COLLEGE OF ENGINEERING & COMPUTER SCIENCE
Florida Atlantic University

# Technical Advisory Group Meeting
# Florida Atlantic University

Funded by the Hinkley Center for Solid and Hazardous Waste Management (HCSHWM)

**DATE:** **Tuesday, March 29, 2022**
**TIME:** **11:00 am**
**WHERE:** **Virtual via Zoom**

**MEETING AGENDA**
**Tuesday, March 29, 2022**

| | | |
|---|---|---|
| **11:00 – 11:15 am** | **Opening Address and Introduction of Participants** | **Daniel Meeroff** |
| **11:15 – 12:00 pm** | **Year 2. Development of a Biosensor for Detecting Odors at Landfills** | **Sharmily Rahman** |
| **12:00 – 12:15 pm** | **Open Forum** | **Participants** |
| **12:15 pm** | **Adjourn, Thank You** | **Daniel Meeroff** |

**Attendance:** Daniel Meeroff, David Binninger, Craig Ash, Nathan Mayer, Masoud Jahandar Lashaki, Sharmily Rahman, Patrick Carroll, Damaris Lugo - BCSD, Daniel Courcy, Mateja Vidovic Klanac, Monica Mejia, Myles Clewner, Rakib Ahmed Chowdhury, Richard Meyers, Bishow Shaha, David Bromfield, Wanda Parker, David Dalton, Joseph Ullo, Jane Gregory, Samuel levin.

**Minutes of Meeting**

1. The opening address was presented by D. Meeroff. He first introduced the project website (labees.civil.fau.edu) followed by a brief overview of the ongoing projects that his research team is currently working on. He mentioned his recent promotion to the Interim Dean position at FAU. Afterwards, he shared some recent updates of the odor detection project which includes a manuscript to be submitted soon for publication in a peer-reviewed journal. He also mentioned his upcoming trip to Las Vegas in May for attending the WasteExpo 2022 conference. He added that he has been able to secure an additional funding of $23,000 from the university to purchase a flow-through spectrofluorometer for the odor detection project. He also talked about the recent success of the FAU team at the Florida Food Future Hackathon. The Hackathon has been arranged as part of Florida Food Waste Prevention Week and the FAU team secured the first place in that competition. Then he gave the floor to the participants of the meeting so that they can introduce themselves. (11:15 pm). Following the introduction of TAG members and guests, D. Meeroff introduced S. Rahman.

2. S. Rahman gave a presentation on the Year 2 study of development of a biosensor for detecting odors at landfills. At the beginning, she outlined the challenges faced by solid waste managers while dealing with odors using state of the art odor detection technology in an absence of objective measurement techniques. Then she talked about how their idea of using human odorant binding protein (hOBP) as a biosensor in detecting odors is one of a kind in addressing those issues. An animation of how human olfaction system works is presented with many details where S. Rahman pointed out how they took the advantage of this

science while developing their novel biosensor technology. The biosensor is prepared using hOBPIIa combined with a fluorophore, 1-AMA, where different odorant gases are introduced to measure their concentration levels by spectrofluorometry. S. Rahman then pointed out the major findings obtained in the Year 1 study of this project. The fluorescence binding assay showed that the protein-fluorophore complex causes a high emission intensity at around 485 nm, proving the effectiveness of the biosensor in Year 1. The optimum concentration ratio of the protein and the 1-AMA was verified to be 1:1. She showed that the peak of the fluorescence intensity curves of the odorant-purged biosensor followed a general decreasing trend with increasing concentration of gases up to a certain point that we refer to as the saturation limit. The biosensor remained unreactive when introduced to inert nitrogen gas. Thus, the biosensor only responds with odorous and hydrophobic compounds. To be able to reuse the spent protein for multiple assays, a regeneration test was performed where nitrogen gas was introduced to the odorant purged solution for a limited amount of time, hoping that this will help the protein re-bind with 1-AMA, but the initial experiments indicated that protein regeneration might take a longer period or different conditions. S. Rahman then outlined the next steps moving forward in the Year 2 study and the latest update on this project to date. As part of the Year 2 study, we plan to introduce more odorant gases and gas mixtures with varying concentrations to the biosensor to further analyze the protein's behavior. We are planning to update the experimental setup very soon by introducing a new flow-through system to miniaturize the current setup and to be able to obtain real-time fluorescence analysis results to increase the accuracy. A number of ideas have been explored in terms of regenerating the biosensor in the Year 2 study. For example, the nitrogen purge time will be extended compared to previous experiments and also adjusting the temperature of the solution more closely to human body temperatures to better replicate a real-world biological scenario. One more experiment where Le Chatelier's principal will be conducted by introducing additional fluorophore (1-AMA) to regenerate the biosensor for another assay. Exploring this idea will shed light on whether this is a truly reversible reaction and whether it is even possible to regenerate the biosensor. (11:54 am)

