# AGENDA

Winter qBio Conference 2026

## Monday, February 16, 2026

**5:00 - 8:00 PM** **Opening Registration and Welcome Reception**
Location: Napili Pool Lawn

## Tuesday, February 17, 2026

**7:00 - 8:30 AM** **Registration and Breakfast**
Location: Sunset Terrace

**8:30 - 10:20 AM** **Opening Remarks and Morning Session I**
Location: Monarchy Ballroom 2-3
*Chair: Gurol Suel*

**Opening Remarks: Hana El-Samad and Wendell Lim**
8:30 - 8:55 AM

**Keynote 1: Hana El-Samad**
9:00 - 9:35 AM

**Ramya Deshpande: Predictive Multicellular Assembly via Cell-Cell Adhesion**
9:40 - 9:55 AM

**Jungmin Lee: Engineering Orthogonal Chemokine-Receptor System to Control Immune Cell Trafficking**
10:00 - 10:15 AM

**10:20 - 10:40 AM** **Coffee Break**
Location: Monarchy Terrace

**10:40 - 12:15 PM** **Kids' Science!**
Location: Sunset Terrace

**10:40 - 12:15 PM** **Morning Session II**
Location: Monarchy Ballroom 2-3
*Chair: Gurol Suel*

**Keynote 2 | Michael Elowitz: Engineering protein circuits for cancer therapy**
10:40 - 11:15 AM

**Mathias Heltberg: Oscillatory Control of Biomolecular Condensates in Regulation of the DNA Damage Response**
11:20 - 11:35 AM

**Silvia Canas Duarte: Revisiting aging in Escherichia coli: When prepping goes awry**
11:40 - 11:55 AM
**Justin Gallivan: Keck Foundation**
12:00 - 12:10 PM

**12:15 - 1:45 PM** **Lunch (on own)**

**1:45 - 3:30 PM** **Main Afternoon Session**
Location: Monarchy Ballroom 2-3
*Chair: Wendell Lim*

**Gege Qian: Remodeling of Cancer Cell Architecture by Chemotherapy**
1:50 - 2:05 PM
**Keynote 3: Silvia Santos**
2:10 - 2:45 PM
**Saki Ozeki: Large-Scale Automated Cell Lineage Analysis for Ploidy Transitions**
2:50 - 3:05 PM
**Shixuan Liu: The Changing Seasonality of Human Reproduction**
3:10 - 3:25 PM

**6:00 - 8:00 PM** **Pau Hana**
A roundtable event with lively discussions that serve to create a positive culture and embrace DEI within the Winter qBio community
Location: Monarchy Ballroom 1

**6:00 - 8:00 PM** **Kids' Movie Night**
Location: Maui Suite 4

## Wednesday, February 18, 2026

**7:00 - 8:30 AM** **Breakfast**
Location: Sunset Terrace

**8:30 - 10:20 AM** **Contributed Session I**
Location: Maui Suite 3

**Akihiro Isomura: A synthetic biology approach to synchronising the segmentation clock**
8:30 - 8:40 AM
**Mohamad Najia: Live-cell transcriptomics with engineered virus-like particles**
8:40 - 8:50 AM
**P C Dave Dingal: Sequence-specific control of embryonic signaling and development using a synthetic protease library**
8:50 - 9:00 AM

**Ayumi Goto: Cooperative Roles of Lfng and Dll3 in Stabilizing Segmentation Clock Synchrony**
9:00 - 9:10 AM
**Cordelia McGehee: Translational Readthrough as a Mechanism of Chemotherapy Resistance**
9:10 - 9:20 AM
**Debalina Datta: Age-Dependent Remodeling of Cellular Stress Response Kinetics: Optogenetic Dissection of the ISR Across the Human Lifespan**
9:20 - 9:30 AM
**Keith Breau: Coupling mathematical modeling with a novel human intestinal stem cell system to understand feedback regulation during planar cell polarity**
9:30 - 9:40 AM
**Alain Bonny: Stromal-Stem Cell Spatiotemporal Coordination in Tissue Repair**
9:40 - 9:50 AM
**Mark Greenwood: Epinephrine oscillation enhances the alertness of target cells to stress**
9:50 - 10:00 AM

## 8:30 - 10:20 AM Contributed Session II

Location: Maui Suite 4

**Hollie Hindley: Heterogeneity in responses to ribosome-targeting antibiotics mediated by bacterial RNA repair**
8:30 - 8:40 AM
**Ksenia Sokolova: Noncoding Regulatory Impact Shapes Cancer Progression**
8:40 - 8:50 AM
**Hyongbum Kim: High-Throughput Functional Annotation of Genetic Variants using Prime Editing and Deep Learning**
8:50 - 9:00 AM
**Pujun Guan: Evaluating Single-Cell Integration without Ground Truth**
9:00 - 9:10 AM
**Cyrus Knudsen: Producing quantitative protein function data at scale to enable protein design**
9:10 - 9:20 AM
**Alexandra Nava: A Machine-Learning Model Predicts Spore Germination**
9:20 - 9:30 AM
**Joseph Slivka: Dissecting the stepping dynamics of dynein using 1- and 2-color MINFLUX**
9:30 - 9:40 AM
**Wei Chen: De novo design of transcription regulators**
9:40 - 9:50 AM
**Ruoyu Wang: Single-molecule sequence model of the human regulatory genome**
9:50 - 10:00 AM
**Wojciech Szpankowski: Detecting Correlated Structures in Biological Networks with Mismatches**
10:00 - 10:10 AM

## 10:20 - 10:40 AM Coffee Break

Location: Monarchy Terrace

**10:40 - 12:15 PM** **Kids' Science!**
Location: Sunset Terrace

**10:40 - 12:15 PM** **Main Morning Session**
Location: Monarchy Ballroom 2-3
*Chair: Jeff Hasty*

**Announcements**
10:40 - 10:50 AM
**Keynote 4 | Kiana Aran: Connecting Semiconductors Chips to the Machinery of Life**
10:55 - 11:30 AM
**Keynote 5 | Michael McManus: Mapping the Language of Cell Communications**
11:35 - 12:10 PM

**12:15 - 1:45 PM** **Lunch (on own)**

**1:45 - 2:55 PM** **Main Afternoon Session**
Location: Monarchy Ballroom 2-3
*Chair: Hana El-Samad*

**Keynote 6 | Wendell Lim**
1:50 - 2:25 PM
**Charilaos Giannitsis: Many behaviors, one regulator: The phenotypic landscape of a single transcription factor**
2:30 - 2:45 PM

**2:55 - 3:15 PM** **Coffee Break**
Location: Monarchy Terrace

**3:15 - 5:00 PM** **Poster Session**
Location: Maui Suite 2

## Thursday, February 19, 2026

**7:00 - 8:30 AM** **Breakfast**
Location: Sunset Terrace

**8:30 - 10:20 AM** **Contributed Session I**
Location: Maui Suite 3

**David Glass: Engineering synthetic multicellularity for understanding consortia and tissue behavior**
8:30 - 8:40 AM
**Xiao Peng: Feedback control of differentiation for ratiometric stability and pattern formation of synthetic multicellular system**

8:40 - 8:50 AM
**Brian Cleary: Fundamental errors in RNA velocity arising from the omission of cell growth**
8:50 - 9:00 AM
**Gene-Wei Li: The defining features of intrinsic transcription terminators**
9:00 - 9:10 AM
**Yujia Liu: Reconstitution of feedback architectures in the cyanobacterial clock that allow growth tolerance**
9:10 - 9:20 AM
**Katie O'Connor: Patient-derived organoids as predictive disease models for therapeutic chassis selection**
9:20 - 9:30 AM
**Kevin YuKai Chao: A Genetically Encoded Device for Transcriptome Storage in Mammalian Cells**
9:30 - 9:40 AM
**Vaibhav Murthy: Decoding Niche Organization and Adaptive Signaling Activation Patterns Underlying Spatiotemporal Osteosarcoma Drug Response Heterogeneity**
9:40 - 9:50 AM
**Victoria Chen: Uncovering the Long-Term Dynamics of Engineered Genes Released to the Environment**
9:50 - 10:00 AM
**Jaeseung Hahn: Two-state pharmacokinetic model simulates systemic delivery of bacterial cancer therapy with dynamic encapsulation system**
10:00 - 10:10 AM

## 8:30 - 10:20 AM Contributed Session II

Location: Maui Suite 4

**Tongli Zhang: Cerebrospinal Fluid Adrenocortical-Brain Steroid Concentrations and Dynamics in Healthy Humans and in Veterans with Posttraumatic Stress Syndrome: Physiologically-based Hormone Dynamic Modeling**
8:30 - 8:40 AM
**Gerald Pao: Explainable data science on low dimensional manifolds**
8:40 - 8:50 AM
**Riley Juenemann: Evaluating Genetic Engineering Trade-offs Through Whole-cell Modeling of Escherichia coli**
8:50 - 9:00 AM
**Meera Prasad: Learning the rules of life by playing multicellular games**
9:00 - 9:10 AM
**Xin Wang: Same-cell profiling of chromatin accessibility and protein**
9:10 - 9:20 AM
**Benjamin Swedlund: Engineering Self-Organized Tissue-Scale Patterns in Mammalian Cells using Synthetic Reaction-Diffusion Circuits**
9:20 - 9:30 AM
**Jiayi Wu: Tuning a Genetic Circuit with Double Negative Feedforward Loops to Approximate Square Waves**
9:30 - 9:40 AM
**Ksenia Zlobina: Reconstructing Biological Dynamics from Transcriptomic**

**Time Series**
9:40 - 9:50 AM
**Filippo Liguori: Dynamic Gene Expression Mitigates Mutational Escape in Lysis-Driven Bacteria Cancer Therapy**
9:50 - 10:00 AM
**Thomas Kuhlman: Human Stress Response Specificity through Bioresonance Selectivity**
10:00 - 10:10 AM

**10:20 - 10:40 AM** **Coffee Break**
Location: Monarchy Terrace

**10:40 - 12:15 PM** **Main Morning Session**
Location: Monarchy Ballroom 2-3
*Chair: Olga Troyanskaya*

**Announcements**
10:40 - 10:50 AM
**Keynote 7: Olga Troyanskaya**
10:55 - 11:30 AM
**Keynote 8 | Michael Shelley: Self-Organization, Flows, and Transport within (and of) Living Cells**
11:35 - 12:10 PM

**12:15 - 1:45 PM** **Lunch (on own)**

**1:45 - 3:10 PM** **Main Afternoon Session**
Location: Monarchy Ballroom 2-3
*Chair: Lev Tsimring*

**Shah Md Toufiqur Rahman: Pooled CRISPRi and live-cell imaging reveal enhancer control of NF-κB signaling dynamics**
1:50 - 2:05 PM
**Keynote 9 | Sujit Datta: Death and chemotaxis: Watching bacterial groups navigate complex environments**
2:10 - 2:45 PM
**Matthew Bennett: Fast, long-range intercellular signal propagation through growth assisted positive feedback**
2:50 - 3:05 PM

**6:30 - 9:30 PM** **Banquet!**
Location: Halona Kai

## Friday, February 20, 2026

**7:00 - 8:30 AM** **Breakfast**
Location: Sunset Terrace

**8:30 - 10:20 AM** **Contributed Session I**

Location: Maui Suite 3

**Mohamed El-Brolosy: ILF3 Links Mutant mRNA Decay to Transcriptional Activation During Transcriptional Adaptation to Mutations—A Process Driven by Novel Trigger RNAs**
8:30 - 8:40 AM

**Taimu Masaki: Reprogramming Müller glia to induce oscillatory Ascl1 expression and neurogenesis in the adult mammalian retina**
8:40 - 8:50 AM

**Chloe Nguyen: Engineering stable cell fate commitment via duplicate-origin cutter plasmids**
8:50 - 9:00 AM

**Gavin Schlissel: Extracellular Hedgehog diffusion, and evolution of morphogen gradients**
9:00 - 9:10 AM

**Ivy Xiong: Dynamic flow-metabolic coupling in renal tubules underlies water and electrolyte conservation by the mammalian kidney**
9:10 - 9:20 AM

**Bassem Al-Sady: Hysteresis measurements reveal a memory gradient in the repressed genome**
9:20 - 9:30 AM

**Paige Steppe: Adaptive Therapeutic Delivery through Plasmid Coupling in Bacteria**
9:30 - 9:40 AM

**Bo Gu: Dissecting the Logic of Signaling Receptor Competition using Multiplexed CRISPRi**
9:40 - 9:50 AM

**Paula Godoy: Divergent Modes of Aging in Yeast and Hematopoietic Stem Cells**
9:50 - 10:00 AM

**Tammy Collins: Insights from Reviewers: Unlocking Success in Applying for Burroughs Wellcome Fund's Career Awards at the Scientific Interface (CASI)**
10:00 - 10:10 AM

**8:30 - 10:20 AM** **Contributed Session II**

Location: Maui Suite 4

**Tammy Collins: Insights from Reviewers: Unlocking Success in Applying for Burroughs Wellcome Fund's Career Awards at the Scientific Interface (CASI)**
8:30 - 8:40 AM

**Mohammad Fallahi-Sichani: AP-1 co-regulatory network organizes discrete, heterogeneous, and reconfigurable cell states**
8:40 - 8:50 AM

**Yuki Maeda: Functional analysis of asynchronous Hes1 oscillations in the neural tube formation**
8:50 - 9:00 AM

**Ljubica Mihaljevic: Membrane protein solubilization and structure determination using WRAPs**
9:00 - 9:10 AM

**Paige Nickerson: The regulatory action of H-NS on gene expression in circular and linear DNA**
9:10 - 9:20 AM
**Lili Yang: Single-molecule Imaging Reveals RNA Polymerase Generates and Confines DNA Supercoiling**
9:20 - 9:30 AM
**Piyush Nanda: Competition between cytosolic and mitochondrial ribosomes produces a metabolic bistable switch**
9:30 - 9:40 AM
**Huyun Chen: Biophysical Requirements for Multicellular $Ca^{2+}$–ROS Wave Propagation**
9:40 - 9:50 AM
**Heath Johnson: Optogenetic construction of de novo integrin-adhesion complexes reveals role for biocondensation in adhesion nucleation**
9:50 - 10:00 AM

**10:20 - 10:40 AM** **Coffee Break**
Location: Monarchy Terrace

**10:40 - 12:30 PM** **Main Morning Session and Closing Remarks**
Location: Monarchy Ballroom 2-3
*Chair: Jeff Hasty*
**Amy Herr: Design of microanalytical tools to understand single-cell biology**
10:40 - 10:55 AM
**Keynote 10: Ellen Zhong**
11:00 - 11:35 AM
**Anthony Fung: Pan-organ profiling of immunosenescence reveals distinct senotypic patterns driven by cell-type and age**
11:40 - 11:55 AM
**Closing Remarks: Jeff Hasty**
12:00 - 12:25 PM

**12:30 PM** **Meeting adjourns**

# Tuesday, February 17, 2026

## Morning Session I

### Hana El-Samad

### Ramya Deshpande: Predictive Multicellular Assembly via Cell-Cell Adhesion

Living organisms can self-assemble into a remarkable variety of controlled multicellular structures; cell–cell adhesion is a key driver of this organization. Yet, we cannot precisely program complex multicellular structures, especially by tuning expression levels of cadherins or combinations of cadherins. In this work, we invert a physics-based model of differential adhesion to design complex assemblies of more than three cell types, and experimentally test the designs by expressing and sorting controlled expression levels of cadherins. We combine quantitative imaging with differentiable programming to explore how tunable adhesion influences collective organization. These results provide a framework for predictive control of multicellular assembly and highlight adhesion as a key design parameter for engineering self-organizing tissues.

