Poster Session Abstracts

1. **Investigating the Flow Response of *Hydra* via Microfluidic Systems**

Ena Ysabella D. Arroyo[1]*, Helen Cardenas-Rios[1]*, Jaimie A. De Guzman[1]*, Maro M. Aziz[1]*, Roquel John Roman[1], Robert E. Steele[2], Siavash Ahrar[1]
[1]Department of Biomedical Engineering, California State University Long Beach, CA, USA
[2]Department of Biological Chemistry, University of California Irvine, CA, USA
* These authors contributed equally.

Hydra has become a popular model system for studying neuronal and corresponding mechanical activities. Flow is a ubiquitous factor in *Hydra's* natural environment. However, the response to flow (particularly in the presence of gravity) has remained underexplored. We used vertically oriented microfluidic chambers to investigate the biomechanical response of *Hydra vulgaris* to fluid flow. Here, flow served as the input, *Hydra* as the biological system, and body column alignment with flow as the measurable output. We quantified alignment by measuring the angle between the organisms (using a vector connecting head to foot) and the flow direction at multiple flow rates (0-100 mL/h). The experiments indicated alignment of the body column with the flow direction at high flow rates (≥ 50 mL/h). Investigating the sensorimotor behaviors is critical for establishing *Hydra* as a tractable model system for studying how mechanical stimuli are linked to behavioral responses.

2. **Central Amygdala Kappa Opioid Receptors Mediate Pain-Related Vulnerability to Stress-Induced Opioid Relapse**

Jason Baker*, Flora D'oliveira da Silva, Catherine Cahill, PhD
University of California, Los Angeles

Chronic pain affects 20.9% of U.S. adults, and up to 25% develop opioid use disorder (OUD) (Goldberg & McGee, 2011). Although these conditions exacerbate one another, the neural mechanisms by which chronic pain increases relapse vulnerability remain unclear. Kappa opioid receptors (KORs) regulate nociceptive and stress-related processes. While systemic KOR activation produces analgesia, it also promotes stress-induced drug seeking. The central amygdala (CeA) integrates affective and pain signals and is required for stress-induced opioid relapse (Taylor et al., 2015). However, the role of CeA KORs under chronic pain conditions is unknown. To test whether right CeA KOR signaling mediates pain-related reinstatement, ($KOR^{flox/flox}$) mice received unilateral Cre infusions into the right CeA. Following chronic constriction injury (CCI) or sham surgery, mice underwent oxycodone conditioned place preference, extinction, and stress-induced reinstatement. Preliminary data suggest that CeA KOR deletion reduces reinstatement selectively in CCI mice, implicating right CeA KOR signaling in pain-enhanced relapse vulnerability.

3. **Predicting Antibiotic Resistance Using Machine Learning and Microbial Data**

Adrian Bautista
California State University, Fullerton

Antibiotic resistance poses a growing challenge to global healthcare systems, requiring faster and more accurate methods for identifying effective treatments. In this project, we present a computational framework for predicting antibiotic resistance using microbial genomic and laboratory-derived data. By applying machine learning classification models (e.g., tree-based and ensemble methods) to identify patterns associated with resistance, our approach aims to support data-driven decision-making in clinical and research settings. The system integrates feature extraction from biological inputs with predictive modeling techniques to estimate resistance probabilities across multiple antibiotics. Results are visualized

through an interactive dashboard, enabling users to interpret predictions alongside confidence scores and key contributing features. This work highlights the potential of combining systems biology and data science to model complex biological responses and improve treatment selection strategies. Future directions include incorporating real-time datasets and expanding model generalization across diverse microbial species.

4. **Balancing Stability and Complexity in Boolean Models of Biological Systems**

Sai Bavisetty[1], Matthew Wheeler[2], Reinhard Laubenbacher[2], Claus Kadelka[3]
[1]University of California, Los Angeles
[2]University of Florida, Gainesville
[3]Iowa State University

Boolean networks were first introduced by Kauffman as models for gene regulatory networks. These models have gained popularity because of their simplicity and ability to capture the complex behaviors of biological systems. Currently, the repository Biodivine Boolean Models contains more than 230 Boolean network models. Systematic investigation reveals these biological networks are remarkably robust to perturbations. Kauffman first showed empirically that network connectivity determines stability, later explained theoretically by Derrida. Subsequent work empirically linked various network parameters to stability. Building on this foundation, we investigate the intrinsic trade-off between phenotypic complexity and network stability. We prove that entropy (a complexity proxy) provides a tight asymptotic upper bound for coherence (a stability measure), extending a conjecture by Willadsen, Triesch, and Wiles. Consequently, we derive the exact Pareto frontier between complexity and stability, determining precisely how much stability is achievable for any given complexity level.

5. **The Effects of Low-Dose Naltrexone on Mice That Have Experienced a Traumatic Stressor**

Owen Bella[1,2,3,5*], **Chloe Z. Sy-Perez**[1,2,3,5*], Jamie E. Mondello[1,3,5], Jeremy M. Trott[1,2,3,4], Michael S. Fanslow[3,4], Catherine M. Cahill[1,2,3,5]
[1]Hatos Center for Pharmacology,
[2]Department of Neuroscience,
[3]Department of Psychiatry and Behavioral Sciences,
[4]Department of Psychology,
[5]Semel Institute,
University of California Los Angeles, Los Angeles California, USA
* These authors contributed equally.

Low-dose naltrexone (LDN) is increasingly used off-label to treat chronic pain, providing therapeutic benefits despite being an opioid receptor antagonist. We hypothesize that stressed mice will spend more time in the LDN-paired context, while non-stressed mice will have no preference for either side. First, we conducted CPP, and then, mice received a traumatic stressor. Days 2-3, conditioning training was conducted using a CPP apparatus, where one context was paired with saline and the other with naltrexone. Day 4, the mice had free exposure to the same CPP apparatus. We found that stressed males that received LDN 2 days after the traumatic stressor spent more time in the LDN-paired context than non-stressed males, indicating LDN is rewarding in stressed males. Furthermore, we did not find similar results in females, indicating that LDN is not rewarding in stressed females. Future directions will investigate the mechanism by which LDN exerts its therapeutic effects.

6. **Sleep Irregularity Is Associated with Systemic Inflammation and Depressive Symptoms in U.S. Adults**

Shubhreet Bhullar[1]
[1] Department of Psychology, University of California, Los Angeles

Irregular sleep patterns are increasingly recognized as a behavioral risk factor for both mood disturbance and chronic disease, yet their relationship with systemic inflammation remains incompletely characterized. Using data from the National Health and Nutrition Examination Survey, we examined whether variability in self-reported sleep duration is associated with circulating C-reactive protein (CRP) and depressive symptoms. Sleep irregularity was defined as differences between weekday and weekend sleep duration. Multivariable regression models evaluated associations with log-transformed CRP and PHQ-9 scores, adjusting for demographic, behavioral, and metabolic covariates. Greater sleep irregularity was associated with elevated CRP and increased depressive symptom burden, independent of mean sleep duration. These findings suggest that inconsistency in sleep timing may link behavioral and inflammatory processes. Promoting regular sleep patterns may represent a modifiable target for reducing both psychological and physiological risk.

7. **Genome-wide base editor screening identifies thousands of functional variants in yeast**

James Boocock[1,2,3,4], Lauren Crisman[1,2,3,4], Shivani Patel[1,2,3,4], Cindy Vo[1,2,3,4], Heriberto Marquez[1,2,3,4], Leslie Alamo[1,2,3,4], Joshua Bloom[1,2,3,4]*, Leonid Kruglyak[1,2,3,4]*

1. Department of Human Genetics, University of California, Los Angeles, Los Angeles, CA, USA.
2. Department of Biological Chemistry, University of California, Los Angeles, Los Angeles, CA, USA.
3. Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, CA, USA.
4. Institute for Quantitative and Computational Biology, University of California, Los Angeles, Los Angeles, CA, USA.

A central goal of genetics is to define the relationships between genotypes and phenotypes. However, genetic mapping approaches often lack the resolution to pinpoint causal variants, and deep mutational scanning is difficult to scale across an entire genome. Here, we present a deep genome-wide CRISPR base editor screen in *Saccharomyces cerevisiae*, deploying 356,323 guide RNAs to introduce single and multi-nucleotide variants across coding and regulatory regions of the genome. We identified 8,094 variants with significant impacts on fitness. The rate of significant fitness effects was highest for stop codons in essential genes, followed by stop codons in non-essential genes and non-synonymous variants in both classes of genes, with synonymous variants showing the lowest rate. These results are consistent with well-established patterns of variant impact across species. We identified 918 non-coding variants that influenced fitness, with gene-proximal variants showing larger effects than gene-distal variants, consistent with the expected role of proximal regulatory regions in controlling gene expression. Among 82,230 variants also observed in natural yeast populations, fitness effects were smaller than those of unobserved variants, and of the 1,280 natural variants with significant effects, common variants had smaller effects than rare ones, consistent with purifying selection. We identified 46 naturally occurring variants that significantly increase fitness, representing candidates for positive selection in yeast populations. At the gene level, our screen revealed modulators of base editor efficiency, offering insights into host factors that influence genome modification. Additionally, variants classified as pathogenic in humans at positions conserved between yeast and humans showed larger fitness effects than those classified as benign. This result highlights the potential of yeast variant screens to inform clinical variant interpretation. Together, these results establish a genome-wide map of variant function in a eukaryotic organism and demonstrate that large-scale base editor screens can connect sequence variation to phenotype at nucleotide resolution.

8. **Establishing a Dual Reporter Assay Combining Effector Domain Screening with Readouts of Enhancer-Promoter Communication**

August W. Brookwell[1], Laura Gambaretto[1], Laurah Chau[1,3], Judith F. Kribelbauer-Swietek[1,2]

[1]Department of Biological Sciences, University of Southern California, Los Angeles, California, USA

[2]Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, California, USA

[3]Department of Cancer Biology, Keck School of Medicine of USC, Los Angeles, California, USA

Enhancers regulate gene expression by recruiting sequence-specific transcription factors (TFs). Once bound, TFs recruit cofactors via their effector domains (EDs), which in turn facilitates enhancer-promoter communication. Despite their importance, many EDs lack functional annotation. While screening approaches have assessed transcriptional activator function of EDs, their role in mediating enhancer–promoter communication remains largely unexplored. To address this limitation, we developed a novel Massively Parallel Reporter Assay (MPRs) that combines existing ED screening with a readout of enhancer-promoter communication. As a proof of concept, we probed whether overexpression of chimeric TFs, combining a fixed DNA-binding-domain with varying EDs of known function, can be paired with the simultaneous readout of reporter transcriptional activity. The approach will enable a systematic assessment of whether, how, and to what extent EDs mediate enhancer-promoter communication, providing mechanistic insight into what remains a black box: Which protein interactions guide enhancer-promoter communication and thus exert transcriptional control.

9. **AI-Inferred Spatial Transcriptomics Unlocks Large-Scale Breast Cancer Biomarker Discovery from Histopathology**

Emma M. Campagnolo[1,2,*], Eldad D. Shulman[1,*], Roshan Lodha[1], Amos Stemmer[1], Thomas Cantore[1,2], Simon Knott[2], Kenneth Aldape[3], Joo Sang Lee[4], Danh-Tai Hoang[1], Eytan Ruppin[1,2]

[1] Cancer Data Science Laboratory, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA;

[2] Cedars-Sinai Medical Center, Los Angeles, CA, USA

[3] Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA
[4] Sungkyunkwan University, Seoul, South Korea
* These authors contributed equally: Eldad D. Shulman, Emma M. Campagnolo.
Spatial transcriptomics (ST) assays are transforming our understanding of tumor heterogeneity, but their high cost limits their application in large-scale biomarker discovery. Here we present Path2Space, a deep learning model that predicts spatial gene expression directly from histopathology slides. Trained on extensive breast cancer ST data, Path2Space robustly predicts the spatial expression of over 4,800 genes, significantly outperforming 21 state-of-the-art methods. Charting the tumor microenvironment (TME) of ~1,100 breast cancer TCGA tumors, it accurately infers cell-type abundances and identifies three novel spatially defined breast cancer subgroups with distinct survival outcomes. Notably, the derived low-cost spatial TME landscapes enable more accurate predictions of patient response to chemotherapy and trastuzumab compared to established costly bulk sequencing–measured biomarkers. Path2Space offers a scalable, fast and cost-effective alternative to sequencing-based assays. It opens avenues for large cohort treatment biomarker discovery and translationally relevant insights into tumor biology, with potential applicability across many cancer indications.

