

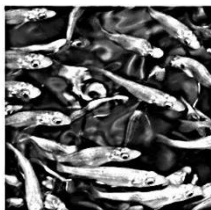
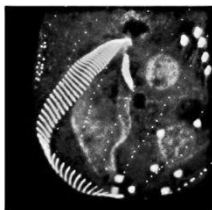
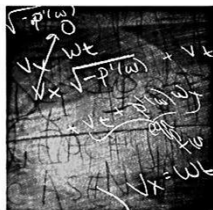
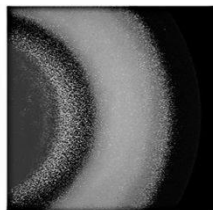
19th Annual Undergraduate Research Symposium in the Chemical and Biological Sciences



Saturday, October 22, 2016

UMBC
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IN MARYLAND

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19th 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*

UMBC
AN HONORS UNIVERSITY IN MARYLAND

Sponsored by:

**National Institute of General Medical
Sciences of the National Institutes of Health
(NIGMS/NIH)**

Schedule of Events

| Time | Event | | |
|---|--|---|---|
| 8:00 am | SYMPOSIUM CHECK-IN & ON-SITE REGISTRATION <i>Lobby, University Center, 3rd Floor</i> | | |
| 8:00 am | LIGHT CONTINENTAL BREAKFAST <i>UC 312, University Center, 3rd Floor</i> | | |
| 9:00 am | OPENING REMARKS & WELCOME ADDRESS Dr. Freeman Hrabowski, President, University of Maryland, Baltimore County (UMBC) Dr. William R. LaCourse, Dean, College of Natural & Mathematical Sciences, UMBC <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i> | | |
| 9:45 am – 11:45 am | MORNING POSTER SESSION Posters # 1-142 A – F: Biochemistry and Molecular Biology, Posters 1 - 38 G – O: Biological Sciences, Posters 39 - 93 P: STEM BUILD at UMBC – Cohort 1, Posters 94 - 100 Q – X: Chemical Sciences, Posters 101 - 142 <i>Ballroom, University Center, 3rd Floor</i> | | |
| 10:30 am | WORKSHOPS <table><tr><td>Master the Art of Making Connections Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i></td><td>A Very, Very Short Introduction to Ethics for Scientists Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i></td></tr></table> | Master the Art of Making Connections Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i> | A Very, Very Short Introduction to Ethics for Scientists Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i> |
| Master the Art of Making Connections Ms. Susan Hindle, Career Services, UMBC <i>UC 310, University Center, 3rd Floor</i> | A Very, Very Short Introduction to Ethics for Scientists Mr. James Thomas, Department of Philosophy, UMBC <i>CASTLE, UC 115D, University Center, 1st Floor</i> | | |
| 11:45 pm | BUFFET LUNCH <i>(gratis for registered guests with symposium name badge)</i> <i>The Commons – Main Street</i> | | |
| 12:45 pm – 2:45 pm | AFTERNOON POSTER SESSION Posters # 143 – 282 Y – DD: Biochemistry and Molecular Biology, Posters 143 - 180 EE – MM: Biological Sciences, Posters 181 - 236 NN – TT: Chemical Sciences, Posters 237 - 275 STEM BUILD at UMBC – Cohort 2, Posters 276 - 282 <i>Ballroom, University Center, 3rd Floor</i> | | |
| 1:30 pm | WORKSHOPS <i>Repeat of workshop titles and locations above</i> | | |
| 3:00 pm | PLENARY TALK “Host-Pathogen Interactions in a Changing Ocean: from Disease Emergence to Solutions?” Dr. Colleen Burge, Institute of Marine and Environmental Technology, UMBC Department of Marine Biotechnology & University of Maryland Baltimore, Department of Immunology & Microbiology <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i> | | |
| 4:00 pm | AWARDS PRESENTATION <i>Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030</i> | | |

Workshops:

Master the Art of Making Connections

University Center, 3rd 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 and how they can advance your professional image as a scientist.



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, 1st Floor, Castle 115D

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



James Thomas is 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

Host-Pathogen Interactions in a Changing Ocean: from Disease Emergence to Solutions?

Meyerhoff Chemistry and Biochemistry Building, Lecture Hall 030

Disease is a natural part of healthy marine ecosystems, however the number and severity of marine diseases is increasing and has been linked to climate change and other anthropogenic factors. We often have a poor understanding of causative agents and infection dynamics. This baseline information is critical to assess the synergisms between human drivers and disease outbreaks, and ultimately better manage disease outbreaks. Disease emergence and epizootics have the capacity to alter both natural and aquaculture-based systems. I will emphasize two host-pathogen systems we are currently focused on in my laboratory: Ostreid herpesvirus 1 infections in Pacific oysters and *Labyrinthula* infections of eelgrass, *Zostera marina*. I will discuss aspects of each host-pathogen relationship including pathogen discovery, diagnostics, and spread as well as potential disease management strategies.



Dr. Colleen Burge is an Assistant Professor at the Institute of Marine and Environmental Technology with dual appointments at University of Maryland Baltimore County (UMBC), Department of Marine Biotechnology and the University of Maryland Baltimore, Department of Immunology & Microbiology. Colleen is a native to the US West Coast where she grew up on the shores of Hood Canal learning marine ecology from a young age. Colleen received her BS (2002) and PhD (2010) in Aquatic & Fishery Sciences at the University of Washington. Colleen held two postdoctoral positions; her first was in the Department of Ecology & Evolutionary Biology at Cornell University and the second in the School of Aquatic & Fishery Sciences at the University of Washington. Colleen's research program, the "Aquatic Animal Health lab" focuses on marine host-pathogen-environment interactions including

disease ecology, organismal physiology and immunology, and development of disease diagnostics. Members of the Aquatic Animal Health lab focus on both temperate and tropical host-pathogen systems, answering both fundamental questions in microbial ecology as well as disease management strategies in a changing ocean. Colleen enjoys mentoring and interacting with students of diverse backgrounds including high school, undergraduate, graduate, and post-graduate. Colleen is a member of the NSF funded Research Coordination Network "RCN: Evaluating the impacts of a changing ocean in management and ecology of infectious marine disease." In 2016, Colleen was a recipient of a UMBC summer faculty fellowship and an Ifremer (Institut Français de Recherche pour l'Exploitation de la Mer) Scientist Fellowship to conduct collaborative research in conjunction with Ifremer scientists on "Biomarkers of disease resistance to an oyster killing virus".

Table of Contents

MORNING POSTER SESSION

Biochemical & Molecular Biology

| | |
|---|-------|
| A | 1-6 |
| B | 7-13 |
| C | 14-19 |
| D | 20-25 |
| E | 26-32 |
| F | 33-38 |

Biological Sciences

| | |
|---|-------|
| G | 39-44 |
| H | 45-50 |
| I | 51-56 |
| J | 57-62 |
| K | 63-69 |
| L | 70-75 |
| M | 76-81 |
| N | 82-87 |
| O | 88-93 |

STEM BUILD at UMBC, Cohort 1

| | |
|---|--------|
| P | 94-100 |
|---|--------|

Chemical Sciences

| | |
|---|---------|
| Q | 101-106 |
| R | 107-111 |
| S | 112-117 |
| T | 118-122 |
| U | 123-127 |
| V | 128-133 |
| W | 134-137 |
| X | 138-142 |

AFTERNOON POSTER SESSION

Biochemical & Molecular Biology

| | |
|----|---------|
| Y | 143-149 |
| Z | 150-155 |
| AA | 156-161 |
| BB | 162-168 |
| CC | 169-174 |
| DD | 175-180 |

Biological Sciences

| | |
|----|---------|
| EE | 181-186 |
| FF | 187-192 |
| GG | 193-198 |
| HH | 199-205 |
| II | 206-211 |
| JJ | 212-218 |
| KK | 219-224 |
| LL | 225-230 |
| MM | 231-236 |

Chemical Sciences

| | |
|----|---------|
| NN | 237-241 |
| OO | 242-246 |
| PP | 247-252 |
| QQ | 253-259 |
| RR | 260-263 |
| SS | 264-270 |
| TT | 271-275 |

STEM BUILD at UMBC, Cohort 2

| |
|---------|
| 276-282 |
|---------|

DISCOVERY OF A RECOMBINATION-DEFECTIVE MUTANT OF ENTEROVIRUS 71

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Enteroviruses are known for their ability to cause neurological damage and paralysis. Among the most famous enteroviruses is the poliovirus (PV), the causative agent of poliomyelitis, a condition characterized by acute flaccid paralysis. A related virus, Enterovirus 71 (EV71), causes similar clinical outcomes in recurrent outbreaks throughout Asia. It is becoming increasingly clear that recombination between circulating strains of EV71 and Coxsackievirus type A produces the outbreak-associated strains of EV71, which exhibit increased virulence and/or transmissibility. While studies on the mechanism of recombination in PV are ongoing in several laboratories, almost nothing is known about recombination in EV71.

We have developed a subgenomic replicon of EV71 expressing a luciferase reporter to monitor EV71 replication in cell culture. In addition, we have developed a cell-based assay to study recombination of EV71. In this assay, we transfect two EV71 subgenomic RNAs into cells. Neither of these RNAs have the ability to produce virus independently. If recombination occurs between the two RNAs, then viable virus will be produced. We have used these assays to determine the extent to which studies of PV recombination pertains to EV71. To do so, we engineered a mutation into the coding sequence of the RNA-dependent RNA polymerase (RdRp) that changes Tyr-276 to His (Y276H), a change known to inhibit RdRp-catalyzed recombination in the PV system.

Using the subgenomic replicon, we showed that Y276H EV71 replicates as well as, if not somewhat better than, wild-type (WT) EV71. Using the recombination assay, we showed that the Y276H substitution in the RdRp impairs EV71 recombination. With these results, we can conclude that mechanisms of recombination used by PV and EV71 are similar. The discovery of the first recombination-defective mutant of EV71 places us in a unique position to determine the role, if any, of recombination in EV71 pathogenesis.

Acknowledgements: This study was funded, in part, by a grant to Dr. Craig E. Cameron from the Human Frontier Science Program. My research was made possible by the 2015 and 2016 summer undergraduate research scholarship sponsored by the endowment of the Eberly Chair in Biochemistry and Molecular Biology.

CHARACTERIZATION OF HUMAN OBSCURIN DOMAINS

Esraa Aldkheil¹, Dr. Nathan T. Wright¹

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Harrisonburg VA, 22807

Obscurin is a cytoskeletal protein implicated in organization and signaling within myocytes. While this giant protein is multifaceted, one of its main functions is to link the contractile cytoskeleton to the surrounding membrane structures. One way it does this is through the specific interactions between obscurin domains Ig58/59 and titin ZIg9/10, and between the obscurin PH domain and various phospholipids. Here, we continue structural studies on these two obscurin regions. PH domain modeling and functional data suggest that this domain binds specifically to PIP₂, however we do not fully understand how this specificity is achieved. To gain better insight into this binding event, we present the initial purification and characterization of the human obscurin PH domain. The high-resolution structure of the obscurin Ig58/59 region has been previously solved in our lab, however molecular mechanism of how obscurin forms a complex with the titin ZIg9/10 region has not been examined thoroughly. To better understand the atomic etiology of this binding event, here we also present initial data mapping the titin ZIg9/10 binding interface of obscurin.

MIMICKING NATURAL PHOTOSYNTHESIS: ULTRAFAST CHARGE TRANSFER IN PpcA-Ru(bpy)₃ COMPLEXES

Matthew O'Malley, Coleman Swaim, Daniel Marzolf, Aidan McKenzie, Oleksandr Kokhan
Department of Chemistry and Biochemistry, James Madison University, 901 Carrier Drive,
Harrisonburg, VA 22807

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)₂(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 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 short covalent donor-acceptor distances play much less significant role.

Acknowledgements: We would like to thank the U.S Department of Energy for Science Undergraduate Laboratory Internship (SULI) funding to MO and the National Science Foundation Research Experience for Undergraduates (NSF-REU) (Grant Number CHE-1461175) to the Department of Chemistry and Biochemistry at James Madison University. In addition, we would like to thank Drs. David Tiede, P. Raj Pokkuluri of the Solar Conversion Group, David Gosztola of the Center of Nanoscale Materials, and Xiaobing Zuo of the Advanced Photon Source for their help and instrument time at Argonne National Laboratory.

STRUCTURAL AND FUNCTIONAL STUDIES OF AQUIFEX AEOLICUS PROTEINS AQ_328, AQ_1359, AND AQ_1482

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Aquifex aeolicus is a thermophilic species of bacteria which contains proteins that resemble eukaryotic histone proteins and modifying enzymes. Histone proteins within nucleosomes can undergo post-translational modification leading to a variety of biological effects. One such modification is acetylation of lysine residues within a histone which has been found to regulate gene expression. Aq_328 is a protein that has been shown to contain a histone fold motif. Aq_1359 and Aq_1482 are hypothetical proteins that may be structurally homologous to known acetyltransferases. In this study we used electrophoretic mobility shift assays to investigate whether Aq_328 binds to DNA as well as implementing size exclusion chromatography coupled with multiangle light scattering to determine Aq_328's stoichiometry. Aq_328 was found to be in monomeric, dimeric, and tetrameric states. Aq_1359 and Aq_1482 were then screened for substrates under the assumption that they behave as acetyltransferases. When Aq_1359 and Aq_1482 were screened for activity with Aq_328 no acetyltransferase activity was observed. These data will provide insight into genome regulation by post-translational modification and the origins of chromatin modification.

We would like to acknowledge the National Science Foundation and their funding of this research with the Research Experience for Undergraduates grant and the Jeffress Memorial Trust for Interdisciplinary research.

INVESTIGATION OF YEAST UBIQUITIN HYDROLASE-1 AND PROUBIQUITIN SUBSTRATES

Shane P. Herr¹

Dr. Christopher E. Berndsen¹

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Ubiquitin is a highly conserved protein in most eukaryotes, most commonly known for tagging proteins for degradation. Ubiquitination cannot occur, however, until ubiquitin is converted from a raw form—termed proubiquitin—to the usable form of ubiquitin. Proubiquitin is a fusion protein made up of a ubiquitin molecule fused in-frame to another ubiquitin, a ribosomal protein, or an amino acid of another protein. Ubiquitin C-terminal Hydrolases (UCHs) cleave the peptide bond of proubiquitin and thus allow ubiquitination to occur. There are currently five UCHs in humans believed to cleave proubiquitin substrates, while only one UCH in *Saccharomyces cerevisiae* is thought to cleave proubiquitin substrates. Our research has focused on purifying proubiquitin substrates and subsequently testing their activity with Yeast Ubiquitin Hydrolase-1 (YUH1). Two of the four proubiquitin substrates have been purified and appear to have activity with YUH1. Future work will include purifying the remaining proubiquitin substrates and determining if YUH1 is capable of cleaving these substrates.

Acknowledgements: We acknowledge the support of an NSF-REU award (CHE-1461175) and the Department of Chemistry and Biochemistry.

AB INITIO QUANTUM MODELING OF HYDROXAMIC ACID METAL CHELATION

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08205-9441

This work sought to understand the energies ab-initio (DFT-B3LYP-631++G, and DFT-B3LYP-321-G) differences between apo/metal chelated modified benzenes and Vorinostat derivatives that includes hydroxamic acids. Hydroxamic acid is an important functional group for chelation of metal ions to inhibit the enzymatic activity of important proteins such as Tumor Necrosis Factor α Converting Enzyme (TACE)/(ADAM-17) and Vorinostat. The inhibition of enzymes which use metals as a catalyst can be an effective inhibitor of enzymatic activity thereby limiting disease such as cancer and HIV. One example is that of TACE which blocks the release of the TNF α into the bloodstream thereby decreasing the inflammation response and disease progression. The understanding of energy differences in metal chelation is an important aspect of metal selectivity. Differences between the apo and zinc chelated molecules sought to understand which biosteric modifications improved the overall bonding efficiency of metal to the hydroxamic acid.

COMBINATION THERAPY TO ENHANCE THE EFFICACY OF RECOMBINANT IMMUNOTOXINS: EVALUATING THE ROLE OF MITOCHONDRIAL TRANSLATION IN TOXIN-MEDIATED CELL DEATH

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Recombinant immunotoxins (RITs) based on *Pseudomonas* exotoxin A (PE) are targeted cytotoxic proteins currently in clinical trials for the treatment of cancer. PE covalently modifies and inhibits eukaryotic elongation factor 2 (eEF2), which halts protein synthesis within the cytosol and ultimately induces apoptotic cell death. Although PE inhibits translation in the cytoplasm, eukaryotes also have a translation system in mitochondria that is similar to translation in bacteria. Recent findings have highlighted the importance of mitochondrial stability and function in inducing tumorigenesis, sustaining cancer cell proliferation, and maintaining cancer cell survival. By inhibiting protein synthesis within the mitochondria through the usage of antibiotics, mitochondria may become unable to properly function, resulting in the inability to meet the energy requirements of the cell and the activation of apoptotic cell death. I propose that combination treatment with both PE-based RITs and antibiotics that inhibit mitochondrial protein synthesis will reduce cancer cell survival compared to independent treatments of either therapeutic alone. To test this hypothesis, combination treatment using RITs and several different antibiotics have been applied to CA46 and HEK293 cell lines followed by the assessment of cell viability through WST-8 assay. This method showed no statistically relevant effects for HEK293 cells, but the combinational treatment of RIT and chloramphenicol in CA46 cells showed a two-fold reduction in cell viability compared to either treatment alone. Combination treatment of specific antibiotics and PE-based RITs warrants further exploration as a possible cancer therapy.

This project was supported by funds from Towson University's Office of Undergraduate Research and the Fisher College of Science and Mathematics, as well as an undergraduate research award from the American Society for Biochemistry and Molecular Biology.

PLOIDY ANALYSIS OF BREAST CANCER CELLS USING CENTROMERE FISH

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Breast cancer is an important cause of cancer in women worldwide. One pathological Ductal carcinoma in situ (DCIS) of the breast is a heterogeneous pre-neoplastic lesion can result in invasive breast cancer or remain contained in the breast duct. The standard of treatment of DCIS can include lumpectomy, or local radiation and adjuvant tamoxifen to prevent recurrence. A better understanding of DCIS disease progression would provide insights on the drivers to malignant disease as well as offer potential targets for reversal of DCIS and progression of early stage disease. Ploidy analysis is a supplemental test that is done to measure the amount of DNA in tumor cells. Ploidy analysis of DCIS breast cells and DCIS breast cells with mutations in the tumor suppressor gene, ANCO1 were analyzed to detect the number of chromosomes in a cell, using centromere FISH for chromosome 17, in particular as a marker. The present study was carried out by hybridizing direct-labeled DNA probes to fixed interphase and metaphase cells. Slides containing metaphase spreads and interphase nuclei are treated and hybridized with direct-labeled DNA probes. Specimens are stained with fluorescent molecules. Fluorescent molecules absorb light of a particular wavelength. After post-hybridization washing dehydrated in a graded ethanol series of 70%, 80%, and 95% for 2 minutes each at room temperature) the slide is counterstained and visualized using a fluorescence microscope. Thirty individual interphase cells were counted for each DCIS cell line by three independent readers. Approximately twenty percent of the cells examined showed additional signals. We found that by using FISH method to determine the number of chromosomes or analyze ploidy. In cells in which there were two 17 chromosome signals, we assumed that the cells were diploid. Diploid suggests that a portion of cancer cells have the same exact number of chromosomes as healthy normal cells. Tumors with greater distribution of aneuploid cells are associated with more aggressive tumors. For that reason, diploid cancer cells are considered to be less combative growing cancers.

I would like to personally thank my mentor and PI, Dr. Jan K. Blacato, for her patience, assistance and guiding me towards the right path of life.

FLUORESCENCE IN SITU HYBRIDIZATION FOR PLOIDY ANALYSIS OF CARCINOMA IN CITU CELLS

Laura B. Garcia¹, Virginie Ory, Ph.D.², and Madihah Fakrudeen¹, Jan K. Blancato, Ph.D.²

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Breast cancer is the most frequently diagnosed female cancer and one of the leading causes of cancer death in the USA. This disease affects as many as 1 in 8 women during their lives. Ductal carcinoma in situ (DCIS) of the breast can give rise to invasive and metastatic breast cancer, but it is difficult to predict which lesions will progress. Today, the standard treatment of DCIS includes lumpectomy or mastectomy, local radiation, and adjuvant tamoxifen to prevent recurrence. However, based on outcome data, it is predicted that only about 20% of DCIS would progress to invasive disease; suggesting that about 80% of the DCIS cases are being over-treated. It is suggested that the loss of expression of the gene ANCO1 in breast cancer cells could give rise to pre-neoplasia as it acts as a tumor suppressor. ANCO1 (=ANKRD11) is a large ankyrin domain repeat protein that binds a number of transcription regulation factors (1, 2). The loss of expression of this protein in breast cancer cells leads to an increase of transcription of genes that could drive pre-neoplasia. Cells in these populations appeared to grow in large clumps and not appear undergo normal cell division. We used FISH with a centromeric probe to determine if the cells were undergoing endoreduplication, that is nuclear replication without cell division. We found that the two control cell lines expressed normal diploid numbers of the FISH probe. The experimental Knocked out lines showed a population of cells that were hyperdiploid. In addition, the study showed that the DCIS cells had difficulty in late metaphase transition, suggesting that the cells did not divide correctly. The specific checkpoint involved in this and the exact point at which the abnormality in anaphase to telophase transition is currently under investigation.

RNA SEQUENCING ANALYSIS OF THE HUMAN RETINAL TRANSCRIPTOME

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The retina is a layered neuronal tissue lining the anterior portion of the eye that converts photons of light into visual images in the brain. Rod and cone photoreceptors are highly specialized light sensitive neurons that initiate this process of phototransduction. Though they are very similar cell types, rods and cones have distinct functionalities, synaptic connection, and are affected by different blinding disease alleles.

In order to better understand the distinct molecular function of human photoreceptors, cone rich central macula and rod rich peripheral retina samples were biopsied from post mortem human donor eyes in biological triplicate. Total RNAs were extracted from retinal samples using a Qiagen AllPrep Mini Kit. RNAs were validated for quality and sent for mRNA-Seq transcriptome analysis using the Illumina NextSeq 500 sequencing platform. Raw sequence quality assessment and high precision mapping to the Human 2013 Hg38 genome assembly were achieved using FastQC and TopHat software respectively. Differentially expressed genes (DEGs) between macula and retina were determined using CuffDiff software analysis of TopHat mapping outputs between the two sample groups. DEGs were filtered for biological and statistical significance yielding 2,240 genes significantly upregulated and 2,387 genes significantly downregulated in the peripheral retina compared to the macula with a p-value of <0.05.

Ongoing pathway analysis is currently being conducted to identify genes with previously unrealized roles in rod and cone-specific visual perception.

Acknowledgements: We would like to thank the Commonwealth Health Research Board (CHRB) and the 4-VA Research Grant for helping support our research.

PREFERENTIAL KILLING OF V-RAS-TRANSFORMED NIH 3T3 CELLS BY INHIBITING ACTIVATED Cdc42 – DEPENDENT KINASE (ACK)

Ruth Opoku, Nadia Burgos, Ijaz Ahmed, Raj Rajnarayanan, and Alam Nur-E-Kamal
Department of Biology, Medgar Evers College of the City University of New York

Hypothesis: The Ras GTPase family has been found to regulate various functions of mammalian cells. Oncogenic mutants of Ras GTPases have been demonstrated to be associated with the development of various types of cancer in human. We propose a hypothesis that cancer cells are more dependent on Ras-Cdc42-ACK signal transduction pathway for their survival. We have previously demonstrated that overexpression of Cdc42 kinase domain of ACK induce death of v-Ras transformed NIH 3T3 cells, but not the parental NIH 3T3 cells. In this report, we studied the effect of tyrosine kinase inhibitors on growth of v-Ras-induced and parental NIH 3T3 cells.

Method: We have seeded v-Ras transformed NIH 3T3 cells into culture dishes and incubate overnight at 37°C in standard culture condition. Then tyrosine kinase inhibitors of MG series were added individually or in different combination at various concentrations. Cell growth was monitored by MTT assay in every 24 hours for three days. We determined IC₅₀ for each inhibitor. We have assayed markers of apoptosis in ACK inhibitor treated cells.

Results: It was found that some inhibitors of activated Cdc42-associated Kinase (ACK) induced Ras-transformed cell death, while some inhibitors did not exhibit v-Ras cell death activity. The cell death activity of these inhibitors exhibited very weak activity against parental NIH 3T3 cells. We also demonstrated that cell death was associated with induction of apoptosis. We will present the results obtained from this study.

Conclusion: Our results indicated that ACK inhibitors induce preferential death of v-Ras transformed cell. Development of ACK kinase inhibitors might lead us to design new experiments to study the potential of such inhibitors for the treatment of Ras-induced cancer.

EFFECT OF AKT INHIBITION ON PROSTATE CANCER CELLS

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Numerous studies have confirmed the energetic requirements for actin filament polymerization and cytoskeletal rearrangement. An interdependent relationship exists between the PI3K/Akt pathway and cell motility in migratory cells and metastatic cancer cells (1). Our study assessed the effect of MK2206, an allosteric Akt inhibitor, on cell velocity in prostate cancer cell line, LnCap. Transfection of the cells with LifeAct-mCherry and mito-BFP allowed for dynamic cell tracking to measure changes in subcellular components. As MK2206 treatment resulted in changes in the cytoskeleton, we hypothesized that cellular metabolism might also be perturbed. Immunofluorescence was used to image the cellular localization and roughly quantify metabolic enzymes GlutI and PFK1, along with actin in treated vs. nontreated cells.

References

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Acknowledgements

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STRUCTURE AND FORCE RESISTANCE OF THE IG35/36 AND IG58/59 DOMAINS OF OBSCURIN

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Obscurin is a large cytoskeletal protein involved in striated muscle organization. Its primary function is to connect the sarcomeric cytoskeleton to the surrounding sarcoplasmic reticulum. Accompanying these long-range interactions, obscurin changes shape as the muscle cell expands and contracts. This motion by obscurin, intrinsically resists force. Here, we begin to ask the related questions of how vigorously obscurin resists force, and what the molecular details of this force resistance have. To do this, we concentrate on previously-solved dual domain structures of obscurin (domains 35/36 and 58/59). By studying these domains in tandem using NMR and SAXS techniques, we gain insight into obscurin's shape and self interactions. With the use of computational techniques, such as molecular dynamics and RDC, we gain understanding of how obscurin resists external force. In comparison, the data suggest different interactions between obscurin dual domains, which supports the notion that the linker region sequence is significant for function. These studies lead the way to more fully probe how obscurin uses its force resistance function to influence cell stretch, signaling, and organization.

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SIMULATION OF VIRAL TETHERING BY MEMBRANE ANCHORED ANTI-VIRAL PROTEIN BST-2

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BST-2/tetherin is a human extracellular transmembrane protein that serves as a host defense factor against HIV-1, HIV-2, and SIV by inhibiting viral budding through a tethering action. Structurally, BST-2 is a flexible helical homo-dimer that is connected to N and C terminal transmembrane anchors by loops. The coiled-coil structure of BST-2 is maintained by three disulfide bonds between cysteine residues of the monomers. The structural mechanism of viral budding and tethering as regulated by BST-2 is not clear. We took a novel approach and simulated viral budding and tethering using steered molecular dynamics to understand the structure function relationship of BST-2. The dynamics and folding of BST-2 in a lipid membrane were analyzed through steered molecular dynamics using the program YASARA. We simulated the viral budding and monitored changes to BST-2 structure over duration of the simulation. We find that non-canonical heptads in the coiled-coil region of BST-2 permit bending of the coiled-coil without full unfolding. These findings support previous mutational analysis and provide a residue level view of the mechanism of viral tethering by BST-2.

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EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF CODON OPTIMIZED DSZB FROM *N. ASTEROIDES*

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Dibenzothiophene (DBT) and its derivatives comprise approximately 60% of the organosulfur contamination of crude oil. 2-(2'-hydroxyphenyl)benzenesulfinate desulfinate (DszB) catalyzes the carbon-sulfur bond cleavage in the final, and rate limiting step in the biodesulfurization of DBT. The DszB gene from *Nocardia asteroides* (A3H1) was codon optimized and overexpressed in *E. coli*, purified, and characterized kinetically and thermally. Expression was examined by FT-IR and activity assays. It was determined that codon optimized DszB (co-DszB) requires chaperone proteins groEL and groES to limit misfolding. Purity of the enzyme was increased by adding additional washes with 10 mM imidazole when eluting from the Ni²⁺ column. Enzyme stored in aliquots containing 15% DMSO was stable at -80 °C over extended periods of time. Temperature stability and optimum were then measured. DszB has optimal activity at 35 °C but is not stable for 30 minutes at temperatures higher than 23 °C.

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HIGH RESOLUTION STRUCTURE OF TITIN ZIG10

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Titin domains ZIg9/10 bind to obscurin domains Ig58/59 during myofibrillogenesis. Mutations in this region lead to hypertrophic cardiomyopathy (HCM) in humans. While the cellular consequences of this interaction are well characterized, the molecular determinants governing this structure are unknown. Previous work from our lab has solved the high-resolution structure of the obscurin domains of the complex. Here, we describe the purification and complete structure characterization of titin domain ZIg10.

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SOLUBILITY STUDIES OF A ZINC-FINGER ANTIVIRAL PROTEIN CLONED WITH AND WITHOUT SOLUBILITY TAGS

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Zinc-fingers are protein folding motifs that are known to use zinc ions in order to stabilize the folding. The Zinc-finger structural motif binds to the DNA (at the major groove). They stabilize and aid the folding of DNA by binding the Zinc atoms to Histidine (His) and Cysteine residues (Cys). The Zinc-Finger Antiviral Protein (ZAP), is known to carry an important antiviral resistance function in the cell against retroviruses such as human immunodeficiency virus (HIV). ZAP contains zinc-finger protein motifs, and the understanding of its binding patterns and exact structure will help to discover the exact ways in which ZAP can be manipulated. In order to find those structural features, the ZAP zinc-finger domain (ZAP-ZFD) must be purified and isolated. Fusion proteins were cloned with two different solubility tags: NusA and Maltose Binding protein (MBP). ZAP-ZFD was also cloned without a solubility tag in order to avoid complications during purification; however the purified soluble fractions of the sample did not contain the protein of interest.

I would like to thank the Hood College, Department of Physics and Chemistry, as well as the Protein Expression Lab at the Frederick National Laboratory for Cancer Research.

EPIGENETIC CHARACTERIZATION OF RETINA-SPECIFIC TRANSCRIPTION FACTORS

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The vertebrate retina is a neuronal tissue of the eye containing rod and cone photoreceptors that make vision possible. Highly regulated gene expression controls differentiation in the developing retina. DNA methylation on cytosine bases of genomic DNA is an epigenetic modification inversely correlated with gene expression. Currently in our lab, the relationship between DNA methylation and the ability of two retina-specific transcription factors to bind in the genome is being studied. These transcription factors, known as cone-rod homeobox (CRX) and neural leucine zipper (NRL), have been shown to act synergistically to control photoreceptor expression in the retina. Preliminary data has supported the hypothesis that DNA methylation is critical for modulating cell-specific binding of CRX and NRL to target recognition sites. DNA binding domain sequences of the human CRX and NRL proteins were cloned into expression vectors, were transformed into competent *E. coli* cells, and transformants were selected for induction of protein expression. Expressed proteins were affinity purified for downstream biochemical analysis. Gel shift assays will be used to determine the ability of CRX and NRL to bind to in vitro unmethylated and methylated oligonucleotides at cytosine residues proposed to be important in DNA methylation and binding of CRX and NRL as determined by bioinformatics analysis. Collectively, these studies will contribute to a better understanding of how epigenetic modifications influence the development, homeostasis and pathology of human retinal neurons.

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EXPRESSION OF MYXOMA VIRUS LEUKEMIA ASSOCIATED PROTEIN IN THE PRESENCE AND ABSENCE OF SOLUBILITY TAGS

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Zinc-fingers were first known for their DNA-binding domains, consisting of multiple fingers that could bind to the major groove of DNA. Now zinc-fingers are additionally known to be a structural motif that stabilizes protein folds through the coordination of zinc atoms with cysteine and histidine residues on the protein. The zinc-finger motif is found in the Myxoma Virus Leukemia Associated Protein (MV-LAP), a protein produced by the Myxoma Virus (MV) which infects the European Rabbit. The MV-LAP protein greatly reduces immune system function in the host's body through the down-regulation of the major histocompatibility complex class I (MHC-1) and remains undetected by the host's immune system. Before the structure of MV-LAP can be characterized, the protein must first be expressed and purified. Two fusion proteins were cloned using two different solubility tags, Maltose Binding Protein (MBP) and NusA, as well as a histidine affinity tag for purification. Both MV-LAP fusion proteins were successfully purified, however, when MV-LAP was cleaved from the tags, purification was unsuccessful. A third fusion protein was cloned with only the histidine tag, and no solubility tag. Without the solubility tag, however, the protein did not appear in the soluble fraction of the cell.

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AF4-AF9 PROTEIN-PROTEIN INTERACTION INHIBITOR: SYNTHESIS AND BIOLOGICAL EVALUATION

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In normal human cells, the Mixed Lineage Leukemia (MLL) gene is believed to encode proteins involved in the transcriptional regulation of developmental processes. However, MLL can undergo chromosomal translocations to join with over sixty partner genes and produce fusion proteins that play key roles in the transformation of hematopoietic cells into leukemia cells. These genetic fusions are observed in eighty percent of infant Acute Lymphoblastic Leukemia patients and sixty percent of infant Acute Myeloid Leukemia patients, and the most common fusion partner is AF4. MLL-AF4 interacts with transcription factor AF9, and it has been shown that inhibiting this interaction leads to cell death. As such, the AF4-AF9 interaction is a target for disrupting the MLL fusion pathway and a potential point of intervention for pediatric leukemia.

This study aims to evaluate and improve upon a small molecule inhibitor of the AF4-AF9 interaction identified from high-throughput screening. Though the compound disrupts AF4-AF9 association, its mechanism of action is unclear and difficult to study. AF4 and AF9 are intrinsically unstable proteins and the initial hit compound is prone to oxidation. In this study, more stable analogs of the hit compound were synthesized. In addition, AF9 was expressed in high yield as a fusion with AF4 containing a proteolytic site in the linker. The fusion protein can be cleaved, but separation of AF4 and AF9 has proved challenging due to strong intermolecular association. Interactions of the complex with the target molecule were evaluated by circular dichroism and fluorescence anisotropy.

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IDENTIFICATION OF NOVEL FOLATE METABOLIZING ENZYMES

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Vitamin B9, also known as folic acid, is a critical nutrient that serves as a cofactor in many metabolic pathways. Currently, there are only assays to detect one form of folic acid in food products. In an effort to identify enzymes that could be used in assays to detect multiple forms, soil bacteria were screened for an organism capable of using folic acid as a single carbon source. A gram-negative bacterium capable of growing on folic acid was isolated and grown up in single-carbon source media with folate as the sole carbon source. 16S-RNA sequencing identified the organism as a strain of *Stenotrophomonas maltophilia*. Cells were harvested, lysed, and the cell lysate was passed through a folate affinity column. Protein eluted off the column using a folate solution was quantified with a BCA assay, concentrated, and analyzed by SDS-PAGE. The resulting bands were excised and digested with trypsin in preparation for LC mass spectrometry. Chromosomal DNA was isolated in preparation for next generation sequencing.

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ALTERNATIVE CONSTRUCTION METHODS OF AN INVITRO BLOOD VESSEL MODEL

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For solid cancerous tumors to grow, angiogenesis, the formation of new blood vessels, must occur to allow for oxygen and other nutrients to enter the tumor. This has been an active area of research in the search for better cancer treatments. Angiogenesis is a complicated biological phenomenon and in order to study it effectively, we need systems that can recapitulate the process, yet are amenable to investigator observation and manipulation. To accomplish this, we have utilized an angiochip, a three-dimensional, in vitro, microtissue model of angiogenesis. The angiochip is only a few square inches and is composed of a top and bottom layer of cured silicone rubber (polydimethylsiloxane, or PDMS). Small circular chambers are created within a 3D collagen gel that allow for the formation of capillaries within the collagen voids. This system is able to generate planar vessels which offer advantages for microscopic imaging and are easily accessible for drug treatment manipulation drug treatment.

Here, we present different methods to construct the angiochip in a manner amenable to our facilities. Rather than using plasma treatment to activate the surface of PDMS to facilitate layer bonding, we have explored the use of sulfuric acid and variations in PDMS curing time. We have found that only partially curing the two layers before combination works well, but painting the layers with uncured PDMS and then curing again was ineffective.

This research is important for our ability to create robust, three dimensional models of angiogenesis. Having such microtissues in vitro will ultimately allow us to investigate more complicated topics like the role of Rho family GTPases in angiogenesis. Having an in vitro platform can allow us to manipulate, observe, and control this process in ways not possible with traditional in vitro angiogenesis model systems.

OPTIMIZING CHARACTERIZATION TECHNIQUE OF PROTEIN POST-TRANSLATIONAL MODIFICATIONS USING ENZYMATIC DIGESTION COUPLED TO HPLC-MS

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This experiment aims to enhance the enzymatic digestion methods to characterize protein with various post-translational modifications (PTMs) (e.g., glycosylation, methylation, phosphorylation, acetylation, ubiquitination, etc.) using High-Performance Liquid Chromatography (HPLC) mass spectrometry (MS). Proteins generated with PTMs play critical roles in cell signaling, cell cycle, apoptosis, monitoring transcription regulation, and many more. Mislabeled proteins as well as inappropriate protein levels can negatively affect body functions causing various health problems/concerns. Consequently, characterizing protein post-translational modifications becomes important especially within medical, biological and environmental research fields. This experiment examines the effectiveness of single and multiple enzymatic digestions in protein fragmentation combined with the use of a HPLC separation with a Dionex Ultimate 3000 Nano system in line with a Bruker 12T Apex IV Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). This technique utilizes ultra-high resolution mass spectrometry detection in conjunction with collision induced dissociation to rapidly and unambiguously identify the sequence and possible PTM attached to the digested peptide. Ultimately, the spectra collected from the instrument are analyzed by computer software called Peaks. The software uses mathematically known equations to reconstruct fragmented peptide sequences and compare the results against available protein databases. This allows for protein identification and sequence coverage to be easily calculated from the data. This powerful combination between enzymatic, HPLC, and MS methods promises an effective technique in characterizing protein PTMs that helps drive the understanding of cellular functions.

The experiment is made possible through the great help and support from the members of the MCAC, who I would like to thank for providing me with essential professional understanding, training and instrumental utilization.

NMR ASSIGNMENT OF THE TAR HAIRPIN IN THE HIV-1 5' LEADER RNA

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In 2015, 1.1 million deaths in the United States were associated with acquired immunodeficiency syndrome (AIDS). The causative agent of this fatal disease is the human immunodeficiency virus (HIV-1). Our research studies the late phase of this virus which characterized by two equilibrium states; the monomer and dimer conformations of the 5' Leader (5'-L) in the RNA genome. A hallmark interaction occurs with the Unique 5 region (U5) which characterizes the respective conformations. In the monomer, the U5 region is bound to the Dimer Initiation Site (DIS), and in the dimer it is bound to the AUG hairpin. Both conformations are important for essential cell functions. The dimer acts as the genome for a new virion during packaging. Both conformations are predicted to contain the TAR hairpin, which is involved in transcription activation. In our experiment, we synthesized a construct known as the locked dimer. This construct was modified to lock the full length leader into the dimeric conformation. It also had a mutation in the DIS sequence that prevented intermolecular dimerization. Using small oligo controls we have been able to confidently assign the entire TAR hairpin in the monomer and upper stem of the TAR hairpin in the dimer. The lower stem TAR signals broaden out beyond detection in the dimeric conformation. It is possible that TAR is taking a new conformation where it is coaxially stacked with the adjacent polyA hairpin in the dimer. To test this hypothesis, we plan to make a GMP labeled sample, which selectively labels the first guanosine of the TAR hairpin. If TAR and polyA are forming a continuous helical structure, there would be a signal produced that shows the interaction between the first guanosine on TAR and G103 on polyA.

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ANALYZING THE STRUCTURAL EFFECT OF 5' START SITE HETEROGENEITY ON THE HIV-1 RNA USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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The human immunodeficiency virus-1 (HIV-1) is currently treated with a medication cocktail, with each drug targeting a specific viral protein. The high rate of mutation of these non-conserved proteins leads to the frequent emergence of resistant strains. The 5' Leader (5'-L) of the HIV-1 RNA genome is a highly conserved region that is constantly in equilibrium between a monomeric and dimeric conformation, which has not been exploited as a therapeutic target. There are discrepancies in the current literature regarding the exact start site of the HIV-1 RNA. *In vivo* studies found that there existed a mixed population of capped viral RNA in the cell with start sites that began with one, two, or three guanosines (Cap1G, Cap2G, and Cap3G respectively). Our goal is to examine the difference in these various start sites and determine its effect on the structure and function of the RNA. Using native gel electrophoresis, it was observed that the Cap1G 5'-L favored the dimeric conformation, while the Cap2G and Cap3G 5'-L favored the monomeric conformation. Applying nuclear magnetic resonance (NMR) spectroscopy and various specific ²H-labeling schemes, we investigated the start site region of the native 5'-L in both its monomeric and dimeric conformations. Incorporating extra guanosines in the 5'-L permits the destabilization of the lower stem of the polyA stem loop. This destabilization releases residues from polyA to base pair with and sequester the palindromic sequence of the dimer-promoting DIS hairpin, thus strengthening the monomeric conformation of the RNA. Further understanding of this novel regulation of the HIV-1 RNA structure and fate can potentially lead to a new highly conserved drug target for HIV therapy.

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ASSIGNING THE SECONDARY STRUCTURE OF THE HIV-1 5'-LEADER MONOMER-DIMER CONFORMATIONS

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The human immunodeficiency virus-1 (HIV-1) is a pandemic that affects thirty-seven million people worldwide, resulting in over 1.2 million AIDS-related deaths yearly. During the late phase of the retroviral replication cycle, the 5' Leader (5'-L) region in the HIV RNA genome can remain as a monomer or base pair with another 5'-L molecule to form a dimeric complex. In this monomer-dimer equilibrium, the monomer allows the RNA genome to be translated into Gag-pol, while the dimer is responsible for assembling an RNA-protein complex to be packaged into new virions. Due to the lack of information, there is currently no drug therapy that targets this highly conserved portion of the viral RNA. We plan to characterize the secondary structure of the two conformations using nuclear magnetic resonance (NMR) spectroscopy. However, studying large RNAs by NMR is difficult because only four residues are present, thus resulting in limited chemical shift dispersion. We overcame this obstacle by using a fragmentation technique. This technique uses smaller RNA samples that mimic structural elements believed to form in the full-length 5'-L. The NMR data from these smaller fragments can be confidently assigned and can be overlaid with the data from the full length 5'-L to confirm the existence of these structural regions. This method allowed us to confirm several structural elements in both the monomer and the dimer conformations. Our future work includes assigning the bottom of the TAR hairpin and the polyA stem loop in the dimer as well as confirm the U5:DIS interaction and the U5:AUG interaction in a native monomer and dimer construct, respectively.

