

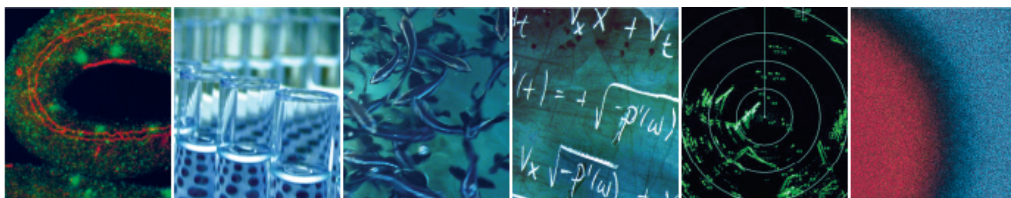
# **21st Annual Undergraduate Research Symposium in the Chemical and Biological Sciences**



**Saturday, October 20, 2018**

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**UMBC**  
AN HONORS  
UNIVERSITY  
IN MARYLAND



# **21st Annual Undergraduate Research Symposium in the Chemical and Biological Sciences**

*The College of Natural and Mathematical Sciences;  
Department of Chemistry and Biochemistry &  
Department of Biological Sciences*





# Schedule of Events

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Time	Event		
8:00 am	<b>SYMPOSIUM CHECK-IN &amp; ON-SITE REGISTRATION</b> <i>Lobby, University Center, 3rd Floor</i>		
8:00 am	<b>LIGHT CONTINENTAL BREAKFAST</b> <i>UC 312, University Center, 3rd Floor</i>		
9:00 am	<b>OPENING REMARKS &amp; WELCOME ADDRESS</b> Dr. Freeman Hrabowski, President, University of Maryland, Baltimore County (UMBC) Dr. William R. LaCourse, Dean, College of Natural & Mathematical Sciences, UMBC <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>		
9:45 am – 11:45 am	<b>MORNING POSTER SESSION</b> (Please see schedule for poster numbers) <i>Ballroom, University Center, 3rd Floor</i>		
10:30 am	<b>WORKSHOPS</b>		
	<table> <tr> <td><b>Master the Art of Making Connections</b> Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i></td><td><b>A Very, Very Short Introduction to Ethics for Scientists</b> Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i></td></tr> </table>	<b>Master the Art of Making Connections</b> Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i>	<b>A Very, Very Short Introduction to Ethics for Scientists</b> Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i>
<b>Master the Art of Making Connections</b> Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i>	<b>A Very, Very Short Introduction to Ethics for Scientists</b> Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i>		
11:45 pm	<b>BUFFET LUNCH</b> ( <i>gratis for registered guests with symposium name badge</i> ) <i>The Commons – Main Street</i>		
12:45 pm – 2:45 pm	<b>AFTERNOON POSTER SESSION</b> (Please see schedule for poster numbers) <i>Ballroom, University Center, 3rd Floor</i>		
1:30 pm	<b>WORKSHOPS</b> <i>Repeat of workshop titles and locations above</i>		
3:00 pm	<b>PLENARY TALK</b> <b>“Development of a self-powered biosensing system”</b> Dr. Gymama Slaughter is the Executive Director of the Center for Bioelectronics. She received her B.S. in Chemistry in 2001, M.S. in Chemical Engineering in 2003, and a Ph.D. in Computer Engineering from the Virginia Commonwealth University in 2005. <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>		
4:00 pm	<b>AWARDS PRESENTATION</b> <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>		



# Workshops:

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## **Master the Art of Making Connections**

*University Center, 3<sup>rd</sup> Floor, Room 310*

In the world of science, we communicate with others many times throughout our day. Effective communication in STEM is crucial and is more than just exchanging information. Effective communication combines a set of skills including nonverbal communication, attentive listening, and the ability to respond appropriately. How well you communicate will determine the impression you make and how others understand your work. It may influence funding and many other opportunities. This workshop is designed to help you learn effective communication skills, as well as how to talk about yourself and your accomplishments in a way that effectively showcases your strengths and passion for science – a skill that will positively impact your professional image as a scientist and ultimately your career.



**Susan Hindle** is the Assistant Director, Internships and Employment for the College of Natural and Mathematical Science at UMBC. Susan has 20 years' experience working with students and alumni in all phases of the career development process. Prior to coming to UMBC in January 2014, Susan worked as a Career Advisor for the both The Johns Hopkins University and the A. James Clark School of Engineering at the University of Maryland. Susan has her undergraduate degree in elementary education from the University of Maryland, College Park and her master's degree in clinical counseling from The Johns Hopkins University.

## **A Very, Very Short Introduction to Ethics for Scientists**

*University Center, 1<sup>st</sup> Floor, Castle 115D*

This workshop will provide a basic overview of the two dominant approaches to thinking about ethical problems. You'll then have a chance to apply these approaches to ethical dilemmas and problems, including some of the sort that might arise specifically for scientists.



**James Thomas** is a Faculty Lecturer in the Department of Philosophy at UMBC. Jim Thomas received a B.A. with honors from the University of Arkansas at Fayetteville with a major in philosophy. He went on to get a Masters degree in philosophy at the University of Arkansas where he received the Philip S. Bashor Award for outstanding graduate student. He earned a second M.A. in philosophy at the University of Washington in Seattle. He is currently a lecturer in the Philosophy Department at the University of Maryland, Baltimore County, where he has been teaching for the last fifteen years. He has also taught courses at the University of Arkansas and the University of Maryland, College Park. His research is focused on Metaphysics, Evolutionary Theory and Philosophy of Humor, and Philosophy of Perception.

# Plenary Talk

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## Development of a self-powered biosensing system

*Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030*

Recent studies on biofuel cells have shown that energy can be harvested from biological compounds. Because of the recent biofuel cell discoveries, it is possible to use biochemical power scavenging design by converting interstitial glucose into energy through the coupling of enzymes and three-dimensional carbon nanotubes (CNTs). This talk will discuss our own contribution to identifying a pathway to embed sensing by eliminating the need for a potentiostat circuit and an external power source required in conventional amperometric glucose sensors. The self-powered biosensing system consists of a massively dense network of 3-D CNTs cell structures fused with an energy amplification circuit that maximizes power and energy densities while maintaining short ion transport distances, thus leading to dramatic improvement in both speed and energy efficiency of biofuel cells. By combining the advantages of porous CNTs and energy amplification circuits, the sensor system exhibited unprecedented performance with high sensitivity, selectivity, and fast response time. Not only is such a paradigm extremely fast because of absence of a potentiostat circuit, but it is also extremely energy-efficient since the device operates at low voltage and current levels. As a result, the biosensing system generates a drive signal in real-time and continuously powers an electrical device by generating and accumulating electrical power as a result of the catalysis of glucose while sensing glucose. We further envision that the self-powered glucose biosensing system could be greatly reduced in footprint by using microsystem techniques and other inexpensive deposition methods to deposit dense mesh network of carbon nanotubes and/ metal wire traces. We believe that this type of high-performance, self-powered glucose biosensing system, combined with low-cost construction shows great potential for use in biotechnology applications relating to medical diagnosis and diabetes management.



**Dr. Gymama Slaughter** is the Executive Director of the Center for Bioelectronics. She received her B.S. in Chemistry in 2001, M.S. in Chemical Engineering in 2003, and a Ph.D. in Computer Engineering from the Virginia Commonwealth University in 2005. She was selected to participate in the Office of Naval Research Sabbatical Leave Program at the Naval Research Laboratory's Center for Bio/Molecular Science and Engineering (CBMSE) where she served as CBMSE

Visiting Scholar and conducted research on the development of flexible biodegradable biological and chemical sensors. Slaughter develops and applies sensor-processor platforms, focusing on innovative contributions to identifying a pathway to embed sensing and processing functions in the same device to eliminate bottlenecks arising from communication between the sensor, transducer and processor, thus, resulting in ultra-fast and ultra-low power devices. Her research has been supported by the National Science Foundation, Department of Army, TEDCO Maryland Innovative Initiative, and the Maryland Industry Partnership. Her research interests include biosensors, microsensors, microfabrication technology, and BioMEMS. She is the recipient of the National Science Foundation's prestigious CAREER AWARD. The award recognizes junior faculty who exemplify the role of teacher-scholar through outstanding research, excellent education and the integration of education and research.

*Please note:*

The page numbers of the abstracts serve as the student poster numbers.

Abstracts are given in alphabetical order of the presenters' last names and are grouped according to the three major disciplines represented at the symposium.

An insert is being distributed with the poster numbers assigned to either the morning or afternoon poster sessions

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# Judges

The annual Undergraduate Research Symposium in the Chemical and Biological Sciences at UMBC is not possible without the efforts of our volunteer judges – faculty members, research mentors, UMBC alumni, and industry or government colleagues who are avid supporters of undergraduate research and have advanced degrees and backgrounds in the sciences.

**Thank you for your contribution to this event and for remembering that this symposium is a professional learning event for new researchers who will benefit from your experience, encouragement, and positive guidance.**

*Please...*

- Remember to wear your name badge throughout the event
- Attend the brief Judges' Meeting before each assigned poster session
- Complete an evaluation for each poster in the group
- Collaborate with other judge(s) assigned to the same group to select first and second place presenters.
- Submit selections for winners to awards coordinator, Mr. Ralph Murphy

**Note:** When judges were assigned groups, efforts were made to avoid conflicts of interest between the presenters and the judges. Judges who find that they have been assigned to a group where there may be a conflict of interest are asked to notify one of the event coordinators promptly and ask to switch assignments with other judges.

*Thank you for volunteering  
today.*

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# Biochemical and Molecular Biology

## ABSTRACTS

**Alphabetical by first author**

**Page number indicates Poster number**

***Confidential***

*Please note that many of the abstracts are not approved for dissemination beyond the student poster sessions and, therefore, are not approved for posting online or distribution beyond the 2018 Undergraduate Research Symposium in the Chemical and Biological Sciences.*



## PURIFICATION AND CHARACTERIZATION OF THE E. COLI COMMON PILUS PROTEIN ECPD

Benjamin Adams

Nathan T. Wright

Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive,  
Harrisonburg, Virginia 22801

*Escherichia coli* (*E. coli*) bacteria cause diarrhea through colonization of the digestive tract. To adhere and remain in the digestive tract, tiny hairs on the bacteria (fimbriae or pili) stick to both other bacteria and host epithelial cells. These pili are encoded by the ECP (*E. coli* Common Pilus) genetic cassette. Within this cassette, EcpA makes up the bulk of the pilus while EcpD comprises the tip, and is crucial for adhesion. While the structure and multimerization of EcpA is well known, virtually nothing is known about the structure EcpD. Here, we describe initial attempts to purify EcpD, in an effort to better structurally and characterize this protein. This work will eventually be used to better define immunogenic regions of EcpD, as well as elucidate the molecular mechanism of bacterial adhesion.

James Madison University Department of Chemistry and Biochemistry  
Research Corporation, NSF REU (CHE-1757874), RUI (MCB-160724)

(1) Garnett, J. A., et. al, Structural insights into the biogenesis and biofilm formation by the *Escherichia coli* common pilus, PNAS 2012, 109, 3950-3955

(2) Kelley, L. A., et. al, Nature Protocols 10, 845-858 (2015)

## DETECTING ANTIBIOTIC RESISTANCE GENES IN LOCAL WATER SAMPLES

Vanessa Adjei<sup>1</sup>, Sara Reagan<sup>1</sup>, Muhammad Al Azizi<sup>1</sup>

Caitlin Kowalewski<sup>2</sup>, Joshua Wilhide<sup>2</sup>

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Hilltop Circle, Baltimore, MD 21250

Antibiotic resistant bacteria have become a prevalent threat to public health. There has been a steady rise in the number of antibiotic resistant infections reported each year. A contributing factor is the overuse of antibiotics by patients and over-prescription of antibiotic drugs by doctors. With the increasing spread of bacteria that can withstand the drugs meant to eliminate them, it is imperative for the medical and scientific community to locate the bacteria that contain such genes. The goal of this experiment was to test for the presence of antibiotic resistance genes *bla* and *ampC* in three local water sources. Water bodies were chosen to see if there was a correlation between the size of the body of water and the number of resistance genes present. UMBC's Library Pond was the small size, Bynum Run Park Pond in Bel Air was the medium size, and Centennial Lake in Ellicott City was the large size. Polymerase chain reaction (PCR) was performed to identify the presence of two  $\beta$ -lactamase resistance genes. The *ampC* gene was found in the library pond. High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used to investigate the presence of ampicillin in the library pond, however ampicillin was found to be below the limit of detection. A Gibson Assembly reaction was performed to clone the *bla* gene to be used in future experiments for  $\beta$ -lactamase protein characterization. A HPLC-MS/MS method was conducted and verified to be used for protein sequencing in future experiments. In the future, this research will likely focus on detecting other antibiotic resistance genes in water samples, and further exploration to determine if a correlation exists between the size of a body of water and resistance gene prevalence.

**Acknowledgements:** This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5RL5GM118987, and 5UL1GM118988). We would also like to thank our peer mentors Ilzat Ali and Courtney Colson.

**INVESTIGATION OF THE PROTEIN-PROTEIN INTERACTIONS AND  
FUNCTION OF THE CHROMATIN ASSOCIATED PROTEIN Set4 IN  
SACCHAROMYCES CEREVISIAE**

Shandon Amos, Eric Garcia, Yogita Jethmalani, and Erin M. Green

Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Set4 is one of the twelve SET domain-containing proteins in *Saccharomyces cerevisiae*, which are known or putative lysine methyltransferases. Under normal conditions Set4 is expressed at very low levels, however, overexpressing Set4 has been shown to adversely affect cell growth and to promote the induction of stress responses. Set4 has both a PHD finger and a SET domain, which are commonly found in proteins that regulate chromatin dynamics. In other proteins, the PHD finger is known to mostly bind histones at modified lysine residues. The PHD finger of Set4 does not have a known binding partner. We hypothesize that the PHD finger is important for the recruitment of Set4 to chromatin and aim to identify its binding partner. We first performed a genetic analysis in which we knocked out several common methyl- and acetyltransferases in order to see if Set4 activity is compromised in the absence of any of these modifications. Our preliminary results show Set4 does not depend on the methylated or acetylated lysine residues that we tested. We are performing additional mutational analysis of the PHD finger to identify binding partners. In previous experiments, Set4 has been shown to play a protective role in the cell under oxidative stress conditions by upregulating stress response genes. Sfl1 is a regulator of stress response genes during oxidative stress. We are performing genetic interaction assays and gene expression analysis to determine whether or not Set4 and Sfl1 regulate similar sets of genes and if they function cooperatively. In the future, we aim to test the role of the PHD finger in gene expression regulation by Set4. This research contributes to the broader understanding of the mechanisms that protect cells during oxidative stress and will identify new molecular roles for the chromatin protein Set4.

This research was supported in part by a grant to UMBC from the Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program.



## **TAGGING THE CD44 INTRACYTOPLASMIC DOMAIN WITH THE FLUORESCENT iLOV DOMAIN**

Wendy Anyona, Karl E. Milette

Department of Biological Sciences, Delaware State University, 1200 N DuPont Hwy, Dover, DE 19901

We have previously tagged the CD44 intracytoplasmic domain (CD44-ICD) with the green fluorescent protein (GFP), and used this fluorescent fusion protein in imaging studies as well as for chromatin immunoprecipitation (ChIP) assays. GFP has been widely used as a fluorescent tag for many different proteins. The GFP length of 238 aminoacids (aa) makes it an ideal tag for a typical eukaryotic protein size (~400 aa) or larger. However, for smaller proteins or polypeptides, the use of this tag is controversial due to the addition of a domain that sometimes is even larger than the original untagged protein. This is the case of the CD44-ICD, which with only 72 aa in length, is about three times smaller than the relatively large GFP protein (238 aa, ~25 kDa). Therefore, the generation of a CD44-ICD tagged with a small fluorescent protein is a wanted improvement. For that purpose we decided to use the iLOV fluorescent protein. The LOV (light, oxygen or voltage) domain, derived from a plant blue light receptor is about half size of GFP (~11 kDa) and with 111 aa long is not much larger than the CD44-ICD. Making use of a considerable large multi-cloning site in a commercially available plasmid carrying the iLOV domain, we will test three different "connecting bridge" lengths between the iLOV domain and the CD44-ICD. We have already PCR amplified and purified the three CD44-ICD inserts, which we expect to clone in the iLOV plasmid by homologous recombination. Ultimately, we expect to identify an iLOV/CD44-ICD fusion protein with enhanced imaging capabilities, which in part will help us validate the GFP/CD44-ICD fusion protein data.

This project was funded by NSF HBCU-UP RIA, Grant No.1700228 and Delaware Economic Development Office, Grant No.103

## ANALYSIS OF ANTIBIOTIC RESISTANCE GENES *AMP<sup>C</sup>* AND *BLA* FOUND IN LOCAL WATER SAMPLES

Shehar Y. Awan<sup>1</sup>, Miglanche Ghomsi Nono<sup>1</sup>, Zulphiya Iskander Harder<sup>1</sup>

Caitlin Kowalewski<sup>1</sup>, Joshua Wilhide<sup>1</sup>

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The abuse of antibiotics through overuse in healthcare, misuse in agriculture, and inconsistent use by patients have allowed bacteria to develop resistance past the capacity to develop new drugs to combat pathogenic microbes. The prevalence of antibiotic resistant infections is no longer confined to health care settings; in fact, community acquired antibiotic resistant infections have been on the rise. As these drugs spill into the environment, there is a growing concern for the development of widespread resistance. Thus, it is necessary to monitor their environmental levels, study their effects on the prevalence of resistant genes, and analyze the proteins produced to understand any underlying correlations. This project focuses on testing several environmental water samples for the presence of antibiotic resistant genes and antibiotics—specifically in the greater Baltimore area. Bacterial DNA was extracted from the water samples and the presence of two  $\beta$ -lactamase producing genes *amp<sup>C</sup>* and *bla*, was determined through polymerase chain reaction (PCR). High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was utilized in order to detect the presence of ampicillin in the Library Pond water sample. Gibson Assembly was used to clone the *bla* gene and HPLC-MS/MS was used to verify a method for protein sequencing in future experiments. The results indicated a possible presence of *amp<sup>C</sup>* and *bla* in UMBC Library Pond, and *amp<sup>C</sup>* in Lake Kittamakundi. Ampicillin was shown to be below the limit of detection in UMBC Library Pond. Based off the collected data, it was hypothesized that other factors were at play between the presence of ampicillin and antibiotic resistance genes. Furthermore, this suggested that continued experimentation would be necessary to draw any conclusions behind the correlation of the presence of antibiotic resistant genes, antibiotics, and the rise of community acquired resistant infections.

**Acknowledgements:** This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5RL5GM118987, and 5UL1GM118988). We would also like to thank our peer mentors Ilzat Ali and Courtney Colson.

## **TAQ ATTACK: ISOLATION, PURIFICATION AND CHARACTERIZATION OF TAQ DNA POLYMERASE I**

Madeleine Beaulieu<sup>1</sup>, Kelly Healy<sup>1</sup>, Elizabeth Hill<sup>1</sup>, Linnea Lundh<sup>1</sup>,  
Shanen Sherrer<sup>1</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, St. Mary's College of Maryland,  
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Polymerases rely on divalent cations like  $Mg^{2+}$  to stabilize negatively charged phosphate groups to catalyze DNA polymerization reactions. *Taq* DNA Polymerase I (*Taq* Pol), a polymerase from the thermophilic bacteria *Thermus aquaticus*, has DNA polymerization as well as 3' to 5' exonuclease activity. Its functionality has been optimized for commercial use by modifying the primary structure of the protein through directed mutations in the *Taq* gene. We approached our research through the focus question, "how does the presence of divalent cations, including  $Mg^{2+}$ , affect the ability of *Taq* Pol to accurately and effectively produce complementary DNA polymers using a template DNA strand?" *Taq* Pol was generated via recombinant cloning using *Escherichia coli*. The resulting protein was then isolated and purified through use of column chromatography and dialysis, yielding approximately 6 mg of *Taq* Pol. The presence and activity of the protein were confirmed by western blot and PCR, respectively. PCR was used to compare the activity of *Taq* Pol in the presence of  $Mg^{2+}$ ,  $Mn^{2+}$ , and  $Zn^{2+}$ . Finally, an EMSA was used to quantify the binding affinity of the isolated *Taq* Pol to DNA. The western blot showed that a His-tagged protein with a molecular weight between 75 and 100 kDa was isolated and became purer as we continued with the purification scheme. PCR revealed that  $Mn^{2+}$  and  $Zn^{2+}$  amplified the exonuclease activity of *Taq* Pol, and the ideal concentration for use in PCR is approximately  $0.5\mu M$ . Future research should focus on the optimization of the concentrations of *Taq* Pol to get the optimal amount of polymerase activity with little to no exonuclease activity in the reactions. Researchers should also focus on the effects of other divalent cations, such as  $Pb^{2+}$  and  $Cd^{2+}$ , on polymerase and exonuclease activity in *Taq* Pol.

Thank you to Dr. Sherrer and her lab staff for a well run laboratory. Thank you to the SMCM Biology department for generous sharing of instruments and equipment. Thank you to all the professors and students who helped us prepare by giving feedback on presentations.

## DESIGNING REDOX SHUTTLES FOR SALT-TOLERANT MICROBIAL FUEL CELLS

Kevin Beaver<sup>1</sup>, Matteo Grattieri<sup>2</sup>, Zayn Rhodes<sup>1</sup>,  
Shelley D. Minteer<sup>2</sup>

<sup>1</sup>Departments of Chemistry and Biology, Lebanon Valley College,  
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Saline wastewater constitutes a sizable fraction of the total amount of wastewater produced worldwide, and industrial developments are expected to increase this proportion in the future. While bacteria are typically used for the biological treatment of wastewater, high salt concentrations can inhibit this process due to cell dehydration, causing bacteria death. However, some bacterial species have developed the capability to tolerate high salinity, opening for their application in saline wastewater treatment. *Rhodobacter capsulatus* (*R. capsulatus*) is a photosynthetic purple-bacterium that may be able to withstand high salinity, due to an evolved mechanism where compatible solutes or ions are accumulated intracellularly to balance osmotic pressure in saline solutions. By establishing electron transfer between this bacterium and electrodes, a microbial fuel cell can be built, allowing the harnessing of electricity while degrading contaminants. As a result, a self-powered treatment for saline wastewater could be envisioned, where the only necessary energy input is light. More remarkably, the generated current could be used to monitor the decontamination process. *R. capsulatus* has not been reported to establish an effective transfer of electrons directly to the electrode of a microbial fuel cell, probably due to its unique membrane structure. However, mediated electron transfer can be performed, where a redox intermediate is used to shuttle electrons from the bacteria to the electrode surface. A wide variety of benzoquinone substitutes can act as a mediator for this process, competing with the quinone center in the *R. capsulatus* electron transfer chain. Herein, we aimed to determine the required properties in a mediator for enhanced electron transfer between *R. capsulatus* and a carbon electrode. Accordingly, several soluble benzoquinone analogs were utilized experimentally to study mediated electron transfer performance. Finally, the data collected were used to create a computational model that predicts the effectiveness of a mediator based on its properties.

National Science Foundation REU Grant

## EXAMINATION OF UV SENSITIVITY IN HAPLOID YEAST CELLS AND SPORES OF SACCHAROMYCES CEREVISIAE

Grace Beecher, Christopher Cortes, Mary Grace Murray, Nathan Navarro, Elizabeth Walton, and Edward Winter, Aikaterini Skokotas

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Yeast spores are known to be resistant to many environmental factors including the mutagenic effects of UV light. This study compares the UV sensitivity of haploid cells and spores of *Saccharomyces cerevisiae*. Both cells and spores were exposed to UV radiation at different time intervals and their ability to survive following UV exposure was examined. Our results suggest that dityrosine present in the outer spore wall protects yeast spores from the mutagenic effects of UV light when contained within the ascus. However, treatment with glusulase to release the spores from the ascus resulted in free spores that were highly sensitive to UV light. Only 1% survival was observed after a 20 sec UV exposure compared to 50% survival for spores that remained in the ascus. This suggests that the ascus provides protection by allowing some spores to hide from UV exposure. Lastly, UV exposure of the haploid strain produced similar survival rates as the spores contained in the ascus.

## EXPLORING THE EXISTENCE OF ANTIBIOTIC RESISTANCE IN FRESHWATER ENVIRONMENTS IN MARYLAND

Daniel Bellanton<sup>1</sup>, Ena Oboh<sup>1</sup>, Victor Omoniyi<sup>1</sup>

Caitlin Kowalewski<sup>2</sup>, Joshua Wilhide<sup>2</sup>

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Antibiotic resistance has become increasingly prevalent in healthcare settings globally. The continuous rise in antibiotic resistance can be due to the mis- and overuse of antibiotics by physicians and patients. *AmpC* and *bla* are common antibiotic resistance genes that produce an enzyme ( $\beta$ -lactamase) that makes them impervious to the effects of penicillin-family antibiotics. This experiment aimed to investigate the spread of antibiotic resistance in freshwater bodies around Maryland. The prevalence of resistance genes was investigated in correlation with populational impact, such as pollution and rural vs. urban locations. It was hypothesized that the bodies of water found in high population areas would show a greater presence of antibiotic resistance genes due to higher pollution, and overall interaction between people and lakes/ponds. Polymerase chain reaction (PCR) was used to detect the presence of *ampC* and *bla* genes from three locations: Friends Park Pond in Forest Hill, Centennial Lake in Ellicott City, and Library Pond at UMBC, Baltimore. Antibiotic resistance was only detected in Friends Park pond. The use of high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) on the Friends Park water sample was conducted to see if ampicillin was present which could have led to antibiotic resistance among the bacteria. Ampicillin was below the limit of detection in the Friends Park water sample. A  $\beta$ -lactam antibiotic resistance gene was then cloned to further characterize the associated  $\beta$ -lactamase enzyme. After the cloning, the HPLC-MS/MS method was conducted for the protein sequencing of a standard  $\beta$ -lactamase enzyme to be used for protein verification in the future. Future directions of this project will include testing the cloned  $\beta$ -lactamase enzyme and its effectiveness against different antibiotic drugs, and conducting further experiments on additional water sources to verify the proposed correlation between highly populated areas and increased presence of resistance genes.

**Acknowledgements:** This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5RL5GM118987, and 5UL1GM118988). We would also like to thank our peer mentors Ilzat Ali and Courtney Colson.

## DEVELOPMENT OF A MITOCHONDRIAL DNA ASSAY FOR SNP SCREENING USING PCR HIGH-RESOLUTION MELT CURVES

Allison Bender, Kelly Elkins

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The analysis of human DNA is vital in the forensic field to identify suspects and human remains. This analysis is often performed on nuclear DNA found at crime scenes, but it is often necessary to analyze mitochondrial DNA when there are only bones, hair, and teeth present at a scene. Mitochondrial DNA (mtDNA) is found on a small, circular chromosome within the mitochondria, and is less likely to degrade when compared to nuclear DNA due to its smaller size. This is ideal for rapid screening where nuclear DNA is too degraded, such as cold cases and natural disaster cases. Current methods for analyzing mtDNA include post-PCR capillary and gel electrophoreses, but these methods can be time consuming and costly. This ongoing study proposes a more rapid and less costly method of analyzing mtDNA single nucleotide polymorphisms (SNPs) via real-time PCR coupled with high-resolution melt analysis. Selected regions within human mtDNA, include the hypervariable I, II, and III regions, have been identified as showing differences within the human population, and have a high SNP prevalence. SNPs are commonly found within the human genome, approximately 1 every 300 bases, and have low mutation rates that make them reliable for human genotyping. SNP primers have been chosen based on their documented high frequency in MITOMAP and they have been tested using purchased mtDNA standards. Melt curves for SNP 16519 showed differentiation with tested DNA standards and clustered in two groups using principal component analysis.

This research is supported by a TU FDRC grant to KME.

## INVESTIGATING THE CORRELATION OF THE PRESENCES OF ANTIBIOTIC RESISTANCE GENES IN DIFFERENT ECOSYSTEMS

Bitania Berhane<sup>1</sup>, Anoosh Rehman<sup>1</sup>, Kaamil Shakir<sup>1</sup>

Caitlin Kowalewski<sup>1</sup>, Joshua Wilhide<sup>1</sup>

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Antibiotic resistant bacteria are bacteria that have grown immune to antibiotics. The rate at which antibiotic resistant bacteria is growing poses a threat to everyone. The amount of antibiotic resistant infections has increased as a result of overprescribing antibiotics to patients, the overuse of antibiotics with livestock, and patient misuse. The presence of antibiotic resistant bacteria in the environment has also increased, exposing more people to the bacteria. The goal of this project was to investigate different freshwater environments that could be potential hosts to antibiotic resistant bacteria. Antibiotic resistance genes were detected in local water sources from Maryland to understand how they spread, and if there is a relationship between the presence of antibiotic resistance genes and different ecosystems. PCR was utilized to detect two  $\beta$ -lactam antibiotic resistance genes (*ampC* and *bla*) in three water samples from around Maryland: Lake Kittamaqundi, Bynum Run Park, and Pig Pen Pond. The *bla* gene was detected in the Lake Kittamaqundi sample. With the help of high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), the presence of ampicillin was analyzed. In Lake Kittamaqundi, ampicillin was found to be below the limit of detection. To further characterize the  $\beta$ -lactamase enzyme produced by *ampC* and *bla*, the *bla* gene was cloned. A HPLC-MS/MS method was conducted and verified for protein sequencing of the  $\beta$ -lactamase protein in future experiments. Further investigations are required to better understand if there is a correlation between the different ecosystems and the spread of antibiotic resistance genes.

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## VIABILITY OF PROBIOTICS IN YOGURT DURING *IN VITRO* DIGESTION

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Yogurt is a cultured milk product made by adding lactic-acid producing probiotics to milk. *Lactobacillus acidophilus* is a common probiotic bacteria found in yogurts. These probiotics not only aid in the culturing of milk but also play a vital role in promoting a healthy digestive tract. Consuming probiotics can help prevent constipation, infections, irritable bowel disease and other colon diseases. If these probiotic bacteria can survive the acidity of the digestive tract then they can better perform their role in digestive health. In this experiment, Dannon® and Great Value® yogurts were digested *in vitro*, prior to, on and after their expiration date. Following gastric and intestinal digestion phases, 0.1 mL aliquots were plated on De Man, Rogosa and Sharpe (MRS) agar plates and incubated for 24 h at 37°C. Undigested yogurt samples (controls) were serially diluted and 0.1 mL of 10<sup>-1</sup> through 10<sup>-6</sup> dilutions were also plated and compared with the digested samples. Colonies were counted and analyzed. A decrease in the colony count in the gastric phase, implies that probiotics were unable to survive gastric conditions. Initial results showed no significant change in the number of *L. acidophilus* in the gastric phase and reduction in the number of *L. acidophilus* counts in the intestinal phase. Conclusions drawn from these results can help consumers decide if brand name and generic yogurt probiotics share similar probiotic viability.

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**CNT FIBER BASED GLUCOSE BIOSENSOR**Jessica Bonilla<sup>†</sup>Jandro L. Abot<sup>‡</sup><sup>†</sup>Trinity Washington University, Michigan Ave NE, Washington, DC 20017<sup>‡</sup>Mechanical Engineering, Catholic University of America, Michigan Ave NE, Washington, DC 20064

Carbon nanotube fibers are made of multiple nano-yarns, bundled and twisted together, and can be used as piezoresistive sensors to measure strain, to detect damage and as biosensors or chemical sensors. They can also serve as reinforcements in composites and used in many other applications. In this specific project, a biosensor was built to sense chemicals, specifically glucose. The CNT fiber end was immobilized with a fresh enzyme, glucose oxidase, to target the glucose molecules in a solution. The biosensor was induced into different concentration amounts of glucose solutions to measure the current through amperometry experiments. The biosensor sensed the glucose inside the solution and its concentration correlated in a linear way with the current. In a real-life application, glucose oxidase helps target the glucose molecules on the end of the fiber which can help to know the glucose levels in the blood. Using the same principles, piezoresistive CNT fibers would be developed into sensors to monitor other substances for a variety of applications.

## THE EFFECT OF POLYCOMB REPRESSIVE COMPLEX 2 INACTIVATION ON MALIGNANT PERIPHERAL NERVE SHEATH TUMOR FORMATION

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Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome associated with malignant peripheral nerve sheath tumor (MPNST) and neurofibromas, a benign precursor lesion. Despite extensive investigation into developing novel MPNST therapies, studies using an Nf1/p53 MPNST genetically engineered mouse (GEM) model have failed to translate to the clinical effects. Recent advances in the molecular genetics of MPNSTs have identified three mutations frequently linked to MPNST progression: (1) NF1, which is associated with neurofibromas, (2) CDKN2A, which encodes INK4A and ARF, and is associated with malignant transformation of neurofibromas to MPNSTs, and (3) SUZ12 or EED, members of polycomb repressive complex 2 (PRC2), associated with MPNST aggressiveness. We predict that developing an MPNST model that targets these three core pathways will produce aggressive MPNSTs that represent a model better recapitulating human disease. To test this prediction, we have developed a Nf1/Cdkn2a GEM model with or without Eed heterozygous conditional deletion. After isolating MPNSTs that form in these mice, we used genetic approaches to demonstrate recombination, and investigate whether Eed loss of heterozygosity occurs. In order to diagnose the MPNSTs, we performed haematoxylin and eosin (H&E) to identify different characteristics associated with MPNSTs, including fasciculation, nucleic atypical, mitotic figures and necrosis. We next performed immunohistochemistry (IHC) to detect S100, a marker of MPNSTs, verifying tumor identity. To assess tumor aggression, we labeled MPNSTs for Ki67, a marker of cell proliferation, and compared proliferation between Nf1/Cdkn2a and Nf1/Cdkn2a/Eed models. Based on qualitative assessment of Ki67 frequency, Eed inactivation did not have a significant effect on MPNST aggression. Based on these data, we propose that PRC2 inactivation may contribute to tumor aggressiveness and provide a novel model for translational studies of human MPNST.

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## **DIETARY POLYPHENOLS AND SULFORAPHANE: IMPACT ON CELL PROLIFERATION, APOPTOSIS, AND METASTASIS IN COLON CANCER**

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The polyphenols resveratrol, epigallocatechin-3-gallate (EGCG) and chrysin are secondary plant metabolites, and are found in many different fruits and vegetables, as well as in beverages including red wine and tea. The dietary compound sulforaphane is an isothiocyanate, and is most commonly consumed via cruciferous vegetables such as broccoli and brussels sprouts. Compelling studies have been conducted which indicate that these dietary compounds potentially play a role in cancer prevention when tissues are directly exposed to them. Our goal is to determine if these dietary compounds impact gene expression, particularly of genes known to influence some of the hallmarks of cancer. Investigation of these preventative traits may lead to targeted cancer therapies and a better understanding of the conditions that regulate the hallmarks of cancer. Murine colon cancer CT26 cells are exposed to 15  $\mu$ M resveratrol, EGCG, chrysin, sulforaphane (5 $\mu$ M), or DMSO vehicle control for 24 hours. Subsequently, mRNA is isolated using Trizol/chloroform extraction, and cDNA is generated using reverse transcriptase. Expression changes of genes relating to cell cycle regulation, apoptosis, and metastasis are quantitated using real-time RT-PCR. Data are analyzed via ANOVA, and  $p < 0.05$  is considered statistically significant. Our preliminary results show interesting trends in gene expression changes related to apoptosis and metastasis for some of the dietary compounds investigated. For example, Sulforaphane appears to slightly increase mRNA expression of the apoptotic regulator bax ( $p < 0.05$ ), as well as dramatically decrease expression of the matrix metalloprotease, mmp9 ( $p < 0.001$ ), suggesting potential cancer preventive properties. We will continue to investigate various commonly eaten dietary compounds and their possible impacts on genes relating to the hallmarks of cancer.

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## FDG AND FTT UPTAKE IN RESPONSE TO PARP INHIBITION AND DNA DAMAGE

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Poly-(ADP)-ribose polymerase (PARP) is an enzyme that catalyzes DNA damage repair. PARP inhibitors (PARPi) have been developed currently to treat breast and ovarian cancers and are especially effective in cancers containing a BRCA mutation. BRCA mutations limit the ability to repair double stranded DNA breaks. In these cells, PARP is heavily relied upon to seal single stranded nicks before lethal double stranded DNA breaks occur. By inhibiting PARP activity, cancer cells must rely on the more error-prone non-homologous end joining to repair breaks in the DNA, leading to a high level of mutagenicity and eventually cell death.

F-18-FluorThanatrace ([18F]FTT) is a radio-labelled analogue of the PARPi rucaparib; it is currently in clinical trials as a tumor imaging agent and predictive marker of clinical response to PARPi therapy. Early trial results have shown some unexpected cases of discordant [18F]FDG (a measure of glucose metabolism) and [18F]FTT uptake in sites of disease in response to treatment. To better understand this, we undertook to measure [18F]FDG and [125I]KX1 (an iodinated analogue of [18F]FTT optimized for in vitro studies) uptake in breast and ovarian cancer cell lines in vitro at baseline, in response to DNA damaging agents cisplatin and doxorubicin and the PARPi rucaparib.

While cytotoxic therapy predictably reduced [18F]FDG uptake and rucaparib reduced binding of [125I]KX1, rucaparib treatment unexpectedly increased [18F]FDG uptake in ovarian cancer cells. These findings are important for understanding the clinical results of [18F]FDG and [18F]FTT scans as well as to better elucidate the mechanism of action and potential resistance pathways for PARPi therapy. Future studies on the topic should focus on the specific metabolic and cell cycle effects of cytotoxic and PARPi therapy, as well as clarifying the ability of [18F]FTT to monitor and predict the response of patients to PARPi therapy.

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## IMPACT OF THE OBSCURIN-PH DOMAIN IN CELL GROWTH, MOTILITY, AND INVASION

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Obscurins are a family of giant cytoskeletal proteins, which, upon loss lead to cellular transformation and tumor potentiation of breast epithelium via deregulation of various cellular mechanisms. One such mechanism is the interaction between the Pleckstrin homology (PH) domain of obscurins and the p85 regulatory subunit of phosphoinositide-3 kinase (PI3K). Upon loss of obscurins and consequently the loss of their PH domain, the PI3K pathway becomes activated resulting in the upregulation of various downstream proteins that promote motility, growth, and invasion. Specifically, previous studies show that over-activation of PI3K results in conversion of epithelial cells to a more mesenchymal phenotype (epithelial-to-mesenchymal transition), which increases the ability of cells to be motile and invade other tissues. We sought to show that restoration of the obscurin PH-domain in obscurin-knockdown cells results in reversion of these characteristics. To test this hypothesis, we transfected shRNA mediated obscurin knockdown MCF10A cells with an expression vector containing the obscurin PH-domain fused to a myristoylation tag (myr-PH) that ensured its proper targeting to the cell membrane. We confirmed successful expression of myr-PH via Western blot and membrane localization via immunofluorescence. In the future, we will conduct an in vitro scratch assay to examine whether expression of myr-PH suppresses cell motility.

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## CHARACTERIZATION OF THE ALLOSTERIC REGULATION OF BAM2 IN ARABIDOPSIS THALIANA

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Starch is an important component of the human diet as it's broken down into glucose to provide energy for metabolic reactions in the body. Plants are the main sources of starch, which is metabolized in chloroplasts for energy production at night.  $\beta$ -amylases (BAMs) hydrolyze starch into disaccharide maltose and are important for day-night metabolism and the response to stress in plants. Understanding starch regulation by BAMs could potentially improve food crop quality and industrial processes like the production of bread and beer. This will benefit those suffering from food insecurity and nutritional deficiencies.

*Arabidopsis thaliana* contains nine BAMs that show distinct catalytic activities and functional regulation based on small sequence variations and domain composition, 4 of which are chloroplastic, including our main focus, BAM2. This enzyme was found to be a tetramer and appears to contain a starch binding site which allosterically regulates BAM2 activity. The goals of this study are to characterize the allosteric regulation mechanism of BAM2 activity by starch and to elucidate its structure. We purified BAM2 and characterized the activity using a novel fluorescence assay involving a maltose binding protein fused to a green fluorophore (MBP-GFP) that we developed. Concomitantly, we homology modeled BAM2, as a tetramer based on previous experimental studies, using YASARA. We performed simulations of the model under 3 conditions, apo, starch-bound, and BAM2 containing a serine to glycine mutation which experimentally appears to change the allosteric mechanism to favor the activated state. We compared the protein dynamics and identified similarities between the starch-bound enzyme and the S to G mutant, supporting the idea that this serine is important in transitioning between active and inactive BAM2 upon starch binding. Future work includes supporting our computational data using kinetic assays to further tease out the regulatory mechanism of BAM2.

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## DETERMINING THE PRESENCE OF *AMPC* AND *BLA* BACTERIAL RESISTANCE GENES IN WATER SAMPLES

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Antibiotic resistance occurs when bacterial cells exhibit an increased defense against drugs used to treat bacterial infections. Resistant bacteria pose a potentially dire situation to worldwide health and medicine. Higher prevalence in resistant bacteria entails greater infection rates and significant increase of disease in populations across the world. Although hospitals have become the epicenter of antibiotic resistant bacteria, bacteria within the surrounding environment are gaining resistance to top-tier antibiotics at terrifying rates. The goal of the project was to see if prevalence of antibiotic resistance genes increases with bodies of water in relation to relative size. Water samples collected from Country Village Pond, Pig Pen Pond, and Centennial Lake were tested using polymerase chain reaction (PCR) in order to identify antibiotic resistance genes. The results revealed that the  $\beta$ -lactamase gene was found in two of the three water samples from Pig Pen Pond and Country Village Pond. Mass spectrometry and cloning procedures were performed for protein characterization of the  $\beta$ -lactamase enzyme. Using high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) the presence of the ampicillin compound was tested in the Centennial Lake water sample, however the compound was determined to be below the limit of detection. The potential directions of the project include testing the enzyme's capabilities against antibiotics and analyzing more resistance genes.

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## **BIOCHEMICAL EVIDENCE OF A NOVEL STRUCTURAL ELEMENT CHARACTERISTIC IN THE DIMER CONFORMATION OF THE HIV-1 GENOME**

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The human immunodeficiency virus (HIV) affects over thirty million people worldwide and is the causative agent for the acquired immunodeficiency syndrome (AIDS). The viral RNA genome of HIV folds to a monomer or dimer conformation contingent on the highly conserved 5' leader and each conformation fulfills a distinct niche in the HIV replication cycle. Monomeric RNA serves as traditional mRNA that is translated into viral protein while the dimeric RNA is packaged as the genomic material for daughter virions. We use nuclear magnetic resonance (NMR) spectroscopy to study the monomer and dimer conformations and our studies suggest a novel stacking interaction characteristic of the dimer conformation. Specifically, NMR spectroscopy studies of the dimer conformation provide evidence for a TAR-polyA stacking interaction in the 5' leader that is believed to be sequestering the 5' guanosine cap. We aim to explore the biochemical implications of the sequestered cap in the dimer-specific stacking interaction by employing a recombinant decapping-exonuclease mechanism that is native to humans. The catalytic subunit of the human decapping complex, hDcp2, was used to remove the 5' guanosine cap, followed by an incubation with XRN-1 5' monophosphate-specific exonuclease. Through gel electrophoresis, we visualized the degradation of each construct. The monomer showed significantly more degradation than the dimer, further supporting the proposed stacking interaction. With this structural information, possible inhibitors could be developed as long-lasting therapies for the HIV-1 virus.

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## **DIFFERENTIAL GENE EXPRESSION IN THE TARDIGRADE TUN STATE: OSMOTIC STRESS VERSUS DEHYDRATION**

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Tardigrades are small organisms that can transform into a cryptobiotic state referred to as a “tun”. In this state, the tardigrades effectively suspend their metabolism and have a high resistance to environmental stressors such as ultraviolet light, gamma rays, high pressures, and the vacuum of space, along with other harsh environmental conditions that would be fatal to other forms of life. Though little is known about the molecular details of the transition into the tun state, a class of intrinsically disordered protein has recently been identified that is thought to protect the animals from external stress and mechanical damage. This research focused on comparing the effects of slow dehydration to those from osmotic stressors (sucrose and sodium chloride) in the tardigrade species *Hypsibius dujardini*. In this project, the expression levels of the genes APQ10, CAHS2, and SAHS8 was determined using qRT-PCR. These results were compared with global expression obtained using RNA sequencing (RNA Seq).

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## ANALYSIS OF ANTIBIOTIC RESISTANCE GENES IN FRESHWATER SAMPLES FROM HARFORD COUNTY, MD

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The rise of antibiotic resistance genes (ARGs) that are found in bacteria has been a prevailing issue across the world. Not only have microbes containing ARGs been associated with higher healthcare costs, but antibiotic resistant microbial infections have been linked to increasing morbidity and mortality rates. ARGs can spread via agricultural, water treatment, tourism, migration, etc. Furthermore, sub-inhibitory concentrations of heavy metals have been associated with increased potential of ARG transfer between bacterial populations. This study aimed to identify the presence of ARGs *ampC* and *bla* in water samples collected from varying sources, all of which were located within Harford County, MD: Friends Park Pond (FPP), Country Village Pond (CVP), and Bynum Run Park Pond (BRP). Identification of aforementioned ARGs was accomplished with the utilization of polymerase chain reaction (PCR). *ampC* was present in FPP and CVP water samples. *ampC* is known to confer resistance to the antibiotic, ampicillin, therefore the presence of ampicillin in FPP was tested via high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Ampicillin was below the limit of detection (LOD). Additionally, sub-inhibitory concentrations of copper in the water samples were investigated via inductively coupled plasma mass spectrometry (ICP-MS) in an effort to discover correlations between heavy metal concentrations and increased levels of ARGs in water samples. The *bla* gene was then cloned to perform protein expression and analysis of the corresponding  $\beta$ -lactamase protein that will be used in future experimentation. HPLC-MS/MS was also utilized to analyze the protein sequence of digested  $\beta$ -lactamase for purposes of protein verification in future experiments. Future directions include the examination of other pond sources to determine the extent of the spread of ARGs, and determining correlations between other metals and resistant bacteria.

