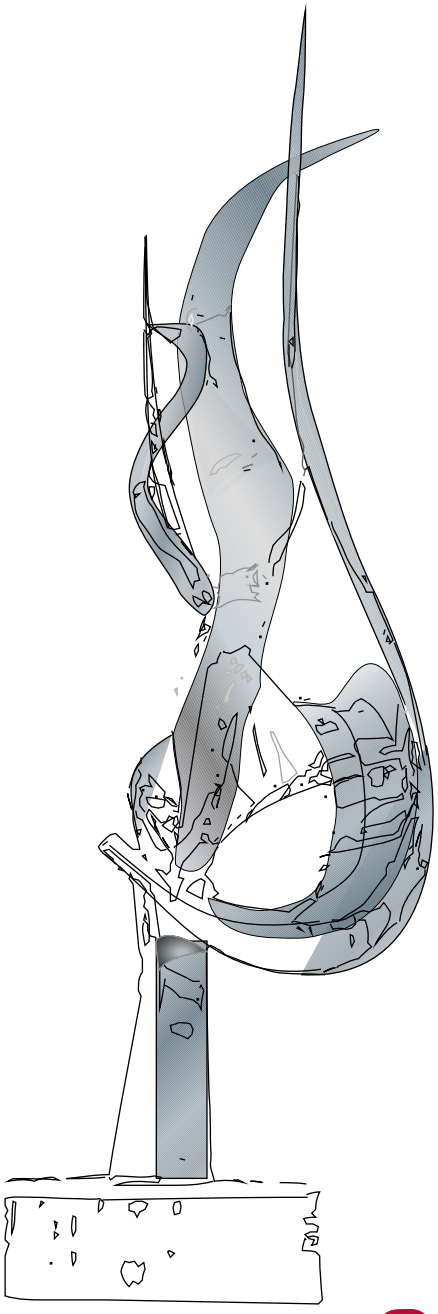
Interdisciplinary Research Collaborative

IRC

Rose-Hulman Institute of Technology



9th Annual IRC Undergraduate Research Symposium

Monday
October 22, 2012

Sponsored by

Edwards Lifesciences

ROSE-HULMAN INSTITUTE OF TECHNOLOGY

Welcome to the

9th Annual IRC Undergraduate Research Symposium

Sponsored by

Monday, October 22, 2012

We are honored to welcome you to the 9th 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 Edwards Lifesciences, 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 some of our colleagues in Indiana.

We are delighted to welcome you to this ninth in the annual event series. 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

Peter Coppinger
IRC Program Coordinator

Rose-Hulman Institute of Technology

Symposium Schedule

Morning Poster Session (8:00 – 8:45 AM)

Construction of an Expression Plasmid for the Human Estrogen Receptor Beta Ligand-binding Domain

Danielle Bauhan, Bianca Maled, and Mark E. Brandt*
Department of Applied Biology & Biomedical Engineering and Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

A PIV Study of Flow Past a Rotating Cylinder

*Jordan Chipka**
Department of Mechanical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

AC Faraday Rotation Analysis of the Iodine Clock Cycle

Thomas Foulkes, John Moore, & Dr. Maarij Syed*
Department of Physics & Optical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Checkmate: A Rapid Yeast Mating Type Detector

Adam Nighswander, Alex Krug*, Ben Deschaine, Robert French, Kristen Schackmann, Devon Trumbauer, David Goulet, Yosi Shibberu, and Richard Anthony*
Department of Applied Biology & Biomedical Engineering and Department of Mathematics, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Halopyridine Bicycles as Intermediates for Alkaloid Synthesis

Laura Major, Cody Prickett*, Lucy Moser, Daniel Dalton and Richard W. Fitch*
Department of Chemistry and Physics, Indiana State University, Terre Haute, IN 47809

Optimized Microwave Assisted Organic Synthesis using Suzuki-Miyaura Cross-Coupling

Kaitlin Schneider, Matthew Welmers, and Rebecca DeVasher*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Morning Session I (8:45 AM – 10:45 AM)

Numerical Simulation of Chromatography and Dimer Exchange

Ted Samore, Mark E. Brandt, and David Goulet*
[1]Department of Applied Biology & Biomedical Engineering, Department of Mathematics, and [2]Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Analysis of Proximal Tibia Strain Response to Resection Alignment in Partial Knee Arthroplasty Tibial Components

Kelli Greenberg, Paige Cook, Amanda Kingman, Kevin Farley, Scott Small, and Renee Rogge*
Joint Replacement Surgeons of Indiana, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Effect of Antiestrogens on Uterine Cell Proliferation

Lauren Gutgesell and Ross Weatherman*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

A New Assay for Assessing Conformational Flexibility in the Estrogen Receptor Ligand-binding Domain

Katherine C. Dial and Mark E. Brandt*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Melanoma Cells Treated with Doxorubicin-loaded PLGA Nano-particles Display Altered Immunogenic Phenotypes

Alex Cochrane[1], Sue E. Blackwell[1], Suresh Veeramani[1], Ken Blackwell[2], and George J. Weiner[1]*
[1]Holden Comprehensive Cancer Center, Department of Internal Medicine, and [2]Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA 52240

Lipids, Metabolism, and Cell Shape in *Caulobacter crescentus*

Alexandra L. Williams, Justin Kern, and Lucy Shapiro*
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

In vitro Investigation of Oxidative Stress in Normal and Malignant Hepatocytes

Esther Kim, Lacy Reynolds, Xiaodong Wen, Cheng Xu Liao, Lance Terada, and Ian Corbin*
Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX

A Partial Ordering of Cellular Metabolic Reactions by Efficiency

Kimberly Boucher and Allen Holder*
Department of Mathematics, Rose-Hulman, Terre Haute, IN 47803

Morning Session II (11:00 AM – 12:30 PM)

Incorporation of Fluorescent Dyes into Polystyrene Latex Microspheres

Max A. Verkamp, Paul Danehy, Patsy Tiemsin, Chris Wohl, and Todd Lowe*
[1]Department of Chemistry & Biochemistry and Department of Physics and Optical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803, NASA Langley Research Center, and Virginia Polytechnic Institute, VA

Role of Selenium Compounds in Coordinating Copper (II) and Decreasing Oxidative DNA Damage

Peter Vannauker and Daniel Morris*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

A Discrete Transmission-Matrix Method for Modeling the Distributed Feedback Arising from Continuously Varying Refractive Index Profiles

Derek S. Heeger, Robert M. Bunch, and Paul O. Leisher*
Department of Physics & Optical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The Effects of Footwear on the Postural Stability of Women

Audrey Niverson, Kevin Farley, and Dr. Rogge*
Joint Replacement Surgeons of Indiana, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Synthesis and Application of Heterogeneous Catalyzed Resorcinol:Formaldehyde Mesoporous Carbon

Caitlin Anderson and Justin Shearer*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Evaluation of "Assay Ready" Frozen Cells and Freshly Cultured Cells for Cell Based Neutralizing Anti-drug Antibody (NAb) Assays

*Emily J. Cottingham**
Department of Chemical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Lunch Break (12:30 – 1:30 PM)

