

BIO481Y5

Summer

Symposium

August 13, 2019
10:00am to 12:00pm
UTM Room



Biology

UNIVERSITY OF TORONTO

MISSISSAUGA

BIO481 –Abstracts and Presentations

10:15 am [Jashandeep Nijjar \(Robert Gerlai\)](#)

10:30 am [Oliver Nazi \(Patrick Gunning\)](#)

10:45 am [Kevin Hoang \(Ingo Ensminger\)](#)

11:00 am Break

11:15 am [Samy Danial \(Jumi Shin\)](#)

11:30 am [Anamika Bhattacharjee \(Robert Gerlai\)](#)

11:45 am [Fabrizio Angeles \(Patrick Gunning\)](#)

Acknowledgements:

Special thanks to Prof. Michael Corrin, Michael Kaler and Jessica Hanley for their presentations this summer.

Also thank you to the BIO481 Reading committee (a group of six Biology graduate students who are tasked with reading the BIO481 papers and selecting a “Best Paper” awardee and the BIO481 presentation judges: Mahmoud Bitar, David Stoker and Julia Gauberg, who will select a “Best Presentation” winner.

Finally thank you to Profs. Joel Levine and Marc Dryer for the responsibility for running this summer course.

10:15 am

Jashandeep Nijjar (Robert Gerlai)

Analyzing the Potential Effects of Weak Electromagnetic Radiation on the Behaviour of Adult Zebrafish (*Danio rerio*)

In the last two decades, the use of micro-wave based devices has dramatically increased, particularly for communication-based use. This increased prevalence in our society has resulted in numerous questions regarding the impact of microwave-based communication on human health. While studies most commonly investigate the influence of radiation on humans and rodents, a recent study has demonstrated that phone radiation may also affect the behaviour of zebrafish. When exposed to mobile phone radiation, it was observed that these zebrafish showed alteration in both anxiety-related behaviour as well as social response. Our laboratory, uses electronic devices, including Wi-Fi based equipment in several of our studies. Thus, the question whether such devices influence brain function and behavior is both an important medical as well as practical experimental question. In the present study, zebrafish were exposed to Wi-Fi radiation using a router. A battery of behavioral tasks was performed in order to test the potential impact of this radiation on zebrafish behaviour. Preliminary results suggest no significant effect of Wi-Fi on anxiety-related behaviour or social behaviour, however, some notable trends were observed that may warrant additional investigation. To investigate these trends, we plan to conduct analysis of cortisol levels and also increase the sample size of the study. This study acts as a preliminary investigation into any potential behavioural alteration of Wi-Fi, which would pose a significant issue for newly developed computer or automated behavioural testing set ups.

10:30 am

Oliver Nazi (Patrick Gunning)

Crystallization trials attempt on the E2 like enzyme UFC1 in its apo form and drug bound form under various conditions

Many proteins from all kind of systems have been crystallized as shown by previous crystallization experiments. Crystallization is a method that forces the protein to compact in an orderly manner and form a crystal-like structure. Protein crystals are very beneficial via X-ray crystallography when it comes to determining how the shape of a specific protein is, what kind of bonds are taking place in the protein, and the locations of any binding pockets for drugs or other types of molecules. Not all proteins are well understood, and neither is their function or contribution to emergence of cancer in certain types of cells. One of the poorly understood proteins is UFC1 (Ubiquitin-fold modifier-conjugating enzyme 1) which is a protein that can be found in almost all tissues in the human body. UFC1 is an E2 like enzyme in biological called ufmylation. Ufmylation is a similar process to ubiquitination where ubiquitin or a ubiquitin-like enzyme to a target protein in order for post translational modifications to take place. Mutations associated with UFC1 are shown by previous experiments to be linked to endoplasmic reticulum (ER) stress and cancer cell proliferation in breast cancer patients. Crystallization screens were synthesized in the lab to attempt and crystallize the UFC1 protein in its apo form and drug bound form. The crystallization trials yielded crystals in some screens and only few were selected to undergo optimization to obtain better shaped crystals. These crystals suggest that the crystal structure of UFC1 can be determined to better understand its molecular structure and its biological relevance to cancer.

