Winthrop University
Summer Undergraduate Research Experience (SURE)
2018 Abstract Book

WINTHROP
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The Winthrop University Summer Undergraduate Research Experience (SURE) is a coordinated effort involving the Departments of Biology, Mathematics, and Chemistry, Physics, and Geology, in which undergraduate students pursue eight to ten weeks of research with faculty mentors. In 2018, the Winthrop SURE Program celebrated its thirteenth year, with a cohort of over 50 students working with more than 20 faculty mentors, examining important questions in biology, chemistry, biochemistry, mathematics, 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. Meir Barak, who worked diligently to assemble, edit, and publish this abstract book.

We also gratefully acknowledge Winthrop's administration, especially President Dan Mahony and Provost Debra Boyd, 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!

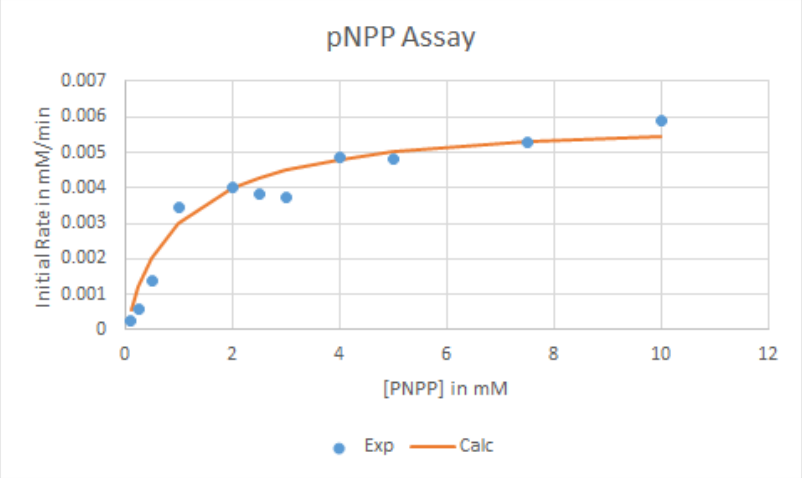
Diana Boyer
SURE Program Coordinator

Robin Lammi
Director of Undergraduate Research

Characterization of the Metal-Dependency of a Streptococcal Phosphatase

Kiera Alexander (2021) **Mentor: Dr. Nicholas Grossoehme**

Protein phosphatase (PhpP) is a metal dependent phosphatase that relies on metals to function and cause dephosphorylation. Specifically, in *Streptococcus pneumonia*, there are three functional proteins within the system: the regulator of iron transport (Rit-R), serine-threonine kinase (StkP), and protein phosphatase (PhpP). In the system, StkP receives an extracellular signal and causes ATP to be converted to ADP allowing for Rit-R to be phosphorylated. PhpP then prevents the creation of iron machinery by dephosphorylating Rit-R. Iron is necessary for the bacteria to be viable, but under high concentrations it is capable of causing oxidative stress through reactive oxygen intermediates (ROIs) and the production of hydroxyl radicals; these radicals are capable of attacking macromolecules within the cell and promoting tissue injury along with cell death. Since iron plays such a critical role in the bacteria, the purpose of this study is to determine whether protein phosphatase is the metal sensor in *Streptococcus pneumoniae* bacteria. The initial steps in this study involved obtaining a reasonable and functional amount of PhpP to conduct kinetic assays with. This process includes growth, harvesting, lysis, and purification through chromatography. We then conducted kinetic assays using para-Nitrophenylphosphate (PNPP) and Manganese as surrogates for Rit-R and iron. With this we were able to assess the rates at which the substrate binds to the enzyme and the number of times each enzyme converts substrate to product, known as the turnover number or the k_{cat}. For Manganese we obtained an average k_{cat} value of 6.241 min^{-1}. This further allowed us to conduct other PNPP assays using iron under anaerobic conditions which gave us a k_{cat} value of 12.38 min^{-1} . With these two data sets we were able to compare the kinetic values and determine how much more efficient iron was at activating the enzyme in the experiment. With these findings the lab anticipates creating more reproducible data, determining how tightly the substrate binds to the enzyme, and analyzing the affinity for iron under physiological conditions.



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 and Antimicrobial Activity of Pyrazole Rings with Varying Substitution

Resa Allen (2021) **Mentor: Dr. Christian T. Grattan**

Antimicrobial drugs are widely known for being able to cure infections caused by microbes. An antimicrobial drug's main method of attack is to break up the synthesis of structural elements in a microbial infection, as well as modifying metabolic functions unique to the cells. The problem is antimicrobial resistance, which is present in every country around the world. Antimicrobial resistance is when microorganisms slowly build up a defense to drugs that were previously effective. When this happens, it increases the cost of healthcare because it takes more time and effort to treat the disease. Defense against antimicrobial drugs also means higher risk medical procedures. My research involved the synthesis of pyrazole rings, commonly found in the structure of some antimicrobial drugs, with variation in the placement of CF_3 and naphthyl groups on the ring and then analyze the rings for inhibition from the synthesized compounds to tell how effective it is. Specifically, I found that 3-(2-naphthyl)pyrazole and 3,5-bis(trifluoromethyl)pyrazole had promising zones of inhibition.

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.

New Species of Platyhelminthes from the Beaches of North Carolina

Mikhail Anfinson (2020) **Mentor: Dr. Julian P.S. Smith III**
Charlie Wolfe (2020)

The Platyhelminth fauna of the North American East coast is under-described. For example, unpublished historical work by the late RM Rieger in the late 1960's and 1970's recorded approximately 10 members of the Order Proseriata and 40 members of the Order Kalyptorhynchia from the NC coast. In the intervening nearly half-decade, fewer than a dozen of these species has been described. Here we provide descriptions of *Antemonocelis andreasi* (Proseriata, Monocelididae) and *Schizorhynchus lupus* (Kalyptorhychia, Schizorhynchidae). *P. andreasi* is interesting because molecular phylogeny (18S/28S) places it as the most primitive species in the family Monocelididae, and its suprataxon (Proseriata) currently occupies an uncertain position in transcriptomic phylogenies, rendering important informed selection of additional Proseriate species from which to obtain transcriptomes. *S. lupus* is interesting because it is only the third known member of the genus *Schizorhynchus*, and appears to have a geographic distribution that extends from New England to North Carolina.

The Authors are grateful to Mr. Joseph Horacek, Winthrop University, for sharing his unpublished 18S and 28S rDNA sequences, to Stefan Gobert, University of Hasselt, for conducting the RaxML analysis of Proseriate species and for sharing his unpublished data on Schizorhynch phylogeny, and to Dr. David Doe, Westfield State University, for providing specimens of S. lupus from Nahant, MA. This project was supported by grant P20GM103499 (SC INBRE) from the National Institute of General Medical Sciences, National Institutes of Health.

Synthesis and Characterization of ZnO Particles in Ethanol-Water Solutions

Tamara Bright (2020) **Mentor: Dr. Maria Gelabert**

This research explores aqueous synthesis of zinc oxide (ZnO) nanoparticles in water, with potential application to water purification applications. Previous work has used a variety of zinc reactants, but the primary focus here was zinc chloride. This exploratory research couples thermodynamic calculations with aqueous synthesis parameters. ZnO particles were synthesized at room temperature in varying water-ethanol solutions with the presence or absence of polyethylene glycol (PEG) capping agent. The addition of PEG was used to inhibit growth and limit particle size. For synthesis, 0.02 M $ZnCl_2$, either in ethanol or water, were added together to prepare varying quantities of ethanol/water solvent. To 25.00 mL of zinc solution, 5.00 mL of 0.50 M NaOH was added slowly over 30 minutes while stirring, then allowed to stir for another 30 minutes before separation and analysis. After centrifuging and decanting with water and ethanol, samples were analyzed by fluorimetry, particle size analysis, and X-ray powder diffraction. X-ray powder diffraction revealed consistent synthesis of zinc oxide, but none of the trials resulted in nanoparticles. Powder data were refined by Rietveld methods, revealing minimum average particles of 15-27 nm. Particle size analysis showed the full distributions, with the smallest sizes of 0.100 microns and typical size ranges of 30-70 microns. The addition of ethanol and PEG did not result in measurable changes in average particle sizes. Individual overlapping peaks of around 397, 450, 470, 480, 500 and 600 nm can partly be attributed to different point defects in zinc oxide that affect surface reactivity for catalytic applications. Broadly, fluorescence measurements for several samples indicated two emission spectra groups with identical peaks: one group with a larger peak around 500 nm and one below 400 nm, and this correlates with pH, where high-pH syntheses at (pH >10) result in lower wavelength (blue) spectral profiles.

