# JOURNAL OF ABSTRACTS OF THE MINNESOTA ACADEMY OF SCIENCE

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*Celebrating 31 Years of Excellence in Undergraduate Research*

UNIVERSITY OF St.Thomas

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The Annual Meeting & Winchell Undergraduate Research Symposium is an opportunity for undergraduate students to present the results of their research to their peers and professional scientists.

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### FORSE

This new program connects students from populations traditionally underrepresented in STEM fields with professional mentors and resources to prepare them to participate in the Minnesota State Science & Engineering Fair and the Junior Science & Humanities Symposium.

# *JOURNAL OF ABSTRACTS OF THE MINNESOTA ACADEMY OF SCIENCE*

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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 hosted by the University of St. Thomas on April 21, 2018, in St. Paul, Minnesota.

# BIOCHEMISTRY

## BIOPHYSICAL CHARACTERIZATION OF G-QUADRUPLEX DNA LIGAND BINDING FOR A FLUORESCENT PROBE N-METHYL MESOPORPHYRIN IX

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A unique noncanonical secondary structure of DNA exists in the form of a four-stranded DNA quadruplex. The quadruplex structure is stabilized by Hoogsten-type hydrogen bonding exhibited by four guanine nucleotides that form a plane. Quadruplex formation has been discovered at the telomere of human chromosomes and is of significant interest in potential anticancer therapies. While the remarkable selectivity of N-Methyl Mesoporphyrin IX (NMM) for Guanine-Quadruplex DNA has been well established, there is still uncertainty in NMM's ability to serve as a fluorescent probe for G-quadruplex when complexed with a transfection agent. Fluorescent ligands for typical double-stranded DNA intercalate within the base pairs and serve as fluorescent probes for DNA in a native conformation. However, when double-stranded DNA is complexed with a transfection agent, there is a quenching event that kicks out the intercalator fluorescent probe. Here we investigate the hypothesized NMM end binding to G-Quadruplexes and its ability to serve as a functional fluorescent probe while the G-Quadruplex is complexed with a transfection agent. We hypothesize that the NMM end-binding to G-Quadruplex will resist the quenching kickoff effect that occurs with double-stranded DNA and intercalator. We will use fluorimetry experiments to see whether there is a notable decrease in the fluorescence of the NMM. The NMM when bound to G-Quadruplex serves as a fluorescent ligand, so we will examine the effect of the transfection agent.

## GLUTATHIONE S-TRANSFERASE EXPRESSION IN EMBRYONIC LIVERS OF *Gallus gallus* EXPOSED TO ATRAZINE IN OVUM

Haley Colton and Debra Martin (Advisor)
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Atrazine (ATR) is one of the most commonly and widely used herbicides in the Midwest. It remains in the soil for long periods of time and has been found to contaminate many different water sources, thus potentially causing developmental effects in agricultural organisms such as chickens (*Gallus gallus*). Many studies have been done to analyze the physiological effects caused by exposure to ATR. Yet, few studies have focused on the *in ovum* effects of ATR exposure. The liver enzymes responsible for the detoxification of ATR are of particular interest and one family of detoxifying enzymes are: glutathione S-transferases (GSTs). Previous studies have analyzed and identified that chronic exposure to ATR in carp leads to a decrease in the enzymatic activity for GST-mu. In order to determine the relationship between *in ovum* exposure to ATR and the enzymatic activity of the three isotypes of GST, fertilized chicken eggs were injected with three different concentrations of ATR: 0 ppb, 3 ppb, and 30 ppb prior to incubation at 37ºC. At 18 days incubation, the embryonic livers were harvested and analyzed utilizing enzyme assays for the three difference GST isotypes (Alpha, Mu, and Pi).

**IDENTIFYING PROTEINS IN ALCOHOLIC FATTY LIVER DISEASE**

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Liver failure due to alcoholism is a problem that is not well understood in the United States. Before the onset of liver failure, the liver accumulates fat in the lipid droplet. This is known as Alcoholic Fatty Liver Disease (AFLD). This research focused on learning more about AFLD, which is an indicator of potential liver failure and yet is reversible if recognized before the disease progresses further. Our experiments aimed to identify proteins present on lipid droplets in a cell culture model of AFLD. We hypothesized that proteins that are more prevalent on lipid droplets in AFLD may play a significant role in the progression of the disease and could help in the diagnosis of AFLD. Previous work in our laboratory has shown that culturing the AML12 mouse liver cell line in choline-deficient media mimics the changes to lipid droplets that are associated with AFLD. We used a protein assay to determine the concentration of total lipid droplet proteins in control and choline-deficient AML12 cells. We used SDS-PAGE and Western blotting to visualize the concentration of candidate lipid droplet proteins. The results of our study show that three proteins—perilipin 1, perilipin 2, and CIDEC—are more prevalent on lipid droplet under cell culture conditions that model AFLD. Future work will assess the role that these proteins play in the development of AFLD and may lead to a more accurate diagnosis or treatment options for this disease.

**INVESTIGATION OF ENZYME ACTIVITY**

Corbin Ketelsen and Heather Sklenicka (Advisor)
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The purpose of this research is to improve the "Investigation of Enzyme Activity" lab that is used by the second semester General, Organic, and Biological Chemistry course (Chem 1118) at RCTC. This lab shows how the functionality of the enzyme sucrase can be changed by manipulating some factors surrounding it. The first week of the lab shows students that by changing factors such as pH, temperature, or enzyme concentration you can affect the activity of the enzyme. The second and third weeks of the lab, students create their own experiment and perform it. The goal of this project is to improve the basic functionality of the first week of the lab. Currently the lab results don't match with the theory of protein structure. An early issue that has been uncovered is that the 4.4 pH buffer is made out of acetic acid that reacts with the sodium carbonate in the Benedict's reagent. The results from converting to another buffer solution and tests of temperature and concentration will be presented.

**LIPOTOXICITY: UNDERSTANDING THE MECHANISM THAT LEADS FROM SATURATED FATTY ACID TO CELL DEATH**

Margaret Brown, Hannah Nilsson, and Laura Listenberger (Advisor)
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An excess of saturated fatty acids causes cell dysfunction and death, which has been linked to obesity-related health problems like heart disease and type II diabetes. In this study, we aimed to determine if the incorporation of saturated phospholipids into the endoplasmic reticulum (ER) membrane would trigger ER stress, leading to cell death. To understand the mechanisms of this pathway, cells were treated with the saturated fat palmitate under conditions that decreased phospholipid synthesis. The effects of palmitate treatment were measured with cell viability assays and fluorescence microscopy. Our experiments show that reducing choline, the main component of the prevalent membrane phospholipid phosphotidylcholine, does not rescue cells from liptoxic effects. Limiting choline caused no significant decrease in the negative symptoms of lipotoxicity when cells were

treated with 400μM palmitate, as quantified by measurements of mitochondrial function and reactive oxygen species levels. Future studies will aim to determine what factors contribute to oxidative stress in the lipotoxic pathway.

## PHOSPHORYLATION PATTERNS IN MOSS WITH VARYING DEGREES OF DESICCATION TOLERANCE

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Desiccation, the state of severe dehydration, is an extreme form of stress few organisms can tolerate. During desiccation, photosynthesis is completely inhibited because of the lack of water, but the tissue is still exposed to light on a daily basis. In order to tolerate desiccation, those plants must use photoprotective mechanisms to cope with excess light while in a desiccated state. Thermal dissipation may be one mechanism desiccation-tolerant (DT) species use to protect against damage while in a desiccated state. An aspect of thermal dissipation regulation involves changes in the phosphorylation of key photosynthetic proteins, including light-harvesting complex II and photosystem II reaction center proteins. Moss is known to have DT qualities. To test DT in moss, a pilot study was conducted to identify twenty moss species as low, medium, or high tolerance. One species from each tolerance level was selected to undergo further desiccation studies. Each species was desiccated for twenty-four hours to two weeks in light and dark environments to examine phosphorylation patterns of the proteins in the photosynthetic apparatus. Western blots were performed on isolated thylakoids from each species to visualize the phosphorylation of the photosynthetic proteins. The pattern of phosphorylation differed between each species and between the light and dark desiccation experiments within each species. This suggests that, depending on environmental conditions, mosses use different phosphorylation patterns when desiccated as an adaptive mechanism to survive the stressful state. The patterns may indicate why moss species vary in degree of DT.

## *Staphylococcus aureus* SUPERANTIGENS: DON'T YOU KNOW THAT YOU'RE TOXIC?

Darian Wisecup, Taylor Mach (Advisor), and Amanda Brosnahan (Advisor)
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*Concordia University, St. Paul, MN*

Protocols were established for identifying the presence of genes for the following enterotoxins from *Staphylococcus aureus*: TSST-1, Alpha, SEC, SEA, and SElX. Genomic DNA amplified via Polymerase Chain Reaction (PCR) and the presence of enterotoxin genes was visualized using DNA gel electrophoresis. Clinical isolates known to be positive and negative for the specific genes were used as controls and genes that did not have known controls have been found.

## VEGF-B AND FABP4 EXPRESSION IN MICE (*Mus musculus*) EXPOSED *in utero* TO ATRAZINE

Jeremy Heinle and Debra Martin (Advisor)
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Atrazine, an herbicide regularly used across the United States, is found in ground water due to its inability to break down. Recently, ground water concentrations higher than the EPA limit of 3 ppb have been detected, raising concerns about possible health issues due to atrazine's prior reputation as an endocrine disruptor. A recent study showed that mice exposed chronically to atrazine had increased serum-free fatty acid levels, indicating issues with fatty acid regulation (Jin et. al., 2014). Two proteins

of interest, Vascular Endothelial Growth Factor-B (VEGF-B) and Fatty Acid Binding Protein-4 (FABP4), are highlighted due to their role in metabolism, specifically within the regulation of fatty acid uptake. Dysregulation of both VEGF-B and FABP4 has been linked to increased risk of metabolic disease. In this experiment, VEGF-B and FABP4 levels was determined by Immunoblot of bicep muscle samples from 9-week-old mice pups that will be exposed to 0 ppb, 3 ppb, and 30 ppb of atrazine *in utero*. A gender difference in expression of VEGF-B was seen with a decreasing expression in males but not in females.

# CELLULAR AND MOLECULAR BIOLOGY

### ABNORMAL WNT/BETA CATENIN SIGNALING IN AN IPSC MODEL OF ARVC ASSOCIATED WITH TRUNCATED PLAKOGLOBIN

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Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a rare inherited heart disease characterized by a progressive fibro-fatty phenotype and arrhythmias primarily affecting the right ventricle. ARVC is connected with desmosomal dysfunction and most of the mutations associated with the pathology are found in genes for desmosomal proteins. A clinically reported nonsense mutation in exon 13 of plakoglobin, a critical component of the desmosome, was investigated in a murine iPSC model to determine the mechanism of this mutation in development of the pathology. Plakoglobin, which shares significant homology with beta-catenin of the Wnt Pathway, has been shown to be differentially spliced between left and right ventricle cardiac progenitors. This study investigated the effect of candidate RNA-binding proteins that lead to alternative splicing of plakoglobin, demonstrating retention of the mutation in exon 13 predominantly in right ventricle cardiac progenitors. We also sought to determine if truncated mutant plakoglobin interferes with canonical Wnt signaling because of its homology with beta-catenin, leading to the fibro-fatty phenotype. Gene expression data of RNA binding proteins and Wnt/beta-catenin transcriptional targets were produced through qPCR. Cardiomyocytes were immunostained to determine the relocalization of plakoglobin. The truncated plakoglobin form was found significantly overexpressed in heterozygous mutatants and accompanied by significant upregulation of the Wnt target transcription factor Lef1, suggesting that Wnt signaling may be abnormally regulated in plakoglobnin mutant cells, contributing to the development of the ARVC phenotype.

