

WINTHROP UNIVERSITY
SUMMER UNDERGRADUATE RESEARCH EXPERIENCE (SURE)
2016 ABSTRACT BOOK



WINTHROP
UNIVERSITY

Table of Contents

Student	Faculty Mentor	Page
Aaron Anderson-Rolfes	M. Gelabert, Chemistry	6
Melissa Barr	M. Stern, Biology	7
Arielle Black	M. Barak, Biology	9
Lindsay Bradley	K. Abernathy and Z. Abernathy, Mathematics	10
Angel Castro	C. Grattan, Chemistry	11
Caitlin Cridland	C. Grattan, Chemistry and J. Hurlbert, Biochemistry	12
Joshua Dasburg	K. Abernathy and Z. Abernathy, Mathematics	13
Brooke Davis	J. Smith, Biology	14
Karyssa Davis	E. Birgbauer, Biology	15
Madeline Diaz	T. Sumter, Chemistry	16
Garrett Driscoll	E. Birgbauer, Biology	17
Rayshon Ellis	A. Hartel, Chemistry	18
Justin Groves	A. Hamm, Mathematics	19
Benjamin Harrison	M. Barak, Biology	20
Dakota Hawkins	C. Calloway, Chemistry	21
Benjamin Hernandez	J. Hanna and R. Lammi, Chemistry	22
Erin Hershelman	J. Hurlbert, Biochemistry	23
Hannah Hopfensperger	M. Stern, Biology	24
Henry Horacek*	J. Smith, Biology	25
Audrey Hughes	J. Smith, Biology	26
Matthew Hurtt	J. Hanna and R. Lammi, Chemistry	27

Douglas Johnson	M. Heard and V. Frost, Biology	29
Christopher Jordan	J. Hanna, Chemistry	30
Ariel Kunde*	M. Barak, Biology	31
Robert Landry	K. Kohl, Biology	32
MaLyn Lawhorn	K. Abernathy and Z. Abernathy, Mathematics	33
Autumn Leggins	N. Grossoehme, Chemistry and M. Stern, Biology	34
Victoria Leroy	E. Birgbauer, Biology	15
Kayla Margin	J. Hurlbert, Biochemistry	35
Elizabeth McAbee	M. Stern, Biology	7
Nicole McMullen	H. Evans-Anderson, Biology	36
Kristen Melton	A. Hamm, Mathematics	37
Natalie Mseis	M. Stern, Biology	39
Dakoda Mullinax	A. Hartel, Chemistry	40
Ansley Nemeth	C. Grattan, Chemistry	41
Jack Nguyen	M. Barak, Biology	42
Carolina Pham	M. Stern, Biology	39
Davis Plasko	J. Hanna, Chemistry	44
Brittney Ramsey	J. Hurlbert, Biochemistry	45
Jake Roberts	J. Hanna and R. Lammi, Chemistry	22
Joshua Sauer	J. Smith, Biology	46
William Schreiber	R. Lammi, Chemistry	27
Evan Schultheis	F. Amir, Physics	47
Andrew Sellers	M. Heard and V. Frost, Biology	29

Hunter Sellers	N. Grosseohme, Chemistry	48
Maryssa Shanteau-Jackson	T. Sumter, Chemistry	49
Megan Smith	C. Tant, Biology	50
Mikala Smith	C. Grattan, Chemistry	51
Jessica Stevens	M. Gelabert, Chemistry	52
Stephen Steward	K. Abernathy and Z. Abernathy, Mathematics	13
Lauren Travis	T. Sumter, Chemistry	16
Sarah Walter	C. Tant, Biology	50
Camerun Washington	K. Kohl, Biology	53
Emily Watson	S. Werts, Geology and M. Gelabert, Chemistry	54
Andrew Williams	K. Kohl, Biology	55

* Graduate student

Winthrop Students Performing Research at Other Institutions

Arielle Black	B. Terry University of Nebraska-Lincoln	9
Justin Hutchinson	J. Sullivan Medical College of Georgia-Augusta University	28
Alexander Middleton	M. Gobbert University of Maryland, Baltimore County	39
Jesslyn Park	E. Glasgow and B. Fox University of Wisconsin, Madison	44

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 2016, the Winthrop SURE Program celebrated its eleventh year, with a cohort of over 50 students working with more than 20 faculty mentors, examining important questions in biology, chemistry, biochemistry, mathematics, and geology. 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.

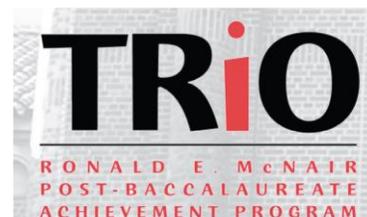
We also gratefully acknowledge Winthrop's administration, especially President Dan Mahony and Provost Debra Boyd, for their ongoing support of SURE and undergraduate research.

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



Synthesis of Zinc Oxide Nanoparticles using Acetate and Sulfate Salts

Aaron Anderson-Rolfes (2017)

Mentor: Dr. Maria Gelabert

The synthesis of zinc oxide nanoparticles has applications in multiples areas ranging from ultraviolet-absorbing skin creams to bacterial remediation. There are many methods to obtain these nanoparticles, and this project investigated variable conditions of aqueous precipitation. The use of water as a solvent is more environmentally benign than current methodology for ZnO nanoparticle synthesis. The solutions with precipitates were prepared in a sonication bath and analyzed with X-ray diffraction and particle size analysis. Zinc oxide particles were synthesized using two different zinc salts, zinc acetate and zinc sulfate, along with sodium hydroxide. To prepared zinc solutions (0.01-0.02 M), fixed volumes of sodium hydroxide (0.0500-0.5014 M) were added over 30 minutes agitation. Relationships between experimental variables and crystal size were examined, where the main variables studied were differing zinc and sodium hydroxide molarities, temperature of the solutions, as well as differences between sonication and stirring. The resulting average particle sizes showed a wide range, with a smallest particle size of 0.60 microns and the largest at 241.3 microns, both obtained with zinc sulfate; acetate samples produces sizes between 5.28 and 172.0 microns. Comparison of the two zinc reagents points to sulfate having the higher capability for smaller particle sizes. The smallest zinc oxide nanoparticles were produced at the high pH of around 12-13.5. Although consistent results were not obtained, two observed trends are that zinc sulfate and high pH produced the smallest particles. These observations are valuable for adding to fundamental knowledge about precipitation from aqueous solution, later leading to reproducible preparations of zinc oxide nanoparticles.

Support for this research was provided by the Research Council of Winthrop University.

Spheroid Culture and Epigenetic Modifiers Alter the Expression of Genes Associated with Developmental Potency and Myogenic Potential in Adipose-Derived Stem Cells

Melissa Barr (2018)
Elizabeth McAbee (2018)

Mentor: Dr. Matthew Stern

Adipose-derived stem cells (ADSCs) are multipotent mesenchymal stem cells obtained from the microvasculature of adipose tissue and represent an attractive source of easily accessible autologous stem cells for therapeutic use. While ADSCs can generate several cell lineages, they cannot match the differentiation potential of pluripotent embryonic stem cells. However, our previous studies have suggested that the non-traditional method of culturing murine ADSCs as three-dimensional spheroids can induce the expression of factors associated with pluripotency, including the transcription factor OCT4. We hypothesized that spheroid culturing in combination with manipulation of the epigenome can upregulate the expression of several genes associated with pluripotency and increase the myogenic differentiation potential of ADSCs. Our results demonstrate that spheroid culturing, treatment with the histone deacetylase inhibitor trichostatin A, and inhibition of DNA methylation with 5-azacytidine all impact the expression of genes associated with developmental potency and/or myogenic potential. Future work will seek to identify the combination of conditions that generates a population of cells most amenable to myogenic differentiation. This would enable ADSCs to serve as a plentiful source of myogenic cells for skeletal muscle tissue engineering and regenerative medicine applications.

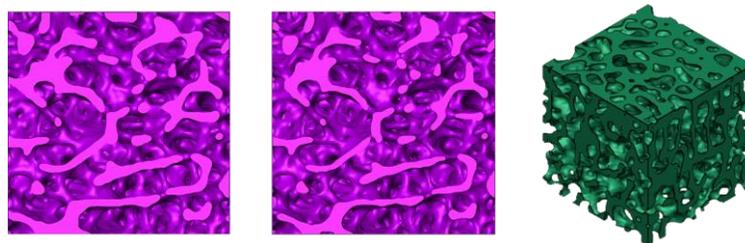
Funding for this project was provided by the South Carolina INBRE Developmental Research Program, The Winthrop University Research Council, Winthrop College of Arts and Sciences, and Winthrop Department of Biology.

Cancellous bone strength and stiffness in health and osteoporosis: A 3d printed model

Arielle Black (2017)

Mentor: Dr. Meir Barak

Cancellous bone structure is important to the mechanical behavior of whole bones. Studies have shown that osteoporosis affects cancellous bone structure, mostly by decreasing trabecular thickness and trabecular number, leading to an increase in fracture risk. One key limitation of determining the exact effect of this structure deterioration is that no two cancellous tissues are identical. Thus, when we compare a group of healthy vs. osteoporotic samples we only get a general trend. 3D-printing is a relatively new technology with promising scientific potential. Here we report a preliminary study that compared a healthy 3D-printed cancellous bone model with the same model after osteoporosis was simulated (i.e. trabecular thickness and number were reduced). Since both models are derived from the same structure it is possible for the first time to directly estimate (percentage wise) the decrease of tissue stiffness and strength as a result of cancellous bone structural deterioration. A cubical volume of interest (4.5x4.5x4.5 mm) was cropped and segmented (Amira 6.0; Fig. 1). To simulate the onset of osteoporosis (loss of about 10% of bone mass), a second model was created (from the same original scan) by raising the gray-scale threshold. The "healthy" and "osteoporotic" models were printed in scale (1:1 to real life size) 30 times each, using a 3D-printer (ProJet 1200). Next, all samples were tested in compression (Instron 5942) until failure and cancellous tissue strength and stiffness was calculated. Our results demonstrate that "osteoporotic" cancellous structure was significantly less strong and stiff than the original intact (healthy) structure ($P < 0.01$). Structural strength decreased by 24%, from an average of 9.14 ± 2.85 MPa to 6.97 ± 2.44 MPa, while structural stiffness decreased by 20% from an average of 282.5 ± 63.4 N/mm to 226.6 ± 37.0 N/mm. Despite the fact that these 3D models are made of printing resin and not bone tissue, they enable us for the first time to compare the exact same cancellous structure before and after the onset of osteoporosis. Our results demonstrate that a relatively small decrease in bone volume (loss of about 10% of bone mass) lead to a dramatic decrease in structural strength (24%) and stiffness (20%). This study demonstrates that 3D-printing is a novel and valuable tool for mechanically testing cancellous structures in order to estimate their mechanical properties. Thus, 3D printing has the potential to advance our understanding of mechanisms, such as osteoporosis, that affect cancellous bone structure.



