Winthrop University
Summer Undergraduate Research Experience (SURE)
Showcase

Friday, September 9, 2011
McBryde Hall

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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 Geology 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.

In Vitro Generation of Cu^{1+} Through Redox Chemistry

Zayed Almadidy (2012) **Mentor: Dr. Nicholas Grossoehme**

The decontamination effort within the body is largely reliant upon the effective capabilities of metalloefflux proteins. These efflux proteins primarily serve in detoxification processes for the elimination of potentially deadly trace metals which can catalytically produce toxic free radicals. It has been repeatedly demonstrated that copper is present in the Cu^{1+} oxidation state *in vivo*. A healthy equilibrium of these copper ions is maintained through efflux protein biosynthesis regulation; homeostatic regulatory mechanisms perceive minute alterations in the concentration of Cu^{1+}, and respond respectively by transcriptionally inhibiting or promoting the translation of additional efflux proteins. The first step in the biophysical characterization of these copper-sensing proteins is the stabilization Cu^{1+} under aqueous conditions—a nontrivial endeavor. Cu^{1+} can be prepared by the comproportionation reaction:

$$Cu^{2+}+Cu^{0}(s) \rightleftharpoons 2Cu^{1+}$$

However, the product of this reaction is short lived due to the high reactivity of Cu^{1+} and readily reverts back to Cu^{2+} and elemental copper. Documented stabilization of Cu^{1+} has been accomplished through addition of bathocuproinedisulfonic acid (BCS) and bicinchoninic acid (BCA) to the disproportion reaction. Upon formation of Cu^{1+}, these ligands form a tetrahedral complex with the element, thereby effectively sustaining its oxidation state:

$$Cu^{+2}+Cu^{0} \rightleftharpoons 2Cu^{1+}+2X \rightleftharpoons (CuX_2)^{1+}$$

Through spectrophotometric analysis of the $Cu^{1+}X_2$ complexes, binding constants and rate laws of the stabilization reaction were calculated. Further experimentation results indicate that Cu^{1+} can be prepared independently, where Cu^{2+} is reduced to Cu^{1+} under basic conditions:

$$4(CuX_2)^{2+} + 4OH^{-} \rightleftharpoons 2H_2O+O_2+ 4\ (CuX_2)^{1+}$$

Furthermore, elemental copper may be oxidized to Cu^{1+}:

$$2Cu^{0} + 2H^{+} + \tfrac{1}{2}O_2 + 2\ X \rightleftharpoons 2\ (CuX_2)^{1+}+H_2O$$

These data indicate that CuX_2 may be generated through multiple mechanisms—a previously unexplored phenomenon.

Further, we have investigated the kinetics of an acetonitrile-stabilized comproportionation reaction; current data is illustrated in Figure 1. Future work encompasses a further exploration of this data.

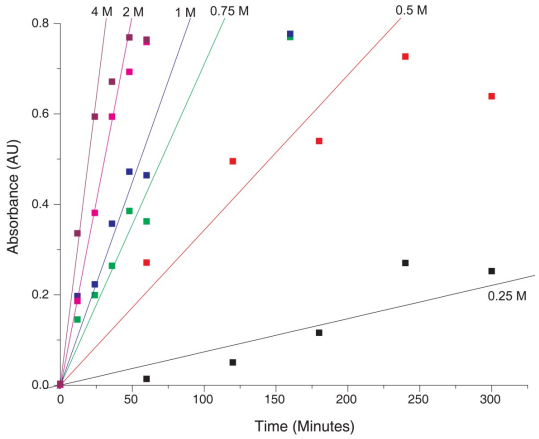


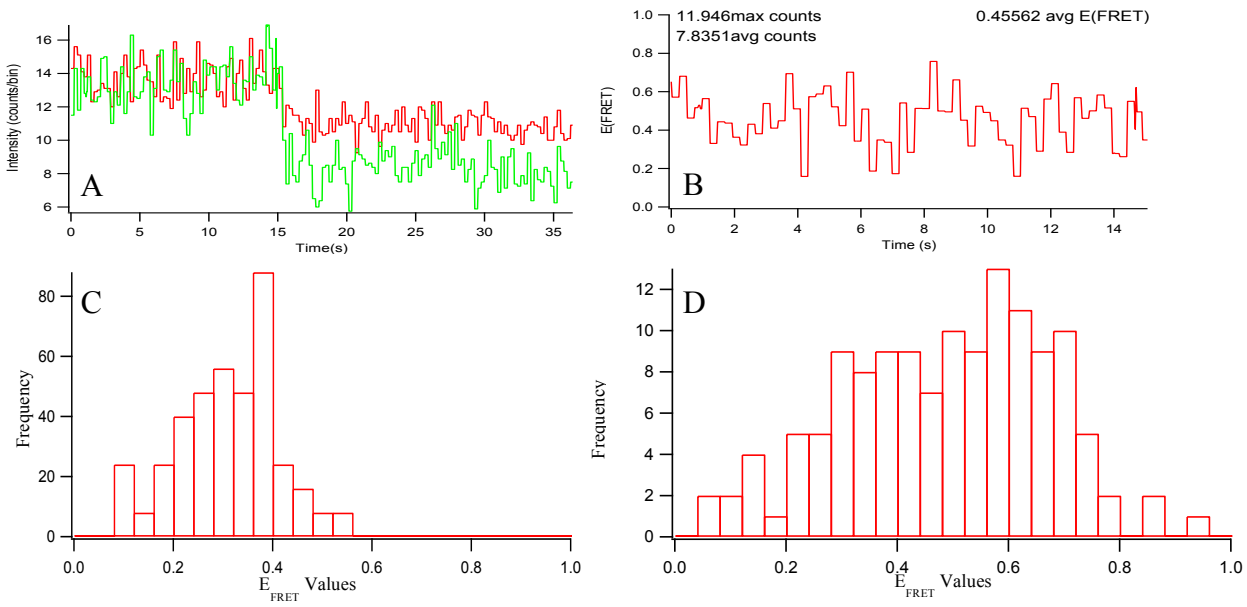
Figure 1: Dependence of various concentrations of acetonitrile on the rate of the comproportionation reaction.

Funding Provided by SC-INBRE

Inquiries on Amyloid-β Dimer Structures

Emily Amenson (2012) **Mentor: Dr. Robin K. Lammi**

Amyloid-β (Aβ) is a self-associating protein linked to Alzheimer's disease (AD) that contains 39-43 amino acids. Recently, it has been discovered that small oligomers of Aβ such as dimers and trimers are closely correlated to the symptoms and development of AD; however, little is known about their structures. We use Förster Resonance Energy Transfer (FRET) to describe characteristic dimer structures, determining FRET efficiencies (E_{FRET}) between associated donor- and acceptor-labeled peptides. Since they are highly sensitive to inter-dye distance, the determination of characteristic E_{FRET} values allows us to probe for preferred structures in Aβ dimers. By examining single, surface-tethered dimers, one at a time, we are also able to investigate time-dependent changes in E_{FRET} and dimer structure. Thus far, we have found two characteristic E_{FRET} values near 0.4 and 0.6, perhaps representing two preferred dimer structures. Approximately two-thirds of dimers exhibit one characteristic E_{FRET} value; the other one-third show two or more characteristic values, likely indicative of structural dynamics. Studies of fluorescence lifetimes in bulk solution are ongoing; these will complement single-dimer results, providing characteristic E_{FRET} values for dimers unaffected by surface tethering.



Single-dimer Results. A) A dimer intensity profile showing donor (green) and acceptor (red) fluorescence. B) Calculated FRET efficiency (E_{FRET}) for the dimer in (A) during the period when both dyes are fluorescing. C) Histogram of E_{FRET} values visited by a single dimer; the presence of one peak suggests one dimer structure. D) Histogram of characteristic E_{FRET} values for dozens of individual dimers. Peaks occur at ~0.4 and ~0.6.

This project was supported by the National Science Foundation (Grant CHE-0848824) and by an NIH grant from the National Center for Research Resources (SC-INBRE)

Crystallization and Preliminary X-ray Diffraction Analysis of *Clostridum papyrosolvens* C7I Xylanase

Elizabeth Bales **Mentor: Dr. Jason Hurlbert**

Clostridum papyrosolvens C7I xylanase catalyzes the hydrolysis of the xylan component plant hemicellulose. Xylans are made of β-1,4-linked xylopyranose units with arabinofuranose and glucuronopyranose substitutions along the backbone that serve to limit xylanase activity by physically blocking access to the xylopyranose backbone. This enzyme has been targeted for structural studies due to the fact that initial functional assays have indicated it may hydrolyze arabinofuranose substituted xylans better than homologues from *Bacillus* and *Erwinia* species, which makes it an interesting candidate for inclusion in the enzymatic repertoire of biocatalysts used in the industrial production of ethanol. An initial crystallization condition was identified from a commercially obtained sparse matrix screen. This condition contained 0.1M ammonium acetate, 0.1M BIS-TRIS pH 5.5 and 17% w/v Polyethylene Glycol 10,000. Crystals of the xylanase as well as co-crytals of the xylanase and the Ligand, X_3, were grown and harvested. Once the crystals were harvested the well solution was exchanged for a cryo-protectant solution made of 50% well solution and 50% glycerine. A Rigaku R-Axis II diffractometer equipped with a rotating anode x-ray source and image plate detectors was used to collect data from the crystals. A 180 degree dataset was collected with 5 minute exposure times and a Phi rotation of 0.5 degrees per image. The resulting data was processed to 2.1 angstroms and the crystal belonged to the orthorhombic space group, C2221 with unit cell parameters: a=66.020, b=76.520, c=150.550 and α=β=γ=90.00.

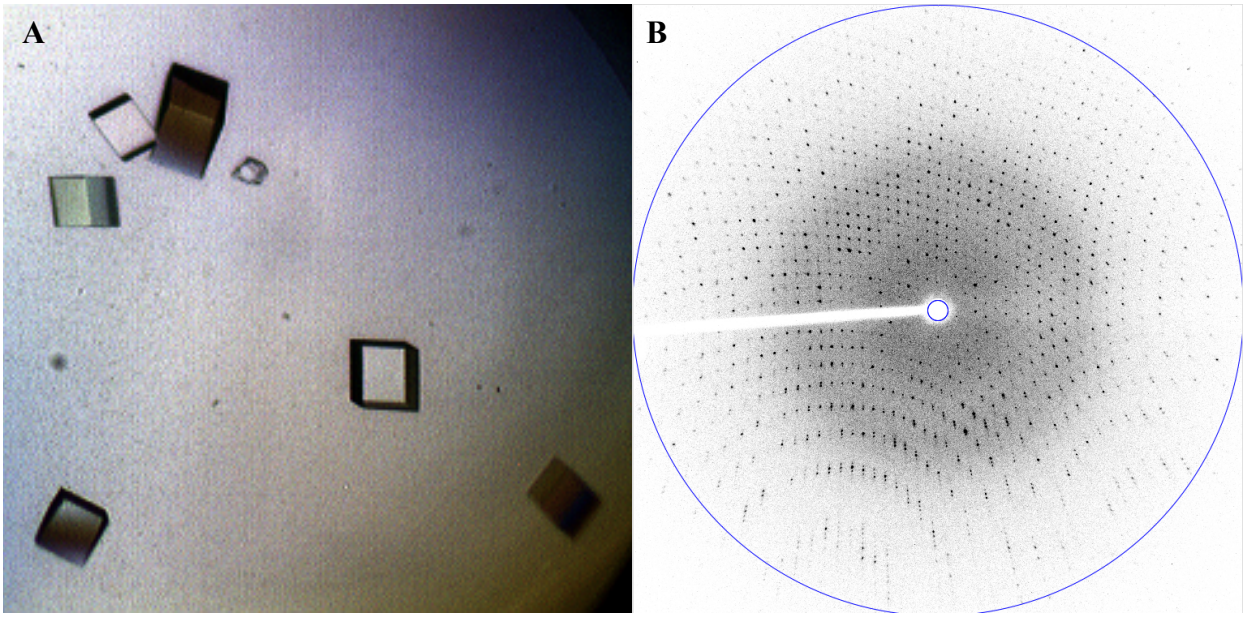


Figure 1: Panel A: Crystals of *Clostridium papyrosolvens* xylanase CpC71 grown in 0.1M $NH_4CH_3CO_2$, 0.1M BIS-TRIS pH 5.5 and 17% PEG 10,000. Panel B: A sample diffraction image that was collected from one of the crystals.