10. **Metabolic Reprogramming and Therapeutic Vulnerabilities in the Tumor Microenvironment Revealed by Multi-scale Network Geometry**

Handan Cetin[1], Paul Bogdan[2], Shannon M Mumenthaler[3,4], Stacey D Finley[1,4,5],
[1] Department of Quantitative and Computational Biology, USC, Los Angeles, CA
[2] Ming Hsieh Dept. of Electrical and Computer Engineering, USC, Los Angeles, CA
[3] Ellison Medical Institute, Los Angeles, CA
[4] Alfred E. Mann Dept. of Biomedical Engineering, USC, Los Angeles, CA
[5] Mork Family Dept. of Chemical Engineering & Materials Science, USC, Los Angeles, CA
The tumor microenvironment comprises diverse cell populations that coordinate metabolic activities to sustain malignant growth, yet the systems-level organization of these interactions remains poorly understood. Here, we present an integrated framework combining single-cell transcriptomics, genome-scale metabolic modeling, and multi-scale network geometry to decode metabolic coordination in colorectal cancer. We show that FAP+ cancer-associated fibroblasts and MARCO+ tumor-associated macrophages undergo extensive metabolic reprogramming and specialization: fibroblasts in amino acid and fatty acid metabolism, macrophages in nucleotide biosynthesis. Applying multifractal geometric characterization and Ollivier-Ricci curvature analysis to flux-weighted metabolic networks, we successfully distinguished tumor from normal phenotypes where conventional metrics failed. Role transition analysis revealed 20–25% of metabolites undergo functional reorganization, with prostaglandin and bile acid derivatives emerging as critical stromal communication hubs. Curvature analysis identified pathway-specific geometric remodeling in fatty acid and leukotriene metabolism. Our findings establish that metabolic adaptation represents ecosystem-level network reorganization, providing a generalizable framework for targeting cooperative metabolic networks therapeutically.

11. **Building a High-Throughput Reporter Assay to Identify Sequence Determinants of Insulator Function**

Laurah Chau[1,2], Laura Gambaretto[2], Christina Girgis[2], and Judith F. Kribelbauer-Swietek[2,3]
[1] Department of Cancer Biology, Keck School of Medicine of USC, Los Angeles, California, USA
[2] Department of Biological Sciences, University of Southern California, Los Angeles, California, USA
[3] Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, California, USA
Gene expression control underlies key biological processes like cell diversification, largely mediated by enhancer-promoter interactions. To prevent inappropriate interactions, the genome contains insulator elements that act as boundaries to restrict regulatory activity. While CTCF is needed for insulator function, not every binding site produces insulator activity, nor does every insulator sequence contain a CTCF binding site. The extent to which DNA sequence and CTCF binding sites determine insulator function across diverse cellular contexts remains incompletely understood. Here, we develop a high-throughput reporter assay to systematically probe insulator activity in both episomal and genome-integrated contexts. Drawing on published literature, we design a library of candidate insulating sequences, focusing on CTCF by varying its binding site number, orientation, and combinatorial configuration, and assess activity in human lymphoblastoid cells. Dissecting the sequence determinants of insulator function will deepen our understanding of gene regulation and how its disruption contributes to diseases such as cancer.

12. **Spatial niches composed of immuno-attractive vasculature and stroma explain the high immune response rate of desmoplastic melanoma**

Daniel G. Chen[1,2], Amanda Truong[2], Nataly Naser Al Deen[2], Cynthia R. Gonzalez, [2] Ignacio Baselga-Carretero[2], Jia Ming Chen[3], Kari L. Kendra[4], Zeynep Eroglu[5], Siwen Hu-Lieskovan[6], Ari M. Vanderwalde[7], David A. Wada[6], Jose A. Plaza[4], Michael C. Wu[8], Philip O. Scumpia[2,] Antoni Ribas[2,3], Katie M. Campbell[2,3]

[1] UCLA-Caltech Medical Scientist Training Program, University of California, Los Angeles, Los Angeles, CA

[2] Jonsson Comprehensive Cancer Center at the University of California Los Angeles (UCLA), Los Angeles, CA

[3] Parker Institute for Cancer Immunotherapy (PICI) at UCLA, Los Angeles, CA

[4] Ohio State University Wexner Medical Center, Columbus, OH

[5] Moffitt Cancer Center, Tampa, FL

[6] University of Utah, Huntsman Cancer Institute, Salt Lake City, UT

[7] The West Clinic, Wolf River, Germantown, TN

[8] SWOG Statistics and Data Management Center, Fred Hutchinson Cancer Center, Seattle, WA

Immune checkpoint blockade (ICB) has revolutionized cancer treatment by inducing remission in patients refractory to traditional-therapies. Interestingly, desmoplastic melanomas (DMs) are highly-responsive to ICB (overall-response-rate=89% with anti-PD1). These findings contrast with prior-work in “cold” tumors where desmoplasia associated with diminished anti-tumor responses. Here, we investigated the intrinsic and extrinsic (microenvironment) signals that position DM tumors for response. We found DM tumor-cells to promote immune-cell recruitment through CCL19/CCL21 secretion, these cytokines attract T/dendritic-cells. Interrogation of vasculature and stroma surrounding DM tumor-cells revealed polarization towards high-endothelial-venule and reticular-like phenotypes, respectively. These cell-states facilitate immune-cell recruitment in non-cancerous tissues; they likely perform similar functions in DM tumors. Spatial analyses confirmed these immuno-attractive cell-types as spatially co-localized hubs that are proximal to proliferative T-cells which, likely, sustain anti-tumor responses. Our findings present a potential explanation for the marked response of patients with DM to ICB; these mechanisms may be leveraged in future immunotherapy engineering strategies.

13. **State-Space Modeling Reveals miRNA-Driven Mechanisms of Chemotherapy Response and Relapse in Acute Myeloid Leukemia**

Ziang Chen[1*], Jennifer Rangel Ambriz[1*], Yu-Hsuan Fu[2], David E. Frankhouser[1,2], Denis O’Meally[1], Sergio Branciamore[1], Jihyun Irizarry[2], Bin Zhang[2], Lianjun Zhang[2], Ying-Chieh Chen[2], Guido Marcucci[2], Russell C. Rockne[1], Ya-Huei Kuo[2]

[1]Division of Mathematical Oncology, Department of Computational and Quantitative Medicine, [2]Department of Hematologic Malignancies Translational Science, Gehr Family Center for Leukemia Research, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA

* These authors contributed equally.

Acute myeloid leukemia (AML) is driven by genetic and chromosomal abnormalities. This study applies a state-space modeling framework, which represents disease progression as movement between “healthy” and “leukemic” states using time-series mRNA and miRNA data. This approach allows researchers to track how gene expression trajectories evolve over time and predict transitions such as treatment response and relapse.In a mouse model, chemotherapy initially shifts trajectories toward the healthy state, but relapse follows, with miRNA responses lagging about two weeks behind mRNA. Network analysis identified a cluster of 30 co-expressed miRNAs—mostly from the DLK1-DIO3 region—linked to miRNA delayed response and AML progression. These miRNAs suppress the PI3K/mTOR pathway, lowering reactive oxygen species and enabling leukemia stem cells to survive treatment. The persistence of these cells likely drives relapse, demonstrating how state-space modeling can reveal dynamic mechanisms behind AML progression.

14. **Differential methylation analysis of genetically diverse mice using Fiber-seq and pyDSS**

Dayeon Cheong[1], Jasmine Sakr[2], Ryan Weber[1], Elisabeth Rebboah[1], Elnaz Abdollahzadeh[1], Ghassan Filimban[1], Ali Mortazavi[1]

[1] Department of Systems Biology, University of California, Irvine, Irvine, CA

[2] Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA

While short-read sequencing struggles to resolve epigenetic modifications within complicated genomic loci and rearrangements, long-read sequencing, specifically Fiber-seq, enables a simultaneous capture of methylation, hydroxymethylation, and open chromatin. Hence, we generated 979.3 Gb of long-read data (average N50 > 30kb, coverage > 20x) from the cerebral cortex of two female and two male replicates of C57BL6/J and CAST/EiJ mouse strains, which serve as bridge samples of the IGVF Consortium. To analyze this, we developed pyDSS, a production-ready Python implementation of Bioconductor DSS package, to identify differentially methylated regions (DMRs) across genotypes,

sex, and cortices. Additionally, we are developing FCAT (Fiber-seq read Cell type Annotation in Tissue) to annotate cell types of individual Fiber-seq reads within bulk tissue samples, using multiome data. This pipeline allows for high-accuracy DMR identification across tissues and cell types, providing regulatory candidates to explain the functional impact of genomic variation.

15. **Accuracy of parameter estimation for a simple GRN model is sensitive to network motif, number of parameters estimated, and regulatory relationships**

Nikki C. Chun[1,2], Kam D. Dahlquist[1], Ben G. Fitzpatrick[2]
[1]Department of Biology,
[1]Department of Mathematics,
Loyola Marymount University, Los Angeles, CA, USA

GRNmap is a MATLAB software package that uses ordinary differential equations to model dynamics of small-scale GRNs. The program estimates production rates, expression thresholds, and regulatory weights for each transcription factor in the network based on gene expression data and then performs forward simulations of model dynamics. While the model has been successfully used to understand medium-scale GRNs, we wanted to closely examine how it works on a smaller scale to determine parameter sensitivity. All 21 possible "toy" networks of 3 nodes and 4 edges were created, and parameters were estimated from simulated data. Comparison of the known to estimated parameters showed that estimating production rates in addition to weights and thresholds reduced the accuracy of the results. To better understand the influence of the weight parameters, we generated all weight permutations for each network motif to better understand why certain network motifs are more prevalent in natural GRNs.

16. **Mapping Neuronal Vulnerability to Amyloid-β and α-Synuclein in Neurodegenerative Disease Models**

Haley De Ocampo[1], Zachary Hemminger[1], Aihui Wang[1], Shujing Zhang[2], Chao Peng[2], Roy Wollman[3,4]
[1] Department of Chemistry and Biochemistry,
[2] Department of Neuroscience,
[3] Department of Bioinformatics,
[4] Department of Systems Biology,
University of California Los Angeles, Los Angeles California, USA

Pathological alpha-synuclein (α-syn) aggregates as Lewy bodies in Parkinson's disease with dementia (PDD), dementia with Lewy bodies (DLB), and some Alzheimer's disease (AD) cases. Although α-syn spreads along neuronal connectivity, distinct brain regions and cell types show variable vulnerability, suggesting molecular mechanisms that remain poorly understood. Amyloid-beta (Aβ) co-pathology further exacerbates susceptibility, underscoring the need for high-resolution approaches to study selective vulnerability. We propose using ATLAS (Atlas-scale Transcriptome Localization using Aggregate Signatures), a novel spatial transcriptomics method, to map neuronal subtypes sensitive to α-syn aggregation. Unlike single-molecule FISH, ATLAS approximates transcriptional states of millions of cells by leveraging gene expression patterns. Using a pre-formed fibril (PFF) injection model in 5xFAD mice, we will examine α-syn propagation in the context of Aβ pathology. Coupling ATLAS with α-syn antibody staining will generate a spatial atlas of α-syn transmission, enabling bioinformatic discovery of candidate genes that drive selective vulnerability and inform therapeutic strategies.

17. **Profiling the Inheritance of Cis and Trans Acting Effects on Immune Gene Expression in Murine PBMCs**

Margaret Duffield, Ryan Weber, Ghassan FIlimban, Andrew Hall, Negar Fattahi, Elisabeth Rebboah, Shimako Kawauchi, Grant MacGregor, Lior Pachter, Brian Williams, Ali Mortazavi
Department of Systems Biology, University of California Irvine, Irvine CA, Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, Transgenic Mouse Facility, University of California Irvine, Irvine, CA

Genomic variation is known to influence gene expression and ultimately phenotypic function, yet the balance between cis regulation, which includes variants that are local to the gene, and trans regulation, which are cis effects in upstream genes, remains unclear. We can use F1 crosses between different genotypes in model organisms to quantify the proportion of cis affecting gene expression. Peripheral blood mononuclear cells (PBMCs) provide an accessible system for dissecting these regulatory modes across diverse immune populations. This project leverages 8 genetically divergent mouse strains and their F1 progeny to map the inheritance of immune gene expression programs at single-cell resolution. Using high-throughput single-cell transcriptomics and allele-specific expression analyses, we will quantify cis and trans regulatory contributions by comparing parental expression differences to allelic imbalance patterns in F1 progeny. Cell-type–resolved expression profiles will allow us to determine how regulatory modes vary across major PBMC subsets, including T cells, B cells, NK cells, and monocytes. Here we describe the cellular annotations of PBMC makeups along the 8 mouse strains and

their subsequent progeny, where cell-type specific expression is specific per mouse model, with unique inheritance patterns per tissue. This analysis will help determine the modeling of immunological diseases relevant per mouse strain and their inheritance patterns.

