Winthrop University
Summer Undergraduate Research Experience (SURE)
2012 Abstract Book

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Table of Contents

Winthrop Students Performing Research at Other Institutions:

ACKNOWLEDGEMENTS

The undergraduate abstract authors were supervised by a vibrant group of faculty in the Biology, Chemistry, and Mathematics Departments. On behalf of the students, faculty, and department administrators, we express our gratitude for the support from the agencies and organizations listed below. The hands-on teaching experiences that faculty provide for these outstanding Winthrop students are only possible through their support.

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Structural Analyses of the GH10 Xylanases Xyn10A and Xyn10C from *Xanthomonas axonopodis* pv. *citri.*

Kelsey Aadland (2013) **Mentor: Dr. Jason C. Hurlbert**

Plant pathogenic species of *Xanthomonas* produce several glycoside hydrolases that catalyze the depolymerization constutent cell wall polysaccharides derived from host tissues, and the participation of these enzymes in the deconstruction of cell walls may contribute to pathogenesis. The sequenced genomes of several plant-pathogenic *Xanthomonas* spp. has revealed genetic orthologs to those encoding well characterized xylanolytic enzymes in other bacteria. These genes are clustered in two adjacent operons that face each other in their transcriptional directions. In *Xanthomonas axonopodis* pv. *citri*, one operon contains *xyn10A*, *xyn10B*, and *xyn10C* genes encoding three endoxylanases of glycoside hydrolase family 10 (GH10) and a second operon contains an *ara43* gene encoding a putative GH43 α-L-arabinofuranosidase and an *agu67* gene encoding a GH67 α-glucuronidase. As part of a multidisciplinary and multi-institutional effort to understand the role of the xylanolytic genes in the pathogenic process, we have undertaken strutural analyses of the functional xylanase proteins encoded in the first operon, xylanase 10A (Xyn10A) and xylanase 10C (Xyn10C). Our current research is split between computational and bench chemistry phases. In the computational phase, we are utilizing homlogy modeling and *ab initio* loop building to generate structures of Xyn10A and Xyn10C. In the bench chemistry phase, we have cloned, expressed and purified Xyn10A to 80% purity and will continue our efforts to purify the protein so that we may begin crystallization trials. Once we have grown crystals of the purified proteins and collected x-ray diffraction data on them, the computationally derived models will be used in the phasing of the x-ray crystallographic data, thereby allowing us to determine the three-dimensional structures of the proteins. This knowledge will be used to design protein-specific inhibitors of the xylanases in order to abrogate the infectious abilities of *Xanthomonas axonopodis* pv. *citri*.

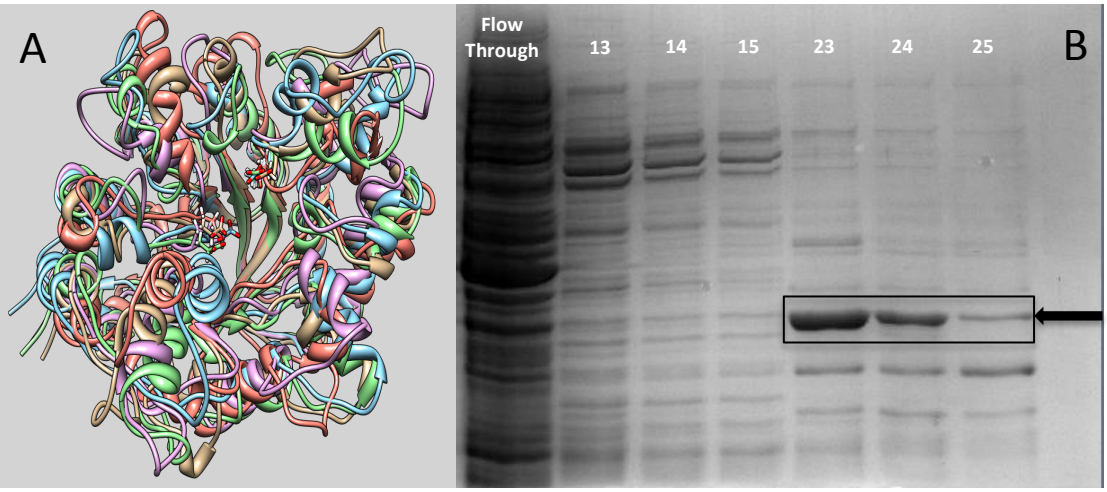


Figure 1: Results of theoretical and computational work on *X. axonopodis* pv. *citri* Xyn10A. In Figure 1A, the five lowest energy structures of Xyn10A generated by homology and *ab initio* loop modeling are superposed. The active site glutamates are rendered as sticks near the central cavity of the TIM barrel. In Figure 1B, an SDS-PAGE analysis of fractions from Ni^{2+}-MCAC purification of a 3L *E. coli* Rosetta2 (DE3) lysate containing recombinant *X. axonopodis* pv. *citri* Xyn10A is shown. The partially purified xylanase is surrounded by a box and marked with an arrow.

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

A colorectal carcinogenesis model incorporating insulin and insulin-like growth factor 1

R. Wayne Anderson (2014), Dane Flinchum (2013), Stephen McFall (2014), and Matthew Neal (2013)

Mentor: Dr. Joseph Rusinko

Edward Giovannucci proposes that variation in insulin and insulin-like growth factor 1 (IGF-1) levels influence colonic carcinogenesis. To study these proposed effects, we develop a system of linear ordinary differential equations to model the human colon on an intracellular level, incorporating insulin and IGF-1 and their effects on mutated cell populations. In particular, we focus on the insulin-dependent and independent intracellular signaling pathways and how they influence programmed cell death and growth. We consider the dynamics of all colorectal crypts using a compartmental approach, accounting for stem cells, transit cells and differentiated cells. With this model in place, we determine how changes in insulin and IGF-1 levels affect mutated cell growth. Using Wolfram *SystemModeler*, we show that high levels of insulin increase the number of cells which resist apoptosis and can lead to the growth of tumors. Our model also tests parameters simulating Familial Adenomatous Polyposis (FAP), a hereditary condition in which stem cells have a mutation at birth. Simulating these conditions, we found that IGF-1 levels noticeably affect the number of mutated cells found after eighty years.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National

Institute for General Medical Sciences

Photocatalytic Splitting of Water Molecules Using Semiconductor Thin Films

Sarah White (2014), Kenisha Barber (2015) and Justin Waller (2015) **Mentor: Dr. Cliff Harris**

Semiconductor photocatalysis shows great promise as a reliable means of water-splitting to be used in the conversion of solar energy to a chemical fuel (H_2) via simultaneous reduction and oxidation of water. A necessary condition of any water-splitting system is that it must effectively absorb visible light. To facilitate the process, the pairing of CdSe quantum dots (QDs) with WO_3 nanoparticles as reducing and oxidizing agents, respectively, has been proposed.

1.92-nm CdSe QDs were synthesized via a reverse micelle collision method using cadmium salt and sodium selenosulfate as aqueous precursors. The QDs were electrodeposited on a fluorine-doped tin oxide (FTO) transparent electrode by applying a 500-volt potential (Figure 1.) After drying, a heated, prolonged soak in DMSO removed adsorbed the AOT surfactant used in the reverse micelle process.

The CdSe QDs and films were characterized by UV-vis at different points during the process showing a well-resolved excitonic peak at around 500 nm. Using AFM, it was found that the CdSe films were approximately 200 nm in thickness. SEM images indicate that the films were non-uniformly coated. To the CdSe films, a WO_3 layer was added using a 1000-volt EPD process under constant sonication. AFM showed this layer to be approximately 60 nm in thickness. A total approximate thickness for both the CdSe and WO_3 film layers was found to be 260 nm.

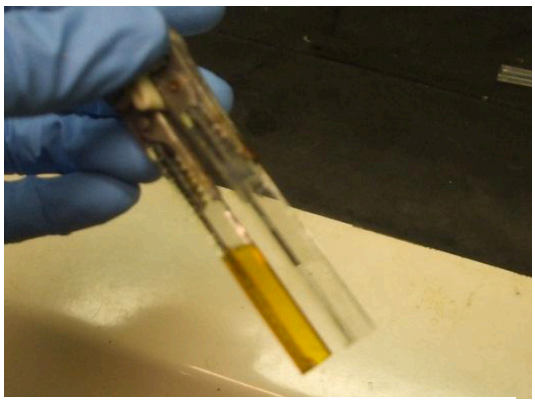


Figure 2: CdSe thin film prepared by EPD

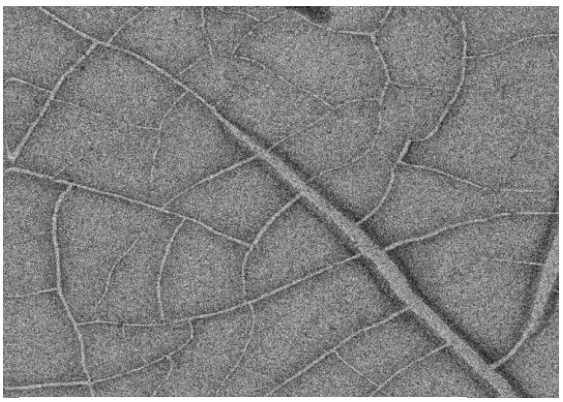


Figure 3: SEM image of CdSe thin film

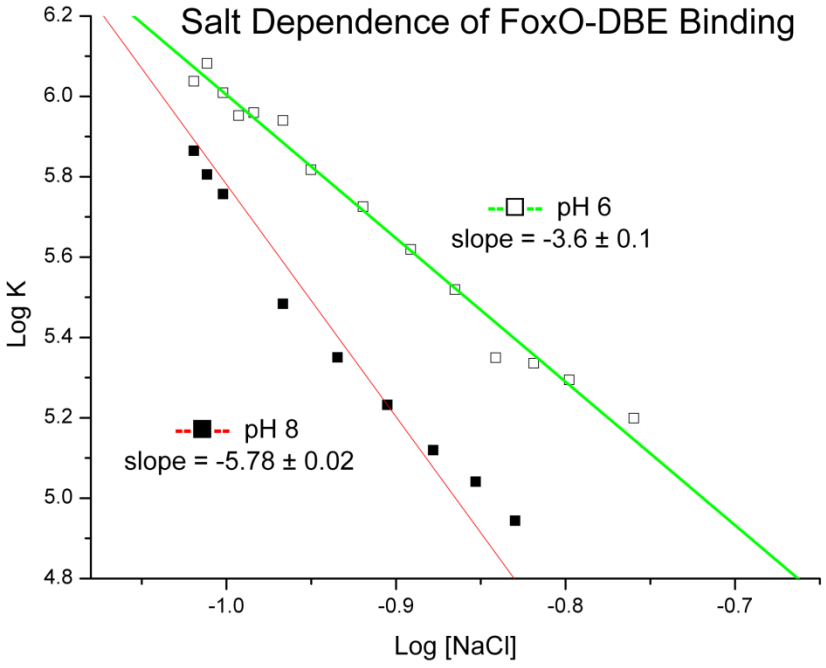
Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences and by the Winthrop University Department of Chemistry, Physics, and

Geology.

