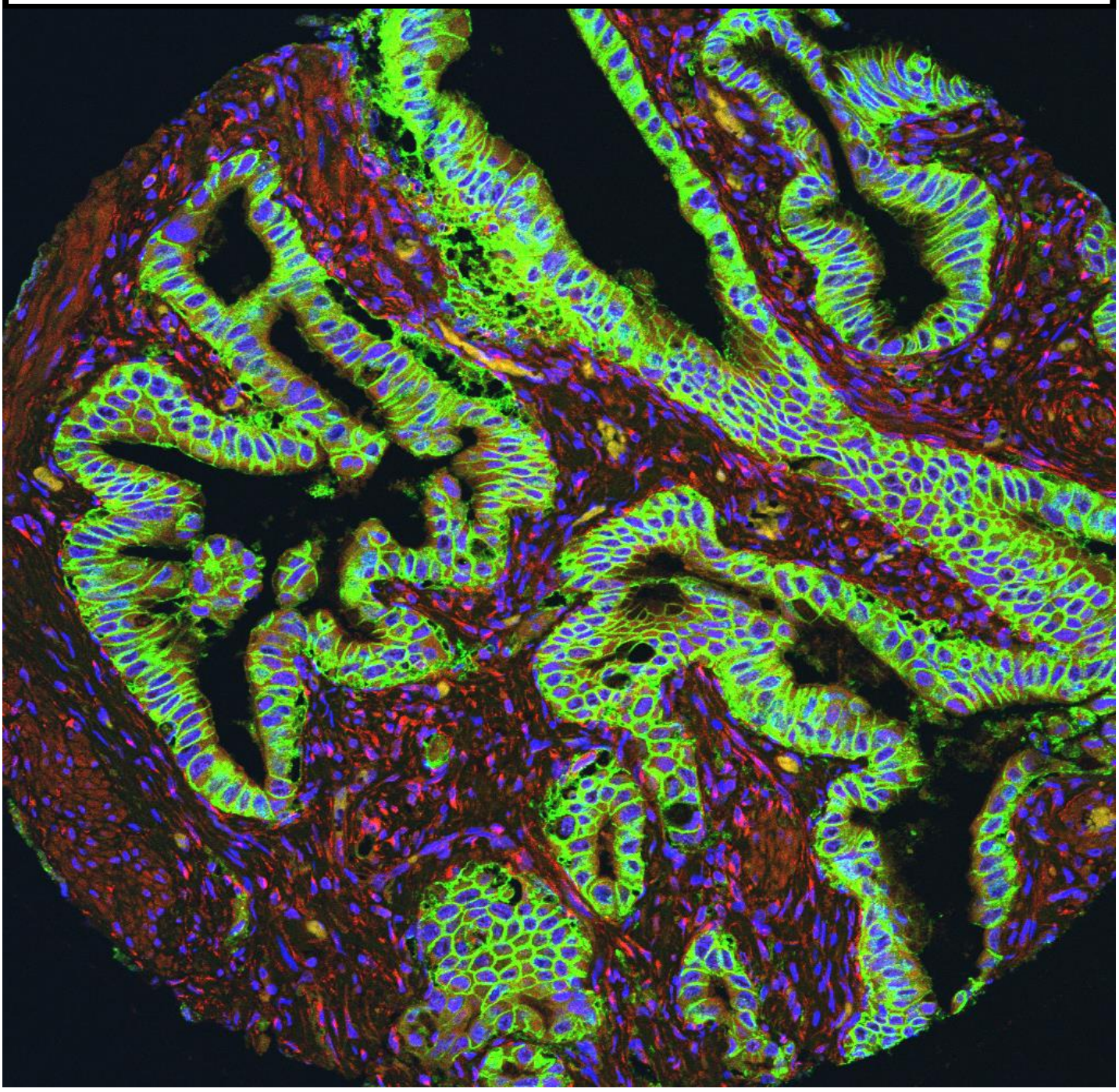
The First Annual

Cell and Molecular Biology Research Symposium



GRAND VALLEY STATE UNIVERSITY

Session 1:

Friday 5:00-8:00 PM

Molecular Implications in Health: Antibiotics | Cancer | Vascular response

Emily McGuffie
Elliot Ensink
Kyle Sugg
Joel Francis
Corbin Gilchrist
Allee VanDine

Session 2:

Saturday 8:30-11:30 AM

Cellular Dynamics: Division, Motility, and Apoptosis

Timothy Gilbert
Rebekah Newman
Daniel Doyle
Zachary Carlson
Heather Zolen
Kelsey Lammers

