Winthrop University
Summer Undergraduate Research Experience (SURE)
2017 Abstract Book

WINTHROP
UNIVERSITY

Table of Contents

Carra Lyons	N. Grossoehme, Chemistry	24
Sara Manore	C. Grattan, Chemistry	25
Shirley Mathur*	M. Barak, Biology	26
Sydney McCall	Z. Abernathy and K. Abernathy, Mathematics	16
Justin McCullough	A. Hamm, Mathematics	27
Eulillian McFadden	C. Grattan, Chemistry	28
Caylie McGlade	A. Hartel, Chemistry	29
Tanisha Moore	T. Sumter, Chemistry	30
Natalie Mseis	M. Stern, Biology	6
Mouskudah Murray	J. Hanna and R. Lammi, Chemistry	19
Jack Nguyen	M. Barak, Biology	31
Jason Peck	F. Amir, Physics	32
Tyler Robbins	D. Boyer, Geology	33
Jennifer Schroen	M. Stern, Biology	11
Hunter Sellers	N. Grossoehme, Chemistry	34
Maryssa Shanteau-Jackson	T. Sumter, Chemistry	30
Mallory Sorenson	J. Hurlbert, Biochemistry	35
Jessica Stevens	Z. Abernathy and K. Abernathy, Mathematics	36
Benjamin Swartz	C. Tant, Biology	7
Timothy Swartz	D. Boyer, Geology	33
Jaime Taylor	S. Werts, Geology	22
Anneke van Eldik	M. Stern, Biology	9
Augustine Vinson	R. Lammi and J. Hanna, Chemistry	12

Alan Way	A. Hamm, Mathematics	27

* High School student, GSSM

Winthrop Students Performing Research at Other Institutions

Sophia Warren	J. Ballato, C. Kucera, A. Vargas Clemson University	37

ACKNOWLEDGEMENTS

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 2017, the Winthrop SURE Program celebrated its twelfth year, with a cohort of over 40 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!

Jay Hanna
SURE Program Coordinator

Robin Lammi
Director of Undergraduate Research

The Combination of Epigenetic Modification with 5-azacytidine and Spheroid Culture of Adipose-Derived Stem Cells Alters the Expression of Genes Associated with Developmental Potency

Melissa Barr (2018) **Mentor: Dr. Matthew Stern**
Natalie Mseis (2018)

Adipose-derived stem cells (ADSCs) are multipotent mesenchymal stem cells found within the microvasculature of adipose tissue. While ADSCs have the potential to differentiate into multiple cell lineages, they cannot match the differentiation potential of pluripotent stem cells such as embryonic stem cells or induced pluripotent stem (iPS) cells. The developmental potency of stem cells is defined by the gene expression profile of the cells and is sensitive to a variety of factors. One method for altering gene expression within a population of cells is via epigenetic modification, which can change the conformation and transcriptional activity of chromatin. Another approach to altering gene expression relies on changing the physical environment in which cells are cultured. *In vivo*, cells reside within a complex three-dimensional environment; however, traditional culture methods place cells in a much simpler and more two-dimensional environment–the surface of a culture plate/flask. Previous work in our lab has shown that treatment with epigenetic modifiers and three-dimensional culture of ADSCs as spheroids both independently alter gene expression, including the expression of genes that regulate developmental potency. This has lead us to the question of whether combing a method of epigenetic modification with three-dimensional culture would produce additional/different changes in gene expression than those observed when each method is used independently. We hypothesized that treating ADSCs with 5-azacytidine, a compound that blocks DNA methylation, while also culturing the ADSCs as three-dimensional spheroids would alter the expression profile of genes associated with developmental potency differently than when either method is used alone. Our data suggest that the combination of epigenetic modification and spheroid culture resulted in a unique gene expression profile of a subset of genes associated with developmental potency. Future work will focus on utilizing these changes to maximize the efficiency of ADSC differentiation into select cell lineages.

SC-INBRE and SC-INBRE Developmental Research Program via NIGMS P20GM103499
Winthrop University Research Council Award SC15017.

Characterizing Freshwater Macroinvertebrate Food Webs at the Winthrop Recreational and Research Complex

Benjamin Swartz (2018) **Mentor: Dr. Cynthia Tant**
Tira Beckham (2020)

Aquatic food webs are complex, and their study can provide valuable information on movement of energy and nutrients in ecosystems. Most food web studies involve microscopic analysis of gut contents that can be time consuming, and many prey species lack features that persist long enough in a predator's gut for taxonomic identification. The application of newer molecular-based approaches has the potential to provide previously unavailable resolution in aquatic food webs. We sampled and identified a variety of benthic macroinvertebrates at the Winthrop Recreational and Research Complex. Individuals from selected predator taxa were used either to create gut content slides to identify prey categories or to extract DNA from gut contents for analysis using NextGen sequencing. These comparative data will ultimately provide baseline taxonomic data on food web components in lake, wetland, and stream habitats at the Complex.

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.

Examining Drug Resistant vs. Sensitive Tumor Cell Populations with Immunotherapy & Chemotherapy

John Brotemarkle (2020) **Mentors: Dr. Kristen Abernathy**
Genia Kennedy (2018) **Dr. Zach 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 conclude with evidence of a limit cycle and conjecture the existence of a Hopf bifurcation.

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.

The Implementation of 3D Printing and 3D Bioprinting in Biomedical Research, Education, and Community Service

Anneke van Eldik (2019) **Mentor: Dr. Matthew Stern**
Chandler Burt (2020)

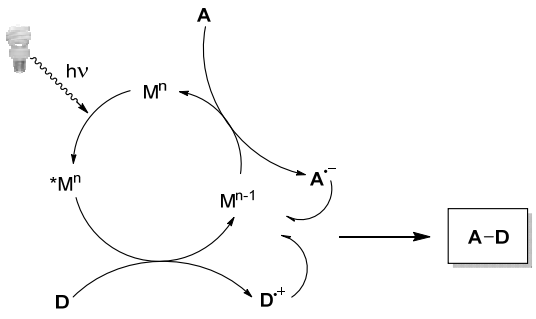
Technologies such as 3D printing and 3D bioprinting are becoming increasingly common in biomedical research. These technologies hold great promise for the production of custom devices, including living bioengineered products, that improve the lives of patients. The production of advanced bioengineered products requires the combined expertise of several fields including engineering and biology. However, introduction to technologies such as 3D printing and 3D bioprinting is not common for undergraduate biology students, particularly those at primarily undergraduate institutions (PUIs). Here, we describe a summer project in which we learned to use a 3D printer and a 3D bioprinter for research purposes while also demonstrating their potential to be used in undergraduate biology education. A relatively inexpensive Flashforge Creator Pro was used to 3D print objects for research and educational use and will serve as the platform to introduce Winthrop biology students to basic 3D printing technology. A BioBot1 was used in our 3D bioprinting work and will also be used to introduce students in select Winthrop biology courses to bioprinting technology. In addition, we have established the Giving Hands student organization, which will be a Winthrop-based chapter of the e-NABLE community–a global organization whose members volunteer to 3D print and distribute mechanical hands for individuals with upper limb differences. Together, these efforts have established the infrastructure required to introduce Winthrop biology students to 3D printing, 3D bioprinting, the workflow involved in each (design, programming/software, troubleshooting), and the many applications of these technologies within the biological sciences.

