The 10th Annual Cell & Molecular Biology Symposium

Book of Abstracts

Winter 2024

Presentation Schedule

Friday Session 1

Chair: Ashley Thompson

Co-CRISPR/cas9 disruption of the Arylalkylamine N-acetyltransferase-like 7 (AANATL-7) gene in *Drosophila melanogaster*
Presenter: Lydia Cruce

Disruption of Genes Involved in Histamine Metabolism to Determine which Metabolite is Present in Male Accessory Glands of *Drosophila melanogaster*
Presenter: Marie Adinolfi

Do Mutations that Disrupt the Arylalkylamine N-Acetyltransferase Like 7 (AANATL-7) Gene in *Drosophila melanogaster* Affect Histamine-like immunoreactivity in the Larval CNS?
Presenter: Ashlyn Tyson

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Understanding how WNT signaling is impacted by FZD2 variants associated with Robinow Syndrome
Presenter: Maddison Marshall

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Saturday Session 1

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Analyzing the Effects of Myocardial Infarction through Spatially-Resolved Single Cell Transcriptomics using Machine Learning Methods
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Using Normal Mode Analysis to Compare the Conformational Changes Induced by Diazepam and Flumenazil in the α1-β2-γ2 GABA(A) Receptor
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Investigating the Effects of Testosterone on Hormone-Responsive Cancer Cells
Presenter: Isabelle Frechette

Reinvigorating Immune Function in High-risk Childhood Leukemia
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Exploring the Importance of Long Chain Fatty Acid Transport in the Mitochondrial Fatty Acid Synthesis Pathway
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Assessing the Accuracy of qPCR (EPA draft method C) for Detecting *Escherichia coli* Contamination in Water Samples.
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Beaches and Feces: the Journey to Solve the #2 Mystery
Presenter: Ashley Thompson

Using PMMoV as a Human Fecal Indicator in Thornapple Lake
Presenter: Hannah Bekius

Detection of Human Pathogenic Viruses through Wastewater-Based Epidemiology
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Saturday Session 4

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Freshwater Algae: a Flora of Common Genera in West Michigan and an Experiment Revealing Interactions between Diatoms and Cyanobacteria
Presenter: Sofia Martinez Martinez

Determining Ideal Laboratory Conditions for Epithemia Growth
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Understanding the Effect of Light and Temperature on Light Regulating BTB (LRB) Proteins in Arabidopsis Thaliana
Presenter: Samantha LaMantia

Co-CRISPR/cas9 Disruption of the Arylalkylamine N-acetyltransferase-like 7 (AANATL-7) Gene in *Drosophila melanogaster*

Lydia Cruce[1]
Mentor: Dr. Martin Burg[1,2]

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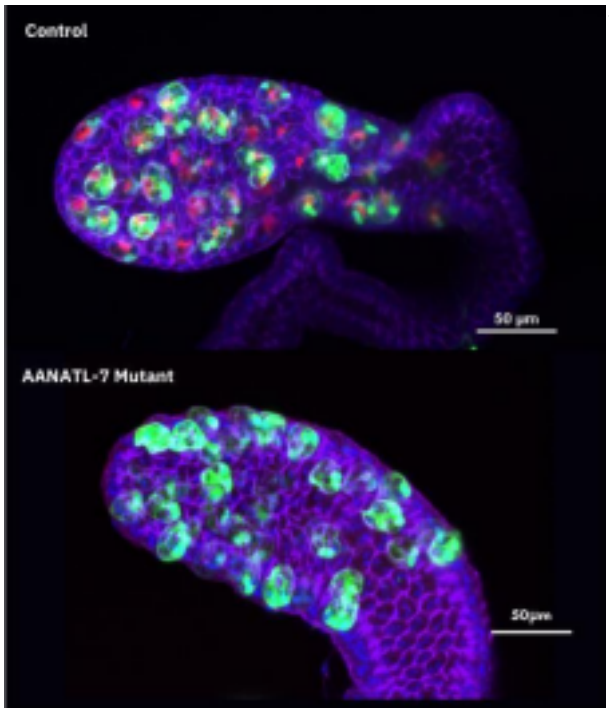

The accessory gland in *Drosophila melanogaster* males is functionally comparable to the human prostate gland. Several substances had been localized to secondary cells in the male accessory gland of *D. melanogaster*, including histamine. Recent results from a number of metabolomics experiments indicated that a metabolite of histamine, N-acetylhistamine (NAH), is present at high levels while histamine was not detected in the accessory gland. Additionally, recent immunocytochemical studies have shown that the histamine antibody that has been used, through several blocking experiments, could be pre-absorbed by not only histamine but also by the histamine metabolites carcinine and NAH. Therefore, to confirm the identity of the substance being detected (carcinine, histamine, or NAH), the gene encoding the enzyme that was predicted to catalyze the synthesis of NAH (*AANATL-7*) needed to be disrupted, as a mutant disrupting this gene had yet to be created. In this project, a co-CRISPER/Cas9 technique was utilized to cause either a synthetic deletion or a point mutation that could disrupt *AANATL-7* gene function. I utilized *ebony* as a visual marker for this approach, which enabled a focused analysis of flies that may bear a mutation in *AANATL-7*. I injected *nos*Cas9 embryos with sgRNAs targeting both the *AANATL-7* and *ebony* genes, which were then mated with *ebony* flies. The resulting F_1 progeny that were *ebony* (induced by CRISPR) were considered to have a higher likelihood of containing the *AANATL-7* mutation and were used to establish single isogenic fly lines. Genomic PCR amplification of these isogenic lines was conducted and the resulting fragment sequenced to identify any point mutations at one of the two sgRNA sites. Of 98 injected flies, 40 produced ebony bearing flies, from which 7 of these isogenic lines contained mutations in the *AANATL-7*. When accessory glands from the AANATL-7 mutants were subjected to immunostaining using the histamine antibody, no immunosignal was present. This indicates that the histamine antibody is detecting NAH, as the other enzymes in histamine metabolism are unaffected. These results will help us further understand the compounds present in the male reproductive system. Future examination of various reproductive related functions can now be carried out using the AANATL-7 mutants.

Disruption of Genes Involved in Histamine Metabolism to Determine which Metabolite is Present in Male Accessory Glands of *Drosophila melanogaster*

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Mentor: Dr. Martin Burg[1,2]

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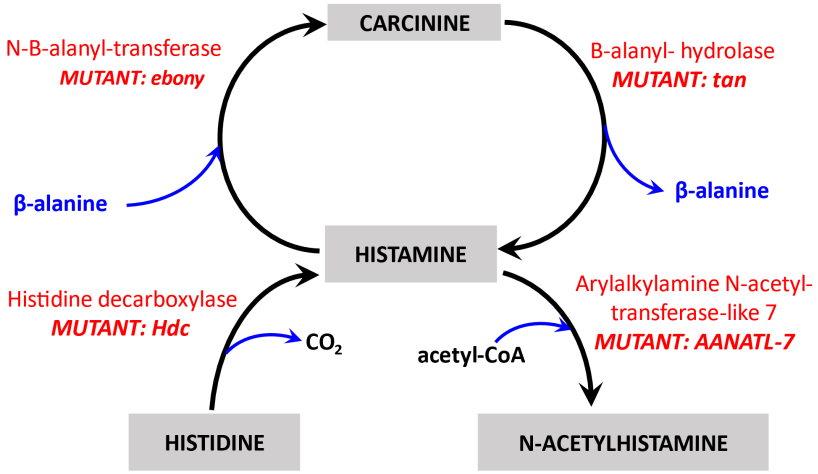

Histamine is a neurotransmitter in *Drosophila melanogaster* that can be metabolized into carcinine (by the enzyme encoded by the *ebony* gene). Carcinine can then be converted back into histamine (by the enzyme encoded by the *tan* gene). N-acetylhistamine (NAH), another histamine metabolite, has been identified in the male accessory gland, which also exhibits high levels of "histamine-like immunoreactivity" (HLI). The histamine antibody was recently shown (by others in the lab) to detect all 3 histamine metabolites, so it is not known which metabolite is being detected in the male accessory gland. The AANATL-7 gene is known to be expressed in accessory glands and is likely responsible for acetylating histamine although genetic evidence has yet to be obtained. Thus, disruption of AANATL-7 expression should reduce the amount of this metabolite in the accessory gland. In this study, AANATL-7 was disrupted using RNA interference (RNAi) to determine its effects on HLI in accessory glands, which resulted in a significant HLI signal reduction. More recently, a parallel study in the Burg lab (L. Cruce), showed that disruption of the AANATL-7 gene using the CRISPR technique confirmed that the HLI detected in the accessory glands was blocked in the AANATL-7 mutant. Currently, accessory glands from *tan* and *ebony* mutants are being examined to determine whether there is any alteration of the HLI in these mutants as was seen in the AANATL-7 mutant. This far, is appears that the *tan* mutation reduces the level of HLI, while *ebony* has little to no effect on HLI levels. These results suggest that the HLI detected in accessory glands is N-acetylhistamine and that the *tan* mutation, which is known to increase amounts of carcinine, may result in less NAH production in the accessory gland, suggesting a role for the *tan* gene in accessory gland function.

