



2016 NIH Common Fund's High-Risk, High-Reward Research Symposium

Natcher Conference Center, NIH campus, Bethesda, MD

December 5-7, 2016

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Oral Presentation Abstracts

Monday, December 5, 2016

Session 1

1.1

THE FABRIC OF THE NEOCORTEX: CANONICAL STRUCTURE AND COMPUTATIONS

Pioneer Award, 2011

Andreas Tolias

Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA; Department of Electrical and Computer Engineering, Rice University, Houston, TX, USA

The neocortex is responsible for human perception, cognition and action, and its malfunction underlies numerous neuropsychiatric disorders. Despite major advances in our understanding of the functional properties of single neurons we still do not know how the cortex works at the circuit level. The essence of the problem lies in understanding how billions of neurons communicating through trillions of connections orchestrate their activities to give rise to our mental faculties. We are far from being able to simultaneously measure the activity of all the myriads of cortical cells and assemble their physical wiring diagram (whole brain connectome). However, if there are underlying principles and rules that govern this complexity, these principles could reduce the impenetrable complexity of the cortex to a manageable scale. One such principle is provided by the hypothesis that the cortex is composed of repeated elementary information processing modules, organized along cortical columns. We combine electrophysiological, imaging, and molecular tools with behavioral and machine learning approaches to determine what constitutes the elementary computational circuit motif in the neocortex and characterize its structure, function and decipher its canonical computation(s). I will describe our work towards those goals from three perspectives. First, from an anatomical perspective where we are mapping the detailed wiring diagram of the canonical cortical microcircuit including identifying all the cell types that comprise cortical circuits. Second, using multi-photon imaging and electrophysiological methods we are characterizing the activity structure of large populations of neurons in the visual cortex during behavioral tasks. Third, we are using machine-learning methods to model these circuit motifs with the goal to decipher the canonical algorithm(s) they implement. Our goal is to compare different cortical areas across species, which we hope will ultimately enable us to also understand the evolution of the neocortical motif at the anatomical and computational level.

1.2

INTRACRANIAL STIMULATION RESTORES PERFORMANCE OF A VISUAL DISCRIMINATION TASK IN ANESTHETIZED RODENTS

Transformative Research Award, 2012

Ken Solt,^{1,2,3} Emery Brown,^{1,2,3} Edward Boyden,³ and Matthew Wilson³

¹*Massachusetts General Hospital, Boston, MA, USA;* ²*Harvard Medical School, Boston, MA, USA;*

³*Massachusetts Institute of Technology, Cambridge, MA, USA*

Abstract author: Jonathan D. Kenny

Massachusetts General Hospital, Boston, MA, USA

Presented by Ken Solt

There are numerous clinical problems associated with emergence from general anesthesia, including short-term complications such as respiratory distress and delirium, and long-term consequences such as cognitive dysfunction. The neural circuits that mediate recovery of consciousness and cognition after general anesthesia are unknown, and there are currently no therapeutic options available to promote active brain recovery after general anesthesia. In a previous study, we reported that optogenetic stimulation of VTA dopamine neurons restores conscious behaviors in anesthetized mice, demonstrating that dopamine release by the VTA plays a critical role in anesthetic emergence. In this study, we used anesthetized rats trained to perform a visual discrimination task to test the hypothesis that VTA stimulation restores cognitive function.

Male Sprague-Dawley rats (n = 8) were trained to perform a touchscreen-based visual discrimination task. The chamber was in a sealed enclosure with ports for gas in/outflow and sampling. Two images were presented simultaneously on the touchscreen, and rats were trained to touch the correct image for a food reward. After reaching > 85% correct responses for at least 3 consecutive days, the animals underwent stereotaxic implantation of bipolar stimulation electrodes in the VTA. After recovery for at least 7 days, they were re-introduced to the testing chambers. Within 3 weeks after surgery, the rats recovered to their baseline performance level. A dose-response relationship for the inhaled anesthetic isoflurane (ISO) was then established by exposing the rats to a steady-state dose of ISO (0.1% - 0.5% in air) while performing the cognitive task. The rats were only exposed to ISO once per week.

After establishing that 0.5% ISO reliably extinguished task performance, once a week (for 5 weeks) the rats underwent electrical VTA stimulation during steady-state 0.5% ISO anesthesia, and task performance was assessed. During week 3, the D1 dopamine receptor antagonist SCH-23390 was administered before VTA stimulation. After completing all experiments, histological analysis of electrode placement was performed. The electrode tip was in the VTA in 5/8 animals. Only these animals were used for further analysis.

At 0.5% ISO, all rats were heavily sedated, rarely moved, and performed no trials. However, during VTA stimulation 5/5 rats performed the task despite continuous 0.5% ISO (weeks 1, 2, 4, 5). Administration of SCH-23390 prior to VTA stimulation (week 3) reversibly abolished task performance. Although VTA stimulation during ISO anesthesia restored task performance, mean accuracy was not restored to baseline.

In anesthetized rodents, VTA stimulation restores task performance, and the effect is abolished by a D1 antagonist. VTA dopamine neurons represent a potential therapeutic target to promote active brain recovery after general anesthesia.

1.3

DEVELOPING MECHANISTICALLY INFORMED NEUROIMAGING MARKERS FOR MENTAL ILLNESS VIA PHARMACOLOGY AND COMPUTATION

Early Independence Award, 2012

Alan Anticevic
Yale University, New Haven, CT, USA

Neuropsychiatric illness such as schizophrenia profoundly alter the structure and function of distributed neural networks and presents a massive health and economic burden. Non-invasive functional neuroimaging tools have evolved sufficiently to allow reliable characterization of large-scale distributed neural systems in humans. Such approaches have been applied to better understand large-scale neural network disturbances in neuropsychiatric disease, particularly using resting-state techniques. Here the focus is placed on emerging neuroimaging findings in schizophrenia using resting-state and task-based neuroimaging, an advantageous approach for biomarker development given its ease of data collection and translational potential. Findings in schizophrenia suggest that disruptions in sensory-thalamic-prefrontal networks may hold promise as a marker for treatment effects in future clinical studies and might constitute a final common pathway of neural system disturbances in schizophrenia. However, human neuroimaging does not yet allow the evaluation of individual neurons within local circuits, where pharmacological treatments ultimately exert their effects. This limitation constitutes an important obstacle to the effort to translate findings from animal research to humans and from healthy humans to patient populations. Integrating new neuroscientific tools may help to bridge these gaps. Two complementary approaches are discussed in the context of understanding cognitive deficits in schizophrenia: First, causal pharmacological manipulations in healthy volunteers that transiently mimic some cardinal features of psychiatric conditions. Specific focus is placed on recent neuroimaging studies using the NMDA receptor antagonist, ketamine, to probe glutamate synaptic dysfunction associated with schizophrenia. Second, human pharmacologic imaging is discussed in combination with biophysically-informed computational models developed to guide the interpretation of functional imaging studies and to inform the development of pathophysiologic hypotheses. This approach is illustrated via recent findings showing how computational modeling has guided inferences drawn from studies in healthy subjects administered ketamine and evaluations of schizophrenia patients. In summary, the argument is presented that linking experimental studies in humans with computational models will advance to effort to bridge cellular, systems, and clinical neuroscience approaches to psychiatric disorders.

**SYNTHETIC BIOLOGY PLATFORMS FOR NATURAL PRODUCT
BIOSYNTHESIS AND DISCOVERY**

Pioneer Award, 2012

Christina Smolke

Stanford University, Stanford, CA, USA

Plants are a rich source of unique scaffolds, including greater than 25% of natural-product-derived drugs. However, the discovery, synthesis, and overall material supply chains for sourcing plant natural products and their derivatives remain ad hoc, biased, and tedious. While microbial biosynthesis presents compelling alternatives to traditional approaches based on extraction from natural plant hosts, many challenges exist in the reconstruction of plant specialized metabolic pathways in microbial hosts. My laboratory has developed approaches to address the challenges that arise in the reconstruction of complex biosynthesis schemes, including spatial engineering strategies to direct the activities and specificities of pathway enzymes and recoding strategies to address folding, processing, and stability issues that may arise with the expression of plant enzymes in heterologous microbial hosts. In addition, coupled with advances in functional genomics, these synthetic biology platforms are leading to more efficient strategies for enzyme discovery and characterization in plant natural product pathways. We have utilized these strategies to develop yeast-based production platforms for an important class of plant alkaloids, the benzyloquinoline alkaloids, including the medicinal opioids and noscapinoids. These synthetic biology platforms are leading to transformative advances in natural product discovery, drug development, and production.

2.2

A HARDWIRED HIV LATENCY PROGRAM

Pioneer Award, 2013

Leor Weinberger

Gladstone Institutes, University of California, San Francisco, San Francisco, CA, USA

To preserve fitness in unpredictable, fluctuating environments, a range of biological systems probabilistically generate variant phenotypes—a process often referred to as ‘bet-hedging’, after the financial practice of diversifying assets to minimize risk in volatile markets. The molecular mechanisms enabling bet hedging have remained elusive. Here, we review how HIV makes a bet-hedging decision between active replication and proviral latency, a long-lived dormant state that is the chief barrier to an HIV cure. The discovery of a virus-encoded bet hedging circuit in HIV (Razooky et al., *Cell* 2015; Rouzine et al., *Cell* 2015) revealed an ancient evolutionary role for latency and identified core regulatory principles, such as feedback and stochastic ‘noise’, that enable cell-fate decisions. These core principles were later extended to fate selection in stem cells and cancer, exposed new therapeutic targets for HIV, and led to a potentially broad strategy of using ‘noise modulation’ to redirect cell fate (Dar et al., *Science* 2014).

2.3

LEARNING FROM TIME

Early Independence Award, 2016

Daniela Witten

University of Washington, Seattle, WA, USA

In statistical machine learning, much interest has focused on the task of learning the relationships among a set of variables—say, genes for which expression has been measured. These relationships are typically represented using a "graphical model", a picture in which each variable is represented by a node, and a relationship between a pair of variables is shown as an edge. The goal is to learn the structure of the graph: that is, to determine which pairs of variables are connected by an edge, and which are not. Most existing methods for this task are suited for the setting in which we have n independent observations of the variables—for instance, we have measured gene expression in n distinct mice. However, in many biomedical experiments, measurements are instead taken over time. For instance, we may measure gene expression in a single mouse, at n distinct time-points. In this setting, existing methods that are intended for the case of independent observations cannot be applied. In this talk, I will present recent work from my research group that is intended to bridge this gap. One piece of work focuses on learning the dependence relationships among a set of p genes measured over time, using an additive ordinary differential equation framework. The other piece of work involves learning the dependence relationships among neural spike trains, using a multivariate Hawkes process model.

