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HIGH-RISK, HIGH-REWARD RESEARCH SYMPOSIUM

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Speaker and Poster Presenter Abstracts

Behavioral and Social Science

Harnessing Mindset in 21st Century Healthcare

Alia Crum, Stanford University (speaker)

Our research focuses on how subjective mindsets can alter health outcomes through behavioral, psychological, and physiological mechanisms. Mindsets are lenses or frames of mind that orient an individual to a particular set of associations and expectations. Our study of mindsets is, in part, inspired by research on the placebo effect, a robust demonstration of the ability of mindsets to elicit healing properties in the body. Our research aims to move beyond the limited notion of the placebo effect as a mysterious and meaningless response to an inert substance and toward a model in which the placebo effect can be systematically understood and deliberately leveraged to promote better health outcomes. To this end, our research strives to (1) develop rigorous experiments that isolate and examine the mind's influence on our health; (2) build and test theoretical frameworks that explicate the mechanisms by which the mind influences health; and (3) use these empirical and theoretical insights to design and test interventions to more effectively harness the mind's influence, with the intent of improving public health and health care. This talk will discuss our theoretical model as well as review representative studies and overarching findings that were made possible by the NIH New Innovator Award.

Market Area Trends in Menthol and Non-Menthol Cigarette Sales in the United States and Associations with Residential Segregation

Daniel Giovenco, Columbia University Mailman School of Public Health (speaker)

Significance: Use of menthol cigarettes in the United States is strongly associated with race. Driven by decades of targeted marketing, approximately 85 percent of Black smokers use menthol cigarettes; conversely, greater than 70 percent of white smokers prefer non-menthol styles. This study uses regional sales data to examine longitudinal changes in menthol and non-menthol cigarette sales and associations with regional racial composition. Methods: Using Nielsen sales data from 30 U.S. market areas, we computed the percent change in per capita menthol and non-menthol cigarette sales between 2016-2018. Demographic characteristics of counties comprising each region were compiled from the U.S. Census Bureau. Correlation analyses assessed the relationship between consumption changes and regional racial composition, including the dissimilarity index to measure the spatial distribution of Black-white residential segregation. Results: On average, menthol cigarettes held a third of the market share across regions (range: 22-49%); market share was highly correlated with the percentage of Black residents (r=0.69). Between 2016-2018, the rate of decline in per capita pack sales was slower for menthol (-8%) versus non-menthol (-11%) cigarettes. No demographic factors were associated with the decline in menthol sales, but the dissimilarity index was negatively correlated with non-menthol sales (r=-0.44, p=0.02). That is, the greater a region's Black-white residential segregation, the faster the decline in non-menthol sales. Conclusion: Given that greater than 80% of non-menthol smokers are white, it is plausible that this group is driving changes in non-menthol consumption. This study suggests that beyond racial composition,

greater residential segregation between Black and white residents is related to declines in non-menthol cigarette sales. Future research should examine individual and structural factors at the local level (e.g., retail marketing, unequal policy impact, treatment access) to identify possible mechanisms of this relationship.

Health Consequences of Shale Gas Development

Elaine Hill, University of Rochester (poster presenter)

Background: Widespread hydraulic fracturing of shale formations has yielded a range of economic and environmental benefits. However, adverse health outcomes associated with shale gas development (SGD) remain uncertain. This study aims to quantify the myriad health risks associated with SGD. Methods: We utilize quasi-experimental methods to assess the causal effects of SGD on health outcomes. We start by building a novel data set that links gas well activity to health outcomes from multiple data sources including birth records, hospitalization records, self-reported well-being surveys, and mortality records. We also link well activity to various environmental databases, such as light pollution, public drinking water sampling data, and air emissions inventories. Using difference-in-differences and controlling for individual and community characteristics over time, we use longitudinal variation to compare health outcomes before and after drilling to estimate the causal effect of SGD on the health outcomes. Results: We find that SGD increases the risk for adverse birth outcomes, both through proximity as well as through contaminated drinking water in Pennsylvania. We find an increased risk for childhood asthma exacerbation in Pennsylvania and Texas. We find an increased risk for AMI hospitalization and mortality in Pennsylvania, comparing to New York where drilling is banned. We find that light pollution from drilling reduces sleep and increases self-reported bad mental health nationally. We find drilling that reduces non-drug mortality and increases opioid overdose mortality nationally and especially in Appalachia. Conclusion: Our paper contributes to an increasing body of research that estimates the causal impacts of SGD on the environment and health in order to weigh the extent of these potential costs against its economic and environmental benefits. In addition, this work demonstrates a unique application of economic methods in a public health setting.

Developing Skin Cancer Education Materials for Darker Skin Populations: Crowdsourced Design, Message Targeting, and Acral Lentiginous Melanoma

Jakob Jensen, University of Utah (speaker)

Despite decreased susceptibility, darker skin individuals who develop melanoma have worse survival. This disparity in melanoma mortality is the largest for any cancer, and partly driven by a lack of educational materials targeted to darker skin populations in whom acral lentiginous melanoma (ALM) is the most common subtype. To address this communication disparity, the current study reports a multi-phase design process that leverages crowdsourcing and message testing to develop ALM-focused educational materials for darker skin populations. Crowdsourced design was utilized to develop a pool of designs (phase 1), the pool was narrowed and thematically analyzed (phase 2), and select designs were evaluated via a message experiment (N=1,877). For darker skin populations, designs that depicted people enhanced

knowledge of ALM through message memorability. The current study engages melanoma disparities by providing ALM educational materials for darker skin populations vetted via a multi-phase process.

Methods for Correcting Inference Based on Outcomes Predicted by Machine Learning

Tyler McCormick, University of Washington (poster presenter)

Machine learning is now being used across the entire scientific enterprise. Researchers commonly use the predictions from random forests or deep neural networks in downstream statistical analysis as if they were observed data. We show that this approach can lead to extreme bias and uncontrolled variance in downstream statistical models. We propose a statistical adjustment to correct biased inference in regression models using predicted outcomes—regardless of the machine-learning model used to make those predictions.

Single-Session Interventions for Adolescent Depression in the Context of COVID-19: A Nationwide Randomized-Controlled Trial

Jessica Schleider, SUNY Stony Brook University (poster presenter)

Background: The COVID-19 pandemic has caused families nationwide extreme financial hardship, social isolation, and distress. These compounding stressors collectively increase risk for adolescent depression—already the leading cause of disability in youth. However, even before the pandemic, less than 50 percent of youth with depression accessed care, and youth do not uniformly benefit from existing treatments. It is thus critical to identify effective, rapidly-scalable strategies to reduce youth depression, during and beyond the COVID-19 pandemic. Method: This randomized-controlled trial tested online single-session interventions (SSIs) designed to improved proximal targets (hopelessness, perceived agency) and 3-month depression, anxiety, and COVID-19-related trauma during the COVID-19 pandemic in adolescents with elevated depression symptoms (N=2,452, ages 13-16). Youth living across the United States recruited via social media were randomized to one of three self-guided SSIs: a behavioral activation SSI (BA-SSI), an SSI teaching growth mindset, that is, the belief that personal traits are malleable (GM-SSI); or a supportive control. We tested each SSI's effects on post-SSI (hopelessness, agency) and 3-month outcomes (depression, generalized anxiety, COVID-related trauma). Results: Compared to the control, both active SSIs reduced 3-month depressive symptoms (BA: d=.18, p<.001; GM: d=.18, p<.001), reduced post-intervention hopelessness (BA: d=.26, p<.001; GM: d=.30, p<.001), and increased post-intervention perceived agency (BA: d=.36, p<.001; GM: d=.18, p<.001). The GM-SSI, but not the BA-SSI, reduced 3-month generalized anxiety symptoms (d=.10, p=.03) and COVID-related trauma (d=.10, p=.03) versus the control. The BA-SSI outperformed the GM-SSI in strengthening agency (d=.16, p=.001); the GM-SSI outperformed the BA-SSI in reducing generalized anxiety (d=.10, p=.04). Conclusions: Results confirm the effectiveness of two free-of-charge, online SSIs for adolescents experiencing depression, even when delivered in the high-stress context of the COVID-19 pandemic.

Bioinformatics and Computational Biology

Discovering Peptidic Natural Products by Integrating Genome Mining and Computational Mass Spectrometry

Hosein Mohimani, Carnegie Mellon University (poster presenter)

Peptidic natural products (PNP) are a major source of signal molecules and drug leads. The existing techniques for PNP discovery require isolation of bioactive molecules and structure elucidation, which are time consuming and expensive. Recent advances in high-throughput mass spectrometry (MS) and next generation sequencing have resulted in large MS/genomic data sets, which are gold mines for PNP discovery. However, currently there is no efficient algorithm to mine these data sets. We have developed computational tools to integrate MS/genomic data for automated discovery of PNPs from environmental isolates/communities. HypoNPAtlas is a database of hypothetical natural products that is readily searchable against MS. Seq2ripp predicts the structure of ribosomally synthesized and post-translationally modified peptides (RiPPs) from microbial genome. MetaMiner (Cao et al., 2019) and NRPminer integrates MS/genomic data to discover RiPPs and non-ribosomal peptides (NRPs) respectively. MolDiscovery is a probabilistic model that efficiently searches small molecules mass spectra. Association networks (Cao, Shcherbin, and Mohimani, 2019) correlates metagenomic and metabolomic features to discover natural products and biotransformations. These tools have enabled discovery of various novel PNPs from public data sets. One of the NRPs discovered has shown anti-parasite activity.

Cao, L., et al. (2019). MetaMiner: A scalable peptidogenomics approach for discovery of ribosomal peptide natural products with blind modifications from microbial communities. *Cell Systems*, 9, 600-608; Cao, L., Shcherbin, E., and Mohimani, H. (2019). A metabolome- and metagenome-wide association network reveals microbial natural products and microbial biotransformation products from the human microbiota. *mSystems*, 4, e00387-19.

Identifying the Entire Mammalian Micro-Proteome

Israel Pichardo, Harvard Medical School (poster presenter)

There are more than 60 million small open reading frames (smORFs), each of which could encode microproteins smaller than 100 amino acids; yet only few have been well characterized. To date, RNA-seq and ribosome profiling have suggested that a few hundred smORFs might be translated. The lack of ultra-sensitive methods of detection, the ambiguity in interpreting mass spectrometry spectra, and the inherent low correlation between RNA and protein levels, however, have hindered the discovery of novel peptides. We have implemented new computational and experimental pipelines based on advanced proteophylogenomics and a combination of low molecular weight fractionation methods and Focused Asymmetric Ion Mobility Mass Spectrometry (FAIMS) to quantify the smORF-encoded peptides (SEPs). Our software identified more than 600 k candidates conserved across mammals, some comprised within 155 families of smORFs. So far, we have detected 4,577 circulating SEPs with gender- and age-dependent differences in expression when analyzing human plasma and serum from healthy men and women, ranging 20 to 50 years old. The putative microproteins, none of which is redundant to previously annotated genes, show a median size of 51 amino acids, more than

half are predicted to be intergenic, and recurrently display ordered secondary structures (alpha helices, beta sheets or both) and an abundance of positive charges. Several smORFs also show genome wide association study (GWAS) hits that correlate with specific types of cancer, longevity, and other traits and diseases. Some candidates were also found in human and mouse primary cell lines and show dynamic regulation under conditions of senescence or iron deficiency, traits also associated with aging. Work is under way to determine the biological functions of the most interesting hits.

Chemical Biology

Synthetic and Systems Biology of Semi-Synthetic Cells with Expanded DNA Alphabets

Steven Benner, Foundation for Applied Molecular Evolution (poster presenter)

This project is engineering strains of bacteria that replicate, evolve, and use DNA built from six independently replicable nucleotides, an Artificially Expanded Genetic Information System (AEGIS). These strains are “Second Examples of Genetics Undergoing Evolution” (SEGUE). By creating living cells capable of Darwinian evolution based on an artificial molecular biology, this project develops biology away from its descriptive roots. Such “grand challenge” synthesis forces us to ask “Why not?” and “What if?” questions as we solve unscripted problems, driving discovery and paradigm change in ways that hypothesis-based research cannot. For technology, a six-letter DNA alphabet offers 216 codons, allowing substantial expansion of the number of encoded amino acids in proteins. The value of such platforms is adumbrated by the $2.4 billion price paid for Synthorx, which added just one unnatural amino acid using hydrophobic pairs from Floyd Romesberg. SEGUE also avoids biohazards intrinsic in genetically re-coded bacteria, which may have no viruses to control their population. SEGUE will be used to manufacture AEGIS aptamers and aptazymes, to replace antibodies and long-sought catalytic antibodies in medicine. This past year, our deepened understand of DNA based on this work allowed us to release two “best in class” COVID-19 diagnostics and surveillance platforms, now being used in India, Europe, and the United States. The scientific impact of this work in biomedical chemistry is also significant. Synthesis is a demonstration of understanding; “What I cannot make, I do not understand.” To get a functioning SEGUE, we must dissect existing systems biology in living cells, and then make our own. This includes systems behind a metabolism to make AEGIS components, systems to manage and repair genetic information, and systems to regulate the new core molecular biology. In each, we learn volumes about how natural life manages the elements of living.

Engineering Glycan Interactions at the Cell Surface to Control Signaling and Differentiation

Kamil Godula, University of California, San Diego (poster presenter)

Cell surface glycans play critical roles in regulating extracellular signaling in embryonic differentiation and tissue development. Glycosaminoglycan (GAG) polysaccharides displayed on membrane-associated proteoglycans contain highly sulfated regions that serve as high affinity binding sites for a variety of growth factors and chemokines and are required for activation of

cognate receptors at the cell surface. Synthetic materials that mimic the architecture and function of GAGs have recently been the subject of intensive research to establish chemical methods for controlling cellular differentiation in the laboratory. We have developed GAG-mimetic materials based on reactive polyacrylamide scaffolds generated by controlled polymerization techniques (e.g., RAFT) and functionalized with sulfated GAG disaccharide building blocks that show avidity for a broad range of growth factors. When introduced to the surfaces of stem cells, these materials were able to promote growth factor signaling and support cellular differentiation in numerous contexts, including neural, mesodermal, or adipogenic differentiation. I will present the development of this concept toward reprogramming the adipogenic differentiation program to produce adipocytes with enhanced glucose clearance capacity. The change in the metabolic activity of the mature adipocytes was insulin-independent and resulted from a switch from fatty acid metabolism toward glycolysis determined early in differentiation. These findings are poised to open a new exciting avenue for the treatment of type 2 diabetes.

Native Mass Spectrometry of Potassium Channels in Complex with Phospholipids

Arthur Laganowsky, Texas A&M University (poster presenter)

Potassium channels, such as G-protein-gated inward rectifying potassium channels and two-pore domain potassium channel, have numerous physiological roles. These channels are regulated by a wide range of physical and chemical stimuli. I will highlight our work using native mass spectrometry to study membrane protein-lipid interactions. From these studies, we show that potassium channels have distinct binding preferences for lipids dependent on acyl chain length and position on the glycerol backbone. We also discovered a potassium channel that can discriminate the fatty acid linkage at the SN1 position. We also draw a correlation with lipid binding affinity and channel regulation. Our results begin to define the molecular requirements for the specific binding of lipids to potassium channels and open new opportunities to better understand how lipids regulate membrane protein structure and function.

Mining Bacterial Genomes for Synergistic Antibiotics

Bo Li, University of North Carolina at Chapel Hill (poster presenter)

Bacterial natural products account for greater than 70 percent of clinically used antibiotics; however, the overuse of these antibiotics has led to a global health crisis for multidrug-resistant infections. Recent advancements in genome sequencing have revealed that bacteria harbor the ability to produce tens of thousands of natural products that are previously uncharacterized. My group develops genome-mining technologies to identify new natural-product therapeutics—synergistic hybrids and combinations of antibiotics—already evolved and optimized by bacteria against antibiotic resistance. In addition, we discover natural products made by pathogenic bacteria with the goals of understanding virulence and pathogenesis and identifying novel antivirulence targets. Our work has the potential to transform antibiotic discovery and yield combination therapies that could avoid or overcome antibiotic resistance.

Targeting Myc/Max with Hyperstable Synthetic Transcriptional Repressors

Raymond Moellering, The University of Chicago (poster presenter)

Transcription factors (TFs) rely on modular DNA-binding domains to recognize specific DNA sequences and regulate gene expression. Despite unequivocal roles in disease, TFs remain largely untapped as pharmacologic targets because of the challenges in targeting protein-protein and protein-DNA interactions. We report a modular strategy to create hyperstable synthetic transcription factor mimetics derived from the bHLH domain of MAX. We developed a convergent synthetic route to create chemically stabilized tertiary domain mimetics that cooperatively bind the MYC/MAX consensus DNA sequence 5'-CACGTG-3' (E-box) with nanomolar affinity, specificity that is equivalent to full-length TFs and directly competes with MYC/MAX protein for E-box DNA binding. We have further established that vigilant synthetic stabilization of secondary, tertiary, and quaternary structural elements in a lead molecule, STR118, resulted in high thermal and proteolytic stability, intrinsic cell permeability, and distribution of the intact molecule throughout the cytosol and nucleus of cells. Chromatin immunoprecipitation studies confirm that STR118 directly binds E-box-regulated genes that are occupied by MYC and MAX in cells and inhibits MYC-dependent phenotypes in cellular models of MYC-driven B-cell lymphoma. Finally, a co-crystal structure of a STR:DNA complex confirmed its non-natural structure, but retention of DNA recognition analogous to full-length bHLH TFs. These data support the potential for STR mimetics to be further developed as MYC antagonists and programmable agents targeting gene expression in disease.

High-Resolution Chemical Imaging of Cells and Tissues

Lu Wei, California Institute of Technology (poster presenter)

Innovations in high-resolution optical imaging have allowed visualization of nanoscale biological structures and connections. However, super-resolution fluorescence techniques, including both optics-oriented and sample-expansion based, are relatively limited in quantification and throughput especially in tissues from photobleaching or quenching of the fluorophores, and low-efficiency or non-uniform delivery of the probes. I will present our recent efforts to develop a general sample-expansion vibrational imaging strategy for label-free high-resolution (to below 100 nm) chemical imaging in cells and tissues. With further adoption of machine learning training, we successfully obtained label-free, multi-component, and volumetric prediction of nucleus, blood vessels, neuronal cells, and dendrites in complex mouse brain tissues.

Clinical and Translational Research

Swab-Seq: Development of a High-Throughput Platform for Massively Scaled Up SARS-Cov-2 Testing and Sequencing

Valerie Arboleda, David Geffen School of Medicine, UCLA (poster presenter)

The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is due to the high rates of transmission by individuals who are asymptomatic at the time of transmission. Frequent, widespread testing of the asymptomatic population for SARS-CoV-2 is essential to

suppress viral transmission. Despite increases in testing capacity, multiple challenges remain in deploying traditional reverse transcription and quantitative PCR (RT-qPCR) tests at the scale required for population screening of asymptomatic individuals. We have developed SwabSeq, a high-throughput testing platform for SARS-CoV-2 that uses next-generation sequencing as a readout. SwabSeq employs sample-specific molecular barcodes to enable thousands of samples to be combined and simultaneously analyzed for the presence or absence of SARS-CoV-2 in a single run. Importantly, SwabSeq incorporates an in vitro RNA standard that mimics the viral amplicon, but can be distinguished by sequencing. This standard allows for end-point rather than quantitative PCR, improves quantitation, reduces requirements for automation and sample-to-sample normalization, enables purification-free detection, and gives better ability to call true negatives. After setting up SwabSeq in a high-complexity CLIA laboratory, we performed more than 100,000 tests for COVID-19 in less than 2 months, confirming in a real-world setting that SwabSeq inexpensively delivers highly sensitive and specific results at scale, with a turn-around of less than 24 hours. Our clinical laboratory uses SwabSeq to test both nasal and saliva samples without RNA extraction, while maintaining analytical sensitivity comparable to or better than traditional RT-qPCR tests. Moving forward, SwabSeq can rapidly scale up testing to mitigate devastating spread of novel pathogens.

Predictive Analytics for Glaucoma using Data from the All of Us Research Program

Sally Baxter, University of California, San Diego (poster presenter)

Purpose: (1) use *All of Us* (AoU)data to validate a previously published single-center model predicting need for surgery among individuals with glaucoma, (2) train new models using AoU data, and (3) share insights regarding this novel data source for ophthalmic research. Design: Development and evaluation of machine learning models. Methods: Electronic health record data were extracted from AoU for 1,231 adults diagnosed with primary open-angle glaucoma. The single-center model was applied to AoU data for external validation. AoU data were then used to train new models for predicting need for glaucoma surgery using multivariable logistic regression, artificial neural networks, and random forests. Five-fold cross-validation was performed. Model performance was evaluated based on area under the receiver operating characteristic curve (AUC), accuracy, precision and recall. Results: The mean (standard deviation) age of the AoU cohort was 69.1 (10.5) years, with 57.3 percent women and 33.5 percent Black, significantly exceeding representation in the single-center cohort ($p=0.04$ and $p<0.001$, respectively). Of 1,231 participants, 286 (23.2%) needed glaucoma surgery. When applying the single-center model to AoU data, accuracy was 0.69 and AUC was only 0.49. Using AoU data to train new models resulted in superior performance: AUCs ranged from 0.80 (logistic regression) to 0.99 (random forests). Conclusions: Models trained with national AoU data achieved superior performance compared to using single-center data. Although AoU does not currently include ophthalmic imaging, it offers several strengths over similar big-data sources such as claims data. AoU is a promising new data source for ophthalmic research.

Bed Bugs: An Emerging Threat to Public Health?

