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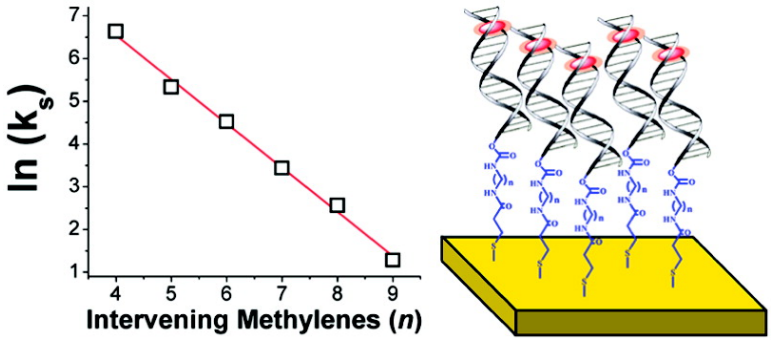

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Electron Transfer Rates in DNA Films as a Function of Tether Length

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Electrochemistry at chemically modified surfaces has been used extensively to examine charge transport (CT) through different media, including straight-chain hydrocarbons, conjugated organic "wires", surfactant films, and proteins.[1−4] We have reported the self-assembly of 15−20 base-pair duplex DNA sequences onto gold surfaces through covalently attached thiol tethers and have exploited the unique characteristics of CT through these assemblies to develop electrochemical assays for DNA intercalation,[5] mutational analysis,[6] lesions,[7] and DNA/protein interactions.[8]

As with DNA CT in solution,[9] DNA films exhibit highly efficient CT over long distances with a remarkable sensitivity to intervening mismatches and lesions.[10] To control precisely the location of intercalating probes, daunomycin (DM) may be site-specifically coupled to the thiol-modified DNA before self-assembly.[11] We previously used this technique to probe the distance dependence of CT by preparing a series of films in which the through-helix DM/gold separations spanned more than 45 Å.[10] Strikingly, the electrochemical response of the intercalated DM did not vary regardless of the DM position along the helix. Given the very shallow distance dependence seen with DNA CT studies in solution,[9] as well as our finding that a single intervening base mismatch caused a complete loss of the electrochemical response,[12] we proposed that tunneling through the (much shorter) σ-bonded tether constitutes the rate-limiting step. To test this hypothesis directly, we have now constructed a homologous series of DM-labeled assemblies featuring thiol-terminated tethers that possess different numbers (n) of methylene units (Figure 1).

DNA sequences (SH-tether-5′-ATCCTCAATCAT**GG**AC-3′ plus complement, where the bold **GG** indicates the DM binding site) were synthesized by solid-phase methods. Changing the length of the diaminoalkane used in the coupling step allowed the synthesis of a series of tethers with n ranging from 4 to 9. After hybridization, the DNA conjugates were covalently labeled with DM by coupling with formaldehyde.[11,13] The resulting DNA−DM conjugates were then self-assembled on a clean gold electrode with excess Mg^{2+} to achieve a dense monolayer.

Extensive physical characterization of DNA monolayers employed for electrochemical measurements is crucial to the proper evaluation of experimental results. In these films, the ratio of DM:DNA determined spectrophotometrically typically varies between 0.85 and 0.95,[14] and the surface coverage of DNA−DM ranges from 30 to 45 pmol/cm^2. These latter measurements are determined both by ruthenium hexammine assay[15] and by integrating the adsorbed DM cyclic voltammetric response.[16] The densities and stoichiometries are consistent with earlier electrochemical-AFM experiments that showed densely packed DNA; in these films, the DNA duplexes adopt an upright orientation along the surface normal at the applied potentials used in the study, due to electrostatic repulsion of the DNA polyanion.[18]

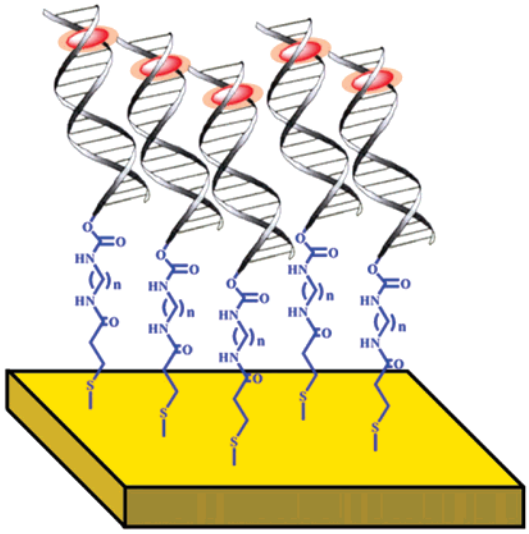

Figure 1. Schematic representation of the DNA−DM modified electrode. The σ-bonded tether is in blue, where n denotes the number of intervening methylene units ($4 \le n \le 9$); DM is in orange. The relative depth of the DNA versus linker region is >3:1 on the basis of AFM investigations.

