

## IMPEDANCE LABELLESS DETECTION-BASED POLYPYRROLE DNA BIOSENSOR

C. M. Li<sup>1</sup>, C. Q. Sun<sup>2</sup>, S. Song<sup>3</sup>, V. E. Choong<sup>4</sup>, G. Maracas<sup>5</sup> and X. J. Zhang<sup>6</sup>

<sup>1,2</sup> School of Electrical & Electronic Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798, <sup>3-5</sup> Motorola Life Science, Tempe, Arizona 85287, USA., <sup>6</sup> Department of Chemistry, University of South Florida, 4202 East Fowler Avenue, Tampa, FL33620-5250, USA

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and Methods
  - 3.1. Materials and solutions
  - 3.2. Apparatus
  - 3.3. Experimental procedure
4. Result and discussion
  - 4.1. Impedance detection of DNA hybridization
  - 4.2. Electrolyte effects on DNA hybridization
  - 4.3. Specificity of impedance labelless detection of DNA
  - 4.4. Results on silicon array chips
5. Conclusions
6. Acknowledgment
7. References

### 1. ABSTRACT

Microelectrodes were fabricated to study impedance labelless detection of DNA hybridization. The probe molecule was attached onto the platinum microelectrode surface by electrochemically copolymerizing pyrrole and the probe oligonucleotides. Measured impedance complexes showed that an electrochemical redox-reaction occurred and the electron-transfer resistance increased after DNA hybridization. It was proposed that the hybridization of DNA in the conductive polymer matrix slowed down the anionic doping/undoping process, resulting impedance changes for the target DNA detection. Impedance measurements were conducted at the complementarily hybridized probe oligomer-attached polypyrrole film electrodes in different anionic solutions to exam the anionic effects. Results showed that higher concentration and smaller size of anions had the lower electron-transfer resistance. The results not only provide further evidence to support the detection mechanism proposed, but also offer a method to improve the signal to noise ratio for the DNA biosensor. The research also tested the specificity of the methods and experimental results, indicating good specificity of the method. A concept array chip was fabricated and used to demonstrate the capability of the labelless detection method. Nano-Molar concentrations were detected and showed fairly linear responses versus the target molecule concentrations. The method is simple and inexpensive. The technique based genosensors could have potential applications in clinical diagnosis, drug discovery, environmental and food analysis.

### 2. INTRODUCTION

DNA hybridization or molecular renaturation was first described by Marmur and Doty (1). This remarkable

property suggested ways to determine specific DNA sequences in biological samples for generic analysis, resulting in great promise of DNA biosensors for important applications in clinical diagnosis, drug discovery, environmental and food analysis (2, 3). Hybridization detection is the key to DNA biosensor, which commonly relies on the immobilization of a single-stranded (ss) DNA probes onto optical, electrochemical or mass-sensitive transducers (4-11) to recognize the complementary target DNA strand in a sample solution. Most of the DNA biosensors detect the DNA hybridization by labeling probes or target DANs in a sample with electrochemical (12, 13) or fluorescent labels (14) for detectable electrical or photonic signals. The advances of these methods had a tremendous influence on the rate of discovery of new genes and stimulated genome-based applications. Among DNA biosensors, electrochemical detection based method provides many advantages inherent in comparison to radioactive or fluorescent labeling techniques to discern molecular interactions. Electrochemical detection techniques are based on the detection of alterations in the electrical properties of an electrode arising from DNA hybridization. This process offers, for example, a detection technique that is safe, inexpensive, and sensitive, not burdened with complex and onerous regulatory requirements, and can be portable devices. Electrochemical DNA biosensor transducers, which convert the hybridization events into an analytical signals, can be amperometric (15-18), potentiometric (19) and impedimetric devices (20). These sensors are mainly based on detecting electrochemically active labels such as organic dyes, metal complexes, enzymes or metal nanoparticles (4).

Despite the advantages of electrochemical DNA biosensors, there are a number of challenges in using

