Winthrop University
Summer Undergraduate Research Experience (SURE)
2023 Abstract Book

WINTHROP UNIVERSITY

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Promoter Methylation Contributes to RYBP Down-Regulation in Glioblastoma Cells

Michelle Aguilar-Gaspar (2024) **Mentor: Daniel B. Stovall, Ph.D.**
Emi Umemoto (2026)

Glioblastoma multiforme (GBM) is a deadly cancer of the central nervous system with a median survival of under 15 months. Dysregulation of gene expression is a major driver of GBM progression. RING1- and YY1-binding protein (RYBP), a member of the Polycomb family of chromatin modifying transcription factors, is crucial in regulating gene expression and maintaining cell identity. However, approximately 50% of GBM patients have reduced RYBP expression, preventing it from exerting the tumor-suppressive effects it has been shown to have in multiple cancer types. Therefore, determining the pathways leading to aberrant RYBP silencing may offer insight into the development of more effective therapeutic strategies for glioblastoma. We hypothesized that methylation of the RYBP gene promoter contributes to the aberrant silencing of RYBP in GBM. U-118 and T-98 glioblastoma cells were treated with a DNA methyltransferase (DNMT) inhibitor, 5-aza-2'-deoxycytidine (5-aza), or DMSO vehicle for 72 hours. Then, protein and RNA were isolated and quantified using a Modified Lowry assay and Nanodrop, respectively. Western blot analysis showed increased RYBP expression in U-118 and T-98 cells at both mRNA and protein levels upon DNMT inhibition with 5-aza. Analysis of the RYBP promoter by methylation-specific PCR revealed the promoter was indeed directly methylated in both cell lines. Therefore, DNA methylation likely directly contributes to RYBP transcriptional silencing in GBM cells. Future objectives include replicating methylation PCR using additional primer pairs to assess the RYBP gene promoter's methylation status in various regions and investigating the impact of other epigenetic modifications on RYBP regulation.

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Antimagic and Magic Labeling-Type Properties of Water Wiggler Graphs

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Matt Brunet (2025)
Juan De Castro Cabrices (2026)
Matthew Feldmann (2027)

A graph with m edges is said to have a magic labeling if the edges can be labeled with distinct numbers from 1 to *m* in which the value of the edges on each vertex sum to the same number. This notion is a kind of generalization of the idea of a "magic square". Alternatively, a graph with m edges is said to have an antimagic labeling if the edges can be labeled with distinct numbers from 1 to *m* in which the value of the edges on each vertex sum to distinct numbers. Antimagic and magic labelings of graphs and all of their generalizations have been given an extensive amount of attention (see Sections 6 and 5, respectively, of *A dynamic survey of graph labeling* by Gallian). Our work focuses on a family of graphs we refer to as "water wiggler graphs". A water wiggler graph can be obtained by starting with a circle, placing *r* vertices on the circle, duplicating arcs of the circle between the *r* vertices as many times as you like, and finally placing vertices along each arc and duplicate arc (with at least one vertex per duplicate arc). In this research we obtain partial and in some cases complete results on whether or not graphs in this family have antimagic labelings, magic labelings, antimagic (*a*,*d*)-face labelings, magic face labelings, antimagic orientations, and magic orientations (with each result depending in some way on the parameters of the graph).

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Can Normal Be Abnormal-Normal?
A Special Case of the *T* -Family Distributions

Gavin Anderson (2024) **Mentor: Dr. Duha Hamed**
Kylie Zangla (2023)

The main aim of this research is to introduce a new flexible distribution which generalizes the normal distribution. The known statistical distributions have different characteristics, but they serve the same purpose of modeling data of different shapes and frequency. For example, the normal distribution is a popular distribution, known for its symmetric, unimodal shape and is often used to model symmetric data. When considering the normal distribution as a base, multiple generalizations of the normal distribution can be defined using the different generalization techniques. The purpose of generalizing distributions is to create more flexible distributions than the base distribution, to model data across a very wide range of applications. Within this paper, a new generalization of the normal distribution is proposed, namely the *T-Normal* {*Cauchy*} class of distributions, using the *T-R*{*Y*} Framework; where *T*, *R*, and *Y* are defined as random variables. For this new class of distributions, some mathematical properties were found. Additionally, three new members of this new class of distributions were studied in more detail. One specific member, the *Normal-Normal* {*Cauchy*} distribution, was applied to five different real-world data sets and its flexibility in fitting these data sets were compared to other competitive distributions. Our newly presented class of distributions is found to be more flexible than normal distribution and capable of fitting unimodal and bimodal data sets.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Investigation of Phage-Host Interaction Using Phenotypic Defense Assays

Alexis R. Atkinson (2026) **Mentor: Dr. Victoria Frost**
Lidia Peralta (2026)

Bacteriophages can destroy their hosts when the lytic cycle is completed, but interestingly, phage can also defend their hosts against further phage infection by producing proteins, often enzymes, to block surface receptors or destroy incoming phage competitors. They defend their hosts either homotypically, meaning against the same phage, or heterotypically, against a different phage, both representing types of superinfection immunity. During the summer of 2023, we investigated the genome of Mycobacteriophage ExplosioNervosa, originally discovered in Winthrop soil by a SEA student alum. Our research is an extension of the SEA-GENES Course Research Experience where each of ExplosioNervosa's genes are cloned to enable downstream investigations of their possible functions. We have been refining a phenotypic defense assay to systematically test select genes from ExplosioNervosa's genome for their defense activity. The genes tested were chosen at random, with the exception of ExplosioNervosa gene 75 that is predicted to be an immunity repressor. These proteins are understood to keep a phage in lysogeny and prevent the switch to the lytic life cycle. They have also been documented to play a pivotal role in defending the host against additional phage infection. We are now in the process of using this particular assay to test a number of ExplosioNervosa's genes, including gp75, to investigate their host defense potential. Measuring the effects of phage gene expression in this way will highlight more details about the processes involved during phage-host interaction.

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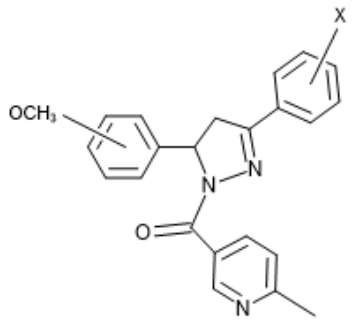
Synthesis of 4,5-dihydro-1H-pyrazole derivatives as BRAF inhibitors

Hannah Bendure (2025) **Mentor: T. Christian Grattan**

BRAF is a human gene that encodes the BRAF protein which stimulates cell growth. BRAF mutations are found in several types of cancer, most notably in 50-70% of melanoma cell lines and tumors. 90% of activating BRAF mutations in cancer cells are a valine to glutamic acid substitution at position 600 (BRAFV600E), which is approximately 500 times more active than the wild-type protein. The constitutive action of the mutated BRAF protein causes constant cellular growth and proliferation, therefore feeding tumor development. Many inhibitors of the mutated protein have been developed and are available as anti-cancer drugs or supplements to cancer treatment. Certain compounds are proven to treat human cancers, including 4,5-dihydropyrazole derivatives and nicotinic acids. Several studies have revealed dihydropyrazoles as good inhibitors for other proteins involved in cancer development. Nicotinic acids are currently used in intravenous cancer therapy with vitamin C and as sensitizing agents for chemotherapy. A promising inhibitor* in the research study on which this project is based contains both of these compounds and has a bioactivity comparable to that of existing inhibitors of BRAF. The purpose of this research is to synthesize similar 4,5-dihydro-1*H*-pyrazole derivatives while comparing the effectiveness of substitution geometry as well as different halogens (such as bromine, chlorine, and fluorine) relative to the starting structure. These inhibitory compounds will be analyzed using bioassay testing to assess their efficacy.



