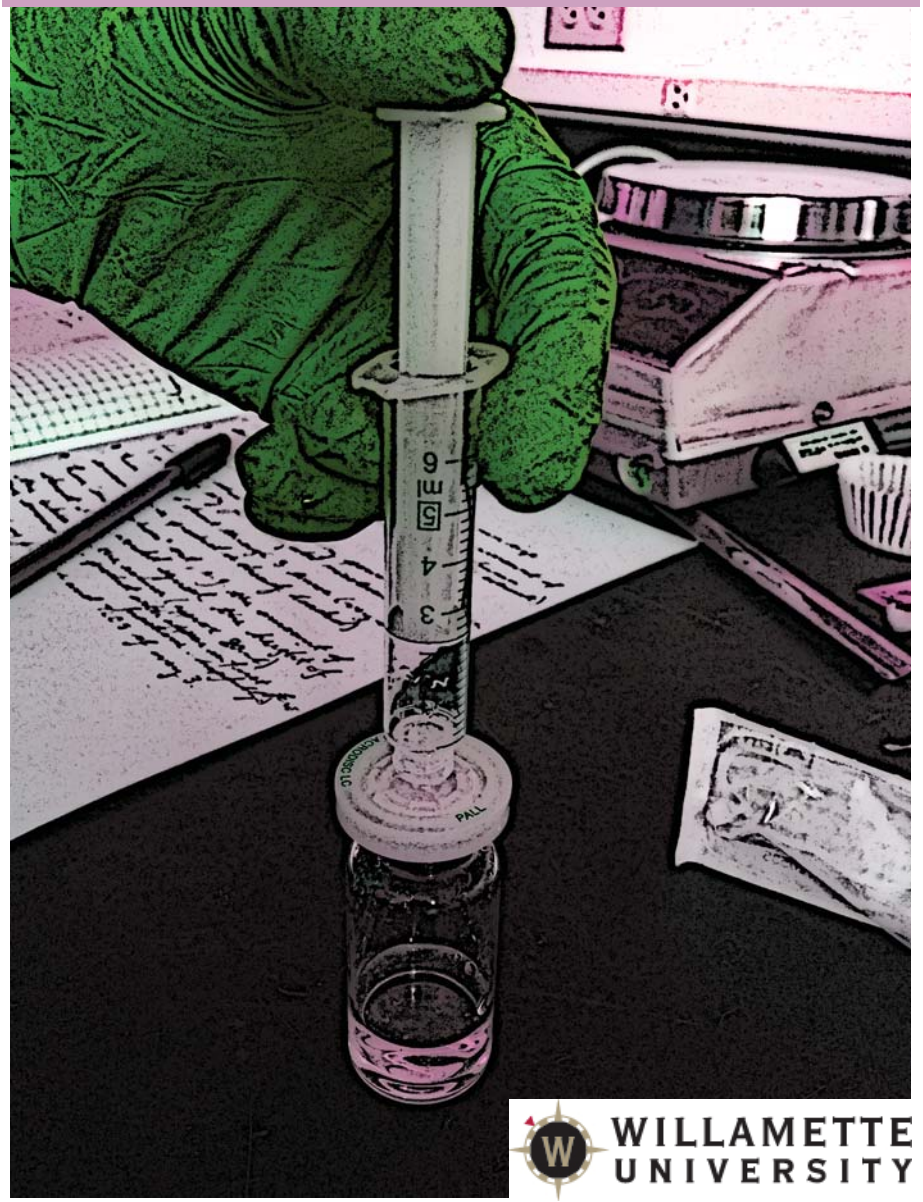
SCIENCE COLLABORATIVE RESEARCH PROGRAM

WILLAMETTE UNIVERSITY SYMPOSIUM

SEPTEMBER 16, 2016 * FORD HALL 122



WILLAMETTE UNIVERSITY

Science Collaborative Research Program
Willamette University Symposium

SEPTEMBER 16, 2016 * FORD HALL 122

2:00 - David Craig - Welcome

2:10 - Ami Boucher - Diabetic Neuropathy on Knee Joint Position Sense (Luke Ettinger Lab)

