

# 20<sup>th</sup> Annual Undergraduate Research Symposium in the Chemical and Biological Sciences



**Saturday, October 14, 2017**

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**UMBC**  
AN HONORS  
UNIVERSITY  
IN MARYLAND



# **20<sup>th</sup> Annual Undergraduate Research Symposium in the Chemical and Biological Sciences**

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



# Schedule of Events

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| Time  | Event  |   |   |
|---|--|---|---|
| 8:00 am   | <b>SYMPOSIUM CHECK-IN &amp; ON-SITE REGISTRATION</b><br><i>Lobby, University Center, 3rd Floor</i>   |   |   |
| 8:00 am   | <b>LIGHT CONTINENTAL BREAKFAST</b><br><i>UC 312, University Center, 3rd Floor</i>  |   |   |
| 9:00 am   | <b>OPENING REMARKS &amp; WELCOME ADDRESS</b><br>Dr. Freeman Hrabowski, President, University of Maryland, Baltimore County (UMBC)<br>Dr. William R. LaCourse, Dean, College of Natural & Mathematical Sciences, UMBC<br><i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>   |   |   |
| 9:45 am –<br>11:45 am   | <b>MORNING POSTER SESSION</b><br><b>Posters # 1- 137</b><br><i>Ballroom, University Center, 3rd Floor</i>  |   |   |
|   | <b>WORKSHOPS</b>   |   |   |
|   | <table><tr><td><b>Master the Art of Making Connections</b><br/>Ms. Susan Hindle, Career Services, UMBC<br/><i>UC 310, University Center, 3rd Floor</i></td><td><b>A Very, Very Short Introduction to Ethics for Scientists</b><br/>Mr. James Thomas, Department of Philosophy, UMBC<br/><i>CASTLE, UC 115D, University Center, 1st Floor</i></td></tr></table>                             | <b>Master the Art of Making Connections</b><br>Ms. Susan Hindle, Career Services, UMBC<br><i>UC 310, University Center, 3rd Floor</i> | <b>A Very, Very Short Introduction to Ethics for Scientists</b><br>Mr. James Thomas, Department of Philosophy, UMBC<br><i>CASTLE, UC 115D, University Center, 1st Floor</i> |
| <b>Master the Art of Making Connections</b><br>Ms. Susan Hindle, Career Services, UMBC<br><i>UC 310, University Center, 3rd Floor</i> | <b>A Very, Very Short Introduction to Ethics for Scientists</b><br>Mr. James Thomas, Department of Philosophy, UMBC<br><i>CASTLE, UC 115D, University Center, 1st Floor</i>  |   |   |
| 11:45 pm  | <b>BUFFET LUNCH</b><br><i>(gratis for registered guests with symposium name badge)</i><br><i>The Commons – Main Street</i>   |   |   |
| 12:45 pm –<br>2:45 pm   | <b>AFTERNOON POSTER SESSION</b><br><b>Posters # 138 – 261</b><br><i>Ballroom, University Center, 3rd Floor</i>   |   |   |
| 1:30 pm   | <b>WORKSHOPS</b><br><i>Repeat of workshop titles and locations above</i>   |   |   |
| 3:00 pm   | <b>PLENARY TALK</b><br><b>“Developing Specific Electrochemical Sensing and Imaging Platforms Inspired by Biology”</b><br>Dr. Ryan White, Associate Professor and Ohio Eminent Scholar at the University of Cincinnati with joint appointments in the Department of Electrical Engineering and Computing Systems.<br><i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i> |   |   |
| 4:00 pm   | <b>AWARDS PRESENTATION</b><br><i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i>   |   |   |

# Workshops:

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

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

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



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

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

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

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



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

# Plenary Talk

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## Developing Specific Electrochemical Sensing and Imaging Platforms Inspired by Biology

*Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030*

Biosensors promise to impact many fields ranging from basic research of biological systems to the development of biomedical devices poised to revolutionize modern healthcare. The number of methods for developing biosensors continues to grow at an impressive rate representing the cutting-edge interface of chemistry and many other fields. This talk takes a step back to understand the fundamentals of sensor performance of a class of bio-inspired electrochemical sensors using functional nucleic acids or membrane proteins. We utilize a combination of electrochemistry, biochemistry, and biomolecular design and engineering to build better biosensors and imaging platforms. By developing models for the electrochemical response and understanding the structure and function of nucleic acids (*e.g.*, aptamers) and proteins (*e.g.*, ion channels) we can tune the response of a sensor based on the bioanalytical application of the sensor. Coupling these sensors with micro- and nanoscale electrodes further enables us to tune sensor performance for applications ranging from molecular flux imaging, single-cell analysis to implantable devices for real-time therapeutic monitoring.



**Dr. Ryan White** is an associate professor and Ohio Eminent Scholar at the University of Cincinnati with joint appointments in the Department of Chemistry and the Department of Electrical Engineering and Computing Systems. He and his research team have developed a research program that lies at the intersection of nanoscience, electrochemistry and the biological interface. Research interests in the group focus on the development of new (bio) analytical methods to probe chemical and biological systems with unprecedented spatial and temporal resolutions afforded by working at the nanoscale.

Ryan began his path as an undergraduate research assistant at the University of North Carolina working with Prof. Royce Murray on the synthesis and characterization of monolayer protected gold clusters. After receiving his BA in Chemistry (2003), Ryan earned his PhD in chemistry at the University of Utah in 2007 followed by an NIH NRSA Postdoctoral Fellowship at the University of California Santa Barbara. Ryan joined the faculty in the Department of Chemistry and Biochemistry at UMBC in 2011 as an assistant professor and was promoted to associate professor in 2016. Ryan recently accepted a position as an Ohio Eminent Scholar in Nano Bio Devices at the University of Cincinnati where he and his team continue to grow an interdisciplinary research program developing electrochemical imaging and sensing platforms.

# Morning Session Judging Groups

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**9:45 a.m. – 11:45 a.m.**

**ALPHABETIZED GROUPS**  **POSTER NUMBERS IN THE CORRESPONDING GROUP** 

## Chemical Sciences Groups A- G

| A   | B   | C             | D  | E   | F   | G   |
|-----|-----|---------------|----|-----|-----|-----|
| 82  | 33  | <del>13</del> | 28 | 9   | 11  | 2   |
| 86  | 49  | 34            | 47 | 14  | 29  | 20  |
| 108 | 62  | 45            | 52 | 64  | 65  | 53  |
| 124 | 77  | 79            | 56 | 102 | 94  | 81  |
| 129 | 121 | 90            | 66 |     | 135 | 104 |
| 134 |     | 96            |    |     | 137 |     |

## Biological Sciences Groups H - O

| H   | I   | J             | K   | L             | M   | N   | O              |
|-----|-----|---------------|-----|---------------|-----|-----|----------------|
| 39  | 6   | 26            | 5   | 46            | 15  | 19* | 59             |
| 48  | 25* | 27            | 10  | <del>50</del> | 17  | 38  | 60             |
| 71  | 30  | <del>74</del> | 70  | 63            | 40  | 58  | 68             |
| 72  | 75  | <del>88</del> | 85  | 92            | 95  | 67  | 83             |
| 73* | 84  | 103           | 112 | 114*          | 100 | 110 | 115            |
| 111 | 126 | 107           |     | 130           | 113 |     | <del>133</del> |

## Biochemical & Molecular Biology Groups P – Z

| P   | Q  | R    | S    | T   | U   | V  | W   | X   | Y  | Z  |
|-----|----|------|------|-----|-----|----|-----|-----|----|----|
| 32  | 3  | 57   | 7    | 37  | 4   | 23 | 18  | 1   | 41 | 36 |
| 98  | 8  | 97   | 89   | 99  | 127 | 42 | 21  | 31  | 43 | 44 |
| 128 | 24 | 101  | 118* | 109 | 132 | 54 | 35* | 61  | 80 | 55 |
| 131 | 76 | 105* | 125  | 120 | 136 | 91 |     | 116 | 87 |    |
|     |    | 119  |      |     |     |    |     | 122 |    |    |

**\*moved to afternoon on day of event due to delayed arrival- transportation issues**



# Morning Session (non-judged)

## STEM BUILD at UMBC Posters

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### **Cohort 3**

12  
22  
51  
69  
78  
93  
106  
117  
123

*Thank you for volunteering  
as a judge today.*

Please remember to wear your name badge  
throughout the entire event.

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# Afternoon Session Judging Groups

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**12:45 p.m. – 2:45 p.m.**

**ALPHABETIZED GROUPS**   
**POSTER NUMBERS IN THE CORRESPONDING GROUP** 

## **Chemical Sciences Groups AA – GG**

| AA  | BB  | CC             | DD  | EE  | FF  | GG  |
|-----|-----|----------------|-----|-----|-----|-----|
| 141 | 185 | 162            | 169 | 184 | 227 | 151 |
| 147 | 206 | 174            | 171 | 193 | 242 | 175 |
| 182 | 221 | 218            | 181 | 196 | 243 | 205 |
| 192 | 233 | <del>225</del> | 247 | 197 | 245 | 213 |
| 232 | 253 | 258            | 255 | 198 | 260 | 229 |
| 262 |     |                |     |     |     | 239 |

## **Biological Sciences Groups HH – OO**

| HH             | II  | JJ  | KK  | LL  | MM  | NN  | OO  |
|----------------|-----|-----|-----|-----|-----|-----|-----|
| <del>138</del> | 158 | 179 | 149 | 166 | 208 | 148 | 150 |
| 153            | 160 | 188 | 155 | 168 | 219 | 152 | 163 |
| 187            | 190 | 195 | 164 | 194 | 223 | 178 | 170 |
| 199            | 214 | 210 | 176 | 211 | 231 | 180 | 186 |
| 261            | 241 | 226 | 202 | 246 | 249 | 204 | 200 |
| 35             | 256 | 234 | 19  | 25  |     | 73  | 250 |

## **Biochemical & Molecular Biology Groups PP – YY**

| PP  | QQ  | RR  | SS  | TT  | UU  | VV  | WW  | XX  | YY  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 16  | 154 | 140 | 142 | 183 | 167 | 146 | 139 | 177 | 114 |
| 191 | 173 | 143 | 207 | 216 | 172 | 159 | 144 | 240 | 118 |
| 237 | 203 | 201 | 220 | 217 | 224 | 161 | 145 | 252 | 250 |
| 238 | 212 | 230 | 236 | 248 | 244 | 189 | 157 | 259 | 254 |
| 251 | 228 | 235 | 105 | 257 |     | 215 | 165 | 156 |     |



# DETERMINATION OF A SAFE DOSE OF MESENCHYMAL STEM CELLS FOR SYSTEMIC ADMINISTRATION

Claire Anne Abijay<sup>1,2</sup>

Daniel N. Darlington<sup>1</sup>, Andrew P. Cap<sup>1</sup>, and Xiaowu Wu<sup>1</sup>

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<sup>2</sup>Department of Chemistry, Georgetown University, 3700 O St. N.W., Washington, D.C. 20057

Mesenchymal stem cells (MSCs) have been widely studied for tissue regeneration, immunomodulation, and treatment of multiple organ failure. Systemic administration of MSCs allows MSC delivery to multiple traumatic sites that regional delivery cannot reach. However, MSCs' expression of tissue factor leads to safety concerns regarding its pro-coagulant capabilities, which can potentially cause inappropriate clot formation and counteract the beneficial effect of MSCs.

Using Sprague Dawley rat bone marrow derived and adipose derived MSCs (BMSC and AMSC), tissue factor expression was measured by immunohistochemistry and ELISA. MSCs were transfused through the rat's femoral vein at doses of 2.5, 5, 10, 20, and 40 million/kg. Citrated whole blood was collected before, immediately after infusion, 1 hour, and 3 hours after infusion. The lung, heart, liver, kidney, spleen, and skeletal muscle were taken immediately after euthanasia and stained for platelets (CD61) and nuclei (DAPI).

At the 20 million/kg dose, rats survived with BMSCs, but died with AMSCs (n=3 per group). All three rats survived infusion of BMSCs at 40 million/kg. Platelet counts were significantly reduced at 1hr after MSC infusion at  $\geq 10$  million/kg BMSC dosage and  $\geq 5$  million/kg AMSC dosage. At 5 and 10 million/kg cell dosages, AMSC transfusion led to lower platelet counts compared to BMSCs. Both AMSCs and BMSCs were found in the lung, along with platelets aggregates that varied proportionally with increasing dosages of MSCs. After infusion of 40 million/kg BMSCs and 20 million/kg AMSCs, platelet aggregates were found in the right ventricle of the heart.

This study suggests that BMSCs have less impact than AMSCs on hemostasis. BMSCs at the dose of 5 million/kg or less have the least risk of causing thrombocytopenia, platelet aggregation and infiltration in lung. The potential risk of systemic administration of MSCs, as characterized by increased thrombosis in the tissue, may be due to tissue factor expression of MSCs.

This research was supported in part by an appointment to the Student Research Participation Program at the US Army Institute of Surgical Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and USAMRMC. We wish to thank the great technical help of Bin Lin, Jeffrey D. Keese, Robbie K. Montgomery, Andres S. Penagosnino, and Josue Garciamarcano. This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

# **DETERMINATION OF IRON AND OTHER METALS IN TRANSPARENT MEMBRANES, USING VISIBLE SPECTROPHOTOMETRY AND CHEMOMETRICS: PRELIMINARY ATTEMPTS AND RESULTS**

Brandin M. Adams<sup>1</sup>, William E. Weller<sup>1</sup>, Nicholas A. Frankos<sup>1</sup>  
Mark T. Stauffer<sup>1</sup>

<sup>1</sup>Chemistry Department, Division of Natural Sciences, Mathematics, & Engineering, University of Pittsburgh – Greensburg, 150 Finoli Drive, Greensburg, PA, USA 15601

A novel approach to determination of iron, aluminum, and other selected metal ions of interest, using common chelating agents in aqueous as well as incorporated into an optically transparent membrane, followed by analysis of the data obtained via univariate and multivariate regression methods, e.g., partial least squares (PLS), to quantify the metals of interest, will be presented and discussed.

Applications of membranes to analyte detection have involved incorporation of the membrane into a sensor device for laboratory and field analyses, or as test “strips” for qualitative and semiquantitative analyses. Using membranes to detect metals involves incorporation of a metal-specific chelator plus necessary reagents in a polymeric gel. The goal of our investigation is to produce optically transparent agarose- and sodium alginate-based membranes impregnated with a chelating agent that ultimately will bind selected metal ions under proper conditions, permitting quantitation of the metal. Eriochrome Cyanine R, Chrome Azurol S, and Pyrocatechol Violet can chelate trivalent forms of aluminum and iron simultaneously in specific pH ranges, and are potential candidate chelators for individual and simultaneous analyte determination. Another chelator of interest for use with membranes is iron(II)-specific Ferene S, which will also be studied as part of this project.

Experiments will be conducted in solution and membrane media to test the feasibility of iron and aluminum determination. The Beer’s law behavior of each chelate of the candidate ligands will be investigated to define suitable experimental conditions. Simultaneous determination of trivalent iron and aluminum concentrations in selected sample types samples will be achieved by applying chemometric methods, e.g., PLS and least-squares minimization, to the spectrophotometric data obtained. The results obtained from our work will be presented and discussed, as will future plans for this research.

# IDENTIFYING NEW TARGETS OF THE LYSINE METHYLTRANSFERASE SET5 AND DETERMINING THE EFFECT OF PHOSPHORYLATION ON ITS METHYLATION ACTIVITY

Assefa Akinwale<sup>1</sup>, Sylvia Min<sup>1</sup>, Marlene Keisha Kontcho<sup>1</sup>, Rashi Turniansky<sup>1</sup>, James Moresco<sup>2</sup>, Julie Wolf<sup>1</sup>, John Yates III<sup>2</sup> and Erin M. Green<sup>1</sup>

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Histones are a group of proteins that are associated with DNA, and they serve an important function to organize and regulate the accessibility of DNA. Histones play a key role during transcription and the post-translational modification of these histones regulates gene expression, allows for responses to environmental stresses, and promotes silencing of genomic regions that should not be expressed. Enzymes post-translationally modify histones, as well as other proteins, with chemical groups such as a in methylation, phosphorylation and acetylation. One such enzyme is Set5, the first discovered H4 methyltransferase in budding yeast that monomethylates lysines 5, 8, and 12. Set5 plays a role in regulating cell growth and stress responses, as well as promoting repression of genes at telomeres in conjunction with Set1, another methyltransferase. In order to understand more about Set5 function, we performed an immunoprecipitation of Set5 coupled to mass spectrometry. This allowed us to identify potential protein interacting partners, as well as post-translational modifications to Set5 itself. We determined that Set5 contains various phosphorylation sites which are likely key in its function within the cell. Using *in vitro* methylation assays with versions of Set5 carrying mutations in the phosphorylation sites, we determined that these phosphorylation sites may affect the methylation by Set5 on histone H4. Additionally, we identified other interacting partners of Set5 that may be methylated by Set5, including Ssa1, Hxt6, Hem14, and Cos6. We will discuss our strategy for verifying whether these are true targets of Set5's methyltransferase activity.

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

## **COPY NUMBER OF TRANSGENIC CALVIN-BENSON CYCLE GENE FBPSBPASE IN THE MICROALGA *CHLAMYDOMONAS REINHARDTII***

Holly Asbury<sup>1</sup>, Rudolph Park<sup>1</sup>

Stephen M. Miller<sup>1</sup>

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As components of the Calvin-Benson cycle of the microalga *Chlamydomonas reinhardtii*, the enzymes FBPase and SBPase are key components of the carbon fixation pathway. However in cyanobacteria, there is a single enzyme which catalyzes both reactions, FBPSBPase. It has been hypothesized that insertion of the FBPSBPase gene into *C. reinhardtii*'s chloroplast genome may increase efficiency of carbon fixation. This project addressed two specific questions regarding the transgene. First, is the gene transformation working? In order to answer this question, the transgene and a control gene encoding actin were analyzed by RT-PCR. RNA was extracted and made into cDNA libraries for both the transgenic strain and the control strain, which were used as the templates for PCR. The results of these experiments led to the conclusion that the transformation worked. The second question that arose was what percentage of the chloroplasts have the transgene? This is an important question because there are approximately 100 chloroplast genomes per *C. reinhardtii* cell, and transgenic protein levels increase with higher copy number. To determine how many of the chloroplast genomes contain the transgene, qPCR was performed using two sets of primers: one that primes both in the 5' region of the transgene and in the endogenous gene, *atpA*, and a second primer that primes in the gene *rbcL* to act as a DNA loading control. The results of these experiments indicate that greater than 100% of chloroplasts contain the FBPSBPase transgene. These results would not make sense except for the fact that the transgene inserts into the inverted repeat region of the chloroplast genome. These results suggest that the transgene inserts into both inverted repeat regions. Based on these results, we can conclude that on average every chloroplast genome contains more than one *FBPSBPase* gene, so that FBPSBPase expression should be optimal.

Acknowledgements: These results were obtained with support from an REU supplement to award NSF-EFRI-1332344 from the National Foundation Directorate for Engineering (ENG) Office of Emerging Frontiers in Research and Innovation (EFRI), made to SMM.

# PKA ACTIVATION IN EARLY *XENOPUS* EMBRYOS CAUSES SEVERE DEVELOPMENTAL DEFECTS

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Georgette N. Jones, Ph.D.<sup>1</sup>

<sup>1</sup>Department of Biology, Hood College, 401 Rosemont Ave, Frederick, MD 21701

The South African clawed frog, *Xenopus laevis*, is an excellent model for developmental research as their embryos are large and easily obtainable in mass quantities. Much is known about the patterns of cell movements and divisions in the early frog embryo, but less is known about the molecular signaling involved in cell maturation, particularly during the period of organ development (organogenesis). We aimed to determine the effect of altered cyclic-AMP (cAMP) dependent protein kinase (PKA) activity on bone and cartilage development in the frog using the frog embryo teratogenesis assay in *Xenopus* (FETAX).

PKA is a protein involved in metabolism and hormonal signaling, as well as cell division, migration, and differentiation throughout development and adulthood. When PKA is over-activated it causes defects in facial bone development in the mouse. In humans, defects in facial bone development (such as cleft palate) are common, and previous findings support the hypothesis that PKA signaling is involved. Furthermore, previous studies demonstrated that PKA activity leads to defective migration of mesodermal cells, which contribute to bone formation, during early development in the frog. We sought to test the hypothesis that altered PKA activity during organogenesis would lead to bone deformation in embryonic frogs.

Using the FETAX approach, we tested two PKA activating drugs, 8-bromo-cAMP and forskolin, for their effect on early frog development. Frog embryos were treated for 96 hours with varying concentrations of each drug starting at 12-18 hours post fertilization. While higher concentrations of each drug resulted in embryonic death, lower concentrations caused severe defects in frog length, spinal curvature, and tissue organization that was not evident in the untreated and vehicle controls. These data support a role for PKA in bone development in the frog, and experiments are ongoing to determine the extent to which the bones are affected in drug-treated embryos.

1. We kindly thank the US Army Center for Environmental Health Research (568 Doughten Drive, Fort Detrick, MD 21702) for their donation of *Xenopus laevis* for use in this study.
2. This work was supported by the Summer Research Institute at Hood College (401 Rosemont Ave, Frederick, MD 21701)

# **BIOINFORMATICS BASED IDENTIFICATION OF CIS-REGULATORY G-QUADRUPLEX MOTIFS IN THE HUMAN PTCHD1 GENE INVOLVED IN AUTISM**

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The human PTCHD1 gene, also known as Patched Domain Containing Protein-1 gene, encodes for a membrane receptor which is involved in early development. Deletions in the PTCHD1 gene are known to be associated with autism.

PTCHD1 gene is mapped to the X-chromosome, and its expression is responsible for organization and morphological development of the embryo. This gene is expressed in cerebellum, spinal cord and pituitary gland during the early development. It is also expressed in the adult brain. At least two alternative isoforms, differing in UTR lengths, have been reported encoding identical proteins. Regulation of its expression at post-transcriptional level needs further investigation.

Studying post-transcriptional regulation of the human PTCHD1 gene expression is expected to enhance our understanding of its function and role in human disease.

G-quadruplexes are three-dimensional structures formed in guanine rich DNA and RNA sequences. G-quadruplexes consist of square coplanar arrays and can be highly stable due to cyclic Hoogsteen bonds. RNA G-quadruplexes have received significant attention because of their importance in biological processes such as regulation of protein synthesis and mRNA turnover.

The goal of this project has been to study the role of G-quadruplex forming motifs in regulating post transcriptional gene expression of human PTCHD1.

We have used a bioinformatics approach to map several evolutionarily conserved G-quadruplexes in three orthologs of the human PTCHD1 mRNA: dog, mouse and rat. Our analysis suggests that the G-quadruplex motifs found in the 5'- and 3'-UTRs of the human PTCHD1 could potentially regulate translation efficiency, mRNA stability, and alternative polyadenylation of the PTCHD1 transcripts.

## MUTATIONAL AND BIOCHEMICAL ANALYSES OF ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE

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Christine Hrycyna<sup>1</sup>

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Ninety percent of pancreatic cancers are attributed to activating mutations in the oncogene K-Ras. Thus, it is important to inhibit the activity of the K-Ras enzyme in cancer cells. This research targets oncogenesis by studying an enzyme that post-translationally modifies K-Ras called isoprenylcysteine carboxyl methyltransferase (Icmt), which transfers a methyl group to the C-terminus of K-Ras. This modification is important for guiding the K-Ras protein to the membrane where it signals cellular growth. Our goal is to understand the mechanism by which Icmt functions in order to develop inhibitors. As a first step, we aim to identify the substrate binding site of Icmt. To this end, site-directed mutagenesis was used to make mutations to residues implicated in substrate binding in the yeast homolog of Icmt, Ste14. Residues L33, L34, L40, L176, L190, and L195 were mutated to alanine and residue F80 was mutated to tyrosine. These mutants were analyzed for structural integrity using mild trypsin digestion. Each mutant demonstrated a similar cleavage pattern to wild-type (WT), indicating that overall, the mutant proteins were folded similarly to the native Icmt protein. When tested with an enzymatic methyltransferase assay, all mutants exhibited some loss of activity (15 to 56%) as compared to the WT enzyme. The mutant protein containing L190A was the most negatively affected, losing 56% WT activity and notably, also demonstrated a different substrate specificity than WT. Together, these data suggest that this residue may be an important determinant in the substrate binding site of Ste14. Photolabeling experiments will be conducted in the future to validate if these residues are important for substrate binding to Ste14. Understanding the molecular nature of the binding site will then be utilized to design more potent and effective drug therapies to minimize K-Ras signaling in cancer cells.

We would like to thank the National Institutes of Health and the Purdue Center for Cancer Research.



## IMPROVED METHODOLOGY FOR THE SYNTHESIS AND POLYMERIZATION OF FUNCTIONALIZED COBALT SULFIDE CLUSTERS

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MD, 21250

Two ligands 2-bromo-5-diphenylphosphinothiophene (1) and 2-diphenylphosphinothiophene (2) were synthesized from the reaction of 2,5-dibromothiophene or 2-dibromothiophene with n-Butyllithium followed by chlorodiphenylphosphine, respectively. The functionalized ligand 1 was serially reacted with cobalt bromide and sodium sulfide to yield  $\text{Co}_6\text{S}_8(\text{P}(\text{Ph})_2(\text{C}_4\text{H}_2\text{SBr}))_6$ , (3). Extended time between the addition of the cobalt bromide and sodium sulfide improved the isolation of 3. Isolation of 3 from the reaction mixture required the development of a new methodology. The most effective of those methods included dissolving 3 in methylene chloride before precipitation in hexanes. Ligands 1 and 2 were recovered in the developed methodology and can be recycled for future reactions to compensate for the relatively low yield of the cluster reaction. Cluster 3 was then polymerized with thiophene co-monomers in various ratios to obtain cluster-containing copolymers with increased polymer chain length between the clusters. Characterization using  $^1\text{H}$  and  $^{31}\text{P}$  NMR, UV-visual spectroscopy, and fluorescence spectroscopy indicated successful incorporation of 3 into the polymer backbone.

Thank you to James Madison University of Chemistry and Biochemistry for providing the facility, instrumentation, and supplies. Funding was received from the Research Experiences for Undergraduate program.

## PLANT-BIOFUEL CELL HYBRID FOR ENERGY PRODUCTION

Becca Drain<sup>1</sup>, Kevin Beaver<sup>1</sup>, Jasmine Olvany<sup>1</sup>  
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As global energy consumption goes up, demand for new resources for sustainable energy increases. One of the alternative mechanisms for energy production is biofuel or solar cells, which utilize the natural energy of the sun or readily available substrates and enzymes to create energy. While both methods can produce renewable energy, combining the two should lead to more efficient and increased energy production. To accomplish this the biofuel cell utilizes the substrates exuded by a plant's rhizosphere, which is a product from photosynthesis, thus combining a solar energy conversion process with an energy production process where the only necessary fuel is light.

The authors would like to thank the Lebanon Valley College Endowed Research fund for generous funding.

## EFFECTS OF AGE ON THE MICROBIOTA OF THE HUMAN BELLY BUTTONS

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Microbes live within and amongst us, and they promote both human health and disease. We were interested in determining the impact of age on the diversity of the human belly button microbiota. We inquired this question because we wanted to determine if there was a correlation between an individual's age and their susceptibility to disease. We hypothesized that there was a greater diversity of microbes in younger individuals than older individuals. To investigate this hypothesis, we used a previously collected metagenomics data set that explored the microbial diversity of the human belly button (Hulcr, et al., 2011). We chose to concentrate our research on individuals living in North Carolina, and its neighboring states: Virginia, Tennessee, Georgia, South Carolina, Kentucky, and West Virginia. We focused on this geographic location because the majority of individuals in the dataset were from the aforementioned states. We divided the dataset into two groups: ages 0-30 (n=101) and 31-60 (n=59). According to the data, there were more microbes present in people between the ages of 0 and 30, than there were in participants between the ages of 31 and 60. The most common bacterial phylum was Firmicutes, a gut microbe. Therefore, our hypothesis was supported, in that we observed a greater microbial diversity in the younger population. Limitations of this work included a small sample size, hygienic factors, and environmental factors. Future directions for this experiment include increasing the sample size, and using gender, birth method, and environment as metadata to evaluate their impacts on the diversity of the belly button microbiota.

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).

## MULTIFUNCTIONAL HYDROXYAPATITES; DESIGN OF MATERIALS FOR BONE AND LASER HOST APPLICATIONS

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And

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The apatite type materials (hexagonal, space group  $P6_3/6$ ) are found in nature and have been explored since past half century for their compositions and crystal structures. Hydroxyapatites are investigated for their applications as the laser host material. Czochralski and flux growth methods have been utilized to achieve single crystals. Since past several years we have developed low temperature processing for preparing multinary oxides for variety of applications including soft and hard bone materials and novel large wide bandgap materials. To achieve desired morphologies, we utilized some organic melt and oriented the grains by the directional solidification method. This organic treated material has different characteristics than coarsened oxide materials. For biological applications calcium based phosphate and silicates are important constituents for teeth, bones and urinary calculi, while their synthesis affects our lives during progressive mineralization of the artery walls. Our approach involved low temperature processing using nano engineered powders of the material system  $MgO-Na_2O-K_2O-CaO-SrO-SiO_2$  and titanate, borates were processed by sintering and grain growth. Our results indicate that substitution of calcium and strontium with some other elements such as gallium and magnesium have great potential for bone weakness. The objective of the present project was to design and develop novel multifunctional materials for bone and laser applications using nano, micro and bulk materials and novel processes. The focus was also understanding the morphology and performance function in human organs that occur because of aging or disease, and responses to interventions. We annealed to determine the changes in morphologies and hence effect of aging. In this presentation, we will discuss correlation between the micromorphology and the performance parameters of these hydroxyapatites based on optical and mechanical characteristics.

# NEURAL REGULATION OF SLEEP-LIKE STATE BY THE GENE *fax-1* IN THE NEMATODE *C. ELEGANS*

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Sleep is a necessary component of life, yet there is little understanding of how it is controlled on molecular and cellular levels. The study of a novel sleep-like state in the model organism *C. elegans* can further our understanding of this essential life function. I am proposing that insulin signaling and interneuron function coordinate to regulate this sleep-like state in the *C. elegans* nematode. The gene *fax-1*, a nuclear receptor, has been implicated in this novel sleep pathway, the insulin-like signaling pathway, and neuron identity. A mutation in *fax-1* in combination with insulin reception results a sleep-like developmental arrest in embryos. The AVK interneuron pair is of interest in the sleep-like pathway, as their identity is controlled by *fax-1*. The neurons' function will be knocked out by expressing a miniSOG construct in them; miniSOG is a fluorescent protein that creates free radicals when activated with blue light, ablating the cell(s) it is expressed in. To direct the construct to the AVK interneurons alone, the *flp-1* promoter will be fused to miniSOG in order to specifically kill these neurons, that have been implicated in an insulin receptor mutant background. Determining the role of *fax-1* in the sleep pathway of this classic model organism will further our understanding of the metabolic and developmental regulations of sleep for higher order systems.

Acknowledgements - Sheila Clever, Funded by NIGMS

## **SPECTROSCOPIC STUDIES OF BUFFER AND METAL ION EFFECTS ON AMYLOID- $\beta$ PEPTIDE STRUCTURE AND AGGREGATION**

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Amyloid- $\beta$  peptide plaques are found in brains obtained from Alzheimer's patients. It is uncertain how the presence of these peptide plaques correlates with the onset of the disease. Previous studies have proven that metal ions such as zinc and copper are co-localized within the same AB plaque samples. Understanding the environmental conditions that affect AB peptide structure and aggregation will provide a greater understanding about the role of metals in disease. Current studies are aimed at understanding how metal ions influence peptide structure, solvation, and aggregation. Infrared spectroscopy was used to monitor aggregation and structural changes of control AB peptide and AB peptide in the presence of metal ions over time. Infrared spectra show that peptide length, buffer and metal content influence AB peptide structure, solvation, and aggregation.

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# **DIFFERENTIAL EXPRESSION OF PKMζ A MOLECULAR MARKER OF MEMORY IN TRAINED ANIMALS TREATED WITH CX5461 AND MTOR ACTIVATOR**

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It was previously shown that Pol I inhibitor CX5461 inhibit for Long-term plasticity (Hernandez 2009 et al Allen 2014) and memory (Allen et al unpublished). Activation of mTOR appears to be relevant for learning memory and perhaps may counteract the effect of CX5461.

This experiment was designed to determine if the effects of mTOR override the effects of CX5461. To test this, mice were trained in active place avoidance (APA) spatial learning task, which is dependent on the hippocampus with 2 training trials and memory 24 hrs later. We used 2 drugs: 1) (mTORa) that was administered by Intraperitoneal (IP) injection; and 2) a Pol I inhibitor (CX5461) injected directly into the dorsal hippocampus. We tested 4 groups of animals; 3 groups were controls. Group 1 (vehicle + No CX) received vehicle in the hippocampus and IP; this group was our base line (we expected to learn OK but their 24 hrs memory is poor). Group 2 (Vehicle + CX 5461) received vehicle IP and Cx5461 injection in the hippocampus. Group 3 received (mTORa + no CX5461) mTORa by IP and vehicle in the hippocampus. Group 4 received both drugs (CX5461 + mTORa). It is known that learning produces an increase in PKMζ protein expression that is sustained over time. Therefore, we used PKMζ levels as a molecular marker of memory and Western blot analysis to determine the effects of the drugs on PKMζ expression levels.



# **Monitoring Electro-osmotic Flow (EOF) and Electrophoretic Mobility (EPM) in Poly(dimethylsiloxane) (PDMS) Channels Fabricated with Varying Channel Dimensions**

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Microfluidics offers the ability to perform benchtop analyses on a micro-scale, with benefits of reduced consumption of resources, cost, and analysis time. Recently in the field, there has been a shift from rigid glass to flexible polymer substrates, of which PDMS is the most common. This work features device molds prepared using a CNC mill to produce raised relief onto which the PDMS is cast, and the resulting PDMS channels are evaluated for their use in electrophoretic separations. The PDMS devices reported here feature single channels, allowing for direct comparison of EOF observed in glass capillaries, and intersecting channels that produce a more complex flow network. In addition, varying channel dimensions, including obstacles to flow, are evaluated, demonstrating the versatility of PDMS devices patterned using machined substrates.

Prior investigation was performed using borate buffers free of analyte in micro-capillary electrophoresis (CE) to determine the efficacy of such buffers in sustaining EOF in un-coated glass capillaries. Sodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) was identified as the best candidate for further analysis, and is featured in this contemporary work as the buffer of choice in both fabricated and glass capillaries. EOF and EPM are monitored visually using a laser-induced fluorescence (LIF) system and fluorescein-doped buffer. The system permits quantitative assessment of qualitative channel properties—providing real-time comparison of EOF and EPM characteristics in varying fabricated channels.

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## CHARACTERIZATION OF EARLY STAGES OF AMYLOID FORMATION BY LIGHT CHAINS INVOLVED IN RENAL AMYLOIDOSIS

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Light Chain (AL) amyloidosis is a rare systemic protein misfolding disease caused by the abnormal proliferation of monoclonal plasma B cells that leads to the overproduction of misfolded immunoglobulin light chain. These excess light chains deposit and aggregate as insoluble fibrils in the extracellular spaces of various organs (the most frequently involved organ is the kidney), resulting in tissue degeneration and organ impairment. Our laboratory has previously characterized the thermodynamic stability and fibril formation properties of four AL proteins involved in renal amyloidosis. This summer, we investigated the early stages of in vitro amyloid formation of 2 of these proteins, AL-T05 variable domain (VL) and AL-T03 VL. Fibril formation of AL-T05 was determined to be extremely fast using sedimentation and ThT fluorescence assays. Additionally, a consistent increase in particle size over time during the initial aggregation stages was observed through light scattering studies. Electron microscopy further confirmed the presence of prefibrillar species that developed into long amyloid fibrils. These observations seemed to match the aggregation pattern of another AL protein involved in cardiac amyloidosis, AL-09 VL, which was previously determined to be a very rapid amyloid former. Our results indicate the presence of some intermediate species during aggregation but their role is still unknown. AL-T03 VL, however, is the most unstable protein characterized in our laboratory to date. We have tested purification protocols taking into account AL-T03's lack of stability by lowering the temperature of expression, increasing the pH of purification buffers and switching refolding buffers. Optimization of AL-T03 VL refolding and purification is ongoing.

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## THE EFFECT OF AGE ON THE PRESENCE OF MICROBIAL COMMUNITIES FOUND IN THE HUMAN BELLY BUTTON

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Microbes are single-cellular prokaryotes that have a variety of functions, including fermentation. Also associated with human and animal health, the numerous amount of microbes can cause disease or promote healthy physiological processes. With over 100 trillion microbial cells in the human body, Proteobacteria is one of the many phyla of bacteria found on the human belly button. Proteobacteria is a gram negative bacterium and previous reports demonstrate increased prevalence in elderly populations. Given this information, the prevalence of Proteobacteria varies amongst different age groups. Using a previously collected metagenomics data set evaluating the microbial diversity of the human belly button (Hulcr, et al., 2012), it was hypothesized that there will be an increased prevalence of Proteobacteria in elderly subjects. We investigated two age groups, 0 to 38 (n=171) and 39 to 67 (n=69). Our results demonstrated that the younger population had a larger abundance of Proteobacteria in their belly button than the older population. This suggests that as one ages, the amount of Proteobacteria decreases. Therefore, our results refuted our hypothesis, as we observed decreased levels of Proteobacteria in older individuals than we did younger. Limitations of this research include the inability to investigate Proteobacteria levels after age 67 and the absence of data for ages 16 and 53. Future directions include using a larger sample size, including all age groups, determining the role of immunity in the levels of Proteobacteria in the belly button, and if the location of the bacterium affects how susceptible an individual is to disease.

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## FUNCTIONAL ANALYSIS OF SEXUALLY DIMORPHIC LONG NONCODING RNAS IN THE DROSOPHILA GERMLINE

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In *Drosophila melanogaster*, sex determination is regulated by the protein SXL which is activated in the presence of two X chromosomes, causing cells to become female in identity. The process by which SXL brings about this fate is well understood in the soma but not in the germline. To identify targets of SXL, RNA sequencing was done using *bam* mutant gonads to enrich early germline and compare *Sxl*-RNAi ovaries to control ovaries. In addition we compared *bam* mutant testes to *bam* mutant ovaries to identify genes with male or female biased expression in gonad. The results of the RNA-seq showed sexually dimorphic expression of roughly 150 predicted long non-coding RNAs (lncRNAs).

To explore the roles of these sex specific lncRNAs in the germline we have made use of multiple loss of function strategies. The Minos mediated integration cassette (MiMIC) is a transposon that incorporates randomly into the genome to create mutations that disrupt proper gene expression. Flies containing these insertions in a lncRNA of interest were aged and their gonads were assessed for sex specific morphological defects using confocal microscopy. To make complete lncRNA gene deletions we are using the CRISPR/Cas9 system. One lncRNA currently of interest is CR43684, which is dramatically enriched, in the male germline. In addition to lncRNAs from the RNAseq data, *Rox1* and *Rox2* are two lncRNAs that are known to be necessary for dosage compensation in the soma. Their function has yet to be explored in the germline, however knockdown of *Rox1* by RNAi in the germline results in a phenotype suggesting there is a previously unknown function for this RNA in germline. In addition, we are using Fluorescence in situ hybridization to investigate localization of *Rox1*, *Rox2*, and CR43684 in the germline as well.

I would like to thank all the members of the Van Doren lab for their tips and advice on starting research. I'd like to thank Caitlin Pozmanter specifically for her mentorship. I'd also like to thank the Chen lab for the use of their equipment. Lastly, I'd like to thank the Woodrow Wilson Fellowship for the opportunity to pursue this research.

