SUMMARY:
DEVELOPMENT OF A BIOSENSOR FOR MEASURING ODORANTS IN THE AMBIENT AIR NEAR SOLID WASTE MANAGEMENT FACILITIES

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David M. Binninger (Co-PI)[2]
Sharmily Rahman[3]

In 2017, the Bill Hinkley Center for Solid and Hazardous Waste Management funded FAU Lab.EES to develop a novel biosensor technology that has the potential to objectively and rapidly measure odor concentrations in real-time, transforming how nuisance odors are monitored and regulated. This is a follow up study to a project entitled, "*Investigation of effective odor control strategies*" that was completed in 2017 that in part studied ways to improve odor detection including development of a novel technology that uses human odorant binding proteins as a biosensor to quantify odors.

Nuisance odor levels produced by solid waste management operations such as landfill facilities are subject to regulatory standards because of their impacts on the quality of life of the residents living within close proximity to the facility. Failure to meet such standards may result in costly fines, litigation, inability to acquire permits, mitigation, and re-siting operations. Since measurement of environmental nuisance odors is currently limited to subjective techniques, monitoring odor levels to meet such standards is often problematic. This is becoming more acute as increasing residential populations begin to encroach on properties adjacent to landfills. Odors can cause relations between the facility and the surrounding population to deteriorate. In order to ensure that nuisance odor issues are minimized, it is necessary to provide an objective measurement. However, until now, we did not have any objective methods of monitoring or recording nuisance odors. Moreover, there are usually a number of odorants that interact with each other, further complicating quantification.

The objective of the current research is to develop a biosensor for providing an objective, standard measurement of odors. Our approach will modify hOBP2A, a human odorant binding protein, isolated using published biomolecular techniques by either fluorescently tagging it with a chromophore functional group or a monoclonal antibody. Then the biosensors will be exposed to selected model odorants (single and mixtures) to determine positive/negative spectrophotometric response and concentration dependence/Beer's Law quantitation for a specific set of odorants typically encountered at solid waste facilities.

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PROGRESS REPORT
(March 2019)

Project Title: DETECTION OF NUISANCE ODORS USING ODOR BINDING PROTEIN SENSOR
Principal Investigator: Daniel E. Meeroff, Ph.D.
Co-Principal Investigator: David M. Binninger, Ph.D.
Affiliation: FAU
Phone number: (561) 297-2658
Project website: http://labees.civil.fau.edu/leachate.html
Student: Sharmily Rahman, MSCV Candidate

Methodology/Scientific Approach

TASK 1. Conduct literature review. Sharmily Rahman continues to update the literature review focused on objective measurement techniques for detecting landfill odors with the main goals of: 1) identifying specific odor causing compounds in solid waste operations to create a database of odorant candidates for testing with several viable candidates found for testing; and 2) identifying literature that would improve the efficiency of the analysis technique proposed. A list of parameters has been identified that can improve said efficiency.

TASK 2. Prepare biosensor molecules. Dr. Binninger has supervised the production of the first large batch of purified hOBP2A with the assistance of Yasmeen Amanza Ampuero and Cynthia Raaijmakers (graduate students in his laboratory). The research team has cultured and induced *E. coli* containing the human odor binding protein gene. The protein was then isolated from the batch and a Bradford assay was conducted to determine the presence of the target protein in the sample. After obtaining a positive result from the Bradford assay, an SDS-PAGE electrophoresis was conducted to confirm the presence of the protein.

Initial Attempt to replicate previous research findings: To replicate previous findings conducted during Julia Roblyer's thesis, Yasmeen Amanza Ampuero and Cynthia Raaijmakers performed the following steps and grew the *Escherichia coli* in an overnight culture of luria broth.

- Three 1000 mL Erlenmeyer flasks containing 500 mL of luria broth in a shaking water bath at 37°C were used to grow the *E. coli* cultures to an optical density of 0.8
- 0.1 mM IPTG was added to the three flasks to induce protein expression and incubated for 1 hour in a shaking water bath at 37°C
- The cells were then centrifuged and resuspended in 2 mL of resuspension buffer (50 mM Tris-Cl pH 7.5, 150 mM NaCl)
- Resuspended cells were then stored at -80°C
- Cells were thawed, sonicated, and centrifuged – supernatant containing protein was aliquoted into separate tubes to be run on a 12.5% PAGE gel (Figure 1)