Afternoon Poster Session (1:30 – 3:00 PM)

Analysis of Proximal Tibia Strain Response to Resection Alignment in Partial Knee Arthroplasty Tibial Components

Kelli Greenberg, Paige Cook, Amanda Kingman, Kevin Farley, Scott Small, and Renee Rogge*

Joint Replacement Surgeons of Indiana, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Synthesis of Tamoxifen Conjugates through Linker Modification

David Harvey and Ross Weatherman*

Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Solid Phase Extraction using Carbon Cryogels

Gregory Horne and Justin Shearer*

Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Binding of Anti-estrogen Derivatives to the Estrogen Receptor Protein in Breast Cancer Therapy

Katherine Moravec, Kati Shearer, and Ross Weatherman*

Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Urinary Bladder Matrix as a biodegradable guide for peripheral nerve damage repair: a functional and tissue analysis

Anna Rector and Jameel Ahmed*

Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

A Low-Temperature Method of Plasma-Enhanced Chemical Vapor Deposition for Carbon Nanotube Growth

Eric Teeman, Daniel Morris[1], and Scott Kirkpatrick[2]*

[1]Department of Chemistry & Biochemistry and [2]Department of Physics and Optical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Afternoon Session (3:00 PM – 4:25 PM)

Detection of Phytoplasma in *Trillium grandiflorum* using 16s rDNA PCR

Nathan D. Wheeler and J. Peter Coppinger.*
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Studying Estrogen Interactions One Receptor at a Time

Bianca Maled, Danielle Bauhan, and Mark E. Brandt*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Alkaloid Constitutents of *Dendrobates auratus* from Oahu, Hawai'i

Ellery Steele,[1] Carly McDonald,[1] Anthea Weng[1] and Ralph A. Saporito,[2] and Richard W. Fitch[1]*
[1]Department of Chemistry and Physics, Indiana State University, Terre Haute, IN 47809.
[2]Department of Biology, John Carroll University, University Heights, Ohio 44118.

Optimized Microwave Assisted Organic Synthesis using Suzuki-Miyaura Cross-Coupling

Kaitlin Schneider, Matthew Welmers, and Rebecca DeVasher*
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Mediator complex requirement for transcription initiation regulation in *S. cerevisiae*

Tanner Reeb, Jason Wong, Suraiya Ansari, and Randall Morse*
Wadsworth Center, New York State Dept. of Health, Albany, NY

Construction of an Expression Plasmid for the Human Estrogen Receptor Beta Ligand-binding Domain

Danielle Bauhan*, Bianca Maled, and Mark E. Brandt
Department of Applied Biology & Biomedical Engineering and Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The estrogen receptor (ER) is a protein that exists in two forms, alpha (α) and beta (β). Several experiments have been performed on ER α, leading to discoveries that link the protein to breast cancer. However, there have not been many experiments done on ER β. In order for us to conduct experiments on ER β, we needed a sufficient amount of the protein. The part of the protein that binds to estrogens is called the ligand binding domain. This is the part of the protein we were interested in producing. In our attempt to make the protein, we took a plasmid (PMAL INGR) and inserted into it the gene that codes for the LBD. We then transformed the new PMAL plasmid with DH5 competent cells in order to grow cells that had the gene to produce the LBD. After incubating the transformations, we purified DNA from the cells and ran samples on an electrophoresis gel to see if the LBD gene inserted correctly. We performed several variations of this experiment using different plasmids and competent cells and none of the gel results came back positive. In order to continue with the research, first we would have to finish the construction of the plasmid with the LBD coding sequence correctly inserted. Once we have the correct sequence of the plasmid, we can then transform more competent cells with the plasmid so that the protein can be produced in sufficient amounts. Then we can purify the protein in order to use it in experiments, such as a dimer exchange assay, to compare ER β characteristics to those of ER α. It is possible that ER β could show significant chemical differences than ER α, which could lead to even more discoveries of the protein's relationship with breast cancer.

This research was funded in part by Edwards Lifesciences under the auspices of the IRC.

A PIV Study of Flow Past a Rotating Cylinder

Jordan Chipka*
Department of Mechanical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Shedding vortices, seen in unsteady flows, are a common occurrence in nature. This flow pattern is examined using particle image velocimetry methods. Specifically, the flow of water past a rotating circular cylinder is studied. The goal of this research is to observe the effect of cylinder rotation rate and free stream velocity on the shedding vortex structures. The experiments were performed at three different values of non-dimensional rotation rates:

$$\alpha = \frac{D\Omega}{2U_\infty}$$

where D is the cylinder's diameter, Ω is the cylinder's angular velocity, and U_∞ is the free stream velocity. The rotation rates at which the experiments were performed are α=0, α=0.8, and α=1.5. Further, each of these experiments were conducted for Reynolds numbers of Re=600, Re=2600, and Re=5100. The results of these experiments are pending.

This research was funded in part by ArcelorMittal under the auspices of the IP/ROP.

AC Faraday Rotation Analysis of the Iodine Clock Cycle

Thomas Foulkes*, John Moore, & Dr. Maarij Syed
Department of Physics & Optical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

While chemists have analyzed the kinetics of reactions for a long time, less emphasis has been placed on the implications of reaction kinetics on the evolution of a solution's magnetic properties corresponding to changes in its molecular structure. Specifically, the classic kinetic study referred to colloquially as the Iodine Clock Cycle involving the evolution of Iodide into Triiodide was altered to yield three unique concentrations of Triiodide. On a chemical level, these species can be differentiated based on three distinct relative concentrations of Iodine and Triiodide thereby allowing distinct amounts of free ions which lead to different magnetic properties for the three solutions.

To investigate the different magnetic (specifically diamagnetic) properties of these differing concentrations we have carried out precise Faraday rotation (FR) measurements in an AC field arrangement. FR refers to magnetically induced birefringence whereby a substance rotates the polarization of the light beam passing through it, in the presence of a magnetic field. This AC experimental setup yields reliable results for rotations as small as one arc minute. Thus, by analyzing the FR of these three unique species of Triiodide, an analysis of the significance of the amount of free ions and their impact on the overall magnetic properties of the solution can be deduced. Supplemented by absorbance and index of refraction measurements for each solution, we also compare our results to various theoretical models that deal with multi-component solutions with the desire of creating a concise model that could be applied to other multi- component liquid systems.

Checkmate: A Rapid Yeast Mating Type Detector

Adam Nighswander*, Alex Krug*, Ben Deschaine, Robert French, Kristen Schackmann, Devon Trumbauer, David Goulet, Yosi Shibberu, and Richard Anthony
Department of Applied Biology & Biomedical Engineering and Department of Mathematics, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Easily manipulated genetics make the yeast *Saccharomyces cerevisiae* a versatile and widely used model eukaryote. To progress, researchers must often determine the mating type of haploid strains, which typically takes days. The goal of our project is to reduce that time to hours. So we designed a novel promoter harboring Ste12 and LexA binding sequences and placed it upstream of an ORF encoding a red fluorescent reporter fused to LexA binding and VP64 activator domains. Others have shown that this fusion protein induces its own expression from a LexA promoter. We propose that Ste12, activated in the pheromone response pathway, will bind the hybrid promoter and induce expression of the fusion protein, which will amplify and maintain its own expression. Therefore, when mating pheromone receptors on a haploid harboring this latch-type circuit are bound and activated, the cell will fluoresce and function as a rapid mating type detector.