Do Mutations that Disrupt the Arylalkylamine N-Acetyltransferase Like 7 (AANATL-7) Gene in *Drosophila melanogaster* Affect Histamine-like immunoreactivity in the Larval CNS?

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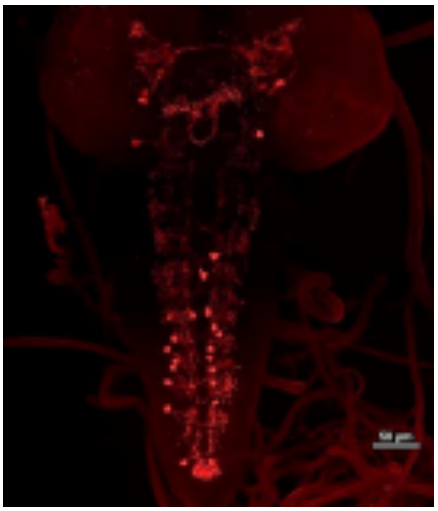

Larval *Drosophila melanogaster* CNS “histamine” staining; (A) wild type versus (B) AANATL-7 mutant

Histamine immunostaining of accessory glands in *Drosophila* males using a “histamine antibody” has indicated the presumed presence of histamine, as mutants defective in histamine synthesis had no signal detected. However, further investigations have determined that this histamine antibody is not specific to histamine but also reacts with two other metabolites of histamine: carcinine and N-acetylhistamine (NAH). Due to this newly discovered cross-reactivity, it is not known which of the three compounds has been detected in previous experiments.

The enzyme, arylalkylamine N-acetyltransferase like 7 (AANATL-7) in *Drosophila melanogaster* is known to catalyze the acetylation of histamine *in vitro*, forming N-acetyl histamine (NAH). Recently, mutations were generated to disrupt the AANATL-7 gene in the Burg lab, resulting in the elimination of histamine-like immunoreactivity (HLI) in the accessory gland, suggesting that the histamine antibody detects NAH in the accessory gland, rather than histamine or carcinine. However, is not known whether the AANATL-7 mutation disrupts or alters HLI in other tissues, such as the larval central nervous system (CNS), which contains 26 “histaminergic” neurons. Therefore, we are investigating whether the CNS immunosignal is altered by mutations in the AANATL-7 gene, by examining the CNS from normal and AANATL-7 mutants using the histamine antibody and imaging using confocal microscopy. Current results indicate a slight immunosignal increase in the mutant brains, suggesting that (1) the substance in the CNS is histamine or carcinine and (2) that AANATL-7 likely functions to remove excess histamine in the CNS.

Understanding how WNT Signaling is Impacted by FZD2 Variants Associated with Robinow Syndrome

Maddison Marshall[1], Cassie Diegel[2], Megan Michalski[2], Alex Zhong[2]
Mentor: Dr. Bart Williams[2]

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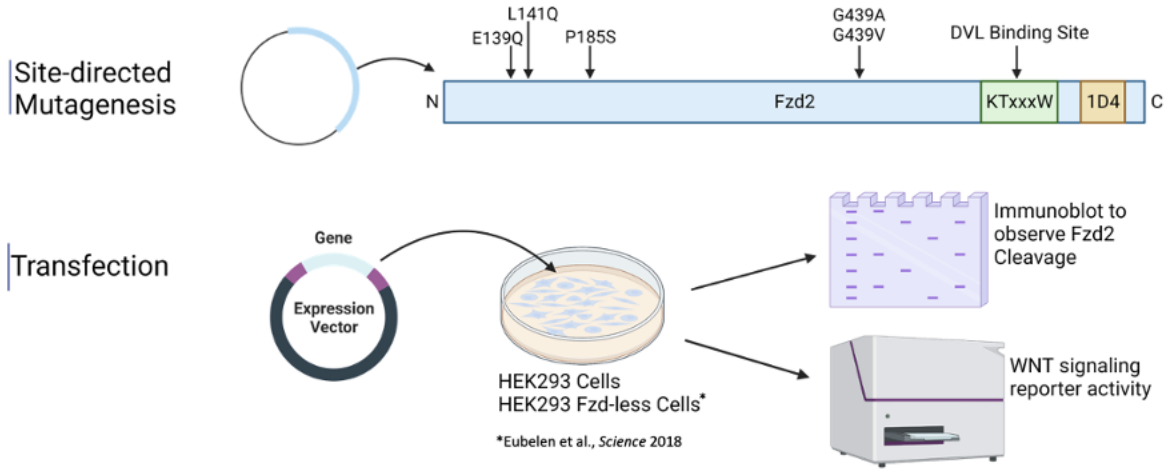

Robinow Syndrome is a rare genetic disorder that is characterized by craniofacial and limb abnormalities. Robinow Syndrome has been linked to variants in *FZD2*, which is a key component in the WNT signaling pathway. *FZD2* variants cause an autosomal dominant form of Robinow Syndrome, meaning one copy of the mutated gene causes the disease. Currently, the mechanism by which *FZD2* variants cause Robinow Syndrome is unknown. *FZD2* is a transmembrane receptor protein upstream in the WNT signaling pathway. Previous studies have shown *FZD2* is cleaved at the intracytoplasmic C-terminal region. The Williams lab previously showed pathogenic Robinow Syndrome *FZD2* variant W553* had a decrease of C-terminal cleavage product. To understand how Robinow Syndrome-linked *FZD2* variants impact the WNT signaling pathway and *FZD2* processing, we used site-directed mutagenesis to generate plasmids containing several Fzd2 mutations. We overexpressed these constructs in HEK293 cells and assessed downstream signaling alterations and *FZD2* processing. Pathogenic Robinow Syndrome variants cells showed a decrease in *FZD2* C-terminal cleavage as well as a decrease in a *FZD2* post translational modification. Downstream signaling of the canonical WNT signaling pathway was increased in two *FZD2* variants. These results indicate a possible mechanistic function of *FZD2* cleavage in causing Robinow Syndrome.

VAIGS Summer Internship funded by the Meijer Foundation

Biochemical and Structural Characterization of ANT(6)-Ib

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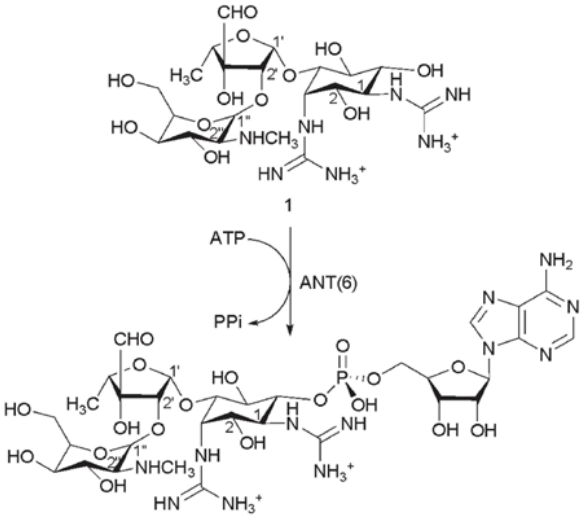
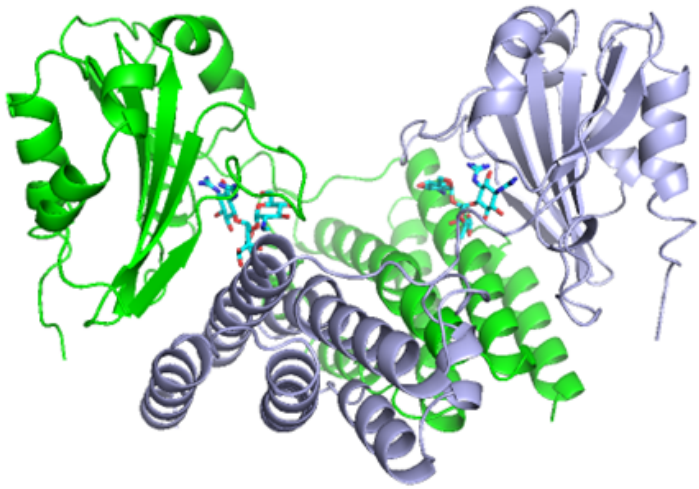


