

# Electronic Anthrax DNA Biosensor Review

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**Abstract-- We introduce preliminary design specifications for an electronic anthrax deoxyribonucleic acid (DNA) biosensor using the principle of DNA displacement. Upon hybridization, the sample DNA displaces a ferrocene-marked signal probe, which induces an increased redox current that is amperometrically measured and relayed.**

## I. INTRODUCTION

The purpose of this article is to propose a design of a Lab-On-Chip DNA biosensor that will be used to detect the presence of bacillus anthracis, more commonly known as b. anthracis or simply anthrax. Anthrax is a form of bacteria found in nature. It is noted for the fact that it exists as a hardy spore until finding a suitable host tissue within which it can become an active, deadly bacteria. It tends to be inhaled by its animal or human victims with no knowledge of ingestion. It can become lethal to humans within days of contact and, therefore, prompt detection of its presence is paramount for an anthrax biosensor. In order to detect the b. anthracis DNA, we will use an electronic DNA (E-DNA) detection scheme to amperometrically confirm matching of sample DNA with probes containing a nucleotide sequence common to only b. anthracis.

## II. DETECTION OF HYBRIDIZATION

An electronic detection mechanism (E-DNA) was chosen to confirm the presence of anthrax DNA due to its relatively simplistic design and straightforward measurement of redox current. The central idea behind the E-DNA method is DNA displacement between two probes and a single strand of sample DNA as introduced in [1]. This method requires two ssDNA probes (capture and signal) that are complementary at both the 3' and 5' termini but are not complementary in the longer middle section. Both capture and signal probes are attached to a gold electrode using gold-thiol chemistry, and the 5' terminus of the signal probe is artificially marked with the reducing moiety methylene blue (MB). In the absence of sample single-stranded DNA (ssDNA), the 3' and 5' ends of the probes will bind to each other, and the signaling probe will produce a limited redox current of about 10nA (with MB marker). However, when a denatured strand of anthrax DNA is introduced, the complementary capture probe will bind to the anthrax strand after the binding between the two probes at

the 5' terminus comes undone. This is a key step in the detection of hybridization between the capture probe and test strand. The signal probe is now able to move much closer to the electrode surface because it is no longer bound to the capture probe. As a result, the rate of electron transfer increases as the number of electrons transferred from moiety to electrode increases exponentially. This redox current is subjected to amperometric detection and the transducer relays

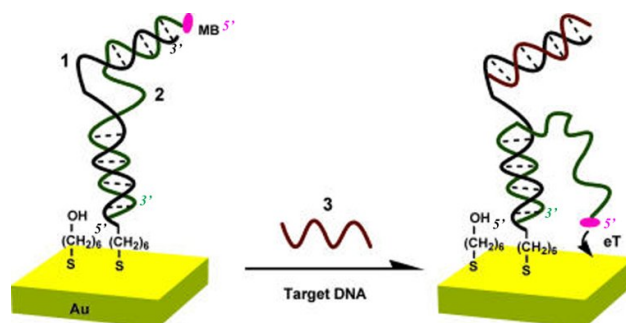


Figure 1. Schematic of DNA detection element [1]

the increased current into a visible reading to an ammeter [1].

The transduction method described above is known as “Signal On,” meaning that hybridization between test sample and capture probe generates a larger current than if no hybridization takes place [1]. This is the opposite of “Signal Off” detection, which occurs when hybridization reduces the amount of redox current. This method is used with Stem-Loop probes as shown in Fig. 2.

The Signal On detection scheme has a number of advantages over Signal Off, such as improved detection limits and an exponential increase in redox current rather than current suppression that occurs with a Signal Off scheme. In Signal Off schemes, suppression can theoretically only reach 100% (meaning no current flow) but often do not reach more than 35% [1].

In order to support the claim that Signal On detection has sufficient signal changes between matched and mismatched DNA, Xiao et al produced graphs of signal strength in terms of both concentration and shift in eV [1]. Alternating current voltammetry was used to amperometrically measure the

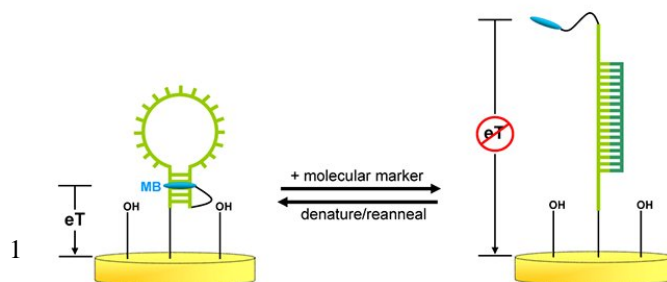


Figure 2. Stem-Loop structure [2]

resulting redox current as shown in Fig. 3. The top of Fig. 3 shows that fully complementary DNA at 20nM concentrations resulted in an energy shift left and increased current while the bottom shows an insignificant signal change in both shift and