18. **Characterizing the cell-type genetic regulatory architecture across multiple mouse tissues at a single-cell resolution**

Ghassan FIlimban[1,2], Ryan Weber[1,2], Margaret Duffield[1,2], Shimako Kawashi[3], Brian Williams[4], Ali Mortazavi[1]
[1] Department of Systems Biology, University of California, Irvine, Irvine, USA
[2] Department of Developmental and Cell Biology, University of California, Irvine, Irvine, USA
[3] Transgenic Mouse Facility, University of California, Irvine, Irvine, USA
[4] Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, USA

Phenotypes are the product of genes and environment interactions, and QTL mapping in model organisms exploits structured strain genetic diversity under controlled environmental conditions to link genomic variation to phenotypic differences. One such phenotype is gene expression across cell types, which can vary between genotypes. The mouse collaborative cross (CC) is a multiparent population (MPP) panel derived from a genetically diverse set of eight founders, five are classical laboratory strains and three are wild-derived. Here, we aim to identify cell-type-specific eQTL (ct-eQTLs) in skeletal muscle using snRNA-seq across the 8 CC founders, 7 F1 hybrid strains generated by crossing C57BL/6J dams with each of the remaining 7 founders, and 33 CC lines, for a total of 48 strains, with 4 male and 4 female replicates. Our ongoing preliminary analyses incorporate kinship-structure-correction and haplotype-resolved mapping, with the goal of generating a genome-wide, cell-type-specific eQTL framework across tissues for the CCs.

19. **Probing Enhancer Cooperation Across Cell Types Using a Massively Parallel Reporter Assay**

Melissa Finegan, Judith F. Kribelbauer
Department of Molecular and Cellular Biosciences,
University of Southern California, Los Angeles, California, USA

Enhancers orchestrate precise patterns of gene expression by recruiting a variety of sequence-specific transcription factors. Some enhancers form cooperative hubs called super enhancers, that interact in 3D to regulate key cell identity genes, thus making their dysregulation a driver of disease states, including cancer. Despite their importance, the mechanisms by which enhancer sequence and transcription factor composition drive super enhancer formation remain poorly understood. Here, we develop a Massively Parallel Reporter Assay to systematically probe the sequence-determinants of enhancer cooperation in both divergent and closely related cell types. By testing enhancers of varying strengths and cell type specificity and comparing transcriptional output of enhancer combinations to that of single-enhancer matched controls, we will produce a detailed map of how enhancer syntax shapes super enhancer formation. The resulting data will provide insights into the mechanisms underlying enhancer cooperativity, a necessary step towards the development of therapies that directly target super-enhancer dysregulation.

20. **Longitudinal single cell RNA-sequencing reveals evolution of micro- and macro-states in chronic myeloid leukemia.**

David E. Frankhouser[1*], Dandan Zhao[2], Yu-Hsuan Fu[2], Anupam Dey[3], Ziang Chen[1], Jihyun Irizarry[2], Jennifer Rangel Ambriz[1], Tiffany Kanesa Ybarra[4], Sergio Branciamore[1], Denis O'Meally[5], Ryan S. Sathianathen[4], Jeffery M. Trent[6], Stephen Forman[2], Adam L. MacLean[3], Ya-Huei Kuo[2], Kathleen M. Sakamoto[4], Bin Zhang[2*], Russell C. Rockne[1*], Guido Marcucci[2*]
[1]Department of Computational and Quantitative Medicine, Beckman Research Institute, City of Hope National Medical Center
[2]Department of Hematologic Malignancies Translational Science, Beckman Research Institute and Division of Leukemia, City of Hope National Medical Center
[3]Department of Quantitative and Computational Biology, University of Southern California
[4]Department of Pediatrics, Stanford University
[5]Department of Diabetes & Cancer Discovery Science, Beckman Research Institute, City of Hope National Medical Center
[6]Translational Genomics Institute

Single cell RNA sequencing (scRNA-seq) has revolutionized our understanding of cancer, yet identifying meaningful disease states from single cell data remains challenging. Here, we systematically explore the chronic myeloid leukemia (CML) specific information content encoded in single cell versus bulk transcriptomics to resolve this paradox and clarify how discrete disease-defining states emerge from inherently noisy single cell data. We demonstrate that, while CML single cell transcriptomes exist along continuous transcriptional microstates, clinically relevant leukemia phenotypes clearly manifest only at the pseudobulk (macrostate) level. By leveraging state-transition theory, we reveal how robust

disease phenotype state-transitions are governed by cell type specific contributions. Our results establish a theoretical framework explaining why discrete disease phenotypes remain hidden at the single cell scale but emerge clearly at the aggregated macrostate level. By resolving how single-cell variation aggregates into macroscopic disease states, we provide a broadly applicable strategy for exploring disease dynamics.

21. **The IL-33/ST2 signaling reshapes the immune landscape and promotes leukemia transformation in acute myeloid leukemia**

Yu-Hsuan Fu[1,2], Man Li[2], Ying-Chieh Chen[2], Lianjun Zhang[2], Wancheng Guo[2], David E. Frankhouser[3], Denis O'Meally[3], Jihyun Irizarry[2], Jennifer Rangel Ambriz[3], Ziang Chen[3], Bin Zhang[2], Sergio Branciamore[3], Guido Marcucci[2], Russell Rockne[3], Ya-Huei Kuo[2]
[1]Irell & Manella Graduate School of Biological Sciences
[2]Department of Hematologic Malignancies Translational Science, Gehr Family Center for Leukemia Research
[3]Department of Computational and Quantitative Medicine,
Beckman Research Institute, City of Hope National Medical Center, Duarte, California, 91010

Acute myeloid leukemia (AML) is an aggressive malignancy with a 5-year survival rate of 30%, largely due to relapse and immune evasion. Using a state-transition modeling framework on time-series RNA-seq from a Cbfb::MYH11 (CM) knock-in AML mouse model, we identified IL1RL1 (ST2), IL-33 receptor, as a leukemia-promoting gene upregulated early in leukemogenesis. ST2 knockout in CM mice (CM-ST2-KO) showed delayed leukemia onset, reduced circulating blasts, and extended survival compared to CM mice (median survival 192 vs. 103 days; $p < 0.0001$). Further scRNA-seq cell-cell communication analysis of mouse bone marrow discovered dysregulation of immunosuppressive pathway crosstalk between ST2-expressing leukemic cells and immune cells in CM mice, such as PD-L1 and ALOX5 signaling pathways, and its reversal in CM-ST2-KO mice. In summary, we identified ST2 as a key regulator of leukemia progression and immune suppression in AML, supporting the therapeutic potential of targeting ST2 to improve immune-based therapies in AML.

22. **CRISPR-Cas9-Mediated Knockout of GPR65 in Jurkat T cells Impacts T cell and Metabolic Function**

Madison G. Gonzalez*[1], Caitlyn Marie Ybanez*[1], Nyla Lopez[1], Delihla Correa[1], Helena Wollam[1], Barbara Bailus[1] and Jeniffer Hernandez[2]
[1]Henry E. Riggs School of Applied Life Science, Keck Graduate Institute, Claremont, CA, USA
[2]School of Pharmacy Keck Graduate Institute, Claremont, CA, USA
* These authors contributed equally.

Autoimmune diseases are an increasing health burden, highlighting the need for targeted therapies. Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS) that is characterized by demyelination, neurodegeneration, and progressive neurological decline. In MS pathogenesis, dysregulated T-helper 17 (Th17) cell activation primarily amplifies inflammatory responses by producing interleukin-17A (IL-17A) and other proinflammatory cytokines. Recent studies in mice have implicated G-protein-coupled receptor 65 (GPR65), an acid-sensing GPCR, as a promoter of Th17 cell pathogenesis, therefore making it a promising therapeutic target. To translate these findings into human systems, we performed a GPR65 knockout in a human T cell line using a CRISPR-Cas9 gene-editing approach. In our study, generating a GPR65 knockout line resulted in decreased T-cell activity as well as reduced cellular metabolism. Collectively, these findings support GPR65 inhibition as a promising therapeutic strategy for the treatment of MS and other Th17-mediated autoimmune diseases.

23. **Macrophage response specificity to ligand mixtures is improved by signaling pathway antagonism**

Xiaolu Guo, Supriya Sen[#], Julian Gonzalez, Alexander Hoffmann*
Department of Microbiology, Immunology, and Molecular Genetics, and Institute for Quantitative and Computational Biosciences, University of California, Los Angeles, 611 Charles E. Young Dr E, Los Angeles, CA 90095
[#] current address: Amgen, One Amgen Center Drive, Thousand Oaks, USA
*Correspondence: ahoffmann@ucla.edu

Macrophages function as immune sentinels that distinguish diverse threats and mount appropriate responses. This stimulus-response specificity (SRS) is partly encoded in the dynamics of the NFκB transcription factor. While most studies examine single ligands, physiological exposures involve complex multi-ligand mixtures. Using a mathematical model that captures heterogeneous single-cell NFκB responses, we generated simulation datasets for mixtures of up to five ligands and validated key predictions with live-cell microscopy. Following iterative refinement of the model, we quantified SRS with Wasserstein distance and machine learning classification and found that that NFκB temporal coding can partially convey the presence of specific ligands within mixtures. By generating simulation datasets across all ligand pairs at doses

spanning the full responsiveness range, we found several cases of synergy and antagonism between stimuli. Antagonism was for example the result of a limited supply of stimulus-cofactor CD14 or endosomal transport capacity. Synergy depended on ultra-sensitive IKK activation in cells with low receptor expression. While synergy does not enhance SRS, antagonism between TLR9 and TLR3 signaling pathway due to endosomal transport competition may enhance the distinguishability of CpG-pIC from ligand mixtures. These studies therefore identified antagonism mechanisms in signaling pathways as key to maintaining immune specificity under complex conditions.

24. **Drug-Metabolizing Enzymes and the Circadian Rhythm: A Multi-Tissue, Single-Nucleus Analysis Across Diverse Mouse Strains**

Andrew Hall[1], Ryan Weber[2], Ghassan Filimban[2], Margaret Duffield[2], Sofia Gagarin[3], Seerith Bal[3], Negar Fattahi[2], Shimako Kawauchi, PhD[4], Ali Mortazavi, PhD[2]
1. School of Medicine, University of California, Irvine
2. Department of Developmental and Cellular Biology, University of California, Irvine
3. Division of Biological Sciences, University of California, Irvine
4. Transgenic Mouse Facility, University of California, Irvine

Genetic variations in drug-metabolizing (xenobiotic clearing) enzymes and proteins (DMEs) can lead to aberrant metabolic phenotypes (e.g., rapid or poor metabolizers) that may positively or negatively impact the efficacy of a pharmacotherapeutic agent. One way genetic variation can modify the action of DMEs is through changes to their gene expression levels. As part of the Impact of Genomic Variability on Function consortium, we aimed to characterize these genetic differences utilizing eight Collaborative-Cross founder strains of mice (Founder Cohort). These strains exhibit a spectrum of innate genetic diversity and are well suited to explore the cell-type-specificity of gene expression variability in DME genes across the eight tissue groups sampled. To further our understanding of the distinct metabolic environments these mice may experience throughout their circadian rhythms (CR), we sampled two highly-divergent founder strains (CR cohort), 24 CAST/J (12M & 12F) and 24 B6/J (12M &12F) mice, across six Zeitgeber Timepoints (ZTs): ZT1, 5, 9, 13, 17, and 21. Here we describe the differential expression profiles of the founder cohort across tissues and examine the liver-specific DME rhythmicity captured thus far.

25. **RCA: An Improved Clustering Tool for single cell analysis**

Timothy Hamilton[1], Juan Vergara Najar[2], Eric J. Deeds[3,4]
[1] Bioinformatics Interdepartmental Program,
[2] Department of Computation and Systems Biology,
[3] Department of Integrative Biology,
[4] Institute of Quantitative and Computational Biology,
University of California Los Angeles, Los Angeles California, USA

The advent of single cell methods has revolutionized the fields of developmental and systems biology by enabling the investigation of complex tissues at the level of individual cells. Supporting these advancements is the fundamental idea that the gene expression of single cells can reveal distinct expression profiles that correspond to biologically distinct and meaningful groups. Unfortunately, recent work belies that notion; if a small percentage of cells is removed from the dataset and the typical analysis pipeline is repeated, the partitioning of remaining cells is significantly changed. We developed a new clustering method based on a resampling based approach to attempt to solve that problem,increasing the robustness of single cell clustering and thus the generalizability of the insights presented by the data.