Following the presentation, David Binninger asked if the slopes of the intensity curves are different enough for different gases so that those can be used in detecting unknown odorants. S. Rahman responded that they will be introducing more odorant gases to the biosensor to increase the spectroscopic library with enough data to analyze the slopes and possibly identify unknown odorants. She added that maybe the slopes along with the decrease in intensity together can be indicative in separating a group of odorants from others. Dr. Meeroff added that the new odorant gases to be tested in Year 2 and the new flow-through system will be able to collect the needed data with greater accuracy. This will likely reduce the scatter in the plots to some extent and that will help in differentiating among the different odorants. Bishow Shaha asked about applying the technology in real-world situations. S. Rahman said that a patent may be submitted by Dr. Meeroff once they successfully demonstrate the technology, and companies who have knowledge in fluorescence spectroscopy can help develop the biosensor to be field-deployed in a handheld platform. Dr. Meeroff added that the team is gradually minimizing the amount of protein required to detect the odors, in the near future they will be able to fit it in a small handheld device. As an added advantage, the lower amount of protein will drive the cost down as well as the analysis time, ensuring that the sensor can perform the detection at a faster rate, possibly within a few seconds. He added that this will provide an opportunity to miniaturize the whole detection platform and connect this to a cellphone-based system to help in hand-held detection which is the ultimate goal of this research.

Wanda Parker asked what kinds of meteorological effects were considered while conducting the experiments. Dr. Meeroff replied that in the Year 1 study, several meteorological characteristics including temperature, humidity, wind direction, and wind speed, among others were considered. He added that since the analysis time while using the field version of this device will be relatively short, the meteorological factors of wind speed and direction may not be a major issue while obtaining the readings even though it is possible to conduct the experiments in the laboratory setting while still changing some of those factors, including the relative humidity or the temperature of the system. Wanda Parker followed up with another question about a case where a number of facilities might be releasing similar odor components with wind blowing from a specific direction and how the technology will fit into identifying the source of odor when complaints are made. Dr. Meeroff responded by mentioning that in such a case, readings will be taken from individual odor source facilities and then a comparison will be made between the fingerprint of the readings obtained from the facilities and the readings taken at the location of the person making the complaint. The dominant signature can be identified and that might lead to more accurate identification of the source. Once the database of different odor signatures is developed, it can be used to map different odorant responses to the biosensor. Dr. Meeroff asked any of the participants if they would like to host the field sampling activities for the project. David Dalton volunteered to provide access to field air sampling to the research team (12:08 pm).

