Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1604 (2003) 67–76
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Conformational reorganisation in interfacial protein electron transfer

Lars J.C. Jeuken *

Physics and Astronomy, University of Leeds, Leeds LS2 9JT, UK

Received 17 October 2002; received in revised form 17 March 2003; accepted 25 March 2003

Abstract

Protein–protein electron transfer (ET) plays an essential role in all redox chains. Earlier studies which used cross-linking and increased solution viscosity indicated that the rate of many ET reactions is limited (i.e., gated) by conformational reorientations at the surface interface. These results are later supported by structural studies using NMR and molecular modelling. New insights into conformational gating have also come from electrochemical experiments in which proteins are noncovalently adsorbed on the electrode surface. These systems have the advantage that it is relatively easy to vary systematically the driving force and electronic coupling. In this review we summarize the current knowledge obtained from these electrochemical experiments and compare it with some of the results obtained for protein–protein ET.

Keywords: Protein–protein electron transfer; Electrochemistry; Self-assembled monolayer; Marcus theory; Gated electron transfer; Azurin; Cytochrome *c*

1. Introduction

Protein-mediated electron transfer (ET) plays a key role in almost all reactions in photosynthesis and metabolism. In general, either the oxidation of fuel (metabolism) or water (after absorption of light energy; photosynthesis) generates electrons with a relatively low reduction potential. Via a series of well-controlled ET reactions, the electrochemical energy is converted into a trans-membrane proton gradient or used to reduce a range of (metabolic and nonmetabolic) substrates. A redox-chain consists of a number of proteins and, consequently, the electron is transported between enzymes, which means that many of the ET reactions occur across the protein–protein interface. Electrons are also transported across protein–domain interfaces. For instance, in mitochondrial cytochrome bc_1 (Complex III), the Rieske iron–sulfur protein domain shuttles between the quinol binding site and cytochrome c_1, while transferring electrons between the two [1–3].

Some interfacial ET reactions are known to be rate-limited by conformational reorganisation or reorientation at the surface interface. Over the last 5 years, a number of electrochemical experiments in which a protein is adsorbed onto an electrode have been reported, which suggests that conformational reorganisation also limits the ET rate across an electrode–protein interface. After a brief introduction into the theory of ET and some examples of ET in protein complexes, the current knowledge of electrode–protein systems will be reviewed.

1.1. Marcus theory

For the last two decades, much of our understanding of ET reactions is based on the Marcus theory [4]. Most protein-mediated ET processes occur over distances well above the van der Waals contact, with the protein matrix providing a weak donor–acceptor mixing, giving generally a non-adiabatic ET mechanism. The semi-classical Marcus theory (as is extensively reviewed in many references, such as Refs. [5–7]) predicts that in this case the ET rate is governed by the driving force of the reaction (ΔG^0), the nuclear reorganisation energy (λ) and the electronic coupling (H_{DA}) between electron donor (D) and acceptor (A) at the transition state, and can be expressed as,

$$k = \frac{2\pi}{\hbar}\frac{H_{DA}^2}{\sqrt{4\pi\lambda RT}}e^{-\frac{(\Delta G^0+\lambda)^2}{4\lambda RT}} \quad (1)$$

Over the last decade, a broad range of theoretical approaches (either (semi-)empirical or electronic structural models) have been developed to predict H_{DA} in proteins [6–10]. The most simple one is the square-barrier model, which treats the protein as an 'organic' glass in which H_{DA} decays

* Tel.: +44-113-343-1675.
E-mail address: L.J.C.Jeuken@leeds.ac.uk (L.J.C. Jeuken).

0005-2728/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0005-2728(03)00026-4

exponentially with distance, where the effectiveness of the protein in mediating ET is given by the tunnelling parameter β, expressed as,

$$H_{DA}^2 = (H_{DA}^0)^2 \exp(-\beta \times (r_{DA} - r_0)) \quad (2)$$

in which H_{DA}^0 is the electronic coupling at the van der Waals distance (r_0) and r_{DA} is the distance between the donor and acceptor [8]. β ranges between 0.8 and 1.6 $Å^{-1}$ and is dependent on the structure of the protein [11,12].

1.2. Gated ET

In many cases the ET reaction is coupled to a non-ET reaction (see Fig. 1). To describe the *kinetics* of a coupled ET reaction, a distinction must be made between *concerted* and *sequential* reactions. In concerted processes, both reactions occur in a single event. To describe the kinetics of concerted reactions, extensions to the Marcus theory have been developed [13,14]. In sequential processes, both reactions occur independently one after the other and the overall rate is controlled by the slowest reaction. When an ET reaction is preceded by a *rate-limiting* non-ET reaction that is *coupled* to the ET, this is generally referred to as gated ET. The black part in Fig. 1 represents a gated reduction going from Ox* to Red if $k_1 \ll k_0$ (note that the reverse reaction is not gated).

There is some divergence in literature about the nomenclature of an ET reaction that is rate-limited by conformational reorientation. Usually, conformational reorientations are *not coupled* to the ET reactions [i.e., the equilibrium constants of the non-ET reactions are independent of the oxidation state of the protein, $K_{OX} = K_{RED}$ (Fig. 1)]. Conformational reorientations can be seen as a probability distribution (i.e., statistical) which will only affect the ET kinetics and not the thermodynamics. Still, interpreting the word 'gate' as a 'barrier that can be opened and closed', I will refer to ET reactions of which the rate is limited by conformational reorganisation as conformationally gated ET.

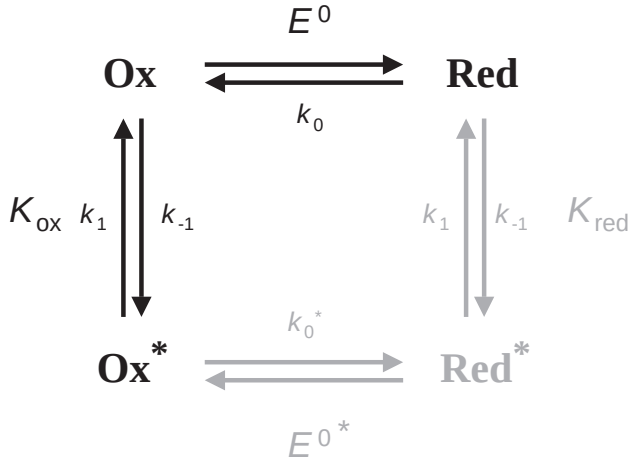

Fig. 1. A scheme of a coupled ET reaction.

2. Protein–protein ET

Examples of conformationally gated ET in protein complexes have been known for over a decade (see for instance Refs. [15–18]). Kostić et al. have been studying the effects of interfacial mobility on the ET rate by varying the solution viscosity [19–23]. A viscous solution decreases the diffusion of (macro)molecules and could influence the rate of configurational rearrangement. Increasing the viscosity indeed lowers the ET rate between preformed complexes of zinc cytochrome *c* and several blue copper proteins. Similar viscosity effects have been found for ET between other proteins or protein domains [24–29].