2:25 - Sophia Wax & Claire DeAngeli - Development of New Drugs as Potential Treatment for Eye Disease (Sarah Kirk Lab)

2:45 - Matt Logan - Cloning Isoprene Synthase from Heath Star Moss (Alison Fisher Lab)

3:00 - Lara Shinsato & Elliot Bullen - Improving Interpretations of δ^{34} Spyrite with Isotopic Measurements of Individual Pyrite Framboids (Katja Meyer Lab)

3:20 - Paige Yeakle - Climate and Dengue Fever in the Southern United States (Melinda Butterworth Lab)

3:35 - Jessenia Chavez & Alexis Carey - Clash of the Caulobacters (Melissa Marks Lab)

4:00-4:25 BREAK & GROUP PHOTO

4:25 - Logan Vine - Estrogen Distributions at the Willow Lake Pollution Control Facility (David Griffith Lab)

4:40 - Reid Milstead - Photolysis of Halogenated Estrogens (David Griffith Lab)

4:55 - Navindi Hewage - The Role of Myosin VI in Retinal Pigment Epithelium Phagocytosis (David Altman Lab)

5:10 - Ellen Rumley & Dylan Tooley - Regulation of Myosin VI Monomers through Force Sensitivity (David Altman Lab)

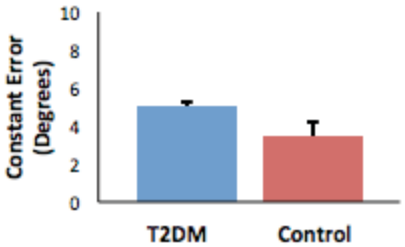
ABSTRACTS

AMI BOUCHER **2:10**

Diabetic Neuropathy on Knee Joint Position Sense

Mentor: Luke Ettinger
Department of Exercise Science

By the year 2040, 642 million individuals are expected to be diagnosed with Type 2 diabetes mellitus (T2DM), which marks a 55% increase from today's prevalence. Of these patients, 60% will experience neuropathic complications involving the lower extremity. Neuropathy involves the degeneration of sensory nerves which may include peripheral proprioception. Proprioception describes the awareness of limb position in space in the absence of visual feedback. The aim of our study was to measure proprioceptive acuity in the lower extremity of diabetic and non-diabetic individuals to assess neuropathic interruption on the proprioceptive system. Type 2 diabetics were matched with healthy controls for age and gender. Knee proprioception was measured with an iPod touch (Apple inc), using a custom JPS application (JPS). Participants were guided to targets in space using auditory feedback. Target positions of 15, 30, 45, and 60 degrees of knee extension were randomly assigned and repeated by 4 trials. Preliminary results indicate a greater magnitude of proprioceptive deficit in the T2DM group; however, our results are inconclusive awaiting further data matching with our control population.



This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from the Mary Stuart Rogers Foundation.

SOPHIA WAX & CLAIRE DEANGELI **2:25**

DEVELOPMENT OF NEW DRUGS AS POTENTIAL TREATMENT FOR EYE DISEASE

Mentor: Sarah Kirk; Department of Chemistry

Retinitis Pigmentosa (RP) is a degenerative eye disease affecting roughly 1 in 4,000 people worldwide. In a normal functioning eye, cyclic nucleotide-gated (CNG) ion channels regulate calcium ion flow into the cell. In a diseased state, a mutation in the channel renders them ineffective and results in an overabundance of calcium accumulating in the cell, leading to cell death. Tetracaine is an FDA approved local anesthetic that has been shown to block CNG ion channels with moderate affinity (Figure 1). Past research has explored using tetracaine derivatives as a treatment for RP by adapting 2 components of the molecule; the body, and tail. It was found that by adding electron withdrawing (EWGs) groups to the aromatic body of the molecule, the binding affinity within the CNG ion channels increased. Increasing the lipophilicity by adding a longer hydrocarbon chain to the tail of the molecule aided in the drugs' ability to enter into the cell and ion channel. The goal of this project is to modify pyridine derivatives to increase the molecules binding affinity and selectivity for CNG ion channels. The purpose of using pyridine derivatives is to bring the EWGs inside of the ring to reduce the risk of intramolecular bonding and sterics. This project also investigates 2 different hydrocarbon tails, butylcaine and octylcaine. We successfully synthesized and purified 3 pyridine derivatives containing an (a) octyl, (b) butyl, and (c) no tail (Figure 2). With yields of; 68%, 77%, and 97%, respectively.