## HOW HIV-1 SELECTIVELY PACKAGES ITS DIMERIC GENOME: MAPPING THE NC BINDING SITES

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Human immunodeficiency virus type-1 (HIV-1) is a retrovirus that is the causative agent of acquired immunodeficiency syndrome (AIDS). There are approximately 36.7 million people in the world infected with HIV. The viral genome is reverse transcribed which is a highly mutagenic process, however the 5'-Leader of the genome is the most conserved region. The 5'-Leader undergoes a dimerization process exposing more than a dozen nucleocapsid (NC) binding sites and is responsible for promoting packaging. Previous studies on the HIV-1 5'-Leader discovered that the minimal region required for viral genome selective packaging is the Core Encapsulation Signal (CES). Our research investigates the binding interactions between the NC domain of the Gag polyprotein and the CES of the dimeric viral genome. The high resolution nuclear magnetic resonance (NMR) structure of the CES revealed weakly base-paired and unpaired guanines, which are characteristic for NC binding sites. Utilizing techniques such as: electrophoretic mobility shift assays (EMSA), isothermal titration calorimetry (ITC), and mutagenesis, we elucidated the specific guanine residues within the CES responsible for NC binding. Ultimately, gaining a greater understanding of the mechanism for selective packaging of the viral genome could eventually translate into successful development of viral inhibitors.

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# IDENTIFICATION OF NOVEL REGULATORS OF INDIVIDUAL AND COLLECTIVE CELL MIGRATION

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Cell migration is a fundamental process essential for embryogenesis, wound healing and pathogenesis. During embryogenesis, groups of cells migrate individually or as a collective. Although there are overlapping features between single and collective cell migration, the collective migration of cells involves mechanisms distinct from that of single cell migration. To better understand the genetic requirements for collective migration of cell groups during embryogenesis, we performed a small-scale RNA interference screen in the *Drosophila* embryonic salivary gland cells that migrate collectively. Of the 36 RNAi lines tested, we identified five that disrupted salivary gland migration, Rac2, MAP kinase activated kinase-2 (MAPK-AK2), Diacylglycerol Kinase (DGK), the PDSW subunit of NADH dehydrogenase and Enabled. Our studies identify Rac2, MAPK-AK2, DGK, Enabled and NADH-PDSW as conserved regulators of cell migration in the *Drosophila* embryonic salivary gland and larval wing disc epithelia.

This project was supported by the NIH-RISE. Thanks to my mentor and the NIH-RISE program for providing laboratory assistance for my research experiment.

## CREATING A NEURONAL EXPRESSION ATLAS OF *CAENORHABDITIS ELEGANS*

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Transgenerational gene silencing by mobile double-stranded RNA (dsRNA) has been documented in the roundworm, *Caenorhabditis elegans* (*C. elegans*). This type of gene regulation occurs when mobile dsRNA made in the soma travels to the germline to silence a germline gene in subsequent generations. Previous work using multicopy arrays of the transgene to express dsRNA targeting a green fluorescent protein (GFP) in all neurons observed silencing of the GFP target for over 25 generations after losing the dsRNA gene<sup>(1)</sup>. However, the usage of multicopy arrays complicates the interpretation of these observations. The dsRNA gene was expressed at unusually high amounts and may have resulted in misexpression because rearrangements are known to occur during array formation. Moreover, a foreign gene may be more susceptible to silencing in the germline. Therefore, we do not know if transgenerational gene silencing of an endogenous germline gene can occur when dsRNA is expressed in typical amounts comparable to other cellular RNAs. Also, by using a pan-neuronal promoter to express dsRNA in all neurons, we do not know if different neurons have different abilities to process and export dsRNA to the germline. To answer these important questions, we need to create transgenic animals that express dsRNAs in subsets of neurons from single-copy genes integrated into the worm genome. Here we present the work of large cohorts of undergraduates in the FIRE-Transgenerational Brain Initiative to systematically test different neuronal promoters to drive expression of a reporter gene. These same validated promoters will be used to express dsRNA from specific neurons. Because these reporters will be integrated into the same locus, we can assemble an expression atlas of neuronal genes including relative promoter strengths. These reporter and dsRNA genes will be used to test if transgenerational gene silencing of an endogenous germline gene can occur under physiological conditions.

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1. Devanpally, S et al., PNAS, 2015



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

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The prevalence of breast cancer in society warrants an immediate need for a cure. Based on data and analysis provided by the American Cancer Society, breast cancer is the most frequently diagnosed and thus poses a serious threat to humanity. Tamoxifen, an antiestrogen drug has been proven effective against hormone receptor positive breast cancers. However, it often causes endometrial cancer and tumor resistance after extended use. Other studies have demonstrated anticancer activity of ferrocifens (tamoxifen analogues) against hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cells. A drawback of these compounds is the possibility of liver damage due to iron overload. Since some rhenium-based compounds have been proven to be less toxic or non-toxic towards normal cells, previous studies in our lab have shown the synthesis and anticancer activity of novel rhenium complexes of the type  $X\text{Re}(\text{CO})_3\text{Z}$  [ $X = \alpha$ -diimines and  $Z = \text{tosylate}$ , 1-naphthalenesulfonate and 2-naphthalenesulfonate] against MCF-7 and MDA-MB-231 breast cancer cells. In this study, we are exploring the optimization of the first step of this synthesis using microwave assisted organic synthesis. This step involves treating dirhenium decacarbonyl with the corresponding  $\alpha$ -diimine in the presence of 1-pentanol and  $\text{CO}_2$  gas to give a pentylcarbonato (PC) complex. Two PC derivatives (PC2 and PC4) were synthesized with 86% and 45% yields. In comparison to conventional methods, the reaction times for PC2 and PC4 were reduced from 12 hrs. to 1.5 hrs. and 24 hrs. to 1.75 hrs. These complexes were characterized using IR,  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR. Further studies on other PC derivatives are ongoing.

## EVALUATING THE NUCLEASE ACTIVITY OF PSEUDOMONAS EXOTOXIN A

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*Pseudomonas* exotoxin A (PE) is a protein toxin secreted by *Pseudomonas aeruginosa*. The toxin is remarkably potent, and believed to be the major virulence factor of *P. aeruginosa*. PE inhibits protein synthesis by inactivating elongation factor 2 (EF2), the protein that advances the ribosome along the mRNA during translation. Cells with inactive EF2 are unable to synthesize proteins, which leads to apoptosis. Recent studies have indicated that, in addition to inhibition of protein synthesis, PE has also functions as a nuclease. The purpose of the experiments described here is to verify this unusual claim and characterize the nuclease activity of PE using only the catalytic fragment of the toxin. We have conducted basic time-course and concentration assays, and have confirmed a low level of nuclease activity from PE. We are currently examining cleavage in the presence of different divalent cations ( $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ ). We have also tested a fluorescence-based assay to observe cleavage of DNA in real-time. Initially experiments using DNase I have been successful, and we hope to use this method to study PE in the near future.

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**BLUNTED CONTRACTILE RESPONSE TO  $\beta$ -ADRENERGIC RECEPTORS  
STIMULATION IS LINKED TO DESENSITIZATION MECHANISMS OF THE  $\beta$  -  
RECEPTORS IN MOUSE LEFT VENTRICLES OVEREXPRESSING BRAIN TYPE  
ADENYLATE CYCLASE**

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Previous studies of Adenylyl Cyclase 8 (AC8) overexpression in the mouse heart have shown increases in heart rate (HR) and left ventricle contractility as well as a reduced response to  $\beta$ -adrenergic receptor ( $\beta$ -AR) stimulation. We verified these results using telemetry *in vivo*, finding that HR was 40% higher in transgenic (TG) models than wild type (WT) models, and the HR response to dobutamine ( $\beta_1$ -AR agonist) infusion was blunted. Echocardiography analysis, under anesthesia, showed a significantly higher fractional shortening (FS%) at baseline and a blunted FS response to dobutamine infusion compared to WT mice. We hypothesized that this reduced response to  $\beta$ -AR stimulation is due to mechanisms that induce un-sensitization of the  $\beta$ -ARs to their ligands. RNAseq analysis of the left ventricle showed alteration of expression in some candidate genes. Utilizing RT-qPCR, we validated these markers of altered  $\beta$ -AR sensitization. Furthermore, AC8 overexpression in an HL-1 cell line (mouse derived atrial cells) showed significantly increased spontaneous beating rate, and the  $\beta$ -AR response to isoproterenol (a nonspecific  $\beta$ -AR agonist) was markedly blunted compared to HL-1 cells expressing a control protein (GFP). We conclude that the reduced response to  $\beta$ -AR stimulation when AC8 is overexpressed is linked to altered sensitization of  $\beta$ -ARs to their ligand and is in part due to receptor internalization.

This research was funded by the National Institute of Health Intramural Research Program and supported by the NIH IRP Summer Internship Program.

## **SUBSTITUENT EFFECTS IN THE SOLVOLYSIS OF ETHYL CHLOROFORMATE ESTERS**

Matthew Dina

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Chloroformates are esters that are reagents in synthetic organic chemistry. Typically, they are precursors in herbicides, pesticides, as well as fungicides and have an adverse effect on human health. In this project, we detail the solvent reactions of 2-methoxyethyl chloroformate and compare its reactivity to 2-ethyl chloroformate. We specifically analyze the impact of the substituent methoxy group which could act as an electron donor or electron withdrawer. We followed the pseudo first-order kinetics of 2-methoxyethyl chloroformate at 25.0°C using the acid-base and conductometric titration methods. During the symposium, I will provide only the initial results obtained.

Financial support was provided through the Delaware INBRE (IDeA Network of Biomedical Research Excellence) program funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences – NIGMS of the National Institutes of Health (NIH) and the State of Delaware DEDO (Delaware Economic Development Office) program under grant number P20GM103446- National Science Foundation S-STEM DUE grant through Wesley College's Cannon Scholar program under award number 1355554 and the State of Delaware DEDO program.

## **DOES MYELIN AND LYMPHOCYTE PROTEIN (MAL) FUNCTION AS *CLOSTRIDIUM PERFRINGENS* EPSILON TOXIN (ETX) RECEPTOR?**

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Epsilon toxin (ETX) is a  $\beta$ -pore forming toxin that is produced by *Clostridium perfringens* type B and type D. While *C. perfringens* is a member of the microbiota present in the human and ruminant gut, historically types B and D have been thought to be ruminant specific. In affected ruminants, ETX targets the central nervous system (CNS), causing symptoms with a striking similarity to human multiple sclerosis (MS). Interestingly, mounting pieces of evidence have recently supported the notion that *C. perfringens* may play a central role in the pathogenesis of MS, and that ETX may be a molecular trigger. Recent data suggest that Myelin and lymphocyte protein (MAL) may serve as a cellular receptor for the toxin. Our lab is interested in knowing if a physical interaction exists between MAL and ETX. To this end, we created a model capable of expressing a large amount of MAL. To accomplish this goal, we use cell culture of the Tni insect cell lineage. These cells were cultivated to >95% viability and to a final concentration of 2 million cells/mL. They were then transfected using varying concentrations of MAL encoding baculovirus and incubated at 2 different temperatures for 48 and 72 hours. Tni cells were transfected with Green Fluorescence Protein (GFP) encoding baculovirus as a positive control. Using fluorescence microscopy and Western blotting, our preliminary results revealed that the Tni cells proficiently express both GFP and MAL proteins. Also, we determined that infecting cells with a viral concentration of 1/20, and incubating for 72 hours at 23°C results in the highest protein yield. In conclusion, our preliminary experiment creates a model capable of proficiently expressing MAL. The next phase of this experiment will be to express and purify MAL for ETX binding studies via Microscale Thermophoresis (MST).

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## THE IDENTIFICATION OF ANTIBIOTIC RESISTANCE GENES IN LOCAL WATER SOURCES

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The development of antibiotics revolutionized healthcare. Bacterial infections that used to kill millions of people per year were almost completely eradicated. However, over the years the development of a new health crisis has been underway. That is antibiotic resistance. The over prescription and general abuse of antibiotics has dramatically decreased their effectiveness. The more antibiotics that are introduced into the environment, the more opportunity there is for bacteria to cultivate and spread resistant genes amongst themselves. This could greatly increase the risk of a resurgence of deadly infectious diseases.

To understand how widespread resistance is, the aim of this experiment was to find evidence of at least one antibiotic resistance gene in a local water source. DNA was isolated from three water sources and PCR was performed to look for the presence of five different antibiotic resistance genes. The gene aminoglycoside-3-*O*-acetyltransferase-I (*aacC1*) was successfully identified in a water sample from Rock State Park in Forest Hill, MD. *aacC1* is a gene that codes for a protein commonly called *Gentamicin 3-N-acetyltransferase*. Gentamicin is an aminoglycoside antibiotic commonly used against a broad spectrum of bacterial infections. To aid in the confirmation of the *aacC1* gene, it was cloned into *E.coli*, the associated protein was expressed, and mass spectrometry along with western blotting were performed. Future directions for this experiment include the optimization of affinity chromatography to better isolate and characterize the protein of interest.

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## CHARACTERIZING THE PATHOPHYSIOLOGY OF *DROSOPHILA* RETINAL DEGENERATION MUTANT, *RDGI*

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The *Drosophila melanogaster* visual system is a well-established model for the study of light-dependent visual signaling. Our laboratory is interested in identifying novel factors that are essential in photoreceptor function and homeostasis. Here we are studying a *Drosophila* mutant called Retinal degeneration I (*rdgI*). The *rdgI* mutant line was first isolated in a large scale mutagenesis screen to identify essential eye-specific genes. As the name indicates, these mutants display severe retinal degeneration in adult flies. Our work shows that young *rdgI* adult flies have a severe reduction in rhodopsin expression which precedes photoreceptor degeneration. In addition, the genetic locus of the *rdgI* mutation is unknown. To identify this site, we use deletion mapping to determine the gene disrupted by the *rdgI* mutation. Through this study, we will identify the gene disrupted in *rdgI* mutant flies and uncover its essential role in rhodopsin expression and photoreceptor function.



## **DATA-MINING IMPACTS OF U.S MORTALITY FROM DISEASES OF THE CIRCULATORY SYSTEM, DIABETES, AND NEOPLASMS**

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Due to an increasing prevalence of diseases of the circulatory system, diabetes, and neoplasms in the United States, the goal of this research is to determine the trends in mortality rates for these three diseases. Variables such as race and age are being examined for each of the diseases in order to evaluate the risk for each demographic. Using data from the Centers for Disease Control and Prevention (CDC) WONDER database from 1999 to 2015, line graphs were created with SAS Programming Software that were able to depict the mortality rate trends for each demographic and disease. In addition, the three Delaware counties are also being examined to determine whether or not a trend exists by location in Delaware. Overall, the graphs show that nationally, the number of people dying from these diseases has continually decreased. In Delaware, the mortality rates fluctuate between counties, as there is no greater risk based off of location.

Financial support was provided through the Delaware INBRE (IDeA Network of Biomedical Research Excellence) program funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences – NIGMS of the National Institutes of Health (NIH) and the State of Delaware DEDO (Delaware Economic Development Office) program under grant number P20GM103446. Financial support was also provided by the National Science Foundation S-STEM DUE grant through Wesley College's Cannon Scholar program under award number 1355554 and the State of Delaware.

## THE ROLE OF THE NON-CANONICAL WNT/CALCIUM PATHWAY IN THE DEVELOPMENT AND FUNCTION OF PRIMARY MESENCHYME CELLS

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The highly conserved non-canonical Wnt/Calcium Signaling Pathway (ncWnt/Ca<sup>2+</sup>) has been shown to regulate cell motility and play a vital role in the developmental processes of many organisms, such as ventral cell fate in frog embryos, gastrulation in zebrafish, and proper organ formation in mice. We use the sea urchin Primary Mesenchyme Cells (PMCs), which give rise exclusively to skeletogenic cells, to examine directed cell migration in response to the ncWnt/Ca<sup>2+</sup> pathway. The working hypothesis is that PMC migration and PMC skeletal function are in part regulated by the ncWnt/Ca<sup>2+</sup> signaling pathway. We have shown that disruption of elements downstream of the ncWnt/Ca<sup>2+</sup> pathway using pharmacological drugs resulted in decreased embryonic skeletal length as well as dramatically altered PMC migration patterns. Activation of Protein Kinase C (PKC) downstream of the ncWnt/Ca<sup>2+</sup> pathway by Phorbol 12-myristate 13-acetate (PMA) also altered the spatial expression of transcripts involved in the PMC gene regulatory network and Vegf3 signaling pathways. We also examined post-transcriptional regulation of Wnt components by microRNAs (miRNAs), which are non-coding regulatory RNAs that repress target gene translation. Specifically, we identified microRNA-1 (miR-1) to have potential target sites within PKC and CDC42. Similar phenotypes were observed in PMA treated embryos and the miR-1 knockdown embryos, suggesting that miR-1 directly suppresses PKC. This research will identify the molecular mechanisms of how the ncWnt/Ca<sup>2+</sup> pathway impacts PMC directed migration and developmental function. Since ncWnt and miRNA regulation are evolutionarily highly conserved, our work can serve as a paradigm to understand the role of ncWnt signaling pathway in other animal systems.

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## **tRNA<sup>Lys3</sup> OUTCOMPETES PI(4,5)P<sub>2</sub> IN MODEL CELL MEMBRANES FOR HIV-1 MATRIX BINDING**

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HIV-1 is a retrovirus that infects CD4+ human immune T-cells. One of the proteins vital for HIV-1 replication is the Gag polyprotein. The gag polyprotein is a protein expressed by the virus and is imperative for HIV-1 replication. The N-terminal domain of Gag, matrix, is essential for Gag targeting and binding to the plasma membrane (PM) to start virion assembly. Matrix binds to phosphatidylinositol 4,5 bisphosphate [PI(4,5)P<sub>2</sub>] in the PM, and it is believed it may also specifically target lipid rafts. Rafts are rigid, liquid order portions of the PM that are high in cholesterol and certain phospholipids. Matrix also binds to tRNAs in the cell, and many believe this binding of tRNA regulates matrix-membrane interactions. According to the current model, tRNA binds to matrix and when this complex reaches the PM, PI(4,5)P<sub>2</sub> will outcompete the tRNA and matrix will bind to PI(4,5)P<sub>2</sub>. To test this theory, we created model membranes (liposomes), representing either raft or non-raft regions of the PM. We used the compositions consistent with earlier experiments in our lab, and also created liposomes using new ratios to mimic MT4 cell PMs and the HIV lipid envelope. For each liposome type we used membranes containing or lacking PI(4,5)P<sub>2</sub>. We performed 1D-<sup>1</sup>H NMR liposome competition assays in which we observed the interaction between the matrix-tRNA<sup>Lys3</sup> complex and these different liposomes. With no tRNA present we saw more binding to rafts and liposomes containing PI(4,5)P<sub>2</sub>, than non-rafts and liposomes lacking PI(4,5)P<sub>2</sub>. This was expected based on the literature and previous experiments. Interestingly, in the competition assays which involved the tRNA-matrix complex, binding did not change regardless of the type of liposome, indicating that PI(4,5)P<sub>2</sub> does not outcompete tRNA<sup>Lys3</sup> for matrix binding. In the future we plan to see if oligomerization of matrix will force tRNA<sup>Lys3</sup> to be displaced by PI(4,5)P<sub>2</sub>.

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## TRANSCRIPTIONAL REGULATION OF THE HIV-1 RNA GENOME

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The human immunodeficiency virus (HIV) uses RNA as its source of genetic material. This RNA can exist as two different conformations, the monomer and the dimer, which ultimately determines the role of the RNA in later steps of the viral life cycle. The monomer is translated into viral proteins while the dimer is packaged into new virions. It was recently discovered that transcriptional start site (TSS) heterogeneity results in multiple viral RNA genome species within an infected cell. These RNA species only differ by the number of guanines on the 5' end beginning with either one, two, or three guanines plus the native cap (Cap-1G, Cap-2G, and Cap-3G, respectively). Surprisingly, it was found that this small change of one guanine affects the structure and function of the viral RNA with Cap-1G favoring the dimer and, therefore, packaged into new virions; while the Cap-2G/3G favors the monomer and thus involved in the process of translation.

Using nuclear magnetic resonance (NMR) spectroscopy and in vivo studies, the existence of TSS, as well as its nature to disrupt the regions around it, has been confirmed. In the monomer structure (Cap-2G, Cap-3G), additional guanosine residues cause the remodeling of polyA and an extended U5-DIS Interaction. In the dimer structure (Cap-1G), polyA is stable and the dimer initiation site (DIS) is no longer sequestered thus promoting dimerization. Our focus now includes assigning all the NOSEY signals for the Cap-1G dimer and Cap-3G monomer conformations in order to solve the three-dimensional structure.

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## HOW HIV-1 SELECTIVELY PACKAGES ITS DIMERIC GENOME: MAPPING THE NC BINDING SITES

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Human immunodeficiency virus type-1 (HIV-1) is a retrovirus that is the causative agent of acquired immunodeficiency syndrome (AIDS). There are approximately 36.7 million people in the world infected with HIV. The viral genome is reverse transcribed which is a highly mutagenic process, however the 5'-Leader of the genome is the most conserved region. The 5'-Leader undergoes a dimerization process exposing more than a dozen nucleocapsid (NC) binding sites and is responsible for promoting packaging. Previous studies on the HIV-1 5'-Leader discovered that the minimal region required for viral genome selective packaging is the Core Encapsidation Signal (CES). Our research investigates the binding interactions between the NC domain of the Gag polyprotein and the CES of the dimeric viral genome. The high resolution nuclear magnetic resonance (NMR) structure of the CES revealed weakly base-paired and unpaired guanines, which are characteristic for NC binding sites. Utilizing techniques such as: electrophoretic mobility shift assays (EMSA), isothermal titration calorimetry (ITC), and mutagenesis, we elucidated the specific guanine residues within the CES responsible for NC binding. Ultimately, gaining a greater understanding of the mechanism for selective packaging of the viral genome could eventually translate into successful development of viral inhibitors.

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## **PURIFICATION OF NADPH-CYTOCHROME P-450 REDUCTASE FROM PIG LIVER MICROSOMES**

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NADPH-cytochrome P-450 reductase is a membrane bound protein found in the liver of mammals. It catalyzes electron transfer from NADPH to cytochromes P-450, and is responsible for the oxidative metabolism of endogenous and exogenous compounds. Based upon this enzyme's molecular structure and mechanism of electron transfer, we anticipated that the activity of this enzyme will be sensitive to macromolecular crowding. Microsomes, fragments of the endoplasmic reticulum, were prepared from the centrifugation of homogenized pig liver to be used for the purification of NADPH-cytochrome P-450 reductase. Three steps were completed to purify the protein starting with the solubilization of microsomes using ionic and nonionic detergents. Next, two chromatographic processes were used: a DEAE-cellulose anion-exchange column followed by a 2', 5'-ADP-Sepharose affinity column. The results of the purification of the enzyme as well as the initial results of macromolecular crowding experiments with this enzyme will be presented.

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## EXPLORING THE REGIOSELECTIVE DIELS-ALDER REACTION SCOPE OF 1,4-NAPHTHOQUINONES

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Altersolanol P (AP), a new member of the altersolanol family of compounds, is the inspiration for multiple synthetic studies in our laboratory.<sup>1</sup> The altersolanols, and structurally similar compounds, exhibit antibacterial activity. Recently, we reported our work toward the regioselective synthesis of intermediates en route to altersolanol derivatives via lewis acid catalyzed Diels-Alder reactions of the natural products isoprene and Juglone (5-hydroxy-1,4-naphthalenedione).<sup>2</sup> Epoxidation or dihydroxylation of the resulting adducts is expected to provide a small library of altersolanol derivatives for antibacterial testing. To further expand the molecular diversity of our library, in this study, we will explore the reactivity of 1,4-Naphthoquinone dienophiles with dienes, such as (2E,4E)-2,4-Hexadienyl acetate and 2,4-Hexadien-1-ol. We eventually hope to substitute Juglone (5-hydroxy-1,4-naphthalenedione) for 1,4-Naphtoquinone to explore regioselectivity of the Diels-Alder reaction. New compounds will be tested for antibacterial activity.

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## **DOES DEAF INTERMARRIAGE LEAD TO “THE FORMATION OF A DEAF VARIETY OF THE HUMAN RACE?”**

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Alexander Graham Bell argued in 1884 against intermarriage amongst deaf individuals, claiming that it would lead to the formation of a deaf race. However, Fay's (1898) study of 4,471 deaf marriages refuted Bell's argument and concluded that that deaf marrying deaf did not appreciably increase the chances of having deaf children. Despite this evidence, during the early-twentieth-century eugenics movement, Germany and 30 United States states passed laws to involuntarily sterilize congenitally deaf individuals. Recently, Nance and Kearsey (2004) have proposed that concentrating signing deaf individuals, such as in deaf schools, may have caused preferential (assortative) mating among deaf individuals based on shared language, which they termed linguistic homogamy. They predicted that linguistic homogamy would increase the frequency of the commonest deafness-causing allele in a population. Nance and Kearsey's predictions were corroborated by Arnos et al. (2008) who showed that deaf couples are now twice as likely to have deaf children than they were 200 years ago. Here, we tested the hypothesis that linguistic homogamy influences the gene pool by conducting forward-time computer simulations with different values for assortative mating and reproductive fitness. Each simulation was run 10,000 times and the results of different simulations were compared via statistical testing. Our results showed that linguistic homogamy increases the number of deaf individuals, corroborating previous findings, but does not increase the allelic frequency. Therefore, our findings are consistent with Wright and Fisher's original treatise on inbreeding. Interestingly, we found that the allele frequency increased more rapidly when increased reproductive fitness was combined with assortative mating. Therefore, our results modify the linguistic homogamy hypothesis in that natural selection and assortative mating act synergistically to change allele frequencies. This finding may explain the rapid fixation of speech and language-related alleles such as *FOXP2* that led to the appearance of modern humans.

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## CHEMICAL EXFOLIATION OF BISMUTH TELLURIDE ( $\text{Bi}_2\text{Te}_3$ )

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Bismuth telluride ( $\text{Bi}_2\text{Te}_3$ ) is the potential generator that can convert thermal heat for electricity.  $\text{Bi}_2\text{Te}_3$  has low thermal conductivity and high electrical conductivity from thermoelectricity figure of merit (ZT), making it an interesting material for thermoelectric applications. The bulk  $\text{Bi}_2\text{Te}_3$  was exfoliated using n-butyllithium and then used deposit nanofilms on cleaned silicon wafers. The wafers were then analyzed using optical microscope and Raman spectroscopy.

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## **HYPERTONIA MACHINE**

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Hypertonia is a movement disorder that affects children with cerebral palsy. It is characterized by tight muscle contractions that decrease the efficiency of muscles, and in some cases, prevent muscle use entirely. Hypertonia has three different subtypes: rigidity, spasticity, and dystonia. Each subtype has different symptoms; however, in clinical studies, there is trouble deciphering each subtype. The goal of this project is to develop a quantitative way to distinguish the three subtypes of hypertonia. The goal for the allotted period was to try and finalize electrical and mechanical components to be ready for initial testing. The mechanical design included adding additional comfort and making sure that the motor was connected onto the machine. Electrical design included designing a PCB (printed circuit board), soldering pin connectors onto the board, connecting wires, and making sure wires were connected. In the future, we plan to collect data from children with cerebral palsy using this device and develop a computer algorithm to distinguish and quantify the three types of hypertonia.

Acknowledgements to NASA DC Space Program for allowing me to participate in this research. Also, to Catholic University of America's Biomedical Engineering Department for allowing me to participate on their research.

## **OVERCOMING DEGRADATION: A NOVEL SYNTHETIC STRATEGY FOR ANTISENSE OLIGONUCLEOTIDE ANALOGS**

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Antisense oligonucleotide analogs (ASOs) are therapeutic agents that consist of short or modified DNA or RNA molecules that bind to messenger RNA (mRNA) and prohibit the synthesis of proteins from translated mRNA. The potential for ASO therapeutic agents is wide, but many toxicological challenges must be overcome before ASO medication can be reliably utilized. Such challenges include poor membrane permeability, poor solubility, and rapid degradation by exonucleases. In order to negate these challenges, removing the sugar-phosphate backbone of DNA and RNA, which is responsible for the rapid degradation of ASOs in the human body, and replacing their backbone with a 9-membered carbon ring was attempted.

I would like to thank the Mohler Lab and James Madison University, Department of Chemistry and Biochemistry for the contributions that have allowed me to complete this research.

## HOW YOUR AGE DETERMINES THE DIVERSITY of MICROBES IN YOUR BODY

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Microbes inhabit the human body. There is a general population of microbes that are common amongst almost all humans. However not all individuals have the same microbiome. Failure to properly understand, tolerate, and control the microbial community may result in several cutaneous diseases. To better understand the diversity among the microbes that live on the human body, we determined the relationship between age and diversity of microbes in the human belly button. We hypothesized that people aged 0-7 would have less microbial diversity on their body because they are less exposed to the environment. To test our hypothesis, we analyzed data from a previously conducted metagenomics experiment (Hulr, et al., 2012). We used this dataset and Phinch.org to analyze the microbial diversity in individuals who were aged 0-7 (n=14) and 8-25 (n=63), focusing on the phylum taxonomic level. We also considered people from North Carolina only because we wanted people who have experienced the same environmental factors. Our results demonstrated increased microbial diversity in the 8-25 age group compared to 0-7 age group. In particular, we observed the Cyanobacteria and Actinobacteria phyla to be most prevalent in our sample. From this we were able to conclude that there are more Cyanobacteria and Actinobacteria present in the belly buttons of people aged 8 and above. The limitations of this research include not having enough data points in each age to accurately assess our hypothesis. Future research directions would be to obtain a higher sample size with all ages 0-99 represented so that we could further compare age groups and their microbes.

### Acknowledgements

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# **SYNTHESIS AND STUDY OF N-TRIAZOLYLPROPANAMIDE DERIVATIVES AND THEIR REACTIONS WITH PALLADIUM(II) AND RUTHENIUM(III) COMPLEXES**

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Five N-triazolylpropanamide derivatives have been synthesized through base catalyzed Michael additions with Triton B as the base catalyst. The reaction of methacrylamide and 1H-1,2,3-triazole produced 1, an asymmetrically substituted triazole with a methylpropanamide moiety. The reaction of N-isopropylacrylamide and 1H-1,2,3-triazole produced two isomeric triazoles, one with the N-isopropylpropanamide substituent bonded to the exterior ring nitrogen (2), the other with the substituent bonded to the central nitrogen (3). Similarly substituted triazole isomers, 4 and 5, were obtained from the reaction of benzotriazole and N,N-dimethylacrylamide. All compounds have been characterized through NMR and IR spectroscopy. Additionally, compounds 1, 2, and 3 have been characterized through elemental analysis. Compound 1 was allowed to react with bisdichloro(1,5-cyclooctadiene)palladium(II) and ruthenium(III)nitrosylchloride mono-hydrate. Compound 2 was allowed to react with bisdichloro(1,5-cyclooctadiene)palladium(II). The products from these reactions are currently being characterized.

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## **DEVELOPMENT OF RASPBERRY PI AND ARDUINO-BASED THERMAL STABILIZER AND DESORBER FOR GC/MS VAPOR ANALYSIS**

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A temperature control and desorption system was developed to assist in collection of vapor phase samples for GCMS analysis. The GCMS utilized for this experiment (Griffin 450) incorporates a direct vapor collection system that bypasses the traditional injection port. Vapor pressure is largely temperature dependent, so an external thermal stabilization system and desorber was constructed using a Raspberry Pi 2 mini-computer and Arduino microcontroller to quickly and accurately control the temperature of samples before transfer to the head of the GC column. Temperature control and stabilization allow for certain chemicals to be vaporized that would not normally be present at meaningful concentrations during ambient room temperature analysis. To achieve this, the system utilizes a braided cartridge heater, a 40 mm DC fan, and an analog temperature sensor that work via a negative feedback loop to reach and maintain the selected heating temperature. Software was programmed in Arduino-variant C++. The system utilizes a small touchscreen monitor for quick and accurate user-input. The controller was able to reach temperatures of up to 320 C with an average error of  $\pm 1.5$  C from the selected input temperature. Future work will focus on construction of a heat sink and in testing the reliability of analysis with the desorber against known vapor analysis data.

## EXPRESSION AND PURIFICATION OF HALOFERAX VOLCANII PYROPHOSPHATASE IN E. COLI

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Haloferax volcanii pyrophosphatase (HvPPi) is an enzyme that catalyzes the hydrolysis of pyrophosphate (PPi) into inorganic phosphate (Pi), and has significant activity in organic solvents of high ionic strength. Enzymes that require ATP hydrolysis to drive their catalytic functions like DNA polymerase or enzymes of the ubiquitin pathway can be assessed for their activity by subjecting the PPi biproduct to colorimetric enzyme-assay. With this goal in mind, we aim to purify HvPPi and use it to indirectly measure the activity of UBA5, a ubiquitin-like protein activating enzyme that is involved in many of the same biological processes as ubiquitin enzymes. In light of the discovery of ubiquitin-like protein enzymes, how these enzymes work is still in question. Whereas HvPPi has only been expressed and purified directly from *H. volcanii* in previous studies, we are currently developing a purification scheme for HvPPi expressed in *E. coli*. Here, we show that *E. coli* expressed HvPPi exhibits little interaction with nickel chelating resins relative to HvPPi isolated directly from *H. volcanii*. Instead, we propose a purification scheme utilizing ethanol precipitation, SDS-PAGE, and malachite-green colorimetric assay, effectively taking advantage of HvPPi's properties in an archaeal solvent to remove impurities from the lysate while maintaining activity. Finally, we hope to characterize the three-dimensional structure of HvPPi via X-ray crystallography.

## **THE ROLE OF DITYROSINE IN THE PROTECTION OF *SACCHAROMYCES CEREVISIAE* SPORES FROM UV LIGHT**

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Yeast spores are known to be resistant to many environmental factors including the mutagenic effects of UV light. Yet, yeast spores are nearly colorless and lack a protective pigment such as melanin. Instead, the outermost spore wall of yeast contains dityrosine, a chromophore that absorbs UV light at 300-320 nm corresponding to the mutagenic range of UV light. To further study the role of dityrosine, a mutant strain, *dit1* that is deficient in dityrosine production was created and the spores were exposed to UV light for 3 minutes. Our preliminary results indicate that 65% of the spores survived in the wild-type strain compared to 43% survival in the *dit1* mutant strain suggesting that dityrosine protects yeast spores from the mutagenic effects of UV light.



## **GREEN CHEMISTRY CATALYSTS FOR TRANSFER HYDROGENATION**

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Novel triazole based N-heterocyclic carbene complexes of Rhodium and Iridium with a bridging or bidentate phosphine ligand have been synthesized and characterized using multinuclear NMR and x-ray studies. The catalytic properties of the complexes were studied in transfer hydrogenation reduction of C=O and C=N bonds, where they prove to be very active.

The authors thank Dr. Andrei Astachkine from the University of Arizona for helping with x-ray structures. K.I. acknowledges funding provided by the dean of the School of Science and Mathematics, the Neimeyer-Hodgson Student Research Grant, the Noonan Endowment Award, and the Student Grants for Research and Creative Activity.

## **MUSCLE SYNERGIES FORMED IN THE SPRAGUE DAWLEY RAT'S TAIL IN RESPONSE TO NOCICEPTIVE STIMULI**

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Noxious stimuli can evoke the nociceptive withdrawal response (NWR), which protects the affected part of the body from injury. The rat tail, because of the large number of joint and muscle degrees of freedom, may present a computational challenge to the central nervous system. Previous studies have revealed that synergies act to reduce the number of degrees of freedom across diverse movements in a variety of animals; however, there is little information in mammals on synergistic control of the tail. The long-term specific aim of this project is to test the hypothesis that during the NWR muscle synergies controlling rat's tail reduce the muscular degrees of freedom by recording the electromyograms (EMGs) from intrinsic tail muscles during heat evoked NWRs. Adult, male Sprague Dawley rats were briefly anesthetized (isoflurane). The tail was marked in thirteen equally spaced locations on dorsal surface of the tail for stimulation and tracking. To record EMG, 15 stainless-steel wires (0.002", Teflon insulated, 7 strands, de-insulated for 2 mm) were inserted subcutaneously with 25 gauge, 5/8" needles. 14 wires were inserted at seven adjacent marks for recording. Heat stimuli (980 nm infrared laser diode) were delivered at the 11 marked locations to evoke a NWR that was captured by high speed video (650 fps). EMG was conventionally amplified and filtered. Robust single and multi-unit EMG recording were obtained. EMG was highly modulated by behavior, typically tonically active at rest and increased significantly during tail movement; in some instances EMG became quiescent. In response to heat stimuli, EMG was briefly activated followed by a variable period of silence and gradual recovery to tonic activity. These results demonstrate that intrinsic tail muscles may contribute physically to the NWR and raise the experimentally addressable question whether synergies of intrinsic tail muscles contribute to control of the tail NWR.

## OVEREXPRESSION OF CALVIN CYCLE ENZYME FRUCTOSE-BISPHOSPHATE ADOLASE TO INCREASE ALGAL GROWTH RATE

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Algae are plant-like organisms that can be used for sustainable production of biofuels and other commercially valuable products. *Chlamydomonas reinhardtii*, a single celled green alga, has been used as a model organism to research algal biofuel production, due to its sequenced genome, ability to be genetically manipulated and its fast growth rate. Algal biofuel production could be a cheaper, greener alternative to fossil fuels. Carbon dioxide (CO<sub>2</sub>) is limiting factor for algal growth, so it is believed that certain enzymes that function in the Calvin cycle, which converts CO<sub>2</sub> into carbohydrates, may be key targets for improving photosynthesis and growth. Fructose-bisphosphate aldolase (FBA) functions in the regeneration phase of the Calvin cycle, and overexpression of this enzyme in higher plants improves growth significantly. We have tested the idea that overexpressing FBA will also increase flux through the Calvin cycle in algae. Using recombinant DNA techniques, we have generated *C. reinhardtii* transformants that contain the coding region for *C. reinhardtii* FBA (myc-epitope tagged) under the control of *psbD* and *psbA* 5' and 3' regulatory sequences, respectively, integrated into the chloroplast genome. We used western blot analysis to determine accumulation of transgenic protein and found that transformants accumulate a myc-reactive protein near the expected size of ~44 kDa. Next, we used an algal multicultivator to compare the growth rate of the best expressing transformants to that of a control/recipient strain cc125. If overexpression of FBA improves *C. reinhardtii* growth, we will apply these methods to other algae, such as the biotechnology production organism *Chlorella*.

These results were obtained as part of the Research Experience and Mentoring (REM) program in the Department of Biological Sciences at the University of Maryland Baltimore County. This program is funded by a grant (REM supplement to NSF-EFRI-1332344) from the National Foundation (NSF) Directorate for Engineering (ENG) Office of Emerging Frontiers in Research and Innovation (EFRI). I would also like to thank the Miller lab personnel: Tsegaye Arficho, Erica Dasi, Ayana Mitchell, Jose Ortega, Rudolph Park, Erin Pueblo, Jacqueline Rivera, Elise Thompson and Jihye Yeon. Faculty Advisor/Mentor: Stephen M Miller, stmiller@umbc.edu

## PRODUCTION OF LATENT VIRUSES FROM NITROGEN-FIXING BACTERIAL SYMBIONTS OF SOYBEAN

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*Bradyrhizobium* is a genus of gram-negative soil bacteria. *Bradyrhizobium elkanii*, *B. diazoefficiens*, and *B. japonicum* form a symbiotic relationship with soybean roots by attaching to root hairs and eventually forming nodules. In the nodules, *Bradyrhizobium* cells fix atmospheric, plant-unavailable dinitrogen gas (N<sub>2</sub>) into a plant-useful form of ammonia (NH<sub>3</sub>) in exchange for sugars and other growth factors from the plant. Some subspecies (strains) of these *Bradyrhizobium* have lysogenic viruses (i.e., latent or temperate viruses) that incorporate their genome into that of the bacterium. In this way the viral genome is maintained as part of the host bacterial genome. It is of interest to determine if these incorporated *Bradyrhizobium* viruses (prophages) again become free viruses either through the addition of inducing agents (e.g., mitomycin C or norfloxacin) or spontaneously without an external chemical cue. To determine the nature of their induction, three species of *Bradyrhizobium* (*B. diazoefficiens* USDA 122, *B. elkanii* USDA 76, and farm isolate *B. japonicum* S06B) were grown in laboratory culture, and both replicates of the treatment group (with the addition of chemical inducing agents) and the control group (non-induced) were sampled after 0, 6, 12, 24, and 48 hours. Non-induced replicates of *B. elkanii* USDA76 demonstrated that associated prophages induced spontaneously given significant increases in free virus abundance over time, and a considerably high virus to bacteria ratio at each sample time. These findings may have important implications for our understanding of *Bradyrhizobium* life cycles and interactions with soybean in agricultural environments. The Viral Populations were 10<sup>1</sup> higher than the bacterial population at least at time 0 (viral was x10<sup>8</sup> and bacterial was x10<sup>7</sup>)

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# **IDENTIFICATION AND DIFFERENTIATION OF HOUSEHOLD PET SPECIES USING REAL-TIME POLYMERASE CHAIN REACTION HIGH RESOLUTION MELT (PCR-HRM) CURVES**

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Application of forensic DNA techniques to detect and identify sources of non-human DNA on forensic evidence submitted as part of criminal investigations can aid investigators in identifying pet attacks on humans, other pets, wildlife and livestock but also determine pet species present at a crime scene or on a victim or suspect that can be used to link a victim and perpetrator. In cases of animal victims and human deaths with no witnesses, investigators must rely solely on physical evidence to solve the case. While microscopy is used to differentiate and identify the species origin of hairs, fur, and bones from pets and other animals by morphology of the submitted evidence, in some cases such as pet bites or attacks, body fluids including saliva or blood (or even urine or feces) may be the submitted evidence and these cannot be differentiated by microscopy. Current DNA techniques employed in species testing includes sequencing a gene for comparison to a database sequence (e.g., GenBank); this method is slow, expensive and time-consuming. In this study, we used DNA gene sequences available in NCBI GenBank to identify loci and design primers to separately target several species of interest; the theoretical specificity for each species was determined using NCBI Blast software. We designed six assays that identify and differentiate common household pets including rabbit, parakeet, rat, hamster, guinea pig, and dog with unique melt temperatures. Using PCR followed by high resolution melt (HRM) curves of the DNA amplicons, we tested the primers experimentally. We also developed a PCR HRM duplex assay that can simultaneously detect parakeet and rat DNA by melt temperatures at  $79.19 \pm 0.042$  °C and  $84.94 \pm 0.062$  °C, respectively. We have performed developmental validation tests for the assays including specificity, robustness, reproducibility, and sensitivity testing.