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UNDERSTANDING THE EVOLUTION OF CIRCADIAN RHYTHM IN CYANOBACTERIA BY KAIABC PROTEIN COMPLEXES

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Synechococcus elongatus, a cyanobacterium, has a circadian clock whose mechanism relies on three proteins: KaiA, KaiB, and KaiC. Of the three, KaiC, a hexameric protein, auto-phosphorylates and dephosphorylates throughout the day; this is the core protein in the complex. KaiA and KaiB are regulatory proteins that activate and suppress phosphorylation causing KaiC to oscillate through different phosphorylation states. As of now, not much is known about the functions of the circadian clock mechanism, its connection to the environment, or how it compares to other bacterium that lack one or both regulatory proteins. In this study, *T. vulcanus* will be compared to *S. elongatus*. We will run an assay composed of purified proteins with a set amount of ATP in a constant temperature. Samples will be collected at specified time points which will be analyzed via HPLC and SDS-PAGE. HPLC will be utilized to analyze the rate ADP production while the SDS-PAGE will allow us to analyze the phosphorylation states of the protein complex. Results of both analysis will be compared to those of *S. elongatus*. We see similar results from the SDS-PAGE, however we require more consistent results to come to a conclusion. *T. vulcanus* is five times faster in terms of ADP hydrolysis than *S. elongatus*. In the future we plan on running a similar assay on two other bacteria: one that lacks KaiA, *Rhodobact_spharoides*, and one that lacks both KaiA and KaiB, *Pyrococcus horikoshii*. Through analysis of these bacterium we hope to have a better understanding of this timing mechanism.

THE ROLE OF SCLERAXIS IN NEUTROPHIL FUNCTION

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Scleraxis, a transcription factor in the basic helix loop helix (BHLH) family, has been shown to regulate the development of embryonic tissue into tendon and endothelium in blood vessels. Recently scleraxis has been linked to activation of fibroblasts and cardiac fibrosis. Organ fibrosis follows inflammation and is characterized by dysregulated and excessive production/release of extracellular matrix proteins—principally collagen. Fibrosis ultimately affects the ability of the organ to function which may lead to prolonged disease. Within such fibrotic organs there are a variety of immune cells, such as Polymorphonuclear Leukocytes (PMN). PMN are first responder cells that respond to injury and inflammation as part of innate immune system and are highly present in organs undergoing fibrosis. PMN have two basic functions, they migrate to areas of inflammation/injury and they destroy pathogenic organisms. They may destroy such pathogenic organisms by the release of myeloperoxidase (MPO) from their primary granules. MPO is a heme protease that is toxic to cells and the release of MPO is heavily dependent on the leukocyte specific integrin CD11b. We hypothesize that scleraxis is involved in the activation of PMN. Immunocytochemistry and Flow Cytometry show that PMN express scleraxis. Detergent lysates used in Western Blot Analysis also reveals that scleraxis is present in PMN. Multiple antibodies to scleraxis show minimal effects on cell attachment of PMN to fibrinogen (CD11b ligand). However, such exposure to anti-scleraxis resulted in the release of MPO, which suggests that scleraxis may be linked to MPO ultimately activating PMN. In summary, these results highlight scleraxis as a potential target protein in the treatment of inflammatory diseases involving PMN.

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RNA-DEPENDENT RNA POLYMERASE-CATALYZED RECOMBINATION IN VITRO

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The viral RNA-dependent RNA polymerase (RdRp) is responsible for transcription and replication of the genomes of RNA viruses. Most RdRps lack a proofreading exonuclease, the consequence of which is a high mutational load in RNA virus populations. One mechanism to purge deleterious mutations and to combine beneficial mutations in a population is recombination. Poliovirus (PV) uses a template-switching mechanism for recombination. During PV RdRp-catalyzed synthesis of RNA on one template, the RdRp-nascent RNA complex "jumps" to another template, the specificity of which is thought to be driven by the sequence of the 3'-end of nascent RNA. Our goal is to establish a robust *in vitro* recombination assay to illuminate the biochemical mechanism of recombination. We have succeeded at creating a primed-template system that can be extended by the PV RdRp, and, in the presence of second template whose sequence contains partial complementarity to the 3'-end of nascent RNA, can "jump" to this new template. Because the template-switching event occurs at the end of the template, this assay mimics outcomes that would occur *in vivo* on templates damaged by nucleases, for example. We find that single nucleotide addition to the blunt-ended product duplex activates the donor RNA for transfer. Donor RNA binds to the end of the acceptor RNA instead of to an internal position. Two base-pairs appear to be sufficient to drive recombination. PV RdRp derivatives with increased capacity to add non-templated nucleotides to donor RNA exhibit increased capacity to catalyze recombination. The converse is also true. We identified concentrations of heparin sufficient to inhibit enzyme that dissociates from donor RNA prior to transfer. When heparin was added to a template-switching reaction, recombination products were observed. Therefore, recombination can occur without the enzyme dissociating from the donor. This assay will prove useful in establishing a detailed mechanism of RdRp-catalyzed recombination.

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PROTEIN:RNA INTERACTIONS THAT NUCLEATE HIV-1 VIRAL ASSEMBLY

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36 million people are currently infected with human immunodeficiency virus (HIV), a retrovirus responsible for the onset of the acquired immunodeficiency syndrome (AIDS). Upon transmission, the virus invades CD4⁺ T cells and integrates its proviral genome into the host genome leading to a life-long infection. During the viral life cycle, interactions between the unspliced viral RNA and its translated product, the Gag polyprotein, initiate the packaging of two copies of the HIV genome. Gag contains three structured domains: Matrix (MA), Capsid (CA), and Nucleocapsid (NC). The NC domain of Gag binds to regions of the 5'-leader (5'-L) within the dimeric HIV-1 genome to initiate genome packaging and viral assembly.

We seek to characterize the Gag-RNA interactions essential to genome packaging using a truncated 5'-L derivative and a hexameric NC protein. The basic unit of the immature viral shell is a hexamer of Gag; mutations at the hexameric interface significantly reduce Gag's selectivity towards dimeric viral RNA. We hypothesize that the hexameric structure of the CA domain contributes to the dimeric RNA genome selection. However, hexamer-hexamer interactions in the C-terminal Domain (CTD) of CA lead to protein aggregation and precipitation in the presence of RNA. To circumvent these problems, we have fused the NC domain of Gag to a hexameric protein scaffold, which mimics hexameric Gag but forms isolated hexamers and does not aggregate in solution. The truncated 5'-L contains only the necessary regions needed for NC binding and packaging. With these RNA and protein constructs, we aim to deduce the qualitative conditions, thermodynamic parameters, and structural information of these Gag-RNA interactions using electrophoretic mobility shift assays, isothermal titration calorimetry, X-ray crystallography, and cryo-electron microscopy. These studies will further our understanding of the mechanism of HIV genome selection, a step of the life cycle that can be targeted with therapeutics.

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INVESTIGATING THE MECHANISM OF TRANS-LESION SYNTHESIS BY HUMAN DNA POLYMERASE KAPPA

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Our DNA is constantly under attack by both exogenous and endogenous chemicals that have the ability to damage it. If not repaired, these DNA adducts can lead to mutations to the DNA or even worse, they can halt replication, eventually leading to aging and/or cancer. Human DNA polymerase kappa (pol κ) is a member of the Y-family of DNA polymerases, known for their ability to conduct translesion synthesis of damaged DNA. Translesion synthesis allows for continued replication past lesions in the DNA that would otherwise stall or halt replication, leading to cell apoptosis. So far, the mechanism for lesion bypass discrimination by pol κ is unclear. Recent studies suggested that, similar to other polymerases, pol κ might cycle between different open and closed conformations upon binding to DNA. Starting with one of the available crystal structures of pol κ , we prepared three different DNA/protein models and we solvated them in an octahedral water box with counter-ions to a final ionic strength of 150 mM, using CHARMMgui. Subsequently, through molecular dynamics simulations, we tested the validity of the conformational change hypothesis by comparing the structural changes undergone by pol κ in the presence of different templating lesions and with correct and incorrect incoming nucleotide. All simulations were performed using the program NAMD, and were run for 10 ns each. Simulation of the binary system (just the protein and DNA without the incoming nucleotide) was also performed as a control system. By analyzing these different structures, we ascertained the residues crucial for fidelity and proposed a mechanism for lesion discrimination.

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STRUCTURAL 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 which 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, I have constructed plasmids encoding Sup35, Rnq1, and Ure2 prion proteins containing an internal hemagglutinin epitope tag and confirmed their expression in yeast. These tools will allow me to examine cotranslational prion aggregate formation on ribosomes using sucrose density gradient centrifugation to purify ribosomes and any associated prion proteins.

I would like to thank my research mentor, Dr. Dale Cameron, for his endless guidance with this project. Additionally, I give my thanks to Usman Baqai, Tom Tessitore and Annie Li for their assistance and mentorship in the laboratory. Finally, I thank Ann Breen for helpful discussion and lab materials assistance. This work was supported by the Ursinus College Department of Biology and the National Institutes of Health Academic Research Enhancement Award Program.

INVESTIGATING THE EFFECTS OF INTERLEUKIN-3 IN AN ANIMAL MODEL OF RETINAL AND CEREBRAL MALARIA

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Malaria is a mosquito-borne disease caused by the protozoan parasite Plasmodium. Common outcomes of infection include anemia, fever, chills, sweats, and headaches. In addition, some strains of Plasmodium induce a potential fatal neurological condition called cerebral malaria in which parasitized RBCs become sequestered in blood vessels of the brain and cause inflammation. Sequestration and vascular damage also occurs in the retina and the degree of damage correlates with the severity of microvascular brain damage. We wished to determine if the presence of the cytokine interleukin-3 (IL-3) affected the progressive microvascular changes that occur in the retina and brain during infection. To investigate these effects, we sacrificed C57BL/10 mice and IL-3 knock out mice that were previously injected intravenously with the fluorescent dye Evans blue. Mouse eyes were then enucleated, fixed, and retinal whole mounts were prepared and examined by confocal fluorescence microscopy. Brains underwent a spectrometry assay to determine levels of leakage. Our results show that mice lacking the IL-3 gene experienced lower levels of leakage in the brain's vasculature. Experiments to determine the effects of the cytokine on retinal vasculature are still underway.

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PARP INHIBITOR SENSITIZES HUMAN TUMORS WITH ALTERATIONS IN THE CHROMATIN REMODELING PATHWAY TO RADIOTHERAPY

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Ovarian cancer is the leading cause of gynecological cancer deaths in the world. Despite of the efforts aiming at early detection and new therapeutic approaches to reduce mortality, approximately 70% of the ovarian cancer patients present advanced diseases when diagnosed and the 4 years' survival rate is less than 30%. The main reason of this poor prognosis is ovarian cancer is highly resistant to standard chemotherapy. Even those patients who have responded to the therapy ultimately develop recurrent cancer. Therefore, more research in finding alternative therapy is an unmet need.

Studies has shown that when cancer cells grow rapidly, DNA (deoxyribonucleic acid) damage can occur as a form of single or double strand breaks. The two major damage repair pathways are homologous recombination and base excision repair. When a chromatin remodeling gene is mutated, the homologous recombination pathway is blocked; therefore, DNA repair is more dependent on the base excision repair known as the PARP pathway. As a result, the mutation of a chromatin remodeling gene will be more vulnerable to the PARP1 inhibitor. Thus, we hypothesize that the combination of PARP inhibitor and DNA damaging agents (eg. irradiation) sensitizes ovarian cancer with mutations in chromatin remodeling genes. We used vivo animal study and in vitro isogenic ovarian cancer cell lines. The chromatin remodeling gene was knocked out to test effectivity of this new combination treatment. A cell viability assay was performed to determine the number of viable cells once the cancer cells were treated with the PARP inhibitor and DNA damaging agent both as a single and combination treatment. In addition, an immunohistochemistry experiment was performed by using a chromatin remodeling gene and Ki67 biomarker. In conclusion, the combination of PARP inhibitor and irradiation significantly sensitized and decreased ovarian tumor growth of the chromatid remodeling mutated gene.

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THE RELATIONSHIP BETWEEN THE 15 KDA SELENOPROTEIN AND COLON CANCER REGULATORY GENES IN COLON TUMORIGENESIS

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Selenium is an essential nutrient that can be co-translationally inserted as selenocysteine into selenoproteins, some of which have been shown to affect mechanisms in cancer prevention and promotion. Our previous study showed that mice lacking the 15kDa selenoprotein (Sep15) developed fewer chemically-induced pre-neoplastic lesions (aberrant crypt foci) than control mice. In this subsequent study, we investigate the ability of Sep15 knockout mice to develop colon tumors in a model of inflammatory carcinogenesis. As expected, most of our control mice developed small to medium-sized tumors when injected with the known colon carcinogen azoxymethane, followed by dextran sulfate sodium administration in drinking water to induce inflammation. However, much to our surprise, many of our Sep15 knockout mice also developed tumors. To explicate these findings, we have isolated mRNA from tumors of both control and Sep15 knockout mice, and we are in the process of quantitatively assessing the mRNA expression of genes typically involved in regulation of sporadic colon carcinogenesis (e.g., *Apc*, *Wnt*, *Ctnnb1*), cell proliferation (*Pcna*, *Ki67*), inflammation (*Nfe2l2*) and selenoproteins shown to be involved in both prevention and promotion of cancer (e.g., *Txnrd1*, *Gpx1*, *Gpx2*), using real-time RT-PCR. We continue to investigate the expression of apropos genes and their pathways to examine what possibly, if anything, makes the tumors in Sep15 knockout mice different from those observed in littermate controls. We hope to expand into the function of Sep15 and what the absence of the functioning protein means for the progression of colon cancer.

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DOES L1CAM PROVIDE CHEMOTACTIC SIGNALS THAT INSTRUCT MIGRATING GLIOBLASTOMA CELLS?

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Glioblastoma multiforme (GBM) is the most common and malignant form of brain cancer. Part of what makes it so dangerous is the tendency for tumors to regrow after tumor resection and kill patients. Currently, there is no effective treatment for such cases, nor is there a consensus on the cause of pervasive GBM cell dispersal. One possible reason for this is the expression and release of the adhesion protein L1CAM (L1). Previously, this lab used modified scratch assays to show that L1 increased motility and proliferation of glioblastoma cells. Since some GBM cells release L1 into their surroundings, it seems possible that L1-responsive cells could be attracted to and/or follow L1 producing cells. However, a chemotactic effect of L1 on glioblastoma cells has never been demonstrated. The possibility of L1CAM attracting and guiding glioblastoma cells along a concentration gradient was investigated. This has been done *in vitro* by modifying an existing scratch assay protocols to examine not only the velocity of cells, but the preferential direction of their movement with and without the presence of cells that released L1. We found that cells tended to move toward a chemotactic gradient of L1. Furthermore, we qualitatively studied a possible chemotactic relationship *in vivo* by injecting a mixture of fluorescently labelled L1+ and L1- glioma cells into the midbrain of chick embryos. After ten days, the brains were dissected, sectioned with a vibratome, and optically sectioned with a confocal microscope. 3D models then were rendered to examine the invasion pathways of the GBM cells. This showed a “leader-follower” relationship between L1+ and L1- cells. Also, the lab has begun culturing living sections of chick brain tissue. It is our hope that by using these different approaches, we will gain an understanding of L1’s potential role in guiding GBM cell invasion.

I would like to thank the University of Delaware’s Summer Scholars Program and the Department of Undergraduate Research, as well as Dr. Galileo, Kyle Plusch, and Reetika Dutt.

THE IMPACT OF HABITAT TYPE ON COMPETITIVE INTERACTIONS BETWEEN ALIEN FIRE ANTS AND ANT SPECIES ASSEMBLAGES ON SAN SALVADOR, THE BAHAMAS

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The imported red fire ant (*Solenopsis invicta*) and the tropical fire ant (*Solenopsis geminata*) are both common, introduced ants throughout most tropical regions of the world. These ants appear to prefer disturbed habitats and frequently interact with humans. Both species are very aggressive, and will attack any animal including ground nesting bees, wasps, and other insects that disturbs their large nests. Studies have shown that the presence of fire ants reduces populations of ground nesting birds, lizards, and mammals. Our studies on San Salvador Island, The Bahamas, examine the habitat preference and competitive interactions of fire ants with other native and introduced ant species. We observed ant interactions at 136 baits across two field seasons (June 2014 and 2015) and in 3 different habitat types (Blackland, Palmetto, and plantations). We found 27 ant species at our baits across both years of this study. The Blacklands had the highest number of native ant species and plantations had the lowest number of native species. Across all habitats, 51% of ant visits observed at baits were by non-native species. Interestingly, competitive interactions at the baits were relatively predictable within each habitat. When alien fire ants were present at baits they would dominate the bait with few exceptions. Many studies have demonstrated that both *S. invicta* and *S. geminata* prefer disturbed habitats and are not frequently found in native undisturbed habitats. We have observed this on San Salvador, which suggests disturbance has a role in alien fire ant persistence on the island. Thus, the clearing of Blacklands may increase the pressure on native ant species from encroachment of alien fire ants.

POTENTIAL TOXICITY OF AMMONIUM AND ITS EFFECT ON THE ANACOSTIA PHYTOPLANKTON COMMUNITY

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The ecosystem health of Anacostia River in the District of Columbia is suffering from nutrients that enter the river as result of anthropogenic activities such as fertilizers from a golf course and people's homes and also through the area's antiquated combined sewer outfalls. As result of these activities, large amounts of nitrogen (N)-containing nutrients such as nitrate and ammonium end up in the waters of the Anacostia. N enters the river through the combined sewer system via overflows when there is a rainfall event. One of the N forms, ammonia, is needed for aquatic life, but may be toxic in high concentrations. For example, high concentrations of ammonia may result in algal blooms. In this study, there were 11 sampling sites chosen because of prevailing land uses along the river. The type of land varies greatly along the river, from wetlands at the first site to a baseball stadium near the last site. In addition to that, there is a higher number of combined sewer outfalls closer to the last site than the first site. Samples were taken every two weeks for chlorophyll *a*, and N concentrations (including ammonium). It was hypothesized there would be a trend where increasing ammonium levels would correlate with a decrease in chlorophyll *a* levels, which would indicate suppression of growth. The results show ammonium levels increased from the first to the last site, and the relationship between ammonium and chlorophyll *a* show a general negative correlation. However, this trend is not consistent over four years. This could be due to differing discharge amounts, proportions of ammonium available to phytoplankton, and adaptation of phytoplankton.

A special thank you goes to the Gordon Brown Foundation, the Anacostia Riverkeeper, Environmental Sciences Research Fund, Maryland Sea Grant, and the DC Water Research and Resources Institute for supporting my research.

RISK DILUTION AND EAVESDROPPER PREFERENCES: THE DYNAMICS OF PREDATION AND PARASITISM RISK IN MIXED-SPECIES CHORUSES

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Eavesdropping predators and parasites, such as the frog-eating bat, *Trachops cirrhosus*, and *Corethrella* midges use mating calls to locate and capture their prey. Eavesdroppers can result in strong selection pressures that influence calling behavior and signal structure. Signaling from aggregations is a tactic used by species of many animal taxa to reduce these predation pressures. However, frogs calling from aggregations are likely to share calling sites with other species and participate in mixed-species mating choruses. When frog species congregate in ponds, the presence and abundance of other species calling close by can affect the predation and parasitism pressures on all individuals participating in these choruses.

In previous work, we demonstrated that male hourglass treefrogs, *Dendropsophus ebraccatus*, signaling for mates near calling túngara frogs experience drastically increased parasitism risk. Túngara frogs, however, produce two distinct mating call variants, a simple ‘whine’, and a complex call that pairs this whine with a broadband ‘chuck’. Thus, these variation in calls may also have a significant effect on the hourglass treefrogs.

Our study used multi-speaker field playback experiments to test whether predator and parasite visitation rates to hourglass treefrog calls are influenced by variation in call-type or density of nearby túngara frogs. Specifically, our questions were: (1) Does variation in the túngara frog call-type alter predation and parasitism risks faced by nearby calling hourglass treefrogs?, and (2) Does the density of túngara calling males influence parasitism and predation risk in hourglass treefrogs? We played pre-recorded exemplars of hourglass treefrog and túngara calls and quantified bat visitation with infra-red cameras and midge visitation with sticky traps. Determining whether individual predation and parasitism risk is influenced by call variation and density of species calling nearby will give us insights into the forces that help shape species composition and spatial arrangement of individuals in breeding aggregations.

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EFFECTS OF AGRICULTURAL RESTORATION PRACTICES ON STREAM HEALTH IN THE SHENANDOAH VALLEY, VIRGINIA

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The Shenandoah Valley encompasses some of the highest agricultural producing regions in Virginia, many of which are large contributors of nutrients and sediment. The Conservation Reserve Enhancement Program (CREP) assists landowners in the installation of riparian restoration projects in which cattle are fenced out or a riparian buffer is planted. We examined the temporal effects of riparian restoration and the impact of upstream land use on water quality for eleven farms participating in the CREP program for various times (from 1 to 14 years). We hypothesized that the length of time that the CREP program has been established would have a positive effect on the water quality of a stream. Water quality was quantified by measuring benthic macroinvertebrate using the Hilsenhoff Biotic Index (HBI), Virginia Stream Condition Index (VA-SCI), Shannon Diversity Index, and total abundance. GIS analysis was also employed to calculate several different upstream land use and stream channel characteristics: land use, canopy cover, slope, impervious surface, relief, road density, and watershed area were assessed for the watersheds and 100-meter stream buffers at each sampling site. Single variable, as well as multiple, linear regressions were performed separately within the watershed and buffer zones. No single variable showed a significant relationship. However, the time since restoration and the percentage of upstream forested land use predicted HBI values, both in the watershed ($p = 0.005$, $R^2 = 0.668$) and in the buffer zone ($p < 0.001$, $R^2 = 0.766$). VA-SCI was predicted by restoration time and upstream impervious surface in the buffer zone only ($p = 0.025$, $R^2 = 0.501$). This shows that CREP efforts are positively affecting water quality, although upstream land use is also an important factor.

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THE SUPPRESSION OF GARLIC MUSTARD BY COMMON CHICKWEED

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Invasive species negatively impact the environment and cause economic harm. Two common invasive plants in eastern deciduous forests are *Stellaria media* L. (common chickweed) and *Alliaria petiolata* L. (garlic mustard). A field study was conducted that investigated soil pH and population traits of *A. petiolata* in the presence and absence of *S. media*. The presence of *S. media* inhibited *A. petiolata* growth, resulting in smaller leaves and biomass. The data suggest that allelopathic chemicals released into the soil by *S. media* acted as a growth inhibitor to *A. petiolata*. While *A. petiolata* has few threats in its invaded habitat, the also invasive *S. media* may restrict its distribution and impact on eastern deciduous forests.

DOES DIET MODULATE GENETIC INFLUENCES ON REPRODUCTION AND LIFESPAN?

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The genetic basis for fitness traits appears to change with environmental context. One example is that with variable diet, which fluctuates depending upon ecological factors (population density, seasonal changes, disruptions in food source), different genes are implicated in certain life history traits such as reproduction and lifespan. In the fruit fly *Drosophila melanogaster*, altering yeast content (primary source of cholesterol and protein for fruit flies) has been shown to affect longevity. A previous genome wide association (GWA) study in this lab identified candidate genes that contributed to variation in age-specific reproduction and longevity. The purpose of this project is to validate the diet-specific influence of these candidate genes using RNA interference (RNAi). Flies with reduced expression of target genes will be reared on either a low- or high-yeast diet, and age-specific reproduction and longevity will be recorded and compared to control genotypes. Reproduction will be measured by the number of eggs laid at one and five weeks of age, and lifespan from eclosion (emergence from pupal case) until death. This information will contribute to our understanding of the genetic basis of these traits in fruit flies and potentially other species as more than 60% of the genes in *Drosophila* are evolutionarily conserved in vertebrates. This project will also test the theory that the genetic basis of phenotypic variation depends on the environmental conditions experienced by the organism. This could explain how genetic variation in fitness traits is maintained in populations.

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IT TAKES TWO TO TANGO: ALGAL AND *AMBYSTOMA* EMBRYO CO-CULTURE EXPERIMENTS REVEAL HOST-SYMBIONT FIDELITY IN ENDOSYMBIOTIC INTERACTIONS

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A unique symbiosis occurs between embryos of the spotted salamander (*Ambystoma maculatum*) and green algae (*Oophila amblystomatis*). Unlike most vertebrate host-symbiont relationships, *A. maculatum* exhibits an endosymbiosis, where the green algal cells enter embryonic tissues as well as individual salamander cells. Past research has consistently categorized this symbiosis as a mutualism, making it the first example of a beneficial microbe entering vertebrate cells. Another closely related species of salamander, *Ambystoma gracile*, also harbors *Oophila* algae in its egg capsules; however, extensive sampling across the *A. gracile* range has consistently shown this to be an ectosymbiotic interaction. In this study we observed the ability of each species of salamander to form an association with host-specific strains of green *Oophila*. Both *A. maculatum* and *A. gracile* embryos were raised in cultures with their own algae or algae from the other host. Under these conditions *A. gracile* never forms an endosymbiosis with either algal strain. While *A. maculatum* algae will enter embryonic *A. maculatum* tissues under these culture conditions, the *A. gracile* algae will not. These data reveal host-symbiont fidelity that allows the unique endosymbiosis to occur in *A. maculatum*, but not in closely related symbioses.

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ROLE OF BTF3/ICD-1 IN CONTROL OF CANCER-ASSOCIATED GENE EXPRESSION IN *C. ELEGANS*

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Cancer is a heterogeneous collection of diseases defined by uncontrolled and invasive cell growth; driving this growth are mutation-driven changes in gene-expression patterns that contribute to tumor cell proliferation and invasion into local tissue. DNA polymerase B transcription factor 3 (BTF3) is overexpressed in numerous malignancies, including prostate, colorectal and gastric cancers. In pancreatic cancer, BTF3 has been shown to affect the expression of Ephrin receptor 2 (EFNB2), a developmental marker expressed in neurons that also plays an essential role in angiogenesis. Specifically, overexpression of BTF3 in pancreatic tumor cells is thought to upregulate EFNB2 expression which, in turn, contributes to cancer development. How the overexpression of EFNB2 contributes to cancer development is unclear. *C. elegans* is a genetically malleable model organism that contains orthologues for both BTF3 (ICD-1) and EFNB2 (Vab-1); depletion of either protein generates robust and reproducible phenotypes in the animal. My goal is to characterize, quantitative as well as qualitative, the relationship between these phenotypes by depleting ICD-1 in wild type and *vab-1* knockout animals. My hypothesis is that certain phenotypes observed in wild type worms depleted of ICD-1 will differ in *vab-1* mutant worms depleted of ICD-1, and that these differences will provide insights into specific ICD-1/Vab-1 interactions. By elucidating the relationship between ICD-1 and Vab-1 in *C. elegans*, we hope to better understand the relationship between BTF3 and EFNB2 in humans, and how the overexpression of these genes might contribute to the development of cancer.

EVIDENCE OF HORIZONTAL GENE TRANSFER BETWEEN *LISTERIA MONOCYTOGENES* AND ITS PHAGES

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Horizontal gene transfer (HGT) is the transfer of genetic material from one organism to another. *Listeria monocytogenes* is a known food-borne pathogen that causes *Listeriosis*. We report on HGT between two *Listeria* phages and 212 *L. monocytogenes* strains. Of these, 54 bacterial strains were found to contain a tail tape measure protein (TMP) that is found in phages. These TMP's in bacteria were compared by multiple sequence alignment and phylogenetic analysis to measure evolutionary distances. Using available metadata, the strains were then grouped into serovars. Serovar information was available for 36 of the 54 strains and the strains grouped in 8 different serovars. Overall our results suggest that HGT does occur between *Listeria monocytogenes* and its phages. Serovar information further suggests that HGT occurs in specific *Listeria* groups.

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IDENTIFICATION OF SECONDARY EXOME VARIANTS RELATED TO MANDIBULAR PROGNATHISM IN THE CLINSEQ COHORT

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Mandibular prognathism is a dentofacial deformity associated with significant Class III skeletal relationship and appears as a concave facial profile, commonly known as “Habsburg jaw.” This condition occurs in ~1% of the US population, and the dysmorphology may be severe enough to warrant surgical reconstruction in ~21% of cases. Understanding the influence of suspected genetic mutations on development of facial structure will help researchers capture the full spectrum of the phenotype-genotype correlation and assist a clinical team to address a patient under study, and affected family members, by facilitating individualized treatment plans through monitoring facial growth patterns.

Our study sample is ClinSeq, an NHGRI project which aims to sequence 1500 participants to improve the way genetic information is stored, shared, and analyzed. We have collected a subset of presently sequenced individuals (n=951) who possess a mutation in at least one of 20 genes, that has been extracted through literature review, to be involved in development of prognathism. Algorithmic notations were constructed to locate variant matches and provide clinical significance in our sample against genomic databases like ClinVar and HGMD. Using the Pandas module, metadata was parsed and matched with refined parameters such as chromosome, position, reference allele, and alternate allele to search for likely causative variants. 7 single nucleotide variants within 4 genes were matched within the ClinSeq subset.

The databases provide researchers with genomic and molecular information to justify correlations between the variants and mandibular prognathism. Identification of individuals from the ClinSeq subset possessing the genotype will lead to matched individuals’ participation in NIDCR protocols involving iterative phenotyping in which individuals with condition-associated genotypes are monitored for clinical signs and symptoms. This approach to bioinformatically analyze the cohort’s exomes for secondary variants has the ability to be applied to studies concerning other disorders with genetic components while using ClinSeq data.

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OVEREXPRESSION OF DUAL FUNCTION FRUCTOSE-1,6-BISPHOSPHATASE/ SEDOHEPTULOSE-1,7-BISPHOSPHATASE (DFS) TO IMPROVE GROWTH IN *CHLAMYDOMONAS REINHARDTII*

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Algal biofuels are an environmentally sustainable alternative to currently used and diminishing fossil fuels because they can be obtained directly from biomass derived from carbon dioxide (CO₂) and sunlight. Increasing the photosynthetic activity of algae can increase their growth rate and biomass. One way to increase photosynthetic activity is to improve the Calvin cycle, a photosynthetic pathway of enzymatic reactions that convert CO₂ and the energy of sunlight into sugars. Dual-function fructose-1,6-bisphosphatase/sedoheptulose-1,7-bisphosphatase (DFS) is a cyanobacterial enzyme that works to regenerate ribulose-1,5-bisphosphate (RuBP) in the regeneration phase of the Calvin cycle. Previously others found that overexpressing this cyanobacterial protein that possesses two Calvin cycle enzyme activities, could significantly improve plant biomass production. The goal of this project is to determine whether overexpressing this dual-function enzyme has the same effect in algae. We have generated *C. reinhardtii* transformants that contain the coding region for DFS under the control of *psbD* and *psbA* 5' and 3' regulatory sequences, respectively, integrated into the chloroplast genome. We analyzed the expression of this protein in transformants by western blot and found that the cyanobacterial protein is expressed. We will proceed by comparing the growth of these transformants with the wild type strain of *C. reinhardtii*. If expression of DFS leads to increased growth rate, then we would conclude that DFS carries out one or more rate-limiting steps in the Calvin cycle. If that is the case, we ultimately will overexpress DFS in a biotechnology production organism like *Chlorella*, in hopes of improving it as a biofuel-producing organism.

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IDENTIFICATION AND CHARACTERIZATION OF *PUMPKIN*, A SPONTANEOUS EYE COLOR MUTATION IN *DROSOPHILA MELANOGASTER*

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For over 100 years, *Drosophila melanogaster* has been a model system for studying genetics. Morgan's discovery of the sex-linked *white* mutation was used to demonstrate the validity of the chromosomal theory of inheritance. Subsequent identification of additional sex-linked mutants enabled the mapping of genes to specific locations on specific chromosomes. Using commercially available stocks, we replicated Morgan's classic mapping experiment as part of the introductory genetics course lab work. The *white*, *miniature* and *forked* triple mutants were used to conduct a standard three-factor cross. However, an unexpected phenotype emerged in the F₁ generation. Nine female siblings exhibited this same phenotype, presumably due to a spontaneous mutation in the germ line of a single male.

The novel phenotype, which we call *pumpkin*, produced flies with orange eyes instead of the expected wild-type red. We have used these founders to establish a stable true-breeding line of *pumpkin* mutants. Crosses between *pumpkin* and wild type demonstrate *pumpkin* is sex-linked. PCR results demonstrate *pumpkin* is not an allele of *white* due to the absence of a Doc transposable element in the promoter region of *white*. The *pumpkin* phenotype resembles *white-apricot*, a known allele of *white*. However, PCR results show *pumpkin* lacks the *copia* element responsible for *white-apricot*. Results of a three-factor cross using *pumpkin*, *miniature*, and *forked* triple mutants show *pumpkin* is located within 0.2 cM of *ruby*, a gene affecting eye color. Complementation studies between *pumpkin* and *ruby* mutants produced wild type females in the F₁ generation, indicating *pumpkin* is not *ruby*. Sequencing of overlapping PCR products covering the entire *ruby* locus in *pumpkin* flies showed no difference at this locus between *pumpkin* and wild type flies. We are currently performing next-generation sequencing to identify the exact location of the *pumpkin* mutation.

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PCR primers used for Doc sequencing were a generous gift of Dr. Oney Smith, Hood College.

**GHOST PUMPKIN: A NOVEL MUTANT PHENOTYPE IN *DROSOPHILA* RESULTING
FROM
AN INTERACTION BETWEEN *RUBY* AND *PUMPKIN***

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The *pumpkin* phenotype is a spontaneous mutation affecting eye color in *Drosophila melanogaster* recently identified at Montgomery College. Mapping experiments place the *pumpkin* locus on the X chromosome within 0.2 cM of the locus for the *ruby* gene. Although *pumpkin* and *ruby* are close to each other, they are distinct. Homozygous *ruby* females crossed with hemizygous *pumpkin* males produce wild type females and *ruby* males. Conversely, homozygous *pumpkin* females and hemizygous *ruby* males produce wild type females and *pumpkin* males. These F₁ results are expected for X-linked genes. F₁ sibling crosses recovered parental phenotypes in F₂ progeny as expected, but they also produced two unexpected phenotypes in the F₂ male progeny (total F₂ progeny 1,249). Wild type males comprised 1.7% of the F₂ progeny. Even more surprising, 1.3% of F₂ male progeny exhibited a phenotype similar to the classic *white* mutant, but distinct from *white*. Sanger sequencing of PCR fragments spanning the promoter region and first exon of the *white* locus demonstrate the absence of the Doc transposable element in these mutants. To distinguish this mutant from *white*, we are naming it *ghost pumpkin*.

Stable, true breeding lines of *ghost pumpkin* have been established. Preliminary F₂ results of *ruby* \times *pumpkin* mating suggests *ghost pumpkin* may be due to a single crossover event between *ruby* and *pumpkin*. We are currently repeating the *ruby* \times *pumpkin* crosses with larger numbers to get a more accurate estimate for the frequency of this potential crossover event.

If this phenotype is due to a crossover as predicted, then we expect to find mutations at both the *ruby* and *pumpkin* loci of affected flies. We are currently obtaining sufficient genomic DNA from the *ghost pumpkin* mutants to determine whether *ghost pumpkin* mutants have these predicted 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.

PCR primers used for Doc sequencing were a generous gift of Dr. Oney Smith, Hood College.

CHARACTERIZING CYTOKINE GENE EXPRESSION IN MOUSE PERIPHERAL BLOOD MONONUCLEAR CELLS USING REAL-TIME PCR IN MICE MODEL OF NEUROFIBROMATOSIS TYPE 1

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Neurofibromatosis Type 1 (NF1) is a genetic disorder that causes tumor on nerve tissues found anywhere on the nervous system. Neurofibromatosis type 1 has mutations in the *NF1* gene. The *NF1* gene regulates the activity of the protein *ras* which promotes cell division. When the *NF1* gene is mutated it leads to shortened version of the neurofibromin protein or an unexpressed neurofibromin that cannot bind to *ras* or regulate its activity. This causes the *ras* protein to be more active causing cells to begin dividing and never told when to stop. Thus, the production of nonfunctional neurofibromin will not be able to regulate the cell growth and division, which will result in tumors such as neurofibromas.

Interferon gamma (IFN- γ) is a pleiotropic cytokine involved in the regulation of nearly all phases of immune and inflammatory responses, including the activation, growth and differentiation of T-cells, B-cells, and macrophages. It has weak antiviral and antiproliferative activity, and potentiates the antiviral and anti-tumor effects of the type one interferon. IFN- γ is held to be more important as an immunoregulator as it enhances the cytotoxic activity of T cells, macrophages and natural killer cells and thus has antiproliferative effects.

Our goal was to test the over-expression of IFN- γ of PBMC cells in WT mice and NF1-/+ p53-/+ mutant mice model of NF1 (Npcis). We applied qPCR in order to check the gene expression of IFN- γ in both stimulated WT mice and stimulated Npcis.

We hypothesize that NF1 mutations impairs the immune response of the mice. We have seen in different trials that the gene expression of IFN- γ in WT was different than those in Npcis. This is an ongoing project, and the observation we have made thus far is that the expression of IFN- γ is lower in NF1 tumor cells after stimulation.

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OPTIMIZATION OF BIOMATERIAL MICROENVIRONMENT FOR MOTOR NEURON TISSUE ENGINEERING

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Injury and disease in the nervous system results in loss of sensory and motor neurons. Tissue engineering strategies have great potential to develop more effective therapeutics by combining appropriate biological cues with responsive biomaterial architectures. We have recently shown that more in vivo-like sensory neuron responses are found in 3D culture vs 2D flat substrates. Unfortunately, primary motor neurons are harder to isolate and culture compared to sensory neurons. Moreover, PC-12 cells are a well-characterized sensory neuron-like cell line and provide an alternative to primary sensory neurons. An analogous motor neuron-like cell line, NSC-34, is available but has not yet been characterized for tissue engineering applications. Thus, our goal was to systematically characterize NSC-34 cells and optimize biomaterial microenvironment and culture conditions for tissue engineering applications. For 2D studies, we cultured NSC-34 cells on coverslips coated with laminin or type I collagen in DMEM with fetal bovine serum (FBS; 3%, 10%), or N2 supplement. In our 3D studies, cells were cultured in gels composed of type I collagen with or without laminin or type IV collagen. We found that laminin substrates were associated with a higher percent of cells expressing neurites and that the neurites were significantly longer than for type I collagen substrates, with greatest results at 3% serum (Fig 2). Medium with N2 was associated with lower cell proliferation vs serum-containing medium regardless of substrate. Although proliferation results showed low percent proliferation for N2 medium, no significant difference was found between type I collagen and laminin. Preliminary results in 3D gels indicate that type IV collagen provides a favorable environment for neurite expression. Ongoing work focuses on rigorously characterizing neuronal morphologies and neurite lengths in 3D cultures.

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THE ROLE OF APONTIC IN CELL MIGRATION AND STEM CELL DYNAMICS

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The dynamics of cell migration and stem cell differentiation are two topics of particular interest because they are normally tightly controlled but, when unchecked, often play roles in pathological conditions. The migratory border cells of the *Drosophila* ovary and the stem cell niche of the *Drosophila* testis, serve as well characterized systems for study of both migration and stem cell differentiation. What we learn in this context is likely to be relevant in many different animals, including humans.

In the fruit fly ovary and testis, a molecular cascade ending in transcription, known as the JAK/STAT signaling pathway, is involved in these two distinct but essential cellular processes. This study focuses on a protein, Apontic, that is present in this molecular pathway. Previous literature showed that Apontic plays a role in suppressing cell migration and motile cell recruitment by regulating STAT activity within *Drosophila* ovaries. Also, in testes, Apontic appears to promote differentiation by antagonizing STAT activity. The mechanism by which STAT and Apontic regulate each other is yet to be elucidated in testes. Here, we look at the MicroRNA 284 (mir284) as a possible means by which STAT down regulates Apontic activity in both the ovaries and the testis.

In addition, we also looked to another protein, Zfh1, that is required for stem cell maintenance in the testes, as a possible regulator of Apontic levels in the stem cell niche. Finally, we sought to mathematically represent the theoretical change in Zfh1 levels using differential equations based on the proposed molecular cascade. We used these equations to develop an understanding of possible cross repression between Apontic and Zfh1.

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DEVELOPMENT OF A CRISPR/CAS9 GENOME EDITING SYSTEM FOR *VOLVOX CARTERI*

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Genome editing is a tool implemented to test gene function through targeted mutations. The Cas9/CRISPR system is simpler and more precise than previously developed genome editing systems. The high precision is due to the CRISPR associated (Cas) endonuclease's ability to bind DNA via associated guide RNAs. Cas endonucleases can delete or add bases to the genome, which permits not only knockouts to determine mutant phenotypes, but also tagging genes with reporters. However, a Cas9/CRISPR system has not been adapted for use with the green alga *Volvox carteri*, an important model organism for investigating the evolution of fundamental developmental processes. In this project, I adapted an existing Cas9 vector for use in *Volvox* using molecular cloning techniques to insert species-specific regulatory sequences and guide RNA sequence targeting a test gene with known mutant phenotype. Biolistic transformation of the vector resulted in viable transformants, which were tested for guide RNA expression and Cas9 protein expression via RT-PCR and Western blots, respectively. RT-PCR confirmed that guide RNA was made. Western blots confirmed that Cas9 was being expressed. Transformants that express both components have not shown any phenotype for the selected mutation, so we are in the process of testing Cas9 functionality as well as making new genomic targets. Ultimately, this system will be used to edit genes related to multicellularity in *Volvox* and then adapted for use in the related alga *Chlorella*, an important biofuels and nutraceuticals production organism.

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CHARACTERIZING THE FUSION OF SPORE-ASSOCIATED PROTEIN A TO AN ANTIGENIC MARKER IN BACTERIAL EXOSPORES AS A METHOD OF VACCINE DELIVERY

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Endospores from unicellular bacteria that express recombinant antigenic markers fused to spore coat proteins (Cot) have been proven an effective vaccine mechanism. Exospores produced by non-pathogenic, filamentous bacteria such as *Streptomyces coelicolor* may also be used. *S. coelicolor* encodes multiple secreted spore associated proteins. The aim was to characterize the secretion and localization of spore associated protein A (SapA) when fused with an antigenic protein at the C-terminus. In *E. coli*, the gene for the *E. coli* β -subunit heat labile toxin (*ltb*) was fused to the 3' end of *sapA* by recombineering and introduced into *S. coelicolor* by conjugation. Proteins from strains expressing SapA-Ltb were extracted and Western Blot analysis demonstrated that the fusion protein is secreted and localized to the spore coat. Now 3' truncations are being made to find the minimum amount of SapA necessary for secretion and localization. This may be a viable vaccine mechanism.

CLONING AND DNA SEQUENCE ANALYSIS OF A PCR AMPLICON FROM A *DROSOPHILA* GENOTYPING ACTIVITY

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The introductory cell biology and genetics course at Hood College includes a lab activity where students conduct the polymerase chain reaction (PCR) to genotype *Drosophila* with red or white eyes. Amplification of the red-eye allele by PCR produces a product of 467 bp, while amplification of the white-eye allele yields a product of 704 bp. Occasionally, samples of DNA from red-eyed flies yield an additional amplicon that approximates 1,800-bp in size. The purpose of this project was to isolate, clone, and sequence this amplicon to help identify its location in the *Drosophila* genome. Agarose gel electrophoresis was used to isolate the 1,800-bp amplicon from samples of six homozygous red-eye flies. Following excision from the gel and purification, the DNA fragment was ligated to plasmid vector pSC-A-amp/kan and transformed into *E. coli* DH5 α cells. Following blue/white color selection, white colonies were transferred to a master plate and screened by EcoR I digestion to confirm the presence of the insert. These results also demonstrated the insert was characterized by an internal EcoR I site that mapped 250 bp from the end of the 1,800-bp amplicon. Two independent clones were selected for plasmid purification by alkaline lysis/DEAE column chromatography. Following quantitation by spectrophotometry and confirmation by agarose gel electrophoresis, both clones were partially sequenced using a BigDye Cycle Sequencing Kit (Applied Biosystems). Analysis of the resulting DNA sequence data using BLAST and the database for *Drosophila* genes (FlyBase) indicates the 1,800-bp amplicon is a DNA element found on more than one fly chromosome.

