Electronic DNA detection on semiconductor surfaces

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ABSTRACT

Electronic biosensors offer a viable alternative to optical biosensors for the rapid, specific, and sensitive detection of DNA. We are utilizing an electrolyte-oxide-semiconductor (EOS) biosensor system on a SiO_2/Si surface for impedimetric detection of DNA. Detection in this system is based on the intrinsic negative charge of DNA. Hybridization of target DNA to the probe-functionalized semiconductor electrode causes a change in the surface charge density, which results in a measurable shift of the impedance response. To covalently bind probe DNA sequences to the biosensor surface, we have activated and functionalized the SiO_2/Si surface with aminosilanes, followed by coupling with activated probe DNA. Efficient and specific hybridization was verified via fluorescence and atomic force microscopy (AFM). To increase the sensitivity of our system, we are coupling a novel exponential isothermal DNA amplification reaction (EXPAR) with impedimetric DNA detection.

Keywords: impedance, silicon, DNA hybridization, Au nanosphere, EXPAR

1 INTRODUCTION

Improved methods for DNA detection hold great promise in enabling the rapid, sensitive and accurate identification of clinical pathogens and biothreat agents, in the early detection of cancer and genetic disease, and in genotyping of patients for personalized medicine. Currently, most DNA assays rely on optical detection methods, which necessitate labeled DNA probes or target, appropriate light sources, filters, and sensitive detectors. Electronic DNA detection offers a viable alternative to optical methods. Electronic transduction of biomolecular recognition events has been implemented on metal and semiconductor electrodes. On metallic surfaces, faradic processes are favored, and DNA detection generally involves electrochemical reactions. On semiconductor electrodes covered by an insulating oxide layer, faradic processes are suppressed, and electronic detection of DNA relies on changes in surface impedance. The largest change in surface impedance upon DNA binding is observed in depletion regime of a semiconductor. The change in surface impedance can be measured either through electrochemical impedance spectroscopy (EIS), as a function of frequency at a constant bias potential [1] or through voltage-dependent impedance measurements as a function of bias potential at a constant frequency [2,3,4]. A more practical approach involves measuring the impedance response at a constant potential and frequency, within the depletion regime of the semiconductor [4,5,6]. We have optimized the conditions for impedance based detection of DNA hybridization. We have further demonstrated that the sensitivity of DNA detection can be increased through coupling with isothermal target amplification via EXPAR.

2 RESULTS AND DISCUSSIONS

2.1 Surface bio-functionalization

Sensitive and reproducible DNA detection requires effective and uniform covalent immobilization of probe DNA to the sensor surface. We have optimized protocols for surface bio-functionalization, as shown in Figure 1. All experiments were performed on silicon wafers (p-doped, 15 Ohms-cm, orientation 100) cut into suitable pieces for the impedance measurements. Silicon substrates were cleaned with Piranha solution (30% H_2O_2: conc. H_2SO_4=1:3) for 30 minutes, rinsed with ultrapure water (Milli-Q, Biocel A10 18.2 MΩ, Millipore Inc., USA) and dried under a stream of Argon. To introduce reactive primary amine groups onto the surface, these Si/SiO_2 substrates were silanized in 1% 3-aminopropyltriethoxysilane (APTES) in toluene at room temperature overnight., followed by rinsing in an aqueous solutions of 1M AcOH to remove the unreacted silane.

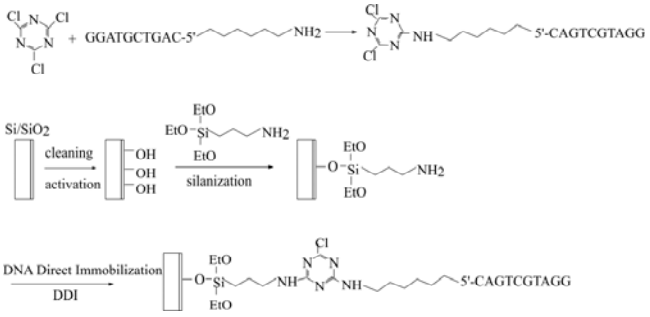

Figure 1: Overview of surface modification process leading to covalent binding of ss probe DNA to Si/SiO_2 substrates.