3. Dr. Meeroff thanked all the participants, and the meeting was adjourned at 12:12 pm.

Welcome
Technical Advisory Group Meeting
Year 2. Development of a Biosensor for Detecting Odors at Landfills
1
Technical Advisory Group Meeting: Tuesday, March 29, 2022
Funded by the Hinkley Center for Solid and Hazardous Waste Management (HCSHWM)


http://labees.civil.fau.edu
Combining Science and Technology
To Protect and Conserve Our Environment
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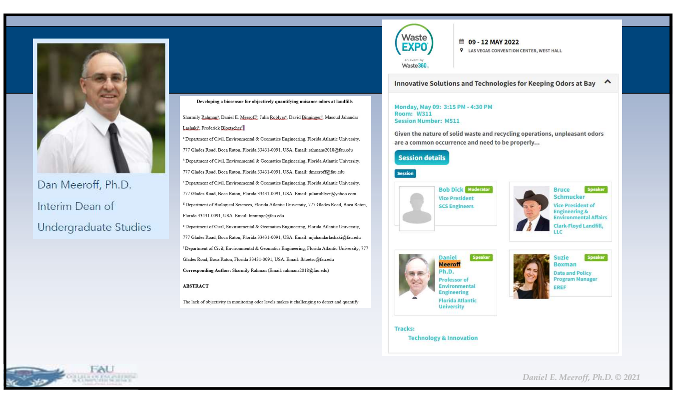
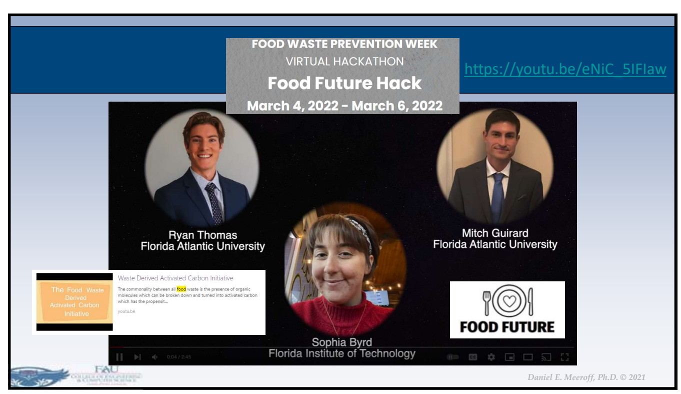
Current Funded Research
Air Quality Biosensor Development
Solid Waste Management/Leachate
Sustainable Materials Management
Algae Control
Food Waste Diversion
Anaerobic Digestion
SARS-CoV-2 Surveillance
Watershed Management/Flood Risk Mitigation
Energy Conservation Technical Assistance
NSF STEM Education Grants


THE
GOOD
NEWS

Dan Meeroff, Ph.D.
Interim Dean of
Undergraduate Studies
Developing a biosensor for objectively quantifying nuisance odors at landfills
Waste EXPO
09 - 12 MAY 2022
LAS VEGAS CONVENTION CENTER, WEST HALL
Innovative Solutions and Technologies for Keeping Odors at Bay
Monday, May 09: 3:15 PM - 4:30 PM
Room: W311
Session Number: M511
Given the nature of solid waste and recycling operations, unpleasant odors are a common occurrence and need to be properly...
Session details
Tracks:
Technology & Innovation
Daniel E. Meeroff, Ph.D. © 2021

FOOD WASTE PREVENTION WEEK
VIRTUAL HACKATHON
Food Future Hack
March 4, 2022 – March 6, 2022
https://youtu.be/eNiC_5IFlaw
Ryan Thomas
Florida Atlantic University
Mitch Guirard
Florida Atlantic University
Sophia Byrd
Florida Institute of Technology
Waste Derived Activated Carbon Initiative
FOOD FUTURE
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Flow Through Spectrofluorometer
QEPro
Daniel E. Meeroff, Ph.D. © 2021


