

WEST MICHIGAN
REGIONAL
UNDERGRADUATE
SCIENCE RESEARCH
CONFERENCE

ABSTRACT BOOKLET

Saturday, November 17, 2012



Van Andel Institute®

333 Bostwick Avenue, NE
Grand Rapids, MI 49503
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**WEST MICHIGAN REGIONAL UNDERGRADUATE
SCIENCE RESEARCH CONFERENCE**

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SCHEDULE OF EVENTS

- 8:30 ARRIVAL AND POSTER SETUP** *Cook-Hauenstein Hall*
- 9:00 WELCOME** *Tomatis Auditorium*
Steve Triezenberg, Ph.D.
President and Dean of Van Andel Institute Graduate School
- 9:15 KEYNOTE ADDRESS** *Tomatis Auditorium*
Elizabeth H. Simmons, Ph.D.
Professor, Physics and Astronomy
Dean of Lyman Briggs College
Michigan State University
"From Asteroid Orbits to the Higgs Boson: Why your Undergraduate Research Experience is so Important"
- 10:00 POSTER SESSION I** *Cook-Hauenstein Hall*
Presenters at even-numbered posters
Refreshments served
- 11:15 FACULTY TALKS** *Tomatis Auditorium*
Greg Fraley, Ph.D.
Associate Professor, Department of Biology
Hope College
"The Neuroprotective Effects of a Plant Hormone, Resveratrol: Implications for End-Stage Parkinson's Disease Therapy"
Jonathan Fritz, Ph.D.
Assistant Professor, Department of Chemistry
Aquinas College
"Regioselectivity of Direct Arylation Reactions"
- 12:00 LUNCH** *Cook-Hauenstein Hall*
- 1:00 POSTER SESSION II** *Cook-Hauenstein Hall*
Presenters at odd-numbered posters
- 2:15 FACULTY TALKS** *Tomatis Auditorium*
Stanley Haan, Ph.D.
Dean for the Natural Sciences and Mathematics
Professor, Department of Physics
Calvin College
"Computer Modeling of Double Ionization of Atoms by High-Intensity Near-Infrared Lasers"
Bart Williams, Ph.D.
Associate Professor and Director, Center for Skeletal Disease Research and Head, Laboratory of Cell Signaling & Carcinogenesis
Van Andel Research Institute
"Genetically Engineered Mouse Models to Study Bone Disease"
- 3:00 CONCLUSION**

Abstract Booklet for Poster Presentations

1. Andrew Grant, Athletic Training

Alma College

(Co-Authors: Marta Perez and John Davis)

“Effect of Altitude of Residence on the Cardiovascular Responses to Dynamic and Isometric Exercise at 4900 meters”

Previous studies have documented the beneficial physiological adaptations to living at high altitude. However, few studies have examined exercise responses for residents from a variety of altitudes at very high altitude. **PURPOSE:** To examine the influence of altitude of residence on cardiovascular responses during dynamic and isometric exercise when exposed to very high altitude. **METHODS:** Thirty subjects voluntarily participated in the study after giving informed consent and completing the Lake Louise survey for acute mountain sickness. Subjects were recruited and tested at the Carroll Hut refugio at Mount Chimborazo in Ecuador located at 4900 meters. This is an optimal location because many Ecuadorians and tourists who reside at a variety of altitudes can easily drive to the refugio. All measurements were taken immediately upon arrival at the refugio. Subjects were divided into three groups based on their altitude of residence: low altitude – LOW (0-1500 m), moderate altitude – MOD (1500-3000 m), and high altitude – HIGH (>3000 m). A maximum voluntary contraction (MVC) using a hand-grip dynamometer was performed for each subject. Each subject then performed 30 contractions on the dynamometer at 50% MVC. Following the dynamic contractions, they performed an isometric contraction at 50% of MVC until they reached 25% of MVC. Heart rate, oxygen saturation, and systolic and diastolic blood pressure were measured before the exercise and during the dynamic and isometric exercise. **RESULTS:** Average heart rates at rest and during both dynamic and isometric exercise were significantly higher in LOW (105±8.2, 112.7±9.5, 112±12.8 bpm) when compared to MOD (84.5±16.2, 91.7±12.6, 91.3±11.8) and HIGH (78.9±17.6, 80.0±10.3, 84.3±11.9). Also, average oxygen saturation was significantly lower at rest and during dynamic exercise in LOW (72.3±15.1, 71.4±10.3%) compared to MOD (78.7±5.5, 81.1±4.1) and HIGH (82.4±3.7, 85.0±3.9). Although changes were observed in systolic and diastolic blood pressure at rest and during dynamic and isometric exercise, these values were not statistically significant between groups ($p>0.05$). This was also the case for oxygen saturation during isometric exercise. **CONCLUSION:** Altitude of residence had an important influence on resting cardiovascular responses at very high altitude. However, the additive effect of the exercise was similar in all three groups.

2. Amanda Bolles, Biochemistry**Kalamazoo College**

(Co-Authors: Erran D. Briggs, Mara R. Livezey, Leslie D. Nagy, Laura Lowe Furge)

“Metoclopramide is not a Mechanism-based Inactivator of CYP2D6”

CYP2D6 is a major drug metabolizing enzyme responsible for metabolism of ~20% of pharmaceutical drugs. Inactivation of CYP2D6 is rare, but - due to its importance in drug metabolism, can be clinically significant. Thus, understanding of inactivation of CYP2D6 is important for devising strategies to avoid undesirable drug-drug interactions. Metoclopramide is a drug that has previously been reported to be a mechanism-based inactivator of CYP2D6 [Desta et al. (2002) Drug Metab Disp 30, 336-343]. In order to better understand its role in inactivation of CYP2D6, we sought to expand that initial finding by determining spectral binding constant, partition ratio, metabolite profile, and performing activity assays. While we found predicted metabolites, our studies did not show metoclopramide to be an inactivator of CYP2D6 (Support: NIH 1R15-GM086767-01 & -01S1).

3. Amanda Porter, Biochemistry**Hope College**

(Co-Authors: Amanda L. Porter, Sarah Colton, Chelsea Campbell, Elizabeth Gerometta, Rachel Haas, Abigail Lindberg, Sara Gallemore, Advisers: Drs. Gregory S. Fraley, Aaron A. Best, & Susan M. Fraley)

“Gut bacterial ecology of developing Pekin ducks in the food industry”

Riemerella anatipestifer (RA), also known as Pasteurella anatipestifer and Moraxella anatipestifer, is a bacterial pathogen causing septicemia in Pekin ducks. This disease generates substantial economic losses for poultry duck producers in the food industry. Little is known about the pathogenesis or the source of this pathogen in ducks. To determine if RA is a natural part of the gut ecology within ducks, we collected the contents of the paired cecae in ducks over a six-week period, isolated the total bacterial DNA from the samples, and analyzed for the presence of RA, E. coli, and Salmonella using PCR. Data shows the absence of RA in samples in the cecae of ducks from hatch to market weight. Given the lack of current knowledge regarding the gut ecology of Pekin ducks we submitted the isolated bacterial DNA for total community ecological analyses, using next generation sequencing technology to sequence bacterial 16S rRNA taxonomic markers. Initial analyses revealed as many as approximately 36,000 unique bacterial taxa present in the gut of developing Pekin ducks. Interestingly, we observed a complete shift in gut bacterial taxonomic composition around day eight post-hatch. This time frame may correlate with observations that maternally derived passive immunity is also lost at about day 8 of age. Thus maternal antibodies may contribute to the gut ecology of Pekin ducks.

(Co-Authors: Abigail Leistra and Kumar Sinniah)

“An Atomic Force Microscopy Study of Riboflavin Receptor Targeting Nanoparticles”

Riboflavin ligands present an alternative pathway for targeted drug delivery as riboflavin receptors are over-expressed in breast and prostate cancer cells. We have examined dendrimers conjugated with riboflavin for targeting riboflavin binding protein, which acts as a model protein for the riboflavin receptor. By characterizing the binding interactions between riboflavin dendrimer gold nanoparticle conjugates and the riboflavin binding protein, the efficacy of this platform for a targeted approach of drug delivery can be predicted more accurately. Atomic Force Microscopy (AFM) was used for biological imaging studies of these riboflavin-dendrimer complexes conjugated with gold-nanoparticles. The size distribution of these nanoparticles was mapped with bound riboflavin binding protein under a number of conditions. This method presents a novel approach to screening the binding of drugs to drug targets. The riboflavin dendrimer conjugates will be used in future studies to target riboflavin receptors for cellular uptake as a potential route for the selective delivery of drug molecules to cancer cells that overexpress riboflavin receptors.

(Co-Authors: Drew Roth, Elizabeth G. Porter, Ryan Martinie, Nathanael M. Myton, Taylor Hegg, David E. Benson)

“Detection Strategies for Tyrosine-Cysteine Crosslinks”

Protein cofactors represent a unique class of redox active posttranslational modifications formed in or by metalloproteins. Of over eighty thousand reported protein structures, only twenty-five are known to contain protein-cofactors. The detection of cofactors, specifically protein crosslinks, is limited by the use of crystallography as the primary identification technique. Our research on tyrosine-cysteine side chains in cysteine dioxygenase and BF4112, both of which are known to form crosslinks, seeks to extend crosslink detection methodology. The wide array of techniques utilized includes optical spectroscopy, fluorescence, square wave voltammetry, proteomic mass-spectroscopy, gel electrophoresis and NMR. Not only do these techniques display usefulness in crosslink detection, they demonstrate wide detection limits and provide crosslink characterization at nano-molar levels. These methods could aid in identifying further protein-derived crosslinks, which would make the study of protein cofactors accessible to a wider scientific community.

(Co-Authors: Ola Alabi, Stephen Gunnink, Dr. Larry Louters)

“Acute Effects of Osthole on Glucose Transporter 1”

The natural coumarin Osthole has been studied as an anti-cancer agent against breast and lung cancer and has been shown to have anti-tumor effects. GluT1 has previously been shown to be acutely activated by a number of different compounds including Angeli’s Salt and Hydroxylamine; activation is thought to be triggered by formation of an internal disulfide bond. We examined the effects of Osthole on glucose uptake activity of GluT1 in L929 fibroblast cells, a cell line that expresses only the GluT1 transporter. Our results showed that Osthole acutely activates the glucose uptake of GluT1 up to 1.5 fold at a concentration of 100 μ M. Furthermore, when cells are pretreated with Osthole, activation by Angeli’s Salt or Hydroxylamine is prevented. Our current model suggests that Osthole may modify the cysteine residues via Michael addition; more research is needed to confirm the model. Osthole may be a viable supplement to existing cancer treatments because it prevents activation of glucose uptake via GluT1 while only slightly affecting the basal uptake.

(Co-Authors: Garrett M. MacLean, Justine M. Travis, Cory M. DiCarlo)

“Reduction Potential Shift in Cytochrome c Peroxidase Mutants D79K and D267K”

Required methodology modifications were determined for the growth and purification of single site mutants of Horse Heart Cytochrome c Peroxidase (CcP) E267K, D79K, and E118K. Two main changes required included significant modification of chromatography conditions and adaptation required by reduced protein expression efficiency in the Escherichia coli bacterial expression system used to obtain these protein mutants. Crystallization was accomplished in each case following method modification, although crystal size ultimately proved suboptimal for x-ray crystallographic structural determination. Further alteration of the method required six-histidine tagging of the protein sequence followed by affinity chromatography for post expression purification. Expression of the six-histidine protein resulted in measurement of the pH 7.0 reduction potential for the D79K and D267K forms.

(Co-Authors: Mujahid Anwar, David Leonard, Rachel Powers, and Bradley Wallar)

“Probing the Binding Site in the Antibiotic Resistance Enzyme, AmpC beta-lactamase”

Beta-lactam antibiotics, such as penicillins and cephalosporins, are widely used to treat bacterial infections, but resistance to these drugs is increasingly becoming a problem. One of the main causes of resistance to these beta-lactam drugs is the bacterial production of beta-lactamase enzymes, such as AmpC. These enzymes are capable of breaking down the drug within their active sites, rendering the antibiotic unable to harm the bacteria. The exact roles that the active site amino acid residues play in the recognition and breakdown of the drug are not fully understood. Here, we investigate the role of the active site residue asparagine-152 (Asn152) in AmpC by mutating it to a glycine, serine, or threonine residue and examining the effect that these mutations have on kinetic and structural properties. We discovered that these mutations are capable of causing a substrate specificity switch, yielding enzymes that have increased activity for one drug but decreased activity for a different drug compared to the wild type enzyme. Crystal structures of the N152G mutant bound to two different drugs are compared and show several differences in orientation and in hydrogen bonding networks. Uncovering the specific role of Asn152 in the function of AmpC will be useful in the development of inhibitors to these enzymes in order to combat bacterial resistance.

“Real Time Analysis of System xc-”

System xc- represents a trans-membrane protein system, composed of proteins xCT and CD98, which exchanges intracellular glutamate for extracellular cystine, ultimately producing the antioxidant glutathione. Thus System xc- has a major role in combating oxidative stress however the mechanism for the relationship between xCT and CD98 remains to be elucidated. Through a process of real time analysis I hope to discover this mechanism of action. By utilizing live cell fluorescence microscopy and future work with fluorescence recovery after photobleaching (FRAP) this goal can be realized. Preliminary results indicate that the proteins xCT and CD98 are colocalized in the endoplasmic reticulum and golgi apparatus but they show separate localizations in the cytoplasm and extracellular regions. Future work with FRAP will yield increased mechanistic information through the use of protein synthesis inhibitors and vesicle formation inhibitors.

10. David Hayes, Biochemistry**Hope College**

(Co-Authors: Maria Burnatowski-Hledin and Vicki Isola)

“The Production of Monoclonal Antibodies to VACM-1/Cullin 5.”

Vasopressin-activated calcium mobilizing receptor 1 (VACM-1/Cullin 5) is a 780-amino acid membrane protein that inhibits cellular proliferation and has been suggested to be a tumor suppressor. We propose to produce monoclonal antibodies to VACM-1/cul5. We will immunize Balb/c mice with purified VACM-1 and a KLH-conjugated peptide corresponding to the amino-terminal sequence of the protein. Spleen cells from mice expressing antibody titers over 1:30,000 will be fused with mouse myeloma cells, and hybrids will be screened for monoclonal antibody secretion using an ELISA assay. To date we have purified VACM-1 protein, prepared the conjugated peptide, and optimized the ELISA assay. Production of monoclonal antibody against VACM-1/cul5 will provide us with a novel tool to better characterize its expression in cancer cell lines and in tumor samples.

11. Elizabeth DeGroot, Biochemistry**Calvin College**

“Investigating Novel Splice Variants in Zebrafish”

Investigating novel splice variants of proteins can help us understand how cells work and can serve as precursors to disease research. This specific project investigated and characterized novel splice variants of a protein from zebrafish (*Danio rerio*). Past research had been done to analyze the genome of zebrafish and several other organisms in order to understand the microscopic properties of the protein of interest and to compare between species. mRNA quantitation discovered that multiple splice variants were transcribed in zebrafish; a goal of this research was to show that the variants were actually translated into protein. The protein of interest was isolated and concentrated from zebrafish and then the technique of gel electrophoresis was employed to separate the proteins by molecular weight. A Western Blot, a biochemical technique using antibodies, was used to highlight the location of the specific protein bands. To give strong evidence that we found the novel splice variants of interest, bacteria cells were transformed with the gene for the protein of interest. After isolating and purifying the protein, it was run on the gel next to the zebrafish samples, thereby serving as a marker showing that the splice variants were actually present in zebrafish. Good evidence was found for the presence of the third variant of interest, as well as some evidence for the first and second variants. Future research should be done to improve on the clarity and specificity of the protein identification method.

(Co-Authors: Neil V. Klinger, Jacob Z. Scheid, Harvey J. Nikkel, Rachel A. Powers, and David A. Leonard)

“Four hydrophobic residues control substrate selectivity in the OXA-24 carbapenemase”

Class D β -lactamases display a wide range of substrate specificities ranging across classical penicillins, early and late generation cephalosporins and carbapenems. Often the acquisition of a new substrate specificity through mutation is accompanied by loss of activity against another class of β -lactam drugs. OXA-24 is a class D β -lactamase with activity against most penicillins and carbapenems, but lacking activity against cephalosporins and the isoxazolyl penicillin oxacillin. We have used site-directed mutagenesis, steady-state kinetics and X-ray crystallography to identify the role of four hydrophobic residues (M223, Y112, M114 and W221) in the selection or rejection of a number of different β -lactam substrates. These residues generally play a positive role in binding the hydrophobic side-chains of the most clinically potent carbapenems including doripenem. M223 discourages the binding of ampicillin and oxacillin, while Y112 has a generally positive effect on the binding of those drugs. X-ray crystallographic analysis of OXA-24 with ampicillin and doripenem bound reveals insights into the nature of the role played by these hydrophobic residues in substrate specificity switches.

(Co-Authors: Allie Bogner and Chad Tatko)

“Metal Binding Catechols”

Many polyphenolic antioxidants are found in natural products such as dark chocolate, black tea, coffee, etc. These compounds are known to react with free radicals and the product is more stable. They are important in our body because it will stop the chain reaction of free radicals, which damage DNA and protein. Also, free metals can be oxidized and occur peroxide to become hydroxyl radical – this is called Fenton type oxidation, which have an effect on neurodegenerative diseases such as Alzheimer’s, and Parkinson’s. Metals can bind with ligands, 3,4-Dihydroxyhydrocinnamic acid (DHCA), which will help stopping Fenton type chemistry. UV-Vis spectrometer was used for the titrations, and the solutions were made with copper, DHCA and MES buffer at pH 5. These samples were then run with a spectral range of 200 – 900nm with a slit width of 1 nm. Within the set of the metal and the ligand, the concentration of copper was set and the concentration of ligand was increased. Copper and the ligands formed catecholate complexes. The catechol binds via hard oxygen catecholates. Depending on the concentration and pH, the complex can contain 1 or 2 ligands interact with copper. Also, the binding of catechol ligands to copper results in the spectroscopic changes and a new band develops in UV-Vis spectragraph. We can see the color change in the solution as the concentration of the ligand increases.

“Cyclophosphamide Metabolism by Cytochrome P450 2B6”

Cyclophosphamide (CPA) is one of the most widely used drugs in cancer treatment, yet, there is considerable interpatient variability with regards to efficacy and toxicity with CPA treatment. This interpatient variability is not well understood, and is the topic of this research. CPA is an oxazaphosphorine prodrug, requiring bioactivation by a cytochrome P450. Cytochrome P450 2B6 (CYP2B6) is the major P450 involved in cyclophosphamide activation. CYP2B6 is known to be highly polymorphic, and these polymorphisms have been demonstrated to effect the metabolism of other known CYP2B6 substrates such as bupropion and efavirenz. We hypothesized that CYP2B6 polymorphisms have varying ability to metabolize cyclophosphamide, resulting in varying amounts of activated cyclophosphamide. This is a potential cause for the efficacy and toxicity discrepancies with regard to cyclophosphamide treatment. We have refined an assay for the measurement of a cyclophosphamide metabolite, and evaluated the metabolism of cyclophosphamide by the wild type CYP2B6. We found a K_m and V_{max} of 2.7 mM and 4.102 pmoles/min/pmoles P450, respectively. Future work will focus on the metabolism of cyclophosphamide by polymorphisms CYP2B6*4, CYP2B6*5, CYP2B6*6, CYP2B6*7, CYP2B6*8, and CYP2B6*9.

(Co-Authors: Neil V. Klinger, Kip-Chumba Kaitany, Robert A. Bonomo, Rachel A. Powers and David A. Leonard)

“A Pro>Ser mutation augments advanced generation cephalosporinase activity in both the OXA-23 and OXA-24 subfamilies”

OXA-23 and OXA-24 are class D β -lactamases that can hydrolyze carbapenem class antibiotics, thus greatly threatening our ability to treat some dangerous infections. Fortunately, advanced generation cephalosporins such as cefotaxime or ceftazidime remain as viable treatment options as these enzymes do not hydrolyze cephalosporin drugs very efficiently. We have investigated the properties and structures of two clinical variants containing the same Pro>Ser mutation (one in the OXA-23 background and the other in the OXA-24 background). Steady-state kinetic measurements show that compared to the parental enzymes both variants have much higher affinities for cefotaxime, ampicillin and most notably ceftazidime. Moreover, the variants maintain strong hydrolytic activity toward carbapenems such as doripenem. X-ray crystallographic analysis of OXA-24 P225S reveals that the mutation causes a deviation of the main-chain atoms of the loop connecting β -strands b5 and b6 thus slightly enlarging the active site. Models of ceftazidime bound to OXA-24 and the P225S variant suggest that this loop deviation provides more room for the binding of the bulky oxyimino side-chain of that drug. These findings warn of the emergence of class D β -lactamases that can provide resistance to carbapenems and advanced generation cephalosporins.

(Co-Authors: Neil V. Klinger, Maddison E. Ramey, Robert A. Bonomo, Rachel A. Powers and David A. Leonard)

“A Class D β -lactamase Clinical Variant with Activity Against Carbapenems, Ceftazidime and Aztreonam”

Like all known class D carbapenemases, OXA-23 cannot bind or hydrolyze the 3rd generation cephalosporin ceftazidime. OXA-146 is an OXA-23 subfamily clinical variant that differs from the parent enzyme by an alanine (A220) duplication in the loop connecting β -strands β 5 and β 6. We have discovered that this insertion enables OXA-146 to bind and hydrolyze ceftazidime with efficiency comparable to other extended spectrum class D β -lactamases. This enzyme also binds aztreonam, cefotaxime and ampicillin with higher affinity than OXA-23. In this study, we report the crystal structures of both the OXA-23 and OXA-146 enzymes. A comparison of the two structures shows that the extra alanine moves a methionine out of its normal position where it forms a bridge over the top of the active site. The insertion also lengthens the b5-b6 loop, moving its main-chain atoms further away from the active site. A model of ceftazidime bound in the active site shows that these two structural alterations are both likely to relieve steric clashes between the bulky R1 side-chain of ceftazidime and OXA-23.

(Co-Authors: Mara R. Livezey, Leslie D. Nagy, Laura E. Diffenderfer, Evan J. Arthur, David J. Hsi, Laura Lowe Furge)

“Molecular analysis and modeling of inactivation of CYP2D6 by four mechanism-based inhibitors”

There are four known and confirmed mechanism-based inactivators of human cytochrome P450 2D6: SCH 66712, EMTPP, paroxetine, and 3,4-methylenedioxyamphetamine (MDMA). SCH 66712 and EMTPP contain piperazine groups and substituted imidazole rings; previous studies with each have indicated that inactivation occurs by reaction with the substituents of the imidazole ring. Paroxetine and MDMA contain methylenedioxyphenyls; the moiety responsible for inactivation is unknown. The current study shows that each inactivator displays Type I binding with K_s values that vary by 2-orders of magnitude. Comparison of K_i and $kinact$ and partition ratio values shows Schering 66712 is the most potent inactivator. Molecular modeling experiments using AutoDock identify Phe120 as a key interaction for all four inactivators with face-to-face and edge-to-edge pi interactions apparent. Also, modeling suggests Thr309 could be a potential site for inactivation. Ligand conformations were scored according to their binding energies as calculated by AutoDock and correlation was observed between molecular models and K_s values. (Support: NIH 1R15-GM086767-01 & -01S1; HHMI [52006304 to Kalamazoo College]).

(Co-Authors: Nichole Michmerhuizen and Christine Timmer)

“Characterizing the Biomolecular Interactions between Insulin and G-Quadruplex DNA”

The formation of guanine (G)-quadruplex structures in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) is linked to transcriptional effects on the insulin gene. Recent studies demonstrate that the ILPR G-quadruplexes can bind to insulin while the energetics of this interaction with the most common ILPR repeat sequences have been characterized. Studies have also measured the transcriptional activity of less common ILPR repeats, and find that it is significantly lower than that of the consensus sequence but can be increased substantially by varying only one or two nucleotides. To determine the potential role of G-quadruplex formation and stability in regulating transcription, we have studied the second and third most common ILPR repeats as well as their variant sequences that exhibit increased transcriptional activity. Circular dichroism (CD) spectroscopy, differential scanning calorimetry (DSC), and isothermal titration calorimetry (ITC) have been used to characterize the binding interaction between insulin and each of the four ILPR repeat sequences. The bulk thermodynamic measurements performed at various temperatures from 20 - 37 degrees Celsius provide insight into these biomolecular interactions.

(Co-Authors: Kim K Colvert)

“Isolation and Characterization of Recombinant Saccharomyces cerevisiae Cytochrome C Peroxidase”

Cytochrome c peroxidase (CCP) is a monomeric heme-containing enzyme located in the mitochondrial inner-membrane space that consists of 294 residues and has an active role in the reduction of peroxides. Equation 1 $CCP + H_2O_2 + 2\text{ferrocycytochrome } c + 2H^+ \rightarrow CCP + 2H_2O + 2\text{ferricycycytochrome } c$ The enzyme is well characterized and classical isolation methods have been published from yeast, and recombinant E. Coli. Assay of its activity can be followed spectroscopically by the change in A550 upon oxidation of ferrocycytochrome c (Equation 1). Incubation of the cells after induced expression was varied from 6-18 hours, and did not give a significant increase in protein yield. Cell lysis was performed by freeze-thaw and either lysozyme treatment or sonication. Lysozyme treatment was rejected due to a precipitant. Sonication was most effective using 1 cm horn at 10 microns for 5 minutes with 10 second pulse and rest intervals. The native holoenzyme of CCP has a 1:1 ratio of heme to protein. Insufficient protein was isolated to perform heme determination by the classic pyridine hemochromogen method. Alternatively the ratio could be determined from the purity index as described by Yonetani. The apoenzyme was isolated and insufficient heme was incorporated during induction. The resulting activity was equally low. The apoenzyme was incubated with bovine hemin. Excess hemin was removed using ion-exchange chromatography. The resulting spectra and purity index showed that heme reincorporation was successful.

