

IN LSAMP Annual Conference

Undergraduate Research Poster Abstracts

1-6 abstracts listed in the conference program under 'Research Showcase'

7. Charles Barrios, Synthesis of New Thermosensitive Copolymers for the Modification of Polysaccharides

The research of stimuli responsive polymers, also referred to as "smart polymers," has recently become an area of interest for research. This is due to the polymers ability to undergo drastic change in physical properties in response to subtle changes in the environment such as temperature, salinity, pH, etc. One kind of smart polymer that has become particularly interesting are thermoresponsive polymers. These polymers are soluble at lower temperatures, however, self-assemble into nangles at higher temperatures due to their lower critical solution temperature (LCST.) These nangles can be used to encapsulate hydrophobic drugs. It has been explored that the carbohydrate hyaluronic acid can be grafted to these polymers. This is beneficial since many cancerous macrophages use this as a fuel source and thus have receptors for it. This grafted polymer can specifically target these macrophages in a controlled manner. In this project we, explore different techniques to functionalize thermoresponsive polymers. The methods involve modifying the polymer in a way that will allow a coupling between the amine and carboxylic acid on hyaluronic acid. One method involved the modification of the of the thermosensitive RAFT polymer poly(diethylene glycol mono methyl methacrylate-co-butyl methacrylate) [poly(DEGMA-co-BMA)]. The end group of the polymer contains a thiocarbonylthio group which underwent an amine acid coupling with the unprotected amine on boc ethylenediamine. This will result in the polymer containing an amine with the boc protecting group. We also explored the functionalization of the polymer poly (diethylene glycol acrylate-co-diacetone acrylamide) with adipic acid dihydrazide to add an amine onto the polymer.

8. Keon Jones, The Effect of EF-P Post-translational Modifications on Cell Physiology in *E. coli*

Elongation Factor P (EF-P) is a universally conserved translation factor that alleviates ribosome pausing at polyproline motifs by facilitating peptide bond formation. Without EF-P, translation elongation becomes the rate-limiting step of protein synthesis and can lead to a wide range of phenotypes. Generally, characterization of *efp* mutants has been performed in conditions optimized for rapid growth for *Escherichia coli*. In this study, we observe that the Δefp phenotype is dependent on growth rate in *E. coli*. In rapid growth conditions, Δefp *E. coli* displays many defects, including increased doubling time, sensitivity to antimicrobial agents, and global ribosome pausing compared to the wildtype. When growth is slowed under conditions such as low temperatures, these defects are abolished. Using polyproline translation reporter assays, we observed the requirement for EF-P in translation of polyproline motifs is greatly diminished under slow growth conditions. Optimization of ribosomes by modified EF-P allows for proficient synthesis of proteins containing proline stretches. EF-P discontinues ribosome stalling for proteins necessary for cellular functions including virulence, motility, cell viability, and growth.

9. Salvador Rodriguez Valle, Electrodeposition of Germanium Telluride

There is always the need of better materials to make our devices go faster, be more efficient, store more energy, among other things. In this case, the Electrodeposition of Materials contribute greatly on the production of Supercapacitors. Capacitors by themselves are already in most of our daily devices. Supercapacitors have been coming more into the picture due to their high-power density. A clear example of Supercapacitors being quite useful is in electric cars. Thanks to their high-power density, electric cars have been able to surpass the velocity and acceleration of gas-based cars. But as they have an advantage in power density, they have a disadvantage in energy density. They are inferior to batteries in that aspect. One focus of the Materials Research Labs at SIU is to investigate the specific capacitance in energy storage applications of certain compounds, e.g. MoS₂. My summer research project was Electrodeposition of Germanium Telluride. The process consisted of Electrodepositing Telluride first by Under Potential Electrodeposition (UPD) to subsequently Electrodeposit Germanium, given that Germanium by itself it is quite difficult to Electrodeposit due to its semiconductor properties. Tellurium on the other side, when in contact with a solution, it dilutes in positive and negative ions. This makes this material easier to Electrodeposit and subsequently deposit Germanium thus making the Germanium Telluride compound. Due to lack of time, we were not able to get to the Co-Deposition part since we also tried to investigate the Electrodeposition of Germanium by itself. The results as expected were not successful, but for Tellurium we did get a potential region where the third cathodic peak characteristic of Tellurium electrodeposition was observed.

10. Monica Appel, Sphero in the Classroom

A collection and analysis of how the Sphero Sprk+ was used to teach computer science to students. Data collected from classes ranging from kindergarten to college. The activities were further divided into different types, and analyzed to see how they related to computer science. The findings indicated that although not all activities done with the Sphero were directly about computer science topics, the activities did tie back to some computer science principles.

11. Joseph Baird, Genome Wide Association (GWA) Studies on Triglyceride Levels Due to the Diet Supplemented with Chili Peppers on *Drosophila melanogaster*

Bodyweight is a complex trait defined by several biological processes. In this report, we describe the genetic approach used for fighting obesity as per the consumption of chili peppers. We also present a web-based pathway simulation that may occur due to consumption of the chili peppers. We show that the bodyweight response to consumption of chili peppers is positive and having them in our diet for a prolonged time will have a significant effect on weight loss than with normal diet. We also show a significant drop in the Triglyceride level with the chili pepper diet than with the normal one. To identify genes affecting bodyweight due to chili pepper diet, we assessed the total dry body weight and triglyceride level of aged flies of the sequenced, wild-derived lines from the *Drosophila melanogaster* Genetics Reference Panel. The flies were fed with the chili pepper diet throughout their life. We performed genome-wide association analyses to identify candidate genes associated with variation in bodyweight and confirmed the effects of 3 mutants tested in these candidate genes. Candidate genes associated with variation in bodyweight to chili peppers form networks of gene involved in purine metabolism, regulation of cell shape, imaginal disc-shaped wing morphogenesis, protein localization, serotonin receptor signaling pathway, regulation of small GTPase mediated signal transduction, signal transduction, regulation of Rho protein signal transduction, and axon guidance for habanero; glycerophospholipid metabolism, glycolysis/gluconeogenesis, signal transduction, sphingolipid metabolism, phototransduction, and protein phosphorylation for serrano and for glycerophospholipid metabolism, protein phosphorylation, Valine, Leucine and Isoleucine degradation, Inositol Phosphate metabolism, glycolysis/gluconeogenesis and G-protein coupled receptor protein signaling pathway for bell peppers. Many of these genes also have human orthologs, highlighting the utility of genome-wide association in *Drosophila* for studying complex human phenotypes.