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## CHARACTERIZATION OF HALOTAG FLUORESCENT LIGANDS IN PLANT AND ANIMAL MODEL SYSTEMS

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The isolation of green fluorescent protein (GFP) from the jellyfish, *Aequorea victoria*, revolutionized cell biology by making it possible to specifically tag proteins to visualize their localization and dynamics by light microscopy. Decades of research led to the availability of a wide variety of fluorescent proteins (FPs) with different spectral characteristics. Despite these efforts, FPs still suffer from low brightness and photostability compared to fluorescent dyes. To overcome this limitation, genetically-encoded tags that bind to fluorescent dyes have been developed. One of the most widely adopted is the HaloTag that is a modified haloalkane dehalogenase that irreversibly binds a ligand. The ligand can be conjugated to a fluorophore for in vivo and in vitro detection or to biotin for protein purification and interaction studies. The HaloTag combines the advantages of FPs and fluorescent dyes. Like FPs, it only requires a single genetically-encoded fusion construct, but it has the superior photophysical characteristics of fluorescent dyes. This powerful approach has been difficult to use in some organisms, such as plants, due to low cell permeability of the fluorescent ligands. Here we show that a newly developed HaloTag fluorescent ligand called JF525 has high permeability in plants compared to Promega TMR ligand that is most often used. This Janelia Fluor (JF), and other variants based on rhodamine with azetidine substituents, were developed by Luke Lavis and were previously shown by his group to have increased cell permeability in mammalian cells. We verified this property of JF525 in HEK293T cells, and other JF dyes with various spectral characteristics. Overall, the JF dyes extend the advantages of HaloTag to plants and provide new, superior fluorescent ligands for researchers using mammalian cells.

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## EFFECTS OF MIR-34A ON NEUROBLASTOMA CELL SENSITIVITY TO RADIATION TREATMENT

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Neuroblastoma (NB) is a neuroendocrine cancer that predominantly affects infants and children under age five. Radiation therapy has been successful in eliminating tumor cells in NB; however, its side effects and long-term health effects after treatment are more pronounced in children. MYCN amplification has been correlated with more therapy resistant forms of NB and poor prognosis. miR-34a has been identified as a tumor suppressor, in part due to its ability to downregulate MYCN translation. However, combined effects of miR-34a and irradiation on NB cells requires further study. Our research investigates the clinical significance of using both miR-34a and radiation treatment to increase NB cell sensitivity to radiation treatment to further reduce NB cell viability and proliferation. The treatment of cells with combined miR-34a and radiation treatment is hypothesized to decrease NB tumor cell viability and proliferation. For all experiments, we used two distinct NB cell lines: SK-N-AS (AS), radiation resistant, and SK-N-DZ (DZ), radiation sensitive. Effects of miR-34a on cell viability were measured to determine the clinical significance of combined miRNA and radiation treatment by MTS assay. Colony formation assays were conducted by transfecting AS and DZ cells with a scramble miRNA sequence, pre-miR34a, and anti-miR34a at two radiation treatment levels of 0Gy (control) and 5Gy, including an empty control without transfected miRNA. The combined use of miR-34a and radiation treatment were found to potentially affect AS cell proliferation and the radiation treatment alone was found to affect DZ cell proliferation; further analysis and verification of mRNA levels by qPCR will be conducted to validate these results.

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**CHARACTERIZATION OF THE HUMAN UFM1 ACTIVATING ENZYME, UBA5**

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Many common diseases, such as diabetes type II and heart disease, are directly affected by endoplasmic reticulum (ER) stress. The ubiquitin-like protein post-translational modification ufm-ylation is linked to ER stress, however the functional details are still being determined. This pathway is initiated by the association of ubiquitin like modifier activating enzyme 5 (Uba5) and ubiquitin fold modifier 1 (Ufm1) to form thioester-linked Uba5-Ufm1 in an ATP dependent reaction. The mechanism for this enzymatic reaction is unknown. Once known there is potential for an ER stress therapy to be developed which can potentially be used for dozens of disease treatments. To understand the mechanism, both human Uba5 and Ufm1 were purified via nickel affinity chromatography and cobalt affinity chromatography, respectively. The activity of this reaction was then tested by gel migration shift assays. Because some experiments require additives which are refractory to these assays we next tested whether the thioester link between Uba5 and Ufm1 was stable in the acidic conditions that occur during trichloroacetic acid (TCA) precipitation. We found that TCA did not affect the thioester, which has not been previously shown. We next performed assays to determine when the protein would be in the steady state for analysis of the kinetics in solvent viscosity assays. These latter experiments will be crucial to find the diffusion limiting steps of the reaction. Interestingly, Uba5 shows kinetics 10-fold slower than E1 enzymes associate with ubiquitin and other ubiquitin-like molecules. These findings will aid in performing advanced mechanistic analysis of Uba5.

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## SECONDARY STRUCTURE ANALYSIS BY SHAPE-MAP OF THE EGFR AND VEGFR2 PRE-MRNA TRANSCRIPTS: UNCOVERING NOVEL REGIONS FOR RNA ANTI-SENSE TARGETED THERAPY

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Glioblastoma multiforme (GBM), a grade IV tumor of the central nervous system, is the most common malignant primary brain tumor, and has a median survival of only 14 months. Poor survival is due to a lack of efficacy in current therapies, which are limited by the blood-brain barrier. A common aberration in GBM is the overexpression and constitutive activation of epidermal growth factor receptor (EGFR), observed in 57% of all GBM. Further, our lab developed a novel therapeutic approach; (Hicks et al. 2016) which delivers a gene directly to the CNS using an adeno-associated virus gene transfer vector to encode either RNA or protein therapeutics. Our current approach is to deliver an RNA molecule with complementarity to critical splicing elements of the EGFR pre-mRNA transcript. Thus, inducing alternative isoforms and driving a reduction in mRNA. Furthermore, alternative splicing is regulated by secondary structure of the pre-mRNA nascent transcript (Soemedi et al. 2017). To improve our therapeutic strategy, we have begun experiments to analyze the EGFR secondary structure using selective 2' hydroxyl acylation and primer extension followed by mutational profiling (SHAPE-MaP). The SHAPE reagent (1M7) reacts with the 2' hydroxyl of RNA molecules when the RNA molecule is in a conformationally flexible position (Weeks et al. 2018) creating a 2' O-adduct. The modified RNA is reverse transcribed, incorporating mismatches at the acylated positions; a comparison of unmodified to modified RNA will allow us to determine RNA nucleotides that are involved in secondary structure, part of RNA-binding-protein complexes, or single stranded. Single stranded RNAs are a preferential target of our therapy.

## CHARACTERIZING THE DYNAMIC CAPSID-SP1 JUNCTION HELIX OF THE HIV-1 GAG POLYPROTEIN AND MATURATION INHIBITOR INTERACTION

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The Human Immunodeficiency Virus (HIV) is a global pandemic affecting millions of individuals world-wide. As a retrovirus, HIV-1's genome consists of RNA, which is reverse transcribed into DNA before being integrated into the host cell's genome. Then, utilizing the host cell's machinery, the newly integrated DNA will be transcribed back into RNA. The unspliced monomeric RNA can then be translated into the Gag polyprotein. The Gag polyprotein has several domains; Matrix, Capsid, and Nucleocapsid, that aid in the proper packaging of the dimeric RNA. Cleavage of the Capsid-Spacer Peptide 1 domain (CA-SP1) serves as a pertinent rate-limiting step during the assembly and maturation process of HIV, in order for the immature virion to become infectious. Before assembly, the CA-SP1 junction is proposed to exist as a random coil. When Gag proteins self-associate to form a 6-helix bundle, the CA-SP1 junction forms a helical structure. Once the maturation process begins, it is hypothesized that the CA-SP1 junction helix exists in a dynamic equilibrium between tight alpha helix and loose random coil in order to mediate the cleavage of the two domains by viral protease. Solution Nuclear Magnetic Resonance (NMR) will be utilized to elucidate the structure and dynamic properties of the CA-SP1 region. By better understanding the dynamic properties in solution, insight can be gained toward the mechanism of maturation inhibitors (MIs) on this region. MIs target the CA-SP1 region to prevent protease from completing the maturation process by stabilizing the junction helix. Through characterizing the dynamic nature of the CA-SP1 junction helix, additional therapies with varied targets can be provided to HIV patients and hopefully increase the effectiveness of treating those infected.

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## ENGINEERING A CYTOCHROME WITH A TUNABLE BANDGAP POTENTIAL

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We are exploring fundamental factors controlling electron flow in proteins and trying to apply these principles to create bio-hybrids that mimic properties of conventional semiconductors. To this end, we developed and extensively characterized 12 point mutations of PpcA, a 3-heme member of the cytochrome c7 family native to *Geobacter sulfurreducens*. These mutations were engineered to influence the redox potential ( $E_m$ ) of the middle heme (heme III) in PpcA by using four different strategies: performing charge reversal mutations, decreasing solvent access to the heme plane with bulky residues, altering the native bis-histidine axial ligation of the heme, and by attempting to form hydrogen bonds with the propionates of the heme. The latter strategy is expected not only to increase  $E_m$  but also to introduce a redox Bohr effect. Out of 12 mutants, 11 were expressed in *E. coli* in sufficient quantities and show thermal stability in temperature-dependent CD experiments comparable to wild-type protein ( $T_m > 90^\circ\text{C}$ ). HPLC-ESI-MS was used to confirm both the purity and the mass of the expressed mutants. Small-angle X-ray scattering confirmed that the mutant proteins were folded correctly and formed the expected compact globular structures. Peroxidase activity assays were used to study flexibility and solvent exposure of heme binding pockets. Optical redox titrations have shown our ability to obtain reliable and reproducible data thereby allowing us to measure the effect of the mutations on the electrochemical properties of all 3 hemes and to understand the underlying principles and viable approaches in tuning relative heme redox potentials. Successful development of this project may lead to biological semiconductors with much smaller footprints and selectively tunable bandgap properties.

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## CHARACTERIZATION OF HUMAN ATPASE P97 – A KEY CONTRIBUTOR TO THE DEGRADATION OF MISFOLDED PROTEINS

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When proteins fold into the wrong three-dimensional structure, the misfolded protein must be degraded before aggregation occurs. Aggregation is the accumulation and clumping together of misfolded proteins and often leads to serious diseases. The ATPase, p97, utilizes the energy from ATP hydrolysis to physically remove the misfolded proteins from cellular membranes. Mutations to p97 have been linked to degenerative disorders such as inclusion body myopathy, Paget's disease of the bone, frontotemporal dementia, and amyotrophic lateral sclerosis (ALS). The study of p97 is of importance because it is a central player in protein quality control and key for effective drug therapy.

Specific mechanisms of interaction between p97 and partner proteins involved in protein quality regulation are still not well understood. Studies of large complexes such as p97 and its protein partners are challenging. Nuclear magnetic resonance (NMR) spectroscopy is a powerful technique to study the interface between p97 and its protein partners. In particular, <sup>77</sup>Selenium NMR is a novel approach that simplifies data interpretation by reducing spectral complexity. To use <sup>77</sup>Se NMR, we aimed to develop a selenium-incorporated p97. By replacing the twenty methionine amino acid residues in p97 residues with selenomethionine, p97 becomes a <sup>77</sup>Se active species. The substitution of selenomethionine has been successfully characterized by mass spectrometry. We describe further optimization of p97 samples for <sup>77</sup>Se NMR spectroscopy.

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**DETERMINING TERMINATION CODON READTHROUGH EFFICIENCY  
PROMOTED BY PREDICTED SIGNALS IN INFECTIOUS RNA ALPHAVIRUS**

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Translation is the process in which a ribosome decodes mRNAs into proteins. When the rules of canonical translation are broken, translational recoding occurs. Translational recoding is used by viruses to regulate synthesis of proteins and increase genome capacity. This work focused on Termination Codon Readthrough (TCR) in a strain of the Ross River Virus, and the Asian/African and Caribbean strains of the Chikungunya Virus. TCR signals have been predicted in the genomes of these viruses, and this project aimed to determine if these signals are functional. In order to test the hypothesis, the predicted TCR signals were cloned into a dual luciferase reporter containing *Renilla* and firefly luciferases, in such a way that firefly luciferase can only be expressed in the event of a TCR event. The resulting plasmids were transfected into HEK293T cells (human embryonic kidney cells). Expression of *Renilla* and firefly luciferases was determined using the dual luciferase assay system. Preliminary data suggest that the predicted TCR signals analyzed are functional.

## BIOCHEMICAL AND STRUCTURAL STUDIES OF A HIV CA-BINDING NANOBODY

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The HIV capsid plays a crucial role in protecting the viral genome from cellular antiviral factors and empowering the virus to take advantage of cofactors. The capsid is composed of capsid protein (CA) monomers, arranged into a lattice of ~250 CA hexamers and 12 CA pentamers to assemble a fullerene cone architecture. Many host protein factors recognize features of the higher order capsid lattice, so that capsid assemblies of defined CA oligomers are needed for biochemical and structural studies of capsid-host factor interactions. However, it has been challenging thus far to overcome the natural polymerization tendency of CA to capture discrete oligomers.

Recently, a novel llama nanobody (“llamabody”) has been developed to bind and block the CA polymerization interface with high affinity. To further establish llamabody as a tool to control CA assembly, we sought to understand the biochemical and biophysical details of the CA-llamabody interaction and characterize mutations that allow us to manipulate this interaction. We used size exclusion chromatography binding assays, isothermal titration calorimetry, and X-ray crystallography to determine that llamabody can bind a variety of CA assemblies, that mutating interface residues on CA allows us to tune llamabody’s affinity, and that the CA-llamabody interface is not rigid and can adjust to environment. Our findings suggest that llamabody can be used to shield polymerization interfaces during the formation of CA oligomeric assemblies—paving the way for future biochemical and structural studies of host factor-capsid interactions.

Llamabody was first described by Gray, et. al. in *ACS Infectious Diseases*. X-ray data was collected at APS beamline ID-24 (NE-CAT). This work was supported by NIH grant P50GM082251 to the Pittsburg Center for HIV Protein Interactions and the Yale College Dean’s Research Fellowship.

**LONG-RANGE REGULATION OF CYTOCHROME *C* BINDING TO *bc1* COMPLEX**

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The cytochrome *bc1* complex (Complex III, ubiquinol-cytochrome *c* oxidoreductase) is a highly conserved multi-subunit protein found in the mitochondria and is a key complex in the electron transport chain. During oxidative phosphorylation, cytochrome (cyt) *c*, a mobile electron carrier, binds to one cyt *c1* subunit of a *bc1* complex dimer and shuttles electrons from Complex III to Complex IV. X-ray crystallographic studies revealed that only one molecule of cyt *c* binds to one *bc1* complex dimer, despite two cytochrome *c1* subunits available for binding, pointing toward the existence of a regulation mechanism preventing the docking of a second cyt *c* substrate. However, the structural basis for such a mechanism of long-range (>30Å) regulation of substrate binding is not clear from static structural studies. We employed all-atom molecular dynamics simulations to uncover a possible mechanism of regulation. Our results reveal that a finger-like extended domain of the vacant cyt *c1* subunit undergoes a conformational change with its tip moving towards cyt *c*, transferring mechanical motion and causing distortion of the vacant cyt *c* binding site. In addition, we explored the role of naturally occurring methylated Lys-72 residue of cyt *c* in substrate binding and its likely role in the regulation of the *bc1* complex activity.

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**CHARACTERIZING TRANSLATIONAL RECODING IN INFECTIOUS RNA VIRUSES**

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Translation is the process of synthesizing proteins by expressing genes within mRNA. Viruses possess small genomes, and as such have adapted several non-canonical methods of regulating gene expression, including translational recoding. Termination Codon Readthrough (TCR) and Programmed -1 Ribosomal Frameshifting (-1 PRF) are two specific mechanisms of translational recoding that viruses employ to synthesize multiple proteins from an mRNA. Both mechanisms result in C-terminal extension of the polypeptide chain, resulting in a fusion protein of two genes. Such recoding happens in small percentages, but is important for viruses to maintain proper ratios of proteins needed to construct mature viral particles. TCR and -1 PRF signals are predicted to be present in Bovine Leukemia Virus (BLV), O'nyong-nyong Virus (ONNV), and Chikungunya Virus (CHIKV). This work aims to determine whether the predicted translational recoding signals in these viral sequences are functional. To test this hypothesis, a dual luciferase reporter system, containing *Renilla* and firefly luciferases, was used. *Renilla* is always expressed, while firefly is expressed only when a translational recoding event occurs. Human Embryonic Kidney (HEK293T) cells were transiently transfected with plasmids containing the viral sequences and the enzymatic protein products of the two luciferases were measured indirectly as a function of substrate usage. The recoding efficiency for each predicted viral signal was then calculated based on the amount of firefly luciferase that was produced. Preliminary data suggest that these viruses have functional TCR and -1 PRF signals.

## SELECTION OF AN APTAMER TO BIND 2-HYDROXYGLUTERATE THROUGH SELEX

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Glioma and Acute Myeloid Leukemia are both cancers that have been linked to the formation of 2-HydroxyGlutarate (2-HG) during the third step in the Krebs Cycle. A point mutation on Arginine 132 of IDH1 enzyme causes a gain of function that converts  $\alpha$ -Ketoglutarate ( $\alpha$ -KG), the correct metabolite, into 2-HG, an inhibitor. The carbonyl group in the molecule is converted into a hydroxyl during the gain of function the enzyme preforms. Up to 86% percent of patients with excess levels of 2-HG have been found to have tumors relating to the above cancers. The goal of the research is to isolate an aptamer, or a single strand of RNA, that can bind to 2-HG with high specificity and accuracy, and then act as a biosensor. Once an aptamer is found, it can then be cloned by use of a plasmid and incorporated into a riboswitch, which acts as a mechanism to turn on and off translation of a desired gene that is placed in the plasmid. In the case of this project, snake venom is a likely candidate to be activated by the binding of the 2-HG metabolite. This will effectively kill any cell that can potentially cause cancer. Through cycles of SELEX, Systematic Evolution of Ligands by Exponential Enrichment, a large pool of randomized RNA sequences is narrowed down, close to Avogadro's number, until eventually the addition of 2-HG to the RNA pool shows a high percentage of cleavage, while also showing little to no cleavage with the addition of random molecules, like magnesium is added to the pool.

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## SYNTHESIS OF MURAMYL DIPEPTIDE DIMERS

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Our bodies house trillions of bacteria that our immune system recognizes as beneficial or pathogenic, misrecognition of which causes autoimmune disorders such as inflammatory bowel diseases (IBDs). Nucleotide-binding oligomerization domain-containing protein 2 (Nod2), an innate immune receptor genetically linked to an IBD called Chron's disease, recognizes fragments of the peptidoglycan. Peptidoglycan, a carbohydrate polymer that can be further cross-linked through its amino acid sidechains, surrounds bacteria to construct its cell wall. Completing the synthesis of Muramyl Dipeptide (MDP) dimers, as well as other disaccharide fragments paves the way for a larger peptidoglycan library. The mechanism of peptidoglycan breakdown in the body is unknown, therefore replicating the possible fragments generated will break ground in understanding their interactions with Nod2. Target MDP dimers will be linked via diamine-alkane linker and Polyethylene glycol (PEG). These compounds will be screened for their ability to initiate a Nod2 dependent immune response in vivo, as well as binding to Nod2 in solution through competition experiments via Surface Plasmon Resonance (SPR). Addition of a free amine linker to these compounds in future studies will allow to study binding through immobilization for SPR, as well as glycan microarray.

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## INVESTIGATING EVOLUTIONARY CHANGES OF INSECT EMBRYOGENESIS GENES USING A CRICKET MODEL?

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There is a broad class of genes, divided into three categories that together control the development of insect body segments. One of these categories is called pair rule genes. Pair rule genes are a class of segmentation genes expressed in a specific series of stripes in the early hours and days after an egg is laid while also defining segments of an insect. We used cricket embryos from stages 1 to 8 which are about 3.5 days old. They were subjected to In-Situ hybridization with DIG probes to localize mRNA fragments in fixed embryos. We isolated most of the pair rule genes by Sanger sequencing. Then we used these copies to design templates for probe for in-situ hybridization. The end goal of this project is to be able to compare the specific spatial pattern of each gene with the *Drosophila* to see if there is any correlation or difference.

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## **REGULATION OF CASPASE 3 EXPRESSION BY CD44 CELL SIGNALING IN MCF-7 BREAST CANCER CELLS**

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The CD44 receptor has been reported to regulate apoptosis in different cell types including breast cancer cells. Because CD44 can regulate gene expression via its intracytoplasmic domain (CD44-ICD) interaction with DNA and/or protein-protein interactions with transcription factors, we hypothesize that this cellular process may be in part regulated via the CD44-ICD interaction with transcription factors or the CD44-ICD response element (CIRE) in apoptosis-related genes. To identify potential apoptosis-related genes regulated by CD44, we carried out a differential expression analysis of 35 apoptosis-related proteins in MCF-7 (CD44 negative) and MCF-7/CD44 (CD44 stable transfected cells) cell lines using the Proteome Profiler Human Apoptosis Array Kit (R & D). We found out that caspase 3 (CASP3) was differentially expressed. MCF-7 cells expressed caspase 3 while MCF-7/CD44 cells did not. Furthermore, a bioinformatics analysis revealed that the promoter region of CASP3 contains two CIREs downstream its transcriptional start site. These findings suggest that CD44 via its CD44-ICD may inhibit caspase 3 expression, and thus has the ability to affect apoptosis. Chromatin immunoprecipitation assays and co-immunoprecipitations will be also carried out to determine whether the CD44-ICD localizes to the CIREs on the CASP3 gene promoter and/or the CD44-ICD interacts with CASP3-associated transcription factors.

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**INVESTIGATING MECHANISMS OF ACTION OF A NOVEL NUCLEOSIDE  
ANALOG TO TREAT PRIMARY EFFUSION LYMPHOMA, COLON AND  
PANCREATIC ADENOCARCINOMAS**

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Primary Effusion lymphoma (PEL) is an HIV and KSHV associated non-Hodgkin's B cell lymphoma. PEL accounts for 4% of all HIV associated non-Hodgkin's B cell lymphomas and is characterized by the formation of lymphomatous effusions in body cavities. The nucleoside analog 6-ethylthioinosine (6-ETI) was identified as a selective inhibitor of primary effusion lymphoma. 6-ETI's ability to inhibit PEL is dependent on the expression of adenosine kinase (ADK). ADK is an enzyme that catalyzes the transfer of gamma-phosphate from ATP to adenosine in order to generate AMP. ADK is overexpressed in PEL, and is necessary for sensitivity to 6-ETI. Knowing ADK's role in effectively inhibiting PEL, our goal was to investigate 6-ETI's ability to hinder other cancers that overexpress ADK. Specifically, we wanted to investigate 6-ETI's ability to inhibit colon and pancreatic adenocarcinomas.

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## CD44 MEDIATED REGULATION OF TRANSCRIPTION FACTORS GENE EXPRESSION

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CD44 is a cell membrane receptor known to play a role in cell migration, invasion, adhesion and signal transduction. In breast cancer cells, the expression of CD44 has been associated with an aggressive cancer cell phenotype. We hypothesize that the signal transduction function of CD44 can affect the cancer cell phenotype, in part by regulating the expression of genes particularly of those encoding cancer-related transcription factors. To test this hypothesis we used a transcription factors-specific profiling plate array (Signosis) to assess the expression of 96 transcription factors in MCF-7 (low in vitro invasion, CD44 negative) and MCF-7/CD44 (high in vitro invasion, CD44 positive stably transfected) cells. Preliminary data from this analysis indicates a differential expression of genes encoding transcription factors in MCF-7/CD44 cells compared to MCF-7 cells. Interestingly, some of the transcription factors differentially expressed at higher than two-fold change, such as Gli-1, ATF2 and FOXO1 are known to be associated with the promotion of breast cancer.

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**A ONE-POT ENZYMATIC SYNTHESIS OF A PHOTOCROSSLINKING CMP-SIALIC ACID DERIVATIVE FOR ACTIVE SITE AMINO ACIDS OF THE *NEISSERIA MENINGITIDIS* SEROGROUP W CAPSULE POLYMERASE**

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*Neisseria meningitidis* is one type of gram-negative bacteria that is a leading cause of bacterial meningitis. Glycoconjugate vaccines are one way to prevent disease caused by this pathogen. However, there is not a complete understanding about the relationship between the vaccine structure and the reaction our immune system produces. The long-term goal is to use *N. meningitidis* capsule polymerase enzymes, which synthesizes capsular polysaccharides that surround the bacteria, as tools to improve glycoconjugate vaccine development. Our current focus is the *N. meningitidis* serogroup W capsule polymerase. This enzyme synthesizes a polysaccharide of galactose-sialic acid repeats by using UDP-galactose (UDP-Gal) and CMP-Sialic acid (CMP-Sia). We aim to optimize this enzyme's activity to make glycoconjugate vaccines of defined sugar lengths to understand how this contributes to the immune response produced. We use CSS (CMP-Sialic Acid Synthetase) and SAS (Sialic Acid Synthase) to produce a compound (CMP-Sialic acid Diazirine) that will help identify the serogroup W capsule polymerase's active site amino-acids by crosslinking to them.

SAS and CSS were used to first make CMP-Sia in a one-pot enzyme reaction in the presence of precursor molecules. To confirm synthesis of CMP-Sia, we used our reaction product as a source of CMP-Sialic acid for the serogroup W capsule polymerase. The serogroup W enzyme successfully added sugars to a fluorescent acceptor as seen by the presence of new peaks on HPLC. Next, we used CSS and SAS to make CMP-SiaDAz. The peaks observed by HPLC analysis are similar in retention time to those observed with CMP-Sia. In conclusion, we successfully used an enzymatic scheme to make CMP-Sia and CMP-SiaDAz. For future work, synthesis of CMP-SiaDAz will be confirmed by mass spectrometry. We will then use this compound for crosslinking to the *N. meningitidis* serogroup W capsule polymerase.

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**Kinetic Studies of 2-(2'-hydroxyphenyl)benzenesulfinate desulfinase substrate analogs**

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2-(2'-hydroxyphenyl)benzenesulfinate desulfinase (DszB) is an enzyme that catalyzes the cleavage of the carbon-sulfur bond as the final step of the desulfurization of 2-(2'-hydroxyphenyl)benzenesulfinate (HPBS) producing sulfite and 2-hydroxybiphenyl (HBP). This reaction is used to study the biodesulfurization of dibenzothiophene, the major organosulfur compound found in fossil fuels/petroleum. Previous studies in the Watkins lab suggested that halogenated analogs of HPBS are competitive inhibitors of DszB, so a coupled assay was used to test their effect on kinetic activity. The relative activity of these analogs was tested measure both products of the reaction. The rate of HBP formation was monitored using a fluorometric assay with an excitation at 288 nm and measuring emission at 414 nm. The rate of sulfite product formation was measured in the coupled assay with sulfite oxidase (SOX) by analyzing a change in absorbance at 540 nm. When testing the analogs HPBS-Cl and HPBS-Br for activity, no product was detected. When measuring enzyme activity in the presence of the halogenated analogs and the substrate HPBS, an increase in DszB activity was observed, with HPBS-Cl and HPBS-Br showing an increase product formation of 475% and 213%, respectively. Individually these HPBS analogs did not act as catalytic substrates or competitive inhibitors, however when coupled with HPBS the analogs acted as activators.

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## DETERMINATION OF THE CONSEQUENCES OF DISRUPTING INTERACTION BETWEEN MYH REPAIR ENZYME AND THE CHECKPOINT CLAMP

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Exposure to reactive oxygen species and radiation leads to DNA damage that can compromise genomic integrity. 8-oxo-guanine (GO) is a frequent and highly mutagenic oxidative lesion, because it mispairs with adenine during DNA replication. The MYH glycosylase increases replication fidelity by removing adenines misincorporated opposite GO. Individuals with a mutation in the *hMYH* gene are at an increased risk of developing colorectal cancer. MYH-directed base excision repair (BER) is tightly coordinated with the DNA damage response (DDR) in order to maintain genomic stability and cell survival. The 9-1-1 complex (Rad9, Rad1, and Hus1 heterotrimer complex), a DDR sensor, is essential for cell viability and development, and is proposed to provide a platform to coordinate BER processes to avoid the accumulation of toxic intermediates. The major Hus1-binding site is localized to residues 295–350 (interdomain connector, IDC) of hMYH. The goal of this project is to interrupt MYH-Hus1 interaction by overproduction of IDC peptides. We propose that the overproduced IDC peptide will inhibit Hus1 binding to hMYH, increasing cell sensitivity to oxidative stress and apoptosis. We amplified the DNA fragments encoding IDC peptides of hMYH using plasmids containing wild-type (WT, V315/Q324) and mutant (V315A and Q324H). After digestion, PCR products were ligated into cleaved pEGFP-N1. Clones were verified by restriction digestion and sequencing, and transfected into 293T cells to express GFP-fusion proteins. We are investigating physical interactions between GFP-IDC (WT, V315A, and Q324H) with Hus1, hoping to demonstrate the biological consequences of expressing IDC in 293T cells. Our preliminary data suggest that expressing GFP-hMYH-IDC (WT), but not mutant IDC (V315A), renders 293T cells more sensitive to H<sub>2</sub>O<sub>2</sub>. These results are consistent with previous research in *S. pombe*, and provide additional evidence that disrupting the links between BER and DDR proteins may provide a new strategy for cancer therapy.

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## IDENTIFYING ANTIBIOTIC RESISTANCE GENES IN FRESHWATER BODIES

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The formulation of the first antibiotics in 1928 by Alexander Fleming paved way for the many other antibiotics today. Yet, the discovery has also brought about the rise of antibiotic resistant bacteria throughout the world due to the misuse of antibiotics within the healthcare system and in agricultural settings. The purpose of this research was to identify the presence of two antibiotic resistance genes, *ampC* and *bla*, that confer resistance to penicillin-family antibiotics. Resistant bacterial strains are becoming more prevalent not only in the healthcare setting, but in the community as well. To explore this problem, water samples of three suburban parks were examined: Pig Pen Pond, Bynum Run and Lake Elkhorn. It was hypothesized that the Bynum Run Park sample in comparison to the other water samples, would have a greater presence of resistant microbial communities because of discoloration of the water due to potential contamination. PCR was used in order to detect the presence of antibiotic resistance genes in the water samples. The outcome yielded a positive result in Lake Elkhorn for *ampC* and a possible positive for *ampC* and in Bynum Run. High performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was then utilized to determine if there was ampicillin in the Bynum Run Park water sample, however the levels of antibiotic were shown to be below the limit of detection. The *bla* gene was cloned in order to characterize the  $\beta$ -lactamase protein associated with the two resistance genes in future experiments. A  $\beta$ -lactamase protein standard was digested and analyzed with HPLC-MS/MS in order to verify a method that could be used for protein sequencing in future experiments. Furthering this research would include an inspection of more community ponds around the Maryland, area as well as expressing the  $\beta$ -lactamase associated with the cloned *bla* gene.

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## THE STRUCTURE AND FUNCTIONAL CHARACTERIZATION OF *LEISHMANIA DONOVANI*'S UFM-YLATION PATHWAY

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Ubiquitin fold modifier 1 (Ufm1) a ubiquitin-like protein and Ubiquitin fold modifier activating enzyme (Uba5, E1) are proteins found in eukaryotic organisms that play a crucial role in cell cycle regulation, signal transduction, and ER stress. *Leishmania donovani* (Ld) is a trypanosomatid parasite that has been shown to have the enzyme homologous proteins to Uba5 and Ufm1. *Leishmania* Ufm1 and Uba5, and the substrate-targeted proteins are associated with the mitochondria which has not been observed in other organisms. This suggests that these *Leishmania* proteins may have physiological roles not yet described in other organisms. *Leishmania donovani* causes leishmaniasis, a disease that is accompanied by sores and lesions that appear at varying depths of the body depending on the type and increases the host's susceptibility to co-infection with other diseases. This disease is prevalent in over 80 countries and continues to spread. Currently there is no effective vaccine for this disease. Therefore, biochemical study of these proteins may provide insight into the molecular basis for leishmaniasis and the fundamental role of this pathway in the parasite. There is no structural information on *Leishmania* proteins involved in Ufm-ylation. LdUfm1 and LdUba5 (E1) are proteins of high interest because these proteins begin the conjugation pathway of Ufm1 to its targets. We first expressed and purified LdUfm1, a truncated form of LdUfm1 and LdUba5. LdUfm1 and LdUfm1(tr.) were successfully purified and were characterized with SEC-MALS. We then performed gel migration shift assays to demonstrate the compatibility of the human and *Leishmania* systems. We found that *Leishmania* Ufm1 but not the truncated form were viable substrates for human Uba5. Additionally, we began setting crystal trays to obtain protein crystals for structure determination. We have preliminarily obtained crystals of truncated Ufm1. Future work will optimize these crystals and explore the cross-reaction between human and *Leishmania* enzymes.

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## INVESTIGATION OF THE EFFECTS OF OPIOIDS ON STAPHYLOCOCCUS AUREUS

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*Staphylococcus aureus* is a microorganism which is commonly found on skin. For this reason, *S. aureus* is often found in wastewater analyses. Opioids are common pain relieving drugs, which have the tendency to induce addiction. Recently, the presence of opiates have also been discovered in wastewater analyses. Due to the presence opioids excreted in urine and their improper disposal, the tracking of opiates in wastewater can be an effective prognosticator for the prevalence of drug use in certain areas. The current study is aimed at determining how opioids effect microbial growth to gain a better understanding of the actual effects of opioids existing in wastewater.

## THE EFFECTS OF ANTI-CANCER DRUGS ON THE REGULATION OF CILIA-ASSOCIATED PDGFRA SIGNALING

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Primary cilia are organelles that project off the surface of nearly every cell in the body. They represent a platform for signaling transduction associated with essential cellular pathways, such as PDGFR $\alpha$ , Hedgehog and Wnt. When ciliation is disrupted, cilia-associated signaling is no longer properly regulated. This results in development of a number of diseases, collectively known as ciliopathies. According to numerous studies, the primary cilia also play an important role in cancer. Intriguingly, tumor development is frequently associated with the complete loss of cilia while normal cells surrounding the tumor maintain cilia, promoting cilia-dependent signaling between cancer and stromal cells. It is not completely understood how anti-cancer drugs may affect this cilia-associated interaction, although there is evidence that several clinically available compounds have a direct effect on cilia assembly and disassembly. To further investigate if there are more anti-cancer compounds affecting ciliary dynamics and cilia-associated signaling, we screened a library of 180 kinase inhibitors, including compounds widely used in clinic. Among the positive hits for ciliary resorption was sunitinib, a clinical compound which targets PDGF and VEGF receptor families. Of those that promote cilia stabilization was an inhibitor of interleukin receptor associated kinases 1 and 4, or IRAK1/4. We next asked if these compounds had an impact on the regulation of cilia-associated PDGFR $\alpha$  signaling. In normal cells PDGFR $\alpha$  signaling is responsible for cell growth, survival, and proliferation, although over-activation of this pathway is commonly associated with cancer. Using Western blot analysis and immunofluorescence we established that, unlike IRAK1/4 inhibitor, sunitinib treatment causes very rapid disassembly of the primary cilium, and effectively inhibits PDGFR $\alpha$  signaling. The findings of this study further support the relationship between cilia-associated signaling and cancer and uncovers novel mechanisms of regulation of ciliary dynamics by anti-cancer drugs.

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## THERAPY RNA G-QUADRUPLEX INTERACTION WITH hnRNPs INDUCING ALTERNATIVE SPLICING IN GBM

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Glioblastoma multiforme (GBM), a grade IV tumor of the central nervous system, is the most common malignant primary brain tumor, having a median survival of only 14 months. Poor survival is due to a lack of efficacy in current therapies which is limited by the blood-brain barrier. GBM tumors are characterized by angiogenesis, which is essential for tumor growth and survival. To develop a new treatment, our lab has designed an RNA therapeutic vector against the pre-mRNA of the pro-angiogenic transcripts, EGFR and VEGFR2. This therapy induces alternative splicing leading to shortened mRNA transcript isoforms, which translate into soluble decoy proteins as opposed to the canonically spliced full-length transmembrane receptor. These soluble decoys competitively bind the EGF or VEGF growth factors, without activation of the intracellular tyrosine-kinase phosphorylation signaling pathway. Targeting of key splicing elements by RNA antisense therapeutics is complemented by the molecular cloning of a heterogenous ribonucleoprotein recruitment domain into the RNA therapeutic vector, effectively silencing the site by artificial intronic redefinition.

By transfection into GBM cell lines, EGFR and VEGFR2 protein expression will be measured by flow cytometry and ELISA RNA expression will be evaluated by MinION nanopore sequencing. Currently, we are developing methods to isolate and purify recombinant poly-histidine/FLAG tagged hnRNP proteins using Ni-Nitriloacetic acid/M2 Affinity resin column. Efficacy of the hnRNP proteins to bind the RNA therapeutic molecule will be tested using the Electrophoretic Mobility Shift Assay (EMSA), and a super-shift EMSA using an antibody against the poly-histidine tagged protein. In addition, we will extract RNA from the EMSA gel and reverse transcribe to confirm therapeutic RNA binding. Transfection of mammalian expression vectors in tissue culture will also be performed and subsequently measured by RNA and protein assays. Once the therapy RNA-hnRNP protein interactions are confirmed, current steps include cloning of a serine-arginine (SR) protein recruitment domain to act as a splicing enhancer, directing the spliceosome to a desired splice site or intronic poly-adenylation site to increase synthesis of EGFR and VEGFR2 soluble decoy isoforms.

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## LOAD-INDUCED CROSSTALK BETWEEN PROSTATE CANCER CELLS AND OSTEOBLASTS IN BONE METASTASIS

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Prostate cancer (PCa) is the second most common type of cancer in men with an estimated 165,000 new cases to be diagnosed in 2018. Approximately 80% of prostate cancer cases experience metastases in bone that induce osteosclerotic lesions that reduce bone strength and increase mechanical strain. I hypothesize that mechanical loading of osteogenic cells increases release of Nerve Growth Factor (NGF) and that NGF directly stimulates proliferation of PCa cells. Alternatively, osteogenic cells also release ATP in response to load and could drive proliferation of PCa cells. To test these hypotheses, we applied fluid shear (5 dynes/cm<sup>2</sup>) to MC3T3 osteoblast-like cells and collected the media. We applied this conditioned media (CM) to C42B4 cells and determined increases in proliferation of these cells using an MTS assay. PCa cells treated with CM showed no difference in proliferation compared to control cells. However, when I observed cell confluency in the groups, it appeared that the CM-treated C42B4 cells were more highly confluent, suggesting a discrepancy between the MTS assay and cell count. To determine whether NGF or ATP mediated this proliferation we treated C42B4 cells with NGF or apyrase, which hydrolyzes ATP, or both daily during the proliferation study. When apyrase and NGF were added together, we saw an increase in proliferation between 24 and 72 hours, comparable to NGF-treated cells alone. These data suggest that NGF stimulates proliferation of C42B4 cells, but the shear-induced release of NGF was not at a high enough concentration to stimulate this response. Future directions include co-culture of C42B4 cells and MC3T3 cells to determine the direct interaction of these cells. These data will further our understanding of the cellular mechanisms involved in prostate cancer and help to identify potential therapeutic markers for the treatment of metastatic prostate cancer in bone.

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## **SYNTHESIS OF EXOSOME-ASSOCIATED AAVS TO DELIVER RNA THERAPEUTIC STRATEGIES TO BLOCK VEGFR2 AND ANGIOGENESIS IN HUMAN GLIOBLASTOMA**

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Glioblastoma multiforme (GBM), a grade IV tumor of the central nervous system, is the most common malignant primary brain tumor, and has a median survival of only 14 months. Poor survival is due to a lack of efficacy in current therapies which is limited by the blood-brain barrier (BBB). GBM tumors are characterized by angiogenesis, which is essential for tumor growth and survival. Endothelial cells form the walls of new blood vessels, bridging the gap between the growing tumor mass and the established vasculature of the circulatory system. The membrane receptor that activates tumors to recruit endothelial cells to promote vascularization is vascular endothelial growth factor receptor 2 (VEGFR2). In our lab, we are developing a novel therapy to alter the expression of VEGFR2. Changes in VEGFR2 expression to block its activation would inhibit the development of new blood vessels. We are designing therapies to bypass the BBB and deliver the genetic sequences of anti-sense RNA molecules to alter the splicing pattern and expression of the VEGFR2 transcript, creating a soluble VEGFR2 decoy. Nine different antisense sequences were designed to target and block critical elements of the VEGFR2 pre-mRNA transcript, and were cloned into two different therapeutic platform vectors, pAAV-U7-smOPT and pAAV-PTM. These vectors direct the antisense RNA therapeutic to spliceosome machinery. U87 GBM cells are being cultured and transfected with the therapeutic vectors, and the total protein and RNA are being collected and analyzed. These therapies will then be delivered using an adeno-associated virus (AAV) due to their non-pathogenic potential and integrative features. AAV, however, does trigger a mild immune response, facilitated by neutralizing antibodies. To circumvent this issue, we are isolating AAV exosomes that can shield the AAV and increase resistance. In addition, these AAV exosomes can easily cross the BBB, and demonstrate a higher transduction efficiency compared to standard AAVs.

## CREATION OF GENETIC KNOCKOUTS OF PUTATIVE MRNA DECAY GENES IN *SACCHAROMYCES CEREVISIAE*

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mRNA degradation modulates eukaryotic gene expression in response to developmental, environmental, physiological, and metabolic signals. *Saccharomyces cerevisiae* is a unicellular eukaryote that is used as a model to study molecular and cellular biology. Previous findings suggested that *S. cerevisiae* *YKL023W* and *PBY1* genes may be involved in mRNA decay pathways. Given this information, the primary aim of this research project was to determine the functions of *S. cerevisiae* *YKL023W* and *PBY1* genes through the creation of genetic knockouts and the analysis of the resulting phenotypes. To create a genetic knockout, we first constructed a deletion cassette by PCR that contained a *URA3* gene surrounded by the flanking genomic regions of either *YKL023W* or *PBY1*. After DpnI digestion of the plasmid template, the *URA3* knockout (KO) cassette was transformed into yeast cells lacking *URA3*. KO candidates that had replaced the target gene with a *URA3* through homologous recombination were selected by growth on *URA3* dropout media. Afterwards, the genomic DNA of the KO candidates was purified and screened by PCR with primers specific to the flanking genomic regions and *URA3*. Agarose gel electrophoresis was performed to verify the KO. We demonstrated the successful creation of the *YKL023W* KO. However, we were not able to isolate the *PBY1* KO due to the absence of colonies on the *URA3* dropout plates, indicating that *PBY1* may play a crucial role in the viability of *S. cerevisiae*. Future directions include *YKL023W* KO reconfirmation and the characterization of growth and mRNA decay phenotypes. For *PBY1*, the creation of hypomorphic alleles may allow for subsequent phenotypic analysis.

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# DECIPHERING AN IKK/NF-KB-MIRNA TRANSCRIPTIONAL REGULATORY NETWORK IN NSCLC CELL PROLIFERATION; THE INTERACTION BETWEEN MIR-342-3P AND FOSB, MAF.

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The NF- $\kappa$ B signaling pathway is a fundamental regulator of cell proliferation. There are two main activation mechanisms: the IKK $\alpha$ -mediated non-canonical and the IKK $\beta$ -mediated canonical pathways. NF- $\kappa$ B signalling has decisive roles in cell growth control and viability. Consequently, it is often deregulated in cancer. We attempted to further decode the mechanisms that control cell growth via the canonical pathway by investigating the miRNA expression patterns regulated by NF- $\kappa$ B. Since miRNAs act post-transcriptionally they may function as tumor suppressors. We knocked-down the upstream canonical NF- $\kappa$ B activating kinase IKK $\beta$  in the human lung cancer cell line A549 using specific novel lentiviral vectors. The pathway induction was succeeded by etoposide (VP16) treatment. The study involved Nanostring<sup>®</sup> nCounter screening of differential miRNA expression. Out of a pool of 800 miRNAs, mir-342-3p was found induced in the IKK $\beta$ <sup>-</sup> A549 cells in response to etoposide. This drug acts by forming a ternary complex with DNA and topoisomerase II leading to DNA damage. Accordingly, we suggest the involvement of mir-342-3p in the DNA damage-induced canonical NF- $\kappa$ B signalling. Based on its seed sequences were identified potential mir-342-3p targets: *maf*, *fosb*, *srcin*, *sox21*, *pdgfra* and *mrfap1*. We continued with the experimental validation using a *Renilla* luciferase reporter gene fused to the 3'-UTR of the predicted target gene sequences, cloned into psiCHECK-2<sup>®</sup> vector. *Maf* and *fosB* emerged as direct targets of miR-342-3p. MAF and FOSB (members of the AP-1 family) are involved in the stimulation of proliferation. Summarizing, miR-342-3p seems to possess a tumour suppressive function while its expression is usually repressed in human cancer. Therefore, we suggest that mir-342-3p suppresses the cell cycle by targeting *fosB* and *maf* and is also associated with cell cycle effectors.