Halopyridine Bicycles as Intermediates for Alkaloid Synthesis

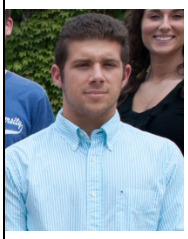
Laura Major*, Cody Prickett*, Lucy Moser, Daniel Dalton and Richard W. Fitch
Department of Chemistry and Physics, Indiana State University, Terre Haute, IN 47809

During our studies toward analogs of the frog alkaloid phantasmidine, we needed to prepare a series of amides of 2,6-dichlorohomonicotinic acid. Several techniques were examined for accomplishing this, including activation of the acid with dicyclohexylcarbodiimide (DCC) or 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), with in-situ reaction with the amine. Otherwise thionyl chloride ($SOCl_2$) conversion of the precursor acid to the it's corresponding acid chloride and subsequent addition to the amine (after removal of excess SOCl2 under vacuum. This latter approach turned out to be most productive, though all produced amide in varying amounts. A significant complication was the presence of isomers and congeners of the precursor acid, which was prepared via an allylation-oxidation protocol. However, this was ultimately overcome by modification of reaction conditions for the two-step sequence. Base-promoted ring closure of the amido alcohols to the bicyclic 3,4-dihydro-2H-pyrido[3,2-g][1,4]oxazocin-5(6H)-ones is currently in progress. Our results to date will be discussed.

Laura Major is a senior ISU Chemistry major from Roachdale, IN, and will graduate in May 2013

Cody Prickett is a senior ISU Chemistry major from Indianapolis, IN, and will graduate in May 2013

Lucy Moser is a junior ISU Chemistry major from Columbus, IN, and will graduate in May 2014



Daniel Dalton is a sophomore ISU Chemistry major from Oakland City, IN, and will graduate in May 2015

Optimized Microwave Assisted Organic Synthesis using Suzuki-Miyaura Cross-Coupling

Kaitlin Schneider*, Matthew Welmers, and Rebecca DeVasher
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The Suzuki cross-coupling reaction was discovered by Professor Akira Suzuki in the late 1970s. This reaction is instrumental in the formation of carbon-carbon bonds between benzene ring structures. The Suzuki reaction, displayed below, couples a phenylboronic acid with an aryl halide to produce a biaryl product.

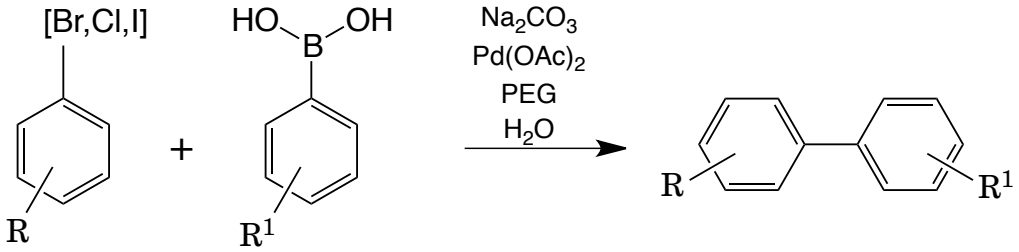


Figure I: The Suzuki-Miyaura cross-coupling reaction where R and R^1 are any organic functional group.

These biaryl products are employed in several growing industries, including pharmaceuticals, herbicides, natural products, conducting polymers, and liquid crystalline materials. An important factor in any industry is optimization, which saves time and ultimately increases profits for a business. The Suzuki reaction is not immune to these economic considerations. As such, scale up of the Suzuki reaction has been an important topic in the current world of green chemistry. This would allow companies to produce the desired biaryl products on a gram instead of milligram scale most efficiently.

Whereas traditional heating methods have been employed in the past, current microwave assisted technologies are being researched. Microwave assisted organic synthesis (MAOS) allows for specificity in control of reaction conditions such as pressure and temperature, as well as reaction time. If optimized, the specificity over control provides the company savings in time and potentially could increase product yields, which would lead to economic benefits.

Due to the above considerations, several reaction variables which potentially could lead to a more optimized Suzuki reaction were studied in laboratory settings. The microwave-promoted, palladium-catalyzed, Suzuki-Miyaura cross-coupling reaction of aryl halides with phenylboronic acid performed in aqueous media was studied to understand the effect of halide lability on final product yield, while also investigating the effect of the presence of a palladium catalyst. To do this, a Suzuki cross-coupling was performed using 5-bromo- and 5-iodo-salicylic acid under the reaction conditions displayed in the figure above. The results indicated that the microwave-promoted Suzuki cross-coupling run in the presence of the palladium catalyst produces significant increase in yield while optimizing reaction time. Currently, the effect of the alkali metal in the base used in the Suzuki cross-coupling is being evaluated, along with various functional groups on the aryl halides, to determine if results can be used to further optimize the microwave-promoted Suzuki coupling reaction.

Numerical Simulation of Chromatography and Dimer Exchange

Ted Samore*, Mark E. Brandt, and David Goulet
[1]Department of Applied Biology & Biomedical Engineering, Department of Mathematics, and [2]Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

The estrogen receptor protein exists in equilibrium between monomers and dimers. Size-exclusion chromatography was performed for the ligand-binding domain of the protein, in order to ascertain the rate parameters governing the dimerization reaction. Work by others indicates that accurate numerical simulation of the chromatography column may lead to more detailed parameter estimates. A numerical simulation of the chromatography column was developed, based on solving systems of partial differential equations. These equations included stiff nonlinear reaction, convection, diffusion, and absorption terms, describing behavior on several spatial and temporal scales. This type of problem is notoriously difficult to solve. Stable and accurate schemes were achieved via operator splitting, which allowed different model components to be treated with specialized numerical techniques. The PDE solvers were coupled to optimization routines and experimental data, allowing initial model parameter estimates to be iteratively refined so as to best approximate the data.

This research was funded in part by a Joseph B. and Reba A. Weaver Undergraduate Research Award and in part by Edwards Lifesciences under the auspices of the IRC.

Analysis of Proximal Tibia Strain Response to Resection Alignment in Partial Knee Arthroplasty Tibial Components

Kelli Greenberg*, Paige Cook, Amanda Kingman, Kevin Farley, Scott Small, and Renee Rogge
Joint Replacement Surgeons of Indiana, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

One major factor in the failing of partial knee replacements is thought to be caused by misalignment of the implant. This misalignment can lead to tibial overload and bony collapse. To prevent this, tibial components should be implanted to promote level strain distributions. New generations of instrumentation and customized guides have allowed for increased accuracy and precision of implant placement. The goal of this study was to determine the effect of bone resection on strain following implantation of the Oxford® unicompartmental knee arthroplasty (UKA) prosthesis.

