UMBC

25th Annual Summer Undergraduate Research Fest

Hosted virtually using the *VoiceThread* platform by the College of Natural and Mathematical Sciences

August 10 to 24, 2022

https://surf.umbc.edu/

A message from the Dean

Welcome to the 2022 Summer Undergraduate Research Fest (SURF) at UMBC. Although many restrictions on campus have relaxed as it relates to COVID-19, the College of Natural and Mathematical Sciences is hosting a virtual and unique SURF event from August 10 – August 24.

This annual event defines the SUMMER STEM experience, where the focus is on high quality STEM classes, opportunities for research and applied learning experiences, and building a strong scholarly STEM community. By practicing and applying the skills of performing research this summer, our students follow in the footsteps of great scientists and researchers – making each a part of a grand scholarly community.

We are delighted to be able to offer this virtual SURF event so that our students who have worked so diligently all throughout the academic year and summer will have the opportunity to participate in our distinctive annual SURF event. During the event, you will have the opportunity to view student research presentations using VoiceThread. As an attendee, you will have the opportunity to leave video, voice, or text feedback for the presenters thus affirming your personal "presence" with our students. Our presenters will be responding to your questions and interacting with you throughout the scheduled event.

We are proud of all that our students have accomplished. They are more knowledgeable, experienced, and skilled – better scientists. Their discoveries, their effort, their willingness to explore have added to the vault of scientific knowledge, which in the end – benefits society through an empowerment - of understanding, prediction, and invention. Their success is also due to the tremendous effort, guidance and support provided by their mentors and across campus by our faculty and staff who support and engage our students every day. Please accept my heartfelt thank you to all of you who work with these outstanding students and help them reach their goals.

I thank you for visiting our virtual SURF event and encourage you to view the many outstanding works of our presenters and to interact with these remarkable students.

Welcome to the **Virtual SURF 2022** event,

William R. LaCourse, Ph.D.
Dean and Professor of Chemistry

ACKNOWLEDGEMENTS

The Summer Undergraduate Research Fest (SURF) is truly a collaborative effort. Each year many people contribute to the success of this event. We would like to recognize the following groups and individuals for their dedication and hard work:

We acknowledge the long-standing support of the College of Natural and Mathematical Sciences (CNMS) and Dean Dr. William R. LaCourse.

We are grateful to the Program directors, Program coordinators and Research mentors who provided research opportunities for undergraduate students.

We are grateful for the Division of Information Technology (DoIT) who provided guidance and direction in hosting this virtual event. Special thanks to Josh Abrams, Instructional Design Specialist, for providing guidance and direction on this project and being such an invaluable resource. We are appreciative of faculty, staff and interns for their willingness to provide assistance.

We would like to recognize family, friends and peers of this year's presenters. Your continued support helps ensure the success of these future researchers.

And most of all, we would like to thank the Student Presenters for SURF 2022. This event would not be possible without you!

Special thanks to Ms. Justine Johnson, Assistant Dean, Inclusive Excellence, CNMS & Executive Director, UMBC STEM BUILD

MENTORS

Special thanks to the mentors who supported this year's SURF presenters. We recognize that there are many types of mentors who support the research training such as student peers, program staff, graduate students, laboratory personnel, and research scientists. While space prohibits inclusion of all the mentors who made SURF 2022 possible, we would like to acknowledge the following primary research mentors:

Dr. Joseph **Bennett** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Michael **Betenbaugh** | Johns Hopkins University | Chemical and Biomolecular Engineering

Dr. Charles **Bieberich** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Rachel **Brewster** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Maria **Cambraia** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | CNMS

Dr. Steven **Caruso** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Angela **Cox** | Johns Hopkins School of Medicine | Molecular Microbiology and Immunology

Dr. Marie Christine **Daniel** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Matthias **Gobbert** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Mathematics and Statistics

Dr. Erin **Green** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Brian **Grossman** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Foad **Hamidi** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Information Systems

Dr. Zahid **Hasan** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Information Systems

Dr. Christopher **Hennigan** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemical, Biochemical, and Environmental Engineering

Dr. Nele **Hollmann** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Jumman **Hossain** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Information Systems

Dr. Erin **Lavik** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemical, Biochemical and Environmental Engineering

Dr. Mark **Lee** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Tara **LeGates** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Weihong **Lin** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Margaret **MacDonald** | US ARMY DEVCOM | Chemical Analysis & Physical Properties

Dr. Victor **Maltzev** | NATIONAL INSTITUTE ON AGING | Biophysics

Dr. Stephen **Miller** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Bradford **Peercy** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Mathematics and Statistics

Dr. Govin **Rao** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemical, Biochemical and Environmental Engineering

Dr. Phyllis **Robinson** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Justin **Rosa-Rojas** | PURDUE UNIVERSITY | Chemical Engineering

Dr. Zeev **Rosenzweig** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Nirmalya **Roy** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Information Systems

Dr. Kathleen **Seley-Radtke** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Aaron **Smith** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Michelle **Starz-Gaiano** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Michael **Summers** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Chemistry and Biochemistry

Dr. Blair **Taylor** | TOWSON UNIVERSITY | Computer and Information Sciences

Dr. Fernando **Vonhoff** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Biological Sciences

Dr. Jianwu **Wang** | UNIVERSITY OF MARYLAND BALTIMORE COUNTY | Information Systems

PARTICIPATING PROGRAMS

Research programs, both grant-funded and university-supported, provide career-focused training that supports undergraduate researchers during summer semesters and/or academic years.

The SURF team would like to recognize the support given by these research programs to novice researchers for early professional research and presentation opportunities. These programs are listed as follows.

COEIT Summer Research Experience Program | *UMBC College of Engineering and Information Technology* | https://coeit.umbc.edu/nsf-reu/

HHMI Scholars Program | *Howard Hughes Medical Institute* | https://meyerhoff.umbc.edu/

Louis Stokes Alliance for Minority Participation Research Programs | *UMBC & University System of Maryland* | https://lsamp.umbc.edu/

U-RISE Program | *National Institute of General Medical Sciences (NIGMS) at the NIH* | https://urise.umbc.edu/

McNair Scholars | *U.S. Department of Education TRIO Program* | https://mcnair.umbc.edu/

Meyerhoff Scholarship Program | *Supported by a network of institutional partners and friends* | https://meyerhoff.umbc.edu/

National Institute on Drug Abuse | https://nida.nih.gov

NSF Research Experiences for Undergraduates | *National Science Foundation* | https://www.nsf.gov/crssprgm/reu/

STEM BUILD 2.0 at UMBC | *NIH Common Fund and NIGMS* | https://stembuild.umbc.edu/

SURF 2022

Presenters and Abstracts

Listed in alphabetical order of the first presenter's last name

Consistent with its commitment to academic freedom, UMBC does not restrict the topics of inquiry that can be accepted for SURF, the conclusions that are reached in student work, or the representations chosen by the student and mentor for that work. A mentor-approved abstract describing the work and its intellectual context is required. Approval to present work at SURF is given by the faculty mentors of the students. All presenters have some connection to UMBC, its faculty, and/or its programs.

DETERMINATION OF THE INTERACTIONS BETWEEN NUCLEOCAPSID OF HIV-1 GAG POLYPROTEINS, NSC260594, AND THE HIV-1 VIRAL RNA

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Treatments for HIV-1 target various aspects of its viral life cycle to terminate reproduction of new virions. However, due to its retroviral nature, as well as social implications that prevent patients from receiving consistent regimens, the virus rapidly mutates to resist current treatments. To compensate, we must determine the structural makeup of the virus's proteins and RNA to identify alternative targets for novel treatment. The HIV-1 viral RNA is selectively packaged for virion assembly via interactions between the nucleocapsid (NC) of the virus's Gag Polyproteins and the Core Encapsidation Signal (CES) of the viral RNA. A region of CES, the Psi Stem-loop (PST), has multiple high-affinity binding sites for NC. NC specifically binds to the PST via non-Watson-Crick base paired guanosines, suggesting that the labile nature of the viral RNA facilitates NC binding. A quinolinium derivative compound NSC260594 (NSC) has been recently identified to potentially inhibit NC binding to the PST. Quinolinium is an ion derivative of quinoline, which inhibits reverse transcriptase in the viral life cycle's early stage. NSC binds to the [UUUU:GGAG] region of the stem-loop, which includes the non-Watson base paired guanosines that NC is so drawn to. The implications of this binding on the structure of the viral RNA are unknown.

To learn the molecular mechanism of this inhibition, I plan to complete gel studies comparing NC and NSC binding to the PST, and thereby determining the binding affinity of NSC to the PST in the presence and absence of NC. Furthermore, I will obtain and compare nuclear magnetic resonance (NMR) data of both the PST and the PST bound to NSC to investigate how the structure of the viral RNA is altered, or stabilized, when bound to NSC. The results of this project may lead to future use of NSC as a novel treatment for the HIV-1 virus.

Funding for this research is supported by the Howard Hughes Medical Institute and the NIH/NIAID #8 R01AI150498-32.

USING MICROBIOLOGICAL DATA TO PREDICT A BACTERIOPHAGE'S MORPHOLOGY

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The purpose of our research is to determine if we can create a set of criteria to predict a phage's morphology by analyzing its microbiological data. We aimed to determine if we could correlate the bacterial strains that the phage infects and the digestion pattern by a restriction enzyme of its genomic DNA with the phage's morphotype. This led us to investigate whether we can predict phage morphology based on what bacterial strains it can/cannot infect and the cuts seen on its restriction enzyme digest. To examine this, we analyzed our archived class data of phages from 2014 to 2019, with a total of 329 phages; including 186 myoviruses, 29 podoviruses, and 148 siphoviruses.

To investigate a correlation between host-range testing, restriction enzyme digest, and phage morphotype, we created a master data table that was split between the three morphotypes. Each column was a different bacterial strain or enzyme that was used to test the phages in the archived class data. If there was at least a 75% match in a column, we considered the finding to be meaningful; thus, we associated that infection, no infection, and range of cuts seen to each certain morphotype.

Our conclusion demonstrates that the particular host range of a phage along with a specific restriction enzyme digest result can be used to predict a specific morphotype. Each morphotype we analyzed has its own set of host-range infections and restriction digest profile, which allows unique predictions to be made between the different bacteriophage morphotypes. With these correlations, we hope that future researchers can apply this method of analysis when researching bacteriophages, despite the potential instance of limited resources.

This research was supported by the Science Education Alliance, Howard Hughes Medical Institute, Chevy Chase, MD, and the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers TL4GM118989, UL1GM118988, and RL5GM118987. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DESIGN OF A STABLE ANTIBODY: HIV-1 RNA COMPLEX SUITABLE FOR STRUCTURAL STUDIES

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Determining macromolecular structures of the Human Immunodeficiency Virus (HIV-1) is vital in developing therapeutic strategies to eradicate the illness. Currently, many large structures of RNA have not been solved; thus, hindering our understanding of the biological roles of RNA in viral assembly. Thus, my role was to develop a method to characterize RNA constructs with Cryo-electron microscopy (cryo-EM), a high-resolution imaging technique. Prior studies confirmed the structure of the Core Encapsidation Signal (CES), a segment on RNA that provides the minimum required signal for genomic packaging. Therefore, we can use CES to create target RNA and compare it to the solution structure. In this study, I am using a recombinantly expressed antibody, Fab BL3-6, as a chaperone that is characterized to recognize and bind to RNA sequences. My goal is to construct a heterodimer with CES constructs with one alignment point for Fab BL3-6 to create clear cryo-EM samples. One construct (CCCC) has an antibody binding site that will bind with another construct (GGGG) that lacks the site; the dimerization tests the efficiency of the chaperone on large RNA structures under cryo-EM. The constructs were labeled with different fluorophores (Cy3 and Cy5) by a 3' end ligation reaction. I monitored the assembly capacity using an electrophoretic mobility shift assay (EMSA). With various RNA sequence ratios (0 to 2), I observed the constructs' binding affinity to one another and Fab BL3-6.

Thus far, I confirmed the binding possibility of a hybridized complex among the three components at a 1 to 2 concentration. I will further homogenize these samples to achieve a stable structure. In the future, I aim to add a large binding protein as a tag on Fab BL3-6 to increase the size of the chaperone to ensure its visibility under cryo-EM.

Funding for this research is supported by the Howard Hughes Medical Institute and the NIH/NIAID #8 R01AI150498-32.

THE EFFECT OF NITROGEN SOURCE ON OXIDATIVE STRESS IN *CHLAMYDOMONAS REINHARDTII*

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Algal biomass can be converted to biodiesel to be used by cars, buses, and trucks. Biodiesel from algae is carbon neutral, with environmental benefits in which the CO_2 released by vehicles serves as an input for the photosynthetic algae. High production of oxidative stress is detrimental to the growth of algae which ultimately impacts algal production. Oxidative stress is the imbalance between reactive oxidative species (ROS) and antioxidant defenses that can damage biomolecules. A major cause of oxidative stress in algae is ROS that is produced during photosynthesis due to excess light. *Chlamydomonas reinhardtii* is used as a model to study mechanisms of stress tolerance in green algae for the purpose of enhancing biofuel production. Previously, a student in the Miller lab found that a closely related alga is much more sensitive to oxidative stress when grown with ammonia vs nitrate as a nitrogen source. There are no published reports describing such an effect on any organism. This study aims to determine how nitrogen sources affect oxidative stress tolerance in *Chlamydomonas reinhardtii.* To this end, we inoculate *Chlamydomonas*, grown to mid-log phase into either NH_4 or NO_3 media containing hydrogen peroxide (H_2O_2) at different concentrations. Finally, we examine growth at different times (24 hrs,48 hrs, and 72 hrs) post-inoculation. We will report the results of several replicate tests. Future directions will include testing other oxidative stress agents such as rose bengal, paraquat, and other algae, including Chlorella, which is a hardier alga that withstands industrial production conditions and naturally makes significant amounts of lipids that can be converted to biodiesel. Our work should lead to improved methods of helping algae to resist oxidative stress, which should have a positive impact on algal biofuel production.

REM supplement to NSF award 1332344

INVESTIGATING THE MECHANISMS OF REVERSE HINGE POINTS DURING NEURAL FOLD FORMATION IN ZEBRAFISH EMBRYOS

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Failure of the neural tube to close often results in neural tube defects (NTDs), such as spina bifida. Neural tube formation (neurulation) involves the bending and folding of the neural epithelial, also known as the neural plate. My research project focuses on a late stage of neurulation when the edges of the neural plate elevate and converge to close the neural tube. Several models have been proposed to explain the cellular basis of neural fold elevation. I am investigating a novel mechanism underlying this process that centers on reverse hinge points (RHPs) formation. These structures are shaped by basal constriction of neural fold cells, which appear tightly linked to neural fold elevation. My research project investigates the general hypothesis that the extracellular matrix (ECM) and more specifically the ECM glycoprotein laminin, provides a spatial cue to attract the actomyosin contractile machinery to the basal pole, possibly via recruitment of the actomyosin binding protein Shroom3. Laminins are ECM glycoproteins that bind to integrins in the plasma membrane of epithelial cells. If my hypothesis is correct, I predict that loss of laminin or/and shroom 3 will prevent the proper formation of the RHPs and hence neural fold elevation and medial convergence. I will test this hypothesis by blocking laminin and shroom 3 functions with translation or splice-blocking morpholinos. The marker emx3 for the edge of the neural plate will be used to measure neural fold convergence through the method of immunolabeling and *in-situ* hybridization. In addition, markers expressed in the forebrain midline will assess whether fusion of the neural folds at the dorsal midline took place. This research is essential to not only understand the pathways forming RHPs, but also to understand further genetic risk factors associated with neural tube defects.