Cloning, Purification and Characterization of Phosphoserine and Phosphoserine Mimics of *Ciona intestinalis* FoxO

Lucas Boncorddo (2015) **Mentor: Dr. Nicholas Grossoehme**

Ciona *intestinalis* has been identified as a model organism for heart regeneration by Dr. Heather Evans Anderson. Human FoxO1 and Ciona *intestinalis* FoxO1 share an area of similarity in amino acid residues. This area corresponded to the DNA binding region, otherwise known as the forkhead domain. To determine the effects a negative charge on the DNA binding affinity between FoxO1 and two sequences of DNA that recognize FoxO1 (DBE and IRE), four mutant proteins were identified as interesting targets. Through standard cloning and techniques, plasmid constructs corresponding to serine to glutamic acid mutants (S282E and S275E) and two serine to phosphoserine (S282Sep and S725Sep) mutants were generated. Purification of S282E was carried out as previously described and its DNA binding properties were characterized by fluorescence anisotropy. Initial DNA binding affinity experiments between the mutant S282E and DBE/IRE were performed at both pH 6 and pH 8 and compared to DNA binding studies between DBE/IRE and wild type FoxO1 at pH 6 and pH 8. Overall, the results suggested that DNA binding affinity was higher for both mutant and wild type FoxO in a solution containing IRE. Also, as the pH of the DNA-FoxO1 solution became more basic, the DNA binding affinity increased. The DNA binding affinity between DBE/IRE and FoxO1 did not appear to significantly change with the mutants. Future experiments would be necessary to fully characterize the DNA binding interactions of FoxO.

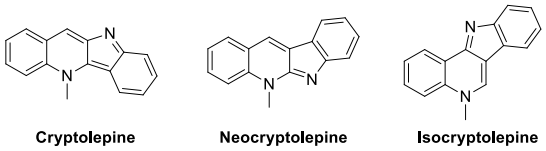


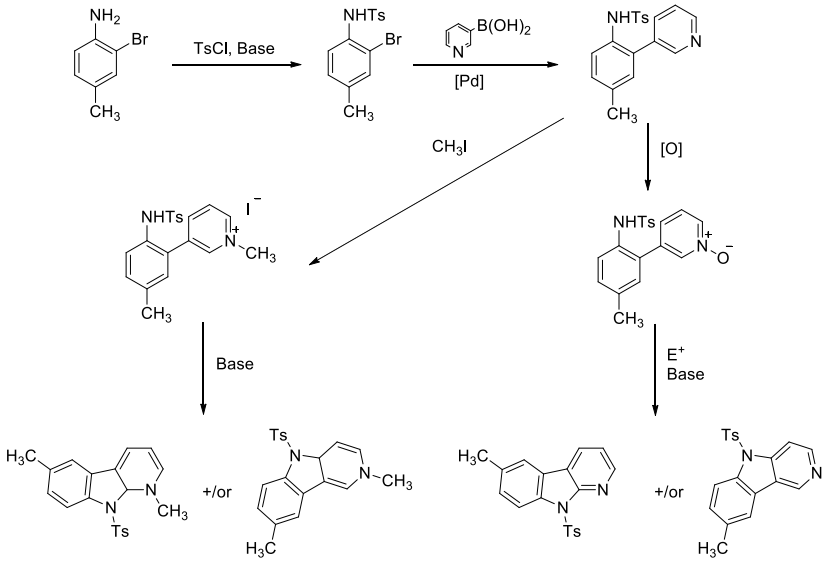
Support was provided by NIH Grant Number 1R15HL104587-01 from the National Heart, Lung, and Blood Institute.

Toward A Synthesis of Cryptolepine Alkaloids. Preparation of Carbolines via Cyclization of 3-(2-Aminoaryl)Pyridine *N*-Oxides

Chelsea Brennan (2013) **Mentor: James M. Hanna, Jr.**

Cryptolepines, shown below, are a series of tetracyclic nitrogen containing compounds that have been isolated from the African plant *Cryptolepis sanguinlenta*. These compounds can function as DNA intercalating agents, which gives them the potential to treat cancer, and thus represent important synthetic targets. Recently, researchers in the Hanna laboratory reported the cyclization of 3-acylpyridine N-oxide tosylhydrazones, and it was hoped that the application of this strategy to the cyclization of 3-(2-aminoaryl)pyridinium salts and 3-(2-aminoaryl)pyridine *N*-oxides could provide a versatile synthesis of compounds related to the cryptolepines. Before attempting to synthesize cryptolepine analogs, however, we decided to test potential reaction paths through the synthesis of the carboline skeleton. Carbolines are medicinally important in their own right, as they can have antimicrobial, antifungal, antitumor, and anticancer properties, among others. The figure to the left shows the proposed reaction path for the synthesis of carboline analogs. The first step in the synthesis, tosylation of the aniline, has been performed under reflux with dichloromethane as a solvent and N-methylimidazole as a base, and afforded a 94% yield. The second step has proven more difficult. In this step, using $PdCl_2$(dppf) as a catalyst in dioxane/H_2O, resulted in a yield of only 25%. Changing the solvents to ethanol and H_2O and using microwave heating has improved the yield to 60%. The biaryl product will now be produced on a larger scale, then the pyridinyl nitrogen will be oxygenated; subsequent ring closure will give the carboline substructure. Alternatively, the pyridinyl nitrogen can be methylated before ring closure is performed to give the *N*-methyl carbolines. Upon optimization of the cyclization conditions, a scope and limitations study varying the substituents on both the carbocyclic and pyridinium portions of the molecule will be carried out.





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

Subcloning and Expression of the Catalytic Core Domain of Human Sphingosine Kinase 1 in *Escherichia coli*

Tyler Couch (2013) **Mentor: Dr. Jason C. Hurlbert**

Ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are lipid signaling molecules that have been shown to control cellular fate. Sphingosine kinase 1 (SK1), an enzyme of the sphingolipid metabolic pathway, has been found to be overexpressed in various types of cancers including breast, colorectal, ovarian, and lung. Increased expression of SK1 has been shown to promote tumor angiogenesis and proliferation while protecting against radiation and chemotherapy[4]. For these reasons, SK1 has become a target in the treatment of cancer for which new therapeutics are being developed. However, there currently is no three dimensional structure of SK1 for which to base these new inhibitors on. Our laboratory aims to express soluble SK1 in *Escherichia coli* so that it may be purified and studied by x-ray crystallography for subsequent structure determination. We have previously cloned a codon-optimized gene for the catalytic core domain of human SK1 into an expression construct. Attempts to express this gene in four different strains of *E. coli* have failed to yield requisite amounts of protein as determined by MALDI-TOF MS. Current efforts are focused on subcloning the SK1 gene from its present construct into other prokaryotic expression constructs in order to boost soluble expression yields to those necessary for x-ray crystallography trials. Determination of the three-dimensional structure of SK1 alone and in complex with sphingosine, ADP or ATP analogues and known inhibitors will allow for a better understanding of the reaction mechanism of this novel family of enzymes.

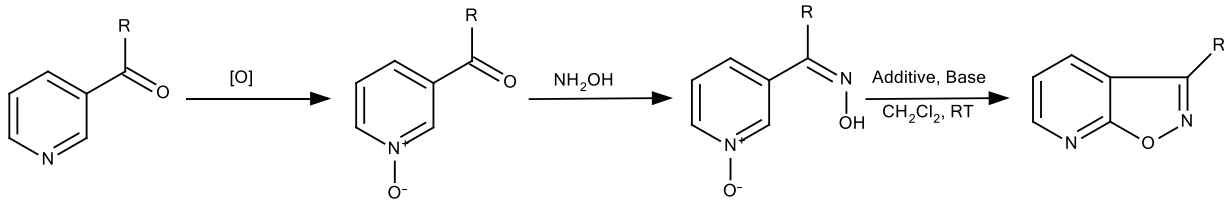
Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences and the Winthrop University Department of Chemistry, Physics, and Geology.

Construction of Isoxazolopyridines via Cyclization of 3-Acylpyridine *N*-oxide Oximes

Kourtland B. Haile (2014) **Mentor: 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 known as cognitive enhancers and could possibly be used as Alzheimer's prevention therapy.

Recently, researchers in our laboratory reported that tosylhydrazones formed using 3-acylpyridine *N*-oxides could be cyclized into pyrazolopyridines. Through the reaction of *N*-oxide tosylhydrazone with a proper electrophile, they were able to form an activated intermediate that allowed nucleophilic attack at C2 on the pyridine *N*-oxide; in the presence of a base an E2 elimination then formed the desired cyclized product. To extend the scope of this cyclization reaction, we investigated the cyclization of pyridine *N*-oxide oximes starting with 3-acetylpyridine as outlined below:



Originally, we began this project trying to cyclize the 3-acetylpyridine *N*-oxide oxime (R = CH_3) into the corresponding isoxazolopyridine. The first two steps of the project resulted in moderate yields of both the *N*-oxide (36%) and the *N*-oxide oxime (76%), but attempts to cyclize this compound have failed to this point. By comparing the ^{13}C-NMR of the stating ketone to the 3-acetylpyridine *N*-oxide oxime (R=CH_3) we found that the oxime was unfortunately in the (*E*)-configuration (δ 44ppm for ketone, δ 28ppm for oxime showed an upfield shift of 16 ppm suggesting the hydroxyl group was syn to the α-carbon) and therefore unable to attack the 2-position of the pyridine ring due to configurational constraints. In an effort to overcome the (*E,Z*)-complication, a different oxime was synthesized (R = *tert*-butyl) in the hope that the steric pressure would force the hydroxyl group into the (*Z*)-position. Thus, 3-pivaloylpyridine *N*-oxide oxime was synthesized in good yield (80%), and found to be the (*Z*)-isomer (Δδ of the α-carbon = -7ppm compared to the starting ketone). Cyclization using the electrophile/base combination of PyBroP and iPr_2EtN was revealed by GC-MS to have produced the cyclized product. The resulting isoxazolopyridine was isolated (41% yield) and characterized using ^{1}H- and ^{13}C-NMR.

