Winthrop University
Summer Undergraduate Research Experience (SURE)
2015 Abstract Book

WINTHROP
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Winthrop Students Performing Research at Other Institutions

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 2015, SURE celebrated its 10th year, with a cohort of 45 students and 21 faculty members on Winthrop's campus and an additional eight students who pursued research elsewhere, 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 as vibrant and successful as 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 want to especially acknowledge Drs. Jason Hulbert and Meir Barak, who worked diligently to assemble, edit, and publish this abstract book.

Finally, on behalf of the students, faculty and department administrators, we would like to express our gratitude for the support from the agencies and organizations listed below. The hands-on teaching experiences that the SURE faculty can provide for these students are only possible through their support.

We invite you to enjoy reading about the excellent research done by our outstanding students this summer!

Jay Hanna
SURE Program Coordinator

Robin Lammi
Director of Undergraduate Research

National Science Foundation
WHERE DISCOVERIES BEGIN

National Institutes of Health

SC INBRE
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RONALD E. McNAIR POST-BACCALAUREATE ACHIEVEMENT PROGRAM

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Quantification of Pseudouridine Modifications through Uridine Specific Cleavage of RNA

Adaeze Aninweze (2016)

Mentors: Dr. Patrick Limbach
Dr. Balasubrahmanyam Addepalli
University of Cincinnati

Pseudouridine (Ψ), an isomer of uridine (U), is the most common post-transcriptional modification found in RNA (1). Detection and quantification of pseudouridine (Ψ) in RNA is challenging. The available chemical detection methods are not quantitative. Recently reported mass spectrometry-based methods in this direction include relative quantification of hydrolysis products (base vs nucleoside) in collision-induced dissociation, and pseudouridine-specific SRM (Selected Reaction Monitoring) transition based absolute quantification. These approaches are effective in dealing with modification at a single sequence location. Our goal is to develop an assay that can quantify multiple modifications in a given oligonucleotide. This is done by uridine specific cleavage through hydrazinolysis. Pseudouridine is immune to hydrazine addition compared to uridine. This assay can be used to understand the functional role of multiple contiguous modifications, such as those in the subunit interface and the peptidyltransferase center of 23S rRNA.

Financial support of this research was provided by the NSF REU Chemistry program (CHE-1156449) and the National Institutes of Health (GM58843)

In Vitro Evaluation of the Anti-Cancer Activity and Mechanism of a Potent Curcumin Analog, EF-24

Sommer Barber (2017) **Mentor: Dr. Takita Felder Sumter**

Colorectal cancer surgery is a primary treatment option and is limited by the presence of undetectable malignant tissues. Thus, this treatment option often has a high risk of recurrence. Chemotherapy is often administered after tumor removal to limit recurrence, but more aggressive, drug resistant tumors often resurface. Curcumin, a component in the spice turmeric, has exhibited both antineoplastic and anti-inflammatory activity in several cancers. 3,5-bis(2-fluoroubenzylidene)piperidin-4-one (EF-24) is a potent curcumin analog that effectively induces apoptosis or cell death *in vitro*. While the improved properties of EF-24 over curcumin coupled with its low toxicity provide the theoretical framework for its use in colon cancer, the exact mechanism of action is not known. The *High Mobility Group A1 (HMGA1)* gene is widely over expressed in human cancer, and high levels portend a poor prognosis in diverse tumors. Overexpression of *HMGA1* drives neoplastic transformation, and recent work from our lab demonstrates its activation in a prominent colon cancer initiation pathway. Emerging data associates colon cancer progression with decreased levels of microRNAs that inhibit *HMGA1* transcription. Interestingly, these microRNAs are also transcriptionally activated by EF24. To determine the role of *HMGA1* in EF24 activity, we investigated the activity of EF24 on HCT116 cells. EF24 significantly inhibited cellular proliferation in HCT116 cells bearing elevated HMGA1. Specifically, less than 50% cell survival was observed at both 8 and 16 μM EF24. Western blot analyses will determine if HMGA1 levels are further elevated upon EF24 treatment. To determine if this effect depends on the presence of the characteristic polyphenol ring system, we assayed a 3, 3'', 4, 4'', tetrehydroxy-m-terphenol and found lower potency than EF24. These results suggest that EF24 exhibits antineoplastic activity on colon cancer cells, potentially via an *HMGA1*-dependent pathway and further support EF24 as a promising chemotherapeutic option.

This work was supported by grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

3D printed model study of the effect of thinned trabeculae on bone mechanical properties

Arielle Black (2017) **Mentor: Dr. Meir Barak**

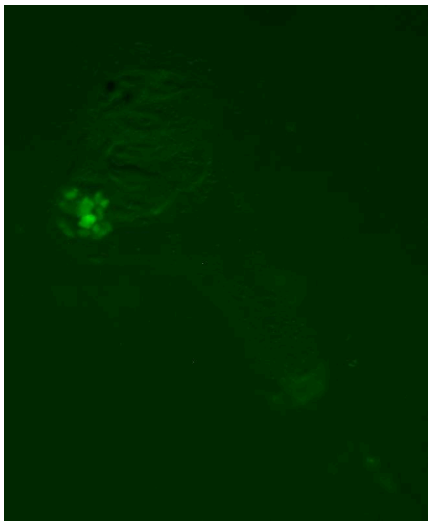
3D-printing is gaining ground in the world of science. This technology enables mass-printing of complex structures with high accuracy at a low price. This is a huge advance in the field of trabecular bone biomechanics. Trabecular structure is complex and unique (i.e. no two tissues are the same) and therefore trabecular samples can be mechanically tested only once before they fail. However, the new 3D-printing approach allows us to test each trabecular structure multiple times. By testing different trabecular structures we can gain insight into which structure is stronger and stiffer and thus we can predict which structure is more prone to fail. In this study we tested in compression (n=30) a cubical 3D-printed sample reconstructed from the metacarpal head of a chimp. The same sample was tested again after we had manipulated the model and thinned the trabeculae to simulate the onset of osteoporosis (decrease of 9.1% in bone volume). Our results demonstrate that the original 'healthy' trabecular structure is significantly stronger than the 'osteoporotic' one (4.13 MPa: 2.202 MPa). This study demonstrates that 3D-printing is a novel and valuable tool for testing the mechanical properties of trabecular structures and the prediction of their failure.

Support for this research was provided by Winthrop University Research Council Grant SC15014

Expression of Heart-Specific Constructs in *Ciona intestinalis* Embryos

Katlyn Brumley (2016) **Mentor: Dr. Heather Evans-Anderson**

Ciona intestinalis is a useful animal model system for studying developmental processes. It is particularly helpful in studies of heart development since many of the developmental steps and genes are conserved in *C. intestinalis.* This system replicates early heart development in other chordates, such as vertebrates. In addition to evolutionary conservation of genes and developmental features, there are many advantages to using this model system including rapid development and simple maintenance. Our main focus is the process of myocardial growth in *C. intestinalis*. In order to monitor the growth of the heart during development, we have constructed an expression vector using a fluorescently-labeled, heart-specific gene (BC030863/Micalcl, transcript model ci0100139114 from the ANISEED database). Previous studies have shown that development of *C. intestinalis* embryos is altered if the PI3K/AKT signaling pathway is disrupted. *C. intestinalis* embryos treated with PI3K- or AKT-specific inhibitory drugs at the larval stage just prior to metamorphosis and heart formation have a reduced heart size and delayed development. We will quantitatively assess heart growth using the reporter plasmid we constructed that contains a heart-specific promoter to generate fluorescently labeled hearts in juveniles. In addition, we also have obtained similar reporter constructs from the *C. intestinalis* transgenic line resource (CITRES, Japan). The requested plasmids, pMiCiTnIG and pMiCiTnIGCiprmG, are specifically expressed in muscle cells, including the heart. Electroporation of these plasmids has been successful and we have generated transgenic juveniles. Currently, we are optimizing the inhibitory drug treatments and will monitor heart growth by fluorescence microscopy.



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) and NIH Grant Number 1R15HL104587-01 from the National Heart, Lung, and Blood Institute.

The Examination of Autotaxin in the Production of Lysophosphatidic Acid as an Axon Guidance Molecule in Retinal Ganglion Cells

Rebecca V. Chopko (2016) **Mentor: Dr. Eric Birgbauer**

Neurons equipped with long axonal extensions facilitate signal transmission resulting in a degree of bodily functions. Axons determine their appropriate location by exhibiting growth cones during neurological development. The finger-like projections detect environmental stimuli, which guide the direction of axonal growth. Subsequently, these stimuli are often referred to as axon guidance molecules. Lysophosphatidic acid (LPA) is suggested as such, and is produced by the exoenzyme, autotaxin (ATX). Evident in the mid-forebrain boundary of the developing embryonic-chick brain, LPA and ATX influence axon growth of retinal ganglion cells. With an interest in this neurological subset, a RCASB-ATXsiRNA virus was injected into the chick brain at day three of development (E3). Through viral infection, the mRNA transcript of ATX is silenced, thereby depleting the production of enzyme and its subsequent product, LPA. Preliminary control data demonstrates normal axonal development in the presence of ATX and LPA. Observation of optic tecti with the ATXsiRNA virus under fluorescence and confocal microscopy may further suggest LPA as an axon guidance molecule. These findings may give insight to neurodegenerative disorders such as glaucoma.

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. (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.)

Next-Gen Sequencing for Community Assessment in the Meiofauna: Developing the Database

Alex Corder (2017) **Mentor: Dr. Julian P.S. Smith, III**

Anthropogenic factors that may influence coastal ecosystems include coastal development and beach nourishment nourishment. We have an incomplete understanding of how these effects may bring about changes in the community composition of organisms that live between sand grains, known as meiofauna. We have carried out DNA isolation, amplification, and sequencing of the 18S gene of marine meiofaunal organisms from samples on a North Carolina beach. We based the sampling methods and locations previously on those previously used for a community inventory completed in 1969 at the same North Carolina site. To date, four organisms that were previously present in 1969 were successfully identified, isolated and sequenced. The future goal is to continue creating this system of genetic markers, ultimately facilitating the use of NexGen sequencing to get a rough estimate of modern meiofaunal community composition. Once these data are collected, any changes that have occurred since 1969 can be examined for possible causation by the anthropogenic factors now influencing this habitat.

Lacunae structural characteristics differ between the femur and the scapula in accordance with Wolff's law

Kesha Daniels (2018) **Mentor: Dr. Meir Barak**

In accordance with Wolff's Law, it is widely accepted that bone structure alters in response to mechanical stimuli. Several recent studies have demonstrated also a similar effect on bone cells, specifically osteocytes. The purpose of our study was to further investigate into this effect by comparing lacunae structural characteristics between a directly loaded bone (femur) and a non-directly loaded bone (scapula, a flat bone suspended by muscle at the shoulder girdle). Multiple slices were cut in the transverse, sagittal and frontal planes from the femur and scapula of a young white-tailed deer. Next the slices were polished to a final thickness of 50-60μm and inspected under high magnification (X400) using a light microscopy (Nikon Instruments). To determine whether lacunae number, size and shape are significantly different between these bones, the size (perimeter) and shape (spherical/ellipsoidal) were measured and compared. As a result of slicing a cross-sectional area, the projection was only a 2D image. Thus, to grasp the full 3D model, the aspect ratio (AR, length to width ratio) of each lacuna was taken. The closer the AR ratio was to one the more circular the 2D image and the more spherical the 3D projection. Our results demonstrated that lacunae in the femur are more numerous than in the scapula, but smaller in size. In addition, the lacunae in the femur were also less spherical (bigger AR ratios) compared to the scapula. Our results are in agreement with previous studies which showed a more spherical lacunae structure in non-loaded bones (e.g. calvaria). Yet this is the first time that this correlation is shown also in non-directly loaded bones such as the scapula.