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

A Time Delay Model of Immune Response with Drug Resistance in Tumor Cells

John Brotemarkle (2020) **Mentors: Dr. Zach Abernathy**
Dr. Kristen Abernathy

Drug resistance, also known as multidrug resistance (MDR), is the leading cause of chemotherapy failure in treating cancer. This drug resistance in cancer cells can be transferred from resistant cancer cells to sensitive cancer cells. Sensitive cancer cells can become resistant through three main methods: direct cell to cell contact with resistant cancer cells, through a membrane, or through exposure to the treatment drug. In our project, we take into account the transfer of drug resistance from resistant to sensitive cancer cells via direct cell to cell contact. We then introduce an immune response and chemotherapy, and establish conditions on treatment parameters in the resulting system to ensure a globally stable cure state. We provide evidence of a limit cycle and conjecture the existence of a Hopf bifurcation. Furthermore, we consider the effects of a delay on the immune response and numerically demonstrate how such a delay can cause further bifurcations of internal equilibria.

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.

Optimization of the FRESH Method of 3D Bioprinting

Tierra Collins (2019) **Mentor: Dr. Matthew Stern**
Chandler Burt (2020)

Advancements in the technology surrounding 3D printing and 3D bioprinting are increasing in the biomedical research field. These technologies hold great promise for improvement of overall wellness of patients through the manufacturing of new highly customizable bioengineered products. This summer, we introduced freeform reversible embedding of suspended hydrogels, also known as the FRESH method of 3D bioprinting to our lab. The FRESH method relies on a gelatin slurry to temporarily support soft biomaterials that are 3D printed into it until they can be crosslinked into a product capable of holding its three-dimensional shape. We aimed to optimize production of the gelatin slurry to ensure that it would allow precise 3D bioprinting and support the complex structures we would print into it. There were many variables in the two-day protocol that required optimization. These included the timing of several steps, the temperature during key steps, and the speed of the blender blade. An optimal gelatin slurry can self heal when a printing needle passes through it and is capable of supporting the bio-ink of interest until it can be crosslinked. We produced and used a sodium alginate bio-ink for testing in these experiments, and we qualitatively assessed each batch of gelatin slurry based on the criteria described above. After optimization of the slurry was completed, we built upon our knowledge of the 3D printing software Pronterface to use our newly acquired R3bel Mini bioprinter, which provides our lab with a second bioprinting system. We successfully printed a child's bronchus using sodium alginate bio-ink, which sets the stage for printing more complex structures and using additional bio-inks as we continue refine our methodology. The next step in our research is to consistently print using the FRESH method on both the R3bel Mini and the Allevi 2 bioprinters with multiple bio-inks and cell types.

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On Game Analogues of Brooks' Theorem and Mycielskians

Chris Chamberlin (2020) **Mentor: Dr. Arran Hamm**
Jacob DeCapua (2019)
Hannah Elser (2020)
Dana Gerraputa (2020)

A graph is a collection of vertices and edges. A proper coloring of a graph is an assignment of color to each vertex so that no edge has the same color on both ends. Two major results in the study of graph coloring are Brooks' Theorem and Mycielskians. The former bounds the number of colors required for a proper coloring of a graph by the maximum number of edges at any of its vertices. The latter is a construction one can apply to a graph so that the resulting graph requires one additional color in order to properly color it.

Shifting gears, consider the following game played by Alice and Bob on a graph. The players will take turns (with Alice going first) coloring the vertices from a common color set so that no edge has its two vertices colored the same color (i.e. after each player's turn, the partial coloring is proper). Alice wins if the entire graph is colored and Bob wins otherwise. Thus the question: given a graph G and a color set C, who has a winning strategy (i.e. can win independently of the other player's moves)? This problem was introduced in the 1980's and is a direct generalization of graph coloring. This summer our work focused on obtaining analogues in the game coloring setting of Brooks' Theorem and Mycielskians and we proved several partial results in each direction.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 18) from the National Institutes of Health.

Visible-Light-Induced Alkylation of Aryl Aldimines with Potassium Organotrifluoroborates Enabled by an Organic Photocatalyst

Brittney Ciesa (2019) **Mentor: Dr. James M. Hanna Jr.**
Evan Thibodeaux (2021)

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. Several organic photocatalysts and solvents were screened; the optimum conditions were found to require the photocatalyst 9-mesityl-10-methylacridinium tetrafluoroborate (***Mes-Acr-Me***) in dichloromethane solvent. Thus, a dichloromethane solution of **1** and **2**, when irradiated with blue LEDs in the presence of ***Mes-Acr-Me*** under argon, resulted in good yields of the desired adducts (**3**).

1 **2** **3**

Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund (58270-UR1) for support of this research. Additional support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (8 P20 GM103499).

The Effect of Semaphorin 3A on Chick Embryo Retinal Growth Cones

Fatoumata Nancy Cisse (2019) **Mentor: Dr. Eric Birgbauer**

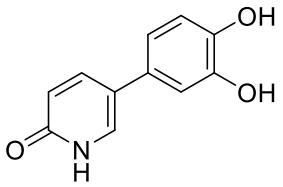
Semaphorin 3A is a crucial axon guidance cue in the nervous system. Axon guidance molecules interact with growth cones, which are the extension of growing or regenerating axons supported by actin extension looking for their synaptic target. Some inhibitory axon guidance molecules are known to cause growth cone collapse and Sema 3A is one of them. When growth cones collapse, they cease to move and then retract. While Sema 3A's importance is known in the nervous system, a study conducted by Luo et al. (1993) demonstrated that Sema 3A causes growth cone collapse on chick embryo dorsal root ganglion cells (DRGs) but not growth cone collapse in chick retinal ganglion cells (RGCs). In Dr. Birgbauer's lab, it was found that Sema 3A does cause growth cone collapse in chick embryo ganglion cells which is inconsistent with the Luo et al. (1993) finding. We are investigating this inconsistency; one hypothesis is that growth factors affect the response of Sema 3A on chick embryo RGCs, as was showed previously with DRG neurons. We are still in the process of testing the effect of Sema 3A in the presence or absence of the growth factors BDNF and CNTF.

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 and Evaluation of (Dihydroxyphenyl)pyridones as Aggregation Inhibitors for Alzheimer's Amyloid-Beta Peptide

Brandy Crenshaw (2019) **Mentors: Dr. Robin K. Lammi**
Mouskudah Murray (2019) **Dr. 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 biphenyltetrols exhibit varying degrees of efficacy as Aβ aggregation inhibitors. 3,3′, 4,4′-biphenyltetrol (3,4-BPT) effectively abrogates Aβ aggregation at stoichiometric concentrations (IC_{50} ~ 1X); other biphenyltetrol isomers were found to be less effective (IC_{50} ~ 2X to >10X), perhaps due to differing abilities to bind to Aβ through hydrogen bonding. 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 pyridone unit into the above-described hydroxybiaryl scaffold may lead to increased inhibition of Aβ aggregation. We therefore synthesized pyridones **1** and **2** (figure) via Suzuki coupling of 3,4-dimethoxybenzeneboronic acid with an appropriate bromomethoxypyridine, followed by demethylation in aqueous HBr. Evaluation of these compounds using a Congo red spectral shift assay gave preliminary IC_{50} values of 3.3±0.3X for **1** and 2.9±0.5X for **2**.