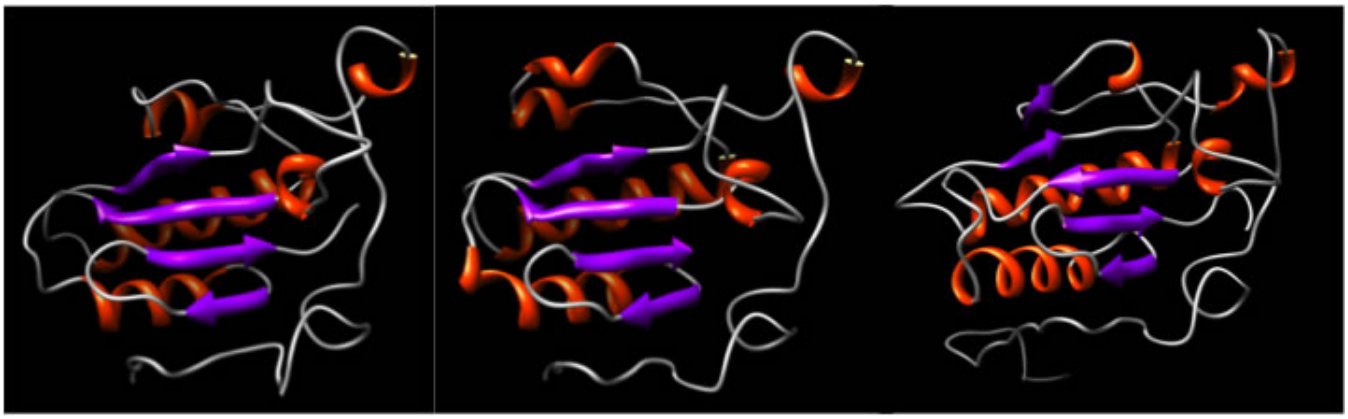
This project is supported by a NIH INBRE grant from the National Center for Research Resources.

The Hedgehog Pathway in the Basal Bilaterian *Isodiametra pulchra* (Acoelomorpha)

Emily Bowie[1], Paul Kaschutnig[2], Arti R. Intwala[1], and Dr. Julian Smith III[1].

[1] Department of Biology at Winthrop University & [2] Institute of Zoology at University of Innsbruck

The goal of this work is to characterize the Hedgehog (Hh) pathway in the acoelomorphan species *Isodiametra pulchra*. This organism was chosen because it is one of the most primitive bilaterians, because we have access to its transcriptome (unpublished), and because it is a completely ciliated animal. This latter feature is important because the primary cilia is known to be an important Hh signaling center during vertebrate development. Using the NCBI protein database, the unpublished transcriptome for *I.pulchra* , BLAST analysis, and ClustalW alignments to identify possible orthologues, our lab has identified and sequenced genes that code for components of this pathway. We have identified the definitive Hedgehog protein, a Kif7 orthologue, one of three possible candidates for Patched (the Hedgehog receptor), and the most likely candidate for the seven-pass-GPCR family member that acts downstream of Patched, Smoothened. In order to determine whether our choice of Patched orthologue is correct, we propose to take advantage of small-molecule-inhibitor effectson Hh signaling. Cyclopamine is an antagonist of the Hedgehog pathway, whereas Purmorphamine is a pathway agonist. Accordingly, we believe that Patched expression, which is upregulated during Hh activation, will decrease under the influence of Cyclopamine, and increase under the influence of Purmorphamine. We want to use RT-PCR methods to measure Patched expression. To date, we have developed and tested RT primers for Patched and for two "housekeeping" genes (beta-Actin and EF1alpha). We have carried out preliminary exposure experiments to groups of 30 *Isodiametra pulchra*. Both groups received 2.4µL of 10µmol concentration of Cyclopamine or Purmorphamine. The animals that were exposed to Purmorphamine looked healthy, but did seem to have developed more eggs after 24 hours than those in the control group. The animals that were exposed to Cyclopamine showed significant phenotypic changes after 24 hours. The worms were lethargic, shrunken, and had a profoundly deformed epidermis. Subsequent work will include RNA isolation from groups of 30 worms, treated as described above, followed by RT-PCR to determine Patched expression levels.



Structural Comparison of Hedgehog homologues. Although the sequence for the Hedgehog signalling ligand in *I. pulchra* is rather divergent at the amino acid level, folding prediction by the Phyre server reveals a probable secondary structure (left) that is more similar to that of *Drosophila* Hh ligand (center) than to that of the model platyhelminth, *Schmidtea mediterranea* (right).

Funded by SC INBRE and a University of Innsbruck graduate travel fellowship to PK.

Parotoplana n. sp. (Proseriata: Otoplanidae) from the Coast of North Carolina, USA

Joseph Bursey[1], Lance Graham[1], Julian P.S. Smith III[1] & Marian K. Litvaitis[2]

[1]Department of Biology, Winthrop University & [2]University of New Hampshire, Durham

Parotoplana n. sp. a new turbellarian species belonging to the family Otoplanidae was found in shoreline sediments collected from swash and shiny zones at low tide on Emerald Isle (Bogue Banks, NC) and near Long Beach (Oak Island, NC). Brightfield and confocal laser scanning microscopy (CLSM) provided a detailed overview of the organism's internal and external anatomy. The habitus of *Parotoplana* n. sp. (Fig. 1) was golden brown with a peripheral translucent zone and an elongated oval body shape averaging 500 µm in length. Patches of ciliation were present in the head region as well as in the creeping sole; in this species, the creeping sole is interrupted at the mouth. The pharynx is ciliated internally and externally. This organism fits into *Parotoplana* by its separate vesicula granulorum, distally connected stylet apparatus, and bursal stalk arising from the dorsal part of the common genital atrium. It differs from other members of the genus by its post-pharyngeal germaria and unique stylet grouping consisting of a closed-circular group of eight winged stylets just anterior and dorsal to a half-circle wreath of eight hook-shaped stylets. A 408bp fragment of the 18s rDNA gene was obtained using universal primers, and aligned (ClustalW) with selected proseriate sequences from GenBank and with other unpublished otoplanid sequences from our lab. Neighbor-joining trees suggest that Parotoplaninae, the subfamily to which our species belongs, is paraphyletic. Our new data on North Carolina otoplanids will contribute to elucidating evolutionary relationships within the family.

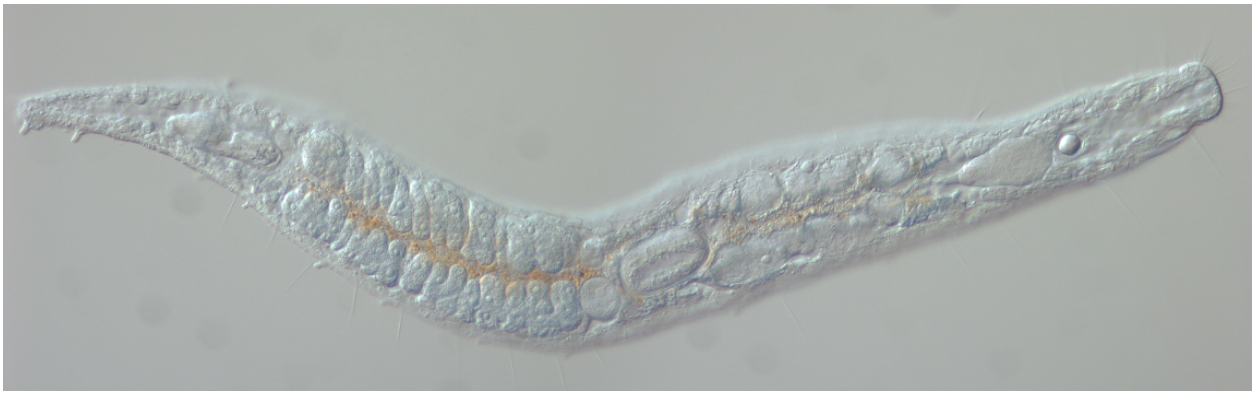


Figure 1. *Parotoplana* n. sp.; dorsal view of live animal.

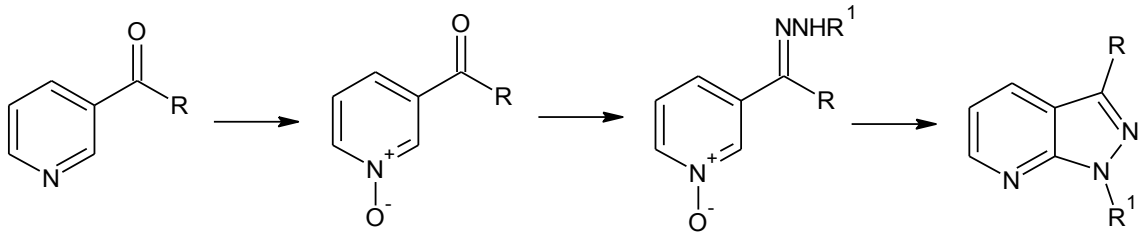
Support for this research was provided by SC INBRE and the Winthrop Research Council to JSIII and by the New Hampshire Agricultural Experiment Station to MKL.

Synthesis of 3-Alkylpyrazolopyridines by Ring Closure of Pyridine *N*-oxide Tosylhydrazones

Megan D'Angelo (2012) **Mentor: Dr. James M. Hanna, Jr.**

Pyrazolopyridines are common substructures seen in a myriad of pharmaceutical compounds. These medications are used in the treatment of inflammation, cancer, viral infections and inhibit the growth of microorganisms. The current syntheses of the compounds require starting with either a pyrazole or pyridine, and fusing the second ring to it. The focus of our research is the development of a more efficient method of fusing a pyrazole onto a pyridine ring. The problem that arises with currently available methods is the need to first functionalize the 2-position of the pyridine ring with a good leaving group, most commonly by chlorination, which can be later used in an intramolecular reaction that allows for the formation of the pyrazole ring.