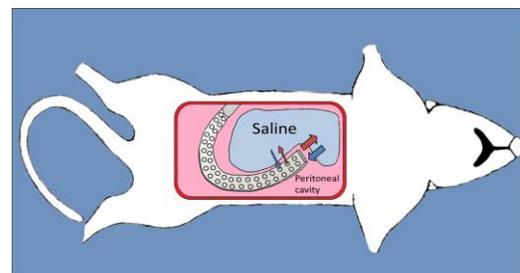
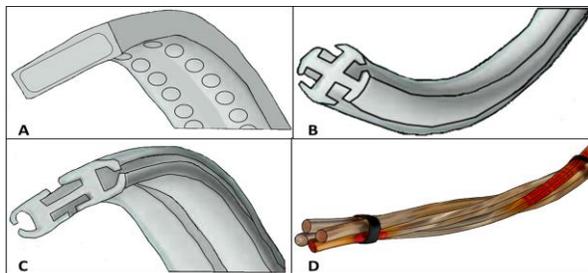
This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

An Effective Catheter for Long-Term Peritoneal Drainage in Rats

Arielle Black (2017)

Mentor: Dr. Ben Terry
University of Nebraska-Lincoln

Patients diagnosed with Acute Respiratory Distress Syndrome (ARDS) are initially treated via mechanical ventilation. Once this technique is no longer adequate, the last resort for treatment currently lies in extra corporeal membrane oxygenation (ECMO). ECMO is an artificial support of the respiratory and cardiac systems by oxygenation and removal of carbon dioxide from the blood. However, this type of supportive treatment carries the need for high technological expertise and is limited in its portability. It also carries inherent risks, such as cranial hemorrhaging due to systemic heparin infusion. A new, novel treatment for such respiratory failures is currently being developed. Oxygen microbubbles (OMBs) are bubbles formed by a lipid monolayer that contain a pure O₂ core. The OMBs are infused into the abdominal cavity where the oxygen will freely diffuse through the lipid layer and into the large vascular system present in the abdominal cavity. Thus, oxygen is provided to the patient with low risk and with the ability to be available in ambulatory situations. However, during long-term studies evaluating the OMB treatment of rats induced with symptoms similar to ARDS, the peritoneal lavage catheters would become occluded with omental tissue forcing an early end to the study. The purpose of this study is to determine the most effective catheter design to prevent tissue growth from blocking the OMB treatments in rats. 4 types of catheters will be tested including a Jackson-Pratt, a 15 Fr. Round Full Fluted, a 7mm Flat ¾ Fluted, and an originally designed Spiral Fusion. They will be surgically placed into the peritoneal cavity of 12 rats (n=3). Saline will be infused and extracted, following a flush method similar to OMB treatments, in order to test the efficacy of each catheter twice daily. Catheters will be scored on a weighted scale based on the amount of time they remained patent, the ease of extraction/infusion, and the amount of saline able to be removed. It was observed that the Round Full Fluted and Flat ¾ Fluted catheters were able to be used for the full length of the study (12 days) compared to the other models failing earlier. This corresponded with a high average of saline able to be extracted daily and a typically easy extraction/infusion. Thus, the Round Full Fluted catheter and the Flat ¾ Fluted catheters hold significant value as the best choice to be used in long-term peritoneal drain studies in rats.



Funding for this work was provided by the National Science Foundation grant EEC-1263181, the DHHS-NHLBI of the NIH under award number 1 R21 HL129144-01, the University of Nebraska Biomedical Research Seed Grant No.507, the Nebraska Research Initiative Grant, the University of Nebraska-Lincoln's Office of Research and Economic Development, the College of Engineering, the Agricultural Research Division, the Department of Biological Systems Engineering, and the Department of Mechanical & Materials Engineering.

A Mathematical Model of Chronic Myeloid Leukemia with Treatment

Lindsay Bradley (2017)

**Mentors: Dr. Kristen Abernathy
Dr. Zach Abernathy**

Chronic Myeloid Leukemia (CML) is a prevalent type of cancer where the presence of cancer stem cells is well studied. In this presentation, we modify existing Gompertzian growth models to study the dynamics of CML and the effects of treatment on CML. In the absence of treatment, we demonstrate that the cure state is always unstable. We then present conditions on treatment parameters to guarantee a locally stable cure state. We conclude with numerical simulations and remaining open questions.

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.

Modification of Zone 1 on a Known Inhibitor of Sphingosine Kinase

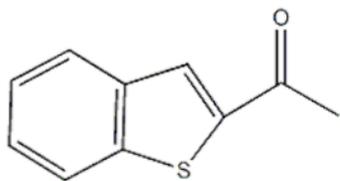
Angel Castro (2018)

Mentor: Dr. T. Christian Grattan

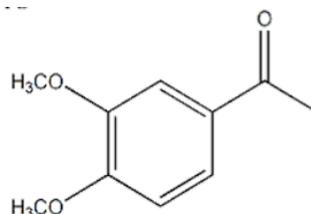
Sphingosine kinase 1 is an enzyme which cancer takes advantage of to cause proliferation in the sphingomyelin pathway and increase the malignancy of cancer. An inhibitor for Sphingosine kinase 1 has been discovered to halt the proliferation of the cell, but is not effective because of the hydrophobicity of the compound. The inhibitor was separated into four zones based on the functional groups found throughout the enzyme. The focus of this research was on the naphthaldehyde ring on the inhibitor which is known as zone one to decrease the hydrophobicity of the inhibitor.

The sphingomyelin pathway is what controls whether a cell proliferates (cell division) or undergoes apoptosis (cell death). The goal of this research is to use the inhibitor to stop the creation of sphingosine-1-phosphate which causes proliferation and create more ceramide as a result which will lead to apoptosis. The way this works is that the inhibitor attaches to sphingosine kinase which causes a build-up of sphingosine resulting in an equilibrium towards making ceramide which leads to cell.

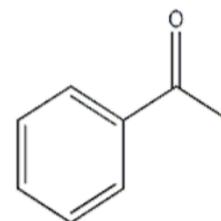
The zone 1 variations of the Sphingosine kinase 1 inhibitor that were made were benzothiophene ring, benzene ring, and 1,2-dimethoxybenzene (starting materials shown). These three derivatives all had the intended effect of lowering the hydrophobicity of the Sphingosine kinase 1 (SK1) inhibitor. The derivative that lowered the hydrophobicity most significantly was the 1,2-dimethoxybenzene as determined by a LogP calculation. The LogP value for the template inhibitor is 5.675 and the derivatives lowered the LogP value from .1 to 1 which made the inhibitor more hydrophilic.



benzothiophene



3,4-dimethoxybenzene



benzene

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

Evaluation of Zone 4 Inhibitors of Sphingosine Kinase Using Sphingosine Kinase Activity Assay

Caitlin Cridland (2017)

**Mentors: Dr. T. Christian Grattan
Dr. Jason Hurlbert**

The sphingomyelin pathway is an essential eukaryotic pathway directing the metabolism and synthesis of sphingolipids within the cell. Sphingolipids are key components of the lipid bilayer and are also involved in cellular signaling, such as cell proliferation and apoptosis. One of the enzymes involved in this pathway, sphingosine kinase 1 (SK1) is responsible for the ATP-dependent phosphorylation of sphingosine to sphingosine-1-phosphate (S1P). SK1 has been shown to be overexpressed in cancerous cells, a fact that may contribute to the resistance of certain cancer cells to chemotherapy and radiation treatments. This resistance is thought to be due to increased intracellular concentrations of S1P which is linked to cell proliferation. Inhibition of SK1 causes a build-up of sphingosine and ceramide; molecules which in high intracellular concentrations trigger apoptosis. Our research objective was to synthesize novel SK1 inhibitors to reduce the synthesis of S1P and promote apoptosis. Inhibitors have been successfully synthesized with modifications to the lead compound. These novel SK1 inhibitors were evaluated as possible antimicrobial agents which would successfully inhibit bacterial growth by interfering with enzymes responsible for fatty acid metabolism. Preliminary tests on numerous derivatives of a lead compound found that aromatic derivatives showed greater bacterial growth inhibition. Additionally, derivatives with substituents in the para position showed increased bacterial growth inhibition in comparison to compounds with ortho and meta substitutions. Further testing of these antimicrobial SK1 inhibitor derivatives to establish optimum substitutions and concentrations will continue.

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

Incorporating the Cancer Stem Cell Hypothesis into a Treatment Model of Glioblastoma Multiforme

Joshua Dasburg (2018)
Stephen Steward (2017)

Mentors: Dr. Kristen Abernathy
Dr. Zach Abernathy

We extend the work of Kronik, Kogan, Vainstein, and Agur (2008) by incorporating the cancer stem cell hypothesis into a treatment model for Glioblastoma Multiforme. Cancer Stem Cells (CSCs) are a specialized form of tumor cell with normal adult stem cell properties. CSCs are believed to be one of the primary reasons for cancer recurrence since they are more resilient to current treatment practices and are able to repopulate the tumor once their own population has regenerated. We present a system of nonlinear ordinary differential equations that describes the interaction between cancer stem cells, tumor cells, and alloreactive cytotoxic T-lymphocytes (CTLs). Under the assumption of constant treatment, we present sufficient conditions for a treatment threshold that ensures a cure state that is globally asymptotically stable. We also explore a more biologically accurate treatment schedule in which CTLs are injected periodically. We consider cases where treatment is applied continuously over varying time intervals, as well as treatment injections using the Dirac Delta function. We conclude with a discussion of biological implications.

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.

Evolution and Biomechanics of the Male Copulatory Organ in Schizorhynchia (Platyhelminthes)

Brooke M. Davis (2018)

Mentor: Dr. Julian Smith III

In the phylum Platyhelminthes there lies a clade called Schizorhynchia that possesses more than 150 different species. Schizorhynchia are predacious hermaphrodites that are known to live between the sand grains at the beach. Their prominent male copulatory organ was used for classifying different species. However, the last review of their organ was based on light microscopy observations only and recent phylogenetic testing did not support the current classifications. Therefore, our research entailed an extensive study of the male copulatory organ using the confocal-laser-scanning microscope; this allows us to see the arrangement of nuclei, musculature, and cilia on the organism, most of which are incredibly difficult to distinguish using light microscopy. We studied the male copulatory organ in *Carcharodorhynchus* and compared it to *Proschizorhynchus* and *Carolinorhynchus*, both of which are evolutionarily more derived than the *Carcharodorhynchus*. We found that even though *Carcharodorhynchus* is in a more basal position in the Schizorhynchia, it still possesses similar construction in the male copulatory organ, suggesting that the anatomy found in the more derived forms extends to the base of the clade.

This work was funded by grant #SC16016 from the WU Research Council.

Investigation of the role of lpar4 in chicken retinal growth cone collapse

Victoria Leroy (2017)
Karyssa Davis (2018)

Mentor: Dr. Eric Birgbauer

The optic nerve fully develops during the embryonic stages of growth. Once damage has been done to the optic nerve, regeneration in adult mammals does not occur naturally. The conditions of chicken embryo development are reproducible, which makes examining the developing nervous system, particularly optic nerve development, in chicken embryos relatively easy. The optic nerve is made up of axons from retinal ganglion cells, or RGCs, which are neurons that transmit visual information from the photoreceptors in the eye to the brain. They travel contralaterally in distinct paths that start in the retina of the eye. The destination of these RGCs is the tectum (which is a structure of the chicken brain that is analogous to the mammalian superior colliculus). They reach their destination by relying on a growth cone, located at the tip of the axon, to detect environmental cues. Lysophosphatidic acid (LPA) may be one such environmental cue because it causes growth cone collapse in culture. This molecule could have a possible role as a repulsive cue to guide the axon to its target in the tectum. LPA receptors are G-protein coupled receptors (GPCR) that work through the $G_{12/13}$ pathway to induce growth cone collapse. This project aims at determining if lpar4, one of the 6 known receptors of LPA, mediates growth cone collapse. In order to test this the expression of the lpar4 receptor must be blocked. First a technique needed to be established that allows us to ensure expression inside of the RGCs. We used a control retrovirus, RCASB-GFP, that was injected into the retinas of chickens at day 3 of embryonic development. Once at day 6 of embryonic development, the retinas were dissected and examined for GFP-expressing growth cones. To date, we have only been able to identify a small number of GFP growth cones. Once we have established a technique that produces strong baseline data for comparison we will begin injecting a virus containing an siRNA to lpar4 that inhibits the lpar4 expression, and a growth cone collapse assay will be performed. We will test whether or not knocking down this pathway inhibits growth cone collapse after LPA treatment.

