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UNDERGRADUATE SYMPOSIA ABSTRACTS

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

HIGHLY STABLE MESOPOROUS SILICA NANOPARTICLES FOR DRUG-DELIVERY APPLICATIONS

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There has been much research exploring the potential of mesoporous silica nanoparticles for biomedical applications such as imaging or drug delivery because of their ordered pore structure and large pore volume/surface area; however, few *in vivo* experiments have been successful because of the large size and low stability of these nanoparticles. Colloidal stability in biological media at physiological temperature is critical if they are to be used for *in vivo* stealth drug delivery because there is rapid unintentional uptake by the reticuloendothelial system if nanoparticles are greater than 100 nm in effective diameter (as individual nanoparticles or agglomerates).

Recent work in the Haynes lab toward the synthesis and purification of multifunctional and size-tunable mesoporous silica nanoparticles for *in vivo* use has shown great potential. Herein, sub-50-nm diameter mesoporous silica nanoparticles with a PEG-silane surface modification and hydrothermal treatment are shown to exhibit high long-term colloidal stability in biological media over 10 days. Specifically, the PEG-silane surface modification reduces hemolysis and macrophage uptake, and increases cell viability compared with bare mesoporous silica nanoparticles. In addition, pegylated mesoporous silica nanoparticles with a secondary inner pore modification show further increase in stability in biologically relevant environments—this will facilitate their application as theranostic nanoparticles. Drug-loading capacity and delivery of doxorubicin (a chemotherapeutic drug) using UV-absorbance is also included. In the future, *in vivo* animal experiments will measure how effective these nanoparticles are in reducing tumor size and their toxicity to the liver and other organs of mice.

TRANSCRIPT AND POLYPEPTIDE ABUNDANCE OF C4 PHOTOSYNTHESIS GENE, PPDK AND ITS REGULATORY PROTEIN, PDRP IN ZEA MAYS LEAVES

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Plants with C4 photosynthesis produce twice as much biomass and grain yield than plants with C3 photosynthesis. Our goal of science is one day to engineer C4 photosynthesis into rice, a C3 plant for improving yield. Before this can be done, much knowledge needs to be gained at the molecular level of how C4 photosynthesis works. One fact that needs to be discovered is the relationship between the key C4 pathway enzyme pyruvate phosphate dikinase (PPDK) and its regulator enzyme PPDK regulatory protein PDRP. Therefore, our goal is to elucidate the precise gene expression ratio for the regulator and regulatee of the C4 pathway. Using Northern blot analysis, we will define the transcript level for PPDK and PDRP in field grown maize leaf. Carrying on, we will use western blot analysis to define the protein levels in the same maize leaves for PPDK and PDRP. With such information, we will be able to define the ratio of transcript and protein for the two genes. The ratio of the regulator and regulatee is important for optimal functioning of the C4 pathway. Such knowledge would be crucial for correctly engineering C4 photosynthesis in to C3 Plants.

SUBLIMATION EXPERIMENT

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Many of the basic skills for working with compounds are developed in the Introductory Chemistry laboratory. Sublimation is one common technique that is usually developed at this stage. We have developed an open-ended sublimation experiment in which students must purify an unknown compound by sublimation. The compound is analyzed by ^{1}H *NMR*, infrared spectroscopy, and melting point. Students then select the identity of the compound from a list of possibilities of inorganic, organometallic, or organic unknowns.

USING BIOINFORMATICS AND SITE-DIRECTED MUTAGENESIS FOR ELUCIDATING AMINO ACID RESIDUES CRITICAL FOR PPDK: PDRP INTERACTION

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C4 plants, such as maize, have a twofold higher rate of photosynthesis as compared with C3 plants because of the higher fluxes of metabolic intermediates in their chloroplast membrane. Pyruvate orthophosphate dikinase (PPDK) is a key intermediate enzyme of the C4 photosynthetic pathway. This enzyme rejuvenates PEP in the first carbon fixation of the cycle by transferring a phosphate from ATP to a pyruvate. Previous studies have

shown PPDK to be regulated by the reversible phosphorylation in the central catalytical domain by the PPDK regulatory protein (PDRP).

Using *in silico* analysis, the region surrounding the conserved active site of PPDK from several species was compared and residues of interest were identified. Based on our analysis, point mutations of PPDK were performed *in vivo*. These mutants were analyzed by LDH assay and regulatory protein immuno-based assay for functionality.

SELECTIVITY BETWEEN BRIDGE N-H AND BRIDGE C-H IN THE H-BOND MOTIFS OF CRYSTALLINE PHENYLHYDRAZONES

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We are using single-crystal X-ray diffraction to examine the solid-state molecular packing arrangements of molecules we have designated "bridge-flipped isomers," pairs of molecules that differ only in the orientation of a bridge of atoms connecting two major portions of the molecule. Examples can be found among the benzylideneanilines, where the isomerism is Ar-CH=N-Ar' vs. Ar-N=CH-Ar', and the phenylhydrazones, where the isomerism is Ar-CH=N-NH-Ar' vs. Ar-NH-N=CH-Ar' (Ar = aryl). Those bridge-flipped isomers that assume identical solid-state packing arrangements might be capable of co-crystallization to form new and useful solid materials. A potential obstacle to this isostructuralism in phenylhydrazones is the presence of the strong H-bond donor N-H in the bridge; changing its position in the bridge could be structure-differentiating between bridge-flipped isomers if it engages in solid-state H-bonding. This differentiation might not occur if the H-bond acceptor were to interact with both the bridge N-H and the bridge C-H.

We are examining cyano-substituted phenylhydrazones to determine whether the nitrile group distinguishes between the strong donor N-H and the weak donor C-H. In contrast to certain of our previous structures, the structure we describe here, that of 1-naphthaldehyde-4-cyanophenylhydrazone, shows a definite preference for H-bonding through the N-H group. Although we have not yet obtained crystals of the bridge-flipped isomer, this result suggests that these two isomers ultimately will be found to assume different packing arrangements because of this H-bonding preference.

ESCALATION OF METHAMPHETAMINE SELF-ADMINISTRATION IN ADOLESCENT AND ADULT RATS

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Adolescence signifies a critical period for addiction formation as many drug-seeking behaviors manifest during this time. Understanding the fundamental patterns of addiction is an essential step in its prevention.

Here we investigate the effects of age on the escalation of i.v. methamphetamine self-administration by comparing adolescent and adult rats during short (ShA, 2-hr) and long (LgA, 6-hr) access to methamphetamine self-administration. On postnatal days 23 (adolescents) and 90 (adults), rats were implanted with intravenous catheters and trained to lever press for intravenous infusions of methamphetamine (0.5 mg/kg) during 2-hr sessions. Once adolescent and adult rats reached a steady rate of infusions, they were allowed to self-administer methamphetamine during ShA or LgA sessions for 16 additional days. Results indicated no age differences in methamphetamine intake during ShA sessions; however, under LgA sessions, adolescents earned more methamphetamine infusions and escalated drug intake at a significantly faster rate than adult rats. These results demonstrate that adolescents are more vulnerable to the escalation of methamphetamine than adults as well as reinforce the social importance of methamphetamine prevention in children. (Work supported by NIDA grant DA19942-01A2.)

PARTITIONING, STOICHIOMETRY, AND DIFFUSION DYNAMICS OF FTY720 (AN IMMUNE MODULATOR) IN TRITON X-100 MICELLES

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FTY720, a synthetic analog of sphingosine, is an immune suppressor that is the first oral drug to be approved by the U.S. FDA for treatment of multiple sclerosis (under the trade name *Gilenya*™). In this contribution, we investigate the partitioning of a newly synthesized derivative of FTY720 that is fluorescent (namely, Bodipy-FTY720) in Triton X-100 micelles, with critical micelle concentration of 0.2 mM. At the nanomolar concentration of Bodipy-FTY720, our fluorescence correlation spectroscopy was used to

estimate the radius of the micelles and the results indicate one molecule per micelle with an estimated micelle radius.

To gain more insights into the micelle-Bodipy-FTY720 interaction, time-resolved anisotropy measurements were carried out and the results suggest a two-step model of a fast-restricted reorientation of Bodipy-FTY720 and a slow overall tumbling motion of the dye-micelle complex. Based on the fast rotational time scale, we conclude that Bodipy-FTY720 is embedded in the hydrophobic core of the micelles. These findings represent a first step toward understanding the mechanism of Bodipy-FTY720 action in living cells as well as its partitioning in biomembranes.

METHANE PRODUCTION RATES FROM A FLOODED PRAIRIE

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Methane is a greenhouse gas of particular concern because of its warming potential, which is 23X greater than that of carbon dioxide. Wetlands alone account for 20-39% of methane emissions. The methane produced from wetlands is the result of a process called methanogenesis. Methanogenesis is the anaerobic respiration of methanogens that produces methane as a by-product. Methanogenesis is the main biotic source of atmospheric methane.

In this study, we take a closer look at how the process of methanogenesis responds to conditions of drought and flooding. The prairie we investigated had been dry for more than two years, and was then flooded for three weeks prior to sampling. Soil cores were taken from varying depths of water and incubated in ideal conditions with gas samples taken every two, 24, and 48 hours. Our results showed the soils from the deepest part of the wetland, which had been submerged the longest, produced methane at significantly higher rates (average 13.8 mg/l/h) compared with the margin of the wetland and the dry prairie. Our results indicate that dry wetlands and prairies have potential to produce methane even under brief ideal conditions. This suggests the possibility that methanogens have the ability to lie dormant for long periods of time, and take little time comparatively to become active. It is difficult to measure and model flux from wetlands, and consequently most estimates are conservative. Our experiment sheds some light on areas of wetlands such as ours that produce methane only at certain times when flooded.

THE EFFECT OF FIBROBLAST GROWTH FACTOR-10 ON SURFACTANT PROTEIN C EXPRESSION IN *Xenopus laevis* EMBRYOS

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Mammalian lung development and branching involves an interaction between the distal lung epithelial tissue and its surrounding mesenchymal tissue. This study focuses on two specific proteins expressed in these tissues: surfactant protein C (SP-C) and fibroblast growth factor-10 (FGF-10). SP-C is a distal lung epithelium specific marker with a functional role of reducing surface tension in the lungs. FGF-10, expressed in the mesenchymal tissue, is essential for the initiation of lung development, serves as a chemoattractant for the distal lung epithelium, and has also been shown to induce SP-C expression in mice. *Xenopus laevis* has proven to be a successful model organism in studying early mammalian lung development due to its development in an extrauterine environment and the conservation of molecular events that govern lung development across species. This study further compares *Xenopus* and mammalian lung development by examining the role of FGF-10 in regulating SP-C expression in the early lung development of *Xenopus*. This was accomplished through a site-specific injection of the mouse FGF-10 gene controlled by a heat shock promoter into *Xenopus* embryos. At developmental stage 43, following the initial events of lung development, *Xenopus* were analyzed for SP-C expression through Quantitative PCR. Results indicated an increase in SP-C expression in FGF-10 injected tissues, although this increase was not statistically significant.

INTRODUCING ONLINE LABORATORIES TO BIOCHEMISTRY AND NUTRITION

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Many schools today are offering online courses to supplement existing curriculum in order to adjust to a growing interest in these fields. Bethel University is currently considering introducing an online section to CHE104 (Biochemistry and Nutrition) to accommodate new transfer, off sequence, and previously unsuccessful students taking the course for their nursing major. The challenge offered in this research is how to create online labs which students can complete at a distance, out of the traditional laboratory, and which are specific to the biochemistry being studied. This research reviews a history of distance education and examines the construction and implementation of five "kitchen chemistry" labs studying amylase, lipids, enzymes, DNA, and digestion.