3.1

STRUCTURE OF HUMAN MEMBRANE TRANSPORTERS BY CRYO-EM AND X-RAY CRYSTALLOGRAPHY

New Innovator Award, 2011

Stephen G. Aller

The University of Alabama at Birmingham, Birmingham, AL, USA

Membrane proteins are encoded by roughly one-third of the human genome and are also targets of ~ 25% of all FDA-approved drugs. A large subset of membrane proteins are embodied by transporters which can utilize primary or secondary energy sources to transport solutes across the plasma membrane or passively conduct substrates through the lipid bilayer down an energy gradient. Due to their positioning in the lipid bilayer of cells, membrane transporters serve as critical gateways to control the uptake of nutrients as well as can serve as the first line of defense against the invasion of toxic small molecules. Despite the critical roles this broad category of proteins play in the underpinnings of life for the cell, far less is known about the structure-function relationship for membrane proteins compared to water-soluble proteins that do not reside in the hydrophobic bilayer. For reasons not entirely clear, this dearth of structural information is particularly true in the case of human membrane proteins. Through Innovator Award funding, we have engaged a novel eukaryotic expression platform together with cutting edge structural techniques in cryo-EM and the crystallization of transporters in the lipidic cubic phase, to accelerate the structure-determination of human membrane proteins specifically. We report strong progress in cryo-EM structure determination of an ABC transporter involved in Stargardt disease as well as the atomic structure of the only high-affinity copper-uptake transporter encoded in the human genome, CTnhr1, which also serves as a gateway for the entry of cisplatin-containing anti-cancer drugs into tumor cells.

3.2

THE IMPACT OF MUTATION ON THE FUNCTION, CONFORMATIONS, AND RECOGNITION OF UBIQUITIN

Early Independence Award, 2011

James Fraser

University of California, San Francisco, San Francisco, CA, USA

Understanding how mutations affect protein structure significantly impacts drug discovery, protein engineering, and the interpretation of individual genome sequences. However, the effects of many mutations, whether they are beneficial or deleterious, cannot be understood from static protein structures alone. To improve our knowledge and understanding of the relationship between mutation, alternative conformations, and phenotypes, we have pursued two parallel studies of the essential protein Ubiquitin, which plays key roles in various cellular stress responses as a post-translational modification of other proteins. First, we employed deep mutational scanning to determine the fitness landscape of all possible single residue mutations of Ubiquitin in the presence of different small molecule perturbations. These perturbations uncover ‘shared sensitized positions’ localized to areas around the hydrophobic patch and the C-terminus. Our data show how chemical stresses can reduce buffering effects in the Ubiquitin proteasome system. Second, we have defined how variants of Ubiquitin can be used to create inhibitors of proteins of the ubiquitin proteasome system. We exploited advances in multiconformer modeling of room temperature X-ray data collection on redesigned ubiquitin variants selected for increasing binding affinity to the deubiquitinase USP7. Initial core mutations disrupt natural packing and lead to increased flexibility. Additional, experimentally selected mutations quenched conformational heterogeneity through new stabilizing interactions. Stabilizing interactions, such as cation- π stacking and ordered waters, which are not included in standard protein design energy functions, can create specific interactions that have long range effects on flexibility across the protein. Our results suggest that increasing flexibility may be a useful strategy to escape local minima during initial directed evolution and protein design steps when creating new functions. Collectively, our results have revealed new connections between the mutational tolerance and structural plasticity of proteins.

3.3

SIGNATURE OF AN AGGREGATION-PRONE CONFORMATION OF TAU

New Innovator Award, 2011

Songi Han

University of California, Santa Barbara, Santa Barbara, CA, USA

Intrinsically disordered proteins (IDP) and their assembly into fibrillar cell inclusions has been at the center of intense interest and debate in the literature because these processes are foundational biophysical problems, and are directly linked to several neuropathological conditions.

- There are many recent and high profile publications on the detailed *structure and packing of fibrils* made of intrinsically disordered proteins that have pathological implications. However, *experimental* insight into the conformational landscape spanned by intrinsically disordered proteins is very limited, let alone into *shifts* in the conformational landscape adopted by IDPs under function-altering environmental factors. This is where our study comes into play that presents, for the first time, direct experimental evidence of the extension of Tau molecules in solution state, in the earliest stages of the aggregation process.
- The very concept of a distinct conformational landscape of an intrinsically disordered protein representing distinct *states* of a protein, such as the “stable” state vs. “aggregation-prone” states of Tau as presented in this study, is a new paradigm for the folding and aggregation of IDP. Hard data is needed to support the validity of such concept, and this is what we have provided, through a combination of state of the art spectroscopic and computational studies.

In this frame of the current state of the art, we present studies that offer direct and clear-cut experimental data, showcasing that an intrinsically disordered protein can populate a distinct conformational landscape, and that this can shift dramatically to reflect a change from a stable to an aggregation-prone state of Tau in solution state, represented by a ~1 nm shift in distances flanking the β -sheet packing PHF6(*) segments of Tau, within minutes of initiating aggregation, well before any fibrils are detectable by any known experimental tool.

The reason why this finding is important is because this strongly suggests that the conformational landscape of IDPs may dictate, or clearly reflect on the stability vs aggregation propensity of a protein. In other words, it may not be the fibrils that play a determining role in promoting aggregation, but the IDP’s solution conformational landscape that may have engrained in it the “fate” of the protein.

Especially in light of the recent designation of Tau as a prion-like protein by the community, in the sense that it traffics from neuron to neuron, identifying the existence and signature of conformational populations of potentially *pathological* Tau that may display different stabilities and/or different aggregation propensities is of enormous interest, but no such experimental data exist to date.

3.4

FINCHES AND SEEDS, PROTEASES AND BEADS: EVOLUTION OF NEW PROTEASE TOOLS FOR HIGH-THROUGHPUT POST-TRANSLATIONAL MODIFICATION MAPPING

New Innovator Award, 2011

Brian M. Paegel

The Scripps Research Institute, La Jolla, CA, USA

With the genome sequences of many organisms complete, we now face the Herculean task of understanding how these genetic instructions translate to cellular protein content, metabolic regulation, homeostasis and the molecular basis of disease. Over the past decade advances in mass spectrometry-based (MS) protein sequencing technology suggest that this grand analytical challenge may soon be within reach. However, while instrumentation capabilities have improved by orders of magnitude, the molecular toolbox for front-end enzymatic sample digestion has remained unchanged for decades and comprises a shockingly small arsenal of just 2–3 viable proteases. We have now developed an in vitro protease evolution platform, consisting of coupled bead-based emulsion PCR/emulsion in vitro translation, for discovering mutants with specificity directed to new, user-defined side chains, such as post-translationally modified side chains (PTMs). Citrulline, a PTM of arginine that is implicated in numerous epigenetic and immunological functions, made an ideal first target. Citrullination eliminates tryptic sites and unambiguous site identification in MS is difficult due to the subtle mass shift of modification and interferences with other chemical modifications that result in an identical mass shift to citrulline. After screening millions of mutants, we identified a 6-error mutant trypsin that exhibited extremely high catalytic efficiency of cleavage at citrulline ($\sim 10^6 \text{ M}^{-1}\text{s}^{-1}$) compared to the wild-type enzyme, which exhibited no detectable citrulline-dependent cleavage. To demonstrate the utility of the newly discovered mutant, we conducted a citrulline-dependent digest of protein arginine deiminase 4 (PAD4, an enzyme that catalyzes citrullination of itself and other proteins) and fibrinogen (FG, a target of PAD4), and analyzed the digests using high-resolution LC-MS/MS. The resulting C-terminally citrullinated peptides fragment in LC-MS/MS sequencing as usual, but fragmentation of the C-terminal amide bond releases a product y_1 ion corresponding to citrulline (176.1030 m/z). The presence of this ion not only flags the peptide as modified but also marks the C terminus as the site of modification. These signatures unambiguously mapped 12 citrullination sites in PAD4 and 25 citrullination sites in fibrinogen (2 previously unknown). With the compartmentalized evolution platform and PTM-dependent digest analysis in hand, we have now generated leads on phosphoserine-dependent and phosphotyrosine-dependent mutant proteases to address similar challenges in phosphorylation site identification. The unique mass spectral features generated using PTM-dependent proteases promise a generalized PTM site mapping strategy based on a toolbox of mutant proteases, which are now accessible by laboratory evolution.

Tuesday, December 6, 2016

Session 4

4.1

TELOMERE EXTENSION USING NUCLEOSIDE-MODIFIED MRNA AS A NOVEL THERAPEUTIC

Transformative Research Award, 2012

Helen M. Blau, Ph.D.

Baxter Laboratory for Stem Cell Biology, Stanford University, Stanford, CA, USA

Telomeres are composed of repetitive TTAGGG sequences located at the ends of chromosomes and are essential for the preservation of genome integrity. Telomere shortening can result in genomic instability and the induction of a DNA damage response that can result in senescence or apoptosis. Telomeres are extended by the enzyme telomerase, which comprises a protein component telomerase reverse transcriptase (TERT) and an RNA component (TR). Mutations in TERT or TR are found in a spectrum of diseases, the telomeropathies, including dyskeratosis congenita (50-60% of patients) and familial idiopathic pulmonary fibrosis (8-15% of patients). More broadly, short telomeres are implicated in diseases of aging including cancer and heart disease and in genetic diseases including Duchenne Muscular Dystrophy (DMD) (Cell, 2010; PNAS, 2016). Indeed, DMD patients have short muscle telomeres. Telomere lengths are shorter in cardiomyocytes of DMD patients lacking the protein dystrophin than in controls (Nat. Cell Biol., 2013). Moreover, telomere lengths are shorter in cardiomyocytes derived from iPS (iPS-CMs) generated from DMD patient cells, compared to control iPS-CMs. Using micropatterned hydrogels bioengineered to have elasticities mimicking those of fibrotic or normal cardiac tissue, we found that telomere shortening is exacerbated in DMD iPS-CMs by mechanical stress and contractile force. In skeletal muscle, short telomeres lead to muscle stem cell (MuSC) replicative exhaustion, and consequent inability to repair damage caused by mutant dystrophin. Telomere extension has been proposed as a means to improve cell culture and tissue engineering, and to treat disease. There is an urgent need for a safe, reliable method to extend telomeres. However, telomere extension by non-viral, non-integrating methods remains inefficient. We have discovered that delivery of modified mRNA encoding TERT to diverse human cell types increases telomerase activity transiently (24-48 h) and rapidly extends telomeres (FASEB J., 2015). Successive transfections over a four-day period extended telomeres up to 0.9 kb in a cell type-specific manner conferring up to 28 ± 1.5 additional population doublings. Unlike immortalized cells, all treated cell populations eventually stopped increasing in number and exhibited senescence markers to the same extent as untreated cells. This technology has utility in extending telomeres in MuSCs to enhance their proliferative and regenerative capacities. We will discuss progress toward therapeutic application of TERT mRNA to prevent or treat diseases and conditions including the cardiac and skeletal muscle phenotypes of DMD and the application of single cell mass cytometry using CyTOF and multiplexed ion beam imaging (MIBI) to telomere length measurement. The ability to rapidly extend telomeres and increase cell proliferative capacity without risk of insertional mutagenesis has broad utility in disease modeling, drug screening, and regenerative medicine.