12. Kierra Adams, Genetic Engineering to Produce DCAs Using *Candida Viswanathii* through CRISPR Modification

Candida viswanathii (*C. viswanathii*) is a relatively unexplored type of pathogenic fungi which is being considered as a new organism for CRISPR mediated genetic engineering. *C. viswanathii* is closely related to *C. tropicalis* which has been known to produce dicarboxylic acids (DCAs). After numerous heat shock trials to determine at what best temperature and time the fungi would uptake the vector of the plasmid pv1495. It has been determined that *C. viswanathii* uptakes that vector best at 42 °C and between 40-50 minutes. Upon receipt of the genome sequence of *C. viswanathii* we are attempting to genetically modify and block the ADE2 gene using CRISPR. We are currently creating guides and repairs specific to *C. viswanathii* in hopes of obtaining genetically modified colonies.

13. Samantha Belcher, Genome Wide Association Studies on Feeding Effects of *Nigella sativa* on *Drosophila melanogaster*

Bodyweight is a complex trait defined by several biological processes. In this report, we describe the genetic approach used for fighting obesity and diabetes as per the consumption of *Nigella sativa*. We also present a web-based pathway simulation that may occur due to consumption of the *Nigella sativa*. We are planning to do a glucose analysis. To identify genes affecting bodyweight due to the *Nigella sativa* diet, we assessed the total dry body weight of aged flies of the sequenced, wild-derived lines from the *Drosophila melanogaster* Genetics Reference Panel. The flies were fed with the *Nigella sativa* diet throughout their life. We performed genome-wide association analyses to identify candidate genes associated with variation in bodyweight. Through our GWAS analysis we found certain genes that explain the effects of change in bodyweight. We also performed pathway analysis using R spider to find the enriched sub-cellular pathways.

14. William Carey, Examining Excessive Ca²⁺/Mg²⁺ in NW Indiana Rainwater

The region of Northwest Indiana experiences abnormally high concentrations of calcium ion and magnesium ion in precipitation when compared with other nearby regions. These data primarily come from the National Atmospheric Deposition Program (NADP), whose broad scope and periodic sampling make investigating the causes for this anomaly difficult. In this work, precipitation samples from multiple storm systems from between May 21, 2018 and May 31, 2018 were collected at eight different sites across Northwest Indiana. These samples were analyzed using cation chromatography to determine the different levels of calcium and magnesium as a function of location and storm system. The relationships uncovered point towards a strong dependence upon location for the specific prevalence of these ions in Northwest Indiana. These relationships can help inform efforts to understand the root causes of the unusual abundance of these ions in the local precipitation.

15. Maurice Dantzer, Herpetological Surveys of Red Tail Land Conservancy Properties

Reptiles and Amphibians are excellent bioindicator species that provide a benchmark for the condition of an ecosystem. Amphibians use cutaneous respiration which allows for direct intake of any liquid or gas it may encounter in an environment. Reptiles being a group of species that have a wide range of diets from small mammals to vegetation, will display a drop in population if their food sources are limited. During the summer of 2018, I used a search and seizure method for the capture of reptiles and amphibians found on four Red Tail Land conservancy properties: Reber Woods, McVey Woods, White River Woods, and Fall Creek Woods. The objectives for these surveys were to 1) obtain information specific to species diversity, 2) species abundance, and 3)

environmental/weather dependent factors that may affect the behavior of reptiles and amphibians. In addition, I created Geographic Information System (GIS) maps to display where species were located on the properties. These surveys are the first to record field data for these four Red Tail Land Conservancy sites. Data from these surveys will provide Red Tail Land Conservancy information on amphibians and reptiles for species-specific management on the properties.

16. Amber Diggs, Overexpression and Purification of T7-RNA Polymerase for RNA In Vitro Synthesis

RNA nanotechnology is a rapidly emerging field and has recently received wide interest in the scientific community. RNA molecules play many important roles in gene expression, and new roles continue to be discovered. Increasing members in gene expression, and new roles continue to be discovered. Increasing numbers of new RNA structures are being solved and deposited each year in the structure databases (PDB and NDB). These structures reveal that RNA molecules form diverse and often intricate 3D structures to carry out their roles. These roles generally involve specific binding to different proteins, nucleic acids (RNA or DNA), or small molecules, including drugs or metabolites. Like proteins, RNA molecules can undergo significant structural rearrangements during function. These RNA features can be implemented to design and fabricate various types of artificial RNA nanoparticles via self-assembly. When a large amount of RNA is desired, (e.g., for making RNA nanoparticles for study therapeutic properties in vivo) it is advantageous to use chemical synthesis based on phosphoramidite technology. However, one of the major limitations of chemical synthesis is the production of long RNA polymers, as it becomes very difficult to synthesize individual RNA strands longer than 50 nucleotides. The transcription reaction using T7 RNA polymerase (T7 RNAPol) is the alternative method that requires DNA template to produce RNA polymer. In optimized conditions, T7 RNAPol can be used in vitro to produce milligram amounts of RNA polymers ranging from 30-30,000 nucleotides. In this study, we describe the overexpression and purification of T7 RNA polymerase enzyme as well as the optimized transcription condition to produce large amounts of RNA nanoparticle.