CLONING AND SEQUENCING A GENE IN THE GLYCOLYTIC PATHWAY OF ARABIDOPSIS THALIANA

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An enzyme that mediates a step in glycolysis, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), is present in all organisms. The gene for GAPDH has a conserved nucleotide sequence because it plays an essential role in energy production; however, the gene is known to vary among species. The purpose of our study was to clone and sequence the GAPDH gene in two species of plants. *Arabidopsis thaliana* is an important model organism and the sequence of the GAPDH gene has been published (National Center for Biotechnology Information). Although the GAPDH sequence is known for some species in the genus *Geum*, GAPDH sequence information is unknown for large-leaved avens, *Geum macrophyllum.* After isolating genomic DNA, a two-step nested polymerase chain reaction (PCR) was used to amplify the gene. We successfully amplified the GAPDH gene from genomic DNA of *A. thaliana*. After repeated unsuccessful attempts to amplify the gene in *G. macrophyllum*, we concluded that the universal primers were not sufficiently similar to the target region in the species. The PCR product from *A. thaliana* has been ligated into a pJet1.2 blunted plasmid vector and *E. coli* have been transformed to uptake the plasmid containing the GAPDH gene. After growing the bacteria on selective media to identify colonies that incorporated the vector and inserted GAPDH gene, restriction enzyme digest will be used to recover the gene. The gene will be sequenced and bioinformatics will be used to compare our sequence to the published sequence.

EFFECT OF STREPTOZOTOCIN ON T-CELL PROLIFERATION AND CYTOKINE SECRETION IN TYPE 1 DIABETES EXPERIMENTAL MOUSE MODEL

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Type 1 Diabetes Mellitus (T1D) is an autoimmune disorder that results from destruction of insulin-producing pancreatic beta cells by auto-reactive T-cells. In the experimental mouse model, autoimmune T-cell-dependent T1D can be induced by streptozotocin (STZ) injected in low doses (LDSTZ model). In contrast, one high dose of STZ (HDSTZ model) directly destructs beta cells, inducing toxic T1D. Different subtypes of T-cells secrete different cytokines. It is believed that IL-2, IFN-γ (secreted by Th1 cells), and IL-17 (secreted by Th1 cells) exhibit diabetogenic effect, in contrast to protective effects of IL-4 and IL-10 (secreted by Th2 and Treg cells).

In this study, we asked whether cytokines, secreted from cells obtained from LDSTZ- and HDSTZ-treated mice, would reflect different nature of those two models of T1D. C57BL/6J male mice were injected with either multiple low doses, or a single high dose of STZ. Proliferative capacity of T-cells was evaluated post addition of mitogen Concanavalin A to a culture of splenocytes (Alamar Blue assay). Cytokine analysis was performed using Th1/Th2/Th17 cytokine kit (BD Biosciences). Our preliminary results showed different cytokine profiles of T-cells, obtained from the spleens of LDSTZ- and HDSTZ-treated mice. Decreased IFN-gamma, IL-6, IL-2, and IL-17, with increased IL-10, were found during the first 7 days in LDSTZ-, while no changes in cytokine profiles were observed in HDSTZ-treated mice. However, increased Th1-, with decreased Th2-/Treg-type cytokines, were not observed in LDSTZ-treated mice. Overall, our data confirmed immune system involvement in LDSTZ, but not HDSTZ model of T1D.

EFFECTIVE VISCOSITY OF ACTIVELY SWIMMING ALGAE SUSPENSIONS

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As the demand for energy soars, the introduction of algae biofuels as a renewable source of energy is receiving much attention. Suspensions of these actively swimming microorganisms exhibit an effective viscosity that may depend on volume fraction, cell shape, and the nature of locomotion (e.g. ``pushers" vs. “pullers”).

Here we report experimental measurements of shear viscosity for suspensions of unicellular green algae (*Dunaliella primolecta*, a biflagellated “`puller”). We use a cone-and-plate rheometer to measure the dynamic shear viscosity for both motile and nonmotile suspensions of *D. primolecta* at concentrations ranging from 0.1% to 10% of volume fraction. Viscosity increases with concentration for both cases, but the active suspensions of ``pullers" have a comparatively higher effective viscosity than passive suspensions. This observation confirms recently proposed theories that predict higher effective viscosity for ``puller" suspensions compared with non-motile suspensions. Our locomotion study reveals that motile algal cells prefer to align and migrate in the direction of positive shear flow vorticity. It is our belief that such a

shear-induced response of the algal cells impacts the resulting effective shear viscosity.

THE EFFECTS OF IRON DEFICIENCY ON THYROID HORMONE-REGULATED BRAIN DEVELOPMENT

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Inadequate dietary intake of micronutrients such as iron (Fe) or iodine during pregnancy and infancy are associated with permanent impairments of brain function. Interestingly, Fe and iodine deficiencies result in similar defects in late brain development including hypomyelination of axons, aberrant hippocampal structure and function, and altered energy metabolism. These similarities suggest a common underlying mechanism associated with these deficiencies contributing to the observed developmental defects. Iodine is required for the production of thyroid hormone, a hormone required for normal mammalian brain development. Interestingly, iron deficiency in adolescents and adults has been associated with altered thyroid hormone status. Therefore, we hypothesized that iron deficiency during the period of late brain development leads to reduced TH levels and that these reductions in TH contribute to the derangements in brain development observed in iron deficient animals. Supporting this hypothesis, we found that Fe deficiency, throughout gestational and early neonatal life, resulted in reduced circulating and brain TH levels in two-week-old rat pups. Additionally, we found that some, but not all, genes regulated by TH in the developing rodent brain are also regulated by Fe deficiency. Interestingly, expression of the TH transporter organic anion transporting polypeptide 1c1 (Oatp1c1) was increased under both FeD and thyroid hormone deficiency, suggesting that increased Oatp1c1-dependent TH transport may help the brain compensate for the reduced circulating TH levels observed in these conditions. These data suggest that altered thyroid hormone levels may contribute to some of the deleterious effects of Fe deficiency on the developing brain.

CLONING AND SEQUENCING THE GAPDH GENE OF PHAGMITES AUSTRALIS

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A plant native to Minnesota, *Phragmites australis*, grows along wetland borders throughout much of the U.S. A non-native Eurasian *P. australis* (haplotype M) has replaced native populations in much of the eastern U.S. and it has been identified in isolated patches in Minnesota (Melchior et al., unpublished data). The objective of our research was to clone and sequence a gene for the enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH) in native and non-native *P. australis*, which have not previously been sequenced. Although native and non-native *P. australis* cannot be reliably distinguished visually, microsatellite analysis can be used to identify plants. DNA from microsatellite-identified plants (Melchior et al.) was used in our study. Using universal primers for the region adjacent to the GAPDH gene, we used polymerase chain reaction (PCR) to amplify the gene and surrounding region along with a plant species known to amplify with the primers, *Arabidopsis thaliana*. Using primers for DNA sequences known to be located within the GAPDH gene, a second round of PCR amplified the portion of the gene that encodes information for the enzyme. The length of the PCR product from native and non-native *P. australis* did not appear to be different (about 1400 base pairs), although larger than we observed in *A. thaliana.* We will next ligate the gene into a plasmid vector for transformation into *E. coli*, isolate colonies that contain the plasmid, and recover the cloned PCR product. The DNA will be submitted to a DNA sequencing facility, and we will use bioinformatics (*Geospiza* software) to annotate the gene and to compare the GAPDH sequence for native and non-native *P. australis*.

STREAM NUTRIENT STOICHIOMETRY FOLLOWING LEAF FALL AND FLOODING

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A major flooding event occurred September 23, 2010, in southeastern Minnesota, providing an opportunity to monitor post-flood water chemistry patterns in streams near the St. Olaf campus. Over a four-week period, we monitored stream nutrient concentrations in Spring Brook, a cold-water stream, and Heath Creek, a warm-water stream, measuring PO_4, NO_3, NH_4, and dissolved organic carbon (DOC). Because flooding hastened the completion of leaf fall, our study considered the impact of both flooding and allochthonous carbon from leaf fall on stream C, N, and P concentrations. In both streams, PO_4 decreased over time while NO_3 was relatively stable and NH_4 fairly variable. Interestingly, the streams exhibited strikingly similar N:P patterns, although absolute NO_3 concentrations were markedly different. DOC similarly declined over time in both streams, suggesting linkage

between DOC concentration and N and P uptake in these streams. We propose the observed trends in N and P uptake were partially derived from a flushing effect caused by major flooding during the post-leaf fall period.

OPTIMIZED SYNTHESIS AND X-RAY CRYSTALLOGRAPHIC STRUCTURE OF BIS[(METHYLTHIO)CARBONYL]DISULFANE

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Bis[(methylthio)carbonyl]disulfane, MeS(C=O)SS(C=O)SMe, was prepared by either of two multi-step routes originally described by Mott and Barany (*J. Chem. Soc. Perkin Trans. I*, **1984**, 2615-2621), but with additional experimental details elucidated for the present study. The final step was either chlorination of *O*-*tert*-butyl *S*-methyl dithiocarbonate or controlled desulfurization, mediated by triphenylphosphine, of pure bis[(methylthio)carbonyl]tetrasulfane, $MeS(C=O)S_4(C=O)SMe$. The title compound was obtained for the first time as a crystalline material, and its molecular structure was confirmed by x-ray crystallography.

IMPROVED PROCEDURE FOR THE SYNTHESIS OF A FUNCTIONALIZED POLYLACTIDE COPOLYMER

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A hydroxyl functionalized polylactide copolymer was successfully synthesized in good yield at both 70:30 and 90:10 ratios of lactide and 3,6-bis(benzyloxymethyl)-1,4-dioxane-2,5-dione. The chain lengths of the copolymers averaged 40 units in length. Improvements to almost all of the synthetic steps were made that both increased yield and decreased the time for synthesis. All new compounds were characterized by H'NMR, C'NMR, and mass spectrometry.

INFLUENCE OF LUNG PHYSIOLOGY DURING POSITIONAL CHANGES AND EXERCISE ON THORACIC IMPEDANCE IN SUBJECTS WITH HEART FAILURE

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Introduction: Optivol (Medtronic Inc.) intrathoracic impedance software provides preemptive disease status information in heart failure (HF) patients. It remains unclear how pulmonary-capillary blood volume (V_C), lung volume (LVol), and interstitial fluid influence impedance. Hypothesis: 1) positional change and exercise result in increased V_C and/or lung interstitial fluid changes, 2) impedance tracks fluctuation in these variables. Methods: Twelve HF patients (age=63±8yr, wt=84±17kg, ejection fraction=41±14%) were recruited. Measures were taken at rest (upright/supine), with exercise and recovery. Measurements included: spirometry, intrathoracic impedance (Z_T), diffusing capacity of the lungs for carbon monoxide and nitric oxide (D_{LCO}, D_{LNO}), alveolar-capillary conductance (D_m-index of interstitial fluid), and V_C. Inspiratory capacity was obtained to provide consistent lung volumes for assessing Z_T. Results: Positional change from upright to supine resulted in an increase in Z_T (5±4%, $p<0.01$), while the change in LVol (RV-TLC) resulted in a 7±2 ohm swing in Z_T (0.2±0.1ohm change/100ml change in LVol). After 30-min supine, V_C increased 28±39% ($p<0.05$) while D_m/V_C and LVol decreased (29±11% and 17±3%, $p<0.05$). During exercise compared to baseline, V_C increased 56±44% ($p<0.01$), D_m/V_c decreased 25±7% ($p<0.05$), and Z_T did not change. During recovery all measures normalized except D_m/V_c, which remained decreased from baseline. **Conclusion:** Acute positional changes and fluctuations in LVol significantly influence intrathoracic impedance however key measures of lung fluid minimally impact this measure despite significant fluctuations. *Medtronic, NIH-HL71478, Mayo SURF program.*

SYNTHESIS AND COMPARATIVE X-RAY CRYSTALLOGRAPHY OF XANTHIC ANHYDRIDES AND RELATED POLYSULFANES

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Xanthic anhydrides, RO(C=S)S(C=S)OR for R = methyl, ethyl, or isopropyl, have been synthesized in one-pot treatments of corresponding xanthates (2 equiv, generated *in situ*) with ethyl chloroformate (1 equiv). Reaction intermediates were isolated and extensively characterized, allowing for optimization of the title compound syntheses. Alternate routes to ethyl and isopropyl xanthic anhydrides involved desulfurization of corresponding dixanthogens with triphenylphosphine; the same reaction in the methyl series gave only *O*,*S*-dimethyl xanthate, which was not the desired product. Attempted desulfurization of dixanthogens by potassium cyanide gave complicated mixtures of unexpected products. Compounds produced in this research were characterized by ^1H and ^{13}C NMR, FT-IR, UV spectroscopy, and elemental analysis. X-ray

crystallographic structures were obtained for the first time for methyl and ethyl xanthic anhydrides, and were compared with the structure of isopropyl xanthic anhydride reported in the literature and independently confirmed here. Furthermore, isopropyl dixanthogen and bis[isopropoxy(thiocarbonyl)]trisulfane were crystallized and solved for the first time, allowing for a definitive comparison of the $[i\text{PrO(C=S)}]_2S_n$ series for n = 1, 2, and 3. These compounds are precursors to reagents that have interesting and important applications to the protection of the sulfhydryl group of cysteine for peptide synthesis.

