

# RI SURF

**11<sup>TH</sup> ANNUAL  
RHODE ISLAND SUMMER UNDERGRADUATE  
RESEARCH FELLOWS CONFERENCE**



*Friday, July 27, 2018  
8:00 AM*

**COLLEGE OF PHARMACY  
AND  
CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES  
UNIVERSITY OF RHODE ISLAND**

*Supported by*



**RHODE ISLAND CONSORTIUM FOR  
Coastal Ecology  
Assessment  
Innovation &  
Modeling**

## RI-INBRE & RI NSF EPSCoR C-AIM

### 11<sup>TH</sup> ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

*FRIDAY, July 27, 2018*

*COLLEGE OF PHARMACY AND THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES*

*UNIVERSITY OF RHODE ISLAND*

*KINGSTON, RI*

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8:00 – 9:00 AM	<b>CONTINENTAL BREAKFAST AND POSTER SET-UP</b>
9:00 – 9:30 AM	<b>WELCOMING REMARKS</b> <ul style="list-style-type: none"><li>- DAVID DOOLEY, PHD, PRESIDENT, UNIVERSITY OF RHODE ISLAND</li><li>- STEFAN PRYOR, RHODE ISLAND SECRETARY OF COMMERCE</li><li>- GEOFFREY BOTHUN, PHD, RI C-AIM PROGRAM DIRECTOR</li><li>- BONGSUP CHO, PHD, RI-INBRE PROGRAM DIRECTOR</li></ul>
9:30 – 11:00 AM	<b>POSTER SESSION A (ODD-NUMBERED POSTERS)</b>
11 AM - 12:30 PM	<b>POSTER SESSION B (EVEN-NUMBERED POSTERS)</b>
12:30 PM	<b>Lunch</b>

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# EXHIBITORS

Located on the 1st Floor of Avedisian Hall (College of Pharmacy)

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## **Cores RI**

A directory of core research facilities, services, and instrumentation in Rhode Island.

[www.coresri.org](http://www.coresri.org)

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## **Rhode Island Consortium of Nanoscience & Nanotechnology (RIN<sup>2</sup>)**

RIN<sup>2</sup>, a joint entity between the University of Rhode Island and Brown University, seeks to enhance the State of Rhode Island's competitiveness as a center of excellence in nanoscience and nanotechnology.

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## **Graduate & Postdoctoral Studies, Division of Biology & Medicine, Brown University**

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# POSTER PRESENTATION SCHEDULE

**\*\* PLEASE NOTE:** Posters are to be set up prior to the welcoming remarks and should remain up until 12:30 PM. Posters are to be presented according to the schedule below.

<b>Session</b>	<b>Presentation Times</b>	<b>Posters</b>
A	9:30 – 11:00	Odd-numbered
B	11:00 – 12:30	Even-numbered

<b>Research Theme</b>	<b>Location</b>
Bioinformatics (BI)	CBLS, 1 <sup>st</sup> Floor, South Lobby
Cell Biology (CB)	CBLS, 1 <sup>st</sup> Floor, North Lobby
Chemistry (CHEM)	Pharmacy, 1 <sup>st</sup> Floor Hallway
Drug Delivery (DD)	Pharmacy, 1 <sup>st</sup> Floor Hallway
Environmental Science (ES)	CBLS, 1 <sup>st</sup> Floor Hallway
Genetics (GEN)	CBLS, 1 <sup>st</sup> Floor South Lobby
Marine Science (MS)	CBLS, 1 <sup>st</sup> Floor Hallway
Microbiology (MICRO)	Pharmacy, Room 105
Molecular Biology (MOL)	Pharmacy, Room 240
Neuroscience (NEURO)	Pharmacy, Room 130

# BIOINFORMATICS

**LOCATED IN THE SOUTH LOBBY ON THE 1<sup>ND</sup> FLOOR OF THE CENTER FOR  
BIOTECHNOLOGY & LIFE SCIENCES**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Gut Microbiomes of Nutrient Enriched Oysters in Pt. Judith Pond

Dana Rojas<sup>1</sup>, Rebecca J. Stevick<sup>2,3</sup>, Ashley P. Hamilton<sup>3</sup>, Serena Moseman-Valtierra<sup>3</sup> & Marta Gomez-Chiarri<sup>4</sup>

<sup>1</sup>Cell & Molecular Biology, University of Rhode Island, Kingston, RI

<sup>2</sup>Graduate School of Oceanography, University of Rhode Island, Narragansett, RI

<sup>3</sup>Biological Sciences, University of Rhode Island, Kingston, RI

<sup>4</sup>Fisheries, Animal & Veterinary Science, University of Rhode Island, Kingston, RI

Oysters have important ecological and economical functions: they remove organic and inorganic particles from water, give shelter to various species, and are consumed by many people. Some bacteria living among oysters can denitrify (the anaerobic reduction of nitrate ( $\text{NO}_3^-$ ) to an inert, dinitrogen gas ( $\text{N}_2$ ) (Knowles 1982)), contributing to improving water quality in eutrophic coastal waters. However, how microbial communities within oysters change in response to environmental change is unknown. Adult oyster samples were taken from Pt. Judith Pond, Rhode Island, where 12 buckets of oysters were kept for three months in the summer of 2017. Six buckets were located at the North end of the pond (Billington Cove) where there is an abundance of nutrients such as nitrogen and phosphorus while the other six were at the Southern end (Bluff Hill Cove) where nitrogen concentrations are lower. Three of the six buckets at each location were given an additional fertilizer treatment to simulate runoff into the water. Initial analysis of nitrogen gas production of the oysters revealed that denitrification rates are dependent on many complex and interacting environmental factors. This suggests that location, and prolonged exposure to localized conditions, rather than stimulated N loading, was the dominant driver in N removal rates. The oysters were collected and dissected, and DNA was extracted from the gut of 36 oyster samples (3 per bucket) and prepared for sequencing by amplification of the V4V5 region of the 16S rRNA to barcode the bacterial communities. An Illumina MiSeq was used to generate overlapping 250 bp paired-end sequencing reads at the Genomics and Sequencing Center at the University of Rhode Island. Through bioinformatic analysis using Quantitative Insights Into Microbial Ecology 2 (QIIME2), the effect of location and nutrient enrichment on microbiome composition in oysters will be determined. Information on the effect of environmental conditions on oyster microbiomes and their relationship with ecosystem services such as denitrification will be useful in improving coastal ecosystem models as well as oyster reef restoration efforts.

## Bioinformatics Pipeline for Transcript-Level Expression Analysis of RNA-seq Experiments Using HISAT2, StringTie and Ballgown

Arianna Alfiero<sup>1</sup> & Christopher Hemme<sup>2</sup>

<sup>1</sup>Bioinformatics & Computer Science, Wheaton College, Norton, MA

<sup>2</sup>Bioinformatics, University of Rhode Island, Kingston, RI

Next generation sequencing (NGS), or high-throughput sequencing, is the process of data generation and analysis which includes sample processing, genetic sequencing and base calling, data quality check, data preprocessing, mapping of reads to a reference genome, and data analysis. Data analysis includes the normalization of the data and is done differently based on the application used. Transcriptomic profiling and splicing variant detection, otherwise known as RNA sequencing (RNA-seq), is the application of next generation sequencing used in this research. RNA-seq is transcriptomic analysis which determines the parts of the genome that are transcribed, or expressed, and how actively they are transcribed, while also being able to differentiate alternative splicing variants, otherwise known as isoforms. In this research, a bioinformatics pipeline was developed in a bash script in order to manage the processing and analysis of RNA-seq data from the Illumina NGS sequencing platform using mouse data as a test sample. Though this data compared a high-fat and low-fat diet of mice, where each of the diets included a sample of mice receiving 45% kcal from fat and 58% kcal from fat, the pipeline is meant to handle all mice and human related data based on the metadata file given. The pipeline includes calls to tools such as FastQC for the quality check, Trimmomatic for the data preprocessing, HISAT2 for the alignment and StringTie for the assembly. This pipeline will also be able to handle both single-end and paired-end reads. At the conclusion of its run, this pipeline will produce a directory used in the R package ballgown, an R script using ballgown to begin the downstream analysis, a few text files used to retrieve the gene names of each transcript, a comma-separated values file that can be used to create count matrices in R in order to run alternative analysis, and some text files storing tables that can be used in IPA for further analysis.

## A Phylogenetic and Genomic Analysis of Lytic Enzymes Across Predatory Bacteria

Joseph Cerra<sup>1</sup>, Laura Williams<sup>1</sup>, Ying Zhang<sup>2</sup> & Jing Wang<sup>2</sup>

<sup>1</sup>Biology, Providence College, Providence, RI

<sup>2</sup>Cell & Molecular Biology, University of Rhode Island, Kingston, RI

Predatory bacteria attack and digest other bacteria. The most well-studied genera of predatory bacteria occur in the delta-proteobacteria and include species found in soil, marine and freshwater environments. These species can attack Gram-negative pathogens, which makes them good candidates for biocontrol agents and alternatives to antibiotics.

The predatory lifecycle depends on lytic enzymes encoded in the predatory bacteria genome. These enzymes allow the predatory cell to enter a prey cell, digest prey cell macromolecules for replication and then burst the prey cell to release progeny. To understand how lytic enzymes impact the evolution and adaptation of predatory bacteria, it is important to analyze the distribution and variation of lytic enzyme gene families across different genera of predatory bacteria.

In this project, we compared multiple predatory bacteria genomes with open source software to generate an organized catalogue of gene families predicted to code for lytic enzymes. We assessed the presence or absence of these gene families across several sequenced predatory bacteria genomes. In addition, we used single copy core genes present in all genomes in our dataset to construct a phylogeny. Moving forward, we will use the lytic enzyme catalog and the phylogeny to explore how lytic enzyme gene families vary in sequence across these different families of predatory bacteria. This research will provide insight regarding the distribution of lytic enzymes across different predatory bacteria families and provide us with strong genetic targets for developing predatory bacteria and their lytic enzymes as biocontrol agents.



## A Genomic-Scale Metabolic Model of *Pyrococcus furiosus*, Strain DSM 3638

Emily Baranowski, Weishu Zhao & Ying Zhang

Cell & Molecular Biology, University of Rhode Island, Kingston, RI

Genome-scale metabolic models (GEMs) are the assembly of all the biochemical information that make up an organism's metabolic network. They can be especially useful when looking at the metabolic phenotype and evolutionary adaptation of a particular species. Over 100 genome-scale metabolic models exist for a variety of bacterial and eukaryotic species. However, only 15 GEMs have been mapped for nine Archaea species. *Pyrococcus furiosus* is a hyperthermophilic marine species of Archaea, meaning it can survive and grow in extremely hot environments. Discovered in 1986, this species is known to have several industrial applications and advantages, which makes it particularly interesting. It may reduce contamination risk, improve mixing and diffusion, and lower cooling costs. Enzymes isolated from *P. furiosus* are also utilized in PCR, which is widely used in molecular biology as a way to amplify DNA and RNA segments for analysis. The KEGG and TCDB transporter databases, supplemented by peer-reviewed literature, were used to obtain reactions and pathways that contribute to the overall metabolism of *P. furiosus*. A template model of a related species, *Thermococcus eurythermalis*, was used as a reference for the construction of the *P. furiosus* network. Of the 614 reactions in the model, 143 were supported by annotations based on peer-reviewed publications, and 119 were added manually upon further annotations of the *P. furiosus* genome. The extended *P. furiosus* network contains 85 transport reactions, 57 of which were added manually from the transporter database based on ortholog gene mapping. A literature-defined, minimal medium was incorporated into the model as a constraint to limit uptake of nutrients and carbon sources, based on published culture experiments. A biomass function was also included to represent the production of DNA, RNA, and protein. By running the *fba* (flux balance analysis) function on PSAMM (a portable system for the analysis of metabolic models) through the command-line interface, the metabolic network of *Pyrococcus furiosus*, strain DSM 3638, was simulated. The simulation showed that *P. furiosus* can utilize maltose as a carbon source for growth, which is in agreement with the literature and the minimal medium created.

## Genome-Scale Metabolic Model for *Haemophilus parainfluenzae*

Michael Pepin<sup>1</sup>, Ying Zhang<sup>2</sup> & Weishu Zhao<sup>2</sup>

<sup>1</sup>Science & Technology, Bryant University, Smithfield, RI

<sup>2</sup>Cell & Molecular Biology, University of Rhode Island, Kingston, RI

Microorganisms being among the smallest living communities makes it difficult to experimentally study. The use of computational analysis and metabolic modelling is becoming more prevalent when attempting to understand that roles of certain bacteria and their functions. *Haemophilus parainfluenzae*, a bacteria found in the oral cavity, is particularly interesting due to its abundance within the human oral microbiome. The aim of this study was to create a model to gain insight into the metabolic and physiological functions of this bacteria. A genome-scale metabolic model (GEMs) was created for *Haemophilus parainfluenzae* ATC33392 through the use of genome annotations and peer-reviewed literature. *Escherichia coli* iJO1336 was used as a template model for the reconstruction of *H. parainfluenzae* metabolism. There was a total of 352 reactions that were mapped from the *E. coli* model based on protein orthology. There was then 452 reactions from the KEGG database that were included based on annotation of enzyme functions. After running a blast on the protein sequence by using TCDB as a reference database, 172 transporter reactions were manually included in the model. The biomass equation was formulated to include the DNA, RNA, and protein based on experimental measurements of the compositions of these molecules in bacterial cells. In total, the *H. parainfluenzae* metabolic model contains 623 genes and 982 reactions. The constraints of exchange reactions in the metabolic model was developed following experimental data of a defined medium for this organism. When analyzing the pathways within this bacteria, it was discovered that the *H. parainfluenzae* genome encodes pathways that are absent from *E. coli*. These include functions in the nitrogen metabolism (i.e. nitrite reductase and nitric oxide reductase) and the inositol phosphate metabolism (i.e. pentose and glucuronate interconversions).

## Textile Pressure Sensors: eMattress

George Humphreys<sup>1</sup>, Nick Constant<sup>2</sup> & Vanessa Kamara<sup>2</sup>

<sup>1</sup>College of Engineering, University of Rhode Island, Kingston, RI

<sup>2</sup>Electrical, Computer, and Biomedical Engineering, University of Rhode Island, Kingston, RI

During the past two months I've worked with a group to improve textile pressure sensors, with the goal of embedding them into a smart mattress. When designing the sensors we focused primarily on improving sensitivity, decreasing background "noise", and minimizing connections. The durability, flexibility, and fiber arrangement (knitted/woven) were also considered when testing and comparing different sensor materials.

We wanted to know the degree of pressure exerted on the sensor and where exactly that pressure was being applied. Velostat™, a synthetic hydrocarbon resin (polymeric foil) infused with black carbon, is actually marketed as a packaging material to protect susceptible devices from electrostatic discharge thanks to its semiconductive properties. Polymeric foil is typically sold as car wrapping vinyl, but when impregnated with black carbon the material becomes selectively conductive. Velostat™ is perfect for our sensors because the electrical permeability of Velostat™ correlates directly with the amount of pressure being exerted on it.

Using conductive thread (sewing thread nano plated with silver), we stitched a circuit for the electricity on two rectangular layers of a knit poly-nylon blend (92% polyester, 8% nylon), making sure that when stacked the circuit formed a four sensor matrix in the one circuit. The Velostat™ was cut into 2cmX2cm squares, and when pressed between the two diagonal conductive thread pieces the entirety of the Velostat™ is turned into a piezoresistive sensor. Using conductive thread, Velostat™, data collection software, and a couple rectangles of fabric, we created flat, stretchable, durable pressure sensors that can sense pressure location, pressure magnitude, and fit seamlessly into a mattress.

We are now prototyping a mattress covered in these sensors. We hope to aid children with disabilities convey their lack of sleep based on a quantitative measurement. The day after their poor sleep they may require a lower stimulus day (studies have found correlations between poor sleep and outbursts by handicapped children). I believe that many healthy adults also may find this useful seeing as there are dozens of popular sleep apps that claim to monitor sleep using cell phone microphones and/or accelerometers. The accuracy of these apps pales when compared to the sensors' ability to monitor near micromovements, which can be analyzed to display the user's sleep cycle, if not even their overall health one day.

# CELL BIOLOGY

**LOCATED IN THE NORTH LOBBY ON THE 1<sup>ND</sup> FLOOR OF THE CENTER FOR  
BIOTECHNOLOGY & LIFE SCIENCES**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Assessment of Hypoxia Markers in Gastrointestinal Cancer Cell Lines Through Immunocytochemistry Analysis

Alicia Cotoia, Karolyne Stimpson, Megan Johnstone, JD Swanson & Anna Radovic

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

Hypoxia is common in solid tumors, where low oxygen conditions require alterations in several functions for cell survival. In low oxygen environments, Hypoxia-Inducible Factor 1 alpha (HIF-1a) avoids degradation and relocates to the nucleus to dimerize with HIF-1b, aiding in the transcription of oxygen independent glucose metabolism, cell proliferation, migration, and angiogenesis. All of these features are prominent in gastric cancer, accounting for its high treatment resistance and mortality rate, ranking it as the third most lethal cancer worldwide. Presently, two immortalized gastric adenocarcinoma cell lines- AGS and MKN28- have been utilized to test potential therapeutics for this disease. Specifically, potential treatments include the phenolic nutraceutical gallic acid, which has been shown to exhibit anti-tumorigenic, anti-oxidative, and anti-proliferative effects on cancerous cells. It is expected that the more malignant AGS cells have increased levels of HIF-1a when compared to MKN28 cells. Additionally, it is expected that gallic acid treatments would initiate a decrease in hypoxia markers due to either apoptosis of the cells or redirected metabolism from oxidative changes. To test this, gallic acid treated AGS and MKN28 cells were cultured and analyzed on microarrays to detect changes in hypoxia gene expressions over 0-24 hour time points at varying doses. Untreated AGS and MKN28 cells were then prepped for immunocytochemistry using HIF-1a antibodies before analysis on a four channeled confocal at the INBRE funded imaging facility at the University of Rhode Island. Expected results include increased staining of HIF-1a in both the cytoplasm and nucleus, with significantly more staining in AGS cells. Future studies would include immunocytochemistry of other protein markers of hypoxia and angiogenesis pathways, including the tumor suppressor Phosphatase and Tensin Homolog (PTEN) and the downstream effector Vascular Endothelial Growth Factor (VEGF). Furthermore, immunocytochemistry would be utilized to differentiate hypoxia markers in AGS and MKN28 cells following gallic acid treatments. By continuing to research the levels of hypoxia over varying states of malignancy in gastric cancer, we increase the potential of discovering noninvasive ways to diagnose and treat those affected by this deadly disease.

## Epithelial to Mesenchymal Transition (EMT) Plays Important Role in Gastric Cancer Progression as Seen in Confocal Images of Two Gastric Tumor Immortalized Lines

Karolyne Stimpson, Alicia Cotoia, Megan Johnstone, JD Swanson & Anna Radovic

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

Most malignant tumors share key transformation features, including the ability to survive in hypoxic (low oxygen) environments, induce epithelial to mesenchymal transitions (EMT), and elevate the expression of long non-coding mRNAs (lnc mRNAs) that regulate gene expression. Gastric tumors take advantage of all of these features, leading to their highly invasive and metastatic nature. This study focuses on the roles of EMT in the progression of gastric cancer by analyzing the expression and localization of selected markers. There are numerous biological markers that signify the passage of a cell through EMT. In this study, four are discussed: E-cadherin, Vimentin, LATS2, and  $\beta$ -actin.

EMT describes the reversible transformation from epithelial to mesenchymal tissue. EMT is achieved through cytoskeletal rearrangement and migration and is often associated with the loss of cell polarity and adhesive properties accompanied by increased invasiveness. This ultimately propagates changes in cell shape, density, and motility. The majority of cancers show high levels of EMT.

Two immortalized gastric cancer cell lines with varying degrees of malignancy, AGS and MKN28 are the primary focus. After culturing both cell lines, selected markers were studied and their influence on the development of malignant properties was determined through immunocytochemistry (ICC). ICC allows for visual sub-cellular localization of proteins and their interactions. Along with ICC data, qPCR data was obtained to follow changes in gene expression levels. Previously constructed heat maps were analyzed as they allow us to visualize gene expression changes of an entire genome over varying time points and doses of gallic acid exposure.

Using these markers in the future will allow us to evaluate the therapeutic potential of gallic acid. Gallic acid (GA), a plant nutraceutical, is associated with anti-proliferative and anti-metastatic effects. It is hypothesized that, upon exposure of cancer cells to gallic acid, the nutraceutical will allow the cancer cells to regain function of E-cadherin, a crucial component in epithelial cells. Furthermore, we expect that GA exposure will decrease levels of Vimentin as well as maintain the functions of both LATS2 and  $\beta$ -actin, resulting in healthy and non-cancerous epithelial cells. Lastly, ICC will allow us to see the effects of treatment with GA within each gastric cell line.

## CRISPR/Cas9 Screen to Identify Genes Required for Zebrafish Melanocyte Stripe Formation

Melanie Cragan, Lee Deorsy, Samuel Restrepo & Larissa Patterson

Biology, Rhode Island College, Providence, RI

Melanin-producing pigment cells called melanocytes originate in the vertebrate neural crest during embryonic development and later migrate to distant locations throughout the body. During early stages of melanoma initiation, melanocytes reactivate an embryonic neural crest signaling profile. Therefore, identifying the signals and cellular behaviors that initiate and terminate embryonic melanocyte migration during normal development may provide insight into melanoma metastasis. The zebrafish, *Danio rerio*, is a valuable model for studying vertebrate development due to high reproduction rates, rapid growth, and genetic similarity to humans. The migration and patterning of melanocytes can be easily observed during zebrafish larval stripe development, but to date, only a handful of mutants with defects in these processes have been isolated. Utilizing CRISPR/Cas9 technology, we performed a screen to identify genes required for melanocyte migration and patterning in larval zebrafish. From a list of 200 candidate genes, we designed gRNAs to knock-out 25 genes, then injected gRNAs and Cas9 protein into 1-cell zebrafish embryos. Phenotypes were analyzed at 3 and 5 days post fertilization and mutagenesis efficiency was verified by Sanger sequencing. Of the candidate genes examined, we identified two with altered melanocyte behaviors and/or stripe formation. This research serves as an essential starting point for understanding the cellular behaviors and molecular signals that both initiate and terminate embryonic melanocyte migration and may facilitate future studies that seek to prevent the spread of mutated melanocytes during melanoma metastasis.

## Yeast Bax Inhibitor (Bxi1p) Is an ER-Vacuole Membrane Localized Protein

Walter Jacob<sup>1</sup>, Camilo Villa<sup>2</sup>, Lukas Ritzer<sup>1</sup> & Nicanor Austriaco, OP<sup>1</sup>

<sup>1</sup>Biology, Providence College, Providence, RI

<sup>2</sup>Blackstone Valley Preparatory High School, Cumberland, RI

Yeast Bax inhibitor-1 (BXI1) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins. Human homologs of yeast BXI1 have been linked to several human cancers. In mammals, the Bax inhibitor family of proteins has cytoprotective properties that are most evident in paradigms of endoplasmic reticulum (ER) stress. Both the crystal structure of a prokaryotic member of the family and our own published studies suggest that the yeast Bax Inhibitor protein, Bxi1p, is a calcium leak localized to the membrane of the endoplasmic reticulum (ER). However, a competing study published by the Madeo Laboratory at the University of Graz has contested our findings. Their work concluded that the protein is localized to the lumen of the yeast vacuole. In response to this challenge, we have shown that yeast Bxi1p is localized to the ER and vacuole membranes during exponential phase. Transport to the lumen of the vacuole occurs during stationary phase suggesting that this may be an autophagy-dependent experimental artifact. Experiments are in progress to demonstrate this claim using yeast cells that carry mutations in different genes that drive the autophagic process.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco, and a Walsh Fellowship awarded to W. Jacob]



## Yeast Bax Inhibitor (Bxi1p) Mutations and the Regulation of Cytosolic Calcium

Michael Bittner, Nicholas Andrews & Nicanor Austriaco, OP

Biology, Providence College, Providence, RI

The Bax Inhibitor (TMBIM) gene family has been linked to different cancers in human patients. Overexpression of the human Bax inhibitor gene, BI-1/TMBIM6 is known to drive the malignant phenotype of prostate cancer cells. Downregulation of BI-1 triggers apoptosis and tumor death suggesting that Bax inhibitor is anti-apoptotic in nature. The budding yeast, *Saccharomyces cerevisiae*, is known to have one TMBIM gene member that we have named BXI1. The protein is localized to the ER and vacuolar membranes. Studies in our lab suggest that it is a calcium channel when overexpressed in *E. coli* yet little is known about its endogenous function in yeast and how it inhibits the killing activity of Bax. To uncover its potential role in regulating calcium dynamics, we have measured the cytosolic calcium levels in yeast cells with loss-of-function and gain-of-function alleles of BXI1. Our preliminary data suggests that Bxi1p functions as a calcium channel in yeast that regulates cytosolic calcium levels.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

## Progress Towards Long-Term Near-Infrared Imaging Probes and Sensors Based on DNA-Wrapped Carbon Nanotubes

Brendan Winne, David Restrepo, Mitchell Gravely & Daniel Roxbury

Chemical Engineering, University of Rhode Island, Kingston, RI

An incomplete understanding in the biocompatibility of single-walled carbon nanotubes (SWCNTs) gives rise to their underdeveloped potential as next-generation bioimaging probes and sensors. Certain types of SWCNTs emit photostable near-infrared (nIR) fluorescence that can be monitored over long periods of time with the use of a nIR hyperspectral fluorescence microscope. When properly functionalized with a biocompatible DNA wrapping, DNA-SWCNT hybrids enter mammalian cells and remain in the endosomal pathway. Lysosomal sensors and multiplexed imaging probes have recently been developed based on the nIR emission of SWCNTs to spatially quantify analytes in live cells and animals. Here, by temporally monitoring the fluorescence from DNA-SWCNTs in live cells over the course of 12+ hours, we report that the nIR emission spectra of these hybrids are progressively shifting towards longer wavelengths. We surmise that this may be due to the aggregation of SWCNTs and/or the degradation of DNA from the surface of the SWCNTs, both occurring as a result of natural endosomal maturation processes within mammalian cells. These results help shed light on the intracellular processing and long-term fate of functionalized SWCNTs for improved applicability with mitigated *in vitro* and *in vivo* toxicity.

