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UNDERGRADUATE SYMPOSIA ABSTRACTS

Abstracts are listed alphabetically by the last name of the first author listed.

SELECTIVE RECOGNITION OF 2,4-DINITROTOLUENE

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2,4-Dinitrotoluene (DNT) is an ubiquitous impurity in 2,4,6-Trinitrotoluene (TNT). If we were able to detect trace amounts of DNT we would effectively be able to detect TNT and therefore test for remnants of explosives which contained TNT. To do this, a molecule that selectively binds DNT would need to be found and successfully integrated into a gas phase sensor. We designed a molecule to selectively bind DNT in solution. My work involved synthesizing this molecule through multi-step synthesis and also determining what solvents dissolve this molecule and DNT. NMR tests determined that the product is only soluble in dimethylsulfoxide (DMSO) which is problematic because the DMSO will saturate the binding sites on the molecule if it is the main solvent, making it unable to bind to DNT. Tests of various combinations of solvents with DMSO showed that DMSO and chloroform is the best combination of solubility and low DMSO concentration for further testing of the molecule's binding strength to DNT. Preliminary NMR results show that binding does occur. This solvent ratio will be used to collect data to be analyzed in Mathematica to find the binding curves and from these curves the binding constant between the synthesized molecule and DNT.

INCENTIVE SALIENCE VERSUS RATS SELECTIVELY BRED FOR HIGH AND LOW SACCHARIN PREFERENCE: A MODEL FOR DRUG ABUSE VULNERABILITY

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Drug addicts show a higher preference for sweetened substances compared to non-addicts. This relationship between sweet preference and addiction vulnerability has been modeled with rats selectively bred for high (HiS) and low (LoS) saccharin intake. One question central to research on addiction pertains to the mechanism driving these phenotypic behavioral differences. Incentive salience is a phenomenon in which contextual stimuli associated with rewarding events begin to function as conditioned stimuli and elicit approach and contact behaviors, and drugs of abuse are thought to accelerate this process. Incentive salience has been modeled with animals with a procedure in which the extension of a lever acts as a cue that is immediately followed by the presentation of a non-contingent food pellet. A percentage of rats exhibit incentive salience by displaying sign-tracking (ST) behavior in which they approach and bite the lever, while other rats show goal-tracking (GT) behavior in which they approach the food delivery receptacle during lever extension. Importantly, the ST rats show greater drug-seeking compared to GT rats. Thus, in this experiment, we examined ST vs. GT behavior in HiS and LoS male and female rats to determine whether rats selectively bred to prefer sweet substances also show elevated incentive salience. Results indicated no differences between ST or GT behaviors in HiS vs. LoS rats; however, females showed more ST and drug seeking than males, suggesting that sex may mediate the incentive salience process and may contribute to our understanding of sex differences in addiction vulnerability.

NATIVE AND EURASIAN PHRAGMITES AUSTRALIS TOLERANCE TO CACL2 AND MGCL2 SALINITIES

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Phragmites australis (*Phragmites*) is a perennial, aquatic grass with world-wide distribution. In invasive Eurasian strain (Haplotype M) has been documented in Minnesota in recent years, but has only been detected in road ditch environments. One hypothesis for this apparent habitat preference is that the salt tolerance of Haplotype M *Phragmites* provides a selective advantage over less tolerant native strains. The Eurasian haplotype M has been shown to be more tolerant sodium chloride in wet soils than the native North American haplotypes. However, the relative tolerance to other common deicing salts such as calcium chloride and magnesium chloride has not been documented. In this study, we grew native and Eurasian strains of *Phragmites* in varying concentrations of calcium chloride and magnesium chloride treated soils to determine whether or not tolerance difference exist between the strains.

EXPLORATORY DEVELOPMENT OF PROCEDURES CONDUCIVE TO THE INVESTIGATION OF Sn WHISKERING

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The formation of sharp spikes on tin on copper substrates is a phenomenon called tin whiskering. In order to investigate the mechanism of formation of tin whiskers, various electroplating and soldering techniques were explored in order to prepare Sn-plated Cu substrate samples. Interface difficulties were encountered in the effort to electroplate Sn onto Cu, which was expressed by Sn unevenly collecting in tree formations over the faces of the Cu coupons and in long needle-like projections at the coupon edge. Efforts to mitigate patchy plating of Sn by utilizing a 1:4 dilution of Sn(II) methanesulfonate electrolyte or by shrinking the diffusion barrier using an ultrasonic bath or both were unsuccessful. Yet, the preparation of a 1:4 diluted electrolyte with 1% vanillin added was found to substantially improve even coverage of Sn plating. The soldering methods used in this study to plate Sn onto Cu also presented interface difficulties in that Sn could not be adhered directly to the Cu coupon surface with sufficient even coverage. In addition, use of a soldering gun demonstrated a lack of control over layering thickness of the Sn onto the Cu surface, which was essential for the purposes of these experiments. The improvements in Sn adhesion of Cu accomplished through the vanillin additive to the electrolyte presented a better surface for observation of Sn whisker growth.

IDENTIFYING THE CORE PROMOTER REGION OF RAP1 GENES USING RT-PCR

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The Rap1 genes, closely related to the Ras oncogenes, have been shown to have numerous functions, including qualities as tumor-suppressor genes. While previous research has provided clues to Rap1's roles in cells, nothing is known about the genes' "core" promoter regions—the minimum nucleotide sequence at or near the Transcription Start Site (TSS) that will initiate transcription. Using bioinformatics, previous research located putative promoter regions of the human Rap1A and Rap1B genes, and plasmid vectors were constructed containing these sequences upstream of a gene for green fluorescent protein. We transiently transfected nine such constructs into a human cell line (HEK-293), lysed the cells, and purified their total RNA. Western blotting and fluorescent microscopy had been able to offer qualitative clues about the proficiency of the putative promoters, but Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) has now allowed us to make quantitative functional comparisons. We have successfully identified functioning promoter fragments for both human Rap1A and Rap1B genes; of special interest is the identification of a 142-base-pair segment upstream of Rap1A that has been shown to significantly hinder transcription. Presently, we are working to confirm this finding by engineering this regulatory DNA segment in front of known, functioning promoters and examining subsequent changes in transcription. By finding the "core" promoter, this project is beginning to answer the question of how Rap1 gene expression is controlled in human cells.

MEASUREMENT OF REGULATORY T CELL ACTIVATION USING FLOW CYTOMETRY

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The purpose of this investigation was to study CD4+CD25+Foxp3+ regulatory T cells to determine what chemical signals will affect their population in human blood. Human peripheral blood leukocytes (PBLs) were obtained via separation through a ficoll extraction. To set a baseline for activation, a generic mitogen, PMA and ionomycin, was used to stimulate the PBLs. After stimulation of the different immune cells, flow cytometry was used to identify CD4, CD25, and Foxp3 regulatory T cells in the population of PBLs. To do so, markers for CD4, CD25, and Foxp3 were fluorescently tagged. The number of CD4+CD25+Foxp3+ regulatory T cells was then counted using a flow cytometer. The number of regulatory T cells found in the test group was then compared to the number of regulatory T cells found in a control group to which no stimulus was added. Our goal in this study was to establish a protocol for extraction and measurement of regulatory T cell numbers from human PBLs. This process can then be repeated with the addition of several potential regulatory T cell effectors to see which will impact their activity within the immune response. This may include natural anti-inflammatory agents or immunosuppressants. In particular, this study plans to continue its focus on the effects of natural supplements on the regulatory response of the immune system.

PHYLOGEOGRAPHY OF MITE HARVESTMEN (GENUS *Austropurcellia*) IN THE WET TROPICS OF AUSTRALIA

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Austropurcellia, a genus of mite harvestman (class Arachnida, order Opiliones, suborder

Cyphophthalmi) endemic to the rainforests of the Wet Tropics of Queensland, Australia, is a dispersal-limited Gondwanan lineage whose diversity and evolutionary history is poorly understood. I identified new morphological species using scanning electron microscopy and reconstructed a molecular phylogeny for the genus, focusing on an upland area with high rainforest stability throughout the Quaternary. In this upland region of approximately 40 km in diameter, I found a total of five distinct species (consistent morphologically and molecularly), including one cryptic species that I have designated as two separate species, *A. daviesae* and *A. tholi* n. sp. The high species richness is explained both by the limited dispersal ability of these animals and climatic history of the Wet Tropics, which experienced dramatic climatic oscillations throughout the Quaternary, resulting in contraction and expansion of rainforest refugia.

A RAPID EFFICIENT METHOD TO DETERMINE IF PLANT TISSUE EXTRACTS POSSESS ANTIFUNGAL ACTIVITY

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Concern for the environment has stimulated interest in plant extracts which possess antimicrobial or antifungal properties that could be used to replace synthetic pesticides currently being used. At present, identification of antifungal compounds requires that extract activity be assayed as amendments to media, which is expensive, slow, and laborious. Colorimetric analysis of biological activity in solution culture is potentially a simpler and speedier process. The objectives of this research are to (1) develop speedy, efficient methodology utilized to test natural compounds for antifungal activity and (2) confirm the presence of antifungal compounds in extracts from a native plant, *Rhus typhina*. The method used is a rapid high-throughput, colorimetric assay which measures respiration of zoospores exposed to various concentrations of extracts when combined in cuvettes. Spectrophotometric analysis of the solutions at 12-hour intervals during a 24-hour period enabled measurement of the effect of the extracts on respiration of *Phytophthora sojae*. A decrease in absorbance of light transmitted through the cuvette and solution at 570 nm with an increased concentration (125 µl, 250 µl, and 500 µl) of natural extracts indicated decrease in respiration by the zoospores. These results indicate that extracts of *Rhus typhina* inhibit respiration by the organism. The limited time and material requirements necessary for the analysis were a considerable improvement over that required for conducting an *in vitro* analysis of the extract's antifungal activity. Application of this method will make future analysis of natural compounds easier and more efficient to facilitate utilization of natural compounds as organic antifungal and antimicrobial pesticides.

CONTROL OF MRNA REGULATORY ELEMENTS THROUGH ALTERNATIVE POLYADENYLATION

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The precise regulation of genetic information is crucial for the immune system to appropriately respond to a challenge. RNA decay is one mechanism used to control the changes in the gene expression pattern in T cells following stimulation. One pathway promoting mRNA decay involves the RNA binding protein CUGBP1 recognizing a GU-rich element (GRE) of the sequence UGU[G/U]UGU[G/U]UGU in the 3'UTR of a network of transcripts involved in proliferation and control of apoptosis. Here we hypothesize that alternative polyadenylation is an important mechanism controlling GRE-mediated decay following T-cell receptor activation. We plan to utilize next generation sequencing and Q-RT-PCR to determine whether there is a shift in mRNA isoform abundance following T cell activation. If the abundance of GRE containing RNA isoforms decreases relative to the abundance of non-GRE containing isoforms throughout T cell stimulation course, this would support a model that mRNA decay is regulated through differential inclusion/exclusion of the GRE from the mRNA transcript, by the process of alternative polyadentylation.

A COMPARATIVE STUDY OF DOLLAR COST AVERAGING VS. VALUE AVERAGING

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My research compares three investment techniques, fixed and variable dollar cost averaging and value averaging to determine if any of the methods yield superior investment returns in the long run. Mutual funds, stocks, and exchange-traded funds were used to test the methods. Value averaging is a formula-based investment technique using a mathematical formula to guide the investment of money into a portfolio over time. With this method investors contribute to their portfolios in such a way that the portfolio balance increases by a set amount, regardless of market fluctuations. Dollar cost averaging invests equal amounts regularly and periodically over specific time periods in a particular investment or portfolio. By doing so more shares are purchased when prices are low, and fewer shares are purchased when prices are high.

After testing many mutual funds, ETFs, and individual stocks, I concluded that the three methods yield

similar annual returns ranging from 6 percent to 8 percent in the long run. The results also indicate that the three methods provide superior investment returns over extended investment time periods with little increase in risk, even if prices are volatile. The only difference between these three formula investment techniques is that value averaging requires larger sums of money to be invested at regular time intervals than fixed or variable dollar cost averaging do.

A MAGNETOENCEPHALOGRAPHIC STUDY OF DEPTH PERCEPTION

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Monocular depth perception relies on a number of visual cues, some of which are based on motion. We were interested in whether depth information generated from moving and non-moving stimuli are processed differently in the brain. Specifically, we hypothesized that depth information derived from motion would be generated in a bottom-up manner (with brain activity associated with depth in this case appearing first earlier in the visual hierarchy), whereas depth information derived from static pictorial cues would be processed first later in the visual hierarchy, i.e. top down. To test this hypothesis, we presented subjects with displays that specified three levels of depth, using one of five different visual cues: three pictorial (static) depth cues and two motion-based depth cues. We recorded the subjects' brain activity using a 248-channel axial gradiometer MEG system while they passively viewed these stimuli. We then used linear discriminant analysis to decode the depth status of each of the stimulus presentations, in 100 ms bins, moving every 10 ms through the trial. We noted the time at which this analysis could successfully classify depth status of a trial's stimulus at levels above chance, and compared the time of first significance across sensor groups. We found that, in both motion- and non-motion- based cues, depth information was first seen in earlier visual areas than later visual areas. This suggests that in pictorial cues, depth information is processed in parallel with object identity information.

DYNAMIC CELL MAINTENANCE OF STEADY STATE IN RESPONSE TO MICROTUBULE-TARGETING CHEMOTHERAPEUTIC AGENTS

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Microtubules are essential for many cell functions, such as directing intracellular transport, determining the position of intracellular organelles, and facilitating mitosis, vesicle traffic, and cell motility. The fact that microtubules are so essential in cell function, particularly mitosis, makes microtubule-targeting therapies very attractive in the treatment of many types of cancer. It is well understood that these microtubule-targeting drugs change the concentration of free tubulin subunits within the cell and suppress microtubule dynamics, but it is less understood how the cell maintains an equilibrium at this new set-free tubulin concentration and how the overall distribution of microtubules changes after drug addiction. This study proposes that the overall microtubule distribution profile within Lewis Lung Cancer Porcine Kidney (LLCPK) epithelial cells can give insight into a mechanism by which these drugs work to change the cell system through alterations in free subunit concentration and how the cell reacts to this perturbation to maintain function and life during interphase. Through microtubule density and distribution analysis, we find that the microtubule-targeting drugs, paclitaxel and nocodazole, maintain an even distribution of microtubules within the cell as compared to the control case and that this can be explained by possible nucleation from noncentrosomal sites. This mechanism gives insight into the ways in which a cell can maintain an equilibrium state even after perturbations to the system.

BLAST-INDUCED TRAUMATIC BRAIN INJURY: A NOVEL METHOD FOR PRECLINICAL INDUCTION

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Blast-induced Traumatic Brain Injury (bTBI) has been coined to be the "signature" injury for today's military conflicts due to the recent wars in Iraq and Afghanistan. As compared to TBI due to blunt-force trauma, bTBI may not present with obvious signs of injury. Current preclinical models use blast chambers, shockwave tubes, or open-field explosions to study the pathophysiology and identify unique signs and symptoms of bTBI. These models, however, are limited by the need for large space, safety concerns, and low reproducibility. Thus, we present here a novel, bench-top model to induce bTBI using shockwave lithotripsy. We used a total of 35 rats and delivered 5 shockwave pulses to the right ride of the brain (prefrontal region). Rats were sacrificed at 1, 3, and 7 days post-bTBI to assess the time-course of injury progression. The injury was characterized by

neurological, motor, and spatial memory assessments as well as angiographic studies of vasospasm. We found that bTBI induction using shockwave lithotripsy caused neurological, motor, and cognitive deficits similar to those observed in the clinical setting. There was significant evidence of vasospasm initiating by day 1, peaking at day 3, and subsiding by day 7 after injury. Our findings suggest that shockwave lithotripsy may be a viable alternative for bTBI induction in preclinical models. Preliminary data from our study demonstrate high reproducibility, ease of use, and clinically relevant outcomes; however, a larger sample size may be needed to further investigate applicability of shockwave lithotripsy as a preclinical bTBI model.

INVESTIGATIONS INTO PHEROMONES IN FRESHWATER DIATOMS (BACILLARIOPHYCEAE)

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Diatoms (Bacillariophyceae) are prolific, unicellular algae and make up the base of the food web in many aquatic ecosystems. They exhibit both sexual and asexual reproduction with the cell predominately reproducing by binary fission progressively decreasing in size from generation to generation. Once they hit their minimum size they switch over to sexual reproduction and form gametes. In the related brown algae (Phaeophyceae), phycologists have discovered various pheromones, and diatoms have been observed to produce these chemicals, but nobody has linked these chemicals to sexual reproduction. We have spent the last few months culturing the freshwater species *Synedra sp.* and *Navicula pelliculosa* and have produced gametes in both, a first for our laboratory. Bioassay experiments have found evidence of a pheromone which induces gametogenesis, but no evidence of a chemotaxic compound.

THE EFFECTS OF ATRAZINE ON BMP4 PROTEIN LEVELS IN LIVER AND SERUM ISOLATED FROM *Phasianus colchisus* AND *Gallus gallus*

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Atrazine is a selective triazine herbicide, known to be one of the most widely used herbicides in the United States. It is used to control broadleaf and grassy weeds, in corn and other crops. Currently the EPA set the maximum contaminant level for atrazine at 0.003 mg/L or 3 ppb. The National Resource Defense Council has estimated that 40% of ground water samples, and 75% of stream samples near agricultural sites contain traces of atrazine. Because of the limitation of drinking water to watersheds, ground water, and rain puddles, wildlife may be exposed to atrazine. Studies have shown that atrazine exposure in amphibians, reptiles, and avians can have a negative effect on the overall health, including decreased pack cell volume in *Gallus gallus,* and preliminary studies show decreased levels of Bone Morphogentic Protein-4 (BMP-4) in liver serum of chickens developing in ova. The purpose of this study was to analyze the effects of atrazine on BMP-4 protein levels in liver and serum isolated from *Phasianus colchicus* (pheasant) and *Gallus gallus* after hatching. The pheasants and chicken chicks were exposed to atrazine for a three-week period. In order to analyze the effects that atrazine had on BMP-4 Proteins, a Western Blot was completed to identify the molecular weight in Daltons of the BMP-4 proteins. Also an ELISA kit (R&D Systems) was used to detect the amount of BMP-4 proteins found in the serum and liver extracts. It was found that the average pg/mL of BMP-4 proteins in pheasant serum decreased as atrazine concentration increased, 30.2 pg/mL for Control, 7.46 pg/mL for 30 ppb, and 6.84 pg/mL for 300 ppb. Interestingly, there was an increased of BMP-4 in liver samples: 22.1 pg/mL for Control, 54.3 pg/mL for 30 ppb, and 75.5 pg/mL for 300 ppb. Data for the chickens were similar. The data support the idea that BMP-4 proteins potentially play a role of development in hematopoietic system.