4.2

UNDERSTANDING AND MODELING AGING

Pioneer Award, 2012

Anne Brunet

Stanford University, Stanford, CA, USA

Age is the greatest risk factor for most diseases, including neurodegenerative diseases, cardiovascular diseases, cancer, metabolic disorders, diabetes, and autoimmune diseases. However, our understanding of aging is still rudimentary because aging is an extraordinarily complex process that defies many conventional rules in biology. My lab aims to discover new, fundamental principles of aging regulation that can ultimately be translated to humans. We have broken new ground by pioneering the naturally short-lived African killifish as a new model to study aging and diseases in the context of aging in vertebrates. This new model has allowed us to generate a high throughput platform to not only model diseases by also screen for the impact of genetic pathways and chemical compounds on disease. In addition to developing this fish, my lab is also using *C. elegans* and mice, as well as cells from mice and humans, to identify genetic and epigenetic mechanisms involved in the regulation of lifespan and understand their mode of action. This approach has already generated new insights on the epigenetic regulation of aging. Our work has the promise to transform our understanding of why aging is at the heart of so many human diseases.

4.3

DIRECT *IN VIVO* LINEAGE REPROGRAMMING WITHOUT LIMITS

New Innovator Award, 2012

P. Duc Si Dong

Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Abstract author: Clyde Campbell

Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Presented by P. Duc Si Dong

The extent to which differentiated cells, while remaining in their native microenvironment, can be converted into unrelated cell types will reveal fundamental insight into cellular plasticity and will impact regenerative medicine. To push the boundaries of lineage conversion and investigate lineage plasticity *in vivo*, we have developed a novel *in vivo* discovery platform for lineage reprogramming. Using this platform, we have identified a cocktail of transcription factors that, when expressed in several non-endoderm lineages including skeletal muscle, are able to cell-autonomously induce the endoderm genetic program. These endoderm induced muscle cells can proceed to express key pancreatic organogenesis genes, including *hnf1b*, *pdx1*, and *ptf1a*, and subsequently form organoid-like tissues. Endoderm markers appearing prior to loss of muscle cell morphology, a lack of dependence on cell division, and a lack of pluripotency gene activation, together, suggests that lineage reprogramming occurred independent of a pluripotent intermediate. Importantly, lineage reprogramming can occur in *oct4* null mutants, providing functional evidence for direct lineage conversion. Our work demonstrates that within a vertebrate animal, differentiated cells originating from one germ layer can be induced to directly adopt lineages of a different germ layer—suggesting that differentiated cells *in vivo* are more amenable for lineage conversion than previously assumed. This discovery may pave the way towards a vast new *in vivo* supply of replacement cells.

4.4

MUSCLE FIBER SIGNALING SCALES THE MYOGENIC STEM CELL POOL

Early Independence Award, 2011

Christoph Lepper

Carnegie Institution for Science, Washington, DC, USA

When unperturbed, quiescent somatic stem cells are poised to affect immediate tissue restoration upon trauma. Yet, little is known regarding the mechanistic basis controlling initial and homeostatic “scaling” of adult stem cell pool sizes relative to their target tissues for effective regeneration. Here, we show that transgenic TEAD1-expressing skeletal muscle features a dramatic hyperplasia of muscle stem cells (i.e., the satellite cells, or SCs) but surprisingly without affecting muscle tissue size. Such super-numeral SCs attain a ‘normal’ quiescent state, accelerate regeneration, and maintain regenerative capacity over several injury-induced regeneration bouts. In dystrophic muscle, the TEAD1 transgene also ameliorated the pathology. We further demonstrate that hyperplastic SCs accumulate non-cell-autonomously via signal(s) from the TEAD1-expressing myofiber, suggesting that myofiber-specific TEAD1 overexpression activates a physiological signaling pathway(s) that determine initial and homeostatic SC pool size. We propose that TEAD1 and its downstream effectors are medically relevant targets for enhancing muscle regeneration.

5.1

UNDERSTANDING SELF VERSUS NON-SELF: IS THAT MY DOUBLE-STRANDED RNA OR YOURS?

Pioneer Award, 2011

Brenda L. Bass

Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

Viruses produce double-stranded RNA (dsRNA) during infection, and long dsRNA has also been detected in animals, including humans, in the absence of infection. Since dsRNA-binding proteins (dsRBPs) are not sequence specific, the question arises as to how our cells discriminate cellular from viral dsRNA. Our “Pioneer” hypothesis postulated that dsRBPs that bind viral dsRNA to initiate an immune response also respond to endogenous dsRNA, possibly explaining the inflammatory component of many diseases.

An important first step in this project was identifying the dsRNAs expressed by animal genomes. To this end we developed a pipeline for the genome-wide identification of expressed, long dsRNA. The RNA editing enzyme ADAR targets dsRNA to convert adenosines to inosines, which are recognized as A to G changes in RNAseq data. The identification of clusters of ADAR editing sites revealed “Editing Enriched Regions” (EERs), and these defined the long dsRNA expressed in cells. We defined > 1,500 dsRNAs in the *C. elegans* dsRNAome,¹ > 300 in the dsRNAome of bone-marrow derived macrophages of mice,² and > 3,400 in the dsRNAome of human peripheral blood monocytes.² Long dsRNA was predominantly in protein coding genes, in introns (*C. elegans* and human), or 3’ UTRs (mouse). Seventy-four mouse EER-associated genes contained an EER in the homologous human gene ($p < 0.0001$, Chi-square test), although nucleotide sequence and position were rarely conserved. While the dsRNAome of *C. briggsae* has not been determined, 12% of *C. elegans* EER-associated genes have a *C. briggsae* homolog with a highly structured intron ($p < 0.0001$, Chi-square test).

These dsRNAomes provide an invaluable resource for future studies and were the starting point for important observations in my laboratory. While proof-of-principle experiments indicate that ADARs antagonize cleavage by Dicer, our studies reveal, for the first time, that this is a natural function of *C. elegans* ADARs. Expression of EER-associated genes is dependent on ADARs, especially in embryos, and using mutant strains and CRISPR technology, we show this regulation is dependent on the presence of ADARs and the EER. Our studies of mouse and human dsRNAomes indicate that a subset of mRNAs containing long dsRNA are enriched in the nucleus, and ongoing studies are designed to reveal whether this is important for precluding induction of an immune response. Finally, we also found that small nucleolar RNAs bind PKR during metabolic stress,³ expanding the definition of cellular dsRNA. While PKR has established roles during viral infection, this result suggests it also mediates response to stress. These observations provide a platform for further understanding of how cells discriminate viral and cellular dsRNA.

¹ Whipple JM, et al., (2015). *RNA*. **21**, 786–800.

² Blango MG, Bass BL. (2016). *Genome Research*. **26**, 852–62.

³ Youssef OA, et al. (2015). *Proc Natl Acad Sci USA*. **112**, 5023–8.

5.2

MECHANISM OF STRAND SPECIFIC DNA MUTAGENESIS DURING ANTIBODY GENE DIVERSIFICATION

New Innovator Award, 2011

Uttiya Basu

Department of Microbiology and Immunology, Columbia University, New York, NY, USA

The distribution of sense and antisense strand DNA mutations on transcribed duplex DNA contributes to the development of immune and neural systems along with the progression of cancer. Within the immunity context, strand specific DNA mutations determine whether programmed DNA rearrangements diversify antigen receptor loci genes; within the cancer context, patients with various malignancies demonstrate DNA mutagenesis skewed toward the sense strand genome-wide. Using single-molecule super-resolution microscopy, we have identified subnuclear compartments in B cells where biologically programmed strand-specific DNA mutagenesis are engineered at focal DNA/RNA hybrid structures. The strand specific distribution of DNA mutations is determined by the coupled activities of two RNA helicases, Mtr4 and senataxin, along with the noncoding RNA processing function of RNA exosome. Our study envisions that the regulatory mechanism of strand specific DNA mutagenesis in subnuclear compartments during programmed and aberrant DNA mutagenesis events will play a major role in other undiscovered aspects of organismic development.

5.3

POLLEN GRAINS AS TROJAN HORSES FOR ORAL VACCINATION

New Innovator Award, 2012

Harvinder Singh Gill
Texas Tech University, Lubbock, TX, USA

Oral edible vaccine is painless and is an attractive alternative to painful needle-based injections. It can also be self-administered, and has potential to induce both systemic and mucosal immune responses. However, oral vaccination is challenging because vaccines degrade in the stomach and have poor permeability through the intestinal lining. We propose to harness the natural toughness of pollens to help transport vaccines into the human body through the oral route. By first removing the native plant biomolecules, which are the root cause of pollen allergies, a non-allergenic empty shell is created, which can subsequently be filled with vaccines. This “trojan horse”-like system is postulated to safely ferry vaccines through the harsh environment of the stomach into the intestines for improved uptake into the body.

- We have developed a novel chemical treatment process to produce clean pollens from different species. The previously reported procedure in the literature is suitable only for a specific pollen species. However, now through our process we can expand the pollen species that can be used for oral vaccination.
- We have shown that vaccine delivery through the oral route with ragweed pollens can induce, serum antibody titers similar to the traditional intramuscular route, and can induce an even stronger mucosal IgA antibody titer than the intramuscular route. Together with the fact that to make a pollen vaccine formulation a simple mixing step of pollens and antigen is needed, this result highlights the strong potential of pollens to be used for oral vaccination.
- We have examined the mechanism of immune stimulation from pollen-based vaccines. Using mouse bone-marrow derived macrophages and dendritic cells we have shown that their incubation with ragweed induces secretion of pro-inflammatory cytokines such as TNF-alpha and IL-6. Further, the expression of CD-40, CD-80, CD-86, and MHC-II is enhanced in dendritic cells after incubation with ragweed, indicating that dendritic cells get activated in the presence of ragweed. Incubation of ragweed with confluent Caco-2 cells (human epithelial cells) also reduces their transepithelial resistance, suggesting that ragweed pollen could increase permeability across the epithelial cells. We have also observed that pollens migrate across the epithelial cells into the intestine. Together these studies offer a potential mechanism by which pollens might induce an immune response.
- To understand the influence of pollen surface on antigen conformation, we studied the effect of incubating lycopodium spores with three different proteins. In all cases it was found that the lycopodium spores do not affect the stability of the proteins.

