Caltech
Student-Faculty Programs

Session B Abstracts

Mathematical models of flexible neuronal participation
Christopher H. Leung
Mentor: Carlos Lois

The mechanisms by which the brain maintains a stable output as the upstream neuronal representation changes remain incompletely understood. The HVC nucleus of the zebra finch song motor circuit demonstrates an ability to restore the original birdsong after lesioning, even though neurons participate stochastically in song generation. We jointly model the song's acoustic features with neuronal calcium imaging on the song syllable scale. Using a hierarchical Bayesian model, we confirm the role of song syntax in neuronal participation. We then statistically decode the syllables to quantify the stability of the neuronal representation even as indivaidual neurons' participations shift after lesion. Finally, we adopt an Ising model to distinguish between network redundancy and rewiring. Our data suggest multiple solutions to the reliability-flexibility tradeoff in motor circuits: the unreliable participation of neurons may provide a flexible manifold for both learning and recovery

Adaptation of sympathetic nervous system in pregnancy
Reina E. Li
Mentors: Yuki Oka and Wongyo Jung

The sympathetic nervous system is a part of the body's autonomous nervous system and plays a critical role in maintaining homeostasis, including the regulation of blood pressure, heart rate, and blood glucose. Pregnancy establishes a new baseline for these physiological parameters by recalibrating the body–brain axis. In this project, we compare sympathetic nerve innervation by performing immunohistochemistry staining on the kidney, pancreas, and duodenum of virgin, pregnant, and lactating mice. Using ImageJ, we obtain area fractions between virgin, pregnant, and lactating kidneys and observe a significant difference between virgin and pregnant area fractions and no significant differences elsewhere. These results are interesting because it implies that sympathetic nervous system activity is elevated specifically during pregnancy, and has interesting implications for diseases that occur during pregnancy such as gestational diabetes. However, our data is still limited and more is needed before fully coming to a conclusion.

Adapting pharmaceuticals for *in vivo* manipulations of rove beetle chemoreceptors
Y Lam Voong
Mentors: Joseph Parker and Hayley Smihula

Manipulating chemoreceptors via pharmaceutical methods can enhance studies on chemosensory contributions to animal behavior. Here, we aim to adapt pharmaceuticals for *in vivo* use in rove beetles, a family of interest due to many species having evolved socially parasitic relationships with ants. Our goal is to establish protocols for treating adult beetles and manipulating TRPA1 and Orco chemoreceptors in *Dalotia coriaria*. We study HC-030031 and VUAA1, compounds that target TRPA1 and Orco, respectively. These chemicals can be delivered via injection, volatilized exposure, topical and oral application. We performed a series of titrations to determine the necessary combination of delivery method, concentration, and persistence time that can disrupt receptor functioning during behavioral assays. Previously established TRPA1 *Drosophila melanogaster* and Orco *Dalotia* mutants were used to evaluate the efficacy of behavior assays for determining successful HC-030031 and VUAA1 treatments. We improved delivery parameters to prevent toxicity that can interrupt behaviors in biological assays. In the future, we hope to translate these protocols to the myrmecophilous rove beetle *Sceptobius lativentris*, which is less genetically tractible due to its lifestyle living inside of ant colonies. Our research on this species would therefore benefit from having a non-genetic method for disrupting chemoreceptor function.

Establishing genetic methods for chemoreceptor manipulation in rove beetles
Conner I. Sakae
Mentors: Joseph Parker and Hayley Smihula

