'Q-wires': Synthesis, electrochemical properties and their application in electro-enzymology
Rossius, S.G.H.

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**Author:** Rossius, S.G.H.
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CHAPTER 4

Electrochemical characterization of gold electrode-bound quinone-terminated oligo(phenylenevinylene) molecular wires

SGH Rossius, MTM Koper, HA Heering
4.1 Introduction

As described in chapter 1, the structure of the ‘Q-wires’ introduced there can be separated into three sections: a gold-electrode-binding terminal thiol; a ‘bridge’ section; and a quinone head group, which can interact with enzymes. Except for $U_0$ and $M_0$ and the decanethiol-tethered $U_{SAT}$, the bridge consists of oligo(phenylenevinylene) (OPV), a highly conjugated moiety, which facilitates non-rate-limiting electron transport between the electrode and the quinone head group [1]. Note that in this study, however, the redox active quinone moiety is not directly coupled to the conjugated system. The (intentional) inclusion of an additional $sp^3$ carbon is expected to preserve the natural (proton-coupled) redox properties of the quinone moiety, and, additionally, to provide flexibility, which is likely required during interactions with enzymes. Binding may be further facilitated by the similarity of the first three carbon atoms (allyl) to the natural isoprenoid tail.

In this chapter, it will be investigated whether the inclusion of an OPV section in the bridges of the ‘Q-wires’ indeed enhances electron transfer rates in comparison with fully saturated bridges, as was claimed in the previous chapters. Additionally, the influence of bridge length on electron transfer kinetics will be assessed. Furthermore, the importance of proton coupling to the electron transfer reaction will be explored.

4.1.1 Bridge length and composition

The composition and length of the ‘bridge’ (i.e. the moiety tethering a redox active molecule of interest to an electrode) can have a great influence on the electron transfer characteristics of self-assembled monolayers (SAMs) modified with a (terminal) redox active molecule [1]. The dependence of the electron transfer rate constant ($k_{ET}$) on the distance between redox center and electrode surface ($r$) and the decay constant ($\beta$), which reflects the composition of the bridge, can be expressed as follows (eqn. 4.1) [2]:

$$k_{ET} = k_{r=0} \exp(-\beta r)$$

(4.1)
Here (eqn. 4.1), $k_{r=0}$ represents the electron transfer rate constant at van der Waals contact and, more precisely, $r$ represents the distance between the centers of the concerning atoms minus the van der Waals radii.

Indeed, fully saturated alkanethiol tethers of different length, carrying a terminal hydroquinone head group, showed an exponential decay in electron transfer rate constants with distance to the electrode – i.e. number of methylene groups – in 0.1 M HClO$_4$ [3]. Typically, $\beta$ values between 0.8 and 1 Å$^{-1}$ or 0.7 and 1.3 per methylene are found for alkanethiol tethers [3, 4]. Hydroquinone-terminated wires containing a short OPV-bridge, on the other hand, showed little influence of the bridge length on the electron transfer rate constants, suggesting enhanced electron tunneling, i.e. a lower $\beta$ value [1]. Moreover, distance-independent rate constants up to 28 Å were found for ferrocene-terminated OPV-wires (suggesting $\beta \approx 0$ Å$^{-1}$) [1, 5, 6]. Efficient electron transfer requires a strong quantum mechanical coupling between both ends of the wire, which is achieved by conjugated bridges through orbital delocalization [7]. At distances below 28 Å, electron transfer through fully conjugated wires is rate-limited by processes other than electron tunneling, while above this distance, electron transfer becomes non-adiabatic and distance dependent [8].

One observation concerning bridge composition is suspected to be of particular relevance to this research: the absence of direct conjugation between an OPV-tether and a terminal hydroquinone moiety – i.e. the substitution of two $sp^2$ carbons with two $sp^3$ carbons – resulted in dramatically lowered (apparent) rate constants (at least 100 times over the whole measured pH range) [9, 10]. As mentioned before, the (intentional) inclusion of an $sp^3$ carbon in the design of the Q-wires is likely to disrupt the coupling between the quinone moiety and the OPV-bridge as well. The aforementioned observation therefore suggests that the rate constants to be found for the Q-wires may be lower than initially foreseen, and perhaps better reflect the tunneling characteristics through uncoupled OPV.
4.1.2 Proton-coupled electron transfer (PCET)

As mentioned previously, apparent rate constants \( k_{app} \) found for alkanethiol-tethered hydroquinones (HQ-(CH\(_2\))\(_n\)-SH, where \( n \) denotes the number of methylene units) decrease exponentially with tether length (i.e. with increasing \( n \)) at acidic pH. Curiously, however, this distance dependence disappears (at least for \( n \leq 12 \)) under highly alkaline conditions (0.1 M NaOH) and rate constants become uniform, implying a \( \beta \) value approaching zero [11]. This would imply \( \beta \) to be a function of pH. In another study, a pH dependence of \( k_{app} \) was observed for an undecanethiol-tethered hydroquinone as well [1]; above pH 9, rate constants rapidly increased with pH, apparently converging with the higher rate constants found for a fully conjugated OPV-methylthiol-tethered hydroquinone of similar length [1].

When subjected to the ILIT (indirect laser induced temperature jump) technique, alkanethiol-tethered ferrocenes (Fc-(CH\(_2\))\(_m\)-SH, where \( m \) denotes the number of methylene units), rate constants in excess of \( 10^4 \) s\(^{-1} \) were found for \( m = 11 \) [12], nearly three orders of magnitude faster in comparison with the aforementioned hydroquinone-alkanethiol wires of similar length under highly alkaline conditions (\( n = 12, k_{app} \approx 26 \) s\(^{-1} \) in 0.1 M NaOH [11]). In addition, for ferrocene-OPV-thiol wires, electron tunneling was found to occur in less than 20 ps over distances below 28 Å [8]. Comparing the rate constants given above, one may conclude that, in the case of short hydroquinone wires, the nature and length of the bridges are of secondary importance only, and that the electron/proton kinetics of the hydroquinone moiety appear to be rate-limiting. Nevertheless, a 100-fold increase in rate constants was found for a fully conjugated hydroquinone-OPV-methylthiol...