Support provided by grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health

Optimization of the Transfer-To Approach for Bottlebrush Polymer Synthesis

Michelle R. Corley (2017)

Mentors: Dr. Scott C. Radzinski
Dr. Jeffrey C. Foster
Dr. John B. Matson
Virginia Polytechnic Institute and State University

Ring-opening metathesis polymerization (ROMP) and reversible addition–fragmentation chain transfer (RAFT) polymerization were employed sequentially to prepare bottlebrush polymers using the transfer-to approach. RAFT transfer-to is a unique strategy for synthesizing bottlebrush polymers wherein a polymeric chain transfer agent (PCTA) is synthesized using ROMP with the Z-group attached to the polymer backbone. In the RAFT step, side chains detach from the bottlebrush backbone, propagate freely in solution, and then return to a new reactive site on the bottlebrush backbone (**Figure 1**). This study focused on optimizing the RAFT transfer-to step for preparing bottlebrush polymers by varying PCTA concentration, radical initiator concentration, and temperature. Size exclusion chromatography (SEC) was used to determine molecular weight and dispersity of the bottlebrush polymers, and aminolysis was conducted to cleave off the side chains to evaluate grafting density as well as the molecular weight and dispersity of the polymeric side chains. Based on SEC, it was concluded that CTA concentration gave control of the molecular weight of bottlebrush polymers as well as the side chains. Lower CTA concentration yielded high molecular weight bottlebrushes (1,600 kDa) of narrow dispersity (1.02). Radical initiator concentration also had an effect on the dispersities of the polymers, with higher radical initiator concentrations leading to a broadening of the bottlebrush molecular weight distribution. Finally, high temperatures (> 75 °C) degraded the PCTA, reducing control over the polymerization. These conclusions will enable the preparation of ultra-high MW bottlebrush polymers with high grafting densities and well-defined side chains.

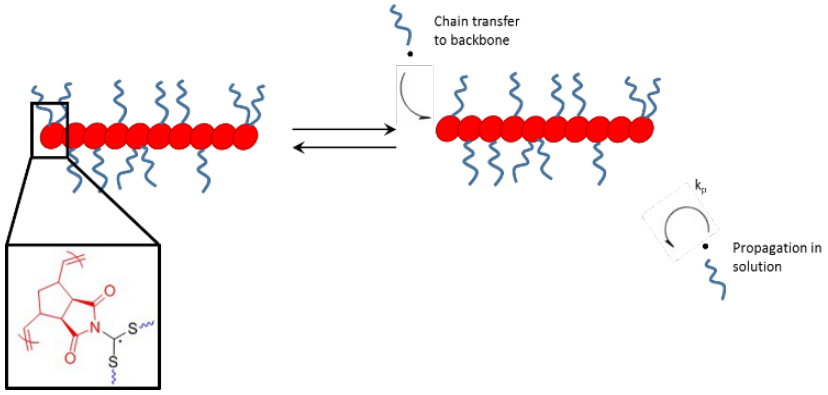


Figure 1. An overview of the RAFT transfer-to equilibrium scheme. This occurs when a polymeric radical detaches from the bottlebrush backbone, propagates freely in solution, and then returns to a new reactive site on the backbone via a chain-transfer reaction.

The authors are grateful for the financial support of the NSF under Contract DMR-1263248.

Toward *ab initio* modeling of CO_2 electroreduction on α-Sn, ß-Sn, and SnO_2 particles

James Dean (2016)

Mentors: Dr. Karthikeyan Saravanan
Dr. John A. Keith
University of Pittsburgh

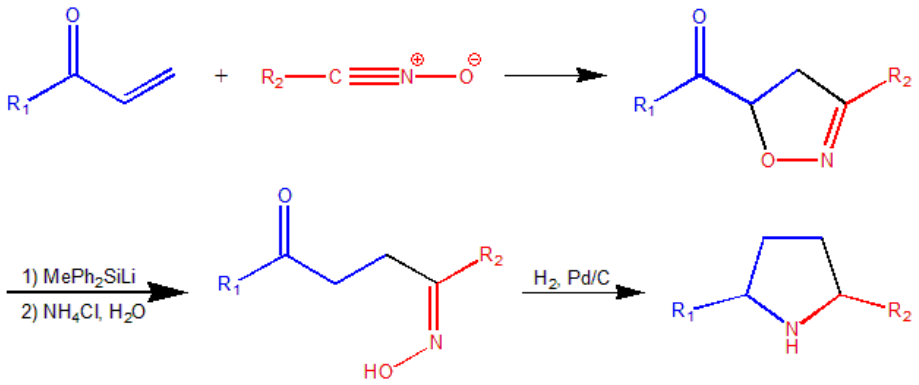
The ever-increasing global production of greenhouse gases prompts the urgent need for conversion into less-harmful substances. Energetically efficient electrochemical conversion of CO_2 into other, more useful products has been a hot topic of recent research. Previous investigations by Bocarsly et al. reported noticeably low overpotentials for CO_2 conversions to formate on polycrystalline tin electrodes. Interestingly, electrochemical conditions were found to promote the formation of tin oxide overlayers on the electrode surface, and these surfaces have been attributed to the catalysis of CO_2. As a starting point toward *in situ* computational modeling of this system, we carried out density functional theory calculations to determine various tin and tin oxide structures that would be energetically accessible at electrochemical reaction conditions via Pourbaix diagrams. We report convergence criteria necessary for ab initio modeling of electrochemical reactions on these surfaces. Future work will address the adsorption energies of CO_2 reduction reaction intermediates and mechanisms.

Synthesis of 2,5-dialkylpyrrolidines from γ-ketooximes derived from isoxazolines

Rayshon Ellis (2017) **Mentor: Dr. Aaron M. Hartel**

γ-Ketooximes 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-ketooxime 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-ketooximes. 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.

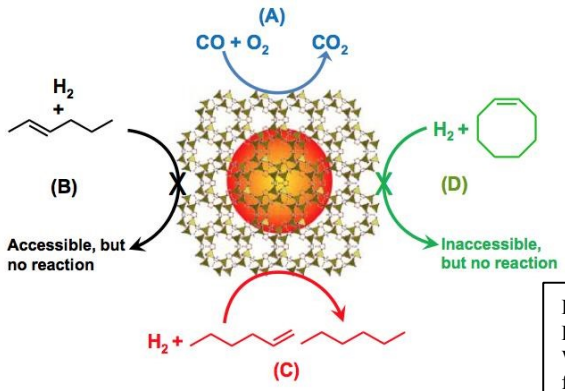
Support was provided the Winthrop University Department of Chemistry, Physics, and Geology

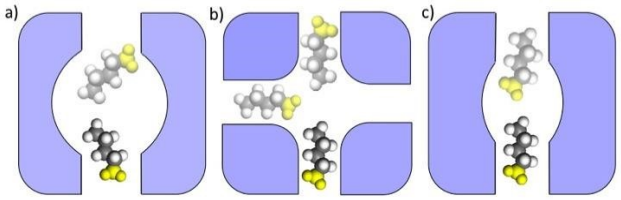
Oxidation of Butane to 1-Butanol with a Metal-Organic Framework (MOF)

Esseabasi Etim (2016)

Mentors: Dr. Rachel Getman
Sean Dix
Clemson University

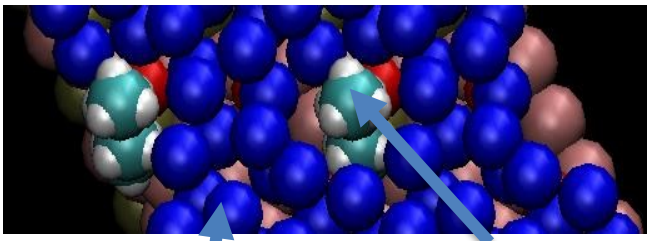
The objective of this project is to model a MOF (nanoparticle catalyst) that selectively oxidates the primary carbon of butane to 1-butanol, and not the favored secondary carbon pathway that produces 2-butanol. From the four carbons in butane, there two are classes of them: - primary/terminal or secondary. Secondary carbons are favored to terminal carbons in a reaction mechanism, resulting in secondary carbons being more reactive than terminal carbons. The desired oxidative product is not the naturally favored product because it follows the terminal carbon pathway. This does not mean that the desired product will not be formed in a traditional oxidative reaction because it will be, however it will not be the main product of the reaction. To make the terminal carbons more reactive than the secondary carbons would involve the use of a catalyst. The catalyst will specifically restrict the oxidation reaction to the terminal carbon, meaning that the catalyst must be regio-selective. A Metal-Organic Framework (MOF) is used as the catalyst to provide high selectivity of terminal carbons for oxidation to 1-butanol. A MOF is a framework is composed of an alloy surface, the pore/ring and an organic molecule. The pore of the framework restricts catalyst access to molecules of correct size, geometry and chemical properties. The framework itself will conform organic molecule to a specific orientation where only the terminal carbon can interact with the catalyst. Below, is a demonstration of a MOF.

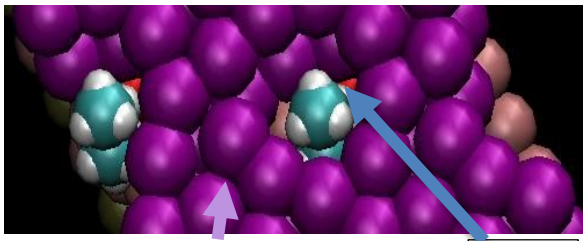




Lu, G.; Li, S.; Guo, Z.; Farha, O. K.; Hauser, B. G.; Qi, X.; Wang, Y.; Wang, X.; Sanyang, H.; Xiangang, L.; DuChene, J. S.; Zhang, H.; Zhang, Q.; Chen, X.; Ma, J.; Loo, S. C. J.; Wei, W. D.; Yang, Y.; Hupp, J. T.; Huo, F.: Imparting functionality to metal-organic framework material by controlled nanoparticle encapsulation. *Nature Chemistry* **2012**, *4*, 310-316.

Two (2) techniques were developed to increase the regio-selectivity of the catalyst:- tilting the Helium ring/pore to restrict butane interaction with catalyst to just the terminal carbon, and substituting Helium with larger Noble Gases to further restrict butane interaction with catalyst to just the terminal carbon which will favor the desired 1-butanol oxidative product. Noble Gas substitution has shown to provide more regio-selectivity so far.





Triangles in Cayley Graphs

Matthew C. Farmer (2016) **Mentor: Dr. Jessie Hamm**

A *group* is a mathematical object that appears in every day life quite often. For instance, the symmetries of a square form a group. The integers, which we all know and love, form a group under the operation of addition, but not under multiplication. To be precise, a group is a set of objects along with a binary operation, *, that satisfies the following properties: (i) there is an identity element, i.e. an element that leaves all others unchanged under the operation, (ii) G is closed under the operation, (iii) the operation is associative, and (iv) there are inverse elements. The integers under multiplication fail property (iv) because 2 has no multiplicative inverse that lives in the integers. Though groups are abstract objects there are many concrete examples, as shown above, and they are very useful.

To any group G we can associate a graph known as the Cayley Graph. Let S be a subset of elements in G. We define $Cay(G,S)$ to be the graph with vertices given by the elements in the group and we have an edge between x and y if $x*a=y$ for some element a in S. Cayley graphs were introduced by Arthur Cayley in 1878 and have been studied extensively since then. Cayley graphs help bridge the gap between abstract group theory and discrete mathematics, and in particular graph theory. They also have applications in biology, cryptography, and computer science.