This research is supported by a grant from the National Eye Institute of the National Institutes of Health under award number R15EY024453 to Dr. Eric Birgbauer as well as the SC INBRE program. (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.)

Evaluation of the potential HMGA1-EF24 nexus in human colon cancer

Madeline Diaz (2018)

Mentor: Dr. Takita Felder Sumter

Lauren Travis (2019)

The architectural chromatinic proteins High Mobility Group A1 (HMGA1) are some of the most overexpressed proteins in malignant cancers and induce neoplastic transformation. The protein is increased as the last step of a prominent colon cancer progression pathway and mediates drug resistance, therefore correlating with a poor patient prognosis. HMGA1-mediated chemoresistance results from a self-protective process called cellular senescence. Analogs of the antioxidant, curcumin, when used in combination with traditional chemotherapeutic agents, are useful treatment options for chemoresistant tumors. This study had two specific aims. The first being to investigate how colon cancer cells HCT116 respond to treatment with EF24. The second aim was to evaluate how hmgal expression changed as a result of treatment with EF24. Our preliminary findings showed that cell viability decreased after 24-hour treatment with low-dose EF24, as indicated by an MTS assay, with notable discrepancies between cells that underwent a pulsed treatment regimen versus those that underwent continuous treatment. Furthermore, we demonstrated that cells exhibited fragmented DNA when treated with pulsed, low-dose EF24, which is characteristic of apoptotic cells. However, at higher, pulsed doses, SA- β -galactosidase activity increased indicating the induction of a senescence pathway. Lastly, gene expression studies indicated that hmgal was significantly down regulated in cells treated with continuous, low-dose EF24. Further investigation of this pathway could lead to decreased toxicity and increased viability of combination cancer therapies.

Support for this project was provided by the SC INBRE grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health with prior support from the National Science Foundation Research Initiation Grant and the NIH Academic Research Enhancement Award. Student support for M. Diaz was graciously provided by the Winthrop Eagle STEM and McNair Scholars Programs. Lauren Travis is funded by the Winthrop CHEM-STEM Scholars Program.

Investigating LPA as an axon guidance molecule in chick retinal ganglion cells by autotaxin siRNA

Garrett Driscoll (2016)

Mentor: Dr. Eric Birgbauer

Growth cones direct axon pathfinding during neurological development through finger-like projections which detect environmental stimuli, commonly referred to as axon guidance molecules. Inhibitory axon guidance molecules have been shown *in vitro* to cause growth cone collapse. Lysophosphatidic acid (LPA) has been shown to cause growth cone collapse, and thus it may act as an inhibitory molecule. LPA is produced by the enzyme autotaxin (ATX), which has been found in the mid-forebrain boundary of the embryonic chick brain, near the target region for retinal axons, the tectum of the midbrain. Through viral expression of an siRNA, we sought to reduce ATX levels in embryonic chick brains prior to retinal axon innervation. Depletion of the enzyme should then reduce the production of LPA. We then label retinal ganglion cell (RGC) axons later to examine retinal axon guidance to the target, the optic tectum. Initial data with control virus show normal retinal axon development to the optic tectum. Preliminary data with the ATX-siRNA suggest aberrant growth in retinal axon development and pathfinding to the optic tectum. Further examination of LPA as a guidance molecule may help to understand neurodevelopment in visual system disorders.

This research is supported by a grant from the National Eye Institute of the National Institutes of Health under award number R15EY024453 to Dr. Eric Birgbauer.

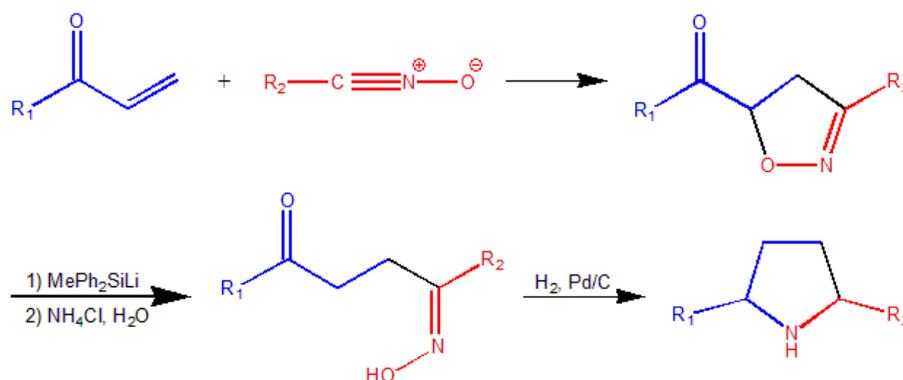
Synthesis of 2,5-dialkylpyrrolidines from γ -keto oximes derived from isoxazolines

Rayshon Ellis (2017)

Mentor: Dr. Aaron M. Hartel

γ -Keto oximes and their cyclic tautomers are versatile synthetic precursors to a variety of important compound classes, including pyrroles, pyrrolidines and γ -diketones. We are currently interested in transforming these valuable intermediates into 2,5-dialkylpyrrolidines, some of which occur naturally in the venoms of various species of *Solenopsis* ants. Related piperidine alkaloids from other *Solenopsis* species have been shown to have significant antiangiogenesis activity and have been investigated as a potential treatment for cancer.

The overall synthetic strategy involves the 1,3-dipolar cycloaddition of a nitrile oxide with an α,β -unsaturated ketone to give an acylisoxazoline. This intermediate is then treated with a silyllithium reagent, triggering a ring-opening Brook rearrangement *via* chemistry previously developed in our laboratory. Excess silyllithium reagent then cleaves the resulting silyl enol ether giving the γ -keto oxime upon workup. Selective reduction of the oxime would then initiate an intramolecular reductive amination to give the target pyrrolidine.



The current focus of the project is the determination of conditions appropriate for the final oxime reduction and reductive amination sequence. Various catalytic hydrogenation conditions have been explored using oximes prepared from cyclopentanone and cyclohexanone as inexpensive and readily available models of the γ -keto oximes. The successful reduction conditions were then applied to mixtures of oximes and ketones to determine if intermolecular reductive amination would also occur under the same conditions.

Support was provided by the Winthrop University Department of Chemistry, Physics, and Geology.

Fractional Matching Number of Divisor Graphs

Justin Groves (2017)

Mentor: Dr. Arran Hamm

A divisor graph is a particular graph whose vertex set consists of positive integers and whose edge set is given by the division relation. This type of graph is relatively unstudied and we have been exploring its properties. Specifically, we have analyzed the fractional matching number of the divisor graph concretely using Mathematica and asymptotically when the number of vertices tends to infinity. We hope to fully determine the asymptotic value of this graph parameter as well as examine related parameters for this family of graphs.

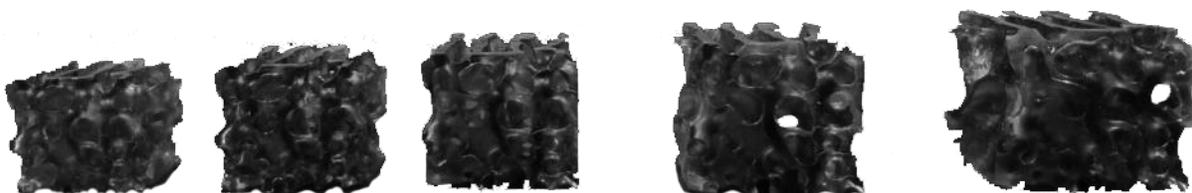
This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

Does the stiffness of trabecular bone 3D-printed model scale linearly with size?

Harrison Ben (May 2018)

Mentor: Dr. Meir Barak

Trabecular bone tissue is a 3D mesh of struts (trabeculae) residing internally to the bone cortex. Despite its seemingly fragile appearance, studies have shown that trabecular bone tissue significantly contributes to the stiffness of the bone (organ). Trabecular bone tissue was also shown to deteriorate extensively after the onset of osteoporosis, a disease which leads to a decrease in bone mechanical properties and an increase in the prevalence of bone fracture - especially during old age. Thus, studying the relation between trabecular bone structure and its function (e.g. stiffness) is a key factor when investigating the increased fracture risk in patients suffering from osteoporosis. Yet, since no two trabecular structures are alike, it is practically impossible to quantify the structural effects of the same tissue in health and disease on its mechanical performance. Using a 3D printer allows one to create as many identical samples as needed (healthy and osteoporotic) to test how the same structure behaves under loading stresses before and after the bone structure has deteriorated. However, since this technology is new, it is necessary to validate the ability of the printer to (i) reproduce accurate replicas of trabecular structures, and (ii) maintain a constant printed material quality (i.e. Young's modulus). Do this end we have used a ProJet 3D printer (3D systems ©) with a spatial resolution of 56µm and a layer thickness of 30 µm to test identical trabecular bone replicas ranging between the size of 15mm and 21.0mm in increments of 1.5mm. Three identical cubes were 3D printed in each size (15, 16.5, 18, 19.5 and 21mm) and then tested in compression using an Instron 5942 machine (Instron ©). Our preliminary results indicate that printed cube volume in each cube size significantly correlated with its measured weight ($R^2=0.9997$), supporting our first postulation. Furthermore, compression tests revealed a relatively constant value of material stiffness (Young's modulus) within our printed sample, supporting our second postulation. These preliminary results demonstrate the potential of harnessing 3D printing to biomedical research and the better understanding of bone diseases such as osteoporosis.



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.

Phytoremediation of copper, chromium, nickel, and zinc using native grasses

Dakota G. Hawkins (2018)

Mentor: Dr. Clifton P. Calloway

Phytoremediation is an environmental cleanup method using green plants to remove contaminants 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.

Bluestem, Twisted Arrow Rush and Lemongrass 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 initially and at two 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 – mass spectrometry (ICP-MS) and a calibration curve method of analysis.

All three species gave concentration ratios greater than one for zinc (1.8 – 6.0) over each test interval, while Twisted Arrow Rush gave concentration ratios greater than one for all four metals (1.5 – 6.0) at each tested interval.

