# JOURNAL OF WINCHELL UNDERGRADUATE SYMPOSIUM ABSTRACTS

*Celebrating 34 Years of Excellence in Undergraduate Research*

## MINNESOTA ACADEMY OF SCIENCE

**SPRING 2022**

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# *JOURNAL OF WINCHELL UNDERGRADUATE SYMPOSIUM ABSTRACTS*

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The abstracts included in this publication describe research presented at the Minnesota Academy of Science Annual Meeting / Winchell Undergraduate Research Symposium held at Hamline University on April 23, 2022

# BIOCHEMISTRY

## INVESTIGATING THE ROLES OF MUTATIONS IN THE MOBILE LOOP OF MALATE DEHYDROGENASE

Fadumo A. Bashir, Nikki Amaya Rapalo, and Betsy Martinez-Vaz (Advisor)
*Department of Biology and Biochemistry Program*
*Hamline University, St. Paul, MN*

Malate dehydrogenase (MDH) is an enzyme that plays an important role in several metabolic pathways such as the TCA cycle. This research looks at the reversible redox reaction catalyzed by MDH where malate is oxidized and NAD+ is reduced to form oxaloacetate and NADH. The purpose of this research was to investigate the effects of MDH mutations D132N and R130E and observe how it might affect the catalytic activity of this enzyme. We hypothesized that these mutations would lower MDH's specific activity due to the changes in overall net charge of the amino acids, which could impact interactions with the substrate at the active site. To test the hypothesis, we purified the wild type and mutant enzymes using His-tag affinity chromatography. Enzyme activity was monitored with a spectrophotometric assay that follows the disappearance of NADH at 340 nm. The results showed that the mutation at position 130 (R130E) reduced enzyme activity by more than 90%. In contrast, the D132N mutation had a 40% reduction in activity compared to the wild type. These findings suggest that the arginine and aspartate residues at positions 130 and 132 in the MDH loop play an important role in substrate stabilization, and catalysis. Future work will focus on studying the effects of the R130E and D132N on MDH substrate binding (Km) and specificity.

## PROLINE TO VALINE CHANGE IN THE ACTIVE SITE LOOP OF MALATE DEHYDROGENASE REDUCES SPECIFIC ACTIVITY BY 85%

Alvina N. Che, Daneisha Bergquist, Mariamu Kamara, and Tamara L. Mans (Advisor)
*Department of Biology*
*North Hennepin Community College, Brooklyn Park, MN*

Malate Dehydrogenase (MDH) catalyzes the oxidation of malate to oxaloacetate by transferring a hydride from malate to NAD+ (Bell et al, 2001). The active site loop of the enzyme is thought to open and close, changing the conformation of the enzyme when the substrate is present. A proline at position 123 of the watermelon glyoxysomal MDH enzyme may bend the loop at this position, enhancing flexibility of the loop. We hypothesized that changing proline to valine at position 123 will lower the specific activity, compared to the wild type MDH. We designed a pair of primers with the valine codon at the nucleotides corresponding to this position and used site-directed mutagenesis on a plasmid construct containing the MDH gene with the QuikChange protocol. We expressed and purified the mutant and the wild type enzymes. We used spectrophotometry to measure absorbance of light by NADH in a kinetic assay. The change in NADH level was used to determine the initial rate and specific activity of the mutant and the wild type enzymes. We found the activity of the P123V mutant enzyme was decreased by 85% compared to the wild type enzyme.

**THE EFFECT OF BULK SOURCE PEPTONE MEDIA, CHEMICALLY DEFINED MEDIA, AND PARTIALLY DEFINED MEDIA ON *Escherichia coli* GROWTH**

Monique C. Demuth, Abigail R. Kisch, Leo R. Pfarr, and Thomas C. Marsh (Advisor)
*Department of Chemistry*
*University of St. Thomas, St. Paul, MN*

Sustainable methods of microbial fermentation are of high interest to produce natural products such as foods, pharmaceuticals, and more. For microbial fermentation to be performed on a mass scale, bulk nutrient sourcing is a more sustainable option. Many animal-based products used in microbial fermentation are sourced using environmentally taxing methods. Plant-based hydrolysates (peptones) have proven to be suitable replacements for animal-derived protein sources in cell mediums. In this study we aimed to examine the impact on cell performance when cultured in LB medias with varying protein sources of soy peptone, pea peptone, a blend of soy and pea peptone, and beef extract. *Escherichia coli* strains EPI-300 and a bioengineered EPI-300 strain containing the plasmid pCC1Fos, otherwise known as F-purple, were tested. Biomass and pCC1Fos plasmid production were assessed via UV-visible spectroscopy and gel electrophoresis, respectively. We found that media formulations containing plant-based hydrolysates resulted in comparable biomass with formulations containing beef extract. Soy peptone media generally resulted in a higher stationary phase OD600 than pea peptone media across both tested *E. coli* strains. Plasmid production was also found to be comparable among media formulations. Plant-based hydrolysate LB media formulations were found to be suitable replacements for beef-extract media formulations for both biomass and plasmid production.

**WATERMELON GLYOXYSOMAL MALATE DEHYDROGENASE: MUTATION OF PHENYLALANINE TO TYROSINE AT AMINO ACID POSITION 134 REDUCES SPECIFIC ACTIVITY**

Esmail El-shekeil, Abdalla Hassan, Riley Reed, and Tamara L. Mans (Advisor)
*Department of Biology*
*North Hennepin Community College, Brooklyn Park, MN*

Malate Dehydrogenase (MDH) is an enzyme that catalyzes the reversible reaction of malate to oxaloacetate by oxidizing NADH to NAD+. This enzyme has an active site loop which is thought to open and close as the substrate enters the active site. We used PyMol to visualize the structure of the watermelon glyoxysomal MDH protein, and focused on position 134 in the active site loop. The phenylalanine at this position is quite close to the substrate and is highly conserved in divergent examples of MDH. We hypothesized that a mutation from phenylalanine to tyrosine would result in decreased activity of the enzyme. We designed primers to change the codon for phenylalanine to the codon for tyrosine on a plasmid construct containing the gene for watermelon glyoxysomal MDH and performed PCR site-directed mutagenesis with the primers. We expressed and purified the mutant enzyme as well as wild type watermelon glyoxysomal MDH protein. A kinetic assay measuring initial rate of reaction showed that the mutant F143Y MDH had one-third of the specific activity of the wild-type MDH.

**PROTEIN ENGINEERING OF A SPECTROSCOPIC PROBE INTO MALATE DEHYDROGENASE**

Equoia S. Gibson, Mary E. Ludwig, Olivia H. Thompson, Genevieve M. Woods, and Ella M. Young, and Lisa N. Gentile (Advisor)
*Department of Chemistry*
*College of Saint Benedict & Saint John's University, St. Joseph and Collegeville, MN*

Malate dehydrogenase (MDH) is an enzyme that has a key role in biological processes, like the Krebs cycle. Specifically, it reversibly catalyzes the interconversion of (S)-malate with NAD+ to oxaloacetate and NADH. Once oxaloacetate is synthesized, MDH dispatches it to citrate synthase, but it is not clear how this happens. One theory is that MDH channels it to citrate synthase by forming a metabolon, a mechanism for direct channeling, preventing diffusion of reaction intermediates into a bulk matrix. There is a lack of research in this area due to the absence of a spectroscopic probe necessary to visualize MDH's conformational changes. Therefore, a method was tested to incorporate a fluorescent landmark into MDH's structure that could be used in future research to reveal the interactions between MDH and citrate synthase. Specific amino acids of MDH were mutated to tryptophan, an amino acid known to fluoresce (V189, I319, A120, I136, P119, G218). The coding sequence for the wildtype MDH and mutant MDHs were incorporated into plasmids and bacterially transformed into *Escherichia coli*. Both wildtype and mutant proteins were over-expressed, then purified by nickel affinity chromatography using a hexahistidine tag on the N-terminus of MDH. We predicted that I136W, I139W, V189W, and A120W would exhibit significantly lower activity than wildtype MDH. We found that I139W and V189W emitted fluorescence at 290 nm, but I136W did not. The mutations P119W and G218W could not be overexpressed or purified. Next steps in design of a fluorescent, active MDH will be discussed.

**3D PHYSICAL MODELS OF THE SARS-CoV-2 SPIKE PROTEIN: EXPLORING MUTATIONS ASSOCIATED WITH HIGH VIRAL TRANSMISSIBILITY**

Patricia Hill, Dean Young, Lauren McDonald, and Betsy Martínez-Vaz (Advisor)
*Department of Biology and Biochemistry Program*
*Hamline University, St. Paul, MN*

The SARS-CoV-2 spike glycoprotein is essential for viral infection because it recognizes and binds to the ACE2 receptor in host cells. The protein can be found in a receptor inaccessible or accessible state based on down and up positions of its receptor-binding domain (RBD). This study's goal was to create a physical 3D printed model of the spike protein to visualize the mutations and molecular changes associated with high transmissibility variants. Protein structure files were downloaded from the Protein DataBank and analyzed using Pymol, a computational program that allows in silico construction of mutants at specific amino acid positions. We conducted structural analyses of the wild-type and Delta variant spike protein (B.1.617.2) using PDB files, 7V7V, 7DDD, and 7DDN. These files were imported into Jmol to create the scripts for 3D printing. Various physical models of the spike protein were created using a ZCorp Projet 660 Pro 3D printer. The models highlight three different subunits of the spike protein, with one chain in the open conformation and two in the closed conformation. The model depicts mutations identified in highly transmissible SARS-CoV-2 strains isolated in India (B.1.617.2), England (B.1.1.7 ) and South Africa (B.1.351) These mutations include amino acid substitutions in the spike's RBD (D614G, N501Y, L452R, T478K, E484K, and K417N) and deletions associated with preventing antibody binding (Δ69-70; Δ156-57). A web-based tutorial was designed to complement the 3D models and help people visualize and better understand how single amino acid mutations can lead to changes in viral transmissibility.