*X = Br, Cl, F

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the Winthrop University Department of Chemistry, Physics, Geology, and the Environment.

Effects of TiO_2 Nanoparticles on Goldfish (*Carassius auratus*) Upper Thermotolerance

Leliana Bohanan (2026) **Mentor: Dr. Salvatore Blair**

Titanium dioxide (TiO_2) nanoparticles can be found in a wide variety of products such as sunscreen, soap, and even foods such as M&Ms. Due to their widespread use, these nanoparticles often find their way to aquatic environments making them a potential threat to aquatic organisms, which has stimulated a surge in research efforts toward understanding the environmental implications of nanotechnology. Our initial objective was to see if TiO_2 inhibited fish's ability to cope with increasing aquatic temperatures as this could present a dual threat when considering the effects of climate change on water temperature. To perform the experiment, we subjected four groups of fish (n = 8) separated into non-injected, saline-injected, polyacrylic acid capsule injected, and TiO2 injected treatments to a critical thermal maximum (CT_{Max}) test and sampled them after loss of equilibrium (LOE). The LOE temperatures for each fish were recorded and an ANOVA test comparing each treatment group to the control group revealed that the TiO_2-injected fish demonstrated a significantly reduced thermal maximum compared to controls (p=0.0262). We now seek to find through what cellular mechanisms TiO_2 harms fish, by conducting molecular analysis of the heart, gills, spleen, kidney, and muscle samples to identify if gene expression was altered, and if so, then which genes are affected.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the U.S. Department of Education McNair Grant (P217A180094).

Uncovering the Diet of Meiofaunal Flatworms with Diagnostic PCR

Abby Bowers (2026) **Mentor: Julian P.S Smith III**

Little is known about the dietary interactions of meiofauna. Meiofauna such as marine meiofaunal flatworms are small invertebrates that live in soil and aquatic sediments. Direct observation is generally the method of choice when recording dietary interactions. However, visual observation of sand dwelling marine flatworms is limited due to the opaque nature of the substrate they live in. Additionally, removal of prey material from predator bodies is not possible due to their microscopic sizes. A molecular approach was used to resolve this issue. The main challenge was to determine a reliable method of prey DNA amplification in a predator DNA saturated environment. Prey specific primers were matched to inferred prey items to understand predator preference. This project was a continuation of a previous project in Dr. Smith's lab, which used prey-taxon- specific primers in PCR to amplify and identify the prey preferences of wild predatory meiofaunal marine flatworms at a species level. The expansion of this project will allow for the creation of the first detailed map of meiofaunal trophic interactions.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and by the SC Governor's School for Math and Science.

Unraveling the secrets of *Amphipora*: Variability in Devonian Marine Communities through space and time

Edward Carter (2025) **Mentor: Diana Boyer**

The Devonian Period (420-360 million years ago), was a time of abundant marine life. There were extensive reef environments, which were composed of different organisms and significantly larger than today. One of the leading players in these ancient reefs were sponge-like organisms called stromatoporoids, more specifically small dendroid organisms known as *Amphipora*. *Amphipora* lived in shallow marine environments and were locally abundant across Devonian reefs, however, not much is understood about their distribution across space and time. To evaluate variability in these extinct organisms, samples were collected from five localities in Utah and Nevada. Density of assemblages, as well as size and orientation data were collected to help characterize these populations. The orientation of individual branches of *Amphipora* are not aligned, suggesting that there was limited post mortem transport and that these rocks provide an approximation of ancient communities. These data also indicate that dense assemblages of *Amphipora* are relatively consistent across over 600 km of ancient shallow marine shelf. Notably, variation within localities is observed in measured density per meter values and average size based on width of branches. Work is ongoing to identify the types of *Amphipora* from these localities, toward understanding life position, and what other organisms co-inhabited these environments in order to unravel the secrets behind these ancient marine organisms.

Fig. 1. Image demonstrating Amphipora in rock sample. meter.

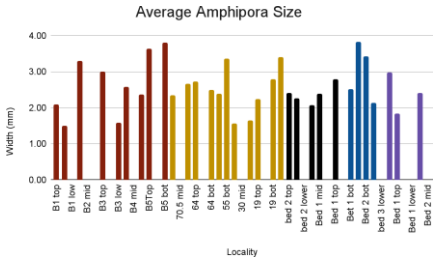


Fig. 3. Graph demonstrating avg. widths of Amphipora versus the density per 1

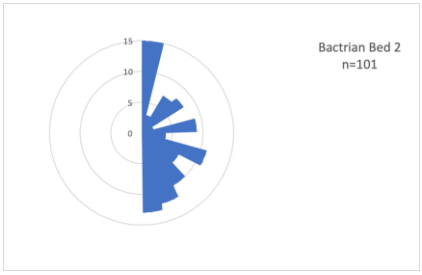


Fig. 2. Rose diagram demonstrating orientation of fossils preserved in branches limestone. No evidence of current alignment observed due to bars being dispersed

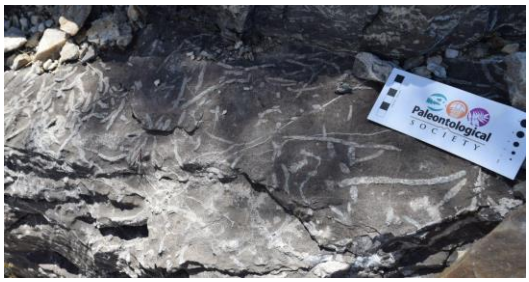


Fig. 4. Image demonstrating orientations of amphipora

Support was provided by an NSF-RUI grant (2044224), the Winthrop Irene Boland Geology fund, and a Winthrop Research Council Grant (SC23005).

Chick RGC and DRG Growth Cone Collapse Response to Semaphorin 3A

Layla GM Carver (2023) **Mentor: Eric Birgbauer**

Visual stimuli from the eye must travel long distances along retinal ganglion cell (RGC) axons in order to be processed in a tangible manner by the brain. RGCs are retinal neurons responsible for sending out axons that are essential for the formation of the optic nerve during neural development. These axons are tipped by growth cones, which are developmental subunits responsible for axon motility and guidance. Growth cones contain many receptors that will respond to both positive and negative guidance cues. *In vitro*, a repulsive cue will cause growth cone collapse. One repulsive guidance cue is semaphorin 3A. Previous studies have proposed that semaphorin 3A is a repulsive cue for chick dorsal root ganglion cell (DRG) growth cones, but not chick RGC growth cones. However, our lab has found that semaphorin 3A causes growth cone collapse in chick RGC growth cones. There are several hypotheses that could explain the differences in results between our lab and prior research. I am testing the hypothesis that there is a difference in sensitivity to semaphorin 3A between RGC and DRG growth cones. To test my hypothesis, I quantified the collapse of both RGC and DRG growth cones treated with the same range of concentrations of semaphorin 3A in vitro to create a dose response curve. Preliminary results show that semaphorin 3A creates a dosage dependent growth cone collapse response in DRGs. Semaphorin 3A induced collapse is evident in RGC growth cones but is not dosage dependent. From this we can suggest that semaphorin 3A may be a repulsive cue for both DRG and RGC growth cones. Differential growth cone sensitivity is not likely to be an explanation for the differences we have observed from previously published results.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) as well as Winthrop University. We also wish to thank Tyson Farms for donating eggs and all current and previous lab members.