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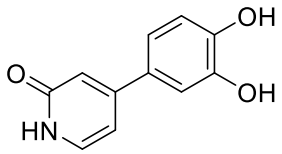

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Support for this project was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (8 P20 GM103499).

Using Deletion Mapping to Locate Mutagen-Sensitivity Gene *mus305* in the *Drosophila melanogaster* Genome

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

All organisms experience DNA damage, and a variety of DNA repair mechanisms exist to combat this damage. The goal of this research was to localize the DNA repair gene *mus305* in the *Drosophila melanogaster* genome. To localize *mus305*, complementation analysis was conducted using three deficiencies while assaying for mutagen-sensitivity. Specifically, two broods were created by crossing virgin female flies containing a known allele of *mus305* to male flies containing one of three deficiencies. Brood 1 offspring were treated with water, which acted as the control, while Brood 2 offspring were treated with MMS, a DNA-damaging agent. Progeny were scored, and then percent relative survival was calculated as the ratio of mutant to control flies in Brood 2, normalized to the same ratio in Brood 1. Relative survival of *Df(3L)BSC443* was 1.22, suggesting that *mus305* is not located in this region. Percent relative survival could not be calculated for *Df(3L)ED223* or *Df(3L)ED4606*, because no $mus305^{D1}$ / *Df* offspring were found in Broods 1 or 2 in either cross. This lethality may indicate that mus305 is an essential gene located within *Df(3L)ED223* and *Df(3L)ED4606*. Future research will test this hypothesis by repeating the deficiency mapping and mutagen-sensitivity assay with a different allele of *mus305*.

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.

Field Study of a Late Devonian Hangenberg Recovery Fauna: Ohio, U.S.A.

Sara Dixson (2020) **Mentor: Dr. Diana Boyer**
Dakota Shope (2020)

The Hangenberg Event was a mass extinction at the end of the Devonian period, marked by a large loss of life recorded in massive expanses of black shale. This extinction is preserved in the Cleveland Formation exposed in Ohio, however, little is known about the cause of this extinction and its recovery. This study presents the first report of fossilized fauna immediately after the Hangenberg Event and compares it to the well-established fauna from the overlying Bedford Formation. We sampled four different creeks around the areas of Cleveland and Columbus, OH in search of fauna that appear after the Hangenberg Event. Using rock hammers, chisels, and hand lenses we carefully extracted pieces of black and grey shale from both the Cleveland and Bedford layers in hopes of finding a diverse and distinct set of fauna. Pyritized recovery fauna within the Cleveland Formation was found at Giant Eagle and Chance Creeks. However, in the same stratigraphic interval, approximately 190.2 km away, the same fauna was absent in this layer. Thus far, we have been able to definitively identify the brachiopods *Lingula, Chonetes,* and *Orbiculoidea* as well as several bivalve species. In total we filled approximately 16 gallon sized bags of shale across the four sampled creeks. The abundance, diversity, and size of fossils preserved of the recovery interval in the Upper Cleveland Formation will be compared to the well documented Bedford Fauna in the lower two meters of the Bedford layer. Our initial results suggest that the recovery fauna is different than the Bedford when considering the initial observation of diversity and abundance.

Funding for this project was received from the Boland Geology Endowment Fund and NSF RUI Grant #1664247

Gut Content Analysis of Aquatic Macroinvertebrates using DNA-based Methods

Evans Dressler (2019) **Mentor: Dr. Cynthia Tant**

With the great deal of complexity associated with aquatic food webs, many questions regarding species interactions remain unanswered. One such question of importance is: Who is eating whom? This is a question that morphological-based analysis techniques have failed to answer with great accuracy. However, with the advent of DNA-based analysis methods, this question can be answered. DNA-based analysis methods allow for greater certainty in taxonomic identification because specific gene regions can be targeted using group-specific primers as a means for DNA detection. This approach was used to analyze dragonfly (Odonata, Anisoptera) gut contents using group-specific primers for midges (Chironomidae) and mosquitoes (Culicidae) as potential prey groups. Specimens were collected in Big Dutchman Creek and Winthrop Lake in Rock Hill, SC. DNA was extracted from gut contents of collected specimens. A polymerase chain reaction was performed on each of the extracted samples in order to amplify DNA concentrations. Gel electrophoresis was used as a presence-absence test for DNA from target prey groups. We found that one individual of the genus *Progomphus* contained DNA from the family Chironomidae, but not Culicidae. A second *Progomphus* individual tested negative for both families, illustrating individual variation in feeding. These refined methods will allow for testing of more *Progomphus* individuals and potential prey groups as well as other aquatic macroinvertebrate predators.

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.

Developing CRISPR/Cas9 Knockout for LPA receptor-4 in Chicken Retinal Ganglion Cells

Garrett Driscoll (2020) **Mentor: Dr. Eric Birgbauer**

Growth cones direct axon pathfinding during neural development by detecting environmental stimuli, known as axon guidance molecules. *In vitro* studies have shown inhibitory axon guidance molecules to cause growth cones to change morphology to what is called a collapsed growth cone. Lysophosphatidic acid (LPA) has been demonstrated to collapse growth cones in culture, and thus it may act as an inhibitory molecule. However, identification of what LPA receptor is responsible for this physiological response is unknown. To investigate which receptor elicits collapse, we focused on LPA receptor-4 (LPAR4) by designing a CRISPR construct to mutate LPAR4 in chick retinal ganglion cells (RGCs). Through retinal injections, we will introduce our CRISPR construct and isolate mutated retinal tissue to observe whether knocking out LPAR4 abolishes RGC growth cone collapse. Preliminary data suggests our injection delivery system can successfully target RGCs. Moreover, we have successfully cloned five guide RNAs (gRNA) that will be transfected into a DF-1 cell-line to analyze the efficiency of LPAR4 mutations before targeting RGCs.

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.

Derivative Design and Synthesis of Sphingosine Kinase 1 Inhibitors to Increase Bioavailability; *In Vitro* Analysis Using Bioassay

Tiffany Dwyer (2020) **Mentors: Dr. Christian T. Grattan**

One of the ways to target cancer therapy is to focus on the pathways in which it exploits the body. This project is focused on the metastasis of cancer through the sphingolipid metabolism pathway. More specifically, this project is focused on the enzyme sphingosine kinase-1 (SK-1). Cells are broken down into ceramide, which is a signaling molecule for apoptosis; they then convert to sphingosine, and through the catalysis of the enzyme sphingosine kinase-1, sphingosine is converted to sphingosine-1-phosphate (S1P), a signaling molecule for proliferation, which is produced by the transfer of a phosphate group from ATP to sphingosine. In cancer patients, overexpression of SK-1 causes metastasis due to increased concentration of S1P formed.

This project is centered around the derivative design and synthesis of a SK-1 inhibitor, sphingosine kinase inhibitor-1 (SKI-1) in one particular zone (zone 1) out of four, which was found to be much more effective *in vitro* than *in vivo.* The goal of this project was to ultimately increase bioavailability and effectiveness of SKI-1 *in vivo*. The derivatives were designed and then analyzed for certain values related to bioavailability such as the Log P value (SKI-1 = 5.6 which is too high). Starting from the parent molecule, SKI-1, several derivatives were synthesized through a pre-determined reaction sequence and confirmed with H-NMR. The SKI-1 derivatives were then tested for effectiveness *in vitro* with a bioassay that measured the amount of unreacted ATP using a luminescence.

In this project, we were able to synthesize a complete set of derivatives of SKI-1 with lower Log P values, thus increasing potential bioavailability of the derivatives that were synthesized compared to SKI-1. In the future, we hope to find the best derivative of each of the four zones and combine them into one molecule.

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.

