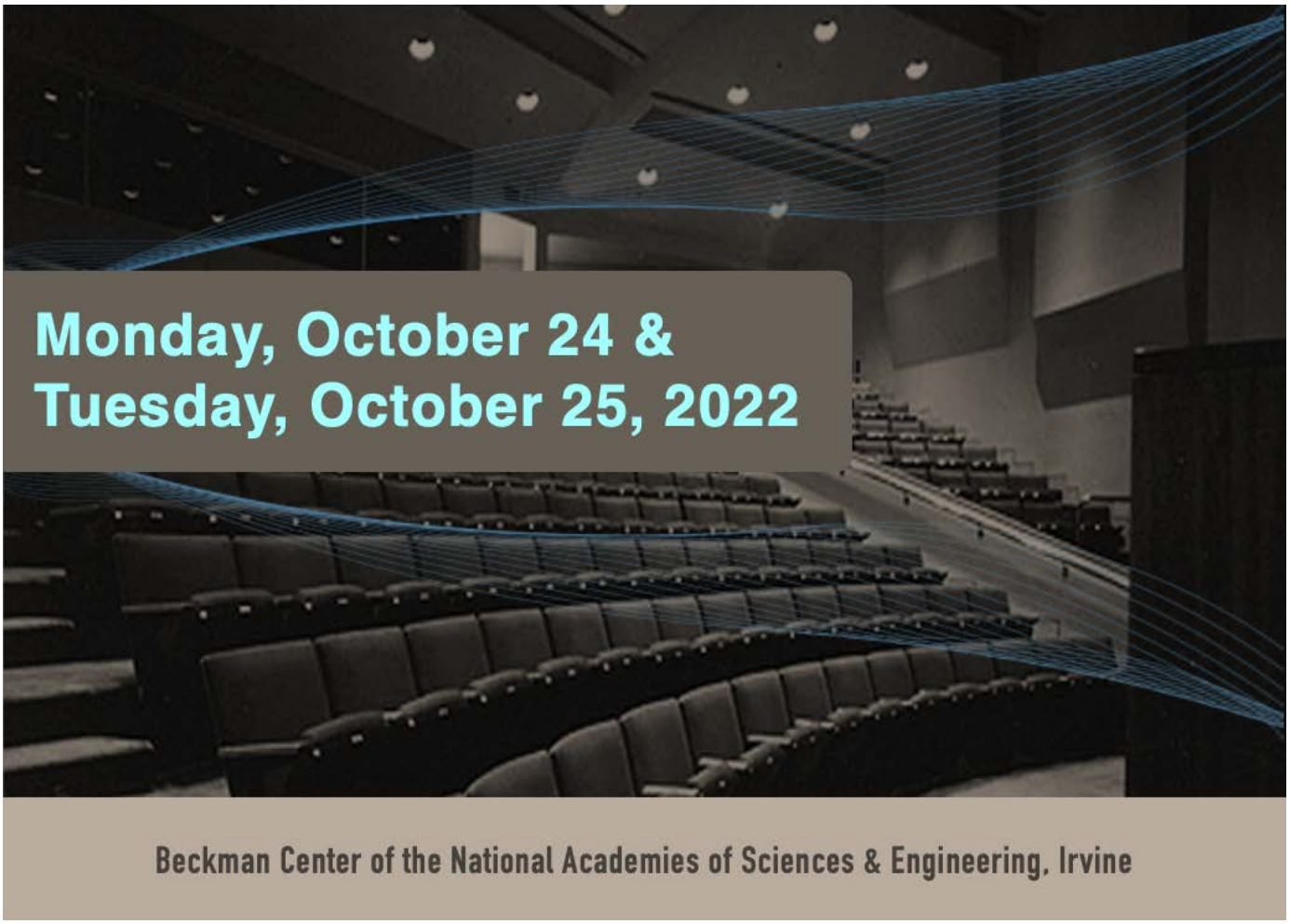
NSF-Simons Center for
Multiscale Cell Fate Research
UC Irvine

5th Annual Symposium on

Multiscale Cell Fate Research



INVITED SPEAKERS

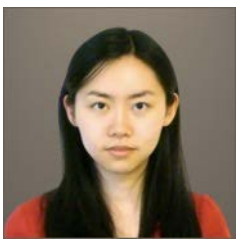
GREG BARSH
HudsonAlpha Institute for Biotechnology

OLIVER BELL
University of Southern California

MICHELLE DIGMAN
University of California, Irvine

ALLON KLEIN
Harvard University

DENISE KIRSHNER
University of Michigan



JINGYI JESSICA LI
University of California, Los Angeles

YANLAN MAO
University College London

WALLACE MARSH
University of California, San Francisco

PADMINI RANGAMANI
University of California, San Diego

SANTIAGO SCHNELL, FRSC
University of Notre Dame

SUZANNE SINDI
University of California, Merced

FENG YUE
Northwestern University

ELAZAR ZELZER
Weizmann Institute of Science

EVENT SCHEDULE

Monday, October 24

07:45 am – 08:25 am	**REGISTRATION & BREAKFAST**
08:25 am – 08:30 am	**OPENING ADDRESS**

SESSION 1 – Session Chair: Arthur Lander

08:30 am – 09:05 am	**SUBCELLULAR CELL FATE, Wallace Marshall**
09:05 am – 09:40 am	**METABOLIC PROFILING AND TRACKING PHENOTYPIC CHANGES IN MITOCHONDRIA IN CANCER CELLS WITH MITOMETER AND THE PHASOR APPROACH TO FLIM, Michelle Digman**
09:40 am – 09:45 am	**IOA ANNOUNCEMENT (Arthur Lander)**
09:45 am – 10:00 am	**COFFEE BREAK**

SESSION 2 – Session Chair: Thomas Schilling

10:00 am – 10:35 am	**STOCHASTICITY AND DYNAMICS OF CELL DIFFERENTIATION, Allon Klein**
10:35 am – 11:10 am	**REVISITING THE MODEL OF LIMB SKELETON DEVELOPMENT, Elazar Zelzer**
11:10 am – 11:45 am	**COPING WITH MECHANICAL STRESS: TISSUE DYNAMICS DURING DEVELOPMENT, HOMEOSTASIS AND REPAIR, Yanlan Mao**
11:45 am – 01:15 pm	**LUNCH**

SESSION 3 – Session Chair: Jin Yu

01:15 pm – 01:50 pm	**MULTI-SCALE MODELING CAN PREDICT FACTORS DRIVING THE IMMUNE RESPONSE AND TREATMENT IN TUBERCULOSIS, Denise Kirschner**
01:50 pm – 02:25 pm	**MULTISCALE MODELING OF MEMBRANE-ACTIN AND ACTIN-CROSS LINKER INTERACTIONS, Padmini Rangamani**

02:25 pm – 03:00 pm **COFFEE BREAK**

SESSION 4 – Session Chair: Jun Allard

03:00 pm – 05:00 pm **IOA AND CONTRIBUTED SHORT TALKS**

SPATIAL PATTERNS, SPATIAL CONTROL

03:00 pm – 03:10 pm Michael Norton, Rochester Institute of Technology

03:15 pm – 03:25 pm Lorenzo Scipioni, UCI IOA Team

03:30 pm – 03:40 pm Axel Almet & Renzi Hou, UCI IOA Team

03:45 pm – 03:55 pm Jinghao Chen & Jesse Holt, UCI IOA Team

CHROMATIN AND CHROMOSOMAL DNA

04:00 pm – 04:10 pm Francesco Palomba, UCI IOA Team

04:15 pm – 04:25 pm Jinsu Kim, POSTECH

04:30 pm – 04:40 pm Ethan Hollingsworth, UCI

04:45 pm – 04:55 pm Kwadwo Bonsu, UCI

05:00 pm – 06:00 pm **RECEPTION AND POSTER SESSION**

06:00 pm – 08:00 pm **DINNER**

EVENT SCHEDULE

Tuesday, October 25

08:00 am – 08:30 am **BREAKFAST**

SESSION 1 – Session Chair: Xing Dai

08:30 am – 09:05 am **DEVELOPMENTAL MECHANISMS OF SELF-ORGANIZING COLOR PATTERNS IN CATS, Greg Barsh**

09:05 am – 09:40 am **IS THE PUBLICLY AVAILABLE DATA OF PHYSICAL-CHEMISTRY CONSTANTS RELIABLE TO BUILD MULTISCALE MODELS IN BIOLOGY? Santiago Schnell**

09:40 am – 10:00 am **COFFEE BREAK AND POSTER SESSION**

SESSION 2 – Session Jing Zhang

10:00 am – 10:35 am **STRUCTURAL VARIANTS, EPIGENOME AND 3D GENOME STRUCTURE IN CANCER, Feng Yue**

10:35 am – 11:10 am **ZFP462 TARGETS HETEROCHROMATIN TO SAFEGUARD NEURAL LINEAGE SPECIFICATION, Oliver Bell**

11:10 am – 11:45 am **ENHANCING RIGOR AND RELIABILITY OF SINGLE-CELL DATA SCIENCE, Jingyi Jessica Li**

11:45 am – 01:15 pm **LUNCH**

SESSION 3 – Session Chair: German Enciso

01:15 pm – 01:50 pm **YEAST PRION PHENOTYPES: A WINDOW INTO COLONY STRUCTURE AND CELL FATE, Suzanne Sindi**

SESSION 4 – Session Chair: Christopher Miles

01:50 pm – 04:00 pm **IOA AND CONTRIBUTED SHORT TALKS**

SIGNALS AND SYSTEM DYNAMICS

01:50 pm – 02:00 pm Amanda Alexander, University of Houston

02:05 pm – 02:15 pm Ali Khalilimeybodi, UCSD

02:20 pm – 02:30 pm Ke Xu, UCI

02:35 pm – 02:45 pm Lingxia Qiao, UCSD

TRANSCRIPTOMICS

02:50 pm – 03:00 pm Sophia Hu, University of Pittsburgh

03:05 pm – 03:15 pm Guanao Yan, UCLA

03:20 pm – 03:30 pm Ping Wu, USC

03:35 pm – 03:45 pm Stephenson Chea, Jesse Krege, Ivy Xiong & Xiaojun Wu, UCI/USC IOA Team

03:50 pm – 04:00 pm Minh Vu & Christian Guerrero-Juarez, UCR/UCI IOA Team

04:10 pm – 04:15 pm **ABSTRACT DUE AND ADJOURN**

ABSTRACTS

Monday, October 24

SUBCELLULAR CELL FATE

Wallace Marshall

We normally think about developmental biology in the context of multi-cellular organisms, but even individual cells undergo basic processes of morphogenesis, pattern formation, and regeneration. The giant ciliate Stentor is a classic model organism for studying development and pattern formation within single cells, owing to its large size, easily visualized patterning, and remarkable abilities of wound healing and regeneration that allow cutting and pasting experiments of the type traditionally employed in classic embryology. This organism was popular a century ago, but was never developed as a molecular model system. We have now sequenced the Stentor genome and developed methods for gene knockdown by RNAi. Using these tools combined with proteomics and transcriptomics, we are beginning to learn some of the molecular mechanisms that underly morphogenesis in Stentor. One of the key findings is that a number of highly conserved molecules, including E2F and Pumilio, with important functions in animal physiology and development, also play a role in regeneration of pattern in Stentor. But developmental biology within a single cell requires different regions of a cell to undergo different morphological processes, and it is challenging to consider how this happens within a single shared cytoplasm that undergoes visible streaming flows. The cortical cytoskeleton of Stentor may provide a stable substrate for establishing and maintaining regional marks of intracellular fate. A set of parallel microtubules runs along the A/P axis, possibly providing a mechanism for polarization on this axis, but experimental evidence also suggests an orthogonal polarity axis that runs around the cell circumference, and the molecular basis for this polarity axis is not known. We have identified a set of fibers composed of SFI1 proteins which may serve to provide marks of longitude.

