7th Annual IRC Undergraduate Research Symposium

Friday
October 22, 2010

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Welcome to the

7th Annual IRC Undergraduate Research Symposium

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We are honored to welcome you to the 7th Annual IRC Undergraduate Research Symposium and we sincerely appreciate your participation. The symposium is coordinated by the Interdisciplinary Research Collaborative (IRC), which is supported by funding from the Lilly Endowment, the Lilly/Guidant Applied Life Sciences Research Center, and Rose-Hulman Institute of Technology. The IRC would like to express its great appreciation for the Symposium sponsorship of Edwards Lifesciences.

The IRC was created to encourage scientific research by undergraduate students and to help them better understand the exciting educational and research opportunities that exist in science and engineering. An appreciation for laboratory research is central to a working understanding of experimental sciences. 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 IRC 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 seventh annual event, we are delighted to welcome you. 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
IRC Program Coordinator

J. Peter Coppinger
IRC Program Coordinator

Rose-Hulman Institute of Technology
Symposium Schedule

8:15 AM Registration

8:30 AM Welcoming Remarks

Morning Session (8:45 – 10:30 AM)

From Waste to Wall; Turning Filter Cake into Drywall
Jessica Zais* and Michael Mueller
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Synthesis and Testing of Tamoxifen Polymer Conjugates
Amanda Jevons* and Ross Weatherman
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Effect of Low Oxygen on Monocyte-Derived Macrophages and Their Susceptibility to Biophysical Regulation
Spencer J. Fox, Lee R. Waite, Henry O. Owegi, Stéphane Egot-Lemaire, and Gabi N. Waite

High Temperature Electrophoresis in Microfluidic Devices
Steven Marczak, Indranil Mitra, and Stephen C. Jacobson
Departments of Chemical Engineering and Chemistry & Biochemistry, Rose-Hulman Institute of Technology, and Department of Chemistry, Indiana University, Bloomington, IN 47405

A Procedure to Quantify Gait Abnormalities after Sciatic Nerve Section in Rats
Julianna Barr, Donna Marsh, and Jameel Ahmed
Department of Applied Biology and Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Trap Grease Based Biodiesel
Othmane Fathi* and Michael Mueller
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The Redistribution of Calpastatin in MCF7 Breast Cancer Cells Induced by Spleen Tyrosine Kinase Syk
Molly Gillam, Bei Fei, Dr. Robert Geahlen

1Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, 2Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907
12:30 PM – 1:30 PM Invited Speaker: Dr. Derek Kingsley, 
Assistant Professor of Physical Education 
Indiana State University 
Fibromyalgia and Resistance Exercise: 
Effects on Autonomic and Vascular Function

Afternoon Session I (1:45 – 3:15 PM)

Development and Assessment of Low-Cost Media for the Production of 
Microalgae-based Biodiesel 
Mitch Braddock* and Peter Coppinger 
Department of Applied Biology, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Angiogenic Provisional Matrix Accelerates Maturation and Healing in Type 
II Diabetic (db/db) Mouse Model 
Bradley A. Herbig*1, Swathi Balaji*2, Rahul D’Mello*2, and Daria A. Narumoneva*2 
1Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 
47803, 2Department of Biomedical Engineering, University of Cincinnati, Cincinnati, OH 
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Examining the Viability of Chlorella vulgaris as a Source of Biofuel and 
Instrumentation for Nile Red 
John Lawrence* and Ric Anthony 
Department of Applied Biology and Biomedical Engineering, Rose-Hulman Institute of 
Technology, Terre Haute, IN 47803

Synthesis of Hyperbranched Polyglycerols to Attach to Tamoxifen Analoggs 
Vince Biondo*, and Ross Weatherman 
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, 
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Non-Classical Estrogen Signaling in Breast Cancer 
Kaci Blumenstock* and Ross Weatherman 
Department of Chemistry and Biochemistry, Rose-Hulman Institute of Technology, Terre 
Haute, IN 47803

Analysis of a Plasmid Encoded Type IV Secretion System in Burkholderia 
Suzanne Kissel*1 and Beth Traxler*2 
1Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, 
IN; 2Department of Microbiology, University of Washington, Seattle, WA 98195

Afternoon Session II (3:30 – 5:00 PM)

