

22nd Annual Undergraduate Research Symposium in the Chemical and Biological Sciences



Saturday, October 19, 2019



UMBC



**22nd 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*



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Schedule of Events

Time	Event
8:00 am	SYMPOSIUM CHECK-IN & ON-SITE REGISTRATION <i>Lobby, University Center, 3rd Floor</i>
8:00 am	LIGHT CONTINENTAL BREAKFAST <i>UC 312, University Center, 3rd Floor</i>
9:00 am	OPENING REMARKS & WELCOME ADDRESS 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	MORNING POSTER SESSION <i>Ballroom, University Center, 3rd Floor</i>
10:30 am	WORKSHOPS
	<div> Exploring Careers in Science: What does your future hold? Ms. Susan Hindle, Career Services, UMBC UC 310, University Center, 3rd Floor </div> <div> A Very, Very Short Introduction to Ethics for Scientists Mr. James Thomas, Department of Philosophy, UMBC CASTLE, UC 115D, University Center, 1st Floor </div>
11:45 am	BUFFET LUNCH <i>(gratis for registered guests with symposium name badge)</i> <i>The Commons – Main Street</i>
12:45 pm – 2:45 pm	AFTERNOON POSTER SESSION <i>Ballroom, University Center, 3rd Floor</i>
1:30 pm	WORKSHOPS
	<div> Beyond the academic journal: The Importance of Sharing Science with the Public, and How to Do It Ms. Sarah Hansen, OIA/CNMS, UMBC UC 310, University Center, 3rd Floor </div> <div> A Very, Very Short Introduction to Ethics for Scientists Mr. James Thomas, Department of Philosophy, UMBC CASTLE, UC 115D, University Center, 1st Floor </div>
3:00 pm	PLENARY TALK “The Genetics of Aging” Dr. Jeff Leips, Professor, Department of Biological Sciences, University of Maryland Baltimore County <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>
4:00 pm	AWARDS PRESENTATION <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>

Workshops:

Exploring Careers in Science: What does your future hold?

University Center, 3rd Floor, Room 310

So you like science and are passionate about research. You've even spent the last summer conducting research and advancing your research skills – and to top it off, you are presenting your first poster to showcase your amazing contribution to science! But what direction will this lead you upon graduation? Where can you use and advance your education, skills, and passion for science that will make an impact? Attend this session to learn about the array of avenues and opportunities to consider when you complete your undergraduate degree.



Susan Hindle is the Assistant Director, Internships & Employment for the College of Natural and Mathematical Science at UMBC. Susan has over 20 years' experience working with students and alumni in all phases of the career development process. Prior to coming to UMBC in 2013, Susan worked as a Career Advisor for both the Clark School of Engineering at the University of Maryland and the Johns Hopkins University. Susan has her bachelor's degree in Elementary Education from the University of Maryland, College Park and her master's degree in Clinical Counseling from Johns Hopkins University.

A Very, Very Short Introduction to Ethics for Scientists

University Center, 1st 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 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.

Beyond the academic journal: The Importance of Sharing Science with the Public, and How to Do It

University Center, 3rd Floor, Room 310

All scientists have a duty to communicate their work—it's the final, crucial step in the scientific method, after all. First, you must learn how to communicate your work effectively within your field. Then, it's time to go further. In this workshop, we'll discuss why it's important to share your research beyond colleagues in your specialty, what you need to think about when sharing your work with a broad range of audiences, and different platforms that will help you communicate your research beyond scientific journals and academic conferences. As emerging scientists, it's particularly important that you make the effort to communicate your work—it will set you on a path to success in your scientific career, and also help ensure that society reaps the benefits of your discoveries.



Sarah Hansen has been a STEM communications manager at UMBC since 2016. Prior to that, she was the communications associate for the Hussman Institute for Autism and the science communications intern at the Smithsonian Environmental Research Center. Sarah served on the D.C. Science Writers Association board from 2014 - 2017. She earned an M.S. in biological sciences from UMBC, and an M.A.T. in secondary education and B.S. in biological engineering from Cornell University. Between Cornell and UMBC, Sarah taught middle school science at the Holton-Arms School in Bethesda, Maryland. When she's not communicating science, Sarah enjoys trail running, singing with the Baltimore Choral Arts Society, gardening, cooking, and traveling.

Plenary Talk

The Genetics of Aging

Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030

Senescence, the age-related decline in physiological traits, is a common feature in most organisms and constitutes a major health risk for the aged human population. Although senescence is nearly universal, in natural populations the rate at which individuals decline in health related traits varies a great deal. Some of the variation is due to the differences in environmental conditions experienced by individuals, such as differences in diet, exercise, and exposure to toxins. However, studies on a number of different species including humans, mice, roundworms, yeast and flies conclude that genetic differences among individuals also play a significant role in producing variation in rates of senescence. While there is a clear genetic component, the actual genes that contribute to the natural variation in rates of senescence are largely unknown. In this talk I will present data from our research focused on identifying the genes that underlie natural variation in the age-specific ability to clear infection and age-specific measures of physical performance like speed and endurance. We use the fruit fly, *Drosophila melanogaster* as they are ideal model systems for research on aging for several reasons. Fruit flies have a short life span, short generation times and are prolific breeders, allowing us to carry out large scale experiments to detect subtle genetic influences on senescing traits. The genome is also completely sequenced and there are a wealth of tools available for genetic manipulation that allow us to validate genetic effects. Finally, flies share over 60% of their genes with humans, and so our work should provide insight into the genetic basis of senescence in humans. Our hope is that our research will not only elucidate the mechanisms that give rise to senescence, but also to nominate candidate genes and signaling pathways that can be targeted to treat or reduce the debilitating effects that accompany aging, and so increase the healthspan of our aging population.



Dr. Jeff Leips is a professor in the Department of Biological Sciences at UMBC. His research uses genomic approaches to elucidate the genetic basis of aging and life span. Specifically his research group is using genome wide association mapping to identify genes that contribute to age-related decline in immune function, physical performance traits like speed, strength and endurance, and reproduction.

Jeff earned his BA, and PhD, in Biological Sciences at Florida State University, with research focused on understanding how evolutionary adaptations influenced population dynamics. After receiving his PhD in 1997, he joined the laboratory of Dr. Trudy Mackay at North Carolina State University as an NIH NRSA Postdoctoral Fellow. His research at NC State was in the field of quantitative genetics and focused on mapping genes contributing to life span, using the fruit fly, *Drosophila melanogaster*, as a model genetic system. He started at UMBC in 2001.

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 Judge's 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 the awards coordinator.

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.

Thank you for volunteering today!

Biological Sciences

ABSTRACTS

Morning Session

*Page Numbers of the Abstracts Serve as
Poster Numbers*

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 2019 Undergraduate Research Symposium in the Chemical and Biological Sciences.

IMPROVED ANALYSIS OF ELECTROENCEPHALOGRAM (EEG) DATA FOR THE COMPARISON OF 432-HZ AND 440-HZ TUNING ON HUMAN BRAINWAVES

Romuald Kenmegne¹, Qi Lu¹

¹Optical Science Center for Applied Research, Delaware State University, College Rd, Dover, DE 19904

In this study, we recorded the brainwaves on a group of male and female volunteers using the electroencephalogram (EEG) function on a BIOPAC interface. The subjects were between 18 and 24 years of age. The EEG was recorded for twenty seconds when pure sine waves of 432 or 440 Hz and a music chord played by guitar tuned at 432 Hz or 440 Hz were played subsequently to each subject. Four types of brainwaves including alpha, beta, delta and theta waves were recorded. For each segment of recording, the average frequency for each wave was obtained by taking five cycles (as opposed to one cycle in past studies) at an approximate interval of five seconds (as opposed to random pick in past studies) for three times. This improved method of analysis will give us a more accurate and objective evaluation of how human brains respond to 432-Hz and 440-Hz music tuning differently. The data collection procedures involving human have been approved by the Internal Review Board (IRB) at Delaware State University.

We acknowledge the funding support from the National Aeronautics and Space Administration (NASA MIRO grant # NNX15AP84A) as well as the resources from the Optical Science Center for Applied Research (OSCAR).

A MACHINE LEARNING APPROACH TO PREDICTING THE POSSIBILITY OF SEIZURES DURING AN ADMISSION FOR INTRACEREBRAL HEMORRHAGE

Marvellous Oke¹, Jason Davies²

¹Department of Psychology, Morgan State University, 1700 E. Cold Spring Lane Baltimore, Maryland

²Department of Neurosurgery, SUNY University at Buffalo, 12 Capen Hall Buffalo, NY 14260

Intracerebral hemorrhage (ICH) usually occurs after a traumatic brain injury, which is known as a TBI or a spontaneous event. Seizures are a common complication after ICH, and they affect long-term functional outcomes for patients. Seizures following ICH contribute significantly to patients' length of stay, which increases healthcare system cost. Our research question is using clinical risk factors data, who will develop long-term seizures during an intensive care unit (ICU) admission for Intracerebral Hemorrhage? We analyzed our patient cohort from the Medical Information Mart for Intensive Care dataset, a single institution dataset consisting of medical records on nearly 40,000 intensive care unit stays (ICU). Using a machine learning model approach, we extracted all ICH patients during their ICU stay using the International Classification Diagnosis code. We identified and extracted patient-level risk factors based on prior literature to generate various random forest prediction models. We extracted n=1307 patients who had ICH; 70% (916) for our train data and 30% (391) for our test data. Based on the Receiver Operating Characteristics (ROC) curve comparison on four different model (SMOTE, ROSE, Up-Sampling and Baseline), the Area Under the Curve was used to identify how well these models were able to predict the positive seizure patients. Respectively, each model performed at 0.5921, 0.5513, 0.5704, 0.6153. Overall, the significant prediction model was the Baseline model (AUC=0.6153) and clinical risk factors of seizures were estimated from this model. Based on the baseline model, indicators for predicting seizures are age, race(white), anemia, sepsis, race(black), race (unknown), gender(male), anticoagulant/antibiotic usage, ethnicity (Latino); with a Mean Decrease Accuracy of 5.8%, 3.9%, 3.0%, 2.0%, 1.6%, 1.5%, 0.8%, 0.7%, 0.5% respectively. Sepsis, anemia and long-term anticoagulant/antibiotic use signify issues with the chemistry of blood for ICH patients who eventually develop long-term seizures. Future tasks include refinement of model.

I would like to thank SUNY University at Buffalo, The National Institutes of Health: U.S. National Library of Medicine Grant #3T15LM012495-02S3, The NIH-BUILD ASCEND Grant #UL1GM118973 and Morgan State University.

LETTING THE LAXKAT OUT OF THE BAG: PACKAGING, SIMULATION, AND NEUROIMAGING DATA ANALYSIS FOR A POWERFUL KERNEL TEST

Jeremy Rubin¹, Simon Vandekar², Lior Rennert³, Mackenzie Edmonson⁴, Russell Shinohara⁵

¹Department of Mathematics and Statistics, University of Maryland, Baltimore County, 1000
Hilltop Cir, Baltimore, MD 21250

²Department of Biostatistics, Vanderbilt University, 2201 West End Ave, Nashville, TN 37235

³Department of Public Health Sciences, Clemson University, 105 Sikes Hall, Clemson, SC
29634

⁴Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania
Perelman School of Medicine, Philadelphia, PA 19104

⁵Penn Statistics in Imaging Visualization Center, Department of Biostatistics, Epidemiology, and
Informatics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

Biomedical research areas including genomics and neuroimaging often have a number of independent variables that is much greater than the sample size. The sequence kernel association test (KAT) and sum of scores tests can offer improved power in this feature setting; however, power is significantly reduced in the presence of a large number of unassociated independent variables. We propose the Linear Maximal KAT (LaxKAT), which maximizes the KAT test statistic over a subspace of linear kernels to increase power. A permutation testing scheme is used to estimate the null distribution of the LaxKAT statistic and perform hypothesis testing. Calculation of the LaxKAT was implemented using a combination of the R and C++ programming languages. We find that this test has power and controls the type I error for different sample sizes and signal distributions. It is expected that the LaxKAT will have competitive power relative to other high-dimensional testing procedures when applied to detect predictors of memory impairment in cortical thickness measurements from the Alzheimer's Disease Neuroimaging Initiative study (ADNI).

This investigation was sponsored by NIH/NIGMS MARC U*STAR T34 HHS 00026 National Research Service Award to UMBC, NIH/NINDS R01NS085211 and NIH/NIMH R01MH112847

NUMERICAL SIMULATION OF CALCIUM WAVES IN A CARDIOMYOCYTE MODELED BY A SYSTEM OF SEVEN COUPLED PARTIAL DIFFERENTIAL EQUATIONS

Gerson Kroiz¹, Carlos Barajas¹, Bradford E. Peercy¹, Matthias K. Gobbert¹

¹Department of Mathematics and Statistics, University of Maryland, Baltimore County (UMBC),
1000 Hilltop Circle, Baltimore MD, 21250

Cardiac arrhythmias affect millions of adults in the U.S. each year. This irregularity in the beating of the heart is often caused by dysregulation of calcium in cardiac muscle cells known as cardiomyocytes. Cardiomyocytes function through the interplay between electrical excitation, calcium signaling via calcium induced calcium release (CICR), and mechanical contraction. This project focuses on a single cardiac muscle cell and uses a mathematical model to represent the electrical excitation, calcium signaling, and mechanical contraction components of a cardiomyocyte. A system of seven coupled, non-linear, time-dependent, partial differential equations (PDEs), which model physiological variables in a cardiac cell, link the processes of cardiomyocytes. A special-purpose code in C with MPI for parallelization on distributed-memory clusters uses a finite volume discretization in space and sophisticated time-stepping by a family of NDFk formulas. Through parameter studies for each component system at a time, we create a set of values for critical parameters that connect the calcium store in the sarcoplasmic reticulum (SR), the effect of electrical excitation, and mechanical contraction in a physiologically reasonable manner. Within these studies, numerical simulations model what impact each of the selected parameters has on the integrated components of a cardiomyocyte for 1,000 ms. This method results in a set of values, for which the overall model shows wave-like behavior. With the set of values determined through the parameter studies, we study the model for a longer duration of time, 5,000 ms. These longer simulations indicate that the calcium store in the SR can be depleted and become the limiting factor in sustaining wave-like behavior in the cell. Additional research in this field is necessary for a better understanding of the fine line between cardiac regulation and dysregulation.

The hardware in the UMBC High Performance Computing Facility (HPCF) is supported by the U.S. National Science Foundation through the MRI program (grant nos. CNS-0821258, CNS-1228778, and OAC-1726023) and the SCREMS program (grant no. DMS-0821311), with additional substantial support from the University of Maryland, Baltimore County (UMBC). See hpcf.umbc.edu for more information on HPCF and the projects using its resources. Author Gerson C. Kroiz was supported by UMBC as URA Scholar. Co-author Carlos Barajas was supported by UMBC as HPCF RA.

PHYSIOLOGICAL CHARACTERIZATION OF ISOLATED STRAINS OF *COMAMONAS TERRIGENA*

Gloria Martinez¹, Kevin E. Shuman¹

¹Department Of Biology, Wesley College, 120 North State Street, Dover, De 19901

Comamonas terrigena was first isolated in 1985 from phenol-contaminated soil in Slovakia. It is a Gram negative, rod-shaped bacterium with no pigmentation. Two independently isolated strains of *C. terrigena* were isolated from the St. Jones River in Silver Lake Park (Dover, DE). The strains were identified using GEN III Biolog plates which characterized the ability of isolates to metabolize various carbon sources and compared this physiological profile against a database of known bacteria. The optimal growth characteristics of the two isolated *C. terrigena* strains were determined in tryptic soy broth media. Both strains were found to be mesophilic with the best growth observed between 27.5°C to 30.0°C. Both strains grew best under neutrophilic conditions (pH=7.0). Neither strain grew with the addition of NaCl. In the presence of various concentrations of sucrose, *C. terrigena* was found to grow best with a sucrose concentration of 0.5%(w/v). This increased the available carbon without altering the osmolarity of the media too significantly. Although sucrose can help increase growth, too much sucrose can lead to dehydration of the cell. By changing and testing different environmental conditions, the optimal growth conditions for the two strains of *C. terrigena* can be determined. Once identified, potential industrial applications for the strains could be determined.

This research was made possible by the National Science Foundation EPSCoR Grant No. 1757353 and the State of Delaware.

IDENTIFYING BACTERIAL ISOLATES ASSOCIATED WITH MICROCYSTIS BLOOMS COLLECTED FROM LAKE TAIHU

Isabella Becker¹, Morgan Steffen¹

¹James Madison University, 800 S Main St, Harrisonburg, VA 22807

Cyanobacteria can form harmful algal blooms, or cHABs, some of which produce toxins. A common genus of cyanobacteria that forms cHABs is Microcystis. These cHABs can bloom all over the world, with the exception for Antarctica, and have the potential to cause negative environmental, financial, and health impacts. Lake Taihu, in China, serves as over two million people's primary water source - which is contaminated by yearlong Microcystis blooms. In the summer of 2018, samples were taken from this lake and isolated in the lab to identify bacteria associated with the bloom. After individual colonies were isolated and gram stained to confirm purity, their DNA was extracted, amplified, and sent off for sequencing. Their 16s ribosomal region was amplified and BLAST was utilized to identify each of the 62 isolates. Biochemical tests were performed on the strains to determine urealytic and nitrogen fixing capabilities. Then plate reader assays were observed with each isolate incubated in conjunction with axenic Microcystis in order to test for either algicidal or growth promoting effects on Microcystis. In the future, these isolates will be used for experiments that will test for nutrient exchange between the bacterial isolates and Microcystis.

I would like to thank Dr. Steffen and Dr. Wurch for being the primary investigators on this project the biology department at James Madison University, all the past and present graduate and undergraduate students in the Steffen and Wurch labs for all of their contributions and assistance, the Eurofins genomics lab for sequencing the isolates, and the National Science Foundation for funding this project.

INVESTIGATING TARDIGRADE METABOLIC AND MORPHOLOGICAL REACTIONS TO OSMOTIC STRESSES

Kory Wolfe¹, Elaine Farkas¹

¹Department of Chemistry and Physics, Mansfield University, 31 S Academy Street, Mansfield, PA 16933

Tardigrades, classified in the phylum Tardigrada, are small, semi-terrestrial animals that have been discovered worldwide, inhabiting some of the most unstable ecosystems. Common semi-terrestrial environments that tardigrades can be found in are soil and moss cushions that can hold water necessary for tardigrade activity. Even though these aquatic animals can be found in many types of environments, they all have similar internal structures: three malpighian tube-like organs that are believed to be involved in osmoregulation, the process of maintaining a constant osmotic pressure in the cellular fluids by controlling the water and salt concentrations. In order to adapt and survive environmental changes, tardigrades enter a phase called cryptobiosis. In this state, metabolic activity is reduced to an undetectable level. This state has been broken down into four subcategories based on the stressor, one of them being osmobiosis caused by osmotic changes in the aquatic environment. Osmobiosis is the least researched type of cryptobiosis and it is unknown how universal this phenomena really is among tardigrade species. The eutardigrade *Hypsibius dujardini* was selected to be used in this investigation of tardigrade response to osmotic stress. Each colony of tardigrades was introduced to one of three solutions (sodium chloride, cupric chloride, and sucrose) at four different osmolalities (0 mOsm/kg, 200 mOsm/kg, 400 mOsm/kg, and 800 mOsm/kg). The colonies were observed using optical microscopy and lipid quantification to determine the effects of the osmotic stress on metabolism and viability.

I would like to acknowledge Mansfield University's Professional Development Committee for funding this research, as well as my colleague, Katelyn Davis, for collaborating on preliminary studies.

THE CATARACT-ASSOCIATED RNA-BINDING PROTEIN CELF1 REGULATES HSPB8, A KEY MOLECULAR CHAPERONE, IN LENS DEVELOPMENT

Sabrina Luther¹, Sandeep Aryal², Bailey Weatherbee², John Rogowsky², Salil A. Lachke²

¹Department of Biological Sciences, University of Delaware, Newark, DE, 19716, USA

²Department of Biological Sciences, Center for Bioinformatics & Computational Biology, University of Delaware, Newark, DE, 19716, USA

The ocular lens is a transparent tissue that focuses light on the retina for high resolution vision. Lens opacification, termed cataract, is a major cause of blindness worldwide and is classified as congenital or age-related. While less common, nearly half of congenital cataract cases are caused by underlying genetic or developmental defects.

To identify high-priority candidate genes associated with lens developmental defects and cataractogenesis, our lab developed a novel bioinformatics-based approach called iSyTE. Using iSyTE we identified *Celf1* (CUGBP Elav-like family member 1) as a potential regulator of lens development. *Celf1* encodes an RNA-binding protein (RBP) that functions in distinct post-transcriptional gene regulatory events such as alternative splicing, mRNA stability, and translational control. We recently reported that *Celf1* lens-specific conditional knockout mice (*Celf1*^{CKO/lacZKI}) exhibit early-onset cataract.

To understand the pathobiology of cataract in *Celf1*-deficient lenses, we performed transcriptome and proteome-based screening. Two transcriptome screens (microarray and RNA-sequencing) as well as a proteome-based screen independently identified a key heat-shock factor, *Hspb8*, to be abnormally overexpressed at both mRNA and protein levels in *Celf1*^{CKO/lacZKI} lenses. *Hspb8* harbors a conserved alpha-crystallin domain and functions as a molecular chaperone, binding partially unfolded polypeptides to maintain them in a refolding-competent state. These new findings are significant, identifying *Hspb8* as a new factor linked to a protein aggregation disease such as cataract.

We performed RT-qPCR and immunofluorescence analysis of *Celf1*-deficient lenses to validate *Hspb8* over-expression. These data provide independent evidence that *Celf1* deficiency causes abnormal *Hspb8* overexpression, in agreement with the high-throughput screens. Further, RNA-immunoprecipitation and cross-linked immunoprecipitation assays demonstrate that *Celf1* protein directly binds *Hspb8* mRNA, suggesting that *Celf1* may post-transcriptionally control *Hspb8* expression in lens development. Because *Hspb8* is involved in multiple cellular processes ranging from cytoskeletal maintenance to repairing damaged proteins, these data provide new insights into the cataract pathology of *Celf1*-deficient lenses.

This work was supported by NIH Grant R01EY021505 (to SAL) and the Jeremie M. Axe, M.D. Summer Scholars Award.

EXPLORING FITNESS AWARENESS MOBILE APPLICATIONS WITH FORMER ATHLETES

Alexander Jean-François¹, Scott McCrickard², Andrey Esakia², Deborah Good³

¹Department of Biology, Wesley College, 120 N State St, Dover, DE 19901

²Computer Science and Center for HCI, Virginia Polytechnic Institute and State University, 925 Prices Fork Road Blacksburg, VA 24061

³Human Nutrition, Foods, and Exercise, Virginia Polytechnic Institute and State University, 1981 Kraft Drive 1020 ILSB Blacksburg, VA 24061

Physical inactivity is a major public health concern around the world. It is the fourth leading risk factor for global mortality, contributing to 6% of deaths globally and approximately 3.2 million deaths each year. It is associated with increased risk of obesity, diabetes, cardiovascular disease, hypertension, some types of cancers, and other disabling conditions. Reduced physical activity and corresponding weight gain and other health issues is notably common in former athletes, as their physical activity decreases after they stop competing, but they do not adjust their food consumption or identify alternative exercise plans. This research is rooted in the FitEx physical activity intervention program that leverages group dynamics to encourage healthy behaviors, and in the FitAware mobile application platform that facilitates increased awareness of individual and team fitness goals and behaviors. The goals of the study are to explore the impacts of the newest version of FitAware in an abbreviated FitEx session, comparing the performance, reactions, and change in behaviors of former athletes and non-athletes. The study examined use of the FitAware mobile application as part of a 3-week FitEx-style physical activity program. 12 people took part in the program, divided into 3 groups: 1 that included former athletes and 2 that included non-athletes. Study results suggest that this type of intervention correlates with increased use among former athletes compared to non-athletes. Fitness programs like FitEx and mobile applications like FitAware seem to be a good match for former athletes due to their competitive nature.

This project was supported by the Virginia Polytechnic Institute and State University Department of Human Nutrition, Foods, and Exercise and the Translational Obesity Undergraduate Research (TOUR) Scholars program with a R25 grant (#R25DK112735) from the National Institute of Health.

PRE- AND POST-TUNNEL COMPARISON OF THE NITROGEN DYNAMICS OF THE ANACOSTIA RIVER, D.C.

Tyler Mitchell¹, Rebekah Officer¹, Gaurav Arora¹, Caroline Solomon¹

¹Department of Science, Technology, and Mathematics, Gallaudet University, 800 Florida NE Ave, Washington, DC 20002

The Anacostia River located in Washington, D.C is one of the most polluted rivers in the United States. To address environmental concerns, DC Water build a storage tunnel beneath the Anacostia River that opened in March 2018 to divert sewage and stormwater from entering the river. To evaluate the effectiveness of the tunnel and examine relationships between flow conditions, nutrient dynamics and microbial communities, samples for nitrogen concentrations were collected over the course of five years (2013-2017) prior and two years after (2018-2019) tunnel implementation. Previous work has shown that flow has an impact on nitrate (NO_3^-) and ammonium (NH_4^+) concentrations and $\text{NH}_4^+:\text{NO}_3^-$ ratios as well as presence of various phytoplankton taxa. We predicted that after tunnel implementation, nitrogen concentrations would decrease and alter $\text{NH}_4^+:\text{NO}_3^-$ ratios. To make a better comparison, we chose a wet year (2014) that compared to years that saw heavy rainfall (2018-2019). The average yearly nitrogen concentrations post-tunnel, especially in 2018, were slightly lower than pre-tunnel concentrations. The year 2018 had lower ratios of $\text{NH}_4^+:\text{NO}_3^-$ than in both 2014 and 2019, suggesting there were higher concentrations of NO_3^- . While spring 2019 had higher concentrations of NH_4^+ , the average nitrogen concentration for that year was reduced. Due to above average rainfall in 2018-2019, the evaluation of the effectiveness of tunnel implementation needs to continue to see if there is any improvement in water quality as indicated by nitrogen levels.

The Gordon Brown Scholarship and Maryland Sea Grant Provided support to Tyler Mitchell. Tyler Mitchell appreciates the support of the Anacostia Riverkeeper who worked with us to get samples.

PLASMIDS CONFERRING RESISTANCE TO LATE-GENERATION CLINICAL ANTIBIOTICS CAPTURED FROM POULTRY LITTER AND STREAM SEDIMENT IN THE SHENANDOAH VALLEY

Emma Eisemann¹, Selena Hise¹, Liam Godbold¹, James Herrick¹

¹Department of Biology, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Antibiotic-resistant bacteria in runoff from the farms may have a significant effect on the reservoirs of resistance genes in bacteria native to soils and streams. These genes, commonly carried on plasmids, can be transmitted by conjugation. Plasmids conferring resistance to tetracycline, a common agricultural antibiotic, were captured from environmental samples of poultry litter. This capture method requires no culturing of plasmid ‘donor’ cells and thus is potentially able to detect plasmids in uncultured bacteria. Plasmids conferring resistance to tetracycline were captured by releasing cells from sediments and conjugating with a rifampicin-resistant recipient *E. coli* cell culture. Six transconjugants were randomly selected and Stokes tests were used to determine susceptibility to nine additional antibiotics. Five transconjugants exhibited phenotypic resistance to piperacillin, a broad-spectrum antibiotic reserved for serious, hospital-acquired infections. Phenotypic resistance to streptomycin and piperacillin/tazobactam (a beta lactam/beta lactamase-inhibitor combination) were also observed. Three transconjugants were intermediately resistant to imipenem. This is of particular concern as resistance to this broad-spectrum antibiotic is rare. We intend to determine the Minimum Inhibitory Concentration of these antibiotics using a Sensititre 96-well test. Additionally, we are developing a method for sequencing multiple transconjugants from a single capture plate using the Oxford Nanopore MinION™ and a bioinformatic method to computationally separate and assemble multiple plasmids simultaneously. The Isolation of plasmids with resistance to late-generation clinical antibiotics from poultry litter is of concern, as it indicates that a reservoir of genes conferring transmissible resistance to these antibiotics may exist in bacteria native to these environments.

James Madison University Department of Biology, Herrick Laboratory, JMU 4VA program

PREFERENCES OF THE INVASIVE SPOTTED LANTERNFLY (*LYCORMA DELICATULA*) AMONG POTENTIAL HOST PLANTS

Suji Kim¹, Anna Kuhn¹, Michael J. Raupp², Holly Martinson¹

¹Department of Biology, McDaniel College, 2 College Hill Westminster MD 21157

²Department of Entomology, University of Maryland, Plant Sciences Building College Park MD 20742

The spotted lanternfly (SLF, *Lycorma delicatula*; Hemiptera: Fulgoridae) is an invasive bug originating from China. This species first became a threat to the mid-Atlantic states around 5 years ago, and its infestation range is rapidly increasing in this region. As of 2019, Pennsylvania, Virginia, New Jersey, and Delaware quarantined counties with severe infestations to prevent the further spread of SLF. Despite the prevention, SLF population is spreading to different regions because of their feeding behavior and lack of general awareness. SLF are feeding generalists, with well-known preferences for tree-of-heaven (TOH, *Ailanthus altissima*), which is also invasive from Asia. Because SLF recently became a threat to the US and suite of available trees differs in their native and introduced ranges, the host plant use of SLF is yet to be understood. The main goal of this research study is to further specify the potential host plant preferences of SLF at different sites in Pennsylvania. The research recorded the relative abundance of eggs and nymphs by timed counts across the function of tree size and different tree species. The abundance of egg masses depended on tree size, whereas nymph abundance differed among plant species. Additionally, host plant use differed among sites. Specifically, SLF were abundant on TOH where it was present and on *Juglas nigra* (black walnut) and *Evodia danielii* (Korean evodia) where TOH was absent. Understanding the behavior and host plant use of SLF in the US will be beneficial for effective regulation and prevention. That egg masses and nymphs responded differently to tree size and species suggest that different management strategies must be employed to manage these different life stages. Future studies, such as investigating chemical similarities among host plants would clarify feeding behaviors and allow more specific management recommendations.

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POPULATION ANALYSIS OF FERAL ISLAND CATS: PREDATION RISK TO ENDANGERED BAHAMA ORIOLE

Breanna Byrd¹, Gabriel Wilkins¹, Sheridan Danquah¹, Emma Kartalia¹, Sierra Barkdoll¹, Janine Antalfy¹, Michael Cove³, Matthew Fagan², Colin Studds², Kevin Omland¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Geography and Environmental Systems, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

³Smithsonian Conservation Biology Institute, 1500 Remount Rd, Front Royal, VA 22630

The Bahama Oriole is a critically endangered species endemic to Andros, The Bahamas. Of the many dangers that the Bahama Oriole faces, introduced mammalian predators, such as feral cats, pose a major threat to the species' survival. Cat predation is one of the largest sources of human related bird mortality, particularly for island species that evolved in the absence of mammalian predators. This study estimated the abundance of feral cats inhabiting the pine forests of Andros. We placed motion sensitive wildlife camera traps at twenty-three locations within our selected six-square-kilometer study plot to detect the presence of feral cats. We found at least five adults as well as three kittens within the plot, indicating about one cat per square-kilometer. This preliminary analysis suggests that, not only are feral cats a likely predator, they are also reproducing successfully in the pine forest and pose a long-term threat to the Bahama Oriole population.

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IDENTIFICATION OF LAMPENFLORA IN BRISTOL CAVERNS

Bonnie Smith¹, Laura Ong¹

¹King University, 1350 King College Rd, Bristol, TN 37620

Since the beginning of time, man has been interested in caves and the underground world. As our knowledge in science has grown, cave exploration has allowed a greater understanding of cavern ecology. Different organisms have adapted to underground environments despite the fact that conditions are cool and damp. Although caves are normally devoid of light, the introduction of artificial lighting systems for public or “show” caves has made it possible for photosynthetic organisms to colonize the areas surrounding the lights. The name “lampenflora” has been given to algae, moss, ferns and sometimes liverworts that grow around artificial cave lighting. The research that I have been conducting is to identify some species of this lampenflora in Bristol Caverns. This research is very preliminary; only two species of algae have previously been identified in this cave. Most existing research on lampenflora has been published in Europe, and very little is known about the ones that are predominant in North America. It is important to understand these species because they have learned to adapt to the harsh conditions of living in a cave. Additionally, the continued growth of these species can eventually damage the underlying rock structures and thus make preservation of the geological features more difficult. The goal of my research is to identify the different types of algal species that are growing in artificially illuminated areas of Bristol Caverns. Identification of these strains will allow for informed decision-making about how they should best be controlled to allow preservation of cave formations.

Ledford and Appalachian College Association

THE EFFECT OF ROADSALT ON DIFFERENT FRESHWATER COMMUNITIES

Anju Ghimirey¹, Rachel Warthen¹, Rebecca Urban¹

¹Department of Biology, Lebanon Valley College, 101 College Ave. Annville, PA 17003

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 native species—and *U. inflata*—a species that has invaded lakes in the northeast United States. We also tested the effect different salt concentration have on zooplankton, a potential food source for these plants. 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 2000 mg/L, while *U. purpurea* declined at 500 mg/L of NaCl. Our results also indicated that daphnia, copepods, and amphipods had a lower percent survival as salt concentrations increased. In correspondence to that, the percentage of organisms in the *Utricularia bladders* also decrease with increasing salt concentration. 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.

Acknowledgements- Thanks to Ryan and Liberty for helping with the experiments. This project was funded by Arnold Experiential Education.

RNA THERAPEUTIC STRATEGIES TO BLOCK VEGFR2 EXPRESSION AND ANGIOGENESIS IN GLIOBLASTOMA MULTIFORM

Kinneret Kanik¹, Martin J. Hicks¹

¹Department of Biology, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

Glioblastoma Multiform (GBM) is an aggressive malignant brain tumor originating in the blood vessels of the brain. Patients with GBM tend to have an over-expression of Tyrosine Kinase Receptor (KDR) Protein, Vascular Endothelial Growth Factor Receptor Type 2 (VEGFR2), which is responsible for the growth of blood vessels. When over expressed, it promotes the development of tumors. Antisense RNA therapy is used to induce an alternative isoform. To reduce VEGF over-expression, we are using this strategy to develop a gene, encoding an antisense RNA, that will create a soluble decoy to block VEGFR activation. This will alter the splicing site of the exons of VEGFR2 pre-mRNA transcript, effectively prohibiting the receptor from binding in the cell. We hypothesize that the secondary structure of VEGF Exon 13_Intron13_Exon14 will determine the most effective way to approach synthetically altering the splicing of the VEGFR pre-mRNA. Through Polymerase Chain Reaction (PCR) quantification, we have successfully treated Glioblastoma cells with anti-VEGFR2 coding sequences directed against 5' splice site of intron 13, which significantly reduced VEGFR2 mRNA and protein expression.

In Hicks Lab, I have learned and carried out basic molecular biology protocols specific to our lab. In order to characterize VEGFR2 and EGFR transcripts, I grew and maintained GBM cell lines, isolated their cytoplasmic and nuclear RNA using trizol, and reverse transcribed the RNA to complementary DNA. To isolate the EGFR isoform, I designed quantitative PCR (qPCR) primers. PCR helps us characterize pre-mRNA structures. qPCR enabled us to monitor VEGFR2 expression in GBM cell lines, HEK293 and SKMG3. To verify the products of qPCR and PCR, I ran an agarose gel electrophoresis. Through these experiments, I have contributed to each of the Hicks Lab projects as well as gained expertise to initiate experiments onto my own individual product directed against KDR Protein, VEGFR2.

THE ACTIVATION OF FIBROTIC EGR1 TARGETS IN LENS CELLS AFTER CATARACT SURGERY

Ananya Garg¹, Samuel Novo¹, Mahbubul Shihan¹, Yan Wang¹, Adam Pater-Faranda¹, Melinda Duncan¹

¹Department of Biological Sciences, University of Delaware, 105 The Green, Newark, Delaware 19716

Cataract surgery is one of the most performed surgeries in the United States. A side effect of this procedure is posterior capsular opacification (PCO), a secondary cataract that develops 3-5 years after initial surgery, resulting from wound healing/scarring in the eye. While it is understood that transforming growth factor-beta (TGF β) contributes to the development of fibrotic PCO, we still do not know what regulates the bioavailability of TGF β post cataract surgery (PCS).

The gene expression profile of naive lens epithelial cells (LECs) was compared to LEC gene expression 6 hour PCS by RNA sequencing as this is more than a day prior to the onset of TGF β pathway activation. Notably, Early Growth Response-1 (EGR1), is one of the most upregulated genes at 6 hours PCS. EGR1 is a transcription factor that upregulates in stressed lens cells and mediates cellular stress responses in other systems, although its function in the lens is less certain.

Interestingly, bioinformatics analysis showed that several of the other genes upregulated at 6 hours PCS have EGR1 binding sites in their promoters including the fibrotic markers, THBS1 and Colla1. Notably, the gene encoding a potential regulator of EGR1 transcription, RUNX1 is also upregulated at 6hours PCS. I wanted to confirm these results to support the hypothesis that EGR1 is a major regulator of the response of LECs to cataract surgery. Thus, I conducted immunofluorescent staining for THBS 1, Colla1, and RUNX1 protein in eyes collected at times ranging from 0 hours to 5 days PCS.

By confocal imaging, I found that the protein expression of both THBS 1 and Colla1 upregulates PCS which mirrors the RNA-seq data, while analyses of Runx1 expression are ongoing. Future work will test the hypothesis that this upregulation is under the control of EGR1 by testing the responses of EGR1 null mice.

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THE VARIATION OF GENERA OF BACTERIA IN INDIVIDUALS WITH INNIE BELLY BUTTONS AND OUTIE BELLY BUTTONS

Kevin Gibbons¹, Caroline Moore¹, Precious Oyinloye¹, Laura Ott^{1,2}

¹STEM BUILD, University of Maryland: Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Natural and Mathematical Sciences, University of Maryland: Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Studying the human microbiota, or the community of microorganisms that exist on and within humans, is essential in understanding human health and identifying new microbes. We conducted a large metagenomic study on the human belly button to explore differences in the diversity of the microbiota in innies vs. outies. In our experiment, we decided to test the variation of bacterial genera in innie versus outie belly buttons. We utilized a large data set of belly button bacteria from previously conducted research and used a computer software program (Phinch) to determine the different bacterial genera within the two different types of belly buttons. While conducting our research, we determined that there were 16 individuals with outie belly buttons and 219 individuals with innies. We also discovered that outie belly buttons contained a combined total of 275 bacterial genera and innie belly buttons had a combined total of 606 bacterial genera. However, many of the bacterial genera found in innies and outies were the same, such as Staphylococcaceae, Corynebacterium, Anaerococcus, and Porphyromonas, which were the top four types of genera found in both types of belly buttons. In addition, after comparing to background research that we'd previously conducted, we discovered that there were many similarities in the microbiomes of the gut, skin, and mouth. The data collected can contribute to our understanding of human health and discovering more about the human microbiota on different morphology.

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QUANTIFICATION OF RELATIVE PEPTIDE INTENSITY: VARIATION IN ANALYSIS METHODS

Ryan Wood¹, Lora Schell¹, Anja Nohe¹

¹Department of Biology, University of Delaware, Newark DE, 19716

To measure changes in protein concentration over time, immunofluorescent samples are often imaged, analyzed, and quantified to determine relative peptide intensity. To ensure accurate readings background noise must be accounted for during analysis. Background removal is done through several methods, which are described in the literature. Threshold analysis is one such way to remove background data. Pixels will have a value between 0 and 255, and the average pixel value in either an area or the total image is recorded. Application of a threshold removes values under the specified value from the image. Thresholds are used to delete irrelevant background information that can harm trends. This only works, however, if no relevant and important information is lost. Whether or not the necessary information was removed was previously unclear. We sought to compare methods to see if data was impacted when using a threshold. Here we compare the analysis of images via the software ImageJ using 3 different thresholds compared to non thresholded images and the influence of changes in the intensity pattern. Our data showed that the use of a threshold radically and unpredictably alters the results and the intensities, and therefore should not be considered a viable method of analysis.

ROLE OF THE C TERMINUS OF THE DROSOPHILA VESICULAR ACETYLCHOLINE TRANSPORTER IN TRAFFICKING

Sophia Gondolfo¹, Sridhar Boppana¹, Hakeem Lawal¹

¹Department of Biological Sciences, Delaware State University, 1200 N Dupont Highway,
Dover, DE 19901

Acetylcholine (ACh) is required for essential neurological functions such as locomotion, cognition, and memory. The molecule is transported and released into the synaptic cleft by the membrane bound Vesicular Acetylcholine Transporter (VACHT). Consistent with an essential role for ACh, the loss of VACHT causes lethality during early development in flies, mice and worms. And while much is known about the system that regulates ACh synthesis and transport, essential questions about how ACh is released remain. One such question is precisely how changes in the structure of VACHT controls its localization and the synaptic release of ACh. Preliminary observations from our lab indicates that the deletion of the entire VACHT C terminus is lethal but we do not know what precisely is responsible for this lethality. Moreover, extensive studies in rodent VACHT has identified residues in the VACHT C terminus as being responsible for the translocation of VACHT to synaptic vesicles. To understand how the trafficking of VACHT is regulated, we inserted both a MYC and the pH-sensitive pHluorin sequence into the first luminal loop of Wt-VACHT c-DNA sequence. We then inserted various other VACHT point mutations into this construct using Site-Directed Mutagenesis to generate five C terminal deletion constructs of progressively short lengths. We transfected these mutant constructs into Drosophila S2 cell lines and measured their trafficking patterns using endocytosis assays. We analyzed and obtained empirical data of the fluorescence produced by these various constructs using the software “Metamorph”. We report the compelling observation that the truncation constructs show a differential effect on the sorting of VACHT to synaptic vesicles. Importantly, our preliminary data show that the VACHT mutant with the deleted C terminus (see above) shows deficits in sorting to synaptic vesicles. Together, these data contribute to our understanding of the intrinsic mechanisms that control trafficking of VACHT.

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IDENTIFICATION OF MUSCLE SYNERGIES BASED ON MATHEMATICAL RECONSTRUCTION OF THE TAIL NOCICEPTIVE WITHDRAWAL RESPONSE IN RATS

Tiffany Tolley¹, Corey L. Cleland¹

¹Department of Biology, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Computational complexity of movement arises in part from muscular redundancy, in which there are more muscles than necessary to produce a movement. The central nervous system may simplify this control of movement by grouping combinations of activated muscle into larger groups, otherwise known as synergies. In order to identify synergies it is necessary to determine the patterns of activity of muscle. Typically, electromyography (electrical recording from the muscle) has been used to characterize muscle activity. The aim of this ongoing study is to develop a new approach by deconstructing the nociceptive withdrawal response movement into linear combinations of tendon primitive movements. Rats were lightly anesthetized to be marked with 11 black bands along the tail to guide heat stimulation and video tracking. The rats were restrained in an acrylic tube and a heat stimulus was applied to the 11 black bands in order to evoke a nociceptive withdrawal response of the tail, which was recorded with high speed video. The following day, a surgical experiment was performed in which the rats were deeply anesthetized and each tendon in the extrinsic tail muscles (about 30-40 tendons) - M. sacrocaudalis dorsalis lateralis (SDL), M. sacrocaudalis dorsalis medialis (SDM), and the M. abductor caudae dorsalis (ACD) - were pulled and subsequent movement of the tail was video recorded. Each behavior was matched to a best-fit linear combination of the tendon pull movements in order to predict which tendons were likely the main contributors to the nociceptive withdrawal movement. Our preliminary results suggest that small movements can be attributed to a single tendon, whereas larger movements involve the recruitment of multiple tendons acting as a unit.

INTEGRITY OF THE OPTIC NERVE ZEBRAFISH FOLLOWING INDUCED HYPERGLYCEMIA USING A NITROREDUCTASE ABLATION MODEL

Jessica Pierce¹, Jessica Clark¹

¹Department of Biological Sciences, Salisbury University, 1101 Camden Ave, Salisbury MD 21801

The central nervous system is composed of sensory and motor nerves that collect, interpret, and respond to sensory information received from the environment. A significant component of the central nervous system is the optic nerve, which functions to relay sensory information received by the retina to the thalamus, where the information is then sent to the occipital lobe to be processed. Nonarteritic Anterior Ischemic Optic Neuropathy (NAION) is a form of damage to the optic nerve, which results in sudden vision loss, and often presents in patients with uncontrolled hyperglycemia associated with Diabetes Mellitus (DM). Here, we propose using zebrafish to investigate how hyperglycemia affects the integrity of the optic nerve using a nitroreductase β -cell ablation model to induce hyperglycemia. Adult Tg(Lumibblue), Tg(mbp;gfpcax);Tg(nbt;dsred) zebrafish will be injected with metronidazole (MTZ) to initiate β -cell ablation. 72 hours later, fish will be anesthetized and the left eye will slightly be pulled out of orbit to expose the optic nerve. The optic nerve will then be transected using forceps (treatment) or pulled out without transection (control). Fish will be allowed to recover for 14 days. On day 15, fish will be euthanized by decapitation, and the brains will be fixed in 4% paraformaldehyde, mounted in agar, and placed in 30% sucrose solution for 48 hours at RT. 25-micron thick sections will be taken, adhered to slides and coverslipped. Images will then be taken using an EVOS microscope. I hypothesize that I will see a change in the integrity of the optic nerve following induced hyperglycemia, specifically a decrease in the number of oligodendrocytes present around the optic nerve. By studying the underlying pathways of NAION in association with DM, effective strategies for prevention and treatments may be developed.

POU6F2 EXPRESSION IN DEVELOPING MOUSE

Samantha Lazo-Contreras¹, Keenan Bartlett¹, Hyunju Cho¹, Susan Wray¹

¹College of Arts and Science, Trinity Washington University, 125 Michigan Ave NE,
Washington DC 20017

¹National Institute of Neurological Disorders and Stroke, National Institute of Health, 49
Convent Dr, Bethesda, MD 20894

Gonadotropin Releasing Hormone (GnRH) neurons originate in the nasal placode and thereafter migrate into the brain where they control reproduction (Wray et al., 1989). GnRH is a hormone released by GnRH neurons and when released it signals the pituitary gland to secrete reproductive hormones that influence sex hormone levels and gamete production. When a defect in GnRH cell migration or hormone secretion occurs, it can lead to Idiopathic hypogonadotropic hypogonadism (IHH) affecting puberty and reproduction. A signal from a cell causes the activation of proteins known as transcription factors. POU (Pituitary-specific Pit-1, Octamer transcription factor, and Unc-86) is a member of the transcription factor family. Some POU proteins regulate GnRH neurons and are involved in the proper development of the HPG (hypothalamic–pituitary–gonadal) axis (shown above). A new POU protein involved in IHH is the mutation of POU6F2 isolated in patient with IHH. Three POU6F2 isoforms have been identified in humans; isoform 1, isoform 2, isoform 3, and is highly expressed in brain. We investigated whether there is a relationship between POU6F2 and IHH by determining the expression of POU6F2 in the main components of the HPG axis. We are currently working on determining the expression of each isoform using in situ hybridization (ISH) with isoform specific probes. We started off by labeling a probe which we then used to hybridize to a known target within a sample enabling us to understand more of their functionality. Resulting in a new Pou6f2 isoform (isoform 2) identified in mice which is expressed in olfactory unsheathing cells, nasal explants, and brain and may be altered by mutation found in IHH patient. We have designed oligonucleotide probes for ISH targeting each isoform in mice and labelled probes using digoxigenin and performed Dot blot assay to confirm the labelling of probes.

Thank you to NIH

LIGHT-DEPENDENT ENDOCYTOSIS OF MOUSE MELANOPSIN

Favour Nwagugo¹, Juan Valdez¹, Phyllis Robinson¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Melanopsin is a unique vertebrate visual pigment expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) of the mammalian retina. These melanopsin-expressing cells regulate non-image forming vision by signaling to the suprachiasmatic nucleus (SCN) and olivary pretectal nucleus (OPN) of the brain, thus impacting behaviors such as circadian photoentrainment and pupillary light response. In M1-type ipRGCs, light-activated melanopsin initiates Gq signaling that results in ipRGCs depolarization. Light-dependent phosphorylation of its C-terminus by GPCR Kinases (GRKs) leads to the binding of β -arrestin and the quenching of signal transduction. β -arrestin possesses clathrin-binding domains, unlike visual arrestin found in rod and cone cells in the retina. Thus, it is possible for melanopsin- β -arrestin complexes to undergo clathrin-mediated endocytosis into the cytoplasm, unlike rhodopsin-visual arrestin complexes in rod cells. Therefore, we hypothesize that light-activated melanopsin bound to β -arrestin will be endocytosed through clathrin-mediated vesicular transport to facilitate melanopsin resensitization and sustained light responses. To test our hypothesis, we transfected HEK293 cell cultures with the PMT3 plasmid containing mouse melanopsin. Following an overnight dark-adaptation, transfected cells were fixed after light exposure, or immediately after dark-adaptation with no exposure to light. Then, we performed immunocytochemistry of transfected cells and examined the localization of our target proteins expression using confocal microscopy. Our preliminary data indicate that melanopsin is endocytosed after light exposure. Specifically, melanopsin fluorescence was observed in the cytoplasm of light-exposed HEK cells, and melanopsin fluorescence colocalized with that of clathrin. In conclusion, our data suggests that melanopsin undergoes a light-dependent endocytosis process, which may contribute to sustained light responses that are unique to the retina.