METABOLIC PROFILING AND TRACKING PHENOTYPIC CHANGES IN MITOCHONDRIA IN CANCER CELLS WITH MITOMETER AND THE PHASOR APPROACH TO FLIM

Michelle Digman

The hallmark of metabolic alteration of increase glycolysis, i.e. Warburg effect, in cancer cells together with atypical extracellular matrix structure may be responsible for tumor cell aggressiveness and drug resistance. While it is it known that tumor cells stiffen the ECM as the tumor progression occurs, a direct relationship between ECM stiffness and altered metabolism has not been explicitly measured. Here we apply the phasor approach technique in fluorescence lifetime imaging microscopy (FLIM) as a novel method to measure metabolic alteration as a function of ECM mechanics. We imaged and compared triple-negative breast cancer (TNBC) cells to non-cancerous cells on various ECM stiffness. Our results show that TNBC exhibit a decreased fraction of bound NADH, (indicative of glycolysis,) with increasing substrate stiffness. All other cell lines showed little to no change in fraction bound NADH on the varying collagen densities. Dysregulation of mitochondrial motion may contribute to the fueling of bioenergy demands in metastatic cancer. To measure mitochondria motion and analyze their fusion and fission events, we developed a new algorithm called “mitometer” that is unbiased, and allows for automated segmentation and tracking of mitochondria in live cell 2D and 3D time-lapse

images. Mitometer shows that mitochondria of triple-negative breast cancer cells are faster, more directional, and more elongated than those in their receptor-positive counterparts.

Furthermore, Mitometer shows that mitochondrial motility and morphology in breast cancer, but not in normal breast epithelia, correlate with fractions of the reduced form of NADH, in its bound form, and features such as speed and displacement, compared to the negative relationships with features such as directionality and branching in both TNBC and ER/PR+ mitochondria, but not in normal breast epithelial mitochondria. Together, the automated segmentation and tracking algorithms and the innate user interface make Mitometer a broadly accessible tool.

STOCHASTICITY AND DYNAMICS OF CELL DIFFERENTIATION

Allon Klein

Stochasticity — apparent randomness — in single cell fate choice fascinates us because the products of cell differentiation are often quite precise in terms of tissue composition and organization. We can try to understand why cell fate is stochastic at two scales: by establishing how individual cells select between distinct and stable cellular states; and by defining how cells coordinate fate choices to pattern tissues. I will describe some efforts to map stochastic cell differentiation by combining single cell genomics and lineage-tracing, and to uncover rules of homeostatic control by live imaging. Cases will be given from bone marrow hematopoiesis and epidermal turnover.

REVISITING THE MODEL OF LIMB SKELETON DEVELOPMENT

Elazar Zelzer

Limb development has long served as a model system for coordinated spatial patterning of progenitor cells. Here, we identify a population of naive limb progenitors and show that they differentiate progressively to form the skeleton in a complex nonconsecutive three-dimensional pattern. Single-cell RNA sequencing of the developing mouse forelimb revealed three progenitor states: naive, proximal and autopodial, as well as Msx1 as a marker for the naive progenitors. In vivo lineage tracing confirmed this role and localized the naive progenitors to the outer margin of the limb, along the anterior-posterior axis. Sequential pulse-chase experiments showed that the progressive transition of Msx1+ naive progenitors into proximal and autopodial progenitors coincides with their differentiation to Sox9+ chondroprogenitors, which occurs along all the forming skeletal segments. Indeed, tracking the spatiotemporal sequence of differentiation showed that the skeleton forms progressively in a complex pattern. These findings suggest a new model for limb skeleton development.

COPING WITH MECHANICAL STRESS: TISSUE DYNAMICS DURING DEVELOPMENT, HOMEOSTASIS AND EPAIR

Yanlan Mao

During growth and development, tissue dynamics, such as tissue folding, cell intercalations and oriented cell divisions, are critical for shaping tissues and organs. However, less is known about how tissues regulate their dynamics during tissue homeostasis and repair, to maintain their shape after development. In this talk, we will discuss how differential growth rates can generate precise folds in tissues. We will also discuss how tissues respond to mechanical perturbations, such as stretching or wounding, by altering their actomyosin contractile structures, to change tissue dynamics, and thus preserve tissue shape and patterning. We combine genetics, biophysics and computational modelling to study these processes.

MULTI-SCALE MODELING CAN PREDICT FACTORS DRIVING THE IMMUNE RESPONSE AND TREATMENT IN TUBERCULOSIS

Denise Kirschner

Tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* (Mtb), is one of the world's deadliest infectious diseases and remains a significant global health burden even in the face of COVID. TB disease and pathology presents clinically across a spectrum of outcomes, ranging from total sterilization of infection to active disease. Much remains unknown about the immunobiology that drives an individual towards various clinical outcomes as it is challenging to experimentally address specific mechanisms driving outcomes. Furthermore, it is unknown whether numbers of immune cells in the blood accurately reflect ongoing events during infection within human lungs. We have utilized a myriad of multi-scale approaches and analyses to study the host immune response to *Mtb* across multiple physiologic and time scales. The models we developed range from molecular scale to whole-host scale and we used a hybrid approach combining differential equations and agent-based modeling at multiple scales. We use extensive analyses to predict mechanisms in the system driving different outcomes via parameters. Additionally, a key goal is to improve antibiotic treatment for TB. Currently multiple drugs are used simultaneously over a 6-9 month period which is a burden on the patient and medical system. We use our models to predict regimens that can improve clinical treatment of TB.

MULTISCALE MODELING OF MEMBRANE-ACTIN AND ACTIN-CROSS LINKER INTERACTIONS

Padmini Rangamani

The interactions between the plasma membrane and the actin cytoskeleton are fundamental to many cellular processes including membrane trafficking, cell shape regulation, and cell motility. These processes are inherently mechanochemical in that they harness complex molecular machineries to drive changes to the physical organization of the cell. As a result, they lend themselves to interrogation using both modeling and experimental approaches. In this talk, I will discuss two such efforts from my group— modeling endocytosis and the associated actin-membrane interactions and modeling actin assembly as a function of cross-linkers. In both these efforts, in addition to the quantitative models, I will also highlight the feedback between experiments conducted by our collaborators and the model predictions we generated, sharing both failures and successes along the way.

ABSTRACTS

Tuesday, October 25

DEVELOPMENTAL MECHANISMS OF SELF-ORGANIZING COLOR PATTERNS IN CATS

Greg Barsh

Chris Kaelin, Kelly McGowan, and Greg Barsh

Department of Genetics, Stanford University, Stanford, CA, 94305, USA.
HudsonAlpha Institute of Biotechnology, Huntsville, AL, 35806, USA.

From leopards to giraffes to zebras, the developmental biology of periodic color pattern in mammals has motivated groundbreaking work in mathematical biology yet remains an unsolved mystery at a molecular level. Starting with fetal skin collected from feral cat spay-neuter clinics, we used scRNA-seq to uncover a molecular pre-pattern of gene expression that predicts and foreshadows the later appearance of spots and stripes. The embryonic pre-pattern becomes evident prior to hair follicle development, weeks before color patterns are apparent, and reveals instructive roles for WNT signaling pathway components, including *Dkk4*, *Wnt10b*, and *Wif1*. Several of the same genes either cause or emerge as strong candidates for morphologic variation in the Bengal cat, a popular breed characterized by ornate color patterns not found in other domestic cat breeds. Taken together, our work provides strong support for a Turing-Gierer-Meinhardt activator-inhibitor system that acts to specify and establish the fate of epidermal cells that later give rise to different colored hairs. Comparative genomics suggests that variation in key components of the system help to explain diversity of color patterns among the Felidae.

IS THE PUBLICLY AVAILABLE DATA OF PHYSICAL-CHEMISTRY CONSTANTS RELIABLE TO BUILD MULTISCALE MODELS IN BIOLOGY?

Santiago Schnell

In 2022, the biological revolution continues unabated. We have multitudinous biological networks and interaction maps at different scales of biological organization. Uncovering these complex networks would have been unimaginable in the era preceding genome sequencing, systems biology and big data. However, if we study these network models and maps statically, they will not get us close to one key aspect of biology: its dynamic behavior. If biology is to be understood as a dynamic process, then we need to develop and implement multiscale models. These models require the use of measured physical-chemistry constants of biological processes described in network models and maps. In this talk, we present an estimate of the experimental uncertainty of physical constants for enzyme catalyzed reactions. Enzymes play critical roles in many biological networks and interaction maps. We discover that the experimental uncertainty of the Michaelis constant – one of the most commonly used enzyme constants in mathematical models - is estimated to yield a mean error of up to 10.8 fold units. Our analysis suggests that there is an undesirable heterogeneity due to biases in experiment design and data reporting leading to irreproducible measurements. The experimental variability of physical constants can create a gap in our ability to develop and implement predictive multiscale models.

