An intercalator film as a DNA–electrode interface†

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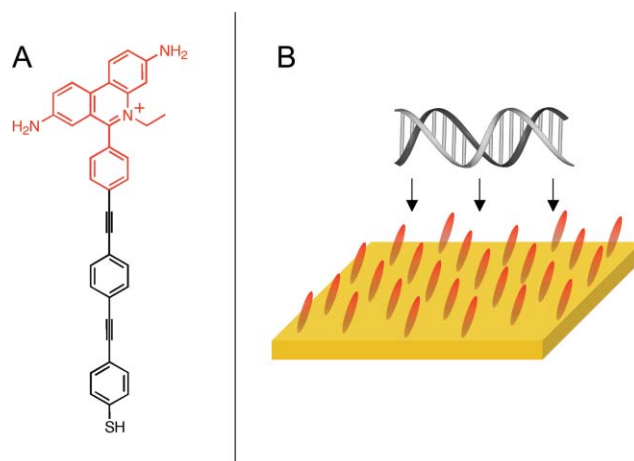**DNA–surface conjugation is achieved through an intercalating molecular wire, resulting in more efficient electron transfer relative to systems utilizing conventional insulating tethers.**

Interfacing DNA and electroactive materials will facilitate the construction of bioelectronic devices and electronic biosensors.[1] Recently, important advances in the development of conductive linkers tethering organic molecules to surfaces were reported,[2] but similar approaches for biomolecules are not currently available. Conventional tethers between electrodes and the DNA bases or backbone typically feature alkane chains[3] that inhibit the transfer of electrons and result in decreased current flow.[4] While efforts have been made to substitute conjugated systems for alkanes, only subtle improvements in electronic coupling are observed.[5] The design of linkers that do not introduce a significant electronic bottleneck inhibiting current flow remains an unsolved problem. In an effort to design a more conductive linker, we considered previous studies suggesting that intercalating molecules participate in more efficient electron-transfer processes in DNA compared to linker molecules attached to a base or backbone site. Here, we present a novel method for attaching DNA to electrode surfaces through a highly conjugated intercalator.[6] This connectivity promotes more efficient electron transfer between the electrode and reporter groups than conventional linkers.[4a,4d,7]

A self-assembled film containing an intercalator derivative was prepared to provide a modified electrode surface that would contact DNA *via* the stacked base pairs (Scheme 1, see ESI†). The intercalator is an ethidium derivative (EtX) featuring a highly-conjugated linkage terminating in a thiol, which can be considered an intercalating molecular wire. One previous report of a thiol-modified ethidium derivative showed that monolayers of this type can be formed.[8] However, the compound used had an alkane tether between the intercalator and thiol. The films we have prepared—featuring the highly-conjugated structure of EtX—are the first to contain an intercalating wire.

To determine whether the EtX film promoted binding of DNA to the electrode surface, gold nanoparticle labeling[9] was used to facilitate visualization by scanning electron microscopy (SEM).[10] To test binding of single-stranded (ss) and double-stranded (ds) DNA, modified PCR products (ds) and synthetic oligonucleotides (ds and ss) were conjugated to gold nanoparticle labels. These conjugates were then incubated with the EtX film and imaged by SEM (Fig. 1). Interestingly, the highest surface coverage was

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Scheme 1 (A) An ethidium derivative, EtX, featuring a conjugated linker terminated by a thiol. (B) Schematic illustration of an EtX monolayer immobilized on a gold surface. Double-stranded DNA, which binds with high affinity to intercalating molecules, would be interfaced with the electrode surface *via* intercalation.

obtained with a ss 15-base DNA oligonucleotide (Fig. 1C, 90 fmol cm^{-2}), indicating that the conformational flexibility of this unstructured oligomer facilitates dense packing on the EtX-modified surface.[11] Consistent with this notion, other studies have revealed higher densities of films composed of ss relative to ds DNA.[5] Indeed, a 15-base-pair (bp) ds duplex also yielded sparse coverage (Fig. 1A, 6 fmol cm^{-2}) when deposited on the EtX film,
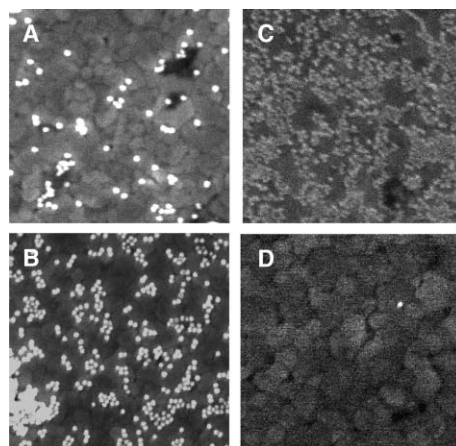

Fig. 1 SEM images (2.25 µm^2) of 5 nm Au-labeled DNA bound to EtX monolayer on Au surfaces. In (A) the DNA is a 15-bp synthetic oligonucleotide duplex, (B) is a 375-bp PCR product, (C) is a 15-base ss oligonucleotide, and (D) is unconjugated nanoparticles. In the absence of EtX, no binding to the surface was observed (data not shown).

while a 375-bp ds PCR product displayed a higher density (Fig. 1B, 24 fmol cm^{-2}). The stronger adsorption of the larger PCR product relative to the 15-bp duplex suggests that higher stability is imparted by the larger number of binding sites on this type of DNA substrate.[12] Addition of benzenethiol as a diluent to the EtX monolayer decreased the surface coverage, indicating that this molecule could be used as a spacer to control the amount of DNA bound to a surface through this type of linkage (see ESI†).

