Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Spectroelectrochemistry of cytochrome *c* and azurin immobilized in nanoporous antimony tin oxide

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Materials and Methods

Preparation of Antimony Tin Oxide

In a typical procedure, 0.132 g of $SbCl_3$ (Sigma-Aldrich, 99.9%) was dissolved in 5.5 g of absolute ethanol. 2.80 g of $SnCl_4 \cdot 5H_2O$ (Sigma-Aldrich, 99%) was dissolved in 7 g of deionized water separately and added into the $SbCl_3$ solution while stirring. Poylethylene glycol (PEG) was then added to the solution and stirred for 30 min to achieve homogeneity of the solution. The amount of PEG was ~8% of the total weight of the final precursor solution. In the solution, 1.0 g of resorcinol (Sigma-Aldrich, 99%) and 1.5 g of 37% formaldehyde solution (Sigma-Aldrich, 7 – 8 % methanol as stabilizer) were added while stirring. In the solution, epichlorohydrin (Sigma-Aldrich, 99%) was added to the clear solution in a molar ratio of (Sn + Sb):epichlorohydrin of 1:7 and stirred for 1 min.

Thin film preparation

The thin films were prepared on fluorine-doped tin oxide (FTO) glass slides (Hartford Glass) by employing the doctor-blade method. The FTO glass slides were first rinsed with water, acetone and ethanol in that order. The slides were then covered with transparent Scotch tape except the area (*ca.* 0.3×2 cm^2) where the thin films were to be cast. About 0.1 ml of the precursor solution was dropped on each slide and quickly spread with a glass pipette. Any excess amount of the precursor solution was removed. The slides were then immersed horizontally in a paraffin oil bath in a petri dish, left for 24 hours at room temperature and finally heated in a laboratory oven at 70 °C for 72 hours. The films on the slides remained transparent but became hard and slightly reddish after the heating. After removal from the oven, the slides were

extracted from the oil bath, thoroughly rinsed with hexanes, the transparent Scotch tape was removed from the slides, and they were left in air at room temperature for 24 hours for drying. Finally, the slides were placed in an ashing furnace at 500 °C for 10 hours for calcination of the films.

Cytochrome *c* spectroelectrochemistry

Bovine heart cytochrome *c* was obtained from Sigma and used without further purification. Biological buffers were of the highest grade commercially available and were also used without further purification. Solutions for electrochemical experiments were prepared using purified water (resistivity 18.2 MΩ cm^{-1}). Electrochemical experiments were routinely conducted in 15 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) with 0.1 M NaCl added as supporting electrolyte. Solutions were adjusted to the desired pH by addition of HCl or NaOH.

Routinely the cleanliness of ATO slides used as working electrodes was electrochemically monitored. Adsorbed species were removed by thorough washing with 1 M NaCl solutions. Following cleaning, cytochrome *c* was adsorbed to the ATO by incubation of the slide in a buffered 150 μM protein solution for thirty minutes at room temperature. After incubation, excess protein solution was withdrawn with a pipette, and the electrode surface was thoroughly rinsed with pure water. Electrochemical measurements were made using a CH Instruments Model 1200A series electrochemical analyzer controlled by CHI1200A software. Simultaneous UV-vis spectroscopy was collected by a Perkin-Elmer Lambda 650 UV/Vis spectrophotometer. The electrochemical cell consisted of a quartz cuvette (5 mm pathlength) in which the ATO coated slide (0.5x 2 cm ATO patch coated on the conductive side of a 3.5x0.75x0.25 cm FTO covered glass slide, Hartford Glass), a 0.5 mm diameter platinum wire

counter electrode, and a Ag/AgCl reference electrode (2 mm diameter, Driref-2, World Precision Instruments) were linearly positioned in 500 µL of protein-free buffered electrolyte. Effects of uncompensated cell resistance were minimized by invoking the positive-feedback iR compensation function of the potentiostat, set at a value slightly below that at which current oscillations emerge. All potentials were corrected to the standard hydrogen electrode (SHE) according to the equation $E_{SHE}=E_{Ag/AgCl}+197$ mV at 25 °C.[1] Electrochemical data were analyzed with SOAS, an electrochemical program freely available for download on the web at http://bip.cnrs-mrs.fr/bip06/software.html.[2] Transmission spectra were recorded through the ATO coated working electrode with the cell potential simultaneously controlled.