Another technique that gave insight into protein-mediated interfacial ET is cross-linking. Cross-linking has been used as a general method to study the relative orientation of proteins within the transient ET complex. For instance, in the cross-linked complex of cytochrome *c* and cytochrome *c* peroxidase or oxidase, the interfacial ET is reported to be retained or only partly perturbed [17,30–32]. However, in some other cases it is found that cross-linking decreases or even abolishes interfacial ET. When cytochrome *f* and plastocyanin are cross-linked the interfacial ET rate drops by more than five orders of magnitude [33]. Recently, the group of Canters used different sized linkers to cross-link two azurin molecules and measured the interfacial ET rate within a dimer and between dimers [34,35]. Crystal structures of the dimers with different linkers are also determined and it could be concluded that short linkers, even when used in an optimal position according to crystal structures of protein complexes, impair surface reorganisation which is necessary to sample the configurational space and optimise ET. Longer linkers allow the complex to retain the interfacial mobility and faster ET rates can be observed.

Recently, results were published that illustrate a third technique which can be used to establish if an interfacial ET reaction is gated. In this study, mutants were made of the Rieske iron–sulfur protein in the cytochrome bc_1 complex [36]. As mentioned above, the Rieske protein domain shuttles between the cytochrome c_1 and the quinol binding domains. The ET reaction between the Rieske protein mutants and the cytochrome c_1 domains was established for the so-called c_1 state, in which the Rieske protein is bound to the cytochrome c_1. Although the mutation alters the reduction potential of the Rieske protein considerably (up to 160 mV), and thus the driving force of the ET reaction, the interfacial ET rate was relatively unaltered. Combined with the fact that no pH dependence was observed, it was concluded that the ET is conformationally gated.

Although viscosity and cross-linking experiments suggest surface reorientation is involved in interfacial ET, they do not provide any detailed structural data. Crystal structures of protein complexes are very informative, but do not provide information about mobility at the protein–protein interface. Mapping protein–protein interactions with NMR spectroscopy is becoming increasingly important and is a perfect tool to study the structure and dynamics of transient protein complexes [37]. ET protein complexes studied with NMR are cytochrome *f*/plastocyanin [38,39], cytochrome b_5/cytochrome *c* [40], cytochrome *c* peroxidase/iso-1 cytochrome *c* [41] and cytochrome *f*/cytochrome c_6 [42]. In three of these five studies [38,40,41], the results indicate that the protein–protein interface is dynamic. An illustrative example is provided by the plant cytochrome *f*/plastocyanin complex in which the NMR data, analysed by restrained rigid-body molecular dynamics, suggest the presence of two distinct complexes. It was proposed that the initial interaction between the redox-partners is directed by electrostatic interactions, but that electrostatic as well as short-range interactions are responsible for a surface reorganisation that results in a complex that is optimal for ET (see Figs. 2 and 3, middle). This is in agreement with earlier studies which showed that when the electrostatic interaction is weakened by increasing the ionic strength from 5 to 40 mM, the ET rate increases [43]. Ionic strengths >40 mM resulted in dissociation of the complex and a decrease in ET rate. In fact, more than a decade ago, similar studies prompted Hazzard et al. [17,18] to suggest that the formation of an electrostatic complex between cytochrome *c* and cytochrome *c* peroxidase is followed by a conformational reorganisation that precedes ET.

Brownian dynamic simulations and ET pathway calculations were used to model the experimental results obtained for the ET reaction between cytochrome b_5 and myoglobin/hemoglobin [44–47]. These simulations provided a model in which the above described two conformations are extended into many different conformations with similar binding energy (see Fig. 3, right). This view has recently been supported by NMR experiments [48]. In the 'dynamic docking' model only one or a few of the many conformations are ET active so that the overall reaction will be

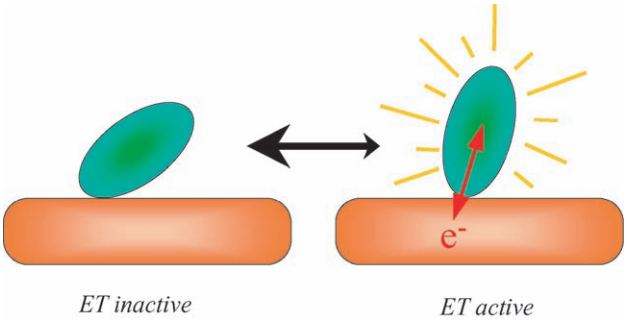

Fig. 2. A schematic representation of gated ET between two proteins or a protein and an electrode.

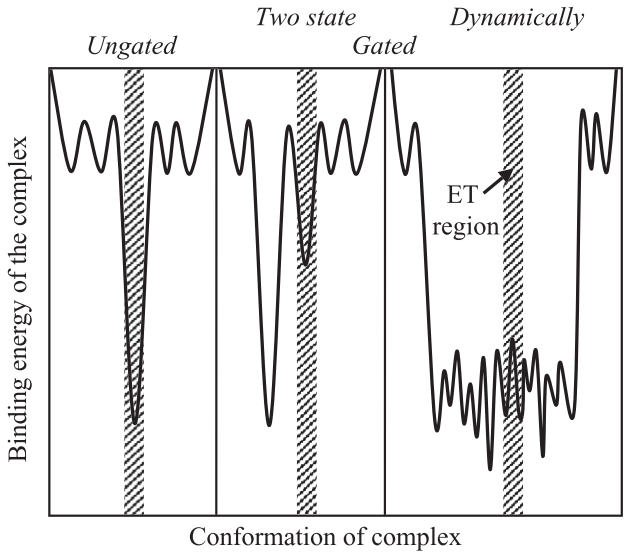

Fig. 3. Schematic energy landscapes, which plot the binding energy as a function of conformational coordinate for two proteins (or a protein and an electrode) which exhibited non-gated ET (left), two-state gated ET (middle) and dynamically gated ET (right). The shaded areas represent the conformation in which the protein complex is ET active. Figure based on Fig. 11 in Ref. [46].

entropically controlled (i.e., small activation energy, E_A, and therefore temperature-insensitive) [46,47].

3. Interfacial ET in electrochemistry

Electrochemical techniques for measuring ET rates are particularly informative when the redox system is adsorbed on the electrode (like protein film voltammetry [49,50] and some types of spectroelectrochemistry [51]). These techniques have the advantage that interpretation of the data is not complicated by diffusion effects. In this way it is similar to the experiments in which a protein–protein complex is formed prior to initiating ET by, for instance, a laser pulse. With electrochemistry, ET to and from the protein can be induced by altering the electrode potential. In order to study ET kinetics between the electrode and the adsorbed proteins, the use of mediators is to be avoided since this would introduce an extra ET reaction. There are two main methods by which ET can be detected: measuring the current (voltammetry) and/or using a spectroscopic method that is sensitive to the oxidation state of the protein (spectroelectrochemistry).

