Tools for Resolving Complexity in the Electron Transfer Networks of Multiheme Cytochromes *c*

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200 A В 200 0 0 Current (nA) -200 200 -400 200 200 -400 -600 0 0 -200 -200 -800 -400 -0.4 0.0 -0.4 0.0 -600-0.3 -0.2 -0.1 -0.5 -0.4 0.0 0.1 -0.5 0.0 -0.4 -0.3 -0.2 -0.1 0.1 Potential (V vs SHE) Potential (V vs SHE)

Electronic supplementary information.

Fig. S1. Electron transfer between redox partners at a PGE electrode in which the voltammogram of the adsorbed protein is shown in red and the adsorbed protein with partner in the cell solution is shown in black. A. CymA is adsorbed with STC. Inset shows the electrochemical response of STC in solution with a blank electrode. B. CymA adsorbed with DmsE in solution. Inset shows the electrochemical response of DmsE in solution with a blank electrode.



Fig. S2. Example of selective electron transfer. A. Voltammogram of STC adsorbed on the electrode (red), adsorbed STC with MtrA in cell solution (black). B. Voltammogram of CymA adsorbed on the electrode (red), adsorbed CymA with MtrC in cell solution (black).



Fig. S3. Examples of unidirectional electron transfer between redox partners at a PGE electrode. A. Voltammogram of MtrA adsorbed on the electrode (red), diffusional voltammogram of MtrC (blue), adsorbed MtrA with $MtrC_{red}$ in cell solution (black). B. Voltammogram of MtrC adsorbed on the electrode (red), diffusional voltammogram of MtrA (blue), adsorbed MtrC with MtrA in cell solution (black).



Fig. S4. SDS-PAGE of anaerobic affinity chromatography. Fraction 1 is CymA-MBP flow through, fractions 2-6 are the wash step, fraction 7 is MtrA, and fractions 8 and 9 are CymA eluting from the column. The slightly fainter lower band for seen in fraction 1 is likely due to CymA clipping.



Fig. S5. UV-Vis spectra from anaerobic affinity chromatography between CymA and c5. CymA_{red} sample loaded onto column (black), $c5_{red}$ fraction rinsed from column (red), CymA_{part.red} fraction eluted from column (green). Inset zooms 500 to 580 nm region of fully and semi-reduced CymA to highlight difference in absorbance at 552 nm.

 $\frac{A_{552}(\text{red})-A_{552}(\text{ox})^{\text{off column}}}{A_{552}(\text{red})-A_{552}(\text{ox})^{\text{dithionite}}} \times 100 = \% \text{ reduced}$

Equation 1 Percentage reduction of MtrA by CymA is equal to the difference in absorbance of the oxidized and reduced spectra at 552 nm of MtrA eluted from column divided by the difference in absorbance of MtrA when reduced with dithionite.