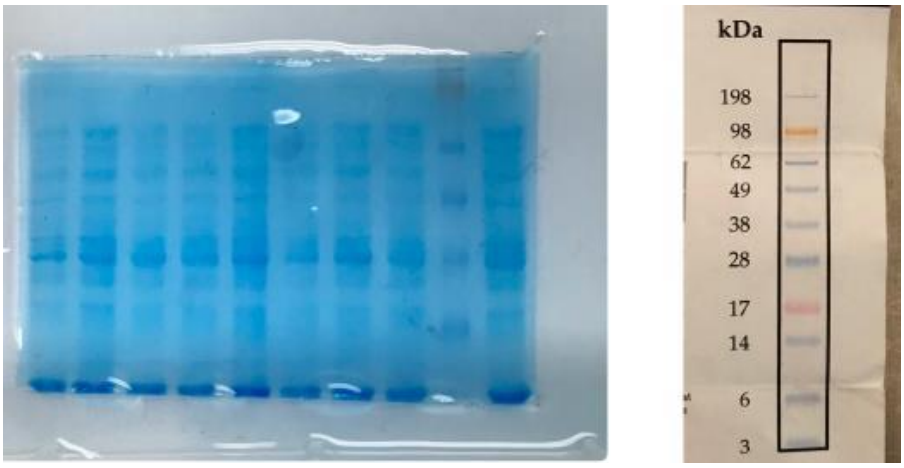
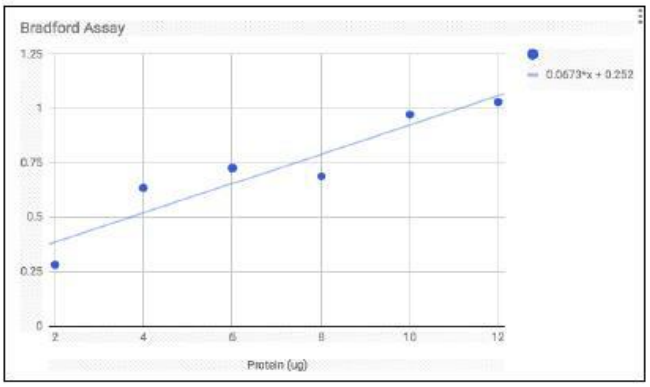


Figure 1: 12.5% PAGE gel run on October 3, 2018 (Lane 1 - 8 and Lane 10 contained induced samples, Lane 9 contained ladder)

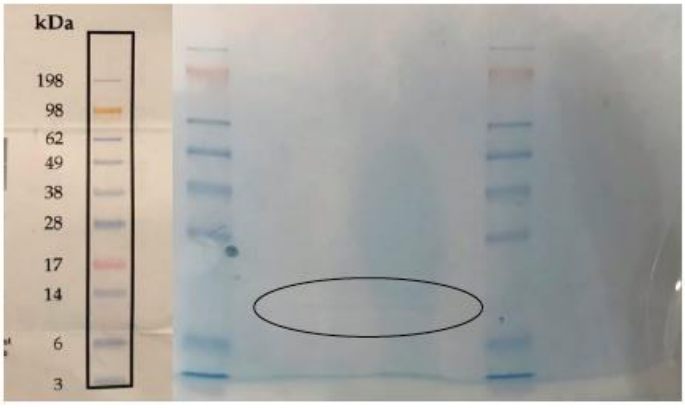
12.5% PAGE gel results: It was speculated that the intense protein band at the bottom of the gel was the protein of interest. Samples were then run through a His SpinTrap Column to purify the isolated protein. After that, a Bradford assay and 12.5% PAGE gel were performed using purified protein samples (Figure 2).



Protein Sample (12uL) measured O.D = 0.080

0.0673x + 0.252 = 0.080

x = 2.5ug/12uL or **0.2ug/uL**



We loaded the gel as follows:

Lane 1 - n/a
Lane 2 - Ladder
Lane 3 - n/a
Lane 4 - sample
Lane 5 - sample
Lane 6 - n/a
Lane 7 - Ladder
Lane 8-10 - n/a

We found two faint band at the circled locations where we loaded the samples.

Around 14kDa

Figure 2: Bradford Curve and results of second 12.5% PAGE gel containing purified protein sample

Bradford Assay and 12.5% PAGE gel results using purified protein: The Bradford Assay suggested an extremely low concentration of protein in the isolated samples, and the PAGE gel showed a faint band at the 14 kDa mark, which indicated a low protein concentration. This low protein concentration may be explained by:

- An issue in the initial induction of the *E. coli*, or
- An issue with the original culture, or
- An issue with the isolation from the His SpinTrap column

Troubleshooting and Attempt 2: Tube #1 containing *E. coli* that had been transformed to contain the gene for the human odorant binding protein was grown in an overnight culture containing kanamycin in a shaking water bath at 37°C, and the following steps were performed:

- The overnight culture was added to two 500 mL Erlenmeyer flasks containing 250 mL of luria broth, and the flasks were incubated in a shaking water bath at 37°C
- The cells were grown to an optical density of 0.4 – one culture was induced with 0.1 mM IPTG for 3 hours and the other culture remained uninduced as a control
- After the incubation period, the cultures were centrifiuged and then resuspended in resuspension buffer (50 mM Tris-Cl pH 7.5, 150 mM NaCl)
- The cultures were then stored at -80°C
- During the next session, the cells were sonicated and centrifuged to remove the pellet
- The crude proteins of the induced and uninduced samples were run on a PAGE gel to identify the protein of interest and compare the concentration of the protein of interest between the induced and uninduced samples (Figure 3)

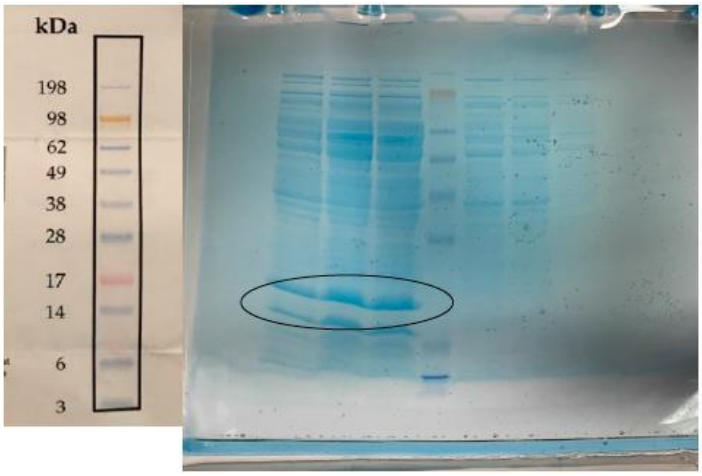
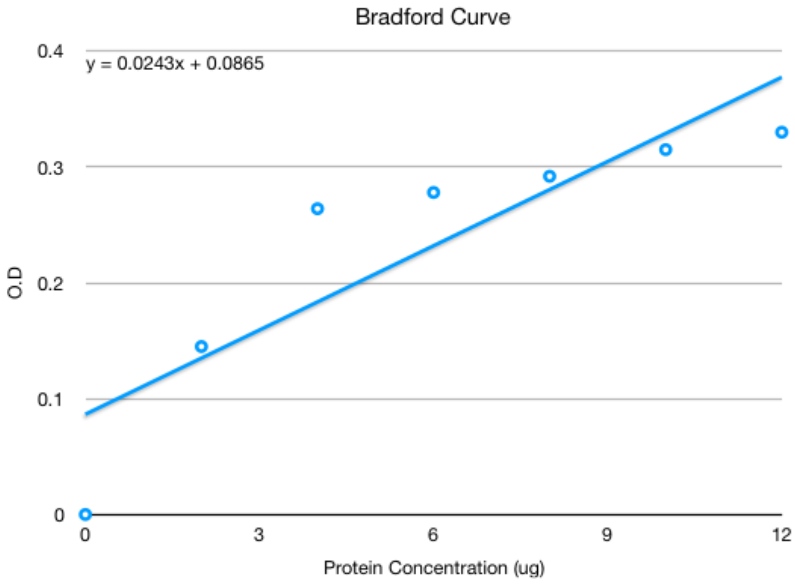


Figure 3: Results of 12.5% PAGE gel containing induced and uninduced protein samples

The proteins from 3 of the 5 samples of both the induced and uninduced were then isolated using a His SpinTrap column. After that, a Bradford Assay was performed, and the purified samples were run on a 12.5% PAGE gel (Figure 4).



Sample	O.D
Induced Sample (2uL)	0.278
Un-induced Sample	0

Induced Concentration = 0.278 O.D = 0.0243x + 0.0865
Induced Concentration = 7.88 ug/2ul or 3.94ug/ul

398ul sample total
398ul x 3.94ug/ul = **1,568ug or 1.568mg in the sample.**

Figure 4: Bradford curve showing a clear increase in protein concentration between induced and uninduced samples