**INVESTIGATING THE GENETIC REGULATION OF THE ENZYMES IN THE METFORMIN DEGRADATION PATHWAY**

Anna Schultz and Betsy Martinez-Vaz (Advisor)
*Department of Biology and Biochemistry Program*
*Hamline University, St. Paul, MN*

Metformin is one of the most prescribed pharmaceuticals, mainly for treating type 2 diabetes. Metformin degrades into guanylurea, a dead-end metabolite, that has been accumulating in waterways at extremely high rates, negatively affecting a variety of aquatic life. In a species known as *Pseudomonas mendocina* strain GU, a mutation arose causing a biuret hydrolase to transform into a guanylurea hydrolase that can now degrade this contaminant. A complete pathway has been proposed for guanylurea degradation including this enzyme and three others. The purpose of this study was to investigate the genetic regulation of two of the enzymes in the guanylurea degradation pathway: guanylurea hydrolase and guanidine carboxylase in order to better understand the degradation of guanylurea. *P. mendocina* was grown in a medium containing the substrate of the target gene or a control, ammonium chloride, as the sole source of nitrogen. Using RT-qPCR, mRNA extracted from these cultures was converted to cDNA, amplified, and quantified using the fluorescent dye, SYBR Green. Levels of gene expression were calculated and both genes were upregulated in the presence of their substrate and were downregulated in the presence of ammonium chloride. The sequence of each upstream region from the target genes was analyzed with bioinformatic programs to predict possible genetic regulation factors. This newfound understanding of the genetic regulation of these two enzymes advances the current knowledge of guanylurea degradation and can help the development of biotechnological applications to reduce the levels of guanylurea in the environment.

**TOTAL PROTEIN AS A MORE RELIABLE AND REPRODUCIBLE INTERNAL CONTROL FOR CANCEROUS LIVER SAMPLE WESTERN BLOT ANALYSIS**

Young Y. Vue and Mong-Lin Yang (Advisor)
*Department of Biology*
*Concordia University - St. Paul, St. Paul, MN*

Traditionally, protein normalization for Western Blot analysis has relied upon housekeeping proteins (HKPs) like β-actin and GAPDH. However, many studies have found HKPs to exhibit saturated signals and varied cellular expression levels that can lead to incorrect conclusions. Our work on comparing protein expressions between liver cancer samples versus normal liver samples encountered similar problems when employing β-actin and GAPDH as internal controls for protein normalization. In this project, we tested whether two commercially available total protein stains, Stain Free and No Stain, serve as better internal control for liver cancer protein analysis. Our result confirmed total protein as the more reliable and reproducible internal control than HKPs. In addition, we identified Stain Free as the preferred method between the two methods tested.

**DYSREGULATION OF PROLINE PATHWAY ENZYMES IN HCC VS FLHCC**

Young Vue, Heenu Kamboj, Gabe Nkumu, Richard Wauer, and Mong-Lin Yang (Advisor)
*Department of Biology*
*Concordia University - St. Paul, St. Paul, MN*

Fibrolamellar Hepatocellular Carcinoma (FLHCC) is a rare type of liver cancer that mainly occurs in teens and young adults under the age of 40. This disease is caused by a deletion between two genes PRKACA and DNAJB1 on Chromosome 19. Preliminary proteomic data suggests dysregulations of the proline pathway enzymes in the FLHCC tumors. This project aimed to verify the proteomic result by employing the Western Blot analysis and comparing data to known Hepatocellular Carcinoma (HCC) proline pathway dysregulation. Our Western Blot data confirmed the downregulation of Glutaminase and the upregulation of Glutamine Synthase and Pyrroline-5-carboxylate synthase within the proline pathway of FLHCC samples when compared to normal liver samples. These results can help provide unique biomarkers for the diagnosis of the disease and help in the development of targeted drugs for treatment.

**SERTRALINE BINDING TO NMDA GLUN2D S1S2 DOMAIN**

Megan A. Wolf and Lisa Gentile (Advisor)
*Department of Chemistry*
*College of Saint Benedict & Saint John's University, St. Joseph and Collegeville, MN*

Ionotropic glutamate receptors help facilitate excitatory synaptic signals between neurons in the central nervous system. These ligand-gated ion channels play an important role in brain function, and the misregulation of them can have a negative neuropsychiatric effect. It is currently believed that antidepressants minimize both the release of glutamate and synaptic transmission. For all antidepressants, the location, and interactions of their binding to ionotropic glutamate receptors is currently unknown. Determining the location where antidepressants, specifically sertraline, bind to the S1S2 domain of the GluN2D subunit on ionotropic glutamate receptors can lead to a better understanding of the mechanism of action and provide insight for further research. We used computational analysis as a foundation to predict the binding location. We analyzed the results based on energy, intermolecular forces, and binding orientation, which lead to the identification the location of sertraline binding to GluN2D subunit of the S1S2 domain as well as the specific amino acids responsible for binding. To confirm the computational results, further research involving purification of the protein and determination of the protein's binding affinity to sertraline will be done. Applying the results of this research will have the potential to develop a better understanding of the mechanism of antidepressants, which can be used to treat depression more effectively.

# CELLULAR AND MOLECULAR BIOLOGY

## DISCOVERING THE TINY EARTH OF ANTIBIOTIC PRODUCING BACTERIA

Talila R. Baskaran and Joanna R. Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

The spread of antibiotic resistance has become a growing problem as more antibiotics cannot combat the bacteria they once were able to treat. This has caused a worldwide outbreak of antibiotic-resistant bacteria. Collaborating with different universities and institutions, The Tiny Earth organization uses students to find antibiotic producing bacteria from soil. We started by collecting soil from St. Paul, MN. The soil was serially diluted and plated on LB media to allow growth of bacteria found in the soil. We then tested 24 bacterial isolates for inhibition of ESKAPE safe-relatives (*Staphylococcus epidermidis, Acinetobacter baylyi, Bacillus subtilis, Escherichia coli, Pseudomonas putida, Enterobacter aerogenes*). We observed one isolate that produced a zone of inhibition on a plate spread with *B. subtilis*, indicating it produces an antibiotic against *B. subtilis*. We proceeded to characterize the isolate by sequencing the 16s rRNA gene, doing a Gram stain, and performing biochemical tests, specialized media tests. Once identified, the isolate may enter the pipeline for further testing to determine if it is viable for pharmaceutical use and help bring a solution to the problem of antibiotic resistant infections.

## DISCOVERING CANDIDATES FOR ANTIBIOTIC PRODUCTION THROUGH THE TINY EARTH PROJECT

Christine V. David and Joanna Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

There is a vital need for the discovery of new antimicrobial medications as antibiotic-resistant bacteria are increasing and the efficacy of current medications are weakening. Unfortunately, most pharmaceutical companies are unwilling to invest resources in developing new antibiotic compounds because there is very little pay-off for them in the end. As such, this experiment was performed in tandem with the Tiny Earth Project to add to the student-sourced global database of soil isolates that have the potential to cure bacterial infections in the future. The purpose of this experiment was to identify specific bacterial strains from local soil samples that demonstrated production of secondary metabolites that could be effective at eliminating pathogens. The procedure began by plating soil samples on potato dextrose agar, then measuring antibiotic production based on the size of the colony's zone of inhibition against common pathogens that exhibit multidrug resistance. For this experiment, the isolate that was selected for further study was effective against an organism that possesses similar characteristics to *Enterococcus faecium*, known to cause neonatal meningitis and endocarditis. The target isolate was then identified by gram-staining, 16s rRNA sequencing, and biochemical media testing. Future study of this isolate may continue to guide our ability to produce antimicrobial medications against resistant strains of disease-causing bacteria.

**THE EFFECT OF FOXF1 OVER AND MISEXPRESSION ON FOREGUT ORGAN DEVELOPMENT IN *Xenopus laevis* EMBRYOS**

Chase A. Hemme and Brian A. Hyatt (Advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

The development and growth of an organism from one cell is a very complex process requiring a variety of different genetic pathways and cellular signals. Many of these differential factors take place in very early development in which three specific layers develop (endoderm, ectoderm, and mesoderm) with the endoderm giving rise to foregut progenitors of which this research focuses on. The sonic hedgehog (shh) gene is known to have wide downstream effects on foregut organogenesis including proper trachea and esophagus formation from the endoderm. Foxf1, a downstream target of shh signaling, is expressed in the lateral plate mesoderm as well as in cells mediating tracheoesophageal differentiation later in development. This demonstrates that there is likely an important role of foxf1 in foregut organ formation, but the exact mechanism and role of this gene are still largely unknown. We used a whole mount *in situ* hybridization for organ specific gene expression to analyze the effects of over and misexpression of foxf1. We found that over or misexpression of foxf1 resulted in foregut organ expression ectopically (as seen through sox2 and nkx 6.2 probes), but we did not find any effects on the liver (hhex), or lung (nkx 2.1) expression.

**EFFECT OF RELATIVE HUMIDITY ON VIABILITY OF EX OVO CHICK EMBRYO CULTURING SYSTEMS**

Dylan Holtmeier, Abigail Lewerenz, Kora Kritzberger, and Mong-Lin Yang (Advisor)
*Department of Biology*
*Concordia University - St. Paul, St. Paul, MN*

The chorioallantoic membrane (CAM) of the developing chick embryo is a highly vascularized system able to be used for many applications. However, published protocols for the ex ovo model were found to be inconsistent and confusing, especially regarding the ideal relative humidity (RH) for chick embryo incubation. Some publications have suggested that a lower RH results in lower embryonic viability compared to that of higher RH, however, no systematic testing regarding the effect of RH on ex ovo chick embryo viability has been reported. The goal of this project was to confirm the importance of RH on embryonic viability in addition to exploring the variables responsible for such importance in order to help standardize the protocol used in the ex ovo CAM field. Trials were conducted by comparing the viability of embryos cultured in previously published novel vessels vs plastic tumbler vessels that were placed in an incubator with either low relative humidity (60% RH) or high relative humidity (80% RH). Our findings suggest that higher RH has a positive impact on embryonic viability. We also note that water evaporation may play a role impacting the viability of the embryo, due to a significant loss of mass over time in ex ovo chick embryos cultured in lower RH compared to higher RH.

