Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Heme bound Amylin Self-Assembled Monolayers on Au electrodes: An efficient Bio-electrode for O_2 reduction to H_2O

Kushal Sengupta, Sudipta Chatterjee, Soumya Mukherjee, Somdatta Ghosh Dey*,

Abhishek Dey*

Department of Inorganic Chemistry, Indian Association for the Cultivation of Science,

2A & 2B Raja S. C. Mullick Road, Jadavpur, Kolkata, 700032

1. Experimental Details

1.1. Materials

All reagents were of the highest grade commercially available and were used without further purification. Amylin peptides (Ay 1-19) (sequence: Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser) and $Arg^{11}Asn$ mutant of Ay (1-19) have been used for this study. All peptides were purchased from GL Biochem (Shanghai) Ltd. with >95% purity. Hemin, Octanethiol (C_8SH), Potassium hexaflurophosphate (KPF_6) and the buffers were purchased from Sigma-Aldrich. Di-Sodium hydrogen phosphate dihydrate (Na_2HPO_4. $2H_2O$) and Imidazole 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 (111) surface). Au and Ag discs for the RRDE and SERRS experiments were purchased from Pine Instruments, USA. Transparent Au wafers (100 Å of Au on 10 Å of Ti) for Absorption experiments were purchased from Phasis, Switzerland.

1.2. 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 rotating ring disk electrochemical (RRDE) set up from Pine Research Instrumentation (E6 series ChangeDisk tips with AFE6M rotor) was used to obtain the RRDE data. The AFM data was obtained at room temperature in a Veeco dicp II (Model no: AP-0100) instrument bearing a phosphate doped Si cantilever (1-10 ohm.cm, thickness 3.5-4.5 μm, length 115-135 μm, width 30-40 μm, resonance frequency 245-287 KHz, elasticity 20-80

N/m). Surface Enhanced Resonance Raman data were collected using a Trivista 555 spectograph (Princeton Instruments) and using 413.1 nm excitation from a Kr^+ laser (Coherent, Sabre Innova SBRC-DBW-K). UV-Vis absorption data were taken in an Agilent technologies spectrophotometer model 8453 fitted with a diode-array detector.

2. Methods

2.1. Construction of the electrodes

2.1.1. Formation of mixed Self Assembled Monolayer (SAM)

Gold wafers were cleaned electrochemically, first by electrolysis where it was hold at a high positive potential (2.1 V) for few seconds and then by sweeping several times between 1.5 V to -0.3 V in 0.5 M H_2SO_4. 0.1 mM Ay (1-19) and its corresponding mutant in 100 mM phosphate buffer were used as the SAM solutions. Freshly cleaned Au wafers or discs were thoroughly rinsed with triple distilled water, and purged with N_2 gas and immersed in the SAM solution for 2 days. After 2 days the substrates were immersed in 0.1 mM C_8SH (in EtOH) solution for 30 mins before electrochemical experiments. In order to study aggregation properties by AFM, surfaces were prepared similarly and kept in the depositing solution of the peptide for 2 months.

2.1.2. Attachment of Heme on to SAM

Gold wafers or discs immersed in the deposition solution were taken out and rinsed with triple distilled deionized water in order to remove any excess adsorbate and dried with N_2 gas to remove residual solvent. The wafers were then inserted into a Plate Material Evaluating Cell (ALS Japan) and the discs were mounted on a platinum ring disc assembly (Pine Instruments, USA). 1 mM Hemin solution in DMSO were used as the incubating solutions. Heme-Ay_{Cys} surfaces were prepared by incubating the modified

Au surfaces with the DMSO solution for 10-15 mins. The surfaces, after incubation with the hemin solution, were rinsed with DMSO for 3 mins to remove excess physiadsorbed heme (if any).

2.2. Atomic force microscopy (AFM) experiments

Freshly cut Au wafers were taken for each AFM analysis where Ay_{Cys} modified surfaces were made as described in section 2.1.1. The surfaces were thoroughly rinsed with triple deionised water before analysis. AFM data were obtained at room temperature in a Veeco dicp II instrument bearing a phosphate doped Si cantilever (1-10 ohm.cm, thickness 3.5-4.5 μm, length 115-135 μm, width 30-40 μm, resonance frequency 245-287 KHz, elasticity 20-80 N/m).

2.3. Electrochemical experiments

All electrochemical (CV and LSV) experiments were done in pH 7 buffer (until otherwise mentioned) containing 100 mM $Na_2HPO_4.2H_2O$ and 100 mM KPF_6 (supporting electrolyte) using Pt wire as the counter electrode and Ag/AgCl as the reference electrode. All the potentials in the text and figures have been adjusted vs. NHE.