Zachary DeVries, University of Kentucky (poster presenter)

Bed bugs (Cimex lectularius) are small, hematophagous ectoparasites of mammals commonly found in close proximity to humans. They are a major indoor pest and notoriously difficult to eradicate from homes. Despite their pest status, bed bugs are widely considered to be of limited medical importance because health issues typically relate to either adverse reactions to their bites, or psychological distress during or following an infestation. Recently, bed bugs were found to produce large amounts of histamine in their feces. Because bed bug populations can attain hundreds or thousands of individuals, it is possible that significant amounts of histamine accumulate in infested homes and pose a potential health risk to residents. Our ongoing work is focused on determining how bed bugs produce histamine, how much histamine accumulates in infested homes, and whether exposure to bed bugs or bed bug-produced histamine constitutes a health risk for humans. When evaluated together, these efforts have the potential to identify a new environmental contaminant that has gone undetected and unabated for almost two decades, and additionally determine whether bed bugs pose a health risk to humans. Furthermore, this project lends itself to collaboration, where there are countless opportunities for entomology to interact and engage with those working in public health, exposure science, immunology, psychology, and others, allowing me to take this line of inquiry further than I could on my own.

Building a Sentient Implantable Neurodevice: Early Progress

Brian Litt, University of Pennsylvania (poster presenter)

Introduction: A major limitation of current implantable devices is that they do not provide feedback or inform how a host's behavior affects his or her health. In many disorders, such as epilepsy, a system that could teach patients to reduce seizure risk while teaching devices which electrophysiologic patterns are associated with ill health or increased seizure likelihood would greatly improve quality of life. Methods: We present early progress on the main components of a sentient, implantable, epilepsy management system. We identify a cohort of 12 patients implanted with stereotactic electrodes to localize epileptic seizures. A Hidden Markov Model (HMM) is used to parse intracranial EEG (iEEG) recordings into discrete states based on covariance across channels. An Ecological Momentary Assessment (EMA) delivered via bi-directional text messaging simultaneously samples patient behavior. We develop a BERT-like NLP algorithm to interpret patient texts chronicling activities of daily living and feelings. We are exploring classifiers and learning algorithms to link these streams, so that patient and device can teach each other to optimize health. Results: We are testing each of the above system components separately for reproducibility, ease of use, and computational efficiency in preparation for an inpatient trial of the closed-loop system. Preliminary results suggest such that a trial is feasible, and a finite number of patient-specific states related to behavior and seizure risk can be identified. Conclusion: We are developing a closed loop system that allows implanted neurodevices and patients to communicate, identify, and modulate behavioral and neural state to improve seizure control and quality of life. We plan to initiate a clinical trial to test a first iteration of this system over the next 6 months.

Potentiated JAK-STAT Signaling Upregulates APOL1 Expression in Patient-Derived iPSC-Podocytes

Opeyemi Olabisi, Duke University (poster presenter)

A recent seminar discovery shows that two coding mutations in the Apolipoprotein L1 (APOL1) gene explains 70 percent of excess risk of focal segmental glomerulosclerosis (FSGS) in African Americans who develop FSGS at four times the rate of European Americans. Compared to Blacks with wildtype APOL1 genotype, carriers of homozygote APOL1 mutants have 17-fold higher odds of developing FSGS. However, kidney disease risk of mutant APOL1 is not completely penetrant: only 20 percent of carriers of homozygote APOL1 mutants develop kidney disease in their lifetime. The basis of this incomplete penetrance is unknown. To address this problem, we generated induced pluripotent stem cells (iPSCs) from cases (n=6)—patients with FSGS who also carry high-risk APOL1 genotype—and controls (n=2)—kidney disease-free Black carriers of high-risk APOL1 genotype. The iPSCs were reprogrammed to become podocytes—the glomerular epithelial cells targeted by disease-causing APOL1. Podocytes were treated or not with disease trigger (interferon gamma) followed by transcriptomic analysis. We discovered that interferon gamma induces higher JAK-STAT activity in podocytes of cases, and that this upregulated activity drives higher expression of cytotoxic APOL1 protein, which in turns drives pathogenesis of FSGS. Our findings identify inhibition of JAK-STAT signaling as therapeutic strategy for APOL1-induced FSGS.

Myocardial Infarction Alters Cardiac Nociception in the Inferior Vagal Ganglia: Implications for Parasympathetic Dysfunction in Heart Disease

Marmar Vaseghi, University of California, Los Angeles (poster presenter)

Background: Myocardial infarction (MI) causes pathological remodeling of the autonomic nervous system, which exacerbates heart failure and predisposes to ventricular arrhythmias. These changes, characterized by sympathetic activation and parasympathetic dysfunction (reduced vagal tone), act in concert to increase risk of death. The primary reasons and mechanisms for vagal withdrawal in this setting are currently unknown. Methods: MI was induced percutaneously in pigs. Six-weeks post-MI (n=11) or age-matched normal animals (n=11) underwent functional nodose ganglia neuronal recordings using linear microarray electrodes in-vivo. After median sternotomy neuronal firing in response to changes in preload/afterload and application of epicardial mechanical or nociceptive stimuli (capsaicin, bradykinin, or vertradine) was assessed by spike sorting. In a different set of normal (n=22) and infarcted animals (n=31), nodose ganglia were rapidly excised for comparison of immunohistological changes. Results: Functional recordings of cardiac chemosensitive neurons in normal animals showed expected increased firing rates in response to nociceptive stimuli. Paradoxically, greater numbers of nociceptive neurons and sensitivity of these neurons (defined as absolute changes in firing rates), infarcted animals demonstrated significant decreases in extracellular neuronal firing in response to nociceptive stimuli. Immunohistochemical analysis revealed increased calcitonin gene-related peptide (CGRP) expression, a surrogate for nociceptive neurons, similar to neural recordings. Both CGRP-positive and CGRP-negative neurons demonstrated increased expression of neuromodulators including glial activation and

nitric oxide synthase post-MI. However, only CGRP-positive neurons showed inhibitory neurotransmission as indicated by increased expression of GABA and its enzymes. Conclusions: Our results, for the first time, indicate significant changes in vagal cardiac afferent neurotransmission post-MII. Nociceptive neurotransmission is decreased, potentially through increased autoreceptor mediated inhibition by GABA. As nociceptive signaling through the vagal ganglia is known to increase vagal tone, decreased nociceptive neurotransmission post-MI may play an important role in occurrence of parasympathetic dysfunction.

High-Throughput and Integrative Biology

mDrop-seq: High Throughput Droplet Single-Cell RNA-Seq of Different Fungal Species Reveal Transcriptomic Differences at Single Cell Level in Homogenous Populations

Anindita Basu, The University of Chicago (poster presenter)

Advances in single-cell RNA sequencing (scRNA-seq) have led to better understanding of heterogeneity in gene expression between individual cells. However, technical challenges like tough cell walls and low RNA quantity have prevented similar profiling of microbial species. We developed microbial Drop-seq (mDrop-seq), a high-throughput scRNA-seq technique on single fungal cells. We demonstrate mDrop-seq's applicability on two yeast species—*Saccharomyces cerevisiae*, a popular model organism, and *Candida albicans*, a common opportunistic pathogen. We benchmarked mDrop-seq for sensitivity and specificity and used it to profile 35,109 *S. cerevisiae* cells to detect variation in mRNA levels between cells. As a proof of concept, we quantified expression differences in heat-shocked *S. cerevisiae*. mDrop-seq detected variations at single-cell resolution in the activation of different stress response pathways within seemingly homogenous populations of *S. cerevisiae*. We also used mDrop-seq to profile single *C. albicans* cells, a clinically relevant yeast species with thick cell walls. 39,705 *C. albicans* cells were profiled using mDrop-seq under different conditions, including exposure to fluconazole, a common anti-fungal agent. We noted differential upregulation in stress response and drug target pathways in *C. albicans* cells with interesting changes in cell cycle patterns. Our experiments are the first single-cell RNA-seq of different yeast species at high throughput, demonstrating mDrop-seq as an affordable, easily implementable, and scalable technique in yeasts.

Live-Cell Transcriptomics via Virus-Like Particles

Paul Blainey, The Broad Institute (poster presenter)

Transcriptional information grants insight into the biological states and responses of living systems. However, available measurement approaches are destructive and prevent continuous retrieval and monitoring of transcriptome-wide RNA information from living cells. We overcame this limitation by repurposing the Gag polyprotein from murine leukemia virus (MLV) to package host transcripts and cause their secretion from living cells in virus-like particles (VLPs). With this RNA self-reporting approach, we collected quantitative transcriptome-wide RNA information from human HT1080 and HEK293 cells and iPSCs, preparing RNA-seq libraries that performed similarly to standard RNA-seq libraries produced from cellular lysates. Further, we

engineered Gag with poly(A)-binding domains to enhance RNA detection, as well as orthogonal affinity purification tags to enable multiplexed RNA detection in co-cultures. Finally, we demonstrated that live-cell transcriptome measurements through RNA self-reporting could time-dependent transcriptional state changes in individual samples responding to TNFα stimulation. RNA self-reporting with VLPs enables faithful live-cell, transcriptome-wide measurements from the same biological samples over time using standard RNA-seq library construction and sequencing procedures.

De Novo Gene Birth

Anne-Ruxandra Carvunis, University of Pittsburgh (poster presenter)

What makes each species unique? This question has fascinated philosophers and scientists alike for centuries. The diversity of life is mediated, at least in part, by protein-coding genes whose sequences are unique to a given species or lineage to the exclusion of all others. These genes are considered evolutionarily novel and often give rise to novel traits and adaptations. Novel genes were long thought to evolve exclusively by divergence from ancestral genes, just as new species descend from ancestral ones. However, it has recently become clear that novel genes can evolve de novo from ancestral sequences that previously lacked coding capacities (e.g., intergenic, regulatory or other “non-genic” sequences). Indeed, the genomic revolution revealed the existence of de novo genes in viruses, bacteria, fungi, plants, and animals. In the human genome, de novo genes are highly expressed in the cerebral cortex, suggesting a contribution to improved cognitive ability. Several de novo genes have been associated with cancer, and ongoing studies in my laboratory have uncovered novel candidate de novo genes in the human genome located at or near disease-associated variants currently thought to land in “gene desert” regions. It is becoming increasingly clear that de novo genes mediate the molecular determinants of species-specificity, including human-specific traits and disease mechanisms. Despite far-reaching implications, a concrete biochemical understanding of the evolutionary transition from a non-genic sequence to a functional protein-coding gene is currently lacking for any species. My laboratory is working to elucidate the molecular mechanisms of the extraordinary paradigm of evolutionary innovation that is the phenomenon of de novo gene birth.

Next-Generation Platforms for Antibody Discovery and Engineering

Brandon DeKosky, The University of Kansas (speaker)

Antibody discovery technologies have led to hundreds of clinical-stage and approved biologics, and potent antibodies are accelerating structure-based vaccine designs. However, functional antibody analyses have mostly been restricted to a small number of high-affinity antibodies, providing limited molecular design feedback and obscuring large-scale data features. To address these issues, we recently developed a suite of high-throughput platforms for paired antibody heavy and light chain sequencing and functional analysis, providing a new window to study human antibody development that informs effective antibody discovery and design. We established an in vitro display system to screen natively paired human antibody heavy and light chains for their function and understand infectious disease responses, including against HIV-1

and COVID-19. We are also performing detailed analyses of the potential antibody mutation landscape to understand the critical features of antiviral antibody potency and neutralization breadth. Finally, we will provide new data that suggest a mechanism for MHC-II-based immune personalization, with high relevance to human antibody development and protein drug design. These high-throughput experimental and computational studies are revealing new quantitative principles that shape human antibody immunity and outline several strategies to accelerate protein drug discovery.

Discovering Axonal Regeneration Genes in the Philippine Mouse *M. castaneus*

Noah Denman, University of Minnesota Stem Cell Institute (poster presenter)

Nerve signals are sent from one region of the body to another along axons, each of which can extend for centimeters. It has been a truism in mammals that once axons get laid down in the central nervous system during development, they do not efficiently regenerate after stroke or trauma. However, neurons of the Philippine mouse *Mus castaneus*, a relative of the laboratory European species *M. musculus*, can regenerate axons far beyond those of any other known mouse system. We are mapping the genetic basis of this trait with a high-throughput screen in a cell culture model. Our approach is to mutagenize neurons of the *M. castaneus* x *M. musculus* hybrid background. In this scheme, a disruptive viral insertion at one parent's allele of a given locus uncovers the function of the wild-type allele of the other parent. In assays of axonal damage and extension in many such mutant cells, we screen for the *M. castaneus* alleles that confer a pro-regeneration phenotype. To lay the groundwork for this design, we have developed methods for high-throughput viral mutagenesis of *M. castaneus* x *M. musculus* hybrid stem cells, and sequencing of viral insertion positions; neuronal differentiation from these stem cells; and axonal extension assays in the resulting neuron cultures. We will show results from these approaches and describe our ramp-up toward a sequencing-based axonal regeneration screen at scale.

Highly Multiplexed Spatial Mapping of Microbial Communities

Iwijn De Vlaminck, Cornell University (poster presenter)

Mapping the complex biogeography of microbial communities in situ with high taxonomic and spatial resolution poses a major challenge because of the high density and rich diversity of species in environmental microbiomes and the limitations of optical imaging technology. In this presentation, I will discuss High Phylogenetic Resolution microbiome mapping by Fluorescence in situ Hybridization (HiPR-FISH), a versatile technology developed with the support of a DP2 award that uses binary encoding, spectral imaging, and machine learning-based decoding to create micron-scale maps of the locations and identities of hundreds of microbial species in complex communities. We have demonstrated the ability of 10-bit HiPR-FISH to distinguish 1023 *E. coli* isolates, each fluorescently labeled with a unique binary barcode. HiPR-FISH, in conjunction with custom algorithms for automated probe design and single-cell image analysis, revealed the disruption of spatial networks in the mouse gut microbiome in response to antibiotic treatment and the longitudinal stability of spatial architectures in the human oral plaque microbiome. Combined with super-resolution imaging, HiPR-FISH revealed the diverse

ribosome organization strategies of human oral microbial taxa. HiPR-FISH provides a framework for analyzing the spatial ecology of environmental microbial communities at single-cell resolution.

Writing and Erasing Epigenetic Memories

Luke Gilbert, University of California, San Francisco (poster presenter)

A general approach for heritably altering gene expression has the potential to enable many discovery and therapeutic efforts. Here, we present CRISPRoff—a programmable epigenetic memory writer consisting of a single dead Cas9 fusion protein that establishes DNA methylation and repressive histone modifications. Transient CRISPRoff expression initiates highly specific DNA methylation and gene repression that is maintained through cell division and differentiation of stem cells to neurons. Pairing CRISPRoff with genome-wide screens and analysis of chromatin marks establishes rules for heritable gene silencing. We identify sgRNAs capable of silencing the large majority of genes including those lacking canonical CpG islands (CGIs) and reveal a wide targeting window extending beyond annotated CGIs. The broad ability of CRISPRoff to initiate heritable gene silencing even outside of CGIs expands the canonical model of methylation-based silencing and enables diverse applications including genome-wide screens, multiplexed cell engineering, enhancer silencing, and mechanistic exploration of epigenetic inheritance.

Dissecting Myeloid-Dependent Signaling Dynamics in the Tumor Microenvironment

Miles Miller, Massachusetts General Hospital (poster presenter)

An expanding atlas of single-cell organization in tumor tissue offers insights into how malignant and stromal cells may communicate with each other to influence disease progression. Nonetheless, it remains difficult to understand functional cause-and-effect relationships between cells from static maps of tissue composition. To overcome this limitation, we have developed strategies for high-resolution in vivo confocal microscopy to directly monitor in situ signaling dynamics and perturbation in live tumor models. We present recent progress in applying this approach to study the spatially-dependent activities of multiple mitogen activated protein kinase (MAPK) pathways, using mouse models of cancer driven by constitutively activating BRAF or KRAS mutations. BRAF mutation is especially common in malignant melanoma, and targeted kinase inhibitors have been developed to inhibit BRAF and downstream ERK signaling activities. However, drug resistance frequently emerges in patients, and new treatment strategies are urgently needed. This work builds on a series of collaborative studies, in which our team found that efficient MAPK-ERK kinase inhibition and resultant cancer cell killing could elicit an immunogenic wound-healing response in tumors, leading to recruitment of innate immune cells (namely macrophages) into the tumor microenvironment. Time-lapse microscopy in mice revealed that macrophages could limit the ability of MAPK-ERK targeted kinase inhibitor to block signaling in adjacent tumor cells, in a highly localized manner. Further analysis implicated bi-directional signaling between tumor cells and macrophages through a family of immunosuppressive receptors involved in clearing dead cell debris from the body. These results helped demonstrate how a feedback of reciprocal tumor-immune signaling

can locally amplify kinase inhibitor resistance in the tumor microenvironment. Furthermore, they set the stage for future work on this project to dissect spatial regulatory relationships and potential therapeutic avenues by locally manipulating immune signaling dynamics.

Combining Population Genomics, Single-Cell Sequencing, and Electrophysiology to Uncover the Genetic Basis of Cognitive Evolution

Michael Sheehan, Cornell University (poster presenter)

How do novel behaviors evolve? What aspects of DNA are changed to influence neural circuits and behavior? We have been investigating these questions in a unique model organism—the northern paper wasp. These wasps have recently evolved individual facial recognition, which they use to mediate conflict on their nests. Using a combination of population genomics, single cell sequencing, and electrophysiology, we are honing in on the changes that have allowed this impressive behavior in a tiny insect brain. This poster provides details on our genomics work and an overview of ongoing work on electrophysiology. Through this work, we aim to uncover basic principles about how the brain and behavior evolve.

Single-Molecule Chromatin Fiber Sequencing Exposes Cell and Haplotype-Specific Chromatin Architectures

Andrew Stergachis, University of Washington (poster presenter)

Gene regulation is chiefly determined at the level of individual chromatin fibers. However, our current understanding of the cis-regulatory architecture of humans is almost entirely derived from the fragmented sampling of large numbers of disparate chromatin fibers across individual cells or bulk tissue. To develop a single-molecule understanding of human gene regulation, we have pioneered an approach for precisely stenciling the structure of individual chromatin fibers onto their underlying DNA templates using non-specific DNA N6-adenine methyltransferases. Single-molecule long-read sequencing of these chromatin stencils enables the nucleotide-precise readout of the primary architecture of multi-kilobase chromatin fibers (Fiber-seq). Fiber-seq exposes widespread plasticity in the linear organization of individual chromatin fibers, and illuminates principles guiding regulatory DNA actuation, single-molecule transcription factor occupancy, and the coordinated actuation of neighboring regulatory elements along individual single-molecule chromatin fibers. Finally, application of Fiber-seq to human primary cells enables the simultaneous mapping of both the primary genetic and epigenetic states of individual regulatory alleles—directly exposing the functional impact of both rare and common regulatory DNA variation. Overall, single-molecule chromatin fiber sequencing opens new vistas on the primary architecture of human gene regulation.

Decoding RNA Dynamics in Single Cells with Time-resolved Metabolic RNA Sequencing

Hao Wu, University of Pennsylvania Perelman School of Medicine (poster presenter)

Single-cell RNA sequencing offers snapshots of whole transcriptomes but obscures the temporal RNA dynamics. Here we present single-cell metabolically labeled new RNA tagging sequencing (scNT-Seq), a method for massively parallel analysis of newly-transcribed (“new”)

and pre-existing ("old") mRNAs from the same cell. This droplet microfluidics-based method enables high-throughput chemical conversion on barcoded beads, efficiently marking newly-transcribed mRNAs with T-to-C substitutions. Using scNT-Seq, we jointly profiled new and old transcriptomes in tens of thousands of mouse primary cortical cells. These data revealed distinct patterns of newly synthesized mRNAs at single-cell level in response to brief or sustained neuronal activation. We further showed that measuring new RNA levels of target genes linked to a neuronal activity regulated transcription factor (TF) can temporally resolve TF regulatory network activity in single neurons. Using a novel computational model that explicitly incorporates metabolic labeling-based single-cell measurements, we computed time-resolved RNA velocity to infer cell state trajectories during the highly dynamic neuronal activation process (minutes to hours). Finally, with pulse-chase experiments, scNT-Seq can more accurately estimate RNA synthesis and degradation rates, revealing RNA regulatory strategies in rare cell populations. High-throughput time-resolved single-cell transcriptomics thus provides a broadly applicable strategy to investigate cell type–specific RNA regulatory mechanisms in dynamic biological processes.

High-Throughput Gnotobiotics: Dissecting the Genetics and Genomics of Microbiome Form and Function in *C. Elegans*

Fan Zhang, Baylor College of Medicine (poster presenter)

The gut microbiome extends the capabilities of its host and alters its physiology. Together with diet, host genetic landscapes shape microbiome form and function in the animal gut. Despite its importance, the essential functions that drive microbiome assembly and stability in remain largely elusive. To address this challenge, we leverage the nematode *Caenorhabditis elegans* to explore how microbiomes assemble in different host genetic backgrounds. This system has several advantages, including (1) a simple microbiome that can be rapidly removed (bleaching) and replaced in high-throughput gnotobiotic experiments; (2) highly conserved intestinal physiology, metabolism, and innate immunity; and (3) shared microbial functions for host gut persistence. To examine the natural variation in acquisition of the microbiome in *C. elegans*, we first established the natural core microbiome and assembled a functionally redundant, model core microbiome of bacteria (BIGbiome). Then *C. elegans* wild strains (38) were made "germ-free" and colonized with BIGbiome to assess strain-level microbiome composition (16S) and levels (CFU) longitudinally using a high-throughput pipeline. The strains clustered into three groups: (1) a highly-selective group that differed greatest from the surrounding environment [Ochrobactrum-dominant]; (2) a "dysbiotic" group [Bacteroidetes-dominant]; and (3) a "non-selective" group. By GWAS-, RNAi-, and RNAseq-based approaches, we identified ~1,000 candidate regulators in highly conserved pathways (>60%). Insulin signaling pathways specifically regulate Ochrobactrum colonization, as impaired daf-2/IGFR signaling (mutants or RNAi) limits its colonization. Last, we next sought to examine alterations in microbiome function. To do this, we sequenced and annotated >100 bacterial genomes. Virtual metagenomes for each *C. elegans* microbiome indicate broad microbiome functions are shared, but also point to many emergent functions among the three host groups. Our study highlights the potential for a robust platform to identify conserved host and microbial determinants that may underlie assembly and stability of the microbiome.

