2018

High Point University Summer Research Program in the Sciences (SuRPS) Final Research Symposium



Thursday, July 26 & Friday, July 27

Phillips Auditorium

2018 Surps Keynote Speaker

"A Journey Through Research: Peptide-Catalyzed Atroposelective Coupling of Arenes and Quinones"



Gavin Coombs, (HPU Biochemistry Class of 2014)

Doctoral Candidate, Department of Chemistry

Yale University, New Haven, CT

ABSTRACT

Conducting summer research is often a very rewarding and integral component of undergraduate education. In addition to exposing students to a wide variety of techniques and unique scientific experiences, they are formative opportunities that can shape the overall trajectory of a student's path through science. Herein will be discussed several research experiences commencing with the visualization of two open reading frames associated with viral nuclear egress in Kaposi's Sarcoma-Associated Herpesvirus (KSHV), continuing through to a spectroscopic and computational analysis of an intramolecular O-H to olefin hydrogen bond, and culminating in the exploration of an asymmetric method for generating BINOL-type, non-C2 symmetric scaffolds employing a Lewis-basic catalyst within a minimal peptide framework.

BIO

Gavin Coombs received his B.S Degree in Biochemistry from High Point University in 2014. During his tenure there, he participated in two summer research opportunities at The Johns Hopkins University before graduating and completing a short post-baccalaureate experience in synthetic chemistry at JHU with Prof. Thomas Lectka. In August 2015, he began his graduate studies at Yale University under the direction of Prof. Scott Miller where he is a rising fourth year PhD candidate. Gavin's current research project involves developing enantioselective arylation reactions using tetrapeptides as small organocatalysts. His awards include being named an NSF Graduate Research Fellow, a UNCF-Merck Undergraduate Fellow, an American Chemical Society Scholar, and has co-authored three publications in several disciplines.

SuRPS Final Symposium

(Thursday, July 26, 2018, Phillips Hall Francis Auditorium)

Session A: Dr. Veronica Segarra, Department of Biology, Presiding

	8:30 – 9:05		COFFEE, TEA RECEPTION (Phillips Hall Lobby)
	9:05 - 9:15	Dr. Brian Augustine	Opening Remarks
Th.1	9:15 - 9:30	Molly Hulver	Analyzing the Role of Tat-SF1 in HIV-1 RNA Stability and Export
Th.2	9:30 - 9:45	Jeremy Muhr	Developing and Validating a Damage Paradigm to Examine Neuronal Regeneration
Th.3	9:45 - 10:00	Molly Penton	Characterization of the Mannose-6-Phosphate Receptor Homology Domain in the Atg27 Protein
Th.4	10:00 - 10:15	Alan Hsueh	Determining Accurate Locations of Fossils Localities of the Bighorn Basin with GIS and GPS
Th.5	10:15 - 10:30	Rachel Berndsen	Synthesis and Evaluation of a Series of Carbazoles as Antibiotic Adjuvants in Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA)
	10:30 - 10:45		BREAK

Session B: Dr. Pamela Lundin, Department of Chemistry, Presiding

Th.6	10:45 - 11:00	Will LeFever	Synthesis of Computationally-Derived ERK2 Substrates to Probe Kinase Activity During Oxidative Stress
Th.7	11:00 - 11:15	Sawyer Lyons	Therapeutic Potential of Cannabidiol in an <i>In Vitro</i> Model of Ischemia
Th.8	11:15 - 11:30	Christopher Goudarzi	Understanding the Mechanism of Photo-Redox Catalysis
Th.9	11:30 - 11:45	James Dew	Using Zebrafish as an Animal Model to Analyze Causes of Miscarriage When Embryos are Treated with Nicotine Using a PCR-Based Approach to Gene Analysis
Th.10	11:45 - 12:00	Jake Schleppy	Drosophila melanogaster as a Model to Examine the Neurological Effects of Cannabinoids
	12:00 - 1:15		LUNCH BREAK

(Note: Thursday, July 26 afternoon session continued on next page)

SuRPS Final Symposium (Thursday, July 26, 2018, Phillips Hall Francis Auditorium)

Session C: Dr. Keir Fogarty, Department of Chemistry, Presiding

Th.11	1:30 - 1:45	Michaela Connors	A Survey of Parasites in Residual Fecal Matter in Woodchucks (<i>Marmota monax</i>) in the Piedmont of North Carolina
Th.12	1:45 - 2:00	Julia Trautman / Abigail North	Investigating Tat-SF1 Interactions With HIV RNA
Th.13	2:00 - 2:15	Isabella Postle	Nanopatterning Conjugated Polymer Growth By Microcontact Printing
Th.14	2:15 - 2:30	Candyce Sturgeon	Characterization of the Functional Relationship Between Atg27 and the YML018C Protein
Th.15	2:30 - 2:45	Kennedy Jackson	Using GIS to Analyze the Effect of Climate Change on Mammalian Fossil Communities During the Early Eocene
Th.16	2:45 – 3:00	Nick Cutrona	Synthesis and Evaluation of Halogenated Tricyclic Compounds for the Treatment of Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA)
	3:00 - 3:15		BREAK

Session D: Dr. Heather Ahrens, Department of Biology, Presiding

Th.17	3:15 - 3:30	Hannah Cozart	Body Size Change and Dental Disparity in <i>Esthonyx</i> (Mammalia)
Th.18	3:30 - 3:45	Soo Min Lee	Molecular Synthesis to Understand the Charge Transfer Between the Donor and Acceptor Components of an Organic Polymer
Th.19	3:45 – 4:00	Katelyn Greer	Hibernation's Effects on the Platelet Counts of Woodchucks (Marmota Monax) in Wisconsin

SuRPS Final Symposium (Friday, July 27, 2018, Phillips Hall Francis Auditorium)

