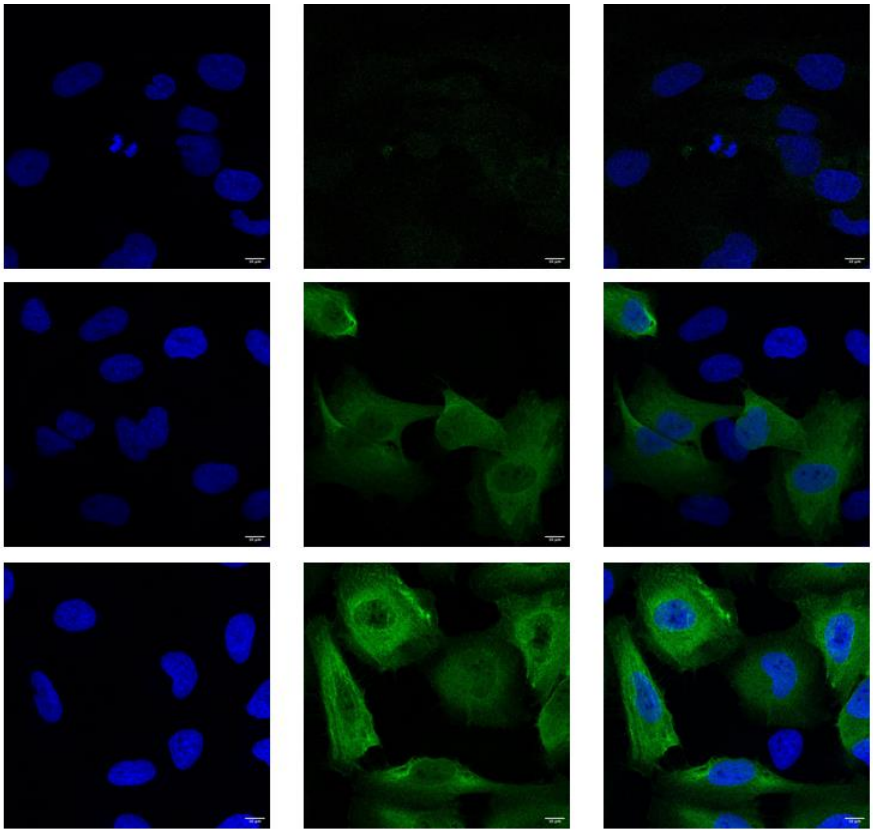
11th Annual Cell and Molecular Biology Symposium



Book of Abstracts

Winter 2025

GRAND VALLEY STATE UNIVERSITY

Presentation Schedule

April 11, 2025

Session I Chair: Noah Tudor

Hemlock Woolly Adelgid (HWA) Monitoring: Testing the Rate of HWA DNA Degradation and New Methods for Processing HWA Field Traps

Presenter: Gabriella Loisch

Creation of a pAANATL7-GAL4 transgenic fly to identify expression of the AANATL7 gene in D. melanogaster

Presenter: Margaret Cubitt

Rapid Identification of Candida auris by Raman Spectroscopy

Presenter: Danielle Percefull

The Effects of Docosahexaenoic Acid on Neural Stem Cell Fate

Presenter: Grace Okros

Session II Chair: Jason Brooks

Effect of Cholesterol on Viability and Proliferation of Breast Cancer Cells

Presenter: Madelyn Maurer

Exploring Potential Binding Sites for an Allosteric Inhibitor, BIBR1532 in Human Telomerase TERT Domain, and Efficiently Analyzing Docking Results with R
Presenter: Noah Tudor

The Cytoprotectivity and Potential Nuclear Localization of Human Arsenite Methyltransferase

Presenter: Emily Seburn

Session III Chair: Danielle Percefull

Mechanosensing and Anesthesia of Single Internodal Cells of *Chara*

Presenter: Manya Rodgers

Degradation Dynamics of LRB1&2 Proteins in Response to Light & Temperature

Presenter: Mitchell Kobayashi

Lady's slipper orchids and fire: Identifying the species of fungal symbionts and the influence of fire in *cypripedium sp.* native to Michigan

Presenter: Jason Brooks

Hemlock Woolly Adelgid (HWA) Monitoring: Testing the Rate of HWA DNA Degradation and New Methods for Processing HWA Field Traps

