Caltech
Student-Faculty Programs

Session A Abstracts

Developing a human tonsil organoid workflow for single-cell antibody discovery using the Beacon optofluidics platform
Arman M. Thoresen
Mentors: Pamela J. Bjorkman and Kate Malecek

Human tonsil organoids recapitulate key features of human adaptive immunity and provide an *ex vivo* model for studying vaccine responses. The goal of this project is to develop a workflow for isolating and analyzing vaccine-induced plasmablasts and plasma cells (PB/PCs) from tonsil organoids using the Beacon, a single-cell optofluidics platform that measures antibody secretion and enables recovery of individual cells for antibody characterization. Tonsil organoids were stimulated with live attenuated influenza vaccine (LAIV) or influenza hemagglutinin nanoparticles, while unstimulated organoids served as controls. Flow cytometry was used to identify antigen-specific B cells and PB/PCs, and magnetic cell separation was used to enrich PB/PCs for Beacon analysis. Initial experiments demonstrated antigen-specific B-cell and PB/PC responses following LAIV stimulation, while unstimulated organoids showed minimal antigen-specific responses. Hemagglutinin nanoparticles also induced antigen-specific responses, although generally at lower levels than LAIV. Magnetic cell separation enriched PB/PC populations, supporting its use for preparing antibody-secreting cells for Beacon analysis. These results establish a foundation for optimizing PB/PC isolation and antibody secretion from tonsil organoids, enabling future single-cell antibody discovery and characterization of responses to next-generation vaccine candidates., enabling future single-cell antibody discovery and characterization of responses to next-generation vaccine candidates.

Can we study growth arrest in diverse bacteria using electrochemistry?
Emil K. Uddevik
Mentors: Dianne K. Newman and Kemal Demirer

Most bacterial research focuses on actively growing cells, but this often isn't how they are found in nature. In chronic infections, bacteria form structured communities called biofilms, whose cores often lack the nutrients and electron acceptors needed for growth. Pseudomonas aeruginosa overcomes this by producing redox-active metabolites (RAMs) called phenazines, which shuttle electrons through the biofilm to distal acceptors such as oxygen. This allows cells to survive but not grow, which we term the maintenance state. Whether this state is unique to P. aeruginosa, or a general feature of bacterial survival is unknown. To test this, we used a high-throughput 96-well electrochemical platform to probe RAM-cycling-dependent anaerobic survival in other clinically relevant bacteria, including Escherichia coli, Burkholderia spp., and Streptococcus parasanguinis. The system applies a constant oxidizing potential and measures current, enabling quantitative, mechanistic study of bacterial growth arrest. We first assessed whether each organism could survive reductive stress by cycling RAMs, then whether cells in this state tolerate antibiotics targeting various cellular processes. Overall, we found that RAM-dependent anaerobic survival is not unique to P. aeruginosa and confers tolerance to certain antibiotics, making it a clinically relevant state that many pathogens may occupy during infection.

How does phenazine retention in *Pseudomonas aeruginosa* biofilms generalize to diverse clinical isolates?
Tasnim Ghoniem
Mentors: Dianne K. Newman, Eloise Masquelier, and Ezekiel J. Johnson

Bacterial biofilms are multicellular communities embedded in a self-produced extracellular matrix. *Pseudomonas aeruginosa* biofilms persist for years within chronically infected hosts, in part because cells in the biofilm core are more tolerant to antibiotics than their actively growing counterparts. Phenazines, small redox-active metabolites, play an essential role in supporting the antibiotic-tolerant cells in the hypoxic core of *P. aeruginosa* biofilms by serving as extracellular electron shuttles. Here, we tested whether the rules governing phenazine retention in the biofilm matrix, previously described

in the model strain PA14, generalize to diverse clinical isolates. Four clinical isolates were grown planktonically and as colony biofilms across several media to characterize phenazine retention. We found that pyocyanin (PYO) and phenazine-1-carboxamide (PCN) were consistently retained within these biofilms across strain and media conditions, while phenazine-1-carboxylic acid showed minimal retention. Preliminary results indicate that the amount of eDNA in the matrix dictates the extent of PYO and PCN retention. Thus, the retention mechanism described in the model strain may be a generalizable feature of *P. aeruginosa* biofilm chemistry, positioning chemical matrix-phenazine interactions as a potential angle for disrupting the persistence of antibiotic-tolerant cells in the biofilm core.

