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Fast electron transfer through a single molecule natively structured redox protein†

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The electron transfer properties of proteins are normally measured as molecularly averaged ensembles. Through these and related measurements, proteins are widely regarded as macroscopically insulating materials. Using scanning tunnelling microscopy (STM), we present new measurements of the conductance through single-molecules of the electron transfer protein cytochrome \( b_{562} \) in its native conformation, under pseudo-physiological conditions. This is achieved by thiol (SH) linker pairs at opposite ends of the molecule through protein engineering, resulting in defined covalent contact between a gold surface and a platinum–iridium STM tip. Two different orientations of the linkers were examined: a long-axis configuration (SH-LA) and a short-axis configuration (SH-SA). In each case, the molecular conductance could be ‘gated’ through electrochemical control of the heme redox state. Reproducible and remarkably high conductance was observed in this relatively complex electron transfer system, with single-molecule conductance values peaking around 18 nS and 12 nS for the SH-SA and SH-LA cytochrome \( b_{562} \) molecules near zero electrochemical overpotential. This strongly points to the important role of the heme co-factor bound to the natively structured protein. We suggest that the two-step model of protein electron transfer in the STM geometry requires a multi-electron transfer to explain such a high conductance. The model also yields a low value for the reorganisation energy, implying that solvent reorganisation is largely absent.

1 Introduction

Long range electron transfer is central to processes essential to life, including photosynthesis, respiration and many enzymatic catalytic events.1,2 Electron transfer (ET) proteins are the components responsible for these processes and perform their function through binding, organising and tuning redox active metals and co-factors. To date, most experimental studies on ET through proteins have been achieved by a combination of chemical modification and spectroscopic measurement.3,4 These have largely been interpreted in terms of transport through a relatively isotropic dielectric medium, well-approximated by widely applicable parameters,5 or a through-bond model in which the detailed protein structure influences electron transport to a significant extent.3,4

High resolution tools, such as mechanically controlled break junctions, scanning tunnelling microscopy (STM)-based methods and conducting atomic force microscopy (cAFM), have greatly advanced our ability to investigate single molecule conduction and have been widely applied to a range of small organic and inorganic single molecules.6–15 However, to apply these tools to measure reliably electronic conductance of structurally complex, and mechanically fragile, single protein molecules, several conditions must be fulfilled. These include (a) effective organisation of protein molecules on a solid surface with sufficient stability against desorbing, (b) good electronic coupling between a protein molecule and external electrodes (i.e. electrical contacts to form molecular junctions), (c) control of molecular orientation and (d) conformation relevant to their biological structure and function under the experimental conditions required for measurements. These conditions have only been partially fulfilled in previous attempts.16–26 Conductance measurements on proteins by scanning probe techniques have nearly all involved a tunnelling barrier between tip and molecule,20–26 or the application of mechanical force to improve the electrical contact between tip and molecule.23 But recently, we have been able to measure STM conductance of protein...
molecules in air, contacted to two metallic electrodes via chemical anchoring groups. In the present study we show the first single-molecule electrochemical-STM conductance measurements of a heme protein, cytochrome \textit{b}$_{562}$ (cyt \textit{b}$_{562}$), of controlled orientation, and contacted directly via two thiol anchoring groups. By operating in solution under electrochemical control, the potential of the tip and substrate can be independently adjusted with respect to a pseudo-reference electrode in the solution. With fixed tip–substrate potential difference, the molecular conductance was found to depend strongly on the substrate potential, with the conductance of the equilibrium potential of the STM tip and substrate can be independently controlled, and contacted directly via two thiol anchoring groups. In the present study we show the first single-molecule electrochemical-STM conductance measurements of a heme protein, cytochrome \textit{b}$_{562}$ (cyt \textit{b}$_{562}$), of controlled orientation, and contacted directly via two thiol anchoring groups. By operating in solution under electrochemical control, the potential of the tip and substrate can be independently adjusted with respect to a pseudo-reference electrode in the solution. With fixed tip–substrate potential difference, the molecular conductance was found to depend strongly on the substrate potential, with the conductance of the equilibrium.

