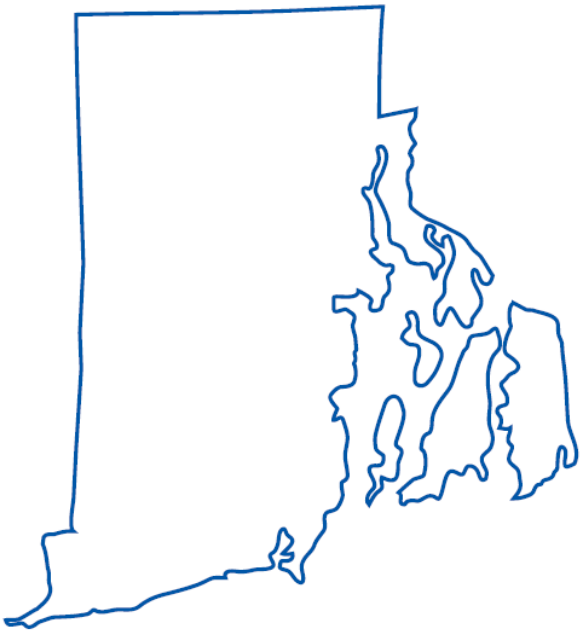
2017 Rhode Island Summer Undergraduate Research Fellowship Conference



Friday, July 28, 2017
8:00 AM

College of Pharmacy
and
Center for Biotechnology & Life Sciences
University of Rhode Island

Supported by

Rhode Island
NSF EPSCoR

RI-INBRE & RI NSF EPSCoR

10TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, July 28, 2017
COLLEGE OF PHARMACY AND THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES
UNIVERSITY OF RHODE ISLAND
KINGSTON, RI

8:00 – 9:00 AM	**CONTINENTAL BREAKFAST AND POSTER SET-UP**
9:00 – 9:30 AM	**WELCOMING REMARKS**
	- DAVID DOOLEY, PHD , PRESIDENT, UNIVERSITY OF RHODE ISLAND
	- STEFAN PRYOR, RHODE ISLAND SECRETARY OF COMMERCE
	- ZAHIR SHAIKH, PHD , RI-INBRE PROGRAM DIRECTOR
	- GERALD SONNENFELD, PHD, VICE PRESIDENT FOR RESEARCH AND ECONOMIC DEVELOPMENT, UNIVERSITY OF RHODE ISLAND
9:30 – 11:00 AM	**POSTER SESSION A (EVEN-NUMBERED POSTERS)**
11 AM - 12:30 PM	**POSTER SESSION B (ODD-NUMBERED POSTERS)**
12:30 PM	**Lunch**

EXHIBITORS

Located on the 1st Floor of the College of Pharmacy Building

Cores RI

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

www.coresri.org

Graduate Programs in Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island

As a leader in Pharmaceutical Sciences graduate education, the URI College of Pharmacy offers you programs and specializations in highly sought after fields available at few other universities in the country.

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Graduate School, University of Rhode Island

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https://web.uri.edu/gso/education/academic-programs53902-2/

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 manned for presentations according to the schedule below.*

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

Research Theme	Location
Cell Biology (CB)	CBLS, 1st Floor, "South Lobby"
Chemistry (Chem)	Pharmacy, 1st Floor Hallway
Genetics (Gen)	CBLS, 1st Floor "North Lobby"
Marine & Environmental Sciences (MES)	CBLS, 1st Floor Hallway
Microbiology (Micro)	Pharmacy, Room 130
Molecular Biology (MB)	Pharmacy, Room 105
Neurosciences (Neuro)	Pharmacy , Room 240

CELL BIOLOGY

LOCATED IN THE "SOUTH LOBBY" ON THE 1ND FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

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

Cell Signaling, Migration and Discrimination in *Entamoeba* spp.

Sara Hunt[1], Matthew Gabrielle[1], Meagan Hackey[1], Guillermo Paz-y-Miño-C[2], & Avelina Espinosa[1]

[1]Biology, Roger Williams University, Bristol, RI
[2]New England Center for the Public Understanding of Science, Roger Williams University, Bristol, RI

Entamoeba spp. are protists that belong to Archamoebae and comprise parasitic, free-living and commensal species. Clone-recognition experiments have been performed with seven *Entamoeba* lineages (*E. invadens* IP-1, *E. invadens* VK-1:NS*, E. terrapinae*, *E. moshkovskii* Laredo, *E. moshkovskii* Snake, *E. histolytica* HM-1:IMSS and *E. dispar*). This study examined whether aggregation and kin-discrimination among *Entamoeba* varieties is based on intra- and inter- strain chemical signaling. Trophozoites secreted extracellular signaling proteins into the growth media, which encouraged aggregation between cells of the same lineage. Based on these results, we hypothesized that migration strategies and signaling molecules secreted by *Entamoeba* varieties might be may be an important factor to pathogenesis. The migration of *Entamoeba histolytica* and *Entamoeba dispar* were analyzed using a three-chamber set up which allows for choice dispersal of trophozoites towards either the like species conditioned media or the conditioned media of another species. The cells were added to the center tube and cell migration was analyzed using cell counts after 24, 48 and 72 hours. The prediction is that the trophozoites would migrate towards the conditioned media of like species due to detection of signaling molecules in the conditioned media of 'kin or kin-like individuals. These experiments will continue with the identification of the signaling proteins released by the cells into the media. Future studies would be designed to block these signaling proteins if they are found to be essential for aggregation and pathogenesis.

Optimizing Chemotherapeutic Temporal Delivery Profiles Using Remotely Activated Biomaterials

Anne Reisch[1], Tania Emi[1], Tanner Barnes[2], Anita E. Tolouei[1] & Stephen Kennedy[2]

[1]Chemical Engineering, University of Rhode Island, Kingston, RI
[2]Electrical, Computer, and Biomedical Engineering, University of Rhode Island, Kingston, RI

Alone in 2016, the American Cancer Society estimates that more than 1.5 million new cases of cancer will be diagnosed. Modern treatments for cancer can involve intravenous cytotoxic drug delivery (i.e., chemotherapy). However, while the cytotoxins kill cancer cells, healthy cells in the body are also exposed to these toxins, often resulting in undesirable side effects. To reduce complications of chemo-therapies, we propose a more direct-to-tumor-site approach using drug-laden bio materials. These bio materials allow for a more direct drug delivery at the tumor site. However, traditional bio materials release drug by diffusion and produce unalterable, sustained tumoral drug concentrations. We hypothesize that these sustained deliveries could be improved upon by using stimuli-responsive bio materials to produce pulsatile deliveries. Thus, we tested to see if pulsatile chemotherapeutic deliveries killed cancer cells more effectively than sustained concentrations. Indeed, we found that pulsatile mitoxantrone deliveries destroyed B16F10 melanoma cells significantly more effectively than sustained deliveries. With these findings, we have altered the frequency of pulses to investigate what the optimal condition could be.

T Cell Proliferation

Zachary Caruolo[1], Jasmine Duong[1], Kirk Haltaufderhyde[2] & Alan Rothman[2]

[1]Cell and Molecular Biology, University of Rhode Island, Kingston, RI
[2]iCubed, University of Rhode Island, Kingston, RI

Peripheral Blood Mononuclear Cells sent in to our lab from the Oklahoma Blood Institute were frozen down in vials and stored in liquid nitrogen. Upon thawing, stimulation of T-Lymphocytes from the vials was hypothesized to result in proliferation that can be detected with a fluorescence measurement technique known as Flow Cytometry and analyzed using FlowJo software.

Methods from our experiment include the staining of PBMC's with Cell Trace Violet fluorescent dye, followed by stimulation of T-Lymphocytes in a 48 well flat bottom plate. The cells were then incubated before being stained again with a conjugate fluorescent antibody.

The findings of this experiment indicate that the stimulants we used were effective at causing proliferation of T-Lymphocytes. From these results we conclude that our stimulant was effective at generating a T-lymphocyte proliferation response

Detecting and Concentrating 4G2 and IgG in HB112

Jasmine Duong[1], Zachary Caruolo[1], Kirk Haltaufderhyde[2] & Alan Rothman[2]

[1]Cellular and Molecular Biology, University of Rhode Island, Providence, RI
[2]Institute of Immunology and Informatics, University of Rhode Island, Providence, RI

Dengue is a tropical and sub-tropical disease that is transmitted by the species *Aedes aegypti* and infects approximately 400 million people a year. With the increasing risk of a dengue infection, the demand to create a vaccine against dengue is exigent; however, due to antibody dependent enhancement, creating a vaccine that neutralizes all four dengue serotypes is problematic. In this experiment, we studied 4G2, a monoclonal antibody that binds to the fusion loop at the extremity of domain II of E protein on all four dengue serotypes, by growing and cultivating the supernatants of HB112 cells. HB112 cells were grown and passaged in a continuous culture of several media conditions and for several incubation periods, including a one, six, fourteen, and twenty-seven day incubation period. The Agilent Bioanalyzer and several ELISAs were used to identify and quantify the concentrations of total IgG and 4G2 being secreted by the hybridomas. After evaluating the antibody concentration value of the HB112 cells, we plan to purify and tag 4G2 for future research.

Exploring the Interactions between Single-Walled Carbon Nanotubes and Supported Lipid Bilayers to Effectively Model the Cell Membrane

Megan McSweeney, Mohammad Moein Safaee & Daniel Roxbury

Chemical Engineering, University of Rhode Island, Kingston, RI

The cell membranes of nearly all living organisms are composed of a complex phospholipid bilayer with embedded transmembrane proteins and carbohydrates. The solid supported lipid bilayer is a popular model used to study the surface chemistry of cells. These bilayer models can be engineered with increasing complexity to mimic the cell membrane, making them ideal tools for nanotoxicology studies. Single-walled carbon nanotubes have garnered significant recent interest in the field of biosensing, due to their intrinsic near-infrared (NIR) and uniquely photostable fluorescence, environmentally-sensitive emission, and enhanced biocompatibility. Here, we explored the physical and optical interactions between fluorescent single-walled carbon nanotubes and supported lipid bilayers comprising 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), one of the most common membrane lipids found in mammals. The quantity and nature of the binding was studied through the use of NIR fluorescence hyperspectral microscopy and intensity based measurements. Additionally, complete cell media containing fetal bovine serum (FBS), and bovine serum albumin (BSA), were introduced to examine the dependence on the nanoparticle protein corona affecting the process of binding. Hyperspectral images showed that the presence of proteins promoted binding to the lipid bilayer. The models will be made more biologically relevant through the addition of various lipids and transmembrane proteins. Understanding the kinetics and binding affinity these carbon nanotubes have to supported lipid bilayers is essential in optimizing future drug delivery and biosensor research.

Characterizing the Biochemical Pathway of Human DNA Polymerase Theta Mutants in Protein Finger and Thumb Domains

Miles Erickson[1], Lauren Gunasti[2], Marat Barnhart[1], Scarlet Santos[1] & Jamie Towle-Weicksel[1]

[1]Biochemistry, Rhode Island College, Providence, RI
[2]Biochemistry, Williams College, Williamstown, MA

Cancer describes diseases characterized by abnormal cell growth and replication. One of the costliest illnesses faced by humans, 15.7% of human deaths in 2015 were due to cancer and $1.16 trillion US dollars were estimated to be its worldwide cost in 2010. Melanoma, the most dangerous form of skin cancer, develops when DNA is damaged from UV radiation. When UV damage creates DNA structure-distorting thymine dimers, the excision repair pathway is one potential method utilized to repair the damaged DNA. If not repaired, these dimers can lead to unintended mutations that multiply as the cell grows and divides, eventually leading to the uncontrolled replication of skin cells. In healthy cells, DNA damage is repaired by highly specialized DNA repair enzymes including DNA polymerases. DNA Polymerase θ, or Polθ, (encoded by the *POLQ* gene) is a low fidelity DNA polymerase that may be involved in multiple DNA repair pathways, including double-strand break and base excision repair pathways, that promote genetic stability. Several *POLQ* mutants were identified from the tumors of patients with melanoma. We hypothesized that Polθ may be involved in the reconciliation of UV damage and that a poorly functioning Polθ may not be repairing the DNA damage correctly, resulting in mutations and eventually melanoma. This project highlights two patient-derived mutants, E2406K and T2161I, found in the finger and thumb domains of the protein, respectively. Mutants were generated using site-directed mutagenesis, expressed and purified in *E. coli*, and assayed for polymerase activity compared to wild-type Polθ to understand better the biological pathway of Polθ through the structural and behavioral differences of its mutants. Our results suggest that residues mutated in these variants play a critical role in DNA polymerase function in that E2406K and T2161I show different biochemical characteristics compared to those of wild-type Polθ under identical conditions.

Investigation of the Effects of Red Maple Leaves Extract and Its Purified Phenolic Compound on NFκB Pathway in Cultured Human Skin Keratinocytes and Fibroblasts

Jillian Higgins[1], Aileen Kraus[1], Matthew Clark[1], Benjamin Gallant[1], Dominic Arruda[1], Hang Ma[2], Navindra P Seeram[2] & Yinsheng Wan[1]

[1]Biology, Providence College, Providence, RI
[2]Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

Our previous studies have demonstrated that UV radiation-induced skin photoaging is associated with collagen degradation, dehydration, and inflammation. While the cellular and molecular mechanisms through which UV induces skin aging are still being unraveled, active compounds, including those natural products, are being sought to attenuate UV-induced skin cell damage and inflammation. Recent studies have shown that maple extracts having active ingredients may have beneficial effects for the development of cosmetics products. In this study, five samples including black cumin extracts (BCE), gennalin A (GA), Maple leave extracts (MAP), Maple syrup extracts (MSX), and thymoquinone (TQ, a major component of black cumin), were investigated whether those abstracts or compounds have effects on cytokine-induced activation of NFκB pathway, which is critical for UV-induced skin inflammation and skin aging. First, we used MTT assay to determine the proper concentration in cultured skin keratinocytes. We found that 5μg/ml is the concentration that does not affect cellular proliferation. Second, we treated both keratinocytes and fibroblasts with TNFα and IL1β (10ng/ml) and measured the degradation of IκB and NFκB subunit, p65 translocation from cytoplasm to nucleus, using confocal microscope and Western blot analysis. The results showed that both TNFα and IL1β induce IκB degradation and p65 translocation in both types of skin cells, while fibroblasts are more responsive to TNFα and IL1β. Under UV radiation, keratinocytes are activated and release cytokines that further activate skin fibroblasts. Third, we tested the effects of all five extracts or compounds on TNFα and IL1β p65 translocation and IκB degradation by confocal and Western blot. The results showed that p65 translocation was inhibited after administering 10μg/ml of the BCE, and inhibition improved as the dosage of BCE increased. IκB level was elevated at the following dosages of BCE 25 and 50μg/ml. In addition, p65 translocation appeared to be inhibited exclusively at 50μg/ml in fibroblast cells. IκB level was observed to be consistent as the dosage of BCE increased, suggesting that BCE may block IκB degradation in fibroblast cells, with a result of inhibition of p65 translocation. Other samples are still being further investigated. Therefore, we conclude that some of those maple extracts may be added to the cosmetics products to attenuate or prevent from UV-induced skin cell damage and inflammation.

Development of Hydrolytically Activated, Oxygen-Generating Biomaterials to Enhance Drug Efficacy on Cultured Ovarian Cancer Cells

Matthew Clark[1], Aileen Kraus[1], Jillian Higgins[1], Benjamin Gallant[1], Dominic Arruda[1], Xioafei Li[2], Wen Di[3] & Yinsheng Wan[1]

[1]Biology, Providence College, Providence, RI
[2]Biology, Brown University/Rhode Island Hospital, Providence, RI
[3]Biology, Renji Hospital/Shanghai Jiaotong University, Shanghai, China

Ovarian cancer remains the most prevalent type in gynecological cancer, with drug resistance still being a challenge. Our previous studies have demonstrated that ovarian cancer cells are resilient due to their active EGFR and AKT/mTOR pathways. Also, abundant data suggests that similar to other cancer types, ovarian cancer cells grow and proliferate remarkably well under hypoxia situation. We hypothesize that the application of oxygen to ovarian cancer cells may render unfavorable conditions, thus leading to the enhanced cell death, when combined with chemotherapy drugs. Recent studies have shown that polydimethylsiloxane (PDMS)-encapsulated calcium peroxide, or PDMS-CaO_2, potently deliver oxygen and eliminate hypoxia-induced cell dysfunction and cell death in normal pancreatic cells and other types of normal cells. In this study, we followed the published protocol, produced PDMS-CaO_2 disks and tested *in vitro* the effects of oxygen on cultured ovarian cancer cells (CaOV3 cells). The results showed that our manufactured disks sustainably release O_2 in a time-dependent manner. MTT assay results indicated that PDMS-CaO_2 disks induce cell death in an oxygen-dependent manner, compared to PDMS disks only. The combination of the inhibitors EGFR, MEK/ERK and AKT/mTOR with PDMS-CaO_2 disks increases cell death. Interestingly, PDMS disks significantly enhance the effects of cisplatin, oxaliplatin, and doxorubicin, but not that of paclitaxel or taxol. PDMS-CaO_2 disks inhibit exosome inhibitor GW4869 induced cellular proliferation. Also, those disks significantly augment ionophore monensin A (for Na^+/H^+ antiporter), and calcium ionophore A23187-induced cell death. Confocal microscope data showed that PDMS-CaO_2 disks alter mitochondria activities. Collectively, our data suggests that PDMS-CaO_2 disks releasing O_2 and inducing cell death may affect cell membrane ion channels and mitochondria activities. The combination of cancer drugs or ionophores or EGFR/MEK/AKT/mTOR inhibitors with PDMS-O_2 disks may provide novel approaches for ovarian cancer clinical management.

CHEMISTRY

LOCATED ALONG THE 1ST FLOOR HALLWAY OF THE PHARMACY BUILDING

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

Investigation of Waste Heat Accumulation and Internal Resistance of AA NiMH Cells for UUV Battery Applications

Daniel Donnelly[1], Charles Patrissi[2] & Thomas Arruda[1]

[1]Chemistry, Salve Regina University, Newport, RI
[2]Naval Undersea Warfare Center, Newport, RI

Nickel metal-hydride (NiMH) cells have been of considerable interest for the powering of unmanned underwater vehicles (UUV), primarily for the outstanding safety of their water-based electrolyte compared to the flammable organic solvents used in Li-ion cells. High energy and power densities, rechargeability, commercial availability, and low cost in the AA form further strengthen the NiMH case for integration into UUVs. While these factors are motivating, secondary cells generate heat during charge, which in excessive amounts is damaging to both the cell and nearby electronics. Our objective is to model the temperature (T) rise of NiMH cells caused by waste heat (Q) accumulation during battery charging according to the following relationship:

$$Q = R\int_{t1}^{t2}(I(t)^2)dt = mc\Delta T$$

Heat generation is a function of charge current (I), time (t), and the internal resistance of the cell (R). The galvanostatic intermittent titration technique (GITT) is being used to investigate resistance as a function of the current , temperature, and state of charge. Calorimetric measurements are ongoing to investigate specific heat of the cells (c). The change in cell temperature (ΔT) can then be modeled for various discharge currents and masses (m) of cells. A Thermal model of NiMH cells configured at the battery level may be used to determine the safety limits for charge rate with respect to heat production during charging in UUVs.

Electron Paramagnetic Resonance Spectroscopy and Electrochemical Investigations into the VO^{2+}/VO_2^+ Redox Couple for all Vanadium Redox Flow Batteries

Sophia Tiano[1], Jamie Lawton[2] & Thomas Arruda[1]

[1]Chemistry, Salve Regina University, Newport, RI
[2]Chemistry, University of Massachusetts, Dartmouth, MA

Redox flow batteries (RFBs) are promising solutions to large scale energy storage, in particular to leverage power generated by intermittent renewable resources such as wind and solar. Vanadium RFBS (VFRBs) work by storing energy in the form of vanadium ions in solution via the following reactions:

$$VO^{2+} + H_2O \rightarrow VO_2^+ + 2H^+ + e^- \quad (1)$$
$$V^{3+} + e^- \rightarrow V^{2+} \quad (2)$$

In the case of the VRFB, crossover of vanadium species from one side of the battery to the other causes significant issues including self-discharge, electrolyte imbalance and transport problems. Understanding the fundamental transport properties is essential to mitigating these issues and bringing the technology closer to market.

This study involves fundamental measurements of $VOSO_4$ solutions in sulfuric acid electrolyte. The effect of sulfuric acid concentration has been studied by electron paramagnetic resonance spectroscopy, electrochemical methods (cyclic voltammetery, chronoamperometry, Levich type treatment of linear sweep voltammetry and electrochemical impedance spectroscopy) as well as other physiochemical characterization techniques including viscosity. Results will include interpretation of the EPR spectra and electrochemical techniques and related values obtained for the hydrodynamic radius of the vanadium species under investigation.

Identifying the Structure of Molecules through MS/MS-Based Molecular Networking and Mass Spectrometry-Guided Isolation

Richard Belisle, Mathew Bertin & Christopher Via

College of Pharmacy, University of Rhode Island, Kingston, RI

Marine filamentous cyanobacteria and other marine microbes continue to be a remarkable source for potently bioactive secondary metabolites. The success of natural products in the therapeutic realm significantly depends on continued access to highly diverse potential lead molecules. Our laboratory is presently addressing this gap in accessing new molecular diversity by profiling the chemical space present in marine cyanobacterial blooms using the cutting-edge technique of MS/MS-based molecular networking in which molecules are clustered into families based on similarities in MS/MS fragmentation patterns. Our current project focused on identifying the structure of molecules within the MS/MS molecular network using mass spectrometry-guided isolation. Our network was comprised of metabolites from a bloom of the cyanobacterium Trichodesmium thiebautii collected from the Gulf of Mexico.. Chemical fractions containing our metabolites of interest were separated using HPLC with a gradient method and collected in time increments. Mass spectrometry was then performed on each sub-fraction, identifying metabolites from the network. NMR experiments partially characterized a chlorinated metabolite. Our results indicate that molecular networking is a useful way to visualize the chemical space in a sample and guide further isolation efforts. Future studies will focus on characterizing the complete structure of our molecule of interest.

Comparative Metabolomics of Gulf Coast *Trichodesmium theibautii*

Samuel Costa & Matthew Bertin

College of Pharmacy, University of Rhode Island, Kingston, RI

Trichodesmium is a genus of cyanobacteria that has previously been under-explored in regard to secondary metabolite composition. Our lab has previously characterized numerous unique metabolites from a 2014 *Trichodesmium theibautii* bloom in the Gulf of Mexico, with some of these metabolites displaying cytotoxicity to cancer cells. In our current study, we analyzed samples from three *Trichodesmium theibautii* blooms collected in 2014, 2015, and 2017 from the Gulf of Mexico off the Texas coast. Comparative 1H NMR revealed several similarities in samples from the two northernmost blooms, while the sample from the southernmost bloom was found to be quite different. Cell assays revealed that all three blooms contained chemical fractions which displayed cytotoxicity against cancer cells. We address the possible implications in the variation in bloom metabolites, as well as further avenues of study.