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## SYNTHESIS OF DUAL-ACTING KDM INHIBITOR-ANDROGEN RECEPTOR ANTAGONISTS

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Prostate cancer (PCa) is the third leading cause of death in men over 50. The overexpression of androgen receptors (AR) and histone lysine demethylases (KDM) are underlying causes of PCa. Current medications designed to treat PCa act as AR antagonists but overtime the drug loses its effectiveness on patients. To develop a more effective treatment, a dual-acting antagonist was designed to target both the AR as a gateway into the cell and the KDM to inhibit transcription of oncogenes. Six synthetically similar compounds were synthesized with varying methylene linker lengths (C2-C7) and were tested on four cell lines; DU145 (AR-), MDA-MB-231 (AR+), LNCaP (AR +), and VERO (AR-, control). *In vitro* cell proliferation assays were performed on each cell line to determine compound potency. The compounds exhibited the most activity on the MDA-MB-231 cell line due to the presence of the AR with C2, C6, and C7 being the most potent.

I'd like to thank James Madison University for funding and Georgia Institute of Technology for providing resources for this research.

## **WHOLE-GENOME EPIDEMIOLOGY OF ENVIRONMENTAL *SALMONELLA*: OPTIMIZATION AND INTEGRATION INTO AN UNDERGRADUATE COURSE**

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Approximately 9.4 million cases of foodborne illness occur annually in the United States, with *Salmonella* infections comprising about 1 million of these cases. Environmental reservoirs of *Salmonella* related to agricultural production and runoff may contribute greatly to the dissemination of these potential human pathogens, though they are not well characterized. The surveillance of environmental *Salmonella* is a critical step in monitoring the incidence of potentially pathogenic *Salmonella*, and can be accomplished through whole genome sequencing of isolates and subsequent analyses. Stream sediment from seven sites and chicken litter from five poultry houses in the Shenandoah Valley were sampled during the winter and Spring of 2017. A modified FDA Bacteriological Analytical Manual method of pre-enrichment, enrichment, and isolation was used to isolate 23 putative *Salmonella*. Eighteen were isolated from stream sediments and five from litter from a commercial chicken house. No *Salmonella* were isolated from the small backyard chicken house litter. Putative *Salmonella* were confirmed by polymerase chain reaction amplification of the *Salmonella*-specific *invA* gene and isolates were distinguished using Rep-PCR BOX fingerprinting. The genomes of the isolates were sequenced using whole-genome shotgun sequencing and are in the process of being typed and annotated. Antibiotic resistance and minimum inhibitory concentrations will be determined using Sensititre 96-well plate assays. These methods will be incorporated into a semester-long module in Bacterial Discovery, a new upper-division laboratory course that follows the Course-based Undergraduate Research Experience (CURE) model, and which will pilot in the Spring 2018 semester. In addition to providing valuable skills in bioinformatics to undergraduate students, this project will supplement the efforts of public health organizations in tracking this major foodborne pathogen.

This research would not be possible without funding from 4-VA in collaboration with Dr. Stephen Turner of the University of Virginia Bioinformatics Core. We would also like to thank Curtis Kapsak for all of his guidance throughout this process.

## THERMAL DEHYDRATION OF MAGNESIUM OXALAT DIHYDRATE

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The reversible chemical reactions have been suggested as a possible way to recover waste heat from industrial burners. Since Calcium Oxalate dihydrate has been shown to be an excellent compound for use in waste heat recovery processes, we hypothesize that the chemically similar Magnesium Oxalate dihydrate may be an even better compound since it has two waters of hydration and a smaller molar mass giving it the potential to exchange more heat per gram than Calcium Oxalate dihydrate. The thermal dehydration of magnesium oxalate dihydrate to form magnesium oxalate was investigated using FT-IR, TGA, DSC, and X-RAY diffraction. Isothermal analysis indicated the thermal dehydration of magnesium oxalate dihydrate followed the Avrami-Erofeev equation with  $n = 2$  with an apparent activation energy of 120 kJ/ mol. However, the activation energies determined using the model free steric method indicated that the apparent activation energy was a function of both the sample mass and the extent of reaction indicating that the reaction mechanism was actually more complicated. The enthalpy of the one-step reaction determined from DCS measurements is  $\Delta H^{\circ}_{rxn} = 121$  kJ/mole. Based upon our measurements, Magnesium Oxalate dihydrate produces more heat per mole than the 75 kJ/ mole produced by Calcium Oxalate dihydrate. However, the complex reaction dynamics observed suggest that it may be harder to design an efficient reactor for Magnesium Oxalate dihydrate than it will be for Calcium Oxalate dehydrate

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## **EXAMINATION OF PRENATAL ZIKA VIRUS INFECTION ON INFLAMMATORY RESPONSE OF MATERNAL & NEONATAL BRAIN**

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Zika virus (ZIKV) is a mosquito-borne flavivirus that is of great global concern due to the association between ZIKV infection in pregnant mothers and the increased risk of microcephaly in the fetus, in addition to other neurological conditions. The 2015-16 Zika virus epidemic in North and South America may be over, but there still remains much to be learned about the virus's pathogenesis and mechanism of infectivity, as well as potential behavioral deficits in infants born to infected mothers. By developing a rat model of prenatal ZIKV infection, our lab works to address some of these unanswered questions.

In order to deepen the current understanding of ZIKV infection, we measured febrile response and sickness behavior in infected and non-infected female Sprague-Dawley rats, as well as neonatal mortality, microglia morphology, apoptosis, brain region-specific volume, and other aspects of brain development in neonatal rats born to pregnant females. Based on our previous work, we know that the immune system undergoes substantial changes during pregnancy, with rats being naturally immunosuppressed during this period, so we also examined the effect of pregnancy on ZIKV infection. Overall, this model will help us to better understand the underlying mechanisms of ZIKV infection in the maternal-fetal interface and the impact of the virus on the developing fetal brain.

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## FUNCTION OF THE *NHR-85*/REVERB GENE IN *C. ELEGANS*

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Sleep is a universal requirement of animals. The nematode *C. elegans* does not sleep according to light/dark changes, but rather enters a sleep-like state, lethargus, prior to molts. Previous research indicates that *nhr-85* expression may be upregulated around the period of lethargus.

Nuclear receptor genes are a class of highly conserved genes that function in DNA binding and transcriptional regulation. While *C. elegans* has experienced an expansion of this gene class, and has hundreds of nuclear receptor genes, there are 18 nuclear receptor gene families broadly conserved across animal phyla. *nhr-85* belongs to one of these broadly conserved gene families, the NR1D gene family. While *nhr-85* isn't well characterized, its vertebrate homolog, RevErb, and *Drosophila* homolog, *E75*, are both implicated in circadian rhythm.

A population of a *C. elegans* strain with a *nhr-85* Green Fluorescent Protein translational reporter was staged, and then allowed to feed. Samples of the population were taken every two hours to observe the expression of *nhr-85* during the first larval stage of the worm using fluorescent microscopy.

Preliminary data analysis indicates that gene expression over time is compatible with previous studies of mRNA expression over the first larval stage, where *nhr-85* appears to be increasingly upregulated prior to lethargus. Gene expression seems to be fairly widespread but overall weak. An understanding of the expression of *nhr-85* could be useful in understanding the function of the gene in the worm, as compared to its vertebrate and fly homologs, in addition to a possible larger understanding of the genetic basis of sleep and arousal states.

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## EFFECTS OF A BELLY BUTTON'S ANATOMY ON ITS MICROBIAL LIFE

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Microbial communities, or microbiota, impact human health and disease in numerous ways. Microbiota provide benefits such as serving as the immune system's first line of defense against diseases. In this study, we explored the impact of the anatomy of belly buttons-- (i.e., an innie or outie) on microbial diversity using a metagenomics approach. We hypothesized that innie belly buttons had greater bacterial diversity than outies. To test our hypothesis, we analyzed data depicting bacterial communities in individuals with either an innie (n=219) or outie (n=16) belly button. Our dataset came from Robert Dunn's research on the diversity of bellybutton microbiota (Hulcr, et al., 2012). Using Phinch.org as our platform, we generated two distinct bubble graphs that illustrated the genera of bacteria found in innies and outies. Our findings revealed that innies had a greater diversity of bacteria than outies by nearly five-fold; there were 32 different genera of bacteria in all innies sampled, and there were only seven genera in the sampled outies. Our research demonstrated that innies had more diverse microbiota than outies, which supported our hypothesis. A possible explanation could be that bacteria prefer the moist environments of innies to outies, which are drier and easier to wash. Possible limitations of this study include a small sample size, particularly for individuals with an outie belly button. Possible future directions research include understanding how the diversity of the microbiota differs at various anatomic locations as well as how antibiotics or probiotics impact the diversity of the human belly button microbiota.

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# DOES INSECT FEEDING INCREASE HEALTHY GLUCOSINOLATES IN COLLARD *BRASSICA OLERACEA L.*?

Zoey Lake<sup>1</sup>

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Glucosinolates are secondary metabolites produced by select plant species, including vegetable crops in the mustard family (Brassicaceae) to protect against herbivore damage. Investigations into the human health benefits of glucosinolates have shown potential positive effects, especially anti-cancer activity. However, the specific magnitude and time course of these effects is not well-known. If glucosinolate-producing crops experience insect feeding in the early stages of development, this could result in a health benefit to human consumers of the crops affected. In this pilot experiment, collard plants (*Brassica oleracea* L.), without any feeding or damage, were assigned one of 3 treatments: insect damage, mechanical damage, and undamaged control. We selected harlequin bugs (*Murgantia histrionica* Hahn (Heteroptera: Pentatomidae)), and cabbage looper caterpillars (*Trichoplusia ni* Hübner (Lepidoptera: Noctuidae)) as representative piercing-sucking and chewing feeders, respectively. For the harlequin bugs, feeding was imitated by mechanical damage with a 00-gauge insect pin, while for cabbage looper, the mechanical damage was accomplished with a triangular hole puncher; both forms of damage were conducted 10 times a day over the span of three (3) days, for a total of 30. Samples were collected from each of the treatments on day 3 (a damaged leaf and an undamaged leaf was collected) and on day 10 (one sample was collected, ideally a leaf that developed after the first sampling). Once all samples were collected, freeze dried, and ground, we then sent them to be tested at our partner facility BARC to be analyzed through high-pressure liquid chromatography and mass spectroscopy (HPLC/MS) to quantitate and measure glucosinolate levels. If early damage to crops results in produce with higher health benefits, then higher pest thresholds under integrated pest management (IPM), and possibly accompanying cosmetic insect damage, might be more widely accepted and implemented in future agricultural fields.

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## EXAMINING THE EFFECT OF NUTRIENTS ON ALGAL COMMUNITIES IN THE ANACOSTIA RIVER, D.C.

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The Anacostia River, also known as ‘the forgotten river’, is polluted from various sources such as sewage (human waste), surface storm runoff and groundwater. The different forms of nitrogen (N) entering the river from the Washington DC’s antiquated combined sewer system is a significant concern and has a serious effect on the aquatic biodiversity and ecosystem health. Other studies have shown that when there is more nitrate ( $\text{NO}_3^-$ ), there are more diatoms present while when there is more ammonium ( $\text{NH}_4^+$ ), there are more chlorophytes and cyanobacteria present. To test this hypothesis and investigate ecosystem concerns, water samples were collected biweekly from 11 designated sites on Anacostia River and analyzed for chlorophyll,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , urea, and composition of phytoplankton communities during summer 2017. Results showed that while the total N concentration remained the same throughout the river during the summer, the percentage of each form of N changed from upstream to downstream, and that correlates with the amount of diatoms present. Also, when the concentration of  $\text{NH}_4^+$  increased, there was a decline in the total phytoplankton community biomass. This study contributes to our understanding of influence of nutrients on phytoplankton communities in overly N enriched ecosystem. By understanding the patterns, we can better predict whether the health of the Anacostia River will change after the Anacostia River Tunnel, that will divert sewage away from the river, goes online in March 2018.

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## UTERINE EXPRESSION OF *NHR-67* IN *C. ELEGANS*

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Nuclear hormone receptors are transcription factors that moderate hormone signaling. Eighteen nuclear hormone receptors are conserved across animal phyla; the study of these genes has contributed to our understanding of organogenesis. There is a great expansion of nuclear hormone receptors in some nematodes. In *C. elegans*, the gene *nhr-67*, which is a conserved member of the NR2E family receptors, is an ortholog of *tailless* and *Tlx*. This gene is required for uterine development, functioning upstream of well-characterized EGF and *Notch* signaling pathways. *nhr-67* is expressed in the pre-VU cells and the AC cell during L2 and L3 larval stages of development. Analysis of the *nhr-67* promoter identified 8 sites that control expression of the gene.

Sites 1 and 7 have been identified as E boxes: their binding activity to homodimer HLH-2 is essential for *nhr-67* expression in the uterus. Site 2 is predicted to be a nuclear hormone receptor binding site. Previous analysis of transgenes with deletions to sites 3,4, and 5 determined that these sites are not necessary for gene expression. I constructed a version of the promoter that is tagged with GFP and lacks sites 3, 4, 5, and 6. This construct was injected into *C. elegans* to create transgenic worms that were visualized under a fluorescent source. I observed significantly reduced GFP expression in pre-VU and AC cells indicating that site 6 is required for uterine expression of the gene. Understanding signaling events and nuclear hormone receptors may provide insight on neural development throughout species.

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Sheila Clever

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# **MOLECULAR PROFILING OF MALIGNANT MELANOMA IN THE STATE OF DELAWARE**

## **DEMOGRAPHIC METADATA CORRELATION ANALYSIS PART II**

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Malignant Melanoma is presenting itself all through the State of Delaware as a leading cause behind increased cancer related morbidity and mortality. While following a national trend of increasing prevalence and incidence, Malignant Melanoma has also proven significantly resistant to conventional cancer treatment. Novel molecular markers have evolved recently in correlation with a high percentage of newly diagnosed cases that serve as a target for personalized treatments. In a recent study focusing mainly on the Sussex and Kent Counties within Delaware we were able to demonstrate by using molecular analysis, not only a following of the national trend but also exceeding it in regards to BRAF mutation in Melanomas. The current study will add another target analyte (NRAS) as well as continuation of the BRAF analysis. This will be correlated all through the state with the aim of inferring better representation of the Melanoma status in Delaware as well as produce tentative guidelines for prognostics and management of Melanoma.

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## THE FORMATION OF DAP LIGAND FROM PHENANTHROLINE AND 4-METHOXYPHENYLBORONIC ACID

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The goal of this project is to identify an efficient method for synthesizing dap (dap = 2,9-bis(p-anisyl)- 1,10-phenanthroline), a commonly used ligand related to bipyridine. Specifically, we wished to alkylate 1,10-phenanthroline using 4-methoxyphenylboronic acid using a microwave reactor. Three procedures were adapted for microwave synthesis to see which one would produce the best results.<sup>123</sup>

To begin, 4-t-butylpyridine was used in place of dap to make sure the procedures would progress under the simplest conditions, and to see which procedure would produce the highest yield of desired product. When the procedures were conducted, it was found that the desired product formed using the Baran<sup>1</sup> and Guchhait<sup>2</sup> procedures, but not using the Zeng<sup>3</sup> procedure. To determine which of the former procedures produced the highest yield, the product from each reaction was run through a gas chromatograph, and the ratio of the peak of desired product to the peak of remaining reactant was measured. It was found that the ratio was greater for the Baran procedure than for the Guchhait procedure, and thus, it was determined that the Baran procedure produced the highest yield.

The Baran procedure was then used with 1,10-phenanthroline in place of dap. The product of this reaction was run through a column to isolate the desired ligand. However, no desired ligand was isolated. It was concluded that this was the case because the ligand was polar and large, and thus was not able to run through the column. Had there been more time to complete the project, a more polar solvent would have been used in future columns, and the columns would have been run for a longer period of time. Had this not worked, the Guchhait procedure would have been tested with 1,10-phenanthroline, and had it worked, the Baran procedure would have been applied to dap.

<sup>1</sup>Seiple IB, Su S, Rodriguez RA, Gianatassio R, Fujiwara Y Sobel AL, Baran, PS\*. 2010. Direct C-H Arylation of Electron-Deficient Heterocycles with Arylboronic Acids. *J Am Chem Soc.* 132(38):13194-13196

<sup>2</sup>Guchhait SK\*, Kashyap M, Saraf S. 2010. Direct C–H Bond Arylation of (Hetero)arenes with Aryl and Heteroarylboronic Acids. *SYNTHESIS.* 7:1166-1170

<sup>3</sup>Zeng Y, Zhang C, Yin C, Sun M, Fu H,\* Zheng X, Yuan M, Li R, Chen H\*. 2017. Direct C–H Functionalization of Pyridine via a Transient Activator. *Org Lett.* 19:1970–1973

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## **GENDER AND BACTERIA: OUR SEX AND NUMBER OF BELLY BUTTON MICROBIOMES**

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Humans have trillions of bacteria inside their bodies. Bacteria plays a vital role in our health by maintaining a healthy metabolism and immune condition. We were interested in the effect of gender on the microbial diversity of the human belly button; therefore we investigated the phyla of bacteria found in the male and female navel. We hypothesized that gender influences the diversity of the belly button microbiota. Microbiota is a community of microorganisms. Using a previously collected metagenomics data set that explored the diversity of the human belly button microbiota (Hulcr, 2012), we investigated our data using Phinch.org. For our analysis, we had 143 females and 106 males and we chose to focus on four common bacterial phyla: Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes. We found that for each bacterium women had a more diverse population than men. For example, Bacteroidetes had a total population of 66.15% of bacterial phyla in women compared to 33.85% in men. In conclusion, women have a more diverse bacterial microbiota. We propose that these differences are due to gender norms and hormones. Some limitations that we faced were sample-size and unknown variables, such as an individual's race, health, and diet. Through further research we hope to discover whether these microbes are the reason why women are likely to live longer and/or are susceptible to specific diseases.

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)

## **RHODAMINE-BASED FLUORESCENT AND COLORIMETRIC CHEMOSENSOR FOR METALS IN AQUEOUS MEDIA**

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Metals are inorganic elements that can cause significant health issues in excess. Metals run off into our water supplies through corrosion of pipes and industrial processes. Certain metals are essential to the body such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$ , however in excess these metals can lead to liver and nerve damage. Fluorescent chemosensors have become an important and widely used tool to detect metal ions in biological samples. Selection, and detection of these metals have potential applications in many fields including chemistry, biology, and the environment. The goal of this experiment is to synthesize a fluorescent chemosensor that will detect metals in aqueous media using Rhodamine B. Rhodamine B is a dye known for its great spectroscopic properties such as high fluorescent quantum yield, long absorption, and emission wavelength and high stability to light.

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Funding Provided By: NIGMS-RISE Program

## THE INTERACTION OF THE CLASS II TRANSACTIVATOR WITH 14-3-3 BETA

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The Class II Transactivator (CIITA) is an important transcription factor of Major Histocompatibility II (MHC II) genes. Defects in CIITA, as occur in Bare Lymphocyte Syndrome, lead to the loss of MHC II expression and subsequent inability to activate an immune response. Accordingly, CIITA has often been termed the master switch of the immune system. Its underactivation results in immunodeficiencies whereas its overactivation may lead to autoimmune disorders. Therefore, the activity of CIITA must be tightly regulated through multiple post translational modifications, including phosphorylation and ubiquitination, to ensure proper levels of activation.

We identified a sequence within CIITA from amino acids 283 to 289 that matches a consensus 14-3-3 binding site motif: RxxxpTxP and decided to explore the effects of the 14-3-3 $\beta$  isoform on CIITA. We have shown through IPs that CIITA interacts with 14-3-3 $\beta$  and that this interaction leads to the proteasome mediated degradation of CIITA. Mutants of the binding site are more stable than wild type CIITA, indicating that they are less prone to degradation. Additionally, phosphorylation at the threonine residue in this site is necessary for the interaction and effects of 14-3-3 $\beta$  upon CIITA.

We have shown that 14-3-3 $\beta$  leads to the degradation of CIITA by scaffolding it to a cellular factor and altering its stability. Much work remains to be done to determine what this cellular factor is and the changes in transactivation potential of CIITA as a result of 14-3-3 $\beta$  interaction.

## **CREATING A 3-D WATERSHED MODEL USING GEOGRAPHIC INFORMATION SYSTEM (GIS) AND 3-D PRINTER**

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A lake's watershed is the land where different routes of water flow towards a lake. The lake's health is important for the environment, especially aquatic animals and plants, and for tourism and recreation, an important source of income for many businesses that have lakeshore properties. ArcGIS (geographic information system) is a program that can analyze different layers of information on the watershed that can be selectively viewed from one layer at a time to all layers overlapped on each other. As an example, to identify possible sources of pollution on the watershed, the following layers would be viewed: orthophotography, watershed boundary, land use, contour elevation, digital elevation media, and subwatersheds. However, there is a limitation: ArcGIS presents data in a two-dimensional image, not a physical three-dimensional model which would be an excellent visual aid in the teaching classroom and at lake association meetings.

To develop a 3-D watershed model, the following programs were used: ArcGIS, Blender, and Cura. The contour elevation (without edge lines) layer created in ArcGIS was exported to Blender, which modified the 2-D file to a 3-D file that can be read by a slicing software program, Cura. The sliced 3-D model in Cura allows for analysis of possible flaws in the model and whether adjustments and supports, such as a brim, are required for a successful printed product. Finally, the Ultimaker 2 Extended printer prints the 3-D model using PLA (polylactic acid) filament. Future work is to create a larger watershed model, which is especially necessary for large chains of lakes with massive watersheds.

Many thanks for the laboratory space during the summers: Mr. Kenton Montgomery and his Natural Resources students Kevin Gohman and Cameron Fleischer (Department of Natural Resources, Central Lakes College, 501 W College Dr, Brainerd, MN 56401) and to the American Sign Language interpreters: Jody Converse and Adam Rademacher. And many thanks for the funding support: Gordon Brown Endowment Scholarship Foundation administered by the Gallaudet University Career Center and Anonymous Donor through the Gallaudet University Development Office.

# DETECTION OF NITROTOLUENES IN CANINE FUR SAMPLES USING GCMS/PSI-PROBE

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In order to further investigate the absorptive properties of hair, canine fur samples were analyzed following exposure to nitrotoluenes. This technique for fur sample analysis minimized sample prep and eliminated the use of solvents. Canine fur samples were exposed to Trinitrotoluene (TNT) degradation products 2,4-Dinitrotoluene (DNT) and 2-Amino-4,6-Dinitrotoluene (Amino-DNT) for variable amounts of time and analyzed using Gas Chromatography-Mass Spectrometry (GCMS) coupled with a PSI probe (Prepress Sample Introduction Probe) to determine their absorptive ability. Both DNT and Amino-DNT were detected using this technique after a period of 24 and 48 hours respectively. The impact of fatty acid content and length of exposure on absorptive properties were also investigated. Canine fur was found to be a viable medium for accumulating and retaining explosives residues upon exposure.

# **POSTURAL MOVEMENTS ACCOMPANY THE HEAT EVOKED NOCICEPTIVE WITHDRAWAL RESPONSE OF THE TAIL AND HIND LIMB IN UNRESTRAINED RATS**

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Noxious stimulation evokes the nociceptive withdrawal response (NWR), in which the part of the body stimulated is moved away from the stimulus. Typically, rats are loosely restrained in boxes or tubes. However, there is limited research that evaluates the NWR of *unrestrained* rats. The impact of restraint is underscored by earlier studies in lizards, which showed that as the heat stimulus location moves proximally closer to the base of the tail, the animal tends to walk forward instead of moving its tail. The specific aim of this study was to identify and characterize postural changes that may accompany the NWR of the foot or tail in unrestrained rats. Male Sprague-Dawley rats (n=8) were briefly anesthetized (isoflurane) to mark their tail (five points distributed evenly along the length of the tail), feet (3 points on each of four feet), and body (rostral, caudal) for tracking and stimulation. Upon recovery from anesthesia, animals were centered on a 3'x3' glass table and heat stimuli were delivered in random order to one of the five stimulus locations on the tail or at a central point on one of the four paws. Movement was recorded through a conventional video camera placed below the glass table and subsequently tracked in software. In addition to the expected tail or foot withdrawal, concomitant body movement occurred in 100% of trials. The direction of body movement consisted of both forward translation and rotation away from the stimulus, neither timing ( $p=0.37$ ) nor magnitude ( $p=0.57$ ) varied with stimulus location. The turning was bimodal with the rats either failed to turn or turned  $\sim 180^\circ$ . Variability in turning arose from a significant ( $p<0.001$ ) difference between rats suggesting different rats utilize different escape strategies. Our results suggest that studying the NWR in restrained rats may miss critical elements of animal's escape response.

## **THE ROLE OF CELL CYCLE CHECKPOINT PROTEINS IN CELLULAR ARREST AND ORGANISMAL SURVIVAL UNDER LOW OXYGEN**

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Oxygen, the last electron acceptor in the electron transport chain, is a critical component of oxidative phosphorylation that is required to generate cellular energy (adenosine triphosphate or ATP). Consequently, in complete absence of oxygen, termed anoxia, most organisms experience an imbalance in ATP production and consumption, which leads to cell death. However, some organisms, such as the zebrafish have evolved adaptive mechanisms to survive oxygen deprivation. Under anoxia, zebrafish embryos can reversibly arrest their development to reduce oxygen demand and ATP depletion. In particular, we have found that mid-blastula (“dome stage”) embryos undergo the most clear and rapid arrest in response to anoxia. We have further shown that cell cycle progression in dome stage embryos appears to arrest in S (DNA Synthesis) and G2 (Gap 2), consistent with previously published work. Based on these observations, we hypothesize that anoxia activates a cell cycle checkpoint in S/G2, leading to accumulation of cells in these phases of the cell cycle. We propose that the proteins Retinoblastoma, (a tumor suppressor that restricts the cell’s ability to replicate DNA), p21 and p27 (cyclin-dependent kinase inhibitors), and p53 (a tumor suppressor activated in response to stress signals) may function as anoxia-induced cell cycle checkpoints. The levels and distribution of these proteins will be analyzed using wholemount immunolabeling of dome stage embryos exposed to different durations of anoxia. Thus far, my preliminary data indicates a reduction in the levels of hyper-phosphorylated Retinoblastoma, which may correlate with arrest in S phase. Identification of an anoxia-induced cell cycle checkpoint would represent a significant advance in this field, as this molecule is likely to be the target of a more proximal signal that mediates the adaptive response to low oxygen.

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## THE EFFECTS OF COMMON CHICKWEED ON GARLIC MUSTARD GROWTH

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The plant species garlic mustard (*Alliaria petiolata*) and common chickweed (*Stellaria media*) are both considered invasive species in the United States. Both compete with native flora for sunlight, nutrients, and space. The purpose of this study was to determine if common chickweed inhibits growth of garlic mustard. We hypothesized that size and frequency of garlic mustard rosettes will be reduced near chickweed plants. To test this hypothesis, a field study was carried out measuring garlic mustard rosette size and frequency immediately surrounding a chickweed plant, 50 cm away from chickweed, and 100 cm away from chickweed. Average biomass of garlic mustard from each distance was also recorded. The results showed that immediately surrounding the base of chickweed plants, there tended to be smaller and fewer garlic mustard rosettes. This suggests a possible allelopathic effect of chickweed on garlic mustard growth. A greenhouse study was also conducted growing garlic mustard in soil treatments of garlic mustard soil, chickweed soil, chickweed plants, and ground chickweed matter. There was not a significant trend between treatment and garlic mustard leaf size. This suggests that the fewer number of garlic mustard rosettes observed near chickweed plants in the field may be caused to inhibition of seed germination of garlic mustard by chickweed.



## **DETERMINATION OF ETHANOL CONCENTRATION USING RAMAN SPECTROSCOPY**

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Raman spectroscopy can be used to identify vibrational and rotational modes of molecules, which constitute their structural fingerprint and hence, can be used for quantification. Two laboratory experiments were designed/improved by using Raman spectroscopy to quantify ethanol in samples. The first laboratory activity was created for quantitative analysis. This lab requires students to measure the percent ethanol concentration in a range of commercial alcoholic beverages using a standard curve they develop from ethanol standards. Students will compare these results to those measured in a related lab using infrared spectroscopy (IR) as well as to the reported ethanol concentration in the commercial beverages. Through this laboratory activity, students obtain knowledge of the similarities and differences between Raman and IR spectroscopy. The second experiment looked to improve a preexisting organic chemistry lab where students perform simple and fractional distillations on fermented molasses. Research was undertaken to determine which combination of yeast strain and which brand of blackstrap molasses would produce the most ethanol. Ethanol concentrations were traditionally measured using density but now students will also use Raman spectroscopy and then compare the results from both methods.

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## DETECTING THE PRESENCE OF *BLA* AND OTHER ANTIBIOTIC RESISTANCE GENES IN FRIENDS PARK POND AND ON THE UMBC CAMPUS

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Antibiotic resistance is a serious issue in today's society. People every year are unable to treat simple infections because of the severe overuse of antibiotics. In order to learn more about antibiotic resistance and how prevalent it is in the environment, local water sources were examined. DNA was extracted from water samples obtained from Friends Park in Forest Hill, Maryland and Pig Pen Pond on the UMBC campus. PCR was then utilized to look for the presence of five different antibiotic resistance genes: *bla*, *ampC*, *nptII*, *cat*, and *aacCI*. In the water sample from Friends Park two antibiotic resistant genes, *bla* and *cat*, were discovered. The *bla* gene was chosen for further analysis and was cloned into *E. coli*. Protein expression of the beta-lactamase enzyme encoded by the cloned *bla* gene was performed and characterized by western blot and mass spectrometry.

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# THE EFFECTS OF COMMON DRUGS ON PROTEASOME ACTIVITY IN CARDIAC CELLS

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The ubiquitin-proteasome system (UPS) plays a critical role in cells and its dysfunction may lead to pathogenic roles in cardiomyopathies. The proteolytic component of the UPS, the proteasome, is critical for the intracellular function of cells. The proteasome is the proteolytic enzyme complex for degradation of intracellular proteins including misfolded or damaged proteins. Nonsteroidal anti-inflammatory drugs (NSAIDs) were previously shown to increase the risk of stroke and cardiovascular death. Other studies suggest that two NSAIDs, diclofenac and meclofenamate, cause proteasome dysfunction in cardiac cells. Ibuprofen, a common NSAID, is suggested to increase the risk of myocardial infarctions, strokes, and cardiovascular deaths. Although not a NSAID, caffeine which is a major component found in NSAID, is a compound regularly consumed by two-thirds of Americans was investigated in this study. Caffeine, used at concentrations typically found in human plasma was found to affect proteasome function in cardiac cells. Ibuprofen was also found to cause proteasome dysfunction. These results suggest that a side effect of many NSAIDs and other commonly used drugs may reduce proteasome activity, which is important because proteasome dysfunction has been linked to many cardiac diseases. Techniques used to study the proteasome activity include, cell culturing, western blotting and proteasome assays with and without inhibitors.

Ms Mowoe would like to thank the University of California MCBGAP Program for funding this research. Funded by the American Heart Association 16GRNT31350040

## **DESIGNING AN ORGANIC CHEMISTRY I EXPERIMENT TO PROVIDE HANDS ON LEARNING WITH ORGANIC FUNCTIONAL GROUPS, LAB TECHNIQUES, INFRARED SPECTROSCOPY, AND MASS SPECTROMETRY**

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Knowledge of organic techniques, chemical structure, and experience with state-of-the-art instrumentation is essential to student success and understanding of fundamental concepts in organic chemistry. For many community college students, limited resources have prevented them from gaining experience with state-of-the-art instruments. This lack of experience may hinder their ability to identify unknown compounds and determine chemical structure; and may negatively impact their ability to perform research in organic chemistry and related STEM fields as they move forward in their education and careers. To provide Organic Chemistry I students with a stronger foundation in critical thinking, problem-solving, and lab skills, an experiment has been developed which introduces an array of functional groups, organic lab techniques, infrared spectroscopy, and mass spectrometry. This experiment also incorporates and reinforces knowledge gained in General Chemistry II such as physical properties and the nature of weak acids and bases.

In this experiment, three unknowns, one solid and two liquids, are assigned to each lab group. The unknowns will be identified from a list of twelve possible knowns that include a range of functional groups. By obtaining a variety of physical property data on each unknown, key pieces of information are determined and compared to those of the listed knowns. Once a proposed identity for their unknown is decided students will compare their unknown to the proposed known using infrared spectroscopy. Comparison of the spectra will determine if the two compounds are the same. To further confirm the identity of their unknown, 3-D models of the compounds will be built using model kits and as pieces are broken off, students will analyze if these “pieces” match up with the fragment patterns shown on mass spectra available online. Building 3-D models will help visual and kinesthetic learners grasp the 3-D structure and flexibility of organic compounds.

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## **SYNTHESIS OF A MINI-REPORTER TO TEST RNA THERAPEUTIC STRATEGIES TO BLOCK VEGFR2 AND ANGIOGENESIS IN HUMAN GBM**

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Glioblastoma multiforme (GBM), a grade IV tumor of the central nervous system, is the most common malignant primary brain tumor, and has a median survival of only 14 months. Poor survival is due to a lack of efficacy in current therapies, including radiation and chemotherapy, which is limited by the blood-brain barrier (BBB). GBM survival depends on the formation of new blood vessels, which is essential for the exchange of wastes and nutrients. Endothelial cells connect with each other and form the walls of new blood vessels, bridging the gap between the growing tumor mass and the established vasculature of the circulatory system. The membrane receptor that activates tumors to recruit endothelial cells to create new blood vessels is vascular endothelial growth factor receptor 2 (VEGFR2). In our lab, we are developing a novel therapy to alter the expression of the VEGFR2 receptor. Changes in VEGFR2 expression to block its activation would inhibit the development of new blood vessels. We are designing therapies to bypass the BBB and deliver the genetic sequences of anti-sense RNA molecules to alter the splicing pattern and expression of the VEGFR2 transcript, creating a soluble VEGFR2 decoy. We have designed and are cloning a mini-reporter-system that contains the regulatory elements of VEGFR2 splicing. This system measures the efficacy of RNA anti-sense therapeutics to alter the splicing of the VEGFR2 transcript. The visual marker, eukaryotic green fluorescent protein is used to mimic the natural splicing product, whereas the red fluorescent protein, mCherry detects changes in the efficacy of our RNA anti-sense therapy.

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# NEXT GENERATION SEQUENCING ANALYSIS OF PUMPKIN, A NOVEL MUTATION IN DROSOPHILA

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The *pumpkin* phenotype is a novel spontaneous mutation affecting eye color in *Drosophila melanogaster* originally identified at Montgomery College. Previous mapping results localized *pumpkin* to the X-chromosome, 2.6 cM away from the *ruby* locus. This report describes the process of variant discovery we are using to identify the genetic basis of this phenotype. Illumina paired-end sequencing-by-synthesis of total genomic DNA from both wild-type and pumpkin flies was used to obtain the data used in all subsequent analyses.

Pre-processed sequences obtained from commercially prepared DNA libraries (Macrogen Labs) were aligned to the reference genome of *Drosophila melanogaster* and used to identify variants that are present in *pumpkin* and distinct from wild-type samples, specifically SNPs and indels. The workflow employed to obtain the resulting variant calls uses a variety of command-line tools: Burrows Wheeler Aligner (BWA), SAMtools, BEDtools, BCFtools, VCFtools, and Variant Effect Predictor (VEP).

Raw variants were identified in wild-type and *pumpkin* samples. We focused on variant calls that met the following criteria:

- 1) located on X- chromosome
- 2) located  $\pm 3$  cM from *ruby*
- 3) homozygous
- 4) good depth of coverage
- 5) located in an exon.

This resulted in a small set of candidates. All variant sites were annotated to filter out known variants as well as synonymous base substitutions. Candidates that meet the above criteria will be further analyzed by Sanger sequencing of PCR fragments containing the candidate mutations.

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

## THE EFFECT OF EXTERNAL TEMPERATURE ON LIFE OF *Mobiluncus* BACTERIA IN THE NAVAL

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In this research project, we analyzed a previously performed experiment done by Hulcr and colleagues (2012) which investigated the microbial diversity of the human belly button. Data in this experiment was collected using a citizen science and metagenomics approach. We used the data set provided by Phinch.org to investigate the presence of *Mobiluncus*, a bacterial genus, in the human bellybutton at different external temperatures. Through data analysis, a trend was found in the number of reads of *Mobiluncus* found in each state. Due to this trend, we decided that *Mobiluncus* would be the focus of our research. We chose to make the external temperature the manipulated variable in our research because of the effect temperature can have on bacterial growth. To address our question, we grouped individuals based on the average climate temperature in the month of January and formed the following groups: 15-30°F (n=84) and 31-90°F (n=26). Based on our analysis, we discovered that *Mobiluncus* was more prominent in people originating from warmer cities as opposed to colder cities. This suggests that *Mobiluncus* is more successful in belly buttons of individuals living in warmer climates. This is likely because *Mobiluncus* is a thermophilic or heat loving bacterium. Future goals of this research would be to increase the sample size to better understand the impact that climate has on *Mobiluncus* growth in the human belly button as well factors such as an individual's occupation.

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).

## INVENTORY PLATFORM MANAGES CHEMICAL RISKS, ADDRESSES CHEMICAL ACCOUNTABILITY, AND MEASURES COST-EFFECTIVENESS

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In order to develop best practices for chemical laboratory safety and for chemical management accountability, the freely-available online platform, *Quartz*, was integrated within our department's storage and handling protocols. In addition, *Quartz* facilitated the digital tracking and dispersal of our hazardous waste inventory.

System implementation facilitated stronger teamwork and an improved collaborative culture between the department faculty, the laboratory manager, and the undergraduate laboratory-assistants. Furthermore, besides the incorporation of an improved *safety & documentation* consciousness, we witnessed greater productivity and efficiencies to monitor chemicals and their associated contaminants, to help reduce our environmental footprint. In addition, there was an annual savings balance of \$12,381.53 (August 2015 to August 2016) on a total billing invoice amount of \$48,878.78.