## Labelless polypyrrole DNA Biosensors

electrochemical detection techniques for analyzing DNA hybridization. One such challenge is the requirement, in some methods, of incorporating an electrochemical label into the target molecule. The excess labels cause inconvenience, which is very time-consuming, labor-intensive, and expensive. They would probably also affect the DNA recognition. Thus, labelless electrochemical DNA biosensors become extremely attractive. Intense investigation has been conducted on answering whether or not DNA is able to conduct electrical charges for direct DNA detection. Although the conductivity of DNA has been assessed from electron transfer as a function of the distance between the donor and acceptor molecules (21-24), the semiconductor-like behavior is not likely to provide sensitive electronic signals for direct detection. Different probe modification or attachment techniques have been studied for enhancing the electronic signal for labelless detection such as hybridized duplex between two electrodes with the vectorial growth of a conductive silver wire for electronic detection (25), loosely packed DNA duplexes covalently crosslinked to a redox-active intercalator for an electrochemical assays (26,27), DNA hybridization with an electroactive, ferrocene-tagged DNA stem-loop structure for labelless-sensing a conformational change (28), and direct recognition of target DNA as a dopant within conductive polymer films (29, 30). Capacitance measurement, electrochemical impedance spectroscopy and constant current chronopotentiometry have been also investigated for the electrochemical detection of DNA at the hanging mercury drop electrode (HMDE) (31) and on silica surface modified by ss homo-oligonucleotides (32). Modulation of ion-exchange kinetics of polypyrrole film also was reported for labelless detection of DNA (33). However, these label-free methods require either tedious probe/target modifications or complicated immobilization technologies. Some of them are difficult to make practical devices. There remains a need to develop alternatives to current labelless detection methods for highly sensitive and inexpensive DNA biosensors.

Impedance labelless detection of DNA hybridization has been reported by Li *et al* (1999) (34) and Lee *et al* (2001) (35). However, there are no detail experimental results to clearly show the mechanism of the label-free detection of DNA hybridization. In addition, dose response of DNA target, i.e. relationship of impedance vs. target concentration has not demonstrated yet. In this paper, we reported the impedance labelless detection approach to monitor the DNA hybridization with oligonucleotide probe attached polypyrrole electrodes. Microelectrodes were employed to investigate the mechanism of the impedance labelless detection of DNA hybridization on the conductive polymers and to examine the electrolyte effects on detection of DNA hybridization. We also fabricated silicon based array chips to test the impedance responses in solutions containing different concentrations of the target DNA.

### 3. MATERIAL AND METHODS

#### 3.1. Materials and solutions

One oligonucleotide probe, its complimentary molecule, noncomplimentary molecule and a 3-mismatched

oligomer were received from Genosys. The oligonucleotide probe bears a terminal amino group on its 5' phosphorylated position as

15-mer NH<sub>2</sub>-5'-C-C-C-T-C-A-A-G-C-A-G-A-G-G-A-3' (1)

and the target complimentary oligonucleotide used in experiments is:

15-mer 5'-T-C-C-T-C-T-G-C-T-T-G-A-G-G-3' (2)

The three-mismatched target is

15-mer 5'-C-C-C-T-C-T-G-G-T-T-G-A-G-G-A -3' (3)

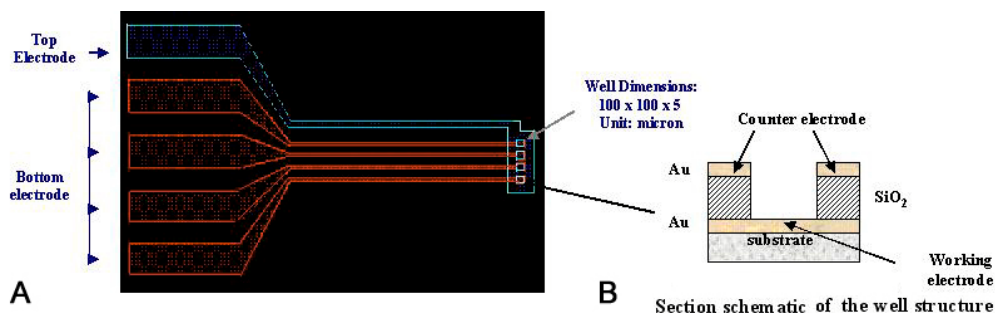
Pyrrole was received from Aldrich, which was distilled and kept in refrigerator prior to use. Different grades of Gamma alumina powder were purchased from CH Instruments, Inc. The deionized water used in all experiments was produced by a Millipore milli-Q water purification system. All pipette tips were sterilized by autoclaving for 2 hours. All other chemicals were of analytical grades and obtained from common commercial supplies.

#### 3.2. Apparatus

EG&G PAR 273 potentiostat was used to conduct cyclic voltametry for copolymerizing oligomer/polypyrrole film. AC impedance measurements were carried out with 1260 Impedance/Gain-Phase Analyser/1287 Electrochemical Interface system (Solartron Inc., Houston, Tex.). A Ag/AgCl, saturated KCl reference electrode was used. A coiled platinum wire was used as the counter electrode. All experiments were conducted under room temperature.