Session E: Dr. Kristin Ackerman, Department of Biology, Presiding

	8:30 – 9:00		COFFEE, TEA RECEPTION (Phillips Hall Lobby)
Fr.1	9:00 - 9:15	Mikaela Seemann	Evaluation of Antibiotic Adjuvant Scaffolds for Mechanism of Action and Broader Therapeutic Potential
Fr.2	9:15 - 9:30	Ricki A. Luongo	Dental Anatomy and Variation in Esthonyx (Mammalia)
Fr.3	9:30 - 9:45	Kendall Ziegler	Cannabidiol Bioavailability in <i>Drosophila melanogaster</i> and Impact on Behavior and Serotonin Levels
Fr.4	9:45 - 10:00	Nathan Grinalds	Photophysical Characterization of Novel Rhodamine B Dimers
Fr.5	10:00 - 10:15	Juliana O'Brien	Synthesis of Carbetocin Using Photochemical Cyclization Conditions
Fr.6	10:15 - 10:30	Kaylee Campbell	Preparation of Surface-Grafted P3HT Brushes Using an Easily Cleavable Self-Assembled Monolayer
Fr.7	10:30 – 10:45	Jacob Dunn	Neuroprotective Effects of Cannabidiol (CBD) Following an In Vitro Oxygen-Glucose Deprivation Model
	10:45 - 11:00		BREAK

Keynote Address: (Introduction by Dr. Heather Miller, Department of Chemistry)

11:00 - 11:50	Gavin Coombs (HPU Class of 2014) Yale University	A Journey Through Research: Peptide-Catalyzed Atroposelective Coupling of Arenes and Quinones
11:50 – 12:00	Dr. Angela Bauer	Closing Remarks
12:15 - 1:45		GROUP LUNCH (Wilson Hall Ballroom)

2018 SuRPS Faculty Participation and Projects

Department of Biology

Dr. Kristin Ackerman	"Effects of Exogenous Nicotine on Gene Expression During Zebrafish Development"
Dr. Heather Ahrens	"Dental Variation and Selection Patterns in the Eocene Mammal Esthonyx"
Dr. Christian George	"Mammalian Community Response to Eocene Climate Change: Investigating Fossils from the Big Horn Basin, Wyoming"
Dr. Michael Grider	"Signaling Mechanisms of Cannabidiol-Mediated Neuroprotection"
Dr. Jackson Sparks	"Cannabinoid Effects on Insect Serotonergic Pathways and Behavior"
Dr. Veronica Segarra	"Dissecting Atg27 Function in Yeast Membrane Traffic and Autophagy"
Dr. Brett Woods	"Life History Traits of Woodchuck (<i>Marmota monax</i>) Populations at Different Sites on an Elevational Gradient"

Department of Chemistry

Dr. Meghan Blackledge	"From Antidepressants to Antibiofilms: Synthesis and Evaluation of Small Molecules that Disrupt Biofilm Formation in <i>Staphylococci</i> "
Dr. Keir Fogarty	"Single Molecule Fluorescence Characterization of Synthetic Dyes and Biomolecular Complexes"
Dr. Pamela Lundin	"Development of the Sonogashira Catalyst-Transfer Polycondensation as a Method to Prepare Covalently Grafted Films with Anti-Microbial Properties"
Dr. Heather Miller	"Investigating Human Tat-specific Factor 1's Role in HIV-1 Gene Expression"
Dr. Andrew Wommack	"Installation of Redox-Inert Disulfide Bioisosteres to Study Thiol-Based Function and Signaling"

Special Thanks:

Dr. Joanne Altman, Director HPU Undergraduate Research and Creative Works Program Rebecca Smoak, Administrative assistant extraordinare Carol Peden. Siyabonga!

David Hayworth College of Arts and Sciences, High Point University for financially supporting the SuRPS Program Dr. Pamela Knippenberg, Chemistry Department Lab Manager

Mr. Luke Dixon and Mrs. Candace Loftis, Biology Department Lab Managers Joint School of Nanoscience and Nanoengineering, Greensboro, NC Janice Foley, Office of Student Financial Accounts

STUDENT ABSTRACTS:

{Note: presenting author is <u>underlined</u>, * denotes faculty advisor(s)}

(Th.5) Synthesis and Evaluation of a Series of Carbazoles as Antibiotic Adjuvants in Methicillin-Resistant Staphylococcus aureus (MRSA)

Rachel Berndsen, Grayson Norris and Meghan Blackledge* Department of Chemistry High Point University

Every year, 90,000 Americans face a battle against methicillin-resistant Staphylococcus aureus (MRSA) and about 20,000 die. MRSA is an extremely contagious bacteria that causes a wide variety of infections almost anywhere in the body. MRSA is one of the hardest infections to treat because there are a plethora of strains which have become resistant to many different antibiotics, including oxacillin. The rate of development of new antibiotics cannot keep up with the rate that bacteria are developing resistance to these new therapeutics. But perhaps it is possible to make antibiotics effective again by using antibiotic adjuvants. The purpose of antibiotic adjuvants is to disarm the resistance mechanisms of the bacteria so that existing antibiotics are once again effective. This project involves the testing and characterization of a variety of compounds to potentiate oxacillin to MRSA. The Blackledge lab previously found that 3,6-dichlorocarbazole could potentiate beta-lactam antibiotics against MRSA. To more fully understand the structural features required for adjuvant activity, we screened commercially available carbazoles to understand the effects of aromatic substitution on biological activity. We have also synthesized additional carbazoles from substituted diphenylamines to complete our structure-activity map of aromatic substitution. Synthetic details and biological data will be presented along with an analysis of our current structure-activity map.

