UNDERGRADUATE REPORT

Label-Free Detection of DNA

by Hoda Eydgahi
Advisor:

UG 2006-3

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Label-Free Detection of DNA

Hoda Eydgahi

Mentors: Mr. Alfred Haas and Mr. Somashekar Prakash

Faculty Advisor: Dr. Pamela Abshire

Integrated Biomorphic Information Systems Laboratory

Department of Electrical and Computer Engineering

Institute for Systems Research

University of Maryland, College Park

August 7, 2006

Introduction:

The discovery of DNA has been described as the “most important biological work of the last 100 years” and the field of research it unlocked “may be the scientific frontier for the next 100” (1). As described by James Watson and Francis Crick in 1953, DNA, which is an acronym for deoxyribonucleic acid, is a right-handed double helix with approximately ten nucleotide pairs per each 3.4 nm helical turn (Figure 1).

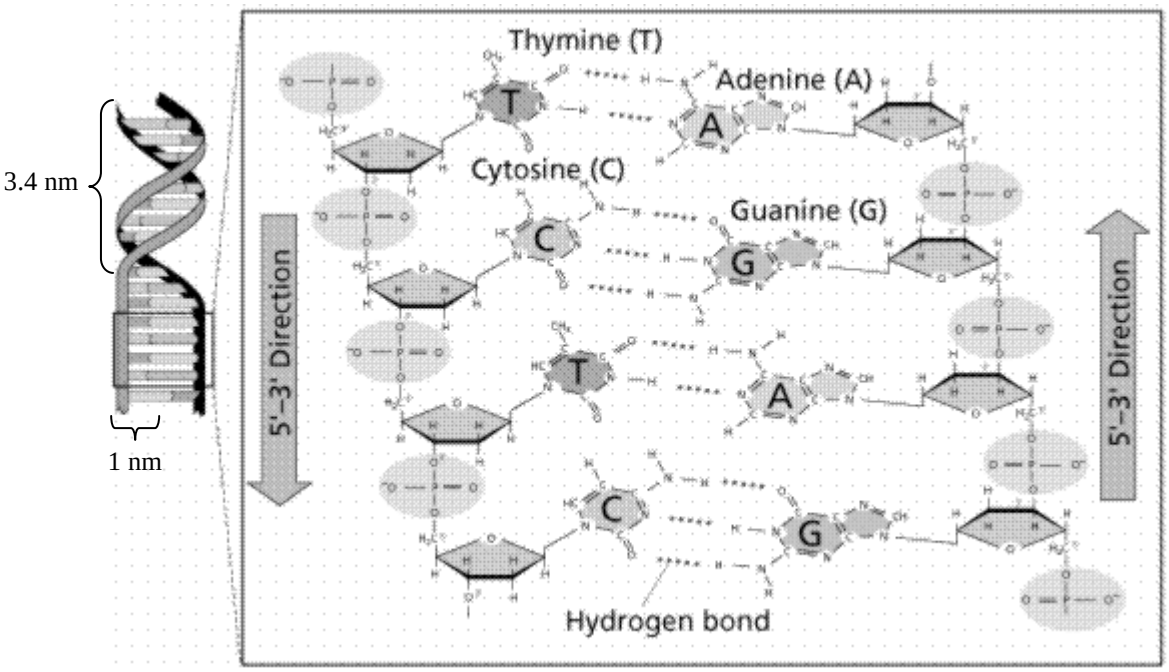

Figure 1: Structure of DNA (5)

Each strand of DNA consists of a sugar phosphate backbone attached to one of four nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C) (2). Thymine and cytosine are pyrimidines, a heterocyclic aromatic organic

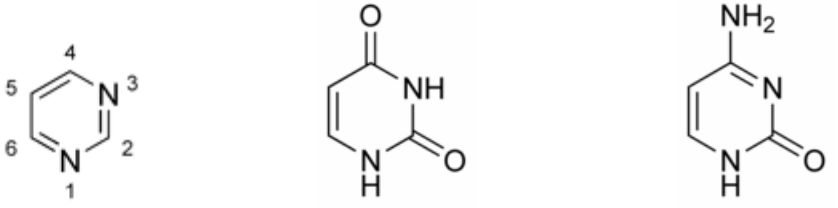

Figure 2: Pyramidines that compose DNA (3)

compound with two nitrogen atoms at positions 1 and 3 of the ring (Figure 2) (3). On the other hand, adenine and guanine are purines, a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring (Figure 3) (4). The double helix consists of a single strand of DNA binding to its complementary strand through hydrogen bonds formed between a purine and a pyrimidine. Specifically, adenine binds to thymine through two hydrogen bonds and guanine binds to cytosine through three (2).