Decoding the Function and Regulation of the Mammalian 12h-Clock

Bokai Zhu, University of Pittsburgh (poster presenter)

Our understandings of biological rhythms have recently expanded beyond the well-characterized ~24h circadian rhythms through our group's recent discovery of a mammalian 12h-clock regulating 12h rhythms of gene expression and metabolism. The mammalian 12h-clock is evolutionarily conserved (likely evolved from the circatidal clock of marine animals), cell-autonomous, and established independently from the circadian clock. However, the exact prevalence, function, and regulation of the 12h-clock are still poorly defined. I recently uncovered widespread 12h transcriptome in mouse BAT, WAT, liver, adrenal gland, skeletal muscle, lung, and aorta, with distinct phase and amplitudes signatures observed in these tissues. I further proposed a tripartite regulatory network comprising of E26 transformation-specific (ETS), Basic Leucine Zipper Domain (bZIP)-containing and Nuclear transcription factor Y (NFY) family of transcription factors (TF) that are responsible for the transcriptional regulation of mammalian 12h-clock, which mainly functions as a vehicle regulating the capacity of genetic information flow from DNA to proteins. Lending further evidence to the vehicle-cargo hypothesis are our recent findings that 12h-clock controls 12h rhythms of nuclear speckle liquid-liquid phase separation dynamics, which in turns leads to 12h rhythmic nuclear speckle spatial chromatin recruitment and gene expression control. Owing to the early stage of the 12h rhythm field, little is currently known about the definitive functions of 12h rhythms in mammals. However, new evidence supports the notion that the endogenous mRNA surveillance and protein quality control systems cycle with a 12h period. I hypothesize that multiple stress conditions can perturb this system from its normal 12h cycle, and if uncorrected, will contribute to increased disease susceptibility. Thus, the identification of novel regulators of the mammalian 12h-clock will most likely uncover new players implicated in stress responses and stress-associated pathologies.

Infectious Diseases and Immunology

Signal Dissemination in the Airway Immune Response

Sam Allon, MIT (poster presenter)

Cascades of communication between neighboring cells underlie some of the most important immune responses in our body. Our field lacks the tools for rapid and systematic discovery of these cascades, and as a result, most of them remain unknown. We have accelerated the discovery and characterization of immune signal dissemination (a three-step cascade of cell-cell communication) in the airway epithelium, the central tissue in immune responses as disparate as asthma attacks and defense against the pandemic coronavirus. We have developed an in vitro platform for rapid and systematic discovery of signal dissemination events, their molecular mechanisms, and their operational role in intercellular circuits. Starting from antiviral immunity in the airways, our advances could find broad application in a variety of airway immune responses as well as in other tissues amenable to in vitro study.

Yellow Fever Is a Consumptive Coagulopathy

Adam Bailey, University of Wisconsin-Madison (poster presenter)

Yellow fever (YF) is a mosquito-transmitted viral disease that causes tens of thousands of deaths each year in endemic areas and threatens to spread into non-endemic areas as climate change intensifies. In its most severe form, YF manifests as a hemorrhagic fever that causes severe damage to visceral organs. Although coagulopathy is a defining feature of severe YF in humans, the mechanism by which it develops remains poorly understood. As hepatocytes are a major target of yellow fever virus (YFV) infection, coagulopathy in severe YF has been attributed to massive hepatocyte infection and destruction that results in a defect in clotting factor synthesis. However, when we analyzed blood from Brazilian patients with severe YF, we found high concentrations of plasma D-dimer, a fibrin split product, which suggests that a consumptive process also contributes to YF coagulopathy. To define the relationship between coagulopathy, hepatocellular tropism, and tissue damage, we compared infection and disease in mice engrafted with human hepatocytes (hFRG mice) and rhesus macaques using a highly pathogenic African YFV strain. YFV infection of macaques and hFRG mice caused substantial hepatocyte infection, liver damage, and coagulopathy as defined by virological, clinical, and pathological criteria. However, only macaques developed a consumptive coagulopathy whereas YFV-infected hFRG mice did not. Thus, infection of cell types other than hepatocytes likely contributes to the consumptive coagulopathy associated with severe YF in primates and humans. These findings expand our understanding of viral hemorrhagic fever and suggest directions for clinical management of severe YF cases.

Role of Innate Immune Dysregulation in the Etiology of Dementia

Annelise E. Barron, Stanford University (poster presenter)

I will investigate a new hypothesis for the etiology of sporadic Alzheimer's disease (AD): that an imbalance of two innate immune peptides may be a key factor modulating the risk of formation, stability, and clearance of AD-associated fibrils and plaques. Human cathelicidin LL-37 is an antiviral, antibacterial host defense peptide deployed by microglia, macrophages, endothelial, T and NK cells. LL-37 is required for immune clearance of bacterial and viral pathogens and infected host cells. Its Vitamin D3-, RXR-agonist-, and butyrate-dependent expression is also stimulated by infection, wounding, exercise, and some vaccines (e.g., BCG vaccine). Certain pathogens, *P. gingivalis* in particular, release virulence factors that degrade LL-37, dysregulating innate immunity. In LL-37's absence, macroautophagy is crippled. Like LL-37, AD-associated peptide Abeta is also a host defense peptide. Brain infections by Herpesviridae or *P. gingivalis* stimulate Abeta production and its accumulation in plaques that co-locate with pathogens. I and collaborators showed that LL-37 and Abeta are expressed in human brains, and bind to each other sequence-specifically. LL-37/Abeta binding prevents Abeta fibrillization. LL-37 degradation thus may allow Abeta to accumulate. Our preliminary in vivo studies show that cathelicidin induction in 5XFAD mice slows AD progression and improves 5XFAD cognition to match wild-type. In this Pioneer project, I aim to tie this finding to infection-associated dementia. Murine studies will be used to demonstrate that degradation of LL-37 by *P. gingivalis* virulence factors may cause brain degradation leading to dementia, which in turn may be

prevented by early-life upregulation of cathelicidin. We will also work to solve the high-resolution structure of the LL-37/ABeta complex.

Cell-to-Cell Transfer of Mitochondria from Adipocytes to Macrophages Regulates White Adipose Tissue Homeostasis and Is Impaired in Obesity

Jonathan Brestoff, Department of Pathology and Immunology, Washington University School of Medicine (poster presenter)

Obesity is characterized by white adipose tissue (WAT) hypertrophy, chronic inflammation, and mitochondrial dysfunction. Macrophages in WAT regulate metabolism by promoting glucose utilization, regulating lipid storage, and increasing energy expenditure. These beneficial immunometabolic processes become dysregulated in obesity through unclear mechanisms. Recent studies suggest that mitochondria can be transferred between cells in the contexts of stroke and cancer to support the survival and proliferation of metabolically compromised cells. However, whether intercellular mitochondria transfer occurs in WAT or regulates metabolic homeostasis in vivo remains unknown. Here, we employ bone marrow transplants and adipocyte-specific mitochondria reporter (MitoFat) mice to demonstrate that macrophages acquire mitochondria from neighboring adipocytes in vivo and that this process defines a transcriptionally distinct macrophage subpopulation. A genome-wide CRISPR-Cas9 knockout screen in a BV2 myeloid cell line revealed that mitochondria uptake depends on the heparan sulfate (HS) biosynthetic pathway, including the gene Exostosin 1 (Ext1), which has been linked to the regulation of lipid metabolism in mice and humans. We found that high fat diet (HFD)-induced obese mice exhibit lower levels of HS on WAT macrophages and markedly decreased intercellular mitochondria transfer from adipocytes to macrophages. Although interferon (IFN)-γ and lipopolysaccharide (LPS) increase the ability of BV2 cells to perform phagocytosis, they substantially decrease mitochondria uptake and downregulate expression of HS biosynthesis genes. Deletion of Ext1 in myeloid cells reduces WAT macrophage HS levels, deceases mitochondria uptake by WAT macrophages, increases WAT mass, lowers energy expenditure, and exacerbates HFD-induced obesity in vivo. Collectively, these findings suggest that adipocytes and macrophages employ intercellular mitochondria transfer as a mechanism of immunometabolic crosstalk that regulates metabolic homeostasis and that is impaired in murine obesity.

Phage-Based Nanomaterials for Antibacterial Therapy

Irene Chen, University of California, Los Angeles (speaker)

While phages have long been considered as potential antibacterial agents, many concerns about phage therapy stem from the fact that phages are replicating, evolvable entities whose biology is often poorly understood. Our goal is to transform phages from an evolving biological entity into a controlled, drug-like reagent. I will describe our research developing phage-based nanomaterials to control the targeting, phenotype, and biosafety of the phages for antibacterial therapy. Further reading: https://doi.org/10.1073/pnas.1913234117

Phosphorothioate Epigenetics in the Human Gut Microbiome

Peter Dedon, Massachusetts Institute of Technology (poster presenter)

Phosphorothioation (PT) is a DNA backbone modification with sulfur replacing a non-bridging phosphate oxygen. As widespread epigenetic marks in bacteria and archaea, PTs are inserted by dnd and ssp genes. Here we show that an abundance of mouse and human gut microbes contain PTs. The discovery of PTs in the microbiome arose in mass spectrometric assays of PT-containing dinucleotides in a limit nuclease digest of fecal DNA. We found 11 of 16 possible dinucleotides in mice and humans. Mice showed more dinucleotide contexts as well as striking similarity and stability among cage mates, while humans showed fewer dinucleotide contexts and significant inter-individual diversity. Further, humans show time-dependent changes in the spectrum and quantities of PTs. We next mined sequenced genomes of ~9,000 bacterial isolates for homologs of dnd and ssp genes, finding ~8 percent of strains containing PT genes. We then used a technique involving iodine-induced cleavage of PTs followed by poly-T tailing at strand breaks to prepare libraries for NGS. Metagenomic alignment of sequencing data revealed a strain distribution similar to isolate genomes. Further, we observed 10 PT motifs: CpsCA from Bacteroidales, GpsAAC/GpsTTC from Enterobacterales, CpsAG and CpsCTC from Bacteroidales, GpsAGC/GpsCTC and CpsCTG from Clostridiales, and GpsTAC and GpsATC from Enterobacterales. PTs were found to be evenly distributed among genetic and intergenic regions in metagenome assembled genomes. Sequenced bacterial isolates, fecal DNA metagenomics, and LC-MS PT analyses showed remarkable epigenetic correlations in the gut microbiome. While much more analysis across larger populations will define the determinants of the PT-possessing microbiome, the redox-active and nucleophilic PT sulfur is known to affect the bacterial fitness during oxidative stress. Future studies will address the effects of inflammatory bowel disease on PT epigenetics in the gut microbiome.

Metabolic Symbiosis between Regulatory T cells and the Tumor Microenvironment

Greg Delgoffe, University of Pittsburgh (poster presenter)

Immunotherapy has changed the treatment paradigm of cancer, but response rates remain low due to several immunosuppressive mechanisms present within the tumor microenvironment. Two major contributors to the tolerogenic environment in cancer are the overrepresentation of suppressive, regulatory T (Treg) cells and the generation of a metabolically dearth landscape. Indeed, we and others have shown that tumor-specific, cytotoxic T cells are rendered metabolically insufficient upon exposure to the tumor microenvironment. Given that regulatory and conventional T cells have distinct metabolic requirements, we hypothesized that the tumor microenvironment may have a metabolic profile that allows regulatory T cells to thrive. We show that regulatory T cells thrive in metabolically active tumors, especially those that deregulate their glucose metabolism. Indeed, the majority of regulatory T cells do not readily take up glucose, and those few that do are poorly suppressive. Rather, regulatory T cells upregulate a transcriptional and metabolic program allowing them to take up and metabolize lactic acid, a byproduct of glucose fermentation that is enriched in tumors. Metabolic flux analysis revealed Treg cells utilize lactate to produce intermediates that support proliferation and suppressor function. Deletion of the lactate transporter MCT1 specifically on Treg cells did

not overtly affect their survival or function in peripheral immune organs and in normal tissues. However, MCT1-deficient Treg cells fail to thrive in tumor microenvironments, resulting in a more inflammatory milieu. Notably, MCT1 deletion on Treg cells synergized with PD-1 blockade in resistant models. Our data highlight that regulatory T cells have a phenotype of metabolic plasticity allowing them to thrive on alternative fuels present in normal and transformed tissues. Further, our study suggest cancer cells evade the immune response not only by starving tumor-specific conventional T cells, but also by feeding immunoregulatory populations.

Immunotherapy Directed against Skin Cancer Precursors Prevents Skin Cancer

Shawn Demehri, Massachusetts General Hospital (speaker)

Skin cancer is the most common type of cancer. Although ultraviolet radiation is its preventable risk factor, the incidence of skin cancer, including squamous cell carcinoma (SCC), has doubled over the last decade in the United States. Besides morbidity and mortality associated with skin cancer, skin cancer treatments represent a rising public health challenge with increasing complications and rising costs. Therefore, skin cancer prevention is urgently needed. To accomplish this goal, we developed topical calcipotriol plus 5-fluorouracil (5-FU) immunotherapy that effectively eliminated SCC precursors called actinic keratosis in a randomized double-blind clinical trial. Since, we performed a blinded prospective cohort study on its participants in order to determine the long-term effectiveness of calcipotriol plus 5-FU treatment for SCC prevention. Calcipotriol plus 5-FU combination induced tissue-resident memory T (TRM) cell formation in face and scalp skin, which was associated with significantly higher erythema scores compared to control groups (Vaseline plus 5-FU, $p<0.01$). Importantly, more participants in the test cohort remained SCC-free over the >1,500-day follow-up period, and significantly fewer developed SCC on the treated face and scalp within 3 years (2 of 30 [7%] versus 11 of 40 [28%] in control group, hazard ratio 0.215 [95% CI: 0.048-0.972], $p=0.032$). Interestingly, we found more epidermal TRM cells persisting in the calcipotriol plus 5-FU-treated face and scalp skin compared with control months to years post treatment ($p=0.0028$). Our findings demonstrate that a short course of a topical immunotherapy that induces T cell immunity and effectively eliminates actinic keratosis can significantly lower the long-term risk of SCC. Our research substantiates a previously unrecognized concept that immunotherapy against premalignant lesions can be used to prevent cancer development and recurrence in high-risk patients.

Structural Parasitology of the Malaria Parasite Plasmodium falciparum

Chi-Min Ho, Columbia University Irving Medical Center (poster presenter)

While most intracellular pathogens export a limited repertoire of effector proteins to co-opt existing host-cell metabolic machineries, the Malaria-causing parasite *Plasmodium falciparum* exports greater than 10 percent of its proteome into host human red blood cells, which are highly specialized for carrying hemoglobin and lack the resources to support the active growth and replication of the parasites. The hundreds of proteins in the *P. falciparum* exportome extensively remodel host erythrocytes, creating the infrastructure needed to import nutrients, export waste, and evade the host immune system. The complexity and breadth of its host-cell

remodeling machinery make *P. falciparum* a rich and exciting system for the study of host-pathogen interfaces. Unfortunately, many of the molecular mechanisms underlying this parasite's ability to hijack human red blood cells remain enigmatic, as much of the *P. falciparum* proteome has proven recalcitrant to structural and biochemical characterization using traditional recombinant approaches. This paucity of high-resolution structural and functional information is compounded by the fact that 50 percent of the *P. falciparum* proteome is novel. To overcome these barriers to structural study of malaria parasites and address the gaps in our understanding of the molecular mechanisms underpinning host-pathogen interactions in parasite-infected red blood cells, our lab develops and implements methodologies for endogenous structure determination from *P. falciparum*. We combine CRISPR-Cas9 parasite gene editing, single particle cryoelectron microscopy (cryoEM), and in situ cryoelectron tomography (cryoET) to determine near-atomic resolution structures of previously intractable protein complexes enriched directly from endogenous *P. falciparum* parasites and directly visualize the host-pathogen interface in intact parasite-infected red blood cells at sub-nanometer resolutions.

Biological Rhythms in Human Health and Infectious Disease

Micaela Martinez, Columbia University Irving Medical Center (speaker)

Biological clocks have evolved on earth in order for organisms to cope with the day-night cycle, lunar cycles, and seasons. I will discuss my lab's research on circadian and seasonal biological rhythms in humans, which has been funded by the NIH Early Independence Award. I will also highlight how we are using the study of infectious disease seasonality and rhythms in the immune system to better understand the transmission of epidemic-prone diseases, inform public health interventions, and develop personalized Darwinian medicine.

Discovery of a New Cue Used by Mosquito Disease Vectors to Find People

Craig Montell, University of California, Santa Barbara (speaker)

Mosquitoes are the most dangerous animal in the world. Diseases spread by one invasive mosquito, *Aedes (Ae) aegypti*, are on the rise and afflict ~400 million people each year. Female Ae. aegypti depend on multiple sensory stimuli such as vision, CO_2, and organic compounds to zero in on humans for blood meals. Over decades, no other cues have been found that mosquitoes use to target people. We discovered that infrared (IR) radiation emanating from people is sensed by *Ae. aegypti* and used as part of their sensory detection arsenal to locate human hosts. The ability to detect IR radiation is dependent in part on the TRPA1 channel. Remarkably, two opsins, which were formerly thought to function exclusively in light sensation are also required for IR detection. We propose that the discovery of IR radiation as a new type of attractive sensory cue used by mosquitoes will enable the development of innovative approaches to limit their ability to locate people, and to devise better mosquito traps to control mosquito populations.

Toward Imaging Bacterial Infection in Humans

Mark Sellmyer, University of Pennsylvania (poster presenter)

In recent years several molecular imaging strategies have been developed to image bacteria in human patients. Nuclear approaches, specifically positron emission tomography (PET), affords both sensitive detection and the ability to non-invasively capture infections deep within the body. Two key radiotracer classes have risen to the forefront, including metabolic approaches, which target bacterial specific biochemical transformations, and antibiotics, which have inherent selectivity for bacteria over mammalian cells. One critical question regarding antibiotic radiotracer clinical application is whether resistance to the antibiotics would abrogate any specific uptake, thus diminishing important features of such a diagnostic test, including the predictive values. We recently developed small molecule PET radiotracers based on the synthetic antibiotic trimethoprim [11C/18F]-TMP and have shown selectivity of these radiotracers for imaging bacteria over other etiologies such as inflammation and cancer in preclinical models. Here, we tested the in vitro uptake of [11C]-TMP in susceptible bacteria and drug-resistant clinical isolates, which are frequent causes of human infection (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*). Both TMP resistant and sensitive bacteria showed similar in vitro uptake, which was surprising. This led us to perform whole genome sequencing of these isolates, identifying the mechanisms of TMP resistance that did not impact radiotracer uptake, and look more broadly into known and annotated whole genome alignments where, despite the designation of TMP resistance, uptake of TMP radiotracers could be putatively maintained by the presence of the target native protein bacterial DHFR. Finally, we present several case vignettes of patients who have both TMP sensitive and drug-resistant infections in our first-in-human experience with [11C]-TMP. This work underscores the ability of select antibiotic radiotracers to image human infection, which may affect our understanding of human bacterial pathogenesis, infection diagnosis, and antimicrobial drug response.

Systemic Dysfunction and Plasticity of the Immune Macroenvironment in Cancer

Matthew Spitzer, University of California, San Francisco (poster presenter)

Understanding of the factors governing immune responses in cancer remains incomplete, limiting patient benefit. In this study, we used mass cytometry to define the systemic immune landscape in response to tumor development across five tissues in eight mouse tumor models. Systemic immunity was dramatically altered across models and time, with consistent findings in the peripheral blood of patients with breast cancer. Changes in peripheral tissues differed from those in the tumor microenvironment. Mice with tumor-experienced immune systems mounted dampened responses to orthogonal challenges, including reduced T cell activation during viral or bacterial infection. Antigen-presenting cells (APCs) mounted weaker responses in this context, whereas promoting APC activation rescued T cell activity. Systemic immune changes were reversed with surgical tumor resection, and many were prevented by interleukin-1 or granulocyte colony-stimulating factor blockade, revealing remarkable plasticity in the systemic immune state. These results demonstrate that tumor development dynamically reshapes the composition and function of the immune macroenvironment.

Single-Cell RNA Sequencing Reveals Cell States and Transcriptional Signatures of Immune and Epithelial Dysregulation That Define Colitis Associated with Immune Checkpoint Inhibitors

Alexandra-Chloe Villani, Massachusetts General Hospital/Harvard Medical School (poster presenter)

Antibodies targeting immune checkpoint inhibitors CTLA-4 and PD-1/PD-L1 have revolutionized the treatment of solid tumors. However, their use is limited by a high incidence of immune-related adverse events (irAEs) affecting all organs, which can mimic autoimmune disease presentations. Beyond improving cancer patient care, studying irAEs represents a unique opportunity to study immune response regulation directly in human subjects. The colon is a frequent target of this immune attack, seen in up to 45 percent of patients on dual PD-1 and CTLA-4 blockade (referred to irColitis). To deeply define cell populations that could be tracked for diagnosis and characterize cell states and transcriptional programs driving irAEs that could therapeutically be manipulated to resolve irAEs, we profiled ~200,000 epithelial, mesenchymal, and immune single cells by RNA sequencing with paired TCR and BCR sequencing from the colon mucosa and blood of irColitis patients on ICI therapy (n=13) and controls that were either healthy (n=8) or on ICI therapy (n=6). Analysis of tissue immune cells from irColitis patients revealed marked expansion of T regulatory cells, effector CD4+ T cells, and three transcriptionally-distinct populations of CD8+ T cells. Single-cell TCR and BCR profiling revealed polyclonal expansion of T and B cell subsets with unique transcriptional signatures. We further showed that tissue resident T cells can transition between different cell states during active irColitis based on the observation of identical TCRs in CD8+ T cells from cytotoxic effector and memory-like cells. scRNAseq analysis of colon epithelial and mesenchymal cells revealed strong inflammatory and interferon signatures, likely driving the tissue damage observed in irColitis patients. Together, these data highlight the molecular mechanisms driving irColitis that may apply to other irAEs and shed light on the specific role of CTLA-4 and PD-1 signaling in maintaining gastrointestinal immune tolerance.

