

The spatial and sequential immobilisation of proteins at adjacent electrodes

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Reagents

1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide (CMC) and ethanol were purchased from Fluka. Potassium phosphate (K_2HPO_4) was purchased from Riedel de Haen. Dielectric paste was purchased from Gwent Electronics. All other reagents were purchased from Sigma-Aldrich. All solutions were prepared using ultrapure water (resistivity of $18.2\text{ M}\Omega\text{ cm}$) prepared using an Elgastat maxima –HPLC (Elga, UK). Gold disc electrodes (diameter 2 mm) were purchased from CH instruments.

Preparation of sputtered gold electrodes

Sputtered gold electrodes were prepared as described previously¹. Two separate gold electrodes were prepared on a gold modified glass slide (2 cm^2) by removal of a narrow strip of gold from the middle of the slide using a circular saw. Electrical connection was provided by soldering a 2 cm length of copper wire (0.2 mm) to the electrodes. The solder was coated with an epoxy resin and the electrode area (disc of area 0.283 cm^2) was defined using a dielectric paint (Gwent Electronics Materials Ltd., UK).

Instrumentation

Electrochemical experiments were conducted using a CHI832 bipotentiostat (CH Instruments, Austin, Texas, USA). Sputtered gold or gold disc electrodes, platinum wire and Ag/AgCl were used as the working, counter and reference electrodes (IJ Cambria Scientific Ltd., UK), respectively.

4. Electrode modification

Commercially available gold disc electrodes were cleaned by immersion in a solution of pirhana (*Caution*) followed by polishing with alumina ($0.05\text{ }\mu\text{m}$). The electrodes were modified with an insulating thiol by immersion in a solution of 1-hexadecanethiol ($5 \times 10^{-4}\text{ M}$ in 20 mM phosphate buffer (pH 7.4) and ethanol (1:1 (v/v))). The potential was scanned over the range 0.4 – 0.6 V for 100 cycles at 10 mVs^{-1} . The integrity of the SAMs was examined by cyclic voltammetry using $K_3(FeCN)_6$ as a redox probe. Cyclic voltammetry experiments for the electrochemical deposition of the binary SAM were performed over a series of potential ranges (0.2 - 0.6 V, 0.4 - 0.8 V, 0.6 - 1.0 V, 0.8 - 1.2 V, 1.0 - 1.5 V) for 25 cycles at a scan rate 50 mVs^{-1} in 1 mM solutions of thiols. Mixed SAMs were prepared from $1 \times 10^{-3}\text{ M}$ thiol solution ($5 \times 10^{-4}\text{ M}$ 11-mercapto-undecanoic acid and $5 \times 10^{-4}\text{ M}$ 6-mercapto-1-hexanol) in phosphate buffer (20 mM, pH 7.4) by applying a single potential scan between 0 and 1.5 V at a scan rate of 50 mVs^{-1} . Electrochemical desorption of 1-hexadecanethiol was performed by scanning between -1.5 and -2 V at a scan rate of 50 mVs^{-1} for 50 cycles. The surface carboxyl groups of the SAM were activated by immersion in 5 mM N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate (CMC), prepared in 100 mM K_2HPO_4 – KH_2PO_4 buffer (pH 7.0) for 30 min at 4 °C. Covalent attachment of the activated carboxyl groups to the lysine residues of *cyt c* was achieved by exposure to a solution of *cyt c* ($35\text{ }\mu\text{M}$) in 4.4 mM phosphate (K_2HPO_4 – KH_2PO_4 , pH 7.0) buffer for 60 min at 4 °C. The surface coverage of *cyt c* was determined by integration of the charge obtained for the oxidation peak.