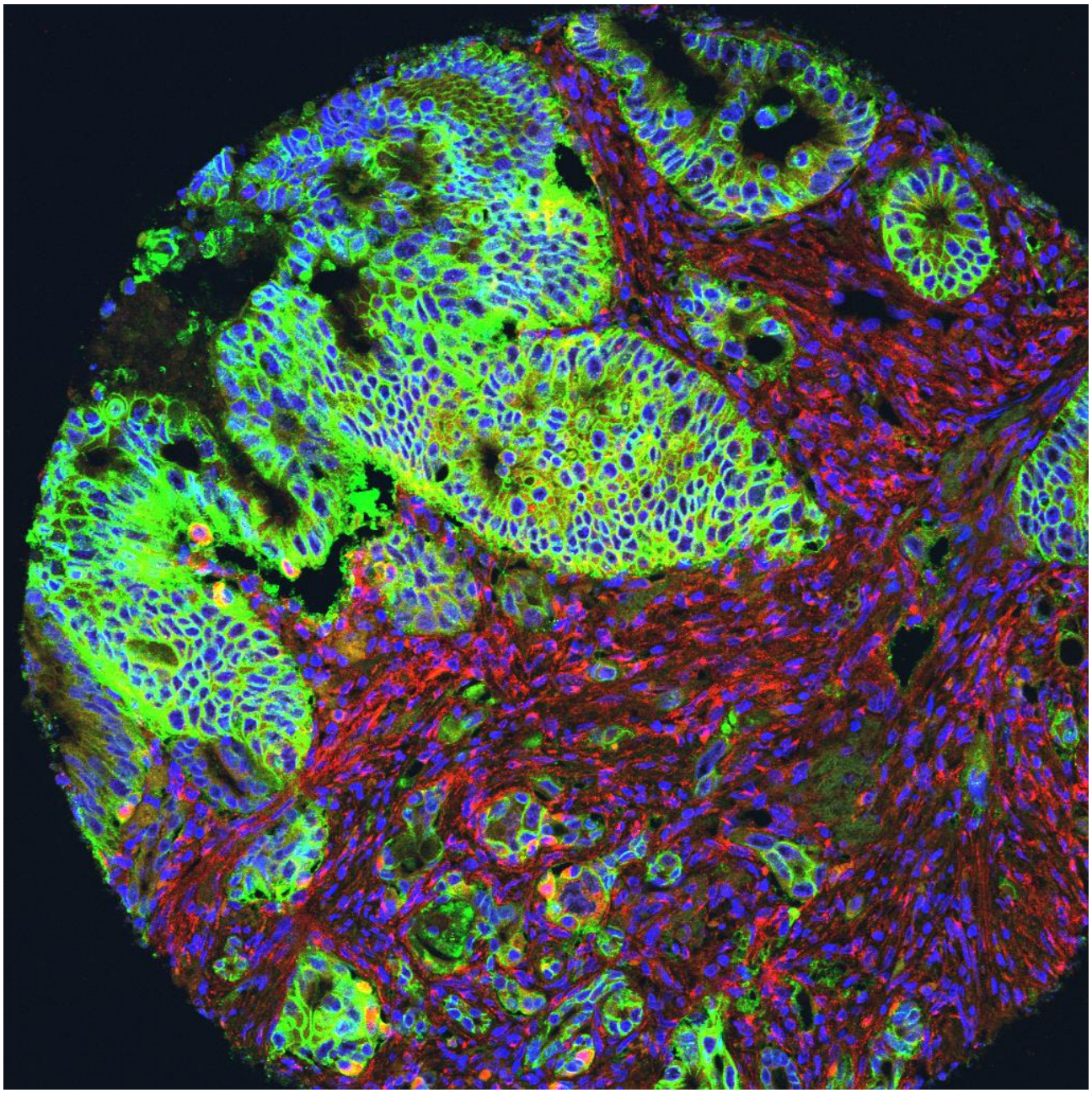
Session 3:

Saturday 12:30-3:00 PM

A Molecular Look at Environmental Interactions

Brooke Prieskorn
Jennifer Grousd
Carly Wiersma
Sarah Brown
Brittany Bunker
Barrett Kyle

Molecular Implications in Health:
Antibiotics | Cancer | Vascular Response



Friday 5:00-8:00 PM

Emily McGuffie
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Allee VanDine

Isolation of 6-Gingerol from ginger root and the synthesis of 6-gingerdiol major metabolites

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[2]Grand Valley State University, Department of Chemistry, Allendale, MI, 49401, USA.

6-gingerol is the most abundant compound in ginger responsible for the pungent smell of the ginger rhizome. The metabolism of 6-gingerol in the body produces 6-gingerdiols, which have been found to have tumor reducing properties. While these compounds have been produced *in vivo* they have yet to be synthesized *ex vivo*. The *ex vivo* synthesis of 6-gingerdiols will allow for further characterizations of these compounds. Due to the difficulty with extractions of 6-gingerol from ginger rhizome, few have tackled the extraction, isolation, and hydrogenation of 6-gingerol. Experiments extracting the 6-gingerol through near supercritical CO_2 fluid extraction, isolation of the 6-gingerol from the extracted ginger oil through HPLC analysis, and hydrogenation of 6-gingerol to produce 6-gingerdiols through hydrogenation with hydrogen gas and ruthenium BINAP were performed. To test possible hydrogenation schemes, hydrogenations were performed on 6-gingerol model compounds. The desired products of the experiment, 6-gingerdiols, are present in shrinking murine tumors which have possible implications in human tumor applications.