### Jungmin Lee: Engineering Orthogonal Chemokine-Receptor System to Control Immune Cell Trafficking

Immune cells' ability to migrate and traffick to specific compartments throughout the body is primarily regulated by a complex network of chemokine ligands and their cognate receptors. The capability to engineer chemokine signaling will provide a critical tool for controlling immune cell trafficking, to target immune cells to specific tissues, to drive new cell-cell interactions, or to recall engineered cells for ex-vivo analysis. However, the native chemokine signaling network is highly redundant and interconnected with overlapping crosstalks and widespread expression in diverse native tissues, making it difficult to predictably direct cells using these native signaling modules. To precisely control immune cell trafficking in vivo, we engineered orthogonal chemokine-receptor pairs that operate independently of endogenous signaling networks. Using structure-guided and machine learning approaches, we constructed a series of orthogonal chemokine-receptor pairs that can trigger Gi protein-mediated chemotactic signaling, without crosstalk with their native counterparts. Among several variants, we focused on two orthogonal receptors ("Ortho-Receptor"), both of which interact with the same orthogonal ligand ("Ortho-Ligand"). These Ortho-Receptor/Ligand pairs were able to induce migration of primary human T cells in an orthogonal manner to the wildtype counterparts in vitro and in vivo. In a preclinical subcutaneous tumor xenograft model, engineered T cells expressing Ortho-Receptor were specifically recruited to the tumor producing the matching Ortho-Ligand, while T cells without Ortho-Receptor localized nonspecifically. More excitingly, when Ortho-Receptor T cells were exposed to two competing chemokine signals within the same animal, they were able to traffick preferentially to the Ortho-Ligand-producing tumor over the wildtype. Finally, we applied orthogonal chemokine-receptor pairs to enhance anti-tumor efficacy of CAR T cells against solid tumors. In preliminary experiments, CAR T cells armored with Ortho-Receptor controlled growth of tumor xenografts secreting Ortho-Ligand, even at a low dose where regular CAR T cells (without chemokine receptor) are not effective. Overall, orthogonal chemokine-receptor pairs provide a new orthogonal communication channel that allows therapeutic immune cells to effectively find target tissues in a complex biological system, independently of the native chemokines and receptors. This synthetic chemokine system has the potential for diverse applications such as selective and controllable delivery of therapeutic cells to the disease tissues, co-localization of multiple cell types to trigger new cell-cell interactions, and retrieval of engineered cells for diagnosis.

## Morning Session II

### Michael Elowitz: Engineering protein circuits for cancer therapy

Cancer therapy requires sensitively and specifically eliminating tumor cells while minimizing harm to healthy tissue. In this talk, I will describe *therapeutic circuits*—engineered proteins that address this challenge by directly rewiring oncogenic mutations to cell death. Focusing on Ras-driven cancer, we show that therapeutic circuits can be delivered as mRNA in lipid nanoparticles, accurately discriminate cancer and normal cells, and suppress tumors in vivo. Further, therapeutic circuits address fundamental limitations of targeted therapies, such as dependence on oncogene addiction and the rapid emergence of resistance. This nascent approach—enabled by advances in synthetic biology and adjacent fields—opens the possibility of rationally programmable therapies for cancer and other diseases.

### Mathias Heltberg: Oscillatory Control of Biomolecular Condensates in Regulation of the DNA Damage Response

Cells must coordinate biochemical processes in crowded environments, and one of their key strategies is the formation of biomolecular condensates. A central question is how these condensates remain stable when many are formed simultaneously as an integral part of the DNA damage response. Passive droplets naturally undergo Ostwald ripening and coarsen over time, which is critical during DNA damage, where multiple repair foci must coexist to ensure accurate genome maintenance. We investigate how oscillations in the transcription factor p53 provide a physical mechanism to control condensate stability. Developing a theoretical framework, we show that the amplitude and period of p53 oscillations modulate material exchange between condensates, preventing coarsening. In this way, oscillations distribute resources across multiple DNA repair foci, thereby circumventing Ostwald ripening. Our model explains how the characteristic timescale of p53 dynamics optimizes the repair process, and we predict that the occurrence of oscillations directly enhances DNA repair, which we confirmed experimentally. We further expanded the model to include spatially dependent addition and removal of condensate material, and analytically derived how this out-of-equilibrium behaviour can stabilize multiple condensates. Finally, we examined whether the p53 network itself exhibits characteristic dynamical properties, and demonstrated that p53 responds to external stimulation with frequency-dependent amplification consistent with resonance. Together, these findings reveal oscillatory signaling as a physical regulatory principle: cells can tune the temporal dynamics of p53 to shape condensate behavior, stabilize DNA repair foci, and enhance functional molecular responses.

### Silvia Canas Duarte: Revisiting aging in Escherichia coli: When prepping goes awry

The lifespan of living cells is generally considered to be finite, with the gradual accumulation of cellular damage leading to fitness reduction, diminished replicative capacity, and ultimately death. Symmetrically dividing microorganisms, including many bacteria, were long regarded as “immortal”. This notion stemmed from the assumption that, at cell division, the progenitor cell (mother) is effectively replaced by two identical newborn cells (daughters), both of which were thought to have no age difference. However, recent studies have challenged this view, revealing age-related differences between daughter cells and highlighting the existence of ageing and age-related patterns within bacterial populations. These new findings further propose that in bacteria like Escherichia coli, “old” cells—identified by inheritance of the old cell pole—exhibit reduced elongation rates, increased cell filamentation frequencies and, overall reduced viability compared to “young” cells. The accumulation of protein aggregates at the old cell pole has also been implicated in bacterial aging. Despite these observations, consensus is lacking due to inconsistent terminology and measurement standards, leaving the defining characteristics and molecular mechanisms of bacterial aging unresolved. Here, we revisit aging in E. coli using microfluidics and advanced cell segmentation algorithms that enable precise measurement of growth and fitness metrics at the single-cell level over hundreds of generations, across diverse growth conditions. Our measurements show that mother cells become increasingly bigger as they get older, while

maintaining their interdivision time constant for over hundreds of generations. This increase in cell size is linked to the preferential accumulation of glycogen at the old pole, and results in the appearance of a reduction in cell elongation rate. Our results suggest that the cell volume occupied by glycogen is not “counted” by E. coli for size homeostasis, similar to membrane-bound organelles in eukaryotic cells. Glycogen-deficient cells not only do not exhibit aging-associated changes in their growth or morphology but also display a significantly reduced frequency of cell filamentation and mortality compared to wild type cells. Finally, we found no significant accumulation of protein aggregates at the old pole for either strain. Instead, aggregates formed during exponential growth are highly mobile and frequently move between cell poles. Overall, our findings highlight discrepancies between growth metrics and highlight the central role of glycogen accumulation in cellular aging phenotypes.

### Justin Gallivan: An Introduction to the W.M. Keck Foundation

The W.M. Keck Foundation has supported fundamental research for nearly 75 years. I’ll describe who we are, what we support, and how to seek funding for your research.

## Main Afternoon Session

### Gege Qian: Remodeling of Cancer Cell Architecture by Chemotherapy

How chemotherapy reshapes tumor cells—and how these changes influence outcomes such as drug resistance—remains largely unclear. Here, we present a multimodal, global characterization of tumor subcellular organization and its reorganization by chemotherapy. We use self-supervised learning to encode protein coordinates across four orthogonal data modalities: proteome-wide size-exclusion chromatography fractionation (before and after treatment with cisplatin or vorinostat), native-state immunofluorescence imaging, affinity purification, and primary sequence information covering 7,579 proteins. This integrated map resolves 174 subcellular components, spanning molecular assemblies from protein complexes to organelles across a size range of ~10–9 to 10–5 nm. 58 components undergo significant remodeling upon treatment, recapitulating known mechanisms of action and revealing previously unrecognized alterations in pathways such as cytoskeletal organization and metabolic rewiring. We systematically validate these “chemotherapy-response assemblies”using genome-wide CRISPR knockout drug-sensitivity profiling, identifying which assemblies confer drug sensitivity versus resistance. Chemotherapy-remodeled components serve as convergence points for cancer mutations that predict therapeutic response—including those involved in homologous recombination repair, chromatin remodeling, and double-strand break repair.

### Silvia Santos

### Saki Ozeki: Large-Scale Automated Cell Lineage Analysis for Ploidy Transitions

Ploidy is the copy number of genome and chromosomes, which plays a crucial role in the viability of organisms. Polyploidization is the process by which a cell acquires more than two basic sets of chromosomes. It occurs in approximately 30% of solid cancers. Cancer therapies targeting polyploidized cells are being explored, and mechanisms of polyploidization are still under investigation. As a previous unpublished study, we found that polyploidized cells were distributed a broad range of ploidy levels (1–6C) after the first division and converged to near-tetraploidy after long-term culture. However, the mechanisms underlying these transitions remain unclear. To elucidate them, tracking ploidy of individual cells is required. Reconstructed cell lineages from the cell tracking can derive ploidy transitions. There are two challenges in cell tracking that are specific to polyploidized cells. Firstly, polyploidized cells often undergo multipolar divisions. Conventional methods that assume bipolar divisions cannot correctly handle the divisions. Secondly, capturing rare events, such as hyper polyploidization, requires extensive manual image analysis, which is time-consuming. To address these challenges, we developed a high-throughput automated cell tracking method that considers multipolar divisions.

For capturing rare ploidy changes, we acquired tiled large-field time-lapse images that can capture even moved cells across fields of view. Then, the cells are tracked, and large-scale lineages are reconstructed by using our customized tracking method. Our method consists of three steps: cell and nucleus detection, cell linking, and lineage reconstruction. In the detection step, we utilized two deep-learning segmentation models, Cellpose and StarDist, to detect cells and nuclei, respectively. In the linking step, we developed a method that can predict multipolar divisions by treating linking as the linear assignment problem. To improve linking accuracy, our method used various motility and morphological features as cost terms to be minimized, such as cell displacement and differences in cellular circularity. After cell linking, we reconstructed cell lineages and estimated ploidy level of each cell based on the nuclear fluorescence intensity. Furthermore, we developed a graphical user interface that allows users to easily correct any mistakes. Our method linked cells with 90% accuracy, and improved division and fusion linking by 15% and 18% over the conventional framework. Using time-lapse images of polyploidized human colorectal cancer cells HCT116, we reconstructed approximately 10,000-cell lineage. In the lineage, we observed that an asymmetrically divided cell with some ploidy level stopped cell divisions at all after several rounds of division. By checking the images, we found that the cell grew rapidly in size, eventually leading to cell death. Our high-throughput automated cell tracking method contributes to identifying such a rare event. Furthermore, our method can track both ploidy levels and cell fates of individual polyploidized cells. This approach leads to discoveries that clarify the mechanisms underlying the heterogeneity in which some polyploidized cells survive as tetraploid cells while others die.

**Shixuan Liu: The Changing Seasonality of Human Reproduction**

Seasonal patterns in human births and deaths have been noted for centuries, yet their global distribution, long-term trends, and underlying causes remain poorly understood. Analyzing longitudinal demographic data from 159 countries, we show that both births and deaths follow remarkably consistent seasonal cycles worldwide. Mortality peaks in winter, and births peak from spring to fall depending on latitude, each varying by ~20% between peaks and troughs. Interestingly, while death seasonality diminishes near the equator, birth seasonality remains strong at low latitudes, often exceeding that of higher latitude regions. The timing (phase) of birth peaks also varies systematically with latitude, from spring and early summer at high latitudes to fall in mid-to-low-latitude zones. Strikingly, high-latitude regions experienced rapid phase shifts in birth peak timing during the 1970s to 2000s, whereas death patterns remained stable. For example, birth peaks in Norway shifted from April to June; and in Switzerland from May to August. Similar shifts occurred across Europe and parts of South America, while most mid-to-low-latitude regions showed little to no change. What drives these rapid shifts in birth seasonality? While photoperiod is arguably the most influential environmental cue for seasonal rhythms, it cannot explain these phase shifts because the annual photoperiod cycle remains constant. A systematic analysis of 13 major socioeconomic and climate variables identified temperature as the predominant predictor of birth season. Warmer regions and years exhibited later birth peaks, even after controlling for latitude. Analysis of the temperature at the estimated time of conception further revealed that conceptions shifted towards colder months, potentially as a response to the adverse effects of rising temperatures on reproduction. This pattern aligns with the accelerated anthropogenic warming in high-latitude regions like Europe, suggesting that climate change may have already reshaped the seasonal rhythm of human reproduction.

# Wednesday, February 18, 2026

## Contributed Session I

**Akihiro Isomura: A synthetic biology approach to synchronising the segmentation clock**

Tight coordination of cell-cell signaling in space and time is vital for self-organization in tissue patterning. During vertebrate development, the segmentation clock drives oscillatory gene expression in the presomitic mesoderm (PSM), leading to the periodic formation of somites. Oscillatory gene expression is synchronized at

the population level; inhibition of Delta-Notch signaling results in the loss of synchrony and the fusion of somites. However, it remains unclear how cell-cell signaling couples oscillatory gene expression and controls synchronization. Here, we report that synthetic cell-cell signaling using designed ligand-receptor pairs can induce synchronized oscillations in PSM organoids. Optogenetic assays uncovered that the intracellular domains of synthetic ligands play key roles in dynamic cell-cell communication. Oscillatory coupling using synthetic cell-cell signaling recovered the synchronized oscillation in PSM cells deficient for Delta-Notch signaling; nonoscillatory coupling did not induce recovery. Our results reveal the mechanism by which ligand–receptor molecules coordinate timings of cell-cell communications and demonstrate a way to program temporal gene expression in organoids and artificial tissues.

**Mohamad Najia: Live-cell transcriptomics with engineered virus-like particles**

Transcriptomic profiling technologies are widely applied to characterize cellular gene expression, yet traditional approaches destroy the biological sample and prevent direct analysis of transcriptional dynamics in the same cells over time. We addressed this limitation by endowing mammalian cells to non-destructively “self-report” their transcriptional states via the export of RNA molecules in virus-like particles (VLPs). Cellular RNAs packaged in VLPs and exported into the extracellular environment faithfully reflected the host transcriptome for diverse types of mammalian cells. Repeated sampling of VLPs captured evolving transcriptional states in complex biological contexts, such as pluripotent stem cell differentiation and primary cell spheroids. Pseudotyping VLPs with epitope-tagged envelope proteins facilitated multiplexed live-cell transcriptomics of distinct cell types from heterogeneous populations. Finally, VLP engineering with RNA binding domains directed RNA packaging preferences, including the accurate reporting of libraries of RNA barcode transcripts. This work establishes a facile and broadly enabling technology for live-cell transcriptome-wide profiling.