2.3.1. Cyclic Voltammetry (CV) experiments

CV data of Heme-Ay_{Cys} and Heme bound R11N mutant were collected in deoxygenated pH 7 buffer under anaerobic conditions using electrodes as mentioned in section 2.3.

2.3.2. Partially Reduced Oxygen Species (PROS) experiment

The platinum ring and the gold disc were both polished with alumina powder (size: 1 μ, 0.3 μ and 0.05 μ) and electrochemically cleaned in 0.5 M H_2SO_4 (as mentioned in section 2.4.1 for Au wafers) and inserted into the Rotating Ring Disc Electrochemistry (RRDE) tip (Figure S14A) which is then mounted on the rotor and immersed into a cylindrical glass cell which is equipped with Ag/AgCl reference and Pt counter electrodes. The collection efficiency of the RRDE set-up is measured in a 2 mM $K_3[Fe(CN)_6]$ and 0.1 M KNO_3 solution at 10 mV/s scan rate and 300 rpm rotation speed. The collection efficiency (CE) generally recorded during these experiments was 20±2% (Figure S14B). The potential at which the ring was held during the collection experiments for detecting H_2O_2 was obtained from literature.[1] The PROS calculations for the species were done at potential 0 V vs. NHE.

2.3.3. Rotating disc electrochemistry (RDE)

The O_2 reduction current increases with increasing rotation rates following the Koutecky–Levich equation, $i^{-1} = i_K(E)^{-1} + i_L^{-1}$, where $i_K(E)$ is the potential dependent kinetic current expressed as $n\text{FA}[O_2]k_{cat}\Gamma_{\text{catalyst}}$, and i_L is the Levich current. i_L is expressed as $0.62n\text{FA}[O_2](D_{O2})^{2/3}\omega^{1/2}v^{-1/6}$, where n is the number of electrons transferred to the substrate, F is the faraday constant, A is the macroscopic area of the disc (0.192 cm^2), $[O_2]$ is the concentration of O_2 in an air saturated buffer (0.26 mM) at 25 ^oC, k_{cat} is the 2nd order rate of catalytic O_2 reduction, Γ_{catalyst} is the catalyst concentration in moles/cm^2, D_{O2} is the diffusion coefficient of O_2 (2.2 x 10^{-5} cm^2 s^{-1}) at 25 ^oC,[2-4] ω is the angular velocity of the disc and v is the kinematic viscosity of the solution (0.009 cm^2 s^{-1}) at 25 ^oC.[5] 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 number of electrons (n) involved in the O_2 reduction by a catalytic species may be calculated from the slope and rate of catalysis (k_{cat}) from the intercept of this linear plot.

2.3.4. Coverage calculation

The coverage for a particular species is estimated by integrating the oxidation and reduction currents of the respective species.[6, 7] Note that, these constructs have large capacitance which is also seen in the background currents (Fig. S15). During coverage calculation these background currents have been taken care of. The marked area indicates the integrated region of the oxidation and reduction currents (Fig. S15).

2.3.5. Double layer Capacitance measurement

C_{dl} can be determined from the measured charging current (I_c) according to the following equation, $C_{dl} = \frac{I_c}{\nu A}$, where ν is the scan rate and, A is the geometric area of the electrode surface.

2.4. Surface enhanced resonance Raman (SERRS) experiments

Ag discs are cleaned using Alumina powder (grit sizes 1, 0.3 and 0.05 microns) and then roughened in 0.1 M KCl solution using reported procedures and immersed in Ay (1-19) solutions to form SAM. The roughened modified Ag discs are then inserted into the RRDE set-up for the collection of SERRS data. Heme are attached to the SAM in a similar manner as described in section 2.1.2. 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 resting/oxidized state the disc is held at 0 V and at -0.5 V to obtain a reduced spectrum.[8]

2.5. Absorption experiments

For the heterogenous absorption experiment covalently attached catalysts on SAM modified transparent Au electrodes were used. These data are collected on a monolayer modified transparent electrode surface and thus appear noisy if compared to solution and in many cases the value of the Soret transition can only be tentatively assigned. However, in spite of such dilution, the very high absorption coefficient of the porphyrin results in resonable absorption features.

2.6. Bulk Electrolysis (BE) and calculation of turnover number (TON)

The bulk electrolysis experiments were performed with the same water jacketed electrochemical cell and same shaft, used for RRDE set-up, fitted with Heme-Ay_{Cys} modified Au discs. The experiment was done in air saturated pH 7 phosphate buffer at -0.250 V vs. NHE. The rotating shaft bearing working electrode was rotated at 100 rpm speed to maintain constant substrate diffusion to the electrode during electrolysis.

From the BE experiment the charge consumed for a period of 5000 sec was noted and the number of O_2 molecules undergoing $4e^-/4H^+$ reduction was calculated. The ratio of the number of O_2 molecules undergoing $4e^-/4H^+$ reduction to the number of active catalysts present in the electrode surface area yielded the TON of the system.

