# 2010 Rhode Island Summer Undergraduate Research Fellowship Conference

*Friday, July 30, 2010*
*8:00 AM*

**The Ryan Center, University of Rhode Island**

*Supported by*

RI EPSCoR
Experimental Program to Stimulate Competitive Research

**RI-INBRE & RI EPSCoR**

**SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE**

*FRIDAY, JULY 30, 2010*
*THE RYAN CENTER CONCOURSE*
*UNIVERSITY OF RHODE ISLAND*
*KINGSTON, RI*

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|---|---|
| 8:00 – 9:00 AM | ***CONTINENTAL BREAKFAST & GROUP A POSTER SET-UP*** |
| 9:00 – 9:15 AM | ***WELCOMING REMARKS*** |
| | ELIZABETH ROBERTS, LIEUTENANT GOVERNOR<br>STATE OF RHODE ISLAND |
| | VINNY BROWNING, SENIOR MANAGER<br>QUALITY ANALYTICAL LABORATORIES, AMGEN |
| | DR. DONALD DEHAYES, PROVOST & VICE PRESIDENT FOR ACADEMIC AFFAIRS, UNIVERSITY OF RHODE ISLAND |
| | DR. PETER ALFONSO, RI EPSCOR PROJECT DIRECTOR & VICE PRESIDENT FOR RESEARCH AND ECONOMIC DEVELOPMENT, UNIVERSITY OF RHODE ISLAND |
| | DR. ZAHIR SHAIKH, RI-INBRE PROGRAM DIRECTOR<br>UNIVERSITY OF RHODE ISLAND |
| 9:15 – 10:45 AM | ***SUMMER UNDERGRADUATE RESEARCH FELLOWS POSTER SESSION*** |
| | GROUP A |
| 10:45 – 11:00 AM | ***INTERMISSION & GROUP B POSTER SET-UP*** |
| 11:00 – 12:30 PM | ***SUMMER UNDERGRADUATE RESEARCH FELLOWS POSTER SESSION*** |
| | GROUP B |
| 12:30 PM | ***LUNCH*** (MORNING PROGRAM ENDS) |

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|---|---|
| 1:00 PM | ***RI-INBRE BUSINESS MEETING*** (RI-INBRE INVESTIGATORS ONLY) |

## LIST OF SUMMER RESEARCH FELLOW POSTERS

***Please note that the poster numbers listed in the following tables also correspond with the page numbers in the abstract book. In instances where the Summer Fellow is from an institution other than where the summer research was performed, the Summer Fellow's home institution has also been listed.*

### University of Rhode Island

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **1** | Rebecca Abramovitz | David Worthen |
| **2** | Cesar Alejo | Stanley Barnett |
| **8** | Correna Blewett | Carol Thornber |
| **22** | Olivia Diprete,<br>Narragansett High School | Angela Slitt |
| **24** | Karen Duong,<br>Brown University | Abraham Kovoor |
| **26** | Maxwell Edmonds | Navindra Seeram |
| **27** | Michael Fagbote | Walter Bessio |
| **32** | Aimee Gagnon,<br>University of New Haven | Keykavous Parang |
| **34** | Meagan Gamache | Tatiana Rynearson |
| **42** | Renae Gupta,<br>Community College of Rhode Island | Roberta King |
| **46** | Wusung Kim | Bongsup Cho |
| **47** | Nelson Knudsen,<br>Moravian College | Angela Slitt |
| **92** | Heather Kumar,<br>Community College of Rhode Island | Geoffrey Bothun |
| **57** | Kimberly Manchester,<br>Community College of Rhode Island | Abraham Kovoor |
| **26** | Chuck Marcotte, Teacher<br>Cranston High School East | Navindra Seeram |
| **62** | Admir Monteiro | Mohammad Faghri |
| **72** | Marissa Raish | Brenton DeBoef |
| **74** | Megan Reidy,<br>Providence College | Niall Howlett |
| **61** | Megan Meloon | Angela Slitt |
| **75** | Adam Reis,<br>Narragansett High School | Angela Slitt |

**University of Rhode Island (Continued)**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **76** | Andrew Roussinos,<br>Providence College | David Nelson |
| **79** | Simon Sarkisian,<br>Providence College | David Rowley |
| **80** | Joseph Schrader | Matthew Stoner |
| **81** | Dante Sciarra,<br>Community College of Rhode Island | Clinton Chichester &<br>Amanda DeAngelis-Chichester |
| **87** | Felicia Strom | Clinton Chichester &<br>Amanda DeAngelis-Chichester |
| **90** | Aimee Welch | Arthur Gold |

**Brown University**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **25** | Anthony Durta,<br>Community College of Rhode Island | Rebecca Page |
| **39** | Michael Gonzalez,<br>University of Rhode Island | Edward Hawrot |
| **51** | Michael Lapadula | Will Fairbrother |
| **53** | Jason Lee | Barbara Stonestreet |

**Providence College**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **40** | Christopher Brennan | Brett Pellock |
| **12, 18** | Gabriella Brum | Yinsheng Wan |
| **14** | James Cebulski | Nicanor Austriaco |
| **15** | Regis Chang | Yinsheng Wan |
| **12, 18** | Vendita Correia | Yinsheng Wan |
| **20** | Shawn Davidson | Nicanor Austriaco |
| **9** | Richard Dell'Isola | Christopher Bloom |
| **40** | James Engel | Brett Pellock |
| **40** | Matthew Goulet | Brett Pellock |
| **41** | Erik Gravel | Nicanor Austriaco |
| **44** | Matthew Hurton | Nicanor Austriaco |
| **9** | Diana Klakotskaia | Christopher Bloom |
| **50** | Christopher Lang | Jack Costello |
| **55** | Elizabeth Lunny | Chrisopher Laperle |
| **60** | Lindsay McHugh | Joseph DeGiorgis |
| **66** | Ari Nalbandian | Yinsheng Wan |
| **77** | Colin Samoriski | Joseph DeGiorgis |
| **9** | Kelly Sheehan | Christopher Bloom |

**Rhode Island College**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **3** | Alexander Amer | John Williams |
| **6** | Angela Bannister | Robin Montvilo |
| **11** | Lily Brown | Karen Almeida |
| **56** | Sathiarith Chau | Thomas Malloy |
| **38** | Katie Cilento | Beverly Goldfield |
| **16** | Erika Clift | Rebeka Merson |
| **19** | Lorenzo Crumbie | John Williams |
| **6, 21, 63** | Jennifer Desjarlais | Robin Montvilo |
| **29, 54, 85** | Xenia Fernandez | Deborah Britt |
| **35** | Chris Gemski | John Williams |
| **37** | Nathan Goff | Karen Almeida |
| **43** | Janis Hall | Rebeka Merson |
| **45** | Michael Jastram | Rebeka Merson |
| **56** | Lorin Kinney | Thomas Malloy |
| **29, 54, 85** | Alise Lombardo | Deborah Britt |
| **11** | Kirsten Mello | Karen Almeida |
| **56** | Tiia Nurmikko | Thomas Malloy |
| **71** | Megan Radka | Sarah Spinette |
| **16** | Daniel Reeves | Rebeka Merson |
| **6, 21, 63** | Erica Russo | Robin Montvilo |
| **56** | Melissa Ryan | Thomas Malloy |
| **29, 54, 85** | Summer Smith | Deborah Britt |
| **3** | Jose Solares | Sarah Spinette |
| **86** | Amanda St. Germain | Sarah Spinette |
| **56** | Marvin Tabares | Thomas Malloy |
| **38** | Renata Veiga | Beverly Goldfield |
| **38** | Lauren Whittle | Beverly Goldfield |
| **91** | Valerie Zabala | Karen Almeida |

**Roger Williams University**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **5** | Nichole Ares | David Taylor |
| **28** | Lisa Fealy | Lonnie Guralnick |
| **30** | Ashley Ferreira | Roxana Smolowitz |
| **36** | Carissa Gervasi | David Taylor |
| **48** | Heidi Kunkel | Marcia Marston |
| **49** | Nicholas Kutil | David Taylor |
| **68, 69** | Colin Latimer | Avelina Espinosa |
| **52** | Garrett LeBlanc | David Taylor |
| **58, 69** | Barbara Mann | Marcia Marston |
| **68, 69** | Monichan Phay | Avelina Espinosa |
| **68, 69** | Lisseth Silva | Lauren Rossi |
| **84** | Noel Sme | Marcia Marston |

**Salve Regina University**

| Poster # | Summer Fellow | Mentor |
|---|---|---|
| **4** | Kendra Andrie | Sandor Kádár |
| **7** | Jessica Barowski | Alison Shakarian |
| **10** | Amanda Borges | Sandor Kádár |
| **13** | Amy Canino | Sandor Kádár |
| **17** | Amy Coffey | Bernard Munge |
| **23** | Paul Diss | Sandor Kádár |
| **17** | Jaimee Doucette | Bernard Munge |
| **31** | David Fraulino | Alison Shakarian |
| **33** | Daneila Galluzzo | Steven Symington |
| **70** | Joel Gluck, Teacher<br>Cranston Public Schools | Sheila Quinn |
| **73** | Carin Heaney | Sheila Quinn |
| **93, 94** | Kylie Kenney | Jameson Chace |
| **93, 94** | Gregory Keras | Jameson Chace |
| **88** | Kara Lombardo | Bernard Munge |
| **59** | Justin Mare | Alison Shakarian |
| **70** | Matthew Maynard | Sheila Quinn |
| **33, 65, 67** | Edwin Mutanguha | Steven Symington |
| **67** | Priscilla Perez | Steven Symington |
| **70** | Lauren Pirrmann | Sheila Quinn |
| **70, 73** | Kaela Rees | Sheila Quinn |
| **78, 82** | Carlos Santos | Alison Shakarian |
| **82** | Connor Shope,<br>Great Bridge High School | Alison Shakarian |
| **88** | Michael Sullivan | Bernard Munge |
| **89** | Zarchary Valentine | Steven Symington |

Purification of *Stevia rebaudiana* extract using foam fractionation

Abramovitz, R. B., Worthen, D., Seeram, N.P.

Department of Biomedical & Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Rebaudioside A (Reb A) is a non-caloric sweetener derived from the leaves of *Stevia rebaudiana*. Reb A must be separated and purified from other *Stevia* components in order to be incorporated into food products. Reb A is currently purified using solvents, resins, and metal chelates. In this study, foam fractionation, an adsorptive bubble seperation technique, was evaluated as a rapid, environmentally-friendly means of separating and recovering Reb A and other *Stevia* glycosides from aqueous dispersions. *Stevia* glycosides foamed under a variety of conditions. A stability indicating HPLC assay revealed that these components remained stable during the foaming process. The ratio of column length to width, foaming temperature, and co-surfactants had the greatest influence on foaming. Foam fractionation may be a green alternative for producing Reb A.

A microfluidic chip to electroporate microalga cells for oil extraction

Alejo, C., Barnett, S.

Departments of Chemical and Electrical Engineering, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Algae cells containing a high percentage of oil can be extracted by different methods and converted to biodiesel. For this experiment we evaluated the electroporation method. Electroporation is the use of high-voltage to create an electric field which causes pores in the cell membrane. These pores allow molecules, ions, and liquids to travel both ways. There are many different types of microalgae cells, for this experiment we used “Dunaliella tertiolecta”. A microfluidic chip was designed and constructed to implement the electroporation of algal cells. This microfluidic chip has a microchannel that gets narrow in the middle to increase the strength of the electric field and open the pores in the cells, at the larger diameter microchannel there is going to be lower electric field strength. That field is going to generate the electro-osmatic flow for the cells to travel from one end of the channel to the other. After running several experiments results came out as expected, we were able to open pores on the cell membrane and cells travel from one end of the channel to the other.

Arylphosphonium salts conjugated to fluorescein show FRET spectra *in vitro* and are taken up by live cells in culture as imaged under fluorescent microscopy

Amer, A.[1], Solares, J.[2], Spinette, S.[2], Williams, Jr., J.C.[1]

[1]Department of Physical Sciences, Rhode Island College, Providence, RI
[2]Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Three examples of arylphosphonium salts conjugated to fluorescein (APS-Fluor) have been prepared and identified by IR, MP and MS. The syntheses were done by conventional bench top methods and using a microwave reactor. The parent compound has shown FRET spectra at 480 nm. It diffuses into live muscle cells in culture from micromolar concentrations in DMSO and the labeled cells are observed by fluorescent microscopy. Observation of differential uptake by normal and malignant cell lines and an SAR for the three APS-Fluor conjugates is underway.

Development and evaluation of a fura-2 fluorescent assay to assess intracellular dynamics of PC12 cells

Andrie, K.[1], Kádár, S.[1], Symington, S.B.[2]

[1]Department of Chemistry, Salve Regina University, Newport, RI
[2]Department of Biology & Biomedical Science, Salve Regina University, Newport RI

RI-INBRE Summer Undergraduate Research Fellowship Program

External stimuli elicited by neurotransmitters and ionic species produce intracellular calcium oscillations that often range from seconds to minutes. These oscillations are propagated throughout a cell by intracellular regulatory mechanisms that include a series of biochemical reactions working in concert to regulate intracellular calcium levels. To better understand the mechanism of intracellular calcium dynamics, we have developed a microplate assay that utilizes PC12 cells loaded with Fura-2, a calcium indicator dye. In this experiment, fura-2 acetylmethoxyester was passively loaded into PC12 cells by incubation at 37°C for one hour, washed, and aliquated to a 96-well microplate. Results indicate that fura-2 was successfully loaded into PC12 cells and changes fluorescence were observed. Preliminary data was subject to Fast Fourier Transfer (FFT) analysis and calcium oscillations were observed. These results indicate that Fura-2 is a viable tool to investigate calcium oscillations in PC12 cells.

Mercury accumulation in brain, muscle, and liver tissue in fish: a tri-species comparison

Ares, N.L., Taylor, D.L.

Department of Marine Biology, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant that negatively affects human health, and exposure occurs mainly through the consumption of finfish. Consequently, previous research has measured Hg levels in muscle filets of edible fish, including bluefish (*Pomatomus saltatrix*), black sea bass (*Centropristis striata*), and tautog (*Tautoga onitis*). However, there is little information on Hg concentrations in other tissues. The brain is a tissue of particular concern because Hg is a neurotoxin, and the liver is also of concern as it plays a role in detoxification. Recent research has suggested that selenium (Se), another heavy metal, may have a mitigating effect on Hg toxicity. While this relationship has been investigated in other organisms, the species in this study have not been investigated. The objectives of this investigation were to: (1) examine Hg bioaccumulation in brain, liver, and muscle tissue of bluefish, black sea bass, and tautog, (2) evaluate the relationship between Hg levels in the three tissue types, and (3) examine the relationship of Hg and Se within the target species. From June to August 2007-2010, target fish were collected from the Narragansett Bay (RI, USA). Length (cm) was recorded for each fish, after which total Hg was measured in excised muscle, liver, and brain tissue using combustion atomic-absorption spectroscopy (ppm dry wt). The tissues were also analyzed for total Se using inductively coupled plasma mass spectroscopy. For tautog, Hg concentrations of muscle was positively correlated with fish length, indicating that Hg bioaccumulates in this tissue. There was also a positive correlation between muscle and brain tissue Hg concentrations in bluefish and tautog, as well as a positive correlation between liver and muscle Hg for bluefish and black sea bass. Bluefish experienced the lowest Hg concentrations in all tissues relative to the other species. Interspecies differences can be attributed to age-at-catch of the different species. Se-Hg relationships were investigated in the bluefish, with molar ratios of 14.1 in muscle, 47.5 in liver, and 78.1 in the brain. All ratios suggest protective properties of Se on Hg toxicity.

The elderly and addiction:  When pastime turns into problem

Bannister, A., Desjarlais, J., Russo, E., Torres, T., Lewis, B., Montvilo, R.

Psychology Department, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

As Baby Boomers are reaching senescence, addictions in this population often go unnoticed, largely due to a lack of screening. Factors contributing to the missed diagnoses of addiction include denial by the individual along with an overwhelming feeling of guilt. Individuals must be encouraged to acknowledge and talk about their issues and physicians must be better trained to screen their patients. A task force on the elderly and addiction was established. In developing best practices, a geriatrician/psychiatrist addressed issues of screening, diagnosis, and treatment of addictions in mature adults and a discussion of SBIRT (Screening, Brief Intervention, and Referral to Treatment) followed. Additionally a questionnaire to detect and diagnose problem gambling in mature adults was developed. Mature adults who utilize senior centers, senior high rises, and assisted living facilities in Rhode Island will be offered screenings and information on problem gambling. For each site, a minimum of 2 INBRE students will conduct the screenings.  A faculty member in the Chemical Dependency/Addiction Studies program will accompany them and do a brief presentation on issues related to gambling. Time will be allotted for questions and answers.

# The effect of metal ions on an expressed secretory Lipase from the human pathogen *Leishmania donovani*

Barowski, J., Shakarian, A.

Department of Biology & Biomedical Sciences, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Previously we identified and began characterization of a secreted lipase (LdLip3) from the human parasite *Leishmania donovani*. Secreted lipases have been implicated as virulence factors in several pathogenic organisms such as fungi, yeast and bacteria. These enzymes catalyze the hydrolysis of fats to form glycerol and fatty acids, which in turn may be used to synthesize complex lipids required for the organism's growth, development and/or survival in its mammalian host. The goal of the current study was to determine if metal ions affected the enzymatic activity of an episomally expressed HA-tagged lipase produced and secreted by transfected *L. donovani* parasites. For these experiments *L. donovani* promastigotes grown in M199 medium without serum and were harvested by centrifugation. The secreted HA-tagged LdLip3 was purified from the cell-free supernatants using an anti-HA protein G affinity matrix column (Roche). The eluted fractions were tested for enzyme activity in McIlvaine buffer at pH of 6.0 for 30 min at 26°C, 37°C, or 42°C using 4-methylumbelliferyl stearate as a substrate. Lipase activity data was analyzed first by comparison to a standard curve constructed with known concentrations of 4-methylumbilliferone to determine nmol of product produced. Subsequently, specific activity of the lipase enzyme was calculated as nmol/min/mg of purified protein. Our results showed that there was approximately a 450 fold increase in enzymatic activity when comparing the purified protein to the 1X (unpurified) supernatant samples from *L. donovani* tranfectants. Metal ions such as Mg and Zn are known to be cofactors of some enzymes, greatly enhancing their activity; therefore, in the current study several metal ions were tested to determine their effect on the specific activity of the purified LdLip3 enzyme. Results showed that the addition of $MgCl_2$, KCL, NaCl, and $ZnSO_4$ to the enzyme assays produced an increase in activity at whereas $MnCl_2$ and $CoCl_2$, showed an inhibitory effect. When the chelating agent EDTA was added, the effect on enzyme activity, whether activating or inhibitory, was reversed. Taken together, our results support the idea that metal ions in general have an activating effect on the activity of the secreted lipase LdLip3 from *Leishmania donovani*.

Effect of tidal height on predation of the Asian shore crab *Hemigrapsus sanguineus* on Prudence Island, Rhode Island

Blewett, C.L., Rohr, N.E., Thornber, C.S.

