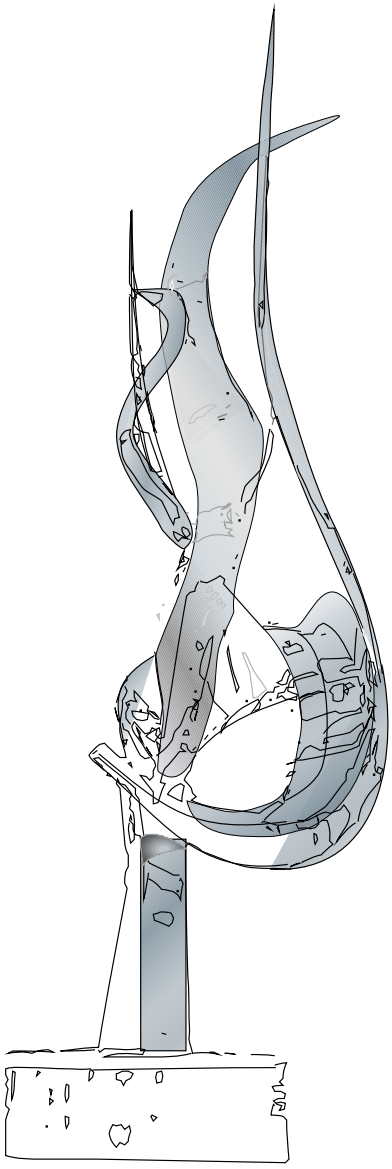
Interdisciplinary Research Collaborative in Biology & Chemistry

IRCBC

Rose-Hulman Institute of Technology



2nd Annual *IRCBC* Undergraduate Research Symposium

Friday
October 7, 2005

MERCK • AAAS
Undergraduate Science Research
PROGRAM

ROSE-HULMAN
INSTITUTE OF TECHNOLOGY

Welcome to the

2nd Annual IRCBC Undergraduate Research Symposium

Rose-Hulman Institute of Technology

Friday, October 7, 2005

We are honored to welcome you to the 2nd Annual IRCBC Undergraduate Research Symposium and we sincerely appreciate your participation. The symposium is coordinated by the Interdisciplinary Research Collaborative in Biology and Chemistry (IRCBC), and is supported by funding from the Merck/AAAS Undergraduate Science Research Program, the Lilly/Guidant Applied Life Sciences Research Center, and Rose-Hulman Institute of Technology.

The IRCBC was created to encourage scientific research by undergraduate students and to help them better understand the exciting educational and research opportunities that lie at the interface of biology and chemistry. An appreciation for laboratory research is central to a working understanding of experimental sciences such as biology and chemistry. By participating in research, students add to current knowledge and, furthermore, they enhance their education and broaden their understanding of the scientific method and its application.

Interdisciplinary research is gaining prominence in both academia and industry, as new techniques from one discipline are applied to problems in other disciplines. By acquiring experience in interdisciplinary research, students become more attractive to potential post-graduate programs and employers. The IRCBC program specifically fosters such interdisciplinary work, and we are pleased to highlight the research of our students, as well as the research of our colleagues in Indiana.

With this second annual event, we are delighted to welcome you, whether you are a member of the RHIT community, or you come from another institution. Our intention in hosting this event is to offer students an opportunity to share their research interests and progress with their colleagues in a nurturing and supportive environment, and to encourage celebration of the undergraduate research experience. We hope you enjoy the dynamic program of speakers.

Mark Brandt
IRCBC Program Coordinator

Ella Ingram
IRCBC Program Coordinator

Symposium Schedule

8:00 AM Registration

8:30 AM Opening Session

– *Welcoming Remarks* – Daniel Jelski, Department of Chemistry, Rose-Hulman Institute of Technology

– Scheduled Presentations

Computational Study of Selective Oxidation on Metals: Gas Phase Reactions of Hydrogen and Methane with Yttrium Monoxide
Shakeel Dalal and Eric Glendening*

Integration of Pathway Datasets Utilizing BioPAX Ontology
Christopher Nash and Keyuan Jiang*

The Tissue Specific Dehydrogenase Activity of 11-Hydroxysteroid Dehydrogenase Type 1
Angela Elsten, **Sean Vaughn***, and Kristy K. Miller*

Proteomic and Genomic Analysis of Leptin Induced Proliferation of Human Mammary Tumor Cells.
*Candida Perera, **Joshua Robertson***, Sulma I. Mohammed, and Ignacio G. Camarillo*

– Break

Site-Specific Fluorescent Labelling of the $GABA_A$ Receptor
Roger E. Wiltfong and Myles Akabas, and David Reeves*

Development of a HPLC Quickscreen for Ractopamine Hydrochloride in Tissues
Ian Dailey and Heather N. Shaw*

Disruption and Refolding of Human Estrogen Receptor Ligand Binding Domain Fusion Protein Aggregates
David Knapp, Carmen Du Vall, and Mark E. Brandt*

Membrane binding activity of the ligand-binding domain of the estrogen receptor under pH controlled conditions.
Carmen Du Vall, David Knapp, and Mark E. Brandt*