**EFFECTS OF NATURAL SUPPLEMENTS ON AN ANXIETY-INDUCED PLANARIA MODEL SYSTEM**

Taylor N. Krueger and Aeisha Thomas (Advisor)
*Department of Biological & Health Sciences*
*Crown College, St. Bonifacius, MN*

Anxiety and PTSD are biological and psychological disorders that many individuals encounter daily and both disorders have genetic components. Most medications for these disorders fall in the category of anti-depressants and benzodiazepines, which tend to have undesirable side-effects. The goal of this study was to identify natural chemicals as potential treatments. Genes common to anxiety and PTSD were identified from the literature. The Drug-Gene Interaction database (DGIdb) was used to determine the most common drugs able to interact with these genes and from those we selected three different natural substances for testing in a planaria model system: tyrosine, tryptophan, and mucuna pruriens. These three supplements have been used to study neurological and mental disorders. Planarians exhibit an avoidant, negative-phototaxis response when introduced to a predator's odor. In preliminary studies, tyrosine was effective in counteracting the planarian anxiety response. The effects of tryptophan and mucuna pruriens were less conclusive but were trending towards effectiveness. Based on these limited results, Tyrosine could be used as a potential alternative treatment for anxiety and PTSD in the future. Further studies could be done with higher concentrations of tryptophan and mucuna pruriens, as well as testing other natural supplements to determine their effects on anxiety in the worms. The planaria model-system was also shown to be an effective testing model for this type of research.

## *Helichrysum italicum* ESSENTIAL OIL PRE-TREATMENT DIMINISHES PRO-INFLAMMATORY ACTIVATION BY IFN-γ/LPS IN THE RAW 264.7 MACROPHAGE CELL LINE

Kenneth J. Kwan and Joyce Doan (Advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

Though inflammation is a normal immune response against invasive pathogens, excessive or chronic accumulation of inflammatory mediators correlates with multiple disease states. In many of these cases, macrophages are a key mediator of the inflammatory pathways and as such may be targeted therapeutically. The interest in non-pharmaceutical interventions for chronic health issues such as inflammation also has been growing for many years. One popular and widely available such alternative is the essential oil of *Helichrysum italicum* (HEO). Previous data from our laboratory suggested that HEO attenuates the activation of RAW 264.7 cells by IFN-γ and LPS when administered after these pro-inflammatory stimuli. The aim of the work presented here was to elucidate the outcomes of HEO pre-treatment on inflammatory markers induced by the M1 macrophage stimuli interferon-gamma and/or lipopolysaccharide using RAW 264.7 macrophage-like cells. HEO pre-treatment significantly attenuated the IFN-γ/LPS-inducible production of reactive nitrogen intermediates in RAW 264.7 cells as determined using the Griess reagent system for nitrite determination ($P=6.11x10-9$). Additionally, HEO pretreatment diminished the IFN-γ-inducible phagocytosis of zymosan A in the same model system ($P=3.48x10-7$). Taken together, these results suggest that further exploration of both the prophylactic and therapeutic anti-inflammatory uses of HEO is warranted both in cell culture and possibly animal model systems.

## DISCOVERY OF ANTIBIOTIC-PRODUCING BACTERIA FROM SOIL

Jade E. Le Bris[1,2], Annalisa M. Jordan[2] (Advisor), Kim N. Ha[2] (Advisor), and Tami R. McDonald[1] (Advisor)
*[1]Department of Biology, [2]Department of Chemistry*
*St. Catherine University, St. Paul, MN*

For the past few decades, antibiotic resistance has become an increasingly significant public health issue in many countries, including the United States where it causes thousands of deaths each year. This project aimed to discover new bacteria with antibacterial properties from nearby soil samples in order to advance the research for new antibiotics. Soil samples were taken before and after a planned burn on a prairie located on St. Catherine University campus. The bacteria from these samples were cultured. Bioassays against ESKAPE-safe pathogens were performed and nine different antibiotic-producing bacteria were isolated. They were identified as members of the *Pseudomonas* genus after sequencing their 16S ribosomal DNA. While these antibiotic-producing bacteria are still being investigated, this project shows the promises that student-sourced projects can have in the search for new viable antibiotics.

**SCREEN FOR ANTIBIOTIC PRODUCING BACTERIA FROM SOIL**

Christophe Niyonkuru and Joanna R. Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

Pathogens evolve at a high-speed compared to the speed at which new antibiotics are produced. Knowing that antibiotics are produced from microbes present in soil, we partnered with the Tiny Earth organization to discover a novel antibiotic. We collected a soil sample from Cottage, Minnesota, hypothesizing that the microorganisms present in that soil will produce an antibiotic against some of the ESKAPE pathogens; *Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus, Enterobacter faecium, Enterobacter species*, and *Klebsiella pneumonia*. The soil sample was diluted in a saline solution, transferred into an agar plate containing PDA and incubated to grow bacteria. We tested the colonies against safe relatives of the ESKAPE pathogens e to see if any of the colonies would produce a zone of inhibition. None of the 24 isolates tested produced an antibiotic. While the soil isolates didn't produce an antibiotic against these six tester strains on this type of media, they may inhibit other strains or when grown under different conditions. To continue work towards the goal of antibiotic discovery, we studied an isolate from a classmate, which *Bacillus subtilis* inhibited, on Lysogeny broth (LB) medium. To identify this microorganism, we performed gram staining, 16s rRNA sequencing, biochemical characterization tests and specialized media tests. Further research on the inhibitory chemical secreted by this isolate may be helpful in the fight against antibiotic resistance.

**SEEKING TO DISCOVER A NEW ANTIBIOTIC FROM SOIL SAMPLES**

Abigail L. Parker and Joanna Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

In the world of microbiology, a growing and widespread challenge has been a lack of effective antibiotics due to antibiotic resistance. While many microorganisms do have innate resistance to certain types of antimicrobial medications, this pressing issue involves the development of acquired resistance in microorganisms. Among the multiple causes of this challenge include the misuse and overuse of antibiotics. The objective of our research was to try to identify bacterial strains that produces inhibitory compounds that could be developed into a new antibiotic. This research was conducted in partnership with the Tiny Earth organization, which supports student-sourced research. Our research began with collecting a soil sample from a garden in Avon, Minnesota and isolating bacterial colonies through serial dilutions. After screening for antibiotic activity using a spread-patch assay with six tester strains, we were successfully able to identify two isolates that exhibited antibiotic activity. We chose one isolate to study further based on its more evident antibiotic activity. Additional studies conducted on this isolate included microscopic, genetic, and biochemical techniques to further classify it. This research adds to the growing body of crowd-sourced data that could lead to the discovery of a breakthrough chemotherapeutic drug and help combat the issue of antibiotic resistance the world is facing.

**ASSESSING THE TINY EARTH ESKAPE MICROBES FOR ANTI-TUMOR ACTIVITY**

Kylie M. Ross, Ayden F. P. Chapman, and Jodi E. Goldberg (Advisor)
*Department of Biology and Biochemistry Program*
*Hamline University, St. Paul, MN*

Bacteria are utilized in the treatment of cancer as vectors to aid in the treatment of tumors. There is limited research investigating the effects of bacteria themselves. The purpose of this research was to examine various inactivated bacterial strains and how their components affected the growth of the DLD-1 human colorectal cancer cell line. This research utilized ESKAPE relative bacteria which have many similar features to six most common ESKAPE pathogens, but are less virulent and not antibiotic resistant. We hypothesized that proteins and molecules present on the surface of a bacteria could interact with colon cancer cell lines in a way that would result in growth inhibition. Bacterial-sized inert microbeads were tested to act as a control for reduced growth due to molecules settling on top of the cancer cells, instead of interactions between the molecules and the cancer cells. We conducted experiments using an MTT assay which indicated how metabolically active the cells were after being incubated with bacteria for 72 hours. Results showed varying outcomes, from no effect with certain bacteria, to hindrance in observed metabolic activity. Specifically, *Erwinia carotovora* and *Enterobacter aerogenes* displayed distinct decreases in metabolic activity in a dose dependent manner while most other bacteria did not. For the bacteria that hindered growth of colon cancer cell lines, further research should be conducted on a non-cancerous cell line to determine potential cytotoxicity in non-cancerous tissues, to determine if the bacteria would be safe to use as a cancer therapy in human systems.

**EFFECTS OF OVEREXPRESSION OF FOXA2 GENE AND DEVELOPMENTAL EFFECTS ON ENDODERMAL ORGAN EXPRESSION IN *Xenopus laevis***

Abigail M. Simpkins and Brian A. Hyatt (advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

The development of the endoderm results in the formation of many organs, and there are a multitude of genes associated with this development. Foxa2, a gene involved in endoderm formation, appears to play a role in multiple organ development processes. By microinjection of mRNA, we were able to over-express foxa2 in two different regions of the developing *Xenopus laevis* embryos. We analyzed of endodermal organ formation using *in situ* hybridization to assay for organ-specific genes. Our results showed reduced sox2 expression, an esophageal and stomach marker, and ectopic or increased expression of ptf1a and hhex, two early pancreatic and liver markers respectively.

**CHARACTERIZING CELL POPULATIONS MOST IMPACTED BY NORMAL MICROBIAL EXPOSURE FOR PRECLINICAL RESEARCH AND HEALTHCARE TREATMENT DEVELOPMENT SUCCESS**

Adhvaith Sridhar and Nathan Schuldt (Advisor)
*Department of Pediatrics*
*University of Minnesota - Twin Cities, Minneapolis, MN*

*Mus musculus* (mice) are often used to model the human immune system and to develop healthcare treatments because mouse immunology accurately recapitulates many aspects of the human immune system. However, differences between the immune systems of lab mice and the true human immune system may decrease the predictive power of treatments developed in mice. One limitation of using mouse models to study human immune conditions is the relative immune immaturity of laboratory mice. Immune maturity is a key component in any organism's ability to resist disease and infection. We hypothesized that normal microbial exposure (NME) mice, which have undergone microbial exposure from conception, have an accelerated immune development relative to their specific pathogen free (SPF) counterparts and thus more accurately model human immune development. We examined tissue samples from 120 mice. The findings supported out hypothesis, indicating that the use of SPF conditions for laboratory mice in preclinical and research studies leaves the immune systems of infant mice underdeveloped and far different from those found in human infants. Our data further showed that infant NME mice develop elevated profiles of numerous immune components during different stages in development and better reflect human infant immunity. Applying *Listeria* monocytogenes challenges or sepsis challenges to NME mice in the future should reveal the physiological changes associated with the changed immune composition. This work is critical in advancing immunology research and accurately developing mouse model-derived healthcare treatments.