We thank the Hood College Summer Research Institute, Department of Biology, and the Hodson Trust for supporting this research experience. We also express appreciation to Melissa Carter of the USDA-ARS for help with DNA sequence analysis.

MAXIMIZING LIPID PRODUCTION IN *FREMYELLA DIPLOSIPHON* VIA NITROGEN STARVATION

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Determining culture conditions that yield optimal lipid content is a crucial factor in large-scale production of cyanobacteria-based biofuel. The effects of nitrogen starvation on *Fremyella diplosiphon* cultures grown for 7, 10, 15 and 20 days were determined using growth and lipid analysis in order to establish conditions necessary for maximum lipid production. Total lipid content in the wild type strain (SF33) grown in standard BG11/HEPES containing 1.5 g/L sodium nitrate (NaNO_3) was compared to SF33 in media amended with 0.25, 0.5 and 1.0 g/L NaNO_3 . Cultures were grown under fluorescent white light adjusted to $30 \mu\text{mol m}^{-2}\text{s}^{-1}$ and an initial optical density of 0.05 at 750 nm. Growth was measured every 48 h and growth rate calculated in doublings per day. Data from three replicated samples were analyzed using one-way ANOVA and Tukey's honest significant difference test. Total lipids were extracted using 2:1 chloroform: methanol phase separation and quantified using gravimetric analysis. No significant difference in culture growth was observed under different concentrations of NaNO_3 . Maximum lipid production of 23.74% and 38.69% was observed in 10 day cultures at 0.5 and 1.0 g/L NaNO_3 respectively. In addition, cultures grown in 1.0 g/L NaNO_3 for 7 days yielded significantly higher amount of total lipids (36.32%). Results of the study suggest that nitrogen starvation leads to an increase in the amount of total lipids in *F. diplosiphon*. Future studies will aim towards determining lipid content using fluorescence spectroscopy and enhancing lipid production in the organism using genetic engineering.

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ANTIMICROBIAL ACTIVITY OF SIX ESSENTIAL OILS AGAINST COMMON SKIN PATHOGENS

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Recently, there has been a growing concern about the effectiveness of antimicrobial treatments and a movement towards natural remedies. Previous research supports that essential oils contain compounds that are successful at preventing the growth of and even killing different pathogenic organisms, including both bacteria and fungi. We hypothesized that the essential oils tested will be as effective as typical antibiotic or antifungal medications at killing common skin pathogens. Six full strength essential oils (oregano, lemon grass, palm rosa, cinnamon, thyme, anise) were evaluated for their ability to inhibit the growth of the following skin pathogens using the disk diffusion method: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Serratia marcescens*, and *Candida parapsilosis*. Oregano, lemon grass, and cinnamon were most effective at growth inhibition among all species. The largest average zone of inhibition (ZOI) observed was 33.9mm (oregano), 30.2mm (lemon grass), and 18.2mm (cinnamon). Out of all of the essential oils tested, oregano produced zones greater than or equal to the antibiotics bacitracin (ZOI \geq 13mm), clindamycin (ZOI \geq 21mm), and mupirocin (ZOI \geq 14mm) among all bacteria tested. Lemon grass produced zones greater than these antibiotics against *S. epidermidis* (30.2mm) and performed better than bacitracin and mupirocin against *S. marcescens* (17.7mm). Cinnamon was more effective than bacitracin and mupirocin against *S. marcescens* (18.2mm) and was equivalent to bacitracin against *S. epidermidis* (13.3mm). Oregano (18.2mm) was the only essential oil that was effective against *C. parapsilosis* with ZOIs slightly greater than the anti-fungal voriconazole (ZOI \geq 17mm). Together these data suggest that oregano, lemon grass, and cinnamon essential oils are effective antibacterials and present an alternative treatment for control of important pathogens.

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THE ISOLATION AND GENETIC IDENTIFICATION OF WILD YEASTS FOR USE IN FERMENTATION

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Inspired by spontaneously fermented beers, such as Belgian Lambic beers, American breweries have moved beyond using exclusively the most common beer-making yeast, *Saccharomyces cerevisiae*, to experiment with a variety of yeasts to produce unique flavors and to promote new acquired tastes. These so-called "funky" and "sour" beers are produced by adding secondary fermenters either during or after the initial fermentation process. Here, we sought to genetically identify yeasts present in old wine barrels used to age Belgium-style ales at Evolution Craft Brewery in Salisbury, MD. In addition, yeasts growing on local wild berries and fruits were captured and identified. Yeasts were identified using microscopy and by DNA sequencing of the 5.8S rRNA gene. The yeasts were identified as *Saccharomyces*, *Pichia*, *Candida*, *Fusarium*, *Torulaspora*, and, most interestingly, *Brettanomyces* genera, a yeast previously known as a winery contaminate but now known to create funky flavors in beer. The next steps in our project include analyzing the growth and fermentation abilities of these yeasts under normal and diverse circumstances such as ethanol concentration or carbon source to help determine the potential for commercial brewing and provide breweries with a wider array of options for making flavorful beverages.

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IMPROVING ALGAL BIOFUEL PRODUCTION BY EXPRESSION OF CARBON CONCENTRATING MECHANISM ENZYMES

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Growing concerns over climate change are driving interest in development of renewable bioenergy to replace fossil fuels. Genetic manipulation of algae can make biofuel production more efficient. The photosynthetic green alga *Chlamydomonas reinhardtii* is a well-studied model organism that is easy to grow and manipulate at the molecular genetic level. This project focuses on a set of genes believed to be important for a carbon-concentrating mechanism (CCM) that acclimates algae to normal, CO₂-limiting conditions. Carbonic anhydrases are components of the CCM that catalyze the interconversion of carbon dioxide and bicarbonate, and thereby make inorganic carbon more accessible to the cell. The purpose of this project is to increase the intracellular concentrations of CO₂ in *C. reinhardtii* by overexpressing periplasmic and thylakoid membrane carbonic anhydrases, CAH1 and CAH3, respectively. *C. reinhardtii* CAH1 and CAH3 coding regions were synthesized with *C. reinhardtii* codon bias and epitope tags and the gene fragments were subcloned into expression vector pARG which contains the ARG7 gene required for arginine biosynthesis. We transformed the CAH1 and CAH3 vectors into an *arg7* mutant strain and selected several ARG survivors for western blot analysis to determine the expression of protein. We will select the best expressing lines for growth curve and dry weight analyses to determine whether the transformants overexpressing CAH1 or CAH3 are able to grow faster than the wild-type *C. reinhardtii* strain. In future both genes could be expressed together. The next step will be to manipulate these methods for microalgae that naturally produce higher lipid levels than *C. reinhardtii*, such as *Chlorella vulgaris*.

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IMPACT OF INTRATUMORAL HETEROGENEITY AND INTERCLONAL-HOST CROSSTALK ON DRUG SENSITIVITY

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Cancer cells are subjected to evolutionary selection of clonal subpopulations in response to fluctuations in the microenvironment and drug treatment. In particular, the interactions between heterogeneous cell subpopulations can effect growth and behavior. To understand the contribution of these subpopulations to malignant progression and drug response, we established a model of tumor heterogeneity from six syngeneic, clonal primary cancer cells isolated from a mutant Kras/P53 mouse pancreatic cancer (KPC). These clonal cells formed invasive and metastatic lesions when grafted into immune competent hosts. The original tumor and clonal cell lines harbored common mutations in 99 genes suggesting their common ancestry. Additional unique mutations in the clonal lines were used to identify and quantitate clones in heterogeneous cell pools. The clones exhibited distinct morphologies, different levels of MAP kinase signaling, and varying rates of growth in vitro and as tumors in immune competent mice. Moreover, the sensitivity to ~200 anticancer drugs revealed an up to 25-fold varying in vitro sensitivity of the clones to signal transduction inhibitors and cytotoxic drugs.

To our surprise, drug sensitivity of individual clones when included in a heterogeneous cell population was strikingly different from their drug sensitivity when grown on their own. In particular, the sensitivity of individual clones to MEK inhibitors or anti-PD1 checkpoint inhibition was not predictive of their sensitivity in a pool with the other clones. The sensitivity to the cytotoxic drug gemcitabine was less impacted by the presence of other clones. Finally, the response of clones grown as heterogeneous tumors from pools of the clones revealed a pattern of drug sensitivity not predicted from the in vitro drug sensitivity.

We conclude that malignant progression and selection of drug resistant cancer cell subpopulations is impacted by the crosstalk between clonal cell populations present in heterogeneous tumors and the host environment.

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ANALYSIS OF HEAT SHOCK PROTEIN 70 MRNA EXPRESSION IN *AIPTASIA PALLIDA*

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Aiptasia pallida is a sea anemone that is used in variety of teaching and research applications and is easily maintained under laboratory conditions. This organism is one of several kinds of cnidarians that harbor algae in a symbiotic relationship. When *A. pallida* is exposed to environmental stress, such as increased temperature or salinity, it produces a protein called heat shock protein 70 (HSP 70). HSP 70 is a chaperone protein with a molecular mass of 70 kDa that helps other cellular proteins sustain a proper folding pattern, and thus function, when organisms are subjected to conditions of stress.

The purpose of this project was to investigate the transcription levels of the mRNA for HSP 70 in *A. pallida* exposed to elevated temperatures. Animals were incubated for four hours at 25°C or 32°C, followed by the extraction of total RNA. After quantitation of total RNA and verification of intact ribosomal RNA by agarose gel electrophoresis, first-strand cDNA was synthesized using 2 µg of total RNA and oligo(dT) as a primer. The cDNA product was used as a template for quantitative PCR (qPCR) using forward and reverse primers for the HSP 70 gene and the house keeping gene RPL11 using a SYBR Green-based protocol (Applied Biosystems). Relative gene expression was computed from the qPCR data employing the comparative C_T ($\Delta\Delta C_T$) method and was based on three technical replicates for each cDNA sample.

Analysis of the data from four animals from the experimental (32°C) group compared to four animals in the control (25°C) group indicated there was a 100-fold increase in expression of the HSP 70 gene. Future work will include the use of western-blot analysis to monitor heat shock protein expression in this organism.

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SEX STEROID HORMONE RECEPTOR EXPRESSION IN GARTER SNAKE SKIN

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Vertebrates display myriad sexually dimorphic traits including size, coloring and behavior. Similarly, the production of differing secondary sexual signals allows individuals to communicate information, such as sex, to conspecifics. It has been shown that many of these signals, including the production of pheromones, are controlled by sex steroid hormones. The garter snake (*Thamnophis sirtalis*) is an ideal model organism for this system because males exclusively use pheromones during courtship in order to identify females with which to mate. Pheromones are chemical signals which, in garter snakes, consist of both saturated and unsaturated methyl ketones. Furthermore, the composition of these lipids varies between males and females and can be directly affected by treatment with sex steroid hormones, an example of which being the production of female pheromones in males after treatment with estrogen. However, little is known as to how hormones incite these changes. Hormone-mediated gene expression and signaling cascades begin with the binding of 17β -estradiol to estrogen receptor α (ESR1) or estrogen receptor β (ESR2) and androgens, such as testosterone, to androgen receptor (AR). The purpose of this study is to establish that the presence of ESR1, ESR2 and AR in the skin of the garter snake can be detected and quantified. This will be achieved by extracting and purifying mRNA from the skin, followed by the production of cDNA from the mRNA using reverse transcriptase. The cDNA can then be used as the template in real-time PCR (qPCR), allowing for the quantification and comparison of mRNA expression levels of the target genes between male and female skin. Both techniques utilize custom, sequence-specific primers designed using the NCBI Primer-BLAST program. While all three receptor types were expressed in the skin of both sexes, ESR1 was more highly expressed in female skin and AR was more highly expressed in male skin.

A NOVEL REPORTER SYSTEM TO QUANTIFY MISSENSE ERRORS DURING TRANSLATION IN *SACHHAROMYCES CEREVISIAE*

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Translation is the final step in the central dogma, in which information is transferred from DNA to mRNA and finally to proteins. The key players during translation include mRNA, tRNA and ribosomes. The ribosomes maintain a fine balance between the rate of protein synthesis and the accuracy with which the proteins are synthesized. Even though the process is highly accurate, errors can occur. A common error that occurs during translation is when an incorrect tRNA binds to the ribosome and adds an incorrect amino acid in the growing polypeptide chain; such an error is called a missense error. We have developed a reporter-based system that can be used to quantify missense errors by a single tRNA in yeast. Such a system can be further employed to understand the mechanism and processes that regulate errors during translation. Errors made during protein synthesis can lead to a non-functional or inactive protein and accumulation of such bad proteins can be deleterious to cells, such as in the patients suffering from Alzheimer's or Parkinson's disease. We have used this reporter system to quantify errors in yeast and found that the eukaryotic ribosome is very efficient in discriminating against most errors, with accuracy as high as one in a million amino acids incorporated. However, certain codons can be misread by the ribosome with a 100-fold higher error rate. These include when the tRNA^{Glu} that normally reads the glutamic acid codons GAA or GAG misreads the glycine codons, GGA and GGG or the aspartic acid codons, GAU and GAC. My goal is to establish whether the activity of the two aspartic acid codons is due to misreading or if glycine or aspartic acid simply partially replace the function of glutamic acid in the protein (functional replacement). I will be using hyperaccurate and error prone mutants of the ribosome to characterize this role.

PRO-INFLAMMATORY EFFECT OF DENGUE VIRUS NS1 AND ENVELOPE PROTEIN IN CULTURED HUMAN ARTICULAR CHONDROCYTES

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Dengue virus (DENV) is transmitted by the *Aedes* mosquito family, and is a leading cause of human illness and death in the tropics, with an estimated 390 million infections occurring annually. It is the causative agent of Dengue Hemorrhagic Fever (DHF), which is characterized by flu-like symptoms and severe arthralgia, and can be fatal, especially in children. There are four distinct serotypes of DENV (DENV 1-4), and subsequent infections with different serotypes significantly increase the risk of developing DHF. Currently, there is no specific treatment for DENV infection or DHF, despite the prevalence and potential severity of the condition.

While the link between DENV infection and arthralgia in the joints has been well established, the pro-inflammatory effect of the virus has not yet been examined in human articular cartilage. This study aims to characterize the pro-inflammatory response of normal human articular chondrocytes (NHACs), the primary cell type comprising articular cartilage, exposed to DENV-derived proteins in order to better understand the severe arthralgia associated with DENV-induced inflammation.

NHACs were cultured with DENV-2-derived surface envelope protein (sE), non-structural protein1 (NS1), bovine serum albumin (BSA), or serum-free media alone, at a range of 0, 0.1 ug/ml, or 1.0 ug/ml protein concentrations, over a short time course of zero to six hours. It was hypothesized that the presence of the viral proteins would induce a pro-inflammatory response, as measured by quantitative Real-Time PCR. While the Dengue sE protein did not induce a significant response in the NHACs, Dengue NS1 protein induced elevated gene expression for several pro-inflammatory cytokines, including IL-1 β , IL-6, IL-8, MCP-1, and TNF- α , among others, in a response that appeared to be dose-dependent. Future investigations will include the characterization of protein expression patterns for select cytokines to determine if the protein levels parallel the observed gene expression patterns.

The authors thank Dr. Tom Voss (SRI) and Dr. Naveen Somanna (SRI) for generously providing the purified DENV-sE protein and the purified DENV-NS1 protein used in this study, as well as the Jeffress Foundation, 4-VA, and the JMU College of CSM for providing funding.

CHARACTERIZATION OF A SPORE-ASSOCIATED PROTEIN TO STUDY ASSEMBLY OF *STREPTOMYCES* SPORES

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Streptomyces coelicolor is a model non-pathogenic, filamentous bacterium that produces chains of spores from aerial hyphae. Linear plasmid SCP1 contains *sapC*, *sapE*, and *sapD*, which are genes that code for spore-associated proteins. Spore-associated protein SapC (17 kDa, no putative conserved domains) does not contain a normal secretion signal nor is the mechanism of secretion known. Sap proteins were identified because they can be extracted from the spore surface using a non-lethal detergent wash and analyzed via SDS-PAGE. In preliminary studies, our strategy to study secretion was to use recombineering to fuse a detectable passenger protein, the β subunit of the *E. coli* heat labile toxin (LTB), to the C-terminus of a spore-coat protein, SapC. Results characterizing this fusion suggested that SapC-LTB was secreted and assembled on the spore surface. A current goal is to analyze several constructed C-terminal truncations of SapC (SapC'-LTB) as well as an N-terminal fusion (LTB-SapC) in an attempt to locate the secretion and/or spore surface assembly signals of SapC. In the future, the use of Sap protein fusions to antigens might lead to an additional method for vaccine delivery by assembling recombinant *Streptomyces* spores with epitopes displayed on the spore surface.

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CHARACTERIZATION OF THE LIN28 PARALOGS THROUGHOUT EMBRYONIC DEVELOPMENT

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Lin28 was first discovered in *C. elegans* as a regulator of the L1 to L2 transition during larval development and is specifically expressed in the hypodermal seam cells. Hypodermal seam cells give rise to the nervous system and skin. Worms breathe through their skin, making it analogous to the mammalian respiratory system. During mammalian development, the lung undergoes the reiterative process of branching morphogenesis to give rise to the complex bronchial tree.

In mammals, there are two Lin28 paralogs that are homologous to the *C. elegans* Lin28: Lin28a and Lin28b. They are highly expressed early in development but decrease as the organism matures into adulthood. Loss of function of Lin28a and b in mammals lead to embryonic lethality. We did an in-depth analysis of several organs from early to late embryonic development in the mouse. We found that Lin28a expression was as previously shown, highly expressed early and decreased throughout maturation. Lin28b expression was high early in development but did not extinguish like Lin28a expression. Instead, it persisted into later embryonic time points. These two points taken together led us to hypothesize a role for Lin28a and b in the developmental process of lung branching morphogenesis.

To study the role of Lin28a/b in lung development, we created various inducible and knockout transgenic mice using a lung-specific Cre. We observed a loss of branching in the lungs of Lin28a/b double knockout mice. We will continue to characterize the role of Lin28a/b in lung branching morphogenesis as well as analyze other organs that undergo branching.

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VAN-GOGH-LIKE 2, FRIZZLED, AND KNYPEK CONTROL DISTINCT ASPECTS OF POLARIZED CELLULAR MIGRATION DURING NEURAL CONVERGENCE

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The planar cell polarity (PCP) pathway plays a significant role in facilitating neural convergence (NC) – the narrowing of the neural plate before the formation of the neural tube. NC in zebrafish requires elongation and midline-directed polarized migration of neural plate cells. Failure of NC or the later stages of neural tube morphogenesis can result in severe neural tube defects (NTDs) which have been observed in all vertebrates studied. Although perturbation of the PCP pathway is associated with NTDs in model organisms and humans, the underlying neural cell behaviors remain elusive.

In order to investigate the cellular effects of the PCP pathway, we use *Knypek* (*Kny*^{fr6}), *Van gogh-like 2* (*Vangl*^{vu67}), and *Frizzled 7a⁺/7b⁻* (*Fzd7a*^{e3-}; *Fzd7b*^{hu3495}), three zebrafish lines carrying null mutations. We confirmed the published result that mutations in the PCP pathway result in delayed NC. Our comparative cellular analysis revealed how cell elongation, membrane dynamics, and trajectory are affected in homozygotes. We show that wild type (WT) neural plate cells elongate and medially restrict membrane protrusions, thus narrowing the neural plate.

Preliminary data show that cells in all mutants initially failed to elongate, specifically *Vangl* and *Fzd* mutant cells extend randomized protrusions while *Kny* mutant cells show temporally restricted protrusive activity. In the PCP pathway, *Kny* is a *Fzd* co-receptor thought to present *Fzd* with various Wnt ligands, suggesting that the differences between these mutants are a ligand-independent aspect of PCP signaling during NC. While current literature understands how PCP genes regulate cell polarity and migration, the effect of these genes is poorly understood in neural tissue as the genes contribute to NTDs. By studying cell behavior in neural tissue, we may reveal how the PCP pathway promotes NC and identify additional genes affecting NTDs.

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SEIZURE-ASSOCIATED CENTRAL APNEA IN A RAT MODEL: EVIDENCE FOR RESETTING THE RESPIRATORY RHYTHM AND ACTIVATION OF THE DIVING REFLEX

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Respiratory derangements, including central and obstructive apnea, are consequences of seizure activity in epilepsy patients and animal models. Irregular, tachypnic breathing during seizure activity indicates an impact of seizure spread on respiratory rhythm generation.

We studied episodes of central apnea, defined as periods > 1 second with no evidence airflow during plethysmography, in rats anesthetized with urethane and given parenteral kainic acid to induce recurring seizures. Our aim was to determine when in the respiratory cycle apneic periods start and if apneic periods end with 1) a reset of the breathing cycle to a common phase at apnea offset, 2) a continuation from the point at which the respiratory cycle was aborted at apnea onset, 3) a continuation from a point predicted by a continuation of the breathing pattern preceding apnea, or 4) a random re-entry into the respiratory cycle.

One hundred ninety central apnea episodes (1.04 to 36.18 seconds, mean: 3.2 ± 3.7 seconds) were recorded during seizure activity from 12 rats. The majority of apneic period onsets occurred during expiration (125/161 apneic episodes, 78%), particularly late expiration (82/125, 66%). Apneic period offsets were more uniformly distributed between early and late expiration (27%, 34%) and inspiration (16%, 23%).

Central apneic periods were associated with a burst of high frequency activity in the EEG. These bursts could be used to identify periods where airflow was maintained, indicating that periods of no airflow were likely due to activation of the diving reflex. Events that were not masked by the diving reflex were completely stereotyped within individual animals, indicating a reset of the respiratory rhythm which was highly variable after about 1 second. We conclude that seizure-associated central apnea is associated with 1) a reset of the respiratory rhythm, and 2) activation of the diving reflex to suppress respiratory behavior.

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LOOMING STIMULI EVOKE A TURNING ESCAPE RESPONSE THAT IS MEDIATED BY BOTH CERCI AND VISION IN CRICKETS

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Crickets preventively escape from predators by turning and running, or jumping, away from wind cues. Previous results indicated that crickets largely utilize the same turning strategy when escaping looming stimuli. In contrast to wind stimuli, looming objects create both wind and visual sensory cues. However, the relative contributions of the two sensory cues – wind and vision – and the receptor modalities – cerci, eyes, antennae, and body mechanoreceptors – to the escape response are unclear. The specific aim of our research was to identify the contribution of each receptor modality to the escape. The crickets' (*Acheta domesticus*) escape from a 3" black polyurethane ball propelled at 45° was captured by a high-speed camera. We designed a series of behavioral lesion experiments to determine if each of the four modalities were necessary or sufficient to produce an escape response. For example, to determine the sufficiency of the eyes, all other relevant sensory organs (cerci, antennae, body mechanoreceptors) were lesioned; alternatively, to determine if the eyes were necessary to elicit the escape, only the eyes were lesioned. Based on 106 crickets, both cerci and eyes, but neither antennae nor body mechanoreceptors, contributed to the escape response. Eyes were necessary and sufficient for anterior looming stimuli, while cerci were necessary and sufficient for posterior looming stimuli. Although cerci have been considered the primary determinant of the escape response, vision may play a significant role, especially for anterior looming stimuli.

IRON DEFICIENCY ALTERS ADENOSINE 2A AND DOPAMINE 2 RECEPTOR LEVELS IN PC12 CELLS AND RAT STRIATUM

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Iron deficiency anemia is a common nutritional disorder that impairs motor and sensory skills in several species, including humans and rodents. Alterations in dopamine neurotransmitter system regulation have been shown to underlie many of these behavioral outcomes. Two key proteins, the dopamine transporter and dopamine 2 (D2) receptor, are both reduced in the brain of rats that were iron deficient during adulthood and in iron deficient PC12 cells. Recent work has focused on determining the mechanisms by which these dopamine-related proteins are altered. There is evidence that implicates the adenosine neurotransmitter system in the connection between iron deficiency and altered dopamine circuitry. Presynaptic adenosine 2a (A2a) receptors are co-localized with and form heterodimers with D2 receptors, and these proteins have an antagonistic interaction. The research presented here is focused on investigating the interaction between A2a and D2 receptors in iron deficiency using PC12 cells and rat brain tissues. In PC12 cells, iron chelation resulted in a dose-dependent increase in A2a receptor protein levels and a dose-dependent decrease in D2 receptor and dopamine transporter protein levels. Striatal A2a receptor levels were also investigated in rats under three diet conditions: 1) iron sufficient throughout gestation and lactation; 2) iron deficient from P4-P21; and 4) iron deficient from G15 to P4. A2A receptors were found to be reduced in striatum from the early iron deficient groups compared to control. These data suggest that compensatory mechanisms in the developing brain can lead to changes in A2A receptor levels that do not mimic changes found in PC12 cells or adult models of iron deficiency.

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INTRACRANIAL DRUG DELIVERY AND BEHAVIORAL TESTING OF MOLECULES THAT MODULATE LEARNING AND MEMORY

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The hippocampus is a brain structure that is important for learning and memory, and is often studied in relation to learning disorders and neurodegenerative diseases such as Alzheimer's and other dementias. However, an obstacle faced by researchers in studying memory is the inability of many molecules or drugs to pass through the blood-brain barrier (BBB), a highly selective barrier that protects the brain from harmful chemicals. In order to study the role of ribosomal RNA (rRNA) synthesis in learning and memory, we needed to apply a drug that selectively blocks RNA Polymerase I (Pol I, the polymerase responsible for the transcription of rRNA); however the drug does not pass the BBB. To overcome the obstacle posed by the BBB, our lab performed Stereotaxic Intrahippocampal Cannulation surgery to deliver the drug directly to the hippocampus. After injection of the inhibitor or vehicle, we trained the animals using the hippocampal dependent Active Place Avoidance (APA) behavioral task to determine whether rRNA synthesis is required for learning and or memory consolidation. In addition, we used immunohistochemistry in conjunction with confocal microscopy to determine whether the drug produced intracellular structural changes. Thus, our methodology provides a powerful tool to test molecules that modulate learning and memory.

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HYPERPOLARIZATION ACTIVATED CATION CHANNELS IN THE COCHLEAR NUCLEUS: PROTEIN EXPRESSION AND PHYSIOLOGICAL PROPERTIES

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Auditory stimuli are processed in parallel frequency-tuned circuits. Auditory nerve fibers convey tonotopic organization onto nucleus magnocellularis (NM), a division of the cochlear nucleus specialized for phase-locking. Phase-locking precision is required for sound localization via a coincidence detection process within nucleus laminaris (NL), the sole target of NM output. NM's primary function is to preserve temporal information from auditory stimuli in output spiking patterns. A number of physiological properties vary systematically along the tonotopic axis and contribute to the ability of NM neurons to integrate and fire precisely across frequencies. Previous findings within NL have shown that there is a gradient of hyperpolarization activated cyclic nucleotide (HCN) gated channels along the tonotopic axis. HCN channels serve as a nonselective voltage-gated cation channels in the plasma membrane. This gradient has been shown to contribute to the differences in firing properties along NL. Recent physiological findings from our lab have determined that HCN conductances are also expressed as a gradient in NM, where HCN channels are weakly expressed at the high frequency end and highly expressed at the low frequency end of NM. For this project, we aim to identify the functional contributions of the gradient of HCN channels within NM. Specially, we recorded HCN currents within NM using an Alexa 488 filled electrode. This arrangement allowed us to visualize the recorded neuron, and to map its location accurately within the tonotopic axis of NM. In order to localize neurons within the tonotopic structure, we optimized our protein labeling protocol to label cell membranes of NM neurons. We completed a dilution series to determine the best concentration for the wheat germ agglutinin (WGA), which fluorescently labels membrane glycoproteins, and determined 1 ug/mL to be the optimal. The results showed that after patch clamping we confirmed that the HCN conductance was greater in the LCF cell in comparison to the HCF cell. These results demonstrate additional novel mechanisms of "tuning" intrinsic neural responses to stimulus frequency.

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THE EFFECT OF NOXIOUS TAIL STIMULATION ON THE NOCICEPTIVE WITHDRAWAL RESPONSE OF UNRESTRAINED RATS

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When animals are exposed to noxious stimuli, they can have varying responses based on the intensity of the stimuli or environmental conditions. Previous studies have observed that when the tail of a *restrained* rat is stimulated using a heat stimulus, the tail moves in the opposite direction from the stimulation site. However, there is no research that evaluates the body movements of rats that are *unrestrained*. Earlier studies in unrestrained lizards have shown that as the stimulus location moves closer to the base of the tail, the animal tends to walk forward instead of moving the tail. This study aims to determine whether an unrestrained rat will move its body when its tail is stimulated using a noxious heat stimulus. Rats were anesthetized to mark the tail, feet, and body. The animal was then centered on a glass table, where a heat stimulus was delivered to one of the five stimulus locations on the tail. Movement was recorded through a video camera placed below the center of the glass table. In addition to tail withdrawal, we observed that concomitant body movement always occurred. The direction of body movement consisted of both forward translation and rotation away from the stimulus. The timing of body movement, based on initial foot movement, lagged tail movement by only 167 ms (median), however, the timing was not dependent on the stimulus location ($p=0.37$). The initial foot movement did depend on stimulus location; when the base of the tail was stimulated, the back left foot moved first, but when the tip was stimulated the front right paw moved first. These results indicate that while the tail movement occurs similarly to previous studies, body movement also plays an integral part in their nociceptive withdrawal responses.

Volumetric Tumor Response Assessment in Patients with Advanced Metastatic HER2+ Tumors Receiving An Autologous AdHER2 Dendritic Cell Vaccine

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The AdHER2 therapeutic vaccine is a personalized cellular immunotherapy using a patient's own dendritic cells that have been transduced to express the extracellular (EC) and transmembrane (TM) domains of HER2. HER2 is an established oncogene overexpressed in ~ 20-25% of breast cancers that is also expressed in other non-breast e.g. bladder, colon, ovarian, NSCLC solid tumors, and is associated with worse clinical outcomes¹. The purpose of this research is to compare Immune-Response Related Criteria (irRC) used on this study and RECIST 1.1 criteria with volumetric segmentations and tumor densities to assess best overall response to vaccination.

With the use of volumetric segmentation tools from PACS (Picture Archiving and Communication System), volumetric segmentation of previously selected target lesions was performed. Time points from baseline to most recent available scan were assessed. 3D view of segmented lesions was obtained. The density (Hounsfield Units) of the lesions is measured using histogram analysis tool. We compared volume lesions densities (Hounsfield units from VOI), Immune-related Response Criteria, and RECIST 1.1 criteria over time and determine true versus pseudo progression of target lesions.

Use of either RECIST 1.1 criteria or Immune-Related Response Criteria (irRC) are only able to document changes in the size of designated lesions in one dimension. In addition, imaging is unable to differentiate increases in lesion size attributable to true disease progression vs. "pseudo progression" that may be related to focal anti-tumor inflammatory responses. The use of Volumetric segmentations, histograms and Hounsfield units from VOI provided a better indication of tumor necrosis associated with vaccine-induced focal anti-tumor responses and is a better method to assess therapeutic response in patients receiving AdHER2 vaccine as an antineoplastic treatment.

MOTHER-INFANT INTERACTIONS: THE EFFECTS OF SEROTONIN DEFICIENCY ON ULTRASONIC VOCALIZATIONS IN NEONATAL *PET-1* KNOCKOUT MICE

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Effective maternal care in rodents depends in part on ultrasonic vocalizations by the pups that trigger survival-promoting behaviors by the dam. The ability of pups to produce effective calls and the ability of dams to hear and respond appropriately to these calls are crucial for survival. Defects in either call transmission or call receipt could lead to sub-optimal maternal care and increased pup mortality. *Pet-1* gene deletion has been associated with a 70% loss of central serotonin neurons and ineffective maternal behavior. Specifically, all pups born to *Pet-1* knockout (*Pet-1*^{-/-}) dams and approximately 25% of *Pet-1*^{-/-} pups born to heterozygous (*Pet-1*^{+/-}) dams die within five days of birth. We hypothesize that abnormal call production and/or call reception may contribute to this mortality. To begin to address this issue we recorded, characterized, and compared ultrasonic vocalizations produced by wild type (*Pet-1*^{+/+}) and serotonin-deficient *Pet-1*^{-/-} pups at two time points during early postnatal development. We found that neonatal mice of both genotypes were capable of producing a range of distinct vocalizations (call types). The distribution of call types was similar between *Pet-1*^{+/+} and *Pet-1*^{-/-} pups shortly after birth (postnatal day 1), but this distribution diverged significantly by the fifth postnatal day. In addition, knockout pups produced fewer calls overall compared to wild type littermates during the first 2 minutes of the 10-minute recording periods. Based on behavioral observations and retrieval assays, the general maternal behavior of *Pet-1*^{+/-} dams was found to be similar to *Pet-1*^{+/+} dams. Nevertheless, our data remain consistent with the possibility that abnormal vocalizations from *Pet-1*^{-/-} pups results in subtle but crucial differences in maternal behavior relative to *Pet-1*^{+/+} littermates, leading to chronic suboptimal care and increased *Pet-1*^{-/-} pup mortality during the early postnatal period.

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QUANTIFYING EMERGENCE IN DYNAMIC NEURAL NETWORKS

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Spatial Light Interference Microscopy (SLIM), Dispersion-relation Phase Spectroscopy (DPS), Fluorescence Correlation Spectroscopy, and Cell Growth and Proliferation Assays are all methods that are currently being used by researchers to quantify neuron behavior. While typical assays such as those conducted with flow cytometry offer easy to interpret statistics, they are rarely able to provide spatial information. Although fluorescence techniques benefit from specificity, they are ill suited for long term imaging due to photo toxicity. To overcome these shortcomings, we seek to develop an automatic analysis process that will allow researchers to study the spatial characteristics of cells within a given culture with improved efficiency. To accomplish this task, we plan to establish a foundation for the cell quantification techniques by providing the underlying morphological characteristics for neuron cultures that have developed over time. Upon the success of our research, we will have a more efficient automatic analysis process that can be used in analyzing the emergent behaviors of neurons such as the change in size, location, and direction of the neurites in a given culture.

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DETERMINING THE RELATIONSHIP BETWEEN L1CAM AND MALIGNANT GLIOBLASTOMA STEM CELLS

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Glioblastoma multiforme (GBM) is the most common and deadly form of brain cancer. Our lab has shown that the L1 Cell Adhesion Molecule increases cancer cell motility and proliferation through autocrine/paracrine stimulation *in vitro* on established cell lines and surgical specimens. This project is focusing on Glioblastoma Stem Cells (GSCs), which often escape surgical resection due to their invasiveness and lead to tumor regeneration. We think that GSCs use the same L1-mediated stimulatory mechanisms and are attempting to demonstrate this with isolated GSCs *in vitro* and in tissue and an *in vivo* model. Time-lapse “super scratch” assays of GSCs have shown that they can influence the motility, speed, and directionality of established GBM cell lines. For *in vivo* injection experiments, GSCs or a mixture of GSCs and normal tumor cells were injected into a chick embryo on day E5. After allowing the embryos to grow for 10 days, the brains are removed and thick-sectioned for viewing with a confocal microscope. By using fluorescent dyes to identify different cell types, we were able to visualize where the cells formed tumors and invaded the brain tissue. GSCs appeared more invasive than established tumor cell lines and invaded areas far away from the main tumor location. Thus, our *in vivo* model appears to recapitulate how GSCs must invade the brains of GBM patients who often experience relapse in areas far from the initial site of tumor growth and resection. In addition, preliminary experiments to show that GSCs appear to have deposited trails of L1 as they invaded the brain tissue. This work has laid the groundwork for myriad future experiments with GSCs over the course of the next few years.

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CELLULAR INTERNALIZATION OF G3 PEGYLATED POLY-L-LYSINE DENDRIMER WITH DOXORUBICIN

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One of the major setbacks of chemotherapeutic drugs is their low specificity. In other words, when the drug enters the body, it distributes within the body rather evenly, killing all actively growing and dividing cells. When large doses of a drug are used during chemotherapy, both cancer cells and healthy cells are killed, hence causing side effects like hair loss and damages to the internal organs. To combat this problem, drug targeting is used due to its cellular selectivity and ability to perform cell penetration. Recent studies have shown cell-penetrable lysine dendrimers as promising carriers for targeted chemotherapeutics delivery because of their unique internalization mechanism and biodegradability. In this project, Generation 3 PEGylated Poly-L-Lysine Dendrimer was chemically conjugated with an anti-cancer drug, Doxorubicin (DOX). Its cytotoxicity and internalization mechanism were then compared with free-DOX. MCF-7 human breast cancer cell line was used. Cytotoxicities were determined by measuring the inhibition of cell growth using WST-1 assays. The percentages of cell viability were then determined. To visualize the internalization, the cells were treated with free-DOX and Lysine-DOX conjugates for 4 and 8 hours respectively. After incubation, the cells were fixed and observed by a confocal microscope. Our result suggested that PEGylated Poly-L-Lysine Dendrimer could be used as a prospective vehicle for sustained drug release targeted to cancer cells.

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CHARACTERIZATION OF BACTERIOPHAGES IN SOIL FROM BOWIE, MD AND REHOBOTH BEACH, DE

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Bacteriophages are viruses that infect bacteria and confer various properties, such as bacterial antibiotic resistance. The purpose of our study was to isolate and characterize novel bacteriophages from the soil microbe *Bacillus thuringiensis* subsp. *Kurstaki* from locations in Maryland and Delaware. Bacteriophage NoSwims was isolated from Bowie, MD and had plaques that were circular and translucent. Bacteriophage FeBro was isolated from Rehoboth Beach, DE and had plaques that were transparent and circular. Transmission electron microscope (TEM) analysis revealed that NoSwims was a Myoviridae and had a medium-sized head and a long tail and FeBro was also bacteriophage Myoviridae and was typically the same structure as NoSwims. DNA was then isolated from both phages and characterized using polymerase chain reaction (PCR) analysis. PCR analysis revealed that FeBro and NoSwims had enough differences between them that if these phages had undergone cluster analysis and the data supported our PCR observations then DNA sequencing could have the potential for promising results. Restriction enzyme digestion determined the rough estimate of base pairs of our Phages. Future directions include sequencing the DNA and annotating the genome of each phage in order to compare these phages to other known soil bacteriophages.

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EXTRACTION AND PURIFICATION OF UNIQUE PHAGES FROM DIFFERENT REGIONS IN MARYLAND

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Phages are a diverse group of viruses that are able to infect bacteria. The world of phages is a vast and unexplored place as only a small percentage of phages are known. Hence programs such as the Phage Hunters through the Howard Hughes Medical Institute have been established to learn more about and discover these unknown viruses. The goal of our research was to isolate and characterize novel *Bacillus thuringiensis* bacteriophages. Bacteriophage Yolk was isolated from Frederick, MD, Bacteriophage Taffo 16 was isolated from Columbia, MD, and Bacteriophage Nickolsky was isolated from Catonsville, MD. Each phage had a unique plaque, with Yolk having large plaques that were mostly equidistant from one another, Taffo16 having plaques that looked like clear rings around a spot of bacteria, and Nickolsky having cloudy plaques with dark centers. Transmission electron microscopy (TEM) was then used to observe the morphology of each phage. Bacteriophages Yolk, Taffo16, and Nickolsky all had the morphology of a Myoviridae phage. Finally, DNA was isolated and characterized by restriction analysis and results demonstrated that each phage was unique based on the restriction enzyme fragment patterns observed. Furthermore, a host range test was applied to each bacteriophage and it was found that bacteriophage Yolk had a very limited range, while bacteriophages Taffo16 and Nickolsky had relatively broad ranges. Of these three bacteriophages, only Taffo16 and Nickolsky were found to be sequenceable. Future research will involve sequencing and annotating the genome of the sequenceable phages to compare them to other known bacteriophages.

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ISOLATION OF BACTERIOPHAGES FROM LOCAL MARYLAND SOIL

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Bacteriophages are a very diverse class of viruses that infect and replicate inside bacteria. There are an approximated 10^{31} phages around the world, of which only a fraction have been classified. Therefore, the purpose of our research was to isolate and characterize novel bacteriophages from local soil. We collected soil samples and isolated bacteriophages from the following cities in Maryland: Frederick, Waldorf, and Catonsville. We then infected separate lawns of *B. thuringiensis* with an enrichment of our soil samples and titrated to isolate and amplify a single phage plaque morphology from each location. The Frederick sample contained plaques that were large and clear with a smooth circumference. The Waldorf sample contained plaques that were small and cloudy with a smooth circumference. The Catonsville sample contained plaques that were large and cloudy with a pinhole sized clearing in the center and a hazy oblong circumference. This is important because different plaque morphologies indicate different types of bacteriophage. Electron microscope (EM) analysis of each bacteriophage indicated that one is in the Myoviridae family due to evidence of a mid-length retractable tail. While the other two are in the Siphoviridae family because of their long to mid-length non-retractable tails. For further analysis, DNA was isolated from each bacteriophage and a Restriction Enzyme Digest was performed to characterize the DNA. Gel Electrophoresis showed replications of different DNA segments for each bacteriophage sample, indicating a diversity in the DNA composition. We have reason to believe that each of our phage samples are different from one another. However, more work needs to be done. Future directions of this research will involve sequencing and annotating the genome of the bacteriophages. The genomes of the phages will then be compared to other known bacteriophages to confirm whether or not the bacteriophages isolated in this study were unique.

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ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES USING *BACILLUS THURINGIENSIS* HOST BACTERIA

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Bacteriophages are viruses that infect and replicate within bacteria. They can be found anywhere in the world, and the genetic information they carry can potentially be used to cure certain bacterial infections. Three phages that infect *Bacillus thuringiensis* subs. *kurstaki* (*Btk*), a bacterium that is used in agriculture as an insecticide, were isolated from Ellicott City, MD (Momo), Hershey Park, PA (Tershka), and East Potomac Park, Washington DC (Kelechi). The goal of this project was to isolate and characterize novel phages to ultimately sequence their genomes. Three phages of interest were isolated from enriched soil samples and characterized using microbiological and molecular biology techniques. They were then characterized based upon their unique plaque morphologies, which ranged from small, distinct opaque circles with a surrounding halo effect to completely clear overlapping circles of differing diameters, host-range infections, various gels, and transmission electron microscopy (TEM) which showed that all three phages were myoviridae. Based on analyses of enzyme-digest gels, it was determined that each of the three phages were unique in their DNA sequences. Further analyses with Quality Control (QC) gels revealed that all three phages had potentially sequenceable DNA. Based on these observations, it was concluded that the isolated phages could be archived for future research purposes. Each novel phage was then archived, and their DNA sent off to be sequenced and further identified. This work has the potential to reveal phage gene sequences that could be used to manipulate phages and implement phage therapy in serious bacterial infections rather than the use of antibiotics that bacteria quickly develop immunity to.