STRUCTURAL VARIANTS, EPIGENOME AND 3D GENOME STRUCTURE IN CANCER

Feng Yue

TBA

ZFP462 TARGETS HETEROCHROMATIN TO SAFEGUARD NEURAL LINEAGE SPECIFICATION

Oliver Bell

ZNF462 haploinsufficiency is linked to Weiss-Kruszka Syndrome, a genetic disorder characterized by a range of neurodevelopmental defects including Autism. Though highly conserved in vertebrates and essential for embryonic development the molecular functions of*ZNF462* remain unclear. We identified its murine homolog ZFP462 in a screen for mediators of epigenetic gene silencing in mouse embryonic stem cells (ESCs). Here, we show that ZFP462 safeguards neural lineage specification by targeting the H3K9-specific histone methyltransferase complex G9A/GLP to silence mesoendodermal genes. ZFP462 binds to thousands of transposable elements (TEs) that are potential enhancers harboring ESC-specific transcription factor (TF) binding sites. Through physical interaction with G9A/GLP, ZFP462 seeds heterochromatin at such enhancers, in turn restricting the binding of pluripotency TFs OCT4, SOX2 and NR5A2. Loss of ZFP462 in ESCs results in increased chromatin accessibility at target sites and ectopic expression of mesoendodermal genes. Taken together, ZFP462 restricts TF binding and subsequent mesoendoderm-specific gene activation by conferring lineage- and locus-specificity to the broadly expressed epigenetic regulator G9A/GLP. Our results suggest that aberrant activation of lineage non-specific genes in the neuronal lineage underlies ZNF462-associated neurodevelopmental pathology.

ENHANCING RIGOR AND RELIABILITY OF SINGLE-CELL DATA SCIENCE

Jingyi Jessica Li

This talk will focus on two directions of statistical method development to enhance the rigor and reliability of single-cell data analysis and tool benchmarking. The first direction aims to address a widespread but largely ignored issue: inflated false discovery rates (FDRs) in single-cell data analysis. We previously reported that several popular bioinformatics tools have unexpectedly large FDRs far exceeding the claimed FDR threshold due to the use of ill-posed p-values. Accordingly, we developed a statistical method, Clipper, to implement p-value-free FDR control and avoid the statistical complications and computational burdens of obtaining well-calibrated p-values. Here we will generalize and adapt Clipper to single-cell data analysis, such as the detection of differentially expressed genes, to ensure valid FDR control. We will also account for the widespread “double-dipping” issue: using the same data twice would complicate statistical inference and inflate FDR (e.g., identifying differentially expressed genes among cell clusters identified from the same data). The second direction aims to develop a versatile simulator to generate realistic single-cell multi-omics data and spatial transcriptomics data with ground truths, thus allowing the single-cell community to perform fair and informative benchmarking of computational tools. We designed the simulator to be by far the most comprehensive, including various cell states (discrete cell types and continuous cell trajectories), technologies (spatial and sequencing omics), experimental factors (cell number, library size, batch effects, and conditions), and data formats (sequencing reads and/or summarized count matrices).

YEAST PRION PHENOTYPES: A WINDOW INTO COLONY STRUCTURE AND CELL FATE

Suzanne Sindi, UC Merced

CONTRIBUTED & IOA TALKS

Monday, October 24

SPATIAL PATTERNS, SPATIAL CONTROL

Control of Active Nematics using Confinement, Optogenetics, Viscoelasticity, and Reaction-Diffusion Systems

Michael Norton, Rochester Institute of Technology

Active nematics are an important class of self-organizing system that include living matter, such as growing bacterial colonies and confluent tissue layers, as well as model systems built from reconstituted cytoskeletal components. Understanding how to shape these nematic flows and structures is a grand challenge in active matter. I present four approaches for shaping nematic dynamics. In the first, I consider how geometric confinement creates a suite of competing stable and unstable flow structures that control the transition from laminar flows to turbulence. Second, I show how optimal control tools and optogenetically enabled nematics can be used to shape active stresses and create desired configurations. Next, I explore how the addition of viscoelastic stresses impacts defect dynamics. Finally, I propose a non-equilibrium mechanochemical feedback system that "detects" +/- 1/2 defects. This scheme lays the foundation for rational engineering of programmable chemomechanical active nematics and understanding phenomena where cell fates are coupled to topology, such as in the hydra, neural progenitor cells, and bacterial films.

A Priori Determination of Neural Stem Cells Transplantation Efficacy Based on Multidimensional Microscopy Physiological Profiling

Lorenzo Scipioni, UC Irvine - IOA Team

TBA

Integrated Hypothesis Generation and Validation to Understand the Avalanching Dynamics of Rapid Epithelial Wound Repair in Axolotl

Axel Almet & Renzi Hou, UC Irvine - IOA Team

TBA

PIEZO1 Regulates Leader Cell Formation and Cellular Coordination During Collective Cell Migration

Jinghao Chen & Jesse Holt, UC Irvine - IOA Team

The collective migration of keratinocytes during wound healing requires both the generation and transmission of mechanical forces for individual cellular locomotion as well as for the coordination of movement across cells. Leader cells initiated along the wound edge transmit mechanical and biochemical cues to ensuing follower cells,

ensuring their uniform polarization and coordinated direction of migration, or directionality. Despite the observed importance of mechanical cues in leader cell formation and governing directionality, the underlying biophysical mechanisms remain elusive. The mechanically activated ion channel PIEZO1 was recently identified to play an inhibitory role during the reepithelialization of wounds through retraction of keratinocytes located along the wound edge. Here, through an integrative experimental and mathematical modeling approach, we elucidate PIEZO1's contributions to collective migration. Time-lapse microscopy reveals that PIEZO1 activity inhibits leader cell formation along the wound edge. To probe the relationship between PIEZO1 activity, leader cell formation and inhibition of reepithelialization, we developed an integrative 2D-multiscale model of wound closure that links observations at the single cell and collective cell migration scales. Through numerical simulations and subsequent experimental validation, we found that directionality plays a key role during wound closure and is inhibited by PIEZO1 activity. We propose that PIEZO1-mediated retraction suppresses leader cell formation which inhibits the coordination of directionality between cells during collective migration.

CHROMATIN AND CHROMOSOMAL DNA

Visualization of Chromatin Remodeling During Nuclear Degradation During Zebrafish Lens Development in vivo

Francesco Palomba, UC Irvine - IOA Team

Transparency of lens optics relies on controlled degradation of light scattering organelles. Primary lens fiber cells first lose their organelles during early development in the lens nucleus, followed by a life-long process of addition of secondary lens fiber cells in the germinal zone undergoing differentiation and organelle degradation. During nuclear degradation DNAses and methyl transferases play important roles in DNA remodeling in fiber cells. Mutants in these processes are associated with pathogenesis of age-related cataract in humans1 and in animal models2. While there has been progress in understanding the biochemical and genetic impact of epigenetic modifications, the complex organization at the molecular scale of the folded chromatin structure remains poorly understood. The main advantage of this characterization would enable researchers to use our platform to leave cells intact while being able to characterize the chromatin architecture in a living cell such that the characterization may provide important diagnostic features and allow for drug screening in real time. For this purpose, we have developed a quantitative imaging method using fluorescence lifetime and hyperspectral imaging of a widely used fluorescent nuclear stain (Hoechst 33342) to assess chromatin architecture in living cells. The spectral and lifetime properties of Hoechst revealed by phasor analyses reveal differences in chromatin density and accessibility, which correlates with transcriptional activity, as we have proven in a cell culture system. In this project we will image chromatin remodeling as a hallmark of epigenetic and architectural DNA changes in living zebrafish lens fiber cells as they undergo nuclear degradation during embryonic development. We hypothesize that chromatin remodeling prior to and during nuclear degradation in central primary lens fiber cells can be described by two models: i) The switch on-off model, whereby prior to nuclear degradation, high transcriptional activity is expected in all lens fiber cells, that then leads to a step-wise switch off of transcription starting at 45 hours post fertilization (hpf) in central fiber cells; or alternatively ii) The gradient model of transcriptional states from the lens periphery to the center of the lens well prior to the onset of nuclear degradation that gradually lead to central fiber cells undergoing nuclear degradation. To test these hypotheses, we will assess overall chromatin accessibility in specific parts of the zebrafish lens prior to, during and post primary lens fiber cell nuclear degradation. We will correlate our results with chromatin accessibility, DNA methylation and transcriptional activity by evaluating changes in the optical properties of Hoechst nuclear staining. We will then challenge in vivo lens fiber cell chromatin architecture accessibility by treatment of drugs that modify chromatin architecture, and therefore gene transcription, as well as in a previously characterized genetic mutant with decreased lens water influx that affects overall lens homeostasis, including the precise

regulated microenvironment required for normal chromatin organization. We will correlate changes in DNA architecture with fiber cell proliferation and differentiation rates as well as transcriptional activity. This approach will enable the first ever visualization of chromatin epigenetic modifications during lens fiber cell differentiation in vivo.

Stochastic Models of Nucleosome Dynamics Reveal Regulatory Rules of Stimulus-Induced Epigenome Remodeling

Jinsu Kim, POSTECH

The genomic positions of nucleosomes are a defining feature of the cell's epigenomic state, but signal-dependent transcription factors (SDTFs), upon activation, bind to specific genomic locations and modify nucleosome positioning. Here we leverage SDTFs as perturbation probes to learn about nucleosome dynamics in living cells. We develop Markov models of nucleosome dynamics and fit them to time course sequencing data of DNA accessibility. We find that (1) the dynamics of DNA unwrapping are significantly slower in cells than reported from cell-free experiments, (2) only models with cooperativity in wrapping and unwrapping fit the available data, (3) SDTF activity produces the highest eviction probability when its binding site is adjacent to but not on the nucleosome dyad, and (4) oscillatory SDTF activity results in high location variability. Our work uncovers the regulatory rules governing SDTF-induced nucleosome dynamics in live cells, which can predict chromatin accessibility alterations during inflammation at single-nucleosome resolution.

Comparative Assessment of Noncoding Variant Activity in vivo Using Limb Polydactyly as a Model

Ethan Hollingsworth, UC Irvine

Human genetics studies implicate a rapidly growing list of non-coding variants in human disease, including congenital heart disease, neurological disorders, and limb malformations. Yet, our understanding of how specific enhancer variants affect gene expression and cause phenotypes observed in patients remains limited, due in large part to a lack of efficient tools for in vivo enhancer variant analysis. Here we developed Dual-enSERT, a highly efficient (~50% integration efficiency) and robust dual fluorescence enhancer reporter system for comparative assessment of different human enhancer variant alleles in the same live mouse. We apply this technology to characterize the effects of rare variants in the ZRS limb enhancer of SHH that are linked to preaxial polydactyly in human patients. We compare enhancer activity across independent ZRS variants and show that all tested variants cause ectopic fluorescent reporter activity in the anterior margin of developing mouse limb bud consistent with their role in causing preaxial polydactyly. Thus, Dual-enSERT provides a highly reproducible, efficient, and customizable mammalian reporter system for the functional assessment of human enhancer variants in vivo.

