Development of a Graphene-based DNA Hybridization Sensor

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ABSTRACT

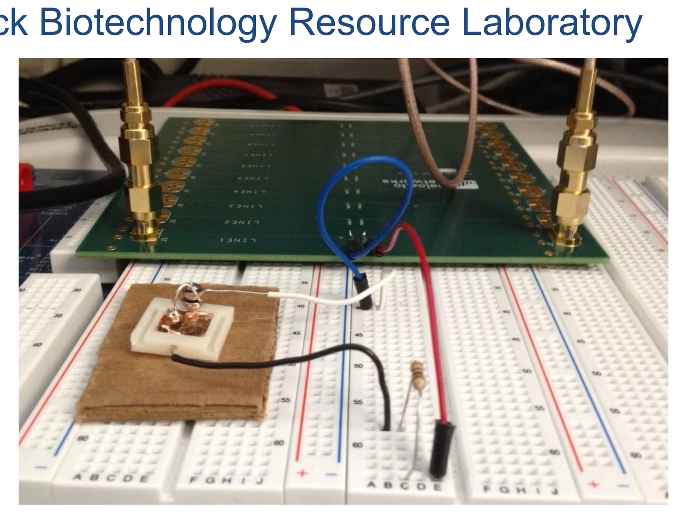
- Hyperchromicity used as basis of hybridization assurance
- DNA assumed to be non-hybridized in water and hybridized in 10x PBS solutions
- 100 ng/μL concentration solutions with hybridized and non-hybridized strands of 20, 40, and 60 nucleotides
- 10 μL volume used on sensor for a testing concentration magnitude of roughly 100 picoMoles
- DNA detected on sensor by monitoring voltage gain
  - Passive hybridization electrical signals were detected in the differing values of voltage gain
  - Active hybridization electrical signals were not detected
- Statistical correlation found between the voltage gain at resonance and the base length of the DNA strand in solution
  - Stronger correlation for oligonucleotides in stock 10x PBS solutions than in deionized water solutions

INTRODUCTION

- Hybridization is a useful phenomena for characterization of deoxyribonucleic acid due to complementary base pairing
- A fast, reliable method for detection of hybridization is crucial for pathogenic DNA/RNA detection
- Current mainstream methods such as Southern Blotting are laborious and time intensive
- Sensor is designed on the fact that DNA has a negatively charged phosphate backbone, which is linked by phosphodiester bonds allowing for a free electron/group
- Electrical conductivity of graphene is 200 times greater than copper and is uniquely adept for detecting minute resistance
  - DNA adheres to graphene's topology better than copper

METHOD & MATERIALS

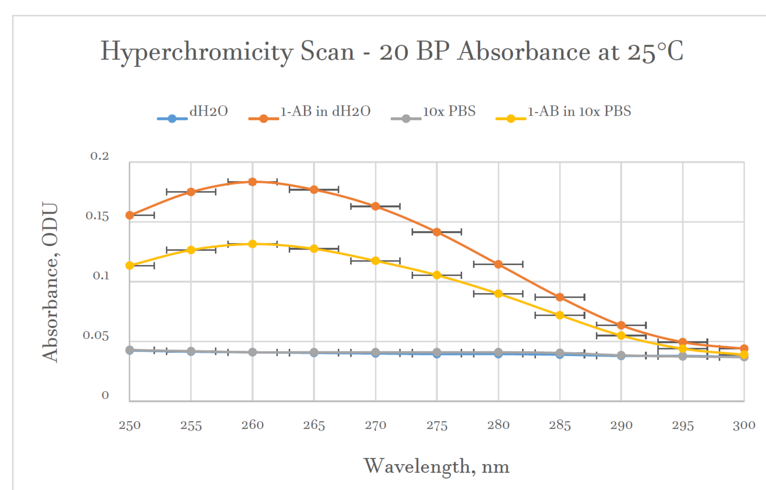
- Method:
  - Model a circuit component as a resistor, inductor, and capacitor (RLC Circuit) for the purpose of measuring the electrical resonance of a solution placed in the component.
- Materials:
  - Graphene deposited on a copper substrate
  - 3D printed ABS plastic housing
  - Palo Alto Networks circuit board
  - Various cables, wires, and solder for circuit prototyping
  - Oligonucleotides synthesized at Yale University, W.M. Keck Biotechnology Resource Laboratory

