

## Session A Abstracts

### **Serotonin dynamics in the VMHvl Esr1 during social interactions**

Zara H. Kanold-Tso

*Mentors: David J. Anderson, Amit Vinograd, and Takumi Itakura*

Serotonin (5-HT) is a neuromodulator which mediates emotional states like fear, safety, and aggression. However the real time dynamics of 5-HT during social behaviors are unknown. Aggression is a highly conserved state that is promoted by estrogen receptor type 1+ (Esr1+) neurons in the ventrolateral part of the ventromedial hypothalamus (VMHvl). Therefore, we did fiber photometry recording of 5-HT dynamics in VMHvl Esr1+ neurons with GRAB\_5HT sensor during social interaction under different social experiences. Using Behavior Ensemble and Neural Trajectory Observatory (BENTO) and deep-learning programs like Deep Action, we correlated 5-HT traces with behavioral data. We found transient and persistent time locked decreases in 5-HT when intruders entered a resident's cage and when resident mice attacked the intruder. These decreases may be related to social fear responses or defensive states in response to the intruder. Further experimentation and social situations are needed to validate these traces and elucidate the meaning of the decreases.

### **Developing a spike S2 domain-based pan-coronavirus vaccine**

Emma L. Titus

*Mentors: Pamela J. Bjorkman and Chengcheng Fan*

Many SARS-CoV-2 and MERS neutralizing antibodies target the spike protein receptor-binding domain, which is subject to frequent mutations, necessitating continual vaccine updates. To overcome this limitation, we are developing a vaccine designed to elicit antibodies that recognize the more conserved S2 domain of the spike protein thereby achieving broader protection. From mice that were immunized with our vaccine candidate, we isolated sequences for over 200 monoclonal antibodies (mAbs) and cloned 27 into expression plasmids. We first evaluated the binding of these 27 mAbs using transfection supernatants to SARS-CoV-2 and MERS spike proteins and S2 domains using an Enzyme-Linked Immunosorbent Assay (ELISA) and found that 19 mAbs showed binding to one or more of the SARS-CoV-2 or MERS constructs. These 19 mAbs were expressed and purified at a larger scale and tested for binding by ELISA at different concentrations. In this ELISA, 8 mAbs showed strong binding to SARS-CoV-2 spike and S2 domain, 5 mAbs showed strong binding to MERS spike and S2 domain, the remaining mAbs showed weak or no binding. Cross-reactive mAbs with strong binding are promising candidates for neutralization and may also target the conserved regions of the spike protein, which will be further studied.

### **Development of mosaic nanoparticle vaccine candidates to protect against diverse sarbecovirus strains**

Indeever Madireddy

*Mentors: Pamela J. Bjorkman and Alexander Cohen*

Global human health is threatened by potential spillovers of zoonotic SARS-like betacoronaviruses (sarbecoviruses). Two well-known sarbecoviruses are SARS-1 and SARS-CoV-2 which were responsible for the 2003 SARS-1 outbreak and the COVID-19 pandemic, respectively. Due to the danger that sarbecoviruses pose to human health, it is pertinent to develop a pan-sarbecovirus vaccine that confers protection against both evolving SARS-CoV-2 variants and any future zoonotic spillovers. Recent work has shown that a mosaic-8 receptor binding domain (RBD) nanoparticle that co-displays 8 distinct RBDs from different viruses can generate cross-reactive and cross-neutralizing antibody responses that target conserved viral epitopes. Mosaic-8 also confers protection against mismatched strains that were not present on the particle suggesting its use as a broadly protective sarbecovirus vaccine.

To improve the responses elicited by mosaic-8, the antibody landscape elicited by a diverse set of sarbecoviruses was used to guide the development of 7 new mosaic RBD nanoparticles. Each displays a different combination of RBDs from different viral clades. The breadth and neutralization potency of the antibodies elicited by these vaccines is currently being characterized. These mosaic vaccine candidates will serve as an updated vaccine against all sarbecoviruses and future emerging variants of concern.

### **Design of synthetic caspase-based circuits for programmable cancer therapy**

Evan Z. Zhang

*Mentors: Michael B. Elowitz, Andrew Lu, and Lukas Moeller*

Therapeutic circuits represent a new potential cancer treatment modality that uses engineered proteins, delivered into cells as mRNA via lipid nanoparticles, to detect if a cell is cancerous and trigger programmed cell death in response. Recent work has shown that engineered protein circuits targeting oncogenic Ras mutants can potently kill Ras mutant cancer cells, with little off-target effect on Ras wild-type cells. However, the current generation of these circuits contain a viral protease-based sensor module, which may raise the risk of a host immune response against the viral components of the circuit itself. Here, we present a remodeled version of the circuit that uses a single human caspase-based module as both a sensor and cell death effector. We demonstrate that the new design maintains potent and specific killing of HEK293 cells with ectopically overexpressed mutant Ras. Overall, these results provide the groundwork for re-engineered circuit designs that preserve the advantages of therapeutic circuits while potentially minimizing immunogenicity.

### **Investigating the role of *Esrrb* in nephron development using zebrafish models**

Kara Z. Lo

*Mentors: Marianne Bronner and Tianli Qin*

The nephron is the functional unit of the kidney. It comprises a blood filter and an epithelial tubule with specialized segments that enable essential physiological functions, including metabolic waste excretion, hormone production, and maintenance of plasma homeostasis. Disruptions in nephron development (nephrogenesis) can lead to nephron dysfunction or loss, contributing to renal disease and broader systemic effects. To investigate genetic regulation of nephron segment patterning, we used the zebrafish pronephros, a model that shares cellular and genetic conservation with the mammalian nephron. We identify and focus on the estrogen-related receptor beta (*Esrrb*) as a candidate regulator of cell fate and patterning of distal segments. Mutants from CRISPR/Cas9-mediated knockouts were analyzed using in situ hybridization chain reactions (HCR). Preliminary findings reveal abnormal pronephric tubule morphology, altered expression patterns of segment-specific markers, and the presence of pericardial edema, suggesting compromised osmoregulatory function.