HOW MUCH DO AMERICAN'S WORK? A STUDY OF THE AVERAGE AMERICAN WORKWEEK IN RELATION TO PART-TIME EMPLOYMENT

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The two most common measures of the American workweek are the Current Employment Statistics (CES) and the Current Population Survey (CPS). These sets of data show different hourly averages of the American workweek due to the different types of employees sampled and the survey measures used. This is an empirical study of the average American workweek supported by BLS data to determine why the workweek has decreased according to the CES and stayed relatively stagnate according to the CPS. Findings suggest the average workweek has decreased in the CES because of the labor market shift in preference from full-time to part-time employment. The Bureau of Labor Statistics shows the highest increase in part-time employment is due to economic hardship. The years 2000-2009 show great increases in part-time employment due to the economic difficulty that began with the 2001 recession. This redistribution caused the average workweek to decrease by CES data. Firms are turning to part-time labor to create more flexibility in meeting fluctuations in demand.

The CPS implies this does not necessarily mean Americans are working less, rather working in different types of employment and household activities not captured by the CES. This research focuses on multiple jobholders, unpaid workers, and the self-employed as examples of these different types of employment. The CES workweek is shortened because it is a measure of the average paid hours an establishment reports per employed position. The CPS has stayed relatively stagnate because it is a measure of overall household employment.

SITE-SPECIFIC MUTATION OF RIM8 IN *Candida albicans*

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Candida albicans is a commensal organism that causes systemic infections in immunocompromised hosts. To survive in neutral-alkaline environments within the host, *C. albicans* and other fungi use the Rim101/PacC pathway, which is required for pathogenesis. Environmental pH is sensed by the surface receptor Rim21, which transduces the pH signal through a number of downstream pathway members, including Rim8, Rim13, and Rim20, to activate the transcription factor Rim101, which promotes changes in gene expression that lead to adaptation. In other fungi, Rim8 is ubiquinated in response to neutral-alkaline pH in a Rim21-dependent manner. However, we found that in *C. albicans*, Rim8 is phosphorylated, and not found to be ubiquitinated. Rim8 hyperphosphorylation occurs concomitantly with and is necessary for Rim101 activation. We propose that phosphorylation of Rim8 is necessary and sufficient for Rim101 activation. However, sequence analysis of *C. albicans* Rim8 protein revealed the presence of a lysine residue in an analogous position to the lysine that is ubiquinated in *Saccharomyces cerevisiae* Rim8, suggesting that Rim8 ubiquitination can occur in *C. albicans* but has not been detected in our assays. To test this hypothesis, we are generating a site-specific mutation within Rim8 to alter this lysine residue. We predict changing the lysine to a negatively charged arginine residue or a neutral alanine residue will not affect Rim8 function. However, if ubiquitination occurs and is important in *C. albicans*, these mutations will disrupt Rim8 function and prevent adaptation to neutral-alkaline pH.

THE EVOLUTION OF LIFE HISTORIES: DIFFERENTIAL SEX DETERMINATION IN THE SCALY PEARL OYSTER *Pinctada longisquamosa*

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Pearl oysters (Pteriidae: Pinctada) are known to be sequential hermaphrodites, generally switching from male to female over the course of a single life history. Strategies of sequential hermaphroditism arise out of differential fitness benefit for each sexual form. This study describes and compares the sex determination trajectories of five populations of the Scaly Pearl Oyster *Pinctada longisquamosa* from inland ponds on San Salvador Island, Bahamas as well as a marine population from the Florida Keys. The mechanisms of population-level sex allocation as well as the plasticity of this

mechanism in the species of *Pinctada* are not well understood. Populations of the Scaly Pearl Oysters differed in sexual allocation trajectory. We suggest that hurricane vulnerability as well as salinity variability may be driving the differential evolution of this life history. Sex determination trajectories in a hurricane-vulnerable population before and after a significant hurricane disturbance were found to be similar, supporting a genetic basis for this life history trait. Potential environmental influences on this life history were also investigated, including the role of algal species diet in oyster sex determination. These findings highlight a pattern of differential life history evolution according to localized selection pressures.

HEMIN ACTIVATES P-SELECTIN EXPRESSION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Sickle cell disease is a hemolytic disease leading to the release of hemoglobin and free hemin from lysed red blood cells into the plasma compartment. We hypothesized that plasma hemin can promote an inflammatory response leading to vaso-occlusion and tissue ischemia in sickle cell disease. Since P-selectin plays a prominent role in the initiation of an inflammatory response, we examined whether hemin can induce P-selectin expression in endothelial cells. Human umbilical vein endothelial cells (HUVEC) in culture were treated with 10 μM Panhematin, a pharmaceutical preparation of hemin, for 2, 5, 10, 15, 30, and 60 minutes and surface P-selectin expression was measured by immunofluorescence microscopy. Expression of P-selectin on the surface of HUVEC was absent after 2 minutes of treatment, but was seen prominently after 5 minutes and appeared to peak after 10 minutes of treatment. P-selectin expression slowly declined after 10 minutes, but could still be seen after 60 minutes. These data suggested hemin can induce an inflammatory response in hemolytic diseases, especially after heme-binding proteins in plasma are depleted as seen in sickle cell disease.

A NOVEL GENE THERAPY IN COMBINATION WITH CHEMOTHERAPEUTIC TO TREAT PANCREATIC CANCER

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A cure for pancreatic cancer is not possible, yet. For more than 80% of U.S. patients diagnosed with unresectable pancreatic cancer, the average length of survival is less than one year. Even with well-established chemotherapeutic agent 5-Fluorouracil (5-FU) treatment, 95% of the patients will not be alive within five years. In recent clinical studies, interferon-α (IFN) therapy in conjunction with 5-FU has emerged as a promising treatment strategy, but systemic toxicity and an unsustainable level of IFN in tumor sites remain serious challenges. Hence, we designed and cloned a novel adenovirus with replication restricted to Cox2-overexpressing pancreatic cancer cells as a vehicle system to deliver high dosage of IFN to only the pancreatic cancer cells, leaving the normal cells intact.

We hypothesize that adenovirus expressing IFN in combination with 5-FU would significantly enhance selectivity and anti-cancer effect of existing IFN-based regimens while reducing toxicity to healthy tissues. The cytocidal effect of the combination therapy on pancreatic cancer cells were analyzed *in vitro* by MTS assay. Eight days post-infection, the lone treatment of adenovirus expressing IFN, the lone treatment of 5uM of 5-FU, and the combination therapy killed 21%, 10%, and 59% of pancreatic cancer cells, respectively. Remarkably, the adenovirus expressing IFN combined with 5-FU exhibited greater oncolysis than either of the treatments alone. Additional experimentation and assessment of the combination therapy may provide new insights into its efficacy. The combination therapy possesses great potential in improving the long-term survival of pancreatic cancer patients.

FACT-CHECKING PRINCIPLES OF ECONOMICS

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Principles of economics textbooks today often ignore the data and statistics underpinning modern economics. Without the nuance afforded by statistical methods, these textbooks are left presenting misleading and sometimes outright false ideas to students, while

also missing teaching opportunities that take advantage of data and statistics-oriented approaches.

This study proposes that where "principles-level" economic theory fails, economic data and corresponding statistical models can be used effectively to convey the appropriate relationships and interactions. In particular, we examine the Phillips Curve and note that the underlying data and relationship changes over time in a fashion suggestive of the Lucas Critique. We also examine the Quantity Theory of Money's assumption that the velocity of money is stable and find it instead to be indistinguishable from a random walk. Next, we examine Okun's Law and note it as an example of good theory that would be better presented through a statistical lens. Finally, we examine the relationship between changes in the term structure of loans and the onset of recessions, viewing this as an example of a recent economic question that is of use in introducing students to statistical thinking in economics. Overall, we find that data-oriented approaches to teaching principles of economics not only correct the errors introduced by old and oversimplified economic theories, but also provide valuable launching points for introducing students to other important ideas in economics, as well as to the statistical ideas and manner of thinking that are of growing importance to the field.

THE EFFECT OF LOWERED ALDOSTERONE ON THE EXPRESSION OF MINERALOCORTICOID RECEPTOR ISOFORMS α, β AND γ.

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Aldosterone is a hormone secreted by the adrenal gland that binds to cytoplasmic mineralocorticoid receptors (MR). The binding of this hormone to its receptor increases the synthesis of the epithelial sodium channel (ENaC) and Na+/K+ATPase proteins, which control the passage of sodium and potassium ions. Mineralocorticoid receptors exist in three isoform structures called alpha, beta, and gamma. Lowering of aldosterone levels can affect blood pressure and hypertension. The objective of this research was to develop three qPCR (quantitative polymerase chain) methods to measure the expression of the three MR isoforms in rat kidneys. The second objective was to test whether lowered aldosterone levels changed the expression of these MR isoforms in the kidneys of treated rats or their male offspring. Total RNA was isolated and reverse transcription was used to make the copy DNAs. Software was used to design TaqMan primers and probes over exon junctions with unique sequences for each isoform, It was found that that the expression of the alpha isoform may be slightly lower in normotensive rat kidneys and in the offspring of spontaneously hypertensive rat kidneys of mothers who had been treated to lower aldosterone levels. This trend is also seen in the kidney samples of SHR male rats. More samples need to be run to verify this trend and to establish significance. The MR B assay was not efficient enough to pursue expression analyses.

TREE GROWTH, MORTALITY, AND REPRODUCTION IN A 20-YEAR-OLD MAPLE-BASSWOOD FOREST RESTORATION

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Much of the maple-basswood forest type, endemic to south-central Minnesota USA, has been fragmented and degraded because of recent human activity such as logging, agriculture, and development.

Data on growth and survival patterns have been collected on more than 1,000 trees in a maple-basswood forest restoration project started in 1990 on former agricultural fields at St. Olaf College, Northfield, Minnesota. As the trees have matured over 20 years, tree sizes have diverged and each tree species has had differential success. Data collected in 2010 showed red oak and American basswood had the largest mean diameter while ironwood and sugar maple had the smallest. Overall mortality was 33.66%, but varied greatly between species from 0 to 60 percent with species such as sugar maple having very high mortality (55%) while American basswood had low mortality (18%). Soil characteristics such as % organic matter, % moisture, and PO4-P content were significantly lower in the restored forest areas than in a nearby mature forest, but have been increasing over time. Implications for management included data showing that (1) tree tube protectors have no long-term benefits for growth and (2) planting grass decreased seedling recruitment.