EFFECTS OF COCAINE ON LOCOMOTOR ACTIVITY IN RATS WITH ADENOVIRAL VECTOR-MEDIATED COCH EXPRESSION

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AIM: Cocaine hydrolase (CocH) is a mutant human-derived butyrylcholinesterase that reduces the physiological and rewarding effects of cocaine by rapidly metabolizing the drug before it is exposed to the brain. Previous studies have shown that adenoviral vector-mediated CocH can block relapse to cocaine-seeking in rats for up to 6 months, indicating the therapeutic potential for CocH in treating cocaine addiction. This study analyzes the effects of CocH delivered by a viral vector on the locomotor-activating effects of cocaine in rats using the cholinesterase inhibitor iso-OMPA to determine whether the behavioral effects are due to enzymatic activity. METHOD: Wistar rats were treated with the adenoviral vector-encoding CocH (VEC). Vector-treated and naïve control rats were then tested for locomotor activity upon receiving intraperitoneal (i.p.) injections of cocaine (10 or 15 mg/kg). To ascertain whether differences in locomotor activity were truly caused by enzymatic destruction of cocaine by CocH, rats

were pretreated with saline or iso-OMPA (1 mg/kg, i.p.), an anticholinesterase that targets and permanently inactivates CocH, 2 hours prior to testing. RESULTS: Vector-treated rats showed significantly less locomotor activity than control rats when cocaine was administered with saline pretreatment. In comparison, upon iso-OMPA pretreatment, both vector-treated and control rats showed significantly increased locomotor activity. However, when CocH levels were allowed to recover, VEC-treated rats again had lower levels of cocaine-induced locomotor activity compared to controls. CONCLUSIONS: Iso-OMPA pretreatment disabled the decreasing effects of CocH VEC on the stimulant effects of cocaine on locomotion. The iso-OMPA pretreatment tests suggest that CocH's effects on locomotor activity are due to its hydrolysis of cocaine. Results support a growing body of evidence that gene therapy that introduces cocaine hydrolases can help reduce the physiological and rewarding effects of cocaine and thereby aid in the recovery process of addiction.

A CARBOHYDRATE-RICH DIET INCREASES SOCIAL IMMUNITY

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Social insects make up a more than half of the world's insect biomass, a dominance that has arisen in spite of inherent costs associated with group living. One social cost is the increased potential for parasite transmission that arises from interactions with nestmates. Some social insects have disease resistance mechanisms that are enhanced in social settings. Here we test whether this "social immunity" can be mediated by nutrition; given that traits underlying immunity require different nutritional mixtures, scarcity of particular nutrients could influence the costs of aspects of immune function in a social setting. In a study in a Panamanian rain forest, we tested whether social immunity in the ant *Ectatomma ruidum* depends on the protein:carbohydrate (P:C) ratio in its diet. In a first part, we reared colonies on either a "high-protein" diet (3P:1C) or "high-carbohydrate" (1C:3P) diet, then created 5-ant ("social") or 1-ant ("solitary") groups. We then exposed half of the social and half of the solitary groups to a parasitic fungus, *Metarhizium anisoplia*. We found that social grouping enhanced individual ant survivorship when challenged with *Metarhizium*, but this effect was particularly strong for ants that had been reared on the high-carbohydrate diet. In a second experiment, we tested how *Metarhizium* exposure affected mortality rates in whole colonies reared on high-protein or high-carbohydrate diets, and found that colony resistance to *Metarhizium* was particularly strong on the high-carbohydrate diet. Combined, our results provide strong evidence that carbohydrate-rich diets enhance social immunity.

DENDRIMER ENCAPSULATED PD NANOPARTICLE CATALYZED DECOMPOSITION OF POLYCHLORINATED BIPHENYLS

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The contamination of many soil and aquatic systems through the bioaccumulation of polychlorinated biphenyls (PCBs) is a major concern due to their high toxicity and slow degradation kinetics. An effective method of reducing PCBs' toxicity is to decompose them into their less toxic biphenyl form. Using magnesium powder as a reducing agent, small amounts of 1.4 nm Dendrimer Encapsulated Palladium Nanoparticles (Pd-DENs) enhanced the rate of decomposition of both a model PCB congener, 2,2',3,3',4,5-Hexachlorobiphenyl (HCB), and a PCB mixture of Aroclor 1260. Reactions were monitored by both a chloride ion selective electrode and gas chromatography. The results indicated that the rate of reaction was moderately enhanced even when microgram amounts of nanoparticle catalysts were used. In comparison to literature reports, it is significant that by using surface active Pd nanoparticles the amount of catalyst required is drastically reduced to achieve the conversion to biphenyl.

SYNTHESIS AND CHARACTERIZATION OF VAPOCHROMIC PLATINUM(II) EXTENDED LINEAR CHAIN MATERIALS

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Vapochromic extended linear chain (ELC) materials consist of one-dimensional molecular arrays of weakly associated coordination complexes that exhibit observable changes in solid-state luminescence upon the incorporation of various organic vapors into their structure. As a result of their unique properties, vapochromic ELC materials are well suited for potential application as volatile organic compound (VOC) sensors. Previously, *cis*-Bis(isopropylisocyanide)dicyanoplatinum(II), $Pt(CN\text{-}i\text{-}C_3H_7)_2(CN)_2$, was synthesized and found to exhibit a significant change in solid-state luminescence upon the selective adsorption of toxic benzene vapor. However, its usefulness as a potential sensor is limited due to its inability to reversibly adsorb and release benzene. By

modifying the alkyl chain of the isocyanide ligands, it is hoped that a vapochromic ELC material might be synthesized that uptakes benzene observably, selectively, and reversibly, so as to be better suited for benzene sensing. With this aim in mind, *cis*-Bis(n-butylisocyanide)dicyanoplatinum(II), Pt(CN-n-$C_4H_9)_2(CN)_2$, and *cis*-Bis(n-propylisocyanide)dicyanoplatinum(II), Pt(CN-n-$C_3H_7)_2(CN)_2$, were synthesized and characterized in order to investigate their vapochromic properties and suitability as benzene sensors.

ZEB 1 REGULATION: A MOLECULAR UNDERSTANDING OF CANCER METASTASIS

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A primary characteristic of cancer is genome instability. In various types of cancer ZEB1 activity has been shown to be a key factor in progression to the metastatic state. ZEB1 protein is encoded by the TCF8 gene and it is thought that mutations in its 3'UTR lead to increased ZEB1 expression, thereby promoting metastasis. Mutations in the 3'UTR are significant as the TCF8 gene is regulated by the binding of mirco-RNA to its mRNA gene products, halting their translation. In this research project the ZEB1 3'UTR sequence of primary and metastatic cancer were compared to a standard healthy sequence. Mutations were identified and further insights into the regulation of ZEB1 by micro-RNA's were gained.

NbzAa AND NbzAb FROM *Comamonas* SP. JS765 AND EXPRESSION OF NITROBENEZE DIOXYGENASE REDUCTASE AND FERREDOXIN COMPONENTS IN *E. coli*

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Comamonas sp. JS765, a bacterium found in the soil surrounding a large nitrobenzene production facility, has been shown to produce a series of proteins that are capable of catalytically degrading nitrobenzene to catechol and nitrite in the presence of O_2. Three of the necessary proteins are encoded by the catabolic operon (nbzAaAbAcAd) found in the genomic DNA of JS765. Together these proteins form a three-component, electron-transfer chain system. The proteins that constitute this system are nitrobenzene dioxygenase (NBDO), in conjunction with $reductase_{NBZ}$ (NBDR), and $ferrodoxin_{NBZ}$ (NBDF). The ultimate goal of this research is to study the mechanistic nature of the reaction, but previous studies have shown that the bacteria are producing insufficient amounts of NBDF in enriched media, and are having difficulties growing to desirable densities in minimal media with nitrobenzene as the sole nitrogen source. A possible solution to this problem is using recombinant DNA technology to produce *E. coli* isolates capable of synthesizing NBDR and NBDF by cloning nbzAa and nbzAb, respectively, and using a pF1A T7 Flexi® Vector capable of inducible expression. Results of the cloning and expression studies will be presented.

EXPRESSION OF LIPOXYGENASE ISOENZYMES IN PEA LEAVES AFTER MECHANICAL WOUNDING AND JASMONATE TREATMENT

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Lipoxygenase (LOX) enzymes participate in plant defenses against environmental and pathogenic stressors. Jasmonates (jasmonic acid and its esters) are signal molecules made in the LOX-related wounding pathway. Previous studies have shown an increase in total lipoxygenase activity in wounded pea plants. Very few studies have shown differences in the relative expression of individual LOX isoenzymes after mechanical wounding or treatment with jasmonates. One recent study has shown a positive correlation between high jasmonic acid and increased expression of LOX-N2. In this present work, multiple Real Time Polymerase Chain Reaction (qPCR) methods have been designed to quantitatively monitor the expressions of the isoforms; LOX 1: PS 5, LOX 1: PS 7, LOX-N3, and LOX-g. RNA was isolated from control and treated pea leaves using the RNeasy Plant Mini Kit (Qiagen). The quantity and quality of the RNA samples were assessed spectrophotometrically. The RNAs were reverse transcribed using a high-capacity cDNA Reverse Transcription Kit (Applied Biosystems). Efficiency curves showed that the qPCR designs were successful. The expressions of the LOX isoenzymes were measured in pea leaves at 0, 3, 6, 12, and 24 hours after mechanical wounding. While LOX-N3, LOX 1: PS 7, and LOX-g expression increased significantly compared to the unwounded controls, LOX 1: PS 5 did not. Prolonged MeJA treatment highly stimulated expression of LOX 1: PS 7, LOX-g, and LOX-N3 at 12 and 24 hours. LOX isoforms showed differences in relative expression both in mechanical wounding and MeJA treatment.

THE ANTI-INFLAMMATORY EFFECTS OF GLYCEROL MONOLAURATE ON TPA-INDUCED INFLAMMATION IN *Mus musculus*

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Glycerol monolaurate has been shown to inhibit production of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α) *in vivo*. Since TNF-α plays a significant role in the symptoms of inflammatory diseases such as rheumatoid arthritis (RA), glycerol monolaurate may be a potential treatment for diseases like RA. Topical treatment with phorbol ester 12-O-tetradecanoylphorbol-13 acetate (TPA) on the ear of a mouse causes inflammation, and therefore serves as an animal model to study inflammatory disease. Studies have shown that the inflammation induced by TPA is largely due to increased production of TNF-α (Murakawa, 2006). The purpose of this study was to determine whether treatment with glycerol monolaurate could reduce TPA-induced inflammation in a mouse. Briefly, the ears of mice were treated with TPA and/or glycerol monolaurate and 6 hours after treatment, the mice were euthanized and tissue from each ear was collected. The tissue samples were then weighed; an increase in weight (due to fluid retention) was indicative of inflammation. Results indicated that while TPA treatment did cause significant inflammation, treatment with glycerol monolaurate did not significantly reduce this inflammatory response. Based on these results, it is questionable whether glycerol monolaurate would be a candidate as a treatment for RA.

THE POPULATION DYNAMICS AND ECOLOGICAL EFFECTS OF GARLIC MUSTARD (*Alliaria petiolata*) IN A MINNESOTA OAK WOODLAND

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Garlic mustard (*Alliaria petiolata*), is an introduced biennial forb that has commonly been referred to as highly invasive and as having substantial negative effects on other plants in the eastern deciduous forests of North America. In a two-year study, we extensively sampled *A. petiolata* in a Minnesota woodland at two spatial scales, including 6.5 km of belt transects in a 6.8-ha study grid (20 x 20 m cells) and 90 small sampling quadrats (1.0 x 0.5 m) within the grid. At the large scale, we compared seed bank abundance and diversity of other herbaceous plants with *A. petiolata* abundance. Using the monitoring data we also investigated whether this population was exhibiting a alternating two-year life-history cycle, previously hypothesized to be driven by intraspecific competition between the stems and the rosettes. At the small scale, we compared *A. petiolata* abundance with the abundance of other plants, including herbs, ferns, shrubs, and tree seedlings. We also conducted an *ex-situ* pot experiment in which we planted seeds of 6 tree species in soil collected from patches dense with and lacking *A. petiolata* and recorded emergence rates and seedling growth over an 8-week period. Overall, we found little evidence that *A. petiolata* was negatively affecting other plant species, suggesting that *A. petiolata* may be more a product than an agent of change in eastern North American deciduous forests. We also documented an alternating two-year life-history cycle, providing evidence that this cycle is at least partly being driven by intraspecific competition.

THE ANTIBACTERIAL EFFECTIVENESS OF VARIOUS MOUTHWASHES ON *Lactobacillus acidophilus* AND *Streptococcus mutans* ON TOOTHBRUSHES

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A variety of bacteria are present in the human mouth, and many of them contribute to dental diseases, including tooth decay. Tooth decay may be prevented if teeth are cleaned properly to remove the dental biofilm, and the toothbrush is disinfected following brushing to eliminate bacteria remaining on the bristles. A variety of solutions are used to eliminate bacteria present on toothbrushes. These include commercial mouthwashes and home remedies. The objective of this study was to test the antibacterial effectiveness of such solutions on oral bacteria present on toothbrushes. To do this, new toothbrushes were immersed in diluted cultures of *Lactobacillus acidophilus* or *Streptococcus mutans* and then disinfected in one of the following solutions: alcohol-free Listerine, original Listerine, 50% white vinegar, or saline (control). The toothbrushes were placed in 10 mL saline to dislodge any remaining bacteria from the bristles. Aliquots of these saline solutions were spread on tryptic soy agar plates, and the plates were incubated at 37^{o}C for 48 hours. The bacterial colonies on the plates were then counted, and the number of bacteria present on each toothbrush was calculated. Statistical analysis was completed on the data to compare the antibacterial effectiveness of each disinfectant. All three disinfectants (alcohol-free Listerine, original Listerine, and white vinegar) significantly eliminated *L. acidophilus* and *S. mutans* from the toothbrushes compared to the control treatment (saline). Interestingly, white vinegar appeared to be the most effective treatment against *L. acidophilus* while alcohol-free Listerine was the most effective treatment against *S. mutans*.

PRODUCTION OF SOOT PARTICLES AND ANALYSIS OF UNBURNED NATURAL GAS

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Soot particles are composed primarily of carbon that is formed by the incomplete combustion of organic

fuels. These particles accumulate in the atmosphere, having direct and indirect effects on the earth's climate and human health. Equipment was set up for the controlled production and collection of natural gas soot. Online heating (RT – 1100°C) within a tube furnace was used as a means of aerosol treatment. The operating pressure, oxygen partial pressure, and gas flow rate were varied to produce soot particles with varied but controlled nano-scale morphologies. Raman spectroscopy and gas chromatography were used to analyze the collected soot particles and exhaust gases, respectively. Results from Raman spectroscopy confirm the reproduction of soot particles consistent with previous work while preliminary gas chromatograms (thermal conductivity detector) indicate there may be unburned natural gas and combustion byproducts in the exhaust gas stream. The implications of the gas chromatograms are explored.

USING STABLE ISOTOPE ANALYSIS ON GOLDEN EAGLE IN WISCONSIN AND MINNESOTA TO DETERMINE BREEDING GROUND LOCATIONS

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Despite its historical presence in the upper Midwest, the Golden Eagle (*Aquilla crysaetos*) has only recently been recognized as an established winter resident of the Upper Mississippi Valley region. Birds of prey are a widely studied avian group, yet researchers often have limited knowledge of the specific geographic ranges of individual birds. Breeding ranges are especially important, as these are the areas where raptors molt every summer. Stable isotope analysis using deuterium (a hydrogen isotope) provides an opportunity to reveal the breeding grounds for this Midwestern population. The amount of deuterium enrichment varies based on climate and elevation of the water source. Because some water is incorporated in feathers, feather growth during the breeding season creates a permanent record of the bird's location during the summer months. Using feathers from museum specimens along with those collected from 2012 winter residents, it is possible to acquire information on the ultimate geographic source of this population.

THE EFFECTS OF GLYCEROL MONOLAURATE ON TNF-α PRODUCTION IN J774A.1 MURINE MACROPHAGE CELLS

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Glycerol monolaurate (GML) has been shown to exhibit anti-microbial properties. Additionally, researchers have shown that GML affects immune cells by modulating their proliferation and cytokine production. For instance, *in vivo* studies have shown that GML decreases production of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), in stimulated human vaginal epithelial cells. The purpose of this study was to analyze the effects of GML on TNF-α production in another type of cell, lipopolysaccharide-stimulated J774A.1 murine macrophage cells. Briefly, macrophage cells were added to a 24-well tissue culture plate and were stimulated with LPS in the presence or absence of various concentrations of GML. At 0, 8, 24, 48, and 72 hours post-stimulation, the numbers of cells were determined and TNF-α levels were measured using a sandwich enzyme-linked immunoabsorbent assay (ELISA). The experiment was completed three times with varying results. Thus, it is not clear whether GML inhibits TNF-α production in these cells. It is possible that GML influences TNF-α production by other cells known to secrete this cytokine. Furthering our understanding of the effect of GML on TNF-α production is of interest. If GML is shown to inhibit production of the pro-inflammatory cytokine TNF-α, then GML could potentially be used in the treatment of inflammatory disease.

TEMPERATURE EFFECTS ON ACTIVITY OF LACTATE DEHYDROGENASE OF THE BLUEGILL (*Lepomis macrochirus*)

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Lactate dehydrogenase was extracted from muscle tissue of *Lepomis macrochirus* to test the enzyme's activity at different temperatures from 5°C to 40°C using a micro-plate reader and spectrophotometer. As temperature rose, the reaction rate increased, but the affinity decreased. The substrate pyruvate inhibited oxidation of NADH at concentrations about 4 times the K_M, while the substrate concentration is usually lower than K_M in normal cells. The inhibition effect lessens at high temperature. Small change of K_M and V_{max} at 15-25°C indicated that the fish regulates LDH activity well at this temperature range, but also it can endure certain temperature change as those values show dramatic change from 5-40°C.

CO_2 PREFERENTIAL ADSORPTION SITES IN MFI-TYPE ZEOLITES

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Sorbents with strong CO_2 adsorption are good candidates for efficient separation of CO_2 from multi-

species gas streams. Zeolites are one attractive option as sorbents.

We have used grand canonical Monte Carlo simulations to investigate the influence of non-framework, mobile cations on CO_2 adsorption behavior in aluminum-substituted MFI-type zeolites.

We have found that the presence of cations changes the location and number of preferential CO_2 adsorption sites. The number of preferential sites depends on the number of cations and the cations' location, as there is a preferred CO_2-cation distance. The number of preferential sites in the straight channel is greatest when cations are evenly spaced and CO_2 loading is low. When evenly spaced, the distance between all cation pairs is such that there are two instead of one preferential CO_2 adsorption sites. When CO_2 loading is low, cations are positioned farthest from the center of the straight channel, thereby increasing the number of preferential sites.

INTERNET USE AND ASSOCIATED RISKS IN A COLLEGE SAMPLE

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The Internet is commonly used among young adults; however, Internet use may become a problematic behavior. Past research has examined Internet behavior in young adults and its relationship to other behaviors and health issues, yet further research needs to be completed to gain a more comprehensive understanding of this relationship. A sample (n = 2108) of college students (56.9% female) was examined using a self-report Internet survey concerning demographic characteristics, Internet use, health behaviors, psychosocial functioning, and psychiatric comorbidities. We found that 237 students (12.9%) met criteria for limited Internet use, 1502 (81.8%) for mild Internet use, and 98 (5.3%) for moderate to severe Internet use. Variables significantly associated with greater frequency of Internet use included lower Grade Point Average ($p = .006$), less frequent exercise ($p = .018$), higher PHQ-9 scores ($p < .0001$) (indicative of greater depression symptoms), and higher Perceived Stress Scores ($p < .0001$). These data indicate that moderate-to-severe Internet use is associated with a range of psychosocial problems in young adults. More research is needed to better understand the relationship between Internet use and physical and mental health, as well as academic variables.

THE EFFECT ON BODY COMPOSITION BY THE ADMINISTRATION OF YOHIMBINE-HCL TO MUS MUSCULUS

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Yohimbine is an alkaloid isolated from the *Pausinystalia Yohimbe* tree and Indian Snakeroot. This natural compound is prescribed as an aphrodisiac for males with erectile dysfunction, although you can find yohimbine as the main ingredient in many weight loss supplements. The objectives of this study were to examine its effects on body composition of *Mus musculus* after the administration of yohimbine HCl. This was performed by randomly separating CD1 male mice into four groups (five in each group), the first group was the control group, another group was exercised and given no yohimbine, a third group was not exercised but was given yohimbine, and the finally group was exercised and given yohimbine. Weight and percent body fat were collected and calculated both pre and post administration of yohimbine. The amount of drug administered to each group was based on an allometric scale down taking into account metabolic rate differences. Based on averages taken from all groups both weight and percent body fat had decreased in the two groups receiving yohimbine. An ANOVA test will be run to determine if the decrease in weight was significantly.

SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF CYCLOMETALLATED GOLD(III) COMPLEXES

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The study of organogold complexes has drawn increasing interest as practical applications of this chemistry including antitumor activity have been discovered. With this in mind, this study explores fundamentals of structure and bonding in gold(III) complexes. Specifically, cyclometallated gold(III) planar complexes were synthesized, incorporating 1,3,7-trithiacyclononane(9S3) ligands. These complexes were accessed through a scheme first involving the preparation of neutral intermediates of the form Au(N^CH)Cl3 (N^CH =2-(p-tolyl)pyridine, 2-(2'-benzothienyl)pyridine). These neutral noncyclometallated complexes were heated as neat solid complexes to induce cyclometallation. The cyclometallated complexes of the form Au(N^C)Cl2 were then reacted with 9S3 and metathesized to access the target complex salts of the form [Au(N^C)(9S3)](PF6)2. These systems were characterized by numerous methods including one- and two-dimensional nuclear magnetic resonance

spectroscopy, electronic spectroscopy, thermogravimetric analysis, differential scanning calorimetry, and X-ray crystallography.

OREXIN A IS NEUROPROTECTIVE AND DECREASES FATTY ACID-INDUCED APOPTOSIS IN HYPOTHALAMIC NEURONS

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Background: Obesity is a growing health concern in today's society. It is estimated that over 30% of the adult population in America is obese. High-fat diets (HFDs) rich in saturated fatty acids such as palmitic acid (PA) increase oxidative stress in both peripheral tissue and in the central nervous system. Recently, HFD-induced neuronal changes in the hypothalamus have been linked with obesity. Orexin A (OxA, also known as hypocretin 1) is a hypothalamic neuropeptide mediating physical activity and eating behavior, and sleep/wake mechanisms. OxA was recently shown to be neuroprotective by decreasing apoptosis, which is programmed cell death. The hypothesis of this project is that OxA protects against PA-induced hypothalamic oxidative stress by increasing cell viability and decreasing apoptosis.

Methods: An *in vitro* hypothalamic cell culture model was used to determine cell viability and caspase 3/7 activity (marker of cell death) following OxA pretreatment and chronic PA exposure in the presence or absence of OxA (50, 100, or 300 nM). Cell viability was determined using a resazurin-based assay and caspase-3/7 activity was determined via a luminogenic caspase substrate assay, DEVD.

Results: All PA-challenged cells that were pretreated with OxA showed a significant decrease in PA-induced cell death and caspase-3/7-induced apoptosis ($P<0.01$).

Conclusions: OxA exerts neuroprotection by decreasing PA-induced cell death and caspase-3/7 activity. This is novel information and can be utilized in defining potential therapeutic treatments for HFD and obesity-related neuronal changes. Further evaluation of mechanisms underlying hypothalamic neuroprotection by OXA is ongoing.

JAK3-DEFICIENT MICE ARE PARTIALLY-PROTECTED FROM STEPTOZOTOCIN-INDUCED TYPE 1 DIABETES

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The streptozotocin (STZ) model of type 1 diabetes (T1D) involves five consecutive daily low doses of STZ to induce immune-mediated pancreatic beta cell death. JAK3 is an essential component of proliferation and differentiation signaling pathways in lymphocytes. As T cells play a critical role in T1D pathogenesis, JAK3-deficient mice were hypothesized to be protected from developing diabetes. The immune responses of wild-type (wt) and JAK3-deficient male C57Bl/6J mice were characterized on days 7, 14, and 28 days post-first STZ injection. While all of the STZ-treated wt mice became diabetic by day 21, 36% of JAK3-deficient mice remained diabetes-free for the entire experiment. T cell immunophenotyping of splenocytes revealed that cell counts were reduced in JAK3-deficient mice, resulting in lower numbers of CD8 positive T cytotoxic cells and CD4 positive T helper cells. JAK3-deficient mice had negligible levels of CD4/CD25/Foxp3 positive regulatory T cells, but high levels of CD4/PD-1 positive suppressor T cells. Cytokine profiles of the two genotypes' splenocytes also revealed a key difference. While the pro-inflammatory cytokines IL-2, IFN-gamma, TNF, and IL-17 were significantly reduced with JAK3-deficiency, there was drastically increased regulatory IL-10 production. Insulitis was examined at the three experimental timepoints and revealed a significant amount of islet infiltration at day 14 only in wild-type mice. Insulin immunohistochemistry was used to determine the functionality of beta cells. In conclusion, the anti-inflammatory immune response of JAK3-deficient mice seems to be responsible for the reduced diabetes incidence, providing additional evidence for the strategy of preventing T1D development by inhibiting JAK3.

CONSTRUCTION OF A RAMAN SPECTROMETER AND APPLICATION TO SOOT STUDY

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Soot particles, made mostly of carbon from incomplete combustion, accumulate in the atmosphere with detrimental effects on human health. Raman spectroscopy is often used as a means to study these soot particles, giving two very strong overlapping Raman peaks near 1350 and 1580 cm^{-1}. The strength of these peaks makes identification of soot a straightforward task. Additionally, the peaks are well characterized as relates to their correlation to specific nano-scale morphologies. The combination of these factors means that Raman spectroscopy is very useful for analysis of soot particles. Optical components and a spectrophotometer were used to construct an apparatus for collecting Raman spectra. The theory of Raman scattering and the rationale for the optical components and architecture of the Raman

spectrophotometer will be explored. The apparatus provides reproducible spectra matching those of various well-known and documented compounds, however, it was designed for soot samples. Various optical components were chosen to ensure fast and efficient analysis of soot nanoparticle films. Soot particles have been analyzed yielding spectra consistent with those collected on other, tested spectrophotometers.

ESTIMATING THE MOLECULAR VOLUMES OF SMALL MOLECULES USING IR SPECTROSCOPY

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Infrared spectroscopic absorbance peaks of moderate to strongly absorbing frequencies of the solutes appear as negative peaks at such positions when solutes are added to it. Such negative peaks are used in this investigation to estimate the molecular volumes of the dissolved solutes. The magnitude of the negative peaks of toluene as the solvent was correlated to the theoretical molecular volumes of the solutes. Using such correlation plots, molecular volumes of some small molecules were estimated and compared to their theoretically calculated molecular volumes. As of now the estimated molecular volumes from the proposed method were within 10-20% of the calculated values.

DETERMINING THE OPTIMAL PASSIVATION TECHNIQUE FOR MP35N

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Cyclic Polarization was utilized to test the rest potential, pitting potential, breakdown potential, passive current density, and corrosion rate of Multiphase alloy system consisting of 35 % nickel, 35 % cobalt, 20 % chromium, and 10 % molybdenum (MP35N). Each MP35N strip was electropolished using a uniform, predetermined procedure prior to passivation. The alloy strips have been passivated under eight different prescribed conditions consisting of three variables (acid used, temperature of acid bath, and time of passivation). These conditions were combined into 32 trials by using a design-of-experiments protocol to determine if there are any correlations between the variables and the properties tested by Cyclic Polarization. Each variable had 2 values that were tested: the acid used was either 35% nitric acid or 4% citric acid, the temperature of acid was either 25 or 65°C, and the time of passivation was either 10 or 60 minutes. Correlations between the variables and corrosion properties of MP35N will be presented, with the ultimate goal of the research to determine the best passivation technique is for this specific alloy.

EFFECTS OF NON-SELECTIVE CYCLOOXYGENASE INHIBITOR, IBUPROFEN, ON GENE EXPRESSION, PROSTAGLANDIN SYNTHESIS AND REPRODUCTIVE BEHAVIOR IN ZEBRAFISH

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Prostaglandins are a class of hormones important for the regulation of gonadal steroidogenesis and ovulation in vertebrates. In fish they are also used as pheromones; they are released by ovulating females and are important for the initiation and synchronization of male reproductive behaviors. Commonly used pharmaceuticals such as nonsteroidal anti-inflammatory pharmaceuticals (e. g, ibuprofen (IB) etc.) inhibit cyclooxygenase (COX) enzymes which catalyze prostaglandin synthesis. Sexually mature zebrafish males (n=14) and females (n=14) were exposed to 50 µg/L of IB for 14 days and their reproductive behaviors were compared to well-water exposed controls (n=14 per sex). The present study also examined effects of IB exposure on prostaglandin synthesis pathway; we measured phospholipase, COX 1 and 2, and prostaglandin synthase mRNA abundance using real time polymerase chain reaction. In addition, the ovarian prostaglandin F2 alpha concentrations and COX enzyme activity were evaluated. Exposure to IB caused decrease in COX activity and was mirrored downstream in the reduced production of PGF-2. Our lack of ability to detect effects on PGF-2□was likely, in part, a result of a high variability due to the asynchronous ovarian maturation. Furthermore, exposed fish may have been able to compensate for the decreased COX activity by increasing abundance of mRNA for COX and prostaglandin synthases (as demonstrated by our gene expression data). Several courtship behaviors were significantly decreased in IB-exposed males, but they were not altered in females. These findings suggest that IB affects prostaglandin synthesis pathway and impairs performance of male sexual behavior and thus has a potential to impair the reproductive success of exposed individuals.

MEASUREMENTS OF ETHANE IN ANTARCTIC ICE CORES

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Ethane is one of the most abundant hydrocarbons in the atmosphere. The major ethane sources are fossil fuel consumption, biofuel combustion, and biomass-burning emissions; the primary loss is via reaction with OH. A paleoatmospheric ethane record could be useful as a tracer of biomass-burning emissions, providing a constraint on past changes in atmospheric methane and methane isotopes.

In this study, we present preliminary measurements of ethane (C_2H_6) in Antarctic ice core samples with gas ages ranging from 0-1900 C.E. Samples were obtained from dry-drilled ice cores from South Pole and Vostok in East Antarctica, and from the West Antarctic Ice Sheet Divide (WAIS-D). Gases were extracted by melting samples under vacuum in an indium-sealed glass vessel and analyzed using high resolution GC/MS with isotope dilution. Ethane levels measured were in the range 100-220 ppt, with a mean of 157 ± 45 ppt (n = 12) with blanks contributing approximately half the amount of ethane in samples. These preliminary data exhibit a temporal trend, with higher ethane levels from 0-900 C.E., followed by a decline, reaching a minimum between 1600-1700 C.E. These trends are consistent with variations in ice core methane isotopes and carbon monoxide isotopes (Ferretti et al., 2005, Wang et al., 2010), which indicate changes in biomass burning emissions over this time period. These preliminary data suggest that Antarctic ice core bubbles contain paleoatmospheric ethane levels. With further improvement of laboratory techniques it appears likely that a paleoatmospheric ethane record can be obtained from polar ice cores.

THE EFFECT OF LAND USE AND AGGREGATION ON EXTRACELLULAR PEROXIDASE ACTIVITY

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Microorganisms are found in virtually every habitat and are essential to soil ecosystems worldwide. Through extracellular digestion, microorganisms return nutrients to the land, promote soil detoxification and provide food for plants and other living organisms. In this study, we examined the effect of cropping system and aggregate size on peroxidase, an extracellular enzyme that aids in the oxidation of lignin and the removal of toxic substances from the environment. We subjected soil plots to three land-use treatments (corn, prairie, and fertilized prairie) and isolated dry aggregate fractions from these plots according to size: Whole Soil (>4 mm), Large Macroaggregates (2-4 mm), Medium Macroaggregates (1-2 mm), Small Macroaggregates (0.25-1 mm), and Microaggregates (<0.25 mm). We conducted enzyme assays to determine peroxidase activity within each aggregate size and within each cropping system. Our results show that land-management practices affect enzyme activity, as peroxidase activity was 2.2 times greater in fertilized prairie plots and 2.7 times greater in fertilized corn plots than in unfertilized prairie plots ($P = .0334$; $P = .0012$). Additionally, we found that peroxidase activity decreases with decreasing aggregate size. Whole-soil activity was 44% greater than medium-macroaggregate activity ($P = .0207$), 67% greater than small-macroaggregate activity ($P=.0230$), and 84% greater than microaggregate activity ($P=.0133$). Overall, we conclude that land use and aggregate size have a significant effect on extracellular peroxidase activity.

SEED MASS AND STORAGE ENVIRONMENT, INITIAL GERMINATION AND PLANT GROWTH, AND COMPETITION HAVE LONG-TERM EFFECTS IN LARGE-LEAVED AVENS

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Large-leaved avens *Geum macrophyllum* is an herbaceous perennial found in moist woods and meadows, which produces spherical infructescences (seed heads) containing several hundred seeds. Each mature fruit (hereafter, seed) has a single elongated burr adapted to animal dispersal. We observed a 10-fold variation in seed mass in the *G. macrophyllum* population studied. The current research is an attempt to understand the potential modes of selection maintaining the variation in seed mass. The null hypothesis of no effect of seed mass on germination and initial plant growth was supported or refuted depending on storage environment of seeds. We hypothesized that seed mass variation may be maintained because seed mass affects persistence in the soil, allowing for prolonged, delayed germination in a "bet-hedging" strategy. Initial germination and growth was greater in seeds stored at higher temperatures than lower temperatures; however, overall seed mass did not have a large effect on germination. In another experiment, plants in two size classes (small and large) were grown for several months. Plants that were initially smaller remained significantly smaller, suggesting a selective advantage to early, rapid growth. Also, plants of similar size grown at low, medium, and high density showed a

strongly negative effect of competition on subsequent plant growth. At the population level microscopic examination and measurements of burrs indicated two classes: seeds with thin burrs and small hooks or thicker burrs with large hooks. Further morphometric examination determined that dimorphic burr morphology occurred within seed heads in plants not among plants in the population.

SYNTHESIS OF SULFUR-BRIDGED TRANSITION METAL ORGANOMETALLIC COMPOUNDS BY ETHYLENE BRIDGE REPLACEMENT

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Many organometallic enzymes that are responsible for redox reactions contain dithiolene ligands. To better understand the importance of the dithio ligands, we have synthesized novel dithiolate transition metal compounds. The dithiolate-bridged compound $[CpMo(\mu\text{-}S_2C_2H_4)]_2$ loses its ethylene bridges on heating, providing reactive sites at its bridging sulfur atoms. We have reacted this molybdenum dimer with group 6-8 transition metal carbonyl complexes to effect replacement of one or both ethylene bridges with transition metal carbonyl fragments. Depending on the transition metal, the metal carbonyl fragments may bridge the sulfurs symmetrically or asymmetrically; one product, $[(CpMo)_2\{Os(CO)_2\}_2(\mu_3\text{-}S)_4]$, adopts a cubane structure. These new complexes have been characterized and identified by X-ray crystallography, NMR, and IR spectroscopy.

SURVEYING *NAD2* RNA EDITING IN DIVERSE MYXOMYCETE SLIME MOLDS

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The myxomycete slime molds use extensive and varied forms of RNA editing on their mitochondrial transcripts. Though four distinct forms of editing were already demonstrated in myxomycete transcripts, a fifth form of editing (A deletions), has recently been described in the *nad2* transcript of *Physarum polycephalum*. We are investigating *nad2* editing in *Didymium nigripes, Semimorula liquescenes*, and *Badhamia gracilis*, three myxomycetes of varying phylogenetic distance from *P. polycephalum.* Since the *nad2* gene sequence is unknown for any myxomycete but *P. polycephalum,* we have aligned its *nad2* amino acid sequence from GenBank with those of several other organisms to identify conserved regions. We also compiled codon usage data from various sequenced mitochondrial genes of myxomycete species. We applied the codon usage data to design degenerate primers in conserved regions of *nad2*. We extracted RNA from *Physarum polycephalum*, reverse transcribed it, and used this cDNA to optimize PCR conditions with the primers. Using similar PCR conditions, we are amplifying cDNA, then DNA from *Didymium nigripes,* and will do the same with the other two species. Comparision of the sequences of cDNA and DNA will identify patterns of RNA editing in these diverse myxomycete species.

OPTIMIZATION OF NITROBENZENE 1,2-DIOXYGENASE EXPRESSION IN *Comamonas* JS765

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Comamonas JS765 isolated from industrial waste sites was previously found to exhibit a novel oxidative pathway for nitrobenzene catabolism. Nitrobenzene 1,2-dioxygenase (NBDO), along with its associated ferredoxin and reductase components, is responsible for the biodegradation. In preparation for future mechanistic studies on the nitrobenzene 1,2-dioxygenase system, *Comamonas* JS765 was optimized for NBDO expression. Oxygraph and gas chromatography methods indicated non-nitrobenzene dependent activity in *Comamonas* JS765 cultures grown on nutrient media. Nitrobenzene-supplemented minimal media were then utilized to induce NBDO expression. Various minimal media and vitamin supplements were analyzed for resulting *Comamonas* JS765 growth. Furthermore, possible limiting growth reagents, culture oxygen concentrations, and culture volumes were investigated for optimal growth conditions. Upon successful culture, *Comamonas* JS765 was harvested and analyzed for NBDO activity via oxygraph and gas chromatography assays.

NOVEL METHOD OF REDUCING AMIDES TO AMINES USING MILD CONDITIONS

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The purpose of this project was to develop a novel method of reducing an amide, acetanilide, to a secondary amine, N-ethylaniline, using mild conditions. Triphenylphosphine, carbon tetrachloride, and the amide were combined and heated in a microwave oven to form an intermediate. This initial reaction was performed in two different solvents, dichloromethane and tetrahydrofuran. The intermediate was characterized using

a multitude of NMR experiments. The intermediate was reduced to form the amine using 5-Ethyl-2-methylpyridine borane at room temperature. The intermediate was also reduced using sodium triacetoxyborohydride at reflux in THF. Initial yields were low and future work will involve optimizing the procedure to maximize the yield.

SYNTHESIS, CHARACTERIZATION, AND ISOLATION OF NOVEL (CARBAMOYL)DISULFANYL CHLORIDES

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Previously unobserved (carbamoyl)disulfanyl chlorides are synthesized by addition of limiting aromatic secondary amine to chlorocarbonyldisulfanyl chloride, Harris reaction of sulfur dichloride with an appropriate *O*-alkyl *N*-methyl-*N*-aryl-thiocarbamate, and asymmetric chlorolysis of a bis(*N*-methyl-*N*-arylcarbamoyl)disulfane. The newly synthesized unstable species was observed *in situ* by ^{1}H NMR and is trapped with alkenes, thiocarbamates, and thiols in methods derived from chemistry of analogous (carbamoyl)sulfenyl chlorides. Furthermore, each of the trapping products is synthesized by an alternate route, reinforcing conclusions about their structures. While (*N*-methyl-*N*-phenylcarbamoyl)disulfanyl chloride is unstable and decomposes quickly or cyclizes intramolecularly, introduction of the *N*,2,6-trimethylphenyl moiety leads to greatly increased stability. This study also led to the discovery of an unexpectedly stable ((carbamoyl)trithio)carbonyl chloride and an interesting 1,2,4-dithiazinone.