Synthesis of Biologically Inspired First Row Transition Metal Complexes Relevant to the Activation of Small Molecules

Julia Brown, Andrew Dillon, Andrew Josling & Daniel Zawacki

Chemistry and Biochemistry, Providence College, Providence, RI

A variety of important chemical reactions cannot be catalyzed by synthetic compounds or require the use of expensive metals, but are catalyzed with high efficiency by metalloenzymes, using less costly first row transition metals. Our research involves bioinspired design and synthesis of transition metal catalysts for the activation of small molecules. We seek to design new catalysts based on the principles used in nature, incorporating features commonly present in enzymes, and use these functional models to gain a better understanding of the mechanism by which enzymes operate and the features that are important in achieving high selectivity and efficiency.

Our first project focuses on the synthesis of new complexes based on the active sites of superoxide dismutase (SOD) enzymes, which catalyze the disproportionation of superoxide, a radical species associated with a number of diseases, to form dioxygen and hydrogen peroxide. The active sites of SOD enzymes all consist of first row transition metal centers, and in many of the active sites imino and thiolate ligands are coordinated to the metal. We will present results of our efforts to synthesize first row transition metal complexes with tridentate imino thiolate and imino thioether ligands.

In our second project, we seek to synthesize new iron □-diimine complexes, in order to evaluate the role of redox active ligands in proton and carbon dioxide reduction. In this case, we are inspired by the carbon monoxide- and formate-dehydrogenase enzymes, which catalyze reduction of carbon dioxide, and hydrogenase enzymes, which catalyze reduction of protons to form dihydrogen. We will present the results of our efforts to synthesize iron □-diimine complexes that incorporate either chloride or carbonyl ligands, as well as, preliminary electrochemical measurements.

HPLC Purification of a 24-mer DNA with FAAF-Carcinogen Adducts

Rachel Carley, Ang Cai & Bongsup Cho

Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

The human body has the capability to repair its own damage DNA. The repair process involves over 30 different proteins. A kind of important protein is XPC-RAD23B (XPC) in humans and Rad4 in yeast, which play a vital role in recognition of damaged DNA. XPC and Rad4, the yeast equivalent to XPC, have a unique function of a beta hairpin which is a thin needle like arm that is used as a DNA sensor when inserted in the DNA strand. These proteins are excellent at detecting bulky organic lesions that have attached to DNA. A common adduct attached to DNA to study cancer is N-(2-deoxyguanosin-8-yl)-2-acetylaminofluorene (AAF) and its fluorine derivative FAAF. These adduct are known to produce cancers in rodents. FAAF is used to produce adducts on the guanines of DNA sequences like the Narl sequence in a 24mer. The 24mer produces three mono-adducts, three di-adducts and one tri-adduct when exposed to FAAF. These adducts are purified with HPLC using a reverse phase column in an acetonitrile and triethylamine acetic acid buffer gradient mobile phase. There is current research on the structure and kinetics of these types of DNA repair proteins. DNA adduct purification is an important first step in X-ray crystallography and surface plasma resonance structural studies.

Competitive Binding of Xenon and a Hyper-CEST Inhibitor to a CB6 Derivative

Adriana Mendieta, David Robinson & Brenton DeBoef

Chemistry, University of Rhode Island, Kingston, RI

In this work, Xenon binding of cucurbit [6] uril (CB6) derivative Benz6C and a competitive inhibitor with respect to pH is studied. The templated synthesis of “open-chain” CB6 was used to form an easily functionalized scaffold for forming CB6 derivatives, in particular Benz6C, which shows greater water solubility than CB6 while retaining xenon binding attributes. The Xenon binding of Benz6C in sodium salt buffers of various pH was measured by Xenon-129 NMR and Hyperpolarized Chemical Exchange Saturation Transfer (Hyper-CEST) to determine the pH where a certain inhibitor leaves the CB6 and allows the Xenon to enter. This molecular switch has the potential for cancer imaging.

Exploring Metal-Binding Affinities of KmtR from *Mycobacterium tuberculosis*

Victoria Surette, Stephanie Lewis, Gina Swanson & Khadine Higgins

Chemistry, 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 was to explore the structure function relationships in KmtR. KmtR is the second Ni(II) and Co(II) responsive transcriptional regulator identified in Mtb. The duration of this summer consisted of metal binding studies to determine the metal-binding affinities of KmtR to cognate and noncognate metals.

Analysis of the Metal Binding Properties of the Metalloregulator KmtR from *Mycobacterium tuberculosis*

Gina Swanson, Victoria Surette, Stephanie Lewis & Khadine Higgins

Chemistry, Salve Regina University, Newport, RI

Mycobacterium tuberculosis (*M. tuberculosis*) is the causative agent of the disease Tuberculosis which infects the respiratory tract of nearly one-third of the world's population annually. Although few cases are reported in the United States, this disease is still prevalent in numerous countries and is responsible for the death of nearly two-million people each year. These deaths are the result of an increase in the number of multi-drug resistant strains of *M. tuberculosis*. Research with the long-term goal of finding new therapeutic strategies to treat Tuberculosis is important for protecting our world from larger-scale infection. *M. tuberculosis* has two metalloregulators that respond to nickel and cobalt indicating the importance of these metals to the bacteria. The metalloregulator being studied in this project is KmtR, the second nickel and cobalt responsive metalloregulator in *M. tuberculosis*, and the specific project goals include determining the coordination number and geometry of the metal sites and measuring the metal binding affinities of metal ions to this protein. The protein was cloned and expressed in *E. coli* BL21 DE3 cells. Metal binding studies are in progress to assess the overall ability of KmtR to bind the noncognate metal zinc as well as the cognate metals nickel and cobalt.

Mechanistic Investigations of Versatile H-Bonding Organic Catalysts for Biocompatible Polymers

Danielle Coderre, Kurt Fastnacht & Matthew Kiesewetter

Chemistry, University of Rhode Island, Kingston, RI

H-bonding urea and thiourea catalysts for ring-opening polymerization have been shown to increase the rate of polymerization and result in polymers with controlled molecular weights (Mn) and low polydispersity indexes while allowing for the incorporation of functional groups into the monomer feed, which is vital for the catalytic synthesis of biologically active polymers. As part of an ongoing effort to develop improved catalysts, fundamental studies were conducted to understand the origin of the catalysts' selectivity. The rate of polymerization of several of these H-bonding catalysts were investigated at temperatures from room temperature to 110^{o}C, showing both Arrhenius and non-Arrhenius behavior, depending on the catalyst. Additionally, the ratio of the rate of transesterification to rate of propagation (ktr/kp) was investigated to determine which catalyst afforded the most controlled ROP. The ratio was determined using a differential equations system in Igor Pro where rate equations were fit to experimental data. The results of these studies can be combined to determine the most controlled catalyst for the ROP systems, showing that it is possible to have both 'fast' rates of polymerization and controlled ROP.

Detection of Bisphenol A Glucuronidation in Enzyme Assays with Human Liver Tissue Fractions

Eric Evans, Adam Auclair & Roberta King

Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

BPA (bis-phenol A) is a plasticizer found in many plastic products. It has been shown to cause breast and prostate cancer, reproductive disorders and heart disease. BPA also effects egg maturation and fetal brain development in humans. BPA can interfere with the function of the hypothalamus and the pituitary gland. Prenatal exposure to BPA can contribute the development of asthma.

There are three methods the human body uses to pass BPA from its system, including glucuronidation, sulfonation, and amidation. These three enzyme-catalyzed reactions make the BPA more soluble and excretable from the body. Glucuronidation attaches a negatively-charged sugar molecule, glucuronide. Sulfonation attaches a sulfate functional group. Amidation attaches an amino acid such as glycine or glutamine.

The purpose of this project is to identify the concentration of BPA-G (bis-phenol A ß-glucuronide) in a human liver tissue sample with an inhibitor. The experiment is to determine whether or not medications interfere with the body's glucuronidation of BPA, resulting in retention of BPA. This will be accomplished by preparing samples using UDPGA, S9, BPA, pH 7.0 phosphate buffer and an inhibitor and quantifying how much of the BPA was converted into BPA-G in the presence of S9 and an inhibitor.

Initial separation of BPA and BPA-G mixture with the HPLC using 65% pH 2.7 buffer and 35% acetonitrile produced inconclusive results because the peaks of the BPA and the BPA-G were indeterminate. The HPLC method was adjusted to 85% pH 2.7 buffer and 15% acetonitrile. The change in method resulted in two distinct peaks with BPA-G eluting around 3.7 minutes while the BPA elutes around 5.5 minutes. The lower limits of detection for BPA and BPA-G were below 500 nM concentrations. Subsequently, the unknown BPA-G concentrations in the enzyme assay were determined by comparing the peak area of BPA-G with the concentration curve of the known standards.

Fluorescence Resonance Energy Transfer Studies of Terpyridine Based G4 Binding Structures

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Guanine rich DNA forms a secondary structure called G4-Quadruplex (G4 DNA) which can be found in the telomeric region. This secondary structure of DNA is known to play a role in the regulation of telomerase, an enzyme often overexpressed in cancer cells. G4 intercalators stabilize the quadraplex, inhibit telomerase activity and their potential as anticancer agents is thus the subject of active research. Phosphonium salts are known to have increased uptake by cancer cells. Using palladium complexes of tolyl-terpyridine that are well known G4 intercalators as our foundation, we explore the effect of a phosphonium tether on G4 DNA binding. The function of the phosphonium salts is to selectively target cancer cells while avoiding neutral healthy cells. Here in we present detailed fluorescence resonance energy transfer (FRET) studies as we try to assess the impact of the different components of our molecule (the metal center, phosphonium group, and tolyl-terpyridine ring) on DNA binding. Furthermore, we attempt to quantify binding affinities through titration studies.

Synthesis and Characterization of Novel Quadruplex DNA Binders Based on a Phosphonium-Tethered Tolylterpyridine Core

Dylan Rodrigues, Gary Marqus & Chin Hin Leung

Chemistry, Rhode Island College, Providence, RI

G-quadruplex DNA (G4 DNA) is a form present in guanine rich regions of our DNA and are known to play a role in the regulation of telomerase. Since telomerase is often overexpressed in cancer cells, G4 DNA binding as a way to kill cancer cells has been an area of active research. Aryl phosphonium salts are known to be very efficient at targeting cancer cells from the healthy cells. The phosphonium moieties also aid in the permeation of the molecule into the cells. Using a tolyl-terpyridine core, we incorporate phosphonium tethers into known G4 DNA binders. In this study we present the synthesis and characterization of a series of noble metal complexes as potential G4 DNA binders. Changing the substituents on the phosphonium and the identity of the metal center, our complexes present a range of steric and electronic properties

Detection of Organochlorine Pesticides in Contaminated Biological Systems via Cyclodextrin-Promoted Fluorescence Modulation

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The sensitive and selective detection of organochlorine pesticides in contaminated biological systems remains a high priority due to the pesticides' toxicity and carcinogenicity. The detection reported herein is facilitated by cyclodextrin, which acts to promote proximity-induced fluorescence modulation of three high quantum yield fluorophores when in close proximity to the pesticide, creating measurable, analyte-specific changes in the fluorescence emission. This method was tested at various temperatures and concentrations, and resulted in 100% success in differentiating between structurally similar organochlorine pesticides. The high selectivity of this method has significant potential in the development of new, practical detection devices for pesticides in complex biological and marine environments.

Detection of BPA in Marine Environments through Use of Conjugated Fluorescent Polymer Systems

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Reported herein is the use of conjugated fluorescent polymers for the detection of Bisphenol-A (BPA). Conjugated polymers were chosen for this detection due to their overall sensitivity and ability to detect organic compounds. The polymers were used both dissolved in solution and as thin films for the fluorescent detection of BPA at different concentrations. Through use of these conjugated polymer thin films, we aim to develop a highly sensitive smartphone compatible device that can be used at the consumer level in order to inform individuals of BPA content in everyday products such as water bottles and food cans.

Development of Drug-Loaded Acetalated Dextran Nanoparticles with Charged Coatings for the Treatment of Pulmonary Diseases

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Diseases affecting the lungs are some of the most common medical conditions in the world. Examples of these diseases include Asthma, Chronic Obstructive Pulmonary Disorder (COPD), and Cystic Fibrosis. The idea of using nanoparticles as drug carriers has gained much attention recently due to the improvements in pharmacokinetics of the loaded therapeutics. Also, targeted drug delivery to specified regions throughout the body can be achieved with the addition of nanoparticle coatings. It has been shown that positively charged coated nanoparticles are uptaken more easily into respiratory epithelial cells. However, once inside of the cell, negatively charged particles are known to traverse more efficiently through the cell cytoplasm than positive or neutrally charged particles. In this project, we developed drug loaded nanoparticles using a tunable biopolymer, acetalated dextran (Ac-Dex). The nanoparticles were coated with polvinyl alcohol (PVA) as the neutrally charged coating, the anionic biological lipid 1,2-dipalmitoyl phosphatidylserine (DPPS) as our negatively charged nanoparticle coating and chitosan as the positively charged nanoparticle coating. Coatings that exhibit different charges were explored and the benefits of each were examined. The nanoparticles were synthesized using a single emulsion technique and were separated by traditional centrifugation and high-throughput tangential flow filtration (TFF). The physicochemical properties of the nanoparticles (size, charge, drug loading) were evaluated using dynamic light scattering for size and charge, whereas drug loading was determined via fluorescence using curcumin as the model drug. So far, neutrally charged nanoparticles that were centrifuged exhibited a size of around 200 nanometers, whereas nanoparticles filtered using TFF showed lower size and dispersion values. Research is ongoing to determine the properties of the other charged particles.

Antagonism of Bacterial Cell-Cell Communication by Phevalin and Derivatives

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Antibiotics, which work by either killing or inhibiting the growth of bacteria, are causing a major problem in today's society. These mechanisms allow bacteria to evolve a resistance against antibiotics, rendering today's techniques of fighting bacterial infections inadequate. This pushed us to dive into the search for new ways to treat infectious diseases. Quorum sensing is the ability of bacteria cells to communicate with one another. Autoinducers, small molecules that help the bacteria communicate, are released into their surroundings sending information to other bacterial cells who are in proximity. These autoinducers bind and stabilize their receptor proteins, creating a ligand-protein complex that initiates transcription of quorum sensing genes, including virulence factor production. This quorum-sensing regulation of virulence and pathogenicity in bacteria creates a new target in fighting antibiotic resistance. Our long-term goal is to develop molecules capable of inhibiting quorum sensing in bacteria. We have developed a four-step synthesis of the cyclic dipeptide, phevalin, a known regulator of virulence factor expression in *Staphylococcus aureus*. Derivatives of phevalin have been synthesized easily by varying the amino acids that are coupled in the first step of the synthesis. To explore the anti-quorum sensing effects of our compounds, we tested their ability to inhibit bioluminescence, a quorum sensing phenotype in *Vibrio harveyi* BB120, and metalloprotease production, a quorum sensing phenotype in *Vibrio coralliilyticus* RE22. Preliminary experiments show that phevalin is an inhibitor of both luminescence and metalloprotease production. Structure-activity studies of the phevalin derivatives are ongoing in our laboratories.

Transesterification and Bacterial Quorum Sensing Inhibition of Beta-Keto Esters

Rachel Ruest & Susan Meschwitz

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A major problem in today's world is the presence of antibiotic resistant bacteria, which have developed due to the overuse of antibiotics. Most antibiotics work by killing the bacteria, which allows for the bacteria that survive to evolve into resistant strains. As such, research is being done to find other ways to fight these resistant bacteria without promoting resistance. One alternative is through the inhibition of quorum sensing (QS) pathways, which is how bacteria communicate with each other. Quorum sensing is mediated through the production and detection of chemicals called autoinducers. Once enough cells produce enough autoinducers, the quorum is met which signals to the bacteria to express certain genes necessary for infection of a host, including virulence factor production, biofilm formation, and swarming. The aim of this research is to synthesize and test molecules similar in structure to autoinducers that interfere with the QS pathway by inhibiting the autoinducers ability to bind to the receptors on bacteria. Previously, our lab has shown that ethyl beta-keto esters containing a substituted phenyl ring are capable of interfering with QS by binding to the bacteria's receptor and blocking the autoinducer. This research has expanded upon these findings by putting the beta-keto esters through a transesterification reaction with various substituted benzyl alcohols, replacing the ethyl group on the ester. These compounds were tested using a broth dilution assay in a wild type strain of *Vibrio harveyi* for their ability to inhibit luminescence, a phenotype controlled through QS, and IC 50s were calculated. These molecules are hoped to be useful tools that can one day lead to the creation of anti-pathogenic medications.

New Methodology Using Nitrogen-Containing Intermediates

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The focus of our research is the development of new methodology in organic synthesis. The first of four projects uses palladium and rhodium to catalyze the formation of beta-carbolines in a minimum number of steps using alkynamides as substrates. We have been screening various factors, such as solvent, ligand, temperature, stoichiometry, and time in order to optimize the reaction yield. A wide substrate scope will be presented. Similarly, a second project will showcase a palladium-catalyzed synthesis of alpha-carbolines, this time involving cyanamides as substrates. A third project will highlight new techniques for the preparation of annulated delta-carbolines. Finally, we will describe the fluorohydration of alkynamides as a new method for the preparation of chiral fluorine-containing molecules.

Multiplex Electrochemical Detection of IL-6 and IL-22 Biomarkers Using a Microfluidic Device

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Rapid, extremely sensitive, and accurate biosensor arrays for clinical measurements of biomarker proteins for early detection and monitoring of cancer are critically important. This development will lead to inexpensive devices for reliable on-the-spot cancer diagnosis, improved therapeutic outcomes at lower costs, decreased patient stress, and new targeted therapies. Herein, we are developing a microfluidic-based immunosensor array coupled with PEG modified multi-labeled magnetic beads for high sensitivity multiplex electrochemical detection of interleukin-6 and interleukin-22 biomarker proteins in calf serum. Higher levels of IL-6 and IL-22 in patients indicate that the patient may have various types of cancer, such as Cutaneous T-cell lymphoma (CTCL), a form of cancer that primarily affects the skin. The sandwich immunosensor relies on attaching a primary antibody on a nanostructured 8-electrode array followed by binding a PEG modified magnetic bead bioconjugate with off-line captured antigen (HRP/MB/Ab2-Ag)-PEG in a microfluidic device. Injecting the microfluidic system with hydrogen peroxide ignites a catalytic reduction between hydrogen peroxide and HRP to create an electrical signal that correlates to the concentration of antigen in the sample. Results show a detection limit of 50 fg/mL for IL-22 and IL-6 in calf serum. These preliminary results show great promise of a point-of-care diagnosis system that will allow simple and early detection of CTCL.

Development of in Field HPLC-Fluorescence Detection of Thiocyanide to Combat Illegal Cyanide Fishing

Holly Eberlin & Steven O'Shea

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Harvesting of tropical ornamental fish by dispersing sodium cyanide into the environment to render fish unconscious, making them more easily collected, is an illegal practice. Upon ingestion by the fish, the primary excretory metabolite is the thiocyanate ion (SCN^-). To address whether cyanide has been unlawfully used a field instrument has to be developed that will have a rapid response to low detection limit of SCN^- in water containing the harvested fish. This research developed a HPLC UV/Vis and fluorescence detection of water-soluble metalloporphyrin SCN^- complex. Simple free metal SCN^- coordination using Fe (III) ion was successful but not sensitive enough to low (ppb) SCN^- concentrations as well as being susceptible to hydrolysis. To circumvent hydrolysis, metalloporphyrins were successful in co-solvent (Saltwater:DMF) coordination of SCN^- and could be characterized by UV-Vis and the more sensitive fluorescence spectroscopy. To enhance the concentration of seawater soluble metalloporphyrin SCN^- complex from field studies, simple SPE (C18 ODS) allows for characterization of both non-coordinated and coordinated metlloporphyrin by HPLC fluorescence detection.

Sediment Core Depth Profile Enhanced Degradation of CFCs by Biotic and Abiotic *in situ* Stimulation

Natalie Gambrell, Colby Masse & Stephen O'Shea

Chemistry, Roger Williams University, Bristol, RI

Though there has been a dramatic decrease in anthropogenic CFCs, they are still having a significant effect on global warming by further compounding the feedback release from their aquatic environment sink. Core samples from marine and terrestrial sites were assessed for the pore water composition and their microbial metabolic *in situ* enhanced oxidant spiked bioremediation of halo hydrocarbons (HCs) in an anaerobic chamber with oxygen and carbon dioxide gas sensors. The headspace gases were elucidated by GC/MS. The mechanistic HCs degradation pathway can be eluded to by oxidation-reduction potential (ORP) of the environment and its pH, shedding light on *in situ* metal oxidation states' and the potential microbial communities principle. Catabolic oxidants and the bacterial succession order, following submergence of a sediment, directly matches the order of decreasing potential for the corresponding redox couples: O_2 /H_2O, NO_3^-/ N_2, $MnO_2(s)$ / $Mn^{2+,}$ $Fe(OH)_3(s)$ $^-$/$Fe^{2+,}$ SO_4^{2-}/HS^- and CH_2O/CH_4. This succession allows the selected spiking of sectioned core sediment microbial oxidant to illicit enhanced microbial activity towards HCs. Release of the halide ion from HCs monitored by HPLC-IC and 9F NMR and GC/MS following the volatile organic degradation by products.

Assessing Legacy Pollution by Shell Surface XRF Analysis of Sedentary Shellfish

Shayne Green & Stephen O'Shea

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The release and leaching of metal pollutants into coastal and estuarine environments has been greatly curtailed, though capped by deposition these contaminated sediments can still reenter the ecosystem by natural perturbation or human activity. To continuously monitor the extent of these pollution events and establish legacy polluted depth profiles would be cost prohibitive. To circumvent this problem, this research investigated the ability of the native bivalve *Mercenaria mercenaria* (quahog) as a natural bioindicator. The quahog has adapted its habitat from open ocean to freshwater environments and because of their abundance, sedentary borrowing habitats, long lifespan (>10yrs) make them ideal *in-situ* biomonitors. The quahog has ability to bioconcentrate heavy metals into their shells and has the ability to exchange/adsorb them on their outer shell surface at levels that exceed those present in surrounding pore-water and benthic sediments. Quahogs were harvested from eight sites in the West Passage of Narragansett Bay and were shells were assessed by X-ray fluorescence spectroscopy for their heavy metal content and their relationship to benthic sediment metal concentrations. The cation exchange capacity of the outer surface shell at various buffered pHs (2.5, 4, 5 and 8) were determined to access heavy metal rerelease into the water column. The pH range reflecting the changing sediment environment profile encountered by the burrowing bivalve were also spiked with various concentrations of Cu, Pb and Cd ions to establish their binding absorption/adsorptive properties. These relationships have further aided in the understanding of the possible bioaccumulation of heavy metals and the health of benthic bivalves in sediments of a dynamic pH changing estuarine environment.