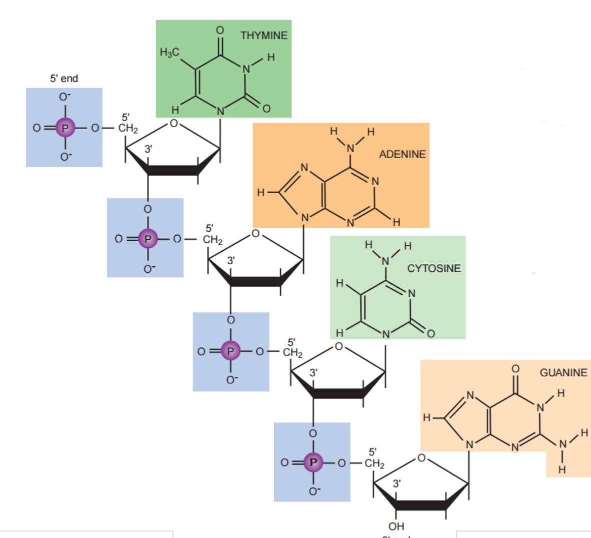


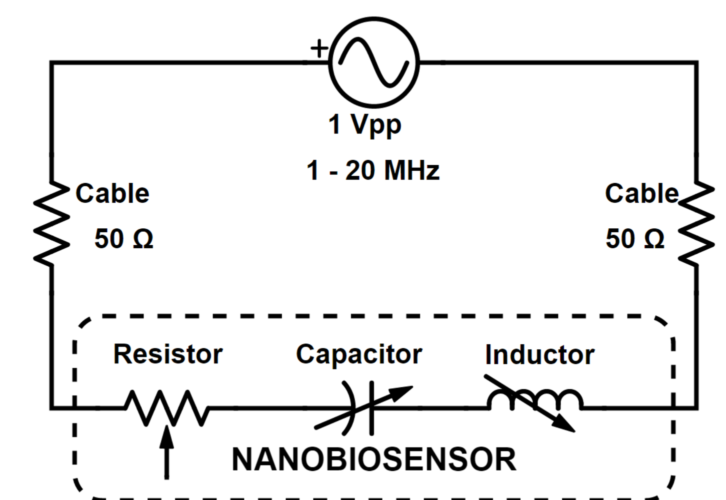
RESULTS

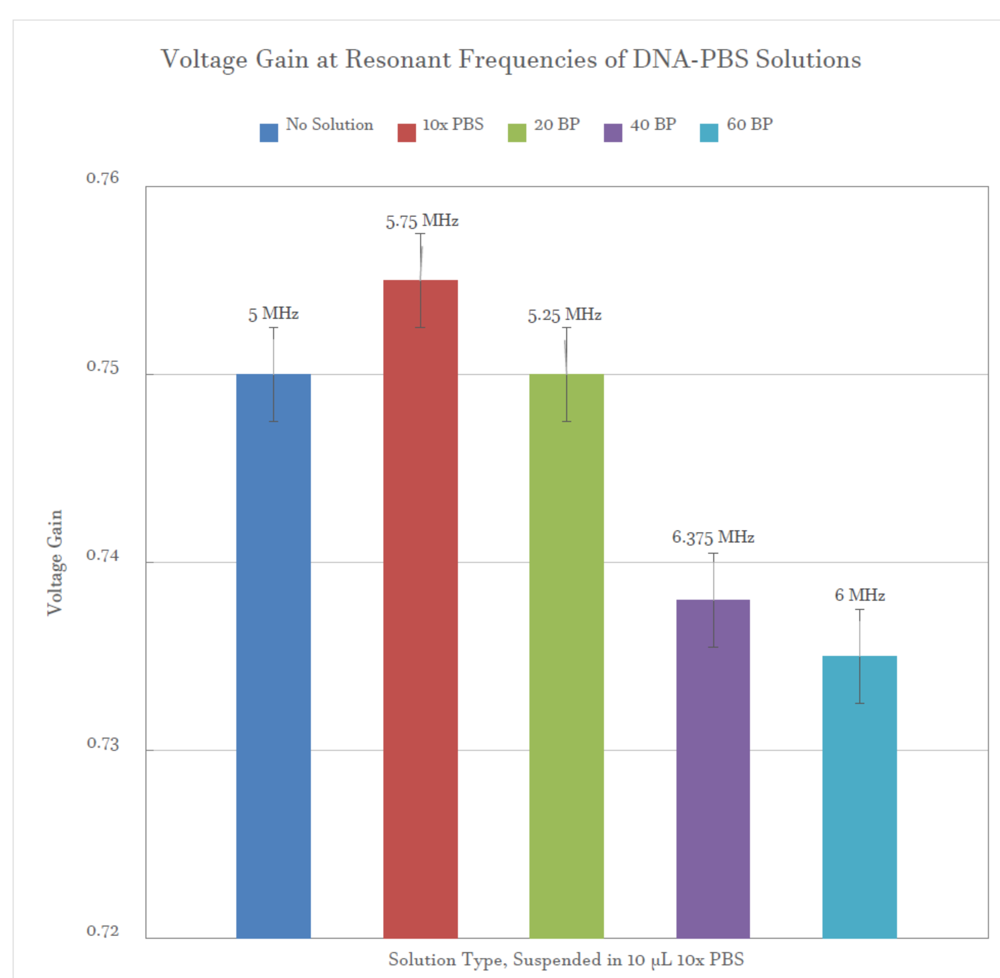
- DNA hybridization tested with spectrophotometer to find the hyperchromic effect
  - decrease in absorbance magnitude by 30-40% from hybridized to non-hybridized
  - DNA not measured to hybridize using a spectrophotometer in deionized water, PerfectHyb™ Plus Hybridization Buffer, and SSC based buffer
    - most successful hybridization buffer was PBS
- Conditions necessary for hybridization were found:
  - 20°C < Hybridization Temperature < 65°C
  - 10x PBS Concentration
  - 0 Minutes < Hybridization Time < 15 Minutes

- Two prototype sensors were developed; salt solutions only and water solutions only
- Prototype is reusable, following a strict protocol established to remove all traces of DNA from the graphene surface
  - sensor for only water solutions showed strong signs of reusability
- Prototype nanobiosensor developed to measure effect of DNA added to an electrical circuit
  - Voltage gain measurements taken in an AC frequency domain of 1 - 20 MHz
- Voltage gain found to indicate electrical resonance when a minimum gain value is measured in a domain of AC frequency
- Data supports the hypothesis that a model may be created to predict the length of DNA based on electrical resistance

