2015

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

Thursday, July 21, 2015
Phillips 120

2015

High Point University

Summer Undergraduate Research Program in the Sciences (SuRPS)

SuRPS KEYNOTE SPEAKER

"Statistical and combinatorial approaches to designing repeat proteins as recognition elements in microbial sensors"

Ms. Rachael Parker (HPU Chemistry Class of 2011)
Department of Chemistry, Virginia Tech University

ABSTRACT

Rachael Parker, Ana Mercedes and Tijana Z. Grove

Repeat proteins have recently emerged as especially well-suited alternative binding scaffolds due to their modular architecture and biophysical properties. Here we present the design of a scaffold based on the consensus sequence of the leucine rich repeat (LRR) domain of the NOD family of cytoplasmic innate immune system receptors. Consensus sequence design has emerged as a protein design tool to create de novo proteins that capture sequence-structure relationships and interactions present in nature. The multiple sequence alignment of 311 individual LRRs, which are the putative ligand-recognition domain in NOD proteins, resulted in a consensus sequence protein containing two internal and N- and C- capping repeats named CLRR2. CLRR2 protein is a stable, monomeric, and cysteine free scaffold that without any affinity maturation displays micromolar binding to muramyl dipeptide, a gram negative bacterial cell wall fragment. To our knowledge, this is the first report of direct interaction of a NOD LRR with a physiologically relevant ligand. We are using CLLR2 as a starting point for development of a synthetic module that specifically binds glycolylated muramyl dipeptide, a cell-wall fragment specific to *Mycobacteria* with the goal of developing inexpensive point-of-care diagnostic devices based on direct recognition of *Mycobacteria* cell wall.

SuRPS Final Symposium

(Thursday, July 16, 2015, Phillips 120)

Session A: Dr. Neil Coffield, Department of Biology, Presiding

	8:00 – 8:45		*CONTINENTAL BREAKFAST (Phillips 120)*
	8:35 - 8:45	Dr. Brian Augustine	Opening Remarks
A.1	8:45 - 9:00	Jimmy Rager	An Investigation into the Effects of 17β-estradiol on Bone Development and Ossification in *Danio rerio*
A.2	9:00 - 9:15	Gabrielle Hayes	Antimicrobial Interactions with Manuka Honey on *Staphylococcus aureus*
A.3	9:15 - 9:30	Elizabeth Reardon	The Effects of UV Radiation on *Diaphanosoma* spp. and Other Cladocerans
A.4	9:30 - 9:45	Matthew Beck	The Role of Rac1 on Cell Migration in ATM Deficient Cells
A.5	9:45 - 10:00	Nicole Bayeur	Effects of Light Environment on New Zealand *Nothofagus* (Southern Beech) Community Ecology
A.6	10:00 - 10:15	Alan Vasquez	There and Back Again?: The Pulsational Changes of CS1246
A.7	10:15 - 10:30	Evan Shaw	Importance of Specific Amino Acids on the *E. coli* MazEF Toxin-Antitoxin System
	10:30 - 10:45		*BREAK*

Session B: Dr. Briana Fiser, Department of Physics, Presiding

B.1	10:45 - 11:00	Maria Valverde	Cloning and Purification of the Transcription Regulator GerE from *Bacillus* Species for In-Vitro Analyses
B.2	11:00 - 11:15	Calla Telzrow	Solid-Phase Peptide Synthesis and Antimicrobial Assessment of a Plant-Derived Cyclic Peptide
B.3	11:15 - 11:30	Sergio Guillen	An Investigation of the Effects of Triclosan on Human Breast Cancer Cells
B.4	11:30 - 11:45	Ryan Casey	The Effects of ATM-Mediated Reactive Oxygen Species Generation on Cell Migration
B.5	11:45 - 12:00	Nicole Wright	Combinatorial Effects of Antibiotics and Manuka Honey on *Escherichia coli*
	12:00 - 1:00		*LUNCH (Wanek Center 2nd Floor)*

Keynote Address: (Introduction by Dr. Todd Knippenberg, Department of Chemistry)

	1:00 - 2:00	**Rachael Parker (HPU Class '11)**	**"Statistical and Combinatorial Approaches to Designing Repeat Proteins as Recognition Elements in Microbial Sensors"**

Session C: Dr. Cindy Viguiera, Department of Biology, Presiding

C.1	2:00 - 2:15	Rodrigo Catalan-Hurtado	Clash of The Titans: A New Blue Supergiant Binary System
C.2	2:15 - 2:30	Tyler Wilson	The Effects of UV Radiation on Calanoid Copepods
C.3	2:30 - 2:45	Halley Watson	Population Structure of the Spotted Salamander (*Ambystoma maculatum*) in North Carolinian Ponds
C.4	2:45 - 3:00	Matthew Carnaghi	Core-Shell Biomimetic Cilia Arrays for Use in Fluid Propulsion
C.5	3:00 - 3:15	Rebecca Ulrich	Probing the Structure-Activity Relationship of *Escherichia coli* Extracellular Death Factor
C.6	3:15 - 3:30	Nicole Clark	The Investigation of the Transcription Regulator GerE in Clostridium Species
	3:30 - 3:45		*BREAK*

Session D: Dr. Meghan Blackledge, Department of Chemistry, Presiding

D.1	3:45 - 4:00	Lisa Nguyen	Extracellular Death Factor and Programmed Cell Death in *Escherichia coli* and *Bacillus subtilis*
D.2	4:00 - 4:15	Max Maurer	Biomimetic Cilia-Driven Fluid Flow in Agarose
D.3	4:15 - 4:30	Christopher Wagner	Comparative Genetics of Parallel De-domestication Events in Weedy Red Rice (*Oryza sativa*)
D.4	4:30 - 4:45	Harris Coley	Biochemical Analysis of Abaxially Variegated Leaves
D.5	4:45 - 5:00	Cailyn Scanlan	A Study into the Effects of Resveratrol on Bone Development in *Danio rerio*
	5:00 - 5:10	Dr. Angela Bauer	Closing Remarks