Developmental Retrotransposon Activation Primes Host Immunity for Future Viral-Clearance

Zhao ZZ Zhang, Duke University (poster presenter)

As one type of transposable elements in the host genome, endogenous retroviruses (ERVs) are the relics of viruses that invaded the hosts in the past. Their uncontrolled activation is linked to sterility, cancer, and other pathological conditions, thereby being largely considered detrimental. Here we report within a strictly defined time window of development, ERV activation can license the host's immune system for future anti-viral responses. We found the Gypsy ERV selectively becomes active during metamorphosis at the Drosophila pupal stage. At this stage, Gypsy activation primes the host's innate immune system by inducing the systemic anti-viral function of the NF-kB protein, Relish. Consequently, adult flies with Gypsy or Relish silenced at the pupal stage are unable to clear exogenous viruses and succumb to viral infection. Altogether, our data reveal that programed activation of ERVs during development endows a long-term benefit in pathogen warfare.

Instrumentation and Engineering

INSITE: Implantable Nanophotonic Sensors for the Immune Tumor microEnvironment

Mekhail Anwar, University of California, San Francisco (poster presenter)

Fluorescence microscopy is instrumental in the lab for cellular visualization of immune activity and disease progression, but is yet to be deployed inside the body. A critical application is cancer immunotherapy, a game-changing therapeutic harnessing the immune system to attack cancer, but which is effective in less than 50 percent of patients. The real-time intratumoral response to therapy in patients remains unknown, and as a result, many patients remain on ineffective therapies for months, missing the cure window while incurring needless toxicity. To provide unprecedented real-time and detailed monitoring of tumor response monitoring both activating and immune suppressing components, we propose a "wireless biopsy," leveraging innovations in optics and CMOS technology to personalize treatment based on the individual patient response. The prototype sensor is under development. Our system aims to provide state-of-the-art fluorescence microscopy-cellular-level resolution, multi-color sensing of different cell types, and 3D image reconstruction—on a millimeter-scale platform compatible with long-term implantation in the body. This requires several key technological innovations: integration of the light source and all optical components on chip, wireless data transmission and power delivery, and advanced image processing techniques. Compact, lens-free imaging is achieved using a custom CMOS image sensor with an integrated micro-laser diode that images tissue directly in contact with its surface. To replace conventional optics, we demonstrate a novel planar filter structure that combines thin-film interference filters with micro-collimators to simultaneously deliver excitation rejection, high resolution, and multi-color sensing. Inspired by current medical implants, an ultrasound power and data communication link is utilized to minimize tissue attenuation, maximize power transfer density and improve robustness for backscattering significant amounts of image data. Additional processing for image enhancement and 3D depth estimation is implemented in software using convolutional neural network models.

High Speed Multi-Channel Imaging of Neural Activity

Yiyang Gong, Duke University (poster presenter)

Understanding the interaction between the activity from different types of neurons requires a tool to simultaneously record the dynamics of multiple genetically targeted cell types. We have developed multiple components of a multi-channel voltage imaging platform. We have developed a red-fluorescent genetically encoded voltage indicator to complement existing green-fluorescent voltage indicators. These indicators could enable spectrally separable recordings of multiple neuron populations simultaneously. We have developed a temporal-multiplexed multi-channel fiber photometry system. This system can independently measure multiple channels of fluorescence indicators with high temporal resolution. We evaluate the fidelity of recordings using these components by comparing the imaging noise structure to the shot-noise limited noise structure.

An Acoustofluidic Avidity Cytometer for Detecting Clinical Multiple Sclerosis

Feng Guo, Indiana University Bloomington (poster presenter)

Multiple sclerosis, the most common of these diseases affecting more than 2 million people worldwide and approximately 400,000 people in the United States, is a chronic inflammatory disease, causing lesions and plaques of demyelination in the brain and spinal cord. However, none of the current clinical tests can confidently predict clinical multiple sclerosis progression or treatment efficacy due to a lack of sufficiently sensitive and effective biomarkers. T cells in multiple sclerosis patients display an activated phenotype with an increased avidity to myelin protein. As a result, we hypothesize that the avidity of autoreactive T cells is a disease marker that can be used to monitor disease progress, judge therapeutic response, or discover new biochemical disease markers for multiple sclerosis. However, none of the current technologies has achieved high-sensitivity and high-throughput measurement of cell avidity, in clinical samples, at the single-cell level. Our engineering advances in “Acoustofluidics,” allowing for precise manipulation of single cells and liquids at the unexpected resolution, shows promising potential for measuring the avidity of highly heterogeneous, clinical autoreactive T cells. Here, I report our progress on the development of a novel “Acoustofluidic Avidity Cytometer” to overcome the barriers in detecting clinical multiple sclerosis.

Molecularly Engineered Model Systems for Studying the Initiation and Progression of Fibrosis

April Kloxin, University of Delaware (poster presenter)

Fibrosis affects almost every tissue in the body, including pulmonary, dermal, and cardiac tissues, and is the pathological outcome of misregulated wound healing or chronic inflammation. For example, idiopathic pulmonary fibrosis (IPF) is hypothesized to be initiated, in part, by repeated micro-injuries to the alveolar epithelium, resulting in deposition and accumulation of scar tissue, increased tissue stiffness, and ultimately loss of lung function. Currently FDA-approved therapeutics for IPF (e.g., pirfenidone and nintedanib) only slow the progression of fibrosis and cannot reverse disease pathology. Development of new therapeutics often is challenged by poor in vivo efficacy despite promising preclinical findings. Improved human model systems are needed to better understand the pathobiology of IPF and improve therapeutic strategies. In this work, we are establishing human, multidimensional culture models that allow probing of dynamic cell-microenvironment interactions that regulate activation and persistence of wound healing cells. A lentiviral reporter system of alpha smooth muscle actin (αSMA) expression has been established for stably transducing a range of human cells, including lung epithelial and fibroblast cell lines and primary cells, and monitoring changes in phenotype in situ and in real time in response to microenvironment changes upon injury. Hierarchically-structured and photoresponsive soft materials that mimic healthy to injured and diseased states have been created, and ongoing work is utilizing these systems for studying cell activation and persistence to identify targets for mitigating maladaptive wound healing responses and developing improved strategies treating fibrosis.

Synthetic Biomarkers: A 21st Century Path to Early Cancer Detection

Gabe Kwong, Georgia Institute of Technology (speaker)

Detection of cancer at an early stage when it is still localized improves patient response to medical interventions for most cancer types. Yet biomarkers shed from early lesions are limited by fundamental biological and mass transport barrier—such as short circulation times and blood dilution—that limit early detection thresholds. I present work on synthetic biomarkers, which are an emerging class of diagnostics that deploy bioengineered sensors inside the body to query early-stage tumors and amplify disease signals to levels that could potentially exceed that of shed biomarkers. I discuss strategies that harness dysregulated protease activity to amplify detection signals, employ tumor-selective activation to improve specificity, and leverage natural processing of bodily fluids (e.g., blood, urine, proximal fluids) for easy detection. These advances will be presented in the context of early detection of acute transplant rejection, and response and resistance to checkpoint blockade immunotherapy. Finally, I discuss work on logic-gated synthetic biomarkers for programmable immune sensing.

Microfluidic Lightsheet Microscopy

Wesley Legant, University of North Carolina (UNC), Chapel Hill/UNC-NCSU Joint Department of Biomedical Engineering (poster presenter)

Nearly 2 meters of DNA are packed into every cell nucleus. Within this volume, gene expression is regulated by the binding of transcription factors, cofactors, and RNA polymerase machinery to the promoters of target genes. However, how a transcription factor navigates through roughly 3.2 billion base pairs to find a 100-1,000 base pair long promoter is an open question. Nor is it clear how the underlying organization, composition, and dynamics of chromatin within the nucleus regulates transcription factor search dynamics. Answering these questions requires technologies that are capable of directly visualizing transcription factors, which diffuse in milliseconds, over the hours to days required for cellular fate specification. With support from the NIH New Innovator Program we are developing a new multifunctional lightsheet microscope. This Multimodal Optical microScope with Adaptive Imaging Correction (MOSAIC) instrument permits rapid cellular imaging under several different types of diffraction limited and super-resolution modalities. In parallel, we are developing microfluidic systems that are compatible with the angled orientation of lightsheet microscopy objectives. We fully characterize the optical performance (aberration, transmission, and polarization effects) of these chips and demonstrate that they are compatible with high-resolution lightsheet, single-molecule imaging, and structured illumination microscopy. Additionally, we demonstrate that they support imaging of both adherent and non-adherent cells, allow for rapid exchange of reagents while imaging, and maintain cell growth and sample sterility over multiple days of imaging. We are applying these advances to visualize the single-molecule dynamics of transcription factor search and directly visualize how they navigate the genome at different stages of cell differentiation. These studies will provide a novel window into how genes are regulated in both normal and disease settings.

Deformable Electronic Materials for Two-Way Communication with Biological Systems

Darren Lipomi, University of California, San Diego (poster presenter)

The goal of this project is to create a class of electronic materials that can measure signals and interface with the nervous system for two-way communication with biological systems. The project is exploring three classes of materials. (1) Semiconducting polymers with properties inspired by biological tissue. The goal of organic bioelectronics is to detect and treat disease by using signal transducers based on organic conductors and semiconductors in wearable and implantable devices. Except for the carbon framework of these otherwise versatile materials, they have essentially no properties in common with biological tissue: electronic polymers are typically stiff and brittle, and do not degrade under physiological conditions. Such properties can be realized in a single-component polymer by incorporating biocompatible subunits. We have synthesized a new type of stretchable, biodegradable polymeric semiconductor whose electronic performance is unaffected by the biodegradable components. Such materials have applications in wearable and implantable sensors. (2) Metallic nanoislands on single-layer graphene for cellular electrophysiology and wearable sensors. We have used these materials to measure the forces produced by the contractions of cardiomyocytes using a piezoresistive mechanism. Separately, we have developed orthogonal methods of stimulating myoblast cells electrically while measuring the contractions optically (a modality we nicknamed as "piezoplasmonic"). We have also used these sensors to measure the swallowing activity of head-and-neck cancer patients who have received radiation therapy and are at risk of dysphagia arising from fibrosis of the swallowing muscles. The combination of strain sensing, surface electromyography, and machine learning can be used to measure the degree of dysphagia. (3) We have developed ionically conductive organogels for haptic feedback. Medical haptic technology has myriad potential applications, from robotic surgery and surgical training, to tactile therapy for premature infants and patients with neurological impairment.

Frugal Science: Democratize Science, Diagnostics and Disease Surveillance

Manu Prakash, Stanford University (speaker)

Science faces an accessibility challenge. Although information/knowledge is fast becoming available to everyone around the world, the experience of science is significantly limited. One approach to solving this challenge is to democratize access to scientific tools. I will briefly discuss a broad philosophy of "Frugal science" that inspires design, development, and deployment of ultra-affordable yet powerful scientific tools for the masses. Using examples from my own work (Foldscope: one-dollar origami microscope, Paperfuge: twenty-cent high-speed centrifuge, Octopi: spectral imaging–based malaria diagnostics, SnapDx: low-cost molecular home diagnostics, Vectorchip: high-throughput molecular surveillance of mosquitoes; Abuzz: citizen science–based mosquito tracking), I will describe the process of identifying challenges, designing solutions, and deploying these tools globally to enable open-ended scientific curiosity/inquiries in communities around the world. By connecting the dots between science education, global health, and environmental monitoring, I will explore the role of "simple" tools in advancing access to better human and planetary health in a resource limited world.

Bioinspired Synthetic Nanobiomaterials for Cardiovascular Immunotherapy

Evan Scott, Northwestern University (speaker)

Atherosclerosis is a chronic vascular inflammatory disorder driven by both innate and adaptive immunity. Anti-inflammatory therapy and immunomodulation are promising strategies for prevention and treatment of atherosclerosis; however, the complex influences of atheroprotective and proatherogenic immune cells necessitates selective targeting of specific cell populations. To address this need, we employed our NIH DP2 New Innovator Award funding to design and validate a range of self-assembled nanobiomaterials that are engineered to achieve specific cellular biodistributions and mechanisms of controlled release to modulate vascular inflammation. Nanobiomaterials are nanoscale (1-1,000 nm in diameter) structures often synthesized and employed for controlled drug delivery applications. Taking advantage of the morphological flexibility of self-assembled systems, we aimed to mimic various structures and biochemical mechanisms of pathogens to enhance cell-selective intracellular delivery and treatment of atherosclerotic inflammation. Synthetic platforms will be presented for enhanced targeting of immune cell subsets, sustained delivery of drug-loaded nanobiomaterials, and highly efficient multi-drug encapsulation of water-soluble payloads such as proteins, peptides, and nucleic acids. In addition to achieving controlled modulation of the immune system for atherosclerosis, our biomimetic approach to rational nanobiomaterial design demonstrated therapeutic applications in a variety of other areas, including vaccination against infectious disease, glaucoma, cancer immunotherapy, and diabetes.

Deconstructing and Reconstructing Human Tissues

Kelly Stevens, University of Washington (speaker)

Over the past several decades, the concept of 3D printed artificial human organs (“bioprinting”) has energized regenerative engineering, capturing attention of both scientists and the general public alike. However, as the bioprinting field has matured, substantive roadblocks have become increasingly apparent. First, it is now clear that our field still needs 3D spatial maps—or “blueprint”—of human organs, down to the cellular and molecular levels and across organ scale. Without a blueprint, it is not possible to accurately build. Second, the field needs integrative technologies for recapitulating these spatial cellular and molecular features within artificial human organs. I will describe my lab’s work in building molecular and cellular “blueprints” of human tissues, as well as in developing new advanced fabrication and cellular methods for tissue construction. Here, I will focus on our efforts to uncover molecular-level information by attaining transcriptomic organ maps, and to develop technologies for encoding spatiogenetic wiring in human artificial tissues. I will also take a moment to reflect and provide data supporting the idea that if we as a profession are to engineer medical advances that equitably improve the lives of all people, our profession needs to prioritize including all people. Diversifying our profession is the engine of innovation and creativity needed to bring us forward into the 21st century.

Highly Multiplexed Fluorescence Microscopy Enabled by Ultrabright Pdot Probes for Interrogation of Complex Tissues

Joshua Vaughan, University of Washington (poster presenter)

In situ proteomic studies of the brain and other complex tissues have been severely hindered by limited technical capabilities. We are developing an approach for highly-multiplexed interrogation of biological specimens based on novel, ultra-bright polymer dots with tunable spectral properties. Polymer dots are luminescent semiconducting polymers that exhibit ultrahigh brightness and versatile spectral tunability of excitation and emission wavelengths that can enable highly multiplexed fluorescence microscopy. The methodology should be applicable to many types of tissues or small organisms, although the final goal of our project is to demonstrate this method by studying development of the mouse visual cortex. We also report the development of a simple, small-molecule-based fluorescent labeling method, termed FLARE (fluorescent labeling of abundant reactive entities), that can rapidly label general protein and carbohydrate groups on biological specimens. FLARE is compatible with a wide range of tissue processing procedures, reveals a wealth of details for volumetric studies of the basic physiology of cells and tissues, and is highly complementary to multiplexed proteomics or transcriptomics approaches.

Multi-Scale Spectroscopic Photoacoustic Image Instrumentation for Prostate Cancer Detection

Haichong Zhang, Worcester Polytechnic Institute (poster presenter)

Prostate cancer (PCa) is one of the most common cancer types and the second leading cause of cancer-related death among men in the United States. Screening and monitoring are critical to both finding PCa in its early stage when it is easier to treat and manage cancer treatment. Although the image-guided needle biopsy has been the gold standard for PCa screening as well as active surveillance, the procedure is non-trivial and fraught with complications including pain, bleeding, and infection. Thus, there is a strong need to develop a non-invasive approach to detect PCa cancer and monitor its growth repetitively. Our imaging approach is based on photoacoustic (PA) imaging, a hybrid modality combining high contrast of optical contrast with high penetration of US imaging. With spectroscopic PA imaging, we envision leveraging the quantification of two distinct and complementary parameters of molecular-targeted contrast agent and oxygen saturation simultaneously for better identification of aggressive PCa. As the first milestone to achieve the goal, we explored three types of PA image instrumentation that are optimized to characterize dissected tissues, small animals, and human organs, respectively. We establish three PA imaging apparatuses to visualize targets with different size scales: microscopic imager for tissues, mesoscopic imager for small animals, and transrectal ultrasound-based imager for organs. The experimental evaluations demonstrate the potential of PA imaging systems to image targets with different size scales in high resolution and can be used for the next step studies to characterize prostate tumors for early detection and active surveillance.

Implantable Cardiac Energy Harvesting Devices Using Geometrically Structured Piezoelectric Thin Films

John Zhang, Dartmouth College (speaker)

Harvesting energy directly from the human body offers a new paradigm to power wearable electronics and implantable biomedical devices without the need to replace batteries. For patients with implantable devices such as cardiac pacemakers, there is an urgent need to improve the quality of patients' post-implantation life by eliminating the risks and costs associated with battery replacement surgeries. Here, we demonstrate the designs, characterizations, and preclinical studies of compact implantable devices showing significant improvement in the electro-mechanical conversion efficiency. The proposed energy harvesting designs combine the thin film piezoelectric materials development with geometric mechanics toward seamless integration with existing medical implants such as the pacemakers. Various energy harvesting device prototypes have been developed using piezoelectric composite films made of mesoporous PVDF-TrFE including Kirigami-inspired energy harvester, bioinspired helical energy harvesting device with cardiac sensing function, multi-buckled-beam energy harvester, dual-cantilever energy harvester, and lead-in-tube design. The results based on those five geometrically designed thin film energy harvesting prototypes showed great promise to provide electrical energy for powering implantable devices. Moreover, in vivo studies demonstrated feasible clinical translation in porcine models. A sealed energy harvesting device was inserted, together with the pacemaker leads, using a standard implantation procedure. Evaluations under different anchoring positions, different heart rates, and drug treatment conditions were performed in right ventricles of porcine models. Both in vitro and in vivo results demonstrate the energy harvesters' capability to provide significant electrical energy directly from the motion of a pacemaker lead. In conclusion, the proposed implantable cardiac energy harvesting strategy can be extended to power other medical implants and wearable devices alike without the limit of the battery.

On-Chip Adhesion Frequency Assay

Yuebing Zheng, University of Texas at Austin (poster presenter)

Receptor-ligand interactions on cells mediate cell-cell and cell-environment communications in many biological processes. Adhesion frequency assay has the unique capability of measuring the receptor-ligand interactions at the single-cell level. The measurement can provide important information on the quality of biological processes and for the selection of potent therapeutic cells. However, current adhesion frequency assay, which uses micropipettes to aspirate cells for both interaction measurement and cell transfer afterwards, is bulky, labor-intensive, manual-operative, and low-throughput. In order to maximize the potential of adhesion frequency assay in biomedical research and clinical applications, it is necessary to develop high-throughput miniature device. Along this line, we aim to develop on-chip adhesion frequency assay by merging optical manipulation and microfluidic technologies. Herein, I will share our progress on the assay.

Molecular and Cellular Biology

Neuromodulation of Germline and Implications for Trans-Generational Effects

Giovanni Bosco, Dartmouth College (speaker)

Germline cells are specialized cells responsible for passing on genetic information from one generation to the next. Until recently, it was believed that information encoded in the DNA of these germ cells is the only determinant of inheritance. However, germline cells develop in the context of other tissues and depend on signals emanating from nearby somatic cells in order to mature and become viable gametes. Recent studies have also suggested that distant tissues, like the brain, and other neuronal cells can signal to germline cells, thus affecting gamete development and potentially contributing to germline reprogramming. An additional intriguing aspect unique to the neuro-germline connection is that environmental inputs into the nervous system could have profound effects on germline information, potentially altering information inherited by offspring. If this were possible, then parental experiences integrated by the brain could affect the traits inherited by offspring or even future generations. To study this brain-germline connection and how it may affect inheritance, we used the *Drosophila* genetic model system to ask how germline physiology is modulated by neuroendocrine signaling originating in the brain. To trigger such effects, we used a potent natural predator, parasitic wasp, that attack different stages of Drosophila offspring. Adults exposed to predators rely on visual and olfactory perception, integrated by the brain, to alter their germline physiology, stem cell proliferation, and development. Strikingly, germline stem cell proliferation can be stimulated or thwarted, depending on the specific types of inputs from different types of wasp predators. A signaling peptide, neuropeptide F (NPF), derived in the adult *Drosophila* brain functions to trigger apoptosis of developing oocytes, while NPF signaling is dispensable for brain signaling that increases germline proliferation. We will discuss how offspring can inherit altered behavior and immunity that is dependent on their parental environment.

An Evolutionary TORtoise: Conservation and Innovation in Plant TARGET OF RAPAMYCIN Signaling

Jacob Brunkard, University of Wisconsin-Madison (poster presenter)

TARGET OF RAPAMYCIN (TOR) is a protein kinase that senses nutrient availability and environmental cues to coordinate metabolism across eukaryotic lineages. TOR is under intense investigation by biomedical researchers because TOR causes or contributes to many diseases, including cancers and age-related disorders, but relatively little is known about TOR in the other major eukaryotic lineages, plants. Recently, my lab discovered a new role for TOR in plants, where TOR regulates cell-cell signaling to organize development and allocation of nutrients. We also discovered that plant TOR is highly sensitive to nucleotides, coordinating ribosome biogenesis (and rRNA synthesis) with purine and pyrimidine availability to promote organismal growth while maintaining cellular homeostasis. Finally, we demonstrated that TOR regulates mRNA translation in plants, in part through a conserved eukaryotic signaling axis via an RNA-binding protein, LARP1, that binds to mRNAs that begin with a stretch of pyrimidines (5'TOP motifs). Although mammalian 5'TOP motifs are most famously associated with ribosome

biogenesis, plant 5'TOP motifs are instead found on a small set of unexpected but deeply conserved mRNAs involved in translation and metabolism. In concert, we have discovered novel and conserved TOR signaling pathways in plants that reveal the evolutionary history of human TOR signaling networks, illuminating unanticipated homology and convergence of this core, "master regulator" of metabolism.