### ANNOTATION OF *Drosophila eugracilis* CHROMOSOME 3L Contig 65

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Annotation identifies the locations and potential functions of genes and other elements in a genome. Once a genome is sequenced, it needs to be annotated to make sense of it. We are annotating a portion of the genome of *Drosophila eugracilis* by adding locations of gene start codons, exons, introns, and stop codons, and hypothesized gene products to the raw sequence data. This sequence annotation is part of the Genomics Education Partnership, in which multiple scientists and many institutions are analyzing homologous sequence data from several *Drosophila* species. By comparing the homologous sequences to the fourth chromosome of *Drosophila melanogaster,* we can better understand expression of genes found in heterochromatin. *D. eugracilis* chromosome 3L's contig 65 (30,000 bases) has five predicted genes, some with multiple isoforms.

## ANTI-INFLAMMATORY EFFECTS OF *Helichrysum italicum* ON RAW 264.7 MACROPHAGES

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Although inflammation is an essential and protective response of the immune system to pathogens and damage, its prolonged presence has been linked to a variety of inflammatory diseases and conditions. With the rise in occurrence of these diseases coupled with a decline in satisfaction with conventional drug treatment options due to their adverse side effects, alternative options are progressively being utilized and studied for their potential to lower inflammation. As highly concentrated plant extracts, essential oils have been used by civilizations across the world for centuries as a remedy for pain and inflammation. In recent years, scientists have endeavored to uncover the biochemical properties and mechanisms by which they function. As the primary modulators of the inflammatory response, macrophages represent an important system for the study of anti-inflammatory properties of essential oils. This experiment sought to investigate the biochemical effects of *Helichrysum italicum* essential oil (HEO) on RAW 264.7 macrophages. The results showed that in cells treated with an interferon-γ (IFN-γ)/LPS combination to produce an M1-like polarization typical of cytotoxic macrophages, HEO lowered levels of toxic nitric oxide and the inflammatory cytokine TNF-α as well as levels of the anti-inflammatory cytokine IL-10 and arginase. When polarized to the repair-promoting M2 phenotype by treatment with IL-4, HEO was also found to lower arginase levels. This collectively suggests that HEO has the ability to attenuate both M1 and M2-activated macrophages, which affirms its potential as an anti-inflammatory agent while suggesting a cautious approach due to the corresponding diminution of M2-associated phenotypes.

## CHARACTERIZING AMMONIUM TRANSPORTERS OF THE CYANOBACTERIUM *Anabaena*

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Most organisms have transmembrane protein channels that transport reduced nitrogen compounds such as ammonia ($NH_3$) or ammonium ($NH_4^+$). Transporters for which either $NH_3$ or $NH_4^+$ act as the substrate compose the AMT/MEP/Rh protein family. Almost all AMT/MEP/Rh proteins transport $NH_3$. However, a small subset of AMTs discovered in flowering plants transport electrogenic $NH_4^+$. Recently, electrogenic AMTs were discovered in the liverwort *Marchantia polymorpha*. The appearance of electrogenic AMTs in such an early land plant as *Marchantia* suggests that the evolutionary origin for electrogenic $NH_4^+$ transport was at least as early as land plants (embryophytes). Certain green algae are the ancestors to land plants. Endosymbiont theory describes the origin of the chloroplast as cyanobacteria that were engulfed by a specific eukaryotic cell and formed an obligate symbiosis. *Anabaena* is a model organism that can represent the ancient cyanobacteria that was engulfed through endosymbiosis. The photosynthetic cyanobacterium *Anabaena* has three identified AMTs. To determine if these AMTs transport $NH_3$ or $NH_4^+$, the *Anabaena* AMT genes were cloned into a yeast expression plasmid and transformed into a mutant strain of yeast lacking functional AMT/MEP/Rh genes. Genes that successfully complemented the mutant were cloned into a *Xenopus* oocyte expression plasmid and will be tested by electrophysiology. Electrophysiology measures changes in resting membrane potential, indicative of electrogenic transport of ions such as $NH_4^+$.

## DIFFERENTIAL PROTEIN EXPRESSION OF FIBROLAMELLAR HEPATOCELLULAR CARCINOMA (FL-HCC)

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Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare hepatic malignancy that is considered a distinct variant of the more commonly seen hepatocellular carcinoma. FL-HCC is not associated with chronic liver disease and has a young age of onset. It is characterized by laminated fibrous layers interspersed between the tumor cells. There are currently no known biomarkers for diagnosis and limited treatment options, generating interest in identifying the etiology of FL-HCC. Genomic screening in recent years has confirmed the presence of a 400-kb deletion that results in the chimeric transcript *PRKACA-DNAJB1* in all tested FL-HCC liver biopsies. Utilizing mass-spectroscopy proteomics, our data revealed possible dysregulation of enzymes implicated in the proline synthesis pathway. In this study, we further confirmed the finding via Western blot protein analysis.

## THE EFFECT OF CANDIDATE GENE CTNNB1 ON HEPATOBLASTOMA

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Background and Aims: Mutations in the beta catenin gene (CTNNB1) that lead to constitutive activation of the Wnt pathway have been detected in a large proportion of hepatoblastoma tumors. The aim of the research is to investigate the effects of CTNNB1 gene knockout (KO) on a hepatoblastoma cell line, HepG2, and analyze cell migration and cellular movement.
Methods: We used the gene-editing tool CRISPR to knock out CTNNB1 in HepG2 cells. HepG2 cells and HepG2 CTNNB1 KO cells were compared in the Transwell Migration Assay and Wound Healing Assay to assess any differences in migration and cellular movement.
Results: A larger count of HepG2 CTNNB1 KO cells were seen in the Transwell Migration Assay which indicated more migration compared to HepG2 cells as well as faster cellular migratory movement by the knockout cells in the Wound Healing Assay.
Conclusion: The greater number of HepG2 CTNNB1 knockout cells counted in the membrane compared to HepG2 cells indicated a greater migratory ability in the Transwell Migration Assay. The knockout cells also closed the wound gap faster which gives insight into HepG2 cancer cell movement and transformation. Overall, the HepG2 CTNNB1 KO showed greater tumorigenic potential, and showed a gain of function mutation that could contribute to proliferation, increased cellular migration, and movement of cancerous cells.
Keywords: hepatoblastoma; CTNNB1; beta catenin; transwell migration assay; wound healing assay

## EFFECT OF TBX2 EXPRESSION ON GROWTH AND APOPTOSIS OF SKBR3 CELLS TREATED WITH HER2 NEUTRALIZING ANTIBODY

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Breast cancer is the most frequently diagnosed cancer in women of all ethnicities, with 1 in 8 women developing the disease during their life time. TBX2 is a transcription factor that is overexpressed in about forty percent of those with breast cancer. In mouse studies, TBX2 has also been linked to cancer by acting as an immortalizing gene and inhibiting p19 ARF and p21 WAP1/C1P1, therefore leading to the bypass of senescence. Previous data from our lab suggest that overexpression of TBX2 leads to increased expression of epiregulin, a member of the EGF family of growth factors. This family of growth factors can activate the HER2 receptor associated with many breast cancers. One treatment for HER2-positive breast cancer is Herceptin. Herceptin works intracellularly by binding to HER2 receptors and blocking HER2 signaling. In this study, TBX2 was overexpressed in the SKBR3 cell line, a HER2-positive breast cancer cell line, by utilizing a lentivirus. The cells were treated with a HER2 neutralizing an antibody similar to Herceptin and assays were completed to measure cell viability and apoptosis. Results of the apoptosis assay suggested there was no effect of TBX2 expression on the cell's response to a HER2 neutralizing antibody.

## HOX TRANSCRIPTION FACTOR REGULATION OF NEURONAL DEVELOPMENT IN *Caenorhabditis elegans*

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Every cell in an organism has the same DNA; however, each type of cell switches on only a small subset of genes, leading to unique cell fates. We investigated how Hox transcription factors determine neuronal cell fate in a model system, the nematode *Caenorhabditis elegans.* Previous work established that Hox transcription factors are responsible for spatial expression of neuronal cell fates in the male nerve cord, binding to specific genes and turning them on or off in different zones. We investigated the function of Hox transcription factor *ceh-13* in cell fates by inhibiting its function using RNA interference. In the absence of *ceh-13* the number of neurons with normal cell fates is decreased, suggesting that its action is required for appropriate neuronal cell fates. To understand *how* Hox transcription factors interact to specify fates, we determined what DNA sequences are required to respond to these signals. We cloned portions of the switch-receptive DNA sequences of the gene *tph-1* into a visual system that indicates if the DNA sequences can be switched on. We found a 486 base-pair DNA sequence that is required to switch on middle zone fates but not first zone fates, suggesting that different DNA sequences are necessary for differential gene expression in the two zones. Our research demonstrates that Hox transcription factors are needed for normal neuronal cell fates. This regulation allows for the development of specific tissues in *C. elegans* and may be important in the neuronal development of all animals.

## INHIBITION OF TBX2 EXPRESSION IN THE T47D BREAST CANCER CELL LINE REDUCES CELL MOBILITY

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Breast Cancer is one of the most prevalent cancers for women in the United States. One gene that has been linked to breast cancer is TBX2, which is a gene within the T-box family of transcription factors. In many cases, TBX2 is amplified or overexpressed in breast cancer. In cells that display overexpression of TBX2, $p19^{arf}$ and $p21^{wap1/cip1}$ expression is suppressed, allowing the cell cycle to progress with limited control. TBX2 has also been found to be associated with increased levels of epithelial to mesenchymal transition (EMT) and metastasis. To further understand the role of TBX2 on cell migration and metastasis, we used the T47D breast cancer cell line and introduced a TBX2 siRNA to knock down TBX2 expression. A scratch assay was performed and the migration rates of the cells were measured to determine the effect TBX2 had on migration rates. We found that knockdown of TBX2 expression inhibited migration rates of the T47D cell line. This supports the idea that TBX2 may play a role in metastasis of breast cancer.

## MI IS IN THE AIR: CHARACTERIZING THE ALLERGIC RESPONSE TO INHALED METHYLISOTHIAZOLINONE IN MICE

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Chronic pain disorders often have a comorbidity of seasonal allergies — of which a major portion comes in contact with the body through the respiratory system. Case studies have shown Methylisothiazolinone (MI) — a common household biocide — to be a potent dermal and respiratory sensitizer. However, previous literature has found that MI is not an asthmagen in inbred mice. This study seeks to analyze MI as a model allergen in the study of chronic pain in outbred ND4 Swiss mice. Mast cell connections to nociceptive neurons and to allergic reactions make this immune cell of particular interest to the study. In order to study this cell in context, two experiments testing MI in the airways were conducted. One experiment looked at optimizing the dosage of MI while the second experiment looked at cytokines characteristic of mast cells in the various regions of the airways. Most response to treatment with MI was seen in the bronchoalveolar lavage and not in the lung tissue itself, indicating that MI was eliciting an inflammatory response in the upper airways rather than in the lower airways. Elevated IgE response in the serum also indicates that a mast cell- and eosinophil-driven allergic reaction might be occurring. These experiments pave the way for testing whether airway allergies may exacerbate chronic pain elsewhere in the body.