### 2 Results and discussion

#### 2.1 Controlled protein orientation, conformation and electrode binding

Cyt \textit{b}$_{562}$ is an ET protein found in the periplasm of the bacteria \textit{Escherichia coli}. The protein binds non-covalently the redox-active iron protoporphyrin IX (heme) via two axial ligands. The central ion can switch between the Fe$^{2+}$ and Fe$^{3+}$ state, with the redox potential defined by heme–protein interactions. Using rational protein engineering, pairs of cysteine residues were introduced that allow cyt \textit{b}$_{562}$ to bind through metal–thiol interactions in defined orientations with respect to the electrode surface and an STM tip. Two sets of cysteine pairs were introduced to sample the two orientations of the approximately prolate spheroidal cyt \textit{b}$_{562}$: (a) the Cys21–Cys50 pair located at opposing ends of protein’s long axis (denoted as SH-LA cyt \textit{b}$_{562}$), leading to a tip-to-surface distance of ~5.2 nm (Fig. 1(a), left) and (b) the Cys5–Cys104 pair binding across the short axis of the protein (SH-SA cyt \textit{b}$_{562}$) leading to a tip-to-surface distance of ~2.4 nm (Fig. 1(a), right). Cyt \textit{b}$_{562}$ with only the Cys50 mutation (DS5C) was constructed as a control to confirm the effects of direct linkage of the cysteine pair protein variants to both tip and substrate. All the cysteine mutants bound heme in the same manner as the original wild-type protein, as verified by UV-visible spectroscopy and hemin titration experiments.

Protein together with the reducing agent 1,4-dithiothreitol (DTT), which prevents formation of disulfide bonds, was absorbed on a gold surface to form a mixed self-assembled monolayer. All the measurements were performed in a pseudo-biological buffer (sodium phosphate, pH 6.2) at room temperature (23 ± 2 °C). These combined experimental conditions create a well-defined environment compatible with the protein retaining its native structure and ET function for single-molecule measurements.

Cyt \textit{b}$_{562}$ deposition on Au(111) substrates produced species with defined molecular orientations in line with positioning of the cysteine residues and with dimensions consistent with retention of their native structure. STM images (Fig. 1(b)) obtained at different surface locations show well-dispersed protein molecules. The observed lateral dimensions of the imaged single molecules of either cyt \textit{b}$_{562}$ SH-LA (circular, with diameter 3.2 ± 0.5 nm, Fig. 1(b), left) or SH-SA (laterally elongated, with a long axis of 4.5 ± 0.7 nm, Fig. 1(b), right) are in good agreement with crystallographic and NMR data for the holo-protein. (ESI, Fig. S1† presents a more detailed analysis of molecular dimensions.) In contrast, the apo-protein did not retain its native structure on adsorption onto the gold surface (ESI, Fig. S3†). The measured equilibrium potential $E^\theta$ of −68 ± 2 mV vs. SCE for both cyt \textit{b}$_{562}$ SH-LA and cyt \textit{b}$_{562}$ SH-SA variants is very close to those reported for the wild-type cyt \textit{b}$_{562}$ adsorbed onto hydrophobically modified silver and on the Au(111) surface modified with a peptide linker monolayer. The electrochemical measurements together with the measured protein dimensions confirm that the protein retains its functional conformation.
when adsorbed onto the Au(111) surface via an introduced cysteine residue.

2.2 Electrochemical gating of single cyt b_{562} protein molecules

The apparent height of individual molecules, obtained from direct STM imaging, was found to depend on redox overpotential. Fig. 2(a) shows a series of STM images of cyt b_{562} SH-SA molecules recorded with the bias voltage $V_b = V_t - V_s$ between the tip (voltage $V_t$) and the substrate (voltage $V_s$) kept constant while adjusting the substrate potential relative to the reference electrode to control the redox state of the proteins. Apparent height versus overpotential $\eta = V_s - E^0_b$ in Fig. 2(b) clearly shows, as the redox state of the protein molecules is shifted relative to the Fermi energy of the metal substrate, a peak in the apparent height for both the SH-LA and SH-SA variants. The maximum in apparent height is, as observed in related experiments, close to the protein equilibrium redox potential $\eta = 0$. No change in lateral dimension was seen. STM-derived heights in proteins are known to be lower than the true height due to a relatively large separation between tip and surface during STM imaging at low set-point current, with only a slight lifting of the tip as it passes over the less conducting molecule. Variation in protein conductance rather than any significant protein conformational change is the likely dominant factor affecting the apparent height, as also confirmed by tunnelling current-distance results discussed below. These results can be described quantitatively with a protein conductance model of ‘two-step’ ET between tip and substrate through the redox level of the protein. In Fig. 3 we schematically illustrate the energy alignment of redox level, substrate and tip in the two-step redox processes. The current $I_t$ can be expressed:

$$I_t = \frac{2e n_{el} k_{stm} k_{mt}}{k_{stm} + k_{mt}}$$  \hspace{1cm} (1)

where $e$ is the electronic charge, $k_{stm}$ and $k_{mt}$ are the rate constants for ET between redox molecule and substrate (i.e. working electrode) and between STM tip and redox molecule, respectively, $n_{el}$ is the number of electrons coherently transferred whilst the redox level relaxes through the energy window $eV_b$ between the Fermi levels of the tip and substrate. The term $n_{el}$ describes the fact that, in contrast to the normally encountered case of redox ET between a metal electrode and a molecular electronic level, in the STM junction there is a strong coupling to two electrodes (tip and substrate). A large number of electrons is thus transferred via the molecular level between the electrodes before the reduced molecular level has time to reach its relaxed state, which may be below the Fermi level of the other electrode. We picture just the initially oxidised state for ease of description, but this applies similarly for an initially reduced state. This transfer can occur because within the energy window $eV_b$ there is a quasi-continuum of available empty states in one metal which can accept an electron, and full states in the other metal which can provide an electron. The rate at which the molecule can be oxidised/reduced by an electrode (which is limited by nuclear reorganisation processes) therefore does not limit the STM current for the strong coupling case, although it does influence the overall current. Using standard expression for rate constants, eqn (1) takes the following form at small overpotentials and bias voltages ($|e\xi\eta|, |e\gamma V_b| \ll \lambda$):

$$I_t = I_{0}\mu_{el}\left\{ \exp \left[ \frac{(\lambda + e\xi\eta + e\gamma |V_b|)^2}{4\lambda k_B T} \right] + \exp \left[ \frac{(-\lambda - e\xi\eta - e\gamma |V_b| + e|V_b|)^2}{4\lambda k_B T} \right] \right\}^{-1}$$  \hspace{1cm} (2)

![Fig. 2](image-url) **Fig. 2** In situ single-molecule mapping of redox state-dependent conductance. (a) Series of STM images for SH-SA molecules obtained at different overpotentials ($I_t = 35$ pA, $V_b = -0.4$ V, $\eta = E_k - E^0_b = -210, -100, -80, +130$ mV from left to right, scan area 45 × 45 nm, z-range 0–1.5 nm). (b) Dependence of apparent contrasts of SH-LA (left) and SH-SA (right) on the overpotentials applied to the working substrate electrode. The solid lines indicate theoretical fitting according to eqn (2) described in the text. Fitted parameters $\xi = 1, \gamma = 0.5, \lambda = 0.10 \pm 0.03$ eV and $\lambda = 0.15 \pm 0.03$ eV for SH-SA and SH-LA respectively. The measured vertical height of the protein in STM scans does not represent the true height of the protein due to the low conductance of biological molecules compared with the substrate, and the properties of the surrounding medium.
where $\lambda$ is the total nuclear (protein and solvent) reorganisation free energy, $\eta$ the substrate overpotential, $k_B$ Boltzmann's constant and $T$ the temperature. The terms $\xi$ and $\gamma$ represent formally the fraction of the substrate potential and bias voltage drops at the site of the heme group redox centre. $I_0$ is a constant, the most important part being the effective nuclear vibrational frequency of all the nuclear modes reorganised. The number $n_\lambda$ (which is proportional to $eV_b$) is large when the electronic coupling between the molecule and the enclosing electrodes is strong and the adiabatic limit of interfacial ET prevails, as in the present case.