12:00 PM Lunch

1:00 PM Afternoon Session

– Scheduled Presentations

Characterization of a Female Rat Model of Childhood Onset Diet Induced Obesity
Chris Gottfried*, Maxine Nichols, and Ignacio Camarillo*

Cytoskeletal Proteins and Mitosis in Soybean Plants
Brayton A. Haag* *and Jeannie T. B. Collins*

The Effects of 3,4-Methylenedioxymethamphetamine (MDMA) and 5-Methoxydiisopropyltryptamine (5-MEO-DIPT) on the Adolescent Rat
Amanda Kleiman*, Charles Vorhees, and Michael Williams*

Soil Phosphorus Availability and Mycorrhizal Associations in the Root System of the Mayapple.
Mercedes Reeder*, **Christina A. Shook****, and Ella Ingram*

Enhancement of Wood Waste Conversion to Ethanol through Extruder and Microwave Pretreatment
Amanda Grantz* *and David J. Dixon*

– Break

Dehydration of Copper II Sulfate 5-hydrate ($CuSO_4 \cdot 5H_2O$) using Thermogravimetric Analysis
Micah Leestma* *and Joseph Bularzik*

The Role of Transition Metal Ion Binding in Oxidative DNA Damage
A. Mae Huehls*, Daniel L. Morris, Jr., Scott D. Noblitt†, and Penney L. Miller*

T cell-catalyzed H_2O_2 production: a novel pathway in inflammation or *in vitro* chemistry?
Derek W. Trobaugh*, Ellen F. Hughes, and Gabi N. Waite*

Use of Solution Phase and Solid Phase Chemistry to Create Substituted Pyrimdine Rings
Emily Maurer* *and* ***Jessica Wojtas****

Fabrication and Characterization of Nanostructured Conducting Polymer Films on the Surfaces of Microfabricated Neural Prosthetic Biosensors
Amber Brannan*, Sarah Richardson-Burns, and David Martin*

– Additional Abstract (not scheduled for presentation)

Fabrication of Microfluidic Devices
Shirley Crenshaw *and Daniel Morris*

Computational Study of Selective Oxidation on Metals: Gas Phase Reactions of Hydrogen and Methane with Yttrium Monoxide

Shakeel Dalal* and Eric Glendening
Department of Chemistry, Indiana State University, Terre Haute, IN 47809

Potential energy surfaces were calculated using computational chemistry methods for the YO+CH_4 and YO + H_2 reactions. While the process is endothermic and the reverse reaction is highly exothermic and therefore kinetically favored, this process has more possible future applications. Coupled cluster calculations with single, double, and triple excitations were performed and a complete basis set extrapolation taken. Zero point energies, spin orbit corrections and core correlation corrections were taken into account. Core correlation corrections were used to account for the variation of the Y frozen core versus the correlated core. Comparisons are made with respect to the synthesis of an alcohol from an oxidized metal atom. Comparisons are made to experimental works. Insertion into a C-H bond, as in CH_4 is kinetically unfavorable relative to insertion into an H-H bond, as in H_2. The two reactions are similar in kinetics, thermodynamics and electron redistribution with the exception of the initial energy barrier involved in both steps. The mechanisms both involve three steps. While a single step mechanism is possible, preliminary calculations show it to be energetically unfavorable.

Integration of Pathway Datasets Utilizing BioPAX Ontology

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Through the development of new technology over the past decades, a massive amount of biological data has been collected and stored in databases throughout the world. A significant amount of this collected data is represented in one way as biological pathways. With the existence of over 180 pathway databases‡, each with their own research focuses, it is becoming increasingly important for researchers to retrieve data from multiple sources. Although these sources make their data readily available, there is very little standardization for methods of modeling and accessing the data. This non-standard approach has made the retrieval of data from multiple sources exceedingly difficult.

Currently, there are very few projects underway to integrate the copious amounts of data and present it in a common format. It is with this in mind that we have developed a system to integrate biological pathway datasets using the proposed BioPAX standard. The PathwayGateway is a web based toolset that facilitates centralized access to various pathway datasets. Utilizing the BioPAX ontology and Web Services, the PathwayGateway exposes several methods which present the requested data in a machine processable format able to be consumed by the application running on different platforms.

As a community-developed ontology based upon the concept of Semantic Web for integrating and exchanging biological pathway data, BioPAX was chosen as a promising standard for integrating and sharing biological pathway data via semantics. An important consideration in determining which format to use is the ability to support machine inference/reasoning. The ability to relate one pathway to another is an invaluable function when searching or mining the available pathway datasets. Since Pathway data represents the interactions occurring in cellular processes, the machine logic must be able to step through those interactions in an efficient manner.

The use of Web Services supports the vision of Semantic Web by allowing the other web resources to access the data available through the PathwayGateway via standard web protocols. Due to the wide variety of system environments utilized by the research community, the use of open standards was an important consideration in the development process. The ability to migrate the data from one system to another underlines the sheer versatility of the PathwayGateway.

Version one of the PathwayGateway is available at http://jlab.calumet.purdue.edu/thegateway/v1/0/pathwaygateway.asmx?wsdl and offers general search functions including single item searches and category searches. Basic search functionality is the foundation for the more advanced features in later versions. Later phases of the PathwayGateway will provide graphics tools for viewing pathway components as well as tools to traverse between datasets. It is our intention that this application provides a centralized location where researchers can retrieve the required data and help to increase the understanding of pathway structures in the field of bioinformatics.