Mathematical Modeling and Bioinformatic Analysis to Investigate Multigenerational Epigenetic Stability of DNA Methylation Landscapes

Kwadwo Bonsu, UC Irvine

Currently, it is not well understood what factors contribute to the stability of the epigenome in different cell types across the lifetime of mammals. Better understanding of these factors would aid development of therapies for age-related diseases, including cancer. DNA methylation is a widely studied epigenetic mark, located primarily on Cytosine-phosphate-Guanine (CpG) dinucleotides, with known associations to transcriptional gene silencing, and variable dynamics across the lifetime of mammalian organisms (replication,

development/differentiation, age, etc.). In this project, I have developed stochastic mathematical models of CpG methylation maintenance, which is the reestablishment of methyl marks following replication. Incorporation of experimentally-derived correlations, probing post-replication re-methylation dynamics, into distance-dependent reactions allows us to model effects such as enzyme processivity, and the influence of different genomic features (i.e., Promoters, Transposable Elements). CpG Islands (CGIs) are regions of the DNA which contain high CpG content, and as such establish regions of hyper-/hypomethylation. Bioinformatic analysis of methylation landscapes in human embryonic stem cells (hESCs) shows that methylation levels of CGIs are inversely correlated with CpG Island size, and suggest a size threshold exists; below which islands are more likely to be methylated. These observations from the data may be used to inform mechanistic mathematical models, as my simulations currently predict that the size and density of CGIs influence overall stability of their methylation patterns, reflected in variable state-switching of island methyl states across multiple replication cycles, but are strongly dependent on the strength of interaction between neighboring CpGs in (de-)methylating reactions.

CONTRIBUTED & IOA TALKS

Tuesday, October 25

SIGNALS AND SYSTEM DYNAMICS

Multi-Stability in a Mathematical Model of T Cell Dynamics in Multiple Sclerosis

Amanda Alexander, University of Houston

A healthy immune system requires a balance between inflammatory signaling to remove pathogens and regulation to avoid autoimmunity. Understanding the mechanisms that facilitate this balance is an ongoing scientific endeavor. Because T cells play an important role in mediating the adaptive immune system, I explore T cell dynamics in a murine model of multiple sclerosis. In collaboration with an immunology lab, I observe that varying initial antigen dose leads to autoimmune symptoms that vary in severity. To elucidate the cell dynamics that mediate these symptoms, I develop and analyze a dynamical system to model interactions between regulatory and inflammatory T cells. My model exhibits three stable steady states, which I interpret to correspond to three observed presentations of autoimmunity. I analyze bifurcations and basins of attraction of the model to develop hypotheses about what mechanisms cause autoimmunity and why there is variability in symptoms between individuals.

Systems Analysis of Controversial Ca2+-YAP/TAZ Relationship in Different Contexts

Ali Khalilimeybodi, UC San Diego

YAP/TAZ proteins have recently attracted researchers' attention as key targets for integrating biochemical and biomechanical signals to regulate cellular functions. However, the temporal multi-scale nature of YAP/TAZ signaling has impeded researchers from fully understanding of YAP/TAZ response. In this study, we developed a network model of Ca2+-mediated YAP/TAZ signaling to examine how temporal dynamics of various pathways contribute to controversial YAP/TAZ responses observed in experiments. By including GPCR, IP3-Ca2+, Kinases, RhoA, F-actin, and Hippo-YAP/TAZ modules and their crosstalk with calcium, we captured both time course and steady-state data on cell response to Ang II and ECM stiffness stimuli. Model results illustrate the role of cell-dependent calcium dynamics and CaMKII bistable response in switching the direction of changes in Ca2+-induced YAP/TAZ activity. Exploring frequency-dependent YAP/TAZ responses revealed how competition between LATS1/2 regulators results in the YAP/TAZ non-monotonic response to periodic GPCR stimulation. Finally, the model predicts the key role of calcium dynamics and CaMKII in the nonlinear relationship between cell size parameters and YAP/TAZ activity.

Enhancement of Car T Cell Activation by Pulsatile Signals Can Be Explained by a Mathematical Model with Homeostatic Feedback, to Predict Improved Activation Protocols

Ke Xu, UC Irvine

T cells are immune cells responsible for identifying and, in some cases, killing pathogenic cells in our bodies. They are activated by receiving an extracellular signal, in the form of antigen, that binds to a receptor on the surface of the T Cell. In the past 10 years, T cells have been engineered with chimeric antigen receptors (CAR) as a potential new class of therapy against a range of disease. Recent experiments found that stimulating a CAR T cell with short pulses of antigen yields a stronger response than stimulating them continuously. Here we aim to show that (Aim 1) a simple model can reproduce the response to pulsing by identifying a previously uncharacterized homeostatic feedback. (Aim 2) We aim to quantitatively learn a model to fit the existing data, crucially, using a neural net to represent the unknown homeostatic feedback, thus obviating the need to assume a particular functional form, as has traditionally been done in mathematical modeling. Finally, (Aim 3) with such a quantitative, predictive model, we aim to design optimal stimulation strategy to maximally activate a CAR T cell.

A Eukaryotic Circuit for Secretion-Coupled Cellular Autonomy

Lingxia Qiao, UC San Diego

Cancers represent complex autonomous systems, displaying self-sufficiency in growth signaling. Autonomous growth is fueled by a cancer cell's ability to 'secrete-and-sense' growth factors: a poorly understood phenomenon. Using an integrated systems and experimental approach, here we dissect the impact of a feedback-coupled GTPase circuit within the secretory pathway that imparts secretion-coupled autonomy. The GTPase circuit is assembled when the Ras-superfamily monomeric GTPase Arf1, and the heterotrimeric GTPase Gi□□□ and their corresponding GAPs and GEFs are coupled by GIV/Girdin, a protein that is known to fuel aggressive traits in cancers. This GTPase circuit ensures the dose information transmission by achieving the dose response alignment behavior of sensing and secretion, leading to self-sustained cell survival by stimulus-proportionate secretion. Findings highlight how enhanced coupling of two biological switches in cancer cells is critical to secretion-coupled autonomy.

TRANSCRIPTOMICS

Epithelial-Mesenchymal Transition Couples with Both G1/S and G2/M Arrest

Sophia Hu, University of Pittsburgh

Epithelial to mesenchymal transition, EMT, is involved in numerous biological processes such as wound healing, tissue fibrosis, and cancer metastasis. Existing literature has debated on the transition paths in EMT as well as how the cell cycle couples to EMT. To address these questions, we first generated a scRNA-seq dataset, where mammary epithelial MCF10A cells were treated with different doses of TGFβ, an EMT inducer. We then analyzed the data with dynamo, a machine-learning based analytical framework we developed to reconstruct single cell dynamical equations (Qiu et al. Cell, 2022). From the obtained vector fields, transition path analyses revealed two unique transition paths, corresponding to either an arrest in the G1/S or G2/M phase, when cells underwent EMT. The existence of these two paths agrees with our previous live cell imaging studies (Wang et al., Sci Adv 2020, eLife 2022), but not with pseudotime analyses reported in the literature. Our analyses demonstrate the importance of including dynamical information in single cell data analyses.

scReadSim: A Single-Cell RNA-seq and ATAC-seq Read Simulator

Guanao Yan, UC Los Angeles

Rapid advances of single-cell RNA-seq and ATAC-seq technologies propelled the development of many computational tools, whose benchmarking demand realistic simulators. However, few simulators can generate sequencing reads, and none of the existing read simulators aim to mimic real cells, hurdling the benchmarking of low-level computational tools that process reads. To fill this gap, we propose scReadSim, a single-cell RNA-seq and ATAC-seq read simulator that generates synthetic cells to mimic real cells. Trained on real data, scReadSim can generate synthetic data in FASTQ and BAM formats. Deploying scReadSim on sci-ATAC-seq and 10x Multiome (ATAC+RNA) data, we show that the scReadSim synthetic data resemble the real data at both read and count levels. As a flexible simulator, scReadSim provides unique molecular identifier (UMI) counts for benchmarking scRNA-seq deduplication tools, and scReadSim can accommodate user-specified open chromatin regions (``ground truths'') to generate single-cell ATAC-seq data. Our benchmark applications of scReadSim show that UMItools is a preferred scRNA-seq deduplication tool, and MACS3 achieves top performance in scATAC-seq peak calling. Moreover, scReadSim can guide experimental design by allowing the cell number and sequencing depth to vary.

Size Control of a Feather: Regulation of Progenitor Cell Zone Enables the Formation of Long Feathers

Ping Wu, University of Southern California

Size control of a feather: Regulation of progenitor cell zone enables the formation of long feathers
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A fundamental question in regenerative medicine is how organ size is determined. The balance of homeostasis of stem cell, TA cells and differentiated cells leads to control of proper organ size. A bird has feathers with different lengths and widths. The molecular and cellular basis of feather size control remains elusive. Here, by comparing different feather types from domestic chickens, we demonstrate that the follicle configuration is related to the feather size. Examining the extremely long feather in Phoenix chickens reveals aspects of specialized stem cell/TA cell homeostasis. Furthermore, transcriptome profiling demonstrates that long feathers are enriched in FGF and IGF signaling pathways. scRNA-seq analysis reveals that longer feathers undergo a longer route to achieve terminal differentiation. Cellchat analysis suggests that longer feathers have increased IGF interactions between epidermal progenitor cells and dermal cells. Functional analysis reveals that FGF pathway inhibition produced shorter feathers, whereas IFG pathway overexpression generated longer feathers. Thus, we discovered principle governing feather size control.