Compartmentalized Regulation of Intracellular NAD+

Lulu Cambronne, University of Texas at Austin (poster presenter)

Cellular bioenergetics both responds to and modulates cellular behaviors. The underlying mechanism involves tight control of metabolic intermediates and pathway enzymes that simultaneously function in metabolism and signaling. As such, the compartmentalization of these components is critical. Nicotinamide adenine dinucleotide (NAD+) is an intermediary metabolite required for central carbon metabolism and as the substrate for a broad class of NAD+-consuming enzymes, including Parp, Sirtuin, and CD-38-related family members that use the adenosine diphosphate ribose moiety as a post-translational adduct, for deacylation, and for signaling, respectively. Diminished intracellular levels of NAD+ can lead to disease. However, there remain multiple unknowns about the regulation of intracellular pools of NAD+, as well as the timing and extent to which depletion may be pathogenic. The challenge has been a technical limitation of methods to study levels in live cells with temporal and spatial resolution that matches it intracellular regulation. To study the intracellular roles of NAD+ my lab developed localizable, genetically-encoded fluorescent sensors for free intracellular NAD+. Most recently, our work with the sensor led to insight into how human mitochondria replenish their NAD+ levels. This critical pool of NAD+ is required for oxidative ATP production and is retained, even at the expense of cytoplasmic or nuclear pools, when a cell is faced with stress. We identified SLC25A51 as a mitochondrial transporter selective for NAD+ in human cells. We determined that SLC25A51 is sufficient to mediate mitochondrial NAD+ import, determined its kinetic parameters in the context of intact mitochondria, and are gaining insight into its translocation mechanism. Because maintenance of mitochondrial NAD+ is important for cell health, the regulation of SLC25A51 may represent new disease etiology and as well as hold potential for interventions.

Genome Folding, Unfolding, and Refolding in Neurological Disease

Jennifer Cremins, University of Pennsylvania (speaker)

The Cremins Lab focuses on higher-order genome folding and how classic epigenetic modifications work through long-range, spatial mechanisms to govern synaptic plasticity in healthy and diseased neural circuits. Much is already known regarding how transcription factors work in the context of the linear genome to regulate brain development. Yet, severe limitations exist in our ability to engineer chromatin in neural circuits to correct synaptic defects in vivo. At the lab's inception, it remained unclear whether and how genome folding could functionally influence cell type–specific gene expression. We have developed and applied new molecular and computational technologies to discover that nested subTADs and long-range loops undergo marked reconfiguration during neural lineage commitment, somatic cell reprogramming,

neuronal activity stimulation, and in repeat expansion disorders. We demonstrated that loops induced by neural circuit activation, engineered through synthetic architectural proteins, and miswired in fragile X syndrome were tightly connected to transcription, thus providing early insight into the genome's structure-function relationship. We are currently focused on understanding how, when, and why 3D genome folding patterns contribute to synaptic plasticity in neural circuits and synaptic dysfunction. Addressing this knowledge gap will provide an essential foundation for our long-term goal to engineer the 3D genome to reverse pathologic synaptic defects in debilitating neurological diseases.

Bidirectional Communication Between Nucleus and Mitochondria Drives Cancer Progression

Abhisha Sawant Dessai, Roswell Park Comprehensive Cancer Center (poster presenter)

Cancer cells have acquired the ability to sense and adapt to varying conditions of nutrient supply. Mitochondria lay at the core of cellular metabolism, whereas nucleus integrates cellular and environmental signals to activate gene transcription. However how mitochondrial enzymes and nuclear factors communicate to modulate gene transcription in response to bioenergetic stress is poorly understood. Biochemical screening identified mitochondrial aconitase (ACO2) enzyme activity as crucial for de novo lipogenesis in tumors. Proteomics studies identified a single acetylation mark lysine258 on ACO2 functioning as a regulatory motif, and the acetylation deficient Lys258Arg-mutant was enzymatically inactive. Acetylation of ACO2 was reversibly regulated by SIRT3, which was predominantly repressed in many tumors including prostate cancer. Mechanistic studies revealed that transcriptional repression of SIRT3 by androgen receptor and coactivator SRC-2 facilitates enhanced ACO2 activity to drive de novo lipogenesis. This genetic regulation was sufficient to drive prostate cancer colonization in the bone microenvironment. Next to investigate the mitochondrial control on gene transcription, we found that a set of enzymes regulating citrate synthesis, the precursor molecule generating acetyl CoA are found in the nucleus. As we know, acetyl CoA availability in the nucleus is critical for acetylation of histone tail for greater chromatin accessibility to the transcription factors. However, acetyl CoA is predominantly synthesized in the mitochondria and remains impermeable to mitochondrial membranes. We found that genetic ablation of ACO2 affects specific histone marks, and genome-wide chromatin accessibility using ATAC-seq showed that depletion of ACO2 resulted in loss of 16626 peaks compared to wildtype cells, suggesting overall closing of chromatin with ACO2 depletion. To conclude, our study identified novel functions of mitochondrial enzymes in the nucleus and a bi-directional inter-organelle communication system regulating tumor progression.

Uncovering the Molecular Landscape of Clonal Hematopoiesis Using Cell Reprogramming

Sergei Doulatov, University of Washington (poster presenter)

Myeloid neoplasms, including myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML), are genetically heterogeneous blood disorders characterized by clonal stem cell expansion. The onset of disease is often preceded by an extended period of age-related clonal

hematopoiesis (CH) during which hematopoietic stem and progenitors (HSPCs) accumulate somatic mutations. CH is defined by the presence of rare oncogenic subclones in healthy individuals and is associated with increased, albeit low, risk of progression to malignancy. A comprehensive molecular description of CH is currently lacking, limiting understanding of why some individuals progress and others do not, and options for early intervention. We have previously shown that reprogramming of MDS/AML patient HSPCs to induced pluripotent stem cells (iPSCs) can identify single cell clonal intermediates (Hsu et al. 2019). Here, we isolated iPSC clones spanning normal, CH, and progression stages of disease in 12 individual MDS or AML patients. Using genome sequencing we mapped the genomic landscape and natural selection shaping somatic genomes during clonal evolution. We simultaneously reconstructed the transcriptome changes during clonal progression by differentiating isogenic iPSCs to HSPCs. We thus use cell reprogramming to for the first time map concurrent genomic and transcriptomic evolution of single cells during life-long progression from normal to pre-malignant and leukemic hematopoiesis in individual patients.

Imaging Cell Lineage with a Synthetic Digital Recording System

Michael Elowitz, Caltech (speaker)

During multicellular development, spatial position and lineage history play powerful roles in controlling cell fate decisions. Using a serine integrase-based recording system, we engineered cells to record lineage information in a format that can be read out in situ. The system, termed intMEMOIR, allowed in situ reconstruction of lineage relationships in cultured mouse cells and flies. intMEMOIR uses an array of independent three-state genetic memory elements that can recombine stochastically and irreversibly, allowing up to 59,049 distinct digital states. It reconstructed lineage trees in stem cells and enabled simultaneous analysis of single-cell clonal history, spatial position, and gene expression in *Drosophila* brain sections. These results establish a foundation for microscopy-readable lineage recording and analysis in diverse systems.

Selective Control of Protein Synthesis by DDX3

Stephen Floor, University of California, San Francisco (poster presenter)

DDX3 is an RNA chaperone of the DEAD-box family that regulates translation. Ded1, the yeast ortholog of DDX3, is a global regulator of translation, whereas DDX3 is thought to preferentially affect a subset of mRNAs. However, the set of mRNAs that are regulated by DDX3 are unknown, along with the relationship between DDX3 binding and activity. Here, we use ribosome profiling, RNA-seq, and PAR-CLIP to define the set of mRNAs that are regulated by DDX3 in human cells. We find that while DDX3 binds highly expressed mRNAs, depletion of DDX3 particularly affects the translation of a small subset of the transcriptome. We further find that DDX3 binds a site on helix 16 of the human ribosome, placing it immediately adjacent to the mRNA entry channel. Translation changes caused by depleting DDX3 levels or expressing an inactive point mutation are different, consistent with different association of these genetic variant types with disease. Taken together, this work defines the subset of the transcriptome

that is responsive to DDX3 inhibition, with relevance for basic biology and disease states where DDX3 is altered.

Non-Refoldability Is Pervasive Across the *E. coli* Proteome

Stephen Fried, Johns Hopkins University (poster presenter)

Decades of research on protein folding have primarily focused on a subset of small proteins that can reversibly refold from a denatured state. However, these studies have generally not been representative of the complexity of natural proteomes, which consist of many proteins with complex architectures and domain organizations. Here, we introduce an experimental approach to probe protein refolding kinetics for whole proteomes using mass spectrometry-based proteomics. Our study covers the majority of the soluble *E. coli* proteome expressed during log-phase growth, and among this group, we find that one-third of the *E. coli* proteome is not intrinsically refoldable on physiological timescales, a cohort that is enriched with certain fold-types, domain organizations, and other biophysical features. We also identify several properties and fold-types that correlate with slow refolding on the minute timescale. Hence, these results illuminate when exogenous factors and processes, such as chaperones or co-translational folding, might be required for efficient protein folding. Finally, we show which features of proteins make them more dependent on chaperonins and the cellular proteostasis machinery, thereby highlighting which proteins are more susceptible to misfolding and initiating proteopathies such as Alzheimer's disease.

Identifying the Common Pathways Implicated by Common Variation Association Studies for Vascular Disease

Rajat Gupta, Brigham and Women's Hospital (poster presenter)

Introduction: Genome-wide association studies (GWAS) have discovered greater than 60,000 loci in the human genome that influence risk for common diseases or traits. Connecting each genetic variant to its disease-relevant functions has proven difficult and inefficient. For vascular disease we seek to identify the common causal biological pathways by profiling the transcriptional effects of gene knock-down in endothelial cells (ECs). Approach: To build maps of which candidate disease genes regulate which transcriptional phenotypes, we use CRISPR perturbations (Perturb-seq/CROP-seq) together with single cell RNA-seq (scRNAseq) to perturb thousands of genes and measure their effects on hundreds of transcriptional programs in parallel. Results: Two immortalized endothelial cell lines (Telo-HAEC and Eahy926) were transfected to express dCAS9-KRAB in response to doxycycline induction. 150 sgRNAs representing 50 genes were lentivirally transfected at a limiting dilution to ensure an average of one sgRNA per EC. Common essential genes such as RPL23A and EIF2S1 resulted in cell death and broad changes in gene expression that spanned multiple pathways. CAD-associated genes such as FLT1, VEGFA, and SWAP70 segregated together with topic modeling of their transcriptional signatures. Conclusions: With this set of single-cell genetic knock-downs we demonstrate that transcriptional pathways can be ascertained from Perturb-seq in ECs. We aim to expand this analysis to the full set of 200+ loci associated with coronary artery disease. These

efforts will help identify new biologic mechanisms and therapeutic approaches to vascular disease.

Active Genetics Comes Alive

Stephen Hedrick, University of California, San Diego (speaker)

Active genetic elements are transmitted during reproduction at greater than expected Mendelian frequencies. Such “super-Mendelian” inheritance can be used for a variety of applications including: gene-drive systems for disseminating beneficial traits throughout populations (e.g., spreading immunizing factors that prevent mosquitoes from transmitting malarial parasites), reversing insecticide resistance, devising elements that can eliminate or inactivate gene drives, creating active genetic modifications that facilitate breeding by bypassing constraints imposed by independent assortment and linkage, developing self-amplifying systems in bacteria to scrub antibiotic-resistance factors from the environment or potentially from patients with chronic bacterial infections, and engineering next-generation genetic circuits for synthetic biology.

In Vivo Single Molecule Analysis of DNA Damage Response

Jens Schmidt, Michigan State University (poster presenter)

The DNA that stores the heritable genetic information in human cells is constantly exposed to endogenous and exogenous insults that can cause DNA damage. To counteract this damage, human cells have evolved a sophisticated machinery to detect and repair DNA lesions, the DNA damage response (DDR). Defects in DNA repair caused by mutations in DDR proteins underlie a wide range of human diseases, including neurological disorders, immunodeficiency, infertility, and cancer. Therefore, understanding the molecular mechanisms of DNA damage repair is highly significant for human health. To systematically define the molecular mechanisms of double stranded break repair in human cells we will analyze the dynamics of the DDR using single-molecule live cell imaging. Single-molecule live cell imaging provides the highest possible sensitivity, spatial, and temporal resolution, making it possible to directly observe dynamic cellular processes that were previously undetectable. To analyze the DDR at the single-molecule level we have generated a collection of genome-edited human cancer cell lines that express fluorescently tagged DDR factors from their endogenous locus. Using these cell lines, we have systematically quantified the absolute protein abundance of these DNA repair factors, defining critical bottle necks that control DDR signaling. In addition, single-molecule imaging revealed a variety of distinct diffusion patterns, which demonstrate that DDR factors can search for sites of DNA damage by 3D-diffusion, chromatin scanning, or a combination of both mechanisms. Together, our approaches provide insight into the molecular mechanisms underlying DNA repair and establish a methodological framework that can be applied to study a wide variety of biological processes at the single-molecule level.

Enhanced Efficacy of Simultaneous PD-1 and PD-L1 Immune Checkpoint Blockade in High Grade Serous Ovarian Cancer

Sarah Hill, Dana-Farber Cancer Institute (poster presenter)

Immune therapies have had limited efficacy in high grade serous ovarian cancer (HGSC), as the cellular targets and mechanism(s) of action of these agents in HGSC are unknown. Here we performed immune functional and single-cell RNA-seq transcriptional profiling on novel HGSC organoid/immune cell co-cultures treated with a unique bispecific anti-PD-1/PD-L1 antibody compared to monospecific anti-PD-1 or anti-PD-L1 controls. Comparing the functions of these agents across all immune cell types in real time identified key immune checkpoint blockade (ICB) targets that have eluded currently available monospecific therapies. The bispecific antibody induced superior cellular state changes in both T and NK cells. It uniquely induced NK cells to transition from inert to more active and cytotoxic phenotypes, implicating NK cells as a key missing component of the current ICB-induced immune response in HGSC. It also induced a subset of CD8 T cells to transition from naive to more active and cytotoxic progenitor-exhausted phenotypes post-treatment, revealing the small, previously uncharacterized population of CD8 T cells responding to ICB in HGSC. These state changes were driven partially through bispecific antibody-induced downregulation of the bromodomain-containing protein BRD1. Small molecule inhibition of BRD1 induced similar state changes in vitro and demonstrated efficacy in vivo, validating the co-culture results. Our results demonstrate that state changes in both NK and a subset of T cells may be critical in inducing an effective anti-tumor immune response and suggest that immune therapies able to induce such cellular state changes, such as BRD1 inhibitors, may have increased efficacy in HGSC.

Spatiotemporal Dynamics of PIEZO1 Localization Controls Keratinocyte Migration During Wound Healing

Jesse Holt, University of California, Irvine (poster presenter)

Keratinocytes, the predominant cell type of the epidermis, migrate to reinstate the epithelial barrier during wound healing. Mechanical cues are known to regulate keratinocyte re-epithelization and wound healing; however, the underlying molecular transducers and biophysical mechanisms remain elusive. Here, we show through molecular, cellular, and organismal studies that the mechanically-activated ion channel PIEZO1 regulates keratinocyte migration and wound healing. Epidermal-specific Piezo1 knockout mice exhibited faster wound closure, while gain-of-function mice displayed slower wound closure compared to littermate controls. By imaging the spatiotemporal localization dynamics of endogenous PIEZO1 channels we find that channel enrichment in sub-cellular regions induces a localized cellular retraction that slows keratinocyte migration. Our findings suggest a potential pharmacological target for wound treatment. More broadly, we show that nanoscale spatiotemporal dynamics of Piezo1 channels can control tissue-scale events, a finding with implications beyond wound healing to processes as diverse as development, homeostasis, disease, and repair.

Single-Cell Metabolic Imaging Reveals a RhoA-Triggered a SLC2A3-Dependent Glycolytic Burst in Motile Endothelial Cells

Jun Huang, The University of Chicago (poster presenter)

Single-cell motility is spatially heterogeneous and driven by metabolic energy. Direct linking cell mobility to cell metabolism is technically challenging but biologically important. Here we implemented a single-cell metabolic imaging assay to measure glycolysis in individual endothelial cells using genetically-encoded biosensors capable of deciphering metabolic heterogeneity with subcellular resolution. We observed that cellular glycolysis fuels endothelial activation, migration, and contraction and that the high lactate production sites co-localize with active cytoskeletal remodeling within an endothelial cell. Mechanistically, we found RhoA induces endothelial glycolysis for the phosphorylation of cofilin and myosin light chain in order to reorganize the cytoskeleton and thus control cell mobility; RhoA activation triggers a glycolytic burst through the translocation of a glucose transporter SLC2A3/GLUT3 to fuel the cellular contractile machinery, as demonstrated across multiple endothelial types. Together, our results discovered that Rho-GTPase signaling coordinates energetic metabolism with cytoskeleton remodeling to regulate the motility of single endothelial cells.

3D Organoids Generated from Human Trophoblast Stem Cells Model Early Placental Development and Susceptibility to Emerging Viral Infections

Rowan Karvas, Washington University School of Medicine (poster presenter)

Trophoblast organoids provide a powerful tool to study human placental development, but obtaining trophoblasts from the first trimester is complicated due to ethical and legal restrictions. Here we report that human trophoblast cells (hTSCs) obtained from primary cytotrophoblasts (CTBs) and naive human pluripotent stem cells (hPSCs) can efficiently give rise to 3D trophoblast organoids. These stem cell–derived organoids recapitulate the villous architecture of placenta-derived organoids containing an epithelial CTB shell with a syncytiotrophoblast (STB) core. Single-cell RNA-sequencing (scRNA-seq) demonstrated that organoids derived from naive and primary hTSCs contained a similar cellular composition, which includes two discrete CTB clusters, two discrete STB clusters, and one extravillous trophoblast (EVT) population. Comparison with scRNA-seq data from human embryos showed a high degree of alignment with post-implantation CTB, STB, and EVT populations. Given emerging evidence regarding pregnancy complications due to COVID-19 infection, we investigated the susceptibility of hTSC-derived organoids to SARS-CoV-2 infection. Our scRNA-seq data indicate that the common entry receptors for the SARS-CoV-2 virus, ACE2 and TMPRSS2, are expressed in a subset of STBs. In accordance with these expression data, infection of trophoblast organoids with a pseudovirus expressing the SARS-CoV-2 spike protein on its surface resulted in limited infection in STBs. By comparison, infection of trophoblast organoids with Zika virus resulted in more robust infection in multiple trophoblast cell types. Our results demonstrate that SARS-CoV-2 has the ability to infect a subset of STBs, yet the underlying CTB population appears resilient to SARS-CoV-2 infection, potentially limiting the vertical transmission of the virus to the fetus. The generation of 3D trophoblast organoids from

hPSCs provides a means of deriving patient-specific organoids to study placental disease and the response to emerging viral infections.

Structural Interiors of Lipid Nanoparticles Regulate RNA Delivery to Cells

Cecilia Leal, University of Illinois at Urbana Champaign (speaker)

We will share our exciting findings that resulted from the 2016 NIH-New Innovator Award entitled: "A New Paradigm in Nanomedicine: can structural interiors of nanoparticles regulate cellular delivery?" The scientific community is witnessing the enormous societal impact of research in lipid-based mRNA cellular delivery with the deployment of the COVID-19 vaccine. These lipid nanoparticle (LNP) materials could be useful in many more therapies, but the technology is not always successful because the delivery of RNA to certain cells fails. Some cell types do not undergo endocytosis, or, most often, LNPs and RNA remain trapped in endosomal compartments. The development of ionizable lipids was an important discovery for LNPs. Ionizable lipids in combination with other lipids in the LNPs have been recently referred to as "structural lipids." This is because as endosomal membrane-proteins pump protons onto the endosomal lumen, these lipids become charged and flip the internal structure of the LNPs to a honey-comb arrangement. This structure has been elusively observed to lead to more efficient RNA release into the cytosol before the onset of the endosome-lysosome degradation pathway. Indeed, LNP internal structure controls RNA delivery efficiency. We have discovered that LNP nanostructure regulates endosomal escape via a process of protein-free fusion between LNPs and endosomal membranes. We have developed a methodology to stabilize LNPs with nanostructures that go beyond the traditional layered lamellar and the hexagonal structure with ionizable lipids. Specifically, we have developed the first bicontinuous cubic LNP for the delivery of RNA to living cells. Depending on the LNP nanostructure, membrane elasticity and curvature properties can be engineered such that LNP-endosome fusion and formation of fusion pores is energetically favorable without the need of ionizable lipids.

Novel Cryo-EM Methods and Applications for Macromolecular Structural Biology

Dmitry Lyumkis, The Salk Institute for Biological Studies (poster presenter)

Recent advances in cryo-electron microscopy (cryo-EM) have enabled routine structural biology applications. However, there is a persistent problem of "preferred specimen orientation"—when only one or a few views of the protein sample is evident within the data. This problem is ubiquitous and affects most cryo-EM specimens prepared using current vitrification technologies, leading to resolution anisotropy, limiting achievable resolution, and often stifling structural biology efforts entirely. We proposed a generalizable experimental strategy to tackle the preferred orientation problem by tilting the stage during data acquisition, which is now frequently utilized by cryo-EM practitioners. We also established quantitative validation procedures to analyze the resolution of cryo-EM reconstructions, which are now incorporated into cryo-EM software packages and available to the community. We are currently developing novel computational tools to visualize distinct effects caused by preferred specimen orientation and to highlight improvements when the stage is tilted in the electron microscope. The ideas provide a general framework for understanding cryo-EM resolution, with specific experimental

recommendations for data acquisition and analysis. We are applying these tools to all our cryo-EM datasets collected in the lab. One principal area of investigation is understanding the structural bases of HIV integration into host chromatin and its inhibition by modern antiretroviral therapeutics. Using tilted data collection strategies, we determined high-resolution cryo-EM structures of HIV integration nucleoprotein assemblies bound to the latest generation clinically approved and developmental drugs. Our structures highlight how small changes in the enzyme active site can have notable implications for drug binding and design and provide mechanistic insights into why a leading drug retains efficacy against a broad spectrum of drug-resistant variants. The structural biology findings have implications for expanding effective treatments available for HIV-infected individuals.

