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Methylene blue as an electrochemical discriminator of single- and double-stranded oligonucleotides immobilised on gold substrates

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Self-assembly of thiol-terminated oligonucleotides on gold substrates provides a convenient and versatile route to DNA-functionalised surfaces. Here we show that the square-wave voltammetric peak position of methylene blue complexed to thiol-terminated single-stranded oligonucleotides immobilised on gold electrodes differs from that of methylene blue complexed to thiol-terminated double-stranded oligonucleotides immobilised on gold electrodes. The peak potential of methylene blue at the single-stranded oligonucleotide array was consistently found to occur at potentials *ca.* 10–15 mV more positive than that at double-stranded oligonucleotide arrays, the precise difference being dependent on the direction of the voltammetry. This voltammetric behaviour mirrors that found for methylene blue bound to freely diffusing single- and double-stranded calf thymus DNA and suggests that the immobilised oligonucleotides retain the methylene blue binding properties of their freely diffusing counterparts. Thus methylene blue provides a simple electrochemical indicator for the status of oligonucleotide-functionalised gold surfaces.

Introduction

DNA-functionalised surfaces provide versatile substrates for a wide variety of applications. Many diagnostic devices exploit arrays of single-stranded (ss) DNA probe sequences. The single-stranded probes hybridise with DNA targets of complementary sequence to generate double-stranded (ds) DNA and these probe arrays lend themselves to applications including the diagnosis of genetic and infectious disease, sequencing by hybridisation and the identification of genes in modified organisms.[1–4] The sensing surface can be regenerated by chemical or thermal dehybridisation and signal transduction may be through optical, electrochemical or mass-sensitive methods. DNA–ligand interactions can also be investigated at DNA arrays. The ligand of interest may be a drug or a protein with DNA repair or expression activities.[5,6] Recently, advances in nucleotide chemistry have allowed the rational design of oligonucleotides with terminal and internal functionalities. These chemistries have facilitated the incorporation of signal transducing and surface anchoring species into oligonucleotides and have led to the use of oligonucleotides as powerful molecular 'scaffolds' in the construction of nanoscale assemblies from solid supports.[7]

Self-assembly of terminally thiol-labelled oligonucleotides on gold surfaces represents a direct and simple method for the formation of oligonucleotide-functionalised surfaces. Covalent attachment of oligonucleotides to the surface occurs through Au–S bond formation and the resulting Au–S–oligonucleotide (Au–S–Oligo) array exhibits thermal stability to at least 80 °C, allowing multiple cycles of thermally directed de/re-hybridisation to be performed.[7,8] Redox transformations of the immobilised oligonucleotides occur outside the potential range of stability for these electrodes, *ca.* −1 to 0.5 V *vs.* SHE.[9,10] However, interactions between oligonucleotides and redox-active molecules of appropriate reduction potential provide an opportunity for voltammetric characterisation of Au–S–Oligo systems. For example, redox molecules which bind preferentially or more numerously to ds- than ss-oligonucleotides give rise to enhanced voltammetric signals from Au–S–dsOligo systems.[5,11,12] Redox molecules which intercalate at remote sites within an Au–S–dsOligo array can also be 'wired' to exchange electrons with the electrode through the π-stack of ds-oligonucleotides.[13–15] In this latter configuration, single base mismatches within the π-stack perturb the conductivity of the Au–S–dsOligo array, allowing for sensitive detection of oligonucleotide sequences with complete complementarity.

Molecules which exhibit distinct reduction potentials when bound to ss- or dsDNA also afford the opportunity to discriminate between Au–S–ssOligo and Au–S–dsOligo surfaces. The reduction potential of benzyl viologen has been shown to afford discrimination between calf thymus ss- and dsDNA immobilised on gold electrodes.[16] The reduction potential of methylene blue allows similar discrimination of calf thymus DNA immobilised on carbon paste electrodes.[17] Here we report that methylene blue provides a rapid and convenient voltammetric probe for discrimination between Au–S–ssOligo and Au–S–dsOligo surfaces. The voltammetric behaviour of methylene blue at Au–S–ssOligo and Au–S–dsOligo arrays mirrors that of methylene blue bound to freely diffusing calf thymus ss- and dsDNA, respectively. Our observations suggest that the immobilised oligonucleotides retain the methylene blue binding properties of their freely diffusing counterparts. Thus methylene blue may have utility as an electrochemical indicator of oligonucleotide strandedness within a DNA biosensor exploiting Au–S–Oligo arrays or for monitoring the status of an oligonucleotide functionalised gold surface during nanostructure assembly.