SC-INBRE and SC-INBRE Developmental Research Program via NIGMS P20GM103499
Winthrop University Research Council Awards CE17010 and SC17014

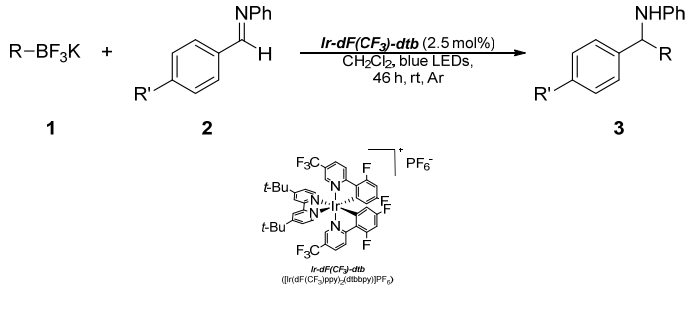
Visible Light-Promoted Additions of Potassium Organotrifluoroborates to Imines

Brittney E. Ciesa (2019) **Mentor: Dr. 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. Visible-light photochemistry has several advantages over traditional ultraviolet (UV) radiation promoted organic photochemistry. For example, many applications of UV photochemistry require quartz vessels to ensure the radiation can penetrate the vessel, and make use of wavelengths which can electronically excite organic substrates, potentially leading to unwanted side reactions. In contrast, visible light passes through ordinary glass, and small organic substrates do not typically absorb wavelengths in the visible range. 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 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). The ability of these photocatalysts to function as both SET oxidants and reductants within the same cycle suggests the possibility of a selective, redox-neutral, radical generation and cross-coupling strategy, illustrated in Scheme 1, where radicals derived from both an acceptor (A) and a donor (D) would engage in productive cross-coupling to form a product (A-D). Our group has successfully employed this approach for the formal 1,2-addition of potassium alkyltrifluoroborates (**1**) to aryl aldimines (**2**), as outlined in Scheme 2. Thus, an argon-sparged dichloromethane solution of **1** (R = benzyl, allyl, 1°-, 2°-, 3°-alkyl) and **2** (R′ = H, OMe, Cl), when irradiated with blue LEDs in the presence of ***Ir-(dFCF$_3$)(dtb)*** at room temperature resulted in moderate to good yields of the desired adducts (**3**). Of note are the successful reactions of primary, secondary, and tertiary alkyltrifluoroborates; we believe they represent the first examples of 1,2-addition of these organometallics to C=N substrates.



Scheme 1.



Scheme 2.

Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund 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).

Production and Characterization of Porcine Acellular Muscle Matrix Scaffolds for use in Skeletal Muscle Tissue Engineering and Regenerative Medicine

Jennifer Schroen (2018) **Mentor: Dr. Matthew Stern**
Tierra Collins (2019)

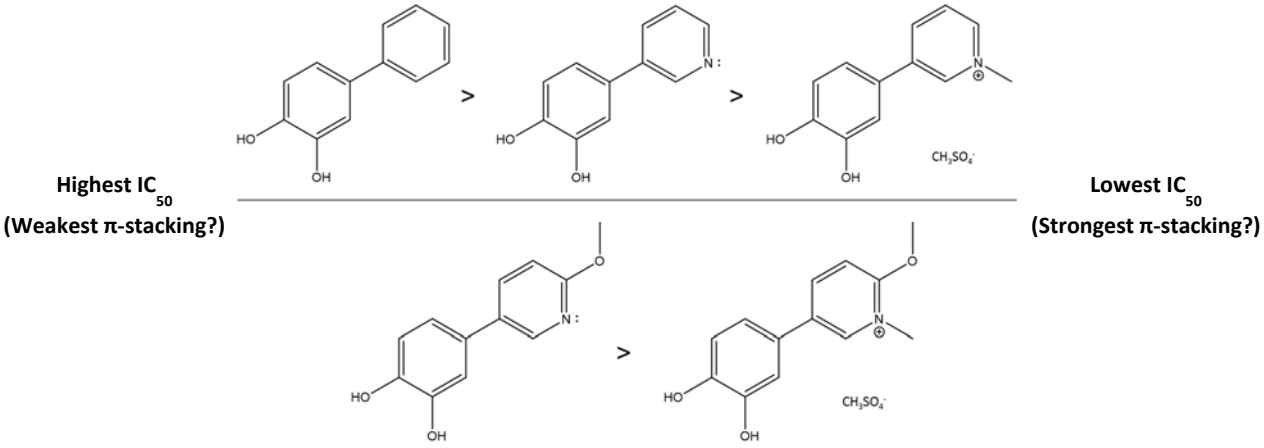
Existing treatment options for patients suffering damage to skeletal muscle are incapable of adequately restoring tissue form and function. Tissue engineering represents a potential solution for these patients. In a tissue engineering-based treatment, a population of stem/progenitor cells with myogenic potential would be seeded within a biomaterial scaffold to create an implantable construct that facilitates muscle repair. Alternatively, the biomaterial scaffold could be implanted alone and recruit endogenous stem/progenitor cells to repopulate it. In either of these cases, the biomaterial scaffold is central to the approach. Our lab is focused on the development of biomaterials from decellularized porcine skeletal muscle. We hypothesized that the porcine longissimus dorsi muscle could be processed into 2mm thick sheets that could be decellularized to serve as biomaterial scaffolds for muscle tissue engineering. Effective decellularization requires the removal of immunogenic cellular materials while leaving behind the tissue's structural framework. We used a combination of histological characterization and DNA content measurement to gauge the effectiveness of our decellularization. DAPI staining revealed that all nuclei were removed during the decellularization process. DNA content measurement confirmed the removal of DNA with the DNA content of decellularized muscle meeting the recognized standard for decellularized tissues. Hematoxylin and eosin staining and gross handling of the acellular tissue demonstrated that the structural framework/extracellular matrix (ECM) of the tissue was largely left in place after decellularization. Furthermore, the orientation of the ECM elements could be controlled by altering tissue orientation during processing. Together, these results demonstrate our ability to produce porcine acellular muscle matrix scaffolds of different architectures from 2mm slices of porcine longissimus dorsi. Future work will focus on optimizing the procedures for recellularizing the scaffolds with populations of myogenic cells and processing the material into alternative forms such as a gel or printable bio-ink.