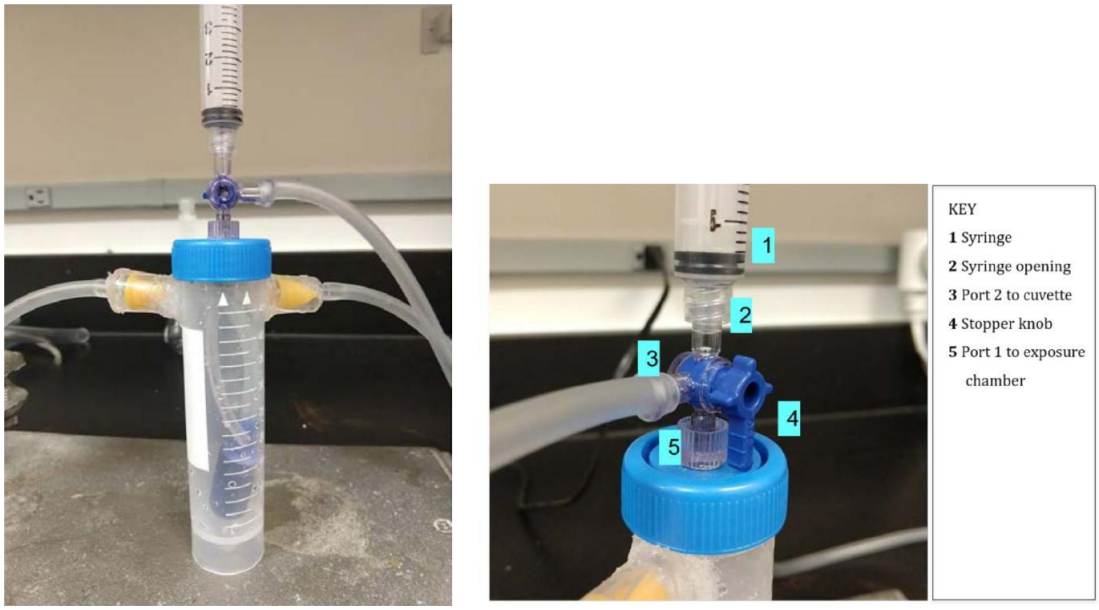
Finally, 1.568 mg of protein was isolated and purified in a sample of 398 µL and provided to S. Rahman in the last week of January for verification testing.

TASK 3. Develop an experimental exposure chamber. In December 2018, S. Rahman updated the prototype reactor, and the following important changes have been made:

- The previous setup consisted of a reactor chamber that had a relatively large base surface area profile. This meant that the exposed surface area allowed odorant molecules to rapidly escape the chamber. In the new setup (Figure 5), the reactor chamber has taken the form of a centrifuge tube. This has a two-fold benefit. The first is that the exposed surface area of the protein complex has been reduced to limit the amount of odorant escaping from the surface. The second benefit comes from the increased length of the centrifuge tube, which should

improve the likelihood of successful binding of the target odorant with the protein complex as it travels a greater distance to reach the surface. These changes in the design of the reactor chamber are expected to increase the efficiency of the process.

- In the previous setup, a pipette was used to collect samples from the exposure chamber for fluorometric tests. This created several issues. Collecting samples using a pipette by opening the cap of the sample bottle may have introduced external contaminants. In the new setup, a 3-way stopcock is attached to the cap of the centrifuge tube. One of the ports (shown as #2 in Figure 5b) is only opened for sample collection using a syringe, which can be screwed on using a Luer-lok mechanism to draw a sample of the solution from the exposure chamber. The other port (shown as #3 in Figure 5b) is used to direct the sample already in the syringe into the cuvette for subsequent fluorometry readings. This setup ensures that the inside of the reaction chamber is never exposed to the external environment, thus eliminating the introduction of any external contaminants. Also, the syringe never needs to be removed during the whole experiment which makes it very convenient. However even if we need to remove the syringe for any reason, the opening has a cap which ensures that it remains sealed.



(a) (b)

Figure 5: (a) Revised reactor chamber using a centrifuge tube **(b)** 3-way stopcock used at the mouth of the exposure chamber. The different ports and other parts are labeled.

The revised setup (Figure 6) was tested using an air pump to check if its functionality and air-tight seal are working as expected.

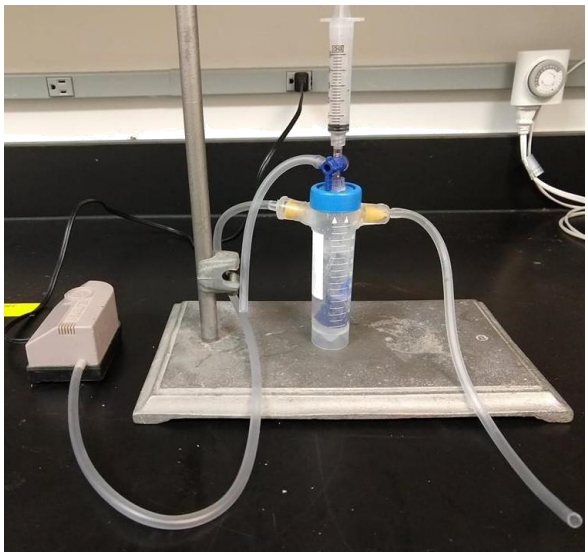


Figure 6: Revised reactor chamber being tested using an air pump. Air was successfully bubbled through a sample of water using a stone bubbler and exited through the valve at the top of the chamber.

