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Fast, long-range electron-transfer reactions of a 'blue' copper protein coupled non-covalently to an electrode through a stilbenyl thiolate monolayer

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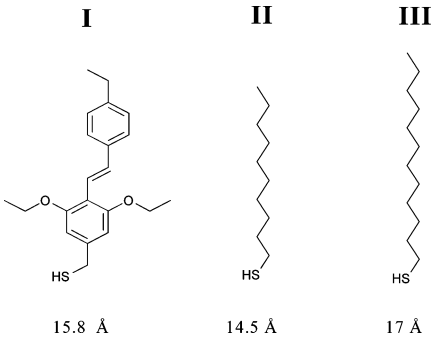
A self-assembled monolayer (SAM), formed by the *in situ* saponification of a stilbenyl thioacetate on a gold electrode, yields fast electron transfer (ET) (the exchange rate at zero driving force exceeds 1600 s^{-1}) with adsorbed molecules of the 'blue' copper protein, azurin, over a distance exceeding 15 Å.

There is great interest in the chemical modification of electrodes to achieve reversible electrochemistry of adsorbed redox enzymes.[1–8] Now, in the quest for electrode surfaces of increasing biological relevance, complexity, and stability, it is becoming highly desirable to achieve fast and specific electron transfer (ET) over a far greater distance than can be achieved with SAMs of short-chain alkane thiols. Recent studies by Creager and Chidsey have identified redox systems that form SAMs on gold electrode surfaces and exhibit remarkably high rates of long-range ET.[9–11] Chidsey has studied the distance dependence of ET rates in systems consisting of ferrocenyl-terminated oligomeric phenylenevinylene (OPV) thiols of varying lengths linked to the electrode and concluded that the electronic coupling distance decay constant β is close to zero.[9] This contrasts with the results obtained for long-chain aliphatic alkyl linkers for which rates decrease exponentially with increasing chain length and β-values are around 1 $Å^{-1}$. Furthermore the β-value for OPVs is lower than those measured for similar molecules based on phenyleneethynyl units.[11] It was thus important to determine whether an OPV thiol could provide such excellent electron coupling when the redox centre is the active site of a *non-covalently* bound protein molecule. In this Communication we report the synthesis and ET properties of a short OPV-type molecule: a stilbenyl thiol (I) designed to bind non-covalently the 'blue' copper protein azurin and give fast electron exchange with the active site over a distance greater than 15 Å.

Azurin is an ET protein containing a single type 1 Cu centre that is located about 6 Å below the surface in a hydrophobic region. We and others have shown recently that azurin can be adsorbed at gold electrodes modified with alkanethiols, the terminal CH_3– functionality being well suited for interacting with the hydrophobic patch close to the Cu.[6–8] Alkanethiols form ordered SAMs, as observed by scanning tunneling microscopy.[6] The ET rate constant varies with the length of the alkyl chain, although evidence suggests[6,7] that a limiting value ($< 10^4$ s^{-1}) is reached either at short chain length or high driving force (similar rate limitations are found in studies with cytochrome c[4]). To establish the magnitude of ET rates possible with long, low-β linkers, we have synthesised a prototype OPV-type molecule, {3,5-diethoxy-4-[(*E*)-2-(4-ethylphenyl)vinyl]-phenyl}methanethiol (**I**), which presents a hydrophobic *ethyl* surface group when assembled as a monolayer on the electrode surface. We have compared the ET kinetics with those obtained for azurin adsorbed at decanethiol (**II**) and dodecanethiol (**III**), the lengths of which are, respectively, slightly shorter and longer.