In conclusion, restored forest soil characteristics have been changing to become more similar to a nearby mature forest, and we expect that this pattern will continue in the future. In areas where the canopy has closed over, the forest is approaching a mature maple-basswood forest.

CULTURING OF *malassezia furfur*, A LIPOPHILIC YEAST

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Malassezia furfur is a lipophilic fungus (yeast), commonly found as part of the skin microbiota, and implicated in skin disease and opportunistic systemic infection in neonatal and immunocompromised populations. An essential nutrient requirement for a lipid source complicates growing conditions of *M. furfur* for *in vitro* experimentation and analysis: serial dilutions methods for susceptibility testing and turbidimetric measurements for evaluating growth curve parameters require that the organism be in liquid suspension culture. We surveyed culture conditions for the growth of *M. furfur* on a variety of solid and liquid media modified with olive oil as a lipid source and sodium dodecyl sulfate (SDS) as an emulsifying agent. When grown in modified liquid media, *M. furfur* grows as globular aggregate at the top of stationary culture tubes. We evaluated agitation methods to obtain *M. furfur* in more uniform suspensions.

This research presents the results of our attempts to grow *M. furfur* on solid media on plates and suspended in liquid media. Results indicate that *M. furfur* will grow on many different substrates of either format provided they contain a lipid source. Static liquid cultures produced poorly dispersed aggregates of the organism. Successful growth of *M. furfur* in more uniform suspension was achieved by continuous stirring closed cultures of modified media incubated at 35°C. Successful uniformly dispersed growth in liquid suspension facilitates growth curve studies and testing the susceptibility of *M. furfur* to antifungal chemotherapeutics *in vitro*.

DISORDER IN THE CO-CRYSTALLIZATION OF 4,5-DICHLOROPHTHALIC ANHYDRIDE WITH 5,6-DICHLOROBENZOFURAZAN OXIDE

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The term "isostere" has been applied classically to pairs of molecules such as benzene and thiophene, those possessing a general molecular size-and-shape similarity. In contrast, we have designated as "strict isosteres" those chemically different molecules possessing a close atom-for-atom correspondence with regard to both van der Waals radii and atomic connectivity. Examples include the organic azides, cyanates, and isocyanates. Certain other molecules qualify as strict isosteres only because disorder in their crystal structures lends them closely similar space-filling requirements in the solid state. We have designated these pairs or groups of molecules as "crystallographic surrogates."

Both strict isosteres and crystallographic surrogates should show extensive mutual solid-state solubility. We are examining those that undergo a solid-state phase transition upon heating or cooling with the goal of modifying or even controlling the phase-transition behavior of one strict isostere or crystallographic surrogate by co-crystallizing it with varied proportions of the other. Using single-crystal X-ray diffraction, we are currently examining the disordered molecular packing arrangement of a crystal containing the crystallographic surrogates 5,6-dichlorobenzofurazan oxide (known to undergo solid-state phase transitions) and 4,5-dichlorophthalic anhydride, and we describe the crystallographic results here. The cell constants and "molecular" geometry obtained are consistent with the formation of a solid solution of the two crystallographic surrogates. Solid solutions such as this will serve as the basis for subsequent phase transition studies.

HPLC ANALYSIS OF CATECHOL FOR NITROBENZENE DIOXYGENASE REACTION SYSTEM

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The bacterial strain *Comamonas* sp. JS765 expresses the nitrobenzene dioxygenase system (NBDOS), an enzyme system that catalyzes the oxidation of nitrobenzene to catechol. In order to develop a purification method for this enzyme system, an effective assay must be developed to determine protein purity. Since catechol is a product of the oxidation reaction, a catechol assay can be used with the NBDOS. The use of an HPLC assay for catechol provides the high resolution necessary to detect low concentrations of catechol in a mixture, so an HPLC catechol assay can be used with the NBDOS. Any HPLC assay for catechol must account for the instability of catechol which arises from its rapid oxidation to *p*-benzoquinone.

The HPLC assay used in this research was adapted from a similar assay used in a green tea system. In the green tea HPLC assay, ascorbic acid was added to catechol standards to preserve their stability. In this research, the addition of ascorbic acid to catechol standards allows one to generate a catechol standard curve. This standard curve can then be used to determine the concentration of catechol in NBDOS solutions.

IDENTIFYING PROTEINS WHOSE LOCALIZATION TO LIPID DROPLETS IS ALTERED BY THE EXPRESSION OF PERILIPIN 2

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Lipid droplets (LDs) function as intracellular lipid storage compartments in all eukaryotic cells, and yet very little is known about their synthesis and breakdown. By exploring the interactions of different proteins that localize to the lipid droplet surface, we aim to understand the mechanisms that govern these dynamic cellular structures. Perilipin 2 (also known as adipocyte differentiation-related protein) localizes to the surface of LDs and is important in LD formation and maintenance. To further understand the function of perilipin 2, we isolated LDs from perilipin 2-expressing and non-expressing HEK 293 cells and used polyacrylamide gel electrophoresis and Coomassie stain to visualize lipid droplet-associated proteins. Previous work from our laboratory has shown that perilipin 2 prevents the lipid droplet localization of a small number of other proteins, including perilipin 3 and adipose triglyceride lipase. In our recent experiments, we have isolated lipid droplets from large numbers of perilipin 2-expressing and non-expressing cells in order to further explore perilipin 2-dependent differences in the lipid droplet-association of other proteins. Proteins that target to lipid droplets only in the absence of perilipin 2 will be identified by mass spectrometry. These experiments will help us learn more about perilipin 2 and its role in the regulation of lipid storage in cells. This in turn may help us to better understand human diseases such as diabetes and obesity in which this process is misregulated.

LOCOMOTOR RESPONSE TO METHAMPHETAMINE IN RATS SELECTIVELY BRED FOR HIGH AND LOW SACCHARIN INTAKE

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Previous research has shown that rats selectively bred for high-saccharin (HiS) intake show a drug-prone profile compared with rats bred for low-saccharin (LoS) intake (Carroll et al. 2003). For instance, previous data suggest that HiS rats display an increase in locomotor response following repeated cocaine injections.

In the present study, locomotor response following methamphetamine (METH) administration was observed in HiS and LoS male rats. Rats received either a METH or Saline injection and were placed on a locomotor activity monitor for 60-minute sessions during a 5-day period. Rats were re-tested 2 weeks later to determine if repeated METH administration induced locomotor sensitization, or an increase in locomotor responding. Results indicated that the LoS METH-treated rats showed a slight trend toward METH-induced sensitization when comparing the first two sessions with the final session 2 weeks later. The METH-treated HiS rats showed consistently higher METH-induced locomotor activity compared with LoS METH-treated rats; however, they showed no increase in activity from the first two sessions to the last session 2 weeks later. Thus, lack of sensitization in the HiS rats may have resulted from ceiling effects. The differences from the previous study that found sensitization with cocaine in HiS (vs. LoS) female rats could be attributed to the different drugs used, sex differences, or different procedures. While additional research is necessary, these results support the HiS and LoS phenotypes as strong animal models for drug-seeking and resisting in human population, and underscore the influence of genes on individual differences in drug-seeking behavior.

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OPTIMIZATION OF AZOBENZENE MOLECULAR SWITCH IN ORGANIC SYNTHESIS

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Photosensitive azobenzene molecules are potential molecular switches for a nanoscale optoelectronic device because of their properties that are based on fast, light-driven processes[1]. An azobenzene derivative, 6,6'-difluoro-2,2'-dinitroazobenzene, is obtained in high yield (almost double) compared with other halogenated derivatives[2]. Also, the fluorines on 6,6'-difluoro-2,2'-dinitroazobenzene can be easily displaced, thus allowing nucleophilic displacement to produce other derivatives**.** Optimizing the synthesis of 6,6'-difluoro-2,2'-dinitroazobenzene from 2-fluoro-6-nitroaniline by studying the effects of several reaction conditions, including heat, solvent, and concentration of oxidizing agent, will eventually and potentially lead to a route that produces the highest yield.

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SYNTHESIS OF AN ANTIVIRAL COMPOUND

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As new viruses continue to mutate, multiply, and threaten the well-being of humanity, new antivirals must be explored and synthesized. Presently, sesquiterpene coumarin ethers are believed to be potential candidates for future antivirals. Progress towards the synthesis of a naturally occurring sesquiterpene coumarin has been made utilizing a unique method for reducing an ester to an alcohol in the presence of an epoxide. This compound is found in the roots of the Ferula assa-foetida plant and its synthesis has never been reported. Extracts containing this compound have been found to be active against influenza A (H1N1) and various human cancer cell lines.

A COMPREHENSIVE STUDY OF THE GENERAL PARAMETERS FOR G-WIRE SELF-ASSEMBLY

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Guanine rich sequences of chromosomal DNA and G-rich oligonucleotides (GROs) demonstrate the capacity to form higher-ordered structures through guanine self-recognition. The fundamental motif that organizes and stabilizes these supramolecular GRO assemblies is a tetrad of guanine nucleosides, dubbed a G-quartet, which interact through Hoogsteen hydrogen bonding. G-quadruplexes (G-DNA) are a higher-ordered continuation of guanine organization that comprises of vertically stacked G-quartets. This GRO formation is additionally stabilized with nucleoside base stacking; however, a crucial feature of G-DNA structures is the coordination of metal cations (K^+, Na^+ and Sr^{2+}) or small molecular cations (NH_4^+) that reside within the central pocket between stacked G-quartets. A cation's ability to strengthen G-DNA is correlated with ionic radius and follows the trend: K^+, Sr^{2+}> Ca^{2+} > NH_4^+>Na^+, >> Li^+> Mg^{2+} . Finally, the presence of counter ions (Mg^{2+}, $Tris^+$) has been shown to create more favorable self-assembly environments due to passivation of the negative charge along the phosphate backbone of DNA. Although the non-covalent forces between cations and individual guanine monomers are relatively weak, the collection of electronic interactions within the entire supramolecular complex has an additive effect, resulting in a highly stable structure.

When utilizing GROs for self-assembly, a supramolecular polymer of continuous parallel G-rich sequences can form. These polymers are coined G-wires. The central goal for this project was to make a broad impact in the field of nucleic acid research by developing general guidelines necessary for successful G-DNA self-assembly. Several different sequences of GROs were utilized under varying conditions: temperature, concentration of coordinating cations and presence of a counter ion (Mg^{2+}). Such a complete, systematic study of GRO sequences with regards to counter ions and coordinating cations has not been undertaken. Analysis of self-assembly reactions with polyacrylamide gel electrophoresis (PAGE) indicated the effectiveness of the coordinating cations for supporting G-DNA structures. The quantification of G-wire formation was performed by measuring and comparatively analyzing the fluorescence of distinct regions on the gel which correspond to a specific degree of higher ordered structure. Our results indicate that good G-wire formation is influenced by thymine units and the number of guanine units within the sequence, the positioning of said nucleosides, the incubation temperature, and the strength of utilized coordinating cations, with strontium being the most successful.