Synthesis and Characterization of Ampicillin-Loaded poly(DL-lactide-co-
glycolide) (PLGA) Nanoparticles for Drug Delivery 
Yile Gu* and Scott J. McClellan 
Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 
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A Biomechanical Analysis of Implantation-Induced Cup Deformation in Acetabular Cup Designs
Mary E. Jones*, S. R. Small, J. B. Meding, and K. S. Toohey
Joint Replacement Surgeons of Indiana Foundation in conjunction with Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Molecular Docking Simulations for Organic Compounds to the Estrogen Receptor Alpha
Melissa Galey*, Mark E. Brandt and Yosi Shibberu
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Synthesis of Carbon Cryogel Microspheres for Use in Chemical Separations
Gregory Blachut1*, and Justin W. Shearer2
1Department of Chemical Engineering and 2Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology Terre Haute, IN, 47803

Electrochemical Adsorption of Metal Cations Using Carbon Cryogel
Daniel Lee1*, and Justin W. Shearer2
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Understanding Antioxidant Activity of Selenium Compounds by Monitoring Production of 8-Hydroxy-2´-Deoxyguanosine from the Nucleoside 2´-Deoxyguanosine-5´-Monophosphate
Cari Harper* and Daniel L. Morris, Jr.
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Detecting Low Levels of Atrial Natriuretic Peptide using nano-LC-MS
Carol Baehr1*, H. Robert Bergen, II1, Carrie Holtz-Heppelman2, Linda Benson2, Denise Heublein3, John Burnett5
1Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN; 2Department of Biochemistry and Molecular Biology and 3Division of Cardiovascular Disease, Mayo Clinic, Rochester, MN

Synthetic Studies Toward Dioicine and Analogs
Breanna L. Wyman* and Richard W. Fitch
Department of Chemistry and Physics, Indiana State University. Terre Haute, IN

Selective Lithiation of 2,6-Dichloropyridine.
Chase A. Buchanan,*, Andrew L. Kast, and Richard W. Fitch
Department of Chemistry and Physics, Indiana State University. Terre Haute, IN

The Autodigestion Hypothesis: Cleavage of the Ischemic Intestine
Hobey Tam*, Marisol Chang2, Geert W. Schmid-Schönbein2
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From Waste to Wall; Turning Filter Cake into Drywall
Jessica Zaiss* and Michael Mueller
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Calcium sulfate di-hydrate, commonly known as gypsum, is a crystalline substance used for the production of drywall. The water in the crystal acts as a fire retardant; as the wall heats up in a fire, the water vaporizes before the wall catches fire allowing more time for an occupant to escape a burning home. Producing gypsum from a calcium based source would provide a means by which a waste product could be transformed to a more useful and potentially profitable substance. The material used is in this study was waste filter cake from Southern Indiana Gas and Electric and is 29.63% by weight calcium oxide. The method involved the dissolution of the calcium oxide into ions in an acidic environment and creating a water soluble calcium salt that would then ionize in water. Sulfate ions were introduced via sulfuric acid and the precipitated calcium sulfate was washed with acetone, filtered, and collected. Once the acetone had evaporated from the sample, analysis by x-ray diffraction determined the final product was calcium sulfate di-hydrate, i.e. gypsum. This method yields over 99% conversion to calcium sulfate based on weight; unfortunately this calculation does not take impurities into account. Further analysis by x-ray diffraction confirmed that the solids that precipitate are mostly gypsum but it is possible that calcium sulfate hemi-hydrate is also present.
Synthesis and Testing of Tamoxifen Polymer Conjugates
Amanda Jevons* and Ross Weatherman
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Tamoxifen is a commonly used drug to treat estrogen receptor positive breast cancer, but a common problem with this treatment is the development of resistance to tamoxifen. Previous work in the Weatherman lab has found that attaching tamoxifen to a polymer overcomes these resistance mechanisms. The purpose of the research done was to synthesize tamoxifen derivatives that could be attached to polymer scaffolds that have a higher potency. The major shortcoming of the current conjugates is that they are prone to aggregation. The hypothesis is that making new conjugates with more hydrophilic linkers would decrease aggregation and increase potency. Analogs with PEG 2 and PEG 5 hydrophilic linkers were created and tested in MCF7 cells to assess their potency. The PEG 2 hydrophilic linker product attained a 0.12 gram yield and an IC 50 value of 2.7 E-08 ± 1.12 E-08 nm after being tested in cell-based assays. Additionally, the PEG 5 hydrophilic linker product yielded 0.10 grams and had an IC 50 value of 1.4 E-08 ± 7.2 E-09 nm. Based upon these values, the tamoxifen derivatives created were able to get into the cell with relatively high potency as desired potency values fall within 1.0 E-08 to 5.0 E-08 nm. Currently, a procedure for attaching larger hydrophilic linkers is undergoing. Furthermore, attachment of these derivatives to polymer scaffolds in order to test their activities via biochemical and cell-based assays and aid in the overcoming of resistance mechanisms is currently in the process of being explored.