## Development of HEPG2 Cell Lines Deficient in Phosphorylation Signaling

Randi Babon, Cole Tindall & Steven Symington

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

This experiment focused on the development and isolation of mutant HEPG2 cell lines with altered phosphorylation signaling. We used CRISPR-Cas 9 technology to specifically make gene knockouts of ERK (gene ID 5594), PKC (gene ID 5580), and PLC (gene ID 5335) in a HEPG2 cell line. HEPG2 cells were maintained in advanced media at 37°C with 5% CO<sub>2</sub> levels, and were propagated every 3-4 days. Specific guide RNA was developed using the E-CRISPR software for ERK, PKC, and PLC and CRISPR-Cas 9 constructs were made by inserting guide RNA sequences into the PX458 vector. The construct was validated through sanger sequencing by comparing it to PX458 alone (control vector). Validated constructs were then transfected into the HEPG2 cells using Lipofectamine transfection reagent in the 6 well plates. Lipofectamine 3000 contains lipid subunits that work to increase transfection efficiency by entrapping the transfected DNA plasmids from formed liposomes in the aqueous environment. Confirmation of successful transfections were determined using a fluorescent microscope to assess GFP fluorescents. The genomic DNA was extracted from the transfected cell lines and mutants were validated by the EnGen Mutation Detection kit. This kit utilizes PCR formation of a heteroduplex formation that is cleaved with endonuclease activity. After knockout validation, the cell lines were then diluted to appropriately and transferred to a 96 well plate in attempts to isolate the transfected individual cell lines. In the future, pyrethroid pesticides will be introduced to the isolated HEPG2 cells with confirmed knockouts in order to observe their metabolic effects with altering phosphorylation signaling.

## Development and Isolation of Ion Channel Knockouts in HEPG2 Cell Lines Using CRISPR-Cas9

Cole Tindall, Randi Babon & Steven Symington

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

The goal of the current research is to establish individual ion channel knockout HEPG2 cell lines to examine their role in metabolic diseases. CRISPR-Cas9 technology was used to generate the mutants by inserting site specific guide DNA in to the PX458 plasmid. Specific constructs responsible for the knockout of Nav1.7 (6335), Cav1.2 (775) and the IP3R (3702) gene. These are all genes responsible for the storage and accumulation of  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  in the cell. Complete constructs were validated by sequence comparison to the vector, and were then transfected in to the HEPG2 cell line using Lipofectamine 3000. Transfections were done in 6 well plates and grown to 80% confluence. After transfection, the cells grew for 3-4 days and then were examined under fluorescent and white lighting to find transfected cells. Results showed that cells were successfully transfected through the presence of GFP markers in the transfected cell, which originates in the PX458 plasmid. Gene knockouts were confirmed using the ENGen T7 Endonuclease Mutation Detection Kit. This kit employs non-matched DNA and a heteroduplex to determine the presence of the knockouts. Once the knockouts in the cells were validated, the HEPG2 cells were diluted to generate isolated cell lines. Future studies will utilize these cell lines to better understand the role in pesticide induced metabolic diseases like obesity and insulin resistance.

# CHEMISTRY

**LOCATED ALONG THE 1<sup>ST</sup> FLOOR HALLWAY OF AVEDISIAN HALL  
(COLLEGE OF PHARMACY)**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Modifying Reported Cages Structures for Selective Imaging Probes; Improving Xenon Enhanced MRI

Adriana Mendieta, Ashvin Fernando, David Robinson & Brenton DeBoef

Chemistry, University of Rhode Island, Kingston, RI

In this work, derivatives of two known molecular cages, cucurbit[6]urils (CB6) and pillar[5]arenes (P[5]A) exhibit optimal exchange interactions with xenon, a phenomena that in conjunction with xenon-129 NMR and Hyperpolarized Chemical Exchange Saturation Transfer, can serve as an MRI contrasting agent. Our group has previously shown that CB6 and pillar[5]arene both have exchange properties with xenon, with the former having previously been used to image a rat, despite it having no selectivity for any region of the body. The goal now is producing a more viable monitoring of Alzheimer's progression by attachment of an affinity tag to a cage so that it will selectively bind and aggregate to beta-amyloid plaques in a brain inflicted with Alzheimer's to effectively image only these regions. The first derivative, known as Benz6C, was a previously created using a reported templated synthesis. Another method of interest is to functionalize CB6 by attaching a single alcohol group by oxidation using a persulfate salt. The -OH group can undergo further reactions to connect a linker through a variety of reaction pathways such as nucleophilic substitution. For the other structures known as pillar[5]arenes, their derivatives are formed by a modification of the functional groups lining the cavity. Subjection to various alterations can tune how well the gas can enter the internal pocket, for how long, and what other substituents can be attached. Depletion spectra of Benz6C and a decamethyl imidazolium P[5]A species have been obtained by our collaborators which have shown promise in exploiting HyperCEST. Generating this spectrum of the desired enclosure can provide an idea of the xenon exchange rate along with the intensity of the signal.

## Microfluidic Immunosensor Array for Electrochemical Detection of Interleukin 17A and Interleukin 17F Cancer Biomarker Proteins in Serum Samples

Cameron Collins, Thorston Brochu, Te'Rell Knox & Bernard Munge

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The detection of cancer biomarker Proteins is a quick and effective means of early diagnosis and disease monitoring and may lead to improved patient health. Interleukin 17A (IL-17A) and interleukin 17F (IL-17F) cancer biomarkers are found in high levels in patients with Cutaneous T cell lymphoma (CTCL) and can be measured as a means to detect cancer. Herein, we report on a microfluidic Immunosensor array based on nanostructured screen printed carbon electrodes (SPE) that simultaneously detect cancer biomarker proteins, IL-17A and IL-17F in serum samples. The sandwich Immunosensor is fabricated by attaching capture antibodies, anti-human IL-17A and anti-human IL-17F on to nanostructured electrode arrays, followed by flowing an offline captured antigens on to a polyethylene (PEG) modified HRP multi-enzyme labeled magnetic bead bioconjugate (PEG-MB/HRP/(Ab<sub>2</sub>)<sub>n</sub>-Ag) in the microfluidic channel. The microfluidic system is then injected with hydrogen peroxide/hydroquinone mixture which triggers a peroxidase iron-heme catalytic reduction of hydrogen peroxide under applied voltage to generate an electrical signal that correlates to the concentration of antigen in the sample. Results show a detection limit of 100 fg mL<sup>-1</sup> for both IL-17A and IL-17 F in diluted serum samples with a linear range from 100 fg mL<sup>-1</sup> to 1000 fg mL<sup>-1</sup>. The disposable Immunosensor array is a simple detection system for fast measurement of interleukin 17A and 17F and shows significant clinical value for application in point-of-care (POC) cancer screening and disease monitoring.

## Nanostructured Sensor for Electrochemical Detection of Nitrites in Sea Water Samples

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Nitrites salts are widely used in industrial manufacturing process and can have detrimental effects to both human health and aquatic life. For example, nitrites can cause the transformation of normal hemoglobin to methemoglobin leading to loss of hemoglobin's ability to transport oxygen. There is an unmet need for portable, reliable and economical sensor for nitrites due to its ubiquitous nature and toxicity. Herein, we demonstrate the use of multi-wall carbon nanotubes screen printed electrodes (MWCNT-SPE) that have the capability for individual determination of nitrite anions at micromolar concentrations in aqueous solutions. Using cyclic voltammetry the MWCNT-SPE displayed activity in the oxidation of nitrite ions at pH = 3.00 with increase in anodic peaks at 0.8 V vs. Ag/AgCl in vast concentrations with a linear range from 10mM to 400mM. The detection limits were further reduced using amperometry. This approach was used to measure nitrites in sea water samples. The disposable MWCNT-SPE offers a low cost, portable and economic approach for nitrite detection in sea water samples



## Nanostructured Sensor for Electrochemical Determination of Phosphates in Narragansett Bay Sea Water

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High phosphate levels are found in the ocean due to excess run off from soil fertilizers, which leads to the eutrophication of sea plant life. Phosphorus is used as a sign to determine if over enrichment of plant life is in fact taking place. These high levels of phosphorus directly correlate with the reduction and loss of oxygen in the ocean leading to depletion of sea life. Herein, we report the use of multi-wall carbon nanotubes screen printed electrodes (MWCNT-SPE) for the electrochemical detection of phosphate in water samples. The method relies on electrochemical reduction of phosphomolybdate in acidic solution at pH 1. This indirect method gave a detection limit of  $5 \text{ mg L}^{-1}$  with a linear range of 5 to  $100 \text{ mg L}^{-1}$ . The electrochemical approach maintains high sensitivity and selectivity of the standard calorimetric method but minimizes detection time and precludes the need for additional reagents. This method shows great promise for a reliable, portable, low cost and economic strategy for determination of phosphates in sea water samples.

## Facile Synthesis of Polyelectrolyte Mediated Gold Nanostructures as Highly Sensitive SERS Substrates

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A particle-templating technique has been developed to synthesize gold nanostructures for surface-enhanced Raman scattering (SERS) applications. A cationic polyelectrolyte Poly-L-lysine(PLL) is first adsorbed on the particles. When these particles are exposed to gold chloride solutions, the interface is enhanced significantly with  $\text{AuCl}_4^-$  anions. When a reducing agent is added, a layer of gold (Au) of thickness of a few nanometers forms on the particles.

The adsorption of (PLL) on different anionic carbon black and fumed silica particles, that act as templates, was studied. Adsorption isotherms indicate the roles played by the underlying particle chemistry on the extent of PLL adsorption, which, in turn affects the morphology a thickness of the gold layer on the particle surfaces. The gold nanostructures were drop cast on a silicon wafer and used for the detection of 4-nitrobenzenethiol (NBT) using SERS. The cusps on the fractal particles as well as the edges of gold surface significantly enhance SERS signals, making these particles as sensitive SERS substrates.

## Development of Nitrite, Nitrate, and Phosphate Detecting Chemosensors for the Marine Environment

Jayden DeCosta, Matthew Leiskau, Connor Sweet, Amanda McCabe & Clifford Murphy

Chemistry & Physics, Roger Williams University, Bristol, RI

Eutrophication is the process in which nutrients, such as nitrates and phosphates, are present in excess amounts, allowing algae and other marine plant life to grow rapidly. Excess eutrophication can lead to hypoxia and other deleterious effects influencing aquaculture and marine ecosystems. This project presents a series of metalloporphyrin functionalized transmissive electrode, which were characterized for their optical and electronic response to nitrate, nitrite, and phosphate ions in filtered seawater from Narragansett Bay. Prior work with similar materials demonstrated high sensitivity to thiocyanate ions in seawater, but with possible interference from nitrate ions. Five different metal varieties (Iron, Zinc, Ruthenium, Cobalt, and Manganese) of metalloporphyrins were tested to determine the sensitivity and specificity of the analytes via cyclic voltammetry and UV-Vis spectroscopy. Preliminary results suggest these electrodes are sensitive to nitrate, nitrite, and phosphate anions in the 1-10 ppb range.

## Lord of the Rings – SAR in Gram-Positive Pathogens

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*Streptococcus pneumoniae* is a Gram-positive pathogen that is the causative agent of pneumonia, meningitis, and other infections in humans. Approximately 30% of these infections are the result from serotypes with resistance to at least one antibiotic, prompting the CDC to elevate the threat of drug resistant *S. pneumoniae* to “serious”. In an attempt to produce a novel antibiotic for the treatment of *S. pneumoniae* infections, our lab has created a panel of compounds that have been found to target the autolysins in the peptidoglycan layer of Gram-positive pathogens, preventing cell growth. These compounds were produced using a modified Ugi reaction in which an acid, aldehyde, amine, and isocyanide were combined. A library of analogs to our *S. pneumoniae* lead compounds fgkc (MIC  $3.8 \pm 1.0 \mu\text{M}$ ) and fgbb (MIC  $11.3 \pm 6.1 \mu\text{M}$ ) were produced using microwave-assisted synthesis, which effectively speeds up the production time while still maintaining moderate yield. These compounds have been screened against *Bacillus subtilis*, *Bacillus cereus*, and target *S. pneumoniae*. The primary objective of this study is to analyze the structure-activity relationships of these compounds based on their inhibitory performance against *S. pneumoniae*. Changing the acid and aldehyde allows us to experiment with different side chains in the R1 and R4 positions. Our lead compound fgkc contains a cyclic alkane in the R1 position. We have synthesized inhibitors containing variable ring structures, as well as branched alkanes, di-substituted arenes, and hetero-arenes. After screening these compounds against our target pathogen, it can be concluded that aromatic rings are not supported in the final compound structure.

## Evaluating Classic Colorimetric Methods for Phosphate and Nitrate

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We have investigated a series of classic colorimetric methods for the detection for phosphate or nitrate . Using standard solutions of phosphate and nitrate, ranging from 10 mM to 10 nM the Griess and Benzidine Lead Iodide Inorganic-Organic Compound methods were explored for nitrate while the Molybdenum Blue, Ionan Molybdate Citrate, and Modified Molybdenum Blue methods were explored for phosphate. Each method was evaluated using a UV-Vis Spectrophotometer to with phosphate and nitrate standards made with deionized water, a 0.1M chloride solution and filtered Narraganset Bay water. The results of this were then analyzed to determine the best limit of detection, Beer's Law linear range, ease of use and suitability of a paper based sensor. The Molybdenum Blue method was determined to be the most effective at detecting phosphate, and nitrate was most effectively detected using the Griess reagent. Using filter paper and crayons, test strips were created and evaluated.

## Exploring Carbon Nanodots for Phosphate Detection

Jenna Kornicki & John Breen

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Carbon nanodots are a relatively new fluorescent sensing platform that are simple to prepare and avoid the chemical safety issues common among other luminescent nanoparticles such as CdSe and CdS. Four different carbon nanodots preparations reported to produce nanodots with carboxylic acid functional groups on their surface were tested for their capacity to detect phosphate. The carbon dots solutions, made from glucose, alginic acid, starch, and citric acid with 11-aminoundecanoic acid, were analyzed using UV-VIS spectrophotometry and fluorescence spectroscopy. All preparations yielded solution with similar UV-VIS spectra in water and pH 7 buffer. Each solution of nanodots in pH 7 buffer was observed to show a decrease in fluorescence intensity with addition of europium(III) ions and a restoration of the fluorescence intensity with addition of phosphate. The nanodots prepared from glucose, alginic acid or starch dots were inferior for phosphate detection relative to the nanodots prepared from citric acid with 11-aminoundecanoic acid, which showed sensitivity to micromolar levels of added phosphate. Experiments seeking to improve the LOD and to develop paper based sensors are underway.

## Organic Synthesis of Trityl Compounds

John Williams, Shawn Contente & Dariusz Puzanowski

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Modification of standard undergraduate organic chemistry experiments were employed to prepare lead compounds for preliminary toxicity studies in bacteria and cell culture, and for DNA binding. The triarylphosphonium salt, a "trityl" moiety, is a common scaffold or structural feature in a variety of anti-cancer agents. Preparation of alkyl and aryl esters were done previously to make variations on the trityl structure. This project was directed to preparing phenolic esters of 4-diphenylbenzylphosphoniobenzoic acid. In particular, we sought to incorporate glucinol as the phenolic component since this plant extract has known antibactericidal activity as do many trityl phosphonium salts. The products were identified by melting point and IR. This reaction sequence gives compounds which can lead to a variety of similar molecules that fall within the Lipinski envelope. Lipinski's Rule of Five was used to select structures with potential drug efficacy. Predicted bioactivity properties of structures to be synthesized were calculated using the proprietary software program MUSE™ and the online program Molinspiration.

## Calculations of Biological Activity of Trityl Compounds

John Williams & Israa Al-Nubani

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Factors to predict bioactivity were analyzed in order to determine suitable triaryl-phosphonium salts ("trityl") to synthesize for potential anti-carcinogenic and anti-bacterial activity. Lipinski's Rule of Five was used to evaluate druglikeness and to determine if the trityl compounds might have biological activity and chemical and physical properties that allow them to be candidates as orally active drugs in humans. Molinspiration, an online drug activity predictor, was used to determine the potential bioactivity of the compound, as well as the chemical and physical properties. The bioactivity score on Molinspiration indicated whether a compound might bind to one or more proteins: GPCR, ion channels, kinase, nuclear receptor, protease, and enzyme. Chemical and physical properties calculated include logP, molecular polar surface area (PSA), and the Rule of Five descriptors. Lipinski's Rule of Five states that an orally active drug must have no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular mass less than 500 daltons, and a partition coefficient log P not greater than 5. Following these standards, HyperChem<sup>®</sup>, a computational program, was utilized, using molecular mechanics calculations, to calculate the optimization energy of molecules complexed with DNA, which an energy decrease indicated favorable DNA binding. Using the QSAR calculator on HyperChem<sup>®</sup>, the surface area, volume, hydration energy, logP, refractivity, polarizability, and mass were calculated for a set of trityl compounds which showed high bioactivity scores in Molinspiration and passed Lipinski's Rule of Five. With this data, plots showing correlations between the logP and the refractivity/polarizability were generated.



## Triaryl Compounds Computational Poster

Dariusz Puzanowski & John Williams

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Triphenylphosphonium (TPP) cations, a subset of triaryl compounds, are of interest because of their ability to passively transfer across cell membranes and permeate cell mitochondria. Both processes are driven by the negative membrane potential. TPP can also act as transfer catalysts to molecules known or predicted to have anti-cancer activity. This research investigates structural effects of TPP scaffold articulation and, moreover, predict bioactivity based on intuition, confining a definite reference structure under Lipinski's rule. The online docking software, Molinspiration, and the proprietary software Muse Invent enable finding reasonable structures that have anti-cancers properties. While both programs calculate physical properties reflective to certain cellular targets, visualization of molecular interaction with the proteins and determining molecular affinity can be done in Muse. The utility of computational analysis reveals potential bioactivity and druglikeness. It is important that many of the active compounds are accessible by laboratory synthesis and the actual compounds can be screened for actual biological activity.

## Nickel, Cobalt, and Zinc Binding to the H110A and H110Q Mutant KmtR Proteins

Aja Pragana, Stephanie Lewis, Sophia Valiente & Khadine Higgins

Chemistry & Biochemistry, Salve Regina University, Newport, RI

Tuberculosis is a deadly respiratory infection that annually infects nearly a third of the world's population. The disease is caused by the bacterium, *Mycobacterium tuberculosis* (*M. tuberculosis*). The *M. tuberculosis* nickel and cobalt responsive metalloregulator KmtR has tighter metal binding affinities for nickel and cobalt than NmtR. Sequence alignment reveals that four of the six residues involved in binding nickel in NmtR are not present in KmtR. H110 is a potential metal binding residue in KmtR. Additionally,  $\beta$ -Galactosidase assay determined that this residue is necessary for Ni(II) and Co(II) recognition. Metal binding competition studies using Mag-Fura 2 are being performed to determine the metal binding affinities. These studies will help to elucidate if H110 is a ligand for the cognate metals, nickel and cobalt, as well as the noncognate metal, zinc. Our research this summer includes continuation of the study of the H110A mutant KmtR protein, as well as the H110Q mutant KmtR protein.

## Metal Binding Studies of H111 Mutant KmtR Protein from *Mycobacterium tuberculosis*

Sophia Valiente, Stephanie Lewis, Aja Pragana & Khadine Higgins

Chemistry & Biochemistry, Salve Regina University, Newport, RI

*Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent of tuberculosis, was involved in 1.7 million deaths worldwide in 2016 and is a leading cause of death in people infected with HIV. The percentage of drug resistant *M. tuberculosis* cases has not decreased in the last 20 years, creating a need for new ways to combat the bacteria. The ability of *M. tuberculosis* to regulate metal concentrations in its cell is vital to its survival in phagosomes. The bacteria use metallosensors to regulate the transcription of proteins involved in influx, efflux, and sequestration of metals. *M. tuberculosis* has 2 metallosensors, NmtR and KmtR, that respond to both nickel and cobalt. This research aims to understand how KmtR binds metals and why there are 2 nickel and cobalt responsive metallosensors. By mutating residues that are known to bind metals and determining the effects on the metal binding affinities, we can elucidate if these residues are involved in binding the cognate metals, nickel and cobalt, as well as the noncognate metal, zinc. This work specifically focuses on the H111 mutations.

## Metal-Binding Affinities of the Metalloregulator KmtR from *Mycobacterium tuberculosis*

Stephanie Lewis

Chemistry & Biochemistry, Salve Regina University, Newport, RI

*Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis, infects close to one-third of the world's population and has surpassed HIV in recent years. The number of multi-drug resistant strains of Mtb continues to increase, thus increasing the importance of developing new therapeutic strategies to target other pathways in Mtb. The objective of this project is to explore the structure function relationships in KmtR. KmtR is the second Ni(II) and Co(II) responsive transcriptional regulator identified in Mtb. This project seeks to determine the coordination number and geometry of the metal sites and to measure the metal binding affinities of both cognate and noncognate metal ions to KmtR. Current work consists of metal binding studies to determine the metal binding affinities to wild type KmtR.

## The Multistep Synthesis of Metal-Pyrrole Derived Complexes and Nitrate Ion Detection

Hannah Theriault & Lauren Rossi

Chemistry & Physics, Roger Williams University, Bristol, RI

Nitrate pollution of the local coastal marine environment can occur via run-off from feeding rivers and streams or the overflow of stressed sewerage treatment systems. The assessment and reduction of nitrate ion contamination within aqueous solutions are important goals aimed toward determining the health of freshwater/ marine environments and impacted communities. A multistep synthesis of tetradentate tris(pyrrolyl- $\alpha$ -methyl)amine ligands has been accomplished. Following iron (II) coordination, these complexes will be evaluated via spectroscopic techniques for nitrate ion binding within organic and aqueous systems.

## Cyanobacteria-Derived Protease Inhibitor

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Our laboratory has been investigating a bloom of *Trichodesmium*, a genus of cyanobacteria, collected from Padre Island in the Gulf of Mexico in May 2014 for bioactive compounds. Mass spectrometry-guided isolation of this bloom was utilized to isolate the metabolite. The structure of this molecule was determined using high-resolution mass spectrometry along with 1D and 2D NMR analysis. The molecule contains interesting chemical features, such as a di-substituted thiazole embedded in a macrolactone ring. Cyanobacteria metabolites have proven to be successful protease inhibitors. When the structure was analyzed using the online platform available at Swiss Target Prediction, the new metabolite was predicted to target multiple membrane receptors and proteases including cathepsin S. A biochemical assay for inhibition of cathepsin S, B and BACE were performed. Cathepsin S is a lysosomal cysteine proteinase which is associated with the degradation of proteins to peptides necessary for the presentation of MHC class II molecules. It is associated with multiple pathological conditions such as coronary heart disease, insulin resistance, and atherosclerosis. Cathepsin S is upregulated following spinal injury and increases the concentration of pro-inflammatory chemokines activating microglial migration to the injury site. The therapeutic development of a cathepsin S inhibitor has the potential to reduce neuroinflammation and neuropathic pain. The isolation and characterization of this molecule adds to our laboratory's pure compound library and provides a new entity to screen for activity against cathepsin proteases.

## Small Molecule Mimics of Superoxide Dismutase Enzymes

Hanna Bovill, Andrew Dillon, Andrew Josling & Maria Carroll

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Our project focuses on the synthesis of new complexes based on the active sites of superoxide dismutase (SOD) enzymes, which catalyze the disproportionation of superoxide to form dioxygen and hydrogen peroxide. High levels of superoxide can lead to oxidative damage, which is associated with a number of diseases, including Parkinson's disease, cancer, and amyotrophic lateral sclerosis. SOD enzymes regulate superoxide levels, but when levels exceed that which can be managed by enzymes, other therapeutic agents are necessary. The active sites of SOD enzymes all consist of first row transition metal centers, and in many of the active sites contain imino and thiolate ligands are coordinated to the metal. We will present results of our efforts to synthesize ligands that contain imino, thiolato, and thioether groups, as well as, first row transition metal complexes that incorporate these ligands.

## Synthesis of Iron $\alpha$ -Diimine Complexes Relevant to Reduction of Protons and Carbon Dioxide

Julia Brown & Maria Carroll

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Traditionally, reactions of transition metal complexes involve the metal and labile ligands, but ancillary ligands do not play an active role. However, specific ligands can be chosen that will participate in reactivity. For example, some ligands are redox active, forming radical species that are stabilized when complexed to a metal center. In complexes of these ligands, the metal center is not the only site of redox reactions, and the use of redox active ligands has proven to be a successful method for conferring reactivity to metals that is not otherwise possible. We propose that iron complexes with redox active  $\alpha$ -diimine ligands can be tuned electronically to be selective for either reduction of protons or  $\text{CO}_2$ , by adjusting the ability of the ligand to store electrons. We will present the results of our efforts to synthesize and characterize iron  $\alpha$ -diimine tricarbonyl complexes and initial reactivity and electrochemical studies on the complexes.



## Cyclodextrin-Promoted Fluorescence Detection of Benzene, Toluene, Ethylbenzene, Xylene, and Cumene (BTEXC) Compounds in Contaminated Snow Environments

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Reported herein is the sensitive and selective cyclodextrin-promoted fluorescence detection of benzene, toluene, ethylbenzene, xylene, and cumene (BTEXC) fuel components in contaminated snow samples collected from several locations in the state of Rhode Island. Cyclodextrin is used to promote analyte-specific, proximity-induced fluorescence modulation of a high quantum yield fluorophore leading to fluorescence responses that are unique to each cyclodextrin-analyte-fluorophore combination. From these responses, unique pattern identifiers were created via linear discrimination analysis. The detection method used is highly sensitive, with sub-micromolar limits of detection, highly selective, with the ability to accurately discriminate between structurally similar compounds, the ortho-, meta-, and para- isomers of xylene, and broadly applicable to chemical composition, pH, and electric conductivity. The qualities of this method indicate significant potential in the development of practical detection devices for aromatic toxicants in complex environments.

## Color Changing Sensors for Ion Detection in Marine Environments

Adelaide Levenson, Teresa Mako & Mindy Levine

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The presence of nutrients, such as nitrate and nitrite, in seawater environments can pose a threat to marine plant life and organisms. The management of these nutrients relies heavily on monitoring their presence; however, detection of such nutrients can be challenging due to the low concentration of these ions in seawater. Current commercial methods of detection are capable of sensing these ions at ppm levels, yet these methods are not proficient at detecting ppb levels. Additionally, current methods cannot fully differentiate nitrate and nitrite. The goal of this research is to create devices that are more selective, sensitive, and robust than current methods. Thus far, new devices for dual nitrate and nitrite detection have been developed that employ surface-bound colorimetric reagents and improved nitrate to nitrite reduction.

## Synthesis of 1,4,8-Trioxaspiro-[4.6]-9-Undecanone (TOSUO) Copolymers for Biomedical Adhesive Applications

Michelle Lee, Jinal U. Pothupitiya & Matthew K. Kiesewetter

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Polymeric materials are playing an ever-increasing role in biomedical applications—from non-mechanical fasteners to tissue scaffolds, and synthetic organic chemistry is the engine that gives rise to new materials. This work focuses on the ring-opening polymerization (ROP) of 1,4,8-trioxaspiro-[4.6]-9-undecanone (TOSUO) to make polyTOSUO. PolyTOSUO has traits desirable in biomedical material; it is: biodegradable, biocompatible, can be copolymerized with a host of cyclic ester monomers to tune physical properties, and it possesses adhesive properties. A selection of TOSUO/lactone (-caprolactone or -valerolactone) copolymers in various architectures—including block- and random-copolymers—was generated and evaluated. The polymer samples were analyzed with NMR, GPC, DSC, and TGA. The adhesive properties of these polymers were assessed through tensile testing, where components such as ultimate tensile strength and breaking strength could be measured.

