Winthrop University
Summer Undergraduate Research Experience (SURE)
2019 Abstract Book

WINTHROP
UNIVERSITY

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ACKNOWLEDGEMENTS

The Winthrop University Summer Undergraduate Research Experience (SURE) is a coordinated effort involving the Departments of Biology, Mathematics, Human Nutrition, and Chemistry, Physics, and Geology, in which undergraduate students pursue eight to ten weeks of research with faculty mentors. In the summer of 2019, a total of 54 students working with more than 20 faculty mentors, explored exciting questions in biology, chemistry, biochemistry, mathematics, human nutrition, geology, and physics. The abstracts in this book represent the culmination of their efforts.

SURE would not be the vibrant, successful program it is without the dedication of the faculty and students involved. Many of these faculty members also coordinated a variety of program activities during the summer, in which the students enthusiastically participated, and we are very grateful for their time and talents. We especially want to thank Dr. Arran Hamm, who worked diligently to assemble, edit, and publish this abstract book.

We also gratefully acknowledge Winthrop's administration, especially President Dan Mahony and Provost Adrienne McCormick, for their ongoing support of SURE and undergraduate research.

Finally, on behalf of students, faculty and administrators, we thank the agencies and organizations listed below for their financial support. The hands-on learning experiences that SURE faculty mentors provide to participating students would not be possible without them.

Please enjoy reading about the excellent research done by our outstanding students this summer!

Diana Boyer
SURE Program Coordinator

Robin Lammi
Director of Undergraduate Research

Synthesis of Zinc Oxide Particles: Biological Systems and Optical Properties

Mikhail Anfinson (2020) **Mentor: Maria Gelabert**

Zinc oxide nanoparticles (ZnO-NPs) have a wide range of applications such as sunscreens, semiconductors, cancer treatments, and antibiotics. They are being employed more frequently, yet their environmental impacts need to be further explored. Properties of nanoparticles are tied to surface structure, and ultimately optical properties. It is important to be able to synthesize ZnO-NPs in a cost effective, environmentally friendly, and a commercially viable way, with full characterization from synthesis to disposal. We conducted experiments to investigate capping agents, at different concentrations, and different ratios of reagents, zinc chloride and sodium hydroxide, and these effects on preparation of ZnO-NPs at room temperature in pure ethanol. Typical syntheses were performed with 25.00 mL of .0200 M $ZnCl_2$ and 5.0 mL of NaOH (0.150 M or 0.500 M), added to the solution in 83-μm aliquots every 30 seconds for 30 minutes. Products were washed and centrifuged, then examined with particle size analysis and scanning electron microscopy (SEM). Optical properties were investigated with ultraviolet-visible (UV-Vis) and fluorescence spectroscopy. Different capping agents and differing ratios of reagents lead to both different defect levels and different morphologies of zinc oxide micro-particles and ZnO-NPs. Guar gum and polyethylene glycol (PEG) seemed to lead to spherical morphologies and oleic acid seemed to lead to needle like morphologies. The median particle size using oleic acid, PEG, and guar gum as capping agents was 1-7, 1-4, and 9-12 μm respectively. UV-Vis spectroscopy revealed broad absorption, around 400 nm, close to the band gap energy. For emission data, synthesis using oleic acid exhibited different fluorescence emission peaks dependent on the ratio of reagents used within the reaction. Products exhibited fluorescence emission peaks typically at 390, 451, and 468nm, with excitation at 350 nm and emission set to 360-680 nm. The higher concentration of NaOH, all else remaining constant, showed peaks at 390 and around 530 nm.

Support for this research was provided by MADE in SC, SC-EPSCoR NSF #1655740

Modeling the Latent Reservoir in the Dynamics of HIV Infection with CTL Memory

Josiah Bauer (2022),
Sarah Fleetwood (2022)

Mentors: Zach Abernathy
Kristen Abernathy

In this project, we model the dynamics of HIV-1 latently infected cells under the effects of a natural immune response. Our purpose in this model is to study the long-term effects of CTL memory on viral load. We establish the existence of equilibria and the global asymptotic stability of the disease-free equilibrium based on the rate that cells are latently infected vs. actively infected. We then perform numerical simulations to illustrate the stability behavior of immune-free and internal equilibria. Furthermore, we demonstrate antiretroviral therapy can stimulate a memory response and reduce the viral load in the case when all equilibria exist.

This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (2 P20 GM103499 15) of the National Institutes of Health.

Global Dynamics of the HIV Latent Reservoir with Latency Reversing Agents and Immune Response

Claire Berchtold (2020),
Hannah Mitchum (2020)

Mentors: Zach Abernathy
Kristen Abernathy

In this project, we model the dynamics of HIV-1 latently infected cells under the effects of latency reserving agents (LRAs) to promote a natural immune response. We establish the existence of immune-free and positive equilibria and then utilize Lyapunov functions to prove the global asymptotic stability of each. Numerical simulations are performed to support and illustrate these results. We conclude with a discussion on the model's predicted threshold for LRA effectiveness to stimulate a natural immune response and decrease the size of the latent reservoir.

This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (2 P20 GM103499 15) of the National Institutes of Health.

Characterization of Metal Dependence of PhpP

David Brandyburg (2022) **Mentor: Nicholas Grossoehme**

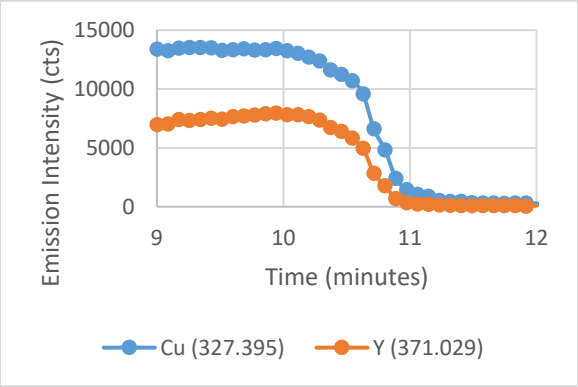
RitR(repressor of iron transport) is an orphan two-component signal transduction response regulator in Streptococcus pneumoniae that has been shown in recent studies to be necessary for iron homeostasis within the bacterium. RitR however lacks the trace amount of Asp upon phosphorylation that appears in most two-component systems and instead contains an Asn residue at the predicted phosphorylation site of RitR. This lead many to wonder if RitR was phosphorylated at all. It was determined in one study that RitR is indeed phosphorylated by the Ser-Thr kinase-phosphatase pair StkP and PhpP. STK is the extracellular sensor, and it is believed that PhpP is an intercellular sensor. The only way to test this would be by testing if PhpP is capable of removing the phosphate from RitR by utilizing pNPP(para-nitrophenylphosphate), which is a substrate that can be used in an assay to determine enzymatic activity. Phosphatases such as PhpP catalyze the hydrolysis of pNPP leading to a phosphate and the conjugate base of pNP(para-nitrophenol). By utilizing this assay, we were able to determine that PhpP is indeed capable of dephosphorylating a substrate. This allowed for us to use another assay called the Malachite Green assay which allows for us to quantify the amount of phosphate that we were able to put on RitR. By combining parts of these assays together we were able to move on and determine how much phosphate was removed from RitR over the course of four hours but unfortunately this needs to be looked back upon and determine what should be revised in order to produce better quality data.