I was prepared and characterised as described below.§ The alkylthiols **II** and **III** were obtained from Aldrich. Electrochemical methods were as described previously[7,8] and the stilbenyl-modified



electrodes were prepared following a recently reported procedure in which the oxidatively unstable thiol was generated by saponification of the corresponding thioacetate immediately prior to modification of the electrode.[12]

Fig. 1 shows voltammograms obtained, at a scan rate of 100 V s^{-1}, for azurin adsorbed on a Au electrode modified with SAMs of **I** (dark line) and **II** (light line).¶ The uniform background has been subtracted to emphasise the faradaic features. At pH 4.0, the redox reaction of the blue Cu site is not complicated by coupling to a proton-transfer reaction.[8] The smaller peak separation shows that ET at **I** is much faster, even though **II** is shorter. At slow scan rates (not shown) the half-height widths are about 100 mV; close to expectations for a one-electron reaction in a homogeneous population of redox couples. The peaks broaden as the scan rate is raised, although, even at 500 V s^{-1} they remain compact at approximately 200 mV in width. Typical electroactive coverages of azurin on the electrode were in the range 8–12 pmol cm^{-2}.

Fig. 2 shows the resulting 'trumpet' plots, in which the peak positions for oxidation and reduction are plotted as a function of log (scan rate). This is a useful method of displaying and analysing the characteristics of protein film ET reactions over a wide time domain.[2] At low scan rates, the oxidation and reduction peak positions are close, and their average is the reduction potential $E^{0\prime}$ of the Cu site. Although the experiments were performed under identical conditions of pH and temperature, the $E^{0\prime}$ value is slightly

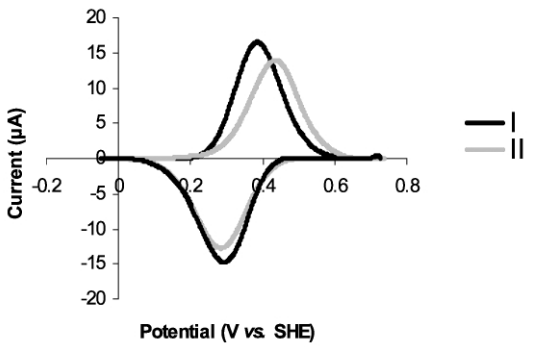


Fig. 1 Voltammograms for azurin adsorbed at Au-SAM electrodes prepared using **I** and **II**. Electrolyte: 0.02 M NaOAc, 0.1 M Na_2SO_4, pH 4.0, 0 °C. Scan rate 100 V s^{-1}. Cycles initiated from high-potential limit.

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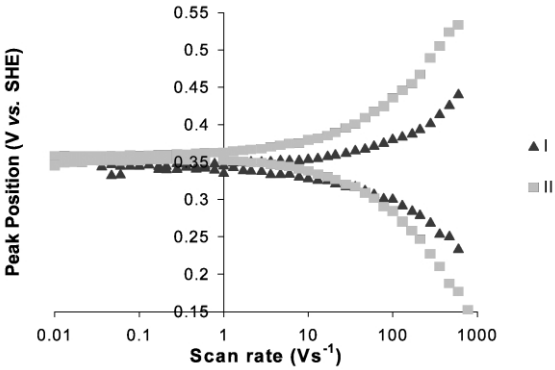

Fig. 2 'Trumpet plots' comparing electrochemical properties of azurin adsorbed on SAMs composed of **I** or **II**. Conditions: 0.02 M NaOAc, 0.1 M Na_2SO_4, pH 4.0, 0 °C. Cycles initiated at high-potential limit.

lower at the stilbenyl surface (347 mV compared to 356 mV). This may reflect a difference in the interfacial potential drop or a small change in the environment of the Cu site, as the stilbenyl surface may be more permeable to water molecules. As the scan rate increases, the oxidation and reduction peak potentials separate, the shape depending on the ET kinetics (the rate constant k_0 is determined from fits to the Butler–Volmer equation) and the participation of reactions coupled to electron transfer. The value obtained for **I** is 1626 s^{-1}, which compares with 481 s^{-1} for **II**, and 60 s^{-1} for **III** (data not shown, but reported earlier[7]). Results were similar regardless of whether the scan was started from the high or low potential limit. Similar results were also obtained when the film was formed using the stilbenyl *disulfide* which can be formed from the oxidative coupling of two molecules of **I**.