In this study, the strain response to distal and medial resection depths of the UKA medial tibial component was analyzed. The metal backed tibial trays were implanted into fourth-generation composite tibial specimens using customized instrumentation. All specimens were axially loaded at the implanted medial condyle to 1.5 kN via an appropriately sized femoral component affixed to the actuator of a materials testing load frame. Loads were applied with the polyethylene tibial bearing in neutral, 4 mm anteriorly translated, and 4 mm posteriorly translated to simulate bearing motion during gait. Strain response was acquired by strain gauges on the antero- and postero-medial aspects of the proximal tibia and digital image correlation. Distal resection and component size was examined through the implantation of three sets of six tibias with 4 mm of medial shift, 4 mm of increased distal resection, and 4 mm medial – 4 mm distal shift.

Increased resection depth and medial shift significantly changed strain response at every strain gage location. Compared to a component implanted with the standard Signature™ resection guidelines, added distal and medial resection also increased variation in strain distribution by 1.2 to 1.7 times. Medial shift of the tibial component resulted in 17-27% increase in strain in the anteromedial tibia. Increased distal resection resulted in a 11-29% increase in medial and distal measurement regions. The combination of distal and medial shifts was cumulative and showed increases in strain of up to 49% in the anterio-medial tibia.

In conclusion, tibial components must be carefully aligned in UKA procedures. Surgeons should remove as little bone as possible when implanting a UKA tibial component in order to avoid increases in bone strain which can lead to failure or painful remodeling.

Effect of Antiestrogens on Uterine Cell Proliferation

Lauren Gutgesell* and Ross Weatherman
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

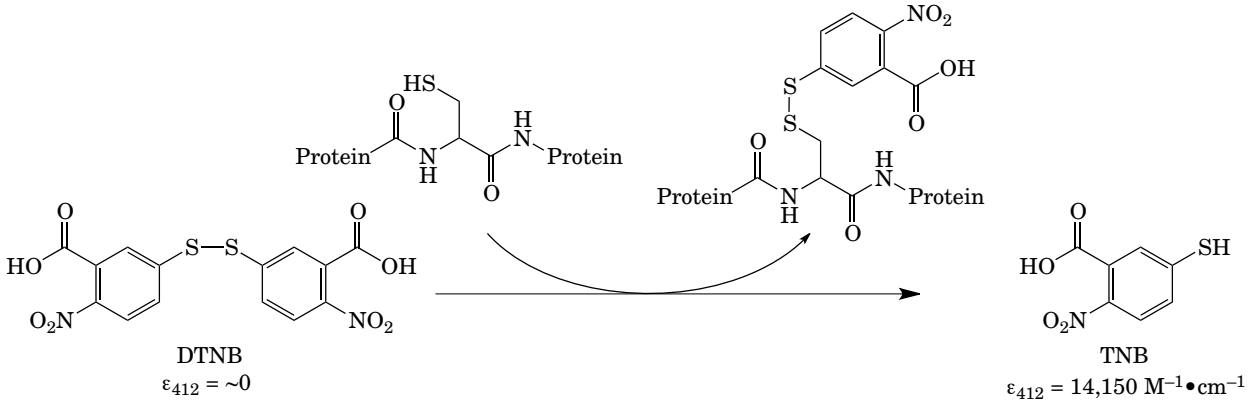
Many forms of breast cancer are exacerbated by increased the presence of estrogen hormones, specifically estradiol. Antiestrogen drugs used to treat estrogen-responsive breast cancer such as tamoxifen and fulvestrant block estrogen action in the breast and prevent the cancer cells from proliferating. Uterine cell proliferation also increases in the presence of estrogen hormones, but may also proliferate in the presence of certain antiestrogens such as tamoxifen. This means that some anti-breast cancer drugs may be pro-uterine cancer drugs. Therefore it is important to test any new antiestrogen developed for breast cancer for possible proliferative activity in the uterus. This is the concept I explored this summer. Using MTT and luciferase assays, I examined the proliferation of uterine cells, specifically the ECC-1 cell line at different concentrations of tamoxifen, fulvestrant, and estradiol. The purpose of the MTT assay was to determine how many ECC-1 cells were present after being dosed with each drug and incubated. The luciferase assay looked at the amount of light produced by a recombinant luciferase gene added to the cells that has been engineered to be regulated by estrogen. These two assays were supposed to be used to examine the proliferation of ECC-1 cells at the cellular and transcriptional level after treatment new drugs developed by the lab that kill breast cancer cells. Though the assays have been successful with MCF7 breast cancer cells, ECC-1 cells were difficult to grow in culture successfully and often suffered from contamination. No usable data was obtained for ECC-1 cells.

This research was funded in part by a grant from the National Institutes of Health.

A New Assay for Assessing Conformational Flexibility in the Estrogen Receptor Ligand-binding Domain

Katherine C. Dial* and Mark E. Brandt
Department of Chemistry & Biochemistry, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Breast cancer is the most common form of cancer in women. Most types of breast cancer are induced by estrogen, acting via the estrogen receptor (ER); many breast cancer patients are treated with tamoxifen, which prevents the binding of estrogen to the ligand-binding domain (LBD) of the ER. Physiologically, the ER exists almost exclusively as a dimer, and the dimerization process is thought to be functionally important to ligand binding. A consequence of ligand binding, generally, is a decrease in the rate of dimer dissociation, and it is hypothesized that ligand binding tightens the 3-dimensional conformation of the protein. In contrast, it has been seen that the rate of the dimerization process of the ER–LBD is increased in the presence of small alcohols (*e.g.,* methanol, ethanol, propanol, and butanol). A hypothesis to explain this effect is that the alcohols cause the ER-LBD to become less tightly folded. In order to test this hypothesis, we are developing a new assay that uses spectrophotometry to monitor a reaction between 5,5´-dithiobis(2-nitrobenzoic acid) (DTNB) and the protein. DTNB undergoes disulfide exchange with cysteine residues in proteins, and releases a compound that absorbs at 412 nm.



Our hypothesis, that the ER-LBD is less tightly folded in the presence of alcohols, predicts that the reaction between the protein and DTNB will be faster in the presence of alcohols, because DTNB will have easier access to the cysteine residues in the protein. When this reaction is carried out, the absorbance at 412 nm will increase proportionally to the reaction rate. Part of the development process for this assay is determining reaction conditions that will produce reproducible, interpretable results. For instance, different conditions have been tested to prevent interference by the alcohols in anything other than affecting the conformation of the protein. Preliminary experiments appear to support the hypothesis. The reaction was observed to proceed to a greater extent for denatured than for folded protein, and the presence of alcohols appeared to increase the rate of the DTNB disulfide exchange reaction. The overall goal of this work is to evaluate possible mechanisms to explain the epidemiological evidence for an association between ethanol consumption and the incidence of breast cancer.