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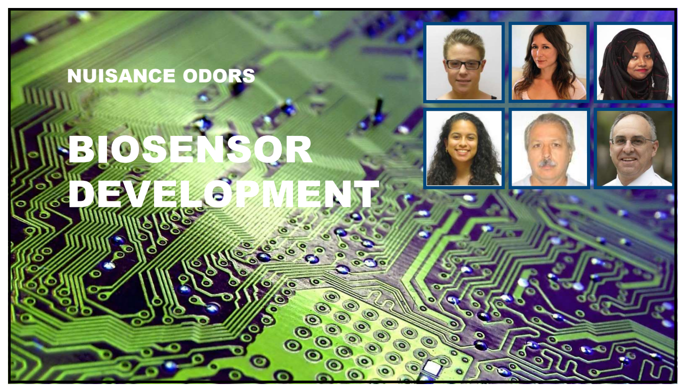
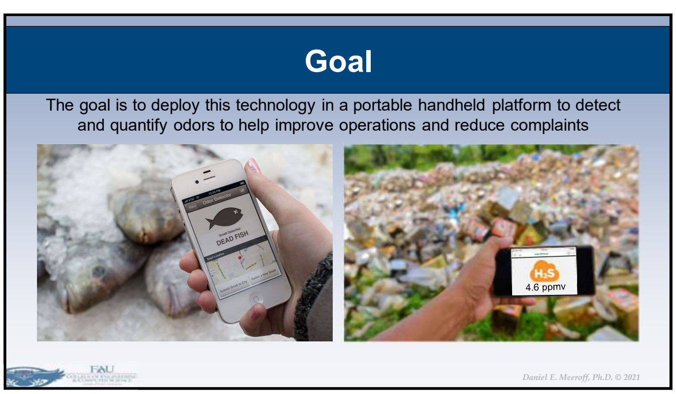
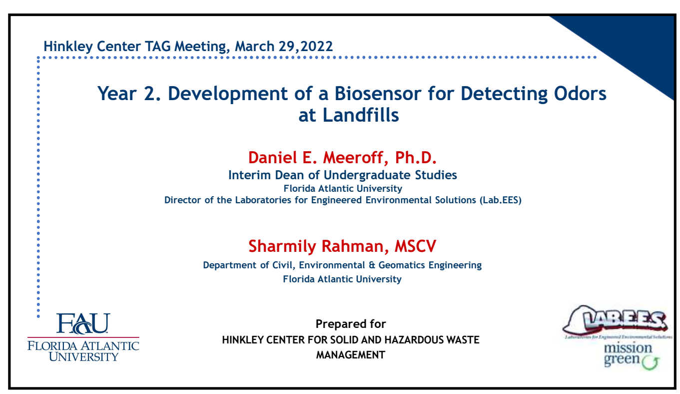
Introductions


NUISANCE ODORS
BIOSENSOR DEVELOPMENT


Goal
The goal is to deploy this technology in a portable handheld platform to detect and quantify odors to help improve operations and reduce complaints
DEAD FISH
H2S
4.6 ppmv
Daniel E. Meeroff, Ph.D. © 2021


Hinkley Center TAG Meeting, March 29,2022
Year 2. Development of a Biosensor for Detecting Odors at Landfills
Daniel E. Meeroff, Ph.D.
Interim Dean of Undergraduate Studies
Florida Atlantic University
Director of the Laboratories for Engineered Environmental Solutions (Lab.EES)
Sharmily Rahman, MSCV
Department of Civil, Environmental & Geomatics Engineering
Florida Atlantic University
Prepared for
HINKLEY CENTER FOR SOLID AND HAZARDOUS WASTE MANAGEMENT
FAU
FLORIDA ATLANTIC UNIVERSITY
LABEES
mission green

## OUTLINE

01 **Background**
Introduction to the issues, currently faced challenges, research inspiration, and objectives

02 **Year 1 Findings**
Experimental tasks, methodology and major findings

03 **Year 2 Experiments**
Proposed tasks and progress made so far



## CHALLENGES

- Subjectivity and different individual perception of odors
- State-of-the-art odor measurement devices:
  - Usually work only for specific odorants
  - Unable to quantify odors
  - Unable to deal with adverse environmental or meteorological conditions (temperature, humidity, etc.)
- Low-cost objective methods are not readily available