The much faster ET rate obtained with **I** compared to that for aliphatic SAMs is consistent with a lower value of β as expected for a conjugated bridge. With the ethyl group as the protein binding functionality, the interfacial interaction should be very similar to that for the aliphatic SAMs, and in each case the electron has to transfer across the remaining distance between the protein surface and the Cu. However, the important point is that the increase in rate constant is *not* several orders of magnitude, as observed for the ferrocenyl-terminated OPVs.[9] This result is important as it supports an emerging model for protein intermolecular and interfacial ET reactions, in which the optimal rate of ET is limited by the probability of achieving, by rapid fluctuations, good electronic coupling through the interfacial assembly of protein and solvent molecules.[4,6,7,13–15] These processes are probably irrelevant for coupling to a small molecule through a covalent attachment as in the ferrocenyl-terminated SAMs, but may be crucial for non-covalent coupling to a protein. Nevertheless, with an electrochemical exchange rate constant exceeding 10^3 s^{-1}, the time domain complies with turnover rates of most enzymes, so that interfacial ET need not be rate limiting.[16] In practical terms, the results demonstrate the feasibility of creating SAMs to provide reversible ET across distances equivalent to those encountered in biological membranes, thereby allowing fast ET to biological structures of greater complexity than has been possible with shorter SAMs.

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Notes and references

§ **I** was prepared in four steps. First, (4-bromo-3,5-diethoxyphenyl)methanol **1** was converted to 2,6-diethoxy-4-(hydroxymethyl)benzaldehyde **2**: *n*-BuLi in hexane (1.6 M, 9.1 cm^3, 23 mmol) was added to a stirred solution of **1** (2.5 g, 9.1 mmol) in dry THF (100 cm^3) at −78 °C under Ar. DMF (7.9 cm^3, 90 mmol) was added to the reaction after 1 h. The reaction mixture was then stirred for 30 min. Aq. HCl (3 M, 50 cm^3) was added and the mixture allowed to warm to room temperature. **2** (1.9 g, 57%) was isolated after work up and purification: m.p. 94–95 °C. (Found: C, 64.3; H, 7.2. $C_{12}H_{16}O_4$ requires C, 64.3; H, 7.2%); ν_{max}(KBr)/cm^{-1} 3494 (OH) and 1684 (C=O); $\lambda_{max}(CH_2Cl_2)$/nm ($\log_{10}\varepsilon$/$dm^3mol^{-1}cm^{-1}$) 276 (4.31) and 323 (3.83); δ_H(400 MHz; $CDCl_3$) 1.43 (6 H, t, *J* 7.0, CH_3), 2.88 (1 H, bs, OH), 4.07 (4 H, q, *J* 7.0, CH_2), 4.64 (2 H, s, ArCH_2), 6.49 (2 H, s, ArH) and 10.42 (1 H, s, CHO); δ_C(100 MHz; $CDCl_3$) 14.6, 64.5, 64.8, 102.2, 113.2, 150.3, 161.6 and 189.5; *m*/*z* [CI+] 225.1 (MH^+, 100%).