Figure 1: Nine-member square scheme, describing the two-electron/two-proton redox reaction of a quinone moiety
wire between pH 4 and 9, in comparison with a hydroquinone-alkanethiol wire of similar length [1]. Therefore, since electron transfer and proton chemistry appear to be intimately coupled in case of quinone-terminated wires, an understanding of the mechanism of the oxidation/reduction reaction of the quinone moiety is crucial.

4.1.3 Modeling PCET

Laviron’s nine-member square scheme (figure 1) [13], describing the two-electron/two-proton reaction for a surface-confined redox species, may be applied to the ‘Q-wires’ as well. The scheme provides a theoretical description of a stepwise electron transfer mechanism, as opposed to a concerted mechanism [14-17], which will not be considered here. The model (extended by Finklea [18]) assumes that protonations are at equilibrium and that no dimerization and disproportionation reactions occur [13]. The overall two-electron/two-proton reaction is divided into 12 steps, representing either an individual electron (governed by a formal potential $E^0_i$) or proton transfer event (governed by a $pK_{ai}$), as depicted in figure 1. Microscopic values for these $pK_{ai}s$ and $E^0_i$s can be obtained by fitting experimental thermodynamic (i.e. $E^0_i$ vs. pH, which forms the basis of a ‘Pourbaix diagram’ [19]) and macroscopic kinetic data (i.e. $k_{app}$ vs. pH) of the overall two-electron/two-proton reaction to a mathematical model described previously [9]. Note that due to thermodynamic coupling, four of the twelve parameters are dependent on the others, and were consequently not independently fitted. Further implementation details can be found in the experimental section. Cyclic voltammetry can provide both the required macroscopic thermodynamic and kinetic data; the former is obtained by averaging the anodic and cathodic peak positions ($E_{pa}$ and $E_{pc}$, respectively) in a voltammogram, measured at a certain pH at a relatively low scan rate. The following section describes how the required kinetic data can be generated. Once the individual (microscopic) $pK_{ai}s$ and $E^0_i$s are produced by the model, they provide insight into the reaction mechanism prevalent at a certain pH, i.e. the order in which the protonations and electron transfers occur. In addition, the model provides a single microscopic ‘pure-electron’ rate constant $k_e$ specific to each wire, which
represents the rate constants pertaining to all six possible single step electron transfers [9] in the square scheme presented in figure 1 (assuming that the pure electron transfer rate constant $k_s$ is independent of protonation state, when it is defined at zero overpotential relative to the actual microscopic reduction potential of that state). Since the $k_s$ rates describe electron transfer only, and are – in principle – separated from proton transfer phenomena, they can be used to determine a value for the decay constant $\beta$, characteristic of each series of wire (either ubiquinone- or menaquinone-terminated). This is in contrast to the use of the macroscopic $k_{app}$ to determine $\beta$, which can also be encountered in the literature (e.g. [3, 11]). In a plot (see figure 11) of $\ln(k_s)$ vs. tunneling distance $L$ (in Å), the slope provides $\beta$, while the intercept $\ln(k_{lim}) = \ln(k_s)_{L=0}$ can be used to calculate the reorganization energy $\lambda$ (in eV), using eqn. 4.2 and 4.3 [20].

$$k_{lim} = k_{max} \rho \sqrt{\frac{4\pi\lambda RT}{F}}$$  (4.2)

$$k_{max} \approx 10^{13} erfc \left( 0.5 \frac{\sqrt{\lambda F}}{\sqrt{RT}} \right)$$  (4.3)

Here, $k_{max}$ (the driving-force-optimized Marcus rate constant for any single, fully occupied state) and $\rho = 0.9$ eV$^{-1}$ (the effective density of electronic states near the Fermi level) were defined as previously described [20-23], $F$ is the Faraday constant, $R$ is the gas constant and $T$ is the temperature.

### 4.1.4 Laviron plots and Butler-Volmer kinetics

A strongly electrode-adsorbed redox species is said to show ideal and reversible electrochemical behavior when its corresponding cyclic voltammogram shows symmetric anodic and cathodic peaks that are not separated at low scan rates (i.e. $\Delta E_p = 0$) since diffusion does not occur. Furthermore, the peak current and scan rate are expected to show a linear relationship and, additionally, the FWHM (full width at half the peak height) should approach a value of $90.6 / n$ mV at 25°C, where $n$ is the number of
electrons being transferred, assuming fully cooperative multi-electron transfer [24].

Increasing scan rates (ν) cause the anodic and cathodic peaks to separate, that is, the peak separation becomes greater than zero (ΔE_p = E_{pa} − E_{pc} > 0). When the midpoint potential E̅₀' (i.e. the average of E_{pa} and E_{pc} at low scan rates) is subtracted from the anodic (E_{pa}) and cathodic (E_{pc}) peak positions, and both are plotted against the logarithm of the scan rate (i.e. (E_p − E̅₀') vs. log(ν) – note that subtracting E̅₀' is not strictly necessary, only convenient), a Laviron plot is obtained (see, for example, figure 7A). Traditionally, the linear parts of both curves (above 200 mV / n) are extrapolated and their points of intersection with the line E_p − E̅₀' = 0 provide critical scan rates ν_a and ν_c, while the slope of the extrapolation lines provide α, the electron-transfer coefficient. These values can be used to calculate the apparent electron transfer rate constant, k_{app}, which represents the kinetic information required for the model described in the previous section [1, 9, 25].