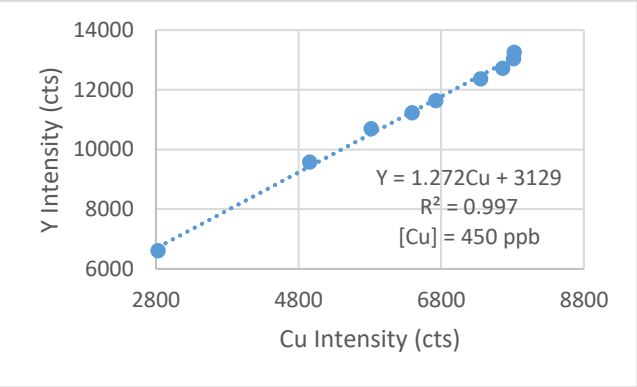
Funding: SC INBRE (NIH 8 P20 GM103499)

Automation of the Standard Dilution Technique for the Determination of Copper in Tap Water using ICP-OES

Tamara A Bright (2020) **Mentor: Cliff Calloway**

Copper is routinely used in society for plumbing potable water, electronic devices, environmental heating and cooling. Copper is essential for several biochemical processes but can cause health problems at elevated concentrations. Copper is routinely determined by ICP-OES using external or internal methods of calibration. Standard Dilution Analysis is a relatively new calibration technique that combines advantages of other calibration methods, reduces the number of solutions that need to be quantitatively prepared. The purpose of this research is to use the standard dilution analysis technique and two programmable syringe pumps to speed and automate the determination. The two pumps were programmed in two ways. In one method, two pumps were infusing and withdrawing solutions at the same, constant flow rate. The second method cycled flow rates with one syringe pump slowing down as the other syringe pump increased, such that the total flow rate remained unchanged. Both methods yielded fast, reproducible and accurate results with minimal sample preparation, simple processing and analysis time of just a few minutes. Recoveries were typically 97-102% with reproducibility less than 5%.





This work was supported by grant P20GM103499 (SC INBRE) from the National Institute of General Medical Sciences, National Institutes of Health and the Wake Forest University Graduate School of Arts & Sciences.

Optimization of RNA Isolation Methodology for Gene Expression Analysis of Self-Organizing Three-Dimensional Tissue Structures

Chandler Burt (2020) **Mentor: Dr. Matthew M. Stern**
Nathan Kidd (2022)

Two-dimensional cell culture has dominated cell biology research for decades and continues to hold great value. However, the current trend is to move towards three-dimensional cell culture models, which can better represent the environments that different cell types encounter *in vivo*. Three-dimensional culture systems allow for more complex cellular interactions and organization than traditional two-dimensional monolayer cell culture. Our collaborators have observed that cells placed on top of collagen hydrogels–a commonly used three-dimensional culture system, organize into a donut-like shape called a toroid. Cells mixed into the hydrogels do not form toroids. Interestingly, when several cancer cell types/lines were tested, they failed to form a toroid when placed on top of the hydrogel. The ultimate goal of this project is to evaluate the signal transduction pathways and cellular mechanisms that are involved in toroid formation and presumably dysregulated in cancer cells. One way to investigate these mechanisms to compare the gene expression profiles of different cells cultured in these environments at different times. Gene expression analysis requires the isolation of high quality RNA in sufficient quantity for the desired experiments. The specific goal of the work described here was to optimize RNA isolation from cells cultured on or in collagen hydrogels–a procedure that is known to be technically challenging, and to use real-time RT-PCR (reverse transcription polymerase chain reaction) to compare the expression of select genes during toroid formation. We tested and compared several different RNA isolation methods and protocols and found that a method based on the use of cetyltrimethylammonium bromide (CTAB), which is typically used in isolation of RNA from plants, proved to be the most effective. Isolation of RNA using the CTAB method yielded RNA with high purity and high concentration. These results allowed us to move onto using real-time RT-PCR to compare the expression of two specific genes, *Cxcr4* and *Cxcl12*, during toroid formation by mouse adipose-derived stem cells. RNA was isolated every two hours over a twelve-hour span and reverse-transcribed into cDNA. We are currently comparing expression of *Cxcr4* and *Cxcl12* across this timecourse and hope to learn more about the timing and importance of their expression in our system. Future work will include analysis of additional genes and a comparison of gene expression in "normal" and cancer cells.

South Carolina EPSCoR/IDeA Collaborative Research Program
South Carolina INBRE

Sphingosine Kinase Inhibition Using Modified Variants of a Sphingosine Kinase Inhibitor

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

In cells, the breakdown of the cell membrane occurs through the sphingomyelin metabolism pathway. Within this pathway, there are 4 four metabolic products: sphingomyelin, ceramide, sphingosine, and sphingosine-1-phosphate. The focus of this research centers around the conversion of sphingosine to sphingosine-1-phosphate using sphingosine kinase 1. If the intracellular concentration of sphingosine 1-phosphate build up in a cancerous cell, the cells signal for anti-apoptotic outcomes spreading the cancer. If sphingosine kinase 1 can be inhibited the intracellular concentration of sphingosine and ceramide would increase and so would the apoptotic outcome.

Sphingosine kinase inhibitor 1 (SKI-1), was identified recently as a potential drug. Despite the drug's success *in vitro*, it failed drastically *in vivo*. It was later discovered that this was due to a violation in Lipinski's Rule of Five. One of the rules states that the LogP value must be below a 5. The LogP value for SKI-1 is calculated to be 5.6, meaning that the drug may not be as effective traveling through the body to the target sites. Because of this, it was decided that synthetically modifying the structure might improve the *in vivo* effectiveness.

Zone 1 and Zone 4 were examined for modification during this research time period. Both of these zones contain two connected benzene rings, but Zone 4 contains a hydroxyl group that is connected to one of the benzene rings. Each inhibitor derivative was produced by a three step process involving microwave synthesis. The purity of each substance was confirmed by ^{1}H NMR and ^{13}C NMR. Each of the new inhibitor compounds were sent off for bioassay testing to determine the effectiveness of these modifications relative to the template compound, SKI-1. Ideally, we will take the best Zone 1 derivative and combine it with the best zone modification in each of the other portions of the structure to prepare a drug that may better realize the potential of this compound *in vivo.*

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.

Remote Sensing and Decomposition Rates of Forest Succession Plots in the Piedmont of South Carolina

Blake Campbell (2020),
Dakota Shope (2020)

Mentor: Dr. Scott Werts

The Piedmont of the US are dominated by ultisol soils, which often contain highly weathered geologic materials. Due to the diverse nature of land development in the southeastern US, these soils are often under a wide range of developmental stages and, especially in the surface horizons, contain a great deal of spatial variability in properties. In this study, we have begun a decompositional study of four forested plots in various stages of succession of former farmland. Litter bags containing native litter at each site and cellulose paper were placed at each site and collected over two-week intervals in order to compare decomposition rates from site to site. Remote sensing stations were also established at each location to record differences in soil temperature and soil moisture. Initial results suggest that there is high variability of decomposition rates in between all the sites, even when controlled for litter type with cellulose paper. Initial decomposition rates were higher for native vegetation than the litter paper. The most recently established plot showed the highest initial rate of decomposition, followed by the more well-established sites. Although soil temperature was higher in the lesser established plots, soil moisture remained lower in all these plots during the decomposition study as well, which may explain the slower decomposition rates.

Funding was received from the Boland Geology Endowment and the Margaret E. Spencer Undergraduate Fellowship

Investigating the Responsiveness of Embryonic Chick Retinal Ganglion Cells to Semaphorin-3A

Fatoumata Nancy Cisse (2020),
Shane Ira Lacanin (2020),
Allison Reed (2021)

Mentor: Dr. Eric Birgbauer

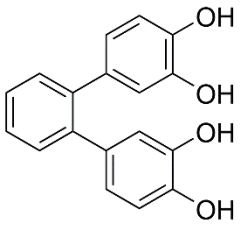
In a developing nervous system, axons need to travel long distances to make interneural connections for the nervous system to function properly. These axons are guided by growth cones, which sense different guidance cues. Semaphorin-3A (Sema-3A) may be a crucial axon guidance molecule in the developing embryonic visual system that is responsible for the direction which axons grow when traveling to their synaptic target, in this case, the tectum of the brain. Growth cones, found on the terminal ends of axons, will either collapse or continue to grow in response to their environment. When a growth cone collapses, it will retract from the axon guidance molecule and grow in a different direction. Previous studies have reported that Sema-3A causes growth cone collapse of dorsal root ganglion cells (DRGs) but not chicken retinal ganglion cells (RGCs) (Luo et al. 1993). However, previous lab work showed that Sema-3A will indeed cause growth cone collapse of chick RGCs. One possible hypothesis for why we found different results is that the retinal explants were treated with Sema-3A for a shorter time period than in the original paper; another possibility is the age of the chick embryos. We investigated chick RGC responses to different concentrations of Sema-3A and found a dose-dependent response. We also examined the responsiveness of different embryonic ages to Sema-3A, and it was found that as the chick embryo develops, the chick RGCs maintain a significant level of responsiveness ($p < 0.05$). We investigated the time response of Sema-3A and found that RGCs exhibited a peak of collapse after 15-minutes, and that they started regrowing at 20-minutes. We have also investigated by RT-PCR the expression of semaphorin-3A receptors, including Neuropilin-1 and Neuropilin-2 as well as their co-receptors Class A plexins, Class B plexins, close homologue of L1, and L1Cam in the chick retina. We found that all of these are expressed except for Class B plexins and Neuropilin-1. In conclusion, our results suggest that Semaphorin-3A induces growth cone collapse in embryonic chick retinal ganglion cells.