‡Pathway Resource List: http://cbio.mskcc.org/prl

The Tissue Specific Dehydrogenase Activity of 11β-Hydroxysteroid Dehydrogenase Type 1

Angela Elsten*, **Sean Vaughn***, and Kristy K. Miller.
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11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) catalyzes the conversion of inactive 11-keto-metabolites (cortisone and 11-deoxycorticosterone) into active glucocorticoids (cortisol and corticosterone) and has been shown to play a key role in metabolic disorders such as central obesity, insulin resistance, and Type II diabetes. Inhibition of 11β-HSD-1 has been proposed as a novel therapeutic in insulin resistant syndromes such as diabetes and obesity. In obese humans, the conversion of cortisone to cortisol by 11β-HSD-1 is impaired in the liver but is increased in adipose tissue. The mechanism for this is not well understood but reflects tissue specific regulation of 11β-HSD-1 activity. Therefore, understanding the tissue-specific functions of 11β-HSD-1 is important to gain insight into the approaches of target experimental and therapeutic manipulations. **The purpose of this study was to determine the tissue-specific metabolism of 11β-HSD-1.** The dehydrogenase activity of 11β-HSD-1 in liver, kidney, and testes tissue fractions was quantified by measuring the production of NADPH at an excitation wavelength of 340 nm and an emission wavelength of 460 nm using several substrates. Rat liver, kidney, and testis microsomal fractions of 11β-HSD-1 metabolized cortisol, however, only kidney microsomal fractions metabolized cortisone and corticosterone. Additionally, kidney microsomal fractions of 11β-HSD-1 exhibited the highest oxidation rate of the C_{27} hydroxysteroids, whereas the liver microsomal fractions of 11β-HSD-1 exhibited the highest oxidation rate of the C_{24} bile acids. Overall, the results indicate that 11β-HSD-1 exhibits tissue specific dehydrogenase activity in metabolizing various bioactive steroids.

Proteomic and Genomic Analysis of Leptin Induced Proliferation of Human Mammary Tumor Cells.

Candida Perera[1], **Joshua Robertson**[1]*, Sulma I. Mohammed[2] and Ignacio G. Camarillo[1].
[1]Department of Biological Sciences, [2]Department of Veterinary Pathobiology, Purdue University, West Lafayette IN, 47906.

Obesity is a risk factor for breast cancer incidence and is positively associated with breast cancer mortality. A characteristic of obesity is elevated circulating levels of leptin, an adipocyte-derived hormone that regulates energy expenditure and food intake. The lack of mammary development in leptin knockout mice and the resistance of these animals in developing mammary tumors suggest leptin plays an important role in normal and cancerous mammary epithelial proliferation. Given that the mechanisms involved in the relationship between obesity and breast cancer are unknown, our goal is to better understand the role of leptin in mammary tumor cell behavior. We hypothesize leptin alters distinct cell signaling pathways and regulates autocrine/paracrine mechanisms to affect tumor cell proliferation and migration. Towards addressing this hypothesis, we have used microarray technology to identify leptin regulated genes associated with mammary tumor proliferation and also real time PCR to confirm our microarray results. Treatment of MCF-7 human mammary tumor cells with leptin for 24 hr resulted in an increase in cell number, compared to controls. Previously, our lab had shown through 2D gel analysis, that secretion of a collagen precursor was upregulated by leptin treatment. Collagens are important regulators of the mitogenic and metastatic potential of tumor cells, this influence of leptin on collagen expression was further studied. MCF-7 cell lysates subjected to Western analysis showed leptin increased protein levels of Collagen I, III and IV. This data suggests leptin alters MCF-7 tumor cell behavior by regulating secreted growth factors as well as extracellular matrix proteins. To evaluate leptin's affect on MCF-7 cell gene expression we used the Eppendorf DualChip™ Cancer microarray. Overall we identified 30 leptin-regulated genes, 14 upregulated, and 16 down regulated, that include growth factors (such as Tumor necrosis factor superfamily 1a, which increases Tumor Necrosis Factor), extracellular matrix proteins (those like BSG, which stimulate matrix metalloproteinase synthesis, tumor progression) and genes associated with cell cycle (Villin 2, Cell surface structure, adhesion, migration and organization), cell proliferation (FOS was shown to regulate cell proliferation, differentiation and transcription), angiogenesis (VIM ECM adhesion), and metastasis. Using real time PCR we have analyzed several genes recognized by the microarray as being up or down regulated. Together, our data suggest leptin may regulate mammary tumor cell behavior through multiple mechanisms. In addition to activating mitogenic signaling pathways, leptin regulates the expression and secretion of proteins and cytokines that may work in an autocrine or paracrine manner to stimulate mammary cell proliferation. The identification of leptin-regulated factors begins to provide mechanistic links involved in the relationship between obesity and breast cancer incidence and morbidity.