I would like to acknowledge my mentors Dr. Rachel Brewster and the rest of the Brewster lab for their support and contributions. In addition, thank you to the Meyerhoff Scholars program and the Howard Hughes Medical Institute (HHMI) undergraduate scholars' program. I was supported by the HHMI grant (52008090); The Brewster lab was supported by funding from the National Institute of Health/NICHD (R21HD089476).

THE IMPORTANCE OF SECURE CODING AND TEACHING IT

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Oftentimes security protocols are not implemented in software programs, leaving many consumers vulnerable to hackers and cyber-attacks. Learning to navigate the online world can be a crucial skill, especially for young people. Secure coding is a fundamental programming technique that enables scientists to develop computer software that guards against the accidental introduction of security vulnerabilities, defects, bugs, and malware that cause security exploitation.

During the research, we used data from the Security Injections @Towson project, developed at Towson, to examine the many security vulnerabilities that can occur when a program is not secured. I examined three injection modules: Buffer Overflow, Integer Error, and Software Development Lifecycle. I examined these three injections by going through each injection and learning what a specific injection can do to a program.

Buffer overflow occurs when data is input or written beyond an object allocated bounds, causing a program to crash or create a vulnerability. Integer Error is when integer values are too large or too small that fall outside the allowable bounds for their data type. The Secure Software Development Life Cycle is a setup of steps implemented to create software applications that include security during all stages of development to avoid adding security afterward.
Through these discoveries, they have left me with a result that each injection can cause a program to malfunction or crash if the code had been input incorrectly on specific terms placed in the program.

We have also discovered through an article called, Teaching High School Students to Code Responsibly, that Secure coding is taught separately from teaching programming. It's also not taught to many students in school. Through this article, we've concluded that Secure coding should be taught early to students learning to program and should continue throughout their educational careers.

I'd Like to personally thank Dr. Blair Taylor, Alexei Kolesnikov, the Department of Computer Information Sciences, and the LSAMP Program for helping me with my research during this time I had during the program. This research was partially funded by the USM LSAMP program, supported by NSF LSAMP Award #1619676

A FEDERATED LEARNING APPROACH TO CAMERA-BASED CONTACT-LESS HEART RATE MONITORING

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rPPG (remote Photoplythysmograpy) data refers to remote contactless heart rate detection using regular video camera sensors. However, privacy concerns arise given the necessity of having multiple patient facial recordings in one location. We identify Federated Learning (FL) approach as a prospective solution to these privacy issues while improving model performance accuracy. Federated Learning is a machine learning model which addresses those concerns. The model has devices only send back updates to the model rather than data, and thus maintains user privacy. This combination of user privacy and functionality is why a federated setup is a promising potential solution to analyzing rPPG data. To the best of our knowledge, a federated learning approach to rPPG data as a potential means to maintain user healthcare privacy has not yet been explored. In this work, we explore a regression Federated Learning setup in the rPPG domain. Our primary goal is to create a comprehensive regression model in a federated setup. Unlike many existing federated learning frameworks, our application is regression based rather than classification based. We will have our centralized model initially train on a randomized selection of patient data. After a sufficient training point has passed, we will send weights of our rPPG model to different nodes (representing local servers) so as to train on the subject's data on their local machine. The corresponding nodes will then send back the updated weights back to the centralized server, with the process repeating until a certain accuracy threshold is met. We also provide detailed analysis and perform ablation studies of the FL methods in the rPPG domain.

This project was funded by the National Science Foundation as part of their Research Experience for Undergraduates Program.

PTEN REACTIVATION IN A MOUSE MODEL OF PROSTATE CANCER

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With recent clinical successes of immune checkpoint inhibitors (ICI), immunotherapy has become standard of cancer treatment, including as the first-line therapies of choice for non-small cell lung cancer and melanoma. However, due to factors including insufficient tumor immunogenicity and an immunosuppressive tumor microenvironment, checkpoint blockade immunotherapy suffers from a limited rate of anti-tumor responses for many cancers, including prostate cancer (PCa). One way to improve these anti-tumor responses is through combination with traditional treatments, such as chemotherapy and radiotherapy, which despite promising preclinical results, tend to cause serious side effects. Therefore, development of a new strategy to exploit immune cell death effectively and safely and synergize with current ICI is of great interest for better cancer treatment. The use of therapeutic mRNA has recently attracted significant attention as a potential means of cancer treatment, as it does not require nuclear entry for activity and has a negligible chance of integrating into the host genome. While the use of therapeutic mRNA seems highly promising, its clinical application in cancer therapy faces a formidable challenge of effective systemic delivery into tumor cells. Nanotechnology has shown promise to improve the cytosolic delivery of various RNA agents, and several nanoparticle (NP) systems have been developed for in vivo mRNA delivery. In this project, we propose to explore whether NP co-delivery of PTEN mRNA can induce anti-tumor immune responses in a genetically engineered mouse model of PCa. To achieve this goal, we will evaluate the anti-tumor efficacy of PTEN mRNA as well as its combination with anti-PD-1 immunotherapy in the immunocompetent BMPC mouse model of PCa. We will also evaluate the in-vivo safety of this combination treatment. We expect that the NP co-delivery strategy could be expanded to other tumor suppressors and adjuvants, which benefits cancer immunotherapy research and development of novel and effective cancer therapies.

I would like to acknowledge and thank the Arnold & Mabel Beckman Foundation for their funding support, and the Beckman program manager, Ms. Caitlin Kowalewski, as well as my research mentor, Dr. Charles Bieberich, and multiple supportive graduate students, including Micheal Rubenstein, and Alexander Chin.

EXAMINING BRAIN REGIONS ACTIVE DURING ACUTE STRESS USING CONDITIONED AVERSION AND RESTRAINT

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The ability to pursue rewarding stimuli and avoid threatening stimuli is necessary to survival, allowing organisms to seek rewards such as food and avoid harm such as predation. This reward and aversion response often requires contextual learning, which is the ability to associate stimuli with contextual cues in the environment. Learning deficits and altered motivation are associated with psychiatric disorders. For example, depression is characterized by decreased reward motivation while addiction is characterized by increased reward motivation. However, the mechanisms by which contextual learning occurs remain unclear. We aim to develop a model of aversive learning to study the neuronal mechanisms that mediate this behavior.

We have previously conditioned mice to associate different environments with an aversive stimulus, physical restraint, by using the conditioned place aversion (CPA) paradigm. During CPA, mice learn to avoid environments associated with restraint over the course of 5 days.

We are now interested in understanding the brain regions that are activated by this acute stress exposure. To determine this, we will develop an acute stress paradigm by allowing mice to freely roam a chamber with visual cues before physically restraining them. We will then mark brain activity directly after exposure to stress using the transcription factor c-fos. To do this, we will perform antibody staining and image the brain slices. During imaging, c-fos, which denotes active cell bodies, will fluoresce green. By quantifying the amount of fluorescing cell bodies, we will determine the level of activity of each brain region. We hypothesize that the nucleus accumbens, hippocampus, and amygdala will be activated during acute stress because these brain regions are involved in learning, behavior, and emotional regulation. These data will determine if our paradigm of physical restraint induces acute stress and further inform us of how limbic brain regions respond to stress during aversive learning.

This research was supported in part by a grant to UMBC from the NIDA through the EDUCATE Scholar Program.

STARVED AND SATIATED *CAENORHABDITIS ELEGANS* ODR-3 MUTANTS RESPONSE TO ATTRACTIVE BACTERIAL ODORANTS

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Caenorhabditis elegans are transparent, free-living nematodes that measure up to 1 millimeter in length. They are self-fertilizing males and hermaphrodites, with a short life cycle of 3 days. The *C. elegans* genome consists of a gene known as odr-3 that regulates olfactory receptor binding activity. A bacterial odor choice assay was used to test if non-functional mutated odr-3 and wild-type *C. elegans* have a preference for certain attractive bacterial odorants.

The bacteria tested were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus.* These are all pathogenic bacteria except for the *E. coli* used. *C. elegans* that had been fed with *E. coli* and those starved were tested. This information is important for the culturing of *C. elegans.*

An initial bacterial odor choice assay was conducted to see if the *C. elegans* travel equally to each quadrant of a plate containing a patch of bacteria on agar attached to the lid. This trial showed that *C. elegans* have a high affinity for *K. pneumoniae* odor, and thus did not travel to the other quadrants as much.

A second bacterial odor choice assay was conducted, where each bacterium was patched individually with *E. coli* as a control to see which odor the *C. elegans* preferred more. This showed that all worms placed on the plate containing *S. aureus* odor were more attracted to *E. coli*, indicating that *S. aureus* has an unattractive odor. Starved worms on the plate containing *P. aeruginosa* showed a stronger affinity for either the *E. coli* or the bacteria, with almost all traveling towards one or the other. The fed worms on this plate hardly traveled at all to either side. Overall, *C. elegans* showed a strong affinity for *K. pneumoniae*, a negative attraction for *S. aureus*, and were neutral towards *P. aeruginosa*.

This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers TL4GM118989, UL1GM118988, and RL5GM118987. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

COPPER-CATALYZED CO2 ELECTROREDUCTION UTILIZING ZEOLITE-TEMPLATED CARBON SUPPORTS

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Global reliance on fossil fuels and the expansion of industry has led to the continuous rise in CO_2 emissions and has directly contributed to climate change since the 19th century. Consequently, global research efforts are now focusing on fuel-production technologies that mitigate CO_2 emissions. One promising alternative is CO_2 electroreduction, which utilizes renewable energy to convert CO_2 to other fuels and chemicals. Previous literature studying CO_2 electroreduction reports the production of light hydrocarbons when using copper-based electrocatalysts. However, data suggests that using microporous carbons may favor the formation of heavier hydrocarbons, which serve as a more efficient fuel source. Zeolite-templated carbons (ZTCs) are microporous carbons that can be synthesized using zeolite templates. The standard practice of ZTC synthesis used includes furfuryl alcohol impregnation, propylene chemical vapor deposition, heat treatment, and hydrofluoric acid etching to obtain the carbon product. Copper nanoparticles were then deposited on the surface of the ZTC via cetrimonium bromide (CTAB) reflux, followed by electrochemical deposition. Electrochemical tests were performed to assess the catalytic activity of the material. Gas diffusion electrodes were then used to reduce CO_2 into alkenes with the copper containing ZTC serving as the catalytic site. Current, hydrocarbon weight, and gas/liquid product was subsequently measured and analyzed. This study aims to interpret the structural integrity of ZTC formation at each step of the synthesis. Moreover, this study looks to clarify how the synthesis and structure of ZTCs relate to their electrochemical performance and reduction of CO_2, in comparison to commercial carbons. The results have provided an understanding of how adjustments to the synthesis protocol correspond to variability in porosity, structural order, and catalytic activity of the ZTCs when used for CO_2 electroreduction.

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AROMATHERAPY OR TOXIC EXPOSURE: AN INVESTIGATION INTO THE EFFECT OF VARIOUS ELECTRONIC CIGARETTE VAPOR EXPOSURE METHODS ON THE OLFACTORY SYSTEM OF AGING MICE

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Electronic cigarette use in the United States is often viewed as an adolescent issue. Usage trend data supports the idea that high school-age adolescents are the group with the highest prevalence of e-cigarette use leading researchers to focus heavily on this target population. This prioritization has led to a gap in the understanding of the effects of e-vapor exposure on the olfactory system of aging populations. As organisms age, the olfactory sensory neurons (OSNs) become desensitized to chemical signals due to accumulated damage, which manifests as anosmia. Aromatherapy has been used to counter olfactory aging through odor stimulation. E-cigarette vapor typically contains strong flavor molecules, nicotine, humectants and other toxicants. Here we plan to study the effect of e-vapor exposure on aging animal populations by comparing olfactory guided behavior of a cohort of both older (14- to 18-month-old) and younger mice (3- to 6-month-old) before and after 4-week e-vapor exposure. We performed pre-exposure T-Maze behavior assays to determine the relative ability of each mouse to discern between competing scents and Buried Food behavioral assays to characterize the ability of each mouse to locate the source of food via olfactory detection. We plan to use our newly developed automatic data analysis tools to extract data from T-maze trials. The use of multiple e-vapor exposure methods allows for data to be collected comparing the short-term effects of aromatherapy-like exposure to that of typical daily use exposure. We hypothesize that the behavior assays conducted post-exposure will show improvement in the highlighted olfactory health markers for the aging mice who underwent occasional aromatherapy-like exposure Ultimately, the data collected will allow us to draw conclusions on any significant differences in the effects of e-vapor exposure on the olfactory systems between younger and aging mice and whether toxic effects outweigh the benefit of aroma stimulation.

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DEFINING THE TOLL-LIKE RECEPTOR REQUIRED FOR SARS-CoV-2 ACTIVATION OF THE INFLAMMASOME

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The inflammasome is an innate sensing system that has antiviral effects, but the cytokines produced in inflammasome activation (interleukin (IL) -1β and IL-18) also mediate inflammation and side effects of viral infection, such as fatigue and fever. Inflammasome activation is initiated by engagement of viral genomic material with a Toll-Like Receptor (TLR). SARS-CoV-2, the virus causing COVID-19, activates the inflammasome robustly and COVID-19 is characterized by the production of very high levels of IL-18. We sought to identify the TLR that initiates SARS-CoV-2 inflammasome activation and examined TLR7 and 8, which form heterodimers in vivo.

Subcloning, DNA extraction, PCR, DNA gel electrophoresis, and Sanger sequencing were used to validate potential TLR knockouts (KOs). Sequencing revealed successful disruption of TLR8 signaling, confirmed by decreases in innate sensing pathway signaling in response to TLR7/8 agonists. Recently, sequencing also revealed successful disruption of TLR7 signaling but these knockouts have yet to be confirmed by innate sensing pathway signaling decreases due to TLR7/8 agonists as well. LDH and IL-18 ELISA assays were used to compare inflammasome activation in TLR KOs versus WT.

There was no significant difference in the ability of SARS-CoV-2 to activate the inflammasome between wild type and TLR8 disrupted THP-1 cells, demonstrating that TLR8 is not required for SARS-CoV-2 inflammasome activation. In future experiments, we will attempt to fully validate TLR7 disrupted THP-1 cells to verify the lack of dependence on TLR7, as well as TLR9 disrupted THP-1 cells. We will also use all this information to generate a useful tool for studying inflammasome activation by other viruses.

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ANALYZING THE EFFECTIVENESS OF PHAGES ENZYMES TOOLS 2.0 (PET) USING *STREPTOMYCES* PHAGE DOXI13 AND RELATED SUBCLUSTERS

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In our research, we are working with *Streptomyces* phage Doxi13 from the BI2 subcluster which was isolated using *Streptomyces scabiei* RL-34. Phages are clustered based on genomic similarity and gene content; they can be further separated into sub-clusters based on particularly high levels of similarity. The criteria that we will be evaluating when analyzing the BI2 sub cluster is the amount of DNA fragments that are shown when enzymes are digested. Our goal is to analyze the accuracy of Phage Enzymes Tool 2.0 (PET) to verify that phages are being clustered properly. We specifically investigated the restriction enzyme digest for Doxi13 because the data conflicted with other phages within the BI2 subcluster. The purpose of a restriction digest is to visualize the number of times an enzyme is cut within an isolated DNA sample. In our experiment, we used multiple databases such as PhagesDB and PET to compare data and make sure the digestion pattern for each enzyme shared correlation with each other. We also used NEBcutter to perform virtual restriction digests to compare with the original digest. The enzyme we specifically focused on was SalI because it cut on every other phage within subcluster BI2, yet no cuts were visible on Doxi13 from PhagesDB. After we conducted our research, we concluded that Doxi13 was clustered accurately despite the discrepancy within the restriction enzyme digests from PhagesDB. Our final takeaway is that when using PET, the results provided are only as accurate as the data taken from the digests, and that using multiple forms of validation is essential before concluding subcluster classification.