26. **Markov Modeling of AML Transcriptome State Transitions Reveals Stochastic Dynamics of Disease Progression**

Danya Hassan[1], David Frankhouser[2], Denis O'Meally[2], Jennifer Ambriz [2], Ziang Chen [2], Yu-Hsuan Fu [3], Jihyun Irizarry [3], Bin Zhang [3], Ya-Huei Kuo [3], Guido Marcucci [3], Barbara Fortini [1], Cesar Espinoza [1], Russell Rockne[2]
[1] Henry E. Riggs School of Applied Life Sciences, Keck Graduate Institute
[2] Division of Mathematical Oncology and Computational Systems Biology, Beckman Research Institute, City of Hope
[3] Department of Hematologic Malignancies Translational Science, Beckman Research Institute, City of Hope

Acute Myeloid Leukemia (AML) is a heterogeneous hematologic malignancy characterized by dynamic changes in gene expression across disease development. We model AML progression in an mRNA state-space as a particle undergoing Brownian motion and extend this framework into a discrete-time Markov model to capture stochastic disease dynamics. Using a transcriptome state-space constructed from bulk RNA sequencing data of AML mouse models, we simulate shifts between three AML disease states under a stochastic state-transition model. This approach captures the evolution of the transcriptome as it progresses toward leukemia. We evaluate transition dynamics across simulated

trajectories, time-to-disease distributions, and stability of intermediate states to assess model consistency. Additionally, gene cluster trajectories and perturbation analyses reveal how coordinated gene expression shifts influence movement through the state-space. Our results demonstrate that AML progression follows a stochastic, state-dependent process, enabling quantification of transition likelihoods and providing insight into gene expression programs associated with leukemic progression.

27. **Developing a mathematical model of TNF production to understand the determinants of single macrophage heterogeneity**

Jasmine Herrera[1], Xiaolu Guo[1], Alexander Hoffmann[1]
[1] Signaling Systems Laboratory, Department of Microbiology, Immunology and Molecular Genetics, and the Institute for Quantitative and Computational Biosciences, University of California Los Angeles, Los Angeles California, USA

Tumor necrosis factor (TNF) is a critical inflammatory cytokine produced by macrophages, mediating immune communication, and is a key coordinator for innate immune responses. While TNF is known to be regulated by NFkB and MAPK signaling, recent studies found TNF secretion to be uncorrelated with NFkB activity. To address the sources of heterogeneity, we developed a mathematical model that integrates both NFkB and MAPK pathways and their regulation of TNF production. Our model simulation can reproduce all key previous experimental observations, from nascent TNF mRNA to secreted extracellular TNF, as well as the key kinases involved, such as MK2 and TTP. Using this validated framework, we analyzed TNF production at sequential biochemical steps - from transcription to secretion - to examine how cell-to-cell heterogeneity of MAPK and NFkB signaling correlates. Our results identify the key determinants that govern TNF heterogeneity.

28. **Uncovering Potential Landscapes for High-Dimensional Biological Systems**

Grady Kestler[1], Sergio Branciamore[1], Andrei Rodin[1]
Department of Computational and Quantitative Medicine
City of Hope, Duarte CA

Traditionally in systems biology, parsimonious dependency models are specified by experts and parameterization are learned from data. However, the extremely large variable space of multiscale biological problems presents an immense challenge for model specification. In rare situations, machine learning techniques have been employed to determine the best-fit model directly from the data, circumventing the model specification entirely. Here, we utilize two machine learning algorithms to determine a mathematical form and parameterization of the underlying statistical landscape directly from large-scale biological data. Bayesian Network (BN) modeling, an interpretable machine learning method, first determines the parsimonious dependency structure followed by sparse regression to uncover a functional form of the probability distribution. We initially apply the method to a well-studied spin-glass Ising model and then extend it to more complex biological applications, such as the analysis of scRNA-seq data from bone marrow cells to uncover a potential energy landscape of stem cell development.

29. **A spatiotemporal model of CD8+ T cell exhaustion dynamics in the tumor microenvironment**

Tyler King[1], Edgar Gonzalez[2], Evanthia T. Roussos Torres[2,3], Stacey D. Finley[1,4,5]
[1] Alfred E. Mann Department of Biomedical Engineering
[2] Norris Comprehensive Cancer Center, Keck School of Medicine
[3] Department of Medicine, Division of Oncology, Keck School of Medicine
[4] Department of Quantitative and Computational Biology
[5] Mork Family Department of Chemical Engineering and Materials Science
University of Southern California

In the solid tumor microenvironment (TME), cell-cell interactions drive CD8+ T cell exhaustion, posing a barrier to clinical interventions like immune checkpoint blockade (ICB). To understand the spatiotemporal regulation of immune activity, we developed an agent-based model of the TME which tracks how contact-dependent and indirect interactions modulate CD8+ T cell behaviors. We obtain initial conditions from multiplexed immunohistochemistry images of lung metastases and simulated for 25 days with and without ICB. Regardless of treatment, most metastases (79%) progressed over all replicates; the remainder displayed a mixture of progression, stable disease, and regression. Sensitivity analysis showed the relative impacts of model parameters on final outcomes correspond to how strongly they affect the dynamics of cancer and CD8+ T cells. Additionally, we observed significant heterogeneity in the temporal exhaustion patterns of CD8+ T cells at the single-cell level. Overall, we demonstrate that ABMs reveal how the TME's spatial features affect outcomes.

30. **Identifying Causes of Donor-Variability in Human Macrophage Preparations**

Vyas Koduvayur[1], Noah Yan[1], Aleksandr Gorin[2], Sarina Lowe[3], and Alexander Hoffmann[1]
[1] Department of Microbiology, Immunology, and Molecular Genetics,
[2] Department of Medicine, Division of Infectious Diseases,
[3] Department of Medicine, Division of Digestive Diseases,
University of California Los Angeles, Los Angeles California, USA

Primary human macrophages are commonly generated from peripheral blood monocytes, but substantial variability across samples can limit the reproducibility of findings. It remains unclear whether this heterogeneity reflects intrinsic differences among donors or arises from technical factors while generating the cultures. Here, we generated and analyzed bulk RNA-seq data from monocytes and MCSF-differentiated macrophages from 19 healthy human donors. We found that transcriptional variability is greater at Day 7 than Day 0, with replicate day 7 cultures appearing highly consistent. This suggests that variability is amplified during differentiation rather than being driven by technical inconsistencies in culture conditions. Principal component–based modeling allowed us to partially predict macrophage states from monocyte profiles. Focusing on outlier donors, we identified a notable interferon signature alongside surprising expression of T-cell marker genes. These results suggest that variable T-cell contamination in monocyte preparations may influence different degrees of interferon-mediated steering of the macrophage differentiation pathway.

31. **Effects of temperature and relative humidity on the behavior of Argentine ants**

Dimitrios-Georgios Kontopoulos[1], Catherine Tran[1], Sofia Barreras[1], Noa Pinter-Wollman[1]
[1] Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA

Ant colonies represent distributed systems in which social interactions among individuals govern the collective actions of the system. Thus, to accurately forecast how climate change will impact ant colonies, it is imperative to understand how their social traits respond to changes in climatic factors. We examined how temperature and relative humidity influence the performance of two key social traits of ants: movement speed and interaction rate in Argentine ants (*Linepithema humile*) from UCLA and UCR. We exposed groups of ants to 10 combinations of temperature and relative humidity and recorded colony behaviors, generating high-resolution trait performance datasets, which we analyzed using novel temperature-humidity performance surface models. We found that as movement speed increases with temperature (up to an optimum), the rate of social interactions decreases. Our results provide insights into how climate change may impact the behavior of this highly invasive social species.

32. **Reaction-Diffusion Systems Generate Dynamical Regimes via Juxtacrine and Paracrine Signaling**

Minnal Kunnan[1], Benjamin Swedlund[1], Soha Ben Tahar[2], Leonardo Morsut[1]
[1]Eli and Edythe Broad CIRM Center, Department of Stem Cell Biology and Regenerative Medicine,
Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[2]Department of Mechanical and Industrial Engineering, Northeastern University, Boston, United States

Reaction-diffusion (RD) systems generate spatial patterns through coupled reaction and diffusion and are widely used to model biological pattern formation. However, classical RD models require differential diffusion, a constraint not always consistent with biological signaling. Here, we present an alternative implementation in which spatial range is encoded through signaling modality - juxtacrine (contact-mediated) and paracrine (diffusive) - rather than just differential diffusion, and show that this architecture is functionally equivalent to classical RD systems. We develop a computational pipeline in 1D and 2D to explore how parameter regimes and initial conditions govern system behavior. Parameter sweeps identify multiple dynamical regimes, including all-on, all-off, Turing, and stalled propagation, with distinct spatial signatures quantified by autocorrelation and Fourier analysis. These findings are validated in 2D finite element simulations and in vitro engineered cell-based circuits, which recapitulate key qualitative features of the patterned and propagation-limited regimes.

33. **Rare tumor cell fusion drives distinct and drug-resistant cancer cell states**

Shinya Kusumoto[1], Maria Sol Recouvrex[1], Sailasya Munamarty[1], Michael Richardson[1], Beth Y. Karlan[1,3], Sandra Orsulic[1,2,3,4]
[1]Department of Obstetrics & Gynecology,
[2]Department of Pathology & Laboratory Medicine,
[3]Jonsson Comprehensive Cancer Center, University of California Los Angeles, CA,
[4]VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Intratumoral heterogeneity drives cancer progression, recurrence, and therapeutic resistance, but the origins of aggressive tumor cell states remain unclear. Tumor cell fusion has been proposed as a source of such diversity, yet fusion-derived cancer cells are rare and difficult to isolate. Here, we developed a Cre/Flexon-mCherry reporter system combined with flow cytometry to identify and isolate fusion-derived tumor cells. mCherry-positive fused cells emerged after co-culture and were purified by FACS. These cells showed increased chromosome number and RNA content relative to parental cells. RNA-seq revealed a distinct transcriptional state enriched for proliferation- and DNA repair-related programs. Functionally, fused cells exhibited enhanced spheroid-forming ability in 3D culture and reduced sensitivity to doxorubicin. Together, these findings show that rare tumor cell fusion events can generate functionally distinct cancer cell states linked to tumor progression and therapy resistance, supporting cell fusion as a potential driver of intratumoral heterogeneity.

34. **Modeling reveals the strength of weak interactions in stacked ring assembly**

Leonila Lagunes[1], Eric J. Deeds[1]
[1]Integrative Biology and physiology, University of California Los Angeles, Los Angeles, California, USA

While cells regulate vital processes with macromolecular machines, they synthesize these machines as individual components that assemble into functional complexes. A common motif is a stacked ring. Insights into stacked trimer assembly are crucial for understanding complex regulation. Here, we developed a mathematical model of stacked trimer assembly that accounts for different binding affinities between and within rings. Our main finding is that deadlock – a severe form of kinetic trapping– can be extremely long. Deadlock is worst when all the interfaces have high binding affinities. We predict that evolution avoids stacked trimers with uniformly strong affinities. Our findings reveal that most solved structures lack such strong interactions. To understand the origins of deadlock, our pathway analyses reveal that strong binding affinities lead to the use of multiple pathways, which consume subunits and intensifies deadlock. Overall, our work provides critical insight into the evolutionary pressures that have shaped stacked ring assembly.

35. **Computational and Structural Investigation of Small Molecules Stabilizing the Extrinsic Tenase Complex**

Kathy Lai, Kenneth Childers, Ph.D. Stevan Pecic, Ph.D.
Department of Chemistry and Biochemistry
California State University, Fullerton.

The tissue factor–factor VIIa (TF: FVIIa) complex is the primary initiator of blood coagulation, yet its ability to sustain hemostasis is limited by its transient nature. To identify small molecules that stabilize this complex, we performed virtual high-throughput screening (ICM Pro) targeting potential binding sites at the TF:FVIIa interface. Representative docking poses, such as ruxolitinib, show predicted interactions with key allosteric sites (M306, G372) and the TF exosite (K165). Top hits were selected based on docking scores, yielding five compounds for experimental testing. We are currently optimizing a colorimetric assay to evaluate the ability of top-scoring compounds to modulate TF: FVIIa amidolytic activity. Preliminary conditions (100 nM FVIIa, 200 nM sTF, 1 mM S-2288) provide a TF-dependent signal suitable for detecting stabilization. This work establishes a screening pipeline to identify small-molecule stabilizers of TF: FVIIa, with the potential to regulate the extrinsic blood coagulation pathway.