Experimental

Samples were prepared and voltammetric experiments performed in 20 mM HEPES, 100 mM NaCl, pH 7.5, unless stated otherwise. All solutions were passed through a 0.2 μm filter prior to use and were prepared with water having resistivity > 18 MΩ cm. Methylene blue (BDH) was used as received and dissolved in water to provide a stock standard solution of *ca.* 1.6

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g l^{-1}. Methylene blue concentrations were determined by absorption spectroscopy using the method of Müller and Crothers.[18] DNA solutions were prepared from the sodium salts of calf thymus dsDNA (Sigma) and calf thymus ssDNA (Fluka) by gently stirring overnight at 4 °C. The absorption ratio $A_{260\,nm}/A_{280\,nm}$ was confirmed to be >1.8 and solutions were used within 2 d of preparation. The concentrations of dsDNA and ssDNA (moles of base l^{-1}) were determined using $\varepsilon_{260\,nm}$ = 6600 l mol^{-1} cm^{-1} and $\varepsilon_{260\,nm}$ = 8250 l mol^{-1} cm^{-1}, respectively.[19] Purified oligonucleotides AT1* 5′-CAGCTAA-CACGACA$(CH_2)_6$–SS–$(CH_2)_6$–OH (MWG Biotech, Germany), AT1 5′-CAGCTAACACGACA$(CH_2)_6$–SS–$(CH_2)_3$–OH and AT2 5′-GTCGTGTTAGCTG-3′ (Sigma-Genosys, UK) were dissolved overnight at 4 °C, then diluted to a concentration of 200 μM bases. Equal concentrations (200 μM bases) of AT1*, AT1 and AT2 were mixed at room temperature to prepare alcohol–disulfide–ds-oligonucleotides. Absorption spectroscopy showed the expected decrease in $A_{260\,nm}$ and hybridisation was essentially instantaneous. Oligonucleotide concentrations (moles of base l^{-1}) were determined using $\varepsilon_{260\,nm}$ (AT1*, AT1) = 1.38×10^6 l mol^{-1} cm^{-1} and $\varepsilon_{260\,nm}$ (AT2) = 1.25×10^6 l mol^{-1} cm^{-1}.

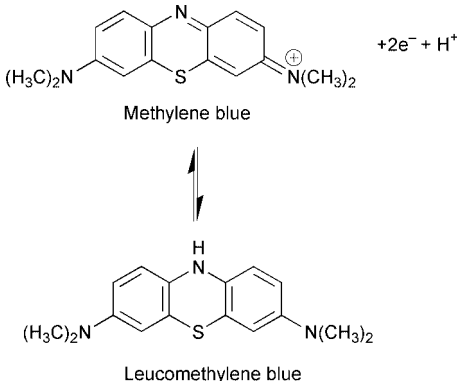
Voltammetry was performed using a three-electrode cell configuration with a platinum counter electrode. A saturated calomel reference electrode (SCE) contacted the sample through a Luggin capillary tip and the sample compartment thermostated at 18 °C, was made anaerobic by a flow of humidified argon gas. Polycrystalline gold electrodes (radius 0.15 mm) were prepared by polishing with an aqueous slurry of 0.3 μm aluminium oxide placed on the cotton wool-covered head of an electric toothbrush (Sunstar, Japan) until a highly reflective 'mirror' finish was achieved. The electrode was then sonicated, rinsed thoroughly with water, dried and placed in 50 mM H_2SO_4. Cyclic voltammetry (0–1.5 V *vs.* SCE, 100 mV s^{-1}) was performed until stable voltammograms were obtained. The voltammograms were in good agreement with those previously reported for clean gold surfaces.[20,21] The electrode surface area and surface roughness factor were calculated from the area of the gold oxide reduction wave and typically found to be 0.17 ± 0.01 cm^2 and 2.44 ± 0.20, respectively. Two-component functionalised electrodes composed of thioalcohol and thiooligonucleotide molecules were prepared by overnight incubation at 4 °C of freshly prepared gold electrodes in freshly prepared solutions of alcohol–disulfide–ss-oligonucleotide (200 μM bases) or alcohol–disulfide–ds-oligonucleotide (200 μM base pairs). Single-component thioethanol-funtionalised electrodes were prepared in a similar manner from solutions of 200 μM 2,2′-dithiodiethanol (Fluka). Functionalised electrodes were rinsed thoroughly with buffer–electrolyte prior to use.