(Fr.6) Preparation of Surface-Grafted P3HT Brushes Using an Easily Cleavable Self-Assembled Monolayer

<u>Kaylee Campbell</u> and Pamela Lundin* Department of Chemistry High Point University

Surface-initiated catalyst transfer polycondensation (SI-CTP) is a chain growth polymerization of conjugated polymers that attaches an initiator to the surface via a self-assembled monolayer (SAM) prior to polymerization. However, removing the grafted polymer from a silica-containing surface like glass requires HF, a highly toxic and corrosive acid. We have designed a new silane containing a functional group that is cleavable under milder conditions. We will present our work to date on our efforts to use this silane to prepare a surface-grafted initiator capable of performing Kumada CTP to prepare poly(3-hexylthiophene) (P3HT) grafted to glass slides and silica nanospheres, as well as our efforts to cleave the P3HT from these surfaces. The steps of this grafting and cleavage sequence are characterized using contact angle measurement, AFM, SEM/EDX, and NMR.

(Th.11) A Survey of Parasites in Residual Fecal Matter in Woodchucks (*Marmota monax*) in the Piedmont of North Carolina Michaela Connors, Katelyn Greer and Brett Woods*

Department of Biology High Point University

Woodchucks (*Marmota monax*) were caught via live trap in Davidson County, NC at 840 ft. above sea level. The woodchucks were processed and released at site of capture. The animals captured were sexed, weighed and their hind feet were measured. Weight and hind foot measurement ranged between 1255-4825 g and 73-85 mm, respectively. The woodchucks were then marked with hair dye in varying patterns to differentiate in the case of recapture. Fecal matter was collected from the traps when present. The stool samples were then brought back to the lab and fecal floats were performed using Feca-Med 35.6% sodium nitrate solution. From the samples collected, contents from the floats were examined under a light microscope and eggs from various parasites were identified based on data and slides published by the Centers for Disease Control. Parasites can influence woodchucks' ability to hibernate. Surveying species richness and diversity in woodchucks before hibernation may provide insight into which species are tolerable and which must be expelled before hibernation. Woodchuck avoid parasites by having a separate area in their burrows for a restroom, replacing nesting materials frequently and by not being social animals. Results from floats may include *Giardia* spp., the pinworm *Citellina triradiata*, and *Entamoeba muris*. Other possible findings may include *Obeliscoides cuniculi*, the tapeworm *Taenia crassiceps*, and the trematode *Dicrocoelium dendriticum*.

(Th.17) Body Size Change and Dental Disparity in Esthonyx (Mammalia)

<u>Hannah K. Cozart</u>, Ricki A. Luongo and Heather E. Ahrens* Department of Biology High Point University

The Big Horn Basin in the western United States contains an abundant fossil record of placental mammals, including Tillodontia. *Esthonyx* is a relatively common member of Eocene communities (56 – 33.9 mya), yet little is known about evolutionary patterns and morphological variation within the lineage. The inferred specialized herbivorous diet of tillodonts allows us to examine mammal evolution in response to climatic fluctuations. In this project, we examined dental disparity and size variation to better describe phenotypic variation within *Esthonyx*. Dental morphology was quantified using geometric morphometric analyses of 20 specimens of *Esthonyx*. Twelve landmarks representing prominent cusps and additional anatomical features of the lower fourth premolar and three molars were digitized in tpsDig2. Principal components analysis was then used to quantify dental disparity along the tooth row in MorphoJ. Though p4 and m3 possessed greater disparity, there were no significant differences in variation along the tooth row. Centroid size was plotted against stratigraphic meter level to assess body size changes through time. All plots showed increasing centroid size over time with the exception of p4; however, the linear relationship was weak. Additional larger sample sizes will aid in drawing conclusions about lineage evolution, as well as species identification.

(Th.16) Synthesis and Evaluation of Halogenated Tricyclic Compounds for the Treatment of Methicillin-Resistant Staphylococcus aureus (MRSA)

<u>Nick Cutrona</u>, Rachel Berndsen, Grayson Norris and Meghan Blackledge* Department of Chemistry High Point University

Methicillin-resistant Staphylococcus aureus (MRSA) is an opportunistic pathogen associated with soft tissue and systemic infections in humans. Unfortunately, the rate of antibiotic discovery has slowed considerably in the last thirty years due to lack of novel drug targets and scaffolds and the poor return on investment for pharmaceutical companies. Alternative approaches to combatting bacterial infections are necessary to effectively stem the growing resistance crisis. We have recently reported that halogenated dibenzoxazepines have the potential to serve as antibiotic adjuvants in potentiating MRSA to commonly used antibiotics. To more completely explore the importance of aromatic halogenation to adjuvant activity, a dibenzazepine compound library has been created using a two-step process with an arylindole intermediate. In addition, we have noted the capability of halogenated carbazoles to also act as antibiotic adjuvants in potentiating MRSA. Other groups have reported N-alkylated carbazoles with antibiotic activity in both bacterial and fungal species. We have prepared alkylated derivatives of 3,6-dicholorocarbazole to evaluate how alkyl derivation affects both adjuvant and antibiotic activity in MRSA. Synthetic details and biological activity data will be presented.

(Th.9) Using Zebrafish as an Animal Model to Analyze Causes of Miscarriage When Embryos are Treated with Nicotine Using a PCR-Based Approach to Gene Analysis

<u>James Dew</u> and Kristin Ackerman* Department of Biology High Point University

Miscarriage is the leading cause of failed pregnancy with around 50% of pregnancies ending in miscarriage. The causes of miscarriage, particularly in early development, are widely unknown with chromosomal abnormalities, lack of maternal factors, and physiological defects being the main causes known. Miscarriage rates are documented to increase with nicotine exposure, yet, genetic and molecular links have not been identified. *Esthonyx* Animal models to study miscarriage mechanisms in combination with nicotine exposure are limited; thus, we aim to characterize and validate zebrafish as a model system of miscarriage. Preliminary data indicate that spontaneous mortality rates in zebrafish range from 2% to 33% +/.082% (n=889) per developing clutch and occurs between 12 and 18 hpf (which correlates with the timing of human miscarriage). Lethal dose of nicotine was discovered and development was monitored specifically at 2, 6, and 12 hpf. Contrary to the hypothesis that nicotine would cause increased death at any concentration, it was found that low concentrations of nicotine are protective and those embryos develop faster. To verify that nicotine penetrated embryonic tissue, we utilized a PCR-based approached and observed an upregulation in both cFos and pax6b. Additionally, we show transient changes in gene expression of a4 and a7 neuronal nicotinic acetylcholine receptors.

