Chemical Communications

SUPPLEMENTARY INFORMATION

Redox tuning of cytochrome b_{562} through facile metal porphyrin substitution

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Materials and Methods

Sample preparation. Construction of the Asp50 to Cys mutant (D50C) of cyt b_{562} has been described previously.[1] Apo-cyt b_{562} D50C was extracted from the periplasmic fraction of *E. coli* and purified as outlined previously.[1]

Copper protoporphyrin IX (Cu-PP) and zinc protoporphyrin IX (Zn-PP) were purchased from Santa Cruz biotechnology, Inc. US. The molecules were dissolved in 1 M NaOH prior to use. To avoid molecular aggregation and photodegradation, Zn-PP and Cu-PP solutions were stored in the dark, kept at 4 °C and centrifuged prior experiments. To reconstitute holo-cyt b_{562} with different metalloporphyrins, excess of protoporphyrin (~ 10 molar equivalents) was added to apo-cyt b_{562} D50C in 10 mM phosphate buffer pH 6.2. The mixture was then incubated in the dark at 4 °C overnight and purified using centrifugal filters (3kDa cut-off). The purity of the resulting samples was verified through SDS-PAGE analysis (Figure S1). Only a single band was observed that corresponds to the molecular weight of cyt b_{562} D50C (12 kDa).[2]

Absorption and circular dichroism spectroscopy. UV-Vis absorption spectra were measured on a Hewlett-Packard (HP) 8450A diode array UV-Vis spectrophotometer at 25°C. Apo-cyt b_{562} D50C concentration was determined by measuring the absorbance at 280 nm (extinction coefficient 3.0 mM^{-1} cm^{-1}).[2] Cu-PP and Zn-PP affinity for cyt b_{562} D50C were performed as described previously.[1] CD spectroscopy was performed in 1.0 cm path length quartz cuvettes on a Jasco J-710 spectropolarimeter. Spectra were collected from 190 to 250 nm with a response time of 1 s and an increment of 0.2 nm. The reported spectra are the average of 5 scans. Base lines obtained from samples containing only buffer were subtracted from all the data reported.

Electrochemical measurements. Au(111) electrodes were electrochemically etched in 1 M H_2SO_4 and washed with 1 M HCl and water, then annealed for 8 hours at 880 °C.[3, 4] Prior to protein deposition the electrodes were annealed with a H_2 flame and the Au(111) single crystal surface was protected with hydrogen saturated water. The electrodes were subsequently incubated in 50-60 µM protein solution at 4 °C overnight. Prior to undertaking the experiments the electrodes were rinsed with water to remove the non-chemically adsorbed molecules. The proteins were checked for stability by measuring the UV-Vis spectra before and after the incubation. Electrochemical measurements were performed using an Autolab potentiostat (Eco Chemie, Netherlands) controlled by a general purpose electrochemical software package (GPES). Cyclic voltammetry experiments were carried out in a single-compartment glass cell consisting of a gold working electrode, a platinum wire gaze auxiliary electrode, and a freshly realized reversible hydrogen reference electrode (RHE). The RHE potential was measured against SCE potential at the end of each experiment and the obtained values used to correct the measured potentials. A constant gas stream of purified Argon was flowed through the electrochemical cell prior and during the experiments to generate an inert atmosphere. The sample was allowed to equilibrate for 5 minutes in the same buffer as the protein, 10 mM phosphate buffer pH 6.2.

Results

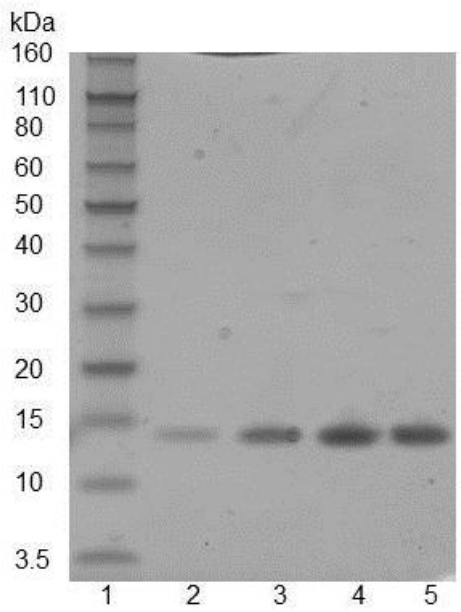

Figure S1. SDS-PAGE analysis of D50C cyt b_{562}. Lane 1, protein molecular weight standards; lane 2 apo-cyt b_{562} D50C; lane 3 D50C cyt b_{562} reconstructed with heme; lane 4 D50C cyt b_{562} reconstructed with Cu-PP; lane 4 D50C cyt b_{562} reconstructed with Zn-PP.