Site-Specific Fluorescent Labelling of the $GABA_A$ Receptor

Roger E. Wiltfong* and Myles Akabas, and David Reeves
Department of Physiology and Biophysics, Albert Einstein College of Medicine of Yeshiva University, New York, NY

The $GABA_A$ receptor ($GABA_A$-R) is a neurotransmitter-gated ion channel of the superfamily including nicotinic acetylcholine receptors (nAchR), 5-HT_3 receptors, and glycine receptors. The receptors consist of 5 subunits, each with a large N-terminal extracellular, ligand-binding domain, and an integral membrane channel domain. The lining of the ion channel is made up from five α–helical M2 transmembrane segments. When neurotransmitters of the superfamily bind to their respective receptors, the proteins undergo a conformational change that opens the ion channel, depolarizing the membrane. The precise mechanism by which this occurs remains unclear. We therefore wish to measure the distance the M2 segment moves in relation to other parts of the receptor. Distances can be measured using fluorescence resonance energy transfer, (FRET) which requires the site-specific attachment of two different fluorophores. The M2 segment may be site-specifically labelled by reaction of engineered cysteines with a thiol reactive fluorophore. To attach the second fluorophore, at the C-terminus of the $GABA_A$-R γ_2 subunit, we have inserted a short amino acid sequence from muscle-type nAchR which has been shown to bind α-bungarotoxin (BTX) with high specificity and affinity (Kd ~10^{-9} M).
Initially, we expressed GABA-R containing the mutated subunit ($GABA_A$-γ_2cBTX) in albino *Xenopus* oocytes by injecting the mRNA directly into the cell. After using two-electrode voltage-clamp recording to determine the presence of functional mutated receptors, we incubated the oocytes with Alexa Fluor 647-BTX, followed by a series of washes to remove unbound BTX. However, a specific fluorescence signal from oocytes could not be distinguished from cellular autofluorescence in either the mutant $GABA_A$ receptor, or nAchR expressed as a positive control. Autofluorescence was reduced significantly by sub-cloning the receptor subunits into the mammalian expression vector pcDNA3.1(+) and expressing the receptors in human embryonic kidney cells (HEK293). Epifluorescence imaging of HEK cells incubated in Alexa Fluor 647-BTX indicated that BTX binds specifically to the nAchR, however there was no visible binding to receptors containing $GABA_A$-γ_2cBTX. We conclude that unmodified $GABA_A$-γ_2cBTX does not bind BTX, and cannot therefore be used for FRET experiments.

Development of a HPLC Quickscreen for Ractopamine Hydrochloride in Tissues

Ian Dailey* and Heather N. Shaw
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Ractopamine HCl is a β-agonist performance enhancer and growth promoter developed by Elanco Animal Health. It is currently approved by the FDA for use in cattle and swine. The existing Elanco method for the determination of ractopamine residues in tissues (Method B03903) provides absolute recoveries of 88% or greater, with relative standard deviation (RSD) of less than 6%. Although this method has been validated and shown to be effective, it is somewhat time-consuming. The utility of B03903 would be improved if it could be adapted to a quickscreen application while still maintaining acceptable levels of recovery and precision. An overall project goal was to completely assay 20 tissue samples for ractopamine HCl in a typical 8-hour workday. Using B03903, this was nearly impossible. Improvements in tissue extraction, sample preparation, and sample analysis were made to reduce the overall method time, making it possible to easily assay 20 samples in 8 hours. Chicken liver was used during development because it was a suitable generic tissue matrix and was readily available. Assaying fortified chicken liver samples using the new method provided recoveries of 99.9% ± 2.1% RSD and required less than half the time, fewer materials, and less waste disposal than B03903.

Disruption and Refolding of Human Estrogen Receptor Ligand Binding Domain Fusion Protein Aggregates

David Knapp*, Carmen Du Vall, and Mark E. Brandt
Department of Chemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The human estrogen receptor ligand binding domain (ER LBD), an independently folding region of the Human Estrogen Receptor, has been successfully expressed in *E. coli* TOPP2 cells as a fusion protein with maltose binding protein (MBP). However, upon initial purification, a significant percentage of the fusion protein is aggregated and inactive, either due to initial misfolding or because of unfolding and subsequent aggregation prior to cell lysis. Under optimum conditions approximately half of the wild type protein remains folded. However, significantly poorer yields of folded protein are observed during purification of less stable mutant constructs, making recovery of the aggregated material potentially more profitable. Disruption of these aggregates was therefore attempted with urea as a denaturant. Slow reduction of the urea concentration via dialysis resulted in the refolding of a substantial percentage of fusion protein, as shown by size-exclusion HPLC analysis. Altering the urea concentration, incubation temperature, and dialysis temperature led to varied levels of recovery. Presence of the ligand, estradiol, also appeared to enhance refolding. Elevated temperatures and high urea concentrations displayed dramatically poorer results, suggesting that MBP plays an important role as a chaperone in LBD refolding. With the currently tested conditions, up to 12% recovery was achieved from samples initially composed entirely of large aggregates. Further research will include the testing of additional ligands and refolding conditions, as well as modification of optimal conditions to scale up throughput. Also, it will be necessary to determine if standard methods of purification, such as centrifugation and anion-exchange chromatography, will be suitable for the removal of mid-sized aggregates formed during the refolding process. The refolding of aggregated protein demonstrated in this study has the potential to significantly increase the yield of mutant ER LBD preparations, making these constructs easier to study. The successful formation of dimers from a completely aggregated state also suggests that the folded structure represents the global energy minimum for the ER LBD protein.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Membrane binding activity of the ligand-binding domain of the estrogen receptor under pH controlled conditions.

