Development of a Graphene-based DNA Hybridization Sensor

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Abstract

The basis of this project is predicated on the research of Dr. Saion Sinha who studied the interaction of Deoxyribonucleic Acid (DNA) on Carbon Nanotube (CNT) nanobiosensors for indications of DNA hybridization using his patented Biological Detection Device (BDD). The nanobiosensor uses electrical frequency resonance to determine DNA hybridization by comparing the resonant frequency of non-hybridized DNA to the resonant frequency of hybridized DNA. This project used the idea of differentiating the resonant frequencies of non-hybridized DNA from hybridized DNA by using graphene substrates as an improvement to CNT substrates to measure electrical impedance. The DNA analyte solutions were biologically optimized for hybridization in a laboratory to ensure that hybridization will occur on the nanobiosensor. The preliminary results indicate that the unique electrical interactions, due to the differences in charge of the DNA molecules, can discern the length of an unknown strand at its resonant frequency.

1. Introduction

The graphene-based nanobiosensor uses principles of physics and biology to detect the presence of DNA and Ribonucleic Acid (RNA). The purpose of developing this sensor is to produce a faster, cheaper and more reliable method for the detection of hybridization in the crucial area of pathogenic DNA/RNA detection. Current mainstream methods of hybridization detection performed in industrial biology labs include Southern Blotting which is laborious, time intensive, and costly. DNA nanobiosensors are a highly active field of research in biology as technology advances and allows for the production of cheaper, more effective equipment. This nanobiosensor utilizes the advancements made in materials science and biology to produce a simple sensor for nucleic acids.

1.1 Biological Principles of Interest

One of the main principles of biology used as the basis for this sensor was hybridization of DNA. Hybridization is a useful phenomenon for the characterization of DNA due to selective complementary base pairing. The strands hybridize due to the base pairing exhibited between the purines, Adenine (A) and Guanine (G), with the pyrimidines: Cytosine(C) and Thymine (T)/Uracil (U) (U is only found in RNA). A binds with T or U, and G binds with C. If a strand has a higher GC content than AT or AU it will exhibit a higher affinity than the opposite.

Another principle of biology used to validate results from the sensor in development was what is known as the hyperchromic effect. The hyperchromic effect is exhibited when spectral absorbance measurements are taken of nucleic acid solutions using a spectrophotometer. The hyperchromic effect is a 30–40 % increase in the amount of light absorbed for a single-stranded DNA (ssDNA) as compared to its double stranded DNA (dsDNA) pairing. The hyperchromic effect was used as a means of characterizing the nucleic acid solutions before depositing samples of these solutions on the graphene-based sensor.

1.2 Principles in Physics of Interest

The topics in physics pertain to electrical phenomena in particular for the purpose of understanding how to interpret the graphene-based sensor. The first principle of physics studied was electrical conductivity of materials. A material involved in the sensing of the DNA had to have very low electrical resistance and contribute little to no noise to a signal analyzer. Very small quantities of nucleic acid were deposited on the graphene-based sensor which meant that the sensing material must be extraordinarily sensitive to ionic disturbances.

Another principle of physics studied was the concept of alternating current (AC) circuit resonance, the same concept behind how the transistor radio works. AC resonance is mathematically quantified by calculating the impedance (i.e., the resistance quantity that accounts for imaginary reactance contributed by circuit elements) of an AC circuit. Resonance was used as a method for determining the presence of the DNA strand on the sensor. Refer to the equation below to find the relation for the resonant frequency in an AC circuit.

$$f_{res} = (2\pi\sqrt{LC})^{-1}$$

In the above equation f_{res} is the resonant frequency measured in Hertz, L is the inductance measured in Henries, and C is the capacitance measured in Farads.

A third principle of physics, used to formulate a hypothesis for the way in which DNA would be manipulated by our sensor, was the concept of treating atoms as point charges. The hypothesis states that the high amount of nega-

tively charged free electrons in the phosphodiester bonds linking the phosphate backbone of DNA together would be electronically attracted to a positively charged surface, or a surface with a positive DC offset applied to the sensor. Refer to Figure 1 to find the detailed molecular structure of a single stranded section of DNA. The hypothesis further implies that the positive charge of the nucleotides in a single strand of DNA would be repelled by the positive charge of the sensor's surface.