3.1. Varying the electronic coupling

The electronic coupling between the adsorbed protein and the electrode can be varied using different self-assembled monolayers (SAMs, see Fig. 4). The most commonly used SAMs are constructed by treating a clean metal surface (usually gold or silver) with a thiol-compound (for instance HS-$(CH_2)_n$-X, in which '*n*' determines the

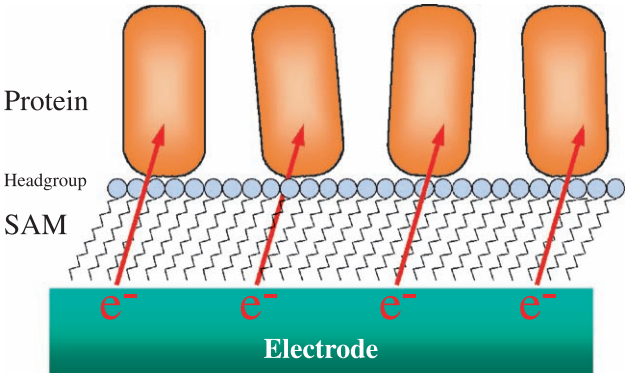

Fig. 4. A schematic representation of ET between an electrode and an adsorbed redox-protein bridged by a SAM.

length of the SAM and X is the 'head' group) [52]. Since the thiol terminus forms a relatively stable bond with the metal, various head groups can be used to optimise protein binding without disturbing the formation of the SAM. One of the first proteins to be adsorbed onto gold electrodes using SAMs of different chain lengths was cytochrome *c* [53,54]. At low ionic strength, cytochrome *c* is strongly adsorbed by SAMs with a carboxylic acid head group ($HOOC(CH_2)_nS$-Au). Initially, relatively slow cyclic voltammetry (up to 200 mV s^{-1}) was used which limited the ET rate that could be measured to $k_0 < 2\ s^{-1}$ (ET rates are usually reported as k_0, which represents the ET rate at $\Delta G^0 = 0$) [54]. Later, Niki et al. continued this work using a faster spectroscopic technique and measured the ET rate for $n = 2-11$ [55,56]. For long chain lengths ($n>9$, i.e., weak electronic coupling), they found that the ET rate exponentially decreased with distance (see Fig. 5, top), in accordance with the square barrier (Eq. (2)) and other models. The tunnelling parameter ($\beta = 1.09$ per CH_2 unit) agreed well with previous work. Similar behaviour has been reported for cytochrome *c* on silver [57,58] although the ET rates are an order of magnitude lower (Fig. 5, top and middle). This difference might be partly due to the fact that different spectroscopic methods were used. Cytochrome *c* adsorbed on a SAM of *N*-acetylcystein showed a rate of $k_0 \approx 800\ s^{-1}$ with voltammetry, while a rate of $2100 \pm 300\ s^{-1}$ was found spectroscopically [59]. ET rates are also known to be dependent on the type of metal used as electrode. Finklea et al. [60] found differences in ET rates for Ru compounds adsorbed on $HOOC(CH_2)_{15}SH$ SAMs on either Pt, Au or Ag. The measured reorganisation energy of the Ru-compounds was the same for the three metals, but k_0 differed. The difference between Au and Ag has been attributed to their difference in the electronic heat constants [60].

For shorter chain lengths ($n<9$) the ET rate for cytochrome *c* on both Au and Ag did not increase exponentially as expected, but levelled off (at $k_0 \sim 3 \times 10^3\ s^{-1}$ for gold). A similar behaviour was observed with a blue copper protein, azurin, for chain lengths $n<9$ (Fig. 5, bottom) [61]. These results are consistent with the notion that the ET to and from

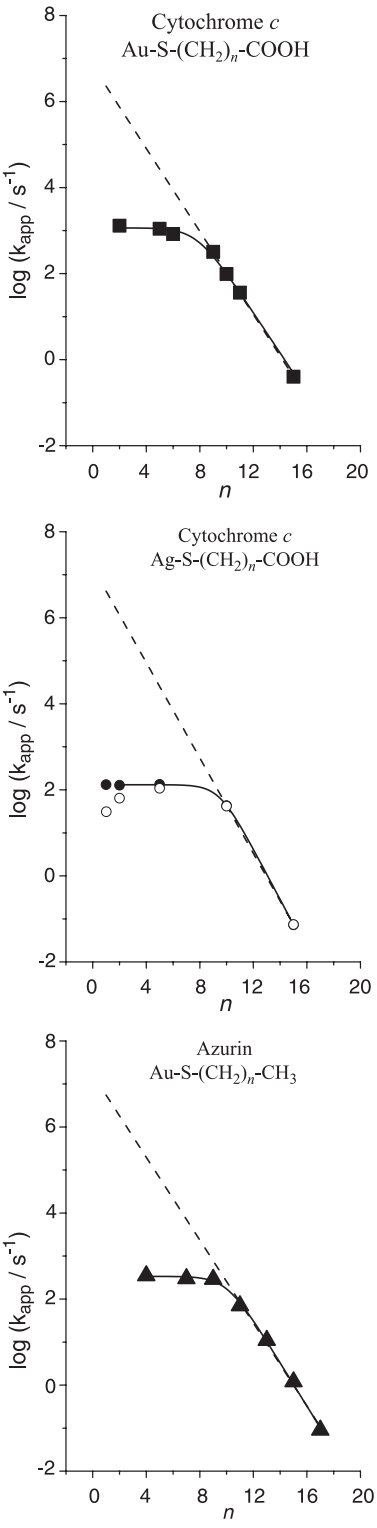

Fig. 5. Logarithmic plot of the observed ET rate constants (at $\Delta G^0 = 0$) of horse cytochrome *c* (top and middle) and azurin (bottom) as a function of the number of methylene groups of indicated alkanethiol SAMs. Data are taken from Refs. [54,55] (top), [57,58] (middle) and [61] (bottom). The dotted lines have a slope between $\beta = 1$ and 1.3 per CH_2. The solid lines represent simulations which take into account that the ET reaction is gated. Closed symbols are measured in H_2O and open symbols in D_2O.

the electrode is gated. When the ET is slow ($n>9$) the gating reaction is relatively fast and does not play a role. When the ET is fast ($n<9$) a preceding non-ET reaction starts to limit the observed ET rate and the reaction becomes gated.

3.2. Varying the driving force

In electrochemistry, one of the partners (the electrode) has a continuum of electronic states. When the Marcus theory (Eq. (1)) is extended to account for the density of states (DOS) of the electrode, the following equation can be derived [52,62–64],

$$k_{ox/red} = k_{max}\sqrt{\frac{RT}{4\pi\lambda}}\int_{-\infty}^{\infty}\frac{\exp\left(-\left(\frac{\lambda \pm F(E-E^0)}{RT}-x\right)^2\frac{RT}{4\lambda}\right)}{\exp(x)+1}dx \quad (3)$$

in which E^0 is the reduction potential of the adsorbed redox site, E is the applied potential and k_{max} is the maximum ET rate observed (at $|\Delta G^0|$ $[=F(E-E^0)]\gg\lambda$) and x is $(E-E_i)F/RT$ for k_{red} and $(E_i-E)F/RT$ for k_{ox} in which E_i is the potential of a specific Fermi level in the electrode. As expected from Eq. (1), $k_{max}\propto H_{DA}^2$ [52,62–64]. Eq. (3) predicts that the ET rate for ΔG^0 close to 0 increases exponentially with driving force and starts to levels off when ΔG^0 exceeds the reorganisation energy, λ, to reach k_{max} (see Fig. 6). Note that for the DOS-Marcus equation, the inverted region (at $\Delta G^0>\lambda$) of the Marcus theory does not result in the well-known decrease in ET rate. Instead, the inverted region in the DOS-Marcus equation results in a levelling off of the ET rate.