S. Rahman has already completed her training for laboratory safety as well as training on the Horiba Jobin Yvon FluoroMax-4 spectrofluorometer (Figure 7) and FluorEssence software interface used to read fluorescence emission needed for testing in Dr. Deguo Du's lab in the FAU Department of Chemistry.

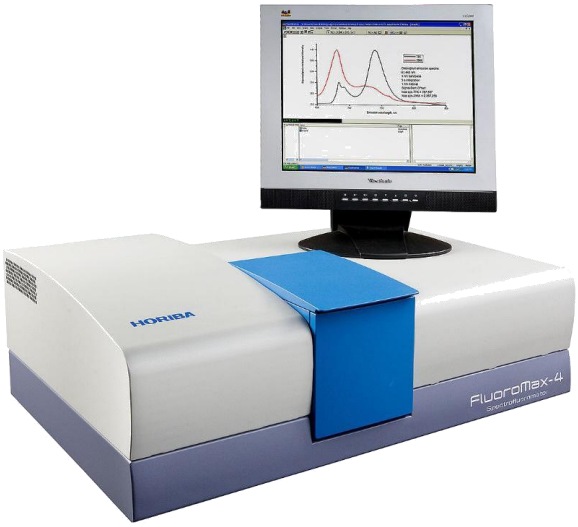


Figure 7: Horiba Jobin Yvon FluoroMax-4 spectrofluorometer and FluorEssence software interface *(image from Horiba website)*

TASK 4. Perform protein sensitivity experiments on model compounds. A 34 L gas cylinder of hydrogen sulfide (H_2S, 25 ppm +/- 5%) and another similar cylinder of ammonia (NH_3, 25 ppm +/- 5%), both in N_2, have been purchased to use as model compounds for experimentation with the biosensor complex. 1-aminoanthracene (1-AMA), which is an intrinsic fluorophore, was obtained from Sigma

Aldrich and has been used in conjunction with hOBP2A to quantify the amount of odorant binding with the protein. Since 1-AMA is hydrophobic, 100% methanol was used to dissolve the powder and later diluted with deionized water to a final methanol concentration of 10%.

Replication of previous experimentation with H_2S: To verify the previous protein sensitivity experiments run by Julia Roblyer on hydrogen sulfide, S. Rahman conducted the first trial experiment in mid-February using the revised reactor chamber (Figure 8). The centrifuge tube used as the reactor chamber has two one-way check valves attached at the top. A Y-connector connects the gaseous cylinder and a flowmeter with one of the check valves at the top of the chamber. A pipe leads from that valve into the chamber and ends in an aquarium-grade, pumice stone bubbler, which is mainly used to increase the surface area of solution exposed to the dispersed influent gas. By means of the second one-way valve, the gas escapes through the top of the chamber. The biosensor complex exists in the buffered solution at the bottom of the reactor chamber as before.