Melanoma Cells Treated with Doxorubicin-loaded PLGA Nano-particles Display Altered Immunogenic Phenotypes

Alex Cochrane[1*], Sue E. Blackwell[1], Suresh Veeramani[1], Ken Blackwell[2], and George J. Weiner[1]
[1]Holden Comprehensive Cancer Center, Department of Internal Medicine, and [2]Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA 52240

Melanoma is an increasingly common and potentially fatal malignancy for which no cure exists. Poly lactic-co-glycolic acid (PLGA) is an FDA approved copolymer that is biodegradable in the presence of water, with minimal human toxicity. We have seen previously that doxorubicin (dox) inhibits proliferation of melanoma cells by G2 phase arrest, but does not efficiently kill the cells. Incorporating dox within the nano-particles allows for control of its rate of release. In the present study, we've shown that doxorubicin causes WM793 cells to secrete a higher level of the chemokine IP-10, with dox NP being more active than free doxorubicin. In addition, activated T cells showed enhanced killing of dox NP pre-treated cells than control NP pre-treated cells. We therefore conclude that IP-10 upregulation by dox NP is a sign of increased immunogenicity of the melanoma cells.

Lipids, Metabolism, and Cell Shape in *Caulobacter crescentus*

Alexandra L. Williams*, Justin Kern, and Lucy Shapiro
Department of Applied Biology & Biomedical Engineering, Rose-Hulman Institute of Technology, Terre Haute, IN 47803

Caulobacter crescentus exhibits a striking dimorphic cell cycle. As a result, it has emerged as an exciting model for studying cellular division and asymmetry in bacteria. We set out to discover the roles of membrane composition in development and division. In other bacteria, including *Bacillus subtilis*, the triterpene squalene and its derivatives are known to play an important role in membrane function. The *C. crescentus* genome contains an open reading frame that is predicted to encode for a squalene synthase. We're seeking to identify lipids produced by the putative squalene synthase by using gas chromatography and mass spectrometry to analyze membrane extracts of *C. crescentus* and of *E. coli* strains engineered to overproduce the gene product. A *C. crescentus* strain lacking the putative squalene synthase exhibits two striking phenotypes: slow growth in minimal media and ectopic pole formation. These phenotypes arise because mutants are incapable of using ammonium as a nitrogen source, and begin to store carbon in large polyhydroxybutyrate (PHB) granules. Because ectopic pole formation indicates a defect in cell division, we hypothesized that strains lacking the squalene synthase and possessing large PHB granules fail to properly localize the FtsZ ring during division. Z-ring formation is crucial to proper cell division, and defects are known to cause ectopic poles in *E. coli*. Overall, these phenomena underscore the close integration of every feature of the cell, and demonstrate that changes in something as straight-forward as nitrogen metabolism can affect seemingly unrelated traits such as overall cell morphology and division.

In vitro Investigation of Oxidative Stress in Normal and Malignant Hepatocytes

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The Oxidation/Reduction (Redox) system is essential in maintaining normal cellular homeostasis. Under physiologic conditions, cells maintain redox balance through the generation and elimination of reactive oxygen/nitrogen species (ROS/RNS). For cancer cells, elevated ROS plays an important role in carcinogenesis, as well as enhancing cell proliferation and avoiding apoptosis. In this study, we have monitored the levels of oxidative stress in normal (TIB-73) and malignant (TIB-75) murine hepatocyte cell lines. Dicholorofluorescin (DCF) fluorescence readings and HyPer-ER fluorescent images showed higher levels of ROS in TIB-75 cells than the TIB-73 cells. In addition, western blot analysis revealed that the TIB-75 cells expressed lower levels of antioxidant enzymes, especially catalase. These data suggest that malignant hepatocytes have greater oxidative stress level than normal cells. This important biological difference between normal and cancer cells may potentially be exploited therapeutically by agents that further increase ROS or weaken antioxidative defenses in cancer cells.

A Partial Ordering of Cellular Metabolic Reactions by Efficiency

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A balanced metabolic system can be represented as a matrix A, whose rows correspond to the nutrients that are necessary for the reactions, and whose columns r_1, r_2, r_3, ..., r_n are the metabolic reactions that the cell can perform, represented as vectors of the stoichiometric coefficients in the reaction. We can also introduce a pseudo-reaction, g taking in metabolites and outputting cell growth (biomass). We consider the minimal-nutrient environment in which the cell can operate. If two distinct (linearly independent) reactions r_i and r_j can be (positively) scaled such that $r_i - r_j \leq 0$, then r_j is less efficient than r_i, because given any environment that allows r_j to run, either r_i can run in the same environment with a higher output, or r_i can run and give the same output in a more restrictive environment. Hence, r_j will never be better than r_i in maximizing cell growth in a minimal (or any other) environment. Generally, however, this can't be used to compare efficiencies between reactions in a cell, because most reactions in the cell don't satisfy $r_i - r_j \leq 0$, where they are identical except for inefficiencies in one reaction. If a set of reactions have none of the same outputs, it doesn't make sense to compare their efficiency, but if reactions have some or all of their outputs in common, then they can be compared on the basis of which reaction or reactions can produce a given amount of the necessary nutrients in the most minimal environment. We construct an ordering to define and express that comparison between reactions.

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Incorporation of Fluorescent Dyes into Polystyrene Latex Microspheres

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The incorporation of fluorescent dyes into polystyrene latex microspheres (PSLs) for use in laser-based wind tunnel measurement techniques was investigated. The goal of adding dye was fluorescence-based thermometry and greater sensitivity near surfaces with current laser velocimetry techniques. An initial literature search was performed to find candidate dyes that met the following criteria: low to no toxicity, non-carcinogenicity, relatively high fluorescence yield, and an excitation maximum that is suitable for use with either an argon ion laser (488 or 514 nm) or frequency doubled neodymium-doped yttrium-aluminum-garnet (Nd:YAG) laser (532 nm). A final qualification for the dyes was that they incorporate well into the PSLs, which was determined experimentally for those that met the other qualifications. The synthesis of batches of PSLs in aqueous solutions was performed via soap-free emulsion polymerization. Several variables, including stabilizing electrolyte and dye concentration, were investigated to determine their effects on particle size and fluorescence signal using the dye 2´,7´-dichlorofluorescein (DCF), the most attractive dye examined. It was determined that particle size increased with increasing electrolyte concentration within a finite range, though the fluorescence was unaffected. Increasing dye content increased the observed fluorescence, though not in a linear fashion. The particle size was unaffected by dye content until high concentrations were used. At this point, the size decreased and incorporation was more variable. Preliminary tests of seeding and sensing the produced PSLs using an argon ion laser at 488 nm and a 532 nm Nd:YAG laser were performed. The fluorescent signal from the particles was observed using both light sources, with the Nd:YAG laser producing a stronger and less noisy signal. The signal produced from the Nd:YAG source was also observed to correlate strongly with the laser scattering, suggesting that the fluorescence can be used in place of scattering near surfaces, where the laser glow interferes.