Gabriella Loisch[1], Carly Thayer[2], Kathryn Geller[3], Syndell Parks[4], Keely Dunham[5], Colleen Black[6]

Mentor: Dr. Charlyn Partridge

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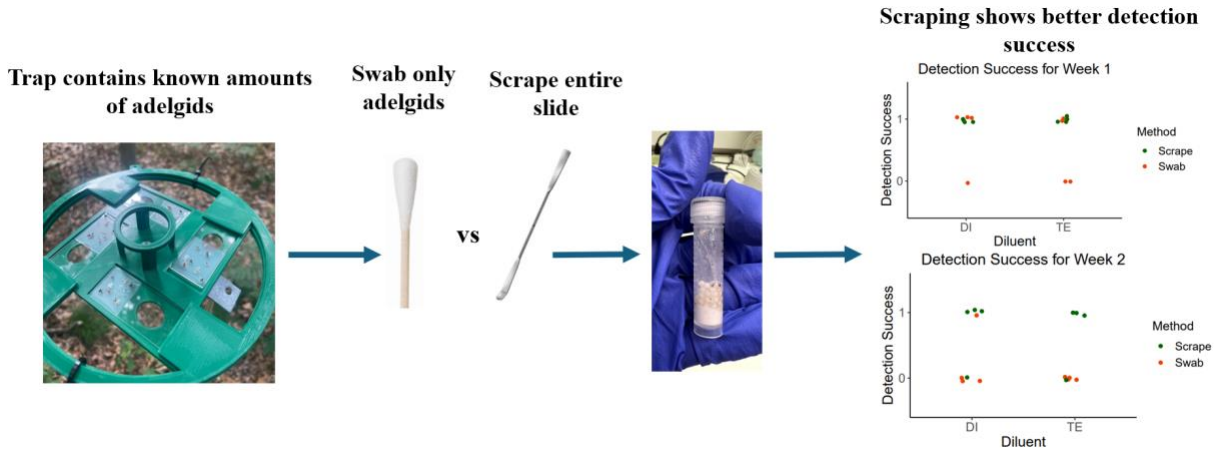

Hemlock woolly adelgid (HWA) is a small, invasive, aphid-like insect that attacks hemlock trees. The adelgids attach to the needles of the tree and suck sap and nutrients, causing starvation and eventual tree death. HWA infestations have caused significant destruction of hemlock forests in Northern America. Monitoring infestations includes the use of special traps that catch adelgids falling from infected trees, and quantifying the adelgid DNA using qPCR which proved to be effective in monitoring severity of infestations. However, use of these traps requires several laborious steps: bi-weekly collection of the caught adelgids, replacement of the collection slides used in the traps, and processing of the collected slides. This project had two goals: 1) to determine whether these traps could be left out for longer periods of time without significant loss of DNA, and 2) to increase efficiency of trap processing methods after collection. We placed 32 traps containing known amounts of adelgid DNA in a non-infested area, collecting 4 traps per week, and comparing the amount of quantified DNA in each week to look at differences in DNA degradation week-to-week. We determined the qPCR adelgid DNA detection success rate in two different collection methods: scraping the entire content of the collected slide into a DNA extraction tube vs. swabbing only for adelgid insects using sterile swabs. Our data show a significant loss of DNA between weeks 1 and 2, indicating the continued need for bi-weekly collections. The less efficient and more time-consuming scraping method was far superior in adelgid DNA detection success, also supporting continued use of this method. Our data indicate that these current methods are the most effective, and further research is needed to develop more efficient methods without sacrificing detection success and accuracy.

Funded by the Annis Water Resources Institute Internship Program

Identification of AANATL-7 Expression Using the UAS-GAL4 System in *D. melanogaster* to Determine Where Histamine Acetylation is Occurring *in vivo*