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Development of a Novel Opsin-Chimera to Explore the G-Protein Signaling of Melanopsin

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Intrinsically photosensitive retinal ganglion cells (ipRGCs) detect environmental light to mediate both image and non-image-forming visual processes. One of ipRGCs' many non-image-forming mediated behaviors include entrainment of circadian rhythm to the day/night cycle. IpRGCs are photosensitive due to the expression of the optically active G-protein coupled receptor (GPCR), melanopsin. Research on melanopsin *in vitro* and *in vivo* has certain constraints due to the need for the control of light conditions.

The field of optogenetics enabled the generation of opsin-chimera GPCRs which maintain light sensitivity but mimic the G-protein activation profile of the spliced receptor. Additionally, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) have been created which are activated by the inert ligand clozapine N-oxide (CNO). Both optogenetic and chemogenetic approaches have improved neuroscience research by enabling the selective excitation or inhibition of any neuronal population with the use of inert stimuli. Here we demonstrate the development of a Melanopsin-DREADD chimera which utilizes the hM3Dq DREADD backbone but replaces the intracellular loops and C-terminus with those of mouse melanopsin. The expected signaling profile will have similarities in its G protein activation to wild type melanopsin without the need for photon activation, but rather will be activated by CNO. This melanopsin-DREADD chimera was constructed and now expresses and signals via G-proteins in response to CNO *in vitro.* Additionally, the chimera maintains the G-protein selectivity of melanopsin but with differential Gq amplitudes compared to WT melanopsin. Further optimization of the chimera is necessary to recapitulate melanopsin Gq signaling amplitudes and will be done through further residue exchange of the transmembrane segments. Creating an effective melanopsin-DREADD chimera will allow the construction of a viral construct for studying the function of melanopsin *in vivo*. Without the need for light, CNO-containing food or injections into mice would induce melanopsin signaling that would ease circadian research.

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THE EFFECTS OF METAL SUPPLEMENT ON DROSOPHILA WITH MUTATIONS IN ALZHEIMER'S GENE

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Alzheimer's is a type of dementia and brain disorder that involves the symptoms of decreasing memory performance and thinking skills, which lead to the difficulty of performing simplest tasks. In this experiment, we are using the animal model Drosophila melanogaster to test whether metal supplements have beneficial effects on flies that have mutations in genes associated with Alzheimer. We measured flight performance and survivorship rates, as those factors are similar to the symptoms of reduced motor ability as observed in humans Alzheimer's patients. We tested different diets with metal in them on flies that have mutations in genes associated with Alzheimer. We specifically used two types of metals Copper (II) sulfate ($CuSO_4$) in three different concentrations (0.5mM, 1.0mM, and 1.5mM), as well as Zinc chloride ($ZnCl$) in three different concentrations (5mM, 10mM, and 20mM) to observe the impact of these diets on flight performance and survivorship rates of both wildtype flies (control flies) and flies that have mutations in genes associated with Alzheimer (APPLd flies). These results might help to provide information on the different benefits of the diverse types of diets that reduce symptoms that we observe in Alzheimer's patients, with the goal of developing better nutritional therapies and treatments.

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ANALYZING THE RELATIONSHIP BETWEEN VARIOUS ENVIRONMENTAL CONDITIONS AND BACTERIOPHAGE MORPHOTYPES

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Bacteriophages, the most abundant biological unit, are being increasingly used in the development of biotechnology. UMBC isolates and characterizes hundreds of phages from soil samples using *Streptomyces* and *Bacillus* species as hosts. Thus, we came to question if there is an ideal soil environment from which phages can be isolated. To test this, we analyzed and organized class data from previous years. We used wet, moist, humid, and dry soil conditions to sort our data. Similarly, we then placed each of the morphotypes from both hosts into our four condition categories. Lastly, a chi-squared test was performed to analyze the observed and expected values of the phage's morphotype compared to all the phages in each host. We identified myoviral, siphoviral, and podoviral morphotypes when *Streptomyces* or *Bacillus* hosts were used and tectiviral morphotypes only when *Streptomyces* host was used. The majority of *Streptomyce*s phages were isolated from dry conditions while most of *Bacillus* phages were isolated from moist conditions ($P > 0.05$). This result was redundant when analyzing the soil conditions of each of the morphotype. This suggests that there is no significant correlation between morphology and the isolation environment. This is likely due to the inconsistent categorization of isolation environments. In addition, we only looked at one environmental condition, the amount of water in the sample, which creates room for confounding variables we didn't account for, including pH and temperature of the soil sample. In the future, test more environmental variables across a broader range of isolated phages for more evidence of a preferred isolation environment and examine a potential relationship between the host and isolation condition range.

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TEST AND EVALUATION OF TEXAS INSTRUMENTS MMWAVE RADAR

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A short wavelength radar system is a method considered for surface object detection due to providing a non-destructive and non-invasive solution for inspection. Most of the previous work which we went through, did not consider the real environment. They had limitations like only working in a favorable setting: not working at night, a foggy environment, etc. The environment of the data collection takes place greatly impacts reflected waves from objects due to their unique physical and chemical properties. These attributes affect an object's frequency response, which can in turn be used to differentiate objects. In order to monitor such behavior, we will first try to integrate a mmWave radar in the TurtleBot3 for object detection. This system emits Frequency Modulated Continuous Waves (FMCWs) as a means of collecting information about the objects present in a scanned range to the UGV. A short wavelength radar's performance is influenced by external factors, but that are not limited to the presence of light, a scanned object's permittivity and material composition, and the radar's distance from a scanned object. This paper will note such limitations of the Texas Instruments AWR2944 Evaluation Model integrated into a Turtlebot3 Burger.

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SELF-ORGANIZATION OF ASYMMETRIC WIRELESS SENSOR NETWORKS ENABLES MULTIPLE DEEP LEARNING ALGORITHMS TO RUN CONCURRENTLY

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Deep learning algorithms implemented within wireless sensor networks is a highly applicable method for data collection. In the military, situational awareness is very important. Unmanned ground and aerial vehicles can offer intelligence and surveillance when combined with deep learning applications. Utilization may be increased by deploying these UGVs and UAVs and allowing soldiers to be stationed elsewhere. Sensors placed on multiple UGVs and UAVs with fast and accurate data processing would be highly advantageous in the battlefield.

We want to run several applications (such as object detection, image segmentation, and face recognition) concurrently on the devices in asymmetric, heterogeneous wireless sensor networks, however, the complexity of the network comes with the resource constraints. The different operations require various amounts of computational power and the many nodes have varying performance characteristics.

The wireless sensor network must be self-organizing, where each node that collects data must be able to decide whether it is able to process the data or not. Additionally, nodes need to be able to initiate another course of action, such as transferring the data to be processed on a stronger node. We must consider what data types need to be sent, and to which device the data should be sent for processing. We study the system resources and process of each individual node of the network before running deep learning algorithms, as well as during. Analyzing latency, accuracy, energy, and other system processes allows us to configure the Network with the optimal organization.

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THE COMPARISON OF PHAGE CHARACTERISTICS TO PREDICT THE CLUSTERS OF A PHAGE WITH THE HOST OF *BACILLUS THURINGIENSIS*

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Bacteriophages (phages) are clustered by comparison of the whole genome sequences. Assigning a phage to a cluster is useful for organizational purposes as the world of phages is still largely untouched and undiscovered. Despite being able to isolate a phage, the resources to have them manually sequenced and compared to other phages are not guaranteed for everyone. The hypothesis of this study was that the clusters of bacteriophages could be predicted by comparing the data collected from the morphology, digest patterns with enzymes, and host range testing of an unsequenced phage to phages that are already sequenced. To test this hypothesis, phages were compiled from BacillusDB that contained similar characteristics to *Bacillus* phage BaxerialKiller, which we isolated this summer, to allow us to make a prediction of the cluster of our unsequenced phage. This research resulted in finding that phages within the same clusters have similar patterns in regards to their morphology, what enzymes cut the DNA of the phage, and what host the phage could use for reproduction. We were able to predict the cluster of our unclustered phage. For confirmation of our results we also predicted the cluster of another unclustered phage from the BacillusDB with the same process used for BaxerialKiller. In conclusion, the findings of this research suggests that those without resources can organize their phages through data comparison as well.

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Structural Basis and Mechanism of HIV-1 Genome Packaging

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Human Immunodeficiency Virus type-1 (HIV-1) weakens the host immune system by destroying CD4+ cells leading to acquired immunodeficiency syndrome (AIDS). Although antiviral therapies exist, drug resistance necessitates novel therapeutic strategies and calls for a better understanding of the viral replication mechanisms. During the late phase of HIV-1 replication, two copies of viral genomic RNA (gRNA) are trafficked to the plasma membrane by a small number of Gag proteins to initiate viral assembly. The genomic recognition stage of the replication cycle is a highly conserved process and better understanding of this process is important for identifying novel drug targets. Our goal is to characterize the structure of the dimeric RNA packaging signal in HIV-1_{MAL} and study RNA-Gag interactions important for genome selection. Using nuclear magnetic resonance techniques, we are able to structurally characterize the CES, which is the minimal unit of gRNA necessary for packaging. However, since there are size constraints for NMR; we segmentally label the gRNA using a novel RNA labeling technique. Segmentally labeling the RNA would result in NMR spectra with decreased signal overlap allowing for unambiguous signal assignment. Once we are able to optimize our protocol for obtaining segmentally labeled RNA, we can go on to studying how the RNA interacts with Gag and how its structure plays a crucial role in selectively binding to Gag to be packaged into new viral particles. These insights should facilitate the development of new antiretroviral therapies targeting Gag/RNA interactions.

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BIOPHYSICAL CHARACTERIZATION OF BQSR, THE RESPONSE REGULATOR OF PSEUDOMONAS AERUGINOSA'S TWO-COMPONENT SYSTEM, BQSR/S

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Pseudomonas aeruginosa (Pa), a ubiquitous and opportunistic Gram-negative bacterium best known as the pathogen that infects late-stage Cystic Fibrosis (CF) patients, can grow either as planktonic or as biofilm. Biofilms are complex microbial structures capable of providing an advantageous protective quality that causes bacteria living within a biofilm to be highly resistant to antimicrobial therapies. Recent studies have unearthed a novel two-component signal transduction system (BqsR/S) that regulates biofilm formation/decay in *P. aeruginosa* through extracellular Fe^{2+} that is present throughout each stage of infection in CF sputum and constitutes a large portion of the iron pool present in advanced stages of lung function failure. *Pa*BqsS has been identified as a transmembrane sensor kinase, while *Pa*BqsR has been identified as a cytosolic response regulator that binds to DNA and is capable of altering transcription. The deletion of either protein gene in *P. aeruginosa* results in a significant increase in biofilm formation. However, neither of these proteins have been structurally characterized, and the details of how and to what extent these proteins interact with Fe^{2+} remains unsolved. In my research, I am exploring the structural and biophysical properties of *Pa*BqsR. In our lab, we have expressed, purified, and partially characterized *Pa*BqsR using NMR structural techniques. We have also solved the structure of the N-terminal domain of *Pa*BqsR to 1.3 Å resolution using X-ray crystallography. Future work aims to determine the DNA binding thermodynamics and metal-binding properties of *Pa*BqsR. To test DNA binding, electrophoretic mobility shift assays (EMSA) are being conducted. To test metal binding, co-crystallization of the protein in the presence of metals along with inductively-coupled mass spectrometry (ICP-MS) are being performed. Once realized, this work will reveal essential properties of *Pa*BqsR that could serve as a foundation for potential novel antibiotics to target biofilms of antibiotic-resistant pathogens.

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EXPLORING THE EFFECTS OF N-LINKED AND O-LINKED GLYCOSYLATION ON MEMBRANE TRAFFICKING AND SIGNALING IN MELANOPSIN

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Melanopsin is a light sensitive photopigment belonging to the opsin family of class A G-protein coupled receptors GPCRs. Melanopsin, like other GPCRs, is located on the plasma membrane and this localization is critical for their function. Glycosylation is a common form of post-translational modification that is responsible for trafficking of proteins to the membrane. N-linked and O-linked glycosylation differentially contribute to a variety of additional functions including signaling, receptor dimerization, and folding. Interference of N-linked glycosylation results in impaired membrane expression, folding, dimerization, and eventual degradation of GPCRs. For both melanopsin and other GPCRs, N-linked glycosylation research has been the dominant focus, with little understood about O-linked glycosylation. This research creates mutant melanopsin receptors lacking consensus glycosylation sites to interrogate the impact of N- vs. O-linked glycosylation.

The first aim of this research is to examine the impact of N-linked glycosylation on melanopsin signaling and expression. N-linked null melanopsin constructs were generated that are unable to be glycosylated. These mutants were expressed in HEK293 cells where their expression was assayed via immunohistochemistry and western blot. Calcium signaling assays and BRET-based G-protein activation assays were used to examine changes in signaling amplitude and kinetics. The second aim of this research used an O-linked prediction tool to identify probable glycosylation sites which were mutated to generate an O-linked null melanopsin. The same set of experiments were employed to dissect the different contributions of N- and O-linked glycosylation. An additional N- and O-linked null melanopsin was generated as well.

Results suggest that lack of N-linked glycosylation clearly impacts membrane localization and calcium signaling amplitude. O-linked glycosylation experiments are ongoing.

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EXPRESSION TESTING OF FEOD, A NOVEL SINGLE-PASS TRANSMEMBRANE PROTEIN OF THE FEO SYSTEM

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Ferrous (Fe^{2+}) iron is utilized as an important cofactor for bacterial metabolic and biochemical processes; however, in order for bacteria to utilize this necessary ion, it must first be acquired from the environment. While multiple Fe^{2+} transporters exist, the ferrous iron transport (Feo) system is the most widely distributed and most prevalent Fe^{2+} transporting system that is found in commensal and pathogenic bacteria alike. The canonical Feo system initially identified in *Escherichia coli* is tripartite and consists of two small proteins (FeoA and FeoC) and one large, polytopic transmembrane protein (FeoB). FeoA is strongly conserved across bacterial *feo* operons, while FeoC is found in less than 20 % of bacteria. Recently, bioinformatics data from our lab have indicated the presence of a single-pass transmembrane protein that may take the place of FeoC in some bacteria. This protein, which we term "FeoD", is predicted to bind an iron-sulfur cluster similar to many (but not all) FeoC proteins. However, there is no biochemical experiments at the protein level on this putative protein. To that end, the *feoD* gene from the organism *Clostridium botulinum* was synthesized, subcloned into the pET-21a(+) expression plasmid, and transformed into the *E. coli* BL21 (DE3) expression cell line. Expression testing in multiple bacterial media and at multiple temperatures is currently being conducted in order to optimize the conditions for protein overproduction. Once optimal conditions are identified, we plan to isolate bacterial membranes, to extract FeoD from the lipid bilayer, and to purify FeoD through several chromatographic methods. A long-term goal is to analyze FeoB-FeoD protein-protein interactions in order to elucidate the function of this novel protein of the Feo system.

