

A principled approach to mouse motor behavior analysis in Parkinson's disease models

Alyssa H. Shin

Mentors: Sarkis K. Mazmanian, Anastasiya Oguienko, and Justin S. Bois

Parkinson's disease (PD) is a progressive neurodegenerative disorder that impairs motor function via alpha-synuclein aggregation followed by death of dopaminergic neurons. To study the underlying mechanisms of the disease, researchers often use mouse models imitating different aspects of PD. Motor behavior assays are used to assess the severity of symptoms, but behavior is often scored using subjective or simplified rating scales, resulting in one or two data points per trial and limiting reproducibility across studies. This project develops a quantitative tool for characterizing behavior in the hindlimb clasping mouse motor assay using an alpha-synuclein overexpression PD mouse model. Mice were recorded throughout the experiment and pose estimation software was used to track individual body parts over time. From these positional data, I developed measurable characteristics hypothesized to distinguish affected mice from healthy controls. Then I used Bayesian inference to develop hierarchical generative models for these behavioral traits, which allowed the models to account for variability across individual mice and experimental cohorts while estimating differences at the genotype level. This approach offers a more rigorous way of quantifying motor behavior in mouse models and could help standardize how motor deficits are measured across labs studying preclinical models of PD.

Investigation of Fis1-mediated regulation of mitochondrial function via proteometabolic profiling

Amarri Z. Robinson

Mentors: David C. Chan and Sangheeta Bhattacharjee

Mitochondria are membrane-bound organelles with vital cellular functions. Beyond the production of chemical energy via oxidative phosphorylation, mitochondria play crucial roles in metabolism, stress response, and apoptosis. Mitochondria are unique in their dynamism: undergoing coordinated cycles of fission, fusion, and self-degradation (mitophagy) to maintain the health of the cell's mitochondrial population. One mitochondrial dynamics protein that has remained elusive is Mitochondrial Fission Protein 1 (Fis1). Bound to the outer mitochondrial membrane, Fis1 recruits numerous protein signalling factors. Overexpression of Fis1 can cause mitochondrial fragmentation in certain cell types, indicating a role in fission. Additionally, Fis1 binds to the TBC1D15 protein, which regulates mitophagy. We aim to investigate the effects of Fis1 gene disruption (knockout) on the mitochondrial proteome and metabolome. This analysis requires effective isolation of the mitochondrial population from a cell lysate. We accomplish this with HA-immunopurification, where HA-tagged mitochondria are isolated using anti-HA antibody conjugated magnetic beads. Following immunopurification, we analyze the proteometabolome of the organelle using Liquid Chromatography- and Ion Chromatography-Mass Spectrometry. This work will provide a higher-resolution view of Fis1's regulatory functions, clarifying its role within mitochondrial dynamics and cellular homeostasis.

Temporal acuity and its perceptual consequences in human intracortical microstimulation

Katelyn A. Sadorf

Mentors: Charles Greenspon and Ueli Rutishauser

Sensory feedback for brain-computer interfaces is typically delivered through intracortical microstimulation of somatosensory cortex, with pulse rate as the primary encoding parameter. This project examined whether human perception is also sensitive to the fine-grained timing of individual pulses, independent of average rate, and whether such timing influences the perceived quality of stimulation. Participants with implanted microelectrode arrays performed two psychophysical tasks: a discrimination task comparing temporally regular and irregular pulse trains matched in mean rate, and an intensity-judgment task comparing trains with identical pulse count and duration but different burst grouping. Discrimination of irregular from regular trains was electrode-dependent and, on the most sensitive electrodes, occurred at intervals substantially finer than a published threshold for conscious

perception. Independently, burst grouping altered perceived intensity despite no change in total stimulation delivered. These results indicate that temporal structure shapes perception through two dissociable channels, explicit discrimination and perceived intensity, operating independently of pulse rate. Fine timing therefore constitutes an additional, controllable dimension for sensory encoding in neuroprosthetic feedback systems, distinct from and complementary to conventional rate-based approaches.

Investigating how gene-environment interaction causes heart defects

Aleen A. Koujakian

Mentors: Duncan Sparrow, Marianne Bronner, and Victoria Rashbrook

Congenital heart disease (CHD) is a non-communicable disease in which the embryonic heart fails to form correctly. CHD is the most common class of human birth defects, but only one-third of cases are attributed to genetic causes. Maternal iron deficiency anemia (IDA) has been identified as an environmental factor that causes CHD in mice. *NOTCH1* haploinsufficiency is also associated with CHD but has high phenotypic variability, which may be explained by gene-environment interaction (GxE) between the *NOTCH1* null allele and maternal IDA. This GxE has been demonstrated experimentally in mouse embryos heterozygous for a global *Notch1* null allele by an unknown mechanism. Here, we investigated the molecular basis of this GxE using whole-mount RNAScope and immunofluorescence to detect changes in expression of genes and proteins required for embryonic heart development. We also sought to identify the cell populations that require *Notch1* expression by phenotyping mouse embryos with conditional *Notch1* deletions in the anterior second heart field, neural crest, or endothelium. High resolution episcopic microscopy (HREM) was used to create 3D models for manual and automated morphology analysis. This work may elucidate how maternal IDA interacts with embryonic genetics to cause CHD and inform future studies aimed at lowering CHD risk.