Department of Biological Sciences, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Invasive species are capable of significantly altering native community structure and biodiversity in their invaded ranges, which often results in decreased marine health and ecosystem services. The introduced *Hemigrapsus sanguineus*, the Asian shore crab, has become established in the intertidal zone of the east coast of North America, and is able to reach high densities. In Rhode Island, *H. sanguineus* out-competes native and established crab species for space, effectively eliminating them from the intertidal zone of cobble beaches. While studies have shown that killifish prey on juvenile *H. sanguineus* in a lab setting, little is known about the predation rate on this invasive crab *in situ*. To determine the effect of tidal height on the predation on *H. sanguineus*, we conducted field studies at Bear Point, Prudence Island during the summer of 2010 at three tidal heights: shallow subtidal, low intertidal, and high intertidal. Determining the predator-prey interactions of *H. sanguineus* is essential to understanding the limiting factors of *H. sanguineus* and how the presence of this invasive crab will affect food web interactions and species composition in the intertidal zone of Rhode Island.

CNS stimulate (caffeine) and environmental stressors in an animal model of non-suicidal self-injury

Sheehan, K., Dell'Isola, R., Klakotskaia, D., Hou, S., Wallin, C., Bloom, C.

Department of Psychology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Self-injurious behavior (SIB) and non-suicidal self-injury (NSSI) are associated with two distinct populations. While SIB is most typically confined to individuals with cognitive or developmental disabilities, NSSI is most common among typically developing adolescents. It is to be expected, then, that these two conditions present in very different ways. SIB often includes stereotypic and repetitive self-harming behaviors, long hypothesized to serve a self-stimulating function for the individual. Typical manifestations include head banging, hitting or striking self, biting, scratching, hair pulling, or hitting arms or legs against other objects. In contrast, the term non-suicidal self-injury (NSSI) has been applied to a wide range of behaviors that result in the immediate damage of one's own body tissue in the absence of intent to die. A recent study found that the most common methods of NSSI cited by university students were cutting and scratching. The primary goal of the present study was to simulate an SIB condition using a supported animal model of self-injury (caffeine injection), and systematically manipulate stress conditions to increase arousal in rodents, and subsequently impact the animals' stress response. In other words, if SIB and NSSI are indeed similar phenomena, application of an external stress condition would likely increase the frequency of SIB in rats. Specifically, we sought to: (1) Determine if a fear evoking environment results in an increase in self-injury; (2) Determine if any increase in self-injury comes at the cost of a decrease in an effective and healthy behavior.

Animals were trained in a high stress (fear conditioning) and low stress (signaled positive reinforcement) and exposed to chronic caffeine injections to investigate the concomitant effects of physiological arousal due to pharmacological intervention and environmental stressor on self-injury in rodents.

Validation of an *in silico* Mathematical Model Used to Predict Internal Calcium Dynamics and Assess the Physiological Consequences of Extracellular Stimuli on PC12 Cells

Borges, A.[1], Salter, D.[2], Kádár, S.[1], Symington, S.B.[2]

[1]Department of Chemistry, Salve Regina University, Newport, RI
[2]Department of Biology & Biomedical Sciences, Salve Regina University, Newport, RI

Calcium ($Ca^{2+}$) is an important second messenger for a variety of cellular processes including muscle contraction, mitosis, and fertilization. Information important in controlling these processes is carried from extracellular signals, and encoded within the frequency of $Ca^{2+}$ oscillations. These processes are enormously complex, involving the fine regulation of several divergent signal cascades. To better understand the $Ca^{2+}$ dynamics of PC12 cells, an *in silico* mathematical model was developed and has the potential to elucidate the complex chemical processes involved with cellular communication. In this experiment, we compared the $Ca^{2+}$ dynamics of PC12 cells that was determined both experimentally, and using our computational model. As expected, extracellular stimuli (dopamine and potassium chloride) altered the $Ca^{2+}$ dynamics of PC12 cells as predicted by computer simulations. Frequency of $Ca^{2+}$ oscillations was extracted from experimental and simulated data through the analytical technique Fast Fourier Transform. Analysis of Fast Fourier Transform results indicates that when external stimuli are added to PC12 cells frequency of $Ca^{2+}$ oscillations remains the same, and Signal to Noise Ratio is decreased. These results demonstrate that the model can be used as a predictive tool to assess $Ca^{2+}$ dynamics. Furthermore, the model predicts that alteration in $Ca^{2+}$ dynamics results in an increase in dopamine release from PC12 cells. Future amperometric experiments will be performed to measure dopamine release from PC12 cells to validate the physiological consequences predicted by the model.

Bloom syndrome facilitates genomic stability through protein-rotein interactions

Brown, L.W., Mello, K., Almeida, K.H.

Physical Sciences Department, Rhode Island College, Providence, RI

RI-INBRE & RI EPSCoR Summer Undergraduate Fellowship Programs

Bloom Syndrome is a rare disorder caused by a mutation in the Bloom Syndrome protein (BLM) gene and characterized by genomic instability and cancer predisposition. BLM protein is a 160 kD protein involved at the DNA replication fork and the DNA repair pathway, homologous recombination (HR). This research investigates the protein-protein interactions between BLM and Flap Endonuclease 1 (Fen1), a protein that removes 5' overhangs and processes Okazaki fragments in DNA repair and synthesis.

Full length BLM open reading frame (ORF) was segmented into smaller overlapping sections and PCR amplified to incorporate a C-terminus FLAG epitope tag. The linear DNA was inserted into pENTR (Invitrogen) vector for *E.coli*. Colonies were verified by restriction enzyme digestion and confirmed by DNA sequencing. BLM protein fragments were expressed from *E.coli* for farwestern analysis, which determined the refined interaction domain as BLM amino acids 1217-1417.

Alternatively, expression vectors for yeast two-hybrid analysis were generated and confirmed via restriction enzyme digest. Preliminary results confirmed that BLM amino acids 1217-1417 contain interaction domains for Fen1. Interestingly, BLM amino acids 642-1290 may also possess a region of weaker association.

The BLM and Fen1 interaction is weak, but can be demonstrated via the methods used here. Additional experiments are required to determine the exact interaction domain.

Hypoxia induces cell migration in CaOV3 cells, but does not induce HIF-1α and LOX expression

Brum, G., Correia, V., Wan, Y.S.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In the presence of hypoxia, ovarian cancer cells are induced to migrate. Because volume grows quicker than the surface area of a cell, inner cells are prone to lower oxygen environments. Under these conditions cancer cells continue to thrive and propagate their species. Previous studies have shown that levels of HIF-1α and LOX increase in the presence of hypoxia. This leads to greater metastatic potential of cancer cells. Our study focused on the migratory potential of ovarian cancer cells in both hypoxic condition as well as the induced hypoxic effect of CoCl2. Levels of LOX and HIF-1α were also tested under similar conditions. Phagokinetic track motility assays demonstrated that the effects of hypoxia and CoCl2 did increase the migration of ovarian cancer cell line, CaOV3. Levels of HIF-1α and LOX were shown to have no change in the presence of hypoxia or CoCl2. This early study of cancer cell migration proposed that hypoxia along with low levels of CoCl2 induced cell migration. The metastatic potential of ovarian cancer cells is thought to be low due to the lack of HIF-1α and LOX expression.

Intracellular calcium: Modeling the biphasic regulation of the IP3 receptor with the DeYoung-Keizer Model

Canino, A., Kádár, S.

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RI-INBRE Summer Undergraduate Research Fellowship Program

Previous models of calcium dynamics use simple biphasic regulation of the inositol-1,4,5-triphosphate ($IP_3$) receptor in order to account for the calcium that is released from the endoplasmic reticulum (ER). This model only accounts for one binding pathway of calcium and does not account for the dynamics of $IP_3$ on the receptor. In order to create a more complete model, a more comprehensive approach to biphasic regulation was added. The Deyoung-Keizer model allows for the activated receptor state to be created by more than one pathway, and it takes the dynamics of $IP_3$ into consideration. The equations for this model were added into a previously written Matlab script. A parameter sweep of the script was run to find that the bifurcation points of $K_{Gp(\beta\gamma)}$ were 0.006 and 0.040. Increasing $K_{Gp(\beta\gamma)}$ values between these points account for increasing frequencies of the calcium oscillations in the cell. Another bifurcation parameter that was altered was $k_{ch}$ which increased the amplitudes of the oscillations proportionally to the increase of the $k_{ch}$ value. The model with simple biphasic regulation saw limited success; however, the frequency of calcium oscillations in experimental data is very similar to data obtained from this theoretical model.

Yeast Bax inhibitor Bxi1p is an ER-localized protein that is involved in the unfolded protein response

Cebulski, J., Malouin, J., Pinches, N., Cascio, V., Austriaco, N.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bax Inhibitor-1 (BI-1) was first identified by its ability to block Bax-induced programmed cell death in the budding yeast, Saccharomyces cerevisiae. Intriguingly, BI-1 is one of only a few cell death inhibitors found in a wide range of fungi, plant, and animal species including S. cerevisiae, D. melanogaster, A. thaliana, M. musculus, and H. sapiens. Significantly, overexpression of BI-1 has been associated with several kinds of cancers in human patients including pulmonary adenocarcinoma. Recent studies in mammalian and plant cells have suggested that BI-1 is involved in the cell's response to endoplasmic reticulum stress (ER-stress). To better understand the function of BI-1, we are characterizing the yeast homolog of BI-1, which we are calling BXI1. We have shown, through fluorescent microscopy that Bxi1p-GFP colocalizes with the ER localized protein Sec63p-RFP. We have also discovered that cells lacking BXI1 have a decreased unfolded protein response as measured with a UPRE-lacZ reporter, suggesting that Bxi1p is involved in the yeast cells response to ER stress. Furthermore, these mutant cells are more susceptible to programmed cell death under a variety of conditions, which in certain cases is exacerbated in the presence of the calcineurin-inhibitor, FK506. Our data suggests that the BXI1 gene functions may be involved in an ER-linked pathway that is linked to calcineurin and the regulation of $Ca^{2+}$ levels within the yeast cell.

UV and infrared radiation down-regulate dendriticity and tyrosinase in cultured human melanocytes

Chang, R., Wan, J., Tian, J., Kan, M., Zheng, D., Wan, Y.S.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Vitiligo is a skin disease with reduced pigmentation. Over the past decade, the incidence of vitiligo has increased. And yet, the causes of the disease remain unclear, with clinical management of such disease limited. Accumulating studies have suggested that increased ultraviolet (UV) radiation may be related to the damage of melanocytes. However, the cellular and molecular mechanisms are largely unknown. Most recent studies also point to the involvement of infrared radiation A (IRA) in skin cell damage. We undertook this study to investigate the effects of UV and IRA on melanocytes. By using confocal microscopy, we observed that UV and IRA impede the dendriticity of melanocytes. Western blot analysis confirmed that finding, indicating that IRA but not UV down-regulates actin and tubulin filaments. Interestingly, both UV and IRA have no effect on those filaments in cultured skin keratinocytes. Further studies have shown that IRA inactivates AKT, 4EBP1 and p70S6K that are known to be associated with protein synthesis. We also observed that both UV and IRA down-regulate tyrosinase expression in melanocytes. Collectively, our data suggest that the loss of dendrites and tyrosinase when exposed to UV and IRA may be a cause of the loss of melanocytes and reduced pigmentation in vitiligo.

An assesment of developmental expression of the AHR in Leucoraja erinacea embryos at various stages

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Although little is understood about the endogenous role of the aryl hydrocarbon receptor (AHR), it appears to play several roles in various biological functions. Compelling evidence demonstrates AHR proteins function in regulating cell differentiation and vascular architecture in mammals. We are developing several approaches to use individual AHR genes of elasmobranchs to test the numerous functions of the one mammalian AHR. The goal of this project is to understand the endogenous role of the AHR in development of the little skate (Leucoraja erinacea). Developmental expression of AHR3 was assessed using in situ hybridization with DIG-labeled specific probes in whole skate embryos at various stages. Negative controls are treated as experimental without the probe. Developmental stages are based on days post-oviposition, rather than staging by development of external structures, as used in previous protocols.

After the first round of in situ hybridizations, specific binding of the AHR3 was observed in the optic lobe, olfactory lobe and a prominent unknown structure located on the dorsal side of the head. These results begin to shed light on localization of gene transcription.We will continue to characterize additional stages and begin to use AHR2-specific probes. Evaluating developmental expression of the AHR2 and AHR3 in the little skate will allow us to make predictions about sensitivity of organs and tissues that may be susceptible to AHR dysregulation by environmental AHR agonists.

Ultrasensitive immunosensor based on gold nanoparticles and magnetic beads multilabel amplification for electrochemical detection of IL-8 cancer biomarker

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Herein we report on an ultrasensitive immunosensor based on glutathione protected gold nanoparticle (GSH-AuNP) for the electrochemical detection of interleukin 8 (IL-8), cancer biomarker in calf serum and proof of concept IL-8 detection in HNSCC cells. GSH-AuNPs were bioconjugated to primary antibodies ($Ab_1$) and used to capture human IL-8 in a sandwich electrochemical immunoassay coupled to horseradish peroxidase enzyme labels. Using the optimized concentrations of the primary and secondary antibodies ($Ab_2$), two sensor approaches were used to measure ultra low (≤ 500 fg $mL^{-1}$) and elevated levels of IL-8. Biotinylated $Ab_2$ bound to streptavidin HRP with 14-16 labels per antigen was used to measure high IL-8 concentration with a DL of 1 pg $mL^{-1}$ in 10 □L calf serum. The second approach greatly amplified the signal using 1 □m magnetic beads coated with ~2 million HRP labels providing the best detection limit of 1 fg $mL^{-1}$ for IL-8 in 10 □L calf serum. This represents a 10,000-fold decrease in the DL over the non-amplified system and the industry standard ELISA for IL-8. The immuonsensors will be used to accurately measure IL-8 in HNSCC cell lines to validate the GSH-AuNP immunosensor. These GSH-AuNP based immuonsensors show great promise for the fabrication of ultrasensitive biosensor microarrays for point-of-care cancer diagnosis.

Hypoxia induces cell migration in CaOV3 cells, but does not induce HIF-1α and LOX expression

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RI-INBRE Summer Undergraduate Research Fellowship Program

In the presence of hypoxia, ovarian cancer cells are induced to migrate. Because volume grows quicker than the surface area of a cell, inner cells are prone to lower oxygen environments. Under these conditions cancer cells continue to thrive and propagate their species. Previous studies have shown that levels of HIF-1α and LOX increase in the presence of hypoxia. This leads to greater metastatic potential of cancer cells. Our study focused on the migratory potential of ovarian cancer cells in both hypoxic condition as well as the induced hypoxic effect of CoCl2. Levels of LOX and HIF-1α were also tested under similar conditions. Phagokinetic track motility assays demonstrated that the effects of hypoxia and CoCl2 did increase the migration of ovarian cancer cell line, CaOV3. Levels of HIF-1α and LOX were shown to have no change in the presence of hypoxia or CoCl2. This early study of cancer cell migration proposed that hypoxia along with low levels of CoCl2 induced cell migration. The metastatic potential of ovarian cancer cells is thought to be low due to the lack of HIF-1α and LOX expression.

Antibiotic arylphosphonium salts attached to polymers by microwave and solid state methods exhibit antibiotic activity

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Aryl phosphonium salts (APS) are a class of lipophilic cation that have antibiotic properties against a variety of mostly gram positive organisms. Derivatives with functional groups that can be polymerized or used to attach the toxic monomers to a polymer have been made using microwave accelerated organic synthesis (MAOS) and solid state methods. The goal is to produce polymers for fabrication of medical devices that would suppress biofilm formation. APS have been attached to cotton fiber, cloth and lint as well as to polyvinyl alcohol (PVA). Solid state reactions using both manual and machine mortar and pestals, and an IR pellet press yield products with APS grafted to cellulose and PVA. Decrease in the IR hydroxyl absorption at ~3300 cm-1 and appearance of an ester carbonyl peak at ~1705 cm-1 indicates the reactions' progress. All reactions, MAOS and solid state, show good yields and dramatically decreased reaction times over traditional bench-top thermal synthesis methods. Kirby-Bauer type screening shows antibiotic activity for APS-treated cotton.

Sulforaphane induces autophagy in *Saccharomyces cerevisiae*

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Sulforaphane (SFN) is a member of a class of antioxidants known as isothiocyanates that are found in broccoli and other cruciferous vegetables. Work from several laboratories has shown that SFN has anticancer and antimicrobial activity though its mechanism of action has not yet been clearly elucidated. Several studies have suggested that SFN may act by causing cell cycle arrest and/or apoptosis. To further elucidate the mechanism of action of SFN, we have initiated studies of its effects on the budding yeast, *Saccharomyces cerevisiae.* We have determined that SFN kills wild type yeast cells. Moreover, we have discovered that sulforaphane induces autophagy and that cells unable to undergo autophagy because of a null mutation in the key autophagic gene, *ATG1*, manifest a heightened sensitivity to the drug.

Storytelling as a therapeutic technique for recovery

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RI-INBRE Summer Undergraduate Research Fellowship Program

While conducting focus groups to ferret out gaps in addiction/recovery system, we spoke with women who were eager to share stories with one another and us. In telling their story, they connected with the world, making themselves and others aware of their relationships with everyone and everything. While using narrative research which emphasized the significance of storytelling as a tool for recovery from addictions, qualitative methods were used to explore the importance of storytelling. Further research indicated that this technique was equally effective for individuals recovering from medical conditions such as stroke or heart disease, as well as for families in the aftermath of divorce or death of a family member. Information obtained using qualitative methods indicated that storytelling is effective as a therapeutic tool for recovery from adverse physical, behavioral, or social situations. Based on these findings, a workshop was held at the Northeast Storytelling Conference (2010). In this workshop, helping professionals were taught to use storytelling as a tool to help clients overcome difficult situations. Using storytelling as a therapeutic tool, it became apparent that people were not being enabled to change their reality, but rather to change perceptions of and reactions to their world. Changing perceptions enables people to better deal with situations, and is an effective way to deal with recovery.

Differential expression of intestinal efflux transporters in mouse models of diabetes

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Diabetes mellitus is a condition in which a person has abnormal hyperglycemia, either because of lack of insulin or insulin resistance. As of year 2007, 23.6 million people in the United States have diabetes, which is roughly 7.8% of the total population (American Diabetes Association). For certain drugs such as antipyrine, diabetics are known to have different pharmacokinetic profiles. Drug pharmacokinetics depends on various factors including Phase-I and -II enzymes, as well as drug transporters (also referred to as “Phase-III”). Drug transporters are membrane proteins that assist in uptake and efflux of chemicals from cells. Specifically, Multidrug resistance protein (MDR1) transporter is present on apical membranes of enterocytes and it pumps chemicals back from enterocytes into the gut lumen. Therefore it is considered to have a protective role in preventing the absorption of harmful chemicals. Other transporters such as Mrp3, Bcrp and Oatp1a5, are also expressed in duodenum and aid in intestinal drug and chemical transport. Overall, little is known about how diabetes affects intestinal absorption, metabolism, and excretion of drugs or chemicals. Therefore, it is necessary to determine whether intestinal expression of transporters differs in diabetics. In this study, transporter expression (mRNA) in the small intestines (duodenum) of male and female db/db mice and diet-induced obese mice (DIO) was determined by the Branched DNA Signal Amplication Assay. It was hypothesized that the expression of numerous transporters would be altered in the intestines of diabetic (db/db) and DIO mice. In general, the expression of the efflux transporter Bcrp (Breast cancer-resistant protein) was downregulated in the duodenum of male db/db mice, as compared to C57BKS mice. Mdr1a and Mrp3 mRNA were expressed more than two fold in duodenum of db/db male mice as compared to duodenum of male C57BKS mice. Female db/db mice did not show any significant alterations in intestinal transporter expression. Bcrp was upregulated in DIO mice as compared to lean mice, whereas Mdr1a was downregulated. As certain transporters work similarly in both mice and humans, it would be practical to determine whether diabetic humans have altered transporter expression in small intestine, as well.