**P C Dave Dingal: Sequence-specific control of embryonic signaling and development using a synthetic protease library**

Cell signaling is essential for coordinating morphogenesis during animal development. Embryonic cells deploy signaling proteins to guide the differentiation of nearby cells, ultimately forming various tissues such as muscle, bone, and blood. Secreted signaling proteins typically undergo proteolytic activation. To precisely control the activity of signaling proteins, we created the Synthetic Processing (SynPro) system, a library of proteases that were engineered to be functional in the secretory pathway of animal embryonic cells. Here we used proteases from the Potyviridae family, a class of enzymes that can cleave a unique seven-amino-acid sequence. More than 3,800 Potyviral proteases are known and are predicted to cleave peptides in a sequence-specific manner. To functionally validate protease-substrate specificity, we constructed the Protease Substrate Specificity Calculator (ProSSpeC), leveraging direct coupling analysis to predict protease performance that were then validated in animal cells in vitro and in vivo. ProSSpeC revealed co-evolutionary sequence features at single amino acid resolution, most importantly in its ability to predict experimental outcomes of any protease-substrate sequence pair. We demonstrate the ability of SynPro proteases to cleave key signaling proteins during early embryogenesis: Gdf1, Nodal, Lefty1/2, and BMP2/4/7, with concomitant phenotypes when these proteins are proteolytically processed. We further engineered the system for optogenetics to artificially induce developmental signaling pathways with light in a developing zebrafish embryo. To develop a light-inducible SynPro system, we fused each half of a protease with iLID and SspB proteins, which dimerize upon blue-light stimulation. We observed light-induced reconstitution of SynPro proteases and downstream activation of secreted proteins. Overall, our studies demonstrate that we can engineer sequence-specific proteases to control secreted proteins and their downstream signaling pathways at the cellular and whole-animal levels.

**Ayumi Goto: Cooperative Roles of Lfng and Dll3 in Stabilizing Segmentation Clock Synchrony**

Somites are metameric structures that later give rise to vertebrae, ribs, and skeletal muscles, and are periodically generated through the segmentation of the anterior presomitic mesoderm (PSM). During this

process, the transcriptional repressor Hes7 shows synchronous oscillatory expression among neighboring PSM cells via Notch signaling, thereby regulating the periodic formation of somites. Lfng and Dll3 are key components of Notch signaling in the PSM, and the loss of either gene leads to severe segmentation defects. Lfng modulates both the speed and amplitude of signal transmission. Although Dll3 is also thought to tune Notch signaling, the mechanism by which Dll3 contributes to Hes7 synchronization remains unclear. To investigate this mechanism, we utilized mouse ES cell–derived PSM-like tissues (iPSM) carrying a Hes7 reporter. These iPSM tissues displayed robustly synchronized Hes7 oscillations and formed somite-like segments. Even in this in vitro system, single knockout of Lfng or Dll3 caused a loss of synchrony, consistent with previous reports. We found that treatment with the γ-secretase inhibitor DAPT restored Hes7 synchronization in Lfng-knockout iPSM but not in Dll3-knockout iPSM, suggesting that Lfng and Dll3 participate in distinct aspects of the signaling process. Furthermore, we discovered that double knockout of Lfng and Dll3 unexpectedly rescued Hes7 synchrony. Reasoning that this resynchronization could provide insight into how Dll3 modulates Notch signaling, we first measured the Delta–Notch coupling delay using an optogenetic system. We observed that Lfng and Dll3 differentially affected the signal transmission speed, but this difference alone could not account for the rescue phenotype. We next performed numerical simulations that incorporated these transmission delays. When both temporal delays and an Lfng-dependent periodic modulation of Dll3 function were included, the model successfully reproduced the synchronized or desynchronized Hes7 dynamics observed in each knockout condition. These findings support the idea that the Delta–Notch coupling delay by both Lfng and Dll3 and periodic modulation of Dll3 by Lfng play an important role in controlling oscillatory synchrony.

**Cordelia McGehee: Translational Readthrough as a Mechanism of Chemotherapy Resistance**

Ovarian cancer is the most lethal gynecologic malignancy and the fifth leading cause of cancer deaths among women. While most ovarian cancer patients with high-grade serous ovarian cancer will initially respond to first line therapy, relapse after treatment is a common and devastating outcome in advanced disease. BRCA2 mutations contribute to treatment sensitivity and pathogenesis of a subset ovarian cancers, and secondary mutations that restore the open reading frame are commonly found in resistant disease. In this talk, we will describe a cell line model of acquired poly(ADP)ribose inhibitor resistance in a BRCA2 mutated ovarian cancer cell line in which full-length BRCA2 protein was restored despite presence of a nonsense mutation. Further analysis demonstrated enhanced codon-specific readthrough of premature termination codons in these cells. This work highlights premature stop codon readthrough as a potential mechanism of therapy resistance in cells with BRCA2 nonsense mutations.

**Debalina Datta: Age-Dependent Remodeling of Cellular Stress Response Kinetics: Optogenetic Dissection of the ISR Across the Human Lifespan**

The Integrated Stress Response (ISR) functions as a central regulatory hub that translates diverse cellular stressors into coordinated decisions governing adaptation, pathology, or cell death. While ISR dysregulation is implicated in age-related diseases including neurodegeneration, the quantitative principles underlying age-dependent ISR remodeling remain elusive. A critical clue lies in stress granules (SGs), the cell's primary condensate-based stress response machinery. SGs have been proposed as upstream regulators that may influence the phase behavior of multiple disease-linked biomolecular condensates, positioning them as potential master regulators of cellular proteostasis during stress. Traditional chemical stressors introduce uncontrolled pleiotropic effects and irreversible cellular damage that obscure intrinsic stress response dynamics. Here, we develop an optogenetic platform enabling precise spatiotemporal control over ISR activation with tunable stress dosage, duration, and critically, reversibility. We apply this system to primary human fibroblasts from donors spanning 17 to 90 years of age, systematically interrogating how aging reshapes stress response plasticity. We hypothesize that age-dependent ISR remodeling will reflect broader principles governing aging and other age-associated neurodegenerative diseases. In this study, we quantify SG assembly

and dissolution kinetics, morphology, and spatial organization under acute and chronic stress paradigms across the age spectrum, coupled with functional analysis of downstream ISR signaling through the ATF4-CHOP pathway and cell viability outcomes. Our measurements reveal striking kinetic defects in aged cells where the older cells exhibit delayed SG assembly following stress onset and significantly impaired SG dissolution upon stress removal. Critically, prior stress exposure differentially modulates subsequent stress responses in an age-dependent manner, revealing that aging erodes not only baseline stress response kinetics but also cellular stress memory and adaptive plasticity. These findings suggest that aging fundamentally alters the cellular stress response setpoint, which is the regulatory threshold governing SG formation and resolution. We propose that age-related pathology reflects maladaptive recalibration of this master regulatory threshold rather than simple component failure. The delayed assembly and impaired dissolution kinetics we observe indicate that aged cells exist in a state of reduced stress response bandwidth. This framework raises the possibility that interventions targeting SG dynamics to restore youthful kinetic parameters could broadly rescue downstream age-related dysfunction across multiple proteostasis-dependent pathways. This work establishes a quantitative framework for dissecting stress signaling across human aging, positioning SG-ISR regulation as a critical node in age-related cellular dysfunction. By defining how stress response capacity erodes with age through kinetic measurements previously inaccessible with traditional methods, we identify potential therapeutic strategies for preserving cellular resilience. Our optogenetic platform offers a versatile approach for mapping stress response networks and identifying targetable nodes in age-related proteostasis collapse and neurodegenerative diseases.

**Keith Breau: Coupling mathematical modeling with a novel human intestinal stem cell system to understand feedback regulation during planar cell polarity**

The Planar Cell Polarity (PCP) complex regulates many diverse phenotypes. While recent literature has elucidated key mechanisms underlying PCP, a mechanistic understanding of how these components function as a system to drive polarity is lacking. Here, we develop a comprehensive multicellular mathematical model centered around key PCP phosphorylation events, directly simulating the protein interactions that drive PCP. Our model confirms key PCP phenotypes, including robust single-junction asymmetry and multicellular polarity alignment in the absence of extrinsic signals. It predicts unique roles for the two known positive feedback mechanisms and predicts that VANGL-mediated DVL phosphorylation may be an underappreciated negative feedback mechanism. To test model predictions, we employ transgenic primary human intestinal epithelium cultured on biomimetic planar-crypt microarrays (PCMs) as a new platform for studying PCP. Together, our model provides novel insights into the mechanisms that regulate PCP, while our experimental results highlight an unappreciated role for PCP in intestinal biology.

**Alain Bonny: Stromal-Stem Cell Spatiotemporal Coordination in Tissue Repair**

Mammalian skin is tasked with protecting the body from an external environment that can induce stress and damage. When skin undergoes an injury, resident stem cells are mobilized to repair and replace the wound; however, the mechanisms by which stem cells identify when and where to repair the skin, and the role of neighboring cells is unclear. To address this, we have utilized a novel in vivo spatiotemporal-labeling approach that allows for contact-based mapping of niche stem cell interactions. Focusing on stromal-stem cell contacts, we identified a transient, compartmentalized feedback loop between the stem cell re-epithelialization front and a stromal cell population along the Gdnf-Gfra1 signaling axis that is critical for proper wound closure. Utilizing lineage tracing mouse models, we have identified resident Schwann cells as a highly dynamic niche cell type that has not been previously described to interact with stem cells. Our results suggest that upon injury Schwann cells dedifferentiate into a transient "repair" state and are guided to the epithelial leading edge by tropic factors expressed by the stem cells where, in return, Schwann cells locally secrete mitogens to induce stem cell migration and proliferation towards timely wound closure. This reciprocal interaction between leading edge stem cells and repair Schwann cells reinforces the role of stem cells as prominent coordinators of their microenvironment.

### Mark Greenwood: Epinephrine oscillation enhances the alertness of target cells to stress

The body uses hormones to coordinate physiological processes such as the stress response. Epinephrine, for example, modulates a range of physiology such as metabolism and airway tone during both homeostasis and stress. However, the receptors that detect hormones like epinephrine can become unresponsive over time, raising the question of how cells remain alert and tunable to changes in stress. Because circulating epinephrine concentrations naturally oscillate in vivo, we investigated whether oscillations help maintain and adjust cellular alertness to stress. Using live-cell imaging of β2-adrenergic receptors together with a cAMP biosensor and mathematical modeling, we compared cellular responses to stress under constant versus oscillatory hormone delivery in airway cells. Constant stimulation blunted receptor signaling, whereas oscillatory input preserved alertness by enabling receptor resensitization between cycles. Oscillations also broadened the signaling range, enhancing tunability to different stress intensities. Inhibiting PKA-mediated desensitization restored both alertness and tunability under constant stimulation, and similar behavior was observed in fibroblasts, which desensitize more slowly. Thus, hormone oscillations preserve cellular alertness while optimizing the tunability of stress signaling, enabling effective signaling during both homeostasis and stress.

## Contributed Session II

### Hollie Hindley: Heterogeneity in responses to ribosome-targeting antibiotics mediated by bacterial RNA repair

RNA repair is critical for cellular function. The Rtc system maintains RNA integrity within the translational machinery of bacteria. In E. coli, Rtc expression enables cells to rescue growth and survive treatment by conferring transient resistance to ribosome-targeting antibiotics, yet the mechanisms underpinning this resistance remain obscure. Here, we present a computational model of Rtc-regulated repair of translational RNAs. Integrating model predictions with experimental validations, we uncover notable cell-to-cell heterogeneity in rtc expression that impacts on translational capacity, indicating that rtc may induce a form of heteroresistance. We moreover identify Rtc targets that may reduce the translational capacity of cells and so potentiate antibiotic effects. Our findings elucidate a complex response underpinning resistance conferred by Rtc, offering alternate routes for addressing resistance in E. coli and other relevant pathogens.

### Ksenia Sokolova: Noncoding Regulatory Impact Shapes Cancer Progression

Somatic noncoding mutations are abundant in cancer genomes, yet their regulatory functional consequences remain poorly understood. To address this gap, we assessed the mechanistic regulatory impact of >35M SNVs within ± 20 kb of transcription start sites across more than 8,000 TCGA samples. Using Sei, a deep-learning model that predicts histone marks, chromatin accessibility, transcription factor binding, and sequence-class regulatory programs (>20,000 features), we quantified the predicted impact of each mutation on local regulatory architecture. We then inferred clonal structure using PyClone-VI to place these noncoding alterations into the tumor evolutionary context. Beyond variant-level annotation, we constructed patient-level representations of regulatory dysregulation. By aggregating predicted effects across genes, we derived a personalized promoter-proximal regulatory disruption score that captures the cumulative functional impact of noncoding mutations in a clinically interpretable form. Promoter-proximal somatic mutations exhibited substantially higher predicted regulatory impact near known cancer genes despite similar mutation burden, revealing a functional dimension invisible to conventional load-based analysis. Critically, this predicted score was a strong and independent predictor of adverse progression (HR = 1.10, 95% CI 1.04–1.15, $P < 0.005$), whereas noncoding mutation count contributed no additional signal. The multivariable Cox model accounted for cancer type, fraction of genome altered (FGA), homologous recombination deficiency (HRD), non-silent mutations per Mb and age at diagnosis. Thus, functional disruption and not noncoding mutational load captures clinically relevant noncoding biology. Dissecting regulatory disruption by evolutionary timing further revealed distinct biological

processes. Early (clonal) promoter-proximal disruption consistently predicted poor pan-cancer outcomes, whereas late-arising (subclonal) burden was prognostic only in select cancers, including uterine corpus endometrial carcinoma (HR = 1.36, P = 0.01). These results suggest that tissue-specific context shapes which regulatory programs become consequential during progression. Finally, Sei sequence classes showed more context-specific vulnerabilities. For example, in lower-grade glioma, somatic mutations disproportionately disrupted brain/melanocyte enhancer programs, while sarcoma exhibited higher sensitivity in polycomb/bivalent stem-cell-associated classes. Together, these results provide a unified, evolution-aware view of noncoding regulatory disruption across cancers. We demonstrate that predicted functional regulatory impact captures the clinically and mechanistically relevant dimension of noncoding variation, revealing regulatory programs that shape tumor evolution and outcome. By scaling deep-learning regulatory modeling to millions of mutations and thousands of patients, we computationally discover underlying noncoding mechanisms that are measurable, interpretable, and directly relevant to cancer biology.

**Hyongbum Kim: High-Throughput Functional Annotation of Genetic Variants using Prime Editing and Deep Learning**

The clinical utility of genomic sequencing is severely limited by the high prevalence of Variants of Uncertain Significance (VUSs), particularly in large genes or in the context of acquired drug resistance. A major challenge in precision medicine is the lack of systematic functional annotation for the entire space of possible genetic variants. To address this, we have developed and applied a suite of high-throughput platforms integrating prime editing (PE) with deep learning to functionally map the variant space at saturation scale. We established PEER-seq, a method using PE to introduce single-nucleotide variants (SNVs) with synonymous marker mutations at endogenous loci, enabling accurate functional profiling via deep sequencing. Using this framework, we created comprehensive resistance maps for critical cancer-related genes. We profiled 97% of all possible SNVs in the ABL1 kinase domain against five tyrosine kinase inhibitors (TKIs) for chronic myeloid leukemia. We also generated a resistance profile for 99% of all possible variants in the EGFR tyrosine kinase domain against afatinib and osimertinib. Furthermore, we functionally assessed all 27,513 possible coding SNVs in the large 63-exon ATM gene. We experimentally evaluated 23,092 SNVs based on cell fitness following olaparib treatment and developed a deep learning model, DeepATM, to accurately predict the functional effects of the remaining 4,421 variants, linking functional status to cancer risk and prognosis using UK Biobank data. Together, this integrated suite of prime editing and deep learning models provides a robust framework for systematically resolving VUSs, understanding complex drug resistance mechanisms, and predicting clinical outcomes, bridging the gap between raw genomic data and clinical actionability.