Overall these results lay the foundation, and facilitate further development of pollens as an innovative approach for painless oral vaccination.

5.4

VACCINATION TO GENERATE PROTECTIVE TISSUE RESIDENT MEMORY T CELLS

Transformative Research Award, 2011

Thomas S. Kupper and Rachael A. Clark

Department of Dermatology, Brigham and Women's Hospital, Boston, MA, USA; Harvard Medical School, Boston, MA, USA

Presented by Thomas S. Kupper

Adaptive immunity relies heavily on memory T cells to mediate successful protective immunity against pathogens. Resident memory T cells (T_{RM}) are a recently described population of memory T cells that are vital to protective immunity. Rather than circulate through blood and secondary lymphoid tissues, they reside in peripheral non-lymphoid tissues. Our work over the past five years has focused on skin and other barrier tissues, and demonstrates that infection generates T_{RM} not only at the site of infection, but throughout the entire tissue. One useful model has been skin infection with Vaccinia virus, which is highly efficient at generating T_{RM} throughout the skin. These T_{RM} do not re-circulate into blood and remain in skin indefinitely. They can provide protective immunity against re-infection in the absence of circulating T cells and antibody. In fact, studies demonstrate that T_{RM} provide superior protective immunity when compared to circulating central memory T cells (T_{CM}). Skin infection also generates T_{RM} in lung that are similarly protective. High throughput sequencing studies demonstrate that T_{RM} and T_{CM} derive from a common precursor naive T cells, demonstrating that the T cell repertoire is shared in tissue resident as well as circulating memory T cells. Transcriptional profiling studies demonstrate that T_{RM} have a gene expression profile that is markedly different from both naive T cells and other subsets of memory T cells. Some of the more marked differences are related to factors relevant to the unique metabolic requirements of these cells. Studies done in human skin and lung demonstrate both CD4 and CD8 T_{RM} in these tissues. Gene expression of human T_{RM} in skin is similar to that in mouse skin. High throughput sequencing of skin, lung, intestine, and lung of the same individual demonstrates that the most abundant memory T cells clones in each tissue are distinct. Thus, T_{RM} in different tissues are specific for pathogens/antigens encountered through each distinct tissue, respectively. Understanding T cell trafficking has implications for therapy. For example, mycosis fungoides, a type of cutaneous T cell lymphoma (CTCL), is a malignancy of T_{RM} , and understanding the non-migratory properties of these cells informs successful therapies. Leukemic CTCL is a malignancy of T_{CM} , and treatment must be directed accordingly. Skin diseases such as allergic contact dermatitis and psoriasis appear to be T_{RM} mediated. We speculate that many autoimmune disorders involving multiple specific tissues are mediated by dysfunctional T_{RM} . Thus, T_{RM} are essential for protective immunity, but their dysfunction is likely causative in several human diseases. At the same time, our findings support the hypothesis that generation of protective T_{RM} should be a goal of vaccination strategies.

5.5

NANOFABRICATED NEURAL PROBES FOR DENSE 3-D RECORDINGS OF BRAIN ACTIVITY

Pioneer Award, 2011

Thanos Siapas

California Institute of Technology, Pasadena, CA, USA

Brain computations such as perception and memory arise from the coordinated activation of populations of neurons that are distributed across brain areas. However, monitoring neuronal activity in the brain of behaving animals with high temporal and spatial resolution has remained a technological challenge. To address this challenge we developed dense, three-dimensional (3-D) electrode arrays for electrophysiology. The 3-D arrays constitute the front-end of a modular and configurable system architecture that enables recordings from behaving animals. I will describe the development and characterization of 3-D arrays using recordings from the hippocampus of head-fixed mice. I will also discuss ongoing efforts towards the development of chronically implantable interfaces for 3-D arrays that can be used with freely behaving rodents. These arrays may enable tracking activity across distributed brain circuits with unprecedented spatiotemporal resolution and scale.

6.1

LIVE-CELL BIOORTHOGONAL CHEMICAL IMAGING FOR BIOMEDICINE

New Innovator Award, 2012

Wei Min

Columbia University, New York, NY, USA

Innovations in light microscopy have tremendously revolutionized the way researchers study biological systems with subcellular resolution. Although fluorescence microscopy is currently the method of choice for cellular imaging, it faces fundamental limitations for studying the vast number of small biomolecules. This is because relatively bulky fluorescent labels could introduce considerable perturbation to or even completely alter the native functions of vital small biomolecules. Hence, despite their immense functional importance, these small biomolecules remain largely undetectable by fluorescence microscopy.

To address this challenge, we have developed a *bioorthogonal chemical imaging* platform. By coupling stimulated Raman scattering (SRS) microscopy, an emerging nonlinear Raman microscopy technique [1-2], with tiny and Raman-active vibrational probes (e.g., alkynes and stable isotopes including ^2H and ^{13}C), *bioorthogonal chemical imaging* exhibits superb sensitivity, specificity, multiplicity and biocompatibility for imaging small biomolecules in live systems including tissues and organisms [3]. Exciting biomedical applications such as imaging fatty acid metabolism related to lipotoxicity, glucose uptake and metabolism, drug trafficking, protein synthesis, DNA replication, protein degradation, RNA synthesis and tumor metabolism will be presented [3-10]. Moreover, further strategies allow for *multicolor* imaging, a valuable technique in the era of “omics”. We envision that *bioorthogonal chemical imaging* platform would do for small biomolecules what fluorescence microscopy of fluorophores has done for larger molecular species, bringing small molecules under the illumination of modern light microscopy.

1. Label-free biomedical imaging with high sensitivity by stimulated Raman scattering microscopy, *Science* (2008).
2. Vibrational spectroscopic imaging of living systems: An emerging platform for biology and medicine, *Science* (2015).
3. Live-Cell Bioorthogonal Chemical Imaging: Stimulated Raman Scattering Microscopy of Vibrational Probes, *Acc Chem Res* (2016).
4. Vibrational imaging of newly synthesized proteins in live cells by stimulated Raman scattering microscopy, *Proc Natl Acad Sci USA* (2013).
5. Live-cell imaging of choline metabolites through stimulated Raman scattering coupled with isotope-based metabolic labeling, *Analyst* (2014).
6. Live-cell quantitative imaging of proteome degradation by stimulated Raman scattering, *Angew Chem Int Ed* (2014).
7. Live-cell imaging with alkyne-tagged small biomolecules by stimulated Raman Scattering, *Nature Methods* (2014).
8. Multicolor live-cell chemical imaging by isotopically edited alkyne vibrational palette, *J Am Chem Soc* (2014).
9. Imaging complex protein metabolism in live organisms by stimulated Raman scattering microscopy with isotope labeling, *ACS Chem Biol* (2015).
10. Vibrational imaging of glucose uptake activity in live cells and tissues by stimulated Raman scattering, *Angew Chem* (2015).

6.2

VISUALIZING MECHANISMS OF MRNA TRANSCRIPTION REGULATION AT THE SINGLE-MOLECULE LEVEL

New Innovator Award, 2012

Alexandros Pertsinidis

Memorial Sloan Kettering Cancer Center, New York, NY, USA

Transcription is the fundamental process by which cells utilize genetic information. The detailed molecular mechanisms as well as the organization and dynamics of the transcription cycle at single genes in live cells remain largely uncharacterized. In my lab at MSKCC we have developed novel single-molecule 3D imaging techniques that can visualize the dynamics of macro-molecular machines in action and we have observed, for the first time, how RNA Polymerase II molecules progress through the transcription cycle at a single gene in live human cells, in real-time.

I will present two new technologies: (i) modulation interferometry, a 3D single-molecule super-resolution imaging approach that achieves dynamic real-time tracking with <2 nanometer axial localization precision, well below the few-nanometer size of individual protein components of large transcription complexes; (ii) target-locking nanoscopy, an ultra-sensitive system that enables single-molecule detection in addressable sub-diffraction volumes, at high background concentrations within crowded intracellular environments.

The new capabilities of modulation interferometry enable probing the dynamics of large, multi-component molecular assemblies. We have visualized the movement of a multi-subunit RNA Polymerase through the complete transcription cycle, dissected the kinetics and conformational changes of the initiation-elongation transition and determined the fate of an initiation factor during promoter escape. Our results validate modulation interferometry as one of few structural biology tools that presently have the combined spatial-temporal resolution and molecular specificity required to capture the movement, conformational changes and sub-unit association-dissociation kinetics of complex macromolecular machines, like the transcription apparatus, in action.

Following the activity of single molecules at specific loci inside live cells remains very challenging - the full potential of single-molecule approaches *in vivo* has yet to be realized. Our newly-developed target-locking nanoscopy system enables us to image, track and count single RNA Polymerase II molecules, in real-time inside live human cells. We discovered ~ 10 Pol II molecules accumulating during active transcription of a tagged mini-gene. Kinetic analysis reveals that mini-gene transcription does not involve transient Pol II clustering at pre-initiation, persistence of accumulated Pol IIs in the absence of transcription or extensive Pol II recycling-related spatial compartmentalization. Rather, single Pol II molecules are stochastically recruited from the nucleoplasm, enter into productive elongation and are predominantly released rather than recycled upon termination. Our results establish a quantitative framework for elucidating Pol II dynamics at single genes, and also demonstrate that complex biological mechanisms can be probed by real-time single-molecule detection in addressable 3D loci inside live cells.

6.3

MECHANICAL SUPER-RESOLUTION: IMAGING STRUCTURE, CHEMISTRY, FORCES, AND VOLTAGE ACROSS BIOMOLECULES AND CELLS

New Innovator Award, 2013

Ozgur Sahin

Columbia University, New York, NY, USA

Advances in imaging technologies are needed more than ever in many areas of biomedical research. Our lab has developed non-invasive mechanical approaches to biological imaging. By exploiting the strong coupling between chemical, electrical, and mechanical properties that are readily present in most biological systems, we have demonstrated imaging of chemical groups within protein complexes with Angstrom scale resolution, determined physiologically relevant intracellular forces in adherent cells, and detected electromechanical activity in synaptic terminals of neurons, all non-invasively and without using labels. We will present how chemical identities and physiological signals are transduced into readily detectible mechanical signatures, and illustrate the potential of these microscopes with challenging imaging problems in structural biology, mechanobiology, and neuroscience.