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PURKINJE CELL EXPRESSION OF MUTANT ATAXIN-1 IN MICE EFFECTS ACTIVITY OF NEURONS IN PREFRONTAL CORTEX AND HIPPOCAMPUS

Adanna Ekekwe¹, Juao-Guilherme Rosa², Marija Cvetanovic²

¹Department of Biological Sciences, 1000 Hilltop Cir, Baltimore, MD 21250 University of
Maryland Baltimore County,

²University of Minnesota, Department of Neuroscience, 3 Morrill Hall 100 Church St. S.E.
Minneapolis MN 55455

Evidence suggests that the cerebellum has anatomical and functional connections with the hippocampus and the prefrontal cortex, which influence cognitive planning. However, the effect of cerebellar dysfunction on the neuronal activity in these brain regions remain unknown. Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by a polyglutamine expansion in the Ataxin-1 protein. This mutation causes a loss of Purkinje cells, which are the only inhibitory output neurons of the cerebellar cortex. SCA1 patients exhibit deficits in motor function as well as in cognition. While the loss of motor function in SCA1 has been studied extensively, cognitive changes in SCA1 remain elusive. We hypothesize that cognitive deficits observed in SCA1 patients are caused, in part, by altered neuronal activity in the hippocampus and prefrontal cortex due to the decreased function of cerebellar Purkinje cells. To test our hypothesis, we used a SCA1 transgenic mouse model expressing ATXN1[82Q] under a Purkinje cell-specific promoter. To investigate how dysfunction and cell loss in the cerebellar cortex affect neuronal activity in the hippocampus and prefrontal cortex, we used immunofluorescence with cFOS as a marker of neuronal activity. cFOS is a protein expressed in neurons following early activation. We found increased cFOS staining in the hippocampus of ATXN1[82Q] mice and no significant difference of cFOS staining in the prefrontal cortex. Our data suggests that ATXN1[82Q] expression in Purkinje cells lead to abnormal activity in the hippocampus, which is heavily associated with cognitive planning and memory and may contribute to cognitive deficits seen in SCA1 patients.

Dr. Marija Cvetanovic's Lab

LSSURP Neuroscience Program, Dr. Robert Meisel & Dr. Timothy Ebner

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CHARACTERIZATION OF GROWTH AND CYST FORMATION OF *AZOSPIRILLIUM BRASILENSE* CHEMOTAXIS MUTANT CHEA4 AS A FUNCTION OF DIFFERENT CONCENTRATIONS OF AMMONIUM CHLORIDE

Julia Rogers¹, Dreek Faust¹, Elena Gansvosa², Gladys Alexandre²

¹Department of STM, Gallaudet University, 800 Florida Avenue Washington, DC 20002

²BCMB Department, University of Tennessee, Knoxville, TN 37996

Azospirillum brasilense, free-living bacteria promoting growth of plants, are motile and able to navigate toward a source of nutrients and away from harmful chemicals using chemotaxis. These bacteria have two major chemotaxis signaling pathways that are coordinated by histidine kinase CheA1 and CheA4 (Mukherjee et al., 2016). These bacteria have also another unique feature – they can fix atmospheric nitrogen (Steenhoudt, 2000). Nitrogen fixation can be initiated only in a microaerophilic environment with no available ammonium or other organic nitrogen source. Evidence in the laboratory suggested that one of the components of the chemotactic signal transduction pathways - Che4 - is functionally linked to the regulation of nitrogen fixation. Moreover, *A. brasilense* cells can form dormant cysts upon unfavorable nutritional environmental conditions (Sadasivan and Neyra, 1987). We characterized growth, cells size and cyst formation in the chemotactic bacteria *A. brasilense* and its mutant CheA4 as a function of different concentrations of ammonium chloride, used as a source of organic nitrogen. We hypothesized that: I) efficiency of growth and survival of the CheA4 mutant will depend on the availability of organic nitrogen; II) cells of the CheA4 mutant strain will form dormant cysts upon nitrogen starvation.

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DEACTIVATION KINETICS OF THE PHOTOPIGMENT MELANOPSIN IN ROD MONOCHROMATS

Eric Upton¹, Haya Algrain¹, Phyllis Robinson¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Due to adaptations to their light environments, mammals can be characterized by the photoreceptor architecture of their retina (i.e. rod monochromats, cone dichromats, and cone trichromats). Intrinsically photosensitive retinal ganglion cells (ipRGCs), which express the photopigment melanopsin, mediate image-forming and non-image forming visual processes. Melanopsin signaling kinetics is well understood in mice, but not in rod monochromat mammals. In a subtype of ipRGCs, melanopsin activates a Gq protein signaling cascade and it is deactivated by the phosphorylation of melanopsin's carboxy tail with subsequent binding of β -arrestin protein. The deactivation kinetics of melanopsin are dependent on the phosphorylation of serine and threonine residues in the carboxy-tail. Sequence analysis of the melanopsin gene *OPN4* has revealed that mammals with different retina architecture have substitutions in important serines and threonines in melanopsin's carboxy-tail. Previous experiments demonstrated characteristic activation and deactivation kinetics linked to these substitutions. In order to isolate the deactivation kinetics of melanopsin in rod monochromats, we created chimeric melanopsin constructs using mouse melanopsin transmembrane region and the carboxy-tail of our mammal of interest. This way, each construct will activate in the same manner due to the interaction of the third intracellular loop with the cognant G-protein, and deactivate according to the phosphorylation sites present in the carboxy-tail. Due to the substitutions in melanopsin's carboxy-tail, we hypothesize that rod monochromat mammals will have slower deactivation kinetics than wildtype mouse melanopsin. To test this hypothesis, chimeras were first cloned into expression vectors, then expressed into HEK293 cells. A calcium signaling assay was used to measure the change in fluorescent calcium as an indicator of melanopsin deactivation kinetics. Our results indicate that melanopsin from rod monochromats deactivates slower than mouse melanopsin. These results suggest that mammals with slow deactivation kinetics have a prolonged pupil constriction response, thus protecting the photoreceptors from photobleaching.

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CHACTERIZATION OF ENVIRONMENTAL BACTERIA ISOLATED FROM THE ST. JONES RIVER

Anthony Baker¹, Kevin E. Shuman¹

¹Department of Biology, Wesley College, 120 North State St Dover, DE 19901

Understanding how bacteria function is an essential part of microbiological lab work. The purpose of this study was to determine the best growing conditions for two different species of bacteria, *Stenotrophomonas maltophilia* and *Bacillus licheniformis*. Both bacteria were isolated from Winogradsky columns made from sediment and water samples obtained from the St. Jones river (Silver Lake Park, Dover, DE). *S. maltophilia* is a Gram-negative opportunistic pathogen commonly found in aquatic environments. *B. licheniformis* is a Gram-positive bacterium commonly found in soils and associated with bird feathers. The optimal growth conditions for both isolates were determined for temperature, pH, and osmolarity. The isolates were cultured in Tryptic Soy Broth (TSB) medium. Both isolates grew best at 32.5oC, under neutral conditions (pH=7.0), and with no additional sodium chloride (NaCl) added to the medium. The results match up with research previously conducted about *S. maltophilia* and *B. licheniformis* and their growth conditions. Characterizing the optimal growth conditions of bacteria is important in determining whether the isolates could be sources of new antibiotics or have potential industrial applications (e.g., enzymes). For example, *B. licheniformis* is a known source of proteases that are used in laundry detergent. *S. maltophilia* is known as a multidrug resistant organism, so identifying environments, from which it can be isolated, may identify potential sources of new antibiotics and bacteriophage that could be used against resistant strains. Further research is needed to determine on whether these isolates are sources of new potential products.

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THE EFFECT OF WASH FREQUENCY ON BACTERIAL PHYLA DIVERSITY

Austin Fowler¹, Josh Ogbenna¹, Sharath Velliyamattam¹, Laura Ott^{1,2}

¹STEM BUILD, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Natural and Mathematical Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Catonsville, MD 21250

Good hygiene is humanity's greatest defense against pathogens. We conducted metagenomic analysis of Robert Dunn's research to focus on how hygiene affected the variety of bacterial phyla in the microbiota of the human naval. Our hypothesis was that individuals with a low wash frequency would have the most bacterial phyla diversity. Sample data of all participants were split into groups based on how frequently the participants washed themselves per week. The grouping and number of the participants was as followed: zero to two (n = 79), three to six (n = 37), seven to fourteen (n = 43), and unknown (n = 112). We analyzed the data using Phinch software to determine the number of participants for each wash frequency group and the top bacterial phyla matches present among each group. The top five bacterial phyla for each wash frequency were Firmicutes (39-48%), Actinobacteria (30-32%), Proteobacteria (11-12%), Bacteroidetes (7-10%), and Cyanobacteria (2-4%). Genomic analysis revealed that as wash frequency increased, bacterial phyla diversity decreased. These results suggested that if people washed themselves more frequently, they would be eliminating a high number of bacteria, both good and bad. The limiting factors in this research included reporting bias because there was a possibility for false testimony and sample bias due to high representation of North Carolina in comparison to other states. Our analysis could inform future researchers in their study of bacterial phyla within the bellybutton by helping determine the effect of certain bacterial phyla on human health as well as discover treatments for skin infections and rashes caused by pathogenic bacterial diseases.

We would like to acknowledge Dr. Robert Dunn (NCSU) for providing the metagenomics data set. We also like to acknowledge Dr. Fredrick Larabee, Dr. Maria Cambraia, and Jamie Denton for constructive feedback on our abstract. This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989.

THE EFFECTS OF STIMULATING THE VENTROMEDIAL PREFRONTAL CORTEX TO BASOMEDIAL AMYGDALA NEURONAL PATHWAY ON CONDITIONED FEAR EXTINCTION LEARNING IN RATS

Sierra Wallace¹, J. Russell Ravenel², Luke Millisor², Cate Levy², Alyssa Fausnaught², Rebecca Rasmussen², Robert Spencer²

¹Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Psychology and Neuroscience, University of Colorado, Boulder, 1905 Colorado Avenue, Boulder, CO, 80309

Individuals with post-traumatic stress disorder (PTSD) have an impairment in conditioned fear extinction learning, which relies on the ventromedial prefrontal cortex (vmPFC) (Woodruff et al. 2018). We believe that stimulating a neuronal pathway involving the vmPFC and the basomedial amygdala (BMA) will improve rats' extinction recall of conditioned fear extinction learning. We used viruses with fluorescent reporters to deliver Cre-dependent Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to the rats in order to manipulate this pathway. Three-day behavioral testing was performed that involved acquisition, extinction training and extinction recall. The rats were given pathway stimulation and behavioral testing during the time of day when they are normally awake (behaviorally active) or asleep (behaviorally inactive). The rats learned to associate a tone with shock during acquisition and then to dissociate the two during extinction training. Postmortem brain sections were examined by fluorescence microscopy to determine if viral expression is in the correct locations. Previous research has shown that rats tested in their active phase perform better than rats in their inactive phase (Woodruff et al. 2015), therefore we expect similar results for rats with no DREADDs. Also, we expect that rats with activated DREADDs will have better extinction learning, and that this effect may differ based on the time of testing.

This investigation was sponsored by NIH/NIGMS MARC U*STAR T34 HHS 00026 National Research Service Award to UMBC and NIH R01 grant MH115947 to the Spencer Lab.

FIBROBLAST GROWTH FACTOR 21 (FGF21) INHIBITED MORPHINE PREFERENCE AND THE DEVELOPMENT OF MORPHINE DEPENDENCE BUT NOT MORPHINE – INDUCED LOCOMOTION

Olufolake Majekodunmi¹, Louben Dorval², Jean M. Bidlack²

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Cir, Baltimore, MD 21250

²Department of Pharmacology & Physiology, University of Rochester School of Medicine and Dentistry

Fibroblast growth factor 21 (FGF21) is an endocrine protein that is released in the bloodstream and regulates glucose homeostasis and ketogenesis. It is expressed in the liver, pancreas, adipose tissues, and neurons. Additionally, FGF21 can cross the blood brain barrier. A previous pharmacological study revealed that elevated FGF21 levels reduced alcohol and sweet preference in mice. Alcohol and sweet substances act on the same dopamine reward pathway as opioids. Therefore, we hypothesized that FGF21 will affect behavior induced by morphine. Through behavioral studies, such as conditioned place preference (CPP), physical dependence, and locomotion, we examined the protein's effects. Wild-type and FGF21-Tg (transgenic mice that expressed a 2,400-fold increase of FGF21 protein in serum) mice were used to study if FGF21 affects preference for morphine. CPP was performed to examine if FGF21 affects morphine preference in mice over a 3-day period. Both male and female FGF21-Tg mice displayed lower preference for morphine compared to wild-type littermates. To determine if FGF21 has an effect on physical opioid dependence, we measured this through naloxone-precipitated withdrawal in mice previously treated with morphine. The number of jumps over a 15-min period was recorded and showed that the development of dependence to morphine was 65% lower in FGF21-Tg mice compared to wild-type littermates. Both male and female transgenic mice displayed attenuation of morphine dependence development. In addition, locomotor activity was examined for 120 min to determine if FGF21 has an effect on locomotion when mice were administered morphine. Mice were treated with 10 or 23 mg/kg of morphine and placed in an open-field activity apparatus and their ambulatory activity was determined. FGF21-Tg mice displayed similar locomotor activity to wild-type littermates. These results demonstrated that FGF21 did not affect locomotion; however, FGF21 inhibited the development of morphine dependence and reduced the preference for morphine. These results suggest that FGF21 has the potential to reduce morphine addiction.

TESTING THE EFFICACY OF A DOPAMINE 1 RECEPTOR IN VITRO

Andrew Hennigan¹, Alexis Rubin¹, Lee Harkless¹, Phyllis Robinson¹

¹Department of Biology, University of Maryland, Baltimore County, 1000 Hilltop Circle,
Baltimore, MD 21250

Melanopsin, a visual pigment expressed in intrinsically photoreceptive retinal ganglion cells (ipRGCs) contributes to circadian photoentrainment and other image forming and non-image forming visual process. It is hypothesized that Protein Kinase A (PKA) has a role in attenuating the signaling of melanopsin. Because PKA activation is part of the dopamine cascade, we are also interested in studying the effect of dopamine on melanopsin signaling. In my work, we are testing the efficacy of a dopamine 1 receptor antibody in HEK cells, as many dopamine receptors are expressed in HEK cells. To test the efficacy of our antibody, we used Immunohistochemistry to determine the localization of the antibody reactivity in the cells. The results suggest that the antibody is effective in labeling D1 receptors, as the localization of the antibody is in the plasma membrane of the HEK cells, which is where we hypothesized the receptor would be localized. In the future, we will use this antibody in vivo, to look at dopamine 1 receptor expression in ipRGC in the retinas of dopamine 1 receptor knockout mice and wild type mice. By determining the efficacy of this antibody, it can be used as a control to determine the quantity of D1 receptors, enabling further experimentation on the role of dopamine and PKA on melanopsin signaling.

CHANGES IN AMINO ACIDS IN RESPONSE TO HERBICIDES IN ARABIDOPSIS

Reid Kelly¹, Mark Edens², Phillip Morgan², Albrecht von Armin²

¹Gallaudet University, 800 Florida Ave NE, Washington, DC 20002

²Department of Biology. University of Tennessee Knoxville, Knoxville, TN 37996

Protein synthesis requires a steady supply of amino acids. In yeast and vertebrates, the GCN2 kinase is known to sense amino acid levels and repress translation globally by phosphorylating the translation factor, eIF2alpha. Whether this is also true in plants remains controversial. In plants, we know that GCN2 is activated rapidly when plants are treated with inhibitors of amino acid biosynthesis. However, it is unknown whether the activation of GCN2 kinase is due to the drop in amino acid levels. Therefore, it was hypothesized that inhibition of amino acid synthesis rapidly trigger GCN2 kinase activity because it rapidly lowers the levels of key amino acids. If the hypothesis is correct then it can be predicted that inhibiting a key amino acid synthesis enzyme, acetolactate synthase (ALS), which is already known to activate GCN2, will cause a rapid drop in free amino acid levels that precedes activation of the kinase. In this project we exposed Arabidopsis seedlings to a time course of the herbicide chlorosulfuron (CSF), which inhibits ALS, the enzyme that catalyzes the first step in the branched chain amino acids (BCAA) biosynthesis pathway. Amino acid levels were measured using a Liquid Chromatography—Mass Spectrometry [LC-MS]. It was discovered that the BCAAs leucine/isoleucine levels remained constant throughout the treatment.

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INVESTIGATING PHENOTYPIC DIFFERENCES BETWEEN THE STP1 K/O AND WT ARABIDOPSIS THALIANA IN RESPONSE TO VARIOUS ENVIRONMENTAL STRESSES

Aya Andos¹, Kristen Connelly¹, Janet Daniel¹

¹Department of Biology, James Madison University, 951 Carrier Dr. MSC 7801 Harrisonburg, VA 22807

Sugar transport protein-1 (STP-1) is an H⁺/monosaccharide co-transporter in model organism *Arabidopsis thaliana*. We are investigating this protein because its impacts on the growth and function of the plant are not well known. For a better understanding of how STP-1 contributes to this plant, we are growing wild type (WT) and STP-1 knock-out (k/o) plants on agar plates to measure their responses to different environmental stressors. Previous work in the lab show that STP-1k/o seedlings grown on agar plates containing 50 -100mM NaCl have increased root length, stalk length and more leaves at 4 weeks. Based on previous results, we hypothesize that STP1 k/o influences plant osmoregulation. Over the last year, we have been characterizing the halotropic response of WT and STP-1 k/o plants by growing seeds on split plates of varying NaCl concentrations (200, 250, and 300mM) to determine genotype specific response to salt exposure. In addition to characterizing the halotropic response we are also measuring root length and the angle of displacement from gravitropic response. We are also using hydroponic systems to develop methods to increase the harvest of roots, shoots and leaf tissue for tissue-specific gene expression studies. These studies will enable us to better understand the role of STP-1 in the plant. We also plan to extend these studies to compare the gene expression patterns of both genotypes in high saline environment.

Supported by 2018 Virginia Academy of Sciences Undergraduate Research Grant.

MID-INFRARED LASER-BASED SYSTEM FOR HIGH RESOLUTION SPECTRAL BIO-CHEMICAL SIGNATURES OF LUNG CANCER IN EXHALED HUMAN BREATH

Minares Ehsani¹, Mohammad Amir Khan¹

¹Department of Engineering Physics, Oscar building, 1200 N Dupont Hwy, Dover, DE 19901

Lung cancer is found to be one of the leading causes of death associated with cancer in the United States. The main cause lung cancers has been found to smoking.

There has been an increased focus on the development of non-invasive biomarkers capable of accurately identifying lung cancer early in its course and characterizing the nature of the cancer. Exhaled breath is a non-traditional source of biomarkers. This research project investigates the state-of-the-art in research on daily health monitoring and early diagnosis of specific diseases via the analysis of exhaled breath biomarkers. Recently, as an emerging breath sensing technique, we highlight laser-based sensors with possibility of bio-sensing medical devices with unique advantages of portability, cost effectiveness, and real-time analysis. Among various diseases, we focus on lung cancer- its pathway and early detection. The purpose of this research is to identify volatile organic compound (VOC) biomarkers that are associated with lung cancer disease. In order to identify the volatile organic compound biomarkers, we investigated 1) three categories of different patient status by looking at what highest concentration molecule is found in their exhaled breath 2) design a system which can detect and measures the acetone molecule. We look for the spectroscopy and the spectrum of the molecule by using a laser diode, temperature control - spherical mirror, and a detector. It was that the VOC molecules with highest concentration found in the patients' exhaled breath was Acetone. The categories with highest concentration of acetone were found in the individuals who smoked or are exposed to polluted environment.

THE DIFFERENCES IN MICROBIOTA VARIATION BETWEEN MEN AND WOMEN

Toni Martin¹, Mia Penn¹, Grace Tugado¹, Laura Ott^{1,2}

¹STEM BUILD, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

²College of Natural and Mathematical Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

There are many physiological differences between men and women, such as hormone production and body pH, that directly impact types of health-related issues. The microbiota that inhabit the human skin can be influenced by a variety of factors, including gender. It is beneficial to further investigate information about the types of bacteria that are more prevalent on each gender in order to gain a better understanding of its impact on human biology. We hypothesized that men had a greater variation of microbiota in the belly button than women. To learn more about the distribution of microbes among the skin, we analyzed data from Phinch, a metagenomics software, comparing the diversity of bacteria between men (n=114) and women (n=151). Based on the data from a previous study, sample sizes generated from the ten most prevalent microbes were analyzed to examine whether gender had an impact on the microbiota variations within the belly button. Examples of the types of microbes studied were: Bacillales, Clostridiales, and Streptophyta. The ratio of the number of microorganisms were calculated between the genders. Moreover, the average amount of microbes found in both men and women were compared. Our hypothesis was refuted, as women showed a slightly greater variation of bacteria than men. These differences are probably due to the benefits of bacteria in the bodies of women, such as with pregnancy and hormones. This study launches future research to further investigate why microbes have a greater variation in one gender over the other, and whether the amount of microbes have a significant impact on health as it relates to physiological differences between genders.

We would like to acknowledge Dr. Rob Dunn (NCSU) for providing the metagenomics data set and Dr. Larabee and Dr. Cambraia for instructive feedback on our abstract.

This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989.

Biochemistry and **Molecular Biology**

ABSTRACTS

Morning Session

*Page Numbers of the Abstracts Serve as
Poster Numbers*

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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 2019 Undergraduate Research Symposium in the Chemical and Biological Sciences.

UNRAVELING THE B TO A CONFORMATIONAL TRANSITION IN DUPLEX DNA AT SINGLE BASE PAIR RESOLUTION

Michal Kalisz¹, Brianna Miller¹, Davis Jose²

¹Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

²Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

The transition of the standard B-form DNA helix to A-form DNA was first seen by X-ray imaging of DNA fibers in 1953. Over time, the structures of B and A DNA have been further characterized with many higher resolution crystal structures. The transition of B-DNA double helix to A-form is essential for biological functions as recognized by the presence of A-form DNA in many protein-DNA complexes. Recently it was proposed that the shorter length of the A-form DNA compared to the B-form DNA might play an important role in duplex DNA packaging in bacteriophages and that this conformational change might itself serve as the source of the large forces generated by the DNA packing motors. Even though it is known that the B to A conformational transition occurs, the specifics like where in the DNA it originates, how it propagates, and the detailed step-by-step mechanism involved is still unknown. By using site specifically positioned fluorescent oligonucleotides, we explored the local and global conformational changes in this highly biologically relevant transition. Our results showed that by using 2-Aminopurine (2-AP), a fluorescent analogue of Adenine, we could monitor the local and global conformational change simultaneously.

Acknowledgements are given to the Summer Research Program 2019 at Monmouth University and Bristol-Myers Squibb for funding this research project.

STRUCTURE AND FUNCTIONAL OVERLAP BETWEEN *LEISHMANIA DONOVANI* AND HUMAN UFM-1 CONJUGATION

Adrianne Lee¹, Gavin M. Lao¹, Christopher E. Berndsen, Ph.D¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive
Harrisonburg, Virginia 22807

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. Ufm1 regulates unknown pathways in *Leishmania donovani* which causes leishmaniasis, a blood-borne disease that is characterized by sores and lesions that will 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 and available treatments lead to corrosive secondary effects. Ufm1 and its conjugating enzymes in *Leishmania* are essential for the proliferation of the disease once inside the host but their structures, biochemical and cellular function are not known. 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. We have successfully purified LdUfm1 and a truncated construct (LdUfm1tr.) and characterized their biochemistry and preliminarily the structure of LdUfm1. E1 charging and fluorescence polarization binding assays were performed to characterize the affinity of LdUfm1 and LdUfm1tr. for their activating enzyme. Circular dichroism and small angle x-ray scattering (SAXS) were used to compare the structures of hUfm1, LdUfm1, and LdUfm1tr. Human and *Leishmania* Ufm1 were found to have similar overall structures despite significant differences in their sequence which we believe may ultimately explain the functional overlap observed between them.

Special acknowledgements to our funding sources NSF REU (CHE-1757874), 4-VA Organization, Jeffrey E. Tickle Scholarship, and the Thomas and Kate Jeffress Memorial Trust. Thank you to the James Madison University Department of Chemistry and Biochemistry for allowing us use of facilities and instrumentation vital to our research.

DNAB ALPHA STRUCTURE ANALYSIS: STUDY OF ALPHA DOMAIN USING FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

Sarah Barndt¹, Meera J. Patel¹, Subhasis Biswas¹

¹Department of Medical and Molecular Sciences, University of Delaware, 16 W. Main St., Newark, Delaware, 19716

DnaB, a bacterial helicase found in *Escherichia coli*, plays a critical role in the genomic DNA replication mechanism of most bacteria. It has been shown to function in ATP hydrolysis, DNA binding, and forming the replisome with other replication proteins. DnaB consists of three domains: Alpha (α), Beta (β) and Gamma (γ). Each of the three domains have a specific and essential role in the overall functional activity of the DnaB protein. Due to the unique attribute of DnaB, not containing the amino acid cysteine, allows insertion of fluorophores by in vitro mutagenesis for the performance of Fluorescence Resonance Energy Transfer (FRET) studies. FRET was used to analyze the structural changes in DnaB protein by site-specific double labeling using a donor fluorophore, fluorescein arsenical hairpin binderethanethiol (FIAsH) and acceptor fluorophore, Alexa568-Maleimide. Conformational changes were assessed through measuring the distance between the fluorophores and calculating the proximity ratios and FRET. In this study, site specific mutagenesis was used to create 3 mutant forms of DnaB that contain cysteine (for Alexa568 labeling) and the CCPGCC sequence (for FIAsH labeling). These mutants were: DnaB B2 (FIAsH at the C-terminus and Alexa at amino acid 181, encompassing the beta-gamma domains), DnaB B2 α C4C (FIAsH at the N-terminus and Alexa at amino acid 157, encompassing the alpha domain), and DnaB CC4 (FIAsH at the C-terminus and Alexa at the N-terminus, containing all three domains). These three mutants, double labeled, were used to perform FRET analysis in the presence of various substrates such as ATP, ADP, and ssDNA and to evaluate how the proximity of the donor fluorophore (FIAsH) and acceptor fluorophore (Alexa568-Maleimide) changes in their presence. This FRET analysis will allow us to evaluate structural changes of various domains of DnaB to better understand how the structure changes in response to various substrates.

I would like to thank Laves Bhatia for the purified protein used in the double labeling process. Additionally, I would like to thank all those in the Biswas Lab who helped me during the summer. This poster was supported by Delaware INBRE program, with a grant from the National Institute of General Medical Sciences- NIGMS (8 P20 GM103446-16) from the National Institutes of Health

INFRARED STUDIES OF AB PEPTIDES WITH COPPER AND ZINC: LINKING INTERACTIONS WITH STRUCTURAL CHANGES

Tewaldemedhine Gebrejesus¹, Keyon J. Carter¹, Gina MacDonald¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, Virginia 22807

The aggregation of Amyloid- β (A β) peptides form insoluble plaques in the brain. Multiple environmental factors most likely influence disease progression. Previous studies have shown that metals are associated with the aggregated A β protein in brains obtained from Alzheimer's patients. Many studies have shown A β peptides interact with metal ions, such as Copper and Zinc and suggest that the metals increase aggregation and A β self-assembly. Additional studies on Copper and Zinc involvement in A β peptides aggregation may provide a greater understanding of A β self-assembly and the development of neurodegenerative diseases. This study uses infrared spectroscopy to investigate metal ion interactions with A β (40) and A β (42). The A β peptides structural changes and aggregation were monitored in the presence and absence of Zn and Cu. Infrared spectra obtained on peptides in the presence and absence of Zn and Cu were analyzed and suggest that metals alter peptide structure and aggregation.

ANAEROBIC BACTERIA CONCENTRATIONS IN INNIES VS OUTIES

Achalefac Akem¹, Gloria Ogordi¹, Laura Ott^{1,2}

¹STEM BUILD, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Mathematical and Natural Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

The human microbiota is comprised of largely unknown bacteria. Understanding the type and effects of bacteria will be helpful to the medical field in terms of identifying diseases. One of the most common forms of bacteria within the skin flora is anaerobic bacteria; these are bacteria that run their metabolic processes in environments with little to no atmospheric oxygen. We hypothesized that there would be fewer counts of anaerobic bacteria in outie belly buttons compared to innies due to their constant exposure to atmospheric oxygen. The anaerobic bacteria we studied falls into two categories: facultative and obligate. Facultative anaerobic bacteria are bacteria that create Adenosine Triphosphate (ATP) by aerobic respiration when oxygen is present and switch to fermentation when there is a lack of oxygen. Obligate anaerobes are bacteria that are killed by atmospheric oxygen, although some can survive in certain levels of oxygen. We performed a metagenomic analysis on previously collected data which included parameters such as type of belly button. Analyzing the data with Phinch software, we decided to isolate the innie and outie group. We examined 6 families of bacteria, 3 facultative: Clostridiaceae, Prevotellaceae, Campylobacteraceae and 3 obligate bacteria: Pseudomonadaceae, Corynebacteriaceae, and Alicyclobacillaceae. The data we found showed that two anaerobe groups had a higher count in individuals with innie belly buttons, Clostridiaceae and Pseudomonadaceae, while the rest had either lower counts or differences that were less than 0.5%: Prevotellaceae, Alicyclobacillaceae, Campylobacteraceae, and Corynebacteriaceae. The results of this study show that there are no apparent correlations between the prevalence of anaerobic bacteria and the type of belly button. Therefore, anaerobic bacteria are able to grow on innie and outie belly buttons.

We would like to acknowledge Dr. Robb Dunn (NCSU) for providing the metagenomic data set. We'd also like to acknowledge Dr. Larabee and Dr. Cambaria for their thoughtful review of our abstract. This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989.

UNDERSTANDING THE EFFECTS OF RETT SYNDROME MUTATIONS ON MECP2 PROTEIN PROPERTIES

Aashni Kamra¹, Tugba Kucukkal¹

¹Department of Science, Technology, and Mathematics, Gallaudet University, 800 Florida Ave NE, Washington, DC 20002

The main goal of the research was to understand the effects of Rett's Syndrome mutations on MECP2 protein properties. Rett Syndrome (RTT) is a neurodevelopmental disorder that affects girls almost exclusively and causes symptoms such as slowed brain and head growth, problems with walking, seizures, and intellectual disability. RTT is caused by mutations in the MECP2 gene, which encodes the MECP2 protein. Through computer simulations, we investigated how each of RTT-causing missense mutations affect protein stability and Hydrogen bonding. Stability changes were quantified in protein folding free energy change calculations upon single point mutations through fast computational methods, including Maestro, I-Mutant, and DUET. In addition, since our previous work showed that these fast methods lack accuracy, we first performed a conformational search for each of the mutant structures by using 3DRobot software. After that, folding free energy changes were calculated for each of the structures that were generated. Then, the results were averaged. Finally, computational results were compared with experimental results. This study is significant in two ways; first, this investigation helps decipher the molecular mechanisms of Rett's Syndrome. This is important because understanding the harmful effects of the mutation on the protein might aid future drug design studies. Second, our results show how conformational sampling affects the accuracy of fast computational methods for folding free energy calculations.

THE EFFECT OF NOVEL COMPOUND BI 885578 ON KINASE ACTIVITY AND ADAPTOR BINDING TO INSULIN RECEPTOR

Sophia Lookingbill¹, Susan Mertins¹

¹Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Rd, Emmitsburg, MD 21727

Insulin receptor (InsR) is a tyrosine kinase ligand-activated receptor that functions in metabolic regulation and cell growth. When the heterodimeric receptor binds insulin at two sites (of high and low affinity), the receptor becomes disinhibited through several conformational changes that ultimately auto- and transphosphorylates tyrosines located downstream. Small proteins called adaptors bind to the altered tyrosines through unique domains, namely SH2 and PTB domains. They signal downstream and can lead to tumor proliferation. A novel investigative compound, BI 885578, has been shown to bind InsR at its ATP pocket and therefore, prevent essential tyrosine phosphorylation leading to tumor suppression in xenograft models. It was of interest to develop a computational model to determine if loss or decrease of adaptor binding was predicted from the measured rate constants of BI 885578. uleBender, a rule-based biological modeling program, was utilized to capture bimolecular interactions, protein copy number, and rate constants of the InsR pathway described above. The program translates BioNetGen language to ordinary differential equations that allow simulations. It was found that the addition of BI 885578 led to less adaptor binding to InsR. In future studies, the drug affinity will be manipulated in the model to observe if all adaptor binding can be prevented. In conclusion, computational modeling of drug mechanism of action offers a means to treat cancer in a precision-based fashion.

This work was supported by Faculty Development Grants at Mount St. Mary's University.

INVESTIGATING PROTEIN EXPRESSION DATA USING PROTEOMICSDB DATASET

Najib Muhammad¹, Barry Cooperman¹

¹Department of Chemistry, University of Pennsylvania, 231 S 34th St, Philadelphia, PA 19104

ProteomicsDB is a protein-centric database providing mass spectroscopy-based proteomic data from 78 studies analyzed through a standardized pipeline which allows for direct comparison between studies¹. The protein expression levels are organized by tissue-, fluid-, and cell-line which can provide useful information for specific biomedical projects. The database was first released in 2014 and extended functionality was introduced in 2017. Due to this novelty, studies have not yet been conducted investigating the reliability and consistency of the data presented. We set out to use existing knowledge of relative ribosomal protein (r-protein) levels to determine the reliability of this data. r-Protein levels are known to exhibit a 1:1 balance between and within the small and large subunits, and thus we used these relationships to assess the reliability of the ProteomicsDB dataset. Several cell-/tissue-lines were selected for consistent data present across all r-proteins) and the median protein expression for that cell-/tissue-line was compared across “early-” and “middle-” binding r-proteins, which are more tightly bound than late binding proteins and are thus expected to display more consistent 1:1 ratios in mass spectroscopy studies. For many of the cell-/tissue- lines, it appears that this expectation is realized. There are some cell-/tissue-lines selected for which this was not true, however, markedly the ones with fewer studies available. Because cell-/tissue-line data had incorporated several studies together, we next investigated our 1:1 assumption against individual studies using the same method. In doing so, it appeared that the individual studies selected did show variability, counter to our expectation. This may account for the variations seen in the overall cell line data, in particular for cell-/tissue-lines with fewer studies. Finally, we identified a select number of reliable r-proteins from the few cell-/tissue-lines that we’ve thus far investigated to serve as a “reliability index”. We will this set going forward to quantify variation from our 1:1 expectation in the other cell-/tissue-lines and more specifically in other individual studies. We also plan to expand our investigation to include other proteins, such as release factor proteins and associated GTPases, once we can establish the reliability of the database for the r-proteins.

SIMULATION OF THE STRUCTURAL MECHANISM OF UBIQUITIN-LIKE PROTEIN ACTIVATION

Shveta Gupta¹, Christopher Berndsen¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St.
Harrisonburg 22807

Ubiquitination and ubiquitin-like protein modification is a form of post translational modification that designates proteins for re-localization, changes in function or activity, and degradation. Three classes of enzymes work together to modify target proteins with ubiquitin or ubiquitin-like molecules: E1 activating enzymes, E2 conjugating enzymes, and E3 ligating enzymes. E1 activating enzymes are categorized into two classes, canonical and non-canonical depending on the presence of the Cys domain, which contains several proposed catalytic amino acids, and the oligomeric state of the enzyme. Uba5 is a non-canonical E1 activating enzyme that begins the initial step of the Ufm-ulation pathway, a modification with the ubiquitin-like protein, Ufm1. Uba5 activates Ufm1 through adenylation of the molecule's C-terminus followed by the formation of a covalent bond between Uba5 and Ufm1 in a thioesterification step. The E1 enzyme then transfers the ubiquitin to the E2 enzyme in a trans-thioesterification step and the pathway proceeds toward target modification. The details of this pathway are not fully understood, including how Uba5 catalyses each step while lacking the crucial Cys domain. In order to illuminate the structural mechanism of the E1 reaction cycle, we modeled the adenylation, thioesterification, and trans-thioesterification steps within Uba5. Replicate molecular dynamics simulations of 100 ns were run in explicit solvent with the AMBER14 force field and the data were analyzed in R to observe changes in dynamics and pKA which may be associated with catalysis. We are empirically supporting these simulations with Small Angle X-Ray Scattering data to determine the influence of substrates in solution dynamics and with biochemical assays to assess active mutations in function. Through this work we will gain further understanding into the dynamic reaction mechanism of E1 enzymes which may lead to improved treatments for diseases linked to these essentials enzymes.

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ACTINOBACTERIA IN THE BELLY BUTTON MICROBIOME OF DIFFERENT AGE GROUPS

Senali Dansou¹, Emma Lorentz¹, Emily Marinko¹, Laura Ott^{1,2}

¹STEM BUILD, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Natural and Mathematical Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Around us, there are plenty of living and non-living microorganisms which constitute the microbiota. Studying the microbiota is important because it enables us to identify the pathogenic microbes around us. The microbiome is the combined genetic material in an environment, like an ecosystem or an organism. The microbiome of the belly button is very diverse as it is made of various classes of bacteria among which belong Actinobacteria. It would be interesting to study Actinobacteria because they are found elsewhere in the body. In our study, we were interested in determining what age group Actinobacteria is most prevalent in. We hypothesized that this class of bacteria would be more predominant in people over 50 years of age because we thought aging decreases the effectiveness of the immune system, which would allow bacteria to grow more. To test our hypothesis, we used collected data of the belly button microbiome with a sample size of 273 subjects. We classified the subjects into the age groups of 1-20 (n=72), 21-40 (n=105), 41-50 (n=34), and 51-74 (n=31). We then looked at the percentage of Actinobacteria in each group. We discovered that Actinobacteria had a higher percentage in the age group 41-50 which means that the data does not support our hypothesis. However, we believe that some factors such as small sample size in some of the age groups or even washing frequency could have impacted the results. This study furthers our understanding of whether the growth of Actinobacteria in the belly button is related to age.

We wish to acknowledge Dr. Rob Dunn (NCSU) for providing the data set and Dr. Larabee and Dr. Cambraia for their thoughtful critique of this abstract. This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989.

ASSESSMENT OF PROTEIN:DNA INTERACTIONS BETWEEN THE WILD-TYPE CD44-ICD AND THE MMP-9 GENE PROMOTER BY CHROMATIN IMMUNOPRECIPITATION

Jalen Wilcher¹, Holly Miller¹, Karl Milette¹

¹Department of Biological Sciences, Delaware State University, 1200 N Dupont Hwy Dover, De 19901

The CD44 gene encodes a cell membrane receptor which undergoes proteolytic cleavage within the cell membrane to generate 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 (1,2). This transcriptional regulatory mechanism is not well 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 region (3). We hypothesize that non-tagged wild-type (wt) CD44-ICD, similar to the GFP-tagged CD44-ICD, interacts with Runx2 in the MMP-9 gene promoter region. To test this hypothesis, we carried out ChIP assays treating MCF-7/CD44 cells with formaldehyde (a protein-DNA crosslinker) and DSG (a protein-protein crosslinker). The ChIP DNA was first amplified in its totality using a whole genome amplification kit (Sigma) and then 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 or multiple non-specific bands were generated. 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 wt CD44-ICD/Runx2 protein-protein interaction in the same cell line using the same anti-CD44-ICD antibody. Additional ChIP assays are in progress in which DSG is not included.

This project was supported in part by the Delaware INBRE program, with a grant from the NIH National Institute of General Medical Sciences – NIGMS (P20 GM103446) and the state of Delaware, the National Science Foundation HBCU-UP Research Initiation Award Grant No. 1700228 (K.M.) and the Delaware Economic Development Office Grant No. 103 (K.M.).

CHARACTERIZATION OF DNMT1 - MUTSA INTERACTION IN VITRO

Martin Yepes¹, Masayoshi Honda², Shanen Sherrer¹, Maria Spies²

¹Department of Chemistry and Biochemistry, St. Mary's College of Maryland, 16800 Point Lookout Road, St. Mary's City, MD 20686

²Department of Biochemistry, University of Iowa, Carver College of Medicine, Iowa City, Iowa 52242

DNA Methyltransferase 1 (DNMT1) is an enzyme that performs maintenance methylation on hemimethylated DNA, and in doing so preserves methylation patterns through DNA replication. Its dysfunction can lead to tumorigenesis through hyper- or hypomethylation of critical regulatory regions of the genome.

MutS α is a mismatch repair protein that binds to mispairings and insertions produced during replication, and also recruits other proteins to the site of the mismatch. There is a growing body of research that links DNMT1 and MutS α , but how this interaction relates to way that either protein acts on DNA remains unclear.

We developed a fluorescence-based assay to study this protein-protein interaction, which measures the 5mC methylation activity of DNMT1 on a oligonucleotide that contains both a hemimethylated CpG site and a G/T mispair.

Three DNMT1 constructs were expressed and purified, the activities of two of these were tested on the substrate, viable reaction conditions were established, and finally, the effect of adding MutS α to the purified system was tested.

This project was a part of the 2019 BSURF program at the University of Iowa, and funded in part by an NSF grant. On a personal note, I recognize that it could not have been successful without Dr. Spies' generosity with regards to her time and resources.

INVESTIGATION OF THE MECHANISM OF ACTIVATION OF THE RET RECEPTOR USING GDNF-DERIVED PEPTIDES

Victoria Jordan¹, Paula Ortet¹, Melesilika Finau¹, Aaron Beeler¹, Adrian Whitty¹

¹Department of Chemistry, Boston University, 590 Commonwealth Ave, Boston, MA, 02215

The Rearranged during Transfection (RET) receptor tyrosine kinase signaling pathway is important for the regulation of many biological functions, making the development of RET modulators potentially valuable for disease therapy. Glial cell-line derived Neurotrophic Factor (GDNF) is one of five ligands that activates RET, acting via a glycoprophosphatidylinositol-linked co-receptor, GFR α 1. RET is activated when a pentameric complex is formed involving one molecule of the homodimeric GDNF, two of GFR α 1, and two of RET. However, the mechanism by which these components come together to activate RET is not well understood. My project explores whether dimerization of GFR α 1 in the absence of GDNF-RET contact is sufficient for RET activation, through the design of GDNF-derived peptide homodimers incorporating only the regions that bind GFR α 1.

Previous cell-based experiments performed in our lab have shown weak activation of RET signaling by a GDNF-derived linear peptide homodimer, consistent with the weak binding of this peptide to GFR α 1 ($K_D = 120 \mu\text{M}$). We hypothesize that increased RET activation can be achieved by improving the affinity of this peptide for GFR α 1. A library of GDNF-derived peptides has been designed with sequence mutations and structural modifications to serve as a tool to explore the RET mechanism of activation. We report a systematic screen of linear and cyclic GDNF-derived monomeric peptides performed via surface plasmon resonance (SPR), to determine the effect of structural constraints on binding affinity. We show that peptide dimerization significantly improved binding affinity to GFR α 1, and conformational stabilization via cyclization resulted in stronger binders compared to linear peptides.

The optimized peptides will be investigated in cellular assays to determine their ability to activate RET downstream signaling. This work will expand our understanding of RET's mechanism of activation and serve as precursors to the development of the first non-protein RET agonists.

This work was supported by grants from the Boston University Undergraduate Research Opportunities Program (UROP), to VJ and MF, a National Science Foundation Graduate Research Fellowship (DGE-1247312) to PO, and by a grant from Boston University's Kilachand Honors College (KHC) to MF.

QUANTITATIVE DNA METHYLATION ANALYSIS OF PHOTORECEPTOR NEURON CIS REGULATORY ELEMENTS

Calvin Chenault¹, Penelope-Marie Clanor¹, Ray Enke¹

¹Department of Biology, James Madison University, 800 S Main Street, Harrisonburg, VA 22807

DNA methylation is an epigenetic modification that contributes to regulating gene expression by the process of an addition of a methyl group to cytosine nucleotides in genomic DNA. In retinal neurons, the accumulation of DNA methylation on gene regulatory sequences has been identified having an inverse relationship with gene expression. The cone-rod homeobox (CRX) transcription factor is a critical regulator of photoreceptor (PR) neuron differentiation and maturation. CRX functions by binding to PR-specific cis-regulatory elements and recruiting histone acetyltransferases that ultimately remodel local chromatin and initiate transcription. Much less is known about how CRX binding to regulatory elements is regulated and how DNA methylation impacts this affinity. The goal of this project is to quantitatively measure DNA methylation at CRX binding regions (CBRs) that regulate PR-specific genes. DNA collected from cone and rod enriched postmortem human retina was collected and treated with sodium bisulfite (BS) to mutate unmethylated cytosines into uracil, leaving 5mCs in the genome intact. Following BS treatment, CBRs were amplified via PCR, and DNA methylation was measured using quantitative pyrosequencing. Preliminary results demonstrate two different methylation patterns, rod-specific methylation, and an intermediary pattern, suggesting CRX may be acting as a transcriptional repressor at certain loci. Current experiments are focused on validating predicted CRX binding sites using gel shift assays.

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EXPRESSION AND PURIFICATION OF WILD TYPE AND MUTANT HUMAN UBA5

Grant Bradley¹, Chris E. Berndsen, Ph.D.¹

¹Department of Chemistry and Biochemistry, James Madison University, 700 South Main Street, Harrisonburg VA 22801

The human Ubiquitylation pathway is critical for maintenance of cellular homeostasis through multiple different processes, notably for the tagging of proteins for degradation. Ubiquitin activating enzyme 5 (Uba5) is the E1 enzyme responsible for the initial activation step in the human Ubiquitylation pathway. Little research has been done into the mechanism of Uba5. The goal of our project is to identify specific amino acids in Uba5 that are essential to its mechanism of action. We have identified potential catalytic amino acids in the Uba5 dimer using YASARA modeling, and five of these mutations were transformed into BL21 competent cells. The mutations are currently being purified, and mutants will be assessed for activity and structural characteristics using activity assays and circular dichroism (CD). These data will then be compared to the wild type version of Uba5, and, hopefully, conclusions will then be made about the contributions of the affected amino acids to the overall function of the protein. A purification method utilizing fast protein liquid chromatography (FPLC) was developed using an imidazole gradient of 10mM-300mM, and wild type Uba5 has been consistently purified. Samples of wild type Uba5 were sent for small angle X-ray scattering (SAXS), however the data do not provide much clarity to the structure due to aggregation of the protein. In addition, we will focus on efforts to improve SAXS data quality.

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JMU Department of Chemistry and Biochemistry
Berndsen Lab group
Shveta Gupta

SUBCLONING AND TRANSFORMATION OF *CAMPYLOBACTER JEJUNI* SIALIC SYNTHASE INTO A BACTERIAL EXPRESSION

Charlene Shoetan¹, Pumtiwitt McCarthy¹

¹Department of Chemistry, Morgan State University, 1700 East Coldspring Ln, Baltimore MD. 21251

The bacteria *N. meningitidis* is a leading cause of meningitis, which is a fatal infection of the thin lining that surrounds the brain and spinal cord. The current vaccines are polysaccharide-based, glycoconjugate, combined conjugate and protein-based vaccines. These vaccines fight against meningitis by targeting 5 of 6 serogroups of the pathogen (serogroups A, B, C, W, and Y). Glycoconjugate vaccines are made of microbial capsular sugars covalently linked to a carrier protein. This research focuses on serogroup W which contains a polymer of galactose and sialic acid in its capsule. Our goal is to learn more about the serogroup W capsule polymerase enzyme that creates the polymer so that we can control sugar length and use these sugars in glycoconjugate vaccines. To better understand the capsule polymerase, modified versions of sialic acid are enzymatically synthesized to provide information about how the enzyme works. An enzyme essential to this is Sialic Acid Synthase. Here we describe progress in subcloning of this gene (derived from *Campylobacter jejuni*). The desired DNA sequence was obtained commercially. Primers were designed to add restriction enzyme sites, NotI and PvuI for PCR was performed, and amplification was successful as determined by DNA gel electrophoresis. The band was isolated, and the DNA was extracted from it. Concentration of the PCR product (51.1 ng/μl) was determined by UV-Vis spectroscopy. A restriction enzyme digest was performed on the vector (pH6HtN-His6 Halo-Tag) and the DNA was purified in the same way as the PCR product. The concentration was determined to be 48.4 ng/μl. The PCR DNA and vector were ligated to form the recombinant plasmid, and this was transformed into KRX cells. The colonies grew overnight as observed on LB-Amp plates. In future work, the DNA will be isolated from these colonies and sequenced.

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AN ANTI-MESOTHELIN RECOMBINANT IMMUNOTOXIN BASED ON DEOXYRIBONUCLEASE-I

Ruby Sprehe¹, Jillian Baker¹, John Weldon¹

¹Department of Biological Sciences, Towson University, 8000 York Rd, Towson, MD 21252

Recombinant immunotoxins (RITs) are fusion proteins consisting of a toxin attached to an antibody fragment. Unlike traditional chemotherapy, RITs used in cancer therapy can be specifically targeted to tumor cells while sparing healthy cells. One shortcoming of RITs is their immunogenicity, which is the immune response of patients to treatment with RITs. Patients with intact immune systems often produce antibodies to the toxin that neutralize its function. For this experiment, we attempted to prepare a RIT using deoxyribonuclease- I (DNase-I) instead of a typical bacterial or plant-derived toxin. DNase-I is an endogenous human protein that catalyzes the degradation of DNA into its component nucleotides. Previous research shows that DNase-I can be toxic to cells if delivered through targeted endocytosis. Furthermore, we predict that DNase-I will be less immunogenic than other toxins, because it is a human protein. We successfully cloned an expression vector joining DNase-I to an anti-mesothelin antibody fragment and expressed it in *E. coli*. The resulting protein, however, was unstable and we were unable to purify it after refolding from inclusion bodies. We concluded that the design of the fusion protein needs improvement to enhance its stability. It is also possible that the refolding conditions need further optimization. Future directions include exploring both of these possibilities.

I would like to thank the Towson Bridges Program and CCBC for the opportunity. Thank you to Dr. Laing, Dr. Snyder, Dr. Berlyn and Trudymae for guidance throughout the program. Thank you to the NIH Bridges Grant 5 R25 GM058264 16 for funding. Special thanks to Dr. Weldon, Jillian Baker and all students that have helped throughout the project.

DETERMINING THE DEPENDENCE OF BRG1 MUTANT LUNG ADENOCARCINOMA ON DNA STRUCTURAL PROTEINS

Tobias Coombs¹, Carla Concepcion², Tyler Jacks²

¹Department of Chemistry and Biochemistry, UMBC, 1000 Hilltop Cir., Baltimore MD. 21250

²Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, 77
Massachusetts Ave, Cambridge, MA 02139

Epigenetic regulation of gene expression is known to play a critical role in the development of different types of cancer. One such regulator is the SWI/SNF chromatin remodeling complex. Loss of function mutations in the Brg1 subunit of this complex have been found in 6-8% of lung adenocarcinoma patients.