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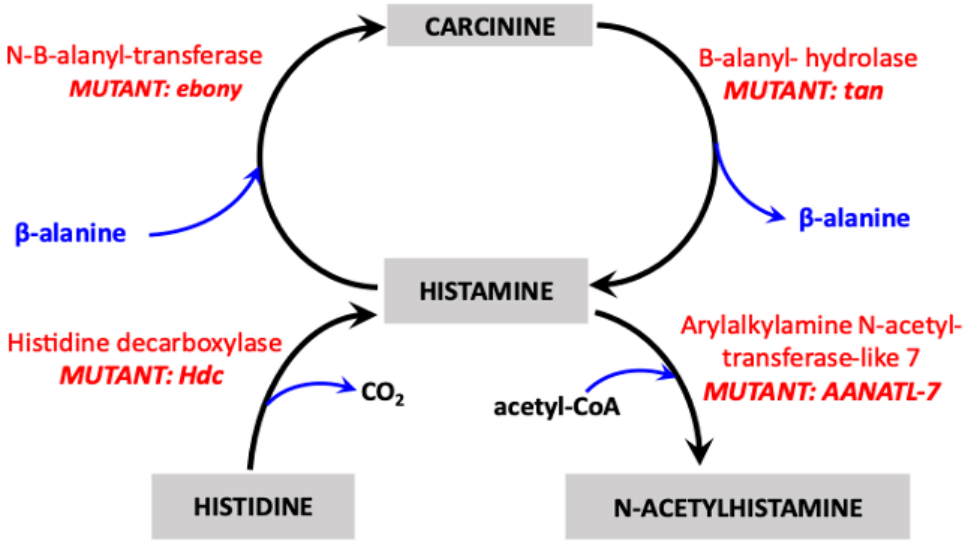

Drosophila melanogaster has previously been used as a model system to study neurotransmitter action and function, including the processes involved in neurotransmitter metabolism. The metabolism of histamine, a neurotransmitter used by *Drosophila*, involves synthesis of an intermediate, carcinine, which can be converted back to histamine. Recently in our lab, mutations were discovered in a gene encoding the enzyme arylalkylamine N-acetyltransferase like-7 (*AANATL-7*), which acetylates histamine and therefore produces N-acetylhistamine (NAH). However, it is unknown in what tissues histamine acetylation is occurring. To identify the tissues that express the *AANATL-7* gene, the UAS-GAL4 heterologous expression system was used. The 2.2 kb *AANATL-7* promoter (control) region was used to induce GAL4 expression in *AANATL7*-expressing cells by first creating the *AANATL7*$^{2.2kb}$*-GAL4* transgene. The *AANATL-7* promoter region was cloned into the pC3G4 plasmid, which contains the w^+ gene marker and the GAL4 gene. Embryos were injected and resulting transgenic flies were identified by eye color conversion from white to red were selected from the injected flies' offspring. After genetically mapping the transgene in each established line to a specific chromosome, flies were mated with UAS-GFP flies to induce GFP expression in the progeny, identified using fluorescence microscopy. By examining tissues from flies containing both the *AANATL7*$^{2.2kb}$*-GAL4* & UAS-GFP transgenes we observed *AANATL-7* expression in adult male accessory gland "main cells" as well as central nervous system neurons in both larval and adult flies. These results can further help us study the importance of the N-acetylation of histamine in these tissues.

Rapid Identification of *Candida auris* by Raman Spectroscopy

S. Kiran Koya[1], Michelle A. Brusatori[1], Sally Yurgelevic[1], Changhe Huang[1], Jake DeMeulemeester[1], **Danielle Percefull[1,5]**, Hossein Salimnia[2,3]
Mentor: Dr. Gregory W. Auner[1,4]

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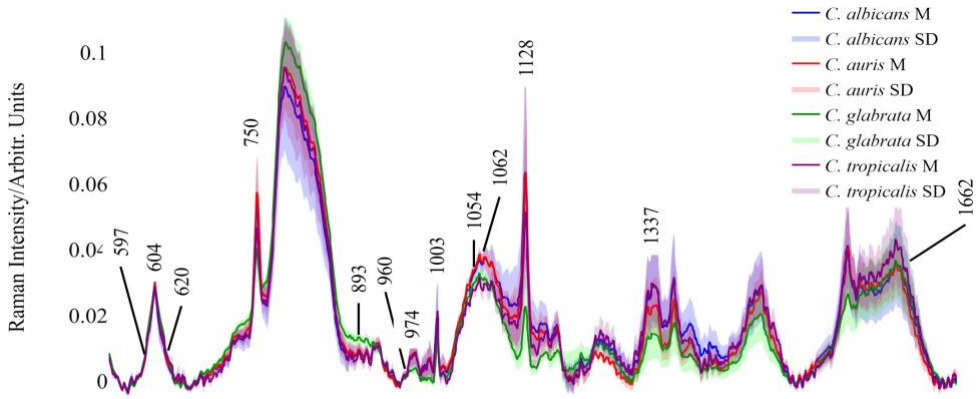