Voltammetry was performed with an Autolab Electrochemical Analyser (EcoChemie, The Netherlands) under the control of GPES software. The parameters for square-wave voltammetry, defined in Fig. 1, were step potential = 0.15 mV, pulse height = 50 mV and frequency ($1/\tau$) = 100 Hz. Prior to initiating the pulse sequence, the electrode was held for 10 s at the starting potential. Quantification of baseline-corrected voltammograms was performed with GPES software to determine the peak potential (E_p), peak height (i_p) and width at half-height (δ) of the voltammetric wave as defined in Fig. 1. The superscript + or − is used to distinguish those parameters derived from a voltammetric sweep to increasingly positive or increasingly negative potentials, respectively. Baseline correction was performed by subtraction of a voltammogram measured in buffer–electrolyte alone or a polynomial base curve fitted to the experimental voltammogram. Analysis by either method produced indistinguishable values of E_p, i_p and δ. The data in Table 1 represent the averaged results from experiments with at least three independently prepared electrodes for each surface functionalisation. All potentials are reported relative to the standard hydrogen electrode (SHE) by addition of 240 mV to the measured value.[22]

Results and discussion

Voltammetry of methylene blue and its complexes with freely diffusing calf thymus DNA

Reduction of methylene blue proceeds by the addition of two electrons and one proton under the experimental conditions to form leucomethylene blue.[23,24]



Voltammetry was performed at naked gold electrodes with both methylene blue and calf thymus DNA freely diffusing in solution to establish whether the electrochemical properties of methylene blue were sensitive to the presence and strandedness of DNA. Cyclic voltammograms of 25 μM methylene blue at 20 mV s^{-1} showed a quasi-reversible wave pair having 36 ± 6 mV separation between anodic and cathodic peak potentials (Fig. 2). The average of the anodic and cathodic peak potentials was 6 ± 11 mV, in agreement with the reduction potential of methylene blue determined under comparable conditions in previous studies.[23,24] The asymmetry of the anodic and cathodic waves

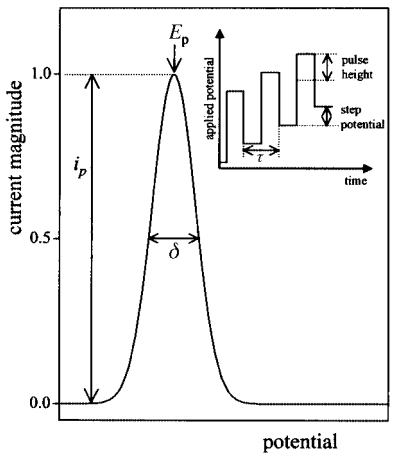


Fig. 1 Definition of the parameters used in this study for quantification of square-wave voltammograms; i_p is the peak height, E_p the peak position and δ the peak width at half-height. Inset: square-wave excitation profile.