This work was funded by NIH (R01DK075376).
Effect of Low Oxygen on Monocyte-Derived Macrophages and Their Susceptibility to Biophysical Regulation

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O2 concentrations normally experienced by mammalian cells in the body are typically between 1 and 13% O2, which is significantly lower than ambient air (21%). There also can be a disparity between O2 levels over time. For instance, monocytes/macrophages encounter a dramatic drop in O2 partial-pressure when leaving the blood vessel and entering inflamed tissue. Hence, it is crucial to study their behavior under varying O2 conditions. In reality, however, most studies use classic cell culture methods that employ 21% O2 and hence simulate hyperoxic conditions. The objective of this study was to analyze the impact of low O2 conditions on the oxidative burst activity of monocyte-derived macrophages and to test their susceptibility to a biophysical therapy with low frequency electromagnetic fields. We used THP-1 cells, treated for 48 hours with PMA to form naive macrophages. Results were validated with untransformed cells from human blood. To create low O2 culture conditions, we used a hypoxia chamber that slowly established hypoxia (several hours) and a two-enzyme system that quickly and flexibly established steady-state low O2 conditions (minutes). The enzyme system consists of glucose oxidase and catalase for which the overall reaction consumes O2. Oxygen conditions were validated using a microsensor (Lazar Research Labs) and modeled using Matlab. The sensing of low O2 by the cells was validated by detection of hypoxia-inducible factor (HIF-1α) as analyzed by Western Blot. Oxidative burst activity was quantified by the cells’ release of H2O2, which was detected by luminol/hypochlorite-enhanced chemiluminescence. Degradation of H2O2 was used to estimate cellular catalase. The RayBio® inflammation antibody array was used to analyze changes in 42 cytokines. To test the sensitivity of low O2 cells to therapeutic electromagnetic fields, cells were exposed to a variety of magnetic fields and controls. We found that macrophages increased their oxidative burst activity up to 3-fold when cultured under quickly induced hypoxia, and that slowly induced hypoxia did not provide the same trigger. In both cases, cells responded with upregulation of HIF. Cells protect themselves from oxidative stress by increasing their endogenous catalase activity. Low O2 conditions significantly increased several inflammatory cytokines (IL-6, IP-10, MIP and MCP families, RANTES), but did not affect macrophage polarization (GM-CSF, G-CSF, M-CSF, INF-γ, IL-4). Cells cultured under low O2 conditions were more susceptible to electromagnetic fields, which caused an additional increase (up to 3-fold) in oxidative burst activity. This effect was not seen in any of the control cultures. These findings clearly indicate that macrophages at low O2 conditions, like those present in inflammatory tissue, increase their biological activity and become more susceptible to biophysical regulation.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.
High Temperature Electrophoresis in Microfluidic Devices

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Recent advances in microfluidic devices have led to improved efficiencies during electrophoretic separations. New designs allow longer channels to be fabricated on microchips, which lead to better performance. This experiment studied the effect of raising the temperature in microfluidic devices to see if these separation efficiencies could be pushed even farther. Using a 22 cm serpentine channel design and an electric field strength of 250 V/cm, samples of fluorescein and dextran were separated independently while the temperature was varied from 23 °C to 50 °C. As the temperature was raised, separation efficiency improved in both the fluorescein sample and the dextran sample. Separations at 50 °C resulted in a significant decrease in plate height. For the dextran sample, nine to ten peaks that were observed at room temperature were not baseline resolved. At 50 °C these peaks were all well resolved and at least twenty peaks could be seen in the electropherograms. In addition to the decrease in plate height, the analysis time was also reduced. This represents a big advantage for analyzing samples with many components. The results from these experiments indicate that increasing the temperature in microfluidic devices improves the separation efficiency in terms of plate height, resolution, and analysis time.
A Procedure to Quantify Gait Abnormalities after Sciatic Nerve Section in Rats
Julianna Barr*, Donna Marsh, and Jameel Ahmed
Department of Applied Biology and Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The goal of this project was to develop a procedure to analyze gait abnormalities in rats that have had surgical alterations to their sciatic nerves. This procedure is to be implemented in an ongoing project examining the efficacy of a novel biomaterial as a peripheral nerve repair guide.

Rats were videotaped as they walked along a Plexiglas walking track. Videos were obtained using a digital video camera mounted on a moving track a distance of 20 cm from the edge of the track. Video recordings were uploaded to the computer and still shots were extracted for analysis using Image Grabber software. Still shots were chosen at mid stance, terminal stance, and swing phase points in a rat’s gait cycle. Still shots were imported into a Matlab program that allowed the user to identify key landmarks on the rat leg, and calculated the joint angle using these points.