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# **DECOMPOSITION OF THE TAIL NOCIEPTIVE WITHDRAWL RESPONSE INTO COMBINATIONS OF MOVEMENT PRIMITIVES ASSOCIATED WITH INDIVIDUAL MUSCLES IN THE RAT**

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In response to noxious stimuli, animals withdraw the affected body part using the nociceptive withdrawal response (NWR). The CNS may simplify control by reducing the number of muscular degrees of freedom through muscle synergies. The rat tail, which contains a large number of hyper-redundant degrees of muscular freedom, presents a substantial computational challenge. The specific aim of our study is to identify possible muscle synergies in the distal portion of the tail that reduce the number of degrees of freedom by decomposing the behavioral NWR into combinations of movement primitives arising from contraction of individual muscles. Adult Sprague-Dawley male rats were loosely restrained in an acrylic tube and 18 evenly spaced black marks were placed on the dorsal surface of the distal half of the tail. Heat stimuli were delivered in random order to the lateral surface of the tail adjacent to each of the 18 black marks with a laser diode (980nm) and the NWR was recorded using high-speed video (650 fps). Subsequently, the same rat was anaesthetized with pentobarbital (60 mg/kg i.p) and a 2 cm incision was made on the dorsal-lateral surface of the proximal tail. Individual tendons arising from pelvic muscles (n=20-30) were identified and pulled rostrally to create tail, “movement primitives”, and recorded by video. Computationally, the behavioral movements were decomposed into combinations of movement primitives to identify the mostly likely patterns of muscle activity that could have given rise to the behaviorally recorded movement. Motor primitives were diverse, creating movements that resulted in focused tail bends distributed over the entire length of the distal tail. In contrast, behavioral responses to heat stimuli resulted in complex movements of the tail, suggesting that multiple pelvic muscles contribute to the NWR of the tail. Ongoing studies should distinguish between alternate muscle strategies underlying the tail NWR.

## SYNTHESIS OF TETRACATIONIC AMPHIPHILIC VIOLOGENS

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With a rise in antibiotic resistant bacterial strains, the demand for novel antimicrobial compounds had increased. The goal of this study is to produce a series of tetracationic amphiphilic viologen derivatives and to determine their antimicrobial properties. The synthesis of these compounds consists of two steps to produce a polycationic amphiphile. The synthesis and structural analysis of these compounds will be presented. Ongoing experiments include solubility, reactivity, melting point temperature, mass spectrometry, <sup>13</sup>C NMR, <sup>1</sup>H NMR, critical aggregation concentration, and minimum inhibitory concentration studies.

Dr. Brycelyn Boardman, Dr. Debbie Mohler, Dr. Scott Lewis, Dr. Daniel Ralston, Dr. Jun Yin, Sara Kendrick, Interpreters Lauren Hooten, Victor Blanco, Judy Bradley, and Lisa Spurgeon, James Madison University, National Science Foundation-CHE-1461175

## ACTIVATION OF PATERNAL UBE3A GENE WITH ARTIFICIAL TRANSCRIPTION FACTORS FOR ANGELMAN SYNDROME

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Angelman syndrome (AS) is a neurogenic disorder that arises from the loss of function of the *UBE3A* gene from the maternal allele. The *UBE3A* gene lies within a small portion of chromosome 15q11-q13 region in humans and is exclusively expressed from the maternal allele in a healthy human brain. Whereas, the paternal *UBE3A* gene expression is silenced by a nuclear-localized long non-coding RNA, *UBE3A* antisense (*UBE3A-ATS*) transcript. One of the therapeutic strategies for Angelman syndrome is to activate *UBE3A* from the paternal allele by inhibiting *UBE3A-ATS*.

An artificial transcription factor designed in this lab, S1KRAB (S1K), inhibits the antisense strand. The S1K protein, when injected subcutaneously, has shown to cross the blood brain barrier and increase the expression of paternal *UBE3A* in the brain of the Angelman mouse model. Herein, we compared a variant of S1K protein, S1FOG (S1F), which contains the same properties, except a different transcriptional repression motif from FOG proteins. To compare the effectiveness between the two proteins, *UBE3A*-YFP animals were injected S1K or S1F (or zinc buffer as control) for two weeks. At the end of the study, brains were collected for further immunofluorescence analysis for YFP expression. Preliminary results showed that S1F is able to activate *UBE3A*-YFP in a similar manner in different parts of the brain: the cortex, the hippocampus and the cerebellum. Current work focuses on optimizing the dosage for long-term administration of S1K and S1F. *Funded by the Foundation for Angelman Syndrome Therapeutics.*

## **ROLE OF N-MYC DOWNSTREAM REGULATED GENE 1 (NDRG1) IN ADAPTATION OF THE KIDNEY TO LOW OXYGEN**

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Oxygen deprivation, which occurs in pathological conditions such as stroke and congenital heart disease results in irreparable cellular damage and even death in humans. However, a number of organisms, including zebrafish, seemingly defy such odds. Under anoxia (0% oxygen), zebrafish embryos enter a hypometabolic state characterized by reversible developmental arrest that enables them to conserve cellular energy (ATP) and survive for up to 50 hours. Developmental arrest is manifested by cessation of most ATP-demanding processes. The molecules that sense low oxygen and orchestrate arrest are for the most part unknown, yet knowledge of such signals would be tremendously beneficial for therapeutic purposes. In an effort to identify molecules that promote arrest, the Brewster laboratory performed metabolic profiling and found that lactate is one of several metabolites that are up-regulated in embryos exposed to anoxia. Lactate has previously been shown to bind to NDRG3 in hypoxic cancer cells and to promote cell survival - identifying lactate/NDRG as a candidate signal for adaptation to low oxygen. We have further found that NDRGs are expressed in tissues with high metabolic demand in the zebrafish embryo. My research project focuses on NDRG1 that is expressed in the embryonic kidney and ionocytes (which maintain ionic homeostasis). Preliminary data indicate that NDRG1 is localized to the cytosol of ionocytes under normoxia and shifts to the cell cortex under anoxia, where it downregulates the ATP-demanding Na-K-ATPase pump. The goal of my project is to determine whether this change in cellular distribution also occurs in the kidney and if so, to assess whether it is dependent on lactate binding. To address this, I am employing immunolabeling using NDRG1 antibody and a marker for the plasma membrane to examine NDRG1 localization in presence/absence of oxygen and lactate. If correct, this model represents a novel, rapid response mechanism to low oxygen.

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## COMBINATION PHOTOTHERMAL THERAPY AND PHOTODYNAMIC THERAPY FOR CANCER TREATMENT

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Triple negative breast cancer (TNBC) comprises 15-20% of breast cancer cases, but these patients are not susceptible to standard targeted or hormonal therapies. This is because TNBC cells lack the receptors necessary for available targeted therapies to be effective. We developed a novel method to treat TNBC, and other therapy-resistant cancers, by combining both photothermal therapy (PTT) and photodynamic therapy (PDT) to irreversibly damage cancer cells. In PTT, nanoparticles emit heat in response to near-infrared (which ranges from ~650-900 nm) light irradiation. In PDT, photosensitizers exposed to white light induce the production of singlet oxygen, which is highly toxic to cancer cells. In this work, we used 150-nm silica core/gold shell nanoshells (NS) and palladium-10, 10-dimethylbiladiene (Pd-DMBil) as the mediators for PTT or PDT, respectively. We hypothesized that simultaneous PTT/PDT would be more effective than either therapy alone. The effects of dual therapy were evaluated by treating TNBC MDA-MB-231 cells with media, NS only, Pd-DMBil only, or NS and Pd-DMBil at various concentrations for 24 hours. Cells were irradiated with 808 nm light (to excite NS), 500 nm light (to activate Pd-DMBil), both light sources, or no light to measure the effects of PTT and PDT both individually and in combination. Cell viability following irradiation was assessed by Alamar Blue assays. Our data demonstrate that combined PTT/PDT resulted in the greatest loss in cell viability due to the combined effects of both treatments. Importantly, dual PTT/PDT required lower concentrations of NS and Pd-DMBil compared to either therapy alone, which serves to enhance the safety and specificity of treatment by minimizing off-target effects. Together, our results show that combination PTT/PDT could be an effective strategy against aggressive cancers like TNBC.

This work was supported by the University of Delaware Summer Scholars Program and by a University of Delaware Research Foundation-Strategic Initiatives Grant.

## THE PREPARATION OF PALLADIUM COMPLEXES OF N-PYRAZOLYLPROPANAMIDE DERIVATIVES

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The reaction of 3,5-dimethylpyrazolyl-N-isopropylpropanamide (**L1**) with dichloro(1,5-cyclooctadiene)palladium(II) displaces cyclooctadiene forming  $\text{Cl}_2\text{Pd}(\text{L1})_2$ . This complex has been characterized by NMR and IR spectroscopy, single crystal X-Ray diffraction, and elemental analysis. It is composed of a square planar Pd with trans **L1** ligands attached through a pyrazolyl nitrogen and intramolecular hydrogen bonding between the amide and chloride. Similar reactions were carried out with 3-(1H-benzotriazol-1-yl)-N,N-dimethylpropanamide (**L2**), pyrazolyl-N-isopropylpropanamide (**L3**), N-pyrazolylpropanamide (**L4**), 3-(1H-benzotriazol-1-yl)-2-methylpropanamide (**L5**), 3-methylpyrazolylpropanamide (**L6**), and 3,5-dimethylpyrazolylpropanamide (**L7**) presumably forming analogous complexes. This formulation for  $\text{Cl}_2\text{Pd}(\text{L2})_2$  and  $\text{Cl}_2\text{Pd}(\text{L3})_2$  is supported by NMR and IR spectroscopy and elemental analysis. Single crystals of these complexes are awaiting crystallographic analysis. The products of the reactions of **L4** and **L5** are yellow precipitates that are insoluble in most organic solvents. The reaction products of **L6** and **L7** have been characterized by NMR and IR spectroscopy but single crystals have not yet been obtained. The reaction of **L1** with bis(benzonitrile)dichloropalladium(II) produces  $\text{Cl}_2\text{Pd}(\text{L1})_2$  but no reaction is indicated with **L1** and dichlorobis(triphenylphosphine)palladium(II).

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# IDENTIFICATION OF GENES THAT AFFECT ACETYLCHOLINE SIGNALING AT THE *C. ELEGANS* NEUROMUSCULAR JUNCTION

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Acetylcholine (ACh) signaling at the neuromuscular junction (NMJ) is required for muscle contraction. Many muscular disorders arise from genetic mutations altering either ACh release or abundance of post-synaptic acetylcholine receptors (AChRs). Identifying the genes that affect ACh signaling will lead to a better understanding of such disorders. *Caenorhabditis elegans* serves as an excellent model organism for muscular research as the body-wall muscles are functionally similar to vertebrate skeletal muscles. A *C. elegans* genome-wide RNA interference (RNAi) screen identified 156 gene knockdowns that caused either resistance or hypersensitivity to the AChR agonist levamisole. The altered levamisole sensitivity suggests that these genes are involved in ACh signaling. Based on predicted function and homology, we hypothesize that ten of the 156 genes identified in the screen affect AChR trafficking and abundance at the NMJ. Our first goal was to confirm the levamisole phenotype resulting from knockdown of these ten genes. We performed time course assays and found that knockdown of *arf-3* and *sec-12* led to levamisole resistance, while knockdown of *cogc-4*, *epr-1* and *F54D7.2* resulted in levamisole hypersensitivity. Our second goal was to determine which of the ten genes were expressed in the body-wall muscles. Prior studies had shown that *arf-3*, *F54D7.2*, *epr-1*, *sym-4*, *erd-2* and *unc-73* were muscle-expressed, however, the expression patterns for *D1081.4*, *cogc-4*, *nsf-1*, and *sec-12* were unknown. We made constructs consisting of the regulatory and promoter sequences followed by mCherry for each respective gene and then created transgenic worms. Confocal imaging showed that *cogc-4* and *sec-12* were expressed in the body-wall muscles, while *D1081.4* and *nsf-1* were expressed in neurons. Our final goal was to determine if knockdown of these genes affects AChR localization. We are growing worms that express an AChR tagged with YFP on the RNAi clones and will use confocal imaging to quantitate AChR::YFP abundance.

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Tanis Lab members.

## CONDUCTIVE YARN-LIKE THREAD FOR WIRELESS WOUND SENSING

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Chronic wounds affect up to 6.5 million individuals in the United States, and are the source of a growing economic burden, estimated to cost \$2 billion annually in lost wages and work days. Thread-based sensors have recently been demonstrated for continuous, non-invasive chronic wound monitoring. When combined with wireless technologies, these sensors enable chronic wound healing to be monitored continuously and remotely by a health care professions which may help to improve wound health management practices. Here, thread-based sensors are used in conjunction with a microcontroller and Bluetooth module for demonstration of a system capable of remotely monitoring wound area and closure. Thick yarn-like threads were coated in a carbon polymer and were readout using an Ohmmeter to determine wound area. Use of an alginate/carbon hydrogel coating was explored, but deemed too brittle for long-term use. Base resistance measurements of individual threads were found to be between 100 - 200  $\Omega$ . For initial characterization, thread resistance measurements were taken as various volumes of DI water were pipetted onto the thread sensors to mimic wound exudate. These sensitivity measurements were used as a basis for the readout circuitry design. For demonstration of the final system, threads are sewn in a grid-like arrangement into a commercial bandage and placed over an alginate hydrogel as a wound model. Data is acquired using the designed circuitry and is transmitted wirelessly to a mobile device.



## **STUDIES ON ANIT-CANCER ACTIVITY OF ACTIVATED Cdc42-ASSOCIATED KINASE (ACK) INHIBITORS USING v-Ras TRANSFORMED MAMMALIAN CELLS**

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The Ras GTPase family has been found to control different elements of mammalian cells. Our lab has demonstrated a possible role of Ras-Cdc42-ACK signaling in the survival of v-Ras-transformed cells. We also found that ACK-deficient v-Ras transformed NIH 3T3 cells undergo apoptosis while the parental NIH 3T3 cells grow normally. We proposed a hypothesis that ACK-induced phosphorylation plays a critical role in the survival of Ras-induced transformed cells. Based on three-dimensional structure of ACK, our lab in collaboration with other group screens tyrosine kinases inhibitors and identify potential specific inhibitors for ACK. These inhibitors need to be studied for their anti-cancer activity using v-Ras cell lines. In this report, I will present results obtained from studies on the effect of tyrosine kinase inhibitors on growth of v-Ras-induced transformed and parental NIH 3T3 cells.

V-Ras transformed and parental NIH 3T3 cells were cultured, treated with potential ACK inhibitors and incubated in standard condition. MTT assay was used to determine cell growth inhibitory effect. Dose dependent effect of these inhibitors were determined. morphological changes of v-Ras and parental NIH 3T3 cells were studied by staining cells for actin filament followed by studies under fluorescent microscope. The mechanism of inhibition of cell growth by inhibitory compounds was studied further by induction of apoptosis by ACK inhibitors. Immunostaining, immunoblotting, and polymerase chain reaction were utilized to determine expression of genes associated with apoptosis.

It was found that some of the ACK inhibitors activated apoptotic pathways in v-Ras transformed cells. This indicates that apoptosis may be associated with ACK inhibitor-induced cell death. The cell death activity of these inhibitors exhibited feeble activity against parental NIH 3T3 cells. Our results indicated that ACK inhibitors induce preferential death of v-Ras transformed cell.

Our results indicated that ACK inhibitors induce preferential death of v-Ras transformed cell. Identification of a specific ACK inhibitor might be useful in understanding the role of ACK in cancer development as well as the function of ACK in growth and development of mammalian cells. However, specificity of ACK inhibitors need to be developed and studied to better understand the role of ACK in the development of cancerous phenotype in mammalian cells.

## IMPACT OF WASH FREQUENCY ON MICROBIOTA IN A HUMAN BELLY BUTTON

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Microbes contribute to both health and diseases. Dr. Dunn conducted a study on the microbiome of the human belly button to find any possible pathogens. We were interested in learning more about how the human microbiota is altered when an individual bathes. We hypothesized that the wash frequency would impact the diversity of their microbiota. We analyzed the metagenomics data previously published by Dr. Dunn's lab (Hulcr, et al., 2012) using Phinch.org to find noticeable trends. We divided the wash frequencies into 4 different ranges: 0-1 (n=68), 2-3 (n=20), 4-5 (n=19), and 6-7 (n=45) times per week. We chose to investigate the presence of 3 different bacterial genera at each wash frequency. The *Streptococcus* genus increased as the wash frequency increased, while both *Corynebacterium* and *Agrobacterium* decreased. Our data supported our hypothesis, as increased wash frequency had an effect on the microbiota in a human belly button. Interestingly, further investigation revealed that there are pathogenic species in each of the three genera, with further investigation needed to identify how wash frequency impacts the presence of pathogenic bacteria. Some limitations of this data were that the sample size was small and inconsistent among the wash frequencies.

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## EPIDEMIOLOGY OF MUCOPOLYSACCHARIDOSES

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The aim of this study was to obtain data about the epidemiology of the different types of mucopolysaccharidoses in Japan and Switzerland and to compare with similar data from other countries. Data for Japan was collected between 1982 and 2009, and 467 cases with MPS were identified. The combined birth prevalence was 1.53 per 100,000 live births. The highest birth prevalence was 0.84 for MPS II, accounting for 55% of all MPS. MPS I, III, and IV accounted for 15, 16, and 10%, respectively. MPS VI and VII were more rare and accounted for 1.7 and 1.3%, respectively. The high birth prevalence of MPS II in Japan was comparable to that seen in other East Asian countries where this MPS accounted for approximately 50% of all forms of MPS. Birth prevalence was also similar in some European countries (Germany, Northern Ireland, Portugal and the Netherlands) although the prevalence of other forms of MPS is also reported to be higher in these countries. Birth prevalence of MPS II in Switzerland and other European countries is comparatively lower. The birth prevalence of MPS III and IV in Switzerland is higher than in Japan but comparable to that in most other European countries. Moreover, the birth prevalence of MPS VI and VII was very low in both, Switzerland and Japan. Overall, the frequency of MPS varies for each population due to differences in ethnic backgrounds and/or founder effects that affect the birth prevalence of each type of MPS, as seen for other rare genetic diseases. Methods for identification of MPS patients are not uniform across all countries, and consequently, if patients are not identified, recorded prevalence rates will be aberrantly low.

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## **FACILE RHODAMINE-BASED FLUORESCENT AND COLORIMETRIC CHEMOSENSOR FOR SEQUENTIAL DETECTION OF Al (III) and AZIDE (N<sub>3</sub><sup>-</sup>) IONS**

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Fluorescent chemo-sensors have become an important and widely used tool to detect metal ions in biological samples. Aluminum is important in its use in daily life such as being frequently utilized as pharmaceutical drugs in human and veterinary medicine. Excess of aluminum damages the kidney, central nervous system, and reduces the total bone. Rhodamine B derivatives have received a great deal of attention as chemosensors because of their useful properties such as high absorption coefficient, high fluorescent quantum yield for excitation, and emission wavelength within the visible region. These properties give rhodamine an excellent potential for the development of turn-on fluorescent sensors. The properties of a novel, rhodamine-based derivative, synthesized by reacting rhodamine hydrazide and 2-methoxy-1-naphthaldehyde in ethanol, were investigated in aqueous solution. The sensor displayed selectivity for Al<sup>3+</sup>, which was characterized using UV-vis and fluorescence spectroscopy. Based on the study, Al<sup>3+</sup> has a high absorbance and fluorescence enhancement in the presence of a large excess of competing metal ions. Upon adding Al<sup>3+</sup>, the spiro-lactam ring of the sensor was opened and a 1:1 metal-sensor complex was formed. These form excellent probes for the azide ion (N<sub>3</sub><sup>-</sup>) which quenches the fluorescence of the metal-sensor complex by extracting the Al<sup>3+</sup>, confirming that the process is reversible.

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## AN EVALUATION ON THE EFFECTS OF CARBAMOYL CHLORIDES ON MODEL MICROBES

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Pesticides include many different types of chemicals, such as fungicides, herbicides, and insecticides; these compounds can seep into the soil and run off into the rivers. Contaminated run off could potentially have hazardous effects on the bacteria and organisms. Diisocarbamoyl chlorides and diallyl carbamoyl chlorides are intermediates in the synthesis of some pesticides. Carbamoyl chlorides can be mixed with ammonia to create urea, alcohol or phenol to create urethanes, and water to produce an intermediate that decomposes into a secondary amine. Some of the products have been found harmful, but little research has been done to determine the impact of these intermediates. Model bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* can be used as a basis to test the effects of these carbamoyl chlorides on bacteria growth. A variety of gram-positive and gram-negative bacteria allows testing to determine if the effects, if any, are specified to a type of bacteria. The disc dispersion method allows observation of qualitative effects the compounds could have on the bacterial growth on agar, and a dilution series of the compounds can be used to quantitatively determine changes in growth through a growth curve analysis. These two particular carbamoyl chlorides do not appear to have an effect on the well-studied models' growth curve in TSB or lawn growth on TSA. The next steps are to try a simpler media in case there was interference with the discs and the compounds as well as other methods of adding the chemical in during the log phase.

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# COMPARING TRANSCRIPTOME BIOINFORMATICS PIPELINES FOR HUMAN AND CHICKEN RETINAL RNA-SEQ DATA

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RNA-sequencing (RNA-seq) is an approach to transcriptome profiling that uses deep-sequencing for interpreting functional elements of the genome. Traditional RNA-seq bioinformatics workflows begin with alignment of sequencing reads to a reference genome followed by estimation of transcript abundances between samples. The alignment step is both time consuming and outputs very large files creating limiting factors when processing transcriptome data from large numbers of samples. A recently developed RNA-seq bioinformatics pipeline, Kallisto, utilizes pseudo-alignment of sequencing reads for rapidly determining read compatibility to targets without the need for genome alignment. Kallisto excels in speed and accuracy while also outputting files that are much smaller in size compared to traditional transcriptome analysis pipelines. In this study, we compare the Tuxedo protocol, a traditional alignment-based RNA-seq pipeline, to the Kallisto pipeline using human and chicken retinal RNA-seq data generated in our lab. For the human and chicken retinal data sets, 270.8 million and 733.5 million Illumina short read sequences were mapped to the reference human and chicken genome assemblies respectively using the Tuxedo protocol. This pipeline was compared to kallisto-mediated pseudo-alignment of the human and chicken data sets. Comparing analysis pipelines of the human retinal RNA-seq data, the Kallisto pipeline was 26 fold faster than the Tuxedo protocol and produced output files containing 490 fold less data. Similarly, Kallisto ran the chicken retinal RNA-seq data 19 fold faster producing 1239 fold less data. Collectively our data demonstrate that Kallisto is a more efficient bioinformatics pipeline for analyzing RNA-seq data. Current analysis using the Sleuth pipeline is being conducted to analyze and visualize differential expression between samples in our retinal transcriptome datasets.

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# ALTERING THE EPIGENETIC LANDSCAPE: COUNTERACTING THE EFFECTS OF EARLY STRESS VIA EPIGENOME MODIFICATION

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Early childhood trauma is known to affect later-life emotional and cognitive capability. The brain-derived neurotrophic factor (*Bdnf*) gene plays an important role in brain development and plasticity and is highly responsive to environmental factors. Using a rodent model, previous work in our lab has demonstrated epigenetic alterations of this gene following maltreatment in early development. In the current study we investigate the effect of the epigenome-modifying drug Sodium Butyrate on expression of BDNF and various epigenetic regulators in the prefrontal cortex of infant rats exposed to maltreatment.

Using a within-litter design, infant subjects were separated into three different maternal treatment groups: normal-care (subjects in home cage with biological mother), cross-foster care (subjects exposed to a non-biological, lactating dam given ample nesting resources), or maltreatment (subjects exposed to a non-biological, lactating dam with limited nesting resources, eliciting erratic caregiving behaviors). Each of these groups were placed with their respective mothers for 30 minutes per day for the first week after birth, after which they were returned to their biological mother. Subjects received Sodium Butyrate (NaB, a histone deacetylase inhibitor, HDACi) immediately before being placed in their respective caregiving conditions. Brains were extracted on postnatal day (PN) 8 (24 hours after the last caregiving manipulation) and the prefrontal cortex isolated for nucleic acid extraction. Complementary DNA strands were then constructed from extracted RNA and real-time PCR was performed to quantify expression levels of BDNF and epigenetic regulators.

Preliminary results suggest that NaB influences the expression of BDNF and specific epigenetic regulators in a manner that varies by both sex and infant condition. Future experiments will focus on the effects of NaB on maltreatment-induced behavioral outcomes previously reported by our lab. This investigation improves our understanding of the epigenomic effects of adverse early-life experiences and may one day inform treatment and intervention of psychiatric disorder induced by early adversity.

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## LOCAL ADAPTATION OF THE PEA APHID PHOTOPERIOD RESPONSE

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Aphids are mavericks and do not conform to a single mode of reproduction. Instead, reproduction in species such as the pea aphid, *Acyrtosiphon pisum*, is plastic, changing in response to changes in photoperiod. Typically pea aphid populations alternate between viviparous parthenogenesis in the spring and summer, and oviparous sexual reproduction in the fall. In the latter case, eggs produced by sexual females are frost-resistant, allowing a population to overwinter. Reproductive fate (asexual vs. sexual) is specified pre-natally, during embryogenesis, by the mother in response to photoperiod—in particular, short nights specify asexual fates while long nights specify sexual fates. Juvenile Hormone (JH) likely mediates this process, as JH titer correlates with photoperiod and topical application of JH can induce asexual fate, even under long nights. Populations also have the ability to adapt to geographical variation in the timing of the first frost through modification of the photoperiod response. For example, southern populations that experience frost later in the season are induced by longer nights, manifesting as a delay in the production of sexuals and eggs. At the extreme, more southerly populations that are unlikely to see a winter frost may not produce sexuals at all. Here we address the role of JH in the photoperiod response and describe differences in the response between pea aphid populations from the Northern and Southern United States. We also test at least one hypothesis for the evolution of the photoperiod response by examining JH sensitivity and the expression of genes in the JH pathway.

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## ALTERED MICROGLIA MORPHOLOGY AND AGE ACCUMULATION IN THE RAGE- AND DIAPH1-NULL AGED MURINE SOMATOSENSORY CORTEX

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In the Central Nervous System (CNS), aging is linked to functional impairments in microglia, as well as glucose homeostasis in both mice and humans. The production of Advanced Glycation End Products (AGEs), accumulate in aging mice and humans. These AGEs bind their chief cell surface receptor RAGE, (Receptor for Advanced Glycation Endproducts) with pathological consequences. AGE-RAGE ligand binding induces intracellular signaling cascades, in part via diaphanous-1 (DIAPH1), which leads to increased RAGE expression, and the ignition of a positive feedback loop driving chronic inflammation. Over time, this may contribute to inflammatory changes in microglia, which may be quantified by assessing morphological changes through surface area to volume ratio (SA:V). As well as studying processes and branches of microglia

This study aims to examine the changes in microglia morphology and AGE accumulation in the murine somatosensory cortex in the presence and absence of RAGE or DIAPH1. This experimental design will be accomplished through studying aged (24-36 mo) and young (2 mo) global *Ager*-null (referred to as RAGE KO) and *drf1*-null (referred to as DIAPH1 KO) as compared to WT (C57BL/6 mice) young and old mice (N=3). We hypothesize that in aged mice, the absence of RAGE and DIAPH1 will partially rescue cortical microglia from the progression into amoeboid morphology, with decreased SA:V, decreased peak branchiness and soma distance as measured by sholl analysis. In addition, we hypothesize that RAGE KO and DIAPH1 KO mice will display a decreased production of AGEs during old age as compared to WT aged

From our results, we conclude that the somatosensory cortex in RAGE KO mice undergo altered age-dependent shifts in microglia morphology and AGE accumulation, as observed through significantly improved SA:V ratio and peak branchiness of RAGE KO aged cortical microglia, as well as diminished AGE accumulation in the RAGE KO cortex during aging.

This project was supported by the Summer Undergraduate Research program at the Sackler Institute New York University School of Medicine. As well as Director Dr. Patwary and Dr. Kamal of the NIH-RISE program at Medgar Evers College. Special thanks to the Diabetes Research Program at NYU and special thanks to Julia Derk for providing me guidance through this project.

## **INVESTIGATION OF NEW BIOMARKERS FOR MAMMALIAN CORNEAL DEVELOPMENT**

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The eye is a multifaceted organ that functions to transmit, process and relay light-based information as molecular signals to the brain, where this visual stimuli is interpreted as sight. The eye consists of the cornea, aqueous humor, iris, pupil, lens, vitreous humor, retina, and the optic nerve. The cornea is a transparent tissue located at the anterior part of the eye, where it functions to control and focus light into the eye and as the principle barrier. The cornea is formed from ectoderm and neural crest and is composed of five primary layers; epithelium, Bowman's Layer, stroma, Descemet's basement membrane, and endothelium. In mouse, corneal development begins at embryonic day (E) 8, when the head surface ectoderm is developed. The surface ectoderm subsequently invaginates to form the lens vesicle, in the process separating away from the overlying ectoderm, which forms the presumptive corneal epithelium at E11.5. The cornea is fully differentiated by P42. The purpose of this investigation is to identify new candidate proteins that may serve as markers for specific cells in mouse corneal development. Wild-type mice of ICR strain were bred to obtain embryonic and postnatal tissues at various stages and eye-tissue sections prepared using a Leica CM3050 cryostat were collected on glass-slides for immunostaining with confocal microscopy. Immunofluorescence analysis was performed to determine the antibodies obtained from the Developmental Studies Hybridoma Bank (DSHB – an NIH resource) that were effective in detecting proteins expressed in the cornea. After performing immunostaining for potential corneal marker proteins, the slides were imaged using confocal microscopy. Several new proteins were detected to be highly expressed in the mouse corneal tissue at embryonic or postnatal stages. These findings establish the immunostaining protocol for new antibodies that identify proteins that serve as novel biomarkers to characterize corneal development and its associated defects.

## **SELF-POWERED ENZYMATIC BIOSENSOR FOR SIMULTANEOUS DETECTION OF TWO BIOMARKERS OF PARKINSON'S DISEASE**

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Parkinson's disease is a chronic neurodegenerative disorder which affects 1% of the world population over 60 years of age. There is currently no definitive test to detect Parkinson's disease in patients, thus it is diagnosed through symptoms and patient history. The purpose of this study is to fabricate and analyze a self-powered enzymatic biosensor that has the ability to detect biomarkers of Parkinson's disease prior to an onset of symptoms. A specific range of uric acid and glutathione levels in the plasma are the two biomarkers that indicate the possible presence of the condition. It has been found that in a patient with Parkinson's disease, uric acid levels are lower than normal while glutathione levels are higher.

Biosensors function by registering the amount of electrons donated or consumed through an electric current, produced by redox reactions that occurs directly on, or near, the sensor. In order to detect the relative concentrations of the desired substances in the blood, two biosensors must be developed that are either enzymatically hindered or exacerbated by the presence of the biomarkers. This study utilized amperometry and cyclic voltammetry to explore the effectiveness of a laccase cathode and an uricase anode, which respond to either the presence of glutathione and uric acid, respectively, resulting in a decrease in current output. The changes in current is related to the relative concentrations of the substances in the blood, allowing the tested plasma to be compared to both healthy and diseased blood.

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## THE BELLY BUTTON MICROBIOME: INFLUENCES FROM WASH FREQUENCY

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Bacteria are everywhere, including the human body. In regards to human health, microbes can have a symbiotic relationship with the body to help us maintain equilibrium. Understanding these mechanisms is of importance to human health and disease. The purpose of this research was to determine the effect of wash frequency on the microbiota of the belly button. The hypothesis was that the more a person washed their belly button, the less diverse their belly button microbiota was. To address this hypothesis, we used previously collected metagenomics data (Hulc, 2011) that explored the diversity of the human belly button microbiota. Through Phinch.org, we separated individuals into their reported weekly wash frequencies: 0-1 (n=33), 2-4 (n=10), 5-6 (n=8), and 7(n=42). Our results demonstrated an increase of the microbial diversity as an individual's wash frequency increased. For example, individuals with a wash frequency of 0-1 had a frequency of 33 unique bacterial [phyla]. Further, we evaluated the presence of four common bacterial families; Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes. We found an increased prevalence of Firmicutes and Actinobacteria by twice fold, Proteobacteria stayed relatively the same and Bacteroidetes decreased slightly. Therefore, our results refuted our hypothesis, as we observed increased microbial diversity in individuals who washed more frequently. A possible explanation for this finding is that cleaner surfaces recruit the presence of opportunistic bacteria more readily than unwashed surfaces. Future directions of this work would be to evaluate the impact of water temperature and cleanliness, as well as various cleaning products on the microbial diversity.

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).

## **SCREENING AND IDENTIFICATION OF INHIBITORS FOR ACTIVATED Cdc42-ASSOCIATED KINASE (ACK) TO REVERSE v-Ras- INDUCED CANCEROUS PHENOTYPE OF MAMMALIAN CELLS**

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**Hypothesis:** The superfamily of Ras GTPase has been reported to control different aspects of mammalian cell growth. Our lab demonstrated a possible role of Ras-Cdc42-ACK signaling in the survival of v-Ras-transformed cells. We also found that ACK-deficient v-Ras transformed NIH 3T3 cells undergo apoptosis while the parental NIH 3T3 cells grow normally. We proposed a hypothesis that “ACK-induced phosphorylation plays a critical role in the survival of Ras-induced transformed cells”. Based on three-dimensional structure of ACK, our lab in collaboration with other group screens chemicals to identify potential specific inhibitors for ACK. In this report, I will present computer aided screening protocol and the effect of these inhibitors on growth of v-Ras-induced transformed and parental NIH 3T3 cells.

**Method:** I have used software to screen and identify potential inhibitors for ACK. These inhibitors were then used to treat v-Ras transformed and parental NIH 3T3 cells. The cell growth was monitored by MTT assay. I have further studied the effect of these inhibitors on gene expression using immunostaining, immunoblotting, and polymerase chain reaction.

**Results:** I have identified some compounds that have strong affinity for ACK and inhibit growth of v-Ras transformed cells. Treatment of v-Ras cells with these inhibitors was found to activate apoptosis. I have used several markers of apoptosis. These results will be described in this report.

**Conclusion:** I have found that ACK inhibitors inhibited the growth of v-Ras transformed cell. I am designing novel compounds with more specific binding to ACK. Development of specific ACK inhibitors will be useful in better understanding the role of ACK in the growth of normal and cancer cells.

# GENETIC DELIVERY OF RNA MOLECULES TO ALTER EXPRESSION OF EGFR IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM), the most common central nervous system (CNS) malignancy, is characterized by overexpression of the membrane bound epidermal growth factor receptor (EGFR). Activated EGFR promotes GBM tumor proliferation and growth. Current prognosis for patients receiving standard care is approximately fourteen months due to the aggressive nature of this cancer and the isolating abilities of the blood brain barrier. Our novel approach to deliver DNA encoding anti-sense RNA molecules to alter pre-mRNA splicing of the EGFR mRNA transcript in GBM cells has the potential to bypass this barrier. In the strategy presented, we have designed a pre-trans-splicing RNA molecule (PTRM) to deliver a polyadenylation signal (PAS) into the EGFR pre-mRNA transcript upstream of the exon corresponding to the transmembrane domain, altering the mature EGFR transcript. In our design, optimization of the EGFR antisense binding domain and a U7 snRNA-SmOpt localization signal will enable the PTRM to compete against the downstream 3' splice sites of the EGFR transcript, generating a shortened mRNA transcript. This shortened transcript would translate into a non-membrane bound soluble peptide decoy and inhibit activation of the EGFR pathway. The PTRM therapy construct was cloned into an adeno-associated viral plasmid vector and delivered to GBM cell lines. Total RNA was isolated from cells and reverse transcribed using a random primer mix and target-specific primers to generate cDNA. PCR with specifically pre-designed primer sets was used to detect therapy expression and alternative splicing of EGFR transcripts. Our novel approach to harness the cellular pre-mRNA splicing machinery and gene therapy to generate a targeted therapy may be an effective strategy in the treatment of GBM.

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## NEW SYNTHESIS OF N-3 MODIFIED L-NEPLANOCIN ANALOGUES

Megan Stout, Jacquelyn Stubenrauch

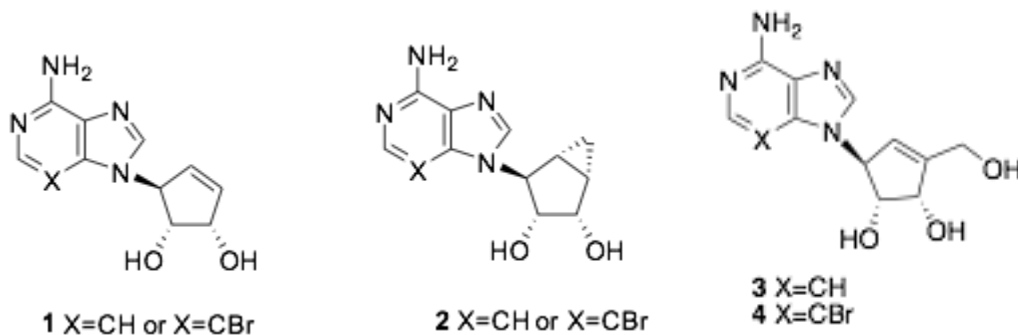
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Previous studies have shown that derivatives of L-carbocyclic nucleosides, such as L-isonoplanocin analogues, possess broad spectrum antiviral activities against Ebola, norovirus, vaccinia, HBV, HCMV, measles, and Dengue. It is noteworthy that replacing the nitrogen atom to a CH or a CBr group at the N-3 position has significant impacts on their biological properties.

Our recent study has also found that L-like, N-3 modified C-4' truncated (DHCDA) (**1**) and 4',6'-methanocarba (MC) (**2**) neplanocin analogues are also effective against norovirus by adopting different conformations.

Following this lead, we have designed and synthesized L-3-deazaneplanocin (**3**) and L-3-deaza-3-bromoneplanocin (**4**) with a newly developed synthesis method, which will further complete the L-like neplanocin collection, and after further comparison with the antiviral activity of well-known D-enantiomers, it will provide further information of the antiviral mechanism for both enantiomers.



## SELECTION OF AN APTAMER THAT BINDS GLUCOSE

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Type 1 diabetes mellitus is a metabolic disease which occurs when the pancreas does not produce enough insulin, resulting in high blood sugar levels. While not life-threatening with proper management, type 1 diabetes continues to affect millions of people. It can be a debilitating and inconvenient condition, as the most popular treatment involves regular injection of insulin.

My project will eventually lead to developing a new form of treatment for diabetes that would be pain free for patients, as opposed to injection. This will be done by converting an aptamer that strictly binds glucose into a riboswitch, which will regulate the production of insulin in the presence of glucose.

To select an aptamer which binds tightly and specifically to glucose, I have used systematic evolution of ligands by exponential enrichment (SELEX). This method begins with amplification of DNA by polymerase chain reaction (PCR). The amplified DNA is then transcribed to RNA to perform both negative and positive selection. Negative selection consists of incubating the RNA with the glucose analogues and magnesium. After incubation, the selection product is analyzed using polyacrylamide gel electrophoresis. Any RNA that has cleaved at this stage is removed, as the aptamer should not cleave without glucose. The next step is positive selection, which consists of incubating the RNA with glucose. At this point, the RNA should cleave because glucose is present, and any RNA that does not cleave is eliminated. Finally, reverse transcription is performed on the positive selection product to prepare for another round of SELEX, starting with PCR.

Ultimately, the goal is to isolate an aptamer which only cleaves in the presence of glucose, then convert it to a riboswitch. Using this technology, blood sugar could be maintained in a less invasive manner, making the lives of diabetes patients substantially easier.

We would like to thank Monmouth University's School of Science for providing the resources necessary to conduct this research. We would also like to thank the Independent College Fund of New Jersey for funding this project. Moreover, we are grateful for the Novo Nordisk scholarship awarded to Emma Stowell.