**A MODEL OF A CHOLANGIOCARCINOMAGENIC PHENOTYPE WITH IDH1 MUTATION**

Jack Strotbeck and Megan Girtman (Advisor)
*Department of Biology*
*Saint Mary's University of Minnesota, Winona, MN*

Cholangiocarcinoma, commonly known as bile duct cancer, encompasses a group of epithelial biliary malignancies. Though it is a rare disease with fewer than 20,000 annual diagnoses, it claims the lives of more than 7,000 individuals each year. The 5-year survival rate is less than 10%. Cholangiocarcinoma presents silently, meaning distinctive symptoms do not appear until late in disease progression contributing to poor overall survival. That combined with the heterogeneous nature of these malignancies and high resistance to chemotherapy makes cholangiocarcinoma particularly challenging to treat. A more extensive understanding of the genetics of cholangiocarcinoma and potential therapeutic targets are urgently needed. Despite this need, few models of cholangiocarcinoma exist. This study aimed to characterize an *in vitro* model of cholangiocarcinoma. This model was created with transfection of isocitrate dehydrogenase 1 (IDH1)$^{R132C}$ mutated plasmid in 293T cells. Initial experiments with this model showed no significant difference in cell proliferation but a 17-fold increase in HIF1A expression consistent with prior predictions that IDH1 mutation does not drive cell proliferation, but instead promotes malignancy in the tumor microenvironment through angiogenesis.

**DISCOVERY AND ANALYSIS OF ANTIBIOTIC PRODUCING *Streptomyces* FROM SOIL**

Nicholas Waughtal and Joanna Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

As antimicrobial resistance to various current antibiotics continues to increase, the number of deaths from infections caused by antimicrobial resistant pathogens could grow to ten million deaths per year by 2050. Some of the greatest antibiotic resistance is found in bacteria known as ESKAPE pathogens which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*. The Tiny Earth Network offers students the opportunity to discover new potential antibiotics by searching for novel antibiotic producing bacteria from the soil that hinder the ESKAPE pathogen safe relatives. Soil from Arden Hills, Minnesota was screened for potential antibiotic producing bacteria. One of the soil isolates tested on Brain Heart Infusion plates showed zones of inhibition against the ESKAPE safe relatives *Bacillus subtilis* and *Staphylococcus epidermidis*. The soil isolate was analyzed further through a genomic analysis of a portion of the 16S rRNA gene. We concluded from bioinformatic analysis of the 16S rRNA sequencing results that the soil isolate was from the genus *Streptomyces*. The isolate was grown on various media and tested against the ESKAPE safe relatives to examine the effect of growth conditions on antibiotic expression. Chemical extraction was performed with ethyl acetate to isolate the bioactive compound which inhibited the growth of *B. subtilis*, *S. epidermidis*, and *Acinetobacter baylyi*. Future work will test the extract against eukaryotic cells to investigate its potential for use as a novel antifungal.

**IDENTIFICATION OF POTENTIAL THERAPEUTIC TARGETS IN AGE-ASSOCIATED TUMORS USING BIOINFORMATICS**

Andy Y. Yang and Aeisha Thomas (Advisor)
*Department of Biological & Health Sciences*
*Crown College, St. Bonifacius, MN*

Aging is a major risk factor for global disease, particularly cancer development. The senescence-associated secretory phenotype (SASP), a part of cellular senescence, is an emerging hallmark of aging. Recent studies have identified SASP factors that could serve as potential therapeutic targets for aging-associated diseases such as cancer. However, these biological factors are poorly understood in age-related differences among different tumor types. To address this knowledge gap, we conducted gene expression analysis to identify differentially expressed SASP genes (DEGs) in age-associated and non-age-associated tumors. We did not find DEGs common to all non-age-associated tumors for either younger or older patients. We did identify four genes for younger patients that were shared by all age-associated tumors and 8 genes for older patients. We conducted additional analyses of these 8 DEGs in older patients with age-associated tumors, including survival analysis, pathway enrichment analysis, and the characterization of mutations and DNA methylation. Further evaluation of these findings could be done to develop therapeutic combinatorial strategies that target key SASP factors in age-associated tumors.

**INVESTIGATION OF *Pseudomonas* STRAINS WITH ANTIBIOTIC ACTIVITY**

Sarah E. Zimmermann and Joanna Klein (Advisor)
*Department of Biology and Biochemistry*
*University of Northwestern - St. Paul, St. Paul, MN*

The issue of antibiotic resistance is a concern throughout the healthcare community since it poses the risk of leaving individuals defenseless against bacterial infections. This is largely due to the misuse of antibiotics, lack of appropriate antibiotics, and a decline in the funding for research dedicated to antibiotic discovery. The aim of this study was to screen a soil sample from Afton, MN for antibiotic producing bacteria by partnering with the Tiny Earth organization. Bacterial isolates found in the soil samples were tested for antibiotic production and one isolate was found to inhibit *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus epidermidis*. A chemical extract prepared from the isolate similarly inhibited the tester strains. Biochemical tests and 16s rRNA gene sequencing identified the isolate as *Pseudomonas*. Effectiveness of the compound against eukaryotic cells will be tested to measure toxicity and/or its potential use against eukaryotic microorganisms. In addition to studying this isolate, we worked to determine the gene causing an inhibition phenotype in a second *Pseudomonas* soil isolate by performing transposon mutagenesis. One mutant was found to have lost the inhibition phenotype. Future work will determine the location of the transposon insertion.

# CHEMISTRY

## EFFECTS OF SILVER NANOPARTICLES BINDING ON THE ESTERASE-LIKE ACTIVITY OF HUMAN SERUM ALBUMIN

Jack Adamietz and M. Abul Fazal (Advisor)
*Department of Chemistry*
*College of Saint Benedict & Saint John's University, St. Joseph and Collegeville, MN*

The tremendous advance of nanotechnology is marked by the large-scale production of diversified nanomaterials with remarkable physical and chemical characteristics. Understanding the interactions of nanomaterials with biomolecules is fundamental to the safe usage of nanomaterials. We investigated the interactions of silver nanoparticles (AgNPs) with human serum albumin (HAS) using various spectroscopic techniques. UV-Vis spectrophotometry indicated strong binding between serum albumin and silver nanoparticles. We used fluorescence quenching experiments to determine the binding constant (Ka). We also studied the esterase-like activity of human serum albumin in presence and absence of silver nanoparticles using 4-nitrophenyl acetate as the substrate. The esterase activity of human serum albumin and HSA-AgNPs complex followed Michaelis-Menten kinetics. The presence of AgNPs significantly affected the esterase activity of human serum albumin.

**CHITOSAN-COATED MESOPOROUS SILICA NANOPARTICLES FOR APPLICATIONS IN AGRICULTURE**

Allamagan Anod, Tana O'Keefe, Wade Elmer, Jason White, and Christy Haynes (Advisor)
*Department of Chemistry*
*University of Minnesota - Twin Cities, Minneapolis, MN*

The world's population is growing at an alarming rate and there is an urgent need for efficient and sustainable agricultural technologies to provide food security. Diseases alone negatively affect 20-40% of agricultural crops each year. For example, *Fusarium* wilt is a soil-borne pathogen that affects various plants by limiting their water consumption. Silica nanoparticles that transform to silicic acid within a plant's root can polymerize and create silica phytoliths, which reinforce plant cell walls to help fight off plant disease. Additionally, chitosan, an organic polymer which has been found to be important in plant disease resistance, serves as another disease defense mechanism through its activation of signaling cascades within plants. Previously, mesoporous silica nanoparticles (MSNs) and chitosan-coated MSNs have increased watermelon defense systems and reduced disease severity by approximately 40% and 27%, respectively. We investigated the use of MSNs and chitosan-coated MSNs to suppress disease, specifically *Fusarium* wilt in wheat, an economically important crop. MSNs were synthesized via a sol-gel method and coated with chitosan. Chitosan-coated MSNs were characterized using dynamic light scattering, zeta potential measurements, and nitrogen physisorption to assess the chitosan coating. Future work will involve performing greenhouse and field studies to assess the impact of chitosan-coated MSNs compared to a control MSN treatment on both healthy and *Fusarium*-infected wheat plants. This work will ultimately demonstrate the potential for MSNs and chitosan-coated MSNs to serve as a sustainable agricultural technology to prevent disease severity in plants while increasing crop yields.

**ANALYZING ROCHESTER TAP WATER FOR PHOSPHATE**

Nasra Arte and Heather Sklenicka (Advisor)
*Department of Chemistry*
*Rochester Community and Technical College, Rochester, MN*

In April of 2014, many residents of Flint, Michigan, were poisoned by lead from tap water due to low phosphate levels. Phosphorus is a prevalent element and, like nitrogen, is an essential ingredient for all living things. Phosphate ($PO_4^{3-}$) is the most frequent type of phosphorus utilized by biological organisms, and it is important for the formation of DNA, cellular energy, and cell membranes in commercial fertilizers. Phosphorus is added to tap water to prevent metal disintegration and, in particular, lead poisoning from the water piping system. To ensure the safety of drinking water, the total phosphate applied should not exceed 10 mg/L in order to prevent lead from entering the water system. The aim of the research was to safeguard the safety of Rochester residents and avoid repeating what happened in Flint, Michigan. In this study, we measured phosphate ion concentrations in water samples from the NE, SE, NW, and SW sections of Rochester, Minnesota. A vanado-molybdate reagent was used to treat water samples that formed a yellow solution when it complexed. Using a spectrophotometer, we created a calibration curve using a set of standards, and the concentration of unknown solutions was determined using the same wavelength of absorption. Preliminary findings show that all samples from the regions of Rochester, Minnesota have a standard phosphate ion concentration of 2 - 10 mg/L.