Modeling intracellular calcium dynamics of a two-cell system with stochastic resonance

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The calcium ion is an important second messenger in cellular activities such as mitosis, ATP synthesis, oxidative phosphorylation, motility, and cellular signaling. An excess of the ion in the cell can cause cell necrosis.

The process modeled in this analysis is the intracellular calcium concentration fluctuations in a modified secondary messenger system.To study the dynamics in the calcium fluctuations, a combined model, comprised of the Cuthebertson-Chay and the Borghans-Dupont-Goldbeter models, was used. The model was then extended to include a second cell. This would provide a better representation of an actual system as the signal received by the cell would be a natural one, not artificial like the first.

Environmental white noise affects all chemical processes in biological systems by creating random fluctuations in the system. As a result of this, stochastic resonance appears in the system. This phenomenon happens when a weak signal that would not cause a reaction in the cell becomes a useable signal in the system.

With the model created, we use MATLAB to preform integrations needed and Microsoft Excel to do most of the data processing. Analyzing both the Fast Fourier Transformation (FFT) and the Signal to Noise ratio (SNR) showed whether the results obtained were mathematically significant. Noise does amplify a normally unusable signal, but only at certain specific levels.

Agonist activation of opioid and dopamine receptors are differentially affected by pH

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INTRODUCTION: G-protein coupled receptors (GPCRs) are 7-pass transmembrane receptors that produce intracellular responses by activating trimeric G-proteins and are a common target of many drugs. Stimulation of a GPCR with an agonist can result in the agonist-induced phosphorylation of the GPCR by G-protein coupled receptor kinases (GRK) and binding of arrestin. Arrestin binding prevents further interaction between the GPCR and G-protein, and serves as a linker between the receptor and the cellular machinery that can internalize the agonist-activated receptor into endocytic vesicles. The endocytotic vesicles are acidified after endocytosis and the decrease in pH which occurs is thought to promote dissociation of the agonist from the receptor so that the receptor can be recycled back to the plasma membrane. Some of the internalized receptor may also be targeted for degradation. We hypothesized that ligand-receptor interactions may vary in their sensitivity to pH which could have important consequences on the fate of a receptor following internalization.

RESULTS: MOR and D2R responses to saturating doses of agonist at pH 7.5 were dramatically decreased when the pH was decreased to 5.0. This was demonstrated for multiple MOR agonists including DAMGO, Morphine, Sufentanyl, and Methadone, and for multiple D2R agonist including, Dopamine, Quinperol, and Apomorphine. In some cases the peak response at pH 7.5 was achieved at pH 5.0 if the agonist dose was increased. This suggests that the decrease in activity at low pH resulted from a decrease in affinity rather than efficacy. In contrast, activation of DOR by the specific DOR agonist, DPDPE, was not effected at pH 5.0.

CONCLUSIONS: Our data confirms that the decrease in pH associated with endocytotic vesicles can dramatically decrease ligand and receptor interactions. Interestingly, this is not the case for all receptors as the activation of DOR by DPDPE was not effected at pH 5.0. We are currently investigating whether pH sensitivity is an important determinant in the rate of receptor recycling or alters the fate of the internalized receptors in some other manner. Such alteration would suggest that pH sensitivity is an important pharmacological property of drugs that target GPCRs and other receptors that undergo internalization – a property that could be exploited to improve drug efficacy or reduce side effects.

Bacterial expression and purification of a constitutively active mutant of the mitogen-activated protein kinase kinase MKK6, MKK6-EE

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The mitogen-activated protein kinase (MAPK) p38 plays a very important role in cellular responses to a number of extracellular stress signals as well as inflammatory cytokines. To fully understand the molecular basis of the regulation of these processes, it is necessary to solve the structure of p38 in both its active and inactive states in complex with its multiple protein interaction partners (kinases, phosphates, and substrates). The goal of this study was to express and purify two constructs of constitutively catalytically active MKK6-EE, the upstream kinase responsible for phosphorylating p38. MKK6-EE was successfully expressed in BL21(DE3) RIL *Escherichia coli* cells using two different expression vectors, RP1B, which contains an N-terminal $his_6$-tag and tobacco etch viral (TEV) protease cleavage site (MGSDKIHHHHHHENLYFQGH) and PTHMT, which contains an N-terminal $His_6$-tag, maltose binding protein and a TEV cleavage site. Both MKK6-EE constructs were purified in three steps. First, the protein was isolated from bacterial lysate using immobilized metal affinity chromatography (IMAC) via its engineered $his_6$-tag. Second, the expression/purification tag was cleaved with TEV protease. Finally, the cleaved protein was isolated using a second IMAC purification step (subtraction purification), in which the untagged, cleaved protein was collected in the flow-through. The MKK6-EE protein was then concentrated and analyzed by SDS-Page to determine purity. Purified MKK6-EE will next be used for p38 phosphorylation assays, which will enable multiple functional and structural studies of active p38 complexes to be performed.

Isolation and structural identification of compounds from Winged Sumac (*Rhus copallinum*) leaves

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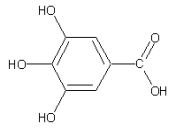
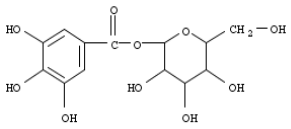
RI-INBRE Summer Undergraduate Research Fellowship Program

Plants from the genus Rhus have been used in traditional medicines but Winged Sumac (*Rhus copallinum*) is yet to be investigated for its phytochemicals. In the current study, a methanol extract of *R. copallinum* leaves was subjected to a series of chromatographic isolation procedures including XAD-16, medium performance liquid chromatography (MPLC) and LH-20 column chromatography, as well as semi-preparative and analysis high performance liquid chromatography (HPLC). Four compounds were obtained and identified by nuclear magnetic resonance (1H-NMR and 13C-NMR) and mass spectroscopy (MS) methods. They include gallic acid (1), glucogallin (2), 4-O-methylgallic acid (3), and pentagalloyl glucose (4). Further work will be done to assess the bioactivity of these compounds.



Compound (1)



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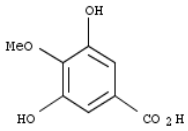
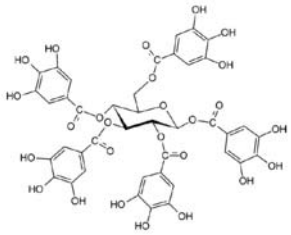


Compound (3)



Compound (4)

# Amplifying the brains electric potentials

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Electroencephalography (EEG) records the electrical activity caused by the firing of neurons within the brain. Low spatial resolution is a major hindrance in the effectiveness of conventional electroencephalography (EEG). The lack of high spatial resolution is primarily due to (1) the blurring affects of the volume conductor with disc electrodes; and (2) conventional EEG signals have reference electrode problems as idealized references are not available with EEG. To resolve the reference electrode problems, (Nunez et al. 1994) proposed a common average reference and concentric electrodes which act like closely spaced bipolar recordings. However, in the common average reference recordings, it is possible that components present in most of the electrodes but absent or minimal in the electrode of interest may appear as "ghost potentials". The application of surface Laplacian (the second spatial derivative of the potentials on the body surface) to EEG can help alleviate the blurring effects and increase the spatial resolution. Dr. Besio has developed a unique, tripolar concentric ring electrode, sensor to acquire Laplacian EEG directly from the scalp surface. However, these sensors are not compatible with currently commercially available EEG amplifiers. Custom designed interfaces (preamplifiers and other instrumentation) are used to adapt the new sensors to the commercial amplifiers. Also, commercially available amplifiers are expensive approximately $1,000 per channel. The new sensors require two channels per sensor doubling the amplifier expense. A practical clinical EEG system requires a minimum of 20 sensors, 40 channels. My work has been to assemble preamplifiers for clinical testing and design a low cost interface to eliminate the need for the costly amplifiers.

Methods: I assembled and tested preamplifiers by soldering surface mount components onto the printed circuit board. The preamplifiers were tested using low amplitude sinusoidal waves and the output was checked on an oscilloscope. I am using Orcad to design and layout a circuit for a low-cost data acquisition system.

Results/product: We now have 30 fully functional preamplifiers. I have most of a printed circuit board designed for the low-cost 24-bit analog to digital converter and controller.

Conclusion: With the preamplifiers that I built and the low cost interface that was designed we are able to effectively lower the cost needed to build new custom EEG that does not have the conventional electrode problems and lack the blurring effect of the volume conductor that were common in conventional EEGs.

Synthesis of a *C*-glycosyl flavones

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RI-INBRE Summer Undergraduate Research Fellowship Program

*C*-Glycosyl flavones have been shown to treat hypertension, arteriosclerosis, cardiovascular disease, and cancer due in part from their antioxidant properties. Antioxidants protect cells from oxidative stress and cellular damage that lead to deteriorative diseases. Natural and synthetic flavonoids as antioxidants may protect cells, thereby being viable treatments for such diseases. Cucumerin A, an 8-*C*-glucosyl flavone isolated from cucumber leaves, will be synthesized through a multistep route. Initial synthetic steps, including *C*- alkylation and acylation of phloroglucinol, will be described.

The role of Bcp1 in cell cycle progression

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The cell cycle is a complex process involving multiple proteins every step of the way, whether it be repairing DNA damage, halting the cycle at a checkpoint, or initiating another phase in the cycle. Through interaction, they can behave as inhibitors, form complexes, and aid other proteins. Learning more about these proteins will provide more insight into their functions. For example, BCCIP is a protein in human cells that acts as a tumor suppressor and is necessary for cytokinesis to occur. Additionally, it interacts with other tumor suppressors such as BRCA-2.

In *Saccharomyces cervisiae*, the fungal homolog for BCCIP is an essential gene called BCP1. The purpose of this study was to define a role for the Bcp1 protein in the progression of the cell cycle. A mutant strain containing a temperature-sensitive version of the protein was used. The progression through the cell cycle was observed in synchronized parental and mutant strains via budding analysis. The mutant strain had more cells with the G2/M phenotype than the parental strain when grown at the non-permissive temperature. The results suggested that Bcp1 was required for completion of the cell cycle. Future experiments will include Western blots of Pds1 and Sic 1, two proteins found in the cell cycle, to further determine the connection between the Bcp1 protein and cell cycle progression. DNA content would also be analyzed via flow cytometry. By defining the role Bcp1 has in *S. cerevisiae*, we could discover more about the functions of BCCIP in human cells. The more we learn about these proteins, the closer science can get to understanding how to correct these proteins when they fail. In turn, that information could give us the key in improving cancer therapies.

The spread of Quahog parasite unknown (QPX) in sediment around an infected *Mercenaria mercenaria* culture site

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Quahog parasite unknown (QPX), a protozoan parasite infecting northern quahogs *Mercenaria mercenaria,* has plagued Massachusetts quahog farmers for years. This study was conducted to determine if and where QPX still exists within Barnstable Bay, MA. Also, we researched the possibility of the spread of QPX from previously infected sites. Sediment and water samples were collected from 10 locations across the bay for our bay wide samples. Clams, sediment, and water samples were also collected at one of the bay wide sample locations determined to have QPX disease in the recent past serving as our local sampling site. This site has been sampled three times so far this summer (6/14, 6/28, 7/15). A nested polymerase chain reaction (PCR) technique was applied involving a series of 2 successive PCR runs using 2 different sets of primers in order to obtain a more specific product, amplifying QPX DNA. Gel electrophoresis of the final PCR products was used to determine the presence or absence of QPX within the sediment sample. From the June 14, 2010 sampling at our local sampling site, all water and sediment samples were negative, and 3% of the clams tested were positive for QPX. For the June 28, 2010 sampling, 22% of the water samples were QPX positive, and the sediment samples are currently being processed. All of the July 15 samples are also currently being processed. We have determined that QPX is still present within the local sampling site as well as within Barnstable Bay, however we do not have enough conclusive data at this time to make further conclusions in regards to the spread of QPX.

Identification of the 5'- splice leader acceptor site and 5'- and 3'- UTRs in *LIP3* mRNA from *Leishmania tarentolae*

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RI-INBRE Summer Undergraduate Research Fellowship Program

*Leishmania* is a trypanosomatid protozoan parasite that has a unique ability to survive in mammalian host macrophages. It is hypothesized their growth and survival results in part from a secretory lipase capable of hydrolyzing lipids into simple fatty acids. In *Leishmania* this secretory lipase is encoded by the *LIP3* gene. Previously we cloned and sequenced the *LtLIP3* gene homologue from *Leishmania tarentolae*. BLASTP searches and Clustal W Alignment showed high aa similarity of *LtLIP3* with known lipases from other *Leishmania* species (i.e. 99% *L. donovani,* 90% *L.infantum,* and 89% *L. major*). Since a PCR based strategy was used to obtain the *LtLIP3* ORF from *L. tarentolae* genomic DNA, the goal of the current study was to verify the 5' and 3' ends of the *LIP3* ORF and to characterize the 5'- and 3'- UTRs using Reverse Transcriptase (RT)-PCR with total RNA from *L. tarentolae*. In addition, the 5' splice site for the unique 39nt capped spliced leader sequence found at the 5'-end of every mature mRNA in all species of trypansomatid protozoa should be identified using this approach. First, cDNA was synthesized from total RNA using an anchored oligo dT primer and RT. To obtain the 5'- splice site, 5'-UTR and to verify the 5' end of the *LtLIP3* ORF, a spiced leader forward oligo and internal lipase reverse oligo were used as primers in PCR amplification reactions with the *L. tarentolae* cDNA synthesized above as template. The resulting amplicon was cloned and subjected to sequenced analysis. Similarly, the 3' end of the *LtLIP3* ORF and 3' UTR will be obtained using anchored oligo dT(reverse primer) and a second internal lipase (forward) primer. Taken together, this data will finalize the identification of the *LtLIP3* gene. Further characterization of this gene and protein will lead to a better understanding of the role of secretory lipases within the biology of the primitive group of pathogens.

Design and evaluation of self-assembled compounds as drug delivery vehicles

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RI-INBRE Summer Undergraduate Research Fellowship Program

The cellular delivery of cell-impermeable and water-insoluble molecules remains a major challenge. The objective of this project was to create nanoscale self-assembled molecular transporters from amphiphilic compounds for non-covalent targeted delivery of hydrophobic drugs. Two classes of amphiphilic compounds were synthesized by Fmoc-based chemistry: 1) single positively or negatively charged amino acids (e.g., Arg, Glu) conjugated with hydrophobic fatty acids (e.g., $C_{12}$, $C_{14}$, $C_{16}$) and 2) octapeptides with alternate charged and hydrophobic residues or octapeptides containing four charged amino acids followed by four hydrophobic residues. We hypothesize that mixing of oppositely charged amphiphilic compounds will generate nanostructures through intermolecular hydrophobic and electrostatic interactions (Figure). Lipophilic drugs can be encapsulated within hydrophobic cavities formed by self-assembly. Circular dichroism was used to compare the secondary structures of parent amphiphilic peptides with the corresponding mixture of oppositely charged peptides. While $W_4R_4$, R-$C_{12}$, R-$C_{16}$, and E-$C_{16}$ + R-$C_{16}$ exhibited helical structures, $(EW)_4$, $(WR)_4$, $(EW)_4$ + $(WR)_4$, $E_4W_4$, and E-$C_{16}$ had β-turn or β-sheet secondary structures. Fluorescence-based studies were used to determine the concentration required for self-assembly of compounds. The data suggested that mixture of peptides, $(WR)_4$ + $(EW)_4$ and $W_4R_4$ + $E_4W_4$, self-assembled at a concentration of ~1.5 mM while the corresponding parent peptides, $(WR)_4$ and $W_4R_4$, showed aggregation at ~500 μM. Encapsulation studies of camptothecin, a water insoluble drug, with $(WR)_4$, $(EW)_4$, $(EW)_4$ + $(WR)_4$ demonstrated a blue shift of emission maximum and/or increase in emission intensity, suggesting partitioning of drug into the self-assembled hydrophobic core. These data demonstrate the potential application of these peptides for encapsulation and delivery of hydrophobic drugs.

Differential inhibition of T-type voltage-sensitive calcium channels ($Ca_v2.3$ and $Ca_v3.3$) by deltamethrin

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RI-INBRE Summer Undergraduate Research Fellowship Program

Low voltage-activated (T-type) calcium channels are involved with a variety of physiological processes including pace making activity and repetitive firing in neurons. $Ca_v3.2$ and $Ca_v3.3$ are two different T-type calcium channels that are expressed in different tissues, possess unique molecular pharmacology and have different voltage-dependence kinetics. In this research the effect of deltamethrin, a potent CS-syndrome pyrethroid used both agriculturally and domestically in the control of pests, was evaluated on $Ca_v3.2$ and $Ca_v3.3$ expressed in *Xenopus* oocytes. $Ca_v3.2$ and $Ca_v3.3$ plasmids were successfully cloned and purified using the Midi DNA plasmid purification kit. Plasmid DNA was verified using a combination of restriction enzymes and linearized DNA was then used as a template to transcribe cRNA using the mMessage mMachine *in vitro* transcription kit. Two electrode voltage clamp electrophysiology was used to confirm $Ca_v3.2$ and $Ca_v3.3$ expression. Concentration-dependent response curves were generated on the relative peak current remaining following perfusion of increasing concentrations of deltamethrin. Deltamethrin inhibits the peak current of both $Ca_v3.2$ and $Ca_v3.3$. However, preliminary results indicate that deltamethrin is more potent and efficacious to $Ca_v3.2$ than $Ca_v3.3$ as judged by the Hill equation fit of concentration-dependent response data. Current studies are underway to assess the effects of deltamethrin on the voltage-dependent and steady kinetics of each of $Ca_v3.2$ and $Ca_v3.3$. Nevertheless, our results indicate a structural specific interaction with deltamethrin on $Ca_v3.2$ and $Ca_v3.3$.

An analysis of genetic and physiological characteristics of phytoplankton species collected from the North Atlantic

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RI EPSCOR Summer Undergraduate Research Fellowship Program

There exists great diversity among genetic and physiological characteristics of diatom populations in the North Atlantic Ocean. We here seek to study these characteristics by growing cultures to determine growth rate and using DNA sequencing tools to determine genetic diversity both within a population and a community. Preliminary results have indicated that the growth rates of three strains of *Ditylum brightwellii* collected from Martha's Vineyard increase with respect to temperature while one strain grows at a significantly faster rate than the others. Additional observations will be made with regard to the effect of light on population growth. Our results have also indicated that the growth rates of axenic and nonaxenic cultures of the same strain do not differ significantly. We have begun to further study the differences among the strains by screening for microsatellites that will serve as markers of genetic diversity. In another study on diversity in a community, we have begun to sequence DNA extracted from a sampled collected in a collection jar held at 600 meters deep in the North Atlantic Ocean near Iceland following a spring bloom. Based on morphology, the sample contains predominantly *Chaetoceros diadema* present in cysts, which is an unusual morphology for this species at the time of collection. We seek to confirm this result by sequencing DNA from the field sample and comparing this result to that of *Chaetoceros diadema*. Additionally, we seek to employ the same analysis in order to identify the other species that might be present in the field sample. Both studies should ultimately provide insight into how these diatoms react to different environmental conditions, which may contribute to our understanding of how these species will respond to global warming.