6.4

TECHNOLOGIES FOR VISUALIZING THE GENOME

Pioneer Award, 2012

Ting (C.-ting) Wu

Department of Genetics, Harvard Medical School, Boston, MA, USA

Abstract authors: Brian J. Beliveau,^{1,2,3} Alistair N. Boettiger^{4,5,6} Maier S. Avendaño,^{2,3} Ralf Jungmann,^{2,3,7} Guy Nir,¹ Siyuan Wang,^{4,5} Hiroshi Sasaki,^{2,3} Bogdan Bintu,^{4,5} Huy Nguyen,¹ Jumana AlHajj Abed,¹ Nuno M. C. Martins,¹ Jeffrey R. Moffitt,^{4,5} Ruth B. McCole,¹ Eric F. Joyce,^{1,8} Caroline Kim-Kiselak,¹ Frédéric Bantignies,^{1,9} Chamith Y. Fonseka,^{1,10} Jelena Erceg,¹ Mohammed A. Hannan,¹ Hien G. Hoang,¹ David Colognori,^{1,5,11} Jeannie T. Lee,^{1,5,11} William M. Shih,^{2,12,13} Peng Yin,^{2,3} Xiaowei Zhuang,^{4,5,14} and Ting (C.-ting) Wu¹

¹*Department of Genetics, Harvard Medical School, Boston, MA, USA;* ²*Wyss Institute for Biologically Inspired Engineering, Harvard Medical School, Boston, MA, USA;* ³*Department of Systems Biology, Harvard Medical School, Boston, MA, USA;* ⁴*Departments of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA;* ⁵*Howard Hughes Medical Institute, Cambridge, MA, USA;* ⁶*Department of Developmental Biology, Stanford University, Stanford, CA, USA;* ⁷*Max Planck Institute of Biochemistry and Ludwig-Maximilians-Universität, Munich, Germany;* ⁸*Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA;* ⁹*Institut de Génétique Humaine, Montpellier, France;* ¹⁰*Departments of Genetics and Rheumatology, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA;* ¹¹*Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA;* ¹²*Departments of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA;* ¹³*Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA;* ¹⁴*Department of Physics, Harvard University, Cambridge, MA, USA*

Presented by Ting (C.-ting) Wu

The overarching goal of our research is to elucidate the relationship between chromosome organization and genome function, stability, and evolution and, to this end, we have been developing tools for imaging the genome in situ. Our technologies are based on Oligopaints (Beliveau, Joyce, et al. 2012 PMC3535588), which are bioinformatically-designed oligonucleotides which, when labeled directly or indirectly with fluorophores, can be used for fluorescent in situ hybridization (FISH). Two of the technologies, OligoSTORM and OligoDNA-PAINT, combine Oligopaints with the single-molecule super-resolution methodologies of, respectively, STORM (Stochastic Optical Reconstruction Microscopy; in collaboration with Alistair Boettiger and others in the laboratory of Xiaowei Zhuang) and DNA-PAINT (DNA-based Point Accumulation in Nanoscale Topography; in collaboration with Ralf Jungmann and others in the laboratory of Peng Yin), to achieve ≤ 20 nm resolution (Beliveau et al. 2015 PMC4430122). The third technology (HOPs) harnesses single nucleotide polymorphisms to enable Oligopaints to distinguish maternal and paternal homologous chromosomes. We have applied these technologies to mammalian and *Drosophila* cells, discovering intriguing organizational themes for different types of chromatin (Boettiger et al. 2016 PMC4905822; Wang et al. PMID: 27445307).

Wednesday, December 7, 2016

Session 7

7.1

ENGINEERING TARGETED THERAPEUTICS FOR BREAST CANCER

New Innovator Award, 2012

Debra Auguste

Northeastern University, Boston, MA, USA

Cells sense changes in their environment and respond by altering their gene expression. We investigate how cells manipulate their membrane proteins, which has profound effects on disease progression. Cells orchestrate the density of proteins and lipids to govern adhesion and migration. From this knowledge, we can engineer drug delivery vehicles that complement the molecular patterns observed on cells to achieve strong, cooperative binding. We have employed these strategies in a model system of endothelial inflammation and in breast cancer metastasis. Our lab has identified a new target and biomarker for triple negative breast cancer, examined the role of ligand/receptor cell adhesion by atomic force microscopy, and synthesized targeted drug delivery vehicles that demonstrate that nanoparticle ligand surface density alters gene expression. The intersections of biology, engineering, and medicine is the basis for the targeted drug delivery platform. We have used these vehicles to identify new strategies in the delivery of nucleic acids.

7.2

NEXT-GENERATION RATIONAL ANTI-CANCER POLYTHERAPIES

New Innovator Award, 2012

Trever Bivona
University of California, San Francisco, CA, USA

7.3

GENE-ENGINEERED CAR T-CELLS: A PLATFORM FOR TREATING CANCER

New Innovator Award, 2012

Laura A. Johnson

*Center for Cellular Immunotherapies, Department of Pathology and Laboratory Medicine,
Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Cancer is a multifaceted disease that is difficult to treat because it arises from normal self. Treatments to eliminate cancer walk a fine line between killing the cancer cells and killing the normal cells, and by extension, the patient. Standard therapy for all solid tumor cancers consists of the same three steps, surgery to remove the bulk of tumor followed by radiation and chemotherapy in attempts to eliminate the proliferating tumor cells, while sparing normal patient tissues. Unfortunately, in the case of advanced and metastatic solid cancers, there are few curative therapies, most offer a modestly prolonged survival, however this may also come with a reduced quality of life due to toxicity caused by the non-specific nature of treatment. In contrast, the immune system has evolved over a million years to recognize pathogens and disease-causing agents with exquisite specificity. Unfortunately, too often cancer is not recognized, as it still looks too much like ‘self’. The effector arm of the immune system is led by T-lymphocytes, or T-cells. These cells recognize their target antigens via T cell receptors (TCR), however, it is possible to engineer in artificial receptors, called chimeric antigen receptors (CARs) to retarget them to any antigen of interest. We have generated CARs to target a tumor-specific mutation of epidermal growth factor receptor variant III (EGFRvIII), expressed on 20-30% of glioblastoma (GBM) brain tumors. EGFRvIII CARs demonstrated anti-tumor efficacy *in vitro* against tumor cell lines and *in vivo* in xenograft mouse models of GBM. We have now translated this work into a clinical trial and have treated ten patients with recurrent GBM.

Looking for more prevalent tumor targets, we have evaluated the expression of tumor-associated viral antigens in the form of cytomegalovirus (CMV), which is reactivated in GBM but not surrounding normal brain or other tissues. We developed a CAR to target the CMV surface protein glycoprotein B (gB), and tested it against tumor cell lines *in vitro* and *in vivo*. gB targeted CAR T-cells were able to selectively recognize and destroy gB-expressing tumors and improved survival *in vivo* in a xenograft mouse model. We are continuing to test gB CARs for efficacy against primary GBM, with plans to move into clinical trials.

In searching for non-typical tumor targets, we identified a new class of tumor antigen that occurs when tumor-specific mutations result in defective cell protein glycosylation. Specifically, we generated a CAR that recognizes a hypo-glycosylated version of the tumor-associated MUC1 protein, that is absent in all normal tissues tested. This CAR shows particular promise, as it recognized virtually every cancer tested, providing a potential “universal CAR” to treat cancers ranging from pancreas, ovarian, breast, lung and leukemias. This CAR is currently slated for translation into clinical trials.

7.4

UNDERSTANDING TIPPING POINTS IN BIOLOGY

New Innovator Award, 2012

Jeff Gore

Massachusetts Institute of Technology, Cambridge, MA, USA

Many biological systems and populations can shift suddenly in response to small changes in environmental conditions. Examples of such sudden transitions include disease outbreaks in response to falling vaccination rates and changes in cell state in response to growth factors. Given that these transitions can have substantial health implications, it would be valuable to obtain advance warning that such a “tipping point” is approaching. Theory from nonlinear dynamics argues that these tipping points should be associated with potentially universal changes in the dynamics of the system resulting from an increase in the time to recover from perturbations. We have used laboratory yeast populations to study these proposed early warning signals of impending population collapse.¹⁻⁵ Yeast cooperatively breakdown the sugar sucrose,⁶ meaning that below a critical size the population cannot sustain itself. We have demonstrated experimentally that changes in spatial patterns or in the fluctuations of the population size can serve as an early warning signal that the population is close to collapse.^{1,2,5} Given the universal nature of these sudden transitions, the behavior explored here may be relevant to tipping points in very different biological transitions. For example, we have recently demonstrated that phenotypic memory in cells loses resilience to environmental perturbations near such a critical transition.⁷

¹ Dai, Vorselen, Korolev, and Gore, *Science* (2012).

² Dai, Korolev, and Gore, *Nature* (2013).

³ Sanchez and Gore, *PLOS Biology* (2013).

⁴ Chen, Sanchez, Dai, and Gore, *Nature Communications* (2014).

⁵ Dai et al, *PNAS* (2015).

⁶ Gore, Youk, and van Oudenaarden, *Nature* (2009).

⁷ Axelrod et al, *eLife* (2015).

7.5

GENOMIC SURVEILLANCE OF MICROBIAL THREATS

New Innovator Award, 2010

Pardis Sabeti

Harvard University, Cambridge, MA, USA

We are in the midst of a revolution in the fields of genomics and public health. The availability of genome-wide sequence and variation data and the ability to rapidly generate new data have created unprecedented opportunities to study human biology, evolution, and disease. These same tools are also making it possible to carry out studies in the microbial pathogens that affect humans. With the support of the NIH Innovator award, I have been able to launch a research program that leverages these rapidly emerging resources (i) to develop and apply methods to investigate natural selection in the human genome (ii) study the genomic variation and evolution of the microbial pathogens that affect humans; and (iii) build new computational tools for studies of genomics and public health. This includes work studying some of the world's great microbial threats including Lassa, Ebola, and Zika virus.

8.1

GENOME-WIDE APPROACHES TO CHARACTERIZE APICOMPLEXAN PARASITISM

Early Independence Award, 2013

Sebastian Lourido

Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Apicomplexan parasites are a leading cause of human and livestock diseases worldwide, yet most of their genes remain uncharacterized. Here, we present the first genome-wide genetic screen of an apicomplexan. Using CRISPR/Cas9, we assess the contribution of each *Toxoplasma gondii* gene to parasite fitness during infection of human fibroblasts. This analysis defined ~ 200 fitness-conferring genes unique to the phylum, from which 16 previously uncharacterized proteins were further investigated. Secondary screens identified the novel invasion factor claudin-like apicomplexan microneme protein (CLAMP), which displays similarity to mammalian tight-junction proteins and localizes to secretory organelles. CLAMP is found in all sequenced apicomplexan genomes, and its ortholog is essential during the asexual stages of the malaria parasite *Plasmodium falciparum*. These results provide broad-based functional information on *T. gondii* genes and will facilitate future genetic approaches, expanding the horizon of antiparasitic interventions.