36. **Spatially Informed Motif Analysis of Cell–Cell Communication in the Tumor Microenvironment**

Yingtong Liu[1] , Xiaojun Wu[1], Evanthia T. Roussos Torres[2], Adam L. MacLean[1]
[1] Department of Quantitative and Computational Biology,
[2] Department of Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine,
University of Southern California, Los Angeles, CA, USA

The tumor microenvironment (TME) comprises diverse cell populations whose interactions shape tumor progression. Cell–cell communication (CCC) analysis using scRNA-seq enables the inference of ligand–receptor-mediated signaling networks and the identification of cell-state-specific interactions. Prior work has shown that motif analysis of CCC networks can reveal recurrent signaling circuits associated with biological conditions. However, scRNA-seq lacks spatial context, limiting its ability to capture spatially constrained interactions. Here, we extend CCC motif analysis to spatial transcriptomics by leveraging CellNEST, a graph attention network (GAT)-based framework, to model spatially informed cell–cell interactions. Applied to breast cancer datasets across multiple time points, this approach enables the characterization of spatial CCC patterns and the exploration of motif organization under spatial constraints. Preliminary results suggest that incorporating spatial information refines inferred communication networks and reveals spatially localized signaling features not captured by scRNA-seq alone.

37. **Protein frustration distinguishes strong protein degraders across PROTAC and Molecular Glue-mediated ternary complexes**

Ning Ma[1], Tianyi Yang[1], Wenyuan Wei[1,2], Veerabahu Shanmugasundaram[3], Nagarajan Vaidehi[1]
[1] Department of Computational and Quantitative Medicine, Beckman Research Institute of City of Hope, Duarte, CA, USA
[2] Irell and Manella Graduate School of Biological Sciences, Beckman Research Institute of City of Hope, Duarte, CA, USA
[3] Bristol Myers Squibb, Cambridge, MA, USA

Protein frustration has emerged as a useful descriptor of functionally critical regions in proteins, yet its utility in targeted protein degradation remains incompletely defined. Here, we integrated molecular dynamics simulations, AlphaFold3 modeling, and frustration analysis across PROTAC and molecular glue -mediated ternary complexes to identify computational metrics that distinguish strong from weak degraders. In SMARCA2-PROTAC-VHL complexes, higher interface frustration tracked stronger cooperativity and, together with conformational entropy, captured differences in ternary-complex dynamics. In BRD4-PROTAC-cereblon and GSPT1-glue-cereblon systems, frustration separated strong from weak degraders and was further strengthened by ligand-protein interaction energy. Notably, AF3 ensembles recovered the same frustration-based trends identified from molecular dynamics. These results support frustration as an accessible metric for mechanism-guided degrader evaluation and design, and further suggest that highly frustrated regions mark functionally important interfaces in productive ternary complexes.

38. **Measuring All-Age and Early Onset Colorectal Cancer Incidence using Electronic Health Records (EHR) Data**

Tomas K. D. Manea[1,2], Ahmad Alkasir[1], Mukta Ubale[1], LaRon Hughes[1], Xingyao Chen[1], William Paiva[4], Dursun Delen[4,5], Zheng Han[6], Naim Matasci[1,7], Mitchell E. Gross[1,3], Reva Basho[1], Gabriel Seidman[1]
[1]Ellison Medical Institute
[2]Department of Population and Public Health Sciences,
[3]Department of Medicine,
Keck School of Medicine of USC, Los Angeles, CA, USA
[4]Center for Health Systems Innovation (CHSI), Spears School of Business,
[5]Department of Management Science & Information Systems, Oklahoma State University, Stillwater, OK, USA
[6]Department of Biomedical Engineering, University of Central Oklahoma, Edmond, OK, USA
[7]Department of Engineering,
Harvey Mudd College, Claremont, CA, USA

Cancer registries lag reporting in their trends by 2-3 years, rendering surveillance outdated and hindering accurate disease characterization. This study evaluates the effectiveness of EHRs for surveillance of emerging cancer trends. Using Oracle Health Real-World Dataset (OHRWD), we calculated the age-standardized incidence rate of colorectal cancer—between 2012 and 2024—using the direct method and the US 2000 Standard population. Colorectal cancer incidence data was extracted from OHRWD using diagnostic codes from the ICD 9th and 10th revisions. The negative binomial regression demonstrated a statistically significant increase in CRC incidence—for all ages—of 2.0% per year from 2012–2022 (IRR = 1.020, 95% CI: 1.006–1.035, p = 0.006), compared to a declining trend observed in SEER 8 and 21 registries. As CRC incidence increasingly reflects cohort-specific exposures and lifestyle patterns globally, policy responses should be guided by more timely data, such as from EHR or claims data.

39. **Apoptosis Signaling Drives Intrinsic Drug Resistance in Prostate Cancer**

Diamond Mangrum[1], Stacey Finley[1,2,3]
1 Alfred E. Mann Department of Biomedical Engineering,
2 Mork Family Department of Chemical Engineering and Materials Science,
3 Quantitative and Computational Biology,
University of Southern California, Los Angeles California, USA

Anticancer drug resistance is challenging to overcome because it can arise through both intrinsic and acquired mechanisms, each driven by distinct cellular machinery. In hormone dependent cancers, specifically, there is a sharp need for therapies that target hormone insensitive prostate cancer due to the growing incidence of castration resistant prostate cancer. Therefore, optimizing the pathways that regulate apoptosis in hormone-dependent cancers offers a promising strategy for developing therapies that induce apoptosis to inhibit tumor progression, since these mechanisms do not depend on hormonal signaling. Here, we used several computational tools to design and exploit a caspase-mediated prostate cancer ordinary differential equation model to identify key modalities that increase the propensity toward apoptosis across three separate pro-apoptotic drugs. Overall, we demonstrate that apoptosis dynamics can be accurately

captured in response to each of the three drugs and identify which features of the model represent viable targets for overcoming intrinsic drug resistance.

40. **Interpretable AI for dynamic network mapping of gene dependencies in spatial transcriptomics data**

Babgen Manookian[1], Bohan Zhang[2], David W. Craig[2], Sergio Branciamore[1], Andrei S. Rodin[1]
[1]Department of Computational and Quantitative Medicine
[2]Department of Integrative Translational Sciences
City of Hope, Duarte California, USA

Spatial transcriptomics (ST) maps gene expression within tissue architecture, however, understanding the dynamic rewiring of gene regulatory networks across the tissue remains unsolved. To fill this gap, we developed Dynamically Resolved Omics Mapping Over Space (DROMOS) to identify and localize gene network change-points at tissue boundaries and, subsequently, the gene sets that enable these transitions. DROMOS ranks spatially variable genes using spatial mutual information, which quantifies dependence between expression and location without requiring predefined clusters. It then constructs an interpretable AI model and locally rescores the model across the tissue using a distance-based kernel. In a glioblastoma dataset, DROMOS detected discrete change-points and sequential activation of ACTB, QKI, and CST3. It resolved two distinct boundary transitions: the first centered on QKI and CST3, and the second involving all three genes, with ACTB showing the strongest enabling activity. Future work will extend DROMOS to additional datasets, including triple-negative breast cancer, for cross-sample comparison.

41. **Perturbing MECOM self-activation reveals distinct geometries and transition paths across hematopoietic cell fate landscapes**

Jonathan Martinez [1*], Anupam Dey [1*], MeiLu McDermott [1], Lenhard K. Rudolph [2,3], Adam L. MacLean [1]
[1] Department of Quantitative and Computational Biology, Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, CA 90089, USA
[2] Research Group on Stem Cell and Metabolism Aging, Leibniz Institute on Aging, Fritz Lipmann Institute (FLI), Jena, Germany
[3] Medical Faculty, Jena University Hospital, Friedrich Schiller University, Jena, Germany
[*] These authors contributed equally

Hematopoietic stem and multipotent progenitor cells can be controlled by mutual inhibition of MECOM and CDK6. High MECOM corresponds to a stem state and high CDK6 to a multipotent state. The IGF pathway promotes CDK6 and inhibits MECOM. To investigate stem-to-multipotent transitions, we modeled MECOM-CDK6 dynamics using differential equations. Bifurcation analysis revealed tetrastability suggesting that early stem cell differentiation proceeds by fine-scaled transitions. Bifurcation analysis of MECOM self-activation under perturbation revealed two IGF-dependent tristable regimes with distinct geometries. At high IGF, transitions occurred through a stable intermediate via a cusp geometry. At low IGF, transitions bypassed the intermediate reminiscent of an elliptic umbilic-like catastrophe. Stochastic simulations and minimum action path analysis show that the multipotent state is most stable at high IGF, while the stem state is highly stable at low IGF. Overall, we show how MECOM self-activation reshapes stem cell fate decisions under the influence of diet and metabolism.

42. **Modeling the dynamics of EMT reveals genes associated with pan-cancer intermediate states and plasticity**

MeiLu McDermott[1], Riddhee Mehta[1], Evanthia T Roussos Torres[2], Adam L MacLean[1]
[1]Department of Quantitative and Computational Biology, Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, CA USA
[2]Department of Medicine, Division of Medical Oncology, Keck School of Medicine, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA USA

Epithelial–mesenchymal transition (EMT) is a cell state transition co-opted by cancer that drives metastasis via stable intermediate states. Here we study EMT dynamics to identify marker genes of highly metastatic intermediate cells via mathematical modeling with single-cell RNA sequencing (scRNA-seq) data. Across multiple tumor types and stimuli, we identified genes consistently upregulated in EMT intermediate states, many previously unrecognized as EMT markers. Bayesian parameter inference of a simple EMT mathematical model revealed tumor-specific transition rates, providing a framework to quantify EMT progression. Consensus analysis of differential expression, RNA velocity, and model-derived dynamics highlighted *SFN* and *NRG1* as key regulators of intermediate EMT. Independent validation confirmed *SFN* as an intermediate state marker. Our approach integrates modeling and inference to identify genes associated with EMT dynamics, offering biomarkers and therapeutic targets to modulate tumor-promoting cell state transitions driven by EMT.

43. **Use of the CRISPR/Cas9 system for gene deletion of *CH25H* in Thp-1 cells to assess its role in atherosclerosis**

Youstina M. Meseha[1], Deborah A. Fraser[1], Jennifer M. Johnston[1]
Department of Biological Sciences,
California State University Long Beach, Long Beach California, USA

Cholesterol 25-hydroxylase (CH25H) has been noted to be involved in cholesterol metabolism aiding the complement protein C1q. To determine the role of CH25H in the development of atherosclerosis, we intend to use the CRISPR/Cas9 technology to delete *CH25H* in Thp-1 monocytes. A multiplexing technique will be utilized to guide the Cas9 endonuclease to produce two double strand breaks, one at the beginning of the gene and one at the end. Various bioinformatics tools were used to identify ten optimal single guide RNAs (sgRNAs) based on their efficiency and specificity scores. sgRNAs were ordered as single stranded oligos, annealed, phosphorylated, and inserted into a Cas9 expression plasmid. The ten sgRNAs will then be delivered to Thp-1 cells via nucleofection and assessed for the presence of insertions and deletions. The most efficient sgRNA will then be used to perform the deletion of *CH25H*. Sequencing will be used to confirm gene deletion.

44. **Genome-wide analysis of mRNA expression and modifications profiles in FSHD cell lines**

Negar Mojgani[1,2], Erisa Taghizadeh[1], Xiangduo Kong[3], Jasmine Sakr[4], Kyoko Yokomori[3], Ali Mortazavi[2]
[1]Department of Developmental and Cell Biology, University of California Irvine, Irvine, USA
[2]Department of Systems Biology, University of California Irvine, Irvine, USA
[3]Department of Biological Chemistry, University of California Irvine, Irvine, USA
[4]Department of Neurobiology and Behavior, University of California Irvine, Irvine, USA

Myogenesis is orchestrated by precise post-transcriptional regulation. Dysregulation of these mechanisms drives RNA metabolic defects that contribute to many muscle diseases, causing structural instability, metabolic dysfunction, and loss of muscle mass and function. RNA modifications are widespread regulators of gene expression, influencing alternative splicing, polyadenylation, RNA stability, and other co- and post-transcriptional processes that shape protein levels and phenotype. In skeletal muscle, reversible modifications such as m6A and m5C, along with irreversible changes including A-to-I editing, pseudouridylation, and 2'-O-methylation, regulate myogenesis, homeostasis, and regeneration. We used ONT bulk long-read direct RNA sequencing to characterize the epitranscriptomic landscape of control and engineered facioscapulohumeral dystrophy (FSHD) human skeletal myotubes. Preliminary analysis of FSHD targets identified more than 20 genes with disease-specific differential modification patterns at individual sites, with ZSCAN4 and H3Y1 exhibiting markedly distinct modification profiles between mutant and control samples. Ongoing analyses will investigate transcriptome-wide modification differences, including non-FSHD disease-specific profiles.