2 was then reacted to form {3,5-diethoxy-4-[(*E*)-2-(4-ethylphenyl)vinyl]-phenyl}methanol **3**: Dry THF (5.0 cm^3) was added to a stirred mixture of (4-ethylbenzyl)phosphonic acid diethyl ester[17] (0.69 g, 2.7 mmol) and **2** (0.50 g, 2.2 mmol), under Ar. Potassium *tert*-butoxide (0.55 g, 4.9 mmol) was added and the reaction mixture was stirred for 22 h. **3** (0.40 g, 55%) was isolated after work up and purification: m.p. 103–104 °C. (Found: C, 77.0; H, 8.2. $C_{21}H_{26}O_3$ requires C, 77.3; H, 8.0%); ν_{max}(KBr)/cm^{-1} 3378 (OH) and 1574 (C=C); $\lambda_{max}(CH_2Cl_2)$/nm ($\log_{10}\varepsilon$/$dm^3mol^{-1}cm^{-1}$) 317 (4.40), 329 (4.42) and 345sh (4.21); δ_H(400 MHz; $CDCl_3$) 1.27 (3 H, t, *J* 7.5, CH_3), 1.50 (6 H, t, *J* 7.0, CH_3), 1.83 (1 H, bm, OH), 2.68 (2 H, q, *J* 7.5, $ArCH_2$), 4.12 (4 H, q, *J* 7.0, CH_2), 4.66 (2 H, d, *J* 5.5, $ArCH_2$), 6.57 (2 H, s, ArH), 7.20 (1 H, ½AA′BB′, ArH), 7.44–7.48 (3 H, m, ArH and vinyl H) and 7.68 (1 H, d, *J* 16.5, vinyl H); δ_C(100 MHz; $CDCl_3$) 14.9, 15.6, 28.6, 64.3, 65.6, 103.4, 114.3, 119.2, 126.3, 128.0, 132.0, 137.0, 140.8, 143.1 and 158.1; *m*/*z* [CI+] 327.2 (MH^+, 100%).

3 was converted into thiolacetic acid {3,5-diethoxy-4-[(*E*)-2-(4-ethylphenyl)vinyl]benzyl} ester **4**: Diethyl azodicarboxylate (2.8 cm^3, 18 mmol) in dry THF (5.0 cm^3) was added to triphenylphosphine (4.6 g, 18 mmol) cooled to 0 °C under argon. The mixture was stirred for 20 minutes at 0 °C and then **3** (1.2 g, 3.5 mmol) and thiolacetic acid (1.3 cm^3, 18 mmol) in dry THF (10 mL) were added. The reaction mixture was stirred for 16 h at room temperature. **4** (0.96 g, 71%) was isolated after work up and purification: m.p. 87–88 °C; (Found: C, 71.6; H, 7.6. $C_{23}H_{28}O_3$ requires C, 71.8; H, 7.3%); ν_{max}(KBr)/cm^{-1} 1687 (C=O); $\lambda_{max}(CH_2Cl_2)$/nm ($\log_{10}\varepsilon$/$dm^3mol^{-1}cm^{-1}$) 320 (4.88), 327 (4.89) 331 (4.90) and 346sh (4.69); δ_H(400 MHz; $CDCl_3$) 1.26 (3 H, t, *J*7.5, CH_3), 1.46 (6 H, t, *J* 7.0, CH_3), 2.39 (3 H, s, $COCH_3$), 2.66 (2 H, q, *J* 7.5, $ArCH_2$), 4.09 (6 H, m, $ArCH_2$ and CH_2), 6.50 (2 H, s, ArH), 7.19 (2 H, ½AA′BB′, ArH), 7.40–7.47 (3 H, m, ArH and vinyl H) and 7.66 (1 H, d, *J* 16.5, vinyl H); δ_C(100 MHz; $CDCl_3$) 14.9, 15.6, 28.6, 30.3, 34.1, 64.3, 105.6, 114.2, 119.1, 126.3, 128.0, 132.0, 137.0, 137.3, 143.1, 158.0 and 195.3; *m*/*z* [CI+] 385.4 (MH^+, 37%).

4 was converted to **I** as follows: NH_4OH (10 μL) was added to an Ar purged solution of **4** in DMF (500 μL, 0.5 mM). This mixture was again purged with Ar and left for 10 min before the gold electrodes were immersed.

¶ Electrodes for azurin voltammetry were cleaned using standard procedures,[7,8] then incubated for 6 hours with a 0.5 mM solution of the appropriate thiol in DMF. Electrodes were rinsed consecutively with chloroform, ethanol and water, before azurin was adsorbed by incubating the electrode overnight in a 2.4 μM solution at 20 °C. Na_2SO_4 was used to avoid complications caused by the specific adsorption of chloride ions.

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