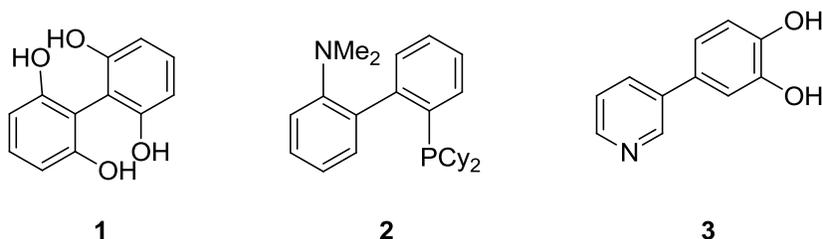
This work was supported by the National Science Foundation's Major Research Instrumentation Program (NSF MRI, grant CHE-1531698). We would also like to thank SC-INBRE (IDeA Network of Biomedical Research Excellence), Dr. Brad Jones, Dean, Wake Forest Graduate School and Wake Forest University URECA (Undergraduate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

Synthesis of Small Molecules for Inhibiting Aggregation of Alzheimer's Amyloid- β Peptide

Jake Roberts (2017)
Benjamin Hernandez (2018)

Mentors: Dr. James M. Hanna, Jr.
Dr. Robin K. Lammi

Amyloid- β peptide ($A\beta$) 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 to enable binding to $A\beta$. We previously demonstrated that 3,3',4,4'-biphenyltetrol (3,4-BPT) effectively abrogates $A\beta$ aggregation at stoichiometric concentrations. To further investigate this molecular architecture and determine how the positioning of the hydroxyl hydrogen-bond donors impacts inhibitor efficacy, we also synthesized four additional symmetrical biphenyltetrols (2,3-, 2,4- 2,5- and 3,5-BPT). However, 2,2',6,6'-tetramethoxybiphenyl, the intermediate for 2,6-BPT (**1**) eluded synthesis using our standard Suzuki coupling chemistry, presumably due to significant steric hindrance in the coupling partners. This limitation was overcome by employing a catalyst comprised of $Pd_2(dba)_3$ and the bulky phosphine ligand **2**; 2,2',6,6'-tetramethoxybiphenyl was thus obtained in 60% yield. Demethylation with BBr_3 afforded a 72% yield of the desired 2,6-BPT (**1**). To expand our investigation into the inhibitory efficacy of biaryls containing multiple hydroxy groups, 4-(3-pyridyl)catechol (**3**) was synthesized via a Suzuki coupling/demethylation protocol. 3-(3,4-Dimethoxyphenyl)pyridine was synthesized in 76% yield from 3-pyridylboronic acid and 4-bromoveratrole using $PdCl_2(dppf)$ as the catalyst; demethylation with BBr_3 produced the desired 4-(3-pyridyl)catechol (**3**).



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.

Expression and Purification of AvrGf2, a Type III Effector Protein from *Xanthomonas fuscans*

Erin Hershelman (2019)

Mentor: Dr. Jason C. Hurlbert

Xanthomonas fuscans subsp. *aurantifolii* B and C group are two of the causal agents of citrus canker. Recent studies have shown that an effector, AvrGf2, belonging to the XopAG family, contributes to a strong hypersensitive response in sweet orange and grapefruit cultivars. Southern blot confirms the presence of AvrGf2 in different strains of the B and C groups. The characteristic feature of AvrGf2 is a cyclophilin activation (CA) domain with the amino acid sequence G³⁵⁷PLL, which is essential for development of an HR in grapefruit (GF) and sweet orange. The protein contains two other domains essential for activity, including an amino terminal chloroplast localization sequence and a carboxy-terminal domain of unknown function containing the amino acid sequence motif CLNAVY. No homologues of AvrGf2 were found in the non-redundant sequence or three-dimensional structure databases, so in order to determine the function of the protein, we need to determine the x-ray crystallographic structure. We have cloned the CA and carboxy-terminal domains of AvrGf2 into a bacterial expression vector and used the resulting construct to transform a strain of *Escherichia coli*. We identified the best conditions for expression of the recombinant protein and attempted to purify it using a combination of metal chelating affinity (MCAC), ion-exchange and size exclusion chromatographic techniques. We were unable to obtain large amounts of purified protein due to an inherent instability in the protein. In the future, we will continue to try and optimize our protocols so that we can purify the protein for structural analyses.

The project described was supported by NIH Grant Number P20 RR-16461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE).

Production of Chemically-Induced Pluripotent Stem Cells from Adipose-Derived Stem Cells is Limited by Toxicity of the Base Medium and Individual Chemicals

Hannah Hopfensperger (2018)

Mentor: Dr. Matthew Stern

Pluripotent stem cells have the ability to differentiate into cells of any tissue or organ and can be obtained from the inner cell mass of a blastocyst as embryonic stem cells. Induced pluripotent stem (iPS) cells are adult cells that have been genetically manipulated to reprogram them to a pluripotent state. Chemically induced pluripotent stem (CiPS) cells are reprogrammed to the pluripotent state through treatment with several small molecules rather than through direct genetic manipulation. CiPS represent an attractive source of stem cells for therapeutic use because they can match the differential potential of embryonic stem cells and iPS cells without the ethical concerns surrounding the derivation of ES cells or the risks and technical challenges associated with the genetic manipulation used to create iPS cells. We hypothesized that a previously published protocol for the generation of CiPS cells (Hou *et al.* 2013) could be used to generate CiPS cells from more developmentally restricted murine adipose-derived stem cells (ADSCs) in our lab. These CiPS cells could then be employed in our development of tissue engineered skeletal muscle. In our attempt to generate CiPS cells, we observed that the ADSCs did not survive the induction protocol. We then tested each element of the protocol independently to determine which component(s) was/were responsible for the observed toxicity. Our results demonstrate that embryonic stem cell culture medium, along with the small molecules CHIR, Rep Sox, and Forskolin each caused abnormal morphology and cell death in ADSCs. Future work will seek to attempt to generate CiPS cells from *Oct-4* GFP ADSCs using a modified version of the published protocol. We will also explore the developmental potential of ADSCs sorted for expression of the cell surface molecule SSEA-1.

Funding for this project was provided by the South Carolina INBRE Developmental Research Program, The Winthrop University Research Council, Winthrop College of Arts and Sciences, and Winthrop Department of Biology.

Cryptic Speciation among Meiofaunal Flatworms

H. Joseph Horacek (2018)

Mentor: Dr. Julian Smith III

In order to investigate cryptic speciation among meiofaunal flatworms, samples were collected from four different locations along the coast of North Carolina, USA. Our target species were two undescribed species of meiofaunal flatworms: *Paramonotus* sp. and *Proschizorhynchella* sp. *Paramonotus* sp. is a mobile species of flatworm that allows itself to be moved by the tide to different locations, and *Proschizorhynchella* sp. is a sit-and-wait predator that resists being swept away by the tide. Our collecting sites were North Topsail Beach, Iron Steamer Pier, Emerald Isle, and Wrightsville Beach. In order to accurately identify species and to see if there is a gradient of genetic differences across populations, we examined the ribosomal DNA of our specimens, including the 18S gene, the 28S gene, and the ITS region. Thus far, we have analyzed the rDNA of four specimens of *Paramonotus* sp., one from each sampling location. The 18S genes from each specimen were 100% identical, 28S genes from each specimen were 99% or 100% identical, and the ITS region of the specimens from Emerald Isle and North Topsail Beach were 99% identical. However, the ITS region of the specimens from Iron Steamer Pier and Wrightsville Beach had unreliable readings, so we cannot give an accurate assessment of that region at this moment. All differences in sequences seem to be due to sequencing error. Unfortunately, only one specimen of *Proschizorhynchella* sp. was found in Onslow Bay, but we hope to find some in Myrtle Beach in the near future. More of the collected specimens will have to be examined in order to create a more concise conclusion.

Funding for this project was provided by grant #SC16012 from The Winthrop University Research Council to Julian Smith III and Joseph Horacek.

Circadian timing of the Cell Cycle in *Aeolosoma hedleyi*

Audrey Hughes (2019)

Mentor: Dr. Julian Smith III

In continuation of a previous study of circadian rhythms performed by Dr. Julian Smith's lab, we made slides of *Aeolosoma hedleyi*, a freshwater annelid, to view and take pictures of using the confocal-scanning laser microscope. Before being put on slides, all annelids were kept in an incubator to acclimate to a 14-hour light and 10-hour dark cycle. One hour before each of the ten designated testing points ten *Aeolosoma* were exposed to ethenyl-deoxyuridine (EdU) that would bind to cells undergoing S-phase of mitosis. At the designated testing time, the group of 10 *Aeolosoma* were euthanized by ejection onto a block chilled by liquid nitrogen. The *Aeolosoma* were then put into a solution of 100% ethanol for 48 hours. Then began a 2-day fixing process to expose the *Aeolosoma* to several antibodies and dyes for view under the confocal microscope. *Aeolosoma* were put on slides mounted in VectaShield, with 4 squares of aluminum foil as spacers, and clear nail polish at corners of coverslip. Data collection, which will continue in the Fall of 2016, consists of counting the number of cells in S-phase and M-phase. Cells were counted in three different areas of the *Aeolosoma*: gut, fission plane, and foregut. Once all the counts are completed, statistical analysis will be used to determine at which part of the circadian cycle S-phase and M-phase occur the most.

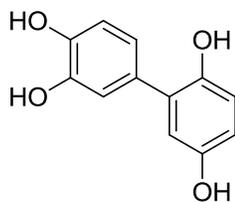
This work was funded by grant #SC16105 from the WU Research Council to Julian Smith III and Audrey Hughes.

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

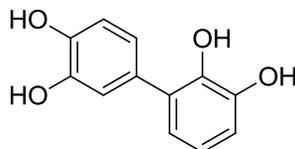
Matthew J. Hurtt (2018)
William J. Schreiber (2018)

Mentors: Dr. James M. Hanna, Jr.
Dr. Robin K. Lammi

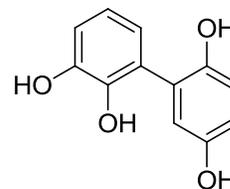
Amyloid- β peptide ($A\beta$) self-assembles into neurotoxic, β -structured aggregates, which are the primary component of the extracellular senile plaques characteristic of Alzheimer's disease. A variety of small molecules have been shown to inhibit the aggregation process; typically, these contain aromatic groups and one or more hydrogen-bond donors to enable binding to $A\beta$. We have previously identified biphenyltetrols (BPTs) as a class of molecules exhibiting promising inhibitory efficacy. 3,3',4,4'-tetrahydroxybiphenyl (3,4-BPT) was the most promising, reducing equilibrium aggregation by 50% when present in stoichiometric concentrations (i.e., $IC_{50} = 1X$); 2,5- and 2,3-BPT were also effective, albeit less so. Other symmetrical BPT's (e.g., 2,4-BPT, 2,6-BPT, 3,5-BPT) failed to exhibit significant inhibition. Based on these results, we hypothesized that "hybrid" unsymmetrical biphenyltetrols combining these arrangements of hydroxyl groups may also be successful inhibitors. 2,3',4',5-BPT, 2,3,3',4'-BPT, and 2,2',3,5'-BPT (figure) were therefore synthesized and evaluated for inhibitory efficacy using the Congo red (CR) spectral-shift assay, which exploits CR's specific binding to β -structured aggregates to enable monitoring of $A\beta$ aggregation as a function of time. Our results indicate that neither 2,3,3',4'-BPT nor 2,2',3,5'-BPT were effective inhibitors; however 2,3',4',5-BPT appeared to be a promising inhibitor of $A\beta$ aggregation ($IC_{50} = 1.8X$).



2,3',4',5-BPT



2,3,3',4'-BPT



2,2',3,5'-BPT

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 temporal effect of DOCA-salt hypertension on renal Tregs in males and females

Justin Hutchinson (2017)

Mentor: Dr. Jennifer Sullivan

Hypertension is the leading risk factor for cardiovascular disease, which is the main cause of death globally. It is known that females have a lower blood pressure (BP) compared to age corrected males prior to the onset of menopause. Therefore, greater understanding of the mechanisms protecting females against chronic increases in BP could improve BP control across both sexes. Hypertension is now considered to be a state of low grade inflammation. Therefore, T-cells are important targets for analysis because of their role in the regulation of inflammatory responses. Moreover, nitric oxide (NO) is a key regulator of cardiovascular function via endothelial dilation and BP control. Previous studies in our lab have shown that there are sex differences in both the NO pathway and T-cells in the spontaneously hypertensive rat (SHR) model, where NO and anti-inflammatory T-regulatory cells are upregulated. Currently, it is our focus to see if the same correlations apply in the salt-sensitive hypertensive rat model. As a result of 3 weeks of DOCA-salt treatment, it was found that Tregs were upregulated in the female model, especially in the group receiving DOCA-salt treatment. There were no sex differences in nitric oxide synthase (NOS) expression. However, statistical analysis via two-way ANOVA revealed a significant difference in NOS3 expression between the control and DOCA-treated group.