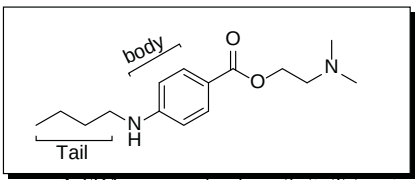


Figure 1. FDA approved local anesthetic Tetracaine.

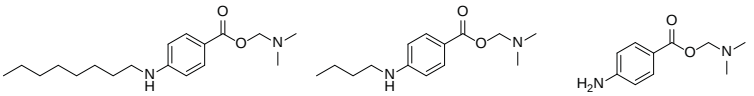


Figure 2. 6-aminonicotinic acid with (a) octyl tail, (b) butyl tail, (c) no tail.

This work was supported by Murdock College Research Program for Natural Sciences - Physical Sciences Grant.

MATT LOGAN **2:45**

Purification of Isoprene Synthase from *Campylopus introflexus*

Mentor: Alison Fisher
Department of Chemistry

Isoprene is a five carbon volatile compound emitted by many land plants in megatons per year. While the function of isoprene in plant systems is unknown and actively disputed, the production pathway of isoprene in plants has been identified and well-studied. Isoprene synthase (ISPS) is the enzyme that catalyzes isoprene production; this enzyme has been characterized and sequenced from several species of angiosperms. The Fisher group has targeted its focus to the earlier diverging plant classes, such as mosses, in hopes to provide insight into the evolution of ISPS and isoprene function in plants. In 2015, the Fisher group successfully isolated and characterized the first moss ISPS from heath star moss (*Campylopus introflexus*). Current efforts are being focused on determining the cDNA sequence of this novel enzyme. This summer, homology-based reverse transcriptase polymerase chain reaction (RT-PCR) was used to clone this cDNA sequence, targeting the metal binding amino acid sequence DDXXD that is a conserved in all sequenced ISPS. Degenerate primers from the literature that target this sequence were used with cDNA from *C. Introflexus*. Cloning attempts resulted in PCR products with sizes that were similar to that of the expected product found in the literature. The most promising product was successfully sequenced and the NCBI online BLAST tool was used to analyze the sequence. Rather than aligning with known ISPS sequences, the sequence was highly similar to H+-pyrophoshatases from several plants. While not the intended result, H+-pyrophosphatase shares the same DDXXD metal binding region as ISPS. With this knowledge we have designed a southern plot protocol to screen a cDNA library of *C. Introflexus* using these same degenerate primers in the hopes of identifying the *C. introflexus* ISPS cDNA.

This work has been supported by the Willamette University Science Collaborative Research Program and supported with generous funding by the M.J. Murdock Charitable Trust and the Mary Stuart Rogers Foundation.

LARA SHINSATO & ELLIOT BULLEN **3:00**

Improving Interpretations of δ^{34} Spyrite with Isotopic Measurements of Individual Pyrite Framboids

Mentor: Katja Meyer
Department Environmental & Earth Sciences

In the rock record, intervals of marine euxinia (anoxic and sufidic conditions) are associated with rapid climate warming, biogeochemical perturbation, and mass extinction. These past episodes of euxinia are of interest because modern anthropogenic climate change is predicted to have similar impacts on marine geochemistry and ecosystems. Sulfur isotope measurements are commonly used to examine the record of ancient euxinia, but interpretation of these data are currently limited by our understanding of how sulfur is incorporated into the sedimentary record. Here, we began an examination of how pyrite-sulfur is incorporated into sediments through high-resolution measurements of S isotope composition and framboidal pyrite diameter. First, we developed a method to separate pyrites from sediments collected from Fayetteville Green Lake (NY), a modern euxinic lake. We then embedded the separated pyrites in 1" round epoxy pucks, and mapped the spatial distribution and diameter of individual pyrites under a scanning electron microscope (SEM). In addition, we mapped the distribution of framboidal pyrites in 1" round thin sections of Early Triassic carbonates deposited in an euxinic environment. Next, we will measure the S isotope composition of these pyrites using secondary ion mass spectrometry (SIMS). We will examine the relationship between framboid size and isotopic composition in these samples to improve our interpretation of bulk S isotope
measurements.