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## CHARACTERIZATION AND REGULATION OF STEROID SULFATASE ACTIVITY IN NIH-3T3 CELLS

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Steroid hormones circulate as inactive sulfated forms, which steroid sulfatase (STS) converts into active forms at the tissue level. We have previously characterized STS activity in human and mouse breast and bone tissues and have shown that STS can provide estrogens to these tissues from circulating sulfated precursors. This study was designed to characterize STS activity in the mouse NIH-3T3 fibroblast cell line. Using a radioactive estrone sulfate conversion assay, we found high levels of STS activity in cultured cells and this activity was blocked by the STS inhibitor EMATE. We also found that microsomes of NIH-3T3 cells had high STS activity, and that cytosols had low activity. Western blotting confirmed the presence of immunoreactive STS in NIH-3T3 microsomes, consistent with the known distribution of STS to the endoplasmic reticulum. Steroid treatments of cultured cells revealed that glucocorticoids decreased STS activity, as we have found for other cell lines. However, estradiol also decreased STS activity, which has not been shown previously. This may reflect negative feedback of estradiol on estrogen formation by STS. Our results may have important implications with regard to local steroid conversions by STS in fibroblasts, which are widely distributed in the body.

## HIDING IN PLAIN SIGHT: STUDY OF POTENTIAL BACTERIAL THREATS AT THE UNIVERSITY GYMNASIUM FACILITY

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Center for Disease Control reports that the emergence of antimicrobial resistance among pathogenic bacteria is widespread in humans both in the community and in the clinical setting. Recent peer-reviewed research publications report that there is a high incidence of Methicillin-resistant *Staphylococcus aureus* infection among athletes who play contact sports such as football or soccer, often sustain skin injuries and hence are susceptible to such infections. The presence of harmful bacteria has been implicated in many college campuses among students using a common athletic gymnasium facility. In our study, we proposed to address the safety of Morgan State University's gymnasium facility, particularly the training room, to identify the potential bacterial threats. To accomplish this objective, we employed microbiological and molecular methods. As a first step, we surveyed the gymnasium facility and identified the most commonly used equipments in the Training room based on the feedback from the training staff at the facility. Then we collected the specimens, by swabbing the various surfaces, (total=43) and processed in our molecular microbiology research laboratory. Our results indicated 5 Gram-positive rods (GPR), 4 Gram-Negative rods (GNR) and 2 Gram-positive cocci (GPC) on 11 different surfaces (treatment tables, entrance tables, AaaA kiosk, treatment pillows, foam rollers, and hand sanitizers). Next, we employed molecular methods by extracting genomic DNA from these isolates and sequenced the 16SrRNA segment. The results of these ongoing molecular analyses will be presented. Future directions will involve the antimicrobial susceptibility profile for these isolates. In conclusion, we have identified bacterial community at our gymnasium facility.

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## CHARACTERIZATION OF THE K5 RING DOMAIN IN KSHV

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Kaposi's sarcoma-associated herpesvirus (KSHV), also known as Human Herpes Virus 8 (HHV-8) is distinct in that it is an oncovirus that can induce the formation of Kaposi Sarcoma (KS), a form of cancer. Growths can occur on the skin, in the mouth, gastrointestinal tract, and respiratory tract, greatly reducing the quality of life for those affected. KSHV is seen mainly in patients who have developed AIDS as an opportunistic infection. K5 is a gene found within the KSHV genome that encodes a membrane-bound ubiquitin E3 ligase. The ubiquitin ligase activity of K5 on human proteins allows the virus to spread more efficiently within the human body by inducing degradation of membrane bound proteins associated with immune function. Similar types of E3 ligases have been identified in humans however there is little structural or functional information these proteins. Our goal was to isolate the K5 protein to perform biochemical and biophysical studies. The protein K5 has never been isolated nor characterized and the acquiring of information on this protein could lead to potential treatments for KS. Moreover, studies on K5 as a model for the analogous human proteins will lead to greater understanding of immune receptor regulation. Initial recombinant protein expression experiments of the K5 E3 ligase domain in both 2XYT and M9ZB media showed successful expression of the protein. To purify the protein, we tried Nickel Affinity Column purification, however, the protein was largely insoluble. To successfully isolate the protein we had to extract the protein directly from the pellet, then refold the protein. Future goals aim to test the protein in an activity assay as well as to characterize other structural components of the protein.

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## TRANSCRIPTION START SITE HETEROGENEITY OF THE HIV-1 RNA GENOME

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In 2016, over forty million individuals were living with the human immunodeficiency virus-1 (HIV-1) worldwide. Additionally, about two million individuals became newly infected and one million died due to late stage of HIV infection. HIV is a retrovirus, meaning it has an RNA genome. The highly conserved 5'-Leader (5'-L) at the beginning of the viral genome plays a crucial role in determining the RNA's function. Structural analysis of the 5'-L can provide crucial information on how the virus regulates its functions and could lead to future therapeutics.

The 5'-L can adopt two conformations: the monomer, which promotes translation of viral proteins, or the dimer, which is packaged as the new genomic material of virions. Previous structural studies have shown that regions of the 5'-L called polyA and trans activation region (TAR) in dimer are stacked together to form a continuous helix. However, polyA of the monomer is unstructured. Furthermore, it was found that adding an extra guanosine shifts the equilibrium to the monomer. Nuclear Magnetic Resonance Spectroscopy revealed that an extra Guanosine, disrupts base pair at the bottom of polyA and strengthens U5:DIS interaction. To further prove this, single mutation was made at the bottom of polyA in the dimer to determine its' effect on the conformation. We hypothesized that this mutation would disrupt the bottom of polyA and hence would disrupt the whole structure, shifting the dimer to a monomer. Another construct was made in a way that two mutations were applied at the bottom of poly A, where it would stay a dimer. Monomer and dimer with no mutations were used as controls and native agarose gel was ran to test if our hypothesis was correct. Gel studies showed that single mutation in the structure of 5' leader of HIV-1 RNA genome can affect monomer dimer equilibrium.

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**RETINOIC ACID EFFECTS ON NEUROBLASTOMA CELLS**

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Neuroblastoma (NB) is a predominantly pediatric cancer deriving from the neural crest resulting in high mortality and relapse rates. Etiologies differ from patient to patient resulting in difficulty with finding a common treatment. Recently it was discovered that high casein kinase-2 (CK2) expression in NB tumors correlated with a worse prognosis. Using lentiviral vectors, SK-N-AS NB cells, which have normal expression of CK2, were transfected to overexpress CK2. This overexpressed cell line was referred to as CK2+ to distinguish it from the untransfected line, CK2. Cells were then treated with retinoic acid (RA) to examine how retinoid therapy would affect the more aggressive overexpressers compared to the normal expressers. Cell viability, proliferation, and neurofilament-M (NF-M) expression were measured in the two cell lines. Utilizing the Trypan Blue Exclusion assay the viability of the cells were tested in response to the retinoid therapy. The therapies did not cause a decrease in cell viability. The CyQuant Proliferation Assay was used to determine the change in rate of proliferation. Decreased proliferation was seen and it was more prominent in the CK2+ cell line. Neurofilament-M, a marker for neuronal differentiation, was measured with western blotting. NF-M was expressed in CK2+ cells after 2 days of stimulation with a low concentration of RA.

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## NUTRACEUTICAL INDUCED ROS SELECTIVELY KILLS ORAL CANCER AND DOWN-REGULATES INFLAMMATORY PATHWAY

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Nutraceuticals are products derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. Pomegranate juice extract (PJE) has anti-proliferative and pro-apoptotic properties in breast cancer and prostate cancer. Another strong nutraceutical is apple extract (AE). They act by metabolite induces Reactive Oxygen Species (ROS) generation which selectively kills glutathione depleted cancer cells at specific concentrations. The association between inflammation and cancer has been studied widely. We want to determine if PJE and AE could inhibit vital signaling of the inflammatory process. NF- $\kappa$ B, a transcription factor, has been a key element in inflammation and its activation has been shown to up-regulate gene expression of other pro-inflammatory cytokines. NF- $\kappa$ B activation can occur in most cell types. Using PJE and AE on the human squamous carcinoma HSC-2 cells and human normal gingival fibroblast cells HF-1, we want to observe if these nutraceuticals could inhibit or slow down the activation of NF- $\kappa$ B and prevent the inflammatory process. To do this, the sub-lethal concentration of AE and PJE was determined using a WST-1 cytotoxicity assay. Once established, EGFR levels were measured by flow cytometry after treatment with the nutraceutical, EGF or both. EGFR is one of the major signaling pathways that activates NF- $\kappa$ B by its signaling ligand epidermal growth factor (EGF). We wanted to quantify the EGF receptors (EGFR) to observe its over-expression in the HSC-2 cells verses HF-1 cells. By quantifying EGFR on the cells, we determined the effect on the cell surface. An ELISA was conducted of treated cells in the same conditions examining activated vs total NF $\kappa$ B. Using this data, current experiments are to perform an ELISA on extracellular secretions of IL-6 and IL-1 $\beta$ , immunofluorescence stain for localization of NF $\kappa$ B and treatment effect time interval EGFR flow cytometry.

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## IOCHEM THE THEOPHYLLINE RIBOSWITCH; ITS DESIGN AND IMPLEMENTATION

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Fluorescence Activated Cell Sorting, or FACS, is a method that was used to convert the Theophylline aptamer into a riboswitch. This method could theoretically be used to convert other discovered aptamers into riboswitches, however it is a costly method and is only available to those with the high-tech, expensive FACS machines. The Theophylline riboswitch was previously discovered by implementing the Theophylline aptamer with random sequences into a specifically designed plasmid and using a FACS machine to sort the cells.

We can structure a new system that would select only the sequence containing the Theophylline riboswitch without the use of a FACS machine. To do so, we place the aptamer with random sequences into a plasmid and transform the plasmid into bacteria cells. Then, the use of replica plating along with screening selects the cells that only contain the correct riboswitch sequence. By doing so, we confirm that this system is efficient in converting aptamers into riboswitches without the need for a FACS machine.

After an aptamer has been successfully converted into its riboswitch, the system of ratiometric fluorescence will allow for testing of the riboswitch's function. This is done by designing a plasmid that contains genes for two fluorescence proteins on either side of the inserted riboswitch. The two fluorescent proteins will provide the ability to measure the riboswitch's function through fluorescence readings.

Both of these systems are the key to innovating the next step in creating synthetic riboswitches.

We would like to thank Monmouth University School of Science for providing the facilities and funding needed for this research project.

## TRANSCRIPTOME ANALYSIS OF THE HUMAN RETINA

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The retina is a layered neuronal tissue lining the posterior portion of the eye that converts photons of light into visual images in the brain. Rod and cone photoreceptors are highly specialized light sensitive neurons that initiate this process of phototransduction. Though they are very similar cell types, rods and cones have distinct functionalities, synaptic connection, and are affected by different blinding disease alleles. In order to better understand the distinct molecular function of human photoreceptors, cone-rich central macula and rod-rich peripheral retina samples were biopsied from post mortem human donor eyes in biological replicate. Total RNAs were extracted from retinal samples using a Qiagen AllPrep Mini Kit. RNAs were validated for quality and sent for mRNA-seq transcriptome analysis using the Illumina NextSeq 500 sequencing platform. To assess and analyze these sequencing data, a novel bioinformatics analysis pipeline was applied to the raw sequencing reads. Sequence quality assessment and pseudoalignment to the human transcriptome assembly were achieved using FastQC and Kallisto software respectively in the Galaxy suite of web tools. The sleuth statistical model and RStudio package was then used to quantify alignments and calculate differential expression of individual transcripts between rod and cone-rich samples. Ongoing analysis is currently being conducted to determine the epigenetic regulation of candidate rod and cone-specific patterns of gene expression.

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## INVESTIGATING THE PRESENCE OF ANTIBIOTIC RESISTANCE GENES IN FRESHWATER SOURCES IN CORRELATION WITH COMMUNITY POPULATION SIZE

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Around the world, antibiotic resistance has become an increasingly prevalent problem. The rise of this problem can be attributed to misuse of antibiotics, lack of new antibiotics and the evolution of bacteria. Not only are these antibiotic resistant bacteria found in healthcare settings, but now they are being found in the community. In order to see if the antibiotic resistant bacteria that are normally found in hospitals had potentially spread into the environment, freshwater samples from locations around Maryland were examined for prevalence of antibiotic resistance. The goal of this experiment was to look at the correlation between the size of the population surrounding the bodies of water and the presence of antibiotic resistance genes. The investigation focused the *bla* and *ampC* genes, which create the protein  $\beta$ -lactamase which allows bacteria to resist antibiotics in the penicillin class of antibiotics. The samples used in this experiment were collected from Lake Kittamaqundi in Columbia, Maryland, Pig Pen Pond in Catonsville, Maryland, and Bynum Run Park in Bel Air, Maryland. Polymerase chain reaction (PCR) was used to identify the presence of *bla* and *ampC* genes. The experiment detected *ampC* in Bynum Run Park Pond. Bacteria that are exposed to antibiotics are more likely to develop resistance therefore, high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used to identify the presence of ampicillin in Bynum Run Park Pond. The *bla* gene was cloned in order to further analyze the associated  $\beta$ -lactamase enzyme in future experiments. Additionally, a HPLC-MS/MS method for protein sequencing of  $\beta$ -lactamase was conducted and verified for future characterization. Further testing will be needed in the future to determine if there is in fact a correlation between population size and spread of antibiotic resistance in the environment.

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## **ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS BETWEEN THE CD44-ICD AND RUNX2 BY CO-IMMUNOPRECIPITATION**

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As part of the CD44 signal transduction pathway, the CD44 intracytoplasmic domain is translocated into the nucleus where it can interact with DNA and/or proteins. Of particular interest is its interaction with transcription factors. This protein-protein interaction (PPI) can regulate gene expression and thus cellular phenotypes. Chromatin immunoprecipitation (ChIP) assays have been used to show a PPI between a GFP-tagged CD44-ICD and the transcription factor Runx2. To validate such findings, we propose to carry out co-immunoprecipitation assays, in which Runx2 will be Flag-tagged and the CD44-ICD will be untagged. Flip-in 293 human embryonic kidney cells, which are normally Runx2 and CD44 negative, will be transiently transfected with constructs expressing Flag-tagged Runx2 and wild type CD44. Primary antibodies against the CD44-ICD and against the Flag tag will be used. This analysis is expected to not only validate previous PPI ChIP assays data but also to provide additional information about the nature of the proposed CD44-ICD/Runx2 nuclear complex.

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## 5-HYDROXYINDOLEACETIC ACID AND KYNURENIC ACID LEVELS IN MOUSE CENTRAL NERVOUS SYSTEM AND PERIPHERY AFTER SYSTEMIC TRYPTOPHAN CHALLENGE

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Approximately 95% of tryptophan (TRP) is metabolized into the kynurenine pathway (KP) while <5% is metabolized into the serotonin pathway. Elevated levels of kynurenic acid (KYNA) have been to cause cognitive deficits in neurological disorders, such as Alzheimer's and schizophrenia. Altered serotonin levels are linked to many psychiatric disorders; 5-Hydroxyindoleacetic acid (5-HIAA) is an inactive metabolite of serotonin. In this study, we compare the kynurenine and serotonin pathway metabolites in the central nervous system (CNS) and periphery by measuring 5-HIAA and KYNA levels in mouse brain, liver, and plasma at 90 minutes and 240 minutes after a TRP challenge or saline.

Brain, liver, and plasma samples were collected from male and female mice after injection of TRP or saline. Plasma and tissues were diluted and homogenized in ultrapure water, deproteinated with perchloric acid, and centrifuged to isolate proteins. The supernatants were collected and subjected to HPLC analysis. KYNA and 5-HIAA were analyzed using fluorometric detection and electrochemical detection, respectively. Statistical analyses were performed using unpaired Student's t-tests.

In the CNS, 5-HIAA and KYNA levels increased at 90 minutes in both males and females. In contrast to CNS, only KYNA increased in liver and plasma at 90 minutes, in both sexes. In females, the ratio of KYNA/5-HIAA increased significantly at 90 minutes in both brain and liver ( $p < 0.05$ ). However, in males we observed that the ratio of KYNA/5-HIAA increased significantly in periphery only.

Our data conforms with the literature that 95% of TRP is converted into the KP in the periphery. However, in the brain 5-HIAA and KYNA increased similarly after an acute TRP challenge. As both metabolites increased significantly at 90 minutes and not at 240 minutes, it would be interesting to measure them, and other metabolites of the TRP pathway, at other time points to examine kinetic differences.

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## CRYSTALLIZATION AND CHARACTERIZATION OF MUTANT *E. COLI* CLPS CONSTRUCTS

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The focus of this research was the structural and biochemical characterization of point mutants of *E. coli* ClpS, a substrate adaptor for the bacterial protease ClpAP. ClpAP helps to enforce protein homeostasis and quality control by using energy acquired from ATP hydrolysis to mechanically unfold and degrade cytosolic protein substrates. The ClpS adaptor recognizes proteins bearing N-terminal Leu, Phe, Tyr, or Trp amino acids and delivers them to ClpAP for destruction, in a pathway termed N-end rule proteolysis. In collaboration with Dr. Aditya Kunjapur at Harvard Medical School, we sought to investigate ClpS variants with altered specificity for non-standard amino acids. We crystallized wild-type ClpS and ClpS-V65I, carrying a single amino acid substitution in the hydrophobic peptide binding pocket. We remotely collected  $\sim 1.5$  Å X-ray diffraction data at the Advanced Light Source synchrotron on ClpS alone and in complex with N-end rule peptides. HKL2000, Phenix, and Coot software packages were used to process raw data and build structural models, which reveal how the subtle Val-to-Ile point mutation alters the shape of the binding pocket. Finally, we measured peptide binding affinity *in vitro* by monitoring fluorescence anisotropy of labeled peptides.

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## FUNCTIONAL COMPARISON OF *MSS11* ALLELES BETWEEN DIVERGENT YEAST STRAINS

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Many genes have multiple versions, called alleles, that can alter the function of the encoded protein and change cellular phenotypes. Using yeast, we study how genotypic differences cause phenotypic differences. One of the phenotypes we have worked on is flocculation. Flocculation is an asexual process where the cell membranes of yeast adhere to each other and form what are called “flocks”, or clumps<sup>1</sup>. We are using two strains of the budding yeast *Saccharomyces cerevisiae* which show two very different flocculation patterns. JAY291 does not flocculate at all whereas S288c flocculates to a much greater extent. We previously determined that this difference was caused by the gene *MSS11*, a transcriptional activator for the expression of flocculation<sup>2</sup>. The only difference between the two alleles is a few nucleotides. The goal was to determine which SNP, or Single Nucleotide Polymorphism, is the cause behind the difference in the flocculation phenotype. We developed a two-pronged flocculation assay to quantitatively measure a spectrum of flocculation abilities. In the future, we will create a strain of yeast in which *MSS11* has been deleted ( $\Delta$ *MSS11*) and various plasmids containing previously-fabricated plasmids with chimeric alleles of *MSS11* will be transformed into the strain. Their ability to complement the function of *MSS11* (and therefore the flocculation phenotype) will be measured to determine which SNP causes the difference in phenotype between the two strains.

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## EFFECT OF HYDROGEN PEROXIDE ON HYDROLYSIS OF PROTEINS

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Hydrogen peroxide is the active ingredient in over-the-counter teeth whitening strips and damages proteins. The goal of this project was to characterize the hydrolysis of proteins with hydrogen peroxide. Various types of albumin protein were mixed with different concentrations of peroxide for one hour. The protease trypsin was added and portions were removed and immediately mixed with trichloroacetic acid to precipitate either unhydrolyzed proteins or proteins that were minimally hydrolyzed. After centrifugation, the supernatant was collected and contains small protein fragments that were released by hydrolysis; the total amount of these were measured using the Lowry assay. The results indicate that even without the protease, there is significant hydrolysis with peroxide and none when the proteins were mixed with water. The amount of hydrolysis correlated with the amount of peroxide. It was important in this method to chemically inactivate peroxide and efficacy of different metals were tested. These results suggest that hydrogen peroxide, even at the concentrations used in whitening strips, can damage protein by promoting hydrolysis. This work was supported by Research and Professional Development Grant from Stockton University.

## EFFECTS OF CURCUMIN ON PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer-related death in the United States. Without an efficient method of diagnosis or treatment plan, the prognosis for PDAC is grim. Plant-derived chemicals that possess a multitude of benefits are becoming increasingly popular as treatment options. For example, curcumin is found in the rhizome of turmeric and has been shown to produce various antitumor effects. Previous studies assessed the effects of curcumin on human PDAC, glioblastoma, lung, breast, cervical, and colon tumors utilizing immunocompromised, xenograft mouse models. However, other studies have shown that some antitumor compounds that appear to be effective in immunocompromised mouse models are not as effective in humans. To address the potential complication of a fully functional immune system on curcumin efficacy, we assessed the efficacy of curcumin treatment on murine PDAC cell lines using syngeneic, immunocompetent mice. Murine PDAC lines were derived from genetically engineered KPC mice, which spontaneously produce metastatic PDAC that closely resembles human PDAC, and these tumor cells were implanted subcutaneously into C57BL/6J mice. Once the tumors were established in the mice, curcumin (or vehicle alone) was administered via intraperitoneal (i.p.) or intratumoral (i.t.) injection. Treatment efficacy was assessed by measuring tumor growth and the overall survival of mice. Further assessment was conducted by analyzing histology and relative expression of various genes via qRT-PCR.

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## UNDERSTANDING THE INTERACTION BETWEEN ESCHERICHIA COLI GENOME AND CUSR TRANSCRIPTION FACTOR

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Two-component systems (TCS) are signal transduction systems commonly found in bacteria. They are comprised of a histidine kinase that responds to stimuli and a response regulator that is responsible for carrying out effector functions. TCSs enable bacteria to sense diverse environmental conditions and carry out appropriate responses. The CusRS TCS system in *Escherichia coli* (*E. coli*) regulates the expression of the *cusCFBA* operon and *cusRS* operon, enabling *E. coli* to respond to toxic levels of copper (Cu) and silver (Ag). Specifically, the CusS histidine kinase is responsible for sensing the presence of Cu and Ag. These metals activate the kinase, causing it to phosphorylate the response regulator CusR. CusR is composed of two domains: a regulator region (RR) and an effector domain (ED). Phosphorylation of the RR domain leads to the activation of the ED. CusR is then able to act as a transcription factor and bind to either the *cusCFBA* promoter or *cusRS* promoter. However, the direct interaction of CusR with the genomic DNA has yet to be studied. We used electrophoretic mobility shift assays to characterize this interaction. Preliminary data suggests that CusR binds only double-stranded DNA and that phosphorylation is not necessary for CusR to bind to its promoters. Understanding this interaction will provide insights into the mechanisms of metal resistance. Since systems used in metal resistance are related to antibiotic efflux complexes this could also contribute to our understanding of antibiotic resistance.

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## PROTECTIVE EFFECTS OF CRANBERRY JUICE ON ALCOHOL-RELATED LIVER DAMAGE

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Global alcohol consumption is a growing concern due to the adverse effects consumption of alcohol can have on the body. The over consumption of alcohol has been linked to the deposition of lipids within liver tissue, tissue scarring, and later development of cirrhosis. When alcohol (ethanol) is consumed in large volumes, the liver is no longer able to convert ethanol to a neutral form (acetyl radical) for further metabolism in the Krebs Cycle. The consequence of the liver being overwhelmed is a buildup of reactive oxygen species that can cause damage to hepatocytes.

In this study, we examined the effects of combining cranberry juice with ethanol during consumption using C57Bl/6 mice to determine whether the antioxidants in the juice had a protective effect on alcohol-related liver damage. Previously, cranberry juice has been shown to have antioxidative effects on damaged liver mitochondria in a non-alcohol-related model. In order to investigate whether cranberry juice has a protective effect when combined with alcohol, we compared three groups of six mice treated as follows: Group 1 – H<sub>2</sub>O (negative control), Group 2 – ethanol (positive control), and Group 3 – ethanol/cranberry juice (experimental group). The mice were dosed for 15 days with an increase in ethanol concentration every seven days and a final “binge” dosage on the 15<sup>th</sup> day. The dose of ethanol administered was relative to body weight. Following euthanasia, blood was harvested and processed for serum, the liver was removed, weighed, and a portion was frozen in OCT for histological examination and a portion was frozen in TRIzol for RNA extraction and gene expression analysis of histone deacetylase (HDAC) genes. HDAC gene expression has been shown to be altered during times of alcohol induced liver steatosis (damage). From these examinations, we were able to quantify the protective effect cranberry juice has on alcohol-related liver damage.

## ENGINEERING MELTING TEMPERATURES OF CARBOHYDRATE BINDING MODULES THROUGH SITE-DIRECTED MUTAGENESIS

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Lignocellulosic biomass comprises the structural elements of plants which in turn is composed of polysaccharides such as pectin, cellulose and hemicellulose. In order to utilize this abundant, renewable resource the polysaccharides must be digested into soluble sugars for downstream uses such as fermentation into bioproducts. The cost of the cellulases and other enzymes required for saccharification of lignocellulosic biomass is one roadblock to cost-competitive biofuels. This research is focused on developing genetically modified cellulases to implement an enzyme recycling strategy in a biorefinery. Cellulases are modular enzymes built of a catalytic module and one or more Carbohydrate Binding Modules (CBM). While the CBMs are necessary for the robust catalytic activity of the holoenzyme, a key obstacle to recycling cellulases is that following the saccharification reaction much of the enzyme remains adsorbed to the residual, undigested biomass through binding interactions with the CBMs. We are developing temperature-tuned CBMs that can be desorbed from the residual biomass by heat-denaturation followed by refolding at a slightly lower temperature for the recycled holoenzyme to be reused in multiple rounds of biomass saccharification. In order to conveniently monitor the binding properties of the CBMs we cloned the sequences encoding CBMs from families 11, 30, and 44 to form CBM-green fluorescent protein superfolder (PGSF) fusions. An assay was developed using fluorescence to determine under which conditions the CBMs bound to various cellulose-based substrates. Using bioinformatics tools we identified potential amino acid substitutions to lower the  $T_m$  of the CBMs while maintaining function. Site-directed mutagenesis was employed to create mutant CBMs to test the effect of various mutations on  $T_m$  and cellulose binding ability. The long-term application of our work may provide an avenue for reducing the cost of biofuel production so that the cost of renewable, carbon-neutral transportation fuels can compete with petroleum-based fuels.

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## A FIRST STEP TOWARD UNDERSTANDING OBSCURIN'S MOLECULAR MECHANISM

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Obscurin is a giant (800-950 kDa) cytoskeletal protein involved in mobility and adhesion signaling pathways. It is the second most mutated gene in breast and colorectal cancers, and in muscles mutated obscurin leads to muscular dystrophy and cardiomyopathy. Obscurin's specific role in these diseases remains an active area of research. In an effort to better understand obscurin's molecular mechanism of action, here we describe an experimental approach to elucidate obscurin binding partners in eukaryotic cells. We have designed a series of constructs containing a promiscuous biotin ligase, and transfected this DNA into MDCK II epithelial cells. The resulting microscopy images and SDS-PAGE gels give us our first understanding of obscurin subcellular localization in non-muscle cells, and provide a proof-of-concept platform to conduct future BioID assays on similar samples to identify specific binding partners.

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## OPTIMIZATION OF A CHIP ASSAY TO ASSESS THE WT CD44-ICD BINDING TO THE MMP9 GENE PROMOTER

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CD44 is a cell membrane receptor which undergoes a proteolytic cleavage within the cell membrane to produce a 74 residues peptide known as the CD44 intracytoplasmic domain (CD44-ICD). This peptide can be translocated into the nucleus where it has the ability to regulate transcription. This transcriptional regulatory mechanism is not completely understood but published chromatin immunoprecipitation (ChIP) data demonstrated that a GFP-tagged CD44-ICD is present in a complex with Runx2 in the MMP-9 gene promoter. We are interested in validating this data by analyzing the wild-type(wt) CD44-ICD peptide and thus we hypothesize that the wt CD44-ICD interacts with Runx2 in the nucleus. To test this hypothesis, we carried out ChIP assays treating the MCF-7/CD44 cells with formaldehyde (a protein-DNA crosslinker) as well as with DSG (a protein-protein crosslinker). The ChIP DNA was PCR amplified with primers flanking the CD44-ICD response element (CIRE) in the promoter region of the MMP-9 gene. The PCR results were not conclusive since the expected band is not consistently present. We have concluded that the crosslinking process in the ChIP assay might be affecting the availability of the CD44-ICD epitope since in Proximity Ligation Assays (PLA) in which no crosslinkers are used, we were able to detect the hypothesized CD44-ICD/Runx2 protein-protein interaction in the same cell line using the same anti-CD44-ICD antibody.

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## CHARACTERIZING TRANSLATIONAL RECODING MECHANISMS IN MAYARO VIRUS

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Mayaro virus (MAYV) is an arthropod-borne, positive sense, single stranded alphavirus. It infects mammalian hosts inducing fever, generalized myalgia, arthralgia, diarrhea and vomiting. It is an emerging virus in tropical America that currently poses threats to children and senior citizens that cannot be treated because currently no FDA approved therapeutics exist.

Translational recoding mechanisms are exceptions to the classic mode of translation. Termination Codon Readthrough (TCR) and -1 Programed Ribosomal Frameshifting (-1PRF) are two examples that are utilized by viruses and eukaryotes for post-transcriptional gene regulation. This work characterizes both the conserved -1 PRF sequence encoded in the structural Glycoprotein 3 (gp3) polyprotein and the predicted RT signal located between nonstructural protein 3 (nsp3) and non-structural protein 4 (nsp4) in MAYV's genome. It is hypothesized that this -1 PRF sequence is important for generating a transframe protein product and the TCR sequence is important for generating nsp4, both of which are essential for viral pathogenicity.

Preliminary validation in bicistronic reporter systems suggests the predicted sequences promote significant rates of translational recoding. Additionally, silent mutations were identified in the slippery site of the -1PRF sequence and in the stop codon by changing it from UGA to UAA or UAG in the TCR sequence. All mutants reduced translational recoding efficiency in bicistronic reporter assays and western blot. The stimulatory RNA secondary structures that are responsible for promoting -1 PRF or TCR were characterized using the biochemical method. Then these structures were used to identify silent structural mutations that ablate the rates of -1 PRF and TCR. We expect that these and other mutations will affect the fitness and hence pathogenicity of the virus. The outcome of the proposed work will aid in identifying viable mutants of the full-length virus that maybe used for developing attenuated live virions.

This research was supported in part by a grant to the University of Maryland from the Howard Hughes Medical Institute through the Science Education Program.



# Biological Sciences

## ABSTRACTS

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***Confidential***

*Please note that many of the abstracts are not approved for dissemination beyond the student poster sessions and, therefore, are not approved for posting online or distribution beyond the 2018 Undergraduate Research Symposium in the Chemical and Biological Sciences.*





**FACTORS ASSOCIATED WITH SPONTANEOUS CLEARANCE OF SEXUAL  
TRANSMITTED HEPATITIS C VIRUS INFECTION AMONG HIV-INFECTED MEN  
WHO HAVE SEX WITH MEN**

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Hepatitis C virus (HCV) infection causes chronic hepatitis, which can lead to liver fibrosis and cirrhosis, and ultimately end-stage liver failure or hepatocellular carcinoma. In the United States, the mortality caused by HCV has surpassed that caused by HIV in recent years. Liver disease progression caused by HCV infection is accelerated and exacerbated in people with HIV coinfection. About 20% of the people with acute HCV infection can clear the virus without developing into chronic/persistent infection. It is not entirely clear as to why some infected people can spontaneously clear HCV, while others fail to do so. The information is even more limited among people with HIV coinfection. The present study is conducted to identify potential host and viral factors that are associated with HCV spontaneous clearance in HIV-infected men who have sex with men (MSM). A total of 98 coinfecting HIV/HCV MSM who received care between 2003-2014 and had anti-HCV positivity, were included for analyses. Demographics, clinical, immunologic and virologic data of these patients were abstracted from the electronic medical records for statistical analyses. We found that spontaneous clearance of HCV was higher among HIV-coinfecting MSM who were younger, obese, had >1 anogenital STI, had HSV and/or HPV infection, had active HIV replication or had chronic hepatitis B (HBV). Thus, BMI, active HIV replication, anogenital STIs and chronic HBV appear to be important factors associated with spontaneous clearance of HCV among HIV-infected MSM. Taken together, persistent HSV/HPV infection, active viral replication, and chronic HBV infection may set several checkpoints throughout the HCV journey, from anogenital mucosa sites to the liver, leading to enhanced spontaneous clearance. Therefore, understanding immune mechanisms that lead to spontaneous HCV clearance will aid in the development of therapeutics and prophylactics for HCV infection.

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## WATER QUALITY ANALYSIS AND MACROINVERTEBRATE COMPOSITION OF AQUATIC ECOSYSTEMS ON ROSEMONT COLLEGE CAMPUS

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The Rosemont College Stream Team has been monitoring the health of the campus stream and ponds since 2013. This monitoring is part of a long term ecological study to determine the impact of storm water run-off on our aquatic ecosystems. We determined that both are in poor health, from assessing macroinvertebrate diversity and water quality testing. Using the Leaf Pack Method to collect macroinvertebrates, we calculated a biotic index of 6.6 and 6.5 for the stream and pond respectively, indicating the presence of mostly pollution tolerant species. The percent EPT was below 1%, indicating a rarity of clean stream macroinvertebrates. Average chloride levels range from 27 mg/L during spring to 113 mg/L in winter, with peaks of 218 mg/L during winters with high precipitation and heavy use of deicers. Phosphate levels averaged  $0.6\text{mg/L} \pm 0.08$ , greater than the recommended maximum of  $0.1\text{mg/L}$ . Nitrate levels averaged  $2.7\text{mg/L} \pm 0.70$ , within the acceptable limits for drinking water but above the maximum tolerance level ( $2\text{mg/L}$ ) for the most pollution sensitive species. Dissolved oxygen levels are also low, ranging from 8-14 mg/L. We conclude that elevated levels of contaminants typically carried in storm water run-off, combined with low dissolved oxygen levels are contributing to the dominance of pollution tolerant macroinvertebrates in the stream and ponds on campus. Our results indicate that our stream and ponds are similar to Mill Creek, ranked as one of the poorest quality streams in the Schuylkill River Watershed.

The Rosemont College Stream Team would like to acknowledge the Connelly Foundation and Rosemont College for their support of this research.

## EVOLUTION OF THE EXPANSIN SUPERFAMILY IN PHOENIX DACTYLIFERA

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Expansins are proteins in the cell wall of plant cells that mediate the ‘loosening’ of the cell wall so that the cell can expand, allowing the plant to grow. Expansins also aid in other developmental processes in plants such as seed germination, fruit softening, water stress response, etc. There are four families of expansins within the superfamily: EXPA, EXPB, EXLA, and EXLB. The EXPA and EXPB families are responsible for cell wall expansion, while the EXLA and EXLB families’ functions are unknown<sup>1</sup>.

Previous studies show that grasses within the monocot taxon contain a different carbohydrate composition in their cell walls compared to all other monocots and dicots<sup>2</sup>. *Phoenix dactylifera*, also known as the Date Palm, is a monocot (but not a grass) whose expansin genes have not been studied<sup>3</sup>. This project aimed to discover whether the expansin superfamily of the Date Palm would relate more closely to grasses (with which it shares a recent common ancestor) or to dicots (with which it shares a more common cell wall composition).

The *P. dactylifera* genome (v 1.0)<sup>4</sup> was mined to collect all *P. dactylifera* expansin genes. After data collection, the genes were trimmed using SeqBuilder Pro software and a phylogenetic analysis was performed to determine relationships between *Phoenix dactylifera*, *Arabidopsis thaliana* (thale cress, a dicot), and *Oryza sativa* (rice, a monocot). The *A. thaliana* and *O. sativa* genomes have been heavily studied, making them effective comparisons for *P. dactylifera*. Results indicate that the expansin superfamily of *Phoenix dactylifera* has a composition more similar to that of dicots than grasses.

Thank you to Dr. Francis T. Lichtner Jr. for supporting this research through his generous gift to the Lebanon Valley College Biology Department.

**CHARACTERIZATION OF SOIL DWELLING BACTERIOPHAGE OREGANO**

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Phage, also known as bacteriophage, are a type of virus that infect and take over bacterial hosts in order to reproduce. The phage population is highly diverse and constantly evolving, so new phages are frequently being discovered. In recent years, scientists have been characterizing and analyzing phage genomes in hopes of being able to use phage for applications such as environmental health gauges and preventative methods for fighting bacterial infections, as phage are essentially harmless to human cells. In this research study, soil dwelling phage were isolated and characterized to understand the variations in phage DNA and how they change over time. Initially, five soil samples were collected from the Clemens Crossing Elementary School Field in Columbia, Maryland and both enrichment and direct isolation methods were used to determine the prevalence of phage in each sample. The bacteriophage Oregon was isolated from the samples and was further purified and characterized. Transmission electron microscopy (TEM) revealed that Oregon is a member of the family *Myoviridae*. PCR indicated that Oregon is a unique phage and appears to share DNA with phages in sub clusters C3A and C3B. Host range tests were conducted and showed that Oregon is capable of infecting *Bacillus thuringiensis* (*B. thuringiensis*) serotype israelensis, *B. thuringiensis* serotype HD1, *B. thuringiensis* serotype HD3 and the control, *B. thuringiensis* serotype kustaki. In the future, the DNA isolated from Oregon will be sequenced and annotated so that it can be further studied and used in potential upcoming research.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 8TL4GM118989, 8UL1GM118988, and 8RL5GM118987).

## Pathophysiology of neurons in the childhood neurodegenerative disease, Mucopolidosis type IV (MLIV)

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Transient receptor potential mucolipin 1 (TRPML1) is a cation channel that is localized to the late endosome and lysosome in all cells. TRPML1 functions to regulate lysosomal pH and ion homeostasis. Loss of function mutations in the human TRPML1 (*MCOLN1*) gene results in a devastating childhood neurodegenerative disease known as Mucopolidosis type IV (MLIV). TRPML1 is an evolutionarily conserved protein with homologs in *Drosophila melanogaster* (*TRPML*) and *C. elegans* (*CUP-5*). In *Drosophila*, *trpml* knockout flies exhibit neurodegeneration and motor defects similar to MLIV patients. Examination of the adult fly brain demonstrates that *trpml* knockout flies have increased accumulation of apoptotic neurons. In this study of neurodegeneration we are identifying cellular mechanisms that are driving increased neuronal cell death in MLIV. Preliminary results show that loss of *trpml* function leads to a significant decrease in an anti-apoptotic factor  $\beta$ -NAC. Our results suggest that  $\beta$ -NAC is required for maintaining neuronal cell viability. Through this study we intend to improve our understanding of neuronal cell pathology in MLIV patients.

## EVALUATION OF A CRISPR-CAS12A-BASED DNA DETECTION PLATFORM TO IDENTIFY PREY SALAMANDER SPECIES IN FECAL SAMPLES OF LOUISIANA WATERTHRUSH NESTLINGS

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Determining the frequency at which the Louisiana Waterthrush provisions novel prey items to its nestlings is critical to conservation efforts. These efforts facilitate a greater understanding of the dietary plasticity exhibited by this primarily insectivorous Neotropical songbird. DNA metabarcoding reveals that the diet composition of this aquatic-specialist is heavily impacted by stream quality. Past research has indicated that adult waterthrush nesting on impacted streams may diversify their foraging efforts, even targeting salamanders in response to the absence of pollution-sensitive arthropods. Although DNA metabarcoding approaches are necessary for accurately detecting hundreds of potential arthropod prey species, it is inefficient when attempting to detect uncommon, novel prey such as stream-side salamanders.

An innovative method known as DETECTR (DNA Endonuclease Targeted CRISPR *Trans* Reporter) has been used in medical diagnostics to identify specific viral strains. This detection platform utilizes the CRISPR-LbaCas12a protein to cleave species-specific target DNA sequences along with a fluorescent report system. LbaCas12a is programmed with taxon-specific gRNAs (guide RNAs) that enable it to bind to these DNA targets. For proper targeting, there must be a protospacer adjacent motif (PAM) on the 5' end of the DNA strand that is complimentary to the target sequence, but this PAM cannot be within the DNA sequence that the gRNA is directly targeting. PCR amplification of the target region enhances the level of detection.

Here, we perform an *in silico* evaluation of the above criteria towards the validation of a novel application of DETECTR on twelve potential prey salamander species present on waterthrush breeding territories in southwestern Pennsylvania. We also developed salamander-specific PCR primers and verified their effectiveness in amplifying DNA for DETECTR applications. Future work will apply these methods to the *in vitro* identification of salamander DNA extracted from both salamander tissue and nestling waterthrush fecal samples.

This project was supported by the Bayer School of Natural & Environmental Sciences through the Summer Undergraduate Research Program at Duquesne University. It is part of a larger ongoing conservation research project supported by Steve Latta and Robert Mulvihill (The National Aviary), focused on studying the life history of the Louisiana Waterthrush at the Powdermill Nature Reserve (Carnegie Museum of Natural History).

## NAVIGATING THROUGH YEAST MEIOSIS USING A COMPASS

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Differentiation requires that cells execute transient transcriptional programs. These programs are regulated by a complex network of post-translational histone modifications. Histone H3 Lys4 methylation (H3Lys4 me), catalyzed by the highly conserved Set1-containing COMPASS complex, is one of the most well-characterized of the histone modifications. H3Lys4 can be methylated up to three times, with each methylation level having a different transcriptional output. Seminal work in the budding yeast *Saccharomyces cerevisiae* has demonstrated that specific members of the COMPASS complex are required for precise methylation levels. While much is known regarding COMPASS function during vegetative growth, less is understood concerning COMPASS during differentiation. Previous studies have demonstrated that Set1 is necessary for yeast to execute meiosis, but have not examined the role of the other COMPASS members. This study focuses on understanding the role of the COMPASS complex during yeast meiosis. Using deletion mutagenesis, we investigated the requirement of each COMPASS subunit to complete meiosis. Surprisingly, we found that SWD1 is important for early meiosis and spindle assembly while SWD3 is not, contradicting their roles during mitosis. Future work will aim to understand the role of the synaptonemal complex protein Zip1 in mediating this process.

**CHARACTERIZATION OF BACTERIOPHAGE CHURROBLAST**

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Bacteriophage are viruses that contain an RNA or DNA genome and are capable of infecting bacteria. Bacteriophage, or phage, have been meticulously studied by scientists and many new species are being discovered. However, scientists do not know how many unique phage there are in the world, which is important given that phages are currently being investigated as therapeutic alternatives to antibiotics, environmental indicators, and food preservatives. To test the prevalence of phage in the environment, five soil samples were collected from Elkridge, Maryland. Both direct and enrichment isolation methods were used to detect any phage present within the samples. Further characterization of the phage was done using a quality control (QC) gel, transmission electron microscopy (TEM), host range testing and polymerase chain reaction (PCR). A myoviridae phage ChurroBlast was isolated from a soil sample collected and preliminary PCR results show the phage shares DNA that is similar to a cluster 3A type. Host range testing indicated the ChurroBlast was capable of infecting hosts *Bacillus thuringiensis* DSM 350, *Bacillus thuringiensis* HD1 and *Bacillus thuringiensis* HD2. The genome of ChurroBlast is currently being sequenced and will be annotated in the future.

Acknowledgements: This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 8TL4GM118989, 8UL1GM118988, and 8RL5GM118987).



## PULSATILE ELECTROMAGNETIC FIELDS REGULATE BONE INTEGRITY THROUGH ACTIVATION OF VOLTAGE SENSITIVE CALCIUM CHANNELS

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Bone cells, such as osteoblasts and osteocytes, are sensitive to mechanical load, incurred through everyday activities such as walking. These mechanical loads increase bone mass and, conversely, disuse can lead to bone loss. This bone loss is similar to that seen in astronauts in a microgravity environment or from disuse due to injury. As we have shown in our lab, voltage sensitive calcium channels (VSCC) are important regulators in bone osteogenesis in response to load. There are two primary types of VSCC in bone: L-types and T-types. L-VSCC are long-lasting, require a higher voltage for activation, and are inhibited by nifedipine. T-VSCC are activated with lower voltages, are primarily open during membrane depolarization, and are inhibited by NNC-55. I hypothesized that these channels can be stimulated by pulsed electromagnetic fields (PEMF) and in turn can stimulate bone formation. I further hypothesized that the L-VSCC regulates this PEMF-induced osteoblastic proliferation. To test this hypothesis, I exposed MC3T3-E1 osteoblasts to PEMF in the presence or absence of nifedipine and NNC-55. PEMF exposure was repeated 2 hours a day for 4 days and proliferation of the MC3T3-E1 cells was determined using an MTS assay. PEMF exposure increased proliferation of these cells compared to non-stimulated controls. Inhibition of the L-VSCC or T-VSCC reduced proliferation, even with the exposure to PEMF. These data suggest that exposure of osteoblasts to PEMF increases the number of osteoblasts available for bone formation and that this is regulated through VSCC. Understanding the effects of PEMF on osteoblast function could allow us to develop wearable technology that can maintain bone density in states of microgravity or immobilization.

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-Dev.

# **DIFFERENTIAL BINDING OF A THYROID HORMONE RECEPTOR BETA SPECIFIC SYNTHETIC FLUORESCENT MOLECULE JZ01 TO VERTEBRATE NEURONAL AND GLIAL CELLS**

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Thyroid Hormone receptors are nuclear hormone receptors and bind to triiodothyronine (T3). There are multiple Thyroid Hormone receptor (TR) isoforms expressed in the brain and identification of the neuronal subtypes that express their different isoforms is essential for further delineating their function. To this end we have embarked upon developing fluorescent compounds that bind to specific isoforms of TR. Our initial binding studies using dissociated mouse cortical cultures and flow cytometry indicated that JZ01 binds to brain cells when incubated for one hour at 10nM concentration. In this project, we are testing the differential binding of the first compound in the series JZ01 which is expected to bind to TR-beta but not TR-alpha. We used fluorescence microscopy to assess the differential binding of JZ01 to different neuronal types as well as glial cells from the well-established vertebrate model system — the developing chick embryo. Chick optic tectum and forebrain from embryonic day 7 (E7) chick embryos were dissected and dissociated with 0.25% trypsin and plated on Matrigel coated coverslips in neurobasal medium. After allowing the cells to develop and differentiate for at least three days, the coverslips were treated with different concentrations of JZ01 – ranging from 10nM to 50nM. Initial results after binding JZ01 for one hour indicate that the optimal concentration for binding of JZ01 to chick optic tectum cells is 25nM. Our results also indicate that JZ01 remains bound to cell even after fixing and washing. We will next identify the neuronal and glial subsets using specific neuronal and glial cell markers like beta-3 tubulin, neurofilament, CamKII, Nestin, Vimentin, GFAP, MAP2 and others. It is expected that JZ01 will bind differentially to neuron and glial cells and will inform us regarding the expression status of TR-beta in these cell types.