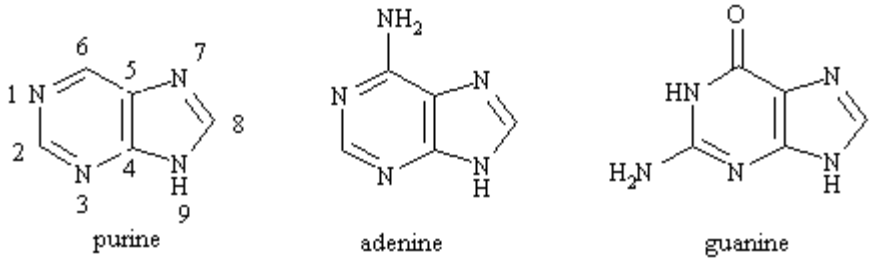

Figure 3: Purines that compose DNA (4)

Within its minute structure, DNA contains the blueprint of the genetic code for all living organisms. Scientists are interested in unraveling the mysteries held within its double helix for it contains answers to a variety of questions with regards to our past, present, and future (7).

In recent years, developing more efficient DNA detection methods has been the center of interest in the growing field of molecular biology. Improved DNA detection methods can benefit the fields of forensic science, risk assessment, medical diagnostics, and genetics, along with a multitude of other technological fields (11).

We are striving to detect the presence of a strand of DNA without the use of any labels. To do so, we must first be able to place DNA on a chip—which is the goal of my summer research project. This project is an excellent example of how the fields of microelectronics and biology are quickly merging into one. The success of this project

will result in the creation of a parallel system that will allow us to analyze of various characteristics of a particular strand of DNA at the same time.

The standard method currently used in molecular biology studies to analyze DNA binding involves fluorescent labeling. Basically, once the strand of DNA in question is labeled with the proper fluorescent tag, it is added to the chip's surface, where it binds with the capture, or known strand of DNA that is bound to the surface. If the oligonucleotide in question is complimentary to the capture DNA, hybridization will occur and they will bind to one another. Fluorescence will be observed when the substrate's surface is viewed under a fluorescence microscope. Although highly sensitive and widely available, fluorescence markers are photochemically unstable and require expensive optical devices for analysis. In addition, they can provide researchers with a false-positive result. For instance, a 25 mer oligonucletide that is complimentary to the capture DNA with the exception of one or two base pairs will still bind to the DNA on the substrate even though it will result in a mismatch error. Fluorescence will be observed regardless—leading the scientist to believe that the two strands are complimentary.

Since both the chemical and electrical characteristics of the substrate will change upon binding of the DNA strand in question, we propose to approach the problem from an electrical standpoint by using Mr. Som Prakash's capacitance sensor to measure the amount of binding between the two strands.

We will initially follow the current protocol that uses fluorescence to measure the presence of DNA. We plan to test fully complementary and non-complementary DNA strands as well as strands containing 1, 5, or 10 mismatches. We will then measure

fluorescence and capacitance levels. The fluorescence method is used purely as a control in this experiment. The purpose of this experiment is to determine whether or not the label-free, electrical approach is more accurate than the current chemical approach. We hope that the capacitance sensor will be able to detect changes in the strength of the bonds present between the purines and the pyrimidines of the double helix formed. Even if the proposed method is just as accurate as the chemical method, it could be used as the method of choice in the future since it is more efficient and will remove the need for optical instruments and fluorescent labeling.

