NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2025 Project Summary

1. Student name & home university: Megan Caserio, University of Notre Dame

2. ND faculty name & department:
Professor Kaiyu Fu, Department of Chemistry & Biochemistry; Professor William Phillip, Department of Chemical and Biomolecular Engineering

3. Summer project title: Biocompatible membrane coated nanoelectrodes for long-term measurements

4. Briefly describe new skills you acquired during your summer research:
I gained many practical laboratory skills this summer as well as how to effectively communicate my research findings and goals. By the end of the summer I was running every step of my experiments independently: functionalizing a methylene blue redox reporter to the aptamer, using spectrometry to quantify the concentration of functionalized aptamer, immobilizing the aptamer and spincoating membranes onto the electrodes, and taking target measurements and analyzing the data. I also presented my findings in lab group meetings and learned how group members help each other determine potential solutions to problems they encounter in their experiments.

5. Briefly share a practical application/end use of your research:
The ultimate goal of my research is the real-time and long-term detection of cadmium and BPA in both freshwater and biological conditions by electrochemical aptamer biosensors (EABs). The data taken with the EABs will be used to track water pollution and prevent harm to ecosystems and human health.

6. 50- to 75-word abstract of your project:
Aptamers are highly versatile molecules with strong sensitivity and specificity, making them ideal for biosensor applications. In electrochemical aptamer biosensors (EABs), aptamers undergo structural changes upon target binding, producing detectable electrochemical signals. This study employs a cadmium-binding aptamer (CBA) and a bisphenol A-binding aptamer (BBA) within an EAB sensing platform. To enhance sensor performance, copolymer membranes are incorporated to both amplify the electrochemical response and protect the aptamer probes from biofoulants and chemical interferents. The integration of these membranes will improve target detection, offering a promising strategy for developing robust, selective, and reusable biosensors for environmental monitoring.

7. References for papers, posters, or presentations of your research:
(1) Lotfi Zadeh Zhad, H. R.; Rodríguez Torres, Y. M.; Lai, R. Y. A Reagentless and Reusable Electrochemical Aptamer-Based Sensor for Rapid Detection of Cd(II). *Journal of Electroanalytical Chemistry* **2017**, *803*, 89–94. https://doi.org/10.1016/j.jelechem.2017.09.022.

(2) Liu, L.; Yu, H.; Zhao, Q. The Characterization of Binding between Aptamer and Bisphenol A and Developing Electrochemical Aptasensors for Bisphenol A with Rationally Engineered Aptamers. *Biosensors (Basel)* **2022**, *12* (11), 913. https://doi.org/10.3390/bios12110913.

One-page project summary that describes problem, project goal and your activities / results:

Aptamers have emerged as a promising basis for biosensors because of their versatility, high sensitivity, and strong specificity in detecting targets. These molecules can be incorporated into electrochemical aptamer biosensors (EABs) that function via target induced binding events which result in structural changes that can be detected electrochemically.

The study I have been working on utilizes an established cadmium binding aptamer (CBA) and bisphenol A binding aptamer (BBA) in an EAB sensing suite and couples it with copolymer membranes to increase target response and prevent foulants and interferents from reaching the aptamer probe. The primary focus of my work this summer has been determining the necessary solution conditions for electrochemical sensing of the environmentally hazardous targets, with the goal of functionality and robust sensor performance in diverse freshwater and biological conditions.

Cadmium Binding Aptamer

I spent most of my time working on the solution conditions necessary for the cadmium binding aptamer. In the paper in which the CBA was developed it was only functional in a 10 mM acetate buffer supplemented with 10mM sodium perchlorate (pH 4.5). These conditions were not practical for real-world applications. Therefore, I began systematically removing solution conditions to find what was strictly necessary for ideal performance.

I first removed the sodium perchlorate. This resulted in such an excessively high and inconsistent signal change that it was likely not due to actual target binding.