Correlations have been observed between Brg1 mutant tumors and increased motif accessibilities for the Ctf and Yy1 transcription factors in a mouse model of lung adenocarcinoma. This led to the hypothesis that Brg1-mutant cells may have increased dependence on Ctf and/or Yy1 function.

To test this hypothesis, we sought to reduce the protein levels of these transcription factors in Brg1 wild-type and mutant cancer cells and measure the competitive advantage of the various cell lines in an in vitro competition assay.

CRISPR single guide RNAs (sgRNAs) were designed to generate loss of function mutations in these genes and cloned into lentiviral constructs. These constructs were then used to produce a lentivirus. Cas9-expressing lung cancer cells were infected with the virus, and a series of western blots were used to identify the most effective guides. These sgRNAs were then subcloned into a lentiviral construct carrying a fluorescent reporter, and once again used to generate virus. Approximately 50% of Brg1 wild type and mutant cancer cells were infected with the virus. The change of the percentage of the guide-expressing cells, measured by the fluorescent reporter, was observed over time by flow cytometry.

This research was supported by the Bridge Project, the KI 5th anniversary fellowship, the ACS fellowship, the MSRP Bio program, and the NIH/NIGMS MARC U*STAR T34 HHS 00026 National Research Service Award.

CORRELATION BETWEEN PREVALENCE OF CORYNEBACTERIUM AND AGE

Tyler Autrey¹, Angela Kim¹, Amir Ledbetter¹, Laura Ott^{1,2}

¹ STEM BUILD, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Mathematical and Natural Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Determining the changes that the human body experiences during the aging process are important for expanding the average life span. An aspect of aging that is poorly understood is how the microbial community changes as humans age. The microbiota or the community of microorganisms on/in a living thing, affects the function of the body. To understand the effect of age on Corynebacterium prevalence, we conducted a metagenomic experiment based off the data from a previous experiment where the microbial communities of different individuals were collected. The application Phinch was utilized to display the 273 samples collected in the best way possible to understand the correlation between age and Corynebacterium. We hypothesized that the amount of Corynebacterium would increase as humans age, with Corynebacterium being most prevalent in the oldest age group. This is due to our assumption that as humans age their immune system weakens. We developed a line graph to compare taxonomy occurrence of different age groups: 0-18, 19-35, 36-50 and 51-99. After analyzing the data, we came to the understanding that the data refuted our hypothesis. This suggests that there is no correlation between the prevalence of Corynebacterium and age. However, age group 0-18 has significantly less amount of Corynebacterium compared to three other groups. This experiment prompts others to look into other factors that may alter the prevalence of Corynebacterium such as region, wash frequency, and hormone production.

This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989. We would also like to acknowledge Dr. Rob Dunn allowing us to use the data from his experiment. We would also like to acknowledge Dr. Maria Cambria and Dr. Fred Larabee for being crucial resources in the creation process of our abstract.

ISOLATION OF BACTERIOPHAGES FOR *STAPHYLOCOCCUS AUREUS* FROM UNIVERSITY GROUNDS

Funmilayo Omole¹, Muyideen Haruna¹, Mathumathi Rajavel²

¹Biology, Morgan State University, 1700 East Cold Spring Lane, Baltimore, Maryland 21251

²Biology/Medical Laboratory Sciences, Morgan State University, 1700 East Cold Spring Lane, Baltimore, MD 21251

University athletic facilities have been the source of community acquired infections among students in many college campuses by sharing a common athletic equipment. Last year, we surveyed our university athletes training room facility, and we had identified the presence of bacterial community including *Staphylococcus aureus* NBRC100910; 12600 and *Staphylococcus cohinii* on selective equipment surfaces confirmed by 16S rRNA sequencing.

In this current study, our objective is to first isolate lytic bacteriophages that are effective against these two specific *Staphylococcal* pathogens and subsequently determine its host range specificity. Toward this, we first collected 15 soil samples (MSU1-15) from various locations at our university grounds. Next, we employed our laboratory standardized methods for phage isolation and determined its efficacy by spot test method. Our preliminary results indicated positive for lytic phages presence, from 5 samples supernatant labeled Mφ-2, Mφ-6, Mφ-8, Mφ-9 and Mφ-13 for the *Staphylococcus aureus* NBRC100910; 12600.

We also performed phage enrichment for this isolate by mixing each of the soil samples with *Staphylococcus aureus* NBRC100910; 12600 and after overnight incubation, samples were centrifuged and the supernatant filtered with 0.45 micron SFCA membrane filters. These supernatants tested by spot test method on the indicator bacteria yielded positive for lytic phages presence from 4 samples labeled Eφ-3, Eφ-5, Eφ-14, and Eφ-15. Additional plaque purification of these phages are warranted prior to further characterization and thus are under investigation at present. Similar efforts to isolate phages using MSU1-15 samples on our lab isolate *Staphylococcus cohinii* yielded no phages.

In conclusion, we used *Staphylococcus aureus* NBRC100910; 12600 as bait and have successfully isolated bacteriophages for this isolate from our university grounds.

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PCR ANALYSIS AND CLONING OF NAP OPERONS PRESENT IN A WICIMICO COUNTY WATERSHED

Justin Bussard¹, Katherine Miller¹

¹Department of Chemistry, Salisbury University, 1101 Camden Avenue, Salisbury, MD 21801

The denitrification process takes excess nitrate and turns it into nitrogen gas through a series of reactions catalyzed by different enzymes within bacterial communities. Nitrate reductases are the enzymes which turn nitrate into nitrite during the first step of this process. The enzyme complex Nap is one of these nitrate reductases, specifically the complex which can be expressed in both aerobic and anaerobic environments. Nap operons code for large periplasmic complexes with multiple subunits that have differing sizes and functions. NapA is the largest structure with the job of binding the nitrate to a molybdenum center as the active reduction site. NapB and napC are both seen as necessary support structures that transfer electrons onto napA through a series of c-type cytochromes and Fe-S complexes that are encased within the protein structures. The operons may come in one of three forms, napAGHB, napABC, and napAGHBC. Each operon structure will be a unique size when isolated using polymerase chain reaction (PCR) and gel electrophoresis. The primers sets used in this experiment were napA349F/ napB393bR and napA349F/ napC665R. These sets should amplify fragments which contained napAB, napAGHB, napABC, and napAGHBC at expected band sizes of 2500 bp, 4200 bp, 3300 bp, and 5000 bp from soil extracted DNA. The results of this experiment were that the napAB and napAGHB fragments were successfully found and isolated with verification by gel electrophoresis. The fragments which were expected from the napA349F and napC665R primer set were not found as it is believed that the rear primer did not successfully bind. Future experiments will be focused on cloning representatives of each operon class into an expression plasmid in order to study the function of the different nap operon.

DISCOVERING THE ROLE OF BIG TAU IN NEURONAL GENOMIC DNA PROTECTION WITH CRISPR-CAS9 CELL LINES

Paulina Bauer¹, Terrie Rife¹

¹Department of Biology, James Madison University, 800 South Main Street, Harrisonburg, VA 22807

Tau proteins are microtubule associated proteins that might be essential for the stability of neuronal cells in the brain. In neurons of those with Alzheimer's (AD), tau proteins are hyperphosphorylated and lose their affinity to microtubules, thereby causing neuronal instability. Because tau has been found to bind to DNA, tau might also play a role in the protection of neuronal genomic DNA from oxidative stress, which further suggests that tau could be related to the DNA oxidative damage observed in the AD brain. Peripheral nervous system (PNS) neurons contain an isoform of tau called "big tau" that is not found in central nervous system neurons. Big tau contains extra inserts in its proline rich region, a region that has been suggested to contribute to DNA binding in non-big tau isoforms. PNS neurons have also been suggested to be more prone to DNA damage, thus we predict that big tau increases protection against DNA double strand breaks. SK-N-MC cells are human cultured neuroepithelioma cells that make a good model to study the role of big tau, as they contain all isoforms of tau and can be made susceptible to DNA double strand breaks through treatment. To study the function of the big tau in DNA protection, CRISPR-gRNAs were designed to induce total and big tau knockouts in SK-N-MC cells. gRNAs that were effective in inducing tau knockouts were identified with surveyor assays and western blots. Effective gRNAs were used to create stable tau knockout cell lines, and the process of verifying other, more effective gRNAs is ongoing. In established cell lines, comet assays will be used to quantitate DNA damage present after oxidative stress. It is expected that cells with total and/or big tau knockouts will have more DNA damage following stress than cells containing all of the tau isoforms.

We would like to thank The Department of Biology at James Madison University for providing the funds and equipment necessary for partaking in this research.

A RICE BETA-AMYLASE GENE WITH TWO PUTATIVE TRANSCRIPTIONAL START SITES APPEARS TO ENCODE A NUCLEAR TRANSCRIPTION FACTOR AND A CHLOROPLAST ENZYME

Claire Ravenburg¹, Christopher Berndsen², Jonathan Monroe¹

¹Department of Biology, James Madison University, 951 Carrier Drive, Harrisonburg, Virginia 22807

²Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, Virginia 22801

In most plants, starch accumulates in plastids during the day to provide the carbon and energy necessary to sustain metabolism at night. Starch hydrolysis is catalyzed by members of the β -amylase (BAM) family, which in *Arabidopsis thaliana* includes nine structurally and functionally diverse members; this work will focus on BAM2 and BAM7. AtBAM2 is a plastid-localized enzyme that is only active in the presence of KCl, and it is a tetramer that exhibits a sigmoidal substrate saturation curve with a Hill coefficient of over 3. Phenotypic analysis of T-DNA mutant plants suggests that AtBAM2 does not play a significant role in leaf starch degradation. AtBAM7 is a catalytically inactive, nuclear-localized transcription factor with an N-terminal BZR1-like domain. Sequence alignments show that the BAM domains of AtBAM7 and AtBAM2 are closely related. Analysis of the genomes of 46 flowering plants revealed 12 including *Amborella*; some monocots; and basal eudicots, that have a BAM7-like gene but lack a BAM2-like gene. Upon closer inspection, the BAM domains of these BAM7 genes are more similar to AtBAM2 than they are to the BAM domain of AtBAM7. They also share all of the functional residues that we identified in AtBAM2, some of which are different in AtBAM7. Moreover, an in-frame methionine near the 3' end of the first intron of these BAM7 genes is the putative N-terminus of BAM2-like proteins that begin with predicted chloroplast transit peptides. We hypothesize that these genes contain two transcriptional start sites that drive the expression of two functionally different proteins. Using rice as a model, we designed cDNAs of the long and short forms of rice BAM7 (OsBAM7) for expression in *E. coli*, and preliminary evidence suggests that the purified short form of OsBAM7 is catalytically active and shares properties with AtBAM2 such as KCl sensitivity.

This work was funded by the National Science Foundation RUI-1616467 grant to JM and CB as well as an American Society of Plant Biologists Summer Undergraduate Research Fellowship to CR.

CHARACTERIZATION AND OPTIMIZATION OF DESULFINASE FOR THE REMOVAL OF RECALCITRANT SULFUR COMPOUNDS IN PETROLEUM

Cameron Dodd¹, David Allely¹, Alanna Hutchison-Lundy¹, Dean Kadir¹, Linette Watkins²

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive
Harrisonburg VA 22807

²Department of Biology, James Madison University, 951 Carrier drive, Harrisonburg Va 22807

Biodesulfurization provides a method to remove the sulfur from organosulfur compounds found in petroleum. This multi-step pathway is able to remove sulfur from dibenzothiophene (DBT), one of the most difficult to remove compounds found in petroleum and crude oil. A critical enzyme in this pathway is an aromatic desulfinase (DszB) that catalyzes the final rate-limiting step of the desulfurization. The DszB enzyme from *Nocardia asteroides* A3H1 was overexpressed in *E. coli*, purified using Ni²⁺-affinity chromatography and characterized kinetically. Kinetic assays revealed a sigmoidal response when the velocity was plotted against [S], calling into question the monomeric structure of the enzyme previously reported by crystallography studies. Size exclusion chromatography and cross-linking experiments were conducted to further investigate the oligomerization of HPBS desulfinase. Attempt to further purify the enzyme using Co²⁺-affinity chromatography provided further evidence that under certain conditions DszB may form multimeric structures.

FUNCTIONAL ANALYSIS OF THE WLS OLIGOMERIC STATE

Nicholas Finelli¹, Erica Selva¹

¹Department of Biological Sciences, University of Delaware, 210 South College Ave. Newark, DE 19716

The Wnt signal transduction pathway plays critical roles in organismal development and the maintenance of tissue homeostasis. Disruptions or abnormalities in the Wnt pathway can lead to various diseases such as developmental defects, osteoporosis, pulmonary fibrosis, type II diabetes and cancer. Therefore, obtaining a better understanding of the molecular mechanism that drives this signaling pathway could have significant implications for human health. This evolutionarily conserved signaling pathway is strictly regulated in both signal-producing and -receiving cells. Wnts, the signaling ligand of the pathway, are trafficked through the secretory pathway by the chaperone protein Wntless (Wls). Recent co-immunoprecipitation studies performed in the Selva laboratory using epitope tagged versions of *Drosophila* Wls and truncations revealed Wls oligomers are required for Wingless (Wg, the prototypical *Drosophila* Wnt ligand) binding and trafficking from the Endoplasmic Reticulum (ER) for release at the Plasma Membrane (PM) for downstream signaling. We also found that Wls oligomers form both in the and absence of Wg. These studies revealed Wls oligomers likely form through intermolecular disulfide bonds between conserved cysteines in Wls. In this study, I used reducing and nonreducing polyacrylamide gel electrophoresis and western blotting to examine oligomerization of full-length WlsHA and the truncation, Wls223F, in the presence and absence of Wg. We found the truncation Wls223F readily formed dimers and higher order structures, while full-length WlsHA also formed dimers, but at a lower level. The influence of Wg on Wls oligomerization will be discussed. These findings support our previous co-immunoprecipitation findings.

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ENGINEERING OF MELTING TEMPERATURES OF CARBOHYDRATE BINDING DOMAINS

Makhalia Aiken¹, Kathleen DeBiasse¹, Craig Laufer¹

¹Department of Biology, Hood College, 401 Rosemont Avenue, Frederick MD, 21701

Biofuels are attractive, sustainable, alternatives to fossil fuels. Second generation biofuels use lignocellulosic materials, such as agricultural wastes, as feedstocks to produce transportation fuels. Although the feedstock is very inexpensive, the processing, particularly the cost of the enzymes to break the biomass down into simple sugars is expensive. One way to notably reduce the cost of this overall process would be to reuse enzymes. This research study is to modify carbohydrate binding modules (CBMs) from cellulases to facilitate their recovery for use in multiple biomass digestion reactions. By engineering a CBM with a melting temperature (T_m) that is below the T_m of the native thermostable enzyme we could thermally control the binding of the enzyme to switch on and off in order to facilitate enzyme recycling. CBMs were fused with super-folder green fluorescent protein and various mutations were introduced that we predicted would lower the T_m but not interfere with ligand binding. These mutants were compared to the wild-type CBM in a quantitative binding assay with cellulose beads as a ligand to test the binding ability of the CBMs at various temperatures.

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XPD HELICASE MUTATIONS AND THE PATHOGENESIS OF PREECLAMPSIA

Gillian Dea¹, Joshua Sokoloski¹

¹Department of Chemistry, Salisbury University, 1101 Camden Avenue Salisbury MD 21801

Preeclampsia is a maternal hypertensive disorder that affects 3-8 percent of women during their second or third trimester of pregnancy and remains one of the leading causes of maternal fatalities. Despite its worldwide impact, neither a cure nor screening test for preeclampsia has been determined. This could be attributed to the lack of animal model and the inability to predict a woman's predisposition for the disease. One characteristic of preeclampsia is the high amounts of reactive oxygen species, which in high amounts inevitably cause DNA damage. XPD is a helicase protein responsible for recognizing DNA damage as it unwinds the helix during the transcription step of DNA replication. A problem or inability to perform this function could result in a cascade of negative effects in the body resulting in preeclampsia. In this study, I am expressing and purifying both *Thermoplasma acidophilum* and human XPD proteins. In previous studies from the literature, common mutations in the genetic structure of XPD were found in women who later developed preeclampsia, and three of these variations are to be tested against the known activity of the unmutated XPD to determine the effect of these mutations on the protein. Wild-type and mutant proteins are being tested with a fluorescence based helicase assay to determine the effects of these mutations on XPD function.

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DIVERSITY OF BACTERIAL SPECIES THROUGHOUT GEOGRAPHIC REGIONS OF THE UNITED STATES

Amanda Chen¹, Bernardo Sola¹, Zachary Willis¹, Laura Ott^{1,2}

¹STEM BUILD, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Natural and Mathematical Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Identifying the bacterial species found on the human body highlights the advantages to human wellbeing, leading to beneficial discoveries regarding human hygiene and health. The problem, however, lies in the many factors that affect the variations of species. External factors pertaining to geographic location can influence the development of varying adaptations. Under the speculation that different bacterial species may arise under varying conditions found in different locations, we hypothesized that the southern region of the United States would have a wider variety of uncommon species in comparison to other regions. Using Phinch, a data visualization software, a previously collected metagenomics data set was analyzed and organized by geographic region: Northeast (n=10), Midwest (n=13), West (n=28), and South (n=196). For each region, the top ten bacterial species were identified and compared. Several bacterial species, such as *Corynebacterium* and *Staphylococcaceae*, recurred across all regions as some of the most commonly observed. Even though *Corynebacterium* was the most common species in three of the four regions, it was found to be the second most prevalent in the South, second only to *Staphylococcaceae*. Furthermore, the South had four species that were unique to its region's top ten, unlike the other regions, which only had two unique species. While the results indicate greater diversity in the South, it is possible that this outcome is due to the uneven distribution of subjects in our study. For a more accurate test, another study should be conducted where the data from every region is distributed equally.

We would like to acknowledge Dr. Rob Dunn (NCSU) for providing the data set and Dr. Larabee and Dr. Cambraia for their constructive review on this abstract. This student research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers RL5GM118987, UL1GM118988, and TL4GM118989.

THE PENN STATE PROTEIN LADDERS: AN INEXPENSIVE SOURCE OF PROTEIN MOLECULAR WEIGHT MARKERS APPROPRIATE FOR BOTH COOMASSIE STAINING AND WESTERN BLOTTING

Ryan Santilli¹, Jack Williamson¹, Rosalie Sowers¹, Yoshitaka Shibata¹, Andrew Fleischman¹
Song Tan¹

¹Department of Biochemistry and Molecular Biology, The Pennsylvania State University,
University Park, Pennsylvania, 16802

Although protein molecular weight markers are among the most commonly used reagents in molecular biology laboratories, simple and inexpensive means to produce such markers are lacking. We have therefore designed and prepared recombinant proteins of defined molecular weights from 10 to 150 kDa that migrate appropriately on SDS-PAGE gel. Each protein was selected for high level expression in *E. coli* and contains a HIS-tag for efficient metal-affinity purification. In addition to detection by Coomassie or silver staining, each protein contains an IgG binding domain and is therefore visible on Western blots without specific second antibodies. The Penn State protein ladder system should provide an inexpensive method to produce protein molecular weight markers for the research laboratory.

This work was supported by Penn State Eberly College of Science Undergraduate Research grants and NIH grants R01GM088236, R01GM111651 and R35GM127034 to S.T.

INHIBITING CALPAIN DEPENDENT DEGRADATION OF DESMOPLAKIN

Kendahl Ott¹, Taylor Albertelli¹, Maegen Ackermann², Heather Manring³, Nathan Wright³

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive,
Harrisonburg, Virginia 22807

²Department of Chemistry and Physics, Mansfield University, 76 Stadium Drive, Mansfield,
Pennsylvania 16933

³Department of Physiology and Cell Biology, Ohio State University, 370 W. 9th Avenue,
Columbus, Ohio 43210

Desmoplakin (DSP) is a large (260 kD) protein found in the desmosome, a subcellular complex that links the cytoskeleton of one myocyte to that of its neighbor. In cardiomyocytes, desmoplakin's main function is to maintain cell-to-cell adhesion and synchronization during heart contractions. A mutation hot-spot, centered around the SH3 domain of DSP, is associated with arrhythmogenic cardiomyopathy, but the underlying mechanism(s) of this association isn't well studied. Here, we show that many of these disease-causing mutations display increased calpain sensitivity. Additionally, structural and computational studies on DSP variants show that this cleavage event is driven not through a gross structural change, but instead through the discrete exposure of a normally occluded calpain cleavage site. In silico and in vitro experiments suggest that through the mutation of a secondary residue- L518Y- reduces calpain sensitivity. This 'molecular band-aid' approach shows promise as a template for future work designing molecules to rescue mutant desmoplakin in vivo.

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PROGRAMMED -1 RIBOSOMAL FRAMESHIFTING OF NANAY AND LAMMI VIRUSES

Julia Jager¹, Dina Elloseily¹, Jenan El-Hifnawi¹, Nuwaryra Mahreen¹, Riya Patel¹, Ridhi Singh¹, Carol Vieira¹

¹The First-Year Innovation and Research Experience, University of Maryland, 4254 Stadium Drive, College Park, Maryland 20742

In order to make an infectious viral particle, a specific ratio of structural and non-structural viral proteins needs to be present. Some viruses rely on programmed -1 ribosomal frameshifting (-1PRF) to control gene expression and ensure the proper protein ratio is obtained. A -1PRF signal consists of a heptameric slippery site, spacer, and secondary structure. When the secondary structure and the transitional ribosome interact, it results in a pause with the A- and P-site tRNAs over the slippery site. This pause allows the tRNA and ribosome to slip one base in the 5' direction causing the ribosome to continue to decode the message in the -1-frame. Therefore, if the efficiency of the -1PRF activity is changed due to a sequence mutation, the virus may become less infectious.

The Nanay Virus (NANV) and the Lammi Virus (LAMV) are transmitted through mosquitoes, infecting humans and mammals. Since NANV is in the same family as the Yellow Fever Virus (Flaviviridae family) it can cause similar symptoms. LAMV can cause encephalitis. We hypothesize that the predicted -1PRF signals in NANV and LAMV promote efficient frameshifting.

To test this, the predicted viral frameshifting sequences were cloned into a dual-reporter plasmid containing Renilla and firefly luciferase. The sequence was placed in between Renilla luciferase, located at the 0-frame, and firefly luciferase, located in the -1-frame. Firefly luciferase is only expressed when a -1 frameshift occurs. The resulting plasmids were transfected into human embryonic kidney (HEK293T) cells. Using a dual-luciferase assay, the efficiency of -1PRF was measured. The -1 PRF efficiencies were 2.87 % and 2.60% for NANV and LAMV, respectively.

Future experiments will include mutating the viral sequences in an effort to decrease -1PRF efficiency and infectivity. If successful, the weakened virus may be used to develop a live attenuated vaccine against the virus.

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SIMPLE AND AFFORDABLE KINETIC ASSAY OF NUCLEIC ACIDS BY GEL STAINING

Danielle Guillen¹, Mika Schievelbein¹, Kushkumar Patel¹, Davis Jose¹, Jonathan Ouellet¹

¹Department of Chemistry, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ, 07764

In an effort to circumvent the pitfalls of using radioactive labeling to measure kinetics in undergraduate labs, a gel staining kinetics assay was developed. The IR-3 enzyme and substrate, a single stranded DNA that cleaves another single stranded DNA only in the presence of Zn^{++} , was used as a simple and inexpensive DNA model. The rate of the IR-3, or D-Zyme, was successfully determined by staining PAGE-Urea gels with SYBR Gold and the viewing them at 302 nm with epi-illumination. The open source software ImageJ (version 1.52K) was utilized to quantify the band intensities, the ratios of cleaved product to the addition of product and substrate gave percent cleavage. When percent cleavage was plotted against time in Minitab (version 18.1, Minitab, Inc.), the rate of the reaction could then be determined by fitting a nonlinear regression to the single exponential. To certify this technique, it was also tested on two other models, one being a modified version of the D-Zyme, while the other was a known Hammer Head Ribozyme, trans RzB. It was demonstrated that the use of SYBR Gold post-migration staining can be used to quantify RNA and DNA bands for cleavage kinetic analysis, without substrate labeling. Although this method is comparatively less sensitive than radioactivity or fluorescence labeling, it has several advantages such as: i) ease of use; ii) cost effective; iii) no half-life decay; iv) absence of substrate labeling; v) precision enabling curve fitting; vi) versatility with non-structured and structured RNAs or DNAs.

RNA APTAMER SELECTION AGAINST THE HUMAN EPIDIDYMIS 4 PROTEIN

Maie Abdel-Wahab¹, Clarice Pamplona¹, Ayse Ecer¹, Brittinie Hughes¹, Wade Winkler¹,
Catherine Spirito¹

¹First-Year Innovation and Research Experience, University of Maryland, College Park,
Maryland 20740

Ovarian cancer is the seventh most common cancer in women, and the fifth leading cause of cancer death in women. Currently, the two most common methods of diagnosis include TVUS (transvaginal ultrasound) and a CA-125 antibody-based blood test. The latter detects the protein CA-125, the clinical “gold standard” for detecting ovarian cancer, yet continues to raise concern for its efficacy. This work focuses instead on utilizing the Human Epididymis 4 Protein (HE4) as a potential biomarker for early detection of ovarian cancer. HE4 is overly expressed by epithelial ovarian cancer cells. This project aims to use RNA aptamers as the basis of detection of HE4. The Systematic Evolution of Ligands by Exponential Enrichment (SELEX) protocol was used to enrich for aptamers for the HE4 protein. Two selections were run in parallel against a 6His-MBP tagged HE4 protein using Ni-NTA magnetic beads. TOPO-TA Cloning and Sanger sequencing of the final selection pool will be used to determine aptamer candidates for further evaluation.

This research was supported by the First Year Innovation and Research Experience program at the University of Maryland.

CHARACTERIZING THE EFFECT OF MANIPULATING GLUTAMINE METABOLISM ON *ESCHERICHIA COLI* AND BACTERIOPHAGE REPLICATION

Daniel Kang¹, Ethan Snider¹, Jessica O'Hara¹

¹FIRE: First Year Innovation & Research Experience, University of Maryland, College Park, 2335 Atlantic Building, College Park, MD 20742-5031

When infecting a host cell, bacteriophage require specific host derived macromolecules in order to successfully replicate. However, the scientific community does not fully comprehend how bacteriophage manipulate certain host cell metabolic pathways during infection. Knowledge about the essentiality of glucose and glutamine for bacteriophage replication in *Escherichia coli* is limited. This pathway has been shown to be critical in other DNA viruses, such as vaccinia virus and human cytomegalovirus, and so we set-out to further explore this in *E. coli* bacteriophage.

In order to investigate the role of host cell glutamine metabolism, we manipulated the *E. coli* host cell by knocking out specific genes: *glsA* and *glsB*. These genes both encode glutaminase enzymes that catalyze the degradation of glutamine into glutamate, which is used to replenish the TCA cycle through anaplerosis. We additionally modified the growth media that the *E. coli* and bacteriophage replicate in by using minimal media and limiting carbon sources to glucose and glutamine. Under these different conditions, we characterized *E. coli* and bacteriophage replication.

Our results further characterize the role of *GlsA* and *GlsB* in *E. coli* metabolism and replication. In minimal M9 media, Δ *glsA* conveyed deficits in replication confirming its significant role in glutamine metabolism. In contrast, Δ *glsB* does not exhibit similar growth deficits in the minimal M9 media confirming a minor contribution in glutaminolysis. Characterization of T-even bacteriophage (T2, T4 and T4r) replication in *E. coli* demonstrated a significant reduction in viral replication in the minimal M9 media supplemented with glucose and glutamine. Our results confirm the role of *GlsA* in *E. coli* host cell glutamine metabolism and suggests the importance of host cell macromolecules for bacteriophage replication. Additional work will aim to further elucidate the importance of glucose and glutamine metabolism for bacteriophage replication using the Δ *glsA* and Δ *glsB* *E. coli* strains.

Financial support for this project was provided by the First-Year Innovation and Research Experience at the University of Maryland, College Park.

THIRD GENERATION SEQUENCING OF EGFR MRNA

Sawyer Hicks¹, Ryan Fink¹, Martin Hicks¹

¹Department of Biology, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults with a mean survival of 14-15 months. 60% of GBM tumors are characterized by the dysregulation, upregulation or constitutive activation of epidermal growth factor receptor (EGFR), leading to tumor cell proliferation. With the advances in nanopore sequencing technology, we have developed a strategy to evaluate alternative splicing and polyadenylation patterns in the EGFR transcript. Our goal is to isolate alternatively spliced and polyadenylated lower abundant EGFR transcripts to characterize the expression of this oncogene. GBM cell lines, SKMG-3, U87MG, U118, and A172, were cultured in multi-layer flasks with total cell confluency of 87,500,000. RNA was isolated using the TRIzol™ Reagent RNA extraction protocol, treated with DNase1 to remove contaminating genomic DNA, and column purified. Biotinylated DNA probes, 26 nucleotides in length, targeting EGFR mRNA exon 2 were used to capture the mRNA transcript. A control RNA was used to determine biotinylated DNA probe efficiency by establishing a standard curve comparing known quantities of in-vitro transcribed EGFR mRNA and their associated cycle threshold (Ct) value. Captured transcripts were pulled down with streptavidin Dynabeads, washed and purified. Generation of cDNA by reverse transcription with random decamer versus EGFR specific primers were compared through qPCR analysis. Captured RNA will be sequenced by Direct RNA nanopore sequencing as well as by Direct DNA sequencing. By identifying lower abundant, less common variants of EGFR transcripts and alternative isoforms of EGFR, we expect to identify candidates for induction through antisense therapeutics to activate alternative splice sites through recruitment of splicing activators or inhibit critical splicing elements through the recruitment of splicing inhibitors.

SCREENING FOR DELAWARE'S ANAL CANCERS WITH AN EMPHASIS ON HPV SEROLOGY ANALYSIS

Osama Mahmoud¹, Fady Gerges², Malcolm D'Souza¹

¹Department of Chemistry, Wesley College, 120 N State Street, Dover, Delaware 19901

²Green Clinics Laboratory , 740 S New Street, Dover, Delaware 19904

During the last decade, HPV related anal intraepithelial neoplasia (AIN) has increased in prevalence and intensity (degree of dysplasia). Several risk factors have been implicated and specific cohorts in Delaware especially the Sussex County population are identified to be at increased risk. Studies have suggested that the rate of progression of high-grade anal intraepithelial lesions to invasive anal cancer is around 5% and anal cytology has been typically used to screen for AIN. However, very little correlation between anal cytology screening to actual pathology was observed.

To develop a reproducible clinical based screening methodology to include both 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.

Dr. Abboud's (S.A) efforts are acknowledged for aiding this research project by providing data, research literature, and laboratory facilities. This undergraduate research project was supported by the Delaware INBRE program, with an IDeA grant from the National Institute of General Medical Sciences- NIGMS (P20GM103446). Financial support was also provided by the State of Delaware.

QUORUM SENSING GENES INVOLVED IN THE REGULATION OF CELLULAR METABOLISM IN THE FISH PATHOGEN *YERSINIA RUCKERI*

Rebecca Hallameyer¹, Anna Jozwick¹

¹Department of Biological Sciences, Goucher College, 1021 Dulaney Valley Rd, MD 21204

Yersinia ruckeri, a gram-negative bacterium, is the cause of Enteric Red-mouth disease (ERM) in salmonid aquaculture worldwide. Due to the high economic losses generated by the pathogen, novel ways to control and/or prevent infection would prove beneficial. This study focused on the mechanism of bacterial communication known as quorum sensing, and how genes *yruI* and *yruR2*, putatively responsible for communication signal production and transcriptional regulation, respectively, within the bacterium to control specific behaviors of *Yersinia ruckeri*. Here, we first compared the wild-type, *yruI* and *yruR2* mutant strains using high-throughput carbon source utilization assays to determine which metabolic pathways are influenced by quorum sensing. Up-regulation of genes within the TCA and glycolysis cycles observed within the mutant strains suggesting a role of quorum sensing in the transcriptional regulation of these loci. To further investigate the transcriptional control, semi-quantitative expression analyses resulted in significant differences in L-aspartate, citrate and succinate metabolism, upon disruption of the intact quorum sensing system. These findings provide evidence that quorum sensing in *Y. ruckeri* may help control metabolic rates, although further analysis of expression at different growth stages and growth studies are still underway. This research provides insights on the basic biology of *Yersinia ruckeri* and may lead to the discovery of new disease-control mechanisms in aquaculture.

This work was funded by Goucher College Summer Science and Start-up Research Funding (A.J.).

ANALYSIS OF POTENTIAL HOUSEKEEPING GENES IN TARDIGRADES

Leanna Hodge¹, Elaine Farkas¹, Kristen Long²

¹Department of Chemistry, Mansfield University of Pennsylvania, 76 Stadium Dr. Mansfield, PA, 16933

²Department of Biology, Mansfield University of Pennsylvania, 76 Stadium Dr. Mansfield, PA, 16933

Tardigrades are interesting micro-animals with unique abilities that have garnered a growing interest from researchers. They are one of the most resilient animals in existence, with the ability to survive extreme conditions by entering a “tun” state when a severe environmental stress is applied. In this state, the tardigrade removes almost all of the water in its body, curling to form a dry husk. They are able to suspend their metabolisms, remaining as such for decades and exiting the tun state only once it comes into contact with water.

Tardigrades produce a variety of proteins that allow them to survive while in the tun state, and to survive exposure to harsh conditions. Understanding how these proteins function to stabilize the tardigrade when exposed to unstable conditions could be beneficial in many research fields. This includes stabilizing vaccines and other sensitive pharmaceuticals, which could be beneficial to people in developing nations and remote areas where lack of refrigeration is a notable problem.

The issue with protein and gene expression analysis lies in the utilization of β -actin as a housekeeping gene by researchers. It has been shown that relying on β -actin for this purpose can be worrisome because it is not constitutively expressed across exposure to different environmental conditions. In this research, expression levels of certain genes are quantified for their ability to act as housekeeping genes. These genes include RPS13; a ribosomal protein coding gene, SCK1; a gene required for trehalase activation, and both RAD50 and XRCC1; genes involved in DNA break repair, as well as Elongation Factor 1-alpha (EF1a) and β -actin. The expression of each gene will be quantified using Sybr Green technology and qRT-PCR. These genes will be quantified in tardigrades that have experienced environmental stress including animals that were exposed to sucrose solutions and NaCl solutions, and dehydration.

ANALYSIS OF THE SOLVOLYTIC RATES OF SYNTHETICALLY USEFUL LAURYL AND HEXADECYL CHLOROFORMATES

Anas Mahmoud¹, Victoir Cahoon¹, Malcolm D'Souza¹

¹Department of Chemistry, Wesley College, 120 N State St, Dover, DE 19901

Today's common analytical derivatization agents, alkyl chloroformates, are very noxious substances that have historically found use as asphyxiating gases during the 19th Century World Wars. This project analyzes their pseudo first-order kinetic rates in a variety of pure and aqueous organic solvent mixtures. The rates of reaction were completed at 25.0°C using the acid-base titration technique, and the esters studied are; ethyl chloroformate (C₂H₅OCOC₂H₅), propyl chloroformate (C₃H₇OCOC₂H₅), butyl chloroformate (C₄H₉OCOC₂H₅), nonyl chloroformate (C₉H₁₉OCOC₂H₅), decyl chloroformate (C₁₀H₂₁OCOC₂H₅), dodecyl chloroformate (C₁₂H₂₅OCOC₂H₅), and hexadecyl chloroformate (C₁₆H₃₃OCOC₂H₅). The project's main objective is to evaluate the impact of the lengthening of the carbon chain on the ester rates of reaction. In the esters studied, our results show that as the (alkyl) chain length is increased, the reaction rates tend to be quicker in the more nucleophilic solvents.

This project was made possible through support from an IDeA award from NIH-NIGMS (P20GM103446, DE-INBRE program), an NSF-EPSCoR award (OIA-1757353), an NSF (DUE) S-STEM grant 1355554 (Wesley College Cannon Scholar program), the NASA Delaware Space Grant Consortium (NNX15AI19H), and the State of Delaware.

THE EFFECT OF CELL CYTOSKELETAL TENSION ON CELL VOLUME REGULATION

Eliana Crentsil¹, Kai Yao², Nicolas Perez³, Shannon Flanary³, Lucia Sablich³, Sean Sun²

¹Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore MD, 21250

²Department of Mechanical Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218

³Department of Chemical and Biomolecular Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218

Cell volume regulation is critical to the maintenance of homeostasis in the body. Dysregulation of cell volume can disrupt organ function and potentially initiate tumorigenesis. Therefore, control of cell volume is vital to organ function. Various mechanosensitive proteins, such as the YAP/TAZ protein that has been found to be upregulated in a number of cancers, have been found to regulate cell volume; however, the mechanism by which cell volume regulation occurs requires further investigation. We hypothesize that cell volume regulation occurs via dynamic assembly and reorganization of the phosphorylated myosin light chain (pMLC), a proxy for cytoskeletal mechanical tension. We seek to determine the relationship between cell tension and cell volume by measuring the changes in cell volume that occur after cell tension is upregulated or downregulated. Y27632, a pMLC inhibitor, was used to downregulate cell tension by inhibiting the phosphorylation and subsequent activation of the myosin light chain. To measure the effect of increasing cell tension, we have developed a platform from polydimethylsiloxane that upregulates cell tension by stretching cells. Quantitative immunofluorescence is used to compare relative amounts of pMLC upon downregulating cell tension with Y27632 and upregulating cell tension via the stretching device. The amount of pMLC is compared, at the single cell level, to cell volume as quantified through a Fluorescence Exclusion Method. Preliminary findings suggest that cells treated with Y27632 have a smaller cell volume, indicating that cytoskeletal tension may play a role in regulating cell volume.

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TRPM1 EXPRESSION AND LOCALIZATION IN MOUSE IRIS

Jeremy Rice¹, Shane Chambers¹, Marquis Walker¹

¹Department of Biology, James Madison University, 800 South Main Street, Harrisonburg, Virginia 22807

In the eye of vertebrates, the iris functions to control the amount of light that irradiates the retina. Pupillary light reflex (PLR) is an involuntary light response in which the muscular tissue of the iris constricts in response to increases in ambient light intensity. Visual signaling that drives PLR is transmitted to the olivary pretectal nucleus by a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs). These light signals are finally transmitted from the brain to the iris sphincter muscle by oculomotor efferent fibers. Recently, it has been shown that isolated mammalian iris tissue can also directly respond to light. This intrinsic light-driven constriction is activated by the melanopsin photopigment. The terminal channel in this light-signaling cascade still remains unclear. Experimental tests from our laboratory strongly suggest that the transient receptor potential cation channel melastatin 1 (Trpm1) is required in iridial cells for pupil constriction. We hypothesize that Trpm1 cation channels are found within iridial cells and play a role in PLR downstream of melanopsin. To test our hypothesis, we have isolated and cultured iris sphincter muscle cells. We have used immunohistochemistry in our study to test the cellular requirement of the light-activated signaling components in cultured primary iridial cells. We hope to use these results as a foundation for identifying the expression of all signaling proteins that are required for iris function in the eye.

Acknowledgements: This research was supported by funds from a 4-VA grant.

IDENTIFICATION OF MICROGLIAL CELLS DURING CHICK EMBRYO BRAIN DEVELOPMENT AND HUMAN XENOGRAFT TUMOR FORMATION

Amy Lin¹, Deni Galileo¹

¹Department of Biological Sciences, University of Delaware, Newark, DE 19716

Glioblastoma (GBM) is the most aggressive and invasive human brain tumor, which despite advancement in surgery and immunology, only 10% of patients survive 18 months after diagnosis. The Galileo lab studies GBM, focusing on the role of the protein L1CAM on increasing the motility, proliferation, and invasiveness of GBM cells using in vitro cell tracking and a novel xenograft chick embryo brain tumor model. My work focused on microglial cells, which are resident brain immune system cells that are not well understood. Previous research in mice showed that microglial cells concentrated in areas with greatest cancer cell invasion. The first aim for my project was to find biomarkers that specifically identify microglial cells in normal chick embryo brain during development. The second aim was to use those markers to examine any potential interaction microglial cells have with xenograft tumors in the chick embryo brain. Chick embryo brains were fixed, frozen, and cut into serial cryostat sections or fixed and vibratome sectioned, followed by immunofluorescent staining using antibodies or lectins that identified microglial cells in other model organisms. The optimal staining protocol required overnight primary antibody/lectin with 0.1% Triton X-100 detergent and 5% normal goat serum in phosphate buffered saline. The antibodies and lectins that I investigated included Isolectin B4, Tomato (Lycopersicon esculentum) lectin, and CD45. Immunofluorescence analysis revealed CD45 to stain microglial cells with the most specificity. CD45 staining reveal microglial cell morphology to progressively change from a compact ameboid shape (E5) into ramified branched shape (E12 and E15). Instead of locating throughout the brain, microglial cells appeared to be located as clusters in certain areas. Also, there appeared to be a greater quantity of ramified microglial cells in chick brains with xenograft human tumors. Further investigations are needed to verify the increased presence of reactive microglial cells and their interaction with tumor cells.

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INVESTIGATING THE ROLE OF HOST CELL GLUTAMINE METABOLISM ON BACTERIOPHAGE REPLICATION

Danielle Gore¹, Haley Harrison-Ellis El¹, Jessica O'Hara¹

¹FIRE: First Year Innovation & Research Experience, University of Maryland, College Park, 2335 Atlantic Building, College Park, MD 20742-5031

During replication viruses take advantage of the host cell metabolism, altering it to enable the propagation of new virions. Previous research in the field has demonstrated the importance of glucose and glutamine for viral replication. However, there has been little work done to determine the preferred carbon source of *E. coli* bacteriophage -- the natural predator of *E. coli*. We set out to investigate the role that glutamine metabolism plays in the replication of lytic bacteriophage (T2 and T4r) using a strain of *E. coli* where one of the glutaminase enzymes, encoded by the *glsB* gene, had been removed. Since this enzyme plays a role in glutaminolysis we predicted that the removal of this gene would hinder bacteriophage replication.

Bacteriophage replication was characterized in both the Δ *glsB* strain and the wild type parent strain of *E. coli*. Our results demonstrated a reduction in T4r phage replication when host cell glutamine metabolism was altered, suggesting the importance of the glutaminase pathway for this bacteriophage. Interestingly, we obtained the opposite result for T2 phage, which appears to replicate at a faster rate in the Δ *glsB* strain when compared to the wild type parent strain. The results of these experiments were unexpected and future work will aim to further determine the role of host cell glutamine metabolism in T-even bacteriophage replication.

Financial support for this project was provided by the First-Year Innovation and Research Experience at the University of Maryland, College Park.

PHENOTYPIC ANALYSIS OF QUORUM SENSING MUTANTS IN *VIBRIO* *PARAHAEMOLYTICUS*

Jisoo Hong¹, Jessica Tague¹, E. Fidelma Boyd¹

¹Department of Biological Sciences, University of Delaware, 105 The Green, 118 Wolf Hall,
Newark, DE 19716

Quorum sensing (QS) is a type of bacterial cell-to-cell communication process, which regulates gene expression to reflect cell density. We used *Vibrio parahaemolyticus* (Vp) as our model organism to study the QS regulation pathway. *Vibrio parahaemolyticus* is a halophilic Gram-negative bacterium found in marine and estuarine environments that causes bacterial gastroenteritis when consumed through raw or undercooked shellfish.

In the canonical *Vibrio* QS pathway, at low cell density, QS response regulator LuxO is phosphorylated and activates sigma factor 54. Sigma factor 54 (also known as RpoN) recruits RNA polymerase to initiate transcription of the five small quorum regulatory RNAs (qrr1-qrr5). The Qrr sRNAs post-transcriptionally stabilize aphA and degrade opaR mRNA transcripts. OpaR is the QS high cell density master regulator that has previously been shown to positively regulate the production of a capsule polysaccharide (CPS) leading to an opaque and rugose colony morphology.

We investigated the role of the Qrr sRNAs in CPS production and motility. We examined four strains: wild type and three deletion mutant strains; Δ opaR, Δ qrr2 and a Δ qrr null mutant and conducted CPS, swimming, and swarming assays. Our results indicate different CPS and motility phenotypes among the four strains. The Δ qrr null and Δ qrr2 mutants presented a similar swimming phenotype but different CPS and swarming morphologies. Further work needs to be done to determine the regulatory role of each Qrr sRNA.

Charles Peter White Fellowship, University of Delaware

DETERMINATION OF TARGET GENES FOR MIRNA-124 AND THEIR FUNCTION IN THE NEUROMUSCULAR REGENERATIVE PROCESS IN *C. ELEGANS*

Scott Korte¹, Michael Turner¹

¹School of Natural Sciences and Mathematics, Mount St. Mary's University, 16300 Old
Emmitsburg Road, MD

Micro RNAs (miRNAs) are found within eukaryotic cells and organisms and are active in the silencing of genes via base pairing with the 3'UTR region of target mRNAs. Due to the irregularity of a miRNA's binding to the 3' UTR target sequence, candidate target genes must be validated experimentally. This project will focus on *Caenorhabditis elegans*, or *C. elegans*, as a model organism. The planarian possesses a rudimentary neuromuscular system, which will be injured via targeted laser ablation in order to measure the genetic response to injury from mir-124. In addition, target genes of mir-124 will need to be validated in order to create mir-124 mutant *C. elegans* populations prior to injury.

Dr. Christine McCauslin, Department Chair, Dr. Michael Turner, Research Advisor, Mount St. Mary's University Honors Program.

MITOCHONDRIAL LONG-CHAIN FATTY ACID β -OXIDATION IS REQUIRED FOR WNT-MEDIATED INCREASES IN BONE VOLUME

Conor Beil¹, Megan C. Moorer¹, Soohyun P Kim¹, Priyanka Kushwaha¹, Ryan C Riddle¹

¹Department of Orthopedic Surgery, Johns Hopkins University School of Medicine, 701-709 Rutland Avenue, Baltimore, MD 21205

Bone formation requires substantial energy-generating capacity especially during periods of rapid bone growth or in response to anabolic stimuli. In our previous work, we demonstrated that long-chain fatty acid β -oxidation is required for normal bone mass accrual and that Wnt signaling enhances fatty acid utilization by osteoblasts. In these studies, we examined the requirement for β -oxidation during Wnt-stimulated anabolism. Ablating the expression of carnitine palmitoyltransferase-2 (Cpt2), an obligate enzyme in mitochondrial fatty acid oxidation, resulted in a 60% reduction in oleic acid metabolism and a modest inhibition of osteoblast differentiation in vitro. However, when cultures were stimulated with Wnt3a (25ng/ml) the effect of Cpt2 loss of function was more profound as Wnt3a-stimulated increases in alkaline phosphatase activity and the expression of Runx2 and Col1a1 were completely abolished. As an in vivo counterpart to these studies, we crossed Sost^{-/-} mice, which exhibit increased skeletal fatty acid uptake, with mice lacking Cpt2 specifically in the osteoblast (Cpt2^{flox}; Ocn-Cre) to generate double knockouts and the appropriate controls. In agreement with our in vitro studies, microCT analysis of bone structure in the distal femur and L5 vertebrae revealed that the loss of Cpt2 function in the context of sclerostin-deficiency greatly diminished the acquisition of trabecular bone volume. Serum P1NP levels were reduced in the double knockouts when compared to Sost^{-/-} mice indicating a defect in bone formation. Appositional growth on cortical bone surfaces was not affected suggesting that substrate selection is compartment specific. Taken together these data reveal an increased requirement for osteoblastic long chain fatty acid oxidation in response to osteo-anabolic signaling.

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SOX17 REGULATES WHITE MATTER DEVELOPMENT THROUGH PROGENITOR CELL SIGNALING

Ashley Garcia¹, Brian McEllin², Elim Hong², Vittorio Gallo², Li-Jin Chew²

¹Department of the Arts and Sciences, Trinity Washington University, 125 Michigan Ave NE, Washington DC, 20017

²Center for Neuroscience Research, Children's National Medical Center, 111 Michigan Ave NW, Washington DC, 20010

Recovery from white matter disease is dependent on the successful maturation of oligodendrocyte progenitor cells to replace lost oligodendrocytes in demyelinating lesions. Understanding cellular factors which promote oligodendrocyte progenitor cell development may benefit strategies to improve cell regeneration in myelin disease. Sox17 is a transcription factor that promotes oligodendrocyte cell formation in cultured cells. Previous studies have shown increased Sox17 expression in newly formed oligodendrocytes of remyelinating brain lesions, suggesting a role for Sox17 in the development of oligodendrocytes. To determine whether Sox17 regulates the amount of white matter by promoting the maturation of progenitor cells we characterized two lines of mutant mice for changes in developing brain white matter. By breeding with the same floxed Sox17 allele (Sox17^{f/f}), CNPCre mice and PDGFRaCreER mice were used to generate two separate strains of Sox17-deficient mice: Sox17 genetically ablated at multiple stages of the oligodendroglial cell lineage (CNPcre) or only at the progenitor stage (PDGFRaCreER) induced postnatally. PCR genotyping is used to identify mutant mice in newborn litters. Once the mice have the gene, we proceeded to inject Tamoxifen into PDGFRaCreER;Sox17^{f/f} mice. Using immunohistochemistry for myelin proteins, white matter structures were observed to be thinner and oligodendrocytes were decreased in number in both mouse strains. Increased oligodendrocyte death was detected in Sox17 mutant mice. We also found Notch signaling to be decreased in Sox17 mutants. Using Western blotting of cultured oligodendrocyte progenitor cells, we determined whether Sox17 regulated the progenitor signaling mediator Notch, and whether Notch regulated survival and signaling. P-Akt(Ser473) was decreased by Notch inhibition, suggesting a role for Notch in supporting the survival of differentiating cells. Our results indicate that Sox17 ablation causes thinning of brain white matter, likely arising from the decreased production of oligodendrocyte cells, as well as their reduced survival. The functions of Sox17 in regulating oligodendrocytes and white matter size may be initiated in the progenitor cell stage through Sox17 regulation of Notch signaling.