## Organocatalyst Mediated Synthesis of Block and Gradient Copolymers for Biomedical Applications

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Biodegradable polymers have found a wide variety of applications in the biomedical field like micelles and nanoparticles for drug delivery. The ability to tune particle properties – degradation, delivery profile, and shape – is ultimately determined by our ability to synthesize precise macromolecular constructs with purposefully chosen building blocks. The focus of this study was to synthesize block copolymers and gradient copolymers via ring-opening polymerization (ROP) of cyclic esters. A series of highly controlled H-bonding organic catalysts were screened to determine the most efficient system for the A-B block copolymerization of two lactones ( $\epsilon$ -caprolactone (CL) and 1,4,8-trioxaspiro-[4.6]-9-undecanone (TOSUO)) initiated from polyethylene glycol (PEG). These ROPs have also been conducted in solvent and solvent-free conditions, and the efficiency of the chosen catalyst system was investigated by a catalyst loading screen. The resulting polymers were analyzed via NMR and gel permeation chromatography (GPC) analysis.

## New Reaction Methodology for the Synthesis of Nitrogen-Containing Small Molecules

Kyle Medas, Robert Lesch, Yazan Al-Issa & Friendship Idioma

Chemistry & Biochemistry, Providence College, Providence, RI

Cyanamides and alkynamides are reactive intermediates that can be elaborated into more complex small molecules. While these functional groups are similar in structure, they have remarkably different reactivities. We have been exploring new methodology that use these intermediates to create small molecules containing one or multiple nitrogen atoms. This research project involving undergraduate students will describe two new directions in this area: 1) palladium-mediated multicomponent reaction methodology to make 2-substituted alpha-carbolines from cyanamides, and 2) the stereoselective synthesis of fluoroimides from alkynamides. A third project closely related to the first two involves the synthesis of neuropharmacological receptor antagonists with a beta-carboline core. Overall, we will report a good substrate scope for these reactions in moderate to good yield, describe our efforts to increase diastereoselectivity in fluoroimide synthesis, and demonstrate that 1-aryl beta-carbolines inhibit the serotonin receptor at nanomolar concentrations.

## Sediment Depth Profile of the Natural Attenuated Remediation of Halogenated Hydrocarbons

Scott C MacConnell, Lyndsay A Marlowe, Colby M Masse & Stephen K O'Shea

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Terrestrial and marine sediments ability to induce transformation of halogenated hydrocarbons (HHCs) is an important aspect of global halogen cycling and their natural attenuated remediation. Comparative solvent extraction techniques employing soxhlet and microwave techniques of whole wet sediments to volatilized solid phase extraction were investigated to determine the volatile and semi-volatile organics present to infer the chemistry of the environment. GC/MS analysis of the total semi-volatile organic array was determined from both field collection and in lab testing. Sediment core samples from these sites were further characterized for their carbon and carbonate content as well as their granular size. A relationship of granular size, carbon content, and surface metals in each of the core depth profiles was determined. Segmented sediment core acid and water extractable metals were determined by ICP and XRF to elucidate potential metal catalyzed surface reactions for HHCs. Sediment efficacy of HHCs transformative potential of autoclaved to oven dried samples were compared deriving *in situ* abiotic or biotic pathways by HPLC-IC GC/MS headspace and  $^{13}\text{C}_2$  NMR.

## Spectroscopic and Instrumental Assay for the Determination of Phosphorus in the Environment as a Prelude to Automated Field Testing

Lyndsay A. Marlowe, Scott C. MacConnell, Colby M. Masse & Stephen K. O'Shea

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Phosphorus is a key limiting element in marine and terrestrial waters integral to primary production. It is incorporated within the ecological fauna cycle from the weathering of the apatite mineral. To elicit the amount of phosphorus in the aquatic environment by remote sensing is a challenging goal, notably in a marine setting. To validate new technologies, a series of classic spectrophotometric tests were used to characterize phosphorus speciation in these water bodies with correlation to surface bound phosphorus at the sediment-water interface. A sediment depth profile of bound phosphorus to surrounding pore water was also derived, elucidating the relationship of bio available phosphorus to that of permanently bound and the potential flux between the two phases. Column and pore water samples were analyzed as is after filtration and Rhizon filtration respectively. Core sediment phosphorus extractions and protocols were compared for their efficacy from terrestrial and marine environments. This research established that basic EDTA/dithionite extraction of marine sediment was superior to that of the acidic ignition technique and was more suitable for NMR analysis. The aqueous spectrophotometric tests employed were molybdovanadate and ascorbic acid tests following the HACH® procedure. These color spot tests were compared to instrumental analysis by HPLC-IC with inline electrochemical detection, phosphorus lines in ICP [@ 214 and 217 nm], vacuum XRF, <sup>31</sup>P NMR and reflectance FTIR.

## *In-Situ* Characterization of HHCs Degradation in Terrestrial and Marine Pore Waters via $^{13}\text{C}$ NMR

Colby M Masse, Scott C MacConnell, Lyndsay A Marlowe & Stephen K O'Shea

Chemistry & Physics, Roger Williams University, Bristol, RI

Segmented, Rhizon filtered sediment core interstitial pore water was characterized to elucidate natural biotic and abiotic attenuated remediation degradation pathways of halo-carbons (HCs). This work has important implications from both a climate perspective and for what it tells us about the HCs biogeochemical cycling and their release into the ecosystem. The mechanistic HCs degradation pathway can be confounded by oxidation-reduction potential (ORP) of the environment and its pH, clarifying *in-situ* metal oxidation states and the potential microbial consortia activity. Catabolic oxidants and the bacterial succession order, following the submergence of sediment pore water, directly matches the order of decreasing potential for the corresponding redox couples:  $\text{O}_2/\text{H}_2\text{O}$ ,  $\text{NO}_3^-/\text{N}_2$ ,  $\text{MnO}_2(\text{s})/\text{Mn}^{2+}$ ,  $\text{Fe}(\text{OH})_3(\text{s})/\text{Fe}^{2+}$ ,  $\text{SO}_4^{2-}/\text{HS}^-$  and  $\text{CH}_2\text{O}/\text{CH}_4$ . Measuring a site's *in-situ* capacity (soil/water) for transformation directly by treating it with a  $^{13}\text{C}$  labeled HC substrate that is capable of undergoing the fundamental processes of oxidation, reduction, and substitution allows the chemistry that occurs to characterize the site. Both the nature and rates of these transformations can be assessed utilizing carbon labeled  $^{13}\text{C}_2$  substrates,  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy analysis and head space gas chromatograph/mass spectroscopy.



## Investigating Beta-Keto Esters as Bacterial Quorum Sensing Inhibitors

Toyosi Akanji & Susan Meschwitz

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Over two million people yearly are infected with bacteria that have developed a resistance to common antibacterial treatments used in modern medicine. This has become an epidemic within today's society because the death toll for those infected with these diseases continues to rise as these bacteria continue to evolve. Traditionally, medical treatments are focused on killing or inhibiting the growth of these bacteria but alternatives like inhibition of quorum sensing pathways can also be used to offset these fatalities. Quorum sensing is known to be the mechanism for cell to cell communication. Bacteria that are capable of quorum sensing, produce auto-inducers or small molecules that are used as chemical signals that can be detected by other bacteria. These chemical signals then allow bacteria to express different genes such as virulence factor production, biofilm formation and swarming, which are all means in which the bacteria infect its hosts. This research has focuses on synthesizing beta-keto ester derivatives as a substitution and a method of binding to receptors instead of the autoinducers the bacteria naturally form. Through transesterification reactions we have been able to synthesize multiple compounds and test them in broth dilution assays. These compounds have been tested for their efficiency in quorum sensing inhibition in *Vibrio harveyi* by measuring the inhibition of luminescence, a phenotype controlled though quorum sensing, and calculating  $IC_{50}$  values. The goal for this project is to expand the library of beta keto ester derivatives, in order to better understand the structure-activity relationships.

## Competitive Antagonism Assay Using *E. coli* JB525 to Test the Quorum Sensing Inhibition of Phenethylamide Derivatives

Nicole Martin & Susan Meschwitz

Chemistry & Biochemistry, Salve Regina University, Newport, RI

In today's world, bacteria are forming a resistance to modern day antibiotics creating a new hurdle for pharmaceutical scientists. Antibiotics are used to kill the bacteria that are making a patient ill, which can lead to the bacteria evolving to form a resistance to these antibiotics. Quorum sensing (QS) inhibition is an alternative method to antibiotics. QS allows bacteria to communicate with one another by the release and acceptance of molecules known as autoinducers. The focus of QS inhibition is to replace these autoinducers with another molecule preventing the bacteria from communicating and spreading virulence. A previous study has shown that the gram-positive marine bacterium, *Halobacillus salinus*, produces a phenethylamide that is capable of QS inhibition. In our lab, a library of phenethylamide derivatives has been synthesized and tested for QS inhibition capabilities. A competitive antagonism assay was one of the methods of testing and used a mutant strain of *E. coli* JB525 (*E. coli* MT102 that contains the recombinant plasmid pJBA132) in order to study the AHL-dependent QS inhibition. The mutant strain links GFP production to the activation of LuxR *V. fischeri* by exogenously added autoinducers (HHL), and can be used to prove that the compounds are actually causing QS inhibition. Fifteen phenethylamide derivatives have been tested using this competitive antagonism assay, of which nine have shown positive QS inhibition. The most active phenethylamide derivatives in this assay have  $IC_{50}$  values of 1.05 $\mu$ g/mL, 3.81 $\mu$ g/mL, and 5.23 $\mu$ g/mL. Ongoing experiments and synthesis of new derivatives are underway in our laboratory in order to expand our library of QS inhibitors.

## Creation of Diphenyl Amides as Inhibitors of Bacterial Quorum Sensing Phenotypes

Ann Mozzer & Susan Meschwitz

Chemistry & Biochemistry, Salve Regina University, Newport, RI

The antibiotic epidemic is at its peak in today's society because antibiotics either kill or inhibit the growth of the bacteria. The destroying of bacteria has led to antibacterial resistance, meaning the mechanisms commonly used to fight off these bacteria are becoming inadequate. This helplessness pushed us to discover new ways to fight against bacterial infections without the bacteria creating a resistance to the drugs. One of the ways bacteria thrive is by communicating with each other, allowing them to eventually overcome and infect the host. This cell-to-cell communication is called quorum sensing (QS). QS utilizes small molecules called autoinducers, which are released into the surroundings sending information to other bacterial cells that are in proximity. Autoinducers then bind and stabilize receptor proteins, which creates a ligand-protein complex that initiates the transcription of QS genes, including virulence factor production. The QS regulation of virulence and pathogenicity in bacteria creates a different direction in fighting antibiotic resistance, which supports our long-term goal of developing molecules capable of inhibiting QS in bacteria. The naturally occurring bacterial metabolite 3-methyl-N-(2'-phenylethyl) butyramide has been found to inhibit QS in the bacteria *Vibrio harveyi*. Our overall goal is to mimic this structure while making slight adjustments to detect if QS inhibition improves. The one-step synthesis involves the coupling of an amine and carboxylic acid to produce an amide. Initial SAR studies showed that decreasing the linker between the nitrogen of the amide and the phenyl ring to zero and adding a phenyl ring with a two-carbon linker to the acyl side chain resulted in a compound with sixteen-fold more potent antagonist activity than the natural product. From there the diphenyl amides were further modified with various substituents on the phenyl rings. So far, fifteen derivatives have been made with the lowest IC50 value of 1.1 mM against *Vibrio harveyi*. Our analogs have also been tested for inhibition of QS phenotypes in *E. coli* JB525 and *Chromobacterium violaceum*.

## Porous Lithium Ion Conductive Glass Ceramics for Enhancing Bond Durability, Strength for Protective Lithium Pouches

Daniel Donnelly<sup>1</sup>, Charles Patrissi<sup>2</sup>, Jennifer Rizzo<sup>2</sup>, Christian Schumacher<sup>2</sup> & Thomas Arruda<sup>1</sup>

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<sup>2</sup>Naval Undersea Warfare Center, Newport, RI

The growth in the capabilities of autonomous underwater vehicles ranging from geophysical mapping to novel defense technology has called for the increase of light weight high energy density batteries that are facile to incorporate into existing systems. Primary lithium (Li)/water batteries are a great candidate providing up to 2MJ/Kg (versus secondary Li-ion with 0.7MJ/Kg) and a theoretical capacity of 5.1-11.4Wh/Kg. The main challenge to realizing the full potential of Li/water is overcoming the incompatibility of the two reactants, as direct contact between the two lead to spontaneous and uncontrollable oxidation of the lithium. PolyPlus Company pioneered the concept of a protected lithium electrode (PLE) which effectively isolates the Li anode from any aqueous cathodic environment by use of a solid electrolyte ceramic separator, conductive only to lithium ions.

The integrity of the PLE and our protective Li anode pouches are dependent on their ability to keep water from intruding into the inner anodic environment. Our investigation focuses on enhancing the adhesion abilities of the seal between the ceramic separator and the laminate pouch material to ensure greater service life for Li/water batteries. This was approached by engineering surface pores into the ceramic membranes to not only increase surface area for adhesion between the laminate and ceramic, but also to add mechanical interlocking sites for the laminate to fill and cure into. SEM studies were conducted to observe the surface morphologies and topographies of the ceramic membranes, and to identify the types of bond failures for 180° peel strength tests before and after immersion of the pouch anodes in water. Finally, electrochemical impedance spectroscopy was used to identify any possible effect that the ceramic surface pores had on battery performance.

## Exploring Concentration Effects on Electrochemical Reversibility, Membrane Phenomena and Crossover of $\text{VO}^{2+}/\text{VO}^{2+}$ Electrolyte Solutions for Vanadium Redox Flow Batteries

Sophia Tiano<sup>1</sup>, Jamie Lawton<sup>2,3</sup> & Thomas Arruda<sup>1</sup>

<sup>1</sup>Chemistry & Biochemistry, Salve Regina University, Newport, RI

<sup>2</sup>Chemistry, University of Rhode Island, Kingston, RI

<sup>3</sup>Cell & Molecular Biology, University of Rhode Island, Kingston, RI

The vanadium redox flow battery (VRFB) is a promising solution for large-scale energy storage. VRFBs consist of positive and negative electrodes operating with  $\text{VO}^{2+}/\text{VO}^{2+}$  and  $\text{V}^{2+}/\text{V}^{3+}$  redox couples separated by an ion exchange membrane such as Nafion. Nafion consists of a perfluorinated carbon backbone with sidechains containing sulfonate end groups. This repeating anion group makes the membrane permeable to cation groups with the intention of conduction of protons but the added problem that the cationic vanadium groups can also enter the membrane and cross to the other side. This crossover effect is driven by two processes: how fast the cation can diffuse in the membrane and more importantly how much cation can enter the membrane at equilibrium (uptake). Understanding the chemistry and processes under which the battery operates, including the reversibility of the redox reactions and unwanted vanadium crossover in the membrane at different states of charge (soc) and electrolyte concentrations allow for the optimization of the battery. Electron paramagnetic resonance (EPR) is a technique that can detect the presence of  $\text{VO}^{2+}$  and can give information about the dynamics of the vanadium environment in solution and imbibed in the membrane. In this study, solutions of  $\text{VO}^{2+}$  and  $\text{VO}^{2+}$  with different concentrations of sulfuric acid and lithium sulfate were analyzed electrochemically to build an understanding the concentration of both  $\text{H}^+$  and  $\text{SO}_4^{2-}$  play in concentration trends observed in both kinetics of the electrochemical reaction and dynamics in the solution interactions with the membrane system. Membranes exposed to vanadium containing solutions were analyzed by EPR. The amount of vanadium absorbed in the membrane was leached from the membrane and measured using EPR. This body of work will help to illustrate the dynamics of vanadium in the Nafion membrane and the role concentration plays on trends that have been observed in both electrochemical reactions and membrane crossover measurements.

# **DRUG DELIVERY**

**LOCATED ALONG THE 1<sup>ST</sup> FLOOR HALLWAY OF AVEDISIAN HALL  
(COLLEGE OF PHARMACY)**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Development of a Novel Cyclodextrin Based Complexing Formulation for Treatment of Pediatric HIV

Ryan Ivone<sup>1</sup>, Ibukunoluwa Oje<sup>2</sup>, Samantha Meenach<sup>1,2</sup> & Jie Shen<sup>1</sup>

<sup>1</sup>Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

<sup>2</sup>Chemical Engineering, University of Rhode Island, Kingston, RI

HIV is a deadly disease that is even more dangerous for people living in countries which lack basic resources. Most anti-HIV therapeutics are associated with poor oral bioavailability due to their poor water solubility. Additionally, children suffering from HIV have a difficult time taking their medication due to the bitter, unpleasant taste profile of these therapeutics. This will lead to a decrease in patient compliance and overall reduce the effectiveness of the medication. Here, we aim to address these key issues and develop a formulation with enhanced bioavailability and improved palatability. To accomplish this, two types of modified cyclodextrins were used with the intent to improve dissolution and palatability of anti-HIV drugs. Cyclodextrins inherently possess a hydrophilic outer shell and can entrap hydrophobic moieties in their core. Using this principle, we are currently investigating ways to complex two anti-HIV drugs, namely Lopinavir and Ritonavir, to the cyclodextrins. These drugs alone have very poor aqueous solubilities, hindering their performance in an oral delivery system. However, when complexed to cyclodextrin, their solubility drastically improves allowing for a much more efficient delivery system. In addition, the palatability of these drugs will be improved when complexed to cyclodextrin. We have also been exploring the technique of spray drying our formulation to achieve stable microparticles with an enhanced dissolution rate. Combining these ideas, we will end up with a stable anti-HIV formulation that exhibits enhanced dissolution as well as improved palatability, thereby increasing pediatric adherence and overall effectiveness of the medication.

## Development of a Novel *In Situ* Gelling Delivery System for the Treatment of Uveal Melanoma

Gabrielle Rozumek<sup>1</sup>, Lingxiao Xie<sup>2</sup>, Khaled Ibrahim<sup>2</sup> & Jie Shen<sup>2</sup>

<sup>1</sup>Biology, Marine Biology & Environmental Science, Roger Williams University, Bristol, RI

<sup>2</sup>Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

Uveal melanoma (UM), commonly referred to as ocular melanoma, is the most common form of ocular malignancy in adults. The 5-year survival rate of UM patients who contract metastatic cancer is about 15%. Unfortunately, there is no effective treatment for UM and this could explain the low 5-year survival rate. In order to improve prognosis and survival of UM patients, it is important for UM to be diagnosed early and to control the spread of the cancer to other parts of the body. The present study focuses on developing an *in situ* gelling drug delivery system using bioinspired materials for the treatment of UM. Curcumin, a well-known anticancer drug, has been chosen as the model drug. The drug was encapsulated into nanoparticles and then incorporated into the optimized *in situ* gelling system. Rheological studies demonstrated that the developed *in situ* gelling system formed a gel within 3 minutes at 37°C, which will be suitable for future animal studies. Furthermore, the developed curcumin nanoparticle/hydrogel composite was able to improve anti-UM effect against the human UM cells (MP38).



## Development and Characterization of Air-Grown 3D Lung Tumor Spheroids

Riley Mather & Samantha Meenach

Chemical Engineering, University of Rhode Island, Kingston, RI

Lung cancer is currently the most deadly form of cancer in the United States. While significant research has been done relating to potential treatments for lung cancer, there is a fundamental issue with the *in vitro* cellular models used to evaluate therapeutics prior to animal studies or clinical trials. Traditionally, cell culture studies are performed in two dimensions (2D) in a cell culture flask, petri dish, or well plates. However, this 2D system does not effectively replicate the microenvironment of tumors. Recently, the use of 3D multicellular spheroids (MCS) has become more mainstream, since it more accurately represents the physiological properties of cancerous tissue. 3D MCS are typically grown and tested in a liquid covered culture environment, which mimics a tumor in most organs, such as the pancreas or liver. In reality, lung cancer tumors are usually found in the airways in the lungs. The purpose of this research is to create an effective procedure which will produce a much more accurate representative model of lung cancer tumors for the *in vitro* evaluation of lung cancer therapeutics. This procedure uses calcium alginate gel scaffolds in which spheroids are formed in liquid covered Transwell culture. Once the spheroids have formed, the gel scaffolds are degraded using EDTA and the liquid is removed through the Transwell membrane, leaving the spheroids in air-interface conditions (AIC). These air-grown 3D MCS will be characterized, and cancer drugs cisplatin and gemcitabine will be tested on them. So far, the procedure which produces AIC 3D MCS has been completed and mostly optimized. Research is ongoing to finish optimization and spheroid characterization using multiple cell lines.

## Enhanced Chemotherapeutic Anticancer Effect via Cell Cycle Synchronization

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<sup>1</sup>Chemical Engineering, University of Rhode Island, Kingston, RI

<sup>2</sup>Electrical, Computer and Biomedical Engineering, University of Rhode Island, Kingston, RI

In 2016, there were an estimated 1.6 million new cases of cancer in the United States, motivating the need for new treatment strategies. Common cancer treatments include the delivery of chemotherapeutics without coordinating deliveries to coincide with specific phases of the cell cycle. This, despite the fact that many chemotherapeutics are most potent when administered at particular phases of the cell cycle. This work aims to explore the concept of (i) first synchronizing cell cycles through sequential deliveries of drugs that arrest cell cycles and then allow them to resume in concert, and then (ii) after a delay time, administer a primary chemotherapeutic whose potency is most effectual when delivered at a specific point during a cell cycle. The hypothesis of this work is that proper timing and dosage of these deliveries can enhance the anticancer effect of the primary chemotherapeutic. Here, the strategy was to use Lovastatin initially in order to arrest B16-F10 and A549 cell cycles in the  $G_0/G_1$  phase. After Lovastatin treatment, Mevalonic Acid would be administered in order to allow cells to resume their cycles, synchronized, starting from the  $G_0/G_1$  phase. Then, time would be allowed for these cells to progress to the S phase. At that time, 5-fluorouracil (5FU) would be administered, which should be most potent during the S phase since its mechanism of action requires access to synthesizing DNA. Our initial experimental approach is to understand how the duration and concentration of Lovastatin exposure impacts cell cycle distribution in B16-F10s (as measured by a DNA-stain-based cell cycle assay). Initial results indicate that Lovastatin exposures of 20 hours at concentrations ranging from 20 to 50  $\mu\text{M}$  arrest higher percentage of B16-F10s in the  $G_0/G_1$  phases as compared to controls (68% in  $G_0/G_1$  compared to 58% in controls,  $p < 0.05$ ). Experiments are underway to do the same with A549 cells. Our next aim was to understand lovastatin release from calcium cross-linked hydrogels. Preliminary experiments of using these ultrasonically responsive gels showed that we can release up to 5 $\mu\text{M}$  of lovastatin. Our next aim will to incorporate multiple drugs into one gel. These studies will be valuable for devising new delivery strategies, particularly from stimuli-responsive hydrogels that are capable of coordinating the sequential delivery of multiple therapeutics.

# **ENVIRONMENTAL SCIENCE**

**LOCATED ALONG THE 1<sup>ST</sup> FLOOR HALLWAY OF THE CENTER FOR  
BIOTECHNOLOGY & LIFE SCIENCES**

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EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Designing a Visual Platform for Exploring Climate Change Impacts on Coastal Plant Populations

Shannon Kingsley<sup>1,2</sup> & Nadia Lahlaf<sup>3,4</sup>

<sup>1</sup>Biology, Brown University, Providence, RI

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As climate change exacerbates hundreds of years of anthropogenic pressures on salt marsh habitats, much of the public remains unaware of the environmental importance of these ecosystems. In order to understand and track the effects of climate change, scientists turn to neglected botanical records in herbaria, and the gap in herbarium records is made apparent. . We have studied the effects of climate change on Tillinghast Place in Barrington, Rhode Island through both the collection and documentation of botanical specimens, as well as through focused investigations into the specimens collected between 1845 and present day as compiled in the Consortium of Northeast Herbaria. In the salt marsh and beach habitats at Tillinghast Farm, we found 14 families of plants, including 15 native species, 7 non-native species, and one invasive species. Among these salt marsh and dune plants, we identified 8 species of grass. Of note, we found an abundant population of *Bromus tectorum*, a non-native species not commonly found in dune habitats. In the disturbed habitat at the transition edge between the upland and wetland, we found 24 families, which includes 20 native species, 18 non-native species. Among these 18 non-native species, 4 of these species are invasive. Although the present species found in the salt marsh and dune habitats at Tillinghast Farm highlight the plant diversity at this site, we found that, when compared with the number of species capable of colonizing these Rhode Island habitats, this present diversity could have been greater. However, although the Tillinghast Farm sand dune does not display the full array of species able to grow in Rhode Island dune habitats, the fact that this created dune displays plant growth beyond the original *Ammophila breviligulata* plantings suggests that future studies may show an even richer plant diversity. We have chosen to creatively represent our research by compiling illustrations, photographs, and cyanotypes in a 50-page book that narrates the declines of salt marshes and herbaria and emphasizes the practical value and visual beauty of these spaces. The main goals of this book are to educate the public about climate change, encourage engagement with nature and natural history, and increase appreciation and understanding of the importance of preserving salt marshes and herbaria. We will distribute 75 copies of our book to Rhode Island colleges and universities, local middle and high schools, and libraries in order to provide free access to the book in hopes of fueling conversation and engagement by informing and exciting people about these topics. We tested the efficacy of this creative vehicle for environmental information by surveying local individuals of various ages and in a range of fields to determine how this book changed their knowledge and perception of salt marshes and herbaria.

## Lattice-Boltzmann Based Simulations of Diffusiophoresis

Jarrett Valenti & Jennifer Pearce

Chemistry & Physics, Roger Williams University, Bristol, RI

We present the results of a series of Lattice-Boltzmann based Brownian Dynamics simulations on diffusiophoresis and the separation of particles within a fluid. A temperature gradient created in a dissolved polymer allows us to separate particles based on their deformability. As shown in previous experiments, the level of deformability of a simulated particle changes how the particle moves within the fluid matrix. We thus conclude, that under the correct circumstances, particles of differing deformability can be separated by the fluid alone. Our simulation was intended to model an oceanic system comprised of three different particles: zooplankton, phytoplankton, and microplastics. The data we collected in our simulations suggest the separation of microplastics from plankton is likely.

## Development and Implementation of the Tracking Portion of a Three-Part System for Improving Grocery Purchase Quality

Carolina de Araujo<sup>1</sup>, Haley Parker<sup>1</sup>, Xintong Guan<sup>2</sup> & Maya Vadiveloo<sup>1</sup>

<sup>1</sup>Nutrition & Food Sciences, University of Rhode Island, Kingston, RI

<sup>2</sup>Marketing, University of Rhode Island, Kingston, RI

**Background:** Diet is a leading modifiable risk factor for 7 of the top 10 causes of death in the US. Current dietary assessment and nutrition intervention methods are imprecise, costly, and rarely overlap with real-time food decisions. Point of sale (POS) grocery purchasing data is a promising source of real-time data that is strongly and positively correlated with dietary patterns. Thus, an increase in grocery purchase quality would likely lead to better health outcomes such as reduced risk of chronic disease. Still, automated methods for classifying and evaluating the quality of POS are needed.