Segment and fit thresholding: a new method for image analysis applied to microarray and immunofluorescence data.

Ensink E[1], Sinha J[1], Sinha A[1], Tang H[1], Calderone HM[1], Hostetter G[1], Winter J[2], Cherba D[1], Brand RE[3], Allen PJ[4], Sempere LF[1], Haab BB[1]
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[2]Thomas Jefferson University, 1025 Walnut Street, Philadelphia, Pennsylvania 19107, USA.
[3]University of Pittsburgh Medical Center , 200 Lothrop Street, Pittsburgh, Pennsylvania 15213, USA.
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Commonly used experiments produce images that require algorithms for high-throughput quantification of the image data. A challenge in the development of such algorithms is to properly interpret signals over a broad range of image characteristics, without the need for manual adjustment of parameters. Currently, most automated image analysis algorithms, such as the popular Otsu's method, perform well when the image has predictable characteristics or conforms to certain assumptions, but not well if the image has unexpected qualities. We predicted that we could use statistical characteristics of small segments of the image and determine the best-fit trends between the statistics to predict appropriate thresholds for signal location. We created a software algorithm, named Segment and Fit Thresholding (SFT), which identifies segments belonging to background regions; analyzes the background to determine optimal thresholds; and analyzes all segments to identify signal pixels. When used for the automated analysis of multicolor, tissue-microarray images, SFT correctly found the overlap of markers with known subcellular localization, and it performed better than a fixed threshold and Otsu's method for selected images.

Funding by the National Cancer Institute (Early Detection Research Network, U01CA152653; Alliance of Glycobiologists for Cancer Detection, U01CA168896) and the Van Andel Research Institute

Characterization of class D β-lactamase, OXA-51, and recently discovered clinical variants yields a greater understanding of β-lactamase structure and function.

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[2]Grand Valley State University, Department of Chemistry, Allendale, Michigan, 49401, USA

Acinetobacter baumannii, a gram-negative bacterium, is one of many organisms to present with resistance to β-lactam antibiotics via β-lactamase enzymes. One such β-lactamase, the carbapenemase OXA-51, is noted for its low levels of hydrolytic activity. However, there is a rising number of OXA-51-like clinical variants that exhibit increased hydrolytic function against carbapenems. Previous literature fails to explain the cause of this due to the recency of these findings. This study focused on three common mutations hypothesized to play a major role in the increased activity, all of which are featured in OXA-51-like β-lactamase – OXA-173. Through the use of enzyme kinetics, the aforementioned mutations found in OXA-173 demonstrated increased hydrolytic activity across all major classes of β-lactams (cephalosporins, penicillins, carbapenems, and monobactams). This work advances the overall understanding of β-lactamase structure and function, and can be utilized for future medicinal research and design.

This research was supported by the National Institute of Health (Grant 1R15AI082416-02); US Department of Energy, Office of Science, and Office of Basic Energy Sciences (Contract DE-AC0206CH11357); and the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor (Grant 085P1000817).

Purification and characterization of BstA, a bacillithiol-dependent transferase

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Since antibiotics became mainstream in the 1940s, antibiotic-modifying enzymes have emerged as a method of resistance. One such pathway revolves around bacillithiol, a low-molecular weight thiol (LMWT) involved in fosfomycin resistance, as well as cellular redox chemistry. This redox chemistry is carried out in part by bacillithiol-dependent BstA, a thiol transferase whose structure and mechanism are yet to be determined. In this study, BstA was purified by ion-exchange chromatography and characterized by enzymatic activity assay and x-ray crystallography. BstA was expressed in *E. coli* BL-21 cells, purified by Ni-column chromatography, characterized in solution with bacillithiol and other LMWTs, and crystals were prepared for structural analysis by x-ray crystallography. Extensive kinetic and x-ray crystallographic analysis is underway, and could provide key insight into the mechanisms of bacillithiol-dependent resistance to fosfomycin.

This project was funded thanks to the Grand Valley State University College of Liberal Arts and Sciences Supplemental Startup Fund.