This work has been supported by the Willamette University Science Collaborative Research Program and supported with generous funding by the Petroleum Research Foundation.

PAIGE YEAKLE **3:20**

Climate and Dengue Fever in the Southern United States

Mentor: Melinda Butterworth
Department of Environmental & Earth Sciences

Dengue fever is the most prevalent mosquito-borne disease in the world, infecting approximately 50 million people annually. Further, the virus has re-emerged in the southern United States after a 70 year absence. The dengue virus is transmitted by *Aedes* genus mosquitoes, a prevalent species in the southern United States. While it is understood that the mosquito and virus are sensitive to temperature and precipitation, less is known about climate induced spatio-temporal variability throughout the region. To address this, we used historic climate data for six cities in the southeastern US to run a dynamic mosquito model (DyMSiM), which simulated daily total and infectious mosquito populations and dengue cases over approximately 120 years. We analyzed the model output to determine the approximate season length for mosquitoes and dengue transmission, and the frequency of continuous winter transmission potential. Our results suggest that, on average, the length of the total and infectious mosquito season has increased for most sites from the late 1800's to 2015. Our results also suggest the potential for continuous mosquito activity during some winters in Houston, S. Florida, and New Orleans, and periodic continuous infectious mosquito activity in Florida. The potential for transmission was notably higher in the southernmost study sites, demonstrating the important climatic constraints on the vector and virus at higher latitudes. Quantifying the spatio-temporal variability of regional dengue transmission in the United States can play an important role in informing public health policy. To our knowledge, this kind of historical modeling with DyMSiM has not previously been conducted, and so our work expands the field of research on mosquito-borne disease and climate.

This work has been supported by the Willamette University Science Collaborative Research Program and supported with generous funding by the Mary Stuart Rogers Foundation.

JESSENIA CHAVEZ & ALEXIS CAREY **3:35**

Clash of the Caulobacters

Mentor: Melissa Marks
Department of Biology

Exopolysaccharide (EPS) production can provide a helpful tool for survival of *Caulobacter crescentus* in environments where bacteriophage infections are common. However, it's not well understood how the production of EPS affects the cellular growth and generation time of EPS producers (EPS+). To assess potential differences between EPS+ and EPS- phenotypes, growth rates were measured in the form of doubling time and later as relative abundance of each strain when in direct competition with each other. A slight difference between the growth rates of EPS+ and EPS- strains was measured, doubling times in EPS- are faster than that of EPS+ by an average of 2.437 ± 1.915 minutes. Though this difference may seem small, when grown in a competitive environment the advantage becomes apparent in the EPS- strain that is seen by a higher relative abundance of EPS- cells compared to EPS+ cells. The small variation of generation time in *Caulobacter crescentus* strains exhibit a relationship between EPS+ and EPS- strains where in specific environments one strain has an advantage over the other. These results represent a potential evolutionary trade-off between being able to grow fast or have partial resistance to infection.

This work has been supported by the Willamette University Science Collaborative Research Program and supported with generous funding by the M.J. Murdock Charitable Trust (Chavez) and the Mary Stuart Rogers Foundation (Carey).