## THE CORRELATION BETWEEN WASH FREQUENCY AND OPPORTUNISTIC BACTERIA

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Bacteria outnumber human cells ten to one and can support healthy physiological processes. However, when these bacteria are disturbed, opportunistic bacteria may predominate. Opportunistic bacteria can be pathogenic or beneficial. However, correlation between wash frequency and opportunistic bacteria in the body has not been investigated. The present study aimed to address this question. Using a metagenomics dataset that investigated the microbial diversity of the human belly button (Hulcr, 2012), we evaluated the impact of wash frequency on the prevalence of opportunistic bacteria. Using Phinch.org, we grouped individuals into four wash frequency per week categories: 0 (n=31), 1-4 (n=41), and 5-7 (n=46). We focused on the presence of the following opportunistic bacterial genera: *Streptophyta*, *Clostridiaceae*, *Anaerococcus*, *Corynebacterium*, *Prevotella*, *Finegoldia*, *Bacilla*, *Staphylococcaceae*, *Campylobacter* and *Streptococcus*. For individuals who washed 0, 1-4, and 5-7 times a week, we found all these bacterial genera in the human belly button. For most of the genera, the prevalence increased in correlation to the wash frequency. However, *Porphyromonas* and *Campylobacter* decreased in prevalence as wash frequency increased. These two bacterial genera are known to be heat sensitive and a possible reason for the negative correlation could be that the bacteria die during warm showers. Limitations of this study were that there was a small sample size and future directions would be to repeat with a greater sample size. These data support the hypothesis that opportunistic bacteria increase as wash frequency increases, and this could be applied to the medical field to reduce opportunistic bacterial infection risk.

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## **A RHODAMINE-BASED FLUORESCENT CHEMOSENSOR FOR SEQUENTIAL DETECTION OF $\text{Al}^{3+}$ AND $\text{N}_3^-$ IONS**

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Fluorescent chemosensors have become an important and widely used tool to detect metal ions in biological samples. Selection detection of these metals has potential applications in many fields including chemistry, biology, and the environment. Aluminum is important in its use in daily life. Aluminum compounds are also frequently utilized as pharmaceutical drugs in human and veterinary medicine. Excess of aluminum damages the kidney, central nervous system causing Alzheimer's disease, reduces the total bone causing osteoporosis and kills fish in acidic water. Rhodamine B derivatives have received a great deal of attention as chemosensors because of their useful properties such as high absorption coefficient, high fluorescent quantum yield for excitation, and emission wavelength within the visible region. These properties give rhodamine an excellent potential for the development of turn-on fluorescent sensors. The optical properties of a novel, rhodamine-based derivative, synthesized by reacting rhodamine hydrazide and 5-bromo-2-hydroxy-3-methoxybenzaldehyde in ethanol, were investigated in aqueous solution. The novel sensor displayed selectivity for  $\text{Al}^{3+}$ , as evidenced by a colorless to pink color change, which was characterized using UV-vis and fluorescence spectroscopy. Based on the study,  $\text{Al}^{3+}$  has a high absorbance and fluorescence enhancement in the presence of a large excess of competing metal ions ( $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ). It was found that  $\text{Cr}^{3+}$  and  $\text{Cu}^{2+}$  has a slight absorbance and fluorescence enhancement while the rest of the metal ions had low or no enhancement. Upon the addition of  $\text{Al}^{3+}$ , the spirolactam ring of the sensor was opened and a 1:1 metal-sensor complex was formed. These form excellent probes for azide ion ( $\text{N}_3^-$ ) which quenches the fluorescence of the metal-sensor complex by extracting the  $\text{Al}^{3+}$ , confirming that the recognition process is reversible.

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## THE ROLE OF RAB35 IN SEA URCHIN MORPHOGENESIS

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Rab35 is a small GTPase conserved in all metazoans, and responsible for membrane trafficking and endocytic recycling. Many studies have shown the cellular function of Rab35, but little is known regarding its physiological role in development. This study examines the role of Rab35 in cellular morphogenesis. The purple sea urchin is an ideal model organism for studying gene regulatory networks; they shed a large number of gametes, undergo external fertilization, have transparent embryos, and have a relatively short, early developmental period. The two major processes during development that are investigated are the endosomal gut formation, and the ingression of primary mesenchyme cells (PMCs) into the blastocoel that later synthesize the larval skeleton. Real time, quantitative PCR was used to determine the temporal expression of Rab35 at major developmental time points. To test the function of Rab35, we used site directed mutagenesis was used to mutate the Rab35 coding sequence into a constitutently active form (Q67L) and dominant negative form (S22N). The resulting mRNA was microinjected into newly fertilized zygotes, and embryos were cultured. Rab35 morpholino (MASO) was also injected to test the function of Rab35. Rab35-eGFP mRNA was synthesized to show the spatial location of the protein in vivo. We hypothesize that Rab35 is involved in primary mesenchyme cell migration, as well as formation of the gut. This study will reveal what function Rab35 GTPase has in development.

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## **HUMAN ENDOGENOUS RETROVIRUS LONG TERMINAL REPEATS ARE ACTIVATED BY RETROVIRAL POTEINS**

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Increased Human endogenous retrovirus (HERV) expression has been detected in several different human cancers including melanoma and lymphomas as well as autoimmune disorders, neurological disorders and HIV-1 infection. HERVs are transposable genetic elements that occupy approximately 8% of the human genome. The genomic organization of HERVs is similar to exogenous retroviruses, the genome includes *gag*, *pro* and *env* genes flanked by 5' and 3' long terminal repeats (LTRs). There are numerous intact HERV-K genes with open reading frames, but due to mutations and deletions there is no known single provirus capable of replicated competent viruses. HERV-Ks are classified as either type 1, capable of producing the accessory protein NP9, or type 2 viruses, which can produce the accessory protein Rec. Rec bind to the Rec Response Element in HERV-K mRNAs with retained introns to promote their nucleo-cytoplasmic export, analogously to the role of Rev in HIV infection. During HIV-1 infection, the regulatory protein Tat enhances the efficiency of viral transcription. Previous studies have shown that the HIV-1 Tat protein may also play a role in activating HERV-K transcription from the LTRs. It is not known if the HIV-1 Rev protein or HERV-K Rec or NP9 proteins are also capable of activating the expression mediated by HERV-K LTRs. We hypothesize that NP9 may enhance the efficiency of HERV-K viral transcription by interacting with the HERV-K LTR, similar to the role of HIV-1 Tat and the HIV-1 LTR.

To experimentally test how these proteins may enhance expression from the HERV-K LTR and the HIV-1 LTR, the LTRs were cloned into the luciferase plasmid pBV-Luc (Addgene) using with Gibson Assembly. Verified plasmid were transfected into 293T/17 cells using the following combinations: 20ng of the HIV-1 LTR-Luc plasmid with 30ng or 100ng each of HIV-1 Tat or Rev or HERV-K Rec or NP9; 100-250ng of the HERV-K LTR plasmid with 30ng or 100ng each of the plasmid expressing HIV-1 Tat or Rev or HERV-K Rec or NP9. Luciferase assays were performed 48 hours after the transfection and Relative Light Units (RLUs) were detected with a Promega GloMax.

Our results confirm that HIV-1 Tat activates the HIV-1 3' LTR. However, when the HERV-K LTR-Luc Plasmid was co-transfected with the plasmid expressing HIV-1 Tat, our results showed no activation of the LTR. Additionally, our results were inconclusive when the HIV-1 Rev, HERV-K Rec or NP9 were co- transfected with the HERV-K LTR-Luc plasmid.

I would like to say thank you to the National Institutes of Health (NIH) for founding my research project. Thank you to the University of Virginia Summer Research Program for giving me an amazing opportunity. Thank you to my mentor Laurie Gray and my principal investigators Dr.David Rekosh and Dr.Hammarskjöld Marie-Louise.

## **LIBRARY GENERATION AND SEQUENCE ANALYSIS OF *NAP A* GENE VARIANTS FROM A WETLAND IN WINTER**

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The nitrogen cycle is crucial to the health of the environment and local economy of the Chesapeake Bay. However, excess intermediates in the nitrogen cycle can disrupt aquatic ecosystems by causing hypoxic conditions. Excess nitrogen causes proliferation of algae that consume oxygen when they die and decompose. The denitrification pathway, an important part of the nitrogen cycle, removes reactive nitrogenous intermediates from the environment. Of the enzymes in this pathway, the gene *nap A*, which encodes for the catalytic subunit of periplasmic nitrate reductase, is the focal point of our research. Characterization of the genetic diversity of the microbial communities that utilize this pathway may allow us to create more proficient solutions to reduce excess nitrogen in the environment. We have cloned the *nap A* gene variants present in sediments collected from the Beaver Dam Creek watershed on February 11, 2013. The cloning was accomplished via PCR, DNA purification, T/A cloning, and transforming competent *E. coli* bacteria. Comparing sequenced gene variants will allow us to characterize the genetic diversity of the microbial communities performing denitrification and potentially quantify the diversity of the gene from the three sample sites (Parker Pond, Schumaker Pond, and Beaver Dam Creek in Salisbury, MD) over the span of two seasons, summer and winter. Of the 15 clones already sequenced from Beaver Dam Creek, eight are identical DNA sequences, resulting in an initial genetic diversity of 46.7%. In addition, those seven unique DNA sequences also produce unique amino acid sequences. These preliminary results suggest significantly less diversity than that found in previously analyzed summer samples where genetic diversity ranged from 85-91% and protein diversity ranged from 74-85%.

Funding for the generation of the winter library and initial sequencing was provided by the Guerrieri Family Foundation and the Henson School of Science and Technology. Continuing support for this project has been provided by the Irving and Sylvia Cort Scholarship Foundation and the Salisbury University Department of Chemistry.

## EXPOSING THE POSSIBLE HAZARDS OF PLUG-IN AIR FRESHENERS

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Have you ever purchased plug-in air fresheners to give your home or office a clean smell? If so, have you ever considered what exactly it is that you are inhaling and if it is hazardous? Plug-in air fresheners are household products that release scents into room air. To determine the chemicals emitted by plug-ins, air samples were analyzed with particular attention to possible hazardous air pollutants (HAPs), carcinogens, and chemicals associated with asthma or other adverse health effects. To determine the chemical composition of initial emissions from a new plug-in, the headspace above the heated liquid was analyzed by GCMS (gas chromatography mass spectroscopy). To generate a picture of how plug-ins affects room air over time, samples were also collected in a home setting over a one-week period using solid-phase microextraction (SPME) field samplers. Our headspace analysis was done on three well-known brands (Glade, Airwick, and Febreze) and one generic brand (Great Value). We tested scents that were similar to each other from all four brands. The samples included compounds with scent (D-limonene, benzaldehyde, linalool, 3-carene), but surprisingly the generic brand also contained alkane hydrocarbons, which are not particularly associated with desirable smell. Alkane hydrocarbons are associated with petroleum and gasoline. The brand used for the at home setting was Glade. Not all of the compounds in the Glade headspace were detected in room air by SPME/GCMS. Concentrations of the detected Glade compounds increased over a period of days after starting plug-in use and decreased after removing the plug-in from the home setting. Compounds that were most prevalent in the headspace show a clear trend of both increasing and decreasing in concentration in room air, while others showed no trend.

We would like to thank the NASA DC Space Grant Consortium for grant funding to support Trinity Summer Internship Opportunities and as well as Dr. Patrice E. Moss, who administered the program at Trinity.

# QUORUM SENSING REGULATORS CONTROL ECTOINE BIOSYNTHESIS GENE EXPRESSION IN THE HALOPHILE *VIBRIO PARAHAEMOLYTICUS*

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*Vibrio parahaemolyticus* is a Gram-negative halophilic bacterium and the leading pathogenic agent for bacterial seafood-related illness worldwide. This bacterium is found in marine and estuarine environments where it must combat osmotic stress due to fluctuations in salinity.

One adaptive strategy the bacterium uses to survive osmotic stress is the uptake and/or biosynthesis of low molecular weight organic compounds called compatible solutes such as ectoine and glycine betaine. How bacteria regulate the expression of ectoine and betaine synthesis is largely unknown.

We investigated the regulation of ectoine biosynthesis genes and its relationship to quorum sensing, a form of bacterial communication that involves the synthesis of auto inducers that trigger a phosphorelay pathway. This pathway controls the quorum sensing master regulators OpaR (LuxR homolog) and AphA. Preliminary and published data suggest that OpaR and AphA play a direct role in the regulation of compatible solute synthesis.

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Research on *Vibrio* stress response mechanisms is supported by National Science Foundation Grant IOS-1656688 to EFB.

# **UNDERSTANDING THE REGULATION OF HISTONE ACETYLTRANSFERASE (*GCN5*) AND DEACETYLASE (*HDA6*) DURING DROUGHT STRESS IN *PANICUM VIRGATUM* L.**

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Switchgrass (*Panicum virgatum* L.) is a perennial grass that is native to most of North America. This crop is important because it can be used for erosion control, food and shelter for wildlife, and as a biofuel. Switchgrass is favorable because it can grow quickly and it can grow in marginal soil. A major abiotic stress that affects all crops including bioenergy crops is drought stress. Not much is understood how genes are regulated in switchgrass during stress. Histone acetylation and deacetylation play a key role in epigenetic regulation of gene expression. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) activate and silence genes respectively, and they are involved in plant development and respond to environmental stresses. Despite their important role in plants, they are yet to be identified and characterized in switchgrass. We have expressed, cloned and sequenced a HAT gene (*GCN5*) and HDAC gene (*HDA6*) from a lowland ecotype of switchgrass; Alamo. Sequence alignment of both genes with homolog sequences reveal highly conserved regions indicating the functional importance of these genes in plants. Preliminary data from quantitative real-time PCR showed that both *GCN5* and *HDA6* are responsive to drought. Future work would include a comparative expression analysis of both genes during salt and cold stress in an upland and lowland ecotypes of switchgrass. Genome-wide analysis would be necessary to identify proteins that interact with these genes. The findings of this work provide the basis to a better understanding of gene regulation in plants.

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# **MTDNA SEQUENCING IN HEAD-AND-NECK SQUAMOUS CELL CARCINOMA AS AN INDICATOR OF INTRATUMORAL HETEROGENEITY AND LYMPH NODE METASTASIS**

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Head and neck squamous cell carcinoma (HNSCC) accounts for 650,000 new cases worldwide. Its notoriously poor prognosis reflects its propensity to present as metastatic disease upon diagnosis. Prognosis and treatment regimens vary dramatically upon the manifestation of lymph node (LN) metastasis. Detection of regional LN metastasis is usually based on clinical examination and imaging followed by post-operational histological analysis. Unfortunately, current tools for detecting tumor cells in surgically resected LN may miss the presence of micrometastasis. Detection of mutations in minuscule metastatic lesions comprised of a few neoplastic cells may fall below detection thresholds of even highly sensitive techniques like next generation sequencing (NGS).

Due to their clonal nature, higher mutation rate, and copy number, assessing tumor-specific mitochondrial DNA (mtDNA) mutations in histologically clean LN may provide a more sensitive diagnostic tool and eventually reduce the false negative rate in patients. Additionally, circular mtDNA is thought to be more stable than linear genomic DNA, and may also be suitable for sequencing formalin-fixed paraffin-embedded (FFPE)-derived genomic material. However, current methods for library preparation are imperfect to detect low-prevalence variants with a high depth of coverage, especially in highly degraded samples.

To this end, we aimed to set up a novel NGS assay for mtDNA analysis in primary and metastatic samples. An amplicon-based NGS library preparation approach, consisting of 149 primer pairs that cover the entire mitochondrial genome (with a dual coverage of 86.18%) was designed in collaboration with Fluidigm. We sequenced mtDNA from 28 HNSCC tumors (fresh-frozen and FFPE), and multiple matched metastatic or histologically clean LN. We obtained over 97% coverage with a median average depth of 4179X. This method can obtain a snapshot of the extent of mitochondrial heterogeneity and may be used to detect low-frequency tumor-associated mtDNA mutations, which may be possible metastatic processes in histologically clean LN.

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## FEMTOSECOND LASER EYEWEAR PROTECTION: MEASUREMENTS AND PRECAUTIONS

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Unlike continuous-wave lasers, femtosecond pulsed lasers have wide spectral bandwidths and extremely high peak power. Lasers such as Ti:Sapphire oscillators also have an adjustable center wavelength. These factors become an issue when selecting eyewear protection, as the eyewear may not protect the user from the entire laser spectrum, and the integrity of the eyewear material may be compromised by the high peak powers. In this study, commercially donated filter samples were tested to characterize their potential modes of failure using a 1 kHz Ti:Sapphire regenerative amplifier which generated ca. 80 fs pulses with various wavelengths, powers, repetition rates, and beam spot sizes. For some filters, the wide bandwidth and variable center frequency of the laser caused the observed optical densities to be significantly lower than the supplier's rating at the center frequency. The observed modes of failure included melting, burning, bleaching, and saturable absorption behavior. Several filters transmitted several orders of magnitude more light than the supplier's suggested optical density ratings without any physical signs of damage. In general, plastic lenses were considerably more likely to fail, while all glass samples tested maintained their integrity under the conditions tested. The results of these experiments indicate that eyewear protection should be tested under the given experimental conditions to determine their efficacy before use.

### Acknowledgements

This experiment was funded by the Hood Summer Research Institute. Measurements were performed at the National Institute of Standards and Technology which provided the lab space and equipment.

## **VOLATILE ORGANIC COMPOUND PROFILE OF A IN CAR CABIN COMMUTE THROUGH DOWNTOWN, WASHINGTON, D.C.**

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Commuting by car exposes drivers and passengers to pollutants such as VOC's (volatile organic compounds) that can affect one's health. The VOCs benzene, toluene, ethylbenzene, and xylenes (BTEX), are particularly harmful for those who suffer from respiratory and cardiovascular diseases. This study was done to examine the identity and levels of VOCs inside the cabin of a vehicle, during a normal commute through the city of Washington D.C. (from Arlington, VA to North East, D.C.). Ten tests were run over a five-week period. A MiniRAE3000 monitored total VOC concentrations, and SPME (solid-phase micro extraction) field samplers collected samples from air. These SPME samples were later analyzed for chemical composition by GC/MS (gas chromatography mass spectrometry). In general, total VOC levels were higher in downtown DC and decreased in suburban areas of Arlington. Chemical compositions of the VOCs collected by SPME in downtown DC were similar for all ten tests. The most prominent VOCs included BTEX. High exposure to these toxins can cause damage to the overall health of the driver and passengers. The National Institute of Occupational Safety and Health short-term exposure limit is 1.0 ppm (3.2 mg/m<sup>2</sup>) for benzene and 151 ppm (560 mg/m<sup>3</sup>) for toluene. The time-weighted average recommended exposure limits for 10-hour workdays during a 40-hour workweek are 0.1 ppm for benzene and 100 ppm for toluene. Average total VOC levels were ~0.3 ppm in isobutylene equivalents; given published correction factors, 0.1ppm benzene is equivalent to 0.2 ppm in isobutylene equivalents. While further calibration is necessary to determine precise benzene concentrations, BTEX levels during the commute were well below short-term exposure limits but could approach exposure limits for those spending 10-hour workdays in consistently high areas.

We thank the NASA DC Space Grant Consortium for grant funding to support Trinity Summer Internship Opportunities, and faculty and staff, particularly Dr. Patrice E. Moss, who administered the program at Trinity.

# **CHANGES IN IRON, MANGANESE, COPPER, ZINC AND ARSENIC LEVELS RESULTING FROM THE APPLICATION OF POULTRY MANURE TO AGRICULTURAL SOILS**

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Poultry manure is often used to fertilize agricultural fields in the Shenandoah Valley. Manure is used because it provides phosphate and nitrogen, essential nutrients for healthy crop production. The trace metals in poultry manure are also added to soil when poultry manure is applied to fields. Until recently, arsenic was an additive to poultry feed, and therefore was present in manure. The fate of trace metals, including arsenic, is not well known when poultry manure is applied to agricultural soils.

We sampled a field that had been treated with poultry manure, and an adjacent field that had never been treated with manure, to study changes to soil levels of Fe, Mn, Cu, Zn and As when manure is routinely used as a fertilizer. Our results show that over a seven-year period Fe and Mn are depleted from the soil, Cu and Zn increase in the soil, and as levels are unchanged.

This work was supported by the National Science Foundation Research Experience for Undergraduates (NSF-REU) grant number CHE-1461175. We also thank Mr. Mike Phillips for permission to use his farm for this study.

## CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES IN LOCAL WATER SAMPLES

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Antibiotic resistance has become an increasing issue in modern society. Bacteria is continuously becoming more and more resistant to the antibiotics we are creating and it has been difficult for the scientific community to keep up. Antibiotic resistance stems from the DNA in the bacteria, in which genes are always rapidly changing and adapting. This experiment aimed to search for antibiotic resistance genes in freshwater from different areas of the state. Samples were taken from Bynum Run Park in Bel Air, MD and the Library Pond at UMBC. DNA was extracted from the collected water samples and PCR was performed to look for five different antibiotic resistance genes. The *bla* gene was found as well as potentially *ampC*. The *bla* gene was found in the water sample from Bynum Run Park while *ampC* was potentially found in the Library Pond at UMBC. The *bla* gene was chosen for further analysis and was cloned into *E. coli*. The beta-lactamase protein expressed by the cloned *bla* gene was then characterized through SDS-Page gel electrophoresis, western blot, and mass spectrometry. Future directions of the project are to further characterize the other potentially present antibiotic resistance gene, *ampC*, and other possible antibiotic resistance genes that may have been missed previously. Other areas could also be surveyed to collect more data about where antibiotic resistance genes are found. This information would be beneficial to the scientific community as well as just the general public.

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## DISSECTING THE CATALYTIC FRAGMENT OF *PSEUDOMONAS* EXOTOXIN A

Alec Ahearn, Nicolas Philip, Earl Brooks, Olabunmi Olakunle, Victor Eromosele, the Towson University Spring 2017 *Biochemistry Lab* class, and John E. Weldon

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*Pseudomonas* Exotoxin A (PE) is a bacterial toxin secreted by the bacterium *Pseudomonas aeruginosa*. The full toxin contains three domains: a receptor-binding domain, an intracellular trafficking domain, and a catalytic domain. The catalytic domain transfers an ADP-ribosyl group to a specific residue in eukaryotic elongation factor 2 (eEF2), which inhibits its ability to translate mRNA in protein, eventually killing the infected cell. PE has been selected as a potential cancer-fighting drug in the form of a recombinant immunotoxin (RIT): chimeric proteins consisting of a toxic fragment and an antibody. Due to the integration of a bacterial toxin in a therapeutic drug, patient immunogenicity is a major limitation in the employment of RITs. We are interested in reducing the catalytic domain of PE to a minimum size that maintains toxicity in an effort to reduce the likelihood of an immune response in the patient. Because deletions have excessively destabilized the protein structure, we have instead introduced a series of Gly-Gly-Ser substitutions into the catalytic domain of PE. By purifying the resulting variants and studying their structure and function, we are determining the residues most essential for normal catalytic activity.

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**A SURVEY OF SELECTED METALS IN TAP WATER FROM SELECTED  
BUILDINGS AT A SMALL COLLEGE CAMPUS: PRELIMINARY RESULTS  
OBTAINED BY ATOMIC ABSORPTION SPECTROMETRY**

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The results of a survey of selected elements (Fe, Cu, Zn, Ca, Cr, Pb, Al, Cd, Hg, As, Na, K) in tap water from selected buildings on the campus of the University of Pittsburgh – Greensburg will be presented and discussed in this paper. This study was inspired by the recent incidences of lead contamination in the municipal water supplies of Flint, Michigan and Pittsburgh, Pennsylvania. In fact, many municipalities across the United States have had problems with contamination of water used for human consumption by toxic metals or metals that, while non-toxic, can still render water supplies unusable. Not only are municipal water supplies susceptible by toxic metals contamination, well water from springs on private lands can be potentially contaminated by such threats as runoff from abandoned coal mines. The EPA as well as state and local regulatory agencies have set allowable limits on the concentrations of various toxic and some non-toxic metals in water supplies designated for human consumption. Thus, the focus of this study is to determine if there are levels of toxic and other relevant metals in the tap water of the Greensburg campus that are cause for concern, as per the allowable limits for each metal in drinking water set by federal, state and local regulatory agencies. The hypothesis is that Pitt-Greensburg tap water will contain mostly Na, K, Ca, and Mg ions, with lower concentrations of Fe and Zn, while the remaining metal ions to be determined will have concentrations below their detection limits.

Sample collection and preparation procedures will be presented and discussed, as will the determination of each element selected by flame atomic absorption spectrometry (FAAS). The results obtained, and their significance, as well as future directions for this study, will be presented and discussed.

## DESMOPLAKIN MUTATIONS' AFFECT ON STRUCTURE AND STABILITY WITHIN THE SH3 DOMAIN

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Desmoplakin is a large (47 kD) structural protein in the desmosome, a subcellular structure that links the cytoskeleton of one myocyte to that of its neighbor. In total, the desmosome works to propagate the contractile force and allows for the synchronized, strong contractions of the human heartbeat. Desmoplakin structure consists of multiple, tandem spectrin repeat (SR) domains, with a single SH3 domain positioned on top of one of the N terminal SR domains. Recent evidence suggests that this SH3 domain may be a hot spot for disease-causing mutations. Here, we examine several specific mutations both *in vitro* and *in silico*. We find that several mutations linked to sudden infant death syndrome (SIDS) significantly perturb the SH3 domain structure and stability. Thus, these studies provide a compelling molecular mechanism of action for at least a subset of SIDS cases nationwide.

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## CHARACTERIZATION OF SUP35, RNQ1, AND URE2 COTRANSLATIONAL PRION AGGREGATION IN SACCHAROMYCES CEREVISIAE

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Prions are a unique class of self-propagating proteins that can adopt self-propagating misfolded conformations and which can spread through templated self-conversion. In mammals, prions are unerringly deadly elements of disease, underlying the fatal transmissible spongiform encephalopathies. In yeast, however, prions may actually serve to benefit the cell under various stress conditions, and have been shown to confer a fitness advantage in diverse extracellular environments. Prions are thought to originate *de novo* from a single misfolding event that could potentially occur either cotranslationally on the ribosome or post-translationally in the cytosol. While post-translational prion formation is known to occur, cotranslational prion formation has not yet been conclusively demonstrated. To test whether prions can form cotranslationally, we have constructed plasmids encoding Sup35, Rnq1, and Ure2 prion proteins containing an internal hemagglutinin epitope tag and confirmed their expression in yeast. Using these tools, we tested for the association of prion-forming proteins with ribosomes and assessed their aggregation state on the ribosome. Previous work from our laboratory has demonstrated that the ribosome-associated complex (RAC), which protects nascent chains from misfolding, antagonizes prion formation. Thus, the potential exists for regulation of co-translational prion formation in response to environmental stress conditions.

I am grateful to many people for their extensive help with this project, principal among them being Dr. Dale Cameron for his mentorship and guidance. I would also like to thank Tom Tessitore and Usman Baqai for their helpful discussion and assistance in the lab. I thank Annie Li, Sophia Lisowski, Dan Selechnik, and Alex van Ooy for their past contributions and mentorship, and the Ursinus College Department of Biology for laboratory materials and funding. Finally, I thank the National Institutes of Health Academic Research Enhancement Award (AREA) for funding of this project.

## ANALYZING ANTIBIOTIC RESISTANCE GENES IN LOCAL FRESHWATER SOURCES

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Antibiotic resistance occurs when an antibiotic loses its ability to effectively eliminate or restrict bacterial growth. Many bacterial infections throughout the world, including in the United States, are increasingly becoming resistant. Studies have shown that there is a potential correlation between the presence of antibiotic-resistance genes in the environment and human health. This study was conducted to characterize the prevalence of antibiotic resistance genes in environmental water samples. Through further research, the presence of the antibiotic resistance gene *nptII* was identified in two different water samples. *NptII* is an antibiotic resistance gene that prevents the efficiency of the antibiotic Kanamycin, which treats bacterial infections such as tuberculosis.

The experiment began by isolating DNA from water samples taken from Bynum Run Park in Bel Air, MD and the AOK Library pond at UMBC. PCR was then utilized to identify five potential antibiotic resistant genes from the extracted DNA. Of the five potential antibiotic resistance genes, *bla* and *nptII* were successfully identified. *NptII* was chosen to further characterize through cloning the gene into *E. coli* and attempting to express the associated protein. Overall this experiment successfully cloned the *nptII* gene and future directions will include optimizing the protein expression.

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## LYSINE ACETYLATION-DIRECTED EPIGENETIC MECHANISMS SUPPORT THE PROLIFERATION OF PROSTATE CANCER CELLS

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Modulation of gene expression, which are independent of DNA sequence is a phenomenon referred as Epigenetics. Nuclear and cellular environment impact the regulatory circuits of gene transcription that ultimately govern cellular fate and biological processes during normal and disease circumstances. Many of the new Drug discovery programs are targeting the epigenetic mechanisms for a positive disease treatment outcome. One of the epigenetics mechanisms “Acetylation” of aminoacid lysine by histone acetyltransferases (HAT) creates acetylated-lysine (AcK) moiety on chromatin and chromatin-associated proteins to regulate nuclear signaling and gene transcription. These AcK sites serve as a docking site for bromodomain-containing proteins. Dysregulation of this acetylation-mediated molecular processes leads to development of many cancers, including Prostate Cancer (PCa). Therefore, blocking acetylation-mediated events could prevent the growth of PCa.

CREB-binding protein (CBP) is the master coactivator that activates gene transcription through site-specific acetylation of lysine residues on chromatin by its HAT domain. One of the members of BET family, the Bromodomain 4 protein (BRD4) is recruited to the AcK site upon CBP-mediated acetylation. Growing literature suggests that inhibition of CBP HAT activity and BRD4 functions abrogate the growth of melanoma cells. Therefore, in this study, we wanted to test whether and if blocking CBP HAT and BRD4 functions affect the proliferation of PCa cells.

CBP is a coactivator of Androgen Receptor that directs the progression of PCa. We previously demonstrated that acetylation plays a pivotal role in development of PCa. Thus, here, we hypothesize that inhibiting CBP HAT and BRD4 will block the growth of PCa cells. Towards this goal, we tested the effects of CM354 and JQ1 which are inhibitors of CBP HAT and BRD4 in blocking the growth of cancer cells. The study demonstrates that CM354 and JQ1 can indeed induce abrogation of PC3 cells proliferation.

## USING X-RAY FLUORESCENCE SPECTROSCOPY APPLICATIONS TO DESIGN AND IMPROVE SCIENTIFIC EXPERIMENTS

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X-ray fluorescence (XRF) spectroscopy identifies elements by illuminating a sample with X-rays. The excitation and relaxation of the electrons from the innermost atomic shells produces a spectrum from which the elements can be easily identified. Incorporating XRF spectroscopy into chemistry, geology, and physics experiments gives STEM and non-STEM majors the opportunity to further understand concepts covered in the classroom.

The addition of XRF spectroscopy to the General Chemistry I precipitation reaction experiment at Frederick Community College (FCC) introduces the modern analytic technique to CH 101 students. In addition to using a solubility rule table to predicate if a precipitate will form, students are also able to confirm their hypothesis by using the XRF spectrometer to identify the elements present in the precipitate.

XRF spectroscopy can be used in non-STEM majors' geology labs to identify the elements present in various minerals. These results, when used in conjunction with other experimental results, will determine the properties of a given mineral.

Moseley's law, an empirical law that shows a linear relationship between the square root of an x-ray emission frequency and the atomic number of an element, is an important concept in modern physics which can be demonstrated using the XRF spectrometer. In an experiment designed for Modern Physics at FCC, the students use the XRF spectrometer to verify Moseley's law with known compounds, then use the plot of their results to identify an unknown compound.

State-of-the-art technology such as the XRF spectrometer, which is both safe and produces straightforward spectra, is a great tool for engaging non-STEM majors into the scientific method. In addition, it will better prepare FCC STEM majors for transitioning to four-year schools. Use of the XRF spectrometer gives students experience with research grade instrumentation at the community college level.

This project was supported by Hood College, Frederick Community College, and Mount St. Mary's University and funded through the National Science Foundation's Improving Undergraduate STEM Education program (DUE-1431522).

## **STIM1, BUT NOT STIM2, IS THE CALCIUM SENSOR CRITICAL FOR SWEAT SECRETION**

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Sweat glands play a critical role for thermoregulation in humans, and calcium is indispensable for sweat secretion. The mechanism of calcium action in sweat secretion remains to be further elucidated. Recent studies showed that as in other exocrine glands, calcium is acquired by two distinctive but related routes in sweat glands, intracellular release and the influx from interstitium. The calcium influx in sweat glands, as in salivary glands or pancreas, is mediated by a mechanism of SOCE (store-operated calcium entry). Two ER transmembrane Stim proteins, Stim1 and Stim2, play a critical role in SOCE; however, it has been unknown if either or both are expressed and functioning in sweat glands. With genetic mouse models, we demonstrate here that Stim1, but not Stim2, is the calcium sensor critical for sweat secretion.

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## IDENTIFYING THE MOLECULAR COMPONENTS OF COLD NOCICEPTION IN *DROSOPHILA MELANOGASTER*

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Nociception refers to an organism's perception and reaction to noxious stimuli. While nociception is a beneficial behavioral response to harmful stimuli, humans suffer from chronic pain in which pain signals abnormally persist months after any form of trauma, injury or infection. This study aims to better understand the molecular mechanisms of pain by researching the potential role of eight *Drosophila* Innexin gap junction proteins in cold nociception. These invertebrate proteins are evolutionarily similar to mammalian Connexins. To screen for a possible role of the Innexin proteins in cold nociception signaling, the expression level of each protein is knocked down by cell-specific expression of *innexin* RNAi constructs in either all of the dendritic arborization sensory neurons (da neurons) or, in separate trials, expression is knocked down in just the class III da neurons.

Wild type third instar *Drosophila* larvae exhibit a characteristic “cringe” response when exposed to noxious cold. Larvae are placed on a cold plate, and their behavior is videotaped. Subsequently, the larval images are processed using Image J software to quantify the “percent cringe” value for statistical analysis. By comparing the percent cringe of the protein-lacking, experimental larvae to the wild type, the involvement of the knockdown protein in the cold nociceptive signaling pathway can be inferred. Controls utilizing Oregon-R wild type larvae (positive for wild type cringe response) and larvae in which tetanus toxin (TNTE; cringe inhibition control) is expressed specifically in da neurons will be described.

To date, the *ogre*, *shaking-B*, *zero population growth* and *innexin 2* have been tested. Down regulation of *ogre* or *shaking-B* in class III da neurons significantly inhibited cringing (Two-Tailed Fisher Exact Test). We will report the results for the other two Innexins and also the effects of down-regulation in all classes of da neurons.

This work was supported by a 4-VA grant to Susan Halsell, the Jeffrey Tickle scholarship and General Biology scholarship to Rachel Barborek, and research stipends from the JMU Department of Biology to Althea Neighbors and Kendyl Combs.

## **DETERMINATION OF NICOTINE CONTENT IN DOKHA BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)**

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Dokha is a Middle Eastern tobacco product known for its euphoric feeling by users, and has claimed to contain up to 5 times as much nicotine as a normal cigarette. Nicotine  $C_{10}H_{14}N_2$ , also known as 3-(1-methyl-2-pyrrolidinyl) pyridine is a highly toxic alkaloid that will reach the central nervous system within 20 seconds of inhalation. While the lethal dose is ~40 to 60 mg for the average size adult, considerable side effects occur at lower doses. There is minimal research in the scientific community dealing with dokha's nicotine content and the affect that dokha can have on the body. It is currently an unscheduled drug. This work sought to compare the nicotine content in three grades of dokha; light, medium, and hot. In order to determine if the dose of nicotine in dokha is abnormal compared to the levels set by the tobacco industry, our results were compared to the nicotine content measured in Newport cigarettes. Nicotine was extracted using a liquid-liquid methanol extraction. A calibration curve was created from standards run on the gas chromatography- mass spectrometry (GC-MS), allowing for the quantification of nicotine in unknown dokha samples. It was found that Newport cigarettes contained the lowest amount of nicotine compared to the dokha samples, and dokha hot contained the highest amount overall. These results will further help the scientific and forensic communities since it can be used to help determine the source of toxicity where dokha use is suspected.

## USING INTERNAL EPITOPE TAGGING TO DETERMINE LOCATION AND ABUNDANCE OF PROTEINS IN *SACCHAROMYCES CEREVISIAE*

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The budding yeast *Saccharomyces cerevisiae* is an exemplary model organism for studying eukaryotic organisms through the manipulation of its genome. Despite being studied for many years, there are still many *S. cerevisiae* proteins whose locations and abundance we are not aware of because their genes are essential to the organism's survival, making it difficult to identify and isolate them without destroying the *S. cerevisiae*. In particular, Stn1 and Mcd4 have not been visualized in the cell because previous attempts to green fluorescent protein (GFP) tag them disrupted the function of proteins. The purpose of this study is to build the genetic tools necessary to internally GFP tag Stn1 and Mcd4 to select functional tagged versions. Once these genetic tools are built, we will use them to study Stn1 and Mcd4 and their location and abundance within the cell.

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## **AN ASSESSMENT ON THE EFFECTS OF CARBOMYL CHLORIDES ON ISOLATED ENVIRONMENTAL MICROBES**

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Pesticides are known to be used for agricultural purposes to control insects, as well as a weed control. A common component in the synthesis of pesticides are carbamoyl chlorides. Reactions of carbamoyl chlorides can result in the production of urea. The carbamoyl chlorides and their derivatives found in pesticides may also be present in local water sources due to run off. In these water sources, many different microbial communities and lifeforms are present that may be effected by these possibly toxic compounds. The presented research aims to address the unknown extent of the effect the carbamoyl chlorides may have on the organisms in the water. To test and measure this, two methods were employed using collected isolates from the St. Jones River, a local public water source. The first method involved the use of a disk infused with the chosen carbamoyl chlorides. The amount of clearing around these disks were measured to determine the change or lack in growth of the bacteria around the concentrated compound. The second method of measurement was a dilution series where the bacteria were exposed to the carbamoyl chlorides to measure and analyze the changes in their growth. The two methods tested yielded both quantitative and qualitative results showing that the bacteria exposed to the carbamoyl chlorides and the unexposed bacteria grew similarly under the conditions. Though the results were not the same in their respective method experiment, the slight differences were not significant enough to conclusively state that the carbamoyl chlorides had any effect on their growth.

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Assistance and time were provided by Rachel Piper.

# **PATTERNS OF HLH-2 BINDING AT AN NHR-67 PROMOTER ELEMENT MEDIATE UTERINE ORGANOGENESIS**

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The tailless family of nuclear receptors is highly conserved across phyla. In humans, it functions in regulating neuronal stem cell differentiation. The *C. elegans* tailless ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells: initially in the 4 pre-VU cells during the L2, then upregulated in the anchor cell (AC) in response to the *lin-12/lag-2* Notch reciprocal signaling system. During the L3 stage, *nhr-67* expression is maintained at high levels in the AC and at low levels in VU descendants that produce the adult ventral uterus. *nhr-67* is required for expression of the *lin-12*/Notch receptor in pre-VU and VU cells and for multiple markers of AC identity, indicating that it functions in differentiation of both uterine cell types. Loss of *nhr-67* in the AC and pre-VU cells leads to a failure of the AC to exit the cell cycle, indicating that differentiation of the AC is compromised.

Deletion of a 276bp region of the *nhr-67* promoter results in a loss of *nhr-67* expression in pre-VU, AC, and VU cells. A 160bp enhancer that includes eight conserved candidate cis elements is necessary and sufficient for *nhr-67* expression during ventral uterine development. The region includes two E box sequences that we propose bind the HLH-2 transcription factor, which functions in AC and pre-VU development. Deletion analysis and site-directed mutagenesis experiments indicate that homodimeric HLH-2 binding sites are required for AC and pre-VU expression, but loss of HLH-2 compromises reporter gene expression in the AC, but not pre-VU cells. Our data demonstrate the primary role of the E box sequences in regulating *nhr-67* in the AC and pre-VU cells; the former apparently via HLH-2 homodimers and the latter via either another HLH protein or HLH-2 heterodimer.

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## IDENTIFYING ANTIBIOTIC RESISTANCE GENES IN WATER SAMPLES FROM THE UMBC CAMPUS

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An emerging dilemma in the world of science has been the growing resistance of bacteria to present-day antibiotics. Ever since the first distribution of antibiotics in the 1940's, they have been the main method of treatment for bacterial infections. Over time, bacteria have been adapting to develop resistance to these antibiotics. Overuse of antibiotics as treatment methods due to a lack of effective alternatives, combined with the over-prescription to patients who do not need them for treatment, have attributed to speeding up the process of bacteria developing resistance.

In order to survey the prevalence of antibiotic resistance genes in freshwater sources, water samples were collected from Pig Pen Pond, a body of water located on the UMBC campus. DNA was extracted from the collected water samples and PCR was used to identify five potential antibiotic resistance genes. The antibiotic resistance gene *ampC*, a beta-lactamase, was identified. A similar beta-lactamase gene, *bla*, was chosen to further characterize by cloning into a puc19 vector, which was used in order to express the protein of interest in *E. coli*. Finally the beta-lactamase protein was purified and analyzed via mass spectrometry and western blotting.