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

Synthesis of Diarylpyridines as Aggregation Inhibitors for Alzheimer's Amyloid-β Peptide

Kendall Claxton (2021) **Mentors: Robin K. Lammi, James M. Hanna Jr.**

Amyloid-β peptide (Aβ) self-assembles into neurotoxic, β-structured aggregates, which are the primary component of the extracellular senile plaques characteristic of Alzheimer's disease. A variety of small molecules have been shown to inhibit the aggregation process; typically, these contain aromatic groups and one or more hydrogen-bond donors. Previous studies in our group have demonstrated that terphenyltetrols exhibit some degree of efficacy as Aβ aggregation inhibitors. For example, *o*-terphenyl-3,3″,4,4″-tetrol (OTT, **1**) is a moderately effective inhibitor of Aβ aggregation (IC_{50} = 2.7±0.3X). Recent modeling studies suggest that binding of small molecules to Aβ may occur via several types of intermolecular interactions, including both hydrogen bonding and π-π interactions (i.e., π-stacking). In addition, other studies indicate that π-interactions between benzene and electron-deficient heterocyclic aromatic rings are stronger than similar benzene-benzene interactions. Based on these observations, we hypothesized that incorporation of a pyridine unit as the central linker in the above-described tetrahydroxyteraryl scaffold may lead to increased inhibition of Aβ aggregation. We therefore synthesized bis(dihydroxyphenyl)pyridines **2** and **3** (figure) via Suzuki coupling of 3,4-dimethoxybenzene-boronic acid with an appropriate dibromopyridine, followed by demethylation in refluxing aqueous HBr. Future work will focus on evaluation of these compounds, as well as 1,3- and 1,4-bis(dihydroxyphenyl)pyridine analogs, using a Congo red spectral shift assay.



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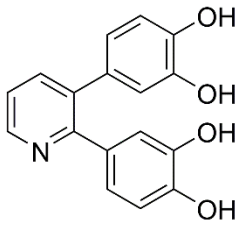

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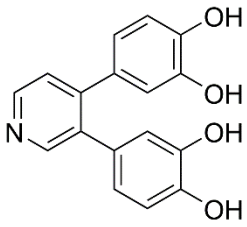

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Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (8 P20 GM103499).

The Effects of DESS on Meiofaunal DNA Yield

Emily Crago (2020) **Mentor: Dr. Julian Smith III**

Several fields have been investigating the effects of using DESS as preservation technique for collected specimens. While there have been several studies on the use of DESS and how it will affect the yield of the specimen's DNA, there has been minimal attention given to Meiofauna, small organisms living in aquatic sediment. Meiofauna can relate to an array of different organisms such as nematoda and copepoda. Samples from Winthrop Lake, Wetlands and an off sector of Winthrop's Lake were collected in June of 2019. One set of 8 samples sat in DESS for one week, while two sets of 3 sat for 24 hours, and another single set of 3 sat for 5 hours. After samples sat for their allotted time, they were processed with a Quaigen DNeasy kit. As data collection concluded, the results showed that samples can remain in DESS for up to a week and that there was no significant difference between DNA yield samples.

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

Recovery Fauna of the Devonian Mass Extinction: Why So Small?

Daniel Croke (2021), **Mentor: Dr. Diana Boyer**
KeeLe Pucket (2021)

The Lilliput Effect proposes a reduction in size of the biota associated with the aftermath of mass extinctions. The Hangenberg event was a widespread bioevent that occurred during the late Devonian period and provides an opportunity to test for the Lilliput Effect in the fossil record. The Hangenberg event is preserved in black shale, which indicates anoxic ocean conditions during the event. Samples were collected from three different late Devonian localites in Ohio from the rock layers above this extinction interval, representing times immediately after the event were analyzed. A total of over 18,77 fossils were analyzed. Data was collected from samples using a caliper to determine size and was then recorded into specified tables for each locality. A total of 749 samples were identified to the genus level. A total of 757 fossils were pyritized and several show delicate features such as spines, indicating that they were not transported. Brachiopods were found to be most abundant in all three localities with bivalves following as the second most abundant group. Fossils ranged from 0.38 mm to 18.32 mm with an average size of less than 5 mm. Although these fossils were all small, additional taxonomic data is needed to confirm the Lilliput effect..

Funding was provided through the Boland Geology Fund.

Validation of Methodology for Use in Detection of Bovine Milk Adulteration in Human Milk Samples

Luke Dawson (2019),
Amy Matsumoto (2019)
Mentor: Hope K. Lima, PhD, IBCLC

Introduction: When mothers have excess breastmilk, they are able to donate it to help feed premature and fragile infants whose mothers cannot provide their own milk. Screening of donor mothers, intake of donor human milk (DHM), and processing of DHM is completed through a milk bank. In the United States there are for-profit and non-profit milk banks. For-profit milk banks provide compensation to the mother for her milk, raising concerns about the purity of the milk that she donates. Professionals in the field are concerned that mothers may be adding bovine milk to the expressed breastmilk prior to donating to increase the compensation received. Currently, at least one for-profit company screens DHM for bovine adulteration; however, methods for screening are considered proprietary and no published protocols exist.

Goal of the Study: Assess current methodology, including ELISA and DNA-based analysis, for the ability to accurately detect the presence of bovine milk in human milk in a cost-effective manner.

Methods: Human milk samples from 40 mothers will be pumped under direct observation of a researcher using a brand new hand pump in order to ensure purity. Additionally, all participants will submit a 24-hour food journal and a food frequency screener to provide information about their diet. Collected samples will be aliquotted and adulterated with either powdered infant formula, whole bovine milk, 2% bovine milk, or skim bovine milk at four different concentrations (1%, 5%, 10%, and 20%). Deidentified samples will then be analyzed using two detection methods. Detection methods include measurement of bovine mitochondrial DNA using quantitative PCR (qPCR) and measurement of β-lactoglobulin using an enzyme-linked immunosorbent assay (ELISA).

Results: Upon completion of qPCR and ELISA testing, samples will be unblinded and analyzed for accuracy, precision, and cost-effectiveness. Use of the 24-hour food recalls and food frequency screeners will provide information about bovine consumption in the human milk samples if unadulterated samples are flagging positive for bovine protein or mitochondrial DNA.

This project was supported by Winthrop University and SC INBRE

Optimizing *mus109* allele-specific PCR Conditions

Jordan DeLoach (2020) **Mentor: Dr. Kathryn Kohl**

All organisms experience DNA damage, and a variety of DNA repair mechanisms exist to combat this damage. The Kohl lab previously identified a candidate gene for *mus109*, an unmapped DNA repair gene in the *Drosophila melanogaster* genome. To confirm the identity of *mus109*, the lab plans to conduct a rescue experiment which requires a molecular tool to quickly and unambiguously identify *mus109* alleles. Therefore, the goal of this research was to design allele-specific primers and to optimize allele-specific PCR conditions for the various alleles of *mus109*. Allele-specific primers were designed using the online WASP program, and optimal allele-specific PCR conditions were obtained by testing a variety of Mg^{2+} concentrations and varying annealing temperatures. Currently, conditions have been established for two out of the three *mus109* alleles, and the final allele is in progress.