Microelectrodes used in all experiments were homemade. Ultra-fine platinum wire having a diameter of 50 micrometers was inserted into a glass capillary tubing (diameter of 2 mm) and sealed by heating to form a solid microelectrode structure. The tip of the structure was then polished with gamma alumina powder to expose a flat disk of the platinum wire. Microelectrodes were initially polished with 0.3 micrometers gamma alumina powder, rinsed with deionized water, and then polished with 0.005 micrometer powder. Following polishing, the microelectrodes were ultrasonically cleaned for 2 min. in deionized water, and then soaked in 1 M HNO<sub>3</sub> for 20 min. following vigorously washing in deionized water. Finally, immersed the microelectrode in acetone for 10 min., and again washed vigorously in deionized water for use.

Silicon array chips used in all experiments were made by the Process Lab of Motorola Research Center, USA as shown in Figure 1a. The chip was constructed on an oxidized silicon substrate with well structure where 4 individual gold working electrodes located in the bottom of 4 wells, and the counter electrode is a square strip gold electrode on the top side of well wall, symmetrically surrounding the 4 well counter electrodes (Figure 1b).



**Figure 1.** Schematic of Silicon array chip. 1a: Silicon chip 1b: Schematic of section diagram of the well structure

### 3.3. Experimental procedure

Electrochemical copolymerization of pyrrole and oligonucleotides can be used to make a probe-immobilized polypyrrole film (36, 37). In our experiments, a solution containing 0.05 M pyrrole, 2.5 microMolar oligonucleotide probe, and 0.1 M LiClO<sub>4</sub> in 95% acetonitrile was prepared as the precursor solution. Cyclic voltametry (CV) method was used for the electrochemical deposition. The potential range for the CV was 0.2 to 1.3 V versus Ag/AgCl for the first cycle and -0.1 to 1.0 versus Ag/AgCl for 10 additional cycles. The scan rate was 10 mV/sec. The precursor solution was purged by nitrogen gas during the entire deposition process. Alternatively, polypyrrole film can be formed by oxidation of pyrrole at a constant current of 0.20 to 0.25 mA/cm<sup>2</sup> in the same solution described herein. This method has an advantage in the fabrication of array-based microelectrodes in that the reference electrode is not required. The polypyrrole electrodes in oxidized form were put into 0.1 M LiClO<sub>4</sub> and cycled over a potential range of -0.1 to 0.8 for 10 cycles. This procedure was used to stabilize the polypyrrole film. The oligo-polypyrrole microelectrodes were washed with TBE buffer (0.89 M Tris-borate, 0.025 M EDTA), rinsed thoroughly in deionized water, and allowed to dry at room temperature for use.

The AC impedance baseline of the oligo-polypyrrole microelectrodes prepared was first determined in the absence of a complementary target molecule. The electrodes were then exposed in a sealed conical tube to 30 milliliters of the complementary target molecule present at concentrations in the micromolar (10<sup>-6</sup> M) to nanomolar (10<sup>-9</sup> M) concentration range. Hybridization of probe and target molecules was performed in 1xSSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at 27°C. for 2-3 hours. Following hybridization, the microelectrodes were thoroughly rinsed in an excess volume of 1xSSC at room temperature and then AC impedance was measured again.

All AC impedance measurements were made at open circuit voltage (OCV) vs. Ag/AgCl in a 0.1 M NaCl over frequency range of 1Hz-1MHz solution except additional description.

## 4. RESULT AND DISCUSSION

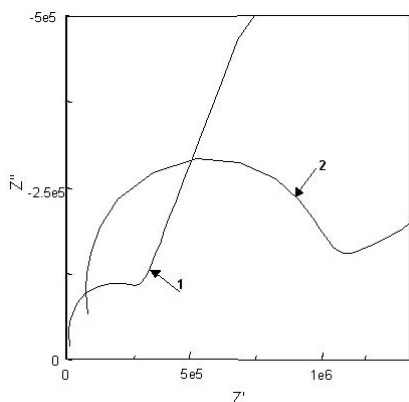
### 4.1. Impedance detection of DNA hybridization

AC impedances were measured using the oligonucleotide-polypyrrole film/platinum microelectrode