Amine-modified ssDNA probe sequences were chemically activated with 2,4,6-trichlorotriazine and purified through a NAP-5 size exclusion column with 0.1M NaBorate as the mobile phase [7,8]. The final concentration of activated probe DNA was adjusted to 1μM. APTES-

functionalized silicon substrates were incubated with this solution at room temperature overnight. The surface was then blocked with succinic anhydride and BSA in PBS to avoid non-specific hybridization. We have observed that this approach results in higher immobilization densities compared to coupling of amino-terminated probe DNA to APTES functionalized SiO_2 surfaces activated via succinic anhydride or 2,4,6-trichlorotriazine.

2.2 DNA hybridization

We have verified specific hybridization to these probe-functionalized sensor surfaces via fluorescence microscopy using fluorescently labeled target DNA, and via atomic force microscopy (AFM) using DNA-functionalized Au nanospheres. Hybridization of 1µM target DNA to the sensor was performed in a standard hybridization buffer used for DNA microarray experiments (4×SSC, 0.2% SDS, 0.1M Tris pH 7.5). After the hybridization, the substrates were carefully rinsed in three steps with 2×SSC, 0.1×SSC and ultrapure water respectively to wash off any unspecifically bound DNA. In this work, we have used the probe oligonucleotide sequence 5'NH_2-CCTACGACTG-3' and its complementary target sequences (both from MWG-Biotech Inc., USA). The sequence of non-complementary target DNA used in this work was 5'-ATAGGCATGCAT-3'. DNA-functionalized Au nanosphere were synthesized as described elsewhere [9].

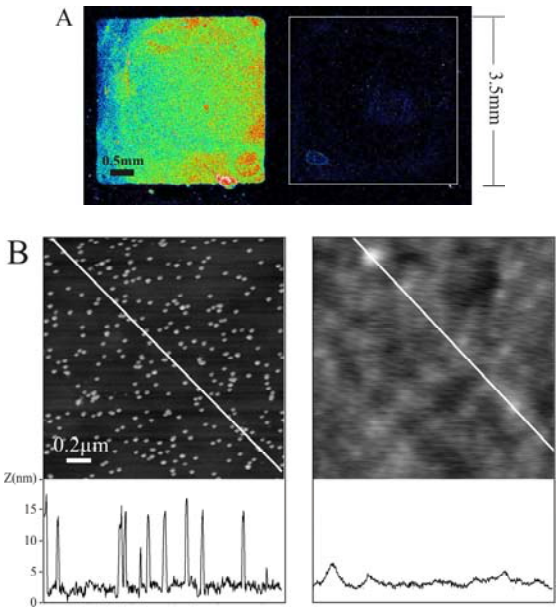

Figure 2: Complementary (left) and non-complementary (right) hybridization of target DNA to probe-functionalized substrates, detected through (A) fluorescence microscopy using Cy-3 labeled target DNA and (B) AFM (2µm×2µm scan area) using DNA-functionalized Au nanosphere.

As shown in Figure 2A, Cy-3 labeled complementary target DNA binds effectively to a probe-functionalized surface (left) with very little binding observed for a non-complementary target oligonucleotide (right). Likewise, AFM images (Figure 2B) reveal a high density of Au nanospheres immobilized through complementary hybridization, but very little gold nanosphere deposition through non-complementary hybridization or non-specific interactions.

2.3 Electrochemical Instrumentation

Our experimental setup shown in Figure 3 enables us to conduct up to eight impedance measurements on a single silicon surface within the same experiment. In this setup, the DNA-functionalized Si/SiO_2 substrate is attached to the backside of an open-bottom 384 well microtiter plate with a adhesive gasket (Grace Bio-labs, USA). In each well, the working electrode consists of the exposed area on the substrate (3.5×3.5 mm^2). Electrical back-contact to the silicon substrate was obtained via conductive silver paint in contact with a copper plate, which as then connected to the impedance analyzer. The volume of detection solution in each well can be as little as ~70µl. A block holding eight individual reference and auxiliary electrodes manufactured from Ag and Pt wire, respectively, were inserted into the top of the microtiter plate.