The blue copper protein azurin adsorbs on methane-terminated SAMs (CH_3-$(CH_2)_n$-S-Au) [65] as well as pyrolytic graphite edge (PGE) electrodes [63]. On methane-terminated SAMs the azurin layer is sufficiently stable to use scanning tunnelling microscopy in air, which shows that azurin is densely packed on the electrode surface [61]. Both Chi et al. [61] and I [66] measured the ET rate of azurin as a function of driving force. Chi et al. [61] measured the ET rates directly by pulse chrono-amperometry, while I [66] used square-wave voltammetry (SWV). In chrono-amperometry a voltage jump is applied to the electrode and the current is measured against time. In SWV, alternating voltage steps are applied with a certain amplitude and frequency. The amplitude can be compared with the magnitude of the voltage jump of chrono-amperometry, while the frequency is analogous to the time trace. SWV is a very sensitive method and has the advantage that it allows for more accurate correction of capacitive-charging currents than chrono-amperometry. However, it has the disadvantage that the analysis procedure is more elaborate and that it is not possible to compare the experimental data directly with theoretical Tafel plots. Nonetheless, with both techniques it was clearly observed that the results did not obey the DOS-Marcus theory, unless very low values for λ ($0.01<\lambda<0.25$ eV) were proposed [61,66]. This was especially true when azurin was adsorbed directly onto a PGE electrode [66]. Increasing the driving force from 0 to 0.3 eV on either side only increased the observed ET rate by a factor of 3 (from $\sim 2\times10^3$ to $\sim 6\times10^3$ s^{-1} at high ionic strength). The DOS-Marcus equation predicts that for $\lambda=0.7$ eV (a reasonable value for azurin [67]), the ET rate should have increased more than 500-fold (see Fig. 6). Again, these results correspond to the notion that the ET is gated by a preceding reaction, which limits the maximum ET rate.

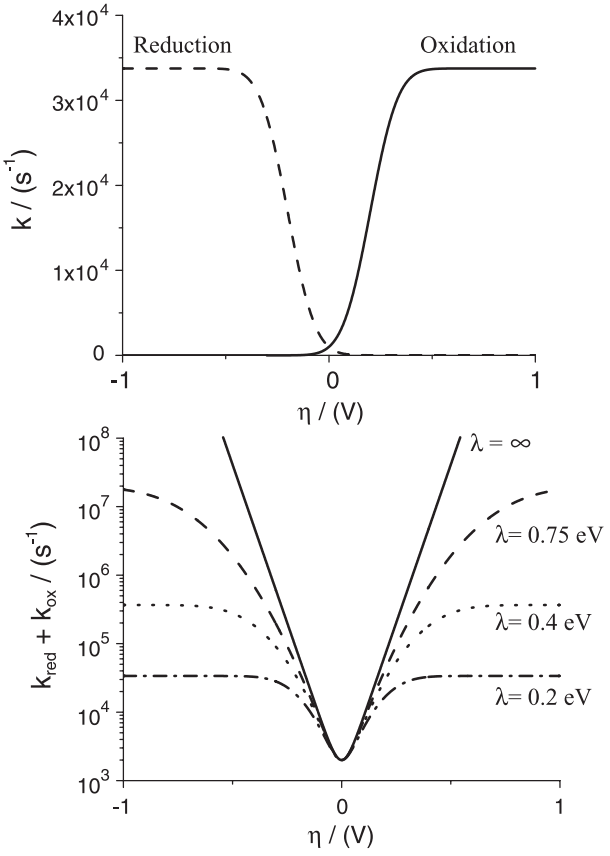

Fig. 6. Top: ET rate (for oxidation and reduction) as a function of overpotential ($\eta=nF\Delta G^0$ (in V)) based on the DOS-Marcus theory with $T=273$ K, $\lambda=0.2$ eV and $k_0=1000$ s^{-1}. Bottom: Theoretical Tafel plots ($[k_{ox}+k_{red}]$ (log scale) versus $\eta=nF\Delta G^0$ (in V)) based on the DOS-Marcus theory with $T=273$ K, $k_0=1000$ s^{-1} and λ as shown.

Using SWV, gating effects were also observed for two fumarate reductases adsorbed on PGE electrodes [68]. Rusling et al. used SWV to determine the ET rate for a system in which proteins are adsorbed on the electrode within thin surfactant films [49,69–71]. Their results with myoglobin indicate that the ET rate levels off at driving forces of 0.2–0.4 V, and good fits could be obtained with the DOS-Marcus theory using reorganisation energies between 0.2 and 0.4 eV [69]. However, these energies are much lower than previously estimated for myoglobin in aqueous solutions. Although the authors attributed the lower λ to the influence of the hydrophobic medium

surrounding the myoglobin (i.e., surfactant film), it might be that the ET rate at high driving force levels off as a result of gating, similar to that observed for azurin. Simple gating models can predict Tafel plots that are remarkably similar to those calculated with the DOS-Marcus theory using low λ values [66]. The ET rate as a function of driving force has also been measured for cytochrome *c* adsorbed on $HOOC(CH_2)_{16}S$(-Ag) [58,72]. Analysis of the data indicates a reorganisation energy of 0.22 eV which is lower than reported for cytochromes in solution ($\sim$ 0.6 eV) [73]. For this system the low reorganisation energy is attributed to the exclusion of water at the electrode–protein interface [58,72]. Similarly to the case of myoglobin just described, the determined reorganisation energy might be at fault if the ET rate at high overpotential is limited by a preceding non-ET reaction. However, in this case it was shown that the maximum ET rate at high overpotential ($\approx 4\ s^{-1}$ with $n=16$) is much lower than the ET rate determined with short SAMs ($\approx 10^2\ s^{-1}$), indicating that either (a) gating does not influences the ET rate in this system (and λ is indeed lower) or (b) electric field effects influence the interfacial ET kinetics (see below).

3.3. Varying the driving force in enzymes

A lot of work has been done with different redox enzymes. Among the enzymes that are studied are laccases [74], peroxidases [75–78], hydrogenases [79–81], reductases [82–84] and dehydrogenases [85,86]. Gorton, Ikeda and coworkers specifically probe the potential application as biosensors [74,77,78,86–88]. Armstrong and coworkers use voltammetry to investigate the catalytic mechanism of several redox enzymes [80–85]. It would exceed the purpose of this review to extensively cover this area. Also, enzymes that are covalently linked (or 'wired') to electrodes or enzymes that are localized onto the electrode by a dialysis membrane are not reviewed here. Neither are systems reviewed in which protein layers are stabilized via affinity interactions, like anti-body recognition. For these systems I refer to another review [89].

The ET rate to or from an enzyme will be dependent on the interfacial ET rate and the turnover number of the enzyme. Were a catalytic site of an enzyme able to produce or consume electrons with a rate that is much faster than the interfacial ET, the measured current would be equal to the interfacial ET rate. However, in most of the studies with enzymes the catalytic turnover seems to be the limiting kinetic step (for at least part of the adsorbed enzyme, see below). Only in few cases is the condition unambiguously met that the catalytic turnover is much faster than the interfacial ET. The group of Dutton adsorbed cytochrome *c* mixed with cytochrome *c* oxidase on SAM-modified gold electrodes [90]. In this system the electrons are initially transferred to cytochrome *c* and, after a second interfacial ET step, consumed by cytochrome *c* oxidase. The ET rate for cytochrome *c* ($20\ s^{-1}$) was much lower than the turnover number of cytochrome *c* oxidase. However, since this is a rather complex system with multiple ET reactions, it is difficult to draw any conclusions about the interfacial ET.

For a fumarate reductase adsorbed on PGE it was shown that the maximum ET rate at high overpotential was similar to the maximum observed turnover number determined in an identical electrochemical setup [68,82]. This could mean that the electrochemical turnover is limited by the interfacial ET. However, turnover numbers in classical enzyme assays are also within the same order of magnitude, making an unambiguous conclusion impossible.