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\*M.D. and T.D. contributed equally to the work

## EVALUATING THE RELATIONSHIP BETWEEN ANXIETY AND PERFORMANCE IN CLINICAL CONCUSSION SCREENINGS

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Anxiety can invoke symptoms such as fear, nervousness, difficulty concentrating, tachycardia, and muscle twitching which all have been shown to impair neurocognitive and biomechanical outcomes. Anxiety can be elevated following concussion, however the relationship between anxiety and common concussion assessments is unknown. Despite common misconception, anxiety does not affect standardized test scores on college level examinations, however, its effect on neurological testing requires further investigation. Therefore, the purpose of this study was to identify if anxiety predicts performance on concussion assessments throughout concussion recovery.

Nineteen student-athletes with diagnosed concussion were enrolled. Anxiety levels were collected using both the Hospital Anxiety and Depression Scale (HADS) and the Brief Symptom Inventory (BSI-18) questionnaires. Participants also completed common concussion clinical measures: SAC (Standardized Assessment of Concussions), CRT (Clinical Reaction Time), KD (King-Devick) test, and the BESS (Balance Error Scoring System) test at four different time points: 1) baseline, 2) acutely post-injury (<48 hours), 3) when asymptomatic, and 4) when cleared for return to play (RTP).

There was no predictive relationship between measured anxiety at baseline and performance on subsequent post-concussion screenings: SAC ( $R^2 = .010$ ,  $p = .921$ ), BESS ( $R^2 = .050$ ,  $p = .664$ ), KD ( $R^2 = .007$ ,  $p = .943$ ), mean CRT ( $R^2 = .081$ ,  $p = .709$ ), best CRT ( $R^2 = .237$ ,  $p = .115$ ). There was a significant main effect for time for all tests except CRT mean ( $p < 0.05$ ). Post-hoc tests identified significant improvements in HADS ( $p = .005$ ), SAC ( $p = .029$ ), and BESS ( $p = .002$ ) baseline to RTP test performance, but did not show significant changes in BSI-18 baseline to RTP ( $p = .804$ ). Anxiety at baseline may not be a symptom of notable importance for clinicians to consider when making their return to play decisions, so other mental health components should be investigated.

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**ISOLATION OF UNIQUE BACILLUS PHAGE, LANDFISH, FROM A SOIL SAMPLE**

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Bacteriophages are one of the most common form of viruses. They are capable of infecting, depending on the phage, a single bacterium or a multitude. Due to the plethora of bacteriophages, many go undiscovered. With research into bacteriophages increasing due to their potential use in medical treatments, it is important to isolate and characterize the phage population. In this study, we took a soil sample from the gps location (39.02687 , -76.685), which is located in Crofton, Maryland, and isolated through an enrichment method. After undergoing serial dilutions and being plated with *Bacillus Thuringiensis Kurstaki* , plaque assay was repeated until a webbed plate was obtained. Once a webbed plate was obtained, the plate was used to create lysate. This lysate was used to create more plates, as well as being used for transmission electron, showing that a singular phage was obtained. Following this host range testing and DNA isolation was performed. Host range testing showed that this particular phage, landfish, had trouble with infection of any other bacteria except the stock *Bacillus Thuringiensis Kurstaki*. DNA from the DNA isolation was then used for a restriction enzyme digestion analysis, which confirmed that another phage wasn't isolated.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 8TL4GM118989, 8UL1GM118988, and 8RL5GM118987).

## EFFECT OF PARKINSON'S DISEASE PATHOPHYSIOLOGY ON DOWNSTREAM TARGETS OF HSF1

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Abnormal  $\alpha$ -synuclein ( $\alpha$ S) aggregation and neurodegeneration are hallmark features of Parkinson's disease (PD). Heat shock transcription factor 1 (HSF1) is a protein responsible for regulating protein folding via heat shock proteins (Hsps) and is transcriptionally activated in response to cell stress and certain disease states. Reports suggest that HSF1 may be degraded in PD via phosphorylation by casein kinase 2  $\alpha'$  (CK2 $\alpha'$ ) and we hypothesized impaired HSF1 function may contribute to PD pathophysiology. We sought to determine whether HSF1 phosphorylation and degradation are increased in PD due to upregulated CK2 $\alpha'$ . To test this, brains from A53T mutant  $\alpha$ S-expressing PD transgenic (Tg) and non-transgenic (nTg) mice were analyzed via immunoblots and immunohistochemistry. We found, compared to nTg and asymptomatic Tg mice, end-stage pre-formed fibril injected (PFF+) transgenic mice showed a reduction in CK2 $\alpha'$  levels, a significant increase in Hsp25 levels, and no change in levels of HSF1 and Phosphorylated HSF1 in the brainstem/spinal cord regions. These results show that the  $\alpha$ S-A53T mutation in our mouse model has no effect on the expression of phosphorylated HSF1 at serine 307 but causes significant upregulation of Hsp25 in astrocytes. Further research is needed to clarify HSF1 inactivity in response to PD cell stress.

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**THE EFFECTS OF COMMON CHICKWEED ON GARLIC MUSTARD**

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Common chickweed is a fast spreading weed that was introduced to the United States from Europe. Previous research shows that this plant uses allelochemicals to outcompete other species. We conducted a series of studies that investigated how common chickweed influenced the common invasive understory plant garlic mustard. First, we documented that garlic mustard was more common ( $P < 0.05$ ), had larger leaves ( $P < 0.01$ ), and greater dried biomass ( $P < 0.001$ ) when found at least 1 m from the root systems of common chickweed. Secondly, we conducted a greenhouse experiment where we grew garlic mustard plants in three treatments: potting soil, potting soil pre-conditioned with chickweed, and potting soil mixed with dried chickweed. Garlic mustard plants tended to have a greater biomass and leaf number when grown in regular potting soil. Finally, we applied extracts of common chickweed to various seeds to investigate if allelochemicals might negatively influence germination success. Our preliminary results showed that germination rates were negatively influenced by higher concentrations of the extract. Based on our studies we believe that the effect of common chickweed on garlic mustard is most effective during seed germination.

**DEVELOPMENT OF A LOW-COST, OPEN SOURCE, MYOELECTRIC ARM**

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The advent of low cost microcontrollers has led to significant advances in hobbyist levels of prosthetic development. Notably, prosthetics utilizing a myoelectric control system allow for greater functionality, mobility, and dexterity than traditional body powered or cosmetic prosthetics. A myoelectric prosthetic picks up electromyogram (EMG) signals that are sent by the brain to control a muscle; this signal must then be translated by a microcontroller and converted to a digital output for servos from the analog input that is the signal from the residual limb. Current commercially available myoelectric prosthetics are heavy, due to metal hardware, as well as, expensive. To solve these problems we used 3D printing to drastically reduce both the weight and cost, allowing a myoelectric prosthetic to be more financially accessible and usable by a wider audience, particularly children, due to the significant cost and weight reductions offered by utilizing 3D printing. In this work, we present our prototype design which utilizes combinations of multiple open source designs, such as the Ada Hand by Open Bionics, and the MyPo by Poparaguay, along with customized Arduino coding to interpret muscle signals from a low-cost arduino-compatible myoelectric detector.

This project could not have been accomplished without the design work, insight, and expertise from Open Bionics and Po Paraguay, as well as, funding from Marymount University's Discover Program.

ESCAPE RESPONSES FROM LOOMING STIMULI IN *PHIDIPPUS REGIUS*Gaudin VA<sup>1</sup>, Cleland CL<sup>1</sup><sup>1</sup> Department of Biology, James Madison University, 951 Carrier Dr, Harrisonburg, VA 22807

Animals use withdrawal and escape responses to retreat from threats. Looming stimuli, which represent the approach of a predator, evoke an escape response in jumping spiders that is mediated primarily by visual cues. The majority of studies have focused on spider predation toward prey; only limited studies have explored the escape response. The goal of our research was to determine the strategy used by jumping spiders, *Phidippus regius*, to escape from looming stimuli. In particular, we sought to determine whether jumping spiders employ jumping as part of their escape repertoire.

Looming stimuli were created by using the controlled projection of a 3” black polyurethane ball (1 m/s, 45 degrees angle, against a white background) toward jumping spiders (*Phidippus regius*, n=9), without actually hitting the spider. The direction of “attack” was varied in 45-degree increments, totaling 8 angles, around the spider. The resulting response was captured from above with high speed video (300 fps) and automated software particle tracking was used to quantify the location and orientation of the spider throughout the escape response. Looming stimuli consistently evoked translation, but not turning. The angle of translation depended significantly on stimulus direction. Typically, following initial translation the spider executed one or two movements that appeared linked to the stimulus, often ending in a position that faced the looming object. Importantly, three of the spiders sometimes jumped away from the looming stimulus, a movement previously reported only associated with prey capture. These preliminary results suggest jumping spiders may use specific, diverse, multi-stage strategies to escape from looming stimuli. Further, their name-sake behavior, jumping, may be employed for both predation and escape.



## DOES ROAD SALT FACILITATE THE INVASION OF A CARNIVOROUS AQUATIC PLANT?

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The application of salts to deice roads has changed the chemical composition of nearby streams and waterways. We conducted a series of experiments that investigated the effects of NaCl on two carnivorous aquatic bladderworts, *U. purpurea*—a common native species—and *U. inflata*—a species that has invaded lakes in the northeast United States. Our results showed that *U. inflata* had a greater tolerance to elevated NaCl concentrations compared to *U. purpurea*. *Utricularia inflata* plants continued to grow in treatments up to 1000 mg/L, while *U. purpurea* declined at 500 mg/L of NaCl. Fragments of *U. inflata* also had higher rates of survivorship compared to *U. purpurea*; 100% of *U. inflata* fragments grew new branches which have the potential to give rise to new individuals, while *U. purpurea* fragments less than 50% survivorship across all NaCl concentrations. Based on our data, we believe that increasing NaCl concentrations due to road salt runoff has the potential to favor the invasive *U. inflata* at the expense of the native *U. purpurea*, causing a major shift in the environmental composition of aquatic plant communities.

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## THE MINIMAL LENGTH FOR AN EFFECTIVE *IN SITU* HYBRIDIZATION PROBE MAY BE GENE-SPECIFIC

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Alternative splicing is a strategy adopted by eukaryotes to increase the number of proteins encoded by a single gene. Because these proteins have different structures and functions, they may be expressed in different parts of an organism. When RNA probes are used during *in situ* hybridization (ISH) to determine such differential expression patterns, however, different mRNA isoforms can potentially all bind to the full-length RNA probe as they share much of the sequence. Because of this, designing an RNA probe that is isoform-unique is critical. Unique sequences can be as short as a single exon, thus the RNA probe can be quite short. However, literature is unclear about how short an effective probe can be. To address this question, we set out to synthesize RNA probes of different lengths and test their efficacy using ISH. We began this endeavor by generating RNA probes of different lengths [190 nucleotides (nt), 500, and 1700 nt (full-length)] for the zebrafish *myoD* gene; these probes were then subjected to ISH. We found that the 500 and 1700 nt probes led to specific staining in the paraxial mesoderm. In contrast, the 190 nt probe led to staining in the entire embryo. Based on these results, we concluded that the minimal effective probe length is between 190 and 500 nt. We also selected a second zebrafish gene, *krx20*, and synthesized probes of 190, 350, and 2037 nt (full-length). Both experimental probes led to specific staining in rhombomeres 3 and 5, as did the 2037 nt probe. This suggested that the minimal probe length for *krx20* is 190 nt or less, and that the minimal ISH probe length may be gene specific. From here, our future plans are to further investigate the gene-dependence of probe length, and further narrow down minimal probe length for *myoD* and *krx20*.

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## THE ROLE OF INSULIN SIGNALING AND NEURON FUNCTION IN DEVELOPMENTAL PROGRESSION

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Insulin signaling pathway relates nutrient levels to metabolism and development in multiple organisms. In *C. elegans*, this pathway is regulated by binding of peptide ligands to DAF-2, the *C. elegans* insulin receptor ortholog encoded by gene *daf-2*. The *fax-1* gene encodes a nuclear receptor that functions in the specification of neuron identities. *fax-1; daf-2* double mutants arrest due to quiescence at the first larval stage (L1), indicating that insulin signaling and neuron function are linked. We can control the level of insulin signaling by temperature. At 15°C, insulin signaling is relatively high and *fax-1; daf-2* double mutants are able to reach adulthood. In contrast, *fax-1; daf-2* double mutants grown at 25°C during embryogenesis arrest as L1 larvae due to low insulin signaling. This allowed us to explore requirements for *fax-1* and *daf-2* at different stages. Raising the temperature to 25°C after hatching results in animals progressing to the L3 dauer stage and arresting at that time, indicating that insulin signaling is not required for progression from L1 to L3. In contrast, raising the temperature to 25°C after the L3 stage resulted in rapid developmental arrest at the L4 or young adult stage. This demonstrates the insulin signaling and neuron function are required for developmental progression to the adult stage.

## PRELIMINARY INVESTIGATION OF RECOVERY IN STREAM ECOSYSTEM FROM ACID MINE DRAINAGE DUE TO PASSIVE TREATMENT

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Coal mining has been active in Pennsylvania since the late-1700s, resulting in the production of industrial waste called Acid Mine Drainage (AMD), which has a negative impact on surface ground waters. AMD is known to lower pH, increase metal concentrations, and result in a loss of macroinvertebrate communities living in streams. To repair these damages, passive treatment is commonly which involves constructing a series of ponds through which the drainage water flow, which leads to much of the iron and sulfur pollutants to be deposited in the wetlands rather than the receiving stream. The purpose of this study the effects of a passive treatment system on an AMD discharge on Sewickley Creek, Westmoreland County, PA. The goal is to quantify the changes in stream chemistry and biology using macroinvertebrate sampling in space (downstream of the treatment site) and time (this is part of a long-term study). We predicted that both biota and chemistry would improve gradually in both space and time. Kick sampling was used to collect macroinvertebrate samples in three one-minute intervals at each sample site. Testing sites included one upstream and three downstream sites, each site being approximately 1 kilometer from the previous site. Water samples were collected at each site and then analyzed using *Lamotte* testing devices. Preliminary results indicate that since 2015, there has been a slight decrease in the presence of iron and sulfate, and a decrease in conductivity. There has been a slight increase in the number of species found at each site, with varying abundances each year. Data collected in previous years indicated improvements at each site with increasing distance from impact. Data collected from this year has been impacted by severe rain and heavy flooding from recent hurricanes, which is believed to have decreased stream life via flood events as well as diluted the stream chemistry, improving many parameters. Further years of research must be done to successfully determine if there is true improvement in stream quality over time.

## CLONING THE CDNA OF THE ZEBRAFISH *NEUREXIN 2A* GENE, A CANDIDATE RECEPTOR GENE FOR *NEUER1*

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Complex life requires coordination and communication between cells, a phenomenon particularly crucial during development. Cells accomplish this task through signal transduction, which often involves a signaling ligand binding to a receptor and triggering downstream effects. *neuer1*, a novel gene that we identified, is critical for proper red blood cell fate specification during zebrafish development. Since *Neuer1* shares a domain with a family of signaling ligands, Neurexophilin, which binds to the Neurexin receptors, we postulated that the same mechanism may be true for *Neuer1*. Zebrafish has an abundance of neurexin genes – six. A major question we are pursuing is which (if any) of the *neurexins* encodes the receptor for the putative *Neuer1* ligand. It is likely for a ligand and receptor to be physically present in proximity in the developing embryo in order to interact during cell signaling. We thus set out to determine the expression pattern of all six neurexin genes, reasoning that the one that is co-expressed with *neuer1* is the likely receptor candidate. Here, we provide a progress report on one of the candidate genes, *neurexin 2a*. In order to determine the expression pattern of *neurexin 2a*, *in situ* hybridization (ISH) using an RNA probe complementary to *neurexin 2a* mRNA needs to be performed. In order to synthesize this RNA probe, we first need to clone the *neurexin2a* cDNA. To this end, we extracted total RNA from zebrafish embryos and synthesized first-strand cDNAs. Using such cDNAs as a template, we amplified a *neurexin 2a* fragment by PCR. The PCR product was then purified, digested by restriction enzymes, and an attempt was made to ligate this PCR product with a pBluescript vector.

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## ALPHA-SYNUCLEIN SUMOYLATION PROTECTS DOPAMINERGIC NEURONS FROM OXIDATIVE STRESS

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Parkinson's Disease (PD) is a neurological disorder characterized by the loss of dopaminergic neurons in the Substantia Nigra pars compacta, due to alpha-synuclein mediated protein aggregates called Lewy bodies. The Small Ubiquitin-like Modifier (SUMO) is a post-translational modification that may regulate protein stability and degradation. We hypothesize that SUMOylation regulate the protein stability/degradation of alpha-synuclein in Parkinson's Disease models. Particularly, we plan to test whether the SUMO conjugase Ubc9 overexpression mitigates the alpha-synuclein A53T mutation induced PD symptoms in mice. For the *in vivo* studies, we crossbreed the Ubc9 overexpressing transgenic (Tg) mice with recently received alpha-synuclein A53T mutant mice that showed PD-related symptoms around 13 months of age. Genotyping has to be conducted to confirm the over-expression of Ubc9 and the mutant alpha-synuclein. My role in the lab is to set up the PCR conditions for genotyping alpha-synuclein mutant, as well as conducting Ubc9 genotyping. For *in vitro* studies, immunoprecipitation was performed using rat N27 dopaminergic cell line overexpressing WT alpha-synuclein and non-SUMOylated alpha-synuclein mutant (K96R & K102R). Currently we are assessing the differential protein interactions between WT alpha-synuclein and non-SUMO form of alpha-synuclein using mass spectrometry-based proteomics. We expect to identify different partner proteins of alpha-synuclein with SUMOylation, compared to non-SUMOylated alpha-synuclein. In the mouse studies, we used the Ubc9 transgenic mice with chronic MPTP injections which cause oxidative stress-induced Parkinson's disease-related symptoms to mice. Our preliminary data showed that the over-expression of Ubc9 increases the solubility of alpha-synuclein and further protects dopaminergic axon tips in the striatum. Using confocal microscopy, we expect to see more alpha-synuclein and synaptophysin labels in the striatum from Ubc9 over-expressing Tg mice with MPTP treatment, compared to wild type with MPTP. This study may reveal SUMO-induced differential protein interactions, which may shed lights on identifying potential regulatory targets in PD pathology.

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## THE ROLE OF FIBRONECTIN IN POST CATARACT SURGERY INFLAMMATION

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Cataract, the leading cause of blindness worldwide, is defined as the clouding of the ocular lens. While cataract surgery has reduced cataract related blindness, it is not without side effects. A short-term side effect is post-surgical inflammation. While this can be treated with steroids, these treatments are uncomfortable for patients and are not always effective. A longer-term side effect is a fibrotic condition called posterior capsular opacification (PCO). PCO, or secondary cataract, results from the proliferation and migration of lens epithelial cells (LECs) remaining post cataract surgery (PCS). While YAG laser treatments are available to treat PCO, these have their own negative consequences, including retinal detachment. Thus, a comprehensive understanding of the molecular mechanisms leading to post-surgical inflammation and lens cell fibrosis must be reached in order to optimize the outcomes of cataract surgery. Our lab has found that fibronectin, a molecule that upregulates in LECs PCS, is required for the sustained fibrotic response seen in PCO. However, the role of fibronectin in post-surgical inflammation is unknown. Thus, fibronectin conditional knockout (FNcKO) mice were generated which lack fibronectin expression in the lens. These animals were subjected to cataract surgery, and their response compared to wild type animals at 24 hours PCS, when robust upregulation of key pro-inflammatory mediators is normally seen. Immunostaining for the three most upregulated mediators, CXCL1, S100A9, and gCSF3, in wildtype mice revealed that FNcKO samples exhibit less protein expression along with a decrease in neutrophil recruitment. Additionally, the enzyme COX2, another key player in inflammation, which upregulated by 5 days PCS, failed to upregulate in the FNcKO lenses, again suggesting that fibronectin has a role in regulating post-surgical inflammation. Since fibronectin also has a role in PCO, inflammation and fibrosis may have mechanistic connections following cataract surgery.

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## THE EFFECTS OF SEAGRASS AND MACROALGAE HABITATS ON GRASS SHRIMP (*PALAEMONETES PUGIO*) BEHAVIOR

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Grass shrimp (*Palaemonetes pugio*) rely on seagrass habitat to provide them with shelter from predators and an area to feed. In the Maryland Coastal Bays system, the extent of seagrass coverage is declining while the abundance of macroalgae habitat is on the rise. I examined how this change in habitat may influence shrimp behaviors using ten gallon aquaria. I measured the frequency of their behaviors under two acclimation periods (30 minutes vs 24 hours) and three different substrates (a red macroalgae, sand and seagrass). Results showed that shrimp were less mobile after 24 hours. I also found a preference for vegetated substrates (i.e. macroalgae and seagrass) over the sand substrate. However, the shrimp that were examined did not show a preference for seagrass over the macroalgae. Therefore, the shrimp may be able to adjust to macroalgae habitat as seagrass habitat declines.

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## TETRACYSSTEINE TAGGED OVA<sub>(323-339)</sub> PEPTIDE AS A NOVEL TOOL FOR TRACKING PEPTIDE MHC-II COMPLEXES IN PRIMARY MOUSE CELLS

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The antigen presenting cell (APC) plays a critical role in the adaptive immune response. In this particular project, the APC consists of the dendritic cell (DC) and the ovalbumin (OVA) peptide-MHC-II complex. The steps for a cell to turn into a professional APC include: having the extracellular peptide enter the DC, digesting the peptide using lysosomes, and then loading the epitopic fragment onto the MHC-II peptide to be presented on the surface of the DC to OTII CD4<sup>+</sup> T-Cells. Typically, protein constructs that contain fluorescent protein tags such as green fluorescent protein (GFP) are used to track and localize proteins inside cells. However, CD4<sup>+</sup> T cell epitopes presented by MHCII consist of fewer than 20 amino acids, whereas fluorescent proteins consist of more than 200 amino acids that has the potential of disrupting the conformation and functionality of the original peptide. Our study tests a six amino acid long motif known as tetracysteine coupled with a labeling reagent known as FLAsH EDT-2 that is used to bind to OVA . The study is divided into roughly three sections that test for antigenic integrity, determining the optimal location to add the tetracysteine and FLAsH tag on the antigen, and using confocal microscopy to track the antigen activity in the APC, as well as monitoring the dynamics between the APC and the OTII CD4<sup>+</sup> T-Cell.

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## THE *C. ELEGANS* REVERB GENE, *NHR-85*, IS EXPRESSED INCREASINGLY OVER THE FIRST HALF OF LARVAL DEVELOPMENT

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The *C. elegans* gene *nhr-85* is a member of the RevErb (NR1D) family of nuclear receptors. Nuclear receptors function as hormonally regulated transcription factors. The well known NR1D family gene members are E75 in *Drosophila melanogaster*, and RevErb in vertebrates. Both these homologs factor in the regulation of circadian rhythm of their respective organisms. The *C. elegans* RevErb, *nhr-85*, is less well understood than its homologs. *C. elegans* does not sleep according to light/dark changes, but experiences a sleep-state, lethargus, that is developmentally regulated with respect to the molt. Since its homologs are regulated according to light/dark changes, it would then follow that *nhr-85* might be regulated according to periods of lethargus. Quantitative PCR experiments were used to determine the changes in *nhr-85* expression as the first two larval stages progressed. It was found that *nhr-85* expression increased progressively over the first two larval stages, but did not appear to be up-regulated or down-regulated at the time of the molt. Since the gene is not regulated in manner consistent with function in lethargus, it would seem as if its functions are exapted while the gene structure is conserved. To determine what functions *nhr-85* might play in the animal, a *nhr-85* Green Fluorescent Protein translational reporter was used to visualize localization of gene expression at different stages in development. I found that *nhr-85* was expressed in several cell types including hypodermal cells and somatic gonad cells. Taken together, these data suggest a possibly diverse range of functions as the gene is expressed increasingly over the first two larval stages.

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# OLIGODENDROGLIAL SOX 17 DEFICIENCY DECREASES PROGENITOR PROLIFERATION AND OLIGODENDROCYTE DEVELOPMENT IN POSTNATAL WHITE MATTER

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Sox17 is a transcription factor that promotes oligodendrocyte cell differentiation in culture. Previous studies have shown increased Sox17 expression in newly generated oligodendrocytes cells of actively remyelinating brain white matter (WM) in experimental demyelinated lesions. However, the function of Sox17 in oligodendrocyte development has not been demonstrated. The present studies test the hypothesis that Sox17 promotes oligodendrocyte production in developing brain WM by analyzing oligodendroglia in Sox17-deficient mice. CNPCre-targeted mutant mice deficient in the Sox 17 gene (CKO) were found to produce significantly fewer oligodendrocytes at postnatal day 30 (P30). We investigated the possibility that Sox17 controls the number of oligodendrocytes by regulating progenitor cells and their proliferation. After PCR genotyping to identify mutant mice, we collected P12 and P18 brains from control and CKO mice, and used dual label immunohistochemistry to detect progenitor cells expressing Sox-2, -9 and NG2 together with the cell cycle marker Ki67. Ki67 was decreased in CKO WM compared with controls. The percentage of total NG2, Sox-2, and -9 cells in the CKO WM was also decreased. Reduced Sox 9 and Ki67 double-labeled cells indicated reduced Sox9 progenitor proliferation. At P18, CC1 oligodendrocytes increased in CKO. These studies suggest that Sox17 regulates progenitor cell proliferation, and a decrease of the progenitor state temporarily increases differentiation. To identify Sox17 targets involved in progenitor expansion, cultured oligodendrocyte progenitor cells were transfected with Sox17 siRNA. Quantitative PCR assays showed that Sox17 knockdown reduced Notch1 RNA, a stem/progenitor cell maintenance factor. However, Sox17 siRNA also decreased TCF7L2, which initiates and sustains differentiation. Reductions in Notch and TCF7L2 were also observed in CKO WM. These results indicate that Sox17 controls a program of progenitor cell expansion and subsequent maturation. This study reveals a novel regulatory role for Sox17 in Notch signaling and TCF7L2 expression in the oligodendrocyte lineage.

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**INSULIN SIGNALING AND NEURONAL  
REGULATION IN AROUSAL IN *C. ELEGANS***

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The *fax-1* gene of *C. elegans* is required for regulation of sleep. *fax-1*, which encodes a nuclear receptor that is also present in humans, is expressed in 20 interneurons in the nematode and functions in specifying neuronal identities. *daf-2* encodes the insulin receptor in *C. elegans*, where it regulates developmental and physiological responses to insulin peptide hormones. *daf-2(e1370);fax-1(gm83)* double mutant animals, which have both compromised insulin signaling and neuronal signaling, result in a quiescent phenotype at hatching that is analogous to sleep. If we selectively express *fax-1* in a subset of neurons in *daf-2(e1370);fax-1(gm83)* double mutants, we can determine which neurons are essential for arousal. We will perform rescue assays with transgenic double mutant animals, injected with constructs that transcribe *fax-1* under the control of promoters of neuron-specific genes (*npr-1*, *glr-2*, *ntc-1*, and *unc-3*). These promoters will express FAX-1 in a limited subset of neurons. If expression of FAX-1 in individuals rescues the animals from arrest, this will reveal which interneurons are involved in the arousal pathway.

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## ANALYSIS OF 3D CHROMATIN CONFORMATION AT *RASGRF1* RELATING TO ITS EXPRESSION IN MOUSE LIVER.

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Genomic imprinting is a regulatory phenomenon in mammals that takes place at a small subset of genes. Imprinting occurs when one parental allele is silenced and the other allele is expressed. Our lab investigates the factors that influence the expression of imprinted genes.

We are currently analyzing *Rasgrfl*, an imprinted gene that follows a unique tissue-specific pattern of monoallelic vs biallelic expression. *Rasgrfl* is differentially methylated on the parental alleles at a region that is upstream of the promoter. In brain, differential methylation of this region is necessary for imprinted expression. However, this region is also differentially methylated in lung, yet *Rasgrfl* is expressed biallelically in this tissue. Tissue-specific enhancers may exist in different locations relative to the differentially methylated region (DMR), which could contribute to the tissue-specific expression.

We hypothesize that the 3D chromatin conformation plays a role in the expression of *Rasgrfl* by bringing different enhancers closer to or farther from the promoter in 3D space. The CTCF protein is able to bind to the unmethylated maternal allele, which allows this region to serve as an enhancer blocker for upstream enhancers, silencing the maternal allele. In contrast, the methylated paternal allele would not bind CTCF and would be expressed. A downstream enhancer would be unaffected by the CTCF protein, and both parental alleles would be expressed.

We are currently executing a comparative study of chromosomal structure at *Rasgrfl* in different tissues to identify enhancers. We are studying the looping of chromatin using a chromatin conformation capture (3C) assay. My project specifically focuses on the chromatin structure around *Rasgrfl* in mouse liver, a tissue with imprinted expression of *Rasgrfl*. I hypothesize that enhancers might be located upstream of the DMR. To date, we have identified several locations of potential enhancers and will continue running 3C experiments to confirm.

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## IDENTIFYING NOVEL REGULATORS OF RETROTRANSPOSON SILENCING IN MOUSE EMBRYONIC STEM CELLS

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Endogenous retroviruses (ERVs) have co-evolved with humans for the past 80 million years. As members of the retrotransposon family, these viral elements make up 5-8% of the human genome and were once thought to be “junk” DNA. However, recent studies have linked the abnormal expression of ERVs with the initiation of a variety of cancers, including acute myeloid leukemia, glioblastoma, and pancreatic cancer. Currently, the proteins that are known to be involved in ERVs silencing are the zinc finger protein 809 (ZFP809), the histone methyltransferase SETDB1, as well as KAP1. More recently, the histone variant H3.3 specific chaperone, DAXX, was also shown to be involved. Nevertheless, little is known about the recruitment and regulation of those proteins. In this study, we aim to identify novel proteins involved in the repression of ERVs using expression of transgenes in cells and protein affinity purification. These findings can bring about insights into the regulatory mechanisms at play and further explain the cellular requirements for the regulation of ERVs in our genome.

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## CLINICOPATHOLOGIC CORRELATION WITH EARLY DETECTION OF ANAL INTRAEPITHELIAL NEOPLASIA (AIN) WITH EMPHASIS ON HPV SEROTYPE IN- SITU HYBRIDIZATION ANALYSIS

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HPV related anal intraepithelial neoplasia (AIN) has increased in prevalence and intensity (degree of dysplasia) within the last 10 years. Several risk factors have been implicated however specific cohorts in Delaware especially in Sussex County are identified to be at increased risk. Several studies have suggested that the rate of progression of high-grade anal intraepithelial lesions to invasive anal cancer is around 5%. Anal cytology has been used for the past 30 years to assess for anal intraepithelial neoplasia aiming at developing a screening recommendation however it has been shown to be of very little correlation to actual pathology. In an attempt to develop a reproducible clinical based screening methodology to include clinical screening and histologic evaluation; patients undergoing colonoscopy underwent targeted biopsy for suspicious anal lesions/changes with or without acetic acid application (vinegar) with subsequent routine histologic evaluation as well as molecular HPV studies. In collaboration with data presented from an ambulatory surgery Center in southern Delaware we identified 31 patients who qualify for inclusion in our study (routine colonoscopy evaluation with positive findings on intraoperative anal exam with or without acetic acid application). They were evaluated retrospectively as well as prospectively to correlate clinical findings with measurable pathology-based identifiers at Green clinics laboratory. These identifiers include data retrieved via regular staining as well as esoteric HPV testing (immunohistochemical analysis). Our pilot study suggested the validity and reproducibility of identifying high-risk population by a simple clinical test as it correlates to test proven high-risk lesions including precancerous and cancerous anal intraepithelial neoplasia.

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## MANUAL GROSS DEXTERITY ANALYSIS OF FOUR POPULAR STYLES OF ENABLE 3D PRINTED PROSTHETIC HANDS USING THE BOX AND BLOCK TEST

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eNABLE, a worldwide volunteer group, working to design and print prosthetics hands, arms, and fingers has found a practical use for 3D printing that helps bring free prosthetics to people throughout the world. The work by eNABLE has gained international media attention, however, the effectiveness of the prosthetic hands has never been formally tested. We tested the manual gross dexterity of the four most popular styles of eNABLE hands, the Unlimbited Phoenix, Phoenix V2, Raptor Reloaded, and Osprey hands using the so-called Box and Block Test (BBT) of Mathiowitz et al. 1985. The box and block test is a clinical evaluation used to measure the manual gross dexterity of test subjects, particularly those with weaker gripping strength, by challenging them to move small blocks over a barrier in the span of a minute. We designed and printed a unique emulator to allow able-bodied test subjects to operate each of these four eNABLE devices in moving the blocks. We found that the Raptor Reloaded hand performed significantly worse in comparison to each other hand style with  $p < 0.05$ . Conversely, there was no statistically significant difference between any of the three other hand styles tested. We also found that there was no statistically significant difference between the order of hands being tested, thus eliminating hand order as a confounding variable. The results show that eNABLE devices still need minor improvements in order to compare favorably with some of the lower performing commercially available prosthetic hands. This work, therefore, establishes a quantitative baseline of performance of current eNABLE designs and provides a clinically validated testing protocol for future assessment of 3D printed prosthetic devices.

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## **AGE-RELATED MACULAR DEGENERATION (AMD): GENOTYPIC STUDY OF THE RETINAL DISEASE AND ITS ASSOCIATED GENES WITH COMMON AND RARE VARIANTS**

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Age-related macular degeneration (AMD) is a progressive ocular disease that affects the central retina, serving as a predominant cause in blindness for elder populations and industrialized countries. AMD is detrimental to retinal pigment epithelial (RPE) cells and photoreceptors, inducing central vision loss and legal blindness. Gaining further knowledge of rare variants may support the relationship of causal genes within the genetic loci and AMD diagnosis. Following DNA extractions of 15 donor human eyes diagnosed with and without AMD, polymerase chain reactions (PCR) and restriction enzyme digestions are being completed to denote genetic variation and correlation in patients. The following variants are being studied as a result of high genetic priority with genes CFH, CFI, TIMP3, and SCLC16A8: rs10922109, rs10033900, rs429358, rs5754227, rs8135665, and rs72802342. Although few genome-wide studies have been completed to highlight the effects of rare and common variants within AMD populations, further research must be completed as risks for developing the disease has increased over the past decade. Lack of effective therapy on the market has yielded deeper and critical insight to AMD-associated genes.

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# CELL-SPECIFIC LESIONING REVEALS THE ROLE OF THE SUBSTANTIA NIGRA IN SLEEP-WAKE CONTROL

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Sleep, which is characterized by low motor function, is critical to survival. In the recent years, the basal ganglia, a brain region known to be involved in motor regulation, has been implicated in the sleep-wake cycle. However, the underlying circuitry mechanism by which it does so remains poorly understood. The main output nucleus of the basal ganglion, the Substantia nigra (SN), is composed of largely GABAergic inhibitory neurons and is known to be involved in inhibiting motor output. In the present study, we investigated whether chronic lesion of SN GABAergic neurons will affect animals' sleep-wake structures using chemogenetic and optogenetic approaches, a previously established protocol that inhibit the SN neurons for a short period of time to test. By injecting virus expressing the diphtheria toxin receptor into the SN of mice, we achieved cell-type specific depletion of SN GABAergic neurons. We then recorded sleep states before and after the lesion and quantified the difference in mouse sleep after the lesion. We also performed immunostaining and fluorescence microscopy to examine the neuron density of SN GABAergic neurons and calculate the lesion efficiency. From my study, I concluded that the lesion was effective in killing the SN GABAergic neurons, killing the neurons decreases sleep, and the SN projects to multiple waking centers. If we can correlate the effect of disrupted sleep and lesion efficiency, it will show a causal link between SN and sleep. Identifying a linkage between the sleep-wake and SN will help develop new clinical methods to improve sleep, especially for alleviating sleep syndrome in Parkinson Disease patients for whom the SN is a main locus of cellular degeneration.

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## GERMINATION ASSAYS FOR RNA SEQ OF MELAMPSORA AMERICANA ON SALIX PURPUREA

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The fungal plant pathogen *Melampsora americana* is the most damaging pathogen of the bioenergy crop *Salix purpurea*. It causes premature defoliation and can significantly reduce crop yield by up to 50%. To better understand the genes associated with initial stages of the plant-pathogen interaction, we will use comparative RNAseq of germinating rust uredospores on several media and time points to deduce what genes have increased transcription during appressoria formation. In order to conduct such an experiment, each medium chosen requires specific germination assays to ensure consistent germination and appressorium formation rates. Since *Melampsora americana* requires a hydrophobic surface with distinct ridges for appressorium production, as found on leaf surfaces, we inoculated polyethylene surfaces (germ tubes only), oil-collodion membranes (germ tubes and appressoria), and *Salix purpurea* leaf tissue with the pathogen. We then counted the number of spores, germ tubes, and appressoria in 20 microscope fields at 40X magnification at four, six, and 18 hour time points. A statistically significant higher percentage of *Melampsora americana* spores germinated at the 18 hour point than the other time points for the polyethylene surface. The 18 hour point was comparable across the three media. No spores formed appressoria on the polyethylene surface, but a statistically significant higher percentage of spores formed appressoria at 18 hours on the other media. Because appressoria do not form in the absence of guard cell-like ridges, RNAseq of the polyethylene treatment can be compared to those of collodion membranes and leaf tissue, where appressoria are expected to form. Elucidating pathogen gene expression during the germination and early infection process will enable the identification of effectors necessary for pathogenicity and will enhance our understanding of the interaction between the pathogen and the plant host.

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## SSRI PRESCRIPTION TRENDS IN THE TREATMENT OF OBSESSIVE-COMPULSIVE DISORDER

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Obsessive-compulsive disorder (OCD) is a chronic neuropsychiatric illness characterized by persistent and uncontrollable thoughts, urges, feelings, and emotions coupled with repetitive behaviors performed to eliminate obsessional distress. There are two first-line treatments for management of OCD: exposure with response prevention (ERP) and pharmaceutical augmentation with a class of drugs known as selective serotonin reuptake inhibitors (SSRI's). There are 6 SSRI's prescribed for OCD: escitalopram, citalopram, fluvoxamine, sertraline, fluoxetine and paroxetine. This survey study identifies the prescription rates and efficacy of these SSRI's as reported by psychiatrists. 2095 psychiatrists were emailed a link to a five question online survey. The survey asked respondents (N=348) to identify: the SSRI medication they most commonly prescribe as a first-line treatment for OCD; the efficacy of their preferred first-line SSRI; and the reason for choosing a specific SSRI as a first-line medication. Results indicate fluoxetine and sertraline are prescribed at a significantly higher rate than the other medications, although the data also suggests there is no significant difference in the reported efficacy for each SSRI. This study compiles the results of 42 published SSRI clinical trial experiments to assess if psychiatrist survey reports are consistent with clinical trial results. In agreement with our survey results, clinical trials suggest there is not a significant difference in efficacy among SSRI's. We hypothesize that prescription rates are linked to the adverse effect profiles and underlying pharmacodynamic and pharmacokinetic properties of each SSRI.

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## INVESTIGATING THE BRAUM BACTERIOPHAGE THROUGH DIRECT ISOLATION

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In this day and age it has become increasingly difficult for antibiotic medications to keep up with the rapid adaptations of bacterial infections. A relatively new solution for antibiotic resistance genes has been found through bacteriophages that can specifically target and infect bacteria. In this experiment a phage was isolated from a soil sample collected near a stream in Laurel, Maryland. The phage was successfully isolated and extracted through direct isolation and the presence of the phage was detected through the use of a plaque assay. To determine the characteristics of Braum, we utilized host range and PCR testing to confirm that Braum was unique from the other samples collected. The host range assay demonstrated that Braum could infect a non-isolated different host. Braum originally was seen infecting BtK and the host range assay confirmed that Braum could also infect hosts such as HD1-HD4. By using PCR, we identified that Braum had a unique base pair length in comparison to the other samples collected by peers. The Transmission Electron Microscope (TEM) was used to produce an image of Braum. This image showed that the phage was octagonal in shape, about 70-100 nm in size, and had a 200 nm tail. After looking at the TEM picture of the phage it was determined that the phage was most likely a morphotype of myoviridae. Even though these tests aided in determining Braum's uniqueness, not enough information is there till the phage has been sequenced. Sequencing the phage would provide more data on Braum and help determine if it is truly unique.

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## ELUCIDATION OF MYCOBACTERIOPHAGE GENE FUNCTION

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Bacteriophage are viruses that infect bacteria; during the course of infection, the bacteriophage genes are transcribed and translated alongside host genes. Several of the resulting phage proteins are capable of commandeering bacterial metabolism to promote the assembly of new phage particles and induce bacterial lysis. While much is known about a subset of these proteins, the functions of many remain uncharacterized. In this study, we attempt to elucidate the function of phage gene products (gps) by investigating their effects on bacterial host phenotypes and their interactions with the host proteome. Genes from four *Mycobacterium smegmatis* bacteriophages (Giles, Waterfoul, Hammy, and Larva) were screened for the ability to interfere with essential host processes using a cytotoxicity assay to observe the effect of gene expression on host growth. Approximately 10% of tested phage genes were found to have varying toxicity to the host. To identify bacterial proteins that interact with these phage gene products, a subset of toxic genes was cloned into a transcriptional-based two-hybrid selection system that allowed us to discover pairwise interactions between our phage gene product and a library of about a million different host protein fragments. Candidate host protein interaction partners were found for Giles gp3 and gp40, and we are currently investigating the potential role of these putative interactions in the phage life cycle.

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## INVESTIGATING THE ROLE OF COSR IN THE OSMOTIC STRESS RESPONSE OF THE HALOPHILE *VIBRIO PARAHAEMOLYTICUS*

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The marine bacterium, *V. parahaemolyticus*, has evolved multiple strategies for adapting to short- and long-term shifts in osmolarity. One such mechanism involves the uptake and/or biosynthesis of compatible solutes (CS), small organic molecules that act to balance the osmolarity of the cell. *V. parahaemolyticus* biosynthesizes the compatible solutes ectoine and glycine betaine. Ectoine gene expression was previously shown in *V. cholerae* to be regulated by CosR, a MarR-type regulator. Here, we characterize a CosR homologue present in *V. parahaemolyticus* through the generation of a *cosR* deletion mutant strain to determine whether it plays a similar role. A *cosR* mutant was generated using the Gibson Assembly protocol to construct a truncated, non-functional *cosR* gene, and allelic exchange to generate a deletion mutant ( $\Delta cosR$ ) in *V. parahaemolyticus* RIMD2210633. We characterized the growth of the mutant in both nutrient rich and minimal media in optimal and low salt conditions. In low salt conditions (1% NaCl),  $\Delta cosR$  had a growth defect when compared to the wild-type (WT) strain. To further investigate the growth defect in low salt, we will perform quantitative NMR to measure production of ectoine in  $\Delta cosR$ , as the over-production of CS can impede growth. The role of CosR in cell motility was investigated through swimming assays. After 20 hours at 37 °C on soft-agar plates, there was no significant difference in the swimming motility of WT and  $\Delta cosR$  strains. To investigate the role of CosR in biofilm formation, both a WT strain and  $\Delta cosR$  were grown overnight, the biofilm stained, and quantified using spectrophotometry.  $\Delta cosR$  was found to produce significantly more biofilm than WT, which will be examined further. Overall, the data show a role for CosR in the osmotic stress response, and differences in the regulation of motility and biofilm formation compared to *V. cholerae* that will be investigated further.

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**CAN ENVIRONMENTAL DNA (EDNA) BE USED FOR DETECTION OF  
ENDANGERED SPECIES IN FRESHWATER?- DEVELOPING SPECIES-SPECIFIC  
MARKER FOR *RHODEUS PSEUDOSERICEUS***

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Environmental DNA (eDNA) is a relatively new, but powerful monitoring tool for the detection of rare, invasive, and endangered species, using genetic material found in water systems. In this study, we designed and validated species-specific primers used in tandem with eDNA samples to confirm the presence of the endangered Hangang bitterling, *Rhodeus pseudosericeus*, which is endemic to the mid-region of the Korean peninsula. Both *in silico* from BLAST methodology and *in vitro* validation tests from ten congeneric species DNA confirmed the specificity of the markers to the species. Subsequently, the sensitivity of the newly designed primers was validated with filtered samples of freshwater collected from three western river systems known to contain Hangang bitterlings. The eDNA was extracted and analyzed by PCR using species-specific mitochondrial cytochrome oxidase subunit I (COI) primers designed to specifically amplify Hangang bitterling DNA. Positive PCR products were purified and sequenced, which confirmed species identity, provided confidence in the results, and demonstrated that higher volumes of eDNA produce clearer identification of the target species. These unambiguous identification results reveal that species-specific surveys are possible and reliable with the eDNA method. However, there are some limitations to this approach, including contamination, false positives, and false negatives, which could be resolved with appropriate procedures in future studies. Overall, this cost-efficient and non-invasive approach can be used as a monitoring tool to aid in effective conservation efforts for this endangered species.