Testing of our analysis technique was done using 3 Harlan Sprague Dawley Rats both before and after surgery to remove a section of their sciatic nerve. In addition to pre-surgical controls, rats’ gait cycles were recorded and 1, 3, and 6 weeks post surgery. Pre- and post-surgical comparisons were made using ANOVA in Minitab software.

Preoperative measurements compared to post operative data show a noticeable decline in angle measurements obtained. The decrease in angle values was significant in the mid stance and terminal position for all three of the rats during the first week post surgery. The average percent decrease in the rats was 16.9% for mid stance and 6.9% for terminal stance. Swing phase data showed greater variability and the difference between pre and post surgery did not show statistical significance. The average percent decrease was 2.24% for swing phase.

In future experiments, sciatic nerve section will be bridged using tubes constructed from Matristem material (ACell, Inc.). The procedure described here will be used to determine functional recovery from nerve regeneration mediated by the nerve guide.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.
Trap Grease Based Biodiesel
Othmane Fathi* and Michael Mueller
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Terre Haute, IN 47803

Food grade vegetable oil based biodiesel is not economically possible at the moment due to the cost of production comparing to diesel fuel. Restaurants trap grease and animal fat and waste cooking oil are considered to be a potential feedstock for biodiesel because they represent one third of the US total fats and oil waste production. This study was conducted to convert trap grease and restaurant waste into biodiesel for two- cycle engines using two different enzymes. Novozym 435 and Amberlyst-15 were used in making biodiesel. The yield of each enzyme and the relationship between the cycles and the yield were examined. Both enzymes gave a yield of 94% to 98%, but Amberlyst-15 stopped working after 13 runs while Novozyme 435 is still working after 17 runs. The results obtained indicate that the process is efficient for converting waste grease and animal fat to a usable source of energy.
The Redistribution of Calpastatin in MCF7 Breast Cancer Cells Induced by Spleen Tyrosine Kinase Syk

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In this study, we attempted to determine the intracellular distribution of calpastatin in MCF7 breast cancer cells with or without stable expression of Syk, a non-receptor tyrosine kinase. Previous data has indicated that Syk positive MCF7 cells have a lower amount of calpain activity; therefore, calpain’s only known endogenous inhibitor, calpastatin, was examined for its distribution and expression by immunofluorescent staining and cellular fractionation. The results demonstrated that there is greater calpastatin expression in the cytoplasm of Syk positive cells than in that of Syk negative cells. Knowing the regulation of Syk on calpastatin can be used to further investigate the signaling pathways related to cancer cell growth and invasion which could aid in determining cellular targets for breast cancer diagnostics and therapeutics.
Development and Assessment of Low-Cost Media for the Production of Microalgae-based Biodiesel
Mitch Braddock* and Peter Coppinger
Department of Applied Biology, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Industrial-scale biodiesel production by lipid-producing microalgae is currently limited by the availability of inexpensive sources of media. This research focuses on the development and assessment of potential media sources from otherwise unusable waste products. *Chlorella vulgaris*, a photosynthetic microalgae currently used for small-scale biodiesel production, requires nutrients such as nitrates, phosphates, and iron for optimal lipid production. Three waste water solutions were examined as potential low-cost nutrient sources for *Chlorella*. Primary effluent form local sewage treatment facility, swine livestock waste, and wetland run-off were collected. *Chlorella vulgaris* was cultured in each solution for two weeks in homeostatic growth chambers under ambient CO₂ concentrations and 24 hour lighting. Cell densities were calculated through optical density measurements and viable counts. Growth analysis comparing the waste waters to defined media was determined by comparing growth rates and total cell counts. Furthermore, the nitrate, phosphate, and iron concentrations were quantified for each media source. Swine waste water provided the best growth environment for *Chlorella vulgaris* based on cell density and growth rate, surpassing defined media. Iron, as well as ortho and total phosphates, were depleted from all media after culturing and approximately half the nitrate and ammonia concentrations were depleted. These results also suggest that microalgae are a viable source for the bioremediation of waste water products containing large concentrations of organic compounds and nutrients.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.
Angiogenic Provisional Matrix Accelerates Maturation and Healing in Type II Diabetic (db/db) Mouse Model