Carmen Du Vall*, David Knapp, and Mark E. Brandt
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The estrogen receptor is a protein that has many beneficial effects for women, including its roles in preserving bone strength and controlling cholesterol production. Unfortunately, the estrogen receptor stimulates cell proliferation that can, if one or more breast cells has mutated, develop into breast cancer. Because estrogen can promote the development of breast cancer, substances that block the action of estrogen might be helpful in preventing or treating breast cancer.
The estrogen receptor is a soluble protein that, when combined with membrane vesicles in a pH controlled buffer, is capable of binding to the vesicles. This particular protein exhibits unusual behavior by physically binding to vesicles in what is still an unknown manner. One way to determine the presence of protein in a solution is to run an SDS-Page Electrophoretic gel. The results of a gel can confirm the presence of protein, as well as give reasonable estimations of the relative amounts of protein in each sample. However, the process of running a gel is slow and high concentrations of protein are required. A very simple, fast and effective method is needed to accurately determine low concentrations of protein in a sample. One such method is the Bradford Assay, a procedure that utilizes dye to form a complex with proteins and then a spectrophotometer to read the corresponding absorbance of the protein in solution. The purpose of this experiment was to determine whether the trend observed in the gel-based assay could be reproduced using the Bradford Assay.
Several control experiments were run at each pH in order to observe an accurate protein concentration. The micro-assay combines the ligand-binding domain of the estrogen receptor with membrane vesicles in 100 mM acetate buffers ranging from pH 5-7 (in increments of 0.5). After incubation and centrifugation, the soluble and membrane-associated fractions were separated and combined with an equal volume of Bradford reagent, and then incubated for ten minutes before their absorbance readings were measured.
The Bradford Assay results indicated that the protein had difficulty staying in the buffered solution, pointing toward solubility (precipitation) problems, or those dealing with the protein "sticking" to the sides of the tubes. The observations obtained from the protein gel indicate that as the pH of the acetate buffer decreases, the protein binds to the vesicles and pellets out, leaving less protein remaining in the supernatant. Therefore, the eppendorf tubes used throughout the experiment were siliconized to prevent the protein from sticking to the sides of the tube. Even after this procedure, however, the data did not follow the predicted pH-dependent protein activity as suggested by the observations from the gel-based assay. Determining the source of the discrepancy between the dye-binding and the gel-based assays will require further work.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Characterization of a Female Rat Model of Childhood Onset Diet Induced Obesity

Chris Gottfried*, Maxine Nichols, and Ignacio Camarillo
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Over the last two decades, obesity has grown to epidemic levels in the United States. Obesity in the population is prevalent not only in adults, but is also increasing among children. Obese individuals (this relationship between childhood obesity and cancer has not been extensively established).suffer an increased risk of developing type 2 diabetes, coronary heart disease, and several forms of cancers. Based on these evidences, we are interested in studying the relationship between childhood obesity and the development of breast cancer. To accomplish this, a rat model of obesity was developed. Briefly prepubertal rats were randomly placed on a high fat (41%), high carbohydrate (29% sucrose) western diet or a normal rat chow diet. Rats were weighed twice a week for approximately 5 weeks. At 53 days of age, the 5 highest and 5 lowest weight gainers of the Western Diet fed animals were designated Obese and Lean Diet Resistant, respectively. With Western Diet animals had a significantly higher increase in % body mass gain , 388.6 + 10.5%, from day 0 of diet as compared to Lean Diet Resistant 333.6 + 8.7% and Lean Rat Chow Control rats, 332.0 + 10.1%. DEXA scans determined the correlation between % body mass gain and % body fat composition. Obese rats had a significant 2-fold higher % body fat mass, 17.4+1.1%, as compared to Lean Diet Resistant, 9.2+1.2%, and Lean Control 9.0+1.6%, rats. RIA's were used to determine differences in comorbidity factors. Obese rats on the Western Diet had significantly higher counts of Leptin, Insulin, Glucose, and Adiponectin, than rats on the Rat Chow diet. Finally, rats on the Western Diet suffered an increase incidence of tumor development upon injection with MNU at 64 days old. These results demonstrate that consumption of Western style diet early in development induces significant changes in various physiological parameters including fat mass, puberty onset and comorbidity factors in Sprague-Dawley female rats. This model exhibits the same characteristics of human obesity and therefore provides an excellent system to study the relationship among Western Diet, early onset obesity and cancer.

Cytoskeletal Proteins and Mitosis in Soybean Plants

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Kinesin is a cytoskeletal protein that acts as an intracellular transport system moving cellular organelles and other cellular material from one point to another within cells. Kinesin is also involved in the separation of the sister chromatids that takes place during cell mitosis. There are a number of kinesin proteins in the kinesin family and which member is involved in which function is being studied. Plant and animal cells replicate in nearly identical ways and use similar proteins to do so. With this knowledge, work was done on *Glycine max*, commonly known as soybean. Using the western blotting technique, samples of soybean plants were taken from areas that were at different developmental stages. Samples were obtained from the apical meristem, the leaves, and two regions of the stem to determine just how much kinesin was present and therefore give insight into kinesin's role in the plant. It is hoped that this information can be used to further our knowledge of the proteins in cellular replication, development, intracellular movement in plants, and to provide insight into a variety of research areas involving these processes.