20. Michael J. Hicks, Biochemistry**Kalamazoo College**

(Co-Authors: Mara R. Livezey, Laura E. Diffenderfer, Leslie D. Nagy, Laura Lowe Furge.)

“Covalent Modification of CYP2D6 by SCH66712 at Thr309”

SCH66712 is a known potent mechanism-based inactivator of CYP2D6. It contains a piperazine group and a substituted imidazole ring; the latter is believed to be the specific reactive element required for CYP2D6 inactivation. Recent modeling studies have suggested that Thr309 could be a potential site for electrophilic inactivation. Using enzyme digestion techniques coupled to LC/MS, labeled peptides from the CYP2D6-SCH66712 inhibitory complex were identified, including a peptide containing Thr309. Mutants of CYP2D6 at Thr309 and other identified residues are being prepared to test for altered inhibition profiles to better understand both the mechanism of CYP2D6 inactivation and the role of potential substrate access and egress channels in the enzyme.

21. Ola-Oluwakiti Alabi, Biochemistry**Calvin College**

(Co-Authors: Dr. Larry Louters, Benjamin Kuiper, Stephen Gunnink)

“Curcumin Inhibits the Glucose Transport Activity of GLUT1”

Curcumin is a compound found in turmeric, a common spice. Curcumin has two possible sites available for 1,4-nucleophilic addition with GLUT1 cysteines. Recent evidence suggests that GLUT1 can be acutely activated by cell stress and other compounds. GLUT1 seems to be highly expressed and activated in several types of cancerous cells. Developing a model of GLUT1 activity and exploring its activation behavior may prove crucial in designing new ways to fight cancer.

22. Riemer Praamsma, Biochemistry**Calvin College**

(Co-Authors: Eric Arnoys)

“Mechanism of Glucose Transporter Protein”

Glucose Transporter 1 (GluT1) is a membrane protein responsible for basal uptake of glucose, one of the most essential cellular energy sources. The activity of GluT1 can be modulated by several compounds which appear to affect the K_m of transport rather than V_{max} . Because diabetes as well as many cancers result in altered glucose transport, a better understanding of GluT1 activation could help us design new approaches to treating them. For our studies, we used fusion proteins of GluT1 to examine the changes in its behavior upon activation or inactivation in two distinct cell types in which the default state of GluT1 was either very active or less active.

“A valine/leucine clamp controls the carbapenemase activity of OXA-24”

The carbapenemase activity of OXA-24 poses an enormous threat to the efficacy of carbapenem therapy against infections caused by Gram-negative bacteria. Current theory suggests that the rotational orientation of the hydroxyethyl moiety of carbapenem drugs is critical in determining whether a class D β -lactamase can effectively hydrolyze these substrates. We have investigated the role of two hydrophobic active site residues (V130 and L168) in orienting the hydroxyethyl group. Mutation of these residues followed by steady-state kinetic analysis reveals that it is possible to specifically deactivate carbapenem hydrolysis, while maintaining activity toward penicillins. Crystal structure analysis of OXA-24 V130T and OXA-24 L168V (both with doripenem bound) provide insight into how these residues influence carbapenemase activity.

(Co-Authors: Anand Divakaran, Elizabeth Porter, Nate Myton, Drew Roth, Taylor Hegg, David Benson)

“Formation Chemistry of Tyrosine-Cysteine Crosslinks”

Crosslinked protein cofactors combined with redox-active metal centers provide proteins with the ability to oxidize a variety of organic substrates. The tyrosine-cysteine crosslink has been shown to form in the active sites of galactose oxidase, cysteine dioxygenase, NrfA nitrite reductase, and NirA sulfite reductase, and the orphan protein BF4112. Formation chemistry of the crosslink has been examined most thoroughly for galactose oxidase, where metal-mediated sidechain oxidation to radical intermediates facilitates carbon-sulfur bond formation. Here, a variety of approaches have been employed to form the Tyr-Cys crosslink in BF4112 and bovine hemoglobin, as assayed by fluorescence spectroscopy and SDS-PAGE. The Tyr-Cys crosslink was formed in BF4112 by CuI-dioxygen chemistry, as well as CuII chemistry, based on methods reported for galactose oxidase. In addition, the crosslink was formed using FeII and L-cysteine, based on methods reported for cysteine dioxygenase. These methods were found to be competent to form the crosslink. Heme peroxidation was also found to form a crosslink between Tyr β -145 and Cys β -93 in bovine hemoglobin. The mechanisms for tyrosine cysteine crosslink formation in these proteins will be discussed.

(Co-Authors: Anne Georges and Dr. Leah Chase)

“Identification of Endocytic Motifs in the C-Terminus of xCT”

System xc⁻ is a heterodimeric plasma membrane transporter that facilitates the stoichiometric exchange of extracellular cystine for intracellular glutamate. Within the reducing environment of the cell, cystine is reduced to cysteine, which along with glutamate and glycine synthesizes glutathione, an antioxidant. Thus, System xc⁻ serves to protect the cell from natural oxidative stress. Although it has been demonstrated that System xc⁻ supports both constitutive and regulated trafficking, the mechanism in which trafficking machinery operates is unclear. Therefore, we have focused on the C-terminus of the xCT protein due to its necessity in basal trafficking, which can be disrupted by altering a specific motif within the terminus. We hypothesize that a tyrosine-based, clathrin-dependent endocytic motif located in a position accessible to intracellular proteins contributes to the basal internalization of xCT. Of the 501 amino acids and 12 putative transmembrane domains in the protein xCT, we are concerned with amino acids 462-468. In this sequence PxxY, YxxΦ and di-tyrosine motifs are found and have been mutated. Thus far, Western blot analyses demonstrated that mutagenesis of these motifs have increased the concentrations of xCT on the plasma membrane, thus revealing a disruption in the constitutive recovery of xCT from the plasma membrane.

(Co-Authors: Ben Kuiper, Ola Alabi, Dr. Larry Louters)

“Detection of Activated GLUT1 in L929 and HCLE cells”

GLUT1 is a membrane spanning transporter protein responsible for the basal uptake of glucose in a wide variety of mammalian cells. Previous studies have shown that GLUT1 can also be acutely activated or inhibited by certain chemical environments, however, the mechanism of activation remains elusive. There are currently two proposed models for the activation of GLUT1. 1) Activation of GLUT1 involves the formation of an internal disulfide bond and subsequent formation of a more active tetramer. 2) Activation of GLUT1 is associated with a movement of the transporter into lipid rafts. To address these two possibilities, we first compared the amount of GLUT1 that can be isolated from lipid rafts in cells where GLUT1 is highly activated (HCLE cells) and cells where GLUT1 has low activity (L929 fibroblast cells). Isolation of lipid rafts by sucrose gradient centrifugation and detection of GLUT1 by Western blotting revealed that in the highly active HCLE over 65% of GLUT1 resides in lipid rafts while less than 40% of GLUT1 resides in lipid rafts in the less active L929 fibroblast cells suggesting the more active form of GLUT1 is associated with lipid rafts. We also treated HCLE cells briefly with formaldehyde in an attempt to covalently fix oligomeric forms of GLUT1. We were unable to detect larger GLUT1 structures suggesting either the tetrameric form is not present in HCLE cells or that the GLUT1 protein does not have histidine or lysine residues close enough to covalently link the tetramer. Future studies will investigate other, longer range, fixative agents.

(Co-Authors: Douglas Vander Griend)

“Solution Phase Self-Assembly of a Cubic Nanocage”

The self-assembly of the supramolecular cube [M8Lnaph₁₂]₁₆₊ in dimethylformamide has been studied via spectroscopic titration and mass spectrometry. The UV-Vis absorbance of forty solutions ranging from zero to three equivalents of Lnaph (two chelating pyrazolyl-pyridine units connected to an aromatic spacer 1,5-diynaphthalene) with Ni(BF₄)₂·6H₂O were measured at three different temperatures (295 K, 305 K, and 323 K). The mathematical structure of each isothermal data set indicates there are at least eight distinct chemical species and up to thirteen. Modeling each data set according to a set of chemical equilibria leads to the identification of these species: [Ni]₂₊, [Ni₂(Lnaph)]₄₊, [Ni_{2n}(Lnaph)_{2n}]_{4n+}, [Ni₆(Lnaph)₈]₁₂₊, [Ni₇(Lnaph)₁₀]₁₄₊, [Ni₈(Lnaph)₁₂]₁₆₊ (cube), [Ni₇(Lnaph)₁₁]₁₄₊, [Ni₆(Lnaph)₁₀]₁₂₊, [Ni₅(Lnaph)₉]₁₀₊, [Ni_{4m}(Lnaph)_{8m}]_{8m+}, [Ni₃(Lnaph)₇]₆₊, [Ni₂(Lnaph)₅]₄₊, [Ni(Lnaph)₃]₂₊. Mass spectrometry was performed with Ni(BF₄)₂·6H₂O and Lnaph in acetonitrile in order to determine the exact size and mass of the species in solution.

(Co-Authors: Neil V. Klinger, Rachel A. Powers, and David A. Leonard)

“Two mutations are necessary to convert class D β-lactamase function to β-lactam sensor function”

Class D β-lactamases (such as OXA-24) and β-lactam sensors (such as BlaR1) share a common topological fold and an acylation mechanism in which a nucleophilic serine is activated by the carbamate of an unusual active site carboxyllysine. β-lactamases are able to complete the hydrolysis of the substrate through activation of a deacylating water, while BlaR1 maintains a persistent sensor function by remaining acylated. It has been shown that an active site valine in the class D β-lactamase family helps ensure persistent carboxylation of the lysine, allowing the carboxy group to activate the deacylating water. The homologous position in β-lactam sensors is a neutral polar residue (asparagine or threonine), which encourages decarboxylation of the lysine and thus deacylation-deficiency. Substitution of asparagine for valine in the OXA-24 β-lactamase greatly decreases the affinity of CO₂ for K84 and reduces hydrolysis rates, but does not completely eliminate catalytic turnover. We have made the double mutant V130N/N87L in OXA-24, thus introducing the β-lactam sensor residues found at these positions into a β-lactamase background. The rate of hydrolysis of ampicillin by the double mutant is very close to background, suggesting that these two residues alone may be responsible for the functional difference of these two proteins. X-ray crystallographic analysis reveals that an active site water bridging N87 to the carboxyllysine in OXA-24 is missing in the double mutant. This provides a possible explanation for the destabilization of the carboxyllysine in β-lactam sensors.

“Tissue-Based comparisons of RNA integrity and purity”

Used to study gene expression, RNA provides key information in research and healthcare. A lack of quality RNA can have extensive effects on downstream applications, leading to unusable results or even false conclusions. This study investigates several steps of tissue-based RNA acquisition and quality-control in order to identify procedures that maximize RNA integrity and purity. First, RNA from nude mouse brain, liver, lung and kidney was extracted using the RNeasy Mini Kit (QIAGEN) implemented both manually and using the QIAcube automated system (QIAGEN), RNA was also extracted using the QIASymphony automated system (QIAGEN). Resulting RNA was tested for integrity using the RNA integrity number (RIN) assigned using data from microcapillary electrophoresis and for purity using 260:230 and 260:280 nm absorbance ratios. Automated extraction methods resulted in RNA with high purity and integrity more consistently than the manual procedure in brain, liver and kidney tissue. Second, the effect of delay in time to snap-freezing of tissue on RNA integrity was tested. Brain, liver, kidney and xenograft samples from nude mice were harvested and left at room temperature; RNA extractions were performed at several time points. Degradation was assessed by RIN. RNA from brain and liver samples showed little to no degradation over the course of 3 hours while RNA from kidney and xenograft samples degraded slowly over time.

(Co-Authors: Dr. Margaret A. Dietrich Professor)

“Abnormal initial cell formation in Physcomitrella patens”

To complete their life cycle, many mosses depend on a switch from filamentous growth to the formation of the leafy gametophyte. Lateral branches form on the filaments via the progressive formation of initial cells (ICs). These are formed via the establishment of polar growth at the apical end of the second subapical (SA) cell of the filament. The filament cell nucleus travels to this site and divides. This nucleus then migrates back and a wall forms to separate the two cells. The IC continues to grow via polar growth to become a lateral branch. An alternative fate depends on the presence of the hormone cytokinin & the length of the IC to initiate the formation of the leafy gametophyte. If the length of the IC is greater than the width of the parent SA cell, it no longer responds to cytokinin and forms a lateral branch. In a *Physcomitrella patens* insertional mutant, with no apparent disruption of protein coding sequence, growth is restricted to the filamentous stage. While the mutant forms ICs in a progressive manner, they are significantly longer than those of the wild type, so they are very rarely competent to respond to cytokinin. It appears that, in some cases at least, the mutant forms the IC on the first SA cell, rather than on the second. Future phenotypic study will include analysis of the rate of polar growth during establishment and growth of the IC itself.

(Co-Authors: Johanna Forst Derek Blok Dr. Katharine Polasek)

“Surface Stimulation for Distal Sensation Threshold”

Phantom Limb Pain (PLP), a pain or discomfort in the missing limb, is experienced by 50-80% of amputees (Darnall). While the exact cause of PLP is not known, a leading hypothesis suggests phantom pain is due to cortical reorganization of the somatosensory cortex of the brain. Our working hypothesis states that by eliciting a “real” sensation in the phantom limb, the progression of cortical reorganization may be reduced or even reversed to decrease or eliminate PLP. We used surface electrical stimulation of distal nerves to achieve sensation in the hand. The median and ulnar nerves were stimulated at the elbow individually using surface electrodes in order to elicit distal sensation in able-bodied subjects. The non-symmetric voltage controlled stimulation pulse train was created using an optically isolated biostimulator and MATLAB software. The adaptive procedure Parameter Estimation by Sequential Testing (PEST) (Taylor and Creelman) was used to determine threshold values for sensation in the hand. PEST was used to calculate threshold values for several set pulse width values while varying amplitude, and several set amplitude values while varying pulse width. These threshold values were used to create a strength-duration curve for threshold values for 28 able-bodied subjects. The strength duration curves were used to determine two parameters of the nerve activation, the rheobase and chronaxie. The rheobase is defined as the lowest threshold voltage required for nerve activation at high pulse widths. The chronaxie is the corresponding pulse width at twice the Rheobase. The chronaxie and rheobase values varied between subjects and therefore a set threshold value cannot be used for all subjects. However, a reliable strength duration curve can be generated from 6 threshold points to produce the strength duration curve that can then be used for further testing. Future work will include studying the location and type of sensation produced when varying frequency, amplitude, and pulse width.

(Co-Authors: Kyle T. Bussis, Paul E. Harper)

“Monosaccharide Inclusion and Exclusion in Lipid-Water Phases”

Phospholipids have been observed to change their conformation depending on temperature. We focused on the transition from lamellar phase to hexagonal phase, $L\alpha$ -HII. This is of particular interest because phospholipids are the building blocks of cells, and this transition is central to endocytosis and exocytosis of cells because membranes, which are in a bilayer, must change shape in order to expel and take in objects encapsulated in vesicles. Using differential scanning calorimetry, we observed the equilibrium temperature, enthalpy, and width of the transition of stearyl-oleoyl-phosphatidylethanolamine (SOPE) in various sugar solutions. Sugars are of significance because they have been observed to play a cryoprotectant role in plants. More specifically, we looked at monosaccharides, such as glucose and fructose, as well as disaccharides. We determined the equilibrium temperature for the lamellar to hexagonal transition decreased with increasing sugar concentration. This relationship may be due to the inclusion and exclusion of sugar in various phases.

(Co-Authors: Jordan T. Presley, Emily M. Brogan, Scott B. Thourson, Shannon J. Timpe, Brian J. Doyle)

“A Quartz Crystal Microbalance Biosensor for Analysis of Herbal Medicine”

1 Departments of Biology and Biochemistry, Alma College, Alma, MI 2 Mechanical Engineering Department, Bradley University, Peoria, IL A Quartz Crystal Microbalance (QCM) is an instrument for measuring mass in the nanogram range. A voltage is applied to the crystal causing it to oscillate at its natural resonant frequency. Due to the piezoelectric nature of Quartz, as molecules adsorb to the surface of the crystal, the decrease in resonant frequency is detected as an electrical signal. The adsorbed mass can be calculated from this change in frequency. When a drug target protein is immobilized to the surface of the QCM, the instrument becomes a biosensor capable of detecting binding of drugs to the target. We are developing a QCM sensor for detecting binding of medicinal plant extracts to bovine serum albumin (BSA). Serum albumin is the most abundant protein in serum and binds a variety of molecules, including drugs, which may affect their bioavailability. BSA was immobilized directly on the gold-coated surface of the QCM or indirectly via a self-assembled monolayer (SAM) of alkanethiols. SAM formation and BSA immobilization were observed in real time by QCM and also analyzed by FTIR. The BSA-functionalized QCM biosensor was then used to detect binding of 8-analino-1-naphthalenesulfonic acid, a known BSA ligand. IR absorbance peaks corresponding to the exposed functional groups of the SAM were observed, as well as the amide peaks of the protein confirming their presence. BSA adsorption to the QCM surface was ~ 450 ng/cm², and the BSA functionalized QCM bound ANS at ~ 300 ng/cm². No significant differences were observed with regard to the method of immobilization. Finally, botanical extracts, including black tea, wild geranium, and St. John's wort were tested for binding to BSA. Binding was detected in all extracts. Further fractionation of the extracts will give insight into the chemical nature of the BSA-binding molecules.

“Effects of UVA on Photorepair in p53 Mutant Zebrafish”

UV light, particularly UVA and UVB, is known to play a role in cancer, though the exact mechanisms are not yet entirely known. One model organism used to study melanoma, the induction of UV damage, and regeneration is zebrafish. However zebrafish, unlike humans, have a repair mechanism known as photorepair. This mechanism is induced by UVA light and repairs damage caused to the fish, including prior UV-induced damage, specifically UVB-induced damage in the form of cyclobutane pyrimidine dimers (CPDs). The overall goal of my research was to determine how effective photorepair is in melanocytes with p53 (an important cellular tumor suppressor known to play a role in human cancers) is nonfunctional. Three important steps to completing this goal have been accomplished thus far: (1) the doses of UVA and UVB to be used were determined based on survivability feasibility tests; (2) histological techniques necessary to this project have been learned and perfected; and (3) a melanocyte count was performed to determine location of tissue for highest number of a to ensure statistically significant results. Currently the immunohistochemical necessary to continue this experiment are being tested and perfected.

35. Abigail Dutkiewicz, Cell and Molecular Biology / Genetics**Ferris State University**

(Co-Authors: Maurisa Flynn Riley and Guillermina Lozano)

“Using Mouse Models to Investigate Chromosomal Abnormalities Resulting from Altering the p53 Pathway”

The p53 pathway is altered in over 90% of human cancers through loss of the tumor suppressor, p53, or overexpression of the p53 inhibitor, Mdm2. Studies in transgenic mice overexpressing Mdm2 indicate that overexpression of Mdm2 is sufficient to cause a tumor phenotype and results in chromosomal abnormalities in a p53-independent manner. Although Mdm2 primarily functions to inhibit p53 activity, Mdm2 has many additional binding partners including the p53 family member, p73. Interestingly, loss of p73 results in a tumor phenotype and cell culture studies show that loss of p73 results in chromosomal aberrations. The hypothesis for this project was that Mdm2 overexpression would cooperate with the loss of p73 to promote tumorigenesis through exacerbating chromosomal defects. This hypothesis was studied through the use of a mouse model to create mouse embryonic fibroblast (MEF) cultures, which were then subjected to chromosomal analysis.

36. Anirudh Chawdhary, Cell and Molecular Biology / Genetics**Grand Valley State University**

(Co-Authors: Anirudh Chowdhary, Thomas Rogers, Anitha Menon, William Schroeder, Robert Smart, Agnieszka Szarecka, and Suganthi Sridhar 1)

“Investigating the Effects of BIBR1532 and Related Analogs on Telomerase Activity in Human Prostate Cancer Cells (PC3-Parental)”

Uncontrolled cellular proliferation of cancer cells is associated with the maintenance of telomeres in DNA. In normal cells the length of telomeres decrease with each successive cell division. When the length becomes too short the cells cannot divide and hence become senescent or die. An enzyme telomerase prevents the degradation of telomeres by adding bases to the ends of the telomere. Cancer cells employ this enzyme telomerase to maintain immortality. Assessing telomerase activity and its inhibition has become an attractive target for new cancer therapeutics. Synthetic telomerase inhibitor, BIBR1532, has shown growth arrest in tumor cells. In our study BIBR1532, a mixed-type competitive inhibitor, and its synthetic analogues (WS6-48, WS4-43A, WS5-29, WS8-3, WS11-41, WS7-6, WS8-9, WS12-16, WS12-43, WS12-44, WS12-45, and WS12-48) were tested for anti-proliferative and migratory activity on metastatic prostate cancer cells. In our preliminary studies we screened 6 of these compounds that are highly active against proliferation. We also carried out docking studies using Swissdock for three of our synthetic analogues with TERT (Telomerase reverse transcriptase) domain of the telomerase in *T. thermophila*. The binding energies of ligands 8-9 and 7-6 are seen to be in the order of -10 J and -9 J, while the binding energies of ligand 8-3 are only on the order of -8 J. This is consistent with the observation from our drug study that WS8-9 and WS7-6 showed high anti-proliferative potential whereas WS8-3 showed comparatively lower anti-proliferative potential. Our next step would be to carry out the TRAP (telomerase repeat amplification protocol) assay to quantify the change in telomerase at different concentrations of the selected 6 compounds in prostate cancer parental cells. If these studies show promising results, we will further research the effect that BIBR1532 and its synthetic analogues have on other metastatic cell lines.

(Co-Authors: Katie R. Martin and Jeffrey P. MacKeigan)

“Myotubularins as PI(3)P Phosphatases in Autophagy and Endocytosis”

Endocytosis and autophagy are both processes dependent upon the signaling lipid PI(3)P. PI is phosphorylated by the well-characterized kinase Vps34 to PI(3)P, but the corresponding phosphatase is unknown. Though myotubularins are known PI(3)P phosphatases, the role of individual myotubularins in autophagy and endocytosis has not been determined. Combinatorial cell-based siRNA screens of sixteen human myotubularins and myotubularin-related proteins were used to identify individual myotubularin genes active in each process. Myotubularin 1 (MTM1) is likely active in autophagy, since a significant increase was seen in PI(3)P-positive vesicles and in autophagy-specific LC3-positive vesicles with MTM1 siRNA knockdown. Myotubularin related protein 6 (MTMR6) is likely active in endocytosis, since an increase in PI(3)P-positive vesicles was seen with no corresponding increase in autophagy with MTMR6 knockdown.

(Co-Authors: Mona Soni, Brittany Gasper, Dr. Virginia McDonough-Stukey)

*“Examining lipid trafficking in eukaryotic cells using the *Saccharomyces cerevisiae* trafficking mutant *mon2Δ*”*

Not much is known about the intracellular trafficking of exogenous lipids in eukaryotic cells. In order to gain insight on this process, we used the yeast trafficking mutant *mon2Δ* in studies of fatty acid uptake and metabolism. Mon2p, the gene product of *MON2*, is a scaffolding protein involved in vesicular budding on the trans-Golgi membrane. The *mon2Δ* mutant has been previously shown in our lab to experience increased growth inhibition when fed undecylenic acid (11:1Δ10), a short chain unsaturated fatty acid (UFA) that is only slightly toxic to wild type cells. We examined wild type W303-1A and mutant *mon2Δ* responses to unsupplemented, 18:2Δ9,12 (linoleic acid, which is an UFA that yeast can grow on) fed, and 11:1Δ10 fed conditions, in order to determine the role of Mon2p in lipid trafficking. Our results suggest that there is a differential response between W303-1A and *mon2Δ* when fed 11:1Δ10. The *mon2* deletion results in 1) differential localization of intracellular BODIPY® 500/510 C1, C12, a fluorescent fatty acid analog; 2) decreased expression of *OLE1*, which encodes delta-9-desaturase, when fed undecylenic acid; and 3) insufficient increase in the synthesis of endogenous UFAs (16:1 and 18:1) when fed 11:1Δ10. Sterol composition was also analyzed, though no significant difference was found between the two strains. We are currently conducting fatty acid uptake assays to determine if there is a difference in the rate of FA uptake in *mon2Δ*.

(Co-Authors: C.M. Dobbins and D.B. Moore)

“ENDOPLASMIC RETICULUM-TARGETTED BCL-2 RESCUES SH-SY5Y NEUROBLASTOMA CELLS FROM ETHANOL TOXICITY”

Ethanol is a well-documented inducer of apoptosis, though the precise cellular mechanism of its toxicity remains unknown. It has recently been demonstrated that endoplasmic reticulum (ER) – localized Bcl-2 is involved in regulating ethanol-induced apoptosis in immortalized CHO695 cells, a non-neuronal hamster cell line. The present study sought to investigate Bcl-2 mediated rescue from ethanol in a more relevant cell line, namely SH-SY5Y human neuroblastoma cells. Ethanol responsiveness of SH-SY5Y cells was first determined. SH-SY5Y cells were cultured in the presence of varying concentrations of ethanol for 24 hours. MTT cell viability assay was performed to quantify surviving cells. Similarly to published studies in CHO cells, relatively high concentrations of ethanol were required to kill this immortalized cell line. A linear ethanol dose response was found, with a 20% reduction in cell viability at 500 mM ethanol, 40% at 750 mM and 80% at 1000 mM. After determining ethanol sensitivity, we next sought to investigate rescue from ethanol toxicity by wildtype Bcl-2, mitochondria-targeted Bcl-2 and ER-targeted Bcl-2. SH-SY5Y cells were cultured for 24 hours then transfected with cDNA constructs encoding GFP-tagged wildtype or organelle-localized Bcl-2. Localization was verified by fluorescence microscopy. Cells were then treated with varying concentrations of ethanol for 24 hours, followed by MTT assay. Overexpression of ER-targeted Bcl-2 rescued SH-SY5Y cells from ethanol at higher rates than wildtype or mitochondria-targeted Bcl-2, even at the highest concentration tested. Indeed, cells treated with 1000 mM ethanol showed only a 20% reduction in cell viability when ER-Bcl-2 was overexpressed, compared with a 40% reduction for wildtype Bcl-2, a 70% reduction for mitochondrial Bcl-2 and an 80% reduction for controls. In conclusion, the present study shows that SH-SY5Y cells are rescued by ER-targeted Bcl-2 much like hamster cells and sets the stage for further investigation of ER-dependent ethanol toxicity mechanisms in a more relevant human neuroblastoma cell line.