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

Expression, Purification, and Initial Crystallization Trials of AvrBs1.1, A Protein Tyrosine Phosphatase from *Xanthomonas euvesicatoria*

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

Brown spots and leaf loss of *Capsicum annum*, a pepper found in the Americas, are caused by prolonged activation of the plant's defense mechanisms to bacterial infection resulting in a phenomenon called the hypersensitive response. In the hypersensitive response, the plant "walls off" the infected tissue with lignin and then a variety of chemical processes occur within the lignified zone which results in the death of both bacterial and plant cells. Certain species-specific bacterial pathogens inject effector proteins with different activities into the cytosol of the host to prevent activation of the plant's defense mechanisms. Our work focuses on the effector proteins AvrBs1.1, a dual specificity protein tyrosine phosphatase produced by *Xanthamonas euvesicatoria*. Recent work has identified the transcription factor WRKY-1 as a possible target for AvrBs1.1. We hypothesize that AvrBs1.1 dephosphorylates WRKY-1 in the cytosol, thereby preventing it from entering the nucleus and activating the genes of the plant's defense response, and determination of the three-dimensional structure of AvrBs1.1 will allow us to visualize the binding site of the protein and deduce complementary amino acids in its binding target. A key first step in this process is the production of purified, recombinant AvrBs1.1 to use for crystallization trials. Using an *Escherichia coli* codon optimized gene encoding AvrBs1.1, we have developed an expression and purification scheme suitable for the production of quantities of the protein suitable for crystallization studies.

Funding: SC INBRE (NIH 8 P20 GM103499)

Measuring heat related to the disassembly and reassembly of ferritin using isothermal titration calorimetry

Brandon Ellison (2022), **Mentor: Nicholas Grossoehme**
Alexandra Perez (2022)

Ferritin is an iron storage protein that is responsible for the accumulation of excess intracellular iron. Native ferritin self-aggregates into a nanocage structure containing a ferroxidase center that regulates the uptake and release of iron. In recent years, researchers have begun to explore the idea of using ferritin as a component in the delivery of drugs throughout the body. Ferritin is an attractive candidate because it is a native human protein that has the ability to encapsulate small molecules. Furthermore, it can be chemically or genetically modified to target very specific cells. One major limitation of drug delivery by ferritin lies in its inherent stability; harshly acidic conditions are needed to drive the disassembly of the nanocage. It was recently discovered that replacing the E-helix of human light chain ferritin with a GALA peptide repeat (hFtnL-GALA) would allow for the pH-induced disassembly to occur at a pH below 6. As a result, ferritin is able to become more applicable as a drug carrier under physiologically relevant conditions.

This project focuses on the expression and purification of modified human L-chain ferritin with a subsequent thermodynamic characterization of the disassembly and reassembly of the hFtnL-GALA nanocage. The chimeric protein is largely localized to the insoluble lysate pellet after sonication; consequently, the published purification protocol failed to produce enough protein for subsequent experiments. An alternate protocol was developed that leveraged 4 M Urea to resuspend the insoluble lysate fraction. The urea was diluted slowly to allow the hFtnL-GALA protein to fold properly. Analysis of the sample by size exclusion chromatography on a sephadex G200 column showed that the protein eluted around 9 mL, consisted with an intact nanocage structure. Currently, the amount of protein that was isolated using this approach is insufficient for a thermodynamic characterization. In the future, the goal is to adjust the most recent purification protocol in an attempt to produce a larger yield of pure protein as well as use isothermal titration calorimetry to explore the heat exchange associated with the dissociation and reformation of ferritn.

Funding: EPSCoR CRP grant

Evaluating the Effects of Detergent Concentration on the Ultrastructure and Recellularization of Porcine Internal Thoracic Artery Scaffolds

Carlos Escoto-Diaz (2022) **Mentor: Dr. Matthew M. Stern**

Heart disease has become the leading cause of death in developed countries, leading to an increase demand for coronary artery bypass graft surgeries. Currently, bypass surgery requires a vessel to be harvested from the patient or a donor graft to be provided. Both of these options can result in complications for the patient. An alternative approach that could alleviate these issues is the use of tissue-engineered vascular grafts. The goal of this study is to evaluate the potential of scaffolds derived from porcine internal thoracic arteries (PITAs) for use in small-diameter tissue engineering. PITA scaffolds can be produced through the process of decellularization, which uses a combination of detergents to remove the porcine (pig) cells while leaving behind the extracellular matrix/framework of the tissue. We hypothesized that increasing detergent concentration during the decellularization would affect the ultrastructure of the scaffolds and be associated with greater residual cytoxicity if the scaffolds are not properly rinsed following decellularization. We evaluated the ultrastructure of the scaffolds using scanning electron microscopy and observed that increasing detergent concentration was associated with greater porosity. We evaluated the residual cytotoxicity of the scaffolds using the alamarBlue assay to quantify the viability of a human endothelial cell line in the presence of scaffold and found that an extensive rinsing protocol is required to eliminate the cytotoxicity. We verified the ability of endothelial cells to grow on/in the scaffolds using a combination of the alamarBlue viability assay and fluorescent microscopy. Taken together, our results show that PITA scaffolds with different ultrastructural features can be prepared and repopulated with endothelial cells as long as the scaffolds are properly rinsed. Establishing a bona fide procedure for successful recellularization of PITA scaffolds will ultimately aid in the development of a clinically relevant alternative to our current options for small-diameter vascular grafts.

South Carolina EPSCoR/IDeA Developmental Research Program
South Carolina INBRE

Optimization of Culture Conditions for the Simultaneous Recellularization of Porcine Internal Thoracic Artery Scaffolds with Multiple Cell Types

Holdyn Ferguson (2021) **Mentor: Dr. Matthew M. Stern**

Approximately one out of every three deaths is linked to heart disease worldwide. Because of the increasing prevalence of ischemic diseases, there is growing need for heart bypass surgeries. There are also approximately 200,000 heart bypass surgeries performed annually. The vascular grafts currently used in bypass surgeries have limitations and can fail or become occluded. The ultimate goal of our research program is to tissue engineer vascular grafts from scaffolds derived from decellularized porcine internal thoracic arteries that can be recellularized with patient specific cells and restore function more effectively than current methods. The ability to recellularize scaffolds with multiple cell types, including endothelial cells (EC), smooth muscle cells (SMC), and possibly mesenchymal stem cells (such as adipose-derived stem cells or ADSCs) is important for imparting function to an engineered vessel. However, an important question that arises in such work is what type of cell culture media should be used to allow the different cell types to grow together during the scaffold seeding/culture process? We hypothesized that mixtures of two media types that support growth of two of the cell types of interest could be identified. To test our hypothesis, we grew ECs in different combinations of 1) EC and SMC medium and 2) EC and ADSC medium. We also grew ADSCs in different combinations of EC and ADSC medium. We used the alamarBlue assay to monitor the viability of the cells over 72 hours of culture. We also used flow cytometry to assess the expression of CD31 on ECs cultured in different media combinations. In all cases, the growth of the cell type of interest was no different in a 50:50 combination of its medium and the other medium than growth in 100% of its own medium. In addition, CD31 expression was maintained in ECs under all experimental conditions tested. These results suggest that the use of 50:50 mixtures of culture medium will support the growth of the cell types of interest following their simultaneous seeding into our vascular scaffolds. Our future work will test this directly.