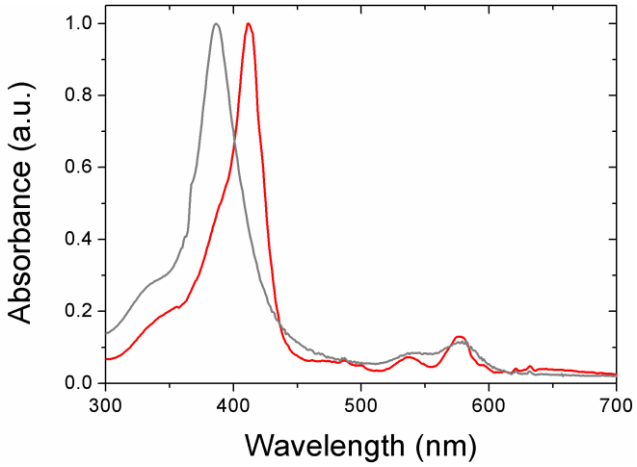

Figure S2. Normalized UV-Vis spectrum of 20 μM of Cu-PP alone (grey line) or 20 μM cyt b_{562} D50C reconstructed with 20 μM Cu-PP (red line). All spectra were recorded in 10 mM phosphate buffer pH 6.2.

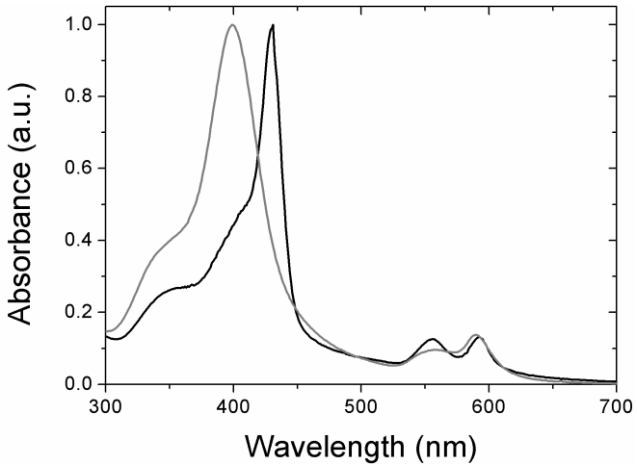

Figure S3. Normalized UV-Vis spectrum of either 20 μM of Zn-PP alone or 20 μM cyt b_{562} D50C reconstructed with 20 μM Zn-PP (black line) (grey line). All spectra were recorded in 10 mM phosphate buffer pH 6.2.

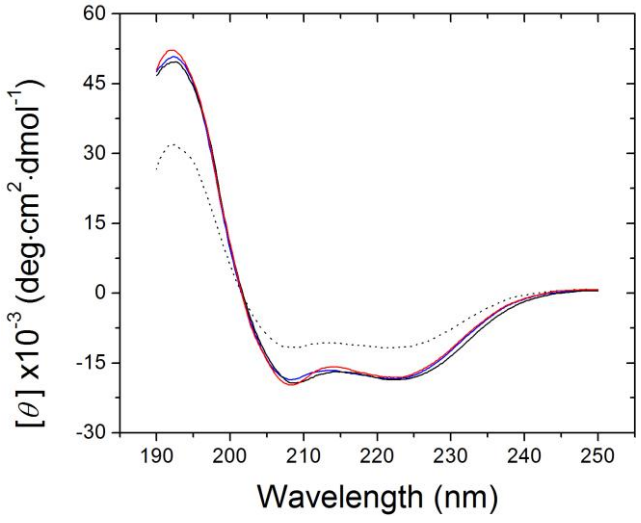

Figure S4. Far UV circular dichroism spectra of apo-cyt b_{562} D50C (dotted line), cyt b_{562} D50C reconstructed with heme (blue), Cu-PP (red) and Zn-PP (black) in 10 mM phosphate buffer pH 6.2 at 25°C. The proteins concentrations were 10 μM.

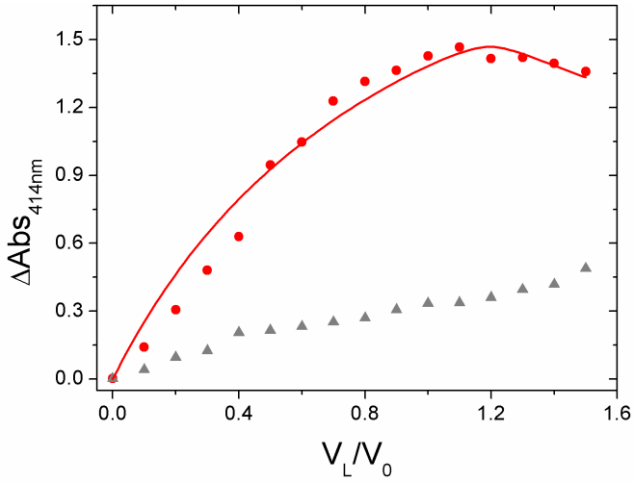

Figure S5. Spectrophotometric titration of apo-cyt b_{562} D50C with Cu-PP (red). Aliquots of Cu-PP sampling a final concentration range of 0 to 15 μM were titrated into 1 ml of 20 μM apo-cyt b_{562} D50C in 10 mM phosphate buffer pH 6.2. A blank (grey) titration of Cu-PP into buffer is also shown (grey). Error bars are not shown to improve the clarity of the figure.