STUDENT ABSTRACTS:

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

(A.5) Effects of Light Environment on New Zealand *Nothofagus* (Southern Beech) Community Ecology

Nicole Bayeur, Kaylyn Carpenter, Kevin Gould, Nicole Hughes*
Department of Biology
High Point University

Two indigenous *Nothofagus* (Southern beech) taxa dominate high elevation forests in the Southern Alps of New Zealand. *Nothofagus solandri* var. *cliffortioides* tends to dominate on the eastern side of the range where the climate is sunny and dry, while *N. menziesii* either dominates or co-dominates with *N. solandri* var. *cliffortioides* on the wetter, western side of the range. Previous research suggests that a greater tolerance for drought may explain dominance of *N. solandri* in drier regions; however, the factors which render *N. menziesii* an effective competitor in wetter climates are unclear. We hypothesized that greater shade tolerance in *N. menziesii* would allow for greater photosynthesis under cloud cover. Photosynthetic measurements were taken on cloudy and sunny days at two sites along the central parts of the range where the species co-exist. Photosynthesis was also compared in the forest understory to determine whether *N. menziesii* would exhibit greater photosynthesis in this habitat as well. Consistent with our hypothesis, *N. menziesii* had significantly higher photosynthesis under cool, cloud-immersed conditions at one site; however, under warmer, more cumuliform cloud cover at the other site, no significant difference was observed. In addition, no significant difference in photosynthesis was observed between the species in either of the forest understories. We suspect that increased shade tolerance of *N. menziesii* may only translate into a photosynthetic advantage under the combination of cooler temperatures and very low sunlight levels, typical of heavy cloud cover and/or shade at high elevations, though further studies under a greater range of cloud regimes and altitudes are needed to corroborate this hypothesis.

(A.4) The Role of Rac1 on Cell Migration in ATM Deficient Cells

Matthew Beck, Melissa Srougi*
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Michaela Rikard
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Ataxia-telangiectasia (A-T) is a genetic condition associated with a lack of ataxia-telangiectasia mutated (ATM) protein, which is crucial in repairing double-stranded DNA breaks (DSBs). A-T patients suffer from neurodegeneration, immunodeficiency and a predisposition to metastatic cancer. The Rho-family of small GTPases including Rho (A, B, C), Rac, and Cdc42 also play key roles in tumorigenesis by controlling cellular migration and adhesion. The activation of Rho proteins is tightly regulated; when bound to GTP they are active, and when bound to GDP they are inactive. Traditionally, only guanine nucleotide exchange factors were thought to assist in GTP loading, however previous work has shown Rho protein activation directly through oxidation by reactive oxygen species (ROS). Work in our lab has shown that loss of ATM results in an increase in ROS that directly activates Rac1. We therefore hypothesize that an increase in activated Rac1 causes greater cell motility and migration in cells lacking ATM kinase activity. In order to test this hypothesis, siRNA was used to knock-down Rac1 in ATM inhibited cells to determine its effects on cell motility. A dose-time response with siRNA specific to Rac1 (siRac1) was performed and its effects on Rac1 protein levels determined. We found that a 25 nM treatment of siRac1 for 72 hours was optimal for Rac1 protein knock-down. We are currently using similar conditions to test for the role of Rac1 in ATM-mediated cell migration using wound healing assays. The findings from our studies will contribute to the mechanism-based understanding of A-T associated tumorigenesis.

(C.4) Core-Shell Biomimetic Cilia Arrays for Use in Fluid Propulsion

Matthew Carnaghi, Briana Fiser*
Department of Physics
High Point University

We are creating biomimetic cilia to model how biological cilia work in the lung. These biomimetic cilia are made by depositing nickel tubes in a porous polycarbonate track-etched (PCTE) membrane and filling the membrane with a flexible polymer. The membrane is then dissolved, and flexible polymer rods with nickel tube caps remain. These rods' responsiveness can be tuned by changing the length of the nickel tubes. They can be moved with magnetic fields, and when placed in fluids, the rods can drive the fluids. The fabrication and characterization of these biomimetic cilia will be discussed.

(B.4) The Effects of ATM-Mediated Reactive Oxygen Species Generation on Cell Migration

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High Point University

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Ataxia-telangiectasia (A-T) is an autosomal recessive disorder whose patients suffer from an increased prevalence of malignant cancers. A-T patients cannot produce the protein ataxia telangiectasia mutated (ATM) which is crucial in the response to double-stranded DNA breaks. Previous data has shown a connection between ATM and the Rho family of small GTPases. Rho proteins are involved in a multitude of cellular events ranging from cell migration, cell cycle arrest, cell division and cell death. Rho GTPases are a broad group of signaling proteins which include the family members Rho, Rac and Cdc42. Work from our lab has shown that loss of ATM increases reactive oxygen species (ROS), which directly activates Rac1. We therefore hypothesize that elevated ROS present in ATM inhibited cells activates Rac1 to increase cellular migration and growth. To test this, we used HeLa cells and inhibited ATM kinase activity using the selective inhibitor Ku55933. Using an immunoprecipitation based assay, inhibition of ATM resulted in elevated Rac1-GTP levels compared to control cells. Interestingly, ATM inhibition led to a delay in wound healing as compared to vehicle alone treated cells without a significant change in cell proliferation. These data may suggest that increases in activated Rac1 may strengthen cell-cell adhesions and thus decrease cell migration. Ongoing work will determine the effects of ROS on cellular migration in ATM inhibited cells following treatment with the scavenging agent N-acetyl cysteine. This work is an important step in understanding the etiology of A-T and provide the foundation for future therapies to ameliorate the disease.