Characterization of *in situ* Marine Core Sediment and Pore Water Degradation of CFCs

Colby Masse, Natalie Gambrell & Stephen O'Shea

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Understanding microbial metabolic and abiotic degradation pathways of natural halo-carbons (HCs) is important not only from a climate perspective but also for what it tells us about the overall balance of HCs biogeochemical cycling and their release into the ecosystem. This research was conducted in order to further understand the *in-situ* transformations of HCs in core ocean sediment and wet terrestrial samples under various oxidation/reduction potential conditions. The analysis inoculated core sectioned samples under inert atmosphere and rhizon filtered pore water by ion chromatography elucidate the potentially primary micro organismal metabolic catabolic and/or abiotic oxidant degradation pathways. Headspace GC/MS analyses give evidence of organic HC degradation byproducts. Development of a new technique, 9F NMR *in situ* determination metabolites and the release of fluoride ion were used to further investigate degradation of alkyl fluorocarbons, the replacement of HCs.

Microwave-assisted Diamide Synthesis for the Construction of a Panel of Antimicrobial Compounds

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Streptococcus pneumoniae is a Gram-positive bacteria that is a causative agent of pneumonia, meningitis, and other infections in humans. Due to the misuse or over-prescribing of common antibiotics, there is an increase in drug resistant strains. The antibiotic resistance of *S. pneumoniae* calls for a need of new compounds to be developed to be used as antibiotic agents for treatment. Research has shown that certain diamides exhibit antimicrobial properties. These compounds are produced using the Ugi reaction, in which an aldehyde, amine, isocyanide, and acid are combined. Using a modified, step-wise Ugi reaction, a library of existing compounds have been cataloged in our laboratories. These diamides have been characterized for their antibacterial properties against Gram-positive bacteria such as *Bacillus subtilis* and *Streptococcus pneumoniae*. The use of a microwave in the synthesis process effectively speeds up the reaction time while maintaining comparable yields to the standard method. Microwave-mediated parallel synthesis also allows for the rapid generation of a library of compounds. It is believed that these benefits make up for the moderate decrease in product yield as compared to the traditional method. The purpose of this research study is to optimize the synthetic process of a known inhibitor fgkc using a microwave, as well as to produce a library of analogs to our *S. pneumoniae* lead compound fgbb (MIC 11.3 ± 6.1 μM, *S.pneumoniae*) through variation of the aldehyde. Synthetic conditions were optimized using the diamide fgkc (MIC 3.1 μM, *B. subtilis*), a lead compound in our laboratory known for its antibacterial properties against *B. subtilis*. A series of experiments tested the order of addition of reactants, temperature, time, and solvent of the reaction. The optimized microwave-assisted method produced the compound fgkc with 54% yield in 1 hour 14 minutes. Conversely, the original method of synthesis makes the same compound at 68% yield in roughly 6 hours. Using this optimized method of synthesis, fgbb analogs, another known inhibitor, were made using aldehyde variables. These compounds have been purified and characterized, and will be screened against various Gram-positive bacteria.

Probing the Internalization and Photoluminescence Response of DNA-Carbon Nanotubes in Multicellular Tumor Spheroids

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Multicellular tumor spheroids serve as useful *in vitro* models by mimicking *in vivo* disease conditions and can help to predict the complex interactions between tumor targeted nanoparticles delivering a chemotherapeutic payload. Biocompatible preparations of single-walled carbon nanotubes can be utilized to observe transient biological processes due to their near-infrared (NIR) tissue-penetrating, environmentally sensitive, and non-photobleaching photoluminescence (fluorescence). Additionally, their surface can be easily functionalized to impart targeting ability and the capability for drug delivery. Here, we investigated the internalization behavior as well as the photoluminescence response of nanotubes interacting with A549 lung carcinoma tumor spheroids. Photoluminescence intensities from the nanotubes within the tumor spheroids were imaged after varying the time of incubation, concentration, and surface functionalization of the nanotubes, providing information regarding the ability of carbon nanotubes to penetrate A549 lung carcinoma tumor spheroids. Due to the dense extracellular matrix, containing large amounts of collagen protein, co-administration of the nanotubes with collagenase enzyme was used to enhance the penetration of nanotubes into the tumor spheroids. In the future, direct conjugation of collagenase to the carbon nanotubes will be explored to create a nanoparticle system capable of penetrating both the extracellular matrix and the tumor, which could significantly enhance drug delivery.

Computational Discovery of 1,1,1-Triarymethyl, ”Trityl,” Compounds as Potential Anticancer Agents

Dariusz Puzanowski, Stephan Gamboa & John Williams

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Triphenylphosphonium (TPP) salts are lipophilic cations that passively transfer across cell membranes and are drawn into mitochondria by the negative membrane potential. TPP’s have shown anticancer activity themselves against human cancer cells. They can also act as carriers for molecules known or predicted to have anti-cancer activity. Neutral non-phosphorus containing triarylmethyl,“trityl,” compounds have been identified as significant and selective anticancer agents that act at the levels of cell cycle arrest, inhibition of tubulin polymerization, by dissociating mitochondrial hexokinase, and inhibition of calcium-dependent potassium ion channels. This research investigates structural variations of the triphenylmethyl scaffold with scoring for bioactivity of molecules with physical properties within the Lipinki parameters. We are using the online docking software, Molinspiration® and Muse Invent,® a proprietary software program (Certara, Inc.). Molinspiration calculates Lipinski properties and scores bioactivity for six classes of protein. Muse Invent applies a genetic algorithm to a starting scaffold and generates molecules that fall within the Lipinski range of “druglikelyness,” generates a synthesis path to each, and docks molecules to proteins from the PDB. These two computational approaches have identified several “compounds of interest” that are feasible synthesis targets. These will be prepared and screened for activity against mouse and human cancer cell lines.

Synthesis of 1,1,1-Triarylmethyl, “Trityl,” Druglikely Molecules

Shawn Contente, Dariusz Puzanowski, Eva Lincoln, Tayla Carvalho & John Williams

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Modification of a standard undergraduate organic chemistry experiment was employed to prepare lead compounds for preliminary toxicity studies in bacteria and cell culture and for DNA binding. The 1,1,1-triarylmethyl, “trityl,” moiety is a common scaffold or structural feature in a variety of anti-cancer agents. We prepared modified triphenylmethanols using Grignard reactions with substituted benzophenones. Ortho and para hydroxy benzophenones were reacted with a 2:1 molar excess of Grignard reagent to give the dibasic trityl compounds which upon acid workup gave the trityl alcohol with a re-protonated phenol. The products were identified by melting point, IR, MS and NMR. These can be modified to make ethers and esters at the trityl alcohol functional group and phenolic ethers or esters at the ortho or para phenolic function. This reaction sequence gives compounds which can lead to a variety of target molecules that fall within the Lipinski envelope and whose predicted bioactivity properties can be calculated with available software.

1,1,1-Triarylmethyl, “Trityl,” Alcohols Bind dsDNA

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The 1,1,1-triarylmethyl, “trityl,” pharmacophore is a ubiquitous feature in a variety of anti-cancer agents. We prepared, by modification of an organic chemistry laboratory experiment, or purchased, triphenylmethanols. Their ΔTm’s for calf thymus dsDNA were determined by monitoring the 260nm absorption band of the DNA. Molecular mechanics calculations using HyperChem showed one or more stable complexes by both or either intercalation and/or major-groove binding. The interactions responsible for these calculated DNA-TPM complexes are presumed to be stabilizing the double strand DNA in solution and thereby raising the melting temperature. DNA binding is our assay for selecting compounds for further study. The compounds which show significant positive shifts of ΔTm’s will be analyzed by DNA gel electrophoresis, Kirby Bauer assays and in cell culture against mouse mammalian normal and cancer cells.

GENETICS

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Genetic Variation and Speciation in the North American Fire Ants Based on Morphology and Mitochondrial DNA

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Evaluating levels of genetic diversity within a species across its geographic distribution is important in understanding speciation and hybridization dynamics. The fire ants (Hymenptera: Formicidae, genus *Solenopsis*) are an economically important group that is widespread in the western hemisphere. Within *Solenopsis*, there are 20+different species in South America, with a subset of several species in North America distributed across various southern and southwestern states Mexico. The North American species are morphologically cryptic and are not well defined. Additionally, one of these putative species, *S. xyloni*, has evolved a unique life history stradegy. In Texas where *S. xyloni* and *S. geminata* overlap exists, there is a hybrid zone with no genetic introgression. Outside of the hybrid zone, *S. xyloni* queens are capable of producing worker and reproductively destined female offspring when mated to a *S. xyloni* male. In the hybrid zone, *S. xyloni* queens mated with *S. geminata* males produce hybrid, sterile workers, but not reproductive offspring. Interestingly, in the hybrid zone *S. xyloni* queens mated with *S. xyloni* males only to produce reproductives, and are unable to produce worker offspring.

The goals of the study were two fold, to determine if patterns of genetic differentiation supports previously published papers, morphological species descriptions, and to evaluate if hybrid zone populations of *S. xyloni* are evolutionarily independent from other populations, with greater genetic divergence than expected due to geographic distance alone. Solenopsis workers were collected from across the southeastern U.S between 2007-2016. The ants were then identified based on morphology by leading taxonomists. To evaluate genetic variation, DNA was extracted from individual workers using chelex, and the mitochondrial genes cytochrome B and cytochrome oxidase 1 were amplified using PCR and the amplicons were then sequenced. Sequences were edited and aligned using Geneious 6.1.8. Phylogenetic trees were produced using the programs POY and Mr. Bayes.

Identifying Key Genes Involved in Halting the Cell Cycle of the Immortal Gastric Adenocarcinoma AGS upon Treatment with Gallic Acid Using Affymetrix Microarrays

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Gastric cancer is the fifth most common cancer and the third most deadly worldwide. Current available treatments, such as chemotherapy, radiation, and surgery, are accompanied by detrimental side effects. Nutraceuticals, naturally occurring plant compounds, offer a promising area of research to develop new treatments. One such phenolic compound found in berries, gallic acid, has been shown to selectively target gastric cancer cells for anti-proliferation and apoptosis while leaving non-cancerous cells unaffected. The goal of our study was to identify key genes and pathways targeted by gallic acid treatments in an immortal gastric cancer cell line. We predict that genes with the highest levels of differential expression will associated with pathways involved in halting cell cycle and apoptosis. In this study, immortal AGS cells were treated with a negative DMSO control and 100 µM concentration of gallic acid for 6, 12, and 24 hours, with each time point replicated in biological duplicates. Affymetrix human transcriptome arrays (4.0) were utilized to compare genome expression profiles between untreated and treated AGS cells across all time points, and heat maps were constructed to visualize fold change in single genes over time using Java TreeView software. Our results suggest that genes involved in cell death and apoptotic pathways exhibiting significant upregulation or downregulation play key roles in mitigating gastric cancer upon exposure to gallic acid. These results suggest that exposure to gallic acid affects expression of genes associated with the cell cycle. In future research, quantitative PCR can support the role of these genes in cell death. Understanding the genetic targets of gallic acid may potentially lead to alternative, non-invasive treatments for gastric cancer.

The Effect of Varying Doses of Gallic Acid on Gene Expression in the Immortal Adenocarcinoma Gastric Cancer Cell Line [AGS]

Megan Johnstone & JD Swanson

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Cancer is a global health issue affecting millions of people, and is one of the leading causes of death worldwide. More specifically, gastric cancer is currently the fifth most common cancer worldwide, and treatments are nonspecific and invasive. Due to common harmful side effects of treatments, there is a large need to find alternatives. Nutraceuticals, naturally occurring plant compounds with human health and medicinal benefits, offer a potential avenue of investigation. One nutraceutical in particular, gallic acid, is a phenolic compound found in high concentrations in berries such as blackberries and raspberries and has been shown to have anti-prolific effects specific to cancer cells. The purpose of this study was to determine the effect of gallic acid on gene expression in the immortal adenocarcinoma gastric cancer cell line AGS. Cultured AGS cells were passaged into 6-well plates, established for 48 hours, serum-starved for 48 hours to sync all cells in the G1 phase of the cell cycle, and then treated with various concentrations of gallic acid (0, 20, and 100µM) for the following time durations: 0, 3, 6, 12, 18, 24, 36, or 48 hours. Following treatment, RNA was extracted using TRIzol and phenol-chloroform. RNA was converted to cDNA, which was standardized to 100 ng/µL, and qPCR was used to determine changes in gene expression compared to the constitutively expressed housekeeping gene *B2M*. Changes in gene expression were evaluated in genes associated with cell cycle regulation (*P21*, *CyclinD1*, *Cdk4*, and *Cdk6*), apoptosis (*Bax*, *RhoB*, and *Bcl2*), and tumor angiogenesis (*MMP9*). We expected that if the response to gallic acid is dose dependent, we would see the largest changes in gene expression in 100uM treated cells compared to 0 and 20µM treated cells. Based off of previously published roles of these genes, it was expected that *P21*, *Bax* and *RhoB* would be up-regulated and *CyclinD1*, *Cdk4*, *Cdk6* and *MMP9* would be down-regulated upon treatment with gallic acid.

Characterizing Branching Pattern in *Drosophila* Motor Neuron

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Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disease that is characterized by the degeneration of the lower and upper motor neurons. Mutations in superoxide dismutase cause familial ALS in humans with the mutations showing a dominant pattern of inheritance. Previously, we introduced ALS disease causing mutations into the *Drosophila SOD1* gene at conserved sites by homologous recombination. Flies recessive for *sodH71Y* alleles live about 15 days post eclosion and exhibit axonal die-back. To study progressive motor neuron changes, the cuticle on the legs were dissected off and the legs and stained HRP a neuronal marker. Our data to be presented quantifies progressive changes in axonal morphology of a previously characterized neuron, from lineage A of the femur, in *H71Y* homozygotes using Sholl analysis, a program that analyzes branch length, number and pattern from digitized images.

Characterizing Axonal Degeneration in a *Drosophila* Model of ALS

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Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disorder that results in paralysis, and ultimately leads to death an average of 3-5 years after diagnosis. In the familial form of ALS, mutations in the superoxide dismutase (*sod*) gene cause the disease. SOD is an enzyme found in many organisms who live in the presence of oxygen and the purpose of this enzyme is to protect the individuals from oxygen toxicity. We have used human disease-causing mutations, such as *H71Y*, in a *Drosophila* model and examined the effects of these mutations across the lifespan of these animals. We are using immunohistochemistry and confocal microscopy to investigate the progressive neurodegeneration seen in 0, 3, 6 and 10 day *Drosophila*. Specifically, we are looking at differences in axonal projections, synaptic bouton size and number as well as overall morphology of the motor neurons in several well characterized motor neurons in their metathoracic legs. Discs-large (*dlg*) is a gene that produces a protein (DLG) which is present at the neuromuscular end plate at the neuromuscular junction. Changes in the neuromuscular junction are characterized by changes in the morphology of synaptic boutons and the reduction of DLG expression post-synaptically. Our results show that *sodH71Y* have a progressively reduced level of DLG expression and show dramatic change in synaptic morphology over time.

Human SOD1 Rescues Disease Phenotypes in a *Drosophila* Model of ALS

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Amyotrophic lateral sclerosis (ALS), is a rapidly progressing neurodegenerative disease. Afflicted individuals have a life expectancy of 3-5 years after diagnosis. Mutations in the superoxide dismutase gene (*SOD1*) cause familial ALS in humans and show a dominant pattern of inheritance. Our previous work developed an ALS model in *Drosophila* by knocking-in mutations into the *Drosophila sod* gene. Mutant *sod* flies show ALS related phenotypes including shortened lifespan, motor neuron degeneration, and oxidative damage. Furthermore, *Drosophila* phenotypes are recessive, while human disease phenotypes are dominant. We are testing the hypothesis that amino acid differences between human and *Drosophila* SOD1 cause the dominant versus recessive patterns of inheritance. To test this hypothesis, we have created a humanized *Drosophila sod* stock. Flies carrying human SOD1 coding sequences replacing the endogenous *sod* gene were characterized as a first step. To assess human SOD1 (hSOD1) protein expression, Western blot analysis probed with a hSOD1 specific antibody confirmed that *Drosophila* were expressing hSOD1. Furthermore life-span analysis showed hSOD1-expressing flies rescued G85R lethal phenotypes. Genetic and biochemical analysis show that humanized SOD1 expressed in *Drosophila* is functional.

Determination of Differential Gene Expression in the Immortal Gastric Cancer Cell Line MKN-28 Treated with the Nutraceutical Gallic Acid Using Microarrays

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Gastric adenocarcinoma (stomach cancer) is the third most deadly cancer, with the five-year survival rate at stage IV being only 4% worldwide. Current treatments include surgery, chemotherapy, and radiation, all of which harm non-cancerous cells. Nutraceuticals, naturally occurring plant compounds, offer a potential area of investigation towards a safe and non-invasive treatment. Gallic acid, a phenolic compound found in raspberries and blackberries, has been shown to target cancer cells by halting cell cycle progress. To test the hypothesis that key genes coding for cell cycle progression in cancer are affected by gallic acid, the changes in gene expression associated with exposure to the treatments in the immortal cell line MKN-28 were analyzed through Affymetrix microarrays. MKN-28 cells were cultured and treated with 0 or 100µM of gallic media and collected at 0, 6, 18, 24, 28, and 36 hours. RNA was extracted, evaluated for quality, then hybridized to microarrays. Differential expression of treated versus untreated cancer cells over the time series was determined using Cluster 3.0 and Java Treeview. Current genes being tested with qPCR are *BAX1*, *MMP9*, *P21*, *BCL2*, *Cyclin D1*, *CdK4*, *CdK6*, and *RhoB*. The heat maps show the extent to which each gene is being up or down regulated by the gallic acid treatments over time. By analyzing the above genes and looking at genes that are co-regulated, the heat maps can be used to identify new molecular targets affected by gallic acid treatments, which can be confirmed in the future using qPCR. Studying the mRNA of gastric cancer cells will allow greater focus on target genes that could trigger cell death in cancerous cells and lead to a non-invasive cure for this deadly disease.

CoresRI.org

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CoresRI (www.CoresRI.org) is a directory of core facilities, services and instrumentation in Rhode Island. Development of CoresRI grew out of a need to maximize awareness and optimize utilization of these important core facility resources within the state. Operational since September 2014 and updated at least annually, CoresRI has grown to include 709 listings in 32 categories at 62 facilities at 15 institutions and 22 centers within Rhode Island. The web portal catalogues instruments (specific makes, models, and uses), services, locations, and contact personnel pertaining to each core or facility. Investigators at Lifespan and all participating institutions have full access to the listed facilities, equipment, and expertise via CoresRI.org. Most of the equipment resides in Core Facilities that are either free-standing or operated within the NIH Centers of Biomedical Research Excellence (COBREs), NIH IDeA Network of Biomedical Research Excellence (INBRE) or NSF Experimental Program to Stimulate Competitive Research (EPSCoR). Besides encouraging equipment sharing and reducing duplication of services, CoresRI.org fosters collaborations and enables investigators to better assess future shared equipment needs. The institutions participating include Brown University, Bryant University, Butler Hospital, Community College of Rhode Island, Providence College, Rhode Island College, Brown University/Rhode Island Hospital, State of Rhode Island, The Providence VA Medical Center, Roger Williams Medical Center, Roger Williams University, Salve Regina University, and the University of Rhode Island.

MARINE & ENVIRONMENTAL SCIENCES

LOCATED ALONG THE HALLWAY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

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

Assessing Benefits Associated With Group Size in *Solenopsis invicta* Exposed to the Fungal Pathogen *Metarhizium anisopilae*

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Although living in a social group is associated with potential benefits, there are also potential costs, such as a risk of increased disease transmission among group-members. In terms of disease, benefits and costs of group size are also controversial; larger groups hypothetically incur a cost as transmission rates may be higher, but also putative benefits, as social interactions may increase survivorship following exposure. Members of the fire ant genus *Solenopsis* are excellent model organisms to observe disease transmission among social organisms, with results easily translatable to human societies. The fire ant species *Solenopsis invicta* is particularly well suited, as this species is characterized by large colony sizes, frequent social interactions, strong division of labor, and continual exposure to soil pathogens in their native habitat. We tested the role of group size on survivorship in the fire ant *Solenopsis invicta* by exposing workers in various group sizes to a generalist entomopathogenic fungus, *Metarhizium anispoliae*. We hypothesized that if group size does not affect disease severity, than mortality rates will not differ significantly among groups of different sizes. However, if group size is associated with reduced disease severity, than ants in groups with more individuals will have lower mortality than those with fewer individuals. We exposed a single small-bodied worker, marked with blue modeling paint, to spores of the fungus *Metarhizium anisopliae* placed the ant into groups of unaffected ants of ants of various sizes (total group sizes: N=1, 3, 7). Each group size was replicated 9 times and control groups that exactly duplicated treatments in all ways except spore exposure were also replicated 9 times. Survivorship was recorded for 7 days, and dead ants were surfaced sterilized, cultured and evaluated for evidence of fungus to determine if death was due to *Metarhizium anisopliae* exposure.

Quantifying Dermo Resistance and Tolerance in Eastern Oysters

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Parasitism is the most common life strategy observed in nature. Since parasites always negatively impact their hosts, there is strong selective pressure for hosts to defend against infection. There are two fundamental strategies for hosts to defend against parasitic infection: resistance, or the prevention of initial infection or parasite proliferation once infection occurs; and tolerance, or the maintenance of host fitness once infected. Since all traits come at some physiological cost, do resistant hosts also tolerate disease? This summer I worked with scientists at the USDA Agricultural Research Service and the University of Rhode Island on laboratory experiments designed to characterize and quantify resistance and tolerance traits in oysters exposed to the protozoan parasite *Perkinsus marinus*. This water-borne parasite is the causative agent of Dermo disease, a significant cause of mortality in wild and cultured oysters along the Gulf and Atlantic coasts. Our research aims to understand how resistance and tolerance traits covary and shape the pathology and spread of Dermo disease.

