Caltech
Student-Faculty Programs

Session A Abstracts

Identifying the circuit for odor-induced visual valence in *Drosophila*
Ava O. Barbano
Mentors: Michael H. Dickinson and Ivo Ros

Multimodal sensory integration is essential for adaptive behavior, yet the neural circuits that mediate cross-modal interactions remain poorly understood. When the fruit fly, *Drosophila melanogaster*, encounters an attractive odor during flight, its behavioral response to visual stimuli can switch from aversion to attraction. This odor-mediated switch in visual valence is also observed in *Aedes aegypti* mosquitoes, suggesting a conserved neural mechanism among dipterans. While the neural circuitry underlying olfactory processing and visual pathways in *Drosophila* are well characterized, far less is known about how olfactory signals modulate visuo-motor pathways. To address this, we measure visual attraction in a psychophysics arena that allows precise control over sensory input by recording behavioral output. We combine optogenetic activation of olfactory receptor neurons using the Q-system with targeted silencing of candidate visual-motor neurons via the orthogonal Gal4/UAS system. Through this genetic approach, coupling fictive odor presentation with neuronal silencing, we can determine the necessity of a candidate neuron to switch the valence of the stimulus response, thus changing the behavioral mode. This approach aims to probe the neural mechanism in the fly brain responsible for odor-mediated changes in stimulus response.

Evaluating and characterising the antibody response to mosaic RBD nanoparticles as novel vaccine candidates
Darshana Marathe
Mentors: Pamela J. Bjorkman, Alexander Cohen, and Jennifer R. Keeffe

Two sarbecoviruses (SARS-CoV and SARS-CoV-2) have been responsible for epidemics in humans in recent years, with SARS-CoV-2 causing the COVID-19 pandemic. The surface glycoprotein, Spike, is the most variable between related coronaviruses, undergoing rapid evolution on a comparable timescale as that of transmission events and ecological dynamics. Mutations in the receptor binding domain (RBD) of the spike protein can give rise to Variants of Concern (VOCs) which show increased transmissibility compared to the original strain and the potential to evade immune responses. The new mosaic nanoparticle vaccine candidates developed by the Bjorkman lab involve the display of multiple RBDs from different sarbecoviruses on a single nanoparticle, with the aim of stimulating a broader response than by a monovalent vaccine. These mosaic vaccines can provide protection against more distantly related SARS-CoV-2 VOCs and importantly against a potential zoonotic spillover event for an as-yet-unidentified animal sarbecovirus. My project involves cloning, expressing, and characterising monoclonal antibodies elicited in response to immunisation with such vaccines. This will ultimately ascertain whether the vaccine candidates effectively elicit monoclonal antibodies that exhibit broad binding to SARS-CoV-2 VOCs and animal sarbecoviruses, and retain broad neutralisation capacity, even against sarbecoviruses not represented by antigens on the vaccine.

Investigating the regenerative capacity of the craniofacial neural crest
Azucena K. Virgen
Mentors: Marianne Bronner and Tatiana Solovieva

The neural crest (NC) is a highly migratory and multipotent embryonic stem cell lineage that contributes to the development of the heart, craniofacial structures, peripheral nervous system, and more. NC disruptions in the chick embryo model can result in defects comparable to human developmental defects, like Treacher Collins and DiGeorge syndrome. Despite the prevalence of congenital craniofacial defects, previous literature suggests that surgically ablated NC populations can regenerate, depending on the timing and extent of surgical ablation. We aim to resolve the spatial and temporal regenerative capacity of the craniofacial NC to further investigate the cellular origin and mechanism of regeneration. We will use the chicken embryo as a model due to its similarity to early

human embryonic development and its accessibility to experimental perturbations. We hypothesize that craniofacial NC has a higher regenerative potential in earlier developmental stages, before migration into surrounding tissues. To test this, we will perform varying surgical ablations of cranial NC at different developmental stages. After ablation, we will use NC-specific antibodies to visualize any NC regeneration 3 to 18 hours post ablation. These findings will inform future studies to identify the cellular mechanism involved in NC regeneration and craniofacial development.

Neural crest cells in jaw regeneration
Pei-yu Kao
Mentors: Marianne Bronner and Miyuki Suzuki

Neural crest cells, which give rise to diverse cell types, are essential for craniofacial development and have been shown to contribute to organ regeneration, including the heart and digit tip. However, their contribution to organ regeneration is not well understood. Urodele amphibians exhibit extraordinary regenerative abilities, and recent findings have revealed upregulation of neural crest-related gene during newt limb regeneration, suggesting the critical role of neural crest cells in regeneration. Newt can also regenerate jaw, and since craniofacial tissues are largely neural crest-derived, the role of neural crest cells in regeneration is especially intriguing. Using Iberian ribbed newt (*Pleurodeles waltl*) as a model, we aim to characterize the role of neural crest cells in jaw regeneration. We examine neural crest cells through *in situ* hybridization chain reaction staining with neural crest cell markers such as *Sox10* and *P0*. We found *Sox10* expression throughout the entire jaw post-amputation. We also utilize live imaging in *Sox10*-Cre vector–injected into Cre responder transgenic newts to trace neural crest cells and assess their role in wound healing, dedifferentiation, proliferation, and redifferentiation during jaw regeneration. Our work may provide valuable insight into jaw regeneration, with potential application to improving regeneration methods in mammals and humans.