**GREEN SYNTHESIS OF CONDUCTING POLYMERS FOR ORGANIC PHOTOVOLTAIC APPLICATIONS**

Hannick Biermeier, Ted Pappenfus, and Daron Janzen (Advisor)
*Department of Chemistry*
*St. Catherine University, St. Paul, MN*

Global energy use relies heavily on fossil fuels and the related emissions are one of the largest contributors to climate change. Consequently, the demand for solar energy, a clean and sustainable energy source, is only increasing. Solar cell devices using organic photovoltaic materials (OPVs) are being widely studied as cost-effective and sustainable alternatives to fossil fuels. The objective of our project was to synthesize new conjugated organic polymers using greener synthetic methods for use in new solar cell materials. Conjugated organic polymer properties are highly tunable and can be manipulated to achieve high power conversion efficiencies in OPV devices. We chose to synthesize monomers (small molecules used as building blocks to make polymers) with high solubility and a rigid planar backbone to engineer desirable optical properties in our goal polymer. We successfully synthesized our novel goal monomer in high yield using a two-step reaction scheme and only a single purification step. In order to verify the structure and purity of the synthesized monomer, we utilized numerous techniques including nuclear magnetic resonance and mass spectroscopy. Using the green method of direct arylation polymerization, we synthesized our desired goal polymer from our new monomer. This method of direct arylation bypasses typical polymerization reactions involving toxic reagents and hazardous procedures. Preliminary optical measurements provide evidence that our new polymer has favorable properties for use in OPV devices including efficient low energy light absorption. The short synthetic pathway, high yield reactions, and green synthesis of this polymer make this attractive to commercial OPV application.

**ELECTROCHEMICAL DETECTION OF REACTIVE OXYGEN/NITROGEN SPECIES: THE EFFECT OF DIFFERENT ELECTRODE COATINGS ON ROS DETECTION**

Astrid C. Hernandez, Eleni M. Spanolios, and Christy L. Haynes (Advisor)
*Department of Chemistry*
*University of Minnesota - Twin Cities, Minneapolis, MN*

Electric cars have become increasingly prominent in recent years due to the need for more eco-friendly transportation. Unfortunately, there is no proper recycling platform established for the batteries of these cars. The batteries are composed of nickel manganese cobalt oxide nanoparticles (NMC NPs). When improperly disposed of, NMC NPs leech into the environment and interact with biological organisms, such as bacteria, producing reactive oxygen and nitrogen species (ROS/RNS). ROS/RNS are highly reactive, short-lived molecules that are produced when a cell is in a toxic or stressed environment. NMC NPs and bacteria can already produce these species on their own, but this interaction produces even more. Our objective was to explore how toxic nanoparticles are to bacteria by determining the major ROS/RNS production mechanism. To do this, we must detect ROS in low concentrations. This project focused on decreasing the limit of detection of carbon-fiber microelectrodes for hydrogen peroxide detection, a common ROS. Different electrode coatings can act as redox mediators on the carbon-fiber surface, making the electrode more sensitive to detecting ROS/RNS. We used cyclic voltammetry and amperometry data collected from bare, cobalt oxide, platinum, and prussian blue coated microelectrodes to detect hydrogen peroxide. Our research showed that the platinum coating performed best for detecting hydrogen peroxide at low concentrations, the limits of detection being 146µM for bare, 86µM for cobalt oxide, and 31µM for platinum.

**MECHANISTIC INSIGHTS OF CATALYST FREE HYDROAMINATION**

Michael L. Le, Max H. Marron, and Eric H. Fort (Advisor)
*Department of Chemistry*
*University of St. Thomas, St. Paul, MN*

Hydroamination is useful in many applications such as pharmaceuticals or total synthesis but often requires a catalyst. Generally, hydroamination is catalyzed using transition metals or strong acids and bases to help the formation of the kinetically unfavorable nitrogen-carbon bond. Theoretical studies suggest two possible mechanisms, and mechanistic experiments have been designed to provide insight into this observed reaction. The objective of this research was to determine the specificity and mechanism of a new catalyst free, trans-aryl-alkyne hydroamination reaction. We conducted experiments using different amines and alkynes to determine the specificity of this catalyst free hydroamination. We found that hydroamination works for both cyclic and aliphatic amines. Current insights and future trajectory for elucidating the mechanism for this hydroamination will be discussed.

**SYNTHESIS AND CHARACTERIZATION OF A FLUORESCENT PROBE FOR THE DETECTION OF HYDROGEN SULFIDE**

Benjamin J. LeBeau, Hunter A. Backholm, and Katie L. Peterson (Advisor)
*Department of Chemistry*
*Bemidji State University, Bemidji, MN*

Hydrogen sulfide is a gasotransmitter used as an intracellular signal transducer at low concentrations but is toxic at high concentrations. Because of this, ways to measure hydrogen sulfide (H2S) in living cells have become an important goal for the diagnosis and understanding of many diseases, including Alzheimer's. To achieve this goal, we synthesized NBD-Coumarin, a dual fluorophore, for H2S and cysteine detection using a nucleophilic substitution reaction. We used 1H NMR and fluorescence spectroscopy to characterized the probe, NBD-coumarin. The fluorescence excitation study showed the probe was best excited at 368 nm. The emission data showed that the NBD-coumarin was very weakly fluorescent in the absence of H2S but fluoresced at the same wavelength as coumarin (450 nm) when reacted with H2S. Further studies investigated the probe's response to cysteine. A kinetics study revealed that NBD-coumarin and hydrogen sulfide continuously react for over an hour. The fluorescence response of NBD-coumarin to H2S evaluated the sensitivity of this probe for its analyte. Future projects will investigate the range at which NBD-coumarin can detect H2S and cysteine. As such, this project contributes to the broader goal of understanding the role of H2S in the body and disease.

**DESIGN AND SYNTHESIS OF SILICA-COATED SWELLING POLYMERS FOR SUSTAINABLE AGRICULTURE**

Andrea T. Ligocki, Beza S. Tuga, Hyunho Kang, and Christy L. Haynes (Advisor)
*Department of Chemistry*
*University of Minnesota - Twin Cities, Minneapolis, MN*

The world's population is expected to rise to 9.7 billion people by 2050, emphasizing the need to increase our food production to accommodate for this rapid population growth. Nanoparticles are increasingly being used to improve agricultural cp production because they can promote nutrient absorption, lower soil and water contamination, and increase the resilience of plants in poor environments. Silica nanoparticles, in particular, are promising due to their synthetic tunability and positive agricultural impacts as a result of their dissolution and release of silicic acid. Unfortunately, the incomplete dissolution behavior of silica nanoparticles leads to a nanoparticle accumulation problem especially present in agriculture. Therefore, this work aims to develop silica-coated swelling polymer nanoparticles using poly-2-(diethylamino)ethyl methacrylate (pDEAEMA) as the core and silica as the external shell. pDEAEMA is a pH-responsive polymer that swells in acidic media and is synthesized via a free-radical polymerization. With the presence of a silica shell, there is a potential for the polymer-core swelling in acidic media to induce silica shell fragmentation to generate small, high-surface area fragments that can serve as a beneficial supply of silicic acid to plants. We used dynamic light scattering and zeta potential measurements to determine the polymer nanoparticle size and surface charge, respectively, as well as the polymer's swelling behavior. Transmission electron microscopy was used to visualize the nanoparticles. Following successful synthesis and characterization, these nanoparticles will be applied to plants using greenhouse and field studies where their impact will be evaluated using various agricultural endpoints.

**NEW ROUTE TO NITROGEN RICH POLYMERS**

Max H. Marron, Michael L. Le, and Eric H. Fort (Advisor)
*Department of Chemistry*
*University of St. Thomas, St. Paul, MN*

We studied the synthesis of nitrogen rich polymers using a Sonogashira-hydroamination reaction series. We completed both the Sonogashira and hydroamination reactions independently, combining them in sequence to synthesize the polymers. The two-step sequence was used to synthesize the polymers containing alternating aromatic and saturated rings. Synthesis of this type of polymer could be useful for ion-exchange applications. Intermediate molecules are feasibly synthesized with current methods, but optimization is still needed. When the synthesis is completed, the physical properties of these polymers will be studied to better understand how they may be used in the future.

**UNIQUE CHEMICAL SIGNATURES ACROSS TWO WILD BERGAMOT (*Monarda fistulosa*) ECOTYPES: A COMMON GARDEN STUDY**

Olivia A. Meyer, Moni C. Berg-Binder[1] (Advisor), and Brett R. Bodsgard[2] (Advisor)
*[1]Department of Biology, [2]Department of Chemistry*
*Saint Mary's University of Minnesota, Winona, MN*

Ecotypes, defined as groups of the same species that are native to different geographic regions and vary in physical characteristics, are the result of evolution and often exhibit local adaptation. Two ecotypes of *Monarda fistulosa* were investigated for evidence of chemical differences unique to each ecotype. The plants were grown using a common garden methodology where all seedlings were grown in identical conditions over the same time period. We used *Monarda fistulosa* in the study because members of the *Monarda* genus have been shown to vary in the chemical signatures of their essential oils within a species. Using GC-MS, the essential oil extracts of these two ecotypes showed seven compounds that were unique to the essential oil of one ecotype. Five out of the 12 significant compounds identified were found in both essential oil extracts from the two ecotypes. Due to the common garden design, the differences in chemical signatures that were observed in *Monarda fistulosa* is likely due to genetic differences. Future research should explore whether these compounds have evolved in response to local selective pressures.

**TUNABLE ORTHOESTER MICELLES FOR USE IN CONTROLLED HYDROPHOBIC DRUG DELIVERY**

Andrew K. Morse, Francesca M. Ippoliti, and Lisa E. Prevette (Advisor)
*Department of Chemistry*
*University of St. Thomas, St. Paul, MN*

Orthoesters hydrolyze at varying rates in an acidic environment depending on their size and functionalization. By synthesizing molecules with a hydrophobic oleoyl group and hydrophilic polyethylene glycol chain on opposite ends of a cyclic orthoester, water soluble micelles can be used to house a hydrophobic drug, such as the chemotherapeutic doxorubicin, and deliver it within the body. By altering the size and functionalization of the ring incorporating the orthoester, the rate of hydrolysis, and thus drug release, from the micelles can be controlled. We synthesized and characterized three orthoester molecules with different ring sizes and functionalization. We found that the critical micelle concentrations of all three different orthoester micelles were below 12 μM, and we determined their hydrodynamic diameters when unloaded to be between 60 and 100 nm. We loaded the micelles with doxorubicin using a dialysis technique and we measured their drug loading efficiencies to be between 3 and 10% at a drug/orthoester molecule feed ratio of 30% (w/w). Orthoester hydrolysis in physiologically relevant pH environments was monitored using 1H NMR, which showed that the rate of hydrolysis varied inversely with the size and functionalization of the orthoester and directly with the acidity of the environment. We measured the release of doxorubicin from the orthoester micelles *in vitro* using dialysis with free doxorubicin as a control. The doxorubicin was released from the micelles at a faster rate in lower pH environments, and in all cases the release from the micelles was at a slower rate than free doxorubicin.