(Fr.7) Neuroprotective Effects of Cannabidiol (CBD) Following an In Vitro Oxygen-Glucose Deprivation Model

<u>Jacob Dunn</u>, Sawyer Lyons and Michael Grider* Department of Biology High Point University

Strokes are the third leading cause of death in the United States. However, there is no currently FDA approved drug to promote neuroprotection once injury occurs. While numerous studies have identified protective effects of Cannabidiol (CBD), other studies have found that under different conditions CBD causes no significant neuroprotective effect. Therefore, we tested CBD in two neuronal cell lines, PC-12 and RN33B, under various injury conditions. Cells were exposed to oxygen-glucose deficient media with 100 μ M cobalt chloride for twenty-four hours. We used various concentrations of CBD (0.125-10 μ M) to potentially act as a neuroprotective treatment against the injury. The cells were then tested for metabolic viability (MTT assay) or acute cellular damage (LDH Cytotoxicity Assay). Preliminary data indicates that our injury model effectively damages cells, however, the conditions under which CBD attenuates injury requires more investigation.

(Th.19) Hibernation's Effects on the Platelet Counts of Woodchucks (Marmota Monax) in Wisconsin

<u>Katelyn Greer</u>, Michaela Connors and Brett Woods* Department of Biology High Point University

The objective of this study is to measure the platelet counts of woodchucks (Marmota monax) before and throughout hibernation. Marmots prepare for hibernation throughout the spring and summer months to store fat as a fuel for the body to burn over extended periods of inactivity. Torpor is known as the "deep sleep" that occurs in hibernation. During torpor, all metabolic function slows to a crawl, almost to the point of death. The heartbeat can slow to as few as 4-10 beats per minute. Blood flow comes almost to a standstill as it pools in different areas of the body. Brain activity decreases significantly. All of these processes occur for long, extended timeframes, possibly weeks. Marmots may experience multiple periods of arousal throughout winter hibernation in which their bodies quickly return to full function and full health with no correlating complications. When comparing the marmot's circulatory system to that of a human's, the marmots are better equipped to adjust to environmental extremes. Due to inactivity, humans consistently face issues of clotting, inflammation, soreness, stiffness, and other symptoms. Woodchucks, however, do not experience these symptoms despite the fact that their bodies are on the verge of death during hibernation. What allows woodchucks to be so adaptable? Through counts of erythrocytes, leukocytes, and platelets of blood samples taken from different woodchucks at different stages in preparation for and during hibernation, data expresses that there are more platelets present and less leukocytes in blood during hibernation.

(Fr.4) Photophysical Characterization of Novel Rhodamine B Dimers

Nathan Grinalds, Pamela Lundin* and Keir Fogarty*
Department of Chemistry
High Point University

Rhodamines are popular and robust fluorescent dyes used in fluorescence microscopy, fluorescent tagging, and flow cytometry. To investigate how the spatial arrangement of fluorophores affect photophysical properties, novel dimers of rhodamine B were synthesized and characterized. The dimers' structure and purity were assessed using NMR, chromatography, and mass spectrometry. The dimers were analyzed in ethanol and water using fluorescence correlation spectroscopy, ultraviolet—visible spectroscopy, fluorometry, and molecular dynamic simulations which revealed interesting spectral shifts and photophysical properties in comparison to the rhodamine B monomer. Containing a 1,4-butanediamine linker, the "flexible" dimer was shown to be redshifted relative to the rhodamine B monomer, likely attributed to more efficient pi stacking enabled by the linker.

(Th.8) Understanding the Mechanism of Photo-Redox Catalysis

<u>Christopher Goudarzi</u> and Keir Fogarty* Department of Chemistry High Point University

Photo-redox catalysis is a phenomenon that uses energy from a light source to catalyze a reaction. Applied in solar chemistry, green chemistry, and synthetic chemistry, photo-redox catalysis is a popular catalysis technique, but the mechanism is yet to be fully understood. The purpose of our experiment is to understand how photo-redox catalysis works. In photocatalysis, light excites the electron of the catalyst. If energy held by the excited electron is not used for a chemical reaction, energy will be lost in the form of fluorescence. The energy obtained by the excited electron can also be used for a chemical reaction, causing the fluorescence to be quenched. The process of quenching and photo-catalysis can be studied using Stern-Volmer plots. Stern-Volmer plots are created by plotting fluorescence intensity versus quencher or reactant concentrations. Linear relationships from a Stern-Volmer plot indicate that simple interactions occur between the catalyst and reactant. Non-linear relationships from a Stern-Volmer plot indicate more complex interactions between the catalyst and reactant. This summer, we have studied a variety of photocatalytic processes of iridium organometallics, and results indicate highly variable mechanistic behavior.

(Th.4) Determining Accurate Locations of Fossils Localities of the Bighorn Basin with GIS and GPS

Alan Hsueh, Kennedy Jackson and Christian George*
Department of Biology
High Point University

Our research consisted of a combination of Geographic Information Systems (GIS) analysis, and fieldwork collecting fossils in the Willwood Formation of the Bighorn Basin, Wyoming. This formation contains abundant fossils from the Early Eocene, and spans the Wasatchian Land Mammal Age which lasted from roughly 56 to 52 million of years ago. The Early Eocene began with rapid global warming which characterizes the Paleocene-Eocene boundary, and had multiple significant climate fluctuations. The climate events are recognized at a number of points through the Willwood formation, and our fieldwork consisted primarily of collecting fossils that were deposited during two of the most dramatic climate events, ETM2 and H2. During these events, there was substantial faunal turnover that has been recognized as Biohorizon B. In order to accurately collect localities that represent these intervals, we needed to assess their stratigraphic position using a combination of GIS and GPS. The challenge of associating each locality to a specific interval is that not every layer is at the same depth across the basin. To assist our fieldwork, we utilized a GIS database and Trimble software for high accuracy GPS positions, and traced the shape of the localities from the real world into our virtual maps.