8.2

ASTROCYTES AND NEURAL CIRCUITS: SIGNALING, FUNCTION, DYNAMICS AND DIVERSITY

Pioneer Award, 2013

Baljit S. Khakh

University of California, Los Angeles, Los Angeles, CA, USA

Glial cells have been studied for as long as neurons, with Cajal's beautiful drawings illustrating the morphology and diversity of both neurons and glia alike. However, twentieth century neuroscience was dominated by neuronal studies, because electrophysiology provided a precise way to study electrical activity in neurons. In contrast, astrocytes (a subclass of glia) are electrically silent, firing no action potentials and rarely receiving fast electrical synaptic inputs. Thus experimental studies of astrocytes lagged behind those of neurons by decades, because electrophysiology was not the appropriate tool with which to study them. Fortunately, recent advances in genetics, protein engineering and optical microscopy provide opportunities to generate the tools needed to study astrocytes and with these a unique opportunity to finally tackle a fundamental and exciting area of neuroscience.

In this presentation, we will present data from our laboratory gathered as part of the Pioneer Award. We will report the development, validation and use of a new method to silence astrocyte intracellular calcium signaling in mature neuronal circuits in order to explore their functions in brain slices and *in vivo*. The development of FRET-based methods to track the subcellular spatial interactions and dynamics of astrocyte branchlets with synapses arising from distinct neural inputs will be described. In addition, we will report the generation, testing and deployment of astrocyte-specific Cre/ERT2 mouse lines that permit selective and pan astrocytic gene expression and determination of astrocyte transcriptomes from distinct brain nuclei, which when combined with proteomics and functional assessments permits direct evaluation of astrocyte diversity in different parts of the brain. Finally, we will also present the design and use of genetically-encoded ATP sensors to image extracellular ATP on the surface of astrocytes in brain slices. The tools we have developed permit exploration of important, open questions in astrocyte biology and neuroscience. Recently gathered data from our laboratory on their use in studies of astrocyte physiology in the adult mouse striatal microcircuit in brain slices and *in vivo* will be presented.

8.3

***IN SITU* TRANSCRIPTION PROFILING OF SINGLE CELLS REVEALS SPATIAL ORGANIZATION OF CELLS IN THE MOUSE HIPPOCAMPUS**

New Innovator Award, 2011

Long Cai

California Institute of Technology, Pasadena, CA, USA

Identifying the spatial organization of tissues at cellular resolution from single cell gene expression profiles is essential to understanding many biological systems. In particular, there exist conflicting evidence on whether the hippocampus is organized into transcriptionally distinct subregions. Here, we demonstrate a generalizable *in situ* 3D multiplexed imaging method to quantify hundreds of genes with single cell resolution via Sequential barcoded Fluorescence *in situ* hybridization (seqFISH).¹ We used seqFISH to identify unique transcriptional states by quantifying and clustering up to 249 genes in 16,958 cells. By visualizing these clustered cells *in situ*, we identified distinct layers in the dentate gyrus corresponding to the granule cell layer, composed of predominantly a single cell class, and the subgranular zone, which contains cells involved in adult neurogenesis. Furthermore, we discovered that distinct subregions within the CA1 and CA3 are composed of unique combinations of cells in different transcriptional states, instead of a single state in each subregion as previously proposed. In addition, we see that while the dorsal region of the CA1 is relatively homogenous at the single cell level, the ventral part of the CA1 has a high degree of cellular heterogeneity. These structures and patterns are observed in sections from different mice, as well as in seqFISH experiments with different sets of genes. Together, these results demonstrate the power of seqFISH in transcriptional profiling of complex tissues.

¹ Lubeck E, Coskun AF, Zhiyentayev T, Ahmad M, Cai L. Single-cell *in situ* RNA profiling by sequential hybridization. *Nat Methods* 2014 April 11(4): 360–1. PMID: PMC4085791.

Also: Shah, Lubeck, Zhou, and Cai. *In situ* transcription profiling of single cells reveals spatial organization of cells in the mouse hippocampus. *Neuron* 2016 Oct 19. <http://dx.doi.org/10.1016/j.neuron.2016.10.001>

8.4

THE BRAIN ACTIVITY MAP OF *HYDRA VULGARIS*

Pioneer Award, 2013

Rafael Yuste

Neurotechnology Center, Columbia University, New York City, NY, USA

The function of neural circuits is an emergent property that arises from the coordinated activity of large numbers of neurons. To capture this, we proposed launching a large-scale, international public effort, the Brain Activity Map Project, which led to the BRAIN initiative, aimed at reconstructing the full record of neural activity across complete neural circuits. We are now achieving this goal with the Cnidarian *Hydra vulgaris*, by making transgenic animals that express genetically encoded calcium indicators in most (or all) of its neurons. This enables us to image the activity of its entire nervous system during its behavior.

Poster Session Abstracts

Monday, December 5, 2016

1.1

NOVEL STUDY DESIGNS TO LEVERAGE LARGE-SCALE POPULATION HEALTH DATA TO QUANTIFY HEALTH IMPACTS OF POLICIES AND PROGRAMS

New Innovator Award, 2013

Jennifer Ahern

University of California, Berkeley, Berkeley, CA, USA

Growing availability of big data on health and increasing demand for evidence-based decision-making are generating unique opportunities and challenges in health research. We present a range of novel study designs we are using to leverage existing large-scale population health data from California to answer important questions about health impacts of policies and programs. We merge data from geocoded statewide birth, hospitalization, and death records together with census, survey, economic, and administrative sources to construct a rich database representing all of California and characterizing both individuals and communities. Taking advantage of these existing data, we are able to conduct time- and cost-efficient studies of the impacts programs and policies such as violence prevention and mental health legislation. Taking advantage of the high degree of geographic and temporal precision in these data, we examine the effects of acute events such as spikes in community violence, or gun show events on a range of sequelae including gestational outcomes, mental health, cardiovascular events, asthma attacks, and injuries. This approach is advantageous because the characteristics of individuals and places are often similar immediately prior and after acute events, thereby reducing confounding and allowing stronger inference. Taking advantage of our data structure that extends across place and time, we apply modern analytic methods such as synthetic control, which are advantageous for estimating program and policy effects in observational settings, when there is no one perfect control for any intervention unit. Taking advantage of the massive sample size, we harness a high degree of statistical power to examine rare outcomes (such as suicide) among important subgroups (such as small racial/ethnic minority groups) for whom previous analyses have been limited. Due to the population based data, estimates are directly applicable to the areas of California under study, and estimates for population subgroups allow understanding of differential program and policy impacts that may have implications for health disparities. Taken together, these approaches use health-related big data to address important health questions and utilize novel analytic methods that overcome key challenges in generating evidence from observational data to inform program and policy development, and may serve as useful models in future research.

1.2

DEVELOPMENT OF HUMAN INTESTINAL SIMULACRA

Transformative Research Award, 2015

Nancy Allbritton, Scott Bultman, Scott Magness, Shawn Gomez, and Yuli Wang
The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract author: Yuli Wang
Presented by Nancy Allbritton

Colonic epithelium consists of an array of crypts with colonic stem cells residing at the crypt base, while their differentiated progeny (enterocytes and goblet cells) migrate to and are located luminally. This tissue architecture is maintained by a gradient of growth factors (Wnt-3A, Notch and BMP), oxygen, and short chain fatty acids (SCFAs). To replicate this geometric complexity, a colon-on-a-chip platform that truly mimics the *in vivo* microenvironments of colonic epithelium has been created from both mouse and human colonic stem cells through a collaboration among biomedical engineers and intestinal biologists. The colonic stem cells were expanded *in vitro* and cultured on microengineered hydrogel scaffolds that possess the same architecture as colonic crypts. The cells were cultured under a gradient of growth factors (Wnt-3A, R-Spondin) to generate polarized crypts with the stem cells confined to the base, dividing cells at the base and along the crypt half, and differentiated non-dividing cells on the luminal surface. The platform contained an array of 200 crypts over a 7 mm² area. The impact of dietary compounds and bacterial metabolites applied to the luminal face of this *in vitro* tissue on the stem cell compartment at the base provided a demonstration of the utility of the platform. Such studies demonstrated that the SCFAs butyrate (5 mM), but not acetate (15 mM) or propionate (5 mM), significantly suppressed the stem cell activity by shortening the length of proliferation zone and reducing the number of S-phase cells per crypt. These findings are consistent with predicted effects of cell proliferation *in vivo*. The platform will enable detailed studies of compounds, such as pre- and probiotics, on primary tissue under controlled conditions.

1.3

THE INITIATION OF ALLERGIC INFLAMMATORY RESPONSES IN THE LUNG

New Innovator Award, 2012

Christopher D. C. Allen
University of California, San Francisco, San Francisco, CA, USA

Abstract author: Xin-Zi Tang
*Cardiovascular Research Institute and Sandler Asthma Basic Research Center,
University of California, San Francisco*

Presented by Christopher D. C. Allen

In the majority of cases of asthma, underlying inflammation in the lung contributes to disease pathogenesis. However, the cellular interactions leading to allergic lung inflammation are poorly understood. We have studied the early events triggering the initiation of allergic lung inflammation in mouse models, focusing on T cell responses and antibody production, by advanced microscopy, flow cytometry, and genetic approaches. Here we report that small populations of myeloid cells are normally located in collagen-rich regions proximal to the bronchial airways, establishing niches that can form inflammatory foci upon induction of allergic airway inflammation. These myeloid cells include both classical dendritic cells (DCs) and a population of previously undefined macrophages, which we term bronchus associated macrophages (BAMs). We found that the BAMs are able to capture allergen from the airway lumen, present peptides to T cells via MHC class II, and interact with effector T cells in the early hours after allergen challenge via the airways. These BAMs stay resident in the lung, where T cell effector responses occur, whereas classical DCs migrate to the lung-draining mediastinal lymph node to prime T cells and initiate antibody responses. The production of IgE antibodies, which can sensitize mast cells and basophils to promote allergic inflammatory responses, occurred primarily in the mediastinal lymph node and was transient. Interestingly, repetitive exposure to allergen via the airways, a feature of many mouse models of allergic airway disease, led to suppression of allergen-specific IgE responses. In contrast, we observed extended production of allergen-specific IgG and IgA antibodies, in part by plasma cells located in the lung tissue. Our findings have important implications for understanding the process by which allergic inflammatory responses are initiated in the lung.