“Isolation and Investigation of Phages from Bacterial Communities of Biomphalaria glabrata Snails”

Biomphalaria glabrata snails are the intermediate host for schistosome parasites which cause disease in humans in Africa and South America. We characterized the bacterial communities in the snail and searched for bacteriophage (viruses that infect bacteria). Phages are extremely diverse and have been found in many environments, but few studies have examined the bacterial communities of invertebrates for phage, and none have been reported from the bacterial communities of snails. The bacterial diversity found in the guts of lab snails was low (5 species), and we attempted to isolate phage from each bacterial species. Our phage isolation techniques included the use of mitomycin C to induce temperate phage to leave the bacterial chromosome and infect more bacteria, and the use of chloroform to assist with the lysis of infected cells. Three phages were isolated; morphologies of two are consistent with filamentous Inoviridae, and the third possesses Myoviridae morphology. Genome sequencing was completed and sequence analysis is ongoing.

(Co-Authors: Daniel Obregon)

“Investigating the Cytotoxic Effects of Mycobacteriophage Vix Gene 80”

A bacteriophage, or phage, is a type of virus that infects and reproduces in bacteria. Phage usurp key metabolic systems of their bacterial host during infection and redirect these processes towards making new phage particles. Identifying the relevant phage and host gene products and understanding how phage exploit their host's weaknesses could lead to new therapeutic options for many bacterial illnesses. In this work, a mycobacteriophage Vix, gene 80, a gene cytotoxic to host strain *Mycobacterium smegmatis*, was studied. Our hypothesis was that an interaction between the Vix80 gene product and a host cell protein, possibly MSMEG_3532, affects host cell metabolism and causes growth inhibition. Vix80 protein shares 68% amino acid identity with the product of gene 77 of mycobacteriophage L5, a gene that has been previously shown to exhibit cytotoxic properties and interacts with MSMEG_3532, a L-serine dehydratase. Both Vix80 and L5_77 gene products contain a conserved domain of unknown function near the N-terminus. The Vix80 gene was dissected and the N-terminal conserved domain was tested separately from the C-terminus domain for cytotoxic activity. The N-terminal 66 residues, encompassing the entire conserved domain of unknown function, was found to be cytotoxic to *M. smegmatis*. Further, the degree of cytotoxicity appeared stronger compared to the full length Vix80 protein. Efforts to express and purify both the Vix80 and MSMEG_3532 proteins in *Escherichia coli* and show a physical interaction in vitro have not succeeded due to extremely low solubility of our T7-antigen tagged MSMEG_3532 protein. Although we continue to explore this approach, an alternative yeast 2-hybrid option is underway.

“Type II MADS-box genes isolated from the gymnosperm cones of Ephedra and Juniper”

Type II MADS-box genes determine the organ identity in angiosperm reproductive structures. Current knowledge of Type II MADS-box genes in gymnosperm cones is limited, but it does support the hypothesis that seed plant reproductive structures (flowers and cones) evolved using a similar genetic toolkit. In this study we focused on isolating and sequencing Type II MADS-box genes from the understudied gymnosperm cones of *Ephedra* and *Juniper*. We isolated 11 unique Type II MADS-box gene sequences that belong to four gene lineages including AGI6, TM3, B-class and C-class. This is the first report of MADS-box genes in *Ephedra*. Our understanding of the evolutionary history of reproductive structures in gymnosperms and seed plants in general will continue to improve with further work on isolating more sequences and gene expression studies with our increased sampling of gymnosperm Type II MADS-box genes. This knowledge may help determine how cone and flowers are similar at the genetic level and ultimately how flowers evolved from cones.

(Co-Authors: Jessica Kozack, Shelby Peterson, Aaron Putzke)

“Fer kinase regulates Notch signaling required for proper vasculature and red blood cell formation in Zebrafish.”

Fer kinase, a protein involved in the regulation of cell-cell adhesion and proliferation, has been implicated in leukemia, gastric cancer, and liver cancer. However, the role Fer plays in the molecular mechanisms of these diseases remains largely unknown. By studying Fer during development, we hope to obtain a better understanding of its involvement in human tumor formation. We have previously shown that FRK-1, a Fer kinase homologue in the nematode *C. elegans*, is involved in regulating a stem cell-like population during development. In this study, we begin to bridge the gap between the invertebrate and vertebrate realms by elucidating the role that Fer kinase plays during zebrafish embryogenesis. Our data indicate that Fer is expressed in zebrafish, and that it is required for normal formation of both the vasculature and red blood cell populations. Elimination of Fer function results in disorganized vasculature and reduced angiogenesis, which prevents any circulation, as well as a significant decrease in red blood cell numbers. We have performed quantitative gene expression and embryonic rescue experiments implicating a requirement for Fer regulation of the Wnt-Notch pathway, which directs formation of the vascular system as well as determines the ratio of vascular to red blood cells during development. This unique regulatory function for Fer kinase would provide valuable information not only to the field of developmental biology, but could also lead to novel therapies in a variety of cancers in which expression of Fer kinase is either misregulated or deleted, such as in myeloid leukemia.

(Co-Authors: Mary Bradley, Daniel A. Smith, NaTasha Schiller, Leah Chase)

“Activation of System xc- Trafficking via an Akt-dependent Signal Transduction Pathway”

System xc- is a heterodimeric plasma membrane transporter involved in the exchange of intracellular glutamate for extracellular cystine. As such, this transporter plays a critical role in the production of the antioxidant glutathione. Previous studies in our lab have demonstrated that there is an increase in cell surface expression within ten minutes of exposure to H₂O₂ in confluent U138MG human glioma cells. The study described herein sought to begin to characterize the mechanism by which H₂O₂ regulates the trafficking of xCT. We hypothesized that Akt signaling is necessary for H₂O₂-mediated trafficking of xCT. A significant increase in Akt phosphorylation was observed in U138MG cells following ten-minute exposure to 3 mM H₂O₂ compared to vehicle-treated cells using western blot analysis. The increase in AKT phosphorylation returns to basal values within 120 min. Treatment with the Akt inhibitor 10-DEBC (2.5 μM) for 30 minutes prior to and during H₂O₂ exposure resulted in a decrease in H₂O₂-induced phosphorylation of Akt at Ser473. Similar inhibition of Akt phosphorylation at Thr308 was observed following treatment of cells with 1.0 μM API-2. Next, we used simultaneous treatment of cultured glioma cells with both inhibitors in the presence of H₂O₂ to determine if such treatment led to a reduction in the trafficking of xCT to the plasma membrane. Our preliminary data suggests that Akt activation is necessary for H₂O₂-induced trafficking xCT to the plasma membrane.

“Does a Phytoplankton Have a Defense Against Its Predator”

Emiliani huxleyi (E. hux) is a coccolithophore. Coccolithophores are single celled phytoplankton with a stage in the lifecycle that forms calcium plates called coccoliths. They play an important role in the food web of the ocean because of their abundance. They also play a key role in the calcium cycle of the ocean due to their coccoliths. They are known for their large blooms that can be seen from space, and their calcium plates are what make up the White Cliffs of Dover. Research has shown that E. hux. has an inducible defense against ciliate predator *Strombidinopsis*. By performing feeding trials, further research was done on predator-prey interactions between E. hux. and ciliate predator *Favella*. Significant evidence was shown for an inducible defense produced by E. hux. against *Favella*.

(Co-Authors: Jordan T. Presley, Emily M. Brogan, Scott B. Thourson, Shannon J. Timpe, Brian J. Doyle)

“A Quartz Crystal Microbalance Biosensor for Analysis of Herbal Medicine”

A Quartz Crystal Microbalance (QCM) is an instrument for measuring mass in the nanogram range. Due to the piezoelectric nature of Quartz, as molecules adsorb to the surface of the crystal, the decrease in resonant frequency is detected as an electrical signal. The adsorbed mass can be calculated from this change in frequency. When a drug target protein is immobilized to the surface of the QCM, the instrument becomes a biosensor capable of detecting binding of drugs to the target. We are developing a QCM sensor for detecting binding of medicinal plant extracts to bovine serum albumin (BSA). Serum albumin is the most abundant protein in serum and binds a variety of molecules, including drugs, which may affect their bioavailability. BSA was immobilized directly on the gold-coated surface of the QCM or indirectly via a self-assembled monolayer (SAM) of alkanethiols. SAM formation and BSA immobilization were observed in real time by QCM and also analyzed by FTIR. The BSA-functionalized QCM biosensor was then used to detect binding of 8-anilino-1-naphthalenesulfonic acid, a known BSA ligand. IR absorbance peaks corresponding to the exposed functional groups of the SAM were observed, as well as the amide peaks of the protein confirming their presence. BSA adsorption to the QCM surface was ~ 450 ng/cm², and the BSA functionalized QCM bound ANS at ~ 300 ng/cm². No significant differences were observed with regard to the method of immobilization. Finally, botanical extracts, including black tea, wild geranium, and St. John's wort were tested for binding to BSA. Binding was detected in all extracts. Further fractionation of the extracts will give insight into the chemical nature of the BSA-binding molecules.

(Co-Authors: Dr. Joseph Stukey Dr. Virginia McDonough-Stukey)

“A Toxic Ride through the Pumpkin Patch: Identification of Cytotoxic Regions in Mycobacteriophage Pumpkin”

Mycobacteriophages are viruses that infect mycobacterium host cells. With more than 200 mycobacteriophage genomes sequenced and available in GenBank, they represent the largest collection of sequenced phages that infect a single host (*Mycobacterium smegmatis*). Surprisingly however, they are genetically diverse and contain many genes of unknown function. This fact begs the question of how the different mycobacteriophages accomplish the host-cell takeover that supports infection and phage propagation. One way to tackle this problem is to first identify phage genes that are cytotoxic when expressed individually in the host cell. We hypothesize that cytotoxic phage genes will encode proteins that interact with and affect the function of critical host cell proteins. We are using this approach on a mycobacteriophage called Pumpkin, which was isolated at Hope College in 2008. We have identified a small genomic region, encompassing genes gp115-120 that is cytotoxic to *M. smegmatis*. Subsequent division of this region further identified gp115 and the region gp117-120 as possessing cytotoxic activity. Future work will include looking at the individual genes within the region gp117-120 and testing all cytotoxic phage gene products for interactions with host cell proteins.

“Investigating Delta-Sarcoglycan N-glycosylation Site Mutants Using Adenovirus Transduced Cell Models”

δ -sarcoglycan is a subunit of the sarcoglycan complex which is a subcomplex within the dystrophin-glycoprotein complex which is located at the sarcolemma membrane of muscle cells. Recessive mutations in δ -sarcoglycan cause Limb Girdle type 2F Muscular Dystrophy while dominant mutations are linked to dilated cardiomyopathy. It has also been shown that δ -sarcoglycan has N-linked glycosylation (at the 60th, 108th, and 284th amino acids) occur before insertion into the sarcolemma. These studies were focused on these N-glycosylation sites and two mutants (R71T and R97Q) that are linked to dilated cardiomyopathy but not skeletal dystrophy. One of these mutants has been shown to have alternative glycosylation patterns in cardiac myocytes and our research showed that the alternative glycosylation patterns is not tissue specific to cardiac muscle. We transduced rat L6 myoblasts, rat L6 myotubes, rat A7r5 smooth muscle, and rat cardiac myocytes with R71T and R97Q mutant adenoviruses. After transduction the protein synthesized by the cells was collected 48-96 hours later and a Western Blot was performed. To investigate N-linked glycosylation sites we transduced cells with adenoviruses (N60Q, N108Q, and N284Q) that eliminated an N-linked glycosylation site. We found that site 60 is not normally glycosylated, site 108 is always glycosylated, and site 284 is sometimes glycosylated. These glycosylation patterns were not tissue specific.

(Co-Authors: Dr. Steve Triezenberg)

“Post-translational modification of a key transcription factor for herpes simplex virus infection”

Herpes simplex virus type 1 (HSV-1) is a highly prevalent virus that causes cold sores. The HSV-1 virion contains VP16, an important multifunctional protein. VP16 is a potent transcriptional activator that recruits host cell proteins, including Oct-1, to initiate immediate early (IE) viral gene expression and therefore the lytic cycle. The VP16 amino acid residue serine 375 resides in a consensus casein kinase II (CKII) site and lies within the region which interacts with Oct-1. We hypothesize that phosphorylation by CKII at serine 375 upon infection activates VP16 to initiate complex formation with Oct-1 and induce IE gene expression. Pharmacological inhibition of CKII with TBCA resulted in a 80% decrease in IE mRNA levels at 2 hours post infection (hpi) as quantified using qRT-PCR. However, phosphorylation at serine 375 could not be detected in infected cell lysates 2-8 hpi using western blotting. In contrast, significant phosphorylation at serine 375 was detected at 20 hpi. Preliminary data suggests that phosphorylated VP16 may be preferentially packaged into the tegument of infectious virions. These data support an alternative mechanism by which late synthesized VP16 is phosphorylated at serine 375, packaged into infectious virions and delivered to the next cell pre-modified for Oct-1 complex formation and IE transcription activation.

(Co-Authors: Mark P. Schotanus, Alex R. Stoddard, John L. Ubels)

“Efficacy of Antioxidants in the Corneal Epithelium”

Introduction: Dry eye disease affects the ocular surface and tear film, increasing risk of oxidative damage. Antioxidants in artificial tear formulations could potentially reduce this risk. Our goal was to test bioavailability of antioxidants in corneal epithelial cells and determine if they reduce reactive oxygen species (ROS) intracellularly and extracellularly. Methods: A cellular antioxidant activity assay was performed to determine the efficacy of epigallocatechin gallate (EGCG), gallic acid, n-propyl, and quercetin. Cells were exposed to antioxidants at varying concentrations then exposed to xanthine oxidase (XO) or co-incubated with XO and an antioxidant. Results: All of the antioxidants were effective at quenching ROS. Intracellularly, quercetin was most effective with an EC₅₀ of 41.3 ± 5.6 μM, followed by EGCG, n-propyl gallate, and gallic acid with EC₅₀s of 56.5 ± 2.9, 70.1 ± 1.4, 337.6 ± 65.4 μM, respectively. When co-incubated, antioxidants were able to quench ROS more effectively. Co-incubation results showed n-propyl gallate to be most efficient, compared to quercetin, EGCG, and gallic acid, with EC₅₀s of 2.7 ± 0.8, 6.3 ± 0.2, 6.5 ± 0.6, and 8.0 ± 1.2 μM, respectively. Conclusions: Results suggest that antioxidants can be taken up by corneal epithelial cells and quench ROS intracellularly and extracellularly. Antioxidants in artificial tears may prove to be effective in protecting the ocular surface of dry eye patients from oxidative damage.

“Let Thy Food be thy Medicine: Investigating Nutraceutical Properties of Cruciferous Vegetables”

Consumption of cruciferous vegetables reportedly has both nutritional and pharmaceutical benefits including decreased risks of cancer, cardiovascular disease, and infectious diseases. These properties are associated with isothiocyanates (ITCs), bioactive compounds that are produced when myrosinase (enzyme present in myrosin cells) hydrolyzes glucosinolates (substrates present in nearby cells) during maceration. Our research focused on optimizing methodologies so that undergraduate students in an introductory cellular biology and genetics laboratory would be able to assess the effects of different food preparation methods on the production of ITCs in cruciferous vegetables. We have developed and optimized a new method for measuring myrosinase activity based on the release of glucose by-product. Our results have demonstrated that different cooking methods have differential effects on myrosinase activity in broccoli and radish sprouts – with harsh methods, such as boiling, significantly reducing myrosinase activities in comparison to raw controls. We also measured the antimicrobial activities of ITCs and concluded that *E. coli* and *S. epidermidis* are more sensitive to benzyl-ITC than to allyl-ITC or phenethyl-ITC. Nevertheless, at 100 μM levels these ITCs significantly inhibited the growth of both species. We infer from these results that cooking methods can have significant detrimental effects on the production of nutraceutical ITCs during the consumption of cruciferous vegetables.

(Co-Authors: Dawn Clifford Hart)

“Characterizing Protein-protein Interactions for Accurate Cell Division in Fission Yeast”

Cell division is a necessary process for growth and development of all organisms. Fission yeast (*S. pombe*) provides a model system for polarity and cytokinetic mechanisms because, like human cells, they grow in a bipolar fashion and divide symmetrically through contraction of an acto-myosin ring. Mid1 is a founding protein of the acto-myosin ring that helps recruit other ring proteins and define the position of division. In cells without functional Mid1, there is incomplete, asymmetrical cell division. One class of *S. pombe* mutants are classified by the apparent loss of cell polarity and round cellular shape due to problems in one of 12 orb genes. To further study whether these polarity defects are related to cytokinetic defects, we have investigated interactions between Mid1 and two orb mutants, Orb5 and Orb6. Analysis of orb5 and orb6 mutants reveal differing localizations of Mid1. Both orb5 and orb6 mutants have also shown a higher prevalence of binucleate cells and most of these binucleate cells have an internal septum. The majority of the orb mutants imaged also show a paired conformation, in which two cells are not completely separated from one another at one end. There is also an overall higher level of Mid1 in cells with defective Orb5 or Orb6 paralleling the more concentrated visualization seen in microscopy. These phenotypes suggest a relationship between cytokinetic defects and the polarity genes, as well as a link between Mid1 and the Orb proteins.

(Co-Authors: Julia M. Santos, Ph.D)

“Temporal Relationship Between Activation of Matrix Metalloproteinase 9 and Mitochondria Damage in the Development of Diabetic Retinopathy”

Purpose: In the pathogenesis of diabetic retinopathy, the dysfunction of mitochondria plays a significant role in the apoptosis of retinal cells. Our previous work has shown that in diabetes, the activation of retinal matrix metalloproteinase 9 (MMP-9), a protease which is regulated by its tissue inhibitor TIMP1, damages retinal mitochondria and activates the apoptotic machinery. The purpose of this study is to investigate the temporal relationship between the activation of retinal MMP-9 and mitochondria damage in the development of diabetic retinopathy. Methods: Gene expression of MMP-9, TIMP1 and of mitochondria DNA-encoded genes ND1 and ND6 were quantified by real-time PCR in isolated bovine retinal endothelial cells incubated in 5 or 20mM glucose medium for 6-96 hours. The expression of MMP-9 in the mitochondria was determined by western blot technique. The results were confirmed in the retina from rats with streptozotocin-induced diabetes for 15 days-12 months. Results: In endothelial cells, MMP-9 mRNA was elevated after 48 hours of high-glucose insult compared to the values obtained from cells incubated in 5mM glucose, but decrease in TIMP1 mRNA was observed as early as in 6 hours of high-glucose insult, and this decrease continued throughout the experiment (96 hours). Although the levels of ND1 and ND6 remained normal during the initial glucose insult, at 96 hours of high glucose exposure their levels were decreased by 30-50%, and this was accompanied by a 35% increase in the mitochondria accumulation of MMP-9 compared to the values obtained from cells incubated in 5mM glucose. Similarly, in rat retina, MMP-9 mRNA was elevated as early as 15 days of diabetes and continued to increase for 6-12 months, a duration in diabetes when retinal capillary cell apoptosis is detectable. There was no change in TIMP1 at 15 days of diabetes, but at 2 months its levels were significantly decreased. In contrast to MMP-9, decrease in ND6 levels was not observed until the duration of diabetes was extended to 6 months. Conclusion: As the duration of diabetes increases, increased MMP-9 accompanied by decreased TIMP1 damage mitochondria, and the electron transport chain system becomes dysfunctional initiating a vicious cycle of free radicals. This further strengthens the importance of early glucose control, and suggests that inhibition of MMP-9 activation using molecular and pharmacological methods could prevent the progression of retinopathy in diabetic patients.

(Co-Authors: Amanda O'Brien, Agnieszka Szarecka, Troy Wymore, Nikolay Simakov)

“Development of CHARMM force field parameters for two different classes of beta-lactam antibiotics”

Beta-lactams are currently the most commonly used group of antibiotics. However, continuously evolving bacterial resistance, mediated by beta-lactam hydrolyzing enzymes (beta-lactamases), limits the clinical efficacy of these drugs. Molecular dynamics (MD) simulations can reveal critical details of the antibiotic binding modes as well as the mechanism of hydrolysis of various beta-lactams by beta-lactamase enzymes. However, MD simulations can be challenging due to the lack of molecular mechanical (MM) force fields parameters for antibiotic molecules, which thus have to be developed rigorously for each compound. In this presentation, we will show our parameterization results for doripenem and ceftazidime beta-lactam antibiotics, which belong to the newest carbapenem and third generation cephalosporin classes, respectively. Our parameterization protocol is consistent with the CHARMM force field and employs a newly developed toolkit called ParamIT (sb.nrbosc.org) containing MM parameter optimization and data management modules. Because doripenem and ceftazidime are large molecules, they had to be divided into several smaller fragments that were parameterized separately and then pieced together to create the final parameter set. The calculated geometry, vibrational modes, dihedral potential energy scans and interactions with water molecules obtained from the developed MM force field, and their agreement with Quantum Chemical calculations will be presented. The force field parameters for both molecules will enable us to simulate their Michaelis complexes with a number of beta-lactamase enzymes.

(Co-Authors: Jodee Hunt)

*“Lonely Boy: Parental Division of Labor & Single Parenting in Convict Cichlids (*Amatitlania nigrofasciata*)”*

Wild convict cichlids provide biparental care with marked division of labor. We investigated whether parental specialization persisted in experimental conditions and if parents continued care if their mate was absent. In separate experiments, we divided broods and either reared half with both parents, but sequestered the other from parental contact or reared half with the female and half with the male. Whether paired or single, females remained near and frequently contacted broods. Paired males were vigilant – similar to parents in wild populations – but single males remained nearer and more frequently contacted offspring compared to paired males. Females (versus males) consistently contacted offspring more frequently. This behavioral flexibility helps parents rear broods to independence.

(Co-Authors: Elizabeth Hidlebaugh, Daniel A. Smith, NaTasha Schiller, Leah Chase)

“Activation of System xc- Trafficking via an Akt-dependent Signal Transduction Pathway”

System xc- is a heterodimeric plasma membrane transporter involved in the exchange of intracellular glutamate for extracellular cystine. As such, this transporter plays a critical role in the production of the antioxidant glutathione. Previous studies in our lab have demonstrated that there is an increase in cell surface expression within ten minutes of exposure to H₂O₂ in confluent U138MG human glioma cells. The study described herein sought to begin to characterize the mechanism by which H₂O₂ regulates the trafficking of xCT. We hypothesized that Akt signaling is necessary for H₂O₂-mediated trafficking of xCT. A significant increase in Akt phosphorylation was observed in U138MG cells following ten-minute exposure to 3 mM H₂O₂ compared to vehicle-treated cells using western blot analysis. The increase in AKT phosphorylation returns to basal values within 120 min. Treatment with the Akt inhibitor 10-DEBC (2.5 μM) for 30 minutes prior to and during H₂O₂ exposure resulted in a decrease in H₂O₂-induced phosphorylation of Akt at Ser473. Similar inhibition of Akt phosphorylation at Thr308 was observed following treatment of cells with 1.0 μM API-2. Next, we used simultaneous treatment of cultured glioma cells with both inhibitors in the presence of H₂O₂ to determine if such treatment led to a reduction in the trafficking of xCT to the plasma membrane. Our preliminary data suggests that Akt activation is necessary for H₂O₂-induced trafficking xCT to the plasma membrane.

(Co-Authors: Pushpaja Dodla, Jared Kaminski, Shambhavi Singh, Suganthi Sridhar, Cindy K. Miranti)

“Identifying a regulatory role for the tumor metastasis suppressor gene KAI1/CD82 in metastatic prostate cancer cell lines”

KAI1/CD82, a metastasis prostate tumor suppressor gene expression is lost when the cancer progresses from a primary to a metastatic stage. CD82 has also been shown to be down-regulated in cancers of the gastrointestinal tract, colon, cervix, breast, lung, pancreas, skin, thyroid and liver etc. As a member of the tetraspanin family of proteins, CD82 interacts with proteins and may act as a master regulator of membrane organization at the cell surface. Even though some of the interacting proteins have been identified, the significance of these associations and its role in metastasis prevention is unclear. By reintroducing CD82 into highly metastatic prostate cells (PC3), we have shown CD82 to regulate c-Met (phosphorylation) and activation. Currently we are focused on studying the exact mechanism by which CD82 regulates c-Met. CD82 does not seem to associate with c-Met nor does it seem to down-regulate c-Met. Preliminary indications are that as a tetraspanin and thus as a molecular organizer it may be redistributing c-Met on the cell surface. It is also highly possible that it may bring a c-Met specific phosphatase (such as DEP-1) to the surface to dephosphorylate and deactivate c-Met. We are currently exploring both possibilities. Even though we have identified c-Met protein to be regulated by CD82, we have reason to believe that there may be more proteins regulated by CD82. Microarray studies done on CD82 (+/-), on both tumor and normal prostate cells suggests that CD82 may be regulating genes involved in cell cycle, growth, and metastatic suppression. To validate the results, we have utilized Q-PCR assays, investigating genes specifically involved in metastasis suppression and growth. These genes include: CXCR4, CXCR7, RUNX3, TFF-3, and MMP10. CXCR4 and CXCR7 are chemokine receptors, RUNX3, a tumor suppressor gene, and MMP10, which encodes the matrix metalloproteinase 10 needed for invasion and continuation of metastasis. Two of the genes involved in metastasis (TFF-3, RUNX-3) have been quantified and the data correlates with the microarray data. MMP10, CXCR4, and CXCR7 are currently being validated. Identifying the proteins regulated by CD82 and deciphering the downstream signaling mechanisms involved in this regulation is the focus of our future studies.