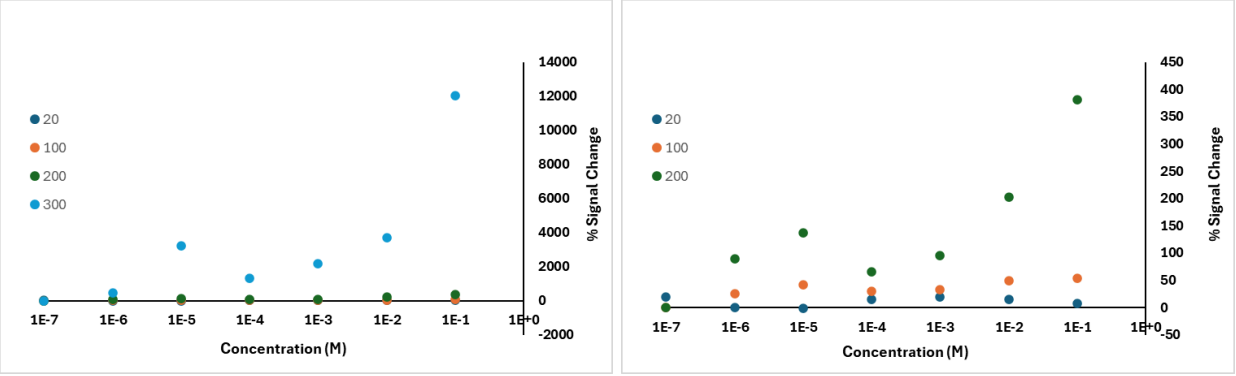


Figure 1. Cadmium Target in 10 mM Acetate Buffer

I then replaced the 10 mM sodium perchlorate with 10 mM sodium chloride. This resulted in a slightly more typical signal change. I then increased the concentration of sodium chloride to 100 mM, achieving a decent but still less than ideal binding curve. From these results and research into the difference between sodium perchlorate and sodium chloride I determined that sodium perchlorate is necessary to destabilize the unbound conformation of the aptamer to promote target binding. While both sodium perchlorate and sodium chloride are denaturants, sodium perchlorate is more effective because it has stronger ion-nucleotide interactions and is chaotropic while sodium chloride is kosmotropic.

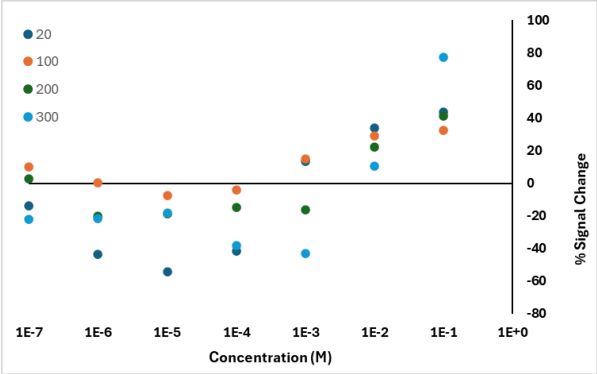


Figure 2. Cadmium Target in 10 mM Acetate Buffer with 100 mM Sodium Chloride

Having determined that the sodium perchlorate was necessary I then removed the 10 mM acetate buffer solution condition and performed a target measurement in nuclease free water with 10 mM sodium perchlorate. This resulted in an ideal binding curve, establishing that the acetate buffer and low pH were not integral to ideal sensor function. Signal change was detected for concentrations as low as 1 nM, which is very promising because the U.S. EPA limits cadmium in drinking water at approximately 44 nM.

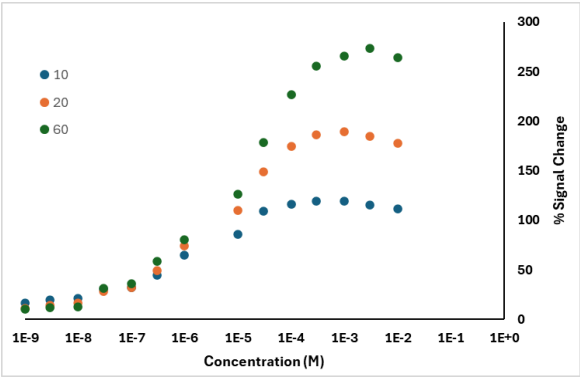


Figure 3. Cadmium Target in Nuclease Free Water with 10 mM Sodium Perchlorate

I then began working on sensor functionality in freshwater solution conditions. I utilized a study conducted by the USDA on the mineral composition of tap water to create a freshwater solution in deionized water containing sodium, potassium, calcium, magnesium, carbonate, and sulfate ions with an ionic strength of 13.7 mM. I again added 10 mM sodium perchlorate.

This resulted in a curve with very little signal change from concentrations of cadmium 1 nM to 300 nM, a sharp decrease from 1 uM to 300 uM, and then a small increase from 1 mM to 10 mM. I determined that this was due to cadmium ions forming an insoluble complex with the carbonate in the solution. At low concentrations of cadmium this results in signal decrease consistent with sensor degradation since there is essentially no cadmium ions in the solution. As the concentration of cadmium increases the solid cadmium carbonate becomes so abundant that it likely gets into the nanopores of the gold electrode, physically blocking the signal transmission from the aptamer, causing the sharp decrease in signal. At 1 mM of cadmium, all the carbonate ions are complexed with cadmium and magnesium. Therefore some cadmium ions are free in solution and can bind to the aptamer resulting in the minor increase in signal. This is stoichiometrically verified given the concentrations of carbonate, magnesium, and cadmium in the solution.