**CHARACTERIZATION OF THE MICROBIOTA OF *CALLOSOBRUCHUS*  
*MACULATUS***

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*Callosobruchus maculatus*, known as bean beetles, is an invasive species known to lay eggs on legumes. During the larval stage, bean beetle larvae eat their way into the center of the legume and undergoes metamorphosis into the adult form. In the adult form, bean beetles do not drink or eat. The approximate two weeks of a bean beetle's adult life span is used to reproduce. Precautionary measures must be taken during the importation of legumes from foreign countries to prevent the introduction of this species in the U.S. Previous research has not been completed on the microbial composition of the bean beetle. Because of this, twenty male and twenty females were isolated from mung bean and black pea habitats. Subsequently, DNA extraction, PCR, NanoDrop 2000 spectrophotometry, and gel electrophoresis was completed to isolate the 16S gene of the microbes on and within the bean beetle samples. After sequencing was complete, the 16S metagenomics application from Illumina Basespace Hub was used to classify the microbiota of the eighty bean beetles. In both bean habitats, at the phylum and species level, male beetles demonstrated a larger richness of bacteria. Both male and female bean beetles exposed to the mung bean habitat demonstrated a greater species richness than bean beetles exposed to the black pea habitat. Characterization of the microbiota demonstrated that bean beetles are an insect vector for a class of mollicutes known for plant pathogenicity. The research provides insight into the variation of an insect microbiota based on gender and bean habitat for nutrient uptake.

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**DISCOVERING THE UNIQUENESS OF BACTERIOPHAGE TOMATO PLANT**

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There is an enormous population of bacteriophages in the environment, which biologists know very little about. Only about 3,000 of which have been characterized genomically. In order to learn more about these phages and how prevalent they are in the environment, soil samples for Ellicott City were collected and examined. The host cell for these experiments was *Bacillus Thuringiensis* (Btk). The soils samples were isolated using enriched isolation, plated with Btk to see if there were phages present. Plates that had plaques had phage. A plaque was picked to be isolated and purified by performing several serial dilutions. There is one pure phage when the plates has plaques that looked similar to each other. Tomato Plant had mostly turbid plaques that were about 2 mm. Stock Lysate, made from the pure phage, was used to extract DNA and for the TEM staining. From TEM staining it was discovered that Tomato Plant is a myoviridae with contracted tail length of 53.6 nm, an uncontracted length of 78.6 nm and capsid length of 44.1 nm. DNA was extracted to perform different tests to characterize the uniqueness of the phages that were found. The restriction enzyme digest was performed. Tomato Plant was cut by EcoR1, hind111, kpn1, sph1. Another test that was carried out was PCR. Tomato Plant had C3 A cluster and was between 300 and 500 bp. A QC gel was run and Tomato Plant has roughly 16 ng/ul of DNA. Host range testing was also done to help classify the phage's uniqueness. Tomato Plant has a narrow host range since it also produced plaques with HD1 which is similar to Btk. All these tests help to prove that Tomato Plant is a unique phage.

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## CORRELATING NEURAL REVERSAL WITH BEHAVIOR IN AN ANATOMICALLY REVERSED MUTANT

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Our cerebrum is split into two hemispheres, the left and right cerebral hemispheres. Typically for right-handers, the left hemisphere is responsible for logic, verbal skills, and right-side motor skills; and the right hemisphere is responsible for creativity, non-verbal skills, and left-side motor skills. Handedness is under contralateral hemispheric control, and the majority ~90%+ of humans are right-handed. Hand preference has been proposed to reflect our ability to learn and remember. The development of L/R laterality is fundamental to development which further influences nervous system function. We are utilizing *Caenorhabditis elegans* to understand the biological correlates of laterality reversal with neuronal functionality. Wild-type *C. elegans* are considered “dextral”, defined by the rightward location of their anterior gonad. Our hypothesis states that laterality reversal leads to atypical neural connectivity that reflects in changes in learning behavior.

Mutations in *gpa-16* expressed in the 4-cell *C. elegans* embryo cause a decrease in frequency of “dextrality” causing a reversal in organ placement, as well as increased embryonic lethality. In terms of the nervous system, *C. elegans* uses specific bilaterally symmetric chemosensory neurons to detect certain chemosensory cues such as food and chemical odors. ASEL/R (gustatory neurons) are used to detect water-soluble attractants. ASEL detects sodium and ASER detects chloride. After standardizing a NaCl based chemosensory associative learning assay, we tested *gpa-16* mutants chemotaxis and associative learning.

Our results indicate that these mutants display a normal gustatory response to NaCl; however, they are deficient in associative learning. This implies that there could be reversed or atypical neuronal connections of ASEL/R with its primary post-synaptic target interneuron AIY. We are also attempting a machine-vision based endo-phenotypic characterization of the mutants’ behavior. In conclusion, our results indicate that anatomic reversal may cause atypical neuronal connectivity which can further affect neural function as assayed in our behavioral assays.

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## CORRELATING GRAMICIDIN ION-CHANNEL FORMATION TO ARTIFICIAL MEMBRANE DYNAMICS

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Numerous studies have found that changes in membrane characteristics like fluidity and thickness correlate to how different sub-types of somatic cells respond to changes in their environment. Investigating the effects of different conditions on membrane dynamics is therefore important to understanding both biological membranes and cellular function. Qualitative and quantitative analyses of membrane structure and dynamics reveal some of these resultant effects.

Neutron scattering techniques reveal structural characteristics in sub-nanometer to tens of nanometers scales. Dynamic features in picoseconds to hundreds of nanoseconds timescales can also be observed. Together these techniques allow us to examine the structure and motion of 5 nm thick lipid membranes. Past experiments using small angle neutron scattering (SANS) and neutron spin echo spectroscopy (NSE) have given insight into membrane response to the presence of protein. In this study, the response of unilamellar vesicle membranes to the addition of increasing amounts of gramicidin is further analyzed. Artificial vesicles composed of varying amounts of gramicidin and a lipid of interest are created via extrusion to obtain vesicles that are 100 nm in diameter. Dynamic light scattering (DLS) was used to confirm the size distribution of suspended vesicles in solution and density measurements were utilized to carry out quantitative characterization.

SANS and NSE experiments allow us to probe the membrane at a nanoscale level. SANS provides us with data from which information about the structure of the membrane can be gleaned. Of particular interest to us is the formation and orientation of the gramicidin channels as well as channel-membrane interactions. To understand more about the dynamics of the membranes, NSE was used to extract aspects like height fluctuations, stretching, and bending in the membrane. Together this data provides a more detailed explanation of how proteins might influence membrane activity on a biological basis, therefore influencing a cells activity.

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**DEVELOPMENT OF *IN VITRO* SPECIES-SPECIFIC ENVIRONMENTAL DNA  
(EDNA) MARKERS FOR *KICHULCHOIA BREVIFASCIATA* (COBITIDAE), SOUTH  
KOREAN ENDANGERED FRESHWATER FISH**

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In an effort to develop more non-invasive tools to monitor and observe species in their natural habitats, we studied environmental DNA (eDNA). eDNA is the genetic material of organisms procured from their habitats in soil, water, and faeces. The genetic identification using eDNA is extremely sensitive, rapid and noninvasive, effective in remote locations, and cost and time efficient. Several organisms, specifically endangered species, were studied using this tool deemed as safer than current biological study methods of extracting DNA from living organisms. We studied one such organism, *Kichulchoia brevifasciata*, the dwarf loach, endemic to the southern Korean peninsula, using eDNA to develop its species-specific marker. The development of the species-specific marker would allow for detection of the species through a more noninvasive means. We hypothesized that if a species-specific marker is developed for the organism, then the genetic identification using eDNA is as effective as the method using the DNA collected from living organisms. To test the species-specificity, we tested 31 primer pairs on 12 eDNA and 2 DNA samples using PCR and gel imaging. Both eDNA (river water collection) and DNA samples (from the live fish) were collected in the natural habitat, Sinpyeong River, for the analyses. The primer pairs were developed by downloading and aligning partial mitochondrial COI gene primer sequences from the NCBI database because mitochondrial DNA is more informative and abundant than nuclear DNA. We found one primer pair species-specific for eDNA samples. However, this requires further testing because it did not exactly match the control DNA image. This research can be beneficial in studying organisms, specifically rare and endangered ones, without causing harm to them or their habitat.

We thank the members of the molecular ecology research group at Yeungnam University, headed by Dr. Ho Young Suk. We are also grateful to Notre Dame of Maryland University and Dr. Hangkyo Lim for his mentorship and support.

## INFLUENCE OF DIFFERENT N, P, AND SI ADDITIONS ON UREA UTILIZATION PATHWAYS IN AN ANACOSTIA RIVER MICROBIAL COMMUNITY

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The Anacostia River, “the forgotten river,” has an overall poor quality caused by several anthropogenic sources including sewage, excess nutrients from runoff, and combined sewer overflows (CSOs). To assess the impact of changing nutrient concentrations and forms on the bacterial and phytoplankton community composition and productivity, samples were collected from the Anacostia River into fifteen cubitainers with different nutrient additions (different combinations of +N, +P, +Si). Our project focused on one aspect: the influence of different nutrient additions on urease activity, an enzyme responsible for the breakdown of urea to ammonium. We hypothesized that there would be more urease activity in the +NO<sub>3</sub><sup>-</sup> and +urea additions because NH<sub>4</sub><sup>+</sup> suppresses urease activity and there will be no difference in +P treatments because urease activity does not require ATP. Urease activity increased in the NH<sub>4</sub><sup>+</sup> treatments once NH<sub>4</sub><sup>+</sup> was exhausted and the microbes were physiologically stressed. Urease activity and NO<sub>3</sub><sup>-</sup> concentrations remained mostly steady throughout the experiment for the other treatments, with the exception of in +P treatments where urease activity varied even though PO<sub>4</sub> concentrations decreased on specific days. Based on these results, changing nutrient dynamics in the Anacostia River will have an influence on urease activity if ammonium levels are reduced due to the new storage tunnel that went on-line in March 2018.

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## INVESTIGATING THE EFFECT OF SODIUM BENZOATE ON VILLUS MORPHOLOGY AND GUT MUCOSAL HEALTH.

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A large volume of evidence show that dietary ingredients can influence the mucosal surface morphology and mucosal immunity of the gastrointestinal tract affecting overall health and wellbeing. Multiple health concerns and behavioral changes have been reported recently which are attributed to consumption of foods containing preservatives and additives. Sodium benzoate (**SB**) is a commonly used food preservative in acidic foods to prevent microbial growth. Our study investigates the effects of SB on the villus morphology and mucosal health of the gastrointestinal tract. The degree of lymphocytic infiltration into the intestinal epithelium as well as the lamina propria and granular density in Paneth cells can be used as evaluators of mucosal immunity. We hypothesize that SB will decrease the PC granular density and lymphocyte infiltration in the ileal mucosa resulting in a reduction in mucosal immunity. Adult C57BL/6 mice were randomly assigned to two groups (n=9). Control group received standard rodent chow and regular drinking water. The treatment group received standard rodent chow and drinking water mixed with 1% SB. Food and drink were available to all animals *ad libitum* for the duration of 21 days. Animals were monitored for weight, activity, and food/drink intake. At the end the experimental period, ileal samples were collected and processed for routine histological evaluation. Villus measurements were determined using ImageJ histomorphometry tools. Paneth cell intensity and lymphocytic infiltration in to the lamina propria were evaluated by double blind scoring system on a scale from 1-4. Intraepithelial lymphocyte index was calculated as a percentage of lymphocytes per total cell counts in the epithelial lining of villi. Data were analyzed using Student T. test at 95% confidence level. Preliminary histological analyses revealed significant reduction in Paneth cell granular density ( $p<0.05$ ) in response to SB treatment indicating possible reduction in mucosal immunity in the gut.

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## REINSERTION OF EXTRACHROMOSOMAL RDNA CIRCLES IN SACCHAROMYCES CEREVISIAE

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The ribosomal DNA (rDNA) locus is a tandem repeat array that codes for ribosomal RNA, a major component of ribosomes. Ribosomes are essential for protein synthesis in all organisms. Due to its repetitive structure, the rDNA array is a dynamic locus that readily undergoes changes in repeat copy number. rDNA copy number changes have been observed in multiple organisms, including yeast and humans. Due to the instability of this locus, rDNA repeats in budding yeast (*Saccharomyces cerevisiae*) can exit the chromosome and form an extrachromosomal rDNA circle (ERC), which is then capable of reinserting into the chromosome. We want to determine how often ERCs reinsert and where they reinsert within the rDNA array. We expect to detect reinsertions at a relatively high rate due to the inherent instability of the rDNA array. Given previous work on transcriptional position effects within the rDNA, we also expect ERC reinsertions to preferentially occur at specific positions within the array. To detect reinsertion events, we used a *ura3* mutant yeast strain harboring an engineered ERC that also carries a *URA3* marker. On media lacking uracil, cells that insert the marked ERC into the chromosomal array form large colonies with smooth edges, whereas cells that do not insert the marked ERC form small colonies with rough edges because the ERC is frequently lost during growth. We have detected potential reinsertion events using this method. ERCs are a frequent hallmark of cancer and this research will help us understand the dynamics of these unusual genetic elements.

Thank you to NYU Biology SURP for promoting diversity in science. We thank our laboratory colleagues and PI for their help and support.



## **LISTERIA MONOCYTOGENES SHOWS EVIDENCE OF CONSERVED DOMAINS TRANSFERRED FROM ITS VIRUS**

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Horizontal gene transfer (HGT) is the transfer of genetic material from one organism to another. *Listeria monocytogenes* is a known food-borne pathogen that causes *Listeriosis*. We report on HGT between two *Listeria* phages and *L. monocytogenes* strains. Our results show that there is transfer of the Tape Measure Protein (TMP) between the phage and the bacteria. Synteny analysis shows that several domains from the transferred region are conserved in the bacteria. The transferred domains are cleaved, rearranged, or conserved in its entirety. The conservation of specific domains, even after modifications suggests a functional role of the phage domain in the bacteria.

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## CONSEQUENCES OF EARLY IRON DEFICIENCY AND LEAD CO-EXPOSURE ON DOPAMINE SIGNALING

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Iron deficiency (ID) and lead exposure are prevalent factors that contribute to neurodevelopmental disabilities in children. Several reports show an association between ID, anemia, and elevated blood lead levels, and given that both ID and lead exposure occur disproportionately in disadvantaged populations, there is significant potential for many children worldwide to be co-exposed to ID and environmental lead during vulnerable periods of brain development. To understand the persistent neurochemical outcomes associated with co-exposure, the aim of this study was to first develop a cell culture model of ID/lead co-exposure, and then to use that co-exposure model to investigate changes in the dopamine system.

PC12 cells were used in this study because they endogenously synthesize dopamine and express dopamine regulatory proteins. In the first experiment, PC12 cells were treated with saline (control) or the iron chelator DFO at concentrations of 25, 50, or 100  $\mu$ M. All concentrations reduced dopamine transporter (DAT) protein levels. In a second experiment, PC12 cells were treated with saline or lead acetate (0.1, 1, 10, 100, 100  $\mu$ M), and a dose dependent reduction in DAT was observed. 50  $\mu$ M DFO and 1  $\mu$ M lead were selected for the co-exposure study because they both showed a 50% decrease in DAT protein levels without altering cell viability. In the co-exposure experiment, PC12 cells were treated with either saline, lead acetate (1  $\mu$ M), or DFO (50  $\mu$ M) /lead acetate (1  $\mu$ M). In cells treated with lead acetate alone, DAT protein levels were reduced, while D1R and D2R protein levels were increased relative to control. In ID/lead co-exposed cells, DAT and D2R protein levels were reduced while D1R and TfR protein levels were elevated. This study will move the field forward by creating a cell culture model that provides an understanding of the combined effects of ID/lead co-exposure on dopamine signaling.

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## THE ROLE OF FATTY ACID OXIDATION IN ASTROGLIAL XENOBIOTIC DETOXIFICATION

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The glycolytic astrocyte possesses a high redox and xenobiotic buffering capacity through glutathione (GSH), neutralizing many forms of reactive oxygen species (ROS) and excreting xenobiotics through GSH-xenobiotic adducts back into the blood stream. Astrocytes are the first line of defense against xenobiotic compounds passing through the blood brain barrier, and provide metabolic and synaptic support for neurons, particularly in the uptake and conversion of the excitatory neurotransmitter glutamate to glutamine. The xenobiotic arsenic is water soluble and readily passes through the blood brain barrier. Arsenic is also prevalent in the Earth's crust and modern industrial processing, and exposure has been linked to the development of neurological degenerative disorders. Previous experiments in the Franco Lab have illustrated that in vitro-exposure of astrocytes to arsenic causes a significant increase in glutamate efflux into the media, and that the entry of carbons from pyruvate and fatty acid oxidation (FAO), but not glutamine, into the mitochondria are vital for astrocyte survival during arsenic exposure. In this study, we seek to both corroborate these findings, and explore the role that fatty acids in particular play in xenobiotic detoxification.

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## THE EFFECT OF TEMPERATURE VARIATION ON BEAN BEETLE ALLELIC FREQUENCY AND THE MICROBIOME

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*Callosobruchus maculatus*, known as bean beetles, is an invasive species known to lay eggs on legumes. Precautionary measures must be taken during the importation of legumes from foreign countries to prevent the introduction of this species in the U.S. The purpose of this study was to determine how the allelic frequency of bean beetles would change due to temperature variation. Previous research has demonstrated that beetles thrive in temperatures between 24°C and 30°C, so the bean beetles were exposed to the following three temperature treatments; 24°C, 30°C, and 40°C. Afterwards, six female and six male beetles were isolated from the 24°C and 30°C temperature treatments. Subsequently, DNA extraction, PCR, NanoDrop 2000 Spectrophotometry, and Gel Electrophoresis was completed to obtain data. An abnormal phenotype was demonstrated in the 24°C temperature treatment. No beetles survived the 40°C temperature treatment, so further testing could be completed with this variable in terms of prevention.

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## DOES $\alpha$ V $\beta$ 8-INTEGRIN INFLUENCE POST CATARACT SURGERY INFLAMMATION?

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Cataract, defined as the clouding of the ocular lens, is the leading cause of blindness in the world, affecting over 90% of people by the age of 65. Cataracts are treated by performing cataract surgery, which replaces the clouded lens with an artificial lens. Although it is very effective, there are still complications post cataract surgery (PCS). The short term side effect is inflammation and the long term is posterior capsular opacification (PCO), also called secondary cataract. Inflammation is currently treated with steroids, although it is unpleasant for patients. PCO is treated by YAG laser, however this also shows unwanted secondary responses, one being retinal detachment. Therefore, it is important to understand the molecular mechanism behind side effects PCS to develop therapeutic treatments. Notably, integrins play an important role in epithelial cell migration, proliferation, differentiation, and apoptosis. Previously, our lab found that  $\alpha$ V $\beta$ 8-integrin plays an important role in PCO pathogenesis, but we do not know if it has any influence on inflammation PCS. So, the objective of this work is to test the hypothesis that alpha V beta integrin influences inflammatory mediator expression PCS. We tested whether the three most upregulated inflammatory mediators in wildtype mice at 24 hours PCS, CXCL1, S100a9, and CSF-3, were equally upregulated in  $\beta$ 8-integrin conditional knockout mice. By immunostaining, we observed no qualitative difference in expression of these inflammatory mediators between wildtype and  $\beta$ 8 conditional knockout mice at 24 hours PCS. Similarly, we have seen neutrophil infiltration in both WT and  $\beta$ 8-integrin conditional knockout mice 24 hours PCS. We also have seen a similar extent of COX-2 expressing macrophage infiltration in both WT and  $\beta$ 8- integrin conditional knockout mice 5 days PCS. As a whole, my findings suggest that the  $\alpha$ V $\beta$ 8-integrin may not have any influence on inflammation PCS.

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## PARASITISM RISK IN MIXED-SPECIES FROG CHORUSES: EFFECTS AND MECHANISMS OF COLLATERAL DAMAGE

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A wide diversity of animals use conspicuous calls to attract mates – these signals come with a cost, however. In addition to attracting females, calls are also used by parasite and predator eavesdroppers to find their prey. The *Corethrella* midge is a common parasite of frogs, eavesdropping on the mating calls of male frogs in order to track them down to obtain a meal (de Silva, Nutter, and Bernal 2015).

Most studies analyzing eavesdropping by parasites and predators have focused on a single species of prey, calling in isolation. Yet, species often call in mixed-species aggregations due to anti-predator benefits attained from increased group size, such as individual reductions in the time spent vigilant for predators, or the dilution predation risk (Coulson 1999; Goodale and Ruxton 2010; Makenbach et al. 2013). We studied parasitism risk in two species of Neotropical frogs which often call from mixed-species choruses. Male hourglass treefrogs (*Dendropsophus ebraccatus*) experience significantly intensified parasitism risks when calling for mates near calling túngara frogs (*Engystomops pustulosus*), a phenomenon known as ‘collateral damage’ (Trillo et al. 2016). Overall parasitism risk faced by hourglass treefrogs, however, results from an interaction between this collateral damage effect and any anti-predator benefits attained from increased group size. The nature of this integration, however, has not been studied.

We used phonotaxis experiments in the field to determine how parasite visitation rates to hourglass treefrogs were influenced by a variation in the presence or density of nearby calling túngara frogs. We found that hourglass treefrogs suffer more parasitism when calling near túngara frogs than when calling alone, but we also found that the nature of this collateral damage changes with the density of heterospecific signalers. Namely, when multiple túngaras are present fewer flies parasitize hourglass treefrogs than when a single túngara frog is present.

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EAU DE CORPSE: VOLATILE ORGANIC COMPOUNDS AND THEIR  
ATTRACTIVENESS TO FLESH- AND FLY-EATING BEETLES

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Forensic entomology is the study of insects and their interactions with carrion as they relate to the time and conditions of a crime. A relatively new field, forensic entomology has only been commonly practiced in North America since the 1970's. Due to the infancy of the field and the recent call for standardization within all scientific fields, especially those referred to in court cases, there is still more that needs to be learned in order to standardize the methods used in forensic entomology, and carrion ecology in general; therefore, it is crucial to know how insects interact with carrion, both human and animal. Previous studies have been conducted to understand these interactions in various fly species, which arrive shortly after death, but very few have attempted the same with carrion-associated beetles, which arrive in the later waves of succession. This series of experiments uses the species *Dermestes maculatus* (Dermestidae), *Dalotia coriaria* (Staphylinidae), and *Carcinops pumilio* (Histeridae) to understand Coleoptera-carrion interactions by measuring each species' affinity to different volatile organic compounds (VOCs) associated with various stages of decay. *D. maculatus* feed primarily on post-bloating carrion and have been used in previous studies. *D. coriaria* and *C. pumilio* are both predatory species that feed on fly larvae. They do not have a strong association with carrion as the dermestid beetles do, but here they are meant to serve as model species for other carrion-associated predatory beetles, as these species have not been studied in the context of carrion ecology with the larger purpose of aiding forensic entomological studies. Using laboratory olfactometer tests, we assess the attractiveness of carrion- and fly-associated VOCs to each species. Differences are found in species responses, particularly their sensitivity to the presence of VOCs. The results of these experiments may be informative to future forensic entomology studies.

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## INCORPORATION OF VISUAL FEEDBACK WITH MYOELECTRIC PROSTHESIS TRAINING

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Loss of a limb can be a traumatic experience for an individual. A variety of issues that are related to limb-loss include the financial burden of medical bills, difficulty of maintenance on complicated myoelectric devices, and frustration associated with an inability to properly use a prosthetic limb. In this work, we aim to address the frustration associated with improper training on how to effectively use a prosthetic limb. We propose to create a virtual reality game where users utilize electrical signals from flexing muscles in their residual arm in order to control a virtual limb. Through this training with a myoelectric system, a user can learn to effectively control a prosthesis as an extension of themselves in an engaging virtual reality environment.

Using a combination of open source platforms like Unity and Oculus Go we can facilitate major improvements to the training mechanisms used by myoelectric devices. We have generated a proof of concept, whereby a user can flex their muscles and observe a virtual hand on a computer screen reacting to this flexion. We will map multiple hand functions to multiple muscle poses through integration of a variety of flexion and extension signals from myoelectric detection. By linking successful flexion and extension to realistic virtual hand motions and corresponding point values in a gamified virtual environment, we hope to make the experience of myoelectric device training fun for both adults and children. Through the combination of an open source developed training tool with a 3D printed, highly available myoelectric arm device; expenses for more functional electronic prosthetics would decrease significantly making the technology available to people all over the world

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## SUBSTRATE STIFFNESS DETERMINES CHONDROCYTE SENSITIVITY TO OSMOLARITY VIA TRPV4 REGULATION

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Osteoarthritis (OA) is the most common joint disorder in the United States, afflicting over 54 million Americans. During development of OA, the stiffness of the extracellular matrix (ECM) of the cartilage decreases due to the degradation of both type 2 collagen and proteoglycans. Because changes in matrix stiffness has been shown to affect the function and phenotype of mesenchymal stem cells, I postulated that the loss of cartilage ECM stiffness would alter the function and phenotype of fully differentiated chondrocytes. The mechanosensitive Transient Receptor Vanilloid 4 (TRPV4) channel conducts Ca<sup>2+</sup> and is central to development of cartilage and chondrocyte phenotype. The goal of this project was to determine how ECM stiffness influences TRPV4 activity, chondrocyte cytomechanics and function. Here we show the decrease in ECM stiffness, modeled by PEG-RGDS hydrogels, reduces the ability of the TRPV4 channel to respond to mechanical load. Previous studies from our lab showed a biphasic calcium response to mechanical loading that suggested there were different calcium sources being activated. The calcium response varied on different stiffness levels suggesting at least one peak was associated with stiffness. The preliminary inhibitor studies on the hydrogel matrix indicate that the biphasic response is due to the first peak of extracellular calcium flow through the TRPV4 channel, and the second peak of intracellular calcium release from the endoplasmic reticulum. Chondrocyte micromasses stained for glycosaminoglycans show that the inhibition of the TRPV4 channel leads to higher proteoglycan levels, while an activation of TRPV4 decreases proteoglycan levels. Further studies will continue to explore the role of TRPV4 and stiffness of the ECM as well as the effect on cell function. Applying these results, we can suggest that an antagonist of TRPV4 in chondrocytes would increase proteoglycan production and could be used to increase the stiffness of osteoarthritic cartilage.

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## EXPLORING ANTIBIOTIC PROPERTIES OF PLANT EXTRACTS AGAINST SELECTED GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Traditionally plant extracts have been known for their potential Antimicrobial and Antioxidant traits. Research data shows the increase rate of antibiotic resistant bacteria known to cause Opportunistic and Nosocomial infections; also known as hospital acquired infections. The rise of antibiotic resistance bacteria warrants the need for alternative ways in controlling microbial growth of these organisms in establishing an infection. The purpose of this project was to explore the potential antimicrobial properties of two plant extracts *Ocimum gratissimum* (OG) and *Emilia coccinea* (EC) against selected gram-positive and gram-negative organisms with a history of antibiotic resistance. The two organisms used were *Staphylococcus aureus* (Gram-positive cocci-shaped bacteria) and *Pseudomonas aeruginosa* (Gram-negative rod-shaped bacteria). We hypothesized that both extracts will inhibit the growth of the organisms. We tested our hypothesis via an Agar Diffusion Assay. As we confirm the results of our preliminary data, the extracts will be further analyzed for future work. This project is a collaborative work between Dr. Alberta Aryee's lab (Department of Human Ecology) and Dr. Aikins' lab (Department of Biological Sciences)

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**TREATING TRAUMATIC BRAIN INJURY WITH A VASOPRESSION V1a  
RECEPTOR ANTAGONIST**

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Arginine vasopressin (AVP) is a chemical signal in the brain that influences cerebral vascular resistance and brain water permeability. Increases in AVP contribute to the pathophysiology of brain edema directly following a traumatic brain injury (TBI). These effects are mediated through AVP V1a receptors that are highly expressed in cortical and subcortical areas of the brain in all mammals. This study features the effects of an innovative, orally active, highly selective V1a receptor antagonist, AVN576, on behavioral and neuroradiological measures after moderate TBI.

Male, adult Sprague Dawley rats were given a moderate concussion using the momentum exchange model developed by The National Football League to study head injury in organized sports. Rats were concussed and given AVN576 intraperitoneal (IP) within 2 hrs of head injury (n= 8), concussed and treated with sterile saline vehicle (n = 8) or unconcussed and vehicle treated. All IP injections were given twice daily for 5 consecutive days. At 6, 12, 24 and 72 hrs post-concussion, all rats were imaged for changes in edema using a T1 weighted protocol. Data was also collected at each time point for resting state functional connectivity using BOLD and changes in gray matter microarchitecture using diffusion weighted imaging. All imaging was done in a 7 tesla small bore, animal scanner (Bruker BioSpec). After imaging, rats were tested for cognitive behavior in the Barnes maze for spatial memory and in the Novel Object Preference test for episodic memory. Concussed rats with vehicle treatment had significant deficits in learning and memory, increases in edema and hypoconnectivity in the hippocampus. There were no significant differences in any behavioral or neuroradiological measures between unconcussed and AVN576 treated concussed rats. These behavioral and neuroradiological measures of brain injury demonstrate the potential efficacy of V1a receptor antagonists for the treatment of TBI in the clinic.

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## INVESTIGATING FIRST RETURNS OF RANDOMWALKS: THE EFFECT OF MULTICOLORED VECTORS

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Dyck paths can be used as models for many concepts. Haug, Prellberg, and Siudem published a paper in early August 2018, titled Area-width scaling in generalized Motzkin paths, this paper explained how different kinds of Dyck paths could be used to analyze different aspects of biology. In the research that we conducted, we analyzed and broke down the different types of paths. A Dyck path is a special combination of random walks. Random walks are a series of up, down, and level steps that enumerate distinct paths from the origin  $(0, 0)$  to a point  $(2n, 0)$ , where  $n$  is the semi-length of the path. We were able to produce generating functions for Catalan, Schröder, and Motzkin paths, by using their first returns. A first return is the immediate moment that a path, using vectors in the Cartesian plane, touches the x-axis after leaving it previously from a given point; the initial point is often the origin. In this case, using certain diagonal and horizontal vectors while restricting the movements to the first quadrant will cause almost every first return to end at the point  $(2n, 0)$ , where  $2n$  counts the equal number of up and down steps in a path. Using the first returns of Catalan, Schröder, and Motzkin paths, which resulted from the lattice paths formed using a combination of diagonal and/or horizontal vectors, we then investigated the effect that coloring (labeling the possible vectors with different colors: Red, Blue, etc.), will have on each of their respective generating functions. Thus, giving us generating functions for those different paths, which give us the ability to be able to produce any level of the specific path. In this research we were unable to find a generalized generating function that worked for all cases. The inability to find a generalized generating function, peaked our interest so, we will continue to work toward a result. We also will continue to look for different ways these paths can be used to model different areas in biology.

## DETECTING THE PRESENCE OF MICROBES NATURALLY OCCURRING ON DELAWARE PRODUCE

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Probiotics are increasing in popularity as many individuals take them to help with regularity/digestion as well as to replace normal flora following treatment with broad-spectrum antibiotics. This leads to the following questions: “Are probiotic bacteria found naturally on produce?” and “Can individuals obtain probiotics by increasing the amount of produce in their diet?” Specifically, samples will be compared that are grown in different conditions, including the Wesley College community garden and organic/inorganic. Additionally, different types of produce will be compared (*ie*, fruits versus vegetables; grows underground versus above ground). Initially, samples will be compared using culture dependent methods using selective, differential, and enriched media as well as the Biolog for species identification. In the long-term, samples will be sent to DBI for sequencing.

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## ISOLATING AND DETECTING BACTERIOPHAGE, JEFE, FOUND IN GROUND SOIL

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Bacteriophages, also known as “phages”, are highly diverse viruses that infect bacteria. Besides their range of bacterial hosts, phages also differ in size and appearance as well as a few other key differences. To find out more about what makes them so different from one another, it is important to examine a variety of phages, which will hopefully shed some light on their diversity. Soil samples were collected from five different locations, one of which was chosen from 39.218159 N, 76.884312 W. That soil sample underwent direct isolation to extract all phage material. The extract went through several rounds of purification to narrow it down to one consistent phage, from which a stock lysate was prepared. From there, DNA was extracted and frozen for further sequencing. The phage, named Jefe, underwent transmission electron microscope imaging and was found to be Myoviridae, with a head diameter of about 65 to 75 nm and a tail of about 155 to 175 nm. PCR was performed, and the phage was also tested against a variety of bacterial hosts. Jefe had no effect on hosts Konkukim, HD4, Al Hakam, and HD3. It was able to lyse and kill BT DSM550, Israelensis, HD1, and HD2. Future continuation on this project would be to finish sequencing the phage and to continue any further analysis to learn more about it. Understanding as much as possible about these mysterious viruses is sure to become more important in the future, as their ability to infect and kill bacteria is a very intriguing topic given the rise of antibiotic resistance.

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## INFLUENCE OF COMMON OPIOIDS ON ESCHERICHIA COLI GROWTH

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Opioids are common pain-relieving drugs that act on opioid receptors in the brain and include drugs such as oxycodone, hydrocodone, and tramadol. Opioids were initially used in cancer related pain but are becoming increasingly prevalent as chronic non-cancer pain relievers. Over the past decade the rate of opioid prescriptions has seen a sharp increase, prompting the need for increased research into their impact on all aspects of life. Both licit and illicit opiates can be detected in wastewater streams due to their excretion in urine/fecal matter or through improper disposal of excess drugs. Wastewater-based epidemiology (WBE) uses the levels of opiates in wastewater to estimate drug use within a specific area. Understanding how microbial communities transform opiates is needed to better understand the fate of opiates in wastewater effluents. This experiment is designed to better understand the interactions which occur during the degradation of these drugs. *E. coli* was grown in minimal media with varying conditions, including with and without glucose and opioid, with the optical density at 600 nm taken at varying time points. The data gathered shows no effects of opioids on *E. coli* growth.

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## IDENTIFICATION AND CHARACTERIZATION OF SMALL PROTEINS IN THE HUMAN PATHOGEN *STREPTOCOCCUS PNEUMONIAE*

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*Streptococcus pneumoniae* (*Spn*) is a Gram-positive bacterium that resides asymptotically in the human nasopharynx. However, it can cause severe disease in the lungs, meninges, blood, and inner ear. Previous studies have identified and characterized small, ~15-50 amino acid proteins encoded by predicted short open reading frames (sORFs) that are important for metabolism and stress response in other bacterial species, such as *B. subtilis* and *E. coli*, but not yet in *Spn*. In this study, we aim to identify intergenic sORFs in *Spn*, determine if they encode small proteins, and elucidate their functions in this respiratory pathogen.

To identify sORFs in *Spn*, we screened the genome for sequences that are  $\leq 90$  base pairs in size and contained predicted start and stop codons. Using the consensus sequence for the *E. coli* ribosome binding site (RBS), we further examined these putative sORFs for RBSs ~8-10 nucleotides upstream of the putative start codon. These analyses revealed ~30 candidate sORFs, which we further examined for predicted transmembrane domains, export signals, and sequence homology in other *Streptococci*. Of these candidate sORFs, our study will investigate five sORFs in more detail. Using a PCR-based method, we generated mutagenic DNA constructs that will be used to transform competent *Spn* cells, thus adding an antibiotic resistance gene and an SPA tag directly upstream of the predicted stop codon. This will allow us to identify transformants via antibiotic selection and track protein expression via Western Blotting. Subsequent experiments will then allow us to determine a) if these predicted small proteins are expressed, and b) under which growth or nutritional conditions they are produced. Ultimately, we aim to identify novel small proteins in *Spn* and elucidate their potential roles in normal *Spn* physiology and disease pathogenesis.



**ANTENNAE POINTING DURING THE ESCAPE RESPONSE TO LOOMING STIMULI  
IN THE CRICKET, *ACHETA DOMESTICUS***

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In the cricket, the escape response is primarily directed by cercal detection of incoming wind stimuli, though previous experiments showed that vision may play a role. For looming stimuli, the direction of the stimuli affect the direction and magnitude of the escape response. The escape response is often accompanied by the pointing of an antennae toward the incoming object. To date, little research has been done on antennae pointing and its relationship to the properties of the stimulus and escape movement. The goal of this research focuses on determining the characteristics and sensory modalities responsible for antennae pointing to looming stimuli in *Acheta domesticus*. Looming stimuli were presented by a 3" black polyurethane ball projected (1 m/s, 45°) toward the cricket from eight circumferential directions. The cricket was placed into a rotatable arena of white canvas surrounded by roof flashing. Escape responses and antennae orientations were recorded using high-speed video (650fps) and manually tracked in motion analysis software. Preliminary findings showed that pointing usually (~95%) accompanied the escape response and occurred for all directions of stimuli. Stimuli presented from the posterior end of the cricket often resulted in running or jumping with little movement of the antennae, while anterior stimuli resulted in a turn and run, with more frequent antennae pointing. When antennae pointing occurred there was an attempt by the cricket to maintain the pointing throughout escape. Additionally, pointing appeared to occur in only one antenna at a time, with a preference for the one ipsilateral to the incoming looming stimulus. Further studies will identify the relative contributions of vision and wind through the use of vision-only (video) and wind-only (white balls against a white background) stimuli as well as more fully characterize the dependence on stimulus direction and correlation to sensory or movement variables.

## Characterization of Fam210b Protein-Protein Interactions with Mitochondrial Heme Synthesis Enzymes

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Heme, the prosthetic group of the oxygen-carrying protein hemoglobin, is produced in the mitochondria via a series of enzymatic reactions. Mitochondrial iron import is an essential component of heme synthesis. Terminally differentiating erythroid cells acquire and utilize 90% of the body's iron. This extreme demand for iron hence require specialized processes for erythroid cells to transport iron into the mitochondria to function as a substrate for heme synthesis. We have identified a novel inner mitochondrial membrane protein, FAM210B, which is essential for transport of iron into the mitochondria during terminal erythropoiesis. To unravel its mechanism, we sought to identify proteins that interacted with FAM210B. We generated a murine erythroleukemia cell line that stably expressed FAM210B with a C-terminal FLAG tag. We then used anti-FLAG antibodies to immunoprecipitate FAM210B to identify its interactions with other mitochondrial proteins. We show that FAM210B FLAG correctly localizes to the mitochondria. Further, our immunoprecipitation experiments revealed that FAM210B interacts with endogenous levels of the the mitochondrial localized heme synthesis enzyme, FECH. Although FAM210B facilitates heme synthesis, it does not interact with the mitochondrial iron transporter, MFRN1. The interaction of FAM210B with FECH suggests that FAM210B may indirectly facilitate the formation of an iron transport complex. Further, the correct localization of FAM210B-FLAG to the mitochondria and its interaction with FECH, which also binds to the mitochondrial inner membrane, suggests that this reagent may be used to identify novel protein interactors of FAM210B.

I would like to say thanks to my principle investigator Dr. Yvette Yien who has helped me both with accomplishing this project and English writing skills. Also, she has provided insightful suggestions and information for my future scientific pathway such as graduation application. Also, thanks for Dr. Olabisi, who has been my academical advisor for three years, introducing me to Yien's lab that offered me exciting chances to learn and think in my professional career. And thanks for other authors who have done their contribution to our published paper "FAM210B is an erythropoietin target and regulates erythroid heme synthesis by controlling mitochondrial iron import and ferrochelatase activit" by Jiahai Shi, Caiyong Chen, Jesmine Cheung, Anthony Grillo, Rishna Shrestha, Liangtao Li, Xuedi Zhang, Martin Kafina, Paul Kingsley, Matthew King, Julien Ablain, Hojun Li, Leonard Zon, James Palis, Martin Burke, Daniel Bauer, Stuart Orkin, Carla Koehler, John Phillips, Jerry Kaplan, Diane Ward, Harvey Lodish, and Barry Paw.

# Chemical Sciences

## ABSTRACTS

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*Please note that many of the abstracts are not approved for dissemination beyond the student poster sessions and, therefore, are not approved for posting online or distribution beyond the 2018 Undergraduate Research Symposium in the Chemical and Biological Sciences.*



## GREEN SYNTHESIS OF GRAPHITIC AND CHALCOPYRITIC QUANTUM DOTS FOR SOLAR CELL APPLICATIONS

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The chief goal of the proposed research plan is to contribute toward the development of green third generation photovoltaic devices. Current solar cell technology is not able to harvest all of the energy available because of the limitations imposed on single-junction devices using bulk semiconductor material. Advances in nanotechnology allow for the conception of third-generation photovoltaic devices involving the use of quantum confined structures, such as quantum dots. Currently, the majority of researchers exploring this path by making quantum dot solar cells have utilized lead and cadmium chalcogenides because they are easily quantum confined; however, these are synthesized in organic solvents at high temperatures under an inert atmosphere with air-sensitive precursors, which is not environmentally friendly, cost-effective, or stable. In contrast, the goals of this project focus on developing an approach to quantum dot solar cells from a green chemistry perspective. Carbogenic quantum dots and copper indium disulfide/zinc sulfide core/shell quantum dots are synthesized in aqueous solution, and a protocol for size control is developed. Optical spectroscopy is used to measure the fluorescence quantum yield of the quantum dots suspended in water. Quantum yield is a measure of the quality of the nanocrystals—high yields mean that the light absorbed by the particles can be efficiently extracted and used to generate electric current. Success in each of these areas will launch the work into the next phase of design and fabrication of devices that will be more environmentally friendly, cost-effective, and stable than the quantum dot solar cells currently presented in the literature.

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**MICROWAVE SYNTHESIS OF RHENIUM PENTYLCARBONATO**

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Cisplatin and other platinum compounds have been the major contributors to the treatment of cancer and are applied in most anticancer chemotherapeutic treatments. Despite their immense success, platinum compounds have two major drawbacks to their use: they are inadequate against platinum resistant tumors and they could lead to side effects such as nephrotoxicity. Hence, other organically synthesized metal complexes, like organorhenium complexes, are being studied and have been shown to exhibit anticancer properties, making them a possible alternative to platinum anticancer complexes. The synthesis of organorhenium complexes is a two-part process, with the first part being the microwave synthesis of Pentylicarbonato complexes (PC). The microwave oven is an enclosed system that functions by the electric field induced phenomenon known as dielectric heating. The highly pressurized system and homogeneous heating drive the reaction to completion.

Microwave synthesis is an effective heating method for synthesizing pentylicarbonato complexes with reduced reaction time, greater yields, reproducibility and enhanced reaction control when compared with the conventional method. Pentylicarbonato complexes are formed by the reaction between dirhenium decacarbonyl and the respective  $\alpha$ -diimine ligand. The reaction is carried out under CO<sub>2</sub> and 1-Pentanol and is heated using microwave radiation. Reaction times are reduced from 12-24 hrs via conventional heating to 2-3.5 hrs via CEM microwave. 22 PC complexes were synthesized and purified with a 60% yield and then characterized using IR and NMR. However, upon examining the IR of the finished products, certain unrecognized peaks were observed. One peak around 2070 cm<sup>-1</sup> was identified as starting material and the other around 1980 cm<sup>-1</sup> is still unidentified. NMR also showed unidentified peaks that may be impurities. After completing preliminary studies, mechanistic studies are being conducted. These include running the reactions using varying microwave times and temperatures and then taking the IR of the product to identify clear peaks.

## THE BENEFITS OF ORGANORHENIUM COMPLEXES AS CANCER DRUGS

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Each year 12.7 million individuals learn they have cancer, and 7.6 million will die from it. Research shows that with proper treatment and early diagnosis, roughly 33% of cases can be cured and 30-40% deaths can be prevented. Current cancer medications e.g. Tamoxifen, the leading drug for hormone receptor positive breast cancer patients, causes endometrial cancer as well as tumor drug resistance after long use. Due to the current drawbacks of current drugs, there is an urgent need to develop an alternative. Previous studies in our lab have shown promising cytotoxicity results for ibuprofen substituted rhenium pentylcarbonato complexes (PC) with IC<sub>50</sub> values as low as 1.059 μM, revealing their strong ability to act as anticancer agents. This study examines the synthesis and levels of cytotoxicity based on IC<sub>50</sub> values, of ten ibuprofen substituted rhenium pentylcarbonato complexes.

Ibuprofen substituted rhenium PC complexes are synthesized in a two-step synthesis: (1) microwave assisted organic synthesis of ten PC (fac-(CO)<sub>3</sub>(α-diimine)ReOC(O)OC<sub>5</sub>H<sub>11</sub>) derivatives (2) treatment of PCs with ibuprofen using traditional heating methods. Seven PC complexes have been successfully synthesized using microwave technology three of which are novel complexes. Re<sub>2</sub>(CO)<sub>10</sub> is combined with a α-diimine ligand and stirred CO<sub>2</sub> for 30 minutes in a microwave vial. The vial is transferred to the CEMTM microwave and irradiated at 170°C for 2 - 4.5 hours. The optimized percent yields for the seven PC derivatives ranged from 75-85%. The yields using conventional heating are as follows: 66% (PC2), 76% (PC4), 66% (PC5), and 83% (PC 7) with reaction times ranging from 24-48 hours. Ibuprofen substituted rhenium PC complex 9 was synthesized in 2 hours resulting in a 100% yield. Utilizing this method, seven precursors and one ibuprofen substituted complex were synthesized with shorter reaction times and higher yields.