Small-Diameter Blood Vessel Tissue Engineering

Nicholle Lewis (2020) **Mentor: Dr. Matthew Stern**
Sydney Frazier (2021)

Approximately 600,000 damaged blood vessels are replaced annually, and while there are effective methods for large-diameter blood vessel repair, the current methods for small-diameter blood vessel replacement are less effective. Thus, there is a dire need for new methods of small-diameter blood vessel repair and/or replacement. Blood vessel tissue engineering involves creating a functional blood vessel *in vitro* through the use of a tubular biomaterial scaffold as a template to create a living, functional vessel that can later be implanted into a patient. For this project, decellularized porcine internal thoracic arteries (PITA) are used as the scaffolds for tissue engineering due to their similarities in size and structure to human thoracic arteries. We used scanning electron microscopy (SEM) to characterize the ultrastructure of both the outer and luminal surfaces of each scaffold. In addition, two cell types crucial to vessel function 1) smooth muscle cells and 2) endothelial cells were isolated from PITA and independently cultured to identify optimal conditions for expansion prior to seeding the cells into scaffolds. For each cell type, we compared growth in two different culture media and on several different extracellular matrix (ECM) proteins/components. The AlamarBlue assay was used as indirect measure of cell viability and numbers. Our results suggest that both cell types experienced higher rates of proliferation in one of the media types it was tested in. In addition, smooth muscle cells showed increased growth on a collagen coated substrate, while endothelial cells were less sensitive to the type of substrate coating. Future experiments will focus on seeding cultured smooth muscle and endothelial cells into decellularized PITA scaffolds in the hopes that they will successfully repopulate the scaffolds and confer the functionality needed to later implant the engineered vessels into living organisms.

SC-EPSCoR/IDEA Stimulus Research Program Grant

Characterization of Zinc-Doped Hematite Thin Films

Cale Gaster (2019) **Mentor: Dr. Clifton Harris**

Zn-doped hematite thin films have been prepared on transparent conductive oxide substrates by a pulsed cathodic electrodeposition technique. The films are comprised of monodisperse nanoparticles, and are strongly adherent, uniform and crack-free. Under illumination, the films exhibit p-type conductivity. By pairing this material with a suitable co-catalsyst, we aim to demonstrate sustainable photocurrent under neutral pH conditions in the absence of sacrificial reagents.

Funded by EPSCoR.

A Mathematical Model of Controlling the Spread of Cholera through Disinfection, Vaccination, and Quarantine

Olivia Greathouse (2020) **Mentors: Dr. Zach Abernathy**
Dr. Kristen Abernathy

Cholera is a water-borne gastrointestinal disease that poses major health concerns and can be fatal. The spread of cholera can be controlled with proper treatment and prevention methods. In this project, we present a mathematical model for the spread of cholera throughout a population, with basic control strategies of disinfection, vaccination, and quarantine. For our proposed model, we calculate the basic reproductive ratio, R_0, and prove global stability of the disease-free and endemic equilibria based on the value of R_0. We conclude with numerical simulations and a discussion of the effectiveness of the control strategies on the spread of cholera

Support for this research was provided by the Ronald E. McNair Scholar's Program.

Skeletal Muscle Tissue Engineering and Regenerative Medicine with Decellularized Scaffolds and Adipose Derived Stem Cells

Sha'Deja Johnson (2019) **Mentor: Dr. Matthew Stern**
Caroline Hammond (2020)

In response to volumetric muscle loss, the body forms scar tissue because the volume of damage is too large for the body to regenerate the lost muscle. Regenerative medicine and tissue engineering could potentially provide a solution to help patients suffering from volumetric muscle loss and could also serve as a model for better understanding muscle regeneration. The process of regenerative medicine relies primarily on inducing a patient's own cells to heal a tissue or organ, while the process of tissue engineering uses a biomaterial scaffold as a template for engineering viable and functional tissue outside of the body that can then be implanted into a patient. Our goal was to further optimize the production of Porcine Acellular Muscle Matrix (PAMM), a biomaterial derived from slices of pig skeletal muscle, for use in regenerative medicine and/or tissue engineering applications. We hypothesized that the hybridization of two previously implemented methods of decellularization that rely on 1) actin depolymerization and osmotic shock and 2) treatment with detergent would result in consistent and effective decellularization as measured by removal of cells/nuclei and retention of the structure of the extracellular matrix (ECM). We also planned to control the orientation of ECM elements within PAMM scaffolds by cutting slices of muscle in parallel or cross sections relative to the long axis of the muscle fibers. Our results indicate that we were able to successfully decellularize 2mm thick slices of porcine muscle, giving us an effective 33% increase in scaffold thickness from previous experiments. There was not a difference in the effectiveness of decellularization due to tissue orientation, and we were able to control the orientation of ECM elements within our scaffolds as planned. In addition, we showed that PAMM scaffolds could be successfully recellularized with human Adipose Derived Stem Cells (ADSCs). Our results show that it is possible to decellularize 2mm thick porcine skeletal muscle slices and recellularize PAMM scaffolds using ADSCs. Future research will focus on inducing myogenesis within ADSC-seeded PAMM scaffolds through mechanical stimulation of the constructs.

South Carolina INBRE Developmental Research Program via NIH/NIGMS P20GM103499

Dynamics of an HIV-1 Virotherapy Model

Connor Hennessy (2019)
Mary McBride (2019)
Gaston Tarque (2018)

Mentors: Dr. Kristen Abernathy
Dr. Zach Abernathy

In this project, we consider the dynamics of the HIV-1 virus under the effects of virotherapy and an immune response. We calculate basic reproductive ratios for the HIV-1 virus and recombinant virus, and use these ratios to establish existence and stability criteria for disease-free, single-infection, and double-infection equilibria. We utilize Lyapunov functions to prove the global asymptotic stability of the disease-free and single-infection equilibria. For the double-infection equilibrium, we explore its stability through numerical simulations and provide evidence of a Hopf bifurcation. We conclude with a discussion on the effects of using a recombinant virus to control HIV-1-infected cell populations.

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.

Effects of Beach Nourishment on the Meiofauna: Not all Bad?

Douglas Johnson (2019) **Mentor: Dr. Julian P.S. Smith, III**
Jeremiah JonesBoggs (2020)

Beach nourishment, or the emplacement of dredged sand to mitigate the effects of erosion, has become a standard method of repairing tourist beaches. One long-term effect of nourishment is a coarsening of the beach, as the finer sediments wash away quickly, leaving behind shell- hash. This is of concern, as sediment grain-size is arguably the major abiotic determinant of meiofaunal community structure. Using sieving granulometry to determine sediment parameters and DNA metabarcoding to characterize the meiofaunal community, we have examined two beaches in North Carolina, USA that differ in nourishment history. Our preliminary findings show that there is a significant difference between the two in sediment parameters, with the nourished beach having a significantly greater mean grain-size (437µm vs 218µm; p=.0013) and a greater (160µm vs 41µm), but non-significant, sorting coefficient. Analysis of alpha diversity from the metabarcoding data shows that the nourished beach exhibits significantly higher diversity in all three measures used (Faith's PD, Chao1, and number of OTU's). It seems possible that the increased proportion of micro-habitats in the nourished beach supports higher community diversity.

This project was supported by INBRE Bioinformatics Pilot Project and INBRE RET grants to JSIII; student stipend support was provided from grant P20GM103499 (SC INBRE) from the National Institute of General Medical Sciences, National Institutes of Health.

Discovery of New Optical Compounds using Hydrothermal Synthesis

Kameron Johnson (2021) **Mentor: Dr. Maria Gelabert**

This project investigated aqueous modeling coupled with hydrothermal methods in order to discover new compounds. Novel materials for optical applications, such as luminescent scintillators, are desired for improvement of properties. Using OLI Systems aqueous speciation software, yield diagrams were developed in the quaternary K-La-Zr-O system, aiming towards discovery of new rare earth optical compounds. Chemical systems such as this one will readily form highly stable thermodynamic binary compounds, notably zirconia (ZrO_2) and lanthanum hydroxide ($La(OH)_3$). Within the yield diagrams, where metal concentrations are plotted against pH, regions just outside of stability regions for $La(OH)_3$ and ZrO_2 were targeted for Zr:La ratios of 1:1 and 4:1. In hydrothermal autoclaves heated to 200 °C, aqueous mixtures of zirconyl chloride, acetylacetone, lanthanum chloride, ethylenediaminetetraacetate and potassium hydroxide produced X-ray powder patterns containing products that are unknown, and thus possibly new stoichiometries. Scanning electron microscopy (SEM) with energy-dispersive X-ray (EDS) analysis revealed polycrystalline morphology with some single crystals (50 microns) of hexagonal geometry that contain significant amounts of lanthanum, zirconium, oxygen with trace levels of alkali metals, leading us to tentatively conclude that these crystals are of a lanthanum zirconate compound.