**Objective:** To develop and implement the first part of an automated system for tracking, evaluating and intervening in grocery purchase quality among a subset of RI households enrolling in a randomized controlled cross-over trial.

**Methods:** POS data containing item codes and descriptions of the most commonly purchased items from 01/17 to 01/18 at Belmont Market in Wakefield, Rhode Island were manually sorted because no system links UPC and PLU coding to nutrition composition and serving size. A dietetics student sorted foods based on composition into the 52 Quarterly Food at Home Price Database (QFAHPD) categories; the system will compare percent spending in these categories against recommendations to evaluate grocery purchase quality. Items that could not be classified by their descriptions or that needed to be grouped as low- or high-fat were researched to determine their composition. Foods that could not be easily classified into one of the QFAHPD categories or for which insufficient composition information was available were classified based on decisions made by a team specialized in nutrition.

**Conclusions and future directions:** UPCs and food descriptions alone provide insufficient data for automated sorting of all POS data. Without better machine learning algorithms, manual sorting and decision making by nutrition specialists was required for 55% of items. Still, the velocity and volume of data obtained from customer purchases is promising. The next steps in the larger study will be to evaluate grocery purchase quality scores based on the sorted data and develop an automated intervention platform that will combine these scores with individual-level data and generate targeted incentives and nutrition education content to improve dietary quality.

## Optical Detection of Heavy-Metal Accumulations in Plants Using Single-Walled Carbon Nanotubes

Caroline Rocchio, Brendan Winne, Mitchell Gravely & Daniel Roxbury

Chemical Engineering, University of Rhode Island, Kingston, RI

Single-walled carbon nanotubes (SWCNTs) are effective optical sensors in biological applications due to their intrinsic near-infrared (nIR) emission that is photostable over long-terms and sensitive to local accumulations in heavy-metal ions. As a model pollutant, cadmium is a heavy-metal ion found in groundwater due to wastewater runoff. Even in microgram quantities, cadmium is toxic to living organisms in soil and is especially dangerous to human health due to accumulations in plants and other animals that we eat. In this study, we investigated the ability of DNA-wrapped SWCNTs to penetrate *Brassica oleracea* (kale) seeds, translocate to the stems and leaves of the plants, and detect accumulations of cadmium due to contaminated soil. Results indicated that kale seeds pre-treated with SWCNTs at a concentration of 5 mg/L and 10 mg/L showed higher growth rates compared to lower concentrations or the control. With the use of near-infrared hyperspectral microscopy, we demonstrated that the SWCNTs were uptaken and translocated to the stems and leaves of the plants, and that the SWCNT emission spectra could be observed and deconvolved from the autofluorescence of the plant. Finally, we observed a detectable shift in stem-localized SWCNT emission as a result of cadmium administration to the soil of the kale seeds.

## Surface Enhanced Raman Spectroscopy (SERS) in Continuous Flow Channel Devices to Detect Low Concentrations of Pollutants

Andrew White, Timo Küster & Geoffrey Bothun

Chemical Engineering, University of Rhode Island, Kingston, RI

Excess amounts of nutrients such as nitrate and phosphate in seawater can lead to rapid algal blooms. These blooms can potentially have negative impacts on coastal ecosystems and pose health risks to the public. Quick and accurate detection of these pollutants is important for rapid mitigation plans to counteract and prevent harmful algae blooms. Current sensing devices predominantly utilize UV-Vis spectroscopy; however, these techniques are incapable of detecting nutrients at the low levels and spatiotemporal scales needed to inform predictive responses. Because of this, a more accurate and precise sensing technique is needed, and Surface Enhanced Raman Spectroscopy (SERS) is a promising platform to fulfill this need. SERS is theoretically capable of single molecule detection, so detection of extremely low concentrations of nitrate and phosphate is expected to be possible. However, there are many challenges to this technique that need to be overcome. SERS is sensitive and can be interfered with easily, so application in seawater may prove difficult due to the organic and inorganic molecules present. Our goal is to engineer a flow through sensor to continuously detect and monitor concentrations of these molecules with SERS for an extended period of time.



## Friar vs. Fire: Surveying Rhode Island for the European Fire Ant (*Myrmica rubra*)

Jonathan Eckel & James Waters

Biology, Providence College, Providence, RI

Ants are among the most ecologically dominant groups of animals on the planet and with climates changing on a global scale and rapid pace, many species are experiencing dramatic range shifts. *Myrmica rubra*, the European Fire Ant, is native to northern Europe but has been expanding its range, first across the Atlantic to Nova Scotia and in recent years down to Maine and even the Boston harbor islands. We have been conducting a multi-year survey of the ants of Rhode Island in collaboration with colleagues at Harvard's Museum of Comparative Zoology and the Rhode Island Natural History Survey. In the early stages of this work, we set out to survey specifically for *M. rubra*, focusing on investigating the habitats and locations most likely for it to colonize. Rhode Island has various viable habitats, which have ideal moisture and temperature for these colonies, but surveying for an elusive ant that may or may not be present entails many challenges. Through the hot, humid summer days follow my adventure as a field entomologist and competitive triathlete as I cycle, crawl, and run along the Rhode Island coast.

# **GENETICS**

**LOCATED IN THE SOUTH LOBBY ON THE 1<sup>ST</sup> FLOOR OF THE CENTER FOR  
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## A Genetic Enhancer Screen to Identify Genes Causing Lethality in a *Drosophila melanogaster* Model of Amyotrophic Lateral Sclerosis

Jocelyn Betancur, Helen Magana, Raquel Villot & Geoff Stilwell

Biology, Rhode Island College, Providence, RI

Amyotrophic Lateral Sclerosis (ALS) is a late-onset, dominant neurodegenerative disease. It is characterized by a progressive degeneration of motor neurons, resulting in paralysis and death. Over 170 point mutations in superoxide dismutase 1 (*sod1*) cause ALS; however, the molecular pathways that lead to disease pathogenesis are not well understood. To better understand mechanisms leading to motor neuron degeneration, we conducted a second-site non-complementation genetic screen to identify interacting genes that produce lethal phenotypes in *Drosophila melanogaster*. The *sod1*G85R allele is adult-lethal in homozygous animals, but heterozygotes show a normal lifespan. In a *sod1*G85R heterozygous background, we targeted genes that are expressed at moderate to high levels in the adult nervous system using RNA interference (RNAi) to knock down expression. RNAi transgenes were transcribed in flies throughout development using a Mifepristone-responsive geneswitch-Gal4/UAS-RNAi system. To identify enhancers, *sod1*G85R/+ heterozygotes were crossed with UAS-RNAi lines and the F1 progeny were scored for lethality. Out of ~600 lines that were tested, ~76 potential enhancers were identified. From this screen, a tRNA-methyltransferase was verified as an enhancer. Additional potential enhancers will be discussed.

## Identification of Cancer-Associated Genes Induced by Gallic Acid Using Affymetrix Microarrays

Julia Gambardella, JD Swanson & Heather Axen

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

Gastric cancer accounts for 38.4% of all cancer diagnoses in the United States. Currently available treatments, such as chemotherapy, radiation, and surgery, are associated with adverse side effects and high cost. Nutraceuticals, naturally occurring plant compounds, offer a promising area of research to develop new treatments. One such phenolic compound found in berries, gallic acid (GA), has been shown to selectively target gastric cancer cells while leaving non-cancerous cells unaffected. This study aims to identify gene targets and metabolic pathways affected by gallic acid in an immortal gastric adenocarcinoma cell line AGS using Affymetrix microarrays. AGS cells were treated with 100  $\mu$ M gallic acid for 6, 12, and 24 hours, with each time point replicated in biological duplicates. The microarray contains a total of 48,226 gene transcripts; of those, 1,049 (2.18%) transcripts were differentially expressed. The 6-hour time point showed the greatest change in expression with 891 (89.4% of 1,049) genes differentially expressed. To verify gene expression in key cancer pathways at this time point, four genes displaying significant up- or down-regulation, MMP1, PRKCA, TNFAIP3, and VEGFA, were investigated using quantitative PCR. Microarray analysis showed oncogenes MMP1 and PRKCA were down-regulated by -13.33 and -3.66-fold change, respectively, during the initial 6 hours of exposure to GA, as was tumor suppressor gene TNFAIP3 by -7.99-fold change. The oncogene VEGFA was up-regulated by 3.25-fold change during the initial 6 hours of exposure to GA. Such gene expression patterns suggest that GA exerts its anti-carcinogenic properties by targeting anti-angiogenic pathways. However, qPCR did not show significant expression changes for these four genes suggesting further work is needed to understand the response of these complex pathways to GA. Understanding the mechanisms in which GA exerts its anti-carcinogenic properties can prompt the development of targeted, less invasive, nutraceutical-based treatments.

## The Role of Group-Level Genetic Variation in Fire Ants (*Solenopsis invicta*) to the Fungal Pathogen *Metarhizium anisopliae*

Rebecca Rhein, Erin Stanley & Heather Axen

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

Social organisms living in dense groups face selection pressure to minimize transmission of pathogens. Ants are highly social insects that can serve as a model for human group dynamics of disease transmission due to their dense colonies and social interactions. Group pathogen defense may be behavioral and/or physiological. Behaviorally individuals may groom themselves or nest mates (allogrooming). Physiological responses depend, in part, on alleles present in an individual, and how they are up or down regulated. Group size can affect the efficiency of pathogen transmission. In large groups there are more individuals to which a pathogen can spread, but more members to groom infected individuals, potentially increasing survivorship. In smaller colonies there are fewer individuals to become infected and pass pathogens, but fewer present to offer grooming behaviors associated with social immunity. A second factor associated with transmission is genetic variation within the group. Groups consisting of genetically similar individuals with low genetic variation may be susceptible to pathogens. Groups with genetically unrelated individuals and high variation may be more resistant. We tested group size and group genetic variation on transmission and survivorship in fire ants (*Solenopsis invicta*) exposed to the common soil-dwelling entomopathogenic fungus *Metarhizium anisopliae*. We set up experimental groups that varied in worker number and genetic composition. Groups were either small (15 workers) or medium (55 workers). These groups contained low, medium, or high levels of genetic variation from mixing workers from 1, 2, or 10 different source colonies. A subset of workers from each colony (1 from small, and 5 from medium size groups) was paint marked and directly exposed to either a solution of *M. anisopliae* conidia or a negative control solution. These were then introduced back into their source group. We observed instances of self and allogrooming for 5 minute periods at 0, 15, 20, 30, 45, 60, and 90 minutes post-introduction. One directly exposed and one secondarily exposed group-mate was collected at 90 minutes, 24 and 48 hours post introduction for expression analyses on genes in the fungal immune pathway Toll using qPCR. All dead ants were surfaced sterilized and cultured at optimal fungal growth conditions. Future studies on heterogeneity and population size within ants, will allow us to further analyze social immune systems within human society.

## Assessing the Effects Colony Size and Exposure to the Fungal Pathogen *Metarhizium anisopliae* on Grooming Behavior and Survivorship in the Pavement Ant (*Tetramorium caespitum*)

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Social organisms, such as humans, undergo frequent interactions favoring pathogen transmission, increasing the potential for disease spread among group members. The highly social ants represent an ideal model system in which to study disease dynamics in social groups because they too face a higher selection pressure from infection transmission due to their dense living conditions and frequent interactions with their nestmates. Most ants are also soil dwelling and encounter pathogens, bacteria, and parasites. Social behaviors such as, social grooming (allo-grooming- cleaning of nestmates) and self-grooming can limit the spread of disease through a colony by removing disease causing agents, promoting survivorship of the individual as well as the group. Group size may affect transmission rates. Smaller groups have fewer individuals, limiting exposure potential, but larger groups have more members that may actively engage in allo-grooming, decreasing transmission potential. Here we investigated the effect of group size and efficacy of grooming on transmission of a common soil-dwelling entomopathogenic fungal pathogen (*Metarhizium anisopliae*) in the common pavement ant (*Tetramorium caespitum*). We set up experimental groupings that contained either 15 (small group) or 50 workers (large group). Experimental groups were exposed to fungal spores via introduced colony-mates that were directly treated with a solution of *M. anisopliae* conidia ( $1 \times 10^7$  conidia/mL) or distilled water with 0.05% Triton (negative control). Directly treated individuals (N=1 or 5, small and large groups respectively) were paint marked to distinguish them from naive nest-mates prior to direct exposure. Post introduction of directly exposed individuals the frequency and duration of antennation, self, and allo-grooming were observed for 5 minute periods at 0, 5, 10, 15, 30, 45, 60, and 90 minutes.. Survivorship was recorded for 7 days, and all dead ants were surfaced sterilized and cultured. Exposed individuals in the small groups (Ngroup= 15) performed significantly more self-grooming than did negative control workers. Humans also use behavioral mechanisms to limit pathogen spread future studies on ants will better inform us of potential novel behaviors to restrict transmission.

## Biodosimetry: The Combination of Radiation and Genetics

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Throughout our experiments, we observed the behavior of specific genes in *Drosophila melanogaster*, also known as the common Fruit Fly, when exposed to varying levels of gamma Radiation. The goal of this project was to be able to determine if there are genes in fruit flies that act predictably under different amounts of radiation. This is helpful because if these genes express radiation exposure linearly, they have human analogues that may also express radiation exposure linearly. If this is the case, then in events of high levels of radiation exposure we are able to measure how much radiation an individual has absorbed without a radiation badge.

## Identification of Chromatin Loops in Metazoan HOX Gene Clusters

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Chromatin is structured in a compact arrangement of proteins (i.e. histones) and DNA within the nucleus. These DNA-protein interactions have been shown to form discrete territories called Topologically Associating Domains (TADs) of high chromatin association. In addition to organizing chromatin, the chromatin looping interactions within TADs promote efficient gene regulation. Though many of the general factors associated with loop formation are known, many specific factors have yet been identified.

To better understand how these loops form and regulate gene activity at the chromatin level we used Chromosome Conformation Capture (3C) to map sites of DNA interaction within HOX gene clusters of adult *c. elegans* and zebrafish embryos. Through our initial efforts we have identified several potential contacts within the HOX genes.

Our future work focuses on creating a complete map of chromatin interactions within the HOX clusters with the ultimate goal of identifying important regulatory factors that modulate chromatin organization. Using CRISPR these identified factors will be mutated and assayed for their contribution to chromatin organization. We hypothesize that these mutations will alter HOX transcriptional activity and localized loop formation but not significantly change the structure of the TAD. By understanding the relationship between gene regulation and chromatin looping, we hope to gain insight into the mechanisms of common developmental disorders associated with HOX gene expression.



# MARINE SCIENCE

**LOCATED ALONG THE HALLWAY ON THE 1<sup>ST</sup> FLOOR OF THE CENTER FOR  
BIOTECHNOLOGY & LIFE SCIENCES**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Comparative Analysis of Bacterial Microbiome of *Ulva rigida* and *Ulva compressa* Observed Through the Blooming Season and at Different Locations

Sara Chlastawa & Anna Radovic

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

Green macroalgae, specifically *Ulva compressa* and *Ulva rigida*, are native to Narragansett Bay waters and are known to harm other aquatic organisms due to their proliferating blooms that take over the coastal regions of Rhode Island. This leaves other marine life with limited recourses and oxygen levels needed for survival. Previous research has shown that these harmful algal blooms (HABs) have been toxic to a number of species living in Narragansett Bay waters. It was proposed by Dr. Marta Gomez-Chiarri of the University of Rhode Island's Fisheries Science Department that the toxicity in oyster larvae possibly arises from a symbiotic relationship between the bacteria living on the macroalgae in the Narragansett Bay. The goal of this study is to identify the bacterial species residing on *U.compressa* and *U.rigida* based on the time of the season and the location in order to help understand which bacterial species might be contributing to excessive blooming. The second goal is to test each bacterial species against a probiotic that kills off bacteria leading to diseased oyster larvae in order to check if the probiotic has similar results when treating the bacteria on Ulva that is presumed toxic. Samples of *U.compressa* and *U.rigida* species were collected at three different locations in Warwick (Sandy Point, Oakland beach, and Chepiwanonex) noting whether the samples were intertidal or subtidal and detached (floating) or attached to rocks/shells. The leaves were swabbed and plated on R2A agar plates in order to collect individual colonies for 16S rRNA PCR amplification and Sangar sequencing. Individual colonies were cultured in isolate with and without the probiotic and plated to identify whether the probiotic works against the bacteria found on both *U.compressa* and *U.rigida*. Results from one collection in June has shown that the probiotic decreased the growth of some of the bacteria based on the number of colonies on the control plate (bacteria only) versus the probiotic plate (bacteria and probiotic). Similar results are expected for future collections in July, August, and September. Future plans for this study is to continue the collections till the end of the blooming season in September and to run an illumina experiment to identify all the bacterial species living on *U.compressa* and *U.rigida*.

## Isolation and Characterization of Marine Microorganisms in Narragansett Bay

Erika Lincoln & Anne Reid

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The marine hydrosphere covers over two-thirds of the planet, however only 0.1% of marine microorganisms have been successfully cultivated within a lab. With the vast majority of microorganisms remaining uncultivable, the ocean contains untapped potential for new antibiotics, enzymes, and compounds from these microorganisms. In order to combat this great plate anomaly, novel techniques have emerged to more closely simulate growth conditions experienced by these microorganisms in their natural habitat. The goal of this study was to identify sampling and isolation conditions conducive to the *in situ* growth of marine microorganisms from Narragansett Bay, RI. Raw seawater was collected from facilities at Roger Williams University and the University of Rhode Island Bay Campus. Microbes were collected by water filtration over a 0.2 $\mu$ m pore size filter or centrifugation, then plated on oligotrophic media (Actinomycete isolation agar and R2A agar) under aerobic and anaerobic growth conditions. Filtration produced a greater abundance and diversity of colonies than centrifugation of the equivalent volume of water. In comparing media, Actinomycete isolation agar was found to recover a greater diversity of colonies than R2A agar, as assessed by colony morphology. Fifteen isolates were prepared for polymerase chain reaction (PCR) amplification of the 16S rRNA and ITS regions for identification of bacteria and fungi respectively. The purified PCR products were Sanger sequenced and isolates were identified to the genus level using nucleotide BLAST. Future research will focus on further subculturing to isolate additional colonies as well as comparing aerobic and anaerobic growth. In summary, through the use of filtration and centrifugation of seawater samples and cultivation on low-nutrient media, bacterial and fungal isolates from Narragansett Bay were cultured and characterized. This research will help to establish protocols for the cultivation of microorganisms which will enable monitoring of changes in marine ecosystems geographically, seasonally, and in response to climate change.

## The Half-Life of Thiocyanate in the Marine Fish *Amphiprion ocellaris*

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Despite being illegal, cyanide fishing continues to be used throughout the Indo-Pacific region to capture reef fish for the marine aquarium and live food industries. In order to combat the practice, there must be an efficient and reliable method to detect if cyanide was used to catch the fish. While there have been cyanide tests adopted, none have proven to be robust enough to be used long term as a means of enforcing the enacted laws. One limitation to developing such a test is that little is known on the toxicokinetics of cyanide up take and metabolism in marine fish. Usually, the major metabolic pathway to expel cyanide is the conversion to thiocyanate. This process is fast in mammals, on the order of hours. The metabolite thiocyanate has a much longer half life, on the order of hours to days, allowing for a longer detection period after capture, making it a more viable marker for cyanide exposure. Here, we report the half-life of thiocyanate in *Amphiprion ocellaris* (common clownfish) from two different exposure methods. For the first exposure *A. ocellaris* were exposed to 100 ppm thiocyanate (SCN) for two weeks. After this exposure, the fish were allowed to depurate and plasma samples were collected at time points across 30 days (ongoing). In the second method, *A. ocellaris* were exposed to a solution of 50 ppm cyanide (CN) for either 45 seconds (n=46) or 20 seconds (n= 47). Plasma samples were collected over 30 days post exposure (ongoing). The samples were analyzed for SCN using HPLC with a pre-pegged C30 column and an UV detector. The half lives were determined by fitting the data to a single exponential using Origin 2018. For both exposure methods, plasma SCN concentrations were observed to initially increase, reaching a maximum of 204 ppm at 4 hours after depuration began for SCN exposure. When exposed to 50 ppm cyanide, the maximum SCN concentration was observed at 12 hours post exposure, and was measured to be 2.3 ppm for 45 second exposure and 1.4 ppm for 20 second exposure. From the data collected so far, the half-life of thiocyanate is  $12 \pm 1$  hours for SCN exposure,  $21 \pm 4$  hours for 45 sec exposure to 50 ppm CN and  $9 \pm 3$  hours for 20 sec exposure to 50 ppm CN. The data show a very fast first order decay, but there is likely a second much slower mechanism for depuration as in all cases, the SCN concentrations did not return to zero as observed for the controls, but rather remained near 500 ppb 14 days after exposure/depuration began.

## Investigating Recent Seasonal Shifts in Diatom Community Composition in Narragansett Bay, RI

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Diatoms are microscopic algae that are essential for basic life in Narragansett Bay, RI as they are nutrient cyclers and an important food source for zooplankton and shellfish. To better understand diatom ecology in the Bay, it is crucial to know which diatom species are present, their respective abundances, and possible relationships with environmental factors. To investigate this, water samples were collected at five locations in the upper and lower Bay beginning September 2017. Water was filtered for phytoplankton biomass ( $> 5.0 \mu\text{m}$ ) and DNA was extracted from the collected cells. Diatom community composition will be determined using diatom-specific primers that target the highly variable V4 region of the conserved eukaryotic 18S rRNA gene via high throughput sequencing on an Illumina MiSeq platform. To predict expected diatom species represented by this molecular analysis, corresponding cell counts were analyzed from the URI Graduate School of Oceanography Long-Term Plankton Time Series. Weekly data from the time series was compiled from August 28, 2017 to July 3, 2018 including corresponding temperature, salinity, and tidal patterns. The most abundant diatom genera counted in this time period fell into three groupings. The first grouping consists of *Thalassiosira*, *Skeletonema*, and *Leptocylindrus*. These genera were most abundant ( $> 10^4$  cells per mL counted in total over the time frame) and each had one dramatic increase in abundance during this time period. The next most abundant, *Chaetoceros* exhibited several spikes over this time period with a total abundance of  $5 \times 10^3$  cells. The third most abundant group includes *Guinardia*, *Asterionellopsis*, and *Pseudo-nitzschia* were each over  $10^2$  cells with moderate increases and decreases throughout the time frame. These observed levels of abundance for the dominant diatom genera occurring in temporal patterns may impact nutrient flux, food webs, and even human health in the case of the toxin-producing *Pseudo-nitzschia*. By using a combination of molecular marker data, cell counts, and environmental metadata, we can better understand and contribute to predictions of diatom community composition in Narragansett Bay.

## Identifying the Composition of Apicomplexans in Narragansett Bay

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Parasites play an important role in marine ecosystems and their diversity is generally understudied. Apicomplexans, a group of parasitic protists in the phylum Alveolata, infect a wide variety of animal hosts and are abundant in ecosystems spanning from Polar Regions to Neotropical rainforests. Previous data generated from marine sediments in Antarctica, Naples Bay, and off the coast of Oslo, exhibit high diversity and numbers of Apicomplexans, which contrasts with sediments collected from Indonesia. Abundance and diversity of apicomplexans is unknown for Narragansett Bay, despite the fact that they infect all commercially important species in the Bay. Both sediment and water samples were collected from ten locations across Narragansett Bay and the Matunuck to Charlestown coastline, ranging from surface water to sediment, 8.5 meters below the water surface. Each sample was then filtered using a peristaltic pump, and total DNA was extracted from each filter. The V3 and V9 region of the 18S rRNA gene was amplified from the DNA using PCR, and the total community amplicons were sequenced on the Illumina MiSeq. Here we compare Narragansett Bay to other areas of the world to determine whether apicomplexan abundance and diversity changes in response to geography.

## Determining Algal Composition with Optical Measurements

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Optical tools can be used to determine phytoplankton community composition, allowing for rapid and continuous assessment of changes in composition. A continuously sampling observatory has been deployed at the Graduate School of Oceanography dock to develop a greater understanding of phytoplankton composition in Narragansett Bay. Along with images of individual phytoplankton cells taken by an Imaging FlowCytobot, the observatory also measures absorption, backscattering, and fluorescence. Here we investigate relationships between chlorophyll and phycoerythrin concentrations, phytoplankton composition determined from the Imaging FlowCytobot, and phytoplankton absorption measured weekly. Covariances are then compared to phytoplankton images around the same times as weekly sampling to determine indicators of algal composition in optical parameters.

## Narragansett Bay Monitoring Network Data Analysis

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The study investigated the change in population size and community structure of zooplankton in Narragansett Bay since the nutrient reduction was completed in 2012. In order to determine if there was a change, samples were collected through replicate tows to find the biomass and abundance each week. At this point, the population of zooplankton appears to have decreased, especially the *Acartia* genus. Changes in zooplankton populations can be the result of changes in water quality, temperature, or nutrients. It is important to note that there is only enough data to extrapolate a preliminary trend. As such, to determine the extent of the change in zooplankton population, monitoring of the bay should be continued.



## Perceptions and Uses in and around Narragansett Bay

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The overall purpose of this project is to collect data on recreational fishing, human uses, interactions, and perceptions of the Narragansett Bay. The data collected will help us determine place based value and fishing behavior. In addition to this, we aim to collect data on views and perceptions from underrepresented communities and diverse ethnic groups. Furthermore, this data can help recreational managers better understand the uses and views of fishermen and recreational users. Ultimately, these findings can be utilized to potentially improve and continue to maintain many of the parks and beaches along the bay.

For Project #34 (Perceptions of Recreational Fishermen), we are interviewing local fishermen from four sites in Warwick, RI: Conimicut Point, Rocky Point Park, Passeonkquis Cove, and Salter's Grove. Each site is visited at least once a week following different time patterns (i.e. tides, morning shifts, post-work shifts). At each site we hope to interview at least ten persons in order to obtain diversity in opinion by diversifying the sites in Warwick. By having two Spanish-Speakers on board, we are able to talk to more fishermen who have historically been excluded in such surveys due to the language barrier. In order to accurately collect data from the interview, all interviews are recorded with consent, and are later transcribed. Each interview is designed to run for a minimum of ten minutes with the maximum of 60 minutes. In Project 1 we focus on place meaning.

For Project #18 (Uses In and Around Narragansett Bay), we survey persons visiting over fifteen sites throughout Rhode Island: from Providence to North Kingston to Newport and beyond. Each site is visited a minimum of two times, with a maximum of four times at different times of the day and different days of the week in order to capture accurate representations of the opinions of all people who visit the sites. At each site, we spend around an hour to an hour and a half surveying people at the sites to understand their usage of the parks and beaches in RI. The surveys are designed to run for a minimum of five minutes and a maximum of ten minutes. In Project 2 we focus on connection to the marine environment, perceptions of water quality, access, and amenities.