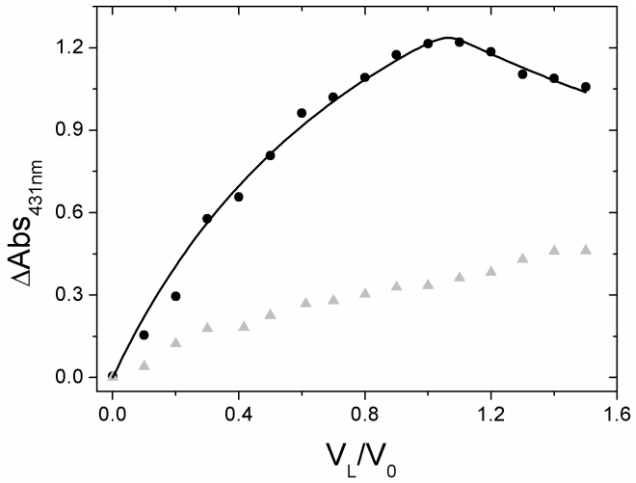

Figure S6. Spectrophotometric titration of apo-cyt b_{562} D50C with Zn-PP (red). Aliquots of Zn-PP sampling a final concentration range of 0 to 15 μM were titrated into 1 ml of 20 μM apo-cyt b_{562} D50C in 10 mM phosphate buffer pH 6.2. A blank (grey) titration of Cu-PP into buffer is also shown (grey). Error bars are not shown to improve the clarity of the figure.

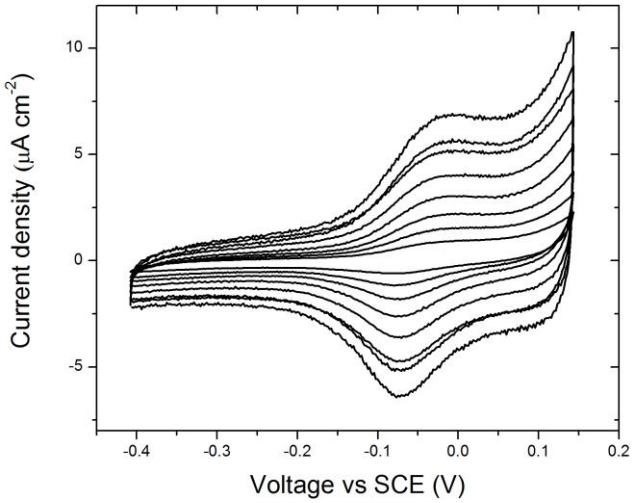

Figure S7. Cyclic voltammetry of Cu-PP in 10 mM phosphate buffer pH 6.2 at various scan rates (from 0.1 Vs^{-1} to 0.9 Vs^{-1}). For performing the experiment 1 ml of 100 µM Cu-PP in 0.1 M NaOH were dissolved in 5 ml of 10 mM phosphate buffer pH 6.2.

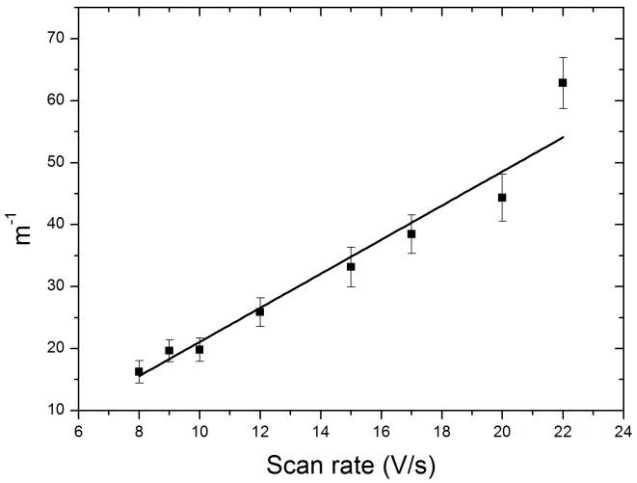

Figure S8. Plot of m^{-1} versus scan rate. The m values were obtained from the peak separations in the cyclic voltammograms. The solid curve is the best linear fit of the experimental data

Notes and references

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[2] Y. Feng, S. G. Sligar and A. J. Wand, *Nat. Struct. Biol.*, 1994, **1**, 30–35.
[3] A. Hamelin and A.M. Martins, *J. Electroanal. Chem.*, 1996, **407**, 13-21.
[4] J. Clavilier, R. Faure, G. Guinet, R. Durand, *J. Electroanal. Chem.*, 1979, **1**, 198- 205.