(Th.1) Analyzing the Role of Tat-SF1 in HIV-1 RNA Stability and Export

Molly Hulver¹, Amanda Goodwin² and Heather Miller^{1,*}

¹Department of Chemistry

²Department of Biology

High Point University

The retrovirus human immunodeficiency virus type 1 (HIV-1) requires various cofactors for transcriptional and post-transcriptional regulation. In previous research, it was determined that Tat-specific Factor 1 (Tat-SF1), a human protein, aids in post transcriptional regulation of the different size classes of HIV-1 RNAs. However, the molecular mechanism behind this is unknown but data would be consistent with roles in RNA splicing, export, and/or stability. We hypothesized that if Tat-SF1 has a role in HIV RNA export, then knocking it down will result in changes to the nuclear and cytoplasmic HIV RNA levels. We also used these cells to better understand the role Tat-SF1 may play in RNA stability, examining the persistence of RNA levels over time. To do this, we first transfected HeLa cells with an shRNA-expressing plasmid targeting Tat-SF1 to knockdown the gene, followed by a transfection with pSG3∆env, a non-infectious HIV-1 plasmid. Treating the transfected HeLa cells with actinomycin D to halt cellular transcription allowed us to analyze viral RNA stability. In addition, fractions containing the cytoplasm and nucleus were purified from the HeLa cells to examine nuclear export. Lastly, reverse transcription quantitative PCR was performed to compare the levels of the three HIV-1 RNA size classes in the knockdown vs. control samples. Our results show that Tat-SF1 selectively stabilizes some of the HIV RNAs. Preliminary results suggest that Tat-SF1 plays a role in exporting the unspliced HIV RNAs to the cytoplasm. Future work will involve quantifying the other two HIV RNA size classes and how they are exported.

(Th.15) Using GIS to Analyze the Effect of Climate Change on Mammalian Fossil Communities During the Early Eocene

Kennedy Jackson, Alan Hsueh and Christian George*

Department of Biology High Point University

The Willwood Formation (upper Paleocene and lower Eocene) of the Bighorn Basin, Wyoming is known for its vast sedimentary deposits containing fossils that spans roughly 56-52 million of years ago. Geologists and paleontologists study the fossils found in the Bighorn Basin because they provide one of the few examples of continuous deposition of fossils over a long time interval that includes several significant climatic events. The purpose of this study was to begin to evaluate a number of different environmental parameters, such as temperature, precipitation, paleosol stage, and their effect on faunal diversity through major climatic events of the Early Eocene, and combine these environmental variables with the locations of fossil sites. The Bighorn Basin has been collected for over a century and this has generated over 2000 fossil localities. These localities have been incorporated into a Geographic Information Systems (GIS) database that allows us to discern spatial patterns in the geographic and chronological distribution of fossils. In order to accurately correlate environmental changes with the changes in mammal fossil communities, we need to verify the stratigraphic position of sites that were deposited during periods of climate change. In Wyoming, we collected a large number of fossils and were able to precisely tie these specimens to their stratigraphic position. These data will enable us to better model the paleoecology of the mammal community of the Early Eocene.

(Th.18) Molecular Synthesis to Understand the Charge Transfer Between the Donor and Acceptor Components of an Organic Polymer

Soo Min Lee and Pamela Lundin* Department of Chemistry High Point University

The demand for renewable energy sources is consistently increasing for the sustainable health of the environment. Solar energy is a promising renewable energy source, and organic-based photovoltaics are particularly attractive due to their flexibility and tunability. Single-component organic photovoltaics incorporate both the donor and acceptor moieties into a single molecule, and have the potential to simplify device processing. The goal of this project is to identify suitable molecules for single-component active materials by understanding the charge transfer process between the donor and acceptor. Computations have identified promising configurations, and we are synthesizing the individual donor and acceptor molecules, as well as the coupled donor-acceptor dyad. The synthetic results were analyzed and confirmed using ¹³C and ¹H nuclear magnetic resonance spectroscopy and mass spectrometry, and will be presented here.

(Th.6) Synthesis of Computationally-Derived ERK2 Substrates to Probe Kinase Activity During Oxidative Stress

Will LeFever¹, Juliana O'Brien¹, Leann Werner¹, Leslie Poole², Ming Dong³, Rob Newman⁴, Anthony Postiglion⁴ and Andrew Wommack^{1,*}

¹Department of Chemistry High Point University ²Department of Biochemistry Wake Forest School of Medicine Winston Salem, NC ³Department of Chemistry ⁴Department of Biology North Carolina A&T State University Greensboro, NC

Extracellular signal-regulated kinase 2 (ERK2) is a mitogen-activated protein kinase and serves as an important relay in phosphorylation signaling cascades. A substrate termed Sub-D was predicted to bind at the D-recruitment site (DRS) on ERK2 through computational modeling studies. Using solid-phase peptide synthesis, we synthesized Sub-D along with anionic and cationic derivatives to compare differences in phosphorylation levels when introduced to ERK2 in the presence or absence of oxidative challenge. Additionally, we will use X-ray crystallography to characterize the predicted interactions between ERK2 and derivatives in order to develop a deeper understanding how oxidative stress affects the phosphorylation efficiency of ERK2.