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IDENTIFICATION OF A SPECIFIC LYSINE SITE METHYLATED BY SET 5 ON THE UBIQUITIN LIGASE CWC24

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Yeast Set5 and human SMYD3 are a part of the SMYD methyltransferase family that are thought to target histone proteins for methylation. Set5 is an enzyme that has methylation activity on lysine side chains through its catalytic domain and is characterized as a histone methyltransferase but is predicted to also target nonhistone proteins. Cwc24 is the first nonhistone substrate for Set5 that has been characterized. Through research conducted in the Green Lab, it was found that Set5 methylates the substrate Cwc24 which is a protein that has an essential splicing factor and contains a RING and zinc finger domain, where the zinc domain is essential for function, but the RING domain is dispensable. The goal of this project is to identify a specific lysine site of Cwc24 methylated by Set5. It was hypothesized that this lysine site is present in the N-terminal domain of Cwc24. To test this hypothesis, the N-terminal domain will be deleted to determine if a methylation site is present in the domain. This will be done using cloning by restriction digest into a vector that expresses a GST fusion protein in *E. coli, which is* used for protein expression and purification. Following this, we will perform protein purification and an *in vitro* methylation assay using recombinant proteins. We were able to make positive clones, as tested by restriction digest, for protein purification and are still working on purification so that we can conduct a methylation assay. Overall, this research will be done to define new methylation sites by Set5, which is similar to SMYD3. SMYD3is implicated in multiple cancer types meaning that discovering new methylation sites can lead to new cancer treatments.

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STRUCTURAL AND BIOCHEMICAL STUDIES OF THE UNSPLICED HIV-1 5'LEADERS

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HIV-1 is the causative agent of acquired immunodeficiency syndrome (AIDS). Although antiretroviral therapies are available, drug resistance prompts the development of novel therapeutic strategies. Our research focuses on comparing the structural differences between monomeric and dimeric HIV-1 5' unspliced Leaders by using SHAPE. HIV-1 uses RNA as its genome. The 5´ leader is an untranslated region located in the 5 end of the genome, which is highly conserved and involved in regulating viral RNA functions and fates. Previous NMR and biochemical studies have shown that the 5'-Leader can adopt two different conformations which are regulated by the heterogeneity of the transcriptional start site. However, these structures were studied under non-physiological conditions due to the limitation of NMR, so we want to know the structural difference between a monomeric leader and dimeric leaders under physiological conditions. Therefore, this prompted us to use a technique called SHAPE (Selective 2' Hydroxyl Acylation by Primer Extension). SHAPE uses an electrophilic reagent that 2' OH groups will attack the RNA backbone in flexible regions of RNA, and this modification will be detected using reverse transcription. SHAPE lets us study the 5'Leader structures in physiological conditions. Unfortunately, in these conditions, the 5'Leader is a mixture between monomer and dimer, and SHAPE requires a homogenous RNA sample. Therefore, we tested different factors and eventually found the optimal condition that allowed the 5' leader to exist as a pure monomer or dimer. Then a SHAPE experiment was performed, and the results indicate that the dimeric leader structure is different from the monomeric structure which is consistent with NMR results. However, the monomeric structure under physiological conditions is not fully consistent with that under the NMR condition. Therefore, our next step is to explore the possible reasons for inconsistencies between the SHAPE and NMR data.

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SENSEBOX V2.0: A ROBUST SMART HOME SYSTEM FOR ACTIVITY RECOGNITION AND BEHAVIORAL MONITORING

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In the past few years, internet-of-things, artificial intelligence, and pervasive computing technologies have contributed to the next generation of context-aware systems with robust interoperability to control and monitor the environmental variables of smart homes. Motivated by this, we propose SenseBox v2.0, a multifunctional automated smart home system for smart home control applications. In addition, SenseBox v2.0 provides a platform for human activity recognition (HAR) and behavioral monitoring. In SenseBox v2.0, we focus on tackling the following challenges: data fragmentation, time desynchronization, network complexity, and system fragility for real-world deployment. To address these challenges, SenseBox v2.0 will integrate Home Assistant (HA) to aggregate data on a local hub device, synchronizing with a server as needed. This is designed to make the system more easily interoperable with future sensor technologies due to broad community support for HA.

We surveyed over 50 commercial off-the-shelf (COTS) sensor solutions and narrowed our selection down to one model of each of the following: a passive infrared (PIR) detection sensor, reed (door) sensor, object tag motion sensor, wearable wrist-attached accelerometer sensor, wearable wrist-mounted accelerometer sensor, and IP camera. The first three sensors use the Zigbee open standard for mesh networking, whereas the wearable sensor (the Empatica E4) uses Bluetooth Low Energy and the IP camera uses WiFi. Lastly, we assembled these devices into a system using HA on an x64-based hub due to the system requirements for the Empatica E4. We evaluate our proposed system by computing data rate loss, network latency, battery life, and computational resource consumption.

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DESALINATION OF LIMESTONE USING AGAROSE/ION-EXCHANGE RESIN HYDROGEL: AN ANALYSIS USING THE PROFILER NMR-MOUSE SPECTROMETER TO PRESERVE CULTURAL HERITAGE

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Soluble salts in artifacts are known to cause degradation—including delamination, powdering, and flaking of the surface—due to the dissolution and recrystallization of salts from fluctuations of an object's environment, such as humidity cycling. Art conservators often desalinate objects as part of the treatment process to prevent further degradation. While there are multiple methods of desalination, one promising technique uses an agarose and ion-exchange resin hydrogel. To monitor the efficacy of the hydrogel, the Profile NMR-MOUSE spectrometer was utilized. Non-invasive depth profiling and T_2 (spin-spin) relaxation experiments were conducted for the *in situ* evaluation of NaCl diffusion from limestone and concrete-based stone samples into the hydrogel. Through these experiments, the efficacy of the hydrogel was shown to be dependent on the physical relationship between pore size of the stone sample and the concentration of the hydrogel mixture. Additionally, the bulk diffusion of NaCl into the hydrogel during desalination impacts the T_2 relaxation and allows for the efficacy of the hydrogel to be assessed over time. Furthermore, the depth profile experiments revealed the exchange of water between the stone and hydrogel during desalination. The culmination of these experiments promotes the use of non-destructive, portable NMR processes to assess treatment methods in the field of cultural heritage.

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REPAIRING PARCHMENT USING HYDROGELS: INCORPORATING CROSSLINKERS TO INCREASE THE STRENGTH OF REPAIR AS MEASURED BY ULTIMATE TENSILE STRENGTH (UTS)

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Since the 2nd century BCE, animal skin parchment has served as a medium for creating art and storing information. Many of the surviving parchments are preserved and studied for their importance to cultural heritage. Parchment is produced from drying animal skin and the subsequent removal of keratin, elastin, and fats, leaving a network structure of collagen. When stored under the right conditions, parchment is long lasting and durable. However, the biological material is susceptible to environmental degradation via acidity, humidity, temperature, mold, and/or microbes/bacteria. There are numerous efforts to preserve and repair parchment; however, many of these methods are fragile or eventually cause further damage to the piece. Here, we used gelatin, collagen, and alginate hydrogels, commonly applied in tissue engineering, to repair damaged parchment. The effect of adding glutaraldehyde (a crosslinker and a preservative) along the edges of the damaged parchment to create a stronger attachment in between the broken collagen fibers and the hydrogel within the parchment was also studied. Using an Instron mechanical testing machine and microscopy, we assessed the repair method, tensile strength, and visual quality of the repairs. The tensile behavior of parchment was tested before (61.7 MPa) and after repair to measure the difference in the strength of the hydrogel formulations. Our most successful repairs resulted when we applied a 4% gelatin hydrogel with glutaraldehyde, in a 1:1 water and ethanol solution, on the parchment with a pipette. After repair, the average tensile value is 14.0 MPa, which is similar to other currently accepted repair methods (20.6 MPa). This study explores an affordable, accessible method to restore torn parchment.

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FOOD PREFERENCE IN *CAENORHABDITIS. ELEGANS* AFTER ETHANOL AND NICOTINE EXPOSURE IN WILD TYPE VS ADR-2 MUTANTS

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Caenorhabditis elegans are a free-living nematode whose life cycle is 3 days. They consist of males and hermaphrodites which are 1 mm in length, making them an ample model organism. Within the *C. elegans* genome is a gene called adr-2 that is involved in positive regulation of chemotaxis. Alcohol and nicotine are two of the most used substances in history. Studies have suggested that habitual smokers have a decreased affinity for sweet-tasting foods while alcohol consumption causes an increase in desire for foods high in sugar. Adr-2 could be tested to determine behavioral changes in drug addiction in relation to its human ortholog protein ADAR. We hypothesized that the adr-2 mutation would influence the food preference of *C. elegans* after pre-exposure to ethanol or nicotine.

Wild-type and adr-2 mutated strains were exposed to nicotine and ethanol for 24 hours and 2.5 hours, respectively. After the separate exposures, a chemotaxis assay determined which food source the worms preferred: *E. coli*, fructose, or sucrose. A preference index was calculated to see the differences between the wildtype and the strain with the non-functional ADR-2 protein.

As a result of the ethanol and nicotine exposures, wild-type and adr-2 mutant *C. elegans* experienced a decrease in affinity for each food source. In comparison to the adr-2 mutant, wild-type *C.elegans* showed a greater decrease in preference after being exposed to ethanol or nicotine. In conclusion, *C. elegans*, like humans, showed a decrease in affinity for food sources after nicotine exposure regardless of strain. In contrast to humans, both *C. elegans* strains showed a decrease in affinity for food sources after pre-exposure to ethanol. Further studies would be necessary to understand the pathways that lead to this difference.

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NANOPARTICLE-BASED SENSOR FOR THE DETECTION OF LEAD IONS IN WATER

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Lead Poisoning is a problem that people all over the world, including the Unites States, are battling. Although medicine has made vast improvements to help fight the side effects and treat lead poisoning, the best way to avoid it is to detect lead and remove it from whatever source the population is drinking from. Lead poisoning occurs when the body intakes too much lead. Just as the body has no use for lead, it also does not have a method for removing lead on its own. As a result, lead is called a cumulative toxicant because it builds up in the body over a period of months or years. The Center for Disease Control permits 15 parts per billion lead in public drinking water and the World Health Organization suggests 10 parts per billion lead in drinking water. Creating a cheap and accurate test is vital to preventing lead poisoning in children and adults.

Gold nanoparticles are particles of gold that range anywhere from 1 nm to 200 nm in diameter. Gold nanoparticles have unique properties such as the ability to be shaped into bipyramids, rods, and many other shapes. Another unique property is that they can be coated with molecules that selectively bind with heavy metal toxins such as lead. In the presence of lead, glutathione-coated gold nanoparticles change color from a shade of red to a shade of blue, thus lead can be detected through color change. In the gold nanoparticle solution glutathione is added because the carboxyl groups attach to the gold and the lead, thus making it more reactive. Additionally, sodium chloride is used to help further induce color change. Thus, we are creating a system of gold nanoparticles, functionalized with glutathione, that change color in the presence of lead with assistance from sodium chloride.

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THE EFFECTS OF PRE-EXPOSURE TO NICOTINE IN *CAENORHABDITIS ELEGANS*

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Nicotine, a highly addictive substance found in tobacco products, has seen a sharp increase in usage, especially with the younger generation. About 35% of children are in households with parents that regularly smoke. The cell biology of *C. elegans* is remarkably well conserved and their attraction to nicotine runs parallel in humans. As a result, *C. elegans* can be used as a model to demonstrate nicotine dependence in humans. The reactions of wild-type and mutant (odr-3) *C. elegans* pre-exposed to nicotine were explored. The odr-3 gene codes for G-protein olfactory receptors in *C. elegans.* The purpose of this research was to understand how *C. elegans* behavior changes when they are pre-exposed to nicotine and if this response is different when the ODR-3 protein is non-functional.

A chemotaxis assay was designed for testing preference index after pre-exposure to nicotine. Wild-type and odr-*3* mutant *C. elegans* were exposed to 0 mM, 0.02 mM and 0.2 mM nicotine for 24 hours. A chemotaxis assay was performed on the non- and pre-exposed worms, and preference for nicotine was measured at 60 minutes.

The non-exposed wild-type and odr-3 mutant *C. elegans* did not show a preference for nicotine. Contrastingly, both pre-exposed strains showed increased affinity to nicotine after 24 hours, with the preference index being exaggerated in odr-3 compared to wild type *C. elegans.* Overall, the data suggests that the pre-treatment with 0.2 mM nicotine seems to be the most effective to increase attraction to nicotine, and the odr-3 gene is likely to have a role in the development of preference to nicotine. The ODR-3 protein is implicated in the function of *C. elegans* olfactory and nociceptive neurons, and the absence of a functional protein could lead to a stronger positive affinity for nicotine.

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BIOPHYSICAL CHARACTERIZATION OF BQSS, THE IRON-SENSING HISTIDINE KINASE OF PSEUDOMONAS AERUGINOSA'S TWO-COMPONENT SYSTEM, BQSR/S

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Pseudomonas aeruginosa (*Pa*) is a ubiquitous Gram-negative bacterium best known for infecting the lungs of cystic fibrosis (CF) patients, is one of the major causes of chronic nosocomial infections, and can grow either as planktonic or biofilm. Biofilms are a serious health threat because bacteria living within these environments are significantly resistant to antibiotic treatments. Recent studies have uncovered a two-component signal transduction system (BqsR/S) that regulates biofilm formation/decay in *P. aeruginosa* through extracellular Fe^{2+} binding. *Pa*BqsS has been identified as a transmembrane sensor kinase and *Pa*BqsR has been identified as a cytosolic response regulator. The deletion of either protein gene in *P. aeruginosa* alters biofilm formation. However, these proteins have not been structurally characterized, and the details of how and to what extent they interact with Fe^{2+} remain unknown. In my research, I am exploring the structural and biophysical properties of *Pa*BqsS. Recent studies have shown that *Pa*BqsS recognizes Fe^{2+} through an RExxE motif in its periplasmic domain. Modeling data suggest that an additional RExxE motif is present in its cytoplasmic domain, which may also be a site of metal binding. However, the metal stoichiometry, ligands, and coordination motif(s) of *Pa*BqsS have yet to be determined. We have expressed, detergent solubilized, and purified *Pa*BqsS. We have also shown that this protein binds one iron ion per dimer. My goal is to alter the RExxE motif through site-directed mutagenesis in order to show that these amino acids are necessary for Fe^{2+} binding. After confirming that the *Pa*BqsS variant has been made, metal-binding experiments will be performed to probe the metal-binding stoichiometry. These results would be an important step towards understanding this two-component system, which could be a future target for novel therapeutics aimed at disrupting biofilm formation.

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THE ROLE OF *RLS1* IN OXIDATIVE STRESS RESISTANCE BY THE GREEN ALGAE *CHLAMYDOMONAS REINHARDTII*

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Biofuels are an important renewable energy source that can help solve both climate and energy security issues. Algae can produce substantial amounts of lipids that can be converted to biodiesel, so are a promising source of biofuels. In this project, we are investigating the model green alga *Chlamydomonas reinhardtii* to learn how to improve algal growth and lipid production by improving growth during conditions of oxidative stress. We are testing the hypothesis that the *RLS1* gene increases stress resistance, specifically against hydrogen peroxide (H_2O_2). It has been observed that under oxidative stress, *RLS1* expression is induced in Chlamydomonas. Improved stress tolerance should improve lipid production and reproductive ability. In this analysis are testing the oxidative stress resistance of two strains, the wild-type (CC-4533) and a closely related strain with an insertional mutation in the *RLS1* gene. To compare their degree of tolerance we are testing their growth at different concentrations of H_2O_2 and with different initial cell concentrations. If our hypothesis is correct, we are expecting the wild-type to have better growth than the *RLS1* mutant in the presence of H_2O_2. If our hypothesis is correct, the application of *RLS1*'s stress resistant attributes could be used to boost lipid production in Chlamydomonas and other algae that are engineered to over-express the gene. *RLS1* function might also be modulated to reduce stress caused by light deprivation.