## OVEREXPRESSION OF TBX2 AND EPIREGULIN HAVE NO EFFECT ON INVASION OF SKBR3 CELLS IN A BOYDEN CHAMBER ASSAY

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Breast cancer is a disease in which cells in the breast tissue grow and divide without their normal control. Most deaths from breast cancer are a result of the cancer cells spreading to other areas of the body, a process called metastasis. The overexpression of TBX2, a member of the T-box family of transcription factor genes, has been shown to be associated with several types of cancer, including breast cancer. Studies have found that overexpression of TBX2 has the ability to suppress senescence,

allowing for continued growth of cells, and promote epithelial-mesenchymal transition (EMT), allowing for invasion of cells. Data from our lab suggest that overexpression of TBX2 can lead to increased expression of epiregulin. Epiregulin is a member of the epidermal growth factor family and it can contribute to the growth of several types of cancer. Due to the effects epiregulin can have on a large range of tissues, the epiregulin signaling pathway may be an appropriate target for therapeutics. To further investigate the effects that TBX2 and epiregulin have on invasion and metastasis, lentiviral vectors were used to express TBX2 in SKBR3 human breast cancer cells. A Boyden Chamber assay was used to determine cell invasion of the cells with or without TBX2 or epiregulin. The data suggests that TBX2 does not have a significant effect on cell invasion indicating that TBX2 may not play a role in this process.

**THE PEPTIDE THAT BROKE THE MOUSE'S BACK: ASSESSING THE ROLE OF THE NEUROPEPTIDE TLQP-21 AND ITS IMMUNE RECEPTOR C3AR1 IN SPINAL CORD INJURY PAIN**

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Chronic pain resulting from spinal cord injuries (SCI) affects millions worldwide, and current therapeutics are largely ineffective, making investigation into the mechanisms behind this condition of paramount importance. Hypersensitivity following SCI occurs due to central sensitization, a process that includes increased membrane excitability, increased synaptic efficacy, and decreased inhibition of neurons in the spinal cord. Recent work has shown that activation of microglia after injury is critical to developing neuropathic pain and may mediate some of these neuronal changes. We have previously shown that the VGF-derived neuropeptide TLQP-21 is upregulated in injured neurons and contributes to the development and maintenance of hypersensitivity after peripheral nerve injury. TLQP-21 acts through the complement 3a receptor (C3aR1) on microglia, and we have shown that it acts to initiate a neuroimmune signaling pathway. This has not yet been studied in SCI, a central model of neuropathic pain. This thesis has used immunohistochemistry and behavioral assays to conclude that microglia and C3aR1 are upregulated in the spinal cord, and that this injury produces mechanical allodynia, thermal hyperalgesia, and spontaneous pain. Examining these novel molecular pathways that occur after injury could help to elucidate new targets to treat pain following SCI.

**TUNNELING NANOTUBE FORMATION IS UPREGULATED IN PANCREATIC CANCER AND MEDIATES A NOVEL LONG-DISTANCE INTERCELLULAR DRUG EFFLUX**

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*University of Minnesota–Twin Cities, Minneapolis, MN*

Background: Intercellular communication plays an important role in cancer progression, yet its correlation with cellular stress responses are largely unknown. Well-established mechanisms of cellular crosstalk such as gap junctions, exosomes, and soluble signaling proteins contribute to the invasive capacity of various malignant cells. However, recent studies have elucidated the role of tunneling nanotubes (TNTs) in enhancing long-distance intercellular communication. TNTs are long, F-actin-based membrane protrusions that facilitate direct cell-to-cell communication between cancerous cells. Methods: We investigated the rate of TNT formation in pancreatic carcinoma in response to varying concentrations of the chemotherapeutic drug doxorubicin. Dosage concentration was optimized such that a physiologically relevant concentration could be utilized. Results: Doxorubicin increased nanotube formation in all dosage concentrations within 48 or 72 hours of exposure ($p < 0.0001$). A peak in TNT index occurred in both cell lines at a concentration of 800 ng/mL Additionally, we discovered that TNTs facilitated drug redistribution between connected cells in both pancreatic and ovarian cancer. Conclusions: Increased TNT formation in response to

doxorubicin implicates chemotherapeutic drugs as potential catalysts for increased intercellular communication due to induction of stress response.

# CHEMISTRY

### AIR-SENSITIVITY PREDICTION OF AMIDE-STABILIZED PRIMARY PHOSPHINES VIA INEXPENSIVE COMPUTATIONAL METHODS

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Primary phosphines ($RPH_2$) are notorious for their stench and pyrophoric behavior. However, several examples of air-stable primary phosphines have been reported. We have designed and explored a class of electronically stabilized phosphines based on the previously reported, air-stable, bis(primary phosphine), $PhN(H)C(O)CH(CH_2PH_2)_2$. Mono-, bis-, and trisphosphines were modeled and tested with the PM6, PM7, and RM1 methods using MOPAC as well as with Hartree-Fock methods with MINI, MIDI, and 3-21G basis sets using GAMESS. All methods were benchmarked against a training set with experimental backing. Several promising targets, with predicted air stability, have been identified.

### BIOPLASTIC: COMBINING SEAWEED AND LOBSTERS TO CREATE A NEW GENERAL CHEMISTRY LABORATORY PEDAGOGY

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Student-driven research projects in the undergraduate teaching laboratory give participants a chance to move beyond the traditional expository style experiments. This allows enhanced development of skills in areas such as experiment design, data collection and analysis, presentation, writing, and critical thinking. There have been many recent reports of multi-week and semester-long projects which either encompass existing faculty research or incorporate real world themes or problems. At Concordia College, Moorhead, Minnesota, we have worked on developing a semester-long research project for our second semester general chemistry II students based around the theme of bioplastics. The goal was to introduce students to both the theory and practice of research by providing an experience that allows students to develop a proposal, design and carry out experiments, collect and analyze their own data, and ultimately share their results with their peers. The semester-long project starts with several weeks of experiments which allow students to prepare their own bioplastic samples and then characterize them by looking at properties such as density and tensile strength. The applicability of these as drug delivery systems is also investigated. The second half of the semester allows students to personalize their project and take it in a direction of their choosing. A particular challenge in this work has been designing experiments that work with existing resources and can be carried out within the time and ability limitations of a general chemistry laboratory. This project also was given the requirement of being green and generating minimal amounts of hazardous waste.

**COMPUTATIONAL INVESTIGATION OF SUBSTITUENT EFFECTS ON THE PREDICTED AIR-SENSITIVITY OF ARYL PRIMARY PHOSPHINES**

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A variety of substituted aryl primary phosphines have been modeled using semi-empirical and *ab initio* methods. Electron withdrawing and donating groups, exhibiting both inductive and resonance effects, are examined solely in the ortho, meta, and para positions in the pursuit of aryl phosphines stabilized by electronic effects alone. The PM6, PM7, and RM1 methods in MOPAC and HF/MINI, HF/MIDI, and HF/3-21G theory levels in GAMESS were all utilized to identify probable air-stable targets. These methods were also benchmarked against an experimentally backed training set at the B3LYP/6-31G* level of theory.

**DETECTION OF HYDROGEN SULFIDE WITH A COUMARIN-BASED FLUORESCENT PROBE**

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Hydrogen sulfide ($H_2S$) is endogenously produced in the body by a few enzymes, allowing it to act as a signaling molecule. Studies of the enzymes responsible linked $H_2S$ to diseases like Alzheimer's disease, Down syndrome, and diabetes. While some estimates about the exact concentration of $H_2S$ in cells have been proposed, a way to directly, accurately, and easily test for $H_2S$ in cells has not been established. Past research has shown promise in using fluorescence to be both selective and sensitive in $H_2S$ detection. Here we are using a fluorescent molecule with a deactivating dinitrobenzene group attached to "turn off" the fluorescence. $H_2S$ will cleave the deactivating group and "turn on" fluorescence. Two fluorescent molecules are proposed, 7-hydroxy-4-methylcoumarin and 7-hydroxycoumarin, as well as two deactivating groups, dinitrobenzene and sulfonyl dinitrobenzene. Both fluorophores will be paired with each of the deactivating groups. The final probes will be evaluated for sensitivity and selectivity to $H_2S$. Excitation and emission profiles will be recorded of the "on" molecule versus the "off" molecule. Once established, increasing concentrations of $H_2S$ will be added to the probe to evaluate the relationship between $H_2S$ concentration and fluorescence intensity. The selectivity of the probes for $H_2S$ over other reactive thiols and anionic species present in living cells will also be investigated.

**EFFECT OF SURFACE TREATMENTS ON THE ELECTROCHEMICAL BEHAVIOR OF NiCr**

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The information obtained from cyclic polarization curves is very important when it comes to metal containing devices that are implanted in the human body, as metal toxicity is of great concern. Cyclic polarization is a process where corrosion information can be obtained by varying the potential applied to a metal sample while measuring the resulting amount of current that flows from the metal. Samples of nickel chromium wire (nichrome) were prepared and then processed via four different methods for electrochemical testing in phosphate buffered saline (PBS) at 37º C, conditions that mimic the ionic character of tissue fluid or blood. The purpose of this research was to determine which cleaning method resulted in a robust oxide layer; those samples would result in the lowest flow of current and have the highest breakthrough potential. The best cleaning method was as follows: two 2-minute ultrasonic cleanings, one in deionized (DI) water and one isopropyl alcohol (IPA); application of Mr. Metal, a

product that dissolves tarnish and grime and leaves a protective coating on the surface of the metal; then a passivation step in 1:8 Citrisurf diluted in DI water at 65 º C, and lastly three DI water rinses, one hot rinse at 65 º C and two at room temperature.

## THE EFFECTS OF MORPHOLOGY ON THE LUMINESCENT PROPERTIES OF UPCONVERTING LANTHANIDE DOPED PHOSPHORS

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The effects of varying the flux on the luminescent properties of $Y_2O_2S$:Yb,Er, $Y_2O_2S$:Yb,Ho, and $Y_2O_2S$:Yb,Tm materials are being investigated. These materials are upconverting phosphors crystals. When excited with 980 nm near-IR laser, these materials give off either red, green, or blue light depending on the dopant. The goal is to determine how morphology affects upconversion efficiency and emission brightness. The flux used include $Na_2SO_4$, $Li_2SO_4$, $K_3PO_4$, $K_2O_3$, $Li_2O_3$, $Li_3PO_4$, $Na_2CO_3$, in a variety of combinations. A total of 86 materials were made by varying the flux and the dopants. It is found that certain fluxes affect different emission colors more significantly than others. The material will also be characterized by X-ray diffraction, scanning electron microscopy, particle sizing, fluorescence spectroscopy, and fluorescence lifetime. Through characterization, the emission intensity of the material can be correlated to the flux effects on crystalline formation.