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RESTORING SENSITIVITY OF RESISTANT MELANOMA CELLS TO KINASE INHIBITORS

Heather Buck¹, Ramon Martinez², Paul Shapiro²

¹Molecular Biology, Biochemistry & Bioinformatics Program, Towson University, 8000 York Road Towson, MD 21252

²Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy and Greenebaum Cancer Center, 20 Penn Street, Baltimore, MD 21201

For individuals diagnosed with melanoma, approximately 50% possess a BRAF kinase mutation that increases the rate of cell proliferation. Persons with stage IV melanoma have a 5-year survival rate of 15-20%, which is decreased by the presence of a mutation in this enzyme. Treatments seeking to inhibit BRAF at the ATP binding site often fail due to development of drug resistance, leading to poor survival rate.

The purpose of this study is to ascertain whether novel, small molecules block substrate binding sites of a down-stream extracellular signal-regulated kinase (ERK1/2) and can restore sensitivity to ATP-inhibiting treatment in melanoma cells that are resistant to the current clinically relevant combination therapies targeting mutated BRAF and MEK1/2.

A375 melanoma cells containing mutated BRAF were generated to develop acquired resistance to a combination BRAF and MEK1/2 inhibitor therapy (AZD6244/PLX4032). Parent and drug resistant cells were treated with various inhibitors in doses ranging from 0.2-25uM in both monolayer and spheroid cell cultures. Monolayer cell cultures were grown via standard practice in 96-well plates. Spheroid cultures were grown in 96-well, round-bottomed, low-attachment plates. Cell viability was determined using Cell Titer Blue assay in monolayer cultures, and via Cell Titer GLO assay in spheroid cultures.

Initial results indicate that cells had acquired resistance to AZD6244/PLX4032 but were still partially sensitive to ATP competitive ERK1/2 inhibitors, and that three of the tested compounds decreased cell viability in drug resistant cells but not in sensitive A375 cells in spheroid culture.

Further evaluation of dose response on resistant cells treated with combination therapy and small molecule ERK1/2 inhibitors may indicate that targeted inhibition downstream of BRAF and MEK1/2 effectors could potentially restore sensitivity to current kinase inhibitor treatment.

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THE ANALYSIS OF PHOSPHODIESTERASE CPDA IN *VIBRIO* *PARAHAEMOLYTICUS*

Chun-kit Shum¹, J. Kent Kwah¹, E. Fidelma Boyd¹

¹Department of Biological Sciences, University of Delaware, 118 Wolf Hall, 105 The Green,
Newark, DE, 19716

In *Escherichia coli*, the preferred carbon source is glucose; and when this carbon source is limited, activation of adenylate cyclase leads to an increase of intracellular cyclic adenosine monophosphate (cAMP). cAMP is a secondary messenger that binds to the global regulator cAMP receptor protein (CRP), together regulating a multitude of genes ranging from motility to central metabolism. cAMP-CRP allows for the utilization of secondary carbon sources when glucose is limited. In order to regulate cAMP levels, a phosphodiesterase, known as CpdA, converts cAMP into adenosine monophosphate (AMP), effectively reducing the levels of cAMP in the cell. The CRP and cAMP regulatory pathway have not been examined in *Vibrio parahaemolyticus*, a marine bacterium that is also a human pathogen.

A homologue of the *E. coli* CpdA, VP0428 is present in *V. parahaemolyticus*. In this study, a mutant strain of Δ cpdA was constructed utilizing Gibson assembly and homologous recombination. The 804-bp cpdA gene was deleted from the wild type strain and replaced with a truncated in-frame 21-bp fragment. The deletion of the cpdA gene in *V. parahaemolyticus* should lead to a build-up of cAMP, causing CRP to be constitutively active. To examine the effects of deleting the cpdA gene, a series of growth curves with different carbon sources were performed as well as motility (swimming and swarming) and capsule polysaccharide production assays.

THE ROLE OF DITYROSINE IN THE UV SENSITIVITY OF HAPLOID SPORES OF *S. CEREVISIAE*

David Kleiner¹, Melissa Gillis¹, Ameer Payton¹, Edward Winter², Aikaterini Skokotas¹

¹Department of Biology, Rosemont College, 1400 Montgomery Avenue, Rosemont, PA 19010

²Department of Biochemistry and Molecular Biology, Jefferson University, 233 South 10th Street, Philadelphia, PA 19107

Yeast spores are known to be resistant to many environmental factors including the mutagenic effects of UV light. The presence of dityrosine in their outermost spore wall may play a protective role by absorbing UV light. This study compares the UV sensitivity of haploid spores in three strains of *Saccharomyces cerevisiae*: a wild-type strain, a dityrosine-deficient *dit1* strain and a heterozygous strain. Treatment with glusulase to release the spores from the ascus resulted in free spores that were highly sensitive to UV light. Only 2% survival was observed after a 30 sec UV exposure compared to 50% survival in the non-glusulized spores. This suggests that the ascus provides UV protection by allowing some spores to hide from UV exposure and that dityrosine may not have a protective function.

ANALYSIS OF DOUBLE-STRANDED BREAK REPAIR IN *SACCHAROMYCES CEREVISIAE* UNDER SPACEFLIGHT CONDITIONS

Joseph Romanowski¹, Matthew Elko¹, Daniel Stoyko², Michael Law²

¹Department of Biology, Stockton University, 101 Vera King Farris Drive, Galloway, NJ 08205

²Department of Biochemistry and Molecular Biology, Stockton University, 101 Vera King Farris Drive, Galloway, NJ 08205

Due to the advancement of space age, all spaceflight-associated health risks should be studied in detail. Mistakes in double-stranded DNA break (DSBs) repair can lead to carcinogenesis. Previous studies reported contradictory results regarding the effect of microgravity on DSB repair. Cells have two major mechanisms that repair DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). HR requires a second, good copy of the damaged DNA for repair, while NHEJ adheres two DNA ends together. The goal of this experiment is to determine how spaceflight impacts NHEJ. To accomplish this goal, we will use the budding yeast *Saccharomyces cerevisiae* as an experimental model. Yeast have several advantages for this line of investigation: 1) Their NHEJ repair mechanisms are well conserved with humans; 2) Their fast proliferation rate generates sufficient material for cellular and molecular analyses; 3) Yeast can live as either diploids or haploids; haploids must exclusively use NHEJ to repair DSBs; 4) DNA damaging agents have been well-studied in this model. Using the confines of a NanoRacks MiniLab, we have engineered an experimental system which will expose proliferating haploid yeast to bleomycin, a DNA damaging agent. Preliminary experiments have identified an optimal bleomycin concentration (1-5 µg/mL) and a sufficient cell density (2.5 x 10⁶ cells/mL). Using these parameters, we will measure NHEJ in both an experimental sample sent to the International Space Station and an Earth control. The results of these analyses will be crucial in forwarding our understanding of NHEJ in space

Dean Peter Straub
Dr. Pamela Cohn
Stockton University School of Natural Sciences and Math
Student Spaceflight Experiments Program (SSSEP)
Stockton University STEM Collaborative
NJ Space Grant Consortium

COMBINATORIAL CONTROL BY THE RNA-BINDING PROTEINS CELF1 AND ELAVL1 IN THE POST-TRANSCRIPTIONAL REGULATION OF KEY FACTORS IN LENS DEVELOPMENT AND CATARACT

Francisco Hernandez¹, Sandeep Aryal¹, Bailey Weatherbee¹, Salil Lachke¹

¹Department of Biological Sciences, University of Delaware, 105 The Green, Newark, DE 19716

The lens, a transparent tissue, together with the cornea focuses light on the retina, rendering high-resolution vision. Loss of lens transparency, termed cataract, is the leading cause of blindness worldwide. Cataract can present at birth, classified as congenital/pediatric or more commonly as an age-related disease.

To identify new genes associated with lens defects and cataract, the Lachke laboratory has developed a systems based tool called iSyTE. iSyTE identified Celf1 (CUGBP Elav-like family member 1) as a novel gene associated with cataract. Our lab recently reported that Celf1 deficiency causes severe lens defects and early-onset cataract across vertebrates.

Celf1 is a conserved RNA-binding protein (RBP) which functions in post-transcriptional gene expression control (PTC) mechanisms such as pre-mRNA alternative splicing, mRNA degradation and translational control of mRNA. Celf1 may interact with other RBPs to mediate combinatorial post-transcriptional control over gene expression. Using protein co-immunoprecipitation, we screened the lens epithelium derived cell line 21EM15 and identified one such RBP, Elavl1 (Embryonic lethal, abnormal vision-like 1), which interacts with Celf1 protein.

Similar to Celf1, Elavl1 regulates gene expression via various PTC mechanisms. Interestingly, using RNA immunoprecipitation (RIP) we identified that both Celf1 and Elavl1 proteins bind to mRNAs of two key lens genes, namely Pax6 and Prox1. Therefore, I hypothesize that Elavl1 is critical for mouse lens development, and in coordination with Celf1 maintains lens transparency.

Using iSyTE, I identified that Elavl1 is expressed in mouse lens. I then independently validated Elavl1 to be lens expressed using RT-PCR, western blot, immunostaining and mass-spectrometry data in mouse lens tissue. To investigate Elavl1 function in mouse lens development, our lab procured an Elavl1 conditional knockout (cKO) amenable mouse line and standardized the genotyping protocol. To generate Elavl1 cKO mice (Elavl1cKO/cKO), I crossed Elavl1 homozygous floxed mice (Elavl1flox/flox) with a lens-specific promoter-driven Cre mouse line that also carried a Elavl1 floxed allele (Elavl1flox/+; Pax6GFPCre).

I then confirmed Elavl1 deletion in mouse lens by genotyping, western blot and RT-qPCR in Elavl1cKO/cKO mouse compared to wild type control. Thus, I generated the lens-specific Elavl1cKO/cKO mice and validated the successful knockout. I will use Elavl1cKO/cKO to further test my hypothesis. Thus far, my data indicates that Celf1 directly binds to Elavl1 and both these proteins directly bind to the mRNAs of two key transcription factors that are dysregulated in the Celf1-deficient lens and are themselves linked to cataract and lens defects. Thus, my data provides new advances in the pathology of cataract.

IDENTIFICATION AND CHARACTERIZATION OF THE ZEBRAFISH SKIN-MUCUS MICROBIOME

Alyssa Long¹, Gabrielle Blazek¹, Jaszmine Wangui¹, Anna Jozwick¹

¹Center for Natural Science, Goucher College, 1020 Dulaney Valley Road, Baltimore, MD 21204

The interplay of a host and their associated microbes is well recognized in many areas of biology. These microbiomes are communities that inhabit a particular environment in or on an organism and play essential roles, such as nutrient provisioning, developmental cues and protection from pathogens. Yet, many communities and their roles are still unexplored. For example, within fish species, few studies have investigated the composition and nature of the skin-mucus microbiome. It is widely accepted that the skin-mucus is part of the innate immunity of zebrafish and contains microbiome (with unknown functional roles). The aim of this study was to culture a diverse array of bacterial members from the zebrafish, *Danio rerio*, skin mucosa using different media types, in order to identify and begin hypothesizing functional roles. Culture-dependent identification was performed on 42 isolates using a high-throughput biochemical fingerprinting (BiOLOG) assay and classical biochemical tests, which resulted in a total of 4 phyla and 12 genera. From these experiments, we can suggest that some of the bacteria may produce antibiotics to form part of the fish's innate immune system. Previous research concludes that the mucus layer also includes antimicrobial peptides and enzymes that contribute to the defense system. In further ongoing research, we aim to establish a phylogenetic map of the cultured zebrafish-associated bacteria with the use of 16S and conserved protein-encoding gene sequencing and compare these findings to 16S metagenomic data. Creating a comprehensive collection bacteria from this community is the first step towards gaining a broader understanding of each member's role and interactions within the fish holobiome.

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Chemistry

ABSTRACTS

Morning Session

*Page Numbers of the Abstracts Serve as
Poster Numbers*

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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 2019 Undergraduate Research Symposium in the Chemical and Biological Sciences.

SYNTHESIS OF UNNATURAL AMINO ACIDS IN PEPTIDE FORMATION

Shannon Rose¹, Anna Will¹, George Lengyel¹

¹Department of Chemistry, Slippery Rock University, 1 Morrow Way Slippery Rock PA 16057

Peptides assemble into complex structures, a characteristic correlated with plaque formation in brains of Alzheimer's patients. We examined the synthesis of unnatural amino acids to observe changes in folding patterns. Utilizing a metal-ion complex, unique side-chains can be built onto backbones using dialkylation processes. After dialkylation, the complex is broken down via acid-facilitated hydrolysis to isolate newly synthesized amino acids. Alternatively, hydrolysis can be facilitated by ethylenediaminetetraacetic acid (EDTA), a chelating agent. Increased water content from hydrolysis results in a dilute product and low yield in subsequent Fmoc protection reactions. To reduce the water content, we are studying the ability of citric acid and PDTA, an EDTA derivative to facilitate hydrolysis. NMR spectroscopy indicated that citric acid is able to disrupt the complex. We incorporated the amino acids into a β -sheet peptide model system using microwave-assisted solid-phase peptide synthesis. 2D NMR will determine the stability of the peptides folded structure.

SYNTHESIS OF A MODIFIED AMIDE-BASED EXTENDED HETEROCYCLIC NUCLEOBASE CAPABLE OF HYDROGEN BONDING TO THE U-A BASE PAIR IN DOUBLE STRANDED RNA

John Talbott¹, Aubrey L. Maryniak¹, James MacKay¹

¹Department of Chemistry and Biochemistry, Elizabethtown College, 1 Alpha Drive,
Elizabethtown PA, 17022

Recent studies have uncovered that noncoding RNA has a multitude of functions including regulating gene expression, catalyzing chemical reactions, and post transcriptional modification. Sequence selectively identifying non-coding RNA has been accomplished with the use of peptide nucleic acid (PNA) through Hoogsteen hydrogen bonding to double stranded RNA (dsRNA) to form a triple helix. However, sequence selectivity has been limited to polypurine strands containing two sites for Hoogsteen hydrogen bonding while only one for pyrimidines which results in poor binding in pyrimidine recognition. Our work aims to prepare synthetic nucleobases that afford better binding and sequence selectivity to pyrimidines. A nucleobase capable of binding across the U-A base pair has been crafted to interact with both uracil and adenine, affording three hydrogen bonds. Ab initio computations suggest that a design with modified uracil connected by an amide at C5 to a benzamide will afford strong binding affinity. We have synthesized this nucleobase, T3, by coupling isoorotic acid to an aminobenzamide moiety. Herein we report the synthesis of T3 and initial binding studies that show strong affinity for the U-A base pair. Consequently, we are currently working on producing more of the T3 monomer for further binding studies.

We gratefully acknowledge the National Science Foundation (CHE – 1708699) and the Elizabethtown College Scholarship, Creative Arts, and Research Projects (SCARP) program for financial support.

SYNTHESIS OF FUNCTIONALLY DIVERSE AND HIGHLY SUBSTITUTED 2,4-DIAMIDO-5-AMINOOXAZOLES

Andrea Cottingham¹, Jeffrey Benner¹, Abhishek Purohit¹, Jenna M. Miller¹, Gianna Falcone¹,
Stephen Habay¹

¹Department of Chemistry, Salisbury University, 1101 Camden Ave, Salisbury, MD, 21801

Oxazoles are important heterocycles, found in natural and synthetic compounds, with an array of biological applications. The ability to regioselectively assemble highly functionalized oxazoles would further facilitate development of pharmaceuticals and allow greater access to biologically relevant compounds. Recently, our group developed a rapid synthesis of highly substituted 2,4-diamido-5-aminooxazoles by reacting an isonitrile amide with an isocyanate in a three-component reaction. A wide variety of isonitrile and isocyanate starting materials were combined to produce a diverse library of di- and tri-substituted oxazoles. In this study, the impact of the amido group of the isonitrile on the multi-component reaction was investigated. It was found that secondary amides led to lower yields of cyclized amino-oxazole products, while tertiary amides produced the highest yields. The influence of the amide group on the relative ratio of constitutional isomer products was also investigated. Additionally, the role of functionalized isocyanates in this reaction were examined. The results demonstrated that electron-dense isocyanates stimulated increased reaction yields. Future work on our mechanistic investigation will be used to further develop our library of highly substituted oxazoles for biological screening.

Research reported in this poster was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R15GM116003.

A ONE-POT ALLYLATION AND CLAISEN REARRANGEMENT OF ACETAMINOPHEN USING MICROWAVE IRRADIATION

Brandon Tessier¹, James A. MacKay¹

¹The Department of Chemistry and Biochemistry , Elizabethtown College, 1 Alpha Drive, Elizabethtown, Pennsylvania 17022

The Claisen rearrangement is a widely applicable organic reaction that involves the shift of a sigma bond across the pi-system of an allyl vinyl ether to produce allylated phenols. In this project, we have developed a microwave assisted allylation followed by subsequent Claisen rearrangement in one pot. The goal of this project is two-fold. First, we aim to design and optimize a one-pot reaction that affords the highest yield of rearranged product from the corresponding phenol. Second, we aim to explore Claisen chemistry using microwave reactors for incorporation into the sophomore-level organic laboratory curriculum. Acetaminophen was chosen as a model phenol to test this chemistry and optimization was performed on the reaction. To date, the time, temperature, and solvent conditions have been explored. Current work is focused on the use of other reagents and stoichiometries. We have developed reaction conditions to obtain greater than 50% isolated yield of rearranged product. Further optimization of the reaction may afford higher yields of rearranged acetaminophen, with the ultimate goal of exploring additional phenols. These studies will be integrated into the Organic 2 laboratory this fall.

We gratefully acknowledge the National Science Foundation (CHE – 1708699) and the Elizabethtown College Scholarship, Creative Arts, and Research Projects (SCARP) program for financial support.

SYNTHESIS OF 3,3 AND 3,4 CROSSLINKED MURAMYL DIMERS

Jared Ramsey¹, Klare Lazor¹, Siavash Mashayekh¹, Catherine Grimes¹

¹Department of Chemistry and Biochemistry, University of Delaware, 163 The Green, Newark, DE 19716

The bacterial cell wall, peptidoglycan (PG), is a key tool for the identification of bacteria by the innate immune system. For the body to function properly, this system needs to be able to accurately dispose of pathogenic threats while retaining commensal bacteria. To accomplish this, immune receptors can recognize PG from a variety of bacterial species and differentiate between them. The immune protein nucleotide oligomerization binding protein 2 (NOD2) has been found to bind a fragment of PG called muramyl dipeptide (MDP), leading to an immune response. MDP is one NOD2 ligand that has been researched extensively due to its commercial availability. However, when bacteria are digested in the body, PG can fragment in a variety of ways aside from MDP. Our synthesis focuses on obtaining a library of muramyl dimers linked through their peptide chain. It is suspected that compounds in this family would be NOD2 ligands based on PG fragmentation patterns. The synthesis of a 2,2 linked MDP dimer was previously displayed in our lab, utilizing a 2:1 coupling as a key step. This work expands on the aforementioned synthesis by replicating peptide linkages found in nature, as well as investigating the differences between α -amides and α -carboxylic acids on the PG peptide chain. This includes primarily 3,3 and 3,4 crosslinking patterns while utilizing both lysine and iE-DAP bridges as opposed to the simpler diamino bridge seen in the 2,2 linkage. The completed synthesis of these compounds will open the doors to an array of applications for muramyl dimers such as biological screening for immune response, or functionalization on the carbohydrate component.

Special thanks to the Sloan Foundation, the University of Delaware Summer Scholars, and the David A. Plastino Alumni Undergraduate Research Fellows for funding this work.

SYNTHESIS OF 1,2-DIHYDRONAPHTHALENES AND INDENES FOR SUBSTRATE SCOPE AND LIMITATION STUDIES OF SUBSTITUTED CYCLOBUTANE FORMATION VIA PHOTOREDOX CATALYSIS

Chanveboll Yun¹, Steven Kennedy¹

¹Department of Chemistry, Millersville University of Pennsylvania, 40 Dilworth Rd,
Millersville, PA 17551

Photoredox catalysis (PRC) provides efficient access of highly substituted cyclobutane rings via [2+2] photocycloadditions. Substituted cyclobutanes are a prevalent structural motif of many biological active natural products; they often exhibit antiviral, anticancer, or antifungal related properties. We have initiated studies to explore and expand the scope and limitations of Ruthenium PRC as a means to access cyclobutanes containing substituents arising from substituted 1,2-dihydronaphthalene or indene precursors. Building on the work of Chen et al., Ru(bpy)₃²⁺ and Ru(bpm)₃²⁺ will be initially screened against a small library of olefins, which can be synthesized in two steps from a variety of commercially available tetralones or 1-indanones. The library will be partially produced by an upper-level advanced organic chemistry laboratory class utilizing a Course-Based Undergraduate Research (CURE) process to produce starting material olefins and preliminary catalysis data.

This work was supported by, Student Grants for Research and Creativity Activity. I would like to thank my mentor, Dr. Kennedy, for his support and guidance, and Dr. Edward Rajaseelan, Chemistry Department Chair, and Mr. Steven Peurifoy, Laboratory Technician.

STUDIES TOWARD 3,4-DIHYDRO-2(H)-QUINOLINONE DERIVATIVES

Yongyu Ou¹, Isaac Whitcraft¹, Steven Kennedy¹

¹Department of Chemistry, Millersville University of Pennsylvania, 40 Dilworth Rd,
Millersville, PA 17551

Hunanamycin A (HA) is a natural product isolated from *Bacillus hunanensis*; it was discovered by MacMillan et al. while investigating sediment from mangrove swamps in the Bahamas. HA exhibits antibacterial activity against various Gram-negative pathogens such as *Salmonella* and *Escherichia coli*. Demand for antibacterial compounds is increasing as bacteria become more resistant to available antibiotics. Nevertheless, only an extremely small amount of HA (ca. 10 mg) can be isolated from a large number of bacteria, not enough for future biological studies. To further explore HA and structurally related compounds, chemical synthesis from readily available starting materials is underway. Our project aims to optimize the synthesis of 3,4-Dihydro-2(1H)-quinolinone derivatives. We have synthesized 2-bromo-4,5-dimethylaniline—our starting material—from 3,4-dimethylaniline, in 33% yield over three steps. We are currently using 2-bromoaniline as a model system to study amide formation followed by Lewis acid catalyzed cyclization as a method to access the 3,4-Dihydro-4,4-dimethyl-2(1H)-quinolinone core structure.

This work was supported by Neimeyer-Hodgson Research Grant, Student Grants for Research and Creativity Activity, and Millersville University Faculty Grant. We would like to thank our mentor, Dr. Kennedy, for his support and guidance, and special thanks to Millersville University Department of Chemistry Faculty and Staff, Dr. Edward Rajaseelan, Chemistry Department Chair, Mr. Steven Peurifoy, and Dean of the College of Science and Technology.

SYNTHESIS OF A POTENTIAL SEROTONIN 5-HT_{2A} AGONIST

Joseph Choquette¹, Zachary Schelmz¹, Connor Ricci¹, Jose Juncosa¹

¹Department of Chemistry, Salisbury University, 1101 Camden Avenue Salisbury MD 21801

The primary goal of this research project is to synthesize a molecule that is predicted to bind selectively to the 5-HT_{2A} receptor, (2S,5S)-2-(4-bromo-2,5-dimethoxybenzyl)-5-(2-methoxyphenyl)pyrrolidine. This compound is an analog of (2S,6S)-2-(4-bromo-2,5-dimethoxybenzyl)-6-(2-methoxyphenyl)piperidine (DMBMPP), whose primary modification is reducing the size of the central amine ring from a piperidine (6 atoms) to a pyrrolidine (5 atoms); this change results in an increase in the bond angles between the critical aromatic groups that we believe could improve binding and selectivity. We have synthesized our desired compound, and after testing we will identify if this structure shows increased potency from DMBMPP while at least retaining selectivity, providing a more in-depth understanding of the conformational requirements and interactions between the 5-HT_{2A} receptor and its substrates. This discovery will allow further, more educated and specific pharmaceutical research to be conducted on the 5-HT_{2A} receptor, with the ultimate goal of discovering new treatments for conditions such as post-traumatic stress disorder (PTSD), learning disabilities, and others.

I would like to acknowledge the Guerrieri Summer Research Grant 2019 for the funding of this project and Henson School of Science and technology for providing the research facilities.

SYNTHESIS OF SUPRAMOLECULAR BORONIC ACID GELATORS FOR ENVIRONMENTAL REMEDIATION

Caroline Walker¹, Gretchen Peters¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 Main Street, Harrisonburg, VA, 22807

Dyes used in textile manufacturing are some of the main sources of industrial pollutants found in aquatic environments. These dyes are typically not biodegradable and have tremendous environmental impacts even at low concentrations. Thus, the ability to efficiently extract dyes from aqueous systems is of great importance. To do this, we envision using a supramolecular gel made from a lithocholic acid (LCA) derivative with a boronic acid handle. The LCA unit has the ability to self-assemble and form a fibrous gel network, while the boronic acid can covalently bind to the diol unit found in industrially relevant alizarin dyes. Upon formation of this boronate ester linkage between the dye and the gelator, physical removal of the gel will allow for efficient extraction of the dye from water. To date, we have successfully synthesized the proposed lithocholic acid-boronic acid conjugate in three steps. We fully characterized this compound and all intermediates by means of ¹H, ¹³C, and ¹¹B NMR. Gelation studies were performed at varying concentrations and in different solvent systems, and Alizarin Red S fluorescence assays were explored to assess diol-binding. In the future, we will continue to optimize this supramolecular gel and explore its viability as an environmental remediation system

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INVESTIGATION OF SOLID-STATE CHANGES OF ITRACONAZOLE SPRAY DRIED DISPERSIONS USING HYDROXYPROPYLMETHYLCELLULOSE ACETATE SUCCINATE (HPMCAS)

Miglanche Ghomsi Nono¹, Asmita Adhikari², James Polli²

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore MD 21250

²Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore MD 21201

Spray-dried amorphous solid dispersions have become a common approach to enable formulation of poorly soluble drugs. HPMCAS has been observed to inhibit the crystallization tendency of supersaturated solution of itraconazole by enhancing the kinetic solubility and thermodynamics of poorly soluble drugs. It has a potential to obtain and maintain supersaturating concentrations of drug to enhance drug absorption, but it tends to precipitate over time. Characterization of amorphous solid dispersions is performed by assessing glass transition temperature (tg). It is indicative of polymer miscibility which is the temperature above which an amorphous material converts from a solid or “glassy” state to a super-cooled liquid or “rubbery” state. HPMCAS is also well - suited as a drug carrier due to its high tg that limits molecular mobility and improves stability. The rank order of the dissolution performance of ITZ spray dried dispersions (SDDs) had already been established for all three grades of HPMCAS in the order of –L>-M>-H. We hypothesize that the performance of spray dried dispersions (SDDs) can be linked to potential changes in solid state during dissolution leading to precipitation of amorphous drug. We aim to characterize the solid-state changes in HPMCAS based spray dried dispersions using various grades of polymer.

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HAZARDOUS VOCs IN AN UNDERGROUND PARKING GARAGE

DaJon McLeese¹, Ozioma Onwuvuche¹, Shizuka Hsieh¹

¹Trinity Washington University, 125 Michigan Ave NE, Washington, DC 20017

Traffic-related pollutants in underground parking garages can possibly affect those who park or work there, for example workers responsible for collecting carts at shopping centers. The purpose of this research was to obtain measurements of volatile organic compounds (VOCs) in the air at an underground parking garage attached to a big-box store. The data give a preliminary answer to whether workers might be exposed to unsafe levels of VOCs in this situation. Data were obtained by GCMS (gas chromatography mass spectroscopy) headspace analysis of VOCs from the air that were adsorbed onto a stationary phase. One-hour sampling on two separate days yielded concentrations of 17 different VOCs, including benzene, which is a carcinogen. The VOC concentrations were found to be well below the OSHA (Occupational Safety and Health Administration) maximum guidelines. These preliminary findings indicate that it is likely that workers at the underground parking garage in question are not being exposed to dangerous levels of VOCs.

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BROMINATION KINETICS OF HALOSALICYLATES: IMPLICATIONS FOR WATER CHLORINATION

Olivia Driessen¹, John Sivey¹

¹Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252

In the presence of free chlorine, bromide can be oxidized to form free bromine species. Free chlorine and bromine can react with natural organic matter to form a variety of chlorinated and brominated disinfection byproducts (DBPs). This process is common in aqueous systems such as drinking water and wastewater, in which free chlorine is used as a disinfectant. Many DBPs pose hazards to the environment and human health, with brominated DBPs generally found to be more genotoxic and carcinogenic than their chlorinated counterparts. Although HOBr is the most abundant free bromine species in aqueous solutions at approximately neutral pH, it is not necessarily the most reactive halogenating agent in reactions with DBP precursors. Other species, such as BrCl (formed via the reaction of HOBr and chloride) should also be considered. Salicylic acid (SA) is a DBP precursor that can enter the environment as a human metabolite of aspirin, skin peeling agent, and food preservative. In the presence of free chlorine and free bromine, SA can form four distinct monohalogenated products, which can then react further with free chlorine/bromine to form dihalogenated salicylates and additional uncharacterized products. The goal of this project is to identify the effect of chloride concentration on the bromination rates of monohalo- and dihalosalicylates. Batch kinetic experiments were performed in buffers at pH 7 and 7.6 to assess reaction rates. Time course samples were analyzed by reversed-phase high-performance liquid chromatography (HPLC). The results showed an increase in pseudo-first-order rate constant as chloride concentration increased, at $\text{pH} \leq 7$. These findings implicate BrCl as an active brominating agent in systems at $\text{pH} \leq 7$. Furthermore, we expect the contribution of BrCl to DBP formation to increase as the chloride concentration in sources of drinking water rises due to human activity such as road salting and hydraulic fracturing.

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CHEMICAL CHARACTERIZATIONS OF EXTRACTS AND THEIR APPLICATIONS ON TEXTILES

Yeihawa Kulanda¹, Hernan Osorio¹, Mark Joseph¹, Victoria Volkis¹

¹Department of Natural Sciences, University of Maryland Eastern Shore, 1 Backbone Road, Princess Anne, MD, 21853

Herbs and various plants have been used in non-traditional medicine for years. Countless studies have focused on isolating and determination of the active components that are responsible for their medical benefits. Holy basil (Tulsi) is a medical herb that has been used for thousands of years. Its highly concentrated essential oils have anti-inflammatory, antipyretic, analgesic, anti-arthritis, antioxidant, anti-ulcer, anti-microbial properties. Another key species native to North America is the *Aronia mitchurinii*. This is a plant that produces fruits with extremely high content of antioxidants. It has been used as medication to treat colds and hemorrhoids. Its other health benefits are under investigation.

This project has two major goals: 1) to extract and study the compounds from different species of holy basil and aronia species; 2) to apply extracted compounds on textile, using encapsulation within biodegradable polymers, in order to produce bands with antibacterial and anti-inflammation properties. In order to isolate extracts, steam distillation and extraction are conducted. Extracted samples are characterized using GCMS and UV/Vis spectroscopy. To incorporate the essential oils into textiles, they are encapsulated within cyclodextrin, polylactide, or polyglycolide.

**THE SYNTHESIS AND CHARACTERIZATION OF
CETYLTRIMETHYLAMMONIUM-4-CHLOROBENZOATE AND
CETYLTRIMETHYLAMMONIUM -3,5-DICHLOROBENZOATE FROM
CETYLTRIMETHYLAMMONIUM BROMIDE**

Zach Lawson¹, Michael Hochstein¹, Patricia Kreke¹

¹Department of Chemistry, Mount St. Mary's University, 16300 Old Emmitsburg Rd
Emmitsburg, MD 21727

This research developed a protocol to synthesize the surfactants cetyltrimethylammonium 2,6-dichlorobenzoate (CTA2,6), cetyltrimethylammonium 4-chlorobenzoate (CTA4) and cetyltrimethylammonium-3,5-dichlorobenzoate (CTA3,5) from cetyltrimethylammonium bromide (CTAB). The surfactants and their micelles were characterized through IR, Uv-Vis, NMR and DLS.

HOW STRUCTURE IMPACTS FUNCTION IN NEW FLUORENE BASED SMALL MOLECULE ACCEPTORS

Sarah Brock¹, Dariia Yehorova¹, Brycelyn Boardman¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S. Main St, Harrisonburg, VA 22807

Small molecules with electron deficient regions have been gaining popularity as acceptor materials in organic photovoltaics because they are effective and easily modified, making them an attractive alternative to traditional fullerene acceptors. Two novel small molecule acceptors with fluorene-based cores were synthesized with terminal phenyl and furyl malononitrile groups. The products were obtained from a two-step reaction of 9,9-dioctylfluorene-2,7-diboronic acid with 3-bromobenzaldehyde or 5-bromo-2-furaldehyde under Suzuki conditions followed by a reaction with malononitrile to yield 9,9-dioctylfluorene -2,7 (1-phenyl-3-methylene malononitrile) (FPM) and 9,9-dioctylfluorene -2,7 (2-furyl-5-methylene malononitrile) (FFM) respectively.

Structural characterization of the compounds was performed using ¹H NMR and ¹³C NMR. Optical and electronic characterization was done using, UV-Vis and fluorescence spectroscopy, cyclic voltammetry, and DFT calculations. A series of quenching experiments were performed to observe donor/acceptor interactions between poly-3-hexylthiophene (P3HT) and FPM and FFM. A large reduction in P3HT emission was observed in the presence of both FPM and FFM with maximum percent emission reduction of 99.2% and 96.0 % respectively, indicating favorable donor/acceptor interaction. Films of P3HT and the small molecule acceptors were cast from chlorobenzene in 1:1 and 1:4 ratios respectively and were analyzed via AFM. All mixtures indicated the formation of continuous films. At low small molecule concentration FPM shows uniform phase separation on a small scale while minimal phase separation is observed in films containing FFM when compared to pristine P3HT films. However, in the high concentrations of small molecule, both FPM and FFM containing films show the formation of more network like morphologies. The combined results indicate that both FPM and FFM have the potential to be effective small molecule acceptors.

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MONITORING ANTIOXIDANT CONTENT OF ARONIA MITCHURINII JUICE DURING FERMENTATION PROCESS

Awaab Elkarim¹, Victoria Volkis¹

¹Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853

Aronia mitchurinii, also known as “black chokeberry”, is considered by many as one of the super- fruits with abnormally high concentration of antioxidants, especially anthocyanins. Native to North America, this super berry was cultivated mostly in Eastern Europe. Current *Aronia* applications include, but are not limited to: 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. Sugars of any fruits can be transformed into alcohol during the yeasts-initiated fermentation process, if the initial content of sugars is high enough. However, many side processes are involved, and some cause significant changes in pH. Presence of oxygen is unwanted and may cause formation of vinegar instead or in addition to alcohol. Through the *aronia* winemaking process, US farmers have noticed that originally deep red-wine colored *aronia* juice often changes its color colorless or deep blue. The color change is most likely associated with the anthocyanins changing their structure with the changing in acidity, the presence of traces of oxygen and some other factors. Other possible side process is degradation of antioxidants. Processes like that not only decrease the quality of wine, but also cause significant reduction in anthocyanins content. However, the detailed research of chemical processes happening to antioxidants during the fermentation of *aronia* juice has never been performed. Here we present detailed characterization of pH, totals of anthocyanins, flavonoids, total phenolics, alcohol and acetic acid at all steps of wine making fermentation of *aronia* juice.

MONITORING METAL-INDUCED CHANGES IN PROTEIN AGGREGATION BY FT-IR

Erica Lam¹, Gina MacDonald¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Protein or peptide aggregates have been shown to be associated with redox reactive metals like zinc and copper. Metals have been identified as an environmental factor that could contribute to protein-misfolding disease pathogenesis. However, it is not fully understood how metals initiate and/or alter protein aggregation. These studies investigated if Zn(II) and Cu(II) induced structural changes in β -lactoglobulin and human serum albumin over time. Fourier transform infrared spectroscopy was used to monitor metal induced changes at room temperature and pH 7.5. Our preliminary results suggest that incubating protein with metals can alter protein structure.

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CHARACTERIZATION OF THE INTERACTION BETWEEN UFM1 AND UBA5

Gavin Lao¹, Christopher E. Berndsen¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Dr,
Harrisonburg VA 22807

Ubiquitin fold modifier 1 (Ufm1) is a ubiquitin-like protein (Ubl) that is present in all metazoan organisms. Ufm1 plays an important role in homeostasis and endoplasmic reticulum stress management which contributes to Alzheimer's, other neurodegenerative diseases, and cancer. Understanding the interaction between Ufm1 and Uba5 will provide the basic knowledge for further biomedical research on combating such diseases. The Berndsen Lab has previously shown that Ufm1 binds to Uba5 at two regions; the adenylation domain and the Ufm1-interacting sequence (UIS) of Uba5, which is found at the C-terminus of the protein. However, we want to further explore the interaction with these enzymes with greater detail to examine the hypothesis that the UIS is an allosteric binding site to aid in the binding of Ufm1 to the adenylation domain. Mutants of Ufm1 that may have an effect on allostery and the binding to the adenylation domain of Uba5 have been purified. We measured UIS affinity for Ufm1 using a fluorescence polarization assay and will confirm these measurements with enzyme activity assays. We will then determine whether the mutants have an effect on the adenylation domain interaction and if the allosteric communication between the UIS site and the adenylation binding region of Ufm1 is affected. If the mutants do in fact have an influence on the interaction at the UIS and the adenylation site, then that will induce more biomedical research on possible drug site for treatment or therapies for neurodegenerative diseases.

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WATER SOLUBLE CADMIUM SELENIDE QUANTUM DOT NANOCRYSTALS

Patrick Keane¹, Nico Fair¹, Stephanie Homan¹

¹Chemistry Department, McDaniel College, 2 College Hill, Westminster, MD 21157

Quantum dots (QDs) have unique optical properties as a result of their small size. In fact, the color in which quantum dots absorb and fluoresce is determined by their size. This property makes QDs an ideal dye for flow cytometry and cell and tissue imaging, due to the variety of colors that can be readily made. Here, we synthesized cadmium selenide (CdSe) QDs that were modified in order to make the QDs compatible with biological systems. The CdSe QDs were shelled using a single molecule precursor for zinc sulfide (ZnS) and transferred into water through ligand exchange. The ligand exchange into water weakened the emission, but it was enhanced through the addition of zinc acetate to passivate the ZnS shell. Overall, we created materials optimal for biological imaging by coating in a nontoxic shell, suspending in water, and optimizing for a maximum emission intensity. The next step is to exchange the ligands with precursors for click chemistry reagents in order to attach QDs to cell antibodies for in vivo imaging and flow cytometry.

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ANION EFFECTS ON THE PARTITIONING OF BENZO[B]THIOPHENE-2-METHANOL BETWEEN OIL AND AQUEOUS PHASES

Sandra Fleku¹, Yanjie Zhang¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main Street, Harrisonburg, VA 22807

The Hofmeister series are a series of ions that determine the effectiveness of causing proteins to precipitate out of solution. The interactions of the Hofmeister series ions with heteroatomic compounds containing sulfur, oxygen and nitrogen in crude oil have not been thoroughly understood. In this study, we investigated the effects of four different sodium salts including Na₂SO₄, NaCl, NaClO₄, and NaSCN at concentrations between 0.2 M to 1.0 M on the partitioning of benzo[b]thiophene-2-methanol between isooctane and aqueous phases using UV-vis spectroscopy. Weakly hydrated thiocyanate slightly increased the standard Gibbs free energy for benzo[b]thiophene-2-methanol transfer from the aqueous phase to isooctane. Strongly hydrated sulfate decreased the standard Gibbs free energy and promoted benzo[b]thiophene-2-methanol transfer. The ordering of the standard Gibbs free energy for benzo[b]thiophene-2-methanol transfer from the least negative to the most negative is SCN⁻ > ClO₄⁻ > Cl⁻ > SO₄²⁻. More experiments on other anions in the Hofmeister series will be performed in the future.

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ANALYZING AND QUANTIFYING THE PERVASIVENESS OF MICROPLASTIC BEADS IN MOUSE LIVERS, SPLEENS AND KIDNEYS

Katherine Thompson¹, Kristen Long², Elaine Farkas¹

¹Department of Chemistry and Physics, Mansfield University, 31 S Academy St, Mansfield, PA 16933

²Biology Department, Mansfield University, 31 S Academy St, Mansfield, PA 16933

Microplastics are defined as plastic particles with a size less than 5 mm. Microplastics have been recognized as a global environmental threat and a potential health hazard to humans. Humans are being exposed to microplastics directly from the environment and through food sources; these particles have been found in water, sediment, animals, and even human excrement. Fish caught in the wild have been found with microplastics in their livers, intestines, and gills. The potential health effects caused by the ingestion of these particles by humans are unknown. To determine whether microplastics persist in mammals after oral ingestion, a mouse study was undertaken. The study included two groups of mice that ingested spherical polystyrene beads containing a fluorescent dye: one group was fed microplastic beads and the other group was fed nanoplastic beads. Two different sizes of beads were tested in this study to determine whether one size was more susceptible to persistence in the animals. The livers, spleens, and kidneys of the mice were processed into histological slices, and the slices were observed under a fluorescent microscope. The fluorescent microscope used contains three filter sets to allow for fluorescence excitation at 434 nm, 514 nm, and 568 nm wavelengths of light. The microplastic beads have very narrow absorption and emission bands, so any fluorescence observed under the 434 nm excitation wavelength that was not present under the 514 nm and 568 nm illumination was assigned to microplastic beads. Sections of the organs were digested to separate the beads from the tissue to quantify the number of beads remaining in the animals after ingestion, and the beads were quantified using a spectrofluorometer. The identification of the microplastic beads within the organs provides insight into the pervasiveness of these materials and displays the need for a remedy to the environmental pollution crisis.

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OPTIMIZATION OF EXTRACTION METHODS FOR LC/MS IN COCAINE DETECTION

McKenzi Weatherhogg¹, Kevin Bennett¹

¹Department of Chemistry and Physics, Hood College, 401 Rosemont Ave., Frederick, MD 21701

Liquid chromatography- mass spectrometry (LC/MS) is used to help identify substances based in both physical separation and determination from mass. LC/MS is often used in forensic chemistry to aid in the determination of substances of abuse. Cocaine is a drug of abuse known to be found in trace amounts in United States paper currency. This lab focuses on what conditions work the best for extracting cocaine from currency and what conditions are optimal for detecting the substance using LC/MS. By varying the conditions of extraction, such as length of extraction, type, and filters, as well as LC/MS conditions (capillary and cone voltages). Cocaine was detected in a range of currency.

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VALIDATION OF THE COMPARATIVE METHOD OF QUANTUM YIELD MEASUREMENT USING ALEXA 488 AND FLUORESCEIN

Keyanna Ryan¹, Brendan Cook¹, Robin Helburn¹, Hacene Boukari¹, Qi Lu¹

¹Department of Physical and Computational Sciences, Delaware State University, 1200 N DuPont hwy Dover, DE 19904

The efficiency of the fluorescence process is measured by the quantum yield. The quantum yield of fluorescence is defined as the number of photons emitted over the number of photons absorbed, which is related to the probability of deactivation of the excited molecules through radiative mechanism (fluorescence). The comparative method has been shown as the most reliable method for determining the fluorescence quantum yield (Φ_F) of an unknown dye through comparison to a well-characterized standard dye. It is important that the two samples have identical absorbance at the same excitation wavelength to ensure that the solutions are absorbing the same number of photons. Alexa 488 and fluorescein were the two dyes used to validate this method. The UV-Vis absorbance and the fluorescence spectra were collected at different concentrations for both dyes to yield a linear correlation between the integrated fluorescence intensity and the peak absorbance. The gradients of the linear plots obtained for both dyes were used to calculate each other's quantum yield and compared to the literature values. The method was shown to be effective and will be further used to study the lipid probes.

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FLUORESCENCE QUENCHING OF COUMARIN BY HOFMEISTER ANIONS

Olivia Mumma¹, Yanjie Zhang¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807

Coumarins are building blocks to a variety of pharmaceutical molecules such as the blood thinner warfarin. The effects of nine sodium salts on the fluorescence of coumarin were studied at varied salt concentrations. It was observed that SO_4^{2-} , H_2PO_4^- , F^- , Cl^- , and ClO_4^- slightly enhanced the fluorescence of coumarin but Br^- , NO_3^- , SCN^- and I^- quenched coumarin fluorescence. The ability of anions to quench coumarin fluorescence was evaluated by using Stern-Volmer plots. The fitting parameters for the anions correlated to their ordering in the Hofmeister series with the exception of perchlorate. In addition, the effects of a combination of two anions on coumarin fluorescence quenching were studied. It is interesting to find that the effects of two anions on coumarin fluorescence are not additive. For the Na_2SO_4 combinations, salts like NaSCN and NaI seemed to quench more with the addition of Na_2SO_4 while NaNO_3 (0.2 M) and NaBr seemed to have a slight increase in fluorescence. The NaCl combinations with NaI , NaBr , and NaNO_3 (0.2 M) had a general increase in fluorescence while NaSCN had slightly more quenching.

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Department of Chemistry and Biochemistry, James Madison University

FORMATION OF TRIHALOMETHANES UNDER CONDITIONS APPROXIMATING SEAWATER

Thomas FitzGibbon¹, John D. Sivey¹

¹Department of Chemistry, Towson University, 8000 York Road, Towson, Md 21252

Chlorination of waters containing organic matter can produce chloroform, bromodichloromethane, dibromochloromethane, and bromoform (collectively, trihalomethanes, THMs). The production of THMs during chlorination of drinking water is well established. THMs are monitored and regulated in municipal water systems due to their potential health risks, which include bladder cancer and birth defects.

Chlorination of saltwater swimming pools or ship's ballast waters also generate THMs. High bromide levels in these waters promote the formation of brominating agents (e.g., HOBr) during chlorination. Despite its common classification as a "spectator ion", chloride can contribute to increased rates of chlorination and bromination by enabling the lesser studied halogenating agents Cl₂ and BrCl. Collectively, the elevated bromide and chloride concentrations in seawater may result in unique THM formation profiles when compared drinking water.

The purpose of this investigation is to examine the effects of chloride ion concentration on THM formation with ionic strength and bromide concentrations fixed at levels comparable to seawater. A trace analytical method was developed using gas chromatography coupled to a micro-electron capture detector (GC- μ ECD). Reactions were conducted at pH 7 with fixed concentrations of bromide and an organic precursor (Suwannee River NOM extract). Chloride levels were varied while maintaining ionic strength constant. Reactors spiked with chlorine were held at 20 °C in a water bath and sacrificed at fixed intervals. Production of THMs was quenched by the addition of 1,3,5-trimethoxybenzene which reacted with any remaining free chlorine or bromine. The aqueous solutions were extracted into methyl tert-butyl ether prior to GC- μ ECD analysis.

The most abundant THM formed was bromoform, followed by dibromochloromethane, chloroform and dichlorobromomethane. High ionic strength encouraged the THM's to volatilize from the water phase. 0.5 mL of reactor headspace resulted in a 20% loss of THMs over 6 hours. Under headspace free conditions, product loss was not evident.

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ACID RAIN MITIGATION AND AQUATIC SYSTEMS: STREAM VERSUS WATERSHED LIMING

Madison Roberts¹, Daniel Downey¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Dr, Harrisonburg, VA 22807

Atmospheric acid deposition (acid rain) is a century old problem that is the result of use of fossil fuels for energy production. Title IV of the Clean Air Act (1990) put restrictions on power plant emissions to reduce the amount of SO_x and NO_x and thus the amount of sulfuric and nitric acid deposited on the landscape. Despite significant reductions in acid content of rainfall in the past 20 years, many streams and lakes along the east coast of the U.S. have yet to fully recover. The addition of limestone to acidified streams (liming) in Virginia to temporarily mitigate the effects of acid rain on aquatic systems has been extensively researched since 1989 by JMU and USFS. Limestone has been introduced directly into the stream channel of all streams treated to date either from dump trucks or helicopters. In some other states limestone has been aerially dispersed over the watersheds of target streams to manipulate water chemistry indirectly. This study focuses on the advantages and disadvantages of stream liming versus watershed liming to evaluate Virginia's first proposed watershed liming at Meadow Run (Shenandoah National Park, VA). Case studies from the liming of Little Stony Creek (George Washington National Forest, VA) and Woods Lake (Adirondack Park, NY) were used for this study. Limestone dosage mass was predicted based on charge balance equilibria of base cations (e.g. Ca²⁺) and acid anions (e.g. SO₄²⁻) in the stream versus inputs from rainfall (National Acid Deposition Program VA28).

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EFFECTS OF TUNDRA WILDFIRE ON INFILTRATION RATES AND NITROGEN SPECIES IN PORE WATER

Gabriel Duran¹, Paul Mann², Seeta Sistla³, Bianca Rodriguez-Cardona⁴, Aquanette Sanders⁵, John Schade⁶, Sarah Ludwig⁶, Susan Natali⁶

¹Department of Geography and Environmental Science, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Geography and Environmental Sciences, Northumbria University, 2 Ellison Pl, Newcastle, UK

³Department of Natural Science, Hampshire College, Amherst, MA 01002

⁴Department of Natural Resources and Earth Systems Science, University of New Hampshire, 105 Main Street, Durham, NH 03824

⁵Marine Science Institute, University of Texas at Austin, Austin, TX, 78712

⁶Woods Hole Research Center, 149 Woods Hole Road, Falmouth, MA 02540

Wildfires in tundra ecosystems have increased in frequency and extent as climate change progresses. In 2015, for example, the Yukon-Kuskokwim (YK) Delta in Alaska experienced the largest fire season in recorded history. This is a major issue for the hydrology of the YK Delta, as fires alter the structure of the soil and the movement of nutrients to downstream aquatic ecosystems. Here we report on concentrations of ammonium, nitrate, and dissolved organic nitrogen in pore waters collected from burned and unburned watersheds in the YK Delta. We quantified hydraulic conductivity to characterize how fire and landscape characteristics affect the rate at which the water is moving through the landscape. Pore water samples were collected from areas burned in 1972 and 2015 and in unburned sites in the YK Delta at depths from 70-80 cm below the ground surface. We measured falling head hydraulic conductivity with the use of piezometers to record the time it took for water to infiltrate soils at 10 and 20 cm within vegetation types unique to each site and common among all (e.g., *Sphagnum fuscum*, lichen, bare ground). Ammonium concentrations in pore water were two orders of magnitude higher than nitrate, which suggests low nitrification rates in pore water. Total dissolved organic nitrogen (DON) in pore water of the unburned site was less than half the DON concentrations in pore water of the 2015 and 1972 burns, showing that there is a long-term impact of fire on nitrogen cycling in the YK Delta. Hydraulic conductivity was highest in the unburned site and decreased with soil depth. Ammonium concentrations were negatively related to hydraulic conductivity, and nitrate concentrations were positively related to hydraulic conductivity, which may reflect differences in oxygen content and nitrification rates associated with pore water movement.

