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

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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.[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.[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.[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.

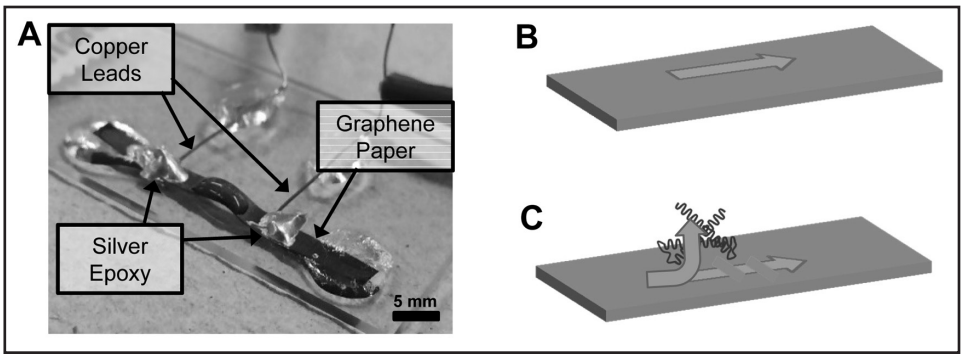

Figure 1. *A two-probe liquid sensor. (A) The two-probe sensor tested by the students. (B) Electrons flow through the graphene paper and when proteins are present (C) some electrons get trapped by the protein and reduce the electrons that flow through the paper, therefore reducing the conductivity.*

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.[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.[14] Typical POC devices are colorimeteric 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.[12,15,16] Graphene is a two-dimensional material that has the same bonding pattern as a carbon nanotube, but in a flat sheet.[17] It has a high electrical and thermal conductivity.[17] While many researchers are exploring pure graphene sensors,[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.[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

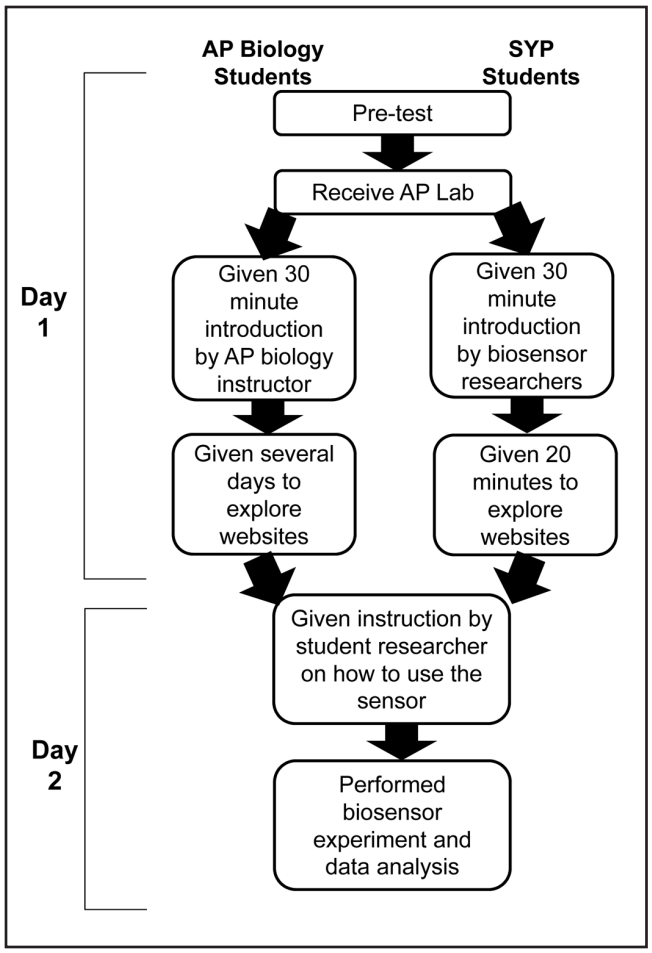

Figure 2. *Workflow of the different student groups. There were several days between Day 1 and Day 2 for the AP Biology Students, whereas the days were contiguous for the SYP students.*

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,[12] 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).[12] 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.[20]

The original biosensor development work was performed on a Keithley multimeter with a 2-probe device.[12] 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.[21] 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

on different protein detection methods and the properties of graphene that could be found on the given websites. Each team only explored one protein detection method, whereas the AP students were given the time to explore all of the websites. The students were then divided into groups of 2-3 students and an undergraduate student described and demonstrated the biosensor use. The protocol the students followed was to measure the resistance of the dry sensor, as given to them. Following the dry measurement, they took a dropper and dropped buffer onto the sensor and measured the resistance. This was followed by removal of the bubble with a Kimwipe. They then dropped the lowest concentration of protein and measured the resistance, followed by bubble removal. This continued until all concentrations were tested, starting with the lowest concentration and proceeding to the highest concentration. The data collected was fit to a Langmuir isotherm[12] and the equilibrium dissociation constants (K_d) were determined through pre-made Excel spreadsheets to assist in the calculations (see Appendix). The students were introduced to the K_d using the chemical definition, as shown in Figure 3, and can be found in the AP lab in the Appendix. From the definition of K_d, it can be seen that as K_d is reduced, more protein is found bound to the sensor. Therefore, a sensor with a lower K_d can detect more protein because the protein wants to stick to the sensor. It is difficult to describe equilibrium to this age group, but we define it as after the sensor sits in contact with the solution for a very long time.