## EXPLORATION FOR NEW, FACILE SYNTHETIC APPROACHES TO BISPHOSPHINE MONOXIDES

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Bisphosphine mono-oxides (BPMOs) are hemi-labile ligands useful for a variety of applications. Current synthetic routes are limited, and standard oxidizers produce a mixture of dioxide and monoxide products with remaining bisphosphine reagent. Current methods used include selective, Pd-catalyzed oxidation and mono-reduction of bisphosphine dioxides using several moisture-sensitive materials. The primary goal of this project has been the discovery of simpler methods to selectively produce BPMOs in high purity. Explored methods, described herein, include using mild, organic oxidizers and using the well-established Wittig reaction to selectively oxidize one phosphorus center. $^{31}P\{^{1}H\}$ NMR and MS were used to characterize the products and determine product distributions for all attempts.

## EXTRACTION AND PURIFICATION OF SAPONINS FROM BLUE COHOSH ROOT

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Blue Cohosh (*Caulophyllum talictoides*) is a plant indigenous to North America. Traditionally its roots were used by Native Americans for a variety of medical purposes including use as a sedative, an anticonvulsive, labor induction, and burn treatment. Today, Blue Cohosh extract is still in use by midwives as a method of inducing labor with varied success. Published reports on the use of Blue Cohosh have presented conflicting results with some reporting the plant successfully induces labor contractions while others claim the root extract causes still-birthing, birth defects, and other complications to the infant. While no direct link has been made between the physiological activity and a specific compound in Blue Cohosh, it is believed that either the alkaloid

or the saponin family of compounds are responsible for the physiological effects. Previous research in our group isolated and tested the alkaloid compounds and found little to no physiological response. The saponins in Blue Cohosh root have been identified as the cauloside family, saponin PE, and leonticin D. The purpose of this study is to develop a methodology for the isolation of individual saponins from powdered Blue Cohosh root. Once isolated, these compounds will be submitted for physiological testing. Here we present the current status of this methodology including Soxhlet extraction of the crude root powder as well as the development of an LCMS protocol for the purification of individual saponins.

## GCMS DETERMINATION OF TERPENE CONCENTRATION IN THE CONTEXT OF THE MOUNTAIN PINE BEETLE

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The mountain pine beetle (*Dendroctonus ponderosae*) is an aggressive bark beetle that can kill mature pines when at outbreak levels. The insect is currently extending its western North American range and threatens to enter eastern pine forests where the response of the native trees in unknown. Here, we quantify the response of a western pine host, Ponderosa pine (*Pinus ponderosa*) and a "naïve" pine exotic species common to eastern North America, Scots pine (*Pinus sylvestris*), to challenge by the beetle-associated fungus *Grosmannia clavigera* in a common garden experiment in the Black Hills, South Dakota, where mountain pine beetle is endemic. We developed an extraction technique for monoterpenes and sesquiteterpenes, common defensive chemicals against insects and fungi, from pine phloem samples using hexane with heptyl acetate as an internal standard. Next, we generated a GC-MS methodology that was capable of separating, identifying, and quantifying approximately 32 terpenes. Finally, we compared terpene levels in phloem samples of the native host, Ponderosa pine, with the naïve host, Scots pine. Scots pine showed a larger induced monoterpene response than ponderosa pine but lower levels of 4-allylanisole, a known deterrent to mountain pine beetles. However, control data suggest induction is a more general chemical response to bark damage not specific to fungal inoculation.

## "GREENER" METHOD FOR THE SYNTHESIS OF PHOSPHINE SELENIDES

Der Vang and Joseph Kent West (Advisor)
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Various aryl, tertiary phosphines were oxidized by elemental selenium in ethyl acetate. In contrast to the standard 20-24 h toluene reflux, microwave irradiation at 170º C for 55 minutes produced the targeted phosphine selenides with high conversion rates and high purity. Products were confirmed by mp IR, MS, and heteronuclear NMR techniques. This procedure proved to be an environmentally friendlier alternative utilizing a less hazardous solvent, and it significantly reduced reaction time.

## INVESTIGATION OF FIRST ROW TRANSITION METAL COMPLEXES USING NITROGEN PHOSPHORUS HYBRID DONOR LIGANDS

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Many industrial processes use transition metal complexes as catalysts. These complexes frequently utilize rare and expensive metals such as palladium or platinum. An exciting area of research investigates the use of more abundant and cost effective first row transition metal complexes to facilitate these reactions. To this end, a new family of ligands with mixed donor atoms has been designed to stabilize low valent first row transition metals. Through the use of a β-diketiminate backbone and pendant phosphine donors, these ligands create a unique binding environment.

## ISOMORPHISM IN HETEROPENTACYCLES: CRYSTAL STRUCTURE OF A 1,2,4-OXADIAZOLE AND COMPARISON TO ITS ISOTERIC ANALOGUES

Maria Neuzil and William Ojala (Advisor)
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Benzonitrile oxides (Ar-C$^{o}$N$^{+}$-O$^{-}$ where Ar = aryl) are useful in solution-phase 1,3-dipolar cycloaddition reactions, but the solid-state chemistry of the benzonitrile oxides and their dimers has been understudied. We have now determined the crystal structure of a compound that we obtained unexpectedly while attempting to prepare a dimer of 4-nitrobenzonitrile oxide, 3,5- bis (4-nitrophenyl)-1,2,4-oxadiazole- N -oxide, by solution-phase dimerization in the presence of a catalytic amount of triethylamine. The compound proved to be 3,5-bis (4-nitrophenyl)-1,2,4-oxadiazole, not its N -oxide. Previous workers report that the deoxygenated product can form upon reaction of the initially formed oxadiazole- N -oxide with the reactant benzonitrile oxide. As part of our concurrent interest in the crystal structures of strict isosteres, chemically different molecules possessing a close atom-for-atom correspondence with respect to atomic coordinates and van der Waals radii, we are comparing our structure to those of its corresponding strict isosteres in the crystallographic literature. Our structure has proved to be isomorphous with the ambient-pressure polymorph (one of four occurring at different pressures) of 2,5- bis (4-nitrophenyl)-1,3,4-oxadiazole (NAGHOT01 in the Cambridge Structural Database). Both structures occur in space group *Pbcn*, with molecules of the published 1,3,4-oxadiazole located on crystallographic twofold axes and molecules of our 1,2,4-oxadiazole disordered about the corresponding crystallographic twofold axes. This is in notable contrast to another pair of strictly isosteric five-membered-ring heterocycles, 3,4-diphenyl-1,2,5-oxadiazole and 1,5-diphenyl-1 H -tetrazole (ZZZTQC/ZZZTQC01 and MOYLAP in the CSD), which occupy general positions and assume different molecular packing arrangements in their respective crystals. Why isomorphism is observed in one case and not the other is under investigation.

## NMR DETERMINATION OF KETO-ENOL EQUILIBRIUM CONSTANTS

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Studying the thermodynamic properties of keto-enol tautomerism is a traditional method implemented in many undergraduate laboratories. In an effort to develop a new experiment for an upper-division thermodynamics course, equilibrium constants for one β-diketone (2,4-pentanedione) and two β-ketoesters (ethyl acetoacetate and diethyl malonate) were determined by investigating their keto-enol forms with $^{1}$H NMR spectroscopy. In addition to varying the substrate, the equilibrium was investigated at several temperatures in various solvents over a range of polarities, including chloroform, methanol, and toluene.

Along with calculating equilibrium constants, a review of Gibbs Free Energy and Van't Hoff Plots was incorporated to determine if results correlate with theoretical values in the literature. The equilibrium constants calculated for the β-diketone imply enol formation in all three solvents. Equilibrium constants calculated for the β-ketoesters indicate keto formation; however, the values are much less varied. In all trials, enol formation was more favored in less polar solvents.

**NOVEL SYNTHESIS OF NEW OXAZOLIDINONE ANTIMICROBIAL AGENT**

Ashley Roux and J. Thomas Ippoliti (Advisor)
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As bacteria become less treatable with currently available antibiotics due to resistance, the creation of new antibiotics is imperative to combat infections. In an issue of Current Opinion in Microbiology, a work titled "New Oxazolidinones" shows the urgency of the matter by stating, "Due to the rapid emergence of resistance to every antibiotic, irrespective of the class or target of the drug, there is a medical need for the discovery of new classes of antibiotics." Former derivatives of Zyvox, an oxazolidinone, have been shown to be effective agents against a multitude of bacterial infections including, but not limited to, tuberculosis. It is the goal of this research to create a new oxazolidinone, Y1-3 with an amine functional group in order to increase hydrogen bonding at the activation site.

**PRIMARY PHOSPHINES: NEW SYNTHETIC METHODS AND NEW TARGETS**

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Primary phosphines, compounds of the type $RPH_2$, are notorious for their reactivity with oxygen — many exhibit pyrophoric behavior. This reactivity has hindered the development of its chemistry despite its potential utility as a synthon and its analogy to well-explored primary amines. Herein, two new approaches for aryl and alkyl primary phosphines are reported. We also report the first aryl, air-stable primary phosphine that is not kinetically stabilized by bulky *ortho* substituents. Phosphines are identified primarily by $^{31}P$ spectroscopy. Experimental air-stability observations are compared with molecular modeling predictions.

**SELF-ASSEMBLY OF PENTAMERIC MACROCYCLES THROUGH ALKENE METATHESIS OF BIS(4-VINYLBENZENE)METHYL DERIVATIVES**

Joseph A. Romo and Dennis D. Cao (Advisor)
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The synthesis of self-assembling macrocycles has recently become an area of intensified research interest. Their unique and symmetric structures enable them to encapsulate other molecules within their structural cavity and can be designed to bind with charged or neutral substrates. Our focus has been to synthesize novel macrocycles by exploiting the 109° angle of $sp^3$ carbons to direct the assembly of pentagonal shapes (interior angle of a pentagon is 108°). Di-topic alkene monomers are exposed to alkene metathesis conditions with the hypothesis that pentagonal macrocycles will be the thermodynamically favored products of the metathesis equilibrium. We are excited to report the successful synthesis of a novel aforementioned molecule that features

a pore size of about 14 Å in diameter. Herein, we present our synthetic pathway and characterization data in support of our hypothesis.

**SOLID-STATE STUDIES OF HALOGENATED BENZONITRILE OXIDES AND THEIR DIMERS**

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*University of St Thomas, St. Paul, MN*

Benzonitrile oxides (Ar-C º N +_ O -, where Ar = aryl) form three possible dimers in solution. We are investigating the solid-state chemistry of halogenated benzonitrile oxides and their dimers, motivated by the published observation that the solid-state stability of benzonitrile oxides depends on the substitution pattern; 2-chlorobenzonitrile oxide and 4-chlorobenzonitrile oxide possess reported stabilities of 3-6 days and 10 days, respectively, while the reported stability of 3-chlorobenzonitrile oxide is only 50-60 minutes. In earlier work we determined the crystal structure of the bis (3-chlorophenyl) furoxan obtained from solution and found it to be isomorphous with our previously examined bis (3-bromophenyl) furoxan. Unlike the crystal structures of many other symmetrically substituted furoxans, these structures show little evidence of twofold disorder about the approximate molecular twofold axis and no halogen···halogen approaches closer than the sum of the van der Waals radii. We have now determined the crystal structure of the bis (4-bromophenyl) furoxan to establish whether it is isomorphous with our previously investigated bis (4-chlorophenyl) furoxan or bis (4-methylphenyl) furoxan, both of which assume unique molecular packing arrangements and undergo solid-state phase transitions on heating. We have found that the 4-bromo isomer assumes a molecular packing arrangement different from those of the 4-chloro and 4-methyl isomers. Unlike its 3-chloro and 3-bromo analogues but like its 4-chloro and 4-methyl analogues, the 4-bromo structure is disordered about the approximate molecular two-fold axis. Like one of the two polymorphs of our 4-chloro structure, the 4-bromo structure lacks close halogen···halogen contacts. Whether this 4-bromo structure also undergoes solid-state phase transitions will be determined in our ongoing studies.

