

Optical, Electrochemical, and Magnetic DNA Biosensors – An Overview

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Abstract— This review paper introduces DNA biosensor technology as it is applied to DNA sequencing, mutation sensing, and hybridization sensing. Following this classification, concrete examples of state-of-the-art DNA biosensors are introduced and reviewed as they apply to each of the three areas of DNA sensors.

I. INTRODUCTION TO DNA SENSORS

The field of biosensors has enlarged greatly over the past few decades through a multitude of technological discoveries and the introduction of new methodologies. Within this broad biosensor field, the sensing of deoxyribonucleic acid (DNA) has also greatly increased. DNA biosensor applications can be classified into three broad categories: sequencing, mutation detection, and matching detection. The objective of this review paper is to describe the general ideas relating to each of the three categories and give concrete examples of state-of-the-art DNA sensor technology. While it would be nearly impossible to present and critique every imaginable DNA biosensor in development, specific sensor examples are reviewed in order to illustrate their basic operation as it relates to sequencing, mutations, or matching. Because we have decided to develop a lab-on-chip anthrax DNA biosensor by testing for hybridization, the latter half of the paper concentrates on sensing techniques explicitly used to determine the matching of anthrax DNA rather than all possible hybridization techniques in general. The first portion of the article introduces the basic concepts of DNA sequencing and mutation sensing and gives explicit examples of such sensors.

While this paper covers only DNA biosensors, the reader should note that all DNA sensors basically operate in the same manner as any biosensor. That is, each sensor design involves immobilization of a biological element on the surface of a transducer, the detection of the resulting signal, and the transduction of this signal into a usable output.

II. SEQUENCING

The purpose of DNA sequencing is to determine the exact order of nucleotides (bases) of a given species when the DNA of that species is obtained. While the sequencing of human DNA is of primary concern, the methods for ordering base

pairs can be utilized for any organism. DNA sequencing and DNA matching are in fact similar fields. While sequencing involves the random compilation of sequential nucleotides in a given DNA molecule, matching involves testing two strands of DNA to observe hybridization (thus giving a match or mismatch). Therefore it is possible to know if a test sample has the same base sequence if hybridization with a known sequence has occurred.

Many techniques used for DNA sequencing are relatively slow, laboratory-based chemical processes, and no single machine or method can input an entire DNA strand and output its DNA map. Such laboratory methods often used include Maxam-Gilbert sequencing, Sanger method, and terminator sequencing. The intent of DNA biosensors in mapping is to enhance sequencing capabilities, and the following examples accomplish this task.

A. DNA Sequencing Using Photocleavable Fluorescent Nucleotides

Seo et al. have developed a method of DNA sequencing genes at a specific location using Sequencing-by-Synthesis (SBS) on a chip by way of four photocleavable fluorescent nucleotide analogues. This technique involves immobilizing ssDNA and performing polymerase chain reaction (PCR) one base at a time. That is, PCR begins at the first nucleotide of the probe strand and grows a complementary base to which only one of the four fluorophores can bind with. The fluorophore contains a photocleavable linker that terminates the PCR process, and a near-ultraviolet Nitrogen laser containing four distinct wavelengths causes only the correct fluorophore to emit light. This light is detected by a scanner/mass spectrometer while the Nitrogen laser concurrently activates an azido-labeled primer to begin the next step of PCR, and the process is repeated. Each distinct step of the process can be summarized by PCR termination, laser pulses, fluorescence, detection, fluorophore removal, and priming. Once all of the components of a single-base step are completed, the process is repeated a maximum of 12 times, thus the mapping of 12 nucleotides is accomplished. Although this process may seem to be miniscule as compared to the number of bases on a chromosome or even within a single gene, the large chip surface area allows for a large

number of parallel processes to occur simultaneously [1]. Fig. 1 shows a visual representation of the process.