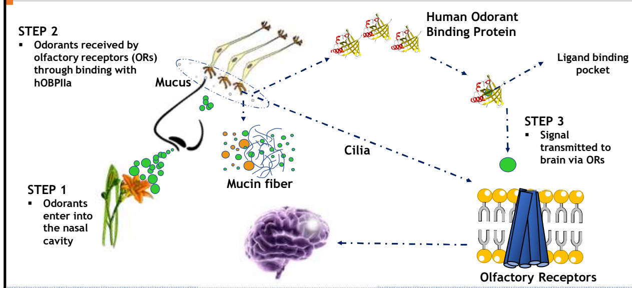


## RESEARCH INSPIRATION

- Turn to biology for inspiration
- In 2013, Silva et al. used porcine Odorant Binding Proteins (pOBP) to mask the smell of cigarettes
- OBPs can bind with odorants in the µM-range and the odor intensity is based on the number of bound receptors
- OBP biosensors can be a potential game-changer for dealing with odors

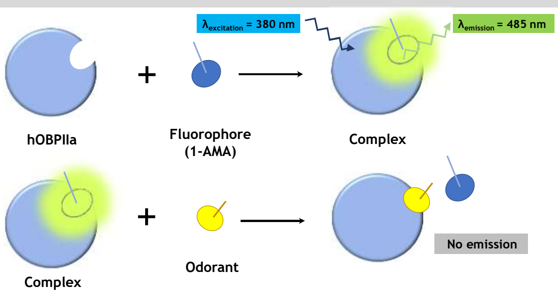


## OLFACTION SCIENCE






## MECHANISM






## HUMAN ODORANT BINDING PROTEIN

- Member of lipocalin super-family
- The ligand binding cavity inside the β-barrel allows binding with a large variety of hydrophobic odorants to deliver them to ORs
- Stable to temperature, organic solvents, and proteolytic digestion
- Broad binding affinity across different ligand structures and sizes
- Can be expressed in bacterial systems at low cost & easily purified

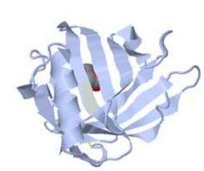
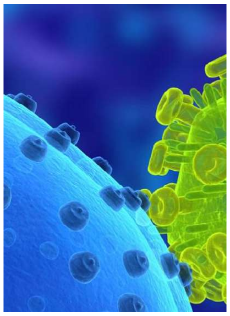


Image source: http://proteopedia.org/wiki/index.php/4run

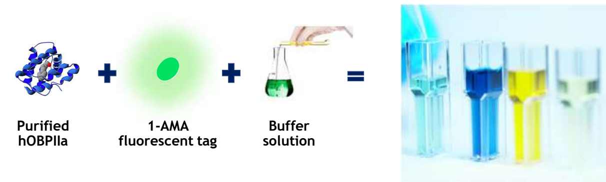


## FLUOROPHORE (1-AMA)

- Widely used as a fluorophore to study interaction of lipocalin proteins
- Hydrophobic in nature
- Shows a strong fluorescence signal when forming complex with OBPs



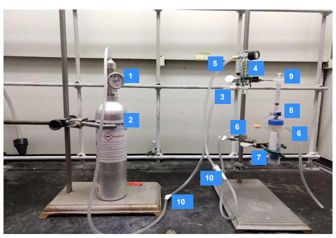
## PREPARION OF THE BIOSENSOR COMPLEX




Biosensor complex



## EXPERIMENTAL SETUP



**KEY**

1. Regulator
2. Gas cylinder
3. Tube connected to cylinder
4. Flowmeter
5. Tube connected to chamber
6. One-way check valve
7. Reactor chamber
8. 3-way stopcock
9. Syringe
10. Tube connector



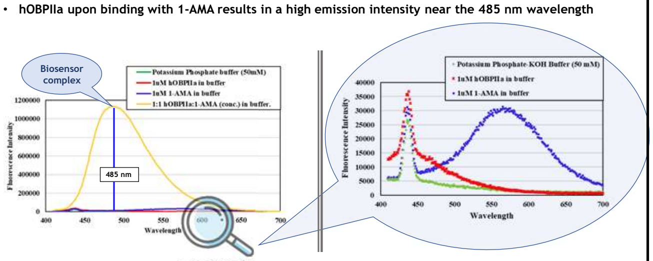
## PURIFIED PROTEIN

- hOBPIIa is procured to be used in the biosensor experiments prepared by Dr. David Binninger



