Winthrop University
Summer Undergraduate Research Experience (SURE)
2021 Abstract Book

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Table of Contents

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Biplanar Subgroup Lattices of Finite Abelian Groups

Josiah Bauer (2021)
Olivia Cantrell (2022)
Gabrielle Cobbina (2021)
John Herndon (2021)

Mentors: Arran Hamm, Jessie Hamm

Given a group G, the *subgroup lattice* is the graph whose vertices are the subgroups of G and whose edges represent direct containment between subgroups. A typical question to examine when looking at the subgroup lattices is: "For which groups G does the subgroup lattice possess a certain graph property?" The case when the graph property of interest is *planarity*, meaning that the graph can be drawn so that no edges cross, was initially studied by Starr and Turner in 2004. Shortly thereafter, the problem of determining all finite groups with a planar subgroup lattice was resolved in 2006 independently by Schmidt and Bohanon & Reid.

There are several well-studied generalizations of the notation of planarity in Graph Theory such as crossing number or genus. Another such example is *biplanarity*. A graph is called biplanar if it can be partitioned into two graphs on the same vertex set so that each of the two graphs is planar. Our work this summer was to answer the question: "For which groups G is the subgroup lattice biplanar?" Although we do not yet have a full characterization of such groups, we obtained a few partial results in this direction.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 20) of the National Institutes of Health.

The Evaluation of Opioid Treatment Programs

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Devin Holder (2021)

Mentors: Joanna Jackson
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Larry Stevens

With the first wave of a national opioid crisis beginning in the 1990s, the United States has seen a steady incline of opioid-overdose-related deaths, nearly 50,000 in 2019 alone. Currently, of the estimated 2.1 million people suffering from opioid use disorder (OUD), 300,000 receive care from in opioid treatment programs (OTPs). OTPs follow complex federal, state, and local regulations, covering six categories: medical service delivery, facility operating requirements, administrative requirements, staffing requirements, social services, and physical facility management. Unfortunately, most of these regulations are not evidence-based; instead, they appear to be based on stigma and the perception of individuals who utilize OTPs. The purpose of this current study is the regulatory burden of state regulations through the lens of OTP administrators. A survey was co-designed by the researchers and piloted with a group of non OTP and healthcare experts and then to a sample of the more the 1500 OTPs in the country. Non-expert and experts found the questionare to be important and timely. However, we determined that the survey was too long. There would be difficulty recruiting a large enough sample size to produce generalizable data if participants were recruited only through email. After modifications to the survey instrument and inclusion of mailers and telephone recruitment methods, the instrument can now be used to examine the impact of state-level regulation on the organization level.

Comparison of Commercial DNA Extraction Kits for Use on Human Breastmilk

Cassidy Butler (2022) **Mentor: Hope Lima**
Amy Matsumoto (2021)

Background: For-profit donor human milk organizations have DNA-based proprietary methodology for testing incoming milk for purity. However, there is currently no standardized methodology for extracting DNA from human milk. Microbiome research has shown that DNA quality can vary depending on the extraction methodology. This study assessed the quality and quantity of DNA extracted from four commercially available DNA extraction kits – including one kit that was specific to human milk.
Methodology: This study was for method validation only. One donor was utilized to provide a 3-ounce sample. The sample was aliquoted into 70, 1-mL microcentrifuge tubes. Aliquots were randomized into one of three categories: fresh extraction, extraction after freezing, and extraction after purification for storage at room temperature. DNA extraction was performed using four commercially available DNA extraction kits and DNA was analyzed for quality and quantity using a NanoDrop Spectrophotometer.
Results: Results confirmed differences in DNA quality and quantity between extraction kits. The Plasma/Serum Circulating DNA Purification Mini Kit (Norgen Biotek, ON, Canada) provided significantly more DNA, consistent purity as measured by 260/280 and 260/230 ratios, and DNA quantity and quality was similar between fresh and frozen human milk samples.
Conclusions: Our results suggest that DNA quality and quantity is highest when extracted using the Plasma/Serum Circulating DNA Purification Mini Kit. To ensure reliable quality assurance protocols for testing donor human milk, standardized methodology for extracting DNA from human milk is necessary. The Plasma/Serum Circulating DNA Purification Mini Kit is consistent, providing high quality and sufficient quantity for downstream analysis.

This project was supported by the Department of Human Nutrition at Winthrop University and SC INBRE.

Sphingosine Kinase Inhibition Using Modified Zone 1 Variants of Sphingosine Kinase Inhibitor-1

Kendarius Butler (2022) **Mentor: Christian Grattan**

Sphingosine kinase is a naturally occurring enzyme involved in the sphingomyelin pathway that is responsible for actively converting sphingosine into sphingosine-1- phosphate (S1P). When the concentration of S1P increases it signals for the proliferation and metastasis of cancerous cells and tumors throughout the body. By inhibiting sphingosine kinase, the concentration of sphingosine increases in the cell leading to an apoptotic outcome. Using a known sphingosine kinase inhibitor, SKI-1, novel derivatives were designed and assessed using UCSF Chimera, Marvin Sketch, Molinspiration, and Autodock Vina. The data collected for the properties and binding energies of the proposed sphingosine kinase 1 inhibitor molecules led to the synthetic approach to prepare and purify these derivatives. The primary focus of this project involved the synthesis and preparation of zone 1 modified sphingosine kinase-1 inhibitors that include a pyridine ring. Once synthesized and purified, these sphingosine kinase inhibitors will be assessed using an *in vitro* assay to compare with the computational binding energies for improved inhibitory activity with the target enzyme.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences as well as the Winthrop University Department of Chemistry, Physics, and Geology.