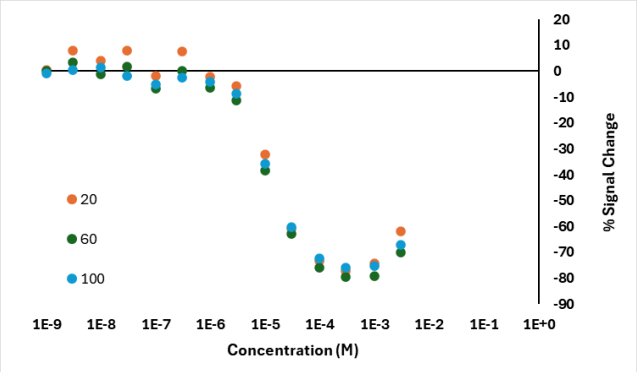


Figure 4. Cadmium Target in Freshwater Ionic Solution with 10 mM Sodium Perchlorate

To solve this problem I decided to pretreat the freshwater ionic solution with another positive ion that creates a more insoluble complex with carbonate than cadmium does. The only two compounds with significantly lower Ksp values than cadmium carbonate are lead carbonate and mercury carbonate. I therefore decided to use lead. I added 1.5x as many moles of lead as moles of carbonate were in the solution and let it sit overnight. A large amount of lead carbonate visually precipitated out of the solution. The target measurement resulted in a somewhat decent binding curve. However, unfortunately the excess lead formed lead sulfate, which is readily reduced within the potential range we take our cadmium target measurements at. This caused lead to be plated onto the electrode each time we took a measurement, which is why the signal begins to degrade at higher cadmium concentrations.

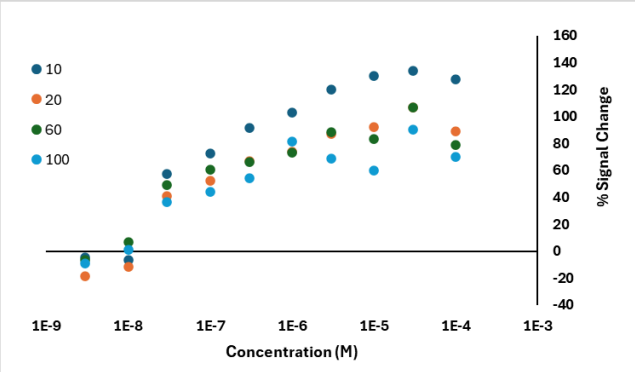


Figure 5. Cadmium Target in Freshwater Ionic Solution with 10 mM Sodium Perchlorate and Lead

Since precipitating the carbonate out did not work, I plan to try adding a small amount of acid to the freshwater ionic solution to lower the pH and prevent cadmium from forming insoluble cadmium carbonate. Additionally, I was unable to achieve a binding curve in phosphate buffered saline to model biological conditions because cadmium also forms an insoluble complex with phosphate. I will therefore run a target measurement in a different solution that also models the pH, ionic strength, and osmolarity of bodily fluids.

Once functionality in freshwater and biological conditions has been confirmed I will begin to integrate the CBA with copolymer membranes to increase target response and prevent foulants and interferents from reaching the probe. I will spincoat a hydroxyethyl methacrylate (HEMA) membrane, which is selectively permeable for small molecules, directly onto the nanoporous gold electrode surface. Next I will functionalize sulfonate groups within the membrane matrix, enabling preferential interaction with cadmium ions. I will ultimately grow a layer of zwitterionic polymers atop the HEMA membrane to resist adsorption of biological macromolecules and prevent biofouling of the EAB.

Bisphenol A Binding Aptamer

The bisphenol A binding aptamer was less difficult to functionalize in various solution conditions. An ideal binding curve was achieved in phosphate buffered saline. It was necessary to add 10% DMSO to fully dissolve the bisphenol A. Unfortunately while the BBA detects the initial addition of bisphenol A at 10 nM, the signal change then remains constant until about 100 uM. Therefore concentrations of bisphenol A between 10 nM and 100 uM are indecipherable from each other.

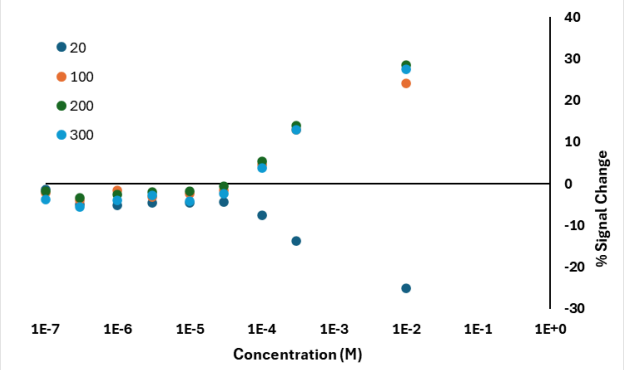


Figure 6. BPA Target in Phosphate Buffered Saline with 10% DMSO

In an attempt to increase sensor sensitivity at low target concentrations I immobilized lower concentrations (0.25 uM and 0.5 uM instead of the normal 1 uM) of aptamer onto the gold nanoporous electrode. By having more space between the aptamers, I theorized structural change would occur more easily and lower concentrations of target could be detected. Unfortunately this resulted in negligibly better signal change at lower concentrations and a messier binding curve. I therefore determined that this would not be a practical solution to improve target detection at low concentrations. I will continue to research potential solutions, but this may simply be a limit of the BBA. I will also begin to integrate the BBA with a copolymer membrane that is selective for bisphenol A.