This work was funded by R01 HL12709.

Examining the diversity and origin of bacteria on South Carolina oceanic beaches

Cameron Sellers (2017)
Douglas Johnson (2017)

Mentors: Dr. Matt Heard
Dr. Victoria Frost

Oceanic beaches are dynamic ecosystems that are home to many different types of microbial species. While most of these bacterial species are not pathogenic to humans, there are some that are of public health concern. As such, these environments are monitored for microbes that may cause disease and illness. The one problem with this approach is that it is difficult to identify and track all species. Therefore, monitoring agencies usually focus on either common pathogens or bacteria which are associated with fecal pollution (Fecal Indicator Bacteria; FIBs). In this study, we assessed bacteria levels for one common pathogen (*Staphylococcus aureus*) as well as two common FIBs (*Escherichia coli* and *Enterococcus spp.*) on three oceanic beaches in South Carolina. These beaches were selected for study because recent research in this area has shown that FIBs may be more commonly found in beach sand than previously thought. In addition, and to help better understand where these bacteria are coming from on these beaches, we also used the molecular technique of phylo-grouping to identify potential sources of *Escherichia coli*. Using this approach, we determined that all three microbial taxa we looked for were present at all study sites, but that there was no significant difference in their distribution patterns. In addition, we found that likely sources of *Escherichia coli* included humans, wild animals, and domesticated animals. Collectively, these findings indicate that pathogens and FIBs may commonly persist on oceanic beaches, but more work is needed to determine if this is of public health concern.

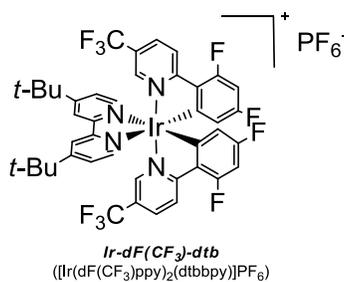
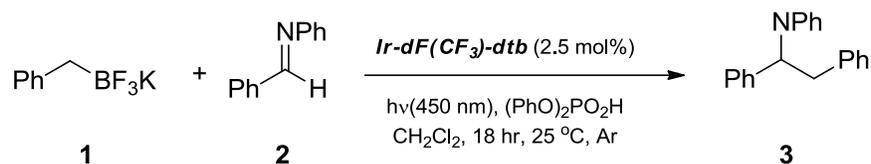
This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

Visible Light-Promoted Additions of Potassium Organotrifluoroborates to Imines

Christopher J. Jordan (2018)

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 many 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 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. This photo-excited catalyst then mediates the formation of radicals from organic substrates through a series of single-electron-transfer (SET) events; the organic radicals thus generated engage in downstream reactions leading to the final product(s). We have begun exploring this strategy as a means to effect a formal addition of potassium organotrifluoroborates to imines. We have found that irradiation of an argon-sparged dichloromethane solution of potassium benzyltrifluoroborate (**1**), benzalaniline (**2**) and diphenylphosphate in the presence of *Ir-dF(CF₃)-dtb* (2.5 mol%) at room temperature using blue LED floodlights (450 nm) resulted in the formation of compound **3** in 76% isolated yield; only traces of homocoupling products could be detected. Control experiments established that catalyst and light are both required for reaction; the elimination of diphenylphosphate led to a slightly lower yield.



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 *Staphylococcus aureus* infection on stiffness of cortical bone

Kunde Ariel (2017)

Mentor: Dr. Meir Barak

Osteomyelitis, a term for bone infection, is a common cause of hospitalization in the United States. Infection leading to osteomyelitis is almost always a product of bacterial origin. Although polymicrobial presence is seen at infection sites of osteomyelitis, *Staphylococcus aureus* is most commonly isolated and found to be the cause of more than 95% of bone infection in adults. This organism is a common commensal of humans that an estimated 60% of the population temporarily carries. *S. aureus* is transferred by infected asymptomatic individuals and its ability to proliferate under a variety of environmental conditions contributes to the organism's role as a pathogen in hosts with compromised immunity. This study examines the effect that infection of *Staphylococcus aureus* has on cortical bone stiffness. Frozen, cortical bone samples were thawed, sterilized with alcohol, inoculated with nutrient broth containing *S. aureus* (ATCC 12600) and then disinfected with chlorhexidine gluconate. Stiffness measurements of each sample were recorded (Instron 5942) before and after bacterial contamination to allow each sample to serve as its own control. One cube out of every testing batch was not inoculated in bacteria to serve as the control. Our preliminary results imply that *S. aureus* infection of bone significantly decrease bone stiffness in the radial and transverse directions. Conversely, bone stiffness in the axial direction decreased but the difference was found to be non-significant. This project is especially interesting as there is little published literature regarding in vitro bacterial infection of bone. This data may also be relevant information for bone graft banks storing donor tissues.

Funding for this project was provided by The Winthrop University Research Council Grant (SC SC16011).

Using allele-specific PCR to verify mutations in a double-mutant *Drosophila melanogaster* stock

Robert Landry (2016)

Mentor: Dr. Kathryn Kohl

A pivotal feature of meiosis is the formation of crossovers between homologous chromosomes. When crossover formation is aberrant, nondisjunction occurs, producing aneuploid gametes. Since aneuploidy is the leading genetic cause of developmental disability in humans, our lab aims to better understand the molecular mechanisms of crossing over. In particular, our lab sought to generate a *Drosophila melanogaster* stock that was double mutant for two genes of interest: *rec* and *su(var)3-9*. The *rec* gene is important in crossover formation (mutants do not localize their crossovers properly) and *su(var)3-9* creates a protein which is necessary for heterochromatin production. Heterochromatin packages the DNA tightly at the centromere discouraging crossovers in this region. For future studies looking at the result of aberrant crossover formation and improper heterochromatin formation, our lab needed to generate a recombinant chromosome with mutations in both genes. This work led to the production of a *Drosophila melanogaster* stock believed to carry both the *rec*² and *Su(var)3-9*² mutations linked on the third chromosome. Phenotypic sorting of nearby genes implied that both mutations were present in the new recombinant fly stock; however, these mutations produce no outwardly visible phenotypic changes. Consequently, the focus of this project was to develop and utilize allele-specific PCR protocols to determine the genotype of the organisms in question. Through this work, I successfully developed these protocols, and by utilizing them in conjunction with Sanger sequencing, I determined that the potential recombinant flies did not contain both mutations.

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.

Using Partial Differential Equations to Model Tumor Growth

MaLyn Lawhorn (2017)

Mentor: Dr. Kristen Abernathy
Dr. Zach Abernathy

Malignant tumors are a collection of cancerous cells that form in various parts of the body. Because a tumor can grow almost anywhere in the body and become particularly problematic after angiogenesis occurs, it is of interest to understand the spatial growth of the tumor. Using an existing PDE model by McGillan et al., we incorporate a stem cell population and explore the behavior of the tumor cells and cancer stem cells as they invade healthy tissue. We also investigate the stability of stationary solutions and numerically demonstrate four different tumor states.

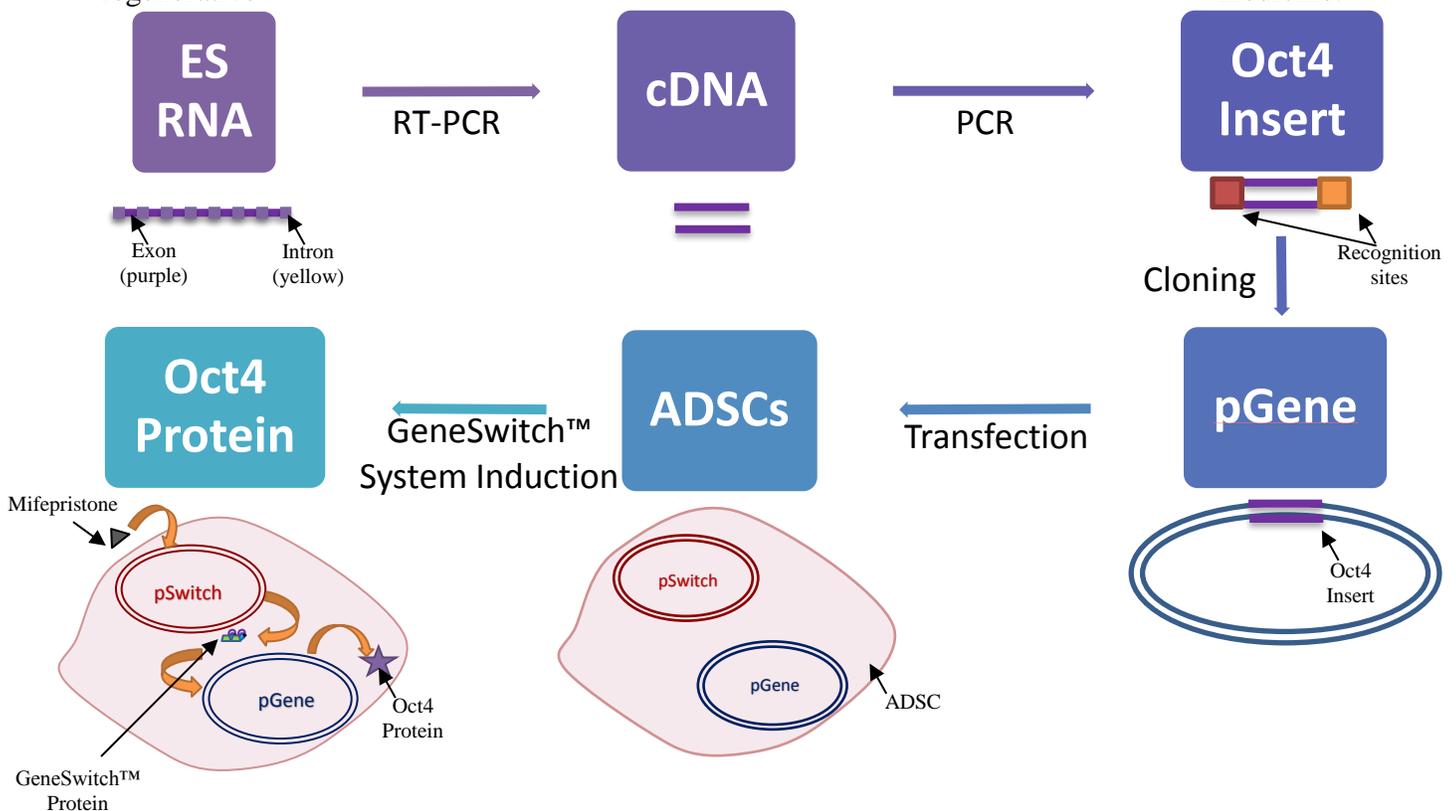
This project was supported by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health.