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Investigating Competition between the HIV-1 Proteins Rev and Gag for Stem 1 of the Rev Response Element

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HIV replication requires the export of full-length RNA genome transcripts from the nucleus to the cytoplasm, where the genome is packaged. Host surveillance mechanisms prevent such RNA export, so HIV uses Rev, a viral protein translated from fully spliced transcripts to enter the nucleus and bind to a highly conserved RNA element of the HIV genome, the Rev Response Element (RRE), which is retained on un/incompletely spliced RNA. The Rev-RRE complex binds to host export machinery, and exits the nucleus to the cytoplasm, where unspliced RNA is available for translation or packaging. Rev binds to the RRE on two stem II binding sites and on one purine-rich bulge in stem 1. The RRE has been shown to also be bound by Gag –a viral protein involved in genome packaging– at the same RRE stem I binding region as Rev. To understand the interaction and biological relevance between Rev and Gag on RRE stem 1, we use a peptide containing the RNA-binding, arginine-rich motif (ARM) of Rev, a protein containing the nucleocapsid (NC) domain of Gag, and truncated RRE stem I fragments in EMSA and ITC studies. Some RNA fragments include mutations that allow us to probe for specific protein binding sites. We find that NC displays tighter binding affinity to our stem 1 constructs than the Rev ARM peptide, which may suggest that Gag binds to the RRE in the cytoplasm and displaces Rev from the stem 1 binding site. This interaction is biologically relevant and may represent a link between nuclear export of the genome and subsequent genome packaging. Future studies are needed to more accurately explore this competitive interaction, such as the usage of full-length RNA constructs, which would contain more Rev binding sites, and full-length protein constructs that would more accurately represent physiological interactions.

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Recovering from hypoxia: The role of ndrg1a in recycling NKA

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As the final electron acceptor in the electron transport chain, oxygen plays a critical role in ATP synthesis. Hence, ischemia (lack of oxygen delivery) is a leading cause of death in the United States. By contrast, zebrafish embryos can survive up to fifty hours in a zero-oxygen (anoxic) environment. The embryos can withstand these harsh conditions by entering into a metabolically suppressed state characterized by reversible metabolic arrest of ATP-demanding processes, such as ion pumping driven by the Na+/K+/ATPase (NKA). The Brewster Lab has previously determined that NKA downregulation in response to anoxia is reversible and is thus an adaptive process. We have also shown that the highly conserved N-myc Downstream Regulated Gene 1 (NDRG1) mediates metabolic suppression, although the exact molecular mechanisms remain unknown. Essentially, NDRG1a is an oxygen sensor that is upregulated in response to low oxygen and works to conserve energy, thus ensuring embryonic survival. Intriguingly, NDRG1a transcript is upregulated nine-fold (especially in ATP-demanding organs) under anoxia, but is only translated post-anoxia, suggesting that Ndrg1a protein is required during reoxygenation. My project aims to test whether NDRG1a is required for the re-insertion of NKA into the plasma membrane post anoxia. I predict that, if this model is correct, these two proteins will co-localize and interact from the earliest time point post-anoxia, as assessed using immunolabeling and the proximity ligation assay (PLA), respectively. Preliminary PLA data suggest an interaction at time zero that increases steadily for several hours post re-oxygenation, consistent with my prediction. Future work will explore the model that Ndrg1a promotes NKA recycling by examining additional interactions with proteins implicated in endosomal trafficking. We anticipate that these studies will further our understanding of hypometabolism as a protective adaptation with great human therapeutic potential.

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OPTIMIZATION OF AN IN VITRO CAPPING REACTION USING THE VACCINIA VIRUS CAPPING ENZYME

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The 5′-Leader of the human immunodeficiency virus type-1 (HIV-1) has many unique properties as it regulates many viral functions such as packaging, assembly, and translation. By focusing on the 5′-Leader and its protein interactions, our laboratory hopes to aid the development of novel therapies that help prevent the packaging of HIV-1. HIV-1 RNA primarily exists as transcripts that begin with a 5′-cap (7-methylguanosine) followed by one or three guanosines. To purify 5′-capped RNAs in our laboratory, we purify and use the vaccinia virus capping enzyme (VVCE) that will add the 5′-cap in an in vitro capping reaction. We are interested in purifying 5′-capped RNAs to study the interactions between the 5′-Leader and a cellular cap binding protein, eIF4E. Our current in vitro capping reaction produces a mixed population of 5′-capped RNA and uncapped RNA. Therefore, we are optimizing the purification of the VVCE and have improved our in vitro capping reaction.

Previous methods of purification used affinity column chromatography which led to an impure product with low yield. When using this protein in the capping reactions, less than 50% of the RNA were successfully capped. To improve the efficiency of VVCE purification we tested purification methods including fast protein liquid chromatography and size exclusion chromatography. We tested different *E. coli* bacterial cell lines. By altering the cell line and the purification method used, we were able to purify an enzyme that can efficiently cap smaller RNA constructs where the one nucleotide shift can be recognized on denaturing polyacrylamide gels. With these findings we will proceed to cap large RNA constructs and complete gel mobility shift assays to determine if our RNAs are capping at ~100% efficiency. Ultimately, these 5′-capped RNAs will be used to study the interactions between eIF4E and the HIV 5′-Leader.

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A NONINVASIVE COMPUTATIONAL DFT INVESTIGATION OF THE PHOTODEGRADATION OF VERMILION

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HgS, the primary component of cinnabar and the pigment vermilion, is known to photodegrade in the presence of chlorides. To uncover the mechanisms of degradation, a noninvasive computational probe called DFT was employed. DFT calculations were used to map out atomistic information about the initial degradation steps of cinnabar and vermilion in the context of art conservation. Variations in surface areas, cleavage planes, and surface terminations were explored through comparing adsorption energies. A test set of adsorbate molecules including pollutants, chloride-containing compounds, and water products, with varying oxidation states of the central atoms, were screened to determine which adsorbates cause surface transformations that coincide with lower adsorption energy. Relevant surface interactions were identified by analyzing atomistic and electronic information such as the making and breaking of bonds, bond distances, surface transformations, changes in oxidation states, and adsorption energy. Multiple surface terminations and cleavage planes provided varying degrees of stability and comparing surface adsorbate interactions on multiple surfaces provided a more complete understanding towards the reactivity of different classes of molecules on cinnabar and vermilion.

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CREATING A 2D SIMULATION OF A CLUSTER OF CELLS MIGRATING IN THE DROSOPHILA MELANOGASTER EGG CHAMBER

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The phenomena of clustered cell migration can be observed in numerous biological processes. The importance of this mechanism is demonstrated in tissue healing and cancer metastasis. We have developed code to simulate this movement of cells through an egg chamber of the *drosophila melanogaster*.

Cell migration was recently modeled imposing an adhesive, repulsive, volume, and spring force in the simulation. However, we observe flattening of the migration cells, a behavior unrealistic for this biological process. To avoid cusp-like corners and sustain a circular cell shape, we have derived an equation for a curvature force. To implement this force, we first measure the angle between the boundary points that make up a cell. If the angle is below a predetermined critical angle, then the curvature force is enacted. Our implementation requires the calculation of vectors between neighboring boundary points. From these vectors, we determine the angle between them using their magnitudes and the dot product. To calculate the curvature force, we find the projection of the force vector onto the vector between the two neighboring points on either side of the specific boundary point. We then balance the curvature force in the two neighboring points if it were to reduce the angle between them. The local curvature constraint may not be sufficient to avoid continued effective flattening, as we observe delayed deflating of the border cells. We are investigating ways in which we can optimize the implementation of this force. The simulation requires a very specific balance of forces which are controlled by various parameters. We find that changing the parameters, even in small magnitudes, has drastic effects on the egg chamber. To further examine the effect of these individual forces, we have simplified the simulation to only contain one migrating cell between two nurse cells, then we plot the radius of interaction and the force vectors of the boundary points to better understand the curvature force before including multiple migrating cells.

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EXPLORATION OF GENETIC COMPENSATION IN ALGAL LIPID PRODUCTION GENES

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Understanding interactions between the algal genome and environmental conditions is fundamental to the goal of generating strains that can become economically beneficial and environmentally sustainable players in the future of energy production. Algae produce the lipid Triacylglycerol (TAG) as a stress response to environmental conditions; the TAG produced is of interest due to its conversion into biodiesel. The purpose of this study is to investigate the mechanism behind the compensation of TAG observed in knockout mutants of TAG synthesis genes by crossing a *upf3* mutation into previously generated mutants of *Chlamydomonas reinhardtii* (Lee et.al, 2020). The UPF3 protein is involved in the nonsense-mediated decay mechanism (NMD) and is proposed to influence genetic compensation. Genetic compensation is observed when a knockout mutation induces the transcription of a different gene that serves the same function, therefore maintaining the needs of the organism. The two genes further investigated in this study are phospholipid: diglycerol acyltransferase (*PDAT*) and the vascular transport chaperone (*VTC1*). PDAT is involved in the acyl-CoA independent pathway of TAG synthesis in *Chlamydomonas*, and the VTC is a cellular mechanism involved in sequestering polyphosphate chains in vacuoles to maintain homeostasis and cellular energy. To generate crosses, mutant strains of *pdat* and Δ*vtc1/pdat* were introduced to a *upf3* mutant. Once *pdat/upf3* and Δ*vtc1/pdat/upf3* mutants are confirmed through PCR analysis, strains will be grown in N and P deprived media to induce TAG gene expression and TAG production. TAG gene expression will be measured by RT qPCR and TAG content by thin-layer chromatography (TLC). The results will be used to assess the involvement of NMD in the genetic compensation of *pdat* and Δ*vtc1/pdat* mutants. Exploration of this genomic response has the potential to aid in the development of profitable algal strains for biofuel production.

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Reference: Lee, Y.-Y., Park, R., Miller, S. M., & Li, Y. (2021). Genetic compensation of triacylglycerol

biosynthesis in the green microalga chlamydomonas reinhardtii. https://doi.org/10.1101/2021.08.12.455529

LIGATION INDEPENDENT CLONING OF HELICOBACTER PYLORI NFEOB

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Ferrous iron (Fe2+) is an important cofactor for bacterial metabolic and biochemical processes; however, in order for prokaryotes to utilize this nutrient, Fe2+ must first be acquired from the bacterial environment. The ferrous iron transport (Feo) system is the most widely distributed and prevalent Fe2+ transport system found throughout the bacterial domain, and pathogenic bacteria make use of this system to establish infection within hosts. The chief component of the Feo system is a large transmembrane protein named FeoB, which contains a soluble N-terminal G-protein domain known as NFeoB that is known to bind and to hydrolyze GTP. Intriguingly, recent studies have shown that select FeoBs are capable of hydrolyzing both GTP and ATP with Michaelis-Menten like kinetics, suggesting that some FeoB proteins are active NTPases. While there are several structures of NFeoB bound to guanine nucleotides, there is currently no structure of NFeoB bound to adenosine nucleotides. To elucidate how NFeoB is able to bind and to hydrolyze both ATP and GTP, our lab has chosen to biophysically characterize the NFeoB domain from FeoB of the pathogenic organism Helicobacter pylori. Our current protein purification protocol of HpNFeoB produces low yields, and we hypothesize this result is due to a disordered region on the C-terminal end of the protein. To increase the protein yield, the C-terminal (His)6 tag is being translocated to the N-terminus via ligation independent cloning (LIC). Once accomplished, the new plasmid will be tested for expression and protein production. Future goals include determining the structure of this domain via X-ray crystallography, which will reveal critical residues that are responsible for GTP and/or ATP binding. These results should shed light on the mechanism of Fe2+ uptake in this pathogen, which could be leveraged for future therapeutic developments.

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PH DEPENDENCE OF BROWN CARBON ABSORBANCE IN CLOUD WATER

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Aerosols are small particles or liquid droplets that accumulate in the atmosphere. They are a concern for air quality and climate forcing. Light-absorbing aerosols, such as black and brown carbon, are those which absorb solar radiation and thus have a warming effect on climate. While black carbon has been widely researched, the effects of brown carbon on the earth's climate are still quite uncertain. The main concern with brown carbon is its ability to absorb in the ultraviolet and visible regions of the spectrum. It is known that clouds contribute to the production of secondary aerosols, and they are likely to play a role in the formation of brown carbon as well. With this assumption, it is possible to use cloud water to characterize brown carbon and determine its effects on the atmosphere. In this work, we characterized brown carbon in cloud water samples from Whiteface Mountain, NY. Specifically, we characterized the pH dependence of brown carbon absorbance in samples with variable cloud water compositions and air mass origins. Absorbance measurements for cloud water were recorded using a liquid waveguide capillary cell (LWCC) coupled with two spectrophotometers and a DH-mini light source. One of the spectrophotometers, the STS-Vis, was used to troubleshoot the light source, whereas the other spectrophotometer recorded the absorbance of the cloud water samples in the LWCC. This approach allows for spectral analysis of low-concentration material, resulting in very precise measurements for absorbance. After absorbance values are recorded, analysis was carried out on the relationship pH has with other variables, such as the mass absorption coefficient (MAC) and the absorption Angstrom coefficient (AAE). Trends suggest that pH has a negative correlation with AAE, and MAC has a positive linear relationship with pH for the samples analyzed to date.

Dr. Christopher Hennigan is the principal investigator of the Cloud Water Project at UMBC. Support is acknowledged from the Department of Energy through grant #DE-SC0022049. This research was partially funded by the USM LSAMP program, supported by NSF LSAMP Award #1619676.

EXPERIMENTATION AND MODELING OF *ASPERGILLUS NIGER* EFFICIENCY IN THE BIODEGRADATION OF HYDROCARBONS

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The exploitation of Earth's petroleum resources in recent decades has led to increased global environmental issues, including the occurrence of harmful industrial oil spills. Novel methods of oil spill cleanup involve bioremediation, in which organisms can utilize petroleum as a main carbon source without producing toxic environmental byproducts. In this study, we explore the potential of *Aspergillus niger* fungi as a hydrocarbon decomposer through experimentation and modeling. Three cultures of *A. niger* strain were prepared in MAG-V media and then grown in BG-11 liquid cultures with experimental carbon sources (motor oil, glycerol, and glucose for control). A MATLAB model was also employed to speculate optimum growth rates of *A. niger* in glycerol and glucose cultures analyzing impacts of environmental factors such as various nitrogen sources. The results of our experimentation highlights the efficiency of *A. niger* as a promising bioremediation solution to oil pollution in novel liquid cultures, and thus water systems.

Our research was conducted as a part of the Emerging Frontiers in Research and Innovations Research Experience and Mentoring (EFRI-REM) program, which was graciously supported and funded by the National Science Foundation (Grant 1332344).

THE EFFECT OF ETHANOL PRE-EXPOSURE ON ETHANOL TOLERANCE IN *CAENORHABDITIS ELEGANS*

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Alcohol use disorders (AUDs) are a predominant problem in our modern world, and much is unknown about the mechanics of AUDs. Studying the effects of alcohol on model organisms can offer insight about the nature of, and possible treatments for, alcohol use disorders. *Caenorhabditis elegans* is a prime model organism for studying the effects of alcohol, in part due to the genetic similarities between *C. elegans* and humans.

The effect of ethanol pre-exposure to ethanol tolerance was observed in *C. elegans*. Two strains of *C. elegans* were chosen for this research, a wild-type (N2) strain, and a npr-1 mutant strain (CX4148). An npr-1 loss-of-function mutation in *C. elegans* is associated with increased ethanol tolerance.