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**THE EFFECT OF DIGESTION METHOD ON QUANTITATION OF SELECTED  
METALS IN TEA TYPES BY FLAME ATOMIC ABSORPTION SPECTROMETRY:  
PRELIMINARY RESULTS FROM A LABORATORY PROJECT FOR  
INSTRUMENTAL ANALYSIS**

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This paper will present and discuss preliminary results from a laboratory project for our chemistry program's Instrumental Analysis Laboratory course that explores and assesses the effect of wet and dry digestion methods of sample preparation on the quantitation of nutritional metals in different types of tea by flame atomic absorption spectrometry (FAAS). So far, this project has been conducted for the past two years (Spring 2017 and Spring 2018). In the Spring 2017 project, Fe, Cu, Zn, and Mn were determined in green and black tea varieties, using sulfuric acid/nitric acid wet ashing and microwave digestion. In Spring 2018, Fe, Cu, Mn, Zn, Cr, and Ca were determined in green, black, white, and oolong tea types, using the two aforementioned sample digestion methods plus a dry ashing method via Meker burner and crucibles.

Tea is one of the most frequently consumed beverages on Earth. The black, green, white, and oolong varieties of tea that are commercially available are grown in locations such as China, Japan, India, Korea, Kenya, and Turkey. Each type of tea has been found to contain varying concentrations of such metals as chromium, iron, zinc, copper, calcium, and manganese – the metals of interest in this study. Each of these metals possesses known nutritional value for humans.

A major objective of this study is to explore and evaluate the effect of each digestion method on the concentration of each analyte. To this end, results obtained for Fe, Cu, Mn, and Zn (Spring 2017), and for Fe, Cu, Mn, Zn, Cr, and Ca (Spring 2018) in the tea types studied will be presented and discussed, along with experimental details for the sample digestion methods and the quantitation of metals by FAAS, statistical assessment of the results obtained to date, and future plans for this project.



**SYNTHESIS OF N-PYRAZOLYLDIACETONEACRYLAMIDE DERIVATIVES AND  
THEIR REACTIONS WITH DICHLORO(1,5-CYCLOOCTADIENE)PALLADIUM (II)**

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The base catalyzed Michael additions of 3,5-dimethypyrazole and 3-methypyrazole with diacetone acrylamide form three N-pyrazolyldiacetoneacrylamide derivatives. These potential ligands have been characterized by elemental analysis, and NMR and IR spectroscopy. X-Ray structures of two reveal that they exist as hydrogen bonded dimers in the solid state. Their reactions with dichloro(1,5-cyclooctadiene)palladium(II) produce complexes by displacement of cyclooctadiene. These have been characterized spectroscopically and by elemental analysis. The X-ray structure of one of these shows the ligand to bond through a pyrazolyl nitrogen and to contain an intramolecular hydrogen bond between an amide hydrogen and a chloride bonded to palladium. There is no indication of further inter- or intramolecular interactions.

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## NICKEL CATALYZED CROSS-COUPPLINGS OF AMINO ACID DERIVATIVES VIA C-N BOND ACTIVATION

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Primary alkyl amines are well appreciated as building blocks for the synthesis of nitrogen-containing molecules. However, their use as alkylating agents via cleavage of the carbon–nitrogen (C–N) bond is largely undeveloped. Recognizing this opportunity to expand the utility of alkyl amines in synthesis, the M. Watson group is developing strategies to convert primary alkyl amines into alkyl arenes via nickel-catalyzed cross-couplings of Katrizky pyridinium salt intermediates. We are now developing this chemistry to enable cross-couplings of  $\alpha$ -amino acids via cleavage of the  $\alpha$ -C–N bond. Pyridinium salts were formed from the N-termini of a number of  $\alpha$ -amino acids and their derivatives. Once formed, the pyridinium salts were allowed to undergo nickel-catalyzed cross-coupling. Our preliminary scope, as well as future work, will be presented.

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## THERMOCHEMICAL ANALYSIS OF SRM FUEL BLENDS USING THE LASER-DRIVEN THERMAL REACTOR

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Biofuels are a class of alternative fuels derived from renewable sources such as animal fat or plant matter. These fuels are of particular interest because of their potential as “drop-in” energy sources; that is, as alternative fuels that can be swapped for or mixed with conventional ones without modification to existing engines or infrastructure. Most “drop-in” biofuel research to date has focused on physical rather than thermochemical properties, considering only factors like viscosity and density while omitting others like energy content and heat release rate. Our current research has focused on the development of a new method for testing such thermochemical properties. The device, referred to as the laser-driven thermal reactor (LDTR), seeks to mimic the environment of a typical combustion engine while maintaining high measurement sensitivity. A laser is used to rapidly heat the sample and obtain signature temperature vs. time plots (thermograms), which are then analyzed to provide information on the liquid sample’s heat release and mass change due to chemical reactions and/or phase changes. The short run time and small sample size required for the LDTR make it a useful preliminary testing method, comparable to DSC, TGA, or bomb calorimetry. Our overall objective was to determine if LDTR thermograms could be used to build a predictive model capable of estimating biofuel blend properties based on a larger database of fuels. Toward this end, we tested varying concentrations of a soy-based biodiesel (NIST SRM 2772) in conventional diesel (SRM 2770) and were able to demonstrate a relationship between observed thermal behavior and biofuel concentration. We also automated our analysis protocol for estimating measurement repeatability and thus the suitability of a given set of data for inclusion in our database. This analysis methodology will be used to further refine the LDTR and model in experiments with additional SRM fuels.

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## STUDYING EDIBLE SEEDS MICROSTRUCTURE USING CONFOCAL MICROSCOPY

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Njangsa seed contain about twice as much oil as soybean (45 - 67%), and high amount of the conjugated linoleic acid: alpha eleostearic acid. Black-eyed peas, and bush and Kent mango kernels are valued for their nutritional quality and potential antioxidant properties, respectively. Very little is known of their microstructure. Microscopy studies may reveal structure, packing, and composition of the cotyledon, which may provide information on the seed's susceptibility to enzymatic attack, stability under various processing conditions and pre-extraction techniques for efficient component recovery. After using a series of unsuccessful sectioning techniques, black-eyed pea, mango kernels, and Njangsa seeds were soaked in springwater to soften the embryonic tissue, fixed in 4% paraformaldehyde and vacuumed for 24 h. The fixed seeds were cut into 30-40 micron sections using a tissue chopper and placed on a small shallow dish with Calcofluor white (a stain that labels cellulose). The fluorescence created by the Calcofour white stain was animated under a 405-laser line on the confocal microscope, and starch granules were strongly polarized. DiOC-18(3) stain marked proteins and lipoproteins under a 488-laser line on the confocal microscope. Various features and cellular constituents, mostly small spherical protein, and oil bodies, and lipoprotein interspersed within the cytoplasmic network were visualized.

Swelling and fixing seed tissue prior to thin sectioning is a feasible method for preserving the lipids in seed tissues. DiOC is a viable stain for identifying total lipids in hydrated fixed plant seeds, while confocal microscopy is a viable modality for localizing macro-molecules in hydrated seed sections. Conventional paraffin embedding techniques for processing seeds would strip away lipids, our method preserve lipids for microscopy analysis. This new method require minimal sample preparation protocols and analysis can be performed on a time scale of seconds.

The authors would like to thank USDA-NIFA and the Department of Human Ecology at Delaware State University for funding this project.

## **AUTOMATED OPTIMIZATION OF INTERVAL-PARTIAL LEAST SQUARES REGRESSION (IPLS) FOR MULTIVARIATE CALIBRATION AND PREDICTION**

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Multivariate calibration models are commonly used in the physical sciences to provide superior quantitative results about a sample with an unknown concentration when compared to those obtained utilizing univariate models. A variety of interval-based multivariate calibration methods have been proposed over the past two decades to further improve the predictive performance of multivariate calibration models. Interval-partial least squares regression (iPLS) attempts to select the one best interval of multivariate data that correlates strongly to the analyte property being studied allowing the rest of the data to be omitted. It has been suggested that this approach could lead to unwanted information leakage about the analyte. Therefore, stacked-partial least squares regression (SPLS) was presented as a way to avoid this information loss. SPLS makes use of all intervals of data and assigns a weight to each interval based on its correlation to the analyte target property (i.e. concentration). Larger weights are assigned to the most correlated data intervals and smaller weights are assigned to intervals not as strongly correlated. It has been reported that SPLS often provides superior results when compared to iPLS when both methods are applied to the same data sets, however, a fully optimized form of iPLS has never been presented.

Here, automated, fully optimized iPLS is presented and tested on 9 independent infrared spectral data sets. All iPLS results are compared to those obtained utilizing both traditional PLS and SPLS. For all 9 data sets, optimized iPLS outperforms traditional PLS and SPLS in terms of the root mean square error of prediction (RMSEP). Optimized iPLS produced smaller RMSEP values in considerably less time when compared to the results and computation time achieved with SPLS, as well as frequently producing calibration models of lower complexity by utilizing fewer latent variable (LVs) in the final optimized model.

Dr. Frank Vogt at the University of Tennessee, Knoxville, Department of Chemistry for the infrared spectral data sets. Salisbury University Henson School of Science and Technology and the Department of Chemistry for financial support.

## TRACE METAL DEPOSITION FROM A COAL FIRED POWER PLANT

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*Gutierrezia sarothrae* samples from five sites near the Navajo Generating Station in northern Arizona were digested and analyzed for the concentrations of copper, zinc, and cadmium. Whole plants, stems, and roots were examined, and a flame atomic absorption spectrometer was used for analysis (FAAS). One way analysis of variance was performed on the data sets, and Fisher's LSD Multiple-Comparison Test and the Tukey-Kramer Multiple-Comparison Test were used to tell if there are statistically significant differences in the data. It was determined that the content of copper is consistently the same in whole plants ( $6.57 \pm 1.19$  ppm), stems ( $6.83 \pm 1.71$  ppm), and roots ( $6.90 \pm 1.36$  ppm). There is a significant difference for zinc concentration in whole plants ( $20.0 \pm 8.13$  ppm) and stems ( $19.5 \pm 8.10$  ppm) versus roots ( $11.7 \pm 4.53$  ppm). Cadmium concentrations are significantly higher in roots ( $0.343 \pm 0.122$  ppm) than stems ( $0.240 \pm 0.106$  ppm) and whole plants ( $0.241 \pm 0.126$  ppm). The samples analyzed in this report were collected in 2007, and this data can be compared with data from before and after the addition of sulfur dioxide scrubbers to the Navajo Generating Station in the 1990s.

## CONTROL OF PROTEIN SELF-ASSEMBLY WITH WATER-SOLUBLE PORPHYRINS

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Interactions between charged porphyrins and complimentary or similarly charged proteins provide important models systems for studies of electron transfer processes, artificial photosynthesis, and control of protein-protein interactions. Typically, the experimental results are analyzed and discussed assuming that the proteins exist in a monodisperse state. Here, we explored interaction of wild-type and 12 mutants of PpcA, a 3-heme c-type cytochrome (cyt) from *Geobacter sulfurreducens*, 2-heme cyt c<sub>4</sub> from *Pseudomonas stutzeri*) with several anionic water-soluble derivatives of tetraphenylporphyrin. Combined small- and wide-angle X-ray scattering experiments revealed formation of multimers with a wide range of complex sizes. Thermodynamic interaction parameters and complex binding stoichiometries were established with isothermal calorimetry.. All-atom molecular dynamics simulations revealed quick complex formation with binding sites well matching the areas identified in our experimental work. The obtained results demonstrate that multimerization of solution-state proteins by large water-soluble ligands can be tuned to control shape and size of the formed complexes. Molecular level mapping of the binding sites allows us to build a theory explaining the size of the formed complexes and provides opportunities for targeted design and assembly of multi-subunit protein complexes.

This work has been supported by NSF RUI grant number MCB-1817488 and NSF-REU grant number CHE-1757874. We also gratefully acknowledge the computing resources provided on Blues, a high-performance computing cluster operated by the Laboratory Computing Resource Center at Argonne National Laboratory. Finally, we would also like to thank staff at the Advanced Photon Source at Argonne National Lab for their help with our X-ray scattering beamtime.

## A KINETICALLY CONTROLLED SYNTHESIS OF Au<sub>25</sub>SG<sub>18</sub> QUANTUM SIZED GOLD NANOCLUSTERS FOR BIOLOGICAL APPLICATIONS

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Within the science community, nanoclusters have been thrust into the spotlight for their wide range of capabilities. Because nanoclusters range less than 2 nanometers, quantum confinement occurs which alters their chemical and physical properties. These alterations allow the clusters to exhibit unique optical, catalytic and electronic properties. The goal of my research is to form stable, robust Au<sub>25</sub>SG<sub>18</sub> (SG-glutathione thiolate) nanoclusters for drug delivery applications. This two-part reaction is accomplished using tetrachloroauric acid trihydrate (HAuCl<sub>4</sub> • 3 H<sub>2</sub>O) and glutathione (GSH). Throughout this reaction, each step is kinetically controlled with temperature and speed to produce both a high yield and uniform nanocluster size dispersity. The formation of Au<sub>25</sub> was confirmed and characterized using UV-Vis Spectroscopy, electron microscopy, and fluorescence spectroscopy. The synthesized nanoclusters will be used for various applications such as theranostic breast cancer treatment. The results of our initial study will be presented.

I would like to acknowledge and thank Dr. Mary S. Devadas for allowing me to join her research lab and for giving me the guidance to further my research. I would also like to thank Towson University's Chemistry Department for providing the necessary equipment to effectively perform research experiments. Lastly, I would like to thank PI's Fisher Endowed Chair grant, Faculty Development Research Committee grant, and NSF MRI for funding.



**AU<sub>25</sub>(C<sub>6</sub>H<sub>13</sub>S)<sub>18</sub> – A KINETICALLY CONTROLLED NANOCUSTER SYNTHESIS**Vincent Brown<sup>1</sup>Dr. Mary S. Devadas<sup>2</sup><sup>1</sup>Department of Biology, UMBC, 1000 Hilltop Circle, Catonsville, MD 21250<sup>2</sup>Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

Gold nanoclusters (NCs) are increasingly becoming a very promising topic of research due to their broad range of applications. NCs show promise in developing targeted cancer therapy and drug delivery methods by use of their ligands and cell labeling and sensing methods by use of their luminescent properties. The aim of this research is to synthesize Au<sub>25</sub>(C<sub>6</sub>H<sub>13</sub>S)<sub>18</sub> nanoclusters and compare the kinetic control of the reaction at room temperature and 0 °Celsius conditions to determine which temperature produces Au<sub>25</sub>(C<sub>6</sub>H<sub>13</sub>S)<sub>18</sub> nanoclusters in higher and purer yields. The formation of the Gold-Thiol polymer intermediate during the synthesis is the most important step in producing Au<sub>25</sub>(C<sub>6</sub>H<sub>13</sub>S)<sub>18</sub> nanoclusters. When the polymer is abundant, Au<sub>25</sub>(C<sub>6</sub>H<sub>13</sub>S)<sub>18</sub> nanoclusters formation will increase after treating the polymer with NaBH<sub>4</sub>. The Gold-Thiol polymer is formed best when the kinetics of the reaction is strictly controlled; whether this calls for a fast-stirred reaction at room temperature or a slow-stirred reaction at 0 °Celsius is to be determined.

I would like to acknowledge and thank Dr. Mary S. Devadas, for her support and guidance throughout my research, Towson University Chemistry Department, Louis Stokes Alliance for Minority Participation (LSAMP), for funding me this summer, and the PI's Fisher Endowed Chair Grant for funding the Devadas Lab.

## ENABLING PEOPLE OF PAKISTAN WITH GRIPPER PROTHESIS MODEL AND VARIOUS MATERIALS

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Millions of disabled people and amputees experience large burdens when faced with normal everyday tasks. This work strives to address the high cost of medical prosthetics by using low-cost, yet functional 3D printed prosthetics designed by eNABLE, a global network of volunteers using 3D printing and design to create open source prosthetics. Through this network, we identified a need for devices in partnership with Grit3D, an eNABLE chapter in Pakistan. However, the environmental conditions (high temperatures and rugged usage environments) precluded use of standard eNABLE plastics and designs. Typical materials used in 3D printing are a form of biodegradable plastic known as Polylactic Acid (more commonly PLA). This plastic is relatively amenable to being printed flat and thermoformed through applying heat in order to make flat pieces formable into cuff shapes to fit over residual limbs. Typically, these thermoformable parts are proven to have higher strength than parts printed with premade curves. However, the intense heat of the environments in Pakistan call for a different material. In this work, we explore some of the thermal properties of a variety of 3D printing plastics (notably, PLA, PLA+, ABS, PETG and NINJAFLEX) to identify suitable materials for printing prosthetic devices for use in high temperature environments. We have conducted various stress tests on the devices (e.g. manual destruction, destruction via tools, exposure to heat) to identify the ideal materials. By obtaining successful combinations of settings and materials, we hope to make the experience of obtaining the prosthetics a more comfortable and friendly approach. With an ideal combination of different 3D printed materials, expenses for more functional prosthetics will be decreased making them more widely available for people all over the world, ensuring the technology remains more cost-friendly for each individual's circumstance.

This research was supported by Marymount University's DISCOVER Research Program and Marymount University, specifically the Department of Biology and Physical Sciences.

**CHEMICAL ANALYSIS AND TOXICITY OF VOC EMISSION FROM HAIR CARE  
PRODUCTS FOR AFRICAN AMERICAN WOMEN**

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To determine what chemicals can be inhaled during an everyday use of hair care products for African American women, shampoos, conditioners, hair lotions, and hair relaxers were analyzed for volatile organic compounds (VOCs) by GCMS (gas chromatography mass spectrometry) with headspace sampling. Over 100 compounds were detected and identified. They included terpenes / terpenoids, alcohols, hydrocarbons, chlorinated hydrocarbons, ethers and esters. Some compounds would not have been expected given the listed ingredients, and they include substances that are suspected carcinogens, for example 1,4-dioxane, styrene, and benzene. Because mixtures of chemicals can have more adverse health effects than individual components, emissions from a subset of products were tested for their toxicity. *Escherichia Coli* and *Bacillus Subtilis* were exposed to product vapors for 24-hours. Preliminary results show no inhibition of bacterial growth.

## AN INFRARED SURVEY OF VOLATILES IN COMET 252P/LINEAR FOLLOWING A NEAR APPROACH TO EARTH

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Cometary nuclei are considered primordial leftovers from the nativity of our solar system. The composition of their native ices encodes traces of the chemical and physical conditions that occurred during the formation of the early solar system – some 4.6 Gyr ago. Comets reside in two dynamical reservoirs: the Oort Cloud and the Kuiper Belt, allowing such information to be preserved throughout the years. Gravitational effects, however, can perturb cometary trajectories, throwing them toward the inner solar system. As these bodies approach the inner solar system, solar radiation triggers the sublimation of volatiles, releasing gas and dust from the icy nuclei that contain the information stored 4.6 Gyr ago. We can study this chemical composition remotely using ground-based infrared telescopes. Among other reasons, a robust catalog of these cometary volatiles is essential to: 1) illuminate what chemistries abounded in the young solar system and 2) probe the role comets played in delivering oceans and organics to the young Earth. In March 2016, Comet 252P/LINEAR enabled unusually sensitive measurements of composition during its historically close approach to Earth, a relatively rare event among comets. In this poster, we present preliminary results obtained for 252P taken in April 2016 using the Keck-2 telescope on Mauna Kea, HI. We will discuss 252P's molecular profile in the context of compositions found for members of the two principal dynamical groupings of comets: short period (ecliptic) comets from the Kuiper Belt and long period comets from the Oort cloud.

This research was supported by the Undergraduate Research Associateship in Astrobiology program at the NASA Goddard Center for Astrobiology.

**SYNTHESIS AND REACTIVITY OF THE DIOSMIUM DIAMIDE CARBONYL  
COMPOUND  $\text{Os}_2(\text{CO})_6(\text{RCONH})_2$  ( $\text{R} = \text{CH}_3, \text{Ph}$ )**

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The diosmium diamide carbonyl complexes  $\text{Os}_2(\text{CO})_6(\text{RCONH})_2$  ( $\text{R} = \text{CH}_3, \text{Ph}$ ) have been synthesized and found to exist as two isomers in which the amides coordinate in a head-to-head or head-to-tail configuration. Synthesis of the acetamide complexes  $\text{Os}_2(\text{CO})_6(\text{CH}_3\text{CONH})_2$  also produces a third compound, which is proposed to be a polymer  $[\text{Os}_2(\text{CO})_4(\text{CH}_3\text{CONH})_2]_n$ . In the presence of carbon monoxide, this polymer converts to the monomer species. In the synthesis of the analogous benzamide complex, no polymer formation is observed. Polymer formation of the known  $\text{Os}_2(\text{CO})_6(\text{COOR})_2$  complex has also been observed under prolonged heat.

The interconversion of the isomers of  $\text{Os}_2(\text{CO})_6(\text{CH}_3\text{CONH})_2$  have been investigated by  $^1\text{H}$  NMR at a range of temperatures and thermodynamic parameters determined. The exchange of the coordinated amide for both isomers with free carboxylic acid or benzamide in solution have been investigated, to better elucidate the mechanism.

**MICROWAVE SYNTHESIS OF *FAC*-TRICARBONYL  
(PENTYLCARBONATO) ( $\alpha$ - DIIMINE) RHENIUM COMPLEXES**

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Cancer's prevalence in society warrants a need for a cure. Based on data from the American Cancer Society, breast cancer is the most diagnosed, which makes it a serious threat to humanity. It is predicted that approximately 266,120 women and 2,550 men will be diagnosed with breast cancer in 2018. Current drugs used to treat breast cancer like Tamoxifen, are effective at destroying hormone-receptor positive breast cancer cells. However, they often cause endometrial cancer and tumor resistance after extended use. Other studies revealed the anticancer activity of ferrocifens against hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cells. However, these ferrocifen compounds can cause liver damage resulting from iron overload. Tamoxifen and ferrocifens, though they're effective anticancer drugs, cause additional damage to the body, thus creating a need for less toxic anticancer drugs. Rhenium-based compounds have proven to be less toxic towards normal cells. The focus of our research is to determine the effectiveness of rhenium complexes against cancer cells without creating additional problems for the patient. In previous studies, rhenium complexes of the type  $XRe(CO)[X = \alpha$  -diimines and  $Z =$  tosylate, 1-naphthalenesulfonate and 2-naphthalenesulfonate] have shown significant anticancer activity against MCF-7 and MDA-MB-231 breast cancer cells. The objective is to optimize the initial step of the two-part synthesis of rhenium complexes using microwave-assisted synthesis. The first step involves treating  $Re_2(CO)_{10}$  with the corresponding  $\alpha$ -diimine in the presence of 1-pentanol and  $CO_2$  then subjecting it to microwave radiation to produce a pentylcarbonato (PC) complex. Three PC derivatives (PC2, PC4 and PC5) were successfully synthesized in 86%, 65% and 84% yields. The conventional yields for these compounds were 66% (PC2), 76% (PC4) and 66%(PC5). Conventional heating times were reduced from 12-24 hrs to 1.5-2.25 hrs using the microwave. Alternative procedures are being investigated to further optimize the yield of these PC's.

## SYNTHESIS OF BORON-DIPEPTIDE BASED GELS AND THE INCORPORATION OF DIOLS

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Diol-containing molecules are ubiquitous both in natural systems and pharmaceuticals. Because of this, the design of supramolecular systems that can selectively bind, detect and transport diol-rich species is a research area of growing interest. For example, dopamine, which contains a catechol unit, is a critical biomolecule that delivers signals to other nerve cells inside the brain and helps to control movement and emotions. Victims of Parkinson's disease lack these important neurotransmitters and therefore struggle to complete everyday tasks. To help alleviate their symptoms, L-3, 4-dihydroxyphenylalanine (L-DOPA), an important precursor to dopamine, is often prescribed. While dopamine is not polar enough to cross the blood brain barrier, L-DOPA can and rapidly decarboxylates to form dopamine. Despite being an important biomolecule, there have been few systems designed to target and bind L-DOPA. In our project, we designed and successfully synthesized a boronic acid-dipeptide gelator in three steps. This molecule has two main features, a diphenylalanine unit to induce gelation upon self-assembly, and a boronic acid unit to recognize and bind diols. We found that this dipeptide forms a transparent, self-supporting gel in a 50:50 ethanol: water mixtures. Furthermore, we have shown that the boronic acid-dipeptide binds diols through a boronate linkage by means of an alizarin red S displacement assay. We are currently optimizing gelation conditions, determining the physical properties of this material, and exploring the incorporation and controlled release of L-DOPA.

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## THE INFLUENCE OF FERMENTATION PROCESS ON THE ANTIOXIDANT CONTENT OF ARONIA MITCHURINII JUICE

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*Aronia mitchurinii*, also known as “black chokeberry”, is considered by many as one of the super- fruits with abnormally high concentration of antioxidants. Aronia is one of the three species of berry-producing plants of the Rosaceae family and is most commonly found in swamps and woodlands along the eastern seaboard of North America. Current Aronia applications include, but are not limited to: fruit juices, syrups, jellies, tea and most notoriously in winemaking. Traditionally, wine has been most associated with grapes, but wine can be made from a wide array of fruits. If the fruit has enough sugar content, it can be turned into alcohol during the fermentation process. Non-grape wine popularity has increased over the years, and with its not so palatable taste without the addition of sugars due to its high tannin concentration, half of all Aronia crops is utilized in winemaking. Through the winemaking process, farmers have noticed that Aronia often changes its color during the fermentation process. The color change is most likely associated with the anthocyanins group of antioxidants, partially or fully decomposing with the changing acidity, the presence of traces of oxygen and some other factors. However, the detailed research of chemical processes happening to antioxidants during the fermentation of Aronia juice has never been performed. This fundamental and very detailed research is an object of the project presented below.



## STUDY OF THE LYSINE DEPROTONATION MECHANISM IN UBIQUITIN CONJUGATING ENZYME UBC13

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Ubiquitin (Ub) is a regulatory protein with the ability to flag proteins to be degraded by the proteasome. Ub is covalently attached to a lysine on the target protein by a series of reactions catalyzed by three types of enzymes: ubiquitin activating enzymes, E1; ubiquitin conjugating enzymes, E2; and ubiquitin ligases, E3. Before Ub is transferred to its target, it is bonded to the E2 via a thioester linkage. In this study, we examine the E2 enzyme, Ubc13, which catalyzes the formation of K63-linked polyubiquitin chains. The chains are formed when a lysine on the target Ub (K63) attacks the thioester bond between Ubc13 and the substrate Ub. To initiate this reaction, K63 on the target Ub must be deprotonated, turning it into an active nucleophile. There are two possible deprotonation sites: a conserved aspartate in Ubc13 (D119) and a conserved glutamate in the target ubiquitin (E64). We are utilizing classical molecular dynamics, Born-Oppenheimer molecular dynamics and single point QM/MM calculations to determine a preference between the sites D119 and E64.

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**SYNTHESIZING PENTADENTATE SCHIFF BASE NICKEL(II) AND COBALT(III)  
COMPLEXES: DOES A MONODENTATE LIGAND BIND TOO?**

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Doxovir is a cobalt-based clinically proven antiviral and antibacterial that acts by binding to the coordinating histidine residues of Zinc fingers, through labile ligand exchange. We have prepared a further thirteen potential Zinc finger disruptors. These comprise two series of prodrugs incorporating either a Nickel(II) or Cobalt(III) ion and a pre-assembled pentadentate Schiff base ligand. Depending on the coordination complex synthesis (solvent, reactants and order added, counter ions present), we have found that the remaining sixth coordination site can be occupied or vacant. The pentadentate Schiff base ligands comprise tris(-2-aminoethyl)amine condensed with an aromatic aldehyde or aliphatic ketone. The monodentate ligands incorporated include aqua, THF, chlorido, or 2-methylimidazole. The complexes prepared were characterized using proton nuclear magnetic resonance spectroscopy, magnetic susceptibility, elemental analysis, and UV-Vis spectroscopy. In general we found that the cobalt(III) complexes coordinated an additional monodentate ligand more often. However, the incorporation of an aromatic Schiff base could allow the isolation of Nickel(II) complexes with an additional monodentate ligand too.

Thank you to the Jean and Donald Richards Student Research Fund for supporting this research.

## INFRARED SPECTROSCOPIC INVESTIGATION OF CARBONYL SULFIDE HYDRATION IN SUPERFLUID HELIUM NANODROPLETS

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Helium is unique amongst the chemical substances in that it remains liquid down to the lowest possible temperatures. Upon cooling to below 2.2 K helium becomes superfluid, possessing strange properties such as zero viscosity and frictionless flow. Superfluid helium nanodroplets have been referred to as the ultimate spectroscopic “matrix” because of their low temperature ( $T = 0.4$  K) and weakly interacting nature, which leads to greatly simplified spectra relative to the gas phase [1]. They are particularly useful for synthesizing molecular complexes, and several previous investigations have focused on investigating the hydration of atmospherically important molecules, such as the hydroxyl radical [2]. While carbonyl sulfide is the most abundant sulphur containing molecule in the atmosphere, little is known experimentally about how it interacts with water. In this study we focus on isolating  $\text{OCS}-(\text{H}_2\text{O})_N$  complexes in helium nanodroplets, and on uncovering their infrared signatures with quantum cascade laser spectroscopy.

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## DETECTION OF CONTAMINANTS OF EMERGING CONCERN IN WATER AND OYSTERS FROM THE POTOMAC RIVER

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Contaminants of emerging concern (CECs), such as pharmaceuticals and personal care products, are a growing problem within the world's watersheds due to their prevalent use in animal feeding operations and urban waste streams. CECs are chemicals that may exhibit adverse ecological or human health effects; furthermore, these molecules typically are not regulated in wastewater. The purpose of this study was to determine the concentrations of 13 ultraviolet-filters (UV-filters) and 11 hormones in water and oyster samples collected from seven sites in the lower Potomac River. These CECs were chosen because of their hydrophobicity, prevalence in urban and agricultural waste, and potential accumulation in aquatic organisms. Water samples were extracted through hydrophilic-lipophilic balanced solid-phase extraction cartridges and a quick, easy, cheap, effective, rugged, and safe extraction method was developed for the oyster samples. The CEC concentrations in extracts were measured by liquid chromatography with tandem mass spectrometry. Results show ubiquitous CEC detection at all seven sampling sites. The average concentrations in water samples for three representative UV-filters were as follows: 33 ng/L of oxybenzone; 160 ng/L of homosalate; and, 129 ng/L of octisalate. Four other UV-filters were detected at least once in water samples. Sucralose, a wastewater indicator, was also detected in all seven sites, suggesting that the CECs stemmed from upstream wastewater effluent. Oxybenzone, homosalate, octisalate, and octocrylene were also detected in oyster tissue at five out of the seven sites, confirming their accumulation into sensitive Chesapeake Bay organisms. Overall, these results highlight the need for further study of the spatiotemporal distribution of these CECs in the Potomac River to inform management strategies that limit potentially adverse effects on the Chesapeake Bay ecosystem.

**CF<sub>2</sub>H ANALOGS OF TRADITIONAL BIFURCATED HYDROGEN BOND DONORS**

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Incorporation of the difluoromethyl group (CF<sub>2</sub>H) into organic molecules leads to changes in physical, chemical, and biological properties leading to great potential in pharmaceuticals. The ability of RCF<sub>2</sub>-H bonds to hydrogen bond has been reported and used as a bioisostere for drug design. However, strategies to improve the class of CF<sub>2</sub>H hydrogen bond donors for other roles such as hydrogen bond catalysis or host-guest chemistry have been limited. The incorporation of the CF<sub>2</sub>H group into traditional bifurcated hydrogen bond donor scaffolds capitalizes on the additive strength of hydrogen bond interactions. The synthesis, characterization, and hydrogen bonding properties of bifurcated CF<sub>2</sub>H systems as a new class of lipophilic hydrogen bond donors is presented.

This work was supported primarily by the REU Site: Interdisciplinary Programs in the Chemical Sciences award number 1460990 with principal investigator Dr. Brian Coppola & co-principal investigator Dr. Melanie Sanford

**KINETIC ANALYSIS OF DRUG RELEASE FROM GELATIN NANOPARTICLES**

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Nanoparticles are becoming more wide-spread in drug delivery systems because of their ability to yield a higher drug encapsulation, initial controlled release and targeting, and decreased toxicity. Specifying the shape and size of nanoparticles allows for precise drug delivery in vivo, without release in unwanted cells. Gelatin nanoparticles are of interest because they are stable, easily biodegradable, and help to improve solubility of the drug. The functional groups on gelatin also allow for it to be easily chemically modified; simple changes in preparation can greatly facilitate drug incorporation and attachment.

The purpose of this project is to determine the optimal conditions for the synthesis of gelatin nanoparticles loaded with selected amounts of pharmaceuticals and to study the kinetics of drug release under selected conditions. By varying pH levels of the nanoparticles, their efficiency as delivery systems will be determined. After optimizing the synthesis of the nanoparticles, they are loaded with acetylsalicylic acid as a test drug. The nanoparticles are characterized using various analytical methods. The release is followed through the changes in concentration of the drug in solution over a period of time using UV-Vis. The results from these experiments are being applied to varying kinetic equations to determine the best fit for each pH value.

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## GELATION BEHAVIOR OF AMIDE DERIVATIVES OF OLEIC ACID

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Efficient molecular gelators have been synthesized from oleic acid, a naturally occurring, environmentally friendly, inexpensive, and abundant resource. Thus, it has yielded two long-chain alkyl amides, N-isobutyl-9,10-dioxooctadecanamide (DIBA) and isopentyl-9,10-dioxooctadecanamide (DIPA). Their structures were characterized spectroscopically and thermally. They formed gels at low concentrations in several liquids; they are low molecular-mass organic gelators (LMOGs). The critical gelation concentration (i.e., the minimum concentration giving a gel that is able to resist gravity at room temperature) of DIBA in silicone oil was 0.6 wt%. Generally, low polarity solvents formed gels that were stable for up to two months in the absence of external stimuli such as heat, radiation, and mechanical stress. Polarizing optical microscopy (POM) images of the gels that had been formed by ‘fast-cooling’ and ‘slow-cooling’ protocols of their sol phases showed spherulitic structures. The spherulites of gels that were formed from sols by the fast-cooling protocol exhibited shorter fibers than those from the slow-cooling protocol. Slow-cooling allows the fibrous structures to grow more discreetly and with less branching. The slow-cooled gels have stronger interactions among the gelator molecules, higher melting temperatures, and are more stable thermodynamically. Cooling thermograms from differential scanning calorimetry (DSC) measurements displayed exotherms at the transitions from sols to gels that are consistent with the gelator structures being crystalline. Rheological measurements of the gels from DIPA or DIBA in silicone oil showed high storage moduli, again indicating that the gels have high strength. Another significant observation was that the gels are thixotropic (i.e., they recover at least some of their viscoelasticity after the cessation of an applied destructive strain). Additional studies have probed the evolution of some of the gel properties over time. The information gathered demonstrates that DIBA and DIPA are excellent gelators with several potentially interesting uses.

My research would not have been possible without the aid of several people and organizations. Funding was provided by the National Science Foundation (NSF) through grant CHE-1502856. Additionally, I thank my research mentor, Girishma Grover, and my principal investigator, Richard Weiss, who both guided me throughout my research and were gracious enough to allow me to work on this project. Lastly, I thank my high school, Thomas Jefferson High School for Science and Technology, and Georgetown University for providing me with the necessary materials.

**PHOSPHINATE-BASED RHODOL AND FLUORESCEIN DERIVATIVES AND THEIR APPLICATIONS AS BIOPROBES**

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Fluorescence sensing is a useful tool to observe otherwise invisible processes or structures. Fluorophores that absorb and emit light within the near-infrared region (NIR, 650-900 nm) are of particular interest due to the reduced scattering and background observed in biological samples at these wavelengths. However, most known fluorophores lack sufficient brightness and photostability to be used as biological sensors. We hypothesized that replacing the bridging oxygen in rhodamine, fluorescein, and rhodol dyes with phosphorus will alter their spectral and physical properties. Phosphinate-based rhodamine dyes, termed Nebraska Red (NR) fluorophores, were previously synthesized and show fluorescence in the NIR region, improved brightness and photostability, and were developed into functional biological sensors. Encouraged by the robustness of the NR scaffold, we sought to create phosphinate-based rhodol and fluorescein dyes and test their spectral properties. The dyes were then used to formulate probes for biological processes including detection of cellular esterase activity, which is elevated in metastatic lymph nodes.

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## SOLVOTHERMAL SYNTHESIS AND CHARACTERIZATION OF PHASES IN THE AMMINE COPPER OXALATE SYSTEM

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Energy lost as waste heat during the production of electricity can be recovered and reused using materials that are able to capture, store, and release this waste heat through a chemical reaction. This project focuses on ammine-copper-oxalates, which are potential materials for waste heat recovery.  $\text{NH}_3\text{CuC}_2\text{O}_4$  and  $\gamma\text{-(NH}_3)_2\text{CuC}_2\text{O}_4$  were successfully synthesized by a solvothermal process by varying parameters such as the temperature, time, and solvent. The optimal solvent for separation of pure phases was propylene carbonate. Generally,  $\gamma\text{-(NH}_3)_2\text{CuC}_2\text{O}_4$  is produced at lower temperatures and shorter reaction times while  $\text{NH}_3\text{CuC}_2\text{O}_4$  is produced at higher temperatures and longer reaction times. The products were characterized using powder X-ray diffraction (PXRD), infrared (IR) spectroscopy, and thermogravimetric analysis (TGA), while their enthalpies of formation were determined by differential scanning calorimetry (DSC). The  $\Delta H_f$  of  $\gamma\text{-(NH}_3)_2\text{CuC}_2\text{O}_4$  was determined to be -905 kJ/mol while the  $\Delta H_f$  of  $\text{NH}_3\text{CuC}_2\text{O}_4$  was determined to be -795 kJ/mol. While the enthalpies of formation are promising, these compounds are not candidates for waste heat recovery because the loss of ammonia is not reversible without decomposition of the oxalate.

I would like to thank the JMU College of Science & Mathematics, the JMU Center for Materials Science, Isatu Kamara, Dr. Harry Hu, and Dr. Daniel Ralston. This work was supported by the National Science Foundation (NSF-REU CHE-1757874).

## SYNTHESIS AND CHARACTERIZATION OF CU-DOPED TiO<sub>2</sub> NANORODS AS A VISIBLE LIGHT PHOTOCATALYST

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Due to its low cost, brilliant white appearance, and nontoxicity, titanium dioxide (TiO<sub>2</sub>) is widely used in paints, pigments, and cosmetics. TiO<sub>2</sub> is also a promising photocatalyst, meaning that, simply powered by light, titanium dioxide can perform a dazzling array of reactions ranging from the splitting of water into hydrogen and oxygen to the breakdown of pollutants in industrial waste streams. However, the photocatalytic performance and therefore the application of TiO<sub>2</sub> is fundamentally limited by electron-hole recombination. Thus, there is great interest in retarding electron-hole recombination in TiO<sub>2</sub> and narrowing the bandgap to improve photocatalytic performance. In this research, a range of Cu-doped TiO<sub>2</sub> nanorods (NRs) with controlled concentrations of copper were synthesized *via* a nonaqueous surfactant-assisted method. The spatial distribution of Cu was controlled by a two-step injection process. The NRs were characterized *via* transmission electron microscopy, UV-vis spectroscopy, and powder X-ray diffraction. Additionally, the photocatalytic activity of the NRs was determined *via* the degradation of methylene blue dye under visible light illumination and measured using a UV-vis spectrophotometer. Results from these experiments indicate that the photocatalytic performance of Cu-doped TiO<sub>2</sub> NRs increases with decreasing copper doping, with the undoped TiO<sub>2</sub> NRs featuring the greatest performance.

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## DESIGN AND SYNTHESIS OF MECHANO-RESPONSIVE FLUOROPHORES FOR LOCALIZED VISUALIZATION OF DAMAGE IN POLYMER COMPOSITES

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Mechanophores are molecules in which chemical change in response to mechanical stress results in color and/or fluorescence. These have been previously introduced into polymer materials for the purpose of damage-imaging and tensile testing. However, each mechanophore features different physical properties. Current mechanophores in use by the National Institutes of Standards and Technology (NIST) activate through a reversible ring-opening reaction of spirolactam-containing mechanophores, all of which obtain their fluorescence and color through reestablishment of conjugation in a rhodamine fluorescent center. One of the research goals was to synthesize a mechanophore with a lower activation energy than those previously utilized. Additionally, while many different types of mechanophores have been utilized in past research, a correlation between fluorescence intensity and localized mechanical energy has not yet been achieved. Two new mechanophores were synthesized, characterized, and tested against a common mechanophore used at NIST, rhodamine 110 spirolactam. The new mechanophores were designed to reduce the ring-opening activation energy of the spirolactam through steric hindrance or electron density. This was accomplished using diamines such as jeffamine d230 and metaphenylene diamine. These new mechanophores were embedded into an epoxy thermoset matrix and exposed to localized ultrasound mechanical energy to induce fluorescence. By altering the power entering a geometrically well-defined sample, the amount of force required to activate fluorescence can be determined. All three samples were imaged with confocal fluorescence microscopy.

I would like to acknowledge Ilabahen Patel, Huyen Vu, Sindhu Seethamraju, and Bharath Natarajan for their assistance in this research. Special thanks goes to the Summer Undergraduate Research Fellowship Program at the National Institutes of Standards and Technology (NIST) for funding my research, award number 70NANB18H053. All materials and equipment were provided by NIST.

**SYNTHESIS AND CHARACTERIZATION OF PALLADIUM(II) COMPLEXES WITH  
SUBSTITUTED N-PYRAZOLYLPROPANAMIDE AND N-  
TRIAZOLYLPROPANAMIDE DERIVATIVES**

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Four derivatives have been synthesized through base catalyzed Michael additions, with Triton B as the basic catalyst. Reaction of methacrylamide and 1H-1,2,3-triazole produced **1**, an asymmetrically substituted triazole with a methylpropanamide moiety. The reaction of N-isopropylacrylamide and 1H-1,2,3-triazole produced two isomeric substituted triazoles, one with the N-isopropylpropanamide substituent bonded to the exterior ring nitrogen (**2**), the other with the substituent bonded to the central nitrogen (**3**). The reaction of diacetone acrylamide with pyrazole produced **4**, an asymmetrically substituted pyrazole. All compounds have been characterized through NMR and IR spectroscopy as well as elemental analysis. Additionally, compounds **1** and **4** have been characterized through single crystal X-Ray diffraction. Derivative **1** was reacted with dichloro(1,5-cyclooctadiene)palladium(II) and the product was characterized through IR spectroscopy. No reaction occurred when derivatives **2** and **3** were reacted with dichloro(1,5-cyclooctadiene)palladium(II). The reaction of dichloro(1,5-cyclooctadiene)palladium(II) with **4** displaces cyclooctadiene, forming complex **5**. The complex has been characterized through NMR and IR spectroscopy, elemental analysis, and X-Ray diffraction. The chemistry of these derivatives with other transition metal complexes is currently being investigated.

This material is based on work supported by the National Science Foundation Research Experience for Undergraduates (NSF-REU) grant CHE-1757874

## DESIGN AND SYNTHESIS OF LPXC INHIBITORS AS POTENTIAL ANTIBIOTICS AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

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As bacteria become more resistant to modern-day antibiotics, finding new methods to combat bacterial infections is becoming more and more important. Gram-negative bacteria, which are especially resistant to antibiotics, consist of a membrane containing Lipid A, a critical fatty acid and membrane anchor for lipopolysaccharide (LPS). An irreversible step in the synthesis of Lipid A involves deacetylation by an enzyme called LpxC. Because LpxC is integral in the replication and survival of gram-negative bacteria, it has become a target to combat antibiotic-resistant infections. However, the vast majority of LpxC inhibitors contain a hydrophobic, linear aromatic scaffold with a hydroxamic acid functional group. The hydroxamic acid is an essential component for these LpxC inhibitors because it acts as a zinc binding group (ZBG) to bind to the active site zinc of LpxC, thus preventing deacetylase activity. This pharmacophore, however, also inhibits matrix metalloproteinases (MMPs). MMPs are a family of zinc metalloenzymes that are upregulated in many cancers. Several MMP inhibitors have been tested in human clinical trials to no avail, often causing a variety of undesired toxicity. Therefore, the inhibition of MMPs is an undesired side effect of most LpxC inhibitors. The goal of this research study is to design and synthesize LpxC inhibitors that do not inhibit MMPs but still retain their antibiotic properties by removing the hydroxamic acid group and replacing it with different ZBGs.

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## HEXANETHIOLATE PROTECTED SUPER ATOM CLUSTERS OF GOLD - A KINETIC STUDY

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The science of gold nanochemistry has consistently been on the rise over the past decade. However, synthesizing gold nanoclusters with precision is a major challenge in nanotechnology research due to their ultra-small dimensions. This project focuses specifically on the synthesis and characterization of Au<sub>25</sub>L<sub>18</sub> icosahedron nanostructures, and the development of multifunctional metal-based nanostructures for energy responsive and biomedical applications.

Under precise and optimal conditions, synthesized nanoclusters display characteristic superatom behavior; a unique property that allows the core gold atoms to transfer electrons to the ligand attached. This project uses the organic ligand hexanethiol, to synthesize and study magic number, icosahedral, monolayer protected clusters (AuMPCs). These MPCs are particularly stable and have high surface-area-to-volume ratio making them highly applicable to biomedical imaging and photovoltaics. Further, their icosahedral geometric shape is of significant interest since it displays molecule-like optical properties such as multiple absorption peaks and enhanced fluorescence exhibiting optimal optical absorption in the near-infrared region.

Once synthesized, each nanoparticle is extensively characterized using UV-Vis spectrometry, fluorescence spectroscopy, transmission electron microscopy (TEM), and electrochemical analysis to characterize the synthesized particles for size, absorbance wavelengths, and the HOMO-LUMO band gap. Future applications include using anthracene dye to modify the surface of the cluster to make the nanoparticle fluorescent. Results from the synthesis and characterization will be presented.