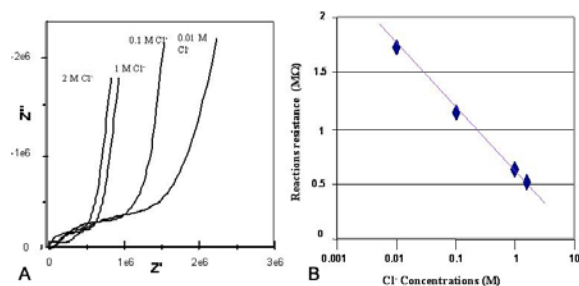
in 0.1 M NaCl solution before and after the 15-mer DNA hybridization. The measured complex impedance ( $Z$ ) versus frequency, known as Nyquist Plot is shown in Figure 2. Well-defined frequency-dependent semicircle impedance curves were observed at the high frequency range following by an straight line, and the diameter of the semicircle after hybridization is much larger than that obtained before hybridization. The complex impedance is the sum of  $Z'$ , the real components mainly from resistance, and  $Z''$ , the imaginary components from capacitance, inductance and other distribution components. Randle equivalent circuit (38) is frequently used to model the complex impedance in an electrochemical cell, which is composed of the ohmic resistance of the electrolyte solution,  $R_s$  in connection in series with parallel elements of double layer capacitance,  $C_{dl}$ , and Faraday impedance,  $Z_f$ . The parallel elements  $C_{dl}$  and  $Z_f$  versus frequencies form a semicircle.  $Z_f$  often comprises serially connected electron-transfer resistance,  $R_{et}$ , and Warburg impedance,  $Z_w$ , resulting from the diffusion of ions from the bulk electrolyte to the electrode surface. Thus, the impedance results in Figure 2 obtained from the oligonucleotide-polypyrrole platinum microelectrode show typical Nyquist plots. Since  $C_{dl}$  and  $R_{et}$  depend on the dielectric and electrocatalytic properties at the electrode/electrolyte interface, the results demonstrate that the electronic properties changed after the hybridization, and electrochemical reactions occurred at the oligonucleotide-polypyrrole platinum electrode surface. Apparently, the great change of the impedance can be used to detect the DNA hybridization for DNA biosensor application.

Willner *et al* have reported in (20, 39) that impedance measurements were used to detect liposomes/biotin labeled target biomolecules such as antibody and DNA for the enhanced amplification of antigen-antibody or oligonucleotide-DNA sensing processes. The mechanism is clear since Fe(CN)<sub>6</sub><sup>+2/+3</sup> was used as reporters to sensing the precipitation of labeled large molecules on electrodes, which could block the redox reaction of Fe(CN)<sub>6</sub><sup>+2/+3</sup> to increase  $R_{et}$  after DNA hybridization.

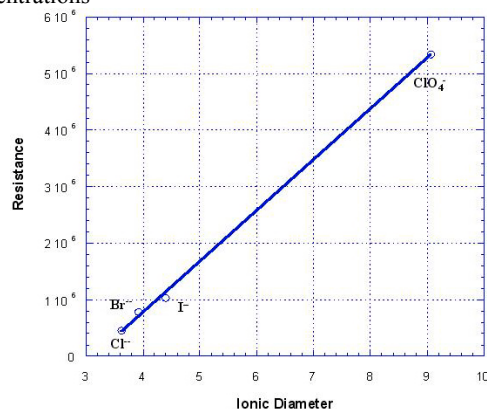
The method in this paper did not use any labeled or non-labeled reporters for signal enhancement. The anionic doping process in electrochemical polymerization of polypyrrole has been extensively studied (40), but the doping/undoping process on a doped conductive polymer has not been carefully investigated. However, an



**Figure 2.** Impedance complex measured at the oligonucleotide-polypyrrole film/platinum microelectrode in 0.1 M NaCl solution, electrode diameter = 50 microns. Curve 1: before hybridization; curve 2: after hybridization in 30 ml of 2 nM complementary oligomer solution.

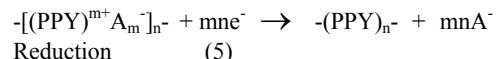
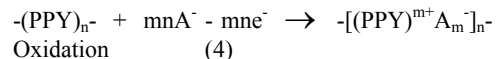


**Figure 3.** Impedance results measured at the hybridized oligonucleotide-polypyrrole film/platinum microelectrode in solutions containing different concentrations of chloride ions as shown in Figure, electrode diameter = 50 microns, hybridization in 30 ml of 2 nM complementary oligomer solution. 3a: Impedance complexes measured in solutions with different anionic concentrations; 3b: Relationship of electron-transfer resistance,  $R_{ct}$  versus anionic concentrations



**Figure 4.** Relationship of electron-transfer resistance,  $R_{ct}$  versus sizes of anions, measured at the hybridized oligonucleotide-polypyrrole film/platinum microelectrode in solutions containing different anions but all with the same cation,  $Li^+$ , electrode diameter = 50 microns, hybridization in 30 ml of 2 nM complementary oligomer solution.