Impact of Multiple Freeze/ Thaw Cycles on Nutritional Integrity, Microbial Content, and Bioactivity of Human Milk

Jaeden Choice (2022) **Mentor: Hope Lima**

The Neonatal Intensive Care Unit (NICU) requires any donor milk that is thawed to be fed to premature infants within a certain timeframe, depending on the storage of the donor milk. These recommendations protect premature infants from potential exposure to contamination from human milk. Current recommendations in the United States and in NICUs do not allow for the re-freezing of human milk once it has been thawed. The aim of this study was to determine the influence of multiple freeze/ thaw cycles on the nutritional integrity and microbial content of donor human milk. Pasteurized donor milk samples were shipped overnight from WakeMed Mothers' Milk Bank (Cary, NC) to Winthrop University. Samples were then subjected to four free-thaw cycles and analyzed for the following components after each thaw: bacterial analysis (total coliform, *S. aureus*, total aerobic), total calories, total protein, total fat, crematocrit, and lactose.

This project was supported by the Department of Human Nutrition at Winthrop University and SC INBRE.

Gastrointestinal Health in Endurance Runners

Eden Crain (2022)

Mentors: Jessie Hoffman
Ashley Licata

Exercise has been positively associated with a more diverse and "beneficial" gut microbiome. However, endurance activities, specifically running, can cause gastrointestinal complications and discomfort in some individuals. This gastrointestinal distress is thought to be related to decreased blood flow to the gut during exercise resulting in intestinal cell damage, increased intestinal permeability, and inflammatory responses, along with altered gut motility and impaired nutrient absorption. Importantly, a variety of factors may modulate the presence of gastrointestinal distress during exercise, including hydration, diet, stress, and exercise intensity. In addition, diet and exercise may alter gut microbiome in ways that impact gut health and the gut's response to exercise. Therefore, this observational study aims to assess the associations between dietary factors, gut microbiota composition, psychological stress, and GI symptoms in a group of recreational endurance runners. This is an ongoing study that is still in the recruitment and data collection phase.

Data collection includes fecal and urine samples, 3-day food and exercise log, and completion of an online survey. DNA from fecal samples will be extracted using QIAmp PowerFecal Pro DNA extraction kits, and extracted DNA will be subsequently analyzed via 16S rRNA gene sequencing of the microbiome. Urine samples will be analyzed using a urinary cortisol ELISA to determine the cortisol level as a biochemical measure of stress. Dietary data will be input into NutriTiming software for analysis of overall dietary and exercise patterns. In addition, the survey is designed to assess running habits, gastrointestinal health using the Gastrointestinal Symptoms Rating Scale (GSRS), and psychological stress using the Perceived Stress Scale (PSS). Data analysis will be conducted using SPSS, GraphPad Prism, and QIIME for 16S rRNA gene sequencing. The study currently has 8 participants who are fully complete, and we are still in the process of recruiting and collecting data from additional participants. Preliminary data analysis of survey data from 8 participants revealed a trend of higher GSRS scores in individuals who reported consuming food 1-4 hours prior to a run, compared to individuals who did not consume food 1-4 hours prior to a run ($n=8$; $p=0.01$). Because our current sample size is low due to still being in the recruitment phase, we hypothesize that this trend may become significant, as dietary intake is a known factor in contributing to gastrointestinal symptoms in runners. Further results may provide a stronger basis for understanding causes of gastrointestinal symptoms during running, thus allowing for the development of improved nutritional recommendations.

Optical Materials in Na-Y-Si-O and Related Quaternary Systems

Meagan Donohue (2022) **Mentor: Maria Gelabert**
Kameron Johnson (2021)

This project utilizes the materials genome approach in order to discover new compounds at an accelerated rate. Novel materials for optical applications, such as luminescent scintillators, are desired for improvement of properties. Using aqueous speciation and density functional theory (DFT) calculations for experimental guidance, potential new scintillating materials may be discovered in the efforts to improve scintillation materials capabilities. Synthesis work targets existing compounds of already-high density, then examines metal substitutions for potential discovery of new compounds with higher density. Na-O-Si-Y and Y-Zr-O systems were investigated with the two existing compounds, $NaY_9Si_6O_{26}$ and $Y_4Zr_3O_{12}$, to investigate if these could be synthesized under mild hydrothermal conditions. Metal salts were mixed with complexing agents, base and water, then enclosed in a hydrothermal vessel at 200 °C for a few to several days. After reaction and isolation by wash/centrifuge, products were analyzed by powder X-ray diffraction (XRD), and scanning electron microscopy with energy-dispersive X-ray analysis (SEM/EDS). For $Y_4Zr_3O_{12}$, XRD and SEM revealed primarily Y_2O_3 with prismatic and hexagonal crystals, and for $NaY_9Si_6O_{26}$, EDS revealed a quaternary phase of approximate composition $Na_5Y_4Si_{12}O_{31}$, with spheroidal crystalline formations. Future work will include further investigation of this quaternary product, as well as continued feasibility and substitution studies using hydrothermal methods.

SEM images for products of $NaY_9Si_6O_{26}$ (left) and $Y_4Zr_3O_{12}$ (right) reactions. Hydrothermal vessels contained deionized water (10 mL), yttrium chloride or oxide, complexing agent $Na_2H_2EDTA{\cdot}2H_2O$ or acetylacetone, $ZrOCl_2$ or $Na_2Si_3O_7$, and KOH or NaOH. EDS analysis indicates stoichiometries of $Na_{1.25}Y_{1.07}Si_{2.90}O_{7.72}$ (left) and Y_2O_3 (right) for hexagonal crystals.

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Expression, Purification, and Characterization of the Cry2 and Per2 homologs in *Isodiometra pultra*

Christine Dunn (2022) **Mentor: Jason Hurlbert**

Our work focused on the expression and purification steps of the Cry2 and Per2 proteins from *I. pulchra*. *E. coli* was used as a host to express the proteins, and purification steps required the use of the FPLC which allowed the proteins to be purified through metal chelating affinity chromatography, gel filtration, and folding on column methods. The goal of protein purification this summer was to collect a concentrated sample of each protein that was approximately 90% pure that can be used for the characterization steps. The purification of the samples was determined using western blotting and gel electrophoresis methods. Concentrated samples of both proteins were collected that can be used in the future for the characterization steps, including quantitation of ligand binding via surface plasmon resonance and crystallization to determine the structure of the complex by x-ray crystallography.