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

Assessing the DNA Binding Interactions of Benzo[4,5]isoxazolo[2,3-a]pyridinium and Benzo[4,5]isoxazolo[2,3-a]quinolinium Tetrafluoroborates

Hua-Wu Huang (2014) **Mentors: Dr. Takita Felder Sumter and Dr. James Hanna**

DNA intercalators are chemical agents that bind directly to DNA in the space between DNA base pairs, resulting in lengthening and deformation of the DNA helix. The structural change inhibits normal DNA processes, and is utilized in treatment of cancers. In a previous study evaluating the activity of a series of benzo[4,5]isoxazolo[2,3-a]pyridinium tetrafluoroborates against human colorectal cancer cells, we observed IC_{50} values for all eight compounds in the micromolar range, possibly as a result of DNA intercalation. In order to confirm the association of these compounds with DNA, DNA binding was assessed through fluorescence competition and molecular docking studies. *In silico* evaluation of the DNA binding activities for the most potent benzo[4,5]isoxazolo[2,3-a]pyridinium tetrafluoroborates confirmed the abilities of these inhibitors to associate with DNA. However, the theoretical trends in binding were not consistent with trends in cytotoxicity. We then evaluated the impact of substituting the pyridinium moiety for a quinolinum group. DNA binding to benzo[4,5]isoxazolo[2,3-a]quinolinium tetrafluoroborates was more thermodynamically favorable than to the pyridinium analogues. In the fluorescence assays, both the pyridinium and quinolinium derivates competed for DNA binding sites that were preoccupied by the known minor groove binder, Hoechst 33258. Similar to the docking results, tetrafluoroborates bearing the quinolinium ring were more potent in these assays than corresponding pyridinium derivatives. These findings imply that quinolinium-based inhibitors may more effectively induce cancer cell death when compared to pyridinium analogues. Collectively, these data confirm the DNA binding interactions of a novel class of compounds that may potentially serve as leads in cancer therapies development.

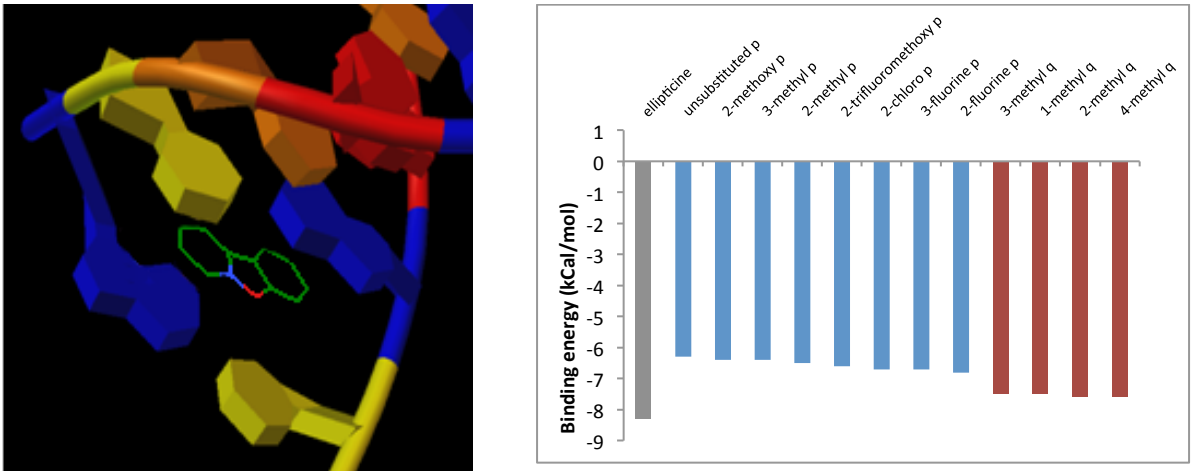


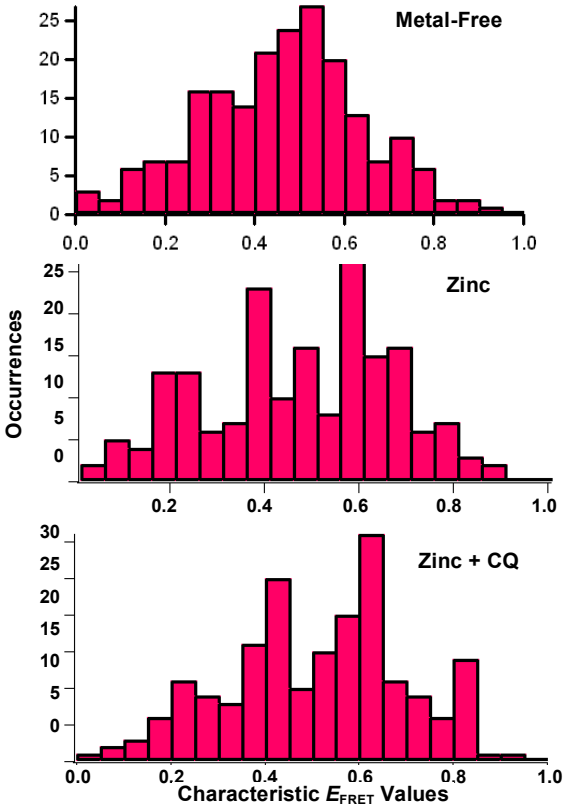
Figure 1. The figure on the left shows unsubstituted benzo[4,5]isoxazolo[2,3-a]pyridinium docked into pre-formed DNA gap. The graph on the right shows the binding energies of compound analogues intercalated with DNA.

This project was supported by the NIH SC-INBRE award (5 P20 RR016461) (8 P20 GM103499).

Structures and Dynamics in Amyloid-β Dimers: Zinc Binding and Chelation

Michelle Humphrey (2015) and Kristen McLaurin (2014) **Mentor: Dr. Robin Lammi**

Amyloid-β (Aβ) is a protein of 39-43 amino acids that self-associates into a diverse array of neurotoxic aggregates linked to Alzheimer's disease (AD). Recently, the smallest Aβ oligomers, dimers and trimers, have been shown to cause memory deficits when they are recruited to the synapse, perhaps due to zinc release during neurotransmission. We have employed single-molecule fluorescence methods to investigate the structures of these smallest oligomers, to understand zinc-induced structural change and determine whether it may be reversed by metal chelation. Single-dimer FRET (Förster Resonance Energy Transfer) measurements were performed using Aβ40 peptides labeled at the N-termini with donor and acceptor dyes; the donor peptide was additionally labeled at the C-terminus with a Lys-biotin moiety to permit tethering to a functionalized cover slip. Time-dependent FRET efficiencies (E_{FRET}) were determined by measuring fluorescence from individual surface-tethered dimers, affording insight into dimer structures and structural dynamics. Characteristic E_{FRET} values were determined for dimers in the presence of Zn^{2+} (1 equiv.), with and without excess clioquinol (CQ, 10 equiv.), a zinc chelator that reached Phase 3 clinical trials for AD treatment; results were compared to previous studies on metal-free samples. Under all sample conditions, dimers appear to exhibit at least two characteristic structures, as evidenced by at least two broad peaks in each of the E_{FRET} histograms at left. Zinc binding causes a slight structural change (indicated by peak shifts toward higher E_{FRET}) that is not reversed by chelation. Strikingly, zinc also severely limits dimer structural dynamics: while 29% of dimers in metal-free samples visit two E_{FRET} values over time, only 12% of dimers in the presence of zinc exhibit structural change. Chelation does not reverse this effect, as only 9% of dimers in the presence of CQ show structural dynamics. These results lend new insights into the role of zinc in promoting Aβ association and the utility of metal chelation as an approach for AD prevention and treatment.



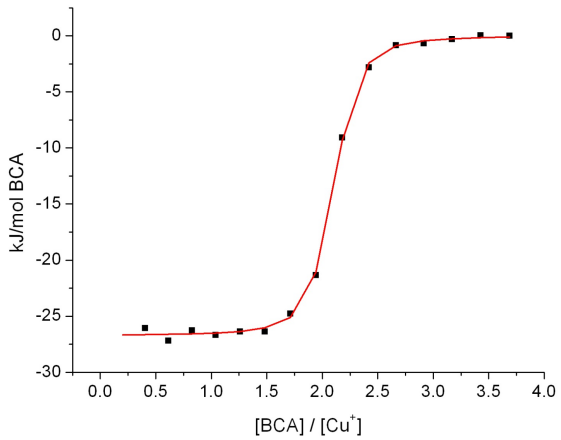
This project is supported by an RUI grant from the National Science Foundation and an NIH-INBRE grant from the National Institute of General Medical Sciences.

Examining the Colorimetric and Calorimetric Properties of Various Copper (I) Complexes

Destinee Johnson (2014) **Mentor: Dr. Nicholas Grossoehme**

Copper (I) is essential for several biochemical processes within enzyme active sites such as electron transfer, dioxygen binding, catalysis, and structural activity. Although the cuprous ion is the physiologically relevant oxidation state of copper, *in vitro* experiments have, in large, been focused on Cu^{2+} because it is stable under typical laboratory conditions. Furthermore, under anaerobic conditions, Cu^{+} participates in a disproportionation process which favors Cu^{2+} by a factor of approximately 1000 relative to Cu^{+}. As such, careful experiment design is necessary to alleviate these complications. This research aims to provide the necessary experimental foundation to directly measure the thermodynamic forces associated with cuprous ion binding energy using isothermal titration calorimetry.