LOGAN VINE **4:25**

Estrogen Distributions at the Willow Lake Pollution Control Facility

Mentor: David Griffith
Department of Chemistry

Estrogens are steroidal hormones used by all vertebrates for proper growth and development, but in high enough concentrations, estrogens can act as endocrine disruptors, leading to the collapse of aquatic populations. Humans excrete free and conjugated estrogens which are concentrated into wastewater treatment plants (WWTPs), where they can be converted into halogenated estrogens, a new family thought to be moderately potent and for which data regarding sources and removal processes is severely lacking. This leads to the potential for unusually high concentrations of estrogens where WWTP effluent is released into the environment. We investigated concentrations of a variety of estrogens, including free, conjugated, and halogenated forms, through the wastewater treatment process at the Willow Lake WWTP (Salem, Oregon) using the method of standard addition and high performance liquid chromatography-tandem mass spectrometry. We detected low levels of brominated estrogens coming from this plant which could indicate that brominated forms are not ubiquitous, and may only be common in the effluent of coastal WWTPs that receive influent with high levels of bromide ion. In addition, our results indicate that the concentration of most forms of estrogen decreased during chlorine-based wastewater disinfection. Together, these findings have important implications for understanding the factors that influence estrogen distributions in WWTPs and environmental loading.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding by the Mary Stuart Rogers Foundation.

REID MILSTEAD **4:40**

PHOTOLYSIS OF HALOGENATED ESTROGENS

Mentor: David Griffith
Department of Chemistry

Halogenated estrogens are potent endocrine disrupting compounds and have been found at relatively high concentrations in wastewater treatment plant effluent. This experiment focused on the photolysis, both direct and indirect, of the halogenated estrogen dibrmo-17β-estradiol (diBrE2) as a way to better understand the fate of halogenated estrogens in aquatic environments. Photolysis experiments were conducted at pH values of 4 and 7 in order to explore the difference in photolytic rates of the protonated and deprotonated forms of the estrogen compound. Our results suggest that diBrE2 degrades relatively quickly at pH 7, which is possibly explained by the high molar absorptivity of the more prevalent deprotonated form at the same pH value. At a pH of 4, samples degraded more slowly. The quantum yield of the compound was calculated to be 4.3 x 10-2 mol Einstein-1. This value is similar to what had been found through previous experimentation. The results of this experiment suggest that diBrE2 degrades relatively quickly at environmentally relevant pH values. Further experimentation will explore photolytic rates of monohalogenated estrogen compounds as well as the identification of photoproducts.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding by the Mary Stuart Rogers Foundation.

NAVINDI HEWAGE **4:55**

The Role of Myosin VI in Retinal Pigment Epithelium Phagocytosis

Mentor: David Altman
Department of Physics

Phagocytic activity is prolific in retinal pigment epithelium (RPE) cells. These specialized cells contribute to engulfment and degradation of rod and cone cell waste in light-sensitive outer segment portions of photoreceptors present in the eye. Described in this presentation is research focused around understanding the involvement of myosin VI in the phagocytosis of microbeads by a human retinal pigment epithelium primary cell line (ARPE-19). In order to study this involvement, two experimental conditions were explored. The first of these consisted of over-expressing in the cells a dominant negative myosin VI construct. This construct only consists of myosin's C-terminal tail domain and not the motor domain. The second condition involved disrupting the actin network with Cytochalasin-D so as to visualize the movement of phagocytized beads in the absence of the acto-myosin interaction. The research successfully demonstrated that myosin VI is involved in nonspecific phagocytosis.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding by the Mary Stuart Rogers Foundation.

ELLEN RUMLEY & DYLAN TOOLEY **5:10**

Regulation of Myosin VI Monomers through Force Sensitivity

Mentor: David Altman
Department of Physics

The purpose of our summer research was to investigate the force sensitivities of motor proteins called myosin VI in their monomer and dimer states in order to understand regulation by forces of the activity of myosin VI motors. We have a implemented experiments that investigate the effects of intramolecular forces on myosin dimers, as well as experiments to determine the coordination and force dependent kinetics of multiple myosin VI monomers. This research has set up a foundation for future work on myosins in the a Altman biophysics research lab.

This work was completed as part of the Willamette University Science Collaborative Research Program and supported with generous funding from Willamette University Carson Undergraduate Research Grant (Rumley) and M.J. Charitable Trust College Research Program for Life Sciences Grant (Tooley).

COVER IMAGE BY SOPHIA WAX