

Electrical Detection of DNA Hybridization using Adjacent Impedance Probing (AIP) Method

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ABSTRACT

Sensitivity and selectivity are two of the most challenging criteria for the development of DNA biosensor devices. These biosensor devices have attracted interest for the rapid identification of pathogens in humans, animals, and plants, for the detection of specific genes in animal and plant breeding and in the diagnosis of human genetic disorders. Traditionally, molecular diagnostic detection has relied on fluorescent or radioactive labels, and signal transduction is performed with equipment that greatly increases size and cost of the whole system. Electronic detection is expected to involve less complicated and smaller instrumentation while detection limits are maintained. Previous efforts on impedance-based DNA biosensors show limitations on repeatability, sensitivity and selectivity. In this work, we introduce the Adjacent Impedance Probing (AIP) technique for DNA hybridization detection. In this novel method, the DNA hybridization site is employed for the bio-recognition event (this site does not necessarily need an underlying conductor surface) and a bare adjacent conductor electrode is employed for generating the largest possible impedance change through the deposition of an insulating material or through chemical passivation induced by the enzymatic reporter reaction. The AIP approach dramatically increases the assay platform's performance vs. the previously employed technique that integrates the impedance electrode and the DNA capture probes. In the case of AIP, the impedance of the bare electrode is lower than that of a conductor surface modified with a self-assembled monolayer (SAM) of probe molecules and will not be subject to the irreproducibility associated with fabrication of such a SAM layer; as a consequence the S/N ratio will increase. The novel detection scheme demonstrated in this work is expected to find real applications for fields in diagnostics and biowarfare agent detection.

Keywords: Biosensor, electrical detection, DNA hybridization, Adjacent Impedance Probing (AIP).

1 INTRODUCTION

The development of DNA sensor devices is continued to be of substantial interest because of its wide applications in

gene analysis, detection of genetic disorders, tissue matching, and forensic applications [1,2,3,4,5]. Optical detection methods where DNA hybridization with fluorescent-labelled complementary strands monitored rapidly by confocal microscopy are the most widely used but normally requires a large and expensive equipment. The increasing demand for low-cost hand held molecular diagnostic devices has lead to the development of portable and easy-to-use biosensors. These hand held devices should be able to perform the diagnostics in a very short time and with a very limited amount of sample. Electrical detection scheme combined with micro-fabricated structures can satisfy these requirements and also allow a high degree of parallelism and sensitivity.

Label free capacitive biosensors have attracted a lot of attention but have been the topic of debate for years [6,7,8]. The debate is on-going since the promise of a simple detection scheme of this nature remains very attractive. The label free capacitive biosensor is based on the theory of the electrical double layer [9], which in principle can be described as a build up of two conducting phases, one consists of a electrode surface and the other of an electrolyte solution. Modification at this interface by immobilization of a recognition element to the conducting surface will lead to a change in capacitance, the size of which will depend on the nature and coverage of the recognition element. Further change in capacitance is expected when analyte binds to the surface. Although there have been many research efforts on the label free biosensors [10,11,12,13,14], they normally show relatively low detection signals.

To obtain a higher detection signal, several groups have used enzymatic amplification scheme to amplify the electrical signal [15,16,17,18,19]. These sensors show higher sensitivity, but the non-specific absorption gives a large background noise thus diminish the signal to noise ratio (S/N) [19]. In this paper, we introduce the Adjacent Impedance Probing (AIP) technique for DNA hybridization detection. In this novel method, the DNA hybridization site is employed for the bio-recognition event (this site does not necessarily need an underlying conductor surface) and a bare adjacent conductor electrode is employed for generating the largest possible impedance change through the deposition of an insulating material or through chemical passivation induced by the enzymatic reporter reaction. The