Machine learning enhanced design of RNA-based fluorescent biosensors for the detection of the neurotransmitter dopamine

Eusebio Chamberlain (2023) **Mentors: Timea Fernandez**
Zach Abernathy

Dopamine (DA) is a neurotransmitter that plays a role in the regulation of physical and emotional well-being. Irregularities in DA production have been linked to several addictive behaviors such as *smoking*, *alcoholism*, and *obesity*, as well as neurodegenerative disorders like *Parkinson's disease*. Early detection of DA abnormalities is paramount for the effective diagnosis and treatment of these ailments, while real-time imaging of DA could assist in the comprehension of their underlying mechanisms. As such, our project aims to design a DA-sensing RNA-based fluorescent (RBF) biosensor for initial *in vitro* experimentation and characterization. Using existing platforms, we can fabricate RBF biosensors that combine a ligand-sensing RNA aptamer with a fluorescent RNA aptamer to indicate the presence of biologically relevant molecules. Previous studies have used electrochemical and protein-based biosensors in the detection of neurotransmitters; yet, to our knowledge, no studies have developed a viable RBF biosensor for the detection of DA *in vitro* or *in vivo*. To date, we have designed, transcribed, purified, and tested the dopamine detection of eight sensor variants.

To aid in the development of viable sensors our project utilized the assistance of machine learning algorithms based on other RNA sensor experiments. The 102 published RNA sensor sequences were cataloged based on the following characteristics: melting temperature of the entire sensor and the length, entropy, change in free energy, hydrogen bonds, and melting temperature of the transducer sequence that connects the dopa and Spinach 2 aptamers. These thermodynamic parameters along with the fluorescence fold increase were input into a decision tree classification model to predict which of these parameters is most influential in producing a good sensor. This dataset was further used to predict the efficacy of novel sensor designs. The distinction between good and bad sensors was made at a 2-fold increase and a classification tree was constructed using 102 sensors, the model accuracy was found to be around .76 as measured by the area under the curve of the Receiver Operating Characteristics or about 76% accuracy rating when the AI tested itself against the 102 sensors that it could test against.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499), Made in SC EPSCoR, NSF (1655740) and the Winthrop University College of Arts and Sciences.

The Expression, Purification, and Preliminary Characterization of a Putative Period 2 homolog found in *Isodiametra pulchra*

Lila Dailey (2026) **Mentor: Jason C. Hurlbert**

Isodiametra pulchra are microscopic marine worms found along the Eastern coast of North America. On the phylogenetic tree, they are placed at the base of the Bilateria branch, making them at least 540 million years old. Studies on aquatic mollusks have proven these organisms to have a circadian rhythm that coincides with the tidal patterns of the ocean. Behaviorally, *I. pulchra* are similar to these mollusks, as both must time their surfacing for air with the tides. This connection prompted us to explore the circadian rhythm of *I. pulchra* with the goal of dating the inheritance of an internal clock back to 540 million years ago. In mammals, the oscillatory nature is governed by transcriptional activators CLOCK and BMAL1 forming a heterodimeric complex and binding to the E-box. In target genes, this activates the expression of Period and Cryptochrome genes. Since Per2 and Cry2 are inhibitors of CLOCK/BMAL1-induced transcription, when enough accumulates in the nucleus, the cell ceases transcription and shifts to its night cycle. After Per and Cry have been used up as inhibitors, the CLOCK/BMAL1 complex goes back to transcription. We took the Per2 protein from rodents and found its homologous protein in *I. pulchra* to study the internal clock of the worm. The expression phase included growing cultures of *E. coli BL21* that had been genetically modified to produce Per2. We would subsequently harvest the cultures so as to examine what proteins had been produced. To purify the sample, we would run metal-chelating affinity chromatography (MCAC) and gel filtration on the fast protein liquid chromatography (FPLC). Between each step on the FPLC, we would run a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-GEL) to ascertain the current purity of the sample. After a relatively pure SDS-GEL had been reached, we would run a Western Blot to further determine the purity. Our goal was to create a solution with a high enough concentration of pure Per2 to be able to test its interactions with Cry2 via crystallography. Future research will test multiple variables to determine the best environment to foster interactions between the two proteins.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Progress Toward Understanding Cadmium Inhibition of Troponin Function

Morgan Dukes (2026) **Mentor: Dr. Nicholas Grossoehme**
Cierra Ari Randolph (2026)
Victoria Williams (2026)

Muscle contraction is a complicated mechanism that involves different protein complexes working synergistically to carry out and regulate the process. One such protein is the troponin complex, which serves as the link between brain signaling and muscle function. This protein is composed of three subunits: troponin-T, troponin-I, and troponin-C. Troponin-T anchors the rest of the complex to tropomyosin, a component of the muscle fiber. Troponin-I is responsible for inhibiting muscle contraction until a surge of calcium is recognized by troponin-C in response to a signal from the brain. Cadmium is a dangerous heavy metal that has carcinogenic properties and is known to impact cardiovascular function. Interestingly, cadmium can bind to troponin-C with equal or higher affinity than calcium. The aim of this research is to understand the impacts of cadmium on troponin function and its implications for muscle contraction. The project focuses primarily on the expression and purification of hexahistidine- or MBP-tagged TnC and TnI (switch peptide and 1-73 regions), respectively. Each construct has been purified to near homogeneity and initial binding experiments were carried out using gel filtration chromatography. We have collected evidence of calcium-loaded TnC binding to the 1-73 region of TnC using gel filtration chromatography. The low Kd for these interaction (> 200 μM) is likely prohibiting us from observing other interactions. The next step for this research is to scale up the concentrations of each protein to confirm binding prior to using quantitative methods to further explore these interactions.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499), the U.S. Department of Education McNair Grant (P217A180094), and an NSF RUI Award (2203467).