(Co-Authors: Aaron Putzke)

“Investigating Fer Kinase Regulation of Gene Transcription During Development”

The *Caenorhabditis elegans* protein FRK-1, an ortholog to Zebrafish (*Danio rerio*) and mammalian Fer kinase, is critical to the proper embryonic and larval development. In humans, aberrant Fer kinase levels have also been implicated in the progression of leukemia and prostate cancer. In addition to roles in the formation of the hypodermis in *C. elegans*, FRK-1 has been shown to localize to the nucleus in a cell-cycle dependent manner (Putzke et al, 2005.). This localization has led us to hypothesize that FRK-1, and its ortholog Fer kinase, are involved in the regulation of transcription factors during development. To discover potential gene targets regulated by Fer kinase activity, we aim to perform microarray analysis in nematodes, Zebrafish and human cells in the absence of Fer/FRK-1. Our microarray results, thus far, indicate that many genes with abnormal levels of transcription at 24 and 48 hours post fertilization in *Danio rerio* are associated with proper neurogenesis and vasculogenesis of the Zebrafish. We are currently pursuing microarray data in *C. elegans* (genomic knockout ok760) and as well as a human cell line utilizing with Fer knock-down (via siRNA). The aim of this research is to determine more about the development of *C. elegans* and *Danio rerio*, and to identify potential therapeutic gene targets for treatment in human cancers.

“The effects of Wnt inhibition on blastema formation and melanocyte re-population on the adult zebrafish.”

In our study we evaluated the effects of inhibition of the canonical Wnt pathway during the process of tissue regeneration in the zebrafish. We performed pilot studies to determine the effects of Wnt pathway inhibition on early regeneration of zebrafish caudal fins as well as regeneration of the adult melanocyte population post chemical ablation. Blastema formation was specifically studied as well as variance in melanocyte regeneration time-lines. Further research along this route may provide detailed information as to the role that the Wnt pathway plays in stem cell function during maintenance, repair, and replenishment of adult cell populations. Blastema formation inhibition may provide information about the signaling pathways and positional information provided through a normally functioning Wnt pathway.

(Co-Authors: John Morris, Anding Shen, Mary Dekker)

“The Role of Cell Cycle Status and Cytokine Influence in HIV Infection of Resting T-cells Co-Cultured with Endothelial Cells”

In vitro, it has been observed that productive infection occurs in activated CD4+ T-cells and that the infection of resting cells is blocked prior to viral integration. However, in vivo studies display HIV infection in resting CD4+ T-cells. One theory as to why this occurs is that T-cells encounter signals from cytokines and antigen-presenting cells. We hypothesize that endothelial cells contribute significantly to HIV infection because they regularly interact in vivo and are able to stimulate T-cells while the T-cells remain in a resting state phenotypically. We propose that endothelial cells also contribute significantly to latency formation and reactivation in resting CD4+ T-cells. Two possible cellular factors that may contribute to the endothelial cell interaction permitting infection to occur are the influence of cytokines and the cell cycle status of the T-cells during interaction with endothelial cells.

(Co-Authors: Gellert Mezei, Isurika Fernando, and Stuart Surmann)

“Selective total encapsulation of anions by neutral, hydrophobic nano-jars”

Selective recognition and encapsulation of anions by artificial receptors is one of the most far-reaching areas of supramolecular chemistry, with implications in chemical, biological and environmental sciences. We have recently developed a new class of anion-binding agents, based on nano-sized toroidal copper(II)-hydroxide/pyrazolate complexes (“nano-jars”), which possess an unprecedented ability to totally encapsulate anions. Lined by H-bond donors on the inside and hydrophobic on the outside, these assemblies selectively extract kosmotropic anions from mixtures with chaotropic anions. Up to twelve hydrogen bonds from the neutral host assembly wrap around and sequester PO₄³⁻, AsO₄³⁻, HAsO₄²⁻, CO₃²⁻, SO₄²⁻, CrO₄²⁻ or Cl⁻ anions, similarly as in their analogs in living organisms, such as the sulfate- and phosphate-binding proteins. Tetrabutylammonium “lids” seal the “nano-jars” and render the encapsulated anion completely buried and inaccessible, so that, for example, sulfate is not precipitated by Ba²⁺ ions. Details of crystallographic, mass spectrometric, nuclear magnetic resonance and selectivity studies will be discussed.

(Co-Authors: Dr. Robert Smart, PhD)

“Combinatorial Synthesis of Semiconductor Oxides for Solar Water Splitting”

Fossil fuels are being depleted at an ever increasing rate while becoming more difficult and costly to acquire. As the cost of the continued use of fossil fuels become more profound, the need for alternative energy development has never been so dire. The most likely source of readily affordable, ‘safe’ and renewable energy is the sun. Clearly effective harnessing of solar energy could move the planet away from the current crisis and to a sustainable future. One approach of collecting and storing the power of the sun is to convert its energy into chemical bonds. The “Holy Grail” of solar energy conversion and storage is solar water splitting using semiconductors as both the light absorber and energy converter, to store solar energy in the simplest chemical bond, H₂. Using a combinatorial approach, a series of multicomponent metal oxides were synthesized. The resulting metal oxides were assessed using a visible wavelength laser measuring the photocurrent generated by excitation. Over 1,500 metal oxides were tested. Metal oxides containing Fe, Cu, Zn, Ag and Mg showed the highest current-voltage readings in comparison to the Fe standard. Successful candidates will be incorporated into nanoparticle films and evaluated as potential semiconductor materials capable of splitting water by the head institutions of CCI Solar.

(Co-Authors: Professor Mark Muyskens)

“The Fluorescence of Aqueous Sycamore Extracts”

The fluorescence properties of wood extracts are only somewhat understood. While it has been found that aqueous extracts from tropical and local trees show evidence of fluorescent activity, this activity has not been thoroughly investigated. Our work involved the further investigation of previous results concerning the fluorescence of sycamore extractives. That work suggested that the sycamore tree is the most fluorescent of local trees, but the cause of this is unknown. Our goal was to identify the fluorescent compound. We had two main lines of work in this investigation. The first was to develop a greater understanding of the properties of the fluorescent extract. The second investigation involved attempting to isolate the fluorescent compound, and obtain a pure sample. Properties investigated were pH dependence, time dependence and temperature dependence. We found that the fluorescent compound shifted from a low pH to a high pH form. The high pH form was the brighter of the two forms. It was also found that the same level of extraction was achieved for extractions taken at all temperatures at or above room temperature and that when left at room temperature for one week degradation occurred, and the apparent fluorescence decreased. Using the method of semi-prep HPLC we isolated the fluorescent fraction. This sample was analysed through mass spectrometry. A mass of 239.1 was determined for the fluorescent compound, which suggests a chemical formula of C₁₃H₂₀O₄.

(Co-Authors: Prof. Mark Muyskens)

“Structure, hydrogen bonding, and barriers to rotation of 1-fluoro-pentane-2,4-dione and related compounds”

Molecular structures, hydrogen bonding, and the barriers to rotation for 1-fluoro-pentane-2,4-dione (monofluoroacetylacetone, MFAA) have been studied using Density Functional Theory (DFT). MFAA is a halogenated derivative of the widely-studied pentane-2,4-dione, (acetylacetone, AA). For comparison's sake, we have also studied other related halogenated derivatives of AA. This research fits into a broader context of understanding molecules that undergo a photoelimination reaction upon excitation with UV light. Since one of MFAA's two methyl groups has a single fluorine atom, MFAA is an asymmetric beta-diketone. The position of the fluorine vis-a-vis the hydrogen bond is of key interest, especially for the photoelimination mechanism. MFAA has two distinct enol isomers: the “carbonyl” isomer, in which the CFH₂ group is proximal to the C=O group, and the “hydroxyl” isomer, in which the CFH₂ group is proximal to the C-OH group. For MFAA and all other comparable molecules we studied, our theoretical calculations at B3LYP/cc-pVTZ show every carbonyl isomer to be more stable than its hydroxyl isomer with an energy difference in the range of 5.1-5.5 kJ mol⁻¹. We find the CFH₂ group rotation in MFAA to be hindered with a double-minima potential in Carbonyl MFAA (maximum barrier height > 18 kJ mol⁻¹) and a triple-minima potential in Hydroxyl MFAA (maximum barrier height > 16 kJ mol⁻¹). All molecules we studied with heterogeneous halomethyl groups show results similar to that of MFAA.

(Co-Authors: Jin Sung)

“Electrochemistry of Catechols”

Naturally occurring polyphenols have been shown to yield favorable health benefits such as the stabilization of free radicals, antioxidant activity, and iron-chelating mechanisms. These polyphenolic compounds can be found in fruits, vegetables, dark chocolate, tea, and red wine. However, for polyphenols containing catechols or gallols there is another pathway in which they prevent the Fenton-type chemistry, which is linked to neurodegenerative diseases such as Parkinson's, through ligand formation between the dihydroxy site of the polyphenol and the metal ion. Specifically looking at 3,4-Dihydroxyhydrocinnamic acid (DHCA), which is a metabolic product and can be absorbed through the colon, and 3,4-Dihydroxy-L-phenylalanine (L-dopa), which is the most widely prescribed drug for Parkinson patients. My research this summer primarily focused on the electrochemical effects of the ligand to metal binding interaction. Utilizing an HCH Instruments Electrochemical Analyzer, I observed the oxidation of the catechol to the quinone due to the change in potential of the system, and then the reduction back to the catechol when the potential was shifted back to the starting potential. This change in oxidation state occurs at and above a specific potential, and with the addition of a metal ion I could observe the change in potential due to the ligand to metal bond formation, indicating a change in the energy required to oxidize to the quinone. This change in energy required to change the redox potential of the ligand suggests the interaction between the ligand and metal ion does affect the redox potential of the metal ion as well, and may aid in preventing Fenton type chemistry.

(Co-Authors: Evan E. Rugen and Mary E. Anderson)

“Synthesis and Characterization of Thermoelectric PbTe Nanoparticles”

Thermoelectric materials are able to convert thermal energy to electricity and vice versa, convert electricity into a thermal gradient. Currently these materials are produced through a “top-down” method using the “bulk” as starting materials. However, this approach is costly and both time and energy consuming. By using a modified polyol synthesis or building from the “bottom-up”, our hope is to increase the efficiency of creating thermoelectric particles. In this type of synthesis, two metal salts are reduced to atoms, heat is applied and the atoms then combine to form intermetallic nanoparticles. These nanoparticles are then characterized by X-Ray Diffractometry, Scanning Electron Microscopy and Electron Dispersive X-Ray Spectroscopy. We have studied the formation as well as the morphology of PbTe nanoparticles while altering variables such as temperature, time, solvents, and size controlling polymers. In the future, we hope to continue studying the formation and morphological changes of thermoelectric nanoparticles. Ultimately, the goal of this research is to optimize the synthesis of nanoparticles for use in applications such as seat warmers, refrigeration, as well as deep space probes.

(Co-Authors: Liyana A. Wajira Ariyadasa and Sherine O. Obare)

“Studying the Charge Transfer Size Dependence between Semiconductor Quantum Dots and Quantized Metal Nanoparticles”

Semiconductor luminescent quantum dots (QDs) have attracted much research attention due to their unique size-dependent optical properties and their photostability. Previous studies in our group showed that QDs can be involved in fluorescence resonance energy transfer (FRET) where they act as donors that transfer energy to nearby fluorophores. On the nanoscale, discrete energy levels arise that significantly influence the inherent properties of metal nanoparticles. Specifically, metal nanoparticles can be synthesized that display discrete energy levels and thus are able to accept energy when brought in close proximity to nearby donors. The purpose of this study was to understand the size effects and the dependence of the QDs to transferring energy. We hypothesized that metal nanoparticles could store the energy received from luminescent quantum dots (QDs) and transfer the energy as needed, thus inducing QD fluorescence quenching. This hypothesis was tested by synthesizing well-defined and monodisperse CdSe QDs and palladium metal nanoparticles with controlled size. We studied the donor-acceptor interactions between the two particles by measuring the fluorescence changes. We found that palladium nanoparticles induced strong quenching of the CdSe QDs. The quenching was size dependent; the smaller the QD the stronger the quenching efficiency, relative to QDs of larger size. Stern-Volmer plots were used to determine the relationship between the QD concentration and the quenching efficiency. It was found that the quenching constant decreased linearly with an increase in palladium nanoparticle core density. These studies provide insight into the properties of metal nanoparticles and their role in charge transfer processes.

(Co-Authors: Nicholas W. Vryhof and Dr. Carolyn E. Anderson)

“Gold(I)-Catalyzed Synthesis of N-Alkyl Pyridones”

The N-alkyl pyridone motif appears in numerous naturally-occurring and pharmacologically important compounds. In previous work, the Anderson lab found that lithium iodide (LiI) catalyzes the transformation of O-propargyl pyridines to N-alkylated pyridones. In addition to the direct migration product, an unexpected N-alkenyl pyridone was also observed in some cases. In order to access this N-alkenyl pyridone in higher yields and to explore its mechanism of formation, O-propargyl pyridine was subjected to gold(I) catalysis. Under gold(I)-catalysis, three pyridones were observed: a methyl ketone, a ketal, and an allylic ether. These pyridones were characterized by ¹H-NMR, ¹³C-NMR and high resolution electrospray mass spectroscopy. The allylic ether pyridone, being an N-alkenyl pyridone, became our target for this summer. Efforts to optimize the synthesis of the allylic ether pyridone are reported herein.

(Co-Authors: Professor Doug Vander Griend, Ph.D.)

“Modeling the Oligomerization of Cationic Methylene Blue in Aqueous Solution”

Methylene Blue (MB⁺) is a cationic dye molecule that is known to aggregate in aqueous solutions. Absorbance data for equilibrated solutions over a concentration range of 1.0×10^{-3} to 2.6×10^{-5} M, in both 0.1 M HCl and 0.1 M HNO₃ was collected. Raw absorbance data was analyzed by a mathematical technique called factor analysis to find that there are at minimum, three distinct molecular chromophores present in solutions of MB⁺. Models of MB⁺ included combinations of monomer, dimer, and trimer all the way up to a septendecomer as well as different numbers of chloride anions. No one model appeared to be better than another. They were indistinguishable qualitatively by how realistic molar absorptivity curves were and quantitatively by the best fit of the data. By modeling simulated data, it was found that systems in which the molar absorptivity curves of the aggregates are mathematically similar, signal noise can obscure the true model from other models. In the case of MB⁺, the aggregation of dye molecules tend to be sufficiently similar so that even the noise of a typical bench top spectrophotometer (0.0001 ABS) obscures the true model.

(Co-Authors: Erran D. Briggs, Amanda K. Bolles, Mara R. Livezey, Leslie D. Nagy, Laura Lowe Furge)

“CYP2D6 is the Major Metabolizing Enzyme of Metoclopramide”

CYP2D6 metabolizes approximately 20% of pharmaceutical drugs. The important role that CYP2D6 plays in drug metabolism makes inactivation events clinically relevant. Thus, it is important to understand the inactivation of CYP2D6 in order to prevent adverse drug-drug interactions. Metoclopramide is a drug commonly prescribed to counteract nausea in chemotherapy patients. It has been reported in the literature to be a mechanism-based inhibitor of CYP2D6 [Desta et al. (2002) Drug Metab Disp 30, 336-343]. In this study we sought to expand this initial finding by constructing a metabolite profile and employing molecular modeling programs in an attempt to better understand the interactions between metoclopramide and CYP2D6. While we did not observe mechanism-based inhibition with metoclopramide, we did find the predicted metabolites of metoclopramide as well as additional metabolites. Furthermore, we were able to establish the relative contribution of specific P450 enzymes to the metabolic profile of metoclopramide. (Support: NIH 1R15-GM086767-01 & -01S1).

(Co-Authors: Cameron F. Holder and Prof. Mary E. Anderson)

“The Synthesis and Characterization of Bi₂Te₃ Nanoparticles”

Due to their ability to convert thermal energy into usable electricity, and vice versa, thermoelectric materials offer an excellent opportunity to improve efficiencies in many applications where energy is lost as heat. However, high production costs and low energy conversion efficiency (ZT) prevent them from being economically viable. By modifying their structure from bulk materials to nanoparticles, their ZT's can be greatly improved, but typical methods for synthesizing nanoparticles are energy intensive, costly, and inefficient. A modified polyol process reduces the production cost and energy consumption of nanoparticle synthesis by using a solution phase “bottom up” approach. This method uses NaBH₄ to reduce metal ions to atoms while mixing them together in a high boiling point solvent to form intermetallic nanoparticles. Many factors such as temperature, reaction time, starting materials, and solvent affect particle morphology. This research has focused on the effect of these variables on Bi₂Te₃ nanoparticle formation as a function of composition and structure. Particles were primarily characterized by X-Ray Diffractometry to characterize crystal structure and indicate composition, Scanning Electron Microscopy to image the morphology of the particles, and Energy Dispersive X-Ray Spectroscopy to analyze elemental composition.

72. Graham Carlson, Chemistry**Hope College**

(Co-Authors: Kara Cousins, Kimberly Brien, Pravin Patil, and Moses Lee)

“DNA Sequence Specific Recognition by Building Block Hx Polyamides I.”

Analogues of the naturally occurring product distamycin bind DNA in stacked anti-parallel dimers with a high affinity and specificity towards their target sequences. Novel polyamides containing Hx moieties exhibit fluorescence, which allows these molecules to be tracked in cells. Hx behaves as two consecutive pyrrole units and is A/T sequence specific. In an effort to optimize the curvature of our compounds we have developed the “Building Block” model in which we have altered the location of the Hx moiety within the molecule to determine the order that produces the most ideal curvature. The present phase of the model involves placing the Hx moiety in the middle of the triamide preceded by a phenyl group and followed by either a pyrrole or imidazole unit. The focus of this research is directed toward the development of Hx polyamides designed to target specific DNA sequences found in the promoters of genes.

73. Hope T. Sartain, Chemistry**Grand Valley State University**

“Exploring the Multi Faceted Ligand Carbamoylmethylphosphine Oxide: In Relation to Nuclear Reprocessing”

The research presented in this poster will include the organic synthesis of multidentate CMPO ligands, as well as their complexes with lanthanides and actinides. One main goal of our research group is to contribute to current strategies involved in nuclear waste remediation. As a part of the TRUEX process, Carbamoylmethylphosphine oxides (CMPO's) have been shown to have an affinity for lanthanides and actinides. Currently, CMPO's are also being used to study luminescence spectroscopy, fundamental f-element coordination chemistry, lanthanide and actinide extraction studies and medical contrast imaging. Metal extraction and structural computational data of the complexes will also be included.

74. Jacqueline Williams, Chemistry**Grand Valley State University**

(Co-Authors: Dr. Matthew E. Hart)

“Synthetic Approach of A Thyronamine Known as the Urea Compound”

The research presented in this poster continues the synthesis of thyronamines, mainly focusing on the urea compound. A general synthesis of the urea compound has led to the production of multiple urea derivatives. However, the incentive of these multiple derivatives arose from the significance of the initial effect the general urea had on the trace amine-associated receptor (TAAR1). These newly developed urea compounds will help with the goal of our research in further studying the TAAR1 receptor and the binding regions of this receptor. Also, we hope that this will lead us to the ultimate goal in developing a pharmaceutical drug to help patients with hyper/hypothyroidism.

75. James Bour, Chemistry**Hope College**

(Co-Authors: Jacob C. Green, Jeffrey B. Johnson)

“Mechanistic Exploration of Palladium Catalyzed Beta-Arylative Elimination of Triarylmethanols”

Due to their inert nature, carbon-carbon δ -bonds are typically unutilized in synthetic processes. However, metal catalysts such as palladium have been shown to activate these inert bonds. One example is the palladium catalyzed β -arylative elimination of triarylmethanols (Miura et. al. J. Org. Chem., 2003, 68, 5236). This project involves investigation into factors affecting the aryl elimination in the aforementioned reaction. Product analysis of electronically disparate aryl halides has shown that not only does the aryl halide participate in the reaction before the elimination, but that it also affects the electronic nature of the metal center enough to provide elimination selectivity in multiple aryl group systems. These data represent progress toward elucidation of the electronic nature of the metal center, which is ultimately useful towards hypothesis driven methodology development.

76. John LaGrand, Chemistry**Calvin College**

(Co-Authors: Carolyn Anderson, Mitch Groenenboom, Emily Rhude)

“Efforts Towards the Synthesis of Amino-Substituted beta-Iodo N-Alkenyl Pyridones”

The N-alkyl pyridone motif has garnered significant interest in the synthetic community, as it is found in naturally occurring and pharmacologically important structures. The Anderson research group has recently disclosed several methods for transforming O-propargyloxypyridines into N-alkyl and N-alkenyl pyridones in the presence of LiI. Previously these migrations had been performed with substrates in which R is an alkyl chain. The dense core of orthogonal functionality found in beta-iodo N-alkenyl pyridone, renders it an important building block for the synthesis of other complex pyridone targets. Adding a nitrogen substituent to this scaffold is expected to significantly extend the range of potential targets that will be accessible.

77. John R. Strikwerda, Chemistry**Calvin College**

(Co-Authors: Eric X. Yu, Roger L. DeKock)

“Atomic size, ionization energy, polarizability, asymptotic behavior, and the Slater-Zener model”

Atomic size is a qualitative concept, fundamental to the study of chemistry. In our work we employ the Slater-Zener model to derive the relationship between the effective radius of an atom and its average valence ionization energy. We find that along a given row in the periodic table, these semi-empirical radii correlate linearly with theoretical Hartree-Fock radii, semi-empirical radii derived from static dipole polarizability, and empirical covalent radii.

"Extent of Reaction"

Nearly 100 years ago de Donder introduced the valuable term "extent of reaction". We build on that work by defining the concept of extrema for an arbitrary chemical reaction, $a A + b B \rightleftharpoons y Y + z Z$. There is an extremum for every reactant and every product in the reaction. The central equation is reaction extrema = $-n/v$, where n represents the initial molar amount of the entity v is its stoichiometric number in the chemical reaction. The extrema for the reactants are positive values (or zero for no initial reactant present) and the least positive of these is the reaction extremum to the right. The extrema for the products are negative (or zero for no initial product present) and the least negative of these is the reaction extremum to the left. We assign the name Reaction Extrema to these extremum values and demonstrate how they comprise an important pedagogical tool for a quantitative understanding of chemical reaction stoichiometry, particularly in regard to limiting reagents.

"The role of textbooks: does the course content or faculty member matter?"

The role of the textbook in college chemistry courses can be evaluated from two perspectives: How do students use the chemistry textbook and how do chemistry professors integrate the textbook into the course? It is also reasonable to think that the role played by the textbook might differ because of the course content. To determine the role the textbook played for faculty and students in general chemistry and organic chemistry, a semi-structured interview protocol was developed and refined by both researchers. Faculty were interviewed by one researcher and students by the other. Each group of interviews, faculty or student, were analyzed and themes emerged. A set of common themes were identified and used to analyze the pooled data. Similarities and differences between faculty and students perceptions of the role of the textbook and the role of the textbook in different courses have been identified. It is hoped that these results will help faculty determine if the money you spent on textbooks is worth the investment.

(Co-Authors: Catherine M. Calyore, Jeffrey B. Johnson)

"Nickel Catalyzed Direct Addition of Diorganozinc Nucleophiles to Substituted Phthalimides"

The direct addition of diorganozinc nucleophiles to a range of N-substituted phthalimides has been observed when a catalytic amount of $Ni(COD)_2$ and triphenylphosphine ligand are used in the reaction. Results have shown that aryl substituted N-phthalimides with alkyl and electron deficient groups consistently produce yields around 80%. Diorganozinc reagents used in the reaction include commercially available diethyl zinc, as well as reagents prepared from a wide range of substituted aryl bromides that are utilized without purification. Twelve diarylzinc nucleophiles have been successfully used in the reaction, while eleven different N-substituted phthalimides have been used. Current work is being done to further expand the complexity of substituents as well as work to incorporate saturated backbones into the reaction.

(Co-Authors: Jessica Priebe, Emily Jutkiewicz, James Woods)

“Characterization of Cholinergic Receptor Agonists Nicotine and Arecoline Using Drug Discrimination”

Smoking is the leading cause of preventable death worldwide partly due to the addictive properties of nicotine. Moreover, nicotine’s discriminative stimulus property makes it easy to relapse after smoking cessation. In this study, the discriminative stimulus property of nicotine was examined by using the two-key and three-key drug discrimination paradigms. Two-key discrimination required training rats to discriminate between nicotine and saline or arecoline - another acetylcholine (ACh) receptor agonist, and saline while three-key discrimination required rats to discriminate between nicotine, arecoline, and saline. Two-key discrimination tests performed on two sets of six rats showed that nicotine does not generalize to arecoline while arecoline may generalize to nicotine at high doses. Varenicline and carbachol were also tested in this two-key discrimination paradigm and results concur with previous findings that they are a nicotinic partial agonist and a muscarinic agonist, respectively. These results also suggest that arecoline may be a muscarinic and nicotinic agonist at high doses while nicotine is solely a nicotinic agonist. A relatively new method of drug discrimination, three-key discrimination, was studied. While still in the initial stages, preliminary findings with three-key discrimination support the validity of the method.