Fostering Increased Awareness and Understanding of Marine Organisms and Ecosystems by Means of Personal, Place-based Narratives and Online Platforms

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Increasing public engagement on issues of marine science and marine conservation is a persistent challenge. Scientists often struggle to effectively broaden awareness and understanding of marine ecosystems and encourage positive shifts in public attitudes. By combining the unique perspectives and talents of both scientists and artists, we expect to achieve greater gains in these efforts. Our approach makes use of strategies grounded in conservation psychology and marketing, namely: 1) taking a place-based approach; 2) using a narrative structure to appeal to our inherent affinity for stories; and, 3) encouraging a personal, creative response to experiences in the marine environment. Across coastal Rhode Island, we installed 10 location-specific posters that guide visitors to a website entitled, “DIVE IN: The Ocean State,” where they can learn more about the marine life at the site they are visiting. Geared towards children aged 8-12 and accompanying adults, the site is accessible on mobile devices and encourages our audience to follow along and then go beyond the creative responses we demonstrate for each site location. Unique to this project, an enthusiastic cartoon character named SURF (the water droplet), guides visitors through our adventures and discoveries around Narragansett Bay. To test the efficacy of our approach, Google Analytics is used to measure public engagement through metrics on website popularity and dwell times.

Bio-Fluid Interactions of Flexible Animal Propulsors

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Diverse organizational arrangements of morphological propulsors give a natural insight to the technique and process of animal movement in various species and fluids. Placement of propulsors and the use of these propulsors to move animals through their individual fluids are found to be quite similar across differing animal phylum. A new approach regarding use of these propulsors to bend and maneuver an animal through its fluid is in direct contrast to past ways of thinking which focus on straight, linear movement dynamics. In comparing the placement of these propulsors in both flying and swimming animals as well as their individual maneuvering abilities through a fluid, we suggest an animal model can be constructed for application of the various fluid forces as a result of the convergence of constrained bending kinematics. We investigate the idea of similar movement patterns between various animal species in different fluids. We propose an idea that there is a predictable pattern to propulsor placement on an animal and its kinematic bending in both swimming and flying animal species. Through studying various animal species of birds, bats, cetaceans, fish, mollusks and insects propulsor placement and their bending kinematics, we will test the hypothesis that certain positions of propulsors predictably determine the movement of animals and propulsive forces through differing fluids.

Reproductive Stress of Marine Invertebrates in the Face of Climate Change

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With climate change largely undeniable at this point, one important question being asked is what is going to happen to local marine ecology? Although it is impossible to see the future, we can predict some of the potential physiological and reproductive impacts by studying simple invertebrate organisms known as sea squirts (*Ciona intestinalis*).

Although they do not have commercial value, sea squirts are ecologically important filter feeders which take up residence where other invasive species could take their place if they were to go extinct. The data collected from these model organisms in the laboratory provides valuable insight into what is likely to occur in sea squirt populations, and for many other species as climate change continues to affect our oceans.

In the Irvine lab, we have endeavored to investigate potential impacts by comparing the viability of *Ciona* offspring hatched at increasing temperatures of 18°C and 22°C compared to a much cooler 14°C environment. We also hatched the larvae at varying levels of acidity and salinity at the three temperatures to determine if the warmer water had an impact on the number of normally developed hatchlings under each stressful condition.

We hypothesize that we will see a decrease in normal larval development in embryos hatched under stressful conditions at 22°C compared to lower temperatures. A positive result would indicate that *Ciona* have more difficulty coping with environmental stress if their climate is warmer than what their species is accustomed to. These findings would suggest that the effects of climate change will take a toll on both the quantity and the health of sea squirt populations, and potentially other local aquatic species as well.

N_2 Uh-Oh! How Warming Waters and Nitrogen Loads from Anthropogenic Climate Change Affect Nitrogen Removal Rates of the Eastern Oyster, *Crassotrea virginica*

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The Eastern Oyster, *Crassostrea virginica*, provides the ecosystem service of nitrogen removal, by aiding in the process of denitrification through their filtration capabilities. As humans continue to alter the marine environment through climate change and pollution, coastal waters are becoming warmer and receiving higher nitrogen loads. These changes to the marine environment will likely alter oyster denitrification rates. This becomes a growing concern as incomplete processes of denitrification can lead to the elimination of this ecosystem service, and/or an accumulation of Nitrous Oxide (N_2O), a powerful greenhouse gas and natural byproduct of denitrification. To investigate this, a two factor test (temperature x nitrogen) was utilized in a controlled laboratory setting. Oyster tanks were maintained under contrasting temperatures (18°C and 24°C), crossed by a gradient of nitrogen levels (20μM, 40μM, 70μM, 100μM), resulting in 8 different combinations of the two factors. An incubation experiment was performed to test how temperature and nitrogen loads may affect *C. virignica*'s nitrogen removal efficiency. Preliminary results from a 2-Way ANOVA analysis suggest that temperature is a significant factor in N_2O production ($F_{23,26}=-2.53$, $p=0.0199$), but short term nitrogen exposure is not ($F_{23,26}=-1.46$, $p=0.1609$). This study can aid in coastal resource management, as well as offer insight as to how to maximize water quality benefits from *C. virginica* especially as it pertains to aquaculture.

Assessing the Effects of Crab Burrows on Green House Gas Fluxes in Rhode Island Salt Marshes

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Salt marshes are important coastal ecosystems that provide multiple beneficial services, including acting as coastal buffers, nurseries, wildlife refuges, and large sinks for green house gases (GHGs). Salt marshes store GHGs within the sediment and can continue to store carbon over long periods of time. Without salt marshes, the carbon that they take up would remain in the atmosphere and exacerbate climate change. Anthropogenic changes to the marsh environment have altered these ecosystems to the point where there are greater abundances of crabs in the area. Previous studies have looked at how crabs affect the marsh landscape through herbivory rates. This study focuses on how the overall abundance of crab burrows affect GHG fluxes in Rhode Island marshes. Crabs are known to significantly alter the marsh landscape through burrowing and foraging behavior when found at higher densities, which could impact GHG fluxes. To determine if crabs increase GHG absorption or decrease GHG absorption, three zones (Sandy Creek, Creekbank, and Spartina alterniflora) were chosen which included differing crab species and densities. At each zone, *in situ* GHG measurements of carbon dioxide (CO_2) and methane (CH_4) were taken using a Picarro G2508 analyzer. Preliminary results show that there was no significant difference between the three zones for methane fluxes. These same results also show that there is a difference between *S. alterniflora* (mean -4.6385) and Creekbank (mean 1.1623), and *S. alterniflora* and Sandy Creek zones (mean 0.8581) for CO_2 fluxes, but no significant difference between Sandy Creek and Creekbank zones for carbon dioxide fluxes. These results suggest that crab presence has a larger effect on carbon dioxide fluxes than on methane fluxes. Further research needs to be done on the effects of crab burrows on GHG fluxes in other salt marshes over a longer period of time to see if there is a similar trend.

Genetic Improvement in Eastern Oysters: Quantifying Variation for Dermo Resistance in a Largescale Breeding Program

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Dermo disease in eastern oysters (*Crassostrea virginica*) is caused by the parasite *Perkinsus marinus*, and is a major problem for both wild and aquaculture populations. Thus, disease resistance is a high priority target for oyster breeding programs. The main purpose of this experiment was to quantify and compare levels of disease resistance among fifty unique families of oysters that are part of the oyster breeding program at the Virginia Institute of Marine Science's Aquaculture Genetics and Breeding Technology Center (ABC). ABC oysters were labeled, injected with either 5×10^6 *P. marinus* cells (Challenged) or sterile seawater (Control), and maintained in separate raceways for the duration of the experiment. At seven and forty-seven days post-injection, oyster tissue was sampled for parasite quantification. DNA was extracted from tissue, diluted to a standard concentration, and Dermo load detected in the DNA using a quantitative PCR (qPCR) assay. Level of Dermo resistance in each oyster family will be inferred from either parasite load measured at day seven post-injection or by the difference in parasite load between days seven and forty-seven. Comparison of resistance levels among oyster families will provide information about the extent to which this trait varies in selectively-bred oysters and should enable more precise selection for Dermo resistance in future generations.

Correlation of Oyster Disease with *Vibrio parahaemolyticus* Accumulation in Oyster Tissues

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As the average water temperature of Narrangansett Bay and other coastal ponds in Rhode Island continue to increase due to climate change, it is probable that the abundance of *Vibrio parahaemolyticus* (Vp) and *Vibrio vulnificus* (Vv) will increase as well. *Vibrio parahaemolyticus* and *Vibrio vulnificus* are gram negative, halophilic bacteria that accumulate within the tissue of the eastern oyster (*Crasssostrea virginica*). While vibrio remains harmless to the oyster itself, some strains of Vp can cause gastroenteritis in humans that consume raw or undercooked oysters. Eastern oysters are also commonly infected with three diseases (Dermo, MSX, SSO) that can cause significant mortality to their host. It is unknown whether oysters infected with these three common diseases will have more Vp and Vv accumulation than non diseased oysters. To determine if this relationship exists, individual oysters as well as composites of 10 oysters were collected during the summers of 2016 and 2017. Following homogenization of oyster tissue, DNA extractions were carried out the same day for Dermo/MSX/SSO and the next day, after a 20 hour incubation, for *Vibrio*. Once the DNA was extracted, previously published primers for Vp and Vv were used to determine the abundance of Vp and Vv using a multiplex quantitative polymerase chain reaction (qPCR). Alongside the duplex, a second multiplex qPCR for Dermo/MSX/SSO was used to determine severity of disease in individual oysters as well as in the composites of 10 oysters. Preliminary results elude to a slight negative correlation between Dermo levels and *Vibrio parahaemolyticus*. However, more sampling will need to be carried out in order to gain a better understanding for Dermo/MSX/SSO infections that range from mild to severe.

Determination of the Location of *Vibrio parahaemolyticus* in Oyster Tissues

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In situ hybridization is a staining method using labeled complementary DNA, RNA or nucleic acid strands to find specific sequences within a tissue sample. The process can be used to obtain important information about the genetic makeup of the specimen based on gene expression, chromosome abnormalities or presence of foreign invader DNA. Visualizing target sequences within specimens can allow scientists to gain more insight into tissue morphology, as well as the regulation and function of genes. This hybridization can be used to determine the location of bacteria within a tissue sample. Methods used to locate the bacteria *Vibrio parahaemolyticus* (Vp) within samples of tissues cut from infected eastern oyster specimens are being developed using digoxigenin-labeled oligonucleotide probes. The process begins by cutting thin tissue samples using a microtome and mounting the sections on slides. Tissues are then rehydrated in ethanol washes and treated with proteinase K to digest membranes allowing labeled probes to penetrate and locate complementary strands. The probes carrying the labels hybridize to their target sequences under specific conditions such as temperature and salt concentrations. Slides are then incubated in an antibody solution to allow binding to the probe. Staining of the antibody is then the final step to allow for visualization of the target sequences using light microscopy. It is expected that the *in situ* hybridization will reveal stained Vp on epithelial surfaces of the mantle cavity or the cilia of the digestive tract, rather than embedded within connective tissues. If *in situ* hybridization shows these expected outcomes, then flushing methods may be utilized to remove the bacteria from the oysters. This would be a significant step in helping oyster harvesters maintain profits when faced with Vp infection, because the bacteria can cause sickness in humans who consume the raw shellfish.

Identifying Bacterial Populations Living Symbiotically on *Ulva compressa* and *Ulva rigida* in Narragansett Bay, RI Using Metagenomics and Next-Generation Sequencing

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Green macroalgae especially *Ulva* spp., have been causing harmful algal blooms (HABs) in coastal ecosystems around the world, including Narragansett Bay, RI. The blooms in the Narragansett Bay are mainly composed of two closely related species of green macroalgae, *Ulva compressa* and *Ulva rigida*. Previous studies suggest that bacterial symbiosis might play a role in algal bloom formation by regulatory factors being released by one or more combinations of bacterial flora. In identifying these populations and regulatory factors we hope to better understand the causative factors involved in rapidly dividing algae. To further test this hypothesis in the two species focused on in this study, we isolated bacterial genomic DNA (gDNA) associated with *Ulva* to identify the bacterial populations that may contribute to rapidly dividing HABs. Six samples, three of each species, were cultured in sterilized seawater and given standard concentrations of vitamins and nutrients. Of these samples two were used for gDNA extraction where the samples were collected in a solution that was then centrifuged down and the pellet was homogenized using the PowerSoil® DNA Isolation Kit. The gDNA for bacterial gene for the ribosomal subunit 16S was then amplified using PCR to isolate bacterial DNA, which was used during Next-Generation sequencing via the Illumina MiSeq platform and sequenced to identify the key bacterial populations that live and sustain a symbiotic relationship with the *Ulva* spp. throughout the blooming season. Such identification can assist in future studies that seek to understand the relationship between associated bacteria and growth patterns of HABs.

Differential Gene Expression in Stress Related Genes Occurring in *Ulva compressa* and *Ulva rigida* in Response to Climate Change

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Over the past decade there has been a noticeable increase of green macroalgae in Narragansett Bay, RI. The two main species found in these blooms include *Ulva compressa* and *Ulva rigida*. The extensive proliferation of these two species have resulted in the formation of Harmful Algal Blooms (HABs) that tend to outcompete other species in the environment for sunlight and nutrients, causing critical economic and ecological damage. This study seeks to identify a link between climate change and gene expression potentially altering the bloom behavior of this specific genus of macroalgae. This was done by obtaining subtidal and intertidal samples from each of our collection sites (Oakland Beach, Chepiwanoxet, or Sandy Point) at key stages in their bloom cycle (May, July, and September). RNA was extracted and converted to cDNA on each sample. Genes that were identified as being implicated in the formation of of HABs were used to evaluate differential gene expression within the species *U. compressa* using qPCR. The identified genes include stress related light harvesting complex (lhcSR), malate dehydrogenase (MDH), Rieske iron-sulfur protein gene (RIE), carotene biosynthesis related gene (CBR), and heat shock protein (HSP). In order to analyze the gene expression of individual samples, we evaluated the change in concentration of fluorescing cDNA over the given time period in relation to the specific gene. This is important because it will allow for the identification of the gene expression at each collection site, tidal zone, and time period to allow further understanding on bloom dynamics.

Chemical Effects of Sea Lettuce on a Co-Occurring Seaweed

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Harmful algal blooms (HABs) are becoming more frequent in our coastal waters due to increased anthropogenic nutrient loading from sources including wastewater treatment plants and run-off from agriculture and lawn fertilizer. “Green tides” are a form of HAB dominating by green macroalgae commonly known as sea lettuce (*Ulva* spp.). Previous laboratory research has shown that *Ulva* can inhibit the growth of co-occurring macroalgae through the release of allelopathic, growth inhibiting, chemicals into the water column. The objective of this study was to determine whether two common bloom-forming *Ulva* species, *U. compressa* and *U. rigida*, compete with the co-occurring macroalga *Chondrus crispus in situ* by conducting field-based experiments. We deployed 15 paired cages at two different field sites in Greenwich Bay during the summer of 2016 and 2017, with 5 paired cages each of the following three treatments: *U. compressa-Chondrus*, *U. rigida-Chondrus*, and *Chondrus-Chondrus* (control). Macroalgae in the cages was changed weekly and the growth rate of *Chondrus* in each treatment was determined. Results to date are consistent with laboratory experiments and show that *Ulva* limits the growth of *Chondrus crispus* via allelopathy. *Chondrus* grown with *Ulva* (0.38-0.85 % per day) had less than half the growth rate of *Chondrus* grown without *Ulva* (1.53-1.87 % per day). Furthermore, *Ulva* had a stronger negative effect on the growth rate of *Chondrus* at our field site with less wave action. We hypothesize that this is because higher wave action increased the flushing of the allelochemicals and *Chondrus* was not exposed to chemicals long enough to reduce the growth rate. Although there was no significant difference in the growth rate of *Chondrus* between the treatments, we have shown strong trends that *Ulva* allelochemicals affect co-occurring macroalgae *in situ*. This study will be replicated in the future to definitively determine whether *Ulva* allelochemicals have widespread impacts in nature.

Cost-Effective Phytoplankton Biomass Sensor

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Oysters play a significant role in the ecosystem, filtering the water column that they inhabit and removing inorganic and organic matter. Changes in water quality can significantly impact the health of oysters. As phytoplankton respond quickly to changes in water chemistry, their biomass can be used as a gauge for assessing water quality in oyster beds. A low-cost, open-source sensor was calibrated and built to monitor phytoplankton biomass. Calibration of the sensor was accomplished by diluting samples of a phytoplankton culture to a range of known concentrations. The intensity of the excitation light and fluorescent light was measured after shining a 405-nm ultraviolet LED onto a sample. The ultraviolet LED excites the chlorophyll of a phytoplankton sample and the resultant red, green and blue light is measured through filters on color sensors, controlled by an Arduino microcontroller. The excitation light is measured by a color sensor with an infrared light filter and the fluorescent light is measured by a color sensor with no filters. The ratio of the fluorescent light to excitation light is used to derive the mass of phytoplankton per liter of water. To create the *in-situ* sensor, the entire apparatus is inserted into PVC piping and secured atop a plastic tube with water flowing through it controlled by a peristaltic pump. In the future, extensive testing will be conducted to determine the capabilities and effectiveness of the sensor. Furthermore, the sensor will have additional components to better predict oyster fitness utilizing measurements such as salinity, temperature, and turbidity.

Open Source Computer Identification of Common Phytoplankton for Harmful Bloom Prevention

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Knowledge of the phytoplankton population composition can be critical to the health and sustainability of the ecosystems they populate. Detecting and recognizing toxic species before they become a harmful bloom is a critical step towards reducing the damage caused by these blooms to both the oyster populations and the people who eat them. Current methods of species identification in use are time-consuming (identified individually by a researcher) and/or prohibitively expensive (flow cytometry combined with proprietary software) for many researchers and aquaculture farmers. A machine learning program was developed to classify species. The program performs image preprocessing, image segmentation, feature extraction and classification/recognition. An input image containing phytoplankton is first filtered to remove noise and phased to greyscale. It is then scanned for general shapes to separate individual phytoplankton from the larger image. Each phytoplankton's features are recorded based on shape, texture, and size. These values are then compared to a large database of species for a specific classification. The main goal of this project is to develop free software that farmers and researchers can use for rapid identification of potentially harmful species. Further implementations and expansions are examined as future work.

Using X-Ray Microtomography to Visualize and Quantify the Three-dimensional Nest Architecture of Acorn Ant Colonies

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Organismal survival is dictated by a basic need for food, water, and shelter. This is true from bacterial colonies to complex social animals, such as ants and humans. For the acorn ant, *Temnothorax curvispinosus* , a physical nest space is crucial to colony survival. However, it has previously been difficult, if not impossible to visualize and quantify the geometry and spatial organization associated with nest architecture in a non-destructive manner. Modern advancements in imaging technologies make it possible to not only see, but analyze the nest spaces of these ants using x-ray computed microtomography. Acorns were collected in Rhode Island without disturbance by manually observing ants coming and going through microscopic pores in the acorn. These acorns were imaged using the Bruker x-ray microtomography center at Union College, NY. Using Bruker CTAn software, virtual cross-sections of the acorn scans were segmented using regions of interest and binarized to remove artifacts. The resulting binary stacks were then used to generate 3D models and estimate the volume and surface area of the interior nest space with precision in the range of 5%. The three-dimensional models of the internal nest regions were visualized using Bruker CTVol software, revealing a strikingly highly partitioned internal nest geometry. Through computer analysis and modeling, we were able to better understand the physical world in which these acorn ants live, offering insight into their unique natural history and also raising questions about the role that physical and built environments play in shaping social interactions in complex societies.

Ontogeny of the Lateral Line System of a Caribbean Reef Goby, *Elacatinus lori*

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Elacatinus lori is a shallow reef-dwelling goby from Belizean coral reefs that lives exclusively in tube sponges. It is a model species for the study of population connectivity and the mechanisms of larval navigation during the pelagic larval phase. The purpose of this study was to analyze the ontogeny of the lateral line (LL) system in larval and juvenile *Elacatinus lori* in order to understand its potential contribution to the sensory biology of pelagic larvae and post-settlement individuals. The LL system of gobies is characterized by a complex proliferation of superficial neuromasts (SN) arranged in numerous lines, which are quite difficult to interpret. However, the ability to rear *E. lori* through settlement provides an opportunity to show how such a complex SN pattern develops, and ultimately how such complex patterns have evolved. Twenty-four individuals (3 mm TL - 62 mm SL) were imaged using 4-di-2-ASP (fluorescent mitochondrial stain) revealing SN distributions on the head, trunk, and tail. Images were supplemented with data from paraffin histology, SEM, and µCT, providing additional information on neuromast and cranial LL canal morphology. SN distribution maps for larvae, juveniles and adults showed that SNs are present in the youngest larvae examined, with the development of simple SN lines, and then SN proliferation and formation of papillae at the time of settlement. SN lines in *E. lori* were compared to those in other *Elacatinus* species, the sister genus *Tigrigobius*, and other gobiids to better understand their organization and evolution. Funded by NSF grant 1459546 to JFW.

Spatio-Temporal Patterns of Knifing Behavior in Mako Sharks (*Isurus oxyrinchus*)

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Satellite telemetry has revolutionized the study of movements of highly mobile marine species, such as sharks, over the past decade. This type of information is critical for managing populations that have undergone substantial decline due to overfishing. The mako shark (*Isurus oxyrinchus*) is an example of a species for which gaps in data limit accurate determination of the status of the population. Satellite transmitters attached to dorsal fins of sharks communicate with Argos satellites each time the shark surfaces and the transmitter is exposed to air as the dorsal fin protrudes from the water (referred to as knifing). Based on the premise that knifing reflects foraging activity, the frequency and timing of knifing events provides a simple method for examining temporal and spatial behavioral patterns of sharks. In this study, knifing events of 32 mako sharks tagged with fin-fixed satellite transmitters in the Western North Atlantic were analyzed to examine spatio-temporal behavioral patterns associated with movements and habitat use of mako sharks. Specific aims were to examine relations between knifing and diurnal period, season, sex, size, and latitude. There was a significant difference in frequency of knifing among diurnal period, and seasons, and between sexes, but not size. A peak in knifing frequency occurred during the dawn period and summer months, with minimum values observed during daytime and spring months. Frequency of knifing for females was significantly higher than males. Frequency of knifing increased with increasing latitude but differences were not significant. This study demonstrates that a relatively simple metric (knifing) can be used to assess spatial and temporal patterns of habitat use of mako sharks in relation to environmental variables. Similar methods may provide information useful for sustainable management of populations of highly migratory marine species.