Understanding contributions of mitochondrial translocase of the outer membrane (TOM) to the integrated stress response under iron deficient conditions
Adeline L. Sun
Mentors: David C. Chan and Yogaditya Chakrabarty

Mitochondria play a dual role in maintaining cellular homeostasis. In response to stress, damaged mitochondria are removed through mitophagy, the selective degradation of damaged mitochondria by lysosome recruitment. In addition, mitochondria also activate global stress responses such as the integrated stress response (ISR). Activation of ISR leads to reduced protein synthesis and increased translation of transcription factors that aid cell survival and recovery. Recent findings suggest that the translocase of the outer mitochondrial membrane (TOM), best known for importing nuclear-encoded mitochondrial proteins, may also stabilize molecules that initiate mitophagy during stress conditions that activate the ISR. We aim to investigate whether TOM-associated proteins contribute to ISR activation. We are generating CRISPR-Cas9 interference knockdowns of TOM20, TOM22, and SAM50 in mammalian cells, treating these cells with stress-inducing agents such as the iron chelator DFP and the mitochondrial membrane depolarizer CCCP, and assessing ISR activation by measuring expression of canonical ISR markers ATF4 and phosphorylated-eIF2α. These experiments will help define the role of TOM in ISR under different stress conditions and offer insights into how mitochondria coordinate both internal quality control via mitophagy and cytosolic stress signaling.

Evaluation of the glycomic profile of p97 disease-associated mutants
Yun-Shan Chen
Mentors: Tsui-Fen Chou and Chia Yen Liew

Protein glycosylation is a fundamental post-translational modification that governs the protein folding machinery. Aberrant glycosylation leading to protein malfunction may contribute to endoplasmic reticulum (ER) stress and disrupt cellular homeostasis. The ubiquitous AAA+ ATPase p97/VCP is an essential protein that plays a central role in protein quality control, involved in diverse ubiquitin-associated pathways such as ER-associated degradation (ERAD) and chromatin-associated degradation. Pathogenic missense mutations in p97/VCP are implicated in rare diseases, multisystem proteinopathy-1 (MSP1), and intellectual disability & developmental delay (IDDD). Although the role of

p97/VCP in protein quality control is well-established, the impact of p97/VCP variants on protein glycosylation remains underexplored. This research employs cutting-edge mass spectrometry to investigate the glycoproteomic and *N*-linked glycan profiles in cellular models expressing p97/VCP variants, thereby advancing our understanding of p97-related disease mechanisms.

Effects of oxytocin receptor knockdown in cholecystokinin A receptor-expressing neurons in the ventromedial ventrolateral hypothalamus on female sexual learning and motivation in female mice

Raquel S. Schlichting
Mentors: David J. Anderson and Emma Boxer

Female sexual learning and motivation are regulated by complex interactions between neuropeptide and neurotransmitter signaling, yet the specific contributions of oxytocin remain unclear. To investigate this, we performed a targeted knockdown of the oxytocin receptor (OxtR-KD) in the ventrolateral subdivision of the ventromedial hypothalamus (VMHvl), focusing on neurons expressing the cholecystokinin A receptor (CCKar), which have been implicated in female sexual behavior. Using an adeno-associated virus (AAV) delivering CRISPR-Cas9, we bilaterally targeted VMHvl CCKar-expressing neurons in sexually naïve female mice (n = 8). Each of the experimental (n=4) and control (n=4) mice underwent two mating assays during proestrus, followed by a week of co-housing with males to increase sexual experience, then were reassessed. Behavioral interactions were annotated using Bento, a MATLAB-based analysis platform, and subsequently quantified. Preliminary data indicate that sexual receptivity was reduced in the OxtR-KD animals, as evidenced by higher rejection rates compared to controls. This suggests that oxytocin signaling in VMHvl CCKar neurons play a role in sexual behavior in female mice. Ongoing experiments will test these findings further by administering an oxytocin injection to wild-type females and performing additional mating assays.

Exploring and predicting the combinatorial effect of cytokines on downstream signaling

Natalie J. Lytell
Mentors: Michael B. Elowitz and Dayeon J. Shon

The JAK-STAT pathway is a crucial mediator of immune responses, translating extracellular cytokine signals into transcriptional programs that shape immune cell behaviors. Over 50 cytokines act through only four Janus kinases (JAKs) and seven signal transducer and activator of transcription (STAT) proteins, creating a many-to-many network where numerous ligands collaborate to influence a fine-tuned reaction carried out by a limited set of transcription factors. Within this network, STAT monomers compete to form DNA-binding homodimers and heterodimers that drive downstream gene expression programs. This STAT dimerization network thus acts as a key layer of signal integration, but it remains unclear how combinatorial cytokine inputs are encoded into specific STAT dimer distributions and how those distributions impact genome-wide target gene activation. The ability to understand and predict how these signaling molecules implement a precise downstream response is necessary for designing and implementing equally precise therapeutic strategies. To address these questions, we generated and characterized reporter cell lines that measure the activities of specific STAT dimers. By analyzing their responses to cytokine combinations, we aim to gain a clearer picture of how these signals are processed by the STAT dimerization network.