It was found, however, that in some instances – especially in case of measurements performed at high pH and high scan rates – the extrapolation procedure proved somewhat arbitrary, and that its outcome was determined by only a few manually selected data points. Additionally, many valid data points were not included in the calculations. An alternative approach was therefore pursued. Instead, the data were fitted to the peak potentials of simulated voltammograms. The underlying rate constants for oxidation (eqn. 4.4) and reduction (eqn. 4.5) were calculated using Butler-Volmer equations [20]:

\[
k_{ox} = k_0 \exp \left( (1 - \alpha)(E - E^{0r}) \frac{nF}{RT} \right) \quad (4.4)
\]

\[
k_{red} = k_0 \exp \left( -\alpha(E - E^{0r}) \frac{nF}{RT} \right) \quad (4.5)
\]

Here, k_0 is the standard rate constant, E is the applied potential, E^{0r} is the equilibrium (midpoint) potential, α is the transfer coefficient, n is the
number of electrons, \( F \) is the Faraday constant, \( R \) is the gas constant and \( T \) is the temperature. Staircase cyclic voltammograms were simulated \([36]\), in which the current at a given time was obtained using eqn. 4.6:

\[
i(t) = nFA(k_{ox}(t)\Gamma_{red}(t) - k_{red}(t)\Gamma_{ox}(t))
\]  \hspace{1cm} (4.6)

Here, \( A \) is the electrode surface area, \( \Gamma_{ox} \) is the surface concentration of the oxidized species and \( \Gamma_{red} \) is the surface concentration of the reduced species at any given potential and time. Values for \( k_0 \) (i.e. \( k_{app} \)), non-integer \( n \) (i.e. \( n_{app} \)) and \( \alpha \) could then be obtained by fitting experimental data to simulated data generated by means of the aforementioned equations. The value of \( n_{app} \) reflects the stability of the semiquinone. When \( n_{app} = 2 \), the semiquinone can be considered fully unstable, while when \( n_{app} = 1 \), the semiquinone can be deemed stable. Intermediate, non-integer values therefore provide a measure for semiquinone stability, which can aid in the elucidation of the mechanism of the quinol/quinone oxidation/reduction reaction \([26]\).

Although straightforward in use and satisfactory for many purposes, the Butler-Volmer expressions for the oxidation and reduction rate constants are essentially empirical, and its parameters have limited physical meaning \([27]\). One may therefore argue whether models based on said expressions are too simplistic to realistically describe the complex two-electron/two-proton reaction of the quinone moiety.

4.2 Results and discussion

The bulk of the experimental work presented here consists of an exploration of the electrochemical behavior of the Q-wires, measured by means of cyclic voltammetry. The kinetic data to be fitted to the aforementioned PCET model is obtained from Laviron plots, i.e. cathodic and anodic peak positions (\( E_{pc} \) and \( E_{pa} \)) measured at increasing scan rates and at different pH values. This section begins, however, with some observations on the SAM preparation process and the relevant characteristics of the measured cyclic voltammograms.
4.2.1 SAM preparation and characterization

To arrive at gold-binding Q-wires, the synthesis pathway outlined in chapter 3 requires one additional step: the conversion of the terminal thioacetate to a thiol. Considering the small amount of product, it was decided to combine this deprotection step with the SAM formation process of the wires; a freshly polished gold electrode was incubated in an appropriate Q-wire solution undergoing relatively mild hydroxylamine-catalyzed thioester hydrolysis, allowing for the liberated thiol groups to bind to the gold surface. After this incubation period, generally lasting 18 hours or more, UV-Vis spectroscopy performed on the deprotection solutions revealed intact π-systems in case of $\text{M}_2$, $\text{M}_3$ and $\text{U}_2$ and $\text{U}_3$ (figure 2). Note that the absorbance maxima ($\lambda_{\text{max}}$) shift to longer wavelengths with increasing length of the OPV system, as observed previously [5]. Additionally, cyclic voltammetry showed characteristic quinone-like electrochemical behavior, suggesting the Q-wires to be undamaged.

As suggested before [9], headgroup interactions among the wires may distort their electrochemical behavior. It was therefore decided to introduce a diluent molecule in order to minimize these interactions. To this end, the electrodes – already modified with Q-wire – were incubated in a heptanethiol solution (butanethiol in case of $\text{U}_0$ and $\text{M}_0$). After several washing steps to remove non-chemisorbed thiols, the prepared working electrode was then mounted in a ‘Hagen cell’ [28], together with the counter and reference electrode. Cyclic voltammetry was then performed in an appropriate buffer under an argon atmosphere.

![Figure 2](image-url) UV-Vis spectra of ‘deprotection solutions’ of $\text{U}_2$ (black dashed line), $\text{M}_2$ (gray dashed line), $\text{U}_3$ (black solid line), $\text{M}_3$ (gray solid line)
Surface coverage (Γ) determination is based on integration of the anodic and/or cathodic peaks in a voltammogram [1, 9]. In these experiments, however, no accurate calculations could be performed since the ‘surface roughness’ – and therefore also the exact surface area – of the electrodes used here is essentially unknown. Although the obtained values varied widely among prepared electrodes, those with an estimated coverage between $3 \times 10^{-11}$ mol/cm$^2$ and $3 \times 10^{-10}$ mol/cm$^2$ (using the geometric electrode surface area) were considered suitable for the generation of Laviron plots.