1,3-dimethylamylamine Effects Renal, Pulmonary and Coronary Arteries Vasoactivity

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1,3-dimethylamylamine (DMAA), a sympathomimetic drug thought to mimic the effects of amphetamine, is used in many dietary and pre-workout supplements. Though DMAA use has become increasingly popular, the vasoactive effects of this compound on the circulatory system have yet to be researched. Few published studies have observed statistically significant physiological effects of DMAA in humans, although these studies only tested changes in vital measurements of heart rate and blood pressure. This experiment focused on the direct effects of DMAA on arteries. In this study, rings of porcine renal, pulmonary, and coronary arteries were excised, attached to a force transducer to measure contractile force, and exposed to varying concentrations of DMAA. Andrergenic receptor blockers were then used to determine if the observed vasoactivity was receptor mediated. It is expected that these results will help us better understand the effects of DMAA on the human circulatory system.

Characterizing novel boronic acid inhibitors to combat antibiotic resistance in *Acinetobacter baumannii*

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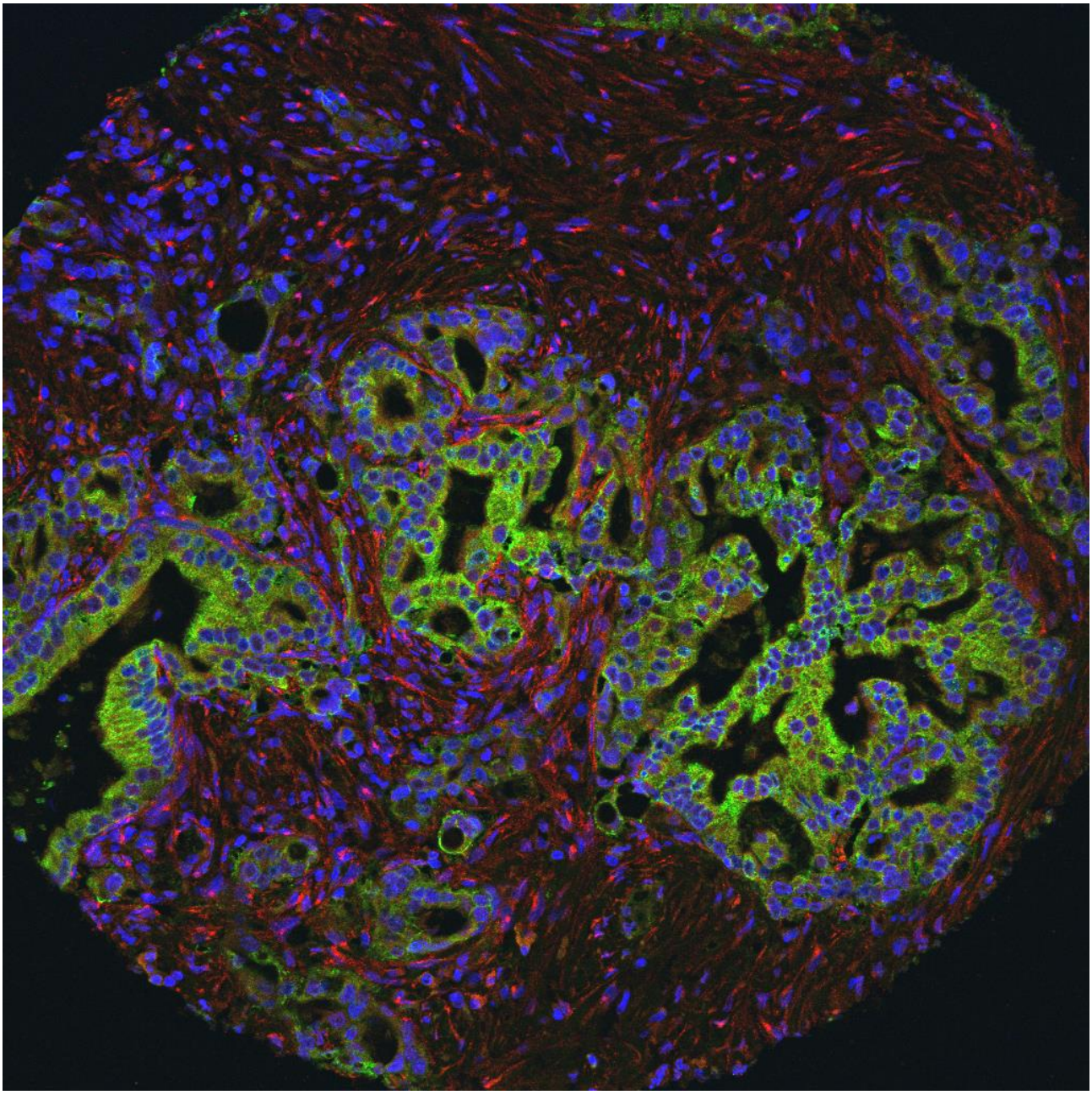
The expression of β-lactamase enzymes in certain bacterial strains such as *Acinetobacter baumannii* has caused an alarming increase in antibiotic resistance. *A. baumanii* express a class C β-lactamase ADC-7 that inactivates β-lactam antibiotics used as a last resort; therefore, a method to inhibit this enzyme is necessary to counter this antibiotic-resistant pathogen. Current approaches in treatment rely on drugs containing a β-lactam ring, which are ineffective against bacterial strains containing ADC-7. Our novel approach utilizes competitive boronic acid inhibitors that mimic the tetrahedral intermediate during the breakdown of substrates in the active site of ADC-7. We conducted kinetic analyses of a variety of boronic acid inhibitors, as well as collected x-ray crystallographic structural data for each enzyme-inhibitor complex. The most effective boronic acid inhibitor will be tested in animal models to progress the treatment of antibiotic resistance in this bacterial strain.