Eqn (2) formally represents well the data from the STM contrast (solid lines in Fig. 2(b)). $\xi$ and $\gamma$ are not known exactly and are correlated in the confined space of the in situ STM tunnelling gap. As an illustration, if the redox center experiences all the substrate potential applied electrochemically ($\xi = 1$) and half of the bias voltage ($\gamma = 0.5$), the reorganisation free energy parameter $\lambda$ can be obtained from fitting, giving $\lambda = 0.10 \pm 0.03$ eV and $\lambda = 0.15 \pm 0.03$ eV for SH-SA and SH-LA, respectively. Even with correlations or non-ideal values of $\xi (<1)$, these fitted values of $\lambda$ represent upper limits on the reorganisation energy. Contributions to $\lambda$ from heme and other porphyrins have been determined experimentally and theoretically to be in the range 0.05–0.15 eV. The low limiting value of $\lambda$ indicates that reorganisation of the heme occurs during ET but much of the reorganisation of the solvent and of the protein scaffold ($\lambda \approx 0.7–1.1$ eV) is decoupled in the molecular scale solvent confinement of the STM tunnelling gap. We also note that transient spectroscopy measurements inevitably involve solvent reorganisation for surface-bound donors or acceptors. In contrast, in the STM measurement electrons are transferred between two free-electron reservoirs (metal substrate and tip, which essentially play the role of donor and acceptor of electrons during electron transfer) and their charge state is fixed by the applied bias. Thus reorganisation of the solvent surrounding the electrodes is not induced during electron transfer.

### 2.3 High single molecule conductance of cyt $b_{562}$

STM imaging gives an indirect measure of the electrochemical gating of the current through the protein via changes in image contrast. A more powerful approach is to quantify the current through the molecule versus applied bias or tip–surface distance. The latter, usually referred to as the $I_z$–$z$ technique, is commonly used for small molecules and allows unambiguous identification of data where a junction is clearly formed between the molecule and both tip and surface. Recently, the $I_z$–$z$ measurements have been applied to extract valuable information on the ET process for the protein azurin bound to the surface through a single cysteine residue present in the protein, but without a defined interaction with the tip. In our present study we take advantage of the free thiol group to make $I_z$–$z$ measurements which provides a defined covalent tip–molecule interaction and avoids a tunnelling barrier between tip and molecule, in the same manner that most small-molecule studies are performed. Furthermore, we ensure that only a single protein is measured at a time by imaging before and after the $I_z$–$z$ scans.

Molecules were first identified by imaging a small scanned area of 10 or 20 nm$^2$, (ESI, Fig. S7) and the STM tip was then positioned above a protein molecule with small set point tunnelling current ($\approx 35$ pA at $-0.1$ V). After disengaging the STM feedback system, the change in $I_z$ was recorded as a function of the tip displacement while the tip was repeatedly moved towards and away from the target molecule. As the tip is withdrawn, a plateau in the measured current may be observed in the $I_z$–$z$ curve just prior to losing contact with the molecule, at which point the current decreases rapidly (Fig. 4(a), left and middle). No current plateaux were observed for D50C proteins having just one cysteine residue, bound to the Au surface (Fig. 4(a), right) or for $I_z$–$z$ measurements away from proteins where the surface is only populated with 1,4-dithiothreitol molecules. Conductance histograms were constructed, without selection, from $I_z$–$z$ data and are shown in Fig. 4(b). Approximately 20% of the $I_z$–$z$ traces...
were non-exponential and exhibited plateaus with length ranging between 0.05 and 0.3 nm. These current plateaus are assigned to the cleavage of individual protein–tip junctions. Around 70% of traces failed to form a molecular junction and showed a monotonic exponential decay, which represents direct electron tunnelling from the STM tip. The remaining 10% of traces were too noisy to classify. The step lengths observed in the \( I_t - z \) traces are small (0.1–0.2 nm) compared with the molecular length, as is expected for these protein molecules since they bind to the surface in defined orientations, rather than lying more nearly flat on the surface as for measurements on conventional larger organic single molecules. In the latter case, there can be a larger range of distance over which the tip binds to one end of the molecule (as the tip is withdrawn from the surface and the angle between molecule and surface is altered) and so lead to a long plateau. One consequence of small step length is that peaks in the histograms are less pronounced.