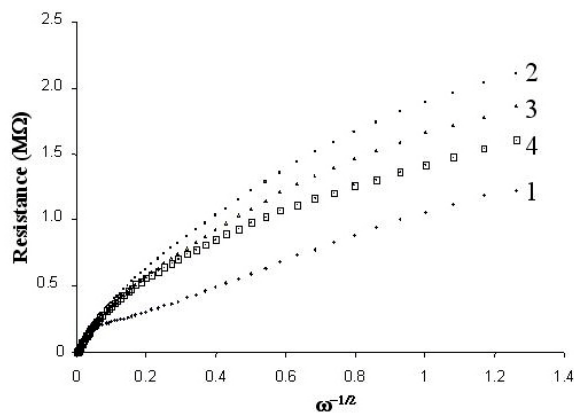
electrochemical redox reaction on the doped polypyrrole film by imposing ac potential could naturally occur for a doping/undoping process, in which the anions serve as mobile dopants moving into and out of the film when the polymer is electrochemically switched between its oxidized and reduced states as follows:



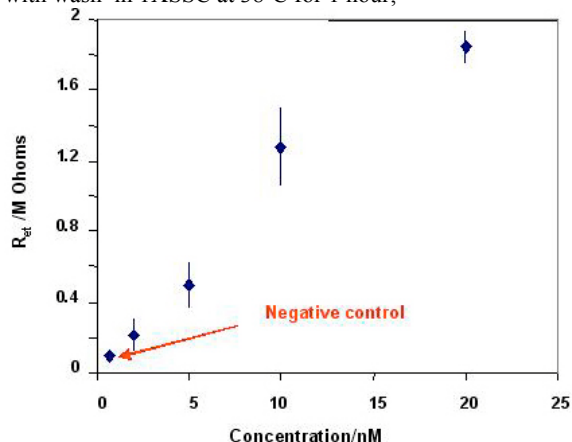
DNA is a negative charged biomolecules. In our experiments, after hybridization the negative charge in the DNA/polypyrrole film electrode was significantly increased and more spatial hurdles were added in the polypyrrole matrix. The increased negative charge pushed the anion away from doping/undoping sites and the physical special hurdle of the target DNA in the film blocked the reaction as well. This could explain the result in Figure 2. Thus, it is proposed that labelless impedance detection of DNA hybridization in a conductive polymer film is based on sensing the change of the doping/undoping redox reaction rate of the polypyrrole films.

#### 4.2. Electrolyte effects on DNA hybridization

AC impedances were measured using the hybridized oligonucleotide-polypyrrole electrodes in solutions containing 0.01, 0.1, 1 and 2 M NaCl concentrations, respectively. The measured complex impedance curves are shown in Figure 3a. Defined frequency-dependent semicircle impedance curves were observed at high frequency range in all cases. The diameters of the semicircles increased with decrease of the concentrations of the anion,  $Cl^-$ . A plot of the reaction resistance,  $R_{ct}$  obtained from the semicircles in Figure 3a versus concentrations of  $Cl^-$ , shows a linear relationship (Figure 3b), indicating that  $R_{ct}$  is inversely proportional to  $Cl^-$  concentrations. Impedance measurements were also conducted with the hybridized oligonucleotide-polypyrrole electrodes in solutions containing 0.1 M  $LiCl$ ,  $LiBr$ ,  $LiI$  and  $LiClO_4$ , respectively. A plot of  $R_{ct}$  obtained from the measured semicircle region of the impedance complex versus the diameter of different anions,  $Cl^-$ ,  $Br^-$ ,  $I^-$  and  $ClO_4^-$  shows a linear relationship (Figure 4), indicating  $R_{ct}$  is proportional to the size of anions in the electrolytes. The diameters of ions were obtained or calculated from (41). These impedance results demonstrate that higher concentration and smaller anion size could result in lower electron transfer resistance of DNA-polypyrrole film electrodes. In terms of equation 4 and 5, the anion is one of the reactant. That is why the electron transfer resistance,  $R_{ct}$  is inversely proportionally to the  $Cl^-$  concentration in Figure 3b. Further, as a mobile dopant, a larger size of the anion could cause greater  $R_{ct}$ , which is in agreement with the results in Figure 4. This further proved the proposed mechanism for the labelless detection scheme. In addition, the results demonstrated that using higher concentration and smaller size of anions could decrease the impedance response of the DNA/pyrrole electrodes, leading to



**Figure 5.** Impedance diagram measured at the hybridized oligonucleotide-polypyrrole film/platinum microelectrode. Hybridization with 30 ml of 2 nM of 3-mismatched target DNA solution, electrode diameter = 50 microns. Curve 1: Baseline measured at the non-hybridized oligonucleotide-polypyrrole film/platinum microelectrode; Curve 2: measured after hybridization in 3-mismatched target DNA with wash in 1XSSC for 1 hour at 25°C; Curve 3: measured after hybridization in 3-mismatched target DNA with wash in 1XSSC at 37°C for 1 hour; Curve 4: measured after hybridization in 3-mismatched target DNA with wash in 1XSSC at 38°C for 1 hour;



**Figure 6.** Relationship of electron-transfer resistance verses concentrations of target DNA molecules, measured on silicon array chips.

increasing the ratio of transducer signals after to before hybridization, which actually improves the signal to noise ratio. This could be used to design optimized assay electrolyte for higher sensitivity.