In this poster we define two new parameters for Cayley graphs. First $Cay^3(G)$ is the largest size we can let S have to guarantee our Cayley graph has no triangles. On the other hand, $Cay\_3(G)$ is the smallest size S can have to guarantee that we do have triangles. We always have $Cay^3(G)<Cay\_3(G)$. In this poster we find $Cay^3(G)$ for all finite groups G and we give some results for $Cay\_3(G)$ with future directions as well.

Studying the Hydrothermal Synthesis of Hydroxyapatite $Ca_5OH(PO_4)_3$

Danielle Gasparik (2016) **Mentor: Dr. Maria Gelabert**

Hydroxyapatite is a natural component of human bone, teeth, and dentin. There are current studies on optimizing the synthesis of hydroxyapatite because it has shown promising results on orthopedic and dental applications. For example, it has previously been synthesized to repair dental and skeletal systems, but has not been synthesized with enough strength to fully replace the systems. The original goals this summer were to study the synthesis of hydroxyapatite over a pH range of 5-9, with increments of 0.5, while changing the chelating agent that coordinates to the calcium ion. DTPA (diethylenetriaminepentaacetate) was chosen as a ligand for study because of the many coordination sites (up to 8), and lactic acid was chosen because it was shown to be successful in previous studies and as a smaller molecule with few coordination sites. The overall shape of the crystals have been established in previous research. It is known that the crystals are acicular, and this is important to note for optical confirmation of the crystals. Experiments were organized by adding amounts of reagent, calculated with OLI Analyzer speciation software, into an autoclave, and placing the autoclaves into a convection oven at 200°C for seven days. Upon completion, the crystals were washed using water and ethanol, dried and viewed under an optical microscope. Visually, the majority of the crystals were acicular, but the sizes varied with the pH. The larger crystals formed at pH values close to biological pH, and other pH values yielded smaller crystals. After optical confirmation, X-ray powder diffraction was performed. Results from the X-ray confirmed that hydroxyapatite was synthesized with DTPA used as the ligand, but not with lactic acid.

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)

Salinity and Temperature Tolerance of Environmental Isolates of *E. coli*

Shiannea Gathers (May 2016) **Mentors: Dr. Victoria Frost**
Jordi Lluch (May 2016) **Dr. Matthew Heard**

Recent studies have shown that *Escherichia coli* is able to survive in sandy beach ecosystems. We were interested in understanding how *E. coli* survives in such a dynamic environment. Several environmental stressors such as light, temperature, salinity and nutrient availability affect the survival of *E. coli* at the beach. We wanted to understand how salinity as well as temperature effects *E. coli* growth. We performed a factorial experiment to observe the combined effects of salinity and temperature on *E. coli* survival. We isolated six different environmental strains from the beach sand of Folly Beach. To test salinity, we exposed our isolates to salt concentrations varying from 0-8% and assessed the colony forming units (CFUs) after an incubation period of 24 hours at 37^{o}C. For temperature, the isolates were placed in a temperature range of 4-50^{o}C for 24 hours. Our data suggests growth completely inhibited in salt concentrations higher than 5% and a growth range between 20^{o}C and 47^{o} C. In our factorial, we subjected a representative environmental isolate to salt concentrations ranging from 0-5% and a temperature range of 20-47^{o}C. Collectively, our findings show that temperature and salinity together are significant factors that influence the growth and survival of *E. coli*.

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)

Establishing a Novel 3D Tissue Culture System to Study Osteogenesis *In Vitro*

Laura Gibbs (2016) **Mentors: Dr. Matthew Stern**
Lisa Baird

The invention of 3D printers has made the process of engineering patent-specific prosthetic limbs less complex and more cost-efficient. While this represents a huge step forward for individuals that are in need of these limbs, several problems arise due to the non-biological nature of the prosthetics. In order for a recipient to obtain a limb that will mature and grow as they do, the fields of tissue engineering and regenerative medicine must make major advances in their ability to deliver clinical scale composite tissues. This requires an improved understanding of how different types of cells behave in a three-dimensional system. However, for decades, the *in vitro* study of cells, including bone cells, has been based on traditional two-dimensional cell culture. We hypothesized that 3D-printed scaffolds produced to the dimensions of sheep trabecular bone could provide a cost-efficient model to study osteogenesis in a three-dimensional *in vitro* system. To test this hypothesis, we printed three-dimensional scaffolds to the precise dimensions of sheep trabecular bone, functionalized the scaffolds with collagen I and hydroxyapatite coatings, and seeded them with a murine osteoblast cell line. Our results show that 1) the scaffolds are biocompatible with the osteoblasts, 2) osteoblasts can adhere to and proliferate on the scaffolds, and 3) approximately 50% of seeded cells are incorporated into the scaffold during an initial round of seeding. These results suggest that this method, with further optimization, can serve as a useful model to better understand the regenerative potential of bone cells and/or populations of stem cells with osteogenic potential in a three-dimensional system.

Funding for this project was provided by the Winthrop University Research Council and the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE) II Program (NIH/NIGMS P20GM103499)

Histone Deacetylase Inhibitor RG2833 Reduces the Viability of Human Malignant Melanoma Cell Lines SK-MEL-5 and SK-MEL-28

Lauren Green (2016) **Mentor: Dr. Matthew Stern**

Histone deacetylases (HDACs) play an important role in the epigenetic control of gene expression in both normal and cancer cells. Previous studies have demonstrated that pharmaceutical inhibition of HDACs can kill and/or suppress the growth of cancer cells. RG2833 is an HDAC inhibitor that targets specific HDACs known to be active in cancer cells. Melanoma cells have previously been shown to respond to HDAC inhibitors that are structurally similar to RG2833. We hypothesized that the inhibition of HDAC activity by RG2833 would result in the reduced growth and/or death of cells from the malignant melanoma cell lines SK-MEL-5 and SK-MEL-28. To test our hypothesis, we exposed SK-MEL-5 and SKMEL-28 cells to increasing concentrations of RG2833. We found that concentrations of RG2833 that effectively inhibited HDAC activity in melanoma cells also resulted in altered gene expression profiles and reduced cell proliferation and viability. These results demonstrate the effectiveness of RG2833 in reducing the growth and viability of malignant melanoma cells *in vitro* and warrant further investigation of the potential therapeutic use of RG2833 and related compounds in the battle against cancer.

Funding for this project was provided by the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE) II Program (NIH/NIGMS P20GM103499) and the Winthrop University McNair Scholars Program.

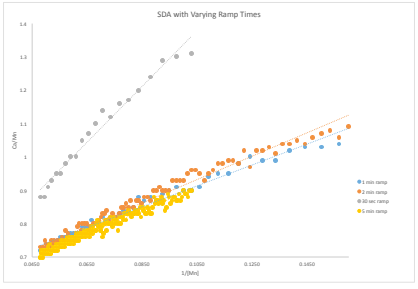
Standard Dilution Analysis for the Determination of Calcium by Flame Atomic Emission Spectrophotometry

Katja A. Hall (2016) **Mentor: Dr. Clifton P. Calloway**
Emily A. Watson (2017)

Standard Dilution Analysis (SDA) is a novel spectroscopic calibration method that can be applied to most instrumental techniques that are capable of monitoring two wavelengths and will accept (or can be made to accept) liquid samples. It combines the traditional methods of calibration curve, standard addition and internal standard, therefore correcting for matrix effects and for fluctuations due to changes in sample size, orientation or instrumental parameters. SDA analysis time requires only about 200 seconds per sample with flame atomic emission spectrophotometry (AES). The preparation of a series of standard solutions and the construction of a universal calibration graph are not required. The analysis is performed by combining two solutions in a single container, the first solution containing 50% sample and 50% of a standard mixture (the analyte + the internal standard); the second solution containing 50% sample and 50% solvent. Data are collected in real time as the first solution is diluted by the second solution. The results are used to prepare a plot of the analyte-to-internal standard signal ratio versus the reciprocal of the internal standard concentration. The analyte concentration in the sample is determined from the ratio of the slope and the intercept of the resulting plot.

Phosphate is a well-known interferent in the determination of calcium in complex samples, such as vitamin tablets and calcium supplements. It is often recommended to add a complexing agent, such as ethylenediaminetetraacetate (EDTA) or lanthanum (La) matrix modifier for the determination of calcium by atomic spectrophotometry. In addition, calcium has been shown to produce non-linear standard addition plots in the extrapolated region when phosphate is present.

SDA method of analysis has been applied to the determination of calcium in over-the-counter vitamin and calcium supplements, containing phosphate, using an inexpensive flame atomic emission spectrophotometer. Accuracy and precision are better than those observed for external calibration, standard addition or internal standard methods of analysis, even in the presence of significant concentrations of phosphate.

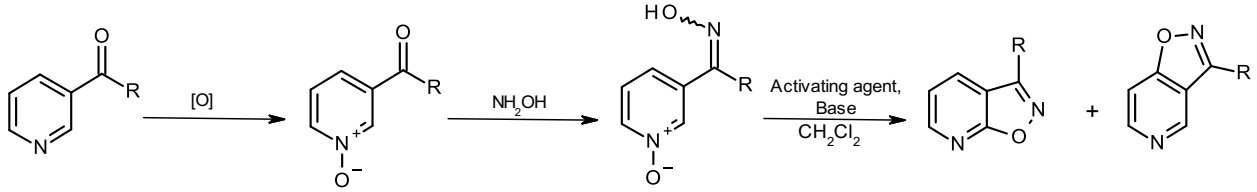


We would like to thank SC-INBRE (IDeA Network of Biomedical Research Excellence), Dr. Brad Jones, Dean, Wake Forest Graduate School and Wake Forest University URECA (Undergraduate Research and Creative Activities) Center for providing support to this project.

Synthesis of Isoxazolopyridines via Cyclization of 3-Acylpyridine *N*-Oxide Oximes

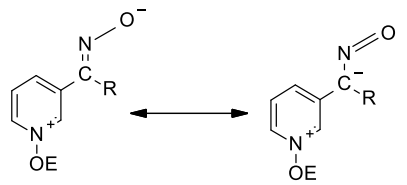
Brandon J. Hicks (2016) **Mentor: Dr. James M. Hanna, Jr.**

Isoxazoles are associated with a wide spectrum of biological functions including antiviral, anthelmintic, anti-inflammatory, anticonvulsant and insecticidal activities. Derivatives of isoxazolopyridines are reported to have cholesterol lowering activities. Recently, the Hanna laboratory reported that tosylhydrazones formed using 3-acylpyridine *N*-oxides could be cyclized into pyrazolopyridines. Reaction of an *N*-oxide tosylhydrazone with a proper electrophile formed an activated intermediate that allowed nucleophilic attack at C2 or C4 on the pyridine *N*-oxide; in the presence of base an E2 elimination then gave the desired cyclized product (Lominac et al. *Tetrahedron Lett*. **2012**. 53, 906-909). We envisioned that this same method could be applied to form isoxazolopyridines from 3-acylpyridines.