Adenylylation of Streptomycin at position 6



ANT(6)-Ib Dimer

Antibiotic drugs have served as essential tools in the treatment of serious bacterial infections since the 1940s and have since revolutionized modern medicine. Aminoglycosides are a class of antibiotics that function by causing translational miscoding during protein synthesis in bacteria. However, the overuse of these drugs has led to a rise in antibiotic resistance severely reducing their efficacy. The most common form of resistance to these drugs is their inactivation by several diverse families of bacterial antibiotic modifying enzymes (AMEs). The AME aminoglycoside nucleotidyltransferase(6)-Ib (ANT(6)-Ib) catalyzes the addition of an adenyl group to position 6 on the antibiotic streptomycin. This modification alters the structure of the drug, thereby hindering its ability to bind to its target, the bacterial ribosome. Despite the significance of this enzymatic activity, ANT(6)-Ib has not been well studied. Here, we used cellular growth assays and steady-state kinetics to biochemically characterize the activity of the ANT(6)-Ib enzyme from *Campylobacter fetus subsp fetus*. Furthermore, we determined the high-resolution crystal structure of the streptomycin bound enzyme. Using this data, we identified key amino acid residues involved in substrate binding and catalysis. These data provide valuable insights into the molecular mechanism of the ANT(6) enzyme family that will be critical to the future development of inhibitory strategies to overcome ANT induced antibiotic resistance.

Supported through funding by the Department of Chemistry and Office of Undergraduate Research and Scholarship (S3 Summer Scholar Grant and Kindschi Fellowship)

Water Quality Testing Strategy for Performance Qualification (PQ)

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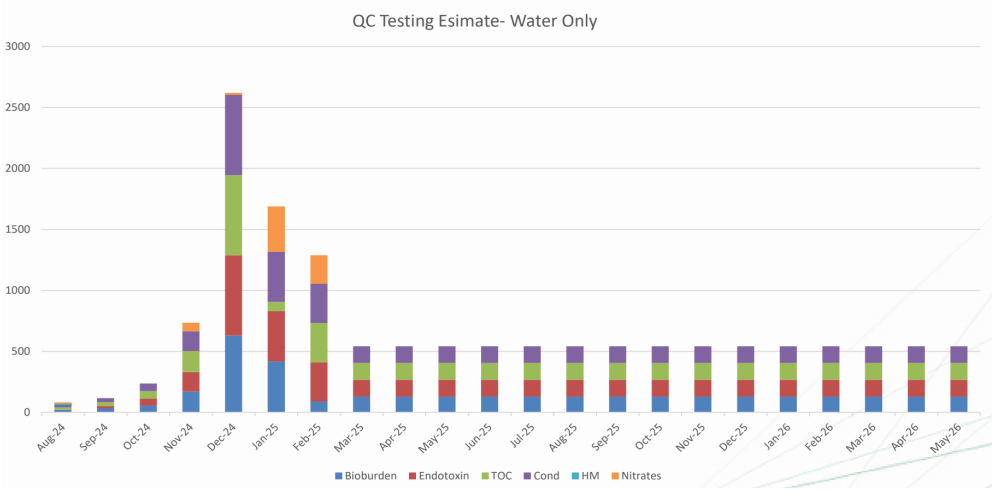

In the pharmaceutical industry, water quality testing is a critical aspect of ensuring the safety and efficacy of drugs. Contract Development and Manufacturing Organizations (CDMO) play a vital role in the production of pharmaceuticals, where adherence to regulatory standards and guidelines is paramount. The performance qualification of water quality testing systems within a CDMO has significant implications, both for the company and for public health. One aspect to consider is insufficient baseline data on the quality of water sources, especially in regions with variable environmental conditions. This lack of data hampers the ability to set benchmarks for water treatment and testing protocols. In Addition, the full spectrum of potential contaminants, including chemical and biological entities, might not be fully characterized. This gap can lead to inadequate testing protocols that fail to detect all potential hazards.

Here we aim to develop and validate a comprehensive water quality testing strategy to identify potential contaminants within the purified water (WPU), Water for Injection (WFI), and Clean Steam distribution systems. To create this strategy, a data collection tool was developed and modified in relation to USP guidelines to formulate a testing plan for microbial, bacterial endotoxin (LAL), total organic carbon (TOC), heavy metals, nitrates, and conductivity tests to be performed during the performance qualification (PQ) phase of startup. This analysis revealed the need for use of off-site laboratory testing as well as an increase in budgetary and personal allocations to water quality testing. The expected date of PQ water quality testing will continue through May of 2026 with many samples being taken between Nov. 2024 and Feb. 2025. By ensuring the highest quality of water in pharmaceutical manufacturing, the industry can minimize the risk of product contamination, thereby enhancing the safety and efficacy of pharmaceuticals.

Analyzing the Effects of Myocardial Infarction through Spatially-Resolved Single Cell Transcriptomics Using Machine Learning Methods

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Mentor: Dr. Zachary DeBruine[2]

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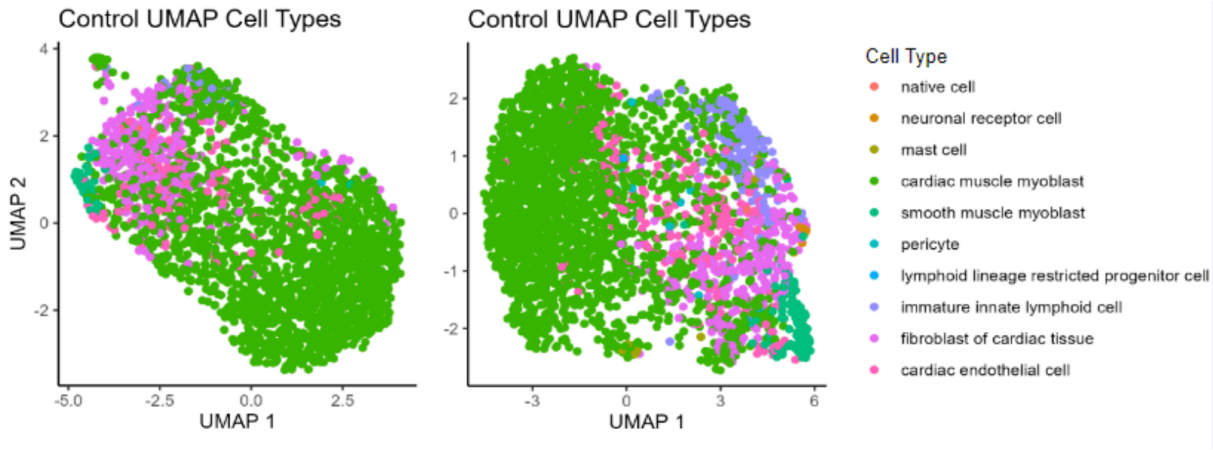

Myocardial infarction is caused by one or more areas of the heart being deprived of oxygen when blood flow through the heart is blocked. This can cause permanent damage to the muscle tissue such as cardiac cell death, impaired signaling and the remodeling/rearrangement of remaining cardiomyocytes/myoblasts. By using Graph Convolutional Non-Negative Matrix Factorization (GCNMF), this study compares spatially relevant cell type patterns to explore rearrangement caused by myocardial infarction. The NMF model is visualized using standard methods to gain qualitative insights into cell type ratios. Finally, a Gene Set Enrichment Analysis is used to break down the NMF factors by the genes that play the greatest roles in factors of interest to analyze what biological processes may play a role in the rearrangement of the cells after a myocardial infarction. GCNMF, along with GSEA will reveal spatially relevant cell type patterns and rearrangements caused by myocardial infarction, and identify marker genes that play a role in the pathology of the disease. The data indicates that myocardial infarction causes a decreased number of cardiac muscle myoblasts, and increased fibroblasts of cardiac tissue.