THE EFFECTS OF EDGE HABITAT ON A CHRISTMAS TREE FARM ON THE NEST ABUNDANCE AND SUCCESS OF NORTHERN TEMPERATE BIRDS

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Edge effects and ecological traps among nesting songbirds have been widely studied but little attention has been paid to these phenomena in agricultural settings. Agricultural plots are quasi-natural but often lack diversity compared to natural habitat. This study was done using data from three monitored Christmas tree farms in Forest Lake and Hugo, Minnesota and one Christmas tree farm near Somerset, Wisconsin. My primary research objectives were to survey use of Christmas tree plots by nesting songbirds, determine whether distance from the edge of the tree plot had an effect on the nest abundance and nest success, document nest success on Christmas tree farms, and compare this data to previous studies done in natural forests. Among the songbirds surveyed, a logarithmic relationship between the distance from the edge and the nest abundance was found (R^2=0.83) suggesting songbirds preferred to nest near the edge of the plot. Nest success was related to distance from the edge to a lesser extent in a linear regression (R^2=0.30). While these two test results do not provide enough evidence that the edges of Christmas tree plots act as an ecological trap, they do indicate that nest abundance was affected by the distance to the edge of the plots. This study also provides evidence for the role of Christmas tree farms as usable habitat for some nesting songbirds. The most common species nesting in the plots were Chipping Sparrows (*Spizella passerina*), American Robins (*Turdus migratorius*), and Song Sparrows (*Melospiza melodia*) which had Mayfield

nest success estimates of 0.65, 0.20, and 0.40 respectively.

EFFECT OF ELECTROPOLISHING ON SURFACE CHEMISTRY AND MORPHOLOGY OF NITINOL

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Nitinol, an innovative equiatomic alloy of nickel and titanium, is currently used in medical implantation devices. This study explored the correlation between electropolishing of Nitinol in a perchloric acid-acetic acid electrolyte and changes in surface morphology of the alloy, as well as some of the mechanistic dynamics of chloride corrosion at the metal-solution interface. The dependence of electropolishing efficacy on the applied voltage and the time was determined by measurement of the mass loss of the wire. The mass loss varies linearly with applied Coulombs, with a loss of linearity after a threshold is reached. To explore this threshold, 60V (3.316A) were applied to the wire for 90s, and spectrophotometry was then used to analyze electrolyte composition. The surfaces after both of these treatments were imaged to determine the effect of each on the surface morphology. It was determined that surface morphology varies with depth of wire in the electrolyte solution. Surface roughness of samples was also examined by profilometry.

DEVELOPMENT OF AN IN VITRO SUSCEPTIBILITY TEST FOR THE LIPOPHILIC YEAST *malassezia furfur*

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Malassezia furfur, a lipophilic yeast common as a member of skin microbiota, is implicated in skin disease and opportunistic systemic infection in neonatal and immunocompromised populations. Current treatment therapy of fungal skin infections includes topical administration of antifungal drugs. Acetic acid and boric acid are often used as carriers for antifungal drugs. We investigated the *in vitro* susceptibility of *M. furfur*, cultured in a modified broth, to various concentrations of acetic acid and for a time course of exposure to a standard (2.5%) concentration to determine what the antifungal properties were for the carrier, in developing a model in vitro susceptibility assay. *M. furfur* was grown in suspension culture in modified liquid media to different growth states and assayed for susceptibility. Kill curves were determined using qualitative and quantitative plating methods (serial dilutions, and spread plating for direct cell counts) to measure the effective dose (concentration) and time of exposure for acetic acid.

This work presents the results of our analyses on the susceptibility of *M. furfur* to acetic acid at various concentrations and length of exposure, and demonstrates an *in vitro* model that may be adapted for use to evaluate other chemical and pharmacological agents for therapeutic effect on *M. furfur.*

GROWTH CURVE ANALYSIS AND STANDARDIZATION OF *malassezia furfur*

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The lipophilic yeast *Malassezia furfur* is a common member of microbial skin communities, and is implicated in skin disease and opportunistic systemic infection in neonatal and immunocompromised populations. Methods for measuring culture turbidity, performing serial dilutions, and examining susceptibility testing *in vitro* require *M. furfur* be grown in suspension culture which is complicated by the need for a lipid source as an essential nutrient requirement in culture media. Culturing conditions using liquid growth media modified to contain olive oil as a lipid substrate, and SDS as an emulsifying agent were used with stir plate agitation to achieve uniform suspension culture to measure growth curve parameters.

This research presents the results of our growth curve analysis of *M. furfur* whereby turbidity measurements (optical density, OD) of absorbance at 630 nm were taken for samples growing in suspension culture at regular intervals in a time course study and correlated with direct cell count determinations using serial dilutions and spread plating methods on modified solid media. Results indicate that *M. furfur* grows remarkably quickly in suspension culture with continuous agitation at 35°C. Our goal is to test cell populations from various stages of the growth curve for susceptibility to potential antifungal chemotherapeutics *in vitro*.

PHOTODECOMPOSITION OF THE ANTIDEPRESSANT SEROQUEL (QUETIAPINE HEMIFUMARATE) IN SURFACE WATER

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Recent studies have shown the presence of human personal care products in streams and rivers which receive water effluent from wastewater treatment plants. There is a growing concern that human personal care products are not broken down completely and removed by current wastewater treatment methods, and have negative

effects on wildlife. Antidepressants are one class of human personal care product which have been identified in waterways receiving treated wastewater effluent. Antidepressants have become an increasingly more common prescription utilized by the American population. This study investigates one of these antidepressants, quetiapine hemifumarate. This project seeks to determine the breakdown rate of this antidepressant by direct and indirect photolysis processes in surface waters. This decomposition process serves as a natural means of eliminating antidepressants and other personal care products from natural waterways thereby limiting their effects on wildlife populations.

ROOM-TEMPERATURE STORAGE OF HUMAN SERUM BY ISOTHERMAL VITRIFICATION

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Biorepositories and biobanks are libraries where biospecimens, including human serum and plasma, are collected from patients and stored to be used for proteomic and genomic analysis, especially in cancer research. Currently, there are hundreds of biobanks across the nation containing millions of biospecimens. All these biospecimens are stored in freezers at -20, -40, -80 ˚C, or in liquid nitrogen for years. These freezers are both expensive to purchase and maintain and also impose freezing stresses on the biospecimens, potentially damaging them. Studies have shown that certain 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 the vitrified state can be reached at -16˚C ± 6.7˚C when 0.4 M trehalose is added and the sample is dried for one week at 2% ± 3% relative humidity. When serum containing 0.4 M trehalose is dried for one month, under the same conditions, it has been shown to reach the vitrified state at 4.2˚C ± .7˚C. This allows serum to be stored in a standard refrigerator (4˚C) rather than a storage freezer, greatly reducing the cost and stresses of frozen state storage.

IMMUNOPHENOTYPING OF T-CELLS IN TYPE 1 DIABETIC JAK3-DEFICIENT MICE

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Type 1 Diabetes (T1D) is an autoimmune disorder which leads to the destruction of the insulin-producing beta-cells in the pancreas by immune cells known as T-cells. There are three main types of T-cells—T-helper (Th), T-cytotoxic (Tc), and T-regulatory cells (Treg)—which are important in the development of T1D. They can be distinguished by their specific cell-surface markers.

The aim of this study was to quantify the T-cell populations involved in immunopathogenesis of chemically [streptozotocin (STZ)] induced autoimmune T1D in C57BL6/J mice which lack expression of the protein Janus Tyrosine Kinase (JAK3). JAK3 plays an important role in the cell signaling of T-cells. When JAK3 is absent, the function of T-cells is severely impaired.

To induce T1D, mice were injected with 40 mg/kg of STZ. The subpopulations of T-cells involved in the T1D development were quantified by flow cytometry using fluorochrome-conjugated antibodies specific for certain cell surface markers (immunophenotyping). It was hypothesized that JAK3-deficient mice that exhibit attenuated development of STZ-induced T1D would have decreased numbers of pathogenic and increased numbers of protective T-cells compared with wild-type mice which express JAK3. The results showed an increase in overall (CD3+), effector (CD4+CD62L+), and Treg (CD4+PD1+) T-cells in JAK3-deficient mice. Interestingly, JAK3-deficient mice did not express common CD4/CD25/FoxP3 markers, typical for Tregs. STZ treatment decreased the CD3+, CD4+ (Th) and CD8+ (Tc) T-cells, while not affecting CD4+CD62L+ and CD4+PD1+ T-cells. Overall, these data suggest that protection against STZ-induced T1D in JAK3-deficient mice can be attributed to unchanged number of Tregs.

COMBINATORIAL LIBRARY SCREENING FOR POSSIBLE INHIBITORS TO THE ENZYME LOW MOLECULAR WEIGHT PROTEIN TYROSYL PHOSPHATASE (LMW-PTP)

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It has been shown that Low Molecular Weight Protein Tyrosine Phosphatase (LMW-PTP) human isoform II has increased expression in certain types of

cancerous cells. Moreover, it has also been shown that increased expression of LMW-PTP can be predictive of more aggressive or invasive cancers.

Our project has been centered on discovering a specific inhibitor for LMW-PTP using analogs of Pyridoxal 5' Phosphate (PLP), a known inhibitor. While PLP is a successful inhibitor, it is not specific to LMW-PTP human isoform II. A combinatorial library of PLP analogs was created using Maestro (Schrodinger, LLC) at the Minnesota Supercomputing Institute. This library was created by linking all the primary amines sold by Sigma-Aldrich to the aldehyde on PLP, creating an amide linker. The modeling was done at a pH 5.5 +/- 2 (the pH at which in-*vitro* biochemical testing is done), and the entire library was docked using High Throughput Visual Screening (HTVS). The 19 compounds which displayed the highest docking scores were then docked to PTP using Standard Precision Docking in both LMW-PTP human isoform I and II, and compared with PLP. Some of these compounds displayed some specificity and good binding affinity. These results were analyzed to determine which intermolecular forces were involved in docking, and which compounds appear to specifically inhibit the Human Isoform II. The predicted compounds will be synthesized and tested using an in-*vitro* screening assay.

PREPARATION OF 1,3,2-OXAZAPHOSPHOLIDINE-2-OXIDE DERIVATIVES AS MODELS FOR THE DEVELOPMENT OF NOVEL CHIRAL AUXILIARIES

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The objective of this research is to prepare novel phosphorus-based chiral auxiliaries for use in asymmetric synthesis. Model 1,3,2-oxazaphospholidine-2-oxide derivatives, patterned after the well-known oxazolidinone and oxazolidinethione auxiliaries of Evans and Crimmins, were prepared from simple reagents. Phosphorus oxychloride was reacted with diethylamine followed by ethanolamine to produce 2-diethylamino-1,3,2-oxazaphospholidine-2-oxide as one of the derivatives. Methylphosphonic dichloride, prepared from phosphorus hexachloride and dimethyl methylphosphonate, was reacted with ethanolamine to prepare the 2-methyl derivative.

This model synthetic pathway could potentially be used to make phosphorus-based chiral auxiliaries that may have complementary utility to the widely applied auxiliaries of Evans and Crimmins.

NUTRIENT AVAILABILITY AND SOIL PROCESSES ALONG TOPOGRAPHICAL GRADIENTS IN A RESTORED PRAIRIE

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Ecosystems are dynamic and respond to environmental changes. Ecosystem gradients influence biogeochemical cycling and microbial stoichiometry, and may also link terrestrial and aquatic systems. Our objective in this project was to characterize resource availability and microbial biomass along transects spanning topographic gradients connecting upland prairies to low-lying wetlands.

We measured labile C, extractable N and P, and microbial C, N, and P in soil samples collected along two topographic gradients in a restored prairie in central Minnesota. Each transect consisted of two to three upland prairie sites and five wetland sites arrayed across a moisture gradient from transitional marginal zones to permanent wetland. We found little spatial variation in nutrient availability within transects, but clear differences in nutrient availability between the two transects. Soil analyses revealed higher labile P and lower microbial N:P ratios in the western transect compared with the eastern transect. The presence of N-fixing legumes (*Petalostemum purpureum*) along the eastern topographical gradient may have released the eastern transect from N-limitation (N:P>7).

