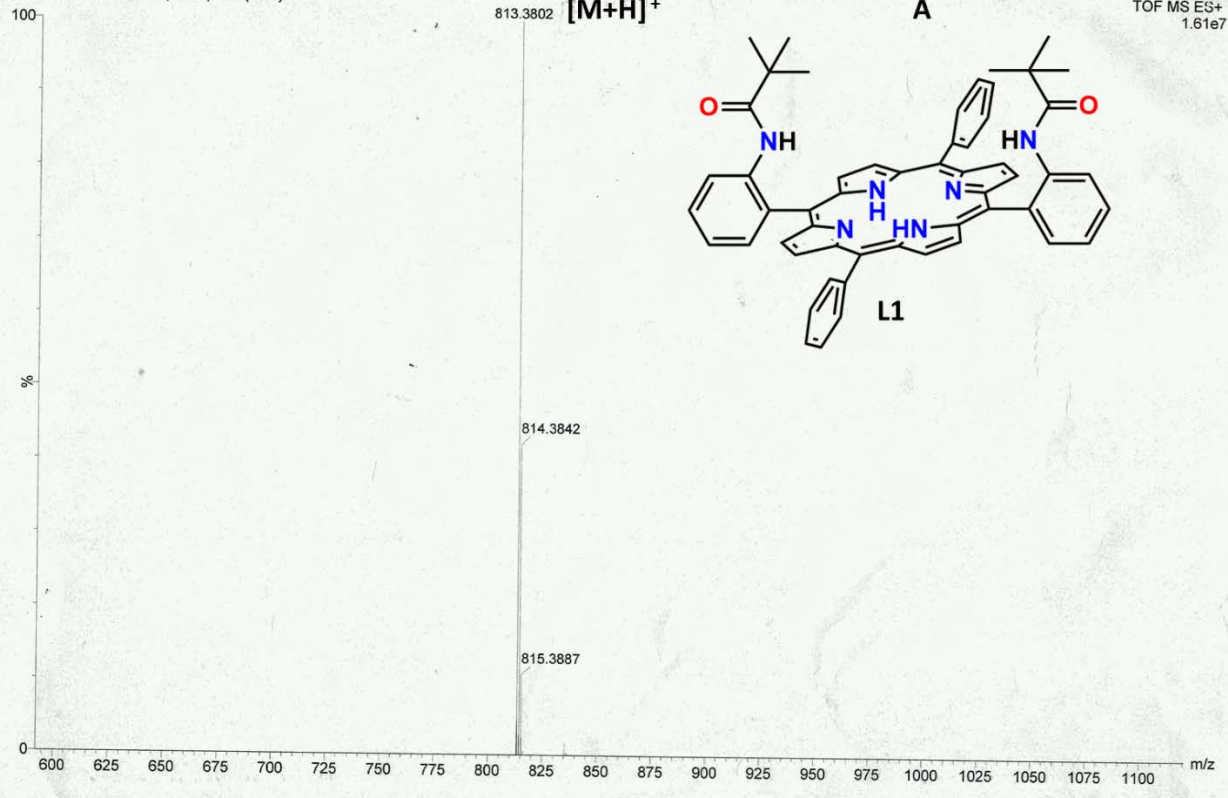
(s, 6H), 0.272 (s, 12H), 7.505-8.761 (m, 18H), 7.408 (s, 2H), 8.822-8.892 (8H). ESI-MS - (+ve ion mode, acetonitrile)  $m/z = 812.8112$ . Then the Ligand (L1) was metallated using 2 equivalent of Lutidine as base and 1.2 equivalent of  $\text{FeBr}_2$  in inert atmosphere.



Scheme S1: Schematic representation of the formation of the Fe-half picketfence.



