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Keywords
spectroscopy, impedance, electrochemical, target, recognition, dna

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Target DNA recognition using Electrochemical Impedance Spectroscopy

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Abstract— **Electrochemical impedance spectroscopy was used to differentiate between different target DNA sequences without the use of a redox label or application of large bias voltages. This method relies on the formation of a mixed self-assembled monolayer (SAMs) composed of single-stranded DNA and a diluent, mercaptoethanol. Recognition of the DNA analytes was achieved through the significant increase in conductance for the double stranded DNA, as compared to single-stranded DNA. Non-complementary and single base-pair mismatches were also able to be differentiated.**

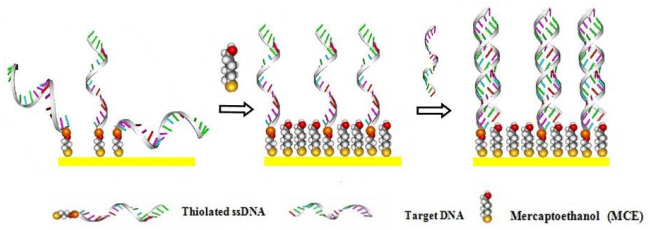
Keywords-DNA, gold-electrodes, electrochemical impedance spectroscopy

I. INTRODUCTION

DNA sequence detection and matching has provided revolutionary benefits to a variety of fields, notably in pharmaceuticals, forensics and clinical diagnostics[1, 2]. Due to the broad applicability to a range of biomedical applications, there has been significant interest in the creation of smaller, faster and more sensitive sensors to expand the range of issues that can effectively be solved. Meeting these requirements calls for sensing technologies with lower detection limits and shorter assay times need to be addressed. DNA biosensors based on electrochemical detection offer several unique advantages over the currently ubiquitous fluorescence-based detection systems which make them a promising candidate for use as more wide-spread and portable biosensing systems in the near future [2].

The charge-transfer properties of DNA that make it electrically conductive have previously been exploited to construct electrochemical DNA hybridization sensors through redox-probe labeling [3-5]. Electrochemical impedance spectroscopy (EIS) provides an alternative sensing method which can eliminate both these requirements, considerably simplifying the sensor interface design[6]. Target DNA analytes are thus detected by directly exploiting the electron transfer properties of DNA.

The ability of DNA to act as a medium for efficient electron transfer relies on the formation of a double helix to provide a pathway for charge transportation [3]. Conversely, single-stranded exhibits poor electron transfer efficiency and conductivity[2, 3, 7-11]. Any conformational changes in the double helix impacts on the charge transfer ability of DNA, hence permitting the detection of mismatches through electrochemical means. [4, 5, 12]



Scheme 1: DNA immobilization and hybridization of electrodes

The purpose of this report, is to explore the application of electrochemical impedance spectroscopy to monitor not only the different conducting properties of single stranded and double stranded DNA but to extend the method to the differentiation of single-base mismatch as well.

II. EXPERIMENTAL METHODS

A. Gold Electrode Modification

The modification of the gold electrodes are described in detail by Wong and Gooding [4] and illustrated in Scheme 1. Briefly, polished polycrystalline gold electrodes were incubated in a 1 μM thiolated probe DNA solution in an immobilisation buffer (1 M KH_2PO_4, pH 4.5) for 90 min. The electrodes were then rinsed with phosphate buffer and immersed in a 1mM mercaptoethanol (MCE) solution for 30 min, followed by rinsing with 75% ethanol and phosphate buffer. The DNA/MCE modified electrodes were then exposed to 4 μM target DNA (Table1) in a hybridisation solution (10 mM Tris-HCl, 1 M NaCl, pH 7.0) for 2.5 hours. Finally, electrodes were rinsed and stored in phosphate buffer (0.05 M KH_2PO_4/K_2HPO_4 + 0.3 M NaCl, pH 7) until electrochemical measurements were carried out.

TABLE I. DNA PROBE AND TARGET SEQUENCES

DNA	Sequence
Probe	5'–GGGGCAGTGCCTCA CAACCT-p-$(CH_2)_3$-SH-3'
Complementary	5'–AGGTTGTGAGGCAC TGCCCC-3'
Non-complementary	5'-GAGTGACTTGATGTGA TTGCC-3'
C-A mismatch	5'–AGGTTGTGAGGCCCT GCC CC–3'

B. *AC impedance measurements*

EIS measurements were performed with an high-resolution INPHAZE (Sydney, Australia) spectrometer system. Spectra were acquired using a 15 mV (rms) sine wave of appropriate angular frequency ω (= $2\pi f$) at open-circuit potential. The frequency f of the potential sine wave was varied between 1 mHz and 1 MHz. Measurements utilized a two-electrode chamber comprising of two identically SAM-modified gold electrodes. Electrodes were immersed in an equimolar (5 mM) potassium ferrocyanide and potassium ferricyanide solution in 300 mM potassium chloride aqueous solution. The electrode set up is shown in Figure 1.