Design of novel nanobodies targeting newly identified conserved regions of HIV gp120 and gp41 glycoproteins

Anthony K. Lindo

Mentors: Stephen L. Mayo and Andres Orta

The acquired immunodeficiency syndrome (AIDS) epidemic has been a global public health concern for over 40 years, infecting 84 million people worldwide and resulting in approximately 40 million deaths total. AIDS is caused by the retrovirus, Human Immunodeficiency Virus 1 (HIV-1), which specifically targets CD4+ T cells resulting in an immune-deficient state over a period of 2-10 years. Recently, the Mayo Lab in collaboration with the Bjorkman Lab discovered an invariant epitope within the HIV Envelope known as the Caldera Region. This presents a potential therapeutic to HIV where a specialized antibody, known as a nanobody, can target the Caldera Region and prevent host cell membrane fusion. Leveraging diffusion models for de novo protein design, this project aims to utilize Boltzgen and Boltz-2 to generate nanobody binders to target the Caldera Region and potentially alternative invariant regions. In order to validate Boltzgen, control nanobodies were designed against the closed HIV envelope which demonstrated predictable binding in deglycosylated regions. Ongoing testing with the fully glycosylated envelope reveals candidate nanobodies targeting a functional pocket that potentially corresponds to the CD4 binding site HIV uses to integrate into CD4+ T cells.

Quantifying the information bottleneck in radiogenomics

David L. Jin

Mentors: Lior S. Pachter and Joseph M. Rich

Radiogenomics is a field in precision medicine that integrates medical imaging data with patient genetic and molecular profiles. It provides a non-invasive way to identify tumor subtypes, predict specific genetic mutations, and choose tailored therapies. However, reported associations between imaging features and mutations in published radiogenomics papers rarely reproduce across hospitals and scanners. Our own Contrastive Language-Image Pre-training (CLIP) model failed to align the two different modalities of data and the model's predictions of the gene mutations remain random. Instead of treating every failed replication as a modeling problem, we asked a more basic question: does a

computed tomography (CT) scan contain enough information about a tumor's mutations to predict them at all, no matter how good the model is?

We instead measure how much biological information survives from a somatic mutation to a CT image. Across roughly ten thousand tumors from The Cancer Genome Atlas (TCGA), mutations explain just 2% of the variation in gene expression above a calibrated permutation baseline. We then used Cohen's d to identify mutations associated with large changes in transcriptomic expression within each cancer type, yielding just 25 gene-cancer type pairs, most involving well-known tumor suppressors or oncogenes (e.g. TP53, KRAS). These genes represent the genes most likely to show up in a CT scan due to their large effect on expression. After training supervised imaging classifiers over the list of genes, we found that every single prediction came back at chance. A separate analysis using a calibrated statistical bound reached the same conclusion: CT contained very little recoverable information about the tumor's gene expression, with an estimated upper bound of 1.7% of transcriptomic variance. The strongest apparent signal was largely associated with patient sex rather than known tumor biology. Together, these results suggest that only a small amount of genetic information survives from the molecular state of a tumor to the macroscopic phenotype represented in a CT image. This provides a possible biological explanation for why many reported radiogenomic associations have been difficult to reproduce.

A framework for inference in spatial transcriptomics models

Jenna Wang

Mentors: Lior S. Pachter and Kayla C. Jackson

Spatial transcriptomics tools measure gene expression while preserving the spatial organization of tissue. While these tools enable the interrogation of diverse biological systems, current analyses fail to address technical processes that introduce spatial bias and confound biological signals. Here, we have implemented a physically motivated model of spatial gene expression in a modular and interoperable Python module. At its core, the package fits a model that explicitly separates biological signals from technical artifacts while encoding assumptions about underlying gene regulatory mechanisms. The package is validated on simulated datasets with known parameters to confirm accurate recovery of the biological parameters. All features of the package are designed with modular architecture that enables future extensions, including improved estimation of variance components and joint analysis of multiple genes.

Phenotypic screening and amplicon-seq genotyping of multiplex CRISPR mutants affecting *Arabidopsis* root development

Erik R. Ledvina

Mentors: Trevor M. Nolan and Xiaohui Li

The transition from proliferation to differentiation is essential to plant root development and thus vital to the rest of the plant's growth. A systematic understanding of regulatory factors involved in this transition will enable scientists to bioengineer more viable crops. However, because of gene redundancy in plants, studying plants via traditional methods of single loci genetic perturbation is ineffective. Thus, in this project, plant lines mutated using a multiplex CRISPR gRNA library that targets multiple genes within a gene family, were used to study fully perturbed gene function. Phenotypic screening was used to identify promising mutants that displayed short root length and next-generation sequencing was used to sequence gRNA containing amplicons to identify the multiplexed target genes. Finally genotyping the target loci of potential novel mutant candidates will be used to support that mutant phenotypes were not the result of off-target effects. Through this forward genetic pipeline, sequencing has revealed mutants with potentially novel or understudied genetic mutations. Future work will pertain to interpreting sequencing results, finishing genotyping, and ordering T-DNA insertion mutants to further support the mutant phenotype.