Research reported in this presentation was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award numbers R01AI100560 and R01AI063517. Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. Department of Energy (DOE) Office of Science by Argonne National Laboratory was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357. Use of the LS-CAT Sector 21 was supported by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor (Grant 085P1000817).

Cellular Dynamics: Division, Motility, and Apoptosis



8:30-11:30 AM Saturday

Timothy Gilbert
Rebekah Newman
Daniel Doyle
Zachary Carlson
Heather Zolen
Kelsey Lammers

Discovery of Cell Cycle Protein Binding Events and Their Role In Fission Yeast Cytokinesis

Timothy Gilbert, Eric Moore, and Dawn M. Hart
Department of Cell & Molecular Biology, Grand Valley State University, Allendale, Michigan, 49401, USA

Protein phosphatase 1 enzymes regulate many essential aspects of the cell cycle through inorganic phosphate abstraction. Substantial eukaryotic homology exists within their catalytic subunit, suggesting a conserved mechanism. Dis2, a catalytic PP1 subunit within fission yeast, modifies the localization and phosphorylation profile of Mid1, a scaffolding protein required for faithful cell division that accumulates midline in late anaphase. Recent studies implicate Dis2 in controlling polarized cell growth, though its cytokinetic function remains unclear. Here we establish Mid1 as a direct Dis2 substrate. Western blot analysis of chimeric Mid1 precipitates on GST fusion resin reveals the presence of Dis2-GFP when probed for by αGFP antibodies. Current work aims to elucidate where Dis2 binds Mid1 by inducing FA mutations along Dis2 within the archetypal RVXF docking motif. Details of the Mid1-Dis2 interaction must be further understood before an accurate account of eukaryotic cytokinesis can be made.

This research is funded by the National Science Foundation (RUI Award #1157997).

Drosophila Protein Kinase N (Pkn) Promoter Mutation Results in Hypomorphic Imaginal Disc Phenotype

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[2]Grand Valley State University, Department of Biology, Allendale, MI, 49401, USA.

A careful balance of proteins is required for the generation of imaginal discs in fruit fly larvae, which form the appendages of the adult Drosophila melanogaster, such as the legs, wings, and eyes. It has previously been suggested that the Drosophila protein kinase N (Pkn) is involved in actin-myosin activity during oogenesis, but little else is known about the protein. Here, we examine the possible hypomorphic effect of a promoter-region insertion of the pkn gene in larval imaginal discs. We characterized the hypomorphic phenotype through visualization of the imaginal discs, cell death and cell proliferation assays of related tissues, and attempted rescue of the wild type phenotype through inducible expression of Pkn using the GAL4 system of expression. The findings indicate that Pkn, a largely understudied protein, may have an important role in the development of Drosophila during the embryonic and larval stages through an influence actin-myosin activity.

Nato3 Overexpression in the Midbrain and Neural Tube Induces Ectopic Expression of Floor Plate Cell Markers

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[2]Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI 49401, USA.

In Parkinson's disease, mesencephalic dopaminergic neurons within the pars compacta region of the substantia nigra die, and the idea of cell replacement therapy to counteract this is being largely investigated. There is no clear answer as to how Nato3, a basic helix-loop-helix transcription factor expressed in the developing midbrain and spinal cord, affects the generation of dopaminergic neurons in the developing nervous system. Previous studies have investigated the necessity of Nato3 in vivo, but have failed to address how sufficiently Nato3 might affect neural stem cell fates. To expand upon the previous results, we explored the effects of an overexpression of Nato3 in the developing chick embryo. The examination resulted in an understanding that overexpressing Nato3 sufficiently induces ectopic expression of the floor plate cell markers Shh and Foxa2 in the developing midbrain, as well as the immature dopamine neuron marker Lmx1b in the developing midbrain and spinal cord. The results were obtained through the use of in ovo electroporation of a bicistronic EGFP reporter expression vector, and immunohistochemistry. With this new information, it is evident that Nato3 affects cells within a dopaminergic neuron lineage. Further characterization of these effects could identify possible uses related to the development of dopaminergic neurons for cell therapy.

This work was supported by the GVSU Student Summer Scholars Program and West Michigan Science and Technology Initiative (WMSTI) Innovation Fund.