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QUANTIFICATION OF CANNABINOIDS IN HEMP AND EXTRACTION INTO AQUEOUS MEDIA

Valerie Nelson¹, Daniel Downey¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 South Main Street
Harrisonburg, VA 22807

Cannabis sativa is a flowering plant used for recreational and industrial purposes that contains a class of compounds called cannabinoids. Industrial hemp is a strain of Cannabis sativa that has been propagated to have a low tetrahydrocannabinol (THC) and a high cannabidiol (CBD) content. Hundreds of farms in Virginia are now growing hemp for fiber or CBD production. Crops with THC content greater than the maximum legal limit of 0.3% must be destroyed. High Pressure Liquid Chromatography (HPLC) separation with ultraviolet detection is being studied for cannabinoid analysis with intent of rapid turnaround time from sample collection to data report. Information thus collected should enable farmers to make modifications during the growing process to reduce THC content. In addition, various aqueous solutions including Earthwater Fulhum™ are being used to extract CBD from hemp oil. Partition coefficients (K_p) vary by pH and time spent in contact with the media. A gas chromatography-flame ionization (GC-FID) method used by the Virginia Department of Consolidated Laboratories (DCLS) is also being studied and compared. Method detection limits for CBD and THC for both HPLC and GC-FID have been measured. Some commercial CBD products and hemp plants have been analyzed.

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PROBING EXTRACTS FROM ARONIA MITSCHURINII AND HOLY BASIL AS POTENTIAL ANTIFOULING AGENTS

Carson Cohen¹, Barry Teemer¹, Evgueni Nesterov², Paulinus Chigbu¹, Baruch Volkis¹, Victoria Volkis¹

¹Department of Natural Sciences, University of Maryland Eastern Shore, 11868 College
Backbone Rd, Princess Anne, MD 21853

²Department of Chemistry & Biochemistry, Northern Illinois University, 1425 Lincoln Hwy,
DeKalb, IL 60115

Biofouling is the accumulation of marine organisms on submerged surfaces, such as ships, buoys, and platforms. The formation of biofilm on the hull of ships can increase the amount of drag, and can greatly reduce the speed of the ship. A consequence of additional drag is the increase in fuel consumption. This poses a major economic issue for maritime travelers whether they be commercial or military. Yet, the major impact of biofouling is on the environment. When a ship travels from one area to another, segments of the biofilm can detach from the surface of the hull causing bacteria to be moved from its natural habitat to foreign areas where they are often invasive. When invasive bacteria are present, extensive blooming and/or death to the surrounding ecosystem can occur. To impede biofilm formation, antifouling biocides are applied to the hull of vessels. Traditional antifouling biocides contain Tributyltin (TBT), which is highly toxic. Use of this biocide is banned by the US, as well as many other countries. Attempts to find an alternative biocide have had limited success thus far. Potential alternatives have failed because they were either too costly or they still were harmful to the environment. Our approach is to use natural sources, such as super-fruits and medical herbs as the basis of an alternative biocide. Biofilm formation consists of five stages, out of which the first stage involves radical reactions. The resulting radicals initiate the polymerization of bacterial metabolites into biofilm. In order to prevent the occurrence of radical processes, we use essential oil extracts of natural crops with an extremely high content of antioxidants (radical scavengers) to limit bacterial growth. Extract-polymer formulations, antifouling tests, bacterial analysis, and surface analysis of thin films coated with the essential oils of *Aronia mitschurinii* and Holy Basil will be presented.

ATR-FTIR STUDIES OF CATION INTERACTIONS WITH CAFFEINE

Samantha Busch¹, Yanjie Zhang¹, Gina MacDonald¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St,
Harrisonburg VA, 22807

The purpose of this research is to elucidate the interactions between Hofmeister cations and a model drug caffeine. Ions are known to interact with proteins and affect their behaviors in aqueous solution, but the complexity of proteins makes these interactions difficult to study. Caffeine shares several common functional groups with proteins, enabling the clarification of specific interactions. ATR-FTIR spectra of caffeine were collected in the presence of nine chloride salts both in solution and in partially dehydrated samples. Divalent cations (i.e. Mg²⁺, Ca²⁺, and Sr²⁺) induced the most significant changes in caffeine vibrations – however, the presence of monovalent ions showed similar but decreased changes, particularly in the carbonyl (~1700cm⁻¹) and ring (~1650cm⁻¹).

EVALUATING CHANGES IN THE EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTORS AND MITONEET UPON TREATMENT

Jenell Gerow¹, Ashley Loe¹

¹Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock PA, 16057

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases. This disease affects over 5 million Americans. Symptoms usually develop slowly and get worse over time. Eventually, these symptoms become severe enough where they interfere with daily tasks. This disease is commonly characterized by the formation of senile plaques of the amyloid beta (A β) peptide and neurofibrillary tangles commonly developed in the hippocampus, prefrontal cortex, cerebellum, and the temporal lobe, thus jeopardizing the patient's memory, thinking, language, planning, and behavior. There have been multiple hypotheses regarding the development of AD, but there is still no known cause or cure. Treatment has been limited to alleviating the symptoms for those affected by AD. Due to this, alternative hypotheses, including mitochondrial dysfunction, have become a part of new efforts of studying AD and developing a potential cure. Mitochondrial dysfunction occurs when the mitochondria, the powerhouse of the cell, releases reactive oxidative species (ROS) that cause changes in expression and damage to tissues, proteins, and genes.

The overarching goal of this research project is to help elucidate the mechanism of AD and identifying potential targets for a cure by evaluating changes in protein expression when exposed to different situations such as a high oxygen environment, A β , and current therapeutics. Our preliminary studies verify that the $\alpha 7$ subtype of nicotinic acetylcholine receptor (nAChR) is upregulated upon exposure to donepezil, a current treatment for AD. Further studies will investigate the effect of donepezil and other treatments on the expression of mitoNEET and other mitochondrial proteins. Changes in protein trafficking and expression will be monitored using fluorescence microscopy in conjunction with immunochemistry and Western blot analysis.

This work was supported, in whole or in part, by Slippery Rock University, the Summer Collaborative Research Experience (SCORE) Grant Program, and the SRU Chemistry Department. We would also like to thank Dr. Christopher Richards (University of Kentucky) for providing cell lines and constructs.

DETECTION OF CHEMICALS USING CARBON NANOTUBE YARNS VIA CYCLIC VOLTAMMETRY

Jessica Bonilla¹, Jandro Abot²

¹Trinity Washington University, 125 Michigan Ave NE, Washington, DC 20017

²Catholic University of America, 620 Michigan Ave NE, Washington, DC 20064

Carbon nanotubes can be spun and twisted together to form a carbon nanotube (CNT) yarn with piezoresistive characteristics that could be tapped to measure strain and detect damage. Under the exposure of chemicals, the electrical current passing through the CNT yarn could be measured through cyclic voltammetry. In this project, we used two approaches to test if the CNT yarn was able to sense the chemicals at different concentrations. One of the approaches was to directly expose the chemical while the second approach was to not directly expose it and instead let it reach the CNT yarn through diffusion. The CNT yarn was able to sense the chemical inside the solution because as the concentration increased, the current peak also increased. The second approach is still being tested to see if the chemical would be absorbed by the non-exposed CNT yarn. The CNT yarn has good sensitivity to detect tiny concentrations of chemicals inside the solution.

COMPARATIVE STUDY ON THE DEVELOPMENT OF LATENT IMPRESSIONS ON GLOVES

Cierra Wiggins¹, Andrew Reitnauer²

¹College of Arts & Sciences, Trinity Washington University, 125 Michigan Ave NE,
Washington, DC 20017

²DC Department of Forensic Sciences, Latent Fingerprint Unit, 401 E St SW, Washington, DC
20024

Latent print evidence is an integral part in the identification process. These impressions are left on various surfaces through the transfer of moisture from the skin onto an object or surface. Gloves are often worn during the commission of a crime, and may be examined for the presence of latent prints. This study examined two types of gloves, laboratory nitrile and latex, and tested various processing methods using: CA fuming/dye stain, CA/WetWop, Gentian Violet, and Magnetic Powder to further develop latent prints. To assess examination methods, results were compared to establish trends and suitability. Based on the data collected, it was observed that there was an overall trend in the development of latent impressions within the types of gloves. When considering the surface of disposable gloves, there may be an increase in moisture from the containment of sweat exuded from the palmar surface of the hand that could compromise the individual characteristics of the friction ridge skin, adversely, if gloves have been changed during use, the surface of the skin may become dry and not have enough matrix to transfer onto the glove to leave a suitable impression. Overall, the nitrile gloves had a more consistent developmental reaction to the reagents used as compared to the latex gloves.

I would like to thank NASA DC Space Consortium for funding my research experience, and the DC Department of Forensic Sciences, Latent Fingerprint Unit for being a great host.

DESIGN AND SYNTHESIS OF SMALL MOLECULES: NLRP3 INFLAMMASOME INHIBITORS

Taylor Dyson¹, Amol Kulkarni¹

¹Department of Pharmaceutical Sciences, Howard University, 2300 4th St NW, Washington DC, 20059

Inflammation resulting from uncontrolled innate immune activation leads to a wide array of disorders, including Alzheimer's disease (AD), type 2 diabetes, multiple sclerosis (MS), atherosclerosis, etc. Nucleotide-binding domain leucine rich repeat protein 3 (NLRP3) has emerged as a druggable molecular target against the innate immune activation. Inhibitors of the NLRP3 inflammasome have shown promising activity in the clinical management of inflammation-associated disorders, such as, MS and AD. There has been a considerable interest in the development of small molecule NLRP3 inhibitors. Our initial research resulted in the identification of tertiary sulfonylurea molecules with good NLRP3 inhibitory activity. The current research involves the development of second generation compounds via refinement of the initial pharmacophore. These compounds were designed using a combination of computational and classical medicinal chemistry principles, synthesized, purified using flash column chromatography, and characterized using spectroscopic and chromatographic techniques. We will discuss the biological screening of our compounds against the NLRP3 inflammasome in central nervous system disorders.

We would like to thank the NASA DC Space Grant Internship and Scholarship for their funding. I would like to thank Dr. Patrice Moss for guiding us through this project.

THE SYNTHESIS OF 5'-NOR-ISONEPLANOCIN

Kathryn Jones¹, Qi Chen¹

¹Department of Chemistry, Slippery Rock University, 1 Morrow Way, Slippery Rock, Pa 16057

The structure of 3-deazaisoneplanocin has been recognized with broad spectrum antiviral activities including Ebola virus. The truncated 5'-nor derivative is unable to obtain for the parent neplanocin due to the unstable enol structure, which makes the designed structure 5'-nor-isoneneplanocin analogues (3, 4) not only interesting derivatives for isoneplanocin, but also an alternative surrogate testing for 5'-nor-3-deazaneplanocin antiviral activities. A novel synthesis route was developed for 5'-nor-isoneneplanocin analogues with a propylidene rearrangement as a key step. The designed target 5'-nor-3-deazaisoneplanocin (3) and its 4'-epimer (4) were accomplished and their L-enantiomers are upcoming.

We are grateful to the Department of Chemistry at Slippery Rock University for their support of this research. Special thanks to Dr. Schneller at Auburn University.

Science Education

ABSTRACTS

Morning Session

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DESIGNING A TEACHING MODULE ON SIGNALING MECHANISMS OF NEURITE OUTGROWTH

Kaitlyn Welch¹, Elizabeth Drda¹, Kristen Boeshore¹

¹Department of Biology, Lebanon Valley College, 101 N. College Ave., Annville PA, 17003

The focus of this project is to design a multi-week lab module for undergraduates. This lab will teach students cell culturing and Western blotting techniques while also demonstrating how researchers elucidate the biochemical signaling pathways that lead to a specific cellular response.

Dexamethasone (DEX), a glucocorticoid, has been shown to inhibit NGF-induced neurite outgrowth from PC12 cells. Specifically, DEX has been shown to inhibit phosphorylation of Akt, thus disrupting signaling downstream of the NGF receptor, trkA (Terada et al., 2014). Our goal was to replicate this finding in our lab. We expected to see less neurite outgrowth in NGF-stimulated cells that had been pre-treated with DEX. We also expected that trkA levels and phosphorylation would be unaffected by dexamethasone pre-treatment, but that phosphorylation of Akt would be decreased. Although it has been postulated that DEX does not directly inhibit the activation of the trkA receptor, this has never been shown. Thus, our demonstration of NGF-induced phosphorylation of trkA despite DEX pre-treatment would establish that DEX's inhibitory effects are in fact mediated downstream of trkA receptor activation.

WESLEY COLLEGE – STEM OUTREACH & SUPPORT SERVICES

Ja'Ki Miles¹, Lily S. Neff¹, Melanie Pritchett¹, Malcolm J. D'Souza¹

¹Department of STEM, Wesley College, 120 N. State St, Dover, DE 19901

This summer, Wesley College expanded its STEM Outreach and Services through its STEM Undergraduate Research Center of Analytics, Talent, and Success (STEM UR-CATS). The expansion included a very diverse set of STEM programming activities that were targeted to Delaware's underserved low, middle, and high school populations. For initial appointments, the schools were first contacted through email or telephone. Then, Wesley College STEM majors, STEM faculty, and UR-CATS staff visited the school locations to communicate their interests in science and math, and to inform participants about Wesley's dynamic STEM program. In addition, this summer, the Wesley College Science Club hosted an annual STEM Maker Fest event at the Dover Public Library. Along with STEM Outreach, STEM UR-CATS launched the Wesley Colleges Success in STEM (SIS) program. The SIS program gives incoming freshman a jump-start in Math, Chemistry, and Scientific Writing. This year's SIS cohort had 29 participants, including 6 high school juniors.

The UR-CATS framework of mentoring models that strengthen student support services and broadens undergraduate research experiences are made possible through support from an NSF-EPSCoR award (OIA-1757353, Grant No. IIA-1301765), an NSF (DUE) S-STEM grant 1355554 (Wesley College Cannon Scholar program), an IDeA award from NIH-NIGMS (P20GM103446, DE-INBRE program), the NASA Delaware Space Grant Consortium (NNX15AI19H), and the State of Delaware.

CHARACTERIZING CLASSROOM PRACTICES IN UNDERGRADUATE INORGANIC CHEMISTRY COURSES USING COPUS

Cole Pate¹, Melissa Kinkaid², Barbara Reisner¹

¹Department of Chemistry and Biochemistry, Cole Pate, James Madison University, 800 S Main St., Harrisonburg, VA 22807

²Department of Biology, Eastern Mennonite University, 1200 Park Rd., Harrisonburg, VA 22802

Previous research has demonstrated that student-centered teaching promotes student learning and retention of material in science, technology, engineering, and mathematics (STEM) courses. Some of the barriers that exist for instructors who wish to transform their courses and adopt student-centered learning practices include resources and community support. To investigate the role of community and instructional materials, eighteen instructors from across the United States who teach a foundations course in inorganic chemistry were selected as the first “VIPeR Fellows” cohort. A total of three cohorts of Fellows will participate in the course redesign process to improve student learning. Fellows recorded four consecutive classes on bonding or solids prior to the course redesign workshop. They will record the same topic the next time that they teach their foundations course. The videos will be analyzed using the Classroom Observation Protocol for Undergraduate STEM (COPUS). Practices observed in the videos before and after the course redesign workshop will be compared to investigate the impact that the community of practice and materials from the VIPeR (Virtual Inorganic Pedagogical Electronic Resource) had on classroom practice. Analysis of videos prior to course redesign reveals that VIPeR Fellows used a mixture of didactic, interactive lectures, and student-centered teaching methods. Trends in COPUS instructional profiles (didactic, interactive lecture, or student-centered) of the Fellows will be compared.

This work is supported by funding from the National Science Foundation (IUSE DUE-1726162/1726133/1725822 and REU CHE-1757874). Any opinions, findings, 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. We would like to acknowledge the VIPeR Fellows and their students and the members of VIPeR Team Research: J. Savage (Hope College), H. Heideman (Willamette University), D. Paunovic (James Madison University), J. Stewart (Hope College), J. Raker (University of South Florida), J. Pratt (University of South Florida), A. Bentley (Lewis & Clark College), S. Lin (United States Naval Academy), S. Smith (University of Michigan-Dearborn). Dr. Blair Schneider (University of Kansas, TRESTLE) and Professor Marilyne Stains (University of Nebraska) are acknowledged for their support with COPUS training.

SOAP-MAKING AS A GENERAL CHEMISTRY LAB: APPLYING A VIRAL TREND TO TEACH CHEMISTRY

Harley White¹, Jonah Ruskin¹, Alena Villanueva¹, Philip King¹, Michael Polen¹
¹Department of Chemistry, McDaniel College, 2 College Hill, Westminster MD 21157

General Chemistry labs are often traditional and inapplicable to everyday life. The goal for this project was to design a new, more modern experiment that would be relevant to students in General Chemistry while teaching them important topics in chemistry. Soap-making is a massively popular social media trend currently. We have designed a two-week experiment that allows students to synthesize their own bars of soap, then analyze their soaps for a number of properties relevant to bar soap. These properties include pH, cleaning efficacy, cost of production, and bar consistency. This lab will be implemented in the second semester of General Chemistry at McDaniel College to teach topics including intermolecular forces, limiting reactants, and organic chemical reactions.

The authors would like to thank The Jean and Donald Richards Student Research Fund for supporting this work.

Biological Sciences

ABSTRACTS

Afternoon Session

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UMBC HARVESTMEN: COLLECTION, PHYLOGENETICS, AND REPRODUCTIVE MORPHOLOGY

Zulekha Karachiwalla^{1,2}, Rahaf Alhabashi², Michael Mercado³, Mercedes Burns¹

¹Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Howard Community College, 10901 Little Patuxent Parkway, Columbia, MD 21044

³Gallaudet University, 800 Florida Ave NE, Washington, DC 20002

Harvestman, or “daddy long-legs”, have survived for over 400 million years on Earth and have evolved unique reproductive traits that have spurred recent scientific interest. Harvestman species are usually identified based on their morphology, which has caused some difficulties in species classification. Sequencing their mitochondrial genomes will allow for genetic species classification and provide more information on their genetic diversity. After PCR and gel electrophoresis, we identified a 600 bp or larger PCR product from the Mt-ND1 gene of 60 harvestmen. These samples were subsequently sequenced so the harvestman species could be genetically barcoded. Ongoing study of the female reproductive system of *Leiobunum* and *Hadrobunus* harvestmen, specifically the spermatheca, allows for understanding of the reproductive and behavioral differences among species. We predict that there should be a larger or more complex spermathecae in species where females mate multiply. A confocal microscope was used to view the spermathecae of each species and capture images for measurement and a stereo microscope was used to measure the body length of each harvestman. By using a T-test to compare body size measurements of 16 harvestman samples and their spermatheca size, it was observed that there was no correlation between the length of the sclerotized chamber of the spermatheca and body length. However, there was a statistically significant difference in spermatheca size between the genera *Hadrobunus* and *Leiobunum*. Ultimately, the combination of traditional morphological features, reproductive characters, and genetic markers has best potential for successful delimitation of species found on UMBC campus and throughout eastern North America.

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).

IDENTIFICATION OF PHOTORECEPTOR-SPECIFIC TRANSCRIPTION FACTORS IN THE DEVELOPING CHICKEN RETINA

Emily Hivick¹, Raymond Enke¹

¹Department of Biology, James Madison University, 951 Carrier Drive, Harrisonburg, Virginia
22807

The retina is a vital aspect of vision that contains sensory neurons, specifically rod and cone photoreceptors (PRs), which absorb light and allow the brain to perceive the outside world. Within the developing retina are specific transcription factors, which guide pluripotent neurons to differentiate into PRs. In mammals, the cone-rod homeobox (CRX) transcription factor largely regulates PR transcriptional networks. The chicken embryo is a reliable model system to study vertebrate retinal development, however, CRX is not coded in avian genomes. This suggests a different transcription factor is responsible for regulating transcriptional networks in chicken PR neurons.

The aim of this study is to identify transcription factors that bind to and regulate expression of PR-specific genes. Conserved regulatory sequences upstream of the chicken Rhodopsin (RHO) gene were amplified using a biotinylated primers. Purified amplicons will be bound to streptavidin affinity columns and used as bait to identify binding partners in protein lysates obtained from mature chicken retinas. Bioinformatics analysis reveals three distinct conserved RHO 5' regions in vertebras species suggesting a regulatory role. Preliminary results demonstrate successful amplification of these regions.

Current experiments are focused on preparing protein lysates from embryonic day 18 chicken retina, which will be utilized to determine protein concentration and thus protein-coding gene for the gRHO gene. These studies will increases our understanding of vertebrate retinal development and provide further insight into the developing and diseased human eye.

EXPANSIN LOSS DUE TO PARASITISM IN *CUSCUTA AUSTRALIS*

James Garraway¹, Robert Carey²

¹Department of Biochemistry and Molecular Biology, Lebanon Valley College, 101 College Ave, Annville, PA 17003

²Department of Biology, Lebanon Valley College, 101 N. College Ave, Annville, PA 17003-1400

Expansins are proteins found in plant cell walls. Major functions of expansins include plant stem elongation, fruit ripening, leaf abscission, and developmental processes. *Cuscuta australis* is a parasitic plant that grafts to its host and steals its water and nutrients. Due to its parasitic lifestyle, this plant is known to lose many organs that are common to land plants such as leaves and roots. Investigating further into how *C. australis*'s parasitic lifestyle affects expansin conservation can help add to a better understanding of expansin function and angiosperm clade characteristics. Interspecies comparisons of expansins in model plants were compared to *C. australis* signifying its lower number of expansins. Further phylogenetic analyses, both Bayesian and Neighbor-Joining, showed an exclusion of *C. australis* for six noteworthy angiosperm clades. Further characterization of these clades is necessary to better understand the function of the expansins that *C. australis* lost. Also, a comparison of *C. australis*'s case to other parasitic plants which may have undergone similar evolutionary losses could add greater insight to the effect of parasitism on expansin superfamily composition.

Thank you to the Lebanon Valley College High Impact Research Fund

BIOINFORMATIC AND MOLECULAR ANALYSIS OF PUMPKIN AND GHOST PUMPKIN, TWO NOVEL MUTATIONS IN *DROSOPHILA*

Susan Searles¹, Katharyne Murphy¹, Elisa Sangriu¹, Anne Mustafa¹, Peter Vos¹

¹Department of Chemical and Biological Sciences, Montgomery College, 20200 Observation Drive, Germantown, MD 20876

The pumpkin phenotype is a spontaneous mutation affecting eye color in *Drosophila melanogaster* first identified at Montgomery College in 2015. Mapping experiments originally placed the pumpkin locus on the X chromosome within 0.2 cM of the ruby gene, however, subsequent complementation studies showed pumpkin and ruby were distinct. Furthermore, males hemizygous for both mutations revealed a second novel eye-color phenotype, which we called ghost pumpkin. True breeding lines of ghost pumpkin were established and subsequently used to map the distance between ruby and pumpkin with higher resolution. The ghost pumpkin data led to more accurate mapping—placing pumpkin 5.7 cM from ruby—and provided an additional data set later used to confirm the variant call identifying pumpkin.

Next Generation Sequencing (NGS) enabled bioinformatic analysis of whole genome (DNA) and whole transcriptome (RNA) sequence data obtained from wild type, pumpkin, and ghost pumpkin flies. Using a variant discovery pipeline derived from GATK best practices, we were able to identify the mutation responsible for the pumpkin phenotype as a novel allele of white. PCR amplification and subsequent Sanger sequencing of DNA from the region of interest within the white gene confirmed the results of our bioinformatic analysis. The mutation is characterized by an in-frame inversion of twelve nucleotides toward the 3' end of exon 6.

The observed DNA sequence change in white is predicted to impact the amino acid sequence in the translated protein, specifically substituting an acidic amino acid for a basic amino acid in this region. Molecular modeling of wild type and pumpkin amino acid sequences indicates the region of interest is located on the extracellular surface of the white gene product, a component of the heterodimeric ABC transporter protein. These results lead us to conclude that we have successfully identified the molecular basis for the pumpkin phenotype.

This research was supported with intramural funding from Montgomery College. Special thanks to Dr. James Sniezek, Instructional Dean for Natural and Applied Sciences, and Dr. Scot Magnotta, Chemical and Biological Science Department Chair, for their support.

THE ROLE OF LACTASE-LIKE (LCTL) IN LENS CELL HOMEOSTASIS

Nicole Rossi¹, Mahbubul H. Shihan¹, Yan Wang¹, Melinda K. Duncan¹

¹Department of Biological Sciences, University of Delaware, Newark DE 19716

The mammalian eye consists of many structures that work together to allow for proper vision. Importantly, the lens, a transparent structure which sits behind the cornea and iris, must develop properly and remain clear, otherwise it can cloud and form a cataract. The pathway most responsible for effective lens function is fibroblast growth factor (FGF) signaling. Some fibroblast growth factors require extra proteins, klothos, to assist in binding their receptors. While alpha and beta family klothos are not expressed in the lens, gamma family klothos (LCTL) is expressed highly in adult lens and LCTL null mice develop mild lens opacities. However, the mechanisms underlying its function in the lens are unknown. Previous RNA sequencing of LCTL null lenses revealed that they upregulate the expression of retinal genes and neural markers, leading to my hypothesis that LCTL plays a role in repressing retinal genes in lens cells. However, this result could also simply be due to contamination of the dissected lenses used in the experiment with retinal tissue. My preliminary protein data is discordant with the upregulation of retinal gene expression observed via RNA sequencing. It is possible that this results from translational control of the upregulated messages, or contamination of the prior experiment with retinal tissue. Future testing of LCTL lenses by RT-PCR, and investigation of the role of LCTL in lens injury responses is required to determine if LCTL plays alternative roles lens development and maintenance.

National Eye Institute grant EY015279, the Delaware Summer Scholars program, and the Department of Biological Sciences

IDENTIFICATION OF MUTATIONS IN TWO STRAINS OF *SERRATIA MARCESCENS*

Maryssa Roberts¹, Kevin E. Shuman¹

¹Department of Biology, Wesley College, 120 North State Street, Dover, DE 19901

Serratia marcescens was discovered in 1819 by a pharmacist named Bartolomeo Bizio when he identified the bacteria as a bloody discoloration in a cornmeal mush. *S. marcescens* is the only pathogenic species in its genus and has been involved in numerous hospital-acquired infections. During this research, three different strains of *S. marcescens* were studied to find phenotypic differences. The KAM and KIDD strains of *S. marcescens* were lab maintained, while a wild-type strain was used as a control. The physiological profiles of each strain were determined using GEN III Biolog plates. Compared to the wild-type strain, the KAM strain exhibited an increase in the number of carbon sources it could metabolize, while the KIDD strain exhibited a significant loss in its ability to use different carbon sources. *S. marcescens* is known to produce a red pigmentation called prodigiosin. This red pigment was extracted from *S. marcescens* using acidified ethanol. There was a significant reduction in prodigiosin production in the KAM strain and an absence of prodigiosin production in the KIDD strain. Identification of phenotypical differences between strains of *S. marcescens* will be used to help identify potential gene mutations that could be identified during the genetics course at Wesley College. There are also plans to sequence the whole genome of each strain.

This research was supported through the Delaware INBRE program funded by the NIH NIGMS IDEA Program Grant #P20 GM103446 and the State of Delaware.

PRE- AND POST-TUNNEL IMPLEMENTATION HAS AN IMPACT ON THE MICROBIAL COMMUNITY DIVERSITY OF THE ANACOSTIA RIVER, D.C.

Rebekah Officer¹, Gaurav Arora¹, Caroline Solomon¹

¹Department of Science, Technology, and Mathematics, Gallaudet University, 800 Florida NE Ave Washington DC 20002

The Anacostia River located in Washington, D.C is one of the most polluted rivers in the United States. To address environmental concerns, DC Water build a storage tunnel beneath the Anacostia River that opened in March 2018 to divert sewage and storm water from entering the river. To evaluate the effectiveness of the tunnel and examine relationships between flow conditions, nutrient dynamics and microbial communities, samples were collected pre-tunnel (2017) prior and post-tunnel (2018) tunnel implementation. Previous work has shown that flow has an impact on nitrate (NO_3^-) and ammonium (NH_4^+) concentrations and $\text{NH}_4^+:\text{NO}_3^-$ ratios as well as presence of various bacteria (16S) and phytoplankton (18S) taxa. We predicted that after tunnel implementation that NO_3^- and NH_4^+ values will increase while the NH_4^+ and NO_3^- ratio will decrease leading to diatoms to increase while cyanobacteria will decrease. Data shows higher counts for bacterial (16S) groups *Burkholderiales*, *Cerasicoccales*, and *Cytophagales* in 2017 compared to 2018. However, there were more transcript reads of eukaryotic (18S) species compared to prokaryotic (16S) species during both years. Overall, the results show that there was a decrease in the composition of the microbial (16S & 18S) communities from pre- to post-tunnel implementation despite changes in nitrogen concentrations and $\text{NH}_4^+:\text{NO}_3^-$ ratio. Next step in data analysis includes making connections between nutrient concentrations and composition of the microbial community and via biodiversity indices and PoCA analyses. Due to above average rainfall in 2018-2019, the evaluation of the effectiveness of tunnel implementation needs to continue to see if there are any changes in microbial biodiversity in the river.

The authors are supported by Maryland Sea Grant, Gordon Brown Endowment Fund, District of Columbia NASA Space, Grant Consortium, and Beverly Taylor Sorenson Student Research Fellowships, and Anacostia Riverkeeper.

THE INFLUENCE OF SEXUAL MATURITY ON THE HERDING BEHAVIOR OF THE *EISENIA FETIDA*: A PILOT STUDY

Prutha Patel¹, Adamary Sanchez-Martinez², Hangkyo Lim¹

¹Department of Biology, Notre Dame of Maryland University, 4701 N Charles St, Baltimore, MD 21210

²Department of Chemistry, Notre Dame of Maryland University, 4701 N Charles St, Baltimore, MD 21210

As composting becomes a more popular method of food waste management, different approaches have arisen to increase its efficiency. One approach involves the use of earthworms to aid in the composting process, referred to as vermicomposting. Specifically, *Eisenia fetida* has become a popular species of earthworms to use in the vermicomposting process. It is important to better understand *E. fetida* because it helps us understand how it behaves while composting. Ultimately, this knowledge can be used to refine the vermicomposting process. In a previous study, this species exhibited a collective movement and participated in group decisions, which would later be referred to as “herding” behavior. However, there were some physiological mechanisms that were not addressed regarding this interesting behavior. Specifically, the sexual maturity of the test subjects and which substrate the *E. fetida* were present in were not factors investigated in causing collective movement. Therefore, the main goal of this present study is a replication of the previous finding, while taking into consideration the sexual maturity of the hermaphroditic species and possible affinity for a certain substrate. The *E. fetida* were placed in a two-choice maze that was lined with soaked filter paper. The two-way choice test was conducted using two sexual maturity groups within 3 different combinations of substrate type. Our results indicate that overall “herding” behavior was influenced by these conditions. After observation, *E. fetida* had an affinity for a certain substrate and the distributions of individuals varied based on sexual maturity. We propose that even if the worms may move toward a certain direction as a group, it still guarantees the further study on whether the behavior is found on an active decision making or a collective result from an individual preference for a common cause.

Special thanks to Benny Erez and the Eco City Farm team for providing us an initial population of *Eisenia fetida*. Thank you also to Pat Bell and Marrisia Moore for providing access to the lab and assisting us throughout the process. As well as a sincere thank you to Dr. Lim for giving us the support and guidance to complete this pilot research

PREDATION OF SPOTTED LANTERNFLY (*LYCORMA DELICATULA*)

Anna Kuhn¹, Su Kim¹, Michael Raupp², Holly Martinson¹

¹Department of Biology, McDaniel College, 2 College Hill, Westminster MD 21157

²Department of Entomology, University of Maryland, College Park, MD 20742

By the necessity of evolution, most prey organisms have specialized methods of defense against predation. However, when a prey organism is introduced to a new environment with previously unfamiliar predators, their defense mechanisms may or may not be effective. Should the prey organism's defenses be effective against the predators of its new environment, this may contribute to the spread and abundance of the new organism. Spotted lanternfly (*Lycorma delicatula*; Hemiptera: Fulgoridae, SLF) have striking aposematic coloration, a powerful jumping reflex, and have been reported to contain alkaloid chemical defenses in their native environment in China. These organisms have spread rapidly in Pennsylvania since their introduction, feeding on trees and bushes and causing significant issues in Pennsylvania's agriculture and forestry. Native predators, save for a select few, have not been observed to be interested in preying upon these insects, and it is unknown if defenses of the SLF are truly preventing predation. Understanding the defenses of SLF could provide a new perspective in developing methods of preventing the spread of SLF. Three studies were conducted to examine the defenses of SLF: (1) plasticine models were used to examine aposematic coloration, (2) samples of SLF examined through Thin Layer Chromatography were used to identify ajmalicine and ailanthone, cytotoxins that SLF reportedly contained in the Chinese and Korean populations respectively, and (3) mesocosm predation experiments were used to identify the impact of SLF jumping reflex and other unknown defenses. The plasticine models contained markings consistent with avian predation, the Pennsylvania population was found to not contain ajmalicine, and green lacewing larvae and Chinese mantises did consume SLF nymphs in mesocosm experiments. This research demonstrates that the effectiveness of prey defenses may change with species introductions, a phenomenon that could be potentially exploited by targeted management interventions of problematic species in their introduced ranges.

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USE OF RADIO TRACKING TO STUDY TERRITORY SIZE OF THE BAHAMA ORIOLE

Sheridan Danquah¹, Breanna Byrd¹, Gabriel Wilkins¹, Matthew Fagan², Colin Studds², Kevin Omland¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Geography Information Systems, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

The Bahama Oriole (*Icterus northropi*) is a critically endangered bird found only in Andros, Bahamas. Previous literature on these orioles suggested they mostly nested in developed areas in Coconut Palms (*Cocos nucifera*). With new knowledge that the Bahama Oriole also nests in pine forests (*Pinus caribaea*), we hope to make more accurate population estimates. Determining the home range of the Bahama Oriole is crucial to making reliable population estimates.

Using radio transmitters placed on three different orioles, we triangulated the position of tagged birds. These positions were used to generate territory maps for the three radio tagged birds. To access the validity of our equipment, and ensure the transmitter was still on sampled birds, we also periodically set out to sight the tagged orioles. The resulting home range maps show that the Bahama Oriole has a larger territory size than was previously assumed. These findings will be crucial in our ongoing efforts to estimate the total number of Bahama Orioles surviving.

OPTIMIZING A COLORIMETRIC NITRATE REDUCTASE ASSAY FOR HARMFUL ALGA *CHATTONELLA SUBSALSA*

Gretchen Johnson¹, Yanfei Wang¹, Kathryn Coyne¹

¹School of Marine Science and Policy, University of Delaware, 700 Pilottown Road, Lewes, DE 19958

In recent years, coastal ecosystems worldwide have experienced increasingly frequent and severe disruption by harmful algal blooms (HABS). *Chattonella subsalsa* is a globally-distributed HAB species implicated in fish kills. In *C. subsalsa*, transcriptomic evidence suggests a phosphoserine and a 14-3-3 protein binding site on a novel nitrate reductase enzyme (NR3). In order to test the putative divalent-cation-dependent regulation pathway of phosphorylation enabling 14-3-3 binding to NR, a colorimetric nitrate reductase assay was optimized for cell extracts from a Delaware inland bay strand of *C. subsalsa*. Optimization experiments manipulated extract homogenization methods (grinding vs. sonication), assay buffers (EPPS, TAPS, Bis-Tris Propane, and Inorganic Phosphate), enzyme stabilizers (Dithiothreitol and Bovine Serum Albumin), and electron sources (NADH vs. NADPH). The optimized assay was then performed with and without chelation of divalent cations by EDTA under various short-term stress regimes to evaluate regulatory dynamics. The protocol developed herein should enable more detailed future studies of *C. subsalsa* NR3, which are merited to improve understanding of the nitrogen metabolism of a ubiquitous HAB species.

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FENCES AND FLOWER FLIES: DETERMINANTS OF FLORAL ABUNDANCE AND FLOWER VISITATION IN A NUTRIENT NETWORK SITE

Kaitlin Murphy¹, Jessie Wyatt¹, Holly Martinson¹

¹Department of Biology, McDaniel College, 2 College Hill Westminster MD 21157

Insect pollinators are important to the health and reproduction of wild and cultivated plants. Factors influencing the number and type of pollinators can therefore be crucial for maintaining pollination services. Old fields are herbaceous plant communities that develop on abandoned agricultural fields and include a diverse set of flowering plants. In this system, insect pollinators may be differentially attracted to these flowering plants, depending on species-specific characteristics or attributes such as plant height or floral abundance. Pollination may also be influenced by biotic interactions such as herbivory. To investigate the determinants of floral abundance and flower visitation, 18 field plots were established in May 2019 in an old field in New Windsor, MD. Fences were installed around six of these plots to investigate the effects of deer on plant-pollinator interactions. Relationships between pollinator visitation, floral abundance, plant species, and fruit set were investigated in repeated 20-minute surveys in open and fenced plots. In total, 27 flowering plant species and 854 pollinator-plant interactions were observed. Of these interactions, 414 were visits by the hoverfly, *Toxomerus marginatus* (Diptera: Syrphidae), and hoverfly visitation was positively related to floral abundance. Investigating the full pollinator community, the number of pollinator visits varied among plant species and with floral unit abundance but did not differ with fencing treatment. Similarly, fruit set varied among plant species and was positively related to floral abundance but unaffected by fencing. In contrast, fencing influenced plant height, and the magnitude of this effect varied among species. These results suggest that non-bee pollinators may be of primary importance to plants at this field site and that retention of native plants with abundant flowers may enhance pollination services. Future studies can investigate the efficacy of particular pollinators and test the responses of plant-pollinator networks to additional environmental disturbances such as nutrient additions.

We gratefully acknowledge the Nutrient Network for providing protocols that we adapted in our field methods for this study. We would like to thank Dr. Martinson for her unconditional guidance from the project's start to finish. She was an invaluable source of knowledge that we are incredibly grateful for. We also thank McDaniel College and the residents of the Singleton-Mathews property for allowing us to use their land throughout the Summer of 2019 to conduct this research. Another thanks to the McDaniel College Biology Department and Lab Facility for always welcoming us to utilize whatever resources we needed. Finally, we would like to thank McDaniel College Academic Affairs and the Milton Hendrickson Student-Faculty Research Fund through the McDaniel College Biology Department, specifically the Jean and Donald Richards Student Research Fund for the financial support to carry out this project.

VALIDATION OF DIFFERENTIALLY EXPRESSED GENES IN AMYLOID BETA-EXPRESSING *C. ELEGANS* FED DIFFERENT DIETS

Charlotte Leslie¹, Andy Lam¹, Jessica Tanis¹

¹Department of Biological Sciences, University of Delaware, 210 South College Ave., Newark, DE 19716

Late onset Alzheimer's Disease (LOAD), a progressive neurodegenerative disease, is one of the leading causes of dementia in the United States. LOAD affects an estimated 5.7 million Americans with approximately 10% of people age 65 and older diagnosed. Accumulation of toxic amyloid-beta (A β) peptides resulting in senile plaques is one of the many pathological hallmarks of LOAD, in addition to abnormalities in mitochondria ultrastructure, and diminished cellular respiration. Utilizing the model organism *C. elegans*, we are identifying factors that impact A β toxicity in vivo. Expression of A β 1-42 in the *C. elegans* body wall muscles results in accumulation of A β and time dependent paralysis upon temperature upshift. We discovered that different *E. coli* diets have a significant effect on the time it takes A β animals to paralyze; animals raised consuming OP50 paralyze significantly faster than animals which are raised on an HB101 *E. coli* diet. We also discovered that A β animals raised on HB101 had higher ATP levels compared to animals raised on OP50, suggesting a link between diet and mitochondrial function. In order to determine the mechanism by which the HB101 diet is protective against A β toxicity we performed RNA-Seq analysis and found several genes that were differentially expressed in A β and wild type (WT) animals, and between the A β animals fed the different diets. To verify the RNA-Seq results we performed quantitative real time PCR (qRT-PCR). We found that *cbs-1*, a cystathionine beta-synthase, was upregulated in A β animals fed HB101 compared to those fed OP50. This is significant because cystathionine beta-synthase plays a role in homocysteine metabolism and hyperhomocysteinemia is one risk factor for LOAD. Our findings suggest that the HB101 diet-induced delay in paralysis of A β animals may result from a protective effect on mitochondrial function and homocysteine metabolism.

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DEVELOPMENT OF ASSAYS TO DETERMINE THE SPECIFICITY OF ANTI-ORTHOHANTAVIRUS ANTIBODIES FROM PATIENTS

Tiara White¹, Annalis Norman¹, Phil Eisenhauer¹, Cecilia Vial^{2,3}, Pablo Vial^{2,4}, Jason Botten^{1,5}

¹Division of Immunology, Department of Medicine, University of Vermont Larner College of Medicine, Burlington, VT 05405, USA

²Programa Hantavirus, Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Avenida Las Condes 12461, Las Condes, Santiago, Chile

³Centro Genética y Genómica, Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Avenida Las Condes 12461, Las Condes, Santiago, Chile

⁴Departamento de Pediatría, Clínica Alemana de Santiago, Avenida Vitacura 5951, Vitacura, Santiago, Chile

⁵Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405, USA

New World orthohantaviruses are carried by rodents in North America and South America and cause hantavirus cardiopulmonary syndrome (HCPS). Andes virus (ANDV) is the primary cause of HCPS in South America and is 30-50% fatal. There are no effective FDA-approved drugs or vaccines available to prevent or ameliorate HCPS. Neutralizing antibodies (nAbs) are the only known correlate of protection from developing severe disease. The goal of this project was to develop an assay to determine the specificity and kinetics the human anti-ANDV antibody response. We transfected HEK293T cells with plasmids expressing different ANDV proteins (nucleocapsid protein (N), glycoprotein (GP), and non-structural protein of the S segment (NSs) that contained a C-terminal HA tag. After 48 hours we collected cellular protein lysates containing the recombinant ANDV proteins. We confirmed the presence of ANDV proteins via western blot by probing for the HA tag. Using the cellular lysates, we employed a strip immunoblot assay (SIA) to test for recognition of ANDV proteins by patient serum from mild and severe HCPS cases. Patient serum collected at either acute (days 1, 3, and 5 post-hospitalization) or convalescent (day 60 post-hospitalization) phases of infection were screened via SIA to determine the kinetics and specificity of the antiviral humoral response. All patients (those with mild or severe disease) generated antibodies against ANDV N by day 1 after hospitalization and these antibodies remained into convalescence. Antibodies that could recognize ANDV NSs were not detected, while a subset (two severe patients and one mild patient) produced antibodies against ANDV GP that were detectable throughout the time course examined. The strip immunoblot assay is an effective means to screen patient sera for antibodies with specificity for orthohantavirus proteins and determine the kinetics of the humoral response during ANDV infection.

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DO TOXIC CYANOBACTERIA LURK IN DELAWARE PONDS?

Alena Brown¹, Thomas Hanson²

¹Department of Biology, Wesley College, 120 North State St. 19901

²School of Marine Science and Policy, Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE

Cyanobacteria are photosynthetic microorganisms that are autotrophic and fix carbon dioxide for growth (photosynthesis: $H_2O + CO_2 \rightarrow CH_2O + O_2$). They are some of the largest bacteria known and can produce large blooms in lakes and ponds that negatively affect water quality. Some types of Cyanobacteria can produce toxins that can poison livestock and humans. In addition, because of the possible harm to surface water normally used as drinking water it is important to know when to treat and remove the toxins. This project aims to examine Cyanobacteria that bloom in Delaware ponds to determine if they are potentially toxic or not. The types of Cyanobacteria and other microbes present in water samples will be determined with Cultivation-Independent DNA sequence analyses while Cultivation-Dependent methods will be used to isolate model organisms for subsequent laboratory experiments. Data from these approaches will be interpreted in light of water quality data collected at the same time. This analysis will allow us to understand what Cyanobacteria are present when in Delaware ponds and what factors cause them to bloom.

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GENOME SEQUENCING OF THE *GROMPHADORHINA PORTENTOSA* AND ITS MICROORGANISMS

Kristina Pickering¹, Kenneth Sossa¹

¹Department of Biology, Notre Dame of Maryland University, 4701 North Charles Street
Baltimore Maryland 21210

There are a growing number of organisms that have their entire genome sequenced in several databases, such as the National Center for Biotechnology Information. The *Gromphadorhina portentosa*, commonly called the Giant Madagascar Hissing Cockroach, is not featured in such databases. The purpose of our work is to not only sequence the genome of the cockroach, but also its gut microbiome. Here we 1) compare efficiency of DNA extraction methods and 2) compare insect and microbe sequences. Overall, the findings in this research will uncover new information about this potentially limitless model organism.

DAPHNIA GLYPHOSATE PROTEOMIC INVESTIGATION

Kerri Corcoran¹, Megan Valentine¹

¹Department of Biology, SUNY Plattsburgh, 101 Broad Street, Plattsburgh, NY 12901

Roundup® is a commercial and domestic herbicide commonly used in the United States and abroad. The active herbicidal ingredient in Roundup®, glyphosate, is marketed to be “environmentally benign” and to have low toxicity. However, runoff from agricultural and commercial settings may provide means by which this chemical can enter waterways (Shambaugh et al., 2016) where this chemical is hypothesized to have sub-lethal effects on non-target species (Battaglin et al., 2014). Recent studies indicate that glyphosate may persist in freshwater environments and may impact *Daphnia*, chemically-sensitive freshwater microcrustaceans that sustain strong ecological interactions and are important ecotoxicological indicators in freshwater environments. To better understand possible changes in the proteins expressed by *Daphnia* in the presence of glyphosate, three *Daphnia pulex* cultures were established. A control culture (unexposed) was used to compare to *D. pulex* exposed to a high dose of RoundUp® (0.050g/L), and exposed to a low dose RoundUp® (0.025 g/L). All cultures were fed with algae (5mL) on a daily basis and collected after 96 hours of exposure. *Daphnia* were harvested and crushed in liquid nitrogen followed by solubilization, after which the proteins were separated by SDS-PAGE. The resulting gel was stained with Coomassie, after which, the entire protein lane of each condition was cut up, trypsinized, and sent to the Vermont Genetics Network Proteomics Facility for peptide sequencing and protein identification. This study presents a semi-quantitative analysis of tryptic peptides identified in low and high dose RoundUp® treatments in an effort to identify proteins whose expression levels have changed in the presence of the RoundUp®. Our findings will lead to a better understanding of how glyphosate based herbicides may be altering the protein expression in microcrustaceans, like *Daphnia pulex*, and the ultimate downstream effect it will have on lake and pond environments, including Lake Champlain.

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A METAGENOMIC ANALYSIS OF THE EQUINE GUT MICROBIOME WITH AND WITHOUT PROBIOTIC SUPPLEMENTATION

Olivia Konen¹, Kristin Peters¹, Petra Tsuji¹

¹Department of Biological Sciences, Towson University, 8000 York Rd, Towson, MD 21252

The intestinal microbiota of mammals consists of a variety of bacteria, archaea, fungi, and viruses. These all play a significant role in the maintenance of homeostasis. Commercially available probiotics are advertised as providing health benefits when consumed, and are utilized to address gastrointestinal disorders and broader systemic issues. The goal of this study is to compare the microbiome of domestic horses with and without probiotic supplementation. Towson University's Institutional Animal Care and Use Committee has reviewed the protocol and granted an exemption, as the horses were not housed on campus and no experimental manipulation occurred. Fecal matter from six privately owned horses maintained on their standard diet were collected. Three of the six horses received a probiotic supplement, SmartDigest, for several years prior to beginning the project. Supplementation ceased for one month, and samples were again obtained. The other three horses never received probiotics. Bacterial DNA was isolated from the fecal samples and microbial diversity was characterized by amplifying the 16S rRNA and tagging it with index primers. The samples were sequenced on the Illumina MiSeq and validated by qPCR. Dominant groups from non-supplemented horses residing on the same property included the phyla Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia. Similarities between horses persist down to the genus level. Interestingly, a greater abundance of Firmicutes was found in the horses who had never received probiotics in comparison to the horses that were off of probiotics for one month. No significance in Firmicute abundance was found between horses on SmartDigest and then taken off for a period of one month.

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THE EFFECT OF NGF RELEASED BY MECHANICALLY LOADED OSTEOCYTES ON PROSTATE CANCER CELL PROLIFERATION AND MIGRATION.