In October labile nutrient pools and microbial stoichiometric ratios in the wetlands reflected the prairies, indicating an ecosystem linkage, possibly because of the downhill flow of nutrients. In November the eastern and western transects were still significantly different, but nutrient availability decreased in the prairies and increased in the wetlands, and microbial N:P increased in the prairie and decreased in the wetland. Our results indicate that resource availability is driven by environmental gradients and exhibits seasonal change.

ELECTRON-RICH BIPYRIDINE COMPLEXES OF RUTHENIUM FOR DYE-SENSITIZED SOLAR CELLS

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The syntheses of new ruthenium-based dyes for use in dye-sensitized solar cell applications will be presented. Nucleophilic aromatic substitution of 4,4'-dichloro-2,2'-bipyridine with piperidine, morpholine, and pyrolidine placed strong electron-releasing

substituents on the bipyridine rings. The modified bipyridine ligands were reacted with $RuCl_3 \bullet nH_2O$ and glucose (reducing agent) to form the corresponding divalent ruthenium complexes, $Ru[(R_2N)_2bpy]_2Cl_2$. These were subsequently reacted with 2,2'-bipyridine-4,4'-dicarboxylic acid, which provides a suitable mode of attachment of the complex to the surface of TiO_2 or ZnO used in solar cells. All complexes were characterized with ^1H NMR spectrometry and electrospray mass spectrometry. Molar absorptivity of all complexes was examined using ultraviolet-visible spectroscopy. Using cyclic voltammetry, electrochemical properties of all complexes will be reported.

CHARACTERIZATION OF THE UNCULTURED CYANOBACTERIAL AND ARCHAEAN COMMUNITIES IN *Sarracenia purpurea* PHYTOTELMATA BY 16S RIBOSOMAL RNA SEQUENCING

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The Pitcher Plant (*Sarracenia purpurea* L.) is a carnivorous angiosperm native to North American acidic peatlands. This species produces specialized leaves (pitchers) that collect rain water in which a microbial community develops. *Sarracenia purpurea* purportedly acquires much of its fixed nitrogen and phosphorous from captured arthropods decomposing in the pitcher fluid (phytotelmata). While evidence exists that the plant's leaves secrete the enzymes responsible for this prey decomposition, less is known about the contribution of the phytotelmata microbial community to this process or as an alternate mechanism of nitrogen fixation.

In recent years, several laboratories have reported on the culturable bacterial and protist communities of *Sarracenia purpurea* phytotelmata. However, less is known about the prokaryotic groups that are currently unculturable or difficult to culture from this environment, particularly the cyanobacteria and the archaea. A more complete characterization of these two sub-populations from *Sarracenia purpurea* phytotelmata is an important step in revealing the role of prokaryotes in the nutrient acquisition process of this plant, and in particular whether nitrogen-fixing cyanobacteria contribute significantly to the process.

We created a clone library of cyanobacterial and Archaean 16S ribosomal RNA genes from the phytotelmata of multiple *Sarracenia purpurea* plants in central Minnesota. The isolated 16S rRNA genes from these clones were then sequenced and analyzed to establish putative classification and identities, which are reported here. The presence of multiple cyanobacterial lineages in the phytotelmata of *Sarracenia purpurea* suggests that these prokaryotes may play a fixed-nitrogen supply role in the life of this host plant.

EXECUTIVE FUNCTIONING AND ITS ROLE AS A MEDIATOR BETWEEN PARENTING AND ACADEMIC SUCCESS AMONG HIGH-RISK CHILDREN

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Studies have shown that quality of parenting, and executive function (EF), which includes inhibitory control (IC), similarly affect children's academic success. I hypothesized that high scores on a scale of Parental Positive Responsiveness (PPR) would be related to IC in children, as coded by a Simon Says task, which would be related to academic achievement at school, and that child IC would mediate the link of PPR to school success. This prediction was based on literature suggesting that positive parenting, which provides warmth and comfort to children, helps young children to inhibit impulsive emotions and actions, which in turn assists children with concentration, facilitating academic success.

My study consisted of 138 children aged four to six and their parents who were living in homeless shelters during the summers of 2008 and 2009 when these families were recruited for a larger study of EF and parenting. Results indicated that IC predicts academic competence at school once age and gender are controlled. However, PPR was not related to academic outcome and therefore a mediating role of IC was not found. The strongest predictors of academic competence were children's IC and age. These results suggest that IC may be particularly important for high-risk children's school success. If we can help disadvantaged children gain IC at a younger age, perhaps we can decrease the achievement gap often found in high-risk children.

MIND THE GAP: SALARY, EXPECATION, GENDER AND EDUCATION

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The class-action law suit by female Wall-mart employees reinforces the gender pay disparity. The employees allege pay gender bias in promotion and pay. This is a disturbing trend for females in the work force. What are the contributing factors, to this salary inequality? Is it based solely on gender and gender

alone? Does an individual's work experience and education level play a part in this? Does this pay gap exist at all levels and in all types of jobs? In this research project our team looked closely at the expectations of the person who is looking for work or/are currently working and the salary these people are receiving or will be receiving in near future. Our team looked closely to see if there is gender discrimination regards to pay and how our current economy affects this data.

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 have already been 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,* a very close relative of *P. polycephalum,* and in *Semimorula liquescenes*, a member of a newly described genus, which may be quite divergent from other myxomycete groups. 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 mitochondrial genes of myxomycete species. We applied the codon usage data to design degenerate primers in three potential conserved regions of *nad2*. We extracted RNA from *Physarum polycephalum*, reverse transcribed it, and are using this cDNA to optimize PCR conditions with the primers. Using similar PCR conditions, we will amplify cDNA, then DNA, from *Didymium nigripes* and *Semimorula liquescenes*. Comparision of the sequences of cDNA and DNA will identify patterns of RNA editing in these diverse myxomycete species.

PHASE BEHAVIOR OF POLY(ETHYLENE OXIDE) IN IONIC LIQUIDS

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We examine lower critical solution temperature (LCST) phase behavior of poly(ethylene oxide) (PEO) of varying molecular weights (Mw=2K, 3.4K, 5K, 20K); dissolved in ionic liquid (IL),1-ethyl-3-methlyimidazolium tetrafluoroborate ([EMIM][BF4]) and 1-ethly-2, 3-methlyimidazolium tetrafluoroborate ([EMMIM][BF4]). Phase transition temperature is determined using cloud point measurements, with critical temperature observed from 200^{o}C to 130^{o}C. Critical composition of PEO is shifted to higher concentrations (approximately 80 wt %) with low PEO-2K and 3.4K systems showing strong dependence on molecular weight. PEO dimethyl ether exhibits lower LCST (as observed with aqueous PEO systems). We are exploring the possibility of hydrogen bonding between the PEO and IL as the cause for unusual phase behavior.

IMPACT OF NA+/H+ EXCHANGER ISOFORM 1 PHOSPHORYLATION BY RIBOSOMAL S-6 KINASE AND RHOA KINASE ON ERM BINDING AND CELL MOTILITY

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Cellular migration involves complex signaling and intricate reorganization of the cytoskeleton. Ezrin/Radixin/Moesin (ERM) proteins play a crucial role in the reorganization of the cytoskeleton by binding to membrane bound proteins including the Na^{+}-H^{+} exchanger (NHE) and anchoring stress fibers to the plasma membrane. During cellular migration, there is a reorganization of the membrane to localize NHE to the leading edge of the cell. Localization of NHE helps to give the cell polarity, which is important in directing stress fiber formation to assist the cell to move forward. The focus of this study was to determine the conditions by which ERM translocates to the membrane to bind to NHE and examine the role of the ERM:NHE binding complex in proper formation of unidirectional cell migration. We have prepared a series of Ser or Thr – Ala mutants at putative RhoA Kinase (Rock) and Ribosomal S-6 kinase (RSK) phosphorylation sites and expressed these in NHE1-null PS120 cells. Co-immunoprecipitation of NHE1 identified ERM-NHE1 interactions for each mutant following activation with lysophopsphatidic acid (LPA). Using wild-type and non-phosphorylatable EFGP-ERM, we investigated the localization of ERM proteins and the impact of NHE1 phosphorylation. Finally we show the effect of NHE1 and ERM phosphorylation on stress fiber formation and cell motility.. This work was supported with funds from NSF-MCB-081778, NSF-RUI-MCB 0930432, and NIH-1-R15-CA135616-01.

THE FLA4 TPR-CONTAINING POLYPEPTIDE LOCALIZES TO THE CELL BODY AND IS REQUIRED FOR FLAGELLAR MAINTENANCE IN *Chlamydomonas*

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Dynamic assembly and disassembly of eukaryotic flagella relies on the coordinated activity of the flagellar intraflagellar transport (IFT) machinery and proper targeting of precursor proteins from the cell body to the flagellum. We have characterized a temperature-sensitive mutation in the *Chlamydomonas* FLA4 gene that permits normal flagellar assembly at 21˚C and 32˚C, but results in flagellar loss at 32˚C. Molecular cloning and sequence analysis demonstrated that the *fla4* flagellar assembly phenotype is the result of a nonsense mutation in exon 2, and the predicted gene product corresponds to a conserved TPR-containing polypeptide with a predicted molecular weight of 78.9 kDa. Flagellar growth after acid-induced flagellar excision in the *fla4* mutant strain was comparable to wild type 137c strain at 21°C and 32°C with exponential growth kinetics. However, flagellar loss after shift to 32˚C showed rapid exponential decay in the *fla4* mutant. Transformation with a FLA4-GFP construct rescued the temperature-sensitive flagellar assembly defect, and immunofluorescence microscopy demonstrated that the majority of FLA4-GFP is localized to the cell body in a punctate distribution, with a minority of cells showing partial GFP fluorescence in the flagellum. These results suggest that FLA4p may play a role in mediating signaling events or protein-protein interactions in the cell body to facilitate the maintenance of flagella. Current experiments focus on the use of cellular fractionation and Western blotting to quantify FLA4p distribution in the flagella vs. the cell body, and to identify other factors that interact with FLA4p.

NUCLEATION INVOLVING H_2SO_4 AND H_2O: PARTICLE NUMBER DEPENDENCE ON H_2SO_4 VAPOR

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The dependence of particle number on sulfuric acid vapor was investigated to get the number of H_2SO_4 molecules involved in the H_2SO_4 /H_2O binary system of nucleation. The experimental apparatus is a flow reactor with nitrogen, water, and sulfuric acid input to make a reaction mixture with total N_2 flow of 6 standard liters per minute (slpm); 0.1% water, and 1 ppb sulfuric acid. The mixture was mixed in a region at 313 K and then it flowed to a flow reactor, where the nucleation took place at 296 K. The particles formed in the flow reactor were counted using modified particle counter 3020(3020M). The power dependencies of particle formation on H_2SO_4 varied for different experimental conditions. The experiment with the mixing region at room temperature and total flow of about 4 slpm produced a power dependency of 9. A power dependency of 6 was obtained with the warm, temperature regulated mixing region. In experiments with total flow of 6 slpm and warm mixing region, a power dependency was found to be between 3.8 and 5.

NEUTROPHIL INFLUX IS NECESSARY FOR MAST CELL-SPECIFIC THERMAL HYPERNOCICEPTION IN MICE

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Mast cells are densely granulated tissue-resident cells capable of releasing a variety of mediators that modulate immune responses. They are involved in allergies, resistance against venoms, and defense against bacterial and parasitic infections; however, their role in inflammatory pain is unclear.

