Session B Abstracts

Understanding the Neural Circuits for Aggression: *Drosophila* Wing Threat Behavior
Mina Mandic
Mentors: David Anderson and Shuo Cao

Innate social behaviors involve complex and functionally relevant movements. However, it remains unclear how these actions are coordinated by the brain. Drosophila display "wing threat" behavior - a complex, multi-motor aggressive behavior, composed of four different actions: wing elevation, pump, turn, and charge. The Anderson lab has identified genetically labeled "AIP neurons" that specifically control wing threat display. To understand the aggression circuitry in the Drosophila brain, it is crucial to identify other connectome-derived neurons that are involved in wing threat and elucidate their connection to AIP neurons. Our methodology entails identifying split-gal4 drivers to label each downstream neuron of interest, followed by gain-of-function and epistasis behavior experiments. Gain-of-function results tell us which behaviors are caused by activation of the neuron of interest. Epistasis results determine whether the effects of AIP neurons are mediated by the inhibited neuron. Our preliminary findings indicate that the neuron CL139 plays a role in wing elevation, and DNp45 induces an augmented velocity response to optogenetic stimulation. Future investigations involve epistasis tests to discern which wing threat behavior DNp45 is responsible for. Additionally, we aim to classify other downstream neurons (DNg39, CL335, CL176) of AIP to gain further insights into the circuitry governing wing threat behavior.

Targeted Recombination in Active Populations of Photo-stimulated Neurons: Developing a Functional Label of Subcircuits Within an Aggression Locus
Mark J. Lewis
Mentors: David Anderson and Amit Vinograd

Targeted recombination in Active Populations (TRAP) is a genetic labelling technique used to fluorescently mark cells expressing activity-dependent $CreER^{T2}$ within a limited timeframe. Anderson et al. have identified and correlated a small population of neurons in the ventrolateral sublocus of the ventromedial hypothalamus (VMHvl) with aggressive behaviors in mice. Inducing aggression states in the presence of injectable tamoxifen results in distinct labelling of VMHvl neurons, as well as its upstream/downstream projections. Photo-stimulation uses optogenetic sensors and actuators to render a cell "active" for a highly controllable period of time. We present a novel combination of these methods to allow for the robust labelling of an aggression subcircuit in the VMHvl with minimal noise.

Investigating the Migration of p97 Into the Nucleus in K562 Cells Due to Treatment With H_2O_2
Berglind Bjarnadóttir
Mentors: Tsui-Fen Chou and Vivian Lai

VCP/p97 protein is an ATPase protein that is involved in various cellular processes, including protein degradation and DNA damage repair. Many mutations in p97 have been linked to several diseases, including cancer. We observed that p97 is more abundant in the nucleus of K562 leukemia cells treated with H_2O_2 than in untreated leukemia cells. However, it is unknown why it is more abundant or how it migrates into the nucleus. Therefore, we have tried to gain a better understanding of this by doing an immunoprecipitation for p97 with treated and untreated K562 cells and sent the result from that for further analysis using mass spectrometry. We are analyzing the data from the mass spectrometry looking for proteins that are upregulated in H_2O_2 treated cells and could be involved in transporting p97 into the nucleus. We are also currently checking one another cell line to see if the same increase of p97 happens in the nucleus under H_2O_2 treatment.

Proteomics Examination and Structural Elucidation and Phospholipase A2 Activating Protein Mutant and Interaction With Valosine-Containing Protein (P97)
Xuan Li
Mentors: Tsui-Fen Chou and Katelyn Radford

PLAA is a regulatory element that modulates phospholipase (specifically PLA2) and its disease mutant was linked to lethal neurodevelopmental disruption and autism spectrum disorder. This project seeks to uncover PLAA mutant structure as potential therapeutic target and further the understanding of PLAA regulated ubiquitination processes, which have been lowly characterized during mammalian neurodevelopmental processes. Additionally, proteomics examination will reveal binding confirmation PLAA and P97, a cancer therapeutic target valosine-containing protein, and trace out related co-factors. Lastly, Cryogenic Electron Microscopy and computationally assisted folding models was adopted to characterize the binding moiety between PLAA-P97 and mutant structure of PLAA.

Optimizing Small Molecule Mitophagy Modulators
Ian Horsburgh
Mentors: Tsui-Fen Chou and William Rosencrans

Macroautophagy, also referred to simply as autophagy, is a cellular system to degrade organelles or protein aggregates via the lysosome. The PINK1/Parkin pathway, which selectively targets damaged mitochondria for autophagy, is often mutated in neurodegenerative disease such as Parkinson's, causing a buildup of damaged mitochondria in neurons. Certain proteins are also known to be capable of initiating autophagy and activating them could help clear damaged mitochondria and thus improve neuronal health in patients with dysfunctional PINK1/Parkin. In this project, I use both computational and in vitro approaches to optimize small molecule binding efficiently to these proteins capable of inducing autophagy, thus enabling targeted degradation of damaged mitochondria.