SC-INBRE and SC-INBRE Developmental Research Program via NIGMS P20GM103499
Winthrop University Research Council Awards SC15017, CE17010, and SC17014

Evaluation of Heterocyclic Biaryls as Aggregation Inhibitors for Alzheimer's Amyloid-β Peptide

Brandy Crenshaw (2019) **Mentors: Dr. Robin K. Lammi**
Augustine Vinson (2019) **Dr. James M. Hanna, Jr.**

Amyloid-β (Aβ) is a peptide of 39-43 amino acids that self-assembles into neurotoxic oligomers and fibrils implicated in 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 to enable binding to Aβ. We have previously demonstrated that biphenyltetrols (BPTs) exhibit varying degrees of efficacy as Aβ aggregation inhibitors. Of nine BPT isomers studied, 3,3′,4,4′-biphenyltetrol (3,4-BPT) is the most successful, effectively abrogating Aβ aggregation at stoichiometric concentrations (IC_{50} ~ 1X); other isomers are significantly less effective (IC_{50} ~2X to >10X), perhaps due to decreased abilities to hydrogen-bond with Aβ. Recent literature suggests that π-π interactions (i.e., π-stacking) may also be implicated in inhibitor binding to Aβ, potentially involving Phe residues in the central hydrophobic region of the peptide. In addition, empirical and theoretical studies with model compounds have shown that benzene-pyridine and benzene-pyridinium interactions are successively stronger than that between two benzene rings. Based on these observations, we have designed and synthesized a series of hydroxybiaryl architectures (Figure) incorporating pyridine or pyridinium rings, hypothesizing that these moieties may confer greater efficacy as aggregation inhibitors due to improved binding to Aβ. IC_{50} values have been determined by means of the Congo red (CR) spectral shift assay, which exploits CR's specific binding to β-structured aggregates to enable quantification of Aβ aggregation. We find successive, measurable improvements in inhibitory efficacy (i.e., decreases in IC_{50} values) when the phenyl ring of 4-phenylcatechol (IC_{50} ~5X) is replaced with a pyridine ring (IC_{50} ~4X) or an N-methylpyridinium (methylsulfate counterion; IC_{50} < 2X). A similar trend is observed between 5-(3,4-dihydroxyphenyl)-2-methoxypyridine (IC_{50} ~10X) and its N-methylated derivative (IC_{50} ~4X). Preliminarily, these results support our hypothesis and may suggest a role for π-stacking in inhibitor binding to Aβ.



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

The Relationship between Osteonal Geometry and Physiological Stresses (Compression vs. Tension) In the Cranial and Caudal Aspects of White-Tailed Deer Proximal Humerus

Michael DeLashmutt (2018) **Mentor: Dr. Meir Barak**

Remodeling refers to the continued biological process of resorbing primary bone tissue (primary osteons) and replacing it with a bone structure known as "secondary osteon" (or Haversian system). When a bone slice is observed in cross section, primary osteons have a brick like structure while secondary look circular in shape. Both structures have a central canal for blood vessels and nerves. The aim of this study was to investigate and quantify bone remodeling in the proximal humerus of white-tailed deer (Odocoileus virginianus). We hypothesized that the cranial and caudal aspects of the proximal humerus, which are subjected to tension and compression respectively, will demonstrate significantly different osteonal geometry such as size, circularity, and angle. Four proximal humeri cross-sections were embed, polished and then inspected with a polarizing microscope and stereoscope to determine areas of remodeling in the cranial and caudal aspects. Next, a scanning electron microscope was used to take high-resolution pictures of the caudal and cranial aspects. Finally, ImageJ© was used to count and assess the secondary osteons geometry. Our results, using a t-test, revealed that the secondary osteons in the cranial aspect, which is subjected to tension, were significantly larger, less circular, angled more medially, and had relatively smaller central canals compared to secondary osteons in the caudal aspect, which is subjected to compression. These results are in line with previous studies that were showing smaller secondary osteons in areas subjected to compression. The conclusion to our study demonstrates the relationship between bone structure and its function, and support the concept of bone functional adaptation.

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 Semaphorin 3A on Retinal and Dorsal Root Ganglion Growth Cones *in Vitro*

Ashley Di Falco (2019) **Mentor: Dr. Eric Birgbauer**

Growth cones are an extension of a growing neurite that responds to its guidance molecules, which are biochemical cues that aid in reaching the neurite's target destination in the developing nervous system. Guidance molecules that growth cones respond to can be positive, in which case cause the developing neuron to grow towards, or negative which cause the growth cone to collapse/retract and regrow in another pathway. The specific molecules that cause this guidance to occur remain unknown, but play a significant role in understanding how the developing nervous system operates. The goal of this study was to understand the key molecules in the developing visual system in chickens that guide growth cones from point A to point B. Semaphorin 3A (Sema3A) is believed to be insignificant according to literature in the collapse of retinal growth cones (RGCs) in embryonic chicks, but is a key guidance molecule in dorsal root ganglion cells (DRGs). The theory that Sema3A does not cause growth cone collapse was then tested on DRG and RGC explants that were extracted from embryonic chicks. DRGs and RGCs were then treated with Sema3A, and collapsed and non-collapsed growth cones were then counted. The DRGs served as a control group that insured the validity of the Sema3A collected. Significant collapse of RGC growth cones was observed in the presence of Sema3A, indicating that it is indeed an important guidance cue in the developing chick's visual system. Although collapse was observed, further research on a dose response curve is in progress. It is suggested that the data observed and reported in past literature showing that chick RGC growth cone collapse does not occur in the presence of Sema3A was due to several factors such as length of Sema3a treatment period or the dose concentration being too high or low

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.

Characterizing Three *UAS::eggless* RNAi Lines of *Drosophila melanogaster* for Future Studies

Rachel Edlein (2019) **Mentor: Dr. Kathryn Kohl**

Meiotic recombination, the crossing over of genetic information between homologous chromosomes, generates genetic variation that is important evolutionarily, and also ensures the proper segregation of homologues. Since proper crossing over is a key step during meiosis for many organisms, it is a highly regulated process. However, the exact regulation mechanisms are currently unknown. Our lab has been studying a gene responsible for heterochromatin formation in *Drosophila melanogaster* called *eggless* to gain more insight into whether the way DNA is packaged could be partly regulating recombination. In this project, I determined which of three different RNAi lines of *eggless* was best for future studies, by completing four assays. They were: qualitative analysis of fly ovary structure, quantification of melanotic tumors in fly ovaries, fecundity, and hatch rate. In characterizing the three RNAi lines using these assays, I identified the line that produced viable offspring and also reduced *eggless* mRNA levels – both necessary components for future studies.

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.

Immunotherapy as a Treatment for Cervical Cancer

Colin Frazier (2018) **Mentors: Dr. Zach Abernathy**
Sydney McCall (2019) **Dr. Kristen Abernathy**

Human Papilloma Virus (HPV) is the known root cause for the vast majority of cervical cancers. Cervical cancer is the fourth most common cancer in women worldwide, and it has become the number one cancer in some developing countries. Immunotherapy is a treatment used to stimulate or restore the ability of the immune system to fight infection and disease. Implementing immunotherapy to slow/eliminate the growth of cervical cancer cells is less harmful to the patient than other treatments such as radiation and chemotherapy. Our model seeks to better understand the dynamics among HPV, cervical cancer, and immunotherapy. Furthermore, through global stability techniques, we provide sufficient conditions on immunotherapy treatment to ensure the eradication of HPV and cervical cancer cells while allowing a positive population of healthy and immune cells to remain.

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.