Methods and Materials Proposed:

DNA Oligonucleotides:

Three 30-mer and one 35-mer set of oligonucleotides were custom synthesized and purified by Sigma Genosys:

Oligo 1: CAT AGA ATC AAG GAG CAC ATG CTG AAA AAA

Oligo 2: CAT AGA ATC AAG GAG CAC ATG CTG AAA AAA

Oligo 3: TTT TTT CAG CAT GTG CTC CTT GAT TCT ATG

Oligo 4: ACT GAC TGA CTG ACT GAC TGA CTG GGC GGC GAC CT

The oligonucleotides were also modified by the company: oligo 1 was alkyl-thiolated at the 5’ end; oligo 2 was alkyl-aminated at the 5’ end; oligo 3 was tagged with fluorescein at the 5’ end; and oligo 4 was not modified.

All other chemicals were purchased from Sigma-Aldrich and used without additional purification.

Procedure Proposed:

To anchor a strand of DNA and conduct hybridization experiments, a modified protocol from Möller et al was proposed (7).

Chip Activation:

Silicon oxide wafers are cut into 10 x 10 mm chips and dry etched with oxygen plasma. The chips are activated by sonication in nitric acid, hydrogen peroxide, and de-ionized water for 10 minutes each. The chips are then dried for 5 min at 80° C and used immediately (7).

Surface Modification:

Silicon oxide chips are placed in a 1% 3'-aminopropyltrimethoxysilane (APTES) solution in 95% acetone/water for 15 minutes. They are then washed five times with acetone; each wash should last 5 minutes. The chips are dried for 45 min at 110° C, incubated for 2 hours in a 0.2% 1,4-phenylenediisothiocyanate (PDC) solution in 10% pyridine/dimethyl formamide, and washed with methanol and acetone. The chips may be stored in a vacuum dessicator containing anhydrous calcium chloride for a

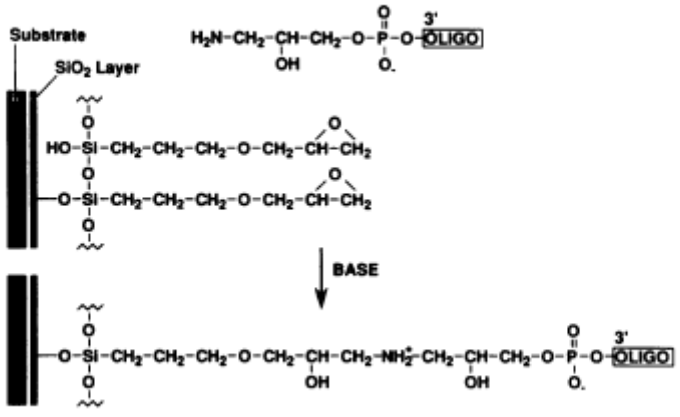

Figure 4: "Probe attachment to SiO2 surface. 3'-Amino modified probes were attached to a thin SiO2 film on the surface of a silicon chip. Attachment occurs by secondary amine formation between an epoxysilane monolayer and the 3' amino linkage" (8).

longer time period without discernible loss of activity (7).

Immobilization of DNA:

Oligo 2 is dissolved at a concentration of 2 mM in 100 mM sodium carbonate/ bicarbonate buffer (pH 9.0). Droplets of this solution are applied directly to the activated chip in the desired pattern. The chip is incubated at 37° C in a covered Petri dish containing small amount of water for 3-4 hours. It is then removed and washed once with 1% ammonium hydroxide (7) to reduce the amount of nonspecific binding of the oligonucleotides to the chip's silane layer (9). The chip is then washed thrice with water and dried at room temperature (7).

Hybridization:

Droplets of a solution containing 1 µM of Oligo 3 in a 5 X SSPE and 0.5% SDS solution are applied to the chip for fluorescent labeling. The chips are then incubated for 3 hours at 30° C in a closed Petri dish containing a small amount of water. After hybridization, the chips are washed twice with washing buffer—2X SSPE and 0.1% SDS. The chips are covered with a microscope cover glass for fluorescence microscopy.

Fluorescent Microscopy:

An FITC-3504B filter set from Semrock's Brightline ZERO series will be utilized in a custom-made fluorescence microscope that is comparable to the Olympus BX-51W series.