The conductance histogram for cyt \( b_{562} \) SH-LA shows a main peak at 12.0 nS accompanied by a smaller peak at 17.8 nS, while only a single distinct peak at 17.8 nS is observed for cyt \( b_{562} \) SH-SA. The observation of two conductance values for cyt \( b_{562} \) SH-LA is unlikely to correspond to one and two protein molecules, since the higher conductance value is not simply double that of the lower and imaging of well-separated protein molecules was achieved. Similar features have been observed in several small organic molecular systems where it is known that the contact configuration is a factor in determining conductance. Another possible interpretation is that the two conductance values correspond to the two possible orientations of the SH-LA molecule (Fig. 1(a), left) on the surface, since the iron redox centre of the heme is asymmetrically positioned along the protein’s long axis. In contrast, the two possible orientations for cyt \( b_{562} \) SH-SA have similar distances between the heme centre and either thiol group, see Fig. 1(a). Further experimental and theoretical work is required to test these interpretations.

The \( I_t - z \) measurements were also performed at various substrate potentials to observe redox-state dependent conductance. Fig. 5 shows the conductance histograms for cyt \( b_{562} \) SH-SA in (a) the fully oxidised form, (b) at the equilibrium redox potential, and (c) in the fully reduced form. It is clear that the protein in its equilibrium state is significantly more conducting than in its fully oxidised and fully reduced form. This strong overpotential dependence of the conductance in \( I_t - z \) measurements confirms that the apparent height dependence on overpotential cannot purely be attributed to conformational changes.

A comparison of our results with those for selected molecules of similar length is summarised in Table 1. Combined with the results for SH-LA, this suggests that the placement of the iron moiety in the context of the protein plays a crucial role in ET. It is immediately noticeable that cyt \( b_{562} \) is highly conductive, even in its oxidised and reduced states. Its conductance is large compared to that for a dsDNA fragment or a conjugated polymer wire of similar length. In particular, the conductance of cyt \( b_{562} \) in its equilibrium state approaches 10 times higher than that of a single zinc porphyrin monomer with two thiol contact groups (ca. 2.2 nS). The conductance values of SH-LA and
substrate potentials applied: (top) the oxidised form (h
redox state. The redox state of the protein was controlled by the working
buffer (pH 6.2) at a fixed bias voltage of
measured molecule and the STM tip increases the measured
the observation that adding a second thiol contact between a
single cysteine linker to the gold electrode. This is consistent with
been reported to change the Au effective work function
52
might arise from a number of factors. For example, solvent has
and 2.7 nS for the SH-LA and SH-SA molecules respectively
b
what lower cyt
Fig. 5
Single-molecule conductance modulation of cyt b_{562} SH-SA by its
redox state. The redox state of the protein was controlled by the working
substrate potentials applied: (top) the oxidised form (\eta = +300 mV),
(middle) the equilibrium state (\eta = 0 mV), and (bottom) the reduced form
(\eta = -300 mV). The measurements were performed in 10 mM phosphate
buffer (pH 6.2) at a fixed bias voltage of -0.1 V.

SH-SA cyt b_{562} are around one order of magnitude greater than
that measured for azurin, another ET protein,\textsuperscript{18,22,50} having a
single cysteine linker to the gold electrode. This is consistent with
the observation that adding a second thiol contact between a
measured molecule and the STM tip increases the measured
conductance by an order of magnitude or more.\textsuperscript{6,54} The somewhat lower cyt b_{562} conductance values measured in air\textsuperscript{27} of 1.5
and 2.7 nS for the SH-LA and SH-SA molecules respectively
might arise from a number of factors. For example, solvent has
been reported to change the Au effective work function\textsuperscript{12} and increase single molecule conductance. The protein charge
distribution and reorganisation energy, whose influence on the
measured conductance is difficult to estimate, may also be affected. Strong solvent effects have been observed in some molecules.\textsuperscript{62}

Cyclic voltammetry measurements on azurin give ET rates\textsuperscript{43,54} around 10\textsuperscript{2} to 10\textsuperscript{3} s\textsuperscript{-1}, which is much lower than might be expected on the basis of the STM measurements. However, our EC-STM measurements involve strong coupling to two electrodes, whereas the measured transfer rate for voltammetry is limited by the lowest value of the ET rate between molecule and electrode, between molecule and solution (necessarily involving solvent reorganisation) and the inverse charging time constant of the double layer. Meaningful comparisons require ultra-fast voltammetry.\textsuperscript{55}