Table 1 Square-wave voltammetric peak parameters for methylene blue at functionalised gold electrodes after immersion for 20 min in 3 μM methylene blue, 20 mM HEPES, 100 mM NaCl, pH 7.5, at 18 °C

	E_p^+/mV	E_p^-/mV	$\lvert i_p^+ \rvert / \lvert i_p^- \rvert$	δ^+/δ^-
Au–S–ssOligo/alcohol	5 ± 6	−10 ± 8	0.58 ± 0.18	0.79 ± 0.07
Au–S–dsOligo/alcohol	−9 ± 7	−21 ± 9	0.45 ± 0.13	0.89 ± 0.04
Au–S–ethanol	−2 ± 5	− 4 ± 6	1.13 ± 0.08	1.00 ± 0.05

has been seen previously and may result from kinetic complexity in the methylene blue ↔ leucomethylene blue transformation.[25] A plot of cathodic peak height, i_{pc}, *versus* (scan rate)$^{1/2}$ was linear from 5 to 200 mV s^{-1}.

Cyclic voltammetry of 25 μM methylene blue in the presence of 0.75 mM bases ssDNA or 0.75 mM base pairs dsDNA showed a large decrease in the amplitude of the voltammetric response compared with that in the absence of DNA (Fig. 2). The smaller peak currents introduced some error into their quantification but within this error the proportionality of i_{pc} and (scan rate)$^{1/2}$ persisted. These observations are in agreement with the results of previous studies where small redox molecules bind to DNA and adopt the diffusion coefficient of the much larger molecule.[26] Voltammetry of potassium hexacyanoferrate(III) or ferrocenemonocarboxylic acid, which do not bind to DNA, was unchanged by the presence of equivalent concentrations of DNA.

Cyclic voltammograms such as those shown in Fig. 2 indicated that the reduction potential of methylene blue was displaced to more negative values on complexation with DNA. However, poor definition of the cyclic voltammetric waveforms prevented confident calculation of the reduction potentials for the methylene blue–DNA complexes. By contrast, square-wave voltammetry afforded clear resolution of the distinct peak potentials arising from the methylene blue–ssDNA and methylene blue–dsDNA complexes (Fig. 3). The peak potentials determined from square-wave voltammetric sweeps performed to increasingly positive potentials (E_p^+) were −27 ± 4 and −35 ± 1 mV for the methylene blue–ssDNA and methylene blue–dsDNA complex, respectively. When voltammetry was performed in the direction of increasingly negative potentials, the

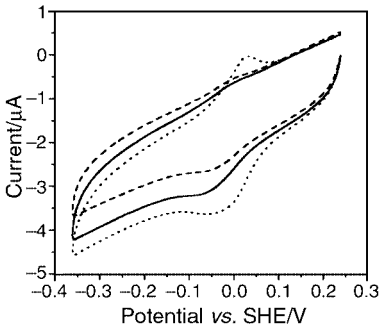


Fig. 2 Cyclic voltammograms of 25 μM methylene blue at a naked gold electrode in buffer–electrolyte (dotted line) containing 0.75 mM bases calf thymus ssDNA (dashed line) and 0.75 mM base pairs calf thymus dsDNA (solid line). The buffer–electrolyte was 20 mM HEPES, 100 mM NaCl, pH 7.5, the scan rate was 20 mV s^{-1} and the temperature was 18 °C.

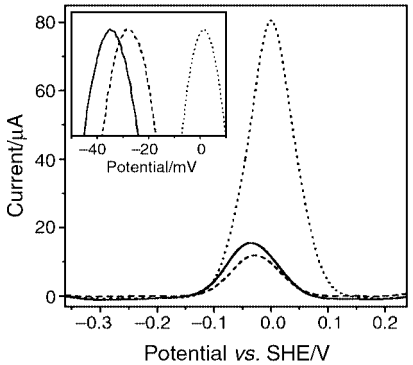


Fig. 3 Square-wave voltammograms from 25 μM methylene blue at a naked gold electrode in buffer–electrolyte (dotted line) containing 0.75 mM bases ssDNA (dashed line) and 0.75 mM base pairs dsDNA (solid line). Inset: magnified view of the voltammetric waves after normalisation. Voltammetry was performed to increasingly positive potentials with step potential = 0.15 mV, pulse height = 50 mV and frequency = 100 Hz. All other conditions as in Fig. 2