## Year1 Results: Effectiveness of the Biosensor

- hOBPIIa upon binding with 1-AMA results in a high emission intensity near the 485 nm wavelength

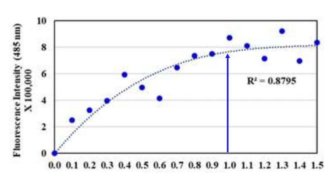



Spectrofluorometric analysis for individual and combination of biosensor components



## Year1 Results: Optimum Ratio

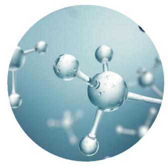
- Verification of optimum concentration ratio of protein-fluorophore complex (1:1)




Titration curve of 1-AMA at different concentrations to hOBPIIa

## Year 1 Results: Gases Tested

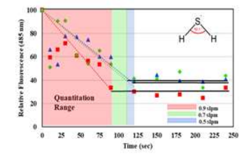
- Pure compounds
  - $H_2S$, 25 ppm balanced with $N_2$
  - $NH_3$, 25 ppm balanced with $N_2$
  - $CH_4$, 25 ppm balanced with Air
  - $CH_4S$, 50 ppm balanced with $N_2$
- Gas mixtures
  - Mixture 1 ($NH_3$, 25 ppm; $CH_4$ 25 ppm) balanced with Air
  - Mixture 2 ($H_2S$, 25 ppm; CO, 50 ppm and $CH_4$ 2.5%) balanced with Air
- Gas flow rates
  - Higher (0.9 slpm)
  - Intermediate (0.7 slpm)
  - Lower (0.5 slpm)
- Exposure times were up to 4 minutes
- Volume: 10 mL protein-fluorophore-buffer solution containing 180 µg of protein





## Year1 Results: Expt. with pure compounds

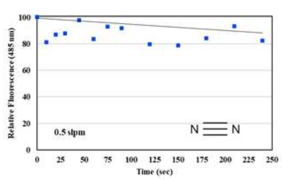
- The fluorescence response curves follow a general trend of decreasing peak fluorescence intensity with time, up until saturation when they become flat
- The biosensor complex saturates faster with an increased gas flow rate
- No mentionable pH change observed during the experiments
- Depending on the gas flow rates, the fluorescence response curves obtained from the experiments can be used to quantify:
  - 35-45 µg of hydrogen sulfide ($H_2S$)
  - 12-18 µg of ammonia ($NH_3$)
  - 83-95 µg of methyl mercaptan ($CH_3SH$)
  - 15 µg of methane ($CH_4$)




Response curve of $H_2S$ for three different flow rates. The lower the flow rate, the higher the time of saturation (i.e., when the curve becomes flat)



## Year1 Results: Expt. with non-odorous $N_2$

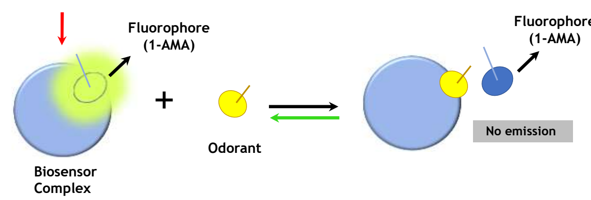



- $N_2$ (0.5 slpm) has no mentionable interaction with the biosensor

- Response was not found in case of non-odorous $N_2$

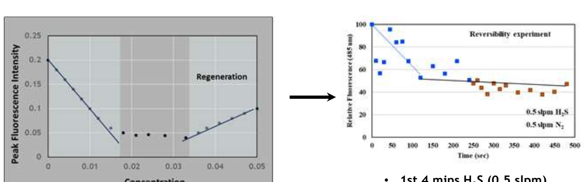


## Year1 Results: Reaction Reversibility






## Year1 Results: Reaction Reversibility




- 1st 4 mins $H_2S$ (0.5 slpm)
- Next 4 mins $N_2$ (0.5 slpm)