Sequence coverage of the beta-lactamase protein product was successfully obtained via mass spectrometry. Future directions include identifying the bacteria from the antibiotic genes present through PCR and sequencing, performing protein expression again to obtain higher coverage on mass spectrometry data, and surveying other locations of differing environments for other antibiotic resistance genes.

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## THE EFFECTS OF EUGENOL ON HELA CELLS

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The ability for eugenol (4-allyl-2-methoxyphenol) to induce apoptosis in cervical cancer (HeLa) cells was examined in this study. Eugenol was dissolved in dimethyl sulfoxide (DMSO) prior to diluting in 10% FBS medium. No mitochondrial or nuclear membrane changes were evident besides cells in apoptosis; the number of cells in apoptosis was consistent for all experimental conditions. Extensive cytoplasmic projections were observed in cells exposed to eugenol and DMSO after 72 hours. Exposure to DMSO and eugenol produced a significant increase in cellular detachment ( $p=0.00701$ ). Treatment with 10  $\mu$ M eugenol produced a significant difference in cell proliferation compared to control ( $p=0.0282$ ); the 10  $\mu$ M eugenol treatment did not differ significantly from DMSO control ( $p=0.905$ ). These results suggest that eugenol does not induce apoptosis in HeLa cells; however, based on the presence of cytoplasmic projections and lack of cell proliferation, it is likely that eugenol in DMSO arrests growth and alters development.

We would like to thank Elmira College's Department of Mathematics and Natural Sciences for funding and supporting this research project.

# ALTERATION OF CIRCADIAN RHYTHM RESULTS IN INCREASED VIABILITY AND GROWTH OF *XENOPUS* EMBRYOS

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The South African clawed frog, *Xenopus laevis*, is an excellent model for developmental research as their embryos are large and easily obtainable in mass quantities. Because embryonic development in *Xenopus* occurs completely externally, they are extremely useful for testing the effects of physical and chemical agents on various aspects of vertebrate embryogenesis. In this study, we sought to determine the effect that varying light sources and changes in circadian rhythm had on embryonic development.

Circadian rhythm is a biological process whereby living organisms oscillate gene expression, metabolic activity, and hormone signaling over a 24-hour period. Circadian rhythm itself is largely driven by changes in light that occur from day to night. Experiments in mice have shown that changes in circadian rhythm are associated with altered metabolism and obesity. We proposed that metabolic changes in *Xenopus* embryos would occur in response to alterations in light exposure, and that this would result in abnormal growth.

*Xenopus* colonies maintained in the laboratory are normally exposed to 12-hour light-dark cycles to maintain proper circadian rhythm. We altered circadian rhythm by exposing embryonic and juvenile tadpoles to 24-hour light, or 24-hour darkness. Additionally, we tested whether the type of light exposure would affect frog size by exposing 24-hour light embryos to either fluorescent, halogen, or LED (light emitting diode) light sources. Interestingly, 24-hour light exposure led to increased viability of early frog embryos, and there was a significant increase in tadpole size when the dark cycle of circadian rhythm was abolished. Additionally, frogs exposed to 24-hour fluorescent or LED light were significantly larger than control frogs exposed to 12-light-dark cycles under fluorescent light. Collectively these data suggest a role for light in the metabolic processes associated with circadian rhythm in the developing frog, and that equilibrations in daytime metabolic activity may result in enhanced growth.

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# **ASSESSING THE ROLE OF VIRAL PROTEINS IN THE CASPASE-MEDIATED REGULATION OF INTERFERON $\beta$ DURING KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS INFECTION**

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Kaposi's sarcoma-associated herpes virus (KSHV), the etiological agent of Kaposi sarcoma (KS), is one of the most common causes of cancer deaths in sub-Saharan Africa. To successfully replicate and establish infection, KSHV must overcome innate immune responses including type I interferon (IFN) responses. The Gaglia lab found that KSHV can inhibit induction of type I IFN, specifically IFN $\beta$ , through a novel mechanism dependent on caspases, cellular proteases involved in apoptosis. Pharmacological inhibition of caspases in KSHV-replicating cells potentiated induction of IFN $\beta$ , suggesting that caspases degrade or process proteins that control IFN $\beta$  induction. However, the identity of these protein(s) is unknown. Two KSHV proteins are known to be cleaved by caspases. KSHV ORF57 is cleaved by caspase-7 and its herpes simplex virus homolog inhibits IFN induction. Additionally, caspase cleavage sites have been identified in the N-terminus of KSHV LANA, and N-terminal truncated isoforms of LANA decrease type I IFN response. I hypothesized that caspase-dependent cleavage of a KSHV factor activates a new function of these proteins, and inhibits immune responses. To test this hypothesis, I tested the effect of over-expressing cleaved form of ORF57 on induction of IFN $\beta$  transcription after caspase inhibition. I also investigated whether caspase inhibition affected the processing of ORF57 and LANA in our system. To study KSHV during replication, I used iSLK.219 cells, an epithelial line latently infected with KSHV that can be lytically reactivated through doxycycline-inducible expression of a viral factor, and the pan-caspase inhibitor IDN-6556. I confirmed that caspase inhibition affected the production of LANA isoforms, while I was unable to specifically detect ORF57 and thus assess its cleavage. Overexpression of the ORF57 cleaved product did not prevent IFN $\beta$  induction in response to caspase inhibition, suggesting that ORF57 cleavage does not play a role in down-regulating IFN $\beta$  transcription.

This work was supported by the Building Diversity in Biomedical Sciences program (R25 HL007785)

# **DENDRITIC COMPLEXITY OF SUPRAGRANULAR EXCITATORY NEURONS BY FUNCTIONAL CORTICAL AREA AND ANATOMICAL DEPTH**

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Dendrites of supragranular excitatory neurons form synapses with neighboring populations of cells to receive sensory or motor information. It is not well understood how the dendritic morphology of neurons of the same subtype and cortical layer vary based on the absolute depth within the cortex and functional cortical area. Since different cortical areas in the brain process distinct sensory and motor information, patterns of synaptic interconnectivity, driven by the complexity of cortical neuronal branching, could be major contributors to the functional specialization of cortical areas. These contributors can be quantitatively measured by analyzing basal dendritic arborization. Our hypothesis is that dendritic complexity in supragranular cortical pyramidal neurons (labeled through in utero electroporation) will change in accordance with functional cortical area and become more complex as cortical depth increases. Here, we use a planar coordinate map normalized to the Allen Brain Atlas to position neurons relative to anatomical landmarks in the brain. This will allow us to relatively position each identified neuron in a distinct cortical region and compare their dendritic complexity to neurons in other functional areas. These comparisons will provide insight into how dendritic organization influences functional processing within distinct cortical areas and by cortical depth.

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## **EFFECTS OF SALT AND ALCOHOL ON TRIBLOCK COPOLYMER PHASE TRANSITION BEHAVIORS**

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In this paper, the effects of salt and alcohol on the aggregation of a triblock copolymer, poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO), were studied by using the OptiMelt automated melting point system. It was found that NaCl promoted aggregation of the polymer and decreased the phase transition temperature of the polymer while NaSCN showed opposite behavior. Introduction of methanol into the salt-polymer mixture enhanced the effects of salts on the aggregation of the polymer.

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Department of Chemistry and Biochemistry, James Madison University



# **THE EFFECT OF UNIVERSAL SCREENING AND TESTING FOR *CHLAMYDIA TRACHOMATIS* DURING PREGNANCY ON *CHLAMYDIA CONJUNCTIVITIS***

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There are no epidemiologic studies of *Chlamydia trachomatis* (CT) eye infections in infants in the U.S. that evaluate the effect of universal screening and treatment (UST) of pregnant women, as recommended by the CDC in 1993.

Hypothesis: There is no current Study of how the burden of eye disease in infants caused by *Chlamydia Trachomatis* has changed in relationship to this intervention. As such, the effects of the screening on this disease will be analyzed.

This is a retrospective observational study of all CT eye cultures submitted to the *Chlamydia* Research Laboratory at SUNY Downstate Medical Center. All culture reports from 1986-2002 were reviewed and analyzed according to calendar year and time period (pre-UST era = 1986-1993; post-UST era = 1994-2002).

Results: During the study period a total of 880 samples were tested by *Chlamydia* culture. Of these 103 were positive (11.7%). The number of submitted samples and positive cultures both declined over time ( $p < 0.001$ ). The culture positivity rate declined from 15.6% during pre-UST (1986-1993) era to 1.8% during post-UST era (1994-1998) ( $p < 0.0001$ ).

The healthcare burden of infant conjunctivitis caused by CT decreased significantly in the study population since the implementation of routine CT screening and treatment of pregnant women. Our results confirm the effectiveness of this important public health intervention in the U.S.

We would like to acknowledge the Research Initiative for Scientific Enhancement (RISE) Grant for funding of this research along with the department of Biology. Most importantly, Dr. Patwary and Dr. Kamal of Medgar Evers.

## **EFFICIENCY OF SILVER NITRATION FILTRATION SYSTEM WITH *E. COLI* AND *S. AUREUS***

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Ceramic water filters (CWF) are the most common type of water filtration system. The addition of silver has been shown to improve the efficiency of eliminating bacteria in the water. There are a few ways to adhere silver to the filter: casein-coated silver nanoparticles (nAg) or silver nitrate (AgNO<sub>3</sub>). In the past, there have been problems in how the silver nitrate is adhered. If it is not properly attached, silver ions can be released which can alter the health of individuals who drink the filtered water (Mittelman, et.al. 2015). However, in this case, the method used was to directly coat the ceramic nanoparticles with silver nitrate. These coated particles were packed into a filtration unit. *Escherichia coli* and *Staphylococcus aureus* were used to determine the number of bacteria present in the final effluent sample after filtered through the material. The results concluded bacteria grew on the filter itself and was present in the effluent water. Future analysis of the silver nitrate adhesion to the surface of the nanoparticles will be investigated.

Dr. Christine Bezotte

*Associate Professor of Biology at Elmira College*

Nicholas Rozard Dosch

*Entrepreneur of Triton Ceramics*

## MIGRATORY RETURN RATES AND BREEDING FIDELITY IN EASTERN BLUEBIRDS (*SIALIA SIALIS*)

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Many migratory birds have low rates of return to their previous nesting locations and mates. These low rates are likely due to the high mortality during migration, making it impossible for reunion to occur. We monitored 25 pairs of Eastern Bluebirds (*Sialia sialis*) across 3 breeding seasons to determine their return and reunion rates. The Birds of North America (BNA) report a range of return rates from 26% in Minnesota to 70% in South Carolina. Eastern Bluebirds are partial migrants, and we suspect the populations in Maryland are short distance migrants. Previous studies of return rates of Eastern Bluebirds have primarily focused on either long distance migrants or permanent residents. Therefore, few studies have focused on the return rates of short distance migrants. The study of migration in short distance migrants is interesting because variation in migration has large effects on the evolution of breeding systems and mate choice. Our data suggest that Eastern Bluebirds in Maryland have high return and reunion rates. Many of the bluebirds that mated together in previous breeding seasons appear to not only reunite the next year but also return to the same territory and nesting cavity.

I would like to express my gratitude to the Honors College for funding my research with the Honors College Research Award. I would also like to thank UMBC for supporting my research with the travel grant that allowed me to present my research at the joint meeting of the American Ornithological Society and the Society of Canadian Ornithologists that was held at Michigan State University in East Lansing, Michigan.

## ANALYSIS OF ANTIBIOTIC RESISTANCE GENES FOUND IN FRESHWATER

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Antibiotic resistant bacteria have become more prevalent in recent years. This poses major problems to the medical field since bacteria are developing resistance to antibiotics faster than new antibiotics are being produced. To investigate the prevalence of antibiotic resistant bacteria in the environment, water samples were collected from the Library Pond on the UMBC campus and Bynum Run Park in Bel Air, Maryland. PCR was used to identify if the antibiotic resistance genes *bla*, *ampC*, *cat*, *aacC1*, or *ntpII* were present in the samples. It was determined that the *ntpII* gene was present in the Library Pond sample. In order to further characterize the *ntpII* gene, it was cloned into *E.coli*. and the associated protein (aminoglycoside 3'-phosphotransferase) was expressed. The results of the experiment showed that the *ntpII* antibiotic resistant gene was successfully cloned into *E.coli*. Future projects will focus on getting more samples from the environment in order to survey the prevalence of antibiotic resistance genes, and optimizing the protein expression of identified genes.

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**DETERMINING THE OCCURRENCE OF SEX-BIASED DISPERSAL AND  
POTENTIAL FOR INBREEDING IN AN ENDANGERED SPECIES, THE SPOTTED  
TURTLE (*CLEMMYS GUTTATA*)**

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The spotted turtle (*Clemmys guttata*) is an endangered species found in small disparate populations in the eastern North America. This species is vulnerable to inbreeding, which is more common in smaller populations, potentially causing inbreeding depression, with low fecundity and high mortality. One way to help better manage conservation efforts for protecting spotted turtles is to determine if they are exhibiting sex-biased dispersal, which can reduce inbreeding. Sex-biased dispersal occurs when one sex remains at its natal home while the other sex is more likely to disperse to a new location where there are few to no relatives present. We hypothesize that spotted turtles will exhibit a female-biased dispersal because this species exhibits sexual dimorphism with females more colorful than males, potentially due to females competing for mates. We compared relatedness among males with relatedness among females by genotyping turtles at 12 microsatellite loci and using this data to analyze spatial genetic structure of males and females, which reflects prior dispersal movements. We found that spotted turtles, both males and females, have positive genetic structure only up to 8 km, meaning that both sexes are unlikely to disperse between populations. However, despite rarely leaving their natal populations, both sexes disperse broadly within those populations. While we did not detect sex-biased dispersal within or between populations, our results show that if turtle populations are large enough, inbreeding will be alleviated by the propensity for within-population dispersal by both sexes. However, because our results show that dispersal of both sexes between populations is very limited, and, as many populations of this endangered species are small, potential for inbreeding depression should be a major concern. An important focus for conservation efforts to reduce inbreeding in this species should therefore be to increase population sizes in existing populations by expanding available habitat.

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## NON-INVASIVE PULMONARY FUNCTION TEST ON MORQUIO PATIENTS

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Morquio patients, in many cases, present with severe tracheal narrowing and restrictive lung problems making them susceptible to high mortality arising from sleep apnea and related complications. Tracheal obstruction with growth imbalance, short neck, adeno and tonsillar hypertrophy, large mandible, and/or pigeon chest also contributes to the challenges in managing the airway with intubation and extubation due to factors intrinsic to Morquio syndrome. Taken together, these issues lead to serious respiratory distress and life-threatening complications during anesthetic procedures. Furthermore, patients with Morquio syndrome frequently cannot perform standard pulmonary function tests as a result of their distinctive skeletal dysplasia and chest deformity, thus making diagnosis of incipient pulmonary disease difficult. In many cases, conventional spirometry is too difficult for patients to complete, deriving from issues with cooperation or clinical circumstance. Therefore, it is an unmet challenge to assess pulmonary insufficiency with standard pulmonary function test (PFT) with minimal effort. Non-invasive PFT such as respiratory inductance plethysmography, impulse oscillometry system, and pneumotachography were described in Morquio patients as compared with spirometry. Findings from our previous study indicate that these non-invasive tests are a reliable approach to evaluate lung function in a larger range of patients, and provide valuable clinical information otherwise unobtainable from invasive tests. In conclusion, the present study describes the utility of non-invasive (PFT) to accommodate a broad range of patients including intolerance to effort-dependent PFT.

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## A COMPUTATIONAL STUDY OF THE ROLE OF ASPARAGINE 79 IN UBC13

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Ubiquitin (Ub) is a regulatory protein with the ability to flag proteins to be degraded. Ub is covalently attached to a lysine on the target protein by a series of reactions catalyzed by three types of enzymes: ubiquitin activating enzymes, E1; ubiquitin conjugating enzymes, E2; and ubiquitin ligases, E3. If ubiquitin is not attached properly, it can lead to various diseases, like Alzheimer's, Parkinson's and anemia. Our lab has recently published data on the mechanism of the E2 enzyme (Ubc13) and contrary to a popular hypothesis, our data shows that it is unlikely that the amino acid asparagine 79 in Ubc13 stabilizes a reaction intermediate. Instead, our results suggest that asparagine 79 plays an important role in maintaining the structure of Ubc13; however, this hypothesis was based on molecular dynamics simulations of a simplified model of the Ubc13~Ub system. The model system consisted of a Ubc13 bonded to a Ub and with a zwitterionic lysine residue as a substrate. This model is incomplete because it is missing the full substrate ubiquitin, an E3 ligase, and a ubiquitin conjugating enzyme variant (UeV). Therefore, we are currently using molecular dynamics to explore the effects of these additional proteins by generating trajectories of Ubc13~Ub complexed with different combinations of E3, UeV, and full substrate Ub. Finally, it has been suggested that amino acid histidine 77 in Ubc13 may also play an important structural role and we are currently conducting simulations to probe its function.

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## BIOANALYTICAL ANALYSIS OF BACTERIAL ANTIBIOTIC RESISTANCE GENES IN WATER SAMPLES

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The prevalence of antibiotic resistant bacteria has increased dramatically since the dawn of penicillin. Practices such as prescribing antibiotics when they are not needed, patients not taking the full dosage, or overuse of antibiotics of patients across the world who self-prescribe, have increased antibiotic resistance in several bacterial species. Many of these species can be found in local environments, such as parks, water treatment plants, and local ponds and lakes. The presence of these antibiotic resistant species are potentially harmful to those living in areas where they are prevalent. Collecting samples and researching these bacterial species helps to preserve the community's health. Local water samples were collected from Lake Artemesia at College Park, Maryland, and the AOK Library pond on the UMBC campus. DNA was isolated from the water samples and PCR was utilized to look for five antibiotic resistant genes in the water. The following antibiotic resistant genes were found in the samples: *bla* and *ampC* from Lake Artemesia and *nptII* from the AOK Library pond. The *bla* gene was selected for further analysis, and was cloned into *E. coli*. The protein product of *nptII* was expressed, purified, and characterized via mass spectrometry and western blotting. Future reproductions of this experiment would be beneficial to the medical and scientific community in resolving the issue of antibiotic resistance as it continues to persist.

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## **DESIGN OF AN IMMUNOGENIC AND ANTI-EGFR RNA THERAPEUTIC TO ALTER RNA AND PROTEIN EXPRESSION IN GLIOBLASTOMA MULTIFORME**

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The most common and lethal malignancy of the central nervous system (CNS) is glioblastoma multiforme (GBM). Due to the blood brain barrier (BBB) and the relatively immunologically privileged status of the CNS, clinical strategies have not improved the standard of care. Epidermal growth factor receptor (EGFR), a type of tyrosine kinase receptor, has been found to be overexpressed in as much as 60% of GBM tumors. Upon binding of its cognate ligand, EGFR promotes tumor growth and proliferation. The glioma-specific antigen, interleukin-13 receptor alpha variant 2 (IL13R $\alpha$ 2) is highly immunogenic, attracting cytotoxic T-cells to the tumor microenvironment. Therapeutically, delivery of this antigen to the tumor has the potential to bypass the BBB and reactivate the immune system toward GBM. In the current study, we have designed and cloned an immunogenic pre-trans splicing RNA molecule (iPTR) against EGFR. In a GBM tissue culture model, we measure the RNA and protein expression of the iPTR and compare to multiple anti-EGFR RNA therapies. In addition, we are developing assays to use ELISA to measure changes in EGFR protein expression. Genetic delivery of our highly immunogenic IL13R $\alpha$ 2 peptide using the iPTR has the potential to redirect the immune system to recognize and induce apoptosis in GBM cells.

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## TARGETED AND UNTARGETED METABOLOMICS PROFILING ON A PALE ALE BREWED WITH GENETICALLY DIFFERENT YEAST STRAINS

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A pale ale was brewed by White Labs (San Diego, CA) and fermented with four genetically yeasts: a California ale yeast, an English ale yeast, a neutral grain yeast, and a Belgium saison yeast. Beer samples were collected during and at the end of fermentation. Each sample was profiled with both positive and negative ion ESI LC q-TOF MS. After molecular feature extraction, metabolites were matched to metabolites in known *Saccharomyces cerevisiae* pathways. Metabolites matched included xanthine, thymidine, riboflavin, ornithine, citric acid, malic acid, 5-methylthioadenosine (5-MTA), tryptophan, and phenylalanine (all confirmed by matching retention times and MS/MS spectra to standards). Malic acid is part of the TCA cycle and 5-MTA is part of polyamine biosynthesis pathway. Both tryptophan and phenylalanine were matched to multiple pathways. The abundance of malic and tryptophan varied little across the samples, with RSDs of 7% and 3%, respectively. More differentiation was observed for phenylalanine (20%) and 5-MTA (48%). Samples collected during fermentation yielded more matches to the *S. cerevisiae* library and known pathways than the final product. A list of confirmed metabolites and compounds commonly found in both sample sets was compiled. We are currently quantifying these compounds, while comparing also comparing them globally (e.g. hierarchical clustering, PCA and Venn diagrams).

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## MEASURING STRESS RESPONSE TO DAILY SELF-WEIGHING

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Daily self-weighing (DSW) is a highly-debated topic; evidence supports DSW for weight control, but concern exists regarding potential negative psychological consequences. To date, no studies have measured biological proxies for mental states in response to DSW. The purpose of this study was to assess college women's biological stress responses to DSW using the salivary biomarkers cortisol (CRT) and alpha-amylase (AA).

6 University of Delaware (18-26yo) women participated. 4 were randomized to DSW and 2 to an active control. Saliva was collected 7 times/day on 4 days: the day prior to starting the intervention (day 0), and days 1, 3 and 7 of the intervention. The passive drool method was used to collect samples immediately after waking (AW), 15 minutes AW, 45 minutes AW, 60 minutes AW, at 12:00pm, 2:00pm, and 6:00 pm.

5 participants returned usable samples. Results showed diurnal CRT and AA curves indicative of poorly collected samples. In all participants, graphed CRT data lacked the distinct morning peak seen in normal diurnal CRT awakening responses. Participant's CRT levels started highest in the morning, and declined throughout the day, indicating that samples were taken post-waking. Of the 2 participants who reported exact collection times, a slightly higher area under the curve (AUC) was visible on the graphed AA results for the DSW participant as compared to the control participant.

Although there is a larger AUC in the DSW participant as compared to control for AA, which would indicate that the DSW participant was under more stress than the control participant, lack of appropriately collected data prohibits conclusions. Process information collected has informed protocols such that future participants will be reminded of the necessity of following directions, such as taking samples on time, filling vials to desired testing volume, and recording exact time of collection.

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## **IMPLEMENTATION OF A PATHWAY TO TRANSITION UNCOMPLICATED SEIZURE PATIENTS SEEN IN THE EMERGENCY DEPARTMENT TO OUTPATIENT CARE**

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A wide variation in care existed for uncomplicated seizure patients seen in the emergency department (ED) at Christiana Care Health System (CCHS). To reduce unnecessary variation and ensure appropriate outpatient (OP) follow-up, CCHS's Neurosciences Service Line designed the Uncomplicated Seizure Pathway. To determine the Pathway's effectiveness, we assessed the number of uncomplicated seizure patients successfully transitioned to OP care after disposition from the ED.

We compared 40 Pathway patients (from September 2016 – May 2017) to 40 control patients (from October 2015 – May 2016) in this retrospective study. Demographic and clinical variables were collected and compared between the two groups. Fisher's exact and 2-sample t-tests were preformed to determine the impact of the Pathway in delivering optimal care to patients.

The mean age of Pathway patients was 42.15 years old; they were 45% female. For subjects entered into the Pathway, those lost to follow-up post ED discharge decreased by 37.5% ( $p<0.001$ ). There was a 37.5% increase in subjects who saw a neurologist (inpatient neurology consult or outpatient office visit) in less than 2 weeks for Pathway patients ( $p<0.001$ ). Subjects who were prescribed anti-epileptic drugs and lost to follow-up in less than 2 months decreased by 36.8% for Pathway patients ( $p<0.001$ ). After analyzing risk factors, including psychiatric illness and history of seizures, we found statistical significance ( $p=0.034$ ) between OP neurology follow-up attendance and subjects on the Pathway with documented psychiatric illness who take psychotropic medication.

The Uncomplicated Seizure Pathway increases the likelihood of seizure patients seeing a neurologist and following-up after they are discharged from the ED. In the future we are going to change the Pathway to include social work consults for patients with documented psychiatric illness in an effort to further reduce variation in delivery of OP care. Further study of the Pathway will be continued to validate these findings.

This project was supported by the Delaware IDeA Network for Biomedical Research Excellence (INBRE) program, with a grant from the National Institute of General Medical Sciences – NIGMS (P20 GM103446) from the National Institutes of Health, and the state of Delaware.

## **INTERNALIZATION DYNAMICS OF THE DISEASE-ASSOCIATED DOPAMINE TRANSPORTER A559V VARIANT**

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When dopamine levels are unregulated, there can be a drastic impact on the human mind and body. The dopamine transporter protein (DAT) regulates the amount of dopamine in a neuronal synapse by reuptake, which facilitates effective communication between neurons. Recently, the dopamine transporter protein A559V variant was discovered in patients diagnosed with disorders such as autism spectrum disorder (ASD), attention-deficit/hyperactive disorder (ADHD), and bipolar disorder. In this effort, we aim to investigate the internalization dynamics of the A559V variant protein. In order to study internalization, HEK293 cells are transiently transfected with two coding variant plasmids- one for the A559V variant and one for the wild type. When the cells express their respective dopamine transporter, the DAT proteins are tagged with DAT specific ligand-conjugated quantum dots. 3D single quantum-dot tracking experiments will be carried out by spinning disk confocal microscopy, which allows us to view the individual transporter proteins in living cells. We hypothesize that the internalization dynamics of the A559V variant protein will distinctly differ from those of the wild-type, which may indicate a correlation between internalization and dysfunction. This information could subsequently be implemented in quantitative diagnosis for behavior disorders.

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## REVISING AN ORGANIC CHEMISTRY EXTRACTION EXPERIMENT PROCEDURE TO INCREASE PRODUCT YIELD

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My research internship focused on revising a current Frederick Community College Organic Chemistry II experiment involving the extraction of the three active ingredients in Extra Strength Excedrin: acetaminophen, caffeine, and aspirin. The goal of the experiment is to use methods such as dissolution, recrystallization, solubility, acid-base reactions and filtration to isolate and purify each active ingredient. To ensure that the correct components have been extracted and purified, students obtain melting points and infrared spectra.

In the past, students have had difficulty extracting pure products in reasonable quantities. The aim of this internship was to improve the procedure by identifying and modifying problem steps. Several obstacles were encountered during the attempts to improve this experiment.

Many of the obstacles involved the gravity filtration and vacuum filtration processes. For example, certain filter papers refused to allow solution through, while others would not collect the solid substances for which they were selected. Additionally, an unexpected complex would form during the addition of sodium hydroxide. This also caused procedural difficulties.

These complications seem to indicate an ingredient inconsistency in the active ingredients between Excedrin before it was recalled in 2012 and after its return to the market later that year. We suspect that the inactive ingredients and binder were altered during that time. To combat any confusion that may occur from the newer inactive ingredients, we have endeavored to create a powder mixture from the three active ingredients and a binder. Beginning with this purified mixture improved our percent yields and eliminated procedural challenges.

Streamlining and increasing the success of this experiment will enhance the Organic Chemistry II experience at FCC. Many of the students who take Organic Chemistry are planning careers in the biomedical sciences or the pharmaceutical industry. This experiment appeals to their career interests while strengthening their laboratory and problem-solving skills.

This project was supported by Frederick Community College and funded through the Frederick County Workforce Services and the National Science Foundation's Improving Undergraduate STEM Education program (DUE-1431522).

# **ESTABLISHING A RELIABLE EXPRESSION AND PURIFICATION SCHEME FOR THE RETINAL TRANSCRIPTION FACTOR CONE-ROD HOMEBOX (CRX)**

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Cone-rod Homeobox (CRX) is a retina-specific transcription factor that plays a key role in the development of photoreceptor neurons. The pathophysiology of a number of retinal diseases, such as Leber's Congenital Amaurosis and Retinitis Pigmentosa, are a result of missense mutations in CRX. Greater understanding of the function of CRX in the developing human retina would allow for better knowledge in photoreceptor degradation. In addition, the effect of epigenetically modified DNA methylation on CRX binding still remains unknown. As stem cell therapies continue to increase as a means of treating photoreceptor degradation, understanding these behaviors within CRX is crucial. Electrophoretic mobility shift assays (EMSA) with differentially methylated rhodopsin enhancer and promoter regions would allow for a better understanding of retinal development. Stability assay for target protein using various buffers was conducted to concentrate protein and further the understanding of its stability longevity. Large stockpiles of target protein and subsequent DNA were prepared, allowing for two column purifications and a protein construct modification to be established.

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# INTERFACE AND SURFACE RELATIONS OF THE GADOLINIUM-DOPED CERIA AND COBALT FERRITE MIXED IONIC-ELECTRONIC CONDUCTOR

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With fossil fuels being limited and environmentally harmful sources of energy, using more abundant resources for energy production is a viable solution. Mixed ionic-electronic conductors have promising futures in energy conversion and storage devices such as solid oxide fuel cells. This experiment explores the intentional formation of a secondary phase in a gadolinium-doped ceria ( $\text{Ce}_{0.8}\text{Gd}_{0.2}\text{O}_{2-\delta}$ ) and cobalt ferrite ( $\text{CoFe}_2\text{O}_4$ ) compound and the effects on electrical properties. At temperatures exceeding  $600^\circ\text{C}$ , this compound operates as a cathode, allowing a flow of  $\text{O}^{2-}$  ions and electrons. Structure and electrical conductivity of these dual-phase semiconductors were measured as they were exposed to different oxygen partial pressures and temperatures. By use of the four-point probes method, electrical resistances were recorded and then converted to values of resistivity on a range of temperatures. The gadolinium-doped ceria and cobalt ferrite were combined in three different volume ratios and exposed to a range of oxygen partial pressures.

With the cobalt ferrite being the predominant contributor of electronic properties in the compound, pure cobalt ferrite was the basis of comparison for all measures of electrical properties. The electrical conductivity of the dual-phase conductor was a function of temperature, cobalt ferrite concentration, and atmospheric conditions. The electrical properties of these compounds are best explained by observing interactions at the cobalt ferrite-air interface. The cobalt ferrite possesses a spinel crystalline structure, which is densely packed and facilitates electron flow. The presence of  $\text{O}_2$  in the air at the interface aided electronic flow by induction within the compound. With measures of conductivity comparable to those of low temperature semiconductors, the gadolinium-doped ceria and cobalt ferrite cathode may be a key component of solid-state energy technology.

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## GREEN CHEMISTRY ONE-POT SYNTHESIS OF CHALCONE EPOXIDES

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Waste minimization is a very important aspect of an environmentally benign protocol. A one-pot consecutive process has been developed for chalcone epoxide synthesis that allows compounds to be prepared without having to isolate and purify the intermediates. The strategy utilizes consecutive Claisen-Schmidt condensation and epoxidation reactions to prepare chalcone epoxides from substituted benzaldehydes and 2-acetylthiophene in good yields

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## ANTIBACTERIAL PROPERTIES OF NOVEL AMPHIPHILES: EXPLORING STRUCTURE-ACTIVITY RELATIONSHIPS

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Overuse and improper usage of antibiotic resistance has led to a recent surge in antibiotic resistance. To address this matter, many research groups are searching for an alternative to antibiotics. One option is the development of amphiphiles, some of which have antibacterial properties. Amphiphiles contain a hydrophilic, polar head group, and a hydrophobic, nonpolar tail which may intercalate into the cell membrane, resulting in cell lysis. Understanding the impact of amphiphile geometry on antibacterial activity allows for the synthesis of potential novel antimicrobial compounds with a variety of applications. Novel bipyridine amphiphiles were synthesized and evaluated for antibacterial properties against seven bacterial strains. Amphiphiles contained two pyridinium headgroups attached by a carbon chain of varying linker lengths. A 12 Carbon tail was also attached to each pyridinium head. An optimal linker length for antibacterial activity was observed for each strain. Amphiphiles with longer and shorter linker lengths were less effective. The rate of killing *P. aeruginosa* was also determined for each amphiphile. The research and development of novel compounds can be used to reduce spread of nosocomial infections and decrease negative impacts of antibiotic resistance.

## ANALYSIS OF BACTERIAL AND ALGAL DIVERSITY IN THE ANACOSTIA RIVER

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Until recently, the Anacostia River, “the forgotten river”, in Washington D.C has been plagued with pollution and toxic waste. Most of the pollution is due to an antiquated combined sewage system that brings excess nutrients that influences the composition of the viral, bacterial, and algal (phytoplankton) communities.

Three sites along the river were sampled during during the summer and fall seasons of 2016 and spring and summer of 2017 for bacteria and during spring and summer of 2017 for algae. The samples were filtered for bacterial and algal DNA, and categorization of bacterial and algal phyla was done using environments such as USEARCH, SILVA, GreenGene, MacQIIME, and PhyloSeq. Two major algal groups, Opisthokonta and SAR, were identified as the most abundant at all three sites, yet the bacterial biodiversity fluctuated across three sites due to seasonal factors such as rainfall that had an impact on the volume of sewage overflow into the river. Analysis of bacterial beta-diversity found that summer 2016 and spring 2017 had the most differences in microbial communities along the river (e.g. more phyla or lack thereof). The mid-river site had the most changes in the microbial community whereas the upriver and downriver sites had more similar communities among seasons. while for the algal beta-diversity, it remained fairly uniform across all sites and seasons observed.

The data from this study will be used as baseline data to help determine the effectiveness of the Anacostia River Tunnel which is predicted to divert nearly 90% of the sewage from the river once it goes online in March 2018. Being able to compare the pre- and post-tunnel microbial communities will help determine whether changes to the ecosystem health and biodiversity has occurred.

We would like to acknowledge the Gordon Brown Fund for funding this research, Juanita College for performing DNA sequencing, DC Water Research and Resources Institute, symbols for diagrams courtesy of the Integration and Application Network ([ian.umces.edu/symbols](http://ian.umces.edu/symbols)). We also would like to thank the Anacostia Riverkeeper and Anacostia Watershed Society for taking us out to take samples.

## EXPLORATION OF NOVEL MARKERS OF POSTERIOR CAPSULAR OPACIFICATION

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Cataract, the leading cause of blindness worldwide, is defined as the clouding of the ocular lens. While cataract surgery is commonly performed by removing the lens and replacing it with an intraocular lens (IOL), it only temporarily cures cataract and actually causes a fibrotic condition called posterior capsular opacification (PCO). PCO, or secondary cataract, results from the migration and proliferation of lens capsule cells remaining post cataract surgery (PCS). While laser treatments can cure PCO, this incurs risks to vision. Thus, a comprehensive understanding of the signaling pathways leading to lens cell fibrosis must be reached in order to prevent PCO. Previous research discovered that over-activation of transforming growth factor beta (TGF- $\beta$ ) signaling leads to increased fibrosis. The bone morphogenetic protein (BMP) signaling pathway is important for normal cell behavior and, in other cell types, controls fibrosis by inhibiting TGF- $\beta$  signaling. This work explores possible relationships between BMP and TGF- $\beta$  signaling during PCO pathogenesis. Immunostaining for alpha smooth muscle actin ( $\alpha$ SMA) found this fibrosis marker upregulated by 48 hours PCS in wild type adult mouse lenses. This upregulation correlates to increases in phosphorylated SMAD3 (pSMAD3) in the TGF- $\beta$  signaling pathway at this time. Additionally, a decrease in pSMAD1/5/8, a major effector of BMP signaling, was observed at the same time point. Previously, RNA sequencing discovered certain genes that changed early in PCO pathogenesis. Notably, the mRNA for Gremlin, a BMP antagonist, upregulates several hundred-fold by 48 hours PCS. This work discovered that Gremlin protein levels upregulate in lens cells sharply within six hours PCS and reach high levels at 48 hours PCS, providing a possible explanation for the attenuation of pSMAD1/5/8 observed. These data suggest that the upregulation of Gremlin PCS may be a critical factor in the upregulation of TGF- $\beta$  signaling necessary for the fibrotic response leading to PCO.

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## **THE EFFECT OF RIBOSOME ACTIVATION ON LEARNING AND MEMORY IN MICE WITH MEMORY DEFICITS DUE TO rRNA REPRESSION**

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Previously, we have determined that inhibition of RNA Polymerase I (Pol I), which explicitly synthesizes new ribosomal RNA (rRNA), disrupts the consolidation of long term memory. Since rRNA are essential component of the ribosomes our data suggests that “existing ribosomes” are not able to compensate for the loss of “new ribosome” synthesis required for memory consolidation. It is known that activation of mTOR pathway produces activation of protein synthesis in existing ribosomes, perhaps activation of those existing ribosomes would be able to compensate for the loss of new ribosome synthesis. To test this, we performed stereotaxic intrahippocampal cannulation surgery on adult mice. This cannulation facilitates the injection of a Pol I inhibitor directly into the hippocampus. We injected mice with Pol I inhibitor intrahippocampus before habituation in the hippocampal dependent active place avoidance behavior task. Then we injected animals with a mTOR activator (or vehicle) intraperitoneally, trained the animals and tested for memory 24h after. We are currently analyzing the data. If our hypothesis is correct, then mTOR activator will reduce the decrease in memory consolidation associated with Pol I inhibition.

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## INVESTIGATING THE *BLA* ANTIBIOTIC RESISTANCE GENE IN UMBC WATER SAMPLES

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In recent years, the amount of antibiotic resistance in bacteria has been increasing at an alarming rate, yet no new antibiotics have been developed. These newly developing strains of bacteria are quickly becoming resistant to antibiotics that are frequently used to treat illnesses such as staph infections and pneumonia. Antibiotic resistant bacteria in the environment are now becoming a greater threat to public health. The purpose of this study was to further investigate the prevalence of antibiotic resistant genes in the environment. Water samples were taken from the UMBC campus at the AOK Library and Pig Pen Pond to determine the presence of antibiotic resistance genes. These samples were tested for several different genes including: *bla*, *ampC*, *cat*, *aacC1*, and *nptII*. PCR showed that the water samples only had traces of the *bla* gene, a gene that codes for beta-lactamase, which is an enzyme that confers resistance to the antibiotic ampicillin. The *bla* gene was then cloned into a strain of *E. coli* to further characterize the protein. The protein was characterized through the use of mass spectrometry and western blots but the final results were inconclusive. The data collected from this study requires further research into which bacterial strains contain the *bla* gene that was expressed in the water samples.

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## INFRARED SPECTROSCOPIC ANALYSIS OF TREE LEAVES FROM THE URBAN ENVIRONMENT: BULK VS. SURFICIAL PROPERTIES

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The surfaces of plant leaves are covered by a hydrophobic lipid membrane, known as the cuticle, that prevents water loss and provides protection from pathogens and contaminants. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy is a non-destructive analytical technique used to identify the chemical and structural properties of materials. Because the penetration depth of the IR beam is on the order of  $\sim 1$  micron, this technique provides a means to examine surface composition, i.e. the cuticle, of plant leaves. We used ATR-FTIR spectroscopy to investigate the structural and compositional properties of intact and homogenized tree leaves from New York City's Central Park. Tree species analyzed include *Platanus x acerifolia* (London plane), *Quercus rubra* (red oak), and *Celtis australis* (European hackberry), among others.

Our results reveal different chemical composition in abaxial (lower) vs. adaxial (upper) surfaces of leaves. Compared with abaxial surfaces, adaxial sides show a smaller  $\text{-OH}$  stretch ( $3340\text{cm}^{-1}$ ), larger methylene ( $\text{CH}_2$ ) peaks ( $2920\text{cm}^{-1}$  and  $2850\text{cm}^{-1}$ ), smaller phenolic peaks ( $1633\text{cm}^{-1}$ ), and larger carbonyl ester peaks ( $1730\text{cm}^{-1}$ ). Adaxial surfaces also show a substantially smaller methyl ( $\text{CH}_3$ ) shoulder at  $2950\text{cm}^{-1}$ . The smaller  $\text{CH}_3\text{:CH}_2$  ratio indicates a longer average aliphatic chain length on adaxial surfaces, consistent with a thicker, waxier cuticle. More esters groups on the adaxial surfaces also reflect a greater contribution from cuticular lipids.