45. **Beyond Static Contacts: Dynamic Network Signatures Define Nanobody Binding**

Elizaveta Mukhaleva[1], Talant Ruzmetov[1], Grigoryi Gogoshin[1], Nagarajan Vaidehi[1], Sergio Branciamore[1], Andrei S. Rodin[1]
[1] Department of Computational and Quantitative Medicine, Beckman Research Institute of The City of Hope, Duarte California, USA

Nanobodies are powerful biotechnological and therapeutic tools due to their small size, stability, and ability to recognize diverse epitopes. While binding specificity is typically attributed to complementarity-determining regions (CDRs), the role of nanobody dynamics remains underexplored. Here, we integrate residue contact analysis with network-based measures of dynamic coupling derived from molecular simulations to investigate how structural and dynamic features jointly govern nanobody–target interactions. We identify distinct classes of nanobodies based on binding modes. One class shows variation in residue-level contacts, particularly in framework region 2 (FR2) and CDR1. In contrast, other classes share similar contact architectures but differ in dynamic coupling, reflected in contributions from CDR1, CDR2, and CDR3. Notably, CDR3 exhibits limited variation in contacts yet plays a dominant role in dynamic allosteric communication. These findings reveal that nanobody binding is shaped by dynamics, not static contacts alone, emphasizing the importance of incorporating protein dynamics into design strategies.

46. **Modeling IFNγ-STAT1 Signaling Reveals Mechanisms of Macrophage Memory**

Darcy Niu[1]**,** Noa Harriott[1], Aleksandr Gorin[2], Xiaolu Guo[1,3], Alexander Hoffmann[1,3]
1. Department of Microbiology, Immunology, and Molecular Genetics, UCLA
2. Department of Medicine, Division of Infectious Disease, David Geffen School of Medicine, UCLA
3. Institute for Quantitative and Computational Biosciences, UCLA

As innate immune sentinel cells, macrophages can acquire trained immune memory following cytokine or pathogen exposure, enabling enhanced responses to subsequent challenges. Interferon-γ (IFNγ), a key macrophage activator, has

been implicated in establishing such memory-like states. Notably, we observe persistent IFNγ-STAT signaling lasting up to four days after cytokine withdrawal, though the underlying mechanism remains unclear. Emerging evidence suggests macrophages sustain signaling through IFNγ sequestered in the extracellular matrix (ECM) and gradually released. We hypothesize that this ECM-mediated retention, together with STAT1-driven positive feedback, underlies sustained signaling. To test this, we developed an ordinary differential equation model incorporating key components of the IFNγ-STAT1 pathway alongside ECM sequestration and release dynamics. Model simulation recapitulates experimental observations, including biphasic pSTAT1 dynamics, disruption and recovery of signaling following JAK inhibition, and attenuation of feedback via protein synthesis inhibition. Together with experimental validation, this framework provides mechanistic insight into key signaling components that underlie macrophage memory.

47. **Computational Model of *Drosophila* Wing Disc Eversion**

Ethan Nowaski[1], Benjamin Speybroeck[2], Weitao Chen[1], Zhiliang Xu[3], Navaira Sherwani[1], Jeremiah Zartman[2], Mark Alber[1]
[1] Department of Mathematics and Interdisciplinary Center for Quantitative Modeling in Biology, University of California, Riverside, CA
[2] Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN
[3] Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN

A critical open question in developmental biology involves how organs form and maintain their shape. The robust formation of organs during development depends on the careful regulation of cellular processes such as adhesion, stiffness, and internal pressure to create tissue-scale architecture. This project utilizes the fruit fly wing imaginal disc, a powerful biological model system, to study how downstream effectors of ecdysone signaling contribute to regulating cell mechanical properties that influence cell shapes and overall tissue structures during wing disc eversion. We report a novel extension of a two dimensional subcellular element computational model of the developing *Drosophila* wing [1]. Candidate mechanisms of wing disc eversion are tested through iterations between biologically calibrated computational simulations and experiments. This work implements and tests how multiple cellular processes coordinate to define organ morphology, which has implications for developmental processes and diseases such as cancers and metastasis.

1. Nilay Kumar, Jennifer Rangel Ambriz, Kevin Tsai, Mayesha Sahir Mim, Marycruz Flores-Flores, Weitao Chen, Jeremiah J. Zartman and Mark Alber [2024], Balancing competing effects of tissue growth and cytoskeletal regulation during Drosophila wing disc development. *Nat Commun* 15, 2477 (2024).

48. **Chromosome Structure Remodeling in Innate Immune Training**

Sohyeon Park[1,2*], Ye Wang[2,3], Aleksandr Gorin[1,4], Frank Alber[1,2], Alexander Hoffmann[1,2]
[1] Department of Microbiology, Immunology, and Molecular Genetics,
[2] Institute for Quantitative and Computational Biosciences,
[3] Department of Pathology and Laboratory Medicine,
[4] Department of Medicine, Division of Infectious Diseases
University of California Los Angeles, Los Angeles California, USA

Macrophages exhibit the capacity for long-lasting trained immune memory after prior stimuli, but the underlying mechanisms that maintain this state are not well understood. Previous studies have largely focused on histone modifications that define the enhancer landscape. Although these marks have been shown to be highly durable, they are, in principle, reversible through the action of eraser enzymes. Two primary mechanisms have been proposed to explain the maintenance of the enhancer landscape: sustained activity of signal-dependent transcription factors (SDTFs) or the engagement of lineage determining transcription factors (LDTFs). Here, we show that immune training also induces changes in chromosome organization and nuclear localization, suggesting a novel memory mechanism for maintaining the trained state of stimulus-exposed macrophages. Specifically, we report that immune training induces large-scale chromosome structural rearrangements that are associated with potentiated gene expression responses. Using high-depth Hi-C sequencing analysis and Integrative Genome Modeling (IGM), we reveal stimulus-specific changes in chromosome structure in human macrophages. By integrating Hi-C data with ATAC-seq and H3K4me1 CUT&Tag profiles, we found that these structural changes coincide with DNA accessibility and poised enhancer activity. Furthermore, by modeling the ensemble of single-cell 3-D genome structures, we identified correlations between structural features, such as radial positioning, proximal enhancer counts, and proximity to nuclear speckles with stimulus specific gene expression. Together, our findings suggest that immune training involves rearrangements of large-scale chromosome structure and nuclear positioning that may contribute to the durability of innate immune memory.

49. **Distinct but Overlapping Sets of Gene Expression Programs Drive Macrophage and Dendritic Cell Differentiation**

Natalie T. Pham[1,5*], Sophia A. Lambrecht[1*], Benancio Rodriguez[2], Aleksandr Gorin[2,4], Alexander Hoffmann[2,3]
[1]BIG Summer Program, Institute for Quantitative and Computational Biosciences, UCLA
[2]Department of Microbiology, Immunology, and Molecular Genetics, UCLA
[3]Institute for Quantitative and Computational Biosciences (QCBio), UCLA
[4]Department of Medicine, David Geffen School of Medicine, UCLA
[5]UC LEADS, Department of Molecular, Cellular, and Developmental Biology, UCSB
[*]These authors contributed equally to the work

Macrophages and dendritic cells originate from hematopoietic progenitor cells in the bone marrow, and mediate related immune system processes, including phagocytosis and antigen presentation. However, the specific genes and regulatory pathways involved in the differentiation of each cell type remain under investigation. We undertook an unbiased RNA-seq transcriptomic profiling approach to track macrophage and dendritic cell differentiation driven by three growth factors over the course of nine days. Using differential gene expression analysis, principal component analysis, and k-means clustering, we identified gene groups that are either common to all or specific to each growth factor pathway. Gene Ontology analysis identified functions associated with these genes. We found that NFkappaB mutants affected each growth factor pathway differently, suggesting that inflammatory dysregulation alters differentiation pathways. These findings serve as a starting point for a systematic analysis of myeloid differentiation to reveal key biomarkers and guide potential therapeutic intervention in inflammatory disease.

50. **Multiomic State-Transition Framework Reveals Chemotherapy-Induced Metabolic Reprogramming in Acute Myeloid Leukemia**

Jennifer Rangel Ambriz[1*], Ziang Chen[1*], Yu-Hsuan Fu[2], David E. Frankhouser[1,2], Denis O'Meally[1], Lianjun Zhang[2], Ying-Chieh Chen[2], Sergio Branciamore[1], Jihyun Irizarry[2], Bin Zhang[2], Guido Marcucci[2], Russell C. Rockne[1], Ya-Huei Kuo[2]
[1]Division of Mathematical Oncology, Department of Computational and Quantitative Medicine, [2]Department of Hematologic Malignancies Translational Science, Gehr Family Center for Leukemia Research, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA
*These authors contributed equally

Acute myeloid leukemia (AML) has poor survival due to relapse driven by metabolic reprograming and treatment failure, highlighting the need for frameworks that characterize disease dynamics and treatment response. We previously applied state-transition theory to model AML evolution as trajectories of mRNA and miRNA transcriptomes in AML state-spaces, characterized by a leukemogenic potential with critical points representing health, transition, and leukemia states. Here, we characterize chemotherapy-induced transcriptomic and metabolic changes using state-transition theory. Longitudinal multiomic profiling of peripheral blood from a murine model of AML treated with chemotherapy revealed a temporal desynchronization between mRNA and miRNA trajectories post-treatment. Extending to a 2D framework captured chemotherapy-induced alterations in the mRNA-miRNA interplay and multiomic potential. Gene Set Variation Analysis showed downregulation of OXPHOS, glycolysis and fatty acid metabolism during remission, with reactivation during relapse. This multiomic state-transition framework provides a systematic approach to characterize treatment response and identify metabolic vulnerabilities in AML.

51. **Modeling the Dynamics and Stability of Bacterial Populations Using Different Methods of Horizontal Gene Transfer**

Jack Root[1*], Shenshen Wang[1*],
[1]Department of Biophysics,
University of California Los Angeles, Los Angeles California, USA
* These authors contributed equally.

To better understand the dynamics and evolution of bacterial populations, it is essential to analyze the role that horizontal gene transfer (HGT) plays. By allowing bacteria to exchange DNA segments, HGT can have two seemingly contradictory roles: (a) facilitate rapid homogenization to a single allele, or (b) maintain a population's diversity. While several papers have analyzed HGT, none have addressed what determines HGT's role within a bacterial population. Using both stochastic and deterministic modeling, we simulate bacterial populations using two different fitness landscapes: one where two equally fittest genes "propagate" similarity to themselves via HGT interactions, and one where the two equally fittest genes don't "propagate" similarity. While HGT serves as a tool for homogenization in the first fitness landscape, it maintains diversity in the second fitness landscape. Exploring what causes these differing roles of HGT can provide insight into bacterial evolution, phylogeny, and the design and implementation of antibiotics.

52. **The (Not So) Sudden Transition to Oscillations in Random Networks of Stochastic Coupled Excitable Units**

Robert J. Rovetti
Dept. of Mathematics Statistics and Data Science, Loyola Marymount University, Los Angeles, CA

Excitable units that can be stochastically activated either by self-activation or by nearest-neighbor recruitment, followed by a refractory period, have been used to model the dynamics of calcium release units in cardiac myocytes. When arranged in a fixed lattice, the resulting network undergoes a bifurcation to oscillatory (period-2) behavior as the activation probability is increased, with a sudden transition to nearly full amplitude post-bifurcation. Here we study the same excitable units on random networks following various degree distribution laws, and observe a qualitative difference in power-law networks, wherein the amplitude increases gradually in a linear fashion, resulting in smooth control over the strength of the oscillations and the loss of sudden phase-transition-like behavior.