We have developed an improved synthesis of this important ring structure based on the hypothesis that initial formation of a pyridine N-oxide followed by treatment with tosylhydrazide to form an N-oxide tosylhydrazone should allow an intramolecular reaction could occur in the presence of an electrophilic reagent and a base, effectively attaching a pyrazole ring to the parent pyridine ring. This approach is outlined below:



Our method eliminates the need to first halogenate the parent pyridine ring. The elimination of this synthetic step will possibly improve the overall yield and result in a more economical synthesis of the target pyrazolo[3,4-*b*]pyridine. Previous results from our laboratory indicate that this method works very well for the synthesis of 3-arylpyrazolopyridines; this project is directed toward demonstrating the suitability of this method for the synthesis of 3-alkylpyrazolopyridines. The first attempts at forming the pyrazolo[3,4-*b*]pyridine utilized 3-acetyl pyridine *N*-oxide tosylhydrazone (R=CH_3 and R^1=Ts) and semicarbazone (R=CH_3, R^1=$CONH_2$) but the cyclizations were unsuccessful. ^{13}C-NMR analysis of the starting compounds indicated that both had the (*E*)-configuration about the imine bond and therefore cyclization was not possible.

Altering the steric bulk of the R group (R=*t*-Bu, R^1=Ts) gave an *N*-oxide tosylhydrazone in the required (*Z*)-configuration about the imine bond as demonstrated by ^{13}C-NMR. Cyclization of this compound proved possible in 27% yield (unoptimized). We believe this success is due to the stereochemistry of the compound about the tosylhydrazone double bond. Further research is planned on optimizing the conditions of this reaction, and also to investigate ways to cyclize alkanoylpyridine *N*-oxide hydrazones where R is a small alkyl group.

This project was supported by a NIH INBRE grant from the National Center for Research Resources and by the Winthrop University Department of Chemistry, Physics and Geology.

LPA and S1P induce retinal growth cone collapse via activating GPCR Intracellular Pathways G_i and $G_{12/13}$

Jarod Fincher (2012) **Mentor: Dr. Eric Birgbauer**

Our research focuses on the molecules involved in axon guidance during the development of the visual system in chicken embryos. Although there have been several axon guidance molecules studied and identified that contribute during the development of the visual system, the full complexity still remains unknown. The axon guidance molecules we are particularly interested in are two lysophospholipids: lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). We have shown that LPA and S1P induce growth cone collapse in chick retinal axons *in vitro*. LPA and S1P bind specifically to G-protein coupled receptors (GPCRs), which activate one of four possible intracellular pathways (G_i, $G_{12/13}$, G_q, G_s). We are investigating exactly which intracellular pathways LPA and S1P mediate. We are using pharmacological inhibitors to block individual intracellular pathways, measuring growth cone collapse by LPA or S1P. The inhibitors used were Pertussis Toxin (PTX), which blocks the G_i pathway, Y-27632, a "ROCK" inhibitor that blocks the $G_{12/13}$ pathway, and U-73122, a Phospholipase C (PLC) inhibitor that blocks the G_q pathway. We have found that the "ROCK" inhibitor Y-27632 prevents growth cone collapse in both LPA and S1P treatments, indicating that the $G_{12/13}$ pathway is involved in growth cone collapse. PTX, an inhibitor of G_i, was found to partially reduce the level of growth cone collapse, indicating that the G_i pathway contributes to growth cone collapse. However, inhibiting the G_q pathway provided no effects of reducing growth cone collapse in the presence of LPA or S1P, suggesting that the G_q pathway is not involved in growth cone collapse. This research is allowing us to identify the intracellular GPCR pathways involved in growth cone collapse with LPA or S1P, gaining a further understanding of visual system development.

Support was received from Winthrop University and from National Institutes of Health Grant Number P20 RR-016461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE).

Thrombomodulin and Endothelial Protein C Receptor in Prostate Cancer

Selah Fredrickson (2012) and Chantelle VanNostern (2012) Mentor: Dr. Laura Glasscock

Prostate cancer (CaP) is the second leading cause of death in men in the United States (Jemal 2011). 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 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 localize TM and EPCR on two CaP cell lines, PC-3 and DU-145. Comparison of the localization of TM and EPCR on these tumor cells to endothelial cells will allow further insight into what role these proteins play in regulating CaP progression. We also investigated how TM on PC-3 and DU-145's regulates adhesion of tumor cells to each other. 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 determined that expression of TM by PC-3 cells affects the ability of these cells to adhere to each other. We are currently determining if TM and EPCR are colocalized on PC-3 and DU-145 cells and if these receptors co-localize with other well-known cell adhesion molecules such as the cadherins. We are also determining what domains of TM are responsible for regulating CaP cell adhesion.

Support was provided by the National Institutes of Health (NIH) through the National Center for Research Resources as part of the South Carolina INBRE award and the McKay Urology Endowment Fund

Construction of siRNA vectors against LPA receptors

Charley Gao (2011) **Mentor: Eric Birgbauer**

The mechanism of how Retinal Ganglion Cells extend their axons to the precise place of the brain is unknown. Recent research reveals that Lysophosphatidic Acid (LPA) can cause the growth cones of the axons to collapse, which suggests LPA may play an important role in the guidance of axon growth. There are five well defined LPA Receptors so far: LPA_1 to LPA_5. We want to determine which of the five receptors are responsible for axon guidance in chicken embryos. We are constructing siRNA hairpins to knock down each of the LPA receptors so that we can investigate the role each receptor plays during the development of chicken visual system. For the hairpin construction, we designed three siRNA hairpins for each LPA receptor. Each hairpin is constructed from two universal primers and two specific primers. Specific primers have the target sequence of the receptor and universal primers have the necessary structure of a hairpin and restriction enzyme sites. Through Polymerase Chain Reaction (PCR), we combined the four primers together to form the hairpin. We cloned the siRNA hairpins into an expression vector: pRFPRNAiC. This vector has a Red Fluorescence Protein tag, an Ampicillin resistance gene, and an RNAi cassette. In the RNAi cassette, there's an U6 chick promoter for siRNA hairpin expression. We transformed the vectors with siRNA hairpins into *E. coli*. By now, we have constructed three siRNA vectors for lpar1 and lpar3 respectively, and two for lpar2, which have all been verified by sequencing. Then we transfected the siRNA vectors into DF-1 cells, a chicken fibroblast cell line, and used real-time PCR to test the efficiency of the knock down. The results of real-time PCR suggest knock down; however, the differences were not statistically significant. One possible reason is that the transfection efficiency was only around 35%, which makes it difficult to distinguish knock down from experimental variability. We are planning either to try to increase the transfection efficiency by using another transfection reagent or to clone the siRNA into a replication competent retrovirus vector, which would allow spread and infection of all cells in the dish.

Support was received from Winthrop University and from National Institutes of Health Grant Number P20 RR-016461 from the National Center for Research Resources for support of the program entitled "South Carolina IDeA Networks of Biomedical Research Excellence" (SC-INBRE).

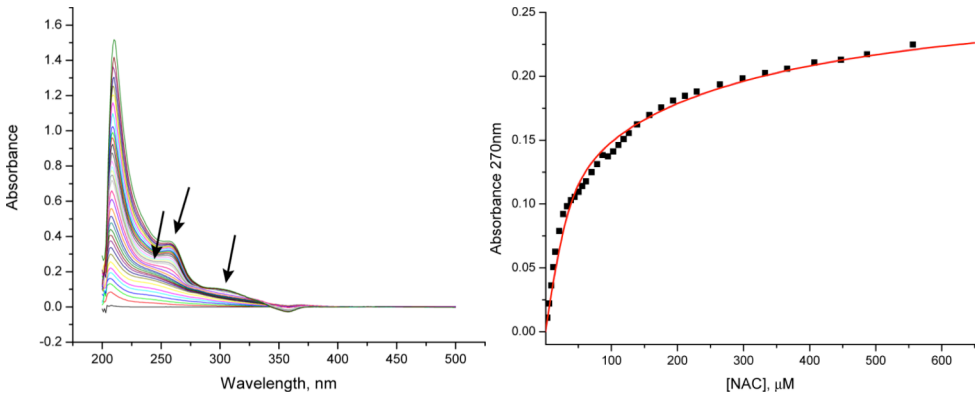
Examining the Colorimetric and Calorimetric Properties of Various Copper (I) Complexes

Sharon Jenkins (2011) **Mentor: Dr. Nicholas E. Grossoehme**

Copper is a transition metal capable of converting between the oxidized, copper (II) and reduced, copper (I) states. This characteristic of copper makes it an important electron transporter, a redox co-factor for a variety of enzymes, and a virulence factor for numerous pathogens. Copper (I) is the physiologically relevant oxidation state; however, most experiments have been conducted *in vitro* on copper (II) due to the unstable nature of copper (I). Strict anaerobic conditions are necessary for work with this oxidation state due to O_2- dependent spontaneous oxidation of copper (I) to copper (II). Copper (I) undergoes a disproportionation reaction which causes a thousand-fold increase in copper (II) in solution as compared to copper (I).

$$2Cu^{+}_{(aq)} \rightleftharpoons Cu^{0}_{(s)} + Cu^{2+}$$

In this process, copper is capable of acting as its own oxidizing and reducing agent so a stabilizing ligand is required in order to maintain the copper (I) oxidation state in solution. These challenges were overcome by manipulating copper in a Coy Lab glove box to completely exclude O_2, and acetonitrile (MeCN) was added to maintain the oxidation state of copper (I) in solution. Equilibrium constants and enthalpy values were extracted from titrations of N-acetyl cysteine (NAC) into copper (I) using colorimetric and calorimetric techniques.



Spectroscopic Data from a Titration of NAC into Copper (I)

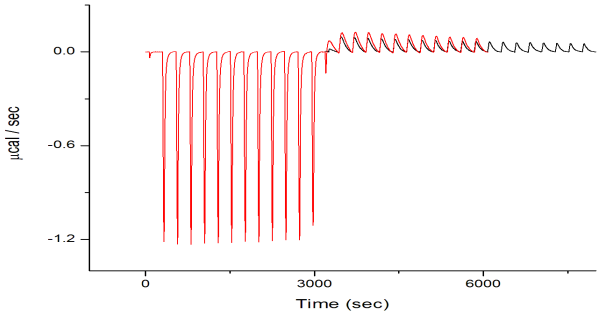
The average K_1 value for colorimetric data was $2 \pm 2 \times 10^5$ while the K_1 extracted from the calorimetric data was $3.0 \pm 0.7 \times 10^5$. The average K_2 value for colorimetric data was $3.2 \pm 0.8 \times 10^3$ while the extracted K_2 from the calorimetric data was $3.0 \pm 2.0 \times 10^3$. The similarities between the data collected colorimetrically and the data collected calorimetrically provides confidence that these values are correct. The enthalpy values associated with the NAC-Cu (I) complex are $\Delta H_1 = -73 \pm 5$ kJ/mol and $\Delta H_2 = -43 \pm 5$ kJ/mol with $\beta_2 = 1.0 \pm 0.4 \times 10^9$ and $\Delta H_1 + \Delta H_2 = -116$ kJ/mol (fixed). These values are critical for understanding the thermodynamic forces that control copper (I) binding and selectivity.

Funding provided by SC-INBRE

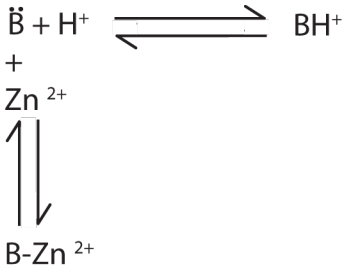
Determination of Metal Binding Constants by Potentiometric Titrations

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

During routine calorimetric titrations of Zn^{2+} into ethylenediaminetetraacteic acid (EDTA) in the presence of N-hydroxy-piperizine-N'-ethanesulfonic acid (HEPES), a series of unexpected endothermic peaks were observed subsequent to the stoichiometric equivalence point. This strongly suggested that the 'dilution' aspect of this titration was not as simple as anticipated and a Zn^{2+}-HEPES complex was hypothesized. The goal of this research was to use potentiometric titrations to extract the metal binding constants of Zn for various buffers.