Controlling Oct4 Expression Levels Using Invitrogen's GeneSwitch™ System

Autumn Leggins (2018)

Mentors: Dr. Nicholas Grossoehme
Dr. Matthew Stern

Oct4 is a protein that is involved in the retention of pluripotency in adipose derived stem cells (ADSCs). Despite this knowledge, Oct4's exact role in the complex system used in maintaining pluripotency is not known. A plausible way of exploring Oct4's role would be through the use of cellular assays to control the expression of Oct4. This can possibly be accomplished by introducing a biological switch and the gene of interest into ADSCs. In this project, the GeneSwitch™ System is used to ultimately induce Oct4 expression. Before the GeneSwitch™ System can be used, the Oct4 gene is extracted from murine embryonic stem cell (ES) RNA. This ES RNA is used as a template to create complimentary DNA (cDNA) that can then be used to create an insert with the Oct4 gene. In addition to the cDNA, recognition sites for endonucleases must be added on to fully create the Oct4 insert. This insert could then be placed into one of the GeneSwitch™ System plasmids that have the same recognition sites and placed into ADSCs along with the plasmid that will act as a biological switch. With this system put into ADSCs, it is expected that Oct4 levels will be successfully controlled. Once controlled Oct4 expression is tested, investigations can be completed to determine how Oct4 expression levels influence pluripotency of ADSCs. This may have significant impact on the creation of regenerative medicine.



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.

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

Kayla Margin (2016)

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 α -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 aurantifolia* 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 and will initiate crystallization trials in the coming weeks to identify solution conditions suitable for growth of crystals for x-ray diffraction experiments.

The project described was supported by NIH Grant Number P20 RR-16461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE)

Targeted editing of FOXO gene in *Ciona intestinalis* using CRISPR/Cas9

Nicole McMullen (2017)

Mentor: Dr. Heather Evans Anderson

The chordate, *Ciona intestinalis*, has become an excellent model organism for the study of cardiac development. *Ciona* is a member of the phylum Tunicata, which is the sister phylum to Vertebrates. Since both *Ciona* and Vertebrates share a common evolutionary ancestor, they also share common genetic regulatory programs that govern developmental events during embryogenesis. FOXO is an orthologous gene shared between *Ciona* and Vertebrates. FOXO1 is known to play an important role in cardiac development in Vertebrates; however, its function in *Ciona* is unknown. This study aims to investigate the function of FOXO in *Ciona* by utilizing the CRISPR/Cas9 system to specifically target and edit the genetic sequence of FOXO during embryogenesis and subsequent heart development in *Ciona*. Our lab has successfully produced viable *Ciona* embryos and transmitted exogenous plasmid DNA via electroporation. Currently, we are in the process of cloning a series of FOXO sgRNA target sequences into expression plasmids that can be electroporated into *Ciona* embryos along with the Cas9 plasmid for targeted FOXO gene editing. We expect that altered FOXO function in *Ciona* embryos will disrupt heart development similar to the phenotypes observed in Vertebrate FOXO1 mutants.

Funding for this project was provided by The Winthrop University Research Council Grant.

The Hadwiger Number of Kneser Graphs

Kristen Melton (2017)

Mentor: Dr. Arran Hamm

Hadwiger's Conjecture is one of the most famous and mysterious open problems in graph theory. This presentation will introduce graph theory topics relevant to this conjecture and the related parameter, the Hadwiger number of a graph. We will state a known lower bound on the Hadwiger number of a graph and in the case of Kneser graphs (with particular parameters) we improve upon it. Using slightly different techniques we make similar improvements in the case of random subgraphs of Kneser graphs (again with particular parameters).

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.

ELECTRICAL EXCITATION, CALCIUM SIGNALING, AND PSEUDO-MECHANICAL CONTRACTION IN CARDIOMYOCYTES: EXTENDING A PARTIAL DIFFERENTIAL EQUATIONS MODEL

Alexander Middleton (2017)

**Mentor: Dr. Matthias K. Gobbert
University of Maryland, Baltimore County**

Calcium dysregulation is a significant cause of fatal cardiac arrhythmias, but it is an incompletely understood phenomenon and difficult to predict. Heartbeat rhythm is governed by periodic membrane depolarization causing the release of calcium ions into the cytosol of individual cardiomyocytes; the reaction of this calcium with contractile proteins triggers the overall contraction of the heart. These calcium wave patterns can be modelled as a system of coupled partial differential equations linking the excitation, signaling, and contraction of individual cardiomyocytes.

The starting point of this research is a model that includes the electrical system of the cell and establishes a one-way link from the electrical system to the chemical system. We extend the model to connect the chemical system to the electrical system and to incorporate a pseudo-mechanical component of calcium dynamics in cardiomyocytes. We extend the model further to include the impact of the efflux of calcium onto the electrical system.

A parallel implementation of a special-purpose numerical code using MPI is necessary to enable the long-time solutions of this large-scale system of partial differential equations. Numerical simulations examine the behavior of the system that arises from the feedback loops between the calcium system, the electrical system, and the pseudo-mechanical system.

These results were obtained as part of the REU Site: Interdisciplinary Program in High Performance Computing (hpreu.umbc.edu) in the Department of Mathematics and Statistics at the University of Maryland, Baltimore County (UMBC) in Summer 2016. This program is funded by the National Science Foundation (NSF), the National Security Agency (NSA), and the Department of Defense (DOD), with additional support from UMBC, the Department of Mathematics and Statistics, the Center for Interdisciplinary Research and Consulting (CIRC), and the UMBC High Performance Computing Facility (HPCF). HPCF is supported by the U.S. National Science Foundation through the MRI program (grant nos. CNS-0821258 and CNS-1228778) and the SCREMS program (grant no. DMS-0821311), with additional substantial support from UMBC. Co-author Uchenna Osia was supported, in part, by the UMBC National Security Agency (NSA) Scholars Program through a contract with the NSA. Graduate assistant Jonathan Graf was supported by UMBC.

Decellularization and Recellularization of Porcine Acellular Muscle Matrix Scaffolds

Natalie Mseis (2017)
Carolina Pham (2018)

Mentor: Dr. Matthew Stern

Traumatic injuries often result in significant damage to skeletal muscle tissue. Current methods for repairing damaged skeletal muscle are inadequate and associated with donor site morbidity as they require a patient's healthy tissue to be harvested in an attempt to repair or replace damaged tissue. A variety of biomaterials that facilitate muscle regeneration/repair are in development; however, few are able to provide the structural and biochemical cues present in the tissue's native scaffolding, its extracellular matrix. Here, we describe the production and initial characterization of a biomaterial we refer to as Porcine Acellular Muscle Matrix (PAMM), which is produced through the decellularization of sheets of porcine skeletal muscle. We also demonstrate that PAMM scaffolds can be efficiently recellularized with murine C₂C₁₂ myoblasts. This work sets the stage for us to 1) use PAMM scaffolds to test the myogenic potential of different stem cell populations in a three-dimensional *in vitro* culture system and 2) test the ability of unseeded or cell-seeded PAMM scaffolds to support skeletal muscle regeneration *in vivo*.

Funding for this project was provided by the South Carolina INBRE Developmental Research Program, The Winthrop University Research Council, Winthrop College of Arts and Sciences, and Winthrop Department of Biology.

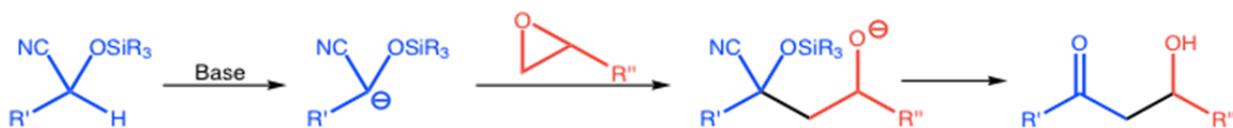
The reaction of O-silylated cyanohydrin anions with epoxides as an alternative for the enantio- and diastereoselective preparation of aldols

Dakoda W. Mullinax (2017)

Mentor: Dr. Aaron M. Hartel

The aldol addition is one of the most important carbon-carbon bond forming reactions in chemical synthesis. The traditional form of this reaction, between an aldehyde or ketone and a second enolized aldehyde or ketone, results in the formation of a β -hydroxycarbonyl (often referred to as an “aldol product”). The reaction can result in the formation of up to two new chiral centers and the absolute and relative stereochemistry of the product can be challenging to control. Modern variations, especially those of Evans and related strategies, have allowed for significant enantio- and diastereoselectivity in the reaction. These methods, while extremely useful, have several drawbacks which include poor atom economy, use of expensive auxiliaries, and the additional synthetic steps required to introduce and remove these auxiliaries.

An alternative potential route for the enantio- and diastereoselective preparation of aldol products is the reaction of O-silylated cyanohydrin anions with epoxides. This method would take advantage of the well-established asymmetric epoxidation procedures currently available, providing an efficient method for the stereoselective formation of aldol products.



The scope and limitations of this method have been investigated with respect to the cyanohydrin structure. The effect of the silyl protecting group was first investigated by reacting mandelonitrile with four inexpensive, readily available chlorosilanes (TMSCl, TESCl, TBSCl and TIPSCl) to prepare the corresponding silylated cyanohydrins. Each was then alkylated with 1,2-epoxybutane and the resulting adducts were deprotected using TBAF to give the desired aldol product. It was found that the more labile protecting groups tended to produce lower yields for the final product. The effect of the cyanohydrin aryl group is also under investigation. Different aldehydes such as *trans*-cinnamaldehyde and 3-pyridinecarboxaldehyde were treated with TMSCN to produce the corresponding TMS protected cyanohydrin. The TMS group was removed by acidic or basic hydrolysis and the resulting cyanohydrin protected with TBSCl. The resulting TBS protected cyanohydrins will undergo the same alkylation-deprotection sequence described above.

This project was supported by the Winthrop University Research Council.

Synthesis of Sphingosine Kinase Inhibitor 1 with Modifications of Zone 2

Ansley Nemeth (2018)

Mentor: Dr. T. Christian Grattan

Sphingosine kinase 1 (SK1) is an enzyme known to catalyze the formation of sphingosine-1-phosphate (S1P) in the sphingolipid metabolic pathway. Within the cell, the formation of ceramide from the sphingolipid triggers apoptosis. If apoptosis does not occur, ceramide is catalyzed to form sphingosine. Following the process, SK1 then catalyzes the formation of S1P, which at high concentrations initiates cell proliferation in cancerous systems. Novel inhibitors for SK1 are needed to stop S1P from being produced. Using a known template of a sphingosine kinase inhibitor (SKI), four new derivatives were created and were intended to improve the oral bioavailability while improving or maintaining interactions with SK1. Modifications were made to the central pyrazole ring of the lead compound. The modifications included the substitution for a 5-naphthylisoxazole (2A) ring, a 3-naphthylisoxazole (2B) ring, a thiophene (2D) ring, and a furan (2G) ring. Through multiple syntheses, the final products of 2A, 2B, and 2G were successfully created after purification and analysis by ¹H-NMR. The new inhibitors are currently being evaluated via enzyme activity assay testing to determine how these modifications impact SKI relative to our lead molecule.

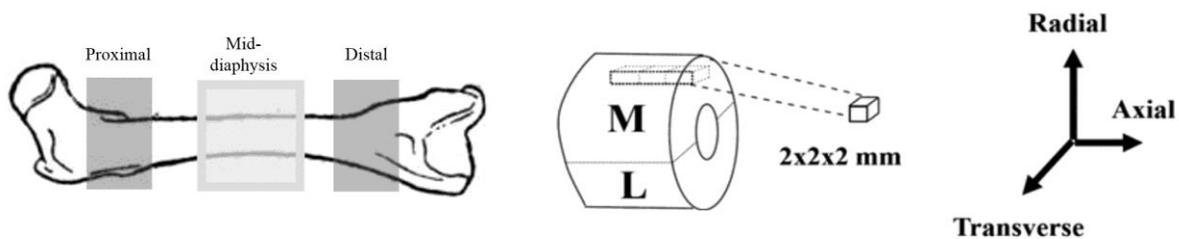
Support was provided by a NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medicine Sciences and the Winthrop University Department of Chemistry, Physics and Geology.