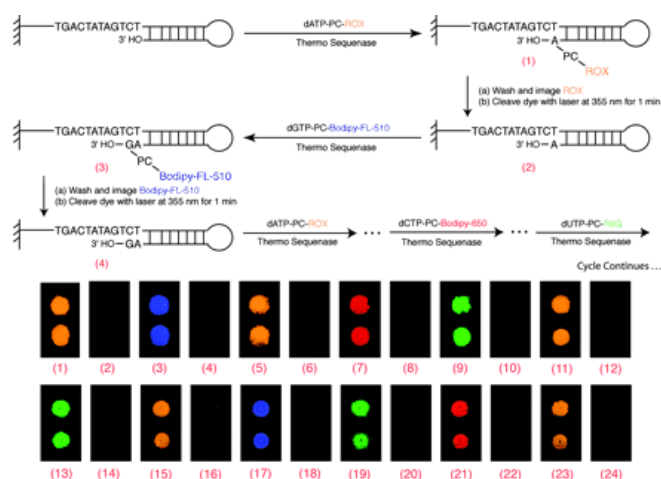


Figure 1. The top half of the diagram shows the basic sequence of reaction while the bottom represents the fluorescent output that is visually recognized by the scanner. The four different colors represent a specific nucleotide base [1].

As with all biosensors, this technique has certain advantages and disadvantages. While this method quickly determines base sequencing using parallel analysis, its chemical limitations lead to a short (12) number of bases that can be analyzed at once. Also, the device is greatly affected by temperature and pH, which leads to an overall accuracy of only 90%.

B. Sequencing using Surface-attached Molecular Beacons

One method for accurate acquisition of DNA sequences is through the use of so-called molecular beacons. These beacons can be used to make electrochemical or E-DNA sensors. The beacon probe uses an oligodeoxynucleotide (ODN) hairpin-like stem-loop structure with a fluorescent label and quencher at each end. Instead of using the fluorescent label as an optical marker, though, an initial monitored signal is applied through the molecular beacon. The molecular beacon uses a gold surface with gold nanoparticles to act as a quenching agent. Once a DNA sequence match is found with a complementary DNA strand, the ODN is broken down and the initial signal greatly decreases or is eliminated. In this sensor, the 27-mer ssODN labeled with ferrocene at its 5' end is immobilized on the gold electrode via its sulfhydryl group at the 3' end, forming the stem-loop structure. With the ferrocene close to the electrode surface, a distinguished cyclic voltammetric redox pair is produced in the absence of the complementary target DNA. Hybridization of the tDNA with the 17 base loop of the 27-mer ODN induces a change resulting in the formation of a rigid, rod-like duplex DNA structure that causes the ferrocene label to move away from the electrode surface [2]. This then causes the decrease or elimination of the initial signal. This E-

DNA sensor is reagentless and reusable. It is well suited to the continuous monitoring of the flow of analyte.

Advantages of electrochemical sensors can include greater sensitivity, faster speed, lower cost, and lower power requirements. Many of the e-sensors can also use simpler designs with smaller dimensions to produce the same or better results than optical DNA sensors. The one major disadvantage would be the lack of experience with E-DNA sensors versus the common fluorescence-based optical sensors. Measuring minute signals can also be a drawback, in terms of uncertainties, when compared to a positive or negative optical result.

III. MUTATION SENSING

DNA mutations are defined as a variation in the normal nucleotide sequence of an organism. These mutations can occur from a variety of causes such as invasion by a pathogen or exposure to radiation. The detection of DNA mutations can lead to the diagnosis of a disease or the prognosis that an individual is susceptible to an ailment. Therefore, the study of DNA mutations is a very important and powerful tool within the field of medical diagnostics and elsewhere.

Various methods have been created to detect mutations within a specific location on a chromosome. These methods mostly involve optical detection of mutations, as is the case with the Fluorescence in situ Hybridization (FISH) technique. Many devices also rely on the use of powerful microscopes that allow the viewer to observe specific sites where mutations have possibly occurred. An example of a biosensor used in the detection of DNA mutation is now presented.