South Carolina EPSCoR/IDeA Developmental Research Program
South Carolina INBRE

Locating *mus305* in *Drosophila melanogaster* using Deficiency Mapping

Tyrice Ferguson (2020) **Mentor: Dr. Kathryn Kohl**

Mutagens are agents that cause DNA damage including single and double-strand breaks. However, there are multiple cellular response pathways that can be used to repair these breaks. The unmapped gene *mus305* is suspected to be involved in DNA repair in *Drosophila melanogaster*. Therefore, my objective was to narrow down the location of *mus305* on the third chromosome from a starting region of ~777kb containing 216 genes. To do that, I used deficiency (deletion) mapping, which determines if a chromosomal deletion overlaps a mutation of interest. If the deletion and the mutation overlap, the mutated phenotype – in this case mutagen-sensitivity – will be uncovered. In this experiment, *mus305 / Sb* females were crossed to *Df / Sb* males in a mutagen-sensitivity assay to locate *mus305*. Visible phenotypes allowed us to count how many flies survived to adulthood in four possible offspring groups in two separate broods – a control brood treated with water and one treated with a DNA-damaging agent. By scoring 5,951 flies within all deficiencies, I have concluded that one of the three deficiencies showed mutagen-sensitivity and therefore uncovers *mus305*. Therefore, we have narrowed the location of *mus305* to ~96 kb and 16 genes (~12% of the starting region). These results provide the foundation for future work to choose a candidate gene within this narrowed region for DNA sequencing.

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

Ramsey and Star-Critical Ramsey Numbers Involving Generalized Fans

Paul Hazelton (2021), **Mentor: Arran Hamm**
Suzanna Thompson (2022)

Ramsey Theory is one of the oldest and most well-studied branches of Combinatorics in which one seeks to find "order amongst chaos". The Fundamental Theorem of Ramsey Theory (for graphs) states that for any two fixed graphs G and H, any large enough red/blue edge-colored complete graph contains a red copy of G or a blue copy of H. The Ramsey number for G and H, then, is the smallest number of vertices of the complete graph where this "unavoidability" property first occurs. Recently, a variant called star-critical Ramsey number was introduced which is a slightly sharper measure on when the unavoidability property first occurs. More precisely, if k is the Ramsey number of G and H, then take Γ_m to be the complete graph on k-1 vertices along with an additional vertex of degree m; the star-critical Ramsey number of G and H is the smallest m for which any red/blue edge-coloring of Γ_m contains a red copy of G or a blue copy of H.

The primary graph of interest for this research is the generalized fan which is a graph formed by taking some number of disjoint copies of a fixed graph H and joining each to a single vertex. Recently, there has been an increased interest in Ramsey and star-critical Ramsey numbers involving this kind of graph which motivated our work. In particular, we computed a few previously unknown Ramsey and star-critical Ramsey numbers involving generalized fans. Specifically, we computed both parameters of a generalized fan versus a complete graph when the fan "blades" are trees, a generalized fan versus a disjoint union of triangles, and a generalized fan with "triangle" blades versus a complete graph on four vertices.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 19) from the National Institutes of Health.

Developing a Multicomponent Three-Dimensional Culture Model of Esophageal Cancer

Connor Hogan (2020), **Mentor: Dr. Matthew M. Stern**
Madeline Linker (2022)

Esophageal cancer is an uncommon form of cancer, making up just 1% of cancer diagnoses in the United States. While rare, a diagnosis of esophageal cancer carries a poor prognosis with only 45% of patients surviving five years. One of the ways to improve cancer treatment is to improve the experimental models used to study cancer and test different treatment strategies. Despite a recent trend towards the use of three-dimensional culture models in cancer research, few such options exist for esophageal cancer. We hypothesized that a three-dimensional multi-component tissue model could be created utilizing commercially available advanced cell culture platforms. We employed 1) cell sheet and 2) RAFT technology to this end. Microscopic observation of cell cultures and alamarBlue viability assays revealed that cell sheet technology was inefficient for layering the cell types tested. In contrast, RAFT technology allowed for the rapid generation of three-dimensional collagen gels embedded with multiple cell types. Fluorescent staining for cytoskeletal actin and cellular nuclei allowed us to visualize the distribution of cells in RAFT cultures. The results demonstrate that the RAFT culture system is promising as a platform for future model development and refinement. Future directions include optimizing the construction of the RAFT gels and investigating the utility of cell-sheet technology for layering esophageal epithelial cells on top of RAFT gels to generate a high-throughput composite model of esophageal tissue. Esophageal cancer tumoroids can then be introduced to the system to model esophageal cancer.

Targeting Cancer Cells Through Modifications of a Known Sphingosine Kinase Inhibitor

Melody Iacino (2020) **Mentor: T. Christian Grattan**

As the cases of cancer continue to rise worldwide, the need for more research in this area has become increasingly important. Of the current treatment types, targeted therapy has shown promising results as it is able to selectively prevent cancer cell proliferation by promoting apoptotic activity of the mutated cells. Responsible for the pro-apoptotic and anti-apoptotic effects on these cancer cells are the metabolites of the sphingolipid metabolic pathway, ceramide and sphingosine-1-phosphate (S1P), respectively. Sphingosine kinase 1 (SK1) is currently responsible for sustaining the biological responses of these metabolites. With targeted inhibition of this enzyme, however, the conversion of SK1 to its anti-apoptotic metabolite, S1P, can be prevented by instead promoting an increase in the ceramide metabolite levels that are responsible for apoptosis. A recently identified SK1 inhibitor has shown successful inhibition results *in vitro*, though it has continued to fail in the environment of the body due to its lack of oral bioavailability. In order to increase the rate of success of the known SK1 inhibitor in an *in vivo* environment, select modifications to the structure must be made.

Synthetic modifications were made to the known SK1 inhibitor using a 3-step process by which a β-enol was converted to a pyrazole hydrazide intermediate and then to the final modified inhibitor. Calculations of the Log P values were performed for each of the modified variants. An analysis of these calculations then allowed for optimization and comparison to the template molecule in order to improve the oral bioavailability of the synthetic SK1 inhibitors. Some of the completed variants were sent in for testing at Select Screen Services (Thermo Fisher Scientific) and put through a preliminary screening. In the future, the remainder of the synthesized variants will be evaluated to assess the success rate of each of the inhibitors interacting with SK1 directly. From there, further modifications will be made to the zones of the template molecule in order to optimize the inhibitor's ability to be used as a potential therapeutic aid in the diagnosis of cancer.

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.

Investigation of an Indirect Defense Mechanism of *Chapmannia floridana* across Florida Scrub Habitats

Mackenzie Jenkins (2021) **Mentor: Dr. Jennifer Schafer**

While many plants have direct defenses, such as thorns or toxic chemicals, that increase their survival and reproduction, recent studies have shown that some plants have indirect defenses. Specifically, these plants have glandular trichomes (sticky hairs) that entrap carrion (dead insects), which attract predators who consume the carrion. These predators then deter herbivores that would harm the plant, leading to less damage to reproductive structures. *Chapmannia floridana* (Florida Alicia) is a perennial plant that is endemic to Florida. Flowering stems of *C. floridana* are covered with sticky hairs. We assessed 84 flowering *C. floridana* individuals across six habitats (southern ridge sandhill, Florida rosemary scrub, scrubby flatwoods, degraded scrub, firelanes, and pastures) to investigate if the sticky hairs act as an indirect defense. We measured the total flowering stem height and the height where trichomes start on the stem, counted the number of fruits, flowers, and buds present, and documented any damage to them. We also counted the number of herbivores, predators, and carrion (per centimeter of the trichome-covered portion of the stem) on *C. floridana* flowering stems. We found that flowering stems were taller in scrubby flatwoods than in pasture and degraded scrub; it is possible that the dense vegetation in scrubby flatwoods requires that *C. floridana* stems grow taller for pollinators to find them. Among habitats, there was no difference in the length of the trichome-covered portion of flowering stems or the number of trichomes at different intervals on the stems; overall, trichome number increased from the bottom to the top of flowering stems. We found a positive correlation between the length of the trichome-covered portion of *C. floridana* flowering stems and the number of carrion trapped, indicating that plants with more trichomes are more likely to trap carrion, potentially making the trichomes more likely to be acting as an indirect defense. Only 10% of fruits, 15% of flowers, and 19% of flower buds we counted on flowering stems had damage. We found predators (i.e., spiders) on 6% of flowering stems and herbivores (e.g., grasshoppers, leafhoppers, caterpillars) on 37% of flowering stems. Overall, our results suggest that herbivory of *C. floridana* reproductive structures is relatively low in Florida scrub habitats and glandular trichomes on *C. floridana* may be acting as an indirect defense.