We plan to group each interview and survey into categories by key phrasing and hope to see trends within the data. We hope to visually present our findings thus far.

## New Technologies for Monitoring the Health of the Narragansett Bay

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The water of Narragansett Bay stratifies in the Summer, which causes the light, warm water on top and cold, salty water to sink to the bottom. It is predicted by model studies that there is a large intrusion of cold salty water into Narragansett Bay from the Rhode Island sound at the mouth of the East Passage. An accurate, cost effective way to test water movement shown by models is to use a Drifter. These Drifters have GPS tracking units that sit at the top of the water to give an hourly location update. By making the PVC pipe 9 meters long this allows the sails to catch the bottom water that is assumed to be flowing into the Narragansett Bay as an intrusion. Our results from the data given by the drifter locations affirmed our hypothesis that there is an influx of cold salty water coming into Narragansett Bay on the bottom section of the stratified water. We expect these results to be further investigated with the data collected from the pump station built at the Castle Hill lighthouse in Newport. This pump station consists of a bundle of tubes run along the seafloor from the Castle Hill lighthouse to a mooring about 120 m out with three vertical pumps to collect samples at three depths in the water column. These pumps work with a bladder inside the pump that will fill with sea water then three of the tubes in the bundle will have compressed air sent from the lighthouse to squeeze all the water sample out of bladder into one of the tubes to send back lighthouse. With the water samples collected from the lighthouse we can measure the amount of nitrogen in the water and determine where in the stratified water is the deep, nutrient rich layer from the Rhode Island sound. With the three depths of the collections we are also able to visualize the layers of water rushing in and out of the mouth of the bay. All of this information will help gain a better understanding of the movements and overall health of the Narragansett Bay.

## Investigating the Plastisphere: The Role of Plastic-Associated Microbes on Microbead Ingestion by the Coral *Astrangia poculata*

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There are currently between 6,350 to 245,000 million metric tons of plastic in the global oceans. An additional 4.8 to 12.7 million metric tons of plastic enters the oceans each year. The macroplastics that enter the ocean often get weathered down into microplastics, which have been found in oceans all across the world, even in the most remote regions and at the deepest depths. Microplastics are consumed by a variety of marine organisms, especially filter feeders and suspension feeders. One such organism, the heterotrophic suspension feeding coral *Astrangia poculata*, is native to Rhode Island. *Astrangia poculata* feeds on zooplankton and other particles in the water column, leaving it vulnerable to incidental microplastics ingestion. It is unknown whether bacterial biofilms on plastics influence ingestion rates and, subsequently, mobility of plastics throughout the food web. This study aims to test if plastics ingestion is influenced by microbes. In controlled feeding trials, *A. poculata* colonies were fed polyethylene microbeads (200 $\mu$ m diameter) biofilmed with laboratory cultures of *Escherichia coli* or incubated in sterile media. Proportion ingestion of beads and retention time of the beads inside the coral were scored from each feeding trial. Proportion ingestion of beads treated with *E. coli* cultures or with sterile LB broth were both significantly higher than that of beads treated with sterile filtered seawater only. Retention time of the beads, however, was not significantly different across treatments. Therefore, ingestion of microplastics by *A. poculata* may be enhanced by elevated nutrient and/or carbon concentrations. Additionally, microbeads were biofilmed in aquarium tanks at Roger Williams University and in seawater at Fort Wetherill State Park (RI) for three weeks. These wild-biofilmed and tank-biofilmed beads were used in suspension feeding assays to test feeding preference of *A. poculata*. Corals were presented equal quantities of biofilmed and non-biofilmed microbeads, and polyps were dissected to score intake of the microbeads. Results of these experiments will further our knowledge of the microbial ecology of microplastics pollution in the coral *A. poculata* and the potential for plastics to vector microbes into organisms and, ultimately, the food web.

## Antimicrobial Activity of Bacteria Isolated from the Coral, *Astrangia poculata*

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*Astrangia poculata* is a temperate coral native to Rhode Island, and its habitat range spans from Florida to Massachusetts. Unlike most tropical corals, this species can withstand dramatic seawater temperature fluctuations. During winter months, when seawater reaches its coldest temperatures, *A. poculata* enters into quiescence, during which the metabolic rates are extremely low. Recent work in the Sharp Lab has demonstrated that there are significant seasonal shifts in the microbiome composition. Notably, during winter quiescence, the microbiome composition mimics that of diseased or disturbed tropical corals. This is thought to reflect a lack of regulation by the coral holobiont, or dysbiosis. In the spring time, the microbiome appears to re-assemble into a specific, core community that resembles healthy tropical corals and includes taxa previously identified as beneficial microbes that protect corals from pathogens. This study tests the hypothesis that there is a shift in the relative proportion of beneficial antibiotic-producing microbes in *A. poculata* mucus across seasons. This is thought to play a role in re-establishing and maintaining the specific core microbiome across different seasons, most notably from winter to spring. *Astrangia poculata* colonies were collected via SCUBA, and mucus was gathered immediately from each colony. Mucus was used as an inoculum to isolate bacteria on 0.1X Marine Agar (2216) during each seasonal time points. Cultures have been isolated and assembled into an archived library. The library of isolates, containing bacteria from *A. poculata* mucus collected in the winter and in the spring, was screened for activity against bacteria isolated from local seawater during the two seasonal time points. To date, 3 of 95 screened isolates from the winter *A. poculata* bacterial library exhibit antibacterial activity (defined by  $\geq 1.0$ mm zone of inhibition) against seawater isolates. Bacteria isolated from *A. poculata* mucus in the spring are currently being screened against seawater isolates. This research identifies mechanisms by which the coral microbiome defends the host against potential pathogens and regulates microbiome composition. Examination of the seasonal shifts in antibiotic production by the *Astrangia* microbiome provides insight into responses of tropical coral microbiomes to changing oceans and temperatures.

## Effect of Seaweed Mats on the Intertidal Invertebrate Community in Narragansett Bay

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When seaweed mats are deposited along the beach the intertidal habitat is altered through the introduction of foreign organisms, change in habitat structure, or even an alteration in habitat chemistry, along with many other factors that have the potential to influence the surrounding environment. The presence of seaweed mats, furthermore, has the potential to reshape the invertebrate community that is living in the sediment. This community is a good indicator for the health of the larger ecosystem and can provide insight on how algal blooms could be affecting coastal ecosystems. In order to determine the impact of seaweed mats on invertebrates living in the sediment, we sampled three locations in and around Greenwich Bay using a sediment grabber, both underneath seaweed mats and in areas where no seaweed mats were present. These samples were then brought to the lab where all invertebrates were isolated and identified to the lowest practical taxonomic unit. Our community analysis at one of the sites (Conimicut Point Park) revealed a potential influence of seaweed mats on the invertebrate community. Underneath seaweed mats, we found a higher abundance of invertebrates in the Phyla Arthropoda and Mollusca; areas with seaweed mats had a mean ( $\pm$ SE) of  $11.33 \pm 8.35$  arthropods and  $39.67 \pm 25.82$  mollusks, while sandy areas were characterized by  $1.66 \pm 0.33$  arthropods and  $5.67 \pm 0.88$  mollusks. Additionally, there was a lower abundance of Phyla Nematoda and Nemertea under seaweed mats with a mean of  $1.33 \pm 1.33$  and  $6.67 \pm 5.23$ , respectively. Sandy areas had a mean of  $20.33 \pm 16.04$  and  $23.00 \pm 17.00$  worms from the Phyla Nematoda and Nemertea, respectively. There was no significant difference observed in the number of worms from the Phylum Annelida between sandy and seaweed mat areas, but within the families of Annelida there was variation between the two treatments. These results show how intertidal invertebrate communities react to the presence of seaweed mats and provide more information on the interactions of organisms in Narragansett Bay. It also provides insight on how invertebrate communities shift in response to a change in their environment.

## Rn-222 Estimates of Groundwater Discharge into Ninigret Pond, Charleston, Rhode Island

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Groundwater is a major source of both fresh waters and dissolved materials, including pollutants, to the coastal zone. Locating the sources of groundwater will shed more light on the sources of pollution. Groundwater is polluted by many different sources such as fertilizers, septic systems, and animal waste. In recent years, municipalities have worked to reduce groundwater nutrient discharge into coastal ponds. To estimate current groundwater associated nutrient contributions, samples were retrieved from Ninigret Pond for measurement of Rn-222, nitrate, temperature and salinity. Rn-222 is used as a unique tracer of groundwater due to its high abundance in underground aquifers, near absence in surface waters, conservative behavior, and short half-life. Samples were collected on 3 separate days and featured both porewater and surface water samples. Porewater samples were typically collected at 10 cm intervals below the sediment water interface down to 50 cm. Porewater profiles were collected in shallow water near Shelter Cove Marina and Ocean House Marina. Surface water was collected from various locations in the pond. Porewater samples contained high levels of radon relative to the surface water samples, with the average porewater sample of Rn-222 valued at 74535 +/- 139000 dpm/100 L and the average surface water sample valued at 43913 +/- 24000 dpm/100 L respectively. Data indicates the deeper the depth of sampling, the higher the amount of radon present in the sample. Nitrate in porewater samples had an average value of 27.3  $\mu\text{M}$  while surface water samples had an average value of 2.7  $\mu\text{M}$ . Temperatures did not vary significantly, with a total range from 19-23°C. Salinity tended to decrease with depth in the profiles, shown by the average porewater value which was found to be 6 and the average surface water value found to be 25. Low salinity values in the sediment suggest that freshwater is being introduced to the pond through underground aquifers. The elevated Rn-222 counts support this inference. Significant levels of nitrate associated with radon found in porewater samples and near zero nitrate concentrations in surface waters suggests the nitrate comes from groundwater discharge and not from the pond itself. The data illustrates that radon levels vary according to depth and location, and are quite patchy in Ninigret Pond.

## Short-Term Temperature Variations Alter Community and Population Structure in the Diatom Genus *Skeletonema*

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Marine phytoplankton are free floating, single celled photosynthetic organisms that are important to the planet. As primary producers they fix approximately 100 million tons of carbon (CO<sub>2</sub>) and produce 50% of the total oxygen we breathe each year. These organisms are subject to currents, which can expose them to varying temperatures and nutrient levels over short periods of time. It is unclear how these short-term environmental changes affect species and population diversity. We utilized molecular techniques to discern whether short periods of temperature fluctuations can drive species composition of a diatom community. Whole seawater samples were collected from Narragansett Bay in March 2017 when the water temperature was 2.6°C and exposed to three temperature treatments over ten days: -0.5, 2.6 and 6°C. Initial data revealed that the diatom genus *Skeletonema* had a strong temperature response, which made it ideal for this population study. Single *Skeletonema* colonies were isolated at experimental onset and post-incubation and identified to the species level with restriction fragment length polymorphism (RFLP) analysis. In total, 300 isolates were analyzed and 96% were identified as *S. marinoi*. Differences in species composition across the three treatments proved to be insignificant (ANOVA,  $p > 0.5$ ) and did not differ from initial species composition (ANOVA,  $p > 0.8$ ) suggesting short term temperature changes do not affect diversity at the species level for *Skeletonema*. Since no variation at the species level was observed we began developing a protocol using microsatellites to see if there are any intraspecific changes across treatments. With this information we will be able to better characterize how short-term temperature fluctuations affect population dynamics and evolution.

## The Effect of Nutrient Concentration on the Growth and Grazing Rates of Microzooplankton

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Single-cell herbivores, microzooplankton, are an essential link in aquatic food webs as they consume 49-77% of daily primary production. Microzooplankton growth and grazing is directly impacted by the availability of phytoplankton, which in turn are dependent upon light and nutrients to grow. However, the effect of varying nutrient concentrations on microzooplankton growth and grazing rates is unknown. This study investigates the impact of nutrient concentrations on the growth and grazing rates of the microzooplankton—*Gyrodinium dominans*, an herbivorous dinoflagellate. Growth and grazing rates of *G. dominans* were examined in three nutrient treatments: filtered seawater (collected from Narragansett Bay; the control), f/2 media (the standard phytoplankton nutrient media), and f/1 media (double the standard nutrient media). Samples were taken in 24-hour intervals over the course of a week and were analyzed by microscopy counting methods. Counts of microzooplankton and phytoplankton were used to calculate growth and grazing rates of *G. dominans*. Initial results of growth and grazing rates in Narragansett Bay water and f/2 media exhibit no significant difference; additional data are currently being evaluated for the double the standard nutrient media, f/1. While it is known that nutrients directly affect phytoplankton populations, the preliminary results of this study indicate that increased nutrient concentrations do not impact microzooplankton growth and grazing rates.



## A Low-Power Mode for Embedded Computing on Oceanographic Moorings and Floats

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Oceanographic mooring and floats often rely on battery power, where size and weight limitations result in a need to optimize power efficiency. Here, I present a solution to markedly improve the longevity of a low-cost data acquisition system (Raspberry Pi Zero) by coupling it to a second system (Arduino Nano) with a deep sleep function that draws 1000x less power than continuous operation of the original system. This solution has been implemented in the construction of a time-lapse camera in a small (>0.5 L) housing that can take a photo every hour for roughly 4 months. The camera will be mounted on the GSO pier alongside a C-AIM supported holographic and stereo camera system to image marine particles in Narragansett Bay.

## Design of C-AIM Instrumentation Buoys

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The goal is to design three instrumentation buoys that will be deployed in two main locations in Narragansett Bay, The Long Time Series (LTS) site east of Wickford Harbor and in Greenwich Bay. The first two buoys will be placed at the LTS location. Buoy 1 will be the biological sensor carrying instruments such as PPS (Phyto-plankton sampler), RAS (remote access sampler), and IFCB (image flow cytobot). Buoy 2 will be the chemical sensor carrying instruments such as SUNA, Hydrocicle P04, Hydrocat EP, and ECO. Buoy 3 will be deployed in Greenwich Bay and this will contain a water sampler instrument. The design of these Buoys with integrated biological and chemical sensors will measure water parameters such as temperature, pH levels, turbidity, etc and also identify chemicals such as nitrogen or phosphate. The purpose of these buoys are to be able to carry these sensors which will collect and communicate data about those chemicals to shore and then to the Narragansett Bay observatory. Moreover it will help scientists come up with ways to respond to potential algae blooms and harmful phytoplankton that may be found in the bay. These is a new opportunity to gather new kinds of scientific information and also sets the foundation for other researchers who may want to test out their sensors in a real environment, the buoys will allow this to be possible.

# **MICROBIOLOGY**

**LOCATED IN ROOM 105 ON THE 1<sup>ST</sup> FLOOR OF AVEDISIAN HALL  
(COLLEGE OF PHARMACY)**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## *Entamoeba histolytica* and *E. dispar* Clone Discrimination and Aggregative Behavioral Analyses

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*Entamoeba* spp. are unicellular protists that have previously shown the ability to recognize kin and discriminate non-kin. The molecules involved in this communication are not yet known. Our laboratory has shown that *E. invadens* IP-1 (Ei-IP1), and *E. invadens* VK1:NS (Ei-VK1:NS), although considered to belong to the same species, form aggregates with members of the same strain (=kin; i.e. Ei-IP1 with Ei-IP1) but not with members of a different strain (=non-kin; Ei-IP1 ≠ Ei-VK1:NS). We were interested in analyzing mobility and aggregative strategies of *E. histolytica* and *E. dispar* using semi-solid agarose plates and a three-chamber apparatus, respectively. Preliminary results on semi-solid agarose plates with fluorescently tagged *Entamoeba* spp. of same and/or different strains allowed us to determine ideal times and distances (in mm) for tracking the motility of *Entamoeba* spp. cell communities. The three-chamber apparatus generated a different setup for similar studies plus the opportunity of identifying potential signaling molecules (e.g. proteins) excreted by the organisms to the milieu. Protists allow us to study cell–cell recognition from ecological and evolutionary perspectives. Modern protistan lineages can be central to studies about the origins and evolution of multicellularity.

## The Role of Flagella in Persistence of *Salmonella enterica* on Red Leaf Lettuce

Emily Jackson & Anne Reid

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*Salmonella enterica* is a Gram-negative bacterium that is the causative agent of Salmonellosis, the leading cause of hospitalizations due to foodborne illnesses in the United States. The objective of this research was to determine the role of flagellar components in attachment, colonization, and survival of *S. enterica* on red leaf lettuce. Flagellar genes *fljB* (flagellin, phase 1), *fliC* (flagellin, phase 2), *flgK* (flagellar hook gene), and *fliB* (flagellin methyltransferase) were targeted for replacement with antibiotic resistance cassettes in *S. enterica* serovars Agona, Javiana, Newport, and Typhimurium using lambda Red homologous recombineering. By deleting the *flgK* gene, *S. enterica* will no longer assemble a flagellar filament. We hypothesize that this will impair the ability of these strains to attach to, colonize, and/or survive on red leaf lettuce. Antibiotic resistance cassettes were successfully amplified for all genes and transformed into *S. enterica* serovars by way of electroporation. Potential mutants were screened by PCR amplification of the target DNA region. To date, deletion mutations in *fljB* have been obtained for all serovars. Phenotypic assays will be performed for mutated strains in order to determine the effect of gene deletion on motility in a standard plate assay. In order to test the effects of these mutations on interactions with red leaf lettuce, wild-type and mutant cells will be spotted onto lettuce and the levels of attachment, colonization, and persistence will be determined. By understanding the role of the flagellum components in plant-bacterium interactions, interventions can be developed to interfere with these interactions and subsequently decrease the frequency of Salmonellosis cases worldwide.

## The Role of Biofilm Components in the Colonization and Persistence of *Salmonella enterica* on Red Leaf Lettuce

Claire Wulfman & Anne Reid

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*Salmonella enterica*, the causative agent of salmonellosis, is a harmful bacterium that is the leading cause of foodborne illnesses in the United States. An understanding of the physical mechanisms which allow this human pathogen to attach to and colonize fresh produce are of critical concern for the food industry. *S. enterica* is able to form biofilms, microbial communities encased in extracellular polymeric substances. The objective of this study was to determine the role of biofilm components in the colonization and persistence of *S. enterica* on red leaf lettuce. We hypothesize that deleting genes contributing to biofilm formation in *S. enterica* will alter the bacterium's ability to adhere to and persist on the surface of red leaf lettuce. Key genes for biofilm formation (*bcsA*, cellulose synthase, and *csgA*, fimbrial subunit) were targeted for chromosomal deletion through the use of lambda Red homologous recombineering. Antibiotic resistance cassettes were amplified with primers designed to introduce 40 nucleotides of DNA from the chromosomal regions flanking the *bcsA* and *csgA* genes. The pKD46 plasmid was introduced into *S. enterica* serovars (Agona, Javiana, Newport, and Typhimurium) via electroporation. Expression of phage genes from this plasmid was induced with arabinose, permitting the production of phage proteins which assist in the homologous recombination of the PCR product onto the chromosome. Mutants were selected by growth on antibiotic-containing media. Putative mutants were confirmed by PCR amplification of the targeted chromosomal region. Biofilm formation in wild-type and mutant strains will be assessed using a standard plate assay to confirm the role of the target genes in the formation of these communities. The ability of these mutants to attach to and persist on red leaf lettuce will also be assessed and compared to the wild-type strains. If the selected biofilm genes are found to contribute to *S. enterica*'s ability to interact with fresh produce, strategies could be designed to disrupt these biofilms in order to remove *S. enterica* from leafy greens before they are sold and consumed.

## Understanding the Role of the O Antigen in Persistence of *Salmonella enterica* on Red Leaf Lettuce

Ryan Senecal

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Salmonellosis affects on average 1.2 million people per year in the United States and is contracted via consumption of fecally-contaminated food such as fruits, vegetables, and poultry. This study focused on understanding the role of the O antigen component of the capsular polysaccharide (CPS) and lipopolysaccharide (LPS) in promoting persistence of *Salmonella enterica* on red leaf lettuce. Lambda Red recombineering was used to create deletion mutants in the *waaB* gene which is responsible for assembling the outer core of the LPS and the *yihO* gene which transports the CPS to the outer cell surface. Antibiotic-resistance cassettes were amplified and tagged with DNA flanking the desired mutation sites. The pKD46 plasmid encoding phage proteins for recombineering was successfully introduced into serovars of *S. enterica* as evidenced by growth on media containing ampicillin. These transformants were then cultured in the presence of arabinose in order to induce expression of the phage proteins and the PCR-amplified antibiotic-resistance cassettes introduced by transformation. Putative mutants were screened by PCR amplification of the targeted chromosomal region. While confirmed mutants have not to date been obtained, efforts to obtain these are ongoing. The expression of CPS and LPS O antigens in the mutants will be assessed by SDS-PAGE analysis of cell lysates followed by silver and Alcian blue staining to visualize polysaccharides. Understanding the molecular mechanisms responsible for *S. enterica* persistence on leafy greens is crucial in order to develop methods for preparing and cleaning these foods so that they can be safely consumed, limiting the number of infected individuals.

## Detection of Dengue NS1 Protein Using Digital ELISA Technology

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Dengue virus (DENV) is a mosquito-borne human pathogen of global medical importance. DENV causes an acute febrile illness that, in some patients, is associated with a life-threatening plasma leakage syndrome, dengue hemorrhagic fever (DHF). Currently, it is estimated that 390 million cases occur each year. These mosquitos thrive in the tropical and sub-tropical regions but as global warming increases the amount of countries affected has the potential to increase. For example, in 2013 there were only a few reported cases in Florida whereas by 2015, 181 cases were reported (WHO.int).

DENV encodes 3 structural proteins (C, prM and E) and 7 non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5). The structural proteins encapsidate the viral genome and can be detected in the blood of an infected patient as part of the viral particle. Conversely, most of the non-structural proteins, which are not part of the viral particle, are undetectable in blood. However, the exception is NS1, which is expressed and secreted out of the cell during viral replication.

Early accurate detection of disease is associated with improved patient outcomes. The advantage of NS1 detection versus other viral proteins is that it can be detected throughout the febrile phase and is more stable in solution than the viral genome. Previous studies have suggested that the levels of NS1 in the blood correlate to severity of disease, suggesting the potential for NS1 to serve as a viral marker for DENV severity.

The goal of this project is to detect NS1 using a digital ELISA assay. Digital ELISAs have shown increased sensitivity in detection of proteins in solution when compared to traditional ELISAs. This would enhance NS1 detection in DENV-infected blood even at relatively low levels of protein, potentially allowing an earlier diagnosis of a DENV infected patient.

The objective of this project is to detect NS1 qualitatively and quantitatively.



## The Search for *Shewanella*: Analyses of Bacteria in Sediment Samples from Oneida Lake

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*Shewanella oneidensis* is a facultative bacterium that can use metals in the place of oxygen when growing under anaerobic conditions. *S. oneidensis* strain MR-1 was isolated in the summer of 1987 from Oneida Lake in New York State. In this project, we evaluated sediment samples collected in 2011 from a cove at the Cornell Biological Field Station located at Shackleton Point for the presence of *S. oneidensis*. Ten sediment samples, which had been stored at 4C for the past seven years, had developed clear stratification, suggesting microbial activity. We extracted sediment cores from each of the ten samples, shook them in saline, then cultured bacteria from each sample. Bacterial colonies that resembled *S. oneidensis* MR-1 were chosen for further analyses. Phenotypic analyses, including Gram staining, will be performed on our colony-purified isolates. The results of these experiments will be used to determine whether *S. oneidensis* is present in our samples.

## Analyses of the Oxidative Stress Hypersensitivity Phenotype of the *Shewanella oneidensis* Hfq Mutant

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Hfq is a bacterial small RNA chaperone that plays an important role in the regulation of gene expression. Small RNA chaperones are responsible for facilitating interactions between messenger RNAs and small regulatory RNAs. We have previously shown that strains of *Shewanella oneidensis*, a dissimilatory metal reducing bacterium, that are lacking Hfq are more sensitive to a variety of stressors. Loss of the RNA chaperone Hfq in *Shewanella oneidensis* results in slow exponential phase growth, a reduced terminal cell density in stationary phase, a loss of colony forming units in extended stationary phase, and a hypersensitivity to hydrogen peroxide and superoxide stress. The hfq mutant is hypersensitive to oxidative stress compared to wild type *S. oneidensis*. Our goal is to investigate the response of *S. oneidensis* to oxidative stress.

To understand the mechanisms by which *S. oneidensis* adapts to oxidative stress, we are analyzing the underlying reasons for hydrogen peroxide hypersensitivity of a mutant strain lacking the hfq gene. We have found that adaptive production of catalase is normal in the hfq mutant, making this an unlikely candidate to explain the mutant's peroxide hypersensitivity. Our current hypothesis is that the hfq mutant is defective in repair of 8-oxo-G oxidative DNA damage. To test this hypothesis, we are analyzing the kinetics of 8-oxo-G repair in both wild type and hfq mutant cells using an 8-oxo-G ELISA assay. If the hfq mutant's peroxide hypersensitivity is due to a deficiency in 8-oxo-G damage repair, then we expect that the hfq mutant accumulates more 8-oxo-G damage than wild type, does not clear 8-oxo-G damage as efficiently as wild type, or both.

## Detection of Dengue Viral Proteins Using Lab On Paper Technology

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Dengue (DENV) is one of the most spread arboviral diseases in the world. DENV has continued to increase its geographic range with nearly half of the world's population at risk of infection. According to World Health Organization (WHO), 390 million infections occur yearly with a death rate near 0.5%.

DENV causes an acute febrile illness that, in some patients, is associated with a life-threatening plasma leakage syndrome termed dengue hemorrhagic fever (DHF).

One of the possible ways to confirm DENV infection is by detecting viral proteins in the blood. DENV envelope (E) protein is part of the viral shell and non-structural 1 (NS1) protein is expressed by infected cells and released into the blood in infected patients. Both of these proteins have been used to detect DENV infections.

Management of patients with fever relies on clinical suspicion and differentiation of febrile illnesses that do not require medical intervention from those that do. Additionally, early accurate diagnosis of disease is associated with improved patient outcomes. Substantial efforts have been made in the development of diagnostic tests for detection of febrile illness at point-of-care for DENV that is relatively inexpensive to use. To this effect, Drs. Anagnostopoulos and Faghri at URI have developed a Lab On Paper technology (LOP), which is a paper-based enzyme-linked immunosorbent assay (ELISA) technology. The LOP to be developed in this project will meet the need for low cost but higher sensitivity rapid diagnostic tests for DENV.

The objective of this project is to 1) optimize conditions and 2) sensitivity for qualitative detection of DENV proteins using LOP technology.

## Development of a $^{13}\text{C}$ Labeling Strategy to Monitor Lipid Upgrading by the Zooplankton *Oxyrrhis marina*

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Lipid molecules are fundamental to many biological systems, including marine ecosystems. These high yield energy sources can be used as biomarkers in assessments of marine food webs. Previous studies on changes in neutral lipid composition in the zooplankton *Oxyrrhis marina* during satiated and starvation conditions showed an accumulation of wax associated fatty alcohols as energy stores, which are mobilized for energy during periods of starvation.