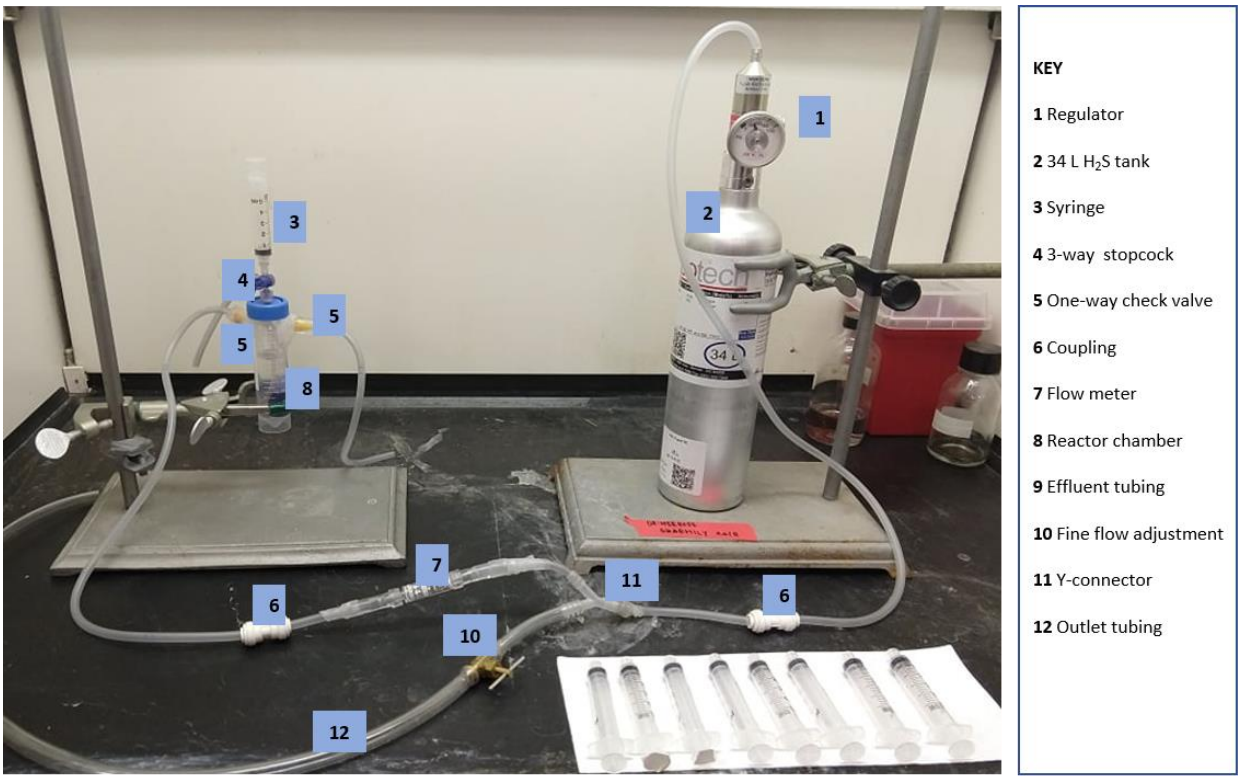


Figure 8: Revised experimental setup for testing with H_2S

50mM Potassium Phosphate-KOH, pH 7.5 solution with 1µM hOBP2A and 1µM 1-AMA (protein to fluorophore1:1 ratio as before) has been used as the biosensor complex. In the previous research, 100 mL buffered solution of the protein-fluorophore complex was used, which is now reduced to 25 mL, as the new reactor chamber has a total capacity of only 45 mL sample volume. The rest of the headspace was utilized for the gas to escape from the chamber through the one-way valve easily without exerting any extra pressure on the chamber. After transferring the 25 mL of biosensor complex to the confined reactor chamber, it was exposed to 25 ppm hydrogen sulfide gas from the pressurized cylinder at a rate of 0.5 standard liters per minute (SLPM) as before.

1 mL samples were drawn at designated times from the solution by means of syringes as described before and transferred to disposable cuvettes each time for spectrofluorometry using the Horiba Jobin Yvon FluoroMax-4. As the sample volume reduced to one-fourth of the starting volume, the 1 mL samples were drawn more frequently than before. This is because a reduced sample volume means that less proteins remain in the system for binding. To perform the spectrofluorometry more rapidly, 1 mL disposable fluorescence cuvettes were used instead of the quartz one used previously. The excitation wavelength was 380 nm, and spectral emission readings were recorded between 410 nm and 700 nm, the same as before. For both excitation and emission, slit width was kept at 5 nm.

Figure 9 shows the measured emission spectra where the emission peaks occurred at ~485 nm for each 1 mL of sample as expected for 1-AMA. The intensity decreases in a linear fashion with increasing exposure to hydrogen sulfide gas same as the previous experimentation with H_2S.