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LOCATING AND ISOLATING DNA FROM BACTERIOPHAGES DISCOVERED IN MARYLAND

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The goal of this research was to successfully locate and isolate the DNA of bacteriophages (phage) found in soil samples around Maryland. A phage is a virus that infects bacteria. Phages are found practically everywhere and are incredibly diverse. There is growing and renewed interest in phages due to their impact on the environment and their ability to confer antibiotic resistance. By better understanding phages, we can use them to develop stronger antibiotics and understand how they impact the environment. Following the extraction of Cleopatra from Catonsville, MD, Joe B from Columbia, MD, and Becky from Brookeville, MD, we began work on isolating each phage from the sample individually. The three *Bacillus* phages were successfully isolated using *Bacillus thuringiensis* subs. *kurstaki* (BTK) as a host. From these samples, microbiological techniques were used to isolate, purify, characterize, and extract the DNA from each phage. A variety of procedures were used to isolate genomic DNA that could be sequenced, which was then compared to other phages within the research team. After comparing our phages to restriction enzymes provided in the lab, we concluded that our bacteriophages were unique. However, host range plaques of each bacteriophage were similar in that they were circular in shape. Though the phages were similar in morphology, Becky and Cleopatra have circular heads attached to a tail while Joe B has a hexagonal head. Cleopatra also showed a contracted outer sheath showing that it was myoviridae. Three samples were then chosen to be sequenced off-site where genome annotation could be completed. From our research, we were able to conclude that phages are abundant and can be found almost anywhere in soil. After successfully locating and isolating the DNA from our research we hope to use this knowledge in better identifying more phages in the future.

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THE IDENTIFICATION OF BACTERIOPHAGES FROM SOIL SAMPLES IN LAWRENCEVILLE, GA AND UMBC

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Bacteriophages are a diverse population of viruses that have a growing presence in the scientific world. Simply known as phages, these viruses inject their genetic material into a bacterial host. This study was conducted in order to isolate and identify phages from the soil in a selected area. We isolated phages from soil samples collected in parts of US using *Bacillus thuringiensis subsp. kurstaki* (Btk) as a host. We then characterized the phages using microbiological and molecular biological techniques to confirm that they were unique in contrast to other phages in the lab. LaFlare, a bacteriophage isolated from Lawrenceville, Georgia, had mostly opaque circular plaques while Lalalita, a bacteriophage isolated from UMBC, had clear circular plaques. After doing a TEM (Transmission Electron Microscopy) analysis, we saw that both phages had a Myoviridae morphology and an unclustered structure. The polymerase chain reaction (PCR) analysis done for LaFlare yielding an inclusive result. However, Lalalita's PCR analysis produced promising results and was one of five phages sent for sequencing and annotation. If indeed the phages are sequencable, that would mean our phage would be able to get archived and serve as a reference in future research. Future directions could include using our sequenced DNA to compare see the similarities and differences with other bacteriophages in the soil residing in urban areas of Maryland and Georgia.

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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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DETERMINATION OF COCOA LIQUOR PROVENANCE USING TRACE ELEMENTAL SIGNATURES

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The purpose of this project was to determine the provenance of cocoa liquor samples from several different countries using trace elemental signatures. A large fraction of the cocoa industry currently suffers from traceability issues. This is problematic because the quality of a finished chocolate product is highly dependent on the growing conditions of the cocoa beans and the manufacturing processes employed. Improving traceability in the cocoa industry would encourage the production of healthier, higher-quality cocoa products.

This project addressed the following questions: 1) Do cocoa liquor samples from the same country have different chemical signatures because of the different roasting and processing conditions (temperature, time, humidity level, etc.) they undergo? 2) Can cocoa liquor samples from more than eight countries be distinguished from each other using elemental signatures? 3) Which elements are most useful in creating a trace elemental signature for cocoa provenance determination?

Cocoa liquor was analyzed in this project because it is the essential component of any finished chocolate product. It undergoes little processing and thus contains a high concentration of trace elements. Cocoa liquors from eleven countries of origin were used in this project; eight replicate samples of each cocoa liquor were prepared and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS). The resulting trace elemental data were analyzed using the discriminant analysis function in SPSS. This software was able to correctly classify the cocoa liquor samples by their country of origin using a cross-validation procedure.

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APPLICATIONS OF HIGHLY SENSITIVE, MASS SPECTROMETRY-BASED, ANALYTICAL TOOLS FOR PROTEOMIC ANALYSIS IN SINGLE CELL NEURONS

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Characterization of gene expression in mammalian neurons provides vital information to uncover molecular pathways that are involved in severe neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Averaging across millions of cells is the methodological norm due to technological limitations. However, averaging fails to provide information on neuronal heterogeneity, which is known to convolute protein identification as averaging obscures the molecular pathways involved with specific proteins. Here, we present a micro-analytical platform that delivers sufficient sensitivity to measure gene expression in small populations of neurons in the mouse cortex, which may extend towards measuring protein content from single cell neurons.

The posterior cortex was isolated from adult mouse brains, trypsin-digested, and reconstituted in 50% acetonitrile containing 0.05% acetic acid. A 1 nL sample volume was analyzed by a custom-built capillary electrophoresis nanoelectrospray ionization platform that we coupled to a high-resolution mass spectrometer (CE-nanoESI-MS). The CE-nanoESI interface used a tapered-tip emitter as a nanoelectrospray source and selected the cone-jet spraying regime to obtain high ionization sensitivity. Peptide ions were sequenced by a hybrid quadrupole orbitrap (QE+) high-resolution mass spectrometer at 35,000 (FWHM) resolution for MS¹, 17,500 (FWHM) resolution for MS². The acquired MS-MS/MS data were searched against the mouse proteome using MaxQuant executing the Andromeda search engine with <1% false discovery rate (FDR). Analysis of standard peptide (Angiotensin II) revealed a ~20-fold sensitivity improvement compared to the previous version (CE- μ ESI), allowing for a ~250 zmol lower limit of detection for this peptide. In addition, CE-nanoESI-MS was able to identify neuron-specific proteins and others involved in neurodegenerative disorders (e.g., synapsin and tau) from <1 ng protein digests.

We envision that high-sensitivity detection by our CE-nanoESI-MS system raises a potential to characterize protein expression in small neuron populations to better understand how molecular processes underlie normal development and neurodevelopmental diseases.

**IN SEARCH OF A MASS BURIAL SITE FROM THE FRENCH & INDIAN WAR:
PRELIMINARY RESULTS FOR ANALYSIS OF SOILS FOR SELECTED ELEMENTS
FROM AN ARCHAEOLOGICAL EXCAVATION NEAR LIGONIER, PENNSYLVANIA**

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Preliminary results for an efficient protocol for extraction of selected elements from soils associated with an archaeological excavation near Ligonier, Pennsylvania, at the site of the Friendly Fire Incident that occurred during the French and Indian War, are presented here. The extraction protocol mimics severe environmental changes in the soil to remove the elements of interest from the soil matrix for subsequent determination by flame atomic absorption spectrometry (FAAS, for Cu, Pb, As, Ca, Cr, Ni) and UV-visible spectrophotometry via the molybdenum blue method (for P). The metals of interest in this part of the study are copper, lead, arsenic, calcium, chromium, nickel, and phosphorus. The hypothesis is that the levels of the aforementioned elements will be higher in the soils surrounding artifacts from the friendly fire incident (e.g., musket balls and buttons from soldiers' uniforms) than in soils containing no artifacts. The ultimate goal of the investigation is to assess whether or not any or all of the elements determined can serve as indicators of a mass burial for casualties of the friendly fire incident. Soil sampling protocols, details of the aforementioned extraction, quantitation of the aforementioned elements, the results obtained, and future plans for this research, will be presented and discussed.

This study is a collaboration between the Pitt-Greensburg Chemistry Program and the Archaeology Program's George Washington Friendly Fire Incident History & Archaeology Project.

ARONIA MITSCHURINII CULTURAL MANAGEMENT SUPPORTED BY PHYTOCHEMISTRY: LESSONS LEARNED SINCE 2006

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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 antioxidant content in Aronia juice samples, yet phytochemical content has never been correlated with cultural management of this crop (fertilizing, mineral additives, irrigation, age of the crop etc.). The pulp is rendered inedible by conventional means, due to its bitter taste and very few is known about its phytochemical content. However, considering recent applications of aronia as a food supplement, agricultural additive, electrolyte for fuel cells and others, use of pulp now has high potential to increase the value of berries and farmers' income. Higher temperatures used in food processing technology for aronia products have three major effects: isomerization, decomposition or loss of water. The first two would negatively impact product quality.

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. We have also studied the effect of temperature and the exposure time on the antioxidant content with an aim to determine the processing technology that would avoid significant decomposition of antioxidants in aronia. All samples are grown in controlled facility of WyeREC.

SYNTHESIS AND ANALYSIS OF BIOORTHOGONAL PHOTOLABILE LINKERS CAPABLE OF TWO-PHOTON UPCONVERSION

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In recent years there has been a significant increase of the study of photosensitive molecules which have the capability of mediating many types of chemical and biological processes *in vivo*. This increase led to the discovery of a specific section of molecules derived from 8-azacoumarin chromophores. These derived molecules show properties which are highly desirable for biorthogonal processes like excellent water solubility and high molar absorptivity. Because of these desirable traits, this research focused on utilizing the parent 8-azacoumarin to synthesize new derivatives with, theoretically, enhanced or more specific traits.

During the eight-week research position, four of the six molecules, needed to advance the project, were synthesized, purified, and analytically confirmed through several methods. The synthesized molecules were projected to be more efficient in upconverting two lower energy photons into a higher energy photon than the parent molecule, and capable of breaking bonds in a coupled nanoparticle system. This has many applications, however, the intention of this research is to couple the linker molecules to nanoparticles which carry a drug which will detach from the system when a certain wavelength of light is concentrated on the photosensitive molecule. A system of this nature is a vast improvement of the current drug delivery system which uses damaging UV light to release drug particles.

The parent molecule was altered in multiple different ways, including differentially reacted brominations, variation of carbon-based substituents, and an alteration between a pyridine ring or natural benzene in the system. While the research conducted in the eight-week period was successful in achieving the above syntheses, it is currently being continued by Dr. Gergely Cserép at MTA-TTK in Budapest.

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

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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. This research overall aims to synthesize a compound using rhodamine to bind the ions and anions in its cavity, conduct UV-Vis and fluorescence studies to detect these ions and anions. To synthesize the compound 1, rhodamine hydrazine was mixed with 2-hydroxy-5-nitrobenzaldehyde and ethanol. When synthesizing the compound for eight trials, the percent yields are 53.8%, 61.5%, 30.8%, 69.2%, 76.9%, 53.8%, 69.2%, and 53.8%. The ligands were confirmed by NMR as pure. The first three trials, the product was a red color. The next four trials, the product was yellow and the last trial, the product was light peach color. Confirmed by the results of the NMR, we have designed and synthesized a rhodamine-based chemosensor. Stock solution was prepared by dissolving approximately 0.048 g of compound 1 with 40 mL acetonitrile. 2 mL of the mixture was placed in a spectrophotometer cell to measure the absorbance and fluorescence. The UV absorbance spectra were recorded on a Cary 60 UV-Vis using acetonitrile as a baseline. Fluorescence spectra were recorded on a Cary 60 Fluorescence spectrophotometer. From the results of measuring the absorbance and fluorescence intensity, Al^{3+} , Cr^{3+} , Cu^{2+} , Ca^{2+} , and Ni^{2+} ions have experienced a pink color change when added with the stock solution while the other solutions remained colorless. $\text{Al}(\text{NO}_3)_3$ has a high intensity with a low absorbance, so this compound can show a significant fluorescent enhancement with Al^{3+} . $\text{Ni}(\text{NO}_3)_2$ has a significant absorption enhancement with a low intensity, so this compound can selectively detect Nickel and Aluminum.

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SYNTHESIS AND CHARACTERIZATION OF POLYMERIZABLE DAPOXYL DYES FOR LUMINESCENCE-BASED SENSING

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Dapoxyl dyes are environmentally sensitive luminophores with unique electronic properties ideally suited for luminescence-based sensing. Opposing electron-donating and electron-withdrawing substituents conjugated through a 2,5-diphenyloxazole core afford a solvatochromic shift in emission wavelength in environments capable of stabilizing the large excited state dipole moment. Recently, Dapoxyl sulfonic acid (DSA) was used in a relative humidity sensor utilizing an analyte-sensitive hydrogel scaffold with DSA suspended in its pores. As the gel expands or contracts based on analyte concentration, the environment around the dye changes altering its emission wavelength. While DSA worked well in a gas phase sensor, leaching of the dye from the hydrogel prevents its use in the aqueous phase. In order to overcome this limitation, we envisioned Dapoxyl dyes containing a functionalized terminus for polymerization into an acrylamide hydrogel.

We have synthesized three derivatives of Dapoxyl dyes. These analogues contain a polymerizable *N*-acrylamido group linked through an electron-donating piperazine. Various electron-withdrawing groups were explored to afford the desired push-pull electronics. The oxazole core was constructed using a Robinson-Gabriel oxazole cyclization. A Boc-protected piperazine was then coupled with an aryl-bromide via a Buchwald-Hartwig amination. Finally, the piperazine was deprotected and acryloylated to give the polymerizable DSA analogues.

A Dapoxyl nitrile derivative (DND) was polymerized into an acrylamide hydrogel. Optical properties of both the DND and the corresponding DND doped hydrogel show solvatochromic shift in emission wavelength with increasing solvent polarity. The dye also displayed no signs of leaching from the polymer. At higher solvent polarity, the emission shift was decreased. The decrease is likely the result of two competing excited states with similar energies but differing environmental sensitivity. These initial results are promising indicating DND and other Dapoxyl dyes could serve as reporter molecules in versatile, sensitive, and reusable aqueous phase luminescence-based sensors.

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ELECTRONIC BEHAVIOR OF FLUOROPHORE LABELLED MAGIC NUMBER GOLD CLUSTERS

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Magic number gold clusters are at the forefront of research owing to their characteristic size dependent optical and electrochemical properties. Of recent interest is their use as biological imaging agents due to their near-IR luminescence (the wavelength of emission is ligand dependent). Magic number Au₂₅L₁₈, and Au₁₄₄L₆₀ clusters were synthesized using a one phase method with L = hexanethiol or dodecanthiol as a stabilizing ligand. The electronic transition states of each particle was observed through optical and electrochemical analysis. The clusters were characterized through observation of documented HOMO/LUMO gap using both optical and electrochemical techniques. Au₁₄₄-clusters indicated quantized double layer charge upon electrochemical analysis. Transmission electron microscopy was employed to determine particle size and dispersity. The MPCs (Monolayer Protected Clusters) with the hexanethiol stabilizing ligand were then labeled with a coumarin dye via directed ligand exchange. The products of the exchange reaction were then compared with the MPC made from the coumarin ligand. The details of the synthesis, characterization and two-photon cross-sections of these clusters will be presented.

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PHYTOCHEMICAL SYNTHESIS OF METAL NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY

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Microbial resistance to antibiotics has been ever increasing and poses serious risks. The goal of this research project is to synthesize stable gold and silver nanoparticles using plant extracts from plants that have medicinal properties. Nanoparticles are excellent substrates to generate high sensitive antimicrobial agents, as they can be tailor made for a specific application and administered in low concentrations. In this project, stable nanoparticles were synthesized phytochemically and antimicrobial studies are being carried out. Using plant extracts rather than commercially available ligands for metal nanoparticles synthesis has benefits in being inexpensive, and bio compatible. To synthesize gold and silver nanoparticles, leaves from the Azalea Indicum plant extracts were used. Characterization was carried out using UV-Vis spectrometry, bright field microscopy, transmission electron and fluorescence. Results have shown that stable gold and silver nanoparticles were successfully formed. Various synthesis strategies and results from antimicrobial activity will be presented.

This project was funded by Towson University Faculty Start-up.

THE EFFECT OF QUANTUM DOT CORE CHEMISTRY ON THE INDUCTION OF APOPTOSIS ON EMBRYONIC ZEBRAFISH (*DANIO RERIO*)

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Quantum dots [QDs] are semiconductor nanocrystals that have unique fluorescent characteristics: broad absorbance, narrow, size-dependent emission peak, high emission quantum yield, and high chemical and photo-stability. Due to these characteristics, a variety of areas have begun to utilize QDs more frequently. Core/shell QDs are synthesized using organic solvents and ligands which prevent their solubility in water. In order to evaluate the biological impact of core/shell QDs, the hydrophobic surface ligands are exchanged with hydrophilic ligands to introduce the QDs to water, thus permitting their bioavailability. The most widely used QDs contain cadmium selenide cores with zinc sulfide shells [CdSe/ZnS]. This composition of QDs is thought to pose a threat on the environment because ionic cadmium is known to be highly toxic. In order to find a safer alternative to CdSe/ZnS QDs, this study aims to compare the biological impact of CdSe/ZnS QDs to that of QDs containing zinc selenide cores and zinc sulfide shells [ZnSe/ZnS]. To do so, we measured the induction of apoptosis in embryonic zebrafish (*Danio rerio*) after environmental exposure to CdSe/ZnS QDs and ZnSe/ZnS QDs solutions. Before QD exposures, however; we first exposed the zebrafish embryos to cadmium chloride [CdCl₂] solutions at different developmental stages and for different exposure time periods. By doing these preliminary exposures, we determined which stage in development was the most sensitive to the exposure and which length of exposure was necessary to cause detrimental effects. These conditions were then replicated with the exposure of the embryos to CdSe/ZnS QDs and ZnSe/ZnS QDs solutions. Induction of apoptosis was measured through Caspase-3 Immunolabeling and a Caspase-3 Activity assay. Caspase-3 Immunolabeling was used along side epifluorescence microscopy in order to localize where apoptosis occurs, while the use of the Caspase-3 Activity assay allowed us to better quantify the induction of apoptosis.

I am grateful for the generous support from the Center for Sustainable Nanotechnology, the University of Maryland, Baltimore County, the Rosenzweig lab, and the Brewster Lab. I am also grateful for tremendous support of Melissa Richardson for guiding me through my research experience.

STABILITY AND ANTI-MICROBIAL PROPERTIES OF GOLD NANOPARTICLES FROM GREEN METHOD

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Gold nanoparticles (GNPs) have received much interest recently in biomedical applications including imaging, diagnostics, and drug delivery. However most synthetic methods of these metallic nanoparticles are not environment friendly and often involve toxic chemicals. This study reports a green synthetic route of GNPs using green tea leaves, and the stability of these GNPs can be greatly enhanced by addition of gum Arabic. Synthesized nanoparticles were characterized by UV-Visible spectroscopy, and the absorbance peak at 560 nm due to surface plasmon resonance confirmed formation of the GNPs. The antimicrobial evaluations of these GNPs against *S. aureus* and *P. aeruginosa* reveal that these gold nanoparticles are not bactericidal.

This project was supported by Rosemont College Connelly Young Faculty Grant. Special thanks to Dr. Aikaterini Skokotas for her help in preparing bacterial cultures.

THE EFFECT OF REACTION TIME AND SOLVENT SYSTEM ON MoS₂ EXFOLIATION

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Our research is interested in developing a process for a large scale synthesis of molybdenum disulfide (MoS₂) nanomaterials. The objective of the current research is to study the effect of reaction time and solvent systems on exfoliation and deposition of MoS₂ films. Samples were prepared on silicon wafer then analyzed by Scanning Electron Microscope (SEM), Energy Dispersive Spectroscopy (EDS) and Raman spectroscopy.

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THE DEVELOPMENT OF FLUORESCENTLY TAGGED SULFATED DEXTRAN-COATED IRON OXIDE NANOPARTICLES FOR MONITORING UPTAKE AND CYTOTOXICITY OF ASTROCYTES AND NEURONS

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Scavenger Receptor-A (SR-A) is a cell surface receptor that recognizes various ligands including modified low density lipoprotein (mLDL), enabling biological functions such as the removal of foreign substances and waste materials in the body by specificity of extensive ligands via endocytosis, phagocytosis, adhesion and signaling. We are particularly interested in SR-A found in brain and spinal cord cells. One ligand for SR-A is dextran sulfate, thereby allowing uptake of sulfated dextran-coated iron oxide (SDIO) nanoparticles. Previous research conducted by the Louie lab revealed that there is uptake of SDIO by SR-A in activated microglia without significant levels of cytotoxicity suggesting the possibility of use in brain imaging during inflammation. The iron oxide content of SDIO nanoparticles makes them a strong T2 MRI contrast agent. The objective of this study was to investigate the uptake and cytotoxicity of SDIO nanoparticles in astrocytes and neurons. These parameters are important because significant levels of cytotoxicity and uptake in non-microglial cells will limit their use in imaging. Due to the cell density of astrocytes and neurons, determining the uptake via T2 measurements with a NMR relaxometer has proven difficult in previous studies. Thus, an SDIO nanoparticle tagged with a rhodamine derivative was synthesized to monitor uptake via fluorescence.

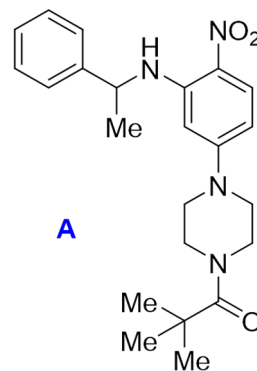
This work was funded, in part, by the UMES-UCD MCBGAP program.

DESIGN & SYNTHESIS OF POTENTIAL NSD1 INHIBITORS FOR PEDIATRIC LEUKEMIA

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The goal of our work is to create potent NSD1 histone methyltransferase inhibitors, for the treatment of pediatric acute myeloid leukemia. Acute myeloid leukemia (AML) is very difficult to treat using the traditional chemotherapy method. The fusion protein known as NUP98-NSD1, occurs in a type of acute myeloid leukemia by the rearrangement of the genes NUP98 and NSD1. The methyltransferase activity of NSD1 induces extreme levels of methylation at lysine 36 on histone 3, and is maintained in the fusion protein leading to oncogene activation. Therefore, NSD1 inhibitors are considered potential drug targets for AML that must be validated with appropriate small-molecules. Preliminary High Throughput Screening conducted at the Nemours Center for Childhood Cancer Research identified 5-(4-pivaloylpiperazine)-2-nitro-N-(1-phenethyl)aniline (**A**) as a low micromolar NSD1 inhibitor. To investigate how variations in its structure might improve activity and selectivity, the piperazine group was modified through chemical synthesis. Several structural analogs were synthesized that modified the benzyl group of (**A**) and were also evaluated for their ability to improve NSD1 potency and selectivity. If successful, these NSD1 inhibitors may be important probes for validating NUP98-NSD1 inhibition as a pharmacological target for leukemia in vivo.



1. Post-Doctoral Researcher
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TOTAL SYNTHESIS OF CLEROBUNGIN A

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A short synthesis of clerobungin A, a natural compound produced by the Chinese plant *Clerodendrum Bungei*, has been developed. Plants of the *Clerodendrum* genus have long been used for their medicinal effects in treating ailments such as dysentery or hemorrhoids. However, the scarcity of this compound has prevented any kind of substantial investigations of its bioactivity. The therapeutic potential of this compound, combined with its complex tricyclic ring structure and its natural occurrence as an unequal mixture of enantiomers (which is unusual in many biological enzyme mediated pathways), makes clerobungin A a prime target for research. Our synthetic approach was largely inspired by the proposed biosynthesis and can be summarized in three key steps: a Williamson ether synthesis, the oxidative dearomatization of a phenol, and an intramolecular hemiacetal formation / conjugate addition sequence. Each intermediate has been fully characterized using modern spectroscopic techniques, and we plan to submit samples of synthetic clerobungin A for further biological testing through the Open Innovative Drug Discovery program at Eli Lilly.

Funding for this project was provided by the Towson University Office of Undergraduate Research (TOUR) and the Fisher College of Science and Mathematics (FCSM) in the form of undergraduate research grants.

DESIGN AND SYNTHESIS OF A 2,5-OXAZOLE NUCLEOBASE FOR TRIPLEX FORMATION WITH WATSON-CRICK A-U BASE PAIRS IN DOUBLE STRANDED RNA

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Molecular recognition of RNA has not been as widely studied as DNA due to the limited knowledge of RNA function until the early 1990s. However, recent research has shown that RNA can act as a regulatory molecule for gene expression, catalyze reactions, and is responsible for a vast number of other cellular processes, making it of great interest for biotechnology. One strategy for RNA recognition involves the formation of a triple helix between dsRNA and a third strand of a nucleic acid. Peptide nucleic acids (PNA) are neutral molecules with amide backbones and appended nucleobases. The nucleobases can interact with the traditional Watson-Crick base pairs through Hoogsteen interactions, forming stable triplexes with dsRNA that may be sequence selective with careful nucleobase design.

Much work has been done using small molecules that are able to recognize a single nucleobase selectively in DNA recognition, but is less explored in RNA chemistry. One design strategy is to extend the nucleobase structure, allowing for recognition of both base pairs. This linker approach has been used for G-C with little success. Less is known about this approach with A-U base pairs.

The energy of a 2,5-disubstituted oxazole containing uracil and a phenol was calculated using PM6 level of theory and showed favorable triplex interactions with A-U base pairs. Our proposed synthesis for this target starts with 2-bromo-3'-methoxyacetophenone. An α -amino ketone has been made utilizing the Delépine reaction. The oxazole was made using the Robinson-Gabriel oxazole synthesis, where isoorotic acid is converted into an acid chloride and reacted with the α -amino ketone to form an amide, followed by cyclization with sulfuric acid. Finally, the product can be alkylated for PNA attachment and demethylated to reveal the tethered phenol that is expected to hydrogen-bond with uracil in the A-U base pair.

We would like to acknowledge the Elizabethtown College Department of Chemistry and Biochemistry, Dr. E. Jane Valas, and the Elizabethtown Summer Scholarship Creative Arts and Research Program (SCARP) for funding this project. We are grateful to the 2015-16 CH 455/456 students for conceiving the idea, and to our collaborator Dr. Eriks Rozners (University of Binghamton).

SYNTHESIS AND REACTIONS OF SOME 1,2-DIACYLDIAZIRIDINES

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Diaziridines are three-membered ring heterocycles that contain one carbon and two nitrogen atoms. In the past century, they have demonstrated exemplary applicability in the following areas: synthesis of heterocyclic systems, photo-affinity labeling experiments, enzyme chemistry, and more. In 1975, Robert A. Izydore and Samuel McLean developed a method for synthesizing diaziridine derivatives via the addition of an alkyl diazoacetate to a cis-diacyl-diimide; however, they did not propose possible N-N bond cleavage. This study investigates the bond-breaking selectivity of the three-membered diaziridine ring through the Quantitative Structure/Activity Relationship (QSAR) method. Bond breaking is monitored by systematically changing substituent electronic effects. Hence, we plan to exploit the use of carbene insertion chemistry with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to give various 1,2-diacyldiaziridines needed for this study. Thus far, various para-substituted diazoacetates have been synthesized. They will be reacted with PTAD, possibly shedding light on the effect that substituent electronics have on the reactivity of the diaziridine ring. The results of this study could also provide different antidepressant, antipsychotic, and anxiolytic pharmaceuticals via N-N bond cleavage, a novel procedure.

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THE SYNTHESIS OF PROPANAMIDE DERIVATIVES OF 1H-1,2,3-TRIAZOLE AND THEIR REACTION WITH PALLADIUM COMPLEXES

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A propanamide derivative of 1H-1,2,3-triazole was successfully synthesized from the reaction between 1H-1,2,3-triazole and acrylamide with Triton B as a catalyst. Two isomers formed: 3-(1H-1,2,3-triazol-1-yl) propanamide (**1**) and 3-(2H-1,2,3-triazol-2-yl) propanamide (**2**). These were separated by fractional crystallization. The two isomers were characterized by NMR and IR spectroscopy. Bis(benzonitrile)dichloropalladium(II) and potassium tetrachloropalladate(II) appear to react with **1** but not with **2**. These reactions are still being characterized.

I acknowledge Prof. Frank T. Edelmann for crystal structure data, and the National Science Foundation Research Experience for Undergraduates (NSF-REU) grant number CHE-1461175 for funding of this research.

EFFECT OF SUBSTITUENT POSITION ON QUINONE METHIDE DNA ALKYLATION

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A quinone methide, or QM, is a highly reactive intermediate that is often formed as a by-product of metabolism. QMs are strongly electrophilic molecules that have the ability to alkylate DNA, and the resulting adducts have the potential to cause cancer if left unrepaired. However, these deleterious effects can also be exploited for use as chemotherapeutics by inducing targeted cell death. By unraveling the mechanisms of QM interactions with DNA, we will be able to capitalize on this process in the future.

The Rokita lab has focused on the unusual reversible nature of the ortho-QM in order to extend the lifetime of these molecules under aqueous conditions. Many variations on mono- and bis-functional QMs have been developed to optimize association with DNA and the kinetics of reversibility. In my project, I am investigating the influence of the placement of the substituent on the stability, and, subsequently, the rate of QM adduct regeneration. While substituents in both the meta and para positions contribute inductive and resonance effects, only the substituent in the meta position can interact directly with the exo-methylene, causing these QM intermediates to be more stable and thus more likely to regenerate.

To explore these effects, I have synthesized a novel mono-functional QM precursor with an electron-rich substituent in the para position, which will be compared to its meta-substituted counterpart. The alkylating efficiency of the para compound was initially tested by incubating the QM with a radiolabeled DNA strand and subsequently treating with piperidine. Alkylation at specific A and G sites along the DNA was then visualized through gel electrophoresis and phosphorimaging. The results of these experiments will be presented alongside results of the meta-substituted compound in order to demonstrate the effect of substituent placement.

We would like to thank the National Science Foundation and the Greer Undergraduate Research Award (IE) from the Johns Hopkins University Department of Chemistry for their support.

STRUCTURE-ACTIVITY RELATIONSHIPS OF MODIFIED CINCHONA ALKALOIDS

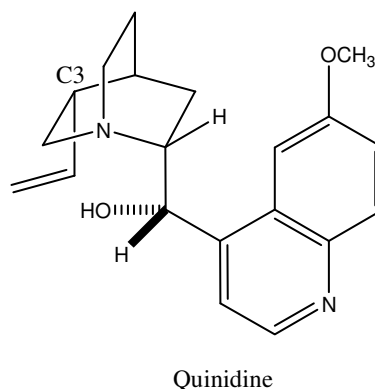
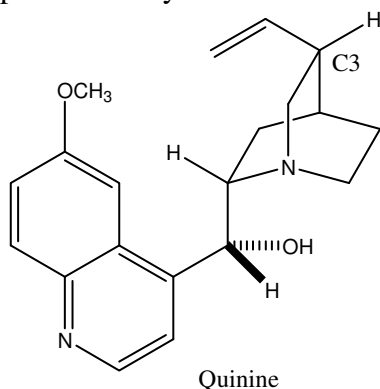
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Cinchona alkaloids, such as quinine and quinidine, are naturally occurring molecules derived from the cinchona tree that have found widespread application due to their medicinal and catalytic properties. The cinchona alkaloids differ in their substitution pattern and stereochemistry. Quinine and quinidine are commonly used as chiral catalysts in organic reactions and are considered pseudo-enantiomers because they have the opposite configuration at the stereocenters closest to the reactive nitrogen. Technically, however, quinine and quinidine are diastereomers (non-mirror image stereoisomers), and this difference causes them to catalyze some reactions with differing levels of stereocontrol.

The goal of this project is to investigate how stereocenters on cinchona alkaloids that are distant from the catalytic site are translated to noticeable differences in stereochemical control. Specifically, we hypothesize that varied substitution patterns on the quinuclidine portion of the alkaloid will cause twisting of the quinuclidine core, resulting in further downstream conformational changes near the active quinuclidine nitrogen.

To test this hypothesis, we are synthesizing derivatives of quinine and quinidine that contain modified substituents at C3 of the quinuclidine ring. We are synthetically modifying the vinyl group of these cinchona alkaloids to make alkyne and triazole substituents. We hope to obtain structural information on these modified alkaloids and correlate the conformational characteristics of the derivatives with their catalytic properties. This poster will summarize our synthetic progress, including our efforts to synthesize the alkyne derivatives via bromination of the vinyl group followed by elimination.



THE EFFECTS OF OSMOLYTES ON CAFFEINE PARTITIONING THERMODYNAMICS AND AQUEOUS INTERACTIONS

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This project illustrates the effects of nine osmolytes on the partitioning thermodynamics of caffeine between aqueous and cyclohexane phases. Gibbs free energy for caffeine transfer was measured with respect to osmolyte concentration and solution temperature. The stabilizers such as betaine and sarcosine decrease the Gibbs free energy for caffeine transfer and enhance caffeine transfer from the aqueous to cyclohexane phase. On the other hand, the denaturants such as urea and guanidinium salts behave the opposite to hinder the caffeine transfer. Gibbs free energy for caffeine transfer was measured at different temperatures to obtain the enthalpy and entropy for caffeine transfer. Caffeine transfer from the aqueous to cyclohexane is entropically driven, though the differences between osmolytes arise primarily from the enthalpy of caffeine transfer. NMR experiments are being conducted to determine how these osmolytes interact with caffeine in solution. The changes in chemical shift of carbon and protons in a caffeine molecule as a function of osmolyte concentration are used to determine the specific interactions of each osmolyte with caffeine.

We would like to acknowledge our funding sources for our research project, the NSF-REU Program (CHE-1461175) and the ACS Petroleum Research Fund (51008-UNI4).

TEMPERATURE DEPENDENT PXRD ANALYSIS OF TUTTON'S SALTS
 $K_2M(SO_4)_2 \cdot 6 H_2O$ (M = Zn, Cu, Ni, Co, and Mg)

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The thermal dehydrations of Tutton's salts with the general formula $K_2M(SO_4)_2 \cdot 6 H_2O$ (M = Zn, Cu, Ni, Co, and Mg) were investigated using thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), temperature dependent powder x-ray diffraction (PXRD), and infrared spectroscopy (IR). Tutton's salts have the potential to be good phase change materials because of their large phase change enthalpies and reversible dehydrations. This isomorphous series of salt hydrates form monoclinic crystals synthesized using two different slow evaporation methods. One method used an equal molar ratio of K_2SO_4 and $M(SO_4) \cdot 6H_2O$ (M=Co, Cu, Mg, Ni, Zn). The second method used a 2:1 molar ratio of K_2SO_4 and $MCl_2 \cdot 6H_2O$ (M=Co, Cu, Mg, Ni, Zn), respectively. Each salt has several pathways that are determined by the conditions of the dehydration such as the heating rate or sample size. These range from a simple one step process where all of the waters of hydration are lost in one step under all conditions (M=Ni) to multistep processes where the apparent intermediate formed depended upon the water vapor pressure in the cell (M=Co, Mg). These investigations show that the removal of all water molecules occurs around 330 K to 500 K. The full dehydrations will be presented in this study.

This material is based on work supported by the National Science Foundation Research Experience for Undergraduates (NSF-REU) grant number CHE-1461175 and James Madison University.

EFFECT OF NITROGEN-ATOM SUBSTITUENTS ON RING-FLIP AND N-INVERSION IN 1*H*-1-BENZAZEPINES

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The 1-benzazepine moiety appears in a wide variety of pharmacologically important molecules. 1*H*-1-Benzazepines contain two elements of chirality, an element of planar chirality due to the puckered azepine ring, plus, if the nitrogen atom is not planar, it may exhibit central chirality. Thus, the molecule may exist in the form of four stereoisomers, two pairs of two enantiomers. Both these elements of chirality are changeable—a ring-flip or an N-inversion converts *R* to *S* configuration. The rates of these changes are important to know, if a particular stereoisomer is to be developed into a pharmaceutical.

Model 1*H*-1-benzazepines were synthesized by reaction of pyruvate esters with 2-fluoroaniline, giving the N-unsubstituted variety. N-Alkylated 1*H*-1-benzazepines were prepared by deprotonation/N-alkylation of 3*H*-1-benzazepines. Computational methods were used to dissect the stereoisomerization process into its components of ring-flip and N-inversion, while variable-temperature NMR spectroscopy was used to determine the Gibbs free energy of activation (ΔG^\ddagger) of the processes by signal-coalescence methods. X-ray crystallography showed the ground-state geometries.

When alkylated, the nitrogen atom was pushed far out of the plane of the benzo group, and became nearly planar, while the azepine ring was highly puckered; thus, ΔG^\ddagger values in this class of compounds were high for ring-flip and low for N-inversion. However, when the nitrogen atom was unsubstituted, it was significantly pyramidal in geometry, while the azepine ring was less puckered. These factors caused ring-flip ΔG^\ddagger values to be lower than their N-inversion barriers in the N-unsubstituted compounds. Interestingly, the ring-flip and N-inversion processes were found to be interdependent in the N-unsubstituted benzazepine series.

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NANO-TRIBOLOGICAL PROPERTIES OF SINGLE AND MIXED MONOLAYERS OF NONADECANOIC ACID AND OCTACOSANOIC ACID SELF-ORGANIZED ON SAPPHIRE SURFACE

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Self-assembled Monolayers (SAMs) are two dimensional molecular arrays that spontaneously form by adsorption of organic molecules onto a surface. Each molecule is composed of a head group that binds to the substrate, an alkyl chain that provides order and stability, and a tail group that determines surface properties. This project focused on single and mixed monolayers of nonadecanoic acid (C19) and octacosanoic acid (C28), formed on sapphire. Single monolayers of C19 and C28 and mixed monolayers with ratios of 75%-25%, 50%-50%, and 25%-75% were formed using solution deposition methods. The binding nature and hydrophobicity of monolayers were analyzed via diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), and water contact angle measurements, respectively. Atomic force microscopy (AFM) was employed to investigate frictional properties of the monolayers in addition to acquisition of surface topography. DRIFT spectra confirmed the formation of highly ordered monolayers with mono/bidentate binding. Water contact angles indicated a hydrophobic surface, and the degree of hydrophobicity increased as C28 composition increased. Single and mixed monolayers exhibited substantially higher friction than uncoated sapphire surface.

PORPHYRIN INDUCED MULTIMERIZATION OF SOLUTION-STATE PROTEINS

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Interactions between charged porphyrins and complimentary or similarly charged proteins provide important model systems for studies of electron transfer processes, artificial photosynthesis, and control of protein-protein interactions. Typically, the experimental results are analyzed and discussed assuming that the proteins exist in a monodisperse state. Here, we explored interaction of four solution-state proteins (horse heart cytochrome c, hen egg-white lysozyme, 3-heme c-type cytochrome PpcA from *Geobacter sulfurreducens*, 2-heme cyt c₄ from *Pseudomonas stutzeri*) with several cationic and anionic water-soluble derivatives of tetraphenylporphyrin. Combined small- and wide-angle X-ray scattering experiments revealed formation of multimers with a wide range of complex sizes. Thermodynamic interaction parameters and complex binding stoichiometries were established with isothermal calorimetry. Locations of porphyrin binding sites were determined with heteronuclear single quantum coherence (HSQC) and total correlation spectroscopy (TOCSY) NMRs. The obtained results demonstrate that multimerization of solution-state proteins by large water-soluble ligands appears to be a wide-spread phenomenon controlled by a delicate interplay of electrostatic and hydrophobic forces. Molecular level mapping of the binding sites allows us to build a theory explaining the size of the formed complexes and provides opportunities for targeted design and assembly of multi-subunit protein complexes.

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PLANT-BIOFUEL CELL HYBRID FOR ENERGY PRODUCTION

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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 EFFECTS OF ALUMINUM IONS ON INTERGRANULAR CORRSION IN AA5083

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Aluminum alloys (AA) of the 5xxx series are common in naval ships due to their low cost, high strength/weight ratio, and great uniform corrosion resistance. Unfortunately, localized corrosion known as intergranular corrosion (IGC) still occurs. When the alloy corrodes, aluminum ions release into the solution the metal is sitting in and this research is aimed to determine if those ions affect the corrosion rate. Initially, AA5083 was tested using cathodic polarization scans in which the Potential (V_{SCE}) vs. the current density (A/cm^2) behavior was determined. From these scans, the diffusion limited current density (DLCD) could be evaluated. The greater the current required for the DLCD to begin, the greater the corrosion rate was. From these initial scans, aluminum ions did not seem to have a direct effect on the corrosion rate, but rather the pH and chloride ions associated with the aluminum chloride seemed to be important. After seeing no effect of the aluminum ions, 100-hour long bulk exposures were conducted in order to physically see the corrosion. The bulk experiments had constant chloride ion concentrations as well as potassium persulfate, but three had no aluminum and varying pH were used. The other three immersions had aluminum ions, two of the exposure contained same concentration (0.022M) as each other, but one had the same pH as the exposure with the greater aluminum concentration (0.1M). From these exposures, it was clear that the exposure with the greater aluminum concentration caused much more damage than any other exposure. It was also observed that the greater the aluminum ion concentration, the more initiation sites occurred. Additionally, electrochemical impedance spectroscopy tests were conducted. These experiments helped prove that the presence of aluminum ions causes AA5083 to corrode at a much faster rate. Hopefully future experiments can explain the cause of aluminum ions negative effects.

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EXPANDING STUDENT ACCESS TO INSTRUMENTATION THROUGH THE INCORPORATION OF PORTABLE INFRARED SPECTROSCOPY INTO THE CURRICULUM

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Hands-on use of modern instrumentation is an integral part of scientific education and research. Infrared (IR) spectroscopy is ideally suited for independent student users because of the ease of use of the instrument, as well as the wealth of quantitative and qualitative information the instrument can provide. This research project focused on the development of laboratory activities for quantitative analysis, instrumental analysis, and quantum mechanics lab using a portable infrared spectrometer. Analysis of ethanol samples was the focus of the quantitative analysis laboratory activity. Students measure the percent ethanol concentration in a range of alcoholic beverage samples using a standard curve they develop. Matrix components within select alcoholic beverages expose student to the idea of interferences within samples. Polymer chemistry was the focus of the instrumental analysis laboratory activity developed. Students use the modeling program Gaussian to predict polymer IR spectra. The IR spectra of the polymers are then collected and compared to the calculated frequencies from the Gaussian simulation. Finally, in Quantum Mechanics, the students look at the Ro-vibrational spectra of HCl and DCl. Using the data obtained from the IR, the students build the equation for the transition energies.

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ENHANCING UNDERGRADUATE EDUCATION WITH RAMAN, INFRARED, AND ULTRAVIOLET SPECTROSCOPY

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Undergraduate students often find gaps in their experience due to a lack of availability of analytical equipment. The National Science Foundation's Improving Undergraduate STEM Education program provides opportunities to enhance undergraduate student learning.

Incorporating Raman spectroscopy into physics and geology labs gives students valuable experience with technology that allows them to relate to their lessons from an interdisciplinary perspective. A lab utilizing the Raman spectrometer was developed for the non-majors Geology class. This lab uses the spectrometer to identify similar appearing rock samples. For Physics (science majors), the Raman spectrometer was used to identify the double and triple bonds in 1-hexene and 1-hexyne and relate the bond strength to the spring constant in a simple harmonic motion lab. These labs provide valuable interdisciplinary education opportunities that previously were unavailable.

Bringing Infrared Spectroscopy (IR) into introductory chemistry classes allows students to begin gaining experience with an advanced analytical tool. Ultraviolet spectroscopy adds an additional analytical tool to the chemistry classroom, allowing for further analysis and identification of products. The use of these advanced instruments better prepares them for upper-level classes. General chemistry classes will be introduced to Infrared spectroscopy through an identification of alcohols lab. Ultraviolet spectroscopy is used in organic chemistry to verify the successful synthesis of diphenylacetone. The Raman spectrometer, with its ability to identify symmetric triple bonds, allows verification of successful synthesis of diphenylacetylene, which was previously impossible with the IR.