In efforts to gain a better understanding of the role of mast cells in hypernociception, we have developed a mast cell degranulation-dependent model of thermal plantar pain in mice. We investigated whether neutrophils (tissue-infiltrating leukocytes that are potently recruited by mast cells) are necessary for this pain response. We found that neutrophil infiltration is necessary for the development of hypernociception in mast cell-mediated inflammation, and both neutrophil influx and pain responses are abrogated when mast cell degranulation is blocked.

USING EGG YOLK TO INVESTIGATE CHARACTERISTICS OF BIOLOGICAL LIPIDS: AN EXPERIMENT FOR UNDERGRADUATE BIOCHEMISTRY STUDENTS

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A laboratory experiment was designed in order for students at Bethel University enrolled in an upper level biochemistry course to study the relationship between relative amount of certain lipids present in an egg yolk and the hen's diet. Eggs from several different sources, a normal store brand and a supplier feeding hens an enriched diet, were taken to create FAME (Fatty Acid Methyl Esters) derivatives. These FAMEs were analyzed

using GCMS, and the students were challenged to identify to which group their sample belonged based on the particular lipid composition observed in their sample and the claims of the egg brands. Specific egg suppliers claim higher levels of essential and other beneficial fatty acids such as omega-3 and omega-6 fatty acids. This experiment exposes students to the applications of instrumentation such as GCMS in the field of Biochemistry, and challenges them to integrate knowledge of the relationship between structure and function of molecules with their own data from sample analysis.

MILD SYNTHETIC REDUCTION OF AN AMIDE TO AN AMINE

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The process of converting an amide carbonyl into a methylene group, also known as a reduction, normally utilizes harsh and extreme conditions and requires the use of relatively dangerous chemicals. This research project entailed the development of a new synthetic methodology to carry out this conversion under mild conditions. To carry out this project, I tested different reagents ability to convert an amide carbonyl into a methylene group. Converting the amide into a more easily reducible entity was the first step. This involved reaction of the amide, triphenylphospine and carbontetrachloride. I then reduced this intermediate with a mild reducing agent, sodium triacetoxyborohydride. Conditions such as concentration, temperature, and solvent will be varied. The conditions that successfully reduced acetamide will be presented as well as characterization of an intermediate.

INTERACTION OF N-ACETYLCYSTEINE AND CYSTEINE IN HUMAN PLASMA

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N-acetylcysteine (NAC), a potent antioxidant, has been investigated as a therapy for numerous diseases including as adjuvant therapy for childhood cerebral adrenoleukodystrophy (CCALD). CCALD is a devastating X-linked disorder affecting boys ages 2-10 years resulting in progressive neurological impairment and death. Preliminary results have been promising; however, NAC's mechanism(s) of action in CCALD and other diseases is poorly understood. Previous research has shown that NAC serves as a precursor to cysteine which increases synthesis of glutathione, a strong, endogenous antioxidant, in erythrocytes. Our preliminary studies in CCALD boys have shown that following NAC IV therapy, red blood cell glutathione levels increased while cysteine concentrations in plasma decreased. We hypothesized that NAC can liberate cysteine bound to plasma proteins, resulting in increased free cysteine that can be used to sustain glutathione synthesis.

The objective of our study was to investigate the effects of NAC on cysteine protein binding in human plasma. Human plasma was incubated for 1 hour with varying NAC concentrations (0-1,000 µg/ml). Unbound and total endogenous cysteine concentrations were measured in the plasma using HPLC-Mass Spectrometry. The time-dependence of this interaction was evaluated by incubating plasma with 100 µg/ml NAC for 5-90 minutes. The results showed significant ($p<0.05$) increases in unbound plasma cysteine and decreases in cysteine plasma protein binding with increasing NAC concentrations. Furthermore, the increase in unbound cysteine was primarily observed within the first 5 minutes of incubation with non-significant increases thereafter. These results demonstrate that NAC liberates endogenous protein-bound cysteine in human plasma at NAC concentrations that are clinically attainable (0-100 µg/ml). A greater understanding of NAC actions in human plasma will allow for the optimization of NAC therapy.

PRODUCTIVITY AND SOIL CHARACTERISTICS AS INDICES OF TALLGRASS PRAIRIE RESTORATION SUCCESS

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Restoration of old fields to tallgrass prairie is implemented to increase nutrient retention, create habitat for native species, and re-establish ecosystem functions. Although some studies suggest that soil organic carbon and nitrogen may take several hundred years to reach remnant prairie levels, research is needed to determine the time frame needed to re-establish much of the prairie ecosystem diversity and function.

In 2010, we sampled from seven prairies planted at St. Olaf from 1989-2004 to assess the effects of restoration on soil and plant communities. Our goals were to determine if soil characteristics or biomass production approach remnant prairie levels as prairies age, and investigate the relationship between soil and plant characteristics in the restored prairies. We measured soil characteristics—including organic matter, bulk density,

nitrate, ammonium, and phosphate—as well as aboveground biomass and species richness. Soil organic matter, all nutrients, and aboveground biomass decreased with prairie age, while the remnant prairie had significantly higher organic matter than six of the restored prairies. Species composition and richness were similar across restored prairies, while the remnant prairie had significantly higher species richness. As residual nutrients from agriculture decrease and competition for nitrates and water increase with prairie age, plant communities are expected to shift the allocation of biomass belowground. While species richness is yet to increase with prairie age, the decrease in soil nutrients may facilitate a future increase in native species richness. We expect ecosystem functions will become more similar to remnant prairies after this species increase occurs.

SEX DIFFERENCES AND EFFECTS OF MODAFINIL AND ALLOPREGNANOLONE ON A RAT MODEL OF METHAMPHETAMINE RELAPSE

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Modafinil (MOD) is an analeptic drug currently being examined as a treatment for stimulant dependence. This experiment examined MOD's potential for use in treatment of methamphetamine (METH) addiction and further investigated the role of sex differences in drug-seeking behavior and treatment receptivity. The effects of allopregnanolone (ALLO), a progesterone metabolite that has been previously shown to reduce drug-seeking behavior in female rats, were also examined. Rats were trained to self-administer IV injections of METH during daily 5-hr sessions, and continued stable METH-seeking behavior over a 10-day maintenance period. Next, METH was replaced with saline, and drug-seeking behavior extinguished over an 18-day period. Following extinction, rats began a reinstatement procedure lasting 9 days in which an ALLO, MOD, or control pre-treatment injection was given 30 minutes prior to daily session, followed by a METH or saline priming injection that was given at the start of session. This reinstatement phase is considered an animal model of human relapse. Females showed greater responding on the previously METH-paired lever during reinstatement compared with males. MOD attenuated METH-seeking behavior equally in males and females, while MOD priming injections did not increase responding compared to saline control. ALLO attenuated METH-seeking behavior in females, but had no effect on males. These results illustrate the potential utility of MOD as a treatment for METH addiction and illustrate the role of gonadal hormones, such as ALLO, in the sex differences observed in drug-seeking behavior. This research was supported by NIDA grants R01 DA018151S1 (TEP), R01 DA003240, R01 DA019942, K05 DA015267 (MEC), and UROP of the University of Minnesota.

DIFFERENTIATING ABO GENOTYPES BY USING QPCR METHODS

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The objective of this research was to develop a genotyping assay in order to identify and distinguish the common allele variants present in the ABO blood system. These include A101, A201, B101, O101, O201 and O303. Previous qPCR (quantitative polymerase chain reaction) genotyping involved the use of single nucleotide polymorphisms (SNP) at nucleotide positions 261 and 297. At the SNP 261, A and B alleles have the guanine nucleotide while the O101 and O201 alleles had the guanine nucleotide deleted. At the SNP 297 type A and variant O101 have an adenine nucleotide while type B and O201 have a guanine nucleotide. Ambiguity was found for heterozygote samples making it necessary to use another SNP to distinguish among the different variants. A qPCR genotyping method was designed for the SNP 930 because it distinguishes the B allele from the A and O303 alleles. The DNA used was obtained using a DNA purification kit and concentrations were estimated using a spectrometer at a wavelength of 260. The designed method was validated with samples of known ABO genotypes and was successfully used to identify genotypes in samples that could not be identified using the SNPs at 261 and 297. The results from the genotyping qPCR run showed that the variants were able to be distinguished and ambiguity was relieved with this additional genotyping procedure.

BRIDGE N-H VS. BRIDGE C-H AS AN H-BOND DONOR IN THE CRYSTAL STRUCTURES OF PHENYLHYDRAZONES

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Molecules we have designated "bridge-flipped isomers" differ only in the orientation of a bridge of atoms connecting two major portions of the molecule. Examples are found among the benzylideneanilines, in which the isomerism is Ar-CH=N-Ar' vs. Ar-N=CH-Ar', and among the phenylhydrazones, in which the isomerism is Ar-CH=N-NH-Ar' vs. Ar-NH-N=CH-Ar' (Ar = aryl).

We are conducting a solid-state study using single-crystal X-ray diffraction to determine whether pairs of these isomers can assume identical molecular packing arrangements. Those that possess this isostructuralism

may be capable of being co-crystallized to produce new materials. A potential structure-differentiating factor between bridge-flipped phenylhydrazones is the difference in H-bond donor strength between the bridge N-H and C-H groups; reversing the bridge orientation would disrupt the packing if an H-bond acceptor on a neighboring molecule interacted more strongly with the strongly donating N-H group than with the weakly donating C-H group.

We are currently examining the crystal structures of cyano-substituted phenylhydrazones to determine how the nitrile group interacts with these two potential H-bond donors. As part of this study, we have determined and describe here the crystal structure of 3-cyanobenzaldehyde-2-bromophenylhydrazone. In contrast to certain of our previous structures, this one features a molecular packing motif at the bridge in which the nitrile group interacts nearly equally with the N-H and C-H groups of a neighboring molecule. Although we have not yet obtained crystals of the bridge-flipped isomer, the nonspecificity of this H-bonding interaction suggests that isostructuralism between these two isomers is possible.

CELLULOSIC ETHANOL FROM CATTAIL LEAVES

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Fuel-grade ethanol can be made from any appropriate source of carbohydrates. Cellulose, an abundant polysaccharide in plant cell walls, can be converted into the readily fermentable sugar glucose. Previous work in this lab demonstrated that cattails (*Typha* species) are a viable source for the production of cellulosic ethanol providing another alternative to the use of food crops to make ethanol. The current project carried cattail biomass through the steps necessary to convert cellulose into ethanol. Leaves were dried and pulverized. This powder was pretreated to break open the cell wall lattice and make the cellulose accessible to hydrolytic enzymes. Pretreated samples were then incubated with cellulase and beta-glucosidase to hydrolyze the cellulose to glucose. Finally, *Saccharomyces cerevisiae* was used to ferment the glucose into ethanol. High Performance Liquid Chromatography was used to measure glucose and ethanol levels. During the course of the project, conditions were varied to improve the yield. Pretreatments using 0.4 M phosphoric acid, 2% sulfuric acid, and water were compared. While the glucose yield was highest with 2% sulfuric acid in the pretreatment, it was not substantially different from the other two pretreatments. Previous work suggested that the enzyme load used in hydrolysis was inadequate. The effect of enzyme load and temperature was investigated to improve glucose yield. Culmination of the project demonstrated the production of ethanol from cattails.

SYNTHETIC ROUTES TO, TRANSFORMATIONS OF, AND RATHER SURPRISING STABILITIES OF (*N*-METHYLPHENYLCARBAMOYL) SULFENYL CHLORIDE, ((*N*-METHYLPHENYLCARBAMOYL)DITHIO) CARBONYL CHLORIDE, AND RELATED COMPOUNDS

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The title compound classes, (carbamoyl)sulfenyl chlorides and ((carbamoyl)dithio)carbonyl chlorides, have been implicated previously as unstable, albeit trappable, intermediates in certain organosulfur chemistry reactions.