Figures

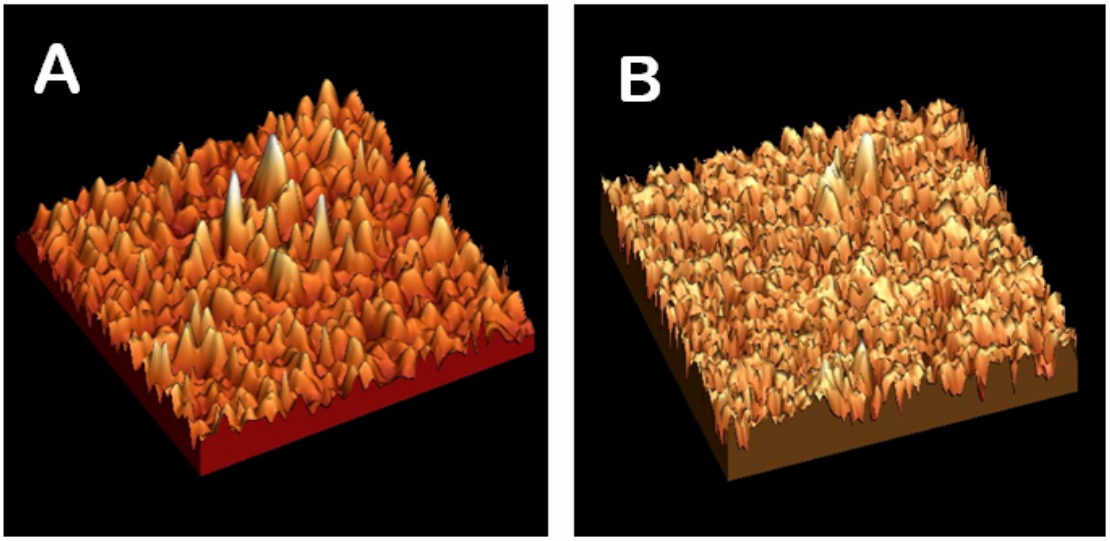

Fig. S1. 3D topology AFM images of Amylin (1-19) modified Au surface kept in the depositing solution for 2 days (A) and for about 2 months (B).

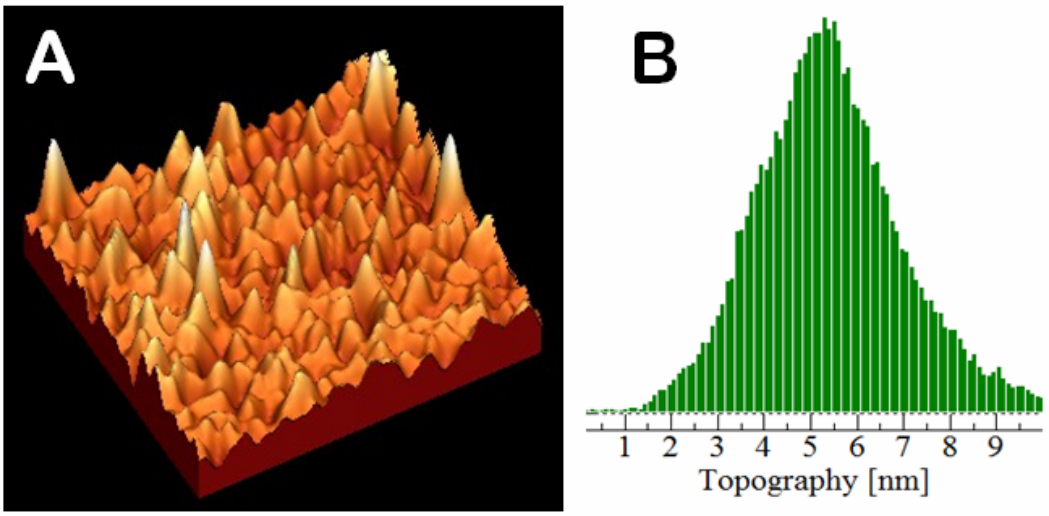

Fig. S2. (A) 3D topology AFM images of Amylin-Arg11Asn (R11N) mutant modified Au surface (Ay_{SAM}-R11N). (B) Height distribution profile diagram of the corresponding surface.