(C.1) Clash of The Titans: A New Blue Supergiant Binary System

Rodrigo Catalan-Hurtado, Brad Barlow*
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High Point University

Blue supergiants are some of the most extreme stars we know of: they have masses up to 100 times that of the Sun and temperatures that are six times hotter. They evolve from massive main sequence stars like the Sun after they have fused all of the hydrogen in their cores to helium. HD 318015 is a newly-discovered blue supergiant binary system with an orbital period of 23 days. Interestingly, it is one of the most luminous binaries ever found. We used the 1.5-m SMARTS telescope in the Chilean Andes to obtain optical spectra of the binary for a time span of 36 days. Using specialized Python code we wrote, we were able to measure the individual velocities of the stars from the Doppler shifts of their atomic absorption lines. Through in-depth analysis of the system's light curve and velocity curves, we were able to solve for many of the binary parameters, including the stellar masses, temperatures, and radii. To our surprise, there seems to be a third star orbiting the binary system.

(C.6) The Investigation of the Transcription Regulator GerE in *Clostridium* Species

Nicole Clark, Dinene Crater*
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High Point University

Organisms of the *Firmicutes* Phyla including *Bacillus* and *Clostridium* are able to undergo a process called sporulation during a time distress. Sporulation involves a lengthy process in which the bacterium undergoes asymmetric cell division resulting in a mother cell and forespore. The mother cell engulfs the forespore and eventually lyses (after eight protective layers surround the forespore) leaving a spore that is almost indestructible. Sporulation in *Bacillus* cannot occur without the help of the transcription regulator GerE, a DNA-binding protein that controls gene transcription in the late stages of sporulation. *Clostridium*, however, is ancestral in comparison to *Bacillus* and sporulation is somewhat different. The transcription regulator SpoIIID is required for sporulation, but we believe that there must be more than one transcription regulator in *Clostridium*. We hypothesize that *Clostridium* species have a GerE-like transcription regulator. GerE from *Bacillus subtilis* binds to DNA, so we used that knowledge to identify homologous proteins in *Clostridium*. We used *Bacillus subtilis* as a control to study four different *Clostridium* species (*C. acetobutylicum*, *C.tetani*, *C. butyricum*, and *C. sporogenes*).We used a bioinformatics approach to conduct a genomic database search and found similar gene sequences amongst different *Clostridium* species. We then designed degenerative primers that were compatible to *gerE*; however, our PCR analysis using those primers was unsuccessful. Future directions will be to redesign the primers using known bioinformatics tools that will allow strong hybridization to *gerE* from related organisms.

Novel Patterning Techniques of Vapor-Deposited Au Thin Films onto Polymeric Substrates

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Special attention has been given to selective patterning of gold thin films onto plastic substrates because of applications in the production of cost-effective biomedical and microfluidic devices. Because gold is a chemically inert metal, it does not generally adhere well to plastic substrates and is difficult to pattern on the micrometer length-scale. In a previous study, gold adhesion to PMMA substrates was demonstrated to be improved through pre- and post-treatment with chloroform. This project examines vapor-deposited Au thin film adhesion to thin films of PMMA dissolved in chloroform and spun-cast as well as bulk PMMA samples. Films were spun-cast from solutions prepared with varying concentrations and molecular weights of PMMA. Additionally, films were prepared with PMMA dissolved in tetrahydrofuran (THF) and toluene for comparison. These films were then characterized with Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, single wavelength ellipsometry, and atomic force microscopy (AFM). Enhanced gold adhesion was observed in solutions prepared with chloroform, THF, and toluene as tested using a standard tape test. This could be explained by nanoporous surface features in films prepared with chloroform and THF; however, further investigation is necessary to understand the interactions between the films and gold. The enhanced adhesion of gold to PMMA by chloroform treatment was also spatially manipulated to develop a gold patterning technique using inkjet printing. Chloroform was deposited into patterns on bulk samples of PMMA using the Microfab 4 Inkjet Printer. After gold was deposited onto the PMMA, the excess gold was physically wiped away, leaving an array of gold dots where the chloroform was deposited. Further research could be conducted to develop arbitrary patterns and to determine the minimum feature size attainable with this technique.

(D.4) Biochemical Analysis of Abaxially Variegated Leaves

Harris Coley, Nicole Hughes*
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High Point University

The objective of our research is to shed light on why some plant species have red pigments (anthocyanins) on the bottom (abaxial) surface of their leaves. Anthocyanins located in upper (adaxial) surfaces of leaves have been shown to attenuate light and dissipate it as heat, essentially acting as a sunscreen. However, this causes lower photosynthetic capacity. Our current hypothesis is abaxial anthocyanins provide photo-oxidative protection, without reducing photosynthetic capacity. We initially examined leaves of *Colocasia esculenta* “Mojito”, which is variegated on both the adaxial (top) and abaxial surface of the leaf. This allowed us to compare the effects of anthocyanins on the top (RG), on the bottom (GR), both (RR), or neither (GG), to relevant photochemical processes in the leaf including xanthophyll pigment de-epoxidation, photosynthetic efficiency, and electron transport rate. However, during data analysis, we realized that many of our results were unreliable, and due to the rare occurrence of leaves suitable for experimentation, repeating the experiments in the time provided was not possible. We have since adjusted our methods and begun a new, similar project using two different (faster-growing) abaxially variegated species-- *Begonia heracleifolia* “Nigricans” and a cultivar of *Impatiens walleriana*. Our hypothesis remains that abaxial anthocyanic tissues contribute to lower photo-oxidative stress, while not compromising photosynthetic efficiency.