(Co-Authors: Samuel Tzou, Vijay Satam, Kimberly Brien, Pravin Patil, Balaji Babu, Matt Gregory, Michael Bowerman, Mia Savagian, Megan Lee, Yang Liu, Joseph Ramos, W. David Wilson, Shicai Lin, Kostantinos Kiakos, John Hartley, and Moses Lee)

“Complete Analysis on the Two Base Pair Sequence Recognition by Hx (p-Anisylbenzimidazole)•Pyrrole and Hx•Imidazole Pairings”

Pyrrole (Py) and Imidazole (Im) polyamide analogs of distamycin are small molecules that bind in the minor groove at specific sequences of DNA and regulate gene function. Despite their potential in drug discovery or as tools in molecular and cell biology, their usefulness is limited by their ability to enter cells and concentrate in the nucleus. There is thus an effort to develop polyamides that are trackable in cells. Thus there is an immediate need for the design of novel heterocyclic DNA sequence cognitive units that exhibit all the positive qualities as Py and Im, and be inherently fluorescent. Our group has recently published a novel class of hybrid Hx-amides, which contain a fluorescent p-anisylbenzimidazole or Hx group. Pairing of Hx with PI, PP and IP provided evidence that it mimics “PyPy” in recognizing two contiguous base pairs in a similar way as polyamides. To complete our examination of the Hx functionality, the remaining molecule in this series, HxII, was successfully synthesized. The DNA binding properties of HxII will be reported along with a discussion on sequence recognition by Hx/polyamide pairings and gene control.

(Co-Authors: Adam C. Boyden Michael T. Peruzzi Eric J. Werner Shannon M. Biros)

“Investigations into a lovely world of multi-faceted chelating agents: potential applications to medical imaging, nuclear remediation, and fluorescence”

The research presented in this poster was spurred from long time developments in nuclear waste remediation. The current TRUEX process used to treat nuclear waste utilizes individual carbamoylmethylphosphine oxides (CMPO's) to chelate radioactive lanthanides and actinides. Our goal is to modify the structure of the current CMPO's by linking them together with a tripodal “cap”. The resultant multidentate ligands can also be modified to present different types of dative bond donors to the metal center. Possible target applications for these compounds include the treatment of nuclear waste, safer medical imaging agents and fluorescent materials.

(Co-Authors: Jacqueline Peacock, Ph.D. and Matthew Steensma, M.D.)

“Effects of Doxorubicin on Neurofibromatosis Type 1 Associated Malignant Peripheral Nerve Sheath Tumor cells”

Neurofibromatosis type 1 (NF1) is a hereditary disorder caused by mutations in the neurofibromin 1 (NF1) gene. The NF1 gene encodes a tumor suppressor protein, neurofibromin, a negative regulator of the Ras signaling pathway. Patients with NF1 develop benign tumors called neurofibromas and have an elevated risk of malignancies including malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs are often treated with Doxorubicin (DOX), an anthracycline chemotherapeutic drug. Resistance to this drug is a common development in patients with the disease. Two experimental approaches were taken to observe the effects of DOX treatments on MPNST cells using three cell lines derived from human MPNSTs, likely pretreated with DOX. In vitro experiments were performed to determine the direct effects of DOX on the living cell count, cellular apoptosis, and proliferation using MTT, BrdU, and TUNEL assays, respectively. A 9-week study was carried out to induce resistance within the cells using long term, low dose treatments of DOX to determine the cellular changes within cells undergoing a resistant transformation. Analysis of the data indicated that there might be a relationship between increased doses of DOX and a decrease in living cell count, though additional analysis is necessary for proliferation and apoptosis effects.

85. Matthew Haveman, Chemistry**Calvin College**

(Co-Authors: Prof. Douglas A. Vander Griend)

“Modeling Complex Solutions: Towards a Web Based Tool”

Sivvu is a program written in Matlab that is used to model how solution phase molecules bind, equilibrate, and absorb light in a UV-vis spectrometer. In order to do this, many absorbance curves from a progression of solutions with changing compositions are measured and input into the program, which can then generate the equilibrium constants that best reproduce the data. In addition, Sivvu calculates the wavelength dependent color of all the chemical species that formed in any of the solutions. Sivvu is a powerful analysis tool, but its use is limited by the necessity of having to purchase a full Matlab license in order to run the program. In light of this limitation, the code was recast so that it could be compiled as a stand-alone executable. The next step is to deploy Sivvu to the web as a Java application.

86. Michael J. Lubben, Chemistry**Calvin College**

(Co-Authors: Douglas A. Vander Griend)

“Spectrophotometric Study of the Metal Complexes of a Tripodal Ligand”

The tripodal ligand tris[3-(2-pyridyl)pyrazol-1-yl]hydroborate (L-) has been shown to self-assemble with Co(II), Mn(II), or Zn(II). This study also examines complexes with Ni(II). Previous work by Prof. Michael Ward of Sheffield University shows that Co(II) crystallizes from methanol as a 1:1 complex with L-, whereas Mn(II) and Zn(II) crystallize as 4:4 complexes (M4L4). For our work, these systems are studied in solution via spectrophotometric titrations and electrospray mass spectroscopy. Equilibrium Restricted Factor Analysis of the absorbance data with the program Sivvu helps to model the self-assembly process and reveals that for the Co(II) and Ni(II) systems other assemblies do indeed exist in solution. Furthermore, no evidence of a solution phase M4L4 supramolecular complex has been found. This is perhaps because the 4:4 complexes are only stabilized in the solid state.

87. Michael Peruzzi, Chemistry**Grand Valley State University***“Synthesis of Novel Tripodal CMPO Compounds for Heavy Metal Chelation”*

The chelation of heavy metals is of great importance due to the wide variety of applications, such as nuclear waste remediation, MRI contrast agents, and chelation therapy. Carbamoylmethyl phosphine oxides (CMPOs) have been shown to be potent bidentate chelating agents in the TRUEX nuclear waste remediation process. However, many metals require a greater denticity for efficient chelation. Our lab's current interest lies in preorganizing these ligands with a tripodal cap to produce more efficient and selective binding agents by taking advantage of the chelate effect. By derivatizing these tripodal CMPOs, we hope to increase these chelating agents' selectivity and affinity for f-series elements as well as the variety of their applications. Current efforts toward the synthesis of these compounds will be described.

(Co-Authors: Kimberly Brien, Pravin Patil, and Moses Lee)

“A Building Block Approach: A New Way of Thinking about Polyamides and Their Impact on DNA Sequence Recognition II.”

Polyamides bind strongly to DNA sequences, including gene sequences involved in DNA replication and transcription of cancerous cells. The pyrrole and imidazole units in the polyamides bind to Adenine/Thymine and Guanine/Cytosine base pairs, respectively. Hx units, derived from Hoechst 33258 behave like two consecutive pyrrole units and bind A/T base pairs. The purpose of this study is to extend the DNA sequence recognition capability of Hx polyamides by incorporating the Hx unit close to the C-terminus end of the molecules. The goal in designing such “building block” Hx polyamides is to optimize the curvature and position of Hx with respect to sequence selectivity and binding affinity. The synthesis and DNA binding properties of two of such Hx polyamides will be described.

(Co-Authors: Laurie Witucki, William Schroeder, Roderick Morgan, Robert Smart)

“Synthesis of Novel Antimicrobial Agents Containing Peptide Bonds”

Antibiotics, produced naturally by microorganisms, have been used for decades in the battle against pathogenic microbes. Bacterial resistance to antibiotics is an ongoing medical issue throughout the world. In an effort to produce novel synthetic antimicrobial agents, amide bond synthesis techniques were used to affix an aliphatic carbon chain and an amino acid residue to an aromatic scaffold. Solution phase organic synthesis was utilized. Thin layer and column chromatography were used to determine reaction completion and purify products, respectively. Infrared and ¹H NMR spectroscopy were employed to characterize the structure of the molecules. The synthesized compounds were assayed for antimicrobial activity using *E. coli* (gram-negative) and *S. aureus* (gram-positive) bacteria. For multiple compounds, bioassay data suggested antimicrobial activity against gram-positive bacteria, and further analysis suggested one compound's low affinity for binding to human serum proteins. Further synthesis is targeted at dipeptide bond synthesis in an attempt to increase this compound's zone of inhibition.

(Co-Authors: Alyssa McNamara and Regina Stevens-Truss)

“Suramin Distinguishes Between the Calmodulin Binding Sequences of Inducible and Neuronal Nitric Oxide Synthase”

Nitric oxide synthase (NOS) catalyzes the production of nitric oxide ($\bullet\text{NO}$) from L-arginine in several tissues in the body. Nitric oxide is believed to play a critical role in cardiovascular disease, stroke, and cancer. For this reason, selective inhibition of NOS isoforms is currently under investigation for their potential as targets in drug design. All known isoforms of NOS require binding of calmodulin (CaM), a calcium binding protein ubiquitously expressed in eukaryotes, for activity. The interactions among the NOS isoforms and CaM vary, and therefore pose a good place to study in differentiating these enzymes. We have previously demonstrated that suramin, a polysulfonated naphthylurea, can distinguish between the CaM binding region of the inducible NOS and the neuronal NOS. When bound to NOS, suramin inhibits its activity by preventing CaM binding and activation. Suramin's inhibition was found not to be time dependent, and IC_{50} values were found to be $\sim 8 \mu\text{M}$ and $\sim 120 \mu\text{M}$ for the inducible NOS and neuronal NOS, respectively. SDS-PAGE and Western analyses demonstrated that suramin is able to inhibit the interaction between NOS and CaM. AutoDoc analysis further demonstrated that suramin most likely interacts with a portion of the NOS molecule believed to be crucial to CaM's interaction. These studies implicate suramin as a potential lead compound.

“MACROMOLECULAR ASSEMBLIES FOR GAS STORAGE FROM DYNAMIC BONDS”

Three-dimensional organic polymers are important materials due to their ability to absorb various gasses including N_2 , CO_2 , and H_2 . Organic polymers are of particular interest to NASA, due to their light-weight nature, high surface area, and potential as a solid-state fuel source. This project focuses on developing new three-dimensional polymers, which fall into a particular class of compounds called covalent organic frameworks (COFs). These COFs are typically prepared using high efficiency reactions in high yields. However, due to their large molecular size, characterization by traditional techniques is difficult if not impossible. This project is designed to elucidate how these COFs aggregate in the solid-state by synthesizing a subunit of a COF. The subunit will allow for greater analysis by NMR, IR, and X-ray crystallography.

(Co-Authors: Dr. Jeffery Johnson, Jessica Simmons)

“Investigating the Scope of Decarbonylative Cross Coupling Reactions of Cyclic Imides Implementing a Nickel Catalyst”

Previous research established that a decarbonylative nickel-mediated cross coupling reaction of N-substituted cyclic imides with diorganozinc reagents is possible with many different functional groups in the R position of the cyclic imide. The scope of the reaction was further expanded with a wide variety of diorganozinc reagents (R1). (J. Org. Chem., 2011, 76, 3588). The focus of this particular research is to expand scope of the reaction by exploring the possibility of a saturated or half-saturated backbone of the imide. In order to find the ideal reaction conditions to promote the cross coupling it was tried under a variety of reaction conditions with varying ligands and solvents.

(Co-Authors: Dr. Elizabeth Jensen)

“A Colorimetric, Paper-Based Test for Deltamethrin and Permethrin”

Insecticide treated bed-netting plays a key role in the defense of the spread of malaria. Such nets, however, only offer the user optimal protection if the incorporated insecticide is present in a large enough concentration to deter mosquitoes. While manufacturers of these nets often provide a range of years, such as three to five years, that a net may provide a user with effective protection, a range of two years is rather considerable when dealing with a disease potentially as impactful as malaria. There is a need for users of such nets to be able to easily and cheaply determine if their net still has sufficient insecticide present. In this study we attempt to identify a qualitative test to detect the presence of two common insecticides incorporated into bed-netting, deltamethrin and permethrin. Such a test could be adapted for use on a paper analytical device (PAD), and might be made quantitative as well. This type of device would allow for cheap and widespread distribution of a user-friendly method to determine if an insecticide was present on a bed net. While we were not able to identify a specific colorimetric reaction for both insecticides, our study examines over ten different tests that serve as a foundation for future study and progress in this area.

(Co-Authors: J. Grant Fahey, Serita M. Nelesen, John T. Wertz)

“HTMAD: Software Design for MALDI-TOF based Microbial Community Analysis”

In the field of microbiology, identifying and comparing species of bacteria from large communities is commonplace. Physically isolating individual microbes and sequencing all or part of their genome is an effective method, but time-consuming and expensive. Proteome analysis via mass spectroscopy can be effective and accurate enough for preliminary analysis, as well as much cheaper and faster. Our novel software program, HTMAD (pronounced hat-mad), is designed to take large amounts of data from MALDI-TOF mass spectrometry and give it a framework for analysis. This includes spectrum graphs, distance matrices, and comparison trees, and can be used to compare individual bacterial samples or whole communities. Though the program is still in developmental stages, it has already been useful in microbiological research at Calvin and we believe could eventually be a useful tool for researchers on a greater scale.

(Co-Authors: Andrew Borgman, Daniel Hodges, Lisa Kefene, Jenea Chesnic, Alison Ruhe, Mark Neff)

“Informatics and the canine model for genetic analysis of complex diseases”

Our lab applies large-scale genetics (genomics) to gain unbiased access to the biology of (i) evolution and adaptation, (ii) brain and behavior, and (iii) complex disease risks. We study natural variation in a unique model organism, the domestic dog, by recruiting and analyzing DNA samples from privately owned pets. Informatics, computation, and applied math are important aspects of this research. We manage and analyze terabytes of digital data; integrate and curate veterinary health records; interface with owners, breeders and clinicians to track long-term health outcomes; logistically ship and receive thousands of DNA collection kits to voluntary participants across the country; and of course, manage with a tailored LIMS the data streams and wet bench workflows that advance our research. Here we describe the computational infrastructure, collectively called Fidelis 3.0, that our team has developed over the past year.

(Co-Authors: Dr. Brian Yurk, Dr. Aaron Putzke, Dr. Airat Bekmetjev, Joseph Adamson, Kristen Bosch, Bennett Riddering, Daniel Faghihnia, Alicia Castillo)

“Predicting Insect Development in Changing Climates: Bean Beetle Phenology Modeling”

The success of a species within their environment is largely dependent upon its developmental timing. In insects, temperature plays a key role in determining rates of growth and development for various life stages including the embryonic stage. Thus time-lapse photography was used to measure the timing of this stage for bean beetles at various temperatures in the laboratory. Mathematical models were developed to fit these data, and statistical analyses were performed to provide a deeper understanding of the relationships between developmental timing and temperature in insect populations.

(Co-Authors: Troy Wymore and Agnieszka Szarecka)

“Multiple Sequence Alignment and Phylogeny of Class D Beta-Lactamases”

Bacterial resistance to antibiotics is currently one of the gravest threats to public health, and a constantly growing challenge in medical practice. Beta-lactamases (enzymes inactivating beta-lactam antibiotics) underly the major mechanism of bacterial resistance, and their four classes: A, B, C, and D, evolve rapidly – in some cases able to acquire a new functional profile via a single amino acid mutation. Among beta-lactamases, class D (also known as OXAs) are the most diverse both in sequence and in substrate selectivity. In particular, the OXA group harbors several enzymes with carbapenemase activity, namely the ability to hydrolyze the newest line of beta-lactam antibiotics. In this study, we created multiple sequence alignments (MSA) and phylogenetic trees of several subgroups of the OXA family that have been proposed in the literature. We show the results of our MSA analysis combined with available structural and biochemical information that help establish sequence-based relationships among the OXAs, as well as the correlation between subgroup-specific residues and carbapenemase activity.

(Co-Authors: Professor Loren Haarsma and Professor Serita Nelesen)

“Pykaryote: A Computer Model of the Evolution of Complexity in Digital Organisms”

Pykaryote is a computer model of the evolution of biological complexity. In the program, digital organisms gather resources from an environment and use them to build proteins and protein-complexes which can speed up the gathering of resources. Each organism has a digital genome that determines which resources it gathers and which proteins it builds. It then reproduces according to its “fitness”, which is based on the amount that it has gathered. Each new offspring’s genome has a chance to experience point mutations, deletion or insertion of genes, or genome-doubling. The purpose of the model is to study how changes to the environment, mutation rate, and protein functionality enhance or inhibit the evolution of complexity. Dozens of settings in the program affect the rate of the development of complexity in the simulated population. This summer, additional code was added that enables a user to run many simulations with different settings and to view the trends in the developing populations. In order to allow the simulations to progress far enough to be interesting, the new code distributes the computation through the Calvin College Unix Lab. Pykaryote was shown to react as expected to changes of settings. Optimal values of some settings for the development of biological complexity were found.

“Smarter Searching Through Knowledge Representation”

This project demonstrates the viability of knowledge representation technologies for enhancing a website search experience. Knowledge representation is a branch of artificial intelligence research focused on storing data in structures that allow computers to reason and infer. Such structures are the basis for data formats that make up the Semantic Web, a layer of the internet for free data interchange, as envisioned by Tim Berners-Lee. The objective of the research project was to use an existing dataset of individuals from the Bible to create an enhanced search experience on the Christian Classics Ethereal Library (CCEL) website, a digital repository of public domain Christian texts. The project involved learning and using semantic web knowledge representation tools, as well as Python, HTML, CSS and JavaScript for web development. The result of this project is a website that demonstrates use of semantic web technology to augment and enhance the CCEL search experience.

“Real-Time Fluid Flow: Solving the Navier-Stokes Equations for Interactive Simulations”

The Navier-Stokes equations give a mathematical description of the behavior of incompressible fluids, including viscosity and turbulence, and are widely used in engineering, computer graphics, and animation. This project explored algorithms and constraints needed to code an interactive, real-time simulation of fluid-flow. The purpose of this research was primarily didactic---the overall algorithmic design was taken from published work, with our goals being three-fold: (a) to study the numerical solution of the Navier-Stokes equations, (b) to understand trade-offs of various optimization techniques to enable real-time interaction, (c) to create a transparent, extensible object-oriented code-base in C++ for future work in fluid flow and computer graphics.

“Effects of a Fungal Endophyte on Resource Allocation in the grass Lolium arundinaceum”

Neotyphodium is a fungus that grows in above ground tissues, also known as tillers, of grasses such as Lolium arundaceum. The fungus reproduces by growing into the seeds of the plant. This sort of symbiont reproduction is called vertical transmission, as it is transmitted to the next generation of the plant. The Neotyphodium and its relationship with the L. arundaceum has been thought to be a defensive mutualism but an alternative relationship known as sexual parasitism has been proposed. Measuring the relative sizes of the reproductive and vegetative tissues from within the grass, as well as counting the amount of overall pollen produced by infected versus uninfected plants, can give us insight on the relationship between the fungus and its host. By studying the resource allocation of male and female reproductive tissues we can find out more information about this system and more accurately determine if it is a defensive mutualism or a sexual parasitism.

(Co-Authors: Dr. Robert Hollister and Tim Botting)

“The Response of the Sedge Genus Carex to Warming”

The Arctic is experiencing significant changes due to global climate change. A study site was established on the North Slope of Alaska outside the town of Barrow in 1994 and a similar site was established outside of the warmer, more southern town of Atqasuk in 1996. The towns both contain wet sites that consist of 24 control plots and 24 experimental plots. The experimental plots are passively warmed 1-3 degrees Celsius using open top chambers. This study used data that was collected in the summer of 2012 to analyze the response to long-term warming of the dominant sedge Carex at the two wet sites. The measurements made were changes in cover, the timing of flowering, growth, and reproductive effort. We found in response to warming that Carex flowered earlier, grew more, and allocated more resources to reproduction resulting in an increase in cover. Furthermore this trend was greater at Barrow than at Atqasuk. These results are consistent with other warming studies conducted across tundra landscapes and suggest that Carex will continue to become more dominant with climate change.

(Co-Authors: Nate Haan and Dr. David Warners)

“Native Plant Propagation, Ecological Restoration, and Management”

Propagating native plants from locally collected seeds provides diverse, relatively inexpensive plant material for landscaping and restoration projects. Native plants convey a host of ecosystem services such as nutrient capture, prevention of soil erosion, water purification, and reduction of run-off. During the first few years after an area has been restored, some management activities are typically needed to ensure proper flourishing. Our goal was to investigate ideal growing conditions of the seedlings from several heavily used native species. We experimented with different combinations of growth media and fertilizer levels to determine the effects on seedling height. In addition, we have been managing and maintaining Calvin’s native tree nursery and some previously established native habitat plantings on campus. We have also planted thousands of seedlings into a large rain garden behind Calvin’s ecosystem preserve house.

“Mantled howler monkey (Alouatta palliata) vocalizations as an intergroup spacing mechanism on Ometepe Island, Nicaragua”

Howler monkeys live in territorial social groups and possess specialized hyoid bones that allow males to emit unique vocalizations critical to their social behavior. This study aimed to understand the function of roars of mantled howler monkeys (*Alouatta palliata*) on Ometepe Island, Nicaragua. I predicted the roar, used by males, would serve as an intergroup spacing mechanism. I conducted 30 minute bouts of instantaneous focal sampling, where I observed responses made to the roar vocalizations of within-group and external males. Adult females and juveniles responded to 40.0% and 37.8% of roars emitted by within-group males, respectively, while males responded 60.0% of the time. Males, females and juveniles rarely responded directly to roars produced by external males. I conclude that males respond more readily to roars made by group members, and that their responses function to augment in-group vocalizations. Overall, roar production and responses to them appear to enhance social group cohesion and intergroup spacing.

105. Geneva Langeland, Ecology and Evolution**Calvin College**

(Co-Authors: Dr. Randy Van Dragt and Dr. David Warners)

“Sustainability @ Calvin.edu”

In 2011, Calvin College received a \$575,000 grant from the Cargill Foundation in support of environmental programs at the college. A small portion of the money was set aside to fund a thorough study and documentation of environmental and sustainability initiatives across the college. Research conducted during the summer of 2012 helped draw together an account of past and present sustainability activities at Calvin. This was done in order to acquaint the campus community with Calvin’s legacy of environmental stewardship; unite those involved in campus sustainability efforts; and gain insight into how the school can best grow its stewardship commitment in the future. A wide range of initiatives were documented, from an influential 1980 book on natural resources, to a 2004 LEED construction project on the campus ecosystem preserve. Such information will be synthesized into a final report, which will also include recommendations for employing the Cargill grant funds, to be published in December 2012.

106. Holly Vander Stel, Ecology and Evolution**Hope College**

(Co-Authors: Austin Homkes, Jeffrey Corajond, James Tufts, Kenneth Brown, Jianhua Li)

“Phylogenetic and phytochemical studies of Apios (Fabaceae).”

Apios is a perennial herbaceous plant genus of 6-10 species, all of which have pinnately compound leaves with milky sap in the petioles and young shoots. Some species have edible tubers that contain high level of protein and isoflavones (e.g., genistein) that may be beneficial to human health and cancer treatment. Geographically, Apios shows a disjunct distribution between eastern North America and eastern Asia. In this study we aimed at examining interspecific relationships of Apios, estimating the time of formation of the intercontinental disjunction, and determining whether genistein occurs in all species of Apios. Our results suggest that the two North American species are more closely related to each other than either is to the Asian species, and their ancestral populations may have migrated from Asia. All species tested produce genistein, which occurs mostly in the tubers.

107. Jamin Wieringa, Ecology and Evolution**Hope College**

“Role of Plant Hormones in the Signaling the Production of Alkaloids by an Endophytic Fungus, Neotyphodium”

Infection of tall fescue with an endophytic fungus conveys resistance to the host plant. Although the response is similar to that of plant hormones, there is no evidence that the fungus is reading a hormonal signal from the host plant. With all of the hormones tested none indicated that it is a signal. With this study there is evidence that direct damage to the fungus stimulates an increase in the production of alkaloids. This increase in the production of alkaloids helps prevent herbivory in the plant and although this also helps the plants, the host plant is not signaling the fungus that it is being damaged.

(Co-Authors: Eric Snyder)

“The Effectiveness of Constructed Wetlands”

Wetland construction represents a vital tool to increase the number and extent of wetlands in the United States. However, there is uncertainty as to how effective constructed wetlands actually are and if they continue to function efficiently as they age. This study’s objective was to evaluate the constructed wetlands on Grand Valley State University’s Allendale campus. The wetlands studied were constructed in both 2009 (n=3) and 2011 (n=5), not specifically to mitigate for wetland loss; rather they are a proactive attempt to reduce erosion from excessive stormwater runoff in the GVSU ravines. We compared these to wetlands constructed in the mid 1980’s (n=3) located at the near-by Bass River Recreation Area. Specifically, aquatic macroinvertebrates were sampled throughout May 2012, following rapid bioassessment protocols used by the Michigan DNR, while water chemistry parameters (specific conductivity, pH, dissolved oxygen, temperature, turbidity, riparian coverage, chloride, and total dissolved solids) were measured bi-weekly throughout the summer. The macroinvertebrate Family richness and diversity were significantly different ($p < 0.05$, ANOVA) and values for each metric ranged from 21.3, 20.67, and 6.6 and 2.31, 2.13, and 1.01 between 1980’s, 2009, and 2011 sites, respectively. These differences in the insect community assemblages were evident in a multivariate test as well (NMDS). Thus, at a community level there was a rapid improvement in the aquatic insects in just three years suggesting these constructed wetlands will rapidly develop into healthier communities.