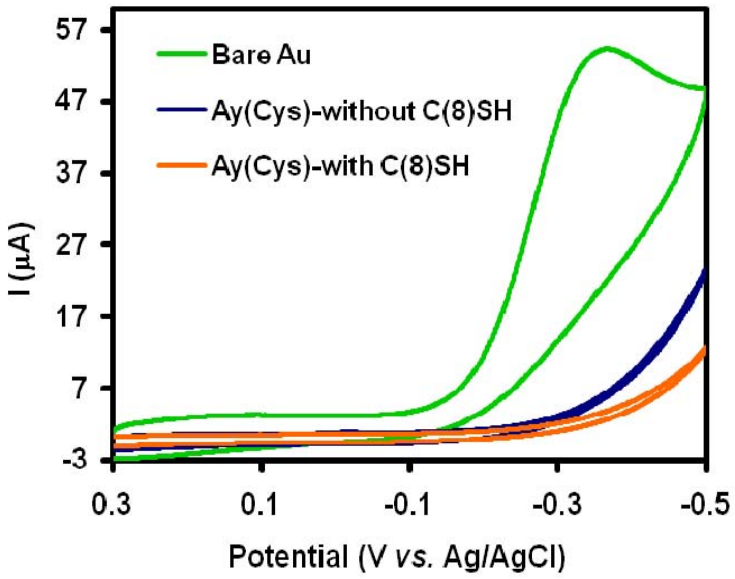

Fig. S3. CV data of bare Au electrode (green), and Ay_{Cys} modified Au electrode with (orange) and without (blue) C_8SH diluent, in air saturated pH 7 buffer.

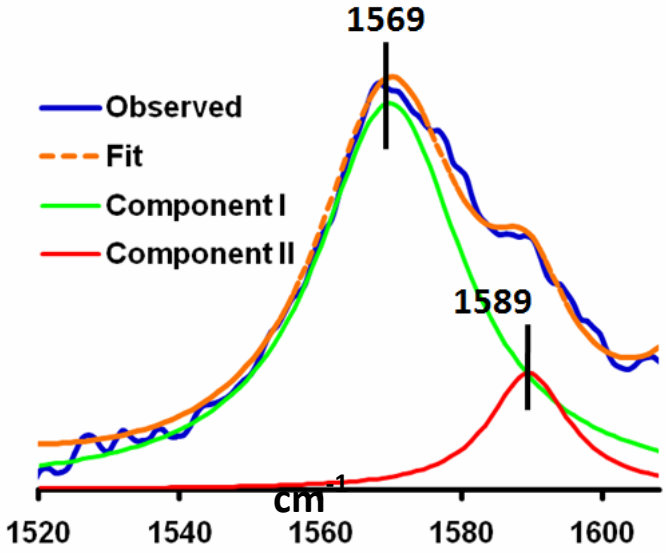

Fig. S4. SERRS data of Heme-Ay_{Cys} (WT) in normal pH 7 buffer showing the υ_2 region along with the Lorentzian fits. The fits show the two different components.

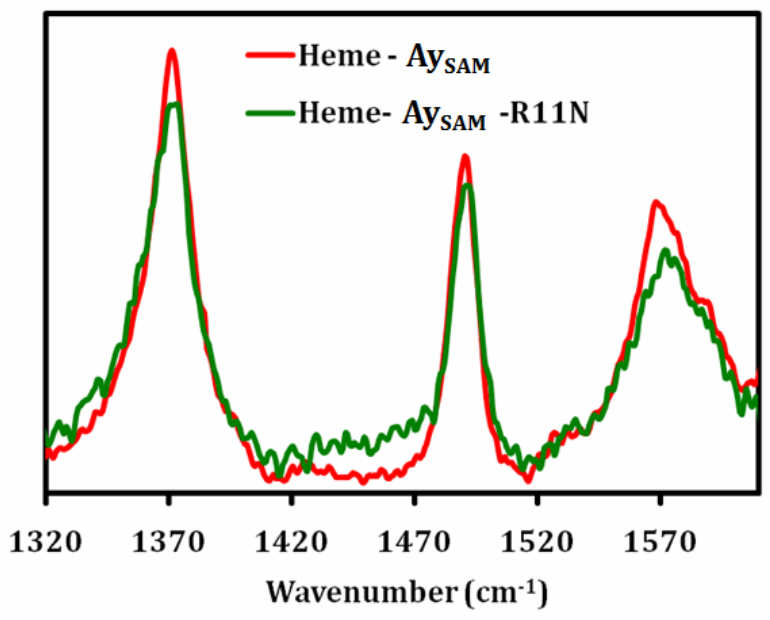

Fig. S5. SERRS data of heme-Ay$_{SAM}$ in pH 7 buffer (red) collected under resting (oxidizing) condition and its corresponding heme bound R11N mutant (heme-Ay$_{SAM}$-R11N) (green).