The progress of the metal-ligand (Zn-buffer) complex formation can be monitored by pH measurements over the course of a titration experiment. The presence of zinc influences pH of the buffer because it binds to the same place that a proton would. Consequently, the proton is displaced and the solution becomes acidic more rapidly (illustrated in the coupled equilibrium below).



Our first goal was to extract the metal binding constant for the Zn-HEPES complex. Upon investigation we found that the titrations in the presence of Zn and the titrations in the absence of Zn were identical, indicating that a metal-ligand complex did not form between Zn and HEPES at our working concentrations. The second goal of this project was to use the same methodology to determine formation constants of the Cu^{2+}-BCS system. This project was approached similarly using pH titrations. Complex formation between Cu^{2+} and BCS did occur and we were able to calculate the formation constant using the Bjerrum method[1]. The equilibrium constant was determined to be $2.07 \pm 0.06 \times 10^{6}$. Our third goal followed the same experimental procedures with pH titrations of Cu^{+} and BCS under anaerobic conditions (due to the strong oxidation potential of Cu^{+}). Very surprisingly, we did witness complex formation of BCS and Cu^{+}, but were unable to obtain a metal binding constant.

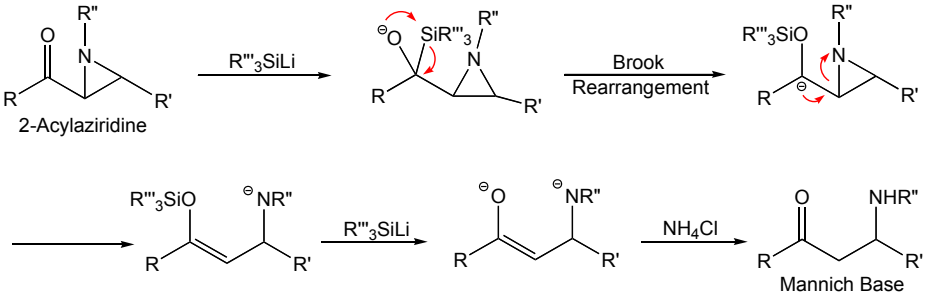
[1.] Rossotti, F. Wavepacket. The Determination of Stability Constants. In *McGraw-Hill Series in Advanced Chemistry;* McGraw-Hill: New York, 1961; pp 127-170.

Support was provided by SC-INBRE

Preparation of Mannich Bases from 2-Acylaziridines Using Silylithium Reagents

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

Mannich bases are very useful intermediates in the synthesis of biologically relevant molecules. We have developed a new method for the preparation of Mannich bases from 2-acylaziridines using silyllithium reagents. The 2-acylazirdine undergoes nucleophilic attack by the silyllithium reagent which triggers a Brook rearrangement. Opening of the aziridine ring results in the formation of a silyl enol ether which is then cleaved by excess silyllithium to form the desired Mannich base product. The reactions of 2-acylaziridines with a phenyl substituent on the carbonyl carbon proceed smoothly. The phenyl group helps to stabilize the negative charge that develops during the Brook rearrangement, encouraging the formation of the corresponding Mannich base. The reactions of 2-acylaziridnes with an akyl substituent on the carbonyl also proceed but with lower yields.



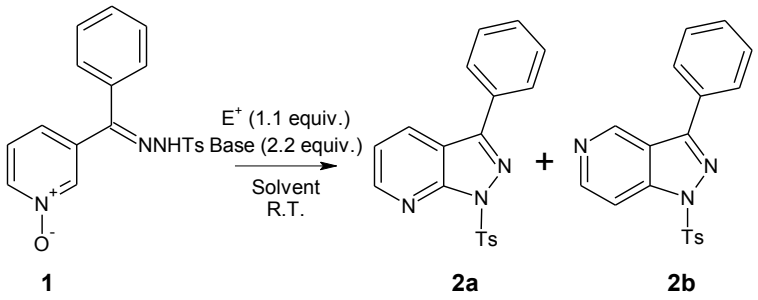
The reactions were performed by the drop-wise addition of methyldiphenlysilyllithium to a stirred solution of the 2-acylaziridine in THF at -50°C under argon. The Mannich bases were then purified via silica gel column chromatography using 5-30% ethyl acetate in toluene as eluent. Product yields for Mannich bases with a phenyl substituent on the carbonyl were fairly high, and showed no signs of decomposition during purification. However, Mannich bases with akyl substituents on the carbonyl showed lower yields. This was due in part to the decomposition of these products during purification. Future research will investigate methods to prevent product decomposition of these sensitive Mannich Bases.

Support was provided by the American Chemical Society Petroleum Research Fund and the Winthrop University Department of Chemistry, Physics, and Geology

Optimizing the Cyclization of 3-Benzoylpyridine *N*-oxide Tosylhydrazone via Manipulation of Electrophile, Solvent, and Base

William Lominac (2012) **Mentor: James M. Hanna, Jr.**

Pyrazolo[3,4-*b*]pyridine is the representative structure for an entire class of molecules collectively called pyrazolopyridines. These molecules have exhibited potential as anxiolytic, or anxiety-relieving pharmaceuticals, in addition to several other medical applications. In precedent literature, the synthesis of pyrazolo[3,4-*b*]pyridines required a halogenation step prior to the final cyclization, which created a more suitable leaving group on the pyridine ring. This halogenation step required harsh reaction conditions which we hoped to eliminate. We envisioned that, by exploiting the increased electrophilicity of the carbons on a pyridine with a capped *N*-oxide, the need for a better leaving group, and thus the entire halogenation step, could be eliminated by employing pyridine *N*-oxide tosylhydrazones as shown in the scheme below. Furthermore, we recognized that under these conditions, two isomeric products could be formed – the pyrazolo[3,4-*b*]pyridine derivative **2a** and the pyrazolo[4,3-*c*]pyridine derivative **2b**, and we were very interested to see if the regioselectivity could be controlled. If so, this approach could lead to an efficient way to produce either isomer from a common intermediate.



1 **2a** **2b**

This synthetic approach has proven successful, and subsequent cyclization reactions in our laboratory have utilized this more direct approach. The focus of the current research has been optimizing the cyclization by varying the reagents used; several electrophiles, bases, and solvents have been evaluated.

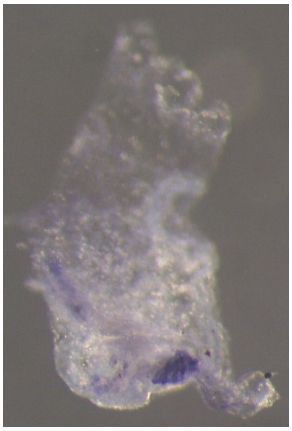
Most of the conditions studied gave good to excellent combined yields of products **2a** and **2b**. We have also found that the regioselectivity of the reaction can be controlled by varying the electrophile. Thus, the use of tosyl anhydride gave primarily compound **2a**, while the use of triflyl anhydride gave mainly **2b**. Electrophile screening experiments indicated that the regioselectivity of the reaction may be related to the stability of the leaving group which results from the reaction of the *N*-oxide with the electrophile, with more stable leaving groups (derived from more electron-poor electrophiles) giving a greater proportion of **2b**. Varying the base and solvent had much less effect on the regioselectivity, except for the use of toluene as a solvent, which gave mainly **2a** even when employing triflyl anhydride. Future work will be focused on a better understanding of the factors which affect the regioselectivity of the cyclization and the application of this novel cyclization method to the preparation of other fused nitrogen heterocycles.

Support was provided by an NIH-INBRE grant from the National Center for Research Resources, 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 **Mentor: Dr. Heather Evans-Anderson**

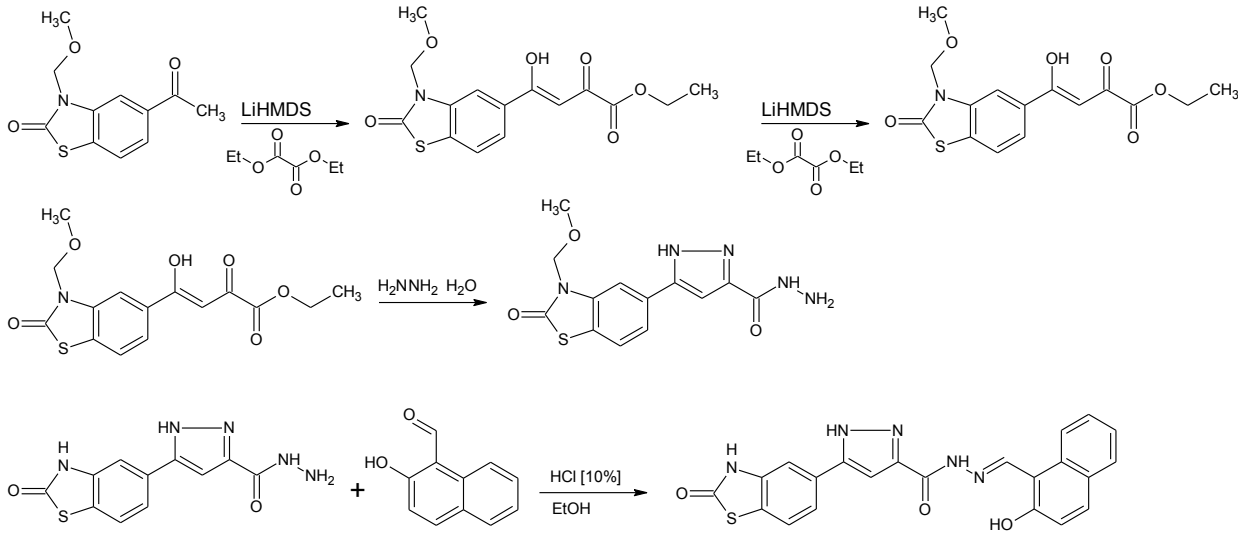
Ciona intestinalis is a useful animal model system for studying developmental processes. It is particularly helpful in studies of heart development since many of the developmental steps and genes are conserved in *Ciona* and replicate early heart development in other Chordates, such as vertebrates. One process that can help define conserved processes and gene expression patterns is in situ hybridization. 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 in situ hybridization than it is to develop new antibodies. There are many preliminary steps to performing an in situ hybridization. Research this 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. Currently we are still in the process of probe preparation. Eventually, more probes will be constructed with other genes of interest to further study gene expression throughout cardiac development in *Ciona intestinalis*.