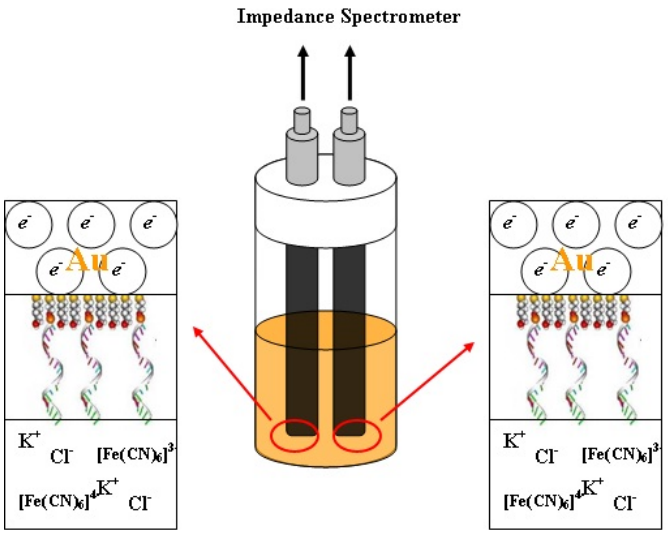


Figure 1: Two identically ssDNA/MCE modified gold electrodes used for analyte DNA detection in an electrochemical cell containing a solution of 5 mM ferrous/ferric cyanide and 300 mM KCl.

III. RESULTS AND DISCUSSION

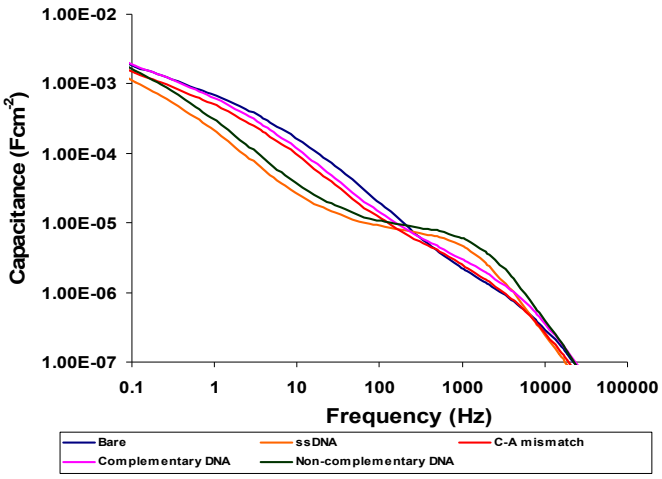
A two electrode system was used to measure the AC impedance as a function of frequency for the different gold-DNA interfaces. Figure 2 a and b show the capacitance and conductance of the gold electrodes respectively. The electrodes are initially bare, then modified with ssDNA, complementary, non- complementary DNA and a single C-A mismatch, located in the middle of the strand. Discrimination between the different target DNA strands is most significant in the middle-range frequencies between 0.1 Hz and 1kHz, where the dispersion of the capacitance is most prominent.

At low and high frequencies, the capacitance converges – at low frequencies, it is governed by the electrical double layer of each interface; at high frequencies the convergence can be attributed to the bulk conductance of the electrolyte. [6]

The trend observed for the conductance of the different target DNA strands is consistent with the conductivity of double stranded versus single stranded DNA. Single-stranded DNA has a low electron transfer efficiency, and hence displays a much lower conductance then the double stranded DNA. A slight decrease in conductance is observed for the C-A mismatch. The signal observed for non-complementary DNA is very similar to that of the single-stranded DNA.

One other factor needs to be considered when accounting for the observed differences in conductance and capacitance of the different target analytes - the orientation of the DNA on the gold surface. The interface is made up of a mixed SAM – the probe ss-DNA and a diluent, mercaptoethanol. The diluent has a distal alcohol moiety which prevented the single stranded DNA from strongly adsorbing to the interface although there is strong evidence to support the hypothesis that the singles strand DNA, being flexible, does not project out into solution but weakly adsorbs onto the surface of the alcohol terminated diluent [13]. The flat-lying DNA would form a physical barrier on the gold surface, inhibiting efficient charge transfer, hence resulting in a lower conductance. Conversely, double stranded DNA is a rigid molecule [14], and would stand upright and project into the solution, independent of the diluent, thus opening up the interface which would lead to a higher conductance.

(a)



(b)

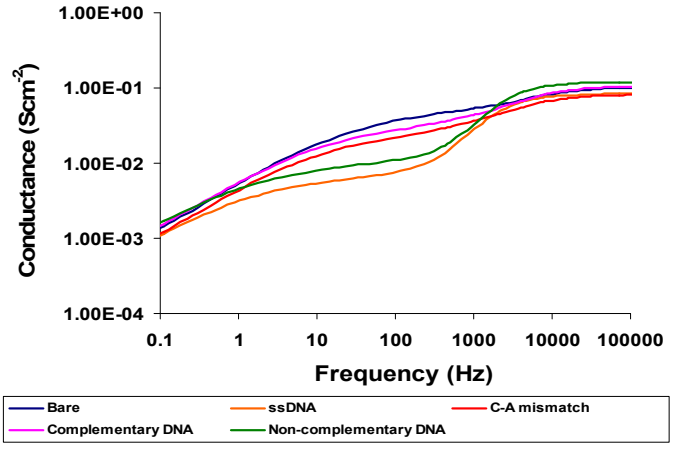


Figure 2: Typical capacitance (a) and conductance (b) data obtained from EIS for a bare Au electrode, ssDNA/MCE modified electrode, and ssDNA/MCE modified electrodes exposed to different DNA target

IV. Conclusion

Electrochemical impedance spectroscopy was successfully applied to differentiate between different DNA target sequences, without the need to apply a large bias voltage and without the use of any redox labeling. Importantly, for the first time the ability to demonstrate single base pair mismatches from complementary sequences was revealed.

Acknowledgements

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