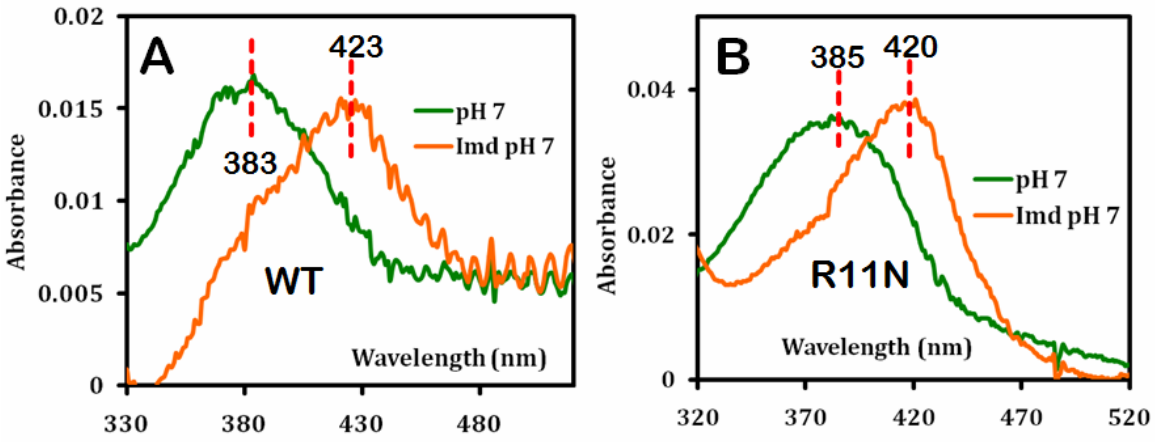

Fig. S6. Absorption spectra of heme-Ay$_{SAM}$ (A) and heme-Ay$_{SAM}$-R11N (B) in pH 7 buffer with (orange) and without (green) the presence of 100 mM imidazole.

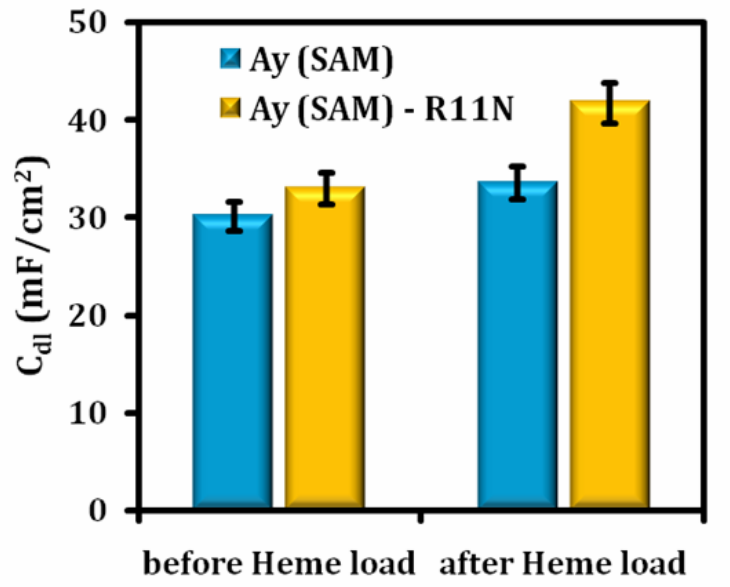

Fig. S7. Double layer capacitance values of Ay_{SAM} (blue) and Ay_{SAM}-R11N (yellow) before and after heme attachment calculated from their respective CV curves at a scan rate of 1 V/s.

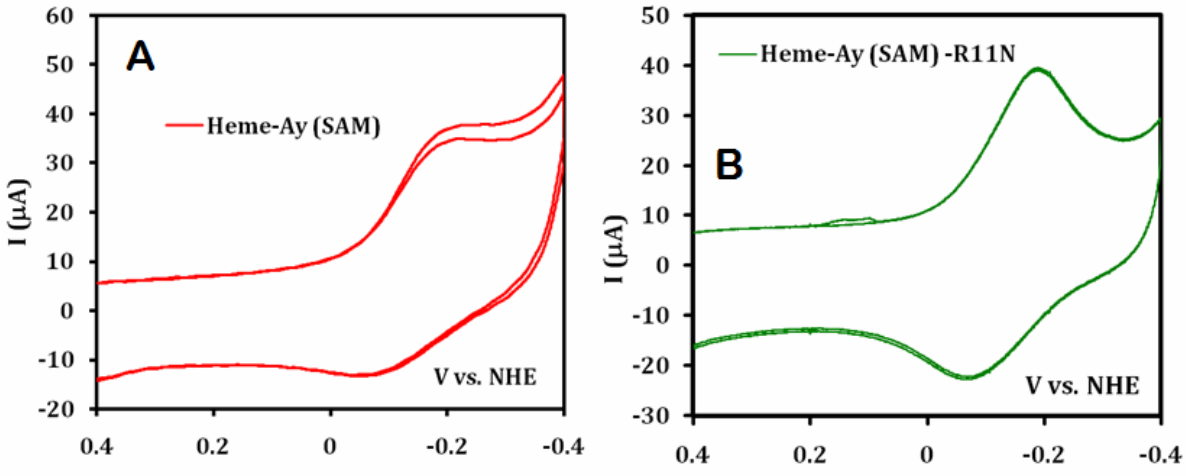

Fig. S8. Multiple segments of the CV data of heme-Ay_{SAM} (A) and heme-Ay_{SAM}-R11N mutant (B) in deoxygenated pH 7 buffer at a scan rate of 1 V/s. No change in the multiple segments confirm the reversibility of the systems.