1.4

CYTOSKELETAL STRUCTURAL PLASTICITY IN FORCE-GENERATION AND MECHANONSENSATION: A CASE STUDY OF THE MYOSIN VI MOTOR PROTEIN

Early Independence Award, 2013

Gregory Alushin

National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

Despite a growing recognition of the impact of mechanical cues on cell fate and behavior in development and disease, the molecular mechanisms underlying the ability of cells to sense and respond to the mechanical properties of their tissue microenvironments (“mechanosense”) remain largely unknown. My research program is directed towards identifying macromolecular machineries which transduce mechanical forces into intracellular signals (“mechanotransduction”) and dissecting their mechanisms of action in atomistic detail. Our long-term goal is to enable the design of drugs which target these processes, analogous to the wide array of highly successful pharmaceutical agents which modulate conventional signal transduction cascades.

Cellular mechanics are orchestrated by the cytoskeleton, a dynamic network of interconnected protein polymers and motor proteins that interface with a broad array of binding partners to facilitate active intracellular organization, control cell shape, and power cell migration and cell division. We and others are accumulating evidence that mechanical forces impinging on one class of cytoskeletal filaments, filamentous actin (F-actin), can evoke conformational transitions in the protein subunits that compose this polymer. We hypothesize that these mechanically regulated conformational states modulate interactions with direct actin binding proteins (ABPs) as an early event in mechanotransduction pathways, analogous to ligand binding allosterically modulating a cell surface receptor’s interaction with a downstream effector. This model predicts that mechanosensitive ABPs will preferentially recognize and bind specific conformations of actin that can be modulated by forces acting on a filament. Thus, to define this structural landscape, we have undertaken high-resolution cryo-electron microscopy (cryo-EM) studies of diverse ABPs bound to F-actin.

Here I present the results of one these studies, characterizing the interface of the myosin VI motor protein with F-actin. We have generated reconstructions of myosin VI in nucleotide-free (rigor, 4.5 Å average resolution) and MgADP (strong-bound ADP, 5.5 Å average resolution) states bound to F-actin, as well as corresponding atomistic models. In addition to defining the interface between this motor and actin, which differs from other myosins, we identify conformational changes in actin that accompany both myosin binding and transitions between states in the force-generation cycle. Furthermore, comparing our models to previous crystal structures of myosin VI in actin unbound states allows us to assemble, to our knowledge, the first detailed “movie” of an actin-based motor in action based entirely on bona fide structural data. Our studies suggest a reciprocal relationship between actin and myosin conformation, and in addition to providing insight into motor mechanism, they lay the groundwork for future investigations of myosin sensitivity to the conformational status of F-actin.

1.5

TARGETING PRIVILEGED FIRST RESPONDERS IN ISOZYME-SPECIFIC REDOX RESPONSE

New Innovator Award, 2014

Yimon Aye

Weill Cornell Medicine, Cornell University, New York, NY, USA

The precisely timed and spatially regulated chemical signals are the essence of biochemical redox signaling. However, defining the precise biological impacts of localized signals that engage with specific protein targets under physiologic conditions has proven to be highly challenging. The Aye Lab presents a unique set of proximity-directed chemical tools that enables powerful interrogation into functional consequences of specific redox events through precision redox targeting in living systems. With this in vivo-validated redox-targeting toolset, we identify bona fide “*first responders*” sufficient to drive phenotypic responses at organismal level. Our unique chemical biology toolset sets the stage for ruling in gain-of-function (or dominant loss-of-function) redox modifications and relating them to phenotype in an unbiased experiment that is not hampered by functional redundancy. The Aye lab’s research and educational activities (www.ayelab.org) are supported by the NIH Director’s New Innovator, the NSF CAREER, the Beckman Young Investigator, and the Sloan Fellowship award programs.

1.6

EXPLORER: CHANGING THE MOLECULAR IMAGING PARADIGM WITH TOTAL-BODY PET

Transformative Research Award, 2015

Ramsey D. Badawi and Simon R. Cherry
University of California, Davis, Davis, CA, USA

Abstract author: Ramsey D. Badawi
Department of Radiology, University of California, Davis, Davis, CA, USA

Presented by Ramsey D. Badawi

Positron emission tomography (PET) is the most sensitive method for non-invasively assaying and imaging biochemical compounds anywhere inside the human body. Furthermore, PET has the ability to quantitatively assay pharmacokinetic and signaling processes *in vivo*. Currently, only a ~ 20 cm segment of the body is contained within the field of view of a PET scanner, leading to two serious drawbacks. Firstly, it is not possible to perform quantitative assays across the whole body for system-level measurements of pharmacokinetics because only part of the body is imaged at any one time. Secondly, < 1% of the radiation emitted from the whole body is collected, which is wasteful in terms of signal and radiation dose.

To overcome these major limitations, and to transform the human molecular imaging paradigm, we are designing and constructing EXPLORER, the first ever total-body PET scanner. Computer modeling predicts a 40-fold gain in effective signal from this scanner which can be used to acquire total body images in just 15–30 seconds, or to dramatically cut the radiation dose (allowing us to study new populations or follow disease trajectories), or to extend the dynamic range (allowing us to study biological processes over much longer timescales). It will permit, for the first time, quantitative total-body molecular imaging of systems biology in humans at unprecedented sensitivity. This step change could revolutionize the way in which PET is used in research and ultimately in the clinic, opening up a wide range of new applications for human molecular imaging.

Progress to date includes the development of a scaled prototype system which is currently undergoing experimental testing, the build of a mock-up of the full-scale system to test the patient/scanner interface, significant progress on the design and build of the scanner components, and engagement with the biomedical research community to develop a range of application ideas.

1.7

TRANSCRIPTIONAL REGULATION OF STEAP1 IN EWING'S SARCOMA

Early Independence Award, 2012

Mona Batish

Rutgers University, New Brunswick, NJ, USA

Abstract author: Fatu Badiane Markey
Rutgers University, New Brunswick, NJ, USA

Presented by Mona Batish

Ewing's sarcoma (ES) is the second most prevalent solid tumor seen in children and adolescents. ES is caused by the chromosomal translocation between the EWSR1 gene and ETS transcription factor family genes. This translocation results in the production of a dysfunctional transcription factor called EWS/FLI1 (EF), which leads to deregulation of a large number of target genes leading to ES. Another endogenous transcription factor, NKX2.2 is upregulated in presence of EF and has been found to deregulate an overlapping set of targets. Since EF is highly disordered, we reasoned that NKX2.2 might be helping to stabilize EF binding on the promoters of target genes and thus cooperatively affecting the transcription regulation of target genes. One of the important target genes encodes for six-transmembrane epithelial antigen of the prostate 1 (STEAP1) protein. STEAP1 is often found to be upregulated in ES and many other cancers and its knockdown leads to significant tumor regression. We used single molecule resolution imaging to study transcriptional profile in ES cell lines and found a positive correlation between the levels of NKX2.2 and STEAP1 mRNAs in individual cells. NKX2.2 knockdown cell lines showed decreased STEAP1 expression at levels comparable to EWS/FLI1 knockdown. Examination of the STEAP1 promoter by ChIP identified conserved NKX2.2 binding domains within the vicinity of the known EWS/FLI1 binding sites. Increased level of STEAP1 in ES has been linked to result in increased reactive oxygen species (ROS) production and the upregulation of several ROS dependent genes (MMP-1, DTX3L, and ADIPOR1). We found that the loss of NKX2.2 in ES cell lines resulted in decreased ROS expression and reduced expression of ROS dependent genes similarly to the effects seen in STEAP1 knockdown cells. These experiments indicate the potential of NKX2.2 to act as an additional regulator of STEAP1 expression. This cooperative interaction of EF with NKX2.2 could be a general mechanism for the transcriptional regulation of EF dependent target genes in Ewing's Sarcoma. The identification of potential molecular targets in Ewing's sarcoma is crucial to the development of new therapeutics in this disease, which currently has very few treatment options. The prevalence of STEAP1 in multiple cancers also indicates that this work could translate into an understanding of the regulation of STEAP1 in other diseases.

1.8

EPIGENETIC REGULATION OF SOCIAL BEHAVIOR IN ANTS

New Innovator Award, 2014

Roberto Bonasio

University of Pennsylvania, Philadelphia, PA, USA

Ants live in sophisticated societies in which morphologically and behaviorally distinct types of individuals (castes) arise from a single genome, carry out different tasks, and respect the societal boundaries so that colonies can thrive. Female embryos become either reproductive queens or various types of workers, and, strikingly, these profound differences in developmental trajectory are independent of their genetic make-up. Hence, the molecular information that specifies the phenotypic differences among castes must be provided at an epigenetic level, that is, without changes in the DNA sequence.

We have sequenced the genome and obtained genome-wide DNA methylation and chromatin structure profiles for the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Camponotus* ants live in large colonies, where only the long-lived queen lays fertilized eggs. In contrast, *Harpegnathos* queens can be replaced by one or few workers, which acquire behavioral and physiological phenotypic traits typical of the queen.

The unique behavioral flexibility of *Harpegnathos* ants offers a natural experimental paradigm to interrogate the role of epigenetics pathways in regulating brain function and behavior. We obtained gene expression profiles for *Harpegnathos* brains before, during, and after their transition to queen status and identified key regulatory genes. In particular, we discovered a neuropeptide that promotes worker identity by stimulating foraging (hunting) behaviors while inhibiting ovary activation and therefore the transition to queen status. This is the first time that a neuropeptide is shown to play a role in caste determination and caste-specific behavior in a social organism.

1.9

VALIDATING THE DEVILSPARC MOBILE-ECOLOGICAL MOMENTARY ASSESSMENT APP FOR EATING AND PHYSICAL ACTIVITY BEHAVIORS

Early Independence Award, 2013

Meg Bruening
Arizona State University, Phoenix, AZ, USA

Abstract author: Michael Todd
Arizona State University, Phoenix, AZ, USA

Presented by Meg Bruening

The majority of nutrition and physical activity assessments methods commonly used in scientific research are subject to recall bias and social desirability, which result in over- and/or under-reporting of behaviors. Real time mobile-based ecological momentary assessments (mEMAs) may result in decreased measurement biases and minimize participant burden.