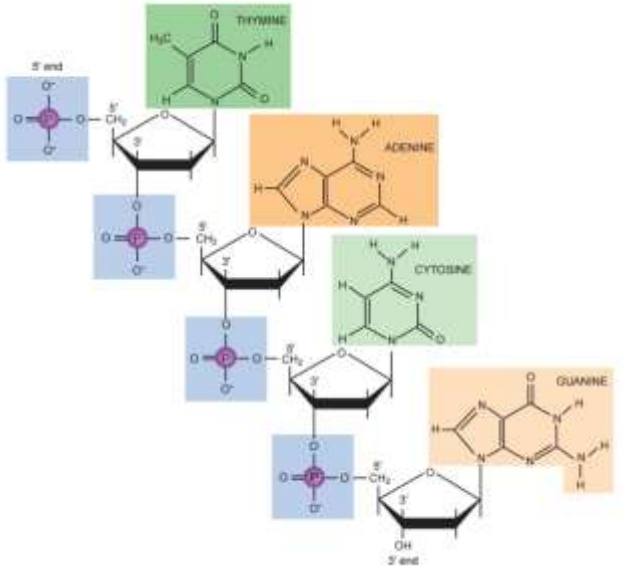


Figure 1: Molecular structure of DNA, note the negatively charged oxygen atoms in the phosphate groups (labelled blue) that form the backbone. The phosphodiester bonds are between the 3' and 5' carbons of the adjacent nucleotides. Credit for figure to Clark [1].

2. Sensor Design

The material choice for the sensor's main interface to the DNA was restricted by the design intent of the sensor. The sensor had to have an interface for the DNA that would contribute very low amounts of unwanted signal noise and offer very high electrical conductivity, while still being very cheap. The obvious choice for the material to serve as the interface was graphene, which was distributed to our research team by Graphenea Inc. in the form of a copper substrate with graphene deposited on one surface through Chemical Vapor Deposition (CVD). Graphene is a two-dimensional, zero-band gap semiconducting material comprised of carbon atoms configured in sp^2 atomic structure. This atomic structure is what makes graphene so electrically conductive because it has four planar carbon atoms and two electrons above and below these planar carbon atoms. An electrical current travels across the top or bottom surface of a graphene lattice with no appreciable resistance [2].

The graphene surface deposited on the copper substrate was housed in a rapid prototyped Acrylonitrile Butadiene Styrene (ABS) plastic piece designed and printed at UNH. There were many iterations of how to properly orientate the sensor to observe the best results with DNA deposited on the sensor, which is why rapid prototyping was chosen as the option to develop the sensor. The design of the sensor started as a flat surface, similar to a Field Effect Transistor (FET), and it ended as this original design due to manufacturing limitations. Other designs explored for the sensor included various forms of capacitors including parallel plate and cylindrical plate which were unsuccessful in detecting the presence of nucleic acid in solution.

The housing with the graphene substrate was then integrated into a circuit board provided by Palo Alto Networks, Inc. using lead based solder and thin gauge electrical wire. The wires were arranged in such a fashion as to allow the current to flow through the nucleic acid solution deposited on the substrate avoiding interference due to the aqueous solutions. The circuit board has gold contacts which offer very low resistance to the flow of current in the circuit which is in line with the design intent of the sensor.

The circuit as seen in Figure 2 has the nanobiosensor modeled as a resistor, capacitor, and inductor to explain the behavior of the DNA near its resonant frequency. The necessity of the capacitor and inductor is that both of these common circuit elements contribute what is known as reactance to an AC circuit. The Euclidean norm of the resistance, reactive capacitance, and reactive inductance is known as the impedance of the circuit element. Refer to the equation below to find the relation for impedance in its most elementary form.

$$Z = \sqrt{R^2 + (2\pi fL - (2\pi fC)^{-1})^2}$$

In the above equation Z is impedance measured in Ohms, R is the resistance measured in Ohms, f is the AC frequency measured in Hertz, L is the inductance measured in Henries, and C is the capacitance measured in Farads.