Role of Selenium Compounds in Coordinating Copper (II) and Decreasing Oxidative DNA Damage

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Oxidative stress is associated with all matters of disease, as well as aging. Understanding the mechanisms behind oxidative stress is required in order to minimize its effects. Oxidative damage to DNA results in production of site-specific base modifications, including production 8-hydroxy-2´-deoxyguanosine (8-OH-dG) from the guanine base. 8-OH-dG is an accepted oxidative damage marker and, elevated levels suggest he presence of certain diseases, including several forms of cancer. Several transition metals react with H2O2 to produce reactive oxygen species (ROS) that result in oxidative damage and produce 8-OH-dG from DNA, and it is well known that species that coordinate these metals act as antioxidants and decrease 8-OH-dG production. Using the reaction of Cu(II) with H_2O_2, this study probed the abilities of selenium dioxide (SeO_2) and sodium selenite (Na_2SeO_3) to coordinate Cu(II) and decrease the levels of 8-OH-dG produced from the mononucleotide deoxyguanosine-5´- monophosphate (dGmp). From these results it was found that these selenium compounds exhibit antioxidant activity similar to the well-known metal ion chelators EDTA and oxalate, suggesting that SeO_2 and Na_2SeO_3 coordinate Cu(II) very effectively and serve as antioxidants in metal-mediated production of ROS.

A Discrete Transmission-Matrix Method for Modeling the Distributed Feedback Arising from Continuously Varying Refractive Index Profiles

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Several methods exist for modeling the Fresnel reflectance arising from arbitrary refractive index profiles. In many cases, the calculation can be done analytically; however, a numerical method must be employed for more complicated scenarios. The transmission matrix is an analytic method which is well suited for modeling reflection at abrupt interfaces. In this work, we develop a numerical approach, relying on the transmission matrix method, which can properly model the reflection and transmission properties of a continuously varying index profile. This approach has been applied to high power semiconductor lasers by modeling the built-in distributed feedback arising from the continuously mismatched wave impedance along the cavity length caused by a non-uniform temperature profile.

The Effects of Footwear on the Postural Stability of Women

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Introduction: Postural stability is the ability to maintain an upright posture and to keep the center of gravity (COG) within the limits of the body's base of support. It is maintained through the dynamic integration of muscle activity and joint position, both of which are influenced by sensory information from visual, vestibular, and somatosensory inputs. The foot, and therefore footwear, also plays a critical role in postural stability. The goal of this study was to determine the effects of footwear on the postural stability of women.

Materials and Methods: Dynamic balance data was collected for 54 women with ages ranging from 18 – 65 (IR# RHS-0160) wearing three types of "footwear": toning shoes, tennis shoes and no shoes (barefoot). The movement of the center of gravity was tracked for 30 seconds for each trial (with eyes open and eyes closed). The movement data was converted into two velocities: velocity in the medial/lateral direction, and velocity in the anterior/posterior directions. The two velocities of each test (toning, tennis, barefoot, eyes open, eyes closed) were plotted, observed, and compared amongst different categories. These different categories include toning vs. tennis, toning vs. barefoot, and tennis vs. barefoot. The role of age on balance was also investigated. Statistical analysis tests were completed using the student's t-test and the two sample t-test.

Results and Discussion: Toning shoes influenced the wearer's balance, particularly in the anterior/posterior direction. The subjects were much more balanced and stable in a barefoot condition; therefore, the barefoot condition was the baseline of comparison when observing the other two footwear types. Subjects experienced a statistically significant increase ($p<0.05$) in anterior/posterior instability when wearing toning shoes as compared to the barefoot data. Age also played a role in subject stability. Women over the age of 40 experienced a statistically significant increase in anterior/posterior instability when compared to women under the age of 40. For all footwear types, stability was negatively impacted ($p<0.05$) when subjects were asked to close their eyes.

Conclusion: In conclusion, the data shows that the toning shoes perform as they are designed and alter the wearer's balance. This can be identified as an increase in the velocity of the center of gravity during balance testing. The data also indicates that the most stable "footwear" for a woman's balance may be her bare feet. While the implications of the effects of footwear on static balance are limited, the increased understanding of how different factors alter a subject's balance can be useful in further studies in static balance due to changes in other factors such as injuries and implants.

Synthesis and Application of Heterogeneous Catalyzed Resorcinol:Formaldehyde Mesoporous Carbon

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Carbon cryogels are unique, porous carbon materials that exhibit high surface area, chemical robustness, and thermal stability. Current research has demonstrated that carbon cryogels serve as an efficient medium to remove heavy metals such as As, Cr, Cu, Pb, and Ti from aqueous solution. However, the ion-exchange capabilities of the carbon cryogels for ions like Cl^- and F^- has been limited. Traditional synthesis of the carbon cryogel material uses an earth metal carbonate catalyst during the resorcinol-formaldehyde polycondensation reaction. Replacing the traditional catalyst with a zeolite catalyst may improve the ion-exchange of the carbon cryogel and the atom economy of its synthesis. Carbon cryogels were synthesized through the polycondensation of resorcinol and formaldehyde in the presence of varying amounts of a zeolite catalyst followed by solvent exchange with acetone and tert-butanol, freeze-drying with liquid nitrogen, and carbonization at 800 °C in an inert atmosphere. The synthesized carbon cryogel samples were then characterized using SEM and their water filtration capabilities were tested using flame atomic absorption and ion probes.

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Evaluation of "Assay Ready" Frozen Cells and Freshly Cultured Cells for Cell Based Neutralizing Anti-drug Antibody (NAb) Assays

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The objective of this project was to evaluate the effectiveness of "assay ready" frozen cells compared to continuously cultured cells used in cell based neutralizing anti-drug antibody (NAb) assays. Maintaining cell cultures for the cell-based NAb assays can be a time and resource consuming process. To minimize delays and to be cost effective, it is desirable to have the means of using "assay ready" frozen cells directly in the cell-based NAb assays. This process was tested with four different conditions on three different cell lines, using two NAb assays, an MSD cyclic AMP kit, and an ELISA cell death assay. After many different tests, it was concluded that condition A was the preferred medium for "assay ready" frozen cells for two of the three cells lines, but did not work for the third cell line. The use of "assay ready" frozen cells would be cell line and assay dependent.

Analysis of Proximal Tibia Strain Response to Resection Alignment in Partial Knee Arthroplasty Tibial Components

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One major factor in the failing of partial knee replacements is thought to be caused by misalignment of the implant. This misalignment can lead to tibial overload and bony collapse. To prevent this, tibial components should be implanted to promote level strain distributions. New generations of instrumentation and customized guides have allowed for increased accuracy and precision of implant placement. The goal of this study was to determine the effect of bone resection on strain following implantation of the Oxford® unicompartmental knee arthroplasty (UKA) prosthesis.

In this study, the strain response to distal and medial resection depths of the UKA medial tibial component was analyzed. The metal backed tibial trays were implanted into fourth-generation composite tibial specimens using customized instrumentation. All specimens were axially loaded at the implanted medial condyle to 1.5 kN via an appropriately sized femoral component affixed to the actuator of a materials testing load frame. Loads were applied with the polyethylene tibial bearing in neutral, 4 mm anteriorly translated, and 4 mm posteriorly translated to simulate bearing motion during gait. Strain response was acquired by strain gauges on the antero- and postero-medial aspects of the proximal tibia and digital image correlation. Distal resection and component size was examined through the implantation of three sets of six tibias with 4 mm of medial shift, 4 mm of increased distal resection, and 4 mm medial – 4 mm distal shift.