(Fr.2) Dental Anatomy and Variation in Esthonyx (Mammalia)

Ricki A. Luongo, Hannah K. Cozart and Heather E. Ahrens*
Department of Biology
High Point University

The extinct clade Tillodontia includes numerous species representing relatively common, specialized herbivores of the Paleogene. The Eocene (56 – 33.8 mya) fossil record of western North America contains abundant fossils representing the tillodont lineage, *Esthonyx*. Due to the abundance of specimens and their specialized diet, *Esthonyx* from the Bighorn Basin, Wyoming, provides a valuable case study in phenotypic variation through an extended temporal scale. Our objective was to obtain both qualitative and quantitative data describing morphological variation between species and through time. Using tpsDig2, 12 landmarks were placed on several dental loci (p4, m1, m2, m3) of twenty specimens using photographs taken in occlusal view. The landmarks were exported into MorphoJ, in which we applied Procrustes superimposition to the geometric morphometric data and ran a principal component analysis (PCA). Based on PC1, the majority of shape variation, though very slight, occurred within the trigonid. When teeth were examined by both species and temporal unit (biochron), distinct species groupings were difficult to distinguish because of the well-conserved dental morphology within the *Esthonyx* lineage. The limited overall sample size and oversampling of the Wa-6 biochron reduced the power to detect species and temporal differences; further sampling should aid in examining patterns of variation.

(Th.7) Therapeutic Potential of Cannabidiol in an In Vitro Model of Ischemia

Sawyer Lyons, Jacob Dunn and Michael Grider*
Department of Biology
High Point University

Cerebral ischemia, stroke as a result as the blockage of blood flow, is a serious condition that leads to the death of brain cells via oxygen and glucose deprivation. To test a potential treatment for ischemia, we used cannabidiol (CBD), a compound derived from the cannabis sativa plant. Some studies have shown promising results with the application of CBD in a model of ischemia. To model cerebral ischemia, PC 12 cells and RN33B cells were placed in glucose free media in an oxygen free chamber for twenty-four hours. Concurrent with injury, cells were treated with varying concentrations of CBD. Cell viability was examined with multiple assays (MTT, LDH) to determine the potential role of CBD in neuroprotection. We are continuing our investigation of CBD under various injury models and in different cell lines to determine its neuroprotective effects.

(Th.2) Developing and Validating a Damage Paradigm to Examine Neuronal Regeneration

<u>Jeremy Muhr</u> and Kristin Ackerman* Department of Biology High Point University

Zebrafish (*Danio rerio*) are a powerful model system in the field of regenerative biology. Compared to mammals, zebrafish possess an innate capacity to regenerate a multitude of tissues/organs including brain, fin, heart, kidney, retina, and spinal cord. The teleost retina is a model for studying cellular and molecular mechanisms underlying retinal regeneration because following neuronal death, Müller glia undergo cell division to yield neuronal progenitors that continue to proliferate, migrate, and differentiate into the lost retinal cell. While avian and mammalian Müller glia exhibit limited proliferation, they cannot regenerate significant numbers of neurons and restore vision. The number of colleges and universities with undergraduate neuroscience programs has greatly risen in last five years, including programs at Primarily Undergraduate Institutions (PUIs). Thus, the goal of this project was to provide a protocol for PUI laboratories to be able to study neuronal regeneration. Due to their low cost, short reproductive cycles, and well-mapped genome zebrafish are ideal organisms for use in small class settings. A new retinal damage paradigm was developed to allow for simple and effective study of the regeneration process modified focused-light lesion damage. Fish were damaged for ninety minutes at 80,000 lux and either allowed a three-day recovery period, or a two-day continuous light damage at 20,000 lux. After each paradigm was completed, the fish were sacrificed, enucleated, and tissue was fixed in 9:1 ethanolic formaldehyde before frozen cryosections were obtained. Damage was assessed through DAPI-labeling to visual cell nuclei and will continue with immunofluorescent-labeled proliferating progenitor cells (PCNA). We propose these models as an inexpensive and manageable way to study regeneration at PUIs.

(Fr.5) Synthesis of Carbetocin Using Photochemical Cyclization Conditions

<u>Juliana O'Brien</u>, Hannah Lee Dixon, Leann Werner, Emma James Barksdale, Olivia Tornow, Melissa Srougi and Andrew Wommack*
Department of Chemistry
High Point University

Oxytocin (OT) is a naturally-occurring human hormone and neurotransmitter that is involved in myriad physiological and psychological phenomenon. Produced in the hypothalamus, OT is a nonapeptide that possesses a single disulfide bond that is critical to both structure and function. In addition to administering OT to induce uterine contractions during childbirth, one common chemotherapeutic use of synthetic oxytocin is for the prevention of postpartum hemorrhage. For these healthcare applications, OT is stable at low temperature for prolonged supply chain storage. However, in areas without access to consistent refrigeration, use of OT is compromised, as disulfide-mediated decomposition occurs rapidly at ambient temperature. Highlighting the need for an improved thermostable OT derivative, Ferring Pharmaceuticals has licensed carbetocin, which contains a disulfide to thioether substitution allowing dramatically improved shelf life. Our research concerning photochemical construction of thioether linkages has facilitated improved synthetic access to carbetocin. Alongside structural characterization, functional analysis of our synthetic carbetocin was determined in collaboration with the Srougi Lab at High Point University.

(Th.3) Characterization of the Mannose-6-Phosphate Receptor Homology Domain in the Atg27 Protein

Molly C. Penton, Candyce M. Sturgeon and Veronica A. Segarra* Department of Biology High Point University

Eukaryotic cells survive stress and starvation by activating a recycling process known as autophagy. Autophagy defects are frequently observed in human disease, however the therapeutic benefits of correcting them remain unknown. A promising target for emerging therapies is the membrane trafficking events leading to the formation of the autophagosome, a unique vesicle that envelops materials destined for recycling. Our research examines the function of Atg27, a single-pass transmembrane protein that helps coordinate this process, using budding yeast as a model system. Atg27 is found in the membranes of the Golgi, early/late endosomes, and the vacuole. While its cytoplasmic domain ensures proper localization, the function of its large lumenal domain remains largely unknownalthough it structurally resembles proteins of the mannose-6-phosphate receptor homology (MRH) domain family, which bind N-glycans and often sort glycosylated proteins through the endomembrane system. We have constructed a MRH-abrogated Atg27 mutant that is stably expressed and continues to localize to the membrane. Using growth assays and biochemical assays we show that cells expressing this mutant are able to carry out autophagy.