Dirty Water: The Physiological Effects of Environmental Turbidity on Bluegill Sunfish (Lepomis macrochirus)

Jada Fogle (2025) **Mentor: Dr. Salvatore Blair**
Gabe Halka (2026)

An environmental disturbance which fish are commonly exposed to (increasingly so with climate change induced extreme weather events: droughts/floods) is an acute shift in environmental turbidity, usually associated with rain runoff events causing waterbodies to become excessively silty, cloudy, or murky. Current literature surrounding the influence of environmental turbidity on Bluegill Sunfish (Lepomis macrochirus) is limited and most of the work is dated. This experiment aimed to assess physiological effects of environmental turbidity on Bluegill Sunfish, specifically at the gill tissue and to observe gene expression at various levels of water turbidity. Two hypotheses were formed 1) the increase in turbidity will cause elevations of the gill ILCM (interlamellar cell mass) due to its protective physiological function observed in response to similar external stimuli and 2) the increase in turbidity will result in triggering differential expression of various cellular genes involved in responding to external stressors. In this experiment four trials were performed with bluegill (n=7) placed in individual identical tanks. The fish were then exposed to different levels of turbidity for 24 hours and tissue samples were collected for histological and molecular analysis. To date gill arches were collected, fixed, processed for paraffin sectioning, and stained for morphological analysis under light microscopy. Tissue samples from the liver, intestines, and gill filaments were sampled and underwent RNA isolation prior downstream molecular methodology for gene expression analysis. This project is ongoing and future data analysis ought to shed light on what cellular mechanisms are employed by fish to cope with high environmental turbidity.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

A Dietary Fiber Educational Program to Improve Nutrition Knowledge and Gut Health

Jackie Gardella (2024) **Mentor: Jessie Hoffman, PhD, RD**
Cameron Scott (2024)

Dietary fiber contributes greatly to cardiovascular, metabolic, and gastrointestinal health.[1] It is well documented that most individuals in the US consistently fall below the recommendation of 14 grams of fiber per 1000 calories eaten.[2] This is further supported by research conducted specifically on young adults and the collegiate population, showing that individuals in these groups also fall below recommended fiber intake levels.[3,4] For many people, college is a time of increasing independence, including more independence with food choices. This presents a unique opportunity to provide education on the benefits of consuming adequate dietary fiber and how to achieve this as a college student. Therefore, we have designed an educational program to assess and fill this knowledge and skill deficit in the college population, with the goal of improving nutritional knowledge and gut health. In this project, we have designed a 6-week asynchronous nutrition education program for college students that provides a specific focus on dietary fiber. This educational program has been designed and verified by a group of registered dietitians prior to implementation with students. Each week, participants will be expected to devote 30 minutes to complete the components of the educational program. Recruitment is set to take place over the fall semester and will continue into the spring semester with a goal of recruiting approximately 50 students to participate in the full program. To assess the effectiveness of the program, participants will take a pre- and post-study survey assessing nutritional knowledge, dietary habits, and gastrointestinal health. Data will be collected using a Qualtrics survey and analysis will be conducted to assess for statistically significant changes between individual pre- and post-study survey measures. We expect that students participating in the program will demonstrate improvements in measures of nutrition knowledge, dietary habits, and gastrointestinal health. The outcomes of this study will fill a gap in the research surrounding dietary habits and gastrointestinal health in the collegiate population. Importantly, the findings of this study may provide a basis for implementing targeted nutrition and dietary fiber educational interventions in the college population, with the ultimate goal of fostering healthy habits that will provide life-long health benefits to participants.

This research was funded by the Winthrop University Biomedical Research Fund.

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G Quadruplex-Stabilizing Ligands Increase RYBP Expression in Glioblastoma Cell Lines

Alexandra Gebbia (2023) **Mentor: Daniel B. Stovall, Ph.D.**
Clara Whitehead (2026)
Farah Tabassum (2025)

Glioblastoma multiforme (GBM) is a malignant cancer of glial cells in the central nervous system, and it has a median survival of only around 12 months. When compared to healthy glial cells, GBM tumors significantly downregulate the Polycomb (Pc) protein RING1- and YY1-binding protein (RYBP), and in various other cancers RYBP exerts antitumor effects, such as promoting cell death and increasing chemosensitivity. However, the molecular mechanisms of RYBP transcriptional silencing in GBM remains unknown. RYBP may be regulated by the formation of G-quadruplexes (G4s), which are guanine-rich sequences that fold in DNA and RNA to create stable secondary structures. When present in gene promoters, G4s can behave as switches that modulate transcription. The complexity and stability of G4s allows them to play a significant role in regulating gene expression and overall genome stability. Therefore, we hypothesized that the aberrant resolution of G4s in the RYBP promoter contributes to RYBP downregulation in GBM cell lines. To determine whether G4s contribute to RYBP silencing, we analyzed the RYBP promoter sequence and identified two putative G4-forming sequences. We treated U-87, U-118, and T-98 GBM cell lines with the G4-stabilizing ligands PHENDC3 and TMPyP4, or vehicle controls (DMSO and sterile water, respectively). After 48 hours, total RNA and protein were isolated and quantified. Isolated RNA was used in reverse-transcription qPCR to detect differences in RYBP mRNA levels. Isolated protein was used to perform SDS-PAGE and Western blot analysis. Our results demonstrate that stabilizing G4 structures increases RYBP transcription in GBM cell lines, although the effects of these ligands on RYBP protein levels remains unclear. Nonetheless, we suggest a regulatory pathway that is readily targetable and worthy of future investigation as a therapeutic application for GBM.

Support was provided by an SC-INBRE DRP grant from the National Institute for General Medical Sciences (5P20GM103499-21).

A New Species of Kalyptorhynch Flatworm From Southeastern Sandy Beaches

Mitchell Hill (2026) **Mentor(s): Julian P.S Smith III**

The sandy high-energy beaches of the U.S. East Coast house a diverse array of Kalyptorhynch turbellarians, most of which are yet to be formally described. Here, we present a detailed description of *Lehardiya spiralis*, a newly discovered Kalyptorynch turbellarian from high-energy sandy beach sites in North Carolina, South Carolina, and Florida. Using differential interference-contrast microscopy, we captured images of the hook apparatus and the unique penis stylet. Using laser-scanning confocal microscopy, we captured detailed images and measurements of the cuticular hooks, hook-supports, images of the male and female genital apparatus, as well as overview images of the entire animal, providing comprehensive morphological characterization. Molecular characterization of the 18S ribosomal gene confirms this as a new species, distinct from the previously-described *Lehardiya alleithoros*. *Lehardiya spiralis* expands the known diversity of Kalyptorynch tubellarians in the marine interstitial environment of North Carolina, raising the reported species count to six. Of especial interest is that this new species is found on the same beaches as the formerly described *Lehardyia alleithoros*, raising ecological questions around niche separation.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the SC Governor's School for Math and Science.

Nucleic Acid Aptamer Au/Ag Nanoparticle Conjugates as Trojan-Horse Drug Delivery Vehicles in the Fight Against Bacterial Infections

Morgan Hunter (2024) **Mentor: Dr. Timea Fernandez**
Jadyn Williams (2024)

Illnesses caused by bacteria are a major public health concern since microorganisms have become increasingly resistant to available antibiotics. At the same time, big pharma has gradually shifted its focus from developing drugs that cure diseases to those that treat chronic conditions. Thus, rediscovering old drugs and using them for new purposes is becoming more important. The ultimate goal of this project is to use nucleic acid aptamer-nanoparticle conjugates as vehicles to deliver antibiotics to cells that are resistant to them.

Currently, we are investigating the therapeutic potency of nucleic acid-gold/silver nanoparticle conjugates as carriers of tetracycline and ampicillin to treat infections caused by E Coli. We hypothesize that by attaching nucleic acids that binds to these antibiotics, to gold nanoparticles, the resulting conjugates will work as a "Trojan-horse" antibiotic-delivery vehicle that smuggles the antibiotic into the cell without being detected by cellular defense systems. Moreover, we reason that gold/silver ions released by the nanoparticles add to the antimicrobial effects of the antibiotics.

To test the viability of this idea we used tetracycline and ampicillin binding DNA aptamers that were developed for detection of these antibiotics . We optimized conditions to attach these DNA aptamers to gold and silver nanoparticles. We are currently testing the antimicrobial effect of these aptamer nanoparticle conjugates using E. coli (ATTC strain 29522) and verify, using MTS assays, that they do not harm mammalian cells.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499), Made in SC EPSCoR, NSF (1655740) and the Winthrop University College of Arts and Sciences.