17. Eva Elmalh, Prevalence of Colistin-resistant Bacteria in Sanitary Sewage and the Environment

Over the past few decades, antibiotic resistance has increased among bacteria because of the frequent use and misuse of antibiotics. Not only is antibiotic resistance acquired by pathogenic bacteria, but also by harmless bacteria found in the human microbiome. Based on our observations of human enteric bacteria obtained from sewage, resistance to many classes of antibiotics is common. Colistin is used as a last-resort antibiotic against bacterial infections in humans, thus resistance to this antibiotic should not be common. In recent studies, it has been found that plasmid-borne colistin resistance among enteric bacteria has emerged, likely derived from animal feeding operations and veterinary use, but spreading to human clinical isolates. To investigate the extent of this resistance, we isolated enteric bacteria from sewage that are specifically resistant to colistin. We then tested for the presence of gene *mcr1* that confers resistance to colistin and the location of the gene (chromosome or plasmid). Thus far, 20 isolates have been obtained from primarily sewage samples, and the results of these isolates have indicated that colistin-resistant bacteria are prevalent in this region. Additional sampling to collect sewage and environmental samples from across the region is in progress in order to process them to allow for isolation and screening of colistin-resistant bacteria; these bacteria will be screened to determine the prevalence of these bacteria in the total bacterial population of sewage samples.

18. Cheikh Ethfagha, Engineering an Acetolactate Synthase Variant Exhibiting Acetohydroxyacid Synthase Activity

Acetolactate synthase (ALS) is an enzyme that catalyzes the formation of (S)-acetolactate in the first step of 2, 3-butanediol synthesis. Similarly, acetohydroxyacid synthase (AHAS) forms (S)-acetolactate for the production of valine and (S)-acetohydroxybutyrate for the production of isoleucine. Though both enzymes can form (S)-acetolactate, only AHAS can form (S)-acetohydroxybutyrate, even though both are thiamin diphosphate (ThDP)-dependent enzymes. It is possible that an ALS variant could be made that has the ability to form the (S)-acetohydroxybutyrate product. In order to test this, ALS variants will be created by site-saturation mutagenesis. Following creation of ALS variants, the plasmids containing these variants will be transformed into knockout *E. coli* strain MI262, a strain lacking AHAS activity. It has already been shown that transformation of a plasmid containing an AHAS gene into this strain allows the cells to grow in minimal media lacking isoleucine and valine. Additionally, it has been shown that transformation of a plasmid containing an ALS gene allows the cells to grow in media lacking valine, but not isoleucine. By transforming the ALS variants into this strain and testing for cell growth in media lacking isoleucine and valine, it can be determined whether the ALS variant is able to form both (S)-acetolactate and (S)-acetohydroxybutyrate. Any variants able to complement growth in this media will be isolated, purified, and analyzed for comparison with wild-type ALS and AHAS.

19. Mahussi Fagnon, Comparing Thromboelastography (TEG) and Turbidity Measurements to Monitor Clot Formation of Native and FITC-tagged Fibrinogen

For this study, thromboelastography (TEG) and turbidity, via a microplate reader, were used to measure fibrin clot strength. TEG results were reported as time to first clot formation (R), clot kinetics (K), rate of formation (Angle), and maximum clot strength (MA). Turbidity measurements were tracked over 20 min at 405 and 550 nm wavelengths in 96 well plates where increases in absorbance were utilized to track clot formation. Fibrin clots were formed by adding physiologically relevant amounts of bovine thrombin (1 U/mL) to bovine fibrinogen (1.5 – 4 mg/mL). Clot formation under conditions such as dialysis and FITC-tagging were used for

fibrinogen preparation while thrombin was held constant. Results demonstrate that fibrinogen, prepared via dialysis, yields an increase in turbidity (61.7% absorbance at 550 nm) while the TEG remained nearly constant (2.2% increase in MA, 18.6 ± 0.3 mm MA, 69.4 ± 0.4 degrees angle). Large differences were observed on both the TEG and microplate reader when comparing native and low FITC-tagged fibrinogen (69.3% decrease in MA and 4.5 times increase in absorbance at 550 nm). Comparing low FITC-conjugated (~ 4) and high FITC-conjugated fibrinogen (~ 10) exposed to the same clot preparation conditions yields measurable differences (73.9% decrease in MA and a large decrease in angle) via TEG while yielding smaller differences (15.6% increase in absorbance at 550 nm, 0.76 ± 0.08 absorbance) via turbidity. Results demonstrate that while turbidity is often the preferred method to track clot formation and fiber thickness, TEG, a direct measurement of clot strength, is a more reliable technique.

20. Giovanna Gorski, vCARE: Application of Geotagging to Improve Delivery of Public Health Services

Over 70 countries have had the Zika (ZKV) virus confirmed by the World Health Organizations. The ZKV virus has the potential for devastating neurological complications and long-term health challenges. The purpose of this project is to leverage information technology to help support the public health organizations manage the Zika health challenge. vCare project provides an integrated software solution that can assist health care professionals to efficiently manage the delivery of healthcare services to at-risk communities, and at the same time, help those whose job is to identify, treat and manage physical sites which pose risk to these populations.

21. Winnie Ihano, Characterization of Adenosine Deaminase (ADA) Mutants in Severe Combined Immunodeficiency (SCID)

Severe Combined Immune Deficiency (SCID) is a genetic disorder caused by mutations in the gene coding for the enzyme adenosine deaminase (ADA). Normally, ADA is involved in purine degradation, converting adenosine to inosine. However, mutations in ADA impair its ability to perform such function. As a result, adenosine levels increase and cause T and B immune cell dysfunction, a sign of an impaired immune system. Hence, the goal of this project is to analyze the mutant enzyme function by isolating and purifying several ADA mutants associated with SCID. Furthermore, the studied ADA mutants will be compared with the wild type ADA enzyme. The long-term goal of this project is to be able to determine if the function of ADA mutants can be reversed in an effort to develop new therapies for SCID.