#### 4.3. Specificity of impedance labelless detection of DNA

Two oligo-polypyrrole microelectrodes were incubated in a solution containing 2 nM of a 15-mer target molecule (5'-T-C-C-T-C-T-G-C-T-T-G-A-G-G-3') that was fully complementary to the attached probe and two other oligo-polypyrrole microelectrodes were incubated in a solution containing 2 nM of a three-mismatched 15-mer target molecule (5'-C-C-C-T-C-T-G-G-T-T-G-A-G-A-3'). Following hybridization, individual microelectrodes were washed at successively higher temperatures in the

order of 25, 37, and 38°C to electrically measure the melting of the duplexes by impedance. Washing was performed by placing the microelectrodes in 1XSSC for 1 hour at every temperature. The impedance curves obtained for the fully complementary target molecule remained unchanged following the three sequential temperature washes, indicating that the melting temperature of the perfect duplex was not exceeded. The impedance curves obtained for the three-mismatched target molecule as shown in Figure 5 moved toward the baseline (the impedance curve obtained from unhybridized oligo-polypyrrole microelectrodes) with the increase of the wash temperatures. This indicated that the melting of this duplex occurred at temperatures near to that mismatched duplexes melting temperature. The results demonstrated specificity of the impedance labelless detection of DNA hybridization, and the ability to discriminate between matched and mismatched nucleic acid sequences demonstrated the applicability of the method described here.

#### 4.4. Results on silicon array chips

The silicon array chip contains four gold microelectrodes located in the bottom of the four wells in the chip. The oligonucleotide immobilized polypyrrole film was deposited on the four gold electrode surface by copolymerization method described above, in which three of them with the probe of 15-mer NH<sub>2</sub>-5'-C-C-C-T-C-A-A-G-C-A-G-A-G-G-A-3' and one of them with the probe of NH<sub>2</sub>-5'-T-C-C-T-C-T-G-C-T-T-G-A-G-G-3', which is fully noncomplementary target molecule. The prepared silicon chips were used for hybridizations in 2, 5, 10 and 20 nM target DNA solutions with the sequence of 5'-T-C-C-T-C-T-G-C-T-T-G-A-G-G-3', which is fully complementary to one of the probes attached on the three electrodes of the chip, but is noncomplementary DNA to another attached probe. Thus, the array electrode with the attached probe sequence of 5'-T-C-C-T-C-T-G-C-T-T-G-A-G-G-3' that is noncomplementary to the DNA to be detected was as a negative control. The results are shown in Figure 6. The standard deviations for the positive responses were calculated from the impedance measured at the three-complementarily hybridized array electrodes. There was no standard deviation showing in the Figure for the negative control since only one array electrode served as the negative control. The result from the silicon chip demonstrated a fairly linear relationship of the impedance measured versus the target concentration. This shows a potential application of this labelless detection technology for high density array DNA chips.

### 5. CONCLUSION AND PERSPECTIVES

A simple impedance labelless detection of DNA hybridization method was investigated for DNA biosensors. The experimental results showed that the detection mechanism is probably due to the change of doping/undoping process of the conductive polymer after the DNA hybridization. The anionic effects were examined in this paper, indicating that higher concentration and smaller size of anions had the lower electron-transfer resistance. The results not only provide further evidence to support the detection mechanism proposed and also offer a

method to improve the signal to noise ratio for the DNA biosensor. The experimental results also showed good specificity of the labelless detection method. Moreover, the method has been demonstrated to detect nM concentrations on a concept silicon array chip. Due to its simplicity and low cost, the techniques could have potential application in clinical diagnosis drug discovery, environmental and food analysis. Literatures (33, 36, 42) reported oligonucleotide-functionalized polypyrrole or covalently immobilized polypyrrole by DNA probes to improve the sensitivity of polypyrrole based electronic detection scheme. The improvement is possibly produced by overcoming the steric and kinetic barriers of entrapped probe DNAs from the surrounding polypyrrole molecules. The authors are also working on techniques to covalently immobilizing probe biomolecules into the conductive polymer matrix for further improvements of the assay sensitivity. Detection of interesting target DNA hybridization in a sample containing different DNAs, particularly in patient samples, needs to be further investigated for non-specific interference, which has been currently studying in the author's lab.

### 6. ACKNOWLEDGEMENT

The authors are grateful to Motorola Process Lab of Motorola Research Center in Tempe, Phoenix, USA for fabricating silicon based array chips for the experiments.