Resolving the Red Algal Diversity of Rhode Island through DNA Barcoding

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Comprehensive species inventories are required to monitor for changes in community structure resulting from global climate change and biological invasions. For marine macroalgae, phenotypic plasticity, morphological convergence, and species hybridization complicate specimen identification, such that molecular tools are required for elucidating species richness. As part of a larger project to establish a comprehensive species inventory for marine macroalgae in Rhode Island, we characterized the morphological and molecular diversity of three genera (*Polysiphonia*, *Melanothamnus*, and *Vertebrata*) of the red algal family Rhodomelaceae, which has a history of challenging identification and problematic taxonomy. Based on morphological and molecular work, nine species have been identified in Rhode Island, including two introduced species. Given prevailing currents and trends of increasing sea surface temperature, some 16 additional species that are known from southern U.S. coastlines, and 11 species that are known to be introduced around the world, might be expected to disperse and establish populations in Rhode Island waters in the future. This work establishes a firm molecular basis for monitoring Rhode Island algal diversity, which is especially important given the disproportionate incidence of species introductions from the Rhodomelaceae.

MICROBIOLOGY

LOCATED IN ROOM 130 ON THE 1ST FLOOR OF THE PHARMACY BUILDING

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

Will a MinD Gene Mutation in *E. coli* Cells Cause a Change in the Formation of a Z-Ring, Therefore Causing a Change in Cell Appearance?

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The Min system in *Escherichia coli* controls the placement of the Z-ring during the cell division pathway. The Z-ring is essential for constriction at mid-cell and septation to produce two daughter cells. There are three main proteins in the Min system, MinC, MinD, and MinE, which work together to prevent Z-ring assembly at the cell poles, thereby promoting Z-ring assembly at mid-cell. MinD is an enzyme that hydrolyzes ATP. ATP binding by MinD leads to association with the phospholipid membrane, and then MinD dissociates from the membrane after ATP hydrolysis is stimulated by MinE. *In vivo*, MinD oscillates from pole-to-pole by the chase and release of MinD from the membrane, which is regulated by MinE. MinC also interacts with MinD and oscillates between the cell poles through MinD binding and directly inhibits FtsZ formation. In this research project, we are studying how membrane association by MinD regulates function *in vivo* and activity *in vitro*. To begin this project, we deleted *minD* from the chromosome and replaced it with an antibiotic resistance marker (*parE*::kan), which gives the *E. coli* cells kanamycin resistance. The *parE*::Kan cassette also contains the gyrase inhibitor, *parE*, under the control of a rhamnose-inducible promoter. In order to check the results, we performed colony PCR, gel electrophoresis, and sequencing. We are also constructing strains containing mutations that impair MinD membrane association and enzyme activity to further understand how MinD oscillates and regulates division.

Inhibitory Effects of Purified Plant Extracts and Homologous Commercial Derivatives on *Entamoeba histolytica* growth

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The unicellular, protozoan parasite *Entamoeba histolytica* is the known cause of amoebiasis, a parasitic infection of the colon. Amoebiasis affects 500,000 people and causes the death of around 100,000 people in the countries of Central and South America, Africa, and the Indian subcontinent. Currently, the only effective treatment against *E. histolytica* is metronidazole. Metronidazole is known to have a high level of toxicity and has been reported to cause nervous system damage. Discovering alternatives to metronidazole is of great interest to improve the management of this disease. Purified extracts from plants and subsequent analogs have shown significant antimicrobial character in previous studies. Both crude and purified plant extracts have been analyzed for their efficacy in inhibiting the growth of *E. histolytica*. Previously, our laboratory identified pomegranate, rhubarb (Rhein), and maple syrup (maplifa) extracts as potential inhibitors of *E. histolytica* growth. This study tested a combination of crude extracts and commercial on healthy, confluent cultures of *E. histolytica*. Each inhibitor was tested at two different concentrations (120µM and 60µM) and observed over 12, 24, and 48 hours. The inhibitors that showed to have promising inhibitory effect on *E. histolytica* trophozoite growth were rhein, rhein extract, sennoside B, unisol blue and maplifa. Future studies will determine the molecular and biochemical mechanism of action for these inhibitors and test their relative toxicity in humans compared to that of metronidazole. Besides showing promising preliminary antimicrobial properties, the anti-inflammatory effect of maple syrup extract could neutralize or defuse the severe inflammatory colitis caused by intestinal amebiasis.

Comparative Analysis of a Novel Microbial Detection System and Gram Staining Utilizing Synovial Fluid

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The ability to quickly and effectively diagnose infections in the operating room remains a pressing challenge for modern surgeons. 0.5-2% of post-surgical sites and up to 28% in open wounds end up in infected. These infections result in healthcare costs, extended hospital stays, and reductions in quality of life. Current methods utilized in hospitals include traditional culturing and PCR, which continue to encounter issues of efficiency and accuracy. This challenge has been the focus of a novel fluorescent-antibody based assay, which aims to create a quicker, more reliable way to visually detect infections. The assay aims to be able to accelerate current diagnostic methods, improve orthopedic interventions, and overall facilitate patient treatment.

This study utilized clinically derived synovial fluid to identify gram positive and negative bacterial infections in heterogenic samples within 40 minutes. A 20-minute gram staining procedure was used as a comparative standard throughout trials.

Through IRB approval, synovial fluid was received from two surgeons at Brown University/Rhode Island Hospital. 20 µL of fluid was fixed with methanol onto microscope slides and labeled using fluorescent-conjugated antibodies (FCBA). Sample was visualized using confocal laser scanning microscopy (CLSM) and analyzed using ImageJ. Results were then compared to trials generated via gram staining. Through a blind, preliminary IRB approved pilot study, 13 samples were tested using the assay and gram staining. Unlike gram stains, the assay proved to be able to differentiate between bacteria and other cells. Samples with high turbidity were diluted 50/50 in 0.5X PBS to remove excess human cells and remove background noise to provide a clearer image. Thus far, the assay was able to label bacterial presence alone and has coincided with gram staining results in each trial. Both procedures agreed that 7.7% (1 out of 13) of samples collected contained bacteria, with the assay raising concern in 15.38% (2 out of 13) samples, having identified a bacterial presence. Further testing will focus on these samples to confirm possible infection.

This methodology uses optimal strategy for intra-operative bacterial detection and visualization to allow surgeons to assess a patient's infection more accurately while still in the operating room.

Phylogenetic Diversity of the Western Antarctic Peninsula Diatom Community

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Diatoms are a highly diverse group of phytoplankton that are responsible for 40% of oceanic primary production and are known for their adaptive responses to harsh environmental conditions. The waters of the Southern Ocean (SO) are rich in macronutrients like nitrogen and phosphorus but are limited in the micronutrient iron. Diatoms utilize iron to maintain their photosynthetic functions. However, SO populations persist and thrive despite iron limitation, making them of ecological interest. Here, we focused on diatoms isolated during an austral spring 2016 cruise in the rapidly changing Western Antarctic Peninsula (WAP). This study aims to identify the important diatom species of the WAP and establish relevant culture representatives for future experiments. Since taxonomic identification of diatoms using morphological features is often difficult due to their size and diversity, the diatom isolates were genotyped to verify species identity. Genotyping is a molecular technique that uses a short fragment of DNA common between species but with species-specific variation, or barcode, to identify an organism. The barcoding regions used were the highly variable V4 region of the 18S rDNA gene and the 18S-23S intertranscribed spacer (ITS) region. The diatom isolates were grown in seawater media, their DNA extracted, and the barcoding regions PCR amplified and Sanger sequenced. The NCBI BLAST database was used to identify the sequences down to species level. In all, species of the genera *Thalassiosira*, *Fragilariopsis*, *Odontella*, *Porosira*, *Chaetoceros*, *Stellarima*, *Pseudo-nitzschia*, and *Actinocyclus* were identified. Phylogenetic trees were also created to compare relationships between genotyped isolates. Additionally, growth rates of selected isolates were determined over a period of two weeks using relative fluorescence to compare physiological differences between species. Future work will focus on iron-limiting different species and comparing their physiological responses. Ultimately, this work will contribute to our understanding of how WAP diatoms persist in an iron limited environment.

Bacteria as Potential Enhancers of Iron Availability for Phytoplankton in the Marine Environment

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Siderophores are secondary compounds widely produced by many bacteria in a variety of aquatic and terrestrial environments. These compounds are released from bacterial cells to bind iron (Fe) in the environment, and the siderophore-bound Fe is taken back up by bacteria increasing Fe bioavailability. Mutualisms between bacteria and other organisms, such as eukaryotic phytoplankton, may exist that enable the eukaryotic partner to take up siderophore-bound Fe and in return, they may release photosynthetically fixed carbon to promote bacterial growth. From recent research cruises in the low-Fe marine environments of the Southern Ocean and North Atlantic Ocean, bacteria have been isolated, purified and screened for siderophore production using the Chrome Azul S (CAS) assay. From each region, about 20 of these strains were isolated directly from phytoplankton. These phytoplankton-associated bacteria have been identified using the highly variable V3 and V4 regions of the 16S rDNA gene. Their growth curves and corresponding siderophore concentrations were recorded. Future work includes identification of the produced siderophores and co-culturing experiments with phytoplankton. This research will further our knowledge of these important bacteria-phytoplankton interactions.

Effect of Cranberry Compounds in Combination on Resistant Strains of *Escherichia coli* and *Klebsiella pneumoniae*

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Background: Urinary tract infections (UTIs) are a prevalent problem, especially among women. Resistant strains of bacteria causing these UTIs are making treatment increasingly difficult. Studies suggest that cranberries have natural, beneficial health effects in regards to UTI prevention, but data is lacking for their use in treatment. The objective of this work was to test several cranberry compounds for antibacterial activity alone and in combination with antibiotics typically used for UTIs.

Methods: Susceptibility of potential cranberry compounds against resistant strains of *E. coli* or *Klebsiella pneumoniae* was done by the Kirby-Bauer method (disk diffusion). Further susceptibility testing was done on promising compounds by determining the minimum inhibitory concentrations (MICs) according to CLSI standards. The MIC is the lowest antibiotic concentration needed to inhibit visual growth of extended-spectrum beta-lactamase (ESBL) or *Klebsiella pneumoniae* carbapenemase-producing (KPC) bacteria. In order to determine the possible synergistic effects of the cranberry/antibiotic combination therapy, both fractional inhibitory concentrations and time-kills were performed. The FICs were performed on four cranberry compounds (HL10, HL11, HL12, and HL13) and were tested against KPC vs. meropenem, KPC vs. colistin, ESBL vs. meropenem, and ESBL vs. ceftazidime. Time-kills were performed on KPC vs. meropenem at 0.25x, 0.5x, 1x, and 2x the MIC and were taken at 0h, 4h, and 24h. Synergy was defined as an FIC index ≤ 0.5 or an increase in activity of ≥ 2 log in time-kill studies.

Results: Activity based on disk diffusion occurred in 3 of the 4 isolates with a mean zone of inhibition of 7mm. Traditional MICs were 2-8mg/mL for the 4 compounds. Based on the FICs, no synergy was found. The average FIC index value was 1.98 (range 1.8-2.6). Statistical analyses on time-kills are in progress.

Conclusion: These cranberry compounds show promise in being active against certain resistant bacteria strains. More research is needed in order to determine the true activities of such compounds and the potential for synergy with standard of care antibiotics.

Impact of Water Temperature and Environmental Conditions on the Diversity and Composition of Marine Viruses in Narragansett Bay

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Cyanophages that infect cyanobacteria such as *Synechococcus* are abundant in coastal waters. This study examines if differences in water temperature and environmental conditions impact the community composition of cyanophages in Narragansett Bay. It is expected that community composition will vary across locations. Water samples were collected from paired sites at three diverse locations within Narragansett Bay: Ninigret Salt Pond (SP1, SP2), Greenwich Bay (GB1, GB2) and the East and West Passage (EP, WP). Water temperature varied at each location, ranging from 20°C to 28°C. Viral abundance was determined using the Most Probable Number Assay and viruses were isolated via extinction dilutions. PCR was conducted to amplify the *g43* DNA polymerase gene that was then sequenced to identify each virus. The diversity of viruses was further analyzed using phylogenic analysis. The number of cyanophages isolated varied at each location, but there were between 9 and 28 isolated from each. The overall abundance of cyanophages also varied at each location, ranging from 46 to 400 viruses/ml. Understanding how water temperature and environmental conditions can influence the interaction between cyanophages and their hosts is important in predicting the future impacts climate change could have on Narragansett Bay.

Immunoinformatic Analysis Relating T cell Education on Prenatal & Postpartum Microbiota to Extragastric Effects of *H. pylori* Colonization

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The bacterium *Helicobacter pylori* colonizes the human gut and infects over half of the world's population. *H. pylori* colonizes during infancy and has been linked to gastric cancer in about 1-2% *of H. pylori*-infected individuals. Infection is variable between different countries, with prevalence in developing nations far greater than developed countries due to lack of antibiotic therapies and greater exposure to bacteria. While eradication of *H. pylori* may be beneficial, recent studies have portrayed that the bacterium provides protection against inflammatory conditions, including asthma and inflammatory bowel disease. It is hypothesized that either natural T-regulatory cells or T-regulatory cells trained on commensal antigens may proliferate and migrate out of the mesentery upon cross-reaction with *H. pylori* antigens presented by MHC Class II molecules on antigen presenting cells.

This study aims to determine the *H. pylori* 9mer frames that are promiscuous in binding to MHC Class II HLA super types, as well as have high cross reactivity with the pre-birth and postpartum microbiome. Recent studies report that the womb is not a sterile environment, and early life colonizing microbiota are essential to priming the immune system by shaping the commensal-specific CD4 T-cell repertoire. To address this aim, pre-birth and postpartum human microbiome databases were curated to compare against *H. pylori* and determine cross-reactivity. *In silico* predictions using the iVax toolkit, including the EpiMatrix, ClustiMer, and JanusMatrix functions, allowed for *H. pylori* 9mer frames that bind promiscuously to Class II HLA super types and have cross-reactivity with the gut microbiota to be determined. Preliminary analysis of *H. pylori* 9mer frames data using statistical analysis methods, including Heat Map Analysis, PCA, and t-SNE, shows that the cross-reactive hits (XR hits) and Janus Homology Score for a 9mer are more correlated than EpiMatrix hits (EPX hits) and Janus Homology Score. It has also been determined that as EPX hits increases, XR hits follows a similar increasing pattern. Upon the completion of further studies to identify and select microbiome homologs of *H. pylori* HLA Class II-binding 9mers, we will investigate the phenotypes and functions of extra-gastric T-regulatory cells that have high cross-reactivity with microbiome homologs of *H. pylori* using peripheral dendritic cell/T cell co-cultures derived from *H. pylori*-infected and uninfected subjects.

Metabolic Analyses of the Biomass Accumulation Defect 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 *S. oneidensis* strains lacking Hfq are more sensitive to a variety of stressors. The *hfq* mutant grows more slowly in exponential phase compared to wild type *S. oneidensis*. When cultures reach stationary phase, mutant *hfq* strains have a lower terminal density and no cells survive through late stationary phase compared to wild type. Our goal is to investigate the lower terminal density of the *hfq* mutant.

To examine this, we have provided the *hfq* mutant and wild type *S. oneidensis* with different concentrations of carbon in a defined media and tested the effects of carbon limitation. We have found that carbon is limiting at low concentrations, however at high concentrations of carbon other factors become limiting for bacterial growth. Additionally, we harvested samples from the hfq mutant and wild type cultures and measured the dry weight of each culture. Overall, *hfq* mutant cultures produce reduced biomass relative to wild type cultures, suggesting that the mutant is deficient in biomass accumulation. We have also begun investigating how heat stress influences *S. oneidensis* growth. We have found that Hfq appears to play a role in adaptation to high temperatures.

We have previously demonstrated that the *hfq* mutant is deficient in production of heme, a chemical compound that is integral to electron flow in the electron transport chain. Therefore, we also plan to complete an ATP assay to determine the quantity of ATP present in wild type and *hfq* mutant cells. This information will help in determining what is happening within the metabolic pathway of the *hfq* mutant that is leading to reduced biomass accumulation relative to wild type.

The Adaptive Response to Oxidative Stress and DNA Damage in *Shewanella oneidensis*

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We are investigating the adaptive stress responses in the bacterium *Shewanella oneidensis* which are controlled by Hfq, a small RNA chaperone protein. Hfq cooperates with many small regulatory RNAs that are produced during times of cellular stress and which facilitate adaptive responses by the bacterium. One specific stress is oxidative stress induced by the presence of hydrogen peroxide. Our lab has previously demonstrated that loss of Hfq in *S. oneidensis* results in hypersensitivity to oxidative stress. Pretreatment with low doses of hydrogen peroxide yield a greater survival in both wild type and *hfq* mutant cells when challenged with a lethal dose. Catalase is the enzyme that is responsible for breaking down hydrogen peroxide. Because other labs have shown that loss of the gene *katB*, which encodes the enzyme catalase, results in hydrogen peroxide hypersensitivity, we hypothesized that deficient adaptive *katB* expression is responsible for the *hfq* mutant's oxidative stress sensitivity. However, our data indicates that loss of Hfq does not compromise the magnitude and timing of adaptive catalase production in the *hfq* mutant when compared to wild type strain. This indicates that adaptive *katB* expression is independent of the presence of Hfq in *S. oneidensis*. Our working hypothesis is that compromised DNA damage response is responsible for the *hfq* mutant's hypersensitivity to oxidative stress, which causes DNA damage. Consistent with this hypothesis, our preliminary data indicates that the *hfq* mutant is significantly more sensitive to short wave UV radiation than the wild type strain. We are currently developing methods to quantify DNA damage and the rate of DNA damage repair to help dissect the role of DNA damage repair in the *hfq* mutant oxidative stress phenotype.

Shewanella oneidensis Hfq Regulates *rpoS* Expression and Stationary Phase Survival

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Hfq is a RNA chaperone that has been shown to have a role in sRNA function in Gram-negative bacteria. sRNAs contribute to adaptive gene expression in response to environmental changes. We are interested in characterizing the role of Hfq in stress responses in the bacterium *Shewanella oneidensis*. We have used a genetic approach to demonstrate that the loss of Hfq in *S. oneidensis* results in slow exponential phase growth, reduced terminal cell density in stationary phase, a striking loss of colony forming units in extended stationary phase, and an exquisite sensitivity to oxidative stress.

To investigate the loss of colony forming units in extended stationary phase, we evaluated the survival of the *hfq* mutant and wild type cells grown in cell free media conditioned by growth of wild type *S. oneidensis* to stationary phase. We determined that conditioned media inhibits the growth of healthy inocula and that the *hfq* mutant is more sensitive to this growth inhibition. In addition, the *hfq* mutant dies more rapidly than wild type after inoculation into the conditioned media. We further investigated the connection between this increased sensitivity to conditioned media and the *hfq* mutant's extended stationary phase death phenotype.

Because Hfq regulates the expression of the stationary phase sigma factor RpoS in other bacteria, and because RpoS generally promotes adaptation to stationary phase stress, we hypothesized that reduced expression of *rpoS* in the *S. oneidensis hfq* mutant is responsible for complete loss of viability in extended stationary phase cultures. Using a *rpoS-lacZ* translational reporter fusion and Western blot analyses, we have found that *rpoS* expression is induced in late exponential phase and continues through stationary phase into death phase. In addition, our data indicates that *rpoS* expression and RpoS protein levels are significantly lower in the *hfq* mutant than in the wild type strain. Importantly, our preliminary data indicates that the survival defect of the *hfq* mutant in extended stationary phase can be rescued by increasing the expression of *rpoS*.

Our working hypothesis is that a subset of cells in stationary phase survive because they express high levels of RpoS. We are currently constructing reagents that will allow us to measure *rpoS* expression on a cell-by-cell basis.

Interactions Between *Corynebacterium* and *Streptococcus* Species within the Human Oral Microbiome

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A recent microscopy-based model of human supragingival, or tooth surface plaque, suggests that there is a “hedgehog” structure present that has long filamentous bacteria bridging the entire structure, which belong to the *Corynebacterium* genus. A majority of individuals’ plaque have *Corynebacterium matruchotii*, but a smaller percentage of people also have *Corynebacterium durum*. The model also shows that various *Streptococcus* species have some sort of spatial interaction with *Corynebacterium*. Previous research conducted in this lab has shown a positive correlation, based on abundance, between *C. matruchotii* and *Streptococcus cristatus*. To better understand the interaction between these bacteria, we are creating fluorescent constructs that will be electroporated into *C. matruchotii* and *C. durum* and observed using fluorescence microscopy. We are also testing physical interactions by designing an aggregation assay, where we determine the rate of aggregation of the bacteria in monoculture and compare it to the rate in coculture. Our two hypotheses are that there is 1) a surface interaction between *Corynebacterium* and *S. cristatus* or 2) both bind to a salivary protein that causes them to aggregate together. These experiments will help increase understanding of how the normal healthy oral microbiome is structured and how it may exclude the presence of pathogenic species.

The Influence of Flagellin Expression and Fitness on the Interaction of *Salmonella enterica* with Leafy Greens

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Salmonella enterica is a Gram negative, facultative anaerobic bacterium that causes food poisoning historically associated with the consumption of undercooked poultry or eggs. Recently, however, frequencies of outbreaks stemming from infected produce are increasing, thus there is increasing pressure to understand the molecular interactions that facilitate survival on leafy greens. *Salmonella* spp. express two phases of flagellins, FliC or FljB, and literature has suggested that the flagellum is involved in its attachment to surfaces. The objective of this study is to examine flagellin expression and motility of seven serovars of *S. enterica* grown in varying conditions (salt concentration, temperature, physical state of media) and to correlate these trends to the behaviors observed on lettuce. Flagellins were sheared from cells through vortexing and were precipitated with acetone. Flagellin levels were compared via Western blot and flagellar fitness was examined by motility plate assays. Serovar-specific variance was observed in all growth conditions for all assays, but in general high salt concentrations yielded higher total percentages of flagellins isolated, whereas flagellins harvested from low salt concentrations represented a minor fraction of the total proteins isolated and had a higher instance of expressing both phases of flagellin. Flagellar fitness was typically greater in high salt over low salt concentrations, and when grown at body temperature as opposed to room temperature. Red leaf lettuce was spot inoculated with serovars grown in the varied growth condition groups and rinsed at time intervals representing attachment, colonization and persistence (30 seconds, 1 hour, 5 days), from which colony counts on XLD media were used to quantify how many cells remained attached. The trends from these data will be compared to flagellar fitness and levels of flagellin expression to determine the role the flagella may play in the interaction with leafy greens. Future studies will focus on qPCR of the *flic* and *fljb* and analysis of flagellar methylation.

