TRANSLATING UNIVERSITY BIOSENSOR RESEARCH TO A HIGH SCHOOL LABORATORY EXPERIENCE

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It is well known that there is a lack of diversity in STEM fields. Between 1999 and 2004, women accounted for only 20% of the national enrollment of undergraduate engineering students.[1] In Fall 2013, there were 26% female students at Michigan Tech,[2] with a 31% female enrollment in chemical engineering and >40% in biomedical engineering. There is a large dispute as to what is causing this low diversity, and just as much debate as to how to bridge the gap. It has been shown that many high school students are not aware of the diversity of engineering fields and how these fields fit into their interests.[3] And exposure to STEM prior to college is a key indicator of students pursuing a STEM degree in college.[4] Once women are drawn to chemical engineering, they persist through to graduation at a greater extent than men.[5] Therefore, it is important to disseminate the message to high school students about the impact and diversity of engineering fields and encourage women and other underrepresented groups to enroll into engineering programs.

A strong method to encourage female participation in engineering programs has been to demonstrate the societal benefit of the field,[6] with female students being drawn to the medical field while male students are drawn to engineering and STEM fields.[7] This may be a reason that chemical and biomedical engineering enroll more female students than most other engineering programs. The cutting edge research programs that have been developed in university laboratories can be one method of attracting not only female, but other underrepresented minority students, to engineering.

The presentation of university-level research to high school students offers a unique opportunity to introduce chemical engineering to currently underrepresented populations in a way that makes an explicit connection between chemical engineering and having a societal impact on areas of health and the environment.[8] Due to safety and cost constraints, it is often difficult to develop meaningful laboratories that can be conducted by high school students that are safe, portable, and can be conducted in a small allotted timeframe, although examples can be found.[9,10] Outreach programs in chemical and biomedical engineering, similar to the one described here, appear to contribute to increased enrollment of students. After the University of Utah created an outreach program in the field of bioengineering, there was a significant increase in the number of female students enrolling in the program.[11] The use of modeling and simulation software can also be used to create virtual laboratories that can provide a more realistic experience for high school students.[12] Finally, the use of social media and online platforms can be used to reach a wider audience and provide information about the opportunities available in chemical engineering.[13]

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Dylan Turpeinen is an undergraduate student in chemical engineering. Mr. Turpeinen is a coach in the Michigan Tech Math Learning Center and a captain of the Men’s Club Soccer Team.

Julia A. King is a professor in the Department of Chemical Engineering. Her research focuses on the creation of carbon composites for thermal and electrical conduction applications. Prior to working at Michigan Tech, Dr. King worked at Exxon USA and DuPont/Conoco for 10 years.

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College of Engineering to demonstrate different types of engineering to high school students, the chemical engineering enrollment increased by >50% over 4 years.\footnote{11}

Our lab has been engaged in biosensor research for several years. We have determined that graphene paper composite materials that were developed for thermal conduction can also be used to detect proteins and be applied as a biosensor.\footnote{12} We then created a laboratory that could quickly and inexpensively be presented to high school students to demonstrate not only engineering research at the university level, but also technology development in the area of biosensors. We presented this biosensor laboratory to two distinct groups of high school students. One group was in an Advanced Placement (AP) Biology course, and this lab was incorporated into a module on technology development. The second group was students who came to a week-long summer camp at Michigan Tech to better understand chemical engineering. Both groups were junior and senior high school students. The objectives of each interaction were (1) to present chemical engineering as a method to study a discipline that has societal impact, (2) to demonstrate technology development at the university level, and (3) to show a connection between math and science classes already taken or soon to be taken by the students and future societal impact that can be found by applying STEM concepts. The laboratory consisted of inquiry-based research that was developed by the College Board for AP laboratories.\footnote{13} Inquiry-based research involves beginning laboratories that have defined procedures and outcomes and then proceeds to have the student explore and test hypothesis on her own in subsequent laboratories. The methodology is less interested in results and more interested in the scientific method. Our lab was student guided and “structured,” as defined by the College Board. We gave the students instructions on how to use our biosensors and then gave them an unknown protein to try to determine what protein they had by using our biosensor and compiling the class results.