Solid state microwave-driven synthesis decreases reaction times to make precursors to a peptidomimetic anti-neurodegenerative polypeptide

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RI-INBRE Summer Undergraduate Research Fellowship Program

Solid state polypeptide synthesis on Wang resin beads has been done in our lab manually at room temperature on a wrist shaker. We are now using a microwave reactor to develop an efficient manual synthesis of a cyclic peptidomimetic small molecule that is effective in a mouse model of a trauma-induced neurodegenerative disorder linked to glutamate toxicity. The synthesis sequence is: swell the resin; $(\text{deprotect, couple, cap})_n$; cleave the polypeptide from the resin. This middle sequence is repeated n-times to give an n-mer polypeptide. The time ratios for room:microwave in minutes are: swelling; 30:2, deprotecting; 10:2, coupling; 90:3, cleavage; 120:2. Efficiency increases with size of the polypeptide. Overall reaction time to the pentamer (n = 5) stage is reduced from 400 to 44 minutes, or about an order of magnitude. Products are identified by mass spectroscopy.

Abundance, growth, and diet of juvenile Summer Flounder (*Paralichthys dentatus*) and Winter Flounder (*Pseudopleuronectes americanus)* In Narragansett Bay RI/MA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Summer flounder, *Paralichthys dentatus*, and winter flounder, *Pseudopleuronectes americanus* utilize estuaries as nursery habitat during early life history stages. In southern New England estuaries, however, little is known regarding the spatiotemporal overlap and potential biotic interactions between the flounder species. The purpose of this research was to assess the abundance, growth, and dietary habits of juvenile summer and winter flounder to determine if predator-prey and/or competitive relationships exist. From May to September 2009 and May to July 2010, flounder in the Seekonk River, RI (5 sites) and Taunton River, MA (6 sites) were sampled biweekly using beach seines. Captured flounder were enumerated, measured for total length (mm), and a sub-sample was preserved for subsequent stomach content analysis. Flatfish abundance was higher in the Seekonk River than the Taunton River for both summer and winter flounder in 2009 and 2010 and summer flounder juveniles were completely absent from the Taunton River in 2010. Summer flounder grew faster than winter flounder both in 2009 and 2010, which may be attributed to differences in dietary habits. Decapods and amphipods comprised the majority of the summer flounder diet (99% IA), while amphipods, nematodes and copepods were favored by winter flounder (99% IA) in 2009 and 2010 combined. Calculation of the Schoener's Index showed that there was no biologically significant competition between summer and winter flounder. Among the identifiable fish prey in summer flounder stomachs in 2009, however, there was evidence of predation on winter flounder, albeit to a limited extent. In order to achieve a better understanding of the diets and trophic positioning of the summer and winter flounder, future work will analyze fatty acids and nitrogen stable isotope signatures of the two species.

Bloom syndrome protein interactions with NBN and H2AFX

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RI-INBRE & RI EPSCoR Summer Undergraduate Research Fellowship Program

Bloom Syndrome is a rare genetic disorder caused by mutation in the Bloom Syndrome Protein (BLM) gene. Patients with Bloom syndrome are characterized by immunodeficiency, growth retardation, sun sensitivity, and a predisposition to a wide range of cancers. The average lifespan of Bloom syndrome patients is approximately 25 years. Bloom Syndrome is rare in the general population, but more common among Ashkenazi Jewish descendants. BLM is a member of the RecQ helicase family of proteins that are highly conserved enzymes to unwind DNA in a 3' to 5' direction. BLM protein is thought to operate in the homologous recombination (HR) repair pathway, which repairs DNA double strand breaks in G2 or S phase. NBN and H2AFX also operate in the HR repair pathway. Interactions have been shown between these proteins and BLM. NBN is a component of the Mre11/Rad50/NBN (MRN) complex. The MRN complex initiates homologous recombination repair by bridging the gap of a double strand break and processing DNA from the 5' end to produce the 3' overhangs necessary for strand invasion. H2AFX is a protein that is phosphorylated immediately after a double strand break, and is used to signal DNA damage. Our lab can observe protein-protein interactions using farwestern analysis and yeast 2 hybrid screening. Overlapping BLM protein fragments spanning the entire open reading frame were analyzed via farwestern analysis against purified NBN and H2AFX. The farwestern analysis results are inconclusive showing multiple nonspecific interactions. Yeast 2 Hybrid screening will be used to confirm the interaction with full length BLM prior to refining the interacting domains. Studying these interactions will help to better understand the HR repair pathway and how cancer develops.

Early comprehension of nouns and verbs

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RI-INBRE Summer Undergraduate Research Fellowship Program

When children learning English begin to talk, parents report many more nouns (e.g., *cookie, shoe*) than verbs (e.g., *eat, hug*) in early vocabularies. This noun bias is also reflected in parental speech, which typically prompts children to produce nouns (e.g., What's this? Can you say *shoe*?). Verbs, on the other hand, are prominent in parental speech used to direct children's non-verbal behavior (e.g., Can you *throw* it to mommy?). This suggests that even young children may comprehend verbs, although they produce few in their own speech. This study compares comprehension of nouns and verbs in children 14, 16, and 18 months of age using two measures, a vocabulary checklist completed by parents (the Bates-McArthur Communicative Development Inventory) and a lab assessment using the Preferential Looking Task (PLT). For the PLT, children are randomly assigned to a noun or verb condition. In the noun condition, children view images of two different objects (e.g., truck and fish) before (baseline trial) and after (test trial) one of the images is labeled (*Look at the truck!*). In the verb condition, children view video depictions of actors performing two different actions (e.g., jump and kick) before (baseline trial) and after (test trial) one action is labeled. Comprehension is measured using eye-tracker technology and is defined as an increase in looking at the labeled image during the test trial when compared to the baseline trial. We examine comprehension of 12 nouns and 12 verbs and predict that (1) children at each age will comprehend verbs and nouns, and (2) at each age, parental reports will more accurately reflect children's comprehension of nouns than they do their comprehension of verbs. Progress to date includes preparation of pictorial, video, and audio testing stimuli, development of a testing protocol, use of the eye tracker technology to calculate visual fixation during baseline and test trials, and pilot testing of 14, 16, and 18-month-olds.

Formation of functional neuronal nicotinic acetylcholine receptor after a site-directed mutagenesis of the Alpha-6 subunit

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Introduction: Neuronal Nicotinic Acetylcholine Receptors (nAChRs) are ligand gated ion channels widely expressed in the Central Nervous Systems (CNS). These receptors play crucial roles in modulating a wide range of higher cognitive functions. Five subunit proteins assemble in a heteromeric pentamer shape composed of alpha and beta subunits to form a functional nAChRs. By mutating five amino acids in the wild type alpha-6 (α6) subunit an α-Bungarotoxin (α-Bgtx) competitive inhibition sensitivity would be introduced in any chimeric α6 subunit containing receptors. This mutation will allow researchers to study the distribution and biological function of nAChRs, which are associated in a wide range of neurological and psychiatric disorders. However, the formation of a functional nAChRs after the mutagenesis must first be ascertained.

Methods:
1) Site Directed Mutagenesis in α6 subunit
2) Bacterial Transformation (Insertion of the mutated plasmid in E-coli bacteria)
3) Amplify the mutated gene (growing the selected bacterial colony then extraction)
4) InVitro Transcription of DNA plasmid to produce mRNA of α6 subunit gene
5) Ascertaining if the chimeric subunit forms a functional nAChR by injecting the mRNA into Zenopus Oocytes and using a Two-voltage Clamp to take Electrophysiological readings

Results: The chimeric α6 Subunit does form a functional heteromeric nAChR with either the beta-2 or the beta-4 subunit that are sensitive to Acetylcholine.

Conclusion and implications: Once sensitivity to α-Bgtx is ascertained, in vivo studies on transgenic mice that express nAChR composed of the chimeric α6 subunit will help understand selective benefit that α-Bgtx in-sensitive nAChR have in the neuronal system. Binding assays of various brain regions with fluorescent and radioactive α-Bgtx can be used to determine the distribution of the different types of nAChRs within the different brain regions. Behavioral mice studies can be performed to determine cognitive and behavior association with nAChR in the CNS. Ascertaining the distribution and biological association of various types of nAChRs expressed in the brain will aid in studies of various neuronal processes including Alzheimer’s disease, Parkinson’s disease, schizophrenia, epilepsy and drug Addiction.

Identification of sRNA genes in the dissimilatory metal-reducing bacterium *Shewanella oneidensis*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Bacterial small, non-coding RNAs (sRNAs) are a class of genes that bacteria use to regulate the expression of other genes in response to changing environmental conditions. sRNAs function by base pairing to their mRNA targets and mediating either positive or negative regulatory outcomes. sRNA genes in bacteria are difficult to identify, since they are relatively small genes and they do not contain the protein-coding signals that typically demarcate protein-coding genes.

We have used a computational approach that integrates multiple indices to predict the existence of 159 sRNA genes in the bacterium *Shewanella oneidensis*. *S. oneidensis* is a member of a class of bacteria known as the dissimilatory metal-reducing bacteria. When grown under anaerobic conditions, *S. oneidensis* can utilize a wide variety of extracellular substrates as terminal electron acceptors, including soluble heavy metals. Of particular interest is that reduction of soluble U(VI) and Cr(VI) converts them into insoluble forms. Thus, it is of interest to explore the mechanisms that control this potentially bioremediative function.

Our preliminary data suggest that our predictions will be very useful in identifying sRNAs, including potential regulators of anaerobic metal-reducing metabolism. Ongoing projects in the lab include: 1) Identifying *S. oneidensis* sRNA genes that are differentially regulated under conditions permissive for anaerobic reduction of Fe(III) or Cr(VI), 2) characterizing a novel sRNA that appears to be specifically downregulated under conditions of Cr(VI) and Fe(III) reduction, 3) investigating the *S. oneidensis* sRNA homologs of the *Escherichia coli spot42* and *ryhB* sRNA genes, 4) characterizing *S. oneidensis* sRNA genes that are strongly expressed during exponential growth in defined minimal medium, 5) screening for mutations that alter the ability of *S. oneidensis* to reduce Fe(III), and 6) searching for a generalized transducing phage for *S. oneidensis* strain MR-1.

A genome-wide screen to identify loss-of-function mutants sensitive to the chemotherapeutic drug sulforaphane in the budding yeast, *Saccharomyces cerevisiae*

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RI-INBRE Summer Undergraduate Research Fellowship Program

Sulforaphane (SFN) is an isothiocyanate found in cruciferous vegetables, particularly broccoli. Studies in human and mouse cancer cell lines have shown that SFN has anticancer properties, and is currently thought to function by inducing programmed cell death (PCD) or triggering cell cycle arrest. However, the mechanisms behind these properties are still poorly understood. In order to identify genes that may be involved in the PCD response to SFN, we have begun a genetic screen of the common budding yeast *Saccharomyces cerevisiae*, searching for loss of function (LOF) mutations using the MAT BY4742 yeast knockout library, a collection of 4,775 individual yeast strains each of which contains a knockout of a single non-essential yeast ORF. Our work thus far has primarily focused on optimizing the parameters of the screen in order to visualize a LOF phenotype by the naked eye. The first step in developing this screen involved identifying positive and negative controls, as well as optimizing experimental conditions such as cell and drug concentration. We tested our developed experimental procedure on a plate from the knockout collection containing both negative and positive controls, and confirmed functional reporting. The screen is currently ongoing, with a dozen potential candidate mutants already identified.

Sulfotransferase activity in mouse models of obesity

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RI-INBRE Summer Undergraduate Research Fellowship Program

Obesity poses significant health risks to humans and has been found to alter expression and activity of enzymes important to drug disposition and to homeostasis of endogenous molecules such as steroid hormones, cholesterol, bile acids and neurotransmitter phenols and catechols. While sulfotransferase is an important regulator of the disposition of these endogenous and exogenous molecules, sulfotransferase activity has not been well-characterized under conditions of obesity or fasting. The objective of this research was to characterize the activity of sulfotransferase enzymes under different mouse models of human obesity. Adult male C57BL/6 mice (wildtype and ob-/ob-) were fed a normal diet or fasted (food taken away) for 24 or 30 hours. Sulfotransferase activity was measured in liver tissue. We found that Sult2a1 activity was undetectable in wildtype mice, but that Sult2a1 activity in ob-/ob- (leptin-knockout) mice was significantly up-regulated (0.010 nmol $min^{-1}$ $mg^{-1}$). In contrast, Sult1a1 activity was high in all animals, irrespective of gene or feeding status (0.10 nmol $min^{-1}$ $mg^{-1}$). These results indicate that steroid hormone (Sult2a1) and catecholamine (Sult1d1) disposition will be altered in human obesity and fasting, but that xenobiotic excretion (via Sult1a1) will not be affected. A second objective was to characterize Bisphenol A sulfonation in these models. Bisphenol A is an endocrine disruptor to which humans are regularly exposed at low levels. Sulfonation is expected to enhance bisphenol A removal from the body, and may be altered in these models of obesity.

Genomic context of shark aryl hydrocarbon receptors

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RI-INBRE Summer Undergraduate Research Fellowship Program

In order to understand the regulation of genes encoding aryl hydrocarbon receptors (AHR), which are involved in numerous physiological processes and the response to persistent environmental chemical pollutants, we investigated AHR loci in the spiny dogfish shark, Squalus acanthias. Resources for molecular biology and evolution of chondricthyians are scarce, so we screened a bacterial artificial chromosome (BAC) library, EST databases, and performed targeted PCR. BAC plasmids from AHR-positive clones were prepared and then probed for other AHRs. Sequences were also obtained by shotgun sequencing of selected BAC clones. Our results support that tandem duplication of AHR genes occurred prior to the divergence of the Class Chondricthyes from the vertebrate lineage. To further investigate these genes and identify regulatory regions, a “genome walking” approach is underway,along with a bioinformatics model approach using different organisms.

Genome reduction in tetraploid *Candida albicans* involves programmed cell death

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Genetic reduction is of great significance in many biological pathways. In particular, cancerous cells, typically with abnormal amounts of genetic material, undergo genetic reduction to obtain a diploid state, a process accompanied by apoptotic programmed cell death. A tetraploid strain of *Candida albicans*, when grown on a diploid specific pre-sporulation media, undergoes random chromosome loss, becoming diploid or close to diploid in DNA content and undergoing significant cell death as part of the completion of a parasexual cycle. To measure this cell death cells were grown on pre-sporulation media plates. Measurements of viability were made with a methylene blue stain, with tetraploid cells on average showing 23% survival by day two compared to 89% for diploid Candida. To test the mechanism of the death, assays were done to measure ROS levels and caspase activity. Cells were stained with dihydrorhodamine 123 and then viewed under a confocal fluorescence microscope, with on average 52% of the tetraploid showing fluorescence whereas only 2% fluorescence was visible in the diploid. A second assay was done using a FLICA protocol for caspase activity, with tetraploids showing around 53% fluorescent cells while in diploids only 2% showed fluorescence. These results indicate high ROS levels and high levels of caspase activity in the tetraploid, suggestive of programmed cell death via apoptosis. Further research will be done with tetraploid strands with knockouts for two apoptosis-related genes, MCA1 and RAS1, to further understand the mechanism of death.

# The Spiny Dogfish: Comparative genomics through bioinformatics

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RI-INBRE Summer Undergraduate Research Fellowship Program

The aryl hydrocarbon receptor (AHR) is a transcription factor known to regulate nearly 400 genes. It plays crucial roles in toxin metabolism, developmental physiology, and reproduction. In humans, these functions are performed by a single protein of complex functionality. But other vertebrates, like sharks, retain multiple AHR genes from genetic duplication events. Each of these AHR genes has shown differentiated function *in vivo*, and we hope that by defining these various functions, we can elucidate the complex functionality of the single remaining AHR gene in *Homo sapiens*. Our model organism is the spiny dogfish.

We took a comparative genetic approach to this research. A BAC clone from the spiny dogfish, known to contain an AHR gene, had been previously shotgun sequenced. Our project was to analyze this genomic data, evaluating for genetic structure, homology, and potential genomic synteny with other organisms in the area surrounding the AHR gene. Additionally, shotgun sequences from the Elephant Shark Genome Project were analyzed for AHR genetic sequence and structure.

Due to the volume of data being analyzed, each analysis approach was constructed as a data pipeline. Although internet-based analysis and search tools proved fruitful, we also built several data processing utilities to assist data flow through these pipelines.

We were able to construct a partial genetic map of a single AHR gene in the spiny dogfish genome. Further mapping awaits the sequencing of more genomic segments. Seven potential putative genes from this region of spiny dogfish genome were also defined, but not definitively characterized. We also constructed partial genomic scaffolds for every AHR gene from the elephant shark genome. One of these genes, known as the AHR repressor protein, has never been successfully cloned from a cartilaginous fish; this may finally be possible.

Conformational and thermodynamic insights into the differential nucleotide excision repair in NarI sequence modified by the aromatic amine carcinogen acetylaminofluorene

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RI-INBRE Summer Undergraduate Research Fellowship Program

Acetylaminofluorene is a prototype arylamine liver carcinogen. Once activated in vivo, it reacts with cellular DNA to form C8-substituted N-acetylated dG-adduct (AAF-dG). AAF-dG adopts three distinct conformations, major groove B-type (B), stacked (S), and wedge (W) depending on the neighboring DNA sequence contexts. It has been reported that in the *E. coli* NarI sequence (5′--$G_1G_2CG_3CC$--3′), AAF-dG adduct at $G_2$ (90%) and $G_3$ (100%) position is more repair prone compared to $G_1$ (40%). We hypothesize that the differences in the repair efficiency are due to sequence dependent S, B, W-conformational heterogeneities and their thermodynamic consequences. In the present study, we prepared three NarI sequences that are site-specifically modified at different guanine positions by the $^{19}$F-labeled acetylaminofluorene (FAAF). We used $^{19}$F-NMR/circular dichroism (CD) and UV/differential scanning calorimetry (DSC) approaches to investigate the structural and thermodynamic basis of the observed repair outcomes. Our results indicate that the adduct in these different sequence contexts (-$CG_1G_2$-, -$G_1G_2C$- and -$CG_3C$-) have all three possible conformations, S, B, and W, but differ significantly in their population ratios. Thermodynamic studies exhibit adduct effects on the thermal stability of the duplexes in comparison to the unmodified controls, but the extent of effects are different and specific to their conformational heterogeneities. Our results provided the conformational and thermodynamic insights into the differences in the repair efficiency of nucleotide excision repair (NER) at different positions in the NarI sequence.