methylene blue–ssDNA complex also showed more positive values of E_p^- than the dsDNA complex, −40 ± 7 and −50 ± 2 mV, respectively. The non-equivalence of E_p^+ and E_p^- noted for each methylene blue–DNA complex was found in square-wave voltammograms of non-complexed methylene blue (E_p^+ = 2 ± 2 mV, E_p^- = −18 ± 1 mV), most likely reflecting the kinetic complexity of the methylene blue ↔ leucomethylene blue transformation. Titration of 25 μM methylene blue with increasing concentrations of ss- or dsDNA showed that the E_p values reported here were recorded under conditions of almost complete complexation of the methylene blue by each form of DNA.

Voltammetry of methylene blue at oligonucleotide functionalised gold electrodes

Non-specific interactions between gold and nucleotide bases cause adsorbed oligonucleotides to lie across a gold surface.[27] Such a situation has a detrimental effect on the efficiency and rate of hybridisation of a probe array and may hinder the use of such an array as a structural scaffold. Formation of a two-component array comprised of terminally thiol-labelled oligonucleotides and alcohols removes the opportunity for non-specific interactions between oligonucleotides and a gold surface.[27] The interactions between immobilised alcohol and oligonucleotide molecules are minimal and homogeneous behaviour of the immobilised oligonucleotides towards solution molecules is expected.

Electrodes functionalised with two-component arrays of thioalcohol and thiooligonucleotide molecules were prepared by spontaneous adsorption of alcohol–disulfide–oligonucleotides onto gold electrodes as described in Experimental. Several experiments were performed to confirm that the voltammetry of methylene blue at these electrodes, termed Au–S–Oligo/alcohol electrodes, was determined by the oligonucleotide rather than the alcohol coadsorbate. Voltammetry was performed at gold electrodes functionalised with propanol– or hexanol–disulfide–oligonucleotides and also at single-component, thioethanol-functionalised (Au–S–ethanol) electrodes. Square-wave voltammetry of the functionalised electrodes in buffer–electrolyte showed no Faradaic response over the potential range of interest (−360 to 240 mV).

In solutions containing 3 μM methylene blue, there were clear differences in the square-wave voltammetric response from each functionalised electrode. The signal magnitude, $|i_p|$, recorded at Au–S–ethanol electrodes immersed in the methylene blue solution showed little variation over 30 min. However $|i_p|$ measured at the Au–S–Oligo/alcohol electrodes increased over *ca.* 15 min to approach a limiting value (not shown). These results are consistent with relatively slow accumulation of methylene blue within a densely packed layer of oligonucleotide immobilised on the gold surface. Typical square-wave voltammograms from each of the electrodes recorded after immersion for 20 min in 3 μM methylene blue are shown in Fig. 4. The magnitude of the voltammograms in this figure has been normalised to emphasise the distinct voltammetric response from methylene blue at each electrode. It is clear that the waveforms arising from methylene blue at the Au–S–Oligo/alcohol electrodes are much broader than that measured at the Au–S–ethanol electrode. Comparison of the square-wave voltammograms obtained for sweeps to increasingly positive and increasingly negative potentials showed further differences in the voltammetry of methylene blue at the Au–S–Oligo/alcohol electrodes compared with the Au–S–ethanol electrodes (Table 1). Non-equivalence of δ^+ and δ^- was observed at the Au–S–Oligo/alcohol arrays with δ^- larger than δ^+ by *ca.* 23 and 15 mV for the ss- and ds-Oligo, respectively. In addition, voltammetry at the Au–S–Oligo/alcohol electrodes yielded

values of $|i_p^+|$ consistently smaller than $|i_p^-|$ whereas for the Au–S–ethanol electrode $|i_p^+|/|i_p^-| \approx 1$.