A. DNA Mutation Detection using Temperature Gradient Gel Electrophoresis

A novel approach to the detection of DNA mutations has been demonstrated by Buch et al. Modifying the gel electrophoresis procedure and adding a temperature gradient allowed them to detect a single nucleotide polymorphism in a DNA strand approximately 500 base pairs long. The DNA is prepared by adding a GC clamp and a fluorescent label during PCR. The GC clamp increases the melting point of the DNA. The increase of the melting temperature is due to the three hydrogen bonds between the guanine (G) and cytosine (C). The GC clamp will literally hold one end of the DNA together as it moves down the channel [3]. Buch et al. varied the temperature of the channel from 70C to 75C and applied an electric field in the channel. In the presence of the electric field the DNA moves from the negative potential towards the positive potential. Shorter strands are more mobile while longer strands are less mobile. As the DNA with the single nucleotide polymorphism moves through the channel it partially melts which also decreases its mobility. This increases the amount of time that the DNA is in the detection area of the sensor and increases the reflected light from the fluorescent marker [3].

This sensor was able to find the mutations in every strand in less than four minutes. Large portions of DNA can be scanned quickly by using multiple micro channels in the polycarbonate substrate [3].

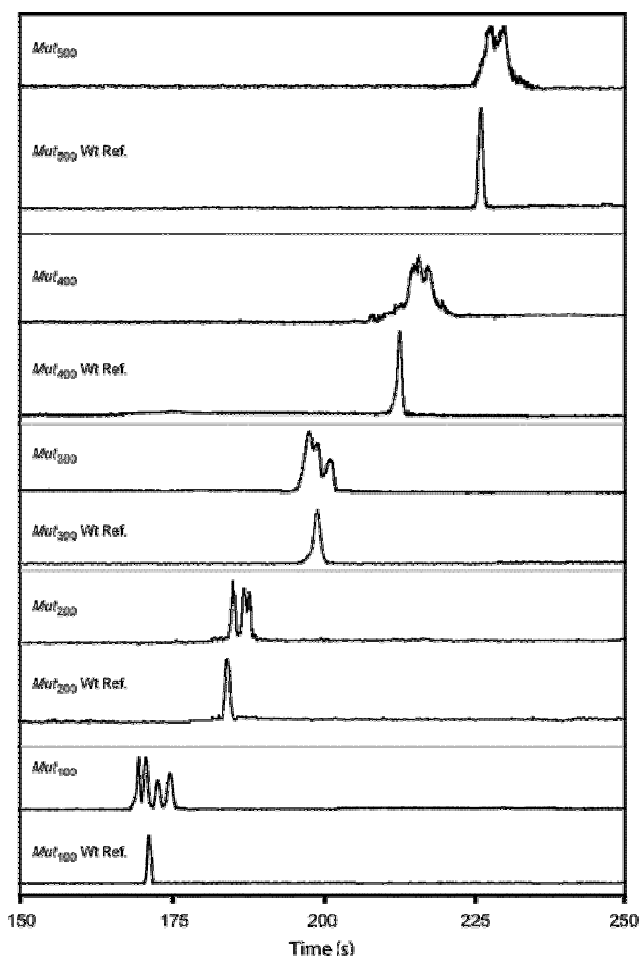


Figure 2. DNA with no mutation (Wt Ref) move quickly through the detection area of the sensor giving one narrow peak of reflected light. DNA with a single mutation (MutX00) partially melts and slows down as it travels through the detection area resulting in a broad peak or multiple broad peaks of reflected light [3].

IV. DETECTION OF ANTHRAX DNA MATCHING

The third category of DNA biosensor technology is the detection of matching between two strands of complementary DNA. As stated previously, we have chosen to emphasize sensors that detect the matching of anthrax DNA. *Bacillus anthracis*, the most potent of this group of bacteria, can be fatal especially if the disease reaches the second stage. The first stage of the disease can be similar to a cold or flu. This stage can last for hours or days depending on the germination rate of the spores inside the lungs. Stage two quickly follows stage one and can include fever, shortness of breath, and shock. Approximately ninety percent of the cases that reach the second stage of the anthrax inhalation illness are fatal.

Bacillus anthracis is a rod shaped bacteria that will grow and divide as long as it is in a nutrient rich environment. Once all the nutrients have been used up the bacteria turns to spores. *Bacillus anthracis* spores are resistant to environmental stress and can survive for decades. When the spores enter the lungs they germinate releasing toxic substances into the body leading to internal bleeding, swelling, and the death of tissue. An infectious dose of *Bacillus anthracis* is exposure to 10,000

spores. *Bacillus anthracis* spores, since they appear readily in nature, can be manufactured easily with very little lab equipment required [4].