C. elegans were pre-exposed to ethanol for 24h, recovered, then placed under an acute ethanol exposure. During this acute exposure, a tolerance assay was performed; thrash rates of *C. elegans* were observed to measure mobility. Differences in mobility when exposed to ethanol was used as an indicator for tolerance.

Our results indicate that pre-exposure to ethanol generally results in increased ethanol tolerance in the wild-type strain of *C. elegans*. In contrast, no clear effect of pre-exposure in the npr-1 mutant was observed. These results were unexpected given previous papers that established a relationship between npr-1 mutants and increased ethanol tolerance. The increase in ethanol tolerance that results from pre-exposure in the wild-type has further implications for a relationship between pre-exposure to alcohol and risk of addiction in humans, as increased tolerance is associated with increased risk of addiction.

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STIMULANT EXPOSURE SIGNIFICANTLY AFFECTS FOOD PREFERENCE IN WILD-TYPE AND ODR-3 MUTANT *CAENORHABDITIS ELEGANS*

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Stimulant abuse is a particularly growing concern in the United States. Understanding the behavior of individuals under the influence of stimulants is paramount to generating better treatment approaches for those with stimulant abuse disorders. With their highly conserved genome to humans, *Caenorhabditis elegans* is a suitable model organism for studying drug abuse and behavior. The ODR-3 protein in *C. elegans* affects the function of olfactory and nociceptive neurons, and odr-3 mutations can lead to reduced responses to volatile and water-soluble odorants. The effect of stimulant use on bacterial food preference was observed in two strains of worms: wild-type (WT) and odr-3 mutant.

C. elegans were pre-exposed to either caffeine, nicotine, or a control NGM plate for 24h. A bacterial choice assay was conducted using *S. aureus*, *S. epidermidis*, *K. pneumoniae*, and *P. aeruginosa* with *E. coli* as the control. A bacterial preference index (PI) was calculated following the assay to determine the attraction or aversion to each bacterial patch.

The non-pre-exposed WT shows attraction to *K. pneumoniae* and *P. aeruginosa*, and aversion to *S. aureus* and *S. epidermidis.* Surprisingly, the non-pre-exposed odr-3 mutant worms had a negative PI for the four bacterial patches. In contrast, caffeine or nicotine pre-exposed odr-3 mutants showed a positive PI to *K. pneumonia* and *P. aeruginosa*, manifesting a behavior for bacterial food preference more similar to the non-treated WT. The caffeine treatment did not change the general attraction/aversion trend for the WT worms, while the major difference observed in the nicotine pre-exposed WT *C. elegans* was an aversion response for *P. aeruginosa.*

The non-functional odr-3 appears to induce an aversion to *K. pneumoniae* and *P. aeruginosa*. However, this negative PI was reverted with the treatment with nicotine or caffeine. Future experiments will be necessary to better understand the pathways involved in these responses.

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Investigating the Correlation Between Host Range and Genomic Content of Bacteriophages

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Host ranges are defined as which host species a virus can infect. For bacteriophages, the host range is determined by attempting to infect multiple strains of bacteria with specific bacteriophages; if the phage is able to infect the bacteria, then it will be included in the phage's host range. Since host range is a common way of characterizing and categorizing bacteriophages, we wanted to investigate the correlation between host range and genomic similarity. We hypothesized that phages with similar genomic content were more likely to infect the same hosts.

In order to find this correlation, we selected eight sequenced *Bacillus* phages from BacillusPhagesDB that have the same morphotype, cluster, and similar genomic lengths as a control. We recorded the host species that each phage had in common with another to compare their host ranges. We entered the sequences of all of the phages into OrthoANI, a data tool that compares the phages' genomes and finds their genomic similarity. We found that phages that had a high genomic similarity were more likely to have more host species in common, after calculating the r-value we found that there was a weak positive correlation between these two variables. In the future, this should be retested on a larger scale in order to rule out possible biases and the results could be further investigated to see which parts of a bacteriophage's genome can determine what host species the phage is able to infect.

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SEQUENCE MODELS FOR CLASSIFICATION OF COMPTON CAMERA IMAGING DATA FOR PROTON BEAM THERAPY

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Proton therapy is gaining popularity as a form of cancer treatment. Most radiation therapies work to damage the cellular DNA of target cancer cells. X-ray therapy delivers dosage at the tumor site, but its radiation continues to travel through the body, potentially harming healthy surrounding tissues with unnecessary radiation. Contrastingly, proton beams deposit the majority of their dose just before they stop. Since almost no radiation is delivered beyond this point, healthy tissue is spared from unnecessary radiation. To leverage the advantages of proton therapy, we must efficiently image the prompt gamma rays in real-time as they travel through the patient's body. A Compton camera can be used to detect the prompt gamma rays, and an algorithm reconstructs the beam's image from the prompt gamma data. Unfortunately, some of the Compton camera data is flawed and the reconstruction algorithm yields noisy and insufficiently detailed images to evaluate the proton delivery for the patient.

Machine learning can be used to clean the raw Compton camera data by identifying and removing false data before image reconstruction. Research efforts to clean the Compton camera data have led to the use of a deep residual fully connected neural network. The use of recurrent neural networks (RNNs) has been proposed. RNNs use recurrence relationships in sequence data sets to make predictions. In this work, RNN architectures using two different recurrent layers are tested, the LSTM and the GRU. The LSTM uses memory cells with gates and a carry track to encode and learn from sequence data. The GRU uses two gating units to encode and learn from sequence data. Although the deep residual fully connected model achieves a slightly higher accuracy in nearly every class, the simplicity of our RNN models containing only 4 hidden layers as opposed to 512 is an advantage. And importantly in a clinical setting, the time to load the model from disk is significantly faster, potentially enabling the use of Compton camera image reconstruction in real-time during patient treatment.

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COULD A BACTERIOPHAGE FOUND IN PHAGE HUNTERS PROGRAM HAVE ENDOLYSINS THAT CAN BE USED IN FUTURE PHAGE THERAPY?

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Phage therapy is a relatively new field that uses bacteriophages (phages) to treat pathogenic bacterial infections. Currently, phages like vB_BanS-Tsamsa (Tsamsa) and vB_BceM-HSE3 (HSE3) are being investigated for their endolysins as possible alternatives to antibiotics. While bacteria evolve to become more resilient to antibiotics, phage and bacteria stay in an evolutionary arms race with each other. A recent focus of phage therapy has been researching phages that can infect *Bacillus cereus* bacteria (*B. cereus*). Some strains of *B. cereus* cause food poisoning in animals and people which prompts an investigation into alternative treatment. The endolysins in phages HSE3 and Tsamsa have been shown to effectively kill strains of *B. cereus*. Our research aimed to determine whether a bacteriophage found in the Phage Hunters program could have the potential to be further investigated for future phage therapy based on its endolysin. We used the National Center for Biotechnology Information (NCBI) and Basic Local Alignment Search tool (BLASTp) to analyze the endolysin protein sequence of several phages from Phage Hunters and compared these endolysin sequences to that of Tsamsa and HSE3. It was found that only the *Bacillus* phage SalinJah had a similar endolysin sequence to both Tsamsa's and HSE3's endolysin sequence. We reviewed the Phage Hunters background data and found that SalinJah was able to infect 75% of the *B. cereus* strains it was introduced to. We then investigated the connection between the endolysin sequences of the SanlinJah and the other phages using phylogenetic inference. We used a One Click, a Phylogeny Tree creating software, to create a tree that showed a potential ancestral link between these endolysins. Based on these results, we can conclude that there is enough homology between the endolysin protein sequences of these phages to suggest further investigation into SalinJah's endolysin and its future in phage therapy.

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USING IMARIS TO CREATE A 3D MODEL OF A DROSOPHILA MELANOGASTER EGG CHAMBER

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Cell migration is an essential process for various biological mechanisms such as wound healing and cancer metastasis. A suitable model for this phenomena can be observed in the *drosophila melanogaster*, where a cluster of cells migrate through the egg chamber during embryonic development. We seek to accurately capture the geometry of this model in three-dimensions to simulate this process computationally.

Using high resolution microscopy, we have acquired a series of 2D slides of the egg chamber, called 'z-stacks'. We construct a 3D model from these slices through automatic rendering by a program, Imaris. We revise this model using various image processing tools available in Imaris. Some of the surface rendering edits involve setting the surface detail radius, threshold, background subtraction (local contrasting), and number of voxels. Through this technique, we have been able to obtain a 3D representation; however, we are currently looking for other ways to optimize the model for our purposes of simulation. One of the issues we encounter with the rendered model is the prevalence of thick walls and many holes (mostly from the nuclei of the epithelial layer), which is less representative of an actual egg chamber. We are investigating the usefulness of another program, Labkit, which uses automatic segmentation and machine learning, and pairs with Imaris. We find that Labkit aids in removing the holes in the model, and we are analyzing ways to obtain walls more realistic to the membranes that make up the egg chamber.

The findings of our research will assist in the creation of a 3D simulation of a cell cluster migrating through the egg chamber using Matlab.

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DETECTING ATMOSPHERIC GRAVITY WAVES WITH TRANSFER LEARNING

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Atmospheric gravity waves are produced when gravity attempts to restore disturbances through stable layers in the atmosphere. Due to their association with weather fronts, wind currents, and extreme weather events, they are an important factor that must be considered when predicting weather. Current methods of gravity wave detection require weather balloons and tedious calculations, which can be limited and inefficient at times. We believe gravity wave detection can become more accessible and effective using machine learning, especially due to the availability and abundance of satellite images. In this project, we aimed to create a model that can classify whether or not images contained gravity waves with good accuracy. First, we preprocessed and denoised various satellite images of airglow from NASA using methods such as Fast Fourier Transform. Then, we used two transfer learning approaches to detect gravity waves in our images. The first approach involved pre-training an autoencoder to recreate thousands of unlabeled satellite images, then using the first half as part of a classification model for the labeled dataset. We created a method for transforming our raw data into the satellite images we needed. The second approach involved taking part of the pre-trained InceptionV3 Model, making certain layers trainable, and adding new layers to create a custom classification model. The autoencoder transfer model achieved a training accuracy of 0.9753, a validation accuracy of 0.7000, a test accuracy of 0.6992, and an F1 score of 0.7296. The custom model achieved a training accuracy of 0.9474, a validation accuracy of 0.9063, a test accuracy of 0.7400, and a F1 score of 0.8000. Our methods and experiments show that certain transfer learning techniques can achieve accurate gravity wave detection on very small, noisy datasets.

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CHARACTERIZING THE DISTRIBUTION OF GADD45b, A CANIDATE MEDIATOR OF HYPOXIA-ADAPTATION

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Oxygen fulfills a critical role in cellular respiration, and when it becomes limited there is usually a change in metabolic priorities. Reduction in ATP production under hypoxic (low oxygen) or anoxic (zero oxygen) conditions can cause cell death and tissue damage rapidly in ischemic stroke or can drive diseases such as chronic kidney disease. Unlike most mammals, zebrafish embryos can survive prolonged periods of anoxia by arresting energy-demanding processes such as development and cell cycle progression and thereby preserving ATP, a response termed metabolic suppression. Metabolic suppression may also involve dramatic decreases in processes such as transcription and translation. Therefore, genes whose transcription increases under hypoxic conditions are likely to play an adaptive role during hypoxia.
RNA-seq provides information on the transcriptome of a cell and using this approach the Brewster lab identified a gene, *gadd45ba*, that is upregulated in response to anoxia. My project aims to investigate the role of gadd45b in hypoxia tolerance by examining its spatial distribution, subcellular localization, and binding partners. Previous studies performed by Chen and others revealed that in 24 hpf embryos, gadd45b mRNA is expressed in the pronephric duct. I performed wholemount immunolabeling on embryos raised under normoxic conditions, using an antibody directed against rabbit Gadd45b. This analysis revealed that the protein has a similar spatial pattern as the mRNA, in cells of the pronephric duct, suggesting that it is specific. Furthermore, preliminary data suggest that Gadd45b protein may be localized to the nucleus, which is consistent with its proposed molecular function in regulating the cell cycle.

Moving forward, I will use this antibody to (1) test whether our gadd45b morpholino knockdown tool is effective and (2) examine the proteins that Gadd45b co-localizes under normoxic and hypoxic conditions. These studies will provide an important first clue on the role Gadd45b in hypoxia adaptation.

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ROBUSTNESS OF UNSUPERVISED DOMAIN ADAPTATION AGAINST ADVERSARIAL ATTACKS AND BLUR

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Domain adaptation has been widely used in semantic segmentation to allow deep learning models to be trained on datasets without labels. However, a largely unexplored field is the robustness of these models. In this work, we introduce distortions from adversarial attacks and Gaussian blur to a target domain of real-world images to create a more robust model. Our source domains are SYNTHIA-SF and Cityscapes, and for our target domain, we collected and partially soft-labeled a dataset of seventeen sequences of images taken on a ROSBot 2.0 and a ROSBot 2.0 Pro around the UMBC campus at noon, sunset, and sunrise. Our models will be constructed using DeepLabV3Plus with a ResNet50 backbone modified for unsupervised domain adaptation using focal loss.

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UNDERSTANDING THE ACCESSIBILITY OF MODELING AND 3D PRINTING CUSTOMIZED ASSISTIVE TECHNOLOGY

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While technologies such as computer-aided design (CAD) modeling, 3D printing, and virtual reality (VR) have opened new doors in the fields of health, education, and entertainment, their accessibility for people with disabilities is not well understood. Consequently, individuals with disabilities are shut out from using these technologies effectively to create customized designs. In this project, participants with disabilities were interviewed to shed insight into their perceptions of the current state of accessibility to assistive technology (AT) created using these emerging technologies (i.e., CAD, 3D printing, and VR). Participants were then assisted in brainstorming personalized AT items that could be modeled and 3D printed. Participants were also asked to give feedback on what features should and should not be included in an AT VR experience, specifically for individuals with difficulty using fine motor skills, which would aid in the design process of these objects. Using study results, designs of the AT items specific to each participant were modeled for initial 3D prints. A VR experience was also created and modified to allow for participants to view their AT in a virtual environment prior to making adjustments and final 3D prints. The contribution of this work is an understanding of a possible process where individuals with disabilities, alongside engineers/designers, are able to collaboratively create AT specific to the individual. This would allow for an easier route to obtaining personalized AT.

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LATERAL GENE TRANSFER OF THE CAPSID MATURATION PROTEASE GENE BETWEEN PHAGE CLUSTERS A & CA

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The rising issue of bacterial resistance to antibiotics, and the study of bacteriophages (phages) has become more prevalent. The genetic diversity of phages allows them to continue infecting and killing bacteria. Some bacteriophages have a gene that codes for the capsid maturation protease (CMP). This enzyme catalyzes the degradation of the scaffolding protein during phage assembly, which allows the mature capsid head to form and DNA packaging to occur. A CMP homologous gene was found in phage cluster A, Mycobacteriophages, and phage cluster CA, *Rhodococcus*. We used VICTOR to compare the entire genome of the phages through Genome Blast Distance Phylogeny. To compare the conservation of the CMP gene, we created a maximum likelihood tree using MEGA. Through analyzing the tree, we observed the clusters to be grouped together. We then created another maximum likelihood tree that compared an essential housekeeping gene, the tape measure protein (TMP), to analyze the conservation of the CMP within these clusters. The TMP tree did not provide a clear insight on the conservation of the protease gene; however, the relationship within this tree presents possible questions about the occurrence of lateral gene transfer between bacteriophages. By analyzing the CMP tree, we observed the *Rhodococcus* phage Bryce to be found within its own group. This suggests it may not share a recent common ancestor with other *Rhodococcus* phages within cluster CA. Furthermore, the CMP gene may have been transferred amongst these clusters through lateral gene transfer. These observations of lateral gene transfer could provide further insight on how the diversity of bacteriophage's genome results from interacting with different bacterial hosts.