Synthesis of Sphingosine Kinase Inhibitors

Casey Kopyc (2024) **Mentor(s): T. Christian Grattan**

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

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the Winthrop University Department of Chemistry, Physics, Geology, and the Environment.

Impact of Multiple Freeze/Thaw Cycles on the Nutritional Integrity of Raw Human Milk

Rachel Layens (2024) **Mentor: Dr. Hope Lima**

Human milk is considered the ideal source of food for infants 0 to 6 months of age and may remain a part of the healthy infant diet for the first two years of life and beyond. Current CDC guidelines dictate that once human milk is frozen and then thawed, it cannot be refrozen for later use. Refreezing human milk that has been previously thawed may help mothers to use the milk for later use. Current studies show that the fat content of raw and pasteurized raw human milk was significantly reduced while lactose and protein were significantly increased after freezing and thawing. However, previous pilot data from our lab indicates that when pasteurized human milk was thawed and refrozen four times, macronutrient content remained stable. To expand on this, our project looked at the changes in protein and fat content of raw human milk after being frozen and thawed four times. An experimental study was performed with 20 raw human milk samples. Samples were thawed in a refrigerator for 24 hours and once thawed, samples were analyzed for total fat, creamtocrit (CRCT), total protein, and total energy content. Upon completion of the sample analysis, all samples were refrozen, and this process was repeated four times with 7 days between thaws.

To measure total protein, 10 mL of each human milk sample was diluted with 90 mL of water and vortexed to mix. After that, standards were prepared based on manufacturer instructions. Standards and diluted samples were added to a 96 well plate in triplicates. 200 uL of Pierce BCA Reagent A and B were added to all wells on the 96 well plate and placed in the incubator for 30 minutes at 35-37 °C, subsequently placed on ice for 10 minutes for cooling, and then read on the Fisher Scientific accuSkan FC. Sample values were compared to the standard curve to determine total protein content. Fat analysis of the raw human milk was measured using a Creamtocrit Plus. The samples were loaded into capillary tubes in triplication then sealed with a HemataSeal and placed in a centrifuge for 10 minutes at 11.2 x 1000 rpm. After centrifuging, samples were loaded into the Creamtocrit Plus for analysis. All results were analyzed using repeated measures one-way ANOVA on GraphPad Prism 9.

Our results revealed that total fat, total calorie content, and total creamtocrit were significantly increased, while total protein was significant decreased after two freeze/thaw cycles. Data analysis for all four freeze/thaw cycles is still underway.

This project was supported by the Department of Human Nutrition at Winthrop University and the Winthrop University Biomedical Research Fund.

Effect of RYBP Overexpression on EMT & Apoptotic Marker Levels in GBM

Dylan Lewis (2023) **Mentor: Dr. Daniel Stovall**
Monica Lopez (2024)

Despite modern advancements, Glioblastoma multiforme (GBM) remains the most common and fatal tumor of the central nervous system. The complexities of treatment arise from its invasive nature, heterogeneous cellular composition, limited drug delivery avenues, and resistance to radio- and chemotherapy. The brief post-diagnosis survival window stresses an urgent imperative for novel therapeutic approaches that can effectively navigate the traditionally complex challenges associated with GBM treatment. This research aimed to identify molecular pathways that may present points of vulnerability in GBM tumors. RING1- and YY1-binding protein (RYBP), a member of the Polycomb group protein family, plays a key role in gene regulation and chromatin modifications, and RYBP mRNA levels are decreased in approximately half of GBM cases. Therefore, we sought to determine whether forced expression of RYBP would antagonize cell survival and trigger apoptosis or suppress the epithelial-to-mesenchymal transition (EMT) and restrict cell invasion. We used U-118 and T-98 GBM cell lines previously transduced with lentivirus expressing green fluorescent protein (GFP) only (as a control) or expressing a RYBP-GFP fusion protein. Cell lysates were isolated and examined by Western blot to measure the levels of molecular markers associated with apoptosis and EMT. We observed a modest decrease in the EMT marker ZEB1, but not ZO1, in RYBP-expressing T-98 cells compared to control. In U-118 cells, RYBP expression appeared to reduce ZO1, but not ZEB1. No changes in beta-catenin were observed in either cell line. The effects of RYBP on the activation of caspase-3, -7, and -9 were inconclusive. Ultimately, this research aids in the ongoing investigation of RYBP's potential role in the governance of EMT and apoptosis in GBM cells. Although early findings suggest possible trends in molecular marker availability, additional research is necessary to definitively uncover RYBP overexpression's effect on these cellular functions.

Support was provided by an SC-INBRE DRP grant from the National Institute for General Medical Sciences (5P20GM103499-21).

Carbon Sequestration in the Timucuan Ecological Reserve

Sydney Elise Lyons (2025) **Mentor: Dr. Scott Werts**

The Florida Department of Natural Resources designates "critical shoreline erosion" based on whether areas adjacent to the Atlantic Ocean have a value for recreation or development. Due to sea level rise and coastal development, many inlet and estuaries in Florida are being eroded at increasing rates, therefore altering carbon cycling, sedimentation rates, and the release of greenhouse gasses into both the ocean and atmosphere. We are investigating carbon concentrations and erosion on Big and Little Talbot Island located in Duval County, Florida on the Atlantic Coast. These two islands are of special significance due rapid erosion caused by a combination of development and sea level rise with the highest rates of erosion occurring along the coast of Big Talbot Island, located in the Nassau Sound. Despite the rapid erosion rates and high concentrations of peat in the soils, they are not included as critical erosion shorelines. The soils here are typical spodosols with thick O horizons, often more than 7 cm thick. Vegetation transitions from old live oak hammock forests in the north to short palmetto dominated in the central portions to coastal dune pines and cedars in the southern part as the elevation descends towards sea level. Our data collection so far indicates that even small islands such as this contain nearly 150,000 tons of soil organic carbon and above ground carbon totaling near 100,000 tons. Current research includes calculations of the density of the carbon content of biomass overlying vegetation, the density of carbon in the soil horizons, and analysis of underlying Pleistocene sediments. Further research includes (vegetation surveys and plans for remote sensing).

Support was provided by a Winthrop Research Council Grant and the Dalton Environmental Endowment.