Differential sulfotransferase expression after fasting and caloric restriction in lean and obese mice

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The CDC estimates that 68% of the American population is overweight or obese. Obesity has multiple comorbidities including Type-II Diabetes Mellitus and Non-alcoholic fatty liver disease (NAFLD). Some studies also indicate that obesity and NAFLD increases the risk for drug-induced liver injury. According to the CDC, more than 2/3 of the population is undergoing fasting or caloric restriction (CR) in an effort to combat obesity and reverse obesity-associated diseases. Sulfotransferases (Sults) are a family of Phase-II biotransformation enzymes, which conjugate hormones, bile acids, drugs, and environmental chemicals to form more water-soluble metabolites for excretion and aid in drug detoxification. Little published information exists regarding how obesity and NAFLD affect Sult expression in liver or how Sult expression changes with nutrient deprivation. It was hypothesized that Sult isoform expression could be altered in liver with obesity, fasting, or CR. In this study, the mRNA expression of 8 Sult isoforms in livers of C57Bl/6 (lean) and ob/ob (obese) mice that were fed or fasted (24 and 30 hours) or placed on CR (40% reduced caloric intake) for 10 weeks was determined by the Branched DNA Signal Amplification Assay. Obesity increased Sult2a1 and 1e1 and decreased 5a1 mRNA expression. At 24 hr, fasting increased Sult1a1 expression (3.2 fold), 1d1 (6.5 fold), and 1e1 (3.7 fold) compared to fed controls in lean mice only. At 30 hours, fasting increased Sult1a1, 1d1 and 1e1 in livers of obese mice by 1.5, 1.5, and 2.3 fold, respectively, over fed controls. CR decreased Sult1b1 and 5a1 mRNA expression in lean mice and increased Sult1e1 mRNA expression in obese mice as compared to ad libitum controls. Together, our data indicate that specific Sult isoform expression in liver is altered with obesity and NAFLD, fasting, and CR. Future studies will address mechanisms by which Sults are regulated during obesity, whether human Sults are regulated by fasting pathways, and whether Sult activity is altered in these models.

Mechanisms of coevolution between Cyanophages and marine Cyanobacteria populations

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RI-INBRE Summer Undergraduate Research Fellowship Program

Coevolution occurs when host species evolve resistance to a predator and the predator subsequently evolves to overcome the resistance. Multiple rounds of coevolution were observed between marine cyanobacteria (*Synechococcus* WH7803) and a bacteriophage (RIM8) in a controlled chemostat experiment. At various time points, viral isolates and cell strains were collected from the experiment. The goals of this study are to examine the different chemostat viral isolates and determine their host range, their adsorption kinetics on resistant and susceptible cells, and their reproductive fitness level. The host range was determined by incubating viral isolates with cell cultures in well plates. The attachment rate for the different isolates was determined using adsorption assays, which measured the number of unattached viruses every 15 minutes for an hour. Viral fitness was determined by measuring the growth rate on each cell type after 24 and 72 hours. The host range data showed that the cells formed additional resistances to each sequential viral isolate. The acquired resistance changed the viral adsorption kinetics. Viruses were unable to bind as efficiently to the later cells as they were to the earlier cells. We are continuing to conduct studies to determine if the fitness of the viruses decreases as the evolutionary arms race progresses. The results of this study could help us understand the evolutionary changes occurring in naturally occurring viral and host populations.

Mercury bioaccumulation in elasmobranch species

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant that bioaccumulates in the tissues of fish, including numerous marine species. Cartilaginous fish of the subclass Elasmobranchii are important ecological constitutients of marine ecosystems, yet the fate of Hg contaminants in their body tissues is largely unknown. In this study, four species of elasmobranchs, little skate (*Raja erinacea*), winter skate (*R. ocellata*), smooth dogfish (*Mustelus canis*), and spiny dogfish (*Squalus acanthias*) were collected from the Rhode Island/Block Island Sound, and the Hg content of white muscle tissue was analyzed using automated combustion atomic absorption spectrometry. Mean Hg concentrations differed significantly among elasmobranch species, with highest levels measured in smooth dogfish (mean Hg = 0.768 ± 0.154, $n$ = 10), followed by spiny dogfish (mean Hg = 0.324 ± 0.049, $n$ = 23) and skates (mean Hg = 0.100 ± 0.012, $n$ = 36 and 0.064 ± 0.005, $n$ = 23 for little and winter skate respectively). The Hg concentration of skate muscle tissue was not affected by body weight, suggesting that Hg does not bioaccumulate in this tissue. Conversely, smooth and spiny dogfish both bioaccumulate Hg with respect to body size, although smooth dogfish have a higher Hg burden relative to spiny dogfish. Mercury toxicity differs in these cartilaginous fish and is species-specific. To look into the accumulation dynamics, future work will include stable-isotope and stomach content analysis to help better understand the trophic levels and eating habits between these marine species.

Reproductive response to starvation of the lobate ctenophore, *Mnemiopsis leidyi*

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(Research conducted at Woods Hole, MA)

RI EPSCoR Summer Undergraduate Research Fellowship Program

The lobate ctenophore *Mnemiopsis leidyi* is a ravenous predator which can greatly influence and eventually completely deplete zooplankton stocks. Previous studies have demonstrated the reproductive capability of *M. leidyi* to be significant in the presence of large amounts of food. In an effort to better understand the population growth and decline patterns of *M. leidyi,* we examined their reproductive response to starvation. Ctenophores were collected and examined at the Marnie Biological Laboratory in Woods Hole, Massachusetts. Individuals would be kept for one week in the lab and inspected under a dissecting microscope daily. It was found that prolonged (>4 d) continuation of egg production in the absence of food seems impossible. It was found that egg production declines starting on the first day of starvation (max. 99.4%, mean 53.3% decline) and continues to decline with time. All specimens ceased egg production after three or four days. This lag period is important because the animals can still reproduce when zooplankton levels dip temporarily. Further lab studies will aim to show how long, once starved, these ctenophores take to begin producing eggs once food is provided. Gaining a firm understanding in both of these patterns will allow us to better understand the total impact that *M. leidyi* can have on the planktonic ecosystem over time.

Identifying anonymous complexes in transcription factor binding

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The transcription factors Oct4, Sox2, and Nanog are known regulators of pluripotency in embryonic stem cells. We examined 316 regions where Oct4, Sox2, and Nanog are known to bind in vivo in order to attempt to identify other potential binding events. We split each region into 30-mer segments, and then scored each segment with position weight matrices obtained from the public databases JASPAR and UniPROBE. These matrices give a measure of how well a particular transcription factor will bind to a specific oligo, and so when we score them across each of our 316 regions, we get a rough estimate of binding affinity at each particular step. These measurements were converted to association constants, and these constants were then used, along with transcription factor abundance data estimated from the Genome Expression Omnibus (GEO) data set, to create a model for transcription factor binding events. In particular, our lab has developed an anonymous assay such that we can measure the degree of protein-DNA binding at various oligos (tiled across a microarray). The assay, however, returns only anonymous measurements (that is, measurements where the identity of the binding partner is unknown), and so the predictive model developed here is used, in conjunction with this assay, to predict binding events.

Habitat effects on mercury bioaccumulation in black sea bass (*Centropristis striata*) and bluefish (*Pomatomus saltatrix*)

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a widespread environmental contaminant that bioaccumulates in fish muscle tissue, and therefore poses a risk to human consumers. Understanding the human risk from Hg exposure requires insight into (1) species-specific Hg concentrations and (2) variability in fish Hg content as a function of life history (e.g., growth, habitat use, and diet). In this study, two important recreational fishes, the black sea bass (*Centropristis striata*) and the bluefish (*Pomatomus saltatrix*) were collected from inshore (Narragansett Bay Estuary) and offshore (Rhode Island/BIS Sound) habitats using trawls and hook & line. For black sea bass (n=36) and bluefish (n=746), white muscle tissue was analyzed for total Hg and results were evaluated relative to fish age and habitat use (inshore vs. offshore). Irrespective of habitat-type, the Hg content of black sea bass muscle tissue was positively correlated with fish age, indicating the bioaccumulation of Hg. Black sea bass collected from inshore habitats, however, had higher Hg levels at a given age than conspecifics from offshore locations. Similarly, bluefish bioaccumulated Hg in muscle tissue, and the inshore populations accumulated Hg at an accelerated rate. Interspecies comparisons further revealed that bluefish have a significantly higher Hg content at a given age relative to black sea bass. This information can be helpful to guide the consumer on what fish to eat. Future work will include stable isotope and stomach content analysis to determine if conspecifics have different diets between habitats.

The effects of ischemia-hypoxia on blood-brain barrier in the fetal ovine model

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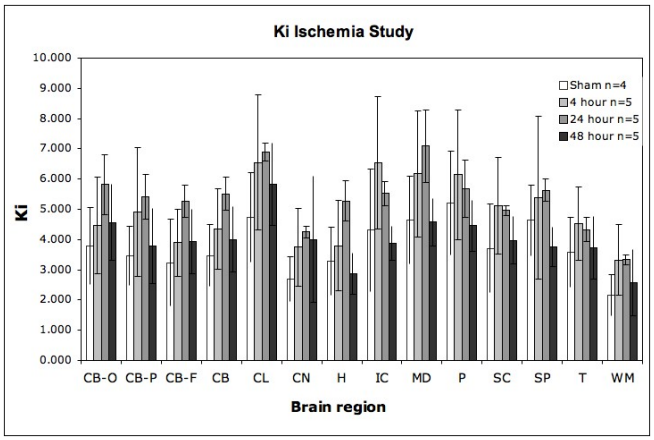
RI EPSCoR Summer Undergraduate Research Fellowship Program

Hypoxic-ischemic brain injury, triggered by a reduction in oxygen and blood flow to various regions of the brain regions, in the perinatal period is closely linked to the pathogenesis of many neurological and developmental impairments. Regulation of blood-brain barrier (BBB) permeability is highly important for preserving homeostasis and preventing entry of harmful substances into the brain of both premature and full term infants. Reperfusion after a period of ischemia restores blood and oxygen supply to the brain, but because of inflammation and oxidative damage, normal blood-brain barrier function may not be restored immediately after brain ischemia. We hypothesized that brain ischemia results in increases in blood-brain barrier permeability in the ovine fetal brain, and that the changes in permeability are a function of the duration of reperfusion after ischemia. Surgery was performed on the ewes to catheterize the fetus and set up the EEG probes and occluders. After six days of recovery, thirty minutes of ischemia was induced via bilateral carotid artery occlusion followed by varying reperfusion times of 4, 24, and 48 hours. By injecting a carbon 14 isotope that slowly crosses the BBB and is absorbed by brain cells, we were able to take beta and gamma radiation readings of various regions of the fetal brain extracted shortly after the permeability study. Preliminary results are shown below with the rate constant for influx ($K_i$, µl / min / gram brain) across the BBB and different regions of the brain for each reperfusion time as well as the sham operated group.




Our preliminary results suggest that the increase in blood-brain barrier permeability is a function of the duration of the reperfusion because there is a marked restoration in blood-brain barrier function 48 hours after exposure to brain ischemia.

The role of Bcp1 in Rad53 activation

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RI-INBRE Summer Undergraduate Research Fellowship Program

Preserving the integrity of the genome is vital for the stability of cells and survival of species. Over time, species have developed mechanisms for DNA repair. Bcp1 is an essential protein found in *S.cerevisiae* and is the homolog of BCCIP in humans. BCCIP is a tumor suppressor involved in cell cycle regulation and DNA repair. Studying Bcp1 in budding yeast could provide information on BCCIP and be applied to cancer therapy. Bcp1 temperature sensitive strain (Bcp1-ts) cells were observed to be more resistant to the DNA damaging agents *methyl* methanesulfonate (MMS) and hydroxyurea (HU) than the parental strain. The purpose of this study was to examine the hypothesis that Bcp1 functions to maintain phosphorylation of Rad53 in the presence of DNA damage. Rad53 is a protein kinase, activated by Rad9, and involved in biological processes including DNA repair, DNA replication initiation, and cell-cycle arrest in response to DNA damage. All experiments were examined at both permissive and non-permissive temperatures. Rad53 dephosphorylation (inactivation) was examined in Bcp1 and Bcp1-ts cells in response to DNA damaging agents. DNA damage sensitivity of both strains was observed by 10-fold spot dilutions on YPD with either MMS or HU. The cell cycle progression was observed by budding index. Localization of Bcp1 was observed by immunoflourescence. Future experiments will focus on Rad53 inactivation by Western blot and flow cytometry.

Solution dynamics of iron pentacarbonyl and ruthenium pentacarbonyl

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Transition metal carbonyls play an important role in many areas of chemistry including catalysis. In contrast to the traditional model of solvation, when group VIII transition metal pentacarbonyls are placed in certain solvents, a complex is formed with one solvent molecule. The formation of this complex potentially impacts both the chemistry of the MPC molecule and the solvent molecule. The equilibrium structure and thermodynamics of iron pentacarbonyl were studied in a variety of ether solvents using FTIR spectroscopy as well as Density Functional Theory calculations. It was found that approximately 50-80% of IPC molecules form a complex in ether solvents at room temperature through an interaction in which the oxygen atom of the ether donates electron density to the iron center of the IPC molecule resulting in stabilization of the solvent molecule. Similar experiments were also performed on iron pentacarbonyl and ruthenium pentacarbonyl in benzene. Approximately 65% of iron pentacarbonyl molecules form a complex with a single benzene molecule while about 80% of ruthenium pentacarbonyl molecules form a complex with benzene. In contrast to ethers, benzene interacts with the metal carbonyl by withdrawing electron density from the metal center through an aryl hydrogen. Ruthenium is more electronegative than iron, suggesting IPC should be a better complexer; however, the Ru-C bonds are also weaker than the Fe-C bonds, so the RPC ligands have more freedom to move which suggests RPC should be a better complexer. The results of these experiments reveal that the fluxional nature of the pentacarbonyl molecule has a greater impact than the electronegativity of the metal center on the complexing ability of the metal carbonyl. The percent complex of IPC was also studied in mixtures of benzene, a complexing solvent, and cyclohexane, a noncomplexing solvent. As the mole fraction of benzene decreased, the percent of complexed IPC molecules decreased; however this relationship was not linear. At a mole fraction of .02, when there is a 1:1 mole ratio of benzene and IPC, 20% of IPC molecules are complexed at room temperature. The fact that 20% of the benzene molecules are fundamentally changed in this solution that contains practical amounts of each species for a synthetic reaction, implies that the presence of this complex could potentially change the reactivity of benzene. Since RPC is a better complexer than IPC, ruthenium pentacarbonyl has an even greater potential to catalyze reactions involving benzene.

Effects of in-group status and out-group stereotypes on reward allocation to an out-group when outcomes are contingent and non-contingent

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RI-INBRE Summer Undergraduate Research Fellowship Program

The Intergroup Relations Model predicts that the relative status of the in- and out-groups, and out-group stereotypes affect behavioral responses of the in-group to the out-group. Groups were formed by giving participants false feedback regarding performance on a dot-estimation task (highly accurate or highly inaccurate); this performance was then liked to contrived research results associating visual perception and career performance. Also manipulated were stereotypes about the out-group. In a positive stereotype condition, the out-group was represented as talented, highly motivated students, whereas in a negative stereotype condition the out-group was represented as untalented, and highly unmotivated. The design was a 2 (high or low in-group status) x 2 (positive or negative out-group stereotype) factorial. Participants allocated practice trials (i.e., reward) on the dot estimation task to in-group and out-group members that would perform the task at a later date. There were no consequences for an in-group as a result of out-group performance on the task. ANOVA revealed a main effect for in-group status; low status members allocated more reward to the in-group, whereas high status members allocated more reward to the out-group. A two way interaction showed that low status group members allocated more reward to the in-group when the out-group was positively stereotyped than when it was negatively stereotyped. Out-group stereotypes did not moderate reward allocation by high status group members. Members of a low status group displayed in-group favoritism, whereas members of a high status displayed out-group favoritism when group outcomes were non-contingent. Study 2 was identical to Study 1except during the reward allocation phase. Participants were told that some participants would be given the opportunity to do the dot-estimation task with feedback on the accuracy of their performance, and that feedback would improve performance. Groups were divided into those ≥ the 50$^{th}$ percentile, and those < the 50$^{th}$ percentile on the DOT. Each participant was a member of one of these groups. Members of the group that improved the most would be entered into a raffle to win a $100.00 gift certificate at the college bookstore. This procedure created a contingency between the outcomes of the in-group and the out-group. In contrast to Study 1 and as expected, low status group members showed in-group favoritism regardless of the out-group stereotype. High status group members showed no bias in reward allocation in the positive out-group stereotype condition and overall, a 52% decline in out-group reward allocation compared to Study 1 when group outcomes were non- contingent. Low status groups display in-group favoritism under contingent and non-contingent outcomes. High status groups provide greater reward to an out-group when outcomes are non-contingent. When outcomes are contingent, high status groups show significantly less out-group favoritism, especially when the out-group is a potential threat to their reward.

DEP domain dependent interaction with the D2 dopamine receptor targets RGS9-2 to detergent resistant lipid rafts

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RI-INBRE Summer Undergraduate Research Fellowship Program

INTRODUCTION: Regulators of G protein Signaling (RGS) proteins modulate G protein coupled receptor signaling (GPCR) by accelerating the GTPase activity of the G alpha subunits of trimeric G proteins. In addition to their RGS domain, the R7 family of RGS proteins share a G Gamma like domain (GGL) and Disheveled/ Engrailed/Plextrin homology (DEP) domain that mediate specific interactions with a variety other proteins. RGS9-2 is specifically expressed in the striatum and is an important regulator of striatal D2 dopamine receptors (D2R). Alterations in the regulation of D2R responses by RGS9 has been implicated in the side effects of the pharmacotherapy of schizophrenia and Parkinson's disease. In the striatum, a significant pool of RGS9-2 is in detergent resistant lipid rafts. However, RGS9-2 is detergent soluble when it is exogenously expressed in mammalian cell lines. We hypothesized that an interaction with D2R may be responsible for targeting RGS9 to lipid rafts leading to the reduced solubility of RGS9-2 in the striatum.

RESULTS: In this study we demonstrate that D2R compared to other related GPCRs like MOR is relatively insoluble in the detergent TX100. RGS9-2 transfected alone or with MOR, was present predominately in the TX100 soluble cell fraction. However, co-transfection of D2R with RGS9 resulted in a significant increase in the amount of RGS9 recovered in the TX100 insoluble cell fraction along with D2R. This effect was specific to RGS9, as D2R co-transfection did not affect the solubility of another cytosolic protein, GFP. Finally, we demonstrated that the interaction between RGS9 and D2R required the DEP domain of RGS9, as D2R did not affect the TX100 solubility of an RGS9 in which the DEP domain was removed.

CONCLUSIONS: In the striatum the DEP domain dependent interaction of RGS9-2 with D2R, is likely responsible for targeting RGS9 to lipid rafts. Furthermore, the specificity of the interaction between D2R and RGS9 further implicates the DEP domain of RGS9 as a D2R targeting motif and provides a mechanism by which different RGS proteins can modulate distinct subsets of GPCRs even though they signal through common G proteins.

Genetic analysis of a host photosynthetic gene in Rhode Island Myovirus 2 isolates

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RI-INBRE Summer Undergraduate Research Fellowship Program

There are hundreds of genotypes of coastal marine cyanophages that infect cyanobacteria, including, *Synechococcus* spp. These cyanobacteria play an important role in the marine ecosystem as primary producers making them an essential part of the oceanic food web. Myoviral communities that infect these cyanobacteria are notably diverse and are important sources of genetic material that can be exchanged with their bacterial hosts. Despite the tremendous viral diversity, Rhode Island Myovirus 2 (RIM2) has repeatedly been isolated from Southern New England coastal waters. In the months of July and September 2009, RIM2 accounted for 40-70% of all viruses isolated making it one of the most common genotypes. RIM2 isolates have been found to have identical or almost identical g43 (DNA polymerase) sequences (>99.5% nucleotide sequence identity). The purpose of this study was to analyze the diversity of host-derived psbA genes in RIM2 isolates collected at different locations and times. The psbA genes from RIM2 viral isolates collected from Roger Williams University, Newport, Colt State Park, Woods Hole and Falmouth water samples between 2004 and 2009 were amplified using PCR and then sequenced. Although these viruses have almost exactly the same g43 sequence, the host-derived photosystem II psbA gene is extremely variable even within RIM2 isolates from the same water sample. The psbA genes were found to differ by up to 15% in nucleotide sequence. Despite the high variability among psbA nucleotide sequences, there is little variability in the amino acid sequence. When compared to known cyanobacteria sequences some of the psbA amino acid sequences were identical to the psbA genes found in cyanobacteria. These results suggest that there can be significant allelic diversity of host-derived genes within a single viral population.