Design and Synthesis of Modified Sphingosine Kinase Inhibitors to Enhance Oral Bioavailability and Overall Effectiveness

Kevin Mays (2012) **Mentors: Dr. T. Christian Grattan and Dr. James M. Hanna, Jr.**

Sphingolipids have historically been known as key components of cellular rigidity and cytoplasmic functionality; recently, however, several sphingolipid derivatives have been characterized as dual structural components and intracellular messengers. Of particular interest are sphingosine-1-phosphate (S1P), an oncogenic and carcinogenic stress-mediated growth factor, and ceramide, a sphingosine derivative believed to mediate apoptotic processes. The phosphorylation of ceramide into S1P is catalyzed by the enzymes Sphingosine Kinase I and II (SPK1/2); the inhibition of this activity has been demonstrated to slow carcinogenesis, metastasis and angiogenesis in cancerous tissues. A number of novel SPK1/2 inhibitors have been identified by Smith *et al* and show promising anticancer effects *in vitro*; this project specifically focused on the design, synthesis and characterization of Zone "4" inhibitor derivatives to improve upon the bioavailability and efficacy of the base SKI1 inhibitor template.



The application of modern technologies to a refined reaction scheme yielded inhibitors in less than half the time of previous syntheses; the design and implementation of several new purification techniques provided inhibitors at purities previously unattainable. Furthermore, a modified reaction scheme has been applied to construct Compound 1J. Finally, a total utilization of modern technology including IR Spectroscopy, one- and two-dimensional Nuclear Magnetic Resonance experiments and other common characterization techniques, has allowed for the complete characterization of "SKI-1", the most promising template identified by Smith *et al.* These analytical techniques have been applied to a myriad of novel Zone "4" derivatives in an attempt to understand and optimize the inhibition and effectiveness of the target enzymes.

This project is supported by a NIH INBRE grant from the National Center for Research Resources and by the Winthrop University Department of Chemistry, Physics, and Geology.

Examining the Effects of Zinc on Amyloid-β Dimers

Rebecca Mitchum (2012) & Iris Strzyzewski (2013) **Mentor: Robin K. Lammi**

Amyloid-β (Aβ) is a self-associating protein linked to Alzheimer's disease that is comprised of 39-43 amino acids. While it was previously believed that the onset of symptoms was caused by large plaques containing amyloid fibrils, recent studies have shown that Aβ oligomers as small as dimers and trimers are more closely related to disease progression. However, there is little experimental data showing the structures of these oligomers. We have used Förster Resonance Energy Transfer (FRET) between donor- and acceptor-labeled Aβ peptides to characterize dimer structures, both in bulk solution and in individual, surface-tethered dimers. The efficiency of FRET can be calculated using equations 1 and 2 below. Here, E_{FRET} is the energy-transfer

$$E_{FRET} = 1 - \frac{\tau_{DA}}{\tau_D} \qquad (1)$$

$$E_{FRET} = \frac{F_A - x(F_D)}{F_A + F_D(\frac{S_A}{S_D}\frac{\Phi_A}{\Phi_D})} \qquad (2)$$

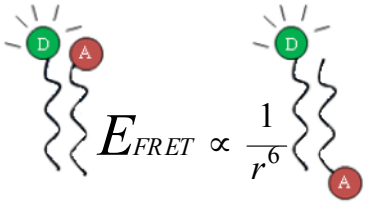


Figure 1. FRET efficiency is used to probe dimer structure. E_{FRET} depends sensitively on the distance (r) between donor and acceptor dyes (D and A).

efficiency, τ_{DA} is the measured lifetime of the donor (HF488AβB) in the presence of the acceptor (HF594Aβ), τ_D is the measure lifetime of the donor alone, and F_D and F_A are the measured fluorescence intensities of the donor and acceptor, respectively. S_D and S_A are the sensitivities of the two detectors, and Φ_D and Φ_A are the donor and acceptor quantum yields. The equations produce efficiency values between 0 and 1, which are directly related to the distance between the dyes and provide insight into dimer structure.

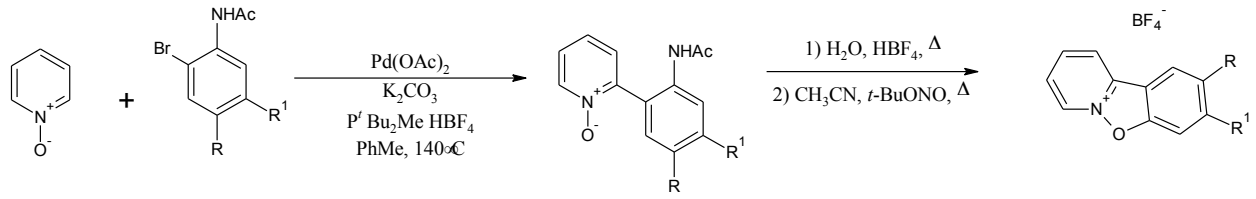
Two characteristic FRET efficiency values have been revealed by both bulk-solution lifetime and single-dimer studies of dimers in the presence of equimolar zinc, indicating that the presence of zinc may allow these dimers to exist in two preferred structures. A comparison study of dimers in the absence and presence of zinc is in progress, to determine whether the presence of zinc affects the observed FRET efficiencies (E_{FRET}) and dimer structures. Ongoing research is also examining the effects of the zinc chelator, 5-chloro-8-hydroxy-7-iodoquinoline (Clioqinol). Clioqinol has previously been studied as a possible therapeutic for Alzheimer's patients; we are interested to determine whether its addition fully or partially reverses any effects of zinc on dimer structures.

This project is supported by a grant from the National Science Foundation (CHE-0848824), as well as an NIH INBRE grant from the National Center for Research Resources (SC-INBRE).

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

Jeffery Myers (2012) **Mentor: James M. Hanna, Jr.**

Benzofuropyridines are the substructures of many pharmaceutical chemicals that have been shown to contain activity against cancer, tuberculosis, osteoporosis, and HIV. Related to these benzofuropyridines are the lesser known benzisoxazolo[2,3-*a*]pyridinium tetrafluoroborates. With only three of these compounds known prior to our work, we became interested in developing a general synthesis of these potentially useful compounds. Research by Abramovitch and coworkers showed that these compounds could be synthesized through an intramolecular phenylation of a 2-(2-aminoaryl)pyridine *N*-oxide. A recent discovery by Fagnou et al. hinted that the starting material required for this reaction could be synthesized by the direct arylation of pyridine *N*-oxide at the 2-position with an *o*-bromoacetanilide. Model studies with bromoanisole led to the observation that the best conditions for the arylation involved microwave heating of pyridine *N*-oxide, *p*-bromoanisole, a phosphonium tetrafluoroborate and potassium carbonate in toluene solvent. This led to 90% conversion of the *p*-bromoanisole to desired product (determined by ^{1}H-NMR). Application of these conditions to the direct arylation of pyridine *N*-oxide with 2-bromo-4-methylacetanilide gave the desired product ((2-acetamino-5-methylphenyl)pyridine *N*-oxide) in an average yield of 35% (after chromatography). In an attempt to improve this yield, multiple ligands and ligand-to-catalyst ratios were screened. This screening found that a 3:1 ligand to catalyst ratio using di-*t*-butylmethylphosphonium tetrafluoroborate and palladium acetate gave an 80% yield (determined by ^{1}H-NMR using an internal standard). We determined the best conditions to be 4 equiv pyridine *N*-oxide, 1 equiv bromoacetanilide, 5 mol % $Pd(OAc)_2$, 15 mol % $^t Bu_2MeP\text{-}HBF_4$, 2 equiv K_2CO_3, in toluene at 140°C for 1 hour using microwave heating. These conditions were then applied to a large scale synthesis, which gave an isolated yield of 65%. This compound was then subjected to hydrolysis, diazotization, and cyclization to produce 2-methylbenzo[4,5]isoxazolo[2,3-*a*]pyridine-6-ium tetrafluoroborate in a 72% yield. This newly developed process, seen in the scheme below, has been used to produce 8 other differently substituted benzisoxazolo[2,3-*a*]pyridinium tetrafluoroborates. The direct arylated products (**3**) were isolated in yields ranging from 45-76%. These products were then converted to the benzisoxazolo[2,3-*a*]pyridinium tetrafluoroborates (**4**) in yields from 71-86%. Future work will involve the application of this method to the synthesis of other novel heterocyclic ring systems and, in collaboration with Dr. Takita Sumter, evaluation of these novel compounds as anti-cancer agents.



1 **2** **3** (45-76%) **4** (71-86%)

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

Evaluating the Impact of Substitutions at Arginine 25 of the High Mobility Group A1b (HMGA1b) Function

Ronald Nelson (2011), Amy Deng (2012) **Mentor: Takita Felder Sumter**

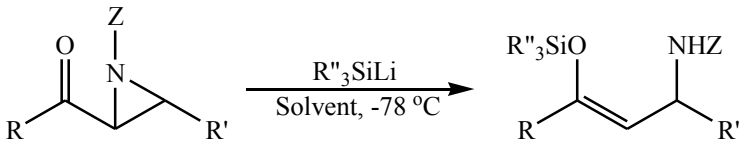
The High Mobility Group A1 (HMGA1) proteins, HMGA1a and HMGA1b, are members of one sub-family of non-histone chromatin binding proteins that orchestrate cellular transformation *in vitro* and *in vivo*. However, the specific mechanisms by which HMGA1 proteins contribute to cancer initiation and progression are not fully defined. The proteins perform key roles in molecular processes including cellular proliferation, differentiation, and apoptosis by binding to various DNA-related substrates including the minor groove of AT-rich duplex DNA structures. Post-translational modifications of HMGA1a and HMGA1b in various tumor types have been widely reported and mounting evidence indicates that these post-translational modifications are important in the progression of HMGA1-dependent tumors. Further, certain modifications occur within highly conserved regions of the protein and are capable of modulating HMGA1 function. In particular, the enzyme, protein arginine methyltransferase 1(PRMT1) methylates arginine 25 (R25), although the significance of this methylation to the protein's oncogenic capacity is unclear. Previously, we evaluated the importance of R25 in binding a previously established A-T rich HMGA1 binding sequence within the *kit ligand* promoter. Conversion of arginine 25 (R25) to alanine (HMGA1b R25A) significantly reduced the protein's ability to bind the target by 40% in our *in vtiro* DNA binding assays. Interestingly, Hmga1b bearing a lysine substitution (HMGA1b R25K) was also unable to bind the *kit ligand* promoter despite the presence of the basic side chain. In the current study, we have generated cell lines overexpressing HMGA1b and the corresponding HMGA1b R25K variant to evaluate the impact of this substitution on the protein's cellular function. We first evaluated the impact of this substitution of cellular proliferation using the MTT assay. The integrity of the MTT assay was confirmed by measuring the absorbance at 550nm using varying cellular amount and subsequent studies showed that ectopic expression of HMGA1b R25K reduced cellular proliferation two-fold when compared to wild-type HMGA1b. Moreover, anecdotal observations of cellular growth rates support this finding. These findings suggest that R25 may orchestrate the formation of Hmga1-DNA complexes commonly formed during cancer initiation and progression provide the basis for continued characterization of novel R25 variants. Ultimately, our work will provide additional insight into the target genes and cellular circuits associated with HMGA1-induced cancers providing critical information for the development of more effective cancer therapies.

Support was provided by National Institutes of Health Grant Numbers P20 RR-016461and 1R15CA137520-01 (to TFS) and from the Winthrop University McNair Scholars Program

Formation of Proximal β-Amino Silyl Enol Ethers from 2-Acylaziridines Using Silyllithium Reagents

Jennifer Schofield (2012) **Mentor: Aaron M. Hartel**

Silyl enol ethers are important synthetic building blocks which can be used in the preparation of other more highly substituted and functionalized molecules. Of particular importance is the Mukaiyama aldol addition between a silyl enol ether and an aldehyde catalyzed by a Lewis acid. We are now developing an indirect method for the stereo- and regioselective preparation of proximal β-amino silyl enol ethers from 2-acylaziridines using silyllithium reagents. These functionalized silyl enol ethers have great potential utility in the synthesis of biologically important compounds, such as pharmaceuticals.