4.2.2 Voltammetric characteristics of the Q-wire SAMs

Figure 3 depicts cyclic voltammograms of Q-wires of different length and type, measured at constant pH and scan rate. A single, asymmetric redox couple can be observed, with increasing peak-to-peak separation and peak broadening as the wire length increases. The broadening of the anodic peak is particularly pronounced, showing non-ideal Nernstian behavior, since at all instances the full width at half maximum far exceeds the theoretical value of 45 mV for a surface-confined two-electron redox couple [1]. In fact, this value is not even found for the shortest wires at high pH, where the electrochemical behavior of the wires becomes more reversible (see, for example, figure 6). Although a single peak is observed, representing the transfer of two electrons, it is in fact the result of the merger of two one-electron peaks. Both one-electron transfers occur at similar potentials, but do not happen simultaneously. Herein lies an explanation for the broadness and the reduced height of the single observed peak [27].
Figure 3 Normalized cyclic voltammograms of Q-wires on a gold electrode at constant pH (6.5) and constant scan rate (50 mV/s), illustrating the effect of OPV bridge length (A) $U_0$ (black solid line), $U_1$ (gray solid line), $U_2$ (black dashed line), $U_3$ (gray dashed line) (B) Same line color/style system for $M_0$ through $M_3$

The distance dependence – i.e. the increase in peak-to-peak distance with increasing wire length at a given scan rate – found for the series of quinone-terminated OPV wires presented here, is (mostly) absent from a set of comparable, but fully conjugated hydroquinone-terminated OPV wires described in the literature [1], which are of similar length to $U_1$ and $U_2$. This suggests that when the conjugation of an OPV system is disrupted by a single $sp^3$ carbon, a distance dependence is introduced.
Figure 4 (A) Linear increase in anodic (filled markers) and cathodic (hollow markers) peak current with increasing scan rate; \( M_1 \) on a gold electrode at pH 6.5 (B) Cyclic voltammograms showing said increase; scan rates: 100, 200, 500, 1000 mV/s

As mentioned previously, an ideal, strongly adsorbed redox species is expected to give rise to a linear relationship between peak current and scan rate. As can be concluded from figure 4, this criterion is indeed met. However, the slope of the line pertaining to the anodic peak is less steep in comparison to the slope of the cathodic line, which can be attributed to the broader shape of the anodic peak, which is consequently less high, yet has roughly the same peak area compared to the cathodic peak.
Figure 5 Normalized cyclic voltammograms showing the effect of increasing scan rates on hysteresis; scan rates: 20, 200, 2000, 20000 mV/s. The arrows indicate the direction in which the anodic or cathodic peaks move with increasing scan rate (A) $M_0$ on a gold electrode at pH 6.5 (B) $M_3$ on a gold electrode at pH 6.5

Figure 5 further illustrates the effects of increasing scan rate; an increase in the peak-to-peak distance – or hysteresis – is again found, accompanied by peak broadening. At a given scan speed, the hysteresis found for a longer wire (figure 5B) is more substantial than that found for a shorter wire (figure 5A). The increasing peak-to-peak distance with scan rate can be used to extract kinetic information, as will be described in more detail below. Plotting the peak positions against the logarithm of the scan rate yields a Laviron plot. The effect of pH on the shape of the Laviron plot is clearly
visible in figure 7A; under acidic circumstances, even at low scan rates, a considerable peak-to-peak separation can be observed, whereas this separation approaches zero under alkaline conditions.

Besides its effect on peak-to-peak distance, the pH also influences the midpoint potential of the quinone head group, as illustrated by figure 6 and 7B; an increase in pH causes a decrease in midpoint potential. Additionally, under increasingly alkaline conditions, the quinone moiety displays increasingly reversible electrochemical behavior, i.e. the voltammogram
becomes symmetric and the peak-to-peak distance approaches zero at low scan speeds. By plotting the midpoint potential against pH, a simplified ‘Pourbaix diagram’ [19] is obtained. Figure 7B shows the midpoint potentials pertaining to all the Q-wires as a function of pH. The slope of the linear part of the lines depicted in this figure approach -60 mV/pH, which corresponds to a two-electron/two-proton transfer process [19]. Also, this suggests that the observed midpoint potentials reflect thermodynamic properties, even though the kinetic behavior is not ideal. The hysteresis thus appears to be nearly symmetrical.

This value (-60 mV/pH) was found for all the Q-wires, suggesting that even under very alkaline conditions (pH ≤ 14), a regime of two-electron/one-proton or two-electron/zero-proton transfer is not encountered. This contrasts with \( pK_a \) values reported in literature (e.g. \( pK_a^{QH_2} = 11.2 \) for isoprenic ubiquinone in water/ethanol [29]). Indeed, increased \( pK_a \) values have been reported for redox species directly adsorbed onto the electrode surface in comparison with their soluble counterparts [1, 3, 9, 30, 31]. Note that the lines in figure 7B, which represent simulated data (using eqn. 4.7 and the \( pK_a \) values produced by the PCET model described below), predict full thermodynamic proton decoupling (slope approaches zero) at even more alkaline pH, i.e. the overall, equilibrated reaction is a two-electron/zero-proton reaction in this regime.
Figure 7 (A) Laviron plot of \( \text{U}_3 \) on a gold electrode at pH 3.5 (gray line) and pH 13 (black line). Note that the lines do not represent model fits (B) Simplified Pourbaix diagram of \( \text{U}_0-\text{U}_3 \) (black line, hollow markers) and \( \text{M}_0-\text{M}_3 \) (gray line, filled markers); marker shapes: \( \text{U}_0/\text{M}_0 \) squares; \( \text{U}_1/\text{M}_1 \) diamonds; \( \text{U}_2/\text{M}_2 \) triangles; \( \text{U}_3/\text{M}_3 \) circles. The solid lines represent the results from the global fit to the PCET model.

An interesting phenomenon can be observed in figure 8A; at high scan rates and alkaline pH (here: 0.5 – 10 V/s and pH 12), a second anodic peak occurs at higher potential. While the second peak develops with increasing scan rate, the first recedes and ultimately disappears. This is likely due to a
‘switch’ in the mechanism of the oxidation reaction, where a slower mechanism is replaced with a faster mechanism at higher scan rates.