Using Normal Mode Analysis to Compare the Conformational Changes Induced by Diazepam and Flumenazil in the α1-β2-γ2 GABA(A) Receptor

Amber Heist[1], Agnieszka Szarecka[1]
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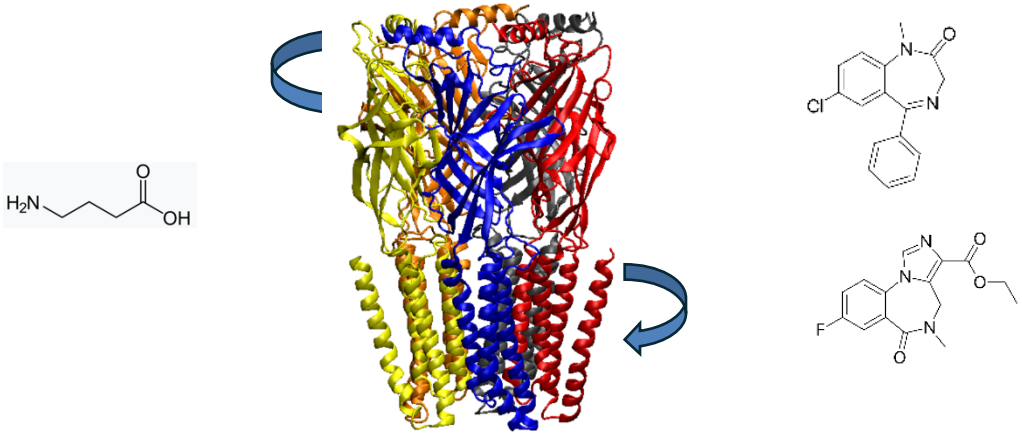

GABA signaling, concentrated in the brain's prefrontal cortex, is important for mental and emotional health. GABA(A) receptors are CYS-loop, neurotransmitter-gated, chloride-specific ion channels. They are pharmacological targets, primarily for benzodiazepines (BZD), Z-drugs, and barbiturates. It has been established that benzodiazepines act as allosteric agonists of GABA(A)R; however, the exact conformational changes that are induced by various allosteric modulators are not well understood. In this study, we used Normal Mode Analysis / Elastic Network Model to analyze the conformational changes induced by diazepam (BZD) and flumenazil (BZD antagonist) in the human GABA(A) α1-β2-γ2 receptor compared to GABA-bound receptor. We have analyzed the three slowest vibrational modes of structures 6x3x (GABA-bound), 6x3z (GABA/diazepam-bound), and 6x3u (GABA/flumenazil-bound). Our data indicates diazepam modulates the slowest mode fluctuations of more residues than flumenazil does. All the affected residues are in the transmembrane domain and two of them, A246 and G254 are on the pore-lining TM helix 2. In contrast, in modes 8-9, diazepam and flumenazil modulate fluctuations of multiple residues in both extracellular and transmembrane domains. These preliminary results contribute to our understanding of the allosteric modulation of GABA(A)R by anxiolytic drugs.

The First European Woolly Rhinoceros Mitogenomes, Retrieved from Cave Hyena Coprolites, Suggest Long-Term Phylogeographic Differentiation

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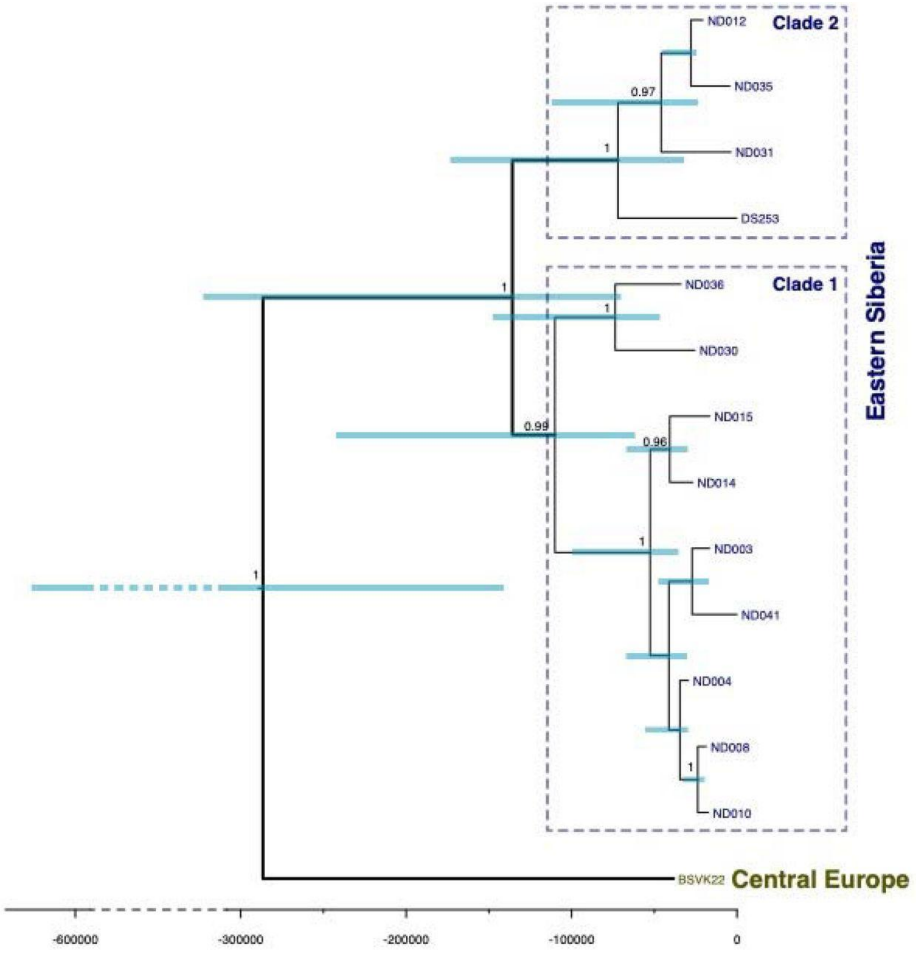

The woolly rhinoceros (*Coelodonta antiquitatis*) is an iconic species of the Eurasian Pleistocene megafauna, which was abundant in Eurasia in the Pleistocene until its extinction, beginning approximately 10,000 years ago. Despite the early recovery of several specimens from well-known European archaeological sites, including its type specimen (Blumenbach 1799), no genomes of European populations were available so far, and all available genomic data originated exclusively from Siberian populations. Using coprolites of cave hyenas (*Crocuta crocuta spelea*) recovered from Middle Paleolithic layers of two caves in Germany (Bockstein-Loch and Hohlenstein-Stadel), we isolated and enriched predator and prey DNA to assemble the first European woolly rhinoceros mitogenomes, in addition to cave hyena mitogenomes. Both coprolite samples produced copious sequences assigned to *C. crocuta* (27% and 59% mitogenome coverage, respectively) and woolly rhinoceros (*Coelodonta antiquitatis*; 27% and 81% coverage, respectively). The sequences suggested considerable DNA degradation, which may limit the conclusions to be drawn; however, the mitogenomes of European woolly rhinoceros are genetically distinct from the Siberian woolly rhinoceros, and analyses of the more complete mitogenome suggest a split of the populations potentially coinciding with the earliest fossil records of woolly rhinoceros in Europe.