Support by MADE in SC, NSF #1655740 and SC EPSCoR GEAR-CRP, U of SC Subaward 20-4041

Fabrication of an RNA-Based Fluorescent Biosensor for the Detection of Dopamine

Brandon Ellison (2022) **Mentor: Timea Fernandez**

Dopamine (DA) is a neurotransmitter that plays a role in the regulation of physical and emotional well-being. Irregularities in DA production have been linked to several addictive behaviors such as *smoking*, *alcoholism*, and *obesity*, as well as neurodegenerative disorders like *Parkinson's disease*. Early detection of DA abnormalities is paramount for the effective diagnosis and treatment of these ailments, while real-time imaging of DA could assist in the comprehension of their underlying mechanisms. As such, our project aims to design a DA-sensing RNA-based fluorescent (RBF) biosensor for initial *in vitro* experimentation and characterization. Using existing platforms, we can fabricate RBF biosensors that combine a ligand-sensing RNA aptamer with a fluorescent RNA aptamer to indicate the presence of biologically relevant molecules. Previous studies have used electrochemical and protein-based biosensors in the detection of neurotransmitters; yet, to our knowledge, no studies have developed a viable RBF biosensor for the detection of DA *in vitro* or *in vivo*. To date, we have designed, transcribed, and purified a total of eight biosensor constructs. In the near future, we plan on assessing their ability to specifically detect DA in a concentration-dependent manner using *in vitro* and *in vivo* studies.

Support for this project was provided by grant P20GM103499-20 (SC INBRE) from the National Institute of General Medical Sciences, National Institutes of Health, MADE in SC, SC EPSCoR, NSF #1655740, and the Winthrop University McNair Scholars Program.

Assessing physical activity using Fitbit technology and measuring well-being following a brief breathing meditation

Leslie Facio (2021) **Mentors: Courtney Guenther, David Schary**

Students, faculty, and staff have experienced increased levels of stress during the COVID-19 pandemic. Both meditation and physical activity have previously been demonstrated to help reduce stress and enhance well-being, however additional research is needed to better understand the intersection of mindfulness, physical health, and well-being, particularly as it relates to changes within higher education during the COVID-19 pandemic. Therefore, this study aimed to use Fitbit technology to examine physical activity and compare this to self-reported measures of well-being after engaging in a brief breathing meditation among students, faculty, and staff. Preliminary conclusions suggest that participants who completed breathing meditation, on average, increased levels of mindfulness and decreased in anxiety scores. Preliminary results also suggest that all participants, on average, displayed a slight decrease in levels of worry and a decrease in depression scores across the two-week study. Preliminary conclusions suggest that brief daily breathing meditations may help improve overall well-being. These findings have broader implications due to the COVID-19 pandemic, which will also be discussed.

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Modernizing the Purification Protocols for Human Cardiac Troponin T and I

Samantha Fritsch (2023) **Mentor: Nick Grossoehme**

The regulation of muscle contraction is contingent on Ca^{+2} binding to a complex protein structure known as troponin. The troponin complex is composed of three distinct proteins: troponin C (TpnC), troponin I (TpnI), and troponin T (TpnT). Each of these proteins serves a distinct and critical role in the regulation of muscle contraction. Briefly, TpnT makes the primary interaction with tropomyosin, TpnI is responsible for the inhibitory interaction that prevents muscle contraction under certain conditions, and TpnC responds to cellular signals by binding Ca^{2+} and triggering a structural change in the complex that abolishes the inhibitory interaction between TpnI and the muscle fiber. The long-term goal of this project is to understand how the toxic heavy metal cadmium impacts the formation and function of this complex; this requires purification of large amounts of each protein. Previous labs have demonstrated successful purification of troponin T and I; however, this was achieved through the use of very high concentrations of urea and ammonium sulfate. The focus of this project is to design a simplified purification protocol using modern techniques that circumvent the need for large amounts of urea and ammonium sulfate. Our strategy involves cloning a maltose binding protein (MBP) affinity tag onto each protein separated by a TEV protease recognition site. Once cloned, the construct is introduced into *E. coli* and for protein expression. The cell mass is then harvested, lysed, and clarified by centrifugation. Affinity chromatography was successfully employed to purify the protein; however, treatment with TEV protease yielded an insoluble protein under the conditions rendered. The solubility conditions of troponin I and troponin T were tested in buffers of potassium chloride and urea independently; however only troponin T showed solubility in the absence of urea, where troponin I achieved solubilization only upon suspension in the presence of urea.

This work was supported by grants from the National Institutes of Health General Medical Sciences (8 P20 GM103499)

Assessment of Well-Being using Fitbit Technology in College Students Performing Breathing Meditation

Lily Garcia (2022) **Mentors: Courtney Guenther, David Schary**

Stress is a common occurrence among college students, and recently, there has been an unprecedented increase in stress and anxiety stemming from the COVID-19 pandemic. Mindfulness has been used as a coping strategy to help reduce anxiety and stress in college students and may be a helpful resource for students during the COVID-19 pandemic. Breath meditation and physical activity may both increase the ability to cope with a range of different mental health stressors. Our study assessed how the use of brief, daily breath meditation in college students impacted their ability to cope with stress and anxiety. Using the Fitbit, a fitness tracking device, allowed for monitoring of physical activity levels, heart rate, and sleeping habits. Participants also completed the Hospital Anxiety and Depression Scale (HADS), Mental Health Continuum Short-Form (MHC-SF), Penn State Worry Questionnaire (PSWQ), and the Mindful Attention Awareness Scale (MAAS) at baseline and immediately after completing the study. Comparisons were made between participants who completed breath meditation exercises and a control group, who wore the Fitbit, but did not perform any breath meditation exercises. Preliminary results suggest that students who participated in breathing meditation appeared to have increased levels of mindfulness, decreased anxiety and depression scores, and increased levels of emotional, social, psychological, and overall well-being. All participants in our study had decreased levels of worry and anxiety scores; however, the treatment group's decrease was more pronounced. Overall, engaging in brief breathing meditation may improve overall well-being among college students.