![Normalized cyclic voltammograms of U₃ on a gold electrode at pH 12, showing the development of two anodic peaks with increasing scan speed, as indicated with the arrows; scan rates: 0.5, 1, 2, 5, 10 V/s (B) Laviron plot showing the coexistence of two anodic peaks (hollow squares) at certain scan rates. The lines represent Butler-Volmer model fits](image)

**Figure 8** (A) Normalized cyclic voltammograms of U₃ on a gold electrode at pH 12, showing the development of two anodic peaks with increasing scan speed, as indicated with the arrows; scan rates: 0.5, 1, 2, 5, 10 V/s (B) Laviron plot showing the coexistence of two anodic peaks (hollow squares) at certain scan rates. The lines represent Butler-Volmer model fits

Given that this behavior is observed at alkaline pH, it is assumed to reflect a kinetic electron-proton (un)coupling effect; the faster proton-uncoupled electron transfer predominant at even higher pH can only be achieved at faster scan rates. A slow step in the mechanism (such as proton transfer) is
outran (‘frozen’) at high scan rates, where only the uncoupled fast steps can occur [37]. Figure 8B, which depicts a Laviron plot illustrating the aforementioned behavior, additionally features a fitting curve resulting from a fit to the Butler-Volmer model described above. Here, although not reflecting reality, both anodic peaks were included in the fit. This minimal modeling approach was chosen because it cannot be excluded that the observed broad single peaks in fact represent two unresolved peaks. In addition, this minimal modeling is implicitly accounted for in the global PCET model analysis (fit) described below.

4.3.4 Kinetic analysis: Butler-Volmer model

As exemplified by figure 8B, the obtained Laviron plots were used to perform fits to the aforementioned Butler-Volmer model, producing output values for $k_{app}$, $\alpha$ and $n_{app}$ (figure 9 and 10). Together with the thermodynamic data ($E'$ vs. pH, see figure 7B), the kinetic data -- $k_{app}$ vs. pH -- then served as input to the PCET model described below. The interpretation of the kinetic data will also be discussed in greater detail.

When inspecting figure 9A, depicting all the $n_{app}$ values found for the wires, it becomes clear that at alkaline pH ≥ 11 the average $n_{app}$ suddenly decreases, after a roughly stable regime, which signifies a decrease in electron cooperativity. Therefore, at alkaline pH, the semiquinone moiety (‘SQ’) is more stable. At low pH, $n_{app} > 1$, which is indicative of cooperativity due to the inversion of $E^{Q/SQ}$ and $E^{SQ/QH2}$. At high pH, $n_{app} \leq 1$, in which case the semiquinone becomes stable and electron transfer occurs in two one-electron steps [27]. At high pH ≥ 14, $E^{Q/SQ}$ and $E^{SQ/QH2}$ may become equal, accounting for the observed approximately 60 mV voltammetric peak width at half-height (FWHM) at high pH [26]. Since $n_{app}$ is in fact a convolution of measures of thermodynamic and kinetic stabilization of the semiquinone, values of $n_{app} < 1$ found at high pH further suggest that, in addition to thermodynamic stabilization of the semiquinone, kinetics may also have to be taken into account.

Although generally somewhat higher than the generic value of $\alpha = 0.5$, figure 9B suggest relatively stable values for the (apparent) transfer
coefficient $\alpha$, obtained from the Butler-Volmer model (expect, perhaps, between pH 11 and 12, where $\alpha$ ranges between 0.4 and 0.8).

Figure 9 Behavior of (A) $n_{app}$ and (B) $\alpha$ vs. pH for $U_{sat}$ (crosses) and $U_0-U_3$ (black dashed line represents average) and $M_0-M_3$ (gray dashed line represents average), obtained from Butler-Volmer model fits. Markers are defined as in figure 7.

4.2.4 Modeling PCET

Figure 10 depicts the kinetic data – i.e. apparent rate constant $k_{app}$ vs. pH – obtained from the Butler-Volmer analysis. The curves represent the global PCET model fit to all data points for one type of Q-wire (menaquinone- or ubiquinone-terminated). Several trends can be observed when inspecting
Firstly, at acidic and neutral pH, the values found for $k_{app}$ are generally low and relatively insensitive to pH change, whereas, at more alkaline pH, a more pronounced increase of $k_{app}$ with increasing pH can be observed. Secondly, a clear dependence of $k_{app}$ on bridge length can be recognized, especially at not too alkaline pH: $k_{app}$ values two orders of magnitude greater than those found for the longest wires ($U_3, M_3$), can be observed for the shortest wires ($U_0, M_0$). Finally, except perhaps in case of $U_3$ and $U_{sat}$, the $k_{app}$ values converge at the highest pH limit, to a value of around 2000 s$^{-1}$. Note that for the shorter wires, the PCET model does not accurately reflect this behavior. It can be concluded that, at very alkaline pH, electron transfer becomes increasingly kinetically uncoupled from proton transfer, but not yet thermodynamically uncoupled, as evidenced by the -60 mV/pH slopes observed in figure 7B, which indicate a (thermodynamic) two-electron/two-proton process.

The disruption of the conjugation in the OPV bridge by a single $sp^3$ carbon introduces a distance dependence, which contrasts with the behavior observed previously for fully conjugated wires of comparable length [1, 8].