Candida auris is a multidrug resistant yeast that can lead to severe illness and outbreaks in healthcare facilities. *Candida auris* detection is challenging using standard laboratory methods since they usually provide genus identification and species are often misidentified. Raman spectroscopy has recently been used for yeast identification and has shown promising results. However, high background and substate signals, interference from untargeted compounds, and variations in sample processing can cause different results. Counter-Propagating Gaussian Beam Raman Spectroscopy (CPGB- RS) is a novel, practical technology that was specifically designed to enhance the sensitivity of RS for detecting low-concentration pathogens. This study utilized the CPGB- RS system to evaluate the accuracy of identifying *C. auris* with other prevalent *Candida* species. *C. albicans* (ATCC 10231), *C. auris* (ATCC CDC B11903), *C. glabrata* (ATCC 2001), and *C. tropicalis* (ATCC 66029) were obtained from the American Type Culture Collection. Samples were prepared from a single colony and inserted into CPGB- RS for testing. Ten spectra per sample were recorded using 100% laser power over a spectral range of 285–1925 cm^{-1}. The Raman spectra data was processed and underwent statistical analysis. This method demonstrated an accuracy of 96%, sensitivity of 96%, and specificity of 99% in classifying the four *Candida* species. This study lays the groundwork for utilizing Raman spectroscopy in *C. auris* identification and has the potential to detect new emerging strains.

Koya, S., Brusatori, M., Yurgelevic, S., Huang, C., DeMeulemeester, J., Percefull, D., Salimnia, H. and Auner, G. (2025), Rapid Identification of *Candida auris* by Raman Spectroscopy Combined with Deep Learning. J Raman Spectrosc, 56: 218.

We acknowledge the Paul Strauss Endowed Chair and M&MI Department of Surgery at WSU for financial support.

Effects of Docosahexaenoic Acid on the Neurogenesis in Developing Chick Model

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Maternal diet plays a crucial role in the development of the fetal brain. Supplements such as those containing docosahexaenoic acid (DHA) are highly recommended for fetal brain development due to deficiencies linked to a variety of brain disorders. DHA is one of many polyunsaturated fatty acids (PUFAs) that are essential for health but must be taken in through our diet. PUFAs are known to modulate gene expression with fetal neurogenesis. DHA has been shown to promote neuronal stem cell differentiation in vitro but the mechanisms are unknown for a developing fetal embryo. We conducted an *in situ* study by injecting chick embryos with PUFAs DHA, linoleic acid (LA), oleic acid (OA), and arachidonic acid (ARA). The embryos were allowed to develop for 24-72 hours with varied dosages of the specific PUFA. The embryos were then cryosectioned and fixed for immunohistochemistry staining to determine the number of neurons. The central hypothesis was that DHA will increase the number of neurons over time. The results showed that an increasing number of neurons persisted over time in a dose dependent manner suggesting that there is an increase in the pool of neuron progenitors, rather than driving neuronal differentiation and cell cycle exit prematurely.

Effect of Cholesterol on Viability and Proliferation of Breast Cancer Cells

Madelyn Maurer

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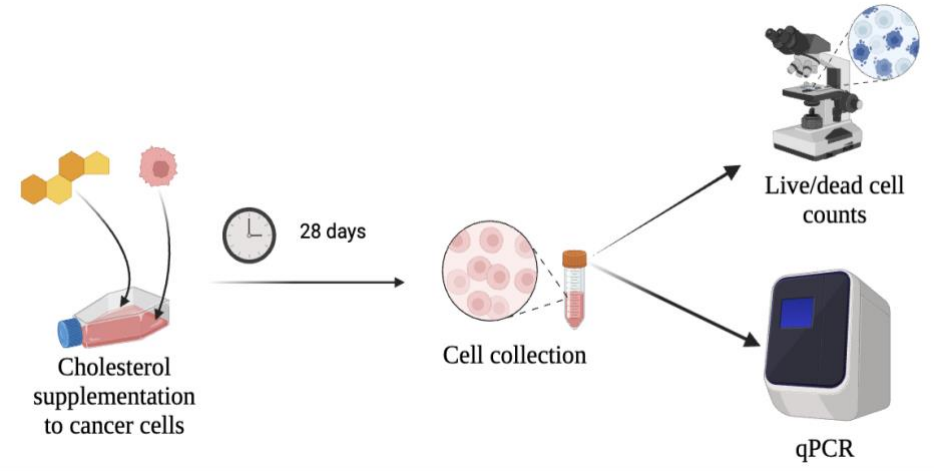