This work was supported by grants from the National Institutes of Health General Medical Sciences (8 P20 GM103499)

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Mutating Lpar4 in the Visual System using CRISPR

Thomas Gonzalez (2023) **Mentor: Eric Birgbauer**

The visual system is developed throughout the embryonic stage and is dictated by axon guidance molecules that tell the growing fibers of nerve cells, axons, where to grow. The ends of axons, called growth cones, continuously sprout out or collapse based on the axon guidance molecules. Research has shown that lysophosphatidic acid (LPA) causes growth cone collapse, so it is hypothesized that it is an axon guidance molecule. LPA binds to specific receptors, known as Lpars of which there are at least five known. Previous research indicates that Lpar 1, 2, and 3 are not required in mice for growth cone responses to LPA. Furthermore, Lpar5 is not expressed in retinal tissue, so our research has focused on Lpar4. Our research is trying to determine how axon guidance molecules help shape the visual system that we have today so we start at the embryonic stage where it begins. By working with an embryo that is somewhat similar to ours, we can further figure out how the system works and how our visual system builds itself. We are working to mutate the receptor that we believe to do a substantial amount of axon guidance by using CRISPR as a tool and then see how growth cones respond to LPA without Lpar4. Chicken eggs are used since we easily could take them out at embryonic ages in an artificial culture system in a cup. We have validated guide RNAs that can mutate chicken Lpar4 by using CRISPR. We will inject plasmids expressing these guide RNAs as well as Cas9 along with a tdTomato fluorescent tag into the developing embryonic chicken eye. After developing further, we dissect the chicken retina to see if retinal cells still respond to LPA with growth cone collapse in the absence of Lpar4. We have been optimizing injection and electroporation of plasmid DNA into early embryonic chicken retina as well as dissecting, and removing embryonic retina tissue to determine if our sample has transfected properly. We have also been obtaining baseline data on growth cone collapse by different concentrations of LPA.

This project was supported by SC INBRE grant from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health

Effect of Increased Salinity Concentrations on Gill Morphology and Gene Expression in Redear sunfish (*Lepomis microlophus*)

Joel Haley (2023) **Mentor: Salvatore Blair**

Salinity levels in freshwater ecosystems continue to rise both around the world and regionally, including the Southeastern United States, resulting from agriculture practices, mining, road de-icing, and transient shifts due to storm surge along the coast. Due to this increase in salinity concentrations among local freshwater regions, it is increasingly important to understand both the overall effects of increased salinity on native species as well as to understand any methods that these native fish are able to employ to survive in these unfavorable osmotic environments. The purpose of this study was to determine both the effects of increased salinity on the osmolality and gill morphology of redear sunfish (*Lepomis microlophus)* as well as the molecular mechanisms by which the sunfish may be able to adjust temporarily to increased salinity. We hypothesized that sunfish would demonstrate an increase in solutes in the blood for the test groups (corresponding to an increase in salt), an increase in the ratio of interlamellar cell mass height (ILCM) to lamellae length, and a higher level of expression for genes related to stress and osmoregulation in test groups compared to controls. Sunfish were divided into four groups ($n = 6$), with one group being exposed to 17 ppt saltwater for 24 hours and another for 96 hours, with two control groups at 24 and 96 hours. After their respective treatments fish were euthanized with blood and tissues sampled for physiological, histological, and molecular analysis. Results from the plasma osmolality tests indicated a significant difference between the test groups and the control groups ($p<0.001$) with an increase in plasma osmolality for test groups. Results from staining of the gill tissue showed a significant increase in the ratio of ILCM height to lamellae length for the test groups when compared to the control groups ($p = 0.0232$). Early molecular analysis indicates the expression of osmotic and stress genes in sunfish exposed to higher saline waters. Collectively, these results suggest that the sunfish employ defensive mechanisms against salinity stresses but are unable to successfully osmoregulate under these conditions and are at increased risk for mortality during long term salinity exposure.

Funding for this project was provided by the Winthrop University Research Council Grant SC21008

The Response of Retinal Ganglion Cells to Semaphorin 3A with BDNF and CNTF

Eva Hermanova (2021) **Mentor: Eric Birgbauer**

In the developing nervous system, axons are guided towards their suitable targets by guidance cues. Here we focus on the guidance molecule Semaphorin 3A, a repulsive axon guidance molecule for many axons including dorsal root ganglion axons, cortical axons, hippocampal axons, motor neuron axons, etc. When originally discovered, Luo et al. (1993) found that Semaphorin 3A did not cause collapse of chicken retinal ganglion cell (RGC) growth cones. Contrary to Luo et al.'s original findings, we found that the addition of Semaphorin 3A induced the collapse of RGC growth cones in a dose-dependent manner. One hypothesis is that the difference in results may be due to the presence of neurotrophins, which in some axons increases the sensitivity of the growth cones to semaphorin. So, we examined the response of chicken RGC growth cones to Semaphorin 3A to see if they can be modulated by their exposure to neurotrophins BDNF and CNTF. RGCs cultured with growth factors BDNF and CNTF did not become more sensitive to Semaphorin 3A; there was no difference in the collapse activity between RGCs cultured with growth factors and RGCs cultured without growth factors. Thus, chicken RGC growth cones collapse when treated with Semaphorin 3A and neurotrophins BDNF and CNTF do not factor into that collapse. These findings indicate that Semaphorin 3A may be a repulsive guidance molecule for RGCs.