**ANALYSIS OF COMMERCIAL PEROXIDE BLEACHES**

Adam E. Pagnano and Heather Sklenicka (Advisor)
*Department of Chemistry*
*Rochester Community and Technical College, Rochester, MN*

In this research, we analyzed three different commercial non-chlorine bleaches to determine their effectiveness as well as the validity of their manufacturer's statements using different tests. We conducted this research so it can be incorporated in the future with RCTC chemistry students to allow them to relate a chemistry topic to a real-world situation. The students will each test their own brands of bleaches expanding a collective database. The first part of the research involved determining the percentage of the active ingredient, hydrogen peroxide, in the bleaches. This was done by redox titration using a potassium permanganate solution. In order to help increase precision, an electronic redox probe was used to alert when the titration was complete. The calculated results from the titrations were compared to the stated percentage on the products Safety Data Sheets (SDS). The results of these tests indicated that the bleaches were relatively close to their specified percentages, although they varied slightly. The second part involved determining the effectiveness of the bleaches at removing stains from cotton fabric. Four different staining solutions were applied to a piece of fabric. Then a small amount of the bleach was applied to the different stains. The bleaches were allowed to sit on the stains for a specific amount of time, after which the fabric was dried in an oven and was visually as well as digitally examined using infrared spectroscopy to see their ability to remove the stains.

**ALKANETHIOL FUNCTIONALIZATION OF GOLD NANOSHELLS**

Ezequiel Pena and Annette F. Raigoza (Advisor)
*Department of Chemistry*
*College of Saint Benedict & Saint John's University, St. Joseph and Collegeville, MN*

Controlling the surface properties of gold nanoshells is important because they have shown promise in their use in biomedical applications, such as cancer treatment and drug delivery systems, as well as catalysis and other areas relying on surface-environment interactions. In these studies, carboxylic acid-, amine-, and alcohol-terminated functional groups were added to gold nanoshells through surface modification with alkanethiols containing these groups. Generally, the functionalization procedures involved mixing the alkanethiol of choice in solution with the nanoshells, stirring for an hour, and then centrifuging to isolate the functionalized nanoshells. Amine-terminated nanoparticles included an additional protonation step with an acid. The resulting functionalized nanoshells were characterized to determine their concentration, effective diameter, and polydispersity using dynamic light scattering (DLS) and UV-Vis. While the alcohol- and carboxylic acid-terminated nanoshells yielded higher concentrations based on their darker blue solution color, the amine-terminated nanoshells yielded lower concentrations. We demonstrate here a solution-based process to consistently produce surface-modified gold nanoshells.

**A GREEN CHEMISTRY APPROACH TO A BIOBASED POLYMER OF CARVACROL**
Swetha Ramaswamy, William McKewan, Marites Guino-o, and Eric H. Fort (Advisor)
*Department of Chemistry*
*University of St. Thomas, St. Paul, MN*

The demand for bio-sourced polymers is increasing due to the environmental toll of fossil fuels. Bio-sourced polymers can be used as an alternative for less sustainable polymers. The polymer poly(2,6-dimethyl-1,4-phenylene oxide) (PPO-DMP) is an important engineering polymer that is sourced from crude oil. In this presentation, we will describe the development of a biobased replacement polymer for PPO-DMP while applying green chemistry principles to nearly every aspect of the synthesis. The replacement polymer is poly(5-isopropyl-2-methyl-1,4-phenylene oxide) (PPO-CAR), which is synthesized from carvacrol commonly sourced from the plant Origanum vulgare. The synthesis involved production of a solvent-free catalyst and a water-based polymerization that yielded a bio-sourced alternative to replace PPO-DMP.

## ECOLOGY AND ENVIRONMENTAL SCIENCE

**SOIL PROPERTIES AND VEGETATION COMPOSITION IN RESTORED GRASSLANDS IN WINNESHIEK COUNTY, IOWA**
Ethan Braun[1], Sophie Epps[1], Logan Olson[2], Molly McNicoll[1] (Advisor), and Laura Peterson[2] (Advisor)
*[1]Department of Biology, [2]Environmental Studies Program*
*Luther College, Decorah, IA*

Grasslands restored from agricultural production, such as those enrolled in the Conservation Reserve Program (CRP), can revitalize soil properties such as erosion resistance and carbon sequestration. Prior research suggests that the rate of carbon storage in soils can be accelerated by highly productive C4 grasses and high species richness. To investigate effects of plant community composition on grassland soil function, we sampled 17 sites across Winneshiek County, Iowa including two conventional agricultural fields, 12 restored prairies (8-22 years old), and three remnant prairies. Plant diversity and abundance were assessed through cover and biomass clipping. Soils were sampled for organic carbon (C), nitrogen (N), bulk density, and aggregate stability. Organic C, N, and bulk density varied considerably among restorations with no association to measured plant community characteristics or age, indicating that initial conditions may be a strong determinant of restored grassland soil properties. Organic C, N, and bulk densities were significantly different between restoration and remnant prairies, supporting existing knowledge that full grassland recovery may take a century, far exceeding typical 10-15 year CRP contract periods. In contrast, within 10 years, aggregate stability of restored grasslands recovered to that of remnant prairies, confirming that erosion control, a key CRP objective, improved on enrolled lands within the contract period. The lack of relationship between plant community diversity or composition on the soil factors investigated suggests that CRP performance is not highly sensitive to the species mix which allows land managers to prioritize other goals, such as pollinator habitat, without compromising soil health.

**QUANTIFICATION OF CYCLIC PEPTIDE AND ALKALOID CYANOBACTERIAL TOXINS IN THE LEAF FLUID OF THE INSECTIVOROUS BOG PLANT, *Sarracenia purpurea***

Jack Jurmu, Hunter Lindsay, Zayid Dakane, and Paul Melchior (Advisor)
*Department of Biology*
*North Hennepin Community College, Brooklyn Park, MN, and Bemidji State University (Twin Cities), MN*

The northern pitcher plant, *Sarracenia purpurea*, is a mixotrophic species native to bog habitats in Minnesota. These plants trap arthropods in their leaf phytotelmata to supplement bioavailable nitrogen and phosphorous compounds that are scarce in the acidic substrate. The phytotelmata microbial community is well studied and contributes to the decomposition of trapped prey organisms. However, little research has been published indicating the role of this community in the killing of trapped insects, which typically drown in the leaf fluid. Numerous cyanobacterial genera are present in *S. purpurea* phytotelmata, including species known to generate and secrete potent neurotoxic compounds. These cyanotoxins may improve the successful capture of insects by *S. purpurea* by accelerating the prey death. In this study, we used competitive enzyme-linked immunosorbent assays to investigate the presence and concentration of both cyclic peptide (microcystin and nodularin) and alkaloid (anatoxin-a cylindrospermopsin) cyanotoxins in the phytotelmata from *S. purpurea* plants in a central Minnesota bog. We detected each cyanotoxin group in varying concentrations in the sampled plants. Further sampling is planned for spring and summer, 2022.

**EXPLORATION FOR THE PRESENCE OF POTENTIAL BIOREMEDIATION GENES IN THE MISSISSIPPI RIVER WATERSHED**

Teagan L. Kuckkahn and Aeisha Thomas (Advisor)
*Department of Biological & Health Sciences*
*Crown College, St. Bonifacius, MN*

The main agricultural pollutants in the Mississippi River Basin are nitrogen and phosphorous. Although efforts have been made to implement water protection acts, bioremediation is another way to address the pollution issue. Genes *nosZ* and *nirK* have been found to be involved in denitrification, and gene *ppk* has been found to decrease phosphorous levels. Therefore, organisms expressing these genes are potential bioremediation agents. Identifying these genes from organisms in the Mississippi River Watershed could open new pathways for cleaning up our waterways. To explore this, DNA was purified from sponge samples collected from rivers that are a part of the Mississippi River Watershed. We used PCR, using gene-specific primers, on the DNA samples to determine if they had the genes *nosZ*, *nirK*, or *ppk*. These experiments showed that the *nosZ* gene is present in DNA samples from multiple rivers. Our results indicate that *nosZ* is present in either bacteria or sponges from the Mississippi River watershed and thus could aid in the denitrification of the river water.

**INVASIVE EARTHWORM RESPONSE TO GRAZING MANAGEMENT OF EUROPEAN BUCKTHORN (*Rhamnus cathartica*)**

Mary M. McTeague and Molly McNicoll (Advisor)
*Department of Environmental Studies*
*Luther College, Decorah, IA*

Earthworms and European buckthorn (*Rhamnus cathartica*) are species invasive to the United States and thought to co-facilitate each other. Goat grazing, a potential invasive management technique, decreases buckthorn abundance, reducing the food source of earthworms. However, little is known about how long it takes for grazing to be effective, and if grazing as management for buckthorn has an effect on earthworm populations. We sampled earthworm abundance, biomass, and species composition, and leaf litter in oak woodlands with three different treatments: buckthorn-invaded and grazed by goats for one summer, buckthorn-invaded and unmanaged, and buckthorn-uninvaded and unmanaged. We extracted worms from 12 plots per treatment, i.e., four replicates at each of three sites. Worms were sampled using liquid mustard extraction and analyzed for relationships between treatment and worm count, biomass, and community composition. Earthworm counts, species composition, biomass, and leaf litter biomass were not significantly different between management and invasion status treatments. The absence of an effect of grazing on earthworm abundance might stem from the short duration of the grazing period, the proximity of sampling transects, and the equal abundances of non-buckthorn leaf litter across treatments. Persistence of earthworms following buckthorn reduction by grazing, suggests that in these woodlands, earthworms are not dependent solely on buckthorn, other food sources are suitable substitutions for earthworms, and buckthorn may have long-lasting ecosystem effects. Based on these limited results, a single grazing application appears to have limited ability to control earthworm populations.