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

Post-Fire Carbon Assimilation Rates in Species with Different Post-Fire Recovery Strategies

Jesse Martin (2021) **Mentor: Dr. Jennifer Schafer**

In many ecosystems where fire is a natural disturbance, it removes aboveground biomass. Fire can kill individuals, but species can maintain populations through the germination of seeds. Some species are only killed aboveground and can grow new shoots, called resprouts, by redistributing carbon resources from belowground to aboveground. Our goal was to determine if carbon assimilation rates of post-fire resprouts vary among species with different post-fire recovery strategies. Specifically, we hypothesized that species that recover only by resprouting would have higher carbon assimilation rates than species that recover by resprouting and/or seed germination because resprouting plants depend solely on their belowground carbon reserves to persist after fire. We measured photosynthesis of post-fire resprouts of 11 species (shrubs and palmettos) in scrubby flatwoods communities in Florida. We measured photosynthetic (i.e., carbon assimilation) rates of five to eight individuals of each species in sites approximately 11 months post-fire. We also measured total leaf area of shoots of four shrub species and counted leaves and measured leaf area of the two palmetto species. There was a significant difference in carbon assimilation rates among species when measured on a leaf area basis. *Palafoxia feayi*, which recovers after fire by resprouting and/or seed germination had the highest carbon assimilation rate, while *Bejaria racemosa*, which recovers by resprouting, had the lowest carbon assimilation rate. When scaled to total plant leaf area, carbon assimilation rates did not differ between the two palmetto species or among four shrub species. Overall, carbon assimilation rates did not differ based on post-fire recovery and were not higher in resprouters; thus, our hypothesis was not supported. This suggests that species that depend only on belowground carbon to support post-fire recovery do not require greater post-fire carbon assimilation to persist in fire-prone habitats than species that can recover via seed germination.

This research was funded by Winthrop University Research Council Grant.

Expression, Purification and Prelimnary Crystallization of a Putative Chaperonin from *Xanthomonas cynarae*

Augustine Vinson (2019) **Mentor: Dr. Jason C. Hurlbert**
Marlin McKnight (2020)

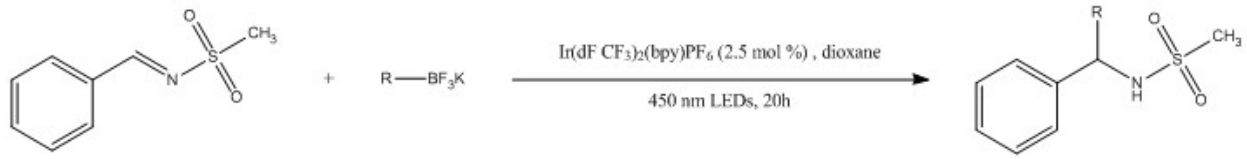
Xanthomonous cynarae is a phytopathogenic bacterium responsible for causing blight on artichokes, resulting in crop loss. The presence of the bacterium on artichoke leaf tissue triggers a hypersensitive response, a type of programmed cell death characterizd by tissue collapse and electrolyte leakage and extensive modifications to the cell walls and surrounding apoplastic tissues. The hypersensitive response is triggered by the interaction of bacterial avirulence proteins (Avr proteins) with plant resistance proteins (R proteins) in the plant cytosol. The Avr proteins are injected into the plant cell via the Type III Secretion System, a modified flagellum that serves as a molecular needle, unfolding the proteins on the bacterial end and injecting them into the plant cytosol. We have recently identified a protein, named XopAZ, that may aid in the proper refolding of these injected Avr proteins. This protein has shown sequence identity to the FK506 binding protein family of peptidyl-prolyl isomerases and the Sensitivty to lysis (Slp) family of chaperononins. We have cloned the gene encoding Xop AZ from *X. cynarae* into a prokaryotic expression plasmid and purified recombinant Xop AZ from the resulting bacterial culture. We purified our protein using metal chelating affinity and gel filtration chromatographic methods. The resulting purified protein has been screened for conditions suitable for crystal growth.

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 Sulfonamides using Visible Light Photoredox Catalysis

Madison Merrill (2021) **Mentor: Dr. James M. Hanna Jr.**

Sulfonamides are commonly found in many pharmaceuticals. Comprised of sulfonyl and amine subgroups, sulfonamides are highly sought after in the medical field for the treatment of bacterial infections, among other applications. Previously synthesized using less robust techniques, our group was specifically interested in using visible light photoredox catalysis to promote the addition of potassium organotrifluoroborates to sulfonyl imines as a way to synthesize these compounds. The photocatalyst involved in this approach is often a ruthenium or iridium polypyridyl complex, which absorbs light in the visible range to give a relatively long-lived excited state, which can 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). Since these photocatalysts can function as both SET oxidants and reductants within the same cycle suggested the possibility of a selective, redox-neutral, radical generation and addition strategy to achieve this goal. Initially, we found that a dichloromethane solution of potassium isopropyltrifluoroborate (**2**, R=*i*Pr) and *N*-benzylidinemethanesulfonamide (**1**), when irradiated at room temperature with 450 nm LEDs in the presence of the photocatalyst ***Ir(dFCF$_3$)$_2$(bpy)PF$_6$*** , resulted in a low yield of the desired sulfonamide (**3**, R=*i*Pr); optimization of solvent and catalyst revealed that 1,4-dioxane, along with ***Ir(dFCF$_3$)$_2$(bpy)PF$_6$*** gave the highest yield of product. We therefore evaluated the reaction of other potassium alkyltrifluoroborates (**2**) with **1** in dioxane in the presence of ***Ir(dFCF$_3$)$_2$(bpy)PF$_6$*** (Scheme), which led to a moderate yield of desired product **3** in each case.



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Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund (58270-UR1) for support of this research. Additional support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (8 P20 GM103499).

Locating Mutagen-sensitivity Gene *mus109* in the *Drosophila melanogaster* Genome Using Deficiency Mapping

Chandani Mitchell (2019) **Mentor: Dr. Kathryn Kohl**

Defects in the DNA repair process can be detrimental. For example, mutations in the *Drosophila melanogaster* DNA repair gene *mus109* cause impairment resulting in larval death. However, little is known about *mus109*, including its genomic location. This study aims to locate *mus109* using deficiency mapping. The $mus109^{D2}$ allele was crossed to four deficiencies covering the 8F10-9B1 region of the genome. Brood 1 and Brood 2 were treated with H_2O and 0.05% MMS, respectively. Offspring were scored based upon the sex and eye shape phenotype, and this data was used in complementation analysis to narrow the probable genomic location of *mus109*.