The proposed mechanism of this preparative method involves nucleophilic attack of the silyllithium reagent on the carbonyl followed by a carbon-to-oxygen migration of the silyl group (Brook rearrangement) with subsequent opening of the aziridine ring. In order to determine the ideal conditions for the reaction, several *N*-substituted 2-benzoyl-3-methylaziridines were reacted with silyllithium reagents in different solvents. The use of methldiphenylsilyllithium in toluene at -78°C was found to give the desired proximal β-amino silyl enol ethers. Further experimentation will be used to develop optimized conditions which will then be applied to several differentially substituted substrates to determine the scope and limitations of the method.

This project was supported by the American Chemical Society Petroleum Research Fund, Grant Number 47986-B1. Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund for support of this research.

Crystallographic Structure Determination of *Clostridium papyrsolvens* C71 Xylanase: Molecular Replacement using Homology Models

Kennon Smith (2012) **Mentor: Dr. Jason C. Hurlbert**

The enzyme CpC71 is a xylanase belonging to Glycosyl Hydrolase (GH) Family 30 cloned from the anaerobic bacterium *Clostridium papyrosolvens.* This enzyme has the ability to bind and hydrolyze beta-1,4-xylan chains bearing arabinose and glucuronic acid substitutions. This feature makes the enzyme of potential value in biocatalytic processes designed to breakdown woody biomass and agricultural waste for conversion to ethanol. The enzyme is only the third xylanase of family GH30 characterized structurally, and previously described structures of *Erwinia chrysanthemi* Xylanase A (XynA) and *Bacillus subtilis* Xylanase C (XynC) are specific to 4-O-methylglucuronate (MeGA) substitutions (PDB entries: 1NOF and 3GTN, respectively), with little tolerance for arabinose substitutions. CpC71's novel specificity garners particular interest in solving the enzyme's structure to determine differences in its binding mechanism. The primary focus of our work was to produce a viable initial molecular replacement (MR) phasing solution for the enzyme using lowest energy homology models. We have previously created homology models of CpC71 using an approach involving two different computational algorithms and used the top five models generated in the phasing process to obtain an initial MR solution. The structure was solved to 2.01 angstroms and the active site was compared to those of XynA and XynC. Structural data indicate three primary mutations to the arabinose-binding subsite along the β8-α8 loop. This initial structure will serve as a basis for mutagenic and functional studies to investigate the role of the putative arabinose binding subsite in the enzyme.

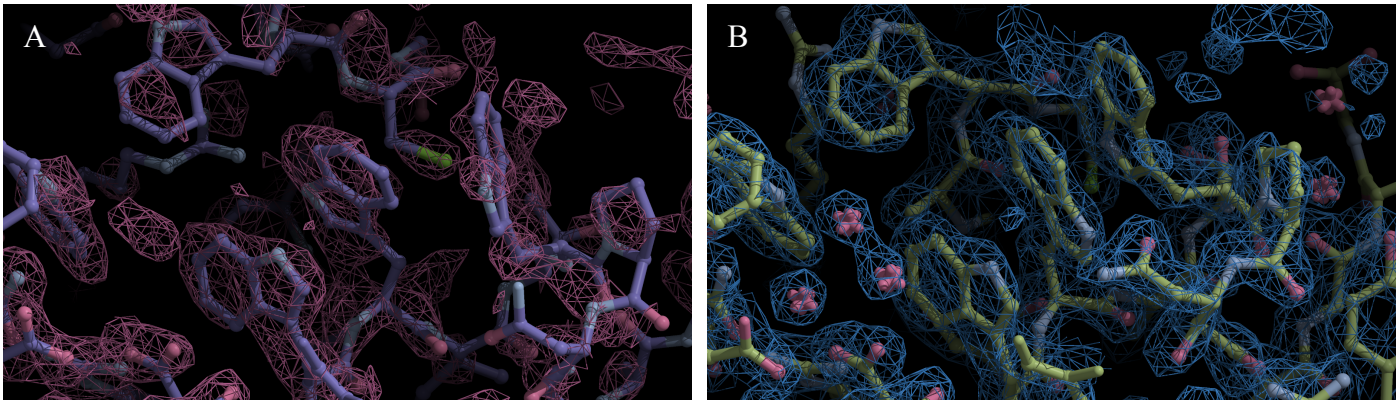


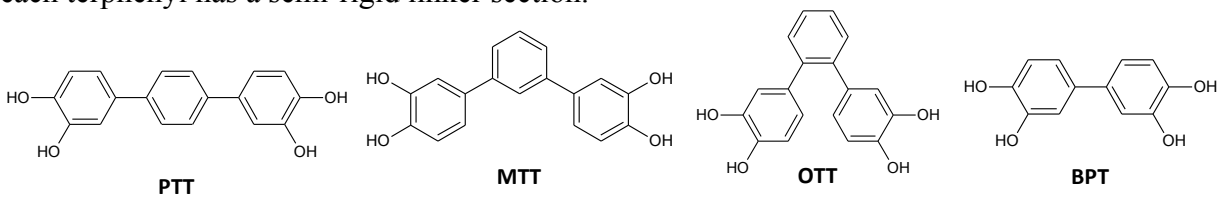
Figure 1: Comparison of initial electron density map and initial model with final electron density map and final model upon completion of rebuilding and refinement. In Panel A, the initial map and model are shown. Note the poor fit between the two. In Panel B, the final, rebuilt and refined model and electron density map are shown. The electron densities corresponding to solvent oxygen atoms and the densities of both main- and sidechain amino acid atoms are clearly observable.

This project is supported by a NIH INBRE grant from the National Center for Research Resources

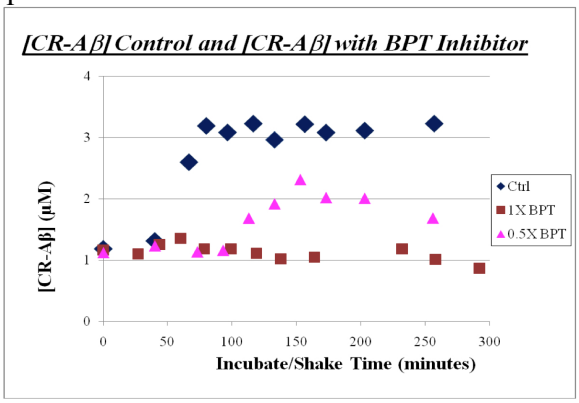
Synthesis and Evaluation of Potential Amyloid-β Aggregation Inhibitors

Craig Stevens (2012) **Mentors: Robin K. Lammi and James M. Hanna, Jr.**

Amyloid-beta (Aβ) is a protein found in human brain plasma and cerebrospinal fluid comprised of 39 to 43 amino acids. It is known to assemble into fibrils containing beta-sheet structure, which are the primary component of the extracellular senile plaques characteristic of Alzheimer's disease. It may be therapeutic to inhibit the aggregation of Aβ so as to block fibril formation. Some natural aromatic molecules, such as resveratrol and curcumin have been shown to inhibit Aβ aggregation. We have synthesized four potential inhibitor molecules – the *para* (PTT), *meta* (MTT) and *ortho* (OTT) isomers of terphenyl-3,3″,4,4″-tetrol and biphenyl-3,3',4,4'-tetrol (BPT). Each molecule contains terminal hydroxy groups to enable hydrogen bonding, and each terphenyl has a semi-rigid linker section.



Evaluation of the inhibitor molecules was made using the Congo Red Spectral Shift Assay, with incubation and shaking of the Aβ. Congo red (CR) binds selectively to Aβ containing beta-sheet secondary structure, resulting in a red shift of its absorbance spectrum. Starting with monomeric Aβ, the aggregation profile is tracked by measuring the CR spectral shifts at specific time intervals, thus enabling quantification of the extent of aggregation. Time courses of Aβ aggregation in the presence of inhibitor are compared to those for Aβ alone to determine the extent of inhibition. Inhibitor addition can typically increase lag time, i.e., the time at which aggregation begins, cause aggregation to proceed at a slower pace and/or reduce the maximum degree of aggregation at equilibrium.



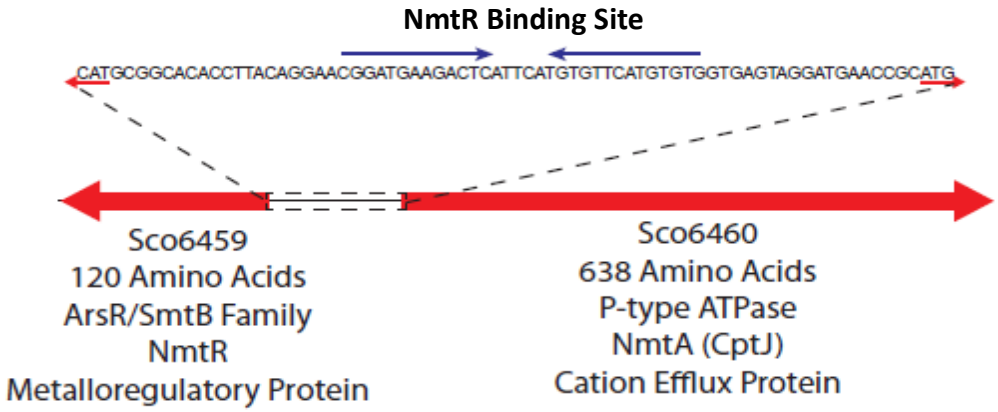
PTT, MTT and OTT provide no inhibition at one molar equivalent. BPT was found to provide complete aggregation inhibition at one molar equivalent, and was found to provide dose-dependent inhibition at sub-stoichiometric equivalents. Future work includes the synthesis and evaluation of other BPT derived inhibitors.

This project is supported by a NIH INBRE grant from the National Center for Research Resources and by the Winthrop University Department of Chemistry, Physics and Geology.

Cloning of Putative Nickel Sensors in *Streptomyces coelicolor*

Becca Toor (2012) **Mentor: Dr. Nicholas Grossoehme**

Streptomyces coelicolor is a gram positive, soil dwelling bacterium that is consumed by humans as a part of a regular diet. The *Actinomyces* genus, of which *S. coelicolor* is a member of, is responsible for producing a majority of the naturally derived antibiotics in use today and it has recently been shown that antibiotic biosynthesis is coupled to metal regulation. In large, the metalloregulatory network of this organism is still unidentified. The goal of this project was to establish the identity of the nickel sensor responsible for nickel efflux in the *Streptomyces* genome and to construct the gene using a multi-round Polymerase Chain Reaction (PCR) based synthetic strategy. Using a bioinformatics approach, the gene SCO6459 was identified as a candidate nickel efflux sensor. Coincidentally, this gene is adjacent to SCO6460, a potential nickel efflux protein. These genes are separated by a 69 base pair intergenic region that contains a very attractive palindrome that we believe is the site of NmtR binding.



Putative Nickel Sensing Operon for Streptomyces coelicolor

Using the *nmtR* gene sequence as a guide, we designed a set of five PCR primers that ultimately allow us to construct the gene through primer elongation. By customization of the primers, the five steps of PCR was utilized to generate an increase in base pair size to an eventual purified replica of SCO6459.