Discussion:

The basic procedure for binding a strand of DNA to a silicon oxide chip first involves modifying the substrate's surface with oxysilanes. This surface modification places the necessary functional groups for the covalent attachment of amino-modified DNA onto the chip's surface. The two principle oxysilanes investigated were APTES and 3'-glycidoxypropyltrimethoxysilane (GOPS). As demonstrated in Figure 5, GOPS's

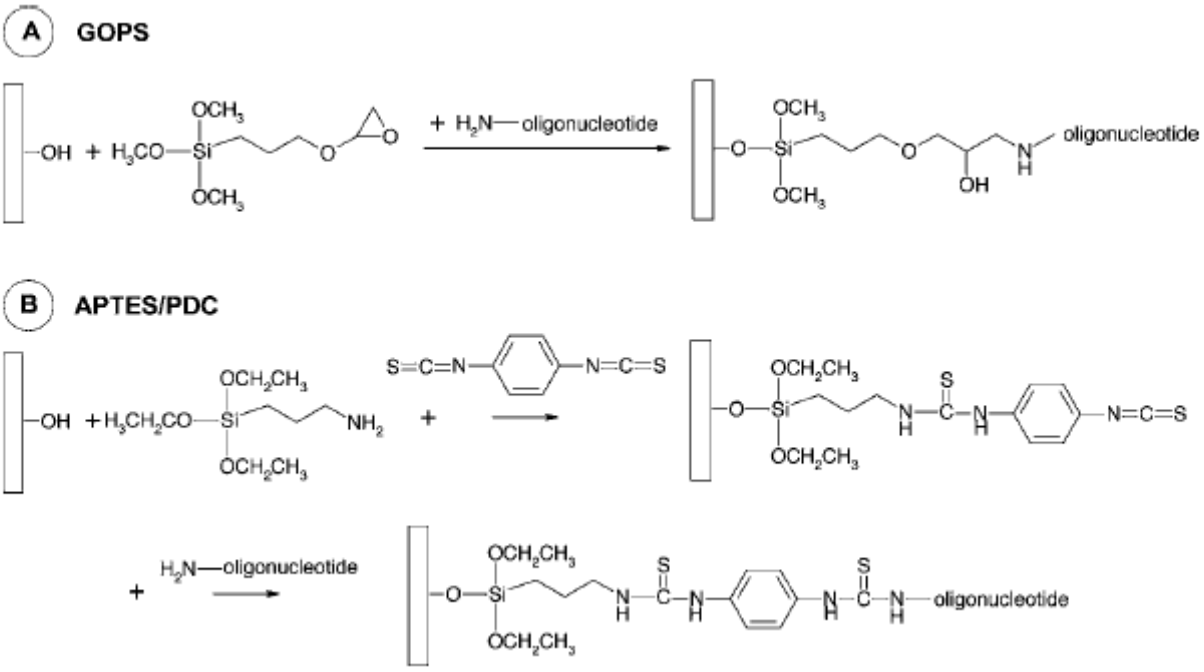

Figure 5: "Suface modification with silanes for DNA attachment. (A) Direct binding of amino-modified DNA by epoxy-groups of 3'-glycidoxypropyltrimethoxysilen (GOPS). (B) Amino attachment chemistry of 3'-aminopropyltriethoxysilane (APTES). The binding of amino-modified oligonucleotides works indirectly by the bifunctional crosslinker 1,4-phenylenedisiothiocyanate (PDC)" (9).

structure includes an epoxy group, which allows direct binding of GOPS to the amino-modified oligonucleotides. On the other hand, APTES's amino-silane structure requires a crosslinker molecule, such as PDC, to bind to the oligonucleotides (9). Even though it requires an additional step to insert the PDC cross linker, the APTES method was chosen to conduct the experiment. The APTES method utilizes aqueous solutions whereas the

GOPS method requires the exclusion of water throughout the experiment. In addition, the silanization of the GOPS method requires a much longer time to complete—4-6 hours for the GOPS silanization compared to the 2.25 hours for the APTES method: 15 minutes for APTES silanization and 2 hours for PDC crosslinker attachment.