This project focused on the determination of a stable isotope labeling strategy for monitoring *O. marina* lipid metabolism. The baker's yeast *Saccharomyces cerevisiae* was used successfully as an alternative prey for the dinoflagellate. *S. cerevisiae* was labeled using carbon-13 sodium acetate as a carbon source. *S. cerevisiae* was grown to an optical density of 1.0 at 600 nm, corresponding to 10<sup>9</sup> colony forming units, and lyophilized. *O. marina* was fed yeast dissolved in sterile filter seawater and monitored for three days to ensure feeding. The heavy carbon was used to track lipid progression through the organisms. Gas chromatography and FAME analysis were used to determine lipid carbon-13 incorporation by comparing samples grown in the presence and absence of the carbon-13 labeled acetate.

Carbon-13 was successfully incorporated into the *S. cerevisiae* cells with a 53% (C18:1) to 95% (C15:0) rate of incorporation, and initial *O. marina* feedings showed carbon-13 incorporation in lipids. Continuation of the characterization of lipid metabolism in marine ecosystems can lead to a better understanding of trophic upgrading and better establish the method of using lipids as biomarkers in microbial predators.

## Toxicological Significance of Diamide Antibiotics

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New antibiotic development is of increased importance due to the prevalence of antibiotic resistant microorganisms in modern day medicine. Resistance is emerging at a faster rate than the discovery and production of novel antibiotics. In comparison to pesticides which act by poisoning the targeted organism, antibiotics act in a relatively non-toxic manner to eukaryotic hosts. However, antibiotics kill all susceptible bacteria, exhibiting selective toxicity. This study was conducted to examine the toxicity of a prospective antibiotic FGKC, a micromolar bacteriostatic inhibitor of *Bacillus subtilis* and the pathogen *Streptococcus pneumoniae*. *Drosophila melanogaster* serves as a suitable model organism due to their rapid generation time and shared homology with the human genome. Survival and dose response curves were used to determine the relative toxicity of the FGKC diamide. FGKC results were compared to a control/non reactive structurally similar diamide, FGTA, Vancomycin and four pesticides (Deltamethrin, DDT, Azinphos-methyl and Permethrin). Varying dose concentrations (0  $\mu\text{g}$  to 400  $\mu\text{g}$ ) of each compound were prepared by serial dilution. Flies were anesthetized, sexed and a contact bioassay was performed in a 20 mL scintillation vial. Each compound consisted of three trials of experimentation, with each trial involving six male vials and six female trials. The number of flies survived was recorded over a seven day period for each vial. Results indicated that the diamides, FGTA and FGKC did not produce a dose response after 48 hours, thus are comparable for the known antibiotic Vancomycin. Thus, the prospective antibiotic, FGKC, did not result in any toxicity at 10X the MIC, a target concentration of an antibiotic at the infection site when an individual is prescribed an antibiotic. In contrast, pesticide exposure resulted in a dose response after 48 hours. Results also showed differing dose response curves between males and females, with male survival percentage affected more drastically across all tested compounds. Therefore, it can be concluded that the toxicity of FGKC is comparable to FGTA and Vancomycin and different from the host toxicity of the pesticides. Further research testing the capacity of the unknown antibiotics to kill bacteria infecting lung epithelial cells will provide additional insight into the biological efficacy of the antibiotic compounds under study.

## Rapid Visual Quantitative Diagnostic for the Detection of Bacterial Bioburden

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Nearly two million fracture fixation devices are inserted yearly. Infection rates for these surgeries approximate 2% for closed fractures and 30% for open fractures (1). In addition, nearly 26,000 prosthetic joint infections occur annually (2). These infections can extend patient hospital stays, increase costs, and decrease quality of life. The current methods used to diagnose these infections are limited to culturing, PCR, and gram staining, but these methods lack efficiency, and accuracy (3,4). This study assesses a rapid visualization assay using fluorescently conjugated antibodies and Confocal Laser Scanning Microscopy (CLSM) to detect bacterial presence on surgical explants, tissue, and synovial fluid in 30 minutes.

Samples were collected through an IRB approved study at Rhode Island Hospital. Synovial fluid samples were fixed onto slides and stained with a cocktail of serums, while explants and tissue were stained in falcon tubes. Fluorescently conjugated anti-LPS (Dylight 594) and anti-LTA (FITC 488) antibodies were added to the samples to mark bacterial presence, as well as DAPI to identify eukaryotic tissue. Positive controls of *S. aureus* and *A. baumannii* and negative controls of FBS are used to determine antibody quality. Images were quantified through CLSM, analyzed with ImageJ (NIH), and compared to hospital data.

43 synovial fluid and 32 hardware/tissue samples were collected. Our assay results agree with hospital data and gram staining trials in 100% of cases for synovial fluid samples. For hardware and tissue samples, hospital data found that 10 of 32 of samples were infected, but our assay showed that 21 of 32 had significant bacterial presence.

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## Detection of Dengue Viral Infection in Live Cells Using a Color Switch System

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Dengue virus (DENV) is a mosquito-borne human pathogen of global medical importance. DENV is predominately found in tropical and subtropical regions around the world and infects 390 million annually. DENV causes an acute febrile illness that, in some patients, is associated with a life-threatening plasma leakage syndrome, dengue hemorrhagic fever (DHF). Given the limitations of clinical studies and existing animal models, cell culture models remain an important approach to studying DENV infection and host responses. Live-cell analysis of virus-infected cells by fluorescence microscopy represents a promising approach to investigate virus-cell interactions. Current methods to detect DENV infected cells use antibody staining, which requires permeabilization and fixation of cells. These methods are inherently destructive and are not amenable to intact cell imaging or sorting. We are developing a cell line that will allow identification of DENV infected cells by fluorescence microscopy or flow cytometry. The objectives of this project are to 1) create a stable cell line that expresses green fluorescent protein (GFP) only when infected and 2) quantitatively identify DENV infected cells by microscopy. The DENV Color Switch Reporter is a promising strategy for identifying live DENV-infected cells by fluorescence microscopy, with potential applications for detection of virus and for studies of virus-cell interactions.

## Comparative Genomics, Prey Range and Plaque Phenotype Variation of *Bdellovibrio* Isolated from a Drain

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Predatory bacteria have been isolated from a range of environments; however, little is known about their association with the built environment. To survey *Bdellovibrio* on manmade structures, we collected swab samples from surfaces at Providence College, extracted metagenomic DNA and performed *Bdellovibrio*-specific PCR. We detected positive hits for multiple independent swabs of a janitorial closet drain. By combining drain swab samples with different prey, we obtained seven predatory bacteria isolates, six using *Raoultella* as prey and one using *E. coli* ML35 as prey. All seven isolates were identical across 1,116 bp of the 16S rRNA gene, which showed 95% identity to *Bdellovibrio bacteriovorus* HD100. To compare the isolates' genomes, we generated MiSeq data for all seven. *De novo* assembly yielded 2-11 contigs per isolate. Using one isolate as a reference, we performed pairwise alignments and found >99% identity across the full length of contigs. This suggests that the isolates are extremely similar across their entire genomes. To further explore this, we assembled a complete genome of one isolate with PacBio data, and we are aligning the MiSeq data to this reference genome. To investigate prey range, we challenged seven of the isolates with eight Gram-negative prey. Each isolate attacked the same two prey strains. This suggests that these *Bdellovibrio* may be prey specialists. To test this, we are isolating bacteria from the drain for further prey range assays. In addition, we are investigating the plaque phenotypes of these isolates, which appear to vary in size, shape and appearance.



## Investigating Variation in Predation via Phenotypic and Genomic Comparisons of *Bdellovibrio* from Bioswale Soil and Type Strain HD100

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Predatory bacteria may play an important role in shaping microbial communities. To investigate variation in phenotype and genotype among predatory bacteria, we isolated *Bdellovibrio* sp. NC01 from a bioswale, an artificial landscape feature that collects and filters stormwater runoff.

We assayed predation efficiency and prey range in comparison to *Bdellovibrio bacteriovorus* type strain HD100. For predation efficiency, we quantified cfu/ml of viable *E. coli* ML35 over 72 hours in the presence of either NC01 or HD100. At 24 hours, HD100 eliminated almost all viable *E. coli*. By contrast, NC01 reduced but did not eliminate viable *E. coli*, and at 48 hours, viable *E. coli* increased even though active NC01 were present. We did not observe *E. coli* population recovery in lysates with HD100. We are testing predation efficiency on a different prey species, a pseudomonas species, to determine if this trend is specific to ML35, or if it is observed in other prey strains that both HD100 and NC01 attack.

For prey range, we tested NC01 and HD100 against eight prey strains. NC01 formed plaques on five prey strains, whereas HD100 formed plaques on all eight. NC01 formed plaques on *E. coli* ML35 but not a different *E. coli* strain, showing that variation within a prey species impacts predation. To further investigate this, we are sequencing both *E. coli* strains and comparing them to catalog genome-wide differences.

To explore variation in predatory bacteria genotype, we are comparing the NC01 genome to HD100 and other available *Bdellovibrio*. We hope to identify genotypic differences that may be useful targets for further study to understand variation in predation phenotype in *Bdellovibrio*.

## Determining Essential Genes of *Haemophilus parainfluenzae*

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Human supragingival plaque, covering the tooth surface above the gumline, is a stable, multispecies community where many species reproducibly live in direct proximity to each other. Little is known about the interactions between these species, both in healthy plaque and in patients with oral disease. Characterizing these interactions will reveal why certain species are found together and how different interactions may prevent or enable the onset of oral diseases. Healthy plaque communities often harbor opportunistic pathogens, typically in very small numbers or in an attenuated state. Interestingly, *Haemophilus parainfluenzae* is one of the most abundant species found in healthy plaque, but it is also an opportunistic pathogen and can enter the bloodstream and cause endocarditis. *H. parainfluenzae* is found in direct contact with various *Streptococcus* species in healthy plaque. Based on coculture data, we determined that *H. parainfluenzae* is killed by *Streptococcus mitis*, but it is not killed by *S. cristatus* or *S. gordonii*, other *Streptococcus* species found within the same environment. We are creating a saturating mariner transposon library in *H. parainfluenzae*, insertionally inactivating every gene. Using TnSeq, which relies on growth and Illumina high-throughput sequencing of this library, we will identify not only essential genes for *H. parainfluenzae*, but will reveal essential genes and conditionally essential genes in the presence of other oral species in coculture growth experiments. With these methods, we will determine the genes that *H. parainfluenzae* needs to compete and survive in its healthy environment.

## Protistan Ecology of Narragansett Bay Benthic Habitats

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Many anaerobic protists host methanogenic symbionts that contribute to nutrient cycling in aquatic ecosystems. Among these are the ciliate classes Plagiopylea and Armophorea. In order to understand the distribution and relative abundance of such protists, as well as to survey the general biodiversity of the protistan community in Narragansett Bay, sediment cores were collected from three locations (Providence River Estuary, Wickford Harbor, and Mid-Bay). Triplicate cores from each site were subsampled into centimeter thick horizons to a maximum depth of six cm. DNA was extracted from the sediment of the top two horizons from all cores, as well as the additional four horizons from the Wickford Harbor cores. The DNA was then amplified using two sets of 18S rRNA gene primers, a universal primer set and a Stramenopile-Alveolata-Rhizaria (SAR) specific primer set. Resulting 18S rRNA gene amplicons were sequenced with Illumina MiSeq. Results will be used to examine the diversity and relative abundance of protists in the benthic habitats of Narragansett Bay according to location and depth.

# **MOLECULAR BIOLOGY**

**LOCATED IN ROOM 240 ON THE 2<sup>ND</sup> FLOOR OF AVEDISIAN HALL  
(COLLEGE OF PHARMACY)**

**ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM  
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## High-Expression and Kinetic Analysis of a Truncated ALDH Domain from the Bifunctional *Entamoeba histolytica* Alcohol-Aldehyde Dehydrogenase 2 (EhADH2)

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*Entamoeba histolytica* is a protist that is the causative agent of the intestinal disease amebiasis. This organism is an amitochondriate and uses a fermentative pathway to process glucose to ethanol, the last two steps are catalyzed by the essential enzyme *E. histolytica* alcohol dehydrogenase 2 (EhADH2). This enzyme contains two domains: an aldehyde dehydrogenase (ALDH), and an alcohol dehydrogenase (ADH). The ALDH portion is located the C-terminus while the ADH is located at the N-terminus. EhADH2 is required for *E. histolytica* trophozoite growth and survival. The ALDH domain size is ~51 kDa, and its function is dependent on the stability of the ALDH domain. A truncated form of EhADH2 was generated by inserting the nucleotide sequence that encodes for amino acids 1-459 in the high expression vector pETRP1B-Nhe1. The purified enzyme will be analyzed kinetically and its crystal structure examined. If successful, this would be the first member of the bifunctional ADHE family with an elucidated structure.

## Inhibition of the Bifunctional *Entamoeba histolytica* Alcohol-Aldehyde Dehydrogenase 2 (EhADH2) by Iron Chelation

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*Entamoeba histolytica* is a unicellular eukaryote that is the causative agent for amebiasis in humans. There are ~100,000 fatalities annually, distributed mostly in developing countries. Cyst-contaminated food and water disproportionately present in conflict zones, lack of resources, clean water, sanitation, and limited access to education and health contribute to the rate of disease worldwide. The current treatment is metronidazole which is effective but has toxic and neurological side effects. The bifunctional alcohol-aldehyde dehydrogenase (EhADH2) has been suggested as a target for anti-amebic inhibitors, because it is essential for *E. histolytica* trophozoite growth via glucose fermentation. *Entamoeba* spp use EhADH2 to convert anaerobically acetyl-CoA to acetaldehyde and acetaldehyde to ethanol. Substrate and cofactor (NADH, Fe) binding sites could be targeted for inhibition. The depletion of Fe has previously been demonstrated to disrupt the pathway for energy derivation. Deferasirox is an Fe chelating agent that decreases *E. histolytica* trophozoite growth and survival *in vitro*. The purification, and kinetic analysis of the deferasirox has shown significant reduction in the activity of EhADH2 in the range of 5-10.25 $\mu$ M. Establishing the mechanism of action of for anti-amebic inhibitors through restriction of EhADH2 activity may provide an alternative treatment to current drugs like metronidazole which have serious side effects.

## Structural Characterization of Stress Response Proteins in Gammaproteobacteria

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In the wild, bacteria encounter a fluctuating and stressful environment, including nutrition deprivation, changes in pH, temperature, or osmolarity. To adapt to these changes, bacteria have developed a variety of stress responses. In Gammaproteobacteria, the general stress response is orchestrated by a single transcription factor called RpoS. RpoS is a promoter specificity subunit of RNAP, which accumulates in the cell under stress conditions to initiate transcription of genes crucial for adaptation and cell survival. Under conditions of active growth, RpoS levels in the cell are barely detectable due to its efficient degradation by the ClpXP protease, which requires the use of an adaptor and response regulator called RssB. In the stationary phase or upon stress, RssB-mediated degradation is inhibited by anti-adaptors IraP, IraD, and IraM, which are induced by different stress signals. Notably, these are thought to use different mechanisms for RssB inhibition, which remain poorly understood due to lack of structural information. We thus set out to elucidate the structure of these proteins and their complexes using X-ray crystallography. We have overexpressed and purified these proteins and their complexes, enabling crystallization screening. Future structural analyses will give us insight into the mechanisms underlying recognition of RssB by RpoS and anti-adaptors. It will provide a framework for interpretation of decades of biochemical and genetic work on RpoS regulation, and will inform future studies aimed at understanding the role of RpoS in bacterial physiology, including the formation and maturation of biofilms, which underlie 80% of all infections.

## Impact of Perinatal Perfluorooctanesulfonic Acid (PFOS) Exposure on Placental Transporter Expression in C57BL/6 Mice

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Per- and Polyfluoroalkyl Substances (PFAS) are a group of man-made bioaccumulative environmental toxicants. PFAS can be found in drinking water, cookware, teflon, fire-fighting films and many household products, and are a potential health hazard. Screening of human serum samples in the USA revealed that perfluorooctanesulfonic acid (PFOS) is present in over 98% of the population. Evidence suggests that PFOS can induce maternal developmental toxicity in humans and in rodents via maternal exposure and is detected in umbilical cord serum and in breast-milk. PFOS exposure is associated with low infant birth weight in humans. In utero rodent PFOS exposure has been shown to cause a dose-dependent reduction in birth weight and developmental abnormalities such as cleft palate, cardiac and lung defects. Although an interim organ, the placenta plays a vital role in fetal development and health. The placenta mediates nutrient uptake, waste elimination, gas exchange via the mother's blood supply, hormone production and development of the immune system for fetal protection during pregnancy. It is well accepted that fetal exposure to xenobiotics can be modulated through the proper function of transporters. The overarching hypothesis of the work is that PFOS exposure impacts placental health and function. The aim of this summer internship project was to evaluate whether PFOS exposure during the perinatal period alters placental transporter expression *in vivo*. At 10 weeks of age, C57BL/6 mice were placed on a standard chow diet. Upon vaginal plug detection at gestational day 1 (GD1) mice dams were assigned to one of the following blinded experimental diets and fed ad libitum: 1) standard chow, 2) 0.0003% PFOS (w/w) or 3) 0.003% PFOS. Dams were euthanized at GD17. Placentas were collected at necropsy and stored at -80°C until extraction. Quantification of relative gene expression by qPCR illustrated that the expression of ABCC2 and ABCC6 efflux transporters were changed. Notably, the efflux transporter, ABCC2 was induced 18 fold by 0.0003% PFOS and 28 fold by 0.003% PFOS. The relative abundance of OAT 1-3, OCT3 and BCRP was similar in placentas from control and treated dams. In conclusion, gestation exposure to PFOS alters placental transporter expression of ABCC2 and ABCC6 in C57BL/6 dams.



## Expression of Long Noncoding RNA Gastric Cancer Markers in the Immortalized Cancer Cell Line MKN28 Following Gallic Acid Treatment

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Gastric cancer is currently the fourth most common cancer in the world and treatments for it are nonspecific and invasive. Due to prevalent and harmful side effects, there is an urgent need to find alternative treatments and a more specific biomarker. Nutraceuticals providing additional health and medicinal benefits offer a potential avenue of investigation for such treatments and mounting evidence has shown that long noncoding RNA's are a promising biomarker for gastric cancer. Long noncoding RNA's (lncRNA's) are a large class of nonprotein-coding transcripts that are more than 200 nucleotides in length. Although they lack protein-coding potential, lncRNA's play critical regulatory roles in a large number of biological processes and have been reported to be abnormally expressed in gastric cancer making them reliable markers of cancer progression. Recent literature has identified five signature lncRNA's associated with the poor prognosis of gastric cancer. In order to test and verify the validity of nutraceuticals as a therapeutic treatment for gastric cancer, the expression of these five signature lncRNA's were treated with varying doses of gallic acid, a phenolic compound shown to have anti-proliferic effects specific to cancer cells. Two gastric cancer cell lines with dissimilar malignant differentiation levels, MKN28 and AGS, were obtained, grown in culture until 80% confluent, and treated with 0, 60, and 100 $\mu$ M doses of gallic acid from blackberries at 0, 18, and 48 hours after serum starvation for 48 hours. RNA was extracted from these cells, converted to cDNA, and primers were designed in order to evaluate the expression levels of the five lncRNA's compared to the housekeeping gene GAPDH using qPCR. It was expected that lncRNA expression would decrease with successful treatment of gallic acid and that expression would be lower in the MKN28 cell line due to its lower metastatic potential.

## Functional Analysis of the Bacterial Cell Division ATPase ZapE

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When bacterial cells divide, a large protein structure called the Z-ring assembles at the division site. The major protein in the Z-ring is FtsZ, a tubulin homolog that hydrolyzes GTP and assembles into polymers to establish the Z-ring. In *Escherichia coli*, many cell division proteins interact with FtsZ and direct Z-ring assembly, while others may modulate constriction or direct cell wall insertion and remodeling. Several accessory proteins that interact with FtsZ are called Z-ring associated proteins (ZAPs). The Zaps (ZapA, ZapB, ZapC, ZapD, and ZapE) are recruited to the division site and may influence Z-ring assembly or stability. ZapE was recently identified to be an ATPase that accumulates during late constriction in *E. coli* and is important for bacterial growth under low-oxygen conditions and high temperatures (Marteyn, et al., 2014). *In vitro* ZapE destabilizes FtsZ polymers suggesting that it may promote Z-ring disassembly *in vivo* (Marteyn, et al., 2014). To evaluate ZapE function and interactions we cloned ZapE into a high-copy inducible expression vector and purified ZapE by metal affinity chromatography. We constructed and purified ZapE with a hexahistidine tag at the N-terminus and, alternatively at C-terminus. We determined that C-terminal histidine tagged ZapE (ZapE-H6) hydrolyzes ATP with an average rate of 0.15 pmol Pi/min/pmol ZapE-H6 under optimized buffer conditions. ZapE contains a putative Walker A (GGVGRGK84T) and B motif involved in ATP coordination and hydrolysis. Site-directed mutagenesis was performed on ZapE-H6 Walker A binding site Lys84 to substitute with Ala. ZapE(K84A)-H6 was tested for ATP hydrolysis under optimal ZapE-H6 hydrolysis conditions. To determine if FtsZ modifies the activity of ZapE, we measured ZapE ATP hydrolysis in the presence of FtsZ, with and without GTP to promote FtsZ polymerization. These studies will help us to further understand the functional role and biochemical activity of ZapE during bacterial cell division.

## Prevalence of *Cardiosporidium cionae* in Rhode Island Marine Tunicates

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Apicomplexa are a large phylum of almost exclusively obligate parasitic protists that infect a large range of animal hosts, including as the causative agent of malaria in humans. In Narragansett Bay, not much is known about the mechanisms of seasonal incidence and transmission of the apicomplexan, *Cardiosporidium cionae*. This species infects several genera of “sea-squirts” including *Ciona intestinalis*, which is invasive in RI and has a higher tolerance for poor water conditions than native species. This advantage makes *C. intestinalis* an ideal candidate for infection prevalence studies due to its abundance in southern Rhode Island. Molecular techniques, including DNA extraction, PCR, and gel electrophoresis, were used to determine the presence of *Cardiosporidium* in *C. intestinalis* samples collected weekly over the summer season. Infection rates of *Cardiosporidium* climb in *C. intestinalis* during the months of May to July, as the water warms. This particular prevalence curve suggests parasite transmission via the water column from live animals, unlike sister taxa *Nephromyces*, which is only able to spread after the death of its host. Dimensions of both whole tunicates and pericardial sacs were also recorded to give an estimation of volume and compared against infection prevalence to explore a potential impact on infection, or vice versa. The data collected thus far suggests a higher prevalence of *C. cionae* as the summer continues, with a possible association between pericardial size and infection prevalence. This will be further explored through statistical analysis.

## Target Identification of Diamide Inhibitors on *Streptococcus pneumoniae*

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Previous testing has shown selective inhibition of *Streptococcus pneumoniae* growth by various diamides synthesized in our lab. Previously it was believed that LytB, a critical autolytic enzyme found in *S. pneumoniae*, was the target. This assumption was based on previous testing that demonstrated inhibition by diamides on homologs of LytB in other organisms. After exhaustive genetic screening and *in vitro* testing, further analysis was required to find a new promising target for the diamides. Using BLAST searches through the NCBI database to identify homologs of *B. subtilis* LytG, the autolytic enzyme identified as the target of our diamides in *B. subtilis*, a number of promising targets were identified and compared to genetic screening results. LytA and LytC, the other two main autolytic enzymes of *S. pneumoniae*, as well as cbpD, a choline binding protein believed to be essential to autolysis, are the current focus. CbpD is an intriguing target that could explain the observed results from the genetic screen, as it has been shown to be essential for activity of LytA and LytC in *S. pneumoniae*. CbpD was cloned from *S. pneumoniae* and ligated into the pET28 vector to generate a N-terminal His-tagged construct. Current work has focused on establishing *in vitro* conditions for measuring enzymatic activity and inhibition. Turbidimetric analysis was performed to measure enzymatic activity by solubilizing isolated peptidoglycan, LytA's substrate, and treating it with both only enzyme as well as enzyme and inhibitor. The amount of light passing through the sample was then measured to assess the degradation of peptidoglycan. Currently, mature and immature peptidoglycan are being assessed as viable substrates.

## Small Molecule Inhibits the Translesion Synthesis of Cisplatin in Mammalian Cells

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Chemotherapy is generally the first line of defense when treating most cancers. However, long-term success rates are decreased due to the eventual accumulation of chemo-resistance in cancer cells. Chemo-resistance is commonly coupled with translesion synthesis (TLS), which promotes a tolerance of DNA damage. Translesion synthesis (TLS) is carried out by TLS polymerases that replicate past DNA lesions in a low fidelity manner, introduce mistakes, and promote the generation of tumors and disease (Yamanaka et al. 2017). REV1 is a protein encoded in humans by the *REV1* gene and behaves as a scaffold protein in TLS by engaging in protein-protein interactions with Pol  $\zeta$  and the REV1-interacting region (RIR) polymerases- POL  $\eta$ , POL  $\kappa$  and POL  $\iota$  (Zhu et al. 2003). The compound JH has shown to inhibit the capacity of TLS to bypass a cisplatin adduct. This study focuses on the effects of JH in the TLS processes on replication bypass and mutagenesis of cisplatin. A TLS assay was performed to determine the activity of TLS on a 1,2-cisplatin containing gapped plasmid DNA. The bypass efficiency and mutation pattern of cisplatin is analyzed by high pressure liquid chromatography coupled with mass spectrometry. The JH-treated bypass efficiency was statistically significantly lower than the DMSO-treated one. The study observed the expected mutation patterns (G>T and G>A) in the HEK293 cells. However, any mutation for the non-lesion containing plasmid were not observed. Overall, JH compound inhibits the TLS capacity for gap filling over the cisplatin adduct in both HEK293 and HT1080 cells.

## Variants Within the Fingers Domain of Human DNA Polymerase $\theta$ Display Altered Polymerase Activity

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Human DNA Polymerase  $\theta$  is a 290kD protein belonging to the A family of DNA polymerases. Like many DNA polymerases, Pol  $\theta$  has a thumb domain which binds DNA, a palm domain which catalyzes the formation of the phosphodiester bond, and a fingers domain which binds the incoming dNTP. Pol  $\theta$ , unlike other A family DNA polymerases though, appears to possess low fidelity; previous studies suggest it plays a role in DNA repair pathways such as microhomology-mediated end joining (MMEJ). Pol  $\theta$  also displays translesion synthesis properties; previous studies indicate an ability to bypass thymidine glycol adducts and abasic sites. Using the National Cancer Institute cBioPortal and the Tissue Resource Core of the Yale SPORE in Skin Cancer, several variants, within critical domains of Pol  $\theta$ , were identified in melanoma patients. Two of these variants, E2406K and N2424I, reside in the fingers domain and were expressed and purified from *E. coli*. Various characteristics of DNA polymerase activity were then assessed. Preliminary data suggests weakened dNTP binding activity which could lead to genomic instability suggesting a mechanism for melanoma.