Investigating Cell Fate Determination in Mouse Model of Cornelia de Lange Syndrome

Stephenson Chea, Jesse Krege, Ivy Xiong & Xiaojun Wu, UC Irvine/ University of Southern California - IOA Team

TBA

Integrating single-cell RNA-sequencing and modeling analyses to delineate cell fate specification in the hair follicle

Minh Vu & Christian Guerrero-Juarez, UC Riverside/UC Irvine - IOA Team

TBA

Poster Presentations

Adam Stabell, UC Irvine
Ali Khalilimeybodi, UC San Diego
Amanda Alexander, University of Houston
Arul Subramanian and Pavan Nayak, UC Irvine
Carmen Al Masri, UC Irvine
Eric Mjolsness, UC Irvine
Ethan Hollingsworth, UC Irvine
Federico Bocci, UC Irvine
Gessner Soto, University of Colorado
Giulia Tedeschi and Francesco Palomba, UC Irvine
Guanao Yan, UC Los Angeles
Jesse Holt, UC Irvine
Jesse Kreger, University of Southern California
Jinsu Kim, POSTECH
Junhao Gu, UC Irvine
Ke Xu, UC Irvine
Kirsten Wong, UC Irvine
Kwadwo Bonsu, UC Irvine
Lingxia Qiao, UC San Diego
Lingyun (Ivy) Xiong, University of Southern California
Lorenzo Scipioni, UC Irvine
Matthew Hur, UC Irvine
Mayte Bonilla Quintana, UC San Diego
Michael Norton, Rochester Institute of Technology
Morgan Dragan, UC Irvine
Ping Wu, University of Southern California
Shannon McElhenney, UC Irvine
Sohyeon Park, UC Irvine
Sophia Hu, University of Pittsburgh
Theresa Loveless, UC Irvine/Rice University
Thomas Beardsley, UC Irvine
Tianyi Sun, UC Los Angeles
Xiaojun Wu, University of Southern California
Yingzi Liu, UC Irvine
Zhuoxin Chen, UC Irvine

Characterization of Human Skin Equivalents Using scRNA-sequencing

Adam Stabell, UC Irvine

Organotypic culture systems are artificial environments designed to model complex 3D biological systems more accurately than 2D cell cultures. They procide a means to study cellular behavior with greater physiological relevance than cell lines, while offering higher throughput than in vivo models. While these organotypic cultures are powerful tools, it is important to know which aspects of them are biologically relevant. In our lab, we have performed single cell RNA-sequencing (scRNA-seq) on fully stratified human skin equivalents (HSEs) that were generated using two distinct in vitro culturing systems. Sequencing experiments coupled with molecular and histological staining elucidated the similarities and differences between in vivo human epidermis and our HSEs. Notably, HSE basal cells are undergoing an epithelial-to-mesenchymal transition (EMT). Additionally, abnormal populations of keratinocytes exist in the HSEs which are not present in homeostatic human epidermis. Xenografting the HSEs rescued many of the abnormalities present in the in vitro culture systems, however, the xenografting process induced a hypoxic response and substantially changed the gene expression patterns of the HSEs. This study moves us closer to generating HSEs that accurately mimic in vivo human skin and highlights areas of improvement for creating better HSEs.

Systems Analysis of Controversial CA2+-Yap/Taz Relationship in Different Contexts

Ali Khalilimeybodi, UC San Diego

YAP/TAZ proteins have recently attracted researchers' attention as key targets for integrating biochemical and biomechanical signals to regulate cellular functions. However, the temporal multi-scale nature of YAP/TAZ signaling has impeded researchers from fully understanding of YAP/TAZ response. In this study, we developed a network model of Ca2+-mediated YAP/TAZ signaling to examine how temporal dynamics of various pathways contribute to controversial YAP/TAZ responses observed in experiments. By including GPCR, IP3-Ca2+, Kinases, RhoA, F-actin, and Hippo-YAP/TAZ modules and their crosstalk with calcium, we captured both time course and steady-state data on cell response to Ang II and ECM stiffness stimuli. Model results illustrate the role of cell-dependent calcium dynamics and CaMKII bistable response in switching the direction of changes in Ca2+-induced YAP/TAZ activity. Exploring frequency-dependent YAP/TAZ responses revealed how competition between LATS1/2 regulators results in the YAP/TAZ non-monotonic response to periodic GPCR stimulation. Finally, the model predicts the key role of calcium dynamics and CaMKII in the nonlinear relationship between cell size parameters and YAP/TAZ activity.

Multi-Stability in a Mathematical Model of T Cell Dynamics in Multiple Sclerosis

Amanda Alexander, University of Houston

A healthy immune system requires a balance between inflammatory signaling to remove pathogens and regulation to avoid autoimmunity. Understanding the mechanisms that facilitate this balance is an ongoing scientific endeavor. Because T cells play an important role in mediating the adaptive immune system, I explore T cell dynamics in a murine model of multiple sclerosis. In collaboration with an immunology lab, I observe that varying initial antigen dose leads to autoimmune symptoms that vary in severity. To elucidate the cell dynamics that mediate these symptoms, I develop and analyze a dynamical system to model interactions between regulatory and inflammatory T cells. My model exhibits three stable steady states, which I interpret to correspond to three observed presentations of autoimmunity. I analyze bifurcations and basins of attraction of the model to develop hypotheses about what mechanisms cause autoimmunity and why there is variability in symptoms between individuals.

Investigating Cranial Tendon Fibroblast Diversity Through Single-Cell Transcriptomics

Arul Subramanian and Pavan Nayak, UC Irvine

Tendons and ligaments are specialized, force-sensitive extracellular-matrix (ECM) rich tissue which function to attach muscles to bones (tendons) or bone to bone (ligaments). Tendon fibroblasts (called tenocytes) constantly sense microenvironment cues and modulate ECM structure and function to maintain tendon homeostasis. As the tendons of the vertebrate head contain diverse tendon attachment regions (ie. Muscle-bone attachments, muscle-muscle attachments etc), we hypothesized that distinct tenocyte subpopulations may underlie tendon tissue level diversity. Given the sensitivity of these fibroblasts, we strived to optimally preserve their transcriptomic signature by developing a cell dissociation protocol which can be performed under cold temperatures. Using this protocol, we explored tenocyte diversity by conducting single-cell RNA sequencing on FACS isolated tenocytes from severed heads of embryonic zebrafish and utilized clustering analysis combined with fluorescent hybridization chain reaction (HCR) to characterize and validate the spatial localization of these transcriptomically distinct populations. Though recent single cell sequencing studies address diversity within individual tendons, few studies have looked at the diversity of global transcriptomic signature profiles of tenocytes populating spatially distinct tendons and ligaments from both an inter- and intra-tendon perspective. From our analysis, we have uncovered putative tendon stem cell populations, as well as novel genes marking distinct connective tissue regions such as articular joints, specific myotendinous junctions, as well as entheses (tendon-bone/tendon-cartilage attachment zones). Further pathway analyses have indicated distinct populations of tenocytes highly expressing WNT signaling, a signaling pathway vastly understudied in tendons. Using a transgenic WNT positive fish in the background of a Tg(scx:mcherry) line marking tendons, we identified a subset of dual-expressing tenocytes localizing to the developing intermandibularis tendon. Furthermore, genetic disruption of WNT signaling causes dramatic tenocyte migration defects at these tissues. Conclusively, our work aims to provide insight into the spatial heterogeneity of tenocytes populating the diverse collection of tendons in the embryonic head. As tendon injuries comprise a large proportion of musculoskeletal injuries worldwide with very few treatment options due to lack of knowledge of tendon biology, our work will provide a developmental perspective for understanding the individual genetic profiles of tenocytes to form a basis for improved tendon therapies.

Non-specific vs Specific Dna Binding Free Energetics of a Transcription Factor Domain Protein Between Search and Recognition

Carmen Al Masri, UC Irvine

Transcription factor (TF) proteins are key in genetic regulation by locating specific sites on the genome. It has been proposed that an optimized search can be achieved by protein alternating between 3D diffusion in the bulk and 1D diffusion along DNA in the facilitated diffusion model. While undergoing 1D diffusion, the protein can switch from a “search” mode for fast diffusion along non-specific DNA, to a “recognition” mode for protein stable binding to specific DNA. Though it was regarded that protein conformation transitions enable the search to recognition transitions, it was recently noticed that for a small TF domain protein, re-orientation of the protein on the DNA, without protein conformational changes, happens between the non-specific and specific DNA binding. We further conducted all-atom molecular dynamics simulations on the TF domain protein bound to a nonspecific or specific DNA site, to energetically confirm that the protein-DNA binding free energetics between the two systems is consistent with the “search” and “recognition”. Using both the Jarzynski equality and umbrella sampling methods pulling protein away from DNA, we obtained protein binding free energetics and a free energy difference of about 10 kBT between the two systems. However, the binding free energy difference estimated from experimental measurements was about 4 kBT on 15-bp DNA. We suggest that the discrepancy between the experimental and computational measurements can be due to DNA sequences

flanking the 6-bp central specific and nonspecific binding sites. Such a proposal was also investigated through a highly simplified spherical protein model along with stochastic simulations, which well supported that flanking DNA sequences impact on overall protein dissociation kinetics and therefore on measuring binding affinity variations with specific or non-specific DNA in the central binding site.

Simulating Actin Networks in Synaptic Spine Heads using Dynamical Graph Grammars

Eric Mjolsness, UC Irvine

Joint work with Padmini Rangamani (UCSD), Terrence Sejnowski (Salk), and Thomas Bartol (Salk).

Comparative Assessment of Noncoding Variant Activity in vivo Using Limb Polydactyly as a Model

Ethan Hollingsworth, UC Irvine

Human genetics studies implicate a rapidly growing list of non-coding variants in human disease, including congenital heart disease, neurological disorders, and limb malformations. Yet, our understanding of how specific enhancer variants affect gene expression and cause phenotypes observed in patients remains limited, due in large part to a lack of efficient tools for in vivo enhancer variant analysis. Here we developed Dual-enSERT, a highly efficient (~50% integration efficiency) and robust dual fluorescence enhancer reporter system for comparative assessment of different human enhancer variant alleles in the same live mouse. We apply this technology to characterize the effects of rare variants in the ZRS limb enhancer of SHH that are linked to preaxial polydactyly in human patients. We compare enhancer activity across independent ZRS variants and show that all tested variants cause ectopic fluorescent reporter activity in the anterior margin of developing mouse limb bud consistent with their role in causing preaxial polydactyly. Thus, Dual-enSERT provides a highly reproducible, efficient, and customizable mammalian reporter system for the functional assessment of human enhancer variants in vivo.

Extracting Cell-State Specific Dynamical Information from Single Cell Transcriptome by Modeling Mrna Splicing

Federico Bocci, UC Irvine

Extracting dynamical information from single cell transcriptomics is a novel task with the promise to advance our understanding of cell state transition and interactions between genes. Yet, theory-oriented, bottom-up approaches that consider differences among cell states are largely lacking. Here, we present spliceJAC, a method to quantify the multivariate mRNA splicing from single cell RNA sequencing (scRNA-seq). spliceJAC utilizes the unspliced and spliced mRNA count matrices to constructs cell state-specific gene-gene regulatory interactions and applies stability analysis to predict putative driver genes critical to the transitions between cell states. By applying spliceJAC to biological systems including pancreas endothelium development and Epithelia-Mesenchymal Transition (EMT) in A549 lung cancer cells, we predict genes that serve specific signaling roles in different cell states, recover important differentially expressed genes in agreement with pre-existing analysis, and predict new transition genes that are either exclusive or shared between different cell state transitions.