This project was supported by the Ronald E. McNair Post-baccalaureate Achievement Program and SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

The Effects of Allophane on Carbon Sequestration and Nutrient Availability in Compost Derived from Food Waste

Dakota Shope (2020) **Mentor: Dr. Scott Werts**
Emily Mitchell (2019)

In America, 21% of all material in landfills is derived from food waste. Nearly all of carbon contained in that waste will be converted to methane under anaerobic decomposition pathways. However, food waste that is composted rather than entering the waste stream contains 35-55% carbon, which can be utilized as soil amendments and fertilizer. There has been much interest in the study of clay and carbon in the soil, slowing decomposition and sequestering carbon from the atmosphere. Allophane, an aluminosilicate clay derived from weathered volcanic ash, is known to create complexed compounds and stabilize the organic carbon for longer periods of time in natural environments. The purpose of this experiment is to determine if allophane effects the carbon loss from compost derived from food waste and if it modifies any of the nitrogen or phosphorus cycling in the compost. Five sets of 3 pots with a compost/topsoil mixture and increasing amounts of allophane ranging from 0-50% by mass were established in a greenhouse and used to grow bush beans (Phaseolus vulgaris). The compost mixture was sampled every two weeks and analyzed for total nitrogen, total carbon, nitrate, phosphate, and ammonium content. Overall, the carbon content of all the compost decreased over time. The carbon nitrogen ratios of the compost remained between 30/1 and 18/1 for the duration of the experiment. While the total amount of nitrogen available decreased over time, the compost with higher amounts of allophane had more available nitrogen in ammonia and nitrate forms. Ammonium availability showed a constant decrease over time in the compost while nitrate showed a large spike followed by a nitrate depression period. The total phosphorus availability increased with increasing allophane and spiked during the nitrate depression period. This spike may be caused by a nitrogen depression period causing the phosphate to be released from microbial matter. Overall, the increasing amounts of allophane are reducing the levels of greenhouse gas emissions from the compost, but are also depressing the nutrient cycling in the compost.

Funding for this project was received from the Boland Geology Endowment Fund and from a Winthrop Research Council Grant.

Microplastics of the Catawba River Basin

Chasity Moore (2018) **Mentor: Dr. Cynthia Tant**

Plastic pollution has become a worldwide ecological and economic issue. Plastic was originally a chemical-based alternative to using finite ecological goods such as wood and ivory. An overproduction of plastic, improper disposal, and faulty recycling practices have caused plastic to be prevalent in the environment at large. Plastic, and more recently microplastic (plastic pieces smaller than 5mm), pollution in the marine environment has become a major area of concern due to the occurrence of plastic in the guts of many aquatic species. Most research has been focused on marine microplastics; however, there has been very little research on inland freshwaters. In this study we focused on the Catawba River and its tributaries to quantify and sort microplastics in surface water, sediment, and invasive freshwater bivalves, *Corbicula fluminea*. The samples were processed and quantified using the NightSea© fluorescent microscope adaptor. We found that two tributaries, Big Dutchman Creek and Manchester Creek, had significant differences in the quantity of microplastics as well as types of microplastics. At Manchester Creek, storm flow increased the quantity of microplastics in surface water samples. There was variation among and within samples but no significant difference between water, sediment, and *Corbicula* samples at Big Dutchman Creek. The data obtained allow for comparisons of microplastics along different areas of the Catawba River basin as well as comparisons to already established marine data.

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.

Areal Capacitance of $Ni_3(HITP)_2$ Supercapacitors Fabricated by Electrophoretic Deposition in $EMIMBF_4$/ACN and Na_2SO_4 Electrolytes.

Darien K. Nguyen (2020) **Mentor: Dr. Amir Fatima**

Supercapacitors offer a promising approach as a relatively new energy storage system due to their high energy density, long-lasting lifetime, and high cycle efficiency. The efficiency of the supercapacitors depends on a number of variables such as porosity and surface area of electrode and the electrolytes used for the fabricated supercapacitor. Herein, we report of electrochemical performance $Ni_3(HITP)_2$ supercapacitor in two different electrolytes $EMIMBF_4$/ACN and Na_2SO_4. The $Ni_3(HITP)_2$ electrodes were fabricated through a low cost process known as electrophoretic deposition. The morphology of the electrodes analyzed using both scanning electron microscopy (SEM) and transmission electron microscopy (TEM), shows an extremely porous surface and two-dimensional nanosheets structure of the $Ni_3(HITP)_2$ electrodes. The structure of the electrodes was also analyzed with x-ray diffraction (XRD), and energy dispersive x-ray spectroscopy. The electrochemical performance of the $Ni_3(HITP)_2$ supercapacitors in both electrolytes was characterized by cyclic voltammetry, electrochemical impedance spectroscopy, and galvanostatic charge-discharge tests. The results indicate the introduction of a new materials for a new generation of supercapacitors fabricated by electrophoretic deposition.

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

The Mechanical Properties of Fabricated Structure When Loaded Off-Axis

Meha Patel (2020) **Mentor: Dr. Meir Barak**

Trabecular bone architectural adjustment to loading (i.e. bone functional adaptation) is thought to increase trabeculae alignment with the principle direction of loading. Yet, since each trabecular tissue is unique in structure, so far it was impossible to test a specific sample multiple times in different directions to find if its optimal mechanical orientation truly correlates with the physiological direction of loading. Recently, we introduced a new approach to determine the stiffness and strength of a single trabecular sample in multiple orientations (on- and off-axis) using 3D printing. Our results revealed that contrary to the accepted paradigm, trabecular structure is significantly stiffer and stronger when loaded off-axis. To validate that these unexpected results are not derived from a limitation of our system (and thus do not represent the true behavior of trabecular tissue), we repeated the experiment with a fabricated 3D symmetrical cube mesh made of beams in three orthogonal axes, which was tested both on-axis (no tilt) and off-axis (10°,20°,30°,40° and 45° tilted to the original axes). Our working hypothesis was that contrary to the trabecular bone 3D model, the fabricated 3D cube will demonstrate the highest stiffness and strength when loaded on-axis. Our results supported our hypothesis, confirming that the unexpected finding we discovered for the 3D printed trabecular structure is not a result of our system limitation but a true unpredicted behavior of trabecular structure, which implies that trabecular bone serve to reinforce the whole bone when it is loaded off-axis (e.g. during falling).
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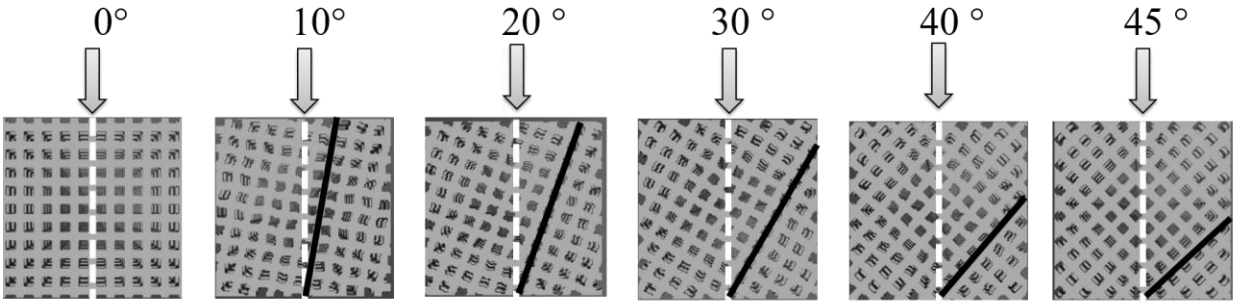
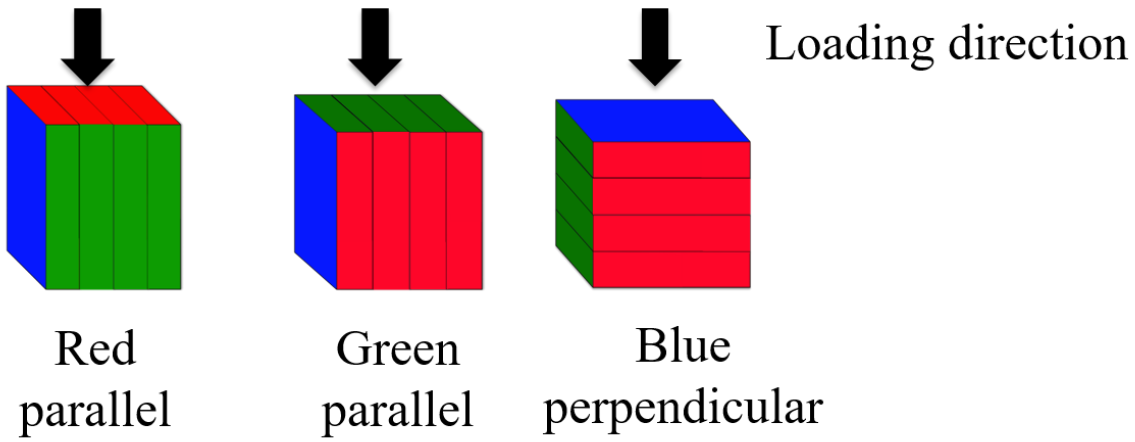