URBAN SURFACE RUNOFF AFFECTS REPRODUCTIVE AND OXIDATIVE STRESS BIOMARKERS IN THE FATHEAD MINNOW

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Many studies have documented the occurrence of environmental estrogens (EEs) in wastewater treatment plant effluents (WWTPEs), but urban aquatic systems may also be impacted by EEs contained in surface runoff. Moreover, untreated wastewater can be released into surface waters as combined sewer overflows (CSOs) during periods of heavy rain or rapid snow melt. We conducted this study within the Metropolitan Water Reclamation District of Greater Chicago (MWRDGC), which receives pollutant loads from seven WWTPs and nearly 240 gravity CSOs, and represents multiple watersheds. The objective was to determine whether surface runoff and CSOs may be contributors of EEs to the MWRDGC watersheds. We conducted 48-h exposures of adult male fathead minnows (n = 8 per treatment) to control water, surface runoff collected from six locations, and one CSO sample. RNA was extracted from the liver and gene expression analyzed using quantitative real time polymerase chain reaction (qPCR). Vitellogenin (VTG) and estrogen receptor (ER) mRNA increases were used as indicators of EEs. In addition, we examined whether runoff has the potential to impact expression of genes involved in reproduction and oxidative and/or metabolic stress responses. Expression levels of ER and VTG, and of several genes indicative of stress, were increased at multiple sites. These results indicate that surface runoff and CSOs may be important contributors of EEs and other micropollutants in urban ecosystems.

ISOLATION OF CD4+T CELLS BY THE ISOLATION KIT II FROM MILTENYI BIOTEC

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The regulation of the cellular immune response in autoimmune type 1 diabetes (T1D) is not yet fully understood. However, it is known that CD4+T cells play a vital role in the immunopathogenesis of T1D. In order to gain detailed knowledge on the effect and function of these cells in the development of T1D, isolating a pure population of CD4+T cells directly from heterogenous splenic cell population becomes an indispensable method. CD4+T Cell Isolation Kit I (Miltenyi Biotec) and EasySep® Mouse CD4 Positive Selection Kit (StemCell Technologies), previously used in our lab for positive isolation of CD4+T cells, yielded 70.6 ± 5.8% and 88.1 ± 2.3% purity, respectively, and recovery of 116.9 ± 34.2% and 60.6 ± 13.0%, respectively. In this study, the purity and recovery of wild-type (WT) and JAK3-deficient (KO) C57BL/6 mice CD4+T cells were obtained by a negative selection method using the CD4+T Cell Isolation Kit II (Miltenyi Biotec). Unlike magnetically labeling CD4+T cells in positive selection, Isolation Kit II isolates CD4+T cells from single cell suspensions of splenocytes by depleting non CD4+T cells. Next, a magnetic column and separator are used to separate CD4+T cells from the labeled non CD4+T cells. Isolated CD4+T cells are bead- and antibody-free and suitable for any downstream *in vitro* application. Our preliminary results showed 87.2 ± 6.9% (WT) and 76.3 ± 6.3% (KO) purity, and recovery of 54.4 ± 29.3% (WT) and 36.3 ± 13.6% (KO), respectively, indicating better purity and recovery rate in WT vs. KO mice.

THE INFLUENCE OF ATTACHMENT ON RELATIONSHIP CLOSENESS

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In this study, we examined the effects of adult attachment, relationship length, and partner behaviors on the odds that one will increase in closeness over the course of a discussion of a problem in the relationship. Married couples with relationship lengths of one year to fifteen years reported individual feelings of closeness and satisfaction prior to and following a discussion of a closeness or jealously problem in their relationship. Using hierarchical linear modeling, it was found that higher anxious attachment levels and longer relationship lengths were associated with an increased odds of increasing in closeness during the discussion, after controlling for avoidance, problem severity, and topic of discussion. Furthermore, stronger expression of negative behaviors by the partner was associated with a greater odds of increasing in closeness given that the participant was in a long relationship and had high anxious attachment. These results indicate that couples may adjust their behaviors and their closeness levels differently with increasing relationship length.

THE IN UTERO EFFECTS OF ATRAZINE ON THE OFFSPRING OF FEMALE *Mus musculus* EXPOSED DURING PREGNANCY

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Atrazine belongs to a triazine group of synthetic organic herbicides that is used in the control of broadleaf weeds, grassy weeds, and corn, and it has been found in groundwater especially in agricultural areas. It has been identified as an endocrine disrupter and several studies have linked it to adverse effects on humans based on studies with animals. In this experiment, *in utero* exposure was tested and its effects on the birth weight, packed cell volume (PCV), blood cell counts, and learning (utilizing a Morris Water Maze) of the exposed pups of the mouse *Mus musculus.* While there was a significant decrease in PCV, the birth weight was not significantly affected by the exposure to atrazine *in utero*. Also, atrazine did not have a significant effect on the learning as assayed by the Morris Water Maze.

ADENOVIRUS-MEDIATED INTERFERON-α THERAPY IN COMBINATION WITH CHEMORADIOTHERAPY TO TREAT PANCREATIC CANCER

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Pancreatic cancer remains as one of the deadliest diseases without a cure. Despite the emergence of adjuvant interferon-α (IFN) in recent clinical studies as a powerful treatment strategy for pancreatic ductal adenocarcinoma (PDAc), the excess toxicity and insufficient level of IFN in tumor site remain as serious challenges. The application of oncolytic adenovirus as a vehicle system enables local production of IFN at therapeutic concentration. We hypothesize that replication-competent adenovirus expressing IFN (Ad-IFN) in combination with either chemotherapy (5-FU) or radiotherapy would significantly enhance anti-cancer effect of existing IFN-based regimens while reducing toxicity. We analyzed potential of IFN as a chemo- and radiotherapy sensitizer and the antitumor effect of combination therapy. *In vitro* assays in human and hamster PDAc cells revealed that combination of Ad-IFN with either 5-FU or radiation killed cancer cells better than either of the single treatments. Furthermore, we established syngeneic pancreatic tumors in hamsters and treated them with a single dose of Ad-IFN followed by radiation (8 and 20 Gy). Ad-IFN combined with radiotherapy showed remarkable decrease in tumor volume and was significantly superior to single treatments of radiation and virus. At day 42, the tumors in the combination group nearly disappeared. Thus, we have the first report of the improved combination effect of Ad-IFN with radiotherapy and 5-FU. These results reinforce the impact of adenovirus-induced IFN expression to sensitize anti-tumor effect of chemotherapy and radiation. Such a strategy may change a paradigm of pancreatic cancer treatment.

NMR IN ADVANCED UNDERGRADUATE LABORATORY EXPERIMENTS: 2D SPECTRA, HETERONUCLEAR COUPLING, PARAMAGNETISM, AND VARIABLE TEMPERATURE EFFECTS

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Advanced laboratory experiments incorporating a wide variety of NMR techniques have been developed for use in chemistry major courses at St. Catherine University. This project is part of a larger effort to reframe the chemistry lab curriculum around four fundamental concepts: structure, mass, energy, and change. These lab experiments were designed to increase student expertise in more complex NMR methods including the use of two-dimensional spectra, selective decoupling, heteronuclear coupling, the Evans method to determine paramagnetism, and variable temperature affects. The structural information gained includes complicated structure assignments, geometry deduced from magnetism, consequences of structure on thermodynamics, and how heteronuclear NMR reveals unique information. The four labs developed will be implemented in advanced analytical, physical, and inorganic chemistry courses. Assessment tools have been created to measure the student outcomes of these experiments, which focus on gradual development of student understanding of how NMR reveals structural information.

WHAT DOES HYDROPHOBIC BEHAVIOR MEAN ON A MOLECULAR SCALE?

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Hydrophobic surfaces repel water, causing the water to bead up rather than spread out. Superhydrophobicity (SHP) is an extreme case of hydrophobic behavior. SHP is defined as a surface on which water drops bead up into nearly perfect spheres and have a droplet surface tangent relative to substrate greater than 150 degrees. This is defined as the contact angle. SHP surfaces repel water by minimizing its interaction with the water. To make a surface that repels water, we need two things: a hydrophobic top layer, and some surface roughness on the nanometer scale. To make our surface structures we attach silica nanoparticles to a glass slide with a sol-gel, and then coat the surface with monolayer of densely packed hydrophobic hydrocarbon chains. When complete the coating should have the transparency of the glass substrate. Using our procedure, we are able to produce SHP slides, with an average contact angle of 160 degrees. We have not been able to reduce the silica coating to a level where the coated slides would be highly transparent and our slides have a slightly frosted appearance. They also exhibit homogenous wetting behavior, also known as a Wenzel surface. Going forward we are interested in probing the molecular interactions of water with our SHP surfaces. Future work will involve studying the structure of the SHP surface, using atomic force measurements to understand exactly how it interacts with water.

BEACH ALMOND (*Terminalia catappa*) SEED RESOURCE SIZE AND RESOURCE PARTITIONING BETWEEN THE SEED PREDATORS SCARLET MACAW (*Ara macao*) AND VARIEGATED SQUIRREL (*Sciurus variegatoides*)

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Little is known of the ecological impacts of the exotic tree species tropical beach almond (*Terminalia catappa*) in the central Pacific of Costa Rica, but studies have found this species to be a potentially important resource for the recovery of threatened scarlet macaws (*Ara macao*). The goal of this study was to quantify the seed resource size provided by beach almond, estimate seed predation by variegated squirrels (*Sciurus variegatoides*) and scarlet macaws, and determine the extent of resource partitioning exhibited by the two predators. Reproductive phenology and seed predation were measured during March and April 2011 on a weekly basis for 111 beach almond trees. Seed productivity was quantified by visual surveys of flower, immature, and mature seed densities while seed predation was measured by collecting and sorting discarded shells by predation type. Seed production was large (about 160,712 seeds) while seed predation was low (about 32% of seeds). Macaw predation made up 67% of the seeds while squirrel predation accounted for 22% of the seeds. Evidence of spatial and temporal resource partitioning of the seed resource between squirrels and macaws was found. Scarlet macaws preferred to feed on the outer edge of trees while squirrels preferred to feed near the trunk. Macaws fed more often on trees with fewer mature seeds than squirrels. The resource size and occurrence of resource partitioning indicates that beach almond is an important food resource for scarlet macaws that should be considered in conservation efforts.

AN ANALYSIS OF ALLOCHTHONOUS AND AUTOCHTHNONOUS CONTRIBUTIONS TO BROOK TROUT (Salvelinus fontinalis) DIET USING HYDROGEN, NITROGEN AND CARBON STABLE ISOTOPES

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A dynamic interdependence exists between a river and its riparian zone. Studies have shown that reciprocal prey subsidies can make up a large proportion of consumer diet, and that these subsidies fluctuate seasonally. This explains, in part, why human induced landscape changes such as deforestation, urbanization and agriculture can significantly affect the survival and health of in-stream consumers like fish. Brook trout (Salvelinus fontinalis) a sensitive species that requires cool, clean and well-oxygenated water, have been particularly devastated by human land use. Stable isotopes of nitrogen, carbon and hydrogen, as well as fish gut contents, were analyzed to characterize the food web and to quantify the spatial and temporal variation in fish diet in a small trout stream in southeastern Minnesota. Contrary to our predictions, d13C and dD values remained relatively constant over time, suggesting that on a large scale brook trout rely on the same subsidies throughout the year. Furthermore, based on the isotope values, fish appear to be feeding on terrestrial prey more than on aquatic prey: dD and d13C values are closer to resources of terrestrial origin than aquatic resources (-125.19‰ and -25.40‰ respectively). Although, on average, fish diet does not exhibit temporal variation, identification of gut contents revealed slight changes in food utilization on a week-byweek basis that were not observed in the time-integrated isotope values. Future research on the turnover time of dD in fish tissue and contribution of environmental water to dD values will help refine this analysis further.

TECHNIQUES FOR OPTIMIZING *Comamonas* SP. STRAIN JS765 GROWTH AND NITROBENZENE DIOXYGENASE EXPRESSION FOR LARGE-SCALE PURIFICATION

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Nitrobenzene (NB), an EPA priority pollutant and carcinogen, is a chemical that is used for industrial production of lubricating oils, drugs, dyes, explosives, pesticides, and synthetic rubber and has been found in soil surrounding many manufacturing plants in the United States. The organism *Comamonas* sp. Strain JS765 was isolated from a New Jersey manufacturing plant and shown to degrade nitrobenzene through the use of the nitrobenzene dioxygenase enzyme system (NBDOS). In order to accomplish the future goal of isolating and studying the three enzymes that constitute this system – reductase (NBDR), ferredoxin (NBDF) and oxygenase (NBDO) – several growth methods were tested to optimize growth of the bacteria and expression of NBDOS. The bacteria were grown in enriched media, enriched media with nitrobenzene, and minimal media containing nitrobenzene. Expression was monitored by SDS-PAGE and NB-dependent oxygen-consumption activity. Although oxygen consumption was detected in extracts grown in enriched media, attempts to quantify NB-dependent activity were prevented due to NB-independent activity. In addition, the SDS-PAGE results from extracts of *Comamonas* grown on minimal media were inconclusive due to insufficient resolution on the gel. Therefore, a GC-based assay was successfully developed for quantifying NB-consumption activity in *Comamonas* extracts, and the components of the NBDOS were partially purified by anion exchange chromatography to facilitate the SDS-PAGE analysis.

AGE, SIZE DISTRIBUTION, AND ABUNDANCE OF GREEN SUNFISH (*Lepomis cyanellus*) IN GILMORE CREEK

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Lower Gilmore Creek is a stretch beginning under U.S. Highway 14 and continuing through the Saint Mary's University of Minnesota campus in southeastern Minnesota. This section of the cold-water stream supports primarily brown trout (*Salmo trutta*) and slimy sculpin (*Cottus cognatus*). Two extreme late summer floods in Gilmore Creek occurred over a ten-year period resulting in large numbers of green sunfish (*Lepomis cyanellus*), which probably moved at least 1.5 km from Boller Lake, a lentic area downstream. Historical data show that after the 2004 and 2007 floods, green sunfish made up roughly 50% of the total catch in Gilmore Creek. The most recent population estimate done in September 2010 as a preliminary survey for the study yielded over 170 green sunfish, composing over 30% of the fish caught. A periodic evaluation of the current green sunfish population in Gilmore Creek will determine the age-length frequency for the sunfish population and establish a timeline for disappearance of green sunfish from the creek in order to analyze the departure rate for each year class present. If the sunfish overwinter in Gilmore Creek, there might be a risk that they consume trout eggs in the fall and trout fry in the spring. A comparison of length distributions to previous flood data will be used to determine the similarity in distributions throughout the years. Since Gilmore Creek is a cold-water trout stream, it is vital to examine the presence of the sunfish due to the danger they may pose to the existing brown trout populations.

3' POLY(U) TAILS ON MYXOMYCETE SLIME MOLD MITOCHONDRIAL mRNAS

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The myxomycete slime molds use several unique forms of RNA modification on their mitochondrial transcripts, including five varieties of RNA editing, plus poly(U) tailing. Prior investigations have demonstrated that poly(A) tails are missing from at least two mitochondrial mRNAs in *Physarum polycephalum* and one mRNA in *Stemonitis flavogenita*. Instead, non-encoded poly(U) tails of varying length were discovered at the 3' ends of these transcripts. Using a modified "anchor" approach, we are investigating this rare phenomenon by amplifying additional mitochondrial mRNAs in two myxomycete species. We have isolated total RNA from *Physarum polycephalum* and *Didymium nigripes*, and then used yeast poly(A) polymerase enzyme *in vitro*, supplied with only GTP nucleotides to add 3' poly(G) extensions to the pools of RNAs. We reverse transcribed with a tagged poly(C) primer to create cDNAs with one known end sequence. We are PCR amplifying selected transcripts from the pools using "internal" forward primers and a reverse primer corresponding to the known "tag" sequence. We will clone and sequence our products to determine the sequence of the 3' UTR of these mRNAs.

THE EFFECTS OF ATRAZINE ON REPRODUCTIVE ORGANS OF *Phasianus colchicus*

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Atrazine is a commonly used herbicide in the United States that is applied to crops in order to provide protection against various species of weeds. Currently, EPA regulations state that levels of this chemical should not exceed 0.003 mg/L or 3 ppb. Due to the nature of the use of this chemical, the application to crops allows for the possibility of contamination of nearby watershed, ground water, and rain puddles, which may lead wildlife to be exposed to atrazine. Research regarding this chemical has shown that atrazine exposure in amphibians, reptiles, and avian species can lead to negative outcomes including decreased pack cell volume and endocrine disruption. The purpose of this study was to determine the effects of atrazine on the reproductive organs of *Phasianus colchicus* (pheasant) and *Gallus gallus* (chicken). The avian species were randomly placed in three groups. The control group received no treatment. The low-dose group was exposed to atrazine at a concentration of 30 ppb and the high group was exposed to 300 ppb. The objectives of this experiment included determination of the genotypic sex of the avian species using PCR amplification of the CDH1 isotype. Phenotypic determination of the reproductive organs was analyzed via dissection and photographic examination. Comparison of the genotypic sex to the phenotypic structure of the reproductive organs was used to examine the impact of the chemical on the reproductive tissues.

INDIRUBIN DERIVATIVES AS INHIBITORS OF THE PARASITE *Toxoplasma gondii* – STRUCTURE-ACTIVITY-RELATIONSHIP OF THE 6-POSITION

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Toxoplasma gondii (*T. gondii*) is an intracellular parasite. Though its definitive host is in cats, humans can serve as an intermediate host. With a functioning immune system, *T. gondii* presents itself through toxoplasmosis with very minor flu-like symptoms. However, in HIV-positive individuals, in patients undergoing chemotherapy, or in organ-transplant recipients, toxoplasmosis can induce severe symptoms including death. Current treatments for toxoplasmosis are not well tolerated. Therefore, new treatments are needed. We found that derivatives of the natural product indirubin inhibit the life cycle of the *T. gondii* in vitro. Subsituents in the 6-position of indirubin appear to be crucial for potency. However, careful structure-activity studies have not been performed. This project aims to synthesize derivatives of indirubin derivatives in order to conclusively characterize the structure-activity relationship of the 6-position of this class of compounds.

SURVEY OF FOSSILS FOUND IN FRANCIS CREEK SHALE NEAR MAZON CREEK, ILLINOIS

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Saint Mary's University of Minnesota has a large collection of Francis Creek Shale fossils from near Mazon Creek, Illinois. Mazon Creek fossils are well known in the field of paleontology because they allow scientists an almost unmatched glimpse into what the Illinois area was like during the Moscovian Pennsylvanian Period approximately 300 million years ago. Unique conditions within the riverine habitat, swamps, and shallow marine bays allowed for both hard and soft tissue to be preserved. Even numerous jellyfish remains are found in the Mazon Creek locations; they are rarely found elsewhere. The Saint Mary's University collection was sorted, identified, counted, and cataloged based on descriptions from relevant literature. There were 912 fossils in the

collection—292 from complete nodules (all pieces present) and 620 from incomplete nodules (at least 10% of nodule missing). The Saint Mary's collection had 26% lycopsids (ancient club mosses), 23% articulates (ancient horsetails), 21% seed ferns (foliage-like true ferns but reproducing through seeds), 15% true ferns (ancient ferns with spores on undersides of leaves), 5% cordaiteans (early conifers), 5% fauna (animal fossils), 1% sphenopterid (an underbrush genus) and 4% unknown or mixed fossils. This differed significantly from the literature's breakdown of fossil types. Several intact nodules were opened using the "freeze-thaw technique," revealing foliage of *Annularia stellata* (an ancient relative of the modern horsetails) and a coprolite (a fossilized fecal pellet).

DEVELOPMENT OF AN IMMUNOLOGICAL LABORATORY EXERCISE MEASURING CELL PROLIFERATION AND CYTOKINE PRODUCTION

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Few immunology lab manuals are available for instructors, leading educators to develop in-house laboratory exercises that best suit their course. As an undergraduate biology major at Saint Mary's University of Minnesota, I was interested in developing a student-friendly laboratory exercise that would allow Saint Mary's immunology students to learn basic immunological techniques using available resources. In particular, my aim was to design a lab exercise in which students would gain expertise in measuring cell proliferation and cytokine production. Murine splenocytes were added to 96-well tissue culture plates. Different concentrations of the T cell mitogen, concanavalin A, were added to the wells, and the plates were incubated at 37^{o}C and 5% CO_2. At 72 hours post-stimulation, supernatant was collected from the wells and assayed for the presence of the T cell growth factor, interleukin 2 (IL-2), using an Immunoassay, and cell proliferation was measured using the TACS XTT Assay. I was able to determine the optimal cell concentration and ConA dose that led to significant cell proliferation and IL-2 production in mitogen-stimulated splenocytes. The procedure I developed has been written up as a laboratory exercise and will be used in Saint Mary's immunology course this spring semester.