A Simple, Low-Temperature Method for Size-Controlled Nanomaterials in Reverse Micelles

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

In this work, a simple, low temperature synthesis of spherical II/VI semiconductor nanoparticles has been developed which allows for precise control of the particle diameter. Nanoparticles are thermodynamically unstable due to unsaturated surface atoms, and will spontaneously aggregate to bulk in the absence of surface ligands. To prevent agglomeration, the nanoparticle synthesis was performed within the pool confines of reverse micelles that acted as templates for nanoparticle growth. Separate micelle solutions were formed containing varying amounts of aqueous metal and nonmetal ions. Dioctyl sulfosuccinate sodium salt (AOT) and heptane were used as the surfactant and oil phase, respectively. The two micelle solutions were mixed dropwise while stirred, causing the contents of the micelles to exchange rapidly, leading to the formation of nanoparticles within the pool. The nanoparticle solution was then heated slightly to improve monodispersivity. Brus' equation was used to estimate the radii of the nanoparticles based on the onset absorbance, while the water-to-surfactant molar ratio (w_o) was used to calculate the radii of the micelle pools. The size of the nanoparticles depended on the ratio of metal/nonmetal, with higher ratios yielding smaller sizes. In the range of ratios from 0.5 to 8, particle diameters ranged from 3.78 nm to 5.92 nm. The diameter of the micelle pools was kept at a constant 10.2nm, indicating that the pool size limits but does not dictate nanoparticle size.

This project was supported by the Winthrop Research Council.

Phytoremediation of Copper, Chromium, Nickel, and Zinc Using Native Grasses

Dakota G. Hawkins (2018) **Mentor: Dr. Clifton P. Calloway**
Matthew J. Hurtt (2018)

Phytoremediation is an environmental cleanup method using green plants to remove containments that lie within the soil. Plants move water from the soil, through the roots to the stems and leaves where it is lost by evaporation, drawing up dissolved substances in the process. While phytoremediation can take longer than other remediation methods, such as excavation, the cost of phytoremediation can be significantly lower with less disturbance to the environment. Excavating a ten-acre site to a depth of one foot would require handling roughly 20,000 tons of material. The goal of this project was to evaluate the metal uptake ability of three varieties of grasses native to the Carolinas. Copper, chromium, nickel and zinc were evaluated because of frequent use in human activities. For example, copper plumbing, chrome and nickel plating and galvanized aluminum. The most effective phytoremediation plants will have stem (leaf) metal concentration to root metal concentration ratios greater than one, indicating the metals are accumulating in the stem (leaf) portion of the plant. A second consideration is the concentration limit for plant survival.

Dwarf Mondo Grass, Rush Blue Dart and Spike Dracaena varieties were evaluated for metal uptake capacity. Grass samples were watered with both tap water and tap water spiked with 100 mg/L copper, chromium, nickel and zinc. Soil, root and stem aliquots were collected at one week intervals. To determine metal concentrations, all samples had to be put into solution form. The soil, root and stem samples were successfully dissolved in 10% nitric acid/6% hydrogen peroxide using microwave digestion, followed by 100-fold dilution. The four test metal concentrations in the resulting solutions were determined using inductively coupled plasma – triple quadrupole mass spectrometry (ICP-QQQ) and calibration curve method of analysis.

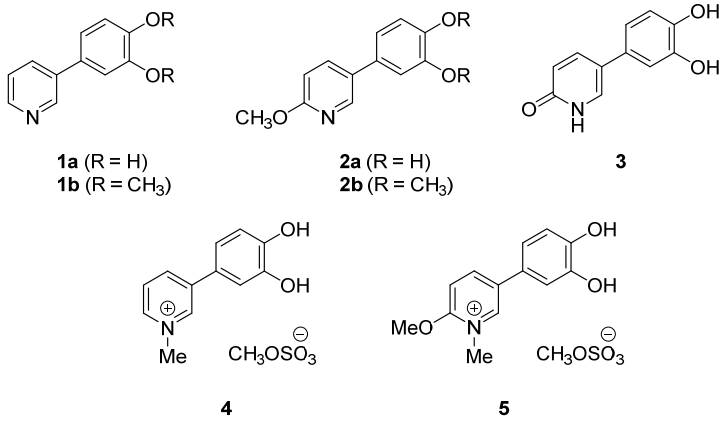
The stem-to-root ratios for the Dwarf Mondo Grass and the Rush Blue Dart initially increased, then slowly decreased after the first week of watering with contaminated water. In fact, the only plant species to survive after four weeks of watering and growth, under elevated concentration conditions, was the Spike Dracaena. The Mondo and Rush plants were overwhelmed by the additional 100 mg/L concentration of metals. However, the Mondo grass was effective at moving metals through the soil in the tap water, where the concentrations of the test metal is lower.

This work was supported by the National Science Foundation's Major Research Instrumentation Program (NSF MRI, grant CHE-1531698). We would also like to thank SC-INBRE (IDeA Network of Biomedical Research Excellence) and Dr. Brad Jones, Dean, Wake Forest Graduate School and the Wake Forest University URECA (Undergraduate Research and Creative Activities) Center for providing support to this project.

Synthesis of Heterocyclic Biaryls as Aggregation Inhibitors for Alzheimer's Amyloid-Beta Peptide

Benjamin Hernandez (2018) **Mentors: Dr. James M. Hanna, Jr.**
Mouskudah Murray (2018) **Dr. Robin K. Lammi**

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). We speculate that this may be due to differences in ability 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 literature data indicate that pyridine-benzene and pyridinium-benzene π-stacking interactions are stronger than those between two benzene rings. Based on these observations, we hypothesized that incorporation of pyridine and/or pyridinium moieties into the above-described hydroxybiaryl scaffold may lead to increased inhibition of Aβ aggregation. Therefore, compounds **1a**, **2a**, and **3** – **5** (figure) were synthesized for evaluation. Dihydroxypyridines **1a** and **2a** were synthesized via a Suzuki coupling/demethylation protocol. An appropriate bromopyridine was coupled with 3,4-dimethoxyphenylboronic acid in ethylene glycol using $Pd(OAc)_2$ as the catalyst; excellent yields of both **1b** and **2b** were obtained. Demethylation of **1b** with BBr_3 gave a respectable yield of the desired **1a**. Surprisingly, demethylation of **2b** under the same conditions did not give the expected product (**3**), but instead afforded a moderate yield of **2a**. Subsequent methylation of **1a** and **2a** with dimethyl sulfate gave good yields of **4** and **5**. Compound **3** was ultimately synthesized by refluxing **2b** in 48% aqueous HBr for several hours. Evaluation of these compounds' inhibitory efficacy is underway.