4. The nature of gating

It is clear that the interfacial ET in many electrode–protein systems does not obey Marcus theory (Eqs. (2) and (3)) and, therefore, seems to be gated. For protein–protein systems, it could be shown by NMR experiments and molecular dynamics simulations that the gating was due to conformational reorganisation at the surface interface. However, these experiments are very difficult for electrode–protein systems and none is reported yet. Still, a range of experiments show that proteins adsorb in a range of conformations at an electrode.

4.1. Thermodynamic dispersion

In an analysis of peroxidase activity on graphite electrodes, it was shown that only part of the adsorbed active enzymes exchanges electrons directly with the electrode [77,91,92]. The total amount of adsorbed peroxidase on the electrode can be determined by supplying two substrates of the enzyme, i.e., peroxide and a one-electron substrate. The latter substrate also acts as a mediator; the peroxidase oxidises the substrate, which is reduced by the electrode. If only peroxide is supplied, the enzyme has to be directly reduced by the electrode. In an approach developed by Lindgren et al., these properties can be used to determine which fraction of the total adsorbed peroxidase is able to exchange electron *directly* with the electrode (and which part needs an mediator to exchange electrons) [77,91,92]. They found that only $\sim$ 50% of the peroxidase was capable of direct ET with the electrode, which suggests that the enzyme is adsorbed in different orientations or conformations, which do not interconvert on the timescale of the experiment.

Recently, this hypothesis was extended into a model which assumes that the adsorbed enzymes exhibit a distribution of orientations, which results in a distribution of distances between the electrode and the redox site of the adsorbed enzyme [93]. Assuming that each distance within certain limits occurs with an equal probability, Eq. (2) can be used to calculate the overall effect on the ET kinetics. For enzymes with redox sites far from the electrode, the

ET kinetics will be slow compared to the catalytic turnover. However, the ET kinetics will increase exponentially with ΔG^0 until λ is approached (see above). In short, increasing the driving force increases the number of proteins that participate in catalytic turnover. It was shown that this model predicts the situation in which the current increases *linearly* with driving force, in contrast to the wave shape of an ideal system. Significantly, this linear behaviour has been found in a number of cases [79,93–95], although in most cases it is found on top of the expected sigmoidal wave, and it is therefore less obvious [82,84,96–101]. This combination of the sigmoidal wave and the linear slope is also predicted by the model. Again, these results suggest that proteins adsorb onto electrodes in multiple orientations, which do not interconvert on the timescale of the experiments.

Myoglobin [49], cytochrome *c* [102,103] and azurin [104] give reduction and oxidation peaks in their cyclic voltammograms that are broader than theory predicts. This has been attributed to a distribution of reduction potentials when adsorbed on electrodes. Furthermore, electrochemical impedance spectroscopy suggests that cytochrome *c* exhibits a distribution of ET rates [102]. Finally, Murgida and Hildebrandt [105] and Rivas et al. [106] performed surface-enhanced resonance Raman experiments and observed two distinctive species (B1 and B2) of cytochrome *c* on both hydrophilic and hydrophobic surfaces.

4.2. Kinetic dispersion

It is clear that many, if not all, proteins adopt a range of conformations or orientations when adsorbed onto an electrode. However, such a distribution of conformations, *thermodynamic* in origin, cannot explain the *kinetic* gating effects described in Section 3. It is important to realize that even if a protein layer on an electrode exhibits a distribution of ET rates, the *observed* ET rate will still exponentially increase with driving force as long as the driving force is below the reorganisation energy. In other words, the Tafel plots will be indistinguishable as to whether or not a distribution of ET rates exists. Only when a second reaction is involved, like catalytic turnover, does a distribution of ET rates influence the observed voltammetry.

In order to explain the gating effects, models have been proposed in which the proteins are adsorbed on electrodes in at least two conformations as depicted in Fig. 1, of which only one is electrochemically active, i.e., $k_0^* = 0$ (see for instance Refs. [55,56]). This model is analogous to those proposed for conformationally gated protein–protein ET (see Figs. 2 and 3, middle).

For Fig. 1 to be consistent with the observed gating effects, k_1 must be $\ll k_{-1}$ and $k_1 \ll k_0$, so that the concentration of the active species (top species in Fig. 1) is low and ET almost only proceeds after the protein is 'activated' by rate k_1. In other words, the inactive species is the thermodynamically favoured state, which has to convert transiently to the active state prior to ET. Note that it will be unlikely that this transient state(s) can be detected spectroscopically.

It has been further suggested that the two conformations in Fig. 1 represent two different orientations in which the proteins are adsorbed on the surface (see Fig. 2) [56]. These *interconvertible* orientations will have to exist in parallel with the already mentioned *non*-exchanging conformations (Section 4.1). This hypothesis is supported by experiments in which the viscosity of the electrolyte solution is increased. For cytochrome *c* adsorbed on $HOOC(CH_2)_nSH$ SAMs ($n < 9$), the gated ET rate decreases when the viscosity is increased [107]. Furthermore, both Arnold et al. [108] and El Kasmi et al. [109] showed that the headgroup of the SAM has a big effect on the observed ET rate at small chain length ($n < 10$). Mixing hydroxy and methyl headgroups with carboxylic headgroups increases the ET rate up to 20 times. Decreasing the charged headgroups will decrease the electrostatic interaction between the electrode and cytochrome *c*. If changes in the orientation involve the making and breaking of salt-bridges, an increase in interfacial mobility is expected. These results are analogous to the ET between plastocyanin and cytochrome *f*, which is faster when the salt concentration is increased from 5 to 40 mM (see above) [43].

With Ag-surface-enhanced resonance Raman spectroscopy, the ET reaction was also found to exhibit a kinetic H/D isotope effect that increases from 1 to 4 [$k_{ET}(H)/k_{ET}(D)$] upon decreasing the SAM chain length (see Fig. 5, middle) [57,58]. The kinetic isotope effect becomes stronger at shorter chain-length, indicating that the energy barrier of a hydrogen-bond reorganisation is raised by the electric field, which increases upon reducing the distance to the electrode. No isotope effect is seen with cytochrome *c* in solution, which implies that this effect is specifically related with protein adsorption. Although the authors favoured the hypothesis that the isotope effect corresponded to the rearrangement of the interior H-bond network in the heme pocket of cytochrome *c*, it could also be connected with a surface-conformational rearrangement. Hydrogen bonds will likely be present between the adsorbed protein and the carboxylic acid headgroups, which will have to be rearranged if the protein reorientates on the surface. For glassy carbon electrodes, evidence has been provided that the orientation (and therefore ET activity) of cytochrome *c* could be influenced by the applied electric field [110].

The nature of the gating reaction has been probed for azurin by varying pH, H_2O/D_2O, ionic strength, viscosity and temperature [66]. Azurin has the rare property that its binding to electrodes is hydrophobic in nature. This allows the use of buffers with a range of ionic strengths (up to 2 M) [66,104]. Increasing the ionic strength from 0.1 to 2.0 M did not significantly change the observed maximum ET rate at high overpotential [66]. At $I = 2$ M it is unlikely that the

double layer or the electric field influences the results. Furthermore, and in contrast to cytochrome *c*, neither the pH, H/D isotopes nor viscosity had a significant effect on the observed ET rate constant.