1 **2** **2'**

Originally we attempted to cyclize 3-pivaloylpyridine *N*-oxide oxime (**1**, R=*t*-Bu) into the corresponding isoxazolopyridine. Previous Hanna group investigations had determined that the *N*-oxide tosylhydrazones cyclized smoothly only when the attacking atom was on the same side as the pyridine ring.. Thus, the reason for starting with 3-pivaloylpyrdine was to force the oxime hydroxyl group to be syn to the pyridine ring. Confirmation of the oxime configuration was obtained by comparing the chemical shifts of the α-carbon of the *t*-butyl group in the starting *N*-oxide to the corresponding carbon in the oxime. ^{13}C-NMR showed an upfield shift of 7 ppm suggesting the hydroxyl group was anti to the α-carbon (i. e. syn to the pyridine ring). Cyclization of **1** (R = *t*-Bu) to the corresponding isoxazolopyridine was accomplished using various electrophile/base combinations, the most effective of which was triisopropylbenzenesulfonyl chloride and diisopropylethylamine in dichloromethane, giving an 86% yield of **2** and a 9% yield of **2′**. We hoped we could apply these conditions to the cyclization of compounds where the oxime hydroxyl was anti to the pyridine ring, so we examined the cyclization of 3-acetylpyridine N-oxide oxime (**1**, R=Me). ^{13}C-NMR confirmed the oxime hydroxyl in this compound was indeed anti to the pyridine ring. Unfortunately, the standard reaction conditions proved to afford no detectable amounts of **2** or **2′** (R = Me). Altering the solvent polarity (i. e. use of acetonitrile or *N*,*N*-dimethylformamide) had no effect. We have since hypothesized that **1** (R = electron withdrawing group) may cyclize when the oxime hydroxyl is anti to the pyridine ring because of the increased contribution of the C-N single bond resonance structure **4** to the overall resonance hybrid of the key intermediate (see below), perhaps allowing rotation about this bond under the reaction conditions. Synthesis of compound **1** (R=CF_3) is underway to test this hypothesis.



3 **4**

Support was provided by NIH-INBRE, the Winthrop University Research Council, and the Winthrop University Department of Chemistry, Physics, and Geology

Expression, Purification and Crystallization of the Catalytic Core domain of human Sphingosine Kinase 1

Justin Hutchinson (2017) **Mentor: Dr. Jason C. Hurlbert**

Sphingosine kinase 1 (SK1) is a lipid signaling enzyme that has been shown to overexpress in certain cancerous tumors. The SK1 enzyme phosphorylates sphingosine into sphingosine-1-phosphate, which is a lipid that plays a role in signaling pathways inside the cell. This makes SK1 an effective target for inhibitors specifically designed to target the enzymatic active site. Our laboratory has previously cloned the catalytic core domain of SK1 into a pMalc-5x plasmid. In the resulting plasmid construct, SK1 is expressed as a fusion protein with maltose binding protein (MBP). In present work, we have expressed the fusion protein in *Eschericha coli* NiCo cells and purified SK1 via a three step protocol. This consists of running an amylose affinity chromatography, which takes advantage of MBP's affinity for amylose, and cleavage of SK1 from MBP with Factor Xa protease. Then the protein was further purified through the use of anion exchange chromatography followed by cation exchange chromatography. We were able to purify MBP and hSK1 to homogeneity, however, we found that MBP associates tightly to SK1 even after cleavage with Factor Xa protease. Future work will utilize the same protocol without Factor Xa cleavage to obtain purified fusion protein which will then be screened for conditions suitable to promote crystal growth.

This project was supported by SC INBRE grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

Fabrication of Sequentially Stacked $TiO_2/CdS/Fe_2O_3$ Thin Films

Christopher Jordan (2018) **Mentor: Dr. Cliff Harris**

The viability of water-splitting catalysts as commercial sources of renewable energy is strongly dependent on the ability of these materials to utilize the visible portion of the solar spectrum, though most narrow band gap materials ($E_g < 3$ eV) are capable of efficiently driving only one half of the water splitting reaction (i.e hydrogen evolution reaction or the oxygen evolution reaction), not both. In the absence of sacrificial reagents, these materials are subject to deactivation or decomposition. Stacked thin films of CdS (Eg 2.4 eV) and Fe_2O_3 (Eg 2.2 eV) have been fabricated by sequential electrodeposition on titania-coated transparent conductive oxide substrates to photocatalytically drive the full water splitting reaction. Under visible irradiation, Fe_2O_3 is known to drive the oxygen evolution reaction, leaving behind an accumulation of conduction band electrons. These electrons can act to suppress the decomposition of a coupled hydrogen evolution catalyst such as CdS, which would otherwise proceed due to oxidation of the nonmental anion by trapped valence band holes. Of course, a high degree of electrical contact between the surfaces of these materials would be needed to ensure rapid charge transfer. This can be achieved by employing multiple electrodeposition steps to grow one material onto the surface of another. CdS films can be grown cathodically from solutions of Cd^{2+} salts and elemental sulfur in hot DMSO. Commonly employed methods of electrodeposition of high quality Fe_2O_3 involve anodic deposition from acidic Fe^{2+}(aq) baths. Unfortunately, the dissolution of CdS working electrodes proceeds quickly under this condition. Therefore, efforts were primarily focused on the development of a method for cathodic deposition of Fe_2O_3 on CdS using a slightly acidic electrolyte bath. The preliminary SEM analysis shown in Figure 1 depicts a catalyst film having three homogenous layers.

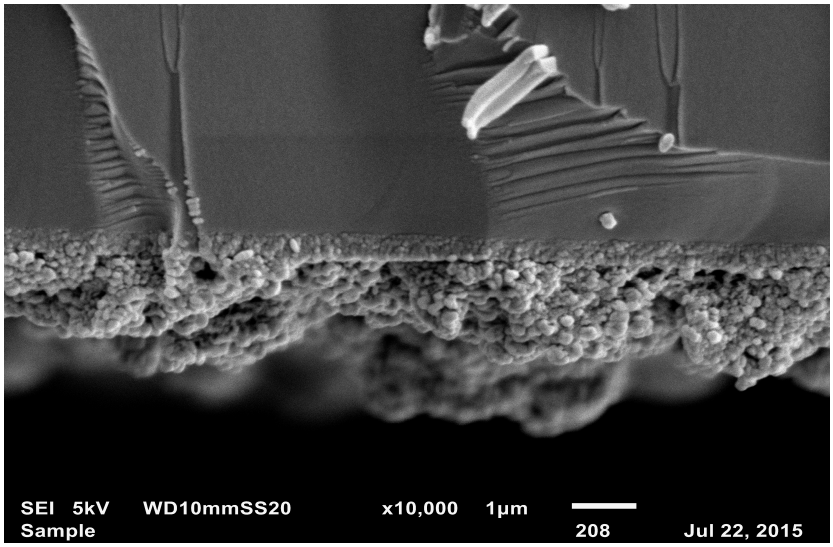


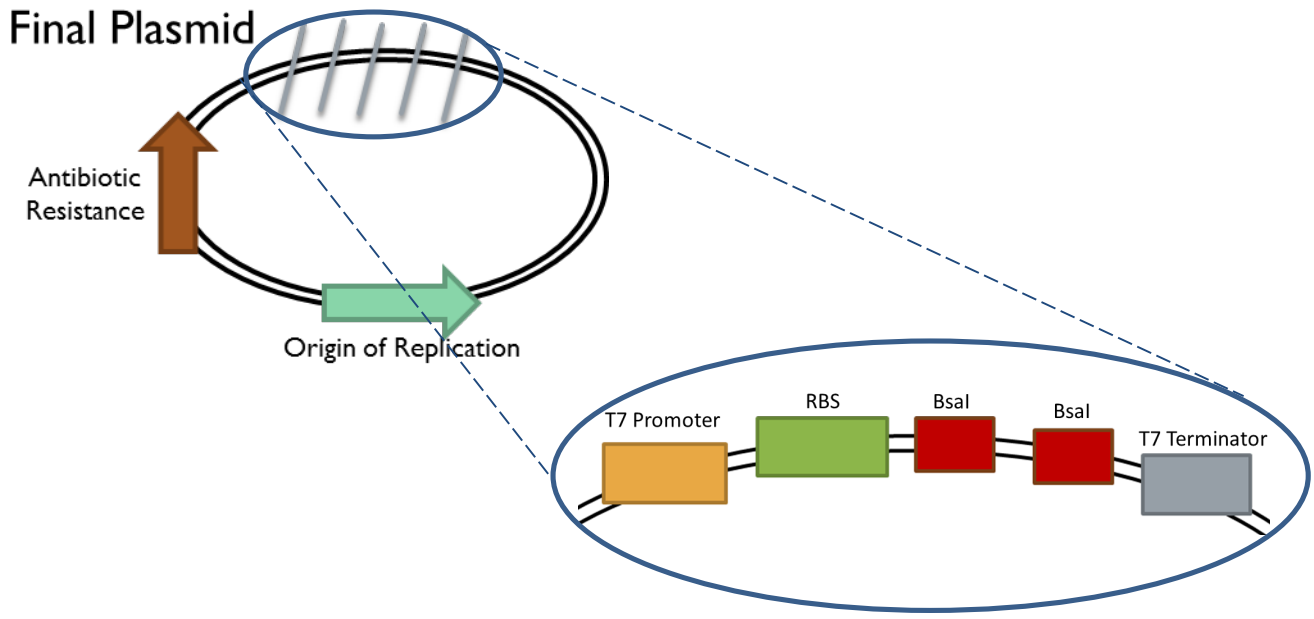
Figure 1. SEM Analysis. Images shows a compact layer of titania on the glass surface, coated with a thin layer of CdS, which is then coated with a non-uniform hematite layer.

This work was supported by The Winthrop University Research Council

Design and Construction of *E. coli* Expression Plasmids That Make Use of the Golden Gate Cloning Strategy

Autumn Leggins (2018) **Mentor: Dr. Nicholas Grossoehme**

Cloning is the alteration of an organism's gene sequence. The traditional method of cloning makes use of one or two Type I endonucleases, at least one gel purification, digestion reactions, ligation reactions, and typically results in a low yield of the desired plasmids. Though this method is very helpful, its low efficiency, high consumption of time and resources, and tendencies for unexplainable failure, there is much room for improvement. The process of cloning has become a faster and more efficient process with newer forms of cloning being created such as the Golden Gate Cloning strategy. Golden Gate Cloning is a one pot reaction that makes use of Type II endonucleases, condenses multiple steps of traditional cloning into one to two steps, and avoids common pitfalls (e.g. self-ligation) due to the lack of palindromic recognition sites in the plasmids going through this process. Biochemists and molecular biologist would greatly benefit from protein expression plasmids that make use of the Golden Gate Cloning approach. The plasmids created needed to have antibiotic resistance, an origin of replication, and the ability to efficiently express the chosen genes in *E. coli* cells using the Golden Gate Cloning strategy. Starting with a commercial plasmid containing an antibiotic resistance site and an origin of replication, this work describes the creation of a plasmid containing a T7 Promoter, Ribosomal Binding Site, T7 Terminator, and BsaI recognition sites so that the so that *E.coli* cells could efficiently express chosen genes once plasmids are introduced to them. By creating two different inserts and running two separate rounds of Golden Gate Reactions, these desired pieces were successfully placed into the plasmids and introduced into *E. coli* cells so that these cells can express the desired genes.



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)

Impacts of Beach Renourishment on the Distribution and Abundance of *Escherichia Coli*

Jordan Lewis (2017) **Mentors: Dr. Matthew Heard**
Dr. Victoria Frost

Erosion on beaches and the replacement of sand through the process of renourishment may impact the survival of many species. In this study, we examined whether a renourishment project at Folly Beach, SC affected the distribution and abundance of *Escherichia coli* (*E. coli*), a common bacteria that often serves as an indicator of pollution and other pathogens. To assess this, we examined differences in the abundance of *E. coli* at field sites over two years. Our findings indicated that renourishment significantly increased *E. coli* abundance over time and that renourishment may also alter pollution and pathogen levels on oceanic beaches.