53. **Artificial Intelligence Driven Nanobody Design**

Talant Ruzmetov[1], Eli Mukhaleva[1], Amin Safdari[1], Fredrik Sadler[2], Ning Ma[1], Sergio Branciamore[1], Andrei Rodin[1], Sivaraj Sivaramakrishnan[2], Nagarajan Vaidehi[1]
[1] Department of Quantitative and Computational Medicine Division of Mathematical Oncology and Computational Systems Biology, Beckman Research Institute, City of Hope
[2] Department of Genetics, Cell and Developmental Biology, University of Minnesota, Twin-Cities

Nanobodies, single-domain antibody fragments derived from camelids, possess favorable therapeutic properties such as high stability, compact size, and low immunogenicity, yet their discovery is limited by inefficient traditional screening methods. Here, we introduce a foundation AI model for nanobody design trained on ~70,000 low-polyreactive, expressed sequences. The model employs a Transformer-based Variational Autoencoder (VAE) to learn latent representations of aligned repertoires, augmented with physics-informed priors. Residue-residue relationships are incorporated into attention via adjacency matrices, enabling capture of coevolutionary and biophysical constraints. We further develop an integrated in silico pipeline combining polyreactivity filtering, framework similarity, frustration profiling, and epitope-specific binding evaluation to prioritize candidates. Experimental validation shows 6 of 8 designed sequences exhibit improved binding over a benchmark. This framework enables scalable exploration of nanobody sequence space and supports the design of novel, high-quality variants with enhanced biochemical and biophysical properties.

54. **Clustering dynamics of elastically interacting biological cells**

Mst Suraiya Akter Shefa, Mykhailo Potomkin
Department of Mathematics, University of California Riverside, Riverside California, USA

Durotaxis, the directed migration of motile cells in response to mechanical cues, plays a central role in the behavior of many biological cell types, including cancer and endothelial cells. Through substrate-mediated elastic interactions, durotactic cells can also exhibit rich collective dynamics that remain incompletely understood. In this work, we employ a computational PDE model of motile durotactic cells to examine how elastic interactions govern clustering and collective motion. We first show that the trajectory of an isolated cell on a substrate with spatially heterogeneous bending stiffness depends strongly on model parameters, resulting in preferential migration toward either softer or stiffer regions. We then demonstrate that elastic coupling between cell pairs can generate persistent rotational motion. Extending the analysis to larger groups of 10–20 cells, we observe robust cluster formation with negligible net cluster displacement despite sustained activity of individual cells within the aggregate. Finally, we quantify how this internal activity influences long-time cluster center displacement and relate the resulting effective diffusion to the properties of individual cells. These results provide new insight into how substrate elasticity can organize cell collectives and drive emergent modes of motion.

55. **Selective packaging of bacterial host DNA by a transposable phage**

Runhang Shu[1], Kehan Ren[3], Tim Downing[3], Albert Siryaporn[1,2]
[1]Department of Molecular Biology & Biochemistry, University of California Irvine
[2]Department of Physics & Astronomy, University of California Irvine
[3]Department of Microbiology and Molecular Genetics, University of California Irvine

During the transition from lysogeny to lysis, phage must package prophage DNA into procapsids in order to produce new progeny. In many phages, the packaging event is initiated by DNA cleavage at a packaging recognition site. One packaging event terminates when the procapsid is “full” of DNA, a process referred to as headful packaging. Because the procapsid can accommodate more DNA than a complete phage genome, typically 2–10% more than the genome length is packaged. Transposable phages, such as Mu, replicate by inserting themselves at random sites in the bacterial genome via

transposition. In each Mu capsid, bacterial DNA is present at both termini. However, it remains unclear whether Mu phage packages its own DNA along with adjacent bacterial DNA through headful packaging. Given Mu phage lacks a specific integration site, DNA packaging could proceed through an alternative mechanism. We investigated packaging of *Pseudomonas aeruginosa* DNA by the transposable Mu-like phage DMS3 through whole genome deep sequencing. We developed a method, called REALong-seq (Restriction Enzyme Assisted Long read sequencing), to sequence the intact single phage DNA from end-to-end. Our analysis identified significant disparities between bacterial DNA contained in the capsid and bacterial DNA flanking prophages in the bacterial chromosome. In particular, bacterial sequences in packaged mature phages are not due to an excision of bacterial DNA flanking the Mu prophage. Rather, our results suggest a mechanism in which the prophage is excised from the bacterial chromosome and concatenated with short leader sequences on the left terminus before packaging into the capsid. We identified consensus sites that are assembled from the bacterial genome that form the leader sequences. Our data identifies separate roles for the leader sequence and the longer transduced bacterial DNA that is packaged in the phage. These results have important implications for how DNA is excised and processed before packaging into the capsid and how Mu-like phage DNA is inserted in the bacterial genome. In addition, the data shed light on the diversity and function of sequence information contained within phage capsids.

56. **Transcriptional tipping points in the frontonasal ectodermal zone**

Kai Silkwood[1,2,3], ChanHee Mok[4], Diane Hu[4], Ralph Marcucio[4], Arthur D Lander[1,2]
[1]Center for Complex Biological Systems
[2]Department of Systems Biology
[3]Mathematical, Computational, and Systems Biology program
University of California, Irvine
[4]Department of Orthopaedic Surgery
University of California, San Francisco

The frontonasal ectodermal zone (FEZ) is a small region in the developing chick beak which regulates spatial coordination through sonic hedgehog (SHH) signaling. The signals that drive the cells to express SHH are not well understood. Here we use BigSur, a method of identifying statistically significant correlations between genes in single-cell RNA-seq data, to monitor gene-gene relationships over time. Using tipping point theory, we identify many candidate cell-state transition driver genes; two of which (PITX2 and NPAS3) have been experimentally validated to be required for normal SHH expression in the FEZ. This work establishes correlation-based tipping point analysis using BigSur as a viable approach for identifying upstream regulators of cell state transitions.

57. **MICAL2 expression promotes invasion and metastasis by cell autonomous and non-cell autonomous mechanisms in Pancreatic Cells**

Katherine Simms[1], Esra T. Karabay [1,2], Andrew Lowy[3], Parag Katira[1]
[1] Department of Mechanical Engineering, SDSU
[2] Department of Bioengineering, UCSD
[3] Department of Surgery, UCSD

Pancreatic ductal adenocarcinoma (PDAC), a lethal cancer with a propensity for early metastasis, highly expresses MICAL (microtubule-associated monooxygenase) proteins, which directly induce actin depolymerization and indirectly cause cytoskeleton reorganization through transcription factors. However, the holistic impact of MICAL2 remains unknown. Here, we have taken a multi-scale modeling approach to connect cytoskeletal gene expression programs exhibited by pancreatic cancer cells to biochemical signaling networks and extracellular matrix conditions that regulate the cellular mechanical state. Our model allows us to determine how the expression of MICAL2 impacts pancreatic cancer cell migration across soft and stiff substrates. Our preliminary results using our modeling framework indicate that MICAL2 activity confined to its direct interaction with the cytoskeletal actin network does not significantly influence cell migration. However, activation of SRF transcriptional factor downstream of MICAL2 activity towards nuclear actin significantly adds to the cytoskeletal changes and increased migration of cells in 3D environments.

58. **From Tissues to Single Cells: Direct Proteoform Profiling Using Orbitrap-Based Single Molecule Mass Spectrometry**

Pei Su

Direct proteoform measurement in biological samples provides a path to proteomics in spatial tissue compartments and single cells at unprecedented sensitivity and throughput. We employ individual ion mass spectrometry (I^2MS), an Orbitrap-based charge detection MS technique in conjunction with direct sampling MS approaches for proteoform profiling in spatial tissue compartments and endogenous single cells directly obtained from tissues. We demonstrate how the

collection of single proteoform molecules or their gas-phase fragments over distinct spatial tissue compartments contribute to our understanding of proteoform identification, quantitation, spatial localization, and posttranslational modifications. In particular, proteoform imaging mass spectrometry (PiMS) using nanospray desorption electrospray ionization (nano-DESI) has enabled highly-multiplexed imaging and identification of kidney and ovarian cancer tissue proteoforms up to 70 kDa at ~20 µm spatial resolution. Such approach has been extended to high-throughput single cell profiling at a speed of >1000 cells per day, enabling proteoform-based cell typing in complex cell mixture from rat brain hippocampus.

59. **Breakdown of quiescence-proliferation transitions in fibroblasts in chronic wounds**

Skylar Tanartkit[1], Mithun Mitra[2,3*], Hilary A. Coller[2,3,4*]
[1] Department of Computational and Systems Biology,
[2] Department of Molecular Cell and Developmental Biology,
[3] Department of Biological Chemistry, David Geffen School of Medicine,
[4] Molecular Biology Institute,
University of California Los Angeles, Los Angeles, California, USA
* These authors contributed equally.

Wound healing is a complex, multi-phase process that restores tissue integrity after injury, with fibroblasts playing important roles in tissue organization and remodeling. Despite its clinical importance, particularly in chronic wounds, the cellular decision-making processes governing effective repair remain not fully understood. Using a time-course human single-cell RNA-seq dataset, we found that proportions of quiescent and proliferating fibroblast populations change across the wound healing stages, with concomitant changes in the activities of state-specific transcription factors(TFs). The early stage was enriched for quiescent TFs, followed by proliferative TFs in intermediate stages, and a combination of both in late stage. In contrast, our analysis of chronic wound data revealed a higher proportion of proliferating fibroblasts with TF activities resembling intermediate to late healing stages. These findings provide insight into fibroblast regulatory behavior and lay a foundation for future studies on how disrupted quiescence dynamics contribute to impaired/chronic wound healing.

60. **Metabolic Modeling and Analysis of Patient-Specific Colorectal Cancer Tumor-Stroma Communities**

Niki Tavakoli[1], Vaidhyanathan Mahaganapathy[5], Handan Cetin[2], Eileen Fung[5], Shannon M. Mumenthaler[1,4,5] & Stacey D. Finley[1,2,3]
[1] Alfred E. Mann Department of Biomedical Engineering
[2] Department of Quantitative and Computational Biology
[3] Mork Family Department of Chemical Engineering and Materials Science
[4] Division of Medical Oncology, Norris Comprehensive Cancer Center University of Southern California, Los Angeles CA, USA
[5] Ellison Medical Institute, Los Angeles, CA, USA

Cancer-associated fibroblasts (CAFs) play an important role in metabolic reprogramming through their interactions with colorectal cancer (CRC) cells. To delineate cell-specific metabolic differences and identify therapeutic targets, we constructed genome-scale metabolic models using transcriptomics data from CRC cells and CAFs. We conducted structural and functional analyses to characterize these metabolic differences, confirming strong distinction between CRC and CAF metabolic phenotypes. Gene deletion analysis revealed genes with enriched effectiveness in each cell type. To capture tumor-stromal crosstalk, we constructed interaction models connecting patient-matched CRC and CAF models through a shared lumen. Co-culture increased CRC growth by 40-68% over monoculture, and CAFs rescued CRC growth following knockouts of CRC-enriched genes, limiting their effectiveness. Conversely, knockout of Complex IV genes in the CAF compartment reduced CRC growth by disrupting this crosstalk. Finally, we found that targeting this gene in both compartments reduced CRC growth to 9-18% of baseline, revealing synergism.

61. **Structure-function relationships in elastic networks trained for nonlinear function**

Stephen J. Thornton[1*], Felipe Martins[2*], Andrea J. Liu[2], Shenshen Wang[1]
[1] Department of Physics and Astronomy, University of California, Los Angeles
[2] Department of Physics and Astronomy and Center for Soft and Living Matter, University of Pennsylvania
* These authors contributed equally.

Proteins with allosteric mechanisms contain within their structure a mechanical coupling between an active and an allosteric site. This coupling is specific, long-ranged, and non-reciprocal. Binding events that drive these allosteric

mechanisms can lead to large conformational changes that require an understanding of the nonlinear response of tuned elastic networks. Evolutionary pressures drive proteins to develop structures that reflect their function. Using the framework of physical learning, we tune elastic networks to perform functions that are inspired by protein allostery. We develop unsupervised measures that allow for the identification of functionally relevant substructures of these artificially tuned elastic networks and relate these measures to the multimodal structure of their response. We show how these techniques can be applied to elastic network models of real proteins, allowing for identification of binding sites and epistatic hotspots.

62. **EmbAlign: Fully Automated Cell Labeling in *C. elegans* Embryo Snapshots**

Miles Tran[1], Pavak Shah[2,3]
[1] Bioinformatics Interdepartmental Program,
[2] Department of Molecular, Cell and Developmental Biology,
[3] Institute for Quantitative and Computational Biosciences,
University of California Los Angeles, Los Angeles California, USA

Lineage-based cell identification in *C. elegans* typically requires intensive live imaging and tracking. Recent methods have been proposed to automate cell identification on the basis of spatiotemporal atlases built from the large number of manually curated datasets the field has generated. We present EmbAlign, a fully automated 3D registration framework that determines lineage identities from single embryo snapshots. Anchoring searches on observed cell counts, EmbAlign retrieves reference templates from a spatiotemporal atlas and refines assignments using an iterative Sinkhorn alignment procedure. This approach robustly handles positional variability and arbitrary orientations in both live and fixed uncompressed embryos. Cross-validation demonstrates that EmbAlign achieves 96.9% accuracy up to the 190-cell stage, with a diagnostic layer providing continuous confidence scores (AUPRC = 0.546). By converting raw spatial data into lineage-aware datasets without requiring timelapse imaging, EmbAlign offers a versatile, complementary solution for high-throughput developmental analysis.