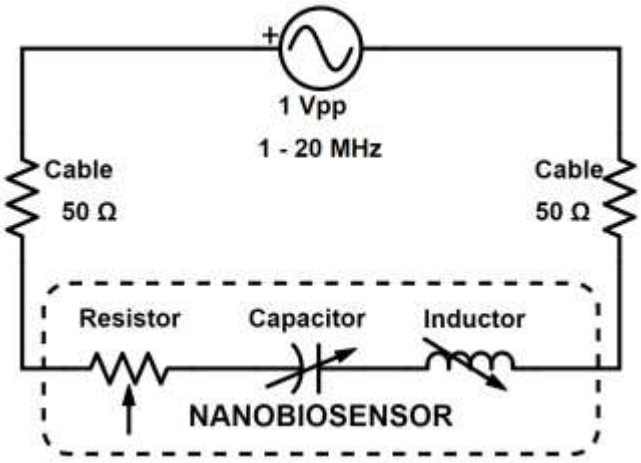


Figure 2: Experimental circuit of variable components including a resistor, capacitor, and inductor in place of the hybridization nanobiosensor. Note

that a positive 50 mV DC offset was applied to the nanobiosensor in addition to 1V peak to peak within an AC frequency domain of 1-20 MHz.

3. Experimental Procedures

The multidisciplinary research required to develop a DNA hybridization sensor is properly conducted when all of the science involved with the development is addressed sufficiently by researchers. There were three laboratories used in the development of this sensor; the biochemistry laboratory in Dodds Hall, the signal processing laboratory in Buckman Hall, and the rapid prototyping laboratory in Buckman Hall. Assistance from professors, graduate students, and technicians while in the laboratories was sought out if uncertainties arose in the procedures performed in each laboratory.

3.1 Biology Laboratory Experiments

During the biological experimentation a few important tests were developed. The standard procedure for preparing a solution to be tested on the sensor involved quantification and a hyperchromicity scan (hyperchromicity is discussed in section 4.1). Single stranded DNA (ssDNA) was ordered through Yale University's Keck Labs where custom molecules are synthesized that are typically used for primers in the Polymerase Chain Reaction (PCR). Stock solutions of each of the six single stranded DNA solutions were prepared by resuspending the ssDNA in cryogenic tubes ideal for freezing, thawing, and refreezing.

From the stock solutions, 100 nanogram per microliter test solutions were prepared by first calculating the concentrations using the information provided by Keck Labs to include the molecular weight of each molecule, and mass of each sample standard. The concentrations were then verified using UNH's EPOCH™ 2 Spectrophotometer. The spectrophotometer was ideal for the testing as it requires only 2 microliters of solution, and can accurately quantify DNA based on absorbance at specific wavelengths using algorithms [3]. The solutions were typically within ten percent error of the 100 ng/μL concentration goal. The error could be accounted for by the micropipettes used, which are often used by students as training tools and may not have been properly calibrated.

After quantification, the analyte solutions underwent a hyperchromicity scan utilizing the EPOCH™ 2. The purpose of the scan was to ensure that the solutions were behaving as expected. Three buffers were tested to find the ideal hybridization buffer for use in the research. The three buffers (a 5x Standard Sodium Citrate (SSC), a PerfectHyb™ buffer from Sigma-Aldrich and a 1x Phosphate-Buffered Saline (PBS) buffer) were tested against deionized water (dH2O). The 5x SSC and PerfectHyb™ buffers performed poorly due to the proteins used in the solutions which are helpful during Southern Blotting to prevent unwanted DNA interactions, but not during the spectrophotometry tests because they contaminate the DNA and effect the absorbance at 260 nm. Therefore, the PBS buffer was chosen as the main solution for the hybridization tests, which underwent further analysis to find the best PBS concentration. Out of 0.1x, 1x, 5x and 10x PBS solutions, the 10x PBS solutions showed a consistently better hyperchromicity curve when compared to the deionized water counterpart and was therefore used for the rest of the testing.