22. Alero Jackson, Quantification of Antibiotic Resistance in Enteric Bacteria Isolated from Untreated Sewage

Antibiotics are widely used as a means of inhibiting bacterial growth and killing bacterial organisms by targeting a variety of metabolic function. Over time, bacteria have developed resistance to nearly all classes of antibiotics, including “superbugs”, which show resistance to many or all classes of antibiotics. Antibiotic resistant bacteria are especially present in sewage where human-associated bacteria, mainly commensal (nonpathogenic), as well as pathogens accumulate and can become resistant. This work focused on analyzing the prevalence of resistance among human enteric bacteria present in sewage. Sewage samples obtained from local plants were filtered and were plated on antibiotic-amended fecal coliform (FC) medium plates. Colonies obtained from the FC plate assays were purified on eosin methylene blue (EMB) medium plates to obtain pure cultures. Antibiotic resistance screening and minimum inhibitory concentration (MIC) tests using 11 different antibiotics across 5 antibiotic classes were performed on to identify highly resistant organisms. Of the 230 isolates that were screened, 13 isolates were extensively drug resistant (XDR), showing resistance to at least 7 of the tested antibiotics. Isolates most commonly showed resistance to augmentin and tetracycline, with 147 and 146 resistant isolates, respectively. Meropenem and azithromycin resistances were least frequent, occurring with 14 and 32 isolates respectively. Future work will explore the mechanisms by which these organisms acquired their resistance.

23. Stacey Jean-Baptiste, Electrocatalytic Oxidation of Aminothiols Using Modified Electrodes with Gold Nanoparticles

Homocysteine (HCys) is an amino acid that contains a thiol group which is a carbon compound bonded to a sulfhydryl group. Cysteine (Cys) which is chemically similar to HCys differing by a methylene bridge, can also be present in biological samples. Elevated levels of homocysteine have been linked to strokes and ischemic heart diseases. Therefore, developing a sensitive method to measure homocysteine in biological samples such as urine is important for prevention and treatment of these diseases. HCys and Cys can both be measured using electrochemical methods because they are both electroactive. In the present work, the electrochemical behavior of HCys and Cys was studied using a glassy carbon electrode modified with gold nanoparticles to enhance the catalytic oxidation of these compounds. Cyclic Voltammetry and chronoamperometry techniques were employed in this work. Preliminary results show that glassy carbon electrode modified with gold nanoparticles show promise for detecting homocysteine and cysteine in aqueous solutions.

24. Crystal Johnson, Spinophilin Protein-protein Interactions in the Striatum Subsequent to Psychostimulant Administration

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopment disorder that can persist into adulthood. Treatments are predominantly psychostimulant-based drugs, which act to increase monoamine concentrations within the central nervous system. Psychostimulants such as amphetamine act at multiple loci to drive increases in synaptic neurotransmitter levels. ADHD along with many neurological diseases including obsessive-compulsive disorder and drug addiction, report perturbations in dopaminergic

regulation and aberrant synaptic transmission. The striatum receives a substantial amount of dopamine inputs. A majority of the neurons within the striatum are medium spiny neurons (MSNs), which contain either dopamine D1 or dopamine D2 receptors. Proper neuronal communication and function is dependent upon the dynamic regulation of reversible protein phosphorylation. Spinophilin, the major protein phosphatase 1 (PP1) targeting molecule, is highly enriched in dendritic spines and has been shown to regulate protein phosphorylation by targeting PP1 to, or inhibiting PP1 activity at, myriad substrates. Our lab previously reported that 6-hydroxydopamine lesioned animals, an animal model of dopamine depletion, leads to global decreases in spinophilin protein-protein interactions in the striatum; however, changes in spinophilin protein-protein associations in a hyperdopaminergic milieu are less understood. Here we report greater increases in spinophilin protein-protein interactions under a psychostimulant-induced behavioral sensitization paradigm.

25. Lyunade Adebowale, LC-MS/MS Detects Urobilinoids from Feces in Fly Guts

Blow flies are suspected to carry pathogens from one location to another due to the consumption of fecal matter leading to the contamination of food sources. Gut extracts of the blow flies were prepared and then analyzed to detect various tetrapyrrole urobilinoids, commonly found in fecal matter, using liquid chromatography-mass spectrometry. The flies analyzed were from three feeding treatments: unfed flies, liver fed flies, and feces fed flies. Confirmatory tests were run on fly extracts where different types of animal feces were consumed to verify that the urobilinoid compounds would be present regardless of the type of fecal matter consumed (omnivore, herbivore, carnivore). The blow flies were analyzed using a Thermo LTQ-XL mass spectrometer (San Jose, CA) coupled to an Agilent 1100 HPLC (Santa Clara, CA). We used reversed phase chromatography on a 100 x 2.1 mm C18 column at a flow rate of 200 μ L/min to separate the urobilinoids of interest. The solvents used were 0.1% formic acid in water (solvent A) and 0.1% formic acid in 70:30 acetonitrile:methanol (solvent B). The data were acquired in positive ion mode using a gradient with an initial 1-minute hold at 30% B along with a 9-minute linear gradient from 30 to 95% B. Two compounds, urobilin and urobilinogen, were used to indicate the presence of the fecal matter with retention times of approximately 6.5min containing the m/z 343 and 8.5min containing the m/z 466 respectively. This was utilized to link the flies to pathogen transmission via fecal matter.

26. Kaylin Laws, Investigation of Reducing Highly Cross-linked Inverse Vulcanized Sulfur Polymers

Sulfur is fairly cheap and there is currently an abundance of it. Elemental sulfur has good electrochemical properties and wide varieties of applications that can be explored. In this experiment, elemental sulfur is polymerized incorporating divinylbenzene creating a highly cross linked polymer via inverse vulcanization. The sulfur cross linked polymer poly(S-DVB) is then reduced to create Thiol functional groups cleaving the hydrogen bonds. The Inverse vulcanization method is used to create poly(S-DVB) in a solvent less and fairly fast polymerization, while still producing a stable crossed-link polymer. Poly(S-DVB) is characterized using gel permeation chromatography and nuclear magnetic resonance. Data indicates a new peak growing in after being reduced to form Thiols. Thiol groups can react with a variety of different functional groups. This will allow the structure of the poly(S-DVB) to change and open the highly cross-linked polymer with intentions to bind other compounds such as Bromoanthracene. A fast reaction will occur removing the Thiol functional group and replacing it with the Bromide from the Bromoanthracene. This reaction is characterized using Ultra violet spectrum instrumentation. Click chemistry will be furthered explored through inverse vulcanized poly(S-DVB) and modifications.