Breast cancer (BC) is the most commonly diagnosed cancer worldwide, and it is a leader of female cancer mortality. Obesity affects over 40% of the US adult population and is a well-established risk factor for BC. Hypercholesterolemia is a dominant factor in the pathophysiology of obesity and is directly linked to the malignancy of endometrial cancer, but its role in the pathogenesis of HR+ BC and HER2+ BC remains to be elucidated. While HER2 has been shown to facilitate intracellular cholesterol transport, the impact of cholesterol on BC subtypes remains unknown. This study evaluates the effects of extended cholesterol supplementation (0 mM - 7.5 mM) on HR+ (MCF-7) and HER2+ (SKBR3) BC cells. Our findings indicate that at a 7.5 mM cholesterol concentration, HR+ BC cell viability decreased by ~50%, with a concurrent increase in apoptotic cells. Conversely, HER2+ BC cells exhibited an increase in proliferation at moderate cholesterol concentrations (5 mM) but showed higher non-viability at 7.5 mM. qPCR analysis revealed that cholesterol exposure suppressed the expression of key genes associated with cell proliferation (cMYC) and the mevalonate pathway (SREBP1, HMGCR, STARD1) in both subtypes, suggesting a conserved cholesterol-mediated regulatory mechanism. These results highlight distinct responses of BC subtypes to cholesterol, warranting further investigation into the mechanistic role of cholesterol in BC progression and potential therapeutic interventions.

Exploring Potential Binding Sites for an Allosteric Inhibitor, BIBR1532 in Human Telomerase TERT Domain, and Efficiently Analyzing Docking Results with R

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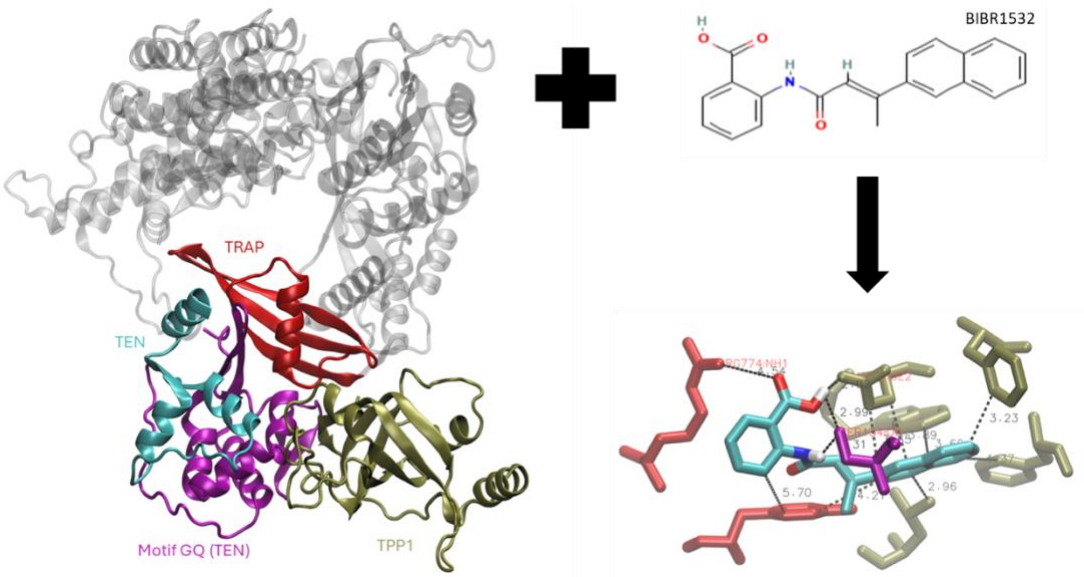