ICAN-HALF-PICKET 7 (0.135) Cm (2:10)



AN-FEPICKET-GB

AN-FEPICKET 19 (0.187) Sm (Mn, 2x3.00); Cm (3:64)

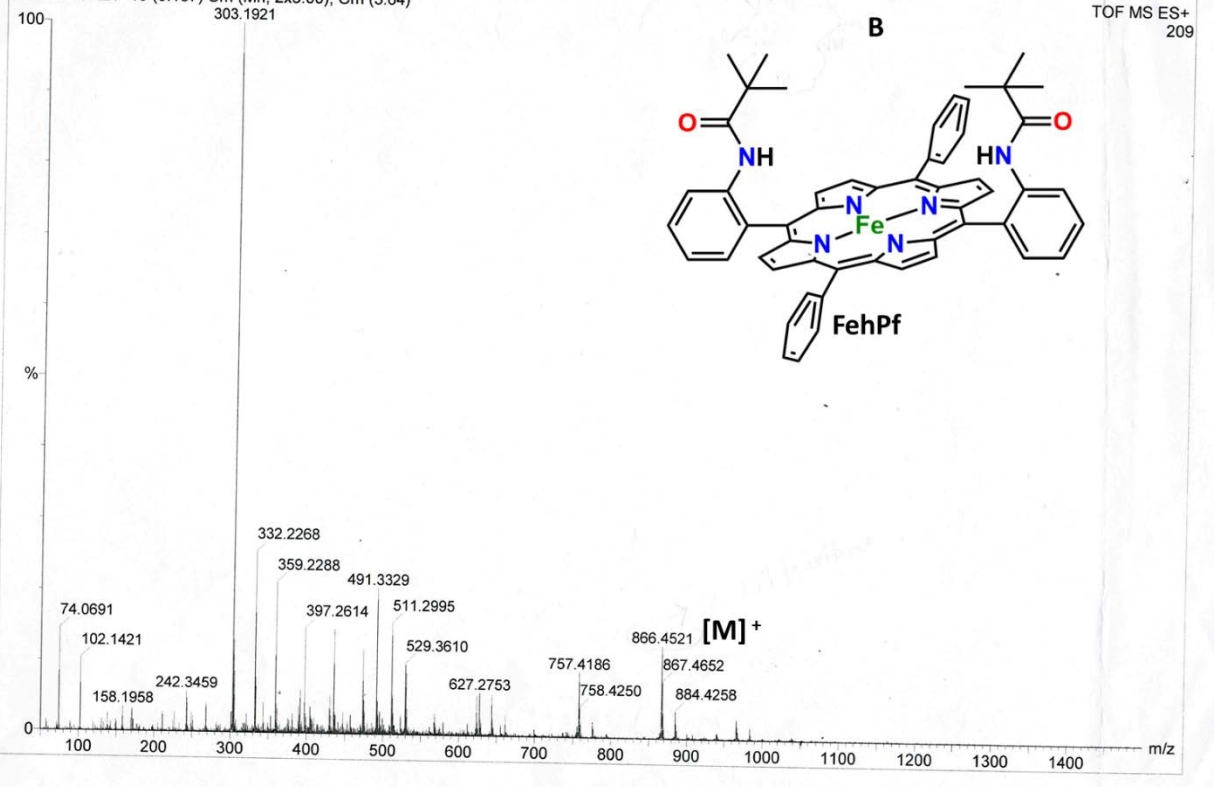


Figure S.6:1: Mass spectra of A. ligand L1 and B. the metallated porphyrin Fehpf. The calculated mass of the ligand L1 is 812.38 mass units where the experimental mass with one proton is 813.3802 mass unit. Similarly, the calculated mass of the B is 866.30 mass units where the experimental mass is 866.4521 mass unit