The secretory Lipase Gene LIP3: generating a Plasmid construct for stable integration and expression in *Leishmania*

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Lipases are enzymes that cleave the ester bonds in fats, separating them into long chain fatty acids and glycerol. Once the hydrocarbon fatty acid chains are released they can be readily transported into a cell and catabolized. *Leishmania* is a genus of protozoan parasites that produces and secretes a lipase. It has been shown that the amastigote forms of *Leishmania* parasites preferentially use Beta-oxidation as their carbon source for energy production where as promastigote forms prefer carbohydrates and amino acids. We hypothesize that the lipase secreted by *Leishmania* is necessary for the parasite's growth, development, and survival in its host. To further investigate this hypothesis and to characterize the leishmanial secretory lipase, an expression plasmid is currently being constructed using the pFX4.1 system. The pFX4.1 plasmid constructs bearing the lipase gene, *LdLIP3,* will be integrated into the ribosomal locus of *Leishmania tarentolae* by transfection. By incorporating the plasmid into the ribosomal genome firstly the parasite's genetics will be permanently changed. Secondly a large amount of the desired protein will be generated; this is due to the fact that DNA transcription enzymes are highly active at the ribosomal loci and this should maximize protein expression. The over expression of the LdLIP3 secretory lipase will make future studies to determine the biological role and significance of the lipase to the biology of *Leishmania* possible.

Pigment granule migration in the squid photoreceptor: A role for microtubules and molecular motor proteins

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RI-INBRE Summer Undergraduate Research Fellowship Program

Within the squid retinal, photoreceptors face the incoming light that enters through the lens of the eye. These cells contain large pigmented granules that move from the base of the cell, near the nucleus, to the distal tips (towards the light) and back in response to light levels and in doing so act as molecular sunglasses to shade the photosensitive and photosensing mechanisms of the cell. Here, we combine techniques in light and electron microscopy to study pigment granule movement and to gain insight into the transport mechanism. By standard EM these granules are found to contact multiple microtubules at discrete foci. Internal to the granule membrane, electron dense specializations are found adjacent to the granule/microtubule contact sites. Each granule appears to contact each microtubule through multiple connections along the length of the granule. In addition to their microtubule association, granules are often found near cortical actin and appear to be bound to the actin network. Antibodies raised against myosin VI decorate structures within the photoreceptors that appear to be the same size as the pigment granules and exhibit a similar distribution. An antibody to a dynein intermediate chain decorates discrete puncta in the regions of pigment granule migration. These data together suggest that pigment granule movement is likely to by microtubule dependant and that actin and myosin also play a role in granule function.

Perinatal bisphenol A exposure alters Abcc 2 and 4 transporter mRNA expression in mouse liver

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Bisphenol A (BPA) is a chemical used in the plastic manufacturing process of hard plastic bottles, baby bottles, plastic-lined cans and food packaging, and water supply pipes. Humans get exposed to BPA through food and beverage containers, dental composites, and many products in the home and workplace because trace amounts of BPA leach from these containers. Multiple animal studies have shown that BPA has endocrine disrupting properties, and is associated with obesity, behavioral changes, diabetes, early onset puberty, asthma, cardiovascular diseases, reproductive disorders, development of prostate, breast and uterine cancer, and transgenerational effects. In mice and rats, perinatal (*in utero* and lactational) exposure to low doses of BPA augments obesity and hormone imbalances. Additionally, perinatal BPA exposure is associated with differential expression of multiple genes in liver and adipose tissue. Epigenetic effects are considered to be altered gene expression that occurs from maternal-fetal exposure during gestation. Virtually no information exists as to whether epigenetic mechanisms contribute to transporter expression. The purpose of this summer research project was to explore effects of perinatal BPA exposure on Abcc transporter expression in liver. Female $A^{vy}$ mice were exposed to two BPA concentrations (50 µg/ kg and 50mg/kg) or vehicle through diet during breeding and lactation. Livers were collected from offspring born to mothers after BPA exposure. Serum glucose, body and liver weights were measured. Total RNA was isolated from liver and Abcc 2 and 4 expression was analyzed using qPCR analysis. Although there was no significant change in body and liver weights, BPA perinatal exposure differentially altered Abcc2 and 4 mRNA transporter expression in livers of male and female offspring. Our data suggests Abcc transporters are likely subject to epigenetic regulation and that perinatal BPA exposure affects Abcc expression in liver.

Development of a CCD contact imaging system for the detection of aggregates in a microfluidic chamber

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RI EPSCoR Summer Undergraduate Research Fellowship Program

The purpose of the research was to develop and engineer an efficient expeditious method to detect a small amount of hazardous bacteria and harmful contaminants in fluid. The applications that this method can be useful for are the detection for bacteria in recreational waters, third world countries, food safety, etc.

The use of qualitative research in dealing with issues of addiction and recovery

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RI-INBRE Summer Undergraduate Research Fellowship Program

BACKROUND AND OBJECTIVES: It has been found in the field of chemical dependency/ recovery there are many issues which go unaddressed. An initial attempt was made to broach these issues with women in treatment and recovery. Qualitative research (narrative recording) was used to serve as impetus for helping overcome problems facing women in recovery.

METHODS: Focus groups were held with women in treatment programs. Information gleaned from these focus groups was analyzed to create a list of problem common to women in treatment/recovery.

RESULTS: Stories obtained from women in the focus groups and lists of problems common to most of these women were presented at a large (100+ people) meeting of treatment providers, women in recovery, and representatives of state agencies. Interactive group sessions were held toward the end of this meeting to generate possible solutions.

DISCUSSION/ CONCLUSIONS: Many excellent ideas for solutions were generated by these interactive groups, ranging from childcare and transportation through employment related issues. Solutions included the implementation of TimeBanks and possible Bicycle Repair and Refurbishment Projects. An actual change in DCYF policy came about as a result of this meeting. Follow-up meetings of a women¹s task force are being held to continue to deal with these issues.

Quantifying cholesterol and triglyceride levels in liver tissue of different mouse models

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Measuring lipid levels in liver tissue is important for determining how different diets or dietary conditions affect the liver. The levels of cholesterol and triglyceride in the tissue can indicate the presence of different diseases (such as hypercholesterolemia or gallstone disease), which can result from the dysregulation of cholesterol and/or bile acid metabolism and excretion. Being able to quantify hepatic lipid levels gives valuable insight into the mechanism by which hyperlipidemia occurs in various mouse models. Different mouse models were tested including C57Bl/6 and Keap1-knock down (Keap-KD) mice fed standard or lithogenic diets for 3 weeks, AKR and C57Bl/6 mice fed standard or lithogenic diets for 2, 4, and 8 weeks, and C57Bl/6 or Keap-KD knockout mice that had been fed or fasted for 24 hours. Hepatic lipids were isolated using chloroform-methanol extraction followed by evaporation and pellets were resuspended in ethanol with 1% Triton-X 100. Cholesterol and triglyceride extracts were tested using reagent kits and it was observed that the lithogenic diet increased cholesterol levels to a great degree in livers of Keap-KD compared to C57Bl/6. In contrast, there was relatively no difference in liver cholesterol levels in the 2, 4 and 8 week AKR and C57Bl/6 mice fed lithogenic diets, whereas the lithogenic diet increased liver triglyceride to a higher degree in AKR mice compared to C57Bl/6 mice. Finally, in fasted mice, there were similar liver cholesterol levels in Keap-KD mice and C57Bl/6 mice, whereas liver triglyceride levels were higher in C57Bl/6 mice compared to Keap-KD mice. Together, these data provide critical information that supports other data for individual studies. Development of this assay provides one more useful tool for the Slitt laboratory to assess various mouse dyslipidemia models.

Competitive inhibition by pyrethroid insecticides on the T-type voltage-sensitive calcium channel $Ca_V3.2$.

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RI-INBRE Summer Undergraduate Research Fellowship Program

The use of synthetic pyrethroids insecticides has been prevalent in vector control programs for more than 30 years. Their low levels of toxicity to humans and relative ease of uptake by plants makes them ideal for use in the control of insect vectors for devastating human and animal diseases especially in urban environments almost ensuring human exposure. Previous research has shown that pyrethroids modify $Ca_V3.2$ in an inconsistent manner classified by three groups, group one pyrethroids show no effect, group two pyrethroids are highly potent and moderately efficacious and group three pyrethroids are the most potent and efficacious. This research was conducted to identify the effect of pyrethroids in binary mixtures on $Ca_V3.2$. Defollicated *Xenopus* oocytes were injected with $Ca_V3.2$ cRNA that was transcribed from cDNA. The expressed human $Ca_V3.2$ currents were then characterized using two-electrode voltage clamp electrophysiology with $Ba^{2+}$ as a charge carrier. Concentration-dependent response curves were generated on the relative peak current and relative indices of toxicity obtained for deltamethrin, fenpropathrin, and permethrin individually and in binary mixtures. Deltamethrin proved to be a potent and efficacious inhibitor of the peak current of $Ca_V3.2$. To the contrary, both permethrin and fenpropathrin did not modify the current characteristics of $Ca_V3.2$. However, when perfused as binary mixtures with deltamethrin, both permethrin and fenpropathrin inhibited the deltamethrin-induced inhibition of $Ca_V3.2$ at the high concentration. These results suggest that the presence of a phenoxybenzyl moiety in the pyrethroid structures is important in the binding of those pyrethroids on $Ca_V3.2$.

ER response to UV radiation in cultured human skin keratinocytes: CRT translocation, EIF2α phosphorylation and mTOR signaling

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RI-INBRE Summer Undergraduate Research Fellowship Program

UV radiation induces apoptosis of skin cells. This preliminary study will seek to investigate the effect of UV on ER stress, looking specifically at calreticulin (CRT), EIF2α phosphorylation, and activation of the mTOR pathway. Previous studies have shown that within the ER, calreticulin (CRT) is a multifunctional protein that binds to misfolded proteins and prevents them from leaving the ER, in addition to having gene regulatory functions within the nucleus. Western blot analysis and Confocal microscopy data showed that UV radiation as well as $H_2O_2$ could induce CRT relocalization outside the ER and/or nucleus to the plasma membrane in cultured human skin keratinocytes. Preliminary studies have also shown that UV induces the phosphorylation of EIF2α, a translation factor, which is involved in ER stress, in addition to causing an increase in mTOR signaling. The mTOR's downstream signals include 4EBP1 and EIF4, two proteins involved in protein translation which could be linked to CRT and EIF2α.

Determination of deltamethrin concentrations extracted from perfused *Xenopus laevis* oocytes

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RI-INBRE Summer Undergraduate Research Fellowship Program

*Xenopus laevis* oocytes have been used as a model system to express voltage-sensitive ion channels and determine the effect of pharmaceuticals and toxicants on these channels. Lipophilic molecules, like pyrethroids, have been examined using this model system for more than 20 years, however, little is known about the tissue distribution of an administered nominal concentration of these compounds in oocytes. Deltamethrin, a highly lipophilic synthetic CS-syndrome pyrethroid, is a widely used insecticide and its effects on various voltage-sensitive ion channels routinely examined in the *Xenopus* oocyte model system. After the perfusion of oocytes with increasing concentrations of deltamethrin, it was found that this compound inhibits human T-type voltage-sensitive calcium channels ($Ca_v3.2$) in a stereo specific and concentration-dependent manner. Furthermore, in experiments conducted with high concentrations, deltamethrin inhibition of this channel was evident following extensive washing. These results suggest that deltamethrin accumulates in oocytes and may be contributing to toxicity, a phenomena that is not well understood. In this study, oocytes were perfused (0.5 ml/min for 6 min) with increasing concentrations of deltamethrin ranging between $1x10^{-16}$ – $1x10^{-6}$ M. In addition, oocytes were also exposed to spiked conditions in the presence and absence of $1x10^{-6}$ M deltamethrin. Perfused oocytes and spiked samples were collected in groups of ten and subjected to centrifugation (15 min at 16,000 x g) to allow for oocyte yolk-membrane separation. Separated oocyte layers were extracted with a 1:1 hexane:acetone solution and concentrations of deltamethrin in oocytes determined by GC/ECD. Preliminary data from spike control samples indicate that less than half of the administered deltamethrin dose accumulates in oocytes and a majority of the deltamethrin is found in the membrane layer. Future research to determine a conversion factor that accounts deltamethrin accumulation in oocytes will aid in the proper assessment of relative indices of toxicity of pyrethroid insecticides.

Synergistic approach to find alternative drugs against amebiasis: 3-Phenyl-1-Phenylcarbamoyl and Thiocarbamoyl-2-Pyrazolines

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RI-INBRE Summer Undergraduate Research Fellowship Program

1) Background and Objective: *Entamoeba histolytica* causes 50 million infections and 100,000 deaths annually. *Entamoeba histolytica* alcohol dehydrogenase 2 (EhADH2) is an essential enzyme in the parasite's glycolytic pathway. Synthesized 1,3,4-triphenyl-1-carbamoyl-2-pyrazoline (series 2) and 1,3,5-triphenyl-1-carbamoyl-2-pyrazoline (series 3) were tested against *E. histolytica* trophozoite growth and enzymatic activities.

2) Methods: *E. histolytica* grown with/without inhibitors (60 and 120 μM) for 48, 72 hrs were counted using a hemocytometer. Episomal expression of amebic *EhADH2* gene in *E. coli* provides a system for EhADH2 inhibitor testing of pyrazolines. Enzymatic alcohol dehydrogenase activity and aldehyde dehydrogenase activities were monitored indirectly by measuring NADH oxidation.

3) Results: A two phenyl substitution of the pyrazoline ring display better inhibition than just one: though only the substitution of phenyl group on the pyrazoline at position 3 and 4 exhibit enzymatic inhibition, whereas the substitution on 3 and 5 position shows no inhibition.

4) Discussion and Conclusions: The position of the third phenyl ring in the pyrazoline ring is crucial for protein binding and inhibition. Preliminary models of series 2 and 3 suggest a potential blockage of the inhibitor binding site by the phenyl ring in position 5.

Chemical synthesis of 3- phenyl-1-(thiocarbamoyl)-2-pyrazoline compounds and evaluation of their biological effectiveness as anti-amebic agents

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RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrazoline derivatives have been shown to effectively inhibit the growth of human parasite *Entamoeba histolytica*. The mechanism of action appears to be through affecting EhADH2, a crucial metabolic enzyme for the parasites' survival. To further understand and elucidate the best structural fit for optimal inhibition, we have prepared novel 3-phenyl-1-(thiocarbamoyl)-2-pyrazoline compounds. These compounds will be evaluated for their capabilities to inhibit *E. histolytica* trophozoite growth and affect EhADH2 enzymatic activities.

Development and generalization of joint attention

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RI-INBRE Summer Undergraduate Research Fellowship Program

Joint attention (JA) is defined as the ability to use eye gaze or facial orientation to discriminate what has captured someone else's attention. It is considered one of the developmentally earliest forms of communication. This poster documents the progress of 3 children with autism through multiple steps of a task analysis to master a functional response to a bid for JA. Generalization of the skill was subsequently observed in unstructured social situations.

Participants in this study (2 boys, 1 girl, aged 6 to 10 years) had been independently diagnosed with autism and severe cognitive impairment. They were referred by the clinical director of an ABA program on the basis on their clinical goals and after consultation with their parents. The study was approved by the Institutional Review Board at Salve Regina University. Pre-testing determined that each of the children would establish eye-contact when a familiar instructor called him/her by name in a structured, reduced- distraction experimental setting. However, none of the children would then follow the instructor's eye-gaze.

A 15-step task analysis for JA beginning with the student turning toward the speaker when his/her name was called and ending with several generalization steps was developed. Each teaching session consisted of 10 discrete trials with the correct choice (right, middle or left) randomly sequenced through the session. The dependent variable was the percent of correct JA responses made each session. Mastery was defined as four out of five consecutive trials with at least 90% correct.

A multiple baseline across participants design was used to assess the children's progress. Because the participants varied in the number of sessions each required to master the subsequent steps in the task analysis (Steps 5 through 15), the design was modified to resemble a changing criterion design, with each child serving as his own control.

All 3 participants developed the ability to discriminate among three choices based on the instructor's facial orientation and eye gaze (Steps 3 and 4). Two children learned to discriminate among the objects placed 15.25 cm apart but the third child has not yet mastered that finer discrimination. The first generalization steps involved teaching outside of the reduced-distraction setting and finally, using various objects in the regular classroom environment. During generalization observations Participant 1 showed three instances of correctly responding to an adult's bid for JA during 2.5 hours of observation over five days. The JA behaviors all occurred during formal academic lessons. Participant 2 was observed for 2.75 hours over seven days and initiated a bid for joint attention on two different occasions, both times when asking an adult for help with her lunch.

# Development of mouse models for investigating the significance of Ufd2a activity in skeletal muscle development and differentiation

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RI-INBRE Summer Undergraduate Research Fellowship Program

Ufd2a is currently recognized as a protein important for the ubiquitylation of certain protein substrates which signals their degradation by the proteosome. Previously, a transgenic mouse model was utilized to examine the effects of inactive Ufd2a. In these mice Ufd2a ubiquitylation activity was shown to be essential for cardiac muscle and neuronal development, since deletion of the catalytic domain of the Ufd2a gene was found to be lethal. While this mouse model offers a valuable tool to assess the role of Ufd2a activity in cardiac and neuronal development, its value for investigations of other organ systems has severe limitations.

Generation of a line of mice in which expression of Ufd2a protein or its active form can be regulated by the additional expression of Cre recombinase, circumvents the lethality of the previous model. To accomplish this, we have obtained mice which have the exon coding for the catalytic domain (U-box) of Ufd2a surrounded with lox-p sites, and also generated mice de novo which have a genetrap cassette (containing loxp sites) inserted in the Ufd2a gene such that no Ufd2a protein would be expressed when Cre recombinase is present. Cre recombinase can then be expressed in cells isolated from these mice lines via adenoviral gene delivery or by cross-breeding homozygotes to various Cre mouse lines.

In the first scenario mice which have the Ufd2a U-box floxed will be bred to mice which carry the Cre-Esr recombinase gene. By adding the drug Tamoxifen to individual fibers isolated from the progeny the Cre recombinase becomes effective and removes the U-box from Ufd2a gene resulting in an inactive protein. The differences in skeletal muscle differentiation can then be observed in the presence or absence of the drug.

A second method will be used to monitor the role of Ufd2a to muscle development *in vivo*. Mice homozygous for the loxp sites will be cross-bred with mice which are homozygous for the Cre-recombinase gene driven by the MyoD promoter such that the recombinase is only expressed in their skeletal muscle. This enables us to observe the effects a lack of UFD2a has exclusively on skeletal muscle cells, while enabling the cardiac muscle and neuronal tissue to develop normally.