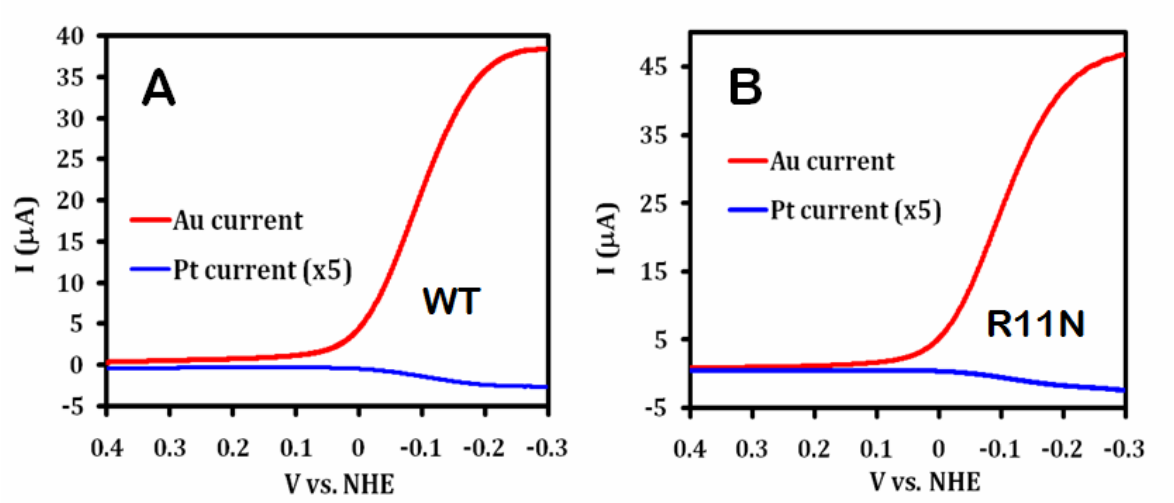

Fig. S9. RRDE plots of heme-Ay_{SAM} (A) and heme-Ay_{SAM}-R11N (B) showing the Au current (red) and corresponding pt ring current (blue) in pH 7 buffer obtained at a scan rate of 10 mV/s and rotation speed of 300 rpm. The Pt ring has been multiplied by a factor for better representation only.

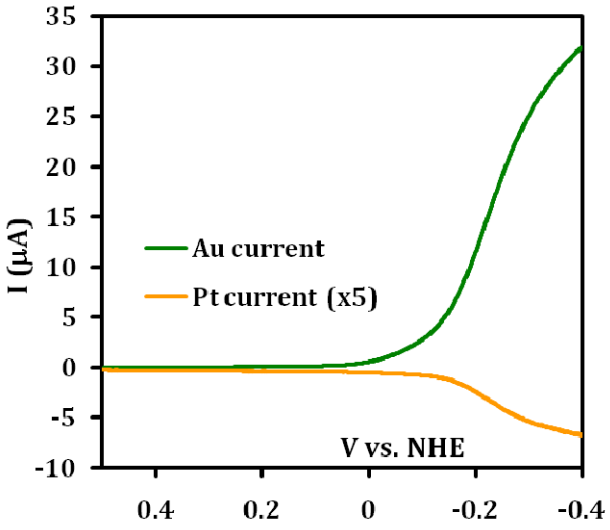

Fig. S10. RRDE plot of heme physiabsorbed on C_8SH SAM, showing the Au current (red) and corresponding pt ring current (blue) in pH 7 buffer obtained at a scan rate of 10 mV/s and rotation speed of 300 rpm.