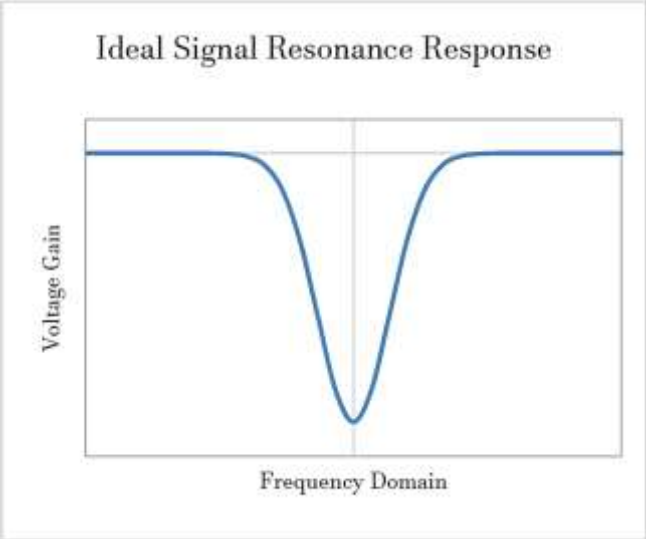
The prepared solutions for testing on the sensor were made with either deionized water or 10x PBS and contained an ssDNA molecule of either a forward or reverse strand in the 20, 40, or 60 length variants. Some of the solutions contained both the forward and reverse strands and were tested as hybridized samples. The forward strands were referred to as the "A" strand, and the reverse strands as the "B" strands, while the 20 nucleotide length strand was numbered "1", the 40 as "2", and the 60 as "3". Thus a deionized water solution containing 1-A would have a concentration of 100 ng/μL of the forward strand of the 20 nucleotide length in deionized water. The solutions each had two more microliters drawn and tested to scan for hyperchromicity. The EPOCH™ 2 was important here as well due to its ability to continuously test the samples from 200 nm to 300 nm. The scans also took place using various incubation temperatures, which was another prompt for using 10x PBS. Furthermore, the absorbance of the 10x PBS is essentially the same as deionized water over the wavelength spectrum of interest.

Many trials were attempted and many erroneous results were had while developing a successful protocol to hybridize laboratory synthesized ssDNA molecules during the course of this past summer. Even more issues were encountered when creating the protocol for the hyperchromicity scan. A big help in the testing was due to the EPOCH™ 2 and the Gen5 software which quickly exported experimental results into an Excel spreadsheet. Three strands of varying lengths, 20, 40 and 60 nucleotides and their respective complementary pairs, were ordered through Keck Laboratory at Yale University. The DNA was ordered as single strands, because using double stranded DNA would have required melting, which would not have been as pure of a sample as having the single strands synthesized. The first hurdle was finding the right buffer and temperature which turned out to be a 10x PBS buffer at room temperature for hybridizing DNA in the lab. Once the experimental results confirmed that the DNA was hybridizing under the conditions that were tested in the biology lab, the experimental testing on the sensor was ready to commence.

3.2 Signal Processing Laboratory Experiments

The procedures developed to be tested in the signal processing laboratory involved designing an experiment for each type of dsDNA and ssDNA, respectively. The experiment was developed to eliminate unwanted electrical noise, control cross contamination of samples, and to find

that the DNA became resonant at some AC frequency to indicate the presence of the DNA. The resonant frequencies of a particular strand were hypothesized to be unique to each strand, based upon the amount of nucleotides present and henceforth, the amount of electrons in each molecule. Each DNA solution was pipetted in samples of 10 µL onto the graphene substrate while it was in a circuit connected to an oscilloscope with a built in function generator (Agilent DSOX3014A). The experiment was designed with the intention of creating a mathematical model to predict the resonant frequency for any given amount of nucleotides. Three different lengths of dsDNA were tested, in addition to three different lengths of ssDNA, all of which were suspended in two different solutions (18 total combinations) to measure the voltage gain of the circuit in Figure 2. The resonant frequency of each of the strands was found when the voltage gain dropped substantially and rose again when



plotted against the frequency domain. Refer to Figure 3 to conceptualize the ideal measurement that was to be taken in the signal processing laboratory.

Figure 3: The idealized measurement for a circuit element resonating in the presence of an AC frequency creating a significant reduction in voltage gain as the element minimally impedes at resonance. The lowest point in this curve occurs at the resonant frequency as a drastic deviation from a standard response.

4. Results

This project resulted in five key findings, some of which are preliminary in nature for the purpose of further development of the sensor in the future when more time and funding is available. These findings were the product of careful considerations made when designing experiments. All findings were as expected and in line with common biological and physical theory.