QUANTIFYING AND EXPLAINING UNCERTAINTY IN CHEMICAL CALIBRATION: METHODS FOR LINEAR AND QUADRATIC DATA

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Calibration of analytical instruments using regression is a frequently used tool in chemistry research. In calibration, the relationship between concentration and response variables is estimated using regression, and then the response of an unknown is used to predict its concentration. Because the response variable is used to calculate the explanatory variable, the associated error in the concentration is complex, particularly when the relationship is nonlinear. We studied and compared several approaches to estimating error in linear calibration, including an analytically derived mathematical expression and bootstrapping. Both methods gave comparable results: the 95% confidence interval of the slope of 1000 bootstrap replications of a calibration line had approximately 89% coverage, and the coverage for the same interval calculated from the mathematical expression was 92.2%. We applied these methods to simulated data to study the effect of the number and range of standards on the uncertainty. Four to five standards, measured at least four times and spanning the range of likely concentrations, were needed to constrain the calibration error to a reasonable level. The bootstrapping method was extended to quadratic calibration curves, as an example of a nonlinear case. We implemented an additional method developed by Tellinghuisen (2000) and Salter (2002) for estimating uncertainty in nonlinear calibration in Excel. This method includes the unknown concentration as a parameter in the regression and extends the covariance and design matrices accordingly. Lastly, we created instruction guides and an informational poster for students in introductory analytical chemistry labs to understand and use these methods.

IN VIVO BISPHENOL-A EXPOSURE MODULATES MURINE INNATE IMMUNE RESPONSES

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Bisphenol-A (BPA) monomer is used in the production of polycarbonate plastics. Oral BPA exposure can be measured in the urine, blood, and amniotic fluids of humans and is widespread due to its presence in the lining of food and beverage containers. BPA's endocrine-

disrupting activity links it to altered physiology in humans and mice, including immune system effects. BPA mediates its effects by binding to estrogen receptors, which are expressed by immune cells. Our previous work showed that low-dose BPA exposure decreased secretion of the TH-1 cytokine IFNγ in both C57BL/6 and autoimmune-prone NZB/WF1 mice, altering the adaptive immune response. We have now examined the effect of *in vivo* exposure on innate immunity in 4-week-old male BL/6 mice fed daily with BPA dissolved in methanol at a dose of 2.5µg, 25µg, or 250µg /kg body weight for 10 to 21 days. We measured splenic LPS-induced COX2 expression levels by real-time RT-PCR. The COX-2 enzyme is involved in the PGE2 synthesis pathway, and mice fed BPA showed an upregulation in COX-2 gene expression and a corresponding increase in PGE_2 release by LPS-stimulated splenic immune cells as measured by ELISA. PGE2 has complicated effects on the immune system; it has been shown to dampen innate immune responses, including the function of neutrophils, and to decrease TH1 cytokine release. We also observed a dose-dependent decrease in the release of KC chemokine, a neutrophil-attracting cytokine. Together our results suggest a modulation of the innate immune response following oral BPA exposure.

INCORPORATION OF GREEN CHEMISTRY METRICS INTO INDEPENDENT SYNTHESIS PROJECTS

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Green chemistry aims to eliminate hazardous substances with less hazardous substances. With reduction of chemical release by humans, ozone depletion, global warming, smog, and water pollution can be decreased. Just as important, completing less hazardous reactions reduces costs to a company's bottom line. Often, green chemistry experiments are included in the organic chemistry curriculum but students are given a reaction to complete along with reasons why the reaction is greener than an alternative. In this curricular study, organic chemistry students are required to propose their own green alternatives by completing a three-step synthesis multiple ways and determining the greenest route. In order to increase the greenness of a reaction, students used alternative solvents and alternative reagents to complete two of the steps of their synthesis. The products were purified, characterized, and compared between the original reaction and the green reaction. A metric system was used to compare the greenness of the reactions. If there were relative decreases in the combination of factors such as cost, environmental factor, yield, and waste produced, the reaction was considered greener. Teaching students the importance and relevance of green chemistry is imperative to the future of chemistry and the environment.

ESTIMATING MEADOW VOLE AND PRAIRIE VOLE POPULATIONS WITHIN FOUR RECONSTRUCTED GRASSLAND TYPES: DOES THE TYPE OF RECONSTRUCTION MATTER?

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Grassland reconstructions can vary significantly in their composition, and vegetation may play an important role in animal diversity. Small-mammal trapping occurred at the Schottler State Wildlife Management Area (Austin, MN) in mid-summer of 2011. Plots consisted of four vegetation types: warm-season grass, cool-season grass, forbs, and tall forbs. Animals captured included meadow vole (*Microtus pennsylvanicus*), prairie vole (*Microtus ochrogaster*), field mouse (*Peromyscus* sp), meadow jumping mouse (*Zapus hudsonius*), and northern short-tailed shrew (*Blarina brevicauda*). Two species, meadow vole (*Microtus pennsylvanicus*) and prairie vole (*Microtus ochrogaster*), were the most frequently captured. The POPAN model in the program MARK was used to estimate population abundances within each plot type. ANOVA tests showed no significant differences between the four vegetation plot types within the meadow vole population ($F = 2.045$, $df = 3, 68$, $P = 0.116$) and a significant difference in prairie vole population within the different vegetation types ($F = 3.814$, $df = 3, 68$, $P = 0.014$). A Tukey HSD Post-Hoc test found the significant difference was between the cool-season grass and forb plot types ($P = 0.008$). My research suggests that vole abundance is not strongly influenced by vegetation type and may vary by species.

REAL-TIME REVERSE TRANSCRIPTION PCR TECHNIQUES AND ANALYSIS

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One of the most effective and powerful methods for analyzing cell gene expression is to quantify the mRNA transcripts within the cell. Real-Time reverse transcription PCR (qRT-PCR) is a method that converts target mRNA into cDNA, amplifies the cDNA template, and proceeds to measure the level of fluorescence using primer-based or probe-based detection systems. By measuring the fluorescence levels of the qRT-PCR reaction in real time (after each PCR cycle) the starting material of template cDNA can be back-calculated using the threshold cycle number (C_t) and compared to a reference sample. The purpose of this project is to present

a reliable qRT-PCR protocol in which a target gene in a cancerous tissue sample is compared to a healthy tissue sample from the same patient. The method involves a one-step qRT-PCR reaction that combines the cDNA synthesis step with the qPCR reaction in one tube. Additionally, the gene of interest in each sample will be amplified along with a normalizer gene to correct for differences in RNA amounts added to each reaction.

RESPONSE OF LIPOXYGENASE EXPRESSION TO WOUNDING AND METHYL JASMONATE IN SOYBEAN LEAVES (*Glycine max*)

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Lipoxygenases (LOXs) are enzymes which catalyze the peroxidation of polyunsaturated fatty acids. They function in development, growth, and response to pathogenic attack including mechanical wounding. There are multiple isozymes of LOX in soybean. It has been reported that each isozyme has a distinct function in different stages of growth. Studies have shown that the expression of some isoforms of LOX is enhanced after wounding or treatment with jasmonic acid (JA) or methyl jasmonate (MJ), a mediator of plant defense mechanism. This project investigated the relationship between wounding, and MJ treatment using Quantitative Polymerase Chain Reaction. Soybean (*Glycine max*) plants were raised in a growth chamber. When the plants reached the bifoliate stage, two sets of experimental plants were used. One set was wounded by crimping the leaves with a clamp. The other set was not wounded. Both sets of experimental plants were sealed in an aquarium containing MJ vapor. Control plants were sealed in an aquarium with no MJ. Leaves were harvested at 0, 3, 6, and 24 hours after treatment. RNA was isolated from the samples using an RNeasy Plant Minikit from Qiagen. RNA quantities were estimated from the absorbance at 260 nm. A High-Capacity cDNA Reverse Transcription kit from Applied Biosystems was used to make cDNA copies of the mRNAs. Relative quantities of the LOX mRNA were measured by qPCR on a Plus One Real-Time PCR System from Applied Biosystems. SYBR green was used to detect the PCR products.

ROOM TEMPERATURE STORAGE OF SERA BIOSPECIMENS

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Biorepositories worldwide collect human serum samples and store them for future research studies. Currently, there are hundreds of biorepositories containing millions of biospecimens. These biospecimens are stored in freezers at -20˚C, -40˚C, -80˚C, or in liquid nitrogen for years. These freezers are expensive to purchase and maintain. Freezers also impose freezing stresses on the biospecimens, potentially damaging them. Studies have shown that certain cancer biomarker proteins are severely damaged by frozen-state storage. To decrease both cost of storage and freezing stresses, research is currently being conducted to use isothermal vitrification in the storage of human serum at room temperature. When serum is vitrified, biochemical reactions can be stopped, the specimen ceases to degrade, and macromolecules become stabilized without exposure to extreme stresses induced by frozen-state storage. Data collected from serum using Fourier Transform Infrared Spectroscopy have shown that the vitrified state can be reached at 3˚C ± 2˚C when 0.8 M trehalose and 0.1 M dextran is added and the sample is vacuum-dried for two hours. Results also show serum, vitrified and stored on glass fiber filter papers, appears to have minimal protein degradation when stored for up to one month. Finally, up to 90% of all proteins can be recovered after stored on glass filter papers, and recovery percentages have not been shown to decrease with time. These results show high feasibility for long-term storage of serum at elevated temperatures.

DEVELOPMENT OF IMMUNOLOGICAL ASSAYS FOR THE FATHEAD MINNOW *(Pimephales promelas*), A MODEL SPECIES OF AQUATIC TOXICOLOGY

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The ubiquitous presence of pollutants in the aquatic environment is of growing concern. Recent studies suggest that in addition to well-established histopathologic, reproductive, and behavioral effects, attenuation of the immune response may result from pollutant exposure. Loss of immunity could be explained by a decrease in functional T or B lymphocytes. The fathead minnow (*Pimephales promelas*), a small fish species, is often used as a bioindicator for pollution. However, there are no established protocols or reagents available for quantifying the immune response in this model. To address this issue, we assessed the efficacy of 11 antibodies (2 α-flounder IgM, 3 α-trout IgM, α-trout IgD, α-striped bass light chain, α-CD3, α-CTLA4, α-LCK, and α-CD11) obtained from the Veterinary Immunology Network for flow cytometric analysis of fathead minnow lymphocytes. Antibodies were tested against lymphocytes obtained from the spleens and anterior kidneys of 110 fathead minnows. Positive staining was observed with two antibodies—α-CD3 and α-striped bass light chain, indicating their ability to bind T and B lymphocytes, respectively. We next examined the proliferative capacity of three mitogens—Concanavalin A (ConA), Lipopolysaccharide (LPS), and

Phytohaemagglutinin (PHA) on fathead minnow lymphocytes. Lymphocytes were isolated from the spleens of ~20 fish and cultured with Con A (1-10 µg/mL), LPS (1-40 µg/mL), or PHA (1-20 µg/mL). Proliferation was measured by Alamar Blue post exposure to mitogens for 3-6 days. Use of these assays in future experiments will greatly augment our understanding of the consequences of aquatic pollution on exposed organisms.

POPULATION GENETIC INSIGHTS INTO SCALY PEARL OYSTER (*Pinctada longisquamosa*)

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The scaly pearl oyster *Pinctada longisquamosa* is a sessile marine bivalve that exhibits protandrous hermaphroditism, maturing as male and switching to female later in the life history. This species can be found in isolated saltwater ponds of San Salvador Island, Bahamas. The oyster populations of different ponds have been found to exhibit different size at sex-switching and, therefore, different sex ratios. Oyster tissues from Oyster Pond, Mermaid Pond, Six-Pack Pond, and a Florida coastal site were collected by colleagues, and DNA was extracted for genetic analysis. Our goal was to assess whether or not there are evolved, genetic differences among the three Bahamian oyster populations that can potentially explain the different sex ratios observed. Polymerase Chain Reaction was used to amplify DNA at several nuclear microsatellite loci and of the mitochondrial protein-coding gene, cytochrome c oxidase I (COI). Nuclear microsatellite loci did not amplify well, perhaps because the primers used were designed from a closely related yet different species. However, we obtained COI sequences for 37 oyster specimens representing all four sites, and analyzed them along with 22 COI sequences obtained in a previous study. The resulting phylogenetic tree provided information on genetic diversity within and among oyster populations.

AZTECA ANTS CONNECT ABOVEGROUND AND BELOWGROUND PROCESSES IN A WET TROPICAL FOREST

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Ecosystem function in terrestrial systems often depends on linkages between aboveground and belowground processes. Links between aboveground and belowground systems can have disproportionate impacts on ecosystem function if nutrients produced or released in one subsystem (e.g., labile carbon through photosynthesis) limit productivity in the other. Organisms that amplify such linkages are thus important research foci, particularly in hyperdiverse systems like wet tropical forests. Here, we report a study conducted on Barro Colorado Island in Panama in which we tested whether refuse generated from colonies of the ant Azteca trigona affects decomposition rates and arthropod community structure in a brown food web. Azteca trigona build large, hanging, conical nests, which funnel refuse from colony activities to the leaf litter community below. We predicted that 1) decomposition rates would be faster under Azteca nests, and 2) this accelerated decomposition would be caused by refuse addition. We found support for the first prediction using an observational study, which showed that cellulose and wood decomposition rates were significantly higher under Azteca nests than in locations 10m away from the nest. We found support for the second prediction in an experimental study in which we added either refuse, soil, or nothing to randomly selected forest plots over the course of six weeks, and measured the effect of these treatments on decomposition. We found that the addition of refuse (but not soil) significantly increased decomposition rates of both cellulose and wood. Effects were large; for example, refuse addition in the experimental study increased cellulose decomposition by 75% after 4 weeks. Refuse effects on decomposition were associated with increased arthropod abundance and changes in arthropod community structure. Given the magnitude and scope of their impact, our results suggest that Azteca ants may be an important connector of ecosystem processes in this system.

IS THE DNA DEAMINASE APOBEC3H AN HIV-1 RESTRICTION FACTOR?

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We are protected from potentially pathogenic viruses by various immune defenses including cellular DNA deaminase proteins called APOBEC3s. One of these proteins in particular, APOBEC3H, is contested as an HIV-1 restriction factor. In previous studies APOBEC3H mRNA has been identified in blood cells, including the main target cells of HIV-1 ($CD4^+$ T cells); however, the resulting protein remains to be detected. To begin to resolve this question of whether APOBEC3H is in a position within the immune system to be an effective inhibitor of HIV-1, we are developing anti-APOBEC3H monoclonal antibodies. Here we provide a comprehensive analysis of four recently developed anti-APOBEC3H monoclonal antibodies: P1H6-1, P1D8-1, P3A1-1, and P3A3-A10. By immunoblotting the entire human APOBEC3 family, we have determined that these antibodies are specific for the APOBEC3H protein. By

immunoblotting a series of human/cow chimeric APOBEC3H proteins, we have shown that P1D8-1 binds to the N terminal region of APOBEC3H and the remaining three antibodies bind the C terminal region. Isotyping of the antibodies by enzyme-linked immunosorbent assay revealed that P1H6-1, P3A1-1, and P3A3-A10 are of the isotype subclass IgG2a with Igκ light chain. Additionally, I tested these antibodies for efficacy in immunofluorescent microscopy, immunoprecipitation, and flow cytometry. Here we present these data that now position us to use anti-APOBEC3H antibodies to test the hypothesis that APOBEC3H is expressed in HIV-1 relevant cells and that it does indeed contribute to HIV-1 restriction.

EXAMINATION OF THE EFFECTS OF *FGF10* OVER-EXPRESSION ON THE DEVELOPMENT OF THE LUNG IN THE FROG *XENOPUS LAEVIS*

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There were two main objectives for this research into Xenopus laevis lung development. The first objective was to determine how over-expression of *Fgf10* affected the expression of the lung specific genes *Spc* and *Spb*. This was done by creating a plasmid containing *Fgf10* that was under the control of an inducible promoter and utilizing microinjection to introduce the plasmid into the frog embryos at the 16-cell stage. This was tested through the use of real-time PCR to determine the expression levels of *Spc* and *Spb*. The second objective was to determine if the over-expressed *Fgf10* changed the size and/or shape of the developing lung through the use of various visualization techniques, including embryonic whole-mount *in situ* hybridization. In conclusion, after creating an inducible plasmid containing *Fgf10* and microinjecting it into developing Xenopus laevis embryos, for some currently unknown reason, it was found that the over-expression of *Fgf10* had no effect on Xenopus laevis lung development.

CARBOXYMETHYL CELLULASE ACTIVITY MEASUREMENT AND ISOLATION IN *Sinorhizobia*

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Carboxymethyl Cellulase is an enzyme used by the bacterium *Sinorhizobia* to break down cellulose into simpler sugars. It has been found to be essential in infecting the root hairs of legume plants in order to stimulate symbiosis (Chen et al.). This type of cellulase must be used in specific concentrations so that it does not adversely affect its plant host (Robledo et al.). Our study looked at the presence of, and level of activity of carboxymethyl cellulase in different strains of *Sinorhizobia.* We found that many of the strains can degrade Carboxymethyl Cellulose "CMC," by using an activity assay in which petri dishes containing media with CMC are streaked with bacteria, and then stained several days later. The stain Congo red binds to CMC, and when it is washed off clearing zones can be seen where CMC has been degraded. These zones were measured and averaged for activity level. The presence of CMCase, coded by the gene Smed_5210, was shown by amplifying the gene using the Polymerase Chain Reaction. The data found using PCR were compared to an online database provided by Genoscope Mage, and strains from our fellow Dr. Michael Sadowsky at the University of Minnesota. A future goal of our study is to over-express the Smed_5210 gene by cloning it into *E. coli.* Once completed, the protein CMCase can be isolated and used in further experimentation.

AN INTRODUCTION TO ACID/BASE CONCEPTS USING KITCHEN CHEMISTRY IN THE HIGH SCHOOL CLASSROOM

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The focus of this experiment was to develop an application-based lab which would use reactions that occur during the cooking process to introduce the concepts of acids and bases to high school students. Acid/base chemistry is visible during the cooking of green fruits and vegetables because chlorophyll, the pigment responsible for the green color, exhibits different coloration in various pH environments. A week-long module was developed using the basis of cooking peas to explain acids and bases. This module was then field tested in two different, but comparable, classes at Stewartville High School in Minnesota. Each class performed three separate experiments: testing the pH of common household items; changing the pH of water; and changing the color of peas. Pre- and post-tests were used to gauge the effectiveness of the module and satisfaction level of the students. The results were analyzed using a Likert-type scale and, overall, the students' understanding of acids and bases increased by an average of 24.4% and their interest level for this module was at 90%. The results from this experiment indicate that using kitchen chemistry to introduce acid/base chemistry is effective and the practical application of this lab engages the majority of students.