Bradley A. Herbig\textsuperscript{a1}, Swathi Balaji\textsuperscript{2}, Rahul D’Mello\textsuperscript{3}, and Daria A. Narmoneva\textsuperscript{2}
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The non-healing diabetic wounds in the US are a significant problem, where slow and ineffective formation of scar tissue leaves diabetic patients with a hindering morbidity and an overall decreased quality of life. Our laboratory has recently developed a new approach to enhance healing of diabetic wounds in db/db (type II) mouse model using wound treatment with RAD16-II peptide nanofibers. In finding how to effectively translate this tissue engineering approach to healing diabetic wounds in human patients, it is imperative to understand the structure, characteristics, and formation of repair tissue following nanofiber treatment. In this research project, we have used microscopy, imaging techniques, antigenic staining, and mechanical testing in order to analyze cell phenotype, distribution, and maturation of the resulting regenerated tissue. The nanofiber treatment has lead to the accelerated maturation of regenerating tissue in type II diabetic mice, a result that has the potential to improve diabetic wound healing via tissue engineering in humans (using materials such as peptide nanofibers). This in situ tissue engineering approach is a promising method for accelerating the overall healing of diabetic wounds.
Examining the Viability of *Chlorella vulgaris* as a Source of Biofuel and Instrumentation for Nile Red

John Lawrence* and Ric Anthony
Department of Applied Biology and Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

*Chlorella vulgaris* is a microalgae with high concentrations of free lipids which can be used for the production for biofuels such as biodiesel. A spectrofluorometer can be used in conjunction with the indicator Nile Red (NR), which binds selectively to free lipid droplets inside of a cell. Once bound, the NR will absorb light at wavelengths around 485nm, and will then emit light at 575nm. The amount of fluorescence can be used to quantify the free lipid in the cells. The goal of this project was to design a high throughput instrumentation method to analyze the free lipid content of microalgae such as *Chlorella vulgaris* using Nile Red and a plate reader spectrofluorometer. Once this instrumentation method was complete, a factorial analysis on *Chlorella vulgaris* could be performed in order to determine the optimal time and conditions for harvesting the algae as a biofuel. With this information a continuous reactor could be designed for the industrial scale production of microalgae for use as a fuel.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.
Synthesis of Hyperbranched Polyglycerols to Attach to Tamoxifen Analogs
Vince Biondo*, and Ross Weatherman
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Terre Haute, IN 47803

Since the 1990’s, tamoxifen has been used to treat estrogen receptor positive breast
cancer. Although tamoxifen has been fairly successful as a Selective Estrogen
Receptor Modulator (SERM), there have been several problems with using																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
to the drug and use usually ends after five years. Past work in the
Weatherman Lab has shown that attaching polymeric scaffolding to tamoxifen
analogs has increased the potency of the drug against tamoxifen resistant breast
cancer cell lines. Past scaffolding molecules faced problems with size, polarity, and
aggregation. Our hypothesis for this project is that the use of hyperbranched
dpolyglycerols (HBP) as a scaffold molecule could solve each of these problems. In
addition, it is hypothesized that controlling the size of the HBP would control
where in the cell the drug can go, increase the potency of the drug and possibly
eliminate some of the side effects. HBP’s could be synthesized to a controlled
molecular weight, so size would not be an issue. The surface of the HBP is
covered with hydroxyl groups, which should make the drug-HBP molecule polar
even to stay water soluble, but hydrophobic enough to still cross the cell
membrane. HBP’s are large and globular, due to the random branching pattern,
virtually eliminating the effects of aggregation. To test this hypothesis, two
conjugates of tamoxifen were synthesized for attachment to HBP scaffolds. Two
types of linkages between the tamoxifen and the scaffold were envisioned– an amine
linkage, as seen in a tamoxifen analog known as endoxifen, and a ether linkage.
Tamoxifen analogs with both types of linkages were synthesized, but still await cell-
based testing. A 30 kDa HBP scaffold has also been synthesized and characterized
by NMR and size exclusion chromatography. Attempts to attach the HBP to the
tamoxifen conjugates are ongoing.

This work was funded by NIH (R01DK075376).
Non-Classical Estrogen Signaling in Breast Cancer
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It is well known that estrogen receptor (ER) plays an important role in several physiological processes through the regulation of transcription of numerous genes. The path the estrogen receptor takes to do this is to bind to estrogen, enter the nucleus, and bind to DNA at DNA binding sites. However, estrogen receptor is believed to participate in other, non-classical pathways during its journey to the nucleus during which it may interact with numerous other proteins throughout the cell. Recent evidence suggests that ER can regulate transcription through processes that are independent of DNA binding. To test the possibility that these non-classical interactions are important in breast cancer treatment, we designed ER mutants that still bound estrogen but were incapable of binding traditional estrogen receptor DNA binding sites to test against a number of non-classical ER promoters. To accomplish this, we first had to design primers that would code for the mutation we desired. Using PCR, we successfully created both an ERα and ERβ mutant. We confirmed that the mutagenesis was a success and that, in the case of ERα, that it is, in fact, expressed in breast cancer cells and is non-functional at the classical DNA binding sites. Some initial testing on other ER regulated promoters has begun, but no conclusive results have been achieved so far. Plans for further research include testing both mutants against a number of ER regulated promoters in varying cell lines.