Increased resection depth and medial shift significantly changed strain response at every strain gage location. Compared to a component implanted with the standard Signature™ resection guidelines, added distal and medial resection also increased variation in strain distribution by 1.2 to 1.7 times. Medial shift of the tibial component resulted in 17-27% increase in strain in the anteromedial tibia. Increased distal resection resulted in a 11-29% increase in medial and distal measurement regions. The combination of distal and medial shifts was cumulative and showed increases in strain of up to 49% in the anterio-medial tibia.

In conclusion, tibial components must be carefully aligned in UKA procedures. Surgeons should remove as little bone as possible when implanting a UKA tibial component in order to avoid increases in bone strain which can lead to failure or painful remodeling.

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Synthesis of Tamoxifen Conjugates through Linker Modification

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As a selective estrogen receptor modulator (SERM), tamoxifen is a successful anti-estrogen treatment for many cases of breast cancer; however, it behaves like estrogen in other tissues. Resistance can also develop among cells after use. While some already existing derivatives, created by attaching 4-hydroxytamoxifen to various organic molecules, have proven to be more potent than tamoxifen and have bypassed some cell resistance, the reason why these derivatives have been successful is still unknown. There are thought to be two reasons that the linkers to the tamoxifen derivatives have effect: the linkers could act as a transportation device for the drug, or the linker could interact with the estrogen receptor directly as an active part of the drug. Four reaction pathways for the synthesis of a tamoxifen derivative that could test the purpose of the linker were attempted. The goal of these reactions was to synthesize an azide derivative of tamoxifen that would break down after delivery. Attempts to link OHT-Br to hydrazine monohydrate, and secondary amines were unsuccessful. Pathways through endoxifen were found to be more promising but have not yet been completed.

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Solid Phase Extraction using Carbon Cryogels

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The concerning pollutants of the modern world tend to be waste of exotic organic materials used in medicine, pest or weed removal. EPA method 525.1 describes a solid-phase extraction (SPE) preconcentration of contaminants in water followed by analysis using gas chromatography with mass spectrometric detection. SPE is an excellent candidate for removing these pollutants as it is effective, does not expose the water to organic solvents, and works at trace concentrations. Carbon cryogels exhibit similar recoveries of trace amounts of 12 pesticides described in EPA method 525.1 when compared to the recoveries of 4 commercially available octadecylsilane (ODS or C18) cartridges. The extractions can also be accomplished using less mass per extraction with carbon versus standard columns.

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Binding of Anti-estrogen Derivatives to the Estrogen Receptor Protein in Breast Cancer Therapy

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Breast cancer, affecting 1 in 8 women in the United States, most often proliferates in the presence of estradiol, a type of estrogen hormone. Many chemotherapy drugs, such as tamoxifen and fulvestrant, work to block estradiol's effects on cancer cells by binding to the estrogen receptor, thus blocking the estradiol. This summer I developed an assay to measure the binding of antiestrogen drugs with human estrogen receptor alpha. The fluorescent polarization assay used a novel fluorescent compound in combination with different recombinant forms of estrogen receptor alpha. Two forms of truncated receptor were found to have similar abilities to bind to the fluorescent compound as full length receptor, but differing degrees of longer-term stability. The potency of the compounds determined by the binding assay reflected the relative functional potency of the compounds seen in luciferase assays in the MCF7 breast cancer cell line. In addition, uptake studies of the novel compound showed stabilized uptake after 24 hours. Current studies are exploring the potential effect of aggregation on the uptake and function of the fluorescent antiestrogen.

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Urinary Bladder Matrix as a biodegradable guide for peripheral nerve damage repair: a functional and tissue analysis

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The peripheral nervous system is the network that links the central nervous system to the rest of the body. Peripheral nerve damage is very prevalent but fortunately peripheral nerves can grow back without assistance. Growth is slow so the implantation of nerve guides can allow for the promotion of faster nerve growth, stabilization, and prevention of neuromas. The current standard for peripheral nerve regeneration is the autologous nerve graft but biodegradable nerve guides show promising results. A biodegradable nerve guide created by ACell has undergone preliminary testing and further research will be used to determine the plausibility of the ACell matristem material as an effective nerve guide. Rats will have 3mm of the dorsal gluteal sciatic nerve on their left side at mid-thigh level removed and the custom guide will be inserted. Recovery will be evaluated both functionally using video gait analysis.

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A Low-Temperature Method of Plasma-Enhanced Chemical Vapor Deposition for Carbon Nanotube Growth

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Carbon nanotubes (CNTs) have many potential applications in academic and industrial settings including structural reinforcement, air and water filtration, electrical circuitry, and detection systems in scientific equipment. Due to the wide variety of applications, research into production and use of CNTs is a very active area with many opportunities for improvement. The primary methods currently available to grow CNTs are arc discharge and chemical vapor deposition (CVD). Chemical vapor deposition requires a very high temperature for the process to work (~700°C). In order to reduce the temperature requirements of CVD, this study evaluated plasma-enhanced chemical vapor deposition (PECVD) for making CNTs. PECVD was performed using a TePla M4L plasma asher for times ranging from 10 to 70 minutes with either carbon dioxide (CO_2) or acetylene (C_2H_2) as the primary carbon source. The flow rates of the carbon source, Argon, and Oxygen gases were varied between 0 and 150 sccm, and the RF power was varied between 200 and 600 W. Scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDX) suggest that carbon growth occurred on the surface of several samples. Other techniques including Raman spectroscopy are currently ongoing to characterize the structure of the carbon growth.

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Detection of Phytoplasma in *Trillium grandiflorum* using 16s rDNA PCR

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Trillium grandiflorum plants in Michigan were found to exhibit symptoms typical of a phytoplasmic infection; using 16SrDNA sequencing infectious agent has been identified to be in the phytoplasm family. *Trillium grandiflorum*, often referred to as White Trillium, is a perennial, monocot flower indigenous to the North Eastern portion of the United States. Trillium seeds are typically spread by ants, who consume the elaiosome of the seed and disperse the seeds; thus raising the issue of vector transmission of the infection. Trillium samples collected in Michigan displayed signs of phytoplasmic infection; namely floral virulence, or the transformation of floral parts to leafy green structures. Since no published information could be obtained concerning the identification of the infectious agent in White Trillium, the primary focus of the project has been to identify the organism causing the symptoms. Phytoplasmic infections are usually confined to the phloem tissue of the plant so using the blank DNA Extraction kit, DNA was isolated from frozen Trillium samples; an uninfected plant control was also used. Using PCR, phytoplasmic DNA was amplified using universal primers. The sequenced DNA matched other phytoplasmic species to 97%. New *Trillium grandiflorum* samples were obtained from a new site in northern Michigan that exhibit symptoms similar to the original samples suggesting a related phytoplasmic infection. The same process used with the original samples was applied to the new samples from Michigan over the summer of 2012. Curiously, subsequent experiments detected the presence of *Erwinia,* a common gram negative plant pathogen. Phytoplasma were not detected. These results have caused a reevaluation in the specificity of the phytoplasma primers and the methods used in the experiments

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Studying Estrogen Interactions One Receptor at a Time

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Estrogen is essential for the growth of breast tissue but also supports the growth of 50% of primary breast cancers. The estrogen ligands involved in estrogen interactions are used to signal other estrogens. There had only been one estrogen receptor (estrogen receptor alpha, ERalpha) known until the late 1990's when a second estrogen receptor had been discovered, ERbeta. It is still unclear just how ERalpha and ERbeta actually interact but it is known that they share 61% of the same ligand binding domain sequence and about 98% of the DNA sequence. Eventually, the main goal of my research is to determine this relationship between the interaction of ERbeta and ERalpha on the molecular level and characterize those interactions. The interaction between ERalpha and other substances (including itself) has already been studied substantially and conclusions have been made, but the study of ERbeta is still in the early stages.