A number of methods have been developed to detect the presence of anthrax by way of antibodies, enzymes, and DNA analysis. One of the most precise detection schemes is DNA analysis that occurs in a laboratory environment, but it is desirable to create small, portable devices to detect *B. anthracis* DNA for on-site confirmation of the presence of anthrax. Four anthrax DNA biosensors are now presented.

A. Magnetic DNA Detection Method

A group of collaborative researchers have developed a method to detect hybridization of anthrax DNA using magnetic microbeads and a giant magnetoresistive (GMR) biosensor. In addition to developing the GMR technology, Tamanaha et al. introduced a cartridge-based MEMS network of microfluidic components in which the removable cartridge greatly reduces the device recovery time. Fig. 3 shows the cartridge that is inserted into the analytical system known as the Bead Array Counter (BARC) system.

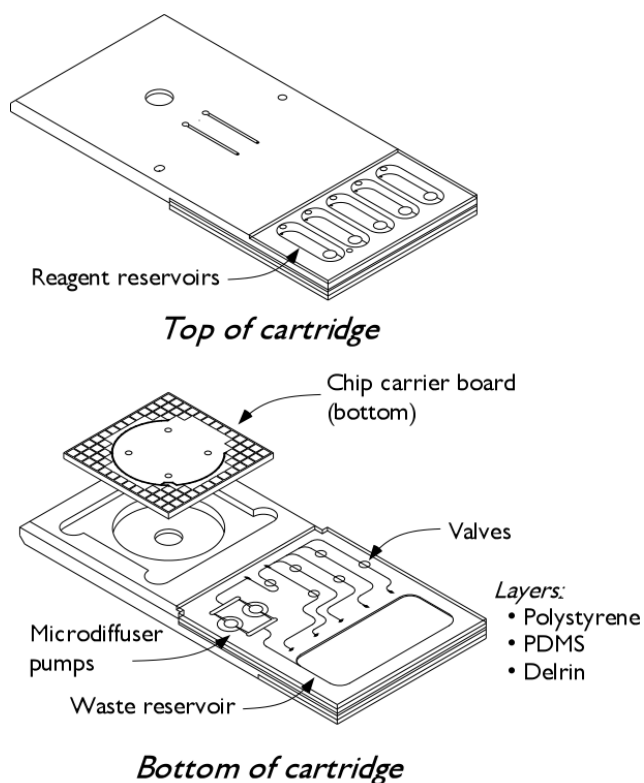


Figure 3. The 5.1cm x 10.2cm cartridge is mounted onto the BARC chip. The cartridge contains a network of 125 micron-deep channels, valves, pumps, and reservoirs that interfaces with an actuator unit on the chip [5].

The device operation can be summarized in a few distinct steps. First, anthrax ssDNA probes are immobilized on the gold electrode surface using gold-thiol chemistry. Second, sample DNA strands are extracted from spores of *B. anthracis*, denatured, and labeled with the magnetic microbeads. The chip is then attached to the BARC system and the sample

strands are carried to the sensor spots via microfluidic channel transport. Next, target DNA is allowed to hybridize with the probes and the sensor spots are then washed to remove any unhybridized strands. At this point, the sensor spots are subjected to an applied magnetic field, and the BARC instrument picks up the resulting bead signal [5]. Although the BARC instrument utilizes complex methods to determine changes in the magnetic field, the basic Hall Effect equation is central to the determination of the microbeads.

$$U_H = R_H \left(\frac{BI}{d} \right) \quad (1)$$

In the above equation, R_H is the Hall coefficient, d is the sample (bead) thickness, B is the applied magnetic field, I is the control current, and U_H is the Hall voltage [6].

The magnetic DNA detection technique has certain advantages and disadvantages. For instance, the disposable chip greatly reduces device recovery time while the sensitive BARC system allows for detection of femtomolar concentrations without optical methods. However, the required device operation time of 40 minutes provides no real advantage over optical techniques and the complex system of MEMS components is still costly and required complicated micromachining.