(B.3) An Investigation of the Effects of Triclosan on Human Breast Cancer Cells

Sergio Guillen, Kristen Bowey, Angela Bauer*, Neil Coffield*
Department of Biology
High Point University

Endocrine disrupting chemicals (EDCs) are environmental contaminants that have the ability to interfere with hormone signaling in the body. The incidence and/or prevalence of health problems associated with endocrine-disruption have increased. Reported adverse effects include (but are not limited to) declines in populations, increases in cancers, and reduced reproductive function. Triclosan is a potent antibacterial and antifungal compound widely used in personal care products, plastics, and fabrics, which has also been shown to alter endocrine function in a variety of species. In this study, we used the E-SCREEN assay to quantitatively detect the estrogenicity of triclosan in an estrogen-sensitive breast cancer cell line MCF-7/BOS. Cell proliferation was tested using a lumogenic ATP assay at different time intervals and a dose response. The upregulation of estrogen receptor alpha (ERα) was also studied by immunofluorescence.

(A.2) Antimicrobial Interactions with Manuka Honey on *Staphylococcus aureus*

Gabrielle Hayes, Patrick Vigueira*
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High Point University

Antibiotic resistance has become a major public health crisis with the rise in cases of methicillin and vancomycin resistant *Staphylococcus aureus* infections, and other resistant bacterial infections. With the increase in these difficult to treat illnesses, efforts to combat this resistance have become a major focus of research. However, due to the expensive and time consuming nature of creating new antibiotics and low economic return of investment, pharmaceutical companies have curbed antibiotic investigations in recent years. As a possible solution to the increasing rates of antibiotic resistance, approved pharmaceuticals and natural compounds have been investigated in conjunction with current antibiotics in an attempt to identify synergistic activity and reinvigorate sensitivity to standing treatments. A natural compound with synergistic potential is Manuka Honey. Manuka Honey has been demonstrated to possess antibacterial properties and is marketed as a possible treatment for wounds and other infections. Our initial screen using disk diffusion on plates containing the honey revealed synergistic relationships with *Staphylococcus aureus* and the following antibiotic discs: bacitracin, cefotaxime, linezolid, minocycline, nitrofurantoin, tetracycline, oxacillin, and sulfamethoxazole/trimethoprim. Interestingly, antagonistic effects were also seen with *S. aureus*, the manuka honey, and fosfomycin. These interactions will be confirmed and further defined through broth dilutions. Methyl glyoxyl, a compound thought to be the active antibacterial component in Manuka Honey, will be utilized independently in future experiments to confirm or deny its relevance as an antibacterial compound.

(D.2) Biomimetic Cilia-Driven Fluid Flow in Agarose

Max Maurer, Briana Fiser*
Department of Physics
High Point University

Mucociliary clearance is a complex process in the lung, which involves the coordination of arrays of biological cilia and the propulsion of the viscoelastic fluid mucus. When this process does not function as it should, diseases such as immotile cilia syndrome or cystic fibrosis can occur. Agarose is a polysaccharide derived from some red algae species, and has been shown to be similar to natural mucus. To further our understanding of biological cilia-driven mucus movement, a technique for creating core-shell rods was utilized to produce highly flexible and magnetically responsive biomimetic cilia with the goal of immersing these cilia in agarose to study the resulting fluid flow. Results on the creation of these rods and their immersion in agarose will be presented.

(D.1) Extracellular Death Factor and Programmed Cell Death in *Escherichia coli* and *Bacillus subtilis*

Lisa Nguyen, Rebecca Ulrich, Meghan Blackledge*
Department of Chemistry
High Point University

Using chemical signals known as quorum-sensing (QS) signal molecules, bacteria are able to communicate with each other. This project focuses on a specific QS molecule called the extracellular death factor (EDF), required for inducing programmed cell death (PCD) in *Escherichia coli* and *Bacillus subtilis*, among other species of bacteria, during periods of stress. Previous research has made it known that the wild-type EDFs of *E. coli* and *B. subtilis* (*Ec*EDF and *Bs*EDF, respectively) are known to be able to act as interspecies death triggers. Moreover, it has been shown which amino acid residues on the *Ec*EDF and *Bs*EDF peptides are required for activity. Using that information, this research lab is making an effort to analyze the structure-activity relationship of the EDF by synthesizing analogues of the peptides and observing their effects on cultures of *E. coli* and *B. subtilis*. Biological assay and peptide synthesis techniques are being improved on in order to effectively determine how the EDF mediates cell death intra-species and interspecies in bacteria.

(A.1) An Investigation into the Effects of 17β-estradiol on Bone Development and Ossification in *Danio rerio*

Jimmy Rager, Angela Bauer*, Neil Coffield*
Department of Biology
High Point University

Studies have demonstrated that estrogen plays a significant role in bone development and ossification in mammals. To date, however, little is known about the sex hormone's role in bone ossification in the zebrafish model, *Danio rerio*. In this study, zebrafish embryos were raised in various concentrations of 17β-estradiol (10^{-11} M - 10^{-9} M) for 14 days. Two staining methods, Alcian Blue/Alizarin Red and calcein were used to determine bone ossification and development. First, embryos treated with 17β-estradiol were stained with Alcian Blue and Alizarin Red, which stain for cartilage and bone, respectively. The specimens were scored for the extent to which their embryonic skeleton had ossified and the length of various endochondral bones and total body length were measured. The second method of staining used calcein dye, which adheres to calcium minerals and fluoresces under GFP-similar-wavelengths, indicating the onset of bone ossification. Lateral/dorsal images of the embryos were obtained from day six through day ten and each of the first fifteen vertebrae were then blindly scored based on the amount of stain present (0= none, 1= x<30%, 2= 30%<x<70%, 3= x>70%). Preliminary data from the first staining method suggests a significant dose-dependent increase in total body length and also an increase in the length of specific endochondral bones compared to untreated controls ($p<0.05$ and <0.005, respectively). Calcein staining data supports the notion that 17β-estradiol increases the rate of vertebral bone ossification, most drastically at day 7. Collectively, this data supports the hypothesis that 17β-estradiol may be responsible for the increased prevalence of skeletal abnormalities seen in animals living in areas contaminated with endocrine disruptors, such as golf courses and their potential impact should be further evaluated.