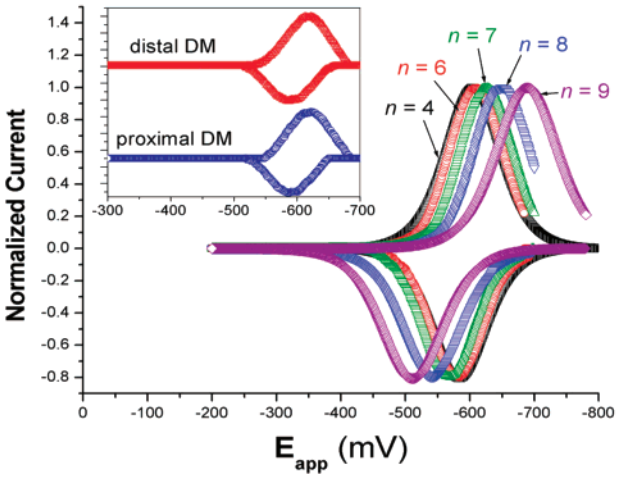

Figure 2. Normalized, background-corrected CVs of DNA−DM monolayers at $\nu = 1$ V/s for tethers with $n = 4$, 6, 7, 8, 9 measured in 5 mM phosphate, 50 mM NaCl, pH 7.5, on a Au electrode (~0.025 cm^2). Inset: normalized CVs ($\nu = 1$ V/s) of DNA−DM films with identical tethers ($n = 6$) but different DM sites: 5′-ATCCT**GG**ATCATCAAC-3′ (blue) and 5′-ATCCTCAATCAT**GG**AC-3′ (red).

Irrespective of tether length, the DNA films exhibit a chemically reversible reduction ca. −604 mV versus AgCl/Ag 1 M KCl (Figure 2). However, the CV response clearly varies with the number of intervening methylene groups in the DNA tether at scan rates (ν) above ~1 V/s. As previously,[10] no variations are apparent upon changing the DM intercalation site along the helix (Figure 2, inset).

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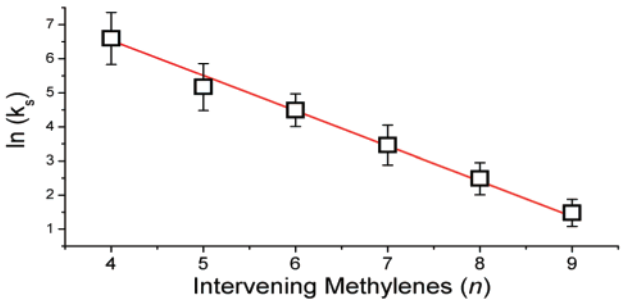

Figure 3. Plot of ln(k_s) versus number of methylene units (n) obtained for different tether lengths.

Plots of the cathodic and anodic peak splitting (ΔE_{pc} and ΔE_{pa}) were analyzed as a function of scan rate according to Laviron's model[19] to yield standard electron-transfer rate constants, k_s ($\Delta G^\circ = 0$), as a function of n (see Supporting Information).

On the basis of these data, values for k_s increase exponentially with decreasing n (Figure 3), consistent with predictions from simple superexchange coupling theory (eq 1), yielding an apparent value for β_n of 1.0 per $-CH_2-$ unit.

$$k_s(n) = k_{n=0} \exp(-\beta_n n) \quad (1)$$

This value is nearly identical to those previously measured for tunneling through an alkylthiol bridge to bound ferrocene ($\beta \sim 1.1$ per CH_2),[1,20] as well as to cytochrome c ($\beta = 1.0-1.1$ per CH_2)[4b,c] and azurin ($\beta \sim 1.0$ per CH_2).[4d] Assigning a β value of 1.0 per bond for the additional eight σ-bonds of the linker that are not included in the methylene chain leads to an extrapolated, zero linker-length rate (k_o) of $\sim 10^8-10^9$ s^{-1}. This value, a lower limit for ET through the DNA−DM conjugate, is comparable to those found using small-molecule redox probes bound directly to the surface,[21] yet here the redox probe appears to be the full ~60 Å long DM−DNA conjugate.

Even though changes in redox probe position within DNA yield no detectable change in ET rate,[22] the variation of rate with distance spanned by the tether is well-behaved. Indeed, on the time scale of CV experiments, the DNA−DM conjugate behaves as a discrete redox-active entity, with an electrochemical response that is independent of the DM intercalation site. This behavior is reminiscent of that seen in STM studies on DNA films where efficient coupling of the tip to the gold surface through the DNA assembly is evident.[24] Here, irrespective of the mechanism for CT through the DNA assembly, CT through the σ-bonded tether follows semiclassical superexchange theory. When both the tether and the DM position are varied, it is clear that CT through the σ-bonds versus π-stack differs significantly, and it is CT through the σ-bonds that limits the rates in DNA films.

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Supporting Information Available: Tabulated electrochemical data for the tether series. Plots of CV peak splitting as a function of scan rate and AFM height measurements of DNA monolayers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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