By cross breeding these mouse lines and confirming their genotypes through tail-snip genomic DNA isolation and PCR, we now have a line of mice which are homozygous for the U-box floxed Ufd2a gene and a line which are homozygous for the MyoD-Cre enzyme. We have also begun to harvest EDL muscles from WT mice and grow the satellite cells in culture to observe the differentiation process and examine Ufd2a expression. These lines of homozygous mice will provide valuable tools with which to determine the significance of Ufd2a in the development and differentiation of skeletal muscle.

Biaryl peptide synthesis using palladium and iron catalysts

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RI-INBRE Summer Undergraduate Research Fellowship Program

This project was designed to improve synthesis of cyclic peptides. The method currently used in large scale synthesis, the Suzuki reaction, creates huge amounts of environmentally detrimental chemicals with low atom economy since an excessive amount of base is necessary in order to drive the reaction. Also, many of the chemicals, with the exception of the palladium, cannot be separated out to reuse with any amount of ease. Our approach is much greener with more limited use of harsh chemicals. The C-H activation step utilizing palladium and iron catalysts may prove beneficial in synthesis of biaryl products. The iron catalyst mimics natural processes so that it may be useful in biomimetrics.

Changes in emotional expression in children involved in Applied Behavior Analysis Program

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RI-INBRE Summer Undergraduate Research Fellowship Program

Joint attention (JA) is the ability to interpret eye gaze to determine what has captured someone else's attention. The 2 boys in this study, who were independently diagnosed with autism and severe cognitive impairment, mastered a JA response within a discrete trial teaching (DTT) program. We hypothesized that the mastery of the JA skill would be accompanied by increases in positive emotions and decreases in negative emotions during the acquisition phase. Videotapes of the DTT program were reviewed and the children's emotional expressions during each trial were recorded. Each session consisted of 10 discrete trials with the correct choice randomly sequenced throughout the session. The emotional expressions examined included; happy, sad, angry/frustrated and neutral. The operational definitions for each of the emotional expressions examined were based on the research of Konstantareas and Stewart (2006) and included; happy, (defined as an upturn of the sides of the mouth from neutral), sad, (a downturn of the sides of the mouth and eyebrows drawn together), angry/frustrated, (characterized by forehead tension, eyebrows pulled downward, saying, 'no,' creating distance from the situation). If none of these emotions were evident during a trial, "neutral" was recorded. Although JA behavior increased from baseline to acquisition, there was no accompanying change in the frequency of any of the emotional expressions targeted by our research. The failure to find changes in emotional expression correlated with JA mastery may be due to the few participants or the lack of customization in defining the emotional expressions. Also, future research may indicate that the participants' later progress in gaining more sophisticated JA skills (interpreting eye gaze rather than facial orientation) is associated with in changes in emotional expression.

Optimizing a BRCA2 immunofluorescence microscopy protocol

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RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi Anemia (FA) is a rare, recessive disease characterized by congenital abnormalities, progressive bone marrow failure and cancer susceptibility. For example, FA patients are highly susceptible to acute myeloid leukemia (AML), squamous cell carcinomas of the head and neck and other cancers at a young age. There are thirteen known FA genes (*FANCA, B, C, D1 (BRCA2), D2, E, F, G, I, J, L, M* and *N*) that encode for proteins crucial in the DNA damage response. The FA proteins together with BRCA1 and BRCA2 act cooperatively in the FA-BRCA Pathway to repair DNA damage and to prevent chromosome instability. Follow exposure to DNA damaging agents (such as mitomycin C, or MMC), the FANCD2 and FANCI proteins become monoubiquitinated, targeting them to discrete nuclear foci. Previous studies have demonstrated that monoubiquitinated FAND2 and FANCI co-localize in these nuclear foci with several known DNA repair proteins, including BRCA2, RAD51 and PCNA. In this study we have attempted to optimize conditions for BRCA2 immunofluorescence microscopy to assess the dynamics of BRCA2 nuclear foci formation.

The role of sirtuin1 ON Abcc2, Abcc3, and Abcc4 induction by the cancer Chemopreventive agnet Oltipraz

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The purpose of this study is to examine whether Sirtuin 1 a necessary intermediary for Oltipraz-mediated induction of ATP-binding cassette transporters 2, 3, and 4. Oltipraz upregulates ATP-binding cassette transporter 2, 3, and 4 (Abcc2, Abcc3, Abcc4), also called multidrug resistance-associated proteins 2, 3, and 4 (Mrp2, Mrp3, Mrp4) expression in mouse liver, hepatocytes, and cells. Oltipraz (OLT) is a drug that was originally developed as an antihelmintic, but more recently has been evaluated as a chemopreventative agent to fight liver cancer. OLT is elicits beneficial effects in various cancer models through activation of the Nrf2-Keap1 transcriptional pathway, and subsequent induction of phase-I and –II biotransformation/detoxification enzymes . ATP-binding cassette transporters are hepatic molecular transporters that efflux glucuronide conjugates of various drugs, as well as endogenous and xenobiotic compounds. OLT induces Abcc expression via Nrf2-dependent mechanisms, but few studies have addressed upstream molecules that mediate this induction. Sirtuin 1 is an epigenetic protein deacetylase. It has been shown to be involved in several cellular functions, including metabolism, senescence, and apoptosis. This study examined whether Sirtuin 1 (Sirt1) is an integral pathway component for Oltipraz to induce Abcc2-4 mRNA expression in liver and hepatocytes. Total RNA was isolated from the livers of wild-type (WT) and liver-specific Sirt1-null (knock out, KO) mice. Basal Abcc2-4 mRNA expression data was collected from analysis of the livers via the Branched DNA Signal Amplification Assay (bDNA), which quantifies mRNA transcript levels. Next, WT and Sirt1 KO mice were administered either OLT (200 mg/kg/day, po) or corn oil vehicle for 4 days. Again, RNA was collected from the livers and data was collected via bDNA. Our preliminary study indicates that Sirt1 KO mice express Abcc transporters in liver more than WT mice, suggesting that under basal conditions, Sirt1 may acts repress Abcc2-4 transcription. As anticipated, Oltipraz increased Abcc2-4 mRNA expression in livers of WT mice by 46%, 92%, and 167%, respectively. In contrast, OLT only slightly induced Abcc2 mRNA expression in livers of Sirt1 LKO mice. Unlike Abcc2, OLT increased Abcc3 and Abcc4 mRNA expression to an equivalent degree in livers of WT and Sirt1 LKO mice, despite the lack of Sirt1. Studies are being conducted to determine whether Sirt1 inhibitors can attenuate OLT induction of Abbc2-4 in hepatocytes and Huh-7 cells. In conclusion, based on data from this study, Sirt1 is not a necessary intermediary in OLT upregulation of Abcc3 and Abcc4 expression, but is a necessary intermediary for Abcc2 upregulation.

Selection of a streptomycin-resistant mutant of *Phaeobacter* S4

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RI-INBRE Summer Undergraduate Research Fellowship Program

The marine bacterium *Phaeobacter* S4, a member of the *Roseobacter* clade, is found in marine ecosystems associated with algae that produce dimethylsulfoniopropionate (DMSP). Members of the Roseobacter clade contribute to the sulfur cycle by degrading DMSP. Previous studies have shown that members of the *Roseobacter* clade produce a biofilm and tropodithetic acid (TDA). TDA has antibiotic activities against bacteria, fungi, parasites, and viruses. Production of TDA is dependent upon the genes *tdaA* through *tdaF*. In this investigation we sought to demonstrate whether *Phaeobacter* S4 contains TDA biosynthesis genes and to construct a streptomycin resistant mutant of S4 in order to have a selectable marker for mutagenesis of this bacterium. Growth experiments demonstrated that S4 cells have an approximate doubling time of 40 min. A streptomycin-resistant mutant of S4 was selected by growing the organism on yeast extract-peptone (YP) agar plates containing increasing amounts of streptomycin. The mutant, designated S4Sm, was able to grow in the presence of 200 μg/ml of streptomycin. During the selection process it was found that S4 produced a yellow pigment during stationary phase. Mutants lacking the ability to produce this pigment were routinely isolated. PCR amplification was used in an effort to detect the *tdaA* gene. Visualization of the PCR products by agarose gel electrophoresis revealed multiple DNA products. Our data suggest that Phaeobacter S4 may contain the TDA biosynthesis pathway and that strain S4Sm can be used in the creation of TDA biosynthesis mutants.

# The amyloid precursor protein (APP) of Alzheimer's disease associates with microtubule-bound vesicles: a mechanism for APP translocation within neurons

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RI-INBRE Summer Undergraduate Research Fellowship Program

Alzheimer's disease (AD) is a neurological degenerative disorder characterized by neuronal damage, memory loss, and decreased mental capacity. The National Institutes of Health estimates that 5.1 million individuals are afflicted with AD in the United States alone at a cost of 146 billion dollars annually. It has been known for decades that familial forms of the disease are caused by mutations in the gene encoding the amyloid precursor protein (APP), a transmembrane protein that co-purifies with clatherin-coated vesicles. While a role for APP in AD has been well established, little is known about the distribution and function of APP in neurons. Here, we use a unique approach to study APP localization by antibody labeling extruded axoplasm from the squid giant axon for examination by electron microscopy. Antibodies raised against the carboxy-terminus of APP decorate the surfaces of a wide variety of membrane-bound vesicles. Most vesicles contain multiple labels and the labeling is distributed over the vesicle surface. Many of the labeled vesicles are found in association with microtubules and are therefore likely to be transported by microtubule-based motor proteins. We hope to determine whether APP and motor proteins of the dynein and kinesin families localize to the same vesicles. Co-localizations studies of APP and these molecular motors are currently underway.

AFLP analysis of cDNA from several species of *Leishmania* reveal polymorphic gene expression profiles

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RI-INBRE-Summer Undergraduate Research Fellowship Program

Despite differences in the form of clinical leishmaniasis that the various *Leishmania* species cause, there is high homology in their overall genome structures and in their genetic profiles. We hypothesize therefore that there must be differences in the gene expression patterns among these organisms that account for their distinct clinical manifestations. The purpose of this research was to use AFLP (Amplified Fragment Length Polymorphism) analysis to identify cDNA markers that could be used to distinguish between four species of *Leishmania: L. donovani, L .mexicana, L. major* (all human pathogens) and *L. tarentolae* (a non pathogenic organism). The cDNA-AFLP technique involves the following steps: 1) cDNA synthesis with RNA from the 4 *Leishmania* species, 2) digestion of the cDNA with restriction enzymes *Mse*I and *Eco*RI, 3) adapter ligation with sticky end *Mse*I and *Eco*RI adapters, 4) pre-selective amplification with primers that have 1 additional nt at the 3' end to decrease the number of amplified fragments by 16- fold, 5) selective amplification with primers that have 3 additional nt at the 3' end to further decrease the number of fragments by 256-fold, and 5) gel electrophoresis to separate amplified fragments for further analysis. Preliminary results with the *Eco*RI AAG-*Mse*I CAC primer pair and *Eco*RI ACG-*Mse*I CAC primer pair revealed monomorphic, polymorphic and unique fragments among the *Leishmania* species. For example, AFLP analysis showed 40 total fragments with the *Eco*RI AAG-*Mse*I CAC primer pair, 29 of which were monomorphic, 11 of which were polymorphic and 4 that were unique to one species only. This indicates that there is a 27.5% polymorphism among the four *Leishmania* species tested with this primer pair. With the *Eco*RI ACG-*Mse*I CAC primer pair showed 83 total fragments, 55 of which were monomorphic, 28 of which were polymorphic and 14 that were unique. This primer pair shows a 33.73% polymorphism among the four *Leishmania* species analyzed. Analysis based on polymorphic cDNA AFLP markers revealed variations in gene expression profiles among these species. Therefore cDNA-AFLP can be used to distinguish between pathogenic *Leishmania* species that cause visceral and cutaneous leishmaniasis and non pathogenic *Leishmania* species. Continuing studies will identify the sequence of the polymorphic fragments for further analysis.

Inhibition of Bacterial Growth & Biofilm Production by Metabolites from *Hypericum* spp.

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Biofilm embedded pathogens such as *Staphylococcus* spp., *E. coli*, *P. aeruginosa*, & *A. baumannii* are difficult to eradicate & are major sources of bacterial re-infections,. New drugs are needed to combat these pathogens. *Hypericum* is a plant genus that contains species known to have antimicrobial properties. However, the specific metabolites responsible for the antimicrobial properties are not entirely known, nor have these compounds been tested as inhibitors of biofilm development. This project was designed to test pure metabolites isolated from the species *H. ellipticum, H. punctatum* & *H. prolificum* as inhibitors of bacterial growth & biofilm (BF) formation.

Methods: The minimum inhibitory concentrations (MIC) & minimum bactericidal concentrations (MBC) of five *Hypericum* spp. metabolites were measured against *A. baumannii*, *P. aeruginosa*, *E. coli*, & *Staphylococcus* spp using Clinical Laboratory Standards Institute (CLSI) methods. MIC is the lowest concentration of an antimicrobial agent that results in no visible bacterial growth. MBC is the minimum concentration required to kill 99.9% of a bacteria inoculum. BF producing reference strains *S. aureus* (ATCC35556) & *S. epidermidis* (RP62A; ATC 35984), as well as a stable BF-negative mutant of *S. epidermidis*, were evaluated. BFs were quantified by staining with crystal violet & measuring optical density at 570nm. Minimum BF inhibitory concentration (MBIC) is defined as the lowest concentration of an antimicrobial agent that inhibits BF formation.

Results: Two of the five *Hypericum* metabolites demonstrated inhibition of staphylococcal growth & BF production. No activity was noted in Gram-negative bacteria. All results are shown as µg/mL

| | Prolifenone B (*H. prolificum*) | | | GHHPu36c (*H. punctatum*) | | |
|---|---|---|---|---|---|---|
| Bacterial Strain | MIC | MBC | MBIC | MIC | MBC | MBIC |
| BF *S. epidermidis* (ATCC 35984) | 15.6 | 15.6 | 3.91 | 7.81 | 15.6 | 1.95 |
| Non-BF*S. epidermidis* | 7.81 | >125 | 3.91 | 7.81 | 7.81 | 1.95 |
| BF *S. aureus* (ATCC 35556) | 15.6 | 31.3 | 7.81 | 7.81 | 7.81 | 1.95 |
| Clinical MRSA strain | 31.3 | 31.3 | 3.9 | 7.81 | 125 | 7.81 |

Conclusion: Of the five *Hypericum* metabolites tested, two demonstrated potent antimicrobial activity against the Gram-positive pathogens. Interestingly, both metabolites also inhibited BF formation at sub-MIC concentrations. These results suggest that these molecules deserve further preclinical evaluation as agents to treat & prevent staphylococcal infections.

Synthesis of anti-epileptic compounds and evaluation of their modulation of nuclear receptor activity

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RI-INBRE Summer Undergraduate Research Fellowship Program

Epilepsy is defined as any of a series of various neurological disorders characterized by sudden recurring attacks of motor, sensory, or psychic malfunction with or without loss of consciousness or convulsive seizures. For reasons as simple as the betterment of quality of life, there is a need for more effective anti-epilepsy drugs, with minimal side-effects, to control epileptic seizures and episodes. Previous research has shown that a series of organic compounds, primarily thiodianilines, exhibit considerable anti-epileptic activity in rodent models; however, these compounds are known carcinogens. The goal of this project was to perform initial in-vitro toxicity screenings of these efficacious compounds for potential undesirable drug-drug interactions, specifically by measuring the modulation of transcriptional activity of drug metabolizing enzyme master regulators: constitutive androstane receptor (CAR), pregnane x receptor (PXR) and aryl hydrocarbon receptor (AhR) in human hepatoma cells using Luciferase reporter assays. Following initial screens, second generation compounds were made using a variety of synthesis methodologies. In summary, a screening of first generation compounds revealed little-to-no potential for this class of therapeutics to cause adverse drug-drug interactions, while the screening of safer second generation compounds is ongoing.

Simulation modeling of Kawasaki disease: Application to nursing practice

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RI-INBRE Summer Undergraduate Research Fellowship Program

INTRODUCTION: Emergency department nurses are the first medical professionals to have contact with a patient. For a patient presenting with possible Kawasaki disease (KD), a rare pediatric disorder that causes widespread inflammation of the blood vessels, time to treatment is an essential component of morbidity and mortality. A complete understanding of the physical and clinical findings associated with KD will enhance the likelihood of accurate and rapid diagnosis. A high-fidelity human patient simulator (HPS) can be used to accurately model classic signs and symptoms of KD and assist nursing students in learning to differentiate criteria associated with KD.

METHODS: The METI PediaSIM HPS was used to model visual and physical symptoms of KD. A primary literature search on KD with emphasis on pathophysiology, clinical presentation, lab values and patient outcomes was completed and the data used to model the pathology of KD.

RESULTS: To accurately model KD-associated pathology, multiple hemodynamic parameters were adjusted including, elevated heart rate, decreased blood pressure (ABP), and the effects of systemic inflammation on the vasculature. These physiologic shifts were made by altering the baroreceptor pressure as well as both extrathoracic and intrathoracic artery pressure. Both the respiratory gain factor and respiratory rate factor were adjusted to simulate respiratory effects. As pyrexia is a common feature of KD, both the body and blood temperature were adjusted to 39.5C and 40.0C respectively. Theatrical make-up and props were employed to create the striking visual symptoms that are consistent with KD. While cervical lymphadenopathy and swelling of the tongue were more easily, produced other symptoms posed more of a challenge. The extensive vasculitis on the trunk and groin were demonstrated by stipple sponging the regions with simulated blood, while clear glue around the lips, hands and feet were applied to express desquamation.

CONCLUSION: Many common pediatric ailments mimic KD increasing the challenge of establishing an accurate diagnosis. This is particularly troubling as early diagnosis and prompt treatment limit the associated cardiac complications. In this scenario the visual and physical symptoms of KD provide a comprehensive learning experience for nursing students that would be difficult to achieve in clinical practice.

AFLP can be used to analyze differences in gene expression patterns between *Leishmania major* and *Leishmania Mexicana*

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*Leishmania* is a single-cell parasitic protozoan that has different clinical presentations varying from skin reactions to mucous membrane infections to organ swelling and fever depending on the species; specifically *Leishmania mexicana* causes weepy, deep cutaneous sores while *Leishmania major* causes shallow, dry cutaneous sores. We hypothesize that gene expression analysis will make apparent the genes that code for these different clinical symptoms. In the current study, we used AFLP (Amplified Fragment Length Polymorphism) analysis with *Leishmania major* and *Leishmania mexicana* mRNA to identify polymorphic fragments among these species. First, we reverse-transcribed cDNA from mRNA; then digested the cDNA with the restriction enzymes *Eco*RI and *Mse*I; used adapter ligation to prepare priming sites; pre-amplified the cDNA; selectively amplified subsets of these fragments using the selective primer sets *Mse*I-CAC with two labeled *Eco*RI primers *Eco*RI-AAG$_{700}$ and *Eco*RI-ACG$_{800}$; and finally denatured and separated the amplified fragments from each of the samples by polyacrylamide gel electrophoresis. Results of our analyses showed 5 polymorphic amplified fragments in *Leishmania mexicana* with the 700 labeled primer set and 12 polymorphic amplified fragments with the 800 labeled primer set when compared to those fragments obtained with *L. major*. AFLP analysis of *L. major* revealed only monomorphic amplified fragments when compared to *L. mexicana*. These results show there was a significant difference in the gene expression profile of *Leishmania mexicana* when compared to *Leishmania major* when using the *Mse*I-CAC -*Eco*RI-AAG$_{700}$ and *Mse*I-CAC- *Eco*RI-ACG$_{800}$ primer pairs for analysis. It will require further characterization to determine what this species' expressed genes encode and if they relate to the specific clinical pathology associated with *Leishmania mexicana*.