Visualization of Chromatin Remodeling During Nuclear Degradation During Zebrafish Lens Development in vivo

Francesco Palomba, UC Irvine

Transparency of lens optics relies on controlled degradation of light scattering organelles. Primary lens fiber cells first lose their organelles during early development in the lens nucleus, followed by a life-long process of addition of secondary lens fiber cells in the germinal zone undergoing differentiation and organelle degradation. During nuclear degradation DNAses and methyl transferases play important roles in DNA remodeling in fiber cells. Mutants in these processes are associated with pathogenesis of age-related cataract in humans1 and in animal models2. While there has been progress in understanding the biochemical and genetic impact of epigenetic modifications, the complex organization at the molecular scale of the folded chromatin structure remains poorly understood. The main advantage of this characterization would enable researchers to use our platform to leave cells intact while being able to characterize the chromatin architecture in a living cell such that the characterization may provide important diagnostic features and allow for drug screening in real time. For this purpose, we have developed a quantitative imaging method using fluorescence lifetime and hyperspectral imaging of a widely used fluorescent nuclear stain (Hoechst 33342) to assess chromatin architecture in living cells. The spectral and lifetime properties of Hoechst revealed by phasor analyses reveal differences in chromatin density and accessibility, which correlates with transcriptional activity, as we have proven in a cell culture system. In this project we will image chromatin remodeling as a hallmark of epigenetic and architectural DNA changes in living zebrafish lens fiber cells as they undergo nuclear degradation during embryonic development. We hypothesize that chromatin remodeling prior to and during nuclear degradation in central primary lens fiber cells can be described by two models: i) The switch on-off model, whereby prior to nuclear degradation, high transcriptional activity is expected in all lens fiber cells, that then leads to a step-wise switch off of transcription starting at 45 hours post fertilization (hpf) in central fiber cells; or alternatively ii) The gradient model of transcriptional states from the lens periphery to the center of the lens well prior to the onset of nuclear degradation that gradually lead to central fiber cells undergoing nuclear degradation. To test these hypotheses, we will assess overall chromatin accessibility in specific parts of the zebrafish lens prior to, during and post primary lens fiber cell nuclear degradation. We will correlate our results with chromatin accessibility, DNA methylation and transcriptional activity by evaluating changes in the optical properties of Hoechst nuclear staining. We will then challenge in vivo lens fiber cell chromatin architecture accessibility by treatment of drugs that modify chromatin architecture, and therefore gene transcription, as well as in a previously characterized genetic mutant with decreased lens water influx that affects overall lens homeostasis, including the precise regulated microenvironment required for normal chromatin organization. We will correlate changes in DNA architecture with fiber cell proliferation and differentiation rates as well as transcriptional activity. This approach will enable the first ever visualization of chromatin epigenetic modifications during lens fiber cell differentiation in vivo.

On Chaotic Non-Stationary Steady-States and Biological Processes

Gessner Soto, University of Colorado

A late-1,970's contribution by Dr. K.-D. Willamowski and Dr. Otto Rössler related to a chemical reaction arrangement that is feasible able to sustain a chaotic type of non-stationary motion is examined. The arrangement of the chemical-reaction network transforms 3 distinct species into 2 distinct species, utilizing 3 transient intermediate species; the concentrations of these 3 intermediate species are the aspects of this arrangement that are able to access this sustained chaotic type of presence. There exist 2 primary objectives with this undertaking: 1) continue developing numerical tools for the analysis of more elaborate collections of non-linear differential-equations, particularly as they relate to reaction networks and 2) lay-out an initial argument for why chaotic-and-beyond type of non-stationary steady-states are not compatible with the type of motion that underlies biological activity.

Monitoring Chromatin Remodeling by Fluorescent Spectral and Lifetime Imaging in Living Systems: From 2D Cells to Zebrafish Embryos

Giulia Tedeschi, UC Irvine

Chromatin architecture and its changes are fundamental for the cell's physiological functions. Chromatin 3D arrangement in the nucleus can influence DNA accessibility to the transcription machinery as well as to regulatory molecules, therefore impacting genome function and gene expression. Moreover, dynamic modification of the nuclear architecture is linked to different biological phenomena including cell differentiation, cell migration and proliferation both in physiological and pathological conditions.

In this work we measured chromatin remodeling in living cells by a quantitative imaging method: we apply fluorescence lifetime imaging microscopy and hyperspectral imaging of a widely used fluorescent nuclear stain (NucBlue/Hoechst 33342) to assess chromatin architecture in living cells. We characterized the response of the nuclear dye biophysical properties in a controlled 2D cellular living system, evaluating different immortalized cell lines signatures and their response to chromatin compaction/decompaction treatments. We applied this method to image chromatin remodeling as a hallmark of epigenetic and architectural DNA changes in more complex 3D living systems, such as in living zebrafish, specifically studying how the lens fiber cells undergo nuclear degradation during embryonic development.

scReadSim: A Single-Cell RNA-seq and ATAC-seq Read Simulator

Guanao Yan, UC Los Angeles

Rapid advances of single-cell RNA-seq and ATAC-seq technologies propelled the development of many computational tools, whose benchmarking demand realistic simulators. However, few simulators can generate sequencing reads, and none of the existing read simulators aim to mimic real cells, hurdling the benchmarking of low-level computational tools that process reads. To fill this gap, we propose scReadSim, a single-cell RNA-seq and ATAC-seq read simulator that generates synthetic cells to mimic real cells. Trained on real data, scReadSim can generate synthetic data in FASTQ and BAM formats. Deploying scReadSim on sci-ATAC-seq and 10x Multiome (ATAC+RNA) data, we show that the scReadSim synthetic data resemble the real data at both read and count levels. As a flexible simulator, scReadSim provides unique molecular identifier (UMI) counts for benchmarking scRNA-seq deduplication tools, and scReadSim can accommodate user-specified open chromatin regions (``ground truths'') to generate single-cell ATAC-seq data. Our benchmark applications of scReadSim show that UMItools is a preferred scRNA-seq deduplication tool, and MACS3 achieves top performance in scATAC-seq peak calling. Moreover, scReadSim can guide experimental design by allowing the cell number and sequencing depth to vary.

PIEZO1 Regulates Leader Cell Formation and Cellular Coordination During Collective Cell Migration

Jesse Holt, UC Irvine

The collective migration of keratinocytes during wound healing requires both the generation and transmission of mechanical forces for individual cellular locomotion as well as for the coordination of movement across cells. Leader cells initiated along the wound edge transmit mechanical and biochemical cues to ensuing follower cells, ensuring their uniform polarization and coordinated direction of migration, or directionality. Despite the observed importance of mechanical cues in leader cell formation and governing directionality, the underlying biophysical mechanisms remain elusive. The mechanically activated ion channel PIEZO1 was recently identified to play an inhibitory role during the reepithelialization of wounds through retraction of keratinocytes located

along the wound edge. Here, through an integrative experimental and mathematical modeling approach, we elucidate PIEZO1's contributions to collective migration. Time-lapse microscopy reveals that PIEZO1 activity inhibits leader cell formation along the wound edge. To probe the relationship between PIEZO1 activity, leader cell formation and inhibition of reepithelialization, we developed an integrative 2D-multiscale model of wound closure that links observations at the single cell and collective cell migration scales. Through numerical simulations and subsequent experimental validation, we found that directionality plays a key role during wound closure and is inhibited by PIEZO1 activity. We propose that PIEZO1-mediated retraction suppresses leader cell formation which inhibits the coordination of directionality between cells during collective migration.

Myeloid-Derived Suppressor Cell Dynamics Control Outcomes in the Metastatic Niche

Jesse Kreger, University of Southern California

Myeloid-derived suppressor cells (MDSCs) play a prominent and rising role in the tumor microenvironment. An understanding of the tumor-MDSC interactions that influence disease progression is critical, and currently lacking. To address this, we developed a mathematical model of metastatic growth and progression in immune-rich tumor microenvironments. We model the tumor-immune dynamics with stochastic delay differential equations, and study the impact of delays in MDSC activation/recruitment on tumor growth outcomes. We find when the circulating level of MDSCs is low, the MDSC delay has a pronounced impact on the probability of new metastatic establishment: blocking MDSC recruitment can reduce the probability of metastasis by as much as 50%. We also quantify the extent to which decreasing the immuno-suppressive capability of the MDSCs impacts the probability that a new metastasis will persist or grow. In order to quantify patient-specific MDSC dynamics under different conditions we fit individual tumors treated with immune checkpoint inhibitors to the tumor-MDSC model via Bayesian parameter inference. We reveal that control of the inhibition rate of natural killer cells by MDSCs has a larger influence on tumor outcomes than controlling the tumor growth rate directly. Posterior classification of tumor outcomes demonstrates that incorporating knowledge of the MDSC responses improves predictive accuracy from 63% to 82%. Our results illustrate the importance of MDSC dynamics in the tumor microenvironment and predict interventions that may shift environments towards a less immune-suppressed state. We argue that there is a pressing need to more often consider MDSCs in analyses of tumor microenvironments.

Stochastic Models of Nucleosome Dynamics Reveal Regulatory Rules of Stimulus-Induced Epigenome Remodeling

Jinsu Kim, POSTECH

The genomic positions of nucleosomes are a defining feature of the cell's epigenomic state, but signal-dependent transcription factors (SDTFs), upon activation, bind to specific genomic locations and modify nucleosome positioning. Here we leverage SDTFs as perturbation probes to learn about nucleosome dynamics in living cells. We develop Markov models of nucleosome dynamics and fit them to time course sequencing data of DNA accessibility. We find that (1) the dynamics of DNA unwrapping are significantly slower in cells than reported from cell-free experiments, (2) only models with cooperativity in wrapping and unwrapping fit the available data, (3) SDTF activity produces the highest eviction probability when its binding site is adjacent to but not on the nucleosome dyad, and (4) oscillatory SDTF activity results in high location variability. Our work uncovers the regulatory rules governing SDTF-induced nucleosome dynamics in live cells, which can predict chromatin accessibility alterations during inflammation at single-nucleosome resolution.