Metabolic contributions of *Lactobacillus brevis* ATCC 367 in adult *Drosophila melanogaster*

Hanna K. Diop
Mentors: Lea A. Goentoro and Judah Bates

This project investigates how the gut bacterium *Lactobacillus brevis* can alter the metabolic profile in *Drosophila melanogaster*. Previous work in the Goentoro lab showed that a combination of leucine, insulin, and glutamine induces partial tibia regrowth in adult flies, which normally do not regenerate. Building on this finding, we focus on *Lactobacillus brevis* ATCC 367 (Lb6). *L. brevis* is heterofermentative, so it produces lactate and acetate. The Lb6 strain also expresses the arginine deiminase (ADI) pathway and secretes ornithine, an amino acid potentially linked to increased protein synthesis and therefore, growth. Since *Drosophila* has low baseline levels of ornithine, we hypothesize that *Lb6* supplementation could increase ornithine levels, signal nitrogen availability to the fly, and

support cell growth. To test this, I am conducting ornithine and acetate assays to quantify metabolite levels in flies treated with *L. brevis* versus untreated controls. This work contributes to understanding how microbe-derived nutrients may support regenerative responses to injury in non-regenerating host models.

Engineering a non-immunogenic drug-inducible vector system for self-replicating RNA vaccine delivery
Ameerah O. Saliu
Mentors: Bruce A. Hay and Thomas A. Adamo-Schmidt

Self-replicating RNA represents a promising platform for the next generation of vaccines. Most currently available mRNA vaccines (such as those used in the SARS-CoV 2 pandemic) utilize an mRNA encoding the target antigen. With these, the lifetime of antigen expression is determined by that of the mRNA. As a result, the dose of antigen may be sub-optimal, representing a limiting factor for inducing a robust immune response. In contrast to simple mRNA vaccines, there has been recent interest in generating self-replicating vaccines, which are able to generate a more significant immune response despite a lower initial titre. Of course, this system carries an obvious drawback, in that it has the potential to allow unregulated replication in host cells, which could have serious health repercussions. To address this, we aim to build a drug inducible, evolutionarily resilient polymerase control switch by which self-amplifying and self-sustaining RNA production can be turned on or off.

In this preliminary investigation, I am using an in vitro assay to assess the safety and efficacy of this novel vector system. Using fluorescence as a marker of replicative activity, I am first testing our ability to achieve replicon amplification only in the presence of an inducer of heterodimerization that brings two otherwise inactive fragments of the RNA-dependent RNA polymerase (RdRp) together. The sensitivity of this system is being assessed through dose-response analysis to identify optimal conditions for replication. In parallel, a second goal I am pursuing is developing a non-immunogenic packaging strategy in which the replicon RNA is linked to immunologically silent membrane proteins to facilitate extracellular vesicle-mediated transfer to neighbouring cells, thereby amplifying antigen production without eliciting anti-vector immunity. Together, these approaches provide the foundations for the use of controllable self-amplifying RNA delivery system to produce more robust vaccine responses and improve patient outcomes.

A DNA-based linear classifier for sequential analog signals
Xiaorui Shi
Mentors: Lulu Qian and Matthew Plazola

Compared to conventional electronic computing, molecular computation offers superior energy efficiency, intrinsic parallelism, and enhanced biocompatibility. Since biomolecules natively exist in living organisms, molecular computing has the potential to enable in vivo intelligent medical diagnosis and therapy. Furthermore, biomolecular concentrations can represent continuous variables, overcoming the inherent binary limitations of electronic computers. In molecular computing, DNA-based neural networks utilize molecular hybridization and strand displacement reactions to emulate neuronal operations, functioning autonomously in bodily fluids or cells without external power sources. This provides inherent advantages for biomolecular diagnostics, such as cancer biomarker classification and concentration analysis.

Most prior DNA computing studies have focused on binary encoding of concentrations (high = logic ‘1’, low = logic ‘0’), with limited exploration of direct analog signal processing. However, analog signal classification is critical for medical diagnostics. Additionally, the irreversible nature of DNA hybridization and strand displacement reactions typically restricts constructed neural networks to single-use operation. Recent advances include the development of DNA-based variable-gain amplifiers that process concentration-dependent analog signals, and the demonstration of thermal energy as a universal reset mechanism for DNA neural networks. These networks maintain stable performance across multiple cycles, enabling iterative computation and unsupervised learning.

In this project, we designed and experimentally realized a reusable DNA-based analog linear classifier. This system processes DNA concentrations as continuous variables through amplification and summation operations, with thermal resetting (heating/cooling cycles) allowing repeated computations for sequential inputs. Our work establishes a foundational step toward DNA computing systems capable of processing time-varying analog signals and adaptive learning.