Azurin Purification and electrochemistry

Purification of azurin followed a previous procedure[3] with the following modifications. The pH of the isolated periplasmic protein solution was adjusted to 4.1 with 20% acetic acid. The first cation exchange column (CM-sepharose, 25 x 20 cm) was equilibrated with 5 mM ammonium acetate buffer at pH 4.1, and the protein was eluted with a pH gradient of 4.1-6.35. The third column, or second cation exchange column, followed the same pH gradient. The gel-filtration column was excluded from the protocol as it was not found to be necessary. Dialysis was used to exchange the buffer following each chromatographic separation. Concentration of the protein was undertaken only after the final column. Protein purity was verified both by SDS-PAGE and the A_{280}/A_{623} via UV-Vis spectroscopy (0.53). Following electrode cleaning, azurin was adsorbed to the ATO by incubation of the slide in a 50 µM protein solution buffered at pH 4 for

thirty minutes at room temperature. After incubation, excess protein solution was withdrawn with a pipette, and the electrode surface was thoroughly rinsed with pure water.

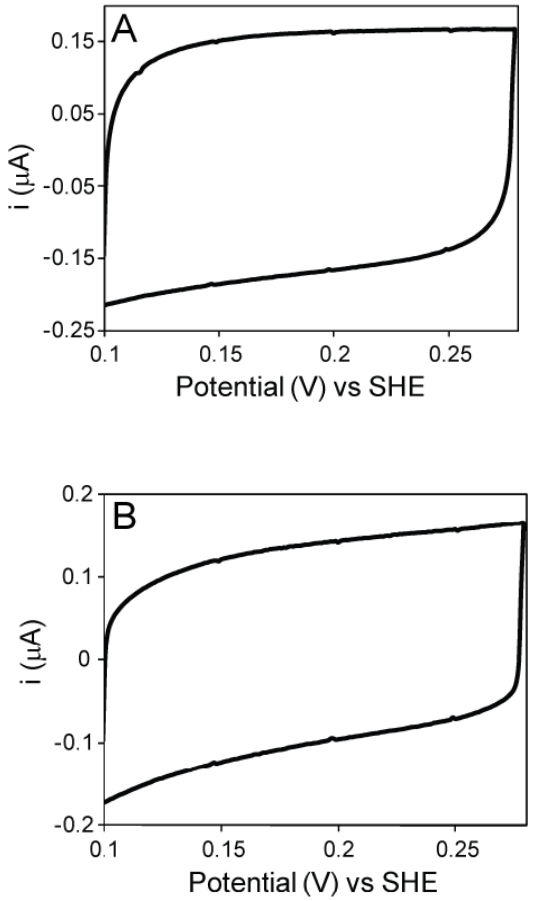

Fig. S1: Cyclic voltammograms from control experiments demonstrating that cyt*C* does not significantly adsorb to a planar (A) ITO or (B) FTO slide. Experimental conditions are as in Figure 2, but slides without ATO coatings were used as working electrodes.

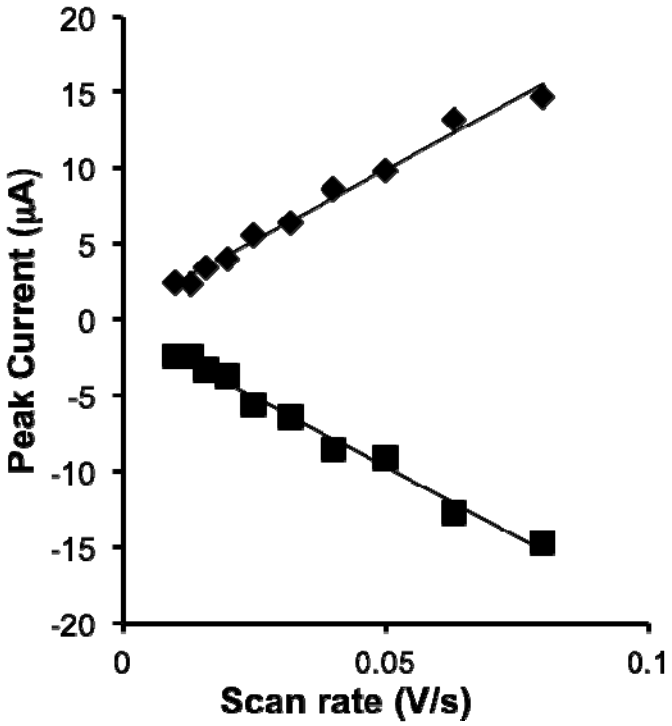

Fig S2: Dependence of cyclic voltammetric peak current from adsorbed cytC on scan rate demonstrating that the electrochemistry arises from an adsorbed species.

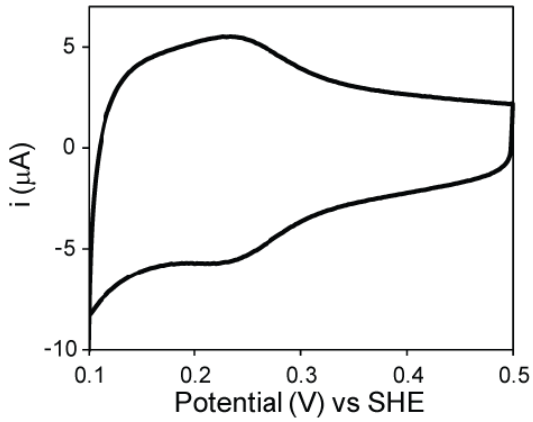

Fig S3: Cyclic voltammogram of an ATO coated slide with adsorbed cyt*C* after storage for twenty-one days in pH 7 buffer (15 mM HEPES, 0.1 M NaCl). The voltammogram was obtained in solution of the same composition.