Distinction between Au–S–ssOligo/alcohol and Au–S–dsOligo/alcohol electrodes was afforded by the square-wave voltammetric peak potentials exhibited by methylene blue at these two surfaces (Fig. 4 and Table 1). Au–S–dsOligo/alcohol electrodes exhibited E_p values more negative than those observed at Au–S–ssOligo/alcohol electrodes and the values of E_p were independent of whether the coadsorbate was thiopropanol or thiohexanol. The difference in E_p^+ values is sufficient to allow for confident discrimination between Au–S–ssOligo/alcohol and Au–S–dsOligo/alcohol electrodes. Thus methylene blue provides a convenient redox indicator with which to confirm the presence and nature of an oligonucleotide array. The relative displacement of peak potentials from Au–S–Oligo/alcohol arrays is the same as for methylene blue complexed to freely diffusing ss- and dsDNA. Comparison of the insets in Figs. 3 and 4 shows that the absolute values of E_p for methylene blue complexed to freely diffusing calf-thymus DNA and the surface-immobilised oligonucleotides differ. This may reflect differences in the nucleotide sequence binding methylene blue in each case or the higher density of nucleotides and/or methylene blue in the electrode-immobilised array. During accumulation of methylene blue at the Au–S–Oligo/alcohol electrodes there were no detectable changes in the parameters describing the waveform aside from an increase in $|i_p|$ which indicates that the oligonucleotides rather than the alcohol molecules determine the voltammetric response at all times. The voltammetric behaviour described above persisted for at least 2 weeks after electrode functionalisation.

Reduction of aqueous methylene blue is accompanied by protonation events which produce a pH dependence of the methylene blue/leucomethylene blue reduction potential.[23,24] To determine whether greater separation of E_p values from Au–S–ssOligo/alcohol and Au–S–dsOligo/alcohol electrodes could be achieved experiments were performed across the pH range 6–8.5 where Au–S–X arrays are stable and dehybridisation of ds-oligonucleotides is not expected.[28] E_p values from each electrode were displaced to more negative potentials on increasing the pH but the difference between E_p values from the ss- and ds-oligonucleotide-functionalised electrodes remained essentially invariant.

Spectroscopic studies have shown that methylene blue interacts with dsDNA by three binding modes: (i) intercalation between successive base pairs with face-to-face binding of the bases and methylene blue; (ii) insertion into the minor groove; and (iii) insertion into the major groove of the double helix.[29–31] Intercalation is predicted as the dominant binding mode for DNA sequences such as those used in this study which contain all four nucleotides. Studies of methylene blue binding to ssDNA are less extensive. Our studies utilising absorption spectroscopy indicate that methylene blue associates with calf thymus ss- and dsDNA with comparable affinities under the conditions of the voltammetric experiments.[32] When methylene blue binds to ssDNA the absorption spectrum experiences a red shift and hypochromism similar to that observed when methylene blue binds to dsDNA.[29–31] Therefore, face-to-face binding of methylene blue and the bases of both ss- and dsDNA is expected although the absence of base pairs and long-range structure in ssDNA will result in distinct environments for the methylene blue bound to each form of DNA. Consequently, the observation of distinct reduction potentials for methylene blue complexed to ss- and ds-nucleotide systems is not surprising.

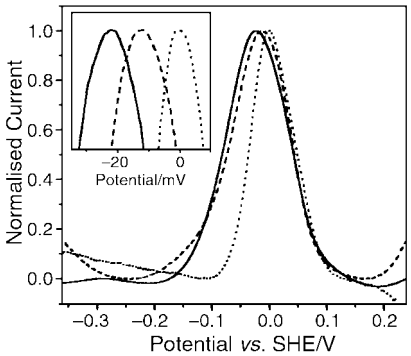


Fig. 4 Representative normalised square-wave voltammograms from Au–S–ethanol (dotted line), Au–S–ssOligo/alcohol (dashed line) and Au–S–dsOligo/alcohol (solid line) electrodes after incubation for 20 min in 3 μM methylene blue. Inset: magnified view of the voltammetric peak positions. Voltammetry was performed to increasingly negative potentials. All other conditions as Fig. 3.