THE CHRONOTROPIC EFFECTS OF FIVE HERBAL UTEROTONICS ON *Rattus norvegicus* IN VITRO

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Herbal supplements such as *Caulophyllum thalictroides* (blue cohosh), *Cimicifuga racemosa* (black cohosh), *Rubus idaeus* (red raspberry), *Mitchella repens* (squaw vine), and *Capsella bursa-pastoris* (shepherd's purse) have traditionally been used as an aid in childbirth due to their apparent uterotonic properties. However, few clinical studies have been performed to determine whether there are any potential side effects associated with the use of these herbs. The purpose of this investigation was to determine if these supplements had any chronotropic effects on isolated cardiac tissue. Rat hearts (n = 24) were isolated and observed in a smooth-muscle bath. Ventricular activity ceased after approximately 3 minutes, and atrial activity continued. If the heart stopped, cardiac massage and/or epinephrine was given to re-initiate pacemaker activity. After atrial rhythmicity was stabilized, herbal aqueous extracts (30 mg/ml) were administered directly to the right atrium. This dose has been demonstrated to strongly contract uterine strips *in vitro*. All treatment hearts stopped by 14 minutes. Two of the three control hearts continued to beat for over 23 minutes, and their decreased heart rate was not significantly dropped over time ($P = 0.9996$). All treatments demonstrated a significant negative chronotropic effect ($P < 0.05$). Red raspberry was the only treatment that exhibited a positive chronotropic effect (at 2 minutes only, $P = 0.0032$). These results may imply that *in vivo* high doses of any of these treatments may result in bradycardia if used to augment labor.

CYCLODEXTRIN-CONTAINING AIR FRESHENERS: A NEW PATHWAY FOR INHALING POLLUTANTS?

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The indoor environment contains many pollutants that can be damaging to health and offensive to human senses. A new generation of air fresheners contains beta-cyclodextrin (β-CD), a cyclic glucose oligomer that is reported by manufacturers to "eliminate" odors—rather than masking them with fragrances—by trapping offensive-smelling molecules in its core, rendering them scent-free. Cyclodextrins engage in non-covalent host-guest interactions with pollutant guest molecules to sequester them from the surroundings. These binding characteristics are well studied in the aqueous phase but not in the aerosol phase, where meaningful interaction between air freshener spray and pollutant molecules occurs in the home. In this study, the equilibrium and mechanism of cyclodextrin-pollutant complexation were studied in solution and in the aerosol phase using spectroscopy and mass spectrometry techniques. Aerosol Time-of-Flight Mass Spectrometry was used to collect mass spectra of aerosolized mixtures of β-CD and simulated indoor pollutants, which were shown to complex in solution using spectroscopic methods. Markers that indicate complexation of the pollutant simulants in cyclodextrin-containing mixtures have been identified in the ATOFMS spectra, leading to the possibility of identifying beta-cyclodextrin-pollutant complexes in real-world particles.

CLONING AND SEQUENCE ANALYSIS OF A GENE IN THE GLYCOLYTIC PATHWAY OF *Arabidopsis thaliana* AND *Phragmites australis*

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An enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), mediates a key step in glycolysis. The GAPDH gene has a conserved nucleotide sequence, which has been examined in a wide range of species due to its presence and importance in all organisms. In the present research, a GAPDH gene was studied in two species of plants, *Arabidopsis thaliana* and *Phragmites australis*. The entire *A. thaliana* genome has previously been sequenced; however, the GAPDH sequence for *P. australis* is not available in the primary repository of gene sequences, the National Center for Biotechnology Information (NCBI). Two haplotypes of *P. australis* were studied: a native North American haplotype and an invasive non-native Eurasian haplotype. The GAPDH gene was amplified via nested polymerase chain reaction, ligated into a plasmid vector and cloned in *E. coli.* Gel electrophoresis verified that the length of cloned fragments of *A. thaliana* agreed with published research. The length of *P. australis* haplotypes was about 1200 base pairs. Following sequencing of the cloned DNA fragments, the *A. thaliana* sequence was found to correspond to the sequence in NCBI. The *P. australis* sequences were similar but not identical. The North American and Eurasian haplotypes became geographically isolated about 10,000 years ago, so the similarity in the highly conserved GAPDH gene was not unexpected.

UV-DERIVED STAR FORMATION RATES IN NEARBY STARBURST DWARF GALAXIES

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Starburst events are periods of unsustainable star formation that will affect the structure, chemical composition, and evolution of the host galaxy. Metal poor and gas rich, nearby dwarf galaxies act as both building blocks of larger galaxies in the hierarchical model and as nearby proxies for young galaxies in the early universe. New examinations show that starburst phases may last much longer than previously thought (200 Myr or longer), propagating throughout the galaxy through mechanisms that are not well understood (McQuinn et al. 2009, 2010). This research presents an derivation of current star formation rates using new and archival GALEX Far-UV and Near-UV images and archival SPITZER MIPS (24 μm, 70 μm, and 160 μm) images of 19 nearby starburst dwarf galaxies from the sample studied by McQuinn et al. (2010a, b). These galaxies all have recent star formation histories derived from Hubble Space Telescope (HST) imaging of resolved stars. We performed background subtractions, cropping to fit HST fields of view, and masking of foreground stars and background galaxies. Through empirical relations, measurements of the UV emission and corrections from IR flux estimates yield current star formation rates. Comparing these current star formation rates to the average star formation rates derived from the optically resolved stellar populations for a variety of timescales, our results show very good agreement for star formation rates averaged over the past 150 Myr.

NICHE SPECIALIZATION AND REDUCED MITOCHONDRIAL INTROGRESSION IN A HYBRIDIZING COMPLEX OF MAP TURTLES

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Because reproductive isolation is a key component of speciation, hybridization between evolutionarily distinct species is a valuable tool to study the origin and maintenance of new species in nature. Previous studies have emphasized genetic and behavioral causes of reproductive speciation. Theory predicts that ecological forces such as feeding niche specialization may also lead to reproductive isolation between species. Specifically, if hybrid offspring exhibit a phenotype intermediate between that of their parents, they may be less effective at exploiting either parental niche, preventing gene flow and promoting reproductive isolation. Despite this strong theoretical prediction, it has been difficult to isolate ecological specialization as a factor driving reproductive isolation because it often co-occurs with genetic and behavioral divergence.

A complex of interbreeding map turtles (*Graptemys geographica* and *G. pseudogeographica*) in the Mississippi River provides a unique opportunity to study ecological speciation. Genetic evidence of introgression has shown that behavioral and genetic barriers to gene flow are absent between the species; however, these sympatric species have remained evolutionarily distinct. Furthermore, if dietary specialization is sexually dimorphic, differential rates of introgression should be expected between mitochondrial and nuclear markers.

By pairing stable isotope analysis with genetic analyses, it is possible to determine how ecological factors can affect the maintenance of reproductive isolation in these species. In particular, stable isotope analysis can elucidate differences between species and between sexes, while genetic analyses are capable of determining if those differences are correlated to differing rates of introgression in the mitochondrial and nuclear genome.

SYNTHESIS OF A NON-NATURAL SUBSTRATE FOR PFTASE

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Protein Farnesyltransferase (PFTase) has numerous natural and non-natural substrates. PFTase covalently links these substrates to proteins and peptides ending in the amino acid sequence CVIA by a prenylation reaction. Three steps of the synthesis of a non-natural substrate have successfully been completed. Tetrazine, which can undergo a click reaction with cyclooctene and norbornene strained rings, was also made but has not yet been purified. Through this research, we are trying to determine the identity of new substrates for PFTase that include analogs with strained rings. Discovering new non-natural substrates for PFTase will create the ability for bioconjugation without using toxic copper catalysts.

DISTRIBUTION OF NORTHERN BROOK LAMPREY (*Ichthyomyzon fossor*) IN THE UPPER IOWA RIVER DRAINAGE

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The northern brook lamprey (*Ichthyomyzon fossor*) is a non-parasitic species with a scattered geographic distribution in the Great Lakes, upper

Mississippi River, and Hudson Bay drainages. The only previous report of this species in Iowa is from Coffins Creek in the Maquoketa River drainage. However, surveys for brook lampreys in southeastern Minnesota have revealed northern brook lampreys in the Upper Iowa River not far upstream from where it enters Iowa. The purpose of this ongoing study is to survey the Iowa portion of the Upper Iowa River drainage for northern brook lampreys. Sampling has been conducted with a backpack electrofisher (pulsed DC), but high water levels during much of the last few years have limited accessibility at some sites. So far, northern brook lampreys have been collected at several locations from Chester upstream to the border, but they have not yet been collected in the Upper Iowa River or tributaries downstream from the dam at the Lidtke Mill. Northern brook lampreys in the Upper Iowa River and in other locations in southeastern Minnesota occur with American brook lampreys (*Lethenteron appendix*) and may be confused with that species in the field. Future sampling at locations where American brook lampreys have been reported may reveal additional populations of northern brook lampreys. As has been revealed for American brook lampreys in southeastern Minnesota, northern brook lampreys in southeastern Minnesota and Iowa tend to reach larger sizes than elsewhere in their range.

RESOURCE AND WATER COMPETITION IN CREOSOTEBUSH *(Larrea tridentata)* AND TRIANGLE-LEAF BURSAGE *(Ambrosia deltoidea)*

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How organisms compete for limited resources is a fundamental question in ecology. Limited in water and nutrients, the desert is an ideal environment to study resource competition among plants. Creosotebush, *Larrea tridentata,* has been studied extensively for its root development and intraspecific spacing, although little is known regarding its interspecific competitiveness. Previous studies indicate that inhibition of growth of neighboring plant roots via allelopathy may be one mechanism by which creosotebush competes for resources. In the desert southwest, we studied plant spacing in washes (water-supplemented environments) versus non-wash environments. We used stable isotope analyses of nitrogen and carbon to compare resource limitation in solitary creosotebush to creosotebushes that were nursing triangle-leaf bursage (*Ambrosia deltoidea*). These metrics are powerful tools for investigating nutrient and water limitation, respectively. We found significantly higher bursage-to-creosote ratios in washes, suggesting bursage requires more water. There were no statistically significant differences between the frequency of nursed bursage in wash and non-wash areas. Stable isotope analysis revealed no evidence of greater water stress or nutrient (N) limitation in creosotebush nursing bursage relative to solitary creosotebush, suggesting that nursing bursage are not competing with creosotebush for water or nitrogen. Previous research suggests that creosotebushes create islands of higher nutrient and water concentration; our results suggest bursage do not, and perhaps cannot, exert competitive pressure on creosotebush for these resources. Our results suggest a commensal relationship, and may force us to reconsider previously held notions about spatial arrangement and competition in desert ecosystems.

COUNTING AND VIABILITY OF THE IMMUNE CELLS: HEMOCYTOMETER VERSUS FLOW CYTOMETER

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The cell-counting technique is a basic method that allows using a particular number of immune cells in an experiment. Historically, the cell counts and viability were obtained by using hemocytometer and trypan blue dye that marks the dead cells. The hemocytometer-based cell quantification is laborious and prone to mistakes. The aim of this study was to compare a flow cytometry-based technique for the quantification and viability of cells to the hemocytometer-based method. Flow cytometer can distinguish live, dead, and injured cell populations by using fluorescent PI and TO dyes, along with quantification of cells by counting beads (BD Cell Viability Kit with Counting Beads, BD Biosciences). Leukocytes isolated from the spleens of C57BL/6 mice were treated with ACK buffer to eliminate erythrocytes. One aliquot of cell suspension was diluted in the trypan blue and counted by hemocytometer, while the other was exposed to the PI and TO dyes, counting beads, vortexed, and analyzed by flow cytometer (FACSCalibur, BD Biosciences). Initial data showed that the cell counts obtained by both techniques are comparable (9.6 X 10^6 by hemocytometer vs. 8.1 X 10^6 by flow cytometer). Yet, the cell viability showed significant discrepancy (the percentage of trypan blue-stained dead cells was 24.2%, compared to 3.2% in PI-stained cells). The difference in the results may be due to the penetration of trypan blue into both dead and live cells. Overall, both data show comparable cell counts. However, the flow cytometric-based method provides precise viability determination compared to the hemocytomer-based method.

WATER-SOLUBLE INDIRUBIN DERIVATIVES AS *Toxoplasma gondii* INHIBITORS

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While the domesticated feline friend, the cat, may bring joy and happiness to its owners, it also is the breeding grounds for disease. *Toxoplasma gondii* is a parasite dispersed to the environment by cats that is highly dangerous to pregnant women, HIV/AIDS patients, and even healthy people. Research has suggested the parasite being the initiator to certain types of schizophrenia, proven it to cause still births or blindness in newborns, and in particular, severe brain damage in HIV-positive individuals by creating lesions. The current pharmacological treatment of the infection with *Toxoplasma gondii* is plagued by toxic side effects. Therefore, there is a need to discover new alternative treatment options. One of the leads that show a promising future is the natural product indirubin. This compound potently inhibits the parasite *in vitro.* Unfortunately, further *in vitro* pharmacological evaluation of indirubin is hampered by its low solubility profile. My plan to address this problem is to add soluble chains onto the compound in the hope of those properties transferring. Indirubin derivatives with such modifications are expected to be effective treatment for *Toxoplasma gondii*.

ION-SELECTIVE ELECTRODES AND SOLUTIONS TO BIOFOULING

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Ion-selective electrodes have numerous applications in medical technology and industry, but are limited by the effects of biofouling, which decreases the electrode's selectivity and increases electrode drift. This project proposes limiting the effects of biofouling by adding a fluorophilic decanol derivative to the membrane of an ion-selective electrode and adding equine serum to the inner filling solution of the electrode. The response of electrodes doped with the fluorophilic decanol and equine serum was observed for cholic acid, octanoic acid, and equine serum. When compared to the response of control electrodes without the fluorophilic decanol and serum, the serum-doped electrodes exhibited the least interference. The selectivity of the doped electrodes was also measured in reference to K^+ and Na^+, and the addition of the alcohol and serum to the electrodes did not appear to change the selectivity relative to the control electrodes.

IRON DEFICIENCY DURING BRAIN DEVELOPMENT INDUCES EXPRESSION OF GENES INVOLVED IN NEOVASCULOGENESIS

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Anemia is a condition in which blood oxygen-carrying capacity is reduced due to low hemoglobin level. According to the World Health Organization, more than one billion people worldwide are affected by anemia. Anemia can lead to tissue hypoxia, a health problem in which there is an inadequate oxygen supply in the body. In the brain, hypoxia induces a compensatory increase in blood vessel outgrowth. The primary cause of anemia is iron deficiency, which results in a nutritional disorder called iron-deficiency anemia (IDA). IDA has its most profound effect on the developing fetus/infant leading to defects in brain development and poor cognitive outcomes. Here, we hypothesize that iron deficiency leads to brain hypoxia and therefore induces new blood vessel growth in the neonatal brain. To test our hypothesis, we measured the mRNA levels of genes associated with hypoxia-mediated neovasculogenesis. Pregnant rats were rendered iron deficient (FeD) from early gestation through postnatal day 10 and 12 (P10 and P12) and mRNA expression of several genes expressed specifically in brain endothelial cells were assessed by qPCR. Fe deficiency increased the expression of Glut1, Cxcl12, Vwf, and Ang2 genes significantly in the whole brain of P12 rats. In the hippocampus and cerebral cortex of P10 rats, Fe deficiency also increased Glut1, Vwf, and Ang2 expression compared to controls. These results suggest that Fe deficiency may lead to new blood vessel growth in the developing neonatal brain. Currently, we are assessing blood vessel density and branching in the neonatal iron-deficient brain. Since delivery of nutrients to the brain is critically important for normal development, these findings reveal a new avenue of research on the impact of Fe deficiency on the developing brain.

SYNTHESIS AND CHARACTERIZATION OF ORGANO-MODIFIED MESOPOROUS SILICA NANOPARTICLES

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Ordered pore structure, large surface area, and substantial pore volume are all definitive characteristics of mesoporous silica nanoparticles (MSNs) that have gained them considerable interest in the biomedical field as drug delivery agents. Modifying the surface of MSNs with derivatives of hydrophilic polyethylene glycol (PEG) has been shown to increase cell viability, reduce hemolysis,

and prevent macrophage uptake relative to bare MSNs. Incorporating a second organosilane, hydrophobic chlorotrimethyl silane (TMS), in a process known as co-modification, has further improved colloidal stability in biological environments such as blood serum. Additionally, TMS contributes to a hydrophobic environment inside the MSN pores, allowing for high retention of hydrophobic cancer therapeutics such as doxorubicin. However, this hydrophobic pore interior is partially compromised in co-modified particles employing the traditional straight-chained PEG due to PEG migration into the pores. Consequently, this migration lowers the available pore volume, limiting drug-loading capacity. Herein, sub-50 nm-diameter co-modified MSNs incorporating TMS and a novel PEGsilane (bis(triethoxysilylpropyl)-polyethylene oxide, i.e. arc-PEG) are shown to have an increased drug-loading capacity and delivery of doxorubicin relative to traditional co-modified particles. This is due to the structure of arc-PEG in that it is bonded to the nanoparticle at two sites, essentially creating an arc on the surface. The bulk and double-anchoring of the arc-PEG prevent its migration into the pores. In addition, these particles exhibit long-term colloidal stability in biological media, indicating their continued biocompatibility. Future studies will include *in vitro* experiments to measure the efficacy of these MSNs against cancer cells.

RAP1A AND RAP1B GENE EXRESSION IN HUMAN CELLS: CELL TYPE- AND CELL CYCLE-SPECIFIC EXPRESSION

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Expression of Rap1A and Rap1B genes in human cell lines was studied using real-time reverse transcription polymerase chain reaction (quantitative RT-PCR). Rap genes are of interest due to their involvement in a multitude of proposed signaling pathways including those affecting cell-to-cell adhesion and apoptosis. Human cell lines were cultured in the absence of growth factors until growth arrest, to relate gene expression to the cell cycle. A pooled RNA sample from human fibroblastic cells was validated for use as a reference point among different runs of quantitative RT-PCR, and among cell lines. Special attention was paid to MCF-7, a breast epithelial carcinoma line, and to MCF-10A, a relatively normal breast epithelial line. We found that expression of Rap1B in the carcinoma line was less than one-fifth of that seen in normal breast epithelial cells. In most cell lines, Rap1A was expressed more highly than Rap1B. In addition to baseline comparisons across cell lines, the effect of growth arrest on expression of Rap1A and Rap1B in individual cell lines was measured. In breast epithelial cells, Rap1A expression appears to be diminished in the absence of growth factors, while Rap1B expression increases with growth arrest. This investigation is ongoing; additional human cell lines are being examined in order to get a wider view of Rap1 gene expression in varying cell types. Chromatin immunoprecipitation (ChIP) will be used in the future to look at proteins that may control the expression of these genes.

INFLUENCE OF ASPARTAME ON INTEGRIN-MEDIATED CELL ADHESION TO SOLID SUBSTRATA IN HUMAN EPITHELIAL KIDNEY CELLS

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Integrin-mediated cell adhesion is vital for a variety of cellular processes including signaling, proliferation, and motility. These transmembrane proteins consist of alpha and beta subunits that recognize arginine-glycine-aspartic acid motifs in the extracellular matrix. The anti-diabetic drug, aspartame, possesses a similar dipeptide recognition sequence and was previously shown to differentially regulate genes in cell adhesion signaling pathways. In this study, aspartame-treated kidney cells were quantitatively assayed for adhesion to substrata in a dose-response format. Results showed no significant difference in the percentage of viable cell attachment, suggesting that soluble aspartame does not inhibit cell adhesion to the extracellular matrix. However, the number of focal contacts for aspartame-treated cells demonstrated a significant dose-dependent increase compared to untreated cells. To test whether focal contact formation is mediated by integrin receptors, we used an Elisa-based assay to screen for differential surface expression of alpha and beta integrins. Notably, the β2 and α5β1 subunits showed a reproducible upregulation of protein expression for treated cells compared to untreated cells. Taken together, these data suggest that soluble aspartame stimulates the formation of focal adhesions through some members of the integrin family of transmembrane receptors, but does not competitively inhibit stable adhesion to solid substrata.