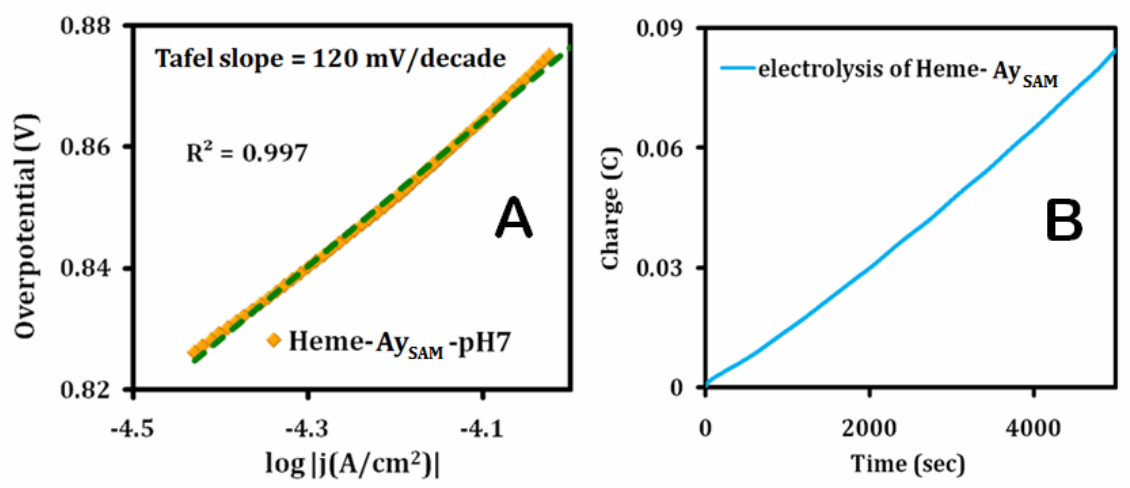

Fig. S11. (A) Tafel plot of the ORR by heme-Ay$_{SAM}$ obtained at 50 mv/s in pH 7 buffer. (B) Plot of charge consumed *vs.* time elapsed in a bulk electrolysis experiment of Heme-Ay$_{SAM}$ in pH 7 buffer. The working was held at -250 mV *vs.* NHE.

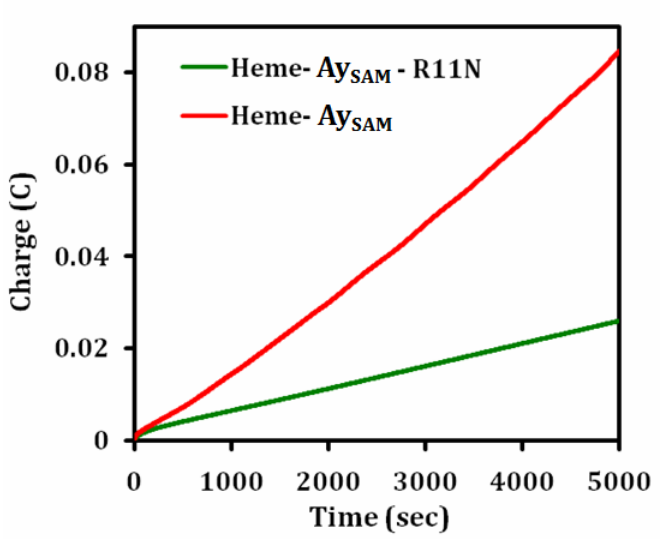

Fig. S12. Plot of charge consumed *vs.* time elapsed in a bulk electrolysis experiment of Heme-Ay$_{SAM}$ (red) and Heme-Ay$_{SAM}$-R11N mutant (green) in pH 7 buffer. The working were held at -250 mV *vs.* NHE.

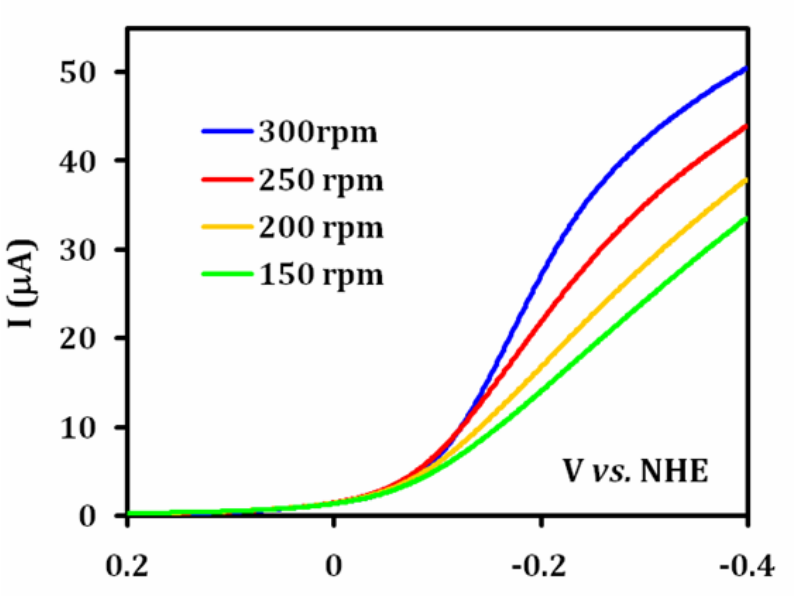

Fig. S13. RDE plots of heme, physiabsorbed on C_8SH modified Au electrode, in air saturated pH 7 buffer at a scan rate of 50 mV/s at multiple rotations.

