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$_2$HPO$_4$) 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 MΩ 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 previously$^1$. 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 μm). The electrodes were modified with an insulting thiol by immersion in a solution of 1-hexadecanethiol (5x10$^{-3}$ 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 1x10$^{-3}$ M thiol solution (5 x 10$^{-4}$ M 11-mercapto-undecanoic acid and 5 x 10$^{-4}$ 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$_2$HPO$_4$–KH$_2$PO$_4$ 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 μM) in 4.4 mM phosphate (K$_2$HPO$_4$–KH$_2$PO$_4$, 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 K$_3$Fe(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 K$_3$Fe(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 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-mercaptoundecanoic acid and 1-hexanethiol deposited at various potential ranges. Scan rate of 100 mV s$^{-1}$.

**References**