This project was funded by a Winthrop University Research Council Grant.

Isolating, Purifying, and Investigating Mycobacterial Lysogens

Allyssa Lewis (2021) **Mentor: Victoria Frost**

Bacteria have shared an entangled evolutionary history with bacteriophages (viruses that specifically infect bacteria) for the past three billion years. Some bacteriophages use a type of infectious pathway that helps maintain their host's viability and hence enable a mechanism of coexistence. To investigate this further, two temperate mycobacteriophages (ExplosioNervosa and Rhynn) were selected. Both these phages are able to form lysogens and exist in the host cell's genome indefinitely as a prophage. Annotation of their genomes revealed the presence of immunity related genes. These particular genes potentially explain how some bacteriophages are able to protect their host and resist superinfection by other related and non-related bacteriophages. Bacterial lysogens were created by incubating bacterial host cells with the phages. Any resulting mesas were a sign that host cell growth had taken place in the presence of a prophage, so provided a sample of lysogenized bacteria. The lysogens were purified and tested against their original infecting phage as well as an unrelated bacteriophage (Haimas) to see if they were able to resist superinfection. Tests showed that both Haimas and the original viruses were still able to infect the lysogens and cause them to lyse. This raised the idea of spontaneous reversion; the prophages could have reverted to the lytic cycle due to a triggering condition in their environment. The ability of the host-phage relationship to respond to certain environmental signals warrants further investigation, as does manipulation of the genes linked with immunity and infection. Unraveling the triggers and mechanisms that fuel coevolution help further our understanding of the host-parasite equilibrium that exists today and highlights opportunities for future applications.

SC INBRE funding, HHMI sponsorship

Optimization of Smooth Muscle Cell Culture for Blood Vessel Tissue Engineering

Nicholle Lewis (2020) **Mentor: Dr. Matthew M. Stern**

Over 1.5 million heart attacks occur annually in the U.S. alone. Vascular bypass surgery is a viable treatment option; however, grafts used in small-diameter bypass surgeries suffer from limitations including decreased patency due to atherosclerotic plaque development, thus creating a need for reoperation after the initial surgery. In order to alleviate these issues, additional optimization is needed. One potential solution is the use of tissue engineered vascular conduits constructed using patient-specific smooth muscle cells (SMC) and endothelial cells (EC). Both cells types are necessary to create functioning blood vessels. This project tested the effects of 1) uncoated vs. collagen coated culture dishes, 2) different SMC media formulations, and 3) different mixtures of both SMC and EC media to identify optimal culture conditions for human aortic smooth muscle cells prior to and during recellularization onto biomaterial scaffolds. We hypothesized that 1) collagen would significantly enhance SMC proliferation based on its abundance in the extracellular matrix, 2) both SMC media formulations would support robust SMC proliferation, and 3) mixtures of SMC and EC media with higher concentrations of SMC media would increase relative levels of SMC proliferation. It is important to note that a parallel project was conducted using ECs cultured in identical mixtures of SMC and EC media to determine optimal conditions for simultaneous seeding with both cell types. Cellular proliferation was monitored using a resazurin reduction assay and quantified using a fluorescence plate reader. Our results show that 1) collagen was not a significant enhancer of proliferation, 2) both types of SMC media tested promoted SMC proliferation, and 3) mixtures of SMC and EC media varied in their effect on SMC proliferation, but a 50:50 mixture showed no negative effect on SMC proliferation after 72 hours. These results indicate that SMCs grow successfully under several conditions, thus providing more leeway for determining simultaneous seeding conditions. Understanding the factors needed to grow SMCs provides valuable information for culturing patient-specific cells for the recellularization and implantation of an "off-the-shelf" vascular conduit for bypass surgery.

South Carolina EPSCoR/IDeA Developmental Research Program
Winthrop University McNair Scholars Program

Multi-Step Deposition of P-type Metal Chalcogenides as Components of Water Splitting Tandems

Blake McCloskey (2020) **Mentor: Clifton Harris**

Thin films of doped, p-type metal chalcogenides have been cast on transparent conductive oxide substrates by a multi-step deposition method. The films have controllable thicknesses, strong adhesion and good uniformity. Photoelectrochemical analysis confirms p-type behavior and reveals photosensitivity under moderate bias. Under suitable conditions and in the presence of proper co-catalysts, these films show promise as components of tandem solar cells for applications to photoelectrochemical water splitting.

This project was supported by EPSCoR.

Developing Microfluidic Devices for Assisted Reproductive Technologies

Darien K. Nguyen (2020) **Mentors: Amir Mokhtare, Dr. Alireza Abbaspourrad**

The gaining popularity of Assisted Reproductive Technologies (ART) such as In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI) calls for the introduction of more affordable and less tedious processes rather than the typical manual operations. In order for ICSI to occur, the Cumulus Oocyte Complexes (COCs) retrieved from the ovaries must be processed in order to remove the tightly-packed cumulus cells surrounding them. As of yet, this tedious and unstandardized process is being done manually by skilled embryologists, which result in variability and unavailability. The focus of this project is to develop microfluidic devices to denude the COCs for ICSI in order to reduce the tyranny of manual operations and push towards automated reproducible operations. These microfluidic devices are fabricated through conventional PDMS microfluidic processes and tested using automated magnetic pumps controlled by a microcontroller. Currently, actual microfluidic devices were developed and were successfully tested using particles similar to COCs.

This project was support in part by the NSF grant no. ECCS-1542081

Cloning and Purification of Human Cardiac Troponin C Mutants

Sophie Nguyen (2021) **Mentor: Nicholas Grossoehme**

There are many protein complexes with several domains involve in the muscle contraction activity – actin site, which is the main compound that connects muscle fibers; tropomyosin, which blocks the binding site when Calcium is not yet added and Troponin. This protein consists of three subunits: Troponin I, troponin T and troponin C, which binds to Calcium via its EF-hands domains. According to the past studies, there are three binding sites in which two of them link to Calcium at all time, but the additional event only happens when Calcium is added. In this study, the aim is to develop an experimental system where scientists can look at an individual binding event by introducing the TEV protease cleavage site to separate the two domains and mutate each EF-hands to study the binding process independently. By DNA cloning and transformation, the sties would be isolated; thus, the study would be done. However, the PCR reactions of the templates did not give the right sequence outcome. Lastly, a particular protein purification protocol including sodium deoxycholate lysis buffer and Ammonium Sulfate with the Phenyl Sepharose Sequence column to get the purest Troponin; yet it also failed. Therefore, with the limited amount of time and unpleasant result, no conclusion was made for the binding event of Calcium at the N-terminal domain.