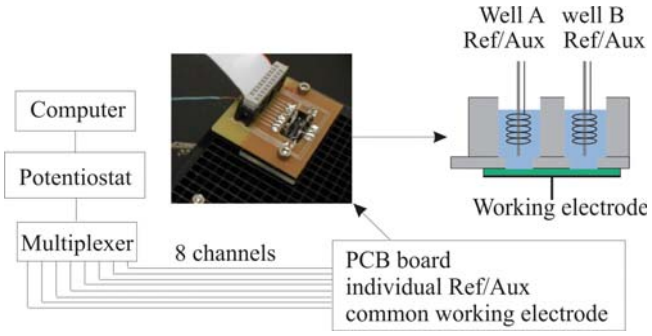

Figure 3: Experimental setup for the impedance measurements.

Impedance measurements were performed with a CHI 604B electrochemical analysis workstation (CH Instruments, Inc., USA) combined with a CHI684 multiplexer. Impedance measurements are not identical from one well to another, most likely due to differences between individual Ag wire pseudo-reference electrodes. However, within each well, the impedance response is stable and reproducible over time, and can be compared before and after hybridization.

2.4 Impedance measurements

The flatband voltage of a Si/SiO_2/electrolyte interface is given by [10]:

$$V_{FB} = E_{ref} - \Psi + \chi_{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ss} + Q_{ox}}{C_{ox}} \qquad (1)$$

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Here, E_{ref} is the reference electrode potential relative to vacuum, Φ_{si} is the workfunction of the silicon substrate, Ψ is the surface potential, χ_{sol} is the surface dipole potential of the solution, Q_{ss} represents the surface state density at the semiconductor-oxide interface, Q_{ox} stands for the charge density at the oxide-electrolyte interface and C_{ox} represents the capacitance of the oxide layer. According to electrochemical convention, potentials are reported relative to the reference electrode, which is equivalent to the gate electrode of a conventional metal oxide semiconductor device.

Covalent coupling of probe DNA to the surface or hybridization of target DNA to the surface-immobilized probe DNA increases the negative charge density at the semiconductor and changes the surface potential. According to equation 1, DNA immobilization causes a shift of the flatband voltage to a more negative potential. In this manner, DNA hybridization can be detected through a shift in the semiconductor's impedance response.

Impedance based DNA detection requires a low ionic strength buffer [11]. The change in charge density upon DNA hybridization can be detected within distances from the surface on the order of the Debye length, which strongly depends on the ionic strength of the solution [12]. Since the ionic strength requirements of DNA hybridization and impedance based detection are diametrically opposed, we have conducted these steps separately in different buffer solutions. First, the impedance response in a low ionic strength detection buffer was stabilized and measured. Next DNA hybridization and subsequent stringency washing was performed as described above. Finally, detection buffer was re-introduced into the well and the impedance response was measured.

We have investigated the impedance response of the DNA-functionalized silicon substrate, before and after hybridization to complementary or non-complementary target DNA, as a function of frequency and ionic strength of the detection buffer solution. For impedance measurements, an AC-modulated DC potential was applied to the silicon working electrode relative to the reference electrode (20mV modulation amplitude, varying frequency from 1Hz-100kHz, varying bias potential as indicated). The imaginary impedance response (Z´´) was acquired either as function of bias potential or as function of frequency.

In general the silicon substrates exhibit normal Mott-Schottky behavior as shown in Figure 4A. The impedance response of the electrolyte-oxide-semiconductor system is determined by the resistive and capacitive properties of its components [13]. The imaginary impedance response is dominated by capacitive components [14] and as such at a higher frequency, the magnitude of the imaginary impedance response is lower.