The nature of this high conductance for cyt b_{562} can be appreciated by the following considerations. Thiol linkers vastly increase conductivity of metal–molecule–metal junctions, and thiolated molecules are now ubiquitous in non-protein single-molecule electronics. The mechanism of the improved conductivity is the stronger quantum mechanical coupling to both tip and substrate of the chemisorbed molecular levels,\textsuperscript{56,57} resulting in a significant broadening \Delta E in their energy. Our results suggest that this is the case for the heme level of the proteins, although this requires verification by detailed electronic structure calculations since \Delta E depends sensitively on the molecular length, details of the contact and nature of the molecule. From the Heisenberg uncertainty relation \Delta E \Delta t \sim h the lifetime of this state \Delta t, which we denote as \tau_{el}, governs the intrinsic ET rate 1/\tau_{el}, as outlined by Datta.\textsuperscript{58} A broadening of around 1 meV, as found for example in DFT calculations\textsuperscript{59} of the porphyrin monomer of Table 1, corresponds to a rate of 10\textsuperscript{12} s\textsuperscript{-1}. A crude estimate based on the \beta factor of the \ell_{z}–\ell'_{z} curves\textsuperscript{59} is also consistent with a broadening of 1 and 10 meV, for the LA and SA molecules. There are uncertainties in such estimates, and this value might easily be exceeded.\textsuperscript{6}

In terms of the two-step model above, when the relevant redox state lies between the Fermi energies of tip and substrate, electrons are transferred at this rate between molecule and tip/substrate. The number of electrons that are transferred in a single vibrational relaxation passage of the molecular redox state through the energy window between the substrate and tip Fermi levels depends on the nuclear environment. This is characterised by a vibrational relaxation time \tau_{rel} that includes all the classical vibrational modes of the solvent that are dominated by low frequency relaxation,\textsuperscript{60} giving altogether \tau_{rel} \sim 10\textsuperscript{-12} s. This value includes a spectrum of values and could be larger if slow protein conformational modes and solvent confinement in the tunnelling gap are also included. If the redox level relaxes slowly enough (large \tau_{rel}) and the broadening is strong enough, then the ratio t_{el} / \tau_{rel} can become large and many electrons are transferred whilst the level relaxes. The diabatic limit of relatively weak interaction corresponds to \Delta E \sim 10\textsuperscript{-4} to 10\textsuperscript{-3} eV or \tau_{el} \sim (10 - 1) \times 10\textsuperscript{-12} s. Larger values take us into the adiabatic range:\textsuperscript{32} in the adiabatic limit we could take \Delta E \sim 10\textsuperscript{-2} to 10\textsuperscript{-1} eV giving \tau_{el} \sim (10 - 1) \times 10\textsuperscript{-12} s. Even for moderately adiabatic transitions the ratio t_{el} / \tau_{rel} is thus likely to assume significant values (orders of magnitude), allowing for the coherent transfer of a large number of electrons in a single \textit{in situ} STM event.
Table 1  Comparison of single-molecular conductance of cyt b₅₆₂ with selected systems