Support provided by Winthrop McNair Scholars Summer Research Experience 2015

Response of Clay Chemistry to Extreme Heating During Fire Events: Applications to Archaeology

Lauren Lintz (2016) **Mentor: Dr. Scott Werts**

Fire in the natural environment has been shown not only to alter the above and below ground carbon stocks, but can also play a role in altering the mineralogy of the geologic material in which it comes in contact. Our work seeks to utilize the nature of these changes in mineralogy on a small scale to seek a relationship between fire intensity and clay mineralogy in the landscape by using locations of past fires and modern analogues. During approximately 1260-1400 AD, the Hopi Indian Tribe had a settlement named Chevelon located near Winslow, Arizona on the high plains of the Sonoran Desert. Due to abundant ash deposits and the highly oxidized nature of some of the walls of this structure, it is thought this location was burned and subsequently abandoned near 1400 AD. To help understand the burning process, archeologists created the Homolovi structure, which is a modern day analogue to the Chevelon structure, which was burned in 2006 by loading with the fuel that would have likely been used by the tribe during that time. Samples were collected from the wall and floor from both structures to help find possible correlations between the structures that could explain the type of fire that occurred at the Chevelon site. Our research is using clay chemistry as a tool to investigate fire intensity in an archeological context. Powder x-ray diffraction and scanning electron microscopy were used to help identify clay mineralogy. Our results suggest that majority of the clay mixture contains quartz, calcite, illite, and an iron oxide. Standards of these minerals were then placed in a muffle furnace for 6 hours at temperatures beginning at 100°C and increasing in increments of 100°C until reaching 600°C to simulate a high fire intensity that would occur from an intentionally set fire. The samples were then studied using a powder x-ray diffractometer and SEM to understand clay chemistry of these minerals at increasing temperatures, and then compared to the samples from the Chevelon and Homolovi structures to get an understanding of both fire intensities. Our work suggests that there are progressive changes in O/Si ratios with temperature in silicates that may be useful in tracing temperature of the sediments during fire events. This may provide additional insight to archeological studies at other sites where fire structures are present.

Support for this research was provided by the Charles A. Boland '08 and Irene Brunson Boland '63 Student Research Assistantship Endowment

A Self-Immolative Nucleic Acid-Drug Conjugate as a Dual-Action Therapeutic Agent

Jessica Logan (May 2016)

Mentors: Dr. Ke Zhang
Xuyu Tan
Northeastern University
Boston, MA

The combined delivery of chemotherapeutics and nucleic acids can have synergistic effects that result in reduced multidrug resistance. The successful co-delivery of hydrophobic drugs and nucleic acids in a carrier-free system has been demonstrated in a self-immolative nucleic acid-drug nanostructure with a photo-labile linker. These nanostructures display spherical nucleic acid properties such as high cellular uptake and increased stability against nuclease degradation. Following application of UV light, the linker is cleaved and the nucleic acids and drugs are released by an irreversible, self-immolative process. The use of the photo-labile linker, however, is not viable for a therapeutic application. Herein, we have designed and synthesized a bio-trigger disulfide linker to be incorporated into the nucleic acid-drug nanostructures. This study is ongoing, and future work will include the demonstration of cell uptake, stability against DNase I, controlled drug release, and cytotoxicity in cancer cells.

Support provided by REU Site Grant NSF CHE-1262734

Metalloregulation by Nur from *Streptomyces coelicolor*

Olivia Manley (2016) **Mentor: Dr. Nicholas E. Grossoehme**

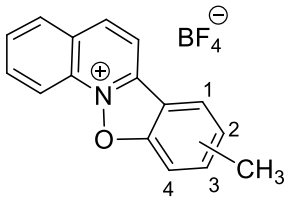
Streptomyces coelicolor is a soil-dwelling actinomycete of medical importance as a result of its ability to produce several antibiotics, including actinorrhodin, undecylprodigiosin, and CDA. These antibiotics, along with many other biologically active compounds, are produced by this organism via a secondary metabolism that is activated nutrient deficient conditions. Investigation of transcriptional regulation, the first level of nutrient regulation in the cell, in *S. coelicolor* is important to further understand the conditions in which this secondary metabolism is activated, as efforts are being made to engineer the regulatory mechanisms of these organisms to optimally produce such antibiotics and other bioactive secondary metabolites. Nur, found only in *S. coelicolor*, regulates nickel homeostasis and contributes to the superoxide response within this organism. Nur is a member of the Fur family of proteins and is dependent upon nickel to be able to bind to DNA, and it is the only member of the Fur family that senses nickel. Nur represses *nikABCDE* and *nikMNOQ*, which encode for proteins responsible for nickel uptake into the cell; directly represses *sodF1* and *sodF2*, which encode an Fe-dependent superoxide dismutase; and indirectly activates *sodN*, which encodes a Ni-dependent superoxide dismutase. Nur is a symmetric homodimer, with each monomer containing two metal binding sites called the Ni-site and the M-site. X-ray crystallographic and mutational studies have shown that the specific amino acids involved in coordinating metal at the Ni-site are His70, His72, and His126, along with three water molecules *in vivo* and that the key amino acids at the M-site are His33, His86, His88, and His90. Additionally, Glu101 forms an electrostatic interaction with the metal at the M-site to further shield it from solvent. The binding of each of these metals is crucial to the regulation of metal homeostasis within *S. coelicolor*. Understanding transcriptional regulation in *S. coelicolor* is of great importance due to the medical relevance of this organism in producing several antibiotics. As nickel accumulation has been proposed to inhibit the secondary metabolism responsible for antibiotic production by *S. coelicolor*, our research seeks to understand nickel regulation within this organism by Nur by uncovering the role of each residue in metal binding and the importance of each binding site in the DNA-binding ability of Nur. A library of relevant Nur mutants has been created to examine the influence of eliminating metal-binding potential of specific residues and entire binding sites on the functionality of Nur. Characterization of these mutants and WT Nur as well aids in understanding the changes in the behavior of Nur caused by the mutations and contributes to a better understanding of nickel homeostasis within *S. coelicolor*. The research conducted this past summer has focused on the requirements for metal of WT Nur in order to induce binding to the *sodF* promoter through fluorescence anisotropy, metal content assays, and titrations in the presence of metal chelators. These experiments have provided new insight on the role and the affinity of each metal-binding site to develop a more thorough understanding of Nur.

Support provided by grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health, Research Corporation Grant 20160, and the Winthrop McNair Scholars Program.

Biological Evaluation of Novel Benzisoxazolo[2,3-a]azinium Tetrafluoroborates as Anticancer Agents

Theresa Melendez (2017) **Mentor(s): Dr. Takita Felder Sumter**
Dr. James M. Hanna Jr.

Ellipticine has been effectively used to treat various types of cancer. This aromatic, planar, antineoplastic drug works primarily by DNA intercalation and its derivatives represent promising options for cancer drug discovery. DNA intercalators are small molecules that can bind to DNA between base pairs, resulting in the inhibition of replication and providing a viable option for cancer treatment. Several novel benzisoxazolo[2,3-a]pyridinium and -quinolinium tetrafluoroborate salts with structural characteristics similar to ellipticine were evaluated and shown to effectively kill colon cancer cells at single digit micromolar concentrations. Previously, the benzisoxazolo[2,3-a]pyridinium compounds were evaluated as possible anticancer agents; various substituents attached to the parent scaffold were tested, and a methyl substituent proved to be the most successful. To expand on this work we evaluated the anti-cancer activity of benzisoxazolo[2,3-a]quinolinium tetrafluoroborate compounds bearing methyl substituents in the 1-methyl, 2-methyl, 3-methyl, and 4-methyl positions (see figure) and testing them against HCT 116 human colon carcinoma cells.



Our preliminary data indicates limited survival of colon cancer cells when treated with 50 μM drug and the cytotoxicity assays used demonstrated an inverse correlation between concentration of drug and cell survival. These findings suggest that benzisoxazolo[2,3-a]quinolinium tetrafluoroborates are an effective lead for better understanding molecular cancer pathways, and additional studies will be aimed at detailed analysis of the DNA binding mechanism of these compounds and expansion of our drug library.

This work was supported by grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health," or "This project was supported by grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

A Reaction-Diffusion Model of Cancer Invasion with Cancer Stem Cells

Alexander Middleton (2017) **Mentors: Dr. Kristen Abernathy**
Dr. Zachary Abernathy

The use of partial differential equations in cancer modeling allows us to describe both how cancer populations grow over time as well as how tumor cells can invade healthy tissue. In 2013, McGillen et al. considered a system of three nonlinear, partial differential equations that modeled the compartmental interactions between healthy cells, tumor cells, and lactic acid concentration. Through numerical simulations and asymptotic approximations, McGillen et al. demonstrated four distinct types of traveling-wave behavior: heterogeneous and homogeneous invasion, nonaggressive (slow-moving) tumor dynamics, and tumor clearance. We extend the work of McGillen et al. by incorporating the cancer stem cell (CSC) hypothesis into the existing model.

CSCs are a specialized subset of tumor cells with normal adult stem cell properties, including high proliferative potential and the ability to self-renew. These cells are a possible culprit for tumor recurrence as they are thought to be highly resistant to standard cancer therapies. By including CSCs into the model, we establish new conditions to achieve a locally stable cure state as well as numerically demonstrate the existence of a recurrence state in which CSCs are able to repopulate a dormant tumor.

Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

Phylo-grouping and analysis of virulence factors of *Escherichia coli* found in beach sand

Savannah Moritzky (2017) **Mentors: Dr. Victoria Frost**
Dr. Matthew Heard

Recent research has shown that *Escherichia coli*, a bacterial species found in the gastrointestinal tract of vertebrates, is able to persist in sand at oceanic beaches. Though the majority of these species do not cause human illness, there are strains that are pathogenic and can cause human sickness. In this study, we isolated *E. coli* from sand collected at Folly Beach, South Carolina in order to study these strains in more detail. Initial characterization helped divide the beach strains into different phylo-groups, indicating their possible origin. The presence of virulence genes was also investigated to help determine their pathogenic potential.

Support for this project was provided by the Winthrop McNair Scholars Program

Construction and analysis of reduced graphene oxide/ruthenium oxide supercapacitor electrodes using electrophoretic deposition

Dakoda Mulinax (2017) **Mentor: Dr. F. Z. Amir**

Novel, low cost, environmentally friendly, and high-performance storage energy systems have been increasingly in demand as a result of the needs of modern society and its emerging ecological concerns. Supercapacitors, or electrochemical capacitors, are a promising candidate for alternative energy storage because of their high power density and their potential to reach relatively high energy densities. Also with their long life cycle and short charge and discharge time, supercapacitors may replace the use of batteries and provide environmentally friendly energy. In this work, we used electrophoretic deposition (EPD) to develop high performance capacitors based on reduced graphene oxide and ruthenium oxide (rGO/RuO_2) composites electrodes. The morphology of fabricated rGO/RuO_2 was analyzed using scanning electron microscopy (SEM), and the structure of the electrodes was analyzed using x-ray diffraction. The electrochemical capacitive properties of rGO/RuO_2 were characterized using a two-electrode cell configuration in H_2SO_4 electrolyte solution. In our research, we fabricated supercapacitors with a performance greater than the ones already existing and we hope to further impact the electrical storage device market and potentially change how electronics are made.