**RESURRECTION ECOLOGY: A TIME TRAVELER'S APPROACH TO STUDYING EVOLUTION**

Marcella G. Meneguzzi, Indigo L. Rowland, and Leif K. Hembre (Advisor)
*Department of Biology*
*Hamline University, St. Paul, MN*

Resurrection ecology is an approach that allows observation of evolutionary change over long periods of time. Researchers use this with aquatic organisms that produce dormant eggs (ephippia) which are deposited in sediments regularly. By extracting ephippia and inducing hatching, researchers can study the organisms as they existed decades ago and evaluate the population for changes over time. Stocking fish (e.g., trout) in lakes can induce trophic cascades that impact the zooplankton composition. As visually-orientated, size-selective predators, rainbow trout (RBT) prey on large-bodied zooplankton grazers (*Daphnia pulicaria*) instead of the smaller-bodied zooplankton (*Daphnia mendotae*). Annual RBT stocking program was initiated in Square Lake in 1981. Researchers determined that mean body size of *D. pulicaria* decreased significantly in Square Lake during stocking moratoriums, while *D. mendotae* remained unchanged. This implies *D. pulicaria* body size decreased after the RBT stocking program began. It's unknown whether the change occurred due to evolution or as an induced response with phenotypic plasticity. The goal of this study was to determine whether body size change was due to evolution. Hatchlings from ephippia extracted from sediments of different depths in lake core samples were cultured to produce clonal offspring able to be observed for life history traits. Initial data show life history traits consistent with evolutionary selective pressures from visually-orientating predators, such as earlier reproduction, reproduction at a smaller size, and overall smaller body size.

**INVESTIGATING THE TROPHIC EFFECTS OF RAINBOW TROUT PREDATION ON *Daphnia* IN SQUARE LAKE USING A PALEOECOLOGICAL APPROACH**

Indigo L. Rowland, Marcella G. Meneguzzi, and Leif K. Hembre (Advisor)
*Department of Biology*
*Hamline University, St. Paul, MN*

Beginning in 1981, the Minnesota DNR began annually adding the non-native zooplanktivorous predator rainbow trout to Square Lake. Past research into the trophic effects of this decision has shown that both the body size and prevalence of the zooplankton *Daphnia* were affected by the addition of the trout. However, most of this research was conducted on the post-1981 era. Last summer, our research focused on analyzing data from the pre-1981 era in both Square Lake and Big Carnelian Lake (our reference lake). We hypothesized that the body sizes and prevalence of the *Daphnia* would remain relatively constant in the pre-1981 era because neither lake experienced any large changes to its ecosystem prior to 1981. To study this, we created microscope slides using sediment from pre-collected sediment cores from both lakes and analyzed these slides for three types of zooplankton: *Daphnia pulicaria*, *Daphnia mendotae*, and *Bosmina* sp. Measurements of the post-abdominal claws of the *Daphnia* correlated with their body size which gave us insight into that life history trait. Findings from this research will complement previous research of the trophic effects of rainbow trout on freshwater ecosystems, and will inform the management of trout stocking programs.

**THE PREVALENCE OF MICROPLASTICS IN NORTH DAKOTA WATERFOWL**

Duong Nguyen and Jennifer Sweatman (Advisor)
*Department of Biology/Environmental Studies*
*Concordia College – Moorhead, Moorhead, MN*

The United States Environmental Protection Agency (EPA) recognizes microplastics to be a growing threat to many organisms in ecosystems due to its high toxicity risk and the current lack of regulation. Therefore, studies focusing on the prevalence of microplastics have become more relevant and prevalent. This study is one of only a few to investigate the presence of microplastic debris in waterfowl populations. Waterfowl gastrointestinal samples (N=102) from the Fall 2020 season were donated by a hunting guide operating out of Devil's Lake, ND. To determine microplastic abundance and distribution within the gastrointestinal tract of waterfowl, we separated samples by organs and analyzed the gut contents under a microscope. Identified microplastics were enumerated and characterized by color, type, and length. We identified a total of 460 microplastics, 69.57% (320 particles) and 30.43% (140 particles) of which were found in dabblers and ground foragers, respectively. Dabblers were dominated (77.5% of samples) by Mallards, and ground foragers were dominated (22.5% of samples) by Canada Geese. Microplastic abundance was significantly greater in ground foragers than in dabblers. Furthermore, within the ground foragers, microplastics were found to be more abundant in the proventriculus than the gizzard, with no significant difference between other organs. Within the subgroup of dabblers, the abundance of microplastics was significantly higher in the intestine than the proventriculus and gizzard. Findings of this study can be used to guide future research and establish foundation for conservation and policy making regarding microplastics.

**DIFFERENCES IN THE PREDATION OF BROWN TROUT BY TERRESTRIAL PREDATORS BASED ON FISH MATURITY**

Karah Stangret and Jennifer L. Biederman (Advisor)
*Department of Biology*
*Saint Mary's University of Minnesota, Winona, MN*

The Brown Trout (*Salmo trutta*) occurs in the cold spring-fed streams of the southeastern Minnesota Driftless Ecoregion. Recent research suggests that Brown Trout populations experience the highest rates of seasonal mortality during fall, which coincides with spawning season, a period when fish may be weak and more vulnerable to predation. To date, there are few studies that document predation of Brown Trout, and no such studies in the Driftless Ecoregion, where such predators are likely to be terrestrial or avian species including herons, eagles, cranes, osprey, and water snakes. The objective of this research was to determine whether the maturity of the fish (juvenile vs. sexually mature) influences the predation rate of Brown Trout. The information gathered could provide a better understanding of why Brown Trout have lower survival rates in the fall and provide valuable information to aid management in improving habitat conditions, including in-stream cover, to increase fish survival during vulnerable life stages. Trail cameras were used to compare predation on Brown Trout ranging from juveniles to adults. In the studied stream we documented frequent predation attempts mainly by avian fauna, with a slight increase in survival among sexually mature Brown Trout.

# NEUROSCIENCE

## EXPRESSION OF PRIMARY CILIA-RELATED GENES IN CHICK CRANIAL NEURAL CREST CELLS

Mai P. Yang and Bridget T. Jacques-Fricke (Advisor)
*Department of Neuroscience*
*Hamline University, St. Paul, MN*

Neural crest cells (NCCs) are vertebrate cell types that are specified in the neural tube and migrate to different areas of the developing embryo to become NCC derivatives such as craniofacial cartilage and bone. We previously demonstrated that the methyltransferase NSD3 is required for NCC development and regulates the expression of several genes. One of these genes, IFT140, is involved with the formation and maintenance of primary cilia which receive and process signals. Primary cilia are required for NCC migration, proliferation, and differentiation. Mutations in IFT140 lead to congenital defects in the craniofacial bone structure such as craniosynostosis and cranioectodermal dysplasia. However, the relationship between IFT140, primary cilia, and NCCs during specification is still unknown. To further define the role of IFT140 and primary cilia earlier in NCC development, we analyzed an RNA-seq data set of potential regulators for NCCs, identified 5 additional genes related to primary cilia, and examined the protein-protein interactions among these genes using STRING and Cytoscape. To examine the spatiotemporal expression of IFT140, *in situ* hybridization was performed. We first cloned a unique region of IFT140 into a plasmid and performed *in vitro* transcription to create an IFT140-specific digoxigenin-labeled RNA probe. We identified IFT140 expression in neural crest cells migrating from the hindbrain region and within the developing eye. By defining the expression profile of IFT140 and examining the potential roles for primary cilia in NCCs, we hope to provide more information on the emergence of craniofacial defects during embryonic development.

**THE IMPACT OF NSD3 KNOCKDOWN AND THE EXPRESSION OF PRDM12 IN CHICK NEURAL CREST CELLS**

Kendra J. Yoder and Bridget T. Jacques-Fricke (Advisor)
*Department of Biology*
*Hamline University, St. Paul, MN*

Neural crest cells (NCCs) are a multipotent migratory cell type that arise in the neural tube during embryonic development of vertebrate organisms. NCC derivatives constitute craniofacial structures, cardiac mesenchyme, melanocytes, as well as most of the peripheral nervous system, including nociceptors. Our previous work found that the methyltransferase NSD3 plays a critical role in the specification and migration of NCCs and impacts the expression of many genes. To examine the functional implications of changes in gene expression after NSD3 knockdown, we performed gene ontology analysis. Among genes upregulated after NSD3 knockdown, genes related to ribosome biogenesis and RNA processing were overrepresented. Prevalent functional categories among downregulated genes included sympathetic nervous system development, sensory organ development, epithelium development and neurogenesis. One gene in the sympathetic nervous system development category was PRDM12. The methyltransferase PRDM12 is required for the creation of the nociceptive lineage, but a requirement for PRDM12 earlier in NCC development, prior to nociceptive differentiation, has not been demonstrated. In this study, we identify the expression pattern of PRDM12 during NCC specification and migration. We conducted *in situ* hybridization, which allows for visualization of PRDM12 expression, throughout early stages of embryonic development. We found that PRDM12 is expressed during NCC specification in the neural folds and in cranial NCCs during migration. We believe that future research on PRDM12 as it relates to NCC development can inform treatment and prevention approaches for diseases and cancers that arise from PRDM12 dysfunction, such as congenital insensitivity to pain and chronic myeloid leukemia.

# ORGANISMAL AND PHYSIOLOGICAL SCIENCES

## CONTRACTILE EFFECTS OF *Matricaria chamomilla* ON *Mus musculus* ISOLATED UTERINE TISSUE

Brittany L. Kothari and Teresa F. DeGolier (Advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

Modern standardized medical practices for labor induction have been shown to be successful, yet sometimes they cause unwanted side effects for mothers and infants. For this reason, some individuals have tried numerous herbal remedies to induce labor in place of current medical practices, such as Pitocin administration. One of these herbal remedies, German chamomile, *Matricaria chamomilla*, has been noted for its ability to both contract and relax uterine tissue. One of *M. chamomilla*'s constituents, a terpene called alpha-bisabolol, is commonly associated with relaxation of pre-contracted smooth muscle. However, one unique study found orally encapsulated *M. chamomilla* to be effective in inducing contractions in labor in vivo. Additionally, a study utilizing isolated rat uterine tissue observed that a hydro-alcoholic extracted of *M. chamomilla* primed with estrogen increased the force of spontaneous contractions. With these studies in mind, the primary goal of this project was to determine whether or not *M. chamomilla* would contract the smooth muscle in isolated uterine horns of mice, and if so, were the contractions concentration dependent. Concentrations (0.07-1.16 mg/mL), *M. chamomilla* produced contractile forces equivalent up to 75% to the tissue's positive control (oxytocin 10-5 M). In addition, *M. chamomilla* produced contractile forces at almost 2.5 times the tissues' own spontaneous motility. These results provide support that *M. chamomilla* may augment labor, however other herbs using a similar protocol have been shown to produce much greater contractile forces.