AIP approach dramatically increases the assay platform's performance vs. the previously employed technique that integrates the impedance electrode and the DNA capture probes [19]. In the case of AIP, the impedance of the bare electrode is lower than that of a conductor surface modified with a self-assembled monolayer (SAM) of probe molecules and will not be subject to the irreproducibility associated with fabrication of such a SAM layer; as a consequence the S/N ratio will increase. As illustrated in Figure 1, single stranded specific DNA with sequence T1 and non-specific DNA with sequence T2 are immobilized on the Au hybridization site. Single stranded biotinylated DNA (cT1') with a sequence complementary to T1 was brought onto the hybridization site. After hybridization, rinse steps, enzyme and substrate introduction, the enzymatic reaction will produce an insoluble precipitate on the chip surface near the hybridization site. The precipitation on the adjacent electrode is expected to introduce a high interfacial electron-transfer resistance that was detected by Electrochemical Impedance Spectroscopy (EIS).

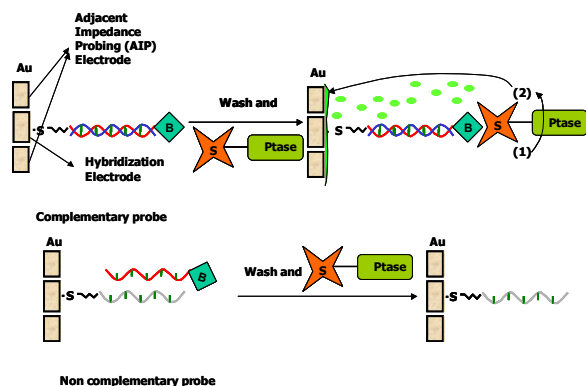


Figure 1 Schematic diagram of DNA hybridization amplified detection by biocatalyzed precipitation of the insoluble product.

2 EXPERIMENTAL

2.1 Materials

All reagents were purchased from Aldrich Chemical and used as received, unless otherwise noted. All solutions were made up using deionized water (18 M Ω -cm resistivity). DNA oligonucleotides were purchased from BioSource Inc. (Camarillo, CA). The two different probes used in this work were T1 and T2, DNA sequences (25mers) with the 5' end thiol-modified and with a 6-mercaptohexyl spacer. The single-stranded target (CT1) is a 25mer complementary to probe T1 and CT1' has the same sequence as CT1 but comes equipped with a biotin label on the 3' end. The sequences and modifications of each oligonucleotide are listed in Table 1.

Table 1 Oligomer nomenclature, modification and sequence

Name	Label	Sequence
T1	5'-SH-(CH ₂) ₆	5'-CACGACGTTGTAACGACGACCAG-3'
T2	5'-SH-(CH ₂) ₆	5'-GATGCCCGGGTCCGGCTAGATGATC-3'
CT1	none	5'-CTGGTCGTCGTTTTACAACGTCGTG-3'
CT1'	3'-biotin	5'-CTGGTCGTCGTTTTACAACGTCGTG-3'

2.2 Electrode preparation

Single-crystal Si (001) covered with a 400 nm thick thermal silicon dioxide was used as the substrate material. The substrates were cleaned using an RCA clean (5 parts deionized water, 1 part NH₄OH, 1 part H₂O₂). A thin layer of positive photoresist (Shipley 1827) is spun at 4000 rpm for 40 seconds onto the substrate, this is followed by a soft bake at 90 °C on a hot plate for 2 minutes. The photoresist is then UV exposed through a patterned iron oxide mask in a Karl-Suss mask aligner and subsequently developed in a MF 319 developer. The Au hybridization and adjacent impedance probing electrodes were patterned by lift-off process. In order to promote adhesion of the gold, a thin Cr adhesion layer (50 nm) was deposited by e-beam evaporation, followed by the e-beam deposition of a 200 nm thick film of gold. The Au adjacent electrode was covered by a layer of Shipley photoresist following the same procedure mentioned above. This layer of photoresist was stripped using acetone after the thiolated-ssDNA self-assembled on the Au hybridization electrode.