Expression, Purification and Crystallization of a novel endoxylanase from the enteric bacterium *Bacteroides vulgatus*

Jesslyn Park (2018) **Mentor: Jason C. Hurlbert**

Sustainable sources of energy are growing in demand as fossil fuels are rapidly expended. One such energy source is fuel ethanol generated from the fermentation of plant biomass by engineered bacterial biocatalysts. The creation of a biocatalyst capable of converting nearly any plant matter to fuel ethanol requires the identification of novel enzymes capable of degrading specific carbohydrate polymers and cloning these enzymes into a bacterial host. This study seeks to structurally characterize a novel xylanase of glycosyl hydrolase family 30 (GH30) from *Bacteroides vulgatus*, a bacterium found in the human gut microbiome, via x-ray crystallography. The gene for *B. vulgatus* GH30 endoxylanase (BvGH30) was cloned into a pET 28b prokaryotic expression vector which was used to transform a culture of *Escherichia coli*, and the resulting bacterial strain was used to express the cloned BvGH30 gene. The recombinant protein produced was then purified to homogeneity via Ni^{2+}-Metal Chelating Affinity Chromatography (MCAC) as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified protein was concentrated to 10 mg/mL and used to screen for solution conditions the promoted crystal growth by sparse matrix screening in hanging drop, vapor-diffusion plates. Single rectangular crystals of defined morphology (less than 0.1mm in length) were obtained in 0.2M $(NH4)_2HPO_4$, 20% (w/v) PEG 3350, pH 8.0 and large numbers of smaller rectangular crystals were obtained in 0.1M Citric acid, 0.8M $(NH_4)_2SO_4$, pH 4.0. Grid screening around these two conditions will be employed to increase crystal size and all crystals larger than 0.1mm in length will be subjected to x-ray diffraction analysis.

Support for this research was provided by the Winthrop University McNair Scholars Program and the Winthrop Univeristy Department of Chemistry, Physics and Geology.

Optimizing Decellularization Protocols for the Production of Porcine Acellular Muscle Matrix Scaffolds

Carolina Pham (2018) **Mentor: Dr. Matthew Stern**
Ariba Naz (2016)

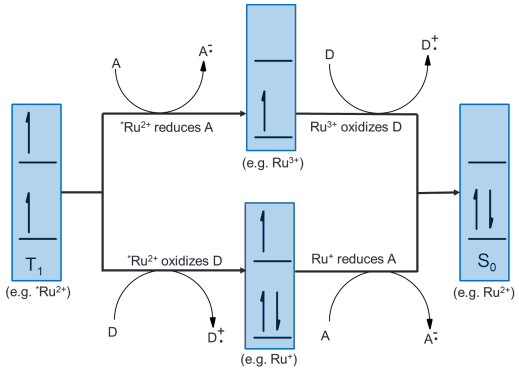
Skeletal muscle tissue is one of the most common sites of traumatic injury in the human body. A variety of biomaterials that facilitate muscle regeneration are in development; however, few are able to provide the structural and biochemical cues present in the tissue's native scaffolding, its extracellular matrix. We hypothesized that the process of tissue decellularization, which removes the cellular content of a tissue while leaving the extracellular matrix intact, could be used to produce biomaterial scaffolds from porcine skeletal muscle tissue. To test this hypothesis, we systematically evaluated the effectiveness of ten decellularization protocols, each of which used a different combination and/or order of decellularization agents. Qualitative histological examination and scanning electron microscopy of the different forms of the material produced revealed a spectrum of effectiveness among the methods tested. Each protocol yielded a different combination of a) removal of cellular content and b) retention of extracellular matrix content and architecture. Future work will seek to quantify these differences as well as the DNA content and mechanical properties of the different forms of the material. Those forms exhibiting sufficient decellularization and retention of extracellular matrix will be termed Porcine Acellular Muscle Matrix (PAMM) and be used in subsequent studies testing their ability to support the growth and differentiation of different populations of myogenic cells.

Funding for this project was provided by the Winthrop University Research Council and the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE) II Program (NIH/NIGMS P20GM103499)

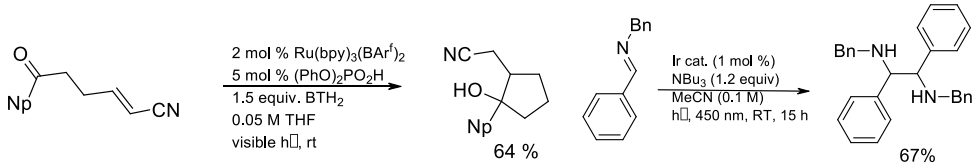
Visible Light Promoted Heterocycle Formation

Davis P. Plasko (2018) **Mentor: Dr. James M. Hanna, Jr.**

Recently, reactions driven by visible light as an energy source have emerged as a valuable synthetic tool, allowing many transformations which often required harsh conditions (such as high reaction temperatures) to be carried out under much milder conditions, increasing product selectivity by reducing the number of side reactions. Typically in these processes, a suitably ligated Ru- or Ir-catalyst, upon excitation with visible light, undergoes metal to ligand charge transfer followed by intersystem crossing to a long-lived triplet excited state (T_1); this excited complex can undergo either oxidative or reductive quenching by an organic molecule (see below) to give an organic radical intermediate, which can interact with other substrates to form new C-C or C-X (X = N, O, S) bonds. The cycle is closed by oxidation or reduction of the metal species to the original (ground state, S_0) catalyst.

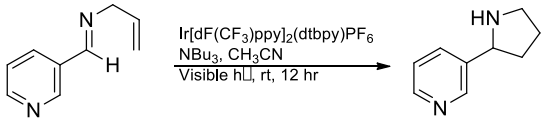


Several groups have reported examples of these visible-light driven reactions; we were attracted to studies by Knowles, et al. and Rueping, et al. who showed that neutral ketyl and aza-ketyl radicals were accessible via visible-light photoredox processes:



Knowles R. et al. *J. Am. Chem. Soc.* **2013**, *135*, 17735. Rueping, M. et al. *Angew. Chem.* **2015**, *54*, 8828

We envisioned merging these two concepts to form nitrogen heterocycles by intramolecular addition of neutral amine radicals formed from imines to unsaturated systems appended to the nitrogen:



Our initial proof-of-concept studies using the above substrate revealed that the desired cyclization did not take place, but instead the dimer (cf. the Rueping example above) was produced as the only isolable product in yields from 16 – 30%. We believe the reason is that the proposed cyclization is a 5-*endo-trig* process, which is stereoelectronically disfavored. In the future, we intend to evaluate other imines and/or hydrazones, the cyclization of which will be more stereoelectronically favored.

Support was provided by the Winthrop University Research Council and grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health

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Injection of ADSCs into Embryonic Chicken Heart Scaffolds as a Model of Cardiac Tissue Engineering

Timothy Raines (2015) **Mentor: Dr. Matthew Stern**

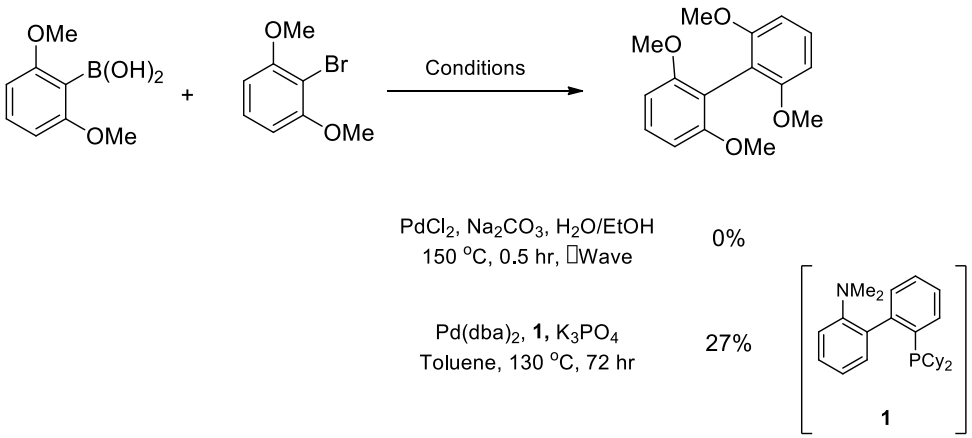
Whole organ tissue engineering represents a potential solution to the problems of organ shortage and rejection; however, the process of producing a three-dimensional functional organ is extremely complex. Therefore, the development of cost-effective, small-scale, three-dimensional model systems for studying cardiac regeneration and engineering *in vitro* represents a useful contribution to the field. We hypothesized that embryonic chicken hearts could be decellularized using previously tested methods and then recellularized with adipose-derived stem cells. To test this hypothesis, we serially injected murine adipose-derived stem cells into decellularized embryonic chicken heart scaffolds. Our results demonstrate that 1) an embryonic chicken heart can be effectively decellularized using common methods, 2) the sterility of these acellular heart scaffolds can be maintained, even when using an open system, 3) sterile acellular heart scaffolds are biocompatible with viable cells, and 4) acellular heart scaffolds can be partially recellularized via injection with murine adipose-derived stem cells. These results suggest that, with further optimization of the recellularization process, acellular embryonic chicken heart scaffolds could be used to study cardiac regeneration and differentiation in the three-dimensional context of the tissue's extracellular matrix. Such a model can provide useful insight into fundamental processes common to the engineering and regeneration of cardiac tissue on a clinical scale.

Funding for this project was provided by the South Carolina IDeA Networks of Biomedical Research Excellence (INBRE) II Program (NIH/NIGMS P20GM103499)

Synthesis of 2,2′,6,6′-Biphenyltetrol via Suzuki Coupling Utilizing Bulky Phosphine Ligands

Jake Roberts (2017) **Mentor: Dr. James M. Hanna, Jr.**
Benjamin Hernandez (2018) **Dr. Robbin K. Lammi**

Amyloid-β peptide (Aβ) self-assembles into neurotoxic, β-structured aggregates, which are the primary component of the extracellular senile plaques characteristic of Alzheimer's disease. A variety of small molecules have been shown to inhibit the aggregation process; typically, these contain aromatic groups and one or more hydrogen-bond donors to enable binding to Aβ. We have previously identified biphenyltetrols (BPTs) as a class of molecules exhibiting promising inhibitory efficacy. Of our symmetrical BPT series, 2,2′,6,6′-biphenyltetrol (2,6-BPT) was the final molecular structure to be synthesized. Previous efforts to prepare the sterically hindered 2,2′,6,6′-tetramethoxybiphenyl intermediate through a typical Suzuki coupling afforded no product. These results prompted a search for an alternative method, and a copper-catalyzed homocoupling of 2,6-dimethoxyphenylboronic acid was found to conveniently give the required 2,2′,6,6′-tetramethoxybiphenyl. However, obtaining reasonable yields of this intermediate required a stoichiometric amount of copper, which would likely complicate tests of inhibitory efficacy. We therefore have re-investigated the Suzuki coupling, this time employing a catalyst designed for coupling sterically hindered substrates. Through a coupling reaction using the catalyst comprised of bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) and the bulky phosphine ligand (2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, **1**), the 2,6-intermediate was obtained in 27% yield after recrystallization. Demethylation of the intermediate afforded the desired 2,6-BPT in 20% yield after recrystallization. Future efforts will include evaluation of 2,6-BPT for its efficacy as an amyloid-β aggregation inhibitor.



Support was provided by the Winthrop University Eagle STEM Program and an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences.