Zeros in Sequencing Data Can Increase Parameter Identifiability for Gene Expression Model

Junhao Gu, UC Irvine

Gene expression is often modeled by a promoter-switching model with different transcription rates, which can give rise to RNA production either in pulsatile bursts, or a Poisson-like accumulation, depending on the parameters. Recently researchers have leveraged this simplified model to understand the gene regulatory dynamics against single-cell RNA sequencing data by analyzing the transcriptomic distributions. Here, we simulated a large parameter space of different variants of the gene expression model and found that drastically different kinetic parameters combinations can result in very similar mRNA distributions, leading to low identifiability of the kinetic parameters. We demonstrated that the "zeros" in mRNA distributions (arising from cells in which no transcripts of a given type are detected) can actually increase parameter identifiability, when the zeros are biological in origin (specifically, when they arise from bimodal expression that occurs in the slow-promoter-switching regime). We further explore parameter identifiability when the zeros arise from a combination of biological and technical noise sources. Our results caution against data processing methods that replace zeros with imputed non-zeros values under the assumption that zeros are purely technical in origin. Despite the limitations we find in parameter identifiability, these results could be useful in revealing dynamics of low-expressed genes and building quantitatively accurate gene network models.

Enhancement of Car T Cell Activation by Pulsatile Signals Can Be Explained by a Mathematical Model with Homeostatic Feedback, to Predict Improved Activation Protocols

Ke Xu, UC Irvine

T cells are immune cells responsible for identifying and, in some cases, killing pathogenic cells in our bodies. They are activated by receiving an extracellular signal, in the form of antigen, that binds to a receptor on the surface of the T Cell. In the past 10 years, T cells have been engineered with chimeric antigen receptors (CAR) as a potential new class of therapy against a range of disease. Recent experiments found that stimulating a CAR T cell with short pulses of antigen yields a stronger response than stimulating them continuously. Here we aim to show that (Aim 1) a simple model can reproduce the response to pulsing by identifying a previously uncharacterized homeostatic feedback. (Aim 2) We aim to quantitatively learn a model to fit the existing data, crucially, using a neural net to represent the unknown homeostatic feedback, thus obviating the need to assume a particular functional form, as has traditionally been done in mathematical modeling. Finally, (Aim 3) with such a quantitative, predictive model, we aim to design optimal stimulation strategy to maximally activate a CAR T cell.

Defining the Role of Lymphocytes in Basal Cell Carcinoma Spontaneous Regression

Kirsten Wong, UC Irvine

Because of UV exposure, our skin accumulates a high mutational load similar to that of many solid tumors, yet can remain phenotypically normal despite the presence of typical cancer driver mutations. Basal cell carcinoma (BCC) is one of the most mutated types of human cancer, but most tumors can be surgically excised from the skin or sometimes spontaneously regress without treatment (20-29% of observed tumors) . The tumor microenvironment interactions that dictate whether aggressive skin tumors will form or not are not well understood. In the inducible Gli1CreERT2; Ptch1 fl/fl transgenic BCC mouse model, microscopic tumors form in the skin and shrink gradually before ever reaching macroscopic sizes, suggesting that a regression mechanism is at play. Using this BCC model of tumor regression in the absence of treatment, we assess the role of immune system interactions in promoting this protective process and as a potential therapeutic target. With case studies

suggesting a role for immune activation in BCC tumor regression, we hypothesize that T cells are recruited to growing BCCs to promote spontaneous tumor shrinkage. Analysis of immune infiltration and bulk and single-cell RNA-sequencing of tumor-bearing skin shows evidence of an innate inflammatory response during tumor growth followed by a transient cytotoxic lymphocyte response. By assessing lymphocyte necessity in inducing anti-tumor responses, we aim to define the immune response against BCC tumors and determine the regulatory role of effector lymphocytes in regression.

Mathematical Modeling and Bioinformatic Analysis to Investigate Multigenerational Epigenetic Stability of DNA Methylation Landscapes

Kwadwo Bonsu, UC Irvine

Currently, it is not well understood what factors contribute to the stability of the epigenome in different cell types across the lifetime of mammals. Better understanding of these factors would aid development of therapies for age-related diseases, including cancer. DNA methylation is a widely studied epigenetic mark, located primarily on Cytosine-phosphate-Guanine (CpG) dinucleotides, with known associations to transcriptional gene silencing, and variable dynamics across the lifetime of mammalian organisms (replication, development/differentiation, age, etc.). In this project, I have developed stochastic mathematical models of CpG methylation maintenance, which is the reestablishment of methyl marks following replication. Incorporation of experimentally-derived correlations, probing post-replication re-methylation dynamics, into distance-dependent reactions allows us to model effects such as enzyme processivity, and the influence of different genomic features (i.e., Promoters, Transposable Elements). CpG Islands (CGIs) are regions of the DNA which contain high CpG content, and as such establish regions of hyper-/hypomethylation. Bioinformatic analysis of methylation landscapes in human embryonic stem cells (hESCs)shows that methylation levels of CGIs are inversely correlated with CpG Island size, and suggest a size threshold exists; below which islands are more likely to be methylated. These observations from the data may be used to inform mechanistic mathematical models, as my simulations currently predict that the size and density of CGIs influence overall stability of their methylation patterns, reflected in variable state-switching of island methyl states across multiple replication cycles, but are strongly dependent on the strength of interaction between neighboring CpGs in (de-)methylating reactions.

A Eukaryotic Circuit for Secretion-Coupled Cellular Autonomy

Lingxia Qiao, UC San Diego

Cancers represent complex autonomous systems, displaying self-sufficiency in growth signaling. Autonomous growth is fueled by a cancer cell's ability to 'secrete-and-sense' growth factors: a poorly understood phenomenon. Using an integrated systems and experimental approach, here we dissect the impact of a feedback-coupled GTPase circuit within the secretory pathway that imparts secretion-coupled autonomy. The GTPase circuit is assembled when the Ras-superfamily monomeric GTPase Arf1, and the heterotrimeric GTPase Gi□□□ and their corresponding GAPs and GEFs are coupled by GIV/Girdin, a protein that is known to fuel aggressive traits in cancers. This GTPase circuit ensures the dose information transmission by achieving the dose response alignment behavior of sensing and secretion, leading to self-sustained cell survival by stimulus-proportionate secretion. Findings highlight how enhanced coupling of two biological switches in cancer cells is critical to secretion-coupled autonomy.

AR Regulates Sex-Dimorphic Gene Expression in the Mouse Kidney

Lingyun (Ivy) Xiong, University of Southern California

Previous studies emphasized sexual dimorphism in renal transcriptome, physiology, response to injury and susceptibility to conditions including hypertension and kidney diseases. In this study, we investigate the regulatory mechanism of dimorphic gene expression in the murine kidney. Whole-kidney bulk RNA-seq from neonates to aged adult C57BL/6 mice revealed that sex differences in gene expression onset at puberty. Gene- and isoform-level analysis highlighted differential functions related to peroxisomal lipid metabolism and nuclear receptor pathways. Gonad removal and sex hormone receptor removal experiments showed that 82.5% of male- and 55.6% of female-biased genes were perturbed upon nephron-specific removal of AR, but not ERα. To investigate the regulatory role of AR in the proximal tubule (PT), a major sex-biased renal population, we performed single-nuclear multimodal analysis on normal and AR KO male kidneys. In the absence of AR, male-biased genes show decreased accessibility, while female-biased genes become more accessible, preluding their altered gene expression pattern in PT segments. Leveraging high-resolution multimodal data, we identified putative AR response elements near sex-biased genes. Lastly, we examined AR-mediated regulation of dimorphic gene expression within the kidney and liver through a comparative approach, uncovering a small but significant set of conserved sex biases. Collectively, this study reports the predominant role of AR in regulating renal sex specificity.

ESPRESSO Phenotyping of Neural Stem Cells for Efficacy Screening for the Treatment of Spinal Cord Injury

Lorenzo Scipioni, UC Irvine

TBA

Simulating Actin Networks in Synaptic Spine Heads using Dynamical Graph Grammars

Matthew Hur, UC Irvine

There is a morphodynamic component to synaptic learning by which dendritic (postsynaptic) spine head size changes can strengthen or weaken the synaptic connection between two neurons, in response to signals from the axon of a presynaptic neuron. These morphological factors are in turn sculpted by the graph-like dynamics of the actin cytoskeleton. In this project, we sought to use Dynamical Graph Grammars (DGGs) implemented within a computer algebra system to model how networks of actin filaments can dynamically grow or shrink and reshape the spine head. We designed and implemented several DGG sub-grammar mathematical models including actin network growth, isotropic/anisotropic filament forces, filament-membrane mechanical interaction, and Hessian Boltzmann sampling, to regulate the generation and deletion of graph objects. From first principles expressed in about a dozen DGG rules we simulate emergent biomechanics of a simplified network of actin polymers and its interaction with membrane, all in two dimensions.

Biophysical Modeling of Actin-Mediated Structural Plasticity

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Neurons encode memory by strengthening their connections (synapse). Such strengthening correlates with dendritic spine enlargement, the postsynaptic part of most excitatory neurons. Upon stimulation, they undergo a series of chemical reactions, and there is a stimulus-triggered influx of actin and actin-binding proteins into the spines. These events translate into a rapid remodeling of the actin cytoskeleton, which allows the spine to transiently enlarge over three times its original size. To investigate this transient enlargement, we developed a minimal 3D model of dendritic spines describing actin, Arp2/3, and cofilin dynamics with partial differential

equations in a moving boundary framework. We found that this reduced model qualitatively reproduces the spine enlargement upon stimulation, and that localization of the stimulus-triggered influx achieves similar spine volume with less protein influx. However, spine growth is limited due to the interaction between the actin polymerization and membrane forces. Thus, further spine expansion is only possible by introducing other mechanical forces, as those exerted by clutch molecules.

Control of Active Nematics using Confinement, Optogenetics, Viscoelasticity, and Reaction-Diffusion Systems

Michael Norton, Rochester Institute of Technology

Active nematics are an important class of self-organizing system that include living matter, such as growing bacterial colonies and confluent tissue layers, as well as model systems built from reconstituted cytoskeletal components. Understanding how to shape these nematic flows and structures is a grand challenge in active matter. I present four approaches for shaping nematic dynamics. In the first, I consider how geometric confinement creates a suite of competing stable and unstable flow structures that control the transition from laminar flows to turbulence. Second, I show how optimal control tools and optogenetically enabled nematics can be used to shape active stresses and create desired configurations. Next, I explore how the addition of viscoelastic stresses impacts defect dynamics. Finally, I propose a non-equilibrium mechanochemical feedback system that “detects” +/- 1/2 defects. This scheme lays the foundation for rational engineering of programmable chemomechanical active nematics and understanding phenomena where cell fates are coupled to topology, such as in the hydra, neural progenitor cells, and bacterial films.

