Supporting Information

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S1. Experimental Details:

S1. 1. Materials and Methods

1-Azidoaundecane-11-thiol from S-(11-bromoundecyl)thioacetate and Hemin-1Fe from Protoporphyrin IX, propergylamine and iron(II) bromide, were synthesized following the previously reported procedures.^{1, 2} Au wafers were purchased from Platypus Technologies (1000 Å of Au on 50 Å of Ti adhesion layer on top of a Si (111) substrate). All electrochemical experiments were done in CH Instruments (model CHI710D Electrochemical Analyzer). Bipotentiostat, reference electrodes, Teflon® plate material evaluating cell (ALS Japan) were purchased from CH Instruments. The rotating ring disk electrochemical set up was purchased from Pine Research Instrumentation (E6 series ChangeDisk tips with AFE6M rotor). An aq. Ag/AgCl and a Pt wire were used as the reference and counter electrode for all of these experiments. Cyclic Voltametry (CV) was measured in buffered solutions (100 mM phosphate) containing 100 mM KPF₆. For resonance Raman experiments, a Kr + laser (Sabre Innova, model SBRC-DBW-K) from Coherent and a spectrograph (model Trivista 555) from Princeton Instruments were used. The excitation wavelength used in the resonance Raman experiments was 413.1 nm and the power applied to the sample was around 50 mW. The silver surfaces were roughened before SERRS experiments following literature protocols.³

S1. 2. Formation of mixed Self Assembled Monolayer (SAM)

Gold wafers or disks were cleaned both by immersing them into a Piranha solution (1:3 H_2O_2/H_2SO_4) and also electrochemically following the reported procedures. ^{4, 5} *Caution!! Piranha solution can be explosive and needs to be handled with extreme caution.* The substrate was rinsed again with deionized water followed by ethanol. Deposition solution was made by dissolving 1-octanethiol (concentration of stock: 10mg/ml in ethanol) and 1-azidoundecane-11-thiol (concentration of stock: 10mg/ml in ethanol) in 9:1 ratio in ethanol. The thiol ratio of 9:1 is not exactly maintained on the surface. The surface coverage determined from the CV of the redox active Hemin-1Fe group is only 49:1 i.e. 1.8 %. The total thiol concentration of the deposition solution was always 0.4mM. Cleaned gold wafers or disks were purged with N₂ and then immersed into the deposition solution for 24 hours.

S1. 3. Formation of Hemin-1Fe Terminated Monolayer

Gold wafers or disks immersed in the deposition solution were taken out and rinsed with ethanol and then deionized water successively in order to remove excess absorbate and dried with Ar to remove residual solvent. The wafer was then clipped into a Plate Material Evaluating Cell (ALS Japan). After measuring the background O_2 reduction current (which was typically less than 2 μ A at -500 mV at a scan rate of 50 mV/ s) i.e. the current produced by the mixed SAM when it is not attached to the rdox active heme moiety, the surface was cleaned with deionized water. A 3:2 DMSO water solution (Click Solution) of 100 μ l 100mM CuSO₄.5H₂O, 200 μ l 100mM ascorbic acid and 40 μ l of Hemin-1Fe taken from 10mg/ml stock solution was poured on top of the gold surface to make Hemin1-Fe terminated SAM on gold surface (Scheme S1.3). Previous reports established, using SERRS, XPS and CV, that the grafting of Hemin-1Fe involves both the alkyne termini of the hemin (Figure. S1.3).^{2, 6}



Scheme S1.3. Covalent attachment of Hemin-1Fe to the dilute monolayer of 1-Azidoaundecane-11-thiol and 1-octanethiol using "click" chemistry (presence of small amount of monoalkyl ligation of the Hemin-1Fe cannot be ruled out (S1.3))



Figure S1.3. Cyclic voltammogram (CV) of Hemin-1Fe clicked electrode surface fabricated with the mixed SAM of 1-octanethiol and 1-azidoundecane-11-thiol, before and after ethynylferrocene click using Ag/AgCl reference electrode and a Pt counter electrode in pH 7 phosphate buffer without imidazole and in presence of O_2 . (No CV for ethynylferrocene is observed at +0.35 V over the Hemin-1Fe clicked surface indicating that both the alkyne termini of the hemin are clicked with the azide terminal functional group, without leaving any free azide for further reaction with ethynylferrocene.)