Agarose Gel Result: Representation of the Increase in Size for PCR Steps 1-3

To date, we have designated SCO6459 as the nickel efflux sensor in *S. coelicolor* and have successfully completed step three of the PCR synthetic strategy and are determined to complete construction of SCO6459.

Funding Provided by SC-INBRE II

Protein Purification and Determination of Metal-Binding Affinities of a Nickel-Uptake Regulator in *Streptomyces coelicolor*.

Paisley Trantham (2012) **Mentor: Dr. Nicholas Grossoehme**

The Streptomyces species is a soil-dwelling, gram-positive bacterium that is particularly notable for its ability to naturally synthesize a vast number of antibiotics. *S. coelicolor* Nur, Nickel-Uptake Regulator of the Fur family of metalloregulatoryproteins, plays a critical role in nickel homeostasis and contributes to the organism's antioxidative stress response. The crystal structure of Nur illustrates the presence of two metal-binding sites, one of which is located in the hinge loop region, and the other at the interface of the dimerization and DNA-binding domains.

Figure 2: Crystal structure of *Sc*Nur in active conformation

Based on a putative functional model suggested for Fur family members, binding of a regulatory metal to a site in the interdomain hinge loop region causes an allosteric transition from the inactive conformation to the active, DNA-binding competent conformation of the protein. As a transcription factor, binding of Nur to a promoter region of DNA prevents transcription from occurring, resulting in the lack of production of nickel-uptake machinery. DNA-binding activity of Nur is regulated by the occupation of the two metal-binding sites by zinc and nickel, as predicted based on the crystal structure. Wild-type Nur was purified using Fast Performance Liquid Chromatography and metal-binding affinities were assessed using UV spectroscopy and isothermal titration calorimetry.

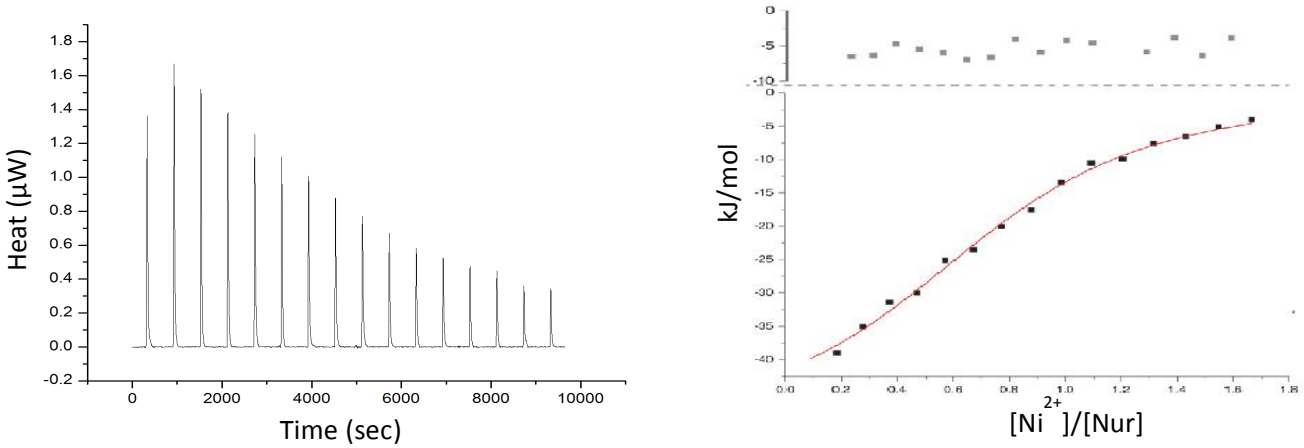


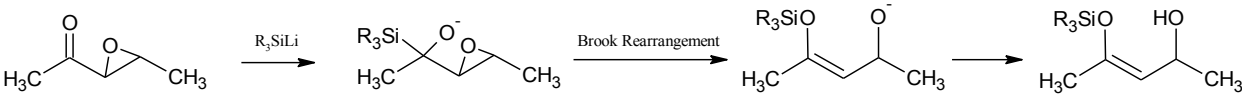
Figure 2. A) ITC titration Raw Data. Titration of 250µM Ni into 50µM apo-Nur in 25mM Tris pH 8 and 100mM NaCl. 3 µL Injections were made every 400 seconds. **B) ITC titration Independent Fit.** Titration of 250µM Ni into 50µM apo-Nur in 25mM Tris pH 8 and 100mM NaCl. 3 µL Injections were made every 400 seconds.

Funding provided by SC-INBRE and Research Corporation for Scientific Advancement Award 20160.

Synthesis and Purification of β-Hydroxy Silyl Enol Ethers Prepared from Epoxy Ketones and Silyllithium Reagents

Amber Wallace (2012) **Mentor: Aaron M. Hartel**

Silyl enol ethers are useful intermediates in reactions such as the Mukaiyama aldol reaction. New methods of synthesizing silyl enol ethers have been successfully developed in our lab using various silyllithium reagents, primarily methyldiphenylsilyllithium. These reactions involve the nucleophilic addition of the silyllithium reagent to a carbonyl followed by the Brook rearrangement. We have recently applied this concept to the reaction of silyllithium reagents with α,β-epoxyketones. In this system, the Brook rearrangement triggers the opening of the adjacent epoxide ring to form the β-hydroxy silyl enol ether. Thus, the treatment of α,β-epoxyketones with silyllithium reagents in toluene at -78°C led to the desired product in moderate to high conversions.



While β-hydroxy silyl enol ethers formed from aryl α,β-epoxyketones can be purified using standard silica gel column chromatography, β-hydroxy silyl enol ethers formed from alkyl α,β-epoxyketones proves problematic under the same conditions. Silyl enol ethers, particularly those formed from alkyl ketones, are known to be sensitive to acid-catalyzed hydrolysis. Thus, the acidic nature of silica gel can promote the decomposition of these sensitive compounds during their purification. In order for this reaction to be useful, a viable means of purification must be determined.

We have investigated two possible solutions to the problem of product decomposition: the alteration of the silyl group and the neutralization of the silica gel. Changing the methyldiphenylsilyl group to the bulkier and less acid sensitive *t*-butyldiphenylsilyl group could diminish acid-promoted decomposition during purification. Additionally, the neutralization of the silica gel by various means should have a similar effect. It was ultimately determined that pre-treatment of the silica gel with triethylamine (TEA) prior to column chromatography resulted in significantly higher recovery of the desired product. Under these conditions, the *t*-butyldiphenylsilyl enol ethers were more stable than the methyldiphenylsilyl enol ethers and could be recovered in much greater yield.

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

Investigating Environmental Influences on the Morphology and Stable Isotope Changes in a Holocene Peat Deposit from Congaree National Park

Nicole Wesselschmidt **Mentor: Dr. Scott Werts**

Congaree National Park, near Columbia, South Carolina, is a floodplain forest that contains the United States' largest contiguous track of old-growth bottomland forest as well as one of the highest natural canopies that remains on Earth. A portion of the park contains a groundwater fed muckswamp and is in the footprint of several ancient meanders of the Congaree River. Within one ancient meander lies an extensive peat deposit assembled over thousands of years from the formation of an oxbow lake. The aim of this study is to investigate the environmental influences affecting the development of this peat deposit and implications on paleoclimate investigation of the southeastern US since the last ice age. From this deposit, a 3.5 meter long core was extracted with a basal date of 21,000 years before present (kyr). We have sampled this core for $\delta^{13}C$ and $\delta^{15}N$ values in both the bulk peat and in monocot macrofossils which were present through most sections of the entire core. The bulk peat was sampled at key changes in morphology and the monocot samples were taken at 8 cm intervals. This data reveals a sawtooth pattern of variation of about 1‰ throughout the lower two meters (-28.5 to -29.5‰) before a much more negative excursion down to -30‰ in the last half a meter. We have also obtained several radiocarbon dates indicating that the initial oxbow lake filled within approximately 1000 years followed by a depositional hiatus or erosional surface occurring between 10,000 and 20,000 kyr. This was followed by a period of slow peat accumulation through 3500 kyr. In more recent years (<160 yrs), an inorganic lens has developed within the upper 10 cm of the core (Figure 1). Further investigation into this section of the core suggest the possibility of vertical leaching and subsequent removal of organic material from this section. Results from this study have implications in further investigations into carbon cycling and storage within Congaree National Park.

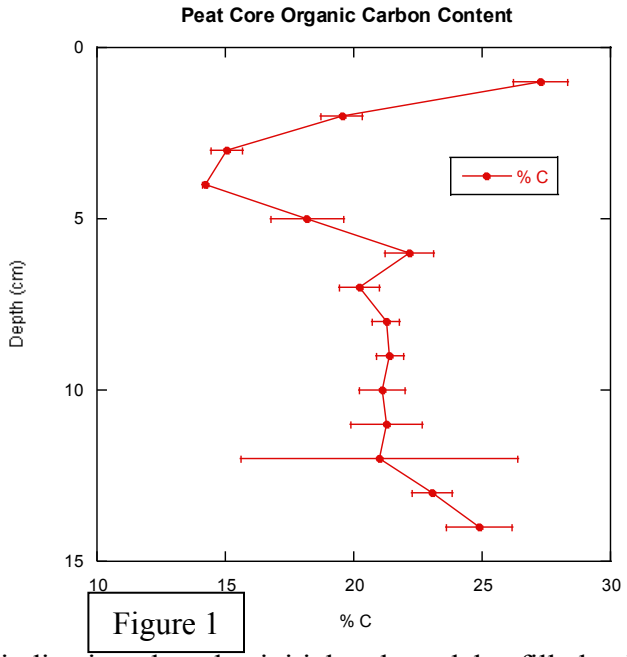


Figure 1

This project was supported by a grant from the Winthrop University Research Council and a grant from the Dalton Endowment

Analysis of Endomycorrhizal Fungal Spores at Elevated Temperatures from Semi-Arid Arizona Soils; Applications to Geoarcheology

Mark White **Mentor: Dr. Scott Werts**

The objective of this study is to investigate the elemental structure of endomycorrhizal fungal spores at temperatures up to 500°C in hopes of establishing a correlation between elemental abundances and fire temperature. Prior research of endomycorrhizal spores from a deciduous forest in Maryland has shown that these spores are structurally resistant to high temperatures and show an increase in the percentage of silicon and aluminum when exposed to increasing temperatures. In this study, we are attempting to investigate whether a similar trend is present in endomycorrhizal fungal spores from arid environments and to possibly apply these results to archeological studies. In the high plains of Arizona, there are numerous archeological sites in which structures from Hopi settlements burned in the 14th century. The potential causes of these fires are still being debated. We collected samples from a structure within Homolovi State Park that was burned in 2006 and designed to simulate these fire events (Figure 1). Samples were collected at 1 cm intervals within individual burned layers in order to investigate any potential changes in chemistry due to changing temperature during the fire. Figure 2 displays the various layers sampled: Rf is composed of the collapsed roof of the structure; Ox is composed of a highly oxidized layer of sediment; Cb is composed of charred biomass compiled on the original surface of the floor; Sf is the original sediment floor of the structure. Several of these samples will be analyzed in a Scanning Electron Microscope (SEM) coupled with Energy-dispersive X-ray Spectroscopy (EDS) to gain insight into their elemental composition. These samples will then compared to the elemental composition of modern fungal spores collected from the same area which were burned at increasing temperatures up to 500°C within the laboratory. Results from this study have implications in the development of a post-fire temperature proxy in both modern and archeological settings.