Mechanistic Connections among Epithelial Plasticity, Skin Barrier, Inflammation, and Whole-Body Metabolism

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Skin epidermis constitutes the outer permeability barrier that protects the body from dehydration and myriad external assaults. Mechanisms that maintain barrier integrity in constantly challenged adult skin and how epidermal barrier dysregulation shapes the local immune microenvironment and whole-body metabolism remain poorly understood. Ovol1 and Ovol2 encode transcriptional repressors in the skin that regulate epithelial differentiation and cell adhesion and inflammation. Here, we generated mice with Ovol1/2 inducibly deleted from epidermal cells in adulthood and found that Ovol1 and Ovol2 are required to promote terminal differentiation, maintain barrier integrity. This is in part due to their overlapping molecular functions of in regulating epithelial-mesenchymal plasticity, cell adhesion, cytoskeleton, and inflammation. Additionally, in both skin and skin-draining lymph node, Ovol1/2-deficient mice show signs of chronic low-grade inflammation featuring precious Langerhans cell activation and aberrant T cell response. Interestingly, we identify whole-body defects in these mice (e.g., lower body weight) and provide functional evidence to suggest that this is in part due to inflammation. Overall, this study aims to elucidate the mechanism by which Ovol1/2 regulate skin barrier function and inflammation.

Size Control of a Feather: Regulation of Progenitor Cell Zone Enables the Formation of Long Feathers

Ping Wu, University of Southern California

Size control of a feather: Regulation of progenitor cell zone enables the formation of long feathers
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A fundamental question in regenerative medicine is how organ size is determined. The balance of homeostasis of stem cell, TA cells and differentiated cells leads to control of proper organ size. A bird has feathers with different lengths and widths. The molecular and cellular basis of feather size control remains elusive. Here, by comparing different feather types from domestic chickens, we demonstrate that the follicle configuration is related to the feather size. Examining the extremely long feather in Phoenix chickens reveals aspects of specialized stem cell/TA cell homeostasis. Furthermore, transcriptome profiling demonstrates that long feathers are enriched in FGF and IGF signaling pathways. scRNA-seq analysis reveals that longer feathers undergo a longer route to achieve terminal differentiation. Cellchat analysis suggests that longer feathers have increased IGF interactions between epidermal progenitor cells and dermal cells. Functional analysis reveals that FGF pathway inhibition produced shorter feathers, whereas IFG pathway overexpression generated longer feathers. Thus, we discovered principle governing feather size control.

Modeling and Simulating Human Mitochondrial RNA Polymerase Nucleotide Addition Cycle Using Computational Microscope

Shannon McElhenney, UC Irvine

Human mitochondrial RNA polymerase (POLRMT) is essential to the transcription of mitochondrial DNA and cell metabolism. This work contributes to an all-atom structural dynamics model for individual steps of the nucleotide addition cycle. We aim to establish a model using all-atom molecular dynamics to reveal regulation of natural and analog nucleotide binding and insertion, and a model for the post-catalytic mechanochemical coupling between product release and POLRMT translocation. The models are constructed combining the high-resolution structures of POLRMT in the open and closed conformations with the bound NTP and Mg2+ ions detected in structurally similar T7 bacteriophage RNA polymerase and using curve interpolation to place missing non-template DNA segment. The models have been evaluated for protein subdomain stability and the appropriate protein conformation, open or closed, via subdomain motion regulation on the active site. Since POLRMT is structurally similar to viral RNA polymerases, the study is particularly important for antiviral design, where nucleotide analog drug candidates are expected to be screened for POLRMT toxicity.

Statistical Learning of Biophysical Factors Controlling Signaling Molecule Localization in Primary Cilium

Sohyeon Park, UC Irvine

Many signaling cascades occur at the primary cilium, and involve dynamic relocalization of signaling molecules. Mislocalization of signaling molecules is associated with a class of diseases called ciliopathies. Even for the most well studied pathway, e.g. Hedgehog, the mechanism which selectively controls the localization of signaling molecules is debated.

There is much prior evidence for a diffusive barrier at the base of the cilium, but 'diffusive barrier' can mean several distinct biophysical phenomena. We develop a method that uses single particle tracks to distinguish local changes in viscosity versus local elastic barriers, and can distinguish how much is due to membrane heterogeneity versus cytoplasmic/cilioplasmic structures. This method is based on advances in Bayesian statistical learning to detect subtle differences between biophysical forces. Here we demonstrate our methods using synthetic data. We plan to apply this method to real single particle tracks.

Epithelial-Mesenchymal Transition Couples with Both G1/S and G2/M Arrest

Sophia Hu, University of Pittsburgh

Epithelial to mesenchymal transition, EMT, is involved in numerous biological processes such as wound healing, tissue fibrosis, and cancer metastasis. Existing literature has debated on the transition paths in EMT as well as how the cell cycle couples to EMT. To address these questions, we first generated a scRNA-seq dataset, where mammary epithelial MCF10A cells were treated with different doses of TGFβ, an EMT inducer. We then analyzed the data with dynamo, a machine-learning based analytical framework we developed to reconstruct single cell dynamical equations (Qiu et al. Cell, 2022). From the obtained vector fields, transition path analyses revealed two unique transition paths, corresponding to either an arrest in the G1/S or G2/M phase, when cells underwent EMT. The existence of these two paths agrees with our previous live cell imaging studies (Wang et al., Sci Adv 2020, eLife 2022), but not with pseudotime analyses reported in the literature. Our analyses demonstrate the importance of including dynamical information in single cell data analyses.

Theresa Loveless, UC Irvine/ Rice University

Detecting Species Interactions in Ecological Systems

Thomas Beardsley, UC Irvine

We investigate methods for detecting interactions between members of an ecological community. Often, qualitative descriptions of interactions are used to infer benefits or consequences for a member's fitness. We develop a purely quantitative model of interactions in an evolving population, and use analytical and numerical tools to determine the existence of interactions among subpopulations. In particular, we use a dynamical model to describe E. Coli serial propagation experiments, develop conditions under which we can reject the null (no interaction) model, and use numerical tools to estimate relative fitness values of genetic subpopulations.

scDesign2: A Transparent Simulator That Generates High-Fidelity Single-Cell Gene Expression Count Data with Gene Correlations Captured

Tianyi Sun, UC Los Angeles

In the burgeoning field of single-cell transcriptomics, a pressing challenge is to benchmark various experimental protocols and numerous computational methods in an unbiased manner. Although dozens of simulators have been developed for single-cell RNA-seq (scRNA-seq) data, they lack the capacity to simultaneously achieve all the three goals: preserving genes, capturing gene correlations, and generating any number of cells with varying sequencing depths. To fill in this gap, here we propose scDesign2, a transparent simulator that achieves all the three goals and generates high-fidelity synthetic data for multiple scRNA-seq protocols and other single-cell gene expression count-based technologies. Compared with existing simulators, scDesign2 is advantageous in its transparent use of probabilistic models and is unique in its ability to capture gene correlations via copula. We verify that scDesign2 generates more realistic synthetic data for four scRNA-seq protocols (10x Genomics, CEL-Seq2, Fluidigm C1, and Smart-Seq2) and two single-cell spatial transcriptomics protocols (MERFISH and pciSeq) than existing simulators do. Under two typical computational tasks, cell clustering and rare cell type detection, we demonstrate that scDesign2 provides informative guidance on deciding the optimal sequencing depth and cell number in single-cell RNA-seq experimental design, and that scDesign2 can effectively benchmark computational methods under varying sequencing depths and cell numbers. With these advantages, scDesign2

is a powerful tool for single-cell researchers to design experiments, develop computational methods, and choose appropriate methods for specific data analysis needs.

Single-Cell CA2+ Parameter Inference Reveals How Transcriptional States Inform Dynamic Cell Responses

Xiaojun Wu, University of Southern California

Single-cell genomics data offer new resources for studying cells, but their potential to inform parameter inference of cell dynamics has yet to be realized. We develop methods for Bayesian inference with data that jointly measure gene expression and Ca2+ signaling dynamics in single cells. The posterior distribution of one is used to inform the prior of its neighbor by transfer learning along a cell chain. In application to Ca2+ dynamics, we fit the parameters of thousands of models of single-cell responses. We show that transfer learning accelerates inference, although building cell chains by transcriptional similarity does not improve over random ordering. Clustering cell posteriors reveals that only using similarity-based chains can we distinguish Ca2+ dynamic profiles. We discover competing intracellular and intercellular sources of cell heterogeneity. Single-cell parameter inference thus offers broad means to quantify relationships between transcriptional states and Ca2+ dynamics.

Single-Cell and Spatial Transcriptomics Reveal Fibroblast Heterogeneity and Pathological Signaling Networks in Human Keloid Scars

Yingzi Liu, UC Irvine

Yingzi Liu, Christian F. Guerrero-Juarez, Qing Nie, Ji Li, Maksim V. Plikus

Keloid, a tumor-like scarring disorder of human skin, is caused by wounding in genetically predisposed individuals with poorly defined pathogenesis. Here, we implemented single-cell RNA-sequencing, spatial transcriptomics, and whole genomic sequencing techniques to uncover the spatial cellular heterogeneity and signaling changes in the context of keloid disease. Integrated analysis revealed that fibroblasts significantly accumulate at the periphery of keloid scar and they express high levels of genes predicted to be ‘causal’ of disease based on their correlation with known keloid-related GWAS loci. Among skin fibroblasts, deep dermal (aka reticular) fibroblasts are significantly expanded in keloid scar compared to normal edge skin. Differential gene expression and regulon analysis revealed that keloid fibroblasts exhibit enrichment in extracellular matrix organization and pro-inflammatory gene expression, driven by unique transcription factors. Thus, we conclude that aberrant activation of reticular fibroblasts drives keloid pathogenesis, in part by producing excessive amounts of extracellular matrix and promoting inflammation. Our findings indicate that targeting reticular fibroblast types might prove as an effective approach for next generation keloid treatments.

Spatial Specificity of Mammalian Enhancer Contacts In Vivo

Zhuoxin Chen, UC Irvine

Developmental genes are regulated by remote enhancers, sometimes located up to two million base pairs away. Enhancers are thought to activate target genes via chromatin looping. Yet, the role and extent of chromatin looping for enhancer function are unclear since some functional enhancer-promoter loops appear to be cell-type specific while others are invariant across cell types. To address this question, we generated high-resolution enhancer-centric contact maps across 10 murine embryonic tissues for nearly a thousand developmental enhancers with characterized in vivo activities. We found that most enhancer loops are tissue-specific, and these loops are significantly stronger when enhancers are active. The invariant loops account for a small fraction (6%) of all examined E-P chromatin interactions and are associated with adjacent CTCF binding but not enhancer activity. We confirmed identified E-P interactions using spatial gene expression information and enhancer

knock-out mice. Our results provide a global view of enhancer loops and highlight the general significance of enhancer-promoter proximity for developmental gene regulation in mammals.