The Effects of 3,4-Methylenedioxymethamphetamine (MDMA) and 5-Methoxydiisopropyltryptamine (5-MEO-DIPT) on the Adolescent Rat

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The abuse of psychostimulants and hallucinogens is a significant problem throughout the world, however little is known scientifically about the effects of many of these drugs. Through neurochemical alterations and activation of the HPA axis, these drugs are believed to disrupt the normal course of development for neurochemical and hormonal systems and result in behavioral abnormalities. The aim of this research is to measure the persistent consequences of neonatal exposure to MDMA and/or 5-MEO-DIPT on brain development. Male and female rats dosed 4 times daily from P11-P20 were observed for changes in weight gain, anxiety, locomotion, cognition, and memory using seven behavioral tests. All experimental groups showed a significant decrease in weight gain with 5-MEO-DIPT having the greatest effect. Animals dosed with MDMA showed heightened anxiety while 5-MEO-DIPT animals demonstrated lowered anxiety. All experimental groups showed decreased spatial learning with MDMA producing the greatest deficit. MDMA animals also displayed reduced path integration learning while other experimental groups showed no significant change in this type of learning.

Soil Phosphorus Availability and Mycorrhizal Associations in the Root System of the Mayapple.

Mercedes Reeder*, **Christina A. Shook***, and Ella Ingram
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Podophyllum peltatum, the common mayapple plant, is a model organism for many traits exhibited among clonal plants. Mayapple has an intricate and complex association in its root systems with arbuscular mychorrhizal (AM) fungi. These fungi colonize the root systems in the generational ramets (*i.e.*, iterated units) of the plant, and it has been suggested that a mutualistic relationship exists between the two, with the plant providing carbon for symbiotic fungi, and the fungi providing inorganic compounds to the plant. Soil phosphorus levels and AM colonization of mayapples were observed to demonstrate the presumed mutualistic aspect of this association. We hypothesized that patterns in soil phosphorus levels could indicate reasons for corresponding AM presence or absence in the roots of individual ramets. We tested two protocols for assessing soil phosphorus level, based on the soil type present in the Western Woods of the Rose-Hulman Institute of Technology campus, where soil and plant samples were obtained. After the initial test run, the Mehlich 3 method of quantifying soil phosphorus was used exclusively because of its higher reliability and the inclusion of less hazardous chemicals in the quantification procedure. The data obtained from the Mehlich 3 method compared well with results obtained from an outside soil testing lab ($r = 0.545$, $p < 0.001$, $n = 35$). These results demonstrate that a small scale bench-top procedure in adequate for soil phosphorus quantification, with considerable savings over agriculturally focused testing services. Analysis of the soil phosphorus data showed that there was no statistically significant relationship between the ramet position and the amount of phosphorus in the surrounding soil ($F_{3,6} = 0.290$, $p > 0.50$, $\eta^2 = 0.126$), contrary to our expectation that soil phosphorus would differ under individual ramets. The colonization patterns we observed in our sample were consistent with previously demonstrated patterns from different times in the year and different locations. However, there was no statistical relationship between soil phosphorus and root colonization, suggesting that factors other than soil nutrient availability may be regulating this mutualistic relationship. Future work will include manipulations of soil phosphorus, soil moisture, and root colonization levels, to experimentally examine the environmental factors influencing this association.

This work was funded in part by the IRCBC under the auspices of the Merck/AAAS Undergraduate Science Research Program.

Enhancement of Wood Waste Conversion to Ethanol through Extruder and Microwave Pretreatment

Amanda Grantz* and David J. Dixon
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A twin screw extruder and traditional countertop microwave oven were explored as means of pretreatment in the conversion of wood waste to ethanol. Enzymatic hydrolysis was employed to quantify glucose recoveries for particular pretreatment schemes, and simultaneous saccharification and fermentation predicted ethanol yields. Independently, extrusion proved more effective than microwave irradiation in enhancing cellulase enzyme recovery of glucose for fermentation. Mechanical breakdown of cell structure in extruded wood produced glucose recoveries 10 to 20 times greater than those obtained from raw feed material. Wood entering the extruder at lower feed rates was eluted as a much finer material and gave an additional two fold increase in recoveries. Maximum recoveries were obtained with a combination of extruder and microwave pretreatments. Microwave experiments on feed and extruded wood were designed and analyzed with Design Expert software. Relationships among time, power, and wood moisture content proved to be complex, calling for further investigation. Scanning electron microscopy revealed significant structural changes in extruded and microwaved wood.