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Length/nm</th>
<th>Conductance/nS</th>
<th>Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyt b₅₆₂ SH-LA (redox protein)</td>
<td>5.2</td>
<td>12.0 (equilibrium state), 5.6 (oxidised), N/A (reduced)</td>
<td>Buffer solution and EC control</td>
<td>This work</td>
</tr>
<tr>
<td>Cyt b₅₆₂ SH-SA (redox protein)</td>
<td>2.4</td>
<td>17.8 (equilibrium state), 11.2 (oxidised), 14.3 (reduced)</td>
<td>Buffer solution and EC control</td>
<td>This work</td>
</tr>
<tr>
<td>6PTTF6 (redox molecule)</td>
<td>2.5</td>
<td>2.5 (on form), 0.5 (off form)</td>
<td>Buffer solution, EC control</td>
<td>Leary et al.42</td>
</tr>
<tr>
<td>Purinon monomer</td>
<td>1.8</td>
<td>2.15</td>
<td>Air</td>
<td>Sedghi et al.43</td>
</tr>
<tr>
<td>Poly-Zn-porphyrin (N = 4)</td>
<td>4.5</td>
<td>0.61</td>
<td>Air</td>
<td>Sedghi et al.43</td>
</tr>
<tr>
<td>Short α-helix polypeptide</td>
<td>2.2</td>
<td>1.7</td>
<td>Buffer solution</td>
<td>Scullion et al.44</td>
</tr>
<tr>
<td>Short polypeptide Cys-Gly-Cys</td>
<td>1.5</td>
<td>0.41</td>
<td>Buffer solution</td>
<td>Xiao et al.45</td>
</tr>
<tr>
<td>Cu-binding polypeptide</td>
<td>1.2</td>
<td>1.77</td>
<td>Buffer solution</td>
<td>Xiao et al.45</td>
</tr>
<tr>
<td>Cu(Cys-Gly-Cys)</td>
<td>3.8</td>
<td>10</td>
<td>Salt solution</td>
<td>Hihath et al.46</td>
</tr>
<tr>
<td>dsDNA fragments</td>
<td>5′GGAGCCCGAGG3′ (11 bp) (dA)₅-5′(dT)₅ (15 bp) (dG)₅-5′(dC)₅ (15 bp) Polyethylene-acryl molecular wire Polyconjugated molecular wires (N = 1–4)</td>
<td>5.0</td>
<td>0.51</td>
<td>Air</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>1.2</td>
<td>Air</td>
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<td></td>
<td></td>
<td>7.0</td>
<td>1.02</td>
<td>Air</td>
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<tr>
<td></td>
<td></td>
<td>3.1 (1-mer)</td>
<td>0.76</td>
<td>Organic solvent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.2 (2-mer)</td>
<td>0.01</td>
<td>Organic solvent</td>
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<tr>
<td></td>
<td></td>
<td>7.3 (3-mer)</td>
<td>0.01</td>
<td>Organic solvent</td>
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<tr>
<td></td>
<td></td>
<td>9.4 (4-mer)</td>
<td>0.004</td>
<td>Organic solvent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>1.6 (equilibrium state)</td>
<td>Buffer solution and EC control</td>
</tr>
</tbody>
</table>

The molecules were selected for their biological relevance or for the dimensions similar to the cyt b₅₆₂ and covalent contact to the electrodes. All the reported conductance values were evaluated by I₋z experiments. Note that comparison of molecular conductance for various systems poses some difficulties, because (i) many factors (some of which are even unknown) affect molecular conductance even for a given system, and (ii) the measurements of single-molecule conductance for various systems have so far been carried out under diverse experimental conditions such as in air, organic solvents, vacuum or in an aqueous solution, and with or without potential control.

3 Conclusions

In summary, we present the first single-molecule conduction measurement of a protein chemically coupled, with controlled protein orientation, to two enclosing electrodes under pseudo-physiological conditions with electrochemical control. Measured electrochemical equilibrium potentials (−68 ± 2 mV vs. SCE) are consistent with the native cyt b₅₆₂ conformation. Electrochemical gating of the molecular conductance was demonstrated both indirectly from apparent height contrast and directly from the I₋z curves. The latter measurements showed that cyt b₅₆₂ has notably higher conductance for two controlled molecular orientations than those of comparable synthetic molecules of similar dimensions. The maximum conductance at the cyt b₅₆₂ electrochemical potential is consistent with its suggested role as an electron shuttle in the inter-membrane region, in which it can be oxidised or reduced according to the electrochemical potential of its interacting molecule. Two possible origins of the high conductance are (i) the solvent contribution to the reorganisation free energy is notably reduced, the main contribution being attributed to the heme, (ii) the strong electronic coupling due to the double metal–sulfur bond gives strongly adiabatic ET at both contacts and a multi-electron transfer via the heme redox level. The observation of ET in which solvent reorganisation is significantly reduced calls for a new systematic study of reorganisation energy in redox proteins, complementing traditional photoexcitation experiments of a surface-bound donor group. The methods developed here hold promise for effective new ways for single-molecule protein ET studies broadly, with prospects for incorporation of single ET protein molecules in its native conformation in an external electronic circuit for future nanoscale bioelectronic devices.

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