The variability of cortical bone stiffness along the femur shaft

Nguyen Jack (2018)

Mentor: Dr. Meir Barak

Cortical bone comprises the rigid outer portion of bone (organ). Based on Wolff's law of bone adaptation, we would expect the cortical bone of white-tailed deer femora to undergo significant modeling in the area which experiences the most loading (i.e. mid-diaphysis, axial direction; assuming the bone is loaded in bending). Therefore, our working hypotheses for this study were that (I) bone samples will have the highest stiffness in the axial direction (along the direction of loading), and (II) bone samples taken from the mid-diaphysis of the femur would have the highest stiffness values when compared against samples taken from other areas. To test these hypotheses, ninety 2mm³ samples were prepared using a low-speed diamond bladed saw from three separate areas of the femur: the proximal end, mid-diaphysis, and the distal end. These cubes were then tested in compression using an Instron 5942 universal testing machine. Tests were repeated a total of three times for each orthogonal direction (axial, radial and transverse). Stiffness values were found to be significantly higher in the axial direction in all three bone locations, thus supporting our first hypothesis. Our results also demonstrated a statistically significant difference in the stiffness values of the mid-diaphysis vs. the other locations in all but the axial direction, thus refuting our second hypothesis. Overall these results support Wolff's law since the axial direction, which normally experiences the most loading in the femur, showed the highest stiffness values. However, the similar stiffness values when comparing samples from the mid-diaphysis and distal end in the axial direction may imply that due to muscle action, the femur is not loaded in bending but in almost pure compression.



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.

Functional characterization of novel GH10 xylanases

Jesslyn Park (2018)

Mentors: Dr. Evan Glasgow
Dr. Brian Fox
University of Wisconsin, Madison

Decreasing availability of fossil fuels has driven an increased demand for the production of sustainable sources of energy such as biofuels. The field of bioenergy research seeks to develop biocatalysts able to break down biomass substrate “waste”-products rather than biomass used for food such as corn. Xylanases are of interest for their ability to hydrolyze hemicellulosic sugars found in all plant cell-walls. In this study, three novel xylanases of glycoside hydrolase family 10 (GH10) were functionally characterized and optimized in regards to temperature and pH, as well as kinetically analyzed in regards to ability to produce xylose monomers and oligomers. It was determined these enzymes, GH10-4, GH10-9, and GH10-15, are active upon substrates xylan and arabinoxylan. Activity is pH and temperature-dependent for the three enzymes. For GH10-4, optimums are approximately 50°C at pH 8 on xylan and 45°C at pH 8 on arabinoxylan. For GH10-9, optimums are approximately 70°C at pH 5 on xylan and 70°C at pH 5 on arabinoxylan. For GH10-15, optimums are approximately 50°C at pH 8 on xylan and 45°C at pH 5 on arabinoxylan. Upon qualitative kinetic analysis, it was determined that GH10-4 produces xylose (X1), xylobiose (X2), xylotriose (X3), xylo-tetraose (X4), and xylopentaose (X5) from xylan, while GH10-9 and GH10-15 produce only X1, X2, and X3. From arabinoxylan, GH10-4 produces X1, X2, X3, X4, while GH10-9 and GH10-15 produce X1, X2, X3. Conclusive kinetic data may suggest applicability of these enzymes to industrial use and will contribute further insight to the functionality of enzymes within GH10.

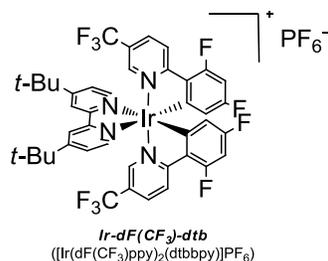
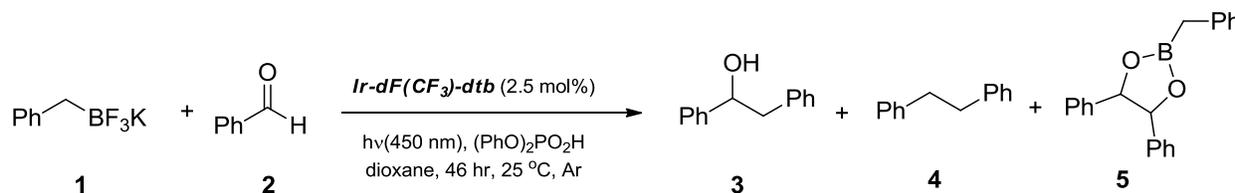
This work was supported by an NSF-REU grant to the University of Wisconsin, Madison

Visible Light-Promoted Additions of Potassium Organotrifluoroborates to Carbonyl Compounds

Davis P. Plasko (2017)

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 many 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 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. This photo-excited catalyst then mediates the formation of radicals from organic substrates through a series of single-electron-transfer (SET) events; the organic radicals thus generated engage in downstream reactions leading to the final product(s). We have begun exploring this strategy as a means to effect a formal addition of potassium organotrifluoroborates to carbonyl compounds. We have found that irradiation of an argon-sparged dioxane solution of potassium benzyltrifluoroborate (**1**), benzaldehyde (**2**) and diphenylphosphate in the presence of *Ir-dF(CF₃)-dtb* (2.5 mol%) at room temperature using blue LED floodlights (450 nm) resulted in the formation of 1,2-diphenylethanol (**3**) in 35% yield; homocoupling products bibenzyl (**4**) and the organoborate **5** (derived from reaction of 1,2-diphenyl-1,2-diol with **1**) were also detected. Control experiments established that light, catalyst, and diphenylphosphate are all required for reaction success. Use of dichloromethane as the solvent resulted in a 48% yield of **3**.



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.

Expression and Purification of the Dimerization and Regulatory Domains of Recombinant Human Phosphodiesterase 11A

Brittney Ramsey (2019)

Mentor: Dr. Jason C. Hurlbert

Phosphodiesterase 11A is responsible for the hydrolysis of the second messenger molecules cAMP and cGMP, which deactivates cellular pathways. This enzyme is found to be important in a variety of human diseases and is expressed in many tissue types in the body, making it an important therapeutic target. PDE11A is 933 amino acids long and has four regions of interest: An amino-terminal domain spanning approximately 200 amino acids that contains several phosphorylation sites, two domains that are implicated in regulating catalytic activity and/or dimerization, named GAF-A and GAF-B respectively, and a carboxy-terminal catalytic domain. We have designed a synthetic gene encoding the GAF-A and GAF-B domains, amino 210-588 and expressed it in recombinant *Escherichia coli* cultures. We attempted to purify the recombinant protein using metal chelating affinity chromatography (MCAC), anion exchange (AIEX) and cation exchange (CIEX) chromatographic methods, but were unable to purify the protein to greater than 70% homogeneity. The protein was inherently unstable and was cleaved between the GAF-A and GAF-B domains. The recombinant protein was determined to be properly folded as monomeric and dimeric species were identified by anti-hexahistidine Western blot. In the future, we will attempt to stabilize the protein by the addition of protease inhibitors to the lysis buffers in hopes of boosting both yield and purity of the recombinant protein.

The project described was supported by NIH Grant Number P20 RR-16461 from the National Center for Research Resources for support of the program entitled "South Carolina IDEa Networks of Biomedical Research Excellence" (SC-INBRE).

An Investigation into the Diet of the *Gastrotricha* Using Diagnostic Polymerase Chain Reaction Analysis

Joshua Sauer (2019)

Mentor: Dr. Julian Smith III

In the past, research into the trophic interactions among meiofaunal organisms among coastal communities have been inhibited for several reasons. Between size, harvesting techniques, cost, habitat or other factors that could have played a role in this inhibition, the result is the same: a lack of easily obtainable, relatively inexpensive and reliable data. However, in recent studies the application of a technique known as diagnostic polymerase chain reaction (PCR) analysis has been employed. In this technique, group specific primers are used to isolate the DNA of potential prey items contained within sample of DNA isolated from a predator, and amplify only the DNA belonging to the biological taxon targeted by the primer. This technique allows for identification of prey DNA contained within a predator and allows for the establishment of trophic interactions among the meiofaunal communities. It is through the employment of this technique that the diet of the *Gastrotricha* is beginning to be understood. Different forms of the *Gastrotricha* were harvested from beaches along the Carolina coast, their DNA isolated, and the diagnostic PCR technique was employed. Upon analysis, data suggests that primers designed to specific hypothesized prey groups are effective in prey identification within the *Gastrotricha* food web. The designing of primers to other hypothesized organismal prey groups and the testing of these primers may help to broaden our knowledge of meiofaunal trophic interactions especially as it pertains to the *Gastrotricha*.

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.

Performance of HRGO-MnO₂ Flexible Solid-State Supercapacitor Electrodes Fabricated by Electrophoretic Deposition

Evan Schultheis (2018)

Mentor: Dr. Fatima Amir

In this work, we report the electrophoretic deposition (EPD) of holey reduced graphene oxide-manganese oxide (HRGO-MnO₂) composite nanomaterials on gold-coated poly-(ethylene terephthalate) (PET) electrodes for flexible solid-state supercapacitors. Scanning electron microscopy and transmission electron microscopy were used to characterize the morphology and composition of the HRGO-MnO₂ hybrid. Cyclic voltammetry (CV) and galvanostatic charge-discharge (GCD) were used to characterize the supercapacitor's performance. The obtained HRGO-MnO₂ supercapacitor displayed excellent electrochemical properties with a specific capacitance ranging from 328 F/cm³ at 0.25 A/cm² to 266 F/cm³ at 2 A/cm² in a PVA-LiClO₄ electrolyte in a two-electrode test cell configuration. The HRGO-MnO₂ supercapacitor also demonstrated a minor capacitive evolution of 8.8% and 8.6% when the supercapacitors were bent over angles as large as 90° and 180° respectively. The excellent performance of the obtained HRGO-MnO₂ supercapacitor can be attributed to a synergistic effect between the double-layer capacitance of the HRGO sheets and the pseudocapacitance of MnO₂ nanosheets deposited across the surface of the HRGO, along with the electrophoretically assembled layer-by-layer structure of HRGO-MnO₂ sheets.

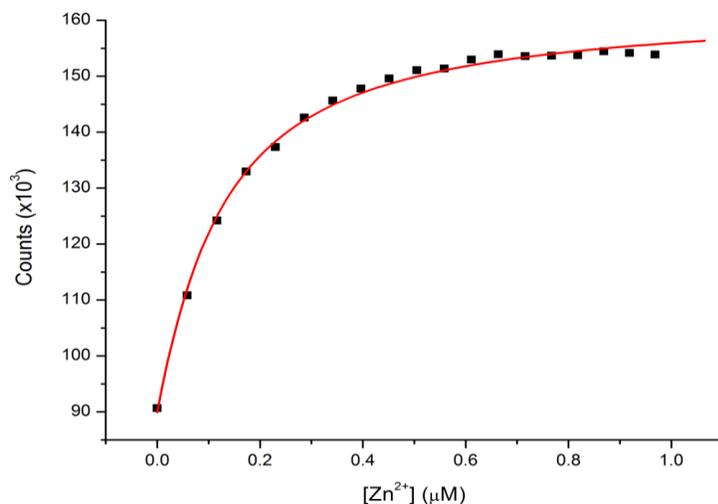
This project was supported by DOE-VFP.