Funnel vision: web characteristics of the Lake Placid funnel-wolf spider (*Sosippus placidus*)

Chiara Meredith (2024) **Mentor: Dr. Jennifer Schafer**

Spiders may prefer to build their webs using specific plant species. Fire can alter plant species composition and vegetation structure, which could affect spider web characteristics. We investigated web characteristics of the Lake Placid funnel-web wolf spider (*Sosippus placidus*), a species that is restricted to pyrogenic scrub habitats in Florida. We assessed the plant species used by *S. placidus* to construct webs and the effect of time-since-fire on web size. We conducted our research in Florida scrub habitats at Archbold Biological Station. We sampled spiders in burn units ranging from 2 to 22 years since fire that included flatwoods, scrubby flatwoods, and rosemary scrub habitats. Each burn unit was searched for web funnels, and the spiders were lured out of their funnels for identification. We measured the height, length, width, and funnel diameter of the webs, and recorded the plant species attached to each web. We used linear regression models to determine if there was a relationship between years since fire and web height, web area, or funnel diameter. We found that spider webs were attached to 26 different plant species, including shrubs, sub-shrubs, and seedless vascular plants. Spider webs were attached to an average of three plant species and were most commonly found attached to the scrub oak and shiny blueberry. There was a negative relationship between years since fire and web height, which suggests that taller webs in more open recently burned sites provide greater access to catch prey. There was no relationship between years since fire and web area or funnel diameter. Previous research found *S. placidus* webs to be associated with areas defined as sand pine or scrub oak, while *Sosippus floridanus*, a more common species, was found frequently in scrubby flatwoods habitats. However, our study indicated that *S. placidus* was found frequently in scrubby flatwoods habitats, which suggests that *S. placidus* may not have been as abundant in scrubby flatwoods previously or could be expanding their habitat range.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Time Series Forecasting Models for Local Light Pollution

Abby Mervine (2025) **Mentor: Dr. Zach Abernathy**

This study aims to build a forecasting model to describe local trends in light pollution: the brightening of the night sky as a result of anthropogenic, artificial light sources. Satellite-based radiance measurements can be used as a proxy for light pollution levels. Specifically, a sample from a dataset representing radiance values from 2012-2022, collected by the Visible Infrared Imaging Radiometer Suite – Day/Night Band (VIIRS-DNB) sensors on the Suomi National-Polar Orbiting Partnership (SNPP) satellite, along with time series forecasting was used to predict future radiance values for Rock Hill, South Carolina, USA. Autocorrelation plots and the augmented Dickey-Fuller test were utilized to select parameters for an Autoregressive Integrated Moving Average (ARIMA) forecasting model. The accuracy of this model, quantified by an Akaike Information Criterion (AIC), was compared to that of models built by Python's auto_arima package, including Seasonal ARIMA (SARIMA) models. The model with the lowest AIC was chosen. A test-train split was then performed on the dataset to cross-validate the chosen SARIMA model. After cross-validation, the chosen model was used to generate an 8-year (2022-2030) forecast with 95% confidence intervals, and the forecast appears to show a decrease in radiance values for the Rock Hill area. We conclude with a discussion on the degree to which these radiance values could correlate with changes in light pollution, including the impact of adopting LED technology for artificial lighting over the period of time used in the training data.

Support was provided by the U.S. Department of Education McNair Grant (P217A180094).

ExplosioNervosa: Discovery and Investigations of a Mutant Mycobacteriophage Population

Jessica E. Morgan (2025) **Mentor: Dr. Victoria Frost**

The relationship between bacteria and bacteriophages has been co-evolving for millions of years to create the dynamic relationships that we see today. The Science Education Alliance (SEA) Program of research at Winthrop University pairs students with their peers and other scientists around the world to better understand these ancient co-evolving interactions. In 2017, a Winthrop student discovered Mycobacteriophage ExplosioNervosa in the soil. Following genome sequencing, a high titer phage sample was stored at -20°C. In the spring of 2023, ExplosioNervosa was reactivated to clone and systematically characterize all 96 putative genes. As investigations progressed, it was realized that the phages in this fresh lysate had evolved to become a mutant strain with a 3,613bp genome deletion. Annotation of ExplosioNervosa's genome indicated that 11 genes should be located within this region. Work began to recover these genes. A lysate saved from ExplosioNervosa's initial isolation was located and investigated for the presence of these "missing" genes. All eleven genes were discovered in the wild-type strain using gene specific amplification techniques. To date, three of the eleven genes in this region have undergone a phenotypic test known as a cytotoxicity assay, which is used as an observable indication of interactions between the host proteome and phage gene products. We are now completing cytotoxicity assays for each gene, and studying the biology of wild-type compared to the evolved mutant version of this phage. Understanding phage-host interactions, and the phenomenon of phage evolution, are fundamental for the advancement of therapeutic phage use and application.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the HHMI.

The Expression, Purification, and Preliminary Characterization of a Putative Cryptochrome 2 homolog found in *Isodiametra pulchra*

Priya Patel (2025) **Mentor(s): Jason C. Hurlbert**

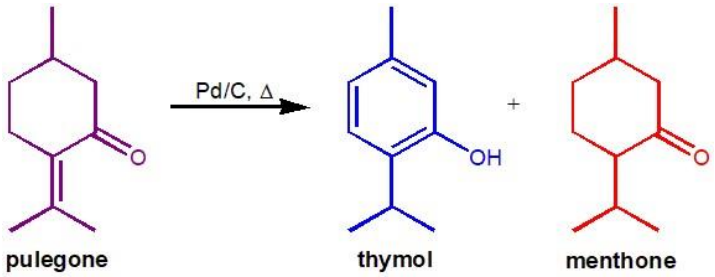
Recently, homologs of the circadian rhythm proteins, Cryptochrome 2 (Cry2) and Period 2 (Per2), were discovered in the transcriptome of *Isodiametra pulchra* by our collaborators, Danny Stanton, and Dr. Julian Smith. These homologs could be the earliest evolutionary appearance of such clock proteins in organisms. Cryptochrome (Cry) and Period (Per) isoforms have been heavily researched in *Drosophila* and murine models to attain a better understanding of possible Cry and Per interactions with each other and with other clock proteins and the role of such protein complexes in the regulation of gene expression of proteins involved in circadian rhythms. Cry2 and Per2 prevent CLOCK: BMAL1 from transcribing target genes during nighttime and show peak expression during the early evening. Our goal is to determine the structure of the *I. pulchra* Cry2 homolog for comparison to homologs found in higher organisms. We placed a synthetic gene encoding Cry2 in a pET28 plasmid and expressed the resulting plasmid construct in *Escherichia coli*. We also developed a multi-step purification protocol to purify the recombinant protein from 6 liter cultures grown in the laboratory. The first chromatographic step employed metal chelating affinity chromatography, using a His-trap nickel column. We then performed gel filtration chromatography and have obtained approximately 80-90% pure recombinant Cry2 at 10 mg/mL. This protein will be used to perform a binding assay with recombinant, purified *I. pulchra* Per2 produced in our laboratory. For future work, we will continue express and purify Cry2, perform crystallization trials on Cry2, and perform binding assays on Cry2 with Per2.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Preparation of the Fragrant Terpenoids Menthone and Thymol via Catalytic Transfer Hydrogenation (CTH) of Pulegone

Mackenzie Paul (2025) **Mentor: Aaron M. Hartel**

Terpenes and terpenoids are important classes of naturally occurring compounds consisting of repeating “isoprene” units. We have developed an experiment for an organic chemistry lab course in which the monoterpenoid pulegone (a fragrant component in catnip) undergoes catalytic transfer hydrogenation (CTH) using a palladium catalyst. The reaction produces two fragrant terpenoids: menthone (found in mint) and thymol (found in thyme).



An acid-base extraction procedure was developed to enable the efficient separation of the two products. The thymol and menthone products could be isolated successfully in moderate to good yields and high purity by GC-MS analysis.