(Th.13) Nanopatterning Conjugated Polymer Growth By Microcontact Printing

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Conjugated polymers have a wide array of potential uses in fields such as renewable energy through their use in organic electronic devices such as organic photovoltaics. Typically, these devices are fabricated with pre-synthesized polymers that are cast as films onto a substrate. However, the nature of the film morphology directly impacts device performance. Being able to grow a conjugated polymer directly from a functionalized surface could lead to higher organization and tighter packing between the molecules, therefore creating more effective devices. Self-assembled monolayers (SAMs) are used to functionalize surfaces with molecules capable of initiating conjugated polymer growth. We are functionalizing SiO_2 and gold surfaces with a new SAM that initiates polymerization, and later can be selectively cleaved to release the polymer. In particular, we are using microcontact printing (μ -CP) to nanopattern our substrates so that we can use atomic force microscopy to show that polymerization only occurs in the presence of our SAM, and then is removed under our specific conditions.

(Th.10) Drosophila melanogaster as a Model to Examine the Neurological Effects of Cannabinoids

<u>Jake Schleppy</u>, Kendall Ziegler and Jackson Sparks* Department of Biology High Point University

Cannabidiol (CBD) is a non-psychoactive component of marijuana known to agonize mammalian serotonin receptors and prevent seizures in humans. The vinegar fly *Drosophila melanogaster* presents functionally conserved serotonergic neurons similar to those of mammals; thus, flies may be a useful model for determining the molecular nature of CBD action. Though larval olfaction appears unaffected by CBD exposure, we have measured behavioral changes in adult and larval flies resulting from CBD ingestion and are working towards determining bioavailability in the adult head. Once we confirm physiologically relevant absorption via feeding, we will similarly administer known serotonin receptor agonists to confirm like behavioral effects. Developmental rate appears to be affected by CBD, but more tests are needed to rule out non-pharmacological effects. We have obtained serotonin receptor knock-out fly lines to demonstrate that behavioral changes are dependent on the activity of this class of receptor. Future efforts include measuring differential expression of all transcripts between CBD-fed and control flies to screen for involvement of other genes not associated serotonergic activity as well as assaying olfactory learning in larvae.

(Fr.1) Evaluation of Antibiotic Adjuvant Scaffolds for Mechanism of Action and Broader Therapeutic Potential

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Department of Chemistry
High Point University

In the United States, over two million people acquire an antibiotic resistant infection every year. As bacterial resistance to antibiotics grows, the search for new methods to kill bacteria becomes increasingly urgent. Some classes of compounds can act as antibiotic adjuvants by re-sensitizing resistant bacteria to existing antibiotics. Adjuvants alone are not toxic to bacteria. Instead, they interfere with bacterial resistance mechanisms thereby rendering the bacteria susceptible to the antibiotics. In the Blackledge lab, several compound classes have been discovered as effective adjuvants in methicillin resistant Staphylococcus aureus (MRSA). To better understand how our compounds potentiate b-lactam antibiotics in MRSA, we screened our compounds against several mutants from the Nebraska Transposon Mutant Library (NTML) to identify potential targets. In addition to understanding the biological mechanisms that these compound classes are using in MRSA, we wanted to determine if these adjuvants are effective in other clinically relevant bacteria. We tested these compounds against a collection of both gram-positive and gram-negative bacteria and evaluated these compounds for both antibiotic and antibiotic adjuvant activity. Results of our screening assays will be presented along with preliminary conclusions about potential adjuvant targets in MRSA and biological activity across other bacterial species.

(Th.14) Characterization of the Functional Relationship Between Atg27 and the YML018C Protein

<u>Candyce Sturgeon</u>, Molly Penton and Veronica Segarra* Department of Biology High Point University

Atg27 is a transmembrane protein important in the catabolic process of autophagy. Autophagy is a cellular method of recycling used when the cell is subjected to stresses like starvation. Atg27 is known to be involved in this process, although not all of its functions have been characterized. Atg27 is found in the membranes of cellular compartments that include the Golgi apparatus, early/late endosomes, and the vacuole. This protein is also known to have a physical interaction with the heretofore uncharacterized protein YML018C. The purpose and timing of these interactions are not known, although it is known that the YML018C protein localizes to the vacuolar membrane. The purpose of our research is three-fold: (1) to confirm YML018C localizes to the vacuolar membrane, (2) to characterize the defects associated with deletion of this protein with unknown function and (3), since Atg27 is currently thought to function as a shuttle protein or trafficking adaptor, to determine whether the ability of the YML018C protein to get to the vacuole is dependent on Atg27. To determine this, we obtained and/or constructed cells lacking specific genes (such as YMML018C\times and cells containing YML018C-GFP, but lacking Atg27.