Analyzing, re-writing, or creating labs to take advantage of new analytical equipment allows the students to learn about these outstanding analytical tools and prepare them for upper-level education. It also allows for non-majors students to have the experience of using and learning about advanced analytical equipment, gaining more insight into the tools used in the scientific community.

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).

ATTENUATED TOTAL REFLECTANCE-INFRARED SPECTROSCOPY SHEDS LIGHT ON A FORENSIC MYSTERY IN CENTRAL PARK

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In the North Woods of Central Park, a hard, dark, shiny material was found adhered to the bark of one of the park's trees. The material was unidentifiable, and therefore a sample of bark that contained the material, as well as a control sample of the bark without the material were extracted. The samples were then analyzed using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy to identify and compare the chemical components of the samples. The resulting spectra were dramatically different, with several peaks only present in the sample with the unknown black substance; the most significant of these peaks represented alkene/aromatic C=C (3010 cm^{-1}), carbonyl C=O (1738 cm^{-1}), and ester (1455 cm^{-1}) functionalities. These functional groups did not appear in the control bark, which displayed characteristic cellulose components, including alkyl C-H (2924 cm^{-1} , 2852 cm^{-1}) and ether C-O (1075 cm^{-1}) bonds. The spectra were compared against a library of standards using linear combination fitting. The best match (78%) was found between the unknown substance and the compound Emuldan hv52. Emuldan hv52 is also called 1-mono-acylglycerol, and is composed of 1-glyceryl, acyl, and unsaturated fatty acid functional groups, which is consistent with the functionalities revealed in the black substance by IR spectroscopy. Emuldan hv52 is an emulsion agent primarily used to make water-based paints, leading to the conclusion that the substance on the tree was the dry remains of a black paint. This conclusion aligns with the chemical information obtained by IR analysis as well as the physical characteristics of the shiny black substance initially found on the Central Park tree. This preliminary study lays the groundwork for further exploration of the chemistry of art materials using ATR-FTIR spectroscopy.

SYNTHESIS OF AMIDE PENDANT RUTHENIUM HOST SYSTEMS

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Key bipyridine containing amide pendant ligands were synthesized and coordinated to ruthenium, a photoactive transition metal. This allows for ion binding studies to determine how efficient and selective a host is towards a specific ion. The photo activity of these host-guest systems can be probed using fluorescence titration studies. Ligands and host complexes were characterized by mass spectrometry and ^1H NMR spectroscopy.

SYNTHESES, CHARACTERIZATIONS, DNA-BINDING, AND CYTOTOXICITY STUDIES OF (TRICARBONYL)RHENIUM(I)(POLYPYRIDINE) MEFENAMATO AND TOLFENAMATO COMPLEXES

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Transition-metal complexes of non-steroidal anti-inflammatory drugs (NSAIDs) are of importance because they possess interesting biological properties including anti-cancer and cancer prevention. Our lab is engaged in the synthesis of organorhenium complexes of a variety of NSAIDs. The objective of this study is to determine the cytotoxicity of tricarbonylrhenium(I)-polypyridyl complexes of mefenamic acid and tolfenamic acid against U-937 lymphoma cells.

NSAIDs are commonly used as pain relievers and fever reduction; however, in cases of prolonged use of NSAIDs, there has been evidence of prevention of some cancers, and other anti cancer properties. Metal complexes of NSAIDs have shown enhanced anticancer properties, by way of polypyridyl ligands used in this study.

We have found that the reactions of tricarbonylrhenium(I)-polypyridyl pentylcarbonato complexes with stoichiometric amounts of mefenamic or tolfenamic acid yield the corresponding mefenamato and tolfenamato complexes, respectively. The complexes were characterized spectroscopically and in some cases crystallographically. They are relatively stable at pH 7.2. The UV titrations of the compounds with CT-DNA indicate that many of these complexes bind to DNA intercalatively. The cytotoxicity studies using MTT assay were completed to determine the cytotoxicity of the compounds against U-937 lymphoma cells. The IC-50 values (half maximal inhibitory concentration) were observed in the range of 2.5 through 5.0 μ M. Therefore, these compounds might find applications in the treatment of various cancers.

This study is supported by the National Institutes of General Medical Sciences of the National Institute of Health under Award Number R25GM058904.

THE SYNTHESIS PATHWAY FOR MOLYBDENUM PYRANOPTERIN DITHIOLENE MODEL COMPLEXES

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The transition metal molybdenum (Mo) plays an important role in living organisms which depend on the ubiquitous molybdenum-containing enzymes for many critical biochemical reactions. Despite this importance to all life, there is little understanding about the role played by molybdopterin, the special ligand on Mo. The goal of this research is to create several pterin precursor molecules, 2-pivaloyl-6-chloropterin and BMOPP, that will be further developed into model complexes for the molybdenum site of the enzymes. Our research group has already successfully synthesized [TEA][Tp*Mo(X)pterin-C(CH₃)₂R-dithiolene], where TEA stands for tetraethylammonium and Tp* is tris(3,5-dimethylpyrazolyl)hydroborate, X is either oxygen or sulfur atom and we have investigated the molybdenum pterin dithiolene chemistry. The quality of the precursor compounds that are the target molecules of this research will be characterized using techniques including NMR, IR, GC-MS, etc. Apart from focusing on reproducing results to examine the complex, we also seek to optimize our current procedures and improve resultant materials.

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LUMINESCENT METAL-ORGANIC FRAMEWORKS CONTAINING OSMIUM AND RHENIUM CARBONYL METAL COMPLEXES: FRAMEWORK STRUCTURE AND SOLVENT DEPENDENT PHOTOPHYSICAL PROPERTIES

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Metal-organic frameworks (MOFs) have been utilized in the fields of catalysis, analytical sensing, and small molecule storage due to their crystalline, porous structure. MOFs are three-dimensional structures formed by the coordination of organic linkers with a central metal ion to form an extended structure. Luminescent metal-organic frameworks (LMOFs) can be created by incorporating a luminescent transition metal complex (TMC) into the framework structure via the organic ligands on the transition metal complex. The LMOF should retain the luminescent properties of the transition metal complex and allow for interactions between analytes of interest and TMC within the pores of the MOF. In the current work, small amounts of luminescent transition metal complexes are incorporated into the known MOF, Zn₃(bpdc)6(bpy) (where bpdc = biphenyldicarboxylate and bpy = 4,4'-bipyridine). The transition metal complexes containing rhenium and osmium metal centers are being used to detect solvents of different polarity while coordinated within the MOF. [Re(CO)₃Cl(dcbpy)] and [Os(CO)₂Cl₂(dcbpy)] have been utilized for this research due to their large dipole moments within the excited state as demonstrated by anisotropy measurements performed on the complexes. The complexes in solution and in the LMOF show sensitivity to solvents of different polarities, which is detected by changes in emission wavelength. By preparing LMOFs, the necessity of dissolving the reporter molecule in the solvent is eliminated, and the range of solvents that can be measured is extended.

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RUTHENIUM TRIS-BIPYRIDINE CAGE COMPLEXES AS HOST SYSTEMS FOR ALKALI AND ALKALINE EARTH GUESTS

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Bipyridine tripodal ligands were synthesized in order to form ruthenium cage complexes, which function as host molecules for cationic guests (Na^+ , Cs^+ , Mg^{2+} , Ba^{2+}). The host properties of the ruthenium cage were investigated using liquid-liquid membrane ion transport studies and UV-visible and fluorescence titration studies, which were performed in order to determine the binding efficiency and selectivity of the hosts for the various cationic guests.

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DISSEMINATION OF BREAST CANCER

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Breast cancer is a leading cause of death in US women. Despite advances in early detection, diagnosis and treatment of breast cancer, metastasis and tumor recurrence due to resistance to both chemo and radiation therapy persist leading to continued morbidity and mortality. Several breast cancer studies have highlighted the role of cyclooxygenase-2 (COX-2) - a mediator of inflammation, produced by cancer cells and their interaction with the tumor microenvironment (TME) in breast cancer progression. The TME consists of stromal cells that include fibroblasts, macrophages, the extracellular matrix (ECM), and several secreted factors that contribute to aggressiveness of the cancer and resistance to therapy. Increase in the density of collagen 1 (Col1) fibers-a major structural component of the ECM in solid tumors and their alignment in tumors frequently result in increased invasion and metastasis of cancer cells. To understand the role of inflammation at the metastatic site (lungs), and establish a relationship between COX-2 and Col1 fibers, studies with COX-2 overexpressing triple negative human breast cancer cells (SUM-149-COX-2) were performed by injecting SUM-149-COX-2 cells through tail vein in SCID mice to compare Col1 fiber patterns in metastatic lesions formed by SUM-149_COX-2 cells with SUM-149 cells expressing empty vector control (SUM-149-EV). We isolated the lungs, processed the tissue, and imaged for Col1 fiber by second harmonic generation microscopy. COX-2 alone did not have a significant effect on Col1 in lung metastatic foci. However, there was a significant difference in fiber density and orientation between lung emboli and micro metastasis.

THE EFFECT OF OXYGEN CONCENTRATION ON MIRNA ABUNDANCE IN TPC-1 CELLS

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microRNAs (miRNAs) are key regulators of many cellular processes in cancer, from proliferation to apoptosis to guiding the response to hypoxia. Of note for the latter, cell cultures are typically maintained at atmospheric oxygen levels and may not reprise the in vivo condition. miRNAs are also considered to be potential diagnostic, prognostic, and treatment response biomarkers for a wide variety of cancers, including thyroid cancer, which has a growing incidence in the United States. Despite low mortality of some types of thyroid cancer, there remains a need for guidance in surgical decisions and for detection of recurrence or development of anaplastic disease. Additional investigations into miRNAs associated with thyroid cancer are required. In this project, we assessed production of several miRNAs in TPC-1 cells, a papillary thyroid cancer cell line, under atmospheric (20.95% oxygen) and hypoxic (1% oxygen) conditions. The specific miRNAs studied were miR-16, miR-221, and miR-146b. Small RNA (snRNA) U6 was used as a reference RNA.

Two T75 flasks were used to grow the cells in each condition. The 1% oxygen condition led to lower confluence, but RNA concentrations measured with NanoDrop were almost twice as much higher for this condition. Cells grown in 1% oxygen also had increased expression of miR-16 by 15% and 18%. For miR-146b expression, a flask in the 1% oxygen condition had a decrease of 3%, while another flask had an increase of 14%; these expression differences could potentially be in the range of error for the qPCR assays. Overall, it is suggested that the 1% oxygen condition can lead to a higher abundance of miRNA, specifically miR-16. However, more work is needed with more samples to validate this conclusion and to better understand how different oxygen conditions can lead to different miRNA expression.

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DIFFERENTIAL COHESION ANALYSIS OF HUMAN ERYTHROLEUKEMIA CELLS IN NORMAL AND VIRUS-TRANSFORMED HUMAN FIBROBLAST CELLS

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The study of human erythroleukemia (HEL) cells is thought to play a major role in establishing effective treatments for cancer. In a stimulated *in vivo* environment, the objective was to examine the attachment of HEL cells when exposed to normal human lung fibroblasts (WI-38) as well as their virus-transformed counterparts (VA13a). Such exposure between the adherent fibroblast cells and the cancer cells growing in suspension could provide insight regarding the formation of tumors and/or tumor metastasis. In an attempt to mimic an *in vivo* environment, suspended HEL cells were added to WI-38 and to VA13a cells. The HEL cells bound to both cell lines, and the bound cells could not be removed by mechanical agitation. These observations would suggest a strong bond between the two cell types.

EFFICACY OF ANTISENSE GUIDED PRE-MRNA *TRANS*-SPLICING ON EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION IN HUMAN GLIOBLASTOMA CELLS

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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. GBM tumors, protected by the blood brain barrier grow aggressively extending into healthy tissue. 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 sequestering extracellular epidermal growth factor and inhibit activation of EGFR. 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 will be 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 therapeutics may be an effective strategy in the treatment of GBM.

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MODELING TUMOR HETEROGENEITY IN PANCREATIC DUCTAL ADENOCARCINOMAS USING 3D STROMAL-ORGANOID CO-CULTURES

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal form of pancreatic cancer with limited treatment options. Additionally, PDAC patients are often diagnosed with late-stage disease, thus lowering their chances to obtain substantial treatments with therapeutics or surgery. Therefore, new therapies must be developed in order to increase survival and treatment options for patients in need. In order to address these limitations, research models such as 3D organoid cultures are being used as an alternative to traditional models such as genetically engineered murine models and monolayer cell lines. Organoid cultures, a type of 3D culture system, contain organ-like units capable of self-organization, mimicking the organ environment. We combined traditional and organoid cell culture methods in order to study the interactions of PDAC cells and pancreatic stellate cells (PSCs), a resident fibroblast population in the pancreas. Studies suggest that PSCs act as an immune surveillance barrier for drug delivery. Thus, understanding the interactions between these cell types is crucial for development of drug therapies for patients. We hypothesized that the development of a PDAC organoid cell co-culture with primary PSCs would provide further insight on the specific metabolites which contribute to tumorigenesis and/or metastasis. In order to examine this concept, we used a transgenic PDAC tumor mouse model containing Kras and p53 mutations (LSL-KrasG12D, p53 fl/fl, Pdx-1-Cre, or “KP-/-C”) to isolate tumor organoids. Tumor cells were resuspended in matrigel and plated in complex organoid media. Murine DNA samples were collected for genotyping and used for PCR analysis to identify mice with the necessary mutations. Results showed that PDAC tumor organoids were highly responsive to co-cultures with PSCs, and displayed increase in cellular proliferation when exposed to metabolites alanine and pyruvate. Future studies will work to characterize metabolites and co-culture PDAC with endogenous fibroblasts in order to further understand tumor heterogeneity.

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WHAT IS THE TRUE ENZYME KINETICS INSIDE THE CELL?

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Cells are crowded with proteins, polynucleic acids, polysaccharides and lipids. Such macromolecular crowding exerts significant effect on the biomolecular interactions. Nevertheless, these interactions are often studied in uncrowded media outside the cell. Several studies have measured the crowding effect on association events such as enzymatic reactions, however the magnitude of such effect is un-predictable and appears to vary from one enzyme-substrate pair to another. For instance, the magnitude of crowding effect on K_m , an enzyme kinetic parameter that describes the binding tightness between an enzyme and its substrate, varies from 30% decrease to 500% increase. Hence, much more research is required to fully profile the enzymes in crowded solution, a closer mimic to the cellular environment.

Our research focuses on an exemplar serine protease, trypsin. We found that in crowded media, trypsin binds to both the substrate and a competitive inhibitor, benzamine, with elevated affinity. The biological implication is that the true kinetics of trypsin in cellular context may involve a tighter binding to its substrate and a slower turnover rate than what we currently believe.

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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. Its function is highly conserved and strictly regulated in signal-producing and -receiving cells. Trafficking of Wingless (Wg), the prototypical *Drosophila* Wnt ligand, through the secretory pathway requires the chaperone Wntless (Wls) in receiving cells. Recent immunoprecipitations studies have shown DWls (*Drosophila* Wls) oligomerization is necessary for Wg binding, whereas Wg binding is not requisite for DWls oligomerization. Fluorescence correlation spectroscopy (FCS), was utilized to examine DWls oligomerization and its role in Wg trafficking and release. FCS measures fluctuation in the concentration of fluorescently tagged molecules in an extremely small observation volume in live cells, allowing real time analysis of what may be a dynamic process of DWls oligomerization. FCS requires a small number of fluorescent molecules within the one-femtoliter observation volume to differentiate the spike associated with monomers or dimers entering or exiting the area. Therefore, low expression is ideal. Using the UAS-gal4 expression system, C-terminal GFP tagged DWls, as well as monomeric and dimeric controls were expressed in transfected S2R+ cells. Under these conditions FCS showed Dwls is a monomer at the plasma membrane and UAS-gal4 yielded excessive expression. The goal of this study is to identify a lower level expression system, determine if high-level endogenous DWls expression in S2R+ cells accounts for the plasma membrane monomer result, and examine whether DWls oligomerization is dynamic within cells. To achieve these goals, the heat shock promoter was tested in S2R+ cells and S1 cells with 7-fold lower DWls expression were examined. Finally, efforts were made to take FCS measurements at endoplasmic reticulum in S2R+ cells to look at dynamic DWls oligomer formation. Optimization of these parameters will enable characterization of dynamic DWls oligomerization and its role in Wg secretion within living cells.

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INVESTIGATION INTO ALLOSTERIC EFFECTS OF UFM1

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Ubiquitin fold modifier 1 (UFM1) is a ubiquitin-like protein found in eukaryotic organisms which plays a crucial role in cell cycle regulation, signal transduction, and more. A three step enzymatic pathway composed of enzymes E1, E2, and E3 is utilized to conjugate UFM1 to its target protein. UFM1 is known to interact with the adenylation domain of the E1 protein. However, it is also hypothesized that a separate portion of the E1 enzyme binds to UFM1 and allosterically regulates the binding to the site in the adenylation domain. Molecular dynamics simulations were run on UFM1 alone and UFM1 with the UBA5-interacting motif peptide non-covalently bound to support the proposed allosteric mechanism of UFM1 binding to UBA5. Groups of residues around P14 and A45 were shown to have less movement and lower RMSD values when the peptide was bound to UFM1. Neither of these amino acids are directly involved in peptide binding supporting allosteric effects. Creation of a dynamic cross correlation matrix showed linked movement between the peptide binding site and these regions, and the sites are highly conserved as shown by ConSurf and STRESS analysis. These data provide insight into a novel mechanism of substrate binding within the ubiquitin field and will serve as the basis for experimental studies to verify this mechanism. Future work includes analysis and comparison of dynamics on other ubiquitin like molecules.

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HIGH-THROUGHPUT, FLUORESCENT ANALYSIS OF REACTIVE OXYGEN SPECIES IN *C. ELEGANS* AFTER KNOCKDOWN OF *MRCK-1*

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Reactive oxygen species (ROS) normally produced in living organisms can generate oxidative stress and contribute to cell damage associated with neurodegenerative diseases, such as Alzheimer's and Parkinson's. Studying responses to oxidative stress in model organisms, like the nematode worm *Caenorhabditis elegans*, may provide insight into basic underlying cellular mechanisms shared with humans. MRCK-1 (myotonic dystrophy kinase-related Cdc42 binding kinase), a highly conserved intracellular kinase, is activated by a G-protein that contains a redox sensitive motif. Previous studies have shown that MRCK-1 regulates cell polarity and cytoskeleton reorganization, particularly during cell division and in response to cellular damage. To determine if MRCK-1 plays a role in other cellular defensive responses, we are testing if reduced levels of MRCK-1 result in the production of more ROS under stressed conditions. To do this, we utilize the cell permeable, carboxymethyl dichlorodihydrofluorescein derivative, CM-H₂DCFDA, a fluorescent ROS indicator, which can be quantified through the use of a SpectraMax microplate reader. Oxidative stress conditions will be induced through heat shock at 35°C, or chemical means by treating the worms with hydrogen peroxide (H₂O₂), in the presence of CM-H₂DCFDA. Two RNA interference (RNAi) constructs, *mrck-m* and *mrck-s*, will be expressed independently in bacteria. Upon ingestion by the worms, the plasmid DNA will be transcribed into dsRNA and used to target *mrck-1* via RNAi. Subsequent *mrck-1* mRNA destruction will reduce the amount of MRCK-1 in the cells, potentially affecting ROS levels. Exploring the levels of ROS in RNAi-treated *C. elegans* may give further insight of the cellular role of MRCK-1 both in worms and in humans.

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SOX17 TRANSGENIC MICE REVEAL FUNCTIONAL SUPPRESSION OF BETA-CATENIN IN NEUROPROTECTION

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Myelin damage and oligodendrocyte loss results in neurological symptoms of Multiple Sclerosis. Oligodendrocytes are myelin-producing cells in the CNS white matter(WM), whose membranes ensheath neuronal axons for rapid neurotransmission. Strategies for oligodendrocyte replacement will improve myelin repair. The transcription factor Sox-17 promotes differentiation of oligodendrocyte progenitor cells (OPCs) into myelinating oligodendrocytes. Transgenic mice overexpressing Sox-17(CNPSox17) show enhanced regeneration of cells and protection from damage in WM lesions. However, the signaling mechanism(s) which promote WM repair is unknown. Wnt/ β -Catenin signaling inhibits differentiation of OPCs, supported by reports of high β -Catenin levels in demyelinating lesions. Sox-17 has been shown to inhibit the Wnt/ β -Catenin pathway and increase WM levels of Hedgehog mediator Gli2. OPCs from CNPSox-17 mice show increased Hedgehog co-receptor clustering, suggesting Gli2 activation. We hypothesize that Sox-17 promotes OPC differentiation through Gli2 to antagonize β -Catenin signaling. To test this, we compared experimental WM lesions in CNPSox-17 and wild-type mice. Lysophosphatidylcholine (Lyso), which disrupts myelin sheaths, was surgically injected into the brain WM to generate a demyelinated lesion and induce spontaneous repair. Using immunohistochemistry, we observed that Lyso increases the number of cells expressing activated β -Catenin in wild type mice, but these did not increase in CNPSox-17 mice. To investigate possible antagonism between the transcription factors Gli2 and β -Catenin, we conducted transfection assays in oligodendroglioma cells. Using the β -Catenin responsive reporter TOPFlash, and a GLI-responsive reporter GliBSLuc, we observed that β -Catenin activated only Topflash, not GliBSLuc. Gli2deltaN (active Gli2) functioned as an activator on GliBSLuc, but as a repressor on Topflash. In contrast, Gli2FL (unactivated Gli2) neither suppressed nor activated Gli or Topflash. This indicates that Gli2 activation is necessary for repressing β -Catenin. We conclude that Sox-17 increases Gli2 activation, resulting in lower activation levels of β -Catenin signaling. This contributes to enhanced regeneration of oligodendrocytes, thus producing a protective effect in disease.

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GLOMERULAR DAMAGE IN THE PROGRESSION OF TYPE 2 DIABETES IN BBZDR/WOR RATS

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Both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus can lead to diabetic nephropathy. During diabetic nephropathy, the glomeruli become scarred, which alters the structure and function of the kidney. The glomeruli can be studied to understand the effects that the progression of diabetes has on the kidney. BBZDR/Wor rats are a new strain of rat that mimics the progression of type 2 diabetes in humans. In this study, trichrome-stained and monoclonal antibody-stained kidney sections were prepared from diabetic and non-diabetic rats ranging in age from 24 days to 13 months. Analysis of the glomerular changes was performed using semi-quantitative measurements of glomerular diameters. Transglutaminase 2, a ubiquitous protein that catalyzes the formation of high molecular weight cross-linked proteins was also studied using a monoclonal antibody. The results indicate that diabetic animals have increased glomerular diameters. Non-parametric t-tests were performed with p-values ≤ 0.05 . Analysis of the monoclonal antibody-stained kidney sections resulted in the conclusion that the expression of transglutaminase 2 around the glomerular capsule becomes more pronounced in diabetic animals as the disease progresses.

CANONICAL WNT/ β -CATENIN SIGNALING IN THE DEVELOPING EYE

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Canonical Wnt/ β -catenin signaling plays an important role during eye development. In the cornea, this signaling participates in corneal stratification and proliferation of limbal stem cells. During lens development, activation of canonical Wnt signaling is required to suppress ectopic lens formation in the periocular ectoderm and aberrant lens activity of Wnt leads to abnormal lens development. In the retina, Wnt activity is linked to the differentiation of neural retinal precursor cells.

It is difficult to evaluate canonical Wnt signaling at the cellular level since its downstream mediator, β -catenin, has dual intracellular functions. It is associated with both cell-cell adhesion, in the cadherin adhesion complex, and gene transcription. After canonical Wnt activation, β -catenin translocates into the nucleus, binds with T cell specific transcription factor/lymphoid enhancer-binding factor 4 (TCF4/Lef), and drives transcription. To study canonical Wnt signaling, it is crucial to differentiate β -catenin from two functionality pools, which can be accomplished by the usage of Wnt reporter mice.

Former canonical Wnt reporter mice that utilized reporter genes, like LacZ, were unable to indicate cell specific activation. Thus, we used a new Wnt reporter mouse strain which can reveal canonical Wnt activation on a single cell basis, by placing TCF/Lef responsive elements and an hsp68 minimal promoter in front of green fluorescent protein (GFP). By visualizing GFP expression via immunofluorescence staining and confocal microscopy, we can more accurately evaluate canonical Wnt activity during eye development. From all selected time points between embryonic day 12.5 (E12.5) to postnatal day 13 (P13), GFP expression is consistently visible in the lens epithelium. GFP expression at E12.5 occurs in the optic cup and developing optic nerve sheath, and at E14.5 occurs in the developing retinal pigmented epithelium. At E16.5, GFP expression appears in the corneal endothelium and the retinal ganglion cell region and is maintained through P13.

This project was made possible through funding by the Charles Peter White Fellowship, the Ethel and Donald Hofmann Research Fund, as well as the National Institute of General Medical Sciences - NIGMS (8 P20 GM103446-13) from the National Institutes of Health and EY12221 from the National Eye Institute.

CHARACTERIZATION AND CATEGORIZATION OF IMMORTALIZED MIXED ASTROCYTE CELL LINES

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Neuroinflammation is understood to be involved in multiple neurodegenerative diseases but its biochemical process within the human brain is yet to be fully grasped. The exposition of the mechanisms involved in glial activation is a pivotal step in improving our understanding, but this requires the generation of a stable and valid model system in which to analyze this progression. Cells were isolated from one-day-old mice cortices then virally infected in prior experiments to generate two immortalized mice glial cell lines, J2B9 and J2B10. In order to determine the growth characteristics and also confirm the identities of our cell lines as mixed microglial/astrocyte cell populations, this project aims to characterize the cell lines via neutral red assays, cell counting assays, cell doubling assays, and antibody immunofluorescence staining. Our results suggest that the doubling time of both populations are stable across multiple weeks in culture. Both cell lines stained positive for GFAP while J2B9 also stained positive for Integrin alpha M. Though further research is required to fully confirm the complete characteristics of our cells, our results support that we have generated stable mixed astrocyte cell lines.

CLONING RFP INTO *PLECTRANTHUS SCUTELLARIOIDES* (COLEUS)

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We aim to generate a transgenic *Plectranthus scutellarioides*, expressing RFP in an effort to employ genetic modification technology into an art exhibit. In this experiment we employ the floral dip technique, using GV3101-pMP90RK *Agrobacterium tumefaciens*. The plasmid containing the mCherry gene was electroporated into *Agrobacterium tumefaciens*, and made into a broth to infect our target organism. *Plectranthus scutellarioides*, a dichotomous plant, is not a typical model system for this procedure, so a procedure known to work in another dichotomous plant, flax, was employed in this endeavour. The mCherry gene has an excitation maxima at 587 nm, and an emission maxima at 610 nm, which will be utilized using LED lights specific to this wavelength. After exposure to the agrobacterium, the target organism will be tested for fluorescence, and then given to the artist for development into an art exhibit.

Funding provided by the Mount Saint Mary's School of Natural Science and Mathematics

PROBING INTRAMOLECULAR INTERACTIONS IN THE HIV-1 5'-LEADER DIMERIC CONFORMATION

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Currently, over 36 million people were infected with human immunodeficiency virus (HIV), which can go on to cause acquired immunodeficiency syndrome (AIDS), a disease associated with 1.1 million deaths in 2015. The late phase of HIV-1 replication cycle is characterized by the equilibrium between the monomer and dimer conformations of the 5' Leader (5'-L) in the RNA genome. The structures of these RNA conformations have never been fully elucidated with native sequences. Our model predicts that in the dimeric conformation, the AUG hairpin unfolds, and binds to the Unique 5 region (U5). This U5:AUG interaction stabilizes the dimeric conformation, which is essential for virus reproduction, binding to nucleocapsid and acting as the genome for a new virion. We synthesized a mutated 5'-L construct with modifications that locked it into the dimeric conformation, but prevented intermolecular dimerization. We called this construct locked dimer (LD). However, using nuclear magnetic resonance (NMR) spectroscopy is particularly challenging for large RNAs such as the 5'-L or LD, which are over 300 nucleotides. We overcame that obstacle by probing a small oligo control which mimics the chemical environment of the U5:AUG interaction, and is easily assigned. We then compared the assigned spectrum from this oligo to the LD construct and the native 5'-L, and found that the signals overlapped, providing the first evidence of the existence of the U5:AUG interaction in a native dimeric conformation. We plan to further characterize this interaction, and characterize the dynamics of the kissing dimer (hairpin loop interaction only) versus extended dimer (hairpin loop and stem interaction).

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CCAAT/ENHANCER BINDING PROTEINS AS POSSIBLE DOWNSTREAM TARGET OF HMGB1 IN NEUROINFLAMMATORY RESPONSE

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Glial cell activation plays a major role in the inflammatory pathway of the central nervous system. High Mobility Group Box protein 1 (HMGB1) is typically found as a non-histone nuclear protein. During a necrotic event, HMGB1 is released into the extracellular matrix to initiate inflammation. HMGB1 then acts as a cytokine or signaling molecule for the activation of immune and inflammatory responses. CCAAT/enhancer binding proteins have also been demonstrated to be involved in the neuroinflammatory pathway. Two C/EBP family members, C/EBP β and C/EBP δ , have been especially noted as transcription factors that are involved in neuroinflammation. C/EBP β is a transcription factor found abundantly in the CNS where it regulates expression of various neurotransmitter and pro-inflammatory genes. CEBP/ δ is also a transcription factor that regulates genes linked to immune and inflammatory responses. C/EBP δ is also found at increased levels in patients possessing neurodegenerative diseases. Because of the demonstrated roles that C/EBP β and C/EBP δ have in the neuroinflammatory pathway, and because HMGB1 is seen as an activator of inflammation, we hypothesize that these C/EBP family members are downstream effectors of HMGB1 signaling. Using CRISPR technology, we will create knockout cell lines for each of the C/EBPs in order to confirm that they are downstream effectors to HMGB1. Other members of our lab are also creating knockout lines for receptors of HMGB1 (TLR-2, TLR-4, and RAGE) in order to determine which receptor, or combination of receptors, is being used to send the signal.

USING A CRISPR-CAS9 KNOCKOUT TO EVALUATE THE ROLE OF FURIN IN THE INTOXICATION PATHWAY OF *PSEUDOMONAS* EXOTOXIN A

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Derivatives of *Pseudomonas* exotoxin A (PE) known as recombinant immunotoxins (RITs) are chimeric proteins being investigated as potential cancer therapies to target and kill cancer cells. RITs gain access to target cells via receptor mediated endocytosis, and traffic through the trans-Golgi network to the endoplasmic reticulum. From there, RITs gain access to the cytoplasm where they inhibit translation elongation factor 2 (EF2) and halt protein synthesis. It is known that the proprotein convertase furin is involved in the pathway, but it is not clear whether furin activates RITs through cleavage, assists intracellular trafficking, or has a combination of both functions.

This project aims to use the CRISPR/Cas9 methodology to create a furin deficient cell line in order to explore the role of furin in the intoxication pathway of RITs. Thus far, a targeting guide RNA has been used to direct the Cas9 endonuclease to the furin gene in HEK293 cells. Endogenous repair mechanisms in the cell corrected the break using non-homologous end joining, which resulted in frameshift mutations in the furin gene and a nonsense product. We plan to introduce mutant furin variants with restricted function into the furin-deficient cells to characterize the role of furin in the RIT intoxication pathway.

I would like to thank the Fisher College of Science and Mathematics (FCSM) as well as the Towson Office of Undergraduate Research (TOUR) for their financial support of this project. I would also like to thank all of the members of Dr. Weldon's lab for their help with this research.

DEVELOPMENT OF A NOVEL SYBR GREEN-BASED REAL-TIME PCR ASSAY FOR QUANTIFYING CCK2R VERSUS CCK2_{i4sv}R IN HUMAN PANCREATIC CANCER CELLS

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Pancreatic adenocarcinoma is an aggressive cancer with low life expectancy and an overall five-year survival of 7.7%. The gastrointestinal hormone, gastrin, and its primary receptor, CCK2R, have been implicated as drivers of tumor growth. Additionally, a receptor splice variant resulting from the abnormal retention of the 4th intron, CCK2_{i4sv}R, results in increased intracellular signaling and greater tumor growth relative to properly-spliced CCK2R. To accurately quantify the relative abundance of each transcript variant in cancer cells and ascertain the causative factors behind intron retention, a reliable real-time PCR assay is essential. Commercially-available Taqman[®] primer/probe sets do not distinguish between the splice variants, and end-point PCR, albeit providing the desired specificity, lacks quantitative accuracy. We report the development of a novel SYBR Green-based Real-Time RT-PCR protocol that allows measurement of total receptor RNA in parallel with selective quantification of CCK2R versus CCK2_{i4sv}R (PPIA, internal calibrator). The forward primer was designed to overlap the intron 3 splice boundary to prevent amplification of genomic DNA or unprocessed RNA. Each primer set was optimized for an amplicon size between 165-199 bp, with melting temperatures 59.1-61.6°C. Specificity against genomic DNA and specificity for each splice variant were confirmed using a panel of plasmid constructs. Optimizations for annealing temperature and primer concentration confirmed ideal amplification at 60°C, demonstrated no primer-dimer interference, and exhibited melt curves with single-peak amplicons. In RNA samples from a panel of human pancreatic cancer cell lines, primer sets for total-receptor and PPIA were tested in parallel with commercial Taqman[®] assays. Both sets exhibited excellent sensitivity, crossing threshold at an average 1.97 (PPIA) and 2.40 (total-receptor) cycles earlier than commercial Taqman[®] assays. Together, these primer sets represent a sensitive and cost-effective means of quantifying CCK2R vs. CCK2_{i4sv}R mRNA in pancreatic cancer cells.

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FLUORESCENCE IMAGE ANALYSIS VIA THRESHOLD ENHANCED ALTERNATIVE MORPHOLOGY GUIDED IMAGE SEGMENTATION

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The recent push towards high-throughput high-content screening has created a growing need for fast, accurate, and automated algorithms for cellular content analysis. However, most image segmentation algorithms pose unique challenges for studying cellular components, including fluorescently labeled proteins. Furthermore, no algorithm is able to correctly segment cells or the cellular compartments as well as visual examination. We are developing such an algorithm to give any user the ability to perform reliable single cell fluorescent microscopy analysis to sample the number, sizes, and areas of the tagged proteins. Our semi-autonomous approach is flexible to account for variance in fluorescent intensity, stochastic cell-to-cell differences, and region-to-region variations introduced during image acquisition processes. The user defined thresholding and geometric morphology parameters enhance the verification and correction of single cell analysis. Thus, the design and the flexibility of the algorithm can be adjusted with ease to fit the requirements of the live cell assays and the fixed cell assays. With our algorithm (version 1.0), we have established to date successful analysis of multiple assays and even different image sets. Further studies must be performed to establish a merit of performance for the algorithm. Images gathered via low magnification objective lenses will be used to establish our algorithm's usefulness for high-throughput high-content work.

ROLE OF TRYPTOPHAN RESIDUES IN UV SENSITIVE OPSIN FUSION PROTEINS

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RhoPDE is a novel UV sensitive rhodopsin-phosphodiesterase fusion protein identified in the genome of the cyanobacterium *Salpingoeca rosetta*, which degrades the phosphodiester bond in the second messenger cGMP. This naturally occurring enzyme has potential for fine-tuned control of cyclic nucleotide signaling pathways, making it an advantageous tool for optogenetics. The microbial transmembrane opsin domain binds retinal making a visible pigment at 490nm, typical of classic opsins. Due to the connection of the phosphodiesterase (PDE) to this opsin domain, it is expected that the activity of PDE would be light-dependent. However, exposure of RhoPDE to visible light has no effect on the PDE, which is constitutively active. RhoPDE exhibits an uncharacteristically high UV absorbance at 280nm which has been localized to the N-terminus of the protein. This phenomenon may be the result of tryptophan residues in this region. This project seeks to determine the resulting functional and spectral properties of RhoPDE upon mutation of all the tryptophans in the UV-sensitive region of the protein. Eight W to F point mutants of RhoPDE were generated via site-directed mutagenesis, expressed in HEK 293 cells, purified, and tested via UV-Vis spectroscopy to determine spectral properties. Absorbance spectroscopy indicated that many of the mutants bound retinal well producing WT spectra, while others did not express as well. One mutant in particular, W63F, showed a double peak in the visible region. All eight mutants retained the abnormally high UV absorbance comparable to that of RhoPDE WT.

CREATION AND CHARACTERIZATION OF RUBRERYTHRIN AND SYMERYTHRIN MODEL PROTEINS

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The ferritin-like superfamily (FLSF) is a class of 4-helix bundle proteins that contain a diiron active site and participate in important biochemical pathways, including fatty-acid desaturation and deoxyribonucleotide formation. The canonical FLSF sequence contains four carboxylate and two histidine metal-binding ligands in the active site. Rubrerythrins (Rbr) and symerythrins (Sym), however, contain one and two additional carboxylate residues, respectively, in their active sites. Interestingly, these proteins exhibit enhanced reactivity with hydrogen peroxide relative to other members of the FLSF, but the correlation between the additional carboxylate residues and the altered functionality is currently not well understood.

To investigate this phenomenon, we created models of Rbr and Sym based on G4DFsc, a small, *de novo*-designed 4-helix bundle protein that mimics the canonical structure and reactivity of FLSF enzymes. Aspartate (D) or glutamate (E) residues were introduced at positions G14, G43, and/or G47 to generate Rbr- and Sym-like active sites within the G4DFsc bundle. The structural and catalytic properties of these systems were investigated using metal-binding, protein-folding, and reactivity assays at pH 7 and pH 7.5. While data show that the double mutants exhibit the weakest metal-binding capacity at pH 7, these proteins also show greater rates of 4-aminophenol oxidation than the original G4DFsc protein. The G47D variant shows the greatest catalytic capacity, increasing rates of 4-aminophenol oxidation by over eight times compared to G4DFsc. G14E and G47E and G14E/G47D demonstrate significantly slower reaction rates, the slowest working at a tenth the rate of G4DFsc. These results provide insight into how particular carboxylate residues in the G4DFsc active site affect its ability to react with dioxygen and hydrogen peroxide.

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RIBOSOMAL PROTEIN BINDING DURING RIBOSOMAL RNA MATURATION

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Genetic and biochemical studies of the ribosome show that ribosome formation is a complex process of synthesis and assembly of individual components, namely ribosomal proteins (rp) and ribosomal RNA (rRNA). However, the exact timing of individual rp addition to ribosome assembly RNA intermediates is not fully understood. This project was designed to develop a method for determining these binding patterns without interrupting ribosome biogenesis. Synthesis of individual tagged rp's were induced and co-immunoprecipitated, along with the specific rRNA segment to which the rp binds. RNA was extracted from the immune precipitate and loaded on slot blots. The blots were then probed with segment-specific oligo-probes loosely corresponding to rRNA cleavage sites (a process we termed "slot-northern"). When a signal showed the slot-northern indicating a probe had bound to the rp/rRNA complex, it was viewed against the known specific probe-segments of the binding probe and used to deduce the location of binding for that individual rp onto the maturing rRNA. By gaining this information about ribosomal proteins, we may better understand the ribosome in its entirety and add to the foundational understanding of one of the most important complexes in life.

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MATRIX BINDING TO tRNA^{Lys3} IN HIV-1 INFECTED CELLS IS pH DEPENDENT

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When entering a host cell, the HIV-1 RNA genome is reverse transcribed into double stranded DNA by reverse transcriptase using human tRNA^{Lys3} as a primer. Packaging of virions starts with the recognition of unspliced viral RNA by Gag and GagPol proteins, which form a complex that is targeted to the plasma membrane (PM) by the matrix domain (MA) on Gag. This binding is mediated through the Highly Basic Region (HBR) and N-terminal myristoyl group on MA and phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] on the PM. In addition to PI(4,5)P₂, MA binds specific tRNAs including tRNA^{Lys3}. MA mutant studies suggest that tRNA^{Lys3} binds to the HBR, which may regulate MA binding to PI(4,5)P₂ in the PM. However, it is unclear whether the N-terminal myristoyl group, which exists in an equilibrium between sequestered and exposed conformations, impacts MA-tRNA^{Lys3} interactions. Isothermal titration calorimetry (ITC) was used to titrate tRNA^{Lys3} into myristoylated (myrMA), unmyristoylated (unmyrMA), and exposure-deficient L8I matrix proteins.

NMR data shows that matrix is predominately in the sequestered form at pH 7, but decreasing the pH will shift the equilibrium toward the exposed conformation. MA's ability to bind tRNA^{Lys3} is pH dependent, as ITC results showed that at pH 5.5, myrMA and L8I binds less strongly to tRNA^{Lys3} than at pH 7. Additionally at pH 7, myrMA behaves like unmyrMA and L8I. This suggests that the myristoyl group is responsible for changes seen at different pHs. The difference seen at pH 5.5 suggests that tRNA^{Lys3} cannot bind exposed myrMA or that myrMA multimerizes in solution and prevents tRNA^{Lys3} binding. These proposed mechanisms can be verified by solving the structure of myrMA-tRNA^{Lys3} complex in solution.

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***IN SILICO* EXPLORATION OF THE MECHANISM OF TRANSLESION SYNTHESIS BY DINB**

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Y-family DNA polymerases are a class of error-prone polymerases able to perform DNA translesion synthesis. *E. coli* DNA Polymerase IV (also called DinB from the name of the gene) is a Y-family DNA polymerase implicated in stress-induced mutagenesis. As other DNA polymerases, DinB has the general architecture of a right hand, with palm, thumb and fingers subdomains. In addition, Y-family polymerases have an extra domain, namely the “little fingers” and have a more solvent accessible active-site than their high-fidelity replicative counterparts. Kinetics studies have shown that DinB is particularly efficient at bypassing damaged guanosines, such as the N²-furfuryl-dG, and even more efficient in lesion bypass on damaged DNA than in replication of undamaged DNA. Recent hydrogen exchange experiments have suggested that the protein undergoes a conformational change in the presence of certain DNA damages on the template strand, but not others, implying a possible mechanism of bypass discrimination. However, the exact nature of such movement is not well characterized. Using molecular dynamics (MD) simulations we explored different DNA/protein/incoming nucleotides combinations to dissect the protein conformational change. 3 systems were simulated: a binary system, ternary system, and an O-methylated system. Results show that without a proper substrate the polymerase was in an open-state conformation, non-active state, which was observed in the binary system. When a proper substrate was introduced the polymerase was seen in a closed position, therefore active, such as in the ternary system. However, in the O-methylate system, a proper substrate was introduced with the exception of a methylation being done on the Guanine 840 residue, leading to an open-state conformation of DinB. Further analysis of the MD trajectories will allow us to identify the key residues for the conformational movement if needed one is observed.

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ISOLATION AND SEQUENCING OF AN APTAMER

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There are many current treatments for cancer such as chemotherapy which have proved to be beneficial but it also comes with many side effects. The long term goal of my project is to develop a cancer-specific therapy for cancer cells by utilizing RNA. The type of RNA that will be used is known as an aptamer and it binds to a small molecule. An aptamer targets a molecule with high affinity and selectivity. The molecule of interest in my project is 2-hydroxyglutarate, (2-HG), found strictly in acute myelogenous leukemia and gliomas.

I had started my project two years ago and since then I have been able to develop a pool of aptamers that bind the oncometabolite in my project, using SELEX with a hammerhead aptazyme. Recently, I have learned the molecular biology techniques to isolate and sequence the most effective aptamers which involved cloning the pool of aptamers. The technique of cloning will be used to effectively isolate the individual aptamers. Then, the individual aptamers will be transcribed and assayed for 2-HG binding. One short term goal for my project is to be able to sequence the most effective aptamers and do bioinformatics analysis.