### 7. REFERENCES

1. Marmur, J. & P. Doty: Thermal renaturation of deoxyribonucleic acids. *J Mol Biol* 3, 585-594 (1961)
2. Skogerboe K: Molecular Biology Techniques. *Anal Chem* 65, 416R (1993)
3. Guiducci C., C. Stagni, G. Zuccheri, A. Bogliolo, L. Benini, B. Samorì, & B. Riccò: DNA detection by integrable electronics. *Biosensors and Bioelectronics* 19, 781-787 (2004)
4. Kerman K., M. Kobayashi & E. Tamiya: Recent trends in electrochemical DNA biosensor technology. *Meas Sci Technol* 15, R1-R11 (2004)
5. Piuino, P.A., U. J. Krull, R. Hudson, M. Damha, H. Cohen: Fiber-Optic DNA Sensor for Fluorometric Nucleic Acid Determination. *Anal Chem* 67, 2635-2643 (1995)
6. Hashimoto, K., K. Ito & Y. Ishimoro: Sequence-Specific Gene Detection with a Gold Electrode Modified with DNA Probes and an Electrochemically Active Dye. *Anal Chem* 66, 3830-3833 (1994)
7. Millan K. M., A. Saraullo & S. R. Mikkelsen: Voltammetric DNA Biosensor for Cystic Fibrosis Based on a Modified Carbon Paste Electrode. *Anal Chem* 66, 2943-2948 (1994)
8. Xu X. H. & A. J. Bard: Immobilization and Hybridization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenerated Chemiluminescent Detection. *J Am Chem Soc* 117, 2627-2631 (1995)
9. Johnston D. H., K. C. Glasgow, & H. H. Thorp: Electrochemical Measurement of the Solvent Accessibility of Nucleobases Using Electron Transfer between DNA and Metal Complexes. *J Am Chem Soc*, 117, 8933-8938 (1995)
10. Okahata Y., J. Y. Matsunobu, K. Ijro, M. Mukae, A. Murakami & K. Makino: Hybridization of nucleic acids immobilized on a quartz crystal microbalance. *J Am Chem Soc*, 114, 8299-8300 (1992)
11. Okahata Y., M. Kawase, K. Niikura, F. Ohtake, H. Furusawa & Y. Ebara: Kinetic measurements of DNA hybridization on an oligonucleotide-immobilized 27-MHz quartz crystal microbalance. *Anal Chem*, 70, 1288-1296 (1998)
12. Healey, B.G., R.S. Matson, R.S. Walt: Fiberoptic DNA sensor array capable of detecting point mutations. *Anal Biochem* 251, 270-279 (1997)
13. Strother, T., W. Cai, X.S. Zhao, R.J. Hamers & L.M. Smith: Synthesis and characterization of DNA-modified silicon (111) surfaces. *J Am Chem Soc* 122, 1205-1209 (2000)
14. Boon, E.M., D.M. Ceres, T.G. Drummond, M.G. Hill & J.K. Barton: Mutation detection by electrocatalysis at DNA-modified electrodes. *Nature Biotech* 18, 1096-1100 (2000)
15. Wang J., E. Palecek, P. E. Nielsen, G. Rivas, X. H. Cai, H. Shiraishi, N. Dontha, D. Luo, and P. A. M. Farias: Peptid Nucleic Acid Probes for sequence-specific DNA biosensor. *J Am Chem Soc*, 118, 7667-7670 (1996)
16. Caruana D. J. & A. Heller: Enzyme-amplified amperometric detection of hybridization and of a single base pair mutation in an 18-base oligonucleotide on a 7-micro diameter microelectrode. *J Am Chem Soc* 121, 769-774 (1999)
17. Wang J., L. Chen & M. Chicharro: Trace measurements of nucleic acids using flow injection amperometry. *Anal Chim Acta* 319, 347-352 (1996)
18. Steel A.B., T.M. Herne & M. J. Tarlov: Electrochemical quantitation of DNA immobilized on gold. *Anal Chem* 70, 4670-4677 (1998)
19. Ozsoz M, A. Erdem, P. Kara, K. Kerman & Ozkan: Electrochemical biosensor for the detection of interaction between arsenic trioxide and DNA based on guanine signal. *Electroanalysis* 15 613-619 (2000)
20. Alfonta L., A. K. Singh & I. Willner: Liposomes labeled with biotin and horseradish peroxidase: A probe for the enhanced amplification of antigen-antibody or oligonucleotide-DNA sensing processes by the



## Labelless polypyrrole DNA Biosensors

precipitation of an Insoluble product on electrodes. *Anal Chem* 73, 91-102 (2001)

21. Lewis F. D., T. Wu, Y. Zhang, R. L. Letsinger, S. R. Greenfield & M. R. Wasielewski: Distance-dependent electron transfer in DNA hairpins. *Science* 277, 673-6 (1997)