Investigating the Effects of Testosterone on Hormone-Responsive Cancer Cells

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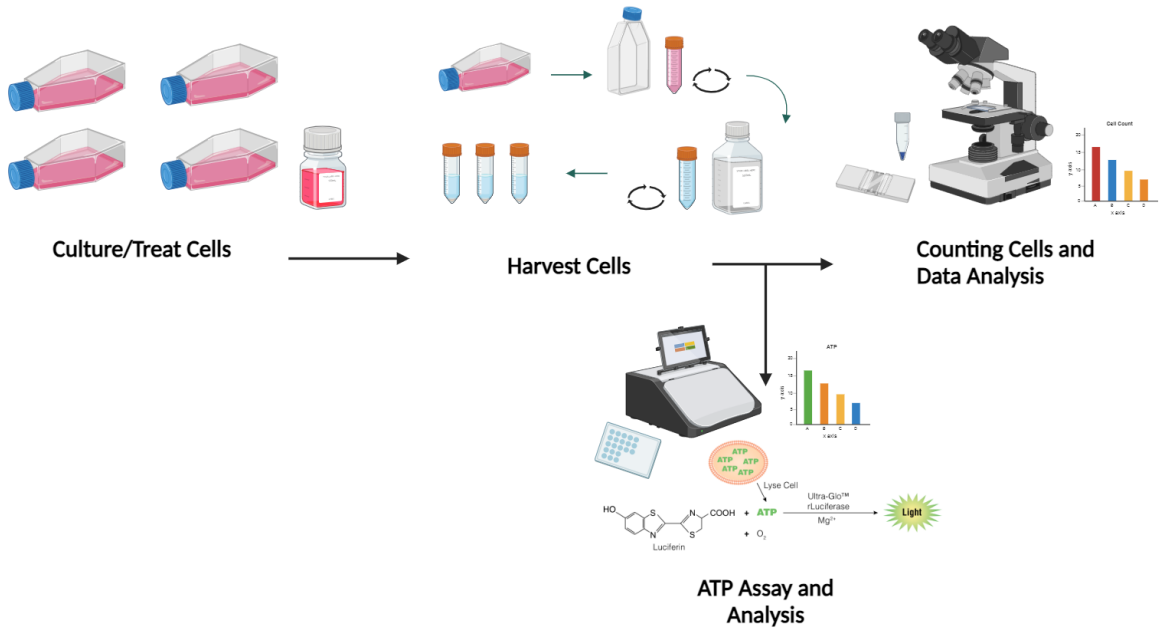

Breast cancer (BC) is now the most diagnosed cancer in women, surpassing lung cancer as of 2020. Research suggests that this increase in BC is likely fueled by hormones; with approximately 75% of new BC diagnoses being hormone-responsive (HR+). The role of estrogens in the development and spread of HR+ BC is well established, leading to the development of targeted therapies. However, the correlation between androgen levels and the risk of female HR+ cancers remain ill-defined. Therefore, this research aims to evaluate the effects of testosterone (T4) on HR+ BC and endometrial cancer (EC) cells. Culture flasks (n=4) were seeded with ~5 x10^5 HR+ BC (MCF-7) and HR+ EC (Ishikawa) cells (n=4) and supplemented with T4 [0 nM (Control), 0.05 nM, 2.5 nM, and 5 nM] for 28 days. Cell viability was assessed by trypan-blue exclusion test, and cellular ATP production was measured using a Bioluminescent assay kit. The cell counts and the intracellular ATP concentration in the 5 nM T4 treated BC group increased by ~51% and ~11% respectively by day 28, compared to the Control. The cell counts in the EC group (5 nM) increased by ~8%, and the intracellular ATP concentration was elevated by ~33% by day 28 of T4 supplementation relative to the Control. These results suggest that testosterone does increase the proliferation rate and ATP production of HR+ BC and EC cells. However, more studies are needed to confirm these results.

Reinvigorating Immune Function in High-risk Childhood Leukemia

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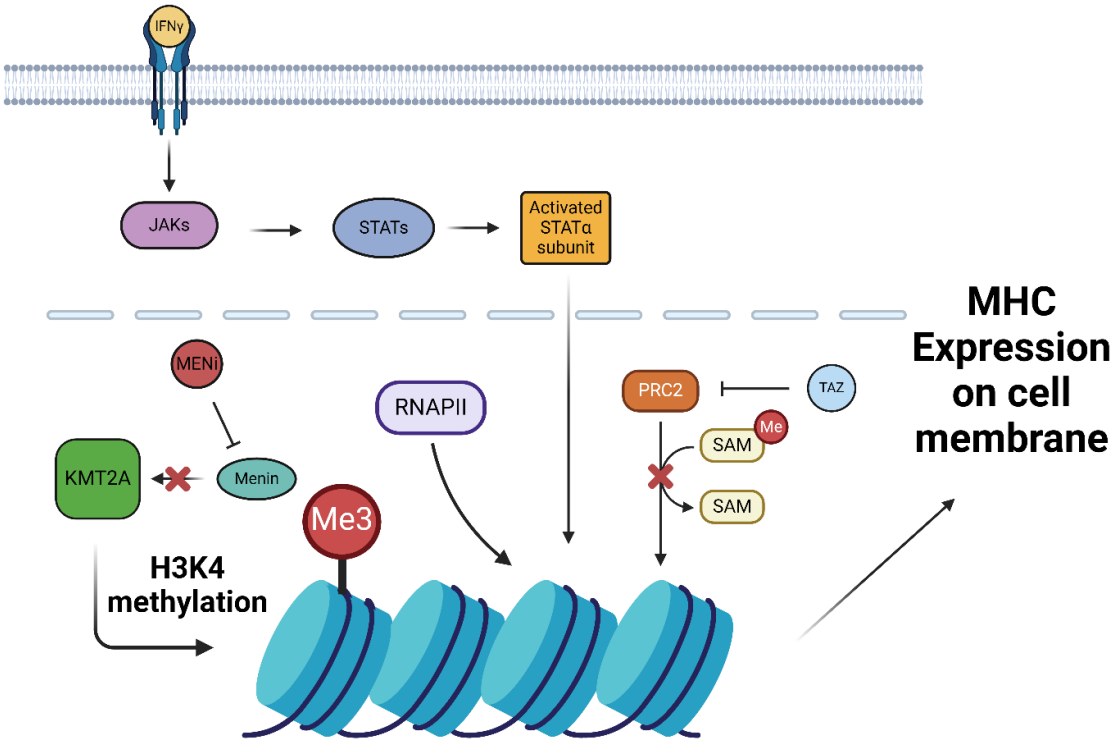

Leukemia patients with a chromosome 16-21 translocation, yielding the FUS::ERG oncoprotein, have extremely poor outcomes and a lack of immunogenicity associated with overexpression of EZH2, a histone methyltransferase. I treated FUS::ERG cell lines with the EZH2 inhibitor tazemetostat, with interferon-gamma, and with the Menin inhibitor VTP50469 to investigate whether patients with this lesion could be rendered immunogenic for stem cell transplantation. Our previous work has demonstrated promising results for the combination of EZH2 inhibition and interferon-gamma (associated with donor lymphocyte activation) in the TSU-1621MT FUS::ERG cell line. Here we report new results from the treatment of an additional FUS::ERG model, the YNH-1 cell line, with the same combination therapy and investigate VTP50469's effect in both models. EZH2 inhibition and interferon-gamma supplementation induce the expression of MHC molecules on the cell membrane of YNH-1 cells while VTP50469 has limited effects on MHC expression in YNH-1 and TSU-1621MT cells. EZH2 inhibition, Menin inhibition, and interferon-gamma supplementation result in a synergistic effect on MHC II expression in TSU-1621MT cells. These results further indicate the efficacy of EZH2 inhibition and interferon-gamma supplementation as a potential treatment for patients possessing the FUS::ERG lesion while highlighting the additional investigation of Menin inhibition needed in leukemia.

Exploring the Importance of Long Chain Fatty Acid Transport in the Mitochondrial Fatty Acid Synthesis Pathway

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Mentor: Dr. Sara Nowinski

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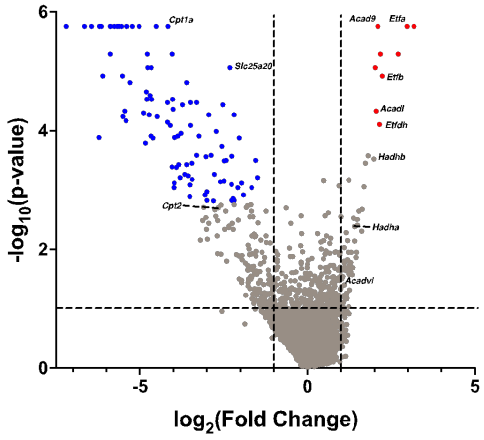