Funding: SC INBRE (NIH 8 P20 GM103499)

Silver Nanoparticle Biosynthesis and Calcite Biomineralization as a Precursor to Hydroxyapatite

Cayla Odom (2020) **Mentor: Maria Gelabert**

Antimicrobial hydroxyapatite (HA) offers preventive measure against surgical implant infection. Embedded silver nanoparticles (AgNP) represent one modification, but reliable synthetic strategies are relatively lacking. This study proposes a novel pathway to synthesize AgNP-doped HA using the fungus *Fusarium oxysporum*. This effort combines AgNP biosynthesis and calcite biomineralization for hydrothermal phosphatization to HA. Calcite biomineralization can be induced by urease positive fungi which catalyzes the hydrolysis of urea into ammonium and carbonate ions. These carbonate ions can bind to introduced calcium metal ions at the surface of the fungal hyphae to form calcite. Fungal quinones allow for the reduction of toxic silver ions in the environment to silver nanoparticles. Combining these two processes will result in silver nanoparticle doped calcite. Through the final step of this process, phosphatization, this product can be hydrothermally converted to hydroxyapatite that has retained the silver nanoparticles. X-ray diffraction and scanning electron microscopy enables confirmation of product and examination of AgNP-HA microstructure, where AgNP are expected to attach to the HA crystallite surfaces. The silver particles produced were not in the nanoscale dimensions, but energy dispersive spectroscopy confirmed successful binding of the silver particles to calcite in half of the test samples. Hydrothermal phosphatization to HA was unsuccessful in all samples. However, the fungal extraction technique used did not allow for a representative diffraction pattern for the samples that contained the largest amount of biomass. Addition extraction techniques will be explored in order to successfully separate the desired product from the fungal surface. Further effort will go into finding the optimum ratio of media to calcium chloride so that optimum fungal growth and calcite formation can be achieved. The second step of the pathway, the addition of calcium nitrate, will be eliminated and another phosphate source will be considered for phosphatization. This work investigates synthetic and analytical steps for production of safe and reliable implant materials.

Support for this research was provided by the Winthrop University McNair Scholars Program and MADE in SC, SC-EPSCoR NSF #1655740

Expression, Purification, and Characterization of XopAZ, a Putative Foldase from *Xanthomonas cynarae*

Alyssa Petty (2021) **Mentor: Jason C. Hurlbert**

Xanthomonas cynarae is a phytopathogenic bacterial species that infects artichokes and causes the formation of water-soaked spots, and eventually necrosis, on the plant. This reaction to bacterial invasion is caused by a hypersensitive response. This response is elicited by the interaction of bacterial avirulence proteins (Avr proteins) with a plant's resistance proteins (R proteins) upon entry of Avr proteins into the plant. Avirulence proteins enter a plant's cytosol by way of a Type III Secretion System (T3SS), a modified flagellum (needle-like) through which unfolded bacterial Avr proteins can travel. Upon emerging from the T3SS into the plant, Avr proteins are returned to their active, refolded state. The interaction of injected Avr proteins with the plant's R proteins results in localized cell death of plant cells (hypersensitive response) as the plant attempts to ward off bacterial infection. Recently, a protein from *X. cynarae* called XopAZ has been identified that, based on sequence similarity to various peptidyl prolyl isomerases and Sensitive to lysis chaperonins, plays a role in Avr protein refolding in the *X. cynarae* invasion process. In this study, XopAZ was expressed via a prokaryotic expression system. The protein was then purified via Ni2+ chelating affinity and gel filtration chromatographic methods, sequentially. Using the purified protein, we have begun initial assays of the ability of XopAZ to prevent aggregation of lysozyme following the dilution of denatured lysozyme into a refolding buffer.

Funding: MADE in SC, SC-EPSCoR NSF #1655740

Diet analysis of juvenile dragonflies using group-specific polymerase chain reaction

Whitney Player (2021), **Mentor: Dr. Cynthia Tant**
Rachael Rowe (2021)

Aquatic food webs are complex and understanding interactions in these food webs can give an indication of ecosystem health and stability as well as movement of energy and nutrients through ecosystems. Previous studies have utilized both microscopic gut content analysis and stable isotopes to aid in constructing food webs in these ecosystems. However, gut content analysis is time consuming, stable isotope analysis can be cost prohibitive, and both methods only identify general categories of food items. The application of newer molecular-based approaches has the potential to provide previously unavailable taxonomic resolution in aquatic food webs (i.e. who is eating whom?). DNA-based methods have been used in other disciplines for diet analyses but have not been widely applied in freshwater ecology. Juvenile dragonflies (Odonata, Anisoptera) were collected in Winthrop Lake in Rock Hill, SC, and gut contents of individuals from three genera were dissected and the DNA extracted. DNA was amplified via PCR using group specific primers for midges (Chironomidae) and mosquitoes (Culicidae) to identify potential prey in gut contents, and gel electrophoresis was used as a presence-absence test for DNA from these prey groups. Occurrence of prey groups in gut contents appeared to vary by individual and by genus of dragonfly examined. With further refinement, these methods have the potential to provide previously unavailable detail on predator-prey interactions in these ecosystems.

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

Investigating the Function of a Novel LPAR-2 Related Gene

Zachary Revert (2020) **Mentor: Dr. Eric Birgbauer**

The human nervous system is a very complex collection of nerves and specialized cells called neurons. The main purpose of the nervous system and these neurons is to send signals across the body from the brain and to the brain from parts of the body. In order for these signals to work, the axon of the neuron must be guided to the right destination of the body. One hypothesized component of this axon guidance is LPA (Lysophosphatidic Acid) and LPA Receptors. These receptors are found in tissues that are present in developing nervous systems while the LPA is in the matrix around the tissue. CHEST973j21 (CHEST) is a possible new LPA Receptor that is the primary interest in this research. It shares a very similar DNA Sequence to LPA Receptor 2 (LPAR 2), which makes us believe that it could possibly be a new LPA Receptor. We cloned CHEST and LPAR 2 into plasmids that would express a Red Fluorescence Protein (RFP) on either the N or the C terminus and we transfected Human Embryonic Kidney Cells with this plasmid. A successful transfection was determined by visualizing the RFP under a fluorescence microscope. We examined the expression of CHEST and LPAR2 by a Western Blot to determine if CHEST and LPAR2 fusion protein with the RFP was present. However, only the RFP tag was detected, suggesting a proper fusion protein was not made.

This project was supported by SC INBRE grants from the National Institutes of Health.

Altering "Zone 2" Structure of a Sphingosine Kinase Inhibitor

Miquela Santoro (2020) **Mentor: T. Christian Grattan**

Cancer is a disease that affects almost all people, if not directly, indirectly. In the near future, cancer cases are projected to increase from 17 million to 21 million by the year 2030. Current popular treatments include: surgery, chemotherapy, and radiation. The method of our research is targeted therapy; this allows the drug to target the cancer cells, and not alter any other normal functioning pathways in the body. The pathway this research focused on was the sphingolipid metabolic pathway; in this pathway, sphingomyelin turns to ceramide, then to sphingosine, and finally sphingosine-1-phosphate via sphingosine kinase. If the intracellular concentration of ceramide increases, it will promote apoptosis, but if the concentration of sphingosine-1-phosphate increases, proliferation of cancerous cells increases as well. So this research focuses on the enzyme that controls the regulation of these two metabolites, sphingosine kinase-1, SK1. Cancer will overexpress this enzyme to cause the concentration of sphingosine-1-phosphate to increase, which leads to the cells proliferating, the cancer to grow, and eventually be able to feed itself via angiogenesis. Our goal was to stop this enzyme from binding, which pushes the equilibrium back up to promote apoptosis. The template drug we worked off was 99% successful *in vitro*, but showed no success *in vivo*; we split this drug into 4 zones and modified them. Zone 2 was the focus on this research. The overall goal was to assess how changes in this heterocyclic ring would change how the drug interacts with the target enzyme. A three-step synthesis process was followed to produce all inhibitor compounds. The first reaction was a Suzuki reaction followed by hydrazine hydrate to form the hydrazide functional group and finally 2-hydroxynaphthaldehyde was added to form each inhibitor derivative. ^{1}HNMR was used after every reaction to confirm the formation of the desired product. Overall, 5 new derivatives of zone 2 were synthesized. Future work includes combining the most effective derivatives from all four zones to essentially create one super drug that is very effective against the target enzyme, SK1, *in vivo*.

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.