**Pujun Guan: Evaluating Single-Cell Integration without Ground Truth**

Integrating heterogeneous single-cell datasets remains a central challenge in computational biology. Despite extensive methodological development, existing benchmarks rely heavily on simulated data or datasets with artificially constructed ground truth. Real-world studies, especially those involving complex disease tissues, provide neither explicit truth labels nor stable references. Consequently, practitioners face substantial uncertainty in choosing algorithms, tuning parameters and distinguishing biological structure from method artifacts. To address this gap, we develop a novel systematic framework for benchmarking integration without requiring ground truth. Our approach begins by constructing a multi-scale proxy truth. We apply multi-atlas label transfer to obtain two complementary levels of proxy labels: (i) coarse aggregated labels, and (ii) fine-resolution transferred labels. Guided by the coarse labels, a global integration reveals the geometry of major lineages and continuous manifolds. We then define geometry-aware integration domains by combining coarse labels with manifold continuity. These domains delineate reliable integration regions for subsequent re-integration, while fine proxy labels support state-level evaluation. In parallel, we curate per-sample expert labels (without integration) to assess the consistency and robustness of proxy-derived annotations. Using this multi-scale proxy, we design a two-step integration protocol that respects the hierarchical organization of single-cell

variation. Step 1 aligns major cell types at the global level. Step 2 re-integrates each domain independently from raw data, using feature selection and graph settings calibrated on development datasets. To ensure methodological rigor, we separate development datasets from held-out validation datasets that never inform parameter selection. Integration methods are evaluated on held-out validation datasets with fixed parameters, providing a genuine test of generalization. We compare the two-step protocol with the baseline: a standard one-step integration that jointly aligns cell types and states. Preliminary results indicate that the two-step strategy improves batch mixing and biological preservation. Importantly, residual local noise in proxy labels has negligible influence on method comparison. Overall, our framework provides a principled pathway for evaluating single-cell integration in settings where true labels are unavailable.

**Cyrus Knudsen: Producing quantitative protein function data at scale to enable protein design**

Designing better enzymes or novel protein biosensors is an exciting prospect of protein design. While high-accuracy prediction of the structure of a protein complex is now feasible, it remains challenging to predict the quantitative functional properties such as catalytic parameters for enzymes or ligand binding affinities for transcription factors. This challenge is in large part due to the intrinsic difficulty in measuring such quantitative protein function in vivo. By integrating mechanistic constraints with large-scale measurements, we demonstrate that we can provide accurate estimates of quantitative functional properties of proteins at a large scale. These functional properties match both in vivo phenotypes and heterogeneous measurements. The integration relies on a novel method based on convex optimization that can effectively train on heterogeneous data while enforcing mechanistic constraints. This enables us to deeply characterize hundreds of key enzymes in E. coli, as a showcase of how this method can be used to generate quantitative protein function data.

**Alexandra Nava: A Machine-Learning Model Predicts Spore Germination**

Predicting the response of an individual cell to a given stimulus remains a fundamental challenge due to the stochasticity associated with the underlying molecular mechanisms. Similarly, germination of bacterial spores has been traditionally understood to be stochastic, even though it is possible to modulate the population-level timing and percentage of germination. In general, it remains unclear whether apparent stochasticity in cellular responses is due to truly random processes, or if there are hidden variables that could mask a more deterministic behavior. We apply data analysis to extract the most predictive features in the germination process. We find that the initial cell size of the cell and changes in electrochemical potential are correlated to germination outcome. Here, we develop a machine-learning model that was trained on these predictive features with thousands of individual spore germination events. We find that our model can predict if a spore will remain dormant or will germinate in the next time frame with high accuracy based on changes in electrochemical potential. The ability to predict the occurrence of an individual event implies that, despite inherent stochasticity, there are deterministic factors that precede a germination event.

**Joseph Slivka: Dissecting the stepping dynamics of dynein using 1- and 2-color MINFLUX**

Cytoplasmic dynein is a dimeric motor that carries intracellular cargoes towards the minus-end of microtubules (MTs). Dynein converts the chemical energy of ATP hydrolysis into coordinated structural changes to step processively. The dynamics of dynein stepping have been poorly characterized by tracking flexible regions of the motor with limited resolution, and how dynein couples ATP hydrolysis to a minus-end-directed step remains controversial. Here, we site-specifically labeled yeast dynein at its MT-binding domain by developing a cysteine-light mutant and tracked its stepping at sub-millisecond and nanometer resolution at physiological ATP concentrations using MINFLUX. We showed that dynein hydrolyzes one ATP per step and takes multitudes of 8 nm steps. Dual color tracking shows dynein steps in an uncoordinated manner though stepping rate can be accelerated at large separations, suggesting intradimer tension offers some communication in dynein stepping. Steps are preceded by a transient movement towards the plus end. These backward “dips” correspond to MT release upon ATP binding and subsequent diffusion of the stepping monomer around its MT-bound partner.

Functional assays showed that dips terminate with a minus-end-directed movement upon ATP hydrolysis. These results provide critical insights into the order of mechanochemical events that result in a productive step of dynein.

**Wei Chen: De novo design of transcription regulators**

Precise regulation of gene expression is essential for maintaining cellular function and homeostasis. During gene activation, transcription factors recognize specific DNA sequences and recruit cofactors via transactivation domains (TADs), which are intrinsically disordered and conformationally flexible. However, the mechanisms governing how TADs recruit cofactors—and whether these interactions are specific or promiscuous—remain largely unknown. This knowledge gap significantly limits our ability to precisely modulate gene expression. Here, we employed AlphaFold to predict the interactions between known TADs and their cofactors. We identified striking specificity in how TADs engage their targets. Leveraging these insights, we designed specific structured binders that exhibit tunable activation capacity and are more potent than existing natural activation domains. This work provides a new avenue for precise gene regulation and therapeutic applications.

**Ruoyu Wang: Single-molecule sequence model of the human regulatory genome**

Genome regulation emerges from dynamic, stochastic events at the single-molecule level, yet most predictive models collapse this complexity into population-level averages. Although sequence-to-function deep learning has substantially advanced our ability to predict mean chromatin and transcriptional outputs, these models are inherently unable to capture the full spectrum of behaviors exhibited by individual molecules. Here, we bridge this gap by integrating single-molecule regulatory genomics with state-of-the-art deep generative AI to develop a sequence-to-distribution framework that predicts the entire distribution of single-molecule chromatin accessibility directly from DNA sequence. Using this framework, we uncover novel and fundamental mechanisms of chromatin regulation encoded in the genome. By quantifying sequence-driven nucleosome-opening and nucleosome-phasing power, we reveal distinct modes through which transcription factors engage nucleosomes. We decode the sequence determinants governing the +1 nucleosome downstream of transcription start sites and identify YY1 as a key factor specifying +1 nucleosome positioning. We further dissect sequence-based mechanisms underlying co-accessibility between distal regulatory elements, pinpointing transcription factors that orchestrate long-range regulatory communication. Extending beyond static single-molecule snapshots, we generate the first simulations of single-molecule chromatin-state trajectories, providing quantitative estimates of nucleosome diffusion dynamics and rapid transcription-factor binding kinetics. Because sequence models are causal, this framework also enables prediction and interpretation of genetic variant effects at single-molecule resolution, revealing how single-nucleotide changes reshape chromatin states molecule by molecule. Together, this sequence-to-distribution framework establishes the foundation for decoding and predicting how genomic sequence encodes single-molecule genome regulation.

**Wojciech Szpankowski: Detecting Correlated Structures in Biological Networks with Mismatches**

In prior work (Q-Bio 2024, Q-Bio 2025), we introduced a framework for detecting significant network-structured signals in biological sequences and designed seed subgraphs for efficiently searching for potentially correlated substructures. In this new effort, we finally resolve the problem of identifying significant structure/ label dependencies for biological networks by developing a novel statistical test. Specifically, we address the fundamental question of whether two biological graphs are statistically correlated in both structure and labeling, reflecting internal similarities in their organization. To quantify this correlation, we propose a structure-weighted label mutual information measure. We present detailed theoretical foundations of the measure and support our framework using computational experiments on networks. Computational and comparative analysis of protein–protein interaction (PPI) networks provides critical insights into the modular organization of the cell by revealing functional modules and protein complexes. Representing protein systems as sparse graphs offers a

principled abstraction for identifying such motifs. The node labels can be composite protein features, incorporating, for example, subcellular localization and signaling cascade annotations. Within this framework, conserved subgraph patterns capture recurring topologies coupled with correlated localization and signaling cascade labels, revealing functional conservation that extends beyond sequence or structural similarity alone. These motifs often correspond to biologically meaningful regions of the network, associated with specific cellular functions. We present a statistical framework for assessing the significance of structural motifs in protein interaction networks and demonstrate its application to the ComPPI interactome. Starting from a seed subgraph that defines a reference motif, our method identifies other subgraphs that are structurally and label-wise similar across the network. For computational efficiency, candidate subgraphs are selected using Breadth-First Search–based procedures that ensure connectivity. We retain protein interactions with a confidence score of at least 0.5, representing the likelihood that the proteins can interact with each other. Each node is labeled with subcellular localization data from ComPPI and signaling cascade annotations from STRING and Gene Ontology (GO). We evaluate multiple pairs consisting of a ComPPI seed subgraph and candidate subgraphs within the network; a representative subset of 100 such pairs is visualized below, with one exceeding the statistical significance threshold and representing the strongest signal. Experiments on the ComPPI network show that the proposed framework effectively detects structurally correlated subgraphs capturing relationships among protein interactions, subcellular localization, and signaling pathway participation.

## Main Morning Session

### Kiana Aran: Connecting Semiconductors Chips to the Machinery of Life

For decades, technology has been the key that unlocks the secrets of our own biological machinery. The invention of tools to decode our genes revealed the fundamental blueprint of life, while further advancements allowed us to identify the intricate functions of proteins and enzymes like CRISPR, which have revolutionized science. We have learned to read the book of life and are now learning how to edit it. However, to truly master this capability, we must move beyond qualitative descriptions and begin to understand biology in a quantitative way, which requires a direct, high-fidelity connection to the biological world. This is where the power of the semiconductor industry becomes essential. The same chip technology that powered the digital revolution provides the foundation for the next great leap in biology. The challenge, and our focus, is to build the physical and electrical bridge between silicon chips and biological machinery. We achieve this by designing novel biosensors that integrate biological molecules directly onto a semiconductor chip's surface. This allows the chip to act as a powerful transducer, converting the rapid, subtle events of biology—like a protein binding or DNA matching—into a clean, measurable electrical signal. By creating these high-throughput "biology-on-a-chip" platforms, we are developing the essential tools to finally quantify biological processes in real time, enabling us to engineer solutions to our most pressing challenges faster and more precisely than ever before.

### Michael McManus: Mapping the Language of Cell Communications

Cell:cell interactions are the core computations of multicellular organisms. They allow cells to recognize specific molecular patterns, integrate context, and commit to durable programs. For example, in the immune system, those programs can look like activation, tolerance, exhaustion, or memory. Yet we still lack a scalable experimental framework that connect three things at once: who interacted with whom, what molecules were recognized in each cell, and what that interaction ultimately encoded in cell state. I will describe platforms from our lab that treat RNA transfer as a measurable, transferable record of cell interaction in vivo. We will explore Relay technologies that quantify barcode RNA transfer during direct cell:cell contact, providing high-resolution maps of interactions between immune cells and antigen-presenting targets. I will then shift to a massively parallel pMHC epitope mapping and TCR specificity mapping program that is designed to scale antigen-resolved recognition, converting sparse, hard-to-measure pairing events into datasets that support inference about the

rules of immune recognition and cell state. More broadly, this platform architecture is intended to be portable across many membrane-protein interaction classes, across cell types, and potentially across species. Together, these high content technologies move toward a quantitative “language model” of interaction, where specificity and context can be mapped to predictable cellular consequences.

### Main Afternoon Session

#### Wendell Lim

#### Charilaos Giannitsis: Many behaviors, one regulator: The phenotypic landscape of a single transcription factor

Cells employ complex gene regulatory networks to perform various information processing tasks and precisely regulate gene expression in space and time. Despite significant advances in our understanding of the dynamics and signal processing of these networks, we still understand little about how they emerged. A likely key step during their evolution is the emergence of simple circuits that produce crude phenotypes that are gradually refined. Here, we take a synthetic biology approach to explore the phenotypic diversity that the simplest network, a single regulator circuit, can produce in E. coli. We find that pleiotropic transcriptional regulation can emerge from small modifications in promoter architecture. We show that a single regulator is sufficient to program complex signal processing, bistable switches, homeostatic feedback control, stripe patterns in morphogen gradients, dynamic temporal oscillations and pulse generators. This work demonstrates that a pleiotropic, single-regulator network can function as a substrate for the evolution of sensing, spatial patterning, adaptation, memory and periodic behaviors and highlights the flexibility of bacterial gene regulation.

## Thursday, February 19, 2026

### Contributed Session I

#### David Glass: Engineering synthetic multicellularity for understanding consortia and tissue behavior

Differentiation – in which stem-like cells produce daughter cells with specialized function but limited growth potential – is essential for multicellular life. However, differentiation is inherently susceptible to mutation because non-differentiating mutants over-proliferate and outcompete normal cells. It remains unclear what mechanisms can resist such mutant expansion. We demonstrate a solution to this fundamental challenge by engineering a synthetic differentiation circuit in Escherichia coli. The circuit generates a controllable differentiation lineage and resists mutations in long-term evolution by coupling differentiation to an essential trait. In solid culture, the lineage structure and selective pressures conspire to form intricate spatial patterns suggestive of developmental patterns in solid tissues. The results provide insight into stability of differentiation and demonstrate a powerful method for engineering evolutionarily stable multicellularity.

#### Xiao Peng: Feedback control of differentiation for ratiometric stability and pattern formation of synthetic multicellular system

Differentiation plays a central role in the development of multicellular organisms and microbial communities. While some microorganisms can differentiate into various cell types in response to environmental stimuli, these behaviors are species-specific and difficult to dissect and control. Synthetic microbial consortia are efficient platforms to study multicellular behaviors. However, despite wide studies on controllable cell interactions and dynamics, there has been no autonomously emerging microbial consortium with programmable differentiation. Here we construct a synthetic bacterial differentiation system that produces permanently differentiated populations in a consortium and regulates the ratio of the two cell types through intercellular signaling. We

show that single-source progenitor cells generate a differentiated daughter population, and the system achieves a stable ratio of the two cell types in a continuous culturing platform. We further show that the feedback strength and growth rate difference of the two cell types collectively determines the steady state ratio. Finally, we show in the heterogeneous environment the system is capable of spatial pattern formation with localized differentiated cell types. This study demonstrates how differentiation coupled with feedback control can be used to create complex synthetic multicellular systems.

**Brian Cleary: Fundamental errors in RNA velocity arising from the omission of cell growth**

New methods to measure “RNA velocity” at the single cell level offer an incredible promise: by estimating kinetic parameters of expression for each gene in many single cells in a population, one can, in principle, piece together observed short-term changes in each cell and map long-term expression trajectories that were never directly observed, charting the paths that single cells take in dynamic processes and providing a foundation for understanding the phenotypic space that cells can occupy. While the ultimate promise of these methods is compelling, their limitations have also been widely documented. Splicing based methods in particular suffer from substantial technical noise and bias (arising in part from the necessary but incidental capture of introns), as well as fundamental modeling and computational challenges. Labeling based methods are generally believed to be significantly more robust, both because they do not rely on incidental capture and because labeling happens in a defined temporal window, allowing for observation of counts of transcripts produced or degraded in defined time periods. Despite the robust and ongoing articulation of limitations and mitigation strategies, consensus RNA velocity frameworks continue to overlook a fundamental aspect of cellular dynamics: cell growth. In a growing population, biomass (including RNA and other macromolecules of the cell) is constantly accumulating. This is true too at the single cell level: biomass accumulates from the beginning of cell cycle to the end before division brings daughter cells roughly back to the same size and state. This implies that to keep up with cell growth we expect a homeostatic velocity (defined in the terms of production and degradation) that is positive, which is at odds with the conventional estimation, interpretation, and uses of velocity. Here, we investigate the consequences of omitting cell growth from the RNA velocity framework. We demonstrate systematic errors in interpretation and estimation that arise from ignoring cell growth. We show how inefficient detection and sampling similarly give rise to systematic artifacts. Analysis of existing datasets confirms near universal presence of such artifacts. Finally, we point the way forward for correcting some of these issues and highlight that explicitly accounting for cell growth in the RNA velocity framework can lead to new biological insights. In particular, this view shows that cell growth rate can be a global regulator of gene inducibility, in the sense that inducing large changes in abundance is “easy” in slow growing and “hard” in fast growing cells.