It may be argued, however, that in case of short, fully conjugated wires (less than 28 Å in length), the OPV system could be considered part of the redox species. In this scenario, the distance between the edge of the OPV system and the electrode – which is constant for a series of wires differing only in the length of the OPV system – is the relevant tunneling distance between the redox species and the electrode, explaining the absence of an OPV length dependence. The Q-wires presented in this study can therefore – unlike the aforementioned fully conjugated wires – be correctly described as ‘a redox species tethered to an OPV system’, while safeguarding its natural electrochemical characteristics by uncoupling the species from said OPV system. Consequently, the midpoint potentials of the quinone moieties of the Q-wires are close to ‘natural’ values at pH 7: -62 mV for menaquinone- (literature: -70 mV [32]) and 122 mV for ubiquinone-terminated wires (literature: 113 mV [33]).
Figure 10 Apparent rate constants $k_{app}$ vs. pH found for the Q-wires on a gold electrode. Markers are defined as follows: $U_0/M_0$ squares; $U_1/M_1$ diamonds; $U_2/M_2$ triangles; $U_3/M_3$ circles; $U_{SAT}$ crosses. The solid gray lines represent a global PCET model fit to all data points of one type of Q-wire – from top to bottom: (A) $U_0$ through $U_2$, $U_{SAT}$, $U_3$ (B) $M_0$ through $M_3$. The dashed lines define pH regimes where a certain electron transfer pathway through the nine-member square scheme (figure 1) is dominant. These pathways are represented schematically by the insets.

As summarized in figure 1, the theoretical model developed by Laviron and Finklea describes the two-electron/two-proton reaction of a surface-associated redox species in terms of 12 possible individual steps, either a proton or an electron transfer, and relates $k_{app}$ and $E^{\circ'}$ values to microscopic parameters, i.e. six $pK_{ai}$ values, six $E_i^{\circ'}$ values and a single $k_s$ value. The latter
value describes all six pure one-electron transfer events, pertaining to a Q-wire of given length and type of quinone head group.

As can be appreciated in figure 11, \( k_s \) decreases exponentially with wire length, i.e. the vector length between the sulfur center and the center of the closest carbon atom in the quinone head group of a 2D-optimized molecular model. The fitted \( \ln(k_s) \) vs. distance yield well-correlated and somewhat parallel straight lines for \( U_0-U_3 \) and \( M_0-M_3 \). This corroborates the assumption that the microscopic pure-electron \( k_s \) does indeed depend on quinone type.

As described above, figure 11 allows for the calculation of Marcus parameters, i.e. the decay constant \( \beta \) and reorganization energy \( \lambda \). The obtained values can be found in table 1.

| Table 1 Marcus parameters \( \beta \) and \( \lambda \) pertaining to \( M_0-M_3 \), \( U_0-U_3 \) and \( U_{SAT} \) |
|-----------------|-----------------|
| \( M_0-M_3 \)    | \( \beta = 0.20 \text{ Å}^{-1} \) |
|                 | \( \lambda = 1.46 \text{ eV} \) |
| \( U_0-U_3 \)    | \( \beta = 0.23 \text{ Å}^{-1} \) |
|                 | \( \lambda = 1.54 \text{ eV} \) |
| \( U_{SAT} \)    | \( \beta = 0.35 \text{ Å}^{-1} \) (estimated) |
|                 | \( \lambda = 1.54 \text{ eV} \) (estimated) |

The very low value for the decay constant \( \beta \approx 0.2 \text{ Å}^{-1} \) reported here, suggests that the conductivity of the Q-wires is not too compromised by the inclusion of an \( sp^3 \) carbon. Compared to wires of comparable length (i.e. \( U_2 \)), \( U_{SAT} \) has – as expected – a lower \( k_s \) value (figure 11) and a higher (estimated) \( \beta \) value (table 1). The latter value, however, contradicts \( \beta \)
values encountered in literature for fully saturated bridges (0.8 - 1.0 Å⁻¹ [1]). It is therefore likely an underestimation, perhaps due to an incorrectly estimated intercept in figure 11, which additionally results in an incorrectly estimated reorganization energy. Additionally, the flexibility of the alkanethiol tether of $U_{\text{SAT}}$, as opposed to the rigidity of the OPV systems of the other wires, may perhaps provide an explanation. In this scenario, coiling could reduce the tunneling distance between the quinone moiety and the electrode, resulting in an overestimation of the value of $k_s$. If the average distance were reduced to the thickness of the supporting heptanethiol SAM, the decay constant would be closer to that expected for a saturated tunneling medium. Furthermore, this microscopic ‘pure-electron’ rate constant was obtained from a model based on a great number of assumptions, and its true significance is therefore uncertain. It may therefore not be possible to fully separate the ‘pure-electron’ rate constants using the method described above, and a comparison between $U_2$ and $U_{\text{SAT}}$ based on $k_s$ may not be very informative. Comparing the values for $k_{\text{app}}$ of both wires, a rate enhancement ranging between a factor of two and ten, averaging around only four, was found for $U_2$, much lower than the 100-fold rate constant increase reported for a fully conjugated hydroquinone-terminated wire in comparison with a fully saturated wire of similar length [1]. As discussed previously, however, when considering the superior rate constants observed for ferrocene-terminated wires of similar length, one may conclude that, in the case of (hydro)quinone-terminated wires, the nature and length of the bridges are of secondary importance only, and that the coupled electron/proton kinetics of the hydroquinone moiety appear to be rate-limiting. The latter may be supported by the observed convergence of the data points in figure 10, which is not accurately reflected by the PCET model; at very alkaline pH, a maximum overall reaction rate constant is reached, which is only weakly dependent on bridge type and length.