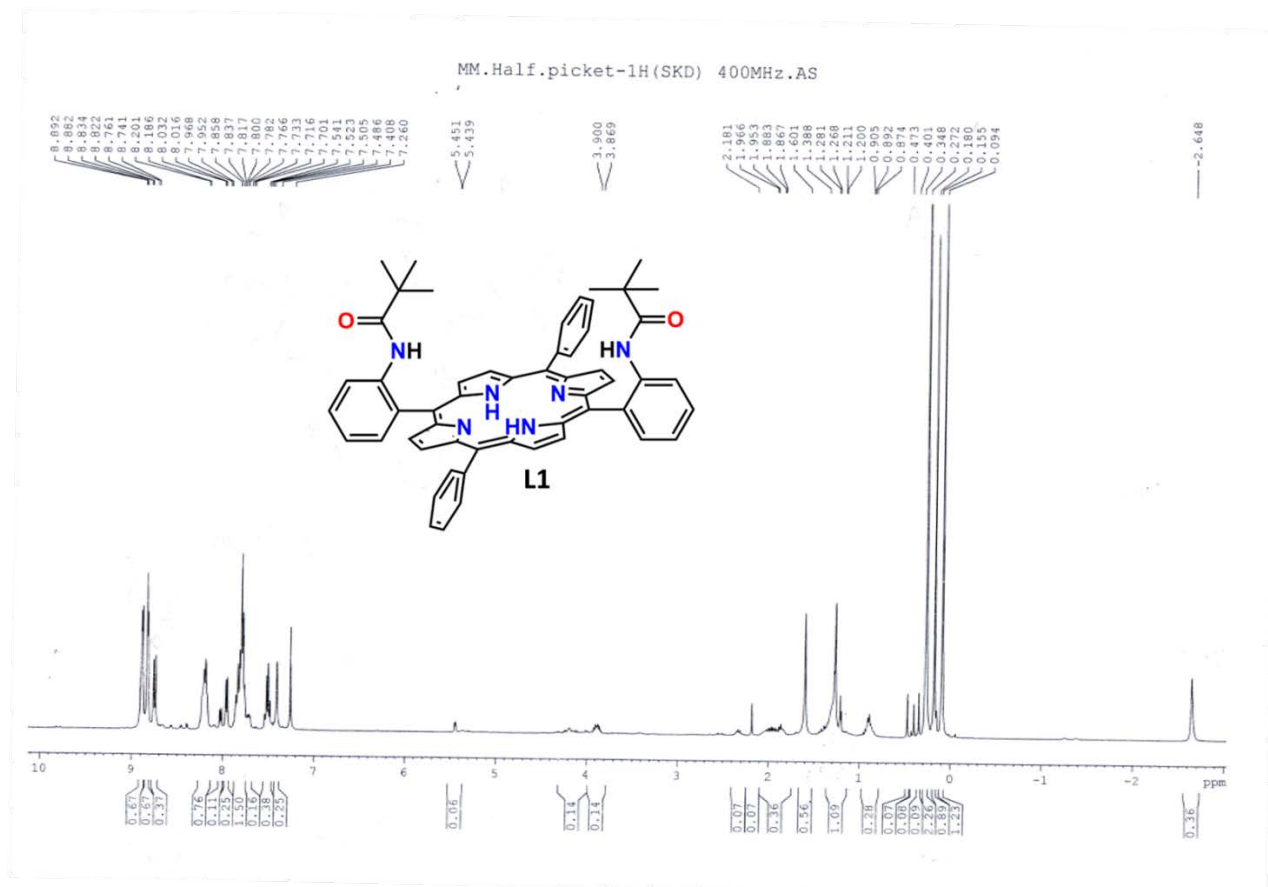


Figure S.6.2: NMR spectra of ligand L1.

Iron picketfence porphyrin (FePf) was synthesised following Collman's method<sup>2</sup>. Characterisation of the ligand was done using NMR spectroscopy and ESI-MS and metallated ligand was characterised with ESI-MS and UV-Vis spectroscopy. All of the data matched exactly with the referred literature.