1a (R = H)
1b (R = CH_3)

2a (R = H)
2b (R = CH_3)

3

4

5

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

The Effects of Physiological Loading on Cortical Bone Stiffness in White-Tailed Deer Proximal Humerus

Naima Jackson (2018) **Mentor: Dr. Meir Barak**

Fibrolamellar bone is a transient primary bone tissue found in fast-growing juvenile mammals such as white-tailed deer. As deer grow, and in direct relation to the load their bones experience, fibrolamellar bone is remodeled into secondary osteonal bone tissue, also known as haversian systems. The structural switch from primary bone to secondary bone affects also the mechanical properties of the bone. While fibrolamellar bone is an orthogonal structure and thus demonstrates three different values of stiffness along its three main axes, osteonal bone is transverse isotropic and thus has just two different values of stiffness (due to the circular nature of the osteon, average stiffness in the two axes normal to the long axis of the osteon are alike). In this project, we examined the stiffness of bone material in the proximal humerus of young white-tailed deer. As the humerus is loaded during locomotion parallel to its long axis (via the humeral head), it is still debated whether it undergoes bending or pure compression (due to muscle action). Investigating the mechanical behavior of the proximal humerus can reveal whether this bone is loaded in compression on both sides like other long bones, or if it is loaded in tension on the cranial side and in compression on the caudal side (i.e. bending). Ten bone cube samples (2x2x2 mm) were machined from the cranial and caudal aspects of the proximal humeri of four young white-tailed deer (total n=20). All cubes were mechanically tested, within their elastic region, in compression in all three orientations – axial, radial, and transverse. Stiffness values in the three orientations were compared between the cranial and caudal aspects of the proximal humerus. Our results revealed that bone in the caudal aspect of the proximal humeri demonstrated a clear orthotropic behavior, while the cranial aspect demonstrated an intermediate behavior between orthotropic and transverse isotropic (i.e. stiffness differences between the radial and transverse directions were much smaller in the cranial aspect). These findings indicate that the cranial aspect of the proximal humeri in white-tailed deer undergoes remodeling earlier and to a greater extent, which in turn suggests that the humerus is loaded in bending.

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.

Analyzing Anthropogenic Effects on Sandy Beaches and Meiofaunal Community Composition Using Metabarcoding

Douglas Johnson (2018) **Mentor: Dr. Julian P.S. Smith III**
Jeremiah JonesBoggs (2020)

Marine meiofauna, comprising sub-millimeter representative from most animal phyla, is ubiquitous in the marine benthos, ranging from the intertidal to the deep ocean. Continuing controversy exists over their relative important in benthic ecosystem processes. Therefore, their importance to the essential ecosystem services provided by marine benthos remains open to question. Although recent research has shown that meiofauna can exert significant effects on sediment structure and stability, nutrient cycling, waste removal, and linkage of microbial production to higher trophic levels, whether or not these results are general is unknown. The question is important because the meiofauna is affected by the same anthropogenic stressors to which marine benthic communities are currently exposed. Therefore, in addition to hypothesis-testing, it is also important to have a baseline for comparison in order to detect future changes in marine meiofaunal communities. Broadly, we propose to establish community metabarcoding as technique at Winthrop University, to use that technique to determine alpha diversity of the meiofaunal communities from two sties differing in degree of anthropogenic stress, and to use a modified version of community metabarcoding to determine trophic connections in these meiofaunal communities.

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

Effects of Allophane on Sequestration of Organic Carbon and Nitrogen, and Nutrient Content of Compost Derived from Food Waste.

McKenzie Kargel (2017) **Mentor: Dr. Scott Werts**
Jaime Taylor (2017)

This study analyzed the effect of allophane on the sequestration of carbon, nitrogen, and phosphorus in compost over time. In the landfill environment, an estimated 40% of all greenhouse gas (GHG) emissions is from the decomposition of food waste. Carbon based GHG concentrations have raised to over 400 ppm (NOAA, 2017) (Eggen, 2001). Compost from food waste is important because the nutrients from food can be used to amend soil, thereby recycling the carbon and other nutrients from food waste back into the environment while also reducing the GHG emissions in landfills. Allophane is a type of clay that weathers from volcanic ash and is known to complex with organic molecules. Allophane has been shown to complex with carbon by protecting it from being chemically or physically weathered, which keeps the carbon in the soil and out of the atmosphere for longer. This study investigated how allophane influences the development and decomposition of the nutrients in compost, specifically total organic carbon (TOC), total organic nitrogen (TON), nitrate, ammonium, and phosphate. It also investigated the effect of allophane in the soil on the growth of green bean (*Phaseolus vulgaris*) plants. 50 pots containing a soil/compost mixture and varying amounts of allophane were established, and *P. vulgaris* seeds were planted in each one. Samples were taken to analyze TOC, TON, nitrate, ammonium, and phosphate every 2 weeks. The *P. vulgaris* plants had a low germination rate, so data on plant growth for each group was not available. Over time, TOC and TON were both shown to gradually decrease with the biggest decrease happening over the first two weeks. For nitrate, ammonium and phosphate, the pots containing 0% allophane decreased in nutrient concentration over time, while pots containing 50% allophane did not decrease over time. However, some of the groups showed an increase in nutrient concentration over time, so it is possible that experimental error lead to this result.

This project was supported by a Research Council Grant from Winthrop University (SC17010) and the Margaret E. Spencer Summer Undergraduate Fellowship from the Environmental Program at Winthrop University.

The Effects of Thermal Stress on the Rate of Nondisjunction in *Drosophila melanogaster*

Olivia Livingston (2017) **Mentor: Dr. Kathryn Kohl**

Meiosis is a process in which a diploid parental cell divides to create four genetically unique haploid cells. However, if the chromosomes improperly segregate (non-disjoin) during this process, aneuploid cells containing the incorrect number of chromosomes result. Aneuploidy is the leading genetic cause of developmental disabilities in humans, emphasizing the importance of understanding the mechanisms by which it occurs. There have been many studies on recombination, the exchanging of genetic material of homologous chromosomes in meiosis, and how this process is altered by stress. It is also known that if these recombinational events in the chromosomes are the incorrect distance from the centromere, the rate of nondisjunction is increased. However, it is unknown whether stress affects the rate of nondisjunction in organisms. Thus, in this experiment, we heat stress *Drosophila melanogaster* females to determine if the rate of nondisjunction is altered. These heat shocked females are paired with males carrying a dominant eye phenotypic marker on their Y chromosome. Scoring the offspring using their eye phenotypes and sex allows us to calculate the rate of nondisjunction occurring in the heat-shocked female parent. Thus far, we have scored 30,500 progeny from 687 parental females that were subjected to one of three temperature treatments. Once scoring is complete, the results of this study will provide insight into the process of nondisjunction and how stress might alter its rate of occurrence in organisms.

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 Identifying the Phosphorylation Site on RitR

Carra Lyons (2018) **Mentor: Dr. Nicholas Grossoehme**

Iron is essential to the survival of nearly all known organisms. However it can also be detrimental to life if there is excess in a cell that is not being used. Free iron in the cell acts as a catalyst, reacting with common oxygen species within the cell creating hydrogen peroxide and hydroxyl radicals. This is a big problem when these free hydroxyl radicle react with other molecules within the cell. This can cause a lot of damage. Therefore every organism has to have a way to control iron uptake, to inhibit excess levels of iron within the cell.