To avoid the potential of Cu^{+} oxidation, all colorimetric and calorimetric experiments were conducted in a Coy Lab glove box. Additionally, acetonitrile (MeCN) was used as a stabilizing ligand to abrogate the disproportionation equilibrium of Cu^{+}. The spectrophotometric data verified that the expected 2:1 BCA-Cu^{+} complex was formed under these experimental conditions. The colorimetric data provided a methodical background for subsequent calorimetric experiments which determined thermodynamic parameters of Cu^{+} binding. In Tris buffer (pH 8) the average binding constant was K= 2.0 ± 0.3 x 10^{6} M^{-1}. The average enthaply value was ΔH = -25.1± 0.6 kJ/mol, and an average reaction stoichiometry (n) of 2.0 ± 0.01. In bis-tris buffer (pH 6) the average enthalpy was -38.3± 0.5 kJ/mol, and at a pH 7 the average enthalpy was -36.8± 0.5 kJ/mol. The difference in enthalpy values may be due to the interactions of the Cu^{+}-BCA complex with different biological buffers. My future research will continue to explore this hypothesis.



Calorimetric binding isotherm for 1.25 mM BCA → 125 μM Cu(I) in 25 mM MeCN at pH 8.0.

Support was provided by the Winthrop University McNair Scholars Program.

METHOD DEVELOPMENT FOR GRADIENT FLOW ANALYSIS

Taisha S. Jones (2014) and Akilah B. Murray (2015) **Mentor: Clifton P. Calloway**

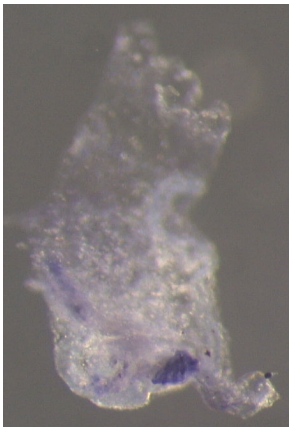
Gradient Flow Analysis is a new calibration method that corrects for matrix interferences and fluctuations in signal levels associated with variations in the sample environment. As such, the method will combine the benefits of the classic standard addition method with those of the internal standard method to a broad array of samples. These corrections will be performed dynamically, in a flowing stream to speed the time of analysis. This will be accomplished by controlling the mixing of three solutions using syringe pumps. The first pump will contain the sample liquid (oil, urine, etc.). The sample will be delivered at a constant rate (0.3 mL/min for example) so the potentially interfering matrix components will be present at a fixed amount. A gradient flow will be established with the other two solutions. One of these will be a simple solvent. The other will be a mixture containing the species to be detected (analyte) and a second species to correct for fluctuations (internal standard). This mixture will have a fixed analyte/internal standard concentration ratio. The mixture solution will be pumped in a gradient of 0-100% while the solvent solution is pumped in an inverse fashion from 100-0%. The combination of the two solutions will be provided at a constant flow rate (0.7 mL/min) so that the overall solution flow remains constant at 1.0 mL/min. The method will plot the ratio of the signals (analyte/internal standard) on the y-axis versus the inverse of the concentration of the internal standard delivered (1/C) on the x-axis. Using a relatively straightforward mathematical treatment, the concentration of analyte in the original sample will be given by the slope of the resulting linear plot divided by the intercept. The method may be applied to any analytical technique that accepts liquid samples. The method will be demonstrated initially using simple UV-VIS absorption spectrometry. The concentration of FD&C Blue Dye No. 1 in a synthetic standard and commercial Listerine® will be determined using classical quantitative methods of analysis and the combination method. Syringe pumps are then applied to various mixing schemes that will be evaluated to produce rapid, uniform solutions in a flowing absorption cell. Results are consistent by each method within the experimental error of the method.

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

In situ hybridization of heart specific mRNA in juvenile and adult *Ciona intestinalis*

Caitlin Manning (2013) **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 *Ciona* and replicate early heart development in other Chordates, such as vertebrates. One process that can help define conserved processes and gene expression patterns is in situ hybridization (ISH). This is a method of localizing mRNA expression in specific cells by hybridizing a gene sequence of interest to a complimentary strand of a labeled nucleotide probe. The advantage of developing a heart specific probe in juveniles is that gene expression and heart formation could be viewed during each stage of development through adulthood. Methods such as immunohistochemistry and immunofluorescence are not ideal for *Ciona* because not many antibodies exist for the organism and it is much less labor intensive to perform an ISH than it is to develop new antibodies. There are many preliminary steps to performing an ISH. Research last summer began with constructing the probe for a heart specific gene labeled J6. A sense and antisense probe must be made to ensure correct hybridization technique. The sense probe for J6 runs in a plus/plus orientation and the antisense probe runs in a plus/minus orientation from the SP6 to the T7 side of the vector. We used the pGEM-T Easy vector system (Promega) and T7 and SP6 polymerases (Roche). In order to generate the J6 sense probe, SacI restriction enzyme was used along with T7 polymerase. NcoI restriction enzyme along with Sp6 polymerase was used for the J6 antisense probe. The sense probe acts as a negative control in that it should not hybridize to the mRNA target sequence. The antisense probe should hybridize the complementary mRNA sequence of interest and allow for visualization of gene expression. J6 and R1 are heart specific probes that were constructed for this project. A tailored ISH protocol for *Ciona intestinalis* has been completed at this time. Trial runs were conducted using this protocol that was adapted from Christiaen L, Wagner E, Shi W, Levine M. lab from the University of California Berkeley. To test the effectiveness of the probes at different concentrations for the ISH protocol, a dot blot test was conducted using FoxO probes in addition to J6 and R1. Currently, troubleshooting for the ISH protocol needs to be further investigated. Eventually, progressive stages of juvenile development can be evaluated using an optimized ISH protocol for *Ciona* and more probes will be constructed with other genes of interest to further study gene expression throughout cardiac development in *Ciona intestinalis.*



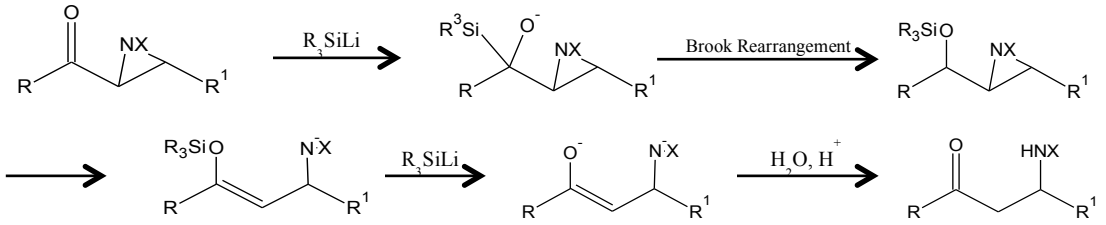
The project described was supported by NIH Grant Number P20 RR-16461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE) and NIH Grant Number 1R15HL104587-01 from the National Heart, Lung, and Blood Institute.

Preparation of Mannich Bases from 2-Acylaziridines Using Silyllithium Reagents

Arthur Korous (2013) **Mentor: Dr. Aaron Hartel**

Mannich bases (β-aminoketones) and their derivatives are an important class of organic compounds with extensive synthetic value, particularly in the preparation of biologically active molecules. The traditional method for their preparation is the Mannich reaction. However, this method has many drawbacks including long reaction times, poor regioselectivity, no enantioselectivity, and competition from unwanted side reactions.

A new method for the preparation of Mannich bases from 2-acylaziridines using silyllithium reagents has been developed. The reaction is believed to proceed via Brook rearrangement assisted by opening of the adjacent aziridine.



A series of 2-acylaziridines were reacted with methyldiphenylsilyllithium to form the corresponding Mannich bases in good to excellent yield. Various 2-acylaziridines were synthesized with different properties to help demonstrate the versatility of the reduction. 2-Acylazridines with a tosyl group attached to the nitrogen and a phenyl group attached the carbonyl carbon produced the highest yield of Mannich base after the reduction. 2-Acylaziridines with an akyl group attached to the carbonyl carbon produced lower yields but were still successfully reduced in appreciable amounts. The use of silyllithium reagents in the reduction of 2-acylaziridines to Mannich bases is a simple effective method that works on a wide variety of 2-acylaziridines.

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

Investigations and Development of Strategies to Effectively Teach Introductory Chemistry through an Online Platform

Amy Moore (2013) **Mentor: Dr. Nicholas Grossoehme**

Education and technology over the past few decades have become increasingly entwined. Everything from iPads® to fully electronic universities have become a norm in teaching. With such a profound influence that these electronic learning environments are having, critical evaluation of effective strategies and best practices are necessary. Unfortunately, these studies about technology and education vary greatly in focus and results and the vastness of what has been published is astonishing. What is most fascinating is how quickly technology has been thrown into education without supporting evidence to show how or if it is effective the classroom. Technology needs to be implemented in an academic way such that it benefits the student and the education system overall. While electronic content to supplement student learning has been implemented in the classroom, much of what has been introduced does not allow students to reach their full potential in learning through technology. This is due mostly to a lack of resources and support at the classroom level and a lack of understanding of how technology can be used to teach more effectively. This research aims to correct this lack of understanding by further studying how students learn introductory chemistry and how technology can be used in teaching to improve student learning. Overall this project will focus on how and if electronic material can replace traditional methods. We hypothesize that this is only possible if the instructor is capable of anticipating student pitfalls; implementing strategies to improve comprehension, problem solving, and analytical skills; and anticipating problems that may arise in an electronic learning environment.

Average Data for Different Features of an Online Learning Environment

Track	Everyone	Have Not Taken Chemistry	Have Taken Chemistry
Examples worked out with explanations	5.68	5.78	5.58
Different methods of problem solving	5.55	5.59	5.48
Navigable lecture notes	5.54	5.55	5.43
Practice problems	5.76	5.54	5.65
Interactive office hours	5.29	5.43	5.13
Interactive activities	5.47	5.36	5.16
Video	5.17	5.23	5.02
PowerPoints	5.00	5.21	4.99
Instant chats/video with professor	5.08	5.07	4.69
Educational apps	4.86	4.97	4.59
Instant chats/video with classmates	4.84	4.93	4.69
Educational games	4.79	4.86	4.45
Podcasts	4.73	4.86	4.27
Electronic group work	4.37	4.48	3.94

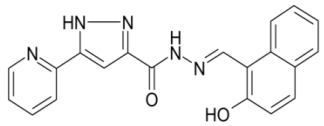
Support was provided by the Winthrop University McNair Scholars Program.