Synthesis of 8-*C*-Glycosyl flavones isolated from *Cucumus Sativus*

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RI EPSCoR Summer Undergraduate Research Fellowship Program

Glycosyl flavones and flavones have received considerable attention due to their beneficial health effects, including antioxidant activity and inhibition of tumor development. *C*-glycosyl flavones isolated from cucumber, *Cucumus Sativus*, leaf are produced by the plant in response to powdery mildew fungus. In order to understand the compounds role in plant physiology and protection, the total synthesis of Cucumerin A, an 8-*C*-glucosyl flavone, was initiated. Initial steps of the multistep synthesis, *C*-acylation and alkylation of phloroglucinol, were attempted using various reaction conditions.

Comparison of Southern New England and Long Island coastal cyanophage communities

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RI-INBRE Summer Undergraduate Research Fellowship Program

Cyanophages are viruses that infect cyanobacteria including *Synechococcus* spp. These viruses are abundant and genetically diverse in coastal environments. Although the cyanophage community in Rhode Island's coastal waters has been extensively studied, little is known about the cyanophages in the Atlantic waters south of Long Island. In this study, we compared the genetic diversity of viruses from Long Island with the diversity of viruses isolated in Southern New England in 2009 and 2010. Seawater samples were collected from two sites on Long Island (Long Beach and Oyster Bay) and ten sites in Southern New England. Viral abundance was determined using the most probable number assay with *Synechococcus* strain WH7803. Individual viral genotypes were isolated using extinction dilution enrichment. For each viral isolate, the DNA polymerase gene was amplified via PCR and then sequenced. The samples were initially screened for myoviruses, and then for podoviruses. The abundance of cyanophages in seawater samples from Long Island varied each month and ranged from 23 virus/mL in spring to 10502 virus/mL in summer. Both myoviruses and podoviruses have been isolated from Long Island seawater samples. To date, twenty-two distinct myoviral and one distinct podoviral genotypes have been detected. Of these DNA polymerase genotypes, six had not been detected in the previous ten years of sampling in Rhode Island. The most abundant type of myovirus in Rhode Island during the fall months matched one of the twelve isolates from Long Island. We are continuing to analyze additional isolates from Long Island to assess the extent to which the Long Island cyanophage community differs from cyanophage communities in Southern New England.

Finding a role for Bcp1 in DNA damage response and Rad53 activation

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RI-INBRE Summer Undergraduate Research Fellowship Program

In response to DNA damage, all cells have a mechanism to arrest the cell cycle to allow repair. The budding yeast *Saccharomyces cervisiae* is an excellent system for studying DNA damage and cell cycle checkpoints because yeast go through their complete cell cycles relatively quickly and are easily manipulated. In this study we examined Bcp1 protein in *S.cerevisiae* to determine whether it plays a role in cell cycle arrest in response to DNA damage. The Bcp1 gene is highly conserved between many species. The human homolog of Bcp1 is BCCIP which has been found to be involved in DNA damage repair, tumor suppression, and maintenance of genomic integrity. Loss of BCCIP is associated with certain types of brain and kidney cancers.

Methods: Because Bcp1 is an essential yeast protein, knock-out strains are inviable, therefore a temperature sensitive mutant strain was obtained. The Bcp1 temperature sensitive mutant strain and a parent strain were treated with DNA damaging drugs MMS and hydroxyurea to observe the DNA damage response in the absence of Bcp1. We found that the mutant strain was more resistant to the drugs than the parent strain. We wanted to determine whether loss of Bcp1 alters Rad53 activation. Our hypothesis was that Bcp1 plays a role in activation of Rad53 in response to DNA damage. Rad53 is a kinase that plays a key part in initiating cell cycle arrest in response to DNA damage. In order to see if loss of Bcp1 affects the timing of arrest we treated the two strains with MMS or hydroxyurea and fixed them then counted large budded cells under the haemocytometer to determine arrest in G2 phase of mitosis. No apparent differences were found in the timing of arrest under these conditions. In future studies we will directly assess Rad53 activation using western blot.

Disparate binding affinity of Ufd2a isoforms with VCP/p97 and its IMBPFD disease causing mutants R155H and A232E

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RI-INBRE Summer Undergraduate Research Fellowship Program

Ufd2a is an E3 enzyme that adds ubiquitin to proteins that will then be sent to a proteosome to be degraded (Koegl 1999, Kaneko 2003, Mahoney 2002). There are three different splice forms of Ufd2a. Ufd2aI is the shortest isoform of the protein, it includes exons one-six and eight-twenty-seven and is expressed in most dividing cells of mammals and in precursor muscle cells called myoblasts. The third splice form, Ufd2aIII, consists of all exons contained in Ufd2aI as well as exon7 and the newly discovered exon 7a. This form is only expressed in later stages of muscle differentiation and in mature, striated, multinucleated muscle cells.

Previous experiments have shown an interaction between Ufd2aI (in yeast and mouse) and the ubiquitous AAATPase, p97/VCP, which binds to ubiquitylated proteins and has many essential functions in the cell which range from mitosis to autophagy. Mutations in the p97/VCP gene resulting in amino acid substitutions R155H or A232E are present in cases of Paget's disease of the Bone with Frontotemporal Dementia (IMBPFD), a type of inclusion body myopathy which presents with an accumulation of autophagosomes (Ju, 2010). We are using a Yeast 2Hybrid assay to compare the binding affinities of Ufd2aI and Ufd2aIII for the wild type and mutant forms of p97/VCP we have. Thus far.Ufd2aI appears to have the strongest affinity for p97/VCP A232E, while its weakest interaction is with wild type p97/VCP. Since p97/VCP is known to bind to a number of diverse molecules in a mutually exclusive manner, we might speculate that a stronger interaction between Ufd2aI and mutant p97/VCP may inhibit other interactions essential to its function in resolving ubiquitylated protein loaded autophagosomes.

Modeling of methadone cardiotoxicity for pharmacy education

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RI-INBRE Summer Undergraduate Research Fellowship Program

INTRODUCTION: Methadone hydrochloride is a long-acting synthetic μ-opioid receptor agonist with the potential for serious side effects including adverse cardiovascular events. As a relatively rare adverse drug event, it is unlikely that Doctor of Pharmacy students will treat this condition in early clinical practice. We modeled a potentially fatal methadone-induced cardiac arrhythmia, torsades de pointes (TdP), using a high-fidelity human patient simulator (HPS).

METHODS: A primary literature search was conducted reviewing the pharmacokinetic and pharmacodynamic properties of methadone as well as a clinical case search focusing on methadone induced cardiac arrhythmias. Physical and clinical parameters yielded from the literature search were used to model a prevalent methadone-induced arrhythmia, torsades de pointes (TdP), in a METI HPS.

RESULTS: Methadone is most frequently administered as the chiral mixture, (R,S)-methadone. The therapeutic effect is mainly due to (R)-methadone, whereas (S)-methadone is responsible for the drug-induced prolongation of the QT-interval by strongly inhibiting the hERG voltage-gated potassium channel. A long QT-interval may lead to potentially fatal ventricular tachyarrhythmia, including TdP. Methadone dosing is dependent on the indication for treatment with doses ranging from 2.5-10 mg every 3-4 hours for pain control, whereas methadone dose is individualized based on withdrawal symptoms in patients with opiate addiction and may exceed 100mg in a single dose. High blood levels of methadone are correlated with the potential for arrhythmias. The scenario begins with a 27-year-old male construction worker presenting to the hospital with syncope due to electrolyte imbalance. Vital signs include elevated heart rate (86 bmp), decreased blood pressure (90/54 mmHg), and increased respiratory rate (18 breaths/per minute). TdP develops due to electrolyte imbalance and methadone administration. Students are expected to recognize the contributing factors that lead to methadone-induced arrhythmia and evaluate treatment strategies.

DISCUSSION: The use of HPS is growing rapidly in the education of pharmacy students nationwide. As of July 2010, the Accreditation Council for Pharmacy Education (ACPE) has acknowledged HPS as an option for students completing their introductory pharmacy practice experiences (IPPE). In this scenario, the demonstration of dose related toxicity of methadone is a valuable learning experience for pharmacy students preparing for clinical pharmacy practice.

Magnetic beads amplification strategy for sensitive electrochemical detection of cancer

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Despite recent advances in treatment, cancer still remains a major leading cause of death in the world. Rapid, specific *early detection* of cancer biomarkers proteins in serum is the only hope to change this fact. Such sensitive detection schemes are expected to greatly improve patient prognoses, treatment success, and even lead to cancer prevention. The broad long-term goals are to develop nanomaterial-based arrays to measure collections of early cancer biomarker proteins for specific forms of cancer. A glutathione protected gold nanoparticle (GSH-AuNP) platform was used to develop a sandwich electrochemical immunosensor for the detection of interleukin 6 (IL-6), cancer biomarker in calf serum. The immunosensor is greatly amplified by using 1 mm magnetic beads coated with horseradish peroxidase and conjugated to the secondary antibody. The amperometric detection is based on peroxidase catalytic reduction of $H_2O_2$. Non-specific binding was minimized using BSA, Tween 20 and optimized concentrations of the primary and secondary antibody. Results gave a remarkably low detection limit at 1 fg $mL^{-1}$ (50 aM). This represents a 10,000-fold decrease in the DL over the non-amplified system and the industry standard ELISA for IL-6. This immunosensor based on SWNT arrays offers great promise for a rapid, simple, cost-effective method for clinical screening of cancer biomarkers and point-of-care diagnosis.

A novel method to increase protein expression of voltage-sensitive ion channels in *Xenopus oocytes*

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The purpose of this experiment was to evaluate the effects of co-injecting an RNAse-inhibitor, SUPERase-In™, to increase the expression of the human t-type calcium channel, $Ca_v3.1$ in *Xenopus* oocytes. Variables examined include percent survivorship to assess relative toxicity, average membrane potential to evaluate overall oocyte health and average peak current and percent expression to determine effects of co-injection of the RNAse-inhibitor on calcium channel expression. Human $Ca_v3.1$ cDNA was transcribed to cRNA using the mMessage mMachine *in vitro* transcription kit and injected into defollicated *Xenopus* oocytes. Channel currents were electrophysiologically characterized using the two electrode voltage clamp system with$Ba^{2+}$ as a charge carrier and confirmed by examining the known voltage-dependent activation and deactivation kinetics of the channel. It was found that co-injection of RNAse-inhibitor increases endogenous calcium peak currents and does not specifically attribute to decreased egg health or survivorship. Channel kinetics also confirmed that the activation and inactivation were not statistically different from values determined in the absence of the inhibitor. These results indicate that SUPERase-In™ can be utilized to increase the expression of $Ca_v3.1$ and possibly other voltage-sensitive ion channels in *Xenopus* oocytes.

Woody debris and biofilm impacts on nitrate-N disappearance in fluvial systems

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Nitrate-nitrogen is an important plant nutrient. However, excess nitrogen (N) can result in algal blooms, anoxia, and fish kills in coastal waters. Watersheds can serve as sinks that retain and remove N through biogeochemical processes, preventing excess N from reaching coastal waters. In this study, we examined the effects of woody debris and biofilm on nitrate-N disappearance in order to better understand N cycling in fluvial systems. Three standardized substrates expected to vary in labile carbon were used in the study: red maple (*Acer rubrum*) wood blocks, bundles of extant woody debris from the stream, and clay-fired blocks (mineral). In the first part of the study in 2009, replicates of each substrate were anchored to bricks and submerged in both a high nitrate agricultural stream in PA and a low nitrate forested stream in RI for 10 weeks. During this time, biofilm accumulated on the substrates. The substrates were collected with minimal disturbance, along with streamwater from the respective streams, for use in lab-based mesocosm assays. Additional mesocosm assays were conducted on the same substrates with no biofilm present, using water from the RI stream in summer 2010. At the start of each mesocosm experiment, nitrate-N was added, oxygen was lowered by bubbling in helium, a water sample was taken and then the mesocosm container was sealed. After approximately 18 hours, the mesocosm was opened and a final water sample was taken. The biofilm on the substrates was quantified and nitrate-N disappearance rates were calculated via mass balance methods. The mesocosms from the high nitrate PA site displayed higher rates of nitrate-N disappearance than those from the low nitrate RI site. The median nitrate-N disappearance rates were two times greater for the wood blocks than the clay-fired blocks while nitrate-N disappearance rates for the wood block substrates and the extant woody debris exhibited similar ranges. Overall, more nitrate-N disappearance was observed with the substrates covered in biofilm than those without biofilm. All 2010 mesocosms with substrate lacking biofilms demonstrated negligible nitrate-N disappearance. Woody debris and biofilm appear to have a synergistic effect on creating nitrogen sinks in fluvial systems. The restoration of forest buffers in riparian zones may serve as an important means for encouraging nitrogen sinks, due to their input of fresh woody debris.

Bloom syndrome protein and Rad51 interactions and the implications of cancer progression

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Background: Genomic instability is a defining feature of cancer and an increase in genomic instability is the diagnostic feature of Bloom Syndrome. Bloom Syndrome is a rare recessive disorder caused by mutations of the Bloom Syndrome protein (BLM), a RecQ helicase involved in the overall stability of the genome. Literature suggests that BLM influences several steps of homologous recombination (HR), the DNA repair pathway for double strand breaks. BLM is implicated in several steps of HR, including resection, strand invasion, and Double Holiday Junction resolution. The N-terminus and C-terminus of BLM interacts physically with Rad51, a HR repair protein involved in homology searching and strand invasion, processes critical for accurate DNA repair. Methods: Fragments of FLAG epitope-tagged BLM protein were expressed in *E.coli* to study the protein binding domains between BLM and Rad51 via Farwestern analysis. These fragments were also used to determine the interaction between BLM and DNA via Southwestern analysis. Results: The binding regions of BLM to Rad51 were refined to the two independent regions of BLM $_{100\text{-}214}$ and BLM $_{1317\text{-}1367}$, with the majority of the interaction at BLM$_{150\text{-}214}$ (>50%). DNA was found to interact with BLM in similar regions, BLM $_{100\text{-}125}$ and BLM $_{1317\text{-}1367}$, with the majority of the interaction at BLM$_{1317\text{-}1367}$. Conclusions: While the specific role of BLM remains unclear, FASTA analysis suggests that these regions may be involved in the regulation and/or processing of transcription products. This implies a greater role for BLM in the regulation of genomic stability and prevention of cancer.

Exploration of ligand size for cancer cell targeting on liposome stability and cell uptake

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The overexpression of folate receptor (FR) on the surfaces of cancer cells provides an effective targeting strategy for the delivery of therapeutic drugs. Liposomes are nanoscale vehicles capable of delivering drugs to cancerous regions in the body using folate-conjugated lipids. This study examined the effects of ligand length on liposome stability, lipid bilayer viscosity, and HeLa cell uptake via FR binding using DSPE lipids with 2000 and 5000 MW PEG spacers linked to folate ligands. The folate-conjugated lipids were combined with cholesterol, four other lipids with varying functions, and a fluorescent agent; the liposomes were formed by self assembly. In one set of samples, fluorescent probe 1,3,5-diphenylhexatriene, or DPH, was used to measure bilayer viscosity based on fluorescence anisotropy. In the second set of samples, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl), or NBD-PE, was used as a fluorescent lipid for imaging. After thin film hydration in phosphate buffer saline (PBS), the liposomes were extruded at 200 nm. Dynamic light scattering (DLS) and zeta potential measurements were taken to determine the size distribution and the liposome surface charge, respectively. As the days progressed, liposomes with DSPE Fol-PEG(2000) aggregated while those with DSPE Fol-PEG(5000) remained stable. Preliminary HeLa cell studies confirmed that the more stable DSPE Fol-PEG(5000) exhibited greater uptake, consistent with greater stability and greater ligand mobility to facilitate FR binding. Further experimentation is being conducted to determine liposome solution structure, quantify liposome uptake into HeLa cells, and to encapsulate doxorubicin to provide valuable insight as to how effective of a platform these liposomes will be in treating cancer cells.

Determining the quality of Aquidneck Island's watersheds

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There are several small watersheds on Aquidneck Island, Rhode Island that contain the main source of drinking water for the 70,000 residents of Newport, Middletown, and Portsmouth. The primary drinking water reservoir for the cities of Newport and Middletown originates from Bailey Brook, and with a secondary supply from the Maidford River. The objective of our study is to monitor various sites along the watersheds to determine their overall conditions and to observe sites of possible nutrient contamination and resulting eutrophic conditions. Beginning in the fall of 2009, Salve Regina University has organized a group of citizen scientists to collect water samples twice per month and measure the levels of dissolved oxygen, nitrates, and phosphates with LaMotte water test kits. We have monitored three sites located at the uppermost, middle, and lowest freshwater reach of the Bailey Brook watershed, and two sites located at the uppermost and lowest reaches of the Maidford river watershed. We have found that dissolved oxygen levels are highest in the winter, and tend to be highest in the middle of the watersheds. We have also found that nitrate and phosphate levels are lowest at the beginning of the watershed and increase as the water flows to the middle and the far reaches. The increase in nutrient levels downstream may be caused by fertilizer runoff as the watersheds change from agricultural areas to residential or commercial areas. Currently, the watersheds are in fair condition and without any visible signs of eutrophication resulting from high nutrient levels.

Riparian buffers and fecal coliform contamination of Aqudineck Island watersheds

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Drinking water is easily the most essential aspect in daily life, and ironically the lowest priority to many. On Aquidneck Island, Rhode Island the 70,000 residents obtain their drinking water from treated surface water. Bailey Brook, located among a commercially zoned area that is highly developed with housing, happens to be the primary source stream for Newport and Middletown residents. The Maidford River the island's secondary source water, flows through agricultural areas with a low density of housing development. Throughout the summer of 2010 we tested the hypothesis that coli-form bacterial contamination is a direct inverse relationship of riparian buffer structural diversity and width. Buffers on Bailey Brook are less wide than those along the Maidford, but the natural habitat is more structurally diverse over most of the reach. Bacteria samples were collected on three dates in July along three locations of both watersheds; upper, middle, and lower reaches. A filter membrane system, paired with a differential and selective media (mTEC agar) was used to color indicate specifically *E. coli.* After 24 hours of incubation we were able to estimate the density of bacteria colonies in a 100 mL sample. Lily Pond had an average of 9-10 colonies/ 100 mL; Aquidneck Center had an average of 2,000-3,500 colonies/ 100 mL; Green End Ave. had an average of 1,240-6,400 colonies/ 100 mL. Geometric means of the bacteria at each location indicates acceptance of the hypothesis. The higher the level of *E.coli* present in the less developed watershed, the Maidford may indicate improper septic management, livestock in the headwaters, and/or pet waste from adjacent housing yards or the Gardner Pond area that are not filtered as well through the more complex buffer of Bailey Brook. Since both streams provide Aquidneck Island residents with drinking water, gaining a better understanding of what is impacting our watershed will hopefully aid in better source protection.

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