S1. 4. Partially Reduced Oxygen Species (PROS)

Platinum ring of 6 mm inner diameter and 8 mm outer diameter Gold disk RRDE (polished by alumina; size: 1 micrometer, 0.5 micrometer and 0.03 micrometer, and electrochemically cleaned before use) having 5 mm x 4 mm is inserted in to the RRDE tip which is then mounted on the rotor and immersed into the cell which is equipped with Ag/AgCl reference and Pt counter electrodes. In order to measure the PROS first the collection efficiency of the rotating ring disk is measured in a 2mM $K_3Fe(CN)_6$ and 0.1M KNO₃ solution at 10 mV/s scan rate and with 300rpm rotation. A 20±2% collection efficiency is generally recorded during these experiments. The potential at which the ring was held during the collection experiments at different pHs are obtained from literature.⁷

S1. 5. Solvent Kinetic Isotopic Effect

The kinetic isotopic effect was studied by generating the PROS in deuteriated buffer (pD ~ 7.01) and normal protonated buffer (pH ~ 7.41). Deuteriated pD~7.01 buffer was prepared by dissolving pH 7 \pm 0.05 buffer capsule in 100 ml of deuteriated water (D₂O) and the pD was measured from pH meter to be 7.01.

S2.1. Nature of Fe^{III} (ox) species between pH 4-9



Figure S2.1. SERRS spectra of Hemin-1Fe modified electrode in oxidized state in the phosphate buffer of pH 5 and pH 9, using Ag/AgCl reference electrode and Pt counter electrode for the electrochemical reduction.

S2.2. Nature of imidazole bound Hemin-1Fe at pH5 (both in oxidized and reduced state)



Figure S2.2. SERRS spectra of Hemin-1Fe modified electrode in both oxidized and reduced state in the phosphate buffer of pH 5 with 100 mM imidazole, using Ag/AgCl reference electrode and Pt counter electrode for the electrochemical reduction.



S3. pH Dependence of the potential of electrocatalytic ORR (E(i)) by

Figure S3. pH dependence of electrocatalytic O_2 reduction by Hemin-1Fe covalently in attached to the mixed SAM of 1-Octanethiol and 1-azidoundecane-11-thiol (in 9:1 ratio) on Au disk in phosphate buffer at 50 mV/s scan rate and at 300 rpm rotation speed using Ag/AgCl reference electrode and Pt counter electrode (A) LSV of O_2 reduction current at

different pH of buffer without imidazole, at 50 mV/s scan rate and at 300 rpm rotation speed. (B) LSV of O_2 reduction current at different pH of buffer with 100 mM imidazole, at 50 mV/s scan rate and at 300 rpm rotation speed. The green arrow head in each case indicates the plot of potential of inflection (E(i)) of the LSV currents (i.e. maxima of the 1st derivative) of the corresponding LSV plot for O_2 reduction at different pH.

S4. The E(i) vs pH experimental plot fitted with theoretical single protonation (pK_a) curve



Figure S4. Plot of variation of E(i) with pH, which does not fit with the theoretical curve for single protonation pathway. The experiments were done under the same physiological condition using Ag/AgCl reference electrode and a Pt counter electrode in pH 7 phosphate buffer without imidazole and in presence of O₂.

A pH equilibrium (pK_a) plot is always sigmoidal and the change of potential occurs within 2 units of pH. This is because Henderson's equation which states $pH = pK_a + \log p$ [base]/[acid]. So, if the pH is 1 unit below the pK_a (i.e. $pH = pK_a$ -1), the ratio of [acid]/[base] = 10 i.e. 91 % acid and 9 % base. Alternatively if pH is 1 unit above the pKa, the ratio [acid]/[base] = 0.1 i.e. 9 % acid and 91 % base. But in the case of PCET the change in potential may or may not occur within 2 units of pH and depending on the range of pH chosen for the experiment, the curve varies from linear to sigmoidal. When the potential varies within a wide pH range and then saturates either at a very high or at a very low pH (beyond the experimental limits), the curve looks linear within a large pH range. For the PCET process, the characteristic feature is ~ 60 mV shift of the formal potential of the redox active species per unit change of pH.