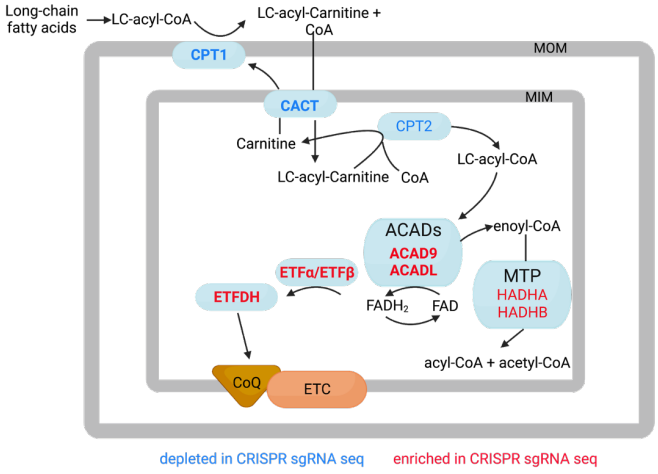

The mitochondria have their own unique pathway for synthesizing long chain fatty acids (mtFAS). This complicated pathway is essential for the electron transport chain, iron sulfur cluster biogenesis, and the TCA cycle. A previous CRISPR screen showed that mtFAS deficient cells are dependent on fatty acid import for growth. To validate this, I Inhibited long chain fatty acid import into the mitochondria in mouse skeletal muscle cells using Etomoxir. I then measured cell proliferation using an incucyte. Etomoxir treatment made the mtFAS mutant cells grow more slowly. In contrast, inhibition of fatty acid oxidation using 3-MPA improved growth of mtFAS-deficient cells. We identified that this did not occur via rescue of protein lipoylation by western blotting for the presence of lipoic acid in cells inhibited with 3-MPA. From this study, we concluded that mtFAS deficient cells grow worse when long chain fatty acids import into the mitochondria is inhibited but better when fatty acid oxidation is inhibited. We also concluded that there is most likely not a scavenging mechanism of long chain acyl-CoA taking place in mutant cells.

Assessing the Accuracy of qPCR (EPA draft method C) for Detecting *Escherichia coli* Contamination in Water Samples

Carter Griffioen[1]

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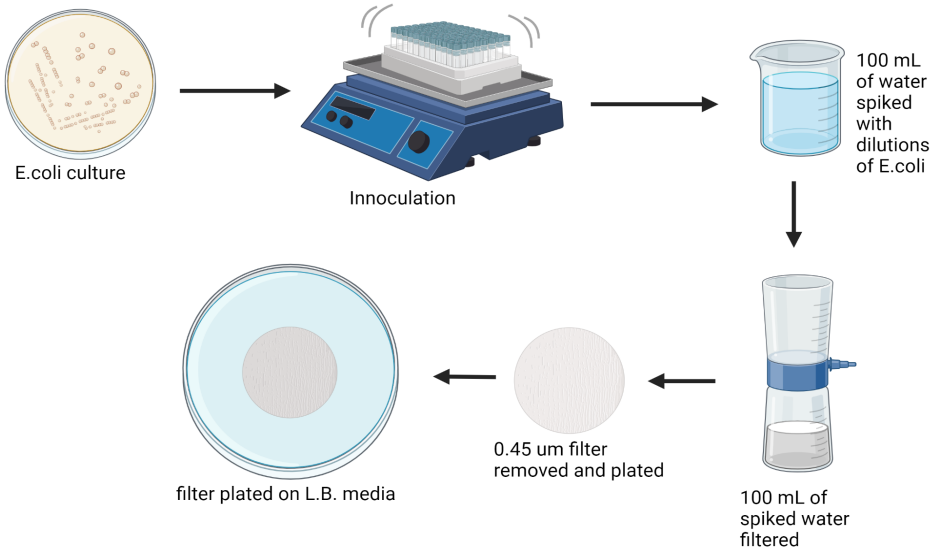

EPA draft method C is a protocol for determining *E.coli* contamination in recreational water samples via qPCR. *E.coli* are the most abundant bacteria in the human gut and constitute 30% of human feces. Because they are so abundant, they are the best marker for fecal contamination of water, and - although most strains of *E.coli* are harmless - some can cause serious sickness. Therefore, determining *E.coli* contamination in freshwater is an important surveillance tool to keep recreational bodies of water safe for people to enjoy and to maintain a healthy environment for wildlife. Most *E. coli* detection methods utilize an overnight culture step. However, the long time required poses a danger that recreational water users could be sickened before beaches are closed. For this reason, Method C, which uses molecular methods, was developed as a rapid method yielding same-day results. To our knowledge, this method has yet to be calibrated with known quantities of bacteria. Therefore, my objective was to test each step of method C for the recovery of *E. coli*. The first step of Method C (water filtration) was tested by filtering water samples spiked with known concentrations of *E.coli* through a 0.45 µm filter to determine how many *E. coli* bacteria were captured and retained by the filter. My results showed that when 100 mL of water spiked with 3 different diluted concentrations of *E.coli* was filtered through a 0.45 µm filter and plated on LB media, there was a significant loss of *E.coli*. This suggests that filtration through the 0.45 µm does not retain all of the bacterial cells. Further work will be aimed at more precisely quantifying potential losses and variability due to filtration and then moving on to test recovery of signal downstream of filtration.

Beaches and Feces: the Journey to Solve the #2 Mystery

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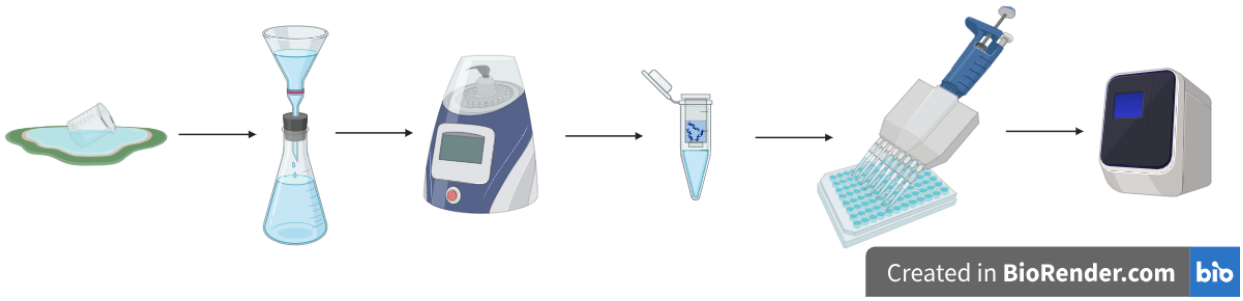

Michigan recreational beaches at risk for water-borne illnesses are routinely monitored during the summer months for *E. coli* – a marker of fecal contamination. Charlton Park beach, located on Thornapple Lake, is frequently closed because *E. coli* exceeds the State threshold forcing the beach to close for 35 days in 2022 and 12 days in 2023. Remediation is not possible until a contamination source is determined. We partnered with Barry-Eaton District Health Department (BEDHD) to determine the source through the molecular detection of host-specific bacteria (MST). We intensively sampled the beach and other surrounding lake and tributary sites for 5 weeks and simultaneously monitored environmental factors. Samples were analyzed for both *E. coli* and MST. Testing showed a correlation between the *E. coli* marker EC23S and the HF183 human marker when the *E. coli* counts are high but proved difficult to correlate them at low levels. We found that contamination: is partially human in origin and originates from two sites upstream of the beach and is most likely to occur when the normal southerly current in Thornapple Lake is slowed. Validation is underway with secondary markers to further confirm human origins, as well as testing the effectiveness of alternative markers. Our work attests to the important role that molecular techniques can play in the field of environmental forensics and the public health safety.

Funded by the Michigan Department of Environment, Great Lakes, and Energy (EGLE) and by Barry and Eaton Counties Health Department "BE in the Swim" Grant

Using PMMoV as a Human Fecal Indicator in Thornapple Lake

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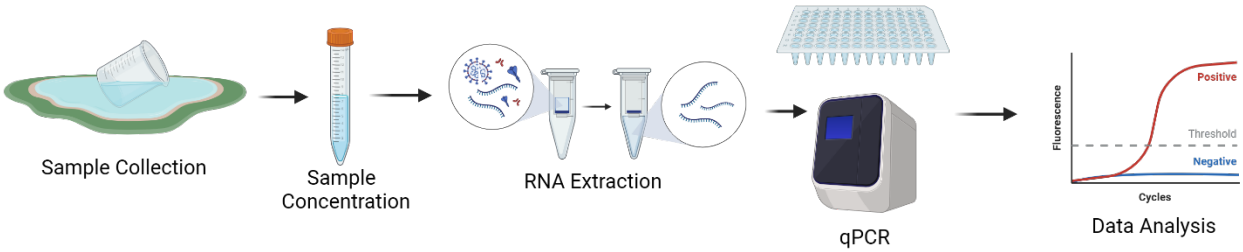