Rethinking Epigenetics

Keith Maggert, University of Arizona (speaker)

"Transgenerational Epigenetic Inheritance" describes phenotypes that are induced in one organism, and transmitted to offspring using "unconventional" modes of inheritance. Much effort has gone into identifying responsible mechanisms, but the best candidates—DNA methylation and histone modification, or other forms of "chromatin structure"—remain hotly debated because none exhibits the necessary features to act as an inducible genetic memory. We proposed a new alternative—a shift in the way we think about, and therefore what we expect of, Transgenerational Epigenetic Inheritance. We challenged our most basic assumptions and found that we could simply bypass the most recalcitrant aspects of Epigenetic Inheritance. Now, near the end of our Transformative Research Award, we show that many cases of Epigenetic Inheritance can be explained by the induction of sequence polymorphisms in sensitive, pleiotropic, and under-studied regions of the genome. We used *Drosophila melanogaster* to show that environmental and mutational stresses destabilize repeat sequences—transposable elements, satellites, and repeat genes such as the ribosomal RNA genes. These induced mutations are rarely considered in studies of Transgenerational Epigenetic Inheritance, but we have shown that they have phenotypic effects on cellular metabolism, heterochromatin function, and genome stability, acting as enhancers or suppressors of many other phenotypes. Thus these induced mutations may account for many or most perceived Transgenerational Epigenetic effects. We have most recently reinvestigated canonical epigenetic systems, such as heterochromatin-induced gene silencing (Position Effect Variegation). We find that heritable gene expression states are likely an artifact of how we think about and investigate the problem, rather than a radically new genetic system.

Regulation of Protein Function by 3'UTRs

Christine Mayr, Memorial Sloan Kettering Cancer Center (speaker)

Approximately half of human genes use alternative polyadenylation to generate mRNA isoforms with identical coding region but with alternative 3' untranslated regions (3'UTRs). We quantified alternative 3'UTRs in more than 100 cell types and observed that hundreds of genes change their 3'UTR isoform expression in a coordinated manner during differentiation. Intriguingly, the majority of these genes (~85%) did not change their mRNA abundance level at

the same time. This indicates that we identified hundreds of genes that have never been implicated in these processes, but for the majority of them it is currently unclear what a change in 3'UTR isoform expression means. Over the past 5 years, we found that 3'UTRs—despite not being part of proteins—regulate protein localization, protein activity, and protein function. In the case of alternative 3'UTRs, we discovered that they diversify protein function without changing the amino acid sequence. Mechanistically, 3'UTR-dependent regulation of protein function occurs through 3'UTR-mediated protein complex formation as we identified protein complexes that can only be established in the presence of specific 3'UTRs. We showed that in some cases 3'UTRs act as scaffolds and recruit potential interactors to the site of translation, thus promoting co-translational protein-protein interactions. In other cases, 3'UTRs enable protein translation in specific phase-separated cytoplasmic compartments thus allowing formation of certain protein complexes that cannot be established upon translation outside of such compartments. Taken together, we found that protein complex formation mediated by 3'UTRs relies less on overall cellular interactor abundance but instead takes advantage of the effective local concentration and possibly a specific solvent environment. Our results indicate that many proteins have several functions. Some of them are autonomous and are predominantly regulated by abundance, whereas other functions are non-autonomous and are dictated by regulatory elements in 3'UTRs.

A Novel Quality Control Mechanism for Muscle Stem Cells during Aging

Prashant Mishra, The University of Texas Southwestern Medical Center (poster presenter)

Age-associated declines in muscle stem cell (MuSC) function are characterized by mitochondrial electron transport chain (ETC) impairment, yet the underlying consequences for MuSCs and existing tissue are unknown. We report here that aged MuSCs accumulate mtDNA mutations while selecting against dysfunctional variants. Using genetic models of ETC-dysfunction, we find that mitochondrial oxidative stress triggers MuSCs to bypass activation and fuse with neighboring myofibers, thereby removing ETC-dysfunctional MuSCs from the stem cell compartment. The MuSC-myofiber fusion event is dependent on the induction of reactive oxygen species, and activates actin-regulatory proteins required for membrane fusion. In the absence of stem cell clearance by MuSC-myofiber fusion, ETC-dysfunctional MuSCs are retained and available to regenerate dysfunctional myofibers. Intriguingly, the absorption of ETC-dysfunctional MuSCs is detrimental to existing myofibers, and initiates chronic myopathic attributes and functional declines in motor performance. We therefore propose a novel model for quality control and homeostasis of MuSCs in which existing myofibers accumulate damage by serving as cellular "sponges" that absorb and remove ETC-dysfunctional MuSCs undergoing oxidative stress.

Transcriptome Analysis of Adult Caenorhabditis Elegans Cells Reveals Tissue-Specific Gene and Isoform Expression/Horizontal and Vertical Transmission of Transgenerational Memories via the Cer1 Transposon

Coleen Murphy, Princeton University (speaker)

The biology and behavior of adults differ from those of developing animals, and cell-specific information is critical for deciphering the biology of multicellular animals. Thus, adult tissue-specific transcriptomic data are critical for understanding molecular mechanisms that control their phenotypes. We used adult cell-specific isolation to identify the transcriptomes of *C. elegans*' four major tissues (or "tissue-ome"), identifying ubiquitously expressed and tissue-specific "enriched" genes, and tissue-specific alternative splicing. These data reveal the hypodermis' metabolic character, suggest potential worm-human tissue orthologies, and identify tissue-specific changes in the Insulin/IGF-1 signaling pathway. Finally, we developed a machine learning-based prediction tool for 76 sub-tissue cell types, which we used to predict cellular expression differences in IIS/FOXO signaling, stage-specific TGF-β activity, and basal vs. memory-induced CREB transcription. Together, these data provide a rich resource for understanding the biology governing multicellular adult animals. Recently, we discovered that exposure to purified small RNAs isolated from pathogenic *Pseudomonas aeruginosa* is sufficient to induce pathogen avoidance in the treated worms and in four subsequent generations of progeny. The RNA interference and PIWI-interacting RNA (piRNA) pathways, the germline, and the ASI neuron are required for avoidance behavior induced by bacterial small RNAs, and for the transgenerational inheritance of this behavior. A single *P. aeruginosa* non-coding RNA, P11, is both necessary and sufficient to convey learned avoidance of PA14, and its *C. elegans* target, maco-1, is required for avoidance. These memories can be transferred to naive animals via Cer1 retrotransposon-encoded capsids. Cer1 functions to transmit information from the germline to neurons and is required for *C. elegans*' learned avoidance ability and transgenerational inheritance. *C. elegans* has co-opted a potentially dangerous retrotransposon to protect itself and its progeny from a common pathogen.

How to Make Microtubules and Build the Cytoskeleton

Sabine Petry, Princeton University (speaker)

How does a cell construct its microtubule cytoskeleton? According to Feynman's principle "what I cannot create, I do not understand," my lab pursues this question by building the chromosome segregation machinery from scratch. I will first discuss how the microtubule framework is generated in a cell. Upon deciphering the function of the most important microtubule accessory proteins, I will present how we use those building blocks to reconstitute a spindle substructure in vitro and determine its building plan. Finally, I will outline how we combine spindle substructures like pieces of a puzzle to assemble and thereby understand a functioning spindle that segregates chromosomes. By studying how the MT cytoskeleton is built, I hope to help explain how hundreds of proteins can self-assemble on the nm scale into a complex molecular machine 1,000-fold larger than its constituents, a challenge for the biochemistry of the 21st century.

Epidemiological Epididymosomal Epigenomics: The Role of the Male Reproductive Tract in Control of the Sperm Epigenome

Oliver Rando, University of Massachusetts Medical School (speaker)

Beyond contributing a haploid genome to the next generation, germ cells also transmit so-called "epigenetic" information with the potential to influence offspring phenotype. Over the past two decades, we and others have shown that ancestral environmental conditions can impact metabolic and other phenotypes in the next generation, a phenomenon clearly related to the once-discredited idea of "inheritance of acquired characters." Our lab has developed several models for transmission of epigenetic information through the male germline in mammals, exploring how paternal diets or drug exposures program metabolism and behavior in offspring. I will discuss our efforts to understand these systems mechanistically, focusing primarily on surprising features of the sperm RNA payload, including (1) a central role for germline accessory tissues in shaping the sperm epigenome; (2) control of preimplantation development and gene regulation by sperm-delivered RNAs, and; (3) functional roles (in the embryo and elsewhere) for an understudied class of regulatory small RNAs derived from mature tRNAs, known as tRNA fragments.

The Structural Basis for Protein Energy Landscapes in a de novo Designed Proteome

Gabriel Rocklin, Northwestern University (poster presenter)

All proteins dynamically sample diverse folded, unfolded, and excited states with differing free energies. Although energy landscapes have been studied for decades, existing methods have been restricted to assaying one protein per sample, limiting the development of quantitative, global models of protein energy landscapes. We developed a new experimental approach to rapidly characterize hundreds to thousands of protein energy landscapes simultaneously by hydrogen exchange mass spectrometry. In this approach, the target protein library is expressed as a mixture from custom-synthesized DNA oligos, and individual intact proteins are resolved by LC-IMS-MS to measure the overall exchange at each timepoint. We applied this approach to examine the energy landscapes of more than 1,000 de novo designed miniproteins (43 residues in length) and found wide variation in landscapes, even among designs with similar topologies. The size of our dataset enabled us to statistically analyze the structural origins of the varied landscapes, revealing how different interaction types modulate both stability and conformational fluctuations. Combining these new large-scale experiments with computational modeling should ultimately lead to a quantitative understanding of the structural determinants of protein energy landscapes.

Observation of Extracellular and Intracellular Conformational Coupling in Membrane Proteins Using Single-Molecule FRET and Nanodiscs

Gabriela Schlau-Cohen, MIT (poster presenter)

Approximately 30 percent of the genes in most genomes encode membrane proteins, and membrane proteins are 60 percent of drug targets. Despite their importance, information about membrane protein function and interaction has been limited due to the challenges

associated with maintaining their mixed hydrophobic/hydrophilic environment while conducting experiments. By combining nanodiscs and single-molecule Förster resonance energy transfer (FRET), we observe conformational coupling across the plasma membrane in two transmembrane receptors, the bacterial chemoreceptor and the mammalian epidermal growth factor receptor (EGFR). In chemoreceptors, periplasmic ligand binding changes the packing and dynamics of the cytoplasmic four-helix coiled-coil bundle >200 Å away. The observed changes are consistent with a transition from a modestly to extended rhomboid organization of the cytoplasmic bundle, which may be a feature shared with other coiled-coil signaling proteins. In monomeric EGFR, extracellular ligand binding induces an intracellular conformational reorganization that is specific to the ligand and lipid composition. These findings demonstrate that the single a-helix of EGFR, and potentially other single-pass membrane proteins, can serve as a minimal yet sufficient system for signal transduction. These examples of transmembrane conformational signaling inform on the mechanistic underpinnings by which cells detect and respond to the fluctuating environment of biological systems.

Inhibition of Acetate Metabolism Enhances Host Anti-Tumor Immunity

Zachary Schug, Wistar Institute (poster presenter)

Acquired resistance to anti-cancer therapy is an enormous challenge. One of the main factors contributing to therapy resistance is tumor hypoxia. The oxygen and nutrient stress imposed by tumor hypoxia forces cancer cells to adapt in order to survive. These metabolically adapted cancer cells are often more invasive, more malignant, and more drug resistant. As a result, the cancer cells that emerge from hypoxic tumor regions are more likely to cause patient relapse. There is therefore a critical need to understand the mechanisms that promote the survival of cancer cells in stressful tumor microenvironments. We previously showed that the enzyme acetyl-CoA synthetase 2 (ACSS2) supports cancer cell metabolism in hypoxic and nutrient-depleted environments. ACSS2 endows cancer cells with the ability to use acetate as an alternative nutrient source to drive acetyl-CoA biosynthesis during nutrient stress, and genetic silencing of ACSS2 inhibits human breast tumor growth in xenograft models. Given the important role of acetate metabolism in breast cancer we expanded upon our studies by using immunocompetent hosts and syngeneic mouse tumor models. Our results revealed a previously unknown role of ACSS2 in modulating host anti-tumor immunity. We found that ACSS2 deficient tumors are unable to grow when host immunity is intact. Depletion of host immunity (Tcells) using genetic or pharmacological models rescues the growth of ACSS2 deficient tumors. Pharmacological inhibition of ACSS2 in tumors in vivo displayed gene signatures associated with immune infiltration and activation within the tumor microenvironment. Our current research demonstrates a novel role for acetate metabolism in supporting tumor extrinsic modulation of host anti-tumor immunity. Since activation of acetate metabolism via ACSS2 is a near universal hallmark of metabolically stressed cancer cells, targeting acetate metabolism represents an unrealized opportunity with significant upside for improving current therapeutic modalities in breast cancer.

Targeted DNA Integration Without Double-Strand Breaks Using CRISPR RNA-Guided Transposases

Samuel Sternberg, Columbia University Irving Medical Center (poster presenter)

Conventional CRISPR—Cas systems maintain genomic integrity by leveraging guide RNAs for the nuclease-dependent degradation of mobile genetic elements, including plasmids and viruses. Here we describe a remarkable inversion of this paradigm, in which bacterial Tn7-like transposons have coopted nuclease-deficient CRISPR—Cas systems to catalyze RNA-guided integration of mobile genetic elements into the genome. Programmable transposition requires CRISPR- and transposon-associated molecular machineries, including a novel co-complex between Cascade and the transposition protein TniQ. Donor DNA integration occurs at a fixed distance downstream of target DNA sequences, accommodates variable length genetic payloads, and functions robustly in diverse bacterial species. Deep sequencing experiments reveal highly specific, genome-wide DNA integration across dozens of unique target sites. The discovery of a fully programmable, RNA-guided integrase lays the foundation for kilobase-scale genome engineering that obviates the requirements for DNA double-strand breaks and homologous recombination.

Opening Windows into the Cell: Bringing Structure to Cell Biology Using Cryo-Electron Tomography

Elizabeth Villa, University of California, San Diego (speaker)

To perform their function, biological systems need to operate across multiple scales. Current techniques in structural and cellular biology lack either the resolution or the context to observe the structure of individual biomolecules in their natural environment and are often hindered by artifacts. My lab builds tools to observe molecular structures in their native cellular environment. Using the power of cryo-electron tomography to image biomolecules at molecular resolution in situ, we are building tools to make compatible with, and directly comparable to, biophysical and cell biology experiments, capturing the structural behavior of macromolecules in action under controlled conditions. I will show how we used these techniques to reveal the structure of LRRK2, the greatest known genetic contributor to Parkinson's disease, and to unveil the molecular architecture of processes in bacterial cell biology.

Healthy Metabolism, Healthy Aging

Meng Wang, Baylor College of Medicine/HHMI (speaker)

Metabolism is fundamental to life. During metabolic reactions, thousands of metabolites are generated, which are directly connected with cellular activities and highly conserved across species. In addition to their best-known functions as structural building blocks and energy resources, research in my group focuses on their novel roles in orchestrating organelle homeostasis, coordinating microbe-host communication, and extending lifespan and healthspan. Our work uncovers several previously unknown metabolite-directed signaling mechanisms and develops new therapeutic compounds to promote longevity and healthy

aging. In particular, we have discovered that bacteria-derived metabolites can actively signal through host mitochondria to prolong lifespan and protect against age-associated pathologies. We further develop an optogenetic strategy to quantitatively manipulate bacterial gene expression and metabolite production inside the gut of live organism to promote longevity. At the same time, new chemical compounds have been generated to directly induce the production of pro-longevity metabolites from the microbiota.

Mechanisms of Ultrafast Endocytosis

Shigeki Watanabe, Johns Hopkins University (poster presenter)

In 1973 John Heuser and Tom Reese demonstrated that neurotransmitter was released from neurons via the fusion of neurotransmitter-filled vesicles with the cell membrane. But at the same time, these experiments launched a controversy that is unresolved today—do vesicles collapse into the membrane and are then recycled slowly on the order of 20 seconds? Or do they retain their existence—and reverse the pore in just 1 second, as proposed in "kiss and run" endocytosis? Since then, molecular pathways for fusion and recycling have been put forward, but the field remains divided. We have used channelrhodopsin to stimulate neurons in intact nematodes and in cultured hippocampal neurons. The specimen is then frozen 15 ms to 20 seconds after the stimulus. To our surprise, we observed a different form of vesicle recycling that is ultrafast, in which membrane is endocytosed at lateral edges of active zones between 30-100 ms after stimulation. The large endocytic vesicles then fuse to form an endosome and are resolved by clathrin into synaptic vesicles. Although rapid, several molecules coordinately mediate ultrafast endocytosis. I will discuss the findings from the original studies and our current work on molecular mechanisms underlying ultrafast endocytosis.

Systemic Injury Responses in Axolotl

Jessica Whited, Harvard University (speaker)

Human limitations in natural regeneration can dramatically affect a patient's life, such as after limb loss. Axolotl salamander limbs are anatomically similar to human limbs, but they can perfectly regenerate throughout life. Understanding how this happens may provide important clues necessary for future therapeutic approaches. While most research has focused on the site of injury, we have discovered that cells throughout the axolotl's body activate and proliferate following amputation. This process of systemic activation is akin to what other researchers have discovered to happen in mice following a local injury. However, mice do not go on to regenerate entire limbs. We will present data demonstrating that some of the molecular mechanisms that enable systemic response in mice are shared with axolotls. Elucidating how the systemic response occurs in axolotl will be key to understanding how cells are initially systemically activated, while only those at the site of injury are converted to blastema cells, those cells are the building blocks for the new limb. Our unpublished work shows that innervation at distantly-responding sites is critical for the systemic injury response in axolotls. This work has led us to consider molecular factors derived from nerves as stimulants for activation pathways in tissue-specific progenitor cells residing throughout the body. A nerve conduit for the response contrasts with the circulation conduit implicated in mouse, raising

questions about how information is transmitted throughout the body in super-regenerators versus animals with more limited natural regenerative abilities. We hope this work will lead to both molecular insights as well as theoretical insights about the differences between species when considering regeneration and, ultimately, how human regeneration might be augmented.

Systemic Regulation of Brain Aging

Tony Wyss-Coray, Stanford University (speaker)

Brain aging leads to cognitive decline and is the main risk factor for sporadic forms of neurodegenerative diseases including Alzheimer's disease. While brain cell- and tissue-intrinsic factors are likely key determinants of the aging process, recent studies document a remarkable susceptibility of the brain to circulatory factors. Thus, blood borne factors from young mice or humans are sufficient to slow aspects of brain aging and improve cognitive function in old mice and, vice versa, factors from old mice are detrimental for young mice and impair cognition. In trying to understand the molecular basis of these observations we found evidence that the cerebrovasculature is an important target and that brain endothelial cells show prominent age-related transcriptional changes in response to plasma. We discovered that plasma proteins are taken up broadly into the brain and that this process various between individual endothelial cells and with aging. We are exploring the relevance of these findings for neurodegeneration and potential applications toward therapies.

Probing Protein Interactions Through Spectral and Diffusional Super-Resolution Microscopy

Ke Xu, University of California, Berkeley (poster presenter)

We discuss our recent efforts to develop and apply spectral and diffusional super-resolution microscopy methods to visualize intracellular protein interactions and phase separation processes. In particular, with spectrally resolved single-molecule localization microscopy (SR-SMLM), we unveil rich, nanoscale functional and compositional heterogeneities in live cells and in in vitro systems using fluorescent probes that exhibit shifts in emission spectra under different local environments. With single-molecule displacement/diffusivity mapping (SMdM), we map out intracellular diffusivity with unprecedented spatial resolution and fidelity, and thus unveil the effects of protein net charge and crowding in diffusion and phase-separation processes.

Circulating Tumor Cells Inform Mechanisms of Breast Cancer Metastasis

Min Yu, University of Southern California (speaker)

Hematogenous metastasis is a complicated and inefficient multistep process by which tumor cells spread via blood circulation to form secondary tumors in distant organs throughout the body. Only a very small fraction of the circulating tumor cells (CTCs) shed into the bloodstream is able to initiate a metastasis. Our research is focusing on understanding the molecular properties of these metastatic "seeds" and their interactions with the local organ microenvironment or "soil." To identify the metastasis-initiating CTCs, we ex vivo expanded

CTCs derived from breast cancer patients and inoculated them into the bloodstreams of immunocompromised mice and identified metastases in the common sites of breast cancer: lung, bone, brain. Importantly, the metastatic patterns in mice reflected those in the corresponding patients. Particularly, one CTC line showed a high capacity for brain metastasis in mice, which preceded the clinical detection of brain metastasis in the corresponding patient by one year. Serial in vivo inoculation showed increased organotropisms, further proving the intrinsic tendency of these cells to initiate metastasis in specific organs. Via genetic, epigenetic, and transcriptional analyses, we revealed genes associated with organotropic features and identified drivers for brain metastasis. Through functional validation, we found that the semaphorin family cell surface receptor SEMA4D and oncogene MYC work together to promote brain metastasis, by promoting blood-brain barrier (BBB) transmigration and colonization, two orthogonal steps of brain metastasis. To further investigate the metastatic capacity of CTCs, our recent research discovered a "hypoxia-exposure memory" that provides a long-lasting effect from the solid tumors on CTCs to promote metastasis. Ongoing research is focusing on elucidating the underlying mechanisms of this novel finding in CTCs, with the goal to uncover vulnerabilities for novel therapies.