## Melanoma-Derived Translesion Bypass Associated Mutant of DNA Polymerase $\theta$ Exhibits Low Binding Affinity to Damaged DNA

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DNA damage can be caused by a variety of factors including, but not limited to ultraviolet radiation, which can result in bulky adducts that can be catastrophic to DNA integrity. These adducts can be repaired through translesion bypass, an alternative DNA repair pathway that is mediated by specialized enzymes, such as Y-family polymerases, and more interestingly Human DNA Polymerase theta (Pol  $\theta$ , POLQ). Pol  $\theta$  is a unique error-prone, A-family DNA polymerase, shown to have translesion bypass activity. The DNA binding domain of Pol  $\theta$  (the thumb domain) tightly interacts with primer backbone, which allows it to bypass damaged lesions and continue DNA extension. Yale SPORE in Skin Cancer center identified a specific mutation of POLQ, T2161I, located in the DNA binding domain. This melanoma-derived mutation was generated by site-directed mutagenesis, purified, and assayed to elucidate its impact on DNA translesion bypass repair. In this research, the binding affinity of T2161I displayed less affinity to damaged DNA when compared to wild-type, thus affecting translesion bypass repair, which can be deleterious to genomic stability.

## Melanoma Derived Mutations of Human DNA Polymerase Theta Exhibit Slow Polymerase Activity

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Melanoma has the highest mortality rate out of all skin cancers. Melanoma is caused by DNA damage in melanocytes through environmental factors such as UV radiation from the sun. The human body has mechanisms to repair such damage through specialized repair enzymes, including DNA polymerase theta (POL $\theta$ , POLQ). POL $\theta$  is an A family polymerase implicated in double strand break repair. DNA polymerases are generally comprised of three functional domains: the fingers, thumb, and palm. The fingers and thumb domains are responsible for binding to dNTP and DNA, respectively. The palm domain contains the DNA polymerase active site, which forms the phosphodiester bond required for extending DNA. The Yale SPORE in Skin Cancer center has identified two POLQ palm domain mutations, V2551D and L2538R, expressed in melanoma tumors. Our study focuses on kinetically characterizing the biochemical pathways of these variants by nucleotide incorporation and binding affinity assays. We hypothesize the cancer associated mutations will affect POL $\theta$ 's ability to extend DNA, thereby disturbing DNA repair pathways. Preliminary data reveals slow DNA polymerase activity compared to the wild-type (WT), suggesting the variants may stalling DNA repair, which can cause genomic instability and be potentially linked to driving melanoma



## Monitoring the Movements of DNA Polymerase Theta Through FRET Analysis

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Cells use specialized enzymes called DNA polymerases to maintain genomic integrity. The tertiary structure of these enzymes exhibits a right-hand form, which is comprised of three main domains: a DNA-binding thumb domain, a nucleotide-binding fingers domain, and an active site in the palm domain. During polymerization, DNA polymerase binds to DNA, “reads” the template, selects and binds to the correct nucleotide. The fidelity of a DNA polymerase is defined by how well it reads the template to correctly insert the complementary nucleotide. The binding of this substrate causes a conformational change in the fingers domain, which results in the alignment of the dNTP to the DNA in the active site. Once the nucleotide is inserted, the extended product is released and the enzyme returns to its original conformation. DNA polymerase Theta is a low fidelity enzyme involved in DNA repair. Even though it is error-prone, little is known about the selection process it uses during nucleotide incorporation. Using Fluorescence Resonance Energy Transfer (FRET), this study allows us to gain insight into this mechanism and how the conformational dynamics of the fingers domain of Pol  $\theta$  affect its fidelity during DNA repair. To internally fluoresce the fingers domain of Pol  $\theta$ , a site-specific labeling protocol was optimized using 5-FAM cadaverine. A Q-tag sequence (GQQQLG) was generated via site-directed mutagenesis in the fingers domain of Pol  $\theta$ . Modified Pol  $\theta$  was expressed in *E.coli* and purified using affinity chromatography. Labeling of Pol  $\theta$  was done concurrently using transglutaminase to catalyze the addition of the FAM dye to the Q-tag sequence. Preliminary results suggest that the generated labeling does not affect the overall activity of the enzyme compared to WT, and Pol  $\theta$  adopts a closed finger conformation when incorporating the correct nucleotide while remaining in an open conformation in the presence of incorrect nucleotide.

## NAMPT Mutant Purification and Activity Analysis

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NAD<sup>+</sup> (nicotinamide adenine dinucleotide) is a crucial biomolecule for life. NAD<sup>+</sup> is a widely known cofactor that has a role in many biological pathways, including as a redox cofactor in metabolism and its consumption in transcription regulation and DNA repair. Thus NAD<sup>+</sup> must be tightly regulated. The importance of NAD<sup>+</sup> is reflected in the fact that there are redundant pathways for regulating NAD<sup>+</sup> concentration. In vertebrates the predominant pathway is the NAD<sup>+</sup> salvage pathway, which provides an efficient two step resynthesis of NAD<sup>+</sup>. Knowledge of NAD<sup>+</sup> regulation is clinically relevant because modulating its concentration in the body may provide new information on metabolic diseases such as diabetes and obesity. In addition NAD<sup>+</sup> regulation has been linked to atherosclerosis, cancer, and rheumatoid arthritis.

The rate limiting enzyme of the salvage pathway, NAMPT (nicotinamide phosphoribosyltransferase), is a 55 kDa homodimer with two active sites at the interface. In addition to NAMPT's transferase activity it also demonstrates cytokine and growth factor properties. Previous work in our lab has determined a potential allosteric binding site for small polyphenic compounds which have shown to increase the activity of NAMPT. Specific focus has been placed on the T391A mutant that is thought to interact directly with a polyphenic modulator. Results from the nickel affinity chromatography and size exclusion chromatography purifications as well as results from the established fluorescence activity assay for the T391A mutant will be presented. Understanding NAMPT's regulation of NAD<sup>+</sup> could provide insights into drug development for an array of diseases.

## Interaction of Polyphenolic Compounds on NAMPT Single Point-Mutations

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NAD<sup>+</sup>/NADH (Nicotinamide adenine dinucleotide) is a coenzyme that is essential to cell survival as a redox cofactor in biochemical processes. NAD<sup>+</sup> is also a substrate for enzymes such as Sirtuins and PARPs (Poly ADP-polymerase) that aid in cell repair, replication and transcription. Dysregulation of NAD<sup>+</sup> levels have been linked to various diseases such as cancer, Alzheimer's, obesity, and many others. For example, in cancer cells NAD<sup>+</sup> levels increase to support cell damage repair, replication and growth. NAD<sup>+</sup> biosynthesis is tightly regulated by two main pathways, where in humans the 2-step NAD<sup>+</sup> salvage pathway predominates.

In the first step, NAM is converted to nicotinamide mononucleotide (NMN) by the enzyme nicotinamide phosphoribosyl transferase (NAMPT). In the second step, NAD<sup>+</sup> is regenerated from NMN using ATP and Nicotinamide mononucleotide adenylyltransferase (NMNAT). In this pathway, NAMPT is the rate-limiting step, and the focus of this work. There are two known active sites that are generated in this stable homodimer of NAMPT. Previous work in our lab has identified polyphenolic compounds that increase NAMPT activity levels, suggesting a new allosteric regulation domain. To confirm the binding site for these compounds, three single-point mutations of NAMPT were created, expressed and purified. An activity assay based on the fluorescent derivative of the NMN product was used to compare each mutation to the endogenous form of NAMPT. By obtaining this information, we will gain an understanding on where these interactions are occurring and how we can apply this knowledge in the potential regulation of NAD<sup>+</sup>/NADH in future research and clinical trials.

## Overexpression of NAMPT and the Effects on NAD<sup>+</sup> Biosynthesis of *C. elegans*

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Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) is an essential coenzyme in cellular metabolism and co-substrate for NAD<sup>+</sup> consuming enzymes (e.g., poly (ADP-ribose) polymerases (PARPs) and Sirtuin proteins), which regulate key biological processes such as DNA damage repair, cell survival, aging, and many others. As a result, the maintenance of an adequate concentration of NAD<sup>+</sup> in the body, is crucial for the survival of the organism. In fact, dysregulation of NAD<sup>+</sup> levels, has been associated to several diseases including cancer, obesity, diabetes, and inflammation. NAD<sup>+</sup> consuming enzymes hydrolyze NAD<sup>+</sup> and generate nicotinamide (NAM) as a byproduct. NAD<sup>+</sup> can be regenerated through multiple pathways, however, the most effective pathway in vertebrate is the NAD<sup>+</sup> salvage pathway. First, NAM is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyl transferase (NAMPT). Second, NAD<sup>+</sup> is regenerated by the adenylation of NMN with ATP by nicotinamide nucleotide adenylyl transferase (NMNAT). NAMPT is the rate-limiting enzyme and our focus of study. NAMPT is a 55-kDa enzyme that forms a homodimer and generates two active sites. Previous work in our lab, suggest that small polyphenolic molecules can increase NAMPT activity. To confirm these findings, we will reproduce these results in *Caenorhabditis elegans*. Unlike vertebrates, *C. elegans* encode nicotinamidases (PNC) instead of NAMPT, which convert NAM to nicotinic acid (NA), creating a longer salvage pathway. Even though NAMPT and PNC-1 are different enzymes, they have equivalent functions in regulating NAM and NAD<sup>+</sup> concentrations. *C. elegans* can be used as a model system to gain more knowledge about genetic and molecular mechanisms of human development and disease. Some of the advantages for using this organism include: the transparency of their body, rapid life cycle, fertility, its entire genome is sequenced, and 40% of its genes have human matches. Our goal is to first induce an alternative vertebrate-like NAD<sup>+</sup> pathway in *C. elegans* by inhibiting the expression of PNC-1 and expressing NAMPT instead. We will then monitor the activity of human NAMPT and changes in NAD<sup>+</sup> levels within the organism. Lastly, we will test the effects that increasing NAD<sup>+</sup> concentration, by this alternative pathway, can have in the lifespan of *C. elegans*. As a result, we will contribute to the understanding of NAD<sup>+</sup> regulation in humans.

## DNA Damage in *Drosophila* Hemocytes and Ovarioles

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Isocitrate dehydrogenase (IDH) is well-conserved metabolic enzyme that has been implicated in specific forms of cancer. IDH is present within the mitochondria and in the cytoplasm of humans and, our model system, *Drosophila melanogaster*. The wild type IDH protein converts isocitrate to alpha-ketoglutarate ( $\alpha$ -KG). When a specific arginine is mutated, the enzyme fails to create adequate amounts of  $\alpha$ -KG due to a newly acquired enzymatic function that converts the catalyzed  $\alpha$ -KG to D-2-hydroxy glutarate (D2HG).  $\alpha$ -KG is an important for not only the citric acid cycle, but also as a cofactor for chromatin remodeling and DNA damage repair enzymes. D2HG competitively inhibits  $\alpha$ -KG resulting in  $\alpha$ -KG being unable to sufficiently fulfill its role in these pathways. We are investigating the impact of this IDH mutation on DNA damage in *Drosophila* hemocytes (blood cells) and the female germline, specifically ovarioles. In hemocytes, we are investigating DNA damage response after treatment with UV and chemical mutagens, such as nitrogen mustard. In the female germline, we are investigating an embryonic lethal phenotype observed when the mutation is driven ubiquitously. To explore this phenotype further, we are using various follicle cell and oocyte drivers to express the IDH mutant protein in order to investigate mutant phenotypes present in female ovarioles that could be leading to our observed lethality. We hypothesize potential developmental and/or recombination repair defects will be observed in IDH mutants. Together these projects will continue to elucidate the cellular and molecular impact of IDH mutations.

## Modeling Parkinson's Disease in the Budding Yeast, *Saccharomyces cerevisiae*

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder worldwide, affecting approximately 1% of people over the age of sixty. In PD patients, inclusion bodies, also known as Lewy bodies (LBs), accumulate within dopaminergic neurons triggering apoptosis and cell death. Studies over the past several decades have revealed that  $\alpha$ -synuclein and tau are two of the major protein components of Lewy bodies though their role in disease progression remains unclear. To better understand the link between protein aggregate formation and cell death, we have overexpressed both human  $\alpha$ -synuclein and human tau in the budding yeast, *Saccharomyces cerevisiae*. Our preliminary data suggests that mannitol, phenylmethylsulfonyl fluoride (PMSF), sulforaphane (SFN), and N-2(chlorobenzyl)-1-(2,5-dimethylphenyl)-1H-benzo[d]imidazole-5-carboxamide (NAB2), may alleviate the aggregation of  $\alpha$ -synuclein within the yeast cell suggesting possible pharmacological interventions that may lower protein aggregation in PD. Unexpectedly, we have also discovered that  $\alpha$ -synuclein aggregation appears to be strain-dependent in yeast, which is reminiscent of the patient-specificity of PD that is observed in the clinic. We are exploring the possibility that this strain differences between W303 and BY4742 may reflect differences in their ability to undergo autophagy. Intriguingly, this may be linked to a single mutation in BY4742 in the heme activator protein gene, HAP1.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

## Yeast Bax Inhibitor (BXI1) Is Involved in Calcium Homeostasis of the ER in *Saccharomyces cerevisiae*

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Yeast Bax inhibitor-1 (BXI1) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins that all contain a transmembrane BAX inhibitor motif. The crystal structure of the prokaryotic member of the family, BsYetJ, has revealed that the Bax inhibitor proteins are pH sensitive calcium leaks. In mammals, the Bax inhibitor family of proteins has cytoprotective properties that are most evident in paradigms of endoplasmic reticulum (ER) stress. Our published studies have shown that yeast Bxi1p is localized to the ER and is involved in the unfolded protein response (UPR) that is triggered by ER stress. BXI1 is thought to act via a mechanism involving altered calcium dynamics. We now show that cells lacking BXI1 accumulate higher levels of calcium in their ER as compared to their wild type counterparts suggesting that Bxi1p is a channel that regulates the outward flow of calcium from the ER lumen. Studies with strains overexpressing Bxi1p and with strains lacking BXI1 and COD1, an ER-localized calcium pump that regulates the inward flow of the cation into the ER lumen are in progress.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

## Yeast Bax Inhibitor ( $\gamma$ Bxi1p) Is a Calcium Leak

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Yeast Bax inhibitor-1 (BXI1/YBH3) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins, which has been linked to different tumor types in human patients. The crystal structure of a prokaryotic member of the family, BsYetJ, has revealed that the Bxi1 proteins are pH sensitive calcium leaks. Our laboratory has shown that Bxi1p is localized to the yeast ER and vacuole and our genetic studies suggest that the protein is a channel that controls the efflux of calcium from the ER. We have also over expressed Bxi1p in *E.coli* and have used a fura-2 based calcium assay to show that the protein facilitates the influx of extracellular calcium into the cell. Further studies have suggested that the influx of calcium can be altered by the pH of the extracellular environment, and that Bxi1p functions as a generalized cation channel. We have initiated a screen to identify small molecule blockers for the channel in the hopes of identifying drugs that would kill cancer cells that are addicted to Bxi1p. Preliminary results suggest that Gadolinium is a potential molecular inhibitor of  $\gamma$ Bxi1p.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco



## Yeast Bax Inhibitor (BXI1) Gene Expression Is Upregulated During the Starvation and the Unfolded Protein Responses.

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Bax Inhibitor (BI-1/TMBIM6) is a transmembrane protein localized to the endoplasmic reticulum membrane that is known to suppresses apoptosis in mammalian cells. Overexpression of BI-1 is associated with a wide range of human cancers, making our understanding of the mechanism of action of this protein of particular importance. The budding yeast, *Saccharomyces cerevisiae*, is known to have one TMBIM gene member that we have named BXI1. The protein is localized to the ER and vacuolar membranes. Though studies in our lab suggest that it is a calcium channel when overexpressed in *E. coli*, little is known about its endogenous function in yeast. Here we use a strain of *Saccharomyces* containing a Bxi1p-GFP fusion driven by the endogenous BXI1 promoter to interrogate its expression profile. Our preliminary data suggests that BXI1 is upregulated during starvation and ER stress, as triggered by the drugs rapamycin and tunicamycin, respectively.

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## The Effects of Acetaldehyde and Ethanol in the Fanconi Anemia Pathway

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Fanconi Anemia (FA) is a rare genetic disease characterized by birth defects, bone marrow failure, cancer, and premature mortality. There are currently 22 known FA genes and more remain to be discovered. The FA proteins work collectively to repair DNA damage and maintain genome stability. The physiological source of DNA damage remains to be determined. Alcohol and its mutagenic oxidation product acetaldehyde represent one potential important source of DNA damage. In this study, we have examined the effects of ethanol and acetaldehyde on the activation of the FA pathway. Activation of the pathway occurs via the site-specific attachment of a single ubiquitin protein to the FANCD2 and FANCI proteins, a process known as monoubiquitination. HeLa cells were exposed to a range of concentrations of ethanol and acetaldehyde for 24 hours, and whole-cell lysates were prepared for immunoblotting. We observed a concentration-dependent increase in FANCD2 monoubiquitination in cells exposed to acetaldehyde, but not ethanol. Using a crystal violet cell survival assay, we also observed increased cell death following exposure to high concentrations of acetaldehyde, while no overt cell death was observed following exposure to ethanol. Our findings indicate that genotoxic aldehydes induce activation of the FA pathway, strongly suggesting that the FA proteins may play a key role in the repair of reactive aldehyde-mediated DNA damage.

## Structural and Functional Analysis of Competence Protein ComEC in *Thermus thermophilus* HB27

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Natural genetic transformation enables bacterial cells to take up exogenous DNA through their cell membrane via a complex system of competence and pilin-related proteins [1]. Such lateral gene transfer is one of the driving forces responsible for genome plasticity, microbial evolution, and the spread of antibiotic resistance in bacteria [2]. *Thermus thermophilus*, an aerobic, rod-shaped, gram-negative bacterium, has been recorded to have the most efficient natural transformation machinery of all competent bacteria [2]. For this reason, *T. thermophilus* HB27 has been studied extensively in an effort to create a foundation for future research in elucidating the competence machinery of other transformable bacteria. Sixteen proteins involved in natural transformation have been identified in *T. thermophilus*. One of these, ComEC, is necessary for natural transformation and is hypothesized to contribute to DNA transport through the membrane of *T. thermophilus* [3]. Three distinct domains have been identified in ComEC: a domain of unknown function (DUF), a competence domain, and a  $\beta$ -lactamase domain. While the specific role of each domain in transformation remains unknown [4], modeling studies suggest a role for the  $\beta$ -lactamase domain in conversion of incoming dsDNA into recombinogenic ssDNA. We have constructed a system for the genetic manipulation of the ComEC protein in *T. thermophilus*. In this study we have found that complete deletion of the comEC gene eliminates competence for transformation, and that competence is restored by expression of ComEC from a plasmid. We are currently in the process of deleting the individual domains and assessing their contributions to transformation. This system will facilitate future studies, including site-directed mutagenesis of putative Zn<sup>2+</sup> binding sites in the  $\beta$ -lactamase domain and structure determination of the  $\beta$ -lactamase domain by X-ray crystallography.

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## The Effects of N-Terminal Acetylation on the Protein Tau

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The microtubule-associated protein tau (MAPT) plays a critical role in many neurodegenerative diseases such as Alzheimer's Disease. Tau functions to stabilize microtubule structures that are essential for transport within the neuron, and loss of transport leads to a loss of neuronal function. Tau binding is regulated by phosphorylation, a post-translational modification (PTM) where a phosphate group is added by a specific kinase. Phosphorylated Tau blocks the microtubule binding sites, then phosphatases remove these phosphate groups which expose the microtubule-binding regions. Tau can also become hyperphosphorylated, which causes Tau to self-assemble into oligomers and higher order aggregates. It is clear that post-translational modification of Tau plays a key role in the dysregulation of neuronal function due to abnormal conformational changes, however not all PTMs are as thoroughly studied as phosphorylation. N-terminal acetylation is the most common PTM of all proteins and Tau is predicted to be a target of N-terminal acetylation. N-terminal acetylation is a co-translational process that is catalyzed by N-terminal Acetyltransferases (NAT), which adds an acetyl group to the N-terminus of a Tau polypeptide, thus neutralizing the positive charge on the N-terminus. Commonly, studies endogenously express Tau in a prokaryotic system that lack PTMs. This project uses a co-expression system that examines the PTM N-terminal acetylation and Tau. Future studies involve examining Tau fibers *in vitro* and the effects of N-terminal acetylation on the function and aggregation of Tau.

## Expression of Tau in *Saccharomyces cerevisiae* to Determine the Effects of N-Terminal Acetylation

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Protein aggregates, which can result in cellular toxicity, are found in the brains of people afflicted with neurodegenerative diseases such as Alzheimer's. One of these proteins found to aggregate is Tau, a protein that stabilizes microtubules in neuronal cells. Based on its amino acid sequence, Tau is likely to be N-terminally acetylated by the complex NatA, which is responsible for the acetylation of a majority of proteins with this modification. Mutations in the NatA complex result in a variety of pleiotropic detrimental phenotypes, showing that acetylation is a crucial modification for many proteins. Without the presence of NatA – and therefore without acetylation – Tau may be more prone to aggregation. *Saccharomyces cerevisiae* is a model system that is easily genetically modified and allows for a straightforward way to examine changes to the cellular level. This project involves expressing Tau in yeast strains with and without the presence of NatA and examining whether there are any noticeable differences in its effects.

## An Effective One-Step Purification of the HSP70 Class Chaperone Ssa1

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Molecular chaperones, also known as heat shock proteins (HSPs), are a diverse and highly conserved class of enzymes that maintain proteostasis through the mediation of protein structure *in vivo* via their up-regulation, allowing for recovery from various cellular stressors. Several families of molecular chaperones are known to exist and are classified by molecular weight (kDa). Ssa1 is a HSP70 class chaperone endogenous to *Saccharomyces cerevisiae* that acts to maintain cellular proteostasis by working in tandem with its HSP40 co-chaperone, Sis1, to bind to non-native polypeptide chains and restore their functional conformations. Studies of Ssa1 often prove limiting due to its transient mechanism of activity and the resource-intensive nature of commonly used purification methods. To address this, we developed a highly efficient, one-step purification of Ssa1 utilizing a previously constructed Protein-A transformant strain. Our results indicate that our method is able to isolate high yields of pure Ssa1 from small amounts of starting material in a short period of time. Furthermore, our method allows for the native elution of Ssa1 without compromising enzymatic activity, allowing for future studies to better understand interactions between Ssa1 and its co-chaperones as well as the effects of post-translational modifications on chaperone function.

## Targeting Immune Checkpoint Genes with CrisprCas9 to Boost Tumor Immunotherapy in Dendritic Cells

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Dendritic cells stimulate antigen-specific T cell immune responses and have shown therapeutic efficacy in anti-tumor immunotherapy in clinics. However, these cells express immunosuppressive genes that have been shown to suppress T cell activation and its effector function. Interleukin 10 (IL10) and the IL10 receptor (IL10R) are immunosuppressive genes that are expressed by DCs. This study investigates whether the depletion of IL10 and IL10R will boost DC-based anti-tumor immunotherapy in preclinical animal models using CRISPR/Cas9, a high-efficient and precision gene-editing tools. Initially, a guide RNA sequence targeting the IL10 and IL10R gene was designed and successfully inserted into a lentiCRISPR vector. Following, Hek293 cells were transfected with the recombinant vector along with two packaging plasmids to produce gene-targeting lentiviruses. These efforts will allow this study to move forward and generate IL10 and IL10R-depleted DCs to test its therapeutic efficacy in preclinical animal models. If successful, the depletion of immunosuppressive genes, such as IL10 and IL10R, may offer as an effective strategy to improve DC-based anti-tumor immunotherapy.

## Investigation of the Role of Glycation of Matrix Proteins in Human Skin Aging

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Chronological skin aging and UV-induced skin photo-aging share certain mechanisms. Our previous studies have shown that collagen degradation and cellular dehydration play important roles in skin aging. Accumulated data have suggested that protein modifications such as glycation is also critical to skin aging, while the mechanism is unclear. Given that the mechanism of glycation of matrix proteins such as collagen remains to be further investigated, and nanoparticles may have potentials for anti-glycation activity, we hypothesize that gold nanoparticles act as deglycase and thus may have potential applications in cosmetics. In this study, we aimed to investigate the factors that affect nonenzyme glycation of matrix proteins such as collagen *in vitro*. Using well established glycation assay with Biotek Cytation imaging reader, we first tested the glycation of bovine serum albumin or BSA and collagen. The results showed that glycation of both BSA and collagen is in dose dependent manner. As expected, aminoguanidine, a known inhibitor of glycation, inhibits the glycation process. Since UV radiation causes skin cell damage and photoaging, we next studied the effect of UV irradiation on glycation of proteins. The data showed that UV radiation at 30mJ/cm<sup>2</sup> in fact inhibits collagen glycation. Interestingly, when PDMS-CaO<sub>2</sub> disks that release O<sub>2</sub> were added to the incubation, UV-enhanced glycation is reduced. Nanoparticles have been used as ingredients of cosmetics. We then studied the effects of nanoparticles on collagen glycation. The results indicated that both Fe<sub>3</sub>O<sub>4</sub> and Gold nanoparticles inhibit collagen glycation. Recently, the extract of *Ganoderma lucidum* attracts attentions and has been added to skin care products. We incubated the methanol extracts of various *Ganoderma lucidum* with collagen and fructose. The results showed that all extracts inhibit collagen glycation *in vitro*. Collectively, we conclude that our *in vitro* glycation assay is valid for screening of compounds that may be used to inhibit skin matrix protein glycation and eventually attenuate skin aging process.



## Transactivation of EGFR and mTOR by H<sub>2</sub>S in Cultured Cancer Cells

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Hydrogen sulfide regulates a variety of pathological processes in various biological systems. Previous studies have suggested that H<sub>2</sub>S plays a significant role as a secondary messenger in cellular signaling. Interestingly, it has been found that this unique gaseous molecule is capable of producing both pro- and anti- apoptotic activity in cultured cells. In order to investigate the effects of H<sub>2</sub>S on cancer cells, we utilized a water-soluble, slow-releasing H<sub>2</sub>S donor, GYY4137, to study the molecular pathways that may cause this pro- and anti- apoptotic activity observed in cancer cells following exposure. Existing data has indicated that molecular targets may include epidermal growth factor receptor or EGFR, ERK and JNK in H<sub>2</sub>S-induced signaling pathways. We hypothesize that when administering GYY the same EGFR pathway that activates ERK, will activate mTOR (mammalian target of rapamycin) and phospho-S6, leading to either cell death or cell proliferation. Four cell lines were cultured and studied in this experiment: CaOV3 (ovarian cancer cells), A375 (melanoma cells), HeLa (cervical cancer cells), and WM 266-4 (melanoma cells). We treated the cells with GYY (200 μM) at various time points in order to determine the effects of GYY on cancer cells when administered at different durations. Using confocal microscope and Western blot analysis we stained for several proteins, phosphor S6 and phosphor-EGFR, to support that GYY activates EGFR and mTOR pathway. Compared to control, we observed that the longer the treatment the more prevalent phosphor S6 and phosphor-EGFR were. Noticeably, we observed a significant difference between 15 minutes and 30 minutes, suggesting that GYY has the largest effect after 30 minutes of treatment. Finally, we studied cell growth and viability using MTT assay to investigate the effects of various concentrations of GYY on the cells. Our data suggests that the stronger the concentration, the lower the cell viability. Similarly, we used Mitotracker<sup>®</sup> Red dye to stain for mitochondrial activity in CaOV3 and WM 266-4 cells treated at various time points. Overall, mitochondrial activity increased when cells were exposed to GYY, suggesting that H<sub>2</sub>S may cause anti-apoptotic activity, and instead favor cell growth at the concentration we used. Collectively, our data suggests that the H<sub>2</sub>S donor, GYY has a significant effect in cell proliferation and survival in several cancerous cells following the activation of EGFR and mTOR pathways.