In S. pneumonia the iron uptake mechanism is activated by extracellular iron, however the sensory mechanism used to inhibit this uptake is not yet well understood. When iron is sense extracellularly a complex known as Stk-P is activated, and in the presence of ATP this molecule will phosphorylate RitR. When not phosphorylated RitR is bound tightly to the DNA of S. pneumonia in close proximity to the piu (pneumococcal iron uptake operon), preventing transcription of that portion of the DNA. When phosphorylated RitR is not bound to the DNA, allowing transcription to occur.

This research focuses on the location of phosphorylation on RitR. This will help understand how this protein functions and how it interacts with the DNA. Additionally, this work explores the difference in binding between RitR in its purified form, and the modified version of RitR.

To analyze these two important questions, first phosphorylated RitR was needed. This was accomplished by purifying RitR and StkP followed by incubation of both proteins in the presence of ATP. This reaction was monitored using ATP assays to ensure that the ATP concentration decreased as expected. As anticipated ATP was consumed at a significantly higher rate when Stk was present with RitR than with RitR or Stk alone, indicating that phosphorylation was occurring.

DNA binding was then tested using fluorescence anisotropy. It was seen that Phosphorylated RitR bound very slowly compared to the purified RitR, indicating that the phosphorylation of RitR plays a role in allowing transcription of the pneumococcal uptake operon.

Purified RitR and modified RitR was then incubated with trypsin allowing the protein to be digested into peptides to be further analyzed using mass spec, as well as being sent away for analysis with and external lab.

Supported by EPSCoR REU Grant and the SC INBRE grant from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health

Synthesis and Analysis of Potential Sphingosine Kinase 1 Inhibitors

Sara Manore (2019) **Mentor: Dr. Christian T. Grattan**

The sphinogmyelin metabolic pathway is a popular target area of research due to the potential apoptosis in cancer cells. In the pathway, sphinogmyelin may be converted to the final product of sphingosine-1-phosphate. Sphingosine-1-phosphate is associated with cell proliferation in cancer cells. This is due to the over-expression of sphingosine kinase 1, an enzyme that catalyzes the phosphorylation of sphingosine to form sphingosine-1-phosphate. Inhibition of sphingosine kinase 1 would prevent proliferation and lead to the desired apoptotic outcome if a potent inhibitor can been identified. Beginning with a promising lead molecule based on *in* vitro studies, synthetic production of a number of structurally modified variations of the inhibitor were prepared to improve the overall hydrophilicity of the lead. These variations have been successfully synthesized, purified and are now being tested against the enzyme for effective *in vitro* activity relative to the template inhibitor.

Using a Sphingosine kinase activity assay kit, the inhibitors were tested in the presence of ATP, sphingosine, and sphingosine kinase at varying concentrations to optimize the results. Our assay results show activities and inhibition results relative to our template structure and we hope to continue to optimize and realize the potential of these inhibitors as a possible treatment option in this cancerous pathway.

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.

Indication for Bone Remodeling in the Lateral Aspect, But Not the Medial Aspect, of Young White-Tailed Deer Distal Femur

Shirley Mathur (GSSM high-school student) **Mentor: Dr. Meir Barak**

Fibrolamellar bone is a transient primary bone tissue found in fast-growing juvenile mammals. As these animal grow, and in direct relation to the load their bones experience, fibrolamellar bone is replaced (remodeled) by secondary osteonal bone tissue, also known as Haversian systems. The structural switch from primary bone to secondary bone affects also the mechanical properties of the bone. While fibrolamellar bone is an orthogonal structure and thus demonstrates three different values of stiffness along its three main axes, osteonal bone is transverse isotropic and thus has just two different values of stiffness (due to the circular nature of the osteon, average stiffness in the two axes normal to the long axis of the osteon are alike). In this project, we examined the innate stiffness of bone material in the distal lateral femora of young white-tailed deer. As the femur is loaded during locomotion parallel to its long axis (via the femoral head), it is still debated whether the femur undergoes bending or pure compression (due to muscle action). Investigating the mechanical behavior of the distal femur can reveal whether this bone is loaded in compression on both sides like other long bones, or if it is loaded in tension on the lateral side and in compression on the medial side (i.e. bending). Thirty-seven bone cube samples (2x2x2 mm) were machined from the lateral aspect of the distal femur of three young white-tailed deer. All cubes were mechanically tested, within their elastic region, in compression in all three orientations – axial, radial, and transverse. Stiffness values in the three orientations were compared to matching data collected previously from the medial aspect of the same distal femora. Our results revealed that axial stiffness in the lateral aspect was significantly lower comparted to the medial aspect. In-addition, bone stiffness in the radial and transverse orientations was close to isotropic, signifying the remodeling of fibrolamellar bone into osteonal bone in the lateral aspect of the distal femur. These results are in contrast to the medial aspect of the bone, where a clear orthogonal mechanical behavior was demonstrated (axial > transverse > radial), indicating that the original fibrolamellar bone was retained. Thus, we now have evidence to suggest that the femur of white-tailed deer is loaded primarily in bending during locomotion. Currently, histological cross-sections are prepared to further investigate the structural differences between the medial and lateral aspects of the distal femur.

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.

Prime Labelings of Graphs and the Coprime Graph

Justin McCullough (2018) **Mentor: Dr. Arran Hamm**
Alan Way (2018)

Graph labeling problems date back to the beginning of Graph Theory itself (see the Four Color Theorem). Roughly 40 years ago the notion of a prime labeling of a graph was introduced; a graph on n vertices has a prime labeling if its vertices can be labeled by the numbers 1, 2, … , n so that each edge spans a coprime pair (i.e. each edge's labels have greatest common divisor one). In the 1980's Entriger conjectured that a certain family of graphs all have prime labelings; our work furthered the progress on this conjecture by giving a prime labeling for several members of this family. Additionally, we studied graph parameters related to the coprime graph. The coprime graph on n vertices is the graph whose vertices are numbered 1, 2, … , n with i~j if and only if i and j are coprime. Using the graph parameters we calculated, we were able to conclude that several classes of graphs are not prime.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 17) from the National Institutes of Health.

Synthesis of Trifluoromethyl-1H Pyrazole Derivatives to Optimize Activity as Antimicrobial Agents

Eulillian McFadden (2019) **Mentor: Dr. Christian T. Grattan**

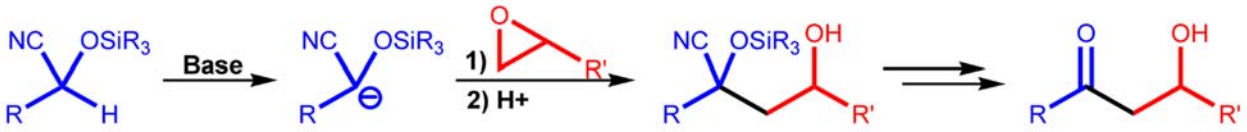
A series of trifluoromethyl-1*H*-pyrazole derivatives with the inclusion of aryl ring systems was synthesized through the condensation of 1,3-diketones with hydrazine under microwave irradiation. The newly synthesized compounds were characterized and confirmed by NMR (^{1}H, ^{13}C, ^{19}F) as well as by melting point, indicating microwave-assisted synthesis as a viable route to the efficient formation of these aryl and trifluoromethyl substituted 1*H*-pyrazoles. The 1*H*-pyrazoles were tested for their antimicrobial properties against *Escherichia coli* via disk diffusion in relation to ampicillin. All of the aryl and trifluoromethyl pyrazoles exhibited significant antimicrobial properties, with the naphthyl and trifluoromethyl substituted compound producing the highest degree of inhibition at 2.32 times that of the streptomycin. Additionally, this compound was further tested against its non-fluorinated analogue as well as against the unsubstituted *1H*-pyrazole, and exhibited similarly enhanced antimicrobial activity in relation, thus indicating 1*H*-pyrazoles incorporating both naphthyl and trifluoromethyl groups as compounds with high potential in antimicrobial drug discovery.