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THE CONNECTION BETWEEN BACILLUS PHAGE CLUSTERING AND ISOLATION LOCALIZATION

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Bacteriophages are viruses that infect bacteria, which are organized into clusters and further into subclusters based on genomic similarities. We investigated the connection between a subcluster and its geographic distribution, specifically in *Bacillus* phages, subclusters C1-C5. By determining a cluster's localization, environmental scientists can predict a region's bacterial landscape based on the bacteria those phages can infect.

We isolated a *Bacillus* phage from Gambrill State Park in Frederick, MD. A restriction digest of the phage's DNA allowed us to preliminarily assign it to the C3-subcluster. We used data from BacillusDB and UMBC class data to map where the C cluster phages were isolated and discerned that most of the cluster was within the DC-Maryland-Virginia (DMV) area. Next, we utilized the Geographic Midpoint Calculator to determine a center of minimum distance and then calculated the distance from that center to each location.

The C3-subcluster had a similar average distance to the entire C-cluster. The C1 and C2-subclusters, however, had dramatically larger average distances than the cluster overall. There were outlying phages isolated from other countries and overseas. The C2-subcluster was also more distributed than others.

The C-cluster is almost entirely isolated by UMBC, VCU, and the University of Mary Washington, so they were primarily collected in the soil near these institutions. Regardless, the cluster does not appear to be localized to the region because there were also cluster members overseas. On the other hand, the C3-subcluster does appear to be localized to the DMV area. Therefore, while we noticed a trend in the C3-subcluster's localization, we cannot definitively conclude that it affects phage isolation due to a lack of significant data. This leaves the possibility to continue studying this hypothesis by studying phages in a larger sample size from various institutions or proving the trend in a different cluster.

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INVESTIGATING THE ROLE OF NDRG1B ON BRAIN AND REGULATING EYE AND BRAIN DEVELOPMENT IN ZEBRAFISH EMBRYOS

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Brain development is a complex process that depends heavily on the interaction of specific genes. Brain development starts with neurulation, the formation of the neural tube from neuroectodermal cells during embryogenesis. In humans, impaired neurulation causes congenital birth defects such as spina bifida, where the neural tube cannot close properly due to mutations and environmental factors. Neural tube convergence is essential for bringing together the neural plate cells at the dorsomedial surface to close the neural tube. To study neurulation, I will use zebrafish whose external development and transparent embryos facilitate live imaging of early embryogenesis. In 24-hour post-fertilization (hpf) zebrafish, we have observed that the knockdown of N-Myc downstream-regulated gene 1b (*ndrg1b)* causes morphological brain and eye deficiencies. These abnormalities are similar to that of "glass onion" mutants in which the cell-cell adhesion molecule N-cadherin (N-cad) is disrupted. A characteristic of these mutants is severe cell adhesion loss in the neural tube and eye. Given that the closely related gene *ndrg1* promotes the recycling of E-cadherin in human cancer cells, my summer research project will focus on investigating if Ndrg1b regulates N-cad turnover in the developing zebrafish nervous system. I will address these goals by utilizing techniques such as morpholino-mediated gene knockdown, *in-situ* hybridization, immunolabeling, and quantitative methods to examine the spatial and temporal distribution of ndrg1b and the specific developmental anomalies that occur when it is knocked down. I predict that the knockdown of *ndrg1b* will result in neural tube defects similar to those observed in N-cad mutants. I plan to use this information to learn more about the importance of cell adhesion regulation during morphogenesis, and gain insight into the role of the ndrg1b human orthologue.

I would like to acknowledge my mentors Dr. Rachel Brewster and Prableen Chowdhary, and the rest of the Brewster lab for their support and contributions. Also, thank you to the Meyerhoff Scholars program and the Howard Hughes Medical Institute (HHMI) undergraduate scholars' program. I was supported by the HHMI grant (52008090); The Brewster lab was supported by funding from the National Institute of Health/NICHD (R21HD089476).

EXPLORING THE INTERACTIONS BETWEEN eIF4E AND HIV-1 RNA GENOME

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The human immunodeficiency virus (HIV) affects approximately 38 million individuals worldwide and is the determinant for the acquired immunodeficiency syndrome (AIDS). Patients with HIV type-1 (HIV-1) are treated with a drug cocktail to target various steps of the viral replication cycle, but high mutation rates lead to drug resistance. Therefore, we study highly conserved regions of the viral life cycle to ultimately develop better treatments for patients. Specifically, we study the 5′-Leader of the viral RNA genome, which exists as a monomer and dimer. Our group investigates how the cellular cap-binding protein, eIF4E, interacts with the monomer and dimer, as eIF4E interacts with 5′-capped RNAs to initiate translation. Recently, our laboratory determined eIF4E interacts with the exposed 5′-cap of the monomer; whereas in the dimer, the 5′-cap is sequestered between the two hairpins, contributing to the dimer being packaged and not translated.

To study the interaction between eIF4E and 5′-capped RNAs, we perform an *in vitro* capping reaction. We tested various conditions that could improve our capping efficiency and determined optimal concentrations of reagents. Through electrophoretic mobility shift assays (EMSAs), we confirm our RNAs are ~100% capped by testing their binding interactions with eIF4E. The monomer has an exposed 5′-cap allowing it to interact with eIF4E and this eIF4E-RNA complex is demonstrated by a shift on the EMSA. Previous data and literature suggest that eIF4E only binds to the 5′-cap of the RNA; however, recent data revealed a second shift, suggesting the 5′-capped RNAs have two binding sites for eIF4E. Preliminary isothermal titration calorimetry (ITC) data highlights the importance of the 5′-capped RNAs for binding eIF4E and understanding thermodynamic parameters. Given these findings, we aim to purify larger constructs of the 5′-Leader to ultimately understand the structural interactions between eIF4E and the HIV-1 RNA genome.

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STRUCTURAL ASSESSMENT OF A TERNARY HIV-1 GAG POLYPROTEIN, CYCLOPHILIN A, AND GENOMIC RNA COMPLEX

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Essential to HIV-1 replication is binding between its genomic RNA (gRNA) and the group specific antigen (Gag) polyprotein. The Gag polyprotein consists of four major domains: the matrix (MA), capsid (CA), nucleocapsid (NC), and p6. The NC domain interacts with the Core Encapsidation Signal (CES) of gRNA, allowing it to be packaged to form an immature virion. However, a high-resolution image of this binding interaction has yet to be obtained. Although the overall complex is large enough for cryogenic electron microscopy (cryo EM), the relatively small size of the individual Capsid-Nucleocapsid (CANC) construct could hinder structural studies.

Therefore, Cyclophilin A (CypA), a human produced protein that regulates protein folding and trafficking within the cell, will be used to increase the overall molecular weight of the complex. CypA aids in HIV-1 replication by binding to the mature CA domain and enabling the uncoating of the capsid, which aids in gRNA being transported into the host cell nucleus. Based on the reported interaction between CypA and the CA, we ultimately plan to form a ternary protein-protein-RNA complex to make cryo EM studies more feasible. For structural studies, this complex of CypA, gRNA, and CANC has to be stable and homogenous.

Crosslinking of CypA and CANC on SDS-Page gels in the presence of glutaraldehyde has successfully shown the formation of a complex. Using analytical size exclusion chromatography, we were further able to isolate the crosslinked complex from homo- or non-crosslinked samples. In the future, nuclear magnetic resonance (NMR) will be used to assess the homogeneity of this sample. Overall, our research will provide structural insights on the process of early virus assembly.

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DEEP MULTI-MODAL TRANSFER LEARNING FRAMEWORK FOR AUDIO ACTIVITY RECOGNITION

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Opulence and incorporation of acoustic devices such as earables, and conversational assistants in our everyday life have boosted a way for audio activity recognition research. Similar to the ImageNet dataset, many large, crowdsourced audio datasets have been curated. Often, these crowdsourced datasets are collected from different sources that contain data samples with background noise, music, and conversations that alter the generated waveform. We hypothesize that such heterogeneity in the data samples will cause a suboptimal performance when deployed in a transfer learning approach. We plan to incorporate wearable accelerometer data to overcome such drawbacks in audio activity classification tasks. In our study, we have collected an in-house dataset for the convenience of relating the time and frequency domain features of accelerometer and the audio data, respectively. We have considered smart-home environment activities that involve repetitive hand movement. We collected an in-house dataset from 10 participants using a wearable sensor called 'eSense' (a pair of earbuds with a microphone and a 6-axis imu sensor) and a Shimmer sensor device to collect the accelerometer data. In the experiment, we have used an acoustic model pre-trained with the AudioSet dataset and evaluated on our dataset. Finally, to boost the audio classification performance, we propose a deep multi-modal transfer learning framework that leverages the labeled accelerometer data and achieve a substantial performance gain.

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HISTOLOGICAL ANALYSIS OF THE TRANSGENIC HOXB13-MCR/TP53-NULL RATS

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One in seven men will be diagnosed with prostate cancer in their lifetime. Men with the less severe form of the disease respond well to front-line therapies, with 5-year survival rates exceeding 95%. Men that develop the more aggressive form of the disease do not respond to therapies, and consequently the 5-year survival rate drops to 29%. Combined prostatic MYC activation and tumor suppressor PTEN loss are the best predictors of poor outcome in men with the disease. We are currently developing a rat model of lethal prostate cancer with prostate-specific loss of *Pten* mediated by a polycistronic transgene with co-expression of MYC and Cre. Our extant rat model of prostate cancer is derived by crossing a transgenic rat line with prostatic MYC activation and constitutive loss of tumor suppressor *Tp53*. We hypothesize that our novel polycistronic transgenic rat line will perform comparably to our extant line with MYC activation. To test this hypothesis, we will analyze histology from rat prostate tissue from both MYC expressing transgenic rat lines bred to *Tp53*-nullity to confirm comparable disease severity. Histological analysis of our novel transgenic rat line will confirm that the expressivity of MYC is sufficient for disease progression in rats, which will yield a more accurate model of human prostate cancer and allow for better drug development, treatment, and prognosis of the disease.

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THE BEHAVIORAL EFFECTS IN THE OFFSPRING OF E-VAPOR EXPOSED PREGNANT MICE

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As there is a rise in electronic cigarette use, many researchers have taken the opportunity to research the effects of e-cigarettes on young adults. However, this has led to the lack of research on the impact on the offspring of pregnant women who express e-cigarette use. In fact, studies show that those who cannot quit smoking, have resorted to using e-cigarettes as a presumed "healthier" alternative. We aim to understand the behavioral differences and motor and olfactory abilities between the offspring of pregnant mice who are exposed to e-vapor and that of pregnant mice who are exposed to air. We hypothesize that the behavioral assays conducted post-pregnancy will show a delayed response in detecting odors and a lack of motor skills in those mice whose mothers were exposed to e-vapor compared to the offspring of pregnant mice who were air-controlled. We will use a Buried Food and T-Maze assay to distinguish the mouse's ability to locate the source of odor and detect competing scents. We will also use reflex and suspension tests to evaluate the pups' motor abilities. The use of multiple exposure methods will allow us to discern the difference in performance between those offspring whose mothers are exposed after pregnancy versus mothers who are exposed only until they give birth. Although the pregnant mice have not given birth yet, we have chosen to conduct experiments on the pregnant mice to locate any behavioral differences. Our preliminary data shows that it takes about five times slower for the exposed mice to locate the odor source than that of the air-controlled mice. Essentially, we will be able to assess the significant differences in the data that is produced on the effects of the offspring on pregnant mice who have been exposed to e-vapor and who have been exposed to air.

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PERIPLASMIC BINDING PROTEIN-BASED AUTOMATIC GLUCOSE MONITORING DEVICE: APPLICATIONS IN BIOPROCESSES AND TRANSDERMAL GLUCOSE SENSING IN HUMANS

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Glucose is the primary currency of energy in many physiological and cellular processes, consequently monitoring glucose is of key importance in both medical applications and bioprocesses. In the microbial system such as E. coli fermentation, maintaining the optimal concentration of glucose is needed to maximize product yields for the development of drugs. These yields can lower the cost of drugs, such as insulin, cancer therapies, and vaccines. Historically, there have been extensive investigations conducted on periplasmic binding proteins as an alternative to enzymatic biosensors for glucose measurement. The binding proteins go through conformational changes when they bind to specific substrates and can be quantified by introducing suitable fluorophores to estimate the substrates' concentration. The expression, characterization, sensitivity, and potential applications of the biosensor have been studied extensively in previous studies. However, an integrated system with an aseptic sampling technique is yet to be reported for automatic and continuous glucose monitoring, especially for bioprocesses. Our research presents a prototype for automatic glucose monitoring based on Glucose Binding Proteins (GBP), capable of monitoring concentrations from a few micromolar to several hundreds of millimolar glucose. The monitoring setup includes a novel sampling system, a specially modified chromatography column to hold the immobilized GBP, and a microfluorometer for efficient fluorescence measurement, all of which are combined into a compact portable device. The device establishes a calibration with standard glucose solutions and can be utilized to monitor the glucose concentration in yeast culture in a mini bioreactor. The sampling and fluorescence measurement technique is versatile. It can easily be adapted to the measurement of additional analytes such as glutamine, branched-chain amino acids, etc., and for healthcare applications such as non-invasive transdermal glucose monitoring in humans.

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SYNTHESIS AND EVALUATION OF FLEX AT-527 NUCLEOS(T)IDE ANALOGUE AS A POTENTIAL ANTIVIRAL THERAPEUTIC

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The COVID-19 pandemic has given renewed cause to the production of novel and modified antiviral therapeutics to combat a broad range of diseases, including SARS-CoV-2. Nucleoside and nucleotide analogues have been an effective class of antiviral therapeutics as they have shown remarkable activity in inhibiting the biological functions of numerous families of viruses.

To overcome the spread of viral infectious diseases, the Seley-Radtke lab has synthesized modified nucleos(t)ide analogues that possess flexible purine base moieties, known as Fleximers. The Fleximer technology involves a single carbon-carbon bond between the pyrimidine and imidazole components of the bicyclic purine nucleobase moiety. The additional flexibility introduced to the bicyclic purine base moiety allows the nucleos(t)ide to bind to enzymes in alternate conformations and interact with secondary amino acid residues within an enzyme's active site, which are not accessible to the rigid, fused purine base of the parent nucleoside. This in turn, can help nucleus(t)ide analogues retain their activity when confronted with point mutations in a binding site. One such analogue with potential to be modified into a Fleximer is AT-527, an experimental antiviral therapeutic in phase III clinical trials that is a double prodrug of a guanosine nucleotide analogue which has shown activity against Hepatitis C Virus (HCV) and several human coronaviruses, including SARS-CoV-2. The aim for the current project is to synthesize and incorporate the Fleximer technology into the AT-527 parent nucleotide analogue and expand upon its biological activity against RNA-dependent RNA polymerases in viruses.

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MAXIMUM COVER DETECTION FOR AUTONOMOUS MANEUVERING IN A SIMULATED MILITARY-RELEVANT ENVIRONMENT

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In military-relevant environments, it is often necessary for autonomous ground robots to find cover to prevent getting detected or attacked by adversaries. A trained machine learning model can play a valuable role in discovering such cover in a real-world setting after receiving information about the environment from autonomous robots. In this regard, we present an object detection-based method for receiving information about a simulated forest environment and attaining maximum tree cover. Our method is focused on detecting the locations and distances to trees to achieve such cover. A forest environment was designed with wooded areas, open paths, water, and bridges. The environment provides a diverse terrain for the robot, including open areas and highly covered areas. A Clearpath Husky robot is simulated in the environment using Army Research Laboratory's (ARL) Unity simulation framework. The Husky robot is equipped with an RGB camera with semantic segmentation and object detection capabilities, and a LiDAR sensor. Our method reads data from these sensors using ROS topics and employs semantic segmentation to detect trees, paths, bridges, and water, as well as the distances to nearby trees. The method uses ROS velocity command topics to move the robot towards trees and remains within a certain distance of the trees to maintain maximum cover.