Dehydration of Copper II Sulfate 5-hydrate ($CuSO_4 \bullet 5H_2O$) using Thermogravimetric Analysis

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Department of Chemistry and Physics, Purdue University Calumet, Hammond, IN 46323

Previous research was conducted at Purdue University Calumet investigating the dehydration of copper II sulfate powder by means of thermogravimetric analysis (TGA). The results showed a mass percent *vs*. temperature dehydration curve similar to the accepted curves from many literature sources. Samples of the powder were then dehydrated at elevated temperatures. TGA results verified the loss of the water. The samples were then left in the open air of the laboratory to re-hydrate. Samples were weighed daily to determine when, and if, all of the lost water mass would be regained. Full re-hydration was achieved in a few days. Once this was achieved, TGA was run on the re-hydrated samples. The results showed that the dehydration curve was different for a re-hydrated sample. The first four water molecules dehydrated at a lower temperature than the original powder, and the fifth dehydrated at the same temperature. X-ray of anhydrous, hydrated, and re-hydrated powders was performed to determine differences in their respective crystal powder patterns. The pattern for the hydrated powder was the same as the pattern for the re-hydrated powder. The anhydrous was different because water was completely removed from the crystal. IR was also done. These results were similar to the X-ray results in that the hydrated and re-hydrated patterns were similar, and the anhydrous pattern was different.

The same dehydration procedure was performed on copper II sulfate crystals. These crystals were grown from $CuSO_4$ solution, and TGA was run on samples. The results showed a mass percent vs. temperature curve similar to that of $CuSO_4$ powder. After complete dehydration, these took over a month on average to fully re-hydrate in open air. TGA was then run on the re-hydrated crystals. The dehydration of re-hydrated crystals was similar to the dehydration of the re-hydrated powder.

Upcoming work with copper II sulfate will be to examine hydrated crystals and re-hydrated crystals by X-ray, and compare the two crystal patterns. This will test our hypothesis that the first four waters in copper sulfate 5-hydrate re-hydrate to a different lattice position, a position that has a weaker bond, than the original crystal, and the fifth dehydrates to the same position. The hypothesis would explain why the first four waters dehydrate at a lower temperature in a re-hydrated crystal, but the fifth dehydrates at the same temperature. Copper II sulfate is being further investigated using the technique of solution calorimetry. This will show the heats of reaction of copper II sulfate dissolving, which can determine the strength of the bonding within the salt. The original, dehydrated and re-hydrated salts are being tested.

The Role of Transition Metal Ion Binding in Oxidative DNA Damage

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Oxidative DNA damage resulting from the actions of reactive oxygen species (ROS) is associated with numerous clinical conditions. Several transition metal ions, including Fe(II), Cu(II), and Cr(III), react with H_2O_2 via the Fenton reaction or "Fenton-like" reactions to produce hydroxyl radicals (•OH), which are most likely the predominant ROS involved in oxidative damage. In the presence of DNA, the Fenton reaction is highly site specific, with guanine being converted to 8-hydroxy-2'-deoxyguanosine (8-OH-dG), an accepted biochemical indicator of oxidative DNA damage. In this study, reactions of Fe(II), Cu(II), and Cr(III) with H_2O_2 were allowed to occur in the presence of the nucleoside 2'-deoxyguanosine (dG) and the nucleotide 2'-deoxyguanosine-5'-monophosphate (dGmp). The roles of metal ion binding to the guanine base and to the associated phosphate in the formation of oxidative damage products were distinguished by varying the order of metal ion addition to the nucleoside/nucleotide solutions, with metal ions being added either before or after the addition of H_2O_2. The amounts of 8-OH-dG produced in the various reactions were quantified with HPLC analysis with absorption detection. For Fe(II), metal ion binding to guanine base was associated with 8-OH-dG production for both forms of the guanine bas. However, comparatively larger 8-OH-dG yields were obtained with dGmp, suggesting that Fe(II)-phosphate interactions may intensify •OH formation. Cu(II) appears to bind with the guanine base for both dG and dGmp. In the case of Cu(II), metal-phosphate interactions, though possibly involved in the mechanism of ROS formation, do not appear to affect the levels of 8-OH-dG formed. For Cr(III), dramatically larger 8-OH-dG yields from reactions of dGmp indicate that oxidation is facilitated by the binding of the metal ion with the phosphate rather than with the guanine base itself. In addition, preliminary efforts were made toward developing a method with which to directly quantify •OH formation. Such an assay would allow for more accurate determination of the extent of the Fenton reactions observed in these systems as well as aid in elucidating the mechanism by which Cu(II) generates oxidative damage products.

References:
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T cell-catalyzed H_2O_2 production: a novel pathway in inflammation or *in vitro* chemistry?