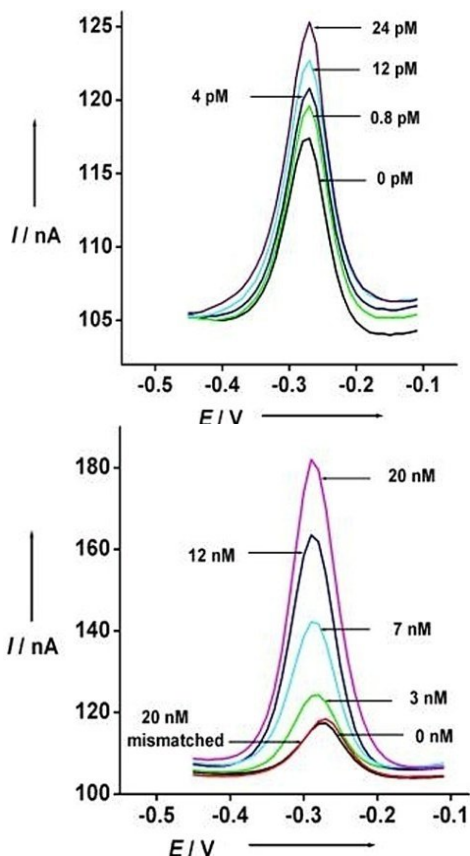


Figure 3. Resulting signal change various test DNA concentrations [1]

current (<5%) for 20nM mismatched DNA.

Since there is a reversible redox reaction between the signal probe and the electrode surface, the peak current is dictated by the Randles-Sevcik equation:

$$i_p = 2.68 \times 10^5 n^{3/2} A D^{1/2} C_{ox} v^{1/2} \quad (1)$$

In Equation 1,  $n$  is the number of electrons transferred after hybridization (58),  $A$  is the area of the electrode,  $D$  is the diffusion constant,  $C$  is the concentration of charges, and  $v$  is the scan rate (10Hz)[1].

### III. ELECTRODE DESIGN

The design in [1] uses a capture probe of 38 base pairs, and a methylene-blue marked signal probe with a base pair length of 32. The signal probe is purposely shorter so that the number of electrons transferred without hybridization is kept limited by the signal probe being taught. Once hybridization occurs, the naturally flexible signal probe is able to move freely closer to the gold surface, increasing the number of electrons transferred. The shorter probe length allows the MB-marked tip to come into contact with the electrode more

frequently, thus increasing the number of reactions between the MB and the gold.

The sensor described in [1] is stable for a period of 24 hours in the absence of target DNA and gives an average current of 10nA. When target anthrax DNA is introduced, the current increases by a factor of seven when the hybridization is allowed to occur for a period of 5 hours at 37° C.

### IV. SENSOR DESIGN IMPROVEMENTS

The single-step E-DNA detection method introduced in [1] served as a basic guide for our design; however a number of important changes are being made in order to improve the detection limits, response time, reusability of electrodes, specificity, and bind strength to meet the desired characteristics of an effective anthrax DNA sensor. The first improvement consists of the utilization of synthetic Peptide Nucleic Acid (PNA) as a capture probe instead of natural DNA. PNA is a synthetic DNA mimic in which the negatively-charged sugar-phosphate backbone of DNA is replaced with a neutral polyamide backbone formed by repetitive units of N-(2-aminoethyl) glycine. The nucleotide bases are then connected to this backbone by way of a two atom carboxymethyl spacer [3]. The basic structure of a PNA

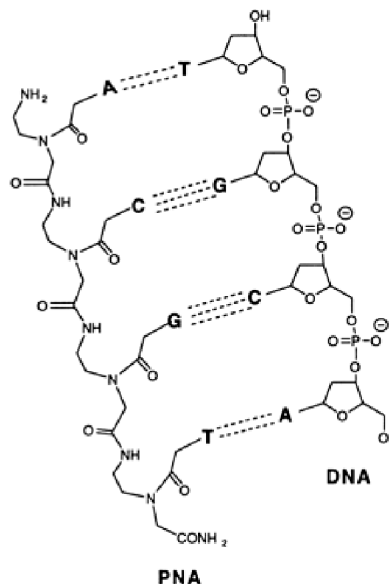


Figure 4. Chemical Structure of PNA as it binds to DNA. [5]

strand is given below in fig. 4.