Effect of Varying Growth Conditions on Initial Attachment, Persistence, and Colonization of *Salmonella enterica* Serovars on Red Leaf Lettuce

Nora Donahue, Kimberly Beaton, Brianna LoCascio, Cristina Marsocci & Anne Reid

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Consumption of *Salmonella enterica* by humans can cause acute gastrointestinal disease; outbreaks have been to contamination of fresh produce by *S. enterica* serovars. Contamination of fresh produce is believed to occur via three stages: initial attachment, colonization, and persistence. The objective of this study is to determine how inoculum growth conditions affect the three stages of contamination in seven serovars to better understand what conditions facilitate bacterial attachment to fresh produce, and to determine whether there are serovar-dependent differences. The serovars were grown in either low-salt LB or Lennox media, prepared as either biphasic media or plates and grown at 37°C or 25°C. After incubation, *S. enterica* was spot inoculated 108 on fresh red leaf lettuce and left for intervals of either 30 seconds (attachment) 1 hour (colonization), or five days (persistence). Leaves were then rinsed with water to remove non-adherent bacteria, massaged in 0.1% tryptone water and serially diluted in tryptone water prior to plating on XLD. After incubation overnight at 37°C, typical *Salmonella* colonies were enumerated, and the log reduction of attached cells was calculated for each time interval and growth condition. Statistical analyses of the log reductions were performed using ANOVA and Tukey tests to determine the effect of varying growth conditions on the attachment, colonization, and persistence of *S. enterica*, and to identify differences between serovars. Statistical analysis for cells grown in biphasic media showed significant variability, with little consistency between serovar fitness in a given medium, temperature, or time interval. This suggests that different molecular mechanisms are involved in the three stages of contamination of fresh produce.

Environmental Conditions Affecting Biofilm Expression in *Salmonella enterica* Serovars

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Salmonella enterica is a Gram negative bacterium that is responsible for diseases ranging from basic food poisoning to typhoid fever. Adhesive structures of *S. enterica* such as fimbriae, surface proteins, and polysaccharides, comprise its biofilm. This community of microorganisms growing together in an enclosed self-produced polymeric matrix exhibits adherent properties and altered gene expression from planktonically grown bacteria, which may include genes responsible for antibiotic resistance. This study was undertaken to determine whether different *S. enterica* serovars form biofilms to similar levels, and if growing temperature and media composition influence biofilm formation in a similar way for different serovars. These findings were then correlated with results from experiments determining conditions in which *S. enterica* attachment, colonization and persistence to lettuce leaves is maximized. Seven *S. enterica* serovars were grown in broth or on plates and cultured in 96-well plates in two types of media (low-salt LB and LB) at two different temperatures (25 and 37°C). Biofilms were then visualized by staining with crystal violet. Results suggest an inherent difference between the serovars' ability to form a biofilm, with Enteritidis having the most biofilm production and *Typhimurium* having the least. In addition, results indicate most strains belonging to the same serovar show similar levels of biofilm production, although some strains (Poona) exhibited notable differences. Future studies will focus on measuring surface hydrophobicity through a microbial adhesion to hydrocarbon assay as well as using qPCR to monitor expression of biofilm genes under the conditions used above.

Correlation Between O Antigen Expression and Interaction of *Salmonella enterica* with Leafy Greens

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Salmonella enterica causes gastrointestinal disease in humans which has recently been linked to the consumption of contaminated fresh produce. Contamination by *S. enterica* relies on three phases: initial attachment, colonization, and persistence. The O antigen is one of multiple outer-surface structures of *S. enterica* that could potentially aid in the bacterium's interaction with produce. A serovar-specific structure, the O antigen is present in two forms: attached to lipid A-core as a lipopolysaccharide (LPS), and independent of lipid A-core as an O-antigen capsule (CPS). Studies have indicated the O antigen is important for environmental persistence of *S. enterica*. The purpose of this research is to determine the effect of growth conditions (temperature, salinity, and physical state of media) on both the expression of O antigen (LPS and CPS) in various serovars, and on each serovar's ability to attach to, colonize, and persist on lettuce. Polysaccharide separation indicating the presence and characteristics of LPS and CPS O Antigen was achieved using SDS-PAGE and staining with Silver, and Silver and Alcian Blue. Attachment, colonization, and persistence of *S. enterica* colonies on lettuce spotted with inoculum and rinsed at 30 seconds, 1 hour, and 5 days respectively, was measured by cell counts on selective XLD media. Preliminary results indicate minor serovar-specific differences in LPS O antigen chain-length distribution and varying size distribution of CPS O antigen expression, as well as significant differences between serovars in measures of attachment, colonization, and persistence on lettuce in response to the temperature, salinity, and physical state of the growth media.

Inhibition of *Streptococcus pneumoniae* LytB by Diamides

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Streptococcus pneumoniae is a common Gram-positive bacteria known to cause moderate to severe symptoms in the event of infection. Recently it has come to the attention of the medical community due increased incidence of drug resistant strains. This antibiotic resistance is of growing concern in a world with high antibiotic reliance and little variation in the mechanisms of antibiotics. Diamides were determined to be a possible new antibiotic whose mechanism varied significantly from most conventional antibiotics. A library of diamides were screened against *S. pneumoniae* and several compounds were identified as having an appropriate level of efficacy. These compounds and their respective MIC values for wild type *S. pneumoniae* are fgbb (11.3 μM), fgbc (7.5 μM), and fgkc (<10 μM). Based on bioinformatics analysis and previous results, these compounds disrupt cell wall metabolism in *S. pneumoniae*. In particular, the N-acetylglucosaminidase LytB was identified as the potential cellular target. LytB aids in the growth and division process by cleaving the bacterial cell wall polymer peptidoglycan, allowing other enzymes to insert new material, increasing the cell's size. The objective of this project is to confirm that LytB is the target of the diamide inhibitors fgbb and fgkc *in S. pneumoniae*. LytB was expressed in *Escherichia coli* BL21 containing the *lytB* gene on a pET28 plasmid in super broth at 16°C. Cells were harvested, lysed, and purified in a 2-step process, first with ion exchange with DEAE cellulose and gel filtration (MWCO 100kDa). In testing inhibition, enzyme activity of various treated solutions was monitored using two complementary assays: remazol brilliant blue labeled peptidoglycan and HPLC analysis of peptidoglycan solubilized products. Preliminary results by the HPLC show inhibition of LytB in presence of 12.5 μM fgbb at approximately 10%. Results from the remazol brilliant blue assay show varying amounts of activity with and without choline, but residual activity in the presence of fgbb remains around 95% at 12.5 μM. Slow binding kinetics of diamide inhibitors was evaluated by preincubation of LytB with inhibitor. Results showed no noticeable increase in efficacy of compounds.

Detection of Fixed DENV-2 Particles Using Multiparameter Flow Cytometry

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Multiparamter Flow cytometry has become an essential tool for the analysis of individual cells (diameter in microns) and subcellular particles (diameter 100-500 nM). However, the fields of virology, immunology, and serology often focus on smaller particles, in the order of nanometers. To determine whether flow cytometry could be used to study flaviviruses (diameter 40-60 nm), we analyzed preparations of dengue virus (DENV) labeled with RNA dye and/or fluorescently labeled antibodies Paraformaldehyde-fixed DENV labeled with an RNA binding fluorescent dye (Quant-IT Ribogreen), which showed fluoresence in the appropriate FITC/B1 spectrum on a flow cytometer (MACSQuant), whereas a control preparation, culture supernatant from uninfected cells did not. To confirm that virus particles were indeed the events detected via flow cytometry, we added a fluorochrome-conjugated anti-DENV antibody (2H2-Dylight 650). The fixed virus showed fluorescence in both R1/APC and FITC/B1 spectra. From these data we conclude that flow cytometry can be used to analyze viral particles. This method may be useful for further study of virion structure and virus-cell interactions.

Persister Cell Formation in Uropathogenic *E. coli* CFT073 in the Presence of Therapeutically Relevant Antibiotics

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Urinary tract infections (UTIs) caused by *Escherichia coli* create a large burden on the healthcare system, especially when causing recurring infections. The bacterial dormancy phenotype, persistence, contributes to the ability of *E. coli* to evade treatment and cause recurrent infection. Persister cells are stochastically forming dormant cells that have a high tolerance to antibiotic treatment. Furthermore, uropathogenic *E. coli* (UPEC) strain CFT073, a clinically relevant UTI strain has been previously shown to form high fractions of persister cells in the presence of ampicillin. In this study, we examined the persister cell fraction of CFT073 in the presence of the relevant antibiotics for uncomplicated cystitis and pyelonephritis: fosfomycin, sulfamethoxazole/trimethoprim, ciprofloxacin, nitrofurantoin, and ampicillin. The goal of this study was to determine what first and second line antibiotics for uncomplicated cystitis, according to the 2011 Infectious Diseases Society of America (IDSA) guidelines for uncomplicated cystitis and pyelonephritis (UTI), were better at reducing persister cell numbers. Of the antibiotics, ciprofloxacin, nitrofurantoin and fosfomycin each showed lower numbers of viable persister cells compared to ampicillin whereas sulfamethoxazole-trimethoprim had higher levels of viable persister cells compared to ampicillin. Ciprofloxacin and fosfomycin had viable persister cells within 2 logs of ampicillin after 24 hours. Nitrofurantoin was tested at several concentrations to determine cell viability of CFT073. 10x the minimum inhibitory concentration (MIC) and 5x MIC of nitrofurantoin resulted in zero persister cells remaining after just 2-4 hours, while 2x MIC and 1x MIC resulted in 1-3 log higher viable persister cells counts when compared to ampicillin. These results suggest that nitrofurantoin, ciprofloxacin, and fosfomycin were better at reducing persister cell numbers, and therefore might be more effective for reducing the recurrence of UTIs.

Evaluating the Effects of Diet in Combination with Environmental Toxicants on the Relative Abundance of *Akkermansia muciniphila* in the Mouse Gut Microbiome

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Alteration in the gut microbiome has been strongly correlated to metabolic disorders, such as obesity and metabolic syndrome. Several bacterial strains in the gut have been shown to be closely linked to the onset of inflammation. It was hypothesized that the relative abundance of several key bacterial strains, in particular *Akkermansia muciniphila*, Firmicutes, and *Bacteroides*, differ based on diet and exposure to different compounds. Perfluoroocatanesulfonic acid (PFOS) and Perfluorohexane sulfonate (PFxHS) are perfluorinated compounds that are bioaccumulative, environmental toxicants. PFOS and PFxHS can be found in water, cookware, and many household products, and are a potential health hazard. The aim of the study was to determine the relative abundance of *Akkermansia* between the different diets in comparison to Firmicutes (*Lactobacillus*) and *Bacteroides*. At 10 weeks of age, C57BL6 mice were placed on a 11% KCal low fat diet or 58% KCal high fat diet with an addition of fructose/sucrose water. Mice were then divided into 3 treatment groups; including diet alone, 0.0003% PFOS in diet, and 0.0003% PFxHS in diet. The end design resulted in a total of 6 groups with an N=6: i) Control LFD, ii) Control HFD, iii) L PFOS, iv) H PFOS, v) L PFxHS and vi) H PFxHS. Fecal pellet samples were collected from mice at weeks 27 and 28. Mice were sacrificed after 29 weeks, upon which cecal content samples were collected. An internal lab method was created and validated for extracting genomic bacterial DNA with a high yield from mouse fecal material (fecal pellets and cecum). Methods to quantify the relative abundance of several bacterial strains from fecal DNA samples were developed. The relative abundance of key bacterial strains in DNA extracted from feces was quantified by real-time quantitative PCR. Data presented will review optimization of DNA extraction from mouse fecal material and relative *Akkermansia muciniphila* abundance.

Variation in Predation and Genotype Among *Bdellovibrio* Predatory Bacteria

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Bdellovibrio are delta-proteobacteria that attack and digest other bacteria. They are the most well studied predatory bacteria and inhabit soil, marine, and freshwater environments. *Bdellovibrio* attack Gram-negative pathogens, which makes them good candidates for biocontrol agents or alternatives to antibiotics.

Most studies of *Bdellovibrio* focus on the type strain HD100. Relatively little information is available about other *Bdellovibrio* strains. Our goal is to investigate variation in predation among different *Bdellovibrio* strains. We studied *Bdellovibrio* isolated from soil and a drain environment. We explored whether these strains were prey specialists or generalists (prey range) and whether *Bdellovibrio* differ in how quickly or effectively they attack prey bacteria (predation efficiency). Furthermore, we investigated how phenotypic differences related to genetic variation among strains.

To understand variation in predation efficiency, we studied *Bdellovibrio* NC01, which was previously isolated from soil. In comparison to type strain HD100, NC01 appeared less aggressive, taking longer to attack prey cells and multiply in a lysate. On a double agar overlay plate, NC01 was slower to form plaques and, in contrast to HD100, plaques of NC01 were not completely clear. To quantify these qualitative observations, we performed a predation efficiency assay to compare NC01 and HD100 rates of predation. We also sequenced the genome of NC01 to explore variation in genotype. The genomes for NC01 and HD100 varied in length, and RAST annotation indicated the NC01 chromosome had more genes from mobile genetic elements such as phage.

To understand variation in prey range, we challenged one of the *Bdellovibrio* (#0059) isolated from a drain with eight Gram-negative prey. This isolate only attacked two of the eight prey, suggesting this strain may be a prey specialist. This contrasts with frequent descriptions of *Bdellovibrio* as a prey generalist. To further explore variation in plaque phenotype among the drain *Bdellovibrio*, we plated all seven isolates on either *E. coli* or *Raoultella*. These seven isolates have identical 16S rRNA gene sequences, but differ in plaque phenotypes, particularly plaque size and appearance. We are using Illumina data to explore how these plaque phenotypes relate to genome variation.

Moving forward, we will continue to investigate variation in these *Bdellovibrio* strains through prey range and predation efficiency assays and genomics.

MOLECULAR BIOLOGY

LOCATED IN ROOM 105 ON THE 1ST FLOOR OF THE PHARMACY BUILDING

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

Interaction of DNA Polymerase IV (DinB) in *Escherichia coli* with Transcription Termination/Antitermination Protein NusA and Transcription Repair Coupling Factor Mfd

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DNA damage in the form of base modifications, DNA adducts, single-stranded and double-stranded DNA breaks is a major cause of genomic instability, and can interfere with essential cellular processes such as transcription and replication. Our goal is to better understand the interplay between these processes and DNA repair using the tools of biochemistry and structural biology. Here, we explore the interactions between three factors involved in three distinct processes: translesion DNA synthesis (the Y-family DNA polymerase DinB), transcriptional regulation (NusA) and transcription-coupled DNA repair (the Mfd ATPase). We hypothesize that these factors collaborate with each other to preserve genomic integrity in a variety of contexts, as suggested by previously reported genetics studies. Here, we describe preliminary work aiming to study NusA/DinB and DinB/Mfd interactions *in vitro* and using X-ray crystallography.

Structural and Biochemical Analyses of ADHE Alcohol Dehydrogenases from *Entamoeba invadens* IP-1, *E. invadens* VK-1:NS and *E. dispar*

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The *Entamoeba* lineage belongs to the Amoebozoa, one of six major divisions of eukaryotes. *Entamoeba* trophozoites, similar to other protists, actively capture ingest and digest bacteria thorough anaerobic pathways that process diverse energy sources. Anaerobic pathogens have evolved adaptive metabolic enzymes that differ from vertebrates, and are ideal targets for novel compounds to improve disease management. The bifunctional alcohol/ aldehyde dehydrogenase enzyme in *Entamoeba histolytica* (EhADH2) belongs to the ADHE iron dependent family, and is essential for trophozoite growth and survival. EhADH2 catalyzes the conversion of acetyl Co-A to acetaldehyde and the final reduction of acetaldehyde to ethanol by its separate ADH and ALDH domains respectively. Several *Entamoeba* spp have homologous genes (*ADHE*) which might share a similar structure and function to that of the *ehAdh2* gene. This study aims to clone, express, and sequence the genes that encode for ADHE in three *Entamoeba* spp. The sequencing identifies evolutionary relationships between the homologs. The transformation of *ADHE* genes into *E. coli* cells will be used for protein purification and kinetic assays for comparison with EhADH2. Kinetic assays will provide Km values for both substrates and information on chemical inhibitors of the ADHE proteins. Inhibitors of this enzyme have promising anti-amoebic capabilities for drugs due to trophozoite growth dependence on ADHE. These studies will further the knowledge about the ecology and evolution of ADHE enzymes and their relevance for host survival and pathogenesis.

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. One of the proteins found to aggregate is Tau, a protein that stabilizes microtubules in neuronal cells. It is predicted that Tau is N-terminally acetylated based on its amino acid sequence by the complex NatA, which is responsible for the acetylation of a majority of proteins with this post-translational 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 and toxicity. *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.

A Highly Efficient, One-Step Purification of the Hsp70 Chaperone Ssa1 from *Saccharomyces cerevisiae*

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In order for a protein to exhibit proper function, it must first fold into a specific three-dimensional structure as dictated by its amino acid sequence. While some proteins fold spontaneously into their native states, others require molecular chaperones to reach their functional state effectively. Molecular chaperones are a diverse class of enzymes that maintains proteostasis through the mediation of protein structure *in vivo* via their up-regulation. This effectively prevents misfolding and aggregation of proteins and allows for cells to recover from various stressors, such as elevated temperatures, oxidative stress, and postischemic stress. In humans, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease are known to be a result of such protein misfolding. In the eukaryote, *Saccharomyces cerevisiae*, there are multiple classes of molecular chaperones known as heat shock proteins (Hsps), which are classified by molecular weight (kDa). Ssa1 is an Hsp70 class chaperone that works in tandem with the Hsp40 co-chaperone Sis1 to bind to non-native polypeptide chains and refold them into a functional conformation. *In vivo* studies of this protein prove limiting due to its transient mechanism of activity, wherein Ssa1 cycles in-between two main conformations as dictated by ATP hydrolysis. *In vitro* and structural studies also prove to be difficult, as they require large amounts of Ssa1 and current purification methods are resource intensive, time consuming and expensive. Here we have developed a one-step purification for Ssa1 by adapting purification methods utilized in large yeast protein complexes which utilizes a Protein-A fusion. This purification will allow for development of novel *in vitro* assays to better understand interactions between Ssa1 and its co-chaperones as well as the effects of post-translational modifications on chaperone function.

Determining the Effects of N-Terminal Acetylation on the Microtubule-Associated Protein Tau

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Neurofibrillary tangles and amyloid plaques characterize Alzheimer's disease, and the aggregation of the protein Tau is the cause of the neurofibrillary tangles. Tau's endogenous function stabilizes polymerized microtubules in neuronal cells. Tau has been characterized to aggregate and malfunction when hyperphosphorylated, a common post-translational modification. Post-translational modifications (PTMs), which change the structure and function of a protein, are the cause of Alzheimer's Disease and the other tauopathies related to Tau. N-terminal acetylation is a PTM that changes the charge and structure of the protein, and also adds a ~40 Dalton shift in molecular weight to the protein. Over 80% of mammalian proteins are N-terminally acetylated, indicating a biological importance to this mechanism. Tau has the correct amino acid sequence to be N-terminally acetylated by the NatA complex and additionally, the N-terminus of Tau has been implicated in altering aggregation rates. However, Tau and N-terminal acetylation have never been studied together. *Escherichia coli* is commonly used to express and purify proteins, including Tau, by utilizing recombinant vector DNA. This system is prokaryotic and cannot post-translationally modify proteins as the endogenous eukaryotic system can. The NatA complex, inserted and expressed in *E. coli*, is able to N-terminally acetylate Tau, even though it is a prokaryote. This prokaryotic system of looking at one PTM is advantageous because it can examine one alteration in a protein, without the other potentially hundreds of PTMs affecting the protein as well. To identify implications of N-terminal acetylation on the protein Tau, a crude purification precipitated out most of the endogenous bacterial protein, leaving ~85% pure Tau. A visible upward shift on a SDS-PAGE of the purified Tau co-expressed with the NatA complex indicates the presence of the acetyl group on the N-terminus. Analysis of our purified protein by mass spectroscopy and isoelectric focusing will definitively determine the molecular weight shift in the N-terminally acetylated Tau. Further goals include polymerization assays of tubulin with Tau with and without the acetyl group, and kinetic and thermodynamic examination of Tau fibers with and without the acetyl group.

Insights into the Role of the FANCD2 Protein in DNA Replication Arrest

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Fanconi anemia (FA) is a rare genetic disease that affects approximately 1 in 160,000 individuals. It is characterized by congenital defects, bone marrow failure, and increased susceptibility to cancers; those affected often are diagnosed with acute myeloid leukemia (AML) and cancers of the head and neck. FA is caused by biallelic mutation of any one of the twenty one FA genes. The proteins encoded by these genes cooperate in the FA/BRCA DNA repair pathway which is responsible for repairing DNA interstrand crosslinks (ICLs). These ICLs lead to DNA damage and act as physical blocks to both DNA synthesis and transcription. A key activating step in the FA pathway is the addition of a single small protein, termed ubiquitin, to the FANCD2 protein, in a process known as monoubiquitination. In addition, FANCD2 monoubiquitination has been linked to cell cycle progression and activation of checkpoint proteins. When cells undergo DNA damage, the CHK1 cell cycle protein becomes activated via phosphorylation of serine 345 (CHK1 pS345) and halts the cell cycle. Previous studies have revealed that phosphorylation of CHK1 is correlated with the ubiquitination of FANCD2. We aimed to gain a better understanding of the relationship between FANCD2 and CHK1, and the kinetics of the DNA damage response to UV irradiation. We induced DNA damage in FA-D2 (FANCD2-/-) patient cells stably expressing wildtype FANCD2 or empty vector using UV irradiation. Cells were collected at various time points post UV treatment, and immunoblotted using antibodies against FANCD2, CHK1 pS345, and other DNA damage response proteins. The results from two replicate experiments imply that robust CHK1 phosphorylation is reliant on the presence of FANCD2 and that CHK1 phosphorylation precedes FANCD2 monoubiquitination, highlighting the link between the DNA damage response and regulation of the cell cycle. In addition, molecular combing was utilized to examine how mutation of FANCD2 affects DNA synthesis. In short, FA-D2 cells were exposed to aphidicolin, a DNA polymerase inhibitor, for 12 hours then pulsed with thymidine analogs. These analogs incorporate into the DNA and can be fluorescently labeled to see differences in DNA synthesis. It was found that the FANCD2 mutant cells may have compromised fork restart following aphidicolin treatment.