Later on, the long term goal is to use the designed aptamer and convert it into a riboswitch for controlling gene expression. The riboswitch will function as a biosensor for 2-HG, allowing early cancer detection. With early cancer detection, this project may be a new platform for developing new cancer-specific therapies for cancer patients.

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MYCOBACTERIUM TUBERCULOSIS PROTEASOME INHIBITORS

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The tuberculosis-causing pathogen *Mycobacterium tuberculosis* (Mtb) has become increasingly resistant to current drugs on the market, making it a prominent infectious disease. This research focuses on molecular targets to suppress the bacterial adapted defense. The Mtb proteasome supports survival of the bacterium by defending it from the host immune response during the latent, non-replicative phase. Therefore, inhibiting the Mtb proteasome would make the pathogen more susceptible to current drug therapies. The objective of this project is to use a validated, high-throughput-amenable, luminescence assay to screen fungal extracts for selective inhibitors of the Mtb proteasome. The basis of the assay is to introduce a crude extract of the Mtb proteasome to a specific peptide designed to produce luminescence when cleaved. This allows the activity of the proteasome to be assessed relative to luminescence produced by uninhibited, inhibited (positive control), and test proteasomes exposed to fungal extracts. The percent inhibition is calculated for each extract. Inhibition >50% is considered a positive hit, and the extract is further analyzed through bioassay-guided fractionation. This analysis includes the extraction, isolation, and characterization of the compound(s) producing the inhibition. The process involves several different chromatographic techniques (TLC, HPLC, flash CC) to fractionate and isolate the inhibiting compound. We aim to discover novel natural-product inhibitors of the Mtb proteasome that potentially can serve as drug leads for combating latent tuberculosis.

PRIMER/PROBE OPTIMIZATION OF RTq-PCR FOR IDENTIFICATION OF DOUBLE-STRANDED (ds) RNA IN RHIZOCTONIA SOLANI

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Rhizoctonia solani is a soil-borne pathogenic fungus with several distinct isolates that have been classified based on their anastomosis groups (AG's). Many isolates of these fungi contain double-stranded viral RNA (dsRNA) that are cytoplasmic and viral in origin. Research in our laboratory has studied the epidemiology and molecular biology of viral RNA in *R. solani*, thus making it a useful biological model in the development of protocols for rapid identification of biological agents.

In the present study the dsRNA from the isolate EGR-4, which is characteristically large at 3.301 Kb was purified. Attempts to clone middle (M)-size dsRNA fragments from *R. solani* have been very difficult primarily due to artifacts that co-purify, including large (L)-size dsRNA in the fungus. Since magnesium is required for DNA polymerase and EGR-4 requires a specific concentration; various MgCl₂ concentrations were tested to optimize full length dsRNA PCR product.

The dsRNA was analyzed by gel electrophoresis. The nuclease-treated dsRNA purified-gel was reverse transcribed into cDNA, ligated into the p-Jet cloning vector, and transformed using *E. coli*. All such clones were sequenced where the forward and reverse primers were generated using BLAST sequence via Biosearch Technology. The plasmids were purified from transformed cultures and amplified using real-time PCR (RTq-PCR) with primers (reverse CCACCGGAAGAGGGAAATCC, forward AGCGCTGACCTTGCTATCGA ATC) and probe (5' Fam-AGTGCCGATCAGCCCTCCACCG-BHQ1 3'). The ideal primer/probe concentration was determined through optimization by comparing the lowest threshold concentration (Ct) values using the plasmid cDNA as a template.

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DETERMINING THE ELASTICITY OF CLOFAZIMINE CRYSTALS

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Elasticity is the ability of an object to be stretched or compressed and return to its normal form. The term is not commonly associated with crystals, but it is known in clofazimine (CFZ), also known by the trade name Lamprene, an FDA-approved leprosy drug. Clofazimine works as an anti-inflammatory agent but bioaccumulates and forms crystals in macrophages. Observations have shown that these biocrystals display curvature, which can slow and even rupture macrophages thereby hindering their critical role in immune responses. The biocrystals' curvature was analyzed using programs in ImageJ to find arc length (L) and radius (r) of the circle that the biocrystal would form. Then a series of formulas including $L = \theta r$ were used to determine the extent of the crystals' curvature. The curve data from the macrophage biocrystals was compared to the control biocrystals and 86% of the internal biocrystals had greater curvature. In order to test the elasticity of CFZ, a large scale hydrochloride (HCl) salt form of CFZ was created. These crystals were subjected to mechanical manipulation by forceps to test the extent of their elasticity. The CFZ-HCl biocrystals were able to bend repeatedly along the frontal plane and return to their normal length an average of 5.38 times prior to fracturing. Future work will involve examination of the associated effects of loss of ordered form in CFZ biocrystals.

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DESIGN OF A GENE TRANSFER VECTOR TO DELIVER A STABILIZED ANTI-EGFR RNA APTAMER TO THE GLIOBLASTOMA MICROENVIRONMENT

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Glioblastoma multiforme (GBM) is an incurable and aggressive type of brain tumor. It is the most common central nervous system (CNS) malignancy with a median survival of only 14 months. The epidermal growth factor receptor (EGFR) is a type of tyrosine kinase receptor (TKR) dysregulated in about 60% of GBM tumors. EGFR amplification and over-expression leads to uncontrolled growth and proliferation of GBM. Although a great deal is known about the biology exhibited by EGFR-activated GBM, the application of therapies against the biologic processes is limited by the blood-brain barrier, which restricts systemically administered therapies from reaching the brain. We are creating an *in vivo* tissue culture model to develop a novel strategy to bypass these barriers by developing a gene transfer vector to deliver the genetic sequences of a known anti-EGFR RNA therapy aptamer that binds with high affinity against EGFR. In this approach, we will use a gene transfer system to modify GBM and CNS cells to express the therapeutic anti-cancer RNA aptamer molecule, and using an extracellular RNA “exRNA” localization element, the RNA aptamer will be transported and spread throughout the tumor microenvironment where EGFR is abundant. In addition, we have added an RNA structural element (an inactivated hammerhead ribozyme) important for the stabilization of the RNA therapeutic molecule.

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CHARACTERIZATION OF CELL-TYPE SPECIFIC RESPONSES TO MISFOLDED PROTEIN STRESS IN *C. ELEGANS*

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Cells experiencing misfolded protein stress can become debilitated and die, contributing to the onset of disease. Different cell types display varying sensitivities to this stress, with neurons being particularly susceptible to death. When a cell experiences misfolded protein stress in the endoplasmic reticulum (ER), the unfolded protein response (UPR) initiates cell-saving mechanisms that mitigate stress and, if the stress cannot be resolved, triggers cell death by apoptosis. The nascent polypeptide-associated complex (NAC) is a heterodimeric chaperone that mediates proper protein folding and localization during translation, and also triggers the UPR when the ER experiences misfolded protein stress. The role of the NAC in relation to the UPR is not well understood, nor is it known if this role is different in different cell types; our goal is to characterize the relationship of the NAC with the UPR in different cell types in the model organism *C. elegans*. We are characterizing how neurons respond to misfolded protein stress in the absence of the NAC by depleting the NAC via RNA interference and quantifying the number of neurons observed in the ventral nerve cord. Generally, depletion of the NAC decreases the number of ventral nerve cord neurons while also leading to the mis-localization of the neurons that remain. In addition, we are characterizing the effects of depletion of the NAC in hypodermal cells, which are relatively more resistant to stress-induced death. Through these experiments, we hope to better understand how different cell types handle misfolded protein stress, and why some cell types are more likely to die in response to this stress while others live.

THE EFFECTS OF SEDIMENT CHARACTERISTICS ON A NATIVE ISOETID

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Isoetids are slow growing, submersed aquatic plants that thrive in oligotrophic water. They are characterized by their small, stiff rosette leaves and extensive root system. This root system is semi-permeable and allows isoetids to take in carbon dioxide and release oxygen, thus altering the sediment biogeochemistry. We conducted a greenhouse experiment to determine if different sediment types and levels of organic matter influenced how the isoetid *Eriocaulon aquaticum* changed its environment. The experiment had nine treatments; each treatment had one *E. aquaticum* plant placed in either sand, pond sediment, or a 50:50 mixture of sand and sediment with varying amounts of organic matter (eastern hemlock branches) added to each substrate type. We hypothesized that plant growth of *E. aquaticum* would be greatest among individuals grown in a mixture of sand and sediment with an addition of a low amount of organic matter. The results indicated that *E. aquaticum* preferred the sand and 50:50 mixture treatments than the nutrient rich pond sediment. The sand treatment exhibited positive redox potentials while the 50:50 mix and pond sediment had negative redox potentials throughout the experiment. The redox potentials of the sand with varying amounts of organic matter treatments began to become negative throughout the course of the experiment. As a result of the nutrient rich pond sediment, some of the plants exhibited mortality likely due to an anaerobic zone around the root system of the plants.

OVEREXPRESSION OF SPECIFIC PROTEINS IN A PANCREATIC CANCER CELL LINE

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Pancreatic cancer is a deadly disease that is ranked third on the list of all cancers among men and women in the United States. Approximately, 53,070 new cases and 41,780 deaths are estimated by the end of 2016. The high volume in death rate is likely due to late diagnosis. The etiology of this disease is poorly understood, however, it is believed that unrepaired DNA damage results in mutations that could affect many cellular processes including gene and protein expression. We therefore assessed the total protein expression in BxPC-3 cells compared to normal cells. We hypothesized that pancreatic cancer cells will overexpress more proteins than normal pancreatic cells. We prepared cell-free extracts from BxPC-3, a pancreatic cancer cell line and HPDE-6, a control cell line. The proteins were separated by SDS polyacrylamide gel electrophoresis and stained using Coomassie blue. Western blot analysis was used to assess protein expression of two proteins, IGFBP5 and CEACAM6. Our results showed that the pancreatic cancer cell line over-expressed a significant number of proteins compared to the normal cell line. Western blot analysis showed that the pancreatic cancer cell line over-expressed IGFBP-5 and CEACAM6 approximately 2-6 times more than the normal pancreatic cells. These result are consistent with our hypothesis and the literature.

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KINOME PROFILING OF GENE KNOCKOUT MUTANTS OF *SALMONELLA* TYPHIMURIUM

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Salmonella enterica strain typhimurium (*S. typhimurium*) is a significant food safety concern in the United States, specifically in the poultry industry, as contaminated food products can cause disease in humans. The goal of my project is to understand the intracellular processes that occur inside chicken macrophage cells in response to various *Salmonella* infections. Experimental infections of cultured chicken HD-11 macrophage cells have been carried out with both mutant and wild type (W.T.) strains of *S. typhimurium* at half hour, 2 hour, 6 hour and 24 hour time points. The mutant strains include *S. typhimurium* that are missing key intermediates in the TCA cycle, either isocitrate dehydrogenase (*icdA*) or aconitase (*acnB*), as well as one with the flagellar biosynthesis gene, flagellin (*fliCfliB*), knocked out. These infections will provide insight into the immune response pathways of infected chickens and ideally will lead to alternative methods to treat *Salmonella* infections more effectively, or develop vaccine strains. To accomplish this, I will be running a peptide array of infected chicken HD-11 macrophage cells against non-infected HD-11 macrophage cells, looking for differences in phosphorylated proteins. These differences would show what proteins or kinases are activated as part of the cellular signal transduction cascade in response to a *S. typhimurium* infection. This past summer has been used to collect data foundational to this experiment. The HD-11 macrophage cells being used for this experiment were found to take approximately 1.5 hours to adhere to flasks and wells while being cultured. W.T. *S. typhimurium* isolated from a mouse was then added to a population of cells at a multiplicity of infection (MOI) of 100 *Salmonella* to 1 macrophage cell. Roughly 1 bacterium infected each HD-11 cell, thus quantifying how much *Salmonella* penetrates the cells upon infection. Finally, a NO Assay was conducted to quantify the amount of nitrite released by the HD-11 cells in the supernatant of the infection assay at the 2 hour time point, as a measure of the cellular immune response. This assay found that all infected samples actively mounted an immune response, with W.T. poultry *S. typhimurium* producing the most nitrite with a value of 50.90ug. The next step in my research will be to run the peptide array to see what the infection of *S. typhimurium* triggers in the immune and metabolic pathways of the cell.

BENTHIC MACROINVERTEBRATES USED AS A BIOMONITORING TOOL TO DETERMINE QUITTAPAHILLA CREEK WATER QUALITY

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Macroinvertebrate species can be used to indirectly measure water quality in creeks, rivers and streams. Certain species have high tolerance to polluted streams, while others are only associated with high water quality. Researchers sampled upstream and downstream from a streambank restoration project at Quittapahilla creek (Annville, PA) both before and after the construction. Organisms were captured in surber nets, identified to the genus level where applicable, and researchers then calculated Family Biotic Index, Biotic Index, species evenness, species abundance, Simpson's Dominance, Simpson's Density, Shannon-Weiner Index, and Coefficient of Community. The number of *Hydropsychidae* strikingly increased from 2013 to 2015, while the number of *Podonominae* dramatically decreased. Two invasive species were also identified in 2015, the rusty crayfish (*Oconectes rusticus*) and the Asian clam (*Corbicula fluminea*). The data suggest a change in environmental conditions occurred from 2013 to 2015 both upstream and downstream of the streambank restoration project site resulting in different macroinvertebrate communities at both sampling locations.

MODELLING A HAWKSBILL SEA TURTLE (*ERETMOCHELYS IMBRICATA*) POPULATION USING NETLOGO

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Hawksbill sea turtles, *Eretmochelys imbricata*, were originally listed as an endangered species in 1968 and have been listed as critically endangered since 1996. This critically endangered status suggests an ominous future for the species and its survival. With the critically endangered classification, conservation biologists often wonder how long the species is expected to survive with its current logistics. However, the classification has brought an increase in conservation efforts, allowing for hope for Hawksbill sea turtles to be removed from the critically endangered list. The rise in conservation begs the question: how much effort needs to be put forth in order to produce a stable population and remove the species from the critically endangered list?

In order to answer this question, a time limit must first be determined to have some perspective on this issue. Conservationists need to know how much longer the population will survive before being able to develop a plan in the proper time frame. In an attempt to estimate the number of years a population is able to survive at its current status, I developed a model, with the agent based modeling program Netlogo, which simulates a Hawksbill sea turtle population using carefully selected and conservatively estimated life history parameters. The model specifically focuses on Belize's population of Hawksbill sea turtles and predicts, with current parameters, the population can survive for about another hundred years. However, after introducing a hatchling release program to the model, the population has the potential to survive for thousands of years. Changing just one small aspect of the population can make a complete difference for the Hawksbill population and possibly other species as well.

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WIDGEON GRASS AND RED ALGAE HABITAT PREFERENCE BY JUVENILE BLUE CRABS

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Seagrasses serve as spawning and nursing habitat for juvenile blue crabs (*Callinectes sapidus*). In the Maryland Coastal Bays the most abundant species of seagrasses are *Zostera marina* and *Ruppia maritima*, Widgeon grass. In recent years the extent of seagrass coverage has been declining, while there have been increases in the amounts of macroalgae. To understand how these changes may impact juvenile blue crabs we used experimental aquaria to examine the effects of two different acclimation periods, substrate use (macroalgae, seagrass and sand), and crab behavior within each substrate. We found that blue crabs better acclimated to the experimental tanks after a 24-hour period compared to 30-minutes. Our results also showed a preference by blue crabs for macroalgae and seagrass substrates over sand substrates. In addition, within those substrates crabs displayed more foraging activity in the macroalgae than in the seagrass and sand substrates. These results suggest that juvenile blue crabs do not display a preference for either seagrass or macroalgae substrates and may readily shift to either as habitat availability changes.

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SUNSCREEN NEGATIVELY AFFECTS THE DEVELOPMENT AND BEHAVIOR OF THE ATLANTIC HORSESHOE CRAB, *Limulus polyphemus*

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Anthropogenic derived environmental impacts are prevalent and increasing rapidly, one of greatest concern is the release of pollutants into the air, water, and sediment. Anthropogenic activity causes increased stress on sensitive coastal environments, sometimes with unintentional consequences to the resident organisms. The Atlantic horseshoe crab, *Limulus polyphemus* is a prehistoric keystone species native to the East Coast, with the densest population found within Delaware Bay. *L. polyphemus* eggs are a dietary necessity to many bird, fish and reptile species. Additionally, due to their unique physiological characteristics, *L. polyphemus* blood is vital to the medical industry. Annually, between May-July on the new and full moon during high tide, *L. polyphemus* engage in a mass spawning event where eggs are deposited into shallow holes dug into the sand along the high tide waterline. During the same time period, Delaware beaches experience a massive influx of human activity due to the tourism season. Recent evidence from coral reef ecosystems has found pollution due to excess sunscreen negatively impacts resident species. In this study we tested the effects of sunscreen on the survivorship, development and behavior of egg, larval and juvenile stages of *L. polyphemus*. Egg clutches were collected and split into four groups, each of which were exposed to different sunscreen concentrations (0ml/L, 0.5 ml/L, 1.0 ml/L, 1.5 ml/L) dissolved in seawater from the Delaware Bay. Horseshoe crabs exposed to sunscreen, at even the lowest concentration tested, did not move through the appropriate stages of development. In comparison, control individuals developed into 1st instar juveniles within a 7-week period. Individuals exposed to all sunscreen concentrations had a significantly higher mortality rate than control individuals when transitioning from the egg to free-swimming larval stage. This study identifies an important pollutant impacting a keystone species within the temperate coastal ecosystem. Impacts to the *L. polyphemus* population could have drastic effects on the ecosystem as a whole as well as future medical advances.

RESPONSE OF THE MICROBIAL COMMUNITY OF THE ANACOSTIA RIVER TO DIFFERENT NUTRIENT TREATMENTS

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The Anacostia River is one of the most polluted rivers in the United States. The main source of pollution is from antiquated sewer systems. Those antiquated sewer systems bring various nutrients such as nitrogen (N) and phosphorus (P) that may fuel the growth of phytoplankton. In order to understand how these excess levels of N and/or P may influence the phytoplankton community, a bioassay experiment lasting four days was conducted to test the response of phytoplankton community to different nutrient treatments (NO₃, NO₃ + P, NH₄⁺, NH₄+P, Urea and P) and to see which nutrients limit or promote the growth of phytoplankton community. Water samples were taken from two different sites on the Anacostia River: Site 1 was located near Bladensburg, Maryland while Site 9 was located near the Nationals Baseball Stadium. Overall, the phytoplankton community responded the best to N + P treatments at both sites, regardless of N source (NO₃ or NH₄). P also limited growth at both sites. The results of this study suggests that the availability of both N and P controls growth of different members of the microbial community, especially nanoplankton that may include cryptophytes and chlorophytes in the Anacostia River. Future efforts need to continue monitoring both N + P levels as well as changes in the microbial community, especially after gray and green infrastructure are put in place to alleviate pollution from the sewer system.

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EVOLUTION OF A POLYPHENISM

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The Pea Aphid, *Acyrtosiphon pisum*, exhibits a remarkable adaptive response to seasonal changes in photoperiod. In spring and summer, aphids reproduce asexually, yielding large numbers of genetically identical female offspring. The longer nights accompanying the fall induce these asexual aphids to produce sexual males and females, which mate to lay frost-resistant eggs. These eggs diapause through the cold winter months, hatch into asexually reproducing females in the spring, and the cycle continues. Pea aphid populations have been shown to exhibit latitudinal variation in this photoperiod response, presumably reflecting local adaptation to variation in the timing of the first frost (e.g., Smith and MacKay, 1990). Populations from the southern United States have been reported to exhibit attenuated photoperiod responses or to have lost the ability to produce sexuals altogether. Here we describe a previously detected difference in the photoperiod response between strains from New York and Arizona. With an eye toward understanding what underlies this difference, we also describe differences in how these strains respond to Juvenile Hormone, which has been implicated in the induction of asexual fate (e.g., Corbit and Hardie, 1985; Ishikawa et al., 2012). Additionally, our attempt to inhibit JH using Methyl Linderone failed to induce sexuals as predicted, but still holds potential for furthering our understanding of the mechanism and evolution of this polyphenism.

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USING DELAY DIFFERENTIAL EQUATIONS TO STUDY CALCIUM ALTERNANS IN A MODEL OF INTRACELLULAR CALCIUM CYCLING

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Cardiac arrhythmias are irregular beatings of the heart caused by disruptions in the electrical activity that triggers contraction. Electrical alternans, a period-2 behavior characterized by long and short action potential durations, is a mild arrhythmia that often degenerates into more life-threatening cardiac arrhythmias such as ventricular fibrillation. Of particular interest is calcium alternans, which is characterized by alternating large and small intracellular calcium concentrations in response to periodic stimuli and can lead to alternans at the cellular and tissue levels. Experimental findings have shown that this calcium cycling can exhibit alternans even when there is no alternans in the electrical dynamics, behavior that can be reproduced with the Shiferaw et al. model (2003) we use here. Despite the need to understand mechanisms for calcium-driven cardiac alternans, however, many ordinary differential equation models of intracellular calcium cycling do not produce alternans, thus restricting the scope of such models for studying alternans behavior. Delay differential equations (DDEs), which in many contexts produce complex dynamics, may be a promising tool for promoting alternans in cardiac models and have been shown previously to enhance alternans in the Shiferaw et al. model in a limited context. We extend this prior work by applying DDEs more broadly to the same model; specifically, we introduce DDEs in the equations for the calcium current gating variables, currents, and the release function. After suppressing alternans in the original model, we show that alternans can be induced by DDEs in certain compartments of the cell. We analyze the changes in the calcium concentrations, currents, and gating variables in response to these DDEs and discuss the mathematical and physiological implications of our findings. Our results provide further insight to understanding the role intracellular calcium cycling plays in the development of alternans.

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INVESTIGATING THE ROLE OF THE CIRCADIAN CLOCK GENES *PRR5*, *PRR7*, AND *PRR9* IN REGULATING PLANT IMMUNITY

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Successful defense against pathogens is critical for plant survival. Recent studies have shown that the circadian clock, the internal time measuring machinery, is involved in disease resistance in addition to its roles in plant development. One clock protein, LUX, binds to the promoters of the clock genes *PRR5*, *PRR7*, and *PRR9*, and we have preliminary results to show that *LUX* and the *PRR* genes are involved in defense. *LUX* was shown to act through defense signaling mediated by salicylic acid (SA). To test if the *PRR* genes also act through the SA pathway, we introduced loss of function mutations in *PRR5*, *PRR7*, and/or *PRR9* into *acd6-1*, a small mutant plant with constitutive SA production whose size change predicts the defense levels. We have isolated the double mutants (*acd6-1prp5*, *acd6-1prp7*, *acd6-1prp9*), two triple mutants (*acd6-1prp5prp9* and *acd6-1prp7prp9*), and the quadruple mutant (*acd6-1prp5prp7prp9*). We are currently assessing plant phenotypes by measuring their sizes, cell death levels, SA levels, and expression of defense genes. Any suppression and the degree of suppression of *acd6-1*-conferred phenotypes would predict whether the *PRR5*, 7, and 9 genes act in the SA pathway and whether they do so in a synergistic manner. Initial results suggest that *PRR7* and *PRR9* promote defense through SA-mediated pathway while *PRR5* plays a lesser role in this pathway.

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HABITAT USAGE AND NESTING BEHAVIOR OF THE CRITICALLY ENDANGERED BAHAMA ORIOLE

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The Bahama Oriole (*Icterus northropi*) is currently listed as a critically endangered species restricted to one island in the Bahamas. It was only recently classified as its own species in 2010, and very little research has been conducted regarding its behavior and population dynamics. In 2011, it was estimated that only 141-254 individuals still survived in the wild, and due to its recent extirpation from Abaco in the 1990's, it is only found on the island of Andros.

In May 2016, a team of UMBC undergraduate researchers led by Dr. Kevin Omland traveled to Andros to conduct preliminary research on the Bahama Oriole. We conducted point count surveys to census their population densities across five distinct habitat classifications: pine forest, hardwood forest, mixed pine/hardwood, agriculture/secondary growth, and developed land. Previous to this study, it was believed that the Bahama Oriole nested mostly in the coconut palm (*Cocos nucifera*) in developed habitats. However, we documented the orioles using all five habitat types. Furthermore, we documented nesting in different tree species, including pine trees (*Pinus caribaea* var. *bahamensis*) and species of understory palm deep in the pine forest. We will return to Andros in January to continue censusing during the non-breeding season.

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UNDERSTANDING TUMOR SUB-CLONAL DYNAMICS USING A COMPUTATIONAL FRAMEWORK

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Cancer is a disease arising from a variety of sub-clonal tumor cells dynamically interacting within themselves and the surrounding environment. This results in heterogeneous cancer phenotypes such as increased tumor volume and growth which are poorly understood. Sub-clonal tumor heterogeneity is a relatively new aspect of cancer, and its understanding is essential for the development of cancer therapeutics. It is shown that different sub-clones have varying impacts on overall tumor growth and their interactions are essential to maintaining the tumor proliferation. Sub-clones have been shown to result in variable phenotypes ranging from decreasing tumor volume to an extreme rise in tumor growth rates. To better understand the dynamics of sub-clonal tumor heterogeneity, we developed a computational framework to construct non-spatial, dynamic mathematical models of tumor heterogeneity. Our method uses high-performance computing to automatically infer models from the data, simulate them through time, and evaluate the *in silico* results in comparison to the results obtained from *in vivo* experiments. The best-fitting model can accurately recapitulate tumor volume and clonal frequency data. Importantly, the reverse-engineered model can be used to predict the results of novel experiments, and hence determine the optimal clone or clones to target for intervention to make the tumor collapse. Additionally, the inferred models may be used to accurately predict tumor growth over a period of time based solely off the profile of the specific tumor. Instead of doctors using inefficient and risky trial and error techniques, *in silico* testing of treatments using the tumor-specific inferred model can potentially predict the best possible therapy for cancer patients. This project will vastly improve the efficacy of cancer therapies with the use of tumor-specific medication as well as expand the knowledge of sub-clonal dynamic interactions.

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SCREENING SOIL BACTERIA FOR ANTIBIOTIC PRODUCTION

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There is an increasing demand for newly discovered antibiotic treatments, as antibiotic resistant infections have become more widespread and new antibiotic discovery has declined. Because the source of most known antibiotics is soil bacteria, soil samples were collected from the New Jersey Pine Barrens to screen for antibiotic producing bacteria. The Pine Barrens environment is unique due to its sandy, acidic, nutrient-poor soil. Of the bacteria isolated in the samples, we focused on three (13, 17, 20), which revealed antibiotic activity against non-pathogenic tester strains. The non-pathogenic tester strains used in this study are related to common pathogenic bacterial threats in health care. The antibiotic activity from the bacterial isolates was successfully extracted using ethyl acetate. These extracts were further analyzed by gas chromatograph mass spectroscopy and revealed potential antibiotic molecules. To identify the soil bacteria, we used the polymerase chain reaction (PCR) to amplify the 16S ribosomal RNA gene. The PCR product was sequenced and then compared to the NCBI database with the BLAST search tool. The preliminary identification revealed both 17 and 20 isolates are most closely related to the genus *Burkholderia*. Continued research is necessary to further characterize the antibiotic compounds, and the bacteria that produce them.

SCREENING FOR ANTIBIOTIC RESISTANCE GENES IN THE *ANOPHELES ARABIENSIS* MOSQUITO

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Anopheles arabiensis, is a mosquito that is commonly found in Ethiopia and transmits malaria. The purpose of this study was to determine whether antibiotic resistance genes are present in the microbiota of this insect vector. There is increasing concern regarding antibiotic resistance in various regions of Ethiopia. The potential spread of antibiotic resistance genes via the mosquito population could impact the health of millions of people. This is because the antibiotic resistance genes in the *A. arabiensis* microbiota could be transferred to pathogens, thereby making it difficult for them to be treated using antibiotics.

The focus of this investigation was the gene *mecA*, which codes for PBP2A (penicillin binding protein 2A). Expression of this gene allows bacteria to be resistant to certain antibiotics, such as penicillin, methicillin, and other penicillin-like antibiotics known as beta-lactams. These antibiotics target penicillin-binding proteins (PBPs) and interfere with their ability to participate in cell wall synthesis.

Polymerase chain reaction (PCR) and gel electrophoresis were used to determine whether or not *mecA* was present in the DNA samples obtained from mosquitoes. The *mecA* amplicon size was expected to be 185bp. Total DNA from 153 salivary glands and 153 abdomens was screened from an archive of mosquitoes collected from Ethiopia in 2014. Out of 306 samples, three were determined to be potential positives. The next step of this research is to try to increase the amount of amplification from those three samples by increasing the amount of DNA used and then confirming the presence of the *mecA* gene by sequencing.

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ANTIMICROBIAL ACTIVITY AND MEAT PRESERVATION OF SPICE EXTRACTS

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Meat, a major part of regular consumer's diet, is among most susceptible food item for microbial contamination. The spoiled meat is known to contain several pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and *Campylobacter jejuni* etc. Increased use of synthetic antimicrobials and preservatives have already raised concerns towards antibiotic resistance and adverse side effects, respectively, that has prompted to revive our efforts towards natural resources of antimicrobials as alternative of preservatives in meat products. We hypothesized that distillation extract from spices will reveal antimicrobial effect against foodborne pathogenic microorganisms and will eventually assist in food preservation at normal room temperature. Dried spices *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric), *Capsicum annum* (Red Pepper), *Cinnamomum verum* (Cinnamon), *Syzygium aromaticum* (Clove), and dried peeling of *Citrus tangerine* (Tangerine) obtained from local grocery stores were subjected to Soxhlet extractor for essential oil extraction using ethanol as polar solvent. The extracts were subjected to antimicrobial activity against 30 pathogenic microorganisms including foodborne pathogens. Among all, the cinnamon and clove extracts revealed highest antimicrobial activity (11-46mm) against most of the pathogenic microorganisms. The GC MS profile of cinnamon and clove extracts revealed variety of compounds including cinnamaldehyde and Eugenol acetate, respectively as antimicrobial compounds. 99% microbial elimination was observed using 3-4% clove extract from meat at 37°C, whereas much lower concentration (0.5-2%) of cinnamon extract revealed 90-99% microbial elimination in meat at 37°C compared to meat samples incubated at similar temperature without clove and cinnamon extracts.

BIOSYNTHESIS OF L-ASPARAGINASE FROM ENDOPHYTIC MICROORGANISMS

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L-asparaginase is a significant enzyme that has commercially being used as a main drug in the treatment of Acute Lymphoblastic Leukaemia (ALL). It is also being used in the food industry to reduce the formation of acrylamide in heat processed food products. A broad variety of microbial L-asparaginase with characteristics compatible with its different applications is needed. We hypothesized that endophytic microorganisms from rhizome of certain medicinal plants of the family Zingiberaceae will reveal L-asparaginase activity. Six endophytic microorganisms designated as EPT1-5 and EPZ1 were isolated from rhizomes of *Curcuma longa* (Turmeric) and *Zingiber officinale*, respectively. The isolates were identified as *Panibacillus* sp. EPT1 and *Lysinibacillus fusiformis* EPZ using 16S rRNA sequencing for over 99% similarity score using EzTaxon search engine. L-asparaginase activity was measured by releases of ammonia accumulation due to hydrolyses of L-asparagine into aspartic acid on Modified Czapek Dox (MCD) agar medium containing phenol red, a pH indicator. The endophytic microorganism EPT1 and EPZ revealed highest activity of L-asparaginase in liquid MCD medium. The fermentation medium was optimized for multiple sugar substrate, varying temperature, and pH for L-asparaginase activity. Microorganism *Lysinibacillus fusiformis* EPZ revealed highest L-asparaginase activity (800-900 U/ml) under optimized fermentation conditions. Current studies provide sufficient grounds to favor the candidature of the enzyme for both its therapeutic and food industrial applications.

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

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Nociception refers to an organism's perception and reaction to potentially damaging noxious stimuli. While nociception is a beneficial behavioral response to harmful stimuli, humans suffer from chronic pain in which the 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, and some of these proteins are hypothesized to be involved in the electrical synapsis between neurons. 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 the dendritic arborization sensory neurons (da neurons) that mediate nociception. The effect of this knockdown is observed in a cold behavior assay.

Wild type third instar *Drosophila* larvae exhibit a characteristic "cringe" response when exposed to noxious cold. Larvae are subjected to the cold behavior assay, 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 knocked down, 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; negative control) is expressed specifically in da neurons will be described.

To date, the *ogre* and *shaking-B* Innexins have been tested. No significant change in cringing was observed for either after RNAi expression (Two-Tailed Homoscedastic TTest, $p > 0.1$). Additional Innexins will be tested and a pan-neuron driver will also be used to drive RNAi expression.

This work was supported by a 4-VA grant to Susan Halsell and the Margaret Poats Fowler '37 and Farrell Biology/Biotechnology Scholarships to Kendyl Combs.

DNA METHYLATION PATTERNS AT *SNRPN*

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Genomic imprinting is an epigenetic mechanism that allows for the expression of one copy of a gene over another. The offspring receives one copy of a gene from its mother and one from its father. In the case of an imprinted gene, only one of those copies is expressed. It is believed that the addition of methyl groups to one of the two alleles tags it such that the copies can be differentiated. This differential methylation results in one copy being expressed while the other is silenced.

It was believed the tagged copy of imprinted genes was invariant and stable because of the importance of maintaining the differential methylation on the two copies. However, it was discovered that some differentially methylated regions (DMRs) show variability in their methylation patterns. Our lab has looked at primary DMRs (inherited at fertilization) and secondary DMRs (acquired during early embryogenesis) on paternally methylated imprinted genes. We found that there is little variability in DNA methylation at primary DMRs, but a significant amount of variability at secondary DMRs examined thus far. Our previous research indicated that high levels of asymmetric methylation on complementary strands of DNA correlates with increased methylation variability.

Thus far, we have only looked at methylation on paternally inherited copies. Now we would like to see if maternally methylated alleles follow similar patterns. I will be looking at the imprinted *Snrpn* gene which has a maternally methylated primary DMR. Previous papers have shown that there is little variability in methylation at the *Snrpn* DMR. I therefore hypothesize that we will find low levels of asymmetric methylation at this gene. The data I obtain will be compared to the data we have obtained from paternally methylated genes to further our understanding of how DNA methylation patterns are maintained at imprinted genes.

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I would like to thank Dr. Tamara Davis for her encouragement, and mentorship. I would also like to thank the other members of the lab, Emma Tunstall; Nana Raymond; and Kristian Sumner, for their advice and support as well as Allison Wightman for her help with minipreps. Support for this research was provided by an award to TLD from NSF grant #1514600.

MICROBIAL AND BIOCHEMICAL ANALYSIS OF YOUNG GOAT POPULATION

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Contagious ecthyma, often called sore mouth or ORF, is a virus that afflicts goats and sheep. Livestock that are infected with this virus present symptoms that include multiple scab buildups on the muzzle and pus discharge. These symptoms inhibit consumption of food and, when left untreated, can lead to other complications and mortality. A population of 28 young goats, ranging in ages from 2 weeks to 4 months were sampled at a Vermont dairy. Swabs of the kids' buckles and/or nasal cavity were obtained and immediately processed.

Mannitol Salt Agar (MSA) results demonstrated positive results for *Staphylococcus aureus* in all collected samples. Further biochemical testing confirmed the presence of *S. aureus*. Kirby Bauer analysis revealed multiple resistant strains.

Conditions of holding pens, feeding equipment, and health of animals all contributed to increased infection rates of sore mouth and the secondary infections leading to mortality in the kid population. The reported results led to alterations of the dairy's sanitation and housing practices. Health of the kids is directly related to implementation of the suggested innovations.

Future DNA studies will explore virulence factors and resistant strain relatedness.

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A METAGENOMIC ANALYSIS OF THE FELINE MICROBIOME IN RELATION TO DIET AND PROBIOTIC SUPPLEMENTATION

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The intestinal microbiota of mammals mostly consists of many different bacteria, and also viruses, and fungi, which play a significant role in their host's physiology and maintaining homeostasis. Variations in the composition of the bacteria in the intestinal microbiome have been observed as a result of diet modifications, such as soluble fiber and macronutrient content. The goal of our study is to investigate alterations in the microbiome of house cats due to diet modifications and probiotic supplementation. Fecal matter from six house cats maintained on a diet of commercially available dry or canned wet cat food with or without added probiotics for 10 days were collected. Bacterial DNA is being isolated from the fecal samples collected after each diet modification. In order to characterize the microbial diversity, the 16S ribosomal RNA gene will be amplified, tagged with index primers, and sequenced on the Illumina MiSeq. From acquired data, we will compare the impact of commercially available dry food, wet food, and probiotic supplementation on the relative composition of the intestinal microbiome of house cats.

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FUNCTIONAL SIGNIFICANCE OF RIBOSOMAL PROTEIN S2 (Rps2) PHOSPHORYLATION DURING TRANSLATION

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Translation is the process in which ribosomes use an mRNA sequence to create a chain of amino acids that is folded to make a protein. It is possible for the ribosome to misinterpret the mRNA sequence and incorporate the wrong amino acid into the protein. This is called a missense error and it can result in a misfolded protein. Many neurodegenerative diseases – such as Alzheimer’s Disease, multiple sclerosis, and amyotrophic lateral sclerosis (ALS) – are the result of misfolded proteins. The purpose of this research project is to gain a deeper understanding of how errors are regulated during translation. One of the proteins that help to regulate the accuracy of decoding during translation is ribosomal protein S2 (Rps2). Rps2 has three serine residues (S176, S181 and S238) that are known to be phosphorylated by C- terminal domain kinase I (Ctk1), a kinase known to be required for maximal translational accuracy, however the functional significance of the three modifications for accuracy is still unknown. This study aims to understand the role that these phosphorylated residues might play during protein translation. Studies have shown that Ctk1 phosphorylates the serine at position 238. We have genetic data, to show that this kinase may also phosphorylate the other two serine residues (S176 and S181). We will use mutational analysis to introduce mutations in *RPS2* gene that will allow us dissect the role of these phosphorylated serine residues in terms of translational accuracy.

TRANS-SPLICING OF INTERLEUKIN-13 RECEPTOR ALPHA VARIANT 2 INTO THE EGFR TRANSCRIPT TO BLOCK GROWTH AND REACTIVATE IMMUNOGENIC POTENTIAL OF GBM CELLS

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Glioblastoma Multiforme (GBM) is one of the most common malignancies of the central nervous system. It is characterized by increased activation of tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR). Overexpressed EGFR, found in as much as 60% of GBM, leads to tumor growth and proliferation. Changes in the GBM microenvironment lead to tumor evasion and current therapies for GBM are limited by the blood brain barrier. RNA Trans-splicing therapeutics offers a novel approach to alter overexpressed oncogene transcripts. Pre-mRNA trans-splicing of an alternative polyadenylation signal into the EGFR transcript has the potential to block expression of the transmembrane domain, generating an extracellular peptide rather than a membrane-bound receptor. This shortened and soluble EGFR decoy isoform has the potential to sequester epidermal growth factor. Here, we describe a pre-trans splicing RNA molecule (PTRM) against EGFR that contains the interleukin-13 receptor alpha chain variant 2 (IL13R α 2). This highly immunogenic isoform is selectively expressed in GBM. Additionally, IL13R α 2 elicits a strong cytotoxic T-lymphocyte (CTL) immune response making itself an effective target for immunotherapy. Insertion of the IL13R α 2 immunogen into the PTRM expression vector has the potential to reactivate an immune response directed specifically toward the GBM tumor microenvironment. Delivery of the PTRM-immunogen hybrid therapy using an adeno-associated virus plasmid vector may synergistically inhibit EGFR expression while reactivating the CD-8 T-cells. We have begun preliminary testing of the PTRM expression in tissue culture and cloning of IL13R α 2 into the PTRM vector. Levels of EGFR expression will be evaluated via Western Blot and ELISA in several EGFR expressing GBM cell lines.

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DEVELOPING A SWITCHABLE SYSTEM TO INDUCE SYNCHRONOUS MEIOSIS IN *S. CEREVISIAE*

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In this study, we are developing a strategy to induce synchronous meiosis in *Saccharomyces cerevisiae*. In yeast, meiosis is regulated by a tightly controlled induction of early, middle, and late sporulation genes. The induction of middle gene expression is the key step that controls exit from prophase I and meiotic commitment. SUM1 is a DNA-binding protein that inhibits middle promoter expression during vegetative growth. SUM1 repression is lifted during prophase I. SUM1 is down-regulated during meiosis by cyclin-dependent kinase (Cdk1) and a meiosis-specific Cdk-like kinase, Ime2. A *sum-i cdk1* analog sensitive strain was used to create a system that will synchronously switch off and on at the meiotic commitment point. This strain lacks the Ime-2 phosphoacceptor site and the meiotic switch can be controlled by the addition of PPI, the Cdk1 inhibitor. Our results indicate that the addition of the inhibitor blocked cells at the pachytene checkpoint right before the commitment point and that subsequent removal of the inhibitor resulted in the synchronous progression through the meiotic commitment point and the production of spores.

UNDERSTANDING THE ROLE OF THE NOVEL HISTONE METHYLTRANSFERASE SET5 IN CHROMATIN ASSEMBLY

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Chromatin is the packaging of histones and DNA in the nuclei of all eukaryotic cells. The post translational modification of histones in chromatin is known to be important for the ability of cells to regulate their gene expression and respond to environmental stresses. Set5 is a yeast histone-modifying enzyme that methylates H4K5, K8, and K12 residues and regulates cell growth and stress responses. However, it is unclear exactly how Set5 does this on a molecular level and an understanding of the proteins it interacts with will shed light on its functions in stress responses. Using the model system, *Saccharomyces cerevisiae*, we performed a proteomic analysis and collected data identifying Set5's protein-protein interactions in the cell. To further characterize the functional role of these interactions, we are performing a biochemical analysis of interacting partners for Set5 and undertaking genetic and molecular investigations of the role of candidate interacting partners of Set5.

Based on the results of the proteomic analysis, we are focusing on the proteins involved in chromatin assembly. We are using co-immunoprecipitations (co-IP), Western blots, and cell fractionations to perform experiments used to verify protein-protein interactions. We are also using yeast molecular genetic approaches and transcription/gene expression assays that investigate the role of Set5 in the process of chromatin assembly. Our initial results regarding Set5's protein-protein interactions and its molecular role in chromatin assembly will be presented.

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EXPLORING THE DIFFERENT TREMATODES FOUND IN THE EASTERN MUDSNAIL, *ILYANASSA OBSOLETA*, THROUGH DNA PROFILING TECHNIQUES

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We investigated the different trematode species present in the mudsnail, *Ilyanassa obsoleta*, collected along the Delaware Bay, Rehoboth Bay, and Indian River Bay. Snail dissections and cercarial release revealed seven different species of parasites. Upon discovery of two morphologically similar parasites, individual cercariae from each species were collected for molecular comparison. In order to better understand these species, PCR amplifications followed by restriction enzyme digests in the cytochrome oxidase 1 (CO1) region verified that the DNA sequences of these species differ. The different band patterns observed on the electrophoretic gel indicate that the restriction enzymes cut at different sites along the DNA sequence. The results from these DNA profiling techniques verify that these two were the most genetically similar out of the seven parasites, and that the cryptic species is different than *L. setiferoides*. Further, trematode morphology and behavior was studied vigorously under a compound microscope to make physical observations. It is important to consider the role and prevalence of these trematodes in populations of *I. obsoleta*. Further studies should investigate how the different effects of this cryptic trematode on *I. obsoleta* varies from *L. setiferoides*, such as the behavior, and explore transmission pathways to second intermediate and definitive hosts.