This work was funded by NIH (R01DK075376).
Analysis of a Plasmid Encoded Type IV Secretion System in *Burkholderia*

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Many *Burkholderia* species are opportunistic pathogens, with the *Burkholderia cepacia* complex (Bcc) strains being notorious for high mortality rates in patients with cystic fibrosis. Past studies show that multiple *Burkholderia* species express two different type IV secretion systems (T4SSs), present on both chromosomes and plasmids, which are thought to be involved in secretion of DNA and/or effector proteins contributing to the bacteria’s pathogenicity. In this study, we compared the genes associated with the plasmid encoded T4SSs of five types of bacteria including *Agrobacterium tumefaciens*, *Escherichia coli*, and three *Burkholderia* strains. Sequence analysis showed that *B. cepacia* strains J2315 and H12424 along with *B. multivorans* strain ATCC 17616 all contain a plasmid encoded T4SS homologous to that of the F plasmid DNA transfer T4SS of *E. coli*. These analyses also suggest that none of these strains contain sequences for the origin of transfer (oriT), the site in a conjugative plasmid like F where the DNA is cut in preparation for transfer; however, the majority of genes needed for conjugative pilus synthesis and mating pair formation are found in these *Burkholderia* strains. We also studied the role of T4S coupling protein (CP) in the various strains of *Burkholderia* to test their involvement in DNA or effector protein transfer. We measured the ability of several CPs (from *Burkholderia* and other T4SS) to act with the F plasmid T4SS in F plasmid transfer and IncQ plasmid mobilization. These analyses and experiments could potentially aid in the identification of a drug target for the treatment of individuals with *Burkholderia* infections.

This project was funded by the Amgen Scholars Summer Research Program.
Synthesis and Characterization of Ampicillin-Loaded poly(DL-lactide-co-glycolide) (PLGA) Nanoparticles for Drug Delivery

Yile Gu* and Scott J. McClellan
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Recently, biodegradable polymer nanoparticles have become popular methods for drug delivery. They can be used to achieve sustained and local drug delivery. The sizes of the nanoparticles are significant because the nanosize provides a large surface to volume ratio in addition to being desirable for some physiological systems. From previous studies of our research group, a rudimentary protocol for producing PLGA nanoparticles without any drug was developed. In this research, ampicillin sodium, a hydrophilic drug, was successfully incorporated into poly(DL-lactide-co-glycolide) (PLGA) nanoparticles by the double-emulsion solvent evaporation method. Shapes of the PLGA nanoparticles were captured by scanning electron microscopy. Particle size analysis was used to obtain the size distributions of the PLGA nanoparticles. Drug loading efficiency was calculated from UV-vis spectrophotometry with an appropriate calibration curve created.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.
A Biomechanical Analysis of Implantation-Induced Cup Deformation in Acetabular Cup Designs
Mary E. Jones*, S. R. Small, J. B. Meding, and K. S. Toohey
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Press-fit implantation in total hip arthroplasty has been shown to cause deformation in acetabular cups. Deformation of cups beyond manufactured tolerances can lead to increased wear and osteolysis. The present study sought to investigate and quantify the deformation of four different acetabular cup designs. A cadaverically validated polyurethane foam model from a prior study was used as the testing medium. Thin and thick-walled CoCrMo metal-on-metal and two Ti6Al4V modular polyethylene-bearing cup designs were compressively implanted with a 1-mm interference fit into polyurethane blocks using a servohydraulic testing machine. Digital image correlation was used to track diametral cup deformation following the implantation of eight specimens of each design. One-way analysis of variance tests (p<0.05) indicated a significantly higher deformation in the porous titanium polyethylene-bearing design (271 microns) than the modular polyethylene-bearing design (106 microns) and thin-walled, metal-on-metal design (102 microns). Additionally, a significantly lower deformation was seen in the thick-walled, metal-on-metal design (9 microns). These results indicate the role that material selection and component design play in the implantation-induced deformation of acetabular components. While prior deformation studies have primarily focused on metal-on-metal components, this comparative study reveals similar or improved deformation resistance in these cups relative to polyethylene-bearing designs.
Molecular Docking Simulations for Organic Compounds to the Estrogen Receptor Alpha