4.1 Hyperchromicity

During experimentation in the biology laboratory, spectrophotometry was the main method of quantifying and organizing the solutions of nucleic acids. Hyperchromicity was tested by spectrally scanning two solutions of the same DNA sequence, one solution of deionized water and another of 10x PBS. The deionized water solution prohibits combined ssDNA from hybridizing at any temperature because of the lack of ions present in the solution. A high ionic content is necessary in a solution to force complementary single strands of DNA to hybridize at some temperature based on the GC base pair content of the sequence.

Using the spectrophotometer with this basic biological fact of DNA in mind, figures were created that show 30-40% increases in absorbance for complementary ssDNA pairs combined in deionized water. Figure 4 is an example of a plot that shows the hyperchromic effect for a sample of DNA with maximum absorbance occurring at 260 nm wavelength light.

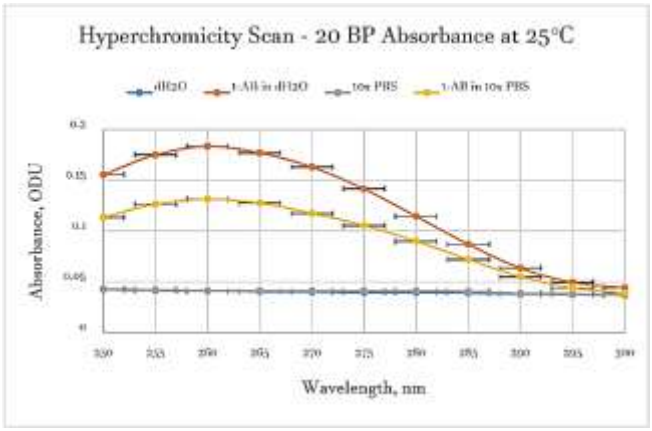


Figure 4: This plot shows a good example of the hyperchromic effect for a sample of DNA illuminated by light of 260 nm wavelength. Note the absorbance values at 260 nm for both the orange (ssDNA) and yellow (dsDNA) lines.

4.2 Hybridization Optimization

During the spectrophotometry experiments in the biology laboratory, it was paramount that the optimum conditions for hybridization be experimentally determined so that the conditions could be replicated when testing the DNA on the nanobiosensor to assure that hybridization is in fact occurring while being sensed with AC signals. It is known that DNA hybridizes at very specific temperatures, in specific solutions, and in a specific amount of time according to theory. The theory was experimentally confirmed in that the optimum hybridization temperature range exists between 20-65°C for the lengths and sequences of DNA used for our experiments. The optimum solution found for hybridization was 10x PBS as superior to 5x, 1x, 0.1x PBS and deionized water as a control. The optimum hybridization time as measured from the moment heating is applied at a fixed temperature was found to be in the range of 0-15 minutes.

4.3 Prototype Sensor Development

Two types of sensors were developed to control the cross contamination of the DNA samples during experiments in the signal processing laboratory. A sensor was made for each solution type, 10x PBS and deionized water. The sensors were designed to be reusable and the deionized water sensor showed the strongest signs of reusability.

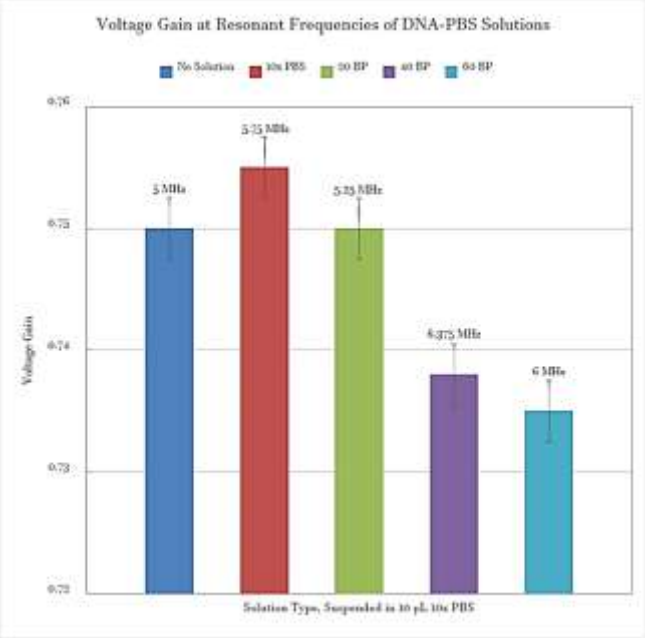


Figure 5: This histogram shows that for an increase in the length of a DNA sequence, there is a corresponding decrease in the voltage gain (i.e. an increase in the impedance). This collection of data is for 10x PBS solutions of varying lengths of DNA and buffer solution at resonance determined by the frequency (above error bars) measured at the lowest voltage gain value.