We also found dramatic differences between intact and homogenized leaf samples. Compared with intact leaves, pulverized samples show smaller alkane contributions, no carbonyl features, more pronounced aromatic peaks at  $1515\text{cm}^{-1}$ , and the emergence of an amide/protein peak at  $1650\text{cm}^{-1}$ . These results demonstrate that the alkane-rich, lipid-based cuticle is diluted in the homogenized (bulk) samples, which are dominated more by protein features. Our future plans are to assess the effects of air pollution in NYC on the leaf cuticle using similar methods.

## AN INVESTIGATION OF THE BACTERIA ON THE PAWS OF SMALL MAMMALS

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Ever since the “Germ Theory” made its initial appearance in laboratories in the 1860’s, the flora and fauna on human skin has been heavily studied, and a war against bacteria seemed to take place. Humans now wash their hands many times each day, and many people carry scented hand sanitizers for on-the-go cleansing. The millions of microbes residing on common surfaces and skin can be difficult to comprehend without being able to see the abundance of tiny organisms. Despite their diminutive size, they have been studied extensively by scientists, and the microbiome of the human skin has since been well defined and categorized. It is amazing, despite the vast array of antibacterial soaps and sanitizers available, that the human hand has such diversity of microorganisms. But what about animals, who do not have conventional hand-washing techniques? Are the microbes found on the paws of beloved house pets similar to our own? Or is there a completely individualized set of microorganisms for each species? Our research is focused on finding out what bacteria is living on the paws of some of man’s best friends, how similar it is to the microbiomes of the human epidermis, and if there are interspecies similarities between our test animals. To study this, the animals’ footprints were sampled onto plates and the different types of bacteria were isolated. Differential testing and DNA barcoding will be utilized to identify the species.

We would like to acknowledge and thank the members of the Department of Natural and Computational Sciences at Immaculata University, particularly Dr. Kelly Orlando, Dr. Jean Shingle, and Deb Tischler, for their guidance throughout the project.



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

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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, as the gut microbiome has been found to affect a host's physiology, impacting nutrient metabolism and immune function. Commercially available probiotics are advertised as providing health benefits when consumed. They are utilized to address gastrointestinal disorders and broader systemic issues such as dermatitis or respiratory infections. 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 there was no experimental manipulation to the horses' feeding implemented. Fecal matter from six privately owned horses maintained on their standard grazing diet were collected. Three of the six horses had received a probiotic supplement, SmartDigest, for several years prior to beginning the project. Bacterial DNA was isolated from the fecal samples and microbial diversity was characterized by amplifying the 16S ribosomal RNA and tagging it with index primers. The samples were sequenced on the Illumina MiSeq, and acquired data allows the comparison of the relative composition of the intestinal microbiome of these six domestic horses.

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

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

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# CHEMICAL EXFOLIATION OF MOLYBDENUM DISULFIDE ( $\text{MoS}_2$ ) USING n-BUTYLLITHIUM

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Molybdenum Disulfide ( $\text{MoS}_2$ ) in its nanoscale form is of big interest to our team for its excellent properties such as high electrical conductivity, direct band gap of 1.8 eV, transparency, and mechanical flexibility. These properties make  $\text{MoS}_2$  an interesting nanomaterial for potential future applications such as flexible and transparent electronic devices, phototransistors, chemical sensors, and more.

Synthesis of Molybdenum Disulfide, as presented in this poster was done by Chemical exfoliation using n-butyllithium. Wafers containing the exfoliated Molybdenum Sulfide were analyzed using Optical Microscopy, Raman Spectroscopy and Scanning Electron Microscope (SEM).

In the future we plan to use other analyses techniques such as Atomic Force Microscopy (AFM). This technique is used to determine the thickness of the nanomaterial.

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## SYNTHESIZING VANADIUM-METAL ALLOY METAMATERIAL SUPERCONDUCTORS

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The goal of this research project is to synthesize and characterize vanadium nanoparticles which can then be turned around to test for superconductivity. The procedure generally involves taking the starting metal dissolved in a solvent and heat it to just below the boiling point of the solvent, adding ligands to stabilize the product, and then adding a reducing agent to form the product. The project, thus far, has varied the starting vanadium chemical, altered the product from elemental vanadium to vanadium- alloys, and changed the gas environment in which the reaction occurs. Recent variations in the experiment shows that vanadium-alloys are likely the key to get the product to exhibit superconductivity because recently synthesized compounds have exhibited superconductivity, albeit at low temperatures. Future experiments involve using a core-shell metamaterial in the hopes of raising the  $T_c$  of superconducting compounds synthesized, in the hopes of creating warmer superconductors which work under higher temperatures ( $T_c > 4K$ ). A potential benefit of this research is that higher temperature superconductors could be constructed and replace other superconductors that operate at temperatures near that of liquid helium. Superconductors have applications in technology that exists today (i.e. maglev trains, MRI scanners, particle accelerators) and in research in that of technology looking to the future (i.e. magnetic confinement fusion reactors).

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# MICRORNA-31 REGULATION OF *EVE* EXPRESSION IN THE EARLY SEA URCHIN EMBRYO

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Proper regulation of microRNAs plays vital roles in the embryogenesis of animals. MicroRNAs are small non-coding RNAs that suppress translation and reduce stability of target mRNAs in animal cells. microRNA-31 (miR-31) has been found to play a role in cancer, bone formation, and lymphatic development. Using the sea urchin as a model organism, we investigate how miR-31 regulates skeletogenic cells in developing sea urchin embryos. Previously, we found that knockdown (KD) of miR-31 resulted in defects in the patterning and function of the skeletogenic cells and skeletal formation. Also, perturbation of miR-31 resulted in aberrant *Vegf3* signaling which is critical for the patterning of skeletogenic cells and skeletal formation. This study tests the hypothesis that miR-31 indirectly suppresses *Vegf3* through its upstream regulators. We found that miR-31 directly suppresses *Eve*. Importantly, removing miR-31 suppression of *Eve in vivo* resulted in similar ectopic *Vegf3* expression as in miR-31 KD embryos, indicating that miR-31 regulates *Vegf3* through suppression of *Eve*. Further, removing miR-31 regulation of *Eve* is sufficient to cause skeletogenic defects. We have identified the molecular mechanism of how miR-31 regulates *Vegf* signaling that impacts skeletogenesis. In addition, we created additional RNA *in situ* probes to further test the involvement of Wnt signaling in this regulatory mechanism. Overall, our results indicate that miR-31 integrates gene regulatory network and signaling pathways to ensure proper development.

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## DEFINING THE TRANSGENERATIONAL BRAIN OF *CAENORHABDITIS ELEGANS*

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Transgenerational gene silencing, in which information is transmitted from the environment to the germline to silence a gene in the next generation, has been observed in many species. In the worm *C. elegans*, this information can be in the form of mobile double-stranded RNA (dsRNA) made in the soma that travels to the germline to silence a germline gene of matching sequence. The study demonstrating this phenomenon used extrachromosomal arrays that overexpressed dsRNA from multiple copies of the transgene. Also, this earlier study used a pan-neuronal promoter to express dsRNA targeting a foreign gene from all neurons making us unable to determine if certain neurons are more effective at triggering transgenerational gene silencing through dsRNA. Because of these limitations, we do not know if transgenerational gene silencing of an endogenous germline gene is possible when dsRNA is made from a single-copy gene and from specific neurons.

To answer these important questions, we will use CRISPR/Cas9-based genome editing to integrate as single copies the transgenes expressing dsRNA into specific loci. Through the work of undergraduates in the FIRE-Transgenerational Brain Initiative we can test many subsets of neurons for their ability to elicit transgenerational gene silencing. Each student will create dsRNA genes under the control of different neuronal promoters to target the germline gene *oma-1*. Silencing of *oma-1* will be scored by looking for viable offspring in *oma-1(zu405)* animals that do not express the dsRNA targeting *oma-1* but had a parent that expressed the dsRNA. Because worms that carry the dominant-lethal *oma-1(zu405)* allele have no viable offspring when grown at 25°C, effective transgenerational gene silencing by mobile dsRNA will result in live progeny from worms with the *oma-1(zu405)* allele at 25°C. By analyzing the subset of neurons that consistently show effective silencing, we will define the transgenerational brain of *C. elegans*.

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## STRUCTURE AND FUNCTION OF THE FERROUS IRON TRANSPORT SYSTEM PROTEIN FEOA

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FeoA is an essential small, soluble protein that is part of the Feo system, which is a series of proteins that is responsible for the transport of ferrous iron ( $\text{Fe}^{2+}$ ) in pathogenic bacteria. Ferrous iron is essential for virtually all organisms, as it is used in processes such as oxygen transport, DNA biosynthesis, and aerobic cellular respiration. Currently little is known about how the Feo system functions, and the role of FeoA within the system is enigmatic. Specifically, previous research indicates that FeoA interacts with membrane-bound FeoB, however the molecular interaction between these proteins is unclear. The ultimate goal of this work is to utilize biophysical and enzymatic assays to characterize FeoAs from two pathogenic bacteria, *Escherichia coli*, (*EcFeoA*), and *Klebsiella pneumoniae*, (*KpFeoA*). This work demonstrates the recombinant expression and purification of *EcFeoA* and *KpFeoA*. Solution circular dichroism spectra of these proteins demonstrate the expected secondary structure, and size-exclusion chromatography indicates that both proteins exist as a mixture of oligomers. The work also reports on the preliminary crystallization of *KpFeoA*, which is actively being optimized.

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## DEVELOPING LONG-ACTING IMPLANTS FOR BREAST CANCER PREVENTION

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This project involves the development of a breast cancer preventative device using anti-estrogen drugs. The two drugs of interest, raloxifene, an Estrogen Receptor Modulator (SERM), and fulvestrant, an Estrogen Receptor Degradar (SERD), are already clinically used in preventing (raloxifene) and treating (fulvestrant) breast cancer. Our goals are to improve the delivery of these drugs for better therapy. The first step of the project includes the creation of short, cylindrical silicone devices infused with each of these select drugs. Standards of both drugs are also created in order to assay the amount of drug released from these silicone-based devices by high performance liquid chromatography (HPLC). Two days of drug release were analyzed, demonstrating that there is appreciable leaching of each drug into an isopropanol:water (50:50 v:v) sink. The second phase involves a one-week release of the drug-infused implants, which will then also be analyzed by HPLC to quantify overall drug release for the longer period. The third step will be a month-long release of the samples. By incorporating preventive devices containing these anti-breast cancer drugs, it is possible that side effects can be limited from the drugs if taken orally. Furthermore, such a device would allow a more direct method of distributing drug to the effected area—the breast. This will also eliminate poor patient compliance and may increase efficacy of these agents in the primary prevention of estrogen-linked breast cancer and in preventing recurrence of ductal carcinoma in situ.

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# INVESTIGATION OF THE BIOLOGICAL PATHWAYS AND INTERACTING PARTNERS OF THE LYSINE METHYLTRANSFERASE SET6 IN *SACCHAROMYCES CEREVISIAE*

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Histone proteins are scaffolds within the nucleus that provide structural and organizational integrity for DNA. Recent studies suggest that histone abnormalities are linked to latent defects in chromatin structure, leading to cancer and other developmental complications. In addition to providing the DNA its structure, histones are also a common site for lysine methylation, which is a post translational modification catalyzed by SET-domain containing chromatin regulatory proteins. These modifications affect DNA templated processes, like chromosome segregation, transcription, and gene repair. In the current experiment, we used the budding yeast, *Saccharomyces cerevisiae*, as a model organism to study the cellular function of Set6 – an uncharacterized SET-domain containing lysine methyltransferase. Therefore, the aim of our project was to investigate biological pathways in which Set6 is involved by performing genetic interaction and protein-protein interaction studies of Set6. According to our preliminary data, cells without Set6 are sensitive to the antimetabolic drug, benomyl. This enhanced benomyl sensitivity possibly indicates a role for Set6 in microtubule function or cell cycle regulation. Furthermore, since we know that lysine methyltransferases usually operate in larger protein complexes, we performed immunoprecipitation followed by mass spectrometry to identify potential protein partners of Set6. We analyzed a candidate protein that showed the strongest interaction with Set6: Gim3. This protein is part of the co-chaperone prefoldin complex that is important for microtubule assembly, chromatin localization and transcriptional elongation. We are using co-immunoprecipitation experiments to further test the interaction between Set6 and these factors. Overall, our data has opened new avenues of investigation for understanding the function of Set6, including determining its potential roles in microtubule assembly and transcription elongation.

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## **HORTICULTURAL PHYTOCHEMISTRY OF *Aronia mitchurinii*: CULTURAL MANAGEMENT AND ITS INFLUENCE ON THE ANTIOXIDANT CAPACITY**

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*Aronia mitchurinii* is a species of berry native to the north-eastern U.S. and naturalized in Eastern Europe. Studies have reported high content of flavonoids, polyphenols, anthocyanin and other phenolic antioxidants in Aronia. Much is known about high antioxidant content in aronia juice, however, its phytochemical content has never been correlated with cultural management conditions such as fertilizing, mineral additives, irrigation, age of the crop etc.

Since 2006 we have been studying the effect of nitrogen treatment, moisture, organic vs. conventional growing, mineral additives and other factors on the antioxidant content of juice and pulp of aronia. The objectives of this study are: 1) to analyze whether variety of factors such as; nitrogen treatment, moisture content, and organic versus conventional fertilizer exposure had an effect on the yield of phenolic, bioactive compounds in *aronia mitchurinii* juice, 2) to develop best practice regarding the growing and fertilization of *aronia mitchurinii*, and 3) to compare the results with data from previous years.

Samples of berries were obtained from experimental plots at Wye Research and Education Center, and total concentrations of flavonoids, anthocyanins and polyphenols have been measured by spectrophotometric procedures. This presentation will be focusing on 2015 and 2016 harvests and juice samples. We have found that the nitrogen rate of 3 g/bush/year is an optimal one and that increasing it to 14 g/bush/year results in significant loss of antioxidants in the harvest. No difference was found with and without the addition of mineral bust, however traditional grown aronia has higher antioxidant content as comparing to certified organic growing.

This project was funded by the Louis Stokes Alliances for Minority Participation (LSAMP).

## EXPANDING THE GREEN SCOPE OF PENTAERYTHRITOL ACETAL FORMATION

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Acetals are germinal diether derivatives of aldehydes formed by the reaction of an aldehyde with two alcohols. Collard et al.<sup>1</sup> have shown that by utilizing temperature control, benzaldehyde and pentaerythritol, when mixed in water with catalytic acid, can selectively form monoacetal derivatives. The goal of our project is to expand the substrate scope of this monoacetal selective reaction to synthesize new monoacetals and to define their structure using 2D NMR techniques. Acetals similar to our target monoacetal products are used in a variety of synthetic applications. They have served as alcohol protecting groups in route to polymer-based adhesives<sup>2</sup> and synthetic glycodendrimers for nanomedicine applications in drug delivery<sup>3</sup> and vaccines.<sup>4</sup> Our work should help to broaden the synthetic utility of this user-friendly and environmentally benign reaction. It is also hoped that this work will produce starting material for multistep synthesis routes optimized for use in advanced-level undergraduate teaching laboratories. Preliminary results based on studies using the NMR internal standard dimethyl sulfoxide indicate that many substituted benzaldehydes will provide a level of selective monoacetal formation.

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## MIMICKING NATURAL PHOTOSYNTHESIS: ULTRAFAST CHARGE TRANSFER IN PpcA-Ru(bpy)<sub>3</sub> COMPLEXES

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

To this end, we developed a series of 22 cysteine mutants of PpcA, a 3-heme cytochrome from *Geobacter sulfurreducens*. These proteins were successfully expressed in *E. coli* and isolated for covalent labeling with Ru(bpy)<sub>2</sub>(bpy-Br). Protein purity and successful posttranslational modifications were confirmed with HPLC-MS. Time-resolved nanosecond and ultrafast transient absorbance characterization was performed at Argonne National Laboratory (ANL) and identified 6 constructs with apparent photo-induced charge transfer time constants of 20 ps or faster, including 2 constructs with 1-2 ps time constants.

The latter is a significant result as up to this point only natural photosynthetic systems demonstrated such a fast initial charge separation, while all artificial covalent biohybrid constructs exhibited charge transfer rates 3 or more orders of magnitude slower. To understand molecular principles responsible for such a dramatic acceleration of electron transfer rates, we started small- and wide angle X-ray scattering data collection at the Advanced Photon Source at ANL.

Further, we are currently attempting to obtain X-ray crystallographic and NMR structures of ultrafast constructs. Finally, we performed triplicate 250-300 ns all-atom molecular dynamics simulations of all 6 ultrafast constructs. Based on the obtained results we conclude that that photo-induced ultrafast charge transfer requires van der Waals contact between heme vinyl groups and photosensitizers while contacts with propionates or a small number of covalent bonds between the donors and acceptors play much less significant role.

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# **EPIGENETIC MODIFICATIONS IN THE OLFACTORY BULB AND ANTERIOR PIRIFORM CORTEX ASSOCIATED WITH INFANT OLFACTORY AND TACTILE EXPERIENCES**

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Rodents are macrosomatic; they depend heavily on their sense of smell to navigate their environment. The olfactory system, including the olfactory bulb and piriform cortex, detects and processes scents. In neonatal pups, the olfactory system is key to survival. Soon after birth, pups learn the scents of the dam, which aid in nipple attachment, orientation to the dam, and overall attachment to the caregiver. This odor preference learning was mimicked in a laboratory-based experimental design where neonatal pups were classically conditioned to associate the novel scent of peppermint with stroking, an action that mimics sensory cues associated with caregiving.

Using an odor-stroke paradigm, Long-Evans rat pups (postnatal days 6 or 7) were assigned to either a paired (peppermint odor paired with stroking), unpaired (peppermint odor and stroking presented at different times), or odor-/stroke-only condition. Half of the subjects were tested for odor-preference 24 hours after conditioning and the brains of the other half of the subjects were harvested 30 minutes post conditioning. Gene expression of *Bdnf* and epigenetic regulators in the olfactory bulb and anterior piriform cortex were quantified through Real-time PCR. Identification of different patterns in gene expression based upon single, paired and unpaired presentations could have implications on attachment-based learning.

Special thanks to Dr. Roth and Tiffany Doherty for their support and guidance along with Lauren Webb's completion of odor-stroke conditioning. This research is funded through grants P20GM103653, R01HD087509, and private donation funds to Dr. Roth.

# GENETIC DIVERSITY AT 6 MICROSATELLITE MARKERS IN THE LONG HORNED CRAZY ANT OF SAN SALVADOR ISLAND, THE BAHAMAS, AND ITS ASSOCIATION WITH HABITAT DOMINANCE

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During the field summers of 2014 and 2015 field observations of ant interactions at baits on San Salvador Island, The Bahamas, showed that *Paratrechina longicornis*, an introduced invasive ant species, appeared to have two distinct behaviors. In some locations *P. longicornis* rapidly recruited massive numbers of foragers to baits and in other locations had very poor competitive abilities with other species (regardless of apparent nest density or local abundance). In order to further examine these outcomes, *Paratrechina* was collected from as many locations as possible, along with behavioral data. Due to the nature of species introductions (often a single queen or nest), populations of introduced ant species often have little genetic variability leading to increased cooperation among nests. It was believed that there may be two distinct genotypes on the island and areas where competitive abilities are low may be a different population from those where *Paratrechina longicornis* has dominated the local ant community.

DNA was extracted from each collected group and each of 6 microsatellites was amplified using a PCR protocol and then visualized on 1xTAE agarose gels. Bands were extracted and then run through another PCR protocol with fluorescently tagged primers before being visualized on a Licor 4300 gel based sequencer. Genotypes were determined by eye and compared to the previously observed behaviors.

Mapping the phylogeny to the behavioral status of each population resulted in no apparent correlation between genotype and aggressive behavior. The 6 microsatellite markers used in this study show a genetically diverse population with high heterozygosity of *P. longicornis* on San Salvador. This is most likely due to multiple introductions. Future research is required to further examine more microsatellite loci and compare to populations around the world.

# **DETECTION OF NUCLEAR AND CYTOPLASMIC PROTEIN-PROTEIN INTERACTIONS OF THE CD44-INTRACYTOPLASMIC DOMAIN WITH RUNX2 BY PROXIMITY LIGATION ASSAY**

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CD44 is a transmembrane receptor involved in signal transduction. CD44 undergoes a proteolytic cleavage by presenilin within the gamma secretase complex, which produces the CD44 intracytoplasmic domain or CD44-ICD. This 72 residues peptide can be translocated into the nucleus where it can regulate transcription. The transcriptional regulatory mechanism(s) is not well understood but published EMSA and chromatin immunoprecipitation data have found the CD44-ICD in a complex with Runx2 in the MMP-9 promoter.

We hypothesize that the CD44-ICD directly interacts with Runx2 in the nucleus. To test this hypothesis we carried out Proximity Ligation Assays (PLA) using MCF-7/vector (CD44 negative) and MCF-7/CD44 (CD44 positive) cells grown on chambered slides. An anti-CD44-ICD antibody (Abcam) and an anti-Runx2 antibody (Santa Cruz Biotechnology) were used to generate the PLA data. This assay not only detects protein-protein interactions (PPI) but also the subcellular localization of such interactions.

The PLA data collected as confocal microscopy images indicate that the CD44-ICD interacts with Runx2 in the nucleus. This data validates our hypothesis. However, we also detected an unexpected similar interaction in the cytoplasm. This cytoplasmic localization for the CD44-ICD/Runx2 PPI interaction suggests a potential novel CD44-mediated mechanism of gene regulation. Future PLA and co-immunoprecipitation experiments will be carry out to confirm the cytoplasmic CD44-ICD/Runx2 PPI interaction.

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## **ANTIFOULING FORMULATIONS INSPIRED BY NATURAL PRODUCTS: HERBS, BERRIES AND ALGAE**

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Marine biofilm formation, also known as fouling, is the accumulation of polymerized metabolites, micro- and macro-organisms on submerged objects. The main environmental trait of this process is spreading of bacteria and barnacle attachment on the bottom of ships and its transportation to other marine areas around the world as a result of cargo and military ships movements. Marine biofilm formation results in significant increase of fuel consumption and damage to ship hulls, petroleum platforms and other under-water objects, as well as some very significant negative environmental effects. To slow or even prevent the biofilm formation, antifouling paint is applied to the bottom of the hull of a ship or boat. While it can decelerate precipitations, traditional antifouling composites contain tributyltin (TBT), which is so toxic , that currently is banned in many countries.

In this project extracts from *Aronia mitschurinii*, a super-berry that contains 15 times more antioxidants than assai berry, holy basil, some algae and sponges and other medical herbs are studied as potential natural organic substitutes for Tributyltin (TBT). Extraction and analysis of potentially active in antifouling ingredients of those berries and herbs, preparation of antifouling formulations and initial antifouling tests will be presented.

Using berries and herbs, grown by local Maryland farms, for additional non-food related applications relevant to military would help to make small farms business in Maryland more sustainable and increase its revenues.



## ASSESSMENT OF A COMPOUND CONVERTED TO ESTRADIOL ONLY IN THE BRAIN ON TRANSMITTER EXPRESSION IN THE HYPOTHALAMUS OF OVARECTOMIZED RATS

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A decline in production of estrogen in females is a hallmark of menopause. Clinical effects of decreased hormone motivated the desire for estradiol replacement treatment. Current methods of treatment deliver the hormone throughout the body, but this treatment can be detrimental to the breasts and uterus, and can lead to cancer. Without estrogen, the brain is at greater risk for neurodegenerative diseases (e.g. multiple sclerosis, stroke, and Alzheimer's disease) when estrogen levels decline. Scientists devised a compound, 10 $\beta$ ,17 $\beta$ -dihydroxyestra-1,4-dien-3-one (DHED), that is converted to estradiol *only in the brain*. In earlier studies, 5 days of treatment led to effects on the brain reducing body temperature, reducing the volume of a stroke, and increasing mRNA for an enzyme that synthesizes acetylcholine. The treatment but did not increase uterine weight. By giving the compound to ovariectomized rats, if the compound acts to generate estrogen, additional transmitter changes should become apparent. Ovariectomized (OVX) rats that received no drug, estradiol (50 $\mu$ g subcutaneously), or the DHED (200  $\mu$ g/kg orally) for 3 days were euthanized on the next day and perfused with 4% paraformaldehyde. The tissue was cut and will be stained for two molecules whose expression is *increased* by estrogen (enkephalin and kisspeptin in the preoptic area), and one whose transmitter is *down-regulated* by estrogen (kisspeptin in the arcuate nucleus). We have now set up the conditions of the assay and determined what will be measured and how. We have begun to cut and prepare tissue for staining.

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## IDENTIFYING THE DYNAMIC OLIGOMERIZATION OF WNTLESS

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The Wnt signal transduction pathway plays a critical role in organismal development and maintaining tissue homeostasis. Disruptions or abnormalities in the Wnt pathway can lead to various diseases such as osteoporosis, pulmonary fibrosis, type II diabetes, and cancer. This highly 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 studies performed in the Selva laboratory have shown DWls (Drosophila Wls) oligomerizes before binding to Wingless (Wg), the prototypical Drosophila Wnt ligand. Crucially, DWls oligomerization has been shown to be necessary for Wg binding and thus, intracellular trafficking. While DWls oligomerization is essential for its interaction with Wg, binding of Wg is not requisite for DWls oligomerization. The goal of this project is to determine the mechanism of DWls oligomerization and its role in the trafficking and release of Wg in vivo. To study this, fluorescence correlation spectroscopy which quantitatively measures the fluctuation in concentration of fluorescently tagged molecules in a small observation volume. The technique is utilized on live cells, allowing for real time analysis of a potentially dynamic process of DWls oligomerization. The observation volume must contain a small number of fluorescent molecules to differentiate between the fluorescent spike associated with a monomer or dimer entering/exiting the observation area. A C-terminal Green Fluorescence Tag was placed on DWls, as well as on monomeric and dimeric controls. Previous studies using S2R+ cells showed DWls is likely a monomer at the plasma membrane. For this project, FCS was utilized to examine the oligomerization dynamics of DWls on the nuclear envelope of S2R+ drosophila cells. A thorough characterization of the Wnt signalling pathway may allow for the generation of more targeted therapies to better treat Wnt associated diseases.

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## EXPRESSION AND PRELIMINARY CHARACTERIZATION OF OMCM

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Outer membrane cytochromes (*Omc*) play an important role in respiration of dissimilatory iron-reducing bacteria. They form extended conduits for charge transfer between cellular metabolism and external electron acceptors such as particles of iron oxide, metal ions, and humic substances. However, very little is known about biophysical, biochemical, and structural properties of this large and diverse family of proteins. Out of more than 20 members of *Omc* family in *Geobacter sulfurreducens*, only the smallest single-heme *OmcF* have been successfully expressed and characterized. To get a better insight into structure-function relationships in this family of proteins, we successfully cloned a gene responsible for *OmcM*, a 6-heme cytochrome with the expected mature size of ~170 amino acids. The protein was expressed in *E.coli* and demonstrated an unexpected propensity to form inclusion bodies. We successfully isolated the recombinant protein under denaturing conditions using a combination of ion exchange and metal affinity chromatographies. The purity, mass, and correct attachment of all 6 hemes were verified with LC-MS. The protein was characterized with UV-Vis and CD spectroscopy. They demonstrated the expected spectral peak positions. We are currently pursuing redox titrations and further optimizing expression and isolation conditions to accumulate sufficient amount of protein crystallization trials.

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## INVESTIGATING THE FOLDING LANDSCAPE OF ALPHA-1-ANTITRYPSIN

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Misfolding of Serpins has been implicated in diseases such as lung disease, emphysema, and dementia. Serpins do not fold into their most thermodynamically stable state which makes them prone to misfolding. The folding landscapes of serpins are complicated by their non-sequential domain structure that suggests the formation of local structures with interdependent stability. The precarious nature of serpin folding raises the question of how serpins avoid misfolding in the cell and one can hypothesize that serpins may fold co-translationally. To investigate the folding pathway of serpins,  $\alpha$ 1-Antitrypsin ( $\alpha$ 1AT) was characterized in-vitro by purifying N-terminal fragments that were predicted to fold autonomously. The fragment 1-190 contains the  $\alpha/\beta$  domain of the protein and was found to be monomeric by analytical ultracentrifugation(AUC), in which a large amount of centrifugal force is applied to the fragments and they diffuse and sediment at certain rates based on their size and shape. Protein denaturation monitored by far UV circular dichroism (CD) reveals that this N-terminal fragment also displays significant amounts of secondary and likely tertiary structure suggesting that this fragment could fold co-translationally. Hydrogen-Deuterium Exchange Mass Spectrometry results suggest the presence of local structure in the 1-190 fragment that is lost over time possibly due to the absence of the C-terminus of the protein. These results suggest that  $\alpha$ 1AT may co-translationally fold in the cell to avoid misfolding and disease.

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## CHARACTERIZATION of CHITIN-BASED SORBENTS FOR CARBON DIOXIDE SEQUESTRATION

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Presently, carbon dioxide ranks among the leading cause in pollution responsible for greenhouse effect and correlated to global warming. Carbon dioxide mostly enters the atmosphere through combustion of fossil fuels (coal, natural gas, and oil), solid waste, trees and wood products, and through certain chemical reactions (e.g., manufacture of cement). It is necessary to produce viable solutions that can capture polluted carbon dioxide and recycle it by re-using in other industrial and biotechnological processes requiring carbon dioxide as a reagent. This idea is implemented in the process named Carbon Capture and Sequestration (CCS).

First attempts in CCS used irreversible sorbents which by itself became a water insoluble waste after sorption of CO<sub>2</sub>. The goal of our project is to find reversible and biocompatible sorbents. Thus, here we present results for chitin and chitosan, extracted through controlled chemical reactions from the exoskeletal waste products of Delmarva seafood industry, as reversible CO<sub>2</sub> capture materials. This research describes the effectiveness of chitin and chitosan and their reversible efficiency in capturing carbon, explains raw shell sample preparation and modification, and deals with chemical characterization of chitin before and after carbon dioxide capture.

## **STIMULTION OF NEURITE OUTGROWTH OF RAT HIPPOCAMPAL NEURONS BY EXTRACELLULAR MATRIX PROTEINS**

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**Hypothesis:** We have previously demonstrated that extracellular matrix (ECM) proteins facilitate neurite extension of rat cerebellar granule neurons (CGNs) in culture. We have also identified the functional domain(s) of these proteins involved in neurite outgrowth. Hippocampal neurons play an important role of storing memory of animals. We propose a hypothesis that “hippocampal neurons can be used to identify genes associated with storage of memory in animals”. In this report we developed a ECM-coated culture surface to growth primary rat hippocampal neurons and determined their neurite outgrowth. We have also compared the neurite outgrowth of CGN and hippocampal neurons.

**Method:** Cerebellar granule neurons and hippocampal neurons were plated onto the cover slips coated with extracellular matrix proteins at a density of 60,000 neurons per ml. The neurons were allowed to extend neurites for 24 hrs in NBM/B27/KCl. The extent of neurite outgrowth was then determined using carboxyfluorescein diacetate (CFDA) labeling. CFDA (Sigma, St. Louis, MO) intensely stains the soma and all processes of cultured, living neurons. Images of the cultures were captured and analyzed with the NIH Image software. A sample of 100 neurons with well-defined processes were studied for each condition.

**Results:** Laminin and fibronectin-coated surfaces induced neurite outgrowth of Cerebellar granule neurons and hippocampal. By immunostaining, immunoblot, and RT-PCR analysis we found alteration of expression of some important genes key genes involved in neurite outgrowth, Results of these studies with be presented in this report.

**Conclusion:** We developed a condition to grow hippocampal neurons on ECM-coated surfaces. These hippocampal neurons will be used to apply different types of stimuli and expression of genes will be studied to find their role in saving memory in neurons. Identification of genes associated with saving memory might be useful in developing a therapy for neurological disorders including Alzheimer's, Parkinson's diseases.

## AMMONIA EVOLUTION REACTION IN AMBIENT CONDITIONS

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Synthesis of ammonia is currently restricted by the absence of a sufficiently active catalyst required to break the strong nitrogen-nitrogen triple bond; therefore, an excessive amount of energy is required to overcome the energy barrier – which is applied in the form of high temperature and pressure. To improve the existing process and exploit the favorable thermodynamics at room temperature, electrochemical ammonia evolution reaction (AER) converts a clean, abundant resource, nitrogen gas, into an incredibly important industrial chemical, ammonia. A reactive ruthenium oxide (RuOx) on carbon nanoparticles provides the catalyst for the AER. This catalyst, commercially available from Premetek, is shown to produce ammonia under alkaline electrolyte and ambient conditions. The flow rate of nitrogen gas and current density are kept constant during the duration of each experiment, while measuring the potential variation with time. The ammonia evolution rate is determined as a function of the steady state potential to provide insight into the kinetics of the ruthenium-carbon catalyst surface. The rate of ammonia evolution is also converted to the partial current responsible for producing the ammonia to determine the fraction of the current going to the desired AER. Further electrochemical experiments, followed by specific analyses and calculations, are still required to completely understand the kinetics of the ruthenium oxide on carbon nanoparticles catalyst, which is an important step towards producing ammonia at ambient conditions.

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# **A MULTI-LEVEL APPROACH TO ASSESS THE EFFECTS OF DEVELOPMENTAL NICOTINE EXPOSURE ON POSTNATAL MATURATION OF CARDIORESPIRATORY CONTROL IN SEROTONIN-DEFICIENT MICE**

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*Pet-1* is a transcription factor that is required for normal development of serotonergic (5-HT) neurons in the mammalian brainstem. Targeted deletion of the *Pet-1* gene results in a 70% loss of central 5-HT neurons that is associated with impaired breathing and high neonatal mortality in newborn knockout (KO) mice. Endogenous 5-HT provides excitatory drive to central neural circuits that generate respiratory rhythm. Central 5-HT deficiency has been linked to Sudden Infant Death Syndrome (SIDS) in humans. We previously tested the effects of developmental exposure to nicotine, a risk factor for SIDS, on breathing behavior in intact and unanesthetized neonatal wild type (WT) and *Pet-1* KO mice. Surprisingly, we found that nicotine exposure resulted in a functional recovery from the breathing deficits that are characteristic of the *Pet-1* KO phenotype, but did not decrease neonatal mortality. These results suggest that the high neonatal mortality of *Pet-1* KO mice may be due to cardiac dysfunction. To better understand the underlying mechanisms for these initial findings, we have taken a multi-level approach to define the cardiorespiratory characteristics of neonatal mice exposed to nicotine in a 5HT-deficient context. Specifically, we have developed a non-invasive method for measuring heart rate that, in combination with plethysmography, allows *in vivo* analysis of cardiorespiratory control in neonatal WT and *Pet-1* KO mice. In addition, we can measure “fictive breathing” in isolated brainstem-spinal cord preparations taken from neonatal WT and *Pet-1* KO mice following nicotine exposure. Neural activity in cervical spinal (C4) rootlets reflects output from the preBötzinger Complex, the site of central respiratory rhythm generation. This “*in vitro*” preparation provides significant experimental control and allows a pharmacological approach to define underlying neural mechanisms that is not possible in whole animal studies. Taken together, we expect our integrated approach to provide significant insight into the etiology of human SIDS.

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## DEVELOPMENT OF A SELECTIVE MEDIA AND METHODS OF DETECTION FOR *SACCHAROMYCES CEREVISIAE* VAR. *DIASTATICUS* IN THE BREWERY.

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*Saccharomyces cerevisiae* is commonly used in the fermentation of beer. A wild yeast strain called *S. cerevisiae* var. *diastaticus* has been identified as a major beer spoilage threat, with contamination behind several costly product recalls. *Diastaticus*-infected bottles or cans may lead to off-flavors, over-attenuation, and over-carbonation, potentially causing exploding packages or non-compliance with federal reporting of Alcohol by Volume (ABV). This re-fermentation by *diastaticus* is caused by the secretion of a glucoamylase, normally absent in brewer's yeast. When present in beer, glucoamylase catalyzes the hydrolysis of any unfermented polysaccharides, thus enabling re-fermentation. There currently exists no easy method for detection of *diastaticus* contamination by the brewery quality control lab. Previous reports have described PCR methods for detection, but the expertise and equipment required for application are not often present in breweries. Here we describe the development of a new microbiological medium, Cupric Sulfate Starch Media (CSSM) for the detection and enrichment of *diastaticus*. CSSM is selective for all *diastaticus* strains tested, inhibiting the growth of traditional brewer's yeast.

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## PARTIAL MOLAR VOLUMES AND VOLUME OF MIXING OF SALTS IN AQUEOUS SOLUTIONS

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The goal of this project was to study ion-water interactions by volumetric analysis. Densities of eight sodium salts at varied concentrations were measured to determine the apparent molar volume of the solute, partial molar volume of solvent and solute, and volume of mixing in aqueous solutions. In general, strongly hydrated salts showed smaller limiting partial molar volume compared to weakly hydrated salts. Volume of mixing values were more negative for strongly hydrated anions suggesting that the interactions of these anions with water were more favorable. Detailed volumetric analysis of salt-water system will be presented.

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Department of Chemistry and Biochemistry, James Madison University

## **ELUCIDATION OF HUMAN CYTOMEGALOVIRUS (HCMV) US27 PROTEIN- PROTEIN INTERACTIONS**

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Human cytomegalovirus (HCMV), a beta herpesvirus, is a threat to neonates and to those whose immune systems are suppressed for medical reasons or have been damaged in any way. HCMV genes encoding potential G protein-coupled receptors, such as US28, UL33 and UL78 have been explored for function more extensively than a fourth, US27, which may also play an key role in viral pathogenesis. Preliminary work identified GABARAP as a possible binding partner for the US27 gene product. This project confirmed the binding of the US27 gene product with GABARAP via GST pull down assay and via bimolecular fluorescence complementation (BiFC). Additional experiments confirmed the requirement for a WTTL motif in the C-terminus of pUS27 by using W306A and W306A/L309A (WALA) US27 mutants. These experiments provide a first step towards better understanding the role of this virus-encoded protein class in HCMV pathogenesis.

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## **THE RELATIONSHIP BETWEEN CALCIUM CONCENTRATION AND ZEBRA MUSSELS IN THE BRAINERD LAKES AREA**

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Zebra mussels are an invasive species that has spread widely across North America. They have a great impact on the environment, economy, and native organisms. A previous study researched the impacts of various concentrations/ranges of calcium, water hardness, total phosphorus, pH, water clarity, alkalinity, conductivity, and chlorophyll *a* on zebra mussels. Results from that study led to the creation of a zebra mussel suitability criteria table. The criteria table helps in determining the risk a water body has to zebra mussel infestation. The aim of our study was to find the relationship between calcium, water hardness, and total phosphorus concentrations to the zebra mussels in the Brainerd Lakes area in north-central Minnesota, and to identify the risk a lake has to an infestation as calcium and phosphorus are important nutrients, for shell formation and for survivability.

Calcium and water hardness concentrations were analyzed using the EDTA titration procedure. The total phosphorus concentrations were analyzed by persulfate digestion and ascorbic acid colorimetric analysis. Water samples were collected using a 2-m integrated sampler at the deepest site of 20 lakes. For all 9 lakes that were infested by zebra mussels, calcium and water hardness concentrations were in the moderate and high risk ranges, while total phosphorus concentrations were mostly in the low-risk range. For the other 11 lakes that did not have zebra mussels, 6 had calcium and water hardness concentrations that fall within a moderate to high risk range that indicates these lakes have a higher potential of infestation, while total phosphorus concentrations were also mostly in the low-risk range.

More research is needed on how zebra mussels impact the total phosphorus concentration. More samples should be collected from different lakes in different geographic regions. The result of this study was shared with the lakes' respective associations.

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## USING PHOTO-CROSS-LINKING TO DISCOVER NEW BINDING PARTNERS OF NON-CANONICAL UBIQUITIN CHAINS

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Post-translational modification of proteins by covalent attachment of a small protein ubiquitin or a polymeric chain of ubiquitins is involved in regulation of vital cellular processes in eukaryotes. Ubiquitin monomers can be linked to each other through any of the seven lysines. The signaling role of poly-ubiquitin depends on its length and linkage composition. Lysine-48-linked ubiquitin chains tag proteins for proteasomal degradation while lysine-63-linked chains are involved in nondegradative processes. The role of the non-canonical chains linked through one of the five other lysines is poorly understood.