(A.3) The Effects of UV Radiation on *Diaphanosoma* spp. and Other Cladocerans

Elizabeth Reardon, Tyler Wilson, Sandra Cooke*
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High Point University

The objective of this study was to examine the effects of ultraviolet radiation (UV) on freshwater zooplankton species, with focus on cladocerans. Zooplankton exposure to UV may induce three responses: a DNA reparation process, known as photoenzymatic repair (PER), behavioral avoidance, and photoprotective pigmentation. The taxonomic group *Diaphanosoma spp.* has been largely understudied in its use of PER in the reparation of its DNA. We examined PER and behavioral avoidance through a series of experiments and field observations. Daytime vertical distribution occurred at two lake sites within North Carolina: low turbidity Reynolds Quarry Lake located in Winston-Salem and high turbidity High Point City Lake located in High Point. The experiments for UV-B exposure and the corresponding UV-A and visible light exposure, components of PER, occurred in a temperature-controlled incubator. Depth distribution data revealed that Diaphanosoma are largely found near the surface of Reynolds Quarry Lake, indicating some defense mechanisms against UV. However, the results of the PER experimentation tests revealed *Diaphanosoma* do not rely on PER. Unexpectedly, *Diaphanosoma* was distributed at more shallow depths in Reynolds Quarry than in City Lake, although weighted mean depths were not significantly different. Furthermore, the results from the vertical migration tests occurring in natural sunlight revealed that *Diaphanosoma* remained at surface level when UV-B was blocked, but migrated downward when UV-B exposure occurred. The results provide new insight into a method of harmful UV-B tolerance by the taxon *Diaphanosoma* and other cladoceran genera.

Investigating Physical Properties of Fuel Components Using Molecular Dynamics

Matthew Sayger, Todd Knippenberg*
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High Point University

The goal of this project was to investigate physical properties of various molecules and combinations of molecules that are potentially useful in renewable fuels. The AIREBO potential is an emipirical bond-order potential that is used to model the interactions between the atoms of individual molecules using computer simulations. This work was done by modeling liquids using molecular dynamics and the AIREBO potential, and calculated the physical properties of density, viscosity, and bulks-modulus. Multiple trials for pure liquids of only one molecule and also binary systems of various ratios of isocetane and dodecane were calculated and simulation results are compared to experimental values. Individual liquid systems of pure, dodecane, dodecene, hexadecane, isocetane, isooctane, methylcyclohexane, methylnaphthalene, octadecane, tetralin, trans-decalin, toluene, and trimethylbenzene were measured. The calculated RMSD (Root Mean Square Derivative) of the twelve pure substances was found to be within 2% of the literature densities. For the binary system of dodecane and isocetane four mole fractions were calculated at two temperatures, and the RMSD of the density at 298K was 1.04% and 1.5% at 353K. The viscosities that were calculated appeared to be qualitatively correct but not quantitatively correct. The bulk modulus that was calculated for the pure systems appeared to be both qualitatively and quantitatively correct when compared to experimental values.

(D.5) A Study into the Effects of Resveratrol on Bone Development in *Danio rerio*

Cailyn Scanlan, Angela Bauer*, Neil Coffield*
Department of Biology
High Point University

Resveratrol, a naturally occurring phytochemical found in grapes and red wine has been shown to have many health benefits as well as increase bone growth in some mammalian species. Resveratrol is thought to exert many of these health effects (including those on bone growth) through its ability to weakly bind and activate the estrogen receptor. Little is known about the effects of resveratrol on bone ossification in *Danio rerio* (zebrafish). This study was conducted to investigate the effects of resveratrol on vertebrae ossification in *D. rerio*. Embryos were raised in five different concentrations of resveratrol (10^{-8} M -10^{-4} M) for 12 days. To determine the effects of resveratrol on ossification, embryos were stained with calcein every other day from day 6 until day 12. Calcein is a fluorescent dye that adheres to calcium ions in the specimen, thereby serving as a marker for ossified bone. Calcein fluoresces under a GFP filter, thus indicating where the process of ossification has begun in the embryos. Specimens were imaged using confocal microscopy and vertebrae were scored according to their degree of ossification. Results indicate that resveratrol accelerates ossification of vertebrae at all concentrations starting at day six compared to untreated control animals. This data supports the hypothesis that resvertatrol can affect bone development in *D. rerio* and merits further investigation into health benefits in various organisms.