27. Adam Lloyd, Synthesis of Substituted Pyrazolines as Inhibitors of Bacterial Invasion

Bacteria are constantly becoming resistant to antibiotics, and as a result, new antibiotics are needed to combat these super bacteria. Staphylococcus aureus is an example of a specific type of bacteria that has shown signs of antibiotic resistance and cellular internalization. A compound known as ML141 has shown to have antimicrobial properties however, is not safe for human use. The central pyrazoline ring gives the compound its activity and allows for binding to Cdc42. When bound to Cdc42, the GTPase is inhibited and Staphylococcus aureus which is bound to Fibronectin, is not internalized. The purpose of this research is to synthesize ML 141 derivatives in ways that increase solubility and reduce cytotoxicity. To make ML 141, the first reaction is an aldol condensation of a ketone and aldehyde to form the chalcone. The second reaction is to make the p-sulfamylphenylhydrazine hydrochloride which reacts with the chalcone to make 4,5-dihydropyrazole, also known as ML 141. The ML 141 structural analogues are biologically tested on human umbilical vein endothelial cells to determine effectiveness of preventing bacterial cellular internalization.

28. Emmanuel Makanjuola, Quantifying ACCH Domain Stability via Differential Scanning Fluorimetry

Angiomotins (Amots) are a family of adaptor proteins with important roles in cell growth, migration, and proliferation. The Amot coiled-coil homology (ACCH) domain, has a high affinity for non- and mono-phosphorylated phosphatidylinositols lending to specificity in membrane association. While this domain is known to target Amot130 to membranes to perform its tumor suppressor function, the N-terminal domain (NTD) has also been shown to regulate ACCH domain membrane association. Furthermore, the NTD is known to associate with actin until undergoing phosphorylation, which lead to membrane association. We hypothesized that interactions with the non-phosphorylated NTD directly causes structural defects in the ACCH domain that prevents membrane

binding. This hypothesis was derived from reports of other coiled-coil domains that undergo structural re-arrangement to allow for protein-actin association. To study this research question, we use differential scanning fluorimetry (DSF) to measure changes in melting temperature because of this interaction. Based on our hypothesis, we expect a decrease in the ACCH domain melting temperature after incubation with NTD constructs reported to reduce membrane affinity vs. an *in vivo* tested NTD phospho-mimetic that restores membrane affinity. Inability of the NTD to affect ACCH domain melting temperature will suggest the need to screen for other potential interactions between the domains that could cause the noted phenomena.

29. Jazmin Marks-Burns, Investigating the Effects of PUS5 Deletion on Mitochondrial Encoded Protein Expression in *Candida albicans* and *Saccharomyces cerevisiae*

While RNA is made of only 4 bases, these bases can be modified in over 100 distinct ways. These modifications play critical roles modulating RNA function. Pseudouridine is the most common modified nucleoside, and is found in all kingdoms of life. However, the role of pseudouridylation in translation and RNA function is not well understood. Pseudouridylation is found at dozens of sites in Eukaryotic cytoplasmic rRNA and cytoplasmic ribosomes translate thousands of proteins. As such, it is challenging to study the effects individual sites of pseudouridylation have on translation. In contrast, in fungi, mitochondrial ribosomes translate only eight genes encoded by the mitochondrial genome and mitochondrial rRNA contains only one highly conserved pseudouridine which is made by the pseudouridine synthase Pus5. We find *Saccharomyces cerevisiae* *pus5*Δ deletion is more sensitive to drugs that inhibit oxidative phosphorylation such as oligomycin, suggesting they have a defect in mitochondrial function. We will investigate the roles of Pus5 mediated pseudouridylation on mitochondrial protein expression. We will use mass spectrometry to determine if mitochondrial rRNA pseudouridylation is required for wild type mitochondrial gene translation. Investigation of this highly conserved RNA modification will lead to a better understanding of how defects in pseudouridylation lead to human disease and could lead to the identification of novel antifungal drug targets.

30. Cancelled

31. Rebeca Mena, Microplate-based Colorimetric Detection of Free Hydrogen Sulfide Produced by mitoNEET and Cysteine
MitoNEET is a protein that contains a [2Fe-2S] cluster in a unique ligation pattern of 3Cys-1His (Figure 1). While iron is an essential element for all life, it can also be highly toxic to living systems when not appropriately sequestered. MitoNEET is an iron-handling protein known to be predominantly localized on the outer mitochondrial membrane. Through previous research it was discovered that the protein mitoNEET likely has a significant role in type-II diabetes and in regulating mitochondrial metabolism. However, the molecular mechanisms are unknown at this time. Previous experiments in the Konkle laboratory a possible enzymatic activity between PLP-modified mitoNEET and L-cysteine (an amino acid with key redox regulatory roles). However, the products of that reaction are still unidentified. We adapted a published assay (Figure 2.) to determine if the thiol group of cysteine was being released as H₂S gas or reacted with an additional cysteine of a protein to form a disulfide bond. If the latter were occurring, then treatment of dithiothreitol (DTT) would release H₂S gas. A standard curve using the positive control of Na₂S was determined. In summary, the reaction of cysteine with PLP-charged mitoNEET does not cleave the thiol group from L-cysteine.