The temperature dependence of the gated ET rate of azurin has been analysed with the Ahrrenius equation (and Eyring equations). Since temperature only had a very minor effect on the observed rate constants, the activation energy, E_A, was calculated to be less than 20 kJ mol^{-1}. These energies suggest that the gating reaction is entropically controlled and that the ET-active conformation of the protein is highly ordered compared to the thermodynamically favoured conformations. This is similar to the dynamic docking model proposed for protein–protein interfacial ET (Fig. 3, right) [46,47]. Since no significant viscosity effect is observed, the ordered ET-active state must have a surface orientation that is only slightly different from the ET-inactive state(s).

For the sake of clarity, the thermodynamic and kinetic dispersions have been separated in the above sections. However, analysis of peak broadening and reduction potentials of azurin and a ferredoxin at different type of electrodes and voltammetric scan rates suggest that both dispersions are mixed [104]. The results suggests that azurin and ferredoxin adsorb in different conformations, each with different reduction potentials. However, these conformations are not completely static but interconvert with rates between $<10^{-3}$ and $>10^{-1}$ s^{-1}. This has been confirmed by recent temperature studies in which azurin and ferredoxin show nearly ideal peak shapes at temperatures above 20 °C, but peak broadening is increasingly introduced at temperatures below 0 °C [111]. A model can be proposed that is even more complex than that shown in Fig. 3 (right). Proteins adsorb in a distribution of conformations that interconvert with rates ranging from $<10^{-3}$ to $>10^{3}$ s^{-1}. Each of these conformations has a different reduction potential as well as a different electronic coupling with the electrode. Most of the conformations are ET inactive. For ET to occur the adsorbed protein has to reconfigure into one of the many ET active conformations, wherein each has a different electronic coupling (and thus different ET rate).

5. Physiological relevance

Almost all *electrochemical* systems that have been thoroughly investigated so far suggest that proteins adsorb in multiple conformations on organic surfaces and that interfacial ET is gated, most likely by conformational reorganisation. This prompts the question as to how the electrochemical systems relate to physiological protein–protein ET reactions since in electrochemistry one of the partners has been replaced by an electrode. In all cases described here, the electrode surface contains organic groups and can, to some degree, be compared to a protein surface. However, specific interactions that exist between protein partners are not present between an electrode and an adsorbed protein (adsorption is a-specific). Mei et al. [26] showed that the physiological ET between yeast cytochrome *c* and cytochrome *c* peroxidase is not gated, but that the same reaction is under the control of configurational gating if the yeast cytochrome *c* peroxidase is replaced by that of horse. Could it be that due to the inherent complexity of proteins, interfacial ET is conformationally gated unless specific interactions are present? In this respect it is important to note that many protein–protein ET rates reported in the literature are not from physiological relevant partners.

Electric field and double layer effects are usually minimized in electrochemical experiments by using ionic strengths higher that 0.1 M, which will usually shield effects for the adsorbed proteins. However, in at least one case (cytochrome *c* on carboxylic-acid terminated SAMs [57,58]) it has been shown that the electric field generated by the electrode can significantly influence the ET kinetics. Except for photosynthetic charge separation, little has been published about electric field effects on protein ET. For cytochrome *c* oxidase, it has been reported that the oxidation state influences the way cytochrome *c* binds and, consequently, the ET kinetics [32], although this remains a point of discussion (i.e., see for instance Ref. [112]). Still, it is conceivable that changes in oxidation states affect the local electric fields and thereby complex formation. More work in this direction is necessary to determine how important the electric field is for interfacial protein–protein ET.

It could be argued that if the interfacial ET is rate-limiting in a redox chain, evolution might have produced specific protein–protein interactions that minimize the control of configurational gating. Cytochrome *c* and cytochrome *c* oxidase, for which a turnover of 2000 electrons/s is observed in the bacterial enzymes (see Refs. [113,114] for reviews), require fast interfacial ET for optimal activity. Indeed, rapid interfacial ET reactions of $>6 \times 10^{4}$ s^{-1} are observed, which are independent of ionic strength, suggesting that this reaction is ungated (no viscosity experiments are reported) [115–117]. Interestingly, in most of these studies the protein partners that are used come from different organisms, arguing against the importance of specific protein–protein interactions to avoid conformational gating. On the other hand, Wang et al. [118] showed that when one of the aspartic acids on cytochrome *c* oxidase is replaced by an asparagine, a dependence on ionic strength is introduced, which suggests that ET to this mutant is conformationally gated. This confirms that care has to taken when analysing ET results from protein partners of different organisms or nonphysiological ET reactions.

Acknowledgements

I am grateful to Fraser Armstrong for his critical reading and helpful discussions, and I would like to thank the UK

BBSRC for supporting this research (David Phillips fellowship).