Telomerase (hTERT) extends telomeric regions of chromosomes and thus prevents chromosomal damage and cell death. Cancer cells overexpress hTERT to divide indefinitely. Although a non-nucleosidic inhibitor BIBR1532 did not pass clinical safety tests, it was shown *in vitro* to inhibit hTERT processivity allosterically. Beetle telomerase structure indicates that BIBR binds in the thumb/CTE domain, but it is not clear if the same region of hTERT is involved. We hypothesized that the allosteric site is located within the hTERT TEN and reverse transcriptase (RT) domains. TEN is an important regulatory segment, interacting with TPP1 (activating protein), TRAP (part of the RT domain), and RNA. The palm and fingers, which form the RT domain, are responsible for nucleotide binding and catalytic site, respectively, and thus are essential for catalytic activity. We used the 7TRE structure to calculate druggable cavities and to dock BIBR. We also developed a code in R to streamline the data analysis of large amounts of docking data. We identified three potential pockets at different domain interfaces. The most promising pocket is formed at the TEN, TRAP and TPP1 interface, which also harbors three out of six conserved residues: P164, Q169, and G172. The other two pockets were part of the TEN/RNA interface and the palm/fingers interface. Our docking data indicate that BIBR can bind relatively strongly to each of these interfaces. This preliminary study allows us to evaluate the potential for the TEN and RT domains to harbor inhibitory allosteric sites.

Protective Function and Nuclear Localization of Human Arsenite Methyltransferase

Emily Seburn
Mentor: Richard Cassidy, Dr. Yvonne Fondufe-Mittendorf

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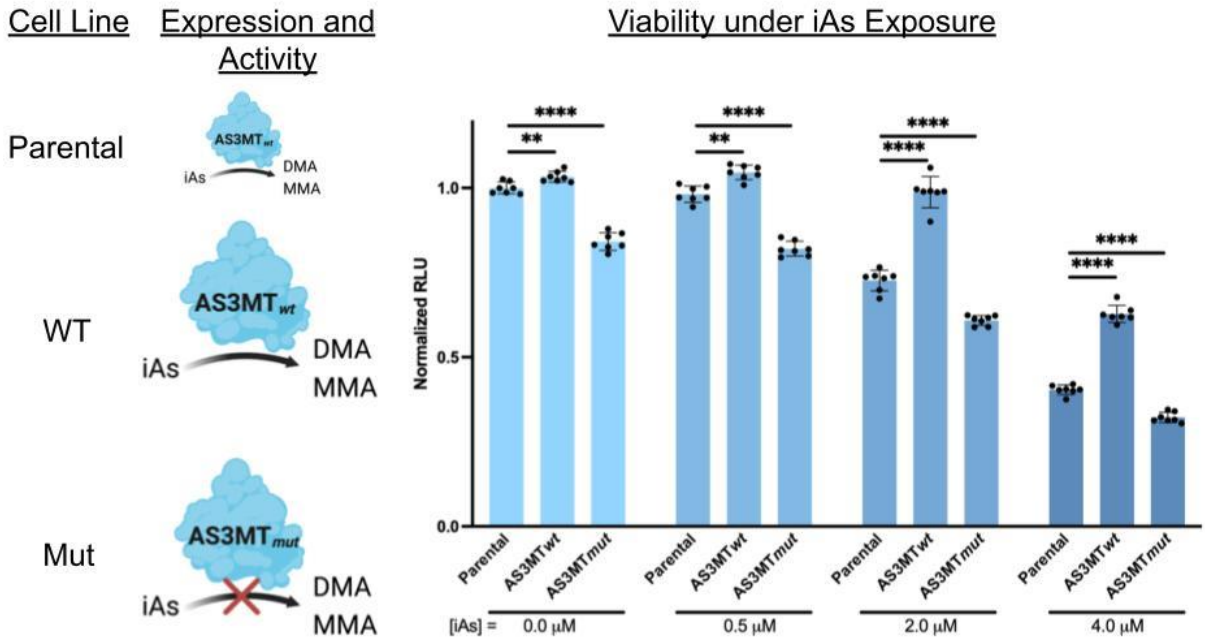