Madeline McGhee¹, Nicholas Trompeter³, Case Gregory¹, Randall Duncan², Mary Boggs⁴,
Kenneth van Golen¹

¹Department of Biological Sciences, University of Delaware, Newark, DE 19716

²Department of Biological Sciences, University of Michigan - Flint, 303 E Kearsley St, Flint, MI 48502

³Department of Biomedical Engineering, University of Delaware, Newark, DE 19716

⁴Delaware Biotechnology Institute, University of Delaware, Newark, DE 19716

Prostate cancer is the second leading cause of cancer death in men. Approximately 80% of prostate cancer cases experience metastases in bone that induce osteosclerotic lesions. These lesions reduce bone strength and increase mechanical strains in the bone. Our lab has already demonstrated that osteocytes release ATP and Nerve Growth Factor (NGF) when under mechanical load. Previous studies have also indicated that exogenous NGF increases proliferation of prostate cancer (PCa) cells. We hypothesize that the NGF released by these osteocytes upregulates purinergic receptors in PCa cells which in turn bind ATP resulting in increased proliferation and migration. Additionally, we hypothesize that NGF acts as a chemotactic attractant which results in migration of PCa cells to bone. C4-2B4 cancer cells were treated with NGF at concentrations of 5ng/mL, 10 ng/mL, and 25 ng/mL in order to determine which concentration is most effective in increasing cell proliferation. Osteocyte-like cells, MLO-Y4, were sheared under fluid flow at 5 dyn/cm², a time and magnitude that we have shown produces maximal release of NGF, and the resulting conditioned medium (CM) was collected. The same fluid shear was applied to osteocytes treated with SIN-1 at 100mmol concentration to determine if oxidative stress increases release of NGF. To determine the effect of NGF on PCa cell migration, C4-2B4 cells were treated with either control, exogenous NGF or MLO-Y4 CM. Based on our data from NGF treated cancer cells, these indicate that the NGF receptor TrkA may be a potential therapeutic target to decrease PCa cell growth.

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USING ELECTRORETINOGRAPHY TO STUDY THE EFFECTS OF VISUAL INPUT, AGE AND SEX ON OCULAR NEURON SPECTRAL SENSITIVITY IN *GROMPHADORHINA PORTENTOSA*

Jeannette Rosales Blanco¹, Kenneth Sossa¹

¹Department of Biology, Notre Dame of Maryland University, 4701 N Charles St. Baltimore, MD. 21210.

The Giant Madagascar Hissing Cockroach (*Gromphadorhina portentosa*) is a species whose size enables its neurological activity to be easily studied in a laboratory setting. Here we will be using the electrophysiological technique known as the Electroretinogram (ERG) to record ocular neuron activity in *G. portentosa*. In particular, ocular neuron activity in response to various light wavelengths will be examined. We will compare ERG responses between normal-reared (NR) (12 hr light, 12 hr dark) and dark-reared (DR) roaches. The dark-reared are a subset of roaches raised in infrared light, which due to the absence of red-light receptors, is not detected by roaches. The varying ERG responses will be analyzed via differences in amplitude and frequency of action potentials. We tested neuronal responses with orange, blue, and UV light. Our preliminary findings show light-induced amplitude and sex-specific differences between NR and DR roaches. These differences are most apparent with UV light responses. Through this research, we hope to detect whether changes in light environment (presumably circadian rhythms) will modulate ocular neuron activity.

TRPM1 CATION CHANNEL HAS A NOVEL ROLE IN LIGHT-DEPENDENT SIGNALING FOR PUPIL CONSTRICTION IN MAMMALS

Tess Leftwich¹, Shane Chambers¹, Jeremy Rice¹, Marquis Walker¹

¹Department of Biology, James Madison University, 800 S Main St. Harrisonburg Va 22807

In mammals, all light detection occurs in the photosensitive retinal tissue of the eye. Photoreceptor cells within the retina collectively provide the visual signaling input necessary for image forming and non-image forming behaviors. Pupillary light reflex (PLR) is an involuntary non-image forming behavior in which iris muscle in the eye constricts in response to increasing ambient light intensity. All retinal photoreceptor classes contribute light-activated signals to drive the PLR behavior, but these signals are exclusively carried from the retina to the olivary pretectal nucleus (OPN) of the brain by intrinsically photosensitive retinal ganglion cells (ipRGCs). Neurons in the OPN control the signaling input that initiates constriction of the iris muscle. Recently it has been demonstrated that the iris muscle can respond directly to light and will constrict independent of neurological signals from the OPN. The light signaling in the iris sphincter muscle is initiated by the photopigment melanopsin. Currently the terminal ion channel in this melanopsin signaling pathway has not been identified. Mice with a loss of function mutation in the transient receptor potential melastatin 1 (Trpm1) cation channel exhibit a severe defect in PLR. Until now the role of Trpm1 in signaling that drives iridial constriction has been unclear. We have tested PLR wildtype and Trpm1 knockout mice at increasing light intensities to demonstrate a novel role for Trpm1 in iridial tissue signaling. We have also localized Trpm1 expression in the iris through RT-PCR. In addition we are able to show that Trpm1 expression in the iris is conserved in other mammals such as rats and humans. Our combined results indicate that Trpm1 has a necessary role in iridial cells and is required for light-driven pupil constriction.

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EFFECT OF COCAINE ON AF5 CELLS

Nadira Abliz¹, Kristen L. Boeshore¹

¹Department of Biology, Lebanon Valley College, 101 College Ave, Annville, PA 17003

We are interested in the effects of cocaine on the embryonic development of the brain. There is evidence that cocaine causes cells to differentiate into neurons too soon in the embryo. AF5 cells are neural progenitors from embryonic rat midbrain. Cells in this region of a cocaine-exposed embryo will give rise to more astrocytes and fewer neurons. It is believed that cocaine results in astrocyte proliferation and premature neuronal differentiation. The imbalance in neurons and astrocytes would potentially cause intellectual defects later in life.

Although we have access to the cell line, we do not yet have access to the cocaine. We first need to provide evidence that we know how to do the assays. The CyQuant assay allows us to quantify the number of cells. Live cells take up the fluorescent CyQuant dye. With a microplate reader we can then read the fluorescence. More fluorescence means there are more cells. With the ROS assay we tried to measure the production of reactive oxygen species (ROS) in cells as a measure of oxidative stress. Cocaine has been shown to increase oxidative stress in cells, and this stress can result in different cell-type specific responses, dependent on downstream signaling pathways that are activated. Eventually, we wish to elucidate the downstream signaling pathways that result in cocaine-induced proliferation (as occurs in astrocytic progenitors) and in cocaine-induced differentiation (as occurs in neuronal progenitors).

PERIPHERAL NERVE REGENERATION FOLLOWING HYPERGLYCEMIC INDUCTION IN *DANIO RERIO*

Sheridan Sargent¹, Jessica Clark¹

¹Department of Biological Sciences, Salisbury University, 1101 Camden Avenue, Salisbury MD
21801

The peripheral nervous system is made up of motor and sensory neurons that allow the brain to sense and respond to the environment. Diabetes mellitus is a chronic metabolic condition that often results in damage to these nerves. Patients with diabetic peripheral neuropathy (DPN) present with numbness, paresthesia, and/or muscle weakness. Because the pathophysiology underlying DPN is not yet clear, treatment is limited to pain management. In the Clark lab, we have shown that incubation of larval zebrafish for five days in 60 mM glucose concentration negatively affects myelination of the peripheral nerves. Due to the known regenerative capabilities of zebrafish, we hypothesize that after removing the larvae from hyperglycemic conditions, myelin will begin to regenerate. Tg(mbp;CAAXegfp) larval zebrafish were incubated in 120 mM glucose solution (treatment), or egg water (control), for seven days beginning five days postfertilization. To allow for regeneration, zebrafish were placed in normal egg water for an additional ten days, and control fish were kept in egg water for an equal amount of time. Currently, we are assessing length of motor nerve myelination in vivo. Understanding the molecular mechanisms of myelination repair in zebrafish would help us characterize the molecular pathways involved in regeneration which may potentially lead us to therapeutics in the future.

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COMBINATORIAL EFFECTS OF PESTICIDE EXPOSURE IN A *DROSOPHILA* MODEL OF PARKINSON'S DISEASE

Crystal Gonzalez¹, Dionne Williams¹, Hakeem Lawal¹

¹Department of Biology, Delaware State University, 1200 N DuPont Hwy, 19901 Dover, DE

Parkinson's Disease (PD) is the preferential loss of dopaminergic neurons in the substantia nigra pars compacta, part of the brain responsible for coordinating motor movement. While vast majority of PD cases are unknown, exposure to environmental factors play a role in PD disease development. However, comprehending the complement of environmental toxins that elevate risk of PD has been challenging due to difficulties in developing suitable models to study epidemiologically relevant exposure paradigms. We hypothesize that combinations of commonly used pesticides will recapitulate key features of PD in a model system, including motor deficiency development. Adult *Drosophila melanogaster* was used to study the synergistic effects of the following pesticides: Alachlor, Atrazine, Acephate, and Diuron. To study pesticides combinatorial effects, the Multi-Worm Tracker program was used to record locomotion and observe any motor deficits due to pesticide exposure. We also included lifespan analysis to record the survival of pesticide-treated flies. This was executed throughout 4 weeks to observe any notable patterns between pesticide exposure and its effect on motor movements. Here we present preliminary data from our on-going studies and expect that our findings will provide critical insight into the risk levels that pesticidal combinations pose to the development of PD.

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IDENTIFYING MUSCLE SYNERGIES IN RAT EXTRINSIC TAIL MUSCLES BY SELECTIVE ELECTROPHYOGRAPHY

Amelia Kahle¹, Corey Cleland¹

¹Department of Biology, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Muscle redundancy, which occurs when multiple muscles create the same mechanical action, complicates the control of movement by the central nervous system (CNS). It is hypothesized that the CNS simplifies the control of movement by activating groups of muscles together, called muscle synergies, rather than activating each muscle individually. Muscle synergies are commonly identified through recording electromyograms (EMGs) during movement, such as the nociceptive withdrawal response (NWR) -- a complex, multi-joint response to noxious stimuli. The rat tail is a particularly appropriate model system because rat tail muscles are hyper-redundant, with dozens of muscles having similar mechanical actions. However, selectively recording EMGs from tail muscles has not previously been successful as a result of their small muscle size and close proximity. Thus, the goal of this study was to develop a selective EMG recording technique to record extrinsic tail muscle activity in order to deduce muscle synergies. Sprague-Dawley rats (4-5 months) were anesthetized and eight bipolar EMG electrodes were placed percutaneously into specific locations along either side of the midline, targeting fascicles of the M. sacrocaudalis dorsalis lateralis (SDL), an extrinsic tail muscle. To identify the muscles being recorded, we electrically stimulated through the bipolar electrodes and video recorded the resulting tail movements. To record extrinsic tail muscle activity following recovery from anesthesia, specific targets along the tail length were heated with a laser to evoke the NWR, and the resulting EMG was recorded. Preliminary results have successfully led to the development of a selective EMG recording technique, which we have used to show significant extrinsic tail muscle activity on both sides of the midline (co-contraction) when stimulating at various specific locations on either side of the tail.

THE ROLE OF ASTROCYTES IN ADRENOCORTICOTROPIC HORMONE'S NEUROPROTECTION AFTER EARLY LIFE SEIZURES

Kelly Lee¹, Willie Curry², Amanda Hernan²

¹Trinity Washington University, 125 Michigan Ave NE, Washington, DC 20017

²The University of Vermont, Given Medical Bldg, E-126, 89 Beaumont Ave, Burlington, VT 05405

Epilepsy is a neurological disorder marked by sudden recurrent episodes of sensory disturbance, loss of consciousness, or convulsions, associated with abnormal electrical activity in the brain. Epilepsy is a common neurological disease affecting approximately 3.4 million people in the US and 65 million people worldwide. Adrenocorticotrophic hormone (ACTH) is a drug that has been known to treat certain types of pediatric epilepsies in patients. We recently showed that early treatment with ACTH can lead to long-term improvements in cognition in several animal models of early life seizures. The mechanism is unknown; however, neuropeptide receptors that bind ACTH (MC4Rs) are present in the brain and may be responsible for some of the effect of ACTH. Activation of MC4Rs located astrocytes dampens neuroinflammation and provides neuroprotection after injury. Dysregulation of astrocytes has recently come to the forefront as a major player in epilepsy-associated injury, making activation of MC4Rs on astrocytes specifically a target for future study. In order to begin to understand whether or not ACTH has actions centrally on astrocytes in our recurrent early life seizure model, we first sought to confirm astrocyte dysregulation. Preliminary immunohistochemistry data suggest there may be alterations in GFAP expression after early life seizures. Future studies will quantify GFAP expression from IHC data, examine whether glutamate transporters are dysregulated after seizures, and determine whether ACTH can ameliorate these changes.

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EFFECTS OF APPLD MUTATION ON THE OLFACTORY SYSTEM IN *DROSOPHILA MELANOGASTER*

Victor Omoniyi¹, Fernando Vonhoff¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Alzheimer's disease (AD) is associated with mutations in the Amyloid Precursor Protein (APP). Previous research has shown that a deletion in the *Drosophila* ortholog APP-like (APPL) gene results in a lack of APPL expression. APPLd mutant flies show developmental defects and aging-dependent neurodegeneration similar to symptoms found in Alzheimer's disease. We hypothesize that APPLd mutants will show developmental defects in the olfactory system. My research project is to examine anatomical defects in the olfactory network of adult APPLd mutants and whether this translates into behavioral consequences in regards to their sense of smell. Using immunohistochemistry methods and confocal microscopy, we observed anatomical differences in the olfactory region (antennal lobe) in brains of young 2 days old APPLd mutants as compared to same-aged controls. This suggests that APPL may play a role during the development of the olfactory network. To test for their olfactory performance, APPLd mutant and wildtype fly's behavior was assayed using a T-maze experiment. We observed a significant difference in the response preference between mutant and wildtype fruit flies towards Apple Cider Vinegar (ACV). For wildtype flies, 50% of ACV has been established as the optimal concentration of ACV to elicit the largest attraction levels. By contrast, APPLd shows a higher preference for 75% than 50% ACV, suggesting an ameliorated repulsion effect. These results indicate that despite aberrant neuroanatomy, APPL fruit flies are able to smell but show subtle, significant changes in the functional properties of the olfactory system. We will test whether the observed defects of APPLd mutants are due to developmental effects or aging-dependent mechanisms. Future experiments will investigate whether the degeneration of the olfactory network is differentially regulated in aging APPLd mutants. Our research will provide insight into the fundamental role of molecules associated with Alzheimer's disease and may provide new pathways for therapeutic targets.

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ARABIDOPSIS STRESS MANAGEMENT 101: HYPOXIA SPECIFIC CML39 IS ALSO EXPRESSED WITH OTHER ABIOTIC STRESSES

Krista Kachnik¹, Sterling Fields², Daniel Roberts², Sterling Fields²

¹Department of Biology, Gallaudet University, 800 Florida Ave NE, Washington, DC, 20002

²Department of Biochemistry, University of Tennessee, Knoxville, TN, 37996

Global climate change has increased food insecurity by impacting crop yields, decreasing availability of food and increasing costs of production. The changing climate can cause more severe stressors for plants, including flooding, heat, cold stress. We can study crop survival and adaptation using the model organism *Arabidopsis thaliana* to withstand environment stress can be applied to other crops.

One critical stress response protein is CALMODULIN-LIKE 38 (CML38), which is essential for flooding survival, but the role of CML38 during other stresses has not been thoroughly investigate. The line of *Arabidopsis* (CML38Pro::GUS) that is being tested has a reporter gene connected to CML38 named GUS (β -glucuronidase). GUS when stained with an X-gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt) solution causes the plant to turn blue where CML38 is expressed, providing an assay to monitor the conditions that result in CML38 expression. By stressing the *Arabidopsis* with different abiotic stressor such as heat, cold, and darkness determine whether CML38 is expressed with hypoxia alone or if applied to other stressors.

Our current hypothesis is that expression of the CML38 gene will be high in flooding and hypoxia, but low in the other stressors. Our data demonstrates CML38 is expressed strongly expressed in abiotic stressors such as cold, darkness, and with the hypoxia accumulating lactic acid stress. In other abiotic stressors such as heat, H₂O₂, and Sodium Azide CML38 was expressed, but did not show as strong of a blue color. Interestingly, expression of CML38 in cold, darkness, and lactic acid was similar to that of hypoxia expression. Ongoing research is using RT-PCR to quantitatively analyze the level of CML38 expression during hypoxia, cold, and darkness and compare to flooding. This research will help us understand the signaling CML38 gene while also experimenting CML38 expression with different abiotic stressors.

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MIR-1 TARGET VALIDATION

Colette Robinson¹, Michael Turner¹

¹Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Rd Emmitsburg, MD 21727

Through research, findings indicate that most, if not all, cellular processes involve microRNA (miRNA). Their main function is to regulate the expression of genes by binding to the 3'-UTR region of its target and thus inhibiting translation. miRNAs are unique because they are able to bind to the 3'-UTR region of its target without perfectly base pairing. Computationally, we are able to predict the targets of each miRNA, however, since the miRNA do not need to base pair perfectly, the algorithms used for prediction may be wrong. Therefore, through experimentation, the genes that miRNA are predicted to regulate are able to be validated. *C. elegans* is an easily manipulated model organism that allows the effective study of the miRNA within various molecular pathways that regulate cellular function. This presentation will focus on the techniques we are using to validate miRNA target interactions using *C. elegans* miRNA mir-1 as a model.

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Biochemistry and **Molecular Biology**

ABSTRACTS

Afternoon Session

*Page Numbers of the Abstracts Serve as
Poster Numbers*

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 2019 Undergraduate Research Symposium in the Chemical and Biological Sciences.

INTERACTIONS BETWEEN PRE-SYNAPTIC MODULATORS OF DOPAMINERGIC TRANSMISSION

Sylvia Okafor¹, Kanisha Clark¹, Harbinder Dhillon¹

¹Department of Biology, Delaware State University, 1200 North DuPont Highway, Dover, Delaware, 19901

Dopamine is a key neurotransmitter that is associated with learning, memory, behavior and locomotion. Dysregulation of dopamine signaling is associated with numerous neurological and mood disorders such as, Parkinson's, Schizophrenia, and ADHD. Understanding interactions between regulators of dopaminergic signaling neurons can reveal a clearer picture of the finely tuned neurotransmitter modulatory processes as well as potentially provide avenues for pharmacological interventions. Specific genetic modulators of dopamine synaptic transmission have previously been described: DAT-1 is a sodium dependent dopamine symporter that is involved in clearing the pre-synaptic cleft of exogenous dopamine. DOP-2 is a dopamine agonist and G-protein coupled receptor that inhibits adenylyl cyclase activation as well calcium channels to regulate dopamine synthesis. ASIC-1 is a subunit for a proton gated sodium channel in a positive feedback mechanism involved in decreasing the pH in the synaptic cleft. A number of behavioral phenotypes can be used to determine interactions between various dopamine regulatory genes to characterize pathway crosstalk in this highly regulated process. Using *asic-1*, *dop-2*, and *dat-1* deletion mutants and their crossed double deletion mutants, we hypothesized that the associative learning and behavioral characteristics displayed in a swimming induced paralysis (SWIP) and NaCl assay will indicate positive genetic interactions between genes of interest. Our results indicate that genes encoding pre-synaptic dopamine regulatory proteins interact via cross-talking pathways that are likely to be critical for precise modulation of dopaminergic transmission.

We acknowledge the support of the National Institutes of Health to the Dhillon lab (NIGMS-1P20GM103653-01A1)

CHARACTERIZING THE BINDING OF RAD3 TO THE CYTOSOLIC IRON SULFUR CLUSTER ASSEMBLY TARGETING COMPLEX

Annelise Hushka¹, Amanda Vo¹, Melissa Marquez², Jessica Cosman¹, Deborah Perlstein¹

¹Department of Chemistry, Boston University, 590 Commonwealth Ave, Boston MA 02215

²Elstar Therapeutics, 840 Memorial Drive, Cambridge, MA 02139

The cytosolic iron sulfur cluster assembly (CIA) system is a highly conserved pathway that assembles and inserts iron sulfur (FeS) cluster cofactors into cytosolic and nuclear proteins. These CIA targets are involved in many important cellular processes including DNA replication and repair, iron homeostasis, and translation. The mechanism by which the CIA substrates, numbering more than 25 proteins, are identified in the last step of the pathway is not understood. However, it is known that the CIA proteins Met18, Cia1 and Cia2 form a large complex, called the CIA targeting complex, which appears to be responsible for substrate recognition in the final step of the pathway.

In this work, we examined how Rad3, a DNA helicase which binds a Fe₄S₄ cluster, associates with two subunits of the CIA targeting complex, Met18 and Cia2. Site directed mutagenesis combined with affinity co-purification assays were utilized to investigate a 10 amino acid region that, when deleted, prevents cofactor acquisition by Rad3 in vivo. To pinpoint which amino acids are critical for recognition of Rad3, site-directed mutants of Rad3 were tested for their ability to bind Met18-Cia2. Deletion of the 10 amino acid region prevented binding of Rad3 in vitro. Although alanine scanning mutagenesis suggested that 3 of the 10 amino acids are required for interaction, our recent results suggest that observation can be explained by a destabilizing effect on tertiary structure. These results indicate that the recognition of Rad3 by the targeting complex depends on the tertiary structure of Rad3 and rule out the possibility that Rad3 is recognized by a short, linear motif. It appears that none of the previously identified 10 amino acids are directly involved with binding to the CIA targeting complex and that the complex recognizes the tertiary structure of a domain far from the FeS cluster binding site.

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PURIFICATION AND BIOPHYSICAL CHARACTERIZATION OF UNUSUAL CLPS PROTEOLYTIC ADAPTERS

Jennifer Vorn¹, Jialiu Jiang², Karl Schmitz³

¹Department of Medical and Molecular Science, University of Delaware, 25 N College Ave,
Newark, DE 19716

²Department of Chemistry and Biochemistry, Department of Biological Science, University of
Delaware, 105 The Green, Newark DE, 19716

³Department of Biological Science, Department of Chemistry and Biochemistry, University of
Delaware, 105 The Green, Newark, DE 19716

Bacterial ATP-dependent proteases contribute to cellular health by recognizing, unfolding, and hydrolyzing damaged and misfolded proteins. Within *Escherichia coli*, the ATP-dependent protease ClpAP works with the ClpS proteolytic adaptor to degrade proteins with specific N-terminal amino acids in a process called N-end rule degradation. *E. coli* ClpS has a special binding pocket with specific affinity for N-terminal Leu, Phe, Tyr, and Trp amino acids. ClpS binds to the N-domain of ClpA and delivers substrates to ClpAP for degradation. ClpS shares strong sequence conservation among many species of bacteria, but ClpS from the human pathogen *Helicobacter pylori* has unusual amino acid substitutions that may change the shape of the binding pocket. We hypothesize that *H. pylori* ClpS has altered specificity for N-terminal amino acids compared to *E. coli* ClpS. We cloned, expressed, and purified *H. pylori* ClpS. We carried out fluorescence anisotropy binding assays to test the preference of *H. pylori* ClpS for N-terminal amino acids. We used proteolysis assays to find out if *H. pylori* ClpS functions as an adaptor for N-end rule proteolysis by ClpAP. We also set up crystallization trials of *H. pylori* ClpS to structurally characterize differences in the shape of its binding pocket that may explain its altered binding specificity. Our results suggest that *H. pylori* ClpS does not recognize standard N-end rule amino acids.

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IN VITRO ANALYSIS OF CRX BINDING AFFINITY TO WILD TYPE AND METHYLATED CBRs

Michael Friedman¹, Sabrina Leicht¹, Christopher Berndsen², Raymond Enke¹

¹Department of Biology, James Madison University, 800 S Main St, Harrisonburg, VA 22807

²Department of Chemistry, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Photoreceptors (PRs) are a specialized type of retinal neuron located in the posterior portion of the eye. PRs are responsible for converting photons of light coming from the world around us into signals that are processed in the visual cortex of the brain. While much is known about the molecular function of PRs, the mechanism responsible for cell type restricted transcription in rod and cone PR neurons has yet to be fully understood. The goal of our project is to determine if cell type restricted transcription is due to epigenetic modifications of cis regulatory elements in the genomes of PR neurons. We hypothesize that cell specific patterns in transcription are due to the methylation of regulatory regions altering the shape of DNA preventing optimal transcription factor binding. To test this hypothesis, we have expressed and purified the human Cone-Rod Homeobox (CRX) protein, the transcription factor responsible for specializing a retinal precursor into PRs. Current experiments with purified CRX focus on in vitro analysis such as DNA binding assay to determine if there is an observable difference in CRX binding affinity to unmodified and epigenetically modified DNA. Structural data from Small Angle X-ray Scattering (SAXS) analysis is also being currently being analyzed to develop a model for the structure of methylated and unmethylated CRX binding regions (CBRs). A novel expression and purification protocol are becoming developed for the full length of the CRX protein. In all, these different assays allow analysis of CRX and CBR structural feature changes in solution. These data will provide essential information on how erroneous binding originating from poor CRX binding affinity lead to diseases such as Cone-Rod Dystrophy.

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POST-TRANSLATIONAL MODIFICATIONS OF HUMAN PHOSPHOFRUCTOKINASE 1 AND THEIR ROLE IN GLUCOSOME FORMATION

Saiprasad Ravi¹, Miji Jeon¹, Songon An¹

¹Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Cancer cells are known to primarily use the glycolytic pathway to generate energy and building blocks for cellular processes. Human phosphofructokinase, liver type (PFKL) catalyzes the third step of glycolysis in which fructose-6-phosphate is converted to fructose-1,6-bisphosphate. This step is an essential regulatory point within the glycolytic pathway as a rate limiting step. Recently, a reversible metabolic complex of glucose metabolism has been discovered in cancer cells, namely the “glucosome,” where PFKL is hypothesized as a scaffold. PFKL has been also known to undergo post-translational modifications such as acetylation, glycosylation, and phosphorylation to regulate its activity and intracellular localization. We investigate if these modifications affect the formation and/or utilization of glucosomes in cancer cells. To understand the effects of these post-translational modifications we have constructed plasmids via site directed mutagenesis that carry several point mutations on PFKL. As of today, we have introduced mutations such as K689Q as an acetylation mimic, K689A to abolish acetylation at this residue, and F638R to disrupt the tetramerization of PFKL. In addition, using fluorescence microscopy, we examine changes in glucosome formation in cancer cells expressing PFKL mutants. These studies would establish a knowledge base to explore the effects of clinically relevant mutations found in cancer patients on glucosome formation. Because PFKL acts as an important regulator of glycolysis, analysis of these post translational modifications will not only help us understand the biochemistry of glucosomes in living cells, but also about its role in cancer cell metabolism.

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DETERMINING THE MINIMUM REQUIREMENTS FOR THE FORMATION OF STRESS GRANULES

Ashley Holloway¹, Christopher Lloyd¹, Joshua Sokoloski¹

¹Department of Chemistry, Salisbury University, 1101 Camden Avenue Salisbury, Maryland 21801

Stress granules are the accumulation of messenger(m) RNA and mRNA binding proteins involved in translation, often in cases of cellular stress. The formation of stress granules is promoted via protein-protein interactions between mRNA-binding proteins involving intrinsically disordered regions of these proteins. To discover the minimum requirement of proteins necessary to form granules, RNA was extracted from yeast and bacterial cells to produce a proto-stress granule with a model peptide. RNA solubility and propensity to aggregate was examined under different solution conditions and in the presence of a model peptide with sequence WWGNAKTRRRERRAEKQAQWKAANKSEPRKSEPR and other proteins. Aggregates with this model peptide were detected fluorescently with Thioflavin T and Proteostat dyes. A variety of other proteins that interact with DNA or RNA were tested as controls and did not exhibit the aggregation response of RNA and the model peptide. Additionally, bacterial cells were grown and lysed with a prepared lysis buffer. RNA was extracted from the lysed bacterial cells and absorbance and fluorescence was measured to conclude how they aggregate. The aggregation data collected from the whole RNA extracted from the bacterial cells was compared to the smaller molecular weight RNA from yeast cells to test the length and source dependence for RNA aggregation.

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KIFC1 CONTRIBUTES TO POLYPLOID GIANT CANCER CELL SURVIVAL

KiChang Kang¹, Laurie Kostecka², Sarah Amend PhD², Kenneth Pienta MD²

¹Department of Biological Sciences, The University of Delaware, 118 Wolf Hall, Newark, DE 19716

²The Brady Urological Institute, Johns Hopkins University School of Medicine, 600 N Wolfe St, Baltimore, MD 21287

Prostate cancer (PCa) is the second leading cause of death in American men. While treatment of localized disease is usually successful with 5 year survival rates of 100%, metastatic PCa remains incurable. A rare subset of cancer cells called polyploid giant cancer cells (PGCCs) may be mediators of metastasis, drug-resistance, and tumorigenesis in PCa. PGCCs are morphologically distinct, behaviorally aberrant, and induced through stress. Previous PCa literature has associated higher KIFC1 expression with poorer prognostic outcomes in patients and docetaxel (DTX) resistance. KIFC1, a minus-end directed kinesin motor protein, is critical for centrosome clustering which may provide one mechanism for PGCC survival. This project aimed to enrich PGCC populations using chemotherapy and characterize the expression of KIFC1 in PGCCs. DU145 and MDA-MB-231 cell lines, positive and negative controls for KIFC1 expression, were plated and treated with IC90 concentration of DTX for 72 hours. Immunohistochemistry staining of DU145 and MDA-MB-231 validated expected KIFC1 expression. Next, PGCCs were enriched using a physical 15 μ m nylon filter, lysed for protein, and KIFC1 protein expression was analyzed via western blot. Interestingly, we found higher KIFC1 expression in both DU145 and MDA-MB231 PGCCs compared to untreated groups. To determine whether KIFC1 plays an essential role in PGCC survival, we used shRNA to knock down KIFC1 expression in PC3 cells, a prostate cancer cell line. Surprisingly, the shRNA knock-down of KIFC1 in PC3 demonstrated decreased proliferation, even without chemotherapy treatment to enrich for PGCCs. These results suggest KIFC1 may be a potential therapeutic target for PCa and a contributing factor to the survival of PGCCs.

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APTAMER SELECTION AGAINST MUTANT BACTERIAL ANTITERMINATOR PROTEIN NASR R340K

Cole Ruoff¹, Emily Wirt¹, Lucas Barry¹, Victor Cao¹, Jonathan Goodson¹, Wade Winkler¹,
Catherine Spirito¹

¹First-Year Innovation and Research Experience (FIRE) Engineering Biosensors, University of
Maryland, College Park, MD 20742

NasR is a bacterial antiterminator protein from *Klebsiella oxytoca* that regulates nitrate metabolism via antitermination of transcription. NasR consists of two domains, the nitrate or nitrite sensing NIT domain and the ANTAR (AmiR and NasR Transcriptional Antiterminator Regulator) domain. The ANTAR domain is widespread across many bacterial species, so understanding how this domain functions will help further understanding of how ANTAR-containing bacteria regulate downstream gene expression. This project focused on a mutant version of the NasR protein, R340K, which exhibits reduced binding to the native RNA substrate. The goal for this project was to determine what RNA sequence-structure motifs this mutant protein can bind. This was done using an aptamer selection process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). We have completed six rounds of RNA aptamer selection against NasR R340K. Next steps include TOPO TA cloning and Sanger sequencing of our final aptamer pools, as well binding affinity assays, to determine which sequence-structure motifs bind strongly and specifically to the mutant NasR R340K protein.

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LONG-RANGE REGULATION OF CYTOCHROME C BINDING TO BC1 COMPLEX

Natalie Simmons¹, Spencer B. Grewe¹, Oleksandr Kokhan¹

¹Department of Chemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807

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. In order to make sure the models done through computational work show the correct mechanism of regulation of the bc₁ complex we will do experimental work. By doing experimental work we can compare it to our computational data.

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GENETIC ENGINEERING OF CARBOHYDRATE BINDING MODULES

Kathleen DeBiasse¹, Makhalia Aiken¹, Craig Laufer¹

¹Department of Biology, Hood College, 401 Rosemont Ave Frederick MD 21701

Biofuels are an attractive alternative to fossil fuels because they are renewable and potentially carbon-neutral. However, at present they cannot compete when it comes to price. The feedstock for second-generation biofuels (agricultural wastes) is relatively inexpensive, however, the enzymes used to break down the biomass are relatively expensive. A way to reduce the cost of biofuels would be to reuse those enzymes. An obstacle to efficient recovery of the enzymes for their reuse is their affinity for lignin and residual cellulose following enzymatic saccharification of lignocellulosic biomass. Our research has focused on genetically engineering carbohydrate binding modules (CBM's) in order to allow for efficient recovery of cellulases for multiple uses. Specifically, this research is focused on engineering the melting temperature of the CBM 11 and CBM 44 of *Ruminoclostridium thermocellum*. These CBMs spontaneously refold to the native state following thermal denaturation. These modifications will facilitate an enzyme recycling strategy where following saccharification, a slight increase in temperature will denature the CBM releasing the whole enzyme from the remaining undigested material to be recovered in the soluble phase. Subsequently the reaction temperature will be set below the TM of the engineered CBM so that fully folded protein with both CBM and catalytic domain can react with the newly added substrate. We have produced mutant CBMs with amino acid substitutions designed to lower the TM, but not interfere with CBM binding, and have developed an assay to test them for their ability to bind and unbind to a cellulose membrane substrate. We will report our results on the temperature-dependent binding activities of these mutants relative to the wild-type CBM.

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EFFECTS OF ACYLDEPSIPEPTIDE FRAGMENTS ON *BACILLUS SUBTILIS* CLPP PEPTIDASE ACTIVITY

Jeffery Hudson¹, Monika Prorok¹, Karl Schmitz¹

¹Department of Biological Sciences, University of Delaware, Newark, DE 19716

Tuberculosis is an infectious disease that is caused by *Mycobacterium tuberculosis*. (Mtb). Although most Mtb infections are treatable, about 5% percent of new infections exhibit resistance to antibiotics, posing a world-wide challenge to public health. Clp proteases are responsible for mediating cell homeostasis and have been shown to be essential for survival of mycobacterium tuberculosis. This makes Clp protease enzymes, such as the ClpP peptidase, promising drug targets for antibiotic resistant bacteria. One such class of compounds are acyldepsipeptides (ADEPs), which are derivatives of naturally occurring compound produced by a soil bacterium, *Streptomyces hawaiiensis*. Since their discovery, various ADEP derivatives and compound fragments have been developed to improve species specificity and efficiency. It has been shown that ClpP in *Bacillus subtilis* is highly susceptible to most ADEPs. While the effects of full ADEPs on *B. subtilis* ClpP (Bsu-ClpP) have been extensively studied, ADEP fragments also have potency against the peptidase activity. Here I present an initial characterization of the in vitro activity of a panel of novel ADEPs and ADEP fragments on Bsu-ClpP. Our results raise new questions about the mode of action of ADEPs, and how their structure can be optimized for use as antibiotics.

This work was funded by NIH, and the University of Delaware. The ADEP compounds and fragments were synthesized by Prof. Jason Sello's Lab at Brown University.

COILIN NTD MUTATIONS INHIBIT PROPER CAJAL BODY FORMATION

Jade Enright¹, Edward Courchaine², Korinna Straube², Karla Neugebauer²

¹Department of Biology, McDaniel College, 02 College Hill, Westminster, MD 21157

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520

The central dogma of molecular biology explains that DNA is used as a template to transcribe RNA and RNA is then used as a template to translate proteins. However, not all RNA is translated. There are many types of noncoding RNAs, most of which play a role in regulating gene expression. Some of these small noncoding RNAs, such as snRNAs and snoRNAs, go through processing in a nuclear compartment called the Cajal body. Cajal bodies consist of an aggregation of RNA and proteins and their formation is not yet well understood. The protein coilin is concentrated in Cajal bodies and acts as a scaffold for their formation by binding itself and other proteins at the coilin N-terminal domain (NTD) region. To further understand the role of this region on Cajal body formation, I observed the effects of point mutations in conserved residues of the NTD on Cajal body formation. HeLa cells were transfected with a variety of NTD mutants and stained to detect coilin and the transfected product. I counted and classified 300 cells of each mutant type based on morphology and quantified the number of normal Cajal bodies if any were present. The effects observed ranged from an altered number to an altered shape, such as fibers, to no Cajal body formation at all, depending on the mutation. These results indicate that the NTD region of coilin is necessary for the stable formation of Cajal bodies. The understanding of Cajal body formation can lead to a further understanding of gene expression regulation.

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MUTATIONS IN THE FURIN CLEAVAGE SITE TO IMPROVE CYTOTOXICITY IN ANTI-MESOTHELIN RECOMBINANT IMMUNOTOXINS BASED ON *PSEUDOMONAS* EXOTOXIN A

Keeley Butters¹, Jillian Baker¹, Danielle Reifer¹, John Weldon¹

¹Department of Biology, Towson University, 8000 York Rd. Towson, MD 21252

A recombinant immunotoxin (RIT) is a fusion protein composed of an antibody and a toxin. The antibody binds to receptors on target cells to promote endocytosis. The toxin kills the cell once it is internalized. RITs based on *Pseudomonas* exotoxin A (PE) have been created to target and kill cancer cells. In order to do this, internalized PE must reach the cytosol through a retrograde trafficking route where it encounters and is cleaved by the protease furin. Furin has been established as an integral part of PE-based RIT intoxication pathways. Mutations in the PE furin cleavage site have been shown to influence cleavage efficiency and RIT cytotoxicity. In order to further explore the role of furin, we have constructed anti-mesothelin/PE-based RITs with mutations on either side of the furin cleavage site. We plan to test cytotoxicity and furin cleavage efficiency of these constructs in tissue culture. We hypothesize that mutations N-terminal to the cleavage site will increase both cleavage efficiency and cytotoxicity in these RITs, while mutations C-terminal to the cleavage site will increase cleavage efficiency without significant improvements to cytotoxicity.

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QUANTIFICATION OF MAGNESIUM ION RELEASE AND UPTAKE BY PROTEIN-DNA COMPLEXES AND CELL SURFACES

Truc Ngo¹, Maiya Robinson¹, Thomas Montag¹, Joshua Sokoloski¹

¹Department of Chemistry, Salisbury University, 1101 Camden Ave, Salisbury, MD 21801

The interactions of metal ions with nucleic acids, proteins, and lipopolysaccharides are significant factors in biological structure/function relationships. Much work has been done on the mechanisms of these interactions, however, the stoichiometry of metal uptake by the protein has yet to be determined. In this project, we developed a fluorescent assay for measuring magnesium ion uptake or release by protein-nucleic acid complexes or by bacterial cells that is suitable for use in a microplate reader using ATP-Mg²⁺ binding as a model system with known stoichiometry. The dye 8-hydroxyquinoline-5-sulfonic acid (HQS) fluoresces only in the presence of Mg²⁺ and therefore can act as a sensor of free magnesium ion in solution. Comparison of the fluorescence signals with or without the presence of a complex that can bind Mg²⁺ leads to the calculation of the stoichiometry associated with this ion binding interaction. Our primary focus was on the helicase RecBCD. RecBCD depends on magnesium ions to disrupt the base pairs of a blunt end DNA, (melting). Blunt end DNA and Pre melted DNA was analyzed using the HQS assay. Using nonlinear least squares fitting, we estimate that two to three Mg²⁺ ions are required for the melting process to occur by RecBCD. We have also extended this assay to interactions of Mg²⁺ ions with the surface of *E.coli* cells, where we probed the effect of ‘heat-shock’ temperatures on Mg²⁺ ion sequestration by the cell surface.

MYCOBACTERIAL CLPS BINDS N-END-RULE PEPTIDES AND MAY BIND THE CLPC1 UNFOLDASE

Thomas Swayne¹, Chris Presloid¹, Karl Schmitz¹

¹Department of Biology, University of Delaware, University of Delaware Newark, DE 19716

N-end-rule proteolytic pathways couple the stability of cytosolic proteins to the identity of their N-terminal amino acid. In proteobacteria, including *E. coli*, proteins bearing N-terminal Leu, Phe, Tyr, or Trp residues are recognized by the ClpS adaptor and delivered to the ATP-dependent ClpAP protease for degradation. No equivalent N-end-rule pathway has been described in actinobacteria, yet many members of this phylum, including the globally important human pathogen *Mycobacterium tuberculosis*, possess a ClpS ortholog. We have solved the X-ray crystal structure of Mycobacterial ClpS, revealing overall structural similarity to its proteobacterial counterpart, including a well-defined binding pocket. Fluorescence anisotropy and co-crystallization experiments show that *Mycobacterial* ClpS can bind peptides bearing canonical N-end-rule amino acids, albeit with weaker affinity than *E. coli* ClpS. These findings suggest that ClpS functions in a mycobacterial N-end rule pathway, which may involve ClpC1P1P2. When the crystal structure of *Mycobacterium tuberculosis* was analyzed, contrary to that of *E. coli*'s structure, there were clearly identifiable and consistent metal ions present. It was initially thought that these metal ions could be contributing to the enzyme's activity, but after conflicting experimental results this does not seem to be the case. ClpS may provide a novel route for disrupting essential proteolytic processes in *Mycobacterium tuberculosis*.

We thank members of the Schmitz lab for help and advice. This work was supported by a pilot grant from the National Institutes of Health National Institute of General Medical Sciences under Award Number P20GM104316, and startup funds from the University of Delaware.

A ONE-POT ENZYMATIC SYNTHESIS OF CMP-SIALIC ACID DERIVATIVES AS SUBSTRATES FOR THE *NEISSERIA MENINGITIDIS* SEROGROUP W CAPSULE POLYMERASE

Nyah Johnson¹, Laleh Moghaddam¹, Pumtiwitt McCarthy¹

¹Department of Chemistry, Morgan State University, 1700 E Cold Spring Ln, Baltimore, Md. 21251

Glycoconjugate vaccines are one way to prevent bacterial meningitis caused by *Neisseria meningitidis*. These vaccines are made of sugars from the bacterial capsule attached to a carrier protein. Typically, there are varied lengths of sugars within these vaccines. The long-term goal is to use *N.meningitidis* serogroup W capsule polymerase enzyme as a tool to improve glycoconjugate vaccine development. We aim to control activity of this enzyme to make defined sugar lengths to determine how this contributes to the immune response produced by such vaccines. The capsule polymerase enzyme synthesizes a polysaccharide of galactose-sialic acid repeats by using UDP-galactose (UDP-Gal) and CMP-Sialic acid (CMP-Sia). To reach this goal, we describe here a one-pot enzymatic synthesis of two CMP-Sialic acid derivatives, CMP-SiaDAz and CMP-4-Ac-Sia. CMP-SiaDAz contains a photocrosslinking diazirine group that will help to identify the important amino acids for activity of the serogroup W enzyme. This will allow control of activity to create desired sugar lengths. CMP-4-Ac-Sia should partially prevent addition of more sugars to an acceptor and will be another angle of control of the W enzyme. In initial results with this derivative, it was observed by HPLC analysis that CMP-4-Ac-Sia was able to increase the presence of shorter sugars and decrease the presence of longer sugars. In future work, we will continue to optimize synthesis of sugar chain lengths using these two CMP-Sialic Acid derivatives and perform further testing to see how they affect the W enzyme.

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OPTIMIZING A BIOSENSOR-BASED ASSAY FOR UBIQUITINATION ACTIVATION

Roma Broadberry¹, Christopher E. Berndsen¹

¹Department of Chemistry and Biochemistry, James Madison University, James Madison University, Harrisonburg, VA 22807

Ubiquitination is a post-translational modification which regulates protein degradation, DNA damage signaling, and inflammatory response. Three transfer enzymes catalyze the reaction's pathway: the E1 activating enzyme, the E2 conjugating enzyme, and the E3 ligating enzyme, respectively. Despite the identification of these enzymes' structure and function, a complete understanding of their catalytic mechanisms remains unclear. Elucidating the ubiquitination mechanism may assist in targeting cancer therapies. Existing assays for ubiquitination include the standard gel-based assay and using radioactive tracers, which have limitations in quantitative accuracy, safety, and cost. We are trying to develop a cheap, continuous fluorescent assay which relies on a genetically-encoded biosensor, known as Perceval, to monitor real-time changes in concentration of ATP during cleavage of ATP to AMP for E1 catalysis. Developed in the Yellen Lab at Harvard Medical School, Perceval was designed to report in vivo sub-second changes in cellular energy as a result of its competitive binding of ATP and ADP. We have worked to adapt the Perceval biosensor toward measuring ATP hydrolysis of E1 in the ubiquitination pathway in vitro. Conditions for the purification and expression of Perceval were tested and set. We examined fluorescent changes of protein solutions to test Perceval's affinity for ATP, and to ensure no competitive behavior is needed or present when AMP and PPi are also in solution. We further tested solutions containing different UbIs and E1s to ensure the ubiquitination enzymes and protein substrates do not interfere with the sensor. When directly applied to ubiquitination, fluorescence readings were taken at single-time points in the ubiquitination pathway and over a continuous duration using Perceval. Future directions will look to further optimize buffer conditions for the assay, as well as combine other analytical and structural techniques to look more in depth at Perceval bound to ATP and AMP.

JMU Department of Chemistry and Biochemistry, NSF REU CHE-1757874, Thomas and Kate Jeffress Memorial Trust, 4-VA Organization

OVERCOMING THE LIMITATIONS OF CONVENTIONAL BACTERIOLOGY USING 16S BACTERIAL IDENTIFICATION

Jade Bowman¹, Colleen Courtney², Rakeiya McKnight², Anthony Tran², Patrice Moss¹

¹Department of Biology, Trinity Washington University, 125 Michigan Ave NE, Washington, DC 20017

²Department of Forensic Sciences, Public Health Laboratory, 401 E St SW, Washington, DC 20024

16s is a genetic code that is located in the 30s subunit of all bacterial species. There are three types of bacteria ribosomes which are 16s rRNA, 23s rRNA, and 5s rRNA. We are studying 16s rRNA because it is conserved amongst all bacteria and allows for the investigation of bacterial phylogeny and taxonomy. In spite of the availability of conventional diagnostic methods such as gram-stain, many bacterial isolates are only identified at the genus level and the species cannot be identified accurately by conventional methods. Previous research has demonstrated that 16s sequencing demonstrates a 90% accuracy or greater for identification of bacterial species. In this study, we predicted that we would be able to classify 20 isolates of *Salmonella* and *Escherichia Coli* (*E. coli*) on the genus and species level through 16s sequencing. To test this prediction, we have isolated the bacterial genome, and amplified the DNA by performing a polymerase chain reaction. In order to confirm the presence of bacterial DNA, we performed gel electrophoresis, which separates DNA present in a sample based on size and charge. To generate the sequence fragment, we utilized the Applied Biosystems-3500, a capillary array based Sanger sequencing platform. Once generated, we analyzed the sequences using the NCBI BLAST database in order to receive an identification of the isolate. We concluded that we were able to identify both *Salmonella* and *E.coli* to the species level of 80% or greater. According to the visualization of the sequence alignments, the assays needs to be optimize to better identify the nucleotides. Utilizing the POP_6 polymer instead of the POP_7 polymer could possibly minimize data caliber. Overall, 16s bacterial identification is a more precise approach to implement when bacterial culture declines.

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MEASURING -1PRF EFFICIENCY IN THE BOVINE LEUKEMIA VIRUS AND EQUINE TOROVIRUS

Nuwayra Mahreen¹, Riya Patel¹, Jenan El-Hifwani¹, Dina Elloseily¹, Julia Jager¹, Ridhi Singh¹, Carol Vieira¹

¹The First-Year Innovation and Research Experience, University of Maryland, 4254 Stadium Drive, College Park, Maryland 20742

Programmed -1 Ribosomal Frameshifting (-1 PRF) mechanism causes the ribosome to slip back one base in the 5' direction and continue translation in the -1 frame. Viruses use -1PRF to control gene expression by increasing the amount of proteins that can be encoded. Often, the -1 PRF is situated between the “gag” and “pol” genes which must be expressed at a specific ratio to make the viral infectious particle. “Gag” encodes the structural protein of the virus and “pol” encodes the polymerase that replicates the genome. The correct ratio of these proteins is necessary for infectivity.

The Bovine Leukemia Virus (BLV) and Equine Torovirus (EToV) are two viruses that are predicted to utilize -1PRF. BLV causes inflammation of the lymph nodes and can lead to leukemia in bovine while EtoV causes gastrointestinal disease in equine. Both viruses can cause massive loss to farmers. To validate if the predicted frameshifting sequences in these viruses are functional, the target sequences were cloned into a dual-luciferase plasmid containing Renilla luciferase in the 0-frame and firefly luciferase in the -1-frame. Therefore, firefly luciferase can only be detected when a frameshifting event occurs. The resulting plasmids were transfected into human embryonic kidney (HEK293T) cells. The dual-luciferase assay was used to determine the frequency at which the frameshift occurs. On average, BLV had a 3% -1 PRF efficiency and EToV had a 12.4% -1 PRF efficiency, suggesting that these viruses rely on -1 PRF. Based on this, future testing will work towards finding mutations to lower -1PRF efficiency and weaken the virus in an attempt to develop a live attenuated vaccine against the virus.

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RING FINGER PROTEIN (RNF11) MODULATES DOPAMINE RELEASE

Maria Yoder¹, Jonah Short-Miller¹, B. Jill Venton², Jeffrey Copeland¹

¹Department of Biology, Eastern Mennonite University, 1200 Park Road, Harrisonburg, VA 22802

²Department of Chemistry, University of Virginia, McCormick Road, PO Box 400319, Charlottesville, VA 22903

Parkinson's disease affects more than ten million people worldwide, with 60,000 new diagnoses each year in the United States alone. Parkinson disease (PD) pathology involves the progressive loss of dopaminergic neurons in the substantia nigra. Recent work has indicated a role for RING finger protein 11 (RNF11) in PD. In this work, we tested the effect of RNF11 RNAi knockdown or overexpression on stimulated dopamine release in the *Drosophila* central nervous system. Using the GAL4-UAS system, RNF11 RNAi and overexpression was specifically activated in dopamine neurons. RNF11 knockdown caused dopamine release to double, but there was no decrease in dopamine from RNF11 overexpression. This effect was specific to dopamine, as knocking down RNF11 in serotonergic or octopaminergic neurons did not alter release. RNF11 knockdown increased the recycled releasable pool of dopamine. Dopamine release was monitored in real-time using fast-scan cyclic voltammetry at an electrode implanted in the isolated ventral nerve cord. Two RNAi fly strains targeting different RNF11 sequences were used to ensure any changes in dopamine release could be attributed to RNF11 knockdown. The current versus time plots were modeled using Michaelis-Menten kinetics to determine the extent to which dopamine release and uptake are affected by RNF11 knockdown. qRT-PCR was used to verify the relative quantities of RNF11 mRNA in *Drosophila* samples. RNF11 expression decreases in human dopaminergic neurons during PD, and that decrease may be protective by increasing dopamine neurotransmission in the surviving dopaminergic neurons.