### S.7. Oxygen reduction reaction (ORR) with thiolate bound iron picketfence porphyrin (FePf):

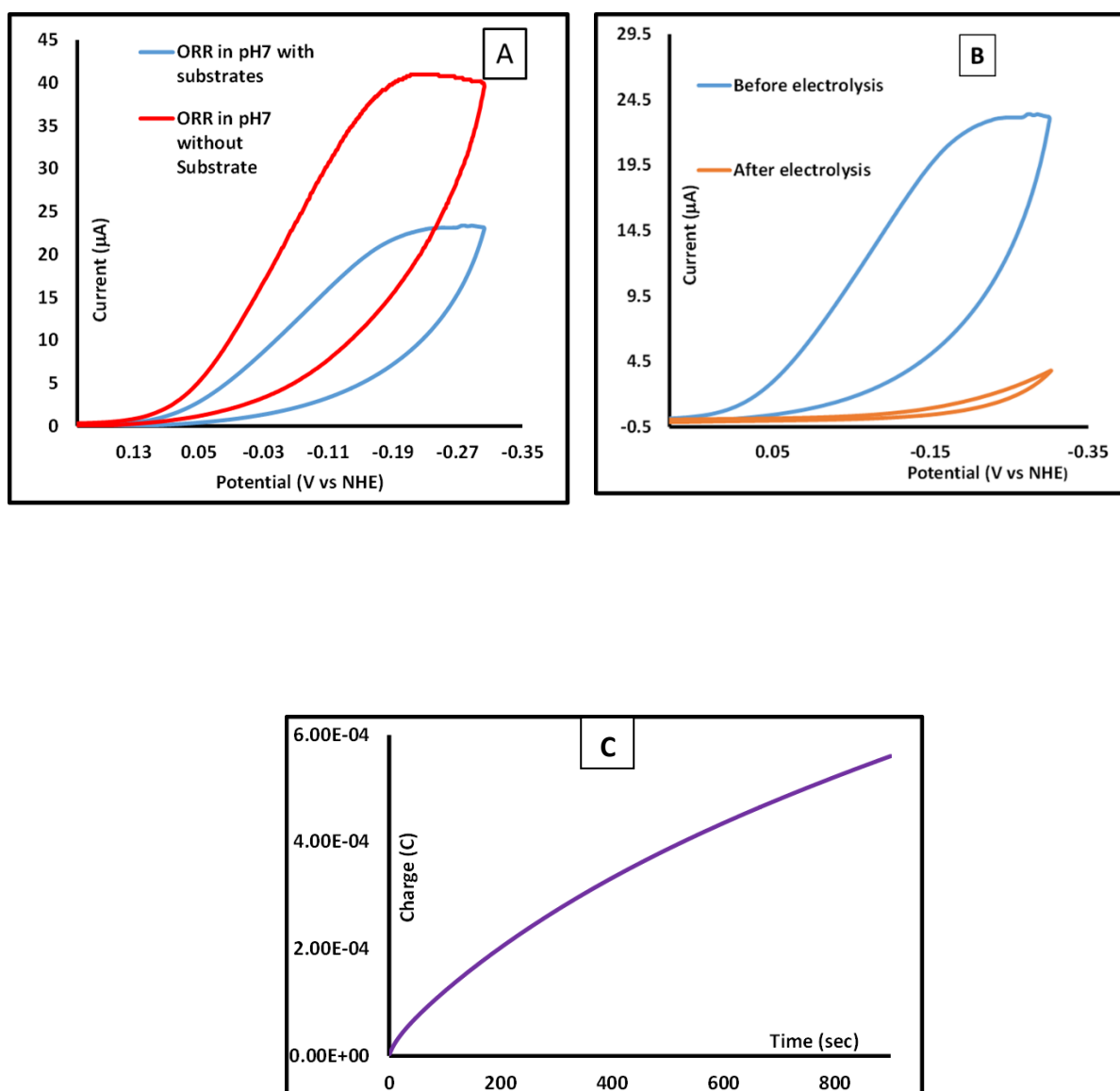


Figure S.7: A) Oxygen reduction reaction (ORR) with the bioelectrode in 100mM pH7 phosphate buffer without (red) and with the substrate (blue) B) ORR with the bioelectrode in substrate saturated pH7 buffer before and after performing the electrolysis at -0.1V potential (vs NHE). C) Electrolysis plot at -0.1V potential.