*“Influence of Fungal Endophytes on Insect Herbivore Defense in Canada Wildrye (*Elymus canadensis*)”*

In the past few decades, the relationship between *Epichloë* and *Neotyphodium* fungal endophytes and cool season grasses has gained increasing attention from botanists, mycologists, and ecologists alike. With hosts displaying traits that increase their competitive abilities and fitness when infected but occasionally suffering from the taxing fungal reproductive structures, an interesting relationship that is both mutualistic and parasitic can be observed. To determine how this dynamic association affects the variation of *Epichloë* infection frequency in *Elymus canadensis* populations across North America, seed samples from multiple populations were taken and tested for endophyte presence via PCR and gel-electrophoresis. Despite predictions, no significant relationship between infection frequency and latitude or longitude could be found, perhaps due to differences in biotic and abiotic factors between tested populations. *Rhopalosiphum padi* and *Spodoptera frugiperda* larvae were utilized to test how a diet of *Epichloë elymi* infected *E. canadensis* blade tissue affected insect herbivore performance. It was believed that since the *E. elymi* endophytes were capable of producing peramine and an intermediate ergot alkaloid that both insect species would suffer negative effects. While the *S. frugiperda* larvae showed less accumulation of dry weight when fed infected tissue, *R. padi* reproduction was not affected by such a diet. The reason for these results may be due to differences in sensitivity to peramine by the insect species.

110. Kristen Zemaitis, Ecology and Evolution**Grand Valley State University***“Sea Turtle Nesting in Guanacaste, Costa Rica: Effects of Temperature and Sea Level Rise”*

Climate change is altering sea level in coastal ecosystems, which will increase an additional 0.6 m by 2100, detrimentally affecting the availability and quality of sea turtle nesting habitat. I used a World Wildlife Fund protocol to monitor temperature and slope of beach habitat used by nesting sea turtles, including green (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), leatherback (*Dermochelys coriacea*), and hawksbill (*Eretmochelys*), on San Miguel Beach, Guanacaste, Costa Rica. During July and August, 2012, I measured beach slope with an Abney level on 5 m transects located in three 100 m sections of beach (N = 20 per section). To model the inundation resulting from predicted sea-level rise, 0.6 m was subtracted from elevation values, resulting in a loss of 3% of the Guanacaste beach area. Beach topography is unstable within and among nesting seasons, and modeled to change in complex ways in response to storm events and human disturbances like tourism, off-road vehicle use and beachfront development. These combined effects will harm sea turtles relative to their body size and timing of nesting. Current beach temperatures support normal sea turtle development, but if the current trajectory of temperature increase continues, feminizing effects will occur by 2100, and lethal temperatures will be reached soon thereafter. If beach temperatures increase faster than modeled in the current study, feminizing and lethal effects will occur even sooner.

111. Leah Sienkowski, Ecology and Evolution**Calvin College***“Effectiveness of Paper Mulch as an Organic Weed Control Method in Lettuce Production”*

This study took place at Fat Blossom Farm, a small USDA certified organic farm in Allegan, Michigan. The scope of the project was a 150 foot square plot in which 100 heads of Pablo Batavian head lettuce was planted. Five replications of five lettuce heads composed each of four weed treatment methods. Weed control is expensive in organic produce production, but is essential to reduce the competition among lettuce and weeds for light, water, and nutrients.

112. Neil Gilbert, Ecology and Evolution**Calvin College***“Floral Inventory and Phenology at Flat Iron Lake Preserve”*

During the summer of 2012, I inventoried and monitored the flowering progress of the flora—particularly forbs (i.e., herbaceous flowering plants, discounting grasses)—at Flat Iron Lake Preserve, a Calvin-owned, sixty-five acre preserve in Oakfield Township. My research was part of an ongoing phenology study that has the goal of documenting year-to-year variation in flowering periods. Once many more years of data have been accumulated, future researchers will examine the results for trends, particularly ones that could be associated with climate change. Between May 30 and August 2, I identified 221 species of forbs on the preserve. The only previous year with sufficient data for comparison was 2010; in that year, on average, forbs flowered three days later than in 2012. Though trends will not become apparent until several more years of standardized data are collected, it is clear that Flat Iron Lake Preserve hosts floral diversity of statewide significance, as measured by standard indices of botanical quality.

(Co-Authors: Cristina Portales, Elizabeth Schultheis, Tomomi Suwa)

“Allelopathic effects of Alliaria petiolata on rhizobia and its implications for native legume performance”

Invasive species, such as *Alliaria petiolata* (Garlic Mustard), can threaten native communities by outcompeting native species, reducing biodiversity, and changing community composition. *A. petiolata*'s success is partially explained by its negative impact on a common soil mutualist for native plants, mycorrhizal fungi. Its allelopathy reduces the performance of this key mutualist, thus indirectly decreasing the performance of their plant mutualist partners. It is possible that these allelopathic effects may extend to other soil biota. In our study, we test if *A. petiolata* allelochemicals have similar negative effects on another important symbiotic organism: rhizobia, a nitrogen fixing bacteria that forms symbiotic relationships with legumes. This was tested by both greenhouse and laboratory experiments. In the greenhouse we manipulated the presence/absence of rhizobia and used stockings made from aboveground plant tissue of *A. petiolata* (GM), *Hesperis matronalis* (DR), *Trifolium pratense* (TR), and a blank control (C). The greenhouse experiment showed no negative effect of the *A. petiolata* on the growth of our focal plant, *Amphicarpaea bracteata* (Hog Peanut). In the lab we grew rhizobia strains with 3 concentrations of *A. petiolata* extract, made by soaking chopped up leaf and stem material in MAG media (0%, 50%, 100%). The lab experiment yielded a negative relationship between an increased concentration of *A. petiolata* extract and growth rates of rhizobia ($F=7.24$, $p=0.002$). *A. petiolata*'s competitive ability against *A. bracteata* may not be explained by its allelopathy on rhizobia mutualists.

(Co-Authors: Loren Haarsma and Becky Haney)

“Economic Growth, Wealth Inequality, Specialization and Trade in an Interdependent Society”

Our modern economy is highly interdependent – each industry depends on many others for materials, equipment, and distribution. Our computer program, SOCIETIES, is an agent-based economic model which simulates how this interdependence self-organizes over time as self-interested agents interact. Each agent gathers, trades, and consumes resources. Then they invent, make, and trade devices that speed up the extraction of resources. When all agents have identical attributes, each agent will specialize in gathering particular resources and making particular devices. They will then trade these with other agents to meet their needs. With this model, we can study how society as a whole acts differently as trading rules, resource properties, or agent abilities are changed. We have now added to this model the ability to have heterogeneous agent types interacting with each other. This enables us to study how unequal agent capabilities or trading power affects wealth inequality, economic growth, specialization and trade, with possible implications for our own economy.

(Co-Authors: Lucas Timmer and Dr. Matthew Heun (Principle Investigator))

“Including Energy in Economic Production Functions: An Emperical Analysis of Developing and Developed Economies”

Neoclassical economic growth theory attributes output growth to growth rates of capital stock and labor factors of production. Some economists use energy as an additional factor of production in the production function. We analyzed three functions that included energy as a factor of production: the Cobb-Douglas, CES, and LINEX production functions. For each of the production functions, three types of energy were considered: thermal energy, exergy, and useful work. Each of these production functions with an energy factor of production describes economic output for nine different countries categorized into four economic types: Developed (United States, Japan, United Kingdom), Developing (China, South Africa), Petrol (Saudi Arabia, Iran), and Malthusian (Zambia, Tanzania). The developed economies were modeled for the years 1980-2011 and the remaining economies were modeled for the years 1991-2011. Our research sought to analyze which energy including production function best describes global economies at different stages along the traditional economic growth path. In comparison to the Cobb-Douglas and LINEX production functions, the CES production function had the highest coefficient of determination (R-squared) values for the countries examined so far. Including energy increases the R-squared value in most cases.

“Infrared Photography in Forensic Science”

Infrared (IR) Photography can be used in Forensic Science in many different ways. It is capable of revealing blood stains in clothing, gunpowder residues, showing changes paper documents, and in surveillance cameras to capture details that regular cameras could not in poorly lit areas. The human eye can see any light in the range of approximately 400nm to 700nm. IR is considered any wavelength above 700nm to about 1200nm. There are several ways a substance may be affected by IR light. The substance could absorb the light which means the substance will remain or turn dark. It could reflect the light so the substance will become lighter. The substance could also transmit the light so the substance will “disappear” and whatever is beneath it will be seen. When using and IRUV camera a range of different filters are placed over the lens, which blocks out different wavelengths of light. Blocking out the wavelengths allows for substances to be seen more or less clearly depending on the filter. In this experiment blood stains and gunshot residue were looked at with IR photography. Once the right filter was found, the blood spatter and gunshot residue were very clearly seen on the different targets that were made. The PECA 914 filter for the Fuji IRUV camera was selected and the results showed that without any aid from the camera only the initial large blood stain was observed. Also the single bullet hole. After viewing the targets with infrared photography the original blood stain and the satellite spatter was observed. Also the gunpowder residue surrounding the original bullet hole was seen clearly. It seemed that the darker the target was and the more patterns it had the better the results of the experiment were. This is partly because on a light target you can already see the satellite spatter or gunpowder residue but with darker targets the substances are not visible and the IR photography allows them to be observed.

117. Owen Selles, Geography**Calvin College**

"Our Piece of Earth: Researching the Environmental History of Calvin College"

Environmental history is important for understanding the context of current ecosystems. This project has initiated an effort to write an environmental history of the Knollcrest campus and Calvin College Ecosystem Preserve. It involved in depth assessments of ecological and anthropogenic changes, which included land use practices, changes in ownership, various construction projects and landscape alterations. The product of this work will be an expanded manuscript that begins to lay out the structure and foundational information for an eventual book project.

118. Melissa Braun, Geology**Calvin College**

"Investigations of Late Cretaceous Paleocology: The Hell Creek Formation Exposed Near Ekalaka, SE Montana"

Vertebrate fossils have been collected from the Late Cretaceous Hell Creek Formation beds in SE Montana since the 1890's, but more recent investigations have permitted us to understand the ancient ecology represented in this sedimentary record more completely. Recent collection efforts by crews from the Burpee Museum (Rockford, Illinois) in this region also have revealed nearly-complete juvenile examples for several taxa, including *Tyrannosaurus rex* and *Triceratops horridus*. Large numbers of several turtle groups, including baenids and trionychids, as well as lowland forest plant remains, are surfacing and are providing us new insights into the ecology of Cretaceous rivers entering into a the large interior seaway of that age. Summer 2012 work included explorative collecting in areas not previously sampled, with new discoveries of hadrosaurian and ceratopsian remains, amplifying this growing database of Late Cretaceous biota.

119. John Morris, Immunology**Calvin College**

(Co-Authors: Anding Shen)

"Murr1 and JNK are not Contributing to HIV-1 Infection of Resting CD4+ T Cells co-cultured with Endothelial Cells"

Resting CD4+ T Cells cannot normally be infected successfully with HIV-1 in vitro but when co-cultured with Endothelial Cells (ECs), a high rate of infection is shown. Using intracellular staining methods, flow cytometry, and western blotting, we investigated what could make this possible. Two ubiquitous proteins, Murr1 and JNK have been shown to have an effect on HIV-1 infection of resting T Cells. We measured levels of Murr1 and phosphorylated JNK (p-JNK) in: resting T cells, activated T cells, resting T cells co-cultured with endothelial cells without IFN- γ (EC-) or with IFN- γ (EC+). In previous studies, increased levels of Murr1 have been shown to impede HIV infection but we determined that the levels of Murr1 were higher in Activated Cells, which are easily infected, than resting T cells or resting T cells co-cultured with ECs, thus showing that Murr1 has no effect on HIV-1 infection. We also observed that Activated T cells had the most p-JNK activity, and resting T cells co-cultured with EC+/- had similar levels as resting T cells alone. We also did not find any difference between then levels of p-JNK in GFP+ populations versus GFP- populations.

120. Elizabeth Hibma, Mathematics**Calvin College**

(Co-Authors: Elizabeth Hibma, Hwa Pyeong Kim, Jonathan Timkovich, Advisor: Professor Todd Kapitula)

“Unstable Eigenvalues in JS Matrices”

When eigenvalues for a matrix have positive real-valued parts, they are considered to be unstable. It is an interesting problem to determine the number of unstable eigenvalues for a matrix without actually computing its eigenvalues. In order to accomplish this task, the matrix must have some kind of structure; for example, it could be of the form JS , where J is a skew-symmetric matrix and S is a symmetric matrix. Without this structure, it can be almost impossible to complete this computation. We began our research this past summer by studying the relationship between eigenvalues of S and the eigenvalues of JS . We then studied the eigenvalue problem for alternating matrix pencils, which are polynomials in the eigenvalue parameter for which the coefficients alternate between skew-symmetric and symmetric matrices. We were able to determine relationships between the number of unstable eigenvalues for these pencils and the eigenvalue structure of one or two of the symmetric matrices in the given pencil.

121. Noah Davis, Mathematics**Aquinas College**

(Co-Authors: Dr. Michael McDaniel)

“Squaring the circle in hyperbolic geometry”

As Bolyai foresaw in 1840, squaring the circle could be accomplished in hyperbolic geometry, although it proved to be impossible in Euclidean geometry. For our summer research project, we developed the construction in the Poincare disk model of hyperbolic geometry and used our construction to prove that the circle and square must be constructed separately.

122. Yeon Hyang Kim, Mathematics**Central Michigan University**

(Co-Authors: Yeon Hyang Kim, Karleigh Cameron, Michael Gustin, John Holden and Stacy Siereveld)

“A lifted Haar basis”

Because of its localization property and fast transform algorithms, a wavelet basis expansion for a vector space has many applications. Special wavelets, known as second generation wavelets are useful in analyzing finite, non-periodic functions. We study the properties of a lifting operator that serves as a tool to construct these second generation wavelets. Using the first generation Haar wavelets, we construct second generation wavelets with higher vanishing moments. We show that these new wavelets form a Riesz basis for $L_2([0,1])$ and we compute the optimal Bessel bound.

(Co-Authors: Timothy Ramnarine and Derek Thomas)

“Analyzing interactions between Candida albicans and other microbes”

Candidiasis represents the fourth most frequent nosocomial infection both in the US and worldwide. *Candida albicans* is an increasingly common threat to human health as a consequence of AIDS, steroid therapy, organ and tissue transplantation, cancer therapy, broad spectrum antibiotics and other immune defects. These infections carry unacceptably high morbidity, mortality rates and important economic repercussions (estimated total direct cost of approximately 2 billion dollars in 1998 in US hospitals alone). The pathogenic potential of *C. albicans* is intimately related to the way this organism senses and reacts to its surrounding environment. *C. albicans* can grow as yeast cells, pseudohyphae or hyphae with its form being dictated by its surrounding conditions. The ability to form hyphae has been fundamentally linked to the disease causing potential of this organism. However, studies have focused on either *C. albicans* in isolation or whilst it alone is infecting a host. In conditions outside of the laboratory *C. albicans* is typically surrounded by, and occupying the host with, other non-related microbes that can be a significant part of the environment *C. albicans* is reacting to. Here we document preliminary studies investigating *C. albicans* interactions with several other microbes found in common environments.

(Co-Authors: Maria Eguiluz)

“Metabolic Modeling of the Genus Shewanella”

Shewanella represent a diverse group of aquatic bacteria that can respire using over 20 known compounds, including some toxic to other organisms (e.g., uranium and chromium). This genus contains organisms that could be used for bioremediation efforts. Genome-scale metabolic models of organisms represent an important computational tool in understanding how organisms will respond to genetic and environmental changes. We generated genome-scale metabolic models for 26 sequenced members of the genus *Shewanella* using the ModelSEED. As part of the model building, we focused on improving accuracy in two areas of metabolism: 1) accurate representation of a *Shewanella* specific LPS structure and 2) expansion and formalization of respiratory capabilities found in the genus. We created a *Shewanella* specific LPS carbohydrate backbone using all *Shewanella* carbohydrate backbone structures available in the literature. This was used to create the LPS biosynthesis reaction used in model generation, and is the most accurate representation of *Shewanella* LPS used in modeling to date. Modeling of respiration was improved by the modularization of common branching points in electron transport chains (ETCs) and expansion of the ETC to include 8 terminal electron acceptors. These models were then used to test electron acceptor use and to run comparative analyses of the models. Each model had on average 1158 reactions, 567 of which were active under aerobic conditions and 5 of which were autocompleted. A core model representing all shared reactions from sequenced *Shewanella* will be determined using comparative analyses of the individual models. This will lead to a better understanding of the metabolic capabilities of this genus.

“Investigating Bacteriophage Maintenance of Microbial Homeostasis in Termite Guts”

Viruses that infect bacteria (bacteriophage or phage) represent the most abundant infectious particle on earth, estimated to be at 10^{31} – 10^X more than the estimated global population of bacteria. In many environments, it appears the relationship between bacteriophage and their hosts is a predator-prey dynamic where the phage will replicate inside the host, rapidly killing it (lytic phage). This sets up an evolutionary “arms race” between the bacteria and the phage. However, in animal-microbial symbiotic systems there is little known about the role of bacteriophage. In these systems, there is a co-dependency between bacteria and the animal where lytic phage may be highly detrimental. These systems may favor temperate bacteriophage, which replicate as DNA-only prophage inside the host without killing it. Additionally, temperate phage provide the host with “immunity” against infection from other similar phage – a mechanism that may help to ensure a stable symbiotic system. Perturbations to the system, such as ingestion of antibiotics or environmental chemicals may induce the temperate phage into the lytic cycle, killing the host bacteria, producing a state of dysbiotic disease in the animal. Here, we use the termite *Reticulitermes flavipes*, several bacterial isolates from its hindgut, and genomic data from the first phage to be isolated from termite guts to begin to answer questions regarding the role of bacteriophage in maintaining homeostasis of the microbial symbiotic system.

(Co-Authors: Julia Hilbrands, William Burmeister, Arlene Hoogewerf)

“The Antimicrobial Effects of the Non-toxic Microbiocide SAFI”

SAFI is a proprietary non-chlorine solution that is manufactured in two formulations: copper sulfate and zinc sulfate. SAFI effectively disinfected water contaminated with *V. cholerae* and *E. coli* and is currently being used as a water treatment in Haiti, Rwanda, and Pakistan. There are roughly 48 million food borne illnesses in the US each year, and a recent outbreak was due to *L. monocytogenes* in cantaloupes. Our study was conducted to determine if SAFI could disinfect cantaloupe rinds contaminated with *L. monocytogenes*. Our tests showed significant reductions in *L. monocytogenes* numbers when the bacteria were suspended in water. The most effective formulations were Cu and Cu 60:40 Zn and the reduction of bacterial counts with these formulations were significantly lower than treatments with water or bleach. SAFI shows promise as a food and water antimicrobial product.

127. Austin DeGross, Microbiology**Calvin College**

(Co-Authors: John Wertz, Ph.D)

“Isolation of Novel Verrucomicrobia and Other Herbivory Associated Microbes from Gut of Cephalotes varians”

Ants in the genus *Cephalotes* have been shown to harbor core gut microbial communities that play a vital role in providing essential nutrients to the ants. Similar core microbial communities are shared between trophically similar species of *Cephalotes* and other closely related herbivorous ants. These core microbial communities consist of five distinct taxa: *Opitutus* (Verrucomicrobia), *Rhizobiales*, *Burkholderia*, *Pseudomonas* and *Xanthomonas*. The vast majority of these microbes are difficult to isolate and have not been cultivated; what is known comes from 16S rRNA sequencing and indirect observations of the effects of antibiotics on the ants. This project involves the cultivation, isolation, and characterization of heretofore-uncultivated microbial inhabitants harbored in the guts of ant species *Cephalotes varians*. Initial cultivation was achieved by removing the abdomens of the ants, homogenizing them and subsequently plating them on three different media in standard atmospheric conditions, under anoxia, and under hypoxic conditions (2% O₂). The most proliferate and diverse growth was observed on 100% tryptic soy agar under hypoxic conditions. Colonies from these plates were subsequently isolated, then characterized and put into a phylogeny via 16S rRNA sequencing. This phylogeny showed interesting results, including a possible novel genus of Verrucomicrobia and *Burkholderia*, while all sixty isolates are novel species of bacteria. Ten isolates were chosen based on the phylogeny for DNA extraction and full sequencing. DNA extraction was achieved using a standard CTAB Phenol/Chloroform extraction. Growth curves under varying oxygen concentration were attained via Spectrophotometric analysis. The results indicate that the selected isolates may not all be microaerophiles as we thought. Rather, they may exhibit characteristics of capnophilia. Future work includes further characterization of possible capnophilic traits, analyzing gene sequences, competition assays, coaggregation studies and electron microscopy.

128. Brandy Hammond, Microbiology**Calvin College**

(Co-Authors: Fabiola Enriquez and Dr. Amy Wilstermann)

“Role of Bacteria in the Premature Rupture of Fetal Membranes”

Bacterial vaginosis, a condition characterized by disruption of the normal vaginal bacterial community, places pregnant women at increased risk for premature rupture of fetal membranes (PROM). To enhance our understanding of the role that bacteria play in PROM, we investigated the ability of *Gardnerella vaginalis*, a BV-associated organism, to directly or indirectly influence the integrity of fetal membranes. Results suggest that *G. vaginalis* does not produce bacterial collagenases that could directly weaken fetal membranes. Previous work indicates that bacteria can disrupt mammalian tissue integrity indirectly by altering the activity and/or expression of mammalian matrix metalloproteinases (MMPs). While our experiments did not allow us to detect changes in MMP activity in a trophoblast-derived cell line exposed to spent bacterial media, we were able to detect changes in MMP expression. These results suggest that BV-associated organisms may secrete substances that trigger changes in MMP expression in fetal membrane tissues.

129. Christopher Stretton, Microbiology**Grand Valley State University**

(Co-Authors: Dr. Roderick Morgan and Dr. William Schroeder, Dr. Robert Smart)

“Antibacterial Activity of GV-1 Chemical Derivatives in the Presence of Human Serum”

Despite advancements in many areas of human medicine, infectious disease continues to be a major cause of mortality worldwide. Improper and excessive use of antibacterial compounds has led to the rise of resistant species of bacteria like Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin Resistant Enterococci (VRE), and Extreme Drug Resistant Tuberculosis (XDR-TB). We have discovered a potential new class of antibiotics that inhibit the growth of Gram-positive bacteria. Upon discovery of inhibition against S. aureus and other Gram-positive bacteria, MRSA, VRE, and other resistant strains were tested. Inhibition by the newly developed compounds on the resistant strains was identical to their inhibition levels against non-resistant strains of these species. We have continued to synthesize and test chemical derivatives of our lead compound in an effort to increase their effectiveness. Overall, these results demonstrate that our carboxylic amide compounds are a novel, non-penicillin based antibiotic that could be used to treat MRSA and other Gram-positive infections.

130. Justin Kelsey, Microbiology**Muskegon Community College**

“Anaerobic Digester (A Look At Potential Substrates)”

A look at biogas production from manure; biogas being a mixture of methane, carbon dioxide, water vapor, and other minute gases(Meyer 2011). Methane is the largest concentration of gas produced in the process of anaerobic digestion, which is used to create the biogas from the manure on a bacterial level. While methane is the most abundant and important as far as industrial applications, our experiment measured total gas production, not just methane. Hopefully it will serve as the groundwork for more advanced experiments in upcoming semesters. Keywords: Anaerobic, digestion, manure, biogas, methane, green energy, renewable energy.

(Co-Authors: Malak Alkanani, Abbey Bell, Amy Bohner, Aaron Burghraef, Jodie DeVries, Thomas Everding, Stacy Hooker, Christopher Jansma, Jessica Lang, Jonathan Lin, Jordan Newhof, Ian Noyes, Lisa Schultz, Shawntavia Stewart, Peter VandeHaar, Josue Vasquez, Kara Venema)

“Everything Will “B” o “K””: The Isolation and Genomic Exploration of Four Novel Mycobacteriophage and the Affinity of Calvin Students for the B and K Clusters”

Twenty students participated in the 2011 - 2012 Phage course at Calvin College and a total of twenty mycobacteriophage were isolated. Of these, Ava3, MacnCheese, Compostia, and Expelliarmus were chosen to be sequenced and annotated based on their plaque morphologies, electron microscopy images, and restriction digests. Ava3 was classified as a member of subcluster C1, Compostia as B3, Expelliarmus as A8, and MacnCheese as K3. Searches were made for integrases, membrane proteins, attP sites, inteins, and G+C content anomalies. In MacNcheese, a tyrosine integrase and an attP site (100% match to *Mycobacterium smegmatis*) were identified. For Ava3, no putative integrases or attP sites were found, however, an intein was identified. Additionally, we calculated genetic and geographic distances for members of subclusters B1, B3, and C1, revealing a strong positive correlation, suggesting that though phages can disperse long distances, geographic distance does produce genetic isolation. Further, analysis of Ka/Ks ratios in these subclusters showed evidence of purifying selection. In previous years, phage isolated and sequenced at Calvin have been members of the B and K clusters. The isolation of Compostia (B3) and MacNcheese (K3) this year prompted us to ask how many B and K cluster phage have been isolated (but not sequenced) by Calvin students since 2009. To answer this question, we designed PCR primers specific to each subcluster within the B and K groups, and used them in amplification reactions with purified DNA or lysate from each of the 64 mycobacteriophage we have isolated over the past three years. In total, 16 B-cluster (3 B1, 5 B3, 8 unknown subcluster) and 19 K-cluster (18 K1 and 1 K3) phage were isolated. The majority (15) of the B cluster phage were isolated in 2009 and 2010, whereas the majority (11) of the K cluster phage were isolated in 2011. This shift from B cluster to K cluster phage is perhaps due to the use of the inducing agent mitomycin c during phage enrichments, or a shift in the local mycobacteriophage community. In total, 55% of the mycobacteriophage isolated at Calvin since 2009 have belonged to the B and K clusters.