**THE CONTRACTILE EFFECTS OF PURPLE HOLY BASIL (*Ocimum tenuiflorum*) ON MOUSE UTERINE MUSCLES**

Madelyn J. Mehr and Teresa DeGolier (Advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

For centuries, herbal agents have been used in the practice of midwifery to induce labor and reduce the stress of post-term labor. Some literature suggests that uterine smooth muscle function may be influenced by some common herbal kitchen ingredients, such as parsley, oregano, and basil. The goal of this research experiment was to provide the broader scientific community with data regarding these claims. This research evaluated purple holy basil (*Ocimum tenuiflorum*), a genus of basil found primarily in tea and prepared with other varieties of basil commonly referred to as tulsi. Several concentrations of this herb were prepared as an aqueous extract and applied to isolated mouse uterine smooth muscle tissues and tested in an organ bath. Upon analysis, it was found that contractile forces produced by the herb were significantly greater when compared to the tissues own spontaneous motility. Higher concentrations of *O. tenuiflorum* (0.274-1.121 mg/mL) were equal to or greater than contractile forces produced by the positive control, oxytocin (10-5M). These results show that purple holy basil does have a contractile effect on isolated strips of uterine smooth muscle. Future studies pursuing this effect with purple holy basil should focus on developing more knowledge of the specific chemical constituents that interact with the receptors found in smooth uterine tissue. Results such as these will have the potential to determine whether purple holy basil truly helps, harms, or even has any medicinal effect during and post-labor.

**COMPARING MORPHOLOGICAL AND MOLECULAR DATA TO DIFFERENTIATE BETWEEN *Peromyscus leucopus* AND *Peromyscus maniculatus***

Kelly M. Lorenz, Lauryn P. Petrich, Vanessa D. Petrich, Taylor M. Devine, Amelia F. Landsverk, Cole R. Nowacki, and Joseph C. Whittaker (Advisor)
*Department of Biology*
*Concordia College-Moorhead, Moorhead, MN*

Similarity in morphological characteristics of white-footed (*Peromyscus leucopus)* and deer mice (*Peromyscus maniculatus*) has led researchers to conclude that they cannot be reliably differentiated live in the field. Inability to identify mice in the field complicates behavioral, ecological, and etiological research. Physical characteristics, such as length of ear, hindfoot, and tail, have been used to attempt to differentiate these two species. With climate change, deer mouse range has diminished as white-footed mouse range has increased, resulting in greater range overlap and regional extirpation of deer mice in Illinois, Michigan, and Wisconsin. Morphological similarity has also increased in many regions. Given such similarity, our goal was to see whether, using molecular techniques for verification, morphological measurements are at all useful to identify these mice rapidly and reliably in the field. We used cellulose acetate electrophoresis of salivary amylase to confirm identification of samples collected from prairies and woodlands from 2004 to 2019. We analyzed 1,262 saliva samples and compared molecular results to corresponding morphological measurements. While differences in ear length in forest habitats, hindfoot length in prairie, and tail length (in both habitats) were statistically significant, the substantial overlap in these measurements makes these characters of limited usefulness. Measurements in individual mice are too variable to allow reliable identification. Thus, rapid identification of these species in the field is complicated by morphological overlap, and accurate identification is only possible through molecular analysis such as salivary amylase electrophoresis.

**PHOTOPROTECTIVE MECHANISMS OF RED VS. YELLOW DECIDUOUS PLANT SPECIES DURING AUTUMN SENESCENCE**

Emma H. Miller and Amy S. Verhoeven (Advisor)
*Department of Biology*
*University of St. Thomas, St. Paul, MN*

While photosystems benefit from sunlight, receiving an excess number of photons produces a highly reactive form of oxygen, leading to premature cell death. To prevent against this, deciduous plants have evolved photoprotective pigments, which give autumn its distinct colors. While all deciduous plants contain the yellow and orange pigments (xanthophyll cycle pigments (VAZ) and alpha/beta carotene), only some significantly upregulate a red pigment (anthocyanin). This study set out to compare red vs. yellow deciduous plants in phylogenetically related pairs, for a total of eight species, to determine whether causal patterns emerged between species of one color. Procedures tested fluorescence, pigment concentration, and nitrogen levels. We found a correlation between color and Lutein per chlorophyll and also between color and AZ/VAZ per chlorophyll within Amur maple, Boxelder, Grape, and Creeper. Yellow species were found to retain higher levels of Lutein late into senescence while losing violaxanthin, as compared to red species. A remarkably strong correlation of chlorophyll loss and neoxanthin loss was found between all four of these species. Lastly, the strongest variation between all four species was seen in VAZ loss per chlorophyll throughout the autumn.

**CAPRYLIC ACID AND ITS RESULTING SIGNIFICANCE ON MALE RAT SPERMATOGENESIS AND REPRODUCTIVE ORGANS**

McKenna M. Stoner and Jonathan Van Berkom (Advisor)
*Department of Biological Sciences*
*Bethel University, St. Paul, MN*

Caprylic acid is a saturated medium-chain fatty acid that is found in the milk of various mammals and is a minor component of coconut oil and palm kernel oil. People have used this compound for various home remedies. When researching the effects of caprylic acid on fertility, previous studies showed that caprylic acid had a negative effect on blastocyte development, hatching rates, developmental kinetics, and total number of cells in murine models while implantation rates were not altered. Alternatively, other research has demonstrated that octanoic acid supplementation does not stimulate the ghrelin-pituitary growth hormone in pregnant rats. This project focused on dosing male rats with caprylic acid for five weeks and analyzing sperm morphology, sperm motility, sperm concentration, testicular weight, epididymal weight, seminal vesicle weight, and prostate weight. We found that Caprylic acid exhibited no significant differences in these areas when compared to the samples that were taken from the rats that served as the control.

# PHYSICS

## OPTIMIZING STRUCTURE AND FABRICATION PROCESS OF THIN-FILM SOLID-STATE LITHIUM-ION BATTERIES

Sydney R. McCutchen and Lifeng Dong (Advisor)
*Department of Physics*
*Hamline University, St. Paul, MN*

Although lithium ion batteries (LIBs) have been broadly used in electric vehicles, laptops, cell phones, and other electronics, few studies have investigated thin film lithium ion batteries for low power electronics. In this study, thin films of aluminum, nickel/lithium cobalt oxide, lithium phosphate, and titanium/copper were constructed by thermal vapor deposition and magnetron sputtering deposition. Various parameters (e.g., deposition time, deposition power, hot-pressing time and temperature, anode electrode materials) were used to optimize the structure and electrical properties of LIBs. A total of 141 batteries were fabricated and tested, and testing results demonstrate that the hot pressing process is critical and copper shows better performances than titanium as anode electrode material. The findings indicate it is promising to further simplify the structure of thin film LIBs through continuous optimizations.

## INCREASED SENSITIVITY FOR LEAD DETECTION IN DRINKING WATER USING SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)

Joshua B. Sedarski, Maddie R. Sowinski, and Lifeng Dong (Advisor)
*Department of Physics*
*Hamline University, St. Paul, MN*

Our project goal was to develop an affordable yet ultrasensitive lead detection method to be owned and operated by a variety of communities. Per the updated EPA Lead and Copper Rule from December, 2020, there is no “safe” amount of lead in drinking water. As such, ultrasensitive detection methods are crucial to maintain the health and safety of communities across the country. Methods exist that utilize atomic absorption spectroscopy, inductively coupled plasma, and DNA-Zyme/biolinker molecules. While these methods are highly sensitive, they require expensive, high-end laboratory equipment that are impractical to use in most communities, especially low-income and rural. Our project focused on a SERS detection method, aiming to utilize more affordable and accessible equipment and materials, such as a portable Raman spectrometer and silver nanoparticles. A layered probe molecule solution was synthesized with the amino acid L-cysteine (Cys), gold or silver nanoparticles (Au/Ag NPs), and the Raman probe 4-aminothiophenol (4-ATP) to trap lead ($Pb^{2+}$) ions. The optimal probe molecule solution that was observed consisted of 925 µL of 5 nM AgNPs with 20 nm diameter, 50 µL of 106 µM Cys, and 25 µL of 50 mM 4-ATP. Upon the addition of 100 µL of 1.02 mM $Pb^{2+}$ solution–with a resulting detection limit of 92.7 µM–4-ATP signals were amplified by nearly 440 times. Despite these promising results, it’s proven difficult to replicate and requires further parameter optimization. Before this detection model can be considered viable, we must be able to consistently replicate Raman signals.

**EXPERIMENTAL OBSERVATION OF HIGH-SPEED FLUCTUATIONS IN SURFACE ENHANCED RAMAN SPECTROSCOPY**

Makayla M. Schmidt, Marit A. Engevik, Sarah E. Bense, Tryg R. Burgau, Eric J. Katsma, and Nathan C. Lindquist (Advisor)
*Department of Physics and Engineering*
*Bethel University, St. Paul, MN*

Surface enhanced Raman spectroscopy (SERS) is a well-known technique that illustrates both the challenges and opportunities inherent to nanophotonics and nanotechnology. This is due to the extremely localized nature of SERS, where intense plasmonic "hotspots" increase Raman scattering by orders of magnitude, generating signals from single molecules. These signals often show significant fluctuations, both in intensity and spectral features due to the dynamic nature of light-matter interaction at the atomic scale. Recent experiments have shown these SERS intensity fluctuations (SIFs) occur over an extremely wide range of timescales, from seconds to micro-seconds. While many mechanisms have been proposed for these fluctuations, such as molecular diffusion or transient plasmonic hotspot generation, the underlying source of these fluctuations are likely to be a complex interplay of several different effects. Furthermore, while high-speed intensity fluctuations provide important information on the overall timing statistics, high-speed spectral information has so far been lacking. In our research, we designed and assembled a system able to trigger the occurrence of SERS, capture both the intensity and spectrum of trials, and process the data to determine the location and type of SIFS observed. Our findings showed initial progress on our high-speed acquisition system capable of taking more than 100,000 spectra per second. We tested various types of SERS samples and determined best experimental conditions to observe these high-speed fluctuations. Characterization of SERS fluctuations at these speeds can provide further clues as to the source of these events.

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