Inorganic arsenic (iAs) is a naturally occurring carcinogen known to cause lung cancer. Millions of people are regularly exposed to iAs, putting them at risk of cancer and other diseases. Thus, elucidating the mechanisms by which cells protect themselves from iAs has widespread repercussions for human health. Human arsenite methyltransferase (AS3MT) methylates iAs for excretion and is believed to be cytoprotective. However, methylated arsenic can be more toxic than iAs, casting doubt on this assumption. We tested the hypothesis that the enzymatic activity of AS3MT is cytoprotective in lung cells exposed to iAs. We overexpressed wildtype (WT) or catalytically inactive (Mut) AS3MT in human lung cells, then measured their viability with or without iAs. We found that across all iAs concentrations, AS3MT WT cells were more viable than parental cells, whereas Mut cells were significantly less viable. Additionally, we demonstrated through cellular localization and fractionation that AS3MT is present in the nucleus. This data suggests that AS3MT may have a secondary function related to cell viability even in the absence of iAs. Ultimately, this research helps explain how AS3MT protects lung cells from iAs exposure, implicating its activity in prevention of iAs-induced carcinogenesis.

Mechanosensing and Anesthesia of Single Internodal Cells of *Chara*

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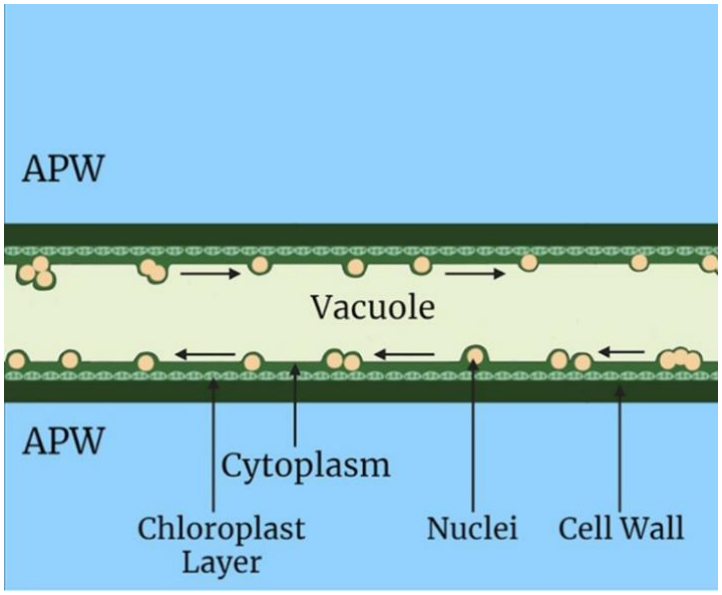

The giant (2-3 × 10^{-2} m long) internodal cells of the aquatic plant, *Chara*, exhibit a rapid (>100 × 10^{-6} m s^{-1}) cyclic cytoplasmic streaming which stops in response to mechanical stimuli. Since the streaming - and the stopping of streaming upon stimulation - is easily visible with a stereomicroscope, these single cells are ideal tools to investigate mechanosensing in plant cells, as well as the potential for these cells to be anesthetized. We found that dropping a steel ball (0.88 × 10^{-3} kg, 6 × 10^{-3} m in diameter) through a 4.6 cm long tube (delivering ca. 4 × 10^{-4} J) reliably induced mechanically-stimulated cessation of cytoplasmic streaming. To determine whether mechanically-induced cessation of cytoplasmic streaming in *Chara* was sensitive to anesthesia, we treated *Chara* internodal cells to volatilized chloroform in a 9.8 × 10^{-3} m^3 chamber for 2 minutes. We found that low doses (15,000-25,000 ppm) of chloroform did not always anesthetize cells, whereas large doses (46,000 and higher) proved lethal. However, 31,000 ppm chloroform completely, and reversibly, anesthetized these cells in that they did not stop cytoplasmic streaming upon mechanostimulation, but after 24 hours the cells recovered their sensitivity to mechanostimulation. We believe this single-cell model will prove useful for elucidating the still obscure mode of action of volatile anesthetics.