Fecal pollution of inland environmental water sources poses a significant risk to human and ecosystem health. Fecal pollution can cause the spread of waterborne diseases and can also have environmental consequences such as algal blooms. *Escherichia coli* levels are the most commonly used indicator to monitor fecal contamination in inland water sources, but *E. coli* is common to all warm-blooded mammals and therefore does not indicate the source of contamination. Microbial source tracking (MST) can identify the source of fecal pollution by using host specific markers. One such fecal marker is pepper mild mottle virus (PMMoV). PMMoV, although it is a plant virus, is the most abundant and stable RNA virus found in human feces and is used frequently as a human fecal marker in wastewater-based epidemiology of RNA viruses. The aim of this research was to determine whether PMMoV could be used to determine fecal contamination in an inland lake. We worked with Barry-Eaton District Health Department to monitor Thornapple Lake, which historically has high *E. coli* counts. We sampled 12 different sites on the lake and surrounding tributaries twice a week for 5 weeks, to test for PMMoV and *E. coli* presence. RNA viruses (including PMMoV) were concentrated using PEG precipitation and viral RNA was extracted and then quantified using qPCR assays. We were able to identify the presence of PMMoV in some sites at low gene copy values (>1000 gene copies/100mL sample). Wastewater was diluted in order to generate a standard curve so that a comparison could be made between the contamination in the lake compared to known dilutions of PMMoV from wastewater. However, the presence of PMMoV did not correlate to HF183 which is another MST marker used to detect human fecal pollution. I conclude that PMMoV is likely not an effective method to detect human fecal pollution in inland water sources, possibly because of environmental inhibitors and UV light.

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Detection of Human Pathogenic Viruses through Wastewater-Based Epidemiology

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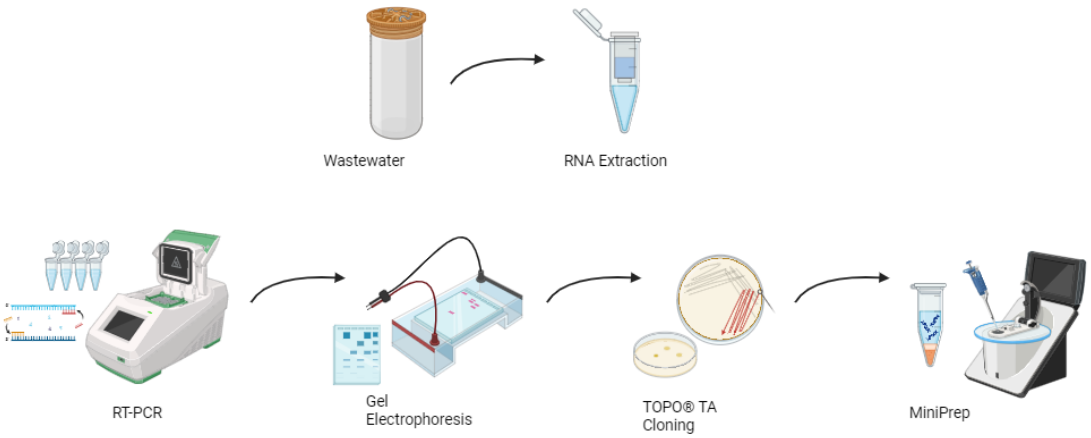

Wastewater contains a mixture of organic matter, and biological and chemical substances, including viral particles that have been shed from individuals infected with pathogenic viruses. Wastewater-Based Epidemiology (WBE) non-intrusively uses this mixture to provide quantitative and qualitative information about the physiologic status of a specific population, including the level of viral exposure in the population. WBE is being used successfully worldwide, including here in Kent County, to detect SARS-COV-2. Our lab (Molecular Monitoring for Health and Environment Lab) is one of 21 labs contributing to a unique publicly funded state-wide initiative initially targeted at monitoring the SARS-CoV-2 pandemic. As the pandemic has waned in urgency, we continue to monitor this virus, but are also expanding our surveillance to other pathogens of concern. In this study, viral particles were precipitated from wastewater collected as part of our routine SARS-CoV-2 monitoring with PEG, and viral RNA was isolated using a Qiagen column extraction procedure. RNA samples were tested with primer sets for 5 different human pathogenic RNA viruses as well as a viral RNA fecal marker (Pepper Mild Mottled Virus), through RT-PCR. Samples that tested positive (demonstrated a discrete band of the correct molecular weight with agarose gel electrophoresis) were cloned with TOPO® TA Cloning and the plasmid with the cloned insert was purified with a miniprep. Preliminary results detected the presence of 1 of the 5 viruses that were tested, Respiratory Syncytial Virus A (RSV A) in Kent County. This work served as proof of concept for detection of RSV in Kent County, a pathogen now being routinely surveyed by our laboratory, and demonstrates the utility of WBE to inform stakeholders so that further action can be taken to mitigate public health risks.

Freshwater Algae: a Flora of Common Genera in West Michigan and an Experiment Revealing Interactions between Diatoms and Cyanobacteria

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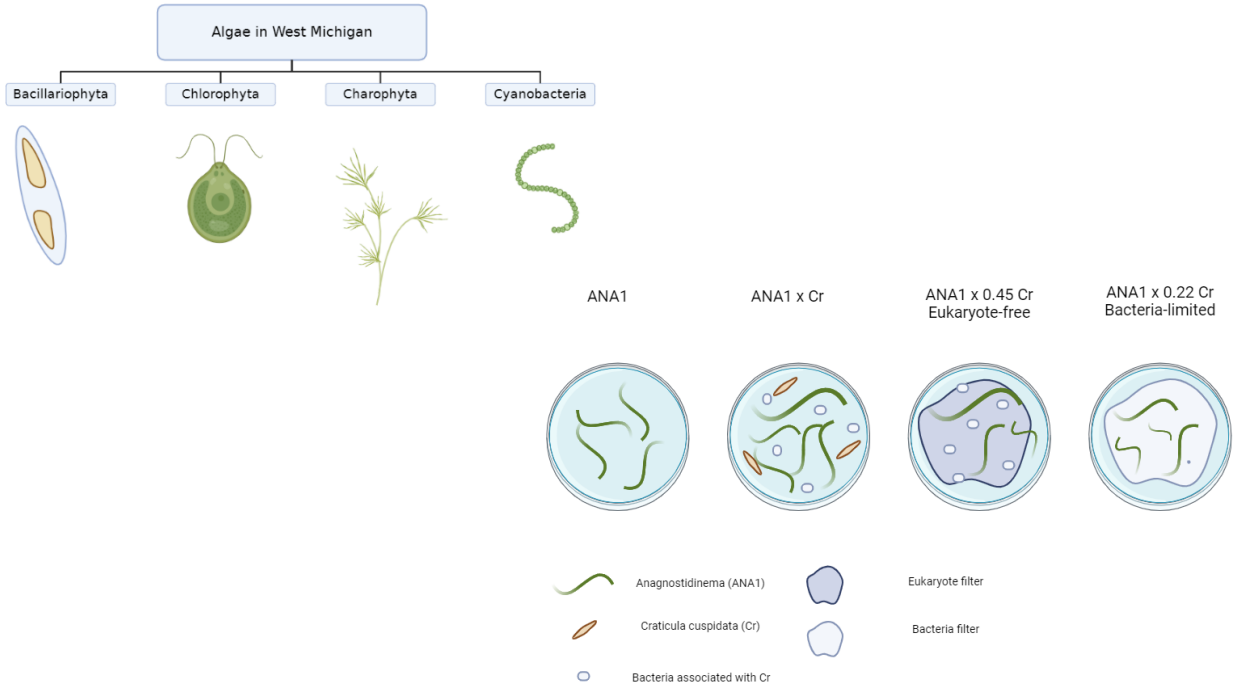

Algae are a polyphyletic group of organisms that photosynthesize and live in environments with adequate light and water. While important to global carbon cycling, algae can be difficult to identify and interactions between them are not well-established. Thus, we designed two projects: 1) a field guide of common algae in the Western Michigan region in winter; and 2) a growth experiment to better understand interactions between Craticula cuspidata (Cr) and an Anagnostidinema (ANA1) strain. For the flora, we depicted the twenty genera most encountered by students in the GVSU Freshwater Algae class. For the growth experiment, ANA1 percent cover was analyzed from images taken over 30 days of ANA1 in co-culture with Cr, in eukaryote-free treatment, and in a bacteria-limited treatment to determine what was responsible for the increase in ANA1 growth. Ultimately, we illustrated commonly encountered algal genera in the Bacillariophyta, Chlorophyta, Charophyta, and Cyanobacteria; and found that ANA1 grew best with Cr cells but grew in the eukaryote-free and bacteria-limited conditions as well. This illustrated guide allows for improved identification in classroom and presents a simple and effective method of understanding interactions between different algal divisions.