Funding for this project was provided by the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE)

An Examination of the Effects of COVID-19 Quarantine on Mental Health in College Athletes

Terrik Johnson (2022)

Mentors: Joni Boyd
David Schary

Mental health has become an emphasis in the well-being of college athletes. Many athletes have reported abnormal levels of depression and anxiety, which may affect quality of life and total mental health. Therefore, the objective of this study was to examine the relationships between depression, anxiety, quality of life, and total mental health among college student athletes through a cross-sectional secondary data analysis. We further examined differences in these relationships among groups of gender and race, and between those that have been quarantined from exposure to COVID-19 and those that have not. From the primary study, a sample of 99 NCAA Division I college athletes completed self-report measures on the variables (66% female; 77% white). Data was analyzed through one-way ANOVA and post hoc means by SPSS. There was a significant inverse relationship between the variables of anxiety and depression on both quality of life and total mental health. Results showed a significantly higher impact of anxiety on total mental health for collegiate student athletes who have to quarantine for COVID-19 versus those that did not ($p<0.05$). There were no significant differences in the relationships between the other groups. These results suggest that while anxiety and depression have a significant impact on quality of life for the group, those that had to quarantine are at an increased risk of lower quality of life and total mental health.

RGC and DRG Growth Cone Response to SEMA 3A

Layla GM Carver (2024) **Mentor: Eric Birgbauer**

To investigate the regenerative abilities of the mature nervous system, the embryonic nervous system is used as a model. Retinal ganglion cells (RGCs) are neurons located in the retina that send out axons to be navigated through the eye and into the brain. These axons are guided by growth cones, the developmental subunits that lie on the tip of an axons, which are essential for the formation of the optic nerve during neural development. Growth cones contain receptors that respond to different guidance cues. *In vitro*, an attractive cue will not cause growth cones to collapse and a repulsive cue will cause growth cones to collapse. An accepted guidance cue is SEMA 3A, a protein in the semaphorin family. Previous studies on chickens have proposed that SEMA 3A is a repulsive cue for dorsal root ganglion cells (DRGs) but not RGCs. However, our lab has found that SEMA 3A causes collapse of RGC growth cones in chick embryos. There are many possible hypotheses that could account for the difference between the results from our lab and previously published results. I am testing the hypothesis that there is a difference in sensitivity to SEMA 3A between RGC and DRG growth cones. To test my hypothesis, I quantified the collapse of both RGC and DRG growth cones treated with the same concentrations of SEMA 3A *in vitro* to create a dose response curve. We have found a similar growth cone collapse response between DRGs and RGCs at different concentrations of SEMA 3A, and thus differential growth cone sensitivity is not likely to be an explanation for the difference we have observed from previously published results.

This work was supported by the SC INBRE grant. Thanks to Tyson Farms for providing eggs for research.

The Inhibition of Sphingosine Kinase Using Modified Variants in Zone 2 of Sphingosine Kinase Inhibitor-1

Jomar Lewis (2022) **Mentor: Christian Grattan**

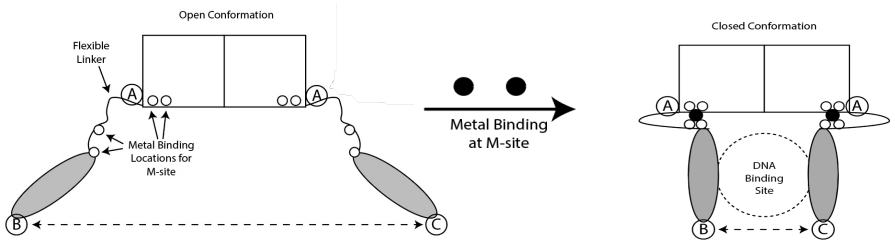
Sphingosine-1-phosphate is a bioactive lipid mediator that has been shown to play a critical role in cell migration, survival, and proliferation. It is phosphorylated from sphingosine, which has the opposite effect on cells. Sphingosine inhibits cell proliferation and causes apoptosis. Sphingosine kinase is a lipid kinase that catalyzes the phosphorylation of sphingosine into sphingosine-1- phosphate. Sphingosine kinase 1 is ubiquitously expressed in most cancer cells where it has been linked to cell proliferation, migration, and survival. Based on this information, sphingosine kinase 1 has become a novel target for anticancer therapy. We analyzed the molecular properties of several zone 2 inhibitors of sphingosine kinase inhibitor-1 using Molinspiration and then uploaded the structures into USCF Chimera to visualize and evaluate the docking analysis using Autodock Vina. The binding energies of each inhibitor were recorded from the docking analysis. The docking energies led to the synthetic development of several zone 2 inhibitor compounds which will ideally lead to an optimized inhibitor of sphingosine kinase. By removing the pyrazole ring and substituting a furan, thiophene, pyridine or imidazole ring we can more accurately assess the inhibitory affinity these derivatives have towards the target enzyme. This will be assessed using assay experiments to determine the optimum substitution moiety in zone 2 for sphingosine kinase inhibitor 1.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences as we as the Winthrop University Department of Chemistry, Physics, and Geology.

Testing the Model of NUR Function: *Incorporation of non-Standard Amino Acids*

Veda Lightfoot (2023) **Mentor: Nicholas Grossoehme**

Nur is a unique member of the Fur family of metalloregulators, in that it is the only Ni-sensing member of the family and, to date, is only present in the bacterium *Streptomyces coelicolor*. Understanding Nur is paramount to understanding how the Fur family has evolved to serve diverse functions. Nur is a symmetric homodimer with monomers bound at the dimerization domain. Each monomer contains two metal binding sites: one M-site and one Ni-site. The dimerization domain is attached to the DNA-binding domain by a linker. Nur functions as a nickel uptake regulator by suppressing nickel uptake systems *nikABCDE* and *nikMNOQ* in excess cellular nickel levels. The previously solved crystal structure of Nur showed a zinc ion bound at the M-site, but the square-planar geometry of the site along with Nur being a Ni-sensing protein, suggests the M-site likely evolved to bind nickel. Previous literature indicates that the Ni-site operate as the regulatory site for Nur, however research in the Grossoehme lab has evinced the M-site as the regulatory site. The proposed conformation model is displayed in the figure below.