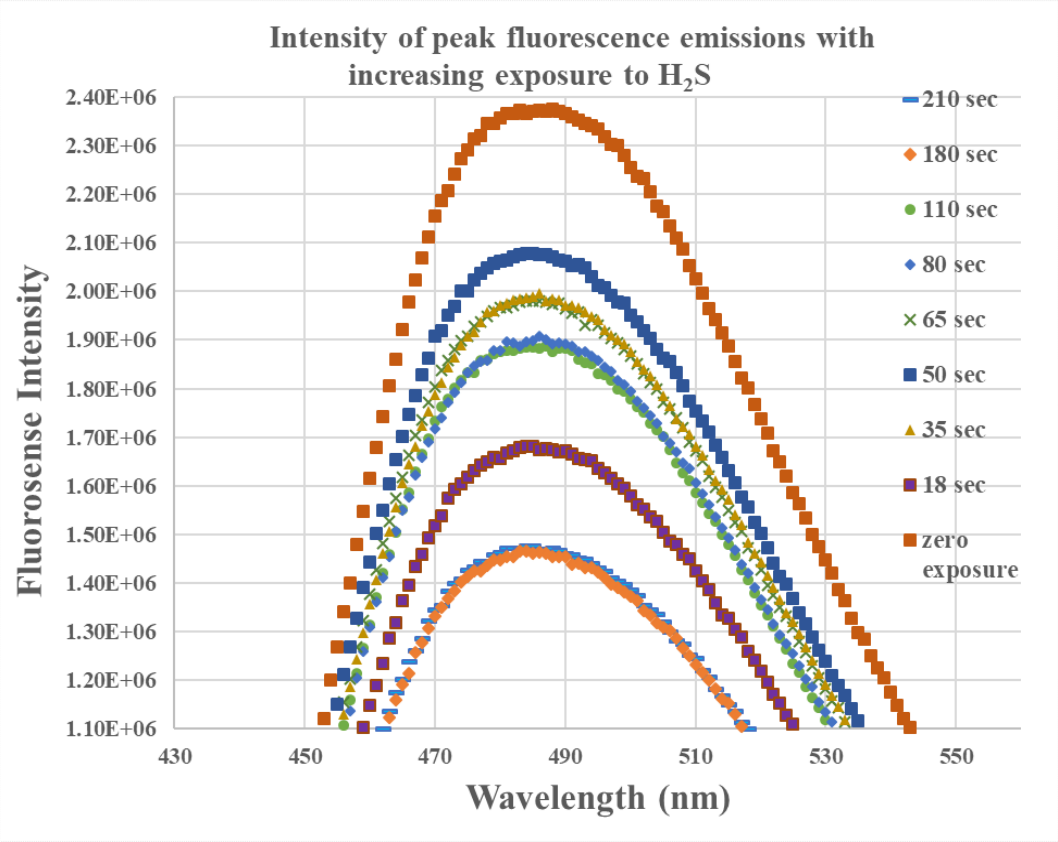


Figure 9: Spectrofluorometry emission spectra at 380 nm excitation

As expected, an inverse relationship between fluorescence intensity and the amount of time the biosensor was exposed to hydrogen sulfide gas (showed in Figure 10) has been revealed. One of the ultimate targets to replicate the previous experiment was to verify the quantitation range of the sensor with H_2S, but it is not well established yet.

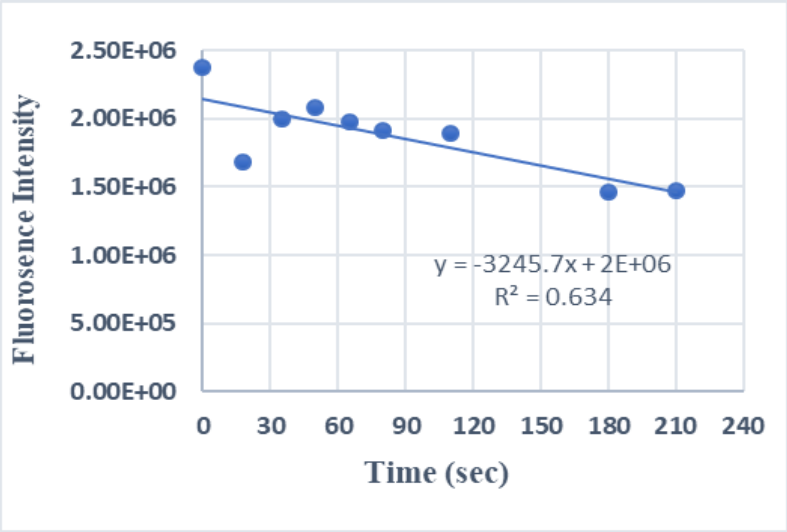


Figure 10: Graph showing inverse linear relationship between fluorescence intensity and exposure time

Depending on the protein availability, 2 or 3 more trial experiments with H_2S will be conducted to be able to establish a quantitation limit with some degree of certainty. A change in the flow rate of the feed gas odorants would in turn change the amount of H_2S in the exposure chamber and would allow formation of stronger model predictions. For the first trial, the flow rate of H_2S (0.5 slpm) was kept same as before, but for future experimentation, different flow rates (0.4 slpm and 0.6 slpm) will be used. A new flowmeter with measuring capacity of 0.1 slpm-1.0 slpm has been purchased for this purpose instead. Also, each time after performing the fluorometry, pH paper will be inserted in the cuvette to identify any large change in pH of the solution, which can impact binding rates, particularly in the case of hydrogen sulfide, which is also a weak acid.