Each team had multiple concentrations of the same protein and the data from each team was aggregated so they could determine which protein they were given. The proteins were chosen for their different sizes and isoelectric points. It was concluded in the original work that in the case of the graphene composite sensor, the K_d was actually a result of the size of the protein and did not reflect an increase in the sensitivity of the probe.[12] The original work used bovine serum albumin (BSA), hen egg white lysozyme (LYS), bovine hemoglobin (HEM), and bovine fibrinogen (FIB). HEM was not used with the students because of its red color, which would be too obvious for the students who were trying to determine an unknown protein. Pre-tests and post tests were used to assess new knowledge of the students.

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

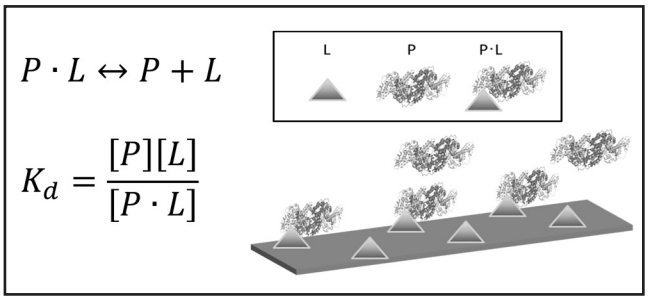

Figure 3. *Definition of K_d. K_d was described as the chemical equilibrium and the lower the K_d, the more sensitive is the biosensor. We use it as a method to compare different papers and device configurations in our research.*

TABLE 1
Knowledge-based survey results

General Subject	Pre-Lab Grade		Post-Lab Grade	
	AP Biology	SYP	AP Biology	SYP
General Biosensor Knowledge	55.6%*	60.5%*	88.3%#	97.6%#
Protein Detection	11.1%+	42.9%+	na	na
Biology	88.9%+	76.2%+	88.9%+	65.0%+
Laboratory Knowledge	na	na	94.4%#	95.2%#

+ 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 protein to protein was a lot lower for the student data than that developed in our lab (see Figure 4), but the trend was the same that the higher the molecular weight, the lower the K_d. This allowed the students to determine the trend of their protein's molecular weight and to identify it when we told them which proteins the group had been given. The students enjoyed the aspect of having to use everyone's data to determine the answer and not just relying on their own experimental data.

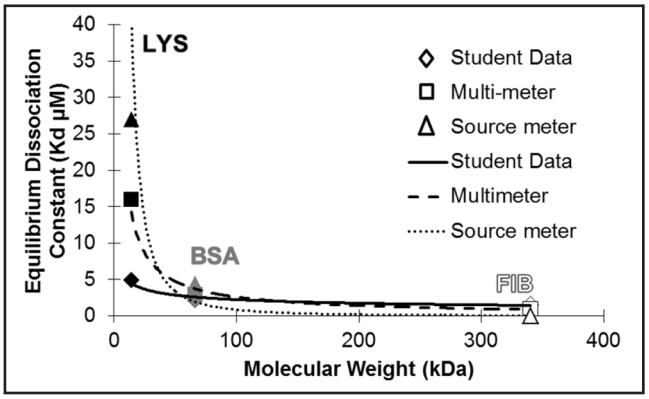

Figure 4. *Graphene composite biosensor results. The source meter data is taken from Reference 12, the multimeter data was conducted in our lab by an undergraduate student and the student data is from the high school students. The source meter shows the greatest difference in K_d for the different proteins. 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
Student impressions of the laboratory

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

After the lab was completed, the students were given a post survey. The results of the technical questions related to the lab can be found in Table 1. The biological knowledge of the students did not change, but they did learn more about biosensors and appeared to understand the technical aspects of the lab. They were also asked how they felt about the lab using a 5-point scale with 1 being strongly disagree and 5 being strongly agree. The students from the different groups felt similarly, except for the introduction to the different protein detection methods, as shown in Table 2. The AP Biology students had more time (several days) to explore all of the websites whereas the SYP students only had less than one hour to work on this part and each group only explored one website. The students enjoyed the lab and learned about biosensors. It does not appear that we encouraged a lot of students to study biosensors, but that was not a goal of the project.

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.

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APPENDIX

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