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QUANTIFICATION OF HYDROXYL RADICALS PRODUCED DURING IN-VITRO FAST PHOTOCHEMICAL OXIDATION OF PROTEINS (FPOP)

Chukwuebuka Eburuoh¹, Raquel Shortt², Alan McKenzie-Coe, Ph.D.², Lisa M. Jones, Ph.D.²

¹Department of Biology, Morgan State University, 1700 E Cold Spring Lane, Baltimore, MD 21251

²Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 200 N Pine St, Baltimore, MD 21201

The normal function of a protein is inherently linked to its three-dimensional structure, in order to develop more efficacious therapeutics, scientists must understand the relationship between protein structure and function. Fast photochemical oxidation of proteins (FPOP), a mass spectrometry based hydroxyl radical protein footprinting (HRPF) method, is a covalent labeling technique, which uses hydroxyl radicals to oxidatively modify the exposed amino acids of a protein. Under relevant biological conditions, the structural confirmation of a protein will change resulting in a difference of exposed amino acids and provides insight into which amino acids are exposed or protected during binding. Despite the advantages of FPOP, determining the effective concentration of hydroxyl radicals delivered to the protein is a major concern. To overcome this, a chemical dosimeter can be added to the FPOP experimental workflow. In the present work, we investigate the potential of terephthalic acid (THA) as a dosimeter for hydroxyl radicals generated from cleavage of hydrogen peroxide by an excimer laser. THA is nonfluorescent but in the presence of hydroxy radicals will produce a single fluorescent product, 2-hydroxyterephthalic acid. We hypothesize that if hydroxyl radicals are present in excess, we can oxidatively modify our protein of interest and THA will react to form fluorescent 2-hydroxyterephthalic acid. To find the optimal concentration of THA for quantifying hydroxyl radicals, varying concentrations of hydrogen peroxide and two conditions of THA (100 μ M and 5 mM) were integrated into an in-vitro FPOP experiment. To determine oxidative modification of the protein, samples were analyzed via intact mass spectrometry where a mass shift of +16 and + 32 were observed. The reaction of THA to 2-hydroxyterephthalic acid was probed using a fluorometer. Preliminary data show that 160 mM of hydrogen peroxide with 5 mM of THA gave the best balance between oxidation and fluorescence.

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EFFICIENCY OF PROGRAMMED -1 RIBOSOMAL FRAMESHIFTING IN BOVINE CORONAVIRUS AND NOUNANÉ VIRUS

Ridhi Singh¹, Jenan El-Hifnawi¹, Dina Eloiseily¹, Nuwayra Mahreen¹, Riya Patel¹, Julia Jager¹, Carol Vieira¹

¹The First-Year Innovation and Research Experience, University of Maryland, 4254 Stadium Drive, College Park, Maryland 20742

Programmed -1 ribosomal frameshifting (-1 PRF) is the process of a ribosome slipping back one nucleotide during translation and rereading that single base, causing the rest of the genetic material to be read in the -1 frame. Programmed frameshifting is a function some viruses use to overcome the limited coding capacity of their small genomes and improve viral infectivity by expressing the necessary ratio of structural and non-structural proteins.

Bovine Coronavirus (BCV) is a member of the Coronaviridae family, that infects cattle, causing calf diarrhea, winter dysentery, and respiratory infections resulting in significant financial losses for cattle farming. Nounané virus (NOUV) is a member of the Flaviviridae family, and although it has not been replicated in mammalian cell culture, it likely has the potential for human infection due to similarities to West Nile Virus, Dengue Virus, and Yellow Fever Virus. In order to determine if BCV and NOUV use -1 PRF, the predicted -1 PRF sequences from each virus were cloned into a dual-luciferase plasmid containing Renilla luciferase in the 0-frame and firefly luciferase in the -1-frame. Therefore, Renilla luciferase is always expressed, but a frameshifting event must occur for firefly luciferase to be expressed. The resulting plasmids were transfected into human embryonic kidney (HEK293T) cells. Dual-luciferase assays were used to measure the luciferase enzymatic activity, determining the percent of the time that a frameshift event occurred. Thus far, NOUV frameshifting efficiency was recorded at 2.7% and BCV at 24.5%.

In the future, the viral -1 PRF sequences will be mutated with the goal of inhibiting -1 PRF and weakening the virus by offsetting the ratio of structural and non-structural proteins, lowering infectivity. The weakened virus can then be used as an attenuated vaccine in the future.

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A CONTINUOUS FLUORESCENT ASSAY FOR B-AMYLASE ACTIVITY

Kenan Ozcan¹, Christopher Berndsen²

¹Department of Integrated Science and Technology, James Madison University, 701 Carrier Drive, Harrisonburg VA, 22807

²Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807

The production and degradation of starch is key for the survival of plants during the day/night cycle. The stored energy is harnessed through the work of many different enzymes including the β -Amylase (BAM) family of proteins. BAMs break down long chains of starch into the disaccharide maltose (4-O- α -D-Glucopyranosyl-D-glucose). The model organism *Arabidopsis thaliana* has 9 β -Amylase proteins with apparently distinct functions, though not all have the ability to hydrolyze starch. Each BAM seems to have its own unique characteristics, although the specificity in these functional differences is not clear. The objective of our research is to create an assay that can show the kinetics of BAM proteins, that is continuous and sensitive while able to work with synthetic and natural substrates of the BAM proteins. We aim to apply this assay to determine the unique biochemical features of the BAMs. To detect BAM activity, we used a maltose binding sensor developed by Marvin and coworkers which is Maltose Binding Protein fused to Green Fluorescence Protein (MBP-GFP). When maltose is bound to MBP it causes conformational changes to GFP resulting in fluorescence. We have tested the assay with maltodextrin chains from 4 to 20 residues in length and with soluble starch finding that we can detect maltose production from all of these substrates with the MBP-GFP biosensor. Moreover, we can detect amylase activity in a continuous measurement format using a plate reader allowing for higher throughput sampling with reduced assay time.

JMU Department of Chemistry and Biochemistry
Dr. Jonathan Monroe
Berndsen Lab
NSF-RUI MCB-1616467 to J.M.
NSF REU CHE-1757874
4-VA Organization

TUDOR DOMAIN CONTAINING PROTEIN 5-LIKE POST-TRANSCRIPTIONALLY REGULATES MATERNALLY DEPOSITED RNAs IN THE *DROSOPHILA* GERMLINE

Sydney Kelly¹, Caitlin Pozmanter¹, Mark Van Doren²

¹Department of Biology, McDaniel College, 2 College Hill, Westminster MD 21157

²Department of Biology, Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218

Tudor domain containing proteins are essential for proper development of germ cells and are conserved across the animal kingdom. tudor domain containing protein 5-like (tdrd5l) is involved in sex determination in *Drosophila*, where it is suppressed in female germline stem cells (GSCs) via Sex lethal (Sxl) but enhanced in male GSCs. However, it functions in the developing germline of both males and females where it localizes into RNA granules similar to Processing bodies (P-bodies.) It was previously shown through antibody staining that tdrd5l may post transcriptionally regulate maternally deposited RNAs in *Drosophila* egg chambers. To determine where this regulation occurs, we tested both gurken (grk) and nanos (nos) RNAs to see if the absence of tdrd5l affected the regulation of each maternally deposited RNA. To investigate grk regulation we optimized the protocol for Fluorescence In Situ Hybridization (FISH) to visualize RNA localization. Since the dorsal side of the egg is specified by grk localization, we also assayed eggs laid by tdrd5l mutants for dorsal appendage (DA) phenotypes. 30% of eggs laid by tdrd5l mutants displayed DA phenotypes suggesting that loss of grk RNA regulation in tdrd5l mutants results in axis specification phenotypes. To investigate the regulation of nos we optimized the MS2 hairpin system which allows us to detect the localization on nos RNAs in the developing nurse cells and oocyte. Currently, we are using both of these systems to assay for changes in RNA localization and expression levels in tdrd5l mutants.

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RIBOSWITCH CONVERSION USING THE THEOPHYLLINE RIBOSWITCH

Mika Schievelbein¹, Lauren Lucia¹, Toni Zangrilli¹, James Tilton¹, Jonathan Ouellet²

¹Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ, 07764

²Department of Chemistry, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ, 07764

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 these high-tech, expensive 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 a pool of sequences containing the aptamer, which is linked to the shine dalgarno with eight random nucleotides, into a designed plasmid and transform the plasmid into bacteria cells. Then, the use of replica plating along with screening selects the cells that only contain the plasmid with 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 red and green fluorescence proteins, mCherry and GFP respectively, on either side of the inserted riboswitch. A PCR product encoding for mCherry, the riboswitch, and GFP will be inserted downstream of the lactose operon in pUC18. Ratios of the fluorescence intensities of the two fluorescent proteins will provide the ability to measure the riboswitch's function through fluorescence readings.

COMPARISON OF RIBOSWITCH REPORTER SYSTEMS FOR LIVE CELL IMAGING OF CYCLIC-DI-GMP DYNAMICS IN *BACILLUS SUBTILIS* POPULATIONS

Yasmine Pierre¹, Silvana Fragano¹, Keren Sneh¹, Susan Kang¹, Cordelia Weiss², Wade Winkler²,
Catherine Spirito¹

¹First-Year Innovation and Research Experience (FIRE) Engineering Biosensors, University of
Maryland, 3216 HJ Patterson, College Park, MD, 20742

²Department of Cell Biology and Molecular Genetics, University of Maryland, 3115 Bioscience
Research Building, College Park, MD, 20742

Fluorescent riboswitch reporters can be used in vivo to monitor metabolite dynamics. Previous work used a fluorescent yfp reporter based on a cyclic di-GMP responsive riboswitch from *Bacillus licheniformis* to monitor cyclic di-GMP levels in individual *Bacillus subtilis* cells. The previous study found that cell fates in *Bacillus subtilis* are not uniform in the presence of varying cyclic di-GMP levels. It is important to further develop tools that enable single-cell imaging in Gram-positive bacteria. Fluorogenic aptamers are single-stranded RNA molecules that have been evolved via in vitro selection to bind strongly and specifically to fluorophore molecules and emit a fluorescent signal. These fluorogenic aptamers can be used instead of fluorescent proteins in riboswitch reporter systems to provide a more dynamic read-out of metabolite dynamics in cells. However, relatively little work has been done to evaluate the use of these fluorogenic aptamers in Gram-positive bacteria. The objective of this project is to evaluate the use of four different fluorogenic aptamers (Mango-III, Broccoli, dimeric Broccoli, and Spinach2) instead of yfp in the cyclic di-GMP responsive riboswitch reporter system in *Bacillus subtilis*. Currently, all plasmids containing the riboswitch reporter have been constructed and successfully transformed into *E. coli* cells and their sequences have been confirmed. Future work involves transforming the riboswitch reporter plasmids into *B. subtilis* cells and evaluating their performance in vivo.

This research was supported by the First Year Innovative and Research Experience program along with Winkler Laboratory in the Department of Cell Biology and Molecular Genetics at the University of Maryland.

EFFICACY DETERMINATION OF *ESCHERICHIA COLI* BACTERIOPHAGES AS POTENTIAL ANTIMICROBIALS

Gianna Montanez¹, Funmilayo Omole², Mathumathi Rajavel²

¹Department of Biology, Morgan State University, 1700 E. Cold Spring Lane Baltimore, Maryland 21251

²Department of Biology/ Medical Technology, Morgan State University, 1700 E. Cold Spring Lane Baltimore, Maryland 21251

The emergence of antibiotics resistance among pathogenic bacteria has posed a great challenge in the field of medicine in the treatment of human diseases caused by these bacteria. In this study, we are using two bacteriophages (ϕ MR1 and ϕ MR2), that were previously isolated in our laboratory and are effective against the clinical isolate *Escherichia coli*.

Our objective in this study is to determine the killing efficacy of these two *E. coli* bacteriophages (ϕ MR1 and ϕ MR2), as a first step towards evaluating them as alternate to antimicrobials for therapeutics against infections caused by *E. coli* and also as environmental decontamination agents. In order to accomplish this, we sub-cultured our host *E. coli* to log phase and infected either with ϕ MR1 or ϕ MR2 with 0.02 multiplicity of infection (MOI) and measured their optical density at 600 nm to determine the time for complete lysis of the phage infected cells compared to our uninfected control *E. coli*. Our preliminary results indicated that both ϕ MR1 infected *E. coli* cells and ϕ MR2 infected *E. coli* cells had a significant lysis within 2 hours and 90 minutes respectively, based on the decline in the optical density measurement.

Next, to quantitate the bactericidal activity with respect to time after phage infection, serial dilutions of uninfected *E. coli* and *E. coli* infected either with ϕ MR1 or ϕ MR2 were plated onto LB agar and incubated overnight. Our results indicated that both ϕ MR1 and ϕ MR2 have a 99.97 \pm 02 % killing efficacy within 2 hours.

In conclusion, we present the preliminary results in this report that *E. coli* bacteriophages ϕ MR1 and ϕ MR2 have the potential to be developed as alternate to antimicrobials for therapeutics and also as environmental decontamination agents. We are currently investigating the minimal bactericidal concentration (MBC) for these phages.

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A SENSITIVE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRIC METHOD FOR THE QUANTITATION OF VEM-BISAMIDE-2 IN SMALL VOLUME OF MOUSE PLASMA: APPLICATION TO PHARMACOKINETIC STUDY

Ahila Moorthy¹, Christina Vedar², Jessie Villanueva PhD³, Jeffrey D. Winkler PhD⁴, Ronen Marmorstein PhD⁵, Athena F. Zuppa, MD²

¹Department of Chemistry and Biochemistry, University of Delaware, 210 South College Ave., Newark, DE 19716

²Center for Clinical Pharmacology, Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, 3401 Civic Center Blvd, Philadelphia, PA 19104

³The Molecular and Cellular Oncogenesis Program, The Wistar Institute, 3601 Spruce St, Philadelphia, PA 19104

⁴Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104

⁵Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104

Malignant melanoma is a type of cancer that develops from melanocytes. Vemurafenib causes programmed cell death in melanoma cell lines by inhibiting the BRAF/MEK/ERK pathway – if BRAF has the common V600E mutation. An obstacle to therapy with vemurafenib is the development of resistance within a year of the start of treatment. One of the mechanisms for drug resistance is transactivation through RAF dimers. Studies from our laboratory demonstrate that the BRAF dimer inhibitor, Vem-BisAmide-2, could be used to inhibit melanoma cell growth. An important obstacle to the development of Vem-BisAmide-2 is the paucity of pharmacokinetic (PK) and pharmacodynamic data. There is a need for a sensitive assay for Vem-BisAmide-2 to conduct preclinical pharmacokinetic studies.

We developed an ultra-performance liquid chromatography-tandem mass spectrometry method (UPLC) for quantification of Vem-BisAmide-2 in small volumes (25 μ L) of mouse plasma. Tandem mass spectrometry (MS-MS) parameters were optimized in the positive ionization mode with sum of two MRM transitions (m/z 1067.2 \rightarrow 468.1 and 361.2) for Vem-BisAmide-2 analysis. A structurally related compound, Vem-BisAmide-4 (m/z 690.3 \rightarrow 468.2) was used as an internal standard. A calibration curve was prepared in mouse plasma (1 – 10,000 ng/mL), and was found to be linear with coefficient of regression, $r^2 > 0.99$. Calibration standards and blanks in mouse plasma were extracted by protein precipitation with 400 μ L of acetonitrile containing 100 ng/mL Vem-BisAmide-4. A 5 μ L extract was injected for UPLC-MS/MS analysis. Vem-BisAmide-2 and Vemurafenib were separated on Phenomenex F5 column using 10 mM ammonium acetate in water and 10 mM ammonium acetate in methanol as aqueous and organic mobile phases with a flow rate of 0.6 mL/min. Analytes were separated using a gradient elution, with retention time of 2.15 min for Vem-BisAmide-2 and 2.24 min for Vemurafenib. This assay has been implemented successfully for pharmacokinetic analysis of Vem-BisAmide-2 in mouse plasma samples.

METHODOLOGY DEVELOPMENT: DETECTION OF PROTEIN FORMATION DISRUPTION CAUSED BY ROUNDUP™

Danielle Spaulding¹, Shanen Sherrer¹

¹Department of Chemistry and Biochemistry, St. Mary's College of Maryland, 47645 College Drive St. Mary's City, MD, 20686

The company Monsanto is facing a civil action lawsuit for their product, Roundup, potentially causing cancer of the lymphatic system. The active ingredient in Roundup is glyphosate. Glyphosate is a man-made chemical that is structurally similar to glycine, a protein building block naturally produced in the body. It is hypothesized that glyphosate can replace glycine during reactions. If this event occurs, it will increase the amount of glycine available and potentially promote protein misfolding with glyphosate interference causing the protein to reorient itself. In this study, an experiment was designed to examine how the methylation of glycine is affected by the presence of glyphosate. The method developed detects glycine and estimates its concentration. The method involves the use of high-pressure liquid chromatography (HPLC) for product separation, fluorometry for glycine detection, and mass spectroscopy for quantification and verification. If glyphosate can replace glycine during a reaction, it may be possible for glyphosate to inhibit protein formation and regulation. The protein formation disruption caused by glyphosate could, therefore, explain the formation of cancer.

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ENGINEERING PROTEIN MULTIMERS WITH WATER-SOLUBLE PORPHYRINS

Tyler Brittain¹, Sam Fontaine¹, Cole Swaim¹, Daniel Marzolf¹, Oleksandr Kokhan¹, Oleksandr Kokhan¹

¹Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807

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. However, combined small- and wide-angle X-ray scattering experiments revealed the formation of multimers with a wide range of complex sizes. Binding interactions were explored using wild-type and 12 mutants of PpcA, a 3-heme c-type cytochrome from *Geobacter sulfurreducens*, with several anionic water-soluble derivatives of tetraphenylporphyrin. Thermodynamic interaction parameters and complex binding stoichiometries were established with isothermal calorimetry. The binding sites of porphyrin were mapped out using HSQC with varying levels of porphyrin concentration to protein. 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 of multi-subunit protein complexes as well as targeted disruption of self-assembled complexes.

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INVESTIGATING THE EFFECTS OF CENTRAL CARBON METABOLISM MUTATIONS ON SOYBEAN SEED COMPOSITION

Cooper Hostetler¹, Shrikaar Kambhampati², Douglas Allen^{2,3}

¹McDaniel College, 2 College Hill, Westminster, MD 21157

²Donald Danforth Plant Science Center, 975 N Warson Rd, St. Louis, MO 63132

³USDA-ARS, 975 N Warson Rd, St. Louis, MO 63132

Soybean (*Glycine max*) is one of the most prevalent crops grown in the world today. Soybean is valuable because of its oil, which is used mainly in biofuel production, as well as its high protein content, which makes it a top choice for livestock feed. However, as oil and protein levels are inversely correlated in mature seeds, changes in one usually come at the expense of the other. Soybean seeds accumulate lipids and protein over the course of development. As the seed matures, the levels of lipids, protein, and sugars change due to differences in carbon partitioning in the seed. Lipid levels decline as the seed matures while levels of indigestible oligosaccharides raffinose and stachyose increase, possibly these two observations are related. For this study, a subset of fast neutron (FN) mutagenized soybean lines with alterations to central carbon metabolism with differences in final biomass composition were investigated for trends in biomass component accumulation over the course of seed development. The oligosaccharide and protein profiles were obtained using liquid chromatography tandem mass-spectrometry. Lipid profiles were obtained using gas chromatography equipped with a flame ionization detector. One line exhibited a predicted decrease in oligosaccharide production and two other lines exhibited increased lipid and protein production. Further study of these lines will indicate a promising avenue for further understanding the precise mechanism of carbon partitioning in seeds that can help improve the overall productivity of soybeans.

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ENHANCED ANTIBIOTIC RESISTANCE IN MOBILE BACTERIA POPULATION

Morgan Powers¹, Yongjin Liu², Paul Straight²

¹Department of Biology, King University, 1350 King College Rd Bristol, TN 37620

²Department of Biochemistry and Biophysics, Texas A&M University, 400 Bizzell St, College Station, TX, 77843

To survive encounters with competitors, bacteria deploy different methods such as changing growth and metabolism. As an example, *Bacillus subtilis* is able to make dormant spores, build biofilms, and produce toxic metabolites for competitive fitness. When exposed to chloramphenicol produced by a competitor, *Streptomyces venezuelae*, a *Bacillus subtilis* population initiates defensive mobility. The mobile population increases expression of a subset of genes that are predicted to convey antibiotic resistance. Therefore, we hypothesized the sliding population would have enhanced antibiotic resistance over a non-motile population. *B. subtilis* also induces mobility when exposed to additional ribosome-targeting antibiotics. This observation raises the question whether the other antibiotic resistance genes were correlated to a specific antibiotic. To study whether these genes contribute to antibiotic resistance and sliding motility, we constructed mutant strains that have deletions in multiple antibiotic resistance genes and tested their sensitivity for growth and induced mobility. We observe differences in sensitivity to some antibiotics (e.g. lincomycin) and not others (e.g. chloramphenicol), among several tested. To determine if a spatial pattern in gene expression could be found, a reporter strain was created. The reporter strain took the promoter from the targeted gene and placed it in front of the luciferase gene to mirror gene expression in luminescence signal. We found that PyxjB-lux demonstrates increase in luminescence signal and translation stress when exposed to chloramphenicol. The luminescence signal can be used to detect spatial patterns of expression within subpopulations of a colony. To explore the possibility that the response in antibiotic resistance genes can function as a sensor to detect the translation stress, we created fusion of relevant promoter fragments to the luciferase operon to use as reporter strains. Based on results from this work we are learning how exposure to antibiotics induces changes to motility and antibiotic resistance to protect *B. subtilis* against competitors.

I would like to acknowledge the Texas A&M Biochemistry REU for allowing me to participate in the summer program. I would also like to thank Yongjin Liu and Dr. Straight for letting me join the research project over summer.

DESIGNING AND TESTING RNA THERAPEUTICS TO BLOCK VEGFR2 AND EGFR ACTIVATION IN HUMAN GLIOBLASTOMA

Flobater Gawargi¹, Martin Hicks¹

¹Department of Biology, Monmouth University, 400 Cedar Ave, West Long Branch, NJ 07764

Glioblastoma multiforme (GBM), is the most common and aggressive malignant primary brain tumor with a median survival of 14 months. Current therapies are limited by the blood brain barrier. Epidermal growth factor (EGFR) and Vascular Endothelial Growth Factor receptor 2 (VEGFR2) are crucial for cancer cell survival. In our lab we are developing an innovative therapy that can bypass the blood brain barrier by developing RNA therapies to alter the splicing mechanism of the EGFR and VEGFR2 gene to reduce or block its activation, thus stop tumor cells from growing. Eleven Antisense sequences were designed to target the EGFR gene and nine Antisense sequences for VEGFR2, to potentially block their activation. The Antisense sequences were cloned into pAAV-U7-smOPT. In addition, multiple cloning strategies and protocols were used to clone the exonic splicing silencer 4G-quadruplex into our therapy vector. In addition, another aspect of this research is to isolate multiple tyrosine kinase receptors mRNA from GBM cancer cells, clone the cDNA into a T7 expression vector to transcribe control RNA to use in our high throughput sequencing experiments. Multiple cell lines including U87 and SK-MG cell line are being cultured and transfected with our novel therapies. Total mRNA was collected, analyzed, and compared to same cell lines without treatment. The collected data will allow the research to move toward a mouse model using adeno-associated virus (AAV) vector which was chosen based on its non-pathogenic and integrative features.

EPIGENETIC THERAPY IN OVERCOMING BONE MARROW MICROENVIRONMENT MEDIATED CHEMOPROTECTION OF LEUKEMIA CELLS

Sharon Kung¹, Anthony Quagliano¹, Anilkumar Gopalakrishnapillai², Sonali Barwe²

¹University of Delaware, Newark, DE 19711

²Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Rd, Wilmington, DE 19803

Acute lymphoblastic leukemia (ALL) is the most commonly diagnosed pediatric malignancy. Although 90% of patients will undergo remission during treatment, around 20% of those patients will have a recurrence of the disease, which is often much more aggressive and difficult to treat. The bone marrow microenvironment, which consists of many different cell types including osteoblasts, plays a key role in protecting leukemic cells from chemotherapy. This “chemoprotection” provided by osteoblasts in the bone marrow microenvironment can contribute to the high relapse rate in ALL. Chemotherapy drugs commonly used to treat ALL are cytarabine and daunorubicin; however, these drugs may not be at maximum efficacy due to osteoblast-induced chemoprotection (Quagliano et al., 2017). The epigenetic drug combination consisting of azacitidine and panobinostat has previously been shown to sensitize leukemic cells to chemotherapy through pretreatment, thereby reducing the effects of the osteoblast-induced chemoprotection. Our goal was to study the effects of azacitidine and panobinostat alone on sensitizing leukemic cells to chemotherapy. Furthermore, we wanted to investigate if the combination of azacitidine and panobinostat that leads to increased sensitivity is due to an additive effect. Our study showed that azacitidine has a greater potential to sensitize leukemic cells to cytarabine through pre-treatment while panobinostat had no effect. In addition, we show that cytarabine pre-treated cells with the addition of azacitidine and panobinostat had no additive effect from azacitidine and panobinostat; therefore, the azacitidine and panobinostat combination does have a potentiating effect in sensitizing leukemic cells to chemotherapy.

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EFFECT OF HIGH-POLYPHENOL SORGHUM EXTRACTS ON GENE EXPRESSION OF SELENOPROTEINS IN COLON CANCER

Ashish Singh¹, Dimitry Smolensky², Petra Tsuji¹

¹Department of Biological Sciences, Towson University, 8000 York Road, Towson, MD 21252

²United States Department of Agriculture, 1515 College Ave Manhattan, KS 66502

Polyphenols are secondary plant metabolites which can be found in many fruits, grains, and vegetables. Thus, they are a part of a normal human diet. Sorghum (*Sorghum bicolor*) is a cereal grain which contains varying amounts of polyphenols. Sorghum polyphenols have previously shown to have strong antioxidant activity in biochemical assays. Many polyphenols have been implicated in the regulation of colon cancer through modulating the antioxidant defense through modulating the antioxidant response, which includes some of the major selenoproteins such as Thioredoxin reductases (TXNRD) and glutathione peroxidases (GPX). Since such redox-active enzymes have been shown to be involved in both tumor prevention and promotion, the goal of our study is to investigate the impact of high-polyphenol sorghum extracts on the expression of selenoproteins. Human colon cancer cells (HT29, HCT116) were incubated with 1.25 mg extract (Control, HP, SC) per mL medium for 24h and 48h. RNA was extracted and reverse-transcribed to cDNA. mRNA expression was quantified using qPCR and normalized to GAPDH. Our preliminary results show that compared to the solvent control, one of the extracts (SC) decreased mRNA expression of GPX1 by a factor of 2.4 and 3.0 in HCT116 cells after 24-hour and 48-hour incubation time respectively. Furthermore, SC extract decreased GPX1 mRNA expression in HT29 cells as well by a factor of 3.19 in 48-hour incubation time. These results suggest that sorghum high polyphenol extracts may influence gene expression of selenoproteins. Further investigation may help explain the effects of these sorghum extracts on expression of selenoproteins which are important in tumor prevention and promotion in colon cancer.

Financial support was provided by Towson University's Fisher College of Science and Mathematics (P. Tsuji) and the USDA (D. Smolensky).

ANALYSIS OF NUTRIENT PROFILE AND FORMULATION OF FINGER MILLET (ELEUSINE CORACANA L.) WITH CHICKPEA (*CICER ARIETINUM*) PROTEIN SOURCE FOR INFANT COMPLEMENTARY FOODS IN KENYA.

Morgan Hall¹, Paul Kimurto, Ph.D.², Cyprian Syeunda², Jayo Manyasi Tracyline², Lillian Jeptanui, Ph.D.², Anne Osano, Ph.D.³, Joshua Ogendo, Ph.D.², Anne Osano¹

¹Department of Biology, Morgan State University, 1700 E Cold Spring Lane Baltimore, MD 21251

²Department of Crops Horticulture, and Soils Department, Egerton University, Nakuru, Kenya

³Department of Natural Science, Bowie State University, 14000 Jericho Park Rd, Bowie, MD 20715

Protein-Energy Malnutrition (PEM) is very prevalent and lethal in Sub-Saharan Africa. In Kenyan children, 26% are stunted, 4% wasted, 11% underweight. Complementary foods that act as substitutes for breast milk for young children and infants are of high demand. In Kenya, United States Agency for International Development (USAID) Feed the Future program in partnership with Egerton University has resulted in improved food security and reduced malnutrition in the country through the promotion of drought tolerant crops like finger millet. To improve protein content and reduce anti-nutrients, finger millet was composited with a protein source (Kabuli Chickpea) and malted to obtain a protein rich complementary infant food.

The main objective of this project was to contribute to the nutritional food security of infants in Kenya through the reduction of malnutrition by formulation of baby weaning food by using finger millet and chickpea.

The study of two finger millet varieties P224 and KNE741 took place at Egerton University in Njoro, Kenya. The field trial sites, Rift Valley, Kenya: Bomet County (medium altitude) and Koibatek, Baringo County (low altitude). Proximate composition involved analysis of moisture content, total ash content, and mineral content.

Based on the mineral results from this study, it is suggested that germination time is dependent on the initial evaluation of the composite(s) to achieve the best nutrient profiles.

In conclusion, it was found that the superior composite for the low altitude finger millet is KNE741 malted for 72-hours in a ratio of 75:25. The best overall, specifically in the medium altitude is P224 malted for 24-hours in a ratio of 70:30. Future tasks include a sensory study to determine the parental feedback on the formulation and soil analysis of the study sites.

This research was supported by the National Science Foundation-REU program by award of Dr. Anne Osano, Bowie State University. I would like to thank Egerton University for hosting this study as well as program coordinator Professor Ogendo PhD

**CHARACTERIZATION OF GROWTH & CYST FORMATION OF *AZOSPIRILLUM*
BRASILENSE CHEMOTAXIS MUTANT CHEA1 AS A FUNCTION OF THE AMOUNT
OF ORGANIC NITROGEN AVAILABLE**

Dreek Faust¹, Elena Ganusova², Kathleen King², Gladys Alexandre²

¹Department of Science, Technology and Mathematics, Gallaudet University, 800 Florida Ave
NE, Washington, DC 20002

²BCMB Department, University of Tennessee, Knoxville, TN 37996

The gram-negative, alpha-proteobacterium bacterium initially discovered by Dr. Dobereiner and his colleagues in Brazil. *Azospirillum B.* is a motile soil bacterium who promote the growth of plants upon inoculation, the extraordinary for fix nitrogen in the atmosphere and convert it into ammonium by the enzyme called nitrogenase. Many bacteria, includes *Azospirillum B.*, use a complicating taxi which called chemotaxis that will regulate them to move towards attracts and away from repellents. Occasionally, chemotaxis called as metabolism-independent behavior. Bacterial chemotactic behavior depends on the transmissions of signals that received by chemoreceptors on the membrane to flagella motors though signal transduction. One of the chemotactic operons, cheA1, is controlling the swimming speed, swimming reversals, and is an insignificant role in chemotaxis. Encystation betided when in stressful states, as such as being exposed abundantly of oxygen, because the enzyme is sensitive to oxygen. Defects in nitrogen signaling should alter nitrogen sensing, and nitrogen fixation was the hypothesis of this task.

I want to thank the foundation, Nation Science Foundation (NSF), for funding. Thanks to Dr. Alexandre, Dr. Ganusova, and Ms. King shall not be forgotten for guidance. Finally, and most importantly, thanks to all the members of the program that helps me prepare to be a better scientist in the future.

PLANARIAN FISSION BEHAVIOR IS MODULATED BY POPULATION DENSITY AND BODY LENGTH

Noor Shaikh¹, Alizay Rizvi¹, Tagide deCarvalho², Daniel Lobo¹

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²College of Natural and Mathematical Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Planarians are freshwater flatworms with an extraordinary regenerative capacity, able to regenerate body parts and organs from almost any amputation. Planarians can reproduce asexually by fissioning transversally, resulting in each fragment regenerating a complete worm. Fission can occur multiple times consecutively and it is inhibited in dense populations. However, a mechanistic model of fission signaling is still lacking.

Here we investigated the hypotheses that fission behavior is affected by population density, pheromones and body length. First, we tested the effect of individual isolation on latency to fission. Thirty large worms were individually placed in well plates and the number of body fragments recorded for nine days. The results showed that worms underwent fission after 1.8 days, on average. Next, we tested whether pheromones, secreted cues in the water environment, inhibit fission. We recorded the number of body fragments of 36 isolated worms for five days, half in water from the high-density colony and half in fresh water. The difference in fission frequency in fresh versus colony water was not significant (4.2 and 3.6 fragments on average, respectively). Finally, we examined the relationship between body length and frequency of fission. Images of 30 worms from a range of sizes (3.2-14.1 mm) were taken on a dissecting microscope, their lengths computed, and the worms isolated for six days. The results showed a positive relationship between body length and fission frequency, and that worms required minimum body length of 5.2 mm to undergo fission.

In summary, this work validated the hypotheses that the population density and body length modulates fission behavior in planaria. However, our data did not support the hypothesis that an inhibitory cue is secreted in the water environment; therefore, a different type of cue must be involved in the suppression of fission behavior when worms are living in dense populations.

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EFFECT OF LOW LIGNIN BAGASSE FROM SUGARCANE CLONE ON PRETREATMENT AND CELLULOLYTIC SACCHARIFICATION

Jordan Specht¹, Rafaela I. S. Ladeira Azar², Sidnei Emilio Bordignon-Junior³, Craig S. Laufer¹,
Daehwan Kim¹

¹Department of Biology, Hood College, 401 Rosemont Avenue, Frederick, MD 21701, United States

²Department of Biochemistry and Molecular Biology, BIOAGRO, Federal University of Viçosa, Viçosa, Minas Gerais 36570-000 Brazil

³Laboratory of Biochemistry and Applied Microbiology, Sao Paulo State University- UNESP, IBILCE, 2265 Cristóvão Colombo, 15054-000, São José do Rio Preto, São Paulo, Brazil

Lignin contributes to the rigid structure of the plant cell wall and is partially responsible for the recalcitrance of lignocellulosic materials to enzymatic digestion. Overcoming this recalcitrance is one the most critical issues in a sugar-flat form process. This study addresses the effect of low lignin sugarcane bagasse on enzymatic hydrolysis after liquid hot water pretreatment at 190°C and 20 min (severity factor: 3.95). The hydrolysis of bagasse from a sugarcane line selected for a relatively low lignin content, gave an 89.7% yield of cellulose conversion to glucose at 40 FPU/g glucan versus a 68.3% yield from a comparably treated bagasse from the high lignin bred line. A lower enzyme loading of 5 FPU/g glucan (equivalent to 3.2 FPU/g total solids) resulted in 31.4% and 21.9% conversion yields, respectively, for low and high lignin samples, suggesting the significance of lignin content in the saccharification process. Further increases in enzymatic conversion of cellulose to glucose were achieved when the bagasse sample was pre-incubated with a lignin blocking agent, e.g., bovine serum albumin (50 mg BSA/g glucan) at 50°C for 1 hr prior to an actual saccharification. In this work, we have demonstrated that even relatively small differences in lignin content can result in considerably increased sugar production. We suggested that the increased glucose yields with the addition of BSA resulted from the inhibition of non-productive binding of enzymes to lignin.

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THE IMPORTANCE OF HOST CELL GLUTAMINOLYSIS ON T-EVEN BACTERIOPHAGE REPLICATION

James Tabet¹, Isabelle Van Benschoten¹, Jessica O'Hara¹

¹FIRE: First Year Innovation & Research Experience, University of Maryland, College Park,
2335 Atlantic Building, College Park, MD 20742-5031

According to the CDC, about 2 million people are infected with antibiotic-resistant bacteria each year in the United States. Due to the rising number of antibiotic-resistant bacteria, phage therapy is being revisited as an emerging method to treat drug-resistant bacterial infections. Bacteriophage infect and hijack a host bacterial cell in order to drive their own replication and as a result are completely dependent on the host cell to provide the building blocks to generate new virions. Viral manipulation of host cell metabolism is critical for successful replication, however, there is a lack of understanding about this critical host-pathogen interaction, especially for bacteriophage. Better characterization of phage metabolic adaptations could allow for potential improvements in phage therapy.

Numerous studies of DNA viruses, such as human cytomegalovirus and vaccinia virus have highlighted the importance of glucose and glutamine for successful viral replication. The aim of our project is to determine the importance of glucose and glutamine for optimal phage growth in the host cell - *Escherichia coli*. We can study the effects of viral replication through the utilization of knockout strains in which a certain gene is removed from the DNA sequence and replaced with a selectable marker that inhibits the production of a certain protein. We obtained an *E. coli* strain where the *glsA* gene was knocked out. This gene encodes a glutaminase enzyme that converts glutamine to glutamate, allowing it to enter the citric acid cycle.

We characterized T-even phage (T2, T4, and T4r) replication in the *glsA* knockout strain. Our results demonstrate a consistent reduction in phage replication when host cell glutamine metabolism is altered, confirming the importance of this metabolic pathway for efficient bacteriophage replication. Future work will aim to further characterize the specifics of viral manipulation of this host cell pathway.

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INVESTIGATION OF REPRODUCTIVE EFFECTS OF BPA AND BPF IN *C. ELEGANS*

Kelly Rivenbark¹, Rebekah Frye², Laura Kelly Vaughan²

¹Department of Chemistry, King University, 1350 King College Road Bristol, TN 37620

²Department of Biology, King University, 1350 King College Road, Bristol, TN 37620

Bisphenol A (BPA) is a commonly used chemical in the synthesis of polycarbonate plastics, which are heavily implemented in consumer-based plastic products. Because of growing concern for the estrogenic and neurological effects, some federal governments (including the US, France, and Canada) have implemented strict regulations on the use of BPA in consumer products to control human exposure to BPA, which have increased the use of other common bisphenol analogues, like bisphenol F. Due to the structural similarity, Bisphenol F (BPF) is hypothesized to induce reproductive toxicity in the same magnitude as BPA as well as have a similar toxicity mechanism.

Previous studies of BPA (and bisphenol) toxicity on model organisms have been conducted in polystyrene petri dishes. While companies (VWR, ThermoFisher) report no levels of BPA in their petri dishes, bisphenol compounds may be leaching into the media or exposure the organisms to bisphenol compounds through direct contact. To eliminate this potential confounding factor, we also utilized glass petri dishes in our study, so that the suitability of plastic lab ware in bisphenol research can be assessed.

Wild type *Caenorhabditis elegans* were chronically exposed to 250µM or 500µM BPA or BPF solutions for 2 days. After 2 days, individual adult hermaphrodites were moved to individual plates for quantification of brood size for each treatment group. After exposure to BPA and BPF, all treatment groups, including those cultured in plastic dishes, displayed a significant decrease in brood size compared to the glass petri dish control group, suggesting that BPA and BPF display estrogenic activity and induce sterility on those exposed. Currently, research suggests that BPF is not a safe alternative to BPA and induces toxic effects in a higher magnitude than that of BPA; further research is needed to determine the extent of the toxic effects on biological systems.

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LIVER-TARGETED AAV8 GENE THERAPY IN MUCOPOLYSACCHARIDOSIS IVA MURINE MODEL

Matthew Piechnik¹, Kazuki Sawamoto², Shunji Tomatsu²

¹Department of Medical and Molecular Science, University of Delaware, 305 Williard Hall 25 N College Ave, Newark, DE 19716

²Skeletal Dysplasia Lab, Nemours Biomedical Research, Nemours/A.I. DuPont Hospital for Children, 1600 Rockland Rd. Wilmington, DE 19803

Mucopolysaccharidosis IVA (MPS IVA) is a lysosomal storage disorder characterized by a deficiency in the GALNS enzyme leading to a systemic accumulation of the glycosaminoglycans (GAGs), keratan sulfate and chondroitin-6-sulfate. MPS IVA manifests clinically with mild to severe skeletal dysplasia, heart valve disease, and upper airway obstruction; some of the leading contributors to mortality. Adeno-associated virus (AAV) mediated gene therapy presents a novel therapy that is non-pathogenic, long-lasting, and able to transduce multiple cell types. Our study investigated a liver-targeting AAV serotype 8 (AAV8) gene therapy vector as a novel therapy in a murine MPS IVA and human GALNS-tolerized (Mtol) model. Treated Mtol mice exhibited significant improvement to GALNS enzyme activity in blood plasma (4x wild type levels), liver (62x wild type levels) and bone (49% of wild type levels) 16 weeks after treatment. Untreated Mtol mice exhibited no detectable enzyme activity. Histopathological analysis of femoral growth plate and myocardium noted reduction in cellular vacuolation in both myocardium and chondrocytes as well as improved column structure within the growth plate. Normalization of chondrocytes however remains an unmet challenge, resulting potentially from a humoral immune response or a need for dose optimization. This AAV8-mediated gene therapy presents itself as a desirable method for achieving sustained and supraphysiological levels of gene expression in MPS IVA mice, leading the way for development of a human clinical therapy.

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DETERMINING THE ROLE OF THE BACTERIAL PROTEIN BEFA IN PROMOTING MAMMALIAN PANCREATIC ISLET EXPANSION

Robin Bailey¹, Melissa Tran², Loc-Duyen Pham², Aleksandar Kostic³

¹Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Microbiology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215 &

³Department of Microbiology & Immunology, Harvard Medical School, 25 Shattuck St, Boston, MA 02115

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of β -cells, pancreatic cells responsible for producing insulin. Those suffering from T1D have little to no insulin production leading to hyperglycemia, which can cause severe side effects in patients. Studies have implicated the gut microbiome as a significant factor in T1D risk. It was recently discovered that certain symbiotic bacteria of zebrafish were required for pancreatic development, and that this effect was mediated by the bacterial protein BefA. The authors then identified BefA homologs in human-associated microbes, proposing that BefA may have a role in the development of human pancreata and in overall T1D risk. These findings raise the possibility that microbiota play a role in preventing or mitigating T1D by promoting β -cell mass expansion and improving innate insulin production in T1D patients. Ex vivo studies of BefA in rat islets have shown to increase β -cell proliferation, suggesting BefA can function in mammals. We aim to observe the effects of the BefA on mammalian pancreatic islet expansion in vivo using 4-week old B6 mice as our model. BefA protein was harvested and purified from *Escherichia coli*, then administered to B6 mice via intraperitoneal injection. We hypothesize that administration of BefA in B6 mice would increase β -cell proliferation, measured using Ki67 as a proxy for cellular replication and staining for Ki67 via immunohistochemistry and confocal microscopy. We predict that this increase in β -cells would increase insulin secretion, resulting in a higher tolerance to glucose, measured via glucose tolerance testing.

Preliminary results suggest that in vivo BefA treatment has no significant effect on β -cell proliferation or glucose tolerance. We plan to investigate if increasing the BefA dosage or immediate tissue harvesting would improve the mice's response to BefA treatment in vivo.

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T CELL-DEPENDENT RESISTANCE TO CURCUMIN AS A TREATMENT FOR PANCREATIC CANCER

Shaun Jackson¹, Adrianna M. Vaskas¹, Joseph A. Fraietta², Kristen Long¹

¹Department of Biology, Mansfield University, 76 Stadium Drive; Mansfield, PA 16933

²Department of Microbiology, University of Pennsylvania, 3400 Civic Center Blvd Philadelphia, PA 19104

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death in the United States. With the mortality rate of PDAC being extremely high, the prognosis is rather grim. PDAC is often undetectable until late stages, and as such, few options for treatment are available, which begs for the development of new and effective treatments.

Plant-derived chemicals are becoming increasingly popular, as they typically possess a variety of benefits and have fewer side effects than conventional treatments. Curcumin has been used for centuries for medicinal purposes. Curcumin has recently shown some promise as a potential therapy when tested in xenograft models of multiple solid tumors and is currently being tested in early clinical trials of some cancers. However, studies have shown that antitumor compounds that appear to be effective in immunocompromised mice are not as effective in humans. To remove this limitation, this study used syngeneic, immunocompetent mice implanted with murine PDAC tumor cell lines that were previously derived from genetically engineered KPC mice. To mimic the athymic mice used in published xenograft models, we used T cell depleting antibodies for a subset of mice, prior to tumor implantation. Once tumors were established, curcumin (or vehicle alone) was administered and treatment efficacy was assessed by measuring tumor growth and the overall survival of mice. For T cell-depleted mice, we found that treatment with liposomal curcumin stalled tumor growth and increased overall survival compared to mock treated mice; this treatment difference was lost in T cell-replete mice. Based on histological evaluation of the tumors at harvest, we believe this difference in efficacy is due, at least in part, to differences in tumor landscape in T cell-depleted vs replete groups. This finding warrants additional studies in order to understand this mechanism and enhance its potential success in the clinic.

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THE EFFECT OF NON-NUTRITIVE SWEETENERS ON *C.ELEGANS*

Macarena Martín Mayor¹, Katelyn Nastasha Gilbert¹, Ashley Reynolds¹, Rebekah Frye¹, Laura Kelly Vaughan¹

¹Department of Biology, King University, 1350 King College RD, Bristol, TN 37620

The increase in the prevalence of overweight and obesity among the population is complex with a multitude of genetic and environmental factors contributing. Artificial sweeteners have been supported as an option to decrease diseases like obesity and type II diabetes, which are crucial health concerns today. However, recent research suggests that consuming them may disrupt both glucose and fat metabolism, which raises concerns as consumption of the sweeteners keeps increasing with the prevalence of diet beverages but also cereals and products aiming a younger generation. Metabolic anomalies in the Insulin Signaling (IGF-1) pathway, which modulates cell proliferation and growth, are expected as an effect of an excessive consumption of artificial sweeteners. The model organism *C.elegans*, which shares 80% of human genes and has a high similarity in the IGF-1 pathway, was treated with glucose, different concentrations (10mM and 30mM) of Sweet and Low® and the sweetener's active ingredient, Saccharin, to evaluate the effect of exposure on lipid accumulation. Oil Red O', a lipophilic dye, was used to quantify the lipid content through image analysis. The images were evaluated and the red pixel count of the nematodes was used as the lipid quantification method. When compared to control, both the active ingredient and the Sweet and Low® exposed worms show significantly greater red pixel count, therefore, greater lipid accumulation. Glucose treated worms on the other hand showed an insignificant amount of lipid accumulation. This data suggests that, unlike glucose, exposure to artificial sweeteners leads to an increase in lipid accumulation in nematodes, indicating that artificial sweeteners may play a role in the obesity epidemic we are currently living.

I would like to thank the Burke Endowment and Dr. Simeon Pickard, Chair of the Chemistry Department and Endowed Chair of the Burke Endowment awarded by Dr Edward W Burke, Jr, Professor in Natural Sciences, for their funding and trust in this project. Moreover, I would like to thank Rebekah Frye, lab manager who helped me and gave me the basics to work on my own. I want to thank Kelly Rivenbark, who supervised and guided me through many steps of the project while doing her own project, and Alexandra Stevik, who with I shared the lab facility and many hours of work. Last but not least, I want to thank Dr. Laura Vaughan, Chair of the Biology Department at King University, who introduced me to the project and to research itself, granted me the ability to access the lab facilities and serves as the Primary Investigator of the Research Lab at King University.

Chemistry

ABSTRACTS

Afternoon Session

*Page Numbers of the Abstracts Serve as
Poster Numbers*

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 2019 Undergraduate Research Symposium in the Chemical and Biological Sciences.

TRANSITION METAL SALT-MEDIATED REACTION BETWEEN BENZALDEHYDE AND ACETOPHENONE

Jaquelyn Lalescu¹, Kameron Kinast¹, Paul Sabila¹

¹Biology, Chemistry & Physics, Department of Math, Science & Technology, Gallaudet University, 800 Florida Ave NE, Washington, DC 20002

The goal of our research group was to develop new and efficient synthesis methodology of coupling reactions and to reduce use of toxic or expensive transition metal catalysts. We studied the coupling of Benzaldehyde and Acetophenone in acetonitrile and toluene. My project explored the use of cheaply available reagents and catalysts in the coupling reaction.

The reaction progress was monitored using HPLC, TLC, and GC-MS instruments. Better results were obtained when silica gel was used.

In conclusion, the best results were obtained using three salts, namely, potassium carbonate, zinc acetate, and potassium bromide.

Center for Integrated Quantum Materials (CIQM), The NASA Science Foundation (NSF CIQM DMR 1231319, The NASA/DC Space Grant Consortium, Center for Science and Technology Research (CSTR), Department of Science, Technology, and mathematics (DSTM), Gallaudet University, The Beverly Taylor Sorensen Student Fellowship

EFFECTS OF VARIOUS TRANSITION METAL SALTS ON THE REACTION BETWEEN BENZALDEHYDE AND ACETOPHENONE

Kameron Kinast¹, Jaquelyn Lalsecu¹, Paul Sabila¹

¹Department Science, Technology, and Mathematics, Gallaudet University, 800 Florida Ave Ne
Washington, DC 20002

Our research is interested in the effects of various transition metal salts on coupling reaction between benzaldehyde and acetophenone. We explore on the factors affecting the transition coupling reactions such as, heat, silica gel, mol % amount of transition metals, reaction time, and solvent. The research goal is to develop a new synthesis methodology that uses less toxic transition metals for coupling reactions.