B. Colorimetric Detection using PNA Sandwich Hybridization

The most common types of DNA sensors utilize DNA probes to hybridize to the target sequences. While useful, these probes require the use of PCR, or polymerase chain reaction, to amplify the existing numbers of DNA sequences so the probes can more easily find the DNA strands. A new solution to this problem involves the use of PNAs, or peptide nucleic acids, to replace the DNA probes. The advantages of PNA use can include resistance to enzyme degradation, increased sequence specificity to complementary DNA and higher stability when bound to complementary DNA. The greatest advantage in the use of PNAs would be the ability to make and easily discernible colorimetric output that readily indicates a positive result for the presence of bacillus anthracis, or anthrax, DNA.

In this colorimetric sensor, PNAalpha is covalently attached to a DNA-bind plate. This is used as a capture probe at the surface. A second PNAbeta probe is used as a detection probe that generates a signal. A biotin labeled PNAbeta, in combination avidin-horseradish peroxidase conjugate (HRP-A) and tetramethylbenzidine (TMB), is used to generate a signal if the target anthrax DNA is present. The HRP-A works to catalyze oxidation of the TMB, and after stopping the reaction with sulfuric acid, a colored product that absorbs at a wavelength of 450nm is produced. All PNA probes are made with a single sequence corresponding to the protective antigen portion of the anthrax genome. The high thermal stability of the PNA-DNA duplexes allows shorter PNA probes when compared to standard DNA probes [7]. Unlike measuring the sometimes uncertain changes in voltage, current

or frequency, the colorimetric outcome provides the user with an easy identification system.

C. Passive DNA sensor using a CMOS Backend Process

The working principle of this device relies on a sensor for electrical detection of DNA is fabricated in a CMOS production line. The sensor principle techniques depend on immobilization of single stranded DNA probe molecules on an array consisting of interdigitated gold lines and subsequent hybridization with labeled target DNA strands. The electrical signal results from an electrochemical redox cycling process [8].

The principle technique of DNA detection is dependent upon single strand DNA molecules (probe molecules, e.g. with about 20 bases) are immobilized on top of the gold electrodes due to gold-thiol coupling. Using a spotting machine, each sensor within a sensor array can contain individual probe molecules. In the following steps, the chip is flooded with an analyte containing labeled target DNA molecules. After that the hybridization takes place: chains of molecules with specific complementary sequence compared to the immobilized probe molecules match and form double-stranded DNA. A suitable substrate is applied to the buffer solution and it is enzymatically cleaved by the label. The resulting species starts an electrochemical redox process at the electrodes. The electron current generated in the redox process is detected and translates the information “matching DNA strand” into electrical signal. Fig. 4 depicts the general idea behind this sensor [9].

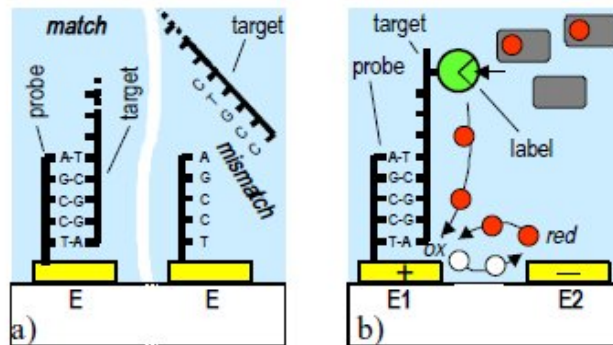


Figure 4. Principle of DNA detection (a) match and mismatch between probe and target molecules (b) redox system.

The CMOS sensor uses amperometric measurements of redox current to sense the occurrence of hybridization. In the ‘match’ case, the current is higher, because the p-Aminophenol phosphate (P-APP) substrate is cleaved at the label at the bound target molecule and more redox pairs are actively generated [10]. Finally the flow of the P-APP is stopped, when the flow stopped, the system begins to supply the data which must be evaluated to distinguish between the match and mismatch case. The mismatch current remains slightly increasing from 20na to 30na. In the match case however, the current increase is by far more pronounced. In this case the P-APP is continuously cleaved by the label and the generation of redox species proceeds. Thus the current raises up to 100 Na within the time of 300 sec. Consequently,

we conclude that use of integrated CMOS circuitry in combination with such sensors enables highly sensitive electrical DNA detection on multi-sensor chips.