It suggests that when the M-site is devoid of metal, the linker between the dimerization domain and the DNA-binding domain is flexible, leaving Nur in an open conformation and lowering its affinity for DNA. However, when metal is bound to the M-site the flexible linker rigidifies, placing Nur in a closed conformation and increasing DNA affinity. EPR spectroscopy and FRET microscopy will be utilized to test this model by attaching EPR probes at the sites labeled A and FRET probes at the sites labeled B and C in the figure. The magnetic interactions of the EPR probes will allow studies on movement of the flexible linker. A broad signal will indicate flexible movement and therefore an open conformation, while a sharp signal will indicate rigid movement and therefore a closed conformation. The energy transfer that occurs between the donor and acceptor probes during FRET microscopy is distance dependent, therefore lower energy transfer efficiency will indicate an open conformation while higher efficiency will indicate a closed conformation. To ensure that the probes are only attached where desired, Nur must contain a completely unique amino acid at the correct locations, making incorporation of non-standard amino acids necessary. In this project, the *nur* gene was cloned into an expression plasmid and mutated at the desired probe locations. This research is ongoing. Transformation into the genetically reprogrammed organism needed for non-standard amino acid incorporation and purification of mutated Nur is underway.

Support provided by SC INBRE and Winthrop University Research Council.

Investigating the Tumor Suppressive Role of RYBP in U-87 Glioblastoma Cells

Mason Linker (2023)
Catherine Moorhouse (2022)

Mentor: Daniel B. Stovall

Glioblastoma (GBM) is the most common and aggressive brain cancer. GBM tumors extend threadlike tendrils throughout the brain, complicating surgical resection. Even with standard chemotherapy and radiation, the median survival rate in adults is only around 14 months. Moreover, GBM displays increased intra- and inter-tumor heterogeneity, which leads to therapeutic resistance and recurrent disease. Thus, there is an urgent need to understand targetable molecular networks that drive GBM progression. Evidence suggests that RING1- and YY1-Binding Protein (RYBP) serves as a tumor suppressor in multiple cancers and its expression is frequently inhibited by small, noncoding microRNAs (miRNAs) (Piunti et al 2021). However, its role and regulation in GBM are not well understood. Therefore, we hypothesized that RYBP exerts a tumor suppressive effect in GBM and is negatively regulated by miRNAs in GBM cancer cells. To test the tumor suppressive effects of RYBP on GBM cells, we generated RYBP-expressing plasmids and co-transfected them into U-87 cells alongside a GFP-expressing reporter. After 24 hours, we isolated total protein from transfected cells and verified RYBP expression by Western blot. Then, we assessed whether microRNAs contributed to reduced RYBP expression in GBM cells. We transfected U-87 cells with specific miRNA inhibitors, then isolated total protein and measured RYBP levels by Western blot. Our data suggest that one of our plasmid clones, pCMV6-XL5-RYBP #3, successfully forced RYBP expression in U-87 cells. However, inhibiting miR-9, miR-125, nor miR-128 was sufficient to restore RYBP expression to detectable levels. In the future, we can use the pCMV6-XL5-RYBP #3 plasmid to transiently manipulate RYBP expression in GBM cells and measure its phenotypic effects.

Using Tetracycline-Binding Nucleic Acid Aptamers as Trojan-Horse Tetracycline Delivery Vehicles in the Fight Against Drug-Resistant Bacteria

Allen Livingston (2023)
Joshua Quarles (2022)
Thomas Sullivan (2021)
Ashley Wood (2022)

Mentor: Timea Fernandez

Illnesses caused by bacteria are now a major public health concern since microorganisms have become increasingly resistant to available antibiotics. As this has occurred big pharma has gradually shifted its focus from developing drugs that cure diseases to those that treat chronic conditions. Thus, rediscovering old drugs and using them for new purposes have become more important. The ultimate goal of this project is to use nucleic acid aptamer-nanoparticle conjugates as vehicles that deliver the antibiotics to cells that are resistant to them.

We investigated the therapeutic potency of nucleic acid- silver/gold nanoparticle conjugates as treatments against bacteria that are resistant to the antibiotic tetracycline. We hypothesized that by attaching nucleic acid aptamers that bind to tetracycline to silver or gold nanoparticles the resulting conjugates will work as a "Trojan-horse" tetracycline-delivery vehicle that smuggles the antibiotic into the cell without being detected by cellular defense systems. In addition we reasoned that the silver or gold ions released by the nanoparticles will add to the antimicrobial effects of tetracycline.

To further prove the viability of this idea we tested three tetracycline binding nucleic acid aptamers. PCR conditions were optimized to make DNA template for RNA synthesis, transcription of RNA aptamers using modified nucleotides was performed, aptamer/linker annealing conditions were optimized, and finally we attached aptamers to gold/silver nanoparticles. In addition, we developed serum stability assays to demonstrate that the RNA aptamers are not degraded by cellular nucleases thus able exert their function in live cells. We are currently testing antimicrobial effect of aptamer nanoparticle conjugates using *E. coli* 5922.

Progress Toward a Soluble pH-sensitive Ferritin Assembly System

Courtney Miller (2024) **Mentor: Nick Grossoehme**

Ferritin is an iron-storage protein that with properties that make it ideal for a number of other applications including a drug delivery systems (DDS). Some of these properties include: (1) it is a small and stable core structure, it has an interior cavity that can be used to transport medicinal drugs in the body, it is naturally found in humans which minimizes the potential for rejection by the body, and the possibility for genetic modifications. For the drug delivery applications, ferritin encapsulated drugs would need to be released based on an external signal or stimuli; however, native ferritin is an extremely stable protein that requires very harsh conditions (e.g. extremely acidic pH or 2.0-3.0) to trigger dissociation. Consequently, the ferritin protein requires modifications to enable the desired stimuli responsiveness. It has been reported that replacing the E-helix of ferritin with six repeating units of EALA (Glutamic acid-alanine-leucine-alanine) results in a pH switch that triggers the reversible disassembly of the nanocage structure in the 6.0-6.5 pH range. Unfortunately, this modification yields a ferritin construct with marginal solubility at neutral pH and very poor solubility below pH 6.8. The goal of the project was to add test the viability of solubility tags additions onto the EALA-modified protein. Two solubility tags, Thioredoxin (Trx) tag and the *E. coli* Biotinylation signal sequence, were selected based on their small size and literature precedent for conferring enhanced solubity to globular proteins. Both constructs were successfully cloned. The Trx tagged protein was tested and failed to enhance the solubility to a meaningful degree.