I would like to acknowledge and thank Dr. Mary S. Devadas for allowing me to join her research lab and for giving me the guidance to further my research. I would also like to thank Towson University's Chemistry Department for providing the necessary equipment to effectively perform research experiments. Lastly, I would like to acknowledge funding from PI's Fisher Endowed Chair grant, Fisher General Endowment grant, Faculty Development Research Committee grant, and NSF MRI for funding.

**LONG-TERM MONITORING OF THE INFLUENCES OF THE CULTURAL  
MANAGEMENT ON PHENOLIC COMPOUND YIELDS IN *ARONIA MITSCHURINII***

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*Aronia mitchurinii* is a species of berry native to the North-Eastern U.S. and a naturalized cultivar in Eastern Europe. Previous studies have reported high content of flavonoids, polyphenols, anthocyanins and other phenolic antioxidants in *Aronia sp.* Much is known about the high antioxidant content in *Aronia* juice, however, its phytochemical content has never been correlated with cultural management conditions. The conditions encompassed include areas such as fertilizing, mineral additives, irrigation, age of the crop, etc. Since 2006, we collaboratively have been studying the effects of nitrogen treatment, soil moisture, organic versus conventional growing, mineral additives and other factors that influence the antioxidant content of the juice and pulp of *Aronia*. The objectives of this study are: 1) to analyze whether the previously listed factors have an effect on the phenolic yields of compounds in *Aronia mitchurinii* juice, 2) to develop best practice regarding the growing and cultivation of *Aronia mitchurinii*, and 3) to compare the results with data from previous harvests.

**THE INFLUENCE OF TEMPERATURE ON THE ANTIOXIDANTS CAPACITY OF  
JUICED *ARONIA MITCHURINII***

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*Aronia mitchurinii*, also known as the black chokeberry, is a fruit-bearing shrub which is native to Maryland. Aronia berries have a dark purple color which can be attributed to the berry's extremely high content of anthocyanins. Antioxidants are an important nutrient needed for capturing naturally formed free radicals in living organisms, and prevention of oxidation and cancer formation. Aronia's reputation of being a super berry entices small farms to use it as a perspective specialty crop. The berry's high content of polyphenols also makes it a likely ingredient in several new products such as, organic teas, wines, power drinks and vitamin supplements. All food applications of any fruit require high temperature pasteurization and sometimes also cooking as major steps during the fruit processing. There are three major effects higher temperatures can have on antioxidants: isomerization, decomposition or the loss of water. Recently we have found that at 120 °C more than half of antioxidants are decomposed already after the first 5 minutes of the process. However, at 80 °C more than 85% of antioxidant content is safe even after two hours of heating. Yet typically such temperature is not high enough for effective pasteurization. There is a need to explore more temperatures and conditions between 80 and 120 °C to determine the optimal processing procedures. This data will be presented. In addition, we will be studying changes in antioxidant profile of anthocyanins, flavonoids, and polyphenols using Liquid Chromatography-Mass Spectrometry (LCMS) before and after heating.



CYCLIZATION OF PEPTOID OLIGOMERS BY [2+2+2] COBALT CATALYZED  
CYCLOADDITION

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This research aims to discover new bioactive molecules that mimic peptide and protein structures. We are developing conformationally ordered “foldamer” compounds. We identified a new strategy to constrain the structure of peptoid oligomers. We used solid phase synthesis protocols to synthesize a peptoid oligomer bearing three alkyne side chains. This molecule was subjected to a cobalt-catalyzed [2+2+2] cycloaddition reaction to form a trisubstituted benzene linkage that constrains the peptoid structure.

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## ISOLATION AND CHARACTERIZATION OF ESSENTIAL OILS IN MEDICINAL HERBS, PLANTS, AND ALGAE; AND THEIR APPLICATIONS IN PEST CONTROL

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Holy basil (*Ocimum teniflorum*), Argan (*Argania spinosa*) and various other herbs, algae and specialty crops are known for their cosmetic and medical use. While there are several studies that show the chemical compositions of essential oils and antioxidants, extracted from these plants, and their benefits, there are limited studies on the insecticidal effects. As well, many of these plants are originally grown in countries of Africa and Asia and currently are on trial to be cultivated in US and in Maryland in particularly. Due to difference in climate and soil, plants grown in US typically would have different oils and nutrients composition, as compared to countries of their origin. For potential applications, the evaluation and comparison for those plants is needed. Here, we hypothesize that the essential oils from Holy basil, argan, *Aronia mitchurinii*, and other medicinal plants and herbs will have either deterrent, attractive or repellent effects on insects. This research has three major aims: 1) To determine the chemical composition of antioxidants and essential oils in commercially available argan oils and the extracts from holy basil leaves, argan seeds and aronia pulp, using plants grown locally; 2) To compare the nutrients of crops locally grown in MD to crops grown in their natural habitats in Tibet, India, Africa etc.; and 3) To test the deterrent, attractive or repellent effects of essential oils and/or antioxidants extracted from plant material against the Japanese beetles and other pests presenting significant tread to local farmers..

# **THERMOCHEMICAL HEAT STORAGE OF SODIUM AND POTASSIUM BIS(OXALATOOXALATO) CUPRATE (II) DIHYDRATE**

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There are considerable amounts of energy lost as waste heat in energy production and other similar chemical processes. Recovering this waste heat would save millions of dollars in fuel costs and reduce the amount of greenhouse gases released into the atmosphere. One way to recover this waste heat is by using a reversible chemical reaction that can capture and store the heat in the endothermic step and release it in the exothermic step of the reaction. We hypothesized that sodium or potassium bis-oxalato cuprate (ii) dehydrate could be good candidates for this process due to their reversibility, thermal stability, high energy density, low toxicity, and low cost. Since the thermal decomposition pathways of sodium and of potassium bis-oxalato cuprate dihydrate are not well established, we have investigated the thermal decomposition using thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC) and powder X-ray diffraction (pXRD). Both compounds were prepared by dissolving 3 to 1 mixture of  $\text{CuC}_2\text{O}_4$  in a concentrated solution of the alkali metal oxalate. The blue crystals that formed on cooling were isolated using vacuum filtration and characterized using pXRD. Both compounds decomposed in three steps in flowing nitrogen. pXRD and Fourier transform infrared spectroscopy were used to establish that the first step was from the loss of the waters of hydration while the second and third step were from the decomposition of the bis(oxalate) cuprate ion. The enthalpies of reaction for each step were established from DSC measurements and used to determine the enthalpies of formation for  $\text{M}_2\text{Cu}(\text{C}_2\text{O}_4)_2 \cdot 2 \text{H}_2\text{O}(\text{s})$  and  $\text{M}_2\text{Cu}(\text{C}_2\text{O}_4)_2(\text{s})$  where  $\text{M} = \text{Na}$  and  $\text{K}$ . The values determined were  $-2743 \pm 12 \text{ kJ/ mole}$  and  $-2695 \pm 10 \text{ kJ/ mole}$  for the hydrated salts and  $-2148 \pm 14 \text{ kJ/ mole}$  and  $-2107 \pm 12 \text{ kJ/ mole}$  for the dehydrated salts of Na and K respectively

James Madison Chemistry and Biochemistry department. This material is supported by the National Science Foundation Research Experience for Undergraduates (NSF REU CHE-1757874). A special thanks to Dr. DeVore for working with me throughout the project. Dr. Black James Madison Chemistry and Biochemistry department.  
Dr.Reisner James Madison Chemistry and Biochemistry department.

## ACIDOLYSIS PRODUCTS OF RHENIUM (I) ALKYL CARBONATO COMPLEXES TO TREAT AND INHIBIT INFLAMMATION IN CANCERS OF THE ORAL CAVITY.

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*Cis-platin*, an organometallic compound with a platinum center, is the most commonly used cytotoxin in cancer treatment. Studies have examined various organometallic compounds with their cytotoxic properties due to the cytotoxic resistance and significant side effects of current therapies. This research focuses on Rhenium(I) centered organometallic compounds supported by one  $\alpha$ -diimine ligand, three carbonyl ligands, and a sixth ligand. In this research there is an emphasis on a rhenium complex with a sixth alkyl carbonate ligand. This ligand arises from the reflux of dirheniumdecarbonyl in pentanol under an atmosphere of carbon dioxide. The alkyl carbonate ligand in the rhenium complex can be substituted in acidolysis reactions with the anions of a variety of acids such as difluoroacetic acid, chloroacetic acid, hexanoic acid, benzene sulfonic acid, tetrafluoroboric acid, benzoic acid, and a pyridinium salt. Acidolysis reaction products are characterized by IR and NMR spectroscopy to verify the compound's structure. The association between inflammation and cancer has been studied widely, so we want to determine if alkyl carbonates could inhibit vital signaling of the inflammatory process. The transcription factor NF- $\kappa$ B has been a key element in inflammation, and its activation has been shown to upregulate gene expression of other pro-inflammatory cytokines. Research has displayed that NF- $\kappa$ B activation may occur in most cell types. Employing carboxylate-supported Rhenium(I) centers on the human squamous carcinoma (HSC-2) cells and human normal gingival fibroblast (HF-1) cells, we want to observe if these organometallic compounds could inhibit or slow down the activation of NF- $\kappa$ B and prevent the inflammatory process. To do this, we needed to determine the sub-lethal concentration of the alkyl carbonates. Evidence of cytotoxicity will lead to studies in the mechanism of action for these cytotoxic properties.

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**CHEMICAL BONDING? IT'S COMPLICATED  
DEVELOPING A QUESTION TO PROBE STUDENT UNDERSTANDING IN A  
FOUNDATION COURSE IN INORGANIC CHEMISTRY**

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The Interactive Online Network of Inorganic Chemists (IONiC) is a community of practice (COP) of inorganic chemists that was established in 2007. The community is working to understand the role of the COP and professional development on faculty practice and student learning. To determine the role of faculty practice on classroom teaching and student learning, a research team will examine classroom practice at twenty universities during the 2018-2019 academic year using the Classroom Observation Protocol for Undergraduate Students (COPUS) and student learning through open-ended free-response questions. The questions will be used to investigate the change in student understanding before and after faculty professional development. This summer, a set of questions about chemical bonding was refined to encourage responses that demonstrate student understanding rather than recall. Multiple preliminary coding schemes were used to attempt to characterize student responses. Preliminary coding schemes and results for the four versions of the bonding question showed a difference in quality and quantity of student responses. Version 2 produced the highest number of ideas per student. However, responses from version 4 invoked deeper understanding and working knowledge of chemical bonding.

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## INTERMETALLIC PtCoAg NANOPARTICLES FOR ELECTROCATALYTIC OXYGEN REDUCTION REACTION

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Modern fuel cells have the potential to revolutionize the transportation industry. They are electrochemical devices that convert chemical energy into electrical energy with high efficiency and no combustion. However, for fuel cells to become commercially viable it is imperative that the rate of the oxygen reduction reaction (ORR) occurring at the cathode is improved. Platinum catalysts can increase the activity of the reaction but are too expensive to be commercially produced and suffers from low durability. In this study, Ag-doped PtCo (PtCoAg) nanoparticle (NP) catalysts are synthesized to reduce the amount of platinum necessary, thus lowering cost, and improve the activity and stability of catalysts with intermetallic structure. Thermal annealing is necessary to transform the crystal structure of PtCoAg NPs from the face-centered cubic (fcc) phase to the catalytically stable face-centered-tetragonal (fct) phase. However, the sintering and grain growth during this process would ultimately reduce catalytic activity. Ideally, the addition of silver would reduce the phase transition temperature, thus decrease the degree of particle aggregation during the annealing process. The PtCoAg NPs with different silver concentrations were synthesized *via* solvothermal method and characterized by transmission electron microscopy (TEM) with high monodisperse in size. Thermally annealing at different temperatures was achieved by rapid thermal annealing and the crystal structure was analyzed with X-ray diffraction (XRD) for the transformation from fcc to fct. XRD showed the lowest conversion temperature at 600°C, which was lower than the binary PtCo system. Results showed that the ternary system could be tuned to different silver concentrations while staying highly monodisperse in size. The higher silver concentrations in PtCoAg NPs resulted in lower phase transition temperatures and this is further correlated with their ORR performance.

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## NEW TRANSITION METAL COMPOUNDS INCORPORATING THE HYDROTRIS(DIMETHYLTRIAZOLYL) BORATE LIGAND

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The hydrotris(triazolyl)borate ligand has been shown to coordinate metal ions to produce structures with multiple dimensionality, including metal-organic frameworks (MOFs). Porous, open-framework MOFs have applications in catalysis, sensing, gas separation, water reclamation, and environmental remediation. In an effort to synthesize new open-framework materials, the coordination chemistry of the hydrotris(3,5-dimethyl-1,2,4-triazolyl) borate anion ( $[\text{BH}(\text{dmtrz})_3]^-$ ) was explored with alkali metal and transition metal cations.  $\text{M}[\text{BH}(\text{dmtrz})_3]_2$  ( $\text{M} = \text{Mn}, \text{Fe}, \text{Co}, \text{Ni}, \text{Cu}, \text{Zn}$ ) were synthesized by a solvothermal reaction in methanol between  $\text{Na}[\text{BH}(\text{dmtrz})_3]$  and  $\text{M}(\text{NO}_3)_2 \cdot n\text{H}_2\text{O}$  or  $\text{MCl}_2 \cdot n\text{H}_2\text{O}$ . Crystal colors were consistent with inclusion of the transition metal: pale pink  $\text{Mn}^{2+}$ , yellow  $\text{Fe}^{2+}$ , yellow-orange  $\text{Co}^{2+}$ , lavender  $\text{Ni}^{2+}$ , blue  $\text{Cu}^{2+}$ , and colorless  $\text{Zn}^{2+}$ .  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  containing compounds were characterized by single crystal X-ray diffraction. All are 0-D coordination compounds with a triclinic cell where the metal ion is chelated by two  $[\text{BH}(\text{dmtrz})_3]^-$  groups. The synthesis, structures, and comparison of these materials to known  $\text{M}[\text{BH}(\text{trz})_3]_2$  phases will be presented.

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**COMPARATIVE SYNTHESIS OF GOLD AND SILVER NANOPARTICLES**

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Nanoparticles have a wide variety of possible applications due to their unique chemical and physical properties. The size dimension of gold and silver nanoparticles causes them to have different properties and usages than bulk gold and silver. Herein, we present a comparative study of synthesizing anisotropic metal nanoparticles. For the gold nanoparticles, synthesis was performed with chloroauric acid and sodium citrate. The silver nanoparticles were synthesized with silver nitrate, sodium citrate, and hydrazine hydrate. The goal of our research was to determine how differences in synthesis procedures affect nanoparticle size. We tested how changes in the amount of sodium citrate affected the size of gold nanoparticles. We also tested how the pH level, temperature, and concentration of hydrazine hydrate affected the size of silver nanoparticles. Potential uses of this research are to create specifically sized nanoparticles for usage in projects related to Surface Enhanced Raman Sensing (SERS). Details of the synthesis procedures and results will be presented.

I would like to thank and acknowledge Dr. Mary S. Devadas for allowing me to join her lab group and introducing me to nanoparticle synthesis. I also want to acknowledge the Towson University Chemistry Department for providing the necessary lab space and equipment to conduct this research. We also acknowledge funding from the NSF-MRI grant which helped us get SEM images and the PI's Fisher Endowed Chair Grant.



## INDUCTIVE EFFECTS IN CARBONYL ADDITION REACTIONS

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2,2,3,3,4,4,4 – Heptafluorobutyl Chloroformate (C<sub>5</sub>H<sub>2</sub>ClF<sub>7</sub>O<sub>2</sub>) is used in treating conditions that are associated with diabetes. The compound aides in controlling the elevations of the beta-1 and beta-2 isozymes within the body. Protein Kinase C isozymes, beta-1 and beta-2, can be inhibited within the body through the use of several known compounds, but specifically 2,2,3,3,4,4,4 – Heptafluorobutyl Chloroformate.

Comparing the solvolysis of 2,2,3,3,4,4,4-Heptafluorobutyl Chloroformate and its parent, Butyl Chloroformate, we conclude that 2,2,3,3,4,4,4-Heptafluorobutyl Chloroformate follows an Addition-Elimination process in all solvents, including the highly ionizing TFE and HFIP mixtures. The large l/m (4.71) ratio for 2,2,3,3,4,4,4 – Heptafluorobutyl Chloroformate signifies a much earlier tetrahedral transition state occurring and a stronger general base catalysis.

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## STUDIES OF IMINE INVERSE ELECTRON DEMAND DIELS-ALDER REACTIONS

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Nitrogen and oxygen containing heterocycles, including benzopyrans, are relevant due to their wide variety of biological applications. The 4-Aminobenzopyrans and their derivatives are of particular interest; they interact with potassium channels, making them valuable anti-hypertensive and anti-ischemic drugs. While there are a wide variety of viable synthesis strategies for preparing these molecules including use of LiBF<sub>4</sub> and Bi(OTf)<sub>3</sub> catalysts, it is our goal to produce more facile methods. A recent study by Kumar *et al.* reports a one pot inverse electron demand Diels-Alder synthesis of fused pyranobenzopyrans and furanobenzopyrans using salicylaldehyde, aromatic amines, and 2,3-dihydrofuran catalyzed with cellulose sulfuric acid. Our long-term goal is to expand the scope of this reaction by utilizing various substituted imines previously synthesized in our lab. In addition, we hope to experiment with various oxygen and nitrogen containing dienophiles to increase the molecular diversity of our library of derivatives.

## CHEMICAL IDENTIFICATION OF COCOA GEOGRAPHIC ORIGIN USING UPLC-MS

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Cocoa and the production of its products is a global industry and the need for a method to chemically discriminate geographical origin is becoming more and more important for quality control and the traceability of fair-trade practices in the industry. Many of the steps in the process of producing cocoa can influence the chemical makeup of the different aspects of the final product including the flavor profile and scent. Since each region where cocoa is grown can contain several variations in soil composition, fermentation process and roasting conditions, the differences in chemical signatures can be used to classify beans by their country of origin. Many studies have employed GC-MS, ICP-MS, and HPLC to quantify to detect the presence of certain compounds in cocoa samples. However, little has been done to group and classify different qualities of a cocoa sample to determine its origin. Previous work by this group has been able to accurately determine the country of origin of a cocoa using ICP-MS to construct a unique elemental profile for each country and identifying unknowns based off the profile. Use of the LC-MS provides an opportunity to use chemical signatures to not only identify country of origin in cocoa samples but potentially differences in genetic strains and classification of some of the compounds that contribute to taste, aroma, and other desirable attributes. This project describes the cocoa sample preparation and conditions for UPLC/ESI-TOFMS used to obtain unique chemical signatures from cocoa liquor samples along with the discriminate analysis used to identify chemical fingerprints for cocoa beans based on one of five countries of origin.

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**SYNTHESIS OF FUNCTIONAL, UNSYMMETRICAL TETRAZINES VIA  
PALLADIUM CATALYZED CROSS-COUPPLINGS USING ORGANOSTANNANES  
AND ORGANOBORANES**

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The development of functional, unsymmetrical tetrazines proves important for a multitude of applications. Current synthesis methods are unsafe, using anhydrous hydrazine at elevated temperature and pressure. Forming unsymmetrical tetrazines via cross-coupling reactions of thioether tetrazines with either organostannanes or organoboranes provides a safer approach to tetrazine production, one that can be used in both industry and academia. As these compounds are incredibly useful in drug delivery and chemical imaging, it is vital to be able to synthesize them safely on a large scale. Tin reagents were synthesized via the palladium-catalyzed stannylation of various functionalized phenyl compounds. These reagents were then utilized in palladium-catalyzed cross-coupling reactions with methyl-thiobenzyltetrazine to produce methylphenyltetrazines. Due to the toxicity of organostannanes, extra measures were taken to ensure safety, including only using a designated toxic rotary evaporator, and difficulty in separating the tin starting material from the reaction's products introduced several additional steps to the purification workup. Since organoboranes are both cheaper and safer than organostannanes, organoborane compounds were used in palladium-catalyzed cross-coupling reactions to yield similarly functional tetrazines. A variety of various functionalized phenyl boron compounds were tested in palladium-catalyzed cross-couplings and produced functionalized methylphenyltetrazines with comparable yields to the tin reagents. These tetrazines play critical roles in bioorthogonal chemistry as they can couple directly to drug candidates or fluorophores via cross-coupling reactions. The accessibility of these tetrazines are likely to improve the efficacy and kinetics of active pharmaceutical ingredients (APIs).

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## UTILIZING HYDROLYSIS TO INCREASE EFFICIENCY OF LOCALLY RECOVERED CHITIN IN REVERSIBLE CARBON CAPTURING

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Atmospheric Carbon Dioxide (CO<sub>2</sub>) has become a leading concern in managing rising greenhouse gas emissions from persistent industrialization. Primary efforts for controlling these gas pollutions focus on reducing the current amount of CO<sub>2</sub> from fossil gases, which is more than 40% of total CO<sub>2</sub> pollutions. Research has focused on materials able to bind atmospheric CO<sub>2</sub>. Prior experiments utilized materials that are irreversible sorbents. Carbon dioxide cannot be released after sorption and the sorbent cannot be reused. Those sorbents were found to be unpractical, because they cause more solid waste needing permanent storage elsewhere. Therefore, effective and practical carbon sequestration should utilize reversible and reusable sorbents. Chitin is a material of high molecular weight that has shown promise as a sorbent material in reversible carbon dioxide capture. This polysaccharide combines with minerals to form the rigid structures of shells. Previous experiments sourced chitin from the shell waste of local seafood industries on the Eastern Shore. Chitin makes weak bonding of CO<sub>2</sub> to its amino groups. Literature for previous generations of sorbents indicated lower molecular weight material demonstrated better sorption properties. We used acidic hydrolysis to produce different smaller molecular weight fractions of chitin from local seafood waste. Our goal is to find a correlation between chitin's molecular weight and sorption properties. A charcoal-celite column, with 5%, 10%, 15%, and 20% ethanol solution respectively was used to separate products by molecular weight. Fractions were concentrated using a rotary evaporator and tested by UV/vis spectrometry for characterization. Using N-Acetylglucosamine (GlcNAc) as a control for the presence of amino groups at  $\lambda_{\text{max}} = 212\text{-}216$  nm, we discovered peaks within control range for 10% and 15% ethanol collections. The various molecular weight fractions were tested for carbon dioxide sorption by measuring FTIR before and after sorption. The hydrolysis and sorption data will be presented.

We would like to thank the University of Maryland Eastern Shore Department of Natural Sciences, Dr. Anthony K. Nyame, Ms. Emily Cable (ACS Project SEED summer 2018), and the Title III Grant.

## USING NUCLEAR MAGNETIC RESONANCE TO EXPLORE THE EFFECT OF SURFACE CHEMISTRY ON THE MAGNETIC SUSCEPTIBILITY OF GOLD NANOPARTICLES

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The electronic properties of gold nanoparticles' (AuNPs) metallic core is affected by the chemisorbed ligands at the particles' surface. This offers a new means to manipulate and tune AuNP properties, rather than the more traditional altering of shape, size, and core composition. Hence, it is of great interest to understand nanoparticle surface chemistry to achieve desired electronic properties for applications such as drug delivery and photovoltaics. AuNPs protected by alkanethiolates of various lengths, from butanethiol to dodecanethiol, were synthesized via the two-phase Brust-Schiffrin method. Particle populations were characterized by transmission electron microscopy (TEM), and the Evans nuclear magnetic resonance method was utilized to calculate the magnetic susceptibility. Analysis revealed that the magnetic susceptibility of the gold nanoparticles decreased with increasing ligand length, indicating that the density of electronic states also decreased. These findings introduce the prospect of a novel method to tailor the properties of nanoparticles.

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## **RHODAMINE-BASED FLUORESCENT AND COLORIMETRIC CHEMOSENSOR FOR DETECTION OF METAL IONS IN AQUEOUS MEDIA**

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Metals are inorganic elements that can cause significant health issues in excess. Metals run off into our water supplies through corrosion of pipes and industrial processes. Certain metals are essential to the body such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Fe}^{3+}$ , however in excess these metals can lead to liver and nerve damage. Selection, and detection of these metals have potential applications in many fields including chemistry, biology, and the environment. The goal of this research project is to synthesize and characterize a fluorescent chemosensor that will detect metals in aqueous media. Rhodamine B is a dye known for its great spectroscopic properties such as high fluorescence quantum yield, long absorption, and emission wavelength and high stability to light. The microwave synthesis of five rhodamine-derived imines is described. The present work involves condensation of rhodamine hydrazide with various aromatic aldehydes in ethanol under microwave irradiation. The results obtained indicate that, unlike classical heating, microwave irradiation results in higher yields, shorter reaction time and simple work-up procedure. The microwave-assisted reactions were conducted at  $100^{\circ}\text{C}$  with yields ranging from 67-81%, as compared to the conventional method which was run under reflux with yields in the range of 42-69%. Rhodamine derivative RD-1 bearing 4-tertbutyl-2,6-diformylphenol unit have been synthesized and utilized towards fluorescence detection of metal ions in aqueous medium. On addition of  $\text{Cu}^{2+}$  ions to a solution of RD-1 in  $\text{CH}_3\text{CN}$ /water medium at  $25^{\circ}\text{C}$ , the systematic increase in chelation-enhanced fluorescence (CHEF) enables the detection of  $\text{Cu}^{2+}$  ions as low as  $32\text{ }\mu\text{M}$ . The compound displayed selectivity for  $\text{Cu}^{2+}$ , which was characterized using UV-vis and fluorescence spectroscopy. Upon adding  $\text{Cu}^{2+}$ , the spiro-lactam ring of the sensor was opened and a 1:1 metal-sensor complex was formed. The structures of synthesized compounds were confirmed by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , FT-IR and high-resolution mass spectrometry.

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## Synthetic Optimization of MCD-66, a Promising Therapeutic for the Treatment of Acute Myeloid Leukemia

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Acute Myeloid Leukemia (AML) is a progressive disease that dismantles the efficiency of the blood through proliferation of immature myeloblasts. When dysfunctional myeloblasts accumulate throughout the bloodstream, overcrowding diminishes the efficiency of the blood to perform its required tasks. As a result, infections become more prevalent and the prognosis exponentially worsens with time. Our labs recently discovered MCD-66, a potential therapeutic agent for the treatment of AML. This novel compound has many of the same characteristics as other naphthoquinone analogs that have shown success against AML cells, specifically tumor reduction in animal studies. In order to obtain significant animal data on the effectiveness of MCD-66, a substantial amount of this compound had to be synthesized. The original synthetic scheme for MCD-66 was costly, involved hazardous reagents, tedious chromatographic purifications, and afforded relatively low yields at each of the steps resulting in a sub-optimal overall yield. The optimization outlined in this poster improved the yields in every step, shortened the synthesis by an entire step, and removed the troublesome chromatography step. As a result, this synthetic optimization has yielded consistently pure samples  $\geq 95\%$  with yields  $\geq 90\%$  at each of the three synthetic steps. This synthetic scheme proved effective in the scale-up synthesis of MCD-66, providing adequate supply for animal studies to be performed, and ultimately, enhancing the viability of MCD-66 as a potential therapeutic agent in the treatment of AML.

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## DIFFERENCES IN THE ENERGY AND REACTIVITY OF MALEIMIDE ENEDIYNES

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Enediynes are antibiotics that are powerful antitumor agents due to their cytotoxic components. Enediynes have a double bond flanked with two triple bonds which can undergo Bergman cyclization to create a diradical that induces double strand DNA cleavage through hydrogen abstraction. However, enediyne cyclization must be thermally induced or photo-activated as it does not occur naturally at room temperature. Enediynes with maleimide moieties are able to undergo cyclization at room temperature. These synthesized enediynes have the potential to be more effective than natural enediynes for cancer treatments as cyclization, diradical formation, and DNA cleavage is more likely to occur at body temperatures. In this project, the electron density and effects of functional groups in maleimide enediynes are examined to investigate their effect on cyclization at room temperature. In order to determine the most viable and energy efficient maleimide based enediyne, Density Functional Theory (DFT) calculations are used which will demonstrate energy differences and how that affects activation energy. Conformers of maleimide enediynes will be examined to determine which functional groups and conformers are the most energetically stable and likely to undergo Bergman cyclization at room temperature.

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## SYNTHESIS AND ANTIBACTERIAL STUDY OF TETRACATIONIC AMPHIPHILES

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Significantly high rates of illness and death caused by antibiotic resistant bacteria is an increasing concern in healthcare, specifically in hospital settings. According to the Center for Disease Control and Prevention, a high volume of patients have been infected with healthcare-associated infections while hospitalized. One approach to addressing this issue, is the synthesis and study of novel antiseptics. To this, end, a series of bis-bipyridinium tetracationic amphiphiles were synthesized with varying tail lengths ( $C_6H_{13} - C_{12}H_{25}$ ) and linker lengths ( $C_5H_{10} - C_{16}H_{32}$ ). Minimum inhibitory concentration (MIC) values were determined against several strains of bacteria for each of the molecules in this study in order to better understand the relationship between surfactant structure and antibacterial activity. Analysis of data collected thus far suggests that amphiphiles in this series with short tails and long linkers demonstrate the highest antimicrobial potency. Ongoing work will focus on fully characterizing this new series of amphiphiles and collecting and analyzing MIC values for recently synthesized derivatives.

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## HIGHLY EFFICIENT HETEROBIMETALLIC AU(I)/AG(I) AND AU(I)/CU(I) PHOSPHORS FOR MODERN DISPLAY, SIGNAGE AND LIGHTING APPS

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Research interest in new emissive materials has increased dramatically due to their crucial role in emissive electroluminescent layer to conquer excellent device performance of solid state lighting, signage and display devices, namely organic light-emitting diodes (OLEDs) and light-emitting electrochemical cells (LEECs). The ability to achieve near unity internal quantum efficiency by incorporating heavy atoms like transition metals has made the development of such emissive materials even more significant. Such emissive materials containing heavy atoms are typically phosphorescent as opposed to fluorescent due to strong spin orbit coupling constant ( $\zeta$ ) associated with them. These phosphorescence materials use the principle of electrophosphorescence to convert electrical energy to light with 100 % internal quantum efficiency, whereby intersystem crossing between singlet and triplet states assists both singlet (25%) and triplet (75%) excitons to decay radiatively. These materials can be used to design and develop cost effective, thinner, lighter, flexible, and energy efficient consumer electronics that are demanded by current technological advancements. Moreover, emissive materials in today's electronic devices (such as iPhone XS, Samsung Galaxy S9, etc.) are using iridium metal that will be replaced by coinage metals in this project to further reduce material costs. To implement our emissive materials in such a profound area of material applications, we will carefully investigate the structural, photophysical and other relevant properties of heterobimetallic coinage metal(I) complexes containing Au(I)/Cu(I) or Au(I)/Ag(I) metal ions bound to pyrazolate and diphenylphosphinopyridyl ligands. Herein, we report experimental results by exploring the synthetic details, single crystal X-ray structural analysis, and broad-range of spectroscopic studies including photoluminescence (steady-state, lifetime, and quantum yield), NMR, FTIR, and UV/Vis/NIR. Ongoing research includes device fabrication in collaboration with a research group in Texas to study the potential of these emissive materials to be used in commercial lighting, signage and display devices.

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**Probing Polymeric Blends with Natural Extracts from *Aronia mitschurinii*, Algae and other  
Natural Extracts as an Effective and Natural Substitutes of Tributyl Tin (TBT) for  
Antifouling Protection**

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Marine biofilm formation is the accumulation of micro/macro- organisms on submerged objects. The main environmental trait of this process is spreading of bacteria and attachment of barnacles to bottom of ships as well as transporting bacteria from its natural habitats to other marine environments. The latter especially affects cargo industry and military. Marine biofilm formation results in significant increase of fuel consumption and damage to ship hulls, petroleum platforms and other submerged objects. To slow the growth of biofilm formation antifouling paint is applied to the bottom of the hull of ships and boats. While it can decelerate the growth of the organisms, traditional antifouling composites contain tributyl tin (TBT), which is an unstable and toxic compound. Use of this additive is banned in many countries including US. It has been found that some marine organisms and algae never have biofilm precipitation due to some natural secrets they have on the surface. In this project we test different specialty crops, herbs and algae as potential natural antifouling agents. Biofilm formation has five stages, in which first two are chemical precipitations involving ox-red radical reactions and bacteria precipitation. Therefore, in our project we focus on crops with high antioxidant content – to prevent radical activities, and high content of essential oils with proven antibacterial properties.

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Environmental Science

**ATMOSPHERIC REACTIONS OF  $\alpha$ -PINENE OXIDE ON THE MINERAL DUST  
KAOLINITE AS A FUNCTION OF RELATIVE HUMIDITY**

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Secondary Volatile Organic Compounds (SVOCs), such as the monoterpene  $\alpha$ -Pinene Oxide ( $\alpha$ -PO), are a major component of the heterogeneous reactions with mineral dust in the atmosphere. In these experiments we focused on the mineral dust kaolinite, as previous research has shown it to be particularly reactive with monoterpenes. FTIR analysis was used to investigate the reactivity of  $\alpha$ -PO as a function of Relative Humidity (RH) on kaolinite mineral dust. Kinetic analysis shows that increasing RH decreases the rate of reaction. The exception to this trend is 50% RH which shows an increase in reactivity from the 0%, 10% and 30% RH reactions. To gain more insight into this anomaly, kinetic analysis was done on the aldehyde levels of each reaction. GC-MS was performed on each sample to identify the major product of the  $\alpha$ -PO and kaolinite reactions.

DETECTION OF N<sup>2</sup>-ETHYLGUANINE USING LIQUID CHROMATOGRAPHY – TRIPLE QUADRUPOLE (LC-TQ) MASS SPECTROMETRY INSTRUMENTATIONAvi M. Newman<sup>1</sup>, Joshua A. Wilhide<sup>2</sup>, and William R. LaCourse<sup>3</sup><sup>1</sup>Department of Chemistry, University of Maryland, Baltimore County,  
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When pregnant women imbibe alcohol, they cause irreversible congenital defects to the fetus. These include small head size, low body weight, and various other mental and physical problems that will adversely affect their entire life. While there isn't a cure for these defects, referred to as Fetal Alcohol Syndrome (FAS), early detection and treatment can prove immensely beneficial in mitigating symptoms and improving the health of the affected individual. The modified nucleoside N<sup>2</sup>-Ethylguanine, an ethylated form of the nucleobase Guanine found in DNA and RNA, acts as a potential biomarker for this condition, since it is produced directly by alcohol consumption. Detection and quantification of this compound could prove instrumental for the quick diagnosis of FAS. The focus of this project is the detection of low levels of N<sup>2</sup>-Ethylguanine using liquid chromatography with triple quadrupole (LC-TQ) mass spectrometry (MS). The TQ utilizes improved scan rates and sensitivities while increasing robustness with a novel clean source design. For initial experiments, Guanine and N<sup>2</sup>-Methylguanine serve as an analog (due to cost and availability) to the compound of interest, providing a baseline for detection and methodology that can then be translated to the detection of N<sup>2</sup>-Ethylguanine. Varying concentrations of standards will be analyzed to determine limit of detection (LOD), limit of quantification (LOQ), and other analytical figures of merit (AFMs). Additional characteristics such as retention time and fragmentation mass will be determined. Future research will focus on the translation to the compound of interest, N<sup>2</sup>-Ethylguanine, and further method optimization.

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## CHLORINATION AND BROMINATION KINETICS OF DICHLOROACETAMIDE HERBICIDE SAFENERS

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Herbicide safeners are a largely uninvestigated group of compounds in terms of their environmental fates. Dichloroacetamide herbicide safeners are one of the most commonly used classes of safeners and are applied to corn and cereal grains. This project focuses on benoxacor, dichlormid, and furilazole, common dichloroacetamide safeners, all of which have been detected in Midwestern U.S. streams near agricultural sites at concentrations up to 192 ng/L. Occurrence in natural waters introduces the possibility these compounds are processed by our water treatment plants. All of these compounds exhibit reactive sites on which halogenation can occur. Experimental research regarding the halogenation rates of these compounds is important to understanding how and to what extent these compounds are being modified during water treatment processes. This research aims to quantify reaction rates of benoxacor, dichlormid, and furilazole under chlorinating and brominating conditions that may be encountered during water treatment processes. Reaction rates will be determined utilizing time course reactors. Time course reactors will also be used to identify the effects pH, chloride, and bromide concentrations have on the chlorination and bromination kinetics. Preliminary data indicate that bromination of benoxacor, chlorination of dichlormid, and chlorination of furilazole, all occur on the order of hours. Chlorination of benoxacor does not appreciably occur at pH = 7.00 (over 96 h) but appears to be undergoing a base-catalyzed hydrolysis under alkaline conditions. Bromination of dichlormid and bromination of furilazole both occur on the order of a few minutes. These results indicate transformations of dichloroacetamide safeners can occur in disinfected waters on environmentally-relevant time scales. Identification of the products of these transformations is ongoing. Products will be characterized via liquid chromatography mass spectrometry quadrupole time-of-flight (LCMS-qTOF) and nuclear magnetic resonance spectroscopy (NMR). Compositional analyses of herbicide formulae will be performed as concentrations of safeners are normally unreported by manufacturers.

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## THE SYNTHESIS OF FORMULA $\text{FAC}-(\text{CO})_2(\alpha\text{-DIIMINE})\text{ReOC}(\text{O})\text{O}(\text{CH}_2)_4\text{CH}_3$ COMPOUND

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Cancer is the abnormal growth of cells and its treatment may include chemotherapy, radiation, and surgery. Different chemotherapeutic drugs have been developed depending on the type of cancer, however, most of these drugs have severe side effect and drug resistance. For example, prolonged use of Tamoxifen, an anti-cancer drug effective on only estrogen receptor-positive breast cancer, has been linked to endometrial cancer. Due to the side effects of some of these cancer drugs, other alternatives are being researched such as organometallics rhenium compounds. Rhenium compounds are known to be nontoxic and exhibit no drug resistance. Previous studies in our lab have shown promising synthesis of a series of organometallic rhenium complexes bearing different  $\alpha$ -diimine ligands. Specifically, this study examines the synthesis of an organometallic rhenium compound bearing the  $\alpha$ -diimine ligand 5,6-dimethyl-1,10-phenanthroline.  $\text{Re}_2(\text{CO})_{10}$  is combined with 5,6-dimethyl-1-10-phenanthroline and  $\text{CO}_2$  and stirred for 30 minutes in a microwave vial. The vial was transferred to the CEMTM microwave and irradiated at  $170^\circ$  and the time varied from 2- 3 hours. The vial was stirred in  $\text{CO}_2$  and hexanes for an additional 23 hours. Subsequent filtration of the mixture afforded yellow particles of PC5 in an optimized yield of 72%.

Acknowledgements Department of Chemistry and Biology, Morgan State University.  
Dr Angela Winstead.



## CHEMICAL CHARACTERIZATION OF ESSENTIAL OILS EXTRACTED OCIMUM FAMILY PLANTS, AND THEIR APPLICATION ON TEXTILES

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Plants from the *Ocimum* family, widely known as Holy basils, have been used as therapeutic plants in non-traditional medicine. Countless studies have focused on isolating and determination of the active components that are responsible for the medical benefits of *Ocimum* plants. From previous studies, it has been determined that the essential oils have anti-inflammatory, antipyretic, analgesic, anti-arthritis, antioxidant, anti-ulcer, anti-microbial properties. As well, multiple lipophilic antioxidants have been isolated from these plants traditionally grown in India, Tibet, Malaysia and other eastern countries. Some farms local to Delmarva are now trying to grow same plants in Maryland and compare their quality to plants in countries of origin. This project has two major goals: 1) to extract and study the essential oils from the *Ocimum* (basil) family: *Ocimum Basilicum*, *Ocimum Gratissimum*, *Tulsi Rama*, *Tulsi Kapoor* and *Ocimum Sanctum*; 2) to apply extracted essential oils to textiles using encapsulation with  $\beta$ -cyclodextrin. To isolate the essential oils wet distillation will be done. Extraction in non-polar solvent will be used for isolation of antioxidants. After extraction samples will be characterized using GCMS, LCMS, and UV/Vis spectroscopy. To incorporate the essential oils to textiles the oils will be encapsulated within  $\beta$ -cyclodextrin to be able to apply to textiles.

**FACILE RHODAMINE-BASED FLUORESCENT AND COLORIMETRIC  
CHEMOSENSOR FOR DETECTION OF METAL IONS**

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Fluorescent chemo-sensors have become an important and widely used tool to detect metal ions in biological samples. Rhodamine B derivatives have received a great deal of attention as chemosensors because of their useful properties such as high absorption coefficient, high fluorescent quantum yield for excitation, and emission wavelength within the visible region. These properties give rhodamine an excellent potential for the development of turn-on fluorescent sensors. The properties of a novel, rhodamine-based derivative, synthesized by reacting rhodamine hydrazide and chromone derivatives in ethanol, were investigated in aqueous solution. The compounds' selectivity, sensitivity and reversibility of metal ions were analyzed and characterized using UV-vis and fluorescence spectroscopy. All compounds are characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR and high-resolution mass spectroscopy. Upon adding a highly selective metal ion, the spiro-lactam ring of the sensor was opened and a 1:1 metal-sensor complex was formed.

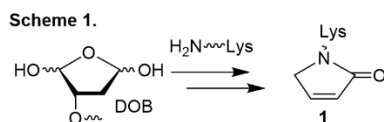
We would like to acknowledge NIGMS RISE Grant R25GM058904.

## SYNTHESIS OF MODIFIED PEPTIDE PROBES FOR STUDYING PROTEIN INTERACTIONS

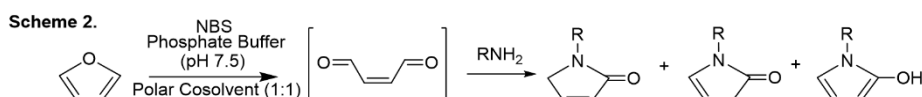
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It is of great interest to investigate the effects of DNA damage on a chemical level because of its effects on cellular stability and function. Our group discovered that certain forms of DNA damage produced by antitumor agents (e.g. DOB) ultimately result in histone modifications (Scheme 1). Histone modifications are important to study because of their role in gene regulation via changes in chromatin organization or interactions with other nuclear proteins such as histone modifiers. We are synthesizing peptides modified with **1**, a 2-pyrrolone, to probe this post DNA damage modification. Our goal is to use these peptides to study the modification's interactions with nuclear proteins. This work is important because it serves as an indicator of how DNA damage of this nature imprints itself into histones and affects the cell.



Recently, we developed a general, one-pot synthesis of 5-methylene-2-pyrrolones, a related family of molecules. Similar conditions were used to synthesize the 2-pyrrolone (Scheme 2). Three isomers of the 2-pyrrolone were generated using this method. The major isomer varies depending on the nature of the amine being modified and the solvent conditions. The procedure was optimized using different cosolvents to isolate individual tautomers using lysine as nucleophile.



The next phase is to synthesize peptides containing **1** to examine the interactions of the isomers with proteins. Two approaches are being pursued to achieve this goal. One strategy involves addition of a DOB modified Fmoc protected lysine onto a growing peptide using solid phase peptide synthesis. In an alternate approach we are carrying out direct addition of the DOB modification to a synthesized protein. The peptides synthesized with these modifications will then be examined as binding partners for proteins.

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**BROOKER'S MEROCYANINE**

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Brooker's merocyanine (1-methyl-4-[(oxocyclohexadienylidene)ethylidene]-1,4-dihydropyridine) is a solvatochromic compound, which exists as a zwitterion in protic solvents and as a neutral molecule. Solvatochromic compounds change color depending on the solvent in which it is dissolved. In more polar substances the shorter wavelengths of light are absorbed. The color, that the dye exhibits, is complementary to the color that it absorbed. Practical applications include pH sensors, transition metal cation indicators, and detection of solvent polarity. Currently, more research is being performed in order to discover new uses. Synthesis of Brooker's merocyanine is achieved by methylating 4-methylpyridine to create 1,4-dimethylpyridinium iodide. A base catalyzed reaction with 4-hydroxybenzaldehyde produces Brooker's merocyanine. Proton, and COSY Nuclear Magnetic Resonance spectra of Brooker's merocyanine (1-methyl-4-[(oxocyclohexadienylidene)ethylidene]-1,4-dihydropyridine) were taken in deuterated dimethyl sulfoxide, methanol and deuterium oxide. C-13 spectra were taken in methanol and deuterium oxide. A NOESY spectrum was taken in deuterated methanol. These results are compared to the calculated chemical shifts determined by the molecular modeling software package GAUSSIAN.

Acknowledgements - Dr. Anil Wagbe from Plymouth State University in Plymouth New Hampshire supplied the compounds. (17 High St, Plymouth, NH 03264)

**RAPID SYNTHESIS OF HIGHLY SUBSTITUTED OXAZOLE COMPOUNDS**

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The purpose of this research project is to develop a quick and inexpensive method of synthesizing multi-substituted oxazole compounds. Oxazole compounds are prevalent in nature, making them an important biological molecule. Both naturally produced and synthetically made oxazole compounds exhibit multiple medically relevant characteristics such as anti-inflammatory and anti-cancer properties. The creation of a simple, one pot synthetic pathway for the creation of multi-substituted oxazole compounds would allow further research to be conducted using these oxazole compounds. The method we have developed is a simple, one step process that creates di-substituted and tri-substituted oxazole compounds from an isonitrile and an isocyanate. The method was refined using solvent and concentration studies in order to find the optimal conditions that favor the production of one multi-substituted oxazole over the other. The benefits of this synthetic scheme are that it uses safer solvents, readily available starting materials, and neutral reaction conditions compared to the established methods of oxazole synthesis. The method we have developed is unique because it creates two multi-substituted oxazole compounds whereas the established methods produce one mono-substituted or di-substituted oxazole compound. The next goal of this research project is to use our refined method to create more di-substituted and tri-substituted oxazole compounds with different substituent groups.