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USING BEHAVIORAL ASSAYS TO STUDY GENES ASSOCIATED WITH AUTISM IN DROSOPHILA FRUIT FLIES

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Autism Spectrum Disorder (ASD) is a complex developmental disability characterized by distinctive, abnormal behaviors. While a direct cause of ASD development has not yet been identified, there are a variety of genetic mutations associated with this disorder. One of the most notable characteristics of this disorder is low levels of sociability. Individuals with ASD typically have little to no desire to socialize, and often have difficulties deciphering the meaning of nonverbal forms of communication. We used the fruit fly Drosophila melanogaster as a powerful animal model to study genes associated with autism at the cellular and behavioral level. We used a previously established behavioral assay in order to assess the effects of gene misregulation on sociability within our fruit fly model. We will compare the social patterns of control flies to those of flies with genetic mutations associated with the development of ASD in humans. Our goal is to determine which candidate genes have an effect on social patterns when misregulated in the fly brain. Future studies will further characterize the molecular role of the identified genes following anatomical and cellular approaches. Developing accurate and predictive animal models may be critical in further understanding the origin of this disorder, as well as developing a more definitive diagnosis test.

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INVESTIGATING THE EFFECTS OF VARYING CONCENTRATIONS OF DILUTED E-LIQUID SOLUTIONS ON MITOCHONDRIAL MORPHOLOGY AND HEALTH OF EPITHELIAL CELLS IN MICE

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Over the past decade, electronic cigarettes (E-cigs) have transformed the tobacco industry. Their usage has many health consequences, including nicotine addiction and heart and lung disease. However, the long-term effects of E-cigs use are incompletely understood, especially concerning cellular health. E-Liquid solutions contain constituents such as flavorants, nicotine, and toxic byproducts. Previous studies have found that exposing respiratory epithelial cells to E-Liquid has induced mitochondrial oxidative stress response. Though, exposure effects on olfactory epithelial cells are understudied. Here we investigate the impact of E-cigs on cellular health by exposing isolated olfactory epithelial cells to a range of diluted E-Liquid solutions. Since cellular health and energetics mechanisms are closely tied to mitochondrial health and morphology, we utilized mitochondrial membrane potential-dependent Mitolite fluorescent dye to analyze the effect of E-cigs exposure on mitochondrial morphological and functional properties in olfactory epithelial cells.

We hypothesized that cells exposed to more highly concentrated solutions of E-Liquid would show greater statistically significant differences concerning fluorescent signal intensity and mitochondrial morphology than control cells. Preliminary data shows a statistically significant dose-dependent effect on mitochondrial fluorescent signal intensity (p-value >.0001) and average mitochondrial area (p-value = .0006) for control vs. high-dose E-Liquid. Although preliminary, current data supports our hypothesis and provides insight regarding the consequences of E-cigs on cellular health and energetics. Furthermore, our findings emphasize the need for continued experimentation. Therefore, future studies are being designed to assess changes in mitochondrial properties in olfactory epithelial cells of mice after E-Vapor condensate exposure, in contrast to the current E-Liquid exposure experiments. As a result, we hope to develop a more comprehensive understanding of the impact of E-cigs on cell and mitochondrial health at the single cell and tissue level.

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INVESITGATING HOW THE STRUCTURAL ELEMENTS OF THE HIV-1 5′-LEADER AFFECT BINDING OF THE CELLULAR CAP BINDING PROTEIN, EIF4E

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The acquired immunodeficiency syndrome is caused by the infection of the human immunodeficiency virus type-1 (HIV-1). Our laboratory focuses on the 5′ untranslated region known as the 5′-Leader which regulates a number of viral functions such as dimerization, packaging, and translation. The HIV-1_{MAL} strain exists between two RNA conformations - a monomer and a dimer. Most RNAs are transcribed with one or three guanosines along with the 5′-cap due to transcriptional start site heterogeneity. In the dimer, the 5′-cap is sequestered between two hairpins which prevents binding of eIF4E, a cellular cap binding protein that initiates translation. In the monomer, eIF4E binds to the exposed 5′-cap, allowing for the initiation of translation. Although it has been determined that HIV-1 genome translation depends on the structural differences of the 5′-Leader, it is unknown whether the structure of the RNA body affects binding of translational machinery such as eIF4E. We study the effects of the 5′-Leader's structure on eIF4E binding using isothermal titration calorimetry (ITC). ITC is a quantitative method that measures thermodynamic parameters between two molecules' interactions. We designed and purified RNA oligos of the 5′-leader, including the TAR hairpin, to determine how the RNA structural elements impact eIF4E binding. Our ITC results revealed that eIF4E binding is ~4-fold tighter than with the 5′-cap. This suggests that the structured RNA is important for binding to eIF4E. Our next steps involve purifying longer RNA segments of the 5′-leader and measuring the binding affinity of those RNAs to eIF4E using ITC. This will allow us to probe the structural elements that are important for eIF4E binding to the 5′-leader to better understand translation regulation during HIV-1 infection.

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On the Way to Solve A Structure: CC Pentamer CA-NC and CES Complex Structural Determination of HIV-1 vRNA and Gag Polyprotein Complex

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Human Immunodeficiency Virus (HIV) is a global pandemic that has infected roughly 36.9 million individuals worldwide. The search for a cure and efficient medication is in high demand. Existing treatments target mutation prone regions of HIV-1, which commonly leads to loss of effectiveness. Due to reverse transcription of RNA to DNA the virus becomes very resistant. As a result, this study focuses on the interaction between the CES region of the highly conserved 5' leader of the viral RNA, and the presumed domains required for packaging within the Gag polyprotein: capsid (CA) and the nucleocapsid (NC), in hopes of discovering consistent therapies.

During the late phase of the replication cycle, the translation of viral RNA develops the Gag polypeptide. Gag aids in packaging viral RNA and orchestrates viral replication. The CA domain allows vRNA to encourage Gag multimerization, and the NC domain interacts with vRNA via zinc-finger binding motifs. In a mature virion, the capsid domain assembles in various complexes that stimulates selective packaging. The majority of these complexes have a hexameric conformation, but some have a pentameric conformation. Pentamers have a very specific shape that allows for the capsid core's conical architecture. The conical structure accommodates viral RNA genomes.

My research focuses on pentameric conformation. To mimic what happens during the assembly process and determine the first capsid conformation, I used an artificial coiled-coiled pentamer that I fused to the capsid sequence. Previous studies have analyzed coiled-coiled hexamers to decide if they are the first confirmation of capsid to begin viral RNA packaging. Likewise, my project utilizes CC Pent CA-NC in hopes of adding to the understanding of selective viral RNA packaging. This will help provide insight into early virus assembly and facilitate the design of novel treatments for HIV-1.

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DETERMINING THE CORRELATION BETWEEN THE HOST RANGE OF A BACTERIOPHAGE AND ITS RESTRICTION DIGEST PROFILE

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Bacteriophages are viruses that infect bacteria, sometimes targeting a range of bacteria that can include pathogenic strains such as anthrax and salmonella. They are highly specific in nature and infect a certain range of bacteria, which is called their host range. Bacteriophages can be classified by their host range, but another way to classify them is based on their restriction digest profile. Their DNA can be cut by enzymes during restriction enzyme digestion and their restriction digest profile shows the results of this digestion. In this experiment, we aimed to determine if there is a correlation between the host range of a bacteriophage and the enzymes that cut its DNA during digestion. To do this, we compiled the class data of the bacteriophages discovered by UMBC students from 2015 to 2017 and analyzed it by looking for patterns in the host range of a bacteriophage and the enzymes that cut it the most during digestion. Our analysis suggests that there are some correlations between host range and restriction digestion pattern. For example, one trend we found was that in 2015, four out of the six bacteriophages that infected the host *B. cereus Gibson 971* were cut by the *Eco*RI enzyme. Based on the trends our analysis revealed, we found that there is a potential correlation between restriction enzyme digestion and host range. This means the host range of a bacteriophage could potentially be used to predict which enzymes would digest their DNA. While more data is needed to confirm these trends, we believe they have the potential to be used to predict the host range of bacteriophages.

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Novel super-resolved SIM imaging and analysis of ryanodine receptors in rabbit sinoatrial node cells reveal emergent hierarchical organization of the receptor network in 3 dimensions.

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The crucial rhythm of life, the heartbeat, is ensured by specialized sinoatrial node cells (SANCs). The rate of the heartbeat is controlled during diastole when calcium is released from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyRs) and interacts with membrane currents. The released calcium takes the form of stochastic, partially periodic, localized calcium release (LCR) events that propagate, wave-like, for limited distances. A computational model of SANCs, including three-dimensional diffusion and buffering of calcium in the cytosol and SR, has been developed in our laboratory, and unexpectedly, numerical simulations of the model revealed the existence of a pathological mode at high RyR sensitivity to calcium, in which the calcium clock loses synchronization with the membrane, resulting in a paradoxical decrease in beating rate in response to β-adrenergic stimulation. The model indicates that the hierarchical clustering of surface RyRs in SANCs may be a crucial adaptive mechanism. Pathological desynchronization of the clocks may explain sinus node dysfunction in heart failure, aging, and heart diseases linked to RyR mutations. The most important limitation of the model is that the true detailed distribution of RyR clusters on the surface of the cell is not known and had to be estimated in an ad hoc manner. In this work, we use a series of novel computational approaches applied to experimental super-resolved SIM data of rabbit SANCs to characterize the true organization of surface RyR clusters in 3D. Our approaches include adapting automated segmentation of nuclei to RyR clusters and approaches in computational geometry to recognize the surface RyR clusters. Our pilot examinations of SANCs using the new methods have revealed a detailed hierarchical architecture of RyR network (including exact positions and sizes of RyR clusters) and provided new key parameters for more realistic numerical modeling of SANC function in our laboratory.

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A SYNCHRONIZING MIDDLEWARE FOR HETEROGENEOUS MULTI-ROBOT SYSTEM WITH LOW LATENCY CO-SIMULATION

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In a heterogeneous multi-robot system, synchronization between each of the agents is one of the most difficult issues to address. We know that different clock and transmission speeds can cause the system to become desynchronized, which would be dangerous in battlefield settings. To address this issue, we propose an extension of the synchronizing middleware, SynchroSim, to minimize these issues and optimize communication between robotic agents. SynchroSim has been tested for co-simulation of physics and network simulators, such as Gazebo and NS-3, as well as running virtual simulations. With these simulations, we are able to then transition our research toward real-life robotic agents and terrain.

In order to test the middleware, we chose a variety of robots to promote potential synchronization issues with a demonstration containing three agents: a Duckiedrone and two slave nodes; Turtlebot3 burger and Duckiebot. The duckiedrone will act as the master node and control communication between the two slave nodes. Currently, communication between each of the nodes uses the Transmission Control Protocol (TCP). Though this protocol is reliable, it can use up a lot of bandwidth and time. To compensate for this, we have been researching the faster but less reliable protocol named User Datagram Protocol (UDP) and analyzing its efficacy in the multi-robot system. To evaluate the performance of our proposed middleware, we plan to employ two of the most important metrics for communication systems: average latency and percentage of packet loss.

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ALTERING TARGET DOMAIN IMAGES ACROSS DIFFERENT LIGHTING CONDITIONS FOR UNSUPERVISED DOMAIN ADAPTATION

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Deep learning models for semantic segmentation have a hard time adapting to new environments other than what the model was trained on. The process of domain adaptation aims to fix this issue, however, as dissimilarities between source and target increase, the performance of the adaptive model tends to decrease. In this paper, we propose a model that aims to fix the issue of performance decrease from non-ideal lighting conditions where the target has higher or lower light intensity than the source. Our solution includes altering the contrast of the target images to resemble that of the source images so that classes are easier to identify. To perform experiments, we have collected our own dataset consisting of 1619 annotated low-angle images taken from a ROSbot 2.0 and a ROSbot 2.0 Pro in a suburban area at different times of day for our target, and we use the SYNTHIA-SF dataset as our source. Cross Entropy loss and Focal loss functions are applied to multiple DeepLabV3Plus models with a ResNet50 backbone to observe an additional effect on performance.

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EXPLORING THE ROLE OF GADD45B IN ADAPTIVE RESPONSES TO HYPOXIA

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Metazoans require oxygen in DNA and RNA synthesis, intercellular signaling, active transport, and muscle contraction. Instances of hypoxia (low oxygen) or anoxia (no oxygen) disrupt these processes and can subsequently cause widespread cell death. However, conserved responses exist that promote organismal adaptation to hypoxia and/or anoxia. One such organism, the zebrafish, is capable of surviving anoxia by entering a state of reversible metabolic suppression. Despite this energy-conserving response to hypoxia, the transcription of genes that promote survival following prolonged oxygen deprivation is up-regulated. Using an RNA-Seq approach, over 1000 genes were differentially increased in embryos exposed to anoxia compared to normoxic controls. *Gadd45ba* was among the up-regulated genes; its expression during development is highly dynamic, with enrichment in the involuting mesoderm, presomitic mesoderm, the eye and kidney (pronephric duct). Gadd45b is known to regulate the cell cycle and survival under hypoxia and other instances of oxidative stress. My project aims to further investigate the role of *gadd45*b by addressing three questions: (1) Is *gadd45b* expression enhanced in the somitic mesoderm (where it is expressed under normoxic conditions) or is it expanded to other tissues in response to hypoxia? (2) Is the expression of somitic genes that have previously been shown to be regulated by *gadd45b* altered in response to hypoxia? , and if so, (3) does *gadd45b* knockdown disrupt somitic gene expression under anoxia? These questions will be addressed using wholemount *in situ* hybridization to analyze the spatial distribution of gene expression, Q-PCR to quantify changes in transcript levels and morpholino-mediated gene knockdown to analyze the function of *gadd45b*. We predict that Gadd45b functions in a protective manner in response to anoxia, possibly by promoting cell cycle arrest and DNA repair in the tissues in which it is expressed or by stabilizing regulatory networks that pattern the mesoderm during somitogenesis.

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MAXIMUM-COVER FOLLOWING TRAJECTORY PLANNING IN UNSTRUCTURED OUTDOOR ENVIRONMENTS WITH DEEP REINFORCEMENT LEARNING

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In a military combat context or in a contested environment, a robot sometimes needs to go undercover to prevent getting detected or attacked by adversarial agents. To move the robot with maximum cover, we present a novel Deep Reinforcement Learning (DRL) based method for identifying covert and navigable trajectories in off-road terrains and jungle environments. Our approach focuses on seeking shelters and covers while safely navigating to a predefined destination. Using an elevation map generated from 3D cloud points, RGB images, robot pose, and goal information as input, our method computes a local cost map that distinguishes which path will grant the maximal covertness while maintaining a low-cost trajectory. The elevation level is another determining factor since the high ground is easily targetable. Besides, maintaining low elevation while traveling allows the robot to be less visible in the environment, as other objects become natural shelters by blocking the view. Our agent learns to go through low elevation via a reward function: we assign punishment when the robot gains elevation. With the assistance of another DRL-based navigation algorithm, this approach guarantees dynamically feasible velocities that match state-of-the-art performance. In addition, we extensively evaluate our model on a Clearpath Husky robot on the TartanDrive dataset in the ARL Unity simulation environment.

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