I do suspect that there is interaction between these two receptors that is extremely relevant to the study of physiological systems. In order to reach my main goal, many experimental protocols need to be tailored to fit some of the unknown properties of the ERbeta. In the short term, the goal is to clone the ERbeta using standard experimental techniques. The ligand-binding domain coding sequence for ERbeta was amplified using PCR, cleaved with restriction enzymes, and inserted into the pMAL plasmid. Cells transformed with the constructed plasmid were then spread on an agarose plate containing ampicillin and colonies were allowed to grow. To be sure that the colonies that grew actually have the correct plasmid, PCR was run using colonies as templates and then run on DNA gels to assure that the size of the amplified DNA was correct. If correct, I used two other tests involving digestions that would be performed to assure that the sequence is the correct size before sending the DNA off for sequencing. During this process, problems occurred mainly during the initial digestion before the ligand-binding domain coding sequence was inserted into the plasmid. Once the ERbeta is successfully cloned, I intend to purify the protein from *E. c*oli containing the plasmid. Then, the properties of this protein will be studied (using dimerization and assay experiments).

Once conclusions can be made on how ERbeta contributes to the interaction of ERalpha with other substances, it can be applied to how these two receptors interact in the human body and we can make assumptions on what we think is going on in the cell. These conclusions can then be tied to breast cancer, other diseases caused by estrogen, and physiological systems of the body.

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Alkaloid Constitutents of *Dendrobates auratus* from Oahu, Hawai'i

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We examined the alkaloid content of 13 specimens of the black and green poison frog *Dendrobates auratus*, collected from three locations in the Manoa highlands of Oahu, HI in 2009. Principal alkaloids included pumiliotoxin 251D as well as its hydroxylated analog, allopumiliotoxin 267A. Other major alkaloids included homologous pumiliotoxins, a series of decahydroquinoline congeners and a variety of minor and trace alkaloids. These profiles were compared to the same species collected in Oahu 1988 as well as in Panama in the 1970s to assess geographic and temporal variations in alkaloid content. Microsympatric arthropods were also collected and analyzed. Our results to date will be discussed.

Ellery Steele is a senior ISU Chemistry major from Linton, IN, and will graduate in May 2013

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Optimized Microwave Assisted Organic Synthesis using Suzuki-Miyaura Cross-Coupling

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The Suzuki cross-coupling reaction was discovered by Professor Akira Suzuki in the late 1970s. This reaction is instrumental in the formation of carbon-carbon bonds between benzene ring structures. The Suzuki reaction, displayed below, couples a phenylboronic acid with an aryl halide to produce a biaryl product.

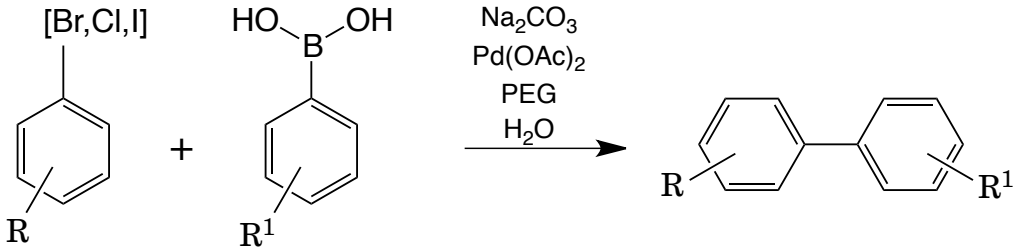

Figure I: The Suzuki-Miyaura cross-coupling reaction where R and R^1 are any organic functional group.

These biaryl products are employed in several growing industries, including pharmaceuticals, herbicides, natural products, conducting polymers, and liquid crystalline materials. An important factor in any industry is optimization, which saves time and ultimately increases profits for a business. The Suzuki reaction is not immune to these economic considerations. As such, scale up of the Suzuki reaction has been an important topic in the current world of green chemistry. This would allow companies to produce the desired biaryl products on a gram instead of milligram scale most efficiently.

Whereas traditional heating methods have been employed in the past, current microwave assisted technologies are being researched. Microwave assisted organic synthesis (MAOS) allows for specificity in control of reaction conditions such as pressure and temperature, as well as reaction time. If optimized, the specificity over control provides the company savings in time and potentially could increase product yields, which would lead to economic benefits.

Due to the above considerations, several reaction variables which potentially could lead to a more optimized Suzuki reaction were studied in laboratory settings. The microwave-promoted, palladium-catalyzed, Suzuki-Miyaura cross-coupling reaction of aryl halides with phenylboronic acid performed in aqueous media was studied to understand the effect of halide lability on final product yield, while also investigating the effect of the presence of a palladium catalyst. To do this, a Suzuki cross-coupling was performed using 5-bromo- and 5-iodo-salicylic acid under the reaction conditions displayed in the figure above. The results indicated that the microwave-promoted Suzuki cross-coupling run in the presence of the palladium catalyst produces significant increase in yield while optimizing reaction time. Currently, the effect of the alkali metal in the base used in the Suzuki cross-coupling is being evaluated, along with various functional groups on the aryl halides, to determine if results can be used to further optimize the microwave-promoted Suzuki coupling reaction.

Mediator complex requirement for transcription initiation regulation in *S. cerevisiae*

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Mediator is a large multisubunit complex composed of four distinct modules: head, middle, tail, and Cdk8-cyclin. Evidence suggests the head module aids in the recruitment of RNA Polymerase II to all protein-coding genes in yeast. The middle module functions primarily as a scaffold to hold the complex together. The tail module however, is required in a gene-specific manner. Different subunits of tail module interact with different activators and coactivators and are also known to interact with the Spt-Ada-Gcn5-Acetyltransferase (SAGA) complex, another regulator of Pol II transcription machinery, for specific genes. The Cdk8-Cyclin module is unique in that it only associates with the rest of the Mediator complex in a gene-specific manner. Cdk8-Cyclin also inhibits genes that require Mediator tail, but some genes, such as *GAL10*, require the Cdk8-Cyclin module for transcriptional activation.

In order to understand the role of tail module subunits in the recruitment of SAGA complex and association of CDK8 module at specific genes, we used a yeast strain defective in the tail module function. ChIP analysis for different subunits of SAGA complex and CDK 8 module indicated that the tail module is required for the recruitment of SAGA complex in a gene specific manner whereas the recruitment of CDK8 module seems to depend on both the tail module and the core Mediator (head and middle modules). We conclude that specific interactions between Mediator tail and other transcriptional regulators may be important for providing gene specificity in transcription regulation.

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