Designing a Protocol to Screen for Optimal Growing Conditions of 70S Ribosome Cystals

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Ribosomes are universal macromolecules composed of two nonequivalent subunits within ribonucleoprotein assemblies that contain translation machinery necessary to power protein synthesis. Solving the atomic structure of complete 70S ribosomes and their subunits has been a particular interest eluding scientist for decades. The structure of total 70S ribosome has been resolved, first in *E. coli*, through the use of X-ray crystallography providing vast insight into ribosome crystallography, prokaryote protein synthesis, antibiotic resistance, and ext. This breakthrough in x-ray crystallography sparked the interest of many scientists and fueled the need for structural data from total ribosomes in additional species. Even though this technique has proven successful in numerous prokaryote species and even in visualizing *S. cerevisiae* 80S ribosomes it has proven difficult due to the relatively large size and complexity of ribosome molecules. Growing crystals can be an exhausting process since it requires the alteration of numerous chemical and physical parameters within growing conditions. The purpose of this study was to design a screening protocol to identify optimal growing conditions for 70S ribosome crystals. Art Robbins Phoenix Crystallization robot was used within this protocol to screen 192 different conditions, collected from Protein Database, prepared in 96-well sitting drop Intelli-plates with relative precision and ease. The optimization protocol designed in this study proved successful in growing 70S ribosome crystals purified from *T. thermophilus* within the following precipitating agent ranges: 2.7-3.5% PEG 20K/ 8-11% MPD, 3-4.5% PEG 20K/ 4-5.5% PEG 550, 3.5-4.5% PEG 20K/ 3.5-4.5% PEG 550, and 3-6% PEG 20K/ 9-12% PEG 200. This protocol could be used as a crucial initial step for the identification and optimization of growing conditions of 70S ribosomes.

Determination of Expression Levels of DENV2 E-Protein in Transfected CHO-S Clones

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DENV E-protein is important for research on dengue virus, but is very expensive. One way to relieve the financial burden is to develop the protein in house using a construct containing the DENV2 E-protein and transfecting these cells to express the protein. The purpose of this experiment is to determine the expression levels of CHO-S transfected Clones 1.0 and 2.2. This was determined through staining intracellularly and running through a flow cytometer as well as lysing samples and visualizing the protein on a western blot. The results of this experiment were that pre-M and E could be detected in Clone 1.0 successfully, but not as well in Clone 2.2. In conclusion, Clone 1.0 appears to have a higher expression of DENV2 E-protein than Clone 2.2.

Assessing the Toxicity of Antimicrobial Diamides Using the Model Eukaryotic Organism *Saccharomyces cerevisiae*

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The development of antibiotics was arguably one of the largest breakthroughs in the medical field. However, due to the continuous usage of these antibiotics, the problem of antimicrobial resistance has emerged. For that reason, it has been of great interest to discover new compounds that demonstrate antimicrobial activity. Several diamide inhibitors have already been successfully identified by our group. The goal of this project is to assess the toxicity of these compounds in eukaryotic systems. The organism *Saccharomyces cerevisiae* was chosen as the eukaryotic model, as it has been successfully used in toxicological assays. *S. cerevisiae* also serves as a model eukaryote, as there is significant preservation of gene function between this yeast strain and humans. As a result, research conducted with *S. cerevisiae* permits us to gain valuable insight into potential eukaryotic toxicity that would otherwise be challenging with larger eukaryotic cell systems. Here, *S. cerevisiae* is used as a simple and rapid screen for evaluating the eukaryotic toxicity of diamide inhibitors of bacterial growth.

In pre-clinical development, toxicology tests are carried out to verify the extent to which a substance can damage a living or non-living entity. More specifically, the cell cytotoxicity testing in this study demonstrated that our diamide lead compounds fgbb and fgkc against *Streptococcus pneumoniae* do not disrupt *S. cerevisiae* cell viability. Subsequently, a hemolysis assay was performed to further assess membrane disrupting properties of the diamide inhibitors. Hemolysis is the process of red blood cells rupturing, leading to the release of the hemoglobin into any surrounding fluid. In this assay, the membrane disrupting properties of the inhibitors were measured by considering the quantity of lysed and intact red blood cells after samples of sheep blood were exposed to them. The results showed that the diamides fgbb and fgkc do not possess membrane disrupting properties at concentrations as high as 125µM. Through these noted toxicity assessments, and the continuation of this research, we shall further assess these compounds as potential antimicrobials.

The Hope Funds for Cancer Research: Experiences and Events

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The Hope Funds for Cancer Research was formed in 2006 to establish a funding vehicle that would take a rational scientific, medical, and investment approach to making grants for the most interesting and promising research efforts to address the most difficult-to-treat cancers, including pancreatic, lung, liver, sarcomas, esophageal, brain, gastric, and ovarian cancers, as well as rare leukemias, lymphomas and MDS. As interns for the Hope Funds, this opportunity has allowed us to experience a variety of events, meet incredible scientists, and has given us the opportunity to interact in today's evolving science field. On a day to day basis, we help organize events such as the annual Gala and Awards Dinner, the Scientific Convening, and the Women and Science luncheon. Hand written invitations to these events and thank-you cards are just small gestures from Hope Funds that show our passion and love for these individuals and the events we put together. Being able to greet and interact with our scientists at the Scientific Convening, held at the International Tennis Hall of Fame, to having a casual sit down conversation with them over lunch overlooking center court, allows for personal connections to be made. As interns, we also contribute to helping with the annual Grants that are given out annually. As first contact, we reassure the applicants that their application had been received and that we will be their point of contact throughout the process. Although our internship may not be directly hands on in a lab, we are creating personal connections and are still interacting directly with these incredible scientists. After only a short time of being a part of this organization, we can honestly say that we have had new, eye-opening experiences, unforgettable conversations, and have connected with individuals, not just scientists, but those who founded this organization, and can say they have strongly impacted our lives already.

The Hope Funds for Cancer Research Foundation: Funding Innovative Research of Understudied Cancers

Emily Riley, Emily Kahler & Leah Cann

Hope Funds for Cancer Research, Newport, RI

The mission of the Hope Funds for Cancer Research is to encourage the investigation of innovative cancer treatment and detection for the most difficult-to-treat and understudied cancers. The foundation supports scientific and medical research programs aimed at increasing knowledge of both cancer care and prevention. The Hope Funds for Cancer Research funds highly innovative research projects that challenge the traditional paradigms of the causes, mechanisms, progression, disease markers or risk factors of the most difficult-to-treat cancers. The primary activity of the foundation is to award fellowships to young researchers who have the highest probability of making an impact in these hardest-to-treat cancers. The foundation hosts annual events, the largest and most known being the Scientific Convening and the Gala, to encourage collaboration in science, and to foster opportunity and recognition of scientific achievement. Hope Funds awards a varying number of three-year postdoctoral fellowships each fall, with a summation of grants throughout the three years equal to $151,500. Hope Funds honors, with an annual medal, individuals or organizations who have made a significant impact in patient care. Honorees are selected for Awards of Excellence in fields of Basic Science, Clinical Development, Medicine, Advocacy, and Philanthropy. The foundation presents the James D. Watson Award at their annual Gala to recognize discoveries that fundamentally change the field of science. Dr. Watson was the first recipient of this award in 2013. The Hope Funds for Cancer Research foundation presents unmeasurable opportunity for young researchers with new ideas and, consequently, encourages groundbreaking discoveries in science and medicine.

Mutagenicity and Replication Block of Guanine DNA Adducts Influenced by Neighboring Epigenetic Biomarkers

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In cell cycle, epigenetic information has been conveyed by epigenetic markers. A prime example includes the DNA base known as 5-methylCytosine (m5C). Cytosine when methylated at the 5 carbon position is an epigenetic regulator and goes through a process known as DNA Methylation in mammals; m5C can be furtherly modified to other epigenetic markers. This process applies a series of oxidation to the derivatives of 5-mC. 5-methylcytosine oxidizes to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC) to which then can be corrected by enzymes that replace the 5-Carboxylcytosine with Cytosine (C). People have shown these epigenetic markers to be very weakly mutagenic and do not inhibit replication. These epigenetic markers appear on CpG (Cytosine-Phosphate-Guanine) islands. Guanine is a major DNA base that can be damaged. Damages occur to guanine and generate DNA adducts 8-oxoguanine (8-oxo-G), O-6methylguanine, along with others. In this project, we will investigate the biological influence of these non-mutagenic epigenetic markers on the mutagenicity of the neighboring DNA adduct 8-oxo-G, which can lead to G-T mutations. The following procedures will be used to achieve synthesis and purification of an oligonucleotide containing both the epigenetic markers and 8-oxo-G. First we'll use DNA synthesizer to synthesize a 16-mer sequence containing both modifications. Second HPLC will be used to purify the synthetic nucleotide product. Third we will use high resolution LC-MS to characterize the identity of the product. For all the other epigenetic markers and the Guanine lesions we will be using the similar procedures. In the future, biochemical and cellular experiments will be carried out to test the replication block and mutagenicity of these oligonucleotides.

Breaking Down Cancer: IDH and Its Impact on Metabolism and DNA Damage and Repair

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Metabolic reprogramming has been detected in many diseases, including cancer. Recently, a small number of metabolic enzymes have been identified as driver mutations for various types of cancer. To better understand how these genes lead to cancer, and to identify molecular targets for treatment, we are investigating the isocitrate dehydrogenase (IDH) mutation implicated in human glioma and acute myeloid leukemia. We are currently using *Drosophila melanogaster* as a model to study both the metabolic changes that occur when IDH is mutated, as well as the potential impact on DNA damage repair pathways. To analyze metabolic changes in the brain of IDH mutant flies we have optimized a protocol for measuring the metabolism of whole brains using the XFe96 metabolic analyzer. We have also detected the expression of D2-HG, a metabolite produced by mutant IDH in human cells, in our fly IDH model. Generation of D2-HG in human cells has been reported to correlate with insufficient DNA repair. To study this further we are testing for increased DNA damage and lack of DNA repair initiation. In particular, we are investigating a hypothesized connection between the Fanconi Anemia DNA repair pathway and IDH mutations.

Palm-Domain Mutations of Human DNA Polymerase Theta Exhibit Altered Polymerase Activity

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Cancer is one of the leading causes of death in the developed world. Melanoma is a type of skin cancer in which melanocytes undergo uncontrolled growth and can spread to other portions of the body. The main cause of melanoma is UV damage to DNA base pairs within melanocytes. DNA damage caused by UV radiation is fixed through various DNA repair pathways using highly specialized repair enzymes, including DNA Polymerase Theta (Pol θ or POLQ). Pol θ is known to maintain genomic stability through microhomology-mediated end joining (MMEJ), but it is also able to perform base excision repair *in vitro*. A number of somatic POLQ variants have been identified in melanoma patients. Much like other polymerases in its family, Pol θ possesses three subdomains within its polymerase domain: a thumb, a finger, and a palm subdomain. It is within the palm subdomain that the polymerase's active site is located. This study identifies three melanoma-derived mutants located in the palm domain, L2538R, Q2537H and V2551D, which could alter the phosphodiester bond formation during nucleotide incorporation. We hypothesize that cancer associated variants of Pol θ may have different DNA repair pathways compared to wild-type, which may act as a driver of cancer. All three variants were generated using site-directed mutagenesis and were purified by affinity chromatography. Polymerase activity, including nucleotide incorporation and DNA binding was assayed and compared to wild-type. Preliminary results show that variants with an amino acid substitution in the palm region display altered polymerase behavior while DNA binding seems unaffected as compared to wild-type. This suggests that the variants may follow alternate polymerization mechanisms, which may impact DNA repair pathways and drive melanoma.

Site-Specific Labeling of DNA Polymerase Theta for FRET

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Environmental factors such as UV light contribute to damaging DNA daily. To overcome those damages, the cell uses key enzymes to repair DNA such as DNA polymerases to maintain genomic stability. A relatively novel DNA Polymerase Theta (Pol θ) is primarily involved in DNA repair of double-strand breaks, and can potentially be used as a base excision repair substitute for Pol β *in vitro*. Although it is the primary DNA polymerase in micro-homology mediated end joining pathway, it is a low fidelity enzyme in DNA repair, unlike Pol β which is highly accurate. This accuracy comes from the enzymes ability to "read" a DNA template and incorporate the correct nucleotide on the opposite strand through a variety of dynamic movements. It is hypothesized a polymerase selects the correct nucleotide complementary to the templating base in the fingers domain, binds to it and positions it opposite the template. If a polymerase has a poor ability to add the correct nucleotide, this can lead to mutations, which in turn leads to genomic instability and potentially cancer as evident by cancer-associated variants of DNA polymerases that are highly mutagenic. While the movements affecting nucleotide choice have been studied in other DNA polymerases, little is known about the global movements of Pol θ and how it chooses a nucleotide. This study aims to elucidate the biochemical mechanism of choice of Pol θ during DNA repair using Fluorescence Resonance Energy Transfer (FRET). In this study, we optimized a site-specific labeling protocol to directly label the fingers domain of Pol θ with fluorescent 5-FAM cadaverine to observe the global movements of the fingers domain as it moves closer to the Dabcyl-labeled DNA during nucleotide incorporation. In order to directly label Pol θ, a Q-tag (GQQQLG) was engineered into the fingers domain via site-directed mutagenesis. The modified protein was expressed in *E. coli* and purified via affinity chromatography. The purified protein was assayed to ensure the modified enzyme conserved the same activity as wild-type Pol θ. Here we present evidence that suggests the Q-tag did not significantly alter the enzyme structure and function as well as a labeling scheme for targeted protein labeling.

Identification of Chromatin Loops in Hox Gene Clusters of Developing Zebrafish Embryos

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Within the nucleus the cell segregates portions of genetic material from one another into large (100KB—5MBs of DNA) physical territories called topologically associating domains (TADs). Within TADs chromatin interactions occur more frequently forming chromatin loops that have been shown to positively or negatively affect gene transcription. To gain a better understanding of the role chromatin organization plays in gene regulation, we have adapted the chromosome conformation capture technique (3C) to identify chromatin loops throughout Hox gene clusters in the developing zebrafish embryo. Hox genes encode a conserved family of homeodomain containing transcription factors that are important in metazoan hindbrain development and have previously been shown to be regulated through chromatin structure. However, many details, such as when during the developmental process these loops form and what specific factors regulating these loops through the Hox clusters, are still unknown. We have potentially identified some interactions within hox gene clusters and are currently validating 3C libraries that we have generated to determine whether or not we have identified true loop formation. Through the identification of chromatin interactions within the hox gene clusters, we aim to gain further insight into the specifics of Hox gene regulation to better understand the role these genes may play in developmental disorders of the hindbrain such as Autism and ataxia.

Graph Analysis of Genome-Scale Metabolic Models Demonstrates Intra-Genus Similarities and Inter-Genus Differences in Network Properties

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Cellular metabolism can be thought of as a series of chemical reactions occurring in a shared space, with enzymes facilitating the conversion of one metabolite to another. Genome-scale metabolic reconstructions are representations of the total set of these metabolic reactions for an organism, which can be used in metabolic simulations, bioengineering, and evolutionary studies. The gram negative bacteria Shewanella are found in a diverse range of aquatic environments and are studied for their ability to metabolize a variety of electron donors and acceptors. These metabolic features make this genus a focus of studies related to microbial fuel cell development, nanowire formation, and genome-scale metabolic modeling. In order to compare a recently published model of *Shewanella piezotolerans* WP3 to another subgroup within the *Shewanella* genus, a previously published genome-scale metabolic model (GEM) of *Shewanella oneidensis* MR-1 was updated to include new gene annotations based on ortholog mapping and cross-referencing several databases, including NCBI protein and literature databases. The metabolic modeling software PSAMM was used to generate network representations of the updated MR-1 model, the *S. piezotolerans* WP3 model, and a model of the gram negative bacteria, *Escherichia coli*. These network representations consist of a set of nodes, representing metabolites, connected by a series of edges, representing enzyme catalyzed reactions, and can be used for the mathematical analysis of network properties and structure. The three metabolic networks were graphically visualized and analyzed using the Cytoscape network mapping software. This analysis revealed minor differences between the three organisms on the full-network scale. However, analysis of metabolic subsystems revealed greater differences in network characteristics between more distantly related species; for example, compared to both *Shewanella* strains, the *E. coli* glycolytic pathway had a higher clustering coefficient combined with lower network diameter, suggesting a more tightly connected pathway. Representing GEMs graphically as interdependent networks, along with comparisons to other genome-scale models, can allow for new types of investigations to be performed and will provide novel insights into the structure and organization of cellular metabolism.

NEUROSCIENCES

LOCATED IN ROOM 240 ON THE 2ND FLOOR OF THE PHARMACY BUILDING

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

Fear of Predator: Automated Home-Cage Monitoring Develops a More Complete Behavioral Profile of Response to Predator Odor

Brandon Couture[1], Joseph Dowling[2], Kaitlin Mulligan[1], Jaime Podracky[1] & Christopher Bloom[1]

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Animal models have become a standard for treating/analyzing human diseases and afflictions. Aside from the ethical implications, animal models are preferred over human testing because they allow researchers the ability to perform more tests at a lower cost. Rodents, for example, have become a favorite model for anxiety studies for this very reason. Using 2,3,5-trimethyl-3-thiazoline (TMT), an extract from fox feces, is an easy and reliable approach to creating an anxiolytic environment (Chakraborty, et al., 2015). Many researchers have coupled TMT with different analytical assays, such as the EPM and Plantar test, in order to understand the effect of anxiety on other behaviors. Bloom (2014), used this approach to indicate a relationship between early life stressors and adolescent anxiety/ pain nociception. Other studies have been conducted which use predator stress to show that high stress events can result in chronic disorders such as PTSD (Janitzky, et al., 2014). Even the most widely accepted assays have a fundamental flaw, their analysis is too far removed from the stressor. Besides that, it is impossible for an assay such as the EPM to analyze every rodent behavior especially since tests seldom run for more than five minutes (Wall & Messier, 2001). The current experiment explores the value of automated home-cage detection software in the continual analysis of behavioral changes resulting from exposure to predator odor. Our results indicate a litany of behavioral changes following exposure to predator odor including changes in resting, mobility, sniffing, grooming & rearing behaviors. This technique may help to develop a more complete view of the complex defensive behavioral cascade and have implications in the understanding, treatment and management of fear-related disorders.

Co-Regulation of Friends' Stress Response

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Relationships are key contexts in which individuals emotionally react to and learn how to regulate stress. During adolescence, peer relationships are perhaps the most salient context in which emotional and physiological responses to stress are regulated and shaped. The reciprocal process by which stress responses may be shared and shaped within relationships has been referred to as co-regulation and can be measured by examining the degree of emotional concordance (e.g., matching arousal level) within a friendship dyad. This co-regulation of a stress response may be particularly strong for girls given the salience of friendships for girls during adolescence and studies suggesting that girls are more attuned to other's emotions than boys. The present study examined if adolescents' friendships provide a context in which emotional responses to stress are shared and shaped and if gender differences exist in the sharing of these stress responses in a sample of 29 friendship dyads. Sixty-one percent of the participants were female and on average were 14.75 years of age (SD =0.75). The majority of the participants were White (79%). Adolescents participated in two interpersonal stressors: one in which they discussed and tried to resolve issues of conflict in their relationship and the other in which they experienced a peer exclusion stressor called the Yale Interpersonal Stressor (YIPS). Measures of stress included peak-baseline scores of heart rate, alpha amylase, cortisol, and self-reports of emotion. Preliminary correlational analyses suggested that adolescents shared emotions following the stressors as indicated by positive correlations between cortisol response and heart rate response between friends. For example, an increased heart rate response to the YIPS stressor for one friend was associated with increased heart rate response to the YIPS stressor for the other friend. Gender differences also suggested that girls were more likely to share physiological responses to stress following the stressors than boys were, particularly in regards to heart rate and cortisol. These findings are consistent with previous research on gender differences. Examination of this topic sheds light on the concordance of emotions at a neurobiological level and if shared emotions serve as a reinforcing dynamic that maintains or amplifies how individuals' regulate stress.

Adolescents' Physiological Response to Stress within in Peer Relationships

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Transitions within peer relationships confront adolescents with new challenges that impact their regulatory abilities and stress response (Zimmerman et al., 2009). Generally, we think of stress occurring when mental, emotional, or physical demands exceed the regulatory capacity of individuals; this may occur when youth experience developmental transitions because these situations are new and unpredictable. Peer relationships, particularly developing intimate relationships with friends, are important throughout adolescence, as youths' need for affiliation and enhanced desire for acceptance are primary developmental concerns. There is a growing body of literature that links peer rejection and exclusion to increased stress reactivity, specifically increased cortisol production and alpha amylase (Byrd-Craven et al., 2011; Stroud et al., 2009). Research, however, has generally neglected to look beyond peer rejection to examine how adolescents handle stress when faced with challenges in friendships (e.g., managing conflicts, giving and receiving support). This is surprising given that problems in friendships and regulating stress within those relationships may be detrimental to functioning (Roisman et al., 2004). Thus, the current study examined the effect of peer rejection and conflict in close friendships on adolescents' stress response as measured by heart rate, cortisol, and alpha amylase. Participants were 60 adolescents (M = 14.75; SD = 0.75) with the majority of youth self-identifying as White (79%). Adolescents participated in two interpersonal stressors: one in which they discussed and tried to resolve issues of conflict in their relationship and the other in which they experienced a peer exclusion stressor called the Yale Interpersonal Stressor (YIPS). One adolescent was randomly assigned to the YIPS and the other was assigned to a control task where they planned a party. Repeated measures ANOVAs indicated that adolescents evidenced an increased alpha amylase response and heart rate response to the friendship conflict task. Participants assigned to the YIPS generally did not evidence a stress response to the YIPS. These results need replication but suggest that conflict experienced within a friendship may have a more pronounced effect on adolescents' stress response than peer rejection, at least as measured in a laboratory setting.

A Computational Approach to Modeling Dopaminergic Neurons with Application to Parkinson's Disease

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More than one million individuals suffer from Parkinson's Disease (PD) in the United States alone, and while the biomedical research community continues to progress neurotherapeutics and enhance our knowledge of the causes and pathogenesis of this disease, a comprehensive understanding of the intracellular mechanisms by which dopaminergic neurons prematurely commit to an apoptosis phenotype in PD remains elusive. To address this issue, we have developed a mathematical model of the intra-cellular signaling pathway of the key proteins in dopaminergic neurons that are directly involved in the progression of PD, including Parkin, PINK1, and alpha-synuclein. To accomplish this, we have completed an extensive search of the PD neurobiological and medical literature and constructed a systems biology based wiring diagram, which in turn was used in developing a kinetics-based system of ordinary differential equations to model this pathway. The model incorporates intracellular species recently found to be central in the pathogenesis of PD, including calcium, the IPAS pathway, and the LRRK2 enzyme which importantly phosphorylates alpha-synuclein. Further, the novelty of this work includes an approach for deducing currently unknown pathway kinetics based upon system phenotype. In particular, we view the premature commitment to a pathological apoptosis phenotype as an irreversible biological switch, and use this perspective to identify those kinetic rate values that enable the mathematical model to support this phenotype. We will present our intra-cellular signaling pathway wiring diagram, mathematical model, numerical approach for identifying kinetics, and preliminary results that showcase the capability of the model to computationally emulate PD pathogenesis. We hope that this work will ultimately help contribute to a greater understanding of the role of the intracellular components responsible for PD progression as well as uncovering potentially new treatment targets and their impact on neuronal survival.