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T cells are important immune cells involved in inflammatory response. During the inflammatory response, T cells are exposed to H_2O_2 that is produced by other inflammatory leukocytes, but they can also produce H_2O_2. H_2O_2 was primarily recognized as a toxin causing oxidative stress and contributing to numerous diseases and aging. But H_2O_2 has another opposing role as an important and beneficial molecule that is involved in cellular signaling. The objective of our study is to investigate the role of the T cell receptor (TCR) for catalyzing H_2O_2 production by comparing the H_2O_2 production of T cells with mutated TCRs, and of T cells with TCRs that are shielded from light, with the H_2O_2 production of normal T cells. We report on a potential mechanism for these effects by showing the light- and singlet oxygen-dependent H_2O_2 production by T cells. T cell membranes (TMs) were purified from parental and mutant Jurkat cells that were cultured in RPMI 1640 plus 10% FBS using a phase separation protocol. To study the biophotonic H_2O_2 production, 50 μl TMs in PBS were placed in borosilicate vessels and exposed in a photomicroreaction to a uniform light flux of ultraviolet light or white light. H_2O_2 concentrations were assayed using the H_2O_2 indicator Amplex Red. As negative controls, samples with PBS, heated and UVC irradiated samples, and samples shielded from light were used. Additional samples contained albumin, myoglobin, aprotinin, beta 2 microglobulin are used as negative controls. We found that Jurkat TMs in PBS produce about 130 nM H_2O_2/min/mg protein for up to 24 hours when pretreated with NaN_3. No H_2O_2 was detected after catalase addition or in samples shielded from light. When we exposed samples, in which we partially shielded TCRs from light by TCR specific antibodies, we obtained about 20% reduction in the H_2O_2 production rate. Interestingly, the H_2O_2 production in TMs from Jurkat cells with a TCR mutation at the postulated catalytic site was not different than the H_2O_2 production of the parental cell line. These results let us hypothesize a different molecular mechanism for the membrane-associated H_2O_2 production in T cells than proposed by others. Our hypothesis is supported by the fact that heating of samples (5 min., 100 °C) and irradiation with UVC did not destroy the catalytic H_2O_2 production as predicted. Our results confirm the novel idea that activated T cells produce H_2O_2 in a membrane-associated event. The TCR seems to contribute to this activity, but additional sources of cell-produced hydrogen peroxide are possible. Preliminary results show when TMs are treated with a general serine protease inhibitor, there is an approximate 40% reduction in H_2O_2. Additional studies are needed to determine which proteins are affected by the inhibitor. We analyzed the contribution of NADPH oxidase, an enzyme known to produce H_2O_2 in phagocytotic white blood cells, to our light-dependent H_2O_2 production of T cells. Preliminary results indicate a potential contribution of this enzyme to our observed H_2O_2 production. Our data provides important insights into the role of H_2O_2 in inflammatory T cells and the mechanism of the singlet oxygen H_2O_2 production. Since light and electromagnetic fields are known to increase singlet oxygen concentration, we anticipate the development of new therapeutic tools for T cell based immune and inflammatory diseases.

Use of Solution Phase and Solid Phase Chemistry to Create Substituted Pyrimdine Rings

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Pyrimidine rings are components of the nucleic acids thymine, cytosine, and uracil. Pyrimidine rings are synthesized by using solution or solid phase synthesis. While solution phase chemistry is done in solution, solid phase chemistry is carried out on a solid support. Wang resin was the solid support used in these experiments. Using solid phase synthesis to create pyrimidine rings allows for better and more efficient methods to synthesize substituted pyrimidine rings. Solid phase chemistry eliminates the need for purification processes and can be easily mechanized. Various methods and reactions to create nitrogen-containing rings were explored in solution and solid phase synthesis. Solution phase synthesis was used to ascertain which types of reactions would be most successful in solid phase synthesis. If more efficient methods of creating these rings were discovered, it would benefit several industries. Pyrimidine rings are components of many pharmaceuticals and photographic chemicals. Several methods in both solution and solid-phase were successful. By using published literature, known experiments could be used and adapted to create the desired molecules, nitrogen containing rings. Various rings not described in these articles were created.

Fabrication and Characterization of Nanostructured Conducting Polymer Films on the Surfaces of Microfabricated Neural Prosthetic Biosensors

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When designing microfabricated neural prosthetic devices for implantation into the Central Nervous System (CNS) it is important to accommodate for differences in mechanical properties at the electrode/tissue interface. Biocompatibility and low electrical impedance at the interface are central to the ability of the device to function. In previous studies from our lab, the conducting polymer poly(3,4-ethylenediocythiophene) (PEDOT) was electrochemically deposited on neural microelectrodes in the presence of cross-linked hydrogels to produce soft, low impedance materials with the potential to improve device performance after implantation. The focus of this project is to evaluate the role of hydrogel concentration and cross-linking on the structure and properties of molecularly-thin PEDOT networks deposited in the hydrogels, alginate and poly(vinyl alcohol) (PVA). The electrical properties of these PEDOT networks were studied using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The EIS results show that a 60 minute deposition of PEDOT in cross-linked 1% PVA gave the lowest impedance at the CNS-relevant frequency of 1 kHz. CV results indicate that this PEDOT structure has the greatest charge capacity. Surface area quantification supports the idea that the lowest impedance and highest charge capacity corresponds to the largest PEDOT network.

Fabrication of Microfluidic Devices

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Microfluidic Devices are on the cutting edge of technology. The have small sample and waste volumes, portable, and multifunctional. In my research, it was demonstrated that solutions can be moved in the microchannel of a glass microfluidic device using an applied voltage. Also, the fabrication of porous frits, that would be used to hold packing in a microchannel, was investigated. The potential goal is to use the microfluidic devices to perform capillary electrochromatography in a microchannel to separate physiologically relevant modified nucleosides that serve as a marker for many diseases and clinical conditions.

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