(A.7) Importance of Specific Amino Acids on the *E. coli* MazEF Toxin-Antitoxin System

Evan Shaw, Meghan Blackledge*
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High Point University

Death of an entire population is not seen as advantageous in the public eye. In bacteria however, large-scale cell death is sometimes necessary for the survival of future generations. Under various types of stress, *E. coli* will undergo programmed cell death via a MazEF-mediated pathway. MazEF, a toxin-antitoxin system, is comprised of two main components, the toxin MazF coded by mazF and the antitoxin MazE, coded by mazE. When bound together, MazE inhibits the enzymatic function of MazF and the cell grows normally. However, when MazE dissociates from the toxin, MazF commences its function as a sequence specific endoribonuclease, cleaving mRNA at ACA sequences and thereby killing the cell. Based on a published crystal structure, the most intimate interaction between these two proteins takes place between a hydrophobic cleft on MazF and residues 71 to 75 of MazE. According to previous publications, it is believed that amongst these 5 residues, IDWGE, the center tryptophan is a key component in the binding of MazE to MazF, and thus a key to its activity. We have synthesized IDWGE, ΔMazE5, to evaluate its utility as an analog for the full length MazE. Initial studies on ΔMazE5 as well as progress toward the cloning, expression, and isolation of both MazE and MazF for enzyme assays will be presented.

(B.2) Solid-Phase Peptide Synthesis and Antimicrobial Assessment of a Plant-Derived Cyclic Peptide

Calla Telzrow, Andrew Wommack*
Department of Chemistry
High Point University

Jatropha, a genus of woody trees and shrubs ubiquitous in the dry tropics, is prevalent in Latin American, Asian, and African ethnopharmacology. Peptides isolated from *Jatropha* species exhibit diverse biological activity, including purgative, wound-healing, antimalarial, and antifungal effects. Cyclogossine A, a cyclic heptapeptide with the primary structure VLATWLG, is isolated from *J. gossypiifolia*. Although Cyclogossine A has been previously synthesized, its biological activity remains unreported. Due to its rigid structure and hydrophobic side chains, Cyclogossine A possesses therapeutic potential. Access to Cyclogossine A was enabled by semi-automated, Fmoc-based solid-phase peptide synthesis. HPLC purification and Q-TOF mass spectrometry were utilized to confirm the synthesis and purity of the primary structure. After optimizing the cyclization reaction, which formed an amide bond between the C-terminus and N-terminus of the linear peptide, the naturally occurring ring structure was created. Interestingly, the identification of a cyclization reaction byproduct provided access to an alkene-containing Cyclogossine A derivative. NMR spectroscopy data confirmed the desired synthesis and cyclization of both the natural and engineered peptides. In future experiments, the antimicrobial effects of Cyclogossine A will be tested on a library of bacteria, parasites, and fungi. Following confirmation of activity, cytotoxicity assays, fluorescence spectroscopy, and electron microscopy may be employed to further investigate the biological mechanisms of Cyclogossine A.

(C.5) Probing the Structure-Activity Relationship of Escherichia coli Extracellular Death Factor

Rebecca Ulrich, Lisa Nguyen, Nickolle Baker, Meghan Blackledge*
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Bacteria use chemical signals for cell-to-cell communication to learn about and respond to their environment and nearby bacteria. This process of behavior modification based on environmental conditions and bacterial populations is called quorum sensing. When dense bacterial populations encounter stress from viral or bacterial invaders, nutrient deprivation, or harsh environmental conditions, quorum sensing allows the larger bacterial community to preserve the integrity of the colony by undergoing programmed cell death. Programmed cell death (PCD) in *E. coli* is mediated by a pentapeptide called *E. coli* extracellular death factor (EcEDF) and occurs when dense cultures of E. coli encounter stress and reduce their populations by up to 80%. EcEDF is a quorum sensing molecule produced by *E. coli* when colonies reach densities of over one million cells per milliliter and induces PCD under stressful conditions. Previous research has determined the amino acid sequence of EcEDF and described the essential and non-essential amino acid residues for EcEDF activity. We seek a more comprehensive understanding of EcEDF to probe the structure-activity relationship of essential residues and more completely describe the necessary functional groups required for activity in *E. coli*. Toward this end, we have begun synthesis of a library of rationally designed EcEDF analogs. These analogs can be screened in cell-based assays for their ability to promote or inhibit PCD in *E. coli*. Efforts towards the synthesis of these analogs as well as assay development and optimization will be presented.

(B.1) Cloning and Purification of the Transcription Regulator GerE from *Bacillus* Species for In-Vitro Analyses

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Organisms belonging to the *Bacillus* genus are gram positive, rod shaped, aerobic soil bacteria. In times of environmental stress, the bacteria form a protective spore which is resistant to desiccation, UV radiation, heat and fire. It remains a spore until the environment is appropriate for germination. Many proteins play a role in sporulation, most interestingly, GerE. The appearance of the regulatory protein GerE is essential at the late stage of spore development because it directly activates the transcription of sigma K (σK)-dependent genes, including cotC and cotX. The goal of this research was to develop a non-radioactive DNA binding assay to analyze the DNA binding characteristics of GerE from different species of *Bacillus* including *Bacillus subtilis*, *Bacillus thuringiensis* and *Geobacillus stearothermophilus*. Toward this aim we cloned *gerE* and were able to overexpress it in *Escherichia coli*. In addition, we were able to amplify regions of the sigma K and Cot X promoters that include a GerE binding site. Having these components will allow us to perform in-vitro DNA binding studies to determine if GerE is able to function as a transcription regulator in these species.

(A.6) There and Back Again?: The Pulsational Changes of CS1246

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Hot subdwarf stars were once main sequence stars, like the sun, that deviated from normal stellar evolution and will directly evolve into white dwarfs. Because hot subdwarfs are the main source of UV light in old galaxies, we want to know what their internal structure is and how they will evolve. While all hot subdwarfs have interesting characteristics, pulsators are the best objects for observing extreme stellar evolution. CS-1246 is a pulsating hot subdwarf discovered in 2009 by Dr. Barlow that exhibits a single, dominant pulsational mode. At the time of its discovery, its pulsational amplitude was one of the largest known, making CS-1246 an ideal candidate for follow up studies. Observations in 2013 implied that the pulsational amplitude had decreased significantly since it was discovered. We have continued monitoring the star over the past few months using the robotic SKYNET telescopes in Chile, in order to further characterize any changes. Our recent observations show that the pulsational amplitude has gone down by a factor of six: CS1246 is barely a pulsator anymore. The decay in amplitude over time is indicative of a damped harmonic oscillator. Here we present possible scenarios that might explain this interesting behavior.