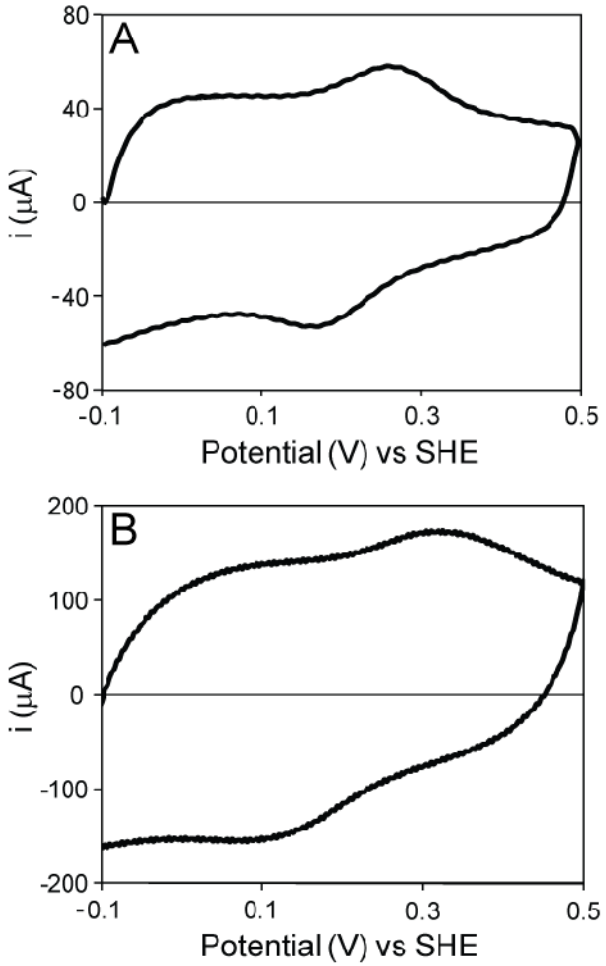

Fig. S4: Cyclic voltammograms of cyt*C* adsorbed to an ATO film at fast scan rates: (A) 100 mV s^{-1} and (B) 316 mV s^{-1}. Other conditions are as indicated in Figure 2.

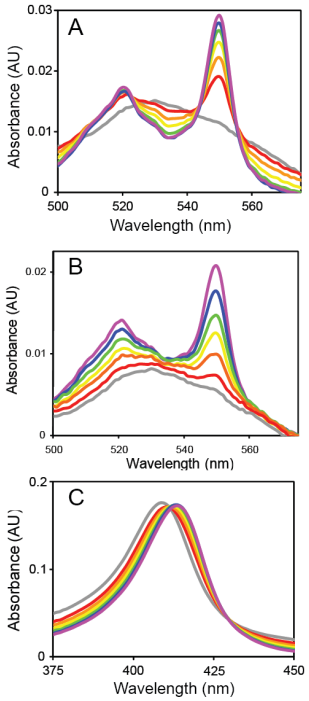

Fig. S5 UV-vis absorbance spectra from cyt*C* adsorbed to an ATO coated slide. (A) Spectra in the Q-band region as the protein is reduced from the air-oxidized state. Spectra are colored as follows: air oxidized (grey), 199 mV (red), 179 mV (orange), 159 mV (yellow), 139 mV (green), 119 mV (blue), 89 mV (violet). Each potential was held for ten minutes before the acquisition of spectra. (B) Analogous spectra obtained starting from the reduced form of cyt*C*. Spectra are colored as follows: 309 (grey), 279 mV (red), 259 mV (orange), 239 mV (yellow), 219 mV (green), 199 mV (blue), 179 mV (violet). (C) UV-vis absorbance spectra in the Soret region as a function of applied redox potentials colored for the same potentials as in panel A. Other experimental conditions are as indicated in the legend of Figure 2.

1. A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, John Wiley & Sons, Inc., New York, 2001.
2. V. Fourmond, K. Hoke, H. A. Heering, C. Baffert, F. Leroux, P. Bertrand and C. Léger, *Bioelectrochemistry*, 2009, **76**, 141-147.
3. G. D. Manetto, D. M. Grasso, D. Milardi, M. Pappalardo, R. Guzzi, L. Sportelli, M. P. Verbeet, G. W. Canters and C. La Rosa, *Chem. Bio. Chem.*, 2007, **8**, 1941-1949.