Decolonizing Education Abroad: Unraveling Colonial Ideologies & Embracing Equitable Practices

Logan Tayler Pender (2024)

Mentor: Heather Haeger, Ph.D.
(University of Arizona, Educational Policy Studies and Practice)

Decolonizing education abroad is a radical approach that confronts colonial practices, assumptions, and values. It requires active engagement from administrators, faculty, and students, requiring intentionality, self-reflexivity, and long-term relationships with local institutions and communities. Postcolonial theory offers methodologies and frameworks to deconstruct Eurocentric perspectives and dismantle colonial ideologies. Collaboration and partnerships with local communities are essential for implementing a decolonizing approach. Decolonization has become an increasingly important topic in education abroad, examining aspects like student learning, access, equity, curricular integration, intervention strategies, and support for underrepresented students. This review explores decolonizing U.S. education abroad in order to examine the intersectionality of education abroad and colonialism. The aim is to provide background on the comprehensive approach to decolonizing America's education abroad and, if so, what it could look like. Conducting a literature review that provides a holistic background on the discussions of the decoloniality of education abroad will enable us to support upcoming research studies that will allow us to create a framework that could be used in programming to prioritize the voices and perspectives of marginalized communities.

Support was provided by the University of Arizona Graduate College, Undergraduate Research Opportunities Consortium - Summer Research Institute and the Winthrop University Ronald E. McNair Post-Baccalaureate Program.

It's a sticky situation: abundance and diversity of arthropods trapped by tarflower (*Bejaria racemosa*)

Sabrina Rocha (2024) **Mentor: Dr. Jennifer Schafer**

Some plants have sticky surfaces that can provide defense against insect herbivores by trapping them. Tarflower (*Bejaria racemosa*), a shrub endemic to the southeastern United States, produces a sticky substance on the bottom of its flower petals. Our research aimed to assess the effects of habitat, reproductive stem height, the number of reproductive branches, and time since fire on the number of arthropods trapped by tarflower. We also investigated which arthropods were most commonly trapped by tarflower. We conducted our research in Florida scrub habitats at Archbold Biological Station. We haphazardly selected 20 tarflower individuals (10 in flatwoods and 10 in scrubby flatwoods) in sites ranging from 2 to 15 years post-fire. We measured the height of the tallest reproductive stem and counted the number of reproductive branches for each individual. We collected reproductive branches and identified entrapped arthropods to order or family. There was no relationship between the number of arthropods trapped and the height of the tallest reproductive stem, the number of reproductive branches, or time-since-fire. We found that over 50% of the trapped arthropods were in the order Hymenoptera, and 33% of the hymenopterans were in the family Formicidae. Our results suggest that the arthropods most commonly trapped by tarflower were not likely to cause damage to the leaves or reproductive structures.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Expression, Purification, and Crystallization of Human Acid Ceramidase 1

Elizabeth Ryerson (2026) **Mentor: Jason C. Hurlbert**

Ceramide, a lipid found in the cellular membrane, is central to many cellular response mechanisms, including the pathway which triggers apoptosis. When the cell is damaged, human acid ceramidase 1 (ASAH1) hydrolyzes the fatty acid tail from ceramide to convert it into sphingosine. Sphingosine kinase then converts sphingosine into sphingosine-1-phosphate. While ceramide and sphingosine favor apoptosis, sphingosine-1-phosphate favors cell survival. An over expression of ASAH1 is found in cancer cells, which increases the concentration of proapoptotic sphingosine-1-phosphate and causes cancer cell proliferation.

It is predicted that an ASAH1 inhibitor will increase the concentration of ceramide and force apoptosis of diseased cells. Therefore, previous research has been done to design such a molecule. To determine its effectiveness, the inhibitor can be added to purified ASAH1, and X-ray crystallography can be used to determine the protein-inhibitor complex. This research focused on the expression, purification, and crystallization of ASAH1 so that it can be analyzed alongside its potential inhibitor.

Escherichia coli cells were transformed with a synthetic gene encoding human ASASH1 in a pET28-based vector and were grown to produce the recombinant protein. The protein was then purified using a variety of chromatographic steps, resulting in purified protein (~90%) being recovered at moderate yield (10 mg/mL). This purified protein sample was combined with an inhibitor synthesized in a collaborator's laboratory and crystallization trials were attempted. Conditions for crystallization were then tested, with three solutions producing crystals. With the discovery of these conditions, the next steps will be to verify that the crystals are protein and further optimize the crystallization conditions to be used for macromolecular x-ray diffraction studies, with the resulting data being used to determine the atomic structure of the enzyme/inhibitor complex.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Investigating the Role of miRNAs towards RYPB Silencing in Glioblastoma Multiforme

Valentine Savioz (2024) **Mentors: Daniel B. Stovall, Ph.D.**
Alex Lee, M.S. Thesis Candidate

Glioblastoma multiforme (GBM) is a devastatingly invasive and deadly tumor of the central nervous system. Survival rates for patients diagnosed with GBM are low, with a median survival of under 15 months. GBM progression involves the alteration of key regulatory epigenetic pathways. Many of these modifications act on Polycomb group (PcG) proteins and contribute to their dysregulation in GBM. Specifically, the RING1- and YY1-binding protein (RYBP) is a PcG protein that is down-regulated in approximately 50% of GBM tumor patients and is a known tumor suppressor in multiple cancers. However, the mechanism of RYBP down-regulation in GBM is unclear. MicroRNAs (miRNAs), small noncoding RNAs that regulate protein synthesis, are thought to contribute to RYBP silencing by complementary binding to the RYBP mRNA transcript at its 3'-UTR. We hypothesized that RYBP is a direct and functional target of multiple oncogenic miRNAs in GBM. To determine which miRNAs are predicted to target the RYBP 3'-UTR, we analyzed the RYBP 3'-UTR using TargetScan and identified miR-9-5p, miR-125b-5p, and miR-128-3p as having the highest probability of functionally targeting the RYBP transcript in GBM. Through inhibition of these miRNAs with a synthetic miR-inhibitor in the U-87, U-118 and T-98 GBM cell lines, RYBP expression increased, as measured by Western blot and RT-qPCR. We also measured the oncogenic effects of miR-9, miR-125b, and miR-128 in U-118 and T98G cell lines using WST-1 viability assays, but these assays were inconclusive. Our findings suggest miR-9, miR-125, and miR-128 contribute to RYBP silencing in GBM, and may offer potential therapeutic targets. Future experiments will be aimed at determining whether RYBP is a direct and functional target of these miRNAs.

Support was provided by an SC-INBRE DRP grant from the National Institute for General Medical Sciences (5P20GM103499-21).

Synthesis of pyrazole derivatives to be used as an anticancer drug

Mackenzie Smith (2023) **Mentor(s): T. Christian Grattan**

Cancer remains a significant target of multiple anti-cancer drug research projects. As new drugs are developed and discovered, the disease seems to evolve and resist the impact these drugs have on curbing the progress of the spread. To combat this drug resistance, molecules containing the heterocyclic pyrazole ring have been developed. This project focuses on the synthesis of novel pyrazole-containing compounds to assess the impact these compounds may have in cancer treatments. The synthetic approach will evaluate how changing the halide, which is para to the diazo group may be introduced by preparing the diazonium salt and reacting with 2,4-pentanedione to substitute the central alpha carbon. The scheme will also focus on how changes in the pyridine ring nitrogen position impacts how the molecule interacts as a cancer therapeutic drug. The preferred halide and pyridine ring will then be combined to further enhance the compounds ability to combat this disease and ultimately the drug resistance.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the Winthrop University Department of Chemistry, Physics, Geology, and the Environment.