(D.3) Comparative Genetics of Parallel De-domestication Events in Weedy Red Rice (*Oryza sativa*)

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Red rice is an aggressive, weedy form of cultivated rice (*Oryza sativa*) that infests crop fields and is a primary factor limiting rice productivity in the U.S. and worldwide. As the weedy relative of a genomic model species, red rice is supremely suited to serve as a model for understanding the genetic and evolutionary mechanisms by which weediness evolves. Previous work has focused on the evolution of weediness in red rice from the southern U.S. (two independent de-domestication events) that is genetically distinct from red rice found in California and Korea. The results from our study reveal that Korean weedy rice contains a genetically distinct japonica-like group that represents a third de-domestication event as well as an indica-like group that may be related to other indica-like weedy rice. Because U.S. weedy rice and Korean weedy rice represent independent de-domestication events, we will be able to examine what genetic changes are required for weedy rice to take hold in an agro-ecosystem using comparative genetics. Three candidate genes that are involved in weedy traits have already been identified and sequenced in crop, wild, and U.S. weedy rice. We have expanded this data set to include sequences from Korean weedy rice. In addition, Sequenced Tag Sites (STS loci) have been sequenced and identified as non-candidate single genes in the genome that can be used to generate an evolutionary timeframe for the de-domestication events in Korean weedy rice. Comparative genetics approaches have allowed for a better understanding of what weedy traits have evolved in independent weedy rice populations and what genetic mechanisms underlie the evolution of these weedy traits.

(C.3) Population Structure of the Spotted Salamander (*Ambystoma maculatum*) in North Carolinian Ponds

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Vernal pools are unique habitats that promote growth, gene flow and variation among demes. In addition to more-developed ponds and lakes, these pools serve as breeding grounds for many amphibians, including the spotted salamander (*Ambystoma maculatum*), which is found in sub-aquatic habitats throughout most of the Eastern United States. Recently, isolation of these habitats has become a concern and research on the genetic diversity and population structure of the spotted salamander could give insight to the long-term health of these populations. We have compared the genomic similarity of *A. maculatum* embryos from different aquatic locations in the Uwharrie National Forest through the comparison of microsatellite markers and Nested PCR. The findings of this study will lead to promoting better conservation methods for this species and a better understanding of the population biology as a whole.

(C.2) The Effects of UV Radiation on Calanoid Copepods

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Ultraviolet radiation (UV) can play a major role in the vertical distribution and survival of organisms in aquatic ecosystems. We looked at these factors in two lakes in North Carolina; a clear, high-UV lake (Reynolds Quarry, Winston-Salem) and a turbid, low-UV lake (City Lake, High Point). The goal of this study was to investigate the effects of UV-B radiation (290-320 nm) on zooplankton species, specifically calanoid copepods, coming from the two lakes of interest. Zooplankton are known to use three methods to combat the harmful effects of UV-B. These strategies include behavioral avoidance of radiation, photoprotective pigmentation, and photoenzymatic repair (PER) of damaged DNA. Our study of vertical depth distribution in each lake gave us insight as to how calanoids may use avoidance strategies for protection against harmful UV-B. We found no difference in calanoid depth distribution between City Lake and Reynolds Quarry. A series of experiments in temperature-controlled incubators was performed to examine the effects of UV-B, UV-A, and PAR on the PER abilities of these organisms. The results of our incubator experiments suggested that calanoids who are exposed to repair radiation (UV-A) after being exposed to UV-B survive longer than individuals exposed to UV-B and given no repair radiation. This study brought insight as to how the varying doses and spectral composition of UV play a role in the diversity of freshwater zooplankton communities.

(B.5) Combinatorial Effects of Antibiotics and Manuka Honey on *Escherichia coli*

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Bacteria are becoming more resistant to antibacterial drugs, and developing new drugs to combat antibiotic resistance is a time consuming and expensive process that yields low economic return. As a possible strategy to overcome antibiotic resistance we combined existing antibiotics with natural products attempting to find synergistic effects. Manuka honey is a monofloral honey that is imported from New Zealand and has been found to possess antimicrobial properties: making it an ideal natural product to test with. We hypothesized that the combination of approved antibiotics with the honey will produce a smaller zone of inhibition (zoi). We used disc diffusion to combine medications that can be used topically or orally, with Manuka honey to determine if the combination had significant effects on the growth of *Escherichia coli*. We found that some of the drugs when combined with Manuka honey produce synergistic effects while others induced antagonistic effects. A sugar surrogate to imitate honey was made to see if the sugar in honey was the reason for the results and we found that the antagonistic effects are sugar mediated more than a result of the components in Manuka honey.