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 Reaction of O-Silylated Cyanohydrin Anions with Epoxides as an Alternative for the Enantio- and Diastereoselective Preparation of Aldols

Caylie A. McGlade (2019) **Mentor: Dr. Aaron M. Hartel**

The Aldol addition is one of the most important carbon-carbon bond forming reactions in organic chemistry. The reaction, which occurs between an enolized carbonyl and an aldehyde or ketone, results in the formation of a ß-hydroxy carbonyl, or "aldol" product. The reaction can generate up to two new chiral centers, making both enantio- and diastereoselectivity important. We have developed a new method for the stereoselective preparation of aldols exploiting the reaction of O-silylated cyanohydrins with epoxides.



The reaction conditions have been optimized and the scope of epoxide substrates has been explored. Current research is focused on investigating the scope of the cyanohydrin component. A series of O-silylated cyanohydrins were synthesized, treated with LiHMDS and the resulting anions reacted with 1,2-epoxybutane to effect alkylation. The crude adduct was then desilylated using TBAF to afford the final aldol product. Overall, variations on the cyanohydrin have been found to be less tolerated than those on the epoxide, however aryl substituents lacking any additional functionality provided the expected aldol in good yield.

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

Probing the Role of *High Mobility Group A1* (*hmga1*) in Chemoresistance Using 5-Fluorodeoxyuridine (FdUrd)

Maryssa Shanteau-Jackson (2018) **Mentor: Dr. Takita F. Sumter**
Tanisha Moore (2019)

Chemoresistance is a major limitation to effective cancer treatment regimens. Specifically, cancer stem cells, self-renewing cells that can differentiate, provide a pathway to escape treatments by targeting rapid cell division pathways. *High mobility group A1 (hmga1)* is implicated in the initiation and progression of various cancers and may be involved in the genetic events leading to the growth of cancer stem cells. Mice bearing the *hmga1* transgene develop aggressive lymphoid malignancies and are less responsive to chemotherapies that have been tested. In fact, somatic cell reprogramming pathways required for cancer stem cell development begin with the Wnt/β-catenin pathway which results in transactivation of oncogenes including *SOX2*, *cMYC*, and *hmga1*. Moreover, hmga1 is both activated by and activates Wnt/β-catenin signaling to amplify the relevant epigenetic pathways. Because high mobility group A1 (HMGA1) is overexpressed in many cancers and is associated with the epigenetic events involved in pluripotency, we explored the role of *hmga1* in chemoresistance using 5-fluorodeoxyuridine (5-FdUrd). 5-FdUrd, is the active antimetabolite of a mainstay in cancer treatment whose activity is based on the misincorporation of fluoropyrimidines into DNA and RNA during their synthesis. Studies were conducted using HCT-116 colorectal cancer cells with high endogenous levels of hmga1 proteins. These cells were treated with varying concentrations of 5-FdUrd and IC_{50} values were determined to be comparable to, but slightly higher than, previously published values. Silencing of *hmga1* expression by siRNA duplexes targeting different genetic regions enhanced sensitivity to 5-FdUrd by greater than 1.5 to 3-fold when compared to native HCT-116 cells. To determine if enhanced sensitivity was related to the ability of HMGA1 proteins to bind DNA, we conducted fluorescence anisotropy in the absence and presence of 5-FdUrd. This investigation is currently in progress and results of this work will may provide mechanistic insights into previous findings. Collectively, we provide data that supports the role of hmga1 in orchestrating the ability of cancer cells to evade the impacts of chemotherapy, particularly those targeting cell division pathways. We expect that this work will contribute to an expanded understanding of cancer initiation and progression and will facilitate development of more effective cancer therapies.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 17) from the National Institutes of Health.

The Effect of Physiological Loading on Cortical Bone Remodeling in White-Tailed Deer Proximal Humerus

Jack Nguyen (2018) **Mentor: Dr. Meir Barak**

Remodeling – the replacement of primary bone with secondary (osteonal) bone was shown to be affected by the type of stress (compression vs. tension) and its magnitude. This study investigated the effect of loading on the morphology and geometry of secondary osteons in the proximal humerus of white-tailed deer. Two cross-sections from the proximal diaphysis of four white-tailed deer humeri were prepared using a low-speed water-cooled diamond saw. One cross-section of each humeri was embedded in an epoxy block and viewed using a scanning electron microscopy (SEM) and the other cross-section was decalcified and viewed with a polarized light microscope. Next, multiple images of each cross-section were captured and then stitched together (PTGUI©) to create a full view of each humerus proximal transverse plane, to determine the areas of bone remodeling. Finally, secondary osteons' geometry and size were measured (ImageJ©) for each humeri in the medial, lateral, cranial and caudal regions. Our results showed that secondary osteons in the cranial region are significantly larger, more angled medially and are less porous (smaller central canal area to osteonal area ratio) than those found in the other three regions. On average, the osteon area in the cranial region is 6369 pixels^2 compared to 4085, 3717, and 4163 pixels^2 in the medial, caudal, and lateral regions respectively. Osteons in the cranial aspect of the humerus are angled on average 105.8º to the frontal plane while osteons in the other three regions are almost perfectly normal to that plane (~90 º). The central canal area to osteonal area ratio is 3.7% for the cranial region, 4.8% for both the medial and lateral regions and 4.7% for the caudal region. These findings are consistent with previous reports in other bones and species. Areas which are subjected to tension (in our case the cranial aspect of the proximal humerus) tend to have larger and less porous secondary osteons. One possible explanation to these findings is that areas which are loaded in compression - usually experience higher magnitude of stresses. Thus, smaller resorption canals (and thus smaller osteons) are advantageous as they will negatively affect, to a lesser extent, the mechanical properties of the bone compared with larger resorption canals.

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.