Pasadena, California is home to *Sceptobius lativentris*, a rove beetle species and obligate symbiont of the ant *Liometopum occidentale*. *Sceptobius'* lifestyle stands in stark contrast to most rove beetles, such as *Dalotia coriaria*, free-living generalists that are prey to ants. We wish to understand how chemosensory systems for ant detection vary between these two beetles. By applying genetic manipulations with RNAi and CRISPR, we disrupted the function of two chemoreceptors involved in pheromone sensing, TRPA1 and Orco, in *Dalotia* and *Sceptobius*. We created and tested RNAi constructs to knockdown TRPA1 in both species, utilized an existing *Dalotia*-Orco CRISPR mutant and have begun validations on a *Dalotia*-TRPA1 CRISPR line. High throughput assays were developed to assess receptor disruption which were subsequently validated with more comprehensive behavioral analysis. Control odors were screened with the goal of producing a consistent behavioral assay that could evaluate the success of genetic manipulations on the phenotypic level. With genetic tools and control assays that can successfully alter and assess chemoreceptor function, we can apply these methods to future work regarding the detection and behavioral response to ant pheromone compounds as a part of ongoing research into the evolution of *Sceptobius'* myrmecophilic lifestyle.

Chromatin accessibility and cofactor rules predict Runt regulatory behavior of the upstream *net* enhancer
Jayden D. Lee
Mentors: Angelike Stathopoulos and Isaryhia M. Rodriguez

Runt is a transcription factor most studied for its role as a segmentation gene critical to *Drosophila melanogaster* development. A few well-studied segmentation genes establish Runt as both a repressor and activator, and indicate this is influenced in part by cofactor presence. However, little is known about Runt regulation across the genome outside of these genes, as well as what rules govern Runt's context-dependent regulatory behavior. Thus, we are interested in whether genomic data and cofactor context can predict Runt site-specific behavior. To answer these questions, we leveraged ATACseq data showing chromatin accessibility differences between Runt mutant and wildtype embryos as well as ChIPseq data showing Runt DNA occupancy to support prediction of genes likely to be directly bound and potentially regulated by Runt. We then used *in situ* hybridization of enhancer-reporter transgenic lines to functionally validate our predictions. Finally, we assessed the genome-wide extent of Runt regulatory behavior and how it is affected by known cofactors. Here we show that genomic data predict Runt activating behavior at the upstream enhancer of *net*. These results support that chromatin accessibility and cofactor context may provide predictive signatures of Runt regulatory behavior at previously uncharacterized loci.

CellSAM3: A promptable foundation model for cell segmentation and tracking
Edmund Tsou
Mentors: David A. Van Valen and Ross Barnowski

Cells are a fundamental unit of biological organization, and most quantitative cellular experiments begin with cell segmentation to identify individual cells in imaging data. Accurate segmentation is essential because morphology, expression, and dynamics are measured per cell, so errors propagate downstream. Although deep learning has enabled generalist models that segment cells accurately across imaging domains, these models provide limited support for user-guided correction at inference time. Here, we present CellSAM3, a promptable foundation model for cell segmentation. CellSAM3 builds on the Segment Anything Model 3 (SAM 3) by jointly fine-tuning a shared backbone for automatic cell detection and promptable segmentation using 49 public datasets spanning fluorescence microscopy, phase-contrast microscopy, and histopathology. We show that a single model can automatically segment all cells in a field of view, respond to point and box prompts, and produce nuclear or whole-cell masks as specified by a text prompt. CellSAM3 outperforms all evaluated generalist models on an internal benchmark and matches the leading method on the held-out NeurIPS 2022 Cell Segmentation Challenge. We adapt CellSAM3 to time-lapse imaging using SAM 3's memory-based video tracker, which preserves cell identities across frames. These results establish CellSAM3 as a unified framework for automatic, interactive, and temporal segmentation.