The Characterization, Synthesis, Purification and Isolation of Sphingosine Kinase Inhibitors

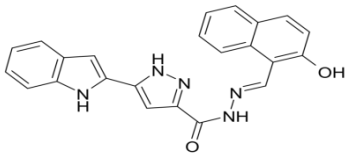
Louise Mount (2014) **Mentor: Dr. Christian Grattan**

Sphingolipids are a family of compounds that, in addition to being structural constituents of cell membranes, play key roles as signaling molecules. In particular two of these sphingolipid metabolites ceramide and sphingosine 1-phosphate (SIP), have recently received considerable attention as integral mediators of cell survival. The regulator of the ceramide/SIP equilibrium is sphingosine kinase-1 (SKI) which phosphorylates sphingosine to form S1P. SKI has been identified as an oncogene and is, therefore, of considerable interest in the treatment of cancer. To this end, a number of novel inhibitors of SKI have recently been identified and evaluated by Smith et al. A concise four step synthesis of SKI-I has been completed, and in vivo studies show the target compound to have a IC50=1uM. Straightforward functional group modification allows for numerous derivatives to be synthesized quickly and concisely in effort to increase the therapeutic effect and oral bioavailability of SKI-I. Following a base catalyzed mixed claisen condensation; two successive microwave reactions produce a family of diverse complexes in relatively high yield. These compounds will be analyzed to determine the change in inhibition of sphingosine kinase-1. Heterocyclic variations in zone 1 can be achieved synthetically. These modifications will be examined to assess the pharmacophoric nature of this portion of the template structure so that an optimized inhibitor may be realized.

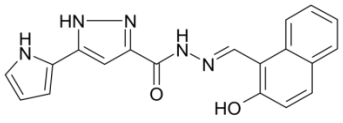
Here are the compounds that I am looking at specifically:



2-Acetylpyridine



3-Acetylindole



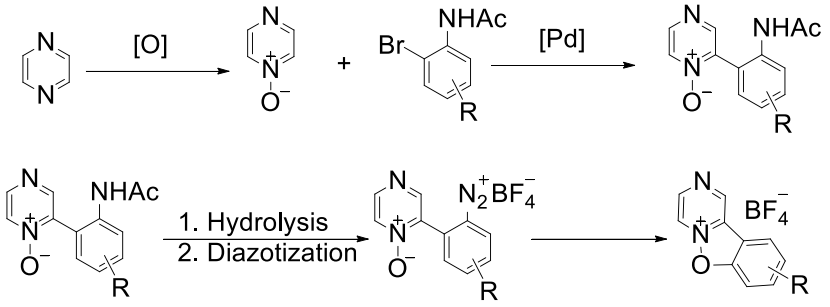
2-Acetylpyrrole

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

Synthesis of Benzisoxazolo[2,3-*a*]pyrazinium Tetrafluoroborates

Jamie Risa Murakami (2015) **Mentor: Dr. James M. Hanna, Jr.**

Recently, researchers in the Hanna lab synthesized several novel benzisoxazolo[2,3-*a*]pyridinium tetrafluoroborates and in collaboration with Dr. Takita Sumter, found these compounds exhibited some activity against HCT 116 colon cancer cells. As part of a structure-activity study aimed at increasing the cytotoxicity of this new class of compounds, we are carrying out the synthesis of the pyrazinium analog via the pathway shown in the figure below.



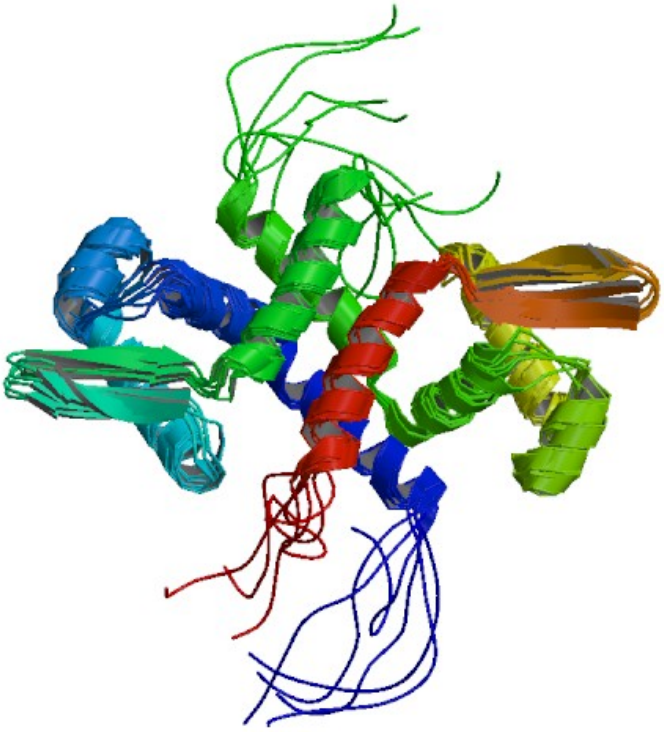
Using a modification of the direct arylation of azine *N*-oxides introduced by Fagnou et al., we have attempted to effect substitution at the 2-position on pyrazine *N*-oxide using two different *o*-bromoacetanilides. Previous work in our lab had found that the direct arylation of pyridine *N*-oxide was successful employing microwave heating, a catalyst composed of di-*t*-butylmethylphosphonium tetrafluoroborate and palladium acetate (3:1, 5 mol % Pd) and potassium carbonate in toluene. Reacting pyrazine *N*-oxide under these same conditions using two different *o*-bromoacetanilides gave the desired products according to GCMS. However, separating these compounds from pyrazine *N*-oxide has proven to be difficult, resulting in isolated yields of only 14 - 41%. In an attempt to simplify the separation, we lowered the molar equivalence of pyrazine N-oxide from 4 to 1.1, which required the time and temperature in the microwave to be increased from 160 °C for one hour to 180 °C for two hours. Unfortunately, complete separation from the unreacted pyrazine *N*-oxide still could not be accomplished, and we are continuing to optimize both the direct arylation and workup conditions to maximize product yield and purity. Upon development of a successful direct arylation process, several differently substituted *o*-bromoacetanilides will be reacted to form these intermediates, which will be cyclized and the resulting products evaluated for their anti-cancer activity.

Support was provided by an 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.

Optimization of Protein Purification Strategy for NmtR from *Streptomyces coelicolor*

Denise Peppers (2015) **Mentor: Dr. Nicholas Grossoehme**

Streptomyces coelicolor is an important organism to study because of its prevalence in antibiotic production. Additionally, *S. coelicolor* is an important model system for pathogenic bacterium in the same phylum such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis, among other things. Within the context of antibiotic production, it has recently been shown that metal regulation is directly coupled with the production of some of the antibiotic that *S. coelicolor* produces. For this reason, understanding the metalloregulatory network of *S. coelicolor* is an important step in identifying key steps to trigger antibiotic synthesis *in vivo*. The Sco6860 gene shares significant similarities to the Ni^{2+} sensing protein NmtR from *M. tuberculosis*. Accordingly, we speculate that this protein in *S. coelicolor* carries out the same or similar functions. The goal for this research project is to characterize this protein as best as possible. To date, we have amplified the *nmtR* gene and inserted it into an *Escherichia coli* expression plasmid. The plasmid was then transformed into the Rosetta 2 strain of *E. coli* for protein production. We are currently in the process of trying to optimize the protein purification strategy to lead to further biophysical studies of this protein



Solution structure ensemble of apo-NmtR from M. tuberculosis (PDBid 2LKP)

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences and a grant from Research Corporation Cotrell College Science Award 20160.

Evaluation of the Potential Role of Arg25 in the HMGA1-STAT3 Cancer Initiation Axis

Derion Reid (2014) **Mentor: Dr. Takita Felder Sumter**

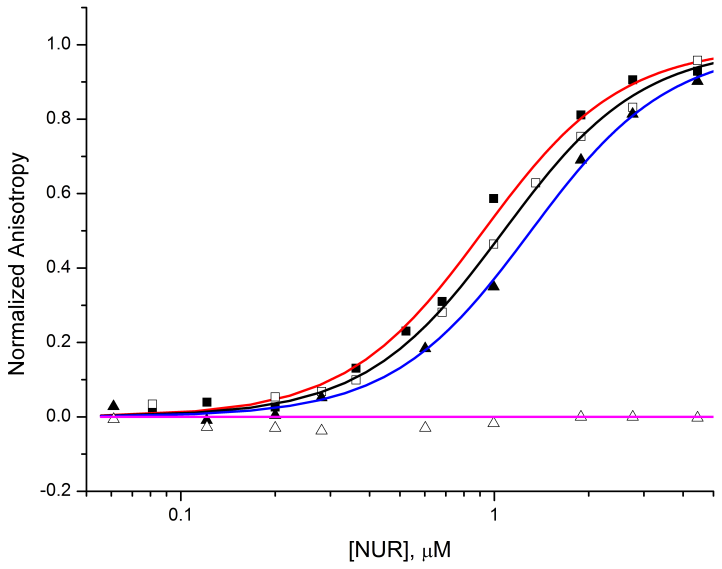
The overexpression and chromosomal rearrangements of the *High Mobility Group A1 (HMGA1*) gene is a well-accepted hallmark of various tumor types. The gene produces two proteins as a result of alternative splicing, HMGA1a and HMGA1b, which play critical roles in neoplastic transformation through mechanisms that are not well understood. In primary breast and prostate tumors, various amino acids within the HMGA1 proteins are extensively modified. Of the three sites of arginine methylation that have been reported, modifications at Arg25 have been independently confirmed. Interestingly, Arg25 (R25) is centrally located within one of the protein's three DNA binding domains suggesting its role in HMGA1-DNA interactions. We describe the effects of a substituting R25 with Lys (mutant referred to as HMGA1b-R25K) in efforts to evaluate the role played by electrostatic interactions between HMGA1a/b proteins and DNA targets. The binding activity of HMGA1b-R25K was assessed for two genes that are transcriptionally regulated by HMGA1b: *kit ligand* and *signal transducer and activator of transcription-3 (STAT3)*. We confirmed previous findings in our lab demonstrating that HMGA1b-R25K binds less tightly to *kit ligand* when compared to native HMGA1b. Additionally, preliminary assessments of HMGA1b-R25K binding to *STAT3* show substantial reduction in binding affinity relative to the wild-type protein. Taken together, our findings indicate that the HMGA1b-R25K mutation affects the capacity of the protein to bind DNA targets and activate their transcription. Further studies to confirm our preliminary findings and evaluate the impact of this substitution on transcription and ultimately, neoplastic transformation are planned as a next step. The findings underscore the role played by individual amino acid residues within HMGA1a/b proteins in oncogenic function and may provide a valuable reagent for better understanding the molecular events associated with cancer initiation and progression.