A global fit over all pH values of both $E^{0'}$ and $k_{\text{app}}$ values for the ubiquinone- and menaquinone-terminated wires, allows for the determination of the aforementioned microscopic parameters, which are summarized in figure
12. These parameters aid in the determination of the dominant kinetic pathway through the square scheme (figure 1) at a given pH [34].

\[
\begin{align*}
Q & \leftrightarrow E_3^{0'} \quad pK_{a_3} = -6.70, -7.26 \\
Q^{-} & \leftrightarrow E_6^{0'} \quad pK_{a_6} = 14.00, 14.00 \\
HQ^{+} & \leftrightarrow 468 \text{ mV}, 262 \text{ mV} \quad pK_{a_1} = -7.17, -8.26 \\
HQ^{-} & \leftrightarrow -290 \text{ mV}, -390 \text{ mV} \quad pK_{a_2} = 9.99, 8.96 \\
H_2Q^{2+} & \leftrightarrow 701 \text{ mV}, 555 \text{ mV} \\
H_2Q & \leftrightarrow 680 \text{ mV}, 552 \text{ mV} \\
\end{align*}
\]

Figure 12 Nine-member square scheme for the Q-wires, showing values for the microscopic PCET model parameters $pK_{ai}$ and $E_i^{0'}$ (upper values $U_0-U_3$, lower values $M_0-M_3$), obtained from a global fit over all pH values of both $E_i^{0'}$ and $k_{app}$ values. The $H_2Q^{2+}$ species is unlikely to be formed (gray area).

From the global fit, it becomes clear that the kinetically preferred electron (e)/proton (H) sequence changes from ‘HeHe’ at the lowest pH values, via ‘eHHe’, to ‘eHeH’, and finally to ‘eeHH’ at the highest pH values, as can be appreciated in figure 10.

Therefore, with increasing pH, the observed trend is that electron transfer increasingly occurs before proton transfer. Above pH 12 ($U_0-U_3$) and pH 11.5 ($M_0-M_3$), both electron transfer steps appear to become kinetically uncoupled from proton transfer (both electrons are transferred before protonation). This yields a pH-independent maximum $k_{app}$, even though thermodynamically, electron and proton transfer only become uncoupled above at least pH 14.
It must be remarked that for the shortest wires, the model predicts a somewhat higher maximum $k_{app}$ than observed (figure 10). This may suggest that electron and proton transfer are not fully uncoupled in the short wires: electron transfer is entangled with proton transfer, i.e. occur over a common electron-proton transition barrier, which is lower than the pure proton transfer barrier, but higher than the pure electron transfer barriers that are assumed in the discrete-step square scheme model. Thus, with increasing electron tunneling rate and decreasing proton transfer rates, proton-coupled electron transfer becomes more favorable when occurring over a trajectory in the energy landscape that lies increasingly towards the diagonal of the scheme. To conclude, in case of longer wires or not too high pH, the used discrete ‘over the corners’ electron and proton transfer square-scheme model is close to reality. However, this model is less applicable to the shortest wires at high pH, where an entangled common trajectory through the energy landscape is a faster alternative to fast electron transfer followed by slow proton transfer.

Quinone-dependent enzymes may face the same dilemma: fast turnover of quinone/quinol is most likely promoted by fast electron transfer to nearby cofactors, but the enzyme must then also catalyze fast proton transfer. The enzyme might achieve this simply by rendering electron transfer fast enough to promote a lower common coupled electron-proton energy barrier.

4.3 Conclusion

In this chapter, an electrode treatment procedure was successfully developed that allows for the preparation of electrodes modified with Q-wire SAMs, capable of withstanding very high scan rates subjected to during certain cyclic voltammetry experiments. These experiments resulted in consistent data sets for both menaquinone- and ubiquinone-terminated wires. For example, $E^{0}$ values consistent with literature values were found, suggesting the inclusion of a single $sp^3$ carbon in the bridge indeed preserves the electrochemical characteristics of the quinone-moieties. However, in the Pourbaix diagrams (figure 7B), slopes of -60 mV/pH were consistently found for pH values up to 14, suggesting an increase in $pK_a$. 

Fitting the obtained Laviron plots (e.g. figure 7A) to a Butler-Volmer model provided values for $\alpha$, $n_{\text{app}}$ and $k_{\text{app}}$ vs. pH, the latter of which (figure 10), together with the Pourbaix diagrams, were used as inputs for a PCET model, which was then used to determine microscopic parameters (figure 11 and 12). This analysis aided in the determination of the prevalent electron/proton transfer pathway through the square scheme (figure 1) followed by the quinone moiety. The general observed trend was that, with increasing pH, electron transfer increasingly occurs before proton transfer. Above pH 12 ($U_0$-$U_3$) and pH 11.5 ($M_0$-$M_3$), both electron transfer steps appeared to become kinetically uncoupled from proton transfer, but not yet thermodynamically until at least pH 14. A value for the decay constant of the Q-wires could also be determined: $\beta \approx 0.2 \, \text{Å}^{-1}$, which signifies that the conductivity of the Q-wires is only mildly compromised by the inclusion of the aforementioned $sp^3$ carbon. A distance dependence was nonetheless introduced, which is not found for fully conjugated wires less than 28 Å in length. Furthermore, comparison between $U_2$ and $U_{\text{SAT}}$ (when assumed to be fully uncoiled) only revealed a moderate 2-10 times enhancement in rate constants for $U_2$.

Considering the low electron transfer rates found at biologically relevant pH (figure 10), it cannot yet be concluded whether the Q-wires can provide non-rate-limiting electron transfer between enzymes and the electrode. It is, however, likely that enzymes facilitate fast proton transfer, enhancing the electron transfer rates of enzyme-associated Q-wires.

4.4 Experimental section

4.4.1 Electrochemistry setup

Electrochemistry experiments were performed using a ‘Hagen cell’ [28] (placed inside a Faraday cage), which accommodates a three-electrode setup: the working electrode (gold disk, 2 mm diameter, CH-Instruments), the reference electrode (saturated KCl/calomel with porous glass junction, Radiometer REF401) and the counter electrode (platinum wire). A small volume of electrolyte (~25 µl) was confined between reference and working electrode. Optionally, additions were injected into the electrolyte by
through an injection port. The composition of the cell’s gas headspace could be regulated; all experiments described in this chapter were performed under argon (Linde gas, quality 6.0). After adjusting the counter electrode to contact the electrolyte meniscus, cyclic voltammetry was performed using a digital potentiostat (Autolab PGSTAT 12, GPES software version 4.9, linear sweep mode, step size 2 mV). The recorded voltammograms were analyzed (i.e. determination of peak characteristics) using the aforementioned GPES software.