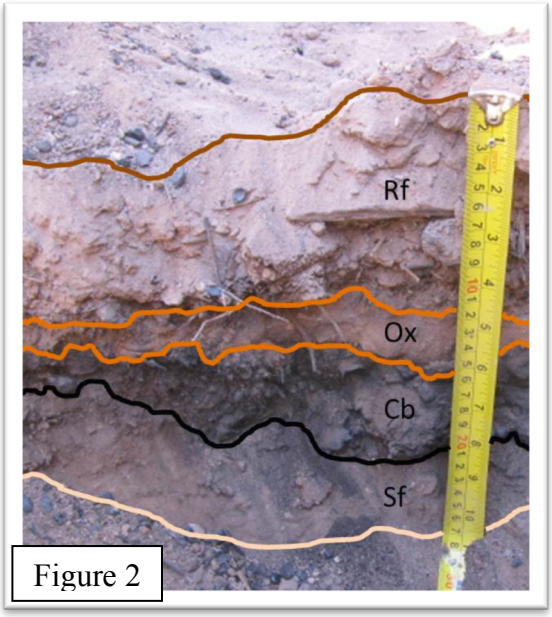


Figure 2

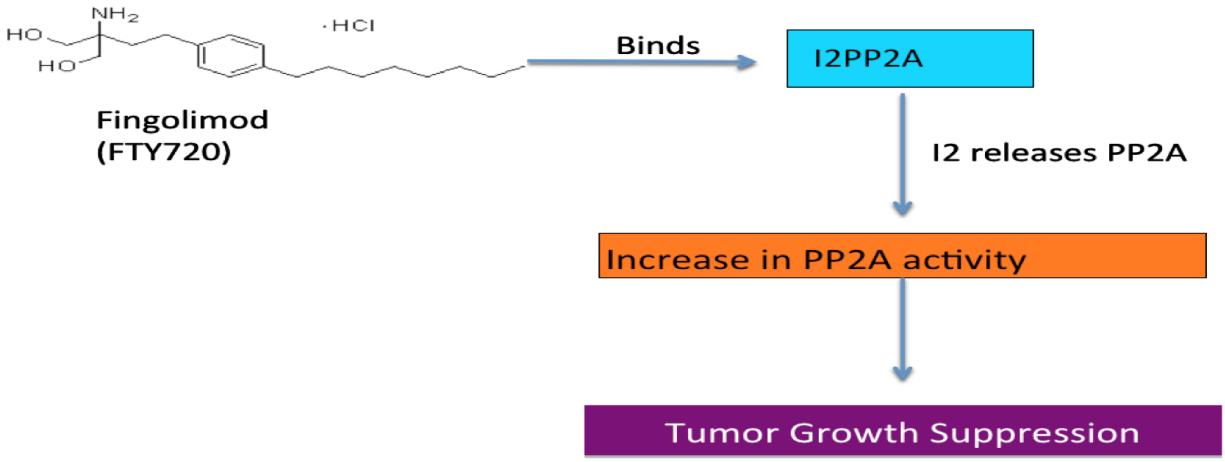

Figure 1

Funding for this research was provided by a Research Council grant and a grant from the Dalton Endowment.

The Effect of Fingolimod (FTY720) on Small Cell Lung Cancer and Non-Small Cell Lung Cancer

Jessica S. Creel (2012) **Mentor: Dr. Besim Ogretmen (MUSC)**

Lung cancer is the second most diagnosed cancer, respectively following prostate and breast cancer. The two main types of lung cancer that exist are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Fingolimod (FTY720) was recently approved by the FDA in August of 2010 for treatment of multiple sclerosis; however, evidence shows that it may have anti-cancer effects. FTY720, a sphingolipid analog for ceramide, counters tumor growth by binding to inhibitor 2 of PP2A (I2PP2A), which is over expressed in lung cancer tissues. The interaction between I2PP2A and FTY720 causes the release of I2PP2A from PP2A, allowing PP2A to dephosphorylate, ultimately resulting in tumor suppression. The purpose of this study is to show that the treatment of lung cancer with Fingolimod (FTY720) will lead to activation of PP2A tumor suppressor signaling by binding/targeting I2PP2A.



In this study, FTY720 is used to treat both H1341 SCLC and A549 NSCLC at various doses, which indicates the IC50 to be 10μM for each cell line. The cell death pathway used by this drug is examined using H1341 SCLC and Necrostatin, a necrotic cell death inhibitor, in combination with FTY720, resulting in a 33% protection from necrotic cell death. Okadaic Acid, a PP2A inhibitor, is also used to treat the cells, resulting in a 30% protection from FTY720, proving that PP2A is utilized in the mechanism with FTY720 and lung cancer cells. The addition of an animal study tests the effect of FTY720 on tumor growth *in vivo* in NSCLC. For this animal study, 2×10^6 A549 NSCLC cells are injected into the flanks of 8 Severe Combined Immunodeficiency (SCID) mice. There are 4 mice in each group: control and 10mg/kg FTY720. The mice are treated orally and their tumors measured for 14 days following their initial tumor development. As the study progresses, the tumors in the control group become significantly bigger than those treated with FTY720. Current studies in progress suggest that FTY720 does promote cell death, and therefore suppresses tumor growth, in both SCLC and NSCLC.

Support provided by College of Graduate Studies and Hollings Cancer Center at the Medical University of South Carolina

The influence of stormwater management practices on denitrification rates of receiving streams in an urban watershed.

Melinda S Cronenberger (2012) **Mentor: Sara K McMillan Ph.D, P.E. (UNCC)**

Increasing urbanization and the subsequent disruption of floodplains has led to the need for implementing stormwater management strategies to mitigate the effects of urbanization, including soil and streambank erosion, increased export of nutrients and contaminants and decreased biotic richness. Excessive stormwater runoff due to the abundance of impervious surfaces associated with an urban landscape has led to the ubiquitous use of best management practices (BMPs) to attenuate runoff events and prevent the destructive delivery of large volumes of water to stream channels. As a result, effluent from BMPs (i.e. wetlands and wet ponds) has the potential to alter the character of the receiving stream channel and thus, key ecosystem processes such as denitrification.

The purpose of this study was to determine the extent to which BMPs, in the form of constructed wetlands and wet ponds, influence in-stream denitrification rates in the urban landscape of Charlotte, NC. Four sites, two of each BMP type, were evaluated. Sediment samples were collected upstream and downstream of the BMP outflow from May-July 2011 to determine the effect of discharge on in-stream nitrogen removal via denitrification. Denitrification rates were determined using the acetylene block method; water column nutrient and carbon concentrations and sediment organic matter content were also measured.

Generally, wetland sites exhibited higher denitrification rates, nitrate concentrations and sediment organic matter content. Our work and others has demonstrated a significant positive correlation between nitrate concentration and denitrification rates, which is the likely driver of the higher observed rates at the wetland sites. Geomorphology was also found to be a key factor in elevated denitrification rates at sites with riffles and boulder jams. Sediment organic matter was found to be higher downstream of BMP outflows at all four sites, but demonstrated no significant relationship with denitrification rates. We are continuing to investigate these spatial (e.g. BMPs, streams) and temporal (e.g. storm pulse, delayed wetland release) patterns, particularly in the context of factors that influence the specific drivers of denitrification. Understanding these patterns is critical to managing stormwater in urban landscapes as we aim to improve water quality while enhancing ecosystem functions.

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Genome Scale RNAi Screen Identifies "Achilles Heels" of Wnt-active Cancer Cells

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A systematic approach to characterize cancer vulnerabilities is to identify genes that are essential for cancer cells. Using this information to find genes that are important for cancer cells with a unique genomic alteration will contribute to the effort in identifying potential therapeutic drug targets of cancer. The recent genome scale high-throughput RNAi screen conducted by our laboratory has identified essential genes for the survival and proliferation of 102 cancer cell lines. Using statistical analysis we identified genes that are essential for Wnt/beta-catenin active cancer cell lines. The Wnt/beta-catenin signaling pathway is one of the most deregulated pathways in malignant cells and its role has been implicated in many types of cancer, especially colorectal cancers. However, it lacks an effective way to target and treat Wnt-pathway dependent tumors. This project is aimed to identify and validate genes that are crucial for the survival and proliferation of Wnt-dependent cancer cells.

One of our top hits for genes that specifically inhibit the proliferation of Wnt/beta-catenin active cell lines was FMS-like tyrosine kinase-3 (FLT3). FLT3 is a member of the class III receptor tyrosine family, which plays an important role in regulating hematopoietic cell survival and proliferation and has been implicated in acute myeloid leukemia (AML) by cooperating with the Wnt-signaling cascade. Other targets that the screen has identified include ring finger protein 1 (RING1) and transcription factor 2 (TCF2). Little is known about the role RING1 plays in cancer currently. Mutation of TCF2, a transcription factor associated with development and metabolism, has been observed in renal cell cancer and ovarian cancer. To validate these targets, we have first measured the mRNA and protein expression levels of FLT3, RING1 and TCF2 in 15 Wnt-active and Wnt-inactive colorectal cancer cells. Subsequently, we conducted loss-of-function phenotype studies using the short hairpin RNAs (shRNAs) targeting FLT3, RING1 and TCF2. Preliminary analysis showed decreased viability and proliferation in Wnt-active cells as a result of FLT3, RING1 and TCF2 knockdown. Ongoing efforts are aimed to elucidate the roles of FLT3, RING1 and TCF2 in Wnt-dependent cancer cells. These analyses can provide important information to further understand the function of the Wnt pathway in cancer and for finding drugs that will inhibit the growth of Wnt dependent tumors.

Funded in part by Grant HL089005 from the National Institutes of Health and the Division of Medical Sciences of Harvard Medical School

The Functional Significance of the Insulin-like Growth Factor Receptor in Adrenocortical Carcinoma

Christine Harvey (2012) **Mentors: Dr. Marion Sewer, Dr. Kai Cai (UCSD)**

Adrenocortical carcinoma is a rare but highly malignant disease. Though other pathways such as adrenocorticotropin (ACTH)-cAMP/protein kinase A and epithelial growth factor (EGF) regulate the proliferation of adrenal cancer cells, the insulin-like growth factor (IGF) pathway is the most studied. IGFI and IGFII are important proteins in cell proliferation with IGFI being expressed in adults and IGFII being expressed during early development. IGFI and IGFII modulate cell growth by binding to the IGFI receptor (IGFIR), a cell surface tyrosine kinase receptor. IGRII and IGFIR overexpression is characteristic of adrenocortical cancer progression. Our laboratory has previously generated an H295R IGFIR knockdown cell line ($IGFIR^{kd}$), which will be used to characterize the role of IGFIR in adrenocortical cancer development. The expression of several proteins involved in adrenal cancer, such as steroidogenic factor 1 and cytochrome P450α17, will be determined after cells are treated with IGFI and IGFII to examine the potential of IGFIR as a target for cancer therapy. mRNA and protein expression levels will be compared between H295R and $IGFIR^{kd}$ cells. Additionally, the role of IGFIR in modifying cell was investigated. Finally, the effect of IGFIR knockdown on the secretion of cortisol and dehydroepiandrosterone (DHEA) was assessed.