Session C Abstracts

Effects of Autism Spectrum Disorder-Associated Mutations on Endosomal Sorting Functions of p97
Bertha Mireles
Mentors: Tsui-Fen Chou and Mengcheng "Windy" Wu

Ubiquitous ATPase p97, also known as valosin-containing protein, is involved in the regulation of a wide range of integral cellular functions, including the regulation of signal pathways and proteostasis. Due to its involvement in the governing of proteostasis, mutations in p97 are directly linked to a variety of neurological diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia. One aspect of proteostasis regulated by p97 is endosomal sorting, a step in the endosomal pathway involving endocytic sorting of lipids and receptors. Previous studies have determined the endosomal pathway is crucial for adequate neural function and linked its dysregulation to autism spectrum disorder (ASD), which is characterized by repetitious sensorimotor behavior and social communication impairment. In this study, I use liquid chromatography mass spectrometry and proteomic analysis to investigate the effect of ASD-associated mutations in p97 on binding of relevant cofactors involved in endosomal sorting regulation. This investigation allows for better insight into the link between p97, endosomal sorting dysfunction, and the genetic etiology of ASD.

Analysis of the Relative Importance of *P. synxantha*'s Gluconate Pathway in Solubilizing Phosphate Minerals
Chris Pope
Mentors: Dianne Newman and Nate Glasser

Overfertilization is a global problem in agriculture. This is especially true for phosphorus, as it is readily consumed by bacteria in the soil, leading to frequent overcompensation in fertilization. An important example of this is the mobilization of phosphate in the soil by the genus *Pseudomonas* in wheat rhizospheres. Understanding more about how much phosphate these bacteria use and how important different gene pathways are to solubilizing said phosphate would help inform more responsible fertilization. To understand more about how a species of *Pseudomonas*, *P. synxantha*, uptakes phosphorus, a genetic approach was used to remove the glucose to gluconate pathway, an important cellular tool for solubilizing calcium phosphate. The mutant was then introduced to soil and plant root environments and the relative impact of the gluconate mutants on the bioavailable phosphate was quantified and compared to wildtype strains of *P. synxantha*. The gluconate mutants were also compared to mutants in the phenazine biosynthesis pathway, another cellular method for solubilizing phosphate minerals that targets iron phosphate in soil. The relative contributions of the phenazine and gluconate pathways for solubilizing phosphorus in the wheat plant rhizosphere were determined.

Denoising Three-Dimensional Bacterial Biofilm Images Using Machine Learning
Mia Mutadich
Mentors: Dianne K. Newman and Georgia R. Squyres

Over the last two decades, the development of high-resolution, three-dimensional imaging has been instrumental in unveiling the structural and dynamical complexities of biofilms, enabling researchers to better understand their development and functions. However, direct observation of individual cell behavior within biofilms has been limited by the low signal-to-noise (SNR) of fluorescence microscopy images. We are applying deep neural networks for image restoration to three-dimensional images of biofilms to reduce noise without blurring the underlying signal – a limitation of previous techniques that I aim to overcome. To develop a neural network for this task, we started with a simpler 2D data set with a known ground truth. Because SNR in 3D biofilms varies with depth, we represented this by acquiring 2D images at various SNR levels. We trained a number of existing neural networks architectures on these images and wrote an algorithm to compare their performance, selecting one that performed best on the full range of representative SNR values and optimizing it accordingly. Finally, after developing a method to simulate noise in three-dimensional biofilm images, we aim to be able to retrain our optimized neural network on our semi-synthetic dataset so that it can denoise three-dimensional images.

Deciphering the Components of *Pseudomonas aeruginosa*'s Response to Nitric Oxide
Emma Isella
Mentors: Dianne Newman and Zach Lonergan

Bacterial infections are of great concern to public health. Some pathogenic bacteria have developed ways of evading the hostile environment of the host's immune system, enabling them to establish chronic infections. One such bacterial species is *Pseudomonas aeruginosa*, which has evolved mechanisms to survive in the presence of nitric oxide (NO), a highly reactive and toxic molecule produced by host immune cells to dispel pathogens. This project aims to discover and characterize novel genes that may be responsible for the bacteria's ability to persist in NO-rich conditions. To do this, we screened a subset of a non-redundant transposon mutant library to find strains that have reduced growth in the presence of NO. Based on their growth characteristics, we compiled a list of genes whose disruption sensitized the bacteria to NO. Bioinformatic methods and secondary screens were employed to

characterize the functions of these genes, including their role in other behaviors such as biofilm formation or response to other stressors, such as iron-limitation or antibiotic exposure. We also created and tested clean deletions of several genes either known or suspected to be involved in the bacterium's NO survival mechanisms using the same methodology. This analysis develops a more comprehensive molecular understanding of components involved in *P. aeruginosa*'s evasion of one of the most important antimicrobial metabolites produced by the host immune system, laying the groundwork for future studies on the specific functions of our flagged genes. Ultimately, this work may inform the development of therapeutics to help combat bacterial chronic infections.