CLONING OF *Trypanosoma brucei* GENES IDENTIFIED AS PUTATIVE LIPID DROPLET METABOLISM AND/OR BIOGENESIS GENES

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Trypanosoma brucei, a bloodborne parasite of both humans and animals, has long been a public health concern in sub-Saharan Africa. *T. brucei* is the causative agent of Human African Trypanosomiasis (HAT), also

known as African Sleeping Sickness, which is transmitted by the bite of the tsetse fly insect vector. Parasites multiply in the blood and lymph, and untreated cases inevitably result in death. Currently, few effective treatments exist, but by obtaining a better understanding of *T.brucei* genetics and physiology it is hoped that potential drug targets can be identified.

T. brucei, similar to most cells, possesses lipid droplet organelles It is unknown whether the function of lipid droplets is essential for the organism's survival, but it is hypothesized that this organelle may be important for the parasite to maintain proper lipid homeostasis as well as other critical metabolic processes. We have recently identified a novel protein kinase (TbLDK), which localizes to lipid droplet membranes (see Fig. 1). In an attempt to learn more about lipid droplet function and biogenesis, we identified and cloned seven genes from the *T. brucei* genome. We predict that these genes and their protein products may be important in lipid droplet function and/or biogenesis. We report here the cloning of these genes from the *T. brucei* genome into a *T. brucei* expression vector designed to express an epitope-tagged version of the expressed proteins. In the future we hope to extend this research toward the identification of potential drug targets in *T. brucei*.

THE CONTRACTILE EFFECTS OF RED RASPBERRY LEAF (*Rubus idaeus*) EXTRACT ON *Mus musculus* UTERINE TISSUE *in vitro*

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An increasing percentage of the population today are looking to holistic medicine for their healthcare needs. Even with increasing use of nutriceuticals, knowledge on efficacy and side effects are relatively unregulated and unknown. The purpose of this research was to collect data that either support or refute claims that red raspberry leaf (*Rubus idaeus*) extracts induce labor and/or promote uterine tone. Red raspberry leaf (RRL) extract applied to isolated mouse uterine tissues induced a contractile response. Dosages ranged from 1.5 mg to 50 mg per 1 mL deionized water. Increasing contractile responses were seen with increasing dosages. However, the increase was not dose-dependent when comparing strength of force (g tension) ($P = 0.33$). When the responses were standardized by comparison to the original acetylcholine (10^{-5} M) response, the increases were significant ($P = 0.005$). Additional RRL applications demonstrated fatigue as compared to their first contractile response ($P < 0.005$). Receptor antagonism was used in an effort to determine the mechanism of action. The cholinergic muscarinic receptor blockers atropine and scopolamine failed to block RRL responses. The cholinergic nicotinic receptor blockers tubocurarine and hexamethonium behaved similarly. This would suggest that the active constituents of RRL do not work through a parasympathomimetic pathway. Atosiban, an oxytocin antagonist, also did not inhibit RRL activity. Results herein do show that at the level of isolated uterine tissues, RRL extracts do induce contraction. This may lend support for the claims that red raspberry leaves can be used as an oxytocic agent.

CLINICAL OUTCOMES OF CATARACT SURGERY ON INTRAOCULAR PRESSURE IN PRIMARY OPEN-ANGLE GLAUCOMA PATIENTS: A RETROSPECTIVE STUDY

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The principal aim of this project was to evaluate the effect of cataract surgery on intraocular pressure (IOP) in trabeculectomized eyes with primary open-angle glaucoma (POAG). A retrospective analysis was performed, consisting of 98 eyes that underwent cataract phacoemulsification following trabeculectomy with mean IOP assessed before surgery, and 1 day, 1-3 weeks, 1-2 months, 6 months, and 1 year after surgery. Preoperative and postoperative IOP measurements were compared using repeated-measures ANOVA. Mean preoperative IOP was 9.9 mm Hg +/- 4.5 (SD), and increased 4.2, 2.0, 1.9, 1.4, and 1.4 mm Hg on the first postoperative day, after 1 day, 1-3 weeks, 1-2 months, 6 months, and 1 year, respectively. At each interval, mean postoperative IOP was significantly higher than mean preoperative IOP ($p < .0001$, $p < .0001$, $p < .0001$, $p = .001$, and $p = .003$, respectively). Stratification into four groups based on preoperative IOP was also performed. Only the lowest group (5.9-1.0 mm Hg) showed significant postoperative IOP increase over preoperative level ($p = 0.023$), with mean IOP 3.7 mm Hg +/- 1.1 and 6.1 mm Hg +/- 2.9, respectively. On average, cataract surgery after trabeculectomy resulted in a small but statistically significant IOP increase in POAG patients. Eyes with lower preoperative IOP showed greatest increase, while those with higher preoperative IOP remained unchanged. Based on these findings, cataract surgery continues to be therapeutically beneficial, but not as a means of reducing IOP, as some studies suggest.

ACCOUNTING FOR ACTIONS: VOCABULARIES OF MOTIVE AND THE CONSTRUCTION OF NARRATIVES IN THE RWANDAN GENOCIDE

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The 1994 Rwandan genocide was one of the worst cases of mass atrocity during the 20th century. By analyzing the transcripts of defendants who testified on

their own behalf for the International Criminal Tribunal for Rwanda, I seek to understand two phenomena. First, I analyze how defendants employ vocabularies of motives, such as justifications or excuses, to rationalize and legitimize their behavior. Second, with the transcripts serving as narratives of history, the project explores how the story of the genocide is told in a particular way through the use of these vocabularies of motive. While results at this time are too preliminary to draw conclusions, I anticipate that defendants will be reluctant to admit any wrongdoing and instead will draw on the genocidal regime's vocabularies of motive to avoid admitting to and serving punishment for the most heinous of crimes. By focusing on individual perpetrators, this project provides important insight into how broad narratives of group differences are translated into individual criminal behavior.

ASSAY AND ISOLATION OF NITROBENZENE DIOXYGENASE FROM *Comamonas* JS765

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Nitrobenzene is a carcinogenic compound produced from many industrial reactions, and it has been declared a priority pollutant by the Environmental Protection Agency. Nitrobenzene is a nitroaromatic compound, which is very difficult to break down into a harmless compound, but a strain of bacteria named *Comamonas JS765* has been found living in toxic levels of nitrobenzene. These bacteria are able to do so because of their ability to break down nitrobenzene into consumable nitrogen and carbon sources by utilizing an enzyme called nitrobenzene dioxygenase (NBDO). NBDO converts nitrobenzene into a *cis*-1,2-dihydrocatechol, making the opening of the benzene ring less energetically demanding for other enzymes. The mechanism by which this enzyme converts the nitrobenzene to the catechol product is currently unknown, and so the goal of this study is to obtain functional enzyme so that the mechanism can be studied. *Comamonas* JS765 cells grown on enriched media were lysed and the proteins were assayed in order to determine if functional enzyme was present in the cells. Negative results led to growing more *Comamonas* JS765 cells on multiple types of media and under different conditions in order to achieve optimal growth. Newly harvested cells were lysed and assayed for activity, and the scale of the bacterial growth was increased to produce enough functional enzymes to begin mechanistic studies.

ANALYZING EXPRESSION OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE(ACC) DEAMINASE IN STRAINS OF *Sinorhizobium*

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Nitrogen-fixing bacteria like *Sinorhiziobia* have the ability to convert nitrogen gas into ammonia, allowing its incorporation into proteins and other biological molecules. These organisms form a mutually beneficial relationship with plants, specifically legumes such as soybeans, peas, and alfalfa. The symbiotic relationship between *Sinorhizobia* and legumes requires the function of many proteins; one is 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase. Bacteria containing ACC deaminase enzymes are more effective in symbiosis due to their ability to lower the concentration of ethylene and bypass the plant defense systems. The goal of this project was to investigate the presence and functionality of ACC deaminase in a collection of *Sinorhizobium* strains from different locations. Upon examination of the complete DNA sequence of forty- eight Sinorhiziobial strains, eighteen sequences similar to known ACC-deaminase gene were identified. These proteins had a range of 33 to 100% sequence identity to ACC deaminases (AcdS) from different rhizobia. The functionality of the newly identified proteins was tested using growth assays. These experiments assessed ACC deaminase activity by examining the bacteria's ability to grow in medium containing ACC as a sole nitrogen source. The results showed that 70% of the strains examined contained functional ACC deaminases. A biochemical assay was developed and tested to determine its effectiveness at detecting AcdS activity. Preliminary data suggest that the amount of ACC deaminase produced by the strains examined is too low to be quantified under the established assay conditions. Research to optimize the biochemical assays is currently in progress.

CONTRACTILE MODULATION OF *Mus musculus* ILEAL TISSUE WITH *Caulophyllum thalictroides* (BLUE COHOSH)

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Caulophyllum thalictroides (blue cohosh) is commonly prescribed by North American midwives as a labor-inducing herbal supplement. The scientific research necessary to substantiate efficacy, however, has been lacking. In 2008, Berger and DeGolier undertook the challenge to quantify the effects of blue cohosh on isolated strips of uterine tissue in mice. They found that increasing contractile force and contractile frequency were induced in a dose-dependent manner. The goal of

this project was to test the effect of similar doses on intestinal tissue, specifically the ileum. Mouse ileal tissue was suspended in a muscle bath and treated with blue cohosh (2.5, 5.0, 10, 20, 30, and 45 mg/15mL bath). Though slight increases in contractile force and contractile frequency were observed, the differences were not statistically significant. These results suggest that when blue cohosh is consumed within the dose range necessary to initiate uterine contractions, ileal motility (at least as sampled *in vitro)* is not affected; fecal contamination due to enhanced ileal activity during labor is, therefore, not a likely side effect of appropriate blue cohosh supplementation.

THE EFFECTS OF XER81 OVEREXPRESSION ON LUNG DEVELOPMENT IN *Xenopus laevis* FROGS

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Mammalian lung development occurs as a ventral outpouching in the foregut endoderm. Reiterative branching and segmentation leads to the formation of bronchi and the alveolar gas exchange system. Mammalian development of the lung involves the coordinated interaction of multiple genes, including *Nkx2.1*, *Spc*, *Spb*, *Wnt7b*, *Wnt5a*, *Shh*, and *Wif1*. These genes have also been found in *Xenopus laevis,* demonstrating the potential of *X. laevis* as a model organism for lung development. Disrupted expression of *Ets*-family transcription factors in mice results in disrupted lung development. The *Ets-related 81* (*Xer81*) transcription factor is highly expressed in the mammalian and *X. laevis* lung, but its role in lung development has not been determined. Overexpression of *Xer81* by microinjection is used to determine its effect on the expression of the lung-specific gene *Spc*, thereby indicating the involvement of *Xer81* in lung development.

AERODYNAMIC ANALYSIS OF THE MINI ULTRA STICK AIRFRAME

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Uninhabited Aerial Vehicles (UAVs) are a versatile platform for many different applications. Their adaptability and relatively low cost make them an ideal research tool for collecting real-time data, especially for autonomous flight. Research in various fields such as controls, navigation, and automation requires accurate mathematical models of the airframe's aerodynamic properties. One method for constructing these mathematical models is to collect and analyze wind tunnel data. The goal of this project is to accurately model the aerodynamic coefficients of the Mini Ultra Stick airframe by collecting data in the Aerospace Engineering and Mechanics (AEM) wind tunnel. The resulting data will then be scaled up and compared to a NASA test aircraft known as FASER, which is a larger version of the Mini Ultra Stick that is currently being used for UAV research within the AEM Department. All data and subsequent analysis shall then be compiled into an easily accessible table and released in the public domain to aid in research.

SYNTHESIS OF A PHOTOACTIVATABLE NON-NATURAL SUBSTRATE FOR PROTEIN FARNESYLTRANSFERASE

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Protein Farnesyltransferase (PFTase) has the ability to site-specifically transfer a variety of non-natural isoprenoid groups to proteins ending in the amino acid sequence CVIA. Here a non-natural isoprenoid was synthesized containing a vinyloxybenzene moiety in six steps. This vinyloxybenzene tag allows for the creation of a fluorescent probe after photoreaction with a diaryl tetrazole. The fluorescent tag can be seen within a cell, thus a protein movement and interactions can be assessed. In the past, protein tags have often been large. The inclusion of a large tag may obscure a protein from it's normal functions, and its ability to interact with other proteins. The vinyloxybenzene modification after reaction with diaryl tetrazole allows for a fluorescent tag that should have less of an impact on protein interactions and functions.

ELEMENTAL STOICHIOMETRY AND NUTRIENT RECYCLING IN AN AQUATIC MICROCOSM FOOD WEB

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The ecological stoichiometry in freshwater food webs is a concept that is becoming more and more relevant with the anthropogenic input of nutrients into these ecosystems. In this experiment, I created a three-trophic-level food chain in laboratory microcosms. These microcosms were set up in three treatments that were provided with different nitrogen:phosphorus ratios (1.7:1, 16:1, and 60:1). The alga that was cultured (*Selenastrum capricornutum*) did not strongly exhibit signs of nitrogen or phosphorus limitation, contrary to the hypothesis that algal cell content would reflect the N:P ratio of the media in each of the three treatments. Similarly, the herbivore, *Ceriodaphnia dubia,* and the predator, *Chaoborus flavicans*, did not differ in body stoichiometry according to treatment. *Ceriodaphnia* showed differences in phosphate excretion among treatments, indicating a

homeostatic response and creating the potential for a positive feedback of phosphorus limitation in a freshwater system. *Chaoborus* did not exhibit differences in body stoichiometry or excretion according to treatment; this supported the evidence of a strong homeostatic response by *Ceriodaphnia* that carried up the food web. This experiment provided evidence for homeostatic responses by algae to differing nitrogen:phosphorus ratios that carried through herbivores and predators in the system. This could have major implications for nutrient cycling and community composition.

IMPACT OF HOST, TEMPERATURE AND NUTRIENTS ON *RHOPALOSIPHUM PADI* FECUNDITY

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To understand how Barley and Cereal Yellow Dwarf Viruses (B/CYDVs) impact grassland species diversity and how host species will contribute to the spread of the B/CYD viruses, it is important to examine the vector species, *Rhopalosiphum padi* (Homoptera; aphididae), that connects these two components. Two separate experiments, conducted at Cedar Creek Ecosystem Reserve in East Bethel, Minnesota, examined the effects of heat, host plant, and nutrients on *R. padi* fecundity. In the first experiment, heated treatments were 5°C above ambient temperatures and aphids were placed on two C3 host species (*Koeleria cristata* and *Poa pratensis)* and two C4 host species (*Andropogon gerardii* and *Schizacharyum scoparium*). In the second experiment, nitrogen, phosphorous, and potassium were added to the soil individually and in all possible combinations. The observed fecundity responses suggested higher *R. padi* reproduction on C3 grasses compared to C4 grasses. Within the heat treatment, fecundity was reduced on *K. cristata* and increased on other hosts. Regarding the fertilization of *S. scoparium*, nitrogen, resulted in a minor increase in fecundity, but only at high concentration, whereas the addition of phosphorus showed a strong reduction in fecundity. Conversely, potassium addition did not affect *R. padi* fecundity positively or negatively. The implications of these data are necessary for the future explorations of how vector fecundity can impact the spread of B/CYD viruses and how the resulting spread will affect grassland composition.

THE SUSCEPTIBILITY OF *Staphylococcus aureus* AND *Escherichia coli* TO THE DISINFECTANTS LYSOL®, THIEVES®, MELALEUCA OIL®, AND VINEGAR

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A number of disinfectant products, both commercial and home remedies, are available to eliminate bacteria from household surfaces. These products differ in terms of both their active ingredients and their costs. The purpose of the present study was to determine the antibacterial effectiveness of commercial disinfectants Lysol, Thieves, and Melaleuca Oil, and the home remedy disinfectant vinegar at eliminating bacteria known to contaminate household surfaces, *Staphylococcus aureus* and *Escherichia coli*. To address this question, minimum inhibitory concentration (MIC) assays were completed to determine the minimum concentration of each disinfectant necessary to inhibit growth of the bacteria 100%. Briefly, *S. aureus* and *E. coli* were exposed to various concentrations of the disinfectants, the bacteria were then incubated at 37°C for 24 hours, and bacterial growth was assessed by measuring the absorbance of the bacterial cultures. All three commercial disinfectants inhibited growth of *S. aureus* and *E. coli* at the dilutions recommended by the manufacturers. Moreover, Lysol and Melaleuca Oil were even effective at inhibiting bacterial growth when diluted 10-fold more than recommended by the manufacturers. Vinegar was found to exhibit antibacterial activity when it was diluted as much as 16-fold. On a per-use basis, Lysol was determined to be the most cost-effective disinfectant analyzed in this study.

INTERACTION OF TAT PEPTIDE AND CELL-SURFACE GLYCOSAMINOGLYCANS TO INCREASE DRUG SPECIFICITY

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The field of drug design and development is an industry in which much time and money have been spent. However, drug efficiency is still limited. Cell-penetrating peptides (CPPs) are positively charged molecules with the ability to cross cell membranes that are often attached to drug molecules to aid their entry into cells. Understanding the interaction between cell-penetrating compounds and glycosaminoglycans (GAGs), negatively charged, linear polysaccharides that exist to different extents on different cell types, may help scientists to better design drugs for maximum specificity. It is hypothesized that the interaction between the positively charged CPPs and the negatively charged GAGs is due to electrostatics. The goal of this study was to compare binding strengths

between the CPP trans-activating transcription factor (TAT) peptide and four GAGs with differing charge density and stereochemistry: heparin, heparan sulfate, chondroitin sulfate A, and dermatan sulfate. First, gel permeation chromatography (GPC) was used to characterize the molecular weight of each GAG polymer. Using isothermal titration calorimetry (ITC), an instrument that is able to measure small heat changes, the binding constant (K_a), enthalpy (ΔH), and stoichiometry (n) of the interactions were simultaneously measured. Differences in binding constants were observed and seemed to increase with charge density on the GAG. The enthalpies obtained were negative but small, indicative of electrostatic interactions that are entropy-driven. The stoichiometries do not seem to follow a distinct pattern. The differences observed may be due to several factors such as GAG stereochemistry or molecular weight.

THE MYSTERY BEHIND THE MECHANISM OF HYDRIDE INDUCED CYCLOPROPANATION

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Almost two decades ago, a graduate student reacted (E)-1-vinyl-1-methoxy-2-(2,4,6-triisopropylbenzenesulfonyl)-4,4-dimethyl-5-cyclohexene with $Li(CH_2CH_3)_3BD$ in an attempt to replace the sulfonate ester with deuterium. Surprisingly, a cyclopropane ring was produced. After stereochemical evidence disproved an S_N2 mechanism, other mechanistic pathways needed to be considered. This research aimed to determine if this reaction proceeds by a hydroboration mechanism. The same starting material was used and reacted with diborane/THF complex or 9-BBN. Diborane/THF conditions were not selective enough, and reduced both double bonds and did not produce the cyclopropane ring. When starting material was treated with 9-BBN no reaction occurred, likely due to steric hindrance. A dialkylborane will be used next to try to mitigate the aforementioned over-reactivity and steric hindrance problems.