References

[1] H. Kim, D. Xia, C.A. Yu, J.Z. Xia, A.M. Kachurin, L. Zhang, L. Yu, J. Deisenhofer, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8026–8033.
[2] Z.L. Zhang, L.S. Huang, V.M. Shulmeister, Y.I. Chi, K.K. Kim, L.W. Hung, A.R. Crofts, E.A. Berry, S.H. Kim, Nature 392 (1998) 677–684.
[3] S. Iwata, J.W. Lee, K. Okada, J.K. Lee, M. Iwata, B. Rasmussen, T.A. Link, S. Ramaswamy, B.K. Jap, Science 281 (1998) 64–71.
[4] R.A. Marcus, N. Sutin, Biochim. Biophys. Acta 811 (1985) 265–322.
[5] G.L. Closs, J.R. Miller, Science 240 (1988) 440–447.
[6] G.W. Canters, M. van de Kamp, Curr. Opin. Struck. Biol. 2 (1992) 859–869.
[7] R.S. Farid, C.C. Moser, P.L. Dutton, Curr. Opin. Struck. Biol. 3 (1993) 225–233.
[8] C.C. Moser, J.M. Keske, K. Warncke, R.S. Farid, P.L. Dutton, Nature 355 (1992) 796–802.
[9] C.C. Page, C.C. Moser, X.X. Chen, P.L. Dutton, Nature 402 (1999) 47–52.
[10] M.L. Jones, I.V. Kurnikov, D.N. Beratan, J. Phys. Chem., A 106 (2002) 2002–2006.
[11] R. Langen, I.J. Chang, J.P. Germanas, J.H. Richards, J.R. Winkler, H.B. Gray, Science 268 (1995) 1733–1735.
[12] H.B. Gray, J.R. Winkler, Ann. Rev. Biochem. 65 (1996) 537–561.
[13] R.I. Cukier, D.G. Nocera, Annu. Rev. Phys. Chem. 49 (1998) 337–369.
[14] Y. Georgievskii, A.A. Stuchebrukhov, J. Chem. Phys. 113 (2000) 10438–10450.
[15] G. McLendon, K. Pardue, P. Bak, J. Am. Chem. Soc. 109 (1987) 7540–7541.
[16] G. McLendon, Acc. Chem. Res. 21 (1988) 160–167.
[17] J.T. Hazzard, S.J. Moench, J.E. Erman, J.D. Satterlee, G. Tollin, Biochemistry 27 (1988) 2002–2008.
[18] J.T. Hazzard, G. McLendon, M.A. Cusanovich, G. Das, F. Sherman, G. Tollin, Biochemistry 27 (1988) 4445–4451.
[19] J.S. Zhou, N.M. Kostić, J. Am. Chem. Soc. 115 (1993) 10796–10804.
[20] L. Qin, N.M. Kostić, Biochemistry 33 (1994) 12592–12599.
[21] M.M. Ivković-Jensen, N.M. Kostić, Biochemistry 36 (1997) 8135–8144.
[22] M.M. Ivković-Jensen, G.M. Ullmann, M.M. Crnogorac, M. Ejdebäck, S. Young, O. Hansson, N.M. Kostić, Biochemistry 38 (1999) 1589–1597.
[23] E.V. Pletneva, D.B. Fulton, T. Kohzuma, N.M. Kostić, J. Am. Chem. Soc. 122 (2000) 1034–1046.
[24] C.J. Feng, R.V. Kedia, J.T. Hazzard, J.K. Hurley, G. Tollin, J.H. Enemark, Biochemistry 41 (2002) 5816–5821.
[25] S. Heimann, M.V. Ponamarev, W.A. Cramer, Biochemistry 39 (2000) 2692–2699.
[26] H.K. Mei, K.F. Wang, N. Peffer, G. Weatherly, D.S. Cohen, M. Miller, G. Pielak, B. Durham, F. Millett, Biochemistry 38 (1999) 6846–6854.
[27] H. Oh-oka, M. Iwaki, S. Itoh, Biochemistry 36 (1997) 9267–9272.
[28] M.R. Harris, D.J. Davis, B. Durham, F. Millett, Biochim. Biophys. Acta, Bioenerg. 1319 (1997) 147–154.
[29] J. Wachtveitl, J.W. Farchaus, P. Mathis, D. Oesterhelt, Biochemistry 32 (1993) 10894–10904.
[30] H.S. Pappa, T.L. Poulos, Biochemistry 34 (1995) 6573–6580.
[31] H.S. Pappa, S. Tajbaksh, A.J. Saunders, G.J. Pielak, T.L. Poulos, Biochemistry 35 (1996) 4837–4845.
[32] V. Sampson, T. Alleyne, Eur. J. Biochem. 268 (2001) 6534–6544.
[33] L. Qin, N.M. Kostić, Biochemistry 32 (1993) 6073–6080.
[34] I.M.C. van Amsterdam, M. Ubbink, L.J.C. Jeuken, M.P. Verbeet, O. Einsle, A. Messerschmidt, G.W. Canters, Chem.-Eur. J. 7 (2001) 2398–2406.
[35] I.M.C. van Amsterdam, M. Ubbink, O. Einsle, A. Messerschmidt, A. Merli, D. Cavazzini, G.L. Rossi, G.W. Canters, Nat. Struct. Biol. 9 (2002) 48–52.
[36] G. Engstrom, K.H. Xiao, C.A. Yu, L. Yu, B. Durham, F. Millett, J. Biol. Chem. 277 (2002) 31072–31078.
[37] E.R.P. Zuiderweg, Biochemistry 41 (2002) 1–7.
[38] M. Ubbink, M. Ejdebäck, B.G. Karlsson, D.S. Bendall, Structure 6 (1998) 323–335.
[39] P.B. Crowley, G. Otting, B.G. Schlarb-Ridley, G.W. Canters, M. Ubbink, J. Am. Chem. Soc. 123 (2001) 10444–10453.
[40] R.D. Guiles, S. Sarma, R.J. DiGate, D. Banville, V.J. Basus, I.D. Kuntz, L. Waskell, Nat. Struct. Biol. 3 (1996) 333–339.
[41] J.A.R. Worrall, U. Kolczak, G.W. Canters, M. Ubbink, Biochemistry 40 (2001) 7069–7076.
[42] P.B. Crowley, A. Diaz-Quintana, F.P. Molina-Heredia, P. Nieto, M. Sutter, W. Haehnel, M.A. De la Rosa, M. Ubbink, J. Biol. Chem. 277 (2002) 48685–48689.
[43] T.E. Meyer, Z.G. Zhao, M.A. Cusanovich, G. Tollin, Biochemistry 32 (1993) 4552–4559.
[44] J.M. Nocek, B.P. Sishta, J.C. Cameron, A.G. Mauk, B.M. Hoffman, J. Am. Chem. Soc. 119 (1997) 2146–2155.
[45] N.R. Naito, H.L. Hui, R.W. Noble, B.M. Hoffman, Biochemistry 40 (2001) 2060–2065.
[46] Z.X. Liang, J.M. Nocek, K. Huang, R.T. Hayes, I.V. Kurnikov, D.N. Beratan, B.M. Hoffman, J. Am. Chem. Soc. 124 (2002) 6849–6859.
[47] Z.X. Liang, M. Jiang, Q. Ning, B.M. Hoffman, JBIC 7 (2002) 580–588.
[48] J.A.R. Worrall, Y.J. Liu, P.B. Crowley, J.M. Nocek, B.M. Hoffman, M. Ubbink, Biochemistry 41 (2002) 11721–11730.
[49] J.F. Rusling, Acc. Chem. Res. 31 (1998) 363–369.
[50] F.A. Armstrong, J. Chem. Soc., Dalton Trans. (2002) 661–671.
[51] J.L. Anderson, E.F. Bowden, P.G. Pickup, Anal. Chem. 68 (1996) R379–R444.
[52] H.O. Finklea, Electrochemistry of organized monolayers of thiols, related molecules on electrodes, in: A.J. Bard (Ed.), Electroanalytical Chemistry, vol. 19, Dekker, New York, 1996, pp. 109–335.
[53] M.J. Tarlov, E.F. Bowden, J. Am. Chem. Soc. 113 (1991) 1847–1849.
[54] S. Song, R.A. Clark, E.F. Bowden, M.J. Tarlov, J. Phys. Chem. 97 (1993) 6564–6572.
[55] Z.Q. Feng, S. Imabayashi, T. Kakiuchi, K. Niki, J. Chem. Soc., Faraday Trans. 93 (1997) 1367–1370.
[56] K. Niki, J.R. Sprinkle, E. Margoliash, Bioelectrochemistry 55 (2002) 37–40.
[57] D.H. Murgida, P. Hildebrandt, J. Am. Chem. Soc. 123 (2001) 4062–4068.
[58] P. Hildebrandt, D.H. Murgida, Bioelectrochemistry 55 (2002) 139–143.
[59] T. Ruzgas, L. Wong, A.K. Gaigalas, V.L. Vilker, Langmuir 14 (1998) 7298–7305.
[60] H.O. Finklea, K. Yoon, E. Chamberlain, J. Allen, R. Haddox, J. Phys. Chem., B 105 (2001) 3088–3092.
[61] Q. Chi, J. Zhang, J.E.T. Andersen, J. Ulstrup, J. Phys. Chem., B 105 (2001) 4669–4679.
[62] C.E.D. Chidsey, Science 251 (1991) 919–922.
[63] J. Hirst, F.A. Armstrong, Anal. Chem. 70 (1998) 5062–5071.
[64] M.J. Honeychurch, Langmuir 15 (1999) 5158–5163.
[65] A.K. Gaigalas, G. Niaura, J. Colloid Interface Sci. 193 (1997) 60–70.
[66] L.J.C. Jeuken, J.P. McEvoy, F.A. Armstrong, J. Phys. Chem., B 106 (2002) 2304–2313.
[67] A.J. Di Bilio, M.G. Hill, N. Bonander, B.G. Karlsson, R.M. Villahermosa, B.G. Malmström, J.R. Winkler, H.B. Gray, J. Am. Chem. Soc. 119 (1997) 9921–9922.