Method Development of measuring Metal/Buffer Equilibria and Enthalpy

Hunter Sellers (2019)

Mentor: Dr. Nicholas Grosseohme

An increasing interest has risen in the biochemical community to better understand the relationship between metal ions and their effect on metalloregulatory protein transcription. To understand these interactions fully, the affinity of these proteins for the metal ions in question must be known. However, biological buffers provide a binding competition for the proteins and therefore make direct measurement of metal/protein affinity difficult to achieve, seeing as proteins must have buffers present to bind effectively. Developing a method of studying these metal and buffer interactions is essential to better understanding the kinetic and thermodynamic relationships between metals and proteins. Due to a fair amount of experimental complications, these metal/buffer interactions are not easily measured directly and therefore must be measured indirectly using a metal binding chromophore. This approach entails first binding a metal to the chromophore in the absence of buffer to enable a comparison with the binding constant when a fixed concentration of buffer is present. This was first tested using UV-spectroscopy; however, the equilibrium between the metal and chromophore could not be seen accurately using the spectrophotometer. To address this problem, the same equilibria were recorded using fluorescence emission spectroscopy which allows for a significantly decreased experimental concentration. The Zn^{2+} - Mag-Fura-2 system was used to pilot this method and resulted in experimental Zn/buffer affinities that match reference values. Metal-buffer enthalpies were also explored. Isothermal titration calorimetry (ITC) was used to observe the heat exchange as metal and buffer were titrated into EDTA, a high affinity ligand. The experimental enthalpies were corrected coupled reactions and the enthalpy of Zn-buffer interactions were determined. While the enthalpies we observed show promise as to the validity of the method developed, there were certain discrepancies in the results of the reaction enthalpies that will warrant further study to validate the method.



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 Novel Variants of HMGA1a to Understand the Role of Arginine Residues in DNA Binding

Maryssa Shanteau-Jackson (2018)

Mentor: Dr. Takita Felder Sumter

Cancer initiation and progression occurs through a series of key molecular steps that lead to aberrations in tumor suppressor, oncoprotein, and signaling functions within the cell. While a number of pathways have been implicated in cancer progression, many mechanistic studies of genes altered as an adenoma progresses to a carcinoma result in upregulation of *high mobility group A1 (hmgal)*. Mice bearing the *hmgal* transgene develop aggressive lymphoid malignancies and *hmgal* overexpression leads to increase drug resistance and self-renewal capacity in a variety of cancers. The gene encodes three products as a result of alternative splicing-HMGA1a, HMGA1b, and HMGA1c-all that preferentially bind DNA sequences rich in Adenine (A) and Thymine (T). While the specific function of these regions is not clearly understood, both DNA-protein and protein-protein interactions depend on the presence of three Arg-Gly-Arg (RGR) motifs. In fact, some studies report the presence of cancer-associated covalent modifications on arginine side chains of HMGA1a. To elucidate the role of highly conserved arginine residues in HMGA1 function, we created single and double mutants of arginines in the first RGR motifs. Because the conversion of Arg at position 25 to Alanine (R25A) and Lysine (R25K) dramatically decreased DNA binding *in vitro*, we engineered mutations encoding both single Arginine to Glutamate (R25E) mutations and Arginine to Glutamate and Alanine (R25E27A). Mutations were generated using Quik-change mutagenesis approaches, specific conditions for gene amplification optimized, and verified by automated sequencing. Future studies will involve expression and purification of the proteins for DNA binding studies using electrophoretic mobility shift assays. From these studies, we hope to gain insights into the molecular networks involved in cancer initiation and progression.

Support for this project was provided by the SC INBRE grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health with prior support from the National Science Foundation Research Initiation Grant and the NIH Academic Research Enhancement Award.

Effects of diphenhydramine and triclosan on aquatic biofilm communities in Lake Wylie, SC

Meg Smith (2016)
Sarah Walter (2017)

Mentor: Dr. Cynthia Tant

Humans use a variety of compounds each day for personal health and hygiene, but these compounds can find their way into freshwater ecosystems through a variety of pathways. Although some research has been done to assess the effects of various compounds on individual species, ecologists still know very little about how they can affect aquatic assemblages and ecosystem function. We measured the effects of a commonly used pharmaceutical (diphenhydramine) as well as an antibacterial ingredient used in many personal care products (triclosan) on autotrophic and heterotrophic activity of aquatic biofilms in Lake Wylie, SC. We measured gross primary production and respiration using pharmaceutical diffusing substrates that contained high and low concentrations of either diphenhydramine or triclosan. Due to high variability within treatments, there were no significant differences in gross primary production or respiration between treatments and controls for either compound. Several factors may have contributed to the lack of a treatment effect including unexpectedly high prevalence of heterotrophic species living within the biofilms, low diffusion rates of each compound, and decreased activity due to stress. Water samples were also taken for nutrient analysis, which suggested enrichment in Lake Wylie. Understanding how these commonly used compounds can affect ecosystem function and how they may interact with other stressors can help inform management of aquatic resources and design of wastewater treatment plants.

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.

Evaluation of Zone 4 Inhibitors of Sphingosine Kinase Using Sphingosine Kinase Activity Assay

Mikala Smith (2017)

Mentor: Dr. T. Christian Grattan

The sphingomyelin metabolic pathway is a target area of research in relation to inducing apoptosis in cancer cells. In the pathway, sphingomyelin is 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 presence of sphingosine kinase, an enzyme that catalyze the phosphorylation of sphingosine to form sphingosine-1-phosphate. Overexpression of sphingosine kinase in cancer cells produce high levels of sphingosine-1-phosphate. To block the increased production of sphingosine-1-phosphate, a template inhibitor has been identified but found to be ineffective *in vivo*. This lead to the production of a number of structurally modified variations of the inhibitor in an attempt to improve the overall hydrophilicity of the compound. These variations were successfully synthesized and are now being tested for effective *in vitro* relative to the template inhibitor and each other.

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, but we hope to continue to optimize and realize the potential of these inhibitors as a possible treatment option in this cancerous pathway.

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

Exploring Variables in Aqueous Synthesis of Zinc Oxide Nanoparticles

Jessica Stevens (2019)

Mentor: Dr. Maria Gelabert

Zinc oxide is a compound most commonly found in sunblock and antibacterial topical creams as well as bacterial remediation and other applications. For these technologies, the habit and size of crystals, connected to its surface properties, are important to be able to reproducibly synthesize. This research into the aqueous synthesis of zinc oxide nanoparticles with water as a solvent is an extension of previous work using ethanol as a solvent, with the eventual goal of water purification application. While previous studies have experimented with reactants such as zinc sulfate and zinc acetate, the focus here was on using zinc chloride. This exploratory research examined many variables: zinc chloride molarities (0.005-0.05), sodium hydroxide molarities (0.05-0.5), presence or absence of guar gum, and time for mixing were all altered in a controlled fashion. The passivating agent guar gum was included as one of the many variables in order to inhibit growth and limit particle size. To gain data on the product, X-ray diffraction and particle size data were collected on the samples. Analysis showed consistent synthesis of zinc oxide, but none of the trials resulted in nanoparticles; smallest sizes were on the order of 1-3 μm , and typical sample size averages were 25-50 μm . Measured pH, dependent on sodium hydroxide amount, showed no correlation to particle size, but smaller particle sizes tended to favor lower molarities of both zinc chloride and sodium hydroxide. With regards to temperature, warmer temperatures (room temperature compared to approximately 70 °C) led to larger particle sizes and thereafter experimentation was restricted to room temperature. Exploratory synthesis with water has enabled the development of fundamental knowledge in the field of crystal growth from aqueous solutions, and the ability to use inexpensive, abundant water as a solvent for technological materials.

Support for this research was provided by the Research Council of Winthrop University.

Investigating X chromosome non-disjunction in *Drosophila melanogaster* *su(var)3-9* mutants

Cameron C. Washington (2017)

Mentor: Dr. Kathryn Kohl

Meiotic recombination is a highly regulated process necessary for promoting proper chromosome disjunction during the first meiotic division. Notably, reduced levels of meiotic recombination are observed in heterochromatic regions of the genome. This study seeks to investigate the molecular mechanisms underlying this observation by examining the effects of reduced heterochromatin on non-disjunction rates in *Drosophila melanogaster*. To accomplish this, we measured non-disjunction in wild-type and reduced heterochromatin mutant *su(var)3-9* flies. To begin, we confirmed the presence of a mutation within *su(var)3-9* via Sanger sequencing. Next, we created allele-specific primers using the WASP tool and designed a PCR protocol to more accurately identify mutant flies at the molecular level. Finally, we measured non-disjunction using a dominant phenotypic marker on the Y chromosome. We discovered that *su(var)3-9* mutants have significantly higher levels of non-disjunction than wild-type flies ($p = 0.001$). We also uncovered a striking sex bias among the non-disjunction progeny, with significantly more males than females ($p < 0.0001$).

Support for this research was provided by the Research Council of Winthrop University, by SC INBRE grants from the National Institute of General Medical Sciences (8 P20 GM103499) of the National Institutes of Health and by Winthrop Ronald E. McNair Scholars Program.

Fire Events and Soil Thermometry: The Applications of Clay Chemistry for Tracing Temperature Changes in Soils and Sediments Below Surface Fires

Emily Watson (2017)

**Mentors: Dr. Scott Werts
Dr. Maria Gelabert**

Fires in the natural environment affect the physical, chemical, and biological properties of soils. However, fires may also alter the mineralogy of the geologic material in which it comes in contact. Previous experiments on high temperature alteration of clays indicate that dehydration, oxidation, and hydroxylation in clay minerals can occur progressively in that order at increasing temperatures up to 500°C. It is also well known that wildfire events can heat soils to these temperature ranges several centimeters deep. In this experiment, alterations in clay chemistry were used as a tool to investigate fire intensity along with the changing morphology of clay minerals. For data collection, small camp fires were set in York County, SC and temperatures were recorded using a datalogger system to 5 cm deep during the fire event. Control samples were taken adjacent to the fires to compare the changing morphology of the minerals when heated. Powder x-ray diffraction and scanning electron microscopy were used to identify the clay mineralogy. The clay from soil samples was identified as hydrous kaolinite, anhydrous kaolinite, and varying types of goethite. To observe the dehydration, oxidation, and hydroxylation of clay minerals, scanning electron microscopy with emission dispersive spectroscopy was used to identify the O/cation ratios present, which would indicate changes in the oxidation state of the clay minerals. By mapping the changes in O/cation ratios with temperature in silicates, we are able to trace the temperature of the sediments during fire events. This research suggests it may be possible to utilize these geochemical trends to aid in soil and sediment temperature investigations in both archeological and modern soil and surface process investigations.

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 the role of heterochromatin on fourth chromosome recombination in *Drosophila melanogaster*

Andrew M. Williams (2017)

Mentor: Dr. Kathryn Kohl

In many organisms, meiotic recombination is essential for proper chromosome disjunction during the first meiotic division. Improper recombination can lead to chromosome nondisjunction and aneuploidy. As aneuploidy is the leading genetic cause of miscarriage and developmental disability in humans, understanding the underlying mechanisms of meiotic recombination is crucial. One poorly understood aspect of meiotic recombination is the regulatory mechanisms controlling where crossover events occur. For example, for unknown reasons, *Drosophila melanogaster* chromosome 4 never undergoes meiotic crossing over in wild-type flies. Since this chromosome is >70% heterochromatic, we hypothesized that the high level of heterochromatin is responsible for suppressing crossing over on chromosome 4. To test this hypothesis, we utilized an existing mutation in *eggless*, a gene responsible for heterochromatin formation of chromosome 4. We observed no meiotic crossing over on chromosome 4 in wild-type or *eggless* heterozygotes. Since *eggless* homozygotes are female sterile, we are unable to score meiotic crossing over in this genetic background. To circumvent this problem, we are currently designing protocols to perform meiotic tissue-specific knockdown of *eggless* to utilize in meiotic crossover assays.

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.