### S.8. GC-MS traces for the oxidation of Cyclohexane, Styrene, Toluene and EthylBenzene:

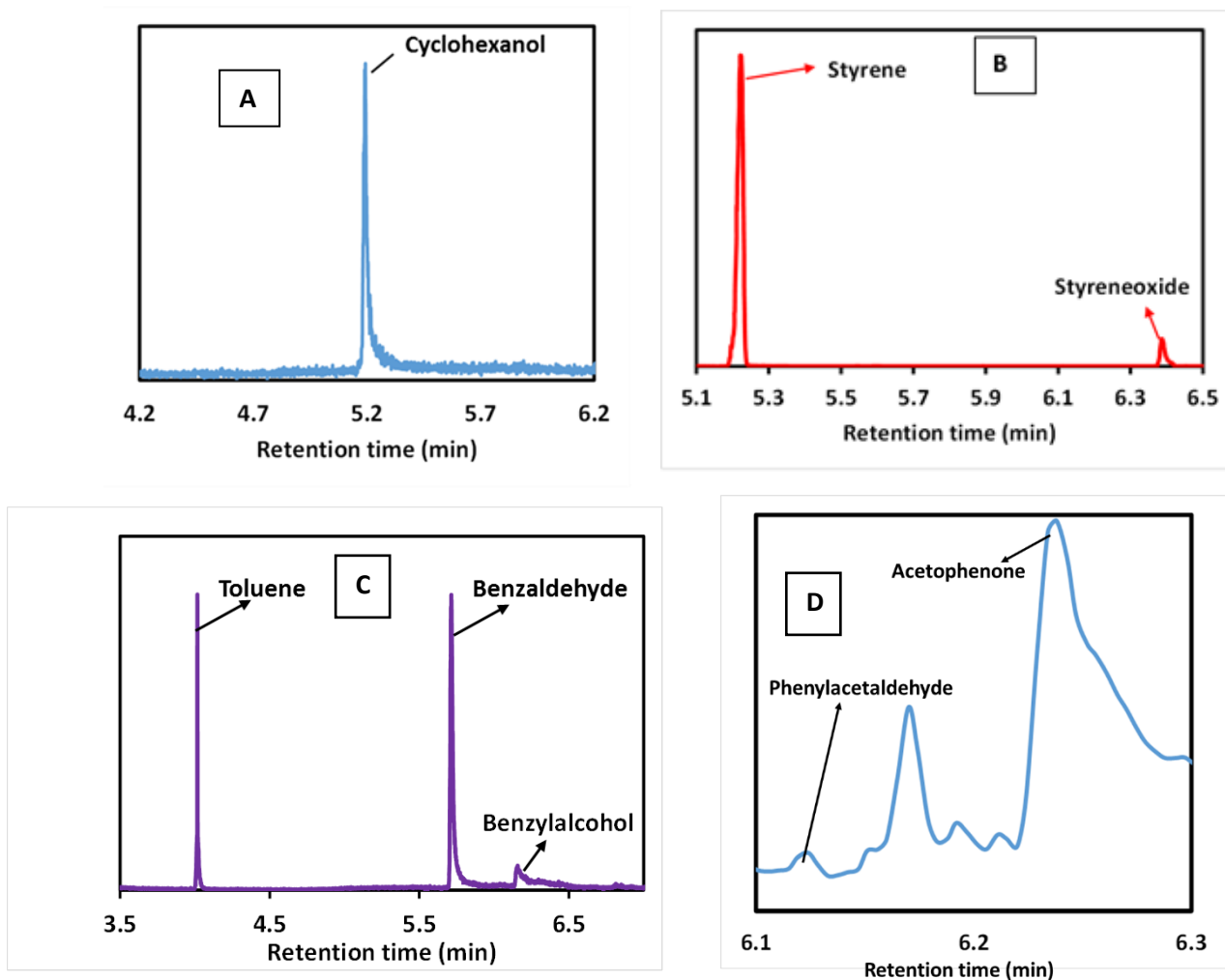


Figure S.8: peaks of A) Cyclohexane, B) Styrene, C) Toluene and D) Ethylbenzene oxidation during the GC-MS analysis.

### S.9. Calibration curves for different oxidised products:

Pure products of various known concentrations were ran in GC and the peak areas were collected. These peak areas were plotted against the known concentrations of the samples and those were used as the calibration curve for individual products.