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Investigating Interactions Between Phage Cain and its Host's Proteome

Dallas Nivens *(2023)* **Mentor: Victoria Frost**
Bethany Wise *(2022)*

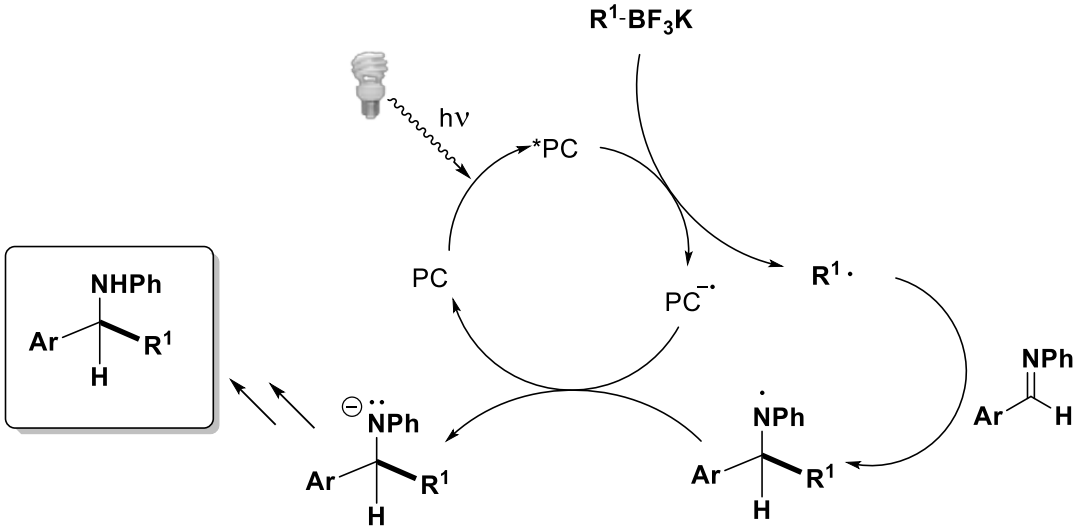
Bacteriophages and their bacterial hosts have been locked in an evolutionary arms race for approximately 3.5 billion years. Although there has been a considerable amount of fundamental research carried out recently, there is still much we do not know. This is particularly true with regards to understanding the biochemical and molecular interactions between the host and its viral parasite. The SEA-GENES Program, and our research, aims to tease apart and understand some of the mechanisms involved at this phage–bacterial interface. To discover how phage proteins interact with the host proteome, we are investigating each individual gene in the bacteriophage Cain, a temperate phage in the K6 subcluster isolated on the host *Mycobacterium smegmatis* (*M. smegmatis*). Previously, SEA-GENES students at Winthrop used phenotypic assays to identify several of Cain's gene products that were able to interact with the host proteome. To investigate this further, a Protein-Protein Interaction (PPI) assay has been developed. This assay specifically reveals which *M. smegmatis* protein interacts with the phage protein being expressed in our system (known as a Bacterial Two-Hybrid, or B2H assay). Cain *55*, a gene without a known function, was the first gene to be cloned into the B2H expression plasmid. With the help of inducible promoters and reporter genes on the plasmid, evidence has shown that Cain *55* interacts with Nus A; a protein known to be an important cellular transcription regulator. Knowing that there is an interaction occurring between these two proteins gives us insight into the possible function of Cain *55* and homologous genes in similar phages. One of these orthologs, Waterfoul *47*, also shows the same ability to interact with Nus A in *M. smegmatis*. In addition, both genes Cain *55* and Waterfoul *47* interact with Nus A in the pathogen, *M. tuberculosis*. This highlights the possible consistent function of these genes across other species of Mycobacteria. Though we cannot come to a definitive conclusion about the function of Cain *55*, this research aims to increase our understanding of the dynamic yet enduring relationship that exists between phages and their bacterial hosts.

SC INBRE and HHMI

Photoredox Mediated Alkylation of Imines with Potassium Organotrifluoroborates in the Presence of an Organic Photocatalyst

Molly Quetel (2023) **Mentor: James M. Hanna Jr.**

During the last 10-12 years, visible-light photoredox catalysis (VLPC) has been developed into a practical method to achieve a wide variety of synthetic transformations. The approach often involves a transition metal complex of ruthenium or iridium, which, upon absorption of visible light, can participate in a series of single-electron-transfer (SET) events with organic substrates, leading to productive chemistry. However, these transition metal catalysts can be quite expensive, which led our group to investigate the use of less expensive organic photocatalysts in our studies of the alkylation of aryl imines with potassium organotrifluoroborates. In this presentation, the application of a widely used organic photocatalyst, 9-mesityl-10-methylacridinium tetrafluoroborate (***Mes-Acr-Me***), will be explored; data from optimization experiments, along with those from the scope and limitations studies including both imines and organotrifluoroborates, will be surveyed. In addition, results from Stern-Volmer quenching studies — carried out to verify the initial electron transfer event of the proposed mechanism (shown in Scheme 1) — will be discussed.



Scheme 1

Support was provided by the Winthrop University McNair Scholars Program and the Donors of the American Chemical Society Petroleum Research Fund. Additional support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences.