2.3 Self-assembled monolayer preparation

Prior to the formation of thiolated-ssDNA self-assembled monolayers on the Au hybridization electrodes, the Au electrodes were cleaned with deionized water (18 M Ω -cm). Next the electrodes were blown dry using pure nitrogen gas and immediately transferred into a solution of 1 μ M of oligonucleotide probes in a potassium phosphate buffer (0.5M, pH=7). For the thiolated-ssDNA self-assembled monolayers to form we allowed reaction for 15 hours or more. Finally, the surfaces were rinsed with deionized water for 5 s and dried under a stream of pure nitrogen.

2.4 Electrochemical measurement

All electrochemical characterizations were performed using an Electrochemical Impedance Spectroscopy Potentiostat from Gamry Inc. (Warminster PA). The working buffer solution (100 mM phosphate (pH 7)) was purged with nitrogen for 10 min to remove dissolved oxygen before the measurements. Electrochemical measurements were performed in a solution of 500 mM [Fe(CN)₆]^{3-/4-} in the working buffer at a scan rate of 100 mVs⁻¹ with an Ag/AgCl reference electrode and a gold auxiliary electrode. The bare gold impedance values were measured in this solution after patterning and cleaning. Hybridization was performed by spotting the CT1 or CT1' solutions (1 μ M DNA in 1X TE buffer plus 1M NaCl) on

the electrode surfaces. After 4 hours, the electrodes were rinsed with 100mM NaCl in 1X TE buffer (10mM Tris, pH 7.4 and 1mM EDTA) buffer for 10 seconds and dried under a stream of pure nitrogen prior to characterization.

3 RESULTS AND DISCUSSIONS

The scheme of electrochemical detection for the DNA hybridization based on the Adjacent Impedance Probing (AIP) technique is shown in Figure 1. As illustrated in the figure, single stranded specific DNA with sequence T1 and non-specific DNA with sequence T2 are immobilized onto the Au hybridization site. Single stranded biotinylated DNA cT1' with a sequence complementary to T1 was brought onto the hybridization site. The enzymatic reaction has been described previously [19]. The biocatalytic enzymatic precipitation on the adjacent electrode is expected to introduce a high interfacial electron-transfer resistance that can be detected by Electrochemical Impedance Spectroscopy (EIS).

EIS is an effective technique to probe the features of surface-modified electrodes. The precipitation of the insoluble and non conductive product on the electrode is anticipated to change the capacitance and electron-transfer resistance at the electrode surface. The complex impedance can be presented as the sum of the real, $Z_{re}(\omega)$, originating from the resistance of the cell, and imaginary, $Z_{im}(\omega)$, originating from the capacitance of the cell. Figure 2(a) represents the EIS spectrum in the form of a Nyquist plot. A typical curve consists of a semicircle region lying on the Z_{re} -axis followed by a straight line. The semicircle portion represented the electron-transfer-limited process at high frequencies and the linear part is characteristic of the lower frequencies range representing the diffusion-limited electron-transfer process. The electron-transfer kinetics and diffusional characteristics can be extracted from the spectra. The semicircle diameter equals the electron-transfer resistance, R_{ct} . This resistance controls the electron-transfer kinetics of the redox-probe at the electrode interface. Any insulating modifier on the electrode is expected to retard the interfacial electron-transfer kinetics and to increase the electron-transfer resistance. Figure 2(a) shows the impedance spectra by Nyquist plot. For non-specific DNA probe T2, the charge transfer resistance R_{ct} only slightly increases, while for specific DNA probe T1, the charge transfer resistance R_{ct} increases dramatically after enzymatic precipitation.

The EIS spectrum represented by Bode plot is shown in Figure 2(b). It is shown as the total impedance data versus frequency, with the third axis the phase angle. The phase angle versus the frequency curve shows that at close to 10kHz it's out of phase, which means the imaginary impedance $Z_{im}(\omega)$ is dominant; while at close to 100Hz it's in phase, which means the real impedance $Z_{re}(\omega)$ (charge transfer resistance R_{ct}) is dominant. Comparing the four impedance curves at 100Hz, we can see that the total impedance for the bare adjacent electrode after T1 and T2

are immobilized is very close, while after enzymatic precipitation, the total impedance for T1 increases dramatically and there is only a slight increase for T2 after enzymatic precipitation reaction.