We have conducted EIS experiments at a bias potential within the depletion regime of the semiconductor to determine the optimal frequency for impedance-based DNA detection. As shown in Figure 4B, we have observed a significant change in imaginary impedance upon DNA hybridization at lower frequencies, in line with literature reports [15]. In the low frequency regime, interfacial phenomena have important contributions to the equivalent circuit. Therefore, we selected a frequency of 1kHz for all subsequent voltage-dependent impedance measurements.

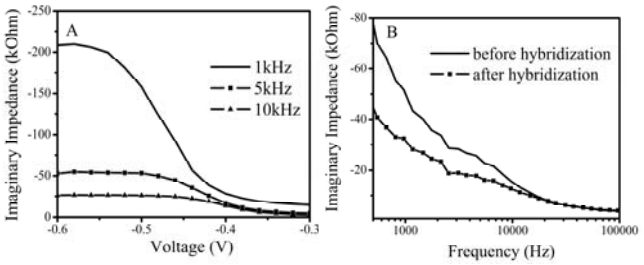

Figure 4: The dependent of surface impedance on voltage at different modulation frequency (A) and EIS for the before/after hybridization.

We then investigated the effect of detection buffer ionic strength on the measurable impedance shift upon DNA hybridization. As shown in Figure 5, a detection buffer containing 1mM NaCl was found to yield a significant measurable shift in the impedance response after complementary DNA hybridization, while little shift was observed using detection buffer of higher ionic strengths.

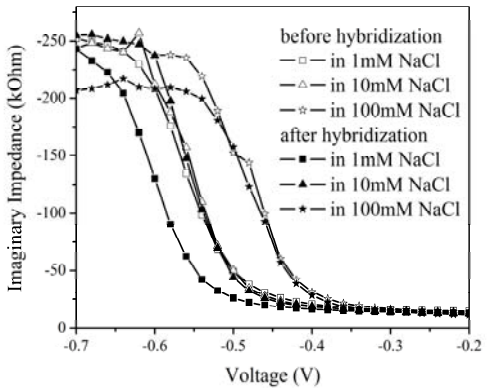

Figure 5: Complementary DNA hybridization measurement in different ionic strength solutions, 1mM NaCl and 100mM NaCl respectively.

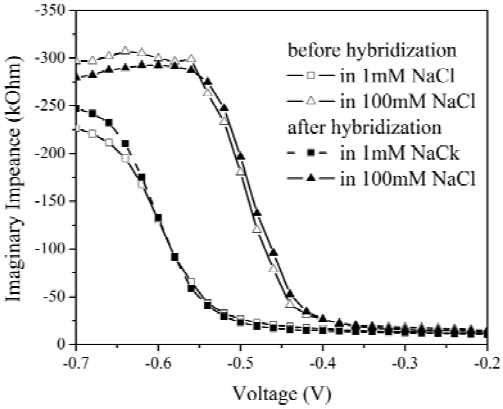

Figure 6: Non complementary DNA hybridization detection in 1mM and 100mM NaCl respectively.

No shift was observed in the impedance response following non-complementary DNA hybridization using detection solutions containing either 1mM NaCl or 100mM NaCl (Figure 6), which verifies the specificity of the process.

2.5 Coupling isothermal DNA amplification with impedimetric DNA detection

To increase the sensitivity, we have coupled impedance based DNA detection with amplification of the complementary target sequence through a novel isothermal DNA amplification reaction called EXPAR [9,16]. We have performed EXPAR amplification either in the presence of 1 pM target DNA (positive), or in the absence of target DNA (negative). Reactions were monitored in real time through a SYBR Green based assay, and were stopped at a time (4mins) at which the positive sample appeared to be efficiently amplified, but at which no amplification was observed for the negative control. These two amplified master mix solutions were then diluted two-fold with 2 × standard hybridization buffers, and incubated with the probe-functionalized silicon substrate for 2 hours, followed by the standard rinsing steps. The imaginary impedance response was again measured in the initial detection solution. As shown in Figure 7, 4mins of EXPAR can amplify 1pM target DNA sufficiently to cause a significant shift of the flatband voltage to a more negative potential, comparable in magnitude to the shift observed in the presence of 1μM un-amplified target DNA. No such shift was observed for the negative control.