Effect of non-native plants on amphibians and reptiles in Winthrop Woods

Luke Reed (2022) **Mentor: Kiyoshi Sasaki**
Braulio Antonio Adorno-Colon (2021)
Ivon Valenciano-Tovar (2022)

Non-native plants can have a negative impact on wildlife habitats. Forests are being lost to human land use, and the remaining forests are increasingly being dominated by non-native plants. Winthrop Woods is one of the largest remaining forests in Rock Hill, South Carolina. This project maps the distribution of the native and non-native plants in Winthrop Woods and determines how non-native plants affect the abundance of reptiles and amphibians. To determine the distribution of plant species, we characterized vegetation in 10 × 10 m plots along transects established every 20 m along the length of Winthrop Woods. In each plot, we recorded the GPS coordinates, identified and scored the abundance of tree and shrub species to species or genus level by size classes. In addition, we recorded variables that likely affect the abundance of amphibians and reptiles: the percentage of the cover by fine woody debris, coarse woody debris, herbs, and lianas. This mapping project is ongoing. We have completed vegetation surveys at 321 plots and 59 transects. Preliminary analyses indicated that most of the understory was dominated by non-native shrubs that originated from Asia. The abundance of amphibians and reptiles will be assessed over the next two years.

Funding for this project was provided by the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE) IV.

Inhibiting Acid Ceramidase with Phenolphthalein Compounds as a Potential Cancer Treatment

Sybil Smith (2023) **Mentor: Christian Grattan**

Cancer is a prevalent issue. The most common current treatments for cancer include radiation therapy and chemotherapy. However, recent developments in cancer treatment have included targeted therapy. Targeted therapy drugs are directed at molecules in cancer cells that promote proliferation. This approach to treating cancer is more specific to cancer cells, allowing healthy cells to be unharmed. The focus of our research was to create a targeted therapy drug to inhibit acid ceramidase, an enzyme that functions in the sphingomyelin pathway. The sphingomyelin pathway creates lipids for the plasma membrane of cells. Acid ceramidase converts ceramide into sphingosine, while later in the sphingomyelin pathway, sphingosine is converted into sphingosine-1-phosphate. A high concentration of ceramide in the plasma membrane promotes apoptosis, while a high concentration of sphingosine-1-phosphate promotes cell proliferation. Many cancer cells have increased proliferation due to an overactivity of acid ceramidase. This makes acid ceramidase a promising target for targeted therapy. The goal of our study was to synthesize an effective inhibitor of acid ceramidase. We designed eight derivatives of phenolphthalein, each with different groups attached to the ortho position on the phenols. We then tested each derivative *in silico*, all of which had promising results. We were able to successfully synthesize all proposed derivatives by reacting 2 equivalents of phenol with phthalic anhydride using an acid catalyst in a microwave. Future works will evaluate the bioavailability of the compounds using tissue culture assays.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences as well as the Winthrop University Department of Chemistry, Physics, and Geology.

The Foibles of Mycobacteriophage Cain

Laela Walker *(2023)* **Mentor: Victoria Frost**
Ma'Liah Maddox *(2024)*

The SEA-GENES program of research at Winthrop University is adding to the growing body of knowledge about the interactions that occur between bacteria and the viruses (phages) that infect them. This past year, students focused on bacteriophage Cain, a K6 subcluster phage, discovered in the soil using the bacteria *Mycobacterium smegmatis* (*M. smegmatis*) as a host. Cain was originally isolated at 24°C, which led to issues with some of our downstream analyses. One of these investigations uses a phenotypic assay to test individual Cain genes, transformed into *M. smegmatis*, for their homotypic defense abilities i.e. whether Cain gene expression results in "protection" of its host cell from attack by external Cain or other similar phages. However, transformed *M. smegmatis* does not grow at 24°C, which is the temperature required when testing active Cain phage to overcome the transformed host's immunity. Rather, the transformed host *M. smegmatis* grows optimally at 37°C. Thus, we devised an array of assays to test the lower limits of temperature for transformed *M. smegmatis* growth, followed by concurrent evaluations of the upper limits of Cain activity. Our results demonstrated that the bacteria were able to successfully form a consistent lawn as low as 30°C, and that Cain was able to infect these cells at the same temperature. However, while Cain and transformed *M. smegmatis* successfully grew on the same plate at 30°C, visual characteristics of a slightly pink, uneven lawn suggest that more replications of the assay need to be carried out. After finding a suitable temperature for both bacteria and phage, as well as testing Cain's defense traits, the next step in our research is a protocol that will further highlight the function of Cain's genes by the use of Protein-Protein Interaction (PPI) experiments. These extensive efforts will collectively aid in the overall studies of how phages interact with their bacterial hosts as they continue to co-evolve over time.

SC INBRE and HHMI

Expression and Purification of GeneM: A Novel Virulence Factor of Unknown Function from the Phytopathogen *Clavibacter michiganensis*

Eric Walters (2023) **Mentor: Jason Hurlbert**

GeneM is a novel virulence factor of unknown structure and function produced by *Clavibacter michiganensis,* the etiological agent of many diseases in agricultural plants. Tomato and potato plants containing the gene have shown signs of symptomatic necrosis, yet plants containing mutants of the gene were shown to be asymptomatic. BLAST analysis of the amino acid sequence has identified homologous proteins belonging to the patain superfamily, however, bioinformatic analysis of the amino acid sequence and homology modeling contradicts this identification. In this work, we describe our efforts to model the structure of GeneM and express it in recombinant *Escherichia coli* cultures. Of the three algorithms used to generate a homology model of GeneM, only one gave us a plausible structure. *De novo* modeling using trRosetta gave a model that is structurally similar to the homology model. Expression trials were performed using different strains of *E. coli* including BL21, NiCo, and Rosetta 2, and based upon the results, cultures of *E. coli* BL21 Rosetta 2 (DE3) had a band at the expected size on SDS-PAGE gels. 6L expression cultures were generated and a chromatographic method was developed, involving MCAC, gel-filtration, and cation exchange, to purify the protein for enzymological studies. Future work includes a phospholipase assay.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences as well as the Winthrop University Department of Chemistry, Physics, and Geology and the Winthrop University McNair Scholars Program.