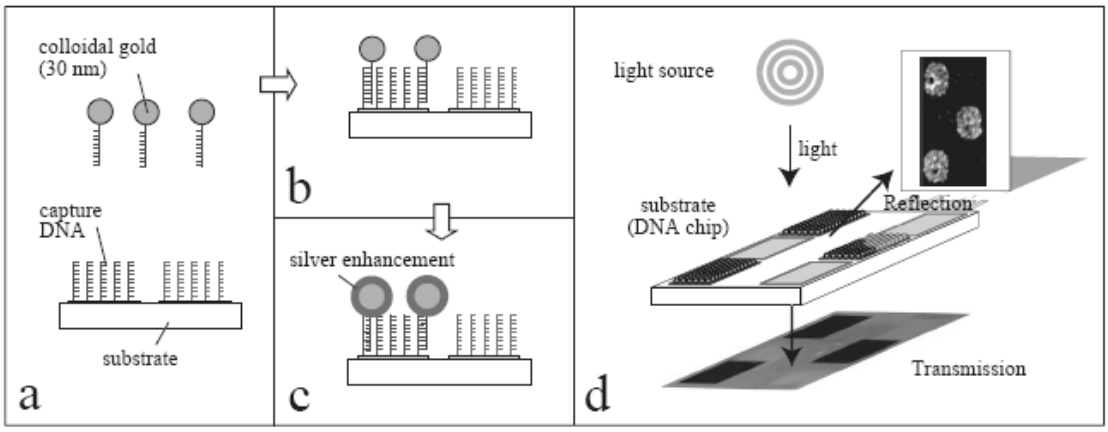

Figure 6: "Scheme of nanoparticle-based labeling for the detection of molecular interactions. a) Molecules of interest are labeled with colloidal gold particles. Arrays of capture molecules, which are complementary to the molecule of interest, are prepared on solid substrates. b) After incubation, the molecules of interest bind to the complementary capture molecules, resulting in surface-bound colloidal particles. c) These particles can be enhanced by selective growth of silver on the nanoparticles. d) A perspective scheme shows a substrate with immobilized nanoparticles. Optical detection in reflection or transmission model is applied for localization of the areas of nanoparticle binding" (11).

After surface silanization, the capture DNA is immobilized on the chip's surface. The oligonucleotide in question is then exposed to the capture DNA to allow the two to hybridize. Oligos 3 and 4 simply serve as fully complementary and non-complementary strands, respectively, to both Oligos 1 and 2. In addition to the various silanization methods, different methods for hybridization were analyzed. Oligo 2 was purchased to explore the validity of the alkyl-aminated DNA procedure described in this paper. Oligo 1 was ordered to explore the hybridization of the two DNA strands through binding of gold and the oligonucleotide's 5' thiol group (10). In addition to the procedures

described, this method also involves coating the surface of the chip with gold. Furthermore, instead of fluorescein, the DNA strand in question is tagged with gold particles that are 30 nm in diameter (10). Once hybridization occurs, the size of the gold particles is enhanced through silver coating. A typical microscope can be used to observe the optical signal produced by the silver particles that cover the chip's surface. Another method to analyze the chip's surface is by measuring the resistance across the silver nanoparticles on the chip's surface. Although the aforementioned two methods are reliable methods of analyzing the hybridized strands of DNA, we decided to use amino-modified Oligo 2 using the procedures described in this paper. The reasons for this decision are threefold: first, we do not have the facilities in the laboratory to be able to coat the chip with gold nanoparticles; second, the amino-modified method is more feasible with regards to both time and financial aspects of the two experiments; finally, the sole purpose of this experiment is to analyze the capacitance properties of DNA, not the resistive ones utilized in the thiol-gold method.

Despite the simplicity of the idea proposed, in reality, a complicated protocol is involved in binding a strand of DNA to a silicon chip as demonstrated by the one suggested in the paper. This complexity arises from the fact that the researcher must take into account the handling procedures and physical properties of both the DNA-based and the silicon-based systems (11).

The procedure proposed was attempted in a laboratory setting and was unsuccessful. This failure is due to the unavailability of the proper biological facilities to the electrical engineering department. For instance, since an oxygen plasma was not readily available, the chips were not properly dry etched, which in turn, effected the

remainder of the experiment. However, upon successful implementation of the proposed method, we plan to test Mr. Prakash's capacitance sensor with not only fully complementary and non-complementary strands of DNA, but also with oligonucleotides that include 5, 10, and 15 mismatches. We hope that the capacitance sensor will be sensitive enough to detect the mismatches and that the proposed label-free, electrical approach to DNA detection is a success.

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