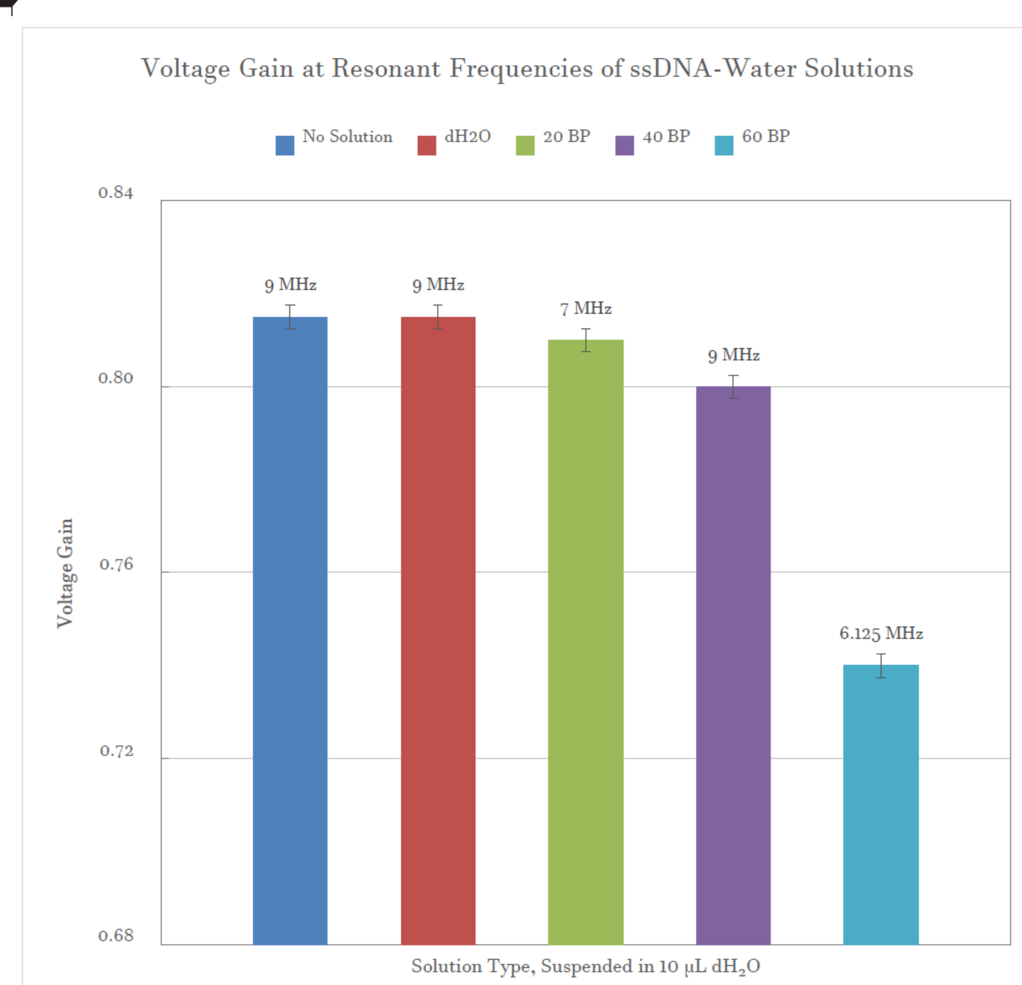








Increase in DNA strand length corresponds to a varying increase in electrical impedance for PBS solutions.



Same voltage gain trend as PBS solutions. DNA resonant frequency still undetermined to have any correlation to length of DNA strands, further studying will ensue.

DISCUSSION

- The results obtained still have much analysis to undergo but there are definitely hidden relationships in the data yet to be uncovered
- Hyperchromicity results are necessary because the absorbance values obtained in the laboratory controlled setting can be used to confirm that the DNA in solution used on the sensor hybridized by comparing absorbance
- There is not enough data as of yet to mathematically model the relationship between DNA strand length and voltage gain
- Further analysis must also be conducted to determine the key components involved with the resonant frequencies of the DNA solutions
  - Mathematical modeling may provide a new way to sequence DNA

CONCLUSIONS

- 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:
  - a parallel-plate capacitance based sensor
  - a cylindrical-plate capacitance based sensor
- Prototype sensor's advantages over a Carbon-Nanotube sensor
  - Used for numerous tests without critical degradation
- A fully developed electrically based DNA Hybridization sensor will have profound effects on diagnostic testing for cancerous miRNAs, viral and pathogenic DNA, and genomics analysis

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