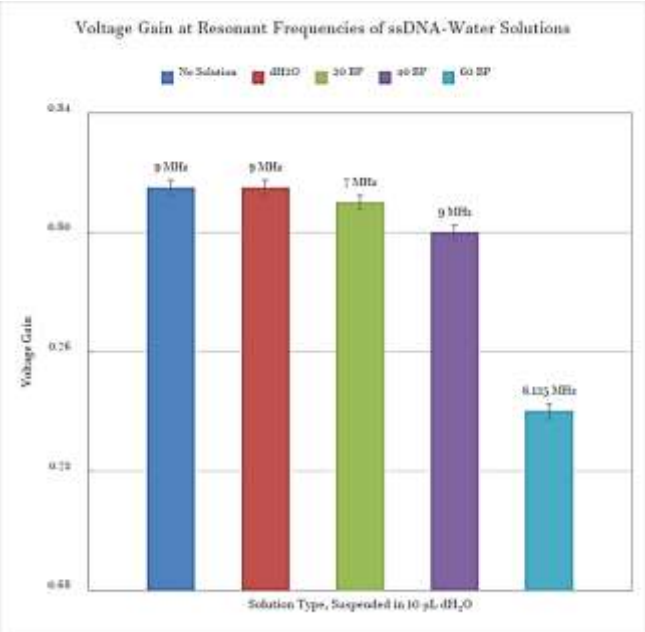


Figure 6: The trend evident in this histogram is the same as the trend observed in Figure 5. This collection of data is for deionized water solutions of varying lengths of DNA and buffer solution at resonance determined by the frequency measured at the lowest voltage gain value.

The sensors were able to measure the different lengths of DNA at the resonant frequency of each strand. The ability of the sensor was not deterred by the change in the solution type. Refer to Figures 5 and 6 to find that the nanobiosensors developed can identify a change in the length of the DNA sequence, for either a 10x PBS or deionized water solution.

4.4 Signal Analysis of Sensor

The quantification of nucleic acids was performed to calculate the approximate amount of free electrons present in each solution. This data was used in addition to the resonant frequency findings to produce Figure 7.

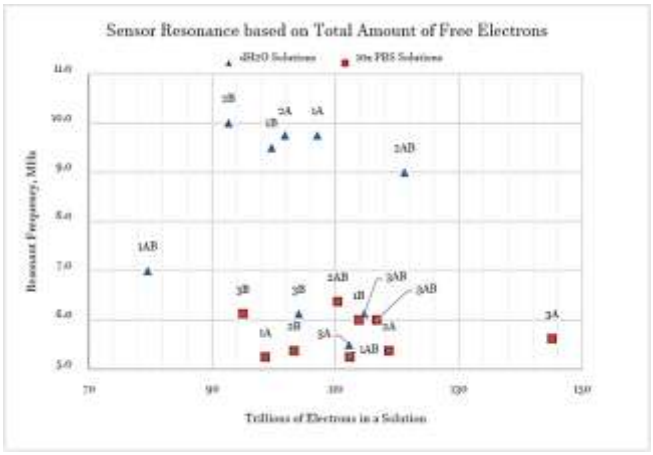


Figure 7: This plot shows that for a given amount of electrons in a molecule there is a corresponding resonant frequency. This ideal trend would be experimentally determined if a red and blue point of the same label are in close proximity to each other (e.g. 3AB). Note that it was not conclusive if the resonant frequency of a DNA sequence is dependent on the amount of electrons in that sequence.