10:45 am

Kevin Hoang (Ingo Ensminger)

Growth performance and chlorophyll fluorescence in response to parental origins in white spruce (*Picea glauca*) seedlings

Future climatic models predict that global warming will have a severe impact on forests in Canada, which white spruce (*Picea glauca* L) is a prominent member. However, intraspecific variation between different white spruce families has the potential to mitigate the impact of climate change by aiding the species to adapt to future climate scenarios. The objective of this experiment was to examine the impact of intraspecific variation, hybridity and parental origin of different white spruce families on their morphological and phenological characteristics. In this study, 10 families of 2-year-old white spruce seedlings originating from differing regions in Quebec and Ontario were examined. A common garden experiment under natural summer growing conditions at the University of Toronto Koffler scientific reserve was used, where the seedlings' height, stem diameter, bud formation and chlorophyll fluorescence were assessed. Significant differences between the families' accumulated height and stem growth was observed. The families also experienced differing timing of bud formation and growth cessation. However, no difference was seen in chlorophyll fluorescence. A significant difference in primary growth was also observed when comparing between families of hybrid and non-hybrid origins. The geographical origin of the seedlings' parents had an effect on the primary and secondary growth between the families, since hierarchical clustering of morphological and phenological parameters grouped together families that originated close to the field site. This would indicate that the proximity of the parental origin to the field site affected the growth performance between the families. Further testing under heat and drought stress is needed to better understand how intraspecific variation between the white spruce families will affect their ability to adapt to future climatic conditions.

11:00 am

Break

11:15 am

Samy Danial (Jumi Shin)

The Intrinsically Disordered Loop of USF-1 modulates DNA binding Specificity in a Gene Involved in Asthma

Upstream Stimulatory Factor 1 is a transcription factor within the ubiquitous b-HLH-Z family that binds to the E-box motif 5' CACGTG to regulate genes involved in a diverse set of biological processes. Of the many genes that are regulated by this transcription factor, USF1 has also been implicated in the transcriptional regulation of plasminogen activator inhibitor 1 (PAI-1), a gene that has been linked to airway remodelling in asthma when abnormally expressed in high levels. Previously, work has been done to identify the factors that influence the level of PAI-1 expression. These same studies have identified polymorphisms flanking the E-box element that is specifically responsible for regulating the PAI-1 gene. Specifically, researchers have found that USF-1 binds to this core E-box motif with higher affinity when there are four guanine nucleotides directly flanking 3' side (4G E-box) compared to five guanine nucleotides downstream (5G E-box). As a result, PAI-1 expression is increased with the 4G E-box compared to the 5G E-box. The first part of this report aims to examine why USF1 is able to bind with higher affinity towards the 4G E-box allele. Based on examination of the crystal structure of USF1 complexed with its E-box motif, it was observed that USF1 has a long disordered loop which allows it to make contacts with nucleotides flanking the 3' end of the E-box motif. Specifically, residues S233 and T234 are seen making contacts with the phosphodiester backbone where the 4G/5G polymorphism occurs. Thus, it was hypothesized that the long intrinsically disordered loop of USF1 is crucial in order to differentiate between the 4G/5G E-box. To test this hypothesis, domain swap experiments were performed in which the USF1 loop was added onto Max. Max is another bHLHZ protein that is hypothesized to not differentiate between the 4G E-box and 5G E-box due to its short intrinsically disordered loop. Utilizing mobility shift assays and bacterial hybrid assays, it was found that native Max was unable to differentiate between the 4G E-box and 5G E-box. However, replacement of the Max loop with the USF1 loop resulted in Max being able to distinguish between the 4G E-box and 5G E-box. This result highlights that the intrinsically disordered loop of USF1 is indeed necessary in order for USF1 to distinguish the 4G/5G E-box polymorphism. To characterize the residues responsible for its 4G/5G E-box differentiation ability, S233A and T234A site directed mutations were performed on the intrinsically disordered loop of USF1. It was found that these mutations diminished the ability of USF1 to differentiate between the 4G E-box and 5G E-box. Taken as a whole, these results provide insight into how these family of transcription factors are capable of targeting only certain E-box motifs and thereby regulate different gene sets despite all having the same 5' CANNTG binding preference. Moreover, because this PAI-1 driven hereditary form of asthma is common among people with asthma, the second portion of this paper will be devoted to discussing potential therapeutic strategies for targeting this form of asthma.