Exploration of Biological Diversity to Discover Novel Molecular Technologies

Feng Zhang, Broad Institute of MIT and Harvard University (speaker)

Many powerful molecular biology tools have their origin in nature, and, often, microbial life. From restriction enzymes to CRISPR-Cas9, microbes utilize a diverse array of systems to get ahead evolutionarily. We are interested in exploring this natural diversity through bioinformatics, biochemical, and molecular work to better understand the fundamental ways in which living organisms sense and respond to their environment and ultimately to harness these systems to improve human health. Building on our demonstration that Cas9 can be repurposed for precision genome editing in mammalian cells, we began looking for novel CRISPR-Cas systems that may have other useful properties. This led to the discovery of several new CRISPR systems, including the CRISPR-Cas13 family that targets RNA, rather than DNA. We developed a toolbox for RNA modulation based on Cas13, including methods for precision base editing. We are expanding our biodiscovery efforts to search for new microbial proteins that may be adapted for applications beyond genome and transcriptome modulation, capitalizing on the growing volume of microbial genomic sequences and building on our bioengineering expertise. We are particularly interested in identifying new therapeutic modalities and vehicles for delivering cellular and molecular cargo. We hope that this combination of tools and delivery modes will accelerate basic research into human disease and open up new therapeutic possibilities.

Next Generation Expansion Microscopy Towards Building Whole Tissue Spatial Molecular Atlas

Yongxin Zhao, Carnegie Mellon University (poster presenter)

Diffraction-limited Optical Microscopy is a powerful tool to observe microscopic structures and processes of biological specimens. However, it is unable to resolve nanoscale configurations of

biomolecules below the diffraction limit, which severely limited its capability of analyzing intricate and subtle biological/pathological changes. Recently, Expansion Microscopy (ExM) has emerged as a ground-breaking new principle for scalable, nanoscale optical imaging of biological specimens. Rather than optically magnifying samples, ExM embeds biological tissue into a water-swellable polyelectrolyte hydrogel, enzymatically homogenizes them, and then isotropically expands the tissue-hydrogel physically in water. Typical ExM protocols expand tissues by ~100 folds in volume, thus enabling nanoscale optical imaging with resolution ~60 nm using diffraction-limited microscopes. However, most ExM methods have to utilize proteinase K that destroys endogenous proteins to facilitate tissue expansion because the majority of tissue types are not expandable otherwise. As a step toward establishing whole tissue spatial molecular atlas, we report a new ExM framework Molecule Anchorable Gel-enabled Nanoscale In-situ Fluorescence MicroscopY (MAGNIFY), which uses a mechanically sturdy gel that enables retention of nucleic acids, proteins, and lipids. MAGNIFY utilizes a non-enzymatic method to expand biological specimens up to 11x—and enables imaging of cells and tissues with 25-nm-resolution on conventional microscopes or with ~15 nm-resolution if combined with Super-resolution Optical Fluctuation Imaging (SOFI). We used MAGNIFY to visualize nanoscopic subcellular features in a broad range of specimens, from synaptic proteins in the mouse brain, to podocyte foot processes in the human kidney, to clustered mitochondria in photoreceptor cells in zebrafish embryos, to defects in cilia and basal bodies in drug-treated human lung organoids. Finally, in combination with reflectance confocal imaging system, we performed large-volume, nanoscale imaging of the whole 5dpf zebrafish embryo.

Stress-Induced RNA-Chromatin Interactions Promote Endothelial Dysfunction

Sheng Zhong, University of California, San Diego (poster presenter)

Chromatin-associated RNA (caRNA) has been proposed as a type of epigenomic modifier. Here, we test whether environmental stress can induce cellular dysfunction through modulating RNA-chromatin interactions. We induce endothelial cell (EC) dysfunction with high glucose and TNFα (H + T), that mimic the common stress in diabetes mellitus. We characterize the H + T-induced changes in gene expression by single-cell (sc)RNA-seq, DNA interactions by Hi-C, and RNA-chromatin interactions by iMARGI. H + T induce inter-chromosomal RNA-chromatin interactions, particularly among the super enhancers. To test the causal relationship between H + T-induced RNA-chromatin interactions and the expression of EC dysfunction-related genes, we suppress the LINC00607 RNA. This suppression attenuates the expression of SERPINE1, a critical pro-inflammatory and pro-fibrotic gene. Furthermore, the changes of the co-expression gene network between diabetic and healthy donor-derived ECs corroborate the H + T-induced RNA-chromatin interactions. Taken together, caRNA-mediated dysregulation of gene expression modulates EC dysfunction, a crucial mechanism underlying numerous diseases.

Natural Display of Nuclear-Encoded RNA on the Cell Surface and Its Impact on Cell Interaction

Sheng Zhong, University of California, San Diego (speaker)

Compared to proteins, glycans, and lipids, much less is known about RNAs on the cell surface. We developed a series of technologies to test for any nuclear-encoded RNAs that are stably attached to the cell surface and exposed to the extracellular space, hereafter called membrane-associated extracellular RNAs (maxRNAs). We developed a technique called Surface-seq to selectively sequence maxRNAs and validated two Surface-seq identified maxRNAs by RNA fluorescence in situ hybridization. To test for cell-type specificity of maxRNA, we used antisense oligos to hybridize to single-stranded transcripts exposed on the surface of human peripheral blood mononuclear cells (PBMCs). Combining this strategy with imaging flow cytometry, single-cell RNA sequencing, and maxRNA sequencing, we identified monocyte as the major type of maxRNA+ PBMCs and prioritized 11 candidate maxRNAs for functional tests. Extracellular application of antisense oligos of FNDC3B and CTSS transcripts inhibited monocyte adhesion to vascular endothelial cells. Collectively, these data highlight maxRNAs as a functional component of the cell surface, suggesting an expanded role for RNA in cell-cell and cell-environment interactions.

Mitochondrial Origin of Cytosolic Protein Aggregation and Proteostasis

Chuankai Zhou, Buck Institute for Research on Aging (poster presenter)

A key process of mitochondrial biogenesis is the import of mitochondrial proteins that are synthesized in the cytosol. These mitochondrial proteins are maintained in unfolded states in the cytosol and cause cytosolic proteostasis stress if not imported into mitochondria. One common consequence of cytosolic proteostasis stress is the formation of protein aggregates that are attached to the mitochondrial outer membrane. It remains unknown why cytosolic protein aggregates are attached to the mitochondria. Here we show that in budding yeast, Tom70, a conserved receptor for mitochondrial import, nucleates the stress-induced aggregation of cytosolic proteins. Anchoring Tom70 or some of its substrates on the vacuole membrane converts this organelle into a primary site for the formation and attachment of cytosolic protein aggregates. This feature is rooted in the misfolding of some, but not all, Tom70 substrates in the cytosol. Cells monitor this weak point of cytosolic proteostasis by adjusting the transcriptional activity of these mitochondrial proteins to match mitochondrial import through a Tom70-mtDNA-FKH1/2 pathway. Tom70's role in transcriptional control of mitochondrial protein is conserved in fruit fly. Our results suggest that Tom70 sits at the crossroad of cytosolic proteostasis and mitochondrial biogenesis by regulating both the synthesis and import of mitochondrial proteins, while nucleating the aggregation of cytosolic proteins and summoning machineries to degrade these misfolded proteins on mitochondrial surface when this balance is disrupted by stresses. The reduction of Tom70 during aging causes age-dependent mitochondrial defects in the biogenesis of mitochondrial proteins and attachment of cytosolic protein aggregates, both can be rescued by overexpressing TOM70. This interdependence between cytosolic proteostasis and mitochondrial biogenesis explains the

observations that mitochondrial dysfunction and protein aggregation are two closely related hallmarks of aging.

Neuroscience

Wiring Logic of the Early Rodent Olfactory System Revealed by High-Throughput Sequencing of Single Neuron Projections

Dinu Albeanu, Cold Spring Harbor Laboratory (poster presenter)

The algorithms used to process sensory information dictate the structure of underlying neuronal circuits. This circuit connectivity in turn often provides insight into the relevant stimulus features, such as topographic location, orientation, or sound frequency. In the olfactory system, anatomical studies have found that principal neurons of the olfactory bulb (OB) innervate the piriform cortex (PC) in a broad and seemingly unstructured fashion. The apparent absence of orderly connectivity from OB to PC together with reports of distributed connections within PC inspired computational models of circuit function that rely on random connectivity. Here we have exploited the high throughput of Multiplexed Analysis of Projections by sequencing (MAPseq) and Barcoded Anatomy Resolved by sequencing (BARseq) to obtain the projections of 5,309 OB and 30,433 PC output neurons in the mouse at single-cell resolution. Analysis of this dataset reveals previously unrecognized spatial structure in the connectivity of both PC inputs and outputs. We identify specific projection gradients in the OB output neurons co-innervating PC and subsets of extra-piriform OB target regions. Distinct populations of narrowly- and broadly-projecting OB cells tile differentially the anterior-posterior (A-P) axis of PC. Furthermore, characteristic groups of PC output neurons, also organized along its A-P axis engage local intra-piriform connectivity and innervate specific sets of brain regions. Strikingly, input-output parallel circuit motifs spanning the A-P axis of PC emerge: a given PC output neuron appears to be wired up such that the strength of its projection to specific extra-piriform OB targets matches the strength of its dominant OB input co-projection to the same region. Our findings suggest an organizing principle of matched, direct and indirect pathways in the olfactory system and challenge the canonical model of piriform cortex as a random network.

Specialized Mechanosensory Epithelial Cells in Gut Intrinsic Tactile Sensitivity

Arthur Beyder, Mayo Clinic (poster presenter)

A major function of the gastrointestinal (GI) tract is to extract nutrients from ingested meals. In addition to nutrients, meals contain toxins and infectious agents. Consequently, most animals conduct the entire digestive process within the GI tract, but luminal contents are entirely outside the body, separated by the tightly sealed GI epithelium. Therefore, like skin and oral cavity, the gut must sense the chemical and physical properties of luminal contents to optimize digestion and nutrient absorption. The gut epithelium contains specialized sensory enteroendocrine cells (EECs) that intimately interact with luminal contents. While chemical sensing is relatively well understood, physical sensing of luminal contents remains unclear. A subpopulation of EECs expresses the mechanically gated ion channel Piezo2 and are

developmentally and functionally similar to the skin's touch sensor, the Merkel cell. We used a range of techniques in single cells to living animals, to determine the function of these sensory cells and their roles in GI physiology. We found that Piezo2+ EECs are synaptically connected mechanosensory enteroendocrine cells and that they rely on the Piezo2 to release serotonin in response to force, and thereby play critical roles in regulating epithelial secretion and motility. Using in vitro and in vivo studies, we determined that the Piezo2+ EECs specialize in detecting small luminal forces and luminal contents' physical properties to regulate GI motility and distribution of luminal contents, which are critical aspects of digestion. In all, our results suggest that the GI tract has intrinsic tactile sensitivity that depends on Piezo2+ EECs.

The Origins of Vocal Learning Circuits During Development

Lomax Boyd, The Rockefeller University (poster presenter)

The neural circuits underlying human speech exhibit a striking level of convergence with other vocal learning species, which all share the ability to produce novel vocalizations acquired through social experience. The presence of a direct cortico-motoneuronal projection—from the primary motor cortex to the brainstem motor neurons controlling the vocal organs—is thought to be a critical and highly derived feature found only in vocal learners. How direct projections for vocal learning form during development or, more broadly, how these novel cortico-motoneuronal circuits for speech evolved from pre-existing motor pathways in the neocortex, are unknown. Recent work has demonstrated that cortico-motoneuronal circuits for limb dexterity, which are highly abundant in humans but depauperate in nonhuman primates, are transiently formed during early postnatal development in rodents. Our group previously discovered that laboratory strains of mice, a species capable of ultrasonic (non-learning) vocalizations, also possess a sparse vocal cortico-motoneuronal (vCM) connection, which argues that the basic constituents of vocal-learning circuits may be more broadly conserved, and open to molecular genetic investigation. Given that projection neurons in the motor cortex are known to extend exuberant collaterals during development, which are subsequently pruned via regionalized and species-specific expression of axon guidance cues, we propose that vCM projections may emerge through developmental co-option of pathways regulating postnatal axonogenesis. We provide preliminary evidence of a population of vCM neurons in the neocortex of mice that make transient connections with motor neurons innervating the laryngeal muscles. Knocking down the axon guidance gene, PlxnA1, in layer V neurons of the neocortex alters juvenile ultrasonic vocalizations and increases the number of vCM neurons in subadults. We argue that interrogation of the largely underexplored diversity of transient neural circuits could contribute significantly to our understanding of pathways critical for human speech.

Elucidating the Molecular and Circuit Consequences of EBF3 Haploinsufficiency in Neuropsychiatric and Developmental Disorders

Hsiao-Tuan Chao, Baylor College of Medicine (poster presenter)

Pathogenic variants and whole gene deletion of EBF3, encoding the Collier/Olf/EBF (COE) transcription factor Early B-cell Factor 3, cause the Hypotonia, Ataxia, and Delayed

Development Syndrome (HADDS) and other neuropsychiatric and developmental disorders including 10q26-deletion syndrome and non-syndromic autism. HADDS is characterized by variable cerebellar-vermian hypoplasia, delayed motor and language development, motor incoordination, altered sensory perception, stereotyped behaviors, and high prevalence of co-morbid neuropsychiatric features including autism and ADHD. The mechanisms mediating these features are poorly understood. To examine the pathogenic role of EBF3 haploinsufficiency, we generated novel mouse Ebf3 null alleles by CRISPR/Cas9 targeted deletion of exons two through four. We found that Ebf3 haploinsufficient mice exhibit delayed growth, altered motor function, repetitive behaviors, and altered social behavior. Cell type–specific Ebf3 haploinsufficiency in either inhibitory or excitatory neurons selectively disrupts developmental processes. In conjunction with fruit fly models of EBF3 pathogenic variants, we found that loss of Ebf3 disrupt molecular pathways regulating cerebellar development. These findings demonstrate that Ebf3 haploinsufficiency perturbs neural circuits in a cell type–specific manner and contributes to neuropsychiatric phenotypes.

The Subcortical Connectome of the Human Default Mode Network

Brian Edlow, Massachusetts General Hospital (poster presenter)

The default mode network (DMN) mediates self-awareness and introspection, core components of human consciousness. Therapies to restore consciousness in patients with severe brain injuries have historically targeted subcortical sites to reactivate cortical DMN nodes. However, the subcortical connectome of the DMN has not been fully mapped, and optimal subcortical targets for therapeutic neuromodulation of consciousness have not been identified. Here, we aimed to create a comprehensive map of the DMN's subcortical connectome by combining ultra-high resolution functional and structural datasets with advanced signal processing methods. We analyzed 7 Tesla resting-state functional MRI (rs-fMRI) data from 84 healthy volunteers acquired in the Human Connectome Project. Cortical and subcortical DMN nodes were identified by applying a Nesterov-Accelerated SCAlable and Robust (NASCAR) tensor decomposition method. The subcortical connectivity map was then overlaid on a 100 micron ex vivo MRI dataset for neuroanatomic analysis. The NASCAR method revealed that the central lateral thalamic nuclei, lateral hypothalamic nuclei, and basal forebrain are highly positively interconnected with the cortical DMN. Multiple brainstem arousal nuclei also connected with the DMN, with the strongest correlations observed within the ventral tegmental area and the dorsal and median raphe. We also found that the putamen and globus pallidus are negatively correlated (i.e., anti-correlated) with cortical DMN nodes, providing rs-fMRI evidence for the "mesocircuit hypothesis" of human consciousness, whereby a striatopallidal feedback system modulates anterior forebrain function via disinhibition of the central thalamus. The DMN subcortical connectome identified here advances understanding of the subcortical regions that contribute to human consciousness and can be used to inform the selection of therapeutic targets in clinical trials for patients with disorders of consciousness.

Unexpected Role for Olfactory Receptors in Neurodegeneration

Paul Greer, University of Massachusetts Medical School (poster presenter)

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that exacts a devastating toll on the individual with the disease, their family, and our society. Nevertheless, despite considerable effort, existing therapeutic strategies targeting AD are limited in both number and efficacy suggesting that novel approaches for preventing or treating AD are needed. Potential unexplored therapeutic targets for treating AD have recently come to light thanks to human genetic studies, which have revealed a relatively small number of genes whose mutation is linked to altered susceptibility to AD. Among the most intriguing of these newly identified AD-associated genes are members of the Membrane Spanning 4a (Ms4a) gene family, whose polymorphisms have repeatedly been shown through dozens of human genome wide association studies (GWAS) to be strongly and reproducibly linked with AD. In fact, current genetic data suggest that up to 10 percent of all AD cases worldwide may be attributable to Ms4a polymorphism. Unfortunately, however, despite the compelling genetic data linking polymorphisms in Ms4a genes with altered susceptibility to AD, as the vast majority of Ms4a polymorphisms are not located in the coding sequences of these genes, it has been largely unclear how these polymorphisms regulate Ms4a gene expression or function. In fact, it is still unknown whether Ms4a loss of function is protective or harmful for AD. Answering this question is critical to understanding the role of MS4As in AD and for ultimately developing AD drugs that target MS4As. We here present data using Ms4a knockout mice that we have bred with two different mouse models of Alzheimer's disease that addresses this fundamental gap in knowledge. This work sets the stage for the rest of our proposed research aimed at elucidating the mechanisms by which Ms4a mutation mediates AD pathogenesis.

Towards Whole-Brain Electrophysiological Readouts of the Mammalian Nervous System: Injectable Electromagnetic Probes for MRI

Aviad Hai, University of Wisconsin-Madison/Department of Biomedical Engineering (poster presenter)

There is currently a surging effort to develop technologies for recording neural activity from the entire volume of the brain in parallel. Whole-brain direct readouts of neural signals will be critical to understanding the elusive cross-regional communication grid underlying brain function and dysfunction. The goal of Dr. Hai's New Innovator Award is the development and application of a new form of brain imaging using electromagnetic circuits that can be deployed throughout the brain and provide parallel volumetric electrophysiological readouts of neural activity. The project relies on advances made by the principal investigator, demonstrating tetherless microelectronic neural interfaces that transduce neurophysiological events wirelessly to detectable magnetic field perturbations that are monitored by magnetic resonance imaging (MRI). By combining the unique three-dimensional capabilities of MRI to obtain functional readouts from the entire volume of the brain, with electromagnetic probes—that can directly record electrophysiological neural activity in-situ and transmit its response to the MRI hardware—this project is aiming to transform the way we acquire brain signals. Dr. Hai's group uses nanofabrication methods to pioneer cell-sized wireless probes, while employing existing

state-of-the-art MRI-compatible microelectrode arrays in rodents for rigorous validation of new technologies and for decoupling electrophysiological readouts from intrinsic fMRI blood-flow signals. The neuroelectronic MRI probes developed under the umbrella of this award will augment current brain recording capabilities, by presenting a different approach whereby minimally invasive devices are powered by the MRI scanner itself without bulky on-board power, and secondly, by interacting with the imaging scanner to transmit electrical neural activity to the detection hardware outside of the brain with no requirement for a tethered connection. The sensors will be used to directly detect electrical neural activity in three-dimensions and tracing the origins of brain physiology.

Infralimbic to Nucleus Accumbens Projections Limit Opioid Addiction

Jasper Heinsbroek, University of Colorado School of Medicine (poster presenter)

High rates of opioid use and associated overdose deaths remain a major health emergency. The neural circuitry controlling relapse to opioid seeking includes a prefrontal cortex to nucleus accumbens circuit, a so-called final common pathway to relapse. Specifically, the ventral component of this pathway arising in the infralimbic (IL) cortex and projecting to the shell of the nucleus accumbens (NAshell) has been shown to limit cocaine seeking. However, to date, no neural circuit has been identified that limits opioid seeking. In addition, no neural circuit has been identified for choice between opioids versus competing natural rewards. We examined the role of IL-NAshell neurons during heroin choice and relapse in a preclinical rat model of heroin addiction. Behavioral economics analyses indicated rats were more motivated for heroin than food reward. When animals were forced to make mutually exclusive choices between heroin or food, subpopulations of heroin versus food preferring rats emerged. However, opioid choice and subsequent relapse to heroin versus food cues were unrelated, suggesting that choice and relapse are distinct behavioral constructs. Supporting this, inactivation of the IL with the GABAA agonist muscimol produced differential effects on opioid choice versus relapse. However, a pathway-specific chemogenetic approach revealed that a subpopulation of IL-NAshell projecting neurons act as a common limiter to both opioid choice and relapse, but not food relapse. In addition, dendritic spine measurements from IL-NAshell neurons correlated with distinct measures of heroin versus food motivation. Collectively, these data support the notion that opioid choice and relapse share a common heroin-limiting circuit in the IL-NAshell pathway.

Reorganization of Cortical Activity Patterns Supports Learning of Tactile Shape Discrimination

Andrew Hires, University of Southern California (poster presenter)

Tactile shape perception is critical for object recognition and tool use. Three-dimensional shape of an object is defined by the contour of object surface, which can be perceived by integrating object surface angles from multiple contact points. Thus, surface angle information is fundamental to object shape perception. We investigated how object surface angle is perceived during active touch using mouse whisker system. Head-fixed mice were trained to discriminate object surface angle using a single whisker. Before and after learning, we tested mice with

seven angles (45° to 135°) to quantify their resolution in angle discrimination. Across training, we recorded the same set of thousands of excitatory neurons in the primary somatosensory cortex (S1) to examine cortical representation of object angle and its change across angle discrimination learning. Mice learned to discriminate two angles in about 2 weeks, and their angle discrimination resolution was at least 15°. Using generalized linear models we extracted the most important sensory inputs in discriminating object angles: vertical bending and slide distance along the object. Encoding of these two sensory inputs were combined to generate object-angle tuning in majority (~80%) of touch-responsive excitatory neurons in S1, both before and after learning. We observed that only about 40 percent of neurons remained active across learning. Despite this high activity turnover rate, object-angle tuning remained stable in both population distribution and persistently active individual neurons. Sensory inputs as well as whisker movements also remained unchanged across learning. However, population activity patterns became better at discriminating object angles after learning, partly due to increased encoding of task-relevant sensory input, vertical bending, in newly recruited touch-responsive neurons. Collectively, the results suggest how primary sensory cortex can adapt to support learned behavior while maintaining a faithful representation of outside world during active sensation.