Degradation Dynamics of LRB1&2 Proteins in Response to Changes in Temperature under Far-Red Light

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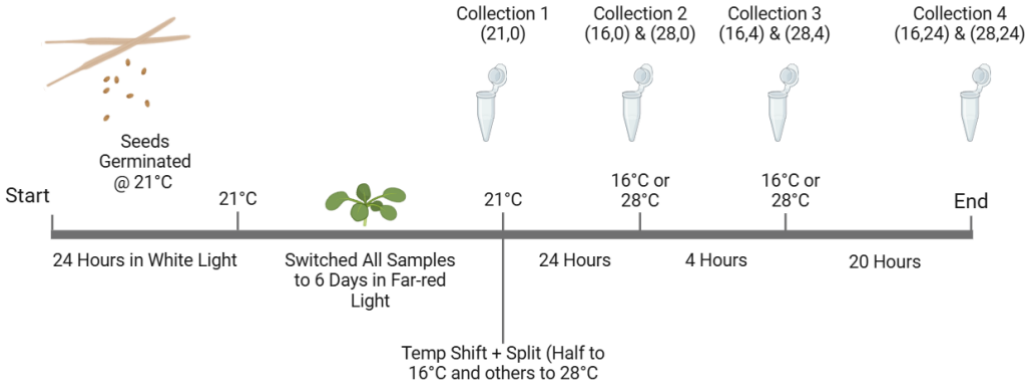

The ubiquitin-proteasome system (UPS) plays a crucial role in regulating plant responses to environmental cues, including temperature fluctuations, by targeting key signaling proteins for degradation. Light Response BTB proteins 1 and 2 (LRB1 and LRB2) function as substrate adaptors for CULLIN3-based E3 ligases, facilitating the turnover of photoreceptors and transcriptional regulators. Among these, phytochromes serve as the primary mediators of red and far-red light signaling in Arabidopsis, playing essential roles in photomorphogenic development and environmental adaptation. While phytochrome stability and activity are known to be tightly regulated by the UPS, less is understood about how temperature influences the degradation dynamics of proteins like LRB1 and LRB2. In this study, we investigated the temperature-dependent degradation of LRB1 and LRB2 using transgenic Arabidopsis lines expressing either free GFP or GFP-tagged versions of LRB1 and LRB2. By subjecting seedlings to alternating temperature conditions and analyzing protein levels over time, we aimed to determine whether LRB1 and LRB2 turnover is modulated by temperature shifts.

Contrary to expectations, results showed that temperature shifts combined with far-red light treatment did not have a significant effect on the expression levels of LRB1 or LRB2. While previous studies have suggested that temperature can influence protein degradation through the ubiquitin-proteasome system, the stability of LRB1 and LRB2 under these conditions suggests that their degradation may be regulated independently of temperature fluctuations or requires additional environmental cues. These findings indicate that LRB1 and LRB2 degradation dynamics remain relatively stable across the tested thermal conditions, implying a level of robustness in their turnover under far-red light. Future work will focus on identifying other regulatory factors that might influence LRB1 and LRB2 stability, including potential interactions with phytochrome signaling components or alternative degradation pathways that respond to light and temperature changes in combination.

Lady's slipper orchids and fire: Identifying the species of fungal symbionts and the influence of fire in *cypripedium sp.* native to Michigan

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Mentors: Drs. Jennifer Winther[2] and Priscilla Nyamai[2]

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Ram's Head Lady Slipper Orchid (*Cypripendium arietinum*) (RHLSO) is a terrestrial orchid species native to Michigan, with limited distribution from Wisconsin to Quebec that is classified as a Species of Special Concern and Regional Forester Sensitive Species due to their declining populations throughout their range. They are a long-lived perennial plant that form mycorrhizal associations with fungi that are required for germination and persist throughout the life of the plant. The aim of this study is to survey current RHLSO populations in Sleeping Bear Dunes National Park and confirm the presence of fungal symbionts in the adult plants. This study is part of a much larger orchid species distribution survey in Sleeping Bear Dunes National Park and detailed analysis of the vegetation and soil characteristics of the habitat where these orchids are found. The larger study consists of areas that have experienced prescribed fire (burned) as part of their management, and areas that have not (unburned). Preliminary results suggest that Ram's head orchids are more common in burned treatment areas and found predominately near white pine trees. DNA has been extracted and fungal DNA sequences PCR amplified from 25 orchids collected in burned and unburned treatment sites and their nearest neighboring white pine tree roots. In addition, RHLSO roots and white pine tree roots have been cleared and stained to characterize the morphology of the fungal associations in their roots. Our morphological results suggest RHLSO form typical orchid mycorrhizal associations in the roots of all orchid roots sampled. We will further characterize these fungal associations after obtaining and analyzing the sequence data. The findings will provide insights into this fungal relationship that is critical for the survival of these orchids, and will be shared with resource managers, contributing to the efforts to manage and protect these beautiful plants.