Characterization of an apoptotic developmental mutation in the outer ommatidia of Drosophila melanogaster.

Zachary J. Carlson[1] & Bruce D. Ostrow[2]
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Genetic crosses have been instrumental in determining the mode of inheritance for traits in Drosophila melanogaster, which provides robust method to pinpoint the source of genetic mutations. The Ostrow lab was given the "55-4; Hdc-jk910" line of flies with a mutant phenotype that caused the outer eye tissue to undergo apoptosis during development. Natural selection normally forces deleterious mutations out of a population, but this line of flies with the "weird-eye" phenotype had been kept for two years and the mutation persisted. To isolate the mutation responsible for the "weird-eye" phenotype, the Ostrow lab performed crosses of the "weird-eye" fly line against other stocks of flies with marker chromosomes. The Canton S, w^{1118}, and E52 stocks (sources of stocks tbd) were crossed against "weird-eye" flies and the results of each generation were used to plan subsequent crosses until the source of mutation was apparent. The "weird-eye" phenotype can be used as a second chromosome marker to allow more rapid sorting of flies during phenotypic observations, improving the accuracy and ease of experimentation for flies with known second chromosome mutations.

Visualization of Prostate Cancer Metastasis Regulation by Tetraspanin CD82 Protein

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[2]Department of Biomedical Sciences, Grand Valley State University, Allendale, MI USA.

The tetraspanin membrane protein CD82 is an important part of a regulatory pathway for prostate cancer metastasis. This study attempts to find additional proteins with an altered expression before and after metastasis of prostate cancer, indicating involvement in the regulatory pathway along with CD82. Previously, research identified changes in other specific intracellular protein expressions, such as c-Met, CD151, and cytoskeletal proteins before and after metastasis, but the specific pathway responsible for this process has yet to be fully analyzed. Fluorescence microscopy of prostate cancer cells with CD82 not expressed (metastatic) and CD82 re-expressed (non-metastatic) was employed with specific fluorescent antibodies for c-Met, Paxillin, and CD151 to determine relative protein levels with and without CD82. The results of these images were overlaid to determine if the proteins were co-localizing with CD82 in the cell to indicate a direct correlation. Different cell lines were made from metastatic prostate cancer cells excised from either brain (DU145) or bone (PC3), and each cell line was observed for differing levels and locations of the four proteins CD82, CD151, c-Met, and Paxillin. Through the results of this study along with others, the intracellular metastatic signaling pathway can begin to be determined and become the target for new pharmaceutical drugs to halt metastasis before it begins.

Loss of Protein Kinase N Affects Programmed Cell Death of Nurse Cells in Drosophila melanogaster During Oogenesis

Kelsey G. Lammers[1], Georgette Sass[2]
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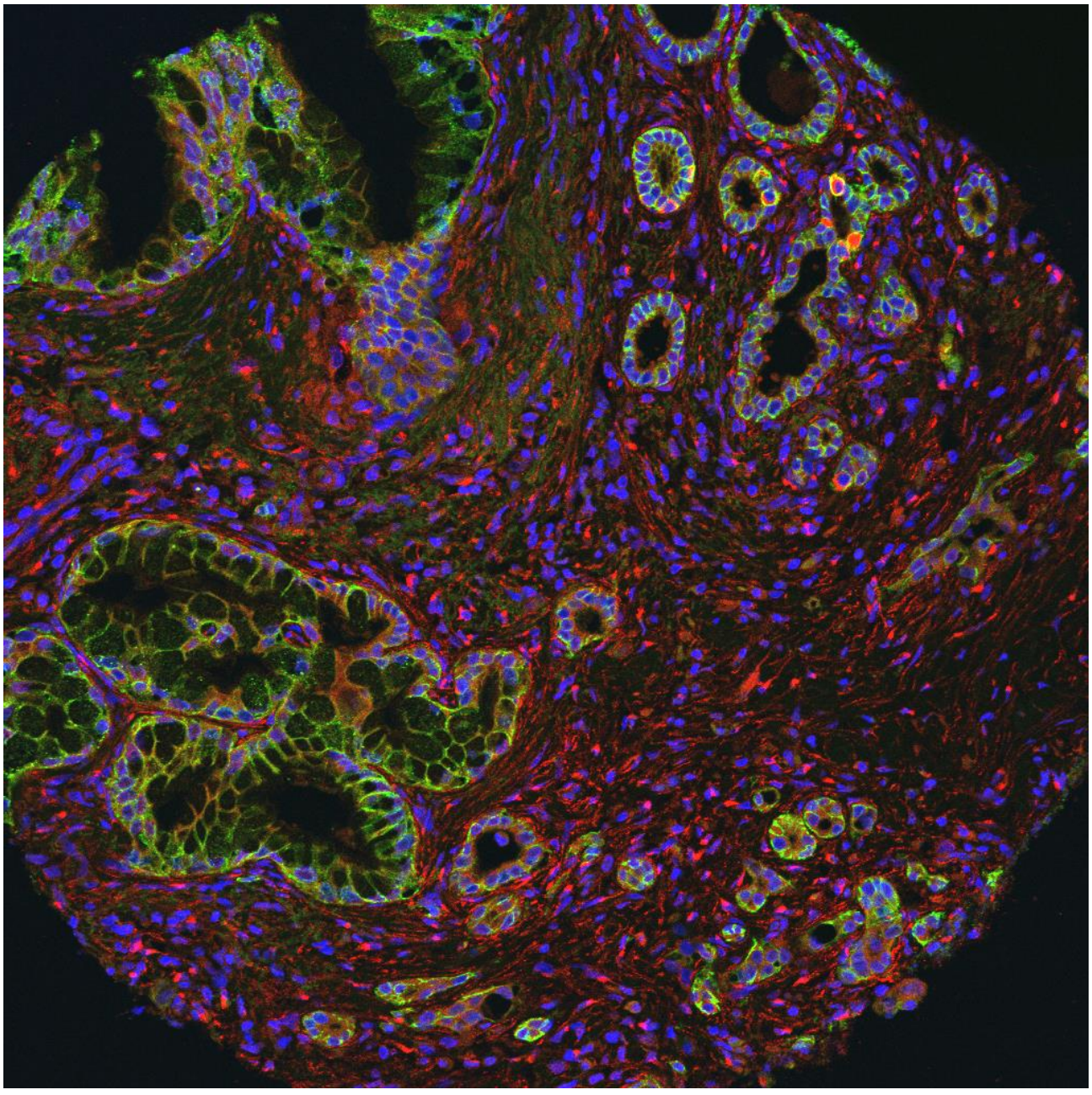
[2]Grand Valley State University, Department of Biology, Allendale, MI, 49401, USA.