This sensor has certain advantages and disadvantages associated with it. While the benefits include high sensitivity and low cost relative to other laboratory-based techniques, drawbacks that must be accounted for include sensitivity to an air gap between the sensor and the sample.

D. DNA Detection using nanoscale ZnO sensor arrays

Kumar et al. developed a sensor using SiO₂ and ZnO. Using various growth methods they were able to build nanorods, nanopillars, and nanosquare sensor arrays. Single stranded DNA could be attached to the ZnO structures through either non-covalent attachment or covalent attachment. Fig. 5 depicts the basic structure of these platforms.

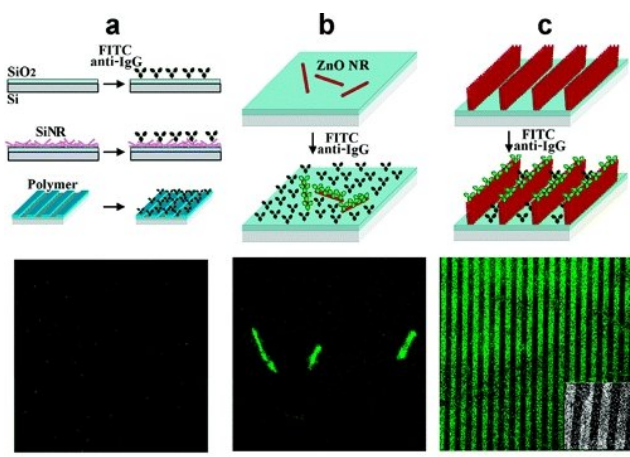


Figure 5. (a) No fluorescence was detected from hybridized DNA using SiO₂, silicon nanorods, or PMMA nanostructures. (b) Fluorescence only detected where hybridized DNA bonded with zinc nanorods. (c) Fluorescence detected in a striped ZnO nanoarray structure [11].

Non-covalent attachment of single stranded DNA could be achieved in five minutes. After five minutes the arrays were rinsed removing any DNA that had not bound to the surface of the sensor. Using this method 20nM concentration of anthrax DNA could be detected with a confocal laser scanning microscope [12]. Covalent attachment of single stranded DNA was a longer process with submersion in GOPS (3-glycidoxylpropyltrimethoxysilane) followed by submersion in Poly-L-lysine. Single stranded DNA was then allowed to incubate on the surface of the sensor at 37C for six hours. Using this method 2fM concentration of anthrax DNA could be detected with a confocal laser scanning microscope. Covalent attachment of DNA to the zinc nanostructures always resulted in more intense fluorescence [12].

Hybridization was detected using an argon laser with a wavelength of 488nm. The fluorescence from the modified DNA was at a wavelength of 510nm. The hybridization occurred over the entire wafer, but only the DNA that bonded

to the ZnO nanostructures resulted in fluorescence. Kumar et al. theorize that the ZnO disables the self quenching traps of the fluorescent molecule, but the exact cause of the fluorescence remains under investigation [12].

V. CONCLUSION

Upon review of the previous examples, it is obvious that each DNA hybridization method has certain advantages and disadvantages. However, the most important characteristic of an anthrax DNA biosensor is the reliable sensing of sample DNA in a short period of time. While each of the previous methods shows significant improvement over laboratory-based analysis, all contain drawbacks that prevent their use in real time. Because the zinc oxide nanostructures show a great deal of promise in reliability and sensitivity without PCR amplification, we have decided to use this as a basis for the development of our own anthrax DNA biosensor. It is our intention to use the basic sensing mechanism while altering various parameters to cut down device operation time. Even though an extreme reduction in operation time may take substantial design improvements, any shortening of the duration while maintaining a concurrent degree of accuracy and reliability will be a welcomed improvement.

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