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The Estrogen Receptor is an intracellular protein that interacts with 17β-estradiol in order to regulate gene expression. Estradiol binds to the estrogen receptor dimer in such a way that it alters the protein structure into an agonist conformation. Our laboratory has shown that the addition of low concentrations of organic solvents (for example, small alcohols) to the protein solution can have a significant effect on estrogen receptor dimer exchange. These solvents have the ability to bind to the surface of the protein or either of the monomer’s cavities, causing the change in the protein’s behavior. Using a molecular docking software package, AutoDock Vina, it is possible to simulate the binding of molecules to the protein. The software determines favorable binding sites by finding the regions with the lowest energy on the protein surface or in one of its cavities. The smaller alcohols, such as methanol and ethanol, primarily bind in a small cavity in the estrogen receptor dimer interface. As the non-polar chain of the alcohol increases in length, we also observed predicted binding in the ligand-binding pocket, the observed site of estradiol binding. There is also a direct correlation found between binding sites and binding affinity. As the size of the mono-functional alcohols increases, the binding affinity that it has to the predicted binding locations increases as well. After analyzing the binding energies for the alcohols tested, it can be shown that the interactions that occur between the solvents and the ligand-binding pocket are more favorable than interactions found in the dimer interface cavity. We have observed a correlation between the predicted binding location of mono-functional alcohols from computer simulation and the degree of experimental disturbance of dimer exchange. It is much more efficient to test compounds using computational methods as this method provides results much quicker than the experimental assays. If a correlation between molecular properties and the predicted binding affinities was demonstrated, it would be possible to predict the effect that an organic solvent would have on the dimer exchange of the estrogen receptor. The future direction of our computer simulation research is to establish these correlations and test them using both experimental methods and predicted binding sites from computer docking simulations. This would allow for the computer simulation predictions to determine which organic solvents would be valuable to collect experimental data for, ultimately evaluating and strengthening the correlation between computational and experiment results.

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Synthesis of Carbon Cryogel Microspheres for Use in Chemical Separations
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Carbon cryogels are an interesting material that exhibit high surface area and chemical robustness. These properties make carbon cryogels a potential material for use as a medium for chemical separations. Carbon cryogels were synthesized using a base-catalyzed sol-gel reaction followed by freeze-drying and thermal cross-linking in an inert atmosphere. The main goals of this project were to reproducibly synthesize carbon cryogels in a spherical particulate motif with controlled diameters between 2-10 $\mu$m. Reverse-micelles were employed to form micro-reaction vessels that would result in particles with the desired dimensions. Dispersions in several surfactants and peanut oil were attempted. The resulting microspheres were characterized using scanning electron microscopy and light-scattering particle analysis. High performance liquid chromatographic columns with various diameters, 0.250-4.6 mm, were produced. The columns were tested to discern the efficacy of the carbon cryogel packed columns.

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Electrochemical Adsorption of Metal Cations Using Carbon Cryogel
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Water pollution by heavy metal ions such as As, Cr, Cu, Pb, and Ti is an environmental concern due to associated health risks. Unlike other types of contamination, heavy metals do not decay; therefore, remediation efforts must be performed to remove heavy metal contamination from the environment. Current research has focused on electrochemical adsorption of metal cations using carbon cryogel as an adsorbent. The carbon cryogel was produced using a resorcinol-formaldehyde gel that was freeze dried with liquid nitrogen and then carbonized at 800°C. Cyclic voltammetry (CV) experiments were performed in order to prove that heavy metal ions could be adsorbed and desorbed from the surface of the carbon cryogel electrode. The CV experiments demonstrated that the carbon cryogel electrode behaved similarly as commercially available electrodes. The electrochemical adsorption of metal cations with carbon cryogel electrodes were analyzed using solutions at 10 ppm concentration. The experiments demonstrated that the carbon cryogel electrode could remove heavy metals at concentration levels which would be experienced in real life remediation efforts.

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Understanding Antioxidant Activity of Selenium Compounds by Monitoring Production of 8-Hydroxy-2’-Deoxyguanosine from the Nucleoside 2’-Deoxyguanosine-5’-Monophosphate