Connectomics Approach Predicts Muscle Activity in *Drosophila* Flight
Deven Ayambem
Mentors: Michael Dickinson and Ivo Ros

During locomotion, the brain transforms sensory and internally generated information into a cohesive set of commands that generate motor output. The architectural logic of connections between networks of neurons in the brain and motor neurons that drive behaviors is an important step in understanding fundamental principles that underlie the actions we humans perform on a day-to-day basis. Using an animal model system such as the fruit fly allows for rigorous study of stereotyped behaviors. For example, when searching their environment, flying flies execute a series of straight flight segments interspersed with rapid turns called flight saccades(Collet and Land, 1975). This work will expand on a Dickinson lab discovery of a sparse network consisting of four descending command neurons that generates these flight saccades. The network consists of two, mirror-symmetric couplets, one for right turns and one for left turns, each containing one excitatory neuron (DNa0X) and one inhibitory (DNb01) that project to the ventral nerve cord. My project maps the downstream connectivity of these descending neurons and predicts how these DNs engage the sparse set of the flies' 12 pairs of steering muscles to saccade. I am developing a principled and robust connectivity analysis to predict functional activation of steering muscles during flight saccades. The overall goal is to develop an analysis framework to characterize the functional organization of premotor circuits that transform descending control commands into concerted movement.

Utilizing Plasmid Machinery to Improve Syn61
Ella Holland
Mentors: Kaihang Wang, Jianyi Huang, and Jolena Zhou

Syn61 is a synthetic E. coli, created in Dr. Wang's previous research, which has a recoded genome with only 61 codons. This organism presents exciting potential for creativity in biological designs that take advantage of such a reprogramming. A current challenge of working with Syn61 in this manner is its relatively slow growth rate in comparison to its wild-type counterpart, MDS42. In order to create a variant with an improved growth rate, a conjugation plasmid is utilized to systematically replace segments of the Syn61 genome with MDS42 DNA from a donor library over multiple rounds of conjugation. This DNA is then integrated into Syn61 by recombineering, and the growth over time of the resulting clones is analyzed. Clones thus far have demonstrated potentially improved growth rate, and the project is moving forward with the goal of conjugating over shorter fragments of MDS42 DNA across multiple rounds to pinpoint a few recoded codons that could have caused the growth defect in Syn61. Additionally, to improve conjugation efficiency, the conjugation plasmid has been sequence optimized and cloned, and tested for conjugation efficiency.

Modulation of the *Drosophila* Microbiome With *Lactobacillus brevis* to Promote Tissue Regeneration
Christian Dimayuga
Mentors: Lea Goentoro and Judah Bates

Only some taxa have evolved the ability to regenerate whole limbs after amputation, and yet continued research by the Goentoro lab suggests that the ability of limb regeneration may be present across wider metazoan species. Regenerative response to injury is influenced by the immune, endocrine, and metabolic state of an organism. Preliminary findings in the lab suggests that the microbiome can influence limb regeneration in the fruit fly *Drosophila*, a genetic lab model used for its simplicity, scalability, and conserved molecular processes. In this project, I investigated how leveraging microbiota within the *Drosophila* gut might prevent typical muscle wasting upon limb amputation and promote a regenerative response. To do this, I exposed young adult flies to *Lactobacillus brevis*, a known gut symbiont in *Drosophila*. After 24 hours, I amputated each fly at the mid-tibia. Brightfield and confocal images taken at 21 days after amputation showed that flies fed *L. brevis* had higher instances of muscle tissue survival and recovery than untreated flies. Therefore, administering probiotics before injury helps muscle tissue survive injury better. My next goal is to characterize how *L. brevis* promotes muscle survival and regeneration using transcriptomic and metabolic modeling analysis. Findings from this research may eventually contribute to identifying new therapeutic avenues for trauma-induced muscle atrophy.

Decoding Transcriptional Regulation: Measuring RNA Interactions in the Nucleus
Tanvi Ganapathy
Mentors: Mitch Guttman and Andrew Perez

The 3-D structure of the nucleus as well as the interactions between nuclear nucleic acids and proteins is key to understanding how differential transcription leads to different cell types and states. Traditional methods, such as Chromatin Immunoprecipitation (ChIP), are limited to testing a single protein per experiment. Previous work includes designing experimental protocols, such as RNA/DNA SPRITE and ChIP-DIP, to multiplex the measurement of protein and nucleic acid interactions in the cell. These methods rely on building a barcode by attaching multiple tags to crosslinked molecules through the split-and-pool method which is then sequenced to match associated proteins and nucleic acids. Currently, these tags are attached to the end of DNA molecules, but unfortunately, is it more difficult and less accurate to use the same method for RNA. An alternate method is to use DNA probes to detect RNA and perform tag extension on the DNA probes using the established protocol. As such, here we present a proof-of-concept experiment using DNA probes designed with the OligoMiner method to show whether DNA probes can be used to detect the transcription level of mRNA and nascent RNA in K562 cells.

Developing an Inner Cell Mass Model Using Extended Pluripotent Stem Cells and Embryonic Stem Cells
Jolie Jones
Mentors: Magdalena Zernick-Goetz and Sergi Junyent

As cells continue to split after initial fertilization, they eventually begin to differentiate into three cell types: Epiblast cells that give rise to all the cells in the body, Primitive endoderm cells (PrE) that give rise to the yolk sack surrounding the organism, and Trophectoderm cells that become the placenta. The Inner Cell Mass (ICM) refers to the collection of Epiblast and PrE cells inside the trophectoderm layer. There is often difficulty in studying the development of the ICM because of this trophectoderm layer, creating the need for a model that mimics ICM development. Extended Pluripotent Stem Cells (EPSCs) were used to create this model by collecting small aggregates of them in cells numbers that resemble the ICM, as EPSCs have been shown to be capable of giving rise to both cell fates within the inner cell mass. Markers specific to PrE and Epiblast cells were used to visualize and justify the components of these models. The optimization of this model is ongoing as data and results are still being collected to verify this model.