Support was provided by National Institutes of Health Grant Numbers P20 RR-016461 and 1R15CA137520-01, the National Science Foundation, and from the Winthrop University McNair Scholars Program.

DNA Binding Studies of NUR, a Nickel Sensing Protein from *Streptomyces coelicolor*

Lauren Rhodes (2014) **Mentor: Dr. Nicholas Grossoehme**

NUR is a nickel uptake regulator in *Streptomyces coelicolor*. NUR is responsible for regulating the transcription of certain genes that affect both the uptake of nickel and how it is used within the cell. This metal sensing protein exists as a symmetrical homo-dimer, which takes on a functional conformation when metal is bound to the two metal binding sites on each monomer. NUR is thought to function through allosteric activation by nickel ions in what is commonly referred to as 'Nickel' sites in the dimeric structure of the protein. The other metal binding sites, the M-sites, are thought to serve an accessory function. Previous experiments carried out in our lab have quantified the metal binding affinities for Zn^{2+} and Ni^{2+} for the Ni-site. The primary focus of these experiments was to characterize the relationship between the *sodF* gene and the protein dimer. This was done through a method called fluorescence anisotropy, which monitors the relative change in mass as increasing concentrations of NUR bind to fluorescent gene fragments. NUR was found to bind to DNA both with and without extraneous Nickel present, suggesting that the M-site, not the Ni-site, facilitated the binding of the protein with DNA. This hypothesis was tested by a series of experiments involving metal chelators of differing affinities. Only metal chelators with sufficient strength to remove metal from the M-site were shown to impede binding. These results show that the M-site is primarily involved in the allosteric activation of the NUR, causing it to bind to the *sodF* gene.



DNA binding isotherms for WT NUR (hollow squares, black line), supplemented with 10 μM Ni^{2+} (solid squares, red line), NTA (solid triangles, blue line) or EDTA (hollow triangles, pink line).

Support was provided by an NIH-INBRE grant from the National Center for Research Resources and the National Institute for General Medical Sciences and a grant from Research Corporation Cotrell College Science Award 20160.

Evaluating the Relationship Between β-catenin Function And *HMGA1* Activation in Colon Cancer

Michelle Rice (2012) **Mentor: Dr. Takita Felder Sumter**

High Mobility A1 (*HMGA1*) as a key transcription factor in the metastatic initiation and progression of cancer and has been identified as one of only four most abundant genes in colon tumors. Emerging evidence support the association of *HMGA1* with key cancer hallmarks based on its role in cellular differentiation, proliferation, apoptosis, and neoplastic transformation; although the processes leading to *HMGA1* overexpression are not well defined. To better understand the events that induce *HMGA1* overexpression in colon cancer, we explored the link between the most commonly deregulated pathway in colon cancers-the APC/β-catenin/TCF4 pathway. This pathway involves the interaction between adenomatous polyposis coli (APC), β-catenin, and transcription factor 4 (TCF4). Normally, APC acts as a tumor suppressor that interacts with a complex group of proteins to regulate β-catenin within the cell by phosphorylating them for proteolytic degradation. This prevents β-catenin from entering the nucleus where it could bind to TCF4 and promote gene expression. In cancerous cells, mutation of either APC or β-catenin promotes the formation of a nuclear β-catenin/TCF-4 complex that promotes oncogene expression. Previous work by our group confirmed the overexpression of *HMGA1* in colon cancer cells and mouse tissues bearing genetic modifications in the APC/β-catenin/TCF pathway. Further, EMSA and ChIP studies confirmed the binding of β-catenin and TCF to the *HMGA1* promoter. In this study, we begin to evaluate the function impact of this interaction. We first aimed to explore the impact of β-catenin silencing on *HMGA1*-mediated cancer progression. Using a Zn-inducible system that regulates the expression of the tumor suppressor, APC, we evaluated correlations in *HMGA1* and β-catenin expression in human colorectal cancer cells. Western analyses confirmed the presence of the two proteins in cells and immunohistochemistry confirms their nuclear co-localization in HCT116 human colorectal cancer cells. Further studies to determine the impact of silencing β-catenin expression will assist in understanding the cellular context of APC/β-catenin/TCF4-induced expression of *HMGA1*. Altogether, our studies may provide insight into the activation pathways associated with aggressive cancers that overexpress *HMGA1*.

Support was provided by National Institutes of Health Grant Numbers P20 RR-016461 and 1R15CA137520-01 (to TFS) and from the Winthrop University McNair Scholars Program. The authors also thank Ronald Nelson for technical support.

Crystallization of CaQ97 Xylanase from *Clostridium acetobutylicum*

Mariam Salib (2014) **Mentor: Dr. Jason C. Hurlbert**

The GH30 xylanase produced by *Clostridium acetobutylicum* can be used to explore a new opportunity of biofuel production, which will lower today's society's dependence on fossil fuels. That is due to its ability to hydrolyze xylan chains at the β-1, 4 positions. Xylan chains are the main component of hemicellulose, which is what holds together the cellulose in plant's cell wall. It is vital to further utilize hemicellulose in the production of biofuel instead of using the plant itself. A few years ago, the world experienced a spike in food prices due to the production of ethanol suing corn plants. In order to solve this problem, the focus needs to be shifted from the corn plant to the husk of the plant. That means using hemicellulose in the production of ethanol progressively. *Clostridium acetobutylicum* Q97 xylanase is being examined because of its ability to degrade xylan polymers decorated with galacturonic acid and arabinose residues. In an effort to make progress towards obtaining a crystallographic structure of the enzyme, our lab has previously cloned and developed a multistep purification protocol for recombinant CaQ97 xylanase. Initial sparse-matrix crystallization screening identified an initial condition that produced clusters of needle-like crystals (0.5M $(NH_4)_2SO_4$, 0.1M Sodium citrate tribasic dihydrate and 1.0M Li_2SO_4, pH 5.6). These crystals were too small to be of use in x-ray diffraction experiments. The goal of my work is to optimize this initial condition so as to obtain diffraction quality crystals of the enzyme. Initial attempts at optimization have focused on employing seeding techniques. In crystal seeding, previously grown crystals are physically crushed and used as seed for crystal growth in a new equilibration experiment. Using such strategies, we have seen a dramatic increase in crystal size and a change in crystal morphology from needle clusters to single hexagonal crystals. Future work will utilize the larger single crystals as seeds in order to further increase crystal size.

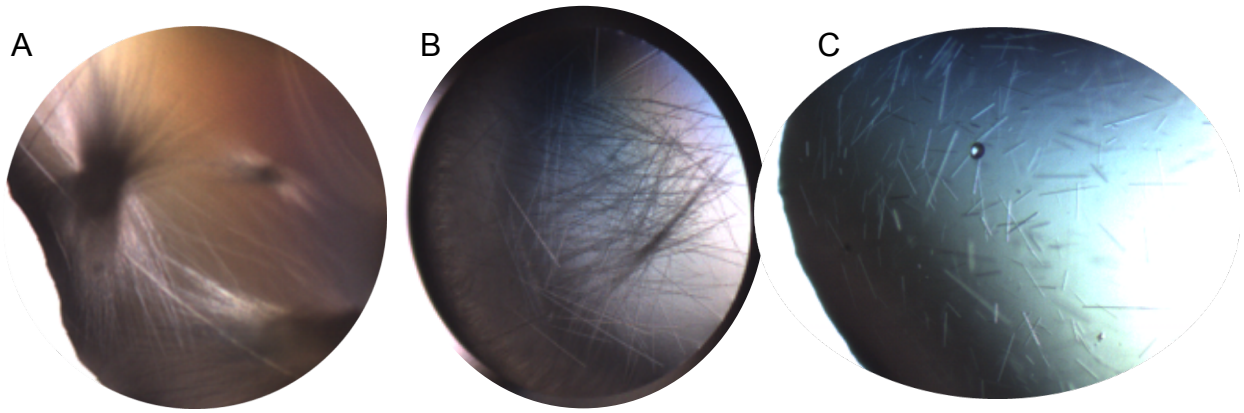


Figure 1: Crystals of *C. acetobutylicum* Q97 xylanase grown in 0.5M $(NH_4)_2SO_4$, 0.1M Sodium citrate tribasic dihydrate and 1.0M Li_2SO_4, pH 5.6. In Figure 1A, the initial needle crystals obtained in the condition are shown. In Figure 1B, the needles that grew from seeds generated from those needles seen in Figure 1A are shown. By slightly changing the harvesting method and the well solution concentrations, seeds generated from the needles in Figure 1A grew larger single crystals seen in Figure 1C.

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

THE PROTEIN C SYSTEM IN PROSTATE CANCER

Allison Stahl (2013) **Mentor: Dr. Laura Glasscock**

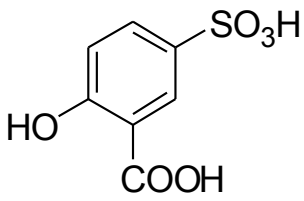
Prostate cancer (CaP) is the second leading cause of death in men in the United States (Siegel 2012). We investigated the expression of two transmembrane receptors, thrombomodulin (TM) and endothelial cell protein C receptor (EPCR), thought to be involved in CaP metastasis. TM and EPCR are normally expressed by endothelial cells in the blood vessel where they function as anti-coagulants. The localization and function of these receptors on endothelial cells is well-documented. Our previous Western blot studies have shown that TM and EPCR are also expressed by CaP cells where they regulate proliferation and invasion by these cells. Our goal was to continue a confocal microscopy project aimed at localizing TM and EPCR on two CaP cell lines, PC-3 and DU-145, using multiple monoclonal antibodies. We determined that the localization of TM and EPCR on PC-3 and DU-145's is similar to that reported for endothelial cells. In fact, this is the first investigation of EPCR's expression by tumor cells. We also began a study investigating the effect of plasminogen activator inhibitor-I on endothelial cell microtubule formation in vitro. Previous preliminary data generated by our lab have shown that individual proteins of the protein C system, including TM, protein C, and activated protein C (APC), affect the ability of human umbilical vein endothelial cells (HUVECs) to form microtubules in Matrigel™. We have re-established this microtubule growth system and are verifying these results. We are currently determining if PAI-I, which inhibits both APC and thrombin, affects how the protein C system regulates microtubule formation. Both the localization and microtubule formation studies will provide further insight into how the proteins of the protein C system, both individually and together, regulate prostate cancer progression.