(Co-Authors: Jennifer Hess)

“The Effect of Garlic on Streptococcus mutans Biofilm Formation on Orthodontic Wire in Mono- and Dual-Species Cultures”

It has long been known that garlic has an inhibitory effect on microbial growth; however, it is not quite understood how this antimicrobial agent affects the formation of biofilms in both mono- and dual-species cultures. In this study, biofilms were grown in both sucrose and non-sucrose media containing varying concentrations of garlic and the relative abundance of growth was qualitatively assessed and recorded. Expression of genes, SpaP, GtfB, and GbpB, known to be associated with bacterial attachment in sucrose and non-sucrose dependent pathways were observed using RT-PCR and presence of banding was recorded. Garlic effectively inhibited bacterial growth of *Streptococcus mutans* and *Streptococcus salivarius* in agar diffusion tests. Biofilm experiments show that garlic may increase biofilm formation in single-species models, but it is unclear if the same is true for dual-species models. Some gene expression in both models was observed but expression did not follow a noticeable trend. Replication of this study is needed in order to eliminate contamination and other sources of error and thereby determining the effect of garlic on microbial biofilm formation in dual-species models. Effective characterization of the relationships among naturally occurring oral flora and the effect of garlic on their adhesion pathways may be important in developing new methods for treating common dental caries and thus reduce the occurrence of associated diseases.

(Co-Authors: Dr. Aaron Best)

“Regulation of hyaluronic acid metabolism in Streptococcus pneumoniae by RegR”

Streptococcus pneumoniae is a strain of bacteria which can cause many diseases such as otitis media, meningitis, and pneumonia. With the negative impact *Streptococcus* can have on human life, it is important to understand how it functions to gain better understanding of its virulence. During colonization and infection, bacteria such as *Streptococcus* must adapt to the changes in their environment. They must be able to establish themselves in the airways of the host and survive to colonize and cause disease. The host and invading organisms interact constantly in the context of changing environments inside the host. One way in which *Streptococcus* interacts with the host environment and helps establish itself is by using hyaluronidase, which degrades hyaluronic acid (HA). HA is a repeating disaccharide found in many human tissues; *S. pneumoniae* utilizes this as a carbon source. Using comparative bioinformatics, we were able to identify predicted three transcription factor binding sites (TFBS) of the transcription factor RegR in *Streptococcus pneumoniae* associate with HA metabolism. We have begun to validate these predictions using fluorescence anisotropy to determine whether or not recombinant RegR is able to bind to DNA oligonucleotide constructs containing the predicted TFBSs in vitro. By understanding the transcriptional regulation of this system, we will gain a deeper understanding of the pathogenicity of *Streptococcus*.

(Co-Authors: Eric Montoye and Dr. Timothy P. Keeton)

“Determination of Antibiotic Resistance in E. coli Isolated from Mid-Michigan Streams Affected by Large Livestock Operations”

Large livestock facilities account for the majority of antibiotic use in this country. Studies have shown that prolific and continual use of antibiotics in these facilities has led to increased resistance in many types of bacteria. Past studies from Mid-Michigan streams have identified antibiotic resistance in fecal coliform bacteria, including *E. coli*. Correlating resistance with nutrient loading data may help identify the proliferating source of resistant strains, when sampling at the livestock facilities themselves is problematic. This study attempted to identify and characterize the presence and possible sources of antibiotic resistant fecal coliform bacteria in surface water around large livestock facilities by testing for and isolating resistant strains and assessing associated nutrient loading at sampling sites. Sediment and pore water samples were collected from streams both upstream and downstream from large livestock facilities and areas of known negative impacts. Pore water extracted from the samples was plated on a media, selective for gram-negative fecal coliform bacteria. Several plates were dosed with different concentrations of Tetracycline Hydrochloride and resistant bacteria were isolated. Tetracycline is an antibiotic widely used in livestock, but rarely used in the human population. Resistant bacteria were analyzed using EStrips, which assess the Minimal Inhibitory Concentration (MIC) of multiple antibiotics. Polymerase Chain Reaction (PCR) and gel electrophoresis were used on resistant isolates to test for the presence of the Tetracycline Hydrochloride resistance Tet(W) gene. Results show fecal coliform bacteria from the Mid-Michigan stream have significant resistance to multiple antibiotics, including Tetracycline, Erythromycin, and Streptomycin. The Tet(W) gene was identified in these resistant isolates, which demonstrates that resistance is most likely mutated from livestock facilities that commonly use Tetracycline Hydrochloride. Corroboration of these results with high nutrient loading and Biochemical Oxygen Demand downstream from these facilities strongly suggests that large livestock operations in the region are the source of these mutations. Additionally, these results show the importance of connecting geochemical and biological techniques to assess environmental impacts of large livestock facilities.

“Cytotoxicity and Neutralization of Clostridium difficile Toxin B”

Clostridium difficile is a human resident microbe that often causes superinfections of the digestive tract in patients who have undergone extensive antibiotic treatments. The main virulence factor of *C. difficile* is an exotoxin called Toxin B from a group of toxins called large clostridial toxins. *Chlamydia muridarum* releases a toxin homologous to large clostridial toxins. Therefore a relationship between the toxicity of toxin B and the toxins in *Chlamydia muridarum* infections was found. Cytotoxicity assays were used to determine the relationship in toxicity between the two. In *C. muridarum* infected mice, the immune system synthesizes antibodies against the chlamydial homologue of toxin B. We hypothesized that these antibodies will neutralize the toxins created by the *Chlamydia* infections and also cross-neutralize the cytotoxic effect of toxin B. Our research found that *C. muridarum* infected mouse serum was able to neutralize some of the cytotoxicity of clostridial toxin B.

(Co-Authors: Sara Gallemore and Dr. Gregory Fraley)

“Involvement of the gpr30 Receptor in Resveratrol's Neuroprotective Mechanism”

During advanced-stage Parkinson's disease (PD), many patients resort to deep brain stimulation (DBS), a treatment in which electrodes are implanted into the subthalamic nucleus (STN). This treatment provides relief from tremors, rigidity dystonias, dyskinesias, and helps in initiating movements. Even though this treatment has proven highly effective for many PD patients, the main side effects are due to a 1 mm³ area of cell death around the electrode. The lesions are due to the physical presence of the electrodes and eventually lead to an ineffectiveness of DBS to ameliorate the signs of Parkinson's disease. Resveratrol (RESV) is a plant flavinoid that is known to have protective effects against cellular degeneration. We previously found that RESV has neuroprotective effects to prevent brain lesions associated with the physical presence of cannulas in the brains of rats. Because of RESV's estrogenic structure we hypothesized that the non-classical estrogen receptor gpr30 may be a part of RESV's neuroprotective mechanism. We used four different treatments to test the action of RESV on gpr30: RESV + Vehicle, RESV + G15 (gpr30 antagonist), Vehicle + G15, and Vehicle + Vehicle. Each treatment was injected bilaterally into the STN and motor coordination was observed using Rotarod testing before and after treatment. 48 hours after surgery, all groups showed a significant reduction in rotarod activity compared to pre-surgical trials except for the RESV group. These data suggest that RESV exerts its neuroprotective effects at least in part via the gpr30 estrogen receptor.

“Determining Intracellular Localization of System xc-: Preliminary Study and Method Development”

A membrane transport system known as System xc⁻ has been shown to traffic constitutively between intracellular vesicles and the plasma membrane, and is known to exchange intracellular glutamate for extracellular cystine. These amino acids compose the oxidant-reducing molecule glutathione. Oxidants rapidly increase membrane expression of System xc⁻ and are thought to manipulate this trafficking mechanism, enabling the cell to rapidly respond to oxidative stress. Oxidative stress plays a key role in many neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's. Using a modified protocol of subcellular fractionation, a technique that separates intracellular components according to their specific densities, specific organelles were probed to identify separation. The current study is focused on method development in order to establish reproducible results. Our initial studies have suggested that a different centrifugation protocol needs to be developed in order to more extensively separate the Golgi compartment from the endoplasmic reticulum compartment. When a reliable and accurate protocol is established, the location of System xc⁻ can be determined. Once the location of System xc⁻ is determined, the trafficking when subject to differing environments of oxidative stress can be studied.

(Co-Authors: Emily Andrews, Daniel Capodilupo, Rebekah Hazel, Johnathon Kisner, Eric Sesselman)

“Detection of Baboon Brain GAP-43 by One and Two Dimensional Gel Electrophoresis”

This investigation seeks to detect the protein, GAP-43 (growth associated protein) in an extract from baboon brain tissue using both one and two dimensional gel electrophoresis. Western blot analysis will be used to specifically detect presence of GAP-43. The major goal of this study is to compare the resolution of GAP-43 isoform separation using two different IPG (immobilized pH gradient) strips with pH ranges from either 3-6 or 4-7. GAP-43 has been reported to contain at least one (and possibly more) phosphorylation sites causing it to create possibly two or more spots in silver stained 2D gels. The various spots are believed to coincide with the phosphorylated isoforms of GAP-43. Since phosphorylation of GAP-43 has been shown to increase in paradigms of learning and memory, we hope to eventually examine changes in quantities of GAP-43 in brain tissue from human patients with Alzheimer’s disease or animal models of Alzheimer’s disease. If changes in quantities of any GAP-43 isoform can be detected, maybe this protein can serve as a potential biomarker of cognition which might be useful for examining effectiveness of new drugs being engineered to treat the profound memory impairment associated with Alzheimer’s disease. Future studies will utilize the IPG strip which reveals a higher degree of resolution.

(Co-Authors: Josiah Sinclair, Paul Moes, Loren Haarsma)

“Probst Bundle Connectivity and AMPA Receptor Kinetics”

Aggenesis of the corpus callosum (AgCC) occurs in humans and mice when developing axons fail to cross the midline to connect left and right cortical areas. These misguided fibers form bilateral pathways, known as Probst Bundles (PBs), which run anterior-to-posterior near the medial cortical surface. Previous research has established that the axons of the PB fibers conduct action potentials, but no studies have determined if PB fibers make functional connections with layer V pyramidal cells. The present study used electrical stimulation of PB axons in mouse brain slices and patch-clamp electrophysiology to verify the existence of monosynaptic connections to cortical cells from PB axons, and to examine the location and nature of excitatory post-synaptic currents. Results verified the existence of functional excitatory monosynaptic responses in layer V pyramidal cells following stimulation of PBs and surrounding intra-cortical tissue rostral to the target cell – similar to responses seen with normal corpus callosum stimulation. Significantly fewer monosynaptic excitatory responses were identified when stimulating caudally to the target cell confirming that the heterotypical pathways are established in an anterior-to-posterior direction. These results confirm that PB fibers make functional intra-hemispheric connections to layer V pyramidal cells that mirror the properties of normal inter-hemispheric synaptic connections.

“The Maintenance of Reproductive Status in Pekin Drakes Requires Both Red and Blue Wavelengths of Light: Relationship to Opsin-Related Proteins in the Hypothalamus”

In birds there is compelling evidence that photoresponsiveness is mediated—at least in part—by neurons that express photosensitive chemicals. These neurons have been referred to as deep encephalic photoreceptors. Photo-responsive pigments all consist of an opsin protein that is a transmembrane, G-couple receptor that transduces light energy into a neuronal signal. Two of these opsin-related proteins, opsin and melanopsin, have been identified in avian brains. Pekin ducks are seasonal breeders and as such, very sensitive to artificial and natural light. The purpose of these studies was to determine if specific wavelengths of light are necessary to maintain plasma luteinizing hormone secretion and to determine the hypothalamic circuitry underlying this effect. First, drakes were exposed to full spectrum, white light or red (~625 nm) or blue (~400 nm) specific wavelengths and blood samples take at intervals around lights on (0300 hrs). We found that neither the red nor blue wavelengths of light could maintain circulating LH levels compared to that of drakes housed under white light. Second, drakes housed under white lights were euthanized and brains processed for immunocytochemistry for opsin and melanopsin. As in other species, opsin-ir (RET-P1) was found in the lateral septal area (LS) and infundibular nuclei (INF) and were colocalized with vasoactive intestinal polypeptide. Melanopsin-ir was observed in the premammillary nucleus (PMM) and colocalized with tyrosine hydroxylase. Immunoreactive fibers for both opsin- and melanopsin were observed throughout the diencephalon and found to be in close contact with GnRH cell bodies. Third, a significant ($p < 0.01$) increase in fos-ir was observed in all three nuclei in drakes exposed to white light compared to dark conditions. These data suggest that multiple opsin-related peptides within the diencephalon are necessary to maintain photoresponsiveness in Pekin drakes.

(Co-Authors: Genevieve Beauvais, Darcy Kaufman, Jennifer Steiner, and Patrik Brundin)

“Differentiation of LUHMES cells into mature dopamine-like neurons”

Lund human mesencephalic (LUHMES) cells are currently being used in the research of Parkinson's Disease (PD). LUHMES cells are a subclone of tetracycline-controlled, v-myc overexpressing human mesencephalic derived from cell line MES2.10. In previous research, (Lotharius et al 2005, Schidknecht et al 2009, Scholz et al 2011), LUHMES cells were differentiated into morphologically and biochemically mature dopamine-like neurons following exposure to tetracycline, GDNF (glial cell line-derived neurotrophic factor) and cAMP. In our research, we aim to use the LUHMES cell line as a model system for studies on PD. First, we standardized LUHMES cells culturing protocol. Then, using a two-step differentiation process, the LUHMES cells entered a post-mitotic state and formed a neurite network. The LUHMES cells morphologically resembled primary neurons. Following differentiation, we found about 1% of the differentiated LUHMES cells expressed the dopaminergic neuronal marker, tyrosine hydroxylase (TH). The culturing method needs to be optimized to yield a larger number of highly differentiated LUHMES. To increase the percentage of cells testing positive for TH, the duration of differentiation will be increased. Further confirmation of the post-mitotic characteristics of LUHMES cells will be done by testing for more dopamine markers, in addition to TH, eg dopamine transporter, VMAT-2 vesicular monoamine transporter-2. Once the protocol for the differentiation of LUHMES cells has been optimized, the cell line may be used as an in vitro model of PD because the cell line possesses characteristics of dopaminergic neurons.

(Co-Authors: John L. Ubels)

“Restoration of Corneal Epithelial Barrier Function Using Artificial Tears Containing Hydroxypropyl-Guar and Hyaluronic Acid”

Introduction: Dry eye is a disease that affects the ocular surface resulting in symptoms of discomfort, visual disturbances, and potential damage to the cornea. To attenuate these symptoms, artificial tears are applied directly to the cornea. The goal of this study was to determine whether an artificial tear containing hydroxypropyl (HP)-guar and hyaluronic acid (HA) would promote recovery of a damaged cornea. Methods: Efficacy of the artificial tear formulations was determined using a 5,6 carboxyfluorescein (CF) uptake assay on intact rabbit corneas. Rabbits were anesthetized and exposed to 0.01% benzalkonium chloride to damage the cornea. After damage, corneas were exposed to artificial tear solutions for 1.5 hours, after which, the rabbits were euthanized and eyes enucleated. The eyes were inverted onto small beakers of CF for 5 minutes before the corneas were removed, weighed, and dialyzed in BSS for 48 hours. Amount of CF uptake was determined by measuring the fluorescence of the dialysate. Results: Corneas exposed to artificial tears without prior damage showed a slight increase in CF uptake. However, damaged corneas exposed to artificial tears containing HP-guar, HA, or both showed significantly less CF uptake than untreated damaged corneas. Conclusions: HP-guar, HA, and the combination solutions were able to promote barrier function recovery. Results using the vehicle suggest that HP-guar and HA are the contributing factors to barrier function recovery.

(Co-Authors: Sarah Colton, Rachel Haas, Elizabeth Gerometta, Erika Coombs, Susan M. Fraley and Gregory S. Fraley)

“Descriptive Analyses of the Development of Gait in Pekin Ducks from Hatch to Market Weight”

During recent studies it was determined that there exist many different opinions among investigators and within literature as to the nature of "normal gait" in Pekin ducks. Of particular importance was a debate as to what degree of metatarsal adduction (MA, aka "pigeon toed") impacts a duck's well-being. Thus, we set out to characterize the range of gait patterns of commercially obtained Pekin ducks in an aviary setting. Day old hatchlings (n=110) were obtained and housed in floor pens under environmental conditions that closely approximate industry standards. Students spent time with the ducks in order to habituate them to investigators and analytical equipment. Beginning on day 3, weekly footprint analyses were completed by allowing ducks to casually walk down a paper-lined runway after their feet were painted with ink. Semi-quantitative analysis revealed that ducks fell into three general categories in a normally distributed manner: 1) wide stance (14%; >1 foot-width between tarsal pads), 2) middle stance (73%; <1 foot width between tarsal pads), and 3) narrow stance (13%; virtually inline placement of tarsal pads). Although all ducks showed MA while casually walking, 9-15% of ducks showed excessive MA in one or both feet (>40° inward rotation) independent of stance width. Observed percentages persisted regardless of age. Also weekly, beginning with day 1, a subset of ducks across gait categories (n=10) were weighed and analyzed for pelvic limb structure and presence of tibial dyschondroplasia (TD). No differences in body weights were observed among gaits, regardless of degree of MA. Each week, approximately 30% of dissected birds showed minimal signs of TD. Although TD may be slightly more prevalent in ducks with excessive MA, TD is also observed in ducks regardless of degree of MA. At this time we conclude that TD may not be a causative factor in the development of excessive MA; and similar to other bipedal species, the presence of MA may not of itself be indicative of lameness or a lack of well-being.

“Biodiversity, Phenology and Thermoregulatory Strategies of Odonates in Barry County, Michigan”

Forty-three species of dragonflies from five families and sixteen species of damselflies from three families were identified at Pierce Cedar Creek Institute in Hastings, Michigan (latitude 42.6459 and longitude -85.2908) between May 7th and August 10th 2012. Our study showed that Pierce Cedar Creek Institute provides good habitat to a much greater number and variety of odonates than formerly expected. The diurnal phenology of the odonates varied by species, with smaller and medium dragonflies generally out earlier in the day and active into the afternoon, and larger dragonflies (such as the aeshnids) more active near dusk. We found that dragonflies and damselflies use a variety of active and passive thermoregulatory strategies. We found that the mean ΔT (the difference between ambient and thoracic temperature) as well as the heating /cooling curves and preferred flight temperatures, vary according to the thorax size of the odonate. In addition, we found that the flow of haemolymph from the wings to the thorax does not function to significantly regulate thoracic temperature.

(Co-Authors: Morgan Bauman, John E. Davis, Maurie J. Luetkemeier)

“No Differences in Wrist Flexion Strength for Isometric, Eccentric, and Concentric Contractions With and Without Venous Occlusions”

There have been published articles stating a significant strength gain with handgrip training in an occluded arm versus a non-occluded arm. Purpose: The purpose of this project is to determine strength gain in an occluded arm and non-occluded arm with exercise using concentric, eccentric and isometric muscle contractions on a Cybex isokinetic dynamometer. Methods: Nine Subjects (Men=4 Women=5, mean age \pm standard deviation= 19 ± 8.5 months) completed 6 trials each in our experimental design. Each subject did one trial each of concentric (at a speed of 90° per second), eccentric (at a speed of 90° per second) and isometric (held contraction for 5 seconds) contractions both occluded and non-occluded. All testing was done using the subject's right arm. The subjects' were seated while an initial reactive hyperemia test was performed. The mercury-filled strain gauge was placed around the subjects' arm at the biggest diameter. A blood pressure cuff placed on the upper arm was inflated to 50 mmHg then released and a reading was taken as blood flow returned to the arm. This was repeated 10 times then the cuff was inflated to 250 mmHg to occlude blood flow for 5 minutes. The cuff was released and 10 more reading were taken with the cuff inflating to 50 mmHg. After the last reading the subject moved to the isokinetic dynamometer and started the exercise after 3 minutes from the time of the last reading. The blood pressure cuff was left on the subject regardless of the condition. The condition was randomly selected. If the subject was doing an occluded condition the cuff was inflated to 80 mmHg for the duration of the exercise. The subject performed 15 repetitions of the assigned condition. The cuff was released as soon as the subject finished the exercise and they moved directly back to the chair. The 10 initial reading followed by the reactive hyperemia were then taken. The Cybex recorded the initial peak torque for the first muscle contraction and the total work done for the 15 repetitions of the exercise. Results: The values for peak torque and total work done showed no statistical significance between the type of contraction or the state of occlusion throughout the 6 trials.

(Co-Authors: Thomas E. Bahl, PhD)

“Sex, Age, and Quantitative Motor Recruitment”

The purpose of this study was to determine if there was a gender difference in the ability of individuals (ages 40 to 55) to quantitatively increase the intensity of grip strength in both their right and left hands. This age group was selected because findings from a previous study conducted at Aquinas College with younger individuals (ages 18-25) found that females were commonly more accurate at quantitatively increasing grip strength. In the current study, there were no significant differences found when comparing men and women who were asked to double or triple their initial grip strengths. Therefore, it was found that sex does not impact quantitative muscle control in fist clenching in 40- to 55-year olds. However, since these findings are not consistent with the previous study, it would seem age does have an impact on muscle control.

147. Jessica Darusz, Organism Biology / Physiology**Calvin College**

(Co-Authors: Huong Tran, Sarah Green, Martina Ralle, and Randall Woltjer)

“Metal content of cerebral white matter in aging and dementia”

Transition metals may function in aging and age-related disease through promotion of free radical-mediated tissue damage. There is controversy surrounding alteration in transition metals in the cortex in Alzheimer's disease (AD), but the published evidence overall suggests a modest decrease in copper. Mixed dementia (MD), which features modest AD changes as well as evidence of vascular ischemic injury to the brain, is characterized by MRI evidence of white matter (WM) damage. We determined transition metal content in postmortem brain tissue affected by AD and MD using inductively coupled plasma mass spectrometry (ICP MS). Similar to reported findings in cerebral cortex, Cu was selectively significantly decreased in AD in WM. However, Mn, Fe, Cu, and Zn were all decreased in WM in MD. These changes were seen only in established MD, and not in age-matched subjects with milder cognitive changes. The findings indicate that transition metal alterations are a late-stage change in MD that point to mechanistic features in WM that distinguish it from AD.

148. Meagan Mc Rae & Monica Langeland, Organism Biology / Physiology**Calvin College**

(Co-Authors: Keith A. Grasman and Sylvia Fuhrman)

“Health and Reproductive Impairments in Colonial Waterbirds in the Saginaw Bay and River Raisin Areas of Concern”

This assessment is part of the Great Lakes Restoration Initiative (U.S. Fish and Wildlife Service), investigating effects of contaminants on reproduction, growth, and immunological health of fish-eating birds in the Saginaw Bay and River Raisin Areas of Concern (AOCs). In Saginaw Bay, field studies were conducted in 2010-12 at two herring gulls colonies (Saginaw Bay Confined Disposal Facility (CDF) and Little Charity Island), two Caspian tern colonies (Saginaw Bay CDF and Charity Reef) and one black-crowned night heron colony (Saginaw Bay CDF). At the River Raisin AOC, herring gulls were studied at the Detroit Edison Power Plant in Monroe on the western shore of Lake Erie. Reference sites were located in the lower St. Mary's River at Pipe Island Twins (gulls) and Two Tree Island (terns), and in Lake Huron at Chantry Island (herons). Embryonic nonviability rates in herring gulls at AOCs (4.5-13%) were higher than at reference sites (<2% Craig Hebert, Environment Canada, personal communication). Herring gull chicks at Monroe in 2010 and 2012, and Caspian tern chicks at Charity Reef in 2010 had poor survival (≤ 0.3 chicks per nest). Mean phytohemagglutinin skin responses for T-cell mediated immunity were suppressed dramatically compared to reference sites in herring gulls (50-57%), Caspian terns (51%), and black-crowned night herons (33%) in the Saginaw Bay AOC. Immune response was suppressed 57% at the River Raisin AOC. Ongoing immunological, developmental, and reproductive impairments in fish-eating birds at these AOCs are consistent with previous studies on the effects of persistent pollutants such as PCBs in Great Lakes birds, although other stressors may be contributing to reproductive effects.

(Co-Authors: M. Makagon, R. Fulton, D. Karcher, PhD)

“The Relationship between Pekin Duck Musculoskeletal Issues and Gait Scores”

In commercial Pekin duck production, lameness is a serious welfare issue. Duck gait has traditionally been scored using a modified method of the system originally developed for broiler chickens and turkeys. The significant, physical differences between duck gait and broiler gait may render this scoring system invalid and cause inaccuracies when assessing gait. To date, no quantitative characteristics of “normal” gait patterns in ducks have been described. Our group has been investigating the relationship between duck gait, musculoskeletal issues, and pathological factors by taking advantage of various technologies to assist in the analyses. The goals are to determine what constitutes “normal” variation in duck gait and to evaluate the validity of the current gait scoring system as a tool for the assessment of lameness. Eighty Pekin ducks per barn (4 barn replicates per age group) at three ages (14, 21, and 32 days old), were assessed on a commercial farm for gait scores, infrared photos, video recordings, and gait pattern and pressure using a Tekscan pressure pad. At each age, 20 ducks per barn were sacrificed for further laboratory analyses, which included CT scans, necropsy, and bone ash analysis. Analyses are still ongoing. The technologies used in this study provide a comprehensive set of tools for the evaluation of gait patterns in Pekin ducks.