Investigating the molecular and neural mechanisms of social behavior in *Drosophila*
Kamarya A. Buckley
Mentors: David J. Anderson, Yousef Ouadah, and Ziqing Zhong

Social behaviors such as aggression and courtship are regulated by neural circuits and pheromone signaling, yet the molecular mechanisms underlying their evolution remain poorly understood. *Drosophila santomea* provides a valuable model because males exhibit increased same sex courtship and produce reduced levels of cis-vaccenyl (cVA), a pheromone that promotes aggression and suppresses male-male courtship in most Drosophila species. This study investigates the cellular basis of cVA production by characterizing the ejaculatory bulb, the organ responsible for cVA biosynthesis, using single-cell RNA sequencing (scRNA-seq). Current work focuses on optimizing tissue dissociation and validating cell viability to generate high-quality single-cell suspensions for sequencing. These data will ultimately identify candidate genes involved in cVA biosynthesis, with particular interest in epithelial cell populations.

In parallel, in *Drosophila melanogaster*, we examined the neural circuitry underlying aggression through an epistasis-based optogenetic approach by activating common aggression-promoting (CAP) neurons while silencing the downstream neuron SMP054; we tested whether SMP054 is required for aggression- related behaviors. Preliminary behavioral observations suggest SMP054 may not be required for CAP-mediated aggression and approach behaviors. Together, these complementary approaches will advance our understanding of the molecular and neural mechanisms that shape the evolution of social behavior in *Drosophila.*

Decoding behavioral states and transition dynamics in human driving task
Deborah R. Fu
Mentors: Markus Meister and Yingxi Jin

The naturalistic approach to human neuroscience study involves the study of human neural and behavioral data during complex tasks. A complex task is defined as one that requires the sequencing of functioning distinct episodes, or "microtasks". Here, we report the behavioral state boundaries of human subjects engaged in a naturalistic, goal-directed complex task: driving a simulated car around a custom figure-8 race track. SVM analysis reveals that microtask decisions become decodable before the microtask boundary is reached. Behavior along the track is modeled as a recurrent switching linear dynamical system. Shared discrete dynamical states and state transitions along the track are identified for neural correlation analysis.

An engineered prodrug-activating enzyme for circuit-based cancer therapy
Evan Z. Zhang
Mentors: Michael B. Elowitz, Andrew Lu, and Lukas Moeller

Enzyme-prodrug therapies, such as yeast cytosine deaminase (yCD) paired with the prodrug 5-fluorocytosine, rely on enzyme-based conversion of a non-toxic prodrug into a potent, diffusible chemotherapeutic within a tumor site to enable greater tumor penetration and clearance. However, existing strategies for restricting enzyme activity to cancer cells, such as tumor-specific promoters or viral delivery, provide limited specificity and cannot directly sense oncogenic signaling within a cell. Therapeutic circuits, which use engineered proteins to detect oncogenic signals and conditionally trigger cell death, offer a potential route to directly couple prodrug activation to the cancer-specific signal. Here, we present the foundation for a mutant Ras-activatable yeast cytosine deaminase, in

which deaminase activity is engineered to depend on a sensor module that is specifically activated by oncogenic Ras. We explore several different strategies for engineering circuit-controlled yCD and evaluate dynamic range using a HEK293-based assay. Overall, these results lay the groundwork for a circuit-based enzyme-prodrug therapy that could combine the tumor-killing efficiency of 5-fluorocytosine conversion with the specificity advantages of Ras-sensing protein circuits.