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

Evaluation of Phenol Derivatives Containing Solubilizing Groups for Potential Inhibition of Amyloid-β Aggregation

Sarah Wicks (2015) and Craig Stevens (2013) **Mentors: Drs. J. Hanna and R. Lammi**

Amyloid-β (Aβ) is a protein of 39-43 amino acids that self-assembles into neurotoxic aggregates causally linked to Alzheimer's disease (AD). Inhibiting this aggregation process is one approach to treating and/or preventing AD. We have previously synthesized and evaluated four small tetrols that successfully limit Aβ association. The most promising of these, biphenyl-3,3′,4,4′-tetrol (BPT), abrogates aggregation near stoichiometric concentrations; however, all four of these compounds are hampered by limited solubility in aqueous solution, necessitating the use of DMSO (10%) as a co-solvent. In designing next-generation inhibitors for synthesis and evaluation, we intend to incorporate carboxylic and/or sulfonic acid moieties to improve water-solubility. To investigate the effects of these functional groups on inhibitor efficacy, we have evaluated several commercially available phenol derivatives using the Thioflavin-T assay. Thioflavin-T (ThT) binds specifically to β-structured Aβ aggregates; fluorescence of the bound dye is proportional to the degree of β-character, such that ThT fluorescence intensity may be used to monitor Aβ aggregation over time. These studies reveal that 5-sulfosalicylic acid is both highly water-soluble and moderately effective in inhibiting Aβ aggregation. As such, the incorporation of both sulfonate and carboxylate groups into a biphenyl tetrol architecture may be particularly promising.



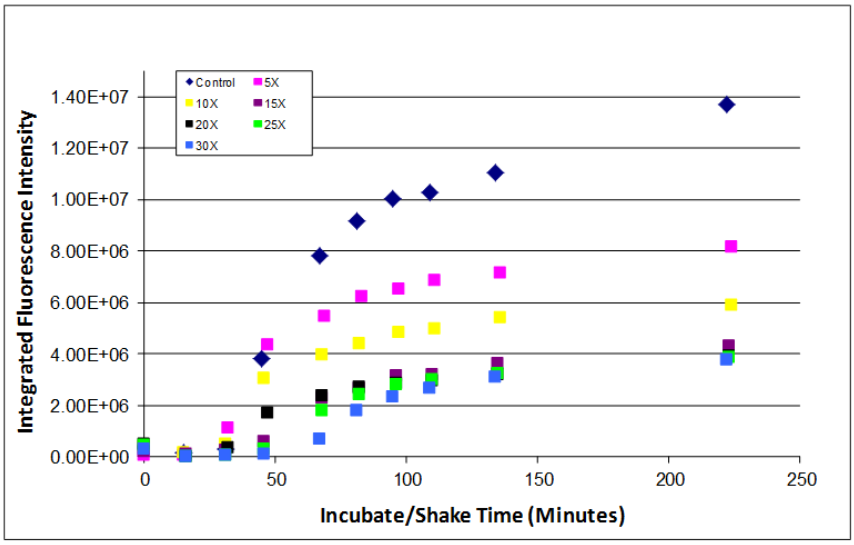


Figure: Structure and inhibitory efficacy of 5-sulfosalicylic acid. At a 10:1 inhibitor:Aβ ratio, the extent of Aβ aggregation is approximately halved (as compared to the inhibitor-free control).

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

CHROMIUM ANALYSIS USING TUNGSTEN COIL ATOMIC EMISSION SPECTROSCOPY

Justin M. Talbert (2013) **Mentor: Clifton P. Calloway**

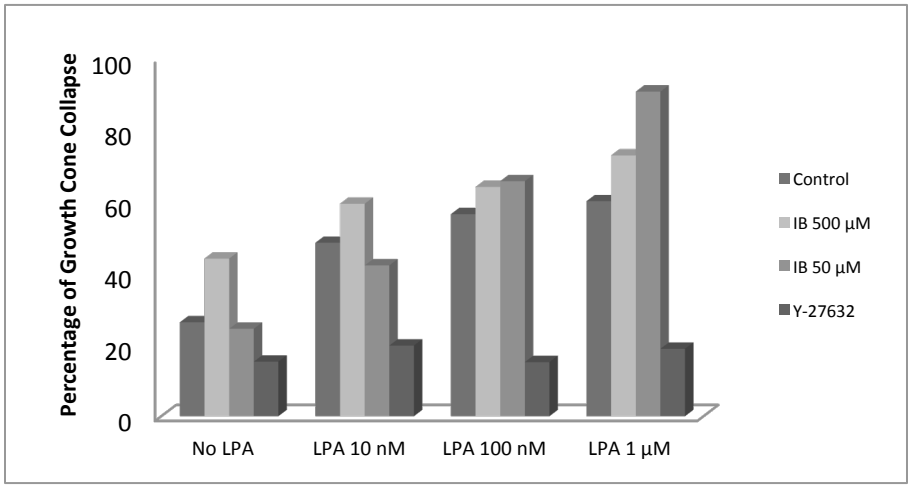
Chromium usually exists in the environment as trivalent and hexavalent forms. Hexavalent chromium is much more toxic than trivalent chromium for both acute and chronic exposure. As a result, hexavalent chromium is strictly regulated in many countries. Although the diphenylcarbazide (DPC) spectrophotometric method has been adopted as a standard method for hexavalent chromium determinations, alternative methods are still required because of its limitations in terms of sensitivity and selectivity. Methods such as electrospray ionization mass spectrometry and ion chromatography coupled with inductively coupled plasma (ICP) mass spectrometry have been studied as alternatives. However, these methods require the use of costly equipment. Traditional elemental techniques such as ICP atomic emission or atomic absorption spectrometry are not capable of distinguishing different oxidation states. However, the two oxidation states of chromium produce salts with dramatically different volatilization temperatures that might be used in separation of the two species. The use a tungsten coil filament as an alternative atomizer for atomic emission spectroscopy has been well described as a low cost alternative to ICP and atomic absorption methods. However, the temperature gradient from the coil center enclosed in glass cells has been shown to be dramatic. By incorporating an aluminum cell to house the coil, improved control of the coil temperature has been realized. Further, using a home-designed heating ramp program for the tungsten coil allows precise coil temperature control and moderate separation of the two primary oxidation states of chromium in a small, rapid analysis. Further improvements in the separation might be achieved through the use of multi-step temperature ramping.

This project is supported by an NIH-INBRE grant from the National Center for Research Resources and the National Institute of General Medical Sciences.

Investigating the Effects of Ibuprofen on Retinal Growth Cones

James A. Vinton **Mentor: Dr. Eric Birgbauer**

Ibuprofen is a non-steroidal anti-inflammatory drug which inhibits pain and inflammation primarily through interactions within the COX-2 pathway, specifically by inhibiting the synthesis of prostaglandins and other proteins associated with inflammation and pain. Fu et al (2007) and Wang et al (2009) suggest that ibuprofen acts specifically on nerve cells through a different pathway not associated with pain or inflammation. These studies suggest that ibuprofen may promote nerve regeneration after injury in mice by reducing the active intracellular RhoA, a GTPase whose pathway is known to induce growth cone collapse. In this study, we investigated the possible inhibitory effects of ibuprofen on embryonic retinal cells, specifically ibuprofen's ability to inhibit growth cone collapse in the presence of lysophosphatidic acid (LPA). Retinal tissue from E6 chick embryos was cultured and treated with a Control, 500μM or 50μM Ibuprofen, or Y-27632 (a known Rho kinase inhibitor), then treated with LPA. LPA induced a concentration dependent growth cone collapse in the absence of ibuprofen (as previously shown). Treatment with Y-27632, a known Rho kinase inhibitor, prevented LPA induced growth cone collapsed (also as previously shown). Treatment with 500μM Ibuprofen alone showed a statistically significant increase in growth cone collapse in the absence of LPA, although that was not seen with 50μM ibuprofen. Furthermore, in the presence of ibuprofen (50μM and 500μM), there was a significant increase in growth cone collapse with the addition of LPA, and thus ibuprofen did not inhibit growth cone collapse. Since the overall results differed from the previously published work, further investigation was warranted. In order to gather real time data, we performed time lapse microscopy. Preliminary results indicate that treatments of 500μM ibuprofen appear to increase the incidence of growth cone collapse. In conclusion, our investigation could not confirm previous literature stating that ibuprofen inhibits growth cone collapse using our model. The continuation of our investigation will entail biochemical and/or qualitative assays of active RhoA in embryonic retinal tissue treated with ibuprofen.



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

Ultrastructure and Immunohistochemical Analyses of a Regenerative Myocardium

Lyndsey Washburn (2013) **Mentor: Dr. Heather J. Evans-Anderson**

Ciona intestinalis is an invertebrate animal model system that is well characterized and has many advantages for the study of cardiovascular biology. A striking difference between most vertebrates and *Ciona* is that the *Ciona* myocardium is capable of regenerating cardiac myocytes throughout its lifespan, which makes the mechanisms of cardiac myocyte proliferation in *Ciona* intriguing. In order to stimulate regeneration, hearts were injured via ligation around the middle of the heart. After a 24 or 48 hour recovery period, damaged and control hearts were fixed for immunohistochemical or ultrastructure analyses. Preliminary TEM studies show degradation of the myofibrils and changes in the organization ofmitochondria in cardiac myocytes. Interestingly, in addition to damaged myocytes, dividing myocytes are also evident and undifferentiated cells populate the damaged myocardium. Furthermore, thickening of the extracellular matrix is apparent, particularly within the lumen of the heart. Histological studies using Mayer's Hematoxylin and Eosin as well as Movat pentachrome stains show basic organization of the matrix components within the *Ciona* heart. Specific labeling of the myocardium using the MF20 antibody (Iowa Hybridoma Bank) shows organization of the cardiac myocytes. Studies using immunohistochemistry to identify proliferation and apoptosis in cardiac myocytes of damaged hearts are currently underway. Taken together, these studies will coordinate differences in cellular organization to ultrastructural changes in cardiac myocytes within the regenerative myocardium of *Ciona*, which will help to elucidate the basic mechanisms of cardiac myocyte proliferation.