The overall goal for this laboratory project is not to teach students the microscopic science of biosensors, but rather to expose them to new and exciting technology that has the potential to improve day-to-day life for millions of individuals. We also tried to highlight that chemical engineering is geared towards societal benefit and gave the students additional information on STEM careers. We also wanted to demonstrate the research that must be conducted to create a marketable biosensor, with examples being a pregnancy test or a glucose sensor. Many students do not have experience with technology development and this proof-of-concept biosensor demonstrates the first steps to commercialization of a novel sensing platform and also demonstrates the amount of research that must go into a marketable product.

**BACKGROUND ON BIOSENSOR RESEARCH**

Point-of-care (POC) medical devices that can help a doctor diagnose a patient before the patient leaves the office are currently in large demand.\footnote{14} The most common POC device is the glucose meter, but others have recently come to market that can diagnose bacterial infections and heart disease with minimal time to diagnosis.\footnote{14} Typical POC devices are colorimetric and give you a yes or no answer, like a pregnancy test, but you can also get concentration data, as in the electrochemical glucose sensor. We are developing a next generation biosensor that could be functionalized to sense biomarkers to stress, cancer, malaria, or a viral disease. Our platform is the electrochemical resistance changes that occur in graphene when in the presence of biomolecules.\footnote{12,15,16} Graphene is a two-dimensional material that has the same bonding pattern as a carbon nanotube, but in a flat sheet.\footnote{17} It has a high electrical and thermal conductivity.\footnote{17} While many researchers are exploring pure graphene sensors,\footnote{18,19} we deviate from this approach by using graphene paper composites.

Graphene has a high conductivity, whereas we discovered that intermediate conductivity functions better for protein sensing.\footnote{12} Thus, the composite structure of graphene and a non-conductive polymer creates a sensor with intermediate conductivity. Our sensors are made up of a 1:1 graphene/cellulose composite by weight and one sensor is shown in Figure 1A. The cellulose swells when in contact with water and this decreases the conductivity as compared to the dry state. As depicted in Figures 1B-C, when protein is present on the graphene surface, some of the electrons flowing through the paper can be trapped within the protein and the conductivity

\begin{figure}
\centering
\includegraphics[width=\textwidth]{biosensor.png}
\caption{A two-probe liquid sensor. (A) The two-probe sensor tested by the students. (B) Electrons flow through the graphene paper and when proteins are present (C) some electrons get trapped by the protein and reduce the electrons that flow through the paper, therefore reducing the conductivity.}
\end{figure}
of the paper is further reduced. The change in conductivity is less sensitive to protein concentration when the graphene concentration is either increased or decreased from the tested 50 wt% graphene composite. We hypothesize that this occurs because a higher background conductivity (i.e., higher graphene content) is less affected by small protein content than a lower background conductivity. However, once you lower the background conductivity too much (i.e., lower the graphene content) then there are not enough graphene contacts to sustain a circuit. We have demonstrated that our sensor is sensitive to protein size, and this is likely due to the protein size being related to the surface area of graphene covered by the protein and the amount of electrons that can be removed from the circuit. We fit the change in resistance versus protein concentration to the Langmuir isotherm to determine the equilibrium dissociation constant ($K_d$). The lower the $K_d$, the more sensitive the sensor is for protein detection. The theory on intermediate conductivity was recently affirmed by another group using a different graphene composite.\[12\]

The original biosensor development work was performed on a Keithley multimeter with a 2-probe device. To make the work more portable, we tested the ability of the sensor to distinguish proteins with a handheld multimeter. The $K_d$ values were grouped closer together with the handheld multimeter as compared to the Keithley, but they were distinguishable enough to carry the project forward to allow high school students to work with the sensors.

