Supporting Information

Protein Expression and Purification

Expression, purification and quantification of the WT and 1-12G heme domains proceeded as previously described.[1]

Electrode Preparation and Voltammetry

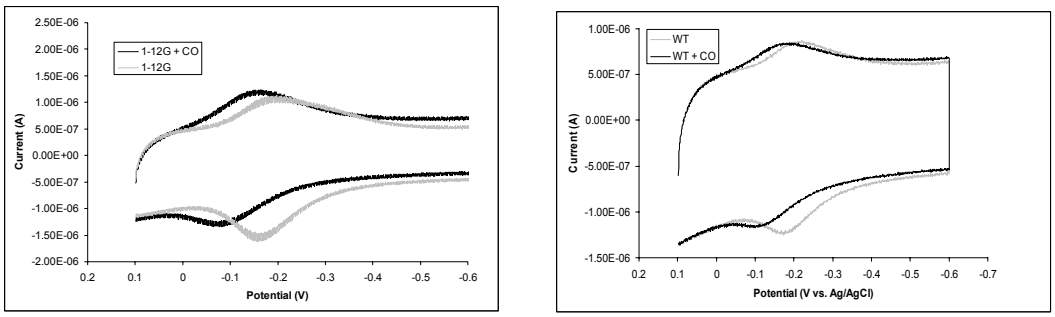
Electrodes for voltammetry (0.07 cm^2) were made using the basal plane of pyrolytic graphite. The surfaces were prepared by sanding briefly with 600-grid sandpaper, followed by polishing with 0.3 and 0.05 μm alumina slurries. The electrodes were then sonicated and dried in air. Protein films were applied by placing 5 μL of 105 μM 1-12G or 21 μM WT in 30 mM KP_i pH 7.4, 5 μL 10 mM DDAB in water, and 5 μL 10 mM NaPSS in 30 mM KP_i pH 7.4 on the electrode surface. The electrodes were covered under water-saturated air overnight, followed by uncovering and drying in air overnight.

A CH Instruments Electrochemical Workstation system was used for the reactions. Voltammetry experiments were performed in a 3-compartment cell, using a platinum wire auxiliary and a Ag/AgCl reference (BAS). All experiments were performed under argon in thoroughly degassed buffer (50 mM KP_i, 20 mM KCl, pH 7) unless otherwise stated.

Rotated-Disk Electrode Experiments

0.5 cm^2 glassy carbon electrodes were used. The electrodes were polished sequentially with 0.3 and 0.05 μm alumina, 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 KP_i / 20 mM KCl / pH 7. All experiments involving oxygen reduction were carried out in air saturated buffer at room temperature (20°C, $[O_2]$ = 290 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. Protein-DDAPSS films were found to be stable up to 1500 rpm.

RDE experiments were conducted by performing bulk electrolyses at -0.5 V (vs. Ag/AgCl) at different rotation rates. Typically, the limiting current used to make the Levich and Koutecky-Levich plots is the steady state value observed during the reaction. However, it was found that degradation of the protein signal 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.



(a) (b)

Figure S1. Voltammograms of a) 1-12G and b) WT in DDAPSS on BPG in the presence and absence of CO in solution.

References

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