32. David Mitchell, Pollinator Diversity and Abundance in Neighboring Urban Wetlands

Urban development and habitat destruction are having a devastating effect on native pollinator species. Pollinators are important because they aid in plant seed production. We conducted three studies in the summer of 2018 exploring the relationships between plants and local pollinators in two restored wetlands on the IU South Bend campus. Visitation rates and visitor diversity were estimated using 15-minute observation periods over 2-5 days per plant species. Mean visitation rates ranged from 1-12 visits/flower/hr, with substantial variation observed between days, between time periods within days, and between plant species. *Asclepias incarnata* and *Iris virginica* received a greater diversity of visitors than *Penstemon digitalis* and *Tradescantia ohiensis*. Bees and flies were the dominant visitors, except in *A. incarnata*, where beetles were also frequent visitors. Using fluorescent dye to track pollen movement, we found that floral visitors move within wetlands, but not between wetlands. Pollen limitation was tested by comparing fruit set and fruit mass in pairs of flowers subjected to supplemental or natural pollination. In *T. ohiensis* we found a trend toward higher fruit set (86% vs 70%) and fruit mass (0.046 g vs 0.037 g) in the supplemental treatment. Fruit set and fruit mass in *P. digitalis* were similar between treatments. Overall, we found fairly high visitor diversity and little pollen limitation, but connectivity between wetlands seems to be low. More studies on plant-pollinator interactions and constructing similar areas of natural vegetation between the wetlands to encourage pollinator movement would contribute to our understanding of how urban landscapes can help support native pollinator species.

33. Carlos Montalvo-Hernandez, All-in-one Pothole Machine

A pothole is a structural failure in a road surface due to water in the underlying soil structure and traffic passing over the affected area. According to the Indianapolis Department of Public Works, there are thousands of potholes yet to be fixed in the first half of 2018, even though \$100 million+ have already been allocated to road projects. Understanding the pothole repairing process through

modeling is key to identifying critical research areas which may lead to new innovations in pothole projects. The objective of our summer project is to build and simulate a typical pothole repairing process model. There are three tasks for the proposed project: interview civil/construction engineers from industry and within IUPUI; build and simulate a block model to represent a typical pothole repair project to recognize bottlenecks and potential optimization spots. We have built a process model which covers an entire pothole repair project. The model was then simulated to help understand the characteristics of the processes and identify potential optimization strategies. The data collection that we have done during the summer is an important first step to our long-term goal, which is to have an all-in-one machine that repairs potholes in a quicker, more cost-efficient way.

34. Christian Moreno, Development of Tool to Operate on Periodic Trends

The main goal of the project is to generate a broad computer program that will be able to provide the trends on the periodic table such as atomic radii and ground state electron-configuration using a model that freshmen can appreciate. The first approach is to begin with the Hydrogen model which is only reliant on one electron but extend it to multiple electrons while ignoring electron-electron repulsion that is present in many-electron atoms. For improvement we must consider some of the electron-electron repulsion. Therefore, when analyzing these multiple electron systems, we replace the distance between the two electrons, r_{12} , with an approximation $r>$, the distance of an electron farthest from the nucleus. This replacement changes the repulsion energy potential from $1/r_{12}$ to an approximated $1/r>$. Although this change is a significant approximation in our analysis we are still successful in replicating the correct trends in our analysis of the periodic table.

35. Pierre-Emmanuel N'Guetta, Engineering a FABulous Fluorescent Glyphosate Biosensor Using Phosphate-binding Protein (PhnD)

Glyphosate is an herbicide also known as Roundup and is used to kill weed by blocking proteins essential to plant growth. Nowadays glyphosate is the most popular herbicide used around the globe. However, very little is known on glyphosate. It has been stated as probable human carcinogen. It also has made recent headlines for its widespread use on genetically modified seeds and research that links it to antibiotics resistance and hormone disruption. Several states are planning to restrict its use, while it has already been banned in the state of California. Hence a successful technique to detect glyphosate can help us determine its concentration and effect in our environment. The idea is to use a protein, Phosphate-binding protein (PhnD), as a biosensor for Glyphosate. PhnD naturally binds to glyphosate, but with a low affinity. Our goal is to increase the affinity of PhnD for glyphosate. PhnD undergoes a conformational change upon binding to its ligand from an open to a closed conformation. We can take advantage of this conformational change to develop PhnD into a fluorescent sensor for glyphosate, where a fluorescent reporter group attached to PhnD changes its emission properties in response to glyphosate binding. We also sought to take advantage of this property by engineering antibody fragments that bind specifically to the closed (glyphosate-bound) form of PhnD. According to Le Chatelier's principle, fabs that bind to the closed form of PhnD would drive the equilibrium towards binding, further enhancing its affinity for glyphosate and resulting in a more sensitive sensor. I generated a set of fabs using a technique known as phage display selection. My project aims are to characterize the fabs to determine: 1. The affinity of each fab for the open and closed form of PhnD, and 2. The effect of the Fabs on the affinity of PhnD for glyphosate.

36. Lionnel Nkurunziza, The Attentional Set Shifting Task as a Measure of Cognitive Flexibility in Wistar Rats and Alcohol- preferring P Rats

The alcohol-preferring P rat is a well-known animal model of genetic predisposition for excessive drinking, which also exhibits elevated impulsivity. (Linsenhardt, Smoker, Janetsian-Fritz, & Lapish, 2017) Here we investigate whether and how such genetic predisposition influences the cognitive flexibility of these animals. Recent research has shown signs that attentional set shifting in animals is dependent on the medial frontal cortex (MFC). (Birrell & Brown, 2000) We report and compare behavioral results from an Attentional Set-Shifting Task performed in both Wistar Rats (a common outbred albino rat) and P rats (Alcohol Preferring Strain of Wistar Rats). The Attentional Set Shifting Task (ASST) is modeled after the interdimensional /extradimensional component of the Cambridge Neuropsychological Test Automated Battery (CANTAB) which is used to identify cognitive dysfunction in humans and non-human primate. (Lapiz-Bluhm, et al., 2008) So far, we have found a few notable distinctions in the performance and training progression of the strains of rats compared. Future findings from this study could improve our understanding of how genetic predisposition to alcoholism is related to impairments in the cognitive flexibility of rodents and suggest directions for future addiction and clinical studies.