$Ni_3(HAB)_2$ MOF high performance supercapacitors fabricated by electrophoretic deposition

Isabella Schepisi (2022),
Sean Wechsler (2020)

Mentor: Dr. Fatima Amir

Supercapacitors are recognized to be one of the most efficient and reliable technologies for energy storage because of their high power density, long cycle-life, and fast charge-discharge. However, the utilization of supercapacitors does have challenges with a major one being low energy density, limiting their applications where high energy is needed. To increase supercapacitor energy density, one approach is to develop new electrodes materials. Metal organic frameworks (MOFs) have emerged as new kind of materials for supercapacitors' electrodes with a remarkable surface area and tunable pore size.

In this work, we report the fabrication of the MOF nickel hexaaminobenzene ($Ni_3(HAB)_2$) supercapacitors' electrodes using electrophoretic deposition. The morphology of $Ni_3(HAB)_2$ electrodes was characterized using field-emission scanning electron microscopy and transmission electron microscopy. The electrochemical performance of the $Ni_3(HAB)_2$ supercapacitors were analyzed using cyclic voltammetry, electrochemical impedance spectroscopy, and galvanostatic charge-discharge tests. The supercapacitor displayed an outstanding electrochemical capacitive performance in Na_2SO_4 electrolyte with an areal specific capacitance of 25.64 $mFcm^{-2}$. These results pave a way for a successful future for this new variety of functional materials for energy storage technologies.

This project was support by the NSF-EPSCoR Award #OIA-1655740

Thermodynamic-based Discovery of New K-La-Zr-O Compounds via Hydrothermal Synthetic Methods

Thomas Sullivan (2021) **Mentor: Maria Gelabert**

This project investigates aqueous modeling coupled with mild hydrothermal methods (≈ 200 °C, 16 atm.) for discovery of new compounds, one specific goal related to advanced materials development outlined in *SC Vision 2025* and NSF *Big Ideas*. Innovative luminescent materials, such as visible-light-emitting scintillators, are needed to improve properties of opto-electronics and other optical material technologies. Exploratory hydrothermal methods were performed, with thermodynamic guidance from aqueous speciation calculations in OLI Studio, in an attempt to synthesize compounds of the K-La-Zr-O quaternary system. This system choice was inspired by several compounds of Na-Y-Si-O and related systems previously synthesized by supercritical hydrothermal methods. By altering the composition of reactants, it is possible to generate trace amounts of crystals that have either never before been synthesized or only been hydrothermally synthesized at more extreme temperatures and pressures. In the discovery of $Zn_2EDTA.2H_2O$, the optimum hydrothermal conditions were just outside of the thermodynamic stability region for ZnO, suggesting that other new compounds are likely to be discovered on the edges of such stability regions. Using OLI Studio Stream Analyzer aqueous speciation software, yield diagrams were constructed for the K-La-Zr-O system, with water-soluble metal salts, chelating agent, and base as reactants to aim towards discovery of new rare-earth optical compounds. Chemical systems such as this one will readily form thermodynamically stable binary/ternary compounds – in this case, zirconia (ZrO_2) and lanthanum hydroxide ($La(OH)_3$). Within yield diagrams of the Zr and La subsystems, where the concentration ratio of metals is plotted against base concentration, locations just outside of the ZrO_2 stability region were targeted for Zr:La ratios of 1:1 and 4:1. Use of scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) revealed polycrystalline morphology with some single crystals (≈ 50 microns) of hexagonal and greater (6+ sides) geometry containing significant amounts of carbon, oxygen, lanthanum, and zirconium, leading us to tentatively conclude that these crystals are a complex lanthanum zirconate compound.

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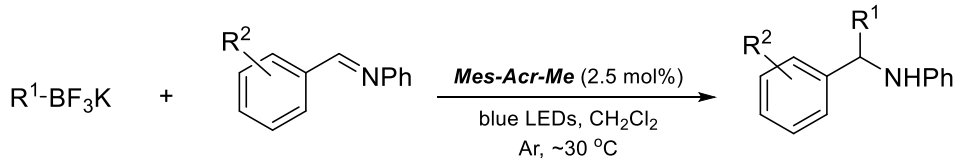
Photoredox-Mediated Alkylation of Imines with Potassium Organotrifluoroborates in the Presence of an Organic Photocatalyst

Evan Thibodeaux (2021) **Mentor: James M. Hanna Jr.**

Recently, the use of visible light combined with a suitable photocatalyst to promote key bond-forming steps in organic synthesis has emerged as a viable strategy to achieve a number of important synthetic transformations. The photocatalyst involved is often a ruthenium or iridium polypyridyl complex, which absorbs light in the visible range to give a relatively long-lived excited state, which may engage organic substrates in a series of single-electron-transfer (SET) events. The organic radicals thus generated participate in downstream reactions leading to the final product(s). Our group has previously employed this strategy for the alkylation of aldimines with potassium organotrifluoroborates using transition-metal photocatalysts. However, because of the much lower cost of organic photocatalysts (~$50/mmol for acridinium-based catalysts vs ~$1000/mmol for Ir-based catalysts), we desired to explore the use of organic photocatalysts in this transformation. Optimization studies using the reaction of potassium isopropyltrifluoroborate (**1**, R^1 = ^{i}Pr) with benzalaniline (**2**, R^2 = H) revealed that the photocatalyst 9-mesityl-10-methylacridinium tetrafluoroborate (***Mes-Acr-Me***) in dichloromethane gave the best yields of alkylation product **3**. A variety of imines and potassium organotrifluoroborates have been found to react according to this protocol, affording moderate to good yields of α-arylamines (**3**).



1 **2** **3**

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Host Range Investigations of Novel Bacteriophages Isolated from the North Catawba River Region

Bethany Wise (2022) **Mentor: Victoria Frost**

Bacteriophages are viruses that infect bacterial cells, using them as a host to express their genetic material and replicate new phage particles. Since phages have the ability to lyse a bacterial cell, it allows the phage to travel to a new host and repeat the process. Some bacteriophages use a specific strain of bacterial host for this procedure, while others have a wider host range, which could be advantageous to the phage under certain conditions. This study aims to help understand this particular area of phage-bacteria interaction by investigating the ability of novel bacteriophages isolated from a specific host to infect and replicate in alternative hosts. To investigate host range, bacteriophages originally isolated from *Microbacterium foliorum* were tested for their ability to infect and lyse *Microbacterium testaceum, Microbacterium paraoxydans, Microbacterium liquefaciens and Mycobacteria smegmatis.* Spot titer assays showed signs of plaque formation on alternative microbacterial hosts by two of the 16 phages (MonChoix and Sirkeiram). Three of the phages (Aries55, BravoCanis and Iann) were able to infect *Mycobacteria smegmatis*. For the next step, two novel phages (Ixel and Nebulous) were isolated from bacterial host *M. liquefaciens*. Using *M. liquefaciens* as the host, the infective ability of Ixel and Nebulous was compared to the infective ability of the *M. foliorum* phages MonChoix and Sirkeiram. The phenotypic measure of infectivity is termed Efficiency of Plating (EOP) and for both the *M. foliorum* isolated phages, the EOP was less (<1) when compared to the host isolated phages. It is likely that the expression of particular genes in the genomes of both phage and bacterial host is able to influence this phenomenon. A number of the phage genomes have been, or are in the process of being, annotated. Comparative analysis of the genomes, and further testing phenotypically, will help elucidate whether specific genes are present that function to enable phage to use a wide host range. As knowledge of the genes and processes involved in the phage-host relationship increases, so do the possible medical and commercial applications of this information.

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Species Description of Schizo 2 Belt

Charlie Wolfe (2020) **Mentor: Dr. Julian Smith III**

Along the North Carolina coast, approximately thirty new species of Kalyptorhynchia have been discovered, however, only two of these have been described, which creates gaps in understanding the origin of certain species. One species that has been described here is *Schizorhynchus lupus*. Formerly known as Schizo 2 belt, his species was isolated and described by looking at their organ structures using light and confocal microscopy. The first being *Schizornychius lupus,* a flatworm species with a finger-like probothesis and a cone shaped stylet that makes it a unique species. Inclusion of a species-name here does not make it formally available for taxonomic purposes.

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