TMED8 selectively promotes kappa opioid receptor biogenesis
Zhenxi Yuan
Mentors: Rebecca M. Voorhees and Aline Milach Teixeira

Kappa opioid receptor OPRK1 (KOR) is a Class A G-protein-coupled receptor (GPCR) involved in mood, anxiety, and addiction. While much attention has focused on how KOR responds to endogenous ligands and drugs, less is known about how cells regulate whether newly synthesized KORs reach the cell surface in the first place. Understanding the biogenesis factors that control KOR abundance could reveal how cells tune receptor availability before signaling and may eventually suggest new strategies for modulating opioid receptor activity in disease. Through a CRISPRi screen for regulators of KOR total abundance in K562 cells, we identified TMED8 as a candidate positive regulator of KOR biogenesis. TMED8 is poorly characterized and atypical relative to canonical p24 family proteins, making its cellular functions unclear. Initial TMED8 knockdown and overexpression experiments both suggest that TMED8 positively regulates KOR total abundance, with a more selective effect on KOR than on other tested opioid receptors and GPCRs. Further preliminary imaging indicates that TMED8 localizes near early secretory pathway compartments, including the ER and ER-Golgi intermediate compartment. These findings support a model where TMED8 promotes KOR biogenesis, potentially by acting within the early secretory pathway. Ongoing work will test this working model using perturbation and trafficking assays to define where and how TMED8 participates in KOR biogenesis.

Single-step megabase addition and replacement in bacteria via conjugative CRISPR-associated transposition
Ian M. Mathews
Mentors: Kaihang Wang and Raymond Zhang

Building chimeric genomes by combining diverse genetic repertoires across distinct bacterial species enables the melding of complex biological functions. However, existing custom genome construction methods depend on double-strand breaks or labor-intensive *in vitro* assembly, making them resource-intensive, time-consuming and unscalable for transferring megabase-sized natural DNA sourced from a diversity of microbial genomes.

To enable genome rewriting on the megabase-scale with natural DNA, we developed a suite of technologies that couple conjugative transfer with CRISPR-associated transposition (CAST) to deliver and integrate targeted genomic fragments in a single step. We evaluated Additive Conjugative-CAST Engineering (ACE) and its donor-engineering extension, Orthogonal ACE Sequential Integration System (OASIS) to transfer 80 Kb and 120 Kb segments of *Pseudomonas protegens* Pf-5 encoding for diverse biosynthetic gene clusters downstream of the *glmS* locus in *Pseudomonas putida* KT2440.

We further benchmarked Replacements with ACE (ReplACE) with cross-genus megabase replacements of 180 Kb, 680 Kb, and 1.08 Mb segments from an attenuated *Shigella flexneri* donor (CFS100) with the corresponding segment of a reduced-genome *Escherichia coli* recipient (MDS42).These results and characterizations establish conjugative-CAST systems as a scalable, programmable platform for rapid, multi-megabase synthetic genome expansion.

Using the Electrophoretic Mobility Shift Assay (EMSA) to test direct DNA binding in dArc1 autoregulation
Faith M. Rosas
Mentors: Kai G. Zinn and Peter H. Lee

Autoregulation is a regulatory mechanism in which a gene produces a protein that binds to its own regulatory DNA sequence, thereby regulating its own expression. The *Drosophila* Arc protein (dArc1) was found to bind specific regions of the genome, referred to as "peaks," which were identified through ChIP-seq analysis. These findings suggest that dArc1 may positively regulate its own transcription by activating expression of its own gene. This project investigates dArc1 autoregulation by testing whether it binds to regulatory DNA sequences that were identified using CHIP-seq analysis. Genetic studies in *Drosophila* were used to evaluate the role of dArc1 in regulating its own expression *in-vivo*. Candidate DNA sequence binding sites were cloned into reporter constructs containing dArc1 protein for S2 cell transfection assays to determine if dArc1 binds to candidate regulatory DNA

sequences regulating its own gene expression. Electrophoretic mobility shift assays (EMSAs) were performed to assess direct binding of dArc1 to candidate DNA sequences *in vitro* by detecting a mobility shift of the DNA–protein complex. Together, these three complementary approaches aim to determine whether dArc1 functions as a transcriptional regulator of its own gene. Understanding the mechanism of dArc1 autoregulation could provide new insights into Arc protein function and the gene regulatory pathways involved in neuronal function.