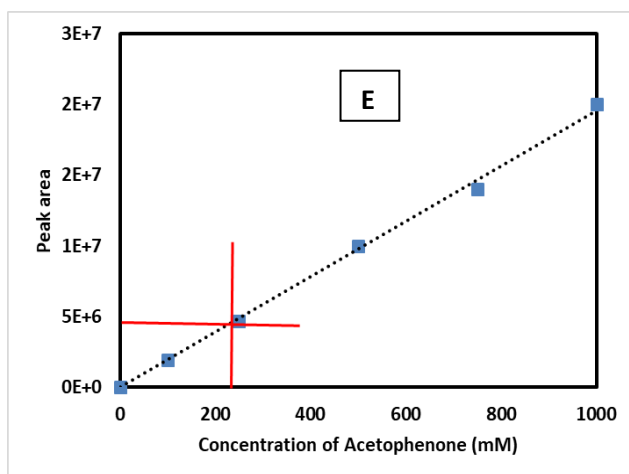
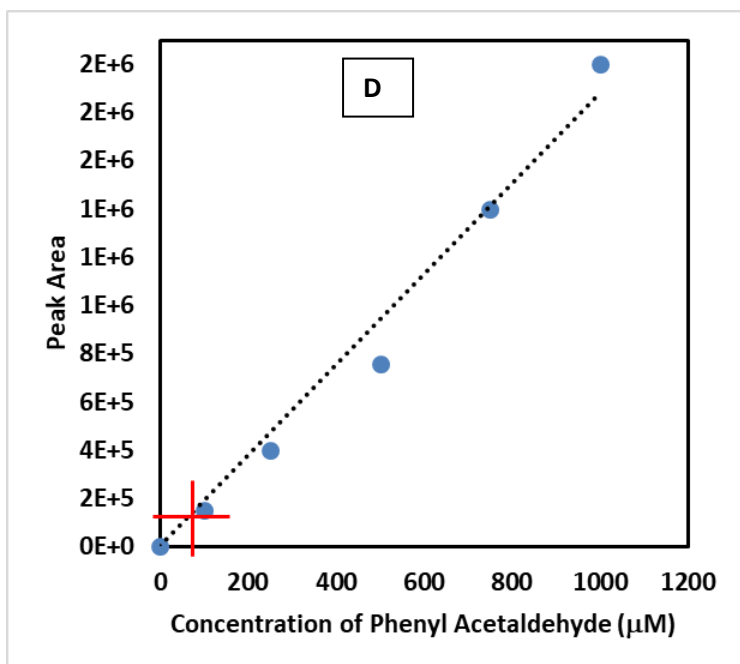
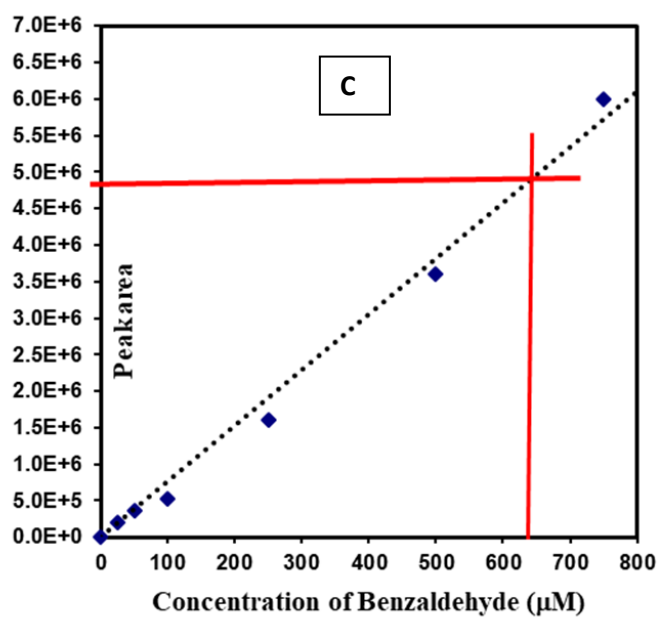
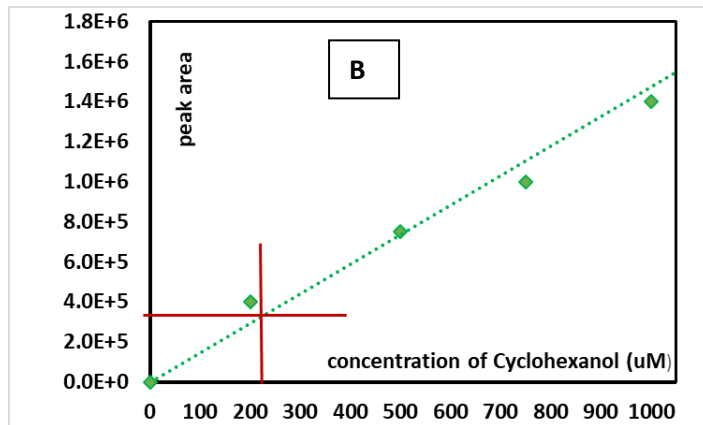
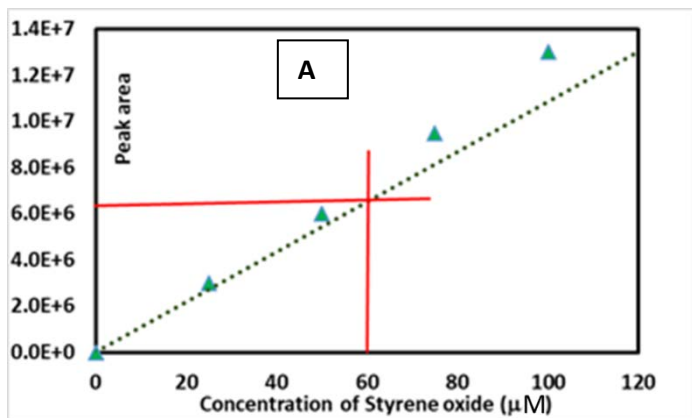