This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

The Effect of 3D Printing Layer Orientation on the Mechanical Properties of the Printed Object

Meha Patel (2020) **Mentor: Dr. Meir Barak**

The purpose of this experiment was to investigate whether the interface between layers of a 3D printed object has a negative effect on its structural stiffness. To this end we 3D printed two samples using a ProJet 3D printer. The first sample was a solid homogenous and isotropic cube (4.5 mm^3, n=5) and the second was a porous homogenous and orthotropic cube fabricated from a trabecular bone micro-CT scan, which was printed in two different orientations, 90° orthogonal in printed layer orientation to each other (4.5 mm^3, n=5 per orientation). Each of the 15 cubes was loaded in compression (Instron 5942) within its elastic region along the 3 principle axes and stiffness was calculated from the stress-strain curve. **Our working hypothesis was that there would be a significantly negative effect on structural stiffness when the sample is loaded parallel to the printed layers orientation. This negative effect would be due to shear stresses generated in the interfaces between printed layers**. Our results supported our hypothesis in the solid isotropic cube. Structural stiffness was significantly higher when the cube was loaded perpendicular to printed layer orientation ($P<0.05$). However, this was not the case for the porous orthotropic cube. In the porous cube the specific orientation of loading (i.e. one of the 3 axes) was much more important ($P<0.05$) than the relation to printed layer orientation, which was found to be non-significant ($P>0.05$). These results demonstrate that in 3D printed porous structures, the inherent orthotropic architecture is the main contributor to structural stiffness. Nevertheless, since our results show that printed layer orientation does effect structural stiffness, 3D printed objects should always be mechanically tested in compression perpendicular to their printed layer orientation.



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.

DNA Barcoding Comes to Winthrop: Developing Pipelines and Exploring Applications

Whitney Player (2018) **Mentor: Dr. Julian P.S. Smith III**

Metabarcoding, or the use of DNA fragments amplified from "all" members of a biological community, has become standard for assessment of community diversity and for community comparison in applications ranging from biomedicine to ecological monitoring. INBRE funding has made possible the establishment of metabarcoding techniques at Winthrop University, and we accordingly present four applications of that here. First, metabarcoding was used to analyze gut contents of a single larval dragonfly (Odonata), employing a so-called "brute force" approach, where the entire gut contents are metabacoded using "universal" 18S primers. In this application, the operational taxonomic units (OTUs) due to the predator are expected to be the most abundant, and the remaining OTUs are expected to be prey (or prey of prey—so-called "secondary predation"). We have developed a pipeline for analyzing these data specifically to be used by another INBRE faculty member and her students working in stream communities. Second, we have developed a pipeline that allows analysis of marine meiofaunal communities, testing it on samples from two beaches that differ in anthropogenic impact. Finally, metabarcoding was used to derive genetic data in support of two long-term class projects (Biol 204H Principles of Biology Honors and Biol 530 Invertebrate Biology) employing morphological biodiversity assessment—one, a census of soil fauna in the Winthrop successional plots, and a second, a census of the fauna of Winthrop Lake. To date we have focused largely on method-development and proof-of-concept with these small projects. Our long-term goal is continue to expand the analyses presented here as well as to assist other faculty and students at Winthrop in taking advantage of this important technique in community assessment.

The authors are grateful to Mr. Jeremiah JonesBoggs for sharing his granulometry data on the two beaches studied here, and to Dr. Cynthia Tant and her students for making the larval dragonfly material available. This project was supported by grant P20GM103499 (SC INBRE) from the National Institute of General Medical Sciences, National Institutes of Health and specifically by SC INBRE Bioinformatics Pilot-Project and INBRE-RET grants to JSIII.

Expression and Purification of a Novel Calcium Binding Protein Necessary for Phytopathogenesis in Xanthomonas strains

Juliana Quay (2020) **Mentor: Dr. Jason C. Hurlbert**

Recently, we have identified a gene whose sequence is conserved in several species of Xanthomonas that, when expressed, elicit a hypersensitive response (hr) in tomato plants. Normally, hr elicitation is limited to very specific bacterial-host pairings, but introduction and expression of this gene by bacterial species that do not normally infect tomato serves to elicit hr, indicating that the encoded protein is crucial to the infectious process. Bioinformatic analysis of the protein, which we have named EfhX (EF-Hand containing protein from Xanthomonas) reveals that the protein is predicted to contain a single transmembrane a-helix, spanning amino acids 60 to 81, and two calcium binding domains, termed EF-Hands, in the carboxy-terminal domain of the protein. In order to better understand the function of this novel protein, we have cloned the efhX gene from *Xanthomonas aurantofolia* and expressed it in Escherichia coli so that we can obtain quantities of the protein sufficient to grow protein crystals and determine the structure of the protein via x-ray diffraction. We have successfully purified the protein to homogeneity (>95%) as determined by SDS-PAGE and anti-hexahistidine Western Blot. In the coming weeks, we will initiate crystallization trials to identify solution conditions suitable for growth of crystals for x-ray diffraction experiments so that the structure of the protein can be determined.

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.

Cloning of LPAR2 and LPAR2 Variant for Expression Studies

Taylor Ries (May 2020) **Mentor: Dr. Eric Birgbauer**

The nervous system is critical for your typical day-to-day life. The system is composed of billions of axons that are guided throughout the body by chemoreceptors. Lysophosphatidic acid (LPA) is a putative axon guidance molecule that has six known chemoreceptors (LPA receptors 1-6). ChEST is a newly discovered receptor that is very genetically similar to LPA receptor 2, so I studied the relationship between ChEST and LPAR2. I hypothesized that ChEST is a seventh LPA receptor similar to LPAR2. In the study, I cut open two different tdTomato vectors (tdTomato-C1 and tdTomato-N1). tdTomato is a red fluorescent protein that can be easily identified when viewed under green light which will be beneficial in my future studies. The project goal was to fuse tdTomato with the different chemoreceptors (ChEST and LPAR2). The difference between tdTomato-C1 and tdTomato-N1 is the location where the vector is cut open. On tdTomato-C1 the vector is cut at the C-terminus, and on tdTomato-N1 the vector is cut at the N-terminus. After opening the vectors, I prepared four different inserts: ChEST to be added to the N-terminus, ChEST to be added to the C-terminus, LPAR2 to be added to the N-terminus, and LPAR2 to be added to the C-terminus. Once all of the vectors and inserts were prepared, I ligated the DNA insert into the corresponding vector. Therefore, the DNA insert closed the tdTomato vector to make it a full vector with the chemoreceptor. The purpose of transforming the different DNA inserts onto the different terminuses of the tdTomato vector is because the location of the tdTomato fusion could result in differences in the functionality of the chemoreceptor. To analyze the results, I had the DNA sequenced and compared the DNA sequence to the expected DNA sequence. The DNA sequence for ChEST on the N-terminus, ChEST on the C-terminus, and LPAR2 on the C-terminus all indicated that the cloning was successful. However, the DNA sequence for LPAR2 on the N-terminus indicates that there was a mutation in the gene sequence. Overall, the successful cloning is beneficial because they can now be used for future studies. To further investigate, the DNA can be transfected into B-103 cells in order to conduct expression studies for the ChEST receptor in relation to the LPA receptor 2. When the DNA is transfected into the B-103 cells, the cells will contain the new DNA and will glow red if the transfection was successful. I will conduct the expression studies by comparing the reaction of each chemoreceptor when LPA is added to the cells. I hope to determine if ChEST reacts the same as LPAR2 or if it exhibits a different response, or no response at all. If ChEST reacts the same as LPAR2, then it is potentially a seventh LPA receptor.