37. Celia Ochoa Medina, UV Nucleotide Binding Site Photocrosslinking of Antibodies at Various Light Intensities

The nucleotide binding site (NBS), found in the Fab variable domain of all antibody isotypes, is utilized in UV photocrosslinking methods for site-specific functionalization of monoclonal antibodies. UV exposure (254nm) to a small molecule, indole-3-butyric acid (IBA), that has high affinity to the NBS can be used to photocrosslink ligands to antibodies. Here, we propose a method to modify antibodies by photocrosslinking with various intensity UV light sources: UV crosslinker XLE-1000 (40-watt), handheld EF-160C (6-watt), and MiniMax UV-5NF (5-watt). The different UV sources possess different power levels and by modulating both time of UV

exposure and distance from source site-specific crosslinking at the NBS, for affinity tags (IBA-Biotin) and fluorescent molecules (IBA-FITC) was optimized. Application of the UV-NBS photocrosslinking technique is possible by first incubating the FDA approved antibodies, Rituximab [chimeric anti-CD20] and Tocilizumab [chimeric anti-IL-6R] (12-15 μ M), with IBA-FITC (300 μ M) followed by 0.1-1.5J/cm² of UV exposure in triplicate experiments. Conjugation efficiency was determined via absorbance/fluorescent measurements for the quantity of conjugated IBA-FITC. The UV-NBS technique is a reproducible method of photocrosslinking antibodies. Optimization of UV energy exposure resulted in an increase of conjugations per antibody with maximized photocrosslinking efficiency, while antibody antigen binding activity and Fc recognition were preserved. This study demonstrates that the UV-NBS site-specific antibody modification technique can be accomplished using UV light sources with differing light intensities expanding its implementation potential through making the technology more accessible. Ultimately, the UV-NBS method is an efficient, practical, and accessible method of functionalizing antibodies in diagnostic, pharmaceutical, and therapeutic settings.

38. Nicholas Olchawa, Me31B Role in mRNA Regulation

The protein Me31B plays a role in RNA regulation by interacting with RNA translation repressor and degrader proteins during oogenesis. If functional Me31B is absent, this causes immature translation of *osk* and *BicD* mRNAs, which suggest that Me31B is needed for the translational repression of the germ granule RNAs. Recent studies have strongly suggested that Me31B plays a conserved role in post-transcriptional RNA regulation, likely by influencing RNA stability. However, whether Me31B causes degradation to germ granule RNAs during oogenesis is not quite yet clear. Moreover, Me31B's RNA binding specificity during oogenesis is unknown. In order to investigate the RNA binding specificity of Me31B in oogenesis, we propose to perform RT-PCR to quantify representative germline RNAs such as *me31B*, and to test the RNA stability in heterozygous *me31B* flies (*me31B Δ 1/+*). We will also use RIP-Seq on the RNAs co-immunoprecipitated with ovarian Me31B. We hypothesize that the defects in germ cell formation in the *me31B* heterozygous flies are caused by the failure to regulate certain germline RNAs like *osk* mRNA.

39. Jose Rodriguez, Investigation of the Impact of Cyano Substituents on the Reactivity of Oxypyridinium Salts

Research mainly focused on how a certain reaction that is primarily done under specific conditions and specific reagents can incorporate a large variety of different reagents and/or conditions that the original reaction was created. The original reactions that is being referred is the synthesis of benzyloxy-pyridine and benzyloxy-pyridinium triflate known as the Dudley salt. The reaction is the creation of a stable benzyl ether that was done under mild conditions and deals with carbocation pathways. The creation of the products showed us how the oxygen based functional groups can be used as stable protecting groups with ether synthesis. Another main objective was to understand the synthetic chemistry behind the creation of the two products. We learned through various articles and group meetings that the salt undergoes S_N1 chemistry since the reaction between the two reagents, benzyl alcohol and 2-chloropyridine, form carbocation intermediates before the final synthesis of the product. The last main objective that needed to be understood was to perform reactions in a wet lab to understand the synthetic chemistry behind the two products.

40. Pedro Sanchez, Initial Studies for a Structure—Functional Analysis of *Phanerochaete chrysosporium* Desaturase

Stearoyl-CoA desaturase (SCD) is an enzyme that is found within all eukaryotes and creates the first double bond between the ninth and tenth carbons of a saturated fatty acid acyl-CoA. Humans have two kinds of SCD, SCD1 and SCD5; mice have four, SCD1-SCD4. The crystal structure of SCD1, deposited in the Protein Data Bank as 4YMK, is the first published structure of a membrane-bound fatty acid desaturase. Using the Phyre2 structure threading web interface and Chimera, a program capable of displaying an interactive three-dimensional structure of proteins, we were able to contrast a hypothetical structure of the *Phanerochaete chrysosporium* desaturase (PchDes), an enzyme that results in a second double bond between carbons 12 and 13, with the experimental three-dimensional structure of SCD1. This improved model allows us to consider possible catalytic and binding sites and any other distinct features. Both SCD1 and PchDes are hypothesized to release the fatty acyl CoA products after the reaction has been catalyzed through reversible hydrogen bonding. Site-directed mutagenesis is being used to test this hypothesis by creating mutants that will replace the amino acids involved in the product release mechanism and potentially affect the locations of the double bonds. Once the site-directed mutagenesis has been completed, the results of yeast expression experiments can be analyzed by gas chromatography–mass spectrometry.