S5. The decay of the monolayer of catalyst with speed of rotation



Figure S5. LSV of the monolayer of Hemin-1Fe catalyst on the mixed SAM of HS-C11-N₃ and HS-C8, at different rotation speed and at 100 mV/s scan rate, using Ag/AgCl reference electrode and Pt counter electrode.





Figure S6. Tafel slope of the ORR current in the pH A) 4, B) 5, C) 9. RRDE measurements were done at 50 mV/s scan rate and at 300 rpm rotation speed, using Ag/AgCl reference electrode and Pt counter electrode.

рН	Tafel slope		
4	164		
5	100		
6	156		
7	129		
8	140		
9	123		

Table S1. Tafel slope at different pH

S7. Cyclic voltammogram of covalently attached Hemin-1Fe in phosphate buffer without and with 100 mM imidazole buffer



Figure S7. Cyclic voltammogram of covalently attached Hemin-1Fe in buffer (A) without (B) with 100 mM imidazole, at 50 mV/s scan rate under completely anaerobic condition and using Ag/AgCl reference electrode and Pt counter electrode.

S8. Theoretical PCET fit of $E_{1/2}$ of $Fe^{III/II}$ reduction process in degassed buffer without and with 100 mM imidazole



Figure S8. Theoretical PCET fit of $E_{1/2}$ obtained from the CV of Fe^{III/II} redox couple in absence of O₂ at different pH of phosphate buffer A) without and B) with imidazole and using Ag/AgCl reference electrode and a Pt counter electrode.



S9. PROS in buffer without and with imidazole

Figure S9. A) PROS produced by covalently attached Hemin-1Fe, with change of pH of the phosphate buffer without and with 100 mM respectively. B) Variation of PROS produced by covalently attached Hemin-1Fe, with change of pH of the phosphate buffer

with 1 mM imidazole. RRDE measurements were done at 50 mV/s scan rate and at 300 rpm rotation speed, using Ag/AgCl reference electrode and Pt counter electrode.

S10. Reproducibility of the data on different days of experiment and for buffer with different pH



Figure S10. LSV for the PROS data collected on different days under the same physiuological condition i. e., at 50 mV/s scan rate and at 300 rpm rotation speed, using Ag/AgCl reference electrode and Pt counter electrode.

S11. Variation of amount of PROS with change of concentration of imidazole in pH 7 phosphate buffer



Figure S11. Plot of variation of PROS (%) with change of imidazole concentration in pH 7 phosphate buffer. RRDE measurements were done at 50 mV/s scan rate and at 300 rpm rotation speed, using Ag/AgCl reference electrode and Pt counter electrode.

S12. Solvent kinetic isotopic effect on O₂ reduction

Electrocatalytic oxygen reductions as well as the PROS production were measured both in pH (protonated) and pD (deuterated) buffers. Electrocatalytic oxygen reduction shifts by 59 mV from -289 mV in H₂O (pH 7.4) to -348 mV in D₂O (pD 7.01) indicative of PCET. PROS in H₂O is half of that in D₂O (Fig. S14, inset). This may indicate the kinetic isotopic effect on PROS formation.



Figure S12. LSV plot of electrocatalytic O_2 reduction in pH 7.41 (protonated, pink) and pD 7 (deuterated, blue) at 50 mV/s scan rate and at 300 rpm rotation speed using Ag/AgCl reference electrode and Pt counter electrode.

S13.

Table S2. pK_a and PA estimation of Fe^{III} -OOH species with or without the trans ligand imidazole

	pK_a		ΔPA		—
			(Kcal/mol)		
	distal	proximal	Distal	Imz (proximal	!-
			(H_2O) vs	distal)	
			Imz)		
H ₂ O	3.5 ±0.1	-	1.027	1.507	_
Imidazole	4.25±0.1	5.35±0.05			

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