**SYNTHESIS AND CHARACTERIZATION OF 1,2,5,6-TETRAMETHYLNAPHTHALENE**

Nhu Nguyen and Dennis Cao (Advisor)
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Naphthalene diimide is an interesting and well-studied molecular scaffold because of its ability to undergo two separate one-electron reductions at modest potentials to reach a radical anion and dianion state. This property is fascinating because it can be leveraged in terms of battery applications as substitutes for toxic heavy metals. However, no one has synthesized or studied its tri- and/or tetra-imide derivatives. In this poster, we present the synthesis of 1,2,5,6-tetramethylnaphthalene, which is a precursor for naphthalene tri- and/or tetra-imide. Synthetic optimization and challenges will be further discussed.

**SYNTHESIS AND CHARACTERIZATION OF NAPHTHALENE TETRAIMIDE AND MELLOPHANIC DIIMIDE**

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Aromatic diimides have been shown to be chemically and thermally stable compounds that can undergo reversible redox reactions to generate radical species that intensely absorb light. This characteristic gives these molecules potential applications

in molecular electronics, optics, and spintronics. One issue with the radical anions of aromatic diimides is they are easily oxidized at low potentials in the presence of $O_2$. We hypothesize that increasing the number of imide groups on the aromatic core will help stabilize the molecule by extending the conjugation of the lowest unoccupied molecular orbital over the molecule. The target molecules for this project are naphthalene tetraimides, which have not yet been reported in the literature. My research over the past summer and fall has focused on the preparation of the naphthalene core for naphthalene diimide. Subsequently, I also worked on the synthesis of a mellophanic diimide, which is the smallest of aromatic diimides. Future work will focus on investigating the reduction potentials of the synthesized mellophanic diimide and improving the synthesis methodology for the naphthalene diimide core.

**SYNTHESIS OF A NOVEL OXAZOLIDINONE ANTIMICROBIAL AGENT**

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The selective force from the ubiquitous use of antibacterial drugs over the past century has pressured bacteria to evolve resistance to their mechanisms of inhibition. This has created a need for the rapid development of new antibacterial drugs that employ novel mechanisms of bacterial inhibition. One such innovation has been the development of Oxazolidinone antibacterials. These drugs target the ribosomes of the bacteria and inhibit protein synthesis, leading to cell death. A novel Oxazolidinone structure incorporating a benzodioxin ring system has displayed incredible potency against Mycobacterium tuberculosis and other drug-resistant strains of bacteria. This research is aimed at synthesizing a new compound with a slightly modified ring system, called a phenoxazine, that incorporates a nitrogen in place of one of the oxygens of the benzodioxin. This change will alter the electron density in the compound, hopefully increasing its effectiveness against drug-resistant tuberculosis. This molecule was synthesized using a ten-step synthesis. The compound, once successfully synthesized, will undergo testing for minimum inhibitory concentration at Johns Hopkins University against several strains of drug-resistant bacteria.

**SYNTHESIS OF Gd-DETA-MAM FOR THE RECYCLING OF PHOSPHATE**

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Excess phosphorus in lakes from fertilizer runoff causes eutrophication, which in turn promotes algal blooms. These algal blooms can pollute drinking water for entire cities. A catch-and-release system for phosphate is proposed that features Gd-complexes that selectively and reversibly bind phosphate in a pH-dependent manner. This project aims to synthesize Gd-DETA-MAM, which is derived from Gd-TREN-MAM. It is hypothesized that this complex will have improved affinity, selectivity, and release for phosphates over Gd-TREN-MAM. The rationale for this hypothesis is that a DETA backbone will have increased accessibility compared with a TREN backbone. Gd-DETA-MAM will be synthesized according to the procedures published for the preparation of Gd-TREN-MAM, with modifications to incorporate the linear backbone. The reactions will be monitored by TLC, and synthetic intermediates will be characterized via $^1$H NMR and mass spectroscopy. Upon completion of the synthesis, the phosphate-binding ability will be evaluated by measuring the relaxivity of Gd-complex solutions in the presence of phosphate ions.

**SYNTHESIS OF HO-farnesyl-OTHP**

Elizabeth Sperry and James Wollack (Advisor)
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*St. Catherine University, St. Paul, MN*

Protein farnesyl transferase (PFTase) is an enzyme that post-translationally modifies proteins by covalently attaching a hydrophobic isoprenoid group to the substrate protein in a process called prenylation. This process of prenylation allows the substrate protein to become embedded in the plasma membrane where it can function in processes such as cell signaling. PFTase is of interest to researchers due to its association with the Ras protein family, a set of small GTPase involved in the regulation of cell growth and division that are often overactive in cancer cells. Understanding the process and targets of prenylation in cells is crucial to gaining a further understanding of cancer pathophysiology. PFTase's natural substrate is farnesyl pyrophosphate (C15-OPP), a molecule consisting of three isoprenoid units covalently bound to a diphosphate. Because PFTase is a promiscuous enzyme, it can tolerate modifications to the farnesyl substrate, especially to the third isoprenoid group. Fluorescent non-natural isoprenoid analogs of C15-OPP have been used to tag prenylated proteins in biological systems. The purpose of this research to synthesize norbornene farnesyl pyrophosphate (Nor-C15-OPP). This analog of C15-OPP will undergo an inverse electron demand Diels-Alder reaction with a fluorescent tetrazine to track prenylated proteins in living cells. The intermediate HO-C15-OTHP was synthesized and purified in a two-step reaction. First, farnesol (C15-OH) was protected with THP. C15-OTHP then underwent a selective oxidation reaction using a selenium-induced 2,3 sigmatropic rearrangement. This yielded multiple alcohol and aldehyde products that were separated by column chromatography and characterized by $^{1}$H-NMR.

**SYNTHESIS OF NEW BISFERROCENYL-SCHIFF BASE COMPOUNDS AND THEIR PT(II) COMPLEXES**

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Schiff bases based on ferrocenecarboxaldehyde and several diamines have been prepared via a "green," aqueous route. These diimines have been utilized as chelating ligands, L, for the synthesis of several $Pt^{II}$ complexes of the form cis-$LPtCl_2$. $^{1}$H NMR, IR, MS, and Mp were used to confirm the successful production of all diimine ligands. These methods along with $^{195}Pt\{^{1}H\}$ NMR were used to verify all synthesized platinum complexes. Additional spectroscopic properties of ferrocenyl diimines and platinum complexes were also explored.

**TECHNIQUES FOR BETTER VISUALIZATION OF TLC**

Lul Sharif and Heather Sklenicka (Advisor)
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Thin-layer chromatography is a technique to separate mixtures on a thin layer of absorbent material such as silica gel. The components each move at a different rate. This chemistry research project concentrates on a thin-layer chromatography lab that provides insight into forensic chemistry; the goal is to find a solvent and stain for clear visualization of different pigments in lipstick. The chosen solvent needs to elute the plate in an appropriate amount of time for the two-hour lab session. It was determined 1-Butanol, the solvent in the sample lab, is not an effective eluting solvent. Using 1-Butanol as the mobile phase, the TLC plate took 45 minutes to develop. The TLC plate took 10 minutes to develop using ethyl acetate and hexane as the

mobile phase. Stains such as potassium permanganate and anisaldehyde were tested to see their ability to visualize the pigments. This lab will provide students the opportunity to attempt an engaging technique for chromatography.

**THREE-CARTRIDGE PORTABLE DIALYSIS SYSTEM**

Jasmin Bretoi and Kenneth Rohly (Advisor)
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*Bethel University, St. Paul, MN*

One of the major functions of a healthy kidney is to filter blood by separating extra chemicals, waste, and fluid from the blood as it moves through the body. In cases of complete or near kidney failure, the only available treatments are a kidney transplant or perpetual dialysis to remove these contaminants. In hemodialysis, blood is run through an artificial kidney in which excess wastes are removed. The cleaned blood is then pumped back into the body. Medtronic has developed a portable, rechargeable, three-cartridge dialysis system utilizing activated carbon, urease enzyme, and zirconium-based ion exchange materials. In the current system, large amounts of materials are used. The goal of the initial project was to see if any metals could be used to directly bind urea - one of the most prominent contaminants in the blood --- in one system and remove it without needing further processing. The binding properties of Fe2+, Fe3+, and Cu2+ with urea were studied by using UV-visible spectrometry. It was determined that these metals were unable to bind urea tightly enough for direct removal of urea to be commercially viable. A second project is now being conducted to better understand the ion-exchange properties of zirconium phosphate (ZP). By modeling this system on a small scale, ion chromatography can be used to better understand the effect of the bicarbonate and other ions on ZP.

**A THREE-STEP SYNTHESIS OF AVOBENZONE**

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Avobenzone is an important component used in many different sunscreen and cosmetic products. It is the most common organic UVA filter used in skin products. Here, a two-step synthesis of avobenzone is presented that highlights green chemistry principles. This includes a solvent free reaction, a 3-step one-pot synthesis, and the use of microwave radiation to save time and energy. The chosen synthetic route highlighted three common synthetic transformations; an Aldol reaction, electrophilic addition of Br2 to an alkene, elimination of a dihalide to form an alkyne, and hydration of an alkyne. Past research has been completed on this route, but the synthesis had not been optimized. Here, LC-MS was used extensively to access reaction progress, while NMR was used to characterize and verify that avobenzone was produced. This led to optimized heating techniques and reaction times that can be incorporated into an Organic Chemistry II teaching laboratory. Thus, an organic chemistry student can complete these reactions within a few lab periods while solidifying their knowledge of key reactions.

## USE OF C-18 SEP-PAK COLUMN with LC-MS TO DETECT MICROCYSTIN-LR IN WATER FROM CYANOBACTERA-RICH STREAMS IN ICELAND

Ange-Gabrielle M. Holm, Paula C. Furey[1] (Advisor), and James W. Wollack[2] (Advisor)
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Aquatic ecosystems are experiencing increase temperatures in response to global warming. Biota, including primary producers, will respond differently to these changing conditions. Cyanobacteria, known to thrive in warm waterbodies, can produce toxins such as the hepatotoxin, mircocystin-LR. Compared with lakes, less is known about microcystin-LR production in streams, so modified analytical methods are needed to study cyanobacterial metabolism. Water and cyanobacteria samples were collected from seven nitrogen-poor, geothermally heated streams (10-20ºC) from Iceland and analyzed for microcystin-LR. Due to the high abundance of cyanobacteria taxa, *Nostoc*, toxins were predicted to be present within the streams. To test for the presence of microcystin-LR within the stream water, liquid chromatography-mass spectrometry (LC-MS) was used. However, since microcystin-LR concentrations can be low (0.03-8 mg/L), samples were run through a C-18 Sep-Pak column to concentrate the toxin. To determine the boundaries of detection, a decreasing gradient of standard concentrations of microcystin-LR at different volumes were run through Sep-Pak purification. Preliminary results suggest that this method can detect microcystin-LR at concentrations of 4nM. Understanding when and if microcystin-LR is produced under varied temperatures will allow better predictions about how streams might respond to climate warming, especially with increase growth of cyanobacteria.