63. **HP-HCR: A High-Throughput Platform for Orthogonal Signal Amplification in Spatial Omics**

Thomas Underwood[1], Haley De Ocampo[1], Aihui Wang[1], Ziwei Qiu[1], Xingbo Shang[2], Kevin Suh[2], Roy Wollman[2]
[1] Biochemistry, Molecular, and Structural Biology Program, University of California Los Angeles, Los Angeles California, USA
[2] Chemistry and Biochemistry Department, University of California Los Angeles, Los Angeles California, USA

Hybridization Chain Reaction (HCR) is a widely adopted signal amplification strategy in molecular imaging, with over 4,500 citations and successful commercialization1,2. Despite its broad utility, HCR is limited by finite hairpin diversity and laborious multiplexing workflows requiring 12+ hour re-amplification cycles (Figure 1A). We introduce High-Plexed HCR (HP-HCR), featuring a redesigned hairpin architecture with cleavable readout probes and a high-throughput screening pipeline that validates hundreds of HCR sets simultaneously. We have screened 192 candidate sets and identified 30 top performers providing 70-200 fold amplification. This research will complete validation of the HP-HCR library and deploy it in spatial transcriptomics applications, including thick tissue ATLAS imaging to extend mouse brain mapping from tens to hundreds of microns depth. HP-HCR addresses critical bottlenecks in multiplexed imaging and establishes a generalizable framework for developing orthogonal amplifier sets, with broad applicability across spatial omics platforms.

64. **Early Detection of Immune Cell Arrest in Hematological Disorders Revealed by Temporally-Resolved AI**

Konstancja Urbaniak[1-2], Greta Zara[3], Kory Harmaine[1], Grady Kestler[1], Nadia Carlesso[2-3], Andrei Rodin[1], Sergio Branciamore[1]
[1]Department of Computational and Quantitative Medicine, City of Hope
[2]California Institute for Regenerative Medicine (CIRM)
[3]Department of Stem Cell Biology and Regenerative Medicine

An interpretable, temporally resolved AI framework is introduced for the early detection of molecular events preceding disease manifestation, such as immune cell developmental arrest in hematological disorders. The approach is domain-agnostic and centers on our novel Bayesian network–based tools, including a network rewiring, reconstruction of network dynamics along developmental trajectories. By leveraging transcriptomic data, early molecular inducers that precede phenotypic changes caused by molecular effectors are identified, addressing limitations of static analyses. The framework provides causal interpretability of evolving gene regulatory dependencies underlying disrupted differentiation. *In silico* network perturbation is further employed to evaluate the effect of inducer(s) and predict their causal impact on effectors

and developmental outcomes. Application to immune cell development reveals early pathogenic alterations and establishes a generalizable strategy for detecting and characterizing developmental dysregulation.

65. **Phased fragility and stability of non-genetic B-cell states accelerate the genetic evolution of antibodies**

Haripriya Vaidehi Narayanan[1,2,*], Mark Y. Xiang[1,2,3,*], Vaibhava Kesarwani[1], Rohan Vanheusden[1,3,4], Tiffany Wang[1], and Alexander Hoffmann [1,2]
[1]Institute for Quantitative and Computational Biosciences,
[2]Department of Microbiology, Immunology, and Molecular Genetics,
[3]Bioinformatics Interdepartmental Program,
[4]Systems Biology Program,
University of California Los Angeles; Los Angeles, California 90095, USA.
*These authors contributed equally.

Vaccine responses depend on the Darwinian genetic evolution of B-cells to generate high-affinity antibodies. However, B-cells gain non-genetic heterogeneity during selection through interactions with antigen and T-cells, and then these non-genetic cell states remain stable when selected cells undergo proliferative clonal bursts. We explored the functional consequence of this dynamic control of non-genetic cell state variability by developing a mathematical model, integrating a wealth of immunological knowledge. We discovered that variability in B-cell fate decisions does not impair, but instead accelerates, affinity maturation by allowing high-affinity outliers to escape plasma cell differentiation and seed further rounds of Darwinian evolution. However, during clonal bursts, non-genetic cell state stability further promotes their amplification. The resulting model correctly predicts emergent vaccine response properties in mouse strains with altered B-cell fate decision profiles. Our work reconciles classical B-cell clonal selection theory with experimentally observed non-genetic variability, providing an interpretable knowledge-based modeling framework to support personalized vaccination strategies.

66. **A graph metric for disentangling the contributions of linear scaling and residual differences between cell lineages**

Charlotte E Weymer[1], Ryan L Johnson[2], Eric J Deeds[3,4], Erik C Andersen[5], Pavak K Shah[2,4]
Affiliations:
[1]Bioinformatics Interdepartmental Program, University of California, Los Angeles
[2]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles
[3]Department of Integrative Biology and Physiology, University of California, Los Angeles
[4]Institute for Quantitative and Computational Biosciences, University of California, Los Angeles
[5]Department of Biology, Johns Hopkins University

Lineal relationships between cells are a major determinant of cell fate and developmental patterning, and quantifying variation between developmental lineages can provide insights into differences that may emerge from genetic, environmental, and stochastic sources. Previously, we developed the branch distance, a novel metric inspired by tree edit distance, to quantify differences between phenotypic measures mapped onto cell lineage trees. Here, we extend this framework by decomposing the branch distance into parallel and perpendicular components, corresponding to scale differences and residual differences between lineages. Applying this approach to *C. elegans* embryonic lineages, where its invariant lineage enables precise cell matching, we find that residual variation dominates differences in cell cycle timing between isogenic embryos, while scale differences are associated with genetic background. These findings provide valuable insights into the genetic sources of variation in development between individuals, and underlying mechanisms that may govern differences in cell lineages.

67. **A Mechanistic Modeling Framework Integrating Bayesian Optimization to Reveal Critical Mechanogenic Regulations in the *Drosophila* Wing Disc**

Emerald Win[1] , Nilay Kumar[2*] , Mayesha S. Mim[2⛌] , Alysha Toomey[1⏀] , Weitao Chen[1] , Mark Alber[1] , Jeremiah Zartman[2]
1 University of California, Riverside - Department of Applied Mathematics
2 University of Notre Dame - Department of Chemical and Biomolecular Engineering
* Purdue University - College of Engineering
⛌ Northwestern University - Feinberg School of Medicine
⏀ The Aerospace Corporation

Tissue morphogenesis emerges from coordinated biochemical and mechanical regulation, yet the quantitative rules linking these processes remain elusive. We used the Drosophila wing disc as a model system to develop an integrative platform combining experiments with computational modeling. Our framework couples Decapentaplegic (Dpp) signaling

to intracellular Rho1 and Cdc42 dynamics through experimentally informed activation and inhibition motifs, including mutual antagonism and integrin-mediated basal activation. By leveraging Bayesian optimization to calibrate a multiscale reaction-diffusion model, we successfully reproduced in vivo spatial distributions of these mechanogens under both wild-type and mutant conditions. Our simulations identified mutual inhibition as a critical requirement for robust patterning during tissue development. This approach demonstrates how coupling mechanistic simulations with data-driven inference reveals the hidden regulatory logic of epithelial morphogenesis, offering a scalable strategy for modeling complex biochemical–mechanical feedbacks.

68. **Engineering an Oral, Modular, Probiotic-Delivered Vaccine Using the MS2 Virus-like Particle**

Jenna Wolfanger,[1] August Dolmatch,[2] Carrie Mills[1,3]
[1]Department of Chemical Engineering
[2]Department of Chemistry
[3]Department of Biological Engineering
University of California, Santa Barbara

Many vaccines require continuous refrigeration to maintain effectiveness and prevent degradation throughout production, shipping, distribution, and storage. This “cold-chain” is expensive, energy-intensive, and often difficult to maintain in areas without sufficient infrastructure, leaving three billion people worldwide without reliable access to life-saving vaccines. Capitalizing on advances in probiotic and virus-like particle (VLP) technologies, I am developing a modular, shelf-stable, orally administered vaccine platform produced and delivered by probiotics, shifting vaccine manufacturing from a factory into microbes that can initiate production in the gut. I will create a subunit vaccine consisting of an antigen of interest symmetrically displayed on a bacteriophage VLP, ensuring a strong immune response. To create modularity, the antigen and VLP will be covalently linked with split inteins, enabling fast response to new pathogens. I will present preliminary data that validates our VLP-intein design, including intein splicing efficiency of over 70% and successfully assembled VLP-intein particles.

69. **Defining the Macrophage Metabolic Response to Oxidized Lipids in a Genetically Diverse Mouse Cohort**

Anne Xu[1,2,3], Michael A. Raddatz[1,2,3], Calvin Pan[1,2,3], Aldons J. Lusis[1,2,3]
[1] Division of Cardiology, Department of Medicine,
[2] Department of Microbiology, Immunology, and Molecular Genetics,
[3] Department of Human Genetics,
University of California Los Angeles, Los Angeles California, USA

Macrophages play a critical role in cardiovascular disease through metabolic adaptations, yet the underlying genetic architecture governing these stress responses remains largely unknown. To investigate the metabolic response of macrophages, we analyzed bulk transcriptomic microarray data of macrophage samples representing 92 inbred strains of the Hybrid Mouse Diversity Panel (HMDP). Using Compass, a flux balance analysis algorithm based on transcriptome data, we predicted continuous biochemical reaction activities under control and oxidized phospholipid (OxPL)-treated conditions. Comparing these predicted fluxes uncovered significant changes in response to OxPL and across the genetic variation of the HMDP. Significantly increased reactions with OxPL treatment included amino acid transport pathways necessary for glutathione synthesis and iron (Fe2+) efflux mechanisms that function to prevent cellular ferroptosis. Ongoing work aims to leverage the genetic variation in the HMDP to identify the genetic drivers of these changes and prioritize candidate genes for further study.

70. **Cohesin cofactor kinetics modulate sister chromatid conformation**

Xingchi Yan[1], Maxim Tortora[1], Anton Goloborodko[2], Geoffrey Fudenberg[1]
[1] Department of Quantitative and Computational Biology, University of Southern California, Los Angeles California, USA.
[2] Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Vienna, Austria.

Cohesion between sister chromatids is required for DNA repair and faithful chromosome segregation. At the molecular level, cohesion is mediated by the cohesin complex, which encircles and tethers sister DNA strands; however, how interactions between cohesin and its cofactors (such as SORORIN and WAPL) regulate cohesion remains unclear. Here, we identify a minimal chemical reaction network governing cohesion maintenance and uncover the rules of engagement between cohesive and loop-extruding cohesin complexes upon encounter. Our model links molecular-scale interactions to the emergent mechanical properties of chromosome cohesion. These quantitative insights have broad implications for

cohesion-dependent processes, including homology search during DNA repair and long-term maintenance during oocyte meiosis.

71. **Beyond affinity: temporal coordination between antigen uptake and T-cell help during germinal center selection shapes B-cell lineage fate**

Patrick H. Yuan[1], Haripriya Vaidehi Narayanan[2,3], Alexander Hoffmann[2,3]
[1]Department of Computational Medicine, David Geffen School of Medicine,
[2]Department of Microbiology, Immunology & Molecular Genetics (MIMG),
[3]Institute for Quantitative & Computational Biosciences (QCBio),
University of California Los Angeles, Los Angeles, California, USA

Germinal center (GC) must generate high-affinity antibodies against foreign antigens while avoiding self-reactive output, even though thymic selection of T follicular helper (Tfh) cells is leaky and self- and foreign-cognate help can overlap. We propose that Tfh help alone is an underdetermined discriminator in this overlap regime, and that antigen acquisition provides an additional axis that reshapes selection stringency. We develop a stochastic, hazard-based model in which B cells in the light zone experience antigen uptake events that both (i) load antigen signals that determine downstream proliferative capacity upon dark-zone return and (ii) incur a time-dependent survival cost through a mechanistically motivated pro-apoptotic pathway. Tfh help controls the stochastic timing of dark-zone return, thereby coupling help availability to both survival and antigen accumulation. The model yields structured fitness landscapes and predicts a physiological “help threshold” separating extinction from expansion that depends on antigen spatial organization. This framework suggests how GC architecture can maintain tolerance without sacrificing foreign responses.