The electronic interface between the electrode and DNA provided by the EtX film was probed using electrochemical methods (Fig. 2). Both ss and ds DNA were used to evaluate the role of the intercalative interaction between the EtX film and DNA. Ss DNA (Fig. 2A, inset) should exhibit electrostatic binding to the positively charged intercalator, while ds DNA (Fig. 2A) will bind *via* intercalation. In addition, a duplex oligonucleotide derivatized with an alkanethiol linker (SH–ds DNA)[5] was used to generate a monolayer that had a direct—but insulating— linkage between the electrode and DNA (Fig. 2B). An intercalating redox-active probe, methylene blue (MB), was bound to the different DNA films, and its electrochemical reduction was monitored to evaluate how charge transport was affected.

The reduction of MB was observed with all three types of films (Fig. 2), and scan-rate studies confirmed that MB was surface bound in all three cases (see ESI†). However, the electron-transfer kinetics were significantly different within the three films. As shown in Fig. 2, the peak separation observed at 50 V s^{-1} with the ds DNA–EtX film (ΔE_{pc} = 98 mV) is significantly smaller than that observed with the SH–ds DNA film (ΔE_{pc} = 199 mV),

consistent with EtX providing better electronic coupling than the alkanethiol linker.[13] Therefore, it appears that intercalative binding of DNA to the EtX film introduces a favorable electronic interface that cannot be provided by an alkanethiol. Furthermore, when ss DNA is deposited on the intercalator monolayer, highly irreversible electrochemistry and large peak separations are observed (Fig. 2A, inset). The much weaker and more irreversible response obtained with ss DNA provides strong evidence that the efficient reduction of MB bound to ds DNA is a result of efficient charge transfer proceeding through DNA. If the fast kinetics resulted from a diffusional pathway facilitated by the enhanced concentration of MB at the surface, equivalent responses should be obtained for ss and ds DNA. DNA that is ss, lacking an ordered and stacked base array for intercalative binding of EtX, does not facilitate efficient electron transfer.

The system we describe features a novel DNA–electrode connectivity that exhibits improved electronic coupling. Intercalative binding of DNA to a self-assembled film presents a means to impart a more direct connection between a biomolecule and an electrode surface that may provide improved sensitivity to electrical biosensing assays. Additionally, the constructs described will assist in the construction of DNA-based electroactive materials and new hybrid bioinorganic heterostructures.

Notes and references

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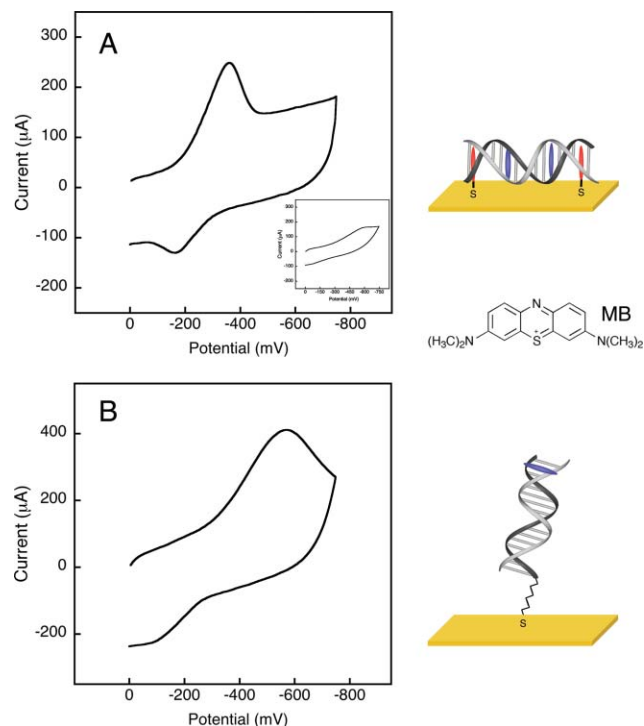**Fig. 2** Cyclic voltammetry of 1 μM MB at gold electrodes modified with (A) EtX monolayer with bound ds DNA, (A, inset) EtX monolayer with bound ss DNA and (B) monolayer of SH–ds DNA. The schematic illustrations include intercalated MB (blue) and EtX (red). Scan rate is 50 V s^{-1} and IR compensation was used to minimize IR drop. The surface coverage of the two ds monolayers was very similar (see ESI Fig. S2†).

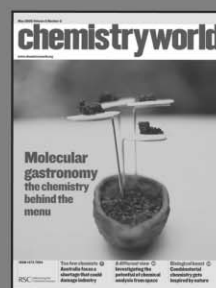R. L. Letsinger, R. Elghanian and G. Viswanadham, *Anal. Chem.*, 2000, **72**, 5535–5541.

10 $FeCN_6^{4-}$ blocking electrochemistry assays were used to monitor DNA binding to EtX-modified electrodes (see ESI figure S2†).

11 Ethidium, which binds to ds DNA *via* intercalation, also binds to ss DNA electrostatically at high concentrations, hence its use as a ubiquitous gel electrophoresis stain for both structural forms (see J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2nd edn, 1989). The high effective concentration of EtX within the immobilized film must therefore drive binding of ss DNA.

12 Binding of DNA to the EtX film was reversible, as washing with high ionic strength buffers or $MgCl_2$ caused dissociation. In addition, the introduction of a diluent into the film (*e.g.* benzenethiol) also decreased surface coverage of all types of DNA (data not shown).

13 Previous measurements of electron-transfer kinetics for MB bound to alkanethiol-linked DNA monolayers yielded a k_{et} value for this system of 150 s^{-1}.[5] For the EtX wire system, the rate was too fast to be accurately measured using conventional electrochemistry.