Supporting Information

Electrode Preparation

Pyrolytic graphite electrodes (0.07 cm^2) were used for voltammetry with the basal plane exposed. First, the electrodes were sanded to expose a fresh surface, and then sonicated briefly in water. Next, the electrodes were polished sequentially with 0.3 μm and then 0.05 μm alumina slurries, followed by sonication and then drying in air. HOPG electrodes were cleaned by removing a few layers from the surface with adhesive tape prior to use.

Mutagenesis and Protein Expression

The single surface cysteine mutant at position 387 was constructed by SOEing as previously described[1]. During cloning, a 6-His tag was introduced at the C-terminus of the protein after Thr^{464}, followed by a stop codon. Cloning, expression, purification, and quantification of the protein proceeded as previously described[2].

Protein and Electrode Modification

N-(1-pyrene)iodoacetamide (Py) was purchased from Molecular Probes. A 20 μM stock solution of the Py probe was made in DMSO. A solution containing 50 μM protein in 30 mM KP_i pH 7.4 buffer was partially degassed by blowing Ar over it for 20 minutes. Next, 15 μL of the Py solution was slowly added to the protein solution with stirring. This mixture was protected from light with aluminum foil and left to stir in the dark under a constant stream of argon at room temperature. After two hours, the solution was passed over a PD 10 column (Pharmacia) equilibrated with 30 mM KP_i pH 7.4 to separate the unreacted probe from the Py-hBM3 conjugate. Electrodes were filmed by suspending them in 200 μL of the Py-hBM3 solution in the fridge (~ 10^oC) overnight.

Fluorescence of the pyrene probe was used to confirm labeling of the protein, as described by Molecular Probes. Emission spectra of the purified pyrene-protein conjugate were recorded after excitation at 340 nm. For the assay, the protein solution was diluted to 2.6 μM with 30 mM KP_i pH 7.4.

The labeling efficiency was determined using Ellman's reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid) for quantifying free sulfhydryl groups as described by Pierce Biotechnology. A purified solution of the protein-pyrene conjugate was diluted to 0.4 μM with 50 mM KP_i pH 8 to a final volume of 1 mL. 50 μL of a 4 mg/mL solution of Ellman's reagent were added to the diluted protein, and the absorption spectrum was recorded. The number of free cysteines was calculated using an extinction coefficient of 14,150 $M^{-1}cm^{-1}$ at Abs_{412}. Based on this, the labeling efficiency was determined to be 50%.

Voltammetry

A CH Instruments Electrochemical Workstation system was used for all electrochemistry. Voltammetry was performed in a three compartment cell, with a frit and Luggin capillary separating the Pt wire auxiliary and Ag/AgCl reference, respectively. The buffer solution, 50 mM KP_i / 20 mM KCl / pH 7, was thoroughly degassed by bubbling argon through it for at least 20 minutes. After degassing, argon was continuously blown through the headspace of the cell to maintain an anaerobic environment.

Rotated-Disk Electrode Experiments

A 0.13 cm^2 pyrolytic graphite electrode with the basal plane exposed was used. The electrode was sanded, polished sequentially with 0.3 μm and 0.05 μm alumina slurries, sonicated, and dried. Experiments were carried out in a two compartment cell, with the working electrode

separated from the Ag/AgCl reference and Pt auxiliary electrodes. The solution was approximately 30 mL of 50 mM KPi / 20 mM KCl / pH 7. All experiments involving oxygen reduction were carried out in air saturated buffer ($[O_2] \approx 250$ μM). A Pine Instruments rotator was used to control the rotation rate, while a CH Instruments Electrochemical Workstation was used to apply the potential and monitor the current response.

BPG-RDE experiments with Py-hBM3 films were conducted by performing bulk electrolysis at -0.5 V at different rotation rates. Typically, the limiting current used to make the Levich plot is the steady state value observed during the reaction. However, it was found that decomposition of the films occurred over the course of the experiment. This was illustrated both by the decay of the current response with time during the rotation experiment, and the reduced current response of the film after rotation compared to before rotation as seen by cyclic voltammetry. Thus, the limiting current was taken to be the initial current after the start of electrolysis with rotation. This was done by plotting the log(current) vs. time, and extrapolating the data back to t = 0 s. Rotation rates above 700 rpm resulted in highly irreproducible data due to rapid film decomposition.

Hydrogen Peroxide Assay

Hydrogen peroxide concentrations were determined using the Amplex Red fluorescence assay supplied by Molecular Probes, using the 96-well procedure precisely as described by the company. Based on the standard curve generated, the detection limit was determined to be ~ 30 nM.

Calculation of Electron Transfer Rates

Rates for the photochemical system were calculated using the equation

$$k_{ET} = A \exp[-(\Delta G^o + \lambda)/(4\lambda k_B T)$$

where λ was approximated to be 0.8 eV at 298 K. The zero-driving force rates ($\Delta G^o = 0$) were calculated by equating the pre-exponential factors (A) for the photochemical experiments with known rate (ΔG^o = -0.98 eV or -1.11 eV for substrate free and substrate bound hBM3, respectively) to the scenario with no driving force and solving for $k_{ET}(\Delta G^o = 0)$.

The standard rate constant (k^o) for the electrochemical system was calculated from Laviron's theory using the theoretical curve for $\alpha = 0.5$. The peak splitting ($E_{p,c} - E_{p,a}$) determined at 100 V/s was 130 mV.

References

(1) Sevrioukova, I. F.; Immoos, C. E.; Poulos, T. L.; Farmer, P. *Isr. J. Chem.* **2000**, *40*, 47-53.

(2) Cirino, P.; Arnold, F. *Adv. Synth. Catal.* **2002**, *344*, 1-6.