A Mathematical Model of the Effects of Neurostimulation Treatments on Neuronal Electrodynamics

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Neurostimulation therapies continue to demonstrate success as a medical intervention for individuals with neurodegenerative diseases. In particular, transcranial direct current stimulation and deep brain stimulation treatments have shown to alleviate movement disorders associated with both early and late stages of Parkinson's disease (PD). Despite promising results from these neuromodulation modalities, the mechanisms by which neurostimulation alleviates PD symptoms remain elusive. Specifically, the influence of an electric current on intracellular and extracellular ion concentrations and subsequent transmembrane electric potentials is not clear. While the equations that govern these bioelectromagnetics are understood, current mathematical models of neurostimulation do not incorporate them, nor do they incorporate biologically-based cellular processes such as transmembrane ion channel gating. This project has focused on developing the first cellular-level mathematical model of neurostimulation, with the goal of better understanding its effects on the electrodynamics of a neuron. To date, we have implemented a numerical solution to the neurologically-inspired Poisson-Nerst-Planck system of partial differential equations using the finite element method, which effectively models intracellular and extracellular electric potential, neuronal transmembrane voltage, as well as sodium, potassium, chloride, and calcium ion concentrations. In addition, we have integrated a Hodgkin-Huxley based scheme to quantify transmembrane ionic flux for all ion species. Further, we have conducted numerical experiments on several idealized two-dimensional neuronal geometries. Our future endeavors will include extending our computational simulations to three-dimensional physiologically-inspired neuron domains, as well as integrating an intracellular signaling pathway model of a dopaminergic neuron. We hope that this work will ultimately help elucidate the principles by which neurostimulation alleviates PD based symptoms.

The Relationship of Parental Speech to 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 early speech is also enhanced when parents label and describe objects and encourage verbal ability (A red truck; Say truck) rather than when parental talk is focused on the child's non-verbal behavior (Sit here). However, although parental speech that refers to the child's non-verbal behavior is negatively associated with language production, it may play a role in children's language comprehension, particularly comprehension of verbs. For example, about one-third of parental speech is directed at child behavior, and verbs in these utterances tend to occur in highly salient positions at the beginning of an utterance, (Sit down; Open your mouth). The present study investigates the relationship of parental use of behavioral directives (BD) 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 comprehended. For the present study, we analyzed transcripts of parent-child interaction during a play session videotaped in the lab following the comprehension assessments. Transcripts were coded for behavioral directives (BD), defined as utterances used to initiate (Let's play ball), maintain (Throw it) or prohibit (Stop that) the child's non-verbal behavior. We examined the relationship of parental speech measures (total number of BD, percentage of all maternal utterances coded as BD, number of verbs in BD utterances) to measures of child verb comprehension (number of verbs parents checked as comprehended by the child on the vocabulary list and the child's score on the laboratory assessment of comprehension). Results indicate a positive relationship between the child's score on the verb comprehension task and the number of verbs parents checked on the vocabulary list, suggesting agreement between the two measures of child verb comprehension. Moreover, children's score on the verb comprehension task was positively related to the percentage of BD in parental speech as well as the number of verbs that appear in utterances coded as BD. These data suggest that parental use of directives may be an important source of information about how words encode action, when assessed by measures of comprehension rather than production.

Early Comprehension of Action-Related Words

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Research suggests that children learning English acquire words for objects (nouns, as in cookie, truck) earlier than words that encode actions (verbs, as in clap, sit). Children begin to comprehend nouns as early as 9 months of age, but verb comprehension is not evident until 16 months. 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 young children comprehend words that label routine actions (e.g., bye), games (e.g., peekaboo), and locative prepositions that encode motion (e.g., up / down). To date, laboratory assessment of early word comprehension has been limited to nouns and verbs. Our current research tests 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 the present study we tested a set of 6 action–related words in children aged 13-15 months of age. Children viewed a slideshow of actors performing pairs of action-related words (e.g., covering/uncovering the face for peekaboo versus an actor lifting a toy bear from the floor for up) and visual attention was recorded for target and distracter images during baseline and test trials. Results indicate that children comprehend these action-related words well before the age typical of verb comprehension. These data suggest that children as young as 13-15 months are capable of the cognitive task of encoding a label that refers to a transient, dynamic event and that the slower course of verb comprehension may be related to other, linguistic factors, such as the frequency and saliency of verbs, compared to nouns, in parental speech.

Patterns of Visual Attention During a Verb Comprehension Task

Esther Quiroz, Briana Poole & Beverly Goldfield

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Research suggests that children learning English acquire words for objects (nouns, as in cookie, truck) earlier than words that encode actions (verbs, as in clap, sit). Children begin to comprehend nouns as early as 9 months of age, but verb comprehension is not evident until 16 months. 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. In a previous INBRE-supported study, we tested verb comprehension in children at 12, 14, 16, 18, and 20 months of age using the Preferential Looking (PLT) with eye tracker technology. Children viewed pairs of video clips in which actors performed actions (e.g., an actor clapping versus an actor dancing) during a sequence of familiarization (each action displayed individually for 5 sec), baseline (both actions displayed side by side for 10 sec), label (a blank screen while the child heard the target word) and test (both actions again displayed side by side for 5 sec) trials. Children's visual attention to target and distracter actions was recorded by a Tobii T60 XL eye tracker system. Comprehension was defined as an increase in attention to the target word during the test trial compared to baseline presentation. We found verb comprehension at 16, 18 and 20 months, but not at 12 and 14 months of age. In the present study, we investigate whether differences in visual scanning of a dynamic event may contribute to younger children's failure on the verb comprehension task. We examined children's visual attention to the actor performing an action during the familiarization trial for 5 verbs (clap, wave, take, break, pull). Results indicate no age differences in (1) duration of visual attention and (2) number of visual fixations to the dynamic event. These data suggest that children between 12 and 18 months of age demonstrate similar overall scan patterns when viewing a dynamic event. Further analyses will examine patterns of visual attention to the varied components that make up the event, such as face versus hands (clap, wave) and face versus hands versus object (take, break, pull).

Interaction of D2-Dopamine Receptors with the Wnt Signaling Pathway

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Schizophrenia is a severely debilitating neurodegenerative disease that affects about 1% of the world's population. The D2-dopamine receptor (D2R) is the major target of all available antipsychotic drugs (APDs) used to treat schizophrenia; however the cellular connections between the blockade of D2R by APDs and suppression of psychotic symptoms have not been identified.

Dact proteins are important regulators of the Wnt signaling pathway, which regulates gene transcription and development, and plays a crucial role in the pathogenesis of schizophrenia. In the adult brain, the Wnt pathway can alter neuronal circuits and synapses to modulate brain function. Here we show that D2R can potently and specifically suppress expression of Dact2. We have identified the structural features of D2R including the 4th transmembrane domain as being important for the suppression of expression of Dact2 and are investigating how treatment with antipsychotic drugs modulates D2R-Dact2 interaction. Investigation of this interaction will provide insight into whether D2R-mediated suppression of Dact2 expression leads to alteration of Wnt signaling by expressing Wnt reporters in appropriate expression systems.

Possibility or Probability: Distinguishing Emotional Reactivity from Sensitivity to Probability in Risk Perception

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This study evaluated the relationship between an individual's risk perception and several behavioral and psychological variables, including anxiety, hopelessness, and risk taking behaviors. . The online survey was distributed to 1200 individuals through MTurk, a web-based labor marketplace. We used several measures in this study. Risk perception was measured with the Possibility/Probability Questionnaire (PPQ), a relatively new measure that is still under development, which is designed to distinguish between emotional reactivity to uncertain events vs. sensitivity to changes in probabilities. Participants also completed the Beck Anxiety Inventory (BAI) and the State Trait Anxiety Inventory (STAI) to assess anxiety levels and specific anxiety symptoms, the Domain Specific Risk Taking Scale (DOSPERT) to measure risk taking behavior, and the Beck Hopelessness Scale (BHS) to measure the specific depression symptom of hopelessness. We predict that the emotional reactivity dimension of the PPQ will have a stronger predictive relationships with anxiety, and that the sensitivity to probability dimension will have a stronger relationship with risky behavior and hopelessness. In addition, we developed a hypothetical scenario describing a low-probability/highly dramatic event, to investigate whether PPQ scores can predict emotional and behavioral reactions to this kind of event.. . The scenario was experimentally manipulated to include either vague or vivid descriptions of a mass-shooting event, and presented equivalent probability information about this risk framed with either a high or low numerator/ denominator format. Based on past research, we predict that, people will be more sensitive to the numerator and perceive the risk more strongly in comparison to those participants given a probability with a low numerator and will perceive the risk more strongly if the scenario is described in vivid imagery. We further predict that the vividness effect will be predicted by the emotional reactivity dimension of the PPQ, while the numerator effect will be predicted by the sensitivity to probability dimension.

Componential Structure of Social Vision

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Social vision is an emerging field that integrates social psychology and vision science. In Study 1, visual attention to faces varying in attractiveness was measured and effects due to the perceiver, the target face and the perceivers' unique responses to specific faces was estimated using variance component analysis. Social vision was determined by individual differences among perceivers in allocation of visual attention to faces, and by perceivers' uniquely high or low visual attention to specific faces. Surprising face effects on visual attention were weak. In Study 2, a face recognition paradigm was used and the effects of visual attention, facial attractiveness and social status of faces were considered. Data analysis is underway.

A Multimodal Body Machine Interface Based on Finite State Machine Principles

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There are an increasing number of individuals in the world suffering from some form of paralysis. A new technology, the body machine interface, is being implemented to improve their quality of life. The concept works by utilizing what capabilities these individuals retain, to compensate for the abilities that they have lost. One of the greatest hurdles that these devices face is feature extraction from biological signals. In the proposed system, a combination of four electromyogram sensors and one electroencephalography sensor were used to generate these signals. In order to simplify the analysis of this data, a magnitude threshold can be set. When this threshold is met or exceeded, the input is a 1. When the threshold is not met, it is a 0. Using a truth table, these binary inputs can be mapped to various outputs, and vice versa. In this way, the desired functional outputs can drive inputs in order to drastically simplify the interfacing process.

Neuroprotective Effects of Amla (*Phyllanthus emblica*) Fruit Juice Powder and Its Purified Constituents in *Caenorhabditis elegans*

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Alzheimer's disease (AD) is a leading cause of death among the elderly population in the United States. One of the characteristics of this neurodegenerative disease is the aggregation of beta amyloid peptide (Aβ) which leads to oxidative stress and cell death in the brain. Most AD treatments address the symptoms of AD while few target the Aβ source of degeneration. The use of natural antioxidants to prevent or delay the onset of AD has gained a substantial amount of attention in recent years. Amla (*Phyllanthus emblica* L.), a fruit commonly grown in India, has been associated with numerous health benefits against cancer, aging, inflammation, diabetes and, neurological diseases, making it a promising candidate for AD treatment. *Caenorhabditis elegans* (*C. elegans*) are nematodes widely used as *in vivo* AD models as they can be transgenically engineered to express human beta amyloid peptide. This study aimed to examine the potential of amla juice powder, and its purified constituents, to delay Aβ induced paralysis using transgenic *C. elegans*. The *C. elegans* were grown, maintained, age synchronized and subsequently treated with amla and its purified constituents. Following an incubation period, the *C. elegans* were counted and incubated every hour until all worms were paralyzed.

Circadian Rhythm in Rats

Dara Cuffe

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Behavioral, physiological, cellular, and metabolic processes adjust throughout the day in approximately 24-hour cycles that coincide with the environmental light dark cycle. This pattern of activity is called a circadian rhythm. Circadian rhythms are internally generated cues that allow an organism to predict environmental events and generate a physiologic response for upcoming events. Cells that are out of synch with the environment are less adaptive than cells that are entrained. The suprachiasmatic nucleus (SCN) is the master biological clock found in the mammalian hypothalamus. The SCN receives environmental cues about time, such as light, but oscillates to a rhythm generated by a cyclical pattern of gene expression, where a negative feedback loop is formed when the protein product of a gene actually turns off production of more protein. These molecular gears seem to effect cell metabolism. As there appears to be a tight connection between circadian rhythms and metabolism, developing a method to evaluate the role of circadian rhythms in cellular energy metabolism could be key to understanding neurological diseases as well as cancer. Here we set out to standardize a method to measure metabolic parameters from SCN tissue in comparison to cortical cells. Tissue punches of SCN and cortex were analyzed using the Seahorse Bioflux analyzer, which measures oxygen and acidity of media surrounding the cells, allowing the derivation of the tissue's oxygen consumption rate (OCR) as well as the extracellular acidification rate (ECARB). Using tissue from different times of day, we measured these parameters. Using this novel method we hope to develop an accurate and simplified means to understand the connection between clock and cellular bioenergetics.

Attentional Set-Shifting Task in Socially vs. Non-Socially Housed Rats

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Attentional Set-Shifting Task (AST) is used to assess top-down processing in animal models to understand higher order executive functioning. We tested 27 Long Evans rats—19 of which were aged (25 months old) that were either socially housed (9) or non-socially housed (10) in enriched housing conditions, and 8 served as young controls (6 months old) that were housed individually in standard shoebox cages. Rats were presented with 6 different perceptual discriminations that featured 2 dimensions, odor and medium. Each discrimination required the rat to cognitively pair a dimension with a reward, while ignoring the irrelevant dimension still present during the task. The Simple Discrimination presented one dimension (medium or odor) and the Compound Discrimination introduced the other dimension. The Interdimensional Shift had two new mediums and odors while its reversal switched which pot was baited. The Extradimensional Shift (EDS) changed which dimension was predictive of the reward and the Extradimensional Shift Control was used as a check to verify that the rats shifted their attention to the new dimension. Rats were evenly divided among two different relevance groups, medium or odor, which the rats would pair one of the two dimensions to a reward. The main measures for each discrimination consisted of trials to criterion and percent correct; both of which showed no significant differences among the housing conditions or age. However, the rats that were randomly chosen to focus on the medium dimension were more accurate during Simple Discrimination than the odor relevant rats, while the odor relevant rats were more accurate during the EDS Control. Because rats made more correct choices when medium rather than odor was the indicator for reward, these results indicate that, in this context, medium is a more salient stimulus than odor. The performance of all rats in the EDS control demonstrates that the rats were not able to shift their attention to the new dimension and disregard the stimuli for which that they have previously been rewarded. The near perfect performances of both the young control rats and the aged rats could be due prior testing experience and possible neural protective benefits from enrichment in aged rats.

Cognitive Flexibility in Aged Socially Housed and Non-Socially Housed Rats

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Twenty-seven Long Evans Rats were tested in the Bi-Conditional Association Task (BAT), a test of cognitive flexibility. Rats were divided into groups based on age and housing conditions: 10 senescent rats (25 months) were individually housed in enriched housing conditions, 9 senescent rats (25 months) were socially housed in an enriched housing condition, and 8 young control rats (6 months) were individually housed in standard shoebox caging. The BAT was tested in a V-shaped maze where two food wells located at the end of each arm were covered by objects so that rats must displace an object to obtain a reward. The task required the subject to select object A but not object B in one arm of the maze but select object B but not A in the opposite arm. Three dependent measures were analyzed: total correct percentage correct, number of working memory errors as defined as a visit to a just-visited arm, and the number of sessions needed to reach criterion. All aged rats were able to perform BAT at the same level as the young rats once criterion was met and no differences in working memory, percentage correct, and number of sessions to criterion were not found between groups in BAT training. 72 hours following BAT, all rats performed behavioral epochs of Alternation (control task with no object displacement) and BAT to prepare for Capture Antibody Targeted Fluorescence *in situ* Hybridization (CAT-FISH). Physiological data made possible by brain extraction immediately after behavioral epoch 2 will reveal which areas of the Medial Temporal Lobe (MTL) and prefrontal cortex (PFC) are active during this task, as activation differences have been found in young vs. old rats. On the behavioral epochs day, there was a significant difference between the three groups: young control rats outperformed aged rats, followed by the social housed aged rats, and then the non-socially housed aged rats. This data suggests that there is a decline in cognitive flexibility with age, but that social housing may preserve this loss of function.

Analyzing the Role of the Microbiome in Neuroinflammation Using Human Cerebral Organoids

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All cells within the central nervous system (CNS), including neurons and glial cells, respond to inflammatory mediators. These responses are important in understanding the pathways by which inflammatory responses are activated within the CNS as well as how various other factors outside of the CNS affect these pathways. As a model of the CNS, cerebral organoids, derived from human pluripotent stem cells, were used to study inflammatory responses since they are a physiologically relevant 3D co-culture of neurons and glial cells. For this experiment, organoids were treated with the inflammatory mediators poly (I:C) and indoxyl-3-sulfate (I3S). Poly (I:C) is a Toll like receptor agonist, and I3S is a metabolite from tryptophan generated by the resident microbiome and an aryl hydrocarbon receptor agonist.. Levels of neuroinflammation were analyzed using immunofluorescence of iNOS and CCL2 co-localization with neurons and astrocytes in tissue sections. qPCR was also used to analyze the expression levels of TNF alpha, iNOS, and IL6. These inflammatory responses have known biological links to many neurodegenerative disorders such as Huntington's and ALS, so the use of human cerebral organoids is pertinent to disease research and drug discovery.

Testing the Effectiveness of Play- and Inquiry-Based Instruction in Early Science Education

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Recently, there has been a call for teachers to implement play- and inquiry-based pedagogies, but research has found conflicting results regarding the effectiveness of these techniques. For example, a study done on shape learning in preschoolers showed that guided play (adult-initiated but child-directed play) was more effective than both didactic instruction and free play (Fisher, Hirsh-Pasek, Newcombe, & Golinkoff, 2013). A related question is whether it is best to let children to find the answers to proposed problems, sometimes called discovery learning, or if it is best to tell them the solution in advance. This study responds directly to these questions by testing the effectiveness of play in early science education as well as investigating the efficacy of giving solutions to children directly or not. To do this, young children are taught two scientific concepts about light that you need light to see things and that light travels in a straight line, in one of four instructional conditions. All participants were in kindergarten or within three months of beginning kindergarten. First, participants are given a pre-test to establish their baseline knowledge of the concepts and then taught one of the four lessons about light. The four conditions are a combination of play, no play, solution, and no solution. The manipulation in the play or no play conditions are whether the child is permitted to manipulate the materials or if the experimenter simply does the demonstration. For the solution and no solution conditions, the child is either told the solution to the tasks in the lesson or is simply given guidance without explicit direction of how to complete the tasks. After the lesson, children's working memory and inhibitory control are measured, and lastly, they are given a post-test with questions that evaluate how deeply they understand the two scientific concepts taught in the lesson. We are interested to see if our manipulations of play and solution guidance will affect children's learning about the two scientific concepts about light. Planned analyses will compare the change from pre-test to post-test scores to see which method(s) of teaching result in greatest learning. Working memory and inhibitory control scores will be used to explore whether certain types of lessons are better for childr

Toxicity and Efficacy Screening Studies of TN1 in SH-SY5Y Neuroblastoma Cells as a Proposed Therapeutic Measure for Tauopathies

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Tauopathies refers to a class of neurodegenerative disorders characterized by the accumulation of the intracellular proteinaceous aggregates called neurofibrillary tangles (NFT), which are primarily composed of hyperphosphorylated tau. Among the various tauopathies; Alzheimer's Disease (AD) is considered the most prominent form of dementia primarily affecting an elderly population; with more than 35 million cases reported across the globe and around 5 million people in the United States. The hyperphosphorylation of tau is responsible for the NFTs found in AD, thus interference in this pathway could be postulated to provide therapeutic benefits. Specificity protein 1 (Sp1) is a zinc-finger transcription factor essential for the regulation of several genes whose products are involved in the phosphorylation of tau. Studies from our lab have provided convincing evidence that treatment of animals with Tolfenamic acid (TA), commercially known as Clotam® in Europe, lowers the expression of Sp1 target genes. Given the already well-defined tau downregulating activity of TA, its impact on pathological and behavioral endpoints in tau transgenic mouse models, along with its previously well-demonstrated clinical use and safety profile, TA serves as an excellent reference against the design and synthesis of novel therapeutics. In the present study we have compared the cytotoxicity and efficacy of such a therapeutic, subsequently known as 'TN1', against TA. Initial *in-vitro* screening of the drug using cytotoxicity assay involving human neuroblastoma cells (SH-SY5Y), revealed a safety profile for it better than that of TA alone. Differentiated SH-SY5Y cells were also used to evaluated the efficacy of TN1 analog in comparison to TA; on tauopathy pathway associated proteins. Prior exposure to lead Acetate (Pb) was used to boost the biomarker expression in order to study the therapeutic effect of the drug. Western blot analysis revealed an ability of TN1 to modulate protein targets in a manner similar to TA.

Therapeutic Potential of Copper–Tolfenamic Acid Complex on Biomarkers of Tauopathy: An *in vitro* Investigation

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Alzheimer's Disease (AD) is a neurodegenerative disease characterized by the accumulation of two proteinaceous aggregates, the extracellular amyloid beta (Aβ) and the intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau. Recent studies have shown tau hyperphosphorylation as a better predictor of AD and other tauopathies compared to Aβ; hence it should be explored as therapeutic targets for such disorders. Tau is a microtubule-associated protein (MTAP) that is pivotal for microtubule assembly. In pathological conditions tau can become hyperphosphorylated due to over-expression of kinases resulting in a loss of its function. Tolfenamic Acid (TA), commercially known as Clotam®, is a non-steroidal anti-inflammatory drug (NSAID, COX inhibitor) approved for acute migraine treatment in Europe. Previous studies from our lab have shown that the therapeutic effect of TA is due to its action on transcription factor Specificity Protein 1 (Sp1), which regulates the expression of Tau and associated kinases. Although TA is designated as safe for human use, it is desirable to limit its known side effects and enhance its use for chronic therapy. Recent studies have suggested that the therapeutic response of NSAIDs is significantly increased when prepared as metal complexes. Copper (Cu) is present in our daily food such as meats with no reported harm to humans. In the present study we examined the efficacy of the Copper-Tolfenamic acid complex (Cu-TA) in a differentiated human neuroblastoma (SH-SY5Y) cell line. Cell viability assays of differentiated SH-SY5Y cells exposed to a series of concentrations of TA and Cu-TA showed that Cu-TA as being less toxic than TA alone. SH-SY5Y cells were also pre-exposed to lead (Pb) to induce Tau-related biomarkers to evaluate Cu-TA's efficacy on modulating Tau-related proteins. Western blot analysis showed that the overall protein concentrations of COX2, tau, GSK3β, CDK5 and SP1 were altered by Cu-TA. Thus, Cu-TA can serve as a potential drug candidate to achieve the same therapeutic goals as TA with a better safety profile.