Conductive $Ni_3(HITP)_2$ supercapacitors Electrodes Fabricated by Electrophoretic Deposition

Jason Peck (2020) **Mentor: Dr. Fatima Z. Amir**

Metal−organic frameworks (MOFs), known as porous coordination polymers, have features such as large surface area, crystalline ordered structure, and highly regularized pores and have emerged as a promising class of materials with a wide spectrum of useful applications. MOFs are also 2D nanosheets materials, which are good candidates for applications in various electrochemical applications such as supercapacitors, batteries, and fuel cells. In this work, we report the fabrication of Ni_3(2,3,6,7,10,11-hexaiminotriphenylene)$_2$ ($Ni_3(HITP)_2$) supercapacitors electrodes by electrophoretic deposition (EPD). The electrodes' surface morphology characterized by SEM shows a honeycomb morphology, which is known for improving the capacitive performance. TEM pictures confirmed the ultrathin two-dimensional structure of ($Ni_3(HITP)_2$). XPS measurement show only the presence of Ni, N, C and O resonance peaks confirming the formation of the monoanionic *o*-diiminobenzosemiquinomate moieties. Zeta measurements show low Zeta potential indicating a low stability of the solution used in EPD and may lead to low electrochemical performance.

This work was supported in part by Winthrop University Research Council

Field Study of the Late Devonian Mass Extinctions; Great Basin, USA

Tyler Robbins (2017) **Mentor: Dr. Diana Boyer**
Timothy Swartz (2018)

The Kellwasser and Hangenberg extinction events in the late Devonian Period were formative in shaping the evolution of life. These events saw massive changes in biodiversity and the duration, mechanism, and prevalence of these events is not fully understood. The Great Basin, specifically Utah and Nevada, provide a natural laboratory to investigate these events. In order to ask first order questions about these biodiversity crises, samples were collected from six localities for fossil, inorganic and organic geochemical analysis. Nearly 150 meters of exposure were described in detail and over 100 individual samples were collected for geochemical and trace fossil analysis to be carried out in the coming years. These samples allow us to characterize ocean conditions before, during and after these biocrises, and ultimately get closer to understanding the kill mechanism(s) of these extinctions. Further, this research will provide a framework to better understand the global signal at this time period.

This research was supported by a WU Research Council Grant (Robbins, Swartz) and RUI NSF-grant 1664247 (Boyer).

Exploring a Possible Moonlighting Role for a Global Phosphatase in *S. pneumoniae*

Hunter Sellers (2019) **Mentor: Dr. Nicholas Grossoehme**

Iron is essential to an overwhelming majority of life on Earth; however, in aerobic conditions it can take on multiple oxidation states and create harmful oxidative species that must be regulated to maintain the health of the cell. Most bacteria use a family of proteins called FUR proteins (ferric uptake regulatory proteins) to maintain safe levels of iron in the cell, but S. pneumonia contains no such proteins. Instead, it uses a system of three proteins StkP, RitR, and PhpP coupled together in a system that controls transcription of iron regulatory and redox stress managing genes through the pneumococcal iron uptake operon. While there is an obvious extracellular iron sensor in StkP, the necessary intracellular iron sensor has yet to be discovered that signals PhpP to dephosphorylate RitR and allow it to suppress the transcription of these iron uptake genes. Knowing that PhpP is a magnesium dependent protein, we hypothesize that perhaps PhpP is activated by intracellular iron in S. pneumonia, thus providing the intracellular iron sensor that it needs. To test this, we first used para-Nitrophenylphosphate assays (a surrogate for phosphorylated RitR) along with manganese as as aerobic condition friendly surrogate to show that PhpP is activated by manganese. The original idea was to vary the concentration of manganese in these PNPP assays in an attempt to extract affinity values of PhpP for manganese; however due to experimental limitations, we moved forward using fluorescence competition experiments with the metal binding fluorophore Mag-Fura-2. Using these experiments, we were able to conclude affinity values of affinity values of 1 μM for manganese and 1 mM for magnesium.

Supported by EPSCoR REU Grant and the SC INBRE grant from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health

Expression and Purification of XopAZ, a Ubiquitous FKBP Found in Phytopathogenic Strains of Xanthomonas

Mallory Sorenson (2018) **Mentor: Dr. Jason C. Hurlbert**

Successful invasion and colonization of host tissues by pathogenic bacteria often requires injection of bacterial proteins directly into host tissues. In phytopathogenic strains of Xanthomonas, this is accomplished via a modified flagellar apparatus called the Type 3 Secretion System (T3SS). Bacterial proteins are unfolded and guided into the T3SS and emerge in the host cytoplasm. In order to ensure proper folding of the bacterial proteins, chaperones and peptidyl-prolyl isomerase (PPIase) are often injected into the host to help refold the bacterial proteins. We have identified a new PPIase found in all strains of phytopathogenic Xanthomonas strains. Bioinformatic analysis reveals significant amino acid identity to the *Escherichia coli* protein, SlyD, a FK506 binding protein (FKBP) consisting of a chepaerone domain and a PPIase domain. As part of our efforts to determine the three-dimensional structure of the protein, this summer we attempted to express and purify the Xanthomonas FKBP from recombinant *E. coli* hosts. We determined the optimal expression temperature to be 25 C and the best recombinant host was found to be *E. coli* NiCo. We then developed a two step purification protocol that allowed purification of the protein to homogeneity. The cell lysate was first passed over a metal chelating resin charged with Co^{2+} and eluted with a linear gradient of 10-500mM Imidazole. Protein containing fractions were then dialyzed against 50 mM HEPES, 50 mM KCl, pH 8 and applied to an anion exchange column (UnoQ, BioRad, Inc.). Purified FKBP eluted at approximately 200 mM KCl. Future work on this protein will involve determining the solution conditions suitable for crystal growth followed by x-ray diffraction analysis of the crystals and structure determination.

This project was supported by an Institutional Development Award from the National Institute of General Medical Sciences (2 P20 GM103499 17) from the National Institutes of Health.

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 ordinary differential equations to model the use of virotherapy as a treatment for cancer. This model specifically describes the interactions among infected tumor cells, uninfected tumor cells, effector T-cells, and virions. Using local and global stability analysis techniques, we establish conditions on model parameters to ensure a stable cure state of the full model as well as various submodels. We illustrate these dynamics through numerical simulations of the model using estimated parameter values from the literature, and we conclude with a discussion on the biological implications of our results.

Support for this research was provided by the Ronald E. McNair Scholars Program.

Stability Study on Eu:LaF_3 Nanoparticles for Optical Fiber Fabrication

Sophia Warren (2018)

Mentors: Dr. John M. Ballato
Dr. Courtney Kucera
Dr. Amber Vargas

Rare earth doped nanoparticles have beneficial properties in optical fibers. The implementation of europium (Eu), as the rare earth, in a lanthanum fluoride (LaF3) host was studied. For the sake of finding optimal synthesis conditions for these nanoparticles, pH is going to be the only varying component in this study. Prior studies indicate that the pH of a solution can affect particle size, stability and agglomeration of the nanoparticles during synthesis. Optimal conditions for the particles create a solution that can be used for incorporating the nanoparticles into a fiber preform for the production of optical fiber. The as-made synthesis solutions will be characterized using photoluminescence (PL), dynamic light scattering (DLS) and ultraviolet-visible spectroscopy (UV-vis).

The authors would like to thank the financial support of the NSF Award OIA-1632881, Center for Optical Materials Science and Engineering Technologies (COMSET) and AFL Telecommunications. The authors would also like to thank Katie Burdette in Foulger's group for help on FTIR and UVVIS and Katie Davis in Mefford's group for the help on DLS, zeta and stability studies.