## UTILIZING PROTEIN PRENYLATION TO MODIFY EpCAM-TARGETING DARPins WITH AN AZIDE-CONTAINING ISOPRENOID ANALOG

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Protein prenylation is the enzymatic addition of isoprenoid substrates to the C-terminus of a “CAAX” peptide sequence, were “C” represents cysteine, “A” represents an aliphatic amino acid, and “X” is a variable amino acid. Design Ankyrin Repeat Proteins (DARPins) are an alternative binding scaffold that can be engineered to recognize specific targets with high affinity and selectivity. A C-terminal CVIA motif was added to the DARPins. This motif allows the DARPins to undergo protein prenylation with the enzyme protein farnesyltransferase (PFTase). PFTase naturally catalyzes the transfer of the farnesyl moiety from its natural substrate farnesyl diphosphate (FPP) to the cysteine of the “CAAX” motif. PFTase has promiscuous substrate specificity. This attribute enables PFTase to accept modifications of its natural substrate FPP. Shown here is the synthesis of a farnesyl analog functionalized with a terminal azide. This azide functionality allows for copper-free azide-alkyne cycloaddition. Utilizing this synthetic azide analog to modify the DARPins allows for the subsequent site-specific labeling with an alkyne-containing fluorophore. The DARPins used in this study were engineered to selectively bind to epithelial cell adhesion molecules (EpCAM). EpCAM is a protein that is overexpressed in the membrane of various cancer cells, including breast or pancreas tumors. By introducing these fluorescently modified DARPins, the visualization of the EpCAM in the cell membrane of these cancer cells becomes possible. This selective targeting method could be used to selectively locate cancerous cells *in vivo* when the azide is coupled with PET imaging reagent instead of a fluorophore.

### ZIRCONIUM PHOSPHATE BEHAVIOR DURING WASHING

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Zirconium phosphate (ZP) can be used as an ion exchange material due to its properties as a zeolite. In order to use ZP in biological applications such as dialysis, the behavior of the material over its lifetime must be predictable and stable. From previous work, ZP must first be washed with water and bicarbonate before the material is stable enough to be predictably used. Our research focuses on the particulars of the chemical behavior of ZP as it is being washed, answering such pertinent questions such as when and where in the washing cycles the material tends to lose phosphate and zirconium ions. By using various methods for detecting zirconium and phosphate bleed such as ion chromatography and atomic absorption, these fundamental questions have been addressed.

## ECOLOGY AND ENVIRONMENTAL SCIENCE

### ATTAINING HIGH SPECIES DIVERSITY IN PRAIRIES WITH LOW INITIAL RESTORATION INVESTMENT

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Remnant prairies are valued for their high species diversity and ability to resist non-native invaders. Research on species composition differences was conducted at Hegg Lake Wildlife Management Area (Minnesota DNR), which contains remnant prairies, restored prairies (varying species diversity), and abandoned agricultural fields. In accordance with previous research, remnant prairies had higher species diversity than either restored prairies or abandoned agricultural fields. Abandoned agricultural fields had higher overall coverage of non-native grasses with averages of more than 80% non-native grass cover. Restored prairies were found to be associated with higher overall coverage of native grasses with averages of more than 70% native grass cover. In comparison, remnant prairies contained a variety of native and non-native grass covers while maintaining high plant diversity overall. These data suggest that although restored prairies have long been thought to be a necessary step in reaching remnant-level diversity, allowing abandoned agricultural fields to self-repopulate native species while utilizing normal land management techniques (burning, grazing, etc.) may be a less expensive and work-intensive method.

### CHARACTERIZATION OF GRAY SQUIRREL (*Sciurus carolinesis*) LEAF NESTS ON AN URBAN COLLEGE CAMPUS

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Concordia College's campus in Moorhead, Minnesota, is home to two diurnal tree squirrels, gray (*Sciurus carolinensis*) and red (*Tamiasciurus hudsonicus*) squirrels. Additionally, the presence of either northern (*Glaucomys sabrinus*) or southern (*G. volans*) flying squirrels on campus was known. One factor that might serve as a limiting resource for all species of campus squirrels could be nesting sites, particularly over the winter months. Squirrel nest characterization of 27 leaf nests was conducted on Concordia College campus. On average, there were 1.2 leaf nests found per tree that possessed a nest. Average

nest height was 9.1 meters and average branch coverage was 72.5%, which is also indicative of total leaf coverage during the spring and summer months. A majority of leaf nests were found in *Ulmus americana* (American elm) trees across campus. Average DBH values for trees with leaf nests were statistically larger than those without, indicating that squirrels are nesting in larger and more mature trees. Spatial mapping and analysis of squirrel leaf nests on Concordia College campus was also performed. Thirteen trapping sessions over a two-month period captured several gray and red squirrels, as well as the first capture of a northern flying squirrel on campus.

## COMPARISON OF SMALL MAMMAL COMMUNITIES ON RESTORED AND REMNANT PRAIRIES IN NORTHWESTERN MINNESOTA

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Prairies are one of the most endangered habitats in North America. There have been numerous restoration efforts of prairie habitat by different private and state organizations in an effort to support and protect native prairie species. The effects of these restored prairies on small-mammal communities are not well studied. We trapped small mammals on various restored and remnant prairies in northwestern Minnesota during the summer of 2017 (May-July), adding to the ongoing dataset from previous years. We live-trapped sites in Becker, Clay, Mahnomen, and Norman counties. The dominant species caught included *Microtus pennsylvanicus, Peromyscus* spp., and *Ictidomys tridecemlineatus;* rare species included *Myodes gapperi, Zapus hudsonius, Sorex* sp., and very rarely *Blarina brevicauda*. It appears through data collected that restored and remnant prairies in this area have similar patterns, though proximity of restored sites to remnant sites influences diversity.

## EFFECTS OF LITTER SIZE AND GENDER IN JUVENILE EASTERN CHIPMUNK (*Tamias striatus*) SURVIVAL

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There are many different factors that contribute to an animal's survival and these differ based on both location and species. Eastern chipmunks (*Tamias striatus*) are a diurnal model species common in Minnesota. Juvenile eastern chipmunks' overall survival can be influenced by maternal investment, environmental challenges, predators, and parasite load. We questioned if litter demographics also impact juvenile survival. Using Tomahawk traps and radio telemetry, we studied the survival of juvenile eastern chipmunks in northern Minnesota. We looked at litter size and gender to determine if these factors influenced the survival of the juveniles. Between 2015-2017, 16.3% of eastern chipmunk juveniles (n = 92) survived to hibernation. Neither litter size (mean = 5.7 juvenile/litter, n = 10; r = -0.27, p = 0.45) nor gender (females = 26.3%, n = 38; males = 11.1%, n = 54; X 2 = 2.609, p = 0.106) were significant determinants in juvenile survival. Known mortality causes include martens, weasels, raptors, and parasites. Litter size was larger than the average size reported elsewhere. Females had only one litter per season and survival based on litter size was similar to that found in ground squirrels. Predator pressure was similar for male and female juveniles.

## FINDING FOSSILS AND THE PALEOENVIRONMENT OF MONTANA'S HELL CREEK FORMATION

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The purpose of our research was to gain insight on the effect that erosion has on the movement of fossil material in sediment. Soil matrices were dried, washed, and filtered to find and identify basic characteristics of fossil material present in the sediment samples. Through the information obtained from these samples we can infer basic traits about species, population densities, and geologic characteristics of the ecosystem. Fossil movement and sediment erosion is a small part of the research being done on the paleoenvironment of the Hell Creek formation in northeastern Montana. Instead of focusing on a particular organism that existed during the Cretaceous period, our research seeks to develop a more holistic idea of a past environment and the community of organisms that existed together at the time.

## IDENTIFYING *Peromyscus leucopus* AND *P. maniculatus* USING SALIVARY AMYLASE

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The white-footed mouse (*Peromyscus leucopus*) and deer mouse (*P. maniculatus*) are two common small mammals in North America. The two species are ecologically important because they serve as controls for insect pests, as vectors and reservoirs for disease, and as environmental indicators of climate instability. Therefore, techniques for distinguishing between the two species are important for monitoring biological control, disease regulation, and climate change. Typically, morphological characteristics have been used to distinguish between *P. leucopus* and *P. maniculatus,* but these measurements have been inconsistent and unreliable due to the substantial overlap between these two species. However, one reliable and novel laboratory technique is cellulose acetate electrophoresis of salivary amylase, which is possible because of the distinct allozymes in *P. leucopus* and *P. maniculatus*'s saliva. Our research is focused on using the cellulose acetate electrophoresis technique to differentiate between *P. leucopus* and *P. maniculatus* from sites throughout northwestern Minnesota between 2004 and 2017. The objective of this research is to definitively identify *Peromyscus* to species in a region with high habitat overlap using salivary amylase identification. Further analysis will compare biochemical results with the commonly used morphological data and cranial characteristics.

## ITASCA IN A BOTTLE: UNDERSTANDING THE ROLE OF BACTERIA IN WOOD DECOMPOSITION REACTIONS

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Every time a tree falls, different fungal and bacterial decomposers begin to break down the wood. The overall goal of breaking down plant material for growth for each individual colony is similar across most species. However, the mechanism by which each of these decomposers function varies greatly. Brown and white rots make up the category of fungal decomposers and differ in the way they break down wood. Brown rot produces soil which is free of carbohydrates while circumventing lignin. White rot, on the other hand, can break down lignin to access sugars. Both fungi are common in northern forests and have various factors which contribute to the type of tree they colonize. Another key contributor in the process of decomposition is bacteria. Although the bacteria associated with decay work along with fungi, bacteria do not typically produce lignin-degrading

enzymes and generally produce very low activities of the enzymes required to decay wood. We have learned in the last few years that 'cheater' bacteria in systems like wood can live productively by stealing sugars from decay fungi, notably brown rot fungi that are 'loose' with their mechanisms. We hypothesized that fungal productivity is limited by bacteria, because of their ability to steal unprocessed sugars. Furthermore, we hypothesized that different bacteria will have a different effect on fungal decomposition. In this home and away study, we measured the decomposition productivity of two brown-rot and two white-rot fungal species in the presence of familiar bacteria versus those found with the opposite fungal group.

**LEAD CONTENT IN THE LIVERS OF SOUTHEASTERN MINNESOTA MALLARDS (*Anas platyrhynchos*)**

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Lead shot deposited into water systems by hunters has plagued waterfowl and related predators. Figures from a 1978 study estimate that 2-3 million waterfowl die worldwide annually from lead poisoning either by the direct toxicity of the lead or secondary effects, which facilitate the predation of the infected individual (Levy and Kelly, 1978). A federal ban enacted in 1991 made using lead shot for waterfowl hunting illegal in an effort to protect the bald eagle, a common predator of waterfowl contaminated with dangerous amounts of lead. Since the ban, little follow-up research has been conducted. The objective of this research was to obtain liver samples of mallards (*Anas platyrhynchos*) harvested in Southeastern Minnesota and analyze them for the presence of lead. Digesting the soft organ in acid and analyzing the samples with an atomic absorption spectrophotometer provided a mean concentration of lead from the mallards to compare with statistics collected by similar studies to help determine if the amount of lead consumed by waterfowl had changed since the ban. The results of this experiment will help determine the effectiveness of the federal action and the current state of waterfowl in southeastern Minnesota.