In Drosophila melanogaster, the late stages of oogenesis are characterized by programmed cell death (PCD) of the nurse cells, which provide critical nutrients to the growing egg. We investigated how protein kinase N (Pkn) plays a role in the process of nurse cell death. Despite the previous finding that loss of Pkn correlates with changes in nurse cell actin-myosin activity, we believe this is a downstream effect of the loss of Pkn in programmed cell death visualized in late stage oogenesis. To show this, multiple ovary specific GAL4 drivers were utilized to reveal the effects from the loss of Pkn on the nurse cell PCD phenotype. The resulting GAL4 ovaries were dissected, stained for nuclei, DNA and actin filaments then visualized for PCD phenotype comparison. By pinpointing Pkn, a known Rho effector, a parallel could be drawn between the actin-myosin activity and lack of PCD interplay that may be one of the first recognizable progenitors in the cancer cascade.

A Molecular Look at Environmental Interactions



12:30-3:00 PM Saturday

Brooke Prieskorn
Jennifer Grousd
Carly Wiersma
Sarah Brown
Brittany Bunker
Barrett Kyle

A disruption in repeated sequence may be responsible for a *P. patens* mutant phenotype

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[2]Department of Biology, Grand Valley State University, Allendale, MI 49401, USA

Plants move between two stages of growth to complete their life cycle: the gametophyte stage (produces sperm and eggs) and the sporophyte stage (produces spores). A novel *Physcomitrella patens* mutant exhibits abnormal initial cell growth that results in an inability to respond to cytokinin and, therefore, the inability to reach the gametophytic stage of development, which is necessary for completion of the life cycle. Although the *P. patens* genome has been sequenced and the indel site mapped, this information alone cannot answer the question of why the mutation caused the observed phenotype. By learning more about the site of the mutation within the genome, the genetic basis for the phenotype may be found. Via Phytozome and Repbase's CENSOR software, the sequence surrounding the insertion/deletion mutation site was mapped. The indel was located in a non-coding region of the genome surrounded by inactive retrotransposons. A greater understanding of the genes and regulatory mechanisms that play a role in the transition from sporophyte to gametophyte in plants will provide a greater knowledge base to apply to pursuits, such as agroscience.

Patterns of Neutral Genetic Variation in the Virginia Big-eared Bat

Jennifer Grousd[1], Marianne Moore[2], Liliana Dávalos[3], and Amy Russell[4]
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Genetic diversity is an important contributor to the fitness of a species. Variation allows species to adapt to changing environments or emerging diseases. Unbiased by selective forces, neutrally evolving microsatellite markers allow for the assessment of recent changes in diversity due to their high mutation rate. Effective population size (N_e), a parameter that reflects a population's rate of genetic drift, can be used to estimate historical events such as bottlenecks. White-nose syndrome (WNS), first detected in North America in 2006, has caused severe population declines in several species of hibernating bats. However, the Virginia big-eared bat (*Corynorhinus townsendii virginianus*), endangered since the mid-20th century, seems to be unaffected by this disease and has been increasing in population size. Due to the recent nature of both of these demographic events, their effects may not be detectable in the genetic diversity of the species. The coalescent-based extended Bayesian skyline plot (EBSP) can determine historical N_e values and previous population size changes using microsatellite loci. By using frequency-based diversity measures and EBSP analyses, we have characterized recent changes in population size of the Virginia big-eared bat. This information may lead to further investigation as to why this species is unaffected by WNS while others are in severe decline.