## The Effect of Hypoxia and Oxygen Supplement on Cultured HeLa Cells

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Our previous data have shown the effect of hypoxia on cultured ovarian cells. CoCl<sub>2</sub>, as well as 2% O<sub>2</sub>, introduces hypoxia and activates transcription factor HIF1- $\alpha$  in the ovarian cancer cells (CaOV3) cell line and in HEP cell line as reported. We hypothesize that this transcription factor is also activated in the HeLa cell line as the concentration of diluted cobalt chloride increases. Initially, we analyzed the morphology of cultured HeLa cells with the addition of 200  $\mu$ M CoCl<sub>2</sub>, also known as our stock solution. Interestingly, these images revealed that 8  $\mu$ M of CoCl<sub>2</sub> treatment differs from the untreated cells because they are less confluent and irregularly shaped. Further, we confirmed these results through Western blot analysis and confocal imaging. We found that high concentration of CoCl<sub>2</sub> directly induces hypoxia leading to the degradation of the cytoskeleton and nuclear membrane. As the concentration of CoCl<sub>2</sub> increases, the activities of HIF1- $\alpha$  and phosphorylated S6 decrease. These added amounts in sequential order are: untreated, 4  $\mu$ M, 40  $\mu$ M and 400  $\mu$ M of 20  $\mu$ M CoCl<sub>2</sub> for the western blot scan. Thirdly, we learned that cell viability with MTT assay showed that there is no significant effect of CoCl<sub>2</sub> on HeLa cells, except the highest concentration tested. We then tested the effect of oxygen supplement by PDMS-CaO<sub>2</sub> disks on HeLa cells with MTT assay as well. The data showed that the viability of HeLa cells is lower when treated with these disks as opposed to CoCl<sub>2</sub> treatment. Taken all together, our preliminary data suggest that manipulation of oxygen level in cancer cells may provide insights into the understanding of the role of oxygen in solid tumors and eventually offer clinic oxygen therapy.

# NEUROSCIENCE

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EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM**

## Comprehending Action Words

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Although children learning English acquire words for objects and entities (nouns, as in cookie, truck) as early as 9 months of age, words that encode actions (verbs, as in clap, sit) are not understood until the second year, as late as 16 months of age. These data suggest that learning words to refer to actions may be a more difficult cognitive task than learning words for objects. Objects are typically stable, static perceptual entities, whereas actions are transient, dynamic events. However, not all words that encode actions are verbs. For example, parents report that children as young as 12 to 14 months comprehend words that label actions in routine events (e.g., waving bye), during games (e.g., hiding the face during peekaboo), and for movement and change of location (e.g., up / down to request getting picked up or put down). However, data from parent report may be biased and unreliable and laboratory assessment of word comprehension has been limited to nouns and verbs. Our current research addresses this gap in our knowledge of children's ability to learn words that encode actions rather than objects. We test children's earliest comprehension of these action-related words using the Preferential Looking Task (PLT) and eye tracker technology. The PLT measures word comprehension by comparing a child's visual gaze to target versus distracter images before (baseline trial) and after (test trial) the target image is labelled. Images are displayed on a computer monitor, and children's visual attention to target and distracter is recorded by a Tobii T60 XL eye tracker system. Comprehension is defined as an increase in visual attention to the target image during test compared to baseline presentation.

In our current research we test a set of six action-related words in children aged 12-15 months. Children view a video slideshow of actors performing pairs of action-related words (e.g., an actor covering/uncovering the face for peekaboo versus an actor lifting a toy bear from the floor for up) and visual attention is recorded for target and distracter images during baseline and test trials. Data analyses examine developmental change as well as individual differences in the types of action words that children comprehend. These data contribute to our understanding of the extent to which perceptual and cognitive factors contribute to early lexical development.

## Parental Speech and Early Verb Comprehension

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Research suggests that the short, highly inflected utterances characteristic of parental speech to children are positively related to language development. Children's comprehension and production of nouns is enhanced when parents label and describe objects (e.g., Truck! That's a red truck). However, we know less about the contribution of parental speech to children's acquisition of verbs, or words that encode actions rather than objects. Verbs may be especially salient in language that directs or encourages the child's behavior (e.g., Sit down; Throw the ball to mommy), which accounts for about one-third of parental speech to children. Thus, the extent to which parental speech highlights verbs, especially verbs that are linked to the child's own behavior, should predict children's ability to comprehend verbs.

The present study investigates the relationship of parental speech to children's verb comprehension. In previous INBRE-supported research, we assessed verb comprehension in 14-month-olds using a laboratory task; we also asked parents to complete a vocabulary checklist of verbs their children comprehend. For the present study, we analyze transcripts of parent-child interaction during a play session videotaped in the lab following the comprehension assessments. Transcripts are coded for total number of verbs (tokens), number of different verbs (types), and the relative position of verbs in parental utterances (initial, medial, final position; or verbs used as single word utterances, as in Look!). We predict that verbs in salient positions (initial position and verbs that occur as single-word utterances) will be positively related to children's comprehension of verbs, as measured by laboratory assessment and parental report data.

## Using Human Neuronal Co-Cultures to Study the Role of the Microbiome in Neuro-Inflammation

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Rapid neuronal responses are critical to a well-functioning central nervous system (CNS). Cells within the CNS, such as neurons and astrocytes, react to inflammatory mediators that can be utilized to better understand the pathways through which inflammatory responses are activated within the CNS. Neuro-inflammation is applicable to neurological disorders that are interrelated with changes of microbiome or microbiota-derived metabolites. As a model of the CNS, 2D neuronal and glial co-cultures, derived from human pluripotent stem cells, were used to study inflammatory responses. These cultures are a physiologically relevant representation of the CNS, as they contain both neurons and astrocytes. For this experiment, 2D terminally differentiated neuron and astrocyte co-cultures were treated for 24 hours with the inflammatory mediators poly (I:C) (10  $\mu\text{g}/\text{mL}$ ) and indoxyl-3-sulfate (I3S) (50  $\mu\text{M}$ ). Poly (I:C) is a toll-like receptor agonist (TLR3), and I3S is a tryptophan metabolite generated by the resident microbiome. I3S also acts as an agonist for aryl hydrocarbon receptors. qPCR was used to analyze the expression levels of TNF alpha, iNOS, and IL-6, which are involved in immune responses to the neuroinflammatory mediators. Many neurodegenerative disorders such as Huntington's and ALS can be linked to these inflammatory responses. The use of these human neuronal co-cultures to examine inflammatory responses is significant for disease research and drug discovery.

## Comparing the Effects of Neurostimulation on Ion Channel Gating and Ionic Flux Using a Computational Approach

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Clinical research has shown neurostimulation to be an efficient neurotherapy for treating a variety of neurological conditions. Transcranial Electrical Stimulation (TES) and Deep Brain Stimulation (DBS) are forms of neurostimulation that, while having very different treatment profiles, have both shown great success in mitigating a range of symptoms associated with neurodegenerative disorders. Despite promising results from these neurostimulation modalities, the cellular-level mechanisms by which they operate are not fully comprehended. In particular, the effects of neurostimulation on ion channel gating and transmembrane ion transport are not known. To address this issue we have developed a biologically-based mathematical model of neurostimulation that operates at the neuron-level. Our approach uses the Poisson-Nernst-Planck model of electrodiffusion coupled with a Hodgkin-Huxley based model of cellular ion transport to quantify electric potential energy, individualized ion species, voltage-gated ion channels, and transmembrane ionic flux. Using a biologically-inspired domain, computational simulations are used to assess the impact of TES and DBS on neuronal electrodynamics. Results show that polarization of membrane voltage from neurostimulation occurs in a location specific manner, where the type and degree of polarization depends on the position on the membrane. This polarization in turn causes ion channel gating variables and transmembrane ionic flux to change in a location specific fashion. Another key simulation finding is that intracellular calcium concentrations increase significantly due to a neurostimulation-induced calcium influx. As cytosolic calcium is critical in intracellular signaling pathways that govern proper neurotransmitter secretion as well as support cell viability, this alteration in calcium homeostasis suggests a possible mechanism by which TES operates at the neuronal level to achieve neurotherapeutic success. Finally, we also present our preliminary results that compare the differences between the ionic fluxes and channel states generated by TES and DBS.

## A Phenotype-Driven Approach for Reverse Engineering a Biomathematical Model of a Dopaminergic Neuron with Application to Parkinson's Disease

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Parkinson's disease is one of the most common neurodegenerative disorders, and affects approximately seven million individuals globally. Despite significant advances through biomedical research and clinical trials, there is no known cure for Parkinson's disease, in part due to a lack of fully comprehending its cellular pathogenesis. Mathematical modeling and in silico experimentation have shown to be highly effective tools in augmenting biomedical research to help formulate a better understanding of the cellular-level mechanisms by which diseases progress. In this light, we have employed a computational based approach to construct a novel mathematical model to help elucidate the neurobiological mechanisms by which dopaminergic neurons prematurely commit to apoptosis in Parkinson's disease. The model emulates the intracellular signaling pathway in dopaminergic neurons, and integrates key proteins which have been found to play a fundamental role in the progression of Parkinson's disease including Parkin, PINK1, DJ-1, IPAS, LRRK2, and  $\alpha$ -synuclein. To begin, an extensive search of the Parkinson's disease neurobiological and medical literature led to the creation of a systems biology based wiring diagram. In turn, this diagram was used to develop an ordinary differential equations based mathematical model using the law of mass action as well as the well-known Michaelis-Menton equation. Though the species of this pathway as well as their interconnections are known, the kinetics governing this system are presently undetermined. To address this issue, a reverse engineering based computational approach was used to estimate these unknown parameter values. This was accomplished by viewing the premature commitment to pathological apoptosis as an irreversible biological switch, and then applying this viewpoint to distinguish those rates that facilitate the mathematical model to predict this phenotype. Then, a strategic parameter space walk in conjunction with k-means clustering, sensitivity analyses, and an eigenanalysis was implemented, thereby enabling a highly robust screening process that culminates with a set of optimal rates for the dopaminergic neuron model. In addition to presenting our signaling pathway wiring diagram, mathematical model, and numerics-based approach for identifying kinetics, we showcase the ability of the model to emulate the intracellular processes of both a healthy dopaminergic neuron as well as one that presents with Parkinson's disease.



## Treat It or Beat It: Possibility Probability Questionnaire to Understand Cancer Treatment Decisions

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Past research has shown that individuals prefer taking action regardless of the risks involved in the measure to treat cancer (Fagerlin, Zikmund-Fisher, & Ubel, 2005). In this study, we aim to understand how individuals differ in this important medical decision. aimed to explain this tendency in terms of individual differences. In hypothetical vignettes, participants imagined a situation in which they were either given the option to undergo surgery or watchfully wait. In a between-subjects experimental manipulation, these vignettes displayed probabilities either portraying surgery as the optimal solution that increases the chances of survival and watchful waiting as the non-optimal solution or surgery as the inferior solution and watchful waiting as the optimal solution. The participants were later asked which treatment option they prefer and how worried they were about each option. Participants completed the Possibility/ Probability Questionnaire (PPQ) a recently developed 20-item measure that was administered through Qualtrics to approximately 1000 participants. The PPQ was developed to examine and differentiate between sensitivity to probabilities and emotional reactivity to possibilities. Participants also completed previously validated scales such as the Intolerance for Uncertainty-Short Scale, the Aversion to Medical Ambiguity, and the Belief in Science and Technology scales. The results will illustrate how individual differences contribute to making medical decisions, and how individuals prefer to taking some degree of action while facing stress even if the action may be known to be more harmful.

## Do You Really Want to Know? The Possibility Probability Questionnaire as a Predictor of Relationship Decision Making

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Past theories in risk perception emphasize a strong roll of emotion, at the expense of carefully weighing probability. In order to further explain this concept, we've developed a 20 item individual difference measure called the Possibility Probability Questionnaire (PPQ). The aim of this newly developed measure is to analyze and distinguish between individuals' sensitivity to probability and the emotional reactivity to possibility. The PPQ has so far been used to predict reactions to health and safety risks. The current study looks to extend this research into a new context of relationship decision making. Using a large internet sample, we asked participants whether they'd want predictive information about their likelihood of divorce in a hypothetical marriage. We found that the PPQ, the Intolerance of Uncertainty scale (IFU), and the Belief in Science and Technology scale (BIST), were significant predictors of the decision to seek divorce predictions. This study shows that the PPQ is not limited to predictions of health and safety, but expands to other contexts as well.

## Testing, Testing 1, 2, 3: Decision Making for Genetic Testing Using the Possibility Probability Questionnaire

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In this age of increasingly available genetic testing, it is important to understand individual differences in the tendency to seek these tests, especially when results will have no bearing on treatment. In order to examine this behavior, a measure known as the Possibility Probability Questionnaire (PPQ), a 20-item scale, was used to examine sensitivities to numerical probabilities and emotional reactivity to possibilities. In a hypothetical vignette, participants imagined a possible diagnosis of the genetic disease Neurofibromatosis (NF1) for which there is no treatment or prevention. In a between-subjects 2x2 experimental design, we manipulated the likelihood of having the disease (low or high) as well as whether the genetic test could provide certain information or only reduced certainty. In each condition, participants were instructed to rate their likelihood of choosing to test for the genetic disease and how worried they were about the disease in general. Other measures including the Intolerance of Uncertainty-Short Scale, the Aversion of Medical Ambiguity Scale, and the Belief in Science and Technology Scale will also contribute to these results. This survey was distributed online, through Qualtrics, to 1000 participants. Anticipated results should demonstrate a relationship between the PPQ and the genetic testing decision making of participants. The results of this study will help to clarify the individual differences and the disease factors that affect decisions about genetic testing.

## Inhibitory Interneurons in SOD1 Mouse Model of ALS Are Hypoexcitable

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Motoneuron hyperexcitability is thought to contribute to the symptomology of ALS. However, the neurons presynaptic to motoneurons have not been well studied, particularly at an early, pre-symptomatic stage. The focus of this study is to assess the parameters of inhibitory spinal interneurons in a mouse model of ALS compared to controls. In this study, anatomical and electrophysiological measurements were performed on inhibitory interneurons in the ventral lumbar enlargement of the spinal cord from 6-12 day old mice. Glycinergic interneurons were targeted based on the expression of GFP driven by glycine transporter 2 (GlyT2). Mice were bred for experiments by crossing GlyT2 EGFP mice with SOD1G93A high expresser mice. Neuron counts and reconstructions were used to ascertain interneuron numbers, soma surface area and volume. Preliminary results show that there was no difference in number of interneurons in the ventral lumbar spinal cord. It was also found that there was no significant difference in neuron soma surface area ( $p= 0.1460$ ) or soma volume ( $p= 0.8087$ ). These findings both suggest that the number and the anatomy of the interneurons is similar in both SOD1 mice and controls. Inhibitory interneurons were found to be less excitable in SOD1 mice. Threshold for firing is more depolarized in SOD1 interneurons ( $-41 \pm 5\text{mV}$  vs  $-38 \pm 5\text{mV}$ ), and the current needed to elicit firing is increased ( $-57 \pm 136\text{pA}$  vs  $-15 \pm 67\text{pA}$ ). This suggests that while spinal interneurons have developed properly in numbers and size, they may fire less frequently, thus leaving spinal motoneurons in SOD1 mice less inhibited.

## Equol, an Isoflavone Gut-Derived Microbial Metabolite, Exhibits Neuroprotection Against Transgenic Models of Parkinson's Disease in *Drosophila melanogaster*

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Parkinson's disease (PD) is the second most prominent neurodegenerative disease after Alzheimer's disease.<sup>1</sup> It is characterized by the death of dopaminergic neurons in the substantia nigra.<sup>1</sup> Neuronal cell death has been linked to neuroinflammation and the presence of  $\alpha$ -synuclein aggregates.<sup>2,3</sup> PD can be caused by both genetic and environmental factors.<sup>2,3</sup> Specific genes, such as leucine-rich repeat kinase 2 (LRRK2) and parkin, have been associated with familial PD.<sup>1,4</sup> Another established risk factor is gender, where the incidence and prevalence of PD is 1.5-2 times higher in men than in women.<sup>4</sup> Furthermore, a positive correlation has been found between the age at onset for PD and the age at menopause.<sup>4</sup> Studies suggest PD may be linked to levels of estrogen, which studies have shown to exhibit neuroprotection.<sup>4</sup>

Therefore, we propose a potential treatment for PD using a nonsteroidal estrogen, namely, equol, a gut microbial metabolite formed from isoflavones by intestinal microflora.<sup>5</sup> To evaluate equol as a treatment for PD, we used *in vivo* techniques in the *Drosophila melanogaster* model. Two common transgenic models of PD were used, LRRK2 and  $\alpha$ -synuclein. Assays were performed using these models to investigate the effects of equol on climbing ability and the abundance of reactive oxygen species (ROS) and nitric oxide synthase (NOS). In conclusion, evidence suggests equol is a potential treatment for PD that reduces neuroinflammation, as exhibited by increased climbing ability and a decrease in ROS and NOS after one week of treatment.

## Electrophoretic Mobility Shift Assay Optimization to Investigate SP1-DNA Binding

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Alzheimer's Disease is characterized by two types of protein aggregates, plaques composed of amyloid-beta ( $A\beta$ ) and neurofibrillary tangles assembled from hyperphosphorylated tau. These biomarkers cause synaptic dysfunction and neuronal loss, leading to the cognitive impairment associated with AD. Expression of the tau and  $A\beta$  genes is regulated by specificity protein 1 (SP1), a zinc finger transcription factor. Tolfenamic acid (TA) has been shown to improve cognitive impairment in transgenic mice carrying the human tau gene through the reduction of total tau levels. In this study, we optimized an electrophoretic mobility shift assay (EMSA), which will be used to evaluate the efficacy of TA analogs as inhibitors of SP1 binding to DNA in future work. Many components of our protocol were optimized to obtain clear and presentable membrane images, including gel percentage, binding reactions, and detection steps. This information will be utilized in future studies which use EMSA.

## Facial Attractiveness, Social Status and Recognition Memory

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Do people recognize others' faces based on facial features and information about their social status? In three experiments we manipulated facial attractiveness of faces generated by software, and in one experiment also included a manipulation of social status. Past results have been mixed; some indicate attractive faces are recognized better than less attractive faces, other results found that unattractive faces were better recognized, whereas other results found no effect of attractiveness. We propose that methodological and analytic concerns may account for the variability of the findings. We instituted methodological and analytic advances in our work. All experiments showed that attractive faces are recognized at beyond chance accuracy. There was also evidence for false positive face recognition for attractive faces; faces that were not seen previously were judged as having been seen. For high status faces, attractive and unattractive faces were recognized with greater accuracy than those of average attractiveness. For low status faces, attractive faces were recognized with greater accuracy than average or unattractive faces. Face recognition is affected by physical features of the face as well as by information that indicates social status.

## The Role of the Posterior Parietal Cortex in Judgments of Physical Distance

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Several rodent studies have implicated the posterior parietal cortex (PPC) as playing an integral role in the representation of spatial relationships between stimuli (Kesner, 2009). More specifically, the PPC may support both the metric and topographical assessments of allocentric space. While a large amount of rodent studies have demonstrated that a lesion of the PPC produces a deficit in a rat's ability to represent topographical space, research on the PPC's role in assessing metric space has been sparse and inconclusive. The aim of this study was to pilot a behavioral task that measured rats' ability to assess differences in the magnitudes of metric space using normal-functioning rats. Six male Long-Evans rats were exposed to a training phase in which the subject was placed into a rectangular apparatus that was divided into two separate arms. At the end of each arm was a novel caged rat that was either "close" or "far" and was separated from the experimental rat by plexiglass. Subjects were divided into two groups, one of which was trained on the near location and the other on the far location. This paradigm could potentially be used to assess whether rats with a lesion of the PPC show a deficit in the ability to analyze metric space when compared to normal-functioning rats.



## Posterior Parietal Cortex: Evidence for Social Functioning

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Sociability is the quality of interactions with conspecifics and can be quantified by determining the frequency and duration of social interactions. The purpose of this study was to determine the extent to which the posterior parietal cortex (PPC) is responsible for maintaining knowledge of social relations and social recognition. 19 male Long-Evans rats were used in this study; 9 rats received neurotoxic lesions of the entire PPC and 10 received sham lesions. After surgeries rats were tested for sociality in Phase 1 of the test in which the subject was exposed to his cage-mate inside a barred chamber that allowed limited physical access and an empty chamber. The sham rats spent more time with their cage-mate as compared to the empty cage but the lesioned rats did not. In Phase 2, using the same sociability apparatus and chambers, we evaluated social novelty by determining if rats would prefer a novel stranger as compared to his cage-mate. The sham group discriminated towards the novel stranger, whereas the lesioned rats displayed no social novelty preference. The sham rats also explored and spent more time with the stranger rat than the cage-mate as compared to the lesioned rats. In other tests we determined that these lesioned rats were unimpaired on object recognition using the same delays and exploration times, so these differences cannot be accounted for by object recognition memory. We therefore conclude that the PPC is necessary for maintaining information about social relationships. Future studies will explore the cognitive mechanism that accounts for the PPC's role in abstract concepts, including, but perhaps not limited to social relationships.

## The Role of the Posterior Parietal Cortex in Memory for Temporal Order

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Nineteen male Long-Evans rats were tested in a temporal order task. The purpose of this task was to investigate the role of the posterior parietal cortex in memory for temporal order. Nine rats received neurotoxic lesions to the posterior parietal cortex (PPC) while ten rats were used as controls. Each rat explored two of the same objects (A1 and A2) in Sample 1 for three minutes and then received a three hour inter-stimulus interval. Sample 2 was then presented in which two novel but identical objects were explored (B1 and B2) for three minutes. After a five minute delay, the test phase was presented where each rat was placed with one object from each sample (A3 and B3) and explored for four minutes. Each phase was counterbalanced across rats for both novel object and side. Due to rats' natural preference for novelty, if the PPC is involved in temporal order tasks, it is expected that the lesion rats would spend equal amount of time exploring both objects at test because there is a deficit in their memory for object order, while sham rats would spend more time exploring the A3 (vs. B3) during test 1 because it is the least familiar. The PPC lesioned rats and the sham rats both discriminated towards the most novel object and there was no significant group difference. There were also no significant group differences for total exploration or number of bouts. However, the sham rats had a significantly higher duration exploring the novel versus the familiar object while there was no significant difference in bout duration for the lesion rats. There was no significant group difference for duration of exploration of novel versus familiar. The results may provide evidence that the posterior parietal cortex does not have a central role in memory of events in relation to time.

## Determining the Neuron Numbers in the Brains of a Transgenic Rat Model of Cerebral Amyloid Angiopathy

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Cerebral Amyloid Angiopathy (CAA) is the accumulation of fibrillar amyloid  $\beta$ -protein ( $A\beta$ ) in the blood vessels of the brain. CAA is a common cerebral small vessel disease that occurs in the elderly and a frequent comorbidity of Alzheimer's disease. Novel transgenic rats (rTg-DI) were developed to study this disease in a living animal model. In rTg-DI rats, fibrillar amyloid accumulates around capillaries of the brain and causes capillary structural alteration, promotes perivascular neuroinflammation, and produces microhemorrhages and small occlusions that are readily detected by magnetic resonance imaging. These novel transgenic rTg-DI rats CAA can be studied to see how it affects the neurons in the brain by comparing rTg-DI rats to wild type rats (RWT). The RWT and rTg-DI rat brains were collected, sliced and placed onto slides, which are then labeled with an antibody to detect the neurons. The tissues were then imaged and neuronal cells were counted and then compared between the rTg-DI rats the RWT rats. Based on this study, the rTg-DI rats have shown to be a new useful model for further research and testing on CAA and Alzheimer's disease.

## Maternal Opioid Use in Pregnancy and Infant Birth Defects

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**Background:** The rapid increase of opioid-related overdoses and deaths has become a public health threat in the United States. The utilization of prescription opioids in pregnant women has increased while the results from teratogenic studies are controversial. **Objective:** To evaluate the association between maternal opioid use during pregnancy and the incidence of congenital malformations in infants.

**Methods:** This retrospective cohort study evaluated Rhode Island Birth Certificate data of live births from 2006 to 2016 for mothers who were enrolled in the Rhode Island Medicaid program. The study cohort included women who had given a live birth and had no opioid use disorders during the study period. Mother's prescription opioid exposure was obtained through pharmacy claims using NDC codes and defined as filling at least one prescription opioid for non-cancer pain during pregnancy. The study outcomes were defined using ICD-9 diagnosis codes and ascertained by reviewing medical records. Continuous variables were presented as mean  $\pm$  standard deviation (SD) and compared using student t test. Categorical variables were presented as frequency (%) and compared using chi-square test or fisher exact test. Statistical analyses were conducted using SAS 9.4 (Cary, NC).

**Results:** Of 25,205 pregnancies included in this study, 1,898 (7.5%) mothers filled prescription opioids and 1,024 (4%) infants were diagnosed with birth defects, either major congenital malformations (MCM) or minor anomalies (MA). Comparing opioid exposed vs unexposed, total birth defects were 9.5% vs 3.6% ( $P < 0.0001$ ), MCMs were 7.0% vs 2.7% ( $P < 0.0001$ ), and MAs were 3.1% vs 1.2% ( $P < 0.0001$ ). Mother's who smoked (52.6%), had a higher number of prenatal care visits ( $13.04 \pm 5.58$ ), and were older ( $27.35 \pm 5.36$ ) were more likely to be exposed to an opioid during pregnancy. Infants with prenatal opioid exposure were more likely to be born preterm (gestational age, mean  $\pm$  SD:  $38.3 \pm 2.3$  vs  $38.6 \pm 2.1$ ,  $P = 0.07$ ; preterm born, N(%): 194(11%) vs 2108(9%),  $P = 0.01$ ). Other factors such as the number of offspring, alcohol consumption, and infant's sex did not prove to have statistical significance in this study.

**Conclusion:** Our results suggest a significant association between opioid use during pregnancy for non-cancer pain and an increased incidence of birth defects. Further investigation is needed to examine the true effects of maternal opioid exposure after adjusting for other demographic and clinical characteristics.