Levy, R.A and J.E. Kelly III. 1978. Preventing Waterfowl Poisoning. Environment 20:25-30.

**SELECTIVE TREE USAGE IN BARK-FORAGING BIRDS: IMPLICATIONS FOR EMERALD ASH BORER**

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Emerald ash borer (*Agrilus planipennis* Fairmaire), a wood-boring beetle native to Asia, has been the cause of widespread ash (*Fraxnius* spp.) mortality across North America. Emerald ash borers (EAB) have faced virtually no population checks with the possible exception of bark-foraging birds (e.g. woodpeckers) which may consume large numbers of EAB from infected trees. However, relatively little is known about the point in the infestation at which the birds alter their foraging preferences. We studied the EAB infestation and the behavior of bark-foraging birds from 2014-2018 in east-central Minnesota, an area with a relatively recent EAB presence. We quantified the level of EAB infestation using primary D-shaped exit holes and EAB galleries) and secondary indicators (crown condition, bark splitting, etc.) and examined the amount of time birds spent foraging on various tree species. We used a chi-square "goodness of fit" test to determine if birds showed a preference for foraging on any tree species. None of the birds showed significant preference towards foraging on ash over other trees. Additionally, the secondary indicators commonly used to identify EAB did not seem to provide conclusive evidence of EAB presence, and thus, we were unable to conclusively determine infestation levels. Our results support the suggestion that an adaptive lag must take place following the initial infestation in order for bark-foraging birds to learn to recognize EAB as a food source.

**TREE GROWTH AND MORTALITY IN A 27-YEAR-OLD MAPLE-BASSWOOD FOREST RESTORATION PROJECT**

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Maple-basswood forests in southeastern Minnesota have declined as land has been increasingly used for agriculture and urbanization since the late 1800s. Restoring these forests helps preserve high levels of biodiversity and facilitates ecosystem resilience to environmental changes. As part of the development of the Natural Lands at St. Olaf College in Northfield, Minnesota, a forest restoration project was started in 1990 on two former agricultural fields. In order to better understand growth patterns in young trees and to increase the effectiveness of restoration projects, over 1,500 trees, including original and colonizing trees, were measured for tree diameter and height in sixteen 0.1-ha transects, divided into four sections. The specific objectives of the study were to (1) compare tree size and mortality patterns among species over time, (2) measure soil physical and chemical characteristics, and (3) make projections about future changes in the forest. The restoration project is dominated by white ash (32%), with black walnut (16%), bur oak (11%), white oak (8%), and sugar maple (5%) being the next most common. Sections one and two had trees with the largest mean diameters, led by red oak (15.6 cm), white oak (14.3 cm), and basswood (11.3 cm), while sections three and four had higher levels of moisture and organic matter, as well as higher levels of mortality. The trees are growing steadily and properties of soil such as moisture and organic matter are increasing throughout the restoration site, indicating that this restoration project is on a healthy trajectory.

**UNDERSTANDING THE RELATIONSHIPS BETWEEN GRAIN YIELD AND POTENTIAL, REALIZED GRAIN YIELD, AND THE ENVIRONMENT USING MAIZE AS A MODEL SYSTEM**

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As a crop plant, maize has seen consistent yield improvements, yet the ceiling for grain yield has not been reached. Further, extreme and unpredictable environmental conditions are resulting in increasingly negative impacts on realized grain yields. To meet the demands for increased crop production in the face of adverse environmental conditions, it is imperative that the mechanisms that drive both yield potential (i.e. the genetic potential of an individual under ideal conditions) and the realized yield (i.e. the yield that results from the interaction of the individual's genome and its growing environment), as well as their relationship with the growing environment, are understood. Phenotypic data are important in evaluating many of these yield component traits. This project aimed to analyze the accuracy of an algorithm designed to extract phenotypic data from high-resolution images. Hand measurements would need to be taken to create the thousands of data points the algorithm extracts, taking both time and labor resources. To evaluate algorithm accuracy, hand measurements of ear and cob circumference, kernel depth, and number of kernels were taken from images of the cross sections of unpollinated ears of corn. The measurements were taken using the imaging platform ImageJ and then evaluated against the data points extracted by the algorithm. It was determined through this project that the algorithm's accuracy is not yet at the desired standard. It is suggested that the algorithm be adjusted and re-evaluated using the hand measurements taken throughout this project.

# ENGINEERING AND PHYSICS

## CRANIAL PROSTHESIS FOR CHRONIC, PAN-CORTICAL TWO-PHOTON IMAGING IN BEHAVING MICE

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Chronic long-term imaging of neurons has become increasingly important in recent years to understand and comprehend how information is encoded in the central nervous system at the single and population level. There have been several technological strides made to help neuroscientist visualize spontaneous and stimulated neuron firing but have been limited to small regions of observation. To address the limitations of current chronic cranial imaging window preparations, we have developed 3D printed morphologically realistic, transparent cranial prostheses that allow cellular resolution optical access to the entire dorsal cortex. We have validated the ability to implant these 3D printed cranial implants for long durations, and preliminary experiments indicate a capacity to perform two-photon (2P) imaging in the chronically implanted Thy1-GCaMP6f mouse and detect Ca2+ activity in individual neurons. The implanted mouse was trained in motorized disc treadmill, while simultaneously carrying out in vivo optical 2P imaging. Future work includes extending 2P to the whole dorsal cortex and histological analysis to assess the inflammatory response of chronic implantation. In addition, we plan to use this technology to learn mesoscale and cellular scale activity of defined cell populations in an animal model. The tottering mice (tg/tg) are a mouse model used to study Episodic Ataxia type 2, a disease caused by a mutation in the CACNA1A gene and exhibit several characteristics, including transient motor ataxia, which can be triggered by stress, caffeine, and alcohol.

## DEVELOPMENT AND TESTING OF AUTOMATED ELECTROMECHANICAL ICE THICKNESS MONITORING SYSTEM

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The goal of this research project was to build an electromechanical device that measures and monitors the thickness of ice. The device, operated by an Arduino Uno, uses a motor to raise and lower magnets to find the underside of the ice. The magnets are enclosed in a PVC pipe that is submerged in water and held in place by the ice. Around the outside of the pipe is a moveable metal ring. As the motor raises the magnets up through the pipe, the ring is lifted up the outside of the pipe. Using a current detector, the system can find the bottom of the ice by detecting an increasing or decreasing current through the motor. Either the motor will stall as the metal ring hits the underside of the ice causing an increase in current or the ring will be pushed off the magnets by the ice causing a decrease in current. An encoder attached to the motor records the position of the magnets as the ring hits the bottom of the ice. The thickness of the ice is stored on a secure digital card along with the date and time of the measurement.

**FEASIBILITY OF PELTIER CHIPS AS THERMOELECTRIC GENERATORS ON HEATSINKS**

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As the demand of computer processing grows, the burden placed on CPUs will increase as well. Computing systems produce heat, which is attracted to a metal heatsink where it is dissipated out of the system by a fan. Liquid cooling can be more effective but is more expensive and increases the risk of damaging leaks. Peltier chips, electronic components which transfer heat into electricity, can be used in an effort to reclaim and reuse some of this waste heat. This experiment tested twelve heatsinks of various designs to maximize the effect of the Peltier chip. Variations in designs of the heatsinks allowed for inspection of designs which are not commonly seen in computer heatsinks to evaluate previously untested heatsink designs for use with Peltier chips. Coupled with collected data, this allowed for inspection of both quantitative and qualitative factors which govern the magnitude of the output voltage from the Peltier chip. In addition, five configurations of the heatsink, Peltier chip, and fan were tested, searching for arrangements that yield higher voltages at the lowest possible temperatures. Data showed that while voltages can be increased by various methods, the same methods have costs which render their widespread use unrealistic. Heatsinks should be redesigned to accommodate Peltier chips and avoid wasted energy. Improvements in heatsink designs were proposed which may help increase the output voltage.

**LOCALIZED SURFACE PLASMON SPECTROSCOPY ON SELF-ASSEMBLED AU@SILICA–PD HETERODIMERS**

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Localized surface plasmons (LSPs) are collective oscillations of electrons along the surface of a metal nanoparticle in response to incident electromagnetic waves. The wavelength that maximizes LSP excitation is known as the LSP resonance (LSPR) and is sensitive to nanoparticle size, shape, composition, and local electromagnetic environment. The later enables spectroscopic study of physical and chemical changes near the nanoparticle surface with single-particle resolution using localized surface plasmon spectroscopy. The plasmonic particles used in this work are gold nanorods (GNRs, length: 74 ± 6 nm, width: 21 ± 1 nm) with an LSPR at 777 nm to maximize sensitivity toward palladium hydrogenation. Coating the GNRs with a thin silica shell (6 ± 1 nm) provided an anionic surface charge that allowed self-assembly with cationic CTAB-ligated palladium nanocubes (13 ± 4 nm, 41 ± 5 nm, and 66 ± 7 nm). Particles were imaged with scanning and transmission electron microscopy (SEM and TEM). Dark-field scattering spectra were taken with a custom in-house setup and correlated with dark-field microscope and SEM images. After further tuning the self-assembly to increase dimer yield, the results presented will enable a large-scale study of how heterogeneities across individual palladium nanoparticles affect bulk properties of the larger ensemble. Understanding this is essential for fully realizing the potential of palladium nanomaterials in catalysis in energy storage.

## A MATHMATICAL MODEL OF SOLAR ENERGY, TEMPERATURE, AND ALTITUDE: AN APPLICATION FOR HIGH-ALTITUDE BALLOONS

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One important application of solar panels is the powering of vehicles or payloads in outer space. Weather balloons can carry payloads to spacelike conditions in the stratosphere at much lower cost and can serve as testbeds for solar-powered systems. At stratospheric altitudes from 60,000 to 120,000 feet, solar panels are exposed to increased sunlight but temperatures that can reach as low as -60°C. Both reduced temperature and increased exposure of sunlight result in increased production of power from solar panels. The purpose of this project is to experimentally determine the mathematical altitude-dependent relationship between temperature, sunlight intensity, and the amount of power produced by solar panels during stratospheric balloon flights. First, a prototype box was constructed to examine solar panel power production under decreasing temperatures with stable lighting conditions. Next, a payload box was constructed which compared the production of power from solar panels at increasing altitudes during a balloon flight in both constant-temperature (i.e. actively-heated) and decreasing-temperature (i.e. exposed) conditions. Nonlinear fits were used to characterize the relationship between the production of power and the altitude from the solar panels. The results demonstrate that the relative impact of changes on solar panel power from temperature and changes in light intensity can be discerned from one another. This project allowed for a better understanding of the correlation between altitude and the production of power from solar panels and may allow for solar-energy-powered payloads on future stratospheric balloon flights.

## NOVEL SHELL-LESS CHICK EMBRYO CULTURE VESSEL FOR THE APPLICATION OF NEURONAL TISSUE ENGINEERING

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This project focuses on improving previously published shell-less culturing methods to better sustain ex ovo chick embryo development. Specifically, the culture vessel backbone was redesigned and custom made with a 3D printer to allow a better surface area for chorioallantoic membrane manipulation as well as experimental observation. Next, to provide needed air permeability and oxygen supply, a thin layer of air permeable polymer mixed with oxygen-releasing chemical was applied as supporting sidings to the vessel. Our results show that this new vessel is able to support chicken embryo development past embryonic day 17 without the need for extra oxygen gas supplementation and provides a repeatable setup for ex ovo chick embryo model.