**Gene-Wei Li: The defining features of intrinsic transcription terminators**

Transcription terminators define RNA boundaries and tune downstream gene expression. However, genome-wide prediction of terminators and their strengths remains challenging. Here we report a missing sequence feature of bacterial intrinsic terminators. By resolving termination at discrete sites for 7,000 terminators in vitro, we identified positions at the edges of the transcription bubble whose sequences strongly influence termination efficiency. These sequences flank a 7-nucleotide U-rich tract, resemble the elemental pause sequence of RNA polymerase, and are characteristic of strong terminators across diverse bacteria. Together with the U-tract and GC-rich hairpin, these defining features account for the majority of variations in termination efficiency. Our findings unlock a key code of the regulatory genome and provide a foundation for quantitative prediction of gene expression.

**Yujia Liu: Reconstitution of feedback architectures in the cyanobacterial clock that allow growth tolerance**

Circadian clocks are cell-autonomous oscillators with a near-24-hour period, ubiquitously found in animals and plants. Surprisingly, circadian clocks were later discovered in cyanobacteria—photosynthetic prokaryotes—which had previously been believed unlikely to have such mechanisms due to their short doubling time. Using a continuous culture device (Cyanotron), we found that the oscillation period remains remarkably stable even when multiple new daughter cells are created within a single circadian cycle (doubling roughly every 7 hours). To understand how cyanobacteria can maintain a precise internal representation of time across generations, we leveraged a purified protein system to reconstitute a growing clock in a test tube using a pipetting robot. In clear contrast to the in vivo clock, the protein oscillator is intolerant of rapid growth, showing period shortening and eventual collapse of oscillations as the simulated growth rate increases. To investigate the mechanisms behind these growth effects, we measured the phase shifts of the in vitro oscillator following a small amount of new protein injection at various oscillation phases, constructing a growth phase response curve (PRC). We found that a simple phase oscillator model, constrained by the growth PRC, quantitatively reproduces the behavior of the growing protein oscillator. Finally, we extended our test tube growing clock system to allow rhythmic protein injection, simulating protein synthesis modulated by clock feedback onto protein expression. Combined with a mathematical model, this approach identified the genetic feedback program implemented by wild-type cells to achieve not only a growth-invariant period but also correct phasing and waveforms, thereby compensating for the growth effects felt by the protein oscillator. Our study reveals the period-shortening effect at high growth rates and details the feedback logic used to compensate for it, which could have broad implications for any system that must function over multiple generation times.

**Katie O'Connor: Patient-derived organoids as predictive disease models for therapeutic chassis selection**

Engineered microbes are emerging as powerful vectors for both drug delivery and immune activation within solid tumors. Standardized models, however, are needed for evaluating strain efficacy, selectivity, and host interactions in order to facilitate translation from in vitro into clinical settings, such as for colorectal cancer therapy. Here, we introduce a physiologically relevant co-culture platform using patient-derived colorectal cancer and normal mucosa organoids (PDOs) to aid in chassis selection, with a comparison between two clinically relevant bacteria for CRC therapy, Salmonella typhimurium and E. coli Nissle 1917, in order to validate the platform. Both strains exhibit greater colonization of CRC organoids compared to matched normal colon organoids. Salmonella demonstrated a greater impact on host response in cancer organoids. Comprehensive phenotypic profiling and correlation analysis revealed strain-specific disruption patterns. Across 2 different patient lines, cancer organoid response is both strain- and genotype-dependent. The utility of PDOs for capturing interpatient variability and cancer-bacterial interactions, and thus chassis personalization, is analogous to PDO drug-screening approaches. These results highlight PDO co-cultures as a scalable and informative platform to help refine chassis selection for microbial cancer therapeutics. Furthermore, this study suggests that attenuated pathogens, such as Salmonella, are worthwhile agents for future engineered bacterial cancer therapies.

**Kevin YuKai Chao: A Genetically Encoded Device for Transcriptome Storage in Mammalian Cells**

A "black box" flight recorder for a cell's history would revolutionize how we study cellular decision making. Yet, building a molecular recorder for a cell's entire transcriptome has remained a major challenge. Here we present TimeVault, a genetically encoded device that repurposes vault particles to store messenger RNAs. TimeVault leverages engineered vault particles as a chassis to encapsulate polyadenylated mRNAs through a genetically engineered poly(A)-binding protein. We demonstrate that TimeVault enables high-fidelity transcriptome-wide recording with minimal cellular perturbation, capturing transient stress responses and revealing gene expression changes underlying drug-naive persister states in lung cancer cells that evade EGFR inhibition. TimeVault is a robust and powerful platform that provides an untargeted, whole-transcriptome recording capability, surpassing

the limitations of existing molecular recorders. By enabling the stable, lineage-retained storage of past transcriptional states, TimeVault functions as a transcriptomic time capsule, revealing the underlying molecular mechanisms that guide cell fate decisions.

**Vaibhav Murthy: Decoding Niche Organization and Adaptive Signaling Activation Patterns Underlying Spatiotemporal Osteosarcoma Drug Response Heterogeneity**

Osteosarcoma lung metastases are poorly responsive to existing therapies and often exhibit significant inter- and intra-lesional drug response variability, the precise mechanisms of which are poorly understood. We have found that lung metastases display significant spatial and temporal ERK signaling and downstream pro-survival target gene expression heterogeneity. To quantitatively investigate how these features contribute to clinically-relevant drug response heterogeneity, we used the Serial Imaging of Tumor and microEnvironment (SITE) platform– a live lung tissue ex vivo platform paired with ERK biosensor-expressing patient-derived osteosarcoma lines. We found that higher ERK signaling at the tumor-lung interface predicted delayed death and increased persistence of osteosarcoma cells, while “ERK-low” core cells died earlier, when challenged with drugs. Cell death itself induced an adaptive, paracrine ERK activation surge in neighboring tumor cells, within hours and across tens of microns, establishing an apparent death-induced survival loop. We applied a data-driven state transition model to better understand how microenvironmental context, such as cell-cell interaction with lung cell types, proximity to cancer cell death events, and ERK signaling status coordinately determine single cell fate. Modeling revealed an adaptive drug-resistance pathway where resistant cells arose from high-cancer-cell-proximity tumor cells with initially low ERK activity and which transitioned to an “ERK-high” tumor boundary proximal niche. In silico prevention of state switching from ERK-low to ERK-high predicted significantly increased drug sensitivity. Motivated by these predictions, we identified a mechanism of drug-induced tumor lysis-mediated growth factor release, that promoted an ERK-high transitions within metastatic lesions. Pharmacological fibroblast growth factor receptor (FGFR) blockade, in combination with cytotoxic drugs, uniquely abrogated this death-induced ERK activation and promoted increased tumor killing. In summary, we identified an FGFR–ERK–driven death-induced adaptive resistance circuit that is spatially enriched at the tumor–lung interface and predicts single-cell persistence. These data highlight how quantitative computational and dynamic ex vivo experimental models, such as SITE, can be coupled to uncover novel mechanisms of drug response heterogeneity from tissue-scale to single cell.

**Victoria Chen: Uncovering the Long-Term Dynamics of Engineered Genes Released to the Environment**

The environmental fate of recombinant DNA remains largely unquantified. In 1989, *Pseudomonas putida* engineered for phenol degradation were released into waterways in northeastern Estonia to remediate pollution from an oil-shale mine fire: one of the first and very few deliberate releases of genetically engineered microbes into an open environment. Thirty-five years later, we sampled ~900 km2 encompassing the release and downstream sites to characterize the persistence, stability, and spread of the released genes. Using nested PCR, qPCR, and long-read metagenomics, we detected sequences identical to the released pheBA operon. We developed a linear mixed-effects model (LME) to distinguish a release-origin detection signal from a natural-origin signal. The model integrates detections across assays and tests whether the spatial pattern is congruent with that of a released gene (i.e., confined to the release site and downstream waterways). The LME supported pheBA descent from the released construct ($p = 0.0003$), with the genes remaining restricted to the release area and downstream rivers rather than simply tracking phenol pollution. We examined horizontal gene transfer using metagenomic data and whole-genome sequencing of pheBA-positive isolates, revealing mobilization across multiple native Pseudomonas species via flanking IS1472 and IS1411 transposable elements onto conjugative plasmid backbones. We quantified plasmid loss dynamics in lab evolution experiments which showed that pheBA-carrying plasmids were maintained in the presence of phenol but lost within 400 generations when grown without phenol, directly linking selective pressure to persistence. Together,

these results reveal the parameters governing the persistence, transfer, and loss of recombinant genes in the wild.

### Jaeseung Hahn: Two-state pharmacokinetic model simulates systemic delivery of bacterial cancer therapy with dynamic encapsulation system

Advances in synthetic biology continue to potentiate bacterial cancer therapy. As living drugs, bacteria behave differently from “passive” pharmaceuticals such as small molecules and biologics. Therefore, it is necessary to establish a pharmacokinetic model that can capture dynamic behavior of this new class of drugs capable of replicating and altering interactions with the host immune system. We developed two-state pharmacokinetic model to simulate the biodistribution of Escherichia coli Nissle 1917 with biosensor-driven encapsulation systems in different biological compartments. Using this model, we identified parameters in gene circuit dynamics and immune clearance that influence tumor colonization and systemic bacterial persistence. In a “humanized” pharmacokinetic model with increased rate of complement-mediated lysis of bacteria in blood, biosensor-driven systems achieved tumor seeding densities comparable to wild-type bacteria while reducing bacterial loads in blood and liver by several orders of magnitude, highlighting their potential for safe systemic delivery. The biosensor-driven systems represent a more effective strategy to control living drugs than inducible systems, and the two-state pharmacokinetic model is a first step to capture the autonomous nature of this new class of therapeutics for clinical translation.

## Contributed Session II

### Tongli Zhang: Cerebrospinal Fluid Adrenocortical-Brain Steroid Concentrations and Dynamics in Healthy Humans and in Veterans with Posttraumatic Stress Syndrome: Physiologically-based Hormone Dynamic Modeling

Abstract. Dehydroepiandrosterone (DHEA) and cortisol are key adrenal hormones whose concentrations dynamically fluctuate in response to stress. These hormones can be measured across multiple physiologic compartments. Measuring them in cerebrospinal fluid (CSF) provides insight into central neuroendocrine regulation but is technically challenging and invasive, whereas saliva sampling offers a noninvasive alternative.
Questions. Understanding the relationship between CSF and salivary concentrations of cortisol and DHEA therefore holds substantial clinical relevance. However, the dynamic and asynchronous nature of these measurements makes it challenging to characterize their interrelationships. Methods. To address this, we conducted both statistical analysis and physiologically based hormone dynamic (PBHD) modeling to account for compartmental exchange dynamics and time lags between CSF and saliva. Result. The statistical analysis extracted correlation of cortisol and DHEA in populations, as well as significant inter-individual heterogeneities. In parallel, PBHD modeling revealed nonlinear, time-dependent relationships between CSF and salivary hormone concentrations, highlighting the utility of combining PBHD modeling with statistical analysis in elucidating complex and dynamical interactions.

### Gerald Pao: Explainable data science on low dimensional manifolds

Quantitative science has been dominated by physics that tries to determine the relationships of natural variables in the form of equations. For these to have closed form analytical solutions requires the relationships among variables to be linear, decomposable and the noise hopefully linear. For this reason for highly nonlinear
systems Physics has not given good solutions with complex systems with Chaos theory as a prime example in complexity where results made little impact in the real world beyond toy models that demonstrated useful principles. Furthermore when systems are highly nonlinear there is an infinity of solutions that is able fit any such distribution relatively well. Deep learning on the other hand has delivered solutions to the prediction of complex systems albeit with little or no explainability. Here we present and alternative framework empirical

dynamic modeling, based on modern extensions of the Takens theorem that produce data driven equation free models that guarantee explainability, allow prediction, causal inference, simulation and experimentally falsifiable predictions of real systems derived from observed data without any latent variables. Here we develop a couple methods that allow gene expression, neuroscience, geophysics and other complex systems.

### Riley Juenemann: Evaluating Genetic Engineering Trade-offs Through Whole-cell Modeling of *Escherichia coli*

Genetically engineered bacteria are increasingly utilized to manufacture products that are difficult, expensive, or impractical to synthesize chemically. These products have potential applications ranging from medicine to sustainability. However, metabolic pathway introduction, extensive feedback mechanisms in the cell, and evolutionary forces complicate the engineering of bacterial strains that are well-suited for the task. We need tools that will enable us to anticipate these challenges, as well as increase efficiency and enable novel design. A recently published large-scale model of Escherichia coli has enabled us to simulate many distinct cellular processes and capture their complex interactions on a system-wide level. This model incorporates decades of heterogeneous data collection from E. coli literature to fit over 19,000 parameters for the mechanistic ordinary differential equations describing processes in the cell. We now introduce components related to genetic engineering, with an initial focus on chromosome modification. In this poster, we describe preliminary work analyzing the trade-offs between maximizing exogenous protein production and preserving cell health. Our numerical experiments varying the expression level of a single gfp gene reveal how exogenous gene products sequester resources in key cellular processes. We anticipate that these methods will set the stage for large-scale computational genetic engineering design tools as they develop and expand.

### Meera Prasad: Learning the rules of life by playing multicellular games

Physiological processes in health and disease arise from coordinated interactions among cells, where cells work together to solve tasks. In immune surveillance, dendritic cells present antigens, T cells mount targeted responses, neutrophils eliminate pathogens, and regulatory T cells resolve the process. Current approaches to understand multicellular dynamics are primarily bottom up efforts to identify molecular components, signaling pathways, and regulatory networks that mediate behaviors. However, they do not reveal the operational logic of how each cell integrates diverse cues, selects among possible actions, and contributes to collective outcomes. Multicellular systems rely on distributed computation: cells act as autonomous agents that send, receive, and react to signals dynamically. Inferring the local rules by which diverse cell types coordinate to produce emergent, multicellular behaviors requires exploring an enormous regulatory and signaling interaction space that is temporally coordinated and context-dependent. To address this, we formulate multicellular behaviors as multi-agent games and use reinforcement learning to learn strategies that solve them. We simulate cell–cell interactions in a multicellular system using an on-lattice, Monte Carlo–based framework in which each cell is defined by three physical parameters—motility, self-adhesion, and cross-adhesion to the substrate and other cell types. Cells are modeled as learning agents with two modules: a communication network that broadcasts signals to neighboring cells, and an action network that interprets incoming signals to dynamically adjust these three parameters, effectively treating them as tunable gene-expression states. Cell networks are optimized with reinforcement learning, iteratively updating their strategies until simulated cells coordinate to reproduce the target multicellular behavior. We apply our framework to a collective movement task where cells begin in a cluster and must migrate and stabilize at a target location. We rank strategies by cumulative reward and measure their robustness to perturbations in signaling noise. We find that cells use a diverse set of strategies to solve the problem, but they fall into two broad classes of solutions: cell-autonomous programs and coordinated communication-based strategies. Autonomous solutions emerge first, as cells can succeed by transiently increasing motility to search, then reducing motility and strengthening adhesion to nucleate and stabilize clusters. Communication-based strategies arise later but are faster and more robust to signaling noise. Our

framework uncovers local rules that govern collective behavior and provides a principled way to explore the design space of multicellular coordination.