Cloning and expression of the DNA binding domain of FoxO from Ciona intestinalis that contains an N-terminal nuclear localization signal

Mikala Smith (2017) **Mentors: Dr. Nicholas Grossoehme**
Dr. Heather Evans Anderson

FoxO proteins are a subgroup of the Forkhead family of transcription factors. FoxO proteins are highly conserved and regulate expression of genes that control a wide variety of cellular processes including: apoptosis, cell differentiation and proliferation, and atrophy. Ciona intestinalis is a useful model system to study developmental biology, particularly heart development since all chordates share a conserved cardiac gene program as well as similar cellular processes during development. Ciona FoxO (ciFoxO) protein is very similar to the FoxO1 protein in humans. In order for ciFoxO to transcriptionally regulate gene expression, it must localize to the nucleus. The major goal of this project is to add a nuclear localization signal (NLS) to an expression vector containing ciFoxO sequence that will be electroporated into Ciona embryos where it will be expressed. The N-terminal NLS will direct the exogenous ciFoxO sequence to the nucleus of cells where it will be able to bind to target DNA sequences in the Ciona genome. The ultimate goal is to express ciFoxO constructs containing a NLS in vivo and then isolate chromatin in order to perform a ChIP-Seq assay to determine ciFoxO target genes. The ciFoxO target genes will be compared to vertebrate FoxO1 target genes to determine the level of conserved function of FoxO family members in chordates during heart development. To date, we have successfully inserted the NLS into the vector and produced dechorinated embryos; electroporation optimization is under way.

Funding source: NIH Grant Number P20 RR-16461 (SC-INBRE), NIH Grant Number 1R15HL104587-01(HJEA) and Institutional Development Awards (EPSCoR/IDeA) Science Affiliate Network grant (NG and HEA).

Creating a microcosm to examine salinity tolerance of *Escherichia coli* in beach sand

Leigha Stahl (2017) **Mentors: Dr. Matthew Heard**
Dr. Victoria Frost

Escherichia coli (*E. coli*) is a bacteria species that thrives in a variety of environments. Due to its widespread prevalence, it is commonly used as an indicator for pollution and other pathogens. One place where it is not often looked for is oceanic beaches because *E. coli* is inhibited by salt. However, recent research has shown that *E. coli* often thrives in sand at beaches. To determine how it persists in sand, we created a microcosm simulating the intertidal zone of a beach. Using this microcosm, we are testing how varying salinity levels affect persistence of *E. coli* in sand. Collectively, our findings suggest that *E. coli* may be able to persist on sandy beaches despite the stress of salinity and may be a useful tool in the future for assessing these ecosystems for fecal contamination levels.

Support provided by Winthrop McNair Scholars Summer Research Experience 2015

Modeling the Dynamics of Glioblastoma Multiforme and Cancer Stem Cells

Stephen Steward (2017) **Mentor: Dr. Kristen Abernathy**
Maria Vølstad (2017) **Dr. Zachary Abernathy**

In this project, 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.

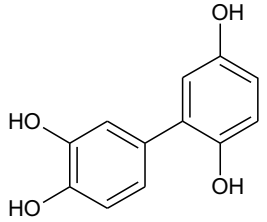
We present a system of nonlinear ordinary differential equations that describes the interaction between cancer stem cells, tumor cells, and alloreactive cytotoxic-T-lymphocytes (CTLs). Under the assumption of constant treatment, we present conditions on the treatment amount that leads to a locally stable cure state. We also explore a more biologically accurate treatment schedule in which CTLs are injected periodically. In the case of periodic treatment, we numerically establish treatment schedules that lead to cancer persistence, cancer recurrence, and cancer eradication. We conclude with a sensitivity analysis of our parameters and a discussion of biological implications.

Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

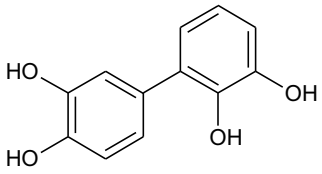
Evaluation of Unsymmetrical Biphenyltetrols as Aggregation Inhibitors for Alzheimer's Amyloid-β Peptide

Andrea Taylor (2017) **Mentor(s): Dr. James M. Hanna, Jr.**
Matthew Hurtt (2018) **Dr. Robin K. Lammi**

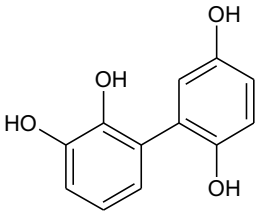
Amyloid-beta (Aβ) is a peptide of 39-43 amino acids that is found naturally in brain plasma and cerebrospinal fluid. The unstructured Aβ monomers formed by enzymatic cleavage of amyloid precursor protein (APP) readily self-assemble into a wide variety of β-structured aggregates, including soluble oligomers and insoluble fibrils, all of which are toxic to neurons. This aggregation process is causally linked to Alzheimer's disease and inhibiting or controlling it is a primary area of research toward Alzheimer's prevention and treatment. We have been investigating a series of isomeric biphenyltetrols (BPTs) as potential inhibitors of Aβ aggregation. Studies of the symmetrical isomers revealed that 3,4-, 2,5-, and 2,3-BPT were the most promising aggregation inhibitors, with IC_{50} values below 10X (where X is equal to the Aβ concentration). To continue probing the effects of hydroxyl positioning on inhibitory efficacy, others in our group synthesized the three unsymmetrical BPTs shown below. We have evaluated 3,4,2,5- 3,4,2,3- and 2,3,2,5-BPT as Aβ aggregation inhibitors by means of the Congo red spectral-shift assay.



3,4,2,5-BPT



3,4,2,3-BPT



2,3,2,5-BPT

Congo red (CR) dye binds specifically to β-structured Aβ aggregates, causing a red-shift in its UV-visible absorbance spectrum: by monitoring dye binding, it is therefore possible to follow Aβ aggregation as a function of time. Inhibitor efficacies are determined by comparing equilibrium levels of aggregation in the presence and absence of BPT compounds. Our results suggest that 3,4,2,3- and 2,3,2,5-BPT are at best minimally effective as inhibitors of Aβ aggregation. In some trials, these compounds seemed to slightly promote aggregation when present at 10X concentrations. In contrast, 3,4,2,5-BPT is a highly effective aggregation inhibitor, completely abrogating Aβ aggregation at concentrations well below 10X. Preliminary results suggest that the IC_{50} value for this compound may be as low as 1.2X, comparable to that of 3,4-BPT, our most promising compound to date. Future work will focus on confirming preliminary IC_{50} results for all three unsymmetrical BPT compounds, and using electron microscopy to investigate Aβ aggregate morphology in the presence and absence of inhibitors.

This project was supported by SC INBRE grants from the National Center for Research Resources (5 P20 RR016461) and the National Institute of General Medical Sciences (8 P20 GM103499) from the National Institutes of Health.

Probing the Activation Mechanism of BAK in Mitochondrial Apoptosis

Michala Tesney (2017)

Mentor: Tudor Moldoveanu
St Jude Children's Research Hospital
Memphis, TN

Proteins of the B-cell Lymphoma 2 (BCL-2) family are the main regulators of mitochondrial outer membrane permeabilization (MOMP), the initiating event of the caspase cascade during mitochondrial apoptosis. Upon activation by BID binding to its trigger site, the BCL-2 effector, Bcl-2 Antagonist Killer (BAK) mediates MOMP at the outer mitochondrial membrane (OMM). The mechanism of BAK oligomerization during MOMP remains poorly understood and controversial. It is postulated to involve sequential dimerization at an extensive interface defined by the trigger site followed by association mediated by α6-α6 interface. Here we probed the oligomerization mechanism using disulfide-bonded dimers of BAK engineered to trap the α6-α6 interface. Using functional assays based on permeabilization large unilamellar vesicles (LUVs) that mimic the OMM, we observed a range of BAK activities imposed through enforced dimerization. The best permeabilization of LUVs was induced by enforced dimerization at the C terminus of α6, whereas a gradual reduction in activity was observed as the disulfide bond was introduced closer to the N terminus of α6. This is the first evidence against the α6-α6 being a preferred oligomerization interface. Locking BAK in the α6-α6 dimeric conformation will serve as an alternate crystallization contact site and will allow access to the trigger site at the BC groove for drug binding. The α6-α6 enforced BAK dimers aid in drug discovery efforts aimed at targeting BAK in cancer biology.

Mimicking Ocean Conditions to Study the Growth of Calcium Carbonate

Danielle Thibault (2016) **Mentor: Dr. Maria Gelabert**

Calcium carbonate is a very prominent biomineral in the ocean that accounts for the skeletons of coral and the shells of other aquatic organisms. In descending stability order, the three polymorphs of calcium carbonate are calcite, aragonite, and vaterite. As a result of recent increases in the atmospheric carbon dioxide pressure, the ocean has been gradually acidifying. As the ocean becomes more acidic, we expect the concentration of carbonate as well as the formation of calcite to decline. This was studied this summer by mimicking ocean conditions and artificial seawater. The role of magnesium partakes in the formation of calcium carbonate was studied at room temperature and a polar temperature of approximately 4 °C. Magnesium is believed to stabilize the metastable polymorphs at high concentrations or incorporate into the crystal lattice at low concentrations, forming dolomite. Through experimental procedure, analysis with optical imaging and scanning electron microscopy (SEM), X-ray diffraction, thermogravimetic analysis, and differential scanning calorimetry, magnesium impacted the morphology of calcium carbonate crystals. The 1:1 magnesium to calcium samples provided a mixture of crystal shapes, while the 8:1 ratio provided solely zinnia-like crystal morphology. Thermal analysis verified the decomposition of calcite at elevated temperatures. As expected, magnesium incorporated into the lattice of the low ratio samples and a less stable polymorph, not calcite, was formed at the higher ratios.

Support for this research was provided by grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health

Synthetic Modification of a "Zone 4" Reaction on a Known Sphingosine Kinase 1 Inhibitor to Improve Oral Bioavailability

Morgan Turnow (2016) **Mentor: Dr. Thomas Christian Grattan**

Targeted therapy is a new and developing technique that has the ability to target a specific molecule and pathway, increasing the efficiency of attacking abnormal cancerous cells. The sphingomyelin pathway is important in cell regulation, signaling, and determining a cells fate (Figure 1).

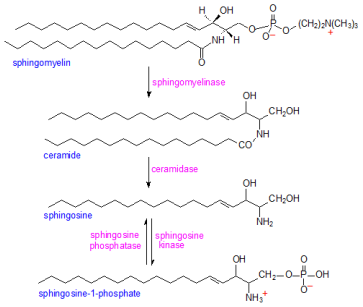


Figure 1: The sphingomyelin pathway

Inhibition of the sphingosine kinase enzyme (SK1) leads to a buildup of sphingosine and ceramide, two molecules directly linked to cell apoptosis. It also decreases the intracellular concentration of sphingosine-1-phosphate (S1P), a molecule linked to cellular proliferation. Therefore, our project objective is to develop inhibitors of SK1 in order to lower the intracellular concentration of S1P and generate apoptosis through the buildup of sphingosine and ceramide molecules. Smith et al. discovered an inhibitor that was successful at inhibiting SK1 *in vitro*, however was unsuccessful *in vivo* (Figure 2). Therefore, this inhibitor must be modified to improve inhibition success *in vivo*.

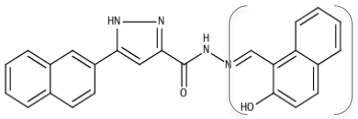


Figure 2: Template inhibitor discovered by Smith et al.
Zone 4 is located in brackets.