TASK 5. Perform protein sensitivity experiments on mixtures. S. Rahman will check whether the protein shows a binding affinity only towards H_2S or whether the same applies to a mixture of "standard landfill gas" containing H_2S, CH_4, NH_3 etc. to confirm the Beer's Law relationship in the presence of other odorants. A 34 L gas cylinder containing Hydrogen Sulfide (H_2S, 25 ppm +/- 5%), Carbon Monoxide (CO, 50 ppm +/- 5%) and Methane (CH_4, 2.5% +/- 2%) has been purchased. Before testing the biosensor complex with this mixed landfill gas, a separate experiment only with methane will be performed so that the behavior of the sensor both towards H_2S and CH_4 separately will be known. S. Rahman will also check the binding capacity of the biosensor complex when exposed to acidic and basic odorants.

Upcoming Research Tasks

- **TASK 1. Conduct literature review.** Continue to update the literature review.
- **TASK 2. Prepare biosensor molecules.** hOBP2A has already been prepared under the supervision of Dr. Binninger. More quantities have been requested of Dr. Binninger's team.
- **TASK 3. Develop an experimental exposure chamber.** Changes would be implemented in case of any problem arising.
- **TASK 4. Perform protein sensitivity experiments on model compounds.** The behavior of the sensor towards different flow rates of H_2S as well as the other model compounds would

be established. Because of the shortage of protein, some strategic changes will need to be implemented for future experimentation.

- **TASK 5. Perform protein sensitivity experiments on mixtures.** After experimenting on model compounds, S. Rahman would analyze the results obtained from mixtures of gases.
- **TASK 6. Develop recommendations and preliminary cost analysis.**
- **TASK 7. Prepare publication materials.**

PROJECT METRICS:

1. List graduate or postdoctoral researchers **funded** by **THIS** Hinkley Center project.

Last name, first name	Rank	Department	Professor	Institution
Rahman, Sharmily	MSCV Candidate	CEGE	Meeroff	FAU
Ampuero, Yasmeen	MS Biology Candidate	BIO	Meeroff, Binninger	FAU
Raaijmakers, Cynthia	MS Biology Candidate	BIO	Meeroff, Binninger	FAU

2. List undergraduate researchers working on **THIS** Hinkley Center project.

Last name, first name	Department	Professor	Institution

3. List research publications resulting from **THIS** Hinkley Center project.

None yet

4. List research presentations resulting from **THIS** Hinkley Center project

None yet

5. List research papers that have cited any publications (or the final report) resulting from this Hinkley Center project (use format for publications as indicated in the Hinkley Center Investigators Guide).

None so far

6. List additional research funding that has been secured due to leveraging the research results from this Hinkley Center project (give project title, funding agency, amount of funding, award date, and award period)

Year two funding from the Hinkley Center for Solid and Hazardous Waste Management was secured. "Development of a biosensor for measuring odorants in the ambient air near solid waste management facilities (this project)," Hinkley Center, $50,487. 12/01/2017 (delayed project start to 08/01/2018) – 05/31/2019.

Additional funding was secured from the Environmental Research and Education Foundation, "*Detection of nuisance odors using odor binding protein sensor,*" Environmental Research and Education Foundation (EREF), $150,000. 12/01/2017 – 11/30/2019.

7. List submitted proposals which leverage the research results from this Hinkley Center project (give the proposal title, funding agency, requested funding, date submitted)

None yet

8. List new collaborations initiated based on this Hinkley Center project

- Deguo Du, Assistant Professor, Chemistry, FAU is allowing us to use his sophisticated fluorometry equipment for this project.
- Dr. Daniela Scheurle, Coordinator for Academic Support Services, Chemistry, FAU is allowing us to use her SpinTrap TALON column to purify the protein.

9. How have the results from this Hinkley Center funded project been used (not will be used) by the FDEP or other stakeholders in the solid waste field? Please note that the term "other stakeholders" is meant to broadly include any party or practitioner in the solid waste field. This includes county solid waste directors and their staff, municipal solid waste directors and their staff, solid waste facility design engineers, local/county/city solid waste management regulatory staff, federal solid waste regulatory staff, landfill owners and operators, waste haulers, waste to energy plant owners and operators, recyclers, composting plant owners and operators, yard waste operators, construction and demolition debris companies and organizations, county recycling coordinators, citizens and members of the academic community, etc. (1 paragraph maximum)

To date, the results have not been used by stakeholders yet.

TAG members:

Mark Eyeington, Mark Maclean, Mark Bruner, Owrang Kashef, Craig Ash, Ravi Kadambala, Ron Schultz, Jeff Roccapriore, André McBarnette, Dan Schauer, Damaris Lugo, Amanda Krupa, Richard Meyers, Amede Dimonnay, Art Torvela, Ted Batkin

TAG meetings:

October 19, 2018 (Joint TAG meeting held at SWA in conjunction with UM)