**Xin Wang: Same-cell profiling of chromatin accessibility and protein**

Measuring multiple molecular modalities across the central dogma (DNA, RNA, and protein) in the same cell can be uniquely powerful by revealing how regulatory events are coordinated. Such rich information improves cell-state resolution beyond what is achievable when each modality is measured in separate batches of cells and the unmatched data are later integrated computationally. In this work, we take chromatin accessibility profiling by Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) as the central axis of our same-cell multimodal design, to profile the footprints of transcription factor programs. The capability complements gene expression data in discerning tumor pathogenesis, and informs potential therapeutic targets to modulate these programs. However, chromatin accessibility alone does not report the downstream protein phenotypes that ultimately shape cell behavior. We introduce same-cell chromatin accessibility profiling and cytoplasmic protein measurement by physically separating intact nuclei while archiving the fractionated cytoplasmic proteome from individual cells. Integrating single-cell ATAC-seq (scATAC-seq) with cytoplasmic protein measurements in the same cell interrogates how accessibility states relate to downstream protein-level phenotypes, and how aligned or heterogeneous these regulatory layers are across a population. To achieve this precision fractionation and analysis capability, we design a multilayer, microwell-based single-cell microfluidic device that couples staged chemical lysis via differential detergent fractionation with microwell geometries that confine all reactions, and supports a throughput of 512 single cells per device. This device is compatible with a deterministic single-cell dispensing system and operates from a starting input of ~30,000 cells, enabling application to scarce patient-derived specimens such as organoids. To fractionate and scrutinize the cytoplasmic proteome, proteins are electrophoretically resolved and immobilized within a polyacrylamide gel array, while nuclei are transferred to a barcoded polydimethylsiloxane (PDMS) microwell layer for a ~6-hr microwell-indexed on-chip scATAC-seq assay, enabling automated reagent loading and localized tagmentation. Our approach is a radical departure from existing same-cell multimodal methods (i.e., ASAP-seq, TEA-seq, DOGMA-seq) that measure proteins via oligo-tagged antibodies against a predefined epitope panel, eliminating the possibility of retrospective analysis of new protein markers. We will describe a customized analysis pipeline optimized for this workflow. To validate barcoding fidelity and compatibility with the scATAC-seq workflow, we performed a human-mouse barcoding-species experiment and the sequencing analysis showed that 76% of the microwells were correctly assigned to the true species, indicating low cross-species contamination in this first implementation. Using MCF7 breast cancer cells, we further demonstrated cytoplasmic protein fractionation and chromatin accessibility profiling from the same cell, obtaining a median of ~10,000 unique fragments per cell in scATAC-seq analysis. Joint analysis of gene scores and protein intensities revealed cell-to-cell heterogeneity in regulation of the chaperone heat shock protein 90 (HSP90). Ongoing studies use this platform to examine how chromatin-protein relationships shift in response to therapy and to identify molecular signatures associated with survivor cell populations. Together, these results establish our device as a platform for robust on-chip scATAC-seq with optional cytoplasmic protein readout from the same single cell.

**Benjamin Swedlund: Engineering Self-Organized Tissue-Scale Patterns in Mammalian Cells using Synthetic Reaction-Diffusion Circuits**

Self-organization refers to the emergence of spatial or temporal patterns from local interactions between the components of a system, in the absence of external instructions. Defining the mechanisms underlying spatial self-organized patterning in natural systems remains challenging due to the complex and interconnected structure of gene regulatory networks. In contrast, theoretical models show that periodic spatial patterns can self-organize from stochastic noise using just two components: short-range activation and long-range inhibition. Such reaction-diffusion (R-D) networks are known to be necessary for tissue patterning in diverse contexts, including digits, hair follicles, and fingerprints. However, whether they are sufficient to drive self-organized

patterning remains unclear. To enhance our understanding and control over spatial self-organized patterning, I engineered synthetic R-D circuits in mammalian cells. I proposed a novel circuit architecture that combines juxtacrine activation with paracrine inhibition. Using synthetic Notch receptors to orthogonally control cell-cell communication, I constructed R-D circuits that generate tissue-scale patterns in mouse fibroblasts, demonstrating their sufficiency for driving spatial self-organization. Guided by a parametrized in silico model, I showed that tuning individual circuit parameters modulates emergent pattern features, highlighting the versatility of simple synthetic designs. Extending this framework, I constructed dual, interacting R-D networks using distinct orthogonal synthetic Notch receptors to generate complex four-state patterns, demonstrating the approach's scalability and modularity. Together, my work establishes a bottom-up framework to study and program self-organized patterning and provides a valuable tool for spatially controlling gene expression.

**Jiayi Wu: Tuning a Genetic Circuit with Double Negative Feedforward Loops to Approximate Square Waves**

Precise temporal control of gene expression is important for programming cellular functions. Existing circuits, however, often couple amplitude and duration. This prevents independent tuning of key dynamic features. We therefore set out to design a genetic system capable of generating square-wave outputs with externally programmable geometry. To achieve this, we constructed a layered regulatory design that separates production and removal into two independently controlled modules. An activating input turns expression on and builds the reporter. A repressing input stops synthesis and removes existing protein. Together, these actions confine the active window and produce square-wave outputs or pulses on demand. Waveform geometry depends on input dose and timing. Analysis of limiting cases reveals that strong repression compresses dynamic range, and finite degradation capacity restricts decay rate. Enhancing removal capacity alleviates this bottleneck and sharpens the off-transition. Together, this work establishes a general framework for programming gene-expression dynamics with independently tunable amplitude and duration.

**Ksenia Zlobina: Reconstructing Biological Dynamics from Transcriptomic Time Series**

Understanding biological dynamics from transcriptomic time series remains a key challenge in modern systems biology. Thousands of gene expression trajectories are recorded, but only a few variables are sufficient to describe the key behavior of the biological system. Deriving these variables directly from transcriptomic data, however, has long been considered a difficult problem. We developed a method that goes beyond traditional clustering: instead of simply grouping genes to interpret biological processes, we convert each cluster into a single dynamic variable – the mean cluster value – capturing the trajectory that the biological system follows over time. This approach allows direct visualization of system dynamics and the identification of major transitions or stable states that may lead to pathological outcomes. Applying this method to transcriptomic time series from acute and chronic wounds in animals and humans, we reconstructed the full trajectories of wound healing. Both healing and non-healing wounds were found not to stall but to follow continuous dynamic trajectories. We also propose that non-healing wounds can oscillate along these trajectories, pointing to a novel possible mechanism of wound chronicity that can be tested in future studies. This discovery provides a new way to study chronic wounds and demonstrates that nonlinear dynamic approach can uncover meaningful patterns in complex omics data.

**Filippo Liguori: Dynamic Gene Expression Mitigates Mutational Escape in Lysis-Driven Bacteria Cancer Therapy**

Engineered bacteria have the potential to deliver therapeutic payloads directly to solid tumors, with synthetic biology enabling precise control over therapeutic release in space and time. However, it remains unclear how to optimize therapeutic bacteria for durable colonization and sustained payload release. We characterize nonpathogenic *Escherichia coli* expressing the bacterial toxin Perfringolysin O (PFO) and dynamic strategies that optimize therapeutic efficacy. While PFO is known for its potent cancer cell cytotoxicity, we present experimental

evidence that expression of PFO causes lysis of bacteria in both batch culture and microfluidic systems, facilitating its efficient release. However, prolonged expression of PFO leads to the emergence of a mutant population that limits therapeutic-releasing bacteria in a PFO expression level-dependent manner. We present sequencing data revealing the mutant takeover and employ molecular dynamics to confirm that the observed mutations inhibit the lysis efficiency of PFO. To analyze this further, we developed a mathematical model describing the evolution of therapeutic- releasing and mutant bacteria populations, revealing trade-offs between therapeutic load delivered and fraction of mutants that arise. We demonstrate that a dynamic strategy employing short and repeated inductions of the *pfo* gene better preserves the original population of therapeutic bacteria by mitigating the effects of mutational escape. Altogether, we demonstrate how dynamic modulation of gene expression can address mutant takeovers giving rise to limitations in engineered bacteria for therapeutic applications.

**Thomas Kuhlman: Human Stress Response Specificity through Bioresonance Selectivity**

In eukaryotes, the mitogen activated protein kinase (MAPK) cascade, a multilayered interconnected network of enzymes, connects external stimuli to gene regulation, determining cellular fate. Environmental stress sensed by a cell starts a complex chain of reactions between MAPK enzymes that ultimately activates the master stress response regulator protein p38 MAPK. Thus activated, p38 must then selectively activate targets from a pool of hundreds to initiate appropriate cellular responses. Mechanisms for how p38 performs this selection remain unclear. Here we show that human p38 target selectivity is based on the same physical principles as modern electronic telecommunications systems, except using waves of chemicals rather than electromagnetic fields or electric currents. p38 encodes information about stimuli as different frequency oscillations of its activation state, and targets are selected through frequency-dependent resonance of oscillating biochemical reactions between p38 and its targets. We demonstrate this mechanism, which we call bioresonance, by activating different responses in human cells by applying only sugar at different frequencies. These results unify observations of oscillating signaling components and altered responses into a coherent framework to understand and control human gene expression.

## Main Morning Session

**Olga Troyanskaya**

**Michael Shelley: Self-Organization, Flows, and Transport within (and of) Living Cells**

Organisms organize their internal contents at the cellular scale through striking dynamical processes. In the nucleus, molecular cross-linkers and nuclear motors work to compartmentalize the genome. In female fruit-fly, self-organized flows transport materials across growing egg cells, establishing functional asymmetries essential for development. And in males, ultralong sperm - as long as the organism itself- are packed and stored in a remarkable state of ordered unrest. I will describe our work aimed at understanding these phenomena by tightly interfacing multiscale mathematical models with quantitative experiment. The theoretical frameworks draw on fluid and nonlinear dynamics, coarse-graining, and active matter concepts, and show how applied mathematics can illuminate the biophysical mechanisms that enable living systems to build, move, and organize themselves.

## Main Afternoon Session

### Shah Md Toufiqur Rahman: Pooled CRISPRi and live-cell imaging reveal enhancer control of NF-κB signaling dynamics

NF-κB is a central regulator of inflammation, immunity, and cellular stress responses. The temporal dynamics of NF-κB activation encode pathogen-specific information and dictate downstream gene expression programs. Recent work suggests that distinct NF-κB signaling profiles can generate epigenetic modifications at enhancer regions, potentially establishing an epigenetic memory that shapes responses to subsequent stimuli. In this study, we investigate how epigenetic perturbations influence NF-κB signaling dynamics. Using CRISPR interference (CRISPRi) in a pooled, genetic-screening format, we systematically disrupt putative enhancer regions surrounding feedback genes of NF-κB. In parallel, we perform quantitative live-cell imaging to measure how these perturbations alter NF-κB signaling dynamics. Preliminary experiments using electroporated tracrRNA–crRNA duplexes in dCas9-KRAB–expressing immortalized ear fibroblasts indicate that targeting enhancer regions near the negative-feedback gene Nfkbia (encoding IκBα) leads to enhanced and sustained NF-κB responses following LPS or TNF-α stimulation. Guided by these findings, we designed a 33-guide RNA (gRNA) CRISPRi library targeting specific regulatory regions around Nfkbia, generated stable cell lines, and validated library representation. We then performed live-cell NF-κB imaging after stimulation with LPS or TNF-α, followed by fixation and optical readout of gRNAs to identify perturbations at single-cell resolution. Together, our optical pooled screening of enhancers at feedback genes will reveal how enhancer elements modulate NF-κB signaling dynamics and provide insights into how tissue-specific NF-κB responses are shaped by the epigenome and how epigenetic memory is formed.

### Sujit Datta: Death and chemotaxis: Watching bacterial groups navigate complex environments

Bacteria in nature inhabit complex, crowded environments like soils, sediments, and biological gels. However, lab studies typically focus on cells in bulk liquid. How do environmental complexities shape bacterial behavior? And how is this behavior altered when bacteria encounter viral predators? In this talk, I will describe my group's work addressing these questions using tools from soft matter, 3D imaging, and biophysical modeling. We have developed the ability to directly visualize bacteria from single cells to entire migrating populations and 3D-print precisely structured collectives in crowded environments that mimic natural soils. I will describe how confined bacteria exhibit hopping-and-trapping motility, how migrating collectives adapt shape to resist perturbations, and, strikingly, how populations escape viral predators by collectively outrunning phage—all autonomously and robustly, by processing chemical information in their local environments.

### Matthew Bennett: Fast, long-range intercellular signal propagation through growth assisted positive feedback

Intercellular signaling in bacteria is often mediated by small molecules secreted by cells. These small molecules disperse via diffusion which limits the speed and spatial extent of information transfer in spatially extended systems. Theory shows that a secondary signal and feedback circuits can speed up the flow of information and allow it to travel further. Here, we construct and test several synthetic circuits in Escherichia coli to determine to what extent a secondary signal and feedback can improve signal propagation in bacterial systems. We find that positive feedback-regulated secondary signals propagate further and faster than diffusion-limited signals. Additionally, the speed at which the signal propagates can accelerate in time, provided the density of the cells within the system increases. These findings provide the foundation for creating fast, long-range signal propagation circuits in spatially extended bacterial systems.

# Friday, February 20, 2026

## Contributed Session I

### Mohamed El-Brolosy: ILF3 Links Mutant mRNA Decay to Transcriptional Activation During Transcriptional Adaptation to Mutations—A Process Driven by Novel Trigger RNAs

A long-standing paradox in genetics is that many engineered mutant animals and individuals carrying loss-of-function variants in critical genes show little or no phenotype. This resilience has renewed interest in mechanisms that buffer genetic perturbations. Many such mutations generate aberrant mRNAs that are degraded by cytoplasmic surveillance pathways such as nonsense-mediated decay (NMD). Transcriptional adaptation (TA) is one such genetic robustness mechanism, in which mutant mRNA decay induces sequence-dependent upregulation of related genes, termed adapting genes. This response can compensate for loss-of-function (LoF) mutations, particularly when the adapting gene is a paralog or the remaining wild-type allele in heterozygous contexts. However, how cytoplasmically generated mRNA fragments influence nuclear transcription has remained poorly understood. Using genome-wide CRISPR screens, we identified ILF3 as an RNA-binding protein that connects mRNA decay to transcription during TA. Comparative Perturb-seq experiments using CRISPR-cutting (to mimic mRNA-destabilizing LoF mutations) and CRISPR interference (as a control that depletes protein without generating mutant mRNA) confirmed a broad role for ILF3 in TA. We show that ILF3 is recruited to adapting gene loci, where it promotes transcription by enhancing elongation and recruiting chromatin modifiers. Accordingly, artificial recruitment of ILF3 using a dCas13 fusion protein is sufficient to induce gene expression. To identify sequences that trigger TA, we developed trigger screens, an oligonucleotide-based screening method in which short fragments tiling a gene with 1-nt increments are cloned into a vector designed to undergo NMD and tested for their ability to activate endogenous genes. Across multiple genes, these screens identified discrete RNA fragments, which we term trigger RNAs, that can induce TA in an ILF3-dependent manner when introduced into cells. Scanning mutagenesis revealed that trigger RNAs activate transcription by base-pairing with and inhibiting antisense RNAs at adapting gene loci. Notably, we show that trigger RNAs can activate disease-relevant genes such as PKD1, implicated in polycystic kidney disease. Together, these results enhance our molecular understanding of TA, defining a pathway in which mRNA decay products can act as information carriers that link cytoplasmic RNA surveillance to nuclear transcriptional activation. The trigger screen approach provides a framework for identifying and designing oligonucleotides that activate gene expression, offering a potential strategy that can be explored as a therapeutic modality for genetic disorders.