Figure S.9: Calibration curves for A) Cyclohexanol, B) Styreneoxide, C) Benzaldehyde D) Phenyl acetaldehyde and E) Acetophenone.

Relative concentration of Adamantane-1-ol, Adamantane-2-ol, Adamantanone and 1-phenylethanol was measured using the relative peak area of the above-mentioned substrates in their GC traces.

### S.10. Kinetic Isotope Effect (KIE) and Kinetic Solvent Isotope Effect (KSIE) for Cyclohexane oxidation:

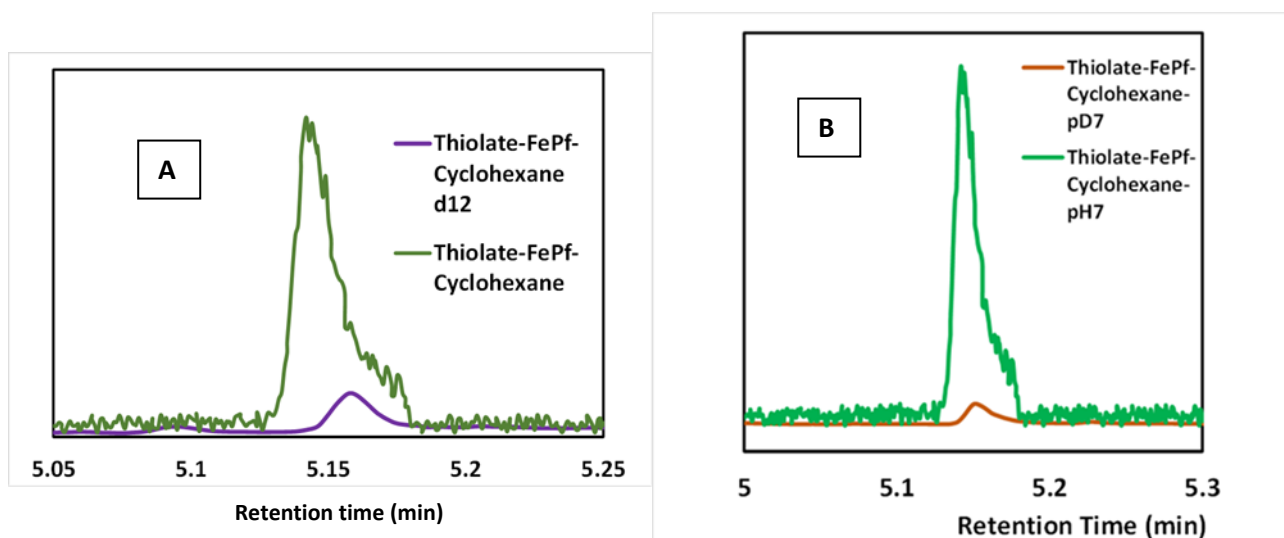


Figure S.10: A) Ratio of the amount of oxidised product produced from cyclohexane (dark green) and cyclohexane-d<sub>12</sub> (purple) B) Ratio of the amount of oxidised product produced for cyclohexane in pH7 and pD7 buffer.

### S.11. Oxygen reduction reaction in absence and presence of Carbon monoxide:

The electrode consisting thiolate ligated FePf forms the high valent intermediate, responsible for substrate oxidation, during the Oxygen Reduction reaction (ORR) in oxygenated