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References

1 J. Wang, *Nucleic Acids Res*., 2000, **28**, 3011.
2 M. Schena, R. A. Heller, T. P. Theriault, K. Konrad, E. Lachenmeier and R. W. Davis, *TIBTECH*, 1998, **16**, 301.
3 M. Chee, R. Yang, E. Hubbell, A. Berno, X. C. Huang, D. Stern, J. Winkler, D. J. Lockhart and M. S. Morris and S. P. A. Fodor, *Science*, 1996, **274**, 610.
4 M. J. O'Donnell-Maloney, C. L. Smith and C. R. Cantor, *TIBTECH*, 1996, **14**, 401.
5 K. Hashimoto, K. Ito and Y. Ishimori, *Anal. Chem*., 1994, **66**, 3830.
6 K. Bondeson, A. Frostell-Karlsson, L. Fagerstam and G. Magnusson, *Anal. Biochem*., 1993, **214**, 245.
7 J. J. Storhoff and C. A. Mirkin, *Chem. Rev*., 1999, **99**, 1849.
8 K. A. Peterlinz, R. M. Georgiadis, T. M. Herne and M. J. Tarlov, *J. Am. Chem. Soc*., 1997, **119**, 3401.
9 J. Wang, G. Rivas and X. Zhang, *Langmuir*, 1999, **15**, 6541.
10 S. O. Kelley, J. K. Barton, N. M. Jackson, L. D. McPherson, A. B. Potter, E. M. Spain, M. J. Allen and M. G. Hill, *Langmuir*, 1998, **14**, 6781.
11 A. B. Steel, T. M. Herne and M. J. Tarlov, *Anal. Chem*., 1998, **70**, 4670.
12 S. Takenaka, K. Yamashita, M. Takagi, Y. Uto and H. Kondo, *Anal. Chem*., 2000, **72**, 1334.
13 S. O. Kelley, J. K. Barton, N. M. Jackson and M. G. Hill, *Bioconj. Chem*., 1997, **8**, 31.
14 S. O. Kelley, N. M. Jackson, M. G. Hill and J. K. Barton, *Angew. Chem*., 1999, **38**, 941.
15 E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill and J. K. Barton, *Nature Biotechnol*., 2000, **18**, 1096.
16 D-W. Pang and H. D. Abruna, *Anal. Chem*., 2000, **72**, 4700.
17 A. Erdem, K. Kerman, B. Meric and M. Ozsoz, *Electranalysis*, 2001, **13**, 219.
18 W. Müller and D. M. Crothers, *Eur. J. Biochem*., 1975, **54**, 267.
19 E. Chargaff and R. Lipshitz, *J. Am. Chem. Soc*., 1953, **75**, 3658.
20 U. Oesch and J. Janata, *Electrochim. Acta*, 1993, **28**, 1237.
21 S. Trasatti and O. A. Petrii, *Pure Appl. Chem*., 1991, **63**, 711.
22 A. J. Bard and L. R. Faulkner, *Electrochemical Methods, Fundamentals and Applications*, Wiley, New York, 2nd edn., 2001.
23 W. M. Clarke, *Publ. Health Rep*., 1925, **40**, 1131.
24 W. M. Clarke, *Oxidation–Reduction Potentials of Organic Systems*, Waverley Press, Baltimore, 1960.
25 H.-H. Yang and R. L. McCreery, *Anal. Chem*., 1999, **71**, 4081.
26 M. T. Carter, M. Rodriguez and A. J. Bard, *J. Am. Chem. Soc*., 1989, **111**, 8901.
27 T. M. Herne and M. J. Tarlov, *J. Am. Chem. Soc*., 1997, **119**, 8916.
28 V. Molinero and E. J. Calvo, *J. Electroanal. Chem*., 1998, **445**, 17.
29 E. Tuite and D. Nordén, *J. Am. Chem. Soc*., 1994, **116**, 7548.
30 E. Tuite and J. M. Kelly, *Biopolymers*, 1995, **35**, 419.
31 R. Rohs, H. Sklenar, R. Lavery and B. Röder, *J. Am. Chem. Soc*., 2000, **122**, 2860.
32 A. Tani, A. J. Thomson and J. N. Butt, unpublished work.