### Taimu Masaki: Reprogramming Müller glia to induce oscillatory Ascl1 expression and neurogenesis in the adult mammalian retina

Retinal neurons possess minimal intrinsic capacity for regeneration. Consequently, injury-or disease-induced loss of retinal neurons is a leading cause of permanent blindness.While no effective treatments currently exist to replace lost retinal neurons, a promising approach involves the reprogramming of endogenous Müller glia cells (MG), which remain quiescent under physiological conditions, into functional retinal neurons. Here, we show that simultaneous Plagl2 induction and Dyrk1a inhibition (iPaD; induction of Plagl2 and anti-Dyrk1a) elicits pronounced MG proliferation in the adult mice retina. Time-lapse imaging and single-cell tracking revealed that iPaD-activated MG acquire key hallmarks of multipotent embryonic neural stem cells, including: (1) interkinetic nuclear migration across the outer nuclear layer accompanied by multiple rounds of cell division, and (2) induction of oscillatory expression of the pro-neural transcription factor Ascl1, conferring neurogenic competence to generate retinal neurons in both the inner nuclear and ganglion cell layers. Single-cell RNA sequencing further demonstrated that iPaD-activated MG transition from quiescence into a proliferative state and subsequently commit to a neural lineage. These findings demonstrate that iPaD-activated MG are robustly reprogrammed into a progenitor-like state with both proliferative and neurogenic competence, an outcome rarely

achieved by previous methods, highlighting its potential as a powerful therapeutic strategy for retinal regeneration in the adult mammalian retina.

**Chloe Nguyen: Engineering stable cell fate commitment via duplicate-origin cutter plasmids**

Cell populations rely on robust decision-making mechanisms to generate stable phenotypes in dynamic environments. However, most synthetic fate-determination tools require continuous induction or rely on complex genetic circuits to maintain commitment. Homing endonucleases instead provide a mechanism for irreversible decision-making because their activity permanently removes genetic material containing their recognition site. Here, we leverage this property to create a two-plasmid system in *E. coli*, in which each plasmid encodes a homing endonuclease and the cut site of the opposite nuclease. When activated, one plasmid permanently eliminates the other, locking the cell into a stable state regardless of changes in environmental signals and therefore, establishing an irreversible cell-fate switch. Overall, we validate homing endonucleases as a modular, irreversible mechanism for cell fate determination. Through independent characterization, duplicate-origin coupling and stress response activation, this work lays the foundation for synthetic fate control systems and allows for new opportunities in long-term memory and adaptive population engineering.

**Gavin Schlissel: Extracellular Hedgehog diffusion, and evolution of morphogen gradients**

Developmental pattern formation relies on secreted signaling proteins to determine cell fates within a patterning field. These signaling proteins must travel through a crowded extracellular matrix, which is composed of insoluble and heavily modified proteins. It has long been appreciated that the extracellular matrix might affect cell-cell communication, however the extracellular matrix is resistant to most legacy biochemical, structural, or genetic analysis and so it has been difficult to understand mechanistically how the extracellular matrix could influence the movement of extracellular signaling molecules. I developed in situ single-molecule microscopy approaches to understand how secreted signaling proteins move through an intact extracellular matrix in live cells and tissues. My work revealed a new model for extracellular diffusion, and identified previously unappreciated mechanisms that evolution has modulated to regulate the size of Hedgehog signaling gradients in tissue-or organism-specific ways. Now, I am using this understanding to discover how animal morphology has evolved, with a special emphasis on understanding the mechanistic cause of the flat-face phenotype in domestic bull dogs, in addition to human bone disorders.

**Ivy Xiong: Dynamic flow-metabolic coupling in renal tubules underlies water and electrolyte conservation by the mammalian kidney**

To maintain a stable environment in the body, the mammalian kidney not only removes waste from the main circulation, but also balances fluid and electrolyte composition. It does so by orchestrating filtration, fluid flow and solute transport from minute to minute within each of its 1 million renal tubules in parallel. While the steady-state behavior of these processes has been well-characterized, little is known about their dynamic coordination in vivo. Applying intravital two-photon microscopy on GCaMP mice, we discovered that in the kidney of living animals, tubular fluid flow and metabolic intermediates (NADH, ATP vesicles, superoxide, and mitochondrial membrane potential) in renal epithelial cells exhibit spontaneous and robust oscillations at characteristic frequency of ~0.03 Hz. This oscillatory behavior is consistent with the action of the tubuloglomerular feedback (TGF) system, an intra-renal negative feedback mechanism for maintaining glomerular filtration rate within a physiological range. Interestingly, we uncovered a temporal synchronization between cell-level metabolic cycles and tissue-level fluid flow oscillations in vivo, mediated by Ca2+ transient spikes in the cytoplasm. Loop diuretics targeting TGF abolished oscillations in tubular fluid flow and metabolic intermediates; and depletion of cytosolic glutathione altered fluid flow dynamics and triggered ferroptosis. Together, these findings suggest that TGF-mediated oscillatory fluid flow in the renal tubule is functionally coupled to epithelial metabolic dynamics, which serves a fundamental role in optimizing cellular bioenergetics to support reabsorption of electrolytes and water in renal tubules. Our work relates to recent reports of

ultra-slow oscillations in cerebral blood flow, oxygenation, and neural activities, bearing wide implications for the principles underlying water and electrolyte conservation by the kidney.

**Bassem Al-Sady: Hysteresis measurements reveal a memory gradient in the repressed genome**

Heterochromatin marked by histone H3 lysine 9 methylation (H3K9me) emerged in the last eukaryotic common ancestor and has been linked to heritable gene repression for a century (1). This connection is reinforced by the discovery of biochemical feedback loops (2–4) that respond to preexisting H3K9 methylation. However, whether H3K9me heterochromatin consistently generates epigenetic memory, and what molecular features enable it, remains untested. We aimed to rigorously probe the association of epigenetic memory with H3K9me heterochromatin in fission yeast. Prior measurements of epigenetic memory rest on laborious time-dependent measurements (5) that do not necessarily report on molecular memory. To address this, we adapted the biophysical concept of hysteresis (6) to quantify epigenetic memory at steady state using a single-cell fluorescent reporter system (7, 8). By engineering the coding gene for the central heterochromatin assembly protein HP1 to depend on estradiol for expression, we modulated heterochromatin to be input-responsive, as required for hysteresis analysis. Using this approach, we observed a striking diversity of hysteresis across the fission yeast heterochromatic genome, ranging from absent to extreme, with the subtelomere showing the broadest variation across the 50 kb domain. These results indicate that the presence of heterochromatic marks and associated proteins does not predict epigenetic memory. Instead, memory strength and regulation are highly locus-specific. Surprisingly, we find that previously identified regulators of epigenetic memory have very divergent impacts on hysteresis at different heterochromatin sites, implying that each heterochromatin locus is governed at least partially by non-overlapping sets of memory pathways. The integrity of multiple heterochromatin nucleation pathways, shown to be redundant for heterochromatin assembly, is also central for retaining memory. This finding suggests a possible explanation for the unexplained phenomenon of redundant nucleation pathways at many loci in fission yeast. They may serve to ensure memory, beyond the initial establishment of silencing. We are now probing how varying the dosages of heterochromatin writers and erasers affects memory, to delineate the H3K9me turnover regimes that are compatible with stable inheritance. Together, these results establish a new hysteresis framework based on single cell reporters for quantifying epigenetic memory. Our measurements reveal that the emergence of epigenetic memory in fission yeast heterochromatin is intrinsically locus dependent and governed by diverse molecular mechanisms.

**Paige Steppe: Adaptive Therapeutic Delivery through Plasmid Coupling in Bacteria**

Live bacterial therapeutics offer unique advantages for cancer treatment, including natural tumor colonization and programmability. However, current designs use fixed genetic architectures that cannot adapt to the spatial and temporal heterogeneity of solid tumors. We present an adaptive framework that enables bacterial populations to autonomously switch between tumor-sensing and therapeutic-delivery modes. Using coupled plasmids sharing a single origin of replication, we demonstrate that bacteria can dynamically adjust plasmid copy number in response to tumor-associated lactate gradients. By integrating lactate-inducible lysis with a synchronized lysis circuit, we achieve cyclical, biomarker-dependent therapeutic release that is spatially localized and self-regulated. Our microfluidic studies show stable phenotypic patterning across lactate gradients, with populations transitioning between a high-sensitivity biosensor mode and an active delivery mode. This work establishes plasmid coupling as a generalizable strategy for engineering adaptive microbial therapies that respond to the heterogeneous tumor microenvironment.

**Bo Gu: Dissecting the Logic of Signaling Receptor Competition using Multiplexed CRISPRi**

The ability to perturb multiple proteins simultaneously within the same cell is essential for understanding and re-engineering biological networks. CRISPR-Cas12a mutants with inactivated DNAse but intact RNAse activity (dCas12a) retain the ability to process large CRISPR RNA (crRNA) arrays, enabling parallel targeting of many genomic loci. When coupled with transcriptional effector domains, these properties make dCas12a a promising

platform for multi-locus transcriptional perturbation. However, existing dCas12a-based transcriptional regulation systems often exhibit low perturbation efficiencies during multi-locus targeting, limiting their practical utility. Here, we combine molecular and architectural engineering to create Pathway Sculptor, a programmable Cas12a-based CRISPRi platform that enables strong, durable, and tunable simultaneous knockdown of seven or more genes in a single cell. Leveraging this capability, we systematically reconfigured TGFβ superfamily receptor profiles to dissect intra- and inter-pathway signaling logic. Our initial findings reveal that receptor competition dictates BMP ligand perception (intra-pathway) while driving context-dependent crosstalk between the BMP and TGFβ pathways (inter-pathway). These results establish Pathway Sculptor as a robust tool for high-order combinatorial genetic perturbations and provide a scalable framework for decoding receptor-level signal processing.

### Paula Godoy: Divergent Modes of Aging in Yeast and Hematopoietic Stem Cells

Replicative lifespan studies in budding yeast have revealed divergent modes of cellular aging characterized by either elevated mitochondrial function or enhanced nucleolar stability. Notably, neither state is intrinsically youthful; rather, the capacity to dynamically oscillate between these modes is associated with extended lifespan. We sought to determine whether analogous aging phenotypes exist in hematopoietic stem cells (HSCs), the stem cell population responsible for lifelong blood and immune system regeneration. HSCs exhibit well-established hallmarks of aging and functional decline in the elderly, making their rejuvenation a promising strategy to improve both healthspan and lifespan. Using tetramethylrhodamine methyl ester (TMRM) to measure mitochondrial membrane potential as a proxy for mitochondrial function, we identified bimodal mitochondrial states in aged but not young HSCs. Elevated mitochondrial membrane potential correlated with nucleolar stress signatures reminiscent of yeast aging phenotypes, as well as increased mTORC1 activity and enhanced clonogenic capacity. Together, these findings suggest that, as in yeast, neither high nor low mitochondrial activity alone defines cellular youthfulness. Instead, future work will test whether restoring dynamic transitions between these states can rejuvenate aged HSCs.

## Contributed Session II

### Tammy Collins: Insights from Reviewers: Unlocking Success in Applying for Burroughs Wellcome Fund's Career Awards at the Scientific Interface (CASI)

Postdoctoral scholars seeking independent funding to support their transition to faculty positions should consider applying for the Career Awards at the Scientific Interface (CASI), a postdoc-to-faculty transition award offered by the Burroughs Wellcome Fund (BWF). CASI supports both a postdoctoral and faculty phase, totaling $560,000 over five years for scientists with backgrounds in physical, computational, mathematical, and engineering fields who apply these approaches to answer important biological questions. The postdoctoral phase of the award provides stipends between $80,000–$85,000 and caregiving expenses of up to $5,000/year. The award is open to non-U.S. citizens and can be used at U.S. or Canadian institutions. BWF especially encourages applications from individuals historically underrepresented in science. This session will focus on what reviewers are looking for in successful applications, and what makes CASI unique compared to grants such as the K99/R00. Anonymized reviewer comments on high- and low-scoring applications will be shared to provide potential applicants with an in-depth perspective on how CASI applications are evaluated.

### Mohammad Fallahi-Sichani: AP-1 co-regulatory network organizes discrete, heterogeneous, and reconfigurable cell states

Responses of cells to signaling perturbations exhibit complex dynamics that vary across diverse cell types or even genetically identical cells exposed to uniform conditions. Heterogeneity arises partly because of the cells' ability to process the signaling input in the context of their states controlled by their epigenome, which although contains heritable information about gene expression programs, remains plastic. Such plasticity enables cells to

transition from one phenotype to another and exhibit fractional responses to perturbations. Despite this knowledge, what remains largely unknown is the nature of these dynamically fluctuating phenotypically consequential cell states, and how they determine the context for signal transduction, and mechanisms that control the diversity of these states, their multi-stability or plasticity, and variation across cell types. Focusing on these key gaps, we recently showed AP-1 transcription factor network serves as a key node in linking MAPK signal transduction to diverse patterns of cell state plasticity. Individual cells can adopt a variety of AP-1 states regulated by a combination of competitive homo- and heterodimeric interactions between individual AP-1 proteins, whose levels and stability are also subject to transcriptional (auto)regulation and posttranslational modification by MAP kinases. We show that the diversity of recurrent AP-1 states and their single-cell frequencies vary across differentiation states and predict their phenotypic plasticity prior to and following MAPK perturbations. However, how the AP-1 network encodes a limited set of discrete states, how their distributions vary across distinct cell states, and what drives phenotypically consequential AP-1 state transitions remain unclear. To address this question, we developed a mechanistic ODE model of the AP-1 network capturing dimerization-controlled, co-regulated, competitive interactions. Calibrated to heterogeneous single-cell data across genetically diverse populations of melanoma cells and combined with statistical learning, the model reveals network features explaining population-specific AP-1 state distributions. These features correlate with MAPK activity across cell types and with variability within clonal populations, linking signaling to AP-1 states. The model predicts—and experiments validate—adaptive AP-1 reconfiguration under MAPK inhibition, inducing a dedifferentiated, therapy-resistant state that can be blocked by model-guided AP-1 perturbations. These results establish AP-1 as a configurable network and provide a general framework for predicting and modulating AP-1 driven cell state plasticity.