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.

Progress toward Quantitative Characterization of the Relationship between StkP and RitR in *Streptococcus pneumoniae*

Lucia Rodriguez (2019) **Mentor: Dr. Nick Grossoehme**

Streptococcus pneumoniae is a pathogenic bacteria that can cause bronchitis, meningitis, and many other life threatening diseases. This bacterium requires iron transport for survival and virulence, which is regulated by a two-component system composed of RitR (regulator of iron transport) and StkP (serine-threonine kinase). RitR binds to DNA when unphosphorylated, repressing iron transport by preventing transcription of the *piu* (pneumococcal iron uptake) operon. Upon sensing iron outside the cell, StkP hydrolyzes ATP and phosphorylates RitR. This causes RitR to dissociate from DNA, allowing transcription of the *piu* operon and expression of iron transport components. Our goal was to quantitatively determine this relationship between StkP and RitR using a reverse-phase HPLC enzymatic assay. RitR was successfully expressed in *Escherichia coli* cells and purified by a combination of ion-exchange and gel filtration chromatography. StkP was expressed in *E. coli* as a fusion protein with glutathione-S-transferase (GST), and purified by glutathione affinity chromatography. Reverse-phase HPLC assays were conducted with ATP standards, which demonstrated that separation of ATP, ADP, and AMP was very dependent on experimental conditions such as temperature, buffer pH, and flow gradient. The optimal parameters were determined to be at 37°C, with mobile phases of 10 mM sodium acetate, pH 5.0 with a slow gradient rising to 100% acetonitrile. When the assay was conducted with StkP, RitR, and ATP, three injections were sampled at 6, 20, and 33 minutes. A decrease was observed in the expected peak for ATP, and an increase was observed in the expected peak for ADP, when comparing retention times to those of the ATP and ADP standards. This indicates ATP-dependent activity of StkP on RitR, although further work is necessary to improve the experimental parameters of the assay.

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.

Spheroid Culture of Human Adipose Derived Stem Cells to Alter Regenerative Potential

Sophia Stefanov (2021) **Mentor: Dr. Matthew Stern**

Adipose Derived Stem Cells (ADSCs) are multipotent mesenchymal stem cells that reside in the microvasculature of adipose tissue. While they are partially defined by their ability to differentiate into multiple cell lineages, their directed differentiation into many lineages of interest remains inefficient. Our lab is specifically interested in the ability of ADSCs to differentiate into skeletal myocytes. The goal of this project is to more efficiently differentiate ADSCs into skeletal myocytes by first moving the cells to a more developmentally potent state through relatively simple manipulation of the cells' culture conditions. Multiple factors have shown promise in enhancing cells' regenerative potential. Specifically, three-dimensional spheroid culture is known for its ability to enhance cells' differentiation potential. We hypothesized that ADSC spheroids generated using micropore well inserts (Figure 1) would show changes in global gene expression indicative of a more developmentally potent state and that the addition of 5-azacytidine, a DNA methylation blocking analogue of cytidine, would provide a synergistic enhancement of the effects of spheroid culture. To test our hypothesis, we generated ADSC spheroids (Figure 2) and compared the transcriptomes of spheroid-cultured ADSCs to ADSCs cultured using the traditional two-dimensional method +/- the addition of 5-azacytidine via RNA sequencing. RNA sequencing results showed differences in the gene expression profiles of all of the experimental and control groups. The expression profile of the plated spheroids was more similar to that of the two-dimensionally cultured cells than to that of the three-dimensional spheroids. The large amount of data generated requires further analysis to determine the relevance of differentially expressed genes to developmental potency. Future work will focus on directing the differentiation of spheroid cultured ADSCs into skeletal myocytes.

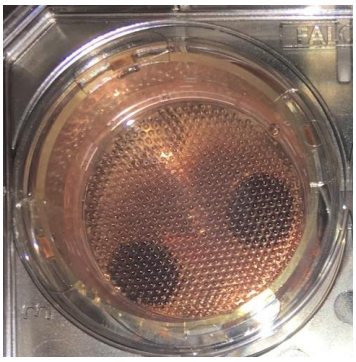


Figure 1: Micropore insert placed inside a 6-well plate for spheroid generation.

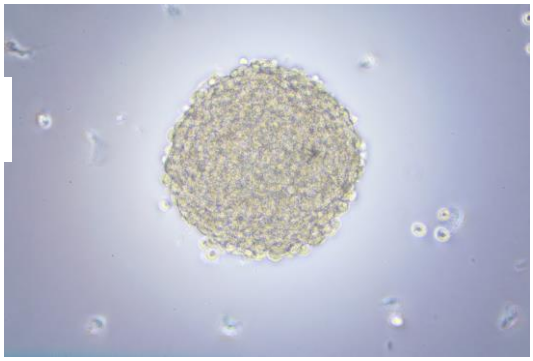


Figure 2: Phase contrast image of an ADSC spheroid 48h after plating onto a micropore insert; 200X.

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A Mathematical Model for Tumor Growth and Treatment Using Virotherapy

Jessica Stevens (2019) **Mentors: Dr. Zach Abernathy**
Dr. Kristen Abernathy

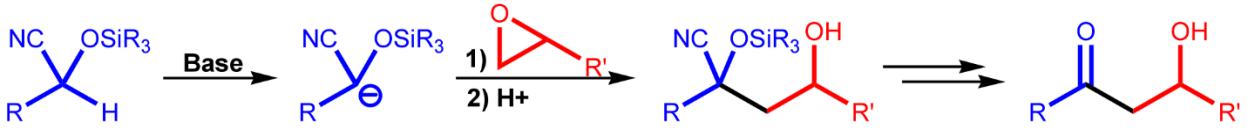
We present a system of four nonlinear differential equations to model the use of virotherapy as a treatment for cancer. This model describes interactions among infected tumor cells, uninfected tumor cells, effector T-cells, and virions. Using various stability analysis techniques, we establish a necessary and sufficient treatment condition to ensure a globally stable cure state. We additionally show the existence of a cancer persistence state when this condition is violated and provide numerical evidence of a Hopf bifurcation under estimated parameter values from the literature. We conclude with a discussion on the biological implications of our results.

Support for this research was provided by the Ronald E. McNair Scholar's Program.

Enantio- and Diasteroselective Preparation of β-Hydroxycarbonyls Using O-silyl Protected Cyanohydrins and Epoxides

Sean Wechsler (2020) **Mentor: Dr. Aaron M. Hartel**

β-Hydroxycarbonyls are formed as the product in the aldol addition, a carbon-carbon bond forming reaction of great importance. In the aldol reaction, an enolized carbonyl reacts with a ketone or aldehyde. The reaction can potentially create as many as two new chiral centers, and enantio- and diasteroselective control of the reaction is often critical. Useful stereoselective variants of the aldol addition have been developed, each with its own set of drawbacks and limitations. We have developed a new alternative method for the formation of a β-hydroxycarbonyls via the reaction of O-silylated cyanohydrins with epoxides.



Previous research on the project consisted of optimizing the reaction conditions for aryl-substituted cyanohydrins (R=Ar) and determining the scope of the reaction with respect to epoxide and cyanohydrin. Current research has been focused on reacting alkyl-substituted cyanohydrins (R=alkyl). Reaction conditions with an alkyl substituent were optimized to avoid an undesired cyclization of product and retro-Brook rearrangement. A subsurface quenching technique was used alongside 0°C reaction conditions to discourage formation of the undesired by-products. Silicon protecting groups such as *tert*-butyldimethylsilyl and triisopropylsilyl were explored to examine the effect of the steric bulk of the protecting group on suppressing the undesired side-reactions. After successful epoxide attack on the cyanohydrin, the silicon protecting group was removed using TBAF to yield a β-hydroxycarbonyl.

This project was supported by EPSCoR.