# MICROBIOLOGY AND IMMUNOLOGY

## COMPARISON OF ANTIMICROBIAL PROPERTIES OF THE CIS AND TRANS ISOMERS OF DICHLOROBIS(ETHYLENEDIAMINE)COBALT(III) CHLORIDE

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Research has shown that compounds containing heavy metals such as copper, silver, and cobalt are capable of inhibiting the growth of microorganisms. This phenomenon is known as the oligodynamic effect of heavy metals. The atomic configurations of these compounds can be both cis and trans isomers. The purpose of this study was to determine whether the cis and trans isomers of the compound dichlorobis(ethylenediamine)cobalt(III) chloride could inhibit the growth of the gram-positive bacterium *Staphylococcus aureus* or the gram-negative bacterium *Escherichia coli*. To do this, bacteria were incubated with varying concentrations of cis or trans dichlorobis(ethylenediamine)cobalt(III) chloride, and bacterial growth was measured. This was done to determine (1) whether cis or trans dichlorobis(ethylenediamine)cobalt(III) chloride could inhibit bacterial growth and (2) the minimum concentration of the metal compounds that could completely inhibit bacterial growth. Preliminary results suggest that the cis isomer of dichlorobis(ethylenediamine)cobalt(III) chloride inhibited the growth of both bacteria, but little to no inhibition of growth was observed when the bacteria were incubated with the trans isomer. This would suggest that cis-dichlorobis(ethylenediamine)cobalt(III) chloride could be an effective antimicrobial agent.

## DEVELOPMENT OF CONJUGATE VACCINES FOR TREATMENT OF OPIOID ABUSE USING *E. coli*-EXPRESSED CARRIER PROTEINS FOR GMP MANUFACTURING AND SCALE-UP

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In the United States, 2.6 million people are dependent on heroin and prescription opioids leading to greater than 50,000 opioid-related fatal overdoses in 2017. Although safe and effective medications are available, only 1 out of 5 opioid addicts benefits from pharmacotherapy. To address this unmet medical need, our group has developed a series of vaccines against heroin and prescription opioids. Vaccines containing opioid-based haptens conjugated to keyhole limpet hemocyanin (KLH) elicit high concentration of high-affinity opioid-specific serum IgG antibodies that block opioid distribution to the brain, and reduce opioid-induced behavioral effects in mice and rats. Lead vaccines do not interfere with endogenous opioids or prevent the use of opioid-based medications. We are currently seeking FDA approval to conduct clinical evaluation of two lead vaccines against heroin and oxycodone abuse.

To ensure vaccine translation, our efforts have focused on GMP components and qualifying assays compliant with FDA release criteria for conjugate vaccines. In this study, we tested and characterized opioid vaccines consisting of an oxycodone-based hapten (OXY) conjugated to several carrier proteins. The leading vaccines consisted of the OXY hapten conjugated to the E. *coli*-expressed (Eco) diphtheria cross-reactive material (EcoCRM™) and nontoxic tetanus toxin fragment (rTTHc). These immunogens effectively induced expansion of hapten-specific B cell lymphocytes, and generated oxycodone-specific serum IgG antibodies, which reduced oxycodone distribution to the brain in mice.

In contrast to KLH, unconjugated and conjugated EcoCRM™ and rTTHc are easily characterized by SEC-HPLC, DLS, and MALDI-TOF. Opioid vaccines containing EcoCRM™ and rTTHc are candidate immunogens for further development.

### STUDYING POLIO TITERS IN INTERNATIONALLY ADOPTED CHILDREN

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Background: Polio is an infectious disease that affects the nerve cells and can lead to paralysis. We analyzed trends of polio-neutralizing antibody levels in internationally adopted children, stratified by region to see if socioeconomic status affected vaccination response. We hypothesized that countries of lower socioeconomic status will have children with a lower likelihood of polio immunity.
Methodology: We included international adoptees that completed their adoption screening at the Adoption Medicine Clinic, University of Minnesota. 215 participants were screened between January 2000 and December 2016. Socio-economic factors from the Human Development Reports database were used to analyze adoptees by gross national income, multidimensional poverty index, life expectancy, under-5-year-old mortality, and inequality in income.
Results: Children from Latin America/Caribbean had 0.215 times the odds ratio of immunity to Polio 1 compared with children from Asia in the adjusted odds ratio. There were no significant relationships with any of the socioeconomic indicators; all had a *p* value greater than 0.5.
Conclusions: Generally, children from Asia had a stronger immune response to all Polio vaccination types than those from Sub Saharan Africa and Latin America/Caribbean. The most important trend is the independent predictor of titers in children from Latin America/Caribbean vs. Asia. Both the adjusted and unadjusted odds ratio find that children from Asia have better immunity to Polio 1 than those from Latin America/Caribbean. Surprisingly, there was no significant correlation to show that specific socioeconomic indicators affect Polio immunity. Further testing with larger data sets is necessary.

## ORGANISMAL AND PHYSIOLOGICAL SCIENCES

### CONTRACTILE EFFECTS OF BLUE COHOSH ON *Mus musculus* DISTAL COLON

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Herbal medicine has been utilized in midwifery for hundreds of years, with little exploration of the mechanisms involved. Blue cohosh (*Caulophyllum thalictroides*) has recently been investigated for its pharmaceutical value to obstetrics, but not its systemic effects on other types of smooth muscle. The investigative goal of this project was to measure the contractile effects of blue cohosh and constituents N-methylcytisine, magnifoline, and saponins on isolated smooth muscle strips of *Mus musculus* distal colon. Contractile forces, frequencies, and changes basal tone were measured in an organ tissue bath. Magnifoline caused a concentration-dependent statistical increase in contractile force, while blue cohosh caused an increase in contractile force, frequency, and basal tone. Both saponins and N-methylcytisine increased smooth muscle motility; however, they were not statistically significant as compared with the tissues endogenous motility. N-methylcytisine and magnifoline have been proposed to act on cholinergic receptors, while saponins as a glycoside created pores to allow calcium influx. The increase in contractile force by blue cohosh would suggest that colonic smooth muscle may be active if blue cohosh were administered as an herbal supplement to induce labor.

## CONTRACTILE RESPONSE OF *Mus musculus* STOMACH TISSUE AFTER APPLICATION OF BLUE COHOSH AND TWO OF ITS CONSTITUENTS

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Blue cohosh is an herbal supplement that has been shown to evoke contractile responses in smooth muscle tissue. Since blue cohosh *Caulophyllum thalictroides* is used commonly to induce labor amongst Native American and midwife populations, this grants the question of what effect blue cohosh has on peripheral tissues surrounding the uterine tissue. This study considers the contractile response of *Mus musculus* stomach tissue to blue cohosh and two of its constituents, saponins and N -methylcytisine. The drugs were administered in vitro to dissected stomach tissue, strung to a force transducer in an organ bath. The contractile responses were recorded using a force transducer and the computer program Lab Chart. It was shown that these drugs did result in significant contractile responses when compared with their resting spontaneous motility. Compared with the contractile responses of uterine tissues, the responses from the stomach tissue were significantly smaller. An interesting observation from this study was that the sum of the average contractile forces for the two constituents was almost equal to the average contractile response from blue cohosh. These data imply that these two constituents tested are responsible for most of the contractile responses seen in smooth muscle tissue following the application of blue cohosh.

## IS *Phytopythium boreale* A PATHOGEN OF SOYBEANS?

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*Phytopythium boreale,* recently isolated from soybeans in Minnesota, is a member of a newly identified genus of oomycete, *Phytopythium*. Morphologically intermediate to the pathogenic oomycete genera *Phytophthora* and *Pythium,* the interaction of *Phytopythium* spp. with soybean plants is not understood. Sometimes referred to as water molds, the oomycetes are eukaryotic microorganisms that are often damaging to soybeans at low soil temperatures (*Pythium* spp.) or in saturated soils (*P. sojae* and *Pythium* spp.) The objective of this study was to determine the interaction of *P. boreale* with soybean. We evaluated the effects of *P. boreale* inoculation on soybean development during germination and early vegetative growth. When inoculated with infested PDA (potato dextrose agar), root growth of germinating seed increased 17.4%. Seedling growth and root development decreased 9.4% and 2.6%, respectively. Further studies are necessary to determine the effect of *P. boreale* on soybean plant development and yield. These studies should consider the effect of *P. boreale* infection on later stages of plant development and consider a range of inoculum concentrations and differing environmental conditions such as soil temperature and soil saturation.

## RICE-INOCULUM DECOMPOSITION AFFECTS VARIETY RESPONSES IN SOYBEAN RESISTANCE TRIALS

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The addition of rice infested with selected plant pathogens, e.g. *Pythium* sp., to soil or vermiculite is a technique used to incorporate pathogens into the plant rhizosphere. The rice inoculum method enables screening of large numbers of soybean genotypes with more than one pathotype of the pathogen in a single assay. Knowing the effect of the rice by itself on the plant is essential to ensure that the observed symptomatology is a result of the organism in question. The objective of this research

was to determine the effect of rice substrate on the early growth of soybean varieties and whether soybean varieties differed in their response to the substrate. In previous studies, soybean seedlings were injured, and growth was often reduced in the presence of uninfested rice. Three soybean varieties were subjected to four treatments: (1) vermiculite only, (2) low soil pH, which often develops in the presence of uninfested rice, (3) uninfested rice, and (4) a *Pythium*-infested rice treatment. Root growth was reduced when inoculated with *Pythium,* in the rice-only treatment, and in the low pH treatment. The response differed by variety. The results indicate that the decomposition of rice inoculum affects soybean, and that varieties differ in their sensitivity to rice breakdown products. Plating of uninfested rice from the experiment yielded bacterial and fungal colonies, indicating that contaminating microorganisms caused decomposition of the rice substrate.

**SHIFTS IN ANTIMICROBIAL GENE EXPRESSION IN MOUSE MODEL OF SELF-ANTIGEN-DRIVEN INFLAMMATORY BOWEL DISEASE**

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are inflammatory conditions affecting the gastrointestinal tract. IBD may be caused by the failure to tolerate intestinal self-antigens or antigens within the microbiota. In this study, we investigated whether self-directed autoimmune responses lead to changes in the expression of antimicrobial genes in the small intestine. Such changes could lead to alterations in the host-microbe interaction and abnormal shifts in the microbiota, worsening the disease. To investigate this question, sections of distal small intestine were obtained from a newly developed mouse model of spontaneous IBD. In these mice, a TCR transgene drives CD4+T cells to become specific to a small intestinal antigen, leading to severe inflammation and weight loss. RNA was purified from biopsies of pre-disease animals and was reverse transcribed to cDNA. Antimicrobial gene expression among IBD-prone and control animals was determined via qPCR. Interestingly, no statistically significant differences between genotypes for the expression of the antimicrobial genes Crypt1, pLYS, or mLYS have been found. This suggests that early localized immune responses to intestinal self-antigens may neither alter the expression of antimicrobial genes nor contribute to subsequent abnormalities in microbial colonization. Future studies will focus on additional antimicrobial genes and mice later in disease development.

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