This project was funded by the U.S. Fish and Wildlife Service as well as the Office of Undergraduate Research and Student Summer Scholars program at GVSU.

Light-Dependent Modification of LRB Complex Members in *Arabidopsis thaliana*

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Upon exposure to red light, Light-Response BTB (LRB) becomes active in the light response pathway of *Arabidopsis thaliana* and targets proteins for degradation via the Ubiquitin-Proteasome System (UPS). LRB is an E3 ubiquitin ligase that associates with the scaffold protein Cullin3 (Cul3) to degrade Phytochrome B and subsequently promote photomorphogenesis of etiolated seedlings. In far-red light, however, the interaction between LRB and Cul3 has not been characterized. Previous research has failed to investigate the mechanism of their interaction and whether it changes in response to far-red light. The presence of a Cul-like (CL) region near the N-terminal end of LRB suggests that it may mediate its interaction with Cul3. LRB was purified and probed for Cul3 and Nedd8 under various light conditions. An understanding of this interaction will help us gain a better understanding of the light response pathway and ubiquitination in *Arabidopsis thaliana*.

Gene Expression Changes in Blood Can Reflect Infection Stages of Typhoid Fever in Children

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Typhoid fever is caused by *Salmonella enterica serovar Typhi* (*S. Typhi*), a human-restricted pathogenic bacteria that is recognized by the World Health Organization as a global health problem. It affects 21 million people each year resulting in 200,000 to 600,000 deaths and current diagnostics are invasive, time-consuming and expensive, with a detection sensitivity of only 40-70%. Current diagnostic procedures fail to address the issue of the time frame in which the disease naturally progresses versus the time a diagnosis can be specified. The aim of this project is to identify genetic signatures for early-diagnostic and stratification of children with typhoid fever. Total RNA extracted from blood at acute, convalescent and recovery phases of infection (patients age from 0 to 5 years old) were processed with gene expression microarrays and qRT-PCR to observe gene expression at the different stages. Preliminary data shows that expression of differentially-expressed genes of the immune system reflect the host immune response according to the infectious stages. By comparing this data to healthy controls and gene expression in patients with other bacteremia, we hope to develop a diagnostic test that can definitively diagnose the disease within a few days as opposed to weeks.

Research reported in this project was supported by *the National Institutes of Health under award number* R01 AI097493-01

Regional Variances of Avian Malarial Infection in *Tachycineta bicolor*

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The species of tree swallow *Tachycineta bicolor* is known to commonly have infection from avian malaria, but it is unknown why there are variances of the infections across three geographic regions. Groups of *T. bicolor* surveyed in New York revealed that approximately 50% of the birds carried malaria, but the same species surveyed in Tennessee showed no apparent infection. Previous work has failed to address why the rate of infection varies across different regions while the rate of infectious mosquitos does not vary. Our hypothesis is the birds that show no blood infection are still positive, but the malaria has not yet progressed to the blood. The research included extracting DNA samples and using malarial primers to test for the presence of infection in West Michigan *T. bicolor* populations. The results of the experiment could imply *T. bicolor* populations that test negative for malaria could still have the infection, but it has not yet progressed to the blood.

Blue Light Yields Clues to the Mechanism of Plant Gravity Sensing

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Since 1900 the most widely accepted model for plant gravity sensing has been the starch-statolith model, which proposes that sedimenting intracellular particles are the gravity sensors. However, some plants do not contain sedimenting statoliths, but do sense gravity. This indicates that there is another mechanism in which these plants and other plants are sensing gravity, which we propose to be the gravitational pressure model. Extensive research has been done into the sedimenting intracellular particles, but we focused on the pressure between the protoplast and the extracellular matrix as a new gravity sensing mechanism. To study our model we increased the density of the external medium to alter the pressure in the cells of rice roots and then exposed the rice roots to blue light so they elicit negative phototropism. While observing the roots using time-lapse imaging, we grew the rice roots perpendicular to the vector of gravity so that the positively-gravitropic growth was antagonistic to the negatively-phototropic growth. Our findings showed that increasing the density of the external medium does inhibit the gravitational curvature, which is consistent with the gravitational pressure model. This allows us to conclude that the gravitational pressure model could be working side-by-side the starch-statolith model to explain plant gravitropism.