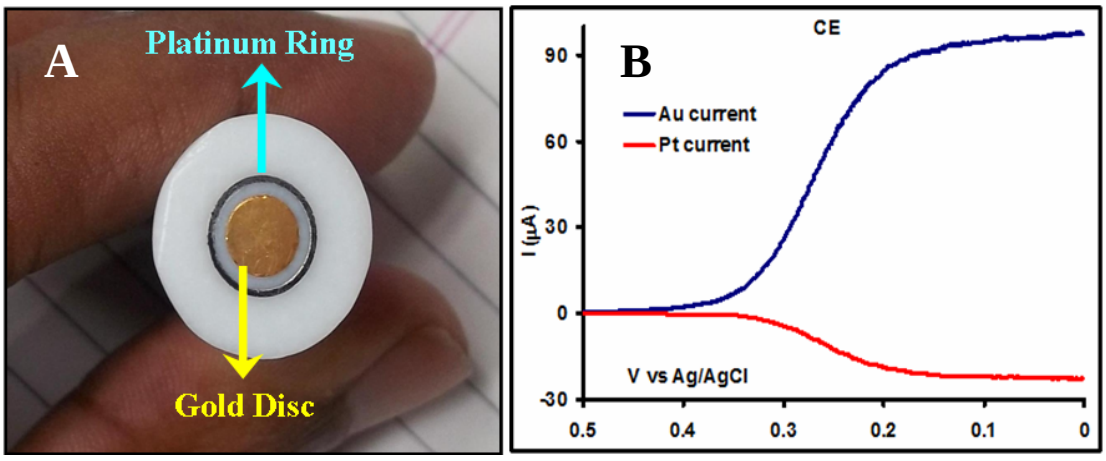

Fig. S14. (A) RRDE assembly showing the Au disc and Pt ring. (B) A Collection efficiency plot.

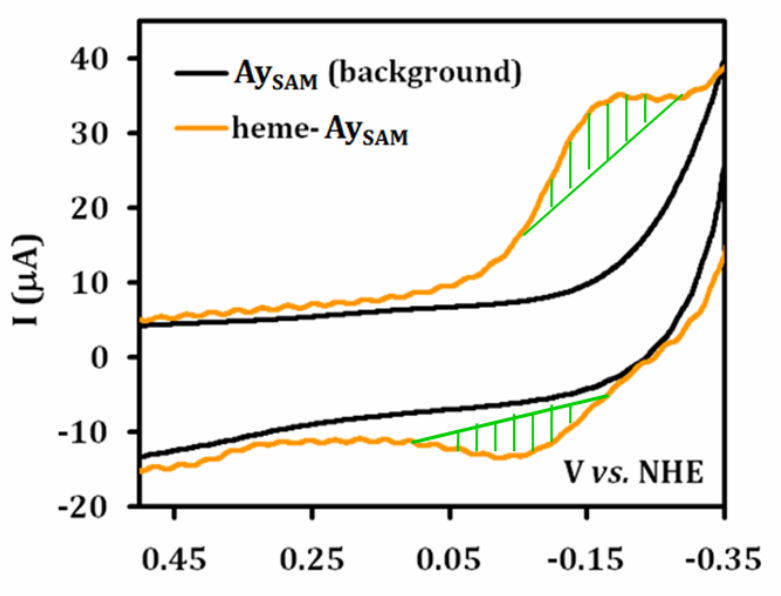

Fig. S15. CV data of Ay_{SAM} (black) and heme-Ay_{SAM} (yellow) in deoxygenated pH 7 buffer under Ar atmosphere at a scan rate of 1 V/s. The marked region indicates the area integrated in order to calculate surface coverages.

References:

1. **Y. Zhang and G. S. Wilson, *J. Electroanal. Chem.*, 1993, 345, 253.**
2. **J. Jordan, E. Ackerman and R. L. Berger, *J. Am. Chem. Soc.*, 1956, 78, 2979.**
3. **M. S. Al-Abdul-Wahid, C.-H. Yu, I. Batruch, F. Evanics, R. g. PomÃ¨s and R. S. Prosser, *Biochemistry*, 2006, 45, 10719.**
4. **S. YÄ±lmaztÃ¼rk, N. Ercan and H. s. DeligÃ¶z, *Applied Surface Science*, 2012, 258, 3139.**
5. **A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications* J Wiley & Sons, New York, 2001.**
6. **J. P. Collman, N. K. Devaraj, T. P. A. Eberspacher and C. E. D. Chidsey, *Langmuir*, 2006, 22, 2457.**
7. **D. Pramanik, K. Sengupta, S. Mukherjee, S. G. Dey and A. Dey, *J. Am. Chem. Soc.*, 2012, 134, 12180.**
8. **K. Sengupta, S. Chatterjee, S. Samanta and A. Dey, *Proc. Natl. Acad. Sci.*, 2013, 110, 8431.**