Determining Ideal Laboratory Conditions for *Epithemia* Growth

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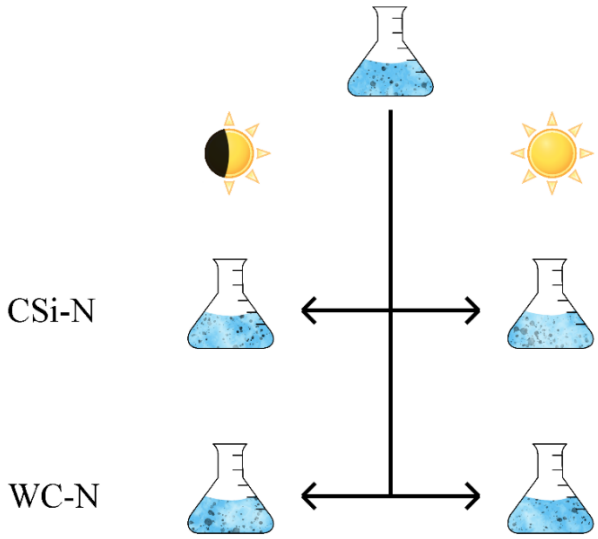

Diatoms are microscopic eukaryotic algae with silica cell walls. *Epithemia*, a genus of diatoms belonging to the Rhopalodiales, have cyanobacterial endosymbionts that allow them to fix atmospheric nitrogen and live in low nitrogen environments. Because of their nitrogen fixation, they are an integral part of the global nitrogen cycle in addition to the carbon cycle. Many diatoms, including *Epithemia,* have a complicated taxonomic history and have been classified using morphology alone, which can underestimate species diversity and does not always reflect evolutionary relationships. To place *Epithemia* spp. in evolutionary context using molecular data, they need to be cultured in a laboratory. Collection of *Epithemia* from the environment indicates they prefer high light environments, but preliminary culturing attempts suggest they grow better in low light conditions. In addition, *Epithemia* have been cultured in two different media in previous studies, WC-N and CSi-N. In this project, we aimed to determine the ideal conditions for laboratory growth of one strain of *Epithemia*. We cultured them in a full factorial design in low (6 µmol m^{-2} s^{-1}) and high (40 µmol m^{-2} s^{-1}) light conditions and in WC-N and CSi-N media. Preliminary results suggest WC-N in high light conditions did not grow as well as other treatments, which displayed similar growth. Better understanding of the growth conditions for this strain will allow us to culture this genus more easily, and ultimately place it more accurately in evolutionary context.

Understanding the Effect of Light and Temperature on Light Regulating BTB (LRB) Proteins in *Arabidopsis thaliana*

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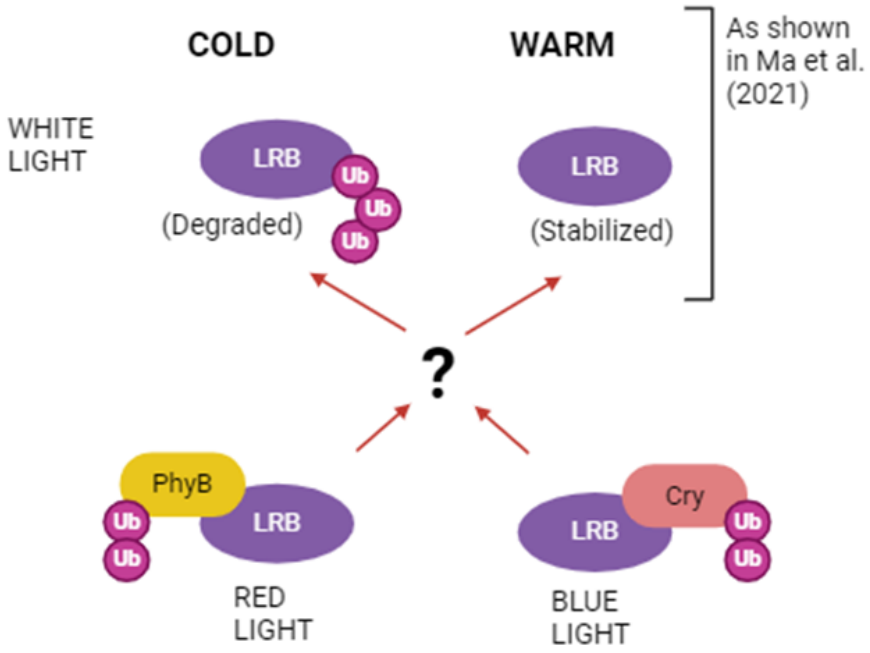

(Created using BioRender)

Light is crucial for photosynthesis, as well as for plant growth and development. Plant development is carefully controlled in responses to varying light conditions, but also other environmental factors, including temperature. Although photoreceptors for various wavelengths of light are known in plants, many questions remain about mechanisms regarding their regulation. Previous research found that the LRB E3 ligase proteins interact with both the cryptochrome (cry, blue light) and phytochrome (phy, red light) photoreceptors and ubiquitinate them. In conjunction with this, temperature has been shown to affect the stability of the LRBs in white light. Since PhyB has been shown to modulate temperature responses in plants, we will investigate the connection between light signaling and temperature stabilization of the LRB proteins. We carried out an assessment of LRB protein expression in different light conditions varying by wavelength as well as under controlled temperature changes. The results of this analysis suggest that red light regulates LRB protein expression in relation to temperature in a new way, as compared to previous findings in white light, however this relationship needs to be studied further. Understanding how plants respond to temperature variations in different light conditions will be extremely beneficial for agricultural applications, given climate change is affecting the quality and quantity of our crop land today.

A Preliminary Flora of the Rhopalodiales (Bacillariophyceae) of Michigan

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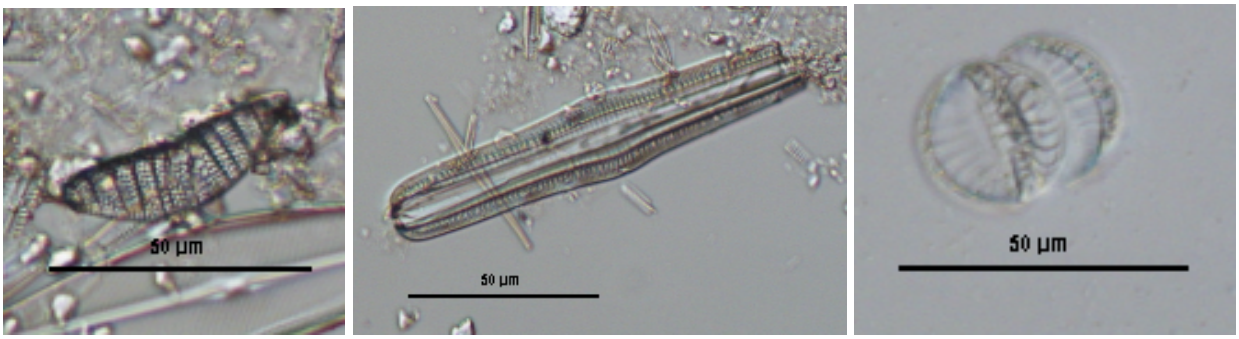

Diatoms are unicellular photosynthetic algae with silica cell walls whose diversity has not been well described. Rhopalodiales are an order of diatoms whose cells contain cyanobacterial endosymbionts that assist them in fixing nitrogen so that they can live in low-nitrogen environments, many of which occur in Michigan. The purpose of this study is to produce a preliminary flora portraying the diversity of this group in Michigan. For the flora, we will utilize previous diatom collections from Southeastern and Northern Michigan and collect new samples from Central and Western Michigan. Any new samples collected will be processed to produce diatom slides. Rhopalodiales in these collections will be observed and documented using light and possible scanning electron microscopy. Through this, Rhopalodiales species will be better documented, easier to recognize in future studies, and contribute to our larger aim to uncover the global diversity of the Rhopalodiales and put these taxa in evolutionary context.

Notes