Personalizing Neuromodulation for Executive Functions: Promise in Brain Networks and Society

John Medaglia, Drexel University (speaker)

Individual differences in cognition and responses to neuromodulation treatments depend on distributed brain network functions and the anatomy that support them. Over 5 years of Early Independence Award support, our laboratory has clarified the basis of executive functions in joint anatomical-functional brain network and linked models of network controllability to variability in neuromodulation outcomes. In addition, we have clarified the psychological mediators of neuroethical choices that the public makes about whether to use neuromodulation. Collectively, these lines of inquiry have revealed the promises and pitfalls in imaging-guided neuromodulation for executive functions and when the public might accept specific uses of technology. Here, we will share the key findings from our research program in personalized neuromodulation for frontal lobe functions and the context of public attitudes in which they exist.

Single Cell Sequencing During Cortical Remodeling Reveals a Conserved Type I Interferon-Responsive Microglial Subset with Enhanced Phagocytic Capacity

Anna Molofsky, University of California San Francisco (poster presenter)

Glia—the non-neuronal cells of the brain—are exquisitely sensitive to dynamic changes in the brain environment. Here we examined cell type–specific transcriptomic responses of glia to physiologic neural circuit remodeling in vivo. Using single-cell transcriptomics, we show that astrocytes and microglia in rodent brain undergo transcriptomic shifts when sensory input from whiskers is deprived, a perturbation that leads to remapping of circuits in the whisker, or “barrel” cortex. Astrocytes in the remodeling cortex increased expression of genes associated

with synaptogenesis and extracellular matrix remodeling. In contrast, microglia exhibited three distinct phagocytic states that shifted in abundance after whisker lesion. Among these, we identified an interferon-responsive microglia population (IMP) that was rare in the resting cortex but expanded 20-fold after whisker deprivation. The top gene candidate in this cluster, Ifitm3, marked a conserved but transient subset of microglia in the remodeling postnatal barrel cortex in vivo. IFITM3+ microglia had a unique elongated morphology and were highly enriched for phagocytic cup formation and lysosomal activity. Interestingly, IFITM3 protein marked a nascent phagocytic cup, rather than a mature phagolysosome, suggesting that interferon signaling is required for a distinct phase of phagocytosis initiation. Consistent with this, mice lacking the type I interferon receptor (IFNAR1-/-) had defective phagocytic cup formation. We identified IFITM3+ phagocytic microglia in two disease models—SARS-CoV-2 infection into the hACE2 mouse, and the 5xFAD model of Alzheimer's disease—suggesting that this is a conserved microglial phagocytic program in disease as well as development. These data reveal coordinated glial responses to developmental circuit remodeling and lead to unexpected discoveries about the mechanisms governing microglial phagocytosis, based on a transient and distinct phase in microglial phagocytic cycle.

Model for Postnatal Interneuron Migration in the Gyrencephalic Brain

Mercedes Paredes, University of California, San Francisco (poster presenter)

Inhibitory neurons (interneurons) are a neuronal subpopulation implicated in the pathogenesis of neurodevelopmental disorders (NDDs) such as epilepsy and autism spectrum disorder (ASD). Interneurons originate in a fetal structure, the ganglionic eminence, which is further divided into medial, lateral, and caudal subregions (MGE, LGE, and CGE, respectively). Each area generates distinct interneuron subtypes with unique functions. We have previously shown that doublecortin (DCX)+ interneurons migrate through a ventricular structure, called the Arc, to areas of the frontal lobe for several months after birth. However, the extent of cortical migration in the infant brain and molecular profile of these neurons remains unknown. We use the piglet brain as a model for human postnatal cortical development given its anatomical and developmental similarities to the human brain. We map distribution of DCX+ neurons in the postnatal day 0 (P0) piglet cortex and identify additional cortical targets for migratory neurons in the postnatal cortex, including the insula and temporal lobe. We also quantify the composition of migratory DCX+ cells in the Embryonic day 89 (E89), E100, and P0 piglet cortex, using regional transcription factors such as LHX6 and NKX2.1 for the MGE and SP8 and COUPTFII for the CGE. Although a subset of these interneurons are derived from the MGE, the piglet Arc contains a majority of interneurons that originated in the CGE; the human Arc had a similar composition. Thus, CGE-derived neurons are the primary contributors to this wave of "late cortical migration" in the human and piglet brains. This study shows that the cellular composition of the postnatal cortex remains dynamic and supports that protracted interneuron development is conserved in gyrencephalic brains. It also provides a novel platform to understand the emergence of NDDs and reveal ways to therapeutically influence how neurons organize themselves, even after a child is born.

Spinal Cord Neural Interface for Neuroprosthetics in a Primate Model

Abhishek Prasad, University of Miami (speaker)

There are more than 270,000 cases of spinal cord injury (SCI) in United States alone with more than 12,500 new cases each year. The average life expectancy of those suffering from high tetraplegia is 36.9 years and for low tetraplegia is 40.5 years after injury. The loss of voluntary control over limb movements results in an inability to perform activities of daily living, creating a dependence on caregivers. Recent advances in brain-controlled neuroprosthetics have shown proof-of-concept and clinical application in paralyzed individuals. Here, we describe a novel neural interface in which signals recorded from the spinal cord can be used for neuroprosthetic applications. We developed a marmoset model as a smaller, nonhuman primate model for behavioral neuroscience studies. The common marmoset has been used in other research types but not for behavioral neuroscience studies. We showed that signals can be recorded from different levels of the motor system in the central nervous system in awake, behaving marmosets. Microelectrode arrays were implanted into the marmoset motor cortex, premotor cortex, and the cervical spinal cord. We demonstrated that both spiking and local field potential activity in cortical structures of the marmoset cortex mirror data from macaques, with subsets of the recorded neuron population illustrating modulation with relevant movement parameters. We further discuss the housing and training details, technical challenges, and surgical details to target motor structures and record chronically for neural interfacing with the cortex and spinal cord in this animal model. Chronic recordings from neurons in the marmoset spinal cord were successful up to 3-months post-implant, and subsets of recorded channels exhibited movement related modulation. It was also possible to classify different phases of the forelimb movement and decode movement direction across all recording sites, demonstrating that the spinal cord presents a viable recording site for future neuroprosthetics.

Communication Between Networks: Context, Inhibition, and Neuromodulation

Caroline Runyan, University of Pittsburgh (poster presenter)

The brain is often bombarded by streams of information from multiple sources simultaneously. The goal of our lab is to understand the mechanisms that underlie the flexibility of information processing in cortical circuits, focusing on how inhibitory neurons gate the flow of information between sensory and association regions in a context-dependent manner. Head-fixed mice voluntarily running on a spherical treadmill are rapidly shifted between behavioral contexts within single sessions. In each context, two-photon imaging of calcium activity is used to monitor the responses of hundreds of genetically labeled inhibitory and excitatory neurons simultaneously. In some experiments, optogenetic stimulation is used to activate specific incoming projections to the imaged region, or to inactivate specific cell types. These tools are combined in three main projects. In the first project, the inhibitory mechanisms gating the flow of information between cortical regions will be dissected, to determine whether canonical rules define inhibitory operations across cortex, or if local specializations allow greater flexibility at different hierarchical levels of the cerebral cortex. In the second project, the roles of inhibitory neurons in setting network dynamics will be determined, and the consequences of shifting network dynamics on signal processing will be defined. In the third project, neuromodulatory

recruitment of inhibitory circuits across the cortical hierarchy will be described, to determine how shifts in brain state affect information processing. To understand the neural underpinnings of perception, attention, and behavioral flexibility, it is critical to study the interaction between brain areas, rather than to focus on single brain regions in isolation. The experiments proposed here will use new tools to answer fundamental questions about how local circuits interact to process information, toward the goal of understanding how the distributed cortical network gives rise to cognitive processes such as attention and perception.

Topological Data Analysis Reveals a Unique Hub-Like Transition State at Rest in Highly Sampled Individuals

Manish Saggar, Stanford University (poster presenter)

Even in the absence of external stimuli, neural activity is both highly dynamic and organized across multiple spatiotemporal scales. The continuous evolution of brain activity patterns during rest is believed to help maintain a rich repertoire of possible functional configurations. Whether these transitions or "explorations" follow some underlying arrangement or instead lack a predictable ordered plan remains to be determined. Although innovative approaches have been previously developed to reveal the temporal structure underlying transitions in the brain at rest, several methodological and data quality issues precluded the development of a clear understanding of brain dynamics at the individual level. Here, we overcame these limitations by examining the dynamic states during intrinsic brain activity using Topological Data Analysis (TDA) on a maximally denoised precision neuroscience dataset—The Midnight Scan Club (MSC). This dataset consists of 10 highly sampled individuals, with greater than 5 hours of resting state fMRI data per individual and individualized parcellations of each brain. Without temporal averaging or sliding windows, our TDA-based approach mapped whole-brain resting state volumes onto a set of individually defined intrinsic dynamical manifolds or "state spaces." For reliability, all reported results were validated using split-half cross-validation and on an independent dataset from the Human Connectome Project. Using our TDA-based approach, we observed a rich topographic landscape in which the transition of activity from one network to the next involved a large shared attractor-like basin, or "transition state," where all networks were represented equally prior to entering distinct network configurations. The intermediate hub-like transition state seemed to provide the underlying structure for the continuous evolution of brain activity patterns at rest. In addition, differences in the manifold architecture were more consistent within than between subjects, providing evidence that this approach contains potential utility for precision medicine approaches.

Refinement of Corticospinal Neuron Activity During Skilled Motor Acquisition

Najet Serradj, Burke Neurological Institute (poster presenter)

The learning of motor skills relies on plasticity of the primary motor cortex as task acquisition drives the remodeling of cortical motor networks. Large-scale cortical remodeling of evoked motor outputs occurs in response to the learning of skilled, corticospinal-dependent behavior, but not simple, unskilled tasks. We determined the response of corticospinal neurons to both skilled and unskilled motor training and assessed the role of corticospinal neuron activity in the

execution of the trained behaviors. Using in vivo calcium imaging, we found that refinement of corticospinal activity correlated with the development of skilled, but not unskilled, motor expertise. Animals that failed to learn our skilled task exhibited a limited repertoire of dynamic movements and a corresponding absence of network modulation. Transection of the corticospinal tract and aberrant activation of corticospinal neurons show the necessity for corticospinal network activity patterns in the execution of skilled, but not unskilled, movement. We revealed a critical role for corticospinal network modulation in the learning and execution of skilled motor movements. The integrity of the corticospinal tract is essential to the recovery of voluntary movement after central nervous system injuries, and these findings should help to shape translational approaches to motor recovery.

Presynaptic Maturation Determines Activity-Dependent Eye-Specific Competition
Colenso Speer, University of Maryland (poster presenter)

The development of eye-specific connections to the brain is a classic model for investigating activity-dependent synaptic competition and refinement. In mammals, axons from the two eyes overlap and compete for target territory in an activity-dependent process driven by patterned spontaneous retinal activity (“retinal waves”). Disrupting or blocking retinal waves causes eye-specific axon segregation defects, but the ultrastructural basis of activity-dependent synaptogenesis and pruning is unknown. Immature eye-specific synapses are difficult to positively identify within electron microscopy images, while diffraction-limited optical imaging methods have low spatial resolution that precludes synapse analysis. Thus, there is a gap in understanding how neural activity shapes the development of synaptic connections from the eyes to the brain. To address this challenge, we used volumetric STochastic Optical Reconstruction Microscopy (STORM), a single-molecule localization super-resolution imaging technique, together with eye-specific anterograde tracing and synaptic immunolabeling to measure nanoscale structural properties of tens of thousands of retinogeniculate synapses during eye-specific competition in the mouse. We found that maturing eye-specific synapses in the “correct” territory show developmental increases in presynaptic active zone (AZ) and vesicle-associated proteins with no change in postsynaptic density (PSD) proteins. In contrast, weaker “incorrect” RGC connections have significantly fewer presynaptic vesicle proteins with no change in the PSD. To examine the role of neural activity in synaptic competition, we analyzed synapses in a mutant model with disrupted cholinergic retinal waves and arrested eye-specific axon segregation. Abnormal retinal wave activity disrupted synaptogenesis, prevented the presynaptic maturation of vesicle pools, and reduced the magnitude of eye-specific synaptic competition with no change in synaptic PSD volume. These results show that presynaptic refinement defines activity-dependent eye-specific synaptic competition and provide support for a synaptotropic mechanism of retinogeniculate axon development.

Assessing the Functional Representation of Vocal Musculature in the Motor Cortex of Mice

Cesar Vargas, The Rockefeller University (poster presenter)

Vocalizations are a complex behavior that can be innate or learned. Vocal learners, animals that learn to imitate or modify vocalizations, use top-down input from the forebrain to provide fine motor control over vocal musculature. These forebrain circuits coordinate sub-cortical networks during, and after, learning to precisely execute these learned behaviors. Currently, we do not have genetically tractable mammalian models for speech and vocal learning. However, behavioral and anatomical findings from our lab and others' have shown that the ultrasonic song system of mice exhibits rudimentary features consistent with advanced features of vocal learners, including multisyllabic structure that varies with social context, and a pool of cortical neurons in posteromedial M1 that projects directly to brainstem vocal motor neurons. To test whether these M1 neurons can functionally activate vocal muscles, and possibly provide fine motor coordination, we used intracortical microstimulation (ICMS) paired with electromyography (EMG) from vocal muscles in the larynx of anesthetized mice. We use latency from stimulation to EMG response to estimate the number of synapses between the cortex and the muscles, with shorter latencies, meaning there are fewer neurons in the circuit (i.e., more direct connectivity). Using this approach, we found a representation of laryngeal musculature in posteromedial M1, consistent with our anatomical tracing, but also a second representation in anterolateral M1. The two regions have distinct latency and stimulation thresholds from one another. The anterolateral region, corresponding to orofacial motor cortex (OFC), has shorter latencies-to-response from stimulation and lower current injection thresholds. Conversely, the posteromedial region has longer latencies and higher thresholds. These two representations possibly underlie different functions of the laryngeal musculature in different behaviors, such as voluntary swallowing, breathing, or vocalizing. These results are the first characterization of the functional representations of laryngeal musculature in the rodent motor cortex.

Synergistic Effector/Environment Decoding in Primate Motor Cortex

Carlos Varrgas-Irwin, Brown University (poster presenter)

Brain computer interface (BCI) technology can be used to bypass damaged motor pathways, allowing people with movement disorders to directly control assistive devices with their thoughts. Current BCI systems are designed to detect information related to intended movements, while excluding information reflecting sensory inputs. However, sensory and motor information are tightly intertwined in cortical circuits, making it difficult to isolate signals encoding motor intention. The goal of this project is to generate new decoding models that simultaneously capture the motor and sensory components of neural activity. Our approach is based on the analysis of three different types of data: single unit neural ensemble activity, 3D tracking of upper limb motion (from shoulder to fingertips), and estimates of the position and shape of objects in the environment. Neural data are collected using microelectrode arrays chronically implanted into the motor cortex of rhesus macaques. Limbs and objects are tracked using a state-of-the-art markerless motion capture system that relies on transfer learning using deep neural networks (DeepLabCut, Mathis lab). These data streams will allow us to generate

models that (1) predict the activity of individual neurons based on upper limb kinematics and object information derived from the visual scene and (2) predict movement kinematics based on neural activity combined with object information, using camera inputs as surrogate sensory data. Our preliminary results validate our approach by demonstrating that models based solely on movement kinematics fail to capture the full variance in firing rates displayed by cortical neurons, and movement prediction significantly improves by taking into account object information derived from the visual scene. Our results will expand our understanding of visuomotor cortical networks, and will also contribute to the development of more effective BCI devices aiming to restore dexterous movement control to people with motor disorders.

Phasic Activity in Serotonin Neurons and Emotional Reactivity

Melissa Warden, Cornell University (speaker)

Survival depends on selecting and executing behaviors that are adaptive for the current environment. For example, a mouse should run from a rapidly looming hawk but should freeze if the hawk is coasting across the sky. Although serotonin has been implicated in adaptive, context-dependent behavior, environmental regulation of its functional role remains poorly understood. In mice, we found that brief stimulation of dorsal raphe serotonin neurons suppressed movement in low- and moderate-threat environments but induced escape behavior in high-threat environments, and that movement-related dorsal raphe serotonin neural dynamics inverted in high-threat environments. Although serotonin neurons appear to switch their functional role in different environments, we suggest an integrative framework in which phasic activity in serotonin neurons provokes a fast, environmentally appropriate emotional reaction—freeze at moderate threat, escape at high threat. This hypothesis cleanly explains our data, and has the potential to encompass a wide range of other environment- and internal state-dependent functions ascribed to this neuromodulatory system. We will discuss how this framework may have relevance for understanding the anti-anxiety effects of chronically administered selective serotonin reuptake inhibitors.

A Novel Oscillator in the Brainstem Synchronizes Neonatal Crying with Breathing

Paul Wei, University of California, San Francisco (poster presenter)

Human speech can be divided into short, rhythmically-timed elements, similar to syllables within words. Even our cries and laughs, as well as the vocalizations of other species, are periodic. However, the cellular and molecular mechanisms underlying the tempo of mammalian vocalizations remain unknown. Here we describe rhythmically-timed neonatal mouse vocalizations that occur within single breaths, and identify a brainstem node that structures these cries, which we name the intermediate reticular oscillator (iRO). We show that the iRO acts autonomously and sends direct inputs to key muscles in order to coordinate neonatal vocalizations with breathing, as well as paces and patterns these cries. These results reveal that a novel mammalian brainstem oscillator embedded within the conserved breathing circuitry plays a central role in the production of neonatal vocalizations.

Chromatin Architecture in Addiction Circuitry Elucidates Biological Mechanisms Underlying Cigarette Smoking and Alcohol Use Traits

Hyejung Won, University of North Carolina at Chapel Hill (poster presenter)

Cigarette smoking and alcohol use are among the most prevalent substances used worldwide and account for a substantial proportion of preventable morbidity and mortality, underscoring the public health significance of understanding their etiology. Genome-wide association studies (GWAS) have successfully identified genetic variants associated with cigarette smoking and alcohol use traits. However, the vast majority of risk variants reside in non-coding regions of the genome, and their target genes and neurobiological mechanisms are unknown. Chromosomal conformation mappings can address this knowledge gap by charting the interaction profiles of risk-associated regulatory variants with target genes. To investigate the functional impact of common variants associated with cigarette smoking and alcohol use traits, we applied Hi-C coupled MAGMA (H-MAGMA) built upon cortical and midbrain dopaminergic neuronal Hi-C datasets to GWAS summary statistics of nicotine dependence, cigarettes per day, problematic alcohol use, and drinks per week. The identified risk genes mapped to key pathways associated with cigarette smoking and alcohol use traits, including drug metabolic processes and neuronal apoptosis. Risk genes were highly expressed in cortical glutamatergic, midbrain dopaminergic, GABAergic, and serotonergic neurons, suggesting them as relevant cell types in understanding the mechanisms by which genetic risk factors influence cigarette smoking and alcohol use. Lastly, we identified pleiotropic genes between cigarette smoking and alcohol use traits under the assumption that they may reveal substance-agnostic, shared neurobiological mechanisms of addiction. The number of pleiotropic genes was ~26-fold higher in dopaminergic neurons than in cortical neurons, emphasizing the critical role of ascending dopaminergic pathways in mediating general addiction phenotypes. Collectively, brain region- and neuronal subtype-specific 3D genome architecture helps refine neurobiological hypotheses for smoking, alcohol, and general addiction phenotypes by linking genetic risk factors to their target genes.

Transcriptional Control of Brain Excitation in Neurodevelopmental Disorders

Bruce Yankner, Harvard Medical School (speaker)

A novel pathway of stress resistance in the brain has recently been described that is mediated by the transcriptional repressor REST/NRSF. We have provided evidence that this pathway is involved in lifespan regulation and the pathogenesis of neurodegenerative disorders (Lu et al., 2014; Meyer et al., 2019; Zullo et al., 2019). REST also plays an essential role in early brain development by regulating neural specification and neuronal differentiation. As such, we asked whether the REST corepressor pathway might play a role in the pathogenesis of neuropsychiatric disorders that may arise from altered brain development. Examination of recent GWAS studies showed that genetic variants associated with bipolar disorder are significantly enriched for REST target genes. Immunofluorescence microscopy showed that nuclear REST levels in neurons of the prefrontal cortex were significantly reduced in patients with bipolar disorder relative to normal controls. Furthermore, studies of gene expression showed that REST target genes, such as ASCL1 and synapsin 1, were upregulated in the prefrontal cortex of patients with bipolar disorder, consistent with loss-of-function of the REST

transcriptional repressor. Reduced REST expression and function were recapitulated in vitro in iPS cell-derived neurons from patients with bipolar disorder, suggesting that loss of REST may be an early event in bipolar disorder. To explore the consequences of loss of REST in the adult brain, we established conditional REST-deficient mice. Neural excitation was globally increased in the cerebral cortex of REST-deficient mice, as determined by PET/CT scanning and electroencephalography. Moreover, loss of REST elevated the sensitivity of cortical neurons to excitatory stimuli. These results suggest that loss-of-function in the REST corepressor pathway can destabilize neural networks predisposing to neural hyperexcitation. Transcriptional dysregulation might therefore lead to loss of neural network homeostasis in the brain, a potential mechanism for mood instability in bipolar disorder.