The present work reports, for each of these compounds in the *N*-methylaniline family: (1) several routes to prepare it; (2) its direct structural characterization by 1H and 13C NMR, IR, and mass spectrometry; (3) its rather unexpected stability, and its ultimate fate upon decomposition; and (4) a series of further chemical transformations that give highly stable derivatives, each of which was in turn characterized thoroughly. Relevant kinetic and mechanistic experiments were carried out, including some with *p*-methyl and 2,6-dimethyl substituted *N*-methylanilines. Given that the title compound classes can be isolated and are relatively stable, they may find applications to the preparation of thiolyzable and/or photo-labile protecting groups for the sulfhydryl function of cysteine, and for the development of new protein synthesis and modification reagents.

LOCAL EXPRESSION OF INFLAMMATORY CYTOKINE RECEPTORS IN MAST CELL-MEDIATED HINDPAW THERMAL PAIN IN MICE

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Mast cells mediate important immune reactions including allergy, host defense, autoimmunity, and cancer. Their contribution to pain responses is an area of active investigation.

Our lab has established mast cell-specific models of thermal hypernociception in mice. One of these models makes use of the polyamine compound 48/80. When injected intraplantary in the hindpaws of mice, this compound causes local non-antigen-triggered mast cell degranulation leading to inflammation and thermal hypernociception. Mast cells can store or

synthesize a number of cytokines and chemokines, which are responsible for triggering different immune responses. We have shown that mast cell-derived pro-inflammatory cytokines such as TNF-α and IL-1β are important in mediating the pain response in the compound 48/80 model.

This study investigates the changes in pro-inflammatory cytokine receptor mRNA levels in the plantar tissue aiming to identify locally relevant signaling pathways activated upon mast cells degranulation in the hind footpads of mice. Using quantitative real time polymerase chain reaction, we show that IL-1β (IL1R1), TNF-α (TNFRsf1) and IL-8 (IL8Rb) receptor mRNA levels are upregulated following mast cell degranulation, indicating the importance of these cytokines and chemokines in mediating thermal hypernociception in mice.

AN EXPLORATION OF MAGNETISM IN THIN FILM STRAINED LACOO3

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LaCoO3 is a non-ferromagnetic insulator that crystallizes in the perovskite structure. Thin film LaCoO3 has recently been shown to exhibit ferromagnetism when grown epitaxially under strain. The origin of this anomalous magnetism is still unknown. This project will seek to understand why thin film LaCoO3 behaves in such a way. The idea is to grow PrCoO3, a closely related oxide material, on the same substrate and gradually alloy in LaCoO3, giving Pr1-xLaxCoO3. Strained PrCoO3 is expected to be diamagnetic, due to the reduced size of Pr ions, which distort the structure, making the non-ferromagnetic phase even more stable. The onset of ferromagnetism with doping will shed some light on the physics behind these materials.

In order to do this, the compounds are first prepared in the form of powders by solid state synthesis, and are checked for structural and compositional phase purity by x-ray diffraction. They are then pressed into pellets and sintered. These pellets serve as targets for physical vapor deposition of thin films, using pulsed laser deposition or sputtering . Finally, the film's magnetic properties are analyzed using a Superconducting Quantum Interfering Device (SQUID) magnetometer.

PrCoO3 has already been successfully synthesized, and it's composition and phase purity has been confirmed by x-ray diffraction.

LIFETIME MEASUREMENTS OF CDSE NANOCRYSTALS IN THIN FILMS

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Optical properties in thin films of CdSe nanocrystals will be important in many types of devices. In this poster, the fluorescence lifetime of CdSe nanocrystals were analyzed though the use of time-correlated single-photon counting as a function of temperature and particle size. Fluorescence lifetime measurements were taken at temperatures from 4 K to 295 K in increments of 50 K while monitoring the emission of different particle sizes. The CdSe nanocrystal sample was transferred into a cryostat, cooled by liquid helium, and irradiated with a 470-nm laser. Larger particles exhibited slower rates of decay than smaller particles, indicating a shorter lifetime for smaller particles at constant temperature. The fluorescence decay for smaller particles were fit with a biexponential function while larger particles were fit with a monoexponential function, indicating more pathways to decay for smaller particles. Higher temperatures resulted in higher rates of decay.

EXPRESSION OF LIPOXYGENASE (LOX) 1 PS:5 AND LOX G IN PEA LEAVES AFTER MECHANICAL WOUNDING

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Lipoxygenase (LOX) enzymes are a class of catalysts that vitally contribute to plant defenses against environmental and pathogenic stressors. Several LOX isoforms like LOX 1 PS: 1, LOX 1 PS: 2, LOX 1 PS: 3, LOX 1 PS: 5, LOX 1 PS: 7, LOX N2, LOX N3 and LOX G have been identified at the DNA level in pea tissues. Previous studies have shown an increase in total lipoxygenase activity in wounded pea plants after the plant had been exposed to biotic stressors. Very few studies have shown differences in the relative expression of individual LOX isoenzymes. In this present work, two real time polymerase chain (qPCR) methods were designed to quantitatively monitor LOX 1 PS: 5 and LOX G expression in wounded pea leaves. RNA was isolated from 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 random hexamers and a high capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers and probes were designed using known sequences and Primer Express software (Applied Biosystems). Efficiency curves showed that the qPCR designs were successful. The expression of the LOX

isoenzymes were measured in pea leaves at 0, 12 and 24 hours after mechanical wounding. While LOX 3 and LOX G expression increased significantly compared to the unwounded control, LOX 1 PS: 5 did not.

CHARACTERIZATION OF THE CEFTAZIDIME RESISTANCE CONFERRING CFT2 BETA-LACTAMASE

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Recent years have seen increase in bacterial drug resistance and the rate of rediscovery of new antibiotics has been steadily decreasing. In fact, 99 out of every 100 antibiotics pulled out of the soil have already been discovered[1]. A gene, *cft2*, conferring resistance to the beta-lactam ceftazidime was isolated from a metagenomic clone. Comparison with the BLAST database revealed a gene encoding a 607 amino acid (AA) multifunctional protein. The sequence of AA 342-602 shows homology to *lra5,* a class A β-lactamase, while AA 40-211 show homology to a sigma factor predicted to be involved in stress response. The AA spanning the middle region of the protein were predicted by HMMTOP to belong to a transmembrane sequence. Western blot analysis yielded more protein in the pellet than the supernatant supporting the idea of an insoluble membrane-associated protein. Therefore the protein was proposed to contain an intracellular sigma factor, a transmembrane domain, and an extracellular β-lactamase. A decrease in function when the β-lactamase domain was expressed alone, random mutagenesis, and hydrolysis assay support the proposed β-lactamase nature of Cft2. Site directed mutagenesis was carried out introducing a four amino acid sequence. Cft2 does not confer resistance to penicillins unlike its homologs that contain the four amino acid sequence. Thus, research is underway to assess the specificity of the Cft2 mutants for various β-lactam antibiotics in an attempt to understand the role that the amino acid sequence plays in β-lactamase activity.

1. Emmert, E.A., A.K.Klimowicz, M.G. Thomas, and J. Handelsman. 2004. Genetics of zwittermicin A production by *Bacillus cereus.* Appl. Environ. Microbiol. **70**:104-113.

MAST CELLS ARE CRITICAL MEDIATORS OF THERMAL PLANTAR PAIN

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Mast cells are key effector cells in allergic and innate immune responses, acting as sentinels at the interface between the external environment and the host. Recent studies suggest a role for mast cell activation and degranulation in the initiation and promotion of inflammatory pain. We have established two models of mast cell-specific plantar pain in mice, mediated by (1) basic mast cell secretagogue, compound 48/80-induced degranulation and (2) passive antibody-sensitization and antigen challenge (IgE/Ag). In these studies we found that compound 48/80-induced plantar pain is abrogated in genetically mast cell-deficient mice and IgE/Ag-induced pain is blocked by administration of histamine receptor antagonists.

USING A MICROPLATE READER TO DETERMINE THE ACTIVITY OF LACTATE DEHYDROGENASE IN RELATION TO TEMPERATURE

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Analyses of temperature-dependent kinetic parameters of enzymes such as lactate dehydrogenase (LDH) are usually carried out within the range of physiological temperatures (0-40 °C). However spectrophotometers with efficient wide range temperature control, require large amounts of sample and long time frame. We adapted an LDH assay for a microplate reader to increase the speed while limiting the amount of sample required. Our biggest limitation with our instrument (BioTek Synergy 2) was temperature control (15-32^{o}C). LDH from rabbit muscle was obtained from Sigma. Pyruvate reduction to lactate was measured by following the oxidation of NADH to NAD at 340 nm. Entire Michaelis Menton curve determinations could be done at once using eight concentrations of pyruvate (0.01 mM to 0.75mM). The Michaelis constant of the substrate pyruvate (K_M) and the, and maximal reaction velocity (V_{max}) were determined by both Michaelis-Menten plot and Lineweaver-Burke plot. The K_M was from 0.089 to 0.030 mM and V_{max} ranged from 0.9×10^{-3} to 1.4×10^{-3} mM/min at 15 and 32^{o} respectively. These values compare well with the previous experiment done my T-microplate on LDH in Atlantiz cod (*G. morhua*) which used Arrhenius and Michaelis-Menten plot to calculate K_m and V_{max} at temperature between 4 to 25^{o}C (K_m=0.01 to 0.3 mM at 25 ^{o}C) . The strategy for mixing and temperature control is described for those microplate readers that lack the ability of mixing and temperature control.

ISOMORPHOUS SUBSTITUTIONS OF CALCIUM BY DYSPROSIUM IN THE STRUCTURE OF SYNTHETIC VANADATE APATITE

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Compounds with the apatite structure had the general composition $M_{10}(EO_4)_6(X)_2$, where M was univalent to trivalent cations (Ca, Sr, Ba, Cd, Eu, Y, La, Na, K, and others); E was tetravalent to hexavalent cations (P, V, As, Si, Ge, S, Cr, and others); and X represented anions (OH^-, F^-, Cl^-, Br^-, I^-, and O^{-2}). Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$,was particularly interesting among them because of its chemical similarity to the principal inorganic constituent of bone tissue. Due to its absolute biocompatibility with living tissues, calcium hydroxyapatite ceramics were widely used as biomaterials in medicine (stomatology, maxillofacial surgery, traumatology, and orthopedy). Apatites with the hydroxovanadate structure, $Ca_5(VO_4)_3OH$, was not studied well. These compounds could be used as luminescent substances, laser materials and catalysts. An important feature of apatite–like compounds was that many different kinds of elements could substitute for the major constituents in their crystal structures. These substitutions, called *isomorphous replacements*, resulted in substances known as *solid solutions*, and presented a very interesting subject matter for investigation in solid state chemistry. In this work, we studied the isomorphic substitution of trivalent dysprosium for calcium in hydroxovanadate with apatite structure under the scheme: $Ca^{2+} + OH^- \rightarrow Dy^{3+} + O^{2-}$. Isomorphic substitutions in system $Ca_{5-x}Dy_x(VO_4)_3(OH)_{1-x}O_x$, were studied by X-ray powder diffraction analysis. Samples were prepared by nitric-tartaric solutions method and calcined at final temperature of 820°C. Solid solutions formed in the systems $Ca_{5-x}Dy_x(VO_4)_3(OH)_{1-x}O_x$ had substitutional limits $0<x<0.20$. The apatite solid solutions coexisted with calcium orthovanadate phase $Ca_3(VO_4)_2$ and unknown X phase in heterogeneous regions of the system. The unit cell parameters were decreased monotonically within homogeneous region of the system corresponding to calcium – rare earth element ionic radii difference.