4.4.2 Electrode preparation

The aforementioned gold working electrodes were polished using aqueous alumina slurries (1 µm, 0.3 µm, 0.05 µm, respectively) on microcloth (Buehler). After each polishing step, the electrode was rinsed and sonicated in water. The electrode was then placed in 80 µl of an appropriate ‘Q-wire’ solution, containing 50 µM of said wire, 5 mM of hydroxylamine and ~1.5 M of water in DMSO. The self-assembled monolayer (SAM) was typically allowed to form overnight, after which the electrode was thoroughly rinsed with water and DMSO. Butanethiol (M₀ and U₀) or heptanethiol (other wires) were used as diluents; incubating the electrode with 0.2% v/v of said alkanethiol in ethanol for ~10 mins ensured the formation of the desired co-SAM. A thorough washing of the electrode with copious amounts of water and DMSO was then performed, after which it was stored in DMSO. Prior to use, the electrode was rinsed with water one final time.

4.4.3 Electrochemistry: Laviron plots

To assess the influence of wire length and pH on the kinetic properties of the ‘Q-wires’, electrodes modified with the appropriate (mixed) SAM were subjected to cyclic voltammetry, performed at increasing scan speeds (10 mV/s – max. 2 kV/s, depending on SAM stability) in buffered solutions with different pH. When necessary, the potential or current range was adjusted. Since the electrodes could not be reused after being exposed to a series of measurements at increasing scan speeds – and with that, increasing overpotentials – freshly prepared electrodes were required for every pH value, duplo and wire type. The following (monovalent) buffers (100 mM)
were used: pyruvic acid (pH 2); formate (pH 3.5); acetic acid (pH 5); Bis-tris methane (pH 6.5); MOPS (pH 7.2); Tris (pH 8); ethanolamine (pH 9.5); piperidine (pH 11); trifluoroethanol (pH 12); potassium hydroxide (pH ‘13’ and pH ‘14’). Where necessary, the ionic strength was adjusted to $I = 0.5$ using KCl. As mentioned previously, GPES software was used to obtain peak characteristics, after which the resulting ‘Laviron plots’ (i.e. $E_{\text{peak}}$ vs log($v$)) were fitted to simulated data obtained from a Butler-Volmer model discussed in the introduction.

### 4.4.4 PCET modeling

In this study, an adaptation of the method described by the Lebedev group was followed [9], which in turn was based on a theoretical analysis by Laviron [13] and Finklea [18]. However, the equation used for the calculation of $E^{Q/QH_2}$ in Lebedev’s work (eqn. 7) was replaced by eqn. 4.7 [35].

$$E^{Q/QH_2} = \frac{E_1^{Q'} + E_4^{Q'}}{2} + \frac{RT}{2F} \ln \left( \frac{10^{-2pH} + 10^{-pH-pKa_5} + 10^{-pKa_5-pKa_6}}{10^{-2pH} + 10^{-pH-pKa_1} + 10^{-pKa_1-pKa_3}} \right) \quad (4.7)$$

The equation implemented in Lebedev’s model was based on the assumption that the fully protonated, oxidized state $H_2Q^{2+}$ and the fully deprotonated, fully reduced state $Q^{2-}$ cannot exist. This assumption yields a truncated apparent Pourbaix equation and an ever-increasing $k_{\text{app}}$ at high pH, which contradicts the clear and consistent leveling off of $k_{\text{app}}$ at high pH observed in this study, which can only be fitted to the given model when the fully deprotonated, fully reduced state $Q^{2-}$ is included as a true kinetic intermediate, even though it is not thermodynamically stable up to pH 14 (i.e., no distinct deviation from the -60 mV/pH slope of $E^{Q'}$ vs. pH was observed up to pH 14 – see figure 7B). Hence, eqn. 4.7 was implemented instead.

For each set of Q-wires (either ubiquinone- or menaquinone-terminated), all $k_{\text{app}}$ and $E^{Q'}$ values as function of pH and wire length were fit globally with
a single set of six $pK_{ai}$ values (thus assuming the proton-affinities to be independent of wire length) and $E_i^{0'}$ values (two independent, four other $E_i^{0'}$ values depend on the former two, as well as on the $pK_{ai}$ values) and one separate free-fitted microscopic $k_i$ for each wire length (thus making the possibly somewhat crude assumption that $k_i$ – i.e. the electron transfer rate at zero overpotential, relative to the microscopic redox potential of the given one-electron step – does not depend on the redox or protonation state of the quinone moiety, but only on its type). Additionally, all microscopic $\alpha_i$ were set to $\alpha_i = 0.5$ [13]. The lowest $pK_a$ ($pK_{a1}$) was not fitted, since it could not be deduced from the data, but was calculated using an equi-distance assumption between the fully oxidized state $Q$ and the fully reduced state $Q^2$- instead: $pK_{a3} - pK_{a1} = pK_{a6} - pK_{a5}$. The other low $pK_a$ values (i.e. $pK_{a2}$ and $pK_{a3}$) were free-fitted since they still influence $k_{app}$ in the acidic pH regime, even though they are below the measured pH range, and thus likely less accurate. The highest $pK_a$ is constrained to be $pK_{a6} \geq 14$, consistent with the observed slope of -60 mV/pH in the Pourbaix traces up to pH 14 (figure 7B). Additional constraints and implementation details can be found in the aforementioned study [9].

4.5 References

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