**Yuki Maeda: Functional analysis of asynchronous Hes1 oscillations in the neural tube formation**

The levels and duration of gene expression both play critical roles in many biological processes. Recent studies have further revealed that the dynamics of oscillatory versus sustained gene expression also provide essential regulatory information; for example, the tumor suppressor p53 induces cell-cycle arrest with oscillatory expression (in response to γ-radiation) but triggers apoptosis with sustained expression (in response to UV). Furthermore, the spatial coordination of these temporal dynamics is equally crucial. In the segmentation clock, Hes7 exhibits synchronized oscillations across the presomitic mesoderm, which are essential for periodic somite formation. Both sustained expression and asynchronous oscillations of Hes7 are known to cause somite defects. This highlights the functional importance of specific dynamic patterns. In contrast, other systems display different dynamics, such as the asynchronous oscillations of Hes1 in the developing nervous system. However, the functional significance of this asynchrony remains unclear. To investigate this question, we employed a blue light-inducible gene expression system to artificially control Hes1 dynamics, inducing either synchronized oscillations or sustained expression in mouse embryonic stem cell (mESC)-derived neural tube organoids (NTOs). We observed that sustained Hes1 expression, in contrast to synchronized oscillations, resulted in a reduction in NTO size. This implies that the oscillatory nature of Hes1 expression is required for normal NTO growth. Additionally, we made an unexpected finding regarding the floorplate marker FoxA2, which typically shows localized expression on one side of the NTO, but both sustained Hes1 expression and synchronized Hes1 oscillations disrupted this pattern, causing FoxA2 upregulation throughout the entire organoid. Taken together, these findings suggest that the asynchronous nature of Hes1 oscillations is critical for two distinct processes: maintaining normal NTO growth and establishing the correct spatial restriction of FoxA2 expression. We are currently conducting real-time imaging of Hes1 and FoxA2 dynamics to further elucidate the underlying mechanisms.

**Ljubica Mihaljevic: Membrane protein solubilization and structure determination using WRAPs**

The development of therapies and vaccines targeting integral membrane proteins has been complicated by their extensive hydrophobic surfaces, which can make production and structural characterization difficult. Here we

describe a general deep learning-based design approach for solubilizing native membrane proteins while preserving their sequence, fold, and function using genetically encoded de novo protein WRAPs (Water-soluble RFdiffused Amphipathic Proteins) that surround the lipid-interacting hydrophobic surfaces, rendering them stable and water-soluble without the need for detergents. We design WRAPs for both monomeric and oligomeric beta-barrel outer membrane proteins and for GPCRs and other helical multi-pass transmembrane proteins, and show that the solubilized proteins retain the binding and enzymatic functions of the native targets with enhanced stability. A 2.95 Å cryo-EM structure of WRAPed Mycobacterial porin demonstrates WRAPs can be used for high resolution structural determination of membrane proteins in solution. As a step towards syphilis vaccine development, which has been hindered by difficulties in characterizing and producing the outer membrane protein antigens, we generate soluble versions of four Treponema pallidum outer membrane beta barrels. WRAPs should be broadly useful for facilitating biochemical and structural characterization of integral membrane proteins, enabling therapeutic discovery by screening against purified soluble targets, and generating antigenically intact immunogens for vaccine development.

**Paige Nickerson: The regulatory action of H-NS on gene expression in circular and linear DNA**

DNA supercoiling, or superhelicity, is the biophysical phenomenon where the degree of twist and writhe in DNA exceeds a basal level. Superhelicity is introduced every time genes transcribe and replicate. In the presence of multiple, transcribing genes, our recent single-molecule experiments strongly suggest that DNA supercoiling is not uniformly distributed across the length of DNA, but rather localized to regions confined by protein-binding events. These findings further suggest that DNA binding proteins can influence gene expression, especially genes that are subject to localized changes in superhelicity. We had previously found that relative gene orientation induces strong changes in superhelicity and gene expression. In this work, we explore the power of the DNA-binding protein H-NS, to modulate gene expression in circular and linearized DNA in cell free expression systems. In our experiments, we isolate, purify, and quantify expression of two fluorescent genes in E. coli cell-free extract, arranged in 3 possible relative gene orientations that are otherwise genetically identical. In each experiment, we co-express H-NS as an auxiliary regulator protein and quantify the measured drop or rise in fluorescence through time-series spectrophotometry. As a control, we also quantify expression of a single gene on linear DNA (and circular DNA), where supercoiling is free to dissipate (or is confined). By expressing H-NS with a linear and circular form of the fluorescent gene, we are able to investigate the extent that H-NS relies on supercoiling to repress expression and pack DNA. We also test other physical constraints on H-NS such as gene layout and interaction with topoisomerases. Our data shows that H-NS repression requires the presence of supercoiling to have a strong effect. Further, we find that the magnitude of H-NS repression (or activation) strongly depends on genetic syntax. In cases where negative supercoiling domains are expected to accumulate, H-NS acts as an effective repressor, with up to 20-fold repression. Most notably, we find that H-NS can sometimes behave as an effective activator for convergently-oriented genes. When we add topoisomerases, we observe that both topoisomerase I and gyrase lift H-NS mediated repression. Separately, H-NS represses expression of a single gene on a plasmid by approximately 2-fold. However, on linear DNA, we observe no H-NS repression, showing that H-NS regulation critically depends on the presence of supercoiling. Finally, we explore and introduce a mathematical model to describe the dynamics of H-NS repression.

**Lili Yang: Single-molecule Imaging Reveals RNA Polymerase Generates and Confines DNA Supercoiling**

DNA supercoiling is critically important as it directly influences essential processes like gene expression, DNA replication, and chromatin organization. For decades, the prevailing paradigm in DNA mechanics has held that significant transcription-driven supercoiling requires topologically constrained DNA, such as circular molecules or fragments with tethered ends. This view, while widely accepted, has never been directly tested by visualizing supercoil emergence in real time on unconstrained DNA. Previous in vitro studies have relied on externally applied torsional stress, leaving a fundamental question unanswered: can the transcription machinery alone

generate and confine supercoiling in a biologically relevant context? Here, using an improved single-molecule imaging platform that allows direct visualization of individual, topologically unconstrained DNA molecules during transcription, we shatter this long-standing dogma. We demonstrate that RNA polymerase (RNAP) dynamically generates and confines supercoiling without any external force or global topological barriers. We reveal that multiple, simultaneous transcription events are critical, creating transient topological domains where plectonemes form and stabilize between RNAP complexes; a single transcription event is insufficient for this process. We confirm these structures are supercoils by demonstrating their specific modulation by topoisomerase activity. Crucially, we establish that the RNAP complexes themselves provide the necessary topological barriers, confining torsional stress and making transcription-induced supercoiling an inherently localized phenomenon. These findings redefine the physical basis of transcription-coupled supercoiling. We provide a new mechanistic framework where supercoiling is not a global property of a DNA domain, but an emergent and localized consequence of multi-polymerase activity. This has profound implications, providing a physical mechanism for feedback and crosstalk between genes. Furthermore, it provides a new evolutionary lens for understanding genome architecture and a predictive framework for the efficiency of genome-editing technologies.

### Piyush Nanda: Competition between cytosolic and mitochondrial ribosomes produces a metabolic bistable switch

In the budding yeast, *S. cerevisiae*, an abrupt shift from glucose to less preferred carbon sources reveals two epigenetic states: Arrestors and Recoverers. Before the change, arrestors, on average, grow faster than recoverers but show a prolonged arrest upon glucose deprivation. In contrast, recoverers adapt to the new carbon source within hours and resume growth. The two states interconvert by stochastic switching at a rate slower than the cell division rate, suggesting bistability. The persistence of these alternative states despite identical external glucose raises the question: what molecular feedback produces this behavior? To uncover the mechanism, we track yeast cells expressing metabolic biosensors to directly measure energy metabolism in single cells. First, we show that arrestors and recoverers are locked in states of fermentation and respiration respectively. We demonstrate that bistability through positive feedback in the mitochondrial translation machinery produces the two states. Specifically, import of nuclear-encoded positively charged mitochondrial ribosome (mito-ribosome) precursors depends on mitochondrial membrane potential, while mito-ribosomes themselves synthesize key electron transport chain components required to maintain this potential, thus establishing a self-reinforcing circuit. We show that cooperative multi-subunit assembly of cytochrome oxidase (Complex IV) leads to non-linear amplification of mitochondrial translation activity resulting in ultra-sensitivity and generating a bistable switch in mitochondrial metabolism. A coarse-grained biophysical model of the circuit suggests global antagonism between cytosolic ribosomes and mitochondrial ribosome in maintain the respiratory machinery emerges as the effective parameter of the bistable switch. Cytosolic ribosome activity promotes cellular growth that constantly dilutes the electron transport chain maintained in part by the activity of mito-ribosomes. Experimentally, reducing cytosolic translation quantitatively circumvents the respiratory defect caused by reduction in mito-ribosome activity in budding yeast and distantly related fission yeast uncovering an underappreciated competition between two distinct ribosomes that sets the mode of energy metabolism.

### Huyun Chen: Biophysical Requirements for Multicellular $Ca^{2+}$–ROS Wave Propagation

Reactive oxygen species (ROS), particularly hydrogen peroxide (H2O2), function as dynamic and reversible regulators of protein signaling. Although H2O2 is traditionally considered locally constrained due to rapid extracellular scavenging and cellular uptake, multiple studies have demonstrated centimeter-scale tissue propagation driven by a mechanism termed the “ROS wave,” in which each downstream cell produces additional ROS in response to elevated intracellular ROS concentration. Despite these observations, the mechanistic requirements that allow a multicellular ROS wave to form, propagate, and persist remain unclear. To address this

gap, we developed a quantitative agent-based model (ABM) that integrates intracellular Ca2+ and ROS reaction kinetics with extracellular and intercellular transport. Each cell contains a redox–calcium system described by coupled ODEs, enabling simulation of H2O2 production and consumption, endoplasmic reticulum release and uptake of Ca2+, and Ca2+- or H2O2-dependent activation of NADPH oxidases (NOXs). The model incorporates two intercellular transport processes: extracellular H2O2 secretion and uptake via aquaporins, and gap junction (GJ) exchange of H2O2 and Ca2+ between neighboring cells. A confluent monolayer of 2000 cells was simulated, with a single cell receiving a bolus H2O2 injection to mimic a localized oxidative injury. Simulations reveal that passive extracellular H2O2 diffusion and GJ-mediated H2O2 transport are insufficient to explain multicellular H2O2 propagation: even moderate intracellular scavenging prevents H2O2 from spreading beyond adjacent cells. Interestingly, we found that the ROS wave propagation rate and feasibility relies upon GJ permeability for Ca2+ and Ca2+-dependent NOX activation, respectively. This work quantitatively delineates the conditions that permit ROS waves to emerge at the tissue scale and highlights the importance of redox-Ca2+ coupled positive feedback. These results establish a computational framework for exploring the cellular mechanisms involved in long-range ROS signaling.

### Heath Johnson: Optogenetic construction of de novo integrin-adhesion complexes reveals role for biocondensation in adhesion nucleation

Integrin-adhesion complexes (IACs) form spontaneously in cells on extracellular matrix substrates, allowing them to sense matrix composition and transduce force. However, IACs often do not form uniformly across a cell, which begs the question: What is required to nucleate an adhesion, and what factors enable the stabilization of an IAC once it has formed? Many factors have been suggested to promote formation and the subsequent stabilization of IACs. It is difficult to explicitly test these factors in vivo as IACs undergo constant remodeling. Here, we employ optogenetics to explicitly test the ability of talin in different activity and phase states to nucleate and stabilize IACs in regions where none are present. We find that fusion of intrinsically disordered regions directly to talin enhances its adhesion nucleation potential and allows new adhesions to be produced in response to optogenetic talin clustering. Similarly, expression of factors previously shown to enhance biocondensation in vitro, such as paxillin, the paxillin N-terminus, or unfolding of talin, allows for adhesion nucleation and biocondensation of talin. We show that these biocondensates of talin can cluster and activate integrins even in the absence of extracellular matrix. By applying optogenetic activation to regions of the cell with or without ventral actomyosin, we demonstrate actomyosin engagement promotes the formation and stability of adhesions. These results are corroborated by theoretical modelling which shows that phase separation of talin is enhanced by differential clutch formation in the presence of actomyosin thus enabling peripheral adhesion formation and stability. This work establishes a model in which increased cooperativity of talin enables IAC nucleation through talin biocondensation, which clusters and activates integrins. In addition to these findings, we generate multiple optogenetic tools that enable local nucleation or enhancement of IACs.

## Main Morning Session

### Amy Herr: Design of microanalytical tools to understand single-cell biology

My lab is interested in design of microanalytical tools to address cellular-resolution questions that are difficult (or impossible) to answer with existing approaches. Our research spans from questions in cancer biology and developmental biology to symbiotic cellular systems and cell biology. We tackle questions where protein expression, state, and function play important biological roles, and we are particularly interested in questions where proteoforms (e.g., protein isoforms) are key molecular players. In this presentation, I will focus on two areas where precision microfluidic tools for molecular and cellular measurements are accelerating biological understanding. First, we begin to expand the possibilities of single-cell proteomics. Single-cell genomics and transcriptomics tools have radically changed the biological sciences and biomedicine. In these areas, microfluidic tools have radically expanded the capabilities of these sequencing tools (e.g., sequencing flow cells

and droplet systems). Our aim is to bring the power of single-cell understanding to proteomics (targeted & discovery) by leveraging the precision of microfluidic design. Using low-volume microfluidic handling and one-pot reactions to minimize loss of proteinaceous lysate, I will describe our single-cell mass spectrometry proteomics workflow design and considerations. With collaborators at Biohub San Francisco and Chicago, I will detail recent results in identifying thousands of proteins per single cell, spanning mammalian (organoid) to cnidarian (symbiotic) systems. Second, I will describe our design considerations for physically linking together multiple, independent measurement modalities in a 'single-cell, same-cell' paradigm. Such so-called "joint analyses" are important to directly correlate different – but interrelated – layers of molecular information. These types of joint analyses may play important roles in generative models of cells and cellular systems, owing to low biological and technical noise. Here, I will describe a suite of approaches that allow us to interrogate the nuclear nucleic acid compartment versus cytoplasmic protein compartment. Our long-term vision is to create tools that allow researchers to ex-post query a unique originating cell for protein-level information, as informed by a priori sequencing-based discovery. Taken together, we strive to introduce tools uniquely equipped to measure both cellular and molecular heterogeneity as a means to more comprehensively understand cellular form and function.

**Ellen Zhong**

### Anthony Fung: Pan-organ profiling of immunosenescence reveals distinct senotypic patterns driven by cell-type and age

Cellular senescence acts as a fundamental safeguard against unchecked proliferation, permanently arresting the cell cycle while maintaining metabolic activity. Although initially sparse, the accumulation of senescent cells is a hallmark of aging, transforming a defense mechanism into a driver of tissue dysfunction and morbidity. This transition is particularly critical within the immune system, where age-correlated dysregulation and chronic low-grade inflammation, or inflammaging, compromise health. While the link between inflammaging and immunosenescence is strengthening, it is often studied in systemic circulation, obscuring the dominant role of tissue-specific microenvironments. Current omics-level investigations face significant hurdles, as variation in immune cell abundance across organs and the artifacts of tissue dissociation often dilute rare senescent populations and destroy crucial spatial context. To resolve these limitations, this study employs a spatially resolved approach to characterize immune cell senescence across diverse tissues, cell lineages, ages, and pharmacologic contexts. Leveraging multiplexed immunofluorescence (CODEX) and spatial transcriptomics (Xenium), we interrogate immune senescence in situ, bypassing the biases of dissociated single-cell datasets. This multi-modal framework allows for the visualization of senescent immune cells within their native cellular neighborhoods, facilitating the analysis of whether these cells distribute diffusely or cluster into focal niches, termed "sen-spots." We examine how local microenvironments shape senescence phenotypes, or "senotypes," and investigate whether these spatial identities predict transcriptional states or responsiveness to therapy. Specifically, this work addresses whether tissue architecture actively propagates aging via paracrine signaling and whether the efficacy of senolytic drugs is context-dependent. By integrating cell identity with spatial biology, this research aims to construct a comprehensive map of immune senescence, elucidating the structural and environmental determinants of immune aging and informing more precise, organ-specific therapeutic strategies.