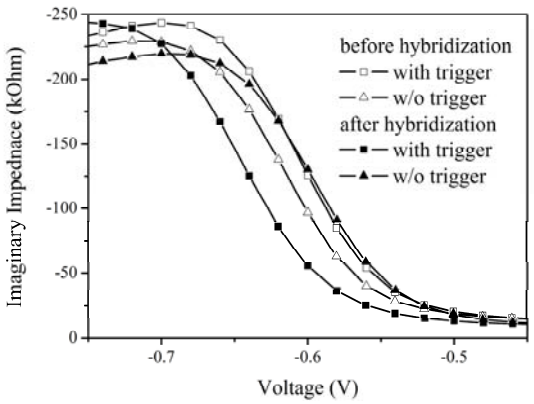

Figure 7: Voltage-dependent Imaginary impedance for EXPAR with or without trigger.

3 CONCLUSIONS

We have performed impedance based DNA detection in a standard 384 well microtiter plate format, using small reaction volumes. By optimizing the measurement frequency and the ionic strength of the detection buffer, we have observed a significant shift in the flatband voltage caused by specific DNA hybridization. The sensitivity of impedance-based DNA detection can be increased through coupling with rapid, isothermal amplification via EXPAR. We are investigating the possibility of further increasing the sensitivity of impedance based DNA detection through co-immobilizing DNA functionalized Au nanospheres to the surface, using a sandwich assay format.

REFERENCES

[1] Katz, E. and Willner, I., Electroanalysis, 15, 913-947, 2003

[2] Cai, W., Peck, J. R., van der Weide, D. W., and Hamers, R. J., Biosensors & Bioelectronics, 19, 1013-1019, 2004

[3] Macanovic, A., Marquette, C., Polychronakos, C., and Lawrence, M. F., Nucleic Acids Research, 32, 2004

[4] Souteyrand, E., Cloarec, J. P., Martin, J. R., Wilson, C., Lawrence, I., Mikkelsen, S., and Lawrence, M. F., Journal of Physical Chemistry B, 101, 2980-2985, 1997

[5] Cooper, E. B., Fritz, J., Wiegand, G., Wagner, P., and Manalis, S. R., Applied Physics Letters, 79, 3875-3877, 2001

[6] Fritz, J., Cooper, E. B., Gaudet, S., Sorger, P. K., and Manalis, S. R., Proceedings of the National Academy of Sciences of the United States of America, 99, 14142-14146, 2002

[7] Vanness, J., Kalbfleisch, S., Petrie, C. R., Reed, M. W., Tabone, J. C., and Vermeulen, N. M. J., Nucleic Acids Research, 19, 3345-3350, 1991

[8] Bandyopadhyay, K., Tan, E., Ho, L., Bundick, S., Baker, S. M., and Niemz, A., Langmuir, 22, 4978-4984, 2006

[9] Tan, E., Wong, J., Nguyen, D., Zhang, Y., Erwin, B., Van Ness, L. K., Baker, S. M., Galas, D. J., and Niemz, A., Analytical Chemistry, 77, 7984-7992, 2005

[10] Bergveld, P., IEEE sensor conference, 1997

[11] Landheer, D., Aers, G., McKinnon, W. R., Deen, M. J., and Ranuarez, J. C., Journal of Applied Physics, 98, 2005

[12] Poghossian, A., Cherstvy, A., Ingebrandt, S., Offenhausser, A., and Schoning, M. J., Sensors and Actuators B-Chemical, 111, 470-480, 2005

[13] Morrison, S. R., 1980

[14] K'Owino, I. O. and Sadik, O. A., Electroanalysis, 17, 2101-2113, 2005

[15] Ingebrandt, S., Han, Y., Nakamura, F., Poghossian, A., Schoning, M. J., and Offenhausser, A., Biosensors & Bioelectronics, in press, 2007

[16] Van Ness, J., Van Ness, L. K., and Galas, D. J., Proceedings of the National Academy of Sciences of the United States of America, 100, 4504-4509, 2003