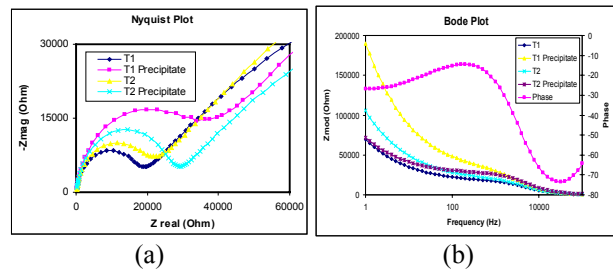


Figure 2 (a) Nyquist diagram (Z_{mag} versus Z_{real}) of the electrochemical impedance measurement for the DNA hybridization process. (b) Bode plot (Z_{mod} versus frequency) of the electrochemical impedance measurement for the DNA hybridization process. The secondary y-axis is the phase angle.

To obtain the statistic data, the impedance measurement was performed on three different chips, and three probes on each chip with five measurements on each probe. The statistical impedance data at 100Hz is presented in Figure 3. It is clear from the figure that the total impedance changes dramatically (100% increase) for the specific DNA probe T1 after the enzymatic precipitation reaction compared to the impedance for both the bare electrode and non-specific probe T2 after the enzymatic precipitation reaction.

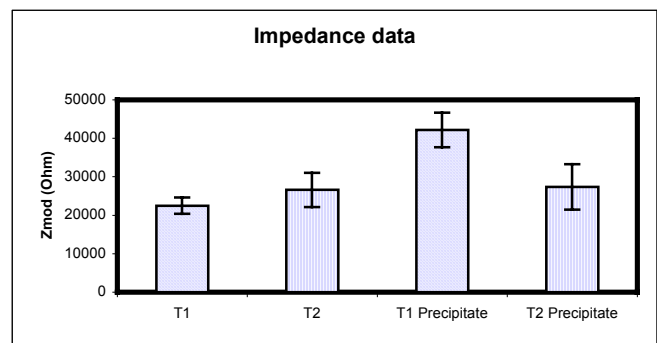


Figure 3 Z_{mod} extracted at 100Hz based on the bode plot. The statistical data is based on three different chips with three probes on each chip and with five measurement on each probe; error bars = 1 standard deviation

The optical image after enzymatic precipitate reaction is shown for both the specific and non-specific probes. For non-specific control experiments, there is no fluorescent light observed. While for specific probes, the green fluorescent light with high intensity was observed. The image was taken after one hour of the enzymatic precipitation reaction and the precipitate has gone a significant distance beyond the hybridization electrode.

This indicates that shorter incubation time can be applied and lower detection limit may be achieved.

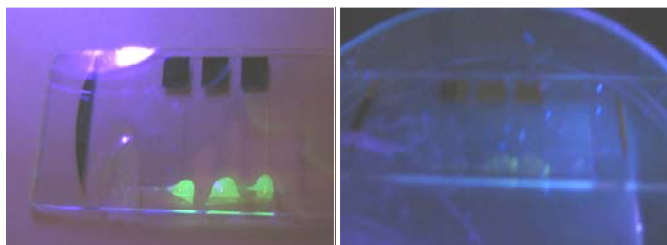


Figure 4 Optical image of the enzymatic precipitate on the hybridization and adjacent electrodes after incubation time of 1 hour.

4 CONCLUSIONS

In this work, we introduce the Adjacent Impedance Probing (AIP) technique for DNA hybridization detection. The AIP approach dramatically increases the assay platform's performance vs. the previously employed technique that integrates the impedance electrode and the DNA capture probes. In the case of AIP, the impedance of the bare electrode is lower than that of a conductor surface modified with a self-assembled monolayer (SAM) of probe molecules and will not be subject to the irreproducibility associated with fabrication of such a SAM layer; as a consequence the S/N ratio will increase. The novel detection scheme demonstrated in this work is expected to find real applications for fields in diagnostics and biowarfare agent detection.

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