**PARTICIPANTS AND METHODS**

Our team reached out to two groups of high school students to demonstrate the societal benefit of chemical engineering. We have engaged participants in Michigan Tech’s Summer Youth Programs (SYP) and an AP Biology class at a local high school. Recent Michigan Tech SYP evaluations showed that 69% of participants were from groups traditionally underrepresented in STEM fields and 49% were female. Hands-on activities were rated as good or excellent by 93% of participants. Longitudinal data shows that of first-year students enrolled at Michigan Tech, 11% were SYP alumni, of that group 95% enrolled in STEM disciplines.\[20\] The AP Biology course was conducted at Calumet High School, which has a total enrollment of 390 students. It is an economically depressed area, where 61% of the students receive free/reduced lunches. All surveys were conducted with Michigan Tech IRB (Internal Review Board) approval including parental permission slips since most students were under 18 years of age.

Our team developed an AP Biology guided-inquiry experiment (see Appendix) that had the students explore protein detection methods and unique properties of graphene. The AP lab in the Appendix has all of the detailed supplies and step-by-step instructions needed to complete this lab. All of the supplies are commercially available except for the graphene paper used. This is a proprietary product from XG Science.

The workflow for the AP biology and SYP students can be seen in Figure 2. Each day in the figure was a 50-minute class period for the AP biology students and a 90-minute class period for the SYP students. Each group started with the pre-test. They were then given the AP Laboratory that can be found in the Appendix. The AP students were given a short introduction to the laboratory by their biology teacher. This introduction focused on protein detection methods and the importance of these methods in current medicine, since this was a biology class. So that the SYP students could be given comparable information, the introduction by the biosensor researchers also focused on current protein detection methods and how they could be improved. Less emphasis was given on the engineering of the device itself, which will be more of the focus in future presentations of this lab.

The AP students were given the lab and spent several days exploring the websites given in the experimental write-up. The SYP students were given 20 minutes where each group explored one website, followed by a student-led discussion.

![Figure 2. Workflow of the different student groups. There were several days between Day 1 and Day 2 for the AP Biology Students, whereas the days were contiguous for the SYP students.](image)
RESULTS AND DISCUSSION

The students began the lab with a pre-test to understand their knowledge of protein detection, sensors, and biology. As shown in Table 1, the general knowledge of the two groups of students was similar, with the AP Biology students scoring slightly better on the biology question, as would be expected, and the SYP students scoring better on the question about protein detection methods. After the survey, the students were presented with the AP Lab in the Appendix. The students were asked to look at several websites on their own covering the topics of mass spectroscopy, electrophoresis, and enzyme-linked immunosorent assays (ELISAs) for protein detection. They were also given the website to XG Sciences, the company that provided the graphene paper composites that we tested. It is shown in Figure 2 that the AP Biology students looked at the websites on their own and had a class discussion of the methods. The SYP students were given 20 minutes to look at the websites, with different groups being assigned to different websites, and then they gave a short oral introduction to the method that they studied. The AP Biology lab manual encourages the students to use outside resources and websites to self-study prior to beginning a lab.

On a different day than the original presentation (for both groups), an undergraduate student and a professor demonstrated the use of the sensor and then helped the students actually determine the change in resistance as they added increasing amounts of protein. The students input their resistance measurements into a pre-programed spreadsheet and the spreadsheet calculated the dissociation constant ($K_d$). It was briefly explained to the students that $K_d$ was a measurement of the sensitivity of the sensor (see Figure 3) and students

\[ P \cdot L \leftrightarrow P + L \]

\[ K_d = \frac{[P][L]}{[P \cdot L]} \]

Figure 3. Definition of $K_d$. $K_d$ was described as the chemical equilibrium and the lower the $K_d$, the more sensitive is the biosensor. We use it as a method to compare different papers and device configurations in our research.