aqueous medium. Now, ORR is completely inhibited CO (1:1) was dissolved in the same buffer solution. This is consistent with CO binding the ferrous reduced state and inhibiting oxygen reduction. Consequently, in the absence of Oxygen reduction, there can not be any substrate oxidation and no oxidized products can be detected.

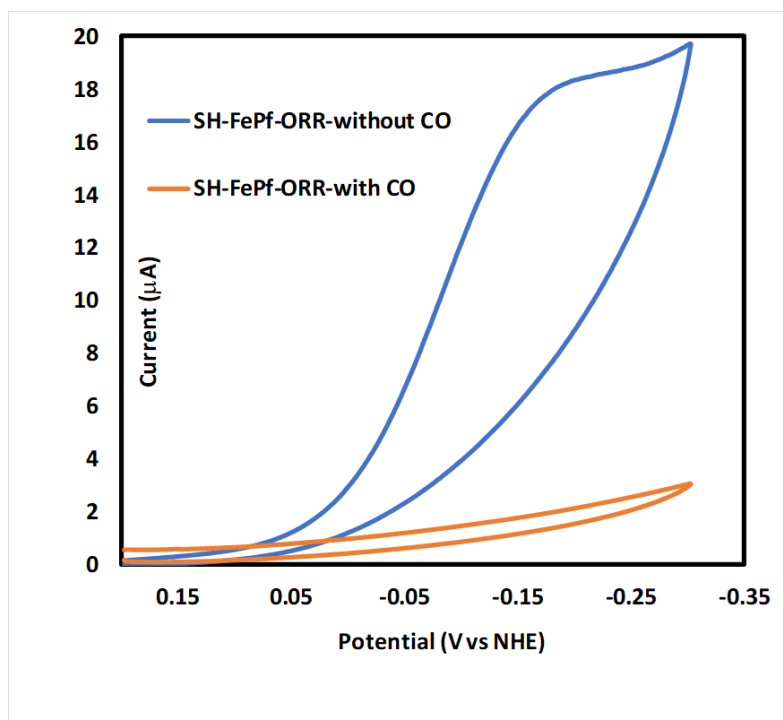


Figure S.11: Overlay of the Oxygen reduction reaction current of the thiolate ligated FePf in absence CO (blue) and in presence of CO (orange).

#### References:

- (1) Littler, B. J.; Ciringh, Y.; Lindsey, J. S. Investigation of Conditions Giving Minimal Scrambling in the Synthesis of trans-Porphyrins from Dipyrromethanes and Aldehydes. *J. Org. Chem.* **1999**, *64*, 2864-2872.
- (2) Collman, J. P.; Gagne, R. R.; Reed, C.; Halbert, T. R.; Lang, G.; Robinson, W. T. Picket fence porphyrins. Synthetic models for oxygen binding hemoproteins. *J. Am. Chem. Soc.* **1975**, *97*, 1427-1439.