41. Princess Walker, Modification of Poly(s-dvb) Using Maleimide

The ability to completely modified poly-sulfides are possible means to broaden the versatility of sulfur base polymers while tackling the huge elemental sulfur mountain occupying landfill and posing a threat to the environment. Elemental sulfur is produced on a million-ton scale annually from crude oil in petroleum refinery; this amount is expected to increase. With this in mind, we embarked upon the job of utilizing as much sulfur into creating polymers with flexible thermo-mechanical properties. Doing so we utilized the inverse-vulcanization method and created a 30:70 sulfur-divinylbenzene polymer (poly(s-DVB)) on a 5gram scale at 185 degrees Celsius for 1 hour. Next, we created a terpolymer by adding maleimide to a sample of the 30:70 poly-sulfide created; this reaction occurs at 100 degrees Celsius for multiple time trials ranging from 0-48 hours in a hot-oil-bath. To aid incorporation, dimethylformamide (DMF) added to the reaction system. This has shown to be effective and produce replicable data. After

completing the reaction, gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) were used to characterize the resulting terpolymer. NMR let us know that as the reaction time increases, more maleimide is being incorporated into the poly(s-DVB); and that is exactly what we're watching out for. Following confirmation that the maleimide is being incorporated into the poly(s-DVB), we ran the sample through GPC to see how the newly formed terpolymer is affecting the structure of the poly(s-DVB). This is determined by looking at changes in the average molecular weight (Mw), with this we noticed that there was no huge change in the molecular weight value, indicating that the backbone of the poly(s-DVB) is still intact as the maleimide is bonded to the structure. Overall, we were able to create a terpolymer by reacting maleimide with poly(s-DVB) with the help of DMF; without destroying the prepolymer.

42. Brooklyn Williams, The Role of Actin Binding Protein in Cellular Protrusions

Mammalian ears are divided into three distinct sections: outer, middle, and inner. The inner ear contains a sensory organ known as the cochlea, which houses the organ of Corti. The organ of Corti contains hair cells which are responsible for hearing. There are two types of highly organized hair cells: inner and outer hair cells. Outer hair cells amplify sound while inner hair cells detect the sound and convert it into neural signals. Protruding from the hair cell are mechano-sensing organelles called stereocilia which respond to fluid motion. At the core of stereocilia are rigid cross-linked, parallel actin filaments, which aid in the stability of these hair-like structures. We hypothesize that uncharacterized actin binding proteins will have novel roles in stereocilia. To this idea, we set out to purify recombinant a candidate protein for use in a reconstitution assay with permeabilized stereocilia. We first sought to subclone flag-tagged protein cDNA from a eukaryotic expression vector to a prokaryotic expression vector. Flag-protein expression will be induced and the protein will be purified by affinity chromatography using the flag affinity tag. Once the protein is purified, we will treat the exposed stereocilia with the flag-tagged protein to determine where the protein localizes. We anticipate this experiment will help better our understanding of what role actin binding proteins play in maintaining stereocilia at a cellular level.

43. Opeibea Aidoo, Differential Regulation of Dyrk1a at the Perinatal Stage in Down Syndrome Mice

Down syndrome (DS), also known as Trisomy 21 is a genetic disorder which arises from the triplication of human chromosome 21 (Hsa21). DS manifests itself with cognitive deficits and skeletal impairments in individuals with DS and occurs in about one per 1000 live births each year. DYRK1A is a gene triplicated in trisomy 21, and has been associated with cognitive and skeletal impairments in DS. It has been hypothesized that the triplication of DYRK1A in DS causes an increase in DYRK1A protein levels. However, studies have shown that Dyrk1a mRNA levels are not overexpressed in trisomic mice at all times and in all tissues. The mechanism of Dyrk1a expression and its effects on cognitive impairments are also not fully understood. The Ts65Dn DS mouse model is a segmental trisomy of mouse chromosome 16 (Mmu16) and carries half the gene orthologs from Hsa21, including Dyrk1a. Ts65Dn is the most widely used mouse model of DS and exhibits similar phenotypes as individuals with DS. The main hypothesis of my research is that differential regulation of trisomic Dyrk1a at the perinatal stage, including temporally and spatially specific expression, leads to the cognitive impairments associated with DS. Preliminary results indicate that Dyrk1a is overexpressed in P15 Ts65Dn males. In this study, the protein and DNA of the mice were isolated at E18.5 and P6. Treatment at these sensitive stages of development when Dyrk1a is overexpressed in trisomy could result in a reduction of cognitive deficits.

44. Sinai Valdez, Short-term Pharmacologic Inhibition of RAGE Suppresses Bone Turnover and Muscle Atrophy in Aging

Osteocytes, cells embedded in the bone matrix, are key in regulating bone turnover by controlling the function of bone-forming (osteoblast) and -resorbing (osteoclast) cells. Research from previous work indicates a specific gap junction protein called connexin43 was observed to be an important component of the signaling pathway controlling osteocyte survival. Further, aging decreases connexin43 and deletion of this protein was found to mimic the skeletal phenotype of old mice. Based on these findings we sought to further examine link between osteocyte apoptosis and osteoclast differentiation. Previous studies have shown that high mobility group box 1 protein (HMGB1), a pro-inflammatory cytokine that activates the receptor for advanced glycation end products (RAGE), is released by dying osteocytes and mediates osteoclast recruitment/differentiation. In order to address the role of these molecules in the skeleton, we injected mice with a small molecule RAGE inhibitor in order to prevent HMGB1-RAGE activation. The data collected so far further confirms the role of RAGE signaling in osteoclast differentiation as evidenced by the decreases in osteoclast number/ bone surface in animals treated with the RAGE inhibitor. Based on preliminary data suggesting that the RAGE inhibitor may also be affecting skeletal muscle in aging. We studied muscle histology and measured the cross-sectional area (CSA) of the muscle fibers and the CSA of specific muscle fiber-types. These measurements allowed us to examine whether aging and/or treatment with the RAGE inhibitor changes the size or distribution of muscle fiber-types. Allowing us to better understand if the RAGE inhibitor is in fact affecting muscle atrophy in aging in addition to the effects that it had on bone (osteoclast number). Through these studies we hope to further understand the molecular signals that link osteocyte apoptosis and osteoclasts recruitment/ differentiation in aging.