Discovering the binding partners for non-canonical ubiquitin chains can reveal their role as cellular signals. In order to determine proteins that bind poly-ubiquitin we use p-benzoyl-L-phenylalanine (BPA) as a photo-activatable cross-linking reagent. When exposed to UV light, BPA cross-links to amino acids within binding distance thus covalently trapping binding partners. BPA was incorporated into recombinant ubiquitin as genetically encoded unnatural amino acid using the amber codon and a tRNA/tRNA synthetase pair. Using this technique, 14 ubiquitin variants with BPA in different positions were made and tested for cross-linking with known ubiquitin-binding partners under UV light. This allowed us to identify optimal BPA locations for cross-linking. Selected photo-reactive monomers were then successfully incorporated into photo-reactive lysine-48-linked and lysine-63-linked ubiquitin dimers. These photo-reactive ubiquitin dimers were tested for cross-linking with the known linkage-selective binding partners. Our results demonstrate both the dependence of photo-cross-linking on the photo-activatable amino acid position and the ability to distinguish between strong and weak binding partners.

Having established that our method works for the lysine-48 and lysine-63-linked dimers we will now apply it to the non-canonical chains, linked via other lysines. After finding new binding partners for non-canonical ubiquitin chains, the role of non-canonical chains will be further classified through detailed experimentation on the interaction between new binding proteins.

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## **WLS TRANSITION FROM OLIGOMER TO DIMER A MECHANISM OF WNT RELEASE?**

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Wnt signaling is essential to regulating cellular homeostasis and the transitions between developmental stages. Mutations and misregulations effecting this pathway can result in cancer or developmental abnormalities. Thus, it is crucial to understand how exactly this signaling occurs. My research focuses on determining whether the functional form of Wntless (Wls), a multi-transmembrane protein dedicated to secretion of Wnt signaling ligands and a conserved component of the Wnt signaling pathway, is oligomeric. Since this process is conserved from humans to *Drosophila* I am using the S2R+ cell culture obtained from *Drosophila*. I examined the oligomeric state of Wls under reducing and non-reducing conditions, because prior results suggest the Wls oligomers are formed by disulfide bonds. To determine whether there are subcellular differences in the Wls oligomerization I compared a secreted and truncated form of Wls, which includes the oligomerization domain, in media and cell lysates. Previous results from the Selva lab suggested the Wls oligomers bind Wnts as an oligomer in the endoplasmic reticulum and escort Wnts to the cell surface for ligand release. Thus, my initial hypothesis was at the membrane the oligomer will revert to a monomer, a potential release mechanism, implying the Wls would be an oligomer in the cell lysate and a monomer in the media under non-reducing conditions. However, preliminary results show that Wls exists as an oligomer in both the cell lysate and media under non-reducing conditions.

Thanks to Erica Selva for all her help and guidance throughout this project. This research was funded by the University of Delaware Summer Fellows grant.

# **METHOD DEVELOPMENT FOR FAST SCREENING OF DRUG EXCIPIENT PROFILES BY ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY – EVAPORATIVE LIGHT SCATTERING DETECTION**

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The Active Pharmaceutical ingredient (API) of drug products tends to be the main focus of both researchers and consumers, however the excipients of drug products have been shown to be a major contributing factor in the efficacy and safety of drugs. Excipients are inactive substances used not only to prepare the drug product formulations, but also to help maintain the stability of the active ingredient and assure its appropriate delivery. With the growing complexity of drug products over the recent years, new methods for measuring and monitoring drug excipients have become increasingly important and may play a greater role in the evaluation of the quality of drug products. This study focuses on the development of methodology for fast screening and quantitation of drug excipients using ultra high pressure liquid chromatography (UPLC) coupled with evaporative light scattering detection (ELSD). The method was developed for a set of sugar standards commonly used as excipients against which unknown prototypical drug excipients were tested. The results from the study show effective separation and efficient identification of drug excipients. Novel approaches utilizing UPLC-ELSD have the potential to advance the regulatory science of product quality for complex drug formulations.

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## **ALZHEIMER'S: CAN A GLASS OF WINE A DAY REALLY KEEP THE DOCTOR AWAY?**

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One of the leading causes of Alzheimer's Disease is the aggregation of a protein plaque known as Amyloid-beta plaque. It has been shown that a stilbenoid molecule known as trans-resveratrol, found in grape vines, has a delaying effect on the progression of amyloid beta plaque. The highest concentrations found naturally are in red wines, specifically the wine from a cabernet grape. The goal of this research is to create and examine the effects of other stilbenoid molecules, relative to resveratrol, on the aggregation of Amyloid- $\beta$  plaques in a fly model of Alzheimer's, in the hopes of finding a more effective compound to be used medically as a preventive or treatment for Alzheimer's. The focus of this past summer was to synthesize several compounds through a greener, solvent-less Wittig reaction. Traditionally the Wittig uses n-butyl lithium as a reagent, which is hazardous since n-butyl lithium is extremely flammable in air. The greener version requires no solvent, and uses potassium hydroxide instead of n-butyl lithium as the deprotonating agent, and the initial waste is aqueous. Additionally, the recrystallization uses an ethanol and water mixture, so the resulting waste is still primarily green/harmless. The percent yield of the reaction depends on the amount of potassium hydroxide. So, currently the reaction is being optimized by varying quantities of potassium hydroxide and differing solvents for recrystallization. Future work shall consist of full characterization of the synthesized compounds via NMR analysis.



## WESTERN BLOTTING OPTIMIZATION

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Protein detection is a key aspect of the analysis of gene expression and function. Western blotting is one of the most commonly used methods to determine protein size and abundance. A major difficulty investigators often have with western blotting is the step after proteins have been transferred onto a membrane via electrical current, when the protein on the membrane is exposed to a primary antibody specific for the protein. Binding efficiency can be low such that the signal is weak or undetectable, background can be high such that signal is obscured, or the signal resolution can be poor. We hypothesize that the conditions of protein/antibody interaction can impact all of these issues. Little has been published on optimization of western blotting conditions. Here, ranges of pH and buffer ionicity were tested to determine their impact on the quality and intensity of the western signal. We tested the standard condition of pH 7.5, in addition to pH 6, 6.5, 7, and 8, and we also tested four different ionicity levels--50mM, 100mM, 150mM, and 200mM NaCl (the standard ionicity is 137 mM NaCl. After testing most combinations of salt and pH conditions, it was found that variations from the standard pH and ionicity conditions increased the amount of background compared to signal and a decrease in band resolution. Future efforts will involve modifications to the blocking agents used (testing powdered milk vs BSA (Bovine serum albumin) as well as testing a Tris-based buffer compared to saline buffered solution.

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## CHARACTERIZATION AND EFFORTS TOWARDS TRAPPING A THIYL RADICAL WITH RLMN I309W

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RlmN and Cfr are two members of the radical S-adenosylmethionine (RS) superfamily of enzymes and catalyze the methylation of carbons 2 and 8 of adenosine 2503 (A2503) in 23S rRNA. RlmN methylates the C2 position, which has been shown to enhance the translational fidelity of the ribosome. RlmN also methylates A37 of different *E. coli* tRNAs.<sup>1</sup> Cfr, a homologous enzyme, methylates the C8 position first and then the C2 position. The methylation of C8 by Cfr confers the bacteria resistance to several types of antibiotics (such as chloramphenicol, Figure 1), and has been isolated from antibacterial resistant bacteria across the globe. A mechanism for RlmN and Cfr has been proposed, in which a covalent cross-link is formed between the enzyme and RNA.<sup>1,2,3</sup> Resolution of the crosslink results in thiyl radical and the enamine tautomer of the methylated product.<sup>1</sup> In order to provide evidence for a thiyl radical, a RlmN I309W variant was generated. Isoleucine 309 is located near the position of the proposed thiyl radical in the active site of RlmN. Upon generation of the thiyl radical in the reaction, the radical may add into the Pi system of the tryptophan and form a cross-link. Here we show that the I309W variant produces methylated product and no radical was detected by EPR, indicating that there was no cross-link formation.

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## **PURIFICATION OF THE CAPPED RNA 5'-LEADER OF HIV-1**

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The production of RNA in eukaryotic cells requires that the RNA has a modified guanosine, native cap, that is added to the 5' end of the RNA. The addition of the native cap prevents the degradation of RNA in the cytosol of the cell, therefore, it is necessary for the survival of the RNA. When analyzing RNA in the lab, it must include the native cap to ensure that the RNA that is analyzed is physiologically relevant. A common protocol that is used for the production and purification of capped RNA, begins with the production of RNA via an in vitro transcription reaction, capping of RNA via the Vaccinia Capping Enzyme Mechanism, and then the purification of capped RNA by gel electrophoresis. Purification of the capped RNA had poor yields that would range from 20% -50% of capped RNA. A different purification process was tested, which purified capped RNA through centrifugation. This new purification process has yields of 80% - 100% of capped RNA.

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## ANALYSIS OF THE SERINE ARGININE-RICH SPLICING FACTOR 1

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The serine arginine (SR) family of proteins play major roles in mRNA splicing, along with genome stabilization in the form of 3' end formation and polyadenylation, the export and translation of mRNA. Serine-arginine-rich splicing factor 1 (SRSF1) is the first protein in the SR family of proteins, which is crucial in the gene expression of the human immunodeficiency virus type 1 (HIV-1). Specifically the SRSF1 protein is responsible for preventing exon skipping which in result ensures alternative splicing is carried out accurately. We plan to examine the binding of SRSF1 to the HIV-1 mRNA along the RNA recognition motifs (RRM) to help in the characterization of the protein RNA complex. Successful characterization of this complex can aid in the potential development of HIV therapeutics; understanding the protein extensively can be used to develop drugs in the near future, which could disrupt the alternative splicing of the HIV-1 genome. In order to analyze these interactions sub-cloning methodology was used to transform the vector containing the sequence that codes for SRSF1 proteins into BL21-DE3 cells. The protein was then expressed using IPTG induction, and the protein was purified using a polyhistidine-tag and a nickel column. We then studied the protein, RNA complex formation by conducting native gel studies. Our results confirmed the protein expression, and the formation of the complex between the protein and our RNA occurred. Based on our data we can conclude that that the protein and RNA bind; however we are unsure of which RRM domain, if not both, are responsible for this complex formation. Future studies will need to be conducted in order to understand the specific domain involved in the complex. To do this we plan to study the structure and the binding affinity of the complex.

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## EXPRESSION AND PURIFICATION OF PHENYLALANINE HYDROXYLASE FOR SENSOR VALIDATION

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Phenylalanine hydroxylase (PAH) deficiency, the minor form of phenylketonuria (PKU), is tested for at the time of birth to prevent any serious complications during development. Without addressing the genetically linked deficiency, individuals suffer from a wide variety of health conditions from osteopenia to mental retardation and many other developmental delays. (1)

A current method to prevent this analyzes phenylalanine levels in blood samples collected from newborns. To further prevent this phenomenon, a simpler biosensor using a paper-based microfluid device could prove faster than current methods. (2) In order to construct a functional device, phenylalanine hydroxylase must be expressed and purified, and then be used to evaluate the sensor.

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## **DETERMINATION OF THE COMPOSITION OF ESSENTIAL OILS IN MEDICINAL HERBS, PLANTS, AND ALGAE AND THEIR APPLICATIONS IN PEST CONTROL**

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Holy basil (*Ocimum teniflorum*), Argan (*Argania spinosa*) and various other herbs and plants are known for their cosmetic and medical use. While there are several studies that show the chemical compositions of these oils and their benefits, there are limited studies on the insecticidal effects. Many of these plants are originally grown in countries of Africa and Asia and currently are on trial to be cultivated in US. For those plants, we are working on the evaluation of their essential oils composition in comparison with grown in native places. Some others are native to US. Here, we hypothesize that the essential oils from holy basil, argan, Aronia mitchurinii, lemongrass and other medicinal plants and herbs will have either deterrent, attractive or repellent effects on insects. To test this hypothesis, we will first isolate essential oils by wet distillation and/or extraction and characterize their composition using GCMS. We will compare results with literature data for similar plants grown in its natural habitats. Then, in order to test the biological effect of the essential oils, a bioassay system will be implemented with controlled pest interference and oil concentrations. Our preliminary tests have shown that essential oils from holy basil leave extracts, that showed mostly monoterpenes and sesquiterpenes on GCMS, demonstrated high deterrence effect against Japanese Beetles with less leaf area damage and high mortality of the beetles observed. This in essence means that holy basil and Argan oil extracts might have potential for pest control in organic produce production.

## UTILIZING A C-TERMINAL HIS-TAGGED VECTOR TO RESCUE HIGH PRIORITY TARGET PROTEINS CONTAINING A SIGNAL PEPTIDE IN VARIOUS PATHOGENS

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The Center for Structural Genomics of Infectious Diseases (CSGID) is a consortium of laboratories using innovative technologies to determine the 3-D structures of proteins and to study their functions from pathogens in the NIAID Category A-C priority lists and organisms causing emerging and re-emerging infectious diseases<sup>1</sup>. At JCVI, we clone and examine the expression and solubility of target proteins for downstream structural and functional analysis. In previous studies N-terminal His-tagged pMCSG7 and/or pMCSG53 vectors were unsuccessful in soluble expression of selected targets in this study. The 6x-His-tag at the N-terminus end blocked the signal peptide activity which prevented protein folding and resulted in poor soluble expression. To recover these targets, the C-terminal His-tagged expression vector pMCSG28 was used to clone the selected high priority targets. Using Ligation Independent Cloning (LIC), the target insert was annealed with vector backbone and then directly transformed into DH5α an *E. coli* cloning strain. The four nicks of an annealed construct were repaired in *E. coli* after transformation. The plasmids were extracted from these cells and were sequence validated before being transformed into the BL21(DE3)/Magic and KRX/pGro7 expression strains. The cells were lysed either by sonication or chemical methods and screened on an SDS PAGE to examine their expression and solubility. In this study, we rescued target proteins, which did not have soluble expression with N-terminal His-tagged vector, using the C-terminal His-tagged vector. Particularly, the targets containing signal peptide were successfully expressed in soluble form. It is critical that in the selection of a vector the signal peptide presence must be considered. The study demonstrated that combination of both vectors improved overall success rate of soluble expression of target proteins. Once these proteins are obtained, X-ray crystallography can be performed to determine their structure and function to help in drug discovery efforts.

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# **ELEPHANT SPECIES IDENTIFICATION FROM IVORY THROUGH POLYMERASE CHAIN REACTION AND SEQUENCING ANALYSIS FOR APPLICATION IN WORKS OF ART**

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Ivory is a material obtained from the tooth or tusk of an animal used for centuries for numerous applications, including art. Species-level identification of ivory works of art is necessary to meet government requirements in travel of exhibitions containing ivory, as well as learning a piece's origins. One of the largest sources of ivory is elephants, of which there are two species: African Elephant (*Loxodonta africana*) and Asian Elephant (*Elephas maximus*). There is currently no known means of visually distinguishing ivory from these species. This effort is made difficult for art pieces due to the artifact's construction and degradation. However, elephants' ivory is distinct morphologically and genetically, suggesting a genetic method of identification.

This study's goal was determining if the genetic identification of ivory is feasible, and, if so, whether it could be applied to artworks. Methods for mitochondrial DNA (mtDNA) extraction from ivory were obtained from published research focusing on forensic analysis for illegal poaching. For positive control and verification of experimental techniques, elephant mtDNA was extracted and amplified from known whole blood and feces samples. Ivory mtDNA was extracted following success with controls. Ivory samples used experimentally did not exceed 0.200g per trial. MtDNA was then sequenced via Sanger sequencing. Sequences were bioinformatically analyzed via BLAST to determine species.

Two samples were successfully identified as *Loxodonta*. These results confirm the possibility of identifying ivory genetically. Due to time constraints, we were unable to determine the minimum mass of ivory necessary to obtain sufficient genetic material for sequencing. A sample of 0.037g of ivory successfully yielded mtDNA, which then underwent PCR amplification. Sequencing failed, however, at the sequencing facility. Without this result and subsequent experiments, we cannot conclude the minimum amount of ivory needed for genetic analysis and, thus, cannot yet approve this method for art works.

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## THE INFLUENCE OF TEMPERATURE ON THE ANTIOXIDANTS CAPACITY OF JUICED ARONIA MITSCHURINII

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*Aronia mitschurinii*, also known as the Black Chokeberry for its taste, is a berry with highest known antioxidant content (15 times more than famous Assai berry future by Dr. Oz). Moreover, this native to Maryland and most of US berries were historically cultivated in Eastern Europe almost without research support. Antioxidants are an important nutrient needed for neutralizing naturally formed free radicals in living organisms, prevention of oxidation, and cancer formation. Those molecules are know to slowly decompose under the influence of higher temperatures, typically used in processes of pasteurization and cooking of aronia products. That is why the research to determine the optimal and safe for antioxidants way to process the berries is so important.

Here we present the data for the antioxidant content of juiced *Aronia mitschurinii* as a function of the variation in temperature and the time of exposure to these temperatures. Detailed measurements and analysis of anthocyanin, flavonoids and polyphenols are presented and discussed, including statistics. The aim of this project is to determine the optimal pasteurization and heating conditions that would avoid significant loss in the antioxidant capacity of aronia.

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## EFFECTS OF HOFMIESTER CATIONS ON SOLVATION OF CAFFEINE USING ATR-FTIR

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Caffeine consists of two fused aromatic rings with four nitrogens and a variety of functional groups, and its structure is similar to many biomolecules. The effects of Hofmeister anions on solvation and aggregation of caffeine have been thoroughly investigated in previous studies. However, the effects Hofmeister cations have on these properties have not been as thoroughly investigated. The Hofmeister series lists cations due to their ability to solvate or desolvate solutes in solutions, and is as follows:  $\text{NH}_4^+$ ,  $\text{Cs}^+$ ,  $\text{Rb}^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Li}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ . ATR-FTIR was used to monitor the cation induced changes in the caffeine absorption spectra. Current infrared studies show that these cations have differential effects on caffeine-caffeine interactions. It was found that divalent cations cause greater solvation of caffeine than the monovalent cations, which have a more subtle, less consistent effect on the solvation. A better understanding of how cation affects caffeine solvation may be relevant to a large number of studies focused on how ions influence the solvation of drugs.

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Dr. Gina Macdonald, Dr. Yanjie Zhang  
Eli and Keyon

## **ANALYSIS OF SPECIFICITY PROPERTIES OF 2-(2'-HYDROXYPHENYL) BENZENESULFINATE DESULFINASE FROM *N. ASTEROIDES* A3H1 AND VARIOUS ANALOGS**

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Biodesulfurization is a metabolically important process that occurs naturally in bacterial cells found near oil and coal deposits. In sulfur-specific biodesulfurization, the dsz pathway, enzymes selectively remove the organic sulfur found in crude oil without disrupting the carbon backbone. The rate-limiting step within this metabolic pathway is the conversion of 2-(2'-hydroxyphenyl) benzenesulfinate (HPBS) to 2-hydroxybiphenyl using HPBS desulfinate (DszB). The DszB enzyme from *Nocardia asteroides* A3H1 was overexpressed in *E. coli*, purified and characterized kinetically. Homology studies identified an active site loop with conserved amino acids that could play a role in the specificity for different organosulfur compounds found in crude oil. Point mutations were generated to study the importance of amino acids 195 and 200 on the specificity of the enzyme. Three mutant enzymes (A195R, A200R and A195/200R) prepared using codon-optimized gene constructs were co-expressed with GroESL and purified from *E. coli* using Ni<sup>2+</sup>-affinity chromatography. Substrate specificity experiments were performed to define the role of the active site loop on specificity and to improve the economic feasibility of using the dsz pathway to decrease sulfur pollution and acid rain production from oil and gas combustion.

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

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

## ***ENTEROCOCCUS* SP. CONTAMINATION OF NEW YORK CITY'S EAST RIVER FROM COMBINED SEWAGE OVERFLOW**

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New York City utilizes a combined sewer system where wastewater, sewage, and runoff are pooled together before being carried to a treatment plant. The system can become overwhelmed during storms, resulting in discharge of excess wastewater into nearby water bodies in a phenomenon known as Combined Sewage Overflow (CSO).

New York's East River is a biologically productive estuary that is used for recreation and fishing. Excess nutrients and bacteria that enter the ecosystem from CSOs negatively affect the river's biodiversity and aquatic health. An important indicator bacterium is *Enterococcus*, which is abundant in human feces. In order to assess fecal contamination from CSOs in the East River, a section of the river between East 63<sup>rd</sup> and East 71<sup>st</sup> Streets in Manhattan was sampled during summer 2017. There are three CSO sites along this stretch, varying in average quantity of outflow. Samples were taken from five locations in a gradient moving away from the dominant CSO outflow in the direction of the river current. Each sample was tested for pH, dissolved oxygen (DO), and temperature before being analyzed using the Enterolert system to count the Most Probable Number (MPN) of *Enterococci* cells in a given volume of sample.

We found that the MPN of *Enterococci* is highest in areas directly downstream of the CSO outflows. Our results also indicate a strong positive correlation between the MPN of *Enterococci* and prior four-day rainfall, consistent with the CSOs acting as the source of bacterial contamination. The data also show a negative correlation between the MPN of *Enterococci* and dissolved oxygen, suggesting that the CSO contamination has a deleterious effect on the overall health of the aquatic ecosystem. We are currently refining a UV/Vis spectrophotometric method for the detection of nitrate and phosphate nutrients in the river samples.

**THERMAL STABILITY OF AROMATIC 2-(2'-  
HYDROXYPHENYL)BENZENESULFINATE DESULFINASE (DSZB) FROM  
*NOCARDIA ASTEROIDES* A3H1**

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2-(2'-hydroxyphenyl)benzenesulfinate desulfinate (DszB) is a desulfinating enzyme that catalyzes the final carbon-sulfur bond cleavage in the biodesulfurization of dibenzothiophene, the major organosulfur compound found in fossil fuels. Homology studies identified an active site loop with conserved amino acids that could play a role in the stabilization of thermophilic homologs. Point mutations were generated to study the importance of amino acids 195 and 200 on the thermal stability of the enzyme. Three mutant enzymes (A195R, A200R and A195/200R) prepared using codon-optimized gene constructs were co-expressed with GroESL and purified from *E.coli* using Ni<sup>2+</sup>-affinity chromatography. Purified enzyme was stored in aliquots containing 15% DMSO and was stable at -80 °C over extended periods of time. Kinetic assays, temperature stability studies, and temperature optimum studies were conducted. Wild-type DszB had optimal activity at 25 °C but is not stable for 30 minutes at temperatures higher than 25 °C. Compared to the wild-type, the A195/200R mutant was more stable at temperatures higher than 25 °C, while the A195R and A200R mutants were less stable at higher temperatures. Thermal stability studies of WT, WT-OPT, and WT without GroES and GroEL enzymes were examined for changes in secondary structure at increasing temperature using circular dichroism. The WT remained folded at very high temperatures (90°C), while the WT-OPT unfolded at temperatures higher than 60 °C. Stability of the WT-OPT can potentially be attributed to co-purification with GroESL. Studies to examine stability in the absence of GroEL and GroES are ongoing.

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## MATRIS-BASED CONTROLLED RELEASE DELIVERY OF ACYCLOVIR FROM SILICONE AND POLY-EVA

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Herpes simplex viruses -1 and -2 (HSV-1 and HSV-2) cause primary and recurrent genital infections in humans. The most common treatment prescribed is oral administration of acyclovir (ACV) or its more readily absorbed prodrug, valacyclovir. ACV is used to treat those infected by HSV-1 and HSV-2, but because of poor oral bioavailability, short half-life, and poor patient compliance oral dosing often shows inefficient viral suppression. We are currently testing the drug release kinetics of 55% (w%) ACV-silicone vaginal rings in cotton rats. We are experimenting with the polymer poly(ethylene) co-(vinyl acetate) (EVA), a non-biodegradable but widely used biocompatible polymer, and its ability to be used as an ACV release platform; we are testing the drug release for two different EVA polymers, one with 25% vinyl acetate (VA) by weight and one with 40% VA by weight. Our lab has fabricated 50% and 65% (w%) ACV EVA rings (with both VA compositions) to test the drug release kinetics alongside those of the 55% (w%) ACV-silicone rings. We conducted one week of drug release in simulated vaginal fluid and quantified ACV through HPLC. The first day of drug release averaged 609 mcg/mL ACV from the 65%(w%) ACV-EVA (25% VA) rings. For days 4-7, 65% (w%) ACV-EVA (25% VA) rings still consistently released approximately 105 mcg/day of drug, whereas 65% (w%) ACV-EVA (40% VA) rings released 6-71 mcg/day and 55% (w%) ACV-silicone rings delivered 4-117 mcg/day. Therefore, the 65%(w%) ACV-EVA (25% VA) rings released the most drug with the highest consistency. These results can now be applied to helping us achieve our short-term goal, use of these rings in experiments with genital herpes in the cotton rat animal model, and our long-term goal, the clinical deployment of vaginal rings in women afflicted with genital herpes.

We would like to thank former and current members of the TUHVL who assisted with this project and the Bridges Program. We would also like to thank Dr. Sivey and Mark Monk for their training on the Agilent 1260 HPLC. This research was funded by grants from the Jess and Mildred Fisher College of Science and Mathematics Undergraduate Research Committee and the Towson Office of Undergraduate Research. This project was also made possible by Grant No. R15A1084069. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

## IMPACT OF LOCAL STRUCTURE CHANGES ON CYTOCHROME ENERGY TRANSFER

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For bio-hybrid mimics of natural photosynthetic systems to be efficient solutions to current energy challenges, the relative bandgap potentials of component energy transfer structures must be optimized. To this end, we developed and extensively characterized 12 point mutations of PpcA, a 3-heme member of the cytochrome *c*<sub>7</sub> family native to *Geobacter sulfurreducens*. These mutations were engineered to influence the redox potential ( $E_m$ ) of the middle heme (heme III) in PpcA by using four different strategies: performing charge reversal mutations, decreasing solvent access to the heme plane with bulky residues, altering the native bis-histidine axial ligation of the heme, and by attempting to form hydrogen bonds with the propionates of the heme. The latter strategy is expected not only to increase  $E_m$  but also to introduce a redox Bohr effect. Out of 12 mutants, 11 were expressed in *E.coli* in sufficient quantities and show thermal stability in temperature-dependent CD experiments comparable to wild-type protein ( $T_m > 90^\circ\text{C}$ ). HPLC-ESI-MS was used to confirm both the purity and the mass of the expressed mutants. Small-angle X-ray scattering confirmed that the mutant proteins were folded correctly and formed the expected compact globular structures while high resolution crystallographic data that has been obtained for A23R and K14E shows unexpected structures. Optical redox titrations have shown our ability to obtain reliable and reproducible data thereby allowing us to measure the effect of the mutations on the electrochemical properties of all 3 hemes and to understand the underlying principles and viable approaches in tuning relative heme redox potentials. Successful development of this project may lead to biological semiconductors with much smaller footprints and selectively tunable bandgap properties.

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## IMINE LIBRARY SYNTHESIS VIA SOLVENT-FREE REACTIONS

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We have initiated studies to expand the scope of Touchette's<sup>1</sup> solvent-free imine formation reaction between ortho-vanillin and para-toluidine. These reactions are cost efficient and exhibit green chemistry properties<sup>2</sup>. The primary goal of this project is to synthesize and characterize a variety of imines. We are taking two related approaches to this study: imine synthesis via para-toluidine and a library of substituted salicylaldehydes or imine synthesis via ortho-vanillin and a library of substituted anilines. Previous studies on structurally similar imine ligands—and their bidentate metal complexes—have revealed multiple biological activities for this class of molecules, including bactericidal properties.<sup>3</sup> We hope to further explore the antibacterial properties of new all compounds produced from our synthetic work. Future studies also include reductive amination of the synthesized imines.

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# ESCAPE BEHAVIOR OF THE *GRAMMOSTOLA ROSEA* TARANTULA AND *PHIDIPUS REGIUS* SPIDER IN RESPONSE TO HEAT STIMULI

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Insects respond to aversive stimuli such as wind, looming objects, and heat by escaping in a direction opposite the stimuli. Spiders, because they have eight legs rather than six, potentially have a greater repertoire of escape responses. However, there are few published studies on their escape responses, especially the effects of stimulus location or direction. The specific aim of this project was to determine the relationship between the stimulus location and direction of response in *Phidippus regius* (Regal jumping spider) and juvenile *Grammostola rosea* (Chilean Rose tarantula) for heat stimuli delivered to the tarsi of the spider's eight legs. Chilean rose tarantulas were chosen because they are docile and readily obtained while jumping spiders are attractive because they have complex predatory strategies. To evoke an escape response, the tarsi of each leg was stimulated in random order at 5 minute intervals with an infrared laser (980 nm). The resulting escape response was captured with high-speed video (300 fps). Following the experiment, movement was tracked allowing quantification of the animals' location and orientation over time. Jumping spiders (n=5) and tarantulas (n=9) displayed both similar and differing responses. For both, the first response was to withdraw the stimulated leg and translate its body directly away from the stimulus, often without turning. Subsequently, both would turn away from the side stimulated and walk varying distances. In contrast to tarantulas, jumping spiders often (~30%) continued to turn until orientating toward the location at which its tarsus was stimulated, and then stopping. These preliminary results demonstrate that tarantulas and spiders, like insects, have well-organized responses to aversive stimuli. The response of the jumping spider, in which it often stopped facing the location of the stimulus, may reflect its reliance on high quality vision and aggressive responses to potential predators.

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## **MODIFYING *DE NOVO*-DESIGNED *DUE FERRI* SINGLE-CHAIN (DFSC) PROTEINS TO MODEL COUPLED BINUCLEAR COPPER PROTEINS**

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Coupled binuclear copper proteins (CBCs) have varying tertiary structures and catalytic properties, despite having a common 6-His copper binding site. It is believed that nuanced structure-function relationships are the cause of this role diversity. The development and characterization of protein-based model system for CBCs allows for in-depth investigation of these relationships. One such protein-based model, the *de novo* designed Due Ferri Single Chain (DFsc) proteins are water-soluble monomeric four-helix bundles optimized for robust mutagenesis, rapid kinetics assays, and promiscuous metal-binding. Several variants on the G4DFsc scaffold, have been shown to mimic natural diiron oxidases. Interestingly, copper titrations have shown 2-, 3-, and 4-His G4DFsc variants bind two copper ions, although they lack the 6-His metal binding site characteristic of CBCs. There is also spectroscopic evidence that the reduced Cu(I):Cu(I) form of these proteins can activate molecular oxygen. The Cu(II)-bound protein variants catalyze the 2-electron oxidation of catechols and 3-amino-4-hydroxybenzoic acid, but lack the phenol monooxygenation activity characteristic of some CBCs. Anaerobic assays suggest 2-electron oxidation is oxygen dependent. Current efforts are focused towards the mutagenesis and characterization of a biomimetic 6-His variant, as well as more in-depth spectroscopic metal-binding and kinetics studies.

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# IDENTIFICATION OF NEW BIOMARKERS IN LENS DEVELOPMENT AND CATARACT

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Lens development in mammals is initiated at early stages in embryogenesis when the presumptive lens ectoderm interacts with the underlying optic vesicle and forms a lens placode that invaginates to form the lens pit. The lens pit pinches off to form a lens vesicle, wherein the anteriorly localized cells form the lens epithelium while the posteriorly localized cells differentiate into elongated primary fiber cells. Throughout life, epithelial cells located near the lens equator exit the cell cycle and differentiate into secondary fiber cells that progressively migrate toward the centrally located core of compact primary fiber cells. Epithelial to fiber differentiation involves expression of specific genes encoding transcription factors, signaling molecules, as well as cytoskeletal, membrane, and crystalline proteins, which are essential for the longevity and transparency of the lens. Misregulation of these factors cause lens defects and cataract, which is defined as an opacification of the lens, and represents the most common cause of blindness.

This study focused on the identification of new proteins in the mouse lens that can serve as biomarkers to assist in the characterization of lens development and cataract. To identify such candidates, we used lens-specific gene expression microarray datasets available in NCBI-GEO. Wild-type ICR mice were bred to obtain embryonic (E)16.5 and post-natal (P)10 lens tissue. Eye tissue was isolated by microdissection, fixed with 4% PFA for 40 min. on ice, and sectioned at 10-14  $\mu$ m thickness on a Leica CM3050 cryostat. Immunofluorescence analysis was performed using primary antibodies specific to candidate proteins from the Developmental Studies Hybridoma Bank (DHSB)- an NIH resource and confocal microscopy. Several proteins were detected to be highly lens-expressed at (E)16.5 and (P)10 and exhibited specific patterns in the lens epithelium, transition zone, and fiber cells. These data establish immunostaining protocols and identify new protein biomarkers for the lens.

Antibodies were provided to the DHSB by the PCRP, the CPTC, the EU Program Affinomics, and A. Kawakami of the Tokyo Institute of Technology. The Developmental Studies Hybridoma Bank (DHSB) –created by the NICHD of the NIH and maintained at the University of Iowa, provided the primary antibodies to be used in our study.

## DIFFERENTIAL BINDING OF C-MYB MUTATNS TO CBP AND P300 KIX DOMAINS

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CBP and p300 are paralogues that function as crucial coactivators of transcription. While similar in sequence and structure, there is mounting evidence that these proteins are not exclusively functionally redundant. Finding a way to specifically inhibit one protein or the other could enable a quick and effective method by which to determine the unique functions of these coactivators. Unfortunately, this task is complicated by the great extent of sequence homology between CBP and p300. We tackled this problem by targeting a single site of distinct residue difference (aspartic acid in CBP and alanine in p300) in the well characterized KIX domain. Peptides that we synthesized to mimic the transactivation domain of c-Myb, mutated at the 303 methionine to promote electrostatic interactions with this site, showed up to a 3-fold difference in affinity for CBP and p300 KIX. Our results indicate that it may be possible to synthesize compounds that exhibit binding specificity for either CBP or p300 KIX.

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# HYDROGEN BONDING IN POLYLACTONES TO IMPROVE INTERMOLECULAR STRENGTH

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Petroleum-based, non-biodegradable plastics, such as LDPE, HDPE, and PETE, are used in storage containers, chairs, water bottles, grocery bags, fuel tanks, car parts, prosthetics and many other applications. They are ideal because they are stable, chemically inert, and strong. However, petroleum-based plastics are bad for the environment because, on average, it takes between 10 to 450 years for decomposition. Due to their durable properties, they can absorb, concentrate, and transport pollutants in the environment; threatening the natural flora and fauna.

Biodegradable plastics are a growing field of interest as a means to replace these petroleum-based plastics. Polyhydroxyalkanoates (PHA) integrate oxygen into the polymer hydrocarbon backbone, which allows it to biodegrade when exposed to three notable bacteria strains; *Bacillus* sp. IBP-V002, *Entrobacter cloacae* sp. IBP-V001, and *Gracilibacillus* sp. IBP-V003. The problem with PHA is that they possess weak intermolecular forces, which leads to a brittle plastic. The integration of oxygen into the polymer backbone renders them vulnerable to certain enzymes. The modification of  $\delta$ -Valerolactone, by  $\alpha$ -substitution with aryl rings, will produce a monomer that will impart more ordered structure to the polymer sample. This increased order will cause the mechanical properties of the polymer to increase and produce a strong biodegradable polymer that can be used in place of commonly used plastics, but which possesses a smaller environmental footprint.

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## EXPERIMENTAL AND COMPUTATIONAL STUDIES OF OBSCURIN'S FLEXIBILITY

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Obscurin is a giant modular muscle protein that functions to connect the sarcoplasmic reticulum to the contractile apparatus. Obscurin is made up of multiple Ig domains in a chain connected by short linkers. Previous structural papers show that short linkers are less flexible, yet MD papers show these linkers to be very flexible. Our research reconciles these divergent data. Here, we test the flexibility of 5 dual obscurin domain systems. Using NMR and SAXS, we show that these domains all adopt an extended architecture. However, MD and SMD data demonstrate obscurin to be significantly flexible. Therefore, we believe obscurin to be an extended, flexible protein, despite its short linkers.

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## LEAVING GROUP EFFECTS IN SOLVOLYSIS REACTIONS

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Substituted ethyl chloroformate esters (EtOCOCl) are alkaloids in oxazolidinones, common components in dyes and antimicrobials. With a chloride substituent on the beta-carbon, we specifically analyzed the chlorine to tosylate leaving group impact in EtOCOX.

To follow the pseudo first-order kinetics of 2-chloro-EtOCOCl and 2-chloro-EtOCOOTS at 25.0 C, we used the acid-base and conductometric titration methods. At this symposium, we will provide the initial results that were obtained.

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## **CHARACTERIZING LONG NON-CODING RNA IN THE ASIAN CITRUS PSYLLID THROUGH GENOME ANNOTATION AND MOLECULAR BIOLOGY**

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Huanglongbing (HLB), or citrus greening disease, is jeopardizing the citrus industry around the world. HLB is an incurable disease of citrus that results in yield loss, decline of tree health and ultimately death of the plant. It is associated with the pathogen *Candidatus Liberibacter asiaticus* (CLas), which is spread by *Diaphorina citri*, or the Asian citrus psyllid (ACP), when psyllids harboring CLas feed on healthy citrus. It is important to note that in order to be effective vectors of CLas, psyllids must acquire CLas as nymphs, the juvenile life stage of the psyllid. My project focuses on interactions between CLas and ACP nymphs, specifically investigating the role of long noncoding RNA (lncRNA). Recent work by the Heck lab identified 83 differentially expressed lncRNAs in the gut of adult ACPs, when exposed to CLas as nymphs, through multi-omics. By identifying and characterizing these lncRNA, we can better understand the relationship between the vector ACP and the pathogen CLas. I identified lncRNA in the ACP genome using multiple bioinformatics pipelines and validated the presence of lncRNA in nymphs with reverse transcription polymerase chain reaction (RT-PCR) and cloning methods. Specific lncRNA found in the adult ACP gut were confirmed to be present in nymphs. Interestingly, expression seemed to vary based on host plant. The end goal of this research is to identify the role of lncRNA in CLas transmission, which will hopefully lead to the development of methods that slow or even stop the spread of HLB.

I would like to thank the Mueller lab and Heck lab at the Boyce Thompson Institute for being so welcoming and supportive of this research. Thank you to my wonderful mentors for providing me with everything I needed to make this project successful. Thank you to everyone at Citrus Greening Solutions for all the support. I would also like to thank Delanie Sickler for being an amazing coordinator this summer. A special thank you to the USDA for funding my research.

## APPLYING AND REVISING ORGANIC CHEMISTRY EXPERIMENTS AT FREDERICK COMMUNITY COLLEGE TO DECREASE COST AND TIME WHILE MAINTAINING YIELD AND SAFETY

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Limitations of resources at Frederick Community College (FCC) has prevented students from successfully performing all aspects of two key organic chemistry experiments in either a reasonable amount of time or with a desirable yield.

Due to a recent increase in the cost of the starting material (*meso*-1, 2-dibromo-1, 2-diphenylethane) for the synthesis of diphenylacetylene, alternate experimental procedures that require less starting material were developed and tested. The synthesis of diphenylacetylene is a simple, straightforward experiment that introduces organic chemistry I students to organic product synthesis. The original procedure was modified to decrease the amount of starting material by one-third. Initial results suggest that the percent yields were more precise and higher than with the larger amount of starting material.

Preserving this experiment allows for the introduction of Raman spectroscopy and ultraviolet (UV) spectroscopy into the Organic Chemistry I lab. These spectroscopies are more suitable than infrared spectroscopy for the analysis of diphenylacetylene due to its conjugation and symmetrical triple bond. Previously, UV spectroscopy had not been integrated into the course material because of the challenges of identifying the appropriate concentration and solvent for analyzing starting material and product.

A key Organic Chemistry II lab involves extracting, separating and assessing the two natural antibiotics present in cloves; eugenol and acetyleugenol. Many students had been unsuccessful in extracting and separating these natural products. After exploring alternate possibilities, it was found that starting with ground cloves requires less whole cloves and decreases distillation time. The revisions improve the safety and success of the experiment. The alterations made to the starting materials still provided sufficient yield for the bioassay and infrared spectroscopy analysis. This experiment can help prepare students for greater success in futures careers in the biomedical field.

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