PNA has a number of favorable characteristics that DNA does not, including stronger and more rapid binding to complementary DNA strands, no electrostatic repulsion (because of its charge neutrality), high binding specificity to DNA, increased breakdown resistance from proteases and nucleases, more rigid physical structure, and a higher temperature resistance when bound to DNA (6 bases PNA/DNA resistant to 31° C while 6 bases DNA/DNA is

only resistant to 10° C) [3]. Because of these benefits, we will use a PNA capture probe while maintaining the DNA signal probe. The breakdown resistance will allow for a longer shelf life as compared to the 24 hours in the design in [1], the temperature resistance will allow for easier denaturing of sample double strand DNA, and the rapid binding to DNA will decrease the required hybridization time. Perhaps the greatest benefit to using PNA is the physical rigidity of the capture probe. With no test DNA present, the signal strand will be held further from the electrode surface, thus decreasing the background current and making the redox current resulting from hybridization much more drastic.

The signal probe must remain as ssDNA since its flexibility is central to the redox reaction once hybridization occurs. However, the increased binding strength between PNA and DNA will also allow us to reduce the number of complementary bases between probes at each terminus. While 7 bases were originally used at the 5' end and 15 used at the 3', we will reduce this number to 3-5 at the 5' terminus and 7-10 at the 3' terminus in order to maintain or improve the displacement ability of target DNA to the capture probe. The number of bases needed to correctly identify lethal b. anthracis DNA was 30 nucleotides in the sequence: 5'- GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT-3' [5]. Therefore, the complementary capture probe must use the sequence: 5'-CTC CCT AAT AAC AAT TTA TAA CTA TTC CTA-3'. Since the number of nucleotides in the test DNA sequence was increased to 30, the total number of bases on the capture probe becomes 57 bases (a target sequence of 15 nucleotides was used) while the total number of bases on the signal probe becomes 43. The use of PNA probes will reduce these values to 40-45 total bases on capture probe and 31-36 pairs using the above PNA reduction ranges. It should be noted that anthrax DNA detection has been done with a smaller number of nucleotide pairs, so an ssDNA sample strand as short as 16-20 bases may be used [5].

A second improvement we will make to the design in [1] is the use of a different redox moiety as the signal marker

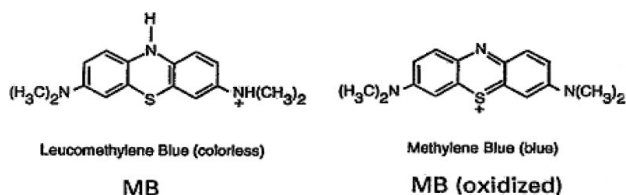
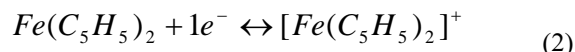


Figure 5. Oxidation of Methylene Blue. [7]

attached to the 5' terminus of the signal probe. The design in [1] used methylene blue as a marker and gives off two electrons in the redox reaction with the electrode surface. Oxidized methylene blue appears in Fig. 5:

Instead of a methylene blue marker, we will use ferrocene as it gets oxidized to its cation ferrocenium. Unlike MB, ferrocene only loses one electron in each reaction. This makes ferrocene more electrochemically stable than MB or any other benzene derivatives while the reaction is still reversible. Also, the background redox current is reduced to a smaller level because of the single electron transfer. This stability makes

ferrocene beneficial to the redox reaction at the electrode[7]. The chemical oxidation of ferrocene is given by:



The transfer of electrons at the electrode is governed by the following equation [8]:

$$i(T) = i_0(T)e^{-\beta d(T)} \quad (3)$$

where: T = Temperature,  $i_0$  = current from bare electrode,  $\beta$  = potential independent electron tunneling coefficient, and  $d(T)$  = the thickness from the redox center to the surface of the electrode.

## V. CONCLUSION

Our original plan for anthrax DNA detection involved the use of optical means to determine hybridization of sample ssDNA to probe DNA using ZnO nanoparticles. Unfortunately, there were serious downfalls to this method because of the time required for hybridization detection, the undesirable solubility in water of ZnO, and the huge cost associated with the electron microscope needed to view the luminescence of the particles. The use of electronic DNA detection by way of DNA displacement effectively addresses these issues, especially with the utilization of peptide nucleic acid and ferrocene. A number of considerations must be made to ensure the rapid detection of anthrax DNA such as the reduction in hybridization time, device complexity and cost, and transduction/amplification of the resulting signal. We aim to implement a microfluidics network and op-amp circuitry to accomplish the complete detection task associated with the basic detection mechanism presented in this article.

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