<table>
<thead>
<tr>
<th>General Subject</th>
<th>Pre-Lab Grade</th>
<th>Post-Lab Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP Biology</td>
<td>SYP</td>
</tr>
<tr>
<td>General Biosensor Knowledge</td>
<td>55.6%*</td>
<td>60.5%*</td>
</tr>
<tr>
<td>Protein Detection</td>
<td>11.1%+</td>
<td>42.9%+</td>
</tr>
<tr>
<td>Biology</td>
<td>88.9%+</td>
<td>76.2%+</td>
</tr>
<tr>
<td>Laboratory Knowledge</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

* 1 question # 2 questions
* 3 questions na – not applicable

Different questions were asked in the pre- and post surveys, and some subjects were not tested.
were shown the data that had been compiled in our lab using the multimeter (see Figure 4) that demonstrated that the $K_d$ was related to the size of the protein. Our previous data demonstrates that the $K_d$ is related to the size of the protein. Additional information was given in the laboratory handout (see Appendix). We didn’t feel it was necessary to explain the details of the Langmuir isotherm to students at this level. The undergraduate then compiled all of the students’ data that the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given. In all cases, the variance of the data from the students needed to make an assessment of which protein they were given.

![Figure 4](image)

**Figure 4.** Graphene composite biosensor results. The source meter data is taken from Reference 12, the multimeter data was conducted in our lab by an undergraduate student and the student data is from the high school students. The source meter shows the greatest difference in $K_d$ for the different proteins. The multimeter, which is less accurate and sensitive than the source meter, showed a compressed difference in $K_d$. The student data had the lowest variability in $K_d$, but the students were still able to identify their proteins from the differences they found (i.e., LYS had the highest $K_d$ and FIB had the lowest $K_d$). Legend – LYS, lysozyme, MW 14 kDa; BSA, bovine serum albumin, MW 66 kDa; FIB, fibrinogen, MW 340 kDa.

**TABLE 2**

<table>
<thead>
<tr>
<th>Qualitative Questions</th>
<th>AP Biology</th>
<th>SYP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I can now accurately give an overview of how at least two different protein detection methods work.</td>
<td>4.00</td>
<td>3.62</td>
</tr>
<tr>
<td>This lab provided a better understanding for biosensors and their real world applications.</td>
<td>4.33</td>
<td>4.19</td>
</tr>
<tr>
<td>The lab was technically challenging but easy to understand what was being done and why.</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>I am more interested in biosensors following this lab.</td>
<td>3.56</td>
<td>3.61</td>
</tr>
<tr>
<td>Overall, this was a great lab experience.</td>
<td>4.33</td>
<td>4.28</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

Our team has taken biosensor research out of a university lab and created a biosensor lab for high school students. We were able to reduce the cost in a safe and effective manner in order to demonstrate the research side of technology development to high school students. We demonstrated this lab to two different groups of students, AP Biology students from a local high school and SYP students who came to Michigan Tech to learn about chemical engineering. Both groups demonstrated that they enjoyed the lab and learned about protein detection. As educators, we need to explore more methods to demonstrate to high school students the societal benefit of biomedical and chemical engineering. This appears to be a fruitful method to increase the enrollment of women and underrepresented minorities in engineering. Only with a diverse engineering workforce can we continue to compete in a global economy and continue to develop novel solutions to societal problems. In the future, we are going to increase the discussion of engineering as a future career to encourage students to enroll in engineering and other STEM fields.

**ACKNOWLEDGMENTS**

We would like to thank XG Sciences for donating the graphene paper composites and Mrs. Jennifer Peters at Calumet High School for working with us to develop the AP Bio Lab and for allowing us to conduct the lab with her students. This work was funded by NSF (CBET-1159425) and the Biotechnology Research Center at Michigan Tech.
REFERENCES
14. NIH, 2010, Fact Sheet-Point of Care Diagnostic Testing

APPENDIX
The AP Bio lab and the Excel sheet for implementation can be found at: <heldtlab.mtu.edu/home/outreach>.