### **Characterizing sacral-derived progenitors of the developing enteric nervous system**

Mandoline H. Nguyen

*Mentors: Marianne Bronner and Jessica Jacobs-Li*

Commonly referred to as the “second brain”, the enteric nervous system (ENS) consists of neurons and glia which regulate gastrointestinal function. The ENS is derived from two distinct subpopulations of the neural crest, the vagal and sacral. While both populations contribute to the ENS of the post-umbilical intestine, their differences remain poorly characterized. Our lab’s single-cell RNA sequencing of post-umbilical sacral neural crest-derived cells revealed an absence of a progenitor cluster in contrast to vagal-derived populations. Despite lacking this progenitor pool, the sacral crest still gives rise to neurons and glia indicating differences in developmental trajectory and timing. These findings, along with prior studies showing that mature enteric glia can revert to a progenitor state, suggest that sacral-derived glia may serve as a neuronal progenitor pool. We hypothesize that sacral-derived

progenitors exhibit a non-canonical state where neuronal, glial, and neural crest markers are co-expressed. To test our hypothesis, we performed indirect antibody staining on transverse sections of the chicken gastrointestinal tract across developmental stages. Additionally, we utilized DiI-mediated lineage labeling of the premigratory sacral neural crest to identify primitive sacral structures and assess co-localization of progenitor and glial markers at earlier stages of development.

### **Developing an approach to assess early misfolding events in Huntington's Disease**

Jaidyn M. Woods

*Mentors: Judith Frydman, Rebecca M. Voorhees, Ivana Bukvin, and Korbin Kleczko*

Huntington's Disease (HD) is a devastating neurodegenerative disorder characterized by the expansion of a CAG repeat (>35) in exon 1 of the Huntington gene (HTT). Aggregation and misfolding of the mutant protein are hallmarks of the disease, although the precise drivers of cellular pathology are unknown. Here, the role of the N17 domain of HTT in early misfolding was investigated. This domain emerges first during translation and has been suggested to initiate HTT misfolding and early oligomer formation. To explore it, the antibody fragment VL12.3, which selectively recognizes the N17 domain, was purified and expressed, allowing for the selective pull-down and purification of ribosomes translating the HTT protein. Furthermore, to structurally characterize misfolding events, 70S ribosomes with an extended 44 helix in their 16S rRNA were purified, allowing for the hybridization of biotinylated oligonucleotides to immobilize them. These immobilized ribosomes will be used to produce ribosome nascent chain complexes (RNCS), in which the conformations explored by the emerging nascent chain can be mapped using single-molecule Forster Resonance Energy Transfer (smFRET). This will characterize the earliest steps in HTT misfolding, potentially uncovering novel targets for therapeutic intervention in HD.

### **Simultaneous mapping of multiway interactions: Understanding the mechanisms of enhancer-promoter interactions through SPRITE data**

Maya C. Yie

*Mentors: Mitchell Guttman, Allen W. Chen, and Isabel N. Goronzy*

DNA, along with RNA and protein, is organized into complex three-dimensional (3D) structures in the nucleus of eukaryotic cells. In particular, enhancer-promoter (E-P) interactions, the contact between the region of a gene where transcription initiates (P, promoter) and a cis-acting region of DNA (E, enhancer), have been shown to be important in regulating gene expression. However, much is still unknown about E-P interactions. Current methods, such as Hi-C, can only map pairwise interactions. To observe whether multiple enhancers and promoters interact simultaneously and in what combinations, we used RNA and DNA Split-Pool Recognition of Interactions by Tag Extension (RD-SPRITE), a method recently developed in the lab that can track genome-wide multiway, long-distance interactions between RNA/RNA, RNA/DNA, and DNA/DNA. We developed an analysis pipeline that identifies enhancers and promoters and determines multiway E-P interactions within the SPRITE dataset. Our results show that simultaneous E-P hubs appear to exist across the genome, with the majority of the multiway interactions being hubs containing one promoter and multiple enhancers. Understanding how enhancers and promoters interact provides valuable insight into gene regulation and its impact on critical cellular processes, including cell fate and other factors in disease.

### **Analytical approaches for multi-modal proteomics for eye disease**

Architesh B. Prasad

*Mentors: Vinit Mahajan and Michael A. Vivic*

Previous work in the Mahajan Lab at Stanford has leveraged proteomics based assays to achieve protein expression analyses from both human and mouse eyes for various diseases. This SURF project utilized techniques in bioinformatics and machine learning to gain insights in these data and guide further experimental work. These datasets considered multiple diseases of the eye including retinal detachment, retinitis pigmentosa, age-related macular degeneration (AMD), and eye senescence. Results include analysis that found certain post-translational modifications were over-represented in extracellular matrix proteins with retinal detachment. In AMD, complement proteins were variably expressed for each disease stage, possibly explaining variable patient outcomes in recent clinical trials

that target different complement proteins in AMD. Other datasets, including one for eye fluid metabolomics data, were compared with previous work to provide justification for expanding sample set sizes in future validation studies. In new applications for this proteomic data, machine learning studies were initiated to construct models for protein diagnostic biomarkers and proteomic clocks.