TIME COURSE OF THE WOUNDING EFFECT ON LIPOXYGENASE EXPRESSION IN SOYBEAN (*Glycine max*) LEAVES

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Lipoxygenases (LOXs) are enzymes that catalyze the addition of molecular oxygen to unsaturated fatty acids to form hydroperoxide products. Soybean (*Glycine max*) plants have several LOX isoenzymes or different proteins that catalyze the same reaction. Soybean seeds contain at least three LOX isoenzymes while at least six different isoenzymes are in the vegetative tissue. Expression of some LOX isoenzymes increases after mechanical wounding in soybean plants. The objective of this project was to explore the time course of the effect of wounding on the expression of Lox mRNAs. Plants were wounded at the bifoliate stage; one leaf was wounded while the other was used as a systemic leaf. Both leaves were harvested separately at 3½, 6, and 24 hours after wounding. Leaves were also harvested from control plants, which were not wounded. RNA was isolated from the samples using the RNeasy Plant Minikit from Qiagen. RNA quantities and quality were assessed by measuring absorbancies at 260 and 280 nm. cDNAs were prepared using a High-Capacity cDNA Reverse Transcription Kit from Applied Biosystems. The Quantitative Polymerase Chain Reaction experiment was done on a Step One Plus Real-Time PCR system from Applied Biosystems using SYBR Green as the fluorescent indicator. Wounded leaves showed a significant increase in the expression of Lox 7 mRNA 3½ hours after wounding. This level was maintained 6 hours after wounding, but significantly increased again 24 hours after wounding. Systemic leaves did not show a significant increase in Lox 7 expression until 24 hours.

MICROWAVE SYNTHESIS OF A FAMILY OF TRIAZOLE SALTS

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From dimethyl sulfoxide, we formed a simple azine, which is then trans-aminated around various aniline derivatives. Mainly, we chose para-substituted phenyl moieties to reflect a range of electron-withdrawing and electron-donating properties, thus creating 4-(4-R)phenyl-1,2,4-triazoles. Some of the triazoles formed were found in literature; from these we developed microwave synthetic methods with comparable yields and much faster reaction times. Next, we added an R' group to the N1 position, forming 1-R'-4-(4-R)phenyl-1,2,4-triazole iodide salts. The R' moiety was chosen to reflect a range of sizes (Me, iPr, *t*-Bu, Ph). In this step we also developed new microwave synthesis. The stereoelectronic effects of this family of triazole salts will be investigated.

THE EFFECT OF ROOT STRUCTURE ON PHOSPHORUS UPTAKE IN ALFALFA (Medicago sativa)

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Nutrient uptake is a multifaceted system which is not fully understood. Many components, such as plant species, root structure, cultivar, nutrient application, nutrient movement within the soil, and mycorrhizal activity all play a part. While it is generally accepted that these mechanisms each play a part, the actual affect of each is not fully known. This study compared phosphorus uptake and plant yield in alfalfa *Medicago sativa* under varying conditions, including: high or low root system branches; alfalfa or alfalfa/grass mixtures; and with and without added phosphorus (P) on low P-testing soil. This experiment indicates a positive correlation between P uptake and yield with added P, as well as an interaction of root structure with P supply. This interaction indicates that under low P conditions P uptake did not differ significantly in the pure alfalfa stand, while in the alfalfa/grass mixture the high branching root systems show greater P uptake. Further research is examining the mycorrhizal componentof uptake and yield through an analysis of nucleotide differentiation of fungi present on the roots. These results will be compared with plant P concentration, P uptake, and yield to evaluate the correlation between P acquisition and mycorrhizal abundance and diversity.

LIPOSOMAL TEMOZOLOMIDE FOR THE TREATMENT OF BRAIN TUMORS

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Progress in the treatment of brain tumors (e.g., gliomas) using chemotherapy has been impeded by the blood brain barrier. Currently, oral administration of temozolomide is the best chemotherapy regiment for the treatment of brain tumors. Only a small fraction of temozolomide reaches the brain because of the drug's low permeability across the blood brain barrier, necessitating higher doses. As a result, the toxicity of temozolomide is elevated due to the high levels of drug reaching normal tissue. Convection-enhanced delivery, combined with liposomal encapsulation and superparamagnetic nanoparticles, may provide improved delivery of temozolomide directly into the brain at a controllable rate. The long-term goal of this research project is to determine whether temozolomide can be effectively encapsulated into liposomes and released in a controlled manner. High-performance liquid chromatography was used to quantify the concentration of temozolomide in the stability, solubility, and liposomal formulation. It was concluded that the degradation of temozolomide follows a pseudo first-order, specific base-catalyzed reaction. In addition, degradation was found to be temperature dependent. From the pH-solubility profile for temozolomide, it was concluded that temozolomide was not ionizable in the pH range considered for active loading of liposomes, meaning that temozolomide cannot be actively loaded into liposomes. Results from passive-loading experiments suggest that temozolomide is released rapidly from DPPC:PEG-2000 liposomes. This may preclude the possibility of using liposomes to control the release of temozolomide for the treatment of brain tumors.

ANTI-ANXIETY AND ANTI-DEPRESSANT EFFECTS OF *Boswellia* EXTRACT GNC ON *Mus musculus*

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Frankincense, or, *Boswellia* (extract form), is a plant that can be used for many reasons, including medical purposes. It has been studied and found that a component of Frankincense, incensole acetate (IA), has an effect on an ion channel in mice brains that affects behavior. An experiment conducted on a sample of 60 mice, 30 male and 30 female, treated for approximately one month, tested the anti-depressant and anti-anxiety effects of *Boswellia* (GNC) and Sertralin (Zoloft®) compared to the non-medicated group. To determine the effects of these drugs, three different tests were performed on each treatment sample: an open-field test, hanging-tail test, and marble-burying test. Once all necessary data were collected, figures were generated and an ANOVA test was ran to analyze the information. Results of the open-field test ($p = 0.323, 0.144$) and marble-burying ($p = 0.658, 0.107$) test were insignificant among gender and across treatment type. ANOVA results for the hanging-tail ($p = 0.387, 0.006$) were found significant between the *Boswellia* and control treatments, indicating anti-anxiety properties of *Boswellia* for both genders. Completing this experiment with a larger sample size may yield more comparable results to previous observations.

DICARBONYL{[2-(DIPHENYLPHOSPHINO)ETHYL]CYCLOPENTADIENYL} GROUP VI METAL HYDRIDES AND HALIDES: PRECURSORS FOR OLEFIN EPOXIDATION CATALYSTS

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Discovering new ways to synthesize catalysts from cheaper and more common metals is one of the overall goals of research in organometallic chemistry, in order to reduce catalyst price as well as preserve the world's stores of rare metals. Examining the potential of molybdenum catalysts for use as olefin epoxidation catalysts is therefore significant, as the current standard primarily employs expensive rhenium complexes. Previous work has been done exploring $(\eta^5\text{-}C_5R_5)Mo\text{-}(CO)_3X$ (X = Cl, Br, I) complexes in carrying out this catalysis, however more studies are emerging that examine the effect of donor-functionalized cyclopentadienyl ligands in fine-tuning the reactivity of these complexes. We have synthesized and characterized (diphenylphosphino)ethyl]cyclopentadienyl (Cp^{PPh})-functionalized molybdenum, chromium, and tungsten catalysts, and have studied their effectiveness as olefin epoxidation catalysts through catalysis studies with cyclooctene and 1-dodecene, allowing us to analyze periodic trends as well as the effectiveness of the phosphine donor ligand. Our results indicate a similar level of activity and similar conversion curves to the reported $(\eta^5\text{-}C_5R_5)Mo\text{-}(CO)_3X$ catalysts.

A MURINE MODEL OF MAST CELL-DEPENDENT MECHANICAL VULVAR PAIN

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Vulvodynia, defined as idiopathic chronic vulvar pain, affects 6-28% of women of reproductive age. Recent clinical studies showed that women with vulvodynia had a significantly greater number of degranulating mast cells in vulvar tissue biopsies compared to controls. Epidemiological studies have shown that vulvodynia patients were more likely to have a history of allergies than controls. Given that mast cells are important orchestrators of allergies, and mediators of inflammatory pain, we sought to determine whether allergen-induced mast cell activation could lead to vulvar pain. Oxazolone-induced contact hypersentivity is a well-established mast cell-dependent model of skin allergies. We have modified it to challenge ND4 mouse labia with oxazolone after sensitization, and have assessed mechanical sensitivity of the anogenital ridge with an electronic pressure-meter. In addition to causing macroscopic labiar edema, vulvar oxazolone challenge following sensitization significantly increased mechanical sensitivity in the vulvar region at 1, 3, 6, and 24 hours post challenge. This mechanical hyperalgesia was accompanied by neutrophil infiltration as confirmed through analyses of labiar tissues of experimental versus control mice. Oxazolone-induced vulvar mechanical pain was partially abrogated by treatment with sodium cromolyn (mast cell granule stabilizer), pyrilamine (histamine-1 receptor antagonist), and amitriptyline (tricyclic antidepressant). Taken together, our findings suggest that oxazolone re-exposure causes vulvar pain and edema, likely mediated by mast cells.

EFFECT OF SHOAL SIZE ON BEHAVIOR OF FATHEAD MINNOWS

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The fathead minnow *Pimephales promelas* is a common shoaling fish, which is widely distributed in North America. Its habitat includes relatively quiet ponds, reservoirs, lakes, and pools in small rivers. The objective of this study was to examine the behavior of fathead minnows in shoals of variable size in the presence and absence of a perceived threatening situation (human movement). Three shoal sizes were examined (10, 20, and 40 individuals) in a 50 cm-diameter basin. All movements and behaviors were recorded vertically over the water surface by a digital video recorder. The videos were captured, analyzed using motion-tracking software, and processed in a video-editor to provide behavioral data. Shoal size appeared to affect fathead minnow behavior. The distance among animals (nearest-neighbor distance), the similarity in orientation (polarity), and overall cohesiveness were variable among replicates; however, in general, cohesion and polarity increased in larger shoals. In the presence of threat, shoals generally formed nearer the center of the arena with shoals more closely approaching the edge of the arena in the absence of threat.

OF POISON, PENS, AND PEER REVIEW

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In Reflections on Gender and Science Evelyn Fox Keller notes that any scientist who is not a man walks a path bounded by inauthenticity on one side and subversion on the other. I examine the subversive identity by examining the work of an "outlaw" scientist; a scientist

that produced work considered anomalous with respect to the current scientific paradigms. Felisa Wolfe-Simon discovered a bacterium adapted to a very unique environment – this bacterium is able to consume and metabolize arsenic for energy, and has also integrated arsenic in place of phosphorous in its DNA. However, this anomaly has encountered severe criticism from scientists and clearly is contrary to the central tenets of biology embedded in the current paradigm. We use Wolfe-Simon's as a case study of a scientific "outlaw" challenging how scientific discoveries are executed, acknowledged, and accepted.

INSULITIS DEVELOPMENT IN STREPTOZOTOCIN-TREATED AUTOIMMUNE TYPE 1 DIABETES IN JANUS TYROSINE KINASE 3-DEFICIENCE MOUSE MODEL

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Type 1 diabetes (T1D) is a disease in which the beta cells of the pancreas have decreased insulin production due to auto-reactive immune cell, primarily T-cell, attack. Insulin is a hormone that functions in the regulation of blood glucose levels. The invasion of the T cells during the autoimmune attack of the beta cells is defined as insulitis. The T cells contain the molecule Janus Tyrosine Kinase (JAK3), which is essential for their function. Therefore, it is hypothesized that the diabetes incidence, as well as insulitis level, will be lower in the experimental model of autoimmune diabetes induced by chemical streptozotocin (STZ) in JAK3-deficient mice, compared to the STZ-treated wild-type mice. The STZ will be administered over the course of a five-day period in a dose of 40mg/kg each day. The mice will be sacrificed on days 7, 14, and 28 following the initial STZ injection, and the pancreata will be collected. The pancreata will then be fixed in formalin and embedded in paraffin. The slide will be stained with a hematoxylin-eosin (H&E) stain and evaluated microscopically for insulitis levels (on a scale from 0-4, based on the size of the T-cell infiltration of the pancreatic islets). Besides the H&E staining, pancreatic slides will be evaluated by immunohistochemistry for the insulin expression, in order to confirm decreased/absent levels of insulin in the islets heavily infiltrated by T cells. These data will allow us to understand the importance of the JAK3 molecule in the development of T1D.

BUMBLEBEE SURVEY IN AND AROUND NORTHFIELD

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Bumblebees (*Bombus* spp.) serve a valuable role in our ecosystem, pollinating flowers and crops. Recently researchers have discovered that *Bombus* populations have declined significantly in North America, with one species believed to be extinct. Despite extensive research nationwide, little research has been done on *Bombus* species richness in southern Minnesota. In order to document *Bombus* species we collected samples from two restored and two native remnant prairies in and around Northfield. We found eight species of bumblebees with *B. bimaculatus* and *B. griseocollis* being the most common. Our research helps document local bumblebee diversity and provides a baseline for future research.

PHOSPHINE SULFIDE COPPER (I) COMPLEXES: EFFECTS ON OXYGEN-SENSING ABILITY

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For many applications photoluminescence oxygen sensors are superior instruments compared to other commonly used techniques due to their use of a lumiphor's emitted light intensity dependence on oxygen. Emitted light from a lumiphor in these sensors is quantitatively reduced by oxygen through quenching events caused by collisions of the lumiphor's excited state with $^3O_2$. The reduction in intensity upon exposure to oxygen is measured to determine ambient oxygen concentrations with great accuracy and precision. Though powerful instruments, photoluminescence oxygen sensors come with some problems including the use of expensive transition metal (Ru and Pt) lumiphors and polymer matrix supports prone to photochemical degradation that leave room for improvement. One possible solution is the use of neat crystalline Copper(I) lumiphors as the sensing material. Our goal is to explore the effects of phosphine ligands (POP=bis[2-(diphenylphosphino)phenyl]ether and Xantphos=4,5-bis(diphenylphosphino)-9,9-dimethlyxanthene) and their sulfide derivatives on the electrochemical and oxygen-sensing properties of a series of [Cu(phosphine/phosphine sulfide)(dmp)]BF_4 complexes. A variety of techniques were employed for structural analysis, characterization, and evaluation of sensors' properties including ^{31}P NMR, mass spectrometry, crystallography, UV-Vis spectroscopy, solid-state emission, and lifetime studies. The best characterized structure, [Cu(POPS)(dmp)]BF_4, showed promise as an oxygen sensor with intense emission, significant, reproducible oxygen quenching, stability to air and light, and long lifetimes.

EFFECTS OF VARYING NITROGEN FERTILIZER TREATMENTS ON SOIL PROPERTIES, PLANT NUTRIENTS, AND ECONOMIC RETURNS IN NO-TILL CORNFIELDS OF SOUTHEASTERN MINNESOTA

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The use of nitrogen fertilizers in agriculture has increased greatly in recent decades, and subsequently so have the problems associated with higher anthropogenic inputs of nitrogen into natural ecosystems. To mitigate further pollution and destruction, there is an urgent need to better understand the dynamics involved and improve management practices with nitrogen fertilizer use in agricultural systems. This study focused on optimizing the level of summer fertilizer application in a no-till system in southeastern Minnesota, USA, by testing a range of summer fertilizer levels (34, 51, 68, 102, and 136 kg N ha^{-1}) in corn (*Zea mays* L.) production. The effects of nitrogen treatment levels on soil properties, plant nutrient properties, yield, and economic returns were analyzed. Levels of soil nitrates (NO3 --N) increased significantly with increasing levels of N inputs. Yield also increased significantly with N inputs; however, there was not a significant difference in the economic returns from the three highest levels of fertilizer applications in either of the experimental fields. Analyses of corn leaves showed (1) a decrease in C:N molar ratios with increased N fertilizer treatment, and (2) the potential use of stable isotopes (15N and 13C) as indicators of nutrient sources and water stress in agroecosystems. This study demonstrates the advantages of local, on-farm research trials by obtaining specific performance data that confirm the ability of farmers to make environmentally conscious decisions while maintaining profitable yields. Such specific feedback for farmers will help promote greater understanding and wider implementation of improved nitrogen use practices.

SHOCKING LIPID PRODUCTION

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During the passage of electrical messages in neurons, calcium ions are introduced to initiate the fusion of neurotransmitter-containing vesicles to the neural cleft, releasing this neurotransmitter to be passed along to the adjacent neural receptor. This process can be activated through electrical stimulation causing the same electric differential created by the calcium ion. In the algal strain Botryococcus Braunii, the process of lipid vesicle fusion can be replicated using a calcium ion gradient. The release of lipids should therefore also be stimulated by electrical impulses at a specific pulsing frequency and voltage. This experiment identifies the necessary voltages and frequencies to stimulate lipid vesicle fusion staying in between lethargic vesicle movement and a cellular state of electrically induced tetany.

SYNTHESIS OF A FLUORESCENT PHOTO-ACTIVATABLE SUBSTRATE FOR THE ENZYME PROTEIN FARNSEYLTRANSFERASE

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The enzyme Protein Farnesyltransferase (PFTase) has the faculty of covalently linking isoprenoid diphosphate analogs to polypeptides. PFTase bonds the non-natural isoprenoid to the C-terminal cysteine in polypeptides containing a four amino acid recognition sequence, cysteine-valine-isoleucine-alanine (CVIA). The CVIA tag can be inserted into peptides via recombinant methods. A potential non-natural substrate for PFTase is presented here. This non-natural substrate represents an isoprenoid linked to a vinyloxybenzene moiety. The synthesized substrate and the natural substrate (farnesyl diphosphate) are both hydrophobic and of comparable size; accordingly, the non-natural substrate stands to have a similar reactivity with PFTase. The alkene based moiety in this non-natural substrate has been reported to induce peptides to become fluorescent following photoinduced cycloaddition of a diaryl tetrazole. The resulting fluorescent tag is advantageous because it is smaller than many preexisting protein tags like GFP. By virtue of its reduced size, this protein tag minimizes interference with the protein's biological function. This tag may also be implemented in the development of a coupled assay that measures protein prenylation rates.

GENE KNOCKDOWN BY TARGET TRANSCRIPTIONAL INTERFERENCE USING DNA-BINDING PROTEINS

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Mammalian cells are commonly used to produce recombinant protein therapeutics. The key to improve these cells for enhanced productivity is through genetic engineering. Several matured techniques available recently have greatly enhanced our ability to genetically manipulate cells by creating gene deletions and insertions. However, for our application, many genes, being essential in function, requires a method to attenuate gene function, rather than complete deletion. Here we attempt to use DNA-binding proteins, Zinc Finger Proteins (ZFP), and Transcription Activator-Like Effector (TALE) proteins to

interfere with our target genes. ZFPs and TALEs can be designed to target any DNA sequence and most designed proteins have a high affinity and specificity to the target sequence. We explored the possibility of using these proteins to attenuate target gene function by blocking the gene function. We find that, while it is easy to knock down genes expressed at a moderate level, suppressing highly expressed genes is still difficult.

GENOTYPE-PHENOTYPE CORRELATIONS IN MUCOPOLYSACCHARIDOSIS I

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Genetic diseases are caused by mutations to genes. Mucopolysaccharidosis I (MPS I) is an autosomal recessive genetic disorder caused by mutations to the alpha-L-iduronidase gene (IDUA). This gene encodes the alpha-L-iduronidase enzyme which is involved in the breakdown of glycosominoglycans. Mutations to the gene result in a deficiency of the enzyme, which leads to a buildup of GAGs throughout the body. There are various mutations to the IDUA that results in MPS I and there is also a wide range of phenotypes associated with the disease; however, the genotype-phenotype correlation is unclear. Here we show that the genotype-phenotype correlation in MPS I patients is clear for nonsense mutations but not as clear for other types of mutations or two different types of mutations.

Our results showed nonsense mutations to be highly correlated with severe phenotypes, as all participants who are homozygous for nonsense alleles or heterozygous for two different nonsense alleles all presented with severe phenotypes. Furthermore, we saw that splice-site mutation and deletions were associated with severe phenotypes in most cases, while missense mutations showed no clear correlation and were seen in both severe and attenuated patients. Additionally, no correlations were found between mutation type and phenotypes involving a specific body system. We anticipate our findings to be helpful in predicting disease outcomes and also in treatment decision making. Correlations will allow physicians to give predictions of not only disease severity but specific symptoms that may be more likely to occur. Knowledge of genotype-phenotype correlations may help to provide better and more personalized treatments for patients.