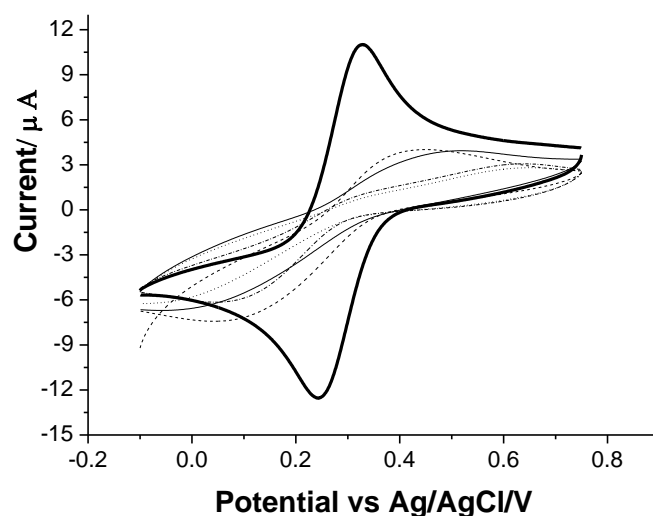


Figure S1. Cyclic voltammograms of $\text{K}_3\text{Fe}(\text{CN})_6$ (2 mM) in 150 mM KCl at gold disk electrodes (2 mm diameter); cleaned electrode (solid and thick line), after 5 min of passive adsorption of thiols (solid line), after 5 minutes of potential assisted deposition at 0.4 V (dotted line), 0.6 V (dot and dashed line) and 0.8 V (dashed line) in a 1 mM solution of 11-mercaptoundecanoic acid and 1-hexanethiol in 20 mM phosphate buffer. Scan rate of 100 mV s^{-1} .

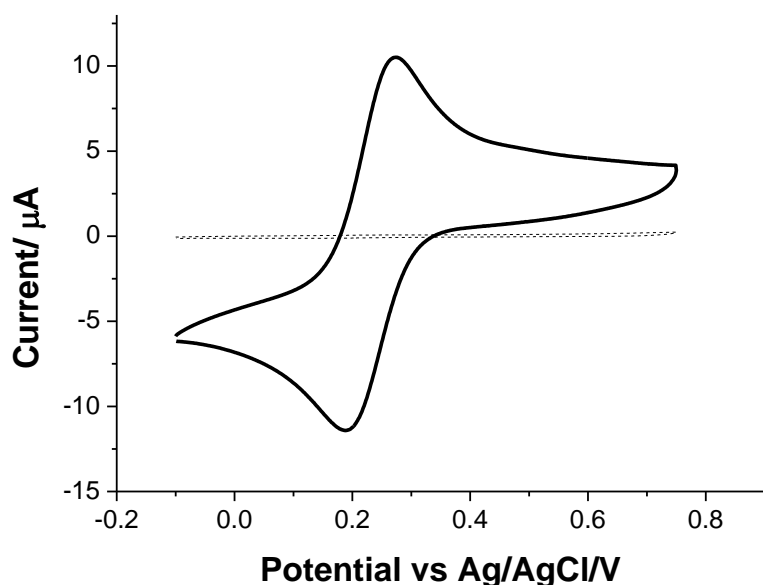


Figure S2. Cyclic voltammograms of $\text{K}_3\text{Fe}(\text{CN})_6$ (2 mM) in 150 mM KCl at (solid line) gold disk electrodes and (dotted line) after deposition of 1-mercaptoundecanoic acid and 1 hexanethiol using a single potential scan ($0 - 1.5 \text{ V}$ at 50 mVs^{-1}) in 20 mM phosphate buffer. Scan rate of 100 mV s^{-1} .

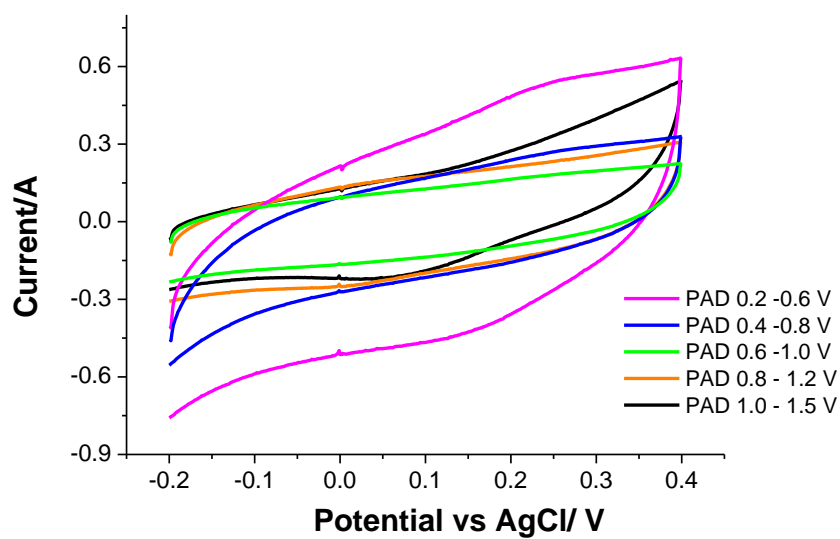


Figure S3. Cyclic voltammograms of *cyt c* covalently immobilized on electrodeposited mixed SAMs of 11-mercaptopundecanoic acid and 1-hexanethiol deposited at various potential ranges. Scan rate of 100 mV s^{-1} .

References

- (1) Scanlon, M. D.; Salaj-Kosla, U.; Belochapkine, S.; MacAodha, D.; Leech, D.; Ding, Y.; Magner, E. *Langmuir* **2012**, *28*, 2251.