(Co-Authors: Allison Desautels, Eric Spencer, Kara Winczkowski, David Kurjiaka)

“Influence of glucose on growth of cultured mouse endothelial cells”

The supplier (ATCC) of cultured mouse endothelial cells states that those cells should be maintained in a high glucose environment. As these are the only cultured endothelial cells available commercially that do not require growth supplements in addition to the FBS, we were interested in whether the media glucose concentration influenced the rates of cell division (growth) in these cells. The growth of mouse endothelial cell (bEnd.3) division was assessed in high (4.5 g/L = 25 mM/L) and low (1 g/L = 5.6 mM/L) glucose with 10% FBS-DMEM. The rate of endothelial cell growth was reduced when glucose was closer to physiological concentrations (5.56 mM/L = 100 mg/dl). When the FBS was lowered/removed from the media, the cells continued to grow (although more slowly than in 10% FBS). As the cells in 5.6 mM grew even slower, high glucose appears to stimulate the release of growth factors from the endothelial cells that are necessary for their growth.

“The Effects of Environmental Enrichment Devices on Feather Picking in Commercially Raised Pekin Ducks”

Similar to other poultry species, Pekin ducks occasionally show feather picking. This self-picking can lead to reduced feather quality and poor overall health of the bird. This picking appears to occur when the ducks are transitioning between downy feathers and adult plumage, between 17 and 22 days of age. We hypothesized that giving Pekin ducks environmental enrichment devices (EED's) during this time would decrease feather picking and improve feather quality and duck well-being. To test this hypothesis, we offered a substitute outlet to minimize feather picking behaviors by providing EED's: plastic wiffle-style balls, each threaded with four zip-ties. In the first study, we set out to determine if placement of EED's would cause harm. Upon placement of the EED's, pens were videotaped for a total of two hours per day and duck behaviors were scored by individuals unaware of treatment groups. Results showed a significant ($p < 0.05$) decrease in both self-picking and conspecific-picking. A second experiment set out to determine if ducks had a color preference for blue/green, red, or white EED's. Again, ducks with EED's showed significantly ($p < 0.05$) reduced feather picking compared to ducks without EED's. By far, ducks interacted significantly ($p < 0.001$) more frequently with blue/green EED's than either red or white EED's. These results suggest that providing environmental enrichment may minimize prevent feather picking and improve feather quality and duck well-being.

(Co-Authors: Laurelin M. Martin, Timothy M. Evans, Neil W. MacDonald)

“Using DNA Barcoding for Plant Identification in a Long-term Prairie Restoration Study”

We studied the effects of site preparation treatments, knapweed removal, and prescribed fire on plant community development in a restored prairie. As part of this study, plants needed to be identified to species in order to perform Floristic Quality Assessments. At times, identification can be difficult using traditional taxonomic keys because of subtle differences among species. We selected 19 plants that had uncertain identifications to conduct a genetic barcoding study to help identify these specimens to species. Chloroplast DNA was extracted from these samples, amplified to sequence the *rbcl*+*matK* plastid coding regions, and the gene sequences were compared to those of species within the public National Center for Biotechnology Information database. Of the specimens sequenced to date, the genera found included *Carex*, *Lespedeza*, *Panicum*, and *Elymus*. Our preliminary results showed that DNA sequencing facilitated plant identification to genus. Compared to traditional taxonomic keys, however, it may be more time consuming and a large degree of uncertainty still remains when trying to identify plants to species using the available information in the public database.

(Co-Authors: Daniel Bergman, PhD)

“Pollutant Effects on Neurophysiological Recordings from Sensory And Motor Neurons Of The Crayfish”

Proper sensory input and motor output relies on constant nervous system activity. We proposed to test the neurological effects of a chemical pollutant on crayfish, *Orconectes propinquus*. Nonylphenol is a chemical used in detergents and pesticides that is commonly concentrated in crayfish, fish, and birds. Crayfish were exposed to 0.20 μ L of nonylphenol for seven days. At the conclusion, crayfish sensory and motor neuron capabilities were tested by allowing crayfish to find food in a Y-maze. Data recorded included percent success finding food, time to find food, time spent motionless, and time spent in the food arm of the Y-maze. In phase two of experiments, primary sensory and motor neurons will be isolated to test changes in membrane potential across axonal membranes. By doing so, we will elucidate any alterations in neuronal signals due to nonylphenol exposure. For example, a reduction in neuronal signaling would indicate the pollutant directly affects the crayfish nervous system.

(Co-Authors: X. Hong, D. Portney, NL. Lehman)

“Combination Treatment focused on Aurora A in Glioblastoma”

Aurora A (STK-15 is a serine-threonine kinase that is critical for mitosis involved in centrosome duplication, mitotic entry, spindle assembly and sister chromatid segregation.¹ In addition, Aurora A can regulate key components of pro-proliferate signaling pathways including Beta-Catenin, Phospho-GSK3b, GSK-3B, HIF-1Alpha and Actin.¹ Transcription of Aurora A is induced by E2F3 in early S phase and its protein expression peaks during G2/M. Aurora A is degraded by the 26S proteasome during anaphase following its ubiquitination by the anaphase promoting complex/cyclosome E3 ubiquitin ligase (APC/C).¹ During the experiment, glioblastoma cell lines U251n, HF2587SP and HF2303 were treated with a combination of radiation and Aurora A inhibitors MLN8237 and Tamizolamide. The cell growth in the cell lines were suppressed in the combination of radiation and drug therapy. Beta-catenin, HIF-1Alpha, Phospho-GSK3B, and GSK-3B from treated U251N and HF2303 cell lines were analyzed using Western Blotting. Furthermore, selective Aurora A inhibitor MLN 8237 is potently cytotoxic to glioblastoma cells and is potentiated by ionizing radiation.¹

(Co-Authors: Shaun Alsum, Margeaux Carter, Jake Lampen, Professor Matt Walhout)

“Producing and Detecting a Beam of Metastable Krypton Atoms”

Our atom-trapping experiments call for a beam of krypton atoms in the metastable $5s[3/2]2$ state, which lies 10 eV above the ground state. Our standard method of producing the metastables employs a D.C. discharge running through the nozzle aperture that admits krypton gas into our vacuum system. The efficiency of this discharge method is known to be $\sim 10^{-4}$. An all-optical method has been predicted to achieve an efficiency of > 0.1 . We aim to compare these two excitation methods, and toward that end we have installed a retractable metastable-atom detector in our atomic-beam apparatus. We still have to improve the frequency stability of our laser system before we can perform a conclusive test of the relative efficiencies.

(Co-Authors: S.K. Remillard)

“Mapping the Nonlinearity of Superconductive Passive Circuits”

The nonlinear response of High Temperature Superconducting (HTS) microwave resonator samples of $Tl_2Ba_2CaCu_2O_8$ (TBCCO) and $YBa_2Cu_3O_7$ (YBCO) on $LaAlO_3$ (LAO) substrates was analyzed around the transition temperature (T_c). Nonlinearity is an undesirable response in commercially-produced superconductors that can potentially be minimized through the understanding of its effects through our investigation. HTS microstrip lines were examined with a travelling microscope and a scanning electron microscope (SEM) to determine the dimensions, geometry, and edge structures of each sample, and Energy-dispersive X-ray Spectroscopy (EDS) was used to verify the material composition. Multi-tone measurements of even and odd order intermodulation distortion (IMD) currents were performed utilizing a simultaneous and synchronous measurement technique developed at Hope College. Around their respective T_c s, resonators of the two material systems exhibited different even and odd order IMD currents. The degree to which the superconducting current breaks time reversal symmetry (TRSB) is revealed by the ratio of the 2nd and 3rd order IMD levels. In YBCO samples, this ratio steadily decreased with increasing temperature, but then rapidly increased through T_c , indicating a considerable amount of TRSB. TBCCO showed a steady decrease in this ratio, as well, with increasing temperature, but did not show any indication of a rise in TRSB whilst approaching T_c . In TBCCO, 3rd order IMD exhibited a peak around T_c which is consistent with the nonlinear Meissner effect. TBCCO also appears to show nonlinearity past T_c much more than does YBCO, indicating the possibility of quantum mechanical fluctuations: a phenomenon associated with anisotropic superconductivity. This material is based upon work supported by the National Science Foundation under NSF-RUI Grant No. DMR-1206149 and the Hope College Division of Natural and Applied Sciences.

(Co-Authors: Stephen Remillard)

“Electromagnetic Dispersion in Periodic Structures”

Photonic crystals are electromagnetic structures that affect the propagation of microwaves, clearly demonstrating nonlinear, band gap dispersion in the band theory of solids. Motivated partly by the development of an advanced physics lab in dispersion, we have compared the dispersion of microwaves in photonic crystals to the dispersion of electrons in semiconducting crystals. The transmission lines were fabricated to achieve periodicity with alternating widths of adjacent copper segments using photolithography. Three identical dispersion diagrams were constructed using different sets of values: the S-parameters measured by the vector network analyzer (V.N.A.), the S-parameters simulated using finite element analysis software, and the delay values measured by the V.N.A. All three methods showed close agreement in the dispersion with a band gap at the Brillouin zone edge. The values from the network analyzer were then used to examine the group velocity of the wave near the band gap. Near the edges of the band gap, the group velocity approached zero; inside the band gap, the evanescent waves tunneled through the crystal with superluminal group velocities. Periodic transmission lines with defects were also constructed; the defects engineered into the photonic crystals produced donor and acceptor states in the band gap. These results indicate that the microwave transmission lines successfully modeled the dispersion from band gaps in photonic crystals.

“T-duality in String Theory”

String theory was devised as an attempt to merge the well-known theories of general relativity and quantum mechanics. T-duality plays a major role in how string theory works. It takes two seemingly different systems and shows that they are physically equivalent allowing us to observe how strings behave in different dimensions. The equivalences found allow the same calculations to be done on two systems, which can provide a way to check our previous work with the other system or it could make the calculations much simpler. We used Mathematica to implement the Buscher rules which convert the geometric description of one system to that of its T-dual system. We studied a system involving D-branes, objects which play an important role in string theory's duality web. In particular we studied whether a D-brane in motion around an extra dimension could be T-dual to a new D-brane combined with a string winding around the extra dimension. Using our Mathematica functions we could test many dual geometries, none of which matched perfectly. We did however find one pair of systems that looked approximately equivalent when viewed from far away.

(Co-Authors: Shaun Aslum, Margeaux Carter, Andrew Hess, Dr. Matthew Walhout)

"Patterns in Lattice-Driven Discharges with Anisotropic Filament Interactions"

The spark-like filaments in a dielectric-barrier discharge (DBD) deposit patches of charge, or "footprints," on the dielectric surfaces. These footprints are deposited and erased with each oscillation of the a.c. driving voltage. In the long term, the average shape and position of each footprint is quasi-stable, and the interactions between adjacent footprints are responsible for the formation of spatial patterns along the lateral dimensions of the discharge gap. This experiment studied DBD patterns formed when a pair of opposed "pin grid" electrodes was used to produce the driving voltage. When a lateral shift was introduced between the grids, so that opposing pins were not aligned but offset in a controlled way, a range of different patterns and footprint shapes were observed. This setup constitutes a new system in which anisotropic interactions can be modified and controlled so as to address general issues in the science of pattern formation.

(Co-Authors: Dr. Karen Gipson)

"Explorations in Sonoluminescence"

Single Bubble Sonoluminescence (SBSL) is the phenomena by which a bubble is levitated in a liquid medium and forced to oscillate using sound waves, which can make the bubble collapse violently enough to produce light. Various material parameters affect the light produced by these bubbles. This project provided an alternative methodology for the production of SBSL, as well as data acquisition. Intensity measurements were obtained using a photomultiplier tube and spectral measurements were obtained using a fiber optic spectrometer. The study focused on the effects of temperature and different liquid compositions on the intensity of the light produced by a SBSL bubble, as well as the spectrum produced by a sonoluminescing bubble. The results contribute to a better understanding of the effects of these material parameters.

(Co-Authors: Loren Haarsma and Paul Moes)

“Electrophysiological evidence for normal proportions of inhibitory (GABA) intra-hemispheric synaptic connections following abnormal development of corpus callosum axons”

Agensis of the corpus callosum (AgCC) occurs in humans and mice when growing axons fail to cross the midline to connect left and right cortical areas. These misguided fibers form bilateral pathways, known as Probst Bundles (PB's), which run anterior-to-posterior near the medial cortical surface. AgCC symptoms overlap with autism-spectrum disorder traits, but researchers have speculated that individuals with PB's, have less severe symptoms than those without. Previous research has established that the axons of the PB fibers conduct action potentials, but no studies have determined if PB fibers make functional connections with layer V pyramidal cells. The present study used electrical stimulation of PB axons in mouse brain slices and patch-clamp electrophysiology to examine the existence of inhibitory post-synaptic currents (e.g., GABA-mediated responses) in cortical cells, and to examine the location and nature of these inhibitory responses. As with normal corpus callosum pathway stimulation, stimulation of PB axons produced very few GABA-mediated responses in layer V pyramidal cells. Together with other outcomes from the present study, the low number of GABA-mediated responses suggests that the resulting intra-hemispheric connections mirror normal inter-hemispheric connections, despite their abnormal growth patterns.

“Characterization of Electrodeposited Nanoporous Ni and NiCu Films”

Nanoporous thin films are interesting candidates to catalyze certain reactions because of their large surface areas. This specific project focuses on the deposition of Ni and NiCu thin films on a Au substrate and further explores the catalysis of the hydrogen evolution reaction (HER). Depositions are created using controlled potential electrolysis, a process where the potential at which the metal alloy deposition occurs is set and the length of time or total charge of the deposition is adjusted. Samples are then dealloyed using either DC potential amperometry with an applied constant potential or cyclic voltammetry for linear sweeping. Before and after the dealloying, all the samples are characterized using multiple techniques. Electrochemical capacitance measurements allow comparisons of sample roughness. HER measurements characterize the reactivity of the sample with respect to the specific catalytic reaction. The Tafel equation is fit to the data to obtain information about the kinetics of the HER of the samples. Other methods for characterizing the samples include scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The use of SEM allows images to be taken of the deposition to determine the change in the structure pre- and post- dealloy of the sample. EDS allows the elemental composition of the deposition to be determined before and after the dealloy stage.

(Co-Authors: Erica Chan and Paul Harper)

“Disaccharide Inclusion and Exclusion in Lipid-Water Phases”

This poster is a continuation of the poster entitled “Monosaccharide Inclusion and Exclusion in Lipid-Water Phases”. Results are given for disaccharide solutions in SOPE, and thermodynamic theory is presented to explain the results. Using this theory, the concentration of sugars in the different lipid phases were able to be calculated. The results of these calculations are presented here, along with physical interpretations. The current physical model has an exclusion region in front of the head groups in each phase. In $L\beta$ and HII this means that sugars are completely excluded from the lipid-water interface. Therefore, no sugar is allowed in the $L\beta$ phase while the HII phase only allows sugar in the water core away from the lipid head groups. In the $L\alpha$ phase there is some lateral space between the head groups, which allows for some sugar to be included next to the head groups. It is again excluded from the area in front of the head groups.

(Co-Authors: Shaun Alsum, Andrew Hess, Jacob Lampen, Prof. Matthew Walhout)

“Dielectric-Barrier Discharges on a Hexagonal Grid”

A Dielectric-Barrier Discharge (DBD) is typically produced when an oscillating high voltage is applied across two insulators separated by a thin layer of gas. As the voltage oscillates, each extreme causes the gas to undergo electrical breakdown and produce spark-like filaments between the faces of the insulators. Discharges occur twice per cycle, jumping across the gap at approximately the same spot due to the memory effect produced by surface charges. When filaments are packed closely, they arrange themselves spontaneously in regularly spaced patterns. While DBD's have historically been used to create reactive chemical species and ultra violet light, it is thought that plasma filament structures could shed light on other pattern-forming systems and interactions. Last year, our lab attempted to stimulate specific patterns by using a square pin grid electrode. This year we hoped to add to those observations by considering a hexagonal pin grid, as well as several other variations.

(Co-Authors: Anderson Peck and Anthony Chang)

“In vitro and in vivo studies of Cerenkov Luminescence Imaging”

Purpose: Cerenkov radiation (CR) is the phenomenon that visible light is emitted when a charged particle, such as a β^+ or β^- , exceeds the speed of light in a medium through which it travels. Detection of the light can be achieved by an optical imager with appropriate sensitivity. The goal of this study is to develop standard protocols testing the feasibility of optical imagers for Cerenkov Luminescence Imaging, then to utilize the protocols to assess if the AMI-1000 is an appropriate device for Cerenkov Luminescence Imaging (CLI). Methods: 2 positron (β^+) emitters (^{18}F , ^{64}Cu) were used for this study. The linearity between optical radiance and radioactivity of these isotopes was investigated. Signal intensity of ^{18}F and ^{64}Cu across a uniform plate was investigated to gain understanding of the field of view (FOV) uniformity. Samples used in these studies were scanned by positron emission tomography (PET) to quantify and verify the correlation between PET and CLI. Lastly, this correlation was assessed for mice injected with ^{18}F , which is a β^+ -emitter. Results: The linearity tests of ^{18}F and ^{64}Cu yielded R^2 values of 0.9988 and 0.9831 respectively. After normalization of experimental data to the Quantum Efficiency of the camera, it matched the expected spectral curve with an R^2 of 0.9532 for ^{18}F . Regressions for PET-CLI correlation in ^{18}F was obtained to be 0.9944. The FOV was found to have 20% variance in regards to ^{64}Cu . Based on our experimental findings in each of these areas, the AMI-1000 was found to be a viable optical imager for Cerenkov Luminescence Imaging.

(Co-Authors: Christian Woolley and Eddy Chen)

“Recollision Excitation in the Quantum Descriptions of Double Ionization of Helium”

Our group uses computer modeling to simulate the double ionization of a helium atom in a strong laser field. Previously, our group has used classical models to show that the double ionization happens primarily through a process called recollision excitation. This process involves 3 steps: (1) one electron ionizes; (2) the oscillating laser field pushes this electron away from the positive ion, then back; (3) the returning electron collides with the other electron and transfers energy to it, allowing it to eventually become ionized by the laser. We now present evidence that the same process of recollision excitation also occurs in our quantum model. We solve the Time Dependent Schrodinger Equation numerically for a one-dimensional quantum model of helium.

“Properties of Pulsating Heat Pipes”

As a result of the ever increasing power demands of modern electronic devices and their simultaneous decrease in size, design engineers are quickly reaching the limits of many conventional heat management techniques. New strategies and innovative methods must be developed to handle the higher heat fluxes present in these harsh thermal environments. Pulsating Heat Pipes (PHP's) represent a newly emerging technology being researched as a potential solution to some of these thermal management challenges. Unlike conventional heat pipes, pulsating heat pipes do not utilize a wicking material to return working fluid from the condenser to the evaporator regions. By contrast, PHP's transfer heat by circulating working fluid interspersed with bubbles, both of which are distributed non-uniformly throughout the serpentine tubing. Experiments were conducted to demonstrate the basic operating principles and measure the thermal characteristics of a copper PHP, using water as the working fluid. Results of a 25% working fluid fill ratio are compared with reference levels of 0% and 100%.

(Co-Authors: Margeaux Carter, Andrew Hess, Jacob Lampen, Dr. Matthew Walhout.)

“Interactions between Plasma Filaments in Dielectric-Barrier Discharges”

Dielectric-barrier discharges (DBDs) comprise a category of industrially useful plasma systems that can produce reactive chemical species and ultraviolet radiation. In the typical DBD, a thin layer of gas is sandwiched between two insulators, and an oscillating high voltage is applied across the narrow gap. With each half-cycle of the voltage, the gas undergoes electrical breakdown and forms spark-like filaments at various positions in the transverse plane. These filaments interact with each other in interesting ways; sometimes repelling one another, and sometimes attracting in order to form clumps we refer to as molecules. We have determined that there is some critical electric field strength in the gap below which filaments can still form, but not bind to each other.

(Co-Authors: Jessica Barboline)

“The evolution of caffeine production in Puallinia cupana”

Although the biological role of caffeine and related purine alkaloids in plants remains unclear, it has been suggested that caffeine plays a role in chemical defense, protecting fruits and young leaves from predators. In humans, caffeine is known to have stimulatory effects when consumed in beverages such as coffee and tea. The biosynthesis of caffeine involves a stepwise methylation of xanthosine derivatives that eventually produce 1,3,7- trimethylxanthine (caffeine). In the first step, xanthosine is methylated to produce 7-methylxanthine. In the second methylation step, the methylation of 7-methylxanthine at the 3-N-position results in theobromine. In the third step, theobromine is methylated and results in the production of caffeine. It is still uncertain how many enzymes perform these methylation steps in various caffeine producing species. The synthesis of caffeine seems to have been the result of convergent evolution, where different plants use different enzymes to perform the same functions. Coffee has three different enzymes, XMT, MXMT, and DXMT that convert xanthosine to caffeine. In the tea plant, *Camellia sinensis*, two caffeine synthase type enzymes have been discovered: TCS1 and TCS2. TCS1 has been shown to carry out the last two steps in the caffeine biosynthesis where it methylates 7-methylxanthine to give theobromine, followed by an additional methylation of theobromine to produce caffeine. Although similar in sequence to TCS1, no activity has been shown in TCS2. In *Puallinia cupana* (guarana), a well-known Brazilian caffeine producing plant, the genes that code for the enzymes responsible for the biosynthesis of caffeine remain unclear. A preliminary phylogenetic investigation, based off of EST sequences from GenBank, show that the genes responsible for caffeine biosynthesis in guarana are similar in sequence to TCS1 and TCS2 in *Camellia sinensis*. The phylogenetic analysis we performed supports the idea that the enzymes responsible for the biosynthesis of caffeine in tea and guarana evolved independently and concurrently from a single common ancestral enzyme. One guarana enzyme that has already been assayed, GCS1, has activity that methylates the xanthosine substrate. For this reason, we hypothesize that the stepwise methylation of xanthosine to produce caffeine will be accomplished using two enzymes, with the second guarana enzyme acting similarly to TCS1 to finish the last two methylations. Alternatively, we suggest that it may be possible for the synthesis of caffeine in guarana to require three enzymes, with each enzyme performing a different methylation step, as in coffee plants. Our work is part of a collaborative effort to better understand the evolution of caffeine, and give insight into the biochemical capacity of the ancestral plants. To test our hypotheses, we synthesized 3 guarana genes based off of similar EST sequences and tested for their activity. The results of the enzyme assays, GCS2 and GCS3, will be discussed in detail.

(Co-Authors: Julie E. Yonker, Laura G. DeHaan)

“Psychological Outcomes as a Function of Religiosity in Adolescents and Emerging Adults”

Previous research indicates that religiosity can serve as a protective factor against negative outcomes such as depression and risk-taking behavior in adolescents. For example, religious adolescents report lower levels of depression than their non-religious peers. Based on this evidence, we examine depression and aspiration (hopes for the future) as additional psychological outcomes of religiosity. The data for our project came from all three waves of the National Study of Youth and Religion (NSYR). Because of the longitudinal nature of our data, we were able to examine how these effects change over time, as adolescents become emerging adults. We used a factorial repeated-measures ANOVA to examine the outcome of depression and aspiration across time as compared to three aspects of religiosity. Overall, females report more depressive symptoms than males, and have a greater sense of aspiration in waves one and two, but in wave three aspiration levels are the same for males and females. In general, reports of religious attendance, religious beliefs, and religious behaviors are similar between the sexes. Furthermore, as religious attendance decreases, aspiration increases. Religious beliefs appear to relate to less depressive symptoms in females, but not in males, indicating that religious beliefs may play a greater role in decreasing females' depressive symptoms than males'.

“Undergraduate Research Grants for the Environment”

Pierce Cedar Creek Institute, a nonprofit environmental education center, offers Undergraduate Research Grants for the Environment to students from our 14 consortium schools including: Aquinas College, Calvin College, Ferris State University Grand Rapids Community College, Grand Valley State University, and Hope College. The poster will showcase past student research projects and provide information on the 2013 program. Students receive a stipend along with room and board to conduct an environmental research project based on ten weeks of field work on our 268 hectare property that features mature forests, prairies, wetlands, and aquatic systems. For details, consult our website: <http://www.cedarcreekinstitute.org/>

(Co-Authors: Paula Kuiper, Simon Veldkamp, Herb Fyneweaver)

“Overcoming the Implementation Gap of Formative Assessment”

What do good professors do that is consistent with formative assessment? What are perceived barriers to use of formative assessment that professors see both inside and outside the classroom? Can teachers' usage of formative assessment be represented by a few idealized types? We created a sketch of four ideal types of teachers based on the professors we studied. Each of these types would have a particular set of techniques, motivations, and barriers that other teachers could identify with and learn from. They could learn to capitalize on their strengths in formative assessment, and better overcome the barriers they perceive.

(Co-Authors: Danielle N. Dremann, and Professor Christine S. Chow, Department of Chemistry, Wayne State University)

“Generation of Peptide Variants for Helix 69 Binding”

An RNA motif known as helix 69 (H69) is found in the large (50S) subunit of the bacterial ribosome, and is noted to play a role in protein synthesis. Peptides are being explored as potential inhibitors of H69 and the translation process for development of new antibiotics. Such antibiotics are necessary due to an increase in bacterial resistance to current drugs. Previously, the phage display technique provided a parent peptide sequence, NQVANHQ, which has moderate binding affinity with H69. Results from previous studies have also shown that histidine (H) at position 6 of the peptide has a role in the binding to H69. Our current studies have focused on further modifications of this peptide, which are created through solid-phase synthetic methods, in order to search for better binding sequences. A number of peptide variants were generated by this approach. After synthesis of the peptides, high-performance liquid chromatography (HPLC) was used to purify them. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry was used to confirm the identity of the peptides. These methods have allowed for alanine and histidine scans to be done on the parent sequence to test for improved binding affinity. Future work will involve testing for binding by using electrospray ionization mass spectrometry (ESI-MS). Using a non-cleavable Tentagel bead to anchor the synthesized peptide, a screening assay is also being developed in order to visualize the binding a fluorescently tagged H69 to the peptide.