Toward absolute protein quantification in the mammalian circadian clock: Evaluating flow cytometry and Western blotting

Qianqian Li
Mentors: Michael B. Elowitz and Gal Manella

The circadian clock is controlled by interacting proteins and genes. Quantitative models of how perturbations alter clock behavior require measurements of relative and absolute protein abundance. This project aimed to measure ectopic protein abundance relative to endogenous protein abundance, while developing a method for absolute quantification. A single-cell flow cytometry assay was evaluated using a fused fluorescent tag to measure ectopic protein and intracellular antibody staining to estimate the total target protein pool. The two signals were compared across induction or depletion conditions. Although the fused tag clearly reflected induction, the antibody signal showed no corresponding increase. Changes to permeabilization and antibody concentrations did not change the result. Western blotting was therefore explored as a population-averaged alternative and as an independent test of antibody specificity. Preliminary experiments revealed variable antibody specificity and identified insufficient loading, low population-averaged target abundance, and suboptimal transfer as barriers to quantitative interpretation. The current flow cytometry assay does not yet support quantitative comparison. Future work will test whether the fused tag alters epitope accessibility using new cell lines and optimize Western blotting for absolute quantification with purified standards.

Building a minimal toolkit for multicellularity: Investigating T cell regulation through synthetic circuits

Kaedan A. Hendrix
Mentors: Michael B. Elowitz, Ethan Richman, and Jacob Parres-Gold

Multicellular organisms utilize intricate biomolecular circuits to coordinate complex behaviors, such as the human immune system's response to infection. Isolating and mastering the minimal design elements of these systems clarifies the functional trade-offs inherent to various circuit architectures. Revealing these design principles is vital to understanding multicellular communication networks and could enable the use of programmable cell circuits for advanced biomedical practices. A critical node and example of this cellular decision-making is the delicate balance between conventional effector T cells, which drive immune defense, and regulatory T cells (Tregs), which actively suppress expansion to protect "self" antigens and prevent autoimmunity.

To uncover the mechanics of this balance, a computational ODE model of alternative circuit designs for T cell regulation by Tregs was constructed in a Python environment. This model is able to systematically explore whether each distinct design can achieve a desired function: permitting conventional T cells to expand in the presence of exponentially increasing stimulation, while remaining strictly controlled under low antigen induction. The model evaluated a multi-axis design space, assessing T cell proliferation drives such as private signal sensing or direct antigen-presenting cell contact. Furthermore, distinct suppression mechanisms including direct private-receptor-facilitated killing, proliferative arrest, and protease-mediated signal cleavage were tested. Computational screening revealed that effective regulation is primarily driven by delayed Treg activation, with direct killing and protease-mediated reduction computationally suggested to be the most robust strategies. By constructing the candidate circuits in mammalian cell lines with synthetic protein components, we are able to test the results of this model *in vitro*. Ultimately, this approach will allow the direct characterization of communication circuits in live mammalian cells, revealing important design principles.

Investigating sex-specific differences in the squid-*Vibrio* symbiosis using 5′-RNA sequencing

Kaifeng Zhang
Mentors: Margaret J. McFall-Ngai and Drew Honson

The symbiotic relationship between the Hawaiian bobtail squid and the luminous bacterium *Vibrio fischeri* provides a well-established model for studying host–microbe interactions. Although this association has been extensively characterized, whether males and females exhibit distinct molecular responses to symbiosis remains largely unknown. The goal of this project is to investigate sex-specific transcriptional differences during symbiosis by combining molecular sex determination with transcriptome analysis. First, genomic DNA was extracted from hatchling squid and quantitative PCR was used to determine the sex of each individual. RNA was then isolated from light organs of squids with different symbiotic states and prepared for 5′ RNA sequencing. Sequencing data were analyzed to compare gene expression patterns between males and females and to identify sex-specific responses to bacterial colonization. These findings provide a foundation for understanding how sex infleunces the establishment and maintenance of beneficial host–microbe interactions.