2015 SuRPS Faculty Participation and Projects

Department of Biology

Dr. Angela Bauer / Dr. Neil Coffield	"Regulation of bone ossification in *Danio rerio* (zebrafish) by 17-estradiol and estrogenic endocrine disruptors"
Dr. Dinene Crater	"The Detection and Characterization of the Transcriptional Regulator GerE in *Bacillus* Species"
Dr. Sandra Cooke	"Effects of ultraviolet radiation on freshwater zooplankton: Examining the tolerance and responses of less well-studied taxa"
Dr. Niky Hughes	"Comparative carboxylation efficiency and photosynthetic capacity of plant tissues with abaxial versus adaxial anthocyanin"
Dr. Cindy Viguiera	"Comparative genetics of parallel de-domestication events in Korean and Californian weedy red rice"
Dr. Patrick Viguiera	Mitochondrial fatty acid synthesis in *Trypanosoma brucei*

Department of Chemistry

Dr. Brian Augustine	"Modification and characterization of polymer surfaces with applications in microfluidic neural circuit development"
Dr. Meghan Blackledge	"Synthesis and evaluation of MazE truncation peptides to probe SAR of mazEF-mediated cell death in *E. coli* "
Dr. Todd Knippenberg	"Investigation of hydrocarbon fuel properties as a function of temperature and pressure changes and changing composition: a computational study"
Dr. Melissa Srougi	"The regulation of RhoGTPases after DNA damage"
Dr. Andrew Wommack	"α-Ketosulfoxonium ylides: diazoalkyl-free cccess to carbenoid chemistry"

Department of Physics

Dr. Brad Barlow	"Characterizing amplitude variations in pulsating hot subdwarf stars"
Dr. Briana Fiser	"Using biomimetic cilia arrays to investigate the flow of mucus in the human lung"

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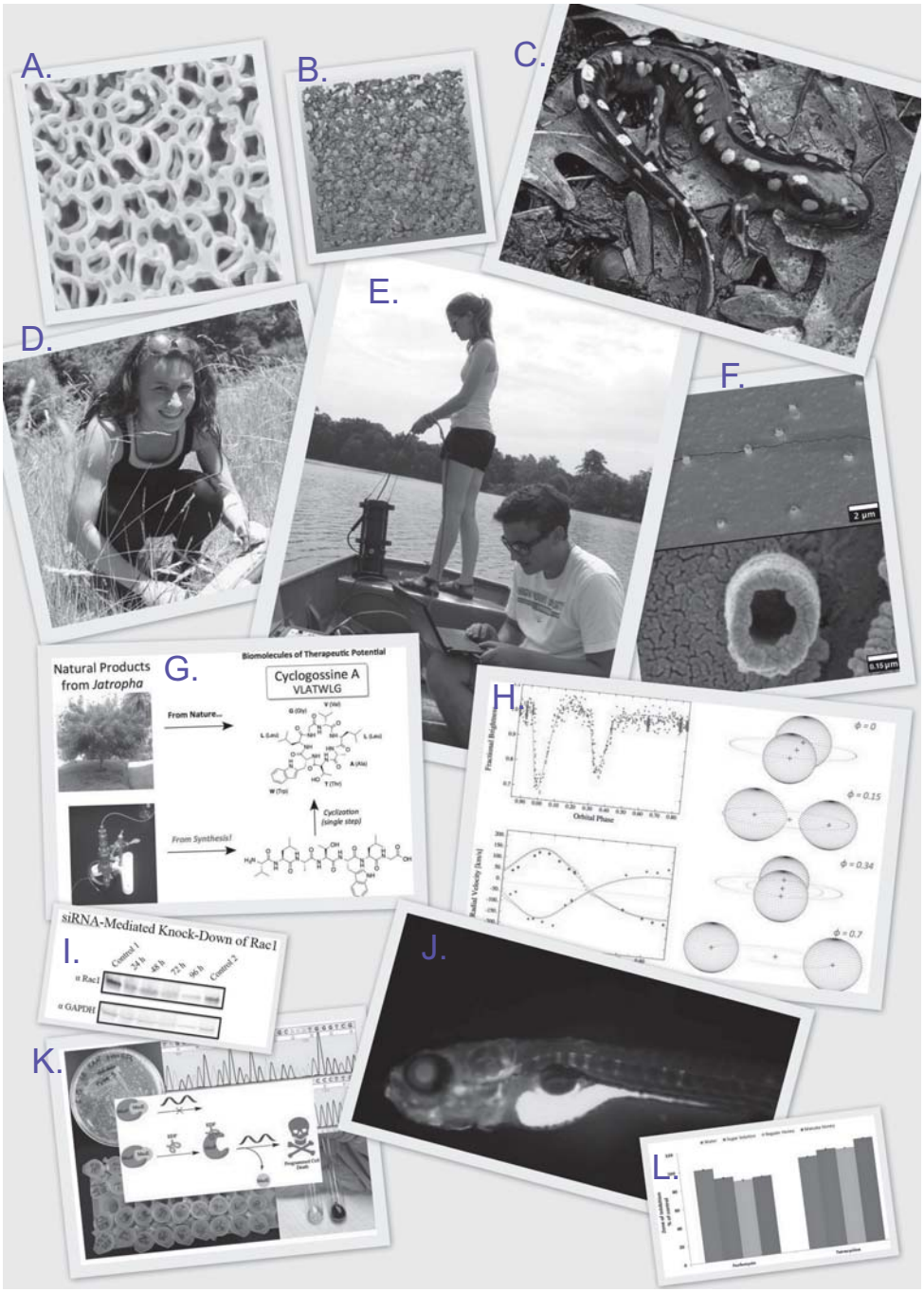

(A.) AFM image from Augustine lab; (B.) Molecular dynamics simulation from Knippenberg Lab; (C.) *A. maculatum* from C. Viguiera Lab; (D.-F.) Field work from the Hughes and Cooke Labs; (F.) SEM image from Fiser Lab; (G.) Schematic of Wommack Lab strategy; (H.) Binary star data from Barlow Lab; (I.) Gel image from Srougi Lab; (J.) Fluorescent microscope image from Coffield/Bauer Lab; (K.) Composite image from Blackledge Lab; (L.) Preliminary results from P. Viguiera Lab.