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Oxidative damage to the nucleotide base is responsible for many DNA mutations associated with cancer, aging, and a host of other disorders. Thus, prevention of oxidative damage is an important factor in cancer prevention and overall health. Two prevalent methods for determining antioxidant activity are strand breakage and observation of the oxidative DNA damage maker 8-hydroxy-2’-deoxyguanosine (8-OH-dG). A key element of interest for its anti-oxidant properties is Selenium, compounds of which have been shown in multiple instances to prevent the oxidative damage caused by reactive oxygen species (ROS) and reduce tissue damage. Several questions remain unanswered about the mechanism through which selenium compounds prevent oxidative damage. We present results from experiments in which we allowed the metal ions Cu(II), Fe(II) and Cr(III) to react with H₂O₂ to generate ROS in the presence of the mononucleotide 2’-deoxyguanosine-5’-monophosphate (dGMP); a simplistic model for nucleic acid polymers. We monitored the production of the oxidative damage marker 8-OH-dG in the presence and absence of selenium dioxide, sodium selenite and sodium selenate using HPLC with electrochemical detection. Our results suggest that the antioxidant properties of selenium compounds are associated with their abilities to bind metal ions and prevent them from forming ROS.
Detecting Low Levels of Atrial Natriuretic Peptide using nano-LC-MS
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Atrial natriuretic peptide (ANP) is a 28-amino acid peptide hormone produced by the heart that acts to decrease blood volume and pressure by increasing water and sodium excretion. ANP is secreted in response to increased atrial blood pressure. In addition to the normal peptide, ANP 1-28, other forms of ANP exist, e.g., ANP-RR, which contains two extra arginine residues at the C-terminal end of the peptide. Additional pro-forms of ANP as well as truncated forms of the peptide with potential biological activity are also thought to exist.

The purpose of this study is to ascertain optimal conditions for ANP analysis from plasma/serum and identify any additional circulating forms of ANP that might have biological activity. Analysis is comprised of two analytical components. The first component is to determine an immunoaffinity extraction procedure for ANP and alternate ANP forms from plasma with sufficient purity for samples to be run on an LC-MS system. The second is to determine optimal nano-LC-MS conditions with sufficient sensitivity (1-10 fmol).

A nano-LC-MS system equipped with a C18 trap (180 μm ID x 20 mm) and a Higgins Targa packed-tip column (75 μm ID x 20cm) with a 0.1% formic acid, 0.1% TFA, 0.0005% Zwittergent 3-16 and 30% ACN in a glass vial afforded femtomole sensitivity. We are currently utilizing 125I-ANP to optimize immunoaffinty extraction of ANP from plasma/serum.
Synthetic Studies Toward Dioicine and Analogs
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Dioicine is a novel prenylated purine from the Kentucky coffeetree (Gymnocladus dioicus, L, K. Koch). The presence of this alkaloid supports the reputed use of this tree as a coffee substitute by early Midwest settlers. On acid hydrolysis the labile prenadienyl side chain is lost, providing a bioisosteric analog of paraxanthine, the major metabolite of caffeine in man. Dioicine presents both interesting structure and biological activity. Collaborators have observed interesting developmental effects in zebrafish, prompting us to examine the semisynthesis of dioicine from paraxanthine as well analogs from theobromine and theophylline. Our synthetic studies to date will be described.
Selective Lithiation of 2,6-Dichloropyridine.
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Lithiation of halopyridines by directed metatation reactions is a useful method for the synthesis of medicinally relevant heterocycles. In accord with prior literature we have observed that 2,6-dichloropyridine may be lithiated at either the 3- or 4-position depending on base and conditions. The reaction is very sensitive to stoichiometry and base. With a slight excess of lithium diisopropylamide (LDA) high selectivity for 3-lithiation is observed. With substoichiometric amounts of LDA or very short reaction times, we observe predominant 4-lithiation, which can rearrange very slowly to the 3-isomer. We have traced this behaviour to the presence of n-butyllithium at very short reaction times. We have also observed direct lithiation of 2,6-dichloropyridine by n-butyllithium in THF at -78°C as opposed to literature reports of alkylation under these conditions. The results of our optimization study of this reaction will be presented.
The Autodigestion Hypothesis: Cleavage of the Ischemic Intestine
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Under ischemic conditions digestive enzymes generate cytotoxic mediators that leak into systemic circulation causing multiple-organ failure. We hypothesize that during ischemic states the intestinal barrier becomes disrupted allowing entry of digestive enzymes into the intestinal wall causing autodigestion. The purpose of this study is to detect fragments of e-cadherin, a common tight junction protein, in the intestinal wall during ischemic and non-ischemic conditions via western blotting. For this study we used an in vivo rat model of splanchnic ischemia via occlusion of the superior mesentery and celiac arteries (SAO) for 15 and 30 min; two additional groups were formed with the previous conditions but following pre-treatment with Cyklokapron (a serine protease inhibitor); a non-ischemic control was also studied in which the arteries were isolated but not ligated. Jejunal sections of the five different groups were collected and homogenized to be later analyzed by in-situ zymography and western blotting. Our findings confirm that during ischemia, e-cadherin fragmentation is well above control samples in as soon as 15 minutes. These results suggest that during early periods of ischemia, powerful pancreatic digestive proteases are transported across the intestinal barrier into the intestinal wall causing injury into the submucosa and subsequently enabling the release of cytotoxic biproducts into systemic circulation.
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