The Synthesis of Diarylpyridines as Inhibitors of Amyloid Beta Aggregation for Alzheimer's Disease

Mary Stegall-Smith (2025) **Mentors: Dr. James M. Hanna Jr.**
Dr. Robin K. Lammi

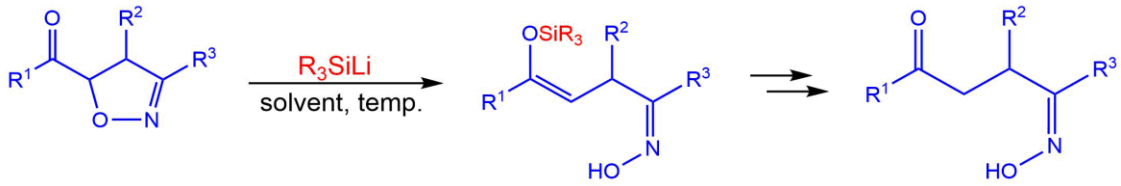
Amyloid-β is a peptide which aggregates with other amyloid-β chains in the brain to form plaques which are correlated to the progression of Alzheimer's Disease (AD). The amino acid phenylalanine plays a key role in aggregation through π-stacking and hydrogen bonding interactions. Previous research in our group has shown that hydrogen bond donors on a biphenyl or terphenyl system can effectively inhibit aggregation, as well as those with a central phenyl linker region. To test how differences in the π-stacking ability in an inhibitor affect aggregation, molecules with a central pyridine linker region, diphenylpyridinetetrol (DPPT) derivatives, were synthesized through Suzuki-Miyaura coupling followed by demethylation with hydrobromic acid (48% aq). Once the small molecules were synthesized, a Congo Red (CR) assay was performed on Aβ-40 to obtain data to assess the efficacy of inhibition. Congo Red assay for 2,3-DPPT showed inhibition, though initially the compound promoted aggregation.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Utilizing the Brook Rearrangement to Form γ-Ketooximes and Their Silyl Enol Ethers from Acylisoxazolines

Jaylin Sypolt (2025) **Mentor: Aaron M. Hartel**
Mackenzie Paul (2025)

γ-Ketooximes are versatile synthetic intermediates. Additionally, the corresponding silyl enol ethers of these useful structures have potential for the preparation of more highly-substituted variants. We have developed a method for the preparation of γ-ketooximes and their silyl enol ethers from acylisoxazolines using silyllithium reagents.



The reaction utilizes a Brook rearrangement: the migration of a silyl group from a carbon to an oxygen. By adjusting reaction parameters such as solvent, silyllithium reagent, and temperature, the reaction can be tuned to favor either the γ-ketooxime or the silyl enol ether product. Optimization experiments have shown the solvent used and the amount of silyllithium added to be the most impactful variables. Upon completion of the reaction, the γ-ketooxime or silyl enol ether can be isolated using column chromatography.

Support was provided by the Winthrop Research Council (SC23004 & SC23007).

Investigating the photocatalytic reduction of alkynes using an organic photocatalyst

Ryan Wernsman (2024) **Mentor: Dr. James M Hanna Jr.**

Alkyne reductions result in the generation of alkenes or alkanes via a number of well-known reactions. In one kind of alkyne reduction, dissolving metal reductions, lithium or sodium metal serves as a reducing agent to effect single-electron transfer (SET) to the alkyne, generating a radical alkene anion; liquid ammonia serves as a proton source. Within the last fifteen years, visible-light photoredox catalysis has been developed into a practical method to generate organic radicals which can engage in productive, downstream processes. A recent report from the Nicewicz group described an organic photocatalyst (9-mesityl-3,6-di-*tert*-butyl-10-phenylacridinium tetrafluoroborate, ***dtb-Mes-Acr-Phe***), which in its reduced, excited state, has an oxidation potential similar to that of lithium metal. This inspired us to investigate its use in the reduction of alkynes. Initial investigations indicate that diphenylacetylene can be reduced to bibenzyl, along with a bibenzyl dimer, by irradiating the solution with a 390 nm LED source, in the presence of ***dtb-Mes-Acr-Phe*** and diisopropylethylamine (DIPEA) in acetonitrile solvent. Both *trans*- and *cis*-stilbenes appear to be intermediates along the reaction pathway, and the addition of thiophenol can eliminate the formation of the bibenzyl dimer. Control experiments show that the reaction can take place without the photocatalyst, albeit in lower yield. In this presentation, we will discuss our efforts to further develop this novel reduction protocol.

We would like to acknowledge and thank the donors of the American Chemical Society Petroleum Research Fund (58270-UR1). Additional support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499).

Exploring the Interplay Between Oxidation, Dimerization, and Regulation of RitR

Jamia White (2024) **Mentor: Dr. Nicholas Grossoehme**
Jacob Rodriguez (2026)

Iron is an essential micronutrient for the fitness of living organisms. Its ability to alternate between the +2 and +3 oxidation states makes it critical for many redox processes; however, it also makes iron accumulation cytotoxic. Surprisingly, the common human pathogen Streptococcus pneumoniae lacks any of the well-characterized iron regulatory systems. The orphan response regulator, RitR, from this organism has emerged as the central component of novel class bacterial iron regulation, which relies on phosphorylation and oxidation/ dimerization to attune transcriptional regulation. This project aims to quantify the relationship between RitR oxidation, phosphorylation, and DNA binding. This project focuses on the expression and purification of RitR with subsequent efforts to stabilize the oxidation-driven dimer. We have successfully created the RitR dimer; however the dimer is sparingly soluble under conditions we have currently tested.

Support was provided by an SC-INBRE grant from the National Institute for General Medical Sciences (P20GM103499) and the U.S. Department of Education McNair Grant (P217A180094).

SDF-1 Decreases the Repellent Activity of LPA on Chicken Embryo Retinal Ganglion Cells (RGCs)

Ana Wingham (2024) **Mentor: Eric Birgbauer**

The nervous system is a complex component of the vertebrate body plan. It is comprised of the central nervous system (CNS) and the peripheral nervous system (PNS). Injury to the CNS is permanent, as these nerves do not naturally regenerate. However, the CNS nerves do extend naturally during development, which could provide clues for regeneration. One aspect of nerve extension is axon guidance to the appropriate target. Axons, which form the nerves, follow a defined pathway to seek their synaptic target, guided by specific molecules during development. We are interested in axon guidance in the nervous system, focusing on the visual system and how axons are sent from the retina to their targets in the brain. Furthermore, many axon guidance molecules have been shown to be repulsive; however, this repulsion may need to be modulated during development. Interestingly, the chemokine SDF-1 was previously shown to reduce repulsion by Slit-2 on retinal axons in vitro (Chalasani et al., 2003). We are investigating whether the modulatory effect of SDF-1 extends to other axon guidance molecules, specifically the bioactive lipid lysophosphatidic acid (LPA). We examined the responses of embryonic chick retinal growth cones to LPA in the presence and absence of SDF-1 in an in vitro retinal explant culture system. We found that LPA causes a dose-dependent growth cone collapse, but treatment with SDF-1 reduces the growth cone collapse by LPA. Thus, SDF-1 appears to modulate the repulsive action of multiple axon guidance molecules and may be used physiologically to stimulate axon growth through a repulsive environment.

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