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RHOA ACTIVATION KINETICS AND LOCALIZATION USING TWO RHOA FRET SENSORS AND TIRF MICROSCOPY

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Neutrophils are initial responders to bacterial infection and other inflammatory stimuli and comprise a key component of the innate immune response. The antimicrobial activity of neutrophils depends upon their ability to migrate rapidly up shallow gradients of attractants released by pathogens. This process, called chemotaxis, is controlled by members of the Rho family of small GTPases, which coordinate intracellular signaling pathways involved in neutrophil gradient detection, cell polarization and motility. Within this family, RhoA is known to regulate actin dynamics toward the cell rear and induce adhesion to promote migration. Active RhoA localizes to the plasma membrane on the trailing edge of polarized neutrophils, while the bulk of inactive RhoA remains cytoplasmic. The cytoplasmic pool of RhoA is maintained by Guanine Dissociate Inhibitors (GDI), which pull inactive RhoA-GDP from the membrane. Intramolecular FRET sensors are used to measure RhoA activity in live cells; however, the RhoA cytoplasmic pool coupled with variations in cell thickness have confounded FRET interpretations. Initial experiments reported that RhoA signaling increases upon stimulation with a chemoattractant but more recent research suggests that it decreases. To understand the spatiotemporal dynamics of RhoA signaling, we measured RhoA activation kinetics and subcellular localization using two RhoA intramolecular FRET sensors, one (SC61) is tethered to the plasma membrane using a KRas-CAAX domain, and the other (SC76) is cytoplasmic, and thus sensitive to GDI activity. Moreover, the SC76 sensor has the native RhoA C-terminus, and therefore it has the native regulation of cytoplasm versus plasma membrane localization. FRET ratios were measured in actively migrating cells using TIRF microscopy to eliminate cell thickness artifacts and distinguish between RhoA pools. Overall, our findings demonstrate that upon chemoattractant stimulation, average cellular RhoA activity decreases.

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INVESTIGATING THE ROLE OF β -NAC ON STRESS RESPONSE IN *Saccharomyces cerevisiae*

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Molecular chaperones are proteins that interact with other proteins to ensure their proper folding and cellular localization. The nascent polypeptide-associated complex (NAC) is a protein complex composed of an alpha and beta subunit that is thought to play a role as a protein chaperone, acting on newly emerging proteins at the ribosome. Research using genetic model organisms, including *Saccharomyces cerevisiae* (baker's yeast), has also indicated a potential role for the NAC in stress response.

Baker's yeast is an excellent model organism; it is a unicellular eukaryote that is especially suitable for basic biological research. The yeast genome has been sequenced and many powerful tools have been developed for manipulation of this organism in the laboratory. It is relatively simple to delete a specific gene in baker's yeast and also a collection exists that contains the deletion of nearly every yeast gene.

We are conducting experiments with yeast strains that have a deletion of the yeast gene encoding β -NAC, to determine whether the yeast NAC mutant has an altered response to stress. We are testing for potential growth defects of the mutant strain in response to heat stress and oxidative stress, and also whether the mutant strain has an altered lifespan.

Many metabolic and cellular pathways are highly conserved and therefore we can learn about basic cellular functions using the yeast model that may be informative for understanding human cells. It is known that proper protein folding and response to stress are important for maintaining a healthy cell, and that defects in these processes have been linked to some human degenerative diseases.

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BIOLOGICAL SEMICONDUCTORS: STRUCTURAL CONTROL OF HEME REDOX POTENTIALS S IN PPCA, A 3-HEME CYTOCHROME

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In an effort to tune and optimize the relative bandgap potentials in biological nanowires formed from multiheme domains of the cytochrome *c*₇ family, we developed and extensively characterized 11 point mutations of PpcA, a 3-heme member of the cytochrome *c*₇ family. These mutations were engineered to influence the redox potential of the middle heme in PpcA by 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, thereby inducing a redox Bohr effect. Formation of propionate hydrogen bonds is supported by all atom molecular dynamics simulations for A19R and A23R, while increased solvent access is supported for A19I, A19R, A23R, H20M, and K60E. 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 compact globular structures as expected. This was further corroborated with CD spectroscopy that revealed negligible changes in protein melting temperature caused by the mutations. Currently, we are performing UV/Vis and NMR redox titrations in order to understand the underlying principles and viable approaches in tuning relative heme redox potentials.

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ISOLATING THE EXPANSIN SUPERFAMILY OF *PICEA ABIES* TO DETERMINE EXPANSIN GENE DIVERSITY IN THE LAST COMMON ANCESTOR OF CONIFERS AND ANGIOSPERMS

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Expansins are a cell-wall loosening proteins found in the plant cell wall that aid in growth and developmental processes.¹ There are four families of expansins within the superfamily; EXPA, EXPB, EXLA, and EXLB.¹ EXPAs and EXPBs are known to influence cell wall loosening, while the function of the EXLA and EXLB families remains unknown.¹ Studies of expansins in angiosperms have resulted in the classification of 17 expansin clades: 12 EXPA clades, 2 EXPB clades, 1 EXLA clade, and 2 EXLB clades.⁷ Previous analyses of both lycophytes and bryophytes have been found to contain only EXPA and EXPB genes.^{3,4} This particular study focuses on assembling the expansin superfamily of *Picea abies*, commonly known as the Norway Spruce. This draft genome was the first of any gymnosperm genome to be released.² The use of this genome reigns difficult, due to its abundance of transposable elements, which can allow for the presence of large introns and an abundance of pseudogenes.² BLAST searches were completed to find expansin sequence matches between *Picea* and angiosperms. After the checking of gene annotations, phylogenetic analysis completed showed that the last common ancestor of spruce and flowering plants contained 4-6 EXPA genes, 2 EXPB genes, 1 EXLA gene, and 1 EXLB gene.

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PAVING THE WAY FOR REGENERATIVE MEDICINE: CURATING PLANARIAN EXPERIMENTS IN A CENTRALIZED MATHEMATICAL DATABASE

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For more than a century, scientists have been captivated by the regenerative capabilities of the planarian flatworm, which can regenerate a full body from almost any type of amputation. To understand the mechanisms controlling this extraordinary ability, research approaches based on surgical, pharmacological, and genetic manipulations have been used extensively to produce a huge dataset of experimental results disseminated through the literature. The rise of sophisticated machine learning algorithms and computational power has brought upon a novel way of studying these fascinating creatures. The Lobo Lab has developed a mathematical ontology for encoding regenerative experiments, together with an artificial intelligence method to automatically infer mechanistic models of regeneration. In this project, we have curated hundreds of additional experiments into a formal database, called Planform, which stores, in a mathematical language, thousands of planarian experiments and results performed in the past decade. The data includes the details of the experimental interventions, such as drug additions, genetic interference, and surgical manipulations, and their effects on the resultant morphologies. This curated dataset not only deal with the growth and shape of the worm, but also show how the presence, or the lack, of key genes, results in different patterns and morphologies in the worm. Planform is a freely-available, centralized collection of information that helps the scientific community search efficiently for experiments and morphologies published in the literature. More importantly, this resource is essential for the application of artificial intelligence methods to automatically reverse-engineer models that explain the regulatory mechanisms controlling the regeneration in these worms. The new dataset is already published and freely available in the dedicated website (<http://lobolab.umbc.edu/planform>), helping human scientists and the automated computational approach to find better models of planarian regeneration. All these efforts will pave the way for the next-generation applications in human regenerative medicine.

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A STUDY OF TOXOPLASMA GONDII IN A 3D MICROENVIRONMENT

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Over 60 million Americans may be infected with *Toxoplasma gondii* (*T. gondii*). Eating infected raw meat or contact with cat feces can allow oocysts containing the parasite to gain entry into an individual's body. Although most infected healthy people are asymptomatic, infection is particularly dangerous for the immunocompromised and the parasite can be transmitted congenitally. *T. gondii* infection is among the most fatal foodborne illnesses; hence, our lab's interest in the parasite's behavior in the human body. We are studying the parasite's behavior in the 3D microenvironment of collagen-coated matrices we have synthesized to mimic the in vivo cellular environment. The specific goals of our project are to characterize cell lysis and cytoskeleton remodeling after parasite infection. Our method has provided a more detailed understanding of cellular behavior, adhesion, and interactions in infected cells. By analyzing the trajectories of individual parasites, we found that the parasites remain within a small radius of the cell after lysis. To analyze the parasite's impact on the cytoskeleton (microtubule dynamics), we transfected VERO cells with an α -tubulin plasmid after infection and performed Fluorescence Recovery After Photobleaching experiments. In the future, we plan to evaluate the influence of cell density and genetics on the spread of infection since our initial data point to changes post-infection. In conclusion, our preliminary findings suggest *Toxoplasma gondii*'s behavior depends on cell geometry and its capacity to remodel the cytoskeleton.

Reference: Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, 14 Dec. 2015. Accessed: 15 July 2016.
<<http://www.cdc.gov/parasites/toxoplasmosis/>>.

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DYE SENSITIZED SOLAR CELLS

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Dye sensitized solar cells are a type of thin film solar cell used to convert sunlight into electrical energy through means different than conventional solar cells. Dye sensitized solar cells are made from materials that are biocompatible and biodegradable. The purpose of these solar cells is to eliminate the use of conventional, silicone, solar cells by creating solar cells that are environmentally friendly and that can product a higher efficiency rate of converting sunlight into electrical energy. Since these solar cells are thin film, this cause them to be very flexible and also very durable. This results in the optimization of the utilizations of these solar cells. They can be implemented in many aspects of daily life such as on windows, backpack, walls, and other things of that nature to generate sufficient amounts of electrical energy. The greatest benefit of these solar cells is that they use renewable resources, so there is no worry about depleting our energy source because our energy source is sunlight. In our research we have extracted anthocyanin from dark colored berries and used commonly available semiconductor titanium dioxide to make our solar cells. We have also investigated the effects of gap widths between the electrodes, effect of pressure and effect of nano-particles and 2D materials like graphene on the efficiency of the solar cells.

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INTERMITTENT PARATHYROID HORMONE ADMINISTRATION IN YOUNG AND OLD MALE FISCHER-344 RATS

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Background: Intermittent parathyroid hormone (PTH) administration improves bone mass and is a treatment for osteoporosis. This study compared the effects of intermittent PTH administration on bone parameters in young (4-6 mon) and old (22-24 mon) male Fischer-344 rats. **Methods:** Rats were divided accordingly: Young Control (YC, n=5), Young PTH (YP, n=5), Old CON (OC, n=5) and Old PTH (OP, n=5). PTH 1-34 was administered intermittently for 2-weeks. Left femora were processed, sectioned (10 μ m) and stained with Goldner's Trichrome. Bone microarchitecture (i.e., bone volume-to-total volume ratio [BV/TV, %], trabecular thickness [Tb.Th, μ m], trabecular number [Tb.N, /mm²], and trabecular separation [Tb.Sp, μ m]) was assessed in the distal metaphysis. Additionally, the following bone static properties were determined: osteoid surface-to-bone surface ratio (OS/BS, %), osteoblast surface-to-bone surface ratio (Ob.S/ BS, %), osteoblast number per total area (N.Ob/T.Ar, /mm²) and osteoblast number per bone perimeter (N.Ob/B.Pm, /mm²). Parameters were quantified utilizing Osteomeasure[®] software. Alpha level was set *a priori* at $p < 0.05$. Data are means \pm S.E. **Results:** Body mass was higher ($p < 0.05$) in OC (434 \pm 14g) and OP (432 \pm 13g) vs. YC (363 \pm 10g) and YP (370 \pm 4g). Bone volume was higher ($p < 0.05$) in YC (15.2 \pm 1.6%) and YP (17.6 \pm 1.7%) vs. OC (7.5 \pm 1.6%). Bone volume for OP (11.2 \pm 2.2%) tended to differ ($p = 0.053$) from YC and YP. Trabeculae were thicker ($p < 0.05$) in YC (18.3 \pm 1.3 μ m) vs. OC (12.8 \pm 1.2 μ m) and OP (13.9 \pm 1.3 μ m) and Tb.N was higher ($p < 0.05$) in YC (8.3 \pm 0.7/mm²) vs. OC (5.7 \pm 1.1/mm²). Tb.Sp was greater ($p < 0.05$) in OC (186 \pm 34 μ m) vs. YP (80 \pm 8 μ m) and tended ($p = 0.056$) to differ from YC (106 \pm 13 μ m) and OP (129 \pm 25 μ m). Additionally, OS/BS was augmented ($p < 0.05$) in OC (8.6 \pm 1.3%) vs. OP (3.6 \pm 0.7%) and tended ($p = 0.06$) to be higher than YP (4.4 \pm 1.3%). No other osteoblast parameter differed among groups. **Conclusion:** Declines in bone volume were observed in old rats and PTH tended to augment bone volume at this age.

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STUDIES ON THE ROLE OF ACTIVATED Cdc42 – ASSOCIATED KINASE ON NEURITE OUTGROWTH DEVELOPMENT IN CEREBRAL GRANULAR NEURONS

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Hypothesis: Extracellular matrix (ECM) proteins facilitate neurite extension of rat cerebellar granule neurons (CGNs) in culture. We have demonstrated that Ras-Cdc42 signaling pathways is associated with neurite outgrowth of cerebellar granule neurons (CGNs). We propose a hypothesis that Cdc42 effector (activated Cdc42 associated kinase, ACK) is involved in transducing Ras-Cdc42 signals in neurite outgrowth of CGN. In this report we studied several ACK inhibitors AU5 and AU6 to determine if they block laminin- and fibronectin-induced neurite outgrowth.

Methods: Extracellular matrix proteins (fibronectin or collagen) were coated on cell culture dishes. Cerebellar granule neurons (CGN) were seeded onto these culture dishes. Neurons were allowed to grow for 4-hours and then tyrosine kinase inhibitors of STK series were added individually in at various concentrations. The neurons were allowed to grow for additional 24-hours, followed by fixation with 4% paraformaldehyde and staining with anti- β tubulin III antibodies. The neurons were visualized under a Zeiss fluorescent microscope and images were captured. Length of neurite outgrowth was determined by using NIH image J software.

Results: We have successfully developed a primary neurite culture condition. Neuronal precursor cells isolated from young rat pups were found to develop neurites in our culture condition. We demonstrated that fibronectin and collagen stimulate neurite outgrowth as measured by their length. We also found that specific inhibitors of ACK blocked ECM-induced neurite outgrowth. Results of these studies will be presented in this report.

Conclusion: We have demonstrated that Ras-Cdc42-ACK signal transduction pathway is involved in ECM-mediated neurite outgrowth of CGN. Understanding the mechanism of Ras-Cdc42-ACK signaling pathway in neurons might be useful in developing a therapy for neurological disorders.

ANALYSIS OF TYROSINE HYDROXYLASE EXPRESSION IN PINK1 KNOCKOUT RATS OF THE SUBSTANTIA NIGRA AND STRIATUM

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Parkinson's Disease is the second most common neurodegenerative disorder affecting millions of people with no known cure. Parkinson's is characterized by: motor dysfunction, the loss of dopaminergic neurons in the substantia nigra (SN), and decreased dopamine (DA). Parkinson's Disease is mostly idiopathic; however, gene mutations produce rare familial forms of the disease. We are focusing on the PINK1 gene, which encodes a protein that provides protection against oxidative stress. PINK1 loss results in an alteration of mitochondrial buffering capacity, increased reactive oxygen species, and impaired cellular respiration; suggesting vulnerability to cell death. Earlier studies in PINK1 knockout rats described loss of DA cells by 8 months of age, with earlier dysfunction of the motor system.

We tested whether at 18 weeks of age PINK1 KO rats would have any change in tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis, in the substantia nigra (SN) and the striatum (ST) compared to the wild type, even if the cells had not yet died.

We examined the brains of 3 wild type (WT) and 3 PINK1 knockout (KO) rats, (18 weeks old). Animals were perfused with 4% paraformaldehyde. Horizontal sections (30 μ m) throughout the SN and ST were stained using immunohistochemistry. We examined the optical density of ST axons, and numbers of SN neurons.

Consistent with earlier findings, we saw no reduction of TH neurons in the SN in the PINK1 knockout animals; however, the differences in TH expression in the ST were striking. KO rats showed lower levels of TH compared to WT rats. We concluded that DA synthesis is likely reduced in ST by 18 weeks of age, even if the neurons were not dead. We plan to study if the decrease in TH is due to instability of the enzyme or deficits in its transport from the cell body.

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NGF REGULATION OF BONE-DERIVED PROSTATE CANCER CELL PROLIFERATION

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Prostate cancer (PCa) is the second leading cause of cancer-related deaths in American men and 180,890 new cases of prostate cancer will be diagnosed this year. Prostate cancer metastasis to bone occurs in approximately 2 out of 3 patients whose cancer has spread to other parts of the body. Previous evidence has suggested that growth-factor controlled pathways are altered during PCa metastasis to bone. One growth factor, nerve growth factor (NGF), is abundant in the human prostate, as well as bone, and the NGF receptors trkA and p75 are believed to play a role in PCa proliferation in bone. We hypothesize that NGF indirectly increases proliferation through upregulation of purinergic receptors of C4-2B4, metastatic PCa cells and increases sensitization to ATP.

The prostate cancer cell line, C4-2B4 (high metastatic), cells were plated on 100 mm dishes at a seeding density of 100,000 cells/dish. C4-2B4 cells were then treated with NGF (50ng/mL) or vehicle control (cell culture media). To determine the effects of NGF on proliferation of C4-2B4s, cells were counted after 24, 48, 72, and 96 hours of treatment. To determine the effect of NGF on the sensitivity of C4-2B4 cells to ATP, calcium imaging was performed. C4-2B4s were treated with NGF or vehicle control for 24 hours and then loaded with calcium chelator, Fluo-4 AM and imaged. Cells were treated with ATP (0.01, 0.1, 1 or 10 μ M) to determine if NGF treatment sensitizes C4-2B4s to ATP.

NGF was found to increase C4-2B4 proliferation by ~32% at day 4 when compared to control. These studies potentially indicate that NGF may play an important role in the growth of metastatic prostate cancer cells in bone. Calcium imaging studies will need to be completed to determine if NGF sensitizes metastatic prostate cancer to ATP to induce growth.

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INVESTIGATING TSC1 AND TSC2 MUTATIONS IN CORTICAL ORGANIDS

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Tuberous Sclerosis (TS) is a multi-system developmental disorder that results in high rates of epilepsy (~90%) and autism spectrum disorder (~50%). Neurological aspects of the disorder are characterized by the presence of cortical tubers, or regions of dysmorphic neurons and glia in the cortex. TS results from mutations in TSC1 or TSC2, genes encoding proteins that function as a heterodimeric complex negatively regulating the mTORC1 pathway. mTORC1 promotes cell growth and proliferation, and the absence of TSC1/TSC2 can lead to reduced autophagy and increased lipid and protein synthesis. Though the mTORC1 pathway is well-studied in dividing cells, it is not well understood in post-mitotic cells such as neurons. We seek to characterize the neurological form of TS through the formation of cortical organoids derived from embryonic stem cells with constitutive homozygous and heterozygous mutations of TSC1 or TSC2. These cortical organoids serve to model the development of the human cortex. Through immunofluorescence, we see proliferative marker Ki-67, and neuronal and glial markers such as Vimentin and PAX6 normally found in the human developing cortex. We then examine the effects TSC1 and TSC2 mutations in two downstream targets of mTORC1, S6 and 4EBP1, through quantification of protein expression and phosphorylation. By looking at different developmental time points in the formation of cortical organoids, we can better understand the consequence of TSC1 and TSC2 mutations in a human neuronal context.

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PRADER-WILLI SYNDROME (PWS) - SNORD116: NON-CODING RNA

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Prader-Willi syndrome (PWS) is a neurodevelopmental disorder caused by the deletion or silencing of the paternal 15q11-13 loci. Within this region, loss of expression of the *SNORD116* gene cluster on the paternal allele is sufficient to cause PWS. *SNORD116* is a non-coding RNA, which is naturally maternally imprinted. When the *SNORD116* gene is spliced, the exons fuse to produce the *SNORD116* Host Gene (*116HG*), which forms an RNA cloud. This RNA cloud regulates the expression of several diurnal genes. Additionally, the introns are processed to become *SNORD116* small nucleolar RNAs (snoRNAs). However, the molecular function of snoRNAs and its relevance to PWS is still unknown.

The formation of an RNA cloud from *116 HG* is a vital concept to creating potential therapies for PWS as well as understanding the pathology of other related disorders. The model mice used in this experiment were genetically modified paternal *SNORD116* knockout Prad mice, Complete transgenic (Ctg) mice, Host Gene transgenic (HGtg) mice, and Sno-Mutant transgenic (SMtg) mice. We hypothesized that restoring RNA cloud formation can rescue some of the symptoms of PWS observed in our PWS mouse model. This purpose of this experiment was to locate the transgene in thirteen Sno-Mutant Transgenic Prad mice by using a method called Inverse Polymerase Chain Reaction (Inverse PCR).

First, a restriction enzyme, DpnII, was used to cut the mice DNA and after ligation with T4 ligase, various primers were used to determine the precise position of the transgene in the genome of the mice samples. Although the location of the transgene in the Sno-Mutant mice is yet to be identified, further research should lead to discovering the factors that tether *116HG* to the DNA to form an RNA cloud; thus, taking us a step further in discovering a potential remedy for PWS.

THE STRUCTURE OF THE RECURRENT LARYNGEAL NERVE IN THE RAT

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Injuring a recurrent laryngeal nerve (RLN) alongside the trachea causes unilateral vocal-fold paralysis, hoarseness, shortness of breath, and possibly bacterial pneumonia. Potential drug treatments that promote RLN regeneration could be tested in rats as animal models. Our previous attempts to damage the RLN in rats, however, caused highly-variable degrees of injury. To improve our surgical approach, we investigated the anatomy of rat RLNs relative to the trachea.

Adult Sprague-Dawley rats were euthanized by deep anesthesia and transcardial perfusion with saline and formaldehyde fixative. The trachea with both RLNs was sectioned transversely at about 50 μ m thickness. Neurons were labeled with anti-neurofilament antibodies, and myelin with a lipophilic fluorophore. Brightfield and fluorescence microscopy were used to image a range of magnifications, from whole sections to individual nerve bundles. Morphological parameters, such as distances and angles between the RLNs, trachea, and esophagus, were measured with image-analysis software.

Our results indicate that the RLNs change their position with respect to the trachea from dorsomedial rostrally to purely medial caudally. The right RLN becomes more accessible on the tracheal surface caudally, whereas the left RLN remains protected in a groove between trachea and esophagus. Along the trachea, both RLNs are associated at different locations with a neurovascular bundle or an overlying fat pad and sprout multiple branches. The RLN bundles contain two classes of neurons that are distinguished by the thickness of their axons and myelin sheaths and are grouped together within the nerve bundle.

We conclude that the structure of the RLN in the rat is more elaborate than previously described. Our result suggests that a reliable procedure for RLN injury requires crushing or bisecting both the neurovascular bundle and the overlying fat pad to ensure that all branches are reliably damaged.

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INHIBITION OF CBP HAT ACTIVITY BY A NOVEL CURCUMINOID ALTERS THE CHROMATIN LANDSCAPE TO ABROGATE CELL PROLIFERATION

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Histone acetyltransferase (HAT) activity and molecular interactions are central to the functions of the transcriptional coactivator CREB-binding protein (CBP), which regulates transcription. The acetylation targets of CBP include histones, transcription factors and cellular proteins that are involved in key cellular processes. Functionally, CBP plays a central role in the activation of downstream target genes. Further, CBP is also involved in normal and disease process, including cancer, neurological disorders and viral infection. Therefore, blocking the acetylation function of CBP could be crucial in averting the disease pathogenesis. However, the biochemical consequences of blocking CBP HAT activity remain unclear. Notably, it has been a challenge to delineate the CBP-directed acetylation-specific function from the molecular interaction ability by its genetic manipulations. More than 2000 mammalian proteins undergo acetylation, but genome-wide CBP acetylation targets remain unknown. Here, we present a small molecule, namely, compound **CM354**, which inhibits CBP HAT activity; treatment of osteosarcoma cells with compound **CM354** led to the reduction in acetylation at lysine 27 on histone H3, which is concomitant with an increase in methylation at lysine 27. Collectively, compound **CM354** has potential to modulate the biological outcomes of CBP-mediated acetylation in normal and disease conditions.

IMPLICATION OF MYH10 IN THE ETIOLOGY OF PANCREATIC CANCER

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Pancreatic cancer is a deadly disease usually diagnosed late after metastasis. American Cancer Society estimates 53,070 new cases and 41,780 deaths in the United States by the end of 2016. Despite the deadly nature of the disease, little is known about its causes. However, altered expression of specific proteins including P53, STK15, HSP70, and SIRT1 has been reported in pancreatic cancer. This implicates these proteins in the etiology of the disease. Other studies have shown that the actin-myosin cytoskeletal protein, MYH10, is involved in the metastasis of cancer. Nevertheless, the specific role of MYH10 in pancreatic cancer is yet to be determined. Since MYH10 is involved in contraction of cells during migration, we hypothesize that its expression level may be important in the etiology of pancreatic cancer. To address our hypothesis, we analyzed the expression of MYH10 in BxPC-3, pancreatic cancer cells relative to the non-malignant HPDE-6 pancreatic cells. The two cell lines were cultured in 5% CO₂ incubators at 37°C. Nuclear extracts were prepared from both cell lines and protein concentrations determined by BCA assay. The proteins were resolved by SDS Polyacrylamide gel electrophoresis and MYH10 level was determined by Western blot analysis. Our results revealed that MYH10 was overexpressed approximately 3-5 times in BxPC-3 cells compared to the nonmalignant pancreatic cells. Furthermore, altered expression of other proteins including 15 kDa and 140 kDa proteins was also observed. These findings are consistent with our hypothesis and implicate the involvement of MYH10 in pancreatic carcinogenesis.

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OVEREXPRESSION OF HUMAN *SLIT1* IN THE ZEBRAFINCH SONG NUCLEUS RA PRODUCES CHANGES IN SINGING BEHAVIOR

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Vocal learning, the ability to learn vocalizations through imitation, is a rare trait found in select mammalian and avian species. Crucial to the development of spoken language in humans, this complex behavior is attributed to the connections of sensorimotor vocal circuits in the forebrain. One of these connections, a direct projection from forebrain vocal motor neurons to brainstem motor neurons, is unique to vocal learners and is associated with fine motor skill and learning. Genetic differences that form and maintain this projection, therefore, is hypothesized to be responsible for the evolution of vocal learning.

In a previous study, we had identified 55 candidate genes that contribute to the shared specialization of this forebrain region in humans, the laryngeal motor cortex (LMC), and in songbirds, the RA song nucleus. One of these genes, the axon guidance gene *SLIT1*, was found to be downregulated in the RA analog of all vocal learning avian species and the human LMC. To test whether the downregulation of this gene contributes to the maintenance of the RA to brainstem connection and the production of learned vocalizations, we overexpressed human *SLIT1* in the RA song nucleus of adult, male zebra finches. We then compared their songs before and after the genetic manipulations. Analysis of the temporal structure of the songs revealed a decrease in song similarity, song accuracy, and song sequential match after human *SLIT1* overexpression. Overall, these results suggest a role for this axon guidance gene in maintaining the stereotyped structure of learned songs.

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THE EFFECT OF OSCILLATORY FLUID SHEAR ON PROSTATE CANCER ATTACHMENT TO BONE MARROW ENDOTHELIAL CELLS

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Prostate cancer (PCa) is both the second most common and the second deadliest cancer among American men. However, most PCa patients die from metastasis of the cancer to the bone, leading us to postulate that metastatic prostate cancer cells preferentially adhere to human bone marrow endothelial (HBME) cells. Because both PCa and HBME cells experience shear forces during metastasis, we sought to determine if fluid shear stress (FSS) increases expression of membrane adherence proteins on either the PCa or HBME cells to increase adhesion of PCa cells to the HBME cells. Using calcein-labeled PCa cells, we examined the attachment of these cells to a monolayer of HBME cells during application of 5 dynes/cm² FSS for 2 hr. Using flow cytometry, we found that PCa cell attachment was higher in our static test groups compared to the sheared groups. Prior studies have indicated that nitric oxide (NO), a signaling molecule released during shear, plays a role in adhesion of PCa cells to HBME cells with reduced levels of NO leading to increased PCa adhesion. To reduce NO signaling, we pretreated both PCa and HBME cells with L-NAME, an inhibitor of NO synthesis. L-NAME Inhibition of NO synthesis increased the number of adherent PCa cells during FSS, but not in our static samples. Since the presence of shear has been shown to increase NO production, the absence of increased adhesion in the static + L-NAME samples may result from decreased NO production. Future studies include experimental design modifications such as increasing the viscosity of the shear medium to increase the wall shear experienced by the cells to better represent the shears resulting from blood flow. Also we will examine if racial differences in NO production could lead to a racial disparity in prostate cancer occurrence and metastasis.

COPPER BINDING AND REACTIVITY OF *DE NOVO* DESIGNED *DUE FERRI* SINGLE CHAIN (DFsc) PROTEIN VARIANTS

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Binuclear copper enzymes such as tyrosinase and catechol oxidase are involved in melanization and sclerotization processes. Understanding the kinetics of the diphenol oxidation activity in these enzymes is crucial for determining the nature of the enzyme-substrate complex and for the design of efficient target drugs and inhibitors. The *de novo* designed *Due Ferri* Single Chain (DFsc) proteins are water-soluble monomeric four-helix bundles that can readily bind metal cofactors. Several variants on this scaffold, namely mutants of G4DFsc and 3-HisG4DFsc, have been shown to mimic diiron oxidase activity. Dicopper binding has been suggested in mutants such as E11H/E44H-G4DFsc and E11H-3HisG4DFsc, which contain high numbers of His residues in their active sites similar to what is found in natural dicopper oxidases. In this study, copper binding was confirmed by circular dichroism spectroscopy. Catalytic oxidase activity for copper-bound DFsc variants was determined using UV-Visible spectrophotometric oxidation assays. Specifically, the formation of a *o*-quinone coupling product with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of the copper-protein complex and trihydroxybenzene (THB) at a rate faster than that of aqueous copper(II) is indicative of catechol oxidase-like behavior, and is supported by initial Michaelis-Menten kinetics studies. Catalytic oxidation was further supported by changes in activity when the assays were conducted under anaerobic and reductive conditions, and in response to active site variations.

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SYNTHESIS AND CHARACTERIZATION OF NEW METAL-ORGANIC MATERIALS INCORPORATING THE [HYDROTRIS(3-5-DIMETHYL-1,2,4-TRIAZOLYL)BORATE] LIGAND

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Six coordination complexes have been synthesized using the hydrotris(3,5-dimethyl-1,2,4-triazolyl)borate anion: $[\text{BH}(\text{dmtrz})_3]^- = \text{dmtris}$. “A(dmtris)” (A = Li, Na, K) were synthesized from the reaction between ABH_4 and excess 3,5-dimethyl-1,2,4-triazole (Hdmtrz) under flux conditions. Powder X-ray diffraction (PXRD) data show that each has a unique structure and may incorporate solvent. $\text{M}(\text{dmtris})_2$ (M = Co^{2+} , Ni^{2+} , Zn^{2+}) compounds were synthesized by the reaction between Na(dmtris) and $\text{M}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in methanol. Yellow-orange “Co(dmtris)₂”, lavender “Ni(dmtris)₂”, and colorless “Zn(dmtris)₂” prisms were isolated from the reaction mixtures between one hour and three days. PXRD data showed that these three compounds were isostructural. The synthesis and characterization of these materials will be presented.

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SYNTHESIS OF METAL COORDINATION COMPOUNDS DERIVED FROM 3-5-DIMETHYL-1,2,4-TRIAZOLE

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In an effort to explore the coordination chemistry of the 3,5-dimethyl-1,2,4-triazolate anion ($\text{C}_4\text{H}_6\text{N}_3^-$, dmtrz $^-$), its reaction chemistry was explored with metal ions including Mg^{2+} , Sr^{2+} , Cr^{2+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pr^{3+} , Nd^{3+} , Gd^{3+} , and Er^{3+} . Evaporation, solvothermal synthesis, and diffusion were used in an effort to crystallize new materials that incorporate this ligand. Single crystal X-ray diffraction (SCXRD) data indicate that two novel coordination compounds, $[\text{M}_3(\text{Hdmtrz})_6(\text{H}_2\text{O})_6](\text{NO}_3)_6(\text{H}_2\text{O})_6$ ($\text{M}=\text{Co}$, Ni), were synthesized by solvent evaporation of solutions of $\text{M}(\text{NO}_3)\cdot 6\text{H}_2\text{O}$ and Hdmtrz from ethanol and methanol. Both contain an $[\text{M}_3(\text{azole})_6(\text{H}_2\text{O})_6]^{6+}$ trimer, which has been observed in divalent metal-azolate systems. The trimer units are held together in the solid state by hydrogen bonding. The synthesis and structure of these compounds will be discussed.

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THE NATURE OF THE BRIDGING ANION CONTROLS THE SINGLE-MOLECULE MAGNETIC PROPERTIES OF DYX₄M 12-METELLACROWN-4 COMPLEXES

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Molecular magnetic properties result from the spin of unpaired electrons and magnetoanisotropy. Single-molecule magnets rely on a thermal energy barrier that separates electron spin states from one another forming a double well. This thermal energy barrier can be described by $E = S^2 |D|$, where E is the height of the barrier, S is the spin of the molecule, and D is the total magnetoanisotropy. Magnetoanisotropy implies that the magnetic moment of the molecule aligns in a single direction. Currently, there is a strong interest in studying single-molecule magnets because of their potential as spintronic devices, magnetic storage devices, and qubits.

One potential synthetic route to prepare single-molecule magnets is to employ a family of inorganic compounds called metallocrowns. Metallocrowns are often thought of as inorganic analogs of crown-ethers. Metallocrowns have proven to be a useful synthetic pathway in the field of single-molecule magnets for their ability to organize metal ions in a predictable geometry and because they allow for easy modification of synthetic parameters.

Here, we present a family of **DyX₄M(12-MC_{Mn}^{III}_{(N)shi}-4)** compounds that were synthesized and magnetically characterized (X = salicylate, acetate, benzoate, trimethylacetate, M = Na^I or K^I). The bridging ligands X , were systematically varied while keeping the remainder of the metallocrown geometry constant. The identity of monovalent cation, necessary for charge balance, was also altered. The dc magnetization and susceptibility of all compounds were similar across the series. Regardless of the identity of the counter-cation, the **Dy(Hsal)₄M 12-MC-4** compounds were the only compounds to show frequency-dependent ac magnetic susceptibility, a hallmark of single-molecule magnetism. This indicates that the nature of the bridging ligand in the **12-MC_{Mn}^{III}_{(N)shi}-4** compounds strongly affects the out-of-phase magnetic properties. The single-molecule magnet behavior appears to correlate with the pK_a of the bridging ligand.

SYNTHESIS OF RUTHENIUM MACROCYCLES

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Our research group works to synthesize and study the ion-binding characteristics of ruthenium host guest complexes. The host guest complexes consist of a "host" molecule that encapsulates a "guest" ion through non-covalent interaction. These complexes have many applications such as chemical signaling or sensing, and the removal of ions from aqueous environments. The goal of this project was to continue previous research done on macrocycles as host guest complexes. Two ruthenium macrocycles were synthesized and are in the process of being purified in order to make stock solutions. In the future, the stock solutions will be used to run fluorometric titrations to test the binding affinities of these host-guest solutions.

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RHODAMINE-BASED FLUORESCENT AND COLORIMETRIC CHEMO-SENSOR FOR METALS IN AQUEOUS MEDIA

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A rhodamine-based chemo-sensor for metal ions, (*E*)-3',6'-bis(diethylamino)-2-(((2-hydroxynaphthalen-1-yl) methylene) amino) spiro[isindoline-1,9'-xanthen]-3-one, was designed and synthesized. Compound 1 is colorless, non-fluorescent compound and was synthesized in microwave via one-step reaction of rhodamine B hydrazide with 2-hydroxy-1-naphthaldehyde. Metal ion complexation triggers formation of a highly fluorescent pink ring open form so it can be used as a 'naked eye' sensor for the presence of metal ions and anions. This research overall aims to synthesize a target compound by using a microwave, conduct UV-Vis and fluorescence studies, and study the sensitivity, selectivity, and reversibility of the compound.

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THE ATMOSPHERE OF CRYSTAL CAVE: UNDERSTANDING SOURCES AND SINKS OF TRACE GASES

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The atmospheric chemistry of cave systems has not been previously studied in depth; however, cave systems are prime locations to study potential sources and sinks for trace gas pollutants. Relatively constant temperatures, humidity, minimal air flow, and lack of sunlight create a stable environment that allows for biogeochemical processes to go on uninterrupted for extended periods of time.

Carbonyl sulfide (OCS) is one of the main contributors to air pollution globally, but many OCS sinks are not fully understood. A preliminary analysis of cave air from Crystal Cave in Sequoia National Park yielded OCS concentrations of 35.2 ± 0.7 pptv, approximately 16 times lower than the average concentration of 568 ± 8 pptv measured outside of the cave. In addition, the concentrations of several other trace gases such as alpha-pinene and methyl bromide were found to be abnormally low (10.5 ± 0.3 pptv inside and 387 ± 8 pptv for alpha-pinene, and 387 ± 8 pptv inside and 11.1 ± 0.4 pptv outside for methyl bromide). The cave air was found to be well-mixed as the concentrations of long lived halocarbons such as CFC 12 were similar inside and outside of the cave (545 ± 5 pptv and 538 ± 4 pptv respectively). This indicates that there may be one or more factors causing the cave to act a sink for several trace gas species. Further sampling and analysis of the atmosphere in the cave is required to draw any concrete conclusions about the unique environment presented here. The information gathered will help elucidate mechanisms for trace gas degradation, which could yield information about global trace gas budgets and their effect on global air quality.

COMPLIMENTARY USE OF RAMAN AND INFRARED SPECTROSCOPIES IN THE IDENTIFICATION OF SYNTHESIZED DIPHENYLACETYLENE

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Infrared spectroscopy (IR) is used for functional group identification in unknown organic compounds by detecting the absorption of light by a molecule. This phenomenon is related to the dipole moment change of the molecule during normal vibration. However, symmetric vibrations do not experience a change in dipole moment, making them IR-forbidden. Raman spectroscopy detects changes in polarizability as a molecule undergoes vibrational motions, making it a powerful detection method for symmetric molecules. Thus, the two spectroscopies are complimentary techniques for the analysis of chemical structures.

The synthesis of diphenylacetylene has proven challenging in the Organic Chemistry I lab. The procedure recommended the use of sand baths, which did not provide controllable heating. If students believed they had synthesized diphenylacetylene, they were unable to confirm their experimental success on the IR due to its symmetric triple bond. With the use of improved heating and recrystallizing methods, students are able to synthesize a larger yield of cleaner crystals. The Raman spectrometer allows them to confirm whether or not their product contains the expected triple bond.

Hands on experience with a multitude of analytical instruments allow STEM students to execute real-life identification of unknown compounds. Working with the Raman provides students with the opportunity to use a complimentary technique when the product structure is undetectable by the IR spectroscopy.

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SECOND-GENERATION METABOLITE SAMPLING BY MICROASPIRATION

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Knowledge of the full suite of metabolites produced in embryonic cells offers the potential to better understand mechanisms of development. As a downstream result of genomic, transcriptomic, and proteomic processes, the metabolome is a powerful descriptor of a cell's phenotype. However, characterization of the metabolome is challenging for single-cells because single cells contain limited amounts of metabolites with high turnover rates, span a broad concentration range, and diverse physico-chemical properties. Previously, we developed a single-cell capillary electrophoresis and electrospray ionization mass spectrometry (CE-ESI-MS) platform, which enabled characterization of single-cell metabolic heterogeneity. Whole-cell microdissection was used to isolate single cells from *Xenopus laevis* (frog) embryos, a popular model for cell and developmental studies. Microdissection becomes laborious when sampling adjacent and/or smaller cells characteristic of cells at later stages of development. In this work, our goal was to overcome this challenge by developing an approach that would allow spatiotemporal microaspiration of cellular content. To this end, micro-capillaries were fabricated from borosilicate capillaries and evaluated to ensure minimal invasion during sampling. This micro-capillary was used to aspirate intracellular content from live, developing embryos. The cellular content was then mixed with metabolite extraction solvent, vortexed, centrifuged, and 10 nL of the resulting supernatant was measured using CE-ESI-MS. Hundreds of signals were detected from the microaspirated cell sample. The data revealed that the developed approach minimized collection of artifacts arising from the sampling media, which in turn improved sensitivity, allowing to detect more metabolites compared to microdissection. Furthermore, the microsampling raises a potential for multiple sampling within the same embryo over different developmental stages. We envision this approach to be adaptable to various cellular models to foster deeper understanding of biological processes in situ and in vivo.

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MICROWAVE MEASUREMENT OF MICELLES IN NONPOLAR SOLVENTS

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Microwave measurements are beneficial in obtaining information about the electrical properties of solutions and molecules. A micelle is an aggregate system of amphipathic molecules which form spontaneously at a critical aggregation concentration (CAC). The study of the microwave properties of micelles, which are commonly used in industry and medicine, would provide valuable information about their formation and their behavior in solution. The prior method of microwave measurement at NIST worked well for polar molecules; however, nonpolar molecules which degrade polydimethylsiloxane (PDMS) channels could not be analyzed. In order to study micelles using the microfluidic microwave devices at NIST, a new system was designed to route fluids through the bottom of an aluminum chuck and into channels made of SU-8 photoresist polymer. A manifold was designed using computer-aided drafting and prototypes were 3-D printed. The manifold was machined and implemented to measure capacitance per unit length and permittivity of nonpolar fluids. This method is applicable for both polar and non-polar solutions and provides a convenient method to route fluids.

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PREFERENTIAL FORMATION OF STRUVITE FROM POULTRY LITTER

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An emerging renewable source for phosphorus-based fertilizers stems from the waste produced by poultry farms in the Delmarva Peninsula. Poultry litter, which is rich in nutrients, has recently become highly regulated under the Phosphorus Management Tool. Our preliminary research has shown that extracting phosphorus from poultry litter in an innovative Phosphorus Extraction And Recovery System (PEARS) can produce a high quality solid product. PEARS utilizes pH as the primary operating parameter to extract phosphorus into solution from solid poultry litter. Through the use of acidification and CO₂ bubbling to lower the pH, up to 85% of the total phosphorus is released into solution then followed by a solid/liquid separation. Thereafter, the pH of the supernatant is raised with the addition of a base (NaOH) and phosphorus is precipitated at high (~90%) recovery efficiencies in bench scale tests. Our aim in this study was to investigate the composition and quality of the mineral precipitates, i.e. organic carbon content, formed in the PEARs process. Three conditions were investigated: (1) dosing magnesium; (2) addition of ethylenediaminetetraacetic acid (EDTA); and, (3) addition of EDTA and magnesium chloride (MgCl₂). By altering precipitation conditions with the introduction of reagents, such as EDTA and MgCl₂, phosphorus-laden solids can be preferentially selected in the form of either struvite (MgNH₄ PO₄ .6H₂O) or hydroxyapatite Ca₁₀ (PO₄)₆(OH)₂. Moreover, the chelating agent EDTA preferentially complexes with calcium over magnesium in solution allowing for selective precipitation of struvite. Furthermore, Scanning Electron Microscopy and Energy-Dispersive Spectroscopy analysis were performed to characterize the composition and morphology of the recovered precipitates. Overall, these results provide insight into the conditions for selective precipitation of phosphorus, which will aid in designing composition specific phosphorus based fertilizers.