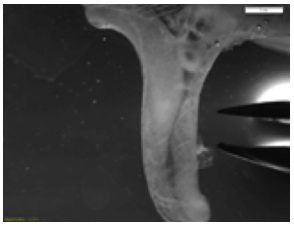


Figure 1: Control-dissected, undamaged *Ciona* heart

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Expression, Purification and Crystallization of the *Xanthomonas euvesicatoria* Avirulence Protein, AvrBs1.1

Laura Wilt (2013) **Mentor: Dr. Jason C. Hurlbert**

Phytopathogenic xanthomonads possess a type III secretion system (T3SS) that serves to secrete effector proteins that interfere with host immunity and disrupt normal cellular function. Avirulence proteins (Avr) are type III effector proteins that are recognized by specific R gene products in resistant hosts and the binding of R gene products by Avr proteins may elicit a hypersensitive resistance (HR) response in some hosts. In the absence of a corresponding R gene, Avr proteins may act as virulence factors contributing to the pathogenicity of the microorganism. A novel Avr protein, AvrBs1.1, from the pepper pathogen *Xanthomonas euvesicatoria* was recently identified to elicit a delayed hypersensitive response on *Capsicum annuum* leaves. Amino acid sequence analysis of AvrBs1.1 has revealed the presence of a Dual-Specificity Protein Tyrosine Phosphatase domain in C-terminus of the protein, meaning that the protein has the potential to bind substrates with phosphorylated Ser, Thr or Tyr residues. Screening for novel Resistance genes has identified a single binding partner of AvrBs1.1, the R gene product Bs7. Our laboratory has constructed homology models of AvrBs1.1 in addition to its homologue AvrBs7 from *X. gardneri* in order to better explain differences in hypersensitive response timings manifest in *C. annuum* after exposure to *X. gardneri* and *X. euvesicatora* cultures. While the computational models have provided some insight into potential differences in activation of Bs7 in *C. annuum*, a better understanding of the mechanism by which the R gene and Avr proteins interact will be gained by x-ray crystallographic studies of the protein complexes. Towards this end, we have begun purifying recombinant AvrBs1.1 in quantities suitable for crystallization trials. The coding sequence of *X. euvesicatoria* AvrBs1.1 was cloned into pET21A (Amp^r/C-terminal hexahistidine tag) which was used to transform *E. coli* BL21 (DE3) cultures. The resulting transformants were used to inoculate 6L of Luria-Bertani medium which were grown at 37°C to an optical density at 600nm of 0.6, were induced by the addition of IPTG and then grown for 18 hours post-induction. The cultures were harvested by centrifugation and the recombinant AvrBs1.1 was purified by a combination of metal chelating affinity and gel filtration chromatographic methods.

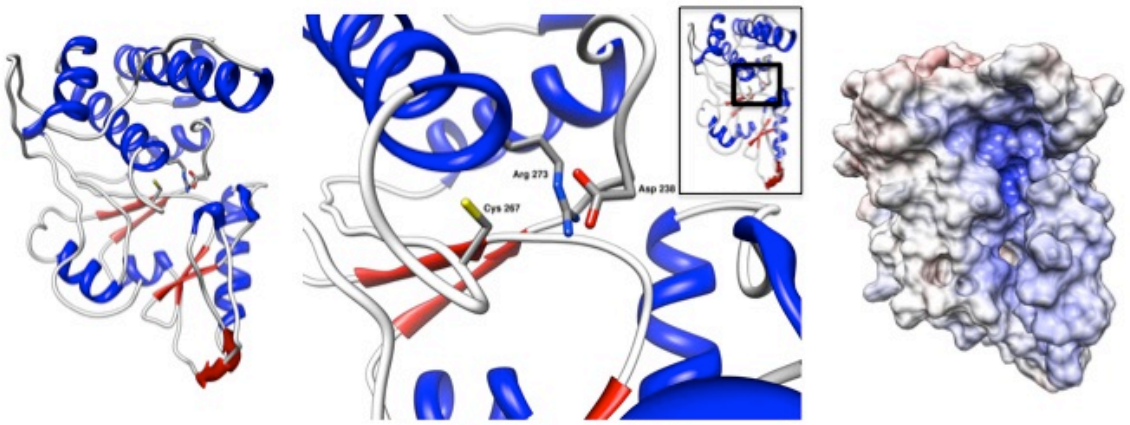

Figure 1: Lowest energy homology model of *X. euvesicatoria* AvrBs1.1. In the left pane, a ribbon diagram of the lowest energy structure generated is shown. The active site residues Cys267, Arg273 and Asp238 are shown in the middle panel. In the right panel, an electrostatic surface has been placed on the protein serving to highlight the active site pocket and binding cleft.

Support for this research was provided by the Winthrop University Research Council (SC120009) and the Winthrop University Department of Chemistry, Physics and Geology.

Design and Synthesis of Zone 1 Modified Sphingosine Kinase Inhibitors

Stephanie Woodson (2013) **Mentor: Dr. Christian Grattan**

Sphingosine Kinase 1 (SK1) is an enzyme overexpressed in cancerous cells which regulates tumor growth; in healthy cells it controls proliferation and cell growth. SK1 is responsible for catalyzing the phosphorylation of sphingosine, which creates sphinosine-1-phosphate (S1P). S1P is a bioactive lipid that regulates proliferation, survival, and motility. The concentration of S1P and ceramide together regulate whether a cell can proliferate or be apoptotic. The enzymes in this pathway give to us pathways in which we may have new anticancer drugs. So far there have been several drugs that have inhibited SK1; however, they were nonselective and also inhibited other kinases that are important in other biochemical processes. A few inhibitors have been identified that are selective and inhibit cancer cell proliferation and stimulate apoptosis, which reduces the levels of S1P. The main goal of this project is to design, synthesize, and characterize pure, potent and selective derivatives of the SK1 template inhibitor that may be orally bioavailable. Specifically, I am synthesizing six zone 1 derivatives by various substitutions and analyzing how the substitutions impact the binding and their overall effectiveness.

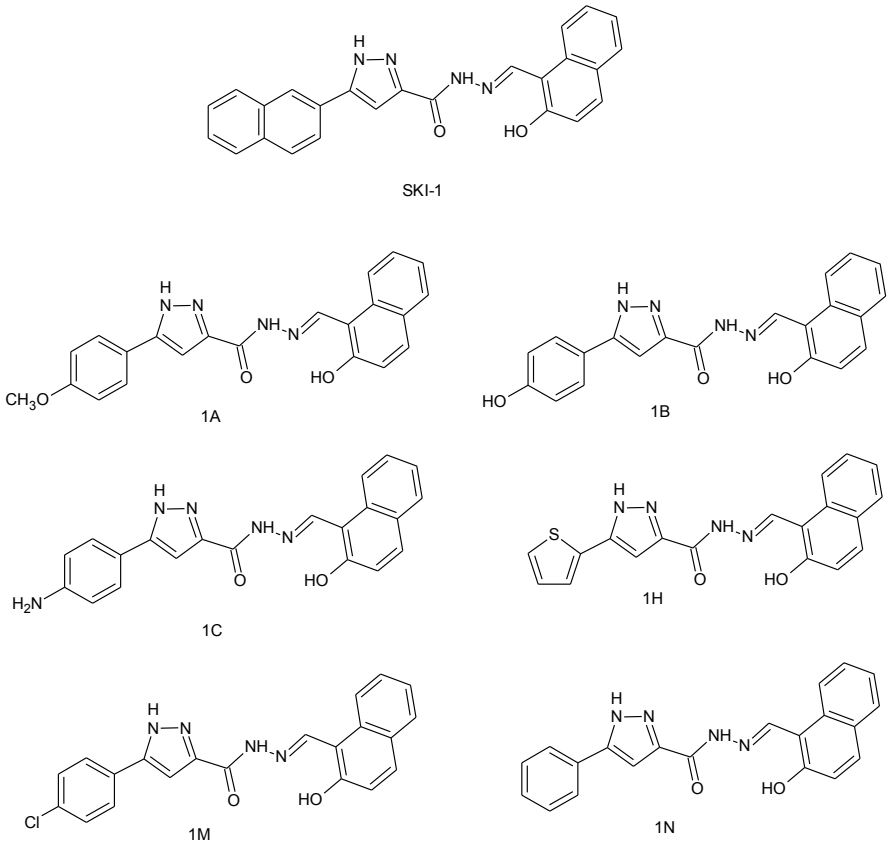

Figure 1: Template and inhibitor derivatives being prepared.

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

The Effect of Ki-16425 on the Action of Lysophosphatidic Acid in the Visual System of the Chick Embryo

Janet Xiao (2013) **Mentor: Dr. Eric Birgbauer**

Axon guidance plays a major role in the development of the visual system. A growing axon is tipped by a structure called a growth cone, which leads the growth of the axon and guides it from the eye to the correct target in the brain. It is critical that these axons reach their appropriate targets in order for a connection to be made between the eyes and the brain. Lysophosphatidic acid (LPA), a lysophospholipid, potentially serves as an axon guidance molecule and has been shown to cause growth cone collapse and neurite retraction in the chick visual system. LPA has five known receptors, LPA receptors 1-5. To try to determine which of these receptors are involved in axon guidance, we used a compound called Ki-16425. Ki-16425, an antagonist to LPA receptors 1 and 3, has been shown to inhibit the effects of LPA on growth cone collapse. A growth cone collapse assay was used to quantify the response to treatment with Ki-16425. Preliminary results show that growth cone collapse decreased, but was still present after treatment with Ki-16425. This incomplete inhibition leads us to believe that other LPA receptors, possibly 2 and/or 4, are also involved in axon guidance. We will conduct further studies in order to conclusively say that Ki-16425 has an effect on inhibiting LPA's actions on growth cones.

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