## Year2: Proposed Approach

**Step 1**
- Perform protein sensitivity experiments on an expanded list of pure odorant compounds.

**Step 2**
- Perform protein sensitivity experiments on landfill gas mixtures.

**Step 3**
- Upgrade the reactor chamber as a flow-through system for improved real-time result accuracy.

**Step 4**
- Perform experiments to explore protein-odorant reaction reversibility.

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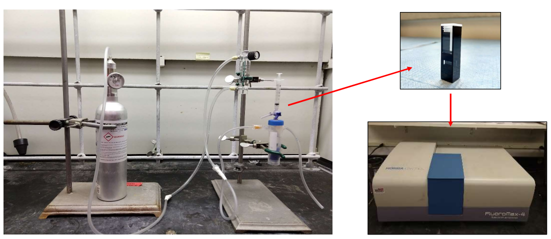
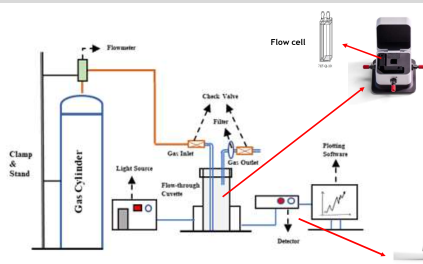
## Current Setup



Horiba Jobin Yvon FluoroMax-4 spectrofluorometer



## Proposed Setup: Step towards miniaturization




Use of flow-through cuvette to act as the reaction chamber for real-time fluorescence analysis and elimination of manual sub-sampling

High Performance Spectrofluorometer, QEPRO-FL from Ocean Insight



## Year2: Proposed Approach

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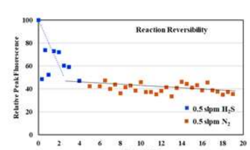
## Year 2: Approach Towards Reaction Reversibility

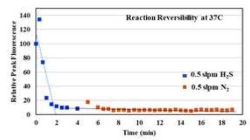
- Purging with inert $N_2$ gas for 4 min X
- Purging with inert $N_2$ gas for an extended period of time




Purge with $N_2$ for 15 min

## Year 2: Approach Towards Reaction Reversibility

- Purging with inert $N_2$ gas for 4 min X
- Purging with inert $N_2$ gas for an extended period of time X
- Adjusting the temperature to human body temp (37ºC) while purge with $N_2$




Purge with $N_2$ while raise the temp to 37C

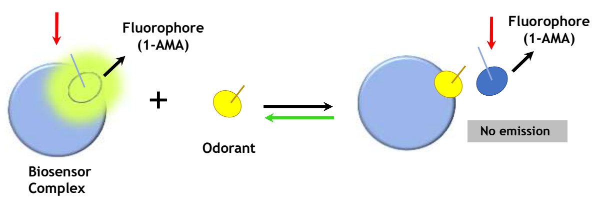


## Year 2: Approach Towards Reaction Reversibility

- Purging with inert $N_2$ gas for 4 min X
- Purging with inert $N_2$ gas for an extended period of time X
- Adjusting the temperature to human body temp (37ºC) while purge with $N_2$ X
- Adding more fluorophore as the reaction reaches equilibrium to shift the equilibrium to the left following La Chatelier's principle ?



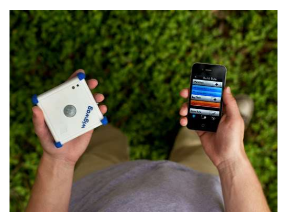
## Year 2: Approach Towards Reaction Reversibility




Adding more 1-AMA at the equilibrium will favor more reactant formation if the reaction is reversible



## FUTURE WORK



- A future handheld device for real-time objective quantification of odors using OBP sensor will have far-reaching benefits for solid waste managers as well as other similar operations management

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