Microwave heating is used to synthesize successful modifications of the template inhibitor in zone 4. It is hypothesized that the addition of polar substituents will increase the hydrophillicity and oral bioavailability of these compounds. There have been fifteen successful inhibitors synthesized, all proven using high-resolution mass spectrometry. The inhibitors will be tested *in vitro* to study their interaction with human SK1 as well as inhibition success rate. It is projected that these inhibitors will be used in pharmaceutical drugs to aid in cancer treatment.

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

Identification and verification of a *su(var)3-9* mutation within *Drosophila melanogaster*

Camerun C. Washington (2017) **Mentor: Dr. Kathryn P. Kohl**

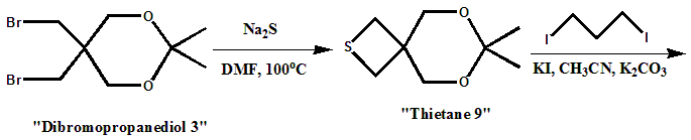
Meiosis is the biological process by which homologous chromosomes are segregated to form four, genetically diverse haploid gametes. In most organisms, for the segregation of homologous chromosomes to occur properly, crossovers must occur on precise, localized regions of each chromosome. Additionally, the phenomenon of the "obligate crossover" ensures that each pair of homologous chromosomes receives at least one crossover (Jones 1984). Observations such as these strongly suggest that crossover formation is a highly regulated process. However, for unknown reasons, in the model organism *Drosophila melanogaster*, meiotic crossover events do not occur on the 4th chromosome. Since Drosophila chromosome 4 is composed primarily of heterochromatin (~70%), in contrast with the other Drosophila chromosomes (~30%) (Adams *et al.* 2000), we hypothesize that the abundance of heterochromatin is responsible for preventing meiotic crossing over on chromosome 4. To test this hypothesis, we seek to reduce the level of heterochromatin in Drosophila using *su(var)3-9* mutants. These *su(var)3-9* mutants are defective for one of the genes responsible for heterochromatin formation. To verify our fly stocks contained the desired mutation in *su(var)3-9*, the DNA from the fly was sequenced and analyzed *in silico.* To accomplish this, *su(var)3-9*-specific primers were designed and used to amplify the gene via polymerase chain reaction. Following agarose gel electrophoresis, DNA bands of the expected size were extracted, purified and Sanger sequenced. The presence of a double peak on the chromatogram at the appropriate location confirmed that the fly stock was heterozygous for the desired *su(var)3-9* single nucleotide polymorphism, which created a missense mutation. With this information, we can now directly test our hypothesis that the high level of heterochromatin on Drosophila chromosome 4 prevents meiotic crossover formation. We will do this by scoring the amount of crossing over on both chromosomes 2 and 4.

*This research was supported by a Winthrop University Research Council Grant **a**nd by Winthrop University Start-Up Funds to Dr. Kathryn P. Kohl*

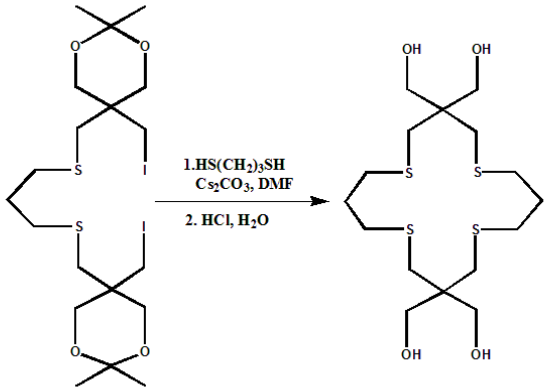
Synthesis of "Thiocrown 12" for the Stabilization of Cu^+ in Titration Experiments

Margaret Whitley (May 2017) **Mentor(s): Dr. Nicholas E. Grossoehme**
Dr. Jay Hanna

Isothermal Titration Calorimetry (ITC) is an ideal method to study binding reactions independently of spectral signatures. The biologically relevant oxidation state of copper, copper (I), is one such "spectroscopically silent" ion. Unlike traditional methods, ITC relies on the generation of a heat signature; as such, it is readily used to quantify thermodynamic properties of Cu^+ binding reactions. However, this metal is difficult to study under aqueous conditions due to its sensitivity to oxygen and disproportionation to Cu^{2+} and Cu^0. This project aims to use a cyclical tetrathioether, "thiocrown 12," for the delivery of chemically stable cuprous ions to systems of interest. The thiocrown synthesized is desirable due to the four coordinate tetrahedral geometry that the cavity presents. Recognition of these desirable qualities led this research to continue in the synthesis of the thiocrown by the method pictured below.



"Dibromopropanediol 3" "Thietane 9"



"Diiodide 10" "Thiocrown 12"

Through the reaction of "dibromopropanediol 3" with Na_2S the cyclized "thietane 9" product was obtained in high yield and purity. The product was confirmed by Gas Chromatography Mass Spectrometry (GC Mass Spec) and comparison to literature ^{1}H-NMR. "Diiodide 10" was produced by reacting "thietane 9" with diiodopropane; however, the literature conditions for this reaction were unusable. To get around this, we optimized a system for the microwave synthesis of "diiodide 10," significantly dampening the time needed for the synthesis. The most effective conditions were found to be 135°C for 10 hours.

Support was provided by grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health, Winthrop University Research Council Grant, and the Winthrop Department of Chemistry, Physics and Geology.

CD8+ T cell Cross-reactivity Following Influenza Infection

Ashley S. Williams (2016)

Mentor: Dr. Paul Thomas
St Jude Children's Research Hospital
Memphis, TN

The seasonal influenza A virus continues to inflict the human population, and emerging novel zoonotic subtypes pose a pandemic threat with risk for severe morbidity and even mortality. Due to a lack of pre-existing neutralizing antibodies to novel subtypes, CD8+ cytotoxic T lymphocytes (CTLs) generated from previous influenza infection play a critical role in facilitating viral clearance in the event of heterosubtypic infection. Here, using a model of subsequent heterosubtypic influenza infection in mice and single cell PCR technology, we characterized the sequences of T cell receptors (TCRs) of CTLs determined to be cross-reactive to different variants of the viral nucleoprotein (NP) peptide (DbNP366–374, NP wt, NP-T8A, NP-N3A). We found that NP wt can generate a population of CTLs that are cross-reactive to NP-T8A or NP-N3A and vice versa; the non-cross-reactive CTLs generated by each peptide showed a highly diverse TCR repertoire, but the cross-reactive CTLs generated by each peptide variant yielded a highly specific and enriched TCR repertoire. Further analysis of the potential molecular patterns in the TCR repertoire of the cross-reactive CTLs may provide insights into the TCR/peptide/major histocompatibility complex class I (MHCI) interaction, as well as into the molecular determinants of CTL cross-reactivity.

Trabecular bone reinforces bone capability to withstand off-axis loading

Zach Wood (2017) **Mentor: Dr. Meir Barak**
Lisa Howard (2016)

Previous studies have shown that trabecular bone responds to external loading by adjusting its architecture to optimize its structure to the principle direction of loading (also known as 'Wolff's law'). Yet, each trabecular bone sample is unique and very delicate (i.e. each sample can be tested only once before it fails). Thus, it was practically impossible to test a sample multiple times in different directions to find its optimal mechanical orientation. Here we present a novel approach to determine the stiffness and strength of a trabecular sample in multiple orientations. In this study we used a 3D printer (ProJet 1200) to create multiple copies of a cubical trabecular bone sample taken from the talus of a sheep. The original templet was reconstructed from a high resolution micro-CT scan along the three principle axes of the bone. Next, the reconstruction was tilted by 10 degrees and a new templet was created (i.e. the principle axes of the cube (axial and radial axes) were tilted by 10 degrees to the principle axes of the bone). This sequence was repeated 9 times, each time the template was tilted by an additional 10 degrees, until the original axial axis became the radial axis orientation (a final tilt of 90 degrees). Each template was printed 10 times to a total of 100 samples. Next each sample was tested in compression using an instron machine (Instron 5942) until failure and sample stiffness and strength were recorded. Our results show that contrary to the accepted paradigm, trabecular structure is significantly stiffer and stronger between 40-80° relative to the axial axis. These results differ from the common belief that trabecular bone optimize its structure along the principle loading axis of the bone and implies that trabecular bone has a major role in maintaining bone integrity when it is loaded off-axis (e.g. a fall or a trauma, hitting the bone from the side). Our study introduces new and unexpected results which may change the way trabecular bone structure-function is understood.

Support for this research was provided by Winthrop University Research Council Grant SC15013

Understanding Iron Regulation in *Streptococcus pneumonia*

Jessica Zielinski (2018) **Mentor: Dr. Nicholas Grossoehme**

Streptococcus pneumonia is a bacterium commonly found within the nasopharynx of humans. It is capable of spreading to other parts of the body and causing many diseases including meningitis, pneumonia, otitis media, and others. The aim of this research is to specifically understand how iron levels are maintained within *S. pneumonia*. A balance has to be stuck between the essential cellular functions that rely on iron and iron induced toxicity. To date, not much is understood on the iron regulatory system in *Streptococcus pneumonia.* However what is known begins with extracellular iron binding to Stkp, or the Serine-Threonine Kinase protein. This results in a conformational change that activates the intracellular kinase domain, which will phosphorylate RitR. RitR, or the regulatory iron transport regulator, represses the expression of the *piu* gene when bound to DNA. In this regulatory process the transmembrane proteins that allow iron within the cell are coded for in the *pneumococcal iron uptake (piu)* gene. For transcription of *piu* to occur, RitR must be phosphorylated which allows it to release from DNA. A third protein is believed to control the reverse of this process, or the dephosphorylation of RitR, to stop the production of iron uptake proteins. This third protein is an enzyme named PhpP or phosphoprotein phosphatase. It is a member of the PP2C class of phosphatases, which are known to be activated by metal binding. If PhpP is activated by iron, this would be a good indication that it is regulating the dephosphorylation of RitR. The project for this summer was to investigate the activity of phpP to better understand its role in the iron regulatory system. A chemical substrate, pNPP or *para*-nitrophenylphosphate was used to test the enzyme activity. By first varying the concentrations of substrate, we were able to determine a substrate affinity to PhpP and maximum velocity with both iron and manganese. These results showed that iron had an overall faster rate versus manganese. To measure the metal affinities to the enzyme itself, we varied the concentrations of metal. These experiments were able to show a higher affinity of manganese to PhpP over iron, however they also showed the same trend as before with iron producing a faster reaction. After conducting these experiments it was determined that PhpP is successfully activated by iron, making it a likely addition to the proposed regulatory system.

Support was provided by grant P20GM103499 from the National Institute of General Medical Sciences, National Institutes of Health

Investigation of the enantioselective catalyzed capture and cyclization of homoallylic alcohols with isocyanates

Jessica Zinna (2016) **Mentor(s): Dr. Jeff Johnston (Vanderbilt University)**

Halocyclizations are a class of reactions that have gained interest in recent years. The ability to synthesize complex heterocycles enantioselectively using relatively simple reagents in a one-pot reaction mixture is enormously beneficial for the advancement of chemical synthesis. The majority of previous studies have focused on intramolecular reactions; the current work focuses on an intermolecular capture of an electrophile followed by an intramolecular cyclization. Achieving high enantioselectivity is possible through the use of a dual Brønsted acid/Brønsted base organocatalyst that has the ability to stabilize the reactive intermediate.

Described is a novel synthetic route to cyclic carbamates utilizing a homoallylic alcohol, an isocyanate, and an iodine source in the presence of a previously described organic catalyst. Future work will focus on the development and utilization of chiral HPLC to determine the enantioselectivity of the reactions performed.

This work was supported by NSF-REU, Department of Chemistry, Vanderbilt University