22. Fink H.W. & C. Schonenberger: Electrical conduction through DNA molecules. *Nature* 398, 407-10 (1999)

23. Okahata Y, T. Kobayashi, K. Tanaka & M. Shimomura: Anisotropic electric conductivity in an aligned DNA cast film. *J Am Chem Soc* 120, 6165-6 (1998)

24. Porath D, A. Bezryadin, S. de Vries & C. Dekker : Direct measurement of electrical transport through DNA molecules. *Nature* 403, 635-8 (2000)

25. Braun E, Y. Eichen, U. Sivan & G. Ben-Yoseph: DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* 391, 775-778 (1998)

26. Long Y. T, C. Z. Li, T. C. Sutherland, M. Chahma, J. S. Lee & H.B. Kraatz: A comparison of electron-transfer rates of ferrocenoyl-linked DNA. *J Am Chem Soc* 125, 8724-8725 (2003)

27. Boon E. M., J E Salas & J K Barton: An electrical probe of protein-DNA interactions on DNA-modified surfaces. *Nat Biotechnol* 20, 282-286 (2002)

28. Fan C, K W Plaxco & A J Heeger: Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *Proc Natl Acad Sci USA* 100, 9134-9137 (2003)

29. Joseph W., M. Miang, A. Fortes and B. Mukherjee: New labe-free DNA recognition based on doping nucleic-acid probes within conducting polymer films. *Anal Chim Acta* 402, 7-12 (1999)

30. Joseph W. & M. Miang: Toward genelectronics: Nucleic acid doped conducting polymers. *Langmuir* 16, 2269-2274 (2000)

31. Hason S, J Dvorak, F Jelen & V Vetterl: Interaction of DNA with echinomycin at the mercury electrode surfaces detected by impedance and chronopotentiometric measurements. *Talanta* 56, 905-913 (2002).

32. Cloarec J P, N Deligianis, J R Martin, I Lawrence, E Souteyrand, C Polychronakos & M F Lawrence: Immobilization of homooligonucleotide probe layers onto Si/SiO<sub>2</sub> substrates: characterization by electrochemical impedance measurements and radiolabelling. *Biosens Bioelectron* 17, 405-412 (2002)

33. Thompson L.A., J. Kowalik, M. Josowicz & J. Janata: Label-Free DNA Hybridization Probe Based on a Conducting Polymer. *J Am Chem Soc* , 324-325 (2003)

34. Li C.M., S. Song, G. Maracas & V-N Choong: Reporterless Genosensors Using Electrical Detection Methods. *US Patent* 20020051975, 1999 filed (2002)

35. Lee T-Y. & Y-B Shim: Direct DNA Hybridization Detection Based on the Oligonucleotide-Functionalized Conductive Polymer. *Anal Chem* 73,5629-5632 (2001)

36. Livache T, A Roget, E Dejean, C Barthet, G Bidan & R Teoule: Preparation of a DNA matrix via an electrochemically directed copolymerization of pyrrole and oligonucleotides bearing a pyrrole group. *Nucleic Acids Rev* 22, 2915-2921 (1994)

37. Saoudi B, N. Jammul, M.-L. Abel, M.M. Chehimi & G. Dodin: DNA adsorption onto conducting polypyrrole. *Synth Met* 87, 97-103 (1997)

38. Bard A. J. & L. R. Faulkner: Equivalent circuit of a cell. In *Electrochemical Methods*. Authors: Bard A.J. & L.R. Faulkner, 2nd ed. John Wiley & Sons, Inc. (2001)

39. Patolsky F, A Lichtenstein & I. Willner: Electron transduction of DNA sensing processes on surfaces: Amplification of DNA detection and analysis of single-base Mismatches by Tagged Liposomes. *J Am Chem Soc* 123, 5194-5205 (2001)

40. Rodriguez J, H. J. Grand & T.F. Otero: Polypyrroles: from basic research to technological applications. In *Handbook of organic conductive molecules and polymers*. Eds: H.S. Nalwa, John Wiley & Sons (1997)

41. Cotton F.A., G Wilkinson, C A Murillo & M Bochmann: *Advanced Inorganic Chemistry*. Eds 6, Wiley International Publication (1999)

42. Korri-Youssoufi H. & A. Yassar: Electrochemical Probing of DNA Based on Oligonucleotide-Functionalized Polypyrrole. *Biomacromolecules* 2, 58-64 (2001)

**Key Words:** Polypyrrole electrode, DNA biosensor, Labelless detection, Impedance measurements

**Send correspondence to:** Dr. Changming Li, School of Electrical & Electronic Engineering, Nanyang Technological University, Nanyang Avenue, Singapore 639798, Tel: 65 6790 4485, Fax: 65 6792 0451, Email: ecml@ntu.edu.sg

<http://www.bioscience.org/current/vol10.htm>