4.5 Nucleotide Resonance Modeling

The most important result that was to be found was perhaps a predictive model to find at what frequency a particular amount of nucleotides in a sequence would become resonant. The model produced is not accurate or experimentally proven and only offered as a possible model generated from

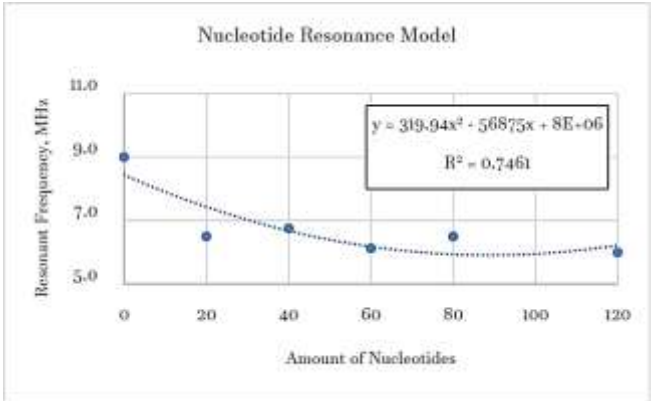


Figure 8: This plot shows a trend that was observed by averaging all voltage gain measurements at resonant frequency for each DNA sequence at our disposal. This model is not accurate enough for prediction as the aver-

age deviation of the experimental data points are too far from the mathematically approximate curve.

measured data. The model must be refined and tested for accurate predictions in the future when more time and funding is available. Refer to Figure 8 to find the plot that was used to determine the predictive model. The importance of this model is that it would be the first of many intellectual challenges conceptualized and experimentally proven that would provide credence for a next-generation DNA sequencer.

5. Discussion

The applications of a DNA Hybridization sensor are far reaching. The ability to rapidly detect the presence of biological molecules in a cost effective and reusable method could be applied at home, in the doctor's office, and even at a crime scene. In a lecture by Dr. Glynn from the Forensic Science department at University of New Haven, she stated that oftentimes fluids from a body are mixed together and that it is hard to discern the origins of samples. However, a promising approach for identification of bodily fluids can be done by analyzing the micro RNAs (miRNA) found in the sample. Currently, a forensic scientist tests a sample in the field using a preliminary test before sending it to the lab for a confirmatory test. A forensic scientist equipped with a hybridization sensor with pre-affixed miRNA probes associated with common tissues of origin could eliminate the need for a preliminary and regard the sensor as an accurate confirmatory test reducing backlogs in the labs, which ties into savings for tax payers.

Another aspect of the hybridization sensor would be to capitalize upon the ever increasing research on miRNA and cancer. Similar to the application above, a person at home would be able to theoretically purchase a kit that had common miRNA probes associated with cancers and deposit a sample of treated saliva onto the sensor. If the sensor detects an elevated presence of a known miRNA that is associated with cancer then that person would be able to go to the doctor and seek screening tests. The sensor would be an effective tool in pre-cancer screening and may be able to help prevent cancer before it becomes symptomatic. Furthermore, the same approach could be used at the doctor's office as a confirmatory test for the presence of pathogenic bacterial and viral DNA/RNA. This would help the medical community more effectively diagnose and prescribe the right medications to fight off diseases and infections.

6. Conclusion

The project accomplished the goals that were originally set out to be completed and therefore was a success. A prototype of the sensor was successfully assembled after many trials of erroneous design types including a parallel plate capacitance based sensor and a cylindrical plate capacitance based sensor. This sensor exhibits advantages and similarities to a carbon nanotube based DNA hybridization sensor including more reusability for testing without critical degradation of the sensor and the ability to detect the presence of nucleic acids of varying lengths. A fully developed electrically based DNA hybridization sensor will have profound effects on diagnostic testing for cancerous miRNA, viral and pathogenic DNA, and genomics analysis.

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Biography

Anthony R. Mastromarino III is a junior majoring in Mechanical Engineering and minoring in Physics at UNH. His interests are in applying quantum mechanics to mechanical engineering to create new technology that harnesses the strangeness of the quantum world. He has been written as an understudy to a publication in the American Physical Society for research in computational fluid dynamics.

Dylan Landry is a current B.S. Chemical Engineering/B.S. Biology conc. Biochemistry student at the University of New Haven. He aspires to be a Ph.D. /P.E. in Chemical and Biomolecular Engineering and hopes to one day be a leader in the field. His interest in Biology and Chemistry started while taking General Chemistry courses instructed by a Microbiologist Dr. Beverly Smith-Keiling at the University of Maryland University College - Europe at Kapaun Air Station, Kaiserslautern, Germany.