10:45 am

Anamika Bhattacharjee (Robert Gerlai)

Acute Embryonic Ethanol Exposure in Zebrafish (*D. rerio*): A Longitudinal Analysis

Alcohol consumption and abuse are pervasive issues worldwide. Alcohol's impact on brain development and other physiological processes are not well understood due to its complex pharmacology. Consumption of alcohol during pregnancy can lead to Fetal Alcohol Spectrum Disorder (FASD), involving dysfunction of the central nervous system, growth deficiencies, facial abnormalities and behavioral disruptions. The phenotype of FASD is variable and highly dependent on the concentration of ethanol ingested, time of exposure, genotype and frequency of consumption. FASD is a preventable mental disorder if pregnant mothers avoid alcohol consumption during pregnancy. In a clinical setting, diagnosing and treating FASD is problematic because mechanism surrounding alcohol's teratogenous effects are not well understood. Animal models, such as zebrafish, can be utilized to explore alcohol's effect on psychological and biological mechanisms. In this project, we aim to classify behavior in quasi-inbred AB derivative strain of Zebrafish embryos exposed at 24-hours post-fertilization to either 0% or 1% (vol/vol) ethanol for 2 hours. Behavior was tested using an open field behavioral testing assay at three key developmental time points: 7-9 days post-fertilization (dpf), 30-32 dpf, and 120 dpf. Subjects were tested for motor function and anxiety-like responses. We found no significance of alcohol tested at the developmental stages we chose, which is contradictory to prior findings. We noticed, however, a high mortality rate in both control and alcohol exposed fish due to suboptimal rearing conditions. We hypothesize that adverse environmental variables made detection of alcohol effects difficult. We have made numerous changes in our rearing and maintenance methods and currently are testing fish that were reared more optimally.

Keywords: Fetal Alcohol Spectrum Disorder (FASD), Zebrafish, *Danio rerio*, alcohol, embryonic development, ethanol, ethanol exposure, behavior

11:45 am

Fabrizo Angeles (Patrick Gunning)

Crystallization and X-Ray Diffraction of HDAC8

Overexpression of HDAC8 has been observed in numerous human cancer types which has made HDAC8 a promising oncological target for pharmacological drug development. Current FDA-approved HDAC inhibitors can non-specifically bind to HDAC8 as well as other HDACs due to structural similarity. Development of HDAC8 selective inhibitors requires structural and functional details regarding the HDAC8-specific binding pocket which can be obtained through crystallization and x-ray diffraction of HDAC8. Purified HDAC8 was screened against 192 crystallization conditions previously prepared. The sitting drop technique was used on 96-well plates with drops per well (HDAC8 apo form and HDAC8 inhibitor-bound form). Optimization plates used hanging drop technique on 24-well plates. Monitoring plates daily for the first week then once a week thereafter lead to obtaining small crystals and optimization trials obtained slightly better defined crystals. HDAC8 continues to be elusive through its oddly specific crystallization conditions and the seemingly low quality crystals that arise. High quality crystals could offer valuable insight into the 3D structure of the protein. More importantly, high quality crystals of drug-bound HDAC8 can lead to structural characterizations of protein-drug interactions which are essential for rational drug design.