- Center for Integrated Quantum Materials (CIQM)
- The National Science Foundation (NSF CIQM DMR 1231319) • The NASA/DC Space Grant Consortium
- Center for Science and Technology Research (CSTR)
- Department of Science, Technology, and mathematics (DSTM) • Gallaudet University
- The Beverly Taylor Sorensen Student Fellowship

THE EFFECT OF SMALL MOLECULES ON THE STABILITY OF G-QUADRUPLEXES

Christopher Bentsen¹, Adriana Zelaya¹, Davis Jose¹

¹Department of Chemistry and Physics, Monmouth University, 400 Cedar Avenue, West Long Branch, NJ 07764

Telomeres are repetitive Guanine rich sequences at the ends of chromosomes that play a crucial role in protecting critical gene coding proteins from getting attacked and lost through cell division. The human telomere consists of a sequence of nucleotides, TTAGGG, that starts near the end of the duplex DNA and continues as a single strand. This single strand intrudes into the duplex DNA to form a T-Loop, which resembles a cap. Inside the cap, the Guanine rich sequence forms a G-Quadruplex structure, which is where our research is focused. The two important abnormalities that are directly correlated to the malfunction of telomeres are cancer and premature aging syndromes. The malfunctioning of telomeres results from many factors and the stability of the G-Quadruplexes is one of them. In the current research, we are introducing several types of small molecules, one of which is the porphyrin-based class of small molecules, to study the effect of ligand-induced stabilization/destabilization of these non-canonical DNA structures.

Incorporation of several classes of ligands have been introduced in two ways. Ligands under study were either incubated with the annealed DNA for 1 hour and or annealed with the DNA. Depending on the way these ligands are introduced has shown variances in the thermal stability of the G-Quadruplex. As the next step, we will introduce fluorescent probes either on the DNA or on the ligands to identify how the ligands bind to the DNA and the structural changes caused by it. The final test will be the introduction of a helicase to analyze the true stability of the G-Quadruplex. This study will be useful in developing new therapeutic methods for treatment of diseases including cancer and genetic diseases such as Bloom syndrome, Werner syndrome, and Fanconi anemia.

We would like to acknowledge the Monmouth University School of Science Summer Research Program

THIOPHENE-BASED COVALENT ORGANIC FRAMEWORKS

Steven Knauss¹, Austin Evans², Kathryn Allen¹

¹Department of Chemistry, Millersville University of Pennsylvania, 1 S George St, Millersville, PA 17551

²Department of Chemistry, Northwestern University, 2145 Sheridan Rd., Evanston, IL 60208

The propeller-like junctions of tetraphenylethylene encourage periodic docking that guides interlayer building between the 2-dimensional layers of covalent organic frameworks (COFs). These produce excellent crystalline COFs.

Incorporating thiophenes into a propeller would allow us to tune the electronic nature of a COF while still theoretically maintaining the periodic docking that guides excellent crystallinity of the tetraphenylethylene propeller-based COF.

A tetrathiophenylbenzene propeller junction was synthesized in three steps from commercially available materials and incorporated into a COF. Upon pairing with a simple benzene linker, the COF suffered from impurities and poor crystallinity. However, incorporating a pyrene linker promoted a crystalline COF, indicating the propeller is unable to guide docking when altered to an asymmetric thiophene ring. XRD confirmed the crystallinity and porosimetry data was consistent with the pore sizes modeled for the COF.

Interestingly, the crystallinity is transient, and may be turned ‘on’ by supercritical CO₂ drying and ‘off’ by storing under ambient conditions. This brings up interesting questions about the freedom of motion of the thiophenes in the propeller in the COF and about the stability of crystalline COFs.

We would like to thank Mrs. Karen Murley for her support through the Murley Summer Research Fellowship. We would also like to thank Millersville University’s support through the Millersville University Faculty Grant.

TUNING THE PROPERTIES OF POLYVINYL ALCOHOL-BORONIC ACID GELS

Holli Scott¹, Gretchen Peters¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main Street, Harrisonburg, VA 22807

Gels formed from polymers are incredibly common in our day-to-day lives. For example, polyvinylpyrrolidone (PVP) is used in many hair gels and creams; the first soft contact lenses were made with the polymer gel formed with poly (2-hydroxyethyl methacrylate) (PHEMA); and sodium polyacrylate (SPA) gels are frequently used as the absorbents in diapers and sanitary napkins. As is clear from these examples, the applications of gels are wide-ranging, and the physical properties needed for these applications are vastly different. That being said, optimization of a gel's material properties to suit a specific application is not a trivial task, and developing new, functional polymer gels can be both labor-intensive and synthetically challenging. Thus, we sought to design a polymer gel system with easily tunable physical properties and functionality. To do this, we envisioned using polyvinyl alcohol (PVA) with a series of commercially available boron-based cross-linkers. In the presence of boric acid/borax or a diboronic acid, PVA forms dynamic boronate ester crosslinks and induces gelation in water and DMSO. Here, we show that using a combination of two or more boron-based crosslinkers changes the material properties of the PVA gels. That is, the properties of PVA gels can be readily manipulated simply by varying the ratios and/or identities of the boron-based cross-linkers. To date, we have fully characterized the material properties of these gels using rheology and explored their viability as separations media. We plan to continue investigating the tunability and function of these materials.

This research was funded by the National Science Foundation Research Experience for Undergraduates (CHE-1757874) as well as the Mr. Jeff Tickle '90 Family Endowment. Additional thanks to James Madison University, Department of Chemistry and Biochemistry.

ADVANCEMENT OF WATER-SOLUBILIZED NON-LINEAR PORPHYRINOIDS FOR PHOTODYNAMIC THERAPY

Molly Warndorf¹, Maxwell Martin¹, Joel Rosenthal¹

¹Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716, USA

Photodynamic therapy (PDT) is a non-invasive treatment for cancer and other diseases that relies on the sensitization of singlet oxygen. PDT is a highly localized type of treatment, and therefore has minimal side-effects so patients are able to recover relatively quickly. A limited number of phototherapeutic treatments are currently used to treat tumors close to the surface of the skin. Since most commercial PDT agents are not activated by the necessary wavelength (phototherapeutic window = 700 nm – 950 nm) required to penetrate deep-tissue tumors, development of improved photosensitizers is required. We have previously reported the structure of a palladium(II) tetrapyrrole complex comprised of a 10,10-dimethylbiladiene ligand (Pd[DMBil]), that displays a very high singlet oxygen quantum yield of 80%. Upon bromination, this porphyrinoid is easily modified using metal-catalyzed cross-coupling chemistry. Such strategies allow the conjugated pi-system of the molecule to be extended, facilitating a shift in the absorbance spectrum of the complex into the phototherapeutic window. We have recently synthesized a suite of 10,10-dimethylbiladiene complexes with arylalkyne substituents that demonstrate promising absorption profiles and high singlet oxygen quantum yields. The synthesis, photophysics and efforts to water solubilize these new derivatives for biological studies will be presented.

This work was supported by NSF CAREER Award CHE1352120 and NIH P20GM104316.

DEVELOPMENT OF PHOTO-SWITCHABLE METAL-ORGANIC FRAMEWORKS (MOFS)

Zachary Clifford¹, Siddhant Warriar², Kate Waldie²

¹Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

²Department of Chemistry and Chemical Biology, Rutgers University, 123 Bevier Road, Piscataway, NJ 08854

Metal-Organic Frameworks (MOFs) are a class of porous materials that have been investigated for several applications in green energy, including the separation and storage of gaseous fuels like hydrogen and catalysis for fuel-forming and energy harvesting reactions. The ability to control the electrical conductivity of MOFs using an external stimulus such as light is of great interest for optical switches and optically switchable catalysts. Photochromic molecules undergo light-triggered isomerization between two different isomeric forms that have different physical properties. It has been reported that certain photochromic organic molecules display conductivity changes between the two isomers. However, the integration of such organic photochromic molecules into MOFs in order to gain optical control of conductivity has only recently been reported, and these few examples displayed very small conductivity values (ca. 10^{-7} - 10^{-6} S•cm⁻¹). In order to make photo-switchable MOFs with better conductivities and stabilities, different photochromic molecules that are more amenable to MOF synthesis are needed, as well as a more fundamental understanding into the modes of conductivity in these structures.

To this end, we are targeting a modified pyrene molecule to use as a photochromic organic ligand in conductive MOFs. The proposed synthesis of the target ligand is an 8-step procedure based upon literature precedent. Using proton nuclear magnetic resonance spectroscopy (¹H NMR), it was confirmed that the products from the first 6 steps were synthesized in high purity. However, the product has only been isolated on a small scale. Current attempts to optimize the synthesis are underway. Upcoming studies will involve completing the ligand synthesis, integrating the new ligand into MOFs, and studying the optical and conductivity properties of these MOFs. Long term goals of this project include creating a database of photochromic MOF structures.

I would like to thank Siddhant Warriar and Dr. Kate Waldie for allowing me to work on this project. I would also like to thank the RISE program and all of its directors for organizing this REU and for all their help this summer. This material is based upon work supported by the National Science Foundation under Grant No. EEC:1659818.

GAS-PHASE REACTIVITY ON THE DECOMPOSITION OF COOKING OILS

Jessica Whittemore¹, Daniel Albert¹

¹Department of Chemistry, Millersville University, 40 Dilworth Road, Millersville, PA 17551

Humans commonly introduce molecules to indoor environments through daily activities, such as cooking or cleaning, which are then subjected to gas phase-reactivity or surface interaction. Indoor environments are characterized by direct and indirect particle-contact reactions. Direct contact is characterized by liquid-liquid or solid-liquid interactions whereas indirect contact refers to gas-phase vapors interacting with a solid or liquid. These reactions decompose thin molecule films and can form products that impose health risks, thus the main goal of this research is to analyze gas-phase formations from contact reactions. A sample cell has been developed to observe the reactive effects of indirect contact between cooking oil and gaseous bleach or hydrochloric acid. Fourier-transform infrared spectroscopy monitors the oil decomposition occurring in real time, revealing spectral changes dependent upon exposure conditions. Gas-phase experiments of direct contact reactions of cooking oil with bleach or hydrochloric acid were analyzed by solid-phase microextraction and gas chromatography-mass spectrometry. It is concluded that gaseous exposure causes minor oil decomposition in the presence of bleach and observable decomposition in the presence of hydrochloric acid. Furthermore, it was found that direct contact reactions stimulate the formation of chlorinated hydrocarbons when exposed to bleach and hydrocarbons in the presence of hydrochloric acid. Implications from these conclusions correlate to indoor environmental conditions and can be applied to improve air quality.

This research is possible under the sponsorship of the Murley Summer Fellowship, Millersville University Department of Chemistry and Millersville University Department of Sponsored Programs and Research Administration.

COMPUTATIONAL AND SYNTHETIC APPROACH TO THE DESIGN OF AN IMIDAZOLE-CONTAINING PNA NUCLEOBASE FOR HOOGSTEEEN RECOGNITION OF AU BASE PAIRS

Emily Kagarise¹, James MacKay¹

¹Department of Chemistry and Biochemistry, Elizabethtown College, 1 Alpha Dr.,
Elizabethtown PA 17022

The central dogma of molecular biology characterized RNA as a passive messenger in the translation of genetic information. It is now understood that RNA transcripts execute a variety of biological functions, including gene expression, catalysis, and post-transcriptional modification. As more RNA transcripts are discovered, the ability to identify and regulate them will become crucial to our understanding of biology. Peptide Nucleic Acids (PNA) are promising tools for RNA recognition due to their potential for sequence-selective recognition. PNA is a synthetic analog of nucleic acids that contains a nonionic peptide backbone rather than the traditional highly charged phosphodiester backbone in RNA. This neutral charge and PNA's easily modifiable sequence promote effective binding with strands of dsRNA through triple helix formation.

This project utilizes computational analysis to guide the design of modified PNA nucleobases that recognize the AU base pair in dsRNA. These computations, performed at the PM6 and Hartree-Fock levels, revealed crude binding energies, molecular geometry, and hydrogen bond lengths in a variety of modified scaffolds containing two 5-membered ring heterocycles extended from the uracil nucleobase. Computational analysis of a 1,2,3-triazole scaffold attached to an imidazole suggests a more favorable binding energy than an isolated uracil nucleobase due to the additional hydrogen bond on the pyrimidine base of the dsRNA. We have successfully synthesized the target monomer, T2, by first alkynylating 5-iodouracil and attaching it to the PNA backbone through amide coupling. The target was obtained through a one-step, 3+2 cycloaddition between 4-azidomethyl-1H-1-triphenylmethylimidazole and the alkynyl uracil derivative, forming the central 1,2,3-triazole. Future work will involve incorporation of the monomer into PNA and binding studies using isothermal titration calorimetry. Strong and selective binding will indicate that this monomer is capable of promoting sequence-selective recognition and regulation of double-helical RNA.

We gratefully acknowledge the National Science Foundation (CHE-1708699) and the Elizabethtown College Scholarship, Creative Arts, and Research Projects (SCARP) program for support.

A COMPUTATIONAL STUDY OF THE EFFECT OF PROTONATION STATES ON THE N79D MUTATION IN UBC13

Robert Gardner¹, Jay-Anne Johnson¹, Kathy Elliott¹, N. Cole Seward¹, R. Hunter Wilson¹, Isaiah Sumner¹

¹Department of Chemistry & Biochemistry, James Madison University, 901 Carrier Drive, Harrisonburg, VA 22807

Post translational modification is the process of changing the function of a protein after it has already been synthesized without changing the entire protein. Ubiquitination is one of these processes and is the covalent attachment of the small protein ubiquitin to a lysine on a target protein through a series of reactions catalyzed by three types of enzymes the E1 (ubiquitin activating enzymes), the E2 (ubiquitin conjugating enzymes), and the E3 (ubiquitin ligases). If this process does not occur properly, it can cause many diseases such as Alzheimer's, Parkinson's and certain anemias. A popular hypothesis is that a conserved asparagine in the E2 enzyme (N79 in UBC13) stabilizes a reaction intermediate in the ubiquitination pathway because of its proximity to the active site, it is highly conserved and mutation studies show it is important for catalysis. MD simulations were run on several structures with the asparagine 79 mutated to a protonated aspartate (N79D), a deprotonated aspartate (N79D-) and an alanine (N79A), from those simulations hydrogen bonding and root-mean-square fluctuation (RMSF) data were obtained. The N79D mutation prefers to hydrogen bond to the substrate, the wild type shows lower fluctuations compared to all the mutations and without more data no other definitive conclusions can be made.

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A COMPUTATIONAL STUDY OF THE ROLE OF THE E3 LIGASE IN THE UBIQUITINATION REACTION

Jay-Anne Johnson¹, Isaiah Sumner¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Post translational modifications can change protein functions without changing the entire protein. Ubiquitination is a type of post translation modification wherein the small protein Ubiquitin (Ub) is covalently bound to a lysine on a target protein. One well-known function of ubiquitination is to flag proteins to be degraded. Ubiquitination occurs by a multitude of reactions catalyzed by three types of enzymes: ubiquitin activating enzymes, E1; ubiquitin conjugating enzymes, E2; and ubiquitin ligases, E3. The E2 enzyme directly catalyzes the ubiquitin transfer, whereas the E3 enzyme improves the efficiency of ubiquitination but it does not affect the chemical mechanism. In the accepted mechanism of the E2 enzyme, Ub is covalently linked to the E2 via a thioester bond and ubiquitination proceeds through a zwitterionic, tetrahedral intermediate. It is suggested that the negative charge on the zwitterion is stabilized by a hydrogen bond to a nearby, highly conserved asparagine (Asn79 in Ubc13). It is hypothesized that the E3 enzyme improves the catalytic efficiency of ubiquitination by placing the E2~Ub conjugate in a “closed” position, which may put bond strain on the thioester linkage. We have analyzed how the hydrogen bond environment changes in the open and closed E2~Ub positions by running molecular dynamics simulations starting from protein databank (PDB) crystal structures 5ait (closed) and 2gmi (open). Our data shows that systems in the closed position increase hydrogen bonding to the substrate. We have also used density functional theory on a model reaction to show that a modest amount of bond strain can result in improved catalysis.

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ULTRAFAST LASER SPECTROSCOPY TO STUDY PHOTODYNAMICS OF ASYMMETRIC HYDROGENASES

Amber Meyers¹, Christopher Stromberg¹, Edwin Heilweil²

¹Department of Chemistry and Physics, Hood College, 401 Rosemont Avenue, Frederick,
Maryland 21701

²Nanoscale Device Characterization Division, Physical Measurement Laboratory, National
Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, Maryland 20899

In nature, some bacteria are able to efficiently produce hydrogen gas. [FeFe]-hydrogenases are the enzymes that generally catalyze the reduction of hydrogen in nature. These enzymes, which contain iron in their active site, are of great interest because of the increasing need to develop a cheaper substitute for platinum as a catalyst for hydrogen production. Symmetric model compounds containing all CO ligands or mixed CO/CN and CO/PMe₃ ligands on both irons in the active site have been studied extensively. However, asymmetric compounds have not been the focus of as much work. This work examined two asymmetric molecules containing both CO and CN ligands: [Fe₂(μ-S₂C₂H₄)(CO)₅(CN)₁]¹⁻ (1) and [Fe₂(μ-S₂C₃H₆)(CO)₅(CN)₁]¹⁻ (2). The asymmetry of these model compounds makes the redox potentials of the two iron atoms different, changing the catalytic properties of the compounds. The molecules were studied using 400 nm and 266 nm UV pump-infrared probe spectroscopy in order to better understand the ultrafast dynamics of the active site.

So far, 1 has been studied using 400 nm pump pulses, and 2 has been studied using 400 nm and 266 nm pump pulses. In 1, distinct bleaches are observed around 1930, 1979, 1999, and 2038 cm⁻¹. There are also new absorptions at 1899, 1964, and 2023 cm⁻¹. The bleaches and absorptions decay with a time constant of 23 ± 6.6 ps. There is some evidence of residual signal remaining out to 300 ps, especially with the bleach at 1979 cm⁻¹. Similar bleaches and absorptions are observed in 2 due to the similarity in structure. Additionally, density functional theory (DFT) calculations are being used to simulate possible photoproducts.

Thank you to the Summer Undergraduate Research Fellowship at the National Institute of Standards and Technology for funding this work and for the use of their TRIR facilities.

OBSERVATIONS OF COMPOUND PENETRATION IN *ESCHERICHIA COLI* USING ETHIDIUM BROMIDE AS A MODEL COMPOUND

Michelle Ramsahoye¹, Ankit Pandeya², Yuguang Cai², Yinan Wei²

¹Department of Physics, UMBC, 1000 Hilltop Circle, Baltimore, Maryland 21250

²Department of Chemistry, University of Kentucky, 161 Jacobs Science Building, Lexington, Kentucky 40506

According to the Centers for Disease Control and Prevention, approximately 2 million people are afflicted with antibiotic-resistant infections annually. One of the contributing factors to a bacterium's resistance to antimicrobials is the presence of multidrug efflux pumps.

By removing various toxins from the host cell (i.e. dyes to antibiotics), these pumps ensure the survival of the pathogen. Little is known regarding the kinetics of these pumps. Found in virtually all types of bacteria, there are multiple families of efflux pumps. This research focuses on AcrAB-TolC, an active efflux pump that is commonly found in Gram-negative bacterium such as *Escherichia coli* (*E. coli*).

The overall objective of this research is the development of a mathematical model to accurately represent compound penetration into *E. coli* and the contribution of efflux in the process.

Using multiple concentrations of the fluorescent compound ethidium bromide (EtBr) and stopped-flow spectrometry, observations of changes in fluorescence intensity over time were made using a genetically altered strain of *E. coli* with a non-functioning AcrAB-TolC pump.

Graphical representations of data revealed four possible distinctive patterns or “stages” of influx of the EtBr compound into the live cells. Data also suggests the Langmuir model of adsorption as being a potential mathematical model for the first diffusion stage.

Development of a working model that allows for accurate determination of the kinetic behavior of the influx of EtBr into the cell allows for a basis of comparison in strains of *E. coli* with fully functioning efflux pumps. This would allow for the quantification of an efficiency value can then be used to help determine which compounds are most effectively removed by the AcrAB-TolC pump.

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FABRICATION OF GREEN QUANTUM DOT SENSITIZED SOLAR CELLS

Brandon Chang¹, Lena Woodis¹

¹Chemistry Department, Salisbury University, 1101 Camden Ave, Salisbury, MD 21801

In today's society, we rely on non-renewable fossil energy to produce electricity. Our team has been working on the development of techniques and methodologies for the design and fabrication of efficient co-sensitized Quantum Dot-Sensitized Solar Cells (QDSSCs) using simple bench-top techniques and the principles of Green Chemistry. We present the fabrication of quasi-solid state solar cells sensitized with copper indium sulfide and carbon quantum dots using a pure carbon counter electrode and a cellulose based polymer electrolyte. The primary aim of this research is to optimize the design and performance of these devices such that they become competitive with the thin film solid-state devices fabricated with expensive chemical vapor deposition and with devices containing harmful lead and cadmium chalcogenides. We have found that the co-sensitized solar cells perform better than the cells made from one type of quantum dot alone, and our continued work will focus on understanding the mechanisms of charge transfer within these co-sensitized devices.

Department of Chemistry, Salisbury University
Henson School of Science and Technology
Office of Graduate Studies and Research
Mr. Paul Clements

DETECTION OF HEAVY METALS BY COLORIMETRIC NANOPARTICLES

Michael Perzel¹, Rachel Molino¹, Lauren Toote¹

¹Department of Chemistry and Biochemistry, Elizabethtown College, 1 Alpha Drive
Elizabethtown PA 17022

Throughout the United States, there are 6.1 million lead water service lines. This poses a danger to many Americans since changes in water, such as pH, can cause lead (Pb²⁺) to leech into their water supply. Long term exposure to lead can result in organ damage and cancer. Lead poisoning is especially threatening to children, due to possible developmental impairments. Current methods to detect heavy metals require sending samples to a highly trained user, whereas the proposed method provides a simple and inexpensive way to test for such metals from a person's own home. This will be made possible by incorporating metal sensitive dye-modified polymer nanoparticles into a lateral flow assay.

In this work, a phenanthroline based probe for Pb²⁺ was synthesized from 1,10-Phenanthroline-5,6-dione and 4-Nitrobenzhydrazide with a yield of 37%. The probe's affinity for Pb²⁺ was evaluated in a variety of solvent systems and with several different lead salts. It was found that with the use of lead perchlorate a visible color change from yellow to orange was observed. This probe response was evaluated by both UV-Vis and fluorescence spectroscopy. Poly(styrene) based nanoparticles were then synthesized to encapsulate the probe for later adherence to a lateral flow assay.

We would like to acknowledge the Elizabethtown Department of Chemistry and Biochemistry and the Elizabethtown Summer Scholarship, Creative Arts and Research Projects Program (SCARP) for support of this project.

Development of a Dynamic Light Scattering System for the Study of Nanoparticles

Taolin Li¹, Qi Lu¹

¹Division of Physics, Engineering, Mathematics and Computer Science Delaware State University, 1200 N Dupont Highway, Dover, DE 19901

Dynamic light scattering (DLS) is an analytical technique for the characterization of size and surface charge of nanoparticles in liquid environment. The fluctuation of light scattered from nanoparticles undergoing Brownian motion is measured and correlated to the fluctuation at a delayed time τ . The correlation decays as τ increases. The size of the nanoparticles can be determined from how fast the correlation decays with τ . Herein, we developed a prototype of DLS system that measures photons scattered 90 degrees from the sample. Using this system, we characterized colloids of monodisperse gold nanoparticles for future study of nanoparticle-integrated drug and delivery systems.

This project was funded by the Delaware INBRE program, with a grant from the National Institute of General Medical Sciences – NIGMS (8 P20 GM103446-16) from the National Institutes of Health and the State of Delaware.

DETECTION OF CHEMICALS USING CARBON NANOTUBE YARNS VIA CYCLIC VOLTAMMETRY

Jessica Bonilla¹, Jandro Abot²

¹Trinity Washington University, 125 Michigan Ave NE, Washington, DC 20017

²Catholic University of America, 620 Michigan Ave NE, Washington, DC 20064

Carbon nanotubes can be spun and twisted together to form a carbon nanotube (CNT) yarn with piezoresistive characteristics that could be tapped to measure strain and detect damage. Under the exposure of chemicals, the electrical current passing through the CNT yarn could be measured through cyclic voltammetry. In this project, we used two approaches to test if the CNT yarn was able to sense the chemicals at different concentrations. One of the approaches was to directly expose the chemical while the second approach was to not directly expose it and instead let it reach the CNT yarn through diffusion. The CNT yarn was able to sense the chemical inside the solution because as the concentration increased, the current peak also increased. The second approach is still being tested to see if the chemical would be absorbed by the non-exposed CNT yarn. The CNT yarn has good sensitivity to detect tiny concentrations of chemicals inside the solution.

COORDINATION OF CU(I) TO 3,6-DIAMINE PHENANTHRENE LIGANDS

Frank MacGregor¹, Walter L. Dorfner², Kimberly Mullane¹

¹Department of Chemistry, Chestnut Hill College, 9601 Germantown Ave, Philadelphia, PA 19118

²Department of Chemistry and Biochemistry, University of the Sciences, 600 S 43rd St, Philadelphia, PA 19104

Methane gas is an integral component of Earth's atmosphere, as it traps heat and allows our planet to retain a habitable temperature. However, the amount of atmospheric methane has increased by 70% due to human activity, such as cattle farming and fossil fuel production, contributing to an unsustainable heating of our planet. In nature, the metalloenzyme, soluble methane monooxygenase (sMMO), is used by bacteria to oxidize methane gas into liquid methanol. Drawing inspiration from the high-valent diiron active site of sMMO, we aimed to synthesize a homobimetallic complex supported by 3,6-diaminophenanthrene ligands, which we hypothesized would create a clamp-like pocket to capture methane due to C-H--- π interactions between methane gas and the ligand, respectively. We propose that these interactions will donate electron density into methane's σ^* molecular orbitals, weakening its C-H bonds, and allowing oxidation to occur. We completed two five-step syntheses of novel 3,6-diaminophenanthrene pro-ligands with p-toluidine and N,N-dimethylethylenediamine substituents. Amine substituents were installed in the final step by a palladium-catalyzed Buchwald-Hartwig amination reaction between the primary amines and 3,6-dibromophenanthrene. Pro-ligands and precursor compounds were characterized by ¹H NMR and IR spectroscopy, and GCMS. We synthesized dicopper complexes of these ligands in preliminary reactions, and aim to target iron metalation reactions in the future.

Sincere thanks to Chestnut Hill College Chemistry Department, University of the Sciences, and the Schelter Research Group at the University of Pennsylvania for allowing us to use their instruments and facilities.

DETERMINING HOW THE 2,2'-DIPICOLYLDITHIOCARBAMATE ANION BINDS ZINC(II), CADMIUM(II) OR MERCURY(II) IONS

Kayla Leonard¹, Peter Craig¹

¹Department of Chemistry, McDaniel College, 2 College Hill, Westminster, MD 21157

We develop and evaluate potential heavy metal recyclable chelators designed to remediate wastewater. The 2,2'-dipicolyl dithiocarbamate anion, [2,2'-dpd⁻tc]- is a multidentate ligand. The likely coordinate bond forming donor atoms within this ligand are the nitrogen atoms of the picolyl substituents and the sulfur atoms of the dithiocarbamate functional group. We successfully synthesized and characterized this ligand, and undertook reactions of this ligand with group 12 iodides to begin the evaluation process: what are the stoichiometries of the complexes formed in the solid state?

We found that [2,2'-dpd⁻tc]- readily formed precipitates on reaction with all group 12 iodides. Evaluation of elemental analysis data indicated that the Zinc(II) and Cadmium(II) complexes of [2,2'-dpd⁻tc]- have a 3:2 metal ion:ligand ratio. For the Mercury(II) complex, this ratio was higher (5:2). ¹H NMR and IR data comparisons between the complexes and the ligand indicated that both the picolyl nitrogens and the sulfur atoms of the dithiocarbamate functional group were all actively involved in binding to the metal ions. We hypothesized that the observed differences in the stoichiometric ratios was a function of the size of the group 12 cation in the respective complex. A single Zn²⁺ or Cd²⁺ ion is small enough to bind to both picolyl nitrogens of a single [2,2'-dpd⁻tc]- ligand whereas Hg²⁺ is not. For Hg²⁺, this means each of the two picolyl nitrogens of a single [2,2'-dpd⁻tc]- ligand can bind a different Hg²⁺ ion, thus increasing the metal ion:ligand ratio beyond 3:2.

Work continues to validate this hypothesis and further evaluate Mercury(II) iodide binding.

I would like to thank the Jean and Donald Richards Student Research Fund for supporting this research.

ZINC(II), CADMIUM(II) OR MERCURY(II) IONS RIDING CHROMIUM(III) AND COBALT(III) FERRIS WHEELS: WHO ARE THE PREFERRED PASSENGERS?

Johnny Schaeffer¹, Brandon Busick¹, Peter Craig¹

¹Department of Chemistry, McDaniel College, 2 College Hill, Westminster, MD, 21157

The 2,2'-dipicolylthiocarbamate anion, [2,2'-dpdtc]⁻ is a multidentate ligand. The likely coordinate bond forming donor atoms within this ligand are the nitrogen atoms of the picolyl substituents and the sulfur atoms of the dithiocarbamate functional group. We have assembled and characterized Chromium(III) and Cobalt(III) tris-chelate complexes of this ligand, denoted as the blue Cr³⁺ and green Co³⁺ Ferris wheels for the purposes of this work.

We proposed two research questions: who amongst the group 12 cations are the preferred passengers of these Ferris wheels, and how many passengers ride each Ferris wheel? We used group 12 iodides in this study owing to their facile and uniform solubility in methanol. We found that Zinc(II) and Cadmium(II) iodide readily bind to both Ferris wheels. Mercury(II) iodide binding was inconclusive. We elucidated that the green Co³⁺ Ferris wheel binds three molar equivalents of ZnI₂ or CdI₂, whereas the blue Cr³⁺ Ferris wheel binds three molar equivalents of ZnI₂, yet four of CdI₂. We hypothesized that the difference in the number of passengers that ride each Ferris wheel was due to bonding preferences. All of the Zn²⁺ and Cd²⁺ ions are tetrahedrally coordinated. Most are bound to the two picolyl nitrogen atoms of a single [2,2'-dpdtc]⁻ ligand and two iodido ligands. For the CdI₂ complex of the blue Cr³⁺ Ferris wheel, two Cd²⁺ ions are bound in this way. The other two Cd²⁺ ions are each bound to one picolyl nitrogen atom, one of the Cr³⁺-coordinated sulfurs, and two iodido ligands.

Work continues to validate this hypothesis and further evaluate Mercury(II) iodide binding.

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

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

Ryan Johnson¹, John Gilje¹, Donna Amenta¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St, Harrisonburg, VA 22807

Our group has studied the coordination chemistry of N-pyrazolylpropanamide derivatives. In order to obtain similar ligands with superior coordination properties we are now seeking N-pyrazolylpropanoate derivatives. The reaction between pyrazole and methyl acrylate forms L1 by a Michael addition. The hydrolysis of L1 produces the carboxylic acid L2, which can easily be converted to the carboxylate. The ligand properties of L1 have been investigated with palladium(II). With Cl₂Pd(COD) a complex Cl₂Pd(L1)₂ can be isolated in good yield. An X-ray crystal structure indicates a trans conformation with the L1 complexing through a pyrazole nitrogen. Similarly, the reaction of 3,5-dimethylpyrazole with methyl acrylate forms L3. With Cl₂Pd(COD) a mixture of isomers appears to form.

Prof. Frank Edelmann and Dr. Felix Engelhardt, University of Magdeburg, Germany.
Department of Chemistry and Biochemistry at JMU

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OPTICAL PROPERTIES OF VARIOUS TWO-DIMENSIONAL LAYERED LEAD HALIDE PEROVSKITES

Dominick Guida¹, Klaus Theopold¹

¹Department of Chemistry and Biochemistry, University of Delaware, 102 Brown Laboratory, Newark, DE 19716

As atmospheric carbon dioxide levels continue to grow at an alarming rate, the development of renewable alternatives to fossil fuels is paramount. One of the most promising fields in renewable energy is photovoltaics, aided by the rapidly increasing efficiencies of solar cells by incorporating metal halide perovskites of the type AMX_3 . This project examined various hybrid organic-inorganic lead iodide perovskites, using a para-substituted benzylamine as an organic spacer. This spacer prevents the molecule from forming three-dimensional crystalline structures, allowing the synthesis of two-dimensional layered perovskites. The para-substitution of the spacer can also be modified to potentially adjust the properties of the material. An array of samples was prepared via solution-based synthesis, where the concentrations of the species in solution were adjusted to achieve varied thicknesses of the layered perovskite. Two different organic spacers were used during the preparation of the samples to help identify its impact on the material's properties. The samples were analyzed using X-Ray Diffraction (XRD) and UV-Vis Spectroscopy to determine the optical properties and unit cell parameters of each material. The data showed a clear effect on the material's properties due to layer thickness, while preliminary results suggest that the electronic character of the organic spacer has, at best, a very modest effect. The findings of this project support the claim that the thickness of layered lead halide perovskites can be used to fine tune its optical properties and invites further investigation of the effects due to the electronic character of the organic spacer.

We thank David Plastino and the University of Delaware's Undergraduate Research Program for funding this project. We would also like to thank Dr. Karl Unruh and Dr. Glenn Yap for data acquisition and analysis.

BAND GAP ENGINEERING IN ALLOYED METAL-OXIDE PEROVSKITES FOR THIN-FILM PHOTOVOLTAICS

Zakiyyah Jones¹, Kaitlin Hellier², Sue Carter²

¹Trinity Washington University, Washington DC, 20017 United States

²Department of Physics, University of California, Santa Cruz, California 95064, United States

BiFeO₃ (BFO) is a multiferroic material capable of a substantial photovoltage and of a greater stability than leading perovskites today. As a reliable metal-oxide-semiconductor, BFO has a vast array of applications, including the potential to act as an affordable active layer for solar cells. However, its application as a photovoltaic (PV) is limited due to a wide bandgap of 1.9-2.7 eV. This project examines the effects of alloying cobalt into BFO to tune the bandgap, to measure the device performance, and to discover the properties of this compound in thin-film PVs to improve application. The BiFe(x-1)Co_xO₃ (BFCO) active layer was first synthesized via the spin coating method, then studied as a neat film and analyzed in a PV device architecture. BFCO thin-films and devices were characterized by UV-visible spectroscopy, x-ray diffraction, and current-voltage measurements to understand the impact of incorporating cobalt into the system, with the end goal of creating a stable alternative to traditional PVs. The results concluded that incorporating different concentrations of cobalt into BFO developed unique phases of the material. The research reflected a decreased band gap of the BFCO compound and showed a photovoltaic current when utilized as an active layer for a PV device. The decreased bandgap supports expanding the application of BFO to solar technology.

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SYNTHESIS AND COORDINATION OF N,N-DIPHENYLPHENANTHRENE-3,6-DIAMINE TO METAL CATIONS

Daisy Owens¹, Walter L. Dorfner², Kimberly Mullane¹

¹Department of Chemistry, Chestnut Hill College, 9601 Germantown Ave., Philadelphia, PA 19118

²Department of Chemistry and Biochemistry, University of the Sciences, 600 S 43rd St, Philadelphia, PA 19104

Methane is a harmful greenhouse gas, emitted during the production of natural gas and biogases, but when converted to methanol finds application in antifreeze, gasoline and diesel fuel production, trans-esterification reactions and waste-water treatment. However, current commercial conversion of methane to methanol requires heat and high pressures. In nature, soluble methane monooxygenase (sMMO), an enzyme with a carboxylate-bridge diiron active site, facilitates the conversion of methane to methanol under mild temperature and pressure conditions. Drawing inspiration from the active site of sMMO, we aimed to synthesize a diiron complex of N,N-diphenylphenanthrene-3,6-diamine through a five-step synthesis, which included Buchwald-Hartwig Cross-Coupling Amination, Horner-Wadsworth-Emmons, and photocyclization reactions as key synthetic steps. N,N-diphenylphenanthrene-3,6-diamine was synthesized in 91% yield and characterized by ¹H and ¹³C NMR, and IR spectroscopy, and precursor compounds were characterized by ¹H NMR spectroscopy and GCMS. The pro-ligand was then deprotonated with n-butyllithium and reacted with copper(I) iodide. Preliminary results indicated that a dicopper complex was synthesized. Metalation reactions of our novel ligand with iron will be targeted in future work.

I would like to acknowledge Chestnut Hill College and University of the Sciences for use of their facilities and instrumentation.

DESIGNING 'INTELLIGENT' MRI CONTRAST AGENTS

Tasneem Elbashir¹, Anthony Rojas¹

¹Department of Chemistry, Salisbury University, 1101 Camden Ave, Salisbury, MD 21801

Historically, transition metals have been used in magnetic resonance imaging (MRI) to diagnose diseases and monitor treatments. These metals act as contrast agents and have been introduced to enhance the quality of MRI images and elucidate hidden anatomical features. Unfortunately, most of the contrast agents are based on the lanthanide metal Gadolinium, which has been implicated as damaging to the liver and neurological systems of patients. Gadolinium's strength is in its ability to act as a strong paramagnetic species and thus, we propose developing MRI contrast agents based on Iron (Fe), which can also exist as a paramagnetic metal but, due to its prevalence in the human body, we predict will be far less toxic to patients.

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Salisbury University
Richard A. Henson School of Science
Department of Chemistry

SYNTHESIS OF N,N-(PHENANTHRENE-3,6-DIYL)BIS(N,N-DIMETHYLETHANE-1,2-DIAMINE) FOR COORDINATION TO DIIRON COMPLEXES

Cailey Stevenson¹, Walter L. Dorfner², Kimberly Mullane¹

¹Department of Chemistry, Chestnut Hill College, 9601 Germantown Ave, Philadelphia, Pennsylvania 19118

²Department for Chemistry and Biochemistry, University of the Sciences, 600 South 43rd Street, Philadelphia, Pennsylvania 19104

Methane is a notorious greenhouse gas that is released into the environment from agricultural and industrial processes; this contributes to global warming. The conventional technique for the conversion of methane gas to liquid methanol is an expensive, high energy two-step process that requires centralized production. Development of a low energy route for this oxidation is desirable because methanol easier to store due to its liquid form and has industrial applications such as fuel, antifreeze, waste-water treatment, and fuel cells. The biological system that converts methane to methanol are soluble methane monooxygenase enzymes (sMMO), which contain a diiron active site. We aimed to synthesize a diiron complex of N,N-(phenanthrene-3,6-diyl)bis(N,N-dimethylethane-1,2-diamine) in an attempt to replicate sMMO's ability to oxidize methane to methanol. The overall synthesis of our pro-ligand was completed in 5 steps. In the final step of the synthesis, 3,6-dibromophenanthrene was reacted with N,N-dimethylethylenediamine through a Buchwald Hartwig amination reaction. Future work for this system will target coordinating the pro-ligand to a diiron complex to test its catalytic activity towards methane oxidation.

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TRACE METAL ANALYSIS OF RICE

Manuel Gamboa¹, Brandy Carter¹, Richard Foust¹

¹Department of Chemistry and Biochemistry, James Madison University, 800 S Main St.,
Harrisonburg, VA 22807

More than half of the world's population depends on rice to account for more than 20% of their daily calories. Unfortunately, clean water is not readily available in arid regions. The lack of clean water often results in the use of water contaminated with trace metals (Fe, Cu, Mn, Zn, Cd, Pb, and As). These metals enter the environment through agricultural, industrial, and domestic waste and negative effects on human health if consumed in excess. The goal of this research project was to determine the amount of trace metal contamination in commercially available rice from nine countries. Rice samples were pulverized, then digested for analysis with a microwave assisted digestion system. Metal concentrations were measured in the digested samples by Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Values for Zn, Cu, Mn, and Fe were successfully determined in 21 rice samples from nine countries.

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DETECTING CELLULAR CONDITIONS USING FLUORESCENT DYES

Skylar Lewis¹, Matthew Krall¹, Joshua Sokoloski¹

¹Department of Chemistry, Salisbury University, 1101 Camden Ave. Salisbury, Maryland 21801

The goal of this project was to use Cyanine 3 (Cy3), a fluorescent dye, to accurately and sensitively measure the changing of the viscosity and temperature in the local molecular environment. Cy3's cis-trans isomerization dependence on local environmental conditions is an effective method for accurately measuring these conditions. We tested the viscosity sensitivity of Cy3 in varied glycerol concentrations as well as several different temperature conditions. We found that the dye has greater fluorescence within a viscous environment. Cy3 exhibits greater fluorescence intensity at lower temperatures. Our results indicate that Cy3's sensitivity to viscosity and temperature is greater than that of other similar dyes. Interestingly, we found that in a viscous environment the dye is more sensitive to temperature changes. Cy3 is therefore a suitable candidate for measuring enthalpy changes for enzymatic reactions. Our results indicate that Cy3 fluorescent intensity can be used to detect the heat released from the reaction catalyzed by glucose oxidase. The condition-dependent fluorescence of Cy3 could prove to be a cost-effective assay in a variety of fields that make use of viscosity and temperature changes. We look to develop a lab screening technique for detecting biofilms as well as quickly measuring the viscosity of blood plasmas or even cellular cytosols.

DETERMINATION OF TRACE METAL CONCENTRATIONS IN SELECT TYPES OF CANNED BEERS VIA FLAME ATOMIC ABSORPTION SPECTROMETRY (FAAS)

Jeremy Hafner¹, Mark Stauffer¹

¹Division of Natural Sciences, Mathematics, and Engineering - Chemistry, University of Pittsburgh - Greensburg, 150 Finoli Dr., Greensburg, PA 15601

This paper will present and discuss preliminary results from an investigation of the heavy metal concentrations determined by flame atomic absorption spectrometry (FAAS) in four varieties of canned beer: lager, light, stout, and India Pale Ale. The focus of this research is on the results obtained for iron, copper, manganese, zinc, and aluminum from the beers analyzed using a nitric acid/hydrogen peroxide microwave digestion. An objective of this study is to achieve a higher analyte sensitivity with the analytical method through developing a more efficient irradiation digestion procedure. Beer is a highly popular and widely consumed beverage in many locations on Earth, particularly in the United States and in many European and Asian countries. Excluding aluminum, each of the metal analytes possesses known nutritional value for humans. There is also a concern with possible leaching of metals, particularly Al, Cu, and Fe, into the beers during the brewing process as well as from storage in cans. Results obtained for Fe, Cu, Mn, Zn, and Al in the beer varieties analyzed will be presented and discussed, along with experimental details for the results, and possible future projects.

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A STUDY OF METAL CONTENTS OF BOTTLED AND CANNED SODA: DOES “MORE EXPENSIVE” IMPLY LOWER METAL CONCENTRATIONS IN SODA?

Brandon Luker¹, Mark Stauffer¹

¹Department of Natural Sciences, Mathematics, & Engineering, University of Pittsburg at Greensburg, 150 Finoli Drive, Greensburg, PA 15601

In this presentation, preliminary results from an investigation of concentrations of selected heavy metals in commercially available brands of soda, determined by flame atomic absorption spectrometry (FAAS) and ultraviolet/visible spectrophotometry (UV/VIS), will be presented and discussed. The metals of interest in this study include iron, lead, nickel, aluminum, and copper. The main objective of the work presented is to examine possible correlations (if any) among cost, brand, and metal concentrations of the sodas. Another objective of the research is to obtain possible evidence of leaching of metals such as aluminum from the cans in which the soda brands are packed by indicating possible statistical differences between the categories of listed above. Also of concern are the nutritional benefits (or lack thereof) of the metal analytes present in the sodas. Aluminum and lead are considered toxic to humans, while iron, nickel, and copper possess known nutritional value to humans.

Results obtained for Fe, Cu, Ni, Pb, and Al in the brands of soda analyzed will be presented and discussed, along with experimental details for sample preparation and quantitation of metals by FAAS and VIS, statistical assessment of the results, and possible avenues for future study.

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ARDUINO-BASED ALTERNATIVE TO GC-TCD

Bradley Owen¹, Nicholas Starvaggi¹, Isaac Mills¹

¹School of Natural Science and Mathematics, Mount St. Mary's University, 16300 Old Emmitsburg Road, Emmitsburg, MD 21727

Thermal conductivity is generally the tool of choice for the quantification of fixed gases, particularly lighter-than-air gasses. Unfortunately, gas chromatography-thermal conductivity detectors (GC-TCDs) are difficult to introduce in undergraduate settings because the necessary space and overwhelming cost of these instruments is often prohibitive. The Mills Lab at Mount St. Mary's University has overcome this barrier through the development of a small, inexpensive Arduino-based thermal conductivity detector (TCD) to quantify lighter-than-air gases for undergraduate teaching and research labs. The device is calibrated through a series of gaseous hydrogen injections and the second-order exponential fit from this calibration can be used to quantify volumes of unknown gases. The total cost of materials is maintained below \$200, including the microcontroller, electronics, TCD pellistor, and the mechanical parts which serve as the housing and analysis chamber. This presentation provides a detailed overview of the development, functionality, operation, initial performance, and overall potential of this instrument.

CONSTRUCTING COBALT(III) ACETYLACETONE OR ORTHOVANILLIN SCHIFF BASE ZINC FINGER INHIBITORS

Vanessa Velez¹, Benjamin England¹, Isaac Mills², Peter Craig¹

¹Department of Chemistry, McDaniel College, 2 College Hill, Westminster MD 21157

²Department of Science, Mount St. Mary's University, 16300 Old Emmitsburg Road,
Emmitsburg, MD 21727

We synthesize cobalt(III)/nickel(II) coordination complexes designed to inhibit zinc finger proteins. The coordination complexes contain a multidentate Schiff base ligand based on acetylacetone or orthovanillin, and a monodentate labile ligand. Exchange of the labile ligand for one of the histidine residues bound to the zinc(II) ion in the zinc finger protein is the proposed point of inhibition. This leads to eventual zinc(II) ion expulsion from the protein. This work is inspired by Doxovir, a related coordination complex undergoing clinical trials as an antiviral metallodrug (Mjos, K. D.; Orvig, C. Metallodrugs in Medicinal Inorganic Chemistry, Chem. Rev. 2014, 114, 8, 4540-4563).

In this work we attempted to prepare coordination complexes from targeted combinations of the following components: the metal ion cobalt(III), the Schiff base made from the 1:1 condensation of tris(2-aminoethyl)amine and acetylacetone or orthovanillin denoted as H[trenacac] or H[trenvan] respectively, and the labile ligands aquo, bromido, chlorido, and 2- or 4-methylimidazole (denoted 2- or 4-MeIm). Characterization techniques employed include elemental analysis, NMR and UV-VIS spectroscopy, and x-ray crystallography.

We were successful in preparing the aquo complexes [Co(trenacac)(OH₂)](ClO₄)₂ and [Co(trenvan)(OH₂)](ClO₄)₂. We were unable to make [CoBr(trenvan)]ClO₄ or [CoCl(trenvan)]ClO₄, isolating instead [Co(trenvan₂)]ClO₄ and [Co(trenvan)(OH₂)](ClO₄)₂ in both cases ([trenvan₂]²⁻ is the Schiff base made from the 1:2 condensation of tris(2-aminoethyl)amine and orthovanillin).

We also prepared [CoCl(trenacac)]ClO₄, [Co(trenvan)(2-MeIm)](ClO₄)₂ and [Co(trenvan)(4-MeIm)](ClO₄)₂. For these three complexes we discovered that they were best isolated after ion exchange chromatography. The identity of the Schiff base and labile ligand is significant to being able to isolate (and purify) these type of coordination complexes.

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INVESTIGATING SOLUTION PROPERTIES OF MANGANESE 12-METALLACROWN-4

Jacob Palmer¹, Thaddeus Boron, III¹

¹Department of Chemistry, Slippery Rock University, 100 Central Loop, Slippery Rock, Pennsylvania 16057

Single-molecule magnets (SMMs) are discrete molecules that have an intrinsic energy barrier to magnetic spin reversal. SMMs show promise as data storage devices. However, work needs to be done before SMMs can be used commercially for data storage. SMMs must be able to maintain a magnetic configuration at ambient temperature for long periods of time to be commercially relevant. It is also crucial that the SMMs are able to be located easily so that the data can be stored or recalled. One possible solution to locating SMMs is to deposit them on surfaces using chemical deposition techniques. By adsorbing SMMs onto a surface, they are assigned a single location.

Metallacrowns (MCs) are inorganic macrocycles analogous to organic crown ethers. The synthesis of MCs can easily be repeated and various properties of MCs can be manipulated, such as magnetic properties, making MCs ideal candidates for studies of SMMs. This project examines the solution properties of $\text{Mn}(\text{OAc})_2[12\text{-MCMn(III)N(shi)-4}]$, a planar, 12-membered MC with known SMM properties. However, this MC has never been deposited on a surface. As a first step towards achieving MC SMMs on surfaces, we chose to study the solution integrity properties of this MC in various polar solvents. Using ESI LC-MS and UV-visible spectroscopy, the solution properties were examined and compared against one another to find a suitable solvent for deposition. Once a solvent is identified that maintains the structural integrity of the MC, deposition of the MC can be studied.

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ETHOXIDE SUBSTITUTION REACTIONS OF $\text{Os}_3(\text{CO})_{10}(\text{OEt})_2$ WITH DIOLS AND AMIDES

Abigail Mullen¹, Mary-Ann Pearsall¹

¹Chemistry Department, Drew University, 36 Madison Ave, Madison, NJ 07940

In our investigations of the alkoxide substitution reactions of $\text{Os}_3(\text{CO})_{10}(\text{OEt})_2$ with 1,3 propanediol and 4-nitrobenzamide, we have found two different pathways depending on the substituting ligand

1,3 propanediol reacts to form a compound believed to be in which the Os_3 triangle is retained with one end of the diol forming an alkoxide bridge such that the alcohol coordinates to form a ring, or “loop” structure. Addition of either CO or PPh_3 displaces the “coordinating” OH to form a pendant product $\text{Os}_3(\text{CO})_8(\mu\text{-O}(\text{CH}_2)_3\text{OH})_2(\text{L})_2$, $\text{L} = \text{CO}$ or PPh_3 .

In contrast with 4-nitrobenzamide, the triosmium cluster fragments and forms a diosmium diamide carbonyl complex, $\text{Os}_2(\text{CO})_6(\text{OCNHC}_6\text{H}_4\text{NO}_2)_2$, which has two isomers with head-to-tail and head-to-head configurations. Characterization of products is based on carbonyl infrared spectroscopy and ^1H -NMR spectroscopy.