[68] L.J.C. Jeuken, A.K. Jones, S.K. Chapman, G. Cecchini, F.A. Armstrong, J. Am. Chem. Soc. 124 (2002) 5702–5713.
[69] T.M. Saccucci, J.F. Rusling, J. Phys. Chem., B 105 (2001) 6142–6147.
[70] Z. Zhang, S. Chouchane, R.S. Magliozzo, J.F. Rusling, Anal. Chem. 74 (2002) 163–170.
[71] Y.L. Zhou, N.F. Hu, Y.H. Zeng, J.F. Rusling, Langmuir 18 (2002) 211–219.
[72] D.H. Murgida, P. Hildebrandt, J. Phys. Chem., B 106 (2002) 12814–12819.
[73] S. Terrettaz, J. Cheng, C.J. Miller, J. Am. Chem. Soc. 118 (1996) 7857–7858.
[74] A.I. Yaropolov, A.N. Kharybin, J. Emnéus, G. Marko-Varga, L. Gorton, Bioelectrochem. Bioenerg. 40 (1996) 49–57.
[75] D.L. Scott, E.F. Bowden, Anal. Chem. 66 (1994) 1217–1223.
[76] T. Ruzgas, E. Csöregi, J. Emnéus, L. Gorton, G. Marko-Varga, Anal. Chim. Acta 330 (1996) 123–138.
[77] L. Gorton, A. Lindgren, T. Larsson, F.D. Munteanu, T. Ruzgas, I. Gazaryan, Anal. Chim. Acta 400 (1999) 91–108.
[78] H. Zimmermann, A. Lindgren, W. Schuhmann, L. Gorton, Chem.-Eur. J. 6 (2000) 592–599.
[79] A.K. Jones, E. Sillery, S.P.J. Albracht, F.A. Armstrong, Chem. Commun. (2002) 866–867.
[80] A.K. Jones, H.R. Pershad, E. Sillery, S.P.J. Albracht, F.A. Armstrong, J. Inorg. Biochem. 86 (2001) 283.
[81] H.R. Pershad, J.L.C. Duff, H.A. Heering, E.C. Duin, S.P.J. Albracht, F.A. Armstrong, Biochemistry 38 (1999) 8992–8999.
[82] A. Sucheta, R. Cammack, J. Weiner, F.A. Armstrong, Biochemistry 32 (1993) 5455–5465.
[83] H.A. Heering, J.H. Weiner, F.A. Armstrong, J. Am. Chem. Soc. 119 (1997) 11628–11638.
[84] K. Heffron, C. Léger, R.A. Rothery, J.H. Weiner, F.A. Armstrong, Biochemistry 40 (2001) 3117–3126.
[85] H.R. Pershad, J. Hirst, B. Cochran, B.A.C. Ackrell, F.A. Armstrong, Biochim. Biophys. Acta, Bioenerg. 1412 (1999) 262–272.
[86] A. Lindgren, T. Larsson, T. Ruzgas, L. Gorton, J. Electroanal. Chem. 494 (2000) 105–113.
[87] K. Kano, T. Ikeda, Anal. Sci. 16 (2000) 1013–1021.
[88] T. Ikeda, K. Kano, J. Biosci. Bioeng. 92 (2001) 9–18.
[89] I. Willner, E. Katz, Angew. Chem., Int. Ed. Engl. 39 (2000) 1180–1218.
[90] A.S. Haas, D.L. Pilloud, K.S. Reddy, G.T. Babcock, C.C. Moser, J.K. Blasie, P.L. Dutton, J. Phys. Chem., B 105 (2001) 11351–11362.
[91] A. Lindgren, F.D. Munteanu, I.G. Gazaryan, T. Ruzgas, L. Gorton, J. Electroanal. Chem. 458 (1998) 113–120.
[92] A. Lindgren, M. Tanaka, T. Ruzgas, L. Gorton, I. Gazaryan, K. Ishimori, I. Morishima, Electrochem. Commun. 1 (1999) 171–175.
[93] C. Léger, A.K. Jones, S.P.J. Albracht, F.A. Armstrong, J. Phys. Chem., B 106 (2002) 13058–13063.
[94] P. Bianco, J. Haladjian, J. Electrochem. Soc. 139 (1992) 2428–2432.
[95] J.N. Butt, M. Filipiak, W.R. Hagen, Eur. J. Biochem. 245 (1997) 116–122.
[96] C. Léger, K. Heffron, H.R. Pershad, E. Maklashina, C. Luna-Chavez, G. Cecchini, B.A.C. Ackrell, F.A. Armstrong, Biochemistry 40 (2001) 11234–11245.
[97] A. Sucheta, B.A.C. Ackrell, B. Cochran, F.A. Armstrong, Nature 356 (1992) 361–362.
[98] J. Hirst, A. Sucheta, B.A.C. Ackrell, F.A. Armstrong, J. Am. Chem. Soc. 118 (1996) 5031–5038.
[99] J. Hirst, B.A.C. Ackrell, F.A. Armstrong, J. Am. Chem. Soc. 119 (1997) 7434–7439.
[100] J.N. Butt, J. Thornton, D.J. Richardson, P.S. Dobbin, Biophys. J. 78 (2000) 1001–1009.
[101] L.J. Anderson, D.J. Richardson, J.N. Butt, Biochemistry 40 (2001) 11294–11307.
[102] T.M. Nahir, E.F. Bowden, J. Electroanal. Chem. 410 (1996) 9–13.
[103] R.A. Clark, E.F. Bowden, Langmuir 13 (1997) 559–565.
[104] L.J.C. Jeuken, F.A. Armstrong, J. Phys. Chem., B 105 (2001) 5271–5282.
[105] D.H. Murgida, P. Hildebrandt, J. Phys. Chem., B 105 (2001) 1578–1586.
[106] L. Rivas, D.H. Murgida, P. Hildebrandt, J. Phys. Chem., B 106 (2002) 4823–4830.
[107] A. Avila, B.W. Gregory, K. Niki, T.M. Cotton, J. Phys. Chem., B 104 (2000) 2759–2766.
[108] S. Arnold, Z.Q. Feng, T. Kakiuchi, W. Knoll, K. Niki, J. Electroanal. Chem. 438 (1997) 91–97.
[109] A. El Kasmi, J.M. Wallace, E.F. Bowden, S.M. Binet, R.J. Linderman, J. Am. Chem. Soc. 120 (1998) 225–226.
[110] B.A. Kuznetsov, N.A. Byzova, G.P. Shumakovich, J. Electroanal. Chem. 371 (1994) 85–92.
[111] J.P. McEvoy, Temperature and solvent effects in protein film voltammetry, PhD thesis, Oxford University, 2002.
[112] V. Drosou, F. Malatesta, B. Ludwig, Eur. J. Biochem. 269 (2002) 2980–2988.
[113] S. Ferguson-Miller, G.T. Babcock, Chem. Rev. 96 (1996) 2889–2907.
[114] H. Michel, J. Behr, A. Harrenga, A. Kannt, Annu. Rev. Biophys. Biomol. Struct. 27 (1998) 329–356.
[115] B.C. Hill, J. Biol. Chem. 266 (1991) 2219–2226.
[116] B.C. Hill, J. Biol. Chem. 269 (1994) 2419–2425.
[117] L.M. Geren, J.R. Beasley, B.R. Fine, A.J. Saunders, S. Hibdon, G.J. Pielak, B. Durham, F. Millett, J. Biol. Chem. 270 (1995) 2466–2472.
[118] K.F. Wang, Y.J. Zhen, R. Sadoski, S. Grinnell, L. Geren, S. Ferguson-Miller, B. Durham, F. Millett, J. Biol. Chem. 274 (1999) 38042–38050.