Engineering Synthetic Allostery for Phosphorylation-Based Protein Circuits
Meryl Liu
Mentors: Michael B. Elowitz and Dongyang Li

Phosphorylation is a ubiquitous post-translational modification, encoding information reversibly and dynamically in cellular signaling pathways. Synthetic protein circuits can reprogram cellular behaviors to function as sensors, switches, and amplifiers. In contrast to protease-based designs, kinase-driven phosphorylation promises to enable fast, reversible protein circuits that can sense and respond to various inputs, including endogenous signaling states and environmental stimuli. Current approaches include split-protein reconstitution and domain insertion, which require extensive fine-tuning for targets while the repertoire of engineered kinases is limited. However, mutating negatively-charged allosteric hotspots to phosphorylatable residues in physically contiguous, co-evolving sectors has previously achieved successful rewiring of yeast MAPK pathways. Here, we extend this strategy to expand the protein circuit toolbox by engineering allosterically-controlled human kinases. We developed a computational pipeline to conduct statistical coupling analysis and identify sector-connected solvent-accessible D/E residues throughout a eukaryotic kinome-wide sequence alignment. We then adapted imaging-based kinase translocation reporters for high-throughput kinase activity profiling using flow cytometry. We are conducting an alanine scan to distinguish functionally-coupled candidate residues for introducing phosphorylation motifs of an input kinase. Our work provides insights into allostery design principles and opens up avenues for the systematic development of phosphorylation-based protein circuits with composable engineered mammalian kinases.

Profiling of Synthetic Transcription Factors to Uncover Design Principles of Combinatorial Gene Regulation
Emily Lin
Mentors: Michael Elowitz and Evan Mun

Multicellular complexity requires the cooperation of thousands of specialized cell states derived from a singular genomic template. Each cell state is characterized by its distinct combination of transcription factors (TFs) which uniquely regulate the genome for that state. While traditional approaches to understanding natural gene regulation demonstrate the necessity of many individual TFs, they lack fundamental models of how a set of TF binding sites proximal to a gene produce a precise regulatory outcome. To uncover the rules that could underly a fundamental model of *cis*-regulation, we utilized a synthetic TF design to modularly recruit combinations of transcriptional effector domains, which are the components of TFs that dictate how the TF impacts the transcriptional output of a gene it is recruited to. Specifically, we examined how TFs respond to a controlled perturbation of the positioning, co-recruitment, and timing of regulatory events. Our initial results quantified an efficacy decay that transcriptional effectors undergo over a shared length scale. Ongoing work intends to characterize synergistic and antagonistic transcriptional effector domain combinations. Our findings highlight the rules and limitations within eukaryotic *cis*-regulation, informing optimal design for synthetic regulatory schemes and providing the core of a fundamental model for predicting regulatory outcomes.

Querying Protein Interactions Software Package Using Eukaryotic Linear Motif Database
Chi Hoang
Mentors: Lior Pachter and Laura Luebbert

Proteins in a cell fulfill their function by interacting with other proteins. One example are transcription factors, which are proteins that regulate gene expression levels. Transcription factors regulate gene expression by binding to DNA regulatory proteins. Similar to a key and lock mechanism, proteins have specific interaction domains for different tasks, which can be recognized from their amino acid sequence. Many human diseases, including most cancers, are caused by mutations of protein interaction domains. Understanding how changes in amino acid sequences disrupt protein interactions is crucial for mapping the potential pathological effects and predicting the consequences of a mutation. Here, I introduce a new Python and command line program, called *gget elm*, which allows researchers to identify protein interaction domains directly from an amino acid sequence or UniProt ID by pulling information from the Eukaryotic Linear Motifs (ELM) database.

Assessing Environmental Factors on Preimplantation Development Using a Stem Cell Embryo Model
Christoph Markus Haefelfinger
Mentors: Magdalena Żernicka-Goetz and Sergi Junyent Espinosa

Human fertility is declining at a concerning pace. With maternal age at conception considered a driver of this trend, novel approaches for preserving maternal fertility are urgently required. Meanwhile, many environmental factors have been shown to impact female fertility, as well as development of the preimplantation embryo, leading to subfecundity. However, the morphological changes and mechanisms impacting early development remain to be uncovered. As access to human embryos is extremely limited, we chose the most extensively studied model organism for mammal development, the mouse embryo. Therefore, we engineered a model of mouse preimplantation embryos from stem cells and exposed them to various xenobiotics determined as relevant from a literature research. Then, embryos were fixed and analyzed using brightfield and immunofluorescence imaging. Treatment with toxins resulted in disruption of several features of normal embryo development. The addition of nutritious compounds however, lead to accelerated development and favorable morphological features. Our study provides a proof-of-concept and framework for future high-throughput studies using human stem cell derived embryo models. Subsequent research could yield unprecedented insights and guidelines for the preservation of human fertility and drug testing.

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.