We would like to thank the National Institute of Health (R15GM116003) for providing funding for this research project. We would also like to thank the Henson School of Science for providing the facilities that made this work possible.

**DESIGN, SYNTHESIS AND EVALUATION OF SELECTIVE INHIBITORS OF THE MONO-(ADP-RIBOSYL)TRANSFERASE, PARP10**

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Poly-(ADP-ribose) polymerases (PARPs) and mono-(ADP-ribosyl) transferases are members of a superfamily of enzymes that catalyze the transfer of ADP-ribose onto target proteins. Inhibitors of PARP1, the parent member of this superfamily, have advanced cancer treatment against tumor types that are deficient in certain DNA repair mechanisms. PARP1, the founding member of the family, has been extensively studied, however, there are currently 17 other members of the PARP family several of which remain uncharacterized. Many of these other members demonstrate intriguing biological activity, but do not have viable chemical probes to validate them as drug discovery targets. One of the other members of the family is the mono-(ADP-ribosyl) transferase, PARP10 (a.k.a. ARDT10). This enzyme has been implicated in the regulation of gene transcription, genomic stability, tumorigenesis and DNA repair. While knockdown data indicate that PARP-10 may be a viable drug discovery target, a selective PARP10 inhibitor could be useful as a probe compound to further validate this target and would provide discovery groups with optimizable leads for cancer therapy. We recently identified a benzamide containing diaryl ether (MCD-72, PARP10 IC<sub>50</sub> = 400nM) that demonstrated good potency against PARP10 without inhibiting PARP1 (IC<sub>50</sub> >100μM). The overall goal of this research was to modify this lead by the addition of nitrogen atoms on to the B-ring, replacing the ether moiety, extending the ether moiety and synthesizing structurally restricted bicyclic amines to improve binding interactions with PARP10. Many of these modifications resulted in loss of potency, but one derivative (MCD-223) demonstrated good PARP10 potency and selectivity over PARP1 and a closely related enzyme PARP14. Future studies will incorporate different bicyclic amines into the side chain of MCD-223 to improve binding potency and drug-like properties.

Acknowledgements: Special appreciation goes to the McDaniel College Chemistry Department, The Jean and Donald Richards Student Research Fund, McDaniel College Student Research and Creativity Grant, and BSE Biosciences for generous support.

**DIFFUSION-CONTROLLED HYALURONIC ACID MICROSPHERES FEATURING  
CLEAVABLE PROTEIN CONSTRUCTS TARGETED BY MATRIX-  
METALLOPROTEINASE ACTIVITY FOR CELL-MEDIATED RESPONSE**

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We report hyaluronic acid hydrogels patterned with cell-cleavable protein constructs targeted by the activity of matrix-metalloproteinases (MMPs) for design of cell-responsive materials. Previously, our group demonstrated that the rapid cycloaddition between tetrazines and trans-cyclooctenes can be used to crosslink hydrogel materials by interfacial, bioorthogonal chemistry. Expanding upon this approach, these hydrogels have been covalently-patterned with selected proteins utilizing the same diffusion-controlled chemistry. Enabled by organic synthesis and techniques for site-specific labeling of model proteins, this method affords microspheres with distinct cell-instructive core or shell features composed of stimuli proteins or other small molecules. Ultimately, we seek to demonstrate that affixing these microspheres with genetically-encoded growth factors featuring MMP-targeted cleavage-sequences promotes proximity-guided cell-response mimicking the ordered heterogeneity of tissues for 3-D cell culture matrices useful for tissue engineering or in vitro models of human disease.

This work was supported by the Office of Undergraduate Research and Experiential Learning through the University of Delaware Summer Scholars program.

Thank you to Jiyeon Song of the Jia Research Group for synthesizing and providing the hyaluronic acid-tetrazine used in this project.

## CATALYTIC ACTIVITY OF Au AND Ni NANOCATALYSTS IN THE REDUCTION OF 4-NITROPHENOL AND NITROANILINES WITH $\text{NABH}_4$

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Noble metals such as gold (Au), platinum (Pt), and palladium (Pd) are excellent catalysts for a wide variety of reactions. There is great interest in the use of nanoparticle forms of these metals due to their high surface area. Mixed metal nanostructures that consist of noble metals and less expensive metals such as nickel (Ni) may show catalytic activity comparable to that of the noble metal catalysts, but with the advantages of reduced cost. We investigated the catalytic activity of Au and Ni nanocatalysts for the reduction of 4-nitrophenol and nitroanilines.

Reduction of aromatic nitro groups to amines is an important reaction in the synthesis of many pharmaceuticals, polymers, dyes, and agricultural products. The compounds studied were 2-nitroaniline, 3-nitroaniline, 4-nitroaniline, and 4-nitrophenol (PNP). Both Au and Ni catalyzed the reduction each nitro compound except 3-nitroaniline. Au was the better catalyst. The easiest compound to reduce with either Au or Ni as catalyst was PNP, followed by 4 nitroaniline, then 2 nitroaniline. The compound that showed the greatest difference between the rates of reduction catalyzed by Au compared to reduction catalyzed by Ni was 2-nitroaniline. This compound will be used to further study the catalytic activity of the mixed metal nanocatalysts.



**N-METHYL CARBAMATE PESTICIDES AND THEIR PHENOLIC DEGRADATION PRODUCTS: HYDROLYTIC DECAY, SEQUESTRATION AND METAL COMPLEXATION STUDIES**

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We report on the rates of decomposition of a group of *N*-methylcarbamate (NMC) pesticides (carbaryl, carbofuran and propoxur) under pre-determined tropical field conditions. Laboratory conditions simulated aquatic conditions determined in field sites in Guayas, Ecuador and Ruiru, Kenya. Both sites are located in agriculturally productive areas with high rates of pesticide use. Kinetic studies on NMC pesticides were conducted by measuring decay rates of parent compounds via UV-vis spectroscopy. Rates of decomposition for the three NMCs were determined at pH 7.08 and T = 20 °C; and pH 7.70 and T = 33 °C respectively as follows: carbaryl (78 days and 69 days); carbofuran (143 days and 83 days); and propoxur (116 days and 79 days). Our results showed that NMCs decomposed faster at higher pH and temperature. In addition, the electronic properties of the NMCs governed decomposition rates via known  $E_{\text{Lcb}}$  mechanisms, and the most electron-poor NMC degraded fastest. Investigations on methods for removal of NMCs and their phenolic decomposition products showed that activated charcoal outperforms zeolite, alumina, diatomaceous earth, cellulose, and montmorillonite clay in the removal of both NMCs and phenols from aqueous solution. A series of metals were also probed for possible complexation to NMCs and their phenolic degradation products. The sequestration studies showed that Fe (III) forms a complex with isopropoxyphenol (IPP) within which the Fe:IPP ratio is 1:3, indicative of the formation of a metal chelate complex with the formula  $\text{Fe(IPP)}_3$ .

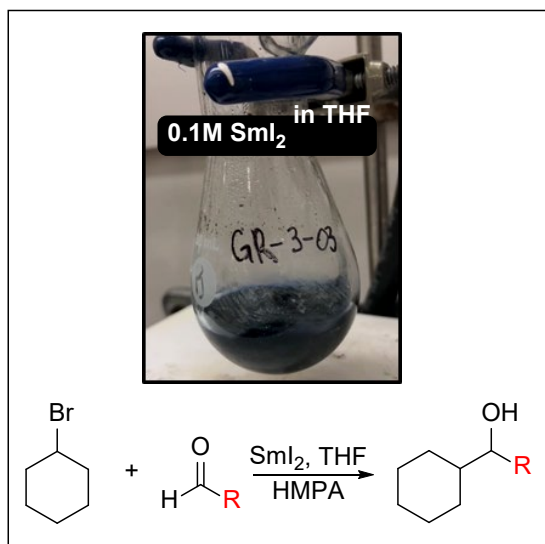
This research was supported by a grant from the Towson University Fisher College of Science and Mathematics Undergraduate Research Fund.

## METHOD DEVELOPMENT OF AIR-FREE TECHNIQUES WITH SAMARIUM DIIODIDE

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Samarium(II) iodide ( $\text{SmI}_2$ ) is a single electron reductant known for its powerful synthetic transformations<sup>1</sup>. The highly air-sensitive reductant is commonly synthesized in a glovebox and requires ultra-dry and air-free solvents typically obtained with a solvent drying system. This type of equipment is not available at all universities, so a systematic approach was used to look at the proper conditions that must be met for the air-free synthesis and running of  $\text{SmI}_2$  reactions. The Samarium-Barbier reaction is a widely used carbon-carbon bond forming reaction, which we used to quantitatively analyze the reduction efficiency of synthesized  $\text{SmI}_2$  with a Lewis base additive, hexamethylphosphoramide (HMPA)<sup>2</sup>. Through our analysis we hope to make the useful reagent,  $\text{SmI}_2$ , an easy-to-handle reagent in other labs even if there is limited equipment.



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**SELF-POWERED ENZYMATIC BIOSENSOR FOR DETECTION OF GLUTATHIONE**

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Several diseases can be indicated by the concentration of glutathione within the blood or plasma of a patient. Designing a sensor for the detection of these levels is highly desirable and can be helpful in diagnosing a patient or monitoring the progression of an illness. The biosensor was designed as a fuel-cell and functions through two enzymes and respective substrates. The first enzymatic reaction, glucose oxidase with glucose, functioned as the anode. For the detection of glutathione, the cathode was modified with bilirubin oxidase. An increase in the concentration of glutathione inhibits the activity of bilirubin oxidase and decreases the overall power of the fuel cell. This sensor could prove to be very helpful in the future for implementation into the medical field by using small biosensors, specifically for tracking the progression of neurodegenerative diseases. By utilizing carbon electrodes and enzymes, this device is much less expensive and more environmentally friendly than other biosensors that utilize heavy metals for the detection of biomolecules. The biosensor produced is cost-effective, sustainable, and is sensitive for the detection of glutathione in a smaller size than other medical devices.

We would like to thank the Lebanon Valley College Endowed Research Fund for general funding in the project's development.

## ADSORPTION OF FORMIC ACID ONTO TiO<sub>2</sub> NANOCCLUSERS USING DENSITY FUNCTIONAL THEORY

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Quantum treatment of periodic model titanium oxide (TiO<sub>2</sub>) surfaces are resource intensive and typically depend upon the physical and chemical model selected. To gain accuracy, TiO<sub>2</sub> nanoclusters have been used to model the surface of TiO<sub>2</sub> and its binding to carboxylic acids, important to scientific and engineering applications. The density functional theory B3LYP with a LanL2DZ basis set was used to scan the potential energies of the many possible binding modes. M06-2X/def2-TZVP was found to more accurately predict formation energies for the nanoclusters and was used to further study the best binding modes. Formic acid has been observed to prefer binding to the lower coordinated Ti sites, resulting in binding enthalpies greater than 80 kcal/mol. This strong binding was found to decrease greatly as Ti coordination approached values found in crystalline TiO<sub>2</sub>, which suggests that surface defects and amorphous regions promote stronger binding of carboxylic acids to the surface. This can serve as a basis for experimentalists to study surface binding as it relates to crystallinity, and improve the prediction of surface modification to create novel materials.

This research was supported in part by grants NSF/REU CHE-1659821, NSF/MRI CHE-1126465, and NSF/MRI CHE-1726824.

## COMPARING THE BROMINATION AND CHLORINATION KINETICS OF THE HERBICIDE DIMETHENAMID IN NATURAL AND IN SYNTHETIC WATERS

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Chlorine used in the disinfection of drinking water can react with bromide and natural organic matter present in water sources, forming toxic by-products (DBPs). Most previous studies examining the kinetics of DBP formation were conducted in synthetic waters. The lack of analogous data obtained in disinfected natural waters presents the question of whether reaction rates calculated for synthetic waters are applicable to natural waters. This research aims to quantify reaction rates of dimethenamid (an herbicide widely-applied to corn fields) in four natural water sources of varying salinity (Susquehanna River, Loch Raven Reservoir, Chesapeake Bay, and Atlantic Ocean). These rates were compared to those previously obtained in synthetic waters to determine under what solution conditions reaction rates of synthetic waters can be applied to natural waters, including those used for drinking water. To determine rates in the natural waters, reactions were performed using each of the four water sources in which pH, chloride and bromide concentration were treated as independent variables. Experiments performed in the absence of dimethenamid displayed consumption of free bromine with half-lives ranging from 3 to 8 hours. Rates of consumption were quantified in all four water sources, so consumption could be considered when calculating reaction rates in synthetic waters to increase the accuracy of the comparison to the reaction rates experimentally determined in natural waters. In high salinity water sources, halogenation reactions were too fast to quantify using the techniques employed in this study. Results show that reactions rates calculated for synthetic waters overpredict those observed in all examined natural waters by up to a factor up 5. The calculated values best approximate the experimental values as bromide concentrations increase.

We extend our thanks to the US National Science Foundation (CBET-1651536), Towson University's Office of Undergraduate Research, Fisher College of Science and Mathematics, and the Department of Chemistry for their financial support. Any findings, opinions, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

## THIOPHENE-BASED COVALENT ORGANIC FRAMEWORKS

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Covalent Organic Frameworks (COFs) are highly crystalline compounds that may be fully conjugated, rendering them useful moieties in organic photovoltaic applications. Polythiophenes are optoelectroactive species that absorb a broad spectrum of light, are chemically stable, and have high charge carrier mobility. We propose the synthesis of a Thiophene-based COF that will marry these two moieties to generate an optimal optoelectroactive organic photovoltaic material. Fused and unfused tetrathiophenylbenzenes were investigated to explore the effect of restricted rotation on crystallinity of the resulting COFs. Ester boronates and imine linkages were explored under heat and pressure to create reversible linkages between tetrathiophenylbenzene and aryl rings. Due to low solubility, ester boronates were difficult to isolate and characterize so they were abandoned, and imine linkages were pursued. We found these syntheses to produce highly soluble tetrathiophenylbenzene carbaldehydes that were readily characterizable in high yield with simple purification techniques. The resulting COFs were analyzed by PXRD for crystallinity, IR for purification, and TGA for thermal stability. We anticipate a fused tetrathiophenylbenzene will possess higher crystallinity than the unfused tetrathiophenylbenzene due to their restricted rotation of the thiophenes.

I would like to thank the National Science Foundation (Grant Number CHE-1757874), Karen Murley Student Research and Travel Fund, Millersville University Faculty Grant, National ACS Travel Grant, Neimeyer-Hodgson Research Grant, Noonan Endowment Fund Grant, SEPSACS Student Travel Grant, Student Research and Creative Activity Grant for funding this research. I would also like to thank the James Madison University Department of Chemistry and Biochemistry for allowing me to participate in their REU program. Finally, I would like to thank Dr. Kathryn Allen for her incredible mentorship these past 2 years.

## OPTIMIZATION OF A NEW NANO-ELECTROSPRAY IONIZATION EMITTER FOR PROTEIN ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY

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Protein analysis, the study of sequence, structure, and function, is a critical component in the fields of biotherapeutics, pharmaceuticals, and forensics, among others. The ability to accurately determine protein sequence is an essential part of protein characterization. Since the advent of softer ionization techniques in the early 1990s, the field of proteomics has been steadily evolving. An established technique is nano-electrospray ionization with high resolution mass spectrometry. Recently, Newomics introduced a new nano-electrospray emitter, the M3 emitter, designed for increased sensitivity and robustness. Previous research determined that operating the emitter as “plug-and-play” as advertised yielded no protein response, while the standard emitter produced protein response and allowed for determination of sequence coverage. However, the improved design of the M3 emitter over the standard emitter has great potential. The established emitter has a singular nozzle, while the M3 emitter splits flow into numerous smaller streams with multiple small silicon tips. Reducing droplet size increases ionization efficiency, and subsequently sequence coverage. The goal of this project is to optimize instrument conditions with the new nano-electrospray ionization emitter to achieve increased sensitivity. The standard used for analyses was Pierce BSA Protein Digest Standard (Product No. 1863078), a commercially predigested standard. The instrumentation used was a Dionex UltiMate 3000 nano high pressure liquid chromatography (HPLC) system with a Bruker 12T high resolution mass spectrometer (HRMS) set up for nano-spray ionization. Conditions studied were flow rate, nebulizer gas flow, and emitter positioning (both distance and angle). The standard was analyzed in triplicate using the varied conditions. To determine the efficiency of the emitter, sequence coverage and protein score were compared. The data was analyzed using PEAKS Proteomics software. Overall, the project examined the effect of various instrument conditions on the efficiency of the M3 emitter.

Acknowledgements: I would like to thank Dr. LaCourse for the opportunity to work in the Molecular Characterization and Analysis Complex (MCAC). I would also like to thank the MCAC staff for all of their help, support, and teaching.

## EFFECT OF MUTANTIONS AND LABELING ON STRUCTURAL STABILITY OF PpcA-Ru(bpy)<sub>3</sub> COMPLEXES

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We are developing biomimetic molecular architectures for efficient solar energy conversion using artificial photosensitizers combined with natural and genetically engineered host systems capable to support long-lived charge-separated states and conduct charges away from the photosensitizers. Converting light energy into its electrochemical equivalent requires precise control and fine tuning of relevant kinetic and thermodynamic parameters, including primary charge separation.

To this end, we developed a series of 22 cysteine mutants of PpcA, a 3-heme cytochrome from *Geobacter sulfurreducens*. Protein mutants were labeled at the engineered Cys sites with Ru(bpy)<sub>3</sub>Br. We verified correct photosensitizer attachment and the extent of labeling with Liquid chromatography- mass spectroscopy (LC-MS). The correctly labeled complexes were analyzed with Circular Dichroism (CD) spectroscopy at various temperature points between 25 and 90°C. Contrary to the expectation that mutations and binding positively charged bulky photosensitizers will cause destabilization of protein structure, we observed relatively small perturbations for the majority of the mutants as well as two complexes more stable than the wild-type protein. Our results also reveal strong preference of individual mutants for binding Ru(bpy)<sub>3</sub> enantiomers from racemic mixture. The obtained results are discussed in context of prior extensive Small-angle X-ray Scattering (SAXS) characterization and all-atom Molecular Dynamics (MD) simulations in explicit solvent.

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## EXPLORING CROSS-COUPPLING REACTIONS OF (2-(4-BROMOPHENYL)-1,3-DIOXANE-5,5-DIYL)DIMETHANOL

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Acetals are geminal diether derivatives of aldehydes formed by the reaction of an aldehyde with two alcohols. Collard et al.<sup>1</sup> have shown that by utilizing temperature control, benzaldehyde and pentaerythritol, when mixed in water with catalytic acid, can selectively form monoacetal derivatives, even in second-year organic chemistry laboratory settings. Our previous work explored the scope and limitations of this reaction using a variety of substituted benzaldehyde derivatives. Our current work is to take a newly formed monoacetal derivative of *para*-Bromobenzaldehyde and pentaerythritol, (2-(4-bromophenyl)-1,3-dioxane-5,5-diyl)dimethanol, and explore a series of cross-coupling reactions, such as Hill's modification of the Suzuki-Miyaura coupling reaction<sup>2</sup>, in order to create a multistep synthesis route to increase molecular complexity. Our work should help broaden the synthetic utility of these extremely user-friendly and environmentally benign reactions. It is also hoped that this work will produce diversity oriented multistep synthesis routes for use in teaching laboratories.

### References

1. Collard, D.M. et al. *J. Chem. Ed.* **2001**, 78, 70–72
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## KINETIC STUDY OF THE CATALYZED REDUCTION OF 4-NITROPHENOL USING GOLD NANOPARTICLES

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The understanding of catalysts is of great importance to many industries such as petroleum, plastics, and medicine. There is therefore a need to find cheaper, more efficient, and less toxic catalysts. Gold is a very good catalyst for the oxidation or reduction of many organic compounds. Gold is, however, rather expensive and is therefore commonly used as a catalyst as nanoparticles which have a much greater surface area per mole than bulk gold. In this work, gold nanoparticles (GNPs) of different sizes are being synthesized. Kinetic studies are then performed in order to determine the effectiveness of different sized GNPs for the reduction of 4-nitrophenol to 4-aminophenol. A reducing agent of NaBH<sub>4</sub> is used in excess so that the reaction behaves under pseudo-first-order conditions. The reduction reactions are monitored using UV-Vis spectroscopy at 400 nm. The data is then used to determine the rate constant for each GNP size. Only spherical GNPs have been tested so far. In the future we hope to make GNPs of different shapes, use TEM to confirm the shape and size of the GNPs produced, and use chemisorption to determine the number of active sites for each GNP solution, which allows for turnover frequency to be calculated. We also hope to determine the more detailed kinetic parameters for this reaction through numerical analysis, which will allow a mechanism to be proposed.

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## COMPUTATIONAL ANALYSIS OF RO-VIBRATIONS OF VINYL ALCOHOL AND THE FORMATION OF 2-CHLOROETHANOL

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The vibrational signatures and reaction pathways of small molecules are of interest when trying to identify a molecule in the interstellar medium, or consider the plausibility of a molecule being present at all. For example, in 2001, Turner and Apponi positively identified vinyl alcohol in the molecular cloud, Sagittarius B2(N). Researchers continue to look for signatures of vinyl alcohol in the interstellar medium since it is an important intermediate in many organic reactions and may therefore play a role in the formation of complex organic molecules in space. We used computational methods to calculate ro-vibrational constants of both syn- and anti-vinyl alcohol and compared them to experiments performed using far-infrared spectroscopy. Specifically, we calculated the anharmonic vibrations using second order vibrational perturbation theory (VPT2) at the CCSD(T)/cc-PVTZ level of theory. The vibrational frequencies, rotational constants and quartic centrifugal distortion constants are in good agreement with experiment and should be useful in identifying anti vinyl alcohol in the interstellar medium. Furthermore, several reaction pathways for the formation of 2-chloroethanol have been investigated. Intrinsic reaction coordinate (IRC) calculations were performed at the MP2/cc-PVTZ level-of-theory for both solvated and gas-phase reactions of oxirane with HCl, and ethylene glycol with HCl. The results of these calculations show that all of these reactions are exothermic with reasonable barrier heights.

## SYNTHESIS OF ULTRA-SMALL CYSTEINE-CAPPED GOLD NANOPARTICLES BY PH SWITCHING OF THE Au(I) CYSTEINE POLYMER

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Ultra-small gold nanoparticles are synthesized by pH jumping the Au(I) polymer. Gold nanoparticles have unique chemical and physical properties, than that of bulk gold, due to their increased surface area to volume ratio. Atoms on the surface of the material are more reactive than those at the center. This property can be harnessed for many applicational uses, such as diagnostics, electronics, and biological purposes. An example of biological use is in chemotherapy. Gold nanoparticles can effectively and specifically deliver chemotherapeutic agents in cancer patients.

A synthetic approach can be utilized for synthesizing gold nanoparticles by using the isoelectric point of the amino acid, cysteine. A Au(I) cysteine polymer that is dependent on pH promotes the growth of these AuNP. Utilizing strict pH exposure enables one to control the size of the nanomaterials synthesized. Ultra-small cysteine capped gold nanoparticles are characterized with UV-Vis spectroscopy and Scanning Transmission Electron Microscopy (STEM ) imaging. This project allows for a reliable way to synthesize gold nanoparticles so that they may aid as drug delivery vehicles with increased payload per kilogram (owing to their nano dimension) in targeted drug delivery for cancer therapy by enabling attachment of protein/molecule with specific activity.

We thank Dr. Mary Sajini Devadas for help with inorganic material studies, Stephen Blama for processing the STEM images, Landon Bechdel for providing assistance on computer editing software, funding from Towson University faculty start up, Fisher Endowed Chair Grant, Fisher General Endowment, and NSF MRI.

**FORMATION OF IMINE-LINKED COVALENT ORGANIC FRAMEWORKS**

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Bonding organic molecular building blocks by strong covalent bonds, such as imine linkages, to make crystals of 2- and 3-D extended structures, produces several new classes of porous materials called covalent organic frameworks (COFs). The construction of porous COFs has gained much attention due to the infinite applications for these species. COF possess low density, large surface area, and tunable pore size and structure. These features play a key role in maximizing the use of COFs for gas trapping, storing, and adsorption ability. This project proposes the synthesis of new classes of COF networks for basic research application. Primarily, we are interested in constructing regioregular, large COF and characterizing them by powder X-ray diffraction, solid-state spectroscopy, and thermogravimetric analysis with collaborative efforts with Northwestern.

Acknowledgements made to Millersville University Noonan Endowment Award, Student Grants for Research and Creative Activity and Neimeyer- Hodgson Research Grant for the funding of this project.

## GREEN NANOPARTICLE SYNTHESIS OF METALLIC AND BIMETALLIC NANOCLUSTERS

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Metal nanoparticles are of increasing interest in the fields of light harvesting, biology and environmental chemistry. The syntheses of nanoparticles are often expensive and require processes that are environmentally not conducive. In order, to look for benign methods and cost effective methods, plant extracts are often used for syntheses. Typically extracts from leaves and flowers are used. Flower-based extracts have been well documented as effective reducing agents in the green synthesis of nanoparticles. Some of the most important organic reducing agents in these flowers are polyphenols, flavonoids, and anthocyanins. In this study, flowers of the Eastern redbud tree were analyzed for their polyphenol, flavonoid, and anthocyanin content, as well as their electronic properties. The organic molecules were extracted using several solvents with varying polarities. The details of the extraction, synthesis and characterization are presented, as well as future plans to use these nanoparticles will be presented.

We acknowledge funding from the Fisher Endowed Chair grant and General Endowment Grant, Faculty Development Research Committee grant, Faculty start-up and NSF MRI for funding.

**CLASSICAL TOSYLATE/CHLORIDE LEAVING GROUP APPROACH CONFIRMS  
TETRAHEDRAL TRANSITION STATE FOR ADDITIONS TO TRIGONAL CARBON**

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The experimentally measured rates of solvolysis of 2-chloroethoxycarbonyl chloride (2-chloroethyl chloroformate, 3), 2-chloroethoxycarbonyl p-toluenesulfonate, and phenoxycarbonyl p-toluenesulfonate were followed at 25.0°C in various pure and binary aqueous-organic solvents with varying degrees of polarity. An analysis of the rate constants for 3, 5, and 6, using the two-term extended Grunwald-Winstein equation was determined, and comparisons are made to the previously published results for ethyl and phenyl chloroformate esters. For the three compounds, the  $k_{\text{OTS}}/k_{\text{Cl}}$  rate ratios and the Grunwald-Winstein  $\rho$  ratios favor the opinion of a dominant bimolecular carbonyl-addition pathway in the more nucleophilic solvents. In 3 and 5, in the strongly hydrogen-bonding, 70% and 50% HFIP mixtures, a side-by-side ionization mechanism is shown to occur.

Support was provided from an IDeA award from an NIH-NIGMS IDeA award (INBRE grant no. P20GM103446, DE-INBRE program), an NSF-EPSCoR award (Grant No. IIA-1301765, DE-EPSCoR), an NSF (DUE) S-STEM grant 1355554 (Cannon Scholar program), the NASA Delaware Space Grant Consortium grant program (NNX15AI19H), and the State of Delaware.

## EXAMINING DENITRIFICATION AGENT PERFORMANCE IN A CLOSED FRESHWATER SYSTEM BY ION CHROMATOGRAPHY WITH UV DETECTION

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Aquaculture, the farming of fish and other aquatic life, is a growing field which aims to overcome global problems of overfishing and disproportionate food distribution. This practice can be done in both freshwater and saltwater and in open bodies of water or within a controlled system. Recirculating Aquaculture Systems (RASs) are emerging as the primary model, eliminating problems of fish escape and reducing disease transfer, water usage, and space requirements. However, one main challenge to RASs is the continuous need for nitrate removal, as fish produce nitrogenous waste in high density tanks and elevated concentrations of nitrate can adversely affect fish health and quality. In natural systems, denitrifying bacteria aid in nitrate elimination, but RAS researchers are exploring alternative, more efficient nitrate removal methods. The goal of this project is to examine the performance of various denitrification agents in a closed freshwater system. For this research, three different non-biological agents were tested: Marineland Activated Carbon, API Nitrazorb, and Seachem Denitrate. Closed freshwater systems of varying known nitrate concentrations, 7.5 ppm, 20 ppm, and 150 ppm, were created using Sigma Aldrich Nitrate Standard for IC and 18 MΩ resistance purity water. After addition of varying amounts of agent, 17.5 g Seachem Denitrate, 6.1 g Marineland Activated Carbon, and 14.5 g API Nitrazorb, small aliquots were removed at recorded time points over two weeks until the system reach equilibrium. These aliquots were analyzed by ion chromatography with UV detection to determine the concentration of nitrate in the water at each time point. Examining the nitrate concentration over time provides an understanding of system dynamics for each agent tested. Future research will examine biological denitrification agents and the performance of various agents in a closed saltwater system.

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## DONOR/ACCEPTER VERSATILITY OF COBALT SELENIDE CLUSTERS IN CLUSTER - SMALL MOLECULE SIMPLE MIXTURE ACTIVE LAYER FOR FLEXIBLE SOLAR CELL APPLICATIONS

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Hybrid organic-inorganic bulk heterojunction (BHJ) photovoltaic devices are an attractive alternative to the common inorganic solar cells potential to offer higher efficiency and lower cost of manufacturing. Typically, these devices consist of a simple mixture of a donor (organic) and an acceptor (inorganic) material. However, the orthogonal properties of the two materials often cause them to phase separate, resulting in decreased device efficiencies. Our lab has previously developed an inorganic cluster  $\text{Co}_6\text{Se}_8(\text{Br}(\text{C}_4\text{H}_2\text{S})\text{P}(\text{Ph})_2)_6$  (1), which has shown promising results as an acceptor as well as reduced phase separation with poly-3-hexylthiophene (P3HT). Interestingly the HOMO/LUMO levels and broad absorption range of 1 suggest that it may also be an effective acceptor material.

The investigation of the donor/accepter versatility of 1 was performed with a small molecule acceptor. A new small molecule acceptor was synthesized in two steps in good yields. A synthetic intermediate, 2,7-Bis(5-formyl-2-thienyl)-9,9-dioctyl-9H-fluorene (2), was made using 2,2'-(9,9-Dioctyl-9H-fluorene-2,7-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (3) and 3-bromo-2-thiophenecarbaldehyde (4) under Suzuki conditions. Malononitrile (5) was added to the compound (1) to obtain a final product, 7,7'-(9,9-dioctyl-9H-fluorene-2,7-diyl) 5-[2-(2-thienyl methylene) malononitrile (6) (FTM). Structural characterization of the material was done with <sup>1</sup>HNMR, <sup>13</sup>CNMR, FT-IR and PXRD. Optical and electronic characterization was done using cyclic voltammetry, UV-vis and fluorescence spectroscopy. A series of fluorescence quenching experiments were performed to observe donor/acceptor interactions of P3HT:FTM, 1:FTM and a control mixture of P3HT :PCBM. The results indicate that FTM has the potential to be an effective and easily modifiable acceptor material. Fluorescence quenching experiments also indicated minor acceptor/donor interactions for the 1: FTM system however additional experiments need to be performed to fully understand these interactions. To compliment the solution state measurements, the solid-state morphologies of the systems were studied using atomic force microscopy.

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**DESIGN AND SYNTHESIS OF DIPEPTIDE-BORONIC ACID GELATORS**

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Supramolecular gels derived from biomolecules are of great interest due to their potential as biocompatible materials for sensing and targeted drug delivery applications. In particular, supramolecular gels derived from dipeptides are known to be suitable for a number of biomedical applications, including tissue engineering. While the gelating potential of dipeptides has been established, there remains an interest in developing new gelating systems and novel ways to incorporate pertinent targets into the gel network. The goal of our project was to synthesize a dipeptide gelator with a boronic acid handle that can be used to bind to different diol-containing biomolecules and drugs. We successfully synthesized a boronic acid dipeptide in three steps with 88% yield and fully characterized the product by  $^1\text{H}$  NMR. This dipeptide gels in ethanol/water mixtures under slightly acidic conditions. The resulting hydrogel is transparent and self-supporting. We are working now to characterize physical properties of this material and to test the boronic acid dipeptide's ability to bind to diol-containing drugs.

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# **STEM BUILD at UMBC**

## **Cohort 4 – BTP Trainee**

### **ABSTRACTS**

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## COMPARISON OF THE DIVERSITY OF THE BELLY BUTTON MICROBIOTA IN INDIVIDUALS FROM COASTAL AND LANDLOCKED REGIONS

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The human microbiota, or bacteria living on or within us, is critically important to human health and disease. We are particularly interested in how geographic features, such as proximity to water, impacts an individual's belly button microbiota. To do this, we analyzed previously collected data from Hulcr, et al. (2012) and hypothesized that there will be a greater biodiversity found in the microbiota of individuals residing in coastal regions. We grouped individuals based on their geographical residence, with coastal individual (n=45) being within 100 miles of a body of saltwater and a landlocked (n=60) city was anything greater than 100 miles from a coast. We then analyzed the data using Phinch metagenomics software focusing on the phylum taxonomic level.

Coastal individuals averaged 7.89 (+/- 3.77) phyla and landlocked individuals average 7.71 (+/- 3.03). We then analyzed our data with a two-sample T-test (p=0.599) and determined that there was not a difference in the diversity of the microbiota between individuals who resided in coastal or landlocked communities. From our data, we conclude that belly button microbial biodiversity was not directly linked to an individual's coastal proximity. In the future, we would like to research the same question but consider a narrower taxonomic classification in an attempt to find a more pronounced difference between the biodiversities in coastal and landlocked regions. This research is important because in the future we will be able to link certain health conditions to the microbes found in the locations that they are prevalent in.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5UL1GM118988, and 5RL5GM118987). We also want to thank Dr. Rob Dunn (NCSU) for providing the metagenomics data set used.

## DIVERSITY OF THE BELLY BUTTON MICROBIOTA IN ADULTS VS. MINORS IN THE STATE OF NORTH CAROLINA

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Everyone has a unique microbiota and knowing how this microbiota grows and changes can provide insight into our health and well being. We were interested in whether there any differences in the diversity of the human belly button microbiota between adults and minors. Our research is important because knowing how the microbiota changes with age could provide insight into certain diseases and conditions. We conducted this research using previously established metagenomics data (Hulcr, et al., 2012). We focused on individuals residing in North Carolina considering the bacterial taxonomic level of class. We hypothesized that in the State of North Carolina, the microbiota in the bellybutton of an adult individual, (ages 21-74 (n=86)), will on average have a greater diversity of bacterial classes, than the belly button of a minor (ages 0-18 (n=54)). We analyzed the data using Phinch metagenomics software and discovered that over 70 unique classes inhabit people's belly buttons. We found 54 different classes of bacteria residing in the belly buttons of minors and 52 different classes of bacteria living in adults. For adults, the average number of bacterial classes inhabiting each person's' belly button was 13 (+/- 8.33) and for minors, the average number was 14.81 (+/-6.19). Our data shows that diversity of microbiota between adults and minors was negligible. Since we could not discern how the differences in the number of classes we cannot confirm our hypothesis. This could mean our belly button microbiota does not change over time. Limitations of our study include a relatively small and uneven sample size, and not controlling for other factors that could affect our results. Future studies would be to expand and diversify the participant population and analyze more aspects of the metadata such as gender and wash frequency instead of only age.

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## EFFECT OF GEOGRAPHIC REGION ON DIVERSITY OF MICROBIOTA IN THE HUMAN BELLY BUTTON

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The environment affects microbial growth negatively and positively due to the chemical and physical characteristics of their surroundings. Thus, we wanted to determine if there was a difference in the diversity of belly button microbiota between individuals who live either above or below the 37° North Latitude line. We hypothesized that individuals who live in the south will have a more diverse belly button microbiota than those living in the north. In order to verify this, we utilized previously collected metagenomic data that sampled the belly button microbiota of volunteers at two different locations. In addition to this, we utilized the results of a questionnaire regarding their gender, age, belly button anatomy, city of residence, and wash frequency (Hulcr, et al, 2012). We identified individuals who live above 37 ° North Latitude (n=51) and those who live below (n=64) and used the genus taxonomic level to compare the diversity of their belly button microbiota. From this, we found that there were 239 different genera from individuals living in the north and 397 different genera from individuals living in the south. We then conducted an independent two-sample t-test and determined that there was a statistically significant difference in the number of bacterial genera in individuals living in the north compared to the south, which supported our hypothesis. Since the south has a greater diversity of microbiota, there may be more beneficial microbes that exist within this region compared to the north. In the future, a study could be conducted to determine the effect of a southern microbe on human health compared to that of a northern microbe to determine if southern microbes actually are more beneficial.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5UL1GM118988, and 5RL5GM118987). We would like to thank Dr. Rob Dunn (NCSU) for providing the metagenomics data set.



## DETERMINING THE DOMINANT PHYLA OF THE HUMAN BELLY BUTTON IN THE UNITED STATES

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There is growing interest in learning how the human microbiota, or the residing microorganisms in our body, facilitate certain functions such as digestion, nutrient absorption, and disease prevention. In our study of the human microbiome, we used previously collected metagenomic data from Hult, et al. (2012) that investigated the diversity of microorganisms living in the human belly button. This data was evaluated to better understand if there is any correlation between an individual's geographic location and the bacteria present within their belly button. We hypothesized that the dominant phyla of bacteria would vary based on the four established geographical regions within the U.S. (East, West, Central, and South) and analyzed the data using Phinch metagenomics software. In the Eastern and Western regions, with 41 and 29 participants respectively, the most prominent bacterial phyla found was Actinobacteria. The bacterial phyla Firmicutes was found to be most prevalent in both the Central and Southern regions, which had 12 and 179 participants respectively. We determined the four most prominent bacterial phyla per geographical area: Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. We did not determine any statistical differences between the phyla in each region, demonstrating that there were consistent bacterial phyla inhabiting belly buttons of individuals throughout the U.S.. In the future, rather than limiting our samples to the United States, we plan to branch out to other nations to increase our understanding of how bacterial phyla in belly buttons has an impact worldwide.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5UL1GM118988, and 5RL5GM118987). We also express gratitude to Dr. Rob Dunn (NCSU) for providing his data set.

## THE EFFECT OF AGE ON THE DIVERSITY OF THE MICROBIOTA IN THE HUMAN BELLY BUTTON

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As humans, we all have bacteria living on and within us that play an important role in how we function. Although these microbes are essential to our health and well-being, we know very little about all of the different species within us. Previous research conducted by Hulcr, et al. (2012), explored the diversity of the microbiota, or community of microorganisms, living in the human belly button. From this, they sequenced the 16s rRNA and used metagenomics analysis to investigate the differences in the microbial populations between individuals.

To further explore Hulcr and colleague's data, we researched whether the variable of age affects the diversity in the human belly button's microbiota. In this experiment we hypothesized that the youngest group would harbor a larger diversity in their microbial population than the middle aged or older groups. In order to determine the microbial diversity, we observed averages of each phyla in each age group. If the averages taken of each individual phyla were close together in value, then there was a high microbial diversity. If prominent phyla groups could be determined and the averages were farther apart in value, then there was a lower microbial diversity.

To answer our question, we divided participants into the following age categories: 0-22 years(n=79), 23-44 years(n=119), and 45-66 years(n=45). We then used the online metagenomics software, Phinch.org, to analyze the data provided by Hulcr, et al., focusing on the phylum taxonomic level. For the 0-22 group, we observed no prominent bacterial phyla. For ages 23-44, three prominent bacterial phyla were observed: Elusimicrobia, Thermicutes, and Acidobacteria. Ages 45-66 had prominent major phyla, and other phyla were not common. Our data supported our hypothesis that as humans get older, the diversity of the microbiota in their belly button decreases. This information will help us better understand how microbial diversity affects the body and its function.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5UL1GM118988, and 5RL5GM118987). We also want to thank Dr. Rob Dunn (NCSU) for providing the metagenomics data set used.

## THE EFFECTS OF BATHING FREQUENCY ON MICROBIAL DIVERSITY OF THE HUMAN BELLY BUTTON

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As humans, we are dependent on microbes for carrying out many daily life processes, for example, digestion. Hulcr, et al. (2012) collected belly button swabs from volunteers at two locations, a Science Convention and a museum both located in North Carolina, to study the diversity of microbial populations in the human belly button. They sequenced the 16s rRNA gene and collected metadata from each participant including the frequency in which they bathed (or washed). We used this data to ask the question: does the frequency of body washing affect the diversity of bacteria in the human belly button? We hypothesized that as a person's wash frequency decreases, microbial phyla would become more diverse. We divided the data collected into three different groups: washing 0-1 (n=58), 2-4 (n=30), and 5-7 (n=52) times per week. We focused on the three most prevalent microbial phyla: Firmicutes, Actinobacteria, and Bacteroidetes. We then performed 18 two-sample unpaired t-tests to determine if there was a significant difference between the phyla prevalence both within and between wash frequency groups. Our results demonstrated that there is not a significant difference between each phylum individually across the wash frequencies. However, there is a statistically significant difference in the diversity of phyla within each wash frequency, showing that individuals who wash only 0-1 times per week had significantly more Actinobacteria than the 2-4 and 5-7 wash groups for example. This data demonstrates that while the diversity of the bacterial phyla does not alter based on wash frequency, there are differences in the population size of bacterial phyla within wash frequencies. In summary, we determined that our hypothesis could not be supported, and the experiment needs to be repeated with a more regulated sample in order to ensure the accuracy of the findings.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 5TL4GM118989, 5UL1GM118988, and 5RL5GM118987). We would also like to thank Dr. Rob Dunn (NCSU) for providing the metagenomics data set.

## THE BIODIVERSITY OF BELLY BUTTON MICROBIOTA ON THE WEST COAST VERSUS EAST COAST

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While considering the implications of the microbiota on human health we investigated whether the geographical location of individuals would affect the makeup of the microorganisms inhabiting their navels. The interconnected relationship between microorganisms and people makes studying the microbiota crucial to reaching a greater understanding of their effects on our health and wellbeing. We focused on the phylum taxonomic level and hypothesized that the phyla of microorganisms inhabiting belly buttons residing in the east coast would be more diverse than that of the west.

In order to test our hypothesis we analyzed a previously collected metagenomic data set (Hulcr, et al., 2012) using Phinch metagenomics analysis software. We categorized two test groups consisting of individuals residing in California, Oregon, and Washington (n = 21) and inhabitants of North Carolina, Maryland, and Virginia (n=181) in the other. The data revealed that there was overall more diversity in the microbial phyla of individuals living on the east coast in contrast to the west coast. There were 31 phyla represented in individuals from the east coast, while there were 21 phyla represented in individuals from the west coast. Our p value of 0.0027 shows a 95% significance which supports our hypothesis. The disparity between sample sizes is a limitation of our study and in future studies we would collect more representative samples with equivalent sample sizes. In the future, we would also consider looking into the effects of wind currents and humidity as a possible explanation for the varying phyla on each coast.

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## THE EFFECT OF AGING ON THE MICROBIAL DIVERSITY OF THE HUMAN BELLY BUTTON

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There is growing interest in the human microbiota (the microscopic organisms which inhabit human beings) because of their role in the functionality and health of the human body. Previous metagenomic analysis based research conducted by Hulcr, et al. (2012) identified the diversity of microorganisms inhabiting the belly buttons of 273 test subjects. We used this data to investigate the impact of age on the diversity of the human belly button microbiota. The initial hypothesis was that as the age of an individual increased, the diversity of the microbiota inhabiting their belly button would also increase. This hypothesis specifically pertained to increased diversity visible on the taxonomic level of family.

We analyzed our data using Phinch metagenomics software and grouped individuals based on the following age ranges: 0-10, 11-20, 21-30, 31-40, 41- 50, and 51-60. Each group consisted of 35, 38, 55, 51, 35, and 21 individuals respectively. Our results demonstrated that the most diverse communities of microbiota occurred in individuals of the age group 0-10, which had an average of 45 families per individual, which is significantly more than the averages of the other age ranges. The second highest average was only 32.7 in the 31-40 age range.

Based on our results we concluded that our original hypothesis was refuted, and that the diversity of an individual's belly button microbial community actually decreases as they age. However, we also determined that further research would be necessary to support said conclusion due to limitations such as a limited number of samples for the age ranges and a lack of direct experimentation. Future directions for this research could include determining if certain physiological changes that are associated with the aging of the skin have an impact on the biodiversity of human belly button microbiota.

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## THE CORRELATION BETWEEN MICROBIAL FAMILIES IN THE BELLY BUTTON AND AGE

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The microbiota is a group of bacteria from a specific region and the microbiome is the DNA from these bacteria. Examining microbiotas could reflect the level of health of individuals as well as give insight to the microorganisms associated with certain age groups or environments. The data presented in this experiment came from previous research (Hulcr, et al., 2012) which explored the diversity of the human belly button microbiota. We were interested on the impact that age had on the diversity of the belly button microbiota. We hypothesized that diversity of the microbiota in the belly button decreases as age increases. Using Phinch metagenomics analysis software and data from Hulcr, et al, we divided the samples into four groups based on the age: 0-25 (n=98), 26-50 (n=116), 51-74 (n=32), and 74 years and older (n=29). We then determined the number of bacterial families in each group using the bubble charts provided by Phinch. We compiled these totals into a bar graph to determine the number of bacterial families. The 0-25 age group had 156 families of microorganisms, making it the most diverse population. The least diverse population was the 51-75 age group, which had 64 families. There were 148 families in the 26-50 age group, and 78 families in the 74+ age group. Our data somewhat supports our hypothesis that as one ages, the diversity of their belly button microbiota decreases. This suggests that there may be a correlation between age and diversity of the human microbiota. The limitations of the data were that the number of individuals per age group was disproportionate and included more younger individuals. Future directions would be to analyze the bacterial families that appear in one age group but diminishes in another age group.

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