(Th.12) Investigating Tat-SF1 Interactions With HIV RNA

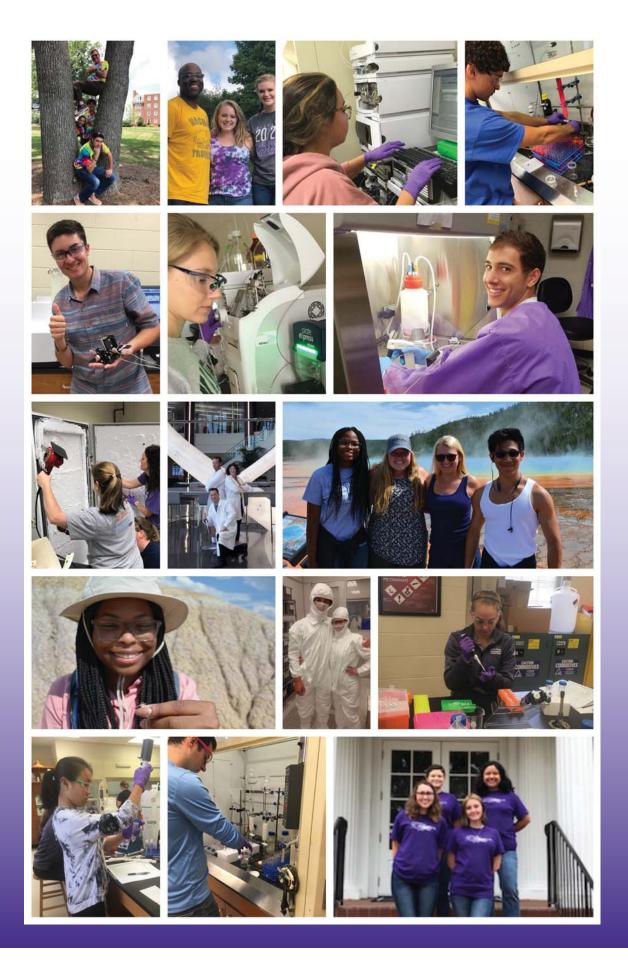
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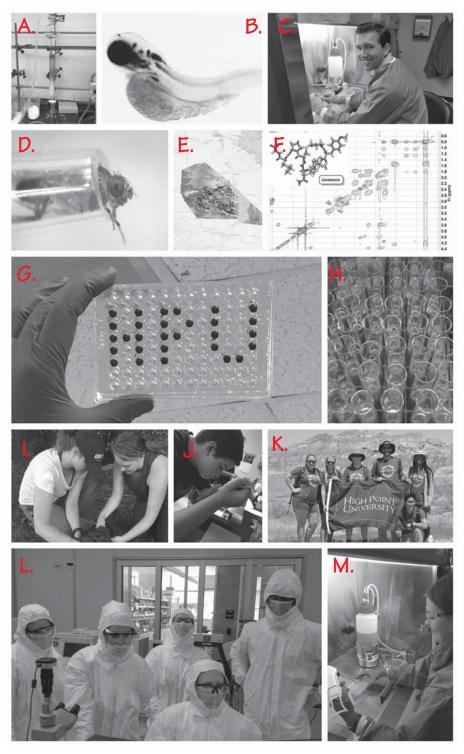
Little is known about the human protein Tat-specific factor 1 (Tat-SF1) and its role in the production and regulation of the Human Immunodeficiency Virus (HIV). It is known that Tat-SF1 is a factor during the transcriptional elongation process. This human protein is also involved with alternative splicing and possesses two RNA recognition motifs typical of RNA binding proteins. Tat-SF1 also upregulates HIV infection and alters the relative levels of HIV RNAs, but exactly how this occurs is unknown. The hypothesis for this study was that if Tat-SF1 helps stabilize, splice and/or export HIV RNA, it must interact with the RNA physically. This work attempts to determine if Tat-SF1 interacts with HIV-1 RNA using in vitro RNA binding assays. Fragments of the HIV-1 genome were made using PCR, followed by DNA sequencing and in vitro transcription. Cell lysates were made using E. coli that expressed Tat-SF1 and glutathione S-transferase (GST). Protein expression was induced by using isopropyl β-D-1-thiogalactopyranoside (IPTG). To confirm protein expression, Western blotting was performed. These cell lysates, purified Tat protein, and the HIV RNA fragment, were used to perform Electrophoretic Mobility Shift Assays (EMSA) and Florescence Polarization (FP), two commonly used binding techniques. Results showed that HIV RNA as well as protein lysates overexpressing Tat-SF1 were successfully made. Preliminary binding data will also be presented.

(Fr.3) Cannabidiol Bioavailability in *Drosophila melanogaster* and Impact on Behavior and Serotonin Levels Kendall Ziegler, Jake Schleppy and Jackson Sparks* Department of Biology High Point University

Cannabidiol (CBD) is the non-psychoactive component of the *Cannabis sativa* plant that has been used to treat seizures in conditions that are resistant to common anti-epileptic agents. CBD may act as a serotonin receptor agonist (5-HT1a receptor), which is highly conserved between *D. melanogaster* and humans. This receptor of the serotonergic pathway can be regulated by various drugs to treat several medical conditions. To observe behavioral differences in *D. melanogaster*, wild type flies were exposed to either acute or chronic feeding conditions. Flies of same sex and age were isolated and starved for 3 hours before acute feeding with either CBD in ethanol/sucrose solution or control ethanol/sucrose solution until satiation. Chronically exposed flies were raised in vials containing either control food or CBD + control food. Climbing assays were performed to observe significant differences in CBD-fed flies' ability to walk in comparison with that of control-fed flies. Following acute feeding, CBD-fed flies took more than twice as long on average as control-fed flies to climb a short distance. We are also measuring larval behavior in response to a small panel of odorants. A serotonin enzyme-linked immunosorbent assay (ELISA) kit will be used to determine the concentrations of serotonin in samples obtained from fly heads of each experimental group in order to determine if CBD is affects serotonin output or reuptake. Mass spectrometry will be used to determine bioavailability.

More Scenes from SuRPS 2018





(A.) Chromatography in the Lundin Lab; (B.) *In-situ* hybridization in zebrafish embryo from the Ackerman Lab; (C.) Cell culture work in the Grider Lab; (D.) A feeding fruit fly in the Sparks Lab; (E.) GIS data of dinasour fossils from the Ahrens/George Labs; (F.) 2D NMR data of carbetocin from the Wommack Lab; (G.) 96 well plate assay from the Blackledge Lab; (H.) Fluorescent samples from the Fogarty Lab; (I.) Field work with woodchucks from the Woods Lab; (J.) Loading sample into PCR for gel electrophoresis in the