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TRACING MARINE PARTICULATE ORGANIC CARBON FROM PRIMARY PRODUCTION TO SEDIMENTATION

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Phytoplankton are primary producers of oceanic organic carbon, most of which is recycled back to CO₂ as their detritus sinks down the water column. A small fraction of organic carbon persists and is ultimately buried in marine sediments, a crucial mode of removing CO₂ from the atmosphere. We analyzed the chemical composition of particulate marine organic matter that persists to sedimentation. Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), we analyzed fresh phytoplankton, partially degraded phytoplankton, and highly degraded sedimentary particles to trace the fate of particulate organic carbon from primary production through sedimentation. We examined isolated membranes of different phytoplankton species, including the coccolithophore *Emiliana huxleyi*, the diatom *Thalassiosira* sp., and the green alga *Tetraselmis* sp. We also analyzed *Tetraselmis* membranes subjected to oxidative treatments, as a representation of partially degraded phytoplankton. Deepwater sediment trap samples from the Arabian Sea represented highly degraded oceanic particulates.

The ATR-FTIR spectra reveal an aromatic peak around 3013 cm⁻¹ in fresh samples of *E. huxleyi* and *Tetraselmis* sp. This peak persists after H₂O₂ oxidation of *Tetraselmis* but disappears upon NaOCl treatment. Alkane C-H peaks at 2800 cm⁻¹ to 3000 cm⁻¹ and 1400 cm⁻¹ to 1450 cm⁻¹ also decrease in intensity with the introduction of oxidants. The FTIR spectra of sediments are dominated by carbonate and silica peaks, reflecting the mineral components of the samples. Acidification to remove carbonate reveals distinct organic carbon features in the sediments, including alkane and protein peaks at 1640 cm⁻¹, 1548 cm⁻¹, and 1459 cm⁻¹. After NaOH removal of biogenic silica, carboxylate and aliphatic peaks appear at 1547 cm⁻¹ and 1411 cm⁻¹, respectively, suggesting that NaOH treatment saponifies fats and implying the presence of lipids in the sedimentary particles. These experiments have thus uncovered remnants of cellular proteins and lipids that are destined for burial in the sediment.

THE USE OF CHITIN, CHITOSAN AND THEIR DERIVATIVES IN REVERSIBLE CARBON DIOXIDE CAPTURE

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Carbon dioxide pollution is among the top challenges facing society due to its suspected effect on global temperatures through the greenhouse mechanism. One approach for reducing carbon emissions is a set of methods called carbon capture and sequestration (CCS), which targets high concentration sources of CO₂ pollution by using chemical means to prevent the CO₂ from entering the atmosphere. Unfortunately, the most successful materials used in CCS react with CO₂ so strongly that it is difficult to separate the material from the captured gas. As a result it is necessary to deposit vast amounts of bound CO₂ and capture material in nature creating a new environmental problem in the process. Other more favorable materials that could be used in CCS are currently too expensive to mass produce. Our lab is preparing and evaluating chitin and chitosan as potential reversible CO₂ capture materials. These are compounds which can be easily isolated from industrial seafood waste and applied to CCS systems. In this work, we quantify the effectiveness of chitin and chitosan in reversible carbon capture, present an efficient method for the extraction of chitin and chitosan from biological sources, and determine the concentrations of chitin in a variety of seafood waste types including crustacean and mollusk shells.

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MACROPOROUS RESINS FOR ANTHOCYANIN EXTRACTION FROM ARONIA MITSCHURINII BERRIES

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Aronia Mitschurinii, commonly called black chokeberry, is an edible member of the Rosacea family native to the north eastern U.S. and naturalized in Eastern Europe. Cultivated as a specialty food crop, it is extremely high in antioxidants, with concentrations significantly higher than even the acclaimed acai berry. Specifically, aronia is high in anthocyanins, which give both the skins and flesh a deep purple color. As antioxidants, anthocyanins serve as potent free radical scavengers. This ability along with their color make them useful in applications such as human health care, pigmentation, food preservation, and antifouling coatings.

Recently, the attempts to extract antioxidants from fruits and berries has been done using polymeric macroporous resins, but it had never been applied to *Aronia Mitschurinii*. This project aimed to investigate this option, not only by developing an extraction method, but also by determining which of the food grade resins available on the market is best suited to the process. To that end, four different resins were treated with juice followed by food grade acidified ethanol to obtain crude anthocyanin extracts. All solutions, pretreated juice, post-treated juice, and final extract were tested for anthocyanin and flavonoid content using UV/Vis spectroscopy to determine extraction efficiencies. ANOVA analysis was performed to verify statistics. Results demonstrated that Amberlite resin FPX66 is the most efficient of the tested resins.

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ISOLATION OF AILANTHONE FROM THE LEAVES OF AILANTHUS ALTISSIMA

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Ailanthone, isolated from the leaves of *Ailanthus altissima*, has been observed in our lab. *Ailanthus altissima* has been shown to exhibit antioxidant, antimalarial antiviral, antifungal, insecticidal, antituberculosis, antiasthmatic, and phosphodiesterase inhibitory activities. Ailanthone, one of most studied biologically active compounds present in *Ailanthus altissima*, belongs to a class of bitter-tasting compound called Quassinoids. Our primary goal was to isolate ailanthone from the leaves of *Ailanthus altissima* by using soxhlet extraction and chromatographic purification. Less than 2 mg of ailanthone was isolated from 38.63 g of leaves. Future work should provide more ailanthone for future biological studies.

The authors acknowledge the Niemeyer-Hodgson Student Research Grant, the Noonan Endowment Award, the Millersville University Student Research and Creativity Grant. These results were obtained during the summer of 2016 as part of an Independent Research program in the Department of Chemistry at Millersville University. We would also like to thank the Chemistry Department Faculty at Millersville University, Mr. Stephen Peurifoy, and the Dean of the College of Science and Technology.

OPTIMIZATION OF NATURAL PRODUCT EXTRACTION FOR INCORPORATION INTO AN UPPER-LEVEL UNDERGRADUATE ORGANIC SYNTHESIS LABORATORY: EFFICIENT ISOLATION AND DERIVATIZATION OF EUGENOL

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A quick and economical pressurized hot water extraction method for the isolation of eugenol from cloves, based on the work of Just and coworkers, is being optimized for incorporation into an upper-level undergraduate organic synthesis laboratory; by the use of a commercially available espresso machine, up to 0.58g of eugenol has been isolated from 15g of cloves. Currently, the synthesis of (E)-4-(4-hydroxy-3-methoxyphenyl)but-2-en-ol, based on the cross-metathesis of eugenol and cis-2-butene-1,4-diol reported by Taber et al., and the dimerization of eugenol, reported by Blackwell et al., are being investigated using a quantitative NMR internal standard. This work will be developed into an upper-level undergraduate research based teaching laboratory focused on the study of natural product extraction, characterization techniques, methods optimization, and synthetic derivatization. Additionally, students will use the cross-metathesis product as the starting material for producing a detailed multi-step synthesis plan based on their prior knowledge of organic reactions, SciFinder searches, and chemical literature.

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MODEL SYSTEMS TO STUDY G-QUADRUPLEX-HEMIN BINDING AND CATALYSIS

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Guanosine and its derivatives self-assemble into ordered structures, known as G-quadruplexes, in the presence of certain cations. These supramolecular structures are held together through an array of ion-dipole, hydrogen bonding and π -stacking interactions. G-quadruplexes can form in DNA sequences rich with repeating G bases, and in both aqueous and organic solutions of guanosine derivatives. The unique self-assembly properties and the π -stacking capabilities of G-quadruplexes make them interesting targets in supramolecular chemistry for building π -stacking based assemblies.

Iron (III) protoporphyrin IX, also known as hemin, is a large planar, aromatic compound, which has been shown to bind to G-quadruplex DNA. This G-quadruplex DNA/hemin adduct has been found to catalyze various oxidation reactions, such as peroxidase and oxygen transfer reactions, at rates comparable with enzymatic hemoproteins. The mechanism by which G-quadruplexes induce this enhanced catalytic activity in hemin remains unsolved.

The goal of my research is to use synthetic G-quadruplex systems to understand how G-quadruplexes interact with hemin and give rise to catalytic activity. I aim to use these synthetic G-quadruplex-hemin complexes to also explore new catalytic applications of these adducts. By using lipophilic guanosine derivatives that form well-defined G-quadruplex structures in organic solution, the G-quadruplex-hemin interaction can be studied by NMR. To explore the catalytic applications of the G-quadruplex-hemin complex, I employ a G-quadruplex based guanosine hydrogel. I have demonstrated that when hemin is incorporated into this gel, it can oxidize several commonly used peroxidase substrates. To our knowledge, this is the first example of a simple monomeric guanosine based G-quadruplex enhancing hemin's catalytic activity.

By understanding the mode of binding of hemin to a G-quadruplex and how this binding induces enhanced catalytic activity in hemin, we may be able to synthetically modify G-quadruplexes to modulate catalytic properties of this complex.

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CHARACTERIZATION OF THE ACTIVE SITE OF VANADIUM HALOPEROXIDASES AND THE F397H MUTANT USING ⁵¹V MAGIC ANGLE SPINNING NMR SPECTROSCOPY

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Vanadium-dependent haloperoxidases (VHPOs) are a class of enzymes found in a variety of marine and terrestrial organisms^{1,2}. VHPOs catalyze a two-electron oxidation of halide ions by hydrogen peroxide, and are named after the most electronegative halide ion that they can oxidize; for example, vanadium chloroperoxidase (VCPO) can oxidize Cl⁻, Br⁻, and I⁻. All members of this family require a vanadate cofactor (VO₄³⁻), which is necessary for catalysis and have very similar active site geometries. Although the active sites of chloroperoxidase and bromoperoxidase (VBPO) differ by only one amino acid (His411 in VBPO is replaced by Phe397 in VCPO), these enzymes exhibit high substrate specificity: VBPO does not oxidize Cl⁻ efficiently³.

Despite several biochemical studies, the catalytic mechanism and the substrate specificity of VHPOs are poorly understood. Vanadium in the vanadate cofactor is in a diamagnetic V(V) state, which prevents its characterization with EPR and UV-vis spectroscopies. A combined approach of ⁵¹V magic angle spinning (MAS) NMR spectroscopy and Density Functional Theory (DFT) calculations has been shown to provide detailed characterization of the electronic and coordination environment of vanadium centers in VHPOs⁴⁻⁶.

In this work we present a ⁵¹V MAS NMR investigation of VCPO, its F397H mutant, and VBPO. The F397H mutation in VCPO is expected to transform its active site into a mimic of the active site of VBPO. Characterization of the electronic and geometric properties of the vanadate cofactor in VCPO, its F397H mutant, and VBPO with ⁵¹V MAS NMR and DFT calculations will help towards the understanding of the substrate specificity and catalytic activity of this class of enzymes.

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IMPACT OF INCORPORATION OF α -ALKYLATED AMINO ACIDS ON β -HAIRPIN FOLDED STABILITY

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Peptides are short sequences of amino acids that can mimic the shape, and therefore function, of larger proteins. Peptide therapeutics can potentially be used for the treatment of a wide range of diseases such as HIV, diabetes, and cancer. Peptides are naturally broken down by enzymes within the body through a process called proteolysis, but the introduction of unnatural amino acids into the sequence can slow this process, potentially increasing the lifetime of peptide therapeutics. We have examined the impact of incorporation of α -alkylated amino acids, unnatural amino acids in which the α -proton is replaced by an alkyl side chain, into short, β -hairpin peptides. Four α -alkylated amino acid derivatives with varying side chain lengths were incorporated into peptides using Fmoc solid-phase peptide synthesis. These peptides were then analyzed using homonuclear multidimensional-NMR spectroscopy to determine the propensity of the unnatural amino acids to promote a β -hairpin folded conformation. These analyses showed that the α -alkylated amino acid with two side chains consisting of one carbon unit completely disrupted the folding population of the model system. However, increasing the side chain length of the α -alkylated amino acids to two or more carbon units restores some of this folded stability.

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INFLUENCE OF MOLECULAR WEIGHTS ON CHARGE TRANSPORT PROPERTIES IN COMPLEMENTARY SEMICONDUCTING POLYMER BLENDS

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Molecular weight plays an important role in polymer's physical properties. It also impacts the morphology of corresponding thin films. This project is aimed to study the effect of molecular weights on the electronic performance of the donor-acceptor type semiconducting polymer, poly(diketopyrrolopyrrole-*co*-thienothiophene) (DPP-TT) and its complementary semiconducting polymer blends.

DPP-TT was synthesized using Stille coupling polymerization. Recycling gel permeation chromatography was utilized to fraction the polymer into different molecular weights. The obtained fractions were characterized using gel permeation chromatography (GPC), UV-Vis spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy. Organic field-effect transistors were fabricated with these polymers and their blends.

CHARACTERIZATION OF BIODEGRADABLE POLYMERS USING TGA-GC/MS

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The goal of this experiment is to analyze standards of three common biodegradable polymers (polybutyl methacrylate, polyvinyl acetate, and polyvinylpyrrolidone) compared to those found in a commercial coronary stent using a PerkinElmer Pyris 1 TGA and a PerkinElmer Clarus 600 GC/MS, a hyphenated instrumental technique to streamline characterization and reduce sample preparation. Polymers play an integral role in the biomedical field, including drug delivery systems, tissue engineering, and heart valve replacements. In order for biodegradable polymers to be utilized in an efficient manner, it is important to know the physical and chemical properties of the compound. In the biomedical field, stents such as drug eluting stents for coronary patients, are beneficial in order for a better quality of life. Stent surfaces are covered in a polymer coating that serves as a drug carrier to permit the release of the drug properly. The stent should be designed appropriately in order to maximize the performance and minimize any fatal consequences. Thermogravimetric Analysis (TGA) allows the mass of a sample to be observed as the temperature changes in a controlled atmosphere as a function of time or temperature. Heating in the TGA causes the sample to degrade and transfer to the Gas Chromatography (GC) instrument. The sample's components are separated in the GC, and the peaks identified by Mass Spectrometry (MS). Coupling TGA with GC/MS allows for a multi-dimensional analysis of the polymers of interest by correctly identifying the broken down monomer for accurate, efficient characterization. Being able to characterize materials used in biomedical research greatly improves the industry and advances future material safety and quality.

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SOLID STATE CHARACTERIZATION OF HYBRID COPOLYMERS WITH PHOTOVOLTAIC APPLICATIONS

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Hybrid organic-inorganic bulk heterojunction (BHJ) photovoltaic devices have recently gained interest within the scientific community for their potential to offer higher efficiency solar cells at a lower cost than those in the current market. Typically, these devices consist of a donor (organic) and an acceptor (inorganic) material that have been combined in a simple mixture to allow for charge transfer at their phase interfaces. However, the orthogonal properties of the two materials cause them to interact poorly, resulting in decreased charge transfer. This has proven to be a large obstacle to developing a more ideal hybrid solar cell.

To overcome this issue, we synthesized a new series of copolymers via Stille coupling with a constant amount of $(\text{Me}_3\text{Sn})_2(\text{C}_4\text{H}_2\text{S})$ and varying ratios of $\text{Co}_6\text{Se}_8(\text{Br}(\text{C}_4\text{H}_2\text{S})\text{P}(\text{Ph})_2)$ (**1**) to $\text{Br}_2(\text{C}_4\text{HS})(\text{CH}_2)_5\text{CH}_3$; these copolymers have been named poly(cluster-co-thiophene-co-hexylthiophene)a-c (**PCLTHTa-c**). Our hypothesis was that by covalently attaching the donor and acceptor materials, it would be possible to increase favorable interactions within the materials. Recently, we successfully showed increased charge transfer within the copolymers in solution compared to representative simple mixtures. However, photovoltaic devices require thin films of the materials to function; therefore, solid state characterization is necessary before these materials can be implemented. Herein, we present recent progress on the thin film characterization of **PCLTHTa-c** using UV-visible spectroscopy, atomic force microscopy, and scanning electron microscopy.

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A COMPUTATIONAL INVESTIGATION INTO THE MECHANISM OF THE HISTONE ACETYLTRANSFERASE, GCN5

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Post-translational modifications (PTMs) can have a profound effect on protein structure and function. One such PTM is the acetylation of a histone (a protein involved in DNA binding). In this reaction, an enzyme catalyzes the transfer of the acetyl group from acetyl CoA to a free lysine on the histone. This transfer neutralizes the positively charged lysine, which allows the DNA to be exposed for transcription. In our study, we focus on the acetyltransferase, Gcn5. Details regarding the reaction mechanism used by Gcn5 remain obscured. However, current mechanistic hypotheses suggest that the reaction occurs through a tetrahedral oxyanion intermediate, which is stabilized by a hydrogen bond to a nearby residue, i.e. an oxyanion hole. We utilize molecular dynamics (MD) simulations and quantum mechanics/molecular mechanics (QM/MM) calculations in order to further probe possible mechanistic schemes of this reaction.

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HOMOCHIRALITY IN METAL AMINO ACID COMPLEXES

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This study investigated whether homochirality could be induced in metal complexes of chiral amino acids. An earlier study showed that, as the number of chiral centers around a crowded metal complex was increased, the tendency for homochiral complexes to precipitate was increased. In this investigation, metal complexes of several amino acids were prepared using a stoichiometry of 1-3: metal to amino acid and partially characterized. Crystalline products will be analyzed by x-ray crystallography. Using the Cache/Scigress program, structures of tris sarcosine complexes of cobalt were constructed. While unbound sarcosine is not chiral, upon binding, the nitrogen atom becomes chiral. The models of the complexes were constructed having the chirality of Δ RRR, Δ RRS, Δ RSS, Δ SSS, and their enantiomers. Calculations of the energies of the various diastereomers show that the most stable isomer has homochiral ligands. At this point, no structural information has been obtained and is pending results of the crystallographer, although infrared spectra indicate complex formation. Results of the modeling study indicate that homochiral tris complexes of amino acids are favored.

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COMPARING FORCE FIELDS WITH DENSITY FUNCTIONAL THEORY IN SMALL, SOLVATED PEPTIDES

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It is well known that a protein's structure is related to its function and that misfolded proteins can cause diseases. Therefore, understanding how proteins fold or misfold is an important topic of research. Simulations often provide critical insight into this process. However, when studying protein folding/misfolding *in silico*, it is critical that the simulation is reliable. The purpose of this research is to test the reliability of molecular mechanics force fields (a common technique used to study protein structure) by comparing force field calculations to density functional theory (DFT). Ultimately, this information will be used to predict the effect of force field errors in the computational determination of protein folding mechanisms. Being able to detect and correct for force field bias before a simulation can make molecular dynamics (MD) a more powerful tool for determining protein-folding mechanisms. Specifically, we use force fields and DFT to calculate the energies of a small, solvated peptide (ACE-ALA-GLY-ALA-NME) in one of five different secondary structures of α_L , α_R , β , β_R and PP-II. The solvation environment for each secondary structure was equilibrated at room temperature while the peptides were held fixed. Five frames were then chosen from each equilibration and the energies of the peptides plus solvation were calculated with the Amber force field (ff12SB), the CHARMM27/CMAP force field, and the M06-2X density functional. M06-2X was performed in combination with various basis sets including: dgdzvp, aug-cc-pvtz, and jun-cc-pvqz. Preliminary results indicate that the solvation environment mitigates the effect of basis set size seen in gas phase calculations.

DFT INVESTIGATION OF METALLA-DIELS-ALDER: TRIGGERING CHEMOSELECTIVITY VIA THE INTRODUCTION OF METAL FRAGMENTS TO 1,3-BUTADIENE

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A DFT study was performed in which the -CH- fragment of 1,3-butadiene was replaced with its with isolobal metal fragments, -Rh(PH₃)₃- (Ru), -Rh(PH₃)₂H- (Rh1), -Ru(PH₃)₃H- (Rh2), to investigate whether Diels-Alder cycloaddition (DA) occurs for each isolobal analogues of 1,3-butadiene. Ru underwent concerted DA cycloaddition, which was the minimum energy path. Rh1 underwent stepwise DA cycloaddition, while the concerted DA cycloaddition was energetically unfavored between the two mechanisms. Rh2 underwent concerted cyclopropanation. By the means of isolobal analogy, we were able to trigger chemoselectivity between DA cycloaddition and cyclopropanation. Herein, the mechanistic and thermodynamic details of each systems will be unpacked.

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DESIGN AND SYNTHESIS OF TRIAZOLE HETEROCYCLES FOR MOLECULAR RECOGNITION OF A-U AND G-C BASE PAIRS IN DOUBLE STRANDED RNA

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DNA and RNA play vital roles in the human body. While DNA carries the genetic code, RNA is useful in transcribing genetic information into proteins. With the aim of molecular recognition, nucleic acids can form triple helices with double-stranded DNA (dsDNA) through selective Hoogsteen binding interactions with the Watson-Crick base pairs, but triple helix formation with dsRNA is a more challenging endeavor. Single-stranded DNA and RNA can bind into the deep and narrow major groove of dsRNA, although both the sequence selectivity and the stability of the triplex are poor. However, peptide nucleic acids (PNA), which contain a charge neutral backbone, selectively bind dsRNA at physiological conditions with increased affinity. This molecular recognition could lead to biomedical advances including anti-gene agents or chromosome mapping through sequence specific oligonucleotides. Our research focuses on the design of novel nucleobases for incorporation into PNA and with the goal of selective recognition of Watson-Crick base pairs through Hoogsteen binding.

In attempt to find the lowest energy species, novel nucleobases were docked with A-U and C-G base pairs. Then, binding energies and hydrogen bond distances were calculated using semi-empirical methods. Two triazole heterocycles were targeted for synthesis based on planarity and energetic favorability. A 1,2,3-triazole was synthesized where a uracil derivative and phenol were linked together by the triazole ring. The Sonogashira reaction was performed to synthesize 5-ethynyl uracil which reacted with an azido phenol in a [3+2] cycloaddition to form the 1,2,3-triazole target. The second molecule selected contained a 5-amino-1,2,4-triazole tethered to a quinoline ring. Our strategy involves first forming 5-amino-1,2,4-triazol-3-ylacetic acid and esterifying. Then, a coupling with 7-bromoquinoline will form the target. After synthesis, these molecules will be incorporated into PNA and binding affinities with dsRNA will be tested.

We would like to thank Elizabethtown College Department of Chemistry and Biochemistry, the Summer Scholarship, Creative Arts Research Program (SCARP), and Dr. Jane E. Valas for funding this project. We would also like to thank the 2015-16 CH 455/456 students for conceiving the idea, and to our collaborator Dr. Eriks Rozners (University of Binghamton).

SYNTHESIS OF N-N-PENTYL-3-PYRIDYL PYRROLIDINE ETHER

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Nicotine is the main addictive chemical that is typically found in tobacco. The Nicotinic Acetylcholine Receptors (nAChRs) are named because they are stimulated by nicotine, which is the basis for their identification. In fact, these receptors are clinically relevant in the regulation of normal neurological pathways and in the management of CNS disorders, such as Alzheimer's disease, Parkinson's disease, Attention Deficit Hyperactivity Disorder (ADHD), schizophrenia, and cigarette addiction. Current available chemical compounds are still unable to adequately discriminate between nAChRs, and it's essential for new chemical compounds to be designed, synthesized, and tested for their ability to discriminate between nAChRs. Additionally, our understanding of nAChRs largely depend on availability of compounds that can discriminate between them. We build our work on 3-pyridyl pyrrolidine ether compounds by attachment of a Pentyl chain to the nitrogen on the pyridine ring. As hypothesized, the attachment of a pentyl chain to the nitrogen in the pyridine ring will change the distribution of electron on the pyridine ring. A change of electron on pyridine ring will affect the binding affinity, selectivity, and activity of the resulting compound at nAChRs. The N-n-pentyl-3-pyridyl pyrrolidine ether was synthesized in two steps using Mitsunobu reaction and by reflux of an intermediate 3-pyridyl pyrrolidine ether with 1-bromopentane. Chromatography and Nuclear Magnetic Resonance (NMR) was used to analyze and characterize the resulting compound of interest. Future work will pharmacologically characterize the new compound at nAChR subtypes.

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DESIGN, SYNTHESIS OF AND EVALUATION OF SELECTIVE INHIBITORS OF THE MONO-(ADP-RIBOSYL)TRANSFERASE, PARP-14

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Poly (ADP-ribose) polymerase-1 (PARP-1) is a well-established drug discovery target for cancer due to its involvement in DNA repair mechanisms. Drug studies over the past three decades have focused on inhibition of PARP-1, whereas many of the other 16 members of the PARP family have not been extensively explored. Research is branching out to other members of this family because it was discovered that most of the PARP-1 inhibitors also non-specifically inhibit other members of the PARP family. The pharmacological effect of inhibiting these other members of the PARP family is unknown, *thus specific inhibitors or probes must be designed for other members of the PARP family in order to delineate the effects of this inhibition*. One of the other members of this family is PARP-14 (a.k.a. ARTD8) a mono-(ADP-ribosyl) transferase. This protein has been studied in recent years and its upregulation has been linked to various diseases including multiple myeloma. A recent study used a docking based virtual screen to identify several weak inhibitors of PARP-14, one of which was (Z)-4-(3-carbamoylphenylamino)-4-oxobut-2-enoic acid, a low MW, modular, lead compound with X-ray co-crystal data. *The goal of this research is to design, synthesize and test a wide variety of compounds with similar key characteristics to this initial lead while eliminating the PARP-1/2 activity. This derivatization focused on replacing the 4-oxobut-2-enoic acid group with a more benign, less toxic isostere and at the same time branching out into the large ADP-ribose binding pocket of PARP-14.* A synthetic route was established for two separate series of compounds: 1) the benzamide lactams; and 2) the diaryl ethers, neither of which should inhibit PARP-1/2 based on design and the known PARP-1 pharmacophore. Several amide-based analogs from the series of diaryl ethers showed >60% inhibition of PARP-14 @ 5 μ M, representing a potential 100 fold selectivity over PARP-1.

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IMPROVING PROPERTIES OF POLY-VALEROLACTONE THROUGH ARYL RING STACKING

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Biodegradable plastics are of increasing interest in the realm of renewable resources. Non-biodegradable plastics use carbon chains and aryl rings as a main component of the polymer plastic backbone. Polyhydroxyalkanoates (PHA) integrate oxygen into the backbone, which makes the polymer biodegradable by certain bacteria and organisms. These molecules have reduced mechanical properties, such as brittleness, which make them unusable for everyday plastic uses. This is primarily due to low molecular weight chains, as the polymerization is limiting.

My research involves taking δ -valerolactone, substituting the α -carbon with aryl rings possessing 4-nitro or 4-methoxy functional groups, and polymerizing the modified valerolactone with ring-opening polymerization. Upon deprotecting the nitro to an amine and the methoxy to a hydroxyl, the product will have increased order and mechanical properties due to the aryl π -stacking and hydrogen bond donors/acceptors. The higher ordered systems will make a stronger polymer plastic that can potentially replace non-biodegradable plastics and still retain the biodegradable characteristics that are valued. I will examine how the conjugated system and differing functionality affect the biodegradable polymer's mechanical properties, including crystallinity, melting point, and tensile strength. GPC will be used to test for molecular weight and degree of polymerization and DSC will be used to determine the new polymer's melting point and ability to absorb heat.

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A NOVEL METHOD FOR THE HYDROLYSIS OF A NICKEL SCHIFF-BASE COMPLEX

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Polypeptides are sequences of amino acids that can fold into secondary structures such as α -helices and β -sheets. Incorporating unnatural amino acids into polypeptides can influence folding of the secondary structures. Many unnatural amino acids can be synthesized using a nickel Schiff-base complex. Standard decomposition techniques of this complex require refluxing with hydrochloric acid. These harshly acidic conditions can remove acid-labile protecting groups from the side chains of the desired amino acids. To circumvent this issue and to provide synthetic access to additional unnatural amino acids with side chain protecting groups, we have devised a novel method of hydrolysis using EDTA, a chemical chelating agent that can sequester metal ions. These conditions prevent the loss of side chain protecting groups while enabling facile hydrolysis of the nickel Schiff-base complex.

SYNTHESIS AND CHARACTERIZATION OF COMPOUNDS TO STUDY CONCERTED PROTON-ELECTRON TRANSFER

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Phenothiazines are a class of chemical compounds that have a wide variety of uses, such as for alternative energy, pharmaceuticals, and tranquilizers. In our study, we focused on the studying pyridine-phenothiazine (Py-PT) radical cations. More specifically, our goal is to synthesize substituted Py-PT radical cations to observe the effect of structure on the mechanism of proton and electron transfer with hydrogen atom donors; a concerted mechanism, concerted proton-electron transfer (CPET), is expected. We expect to investigate the kinetics of these reactions. Currently, we are investigating a tethered compound with a four-methylene group unit between the two rings with the intention of comparing it to the compound with no tether. Three steps of the synthesis of the radical cation precursor are completed and work toward the last step is described. Additionally, we characterized radical cations of previously synthesized Py-PT compounds via UV-vis spectroscopy.

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SYNTHESIS OF SMALL-MOLECULE INHIBITORS FOR BOTULINUM NEUROTOXINS

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Botulinum neurotoxins (BoNTs) are some of the deadliest naturally occurring toxins. BoNTs are produced by a common bacterium found in soil called *Clostridium botulinum* and are commonly prescribed medicinally for their ability to locally paralyze muscles at low doses. Based on previous research, a matrix of small-molecules was created in order to investigate the effectiveness of certain features in inhibiting the activity of botulinum neurotoxin serotype A. Each molecule synthesized has a counterpart, or a series of counterparts, with slight structural variations. Biological assays will be used to measure the BoNT inhibition of these molecules and the structure-activity relationship will be further refined from the results.

BIOCATALYTIC REDUCTION OF KETONES AND IMINES USING *DAUCUS CAROTA* ROOT

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Whole vegetables are currently sought as an alternative to traditional reducing reagents in Organic reactions because of their effectiveness in aqueous solvents, very low environmental impact, availability in developing countries and their impressive stereoselectivity. Furthermore, the reduction of imines, a nitrogen containing analog of ketones, while biologically important, are not well described in the literature. The present study uses the root of the carrot (*Daucus carota*) in water as a biocatalytic reducing agent for reactions of ketones to alcohols and imines to amines. Method optimization for the reduction of ketones showed that untreated, peeled carrots performed reduction reactions many times more efficiently than grated or juiced carrot, as procedures described in the literature. The revised procedure, when applied to the reduction of imines, was ineffective and suggests some sort of failure of the primary active enzyme within the whole carrot parts to properly reduce the imine functional group.

SYNTHESIS OF METHYL BENZOATE AND ETHYL BENZOATE WITH A REDUCED REFLUX TIME

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The synthesis of methyl benzoate and ethyl benzoate is a procedure that all organic chemistry students at Mount Saint Mary's University complete. The first step of this reaction calls for a reflux of 4 hours, and shortening this step would make the experiment less time consuming for the students and professors. This project was an effort to shorten the pre-existing procedure in the synthesis of methyl benzoate. The time of the reflux was reduced to 2 hours to observe changes in the purity and yield of the methyl benzoate. The experiment was conducted using methanol over molecular sieves due to its high purity. Purity was assessed by Infrared Spectroscopy (IR) and distillation temperature. The two-hour reflux with the methanol produced a product that was still as pure as the 4-hour reflux, and their yields were similar. These results indicate that using half of the reflux time, 2 hours, would be acceptable for an adjusted lab procedure as it yields similar results as long as the methanol is of adequate purity. The same procedure was then followed to produce methyl benzoate with a 1-hour reflux. The 1-hour reflux did not produce adequate yields. We are now repeating the experiment with 200 proof ethanol to see if the reflux can again be cut down to 2 hours instead of 4.

ORGANIC SYNTHESIS OF FLUORESCENT CYANINE DYES AND THEIR PRECURSORS

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Heptamethine cyanine (Cy7) dyes are of interest to scientists because of their significant ability to emit luminescence in the near-IR region, 650-900 nm. Pentamethine cyanine (Cy5) dyes are near-IR and are often used as fluorescent probes for pathogen detection. However, Cy5 fluorescence labels suffer from biological interference from molecules that also fluoresce at 650-700 nm. Current synthetic methods utilize excessive amounts of solvent, involve long reaction times (16-18 h), and contain impurities in the products.

This study applies microwave assisted organic synthesis (MAOS) as an eco-friendly, faster, and cleaner approach to synthesizing benzoindolenine (benzo) heptamethine cyanine dyes. The specific aims of this approach are the synthesis of: 1) benzoindolenine heterocyclic salt derivatives; 2) benzoindolenine heptamethine cyanine dyes; and 3) aniline substituted benzoindolenine dyes.

5-bromovaleric acid methyl ester and 6-iodohexanoic acid ethyl ester were synthesized as synthetic reagents to the 5-bromovaleric and the ethyl hexanoate benzo salts, respectively. Several benzoindolenine heterocyclic salt precursors were synthesized with satisfactory yields and reduced reaction times. The benzo-ethyl and -propyl dyes were synthesized with 97% and 85% yield, respectively, and in 30 minutes or less, a reduction from the 18 h stated in the literature. The aniline-substituted benzo-ethyl Cy7 dye has been synthesized in 54% yield. Optimization studies of this reaction are ongoing. Future work includes the synthesis of additional benzoindolenine heterocyclic salts and Cy7 dyes that possess the excellent spectral properties. All structures were determined by ¹H and ¹³C 400 MHz NMR. The dyes were also characterized using the Cary50 UV /Vis spectrometer.

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WOMEN AND MEN HAVE SIMILAR BACTERIAL FAMILIES INHABITING THEIR BELLY BUTTONS

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Microbes exist as natural fauna along every part of the human body and influence a variety of physiological functions, such as digestion and allergies. Using a previously collected metagenomics data set, with samples collected by swabbing belly buttons, we asked if men or women would have more diverse belly button microbiota (Hulcr, *et. al*, 2012). We hypothesized that women would have more bacteria in their belly buttons than men due to various hormonal processes that affect a female's hormonal and chemical balances. Using phinch.org, we filtered the data between men (n=114) and women (n=151) and specifically investigated which bacterial families were found in the belly buttons. We focused on the six largest families of bacteria shared by men and women: Corynebacteriaceae, Staphylococcaceae, Clostridiaceae, Porphyromonadaceae, Pseudomonadaceae, and Alicyclobacillaceae. Women had a higher total count of each of the six bacterial families investigated compared to men. However, the average number of bacterial families showed no difference between men and women, which refutes our hypothesis. The results in the study showed that in the navel, there is no difference in microbiota of men and women. Other studies, however, state that the prevalence of microbes in other body parts, such as hands, differ between men and women. Thus, further research is required to determine whether or not gender influences the human microbiota on various body locations.

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ANALYSIS OF BELLY BUTTON MICROBIOTA IN RELATION TO WASHING FREQUENCIES PER WEEK

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The human microbiota are microbial communities that influence an individual's health or susceptibility to diseases. A previous study investigated the microbial diversity of the human navel by swabbing the belly buttons of 273 individuals and collecting metadata of the individual, such as gender, age, washing frequency per week, and hometown (Hulcr, et al, 2012). Using this data, we asked if the microbial diversity of an individual's belly button was affected by the frequency of washings per week. We hypothesized that individuals who bathe 6-7 times per week would have a smaller total number of microbes compared to individuals who washed less frequently. We divided the washing frequencies into three groups based on how often each individual washed per week: 0-2 times per week, 3-5.5 times per week, and 6-7 times per week. Our results revealed that all of the washing frequencies consisted of the *Corynebacteriaceae* and *Staphylococcus* genera among the three categories. The three groups of washing frequencies all shared the top two bacterial genera, but the third top genera from each category differed. *Corynebacteriaceae* was the most common bacteria shared among all three frequency washes. Our results showed that individuals who bathed 0-2 times per week had the highest prevalence of *Corynebacteriaceae*, individuals who bathed 3-5.5 times per week had the lowest prevalence, and individuals who bathed 6-7 times per week were between those two values. From this, we concluded that our hypothesis was refuted; meaning, those who bathed 3-5.5 times per week had the lowest total reads of microbes than any of the other washing frequencies. It is important to review these findings as it may aid in future experiments focusing on the resistance of microbes to different body washes and washing frequencies.

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NORTH CAROLINA GEOGRAPHY: A REVIEW OF ACTINOBACTERIA, BACTEROIDETES, AND FIRMICUTES IN MALE'S NAVALS

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A microbiome is the genetic material isolated from microbial communities. These communities can thrive in environments such as on and in the human body. Our research specifically investigated the microbiota within males' navels within the state of North Carolina. We asked if men who live across the state of North Carolina would have a similar microbiome distribution. Our hypothesis was that male residents will contain similar percentages of the microbiomes present in their belly buttons. Using a total of thirteen cities, we analyzed the percentages of the three most prevalent bacterial phyla from a previously collected metagenomics data set (Hulcr, et al, 2012). Actinobacteria, Bacteroidetes, and Firmicutes were all phyla found within the navels of sixty-two males in North Carolina. Our results were consistent, as the lowest prevalence was associated with Bacteroidetes. However, there were differences among the prevalence of Firmicutes and Actinobacteria in the North Carolina cities. While most cities had a higher prevalence of Firmicutes, limiting factors which may include non-equal sample sizes, uncertainty about the males' permanent residence, unique geographical sites, and/or weight led to two cities having a higher prevalence of Actinobacteria. Percentages of Firmicutes, Actinobacteria, and Bacteroidetes of the thirteen cities ranged from 20-40, 13-27, and 5-15, respectively. Based on these results, our hypothesis was supported in that males had a similar prevalence of microbial phyla in their belly buttons. These findings could possibly be accredited to the weight of male residents and the geography of North Carolina. Future research is to investigate the specific bacterial species within the three phyla found in the navel microbiota of the sampled residents. With the discovered bacterial species, we can draw more conclusions between the microbiota of the navels and the geography of North Carolina.

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THE IMPACT OF POPULATION DENSITY OF EACH CITY ON THE DIVERSITY OF THE BELLY BUTTON MICROBIOTA

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Microbes are found almost everywhere and are linked to human health and disease. However, there is still a great deal of facts unknown about microbes, and since they greatly impact one's health it is imperative that more research is conducted. Using a previously collected metagenomics data set (Hulcr, et al, 2012), it was investigated how the population density of a city impacts the diversity of microbiota found in belly buttons. It was hypothesized that more diverse microbial communities will be located in areas with a higher population density. To accomplish this, twenty-five cities were selected and broken into two groups, small cities and large cities, based on level of urbanization using the United States National Air Quality Report. The small cities included Baltimore MD, Boulder CO, Alameda CA, San Francisco CA, and Minneapolis MN. Large cities included Raleigh NC, Durham NC, Seattle WA, Greenville NC, and Pueblo CO. Data was analyzed using Phinch.org, a website for collecting and organizing data, and focused on the class taxonomic level. The data was then graphed for a visual comparison between a cities population density and its diversity of microbes. The data revealed that there was no correlation between the diversity of the belly button microbiota in cities of high or low level of urbanization. In conclusion, our data suggests that population density does not play a role in the diversity of microbes in an individual's belly button. Future research should be conducted to determine what other environmental factors impact the diversity of belly button microbiota.

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FEMALES WITH AN INNIE BELLY BUTTON HAVE A LARGER POPULATION OF COMMON MICROBES THAN MALES

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A link between a person's microbiota and their health has been well established through metagenomics research. To further understand how the microbial community affects human health, we analyzed a previously collected metagenomics data set investigating the diversity of the belly button microbiota. We hypothesized that a male subject with an innie belly button will have a different ratio of microbes than a female subject with an innie belly button. By using the Phinch.org analysis tool we were able to analyze the data, which was collected via deep sequencing of bacterial 16s rDNA (Hulcr, *et. al*, 2012). The data was limited to include the microbes in the innie belly buttons of male and female subjects. We focused on six of the most common bacterial genera: *Micrococcus*, *Bacilli*, *Staphylococcus*, *Ananerococcus*, *Prevotella*, and *Clostridiaceae*. For example the ratio for the genus *Bacilli* was 19 Female to 5 Male. From this study we were able to conclude that our hypothesis was supported by the data and demonstrated that there was a difference in the ratio of microbes in the innie belly buttons of females and males within a large population. This data can help to better understand the functions of the microbes in our belly buttons and the role that these bacterial genera play in health and disease. With future research we will be able to determine whether hormonal or physical changes could be a factor as to why the microbe ratio differs between male and female innie belly buttons.

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PHYLUM VARIATION IN THE BELLY BUTTONS OF MALES AND FEMALES WITHIN NORTH CAROLINA

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Microbes are tiny microorganisms that are prevalent on and within our bodies, outnumbering human cells 10-fold. A microbiota is a community of the microbes that reside within a certain area or environment. Using previously collected metagenomics data set investigating the diversity of the belly button microbiome (Hulcr, et al, 2012), we examined the variation between males and female's bacterial phyla within the State of North Carolina. We hypothesized that women would have a greater diversity of bacterial phyla compared to men in North Carolina. Our method of analyzing the data was to observe samples from thirty-five males and forty-five females that were residents of North Carolina. Our findings revealed that men had a dominant bacterial phylum of *Firmicutes* whereas women had two dominant bacterial phyla: *Proteobacteria* and *Firmicutes*. Our results revealed that women have a more diverse belly button microbiota compared to men in the State of North Carolina. Thus, the female belly button appears to be more hospitable to microbes. Our results provided a glance into one of the differences of the male and female microbial communities. We know that the human microbiota does play a role in the initial development of the human immune system, which may determine the immune system efficiency in our bodies. Past research indicated links between gender, diseases and microbiomes. With these results, we have the ability to further research why women tend to respond better to vaccines and have stronger immune systems than men in most cases.

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THE EFFECT OF AGE AND GENDER ON THE DIVERSITY OF BELLY BUTTON MICROBIOTA

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A correlation between the microbiota, or a community of microorganisms, of individuals and human health has been revealed through various research. In this study, we asked if genetic material isolated from a microbial community in the belly buttons of males and females of the same age would differ. Our hypothesis was that the microbiomes of males between the ages of 1-99 will vary from females of the same age group. The data in this study was from a previously collected experiment investigating the diversity of the belly button microbiomes from individuals visiting two locations (Hulcr, et al., 2012). The bacteria were identified using a metagenomics approach and deep sequence analysis of bacterial 16s rDNA. Corynebacteriaceae, Staphylococcaceae, Clostridiaceae, and Porphyromonadaceae were the most abundant bacterial families in the navels of 54 male and 78 female test subjects. Females had a greater proportion of Corynebacteriaceae than males (1%). For Clostridiaceae and Porphyromonadaceae, males had a greater proportion than females (3%). The proportion of Staphylococcaceae was equal in both males and females (94%). We further analyzed the data by investigating a difference in the microbial communities of males and females in the age groups 0-24 and 25-99. The 11 males had a greater proportion for all 4 bacteria than the 7 females in the 0-24 age group. The 15 males in the 25-99 age group had a greater proportion of the Staphylococcaceae than the 36 females, while the females had a greater proportion for Clostridiaceae and Porphyromonadaceae. 100% of the male and female subjects contained Corynebacteriaceae. Through this study, we were able to determine that the microbiota of males and females vary at specific age groupings, but overall remain consistent. This study can be used in further investigations to determine what roles male and female microbiota play in human development and aging.

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