The objective is to examine the effectiveness of an mEMA methodology using the devilSPARC mEMA app, on dietary and physical activity levels with respect to 24-hour dietary recalls and physical activity. Methods: This study was a pilot test of the SPARC (Social impact of Physical Activity and nutRition in College) study, which aims to determine the mechanism by which friendship networks impact weight-related behaviors among young people. As part of the SPARC study, an mEMA app, devilSPARC, branded for the university in which the study took place, was developed to assess weight-related behaviors in real-time. A diverse sample of 107 freshmen and community mentors attending a large southwestern university downloaded the devilSPARC mEMA app onto their personal smartphones. Participants were prompted randomly 8 times per day over the course of 4 days to complete the mEMA. During the same 4 day period, participants also completed up to three 24-hour dietary recalls and/or 4 days of accelerometry. Self-reported mEMA responses were compared to 24-hour dietary recalls and accelerometry measures using comparison statistics such as match rate, sensitivity and specificity, mixed model odds ratios, adjusting for repeated measures within person.

Total dietary intake data reported through the mEMA app was similar to eating choices as measured by the 24-hour recall at the day level. Entrées had the lowest match rate across all the time windows (79%), and fruits and vegetables the highest match rate (94%). Widening the window of aggregation of 24-hour dietary recall data on either side of the mEMA response resulted in increased specificity and decreased sensitivity. For physical activity behaviors, levels of activity reported through mEMA differed for sedentary vs non-sedentary behaviors at the day level activity counts measured by accelerometer ($p < 0.001$), but participants did not accurately report intensity of activity in the mEMA.

The devilSPARC mEMA app is valid for assessing eating behaviors and the presence of physical activity at the day level. This mEMA may be useful in studies examining real-time weight-related behaviors.

1.10

GERMLINE IMMUNE VARIATION RESTRICTS THE CANCER MUTATIONAL LANDSCAPE

Early Independence Award, 2013

Hannah Carter

University of California, San Diego, La Jolla, CA, USA

Established tumors suppress or evade T cells that would otherwise initiate an immune response against neoepitopes generated through genetic mutation. It is unclear, however, how the immune system affects the early stages of disease. We investigated this by systematically analyzing the ability of the Major Histocompatibility Complex class I (MHC-I) to present recurrent cancer mutations. We found that peptide sequences harboring recurrent cancer mutations are presented significantly worse than expected, suggesting that mutation frequency is influenced by ability to evade immune detection through low MHC-I affinity. Furthermore, individuals with MHC alleles that can effectively present a recurrent cancer mutation are less likely to develop tumors with that mutation. These findings suggest that inherited MHC-I alleles restrict the landscape of driver mutations that arise in developing tumors.

1.11

WHY JUVENILE SPERMATOCYTES HAVE CHROMOSOME SEGREGATION ERRORS—PROGRAMMED SUPPRESSION OF TWO DNA REPAIR PATHWAYS IS REQUIRED FOR CROSSOVER ASSURANCE

New Innovator Award, 2015

Francesca Cole

The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Abstract author: Maciej J. Zelazowski

The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Presented by Francesca Cole

Successful chromosome segregation during meiosis requires formation of crossovers (COs), which involve exchange of chromosome arms between homolog pairs. Despite their critical role, the number of COs in mammals is low and regulated by CO assurance. How germ cells achieve this precise outcome is not well understood, but impaired CO assurance likely contributes to infertility and aneuploidies. Epidemiological studies find that pubertal fathers are 5-fold more likely to foster a child with Down syndrome, suggesting an apparent lack of robust CO assurance in juvenile males. Previous studies also showed a decrease in CO numbers as measured by deposition of MutLgamma resolvase protein MLH1 in juvenile male mice. To determine how CO assurance is deregulated in juveniles, we cytologically and molecularly investigated the first three waves of juvenile mouse meiosis. We find that robust CO assurance requires exclusion of both MutLgamma-independent double-Holliday junction resolution and dissolution – two prominent double-strand break repair pathways in mitosis. In juvenile mice, unrestrained activity of these pathways generates unproductive noncrossovers, an alternative recombination product that involves conversion of sequences with no exchange of flanking markers. These noncrossovers are generated at the expense of COs, reducing assurance leading to increased chromosome segregation errors in juvenile spermatocytes and providing a mechanistic basis for the increased aneuploidy observed.

1.12

HOST-DIRECTED STRATEGIES TO CREATE SYNERGISTIC ANTIBACTERIAL THERAPIES

Pioneer Award, 2015

Jeffery S. Cox

University of California, Berkeley, Berkeley, CA, USA

The global rise of antibiotic resistance threatens to return us to the pre-antibiotic age, where treatments for the simplest microbial infections would be ineffective. The current approach of the pharmaceutical industry – attempting to stay one step ahead of resistance by modifying existing antibiotics to old targets – is unsustainable as the development of new antibiotics is unlikely to keep pace with the rate of drug resistance. We propose to take a radically different approach to new antibiotic development by targeting host pathways to treat infectious disease. The potential impact of such “Host-Directed Therapies” could be significant, as they may synergize with traditional antibiotics to re-sensitize drug-resistant strains and shorten the time to eradicate chronic infections. We have focused primarily on tuberculosis (TB), one of the most notorious infections of humankind. The recent identification of humans with heritable resistance to TB, as well as our discovery of genes required to promote TB pathogenesis, indicate that endogenous host resistance mechanisms could be manipulated with drugs to fight infection. However, our understanding of the genes and pathways that would be appropriate for such therapeutic intervention is rudimentary. We seek to bridge the gap between known host-pathogen interactions and the creation of novel antibiotics, and also to create a pipeline for identifying new host factors that are amenable for host-directed therapeutics.

1.13

MOZART: HIGH-RESOLUTION OPTICAL MOLECULAR IMAGING SYSTEM FOR MEDICAL AND FUNCTIONAL BIOLOGICAL APPLICATIONS

Early Independence Award, 2012

Adam de la Zerda
Stanford University, Stanford, CA, USA

We developed a new molecular imaging technology we call MOZART that provides a promising platform for imaging of cellular expression of biomarkers, visualizing intercellular signaling among 100 million cells in living animals with a single-cell resolution. We achieved this by advancing Optical Coherence Tomography (OCT), an existing imaging modality, to have molecular imaging capabilities. OCT allows real-time imaging of living tissues at cell-scale resolution over millimeters in three dimensions. Despite these advantages, functional biological studies with OCT have been limited by a lack of exogenous contrast agents that can be distinguished from tissue. Here we developed a new class of imaging agents for OCT—large gold nanorods (LGNRs)—and show that they exhibit over 100-fold greater signal than traditional gold nanorods for OCT. This signal enhancement, in combination with novel image processing algorithms we developed for OCT, allowed us to image the presence of these imaging agents in living tissues at unprecedented sensitivity of 250 pM. In this presentation, we demonstrate MOZART capabilities including the ability to image small capillaries in tumor xenografts in living mice, highlighting the differences in vascular morphology between healthy and tumor tissue. We also show MOZART's functional abilities by multiplexed imaging of two spectrally-distinct LGNRs and use this to show the dynamics of lymph vessel drainage, including observing the functional states of individual lymphangions and valves in a lymphatic network. In the future we plan to use MOZART to interrogate the spatial and temporal expression patterns of cancer biomarkers in a live tumor during drug response or disease progression.

1.14

PREDICTING RESILIENCE IN THE HUMAN MICROBIOME

Transformative Research Award, 2012

David Relman,¹ Susan Holmes,¹ Jeremy Nicholson,² Elaine Holmes²

¹Stanford University, Stanford, CA, USA; ²Imperial College London, Kensington, London, UK

Presented by Les Dethlefsen

Humans have co-evolved with complex, dynamic microbial communities in the distal gut that play essential roles in nutrition, metabolism, immunity, and other aspects of human physiology. Hence, maintenance and recovery of beneficial microbial activities during and after disturbances to the microbiota are fundamental to human health. However, microbial stability and resilience vary both within and between individuals, and are poorly understood. Our goal is to identify features of the human microbiome and host that predict microbial community stability and resilience following disturbance. We have recruited approximately 100 healthy adult subjects to provide stool and urine samples at daily or weekly intervals over about 34 weeks. Twice during the study, participants experience brief, deliberate perturbations of their gut microbiota: diet supplementation with resistant starch for 5 days at about week 11, then either a 1-day colon cleanout (as if preparing for a colonoscopy) or a short course of the antibiotic ciprofloxacin at about week 22. Responses of the microbiota to these perturbations occur in the context of routine day-to-day variability due to intrinsic factors and host effects such as meal choice, activity level, acute illness, etc. The composition and activity of the gut microbiota are being assessed via 16S rRNA gene sequencing, whole genome ‘shotgun’ metagenomic sequencing, metatranscriptomic assessment of microbial gene expression, and untaraged high-throughput metabolomic analysis.

Aim 1. Profile the composition and activity of the human gut microbiome before, during and after multiple types of perturbation, as well as during unperturbed intervals, in a moderately large cohort of healthy individuals. **Aim 2.** Develop non-linear approaches to integrate complex, heterogeneous data using sparse, multiple-table methods; assess aspects of microbial function and composition that are stable across perturbations, that are resilient (i.e., respond to perturbation but return to baseline), or that display other temporal patterns. Such aspects may include, e.g., the relative abundance of particular taxa, the carriage or expression level of particular metabolic pathways, the concentrations of microbial metabolites, and summary measures such as ecological diversity metrics or pairwise sample dissimilarity. **Aim 3.** Use statistical learning approaches to discover and validate traits of either the gut microbiota or the human host that predict stability and/or resilience of the microbiota. Such traits may include, e.g., features of the microbiota itself such as taxonomic or functional diversity or baseline temporal stability, or characteristics of the host such as the diversity of diet or range of past or current microbial exposure.

1.15

DEVELOPMENT OF PIRNAS FOR TARGET-SPECIFIC DNA METHYLATION

Transformative Research Award, 2015

Dana Dolinoy
University of Michigan, Ann Arbor, MI, USA

Presented by Christopher Faulk

Precision modification of the epigenome holds great promise for our ability to target changes in gene expression, yet is currently out of reach using common techniques (drugs, transgenics, nutrition, etc.). Our Transformative Research Award challenge is to develop a suite of tools, based on the Piwi-interacting RNA (piRNA) system, to advance precision epigenetic editing, while avoiding drawbacks of current technology (e.g. off-target effects), and would be adaptable to basic science and clinical settings. It has been shown that piRNAs interact with three PIWIL proteins (PIWIL 1, 2, and 4) for a variety of functions, most applicable to this challenge is its modification of DNA methylation. Until now, it has been widely believed that PIWIL gene expression was confined to the germ line of animals, and neither PIWILs nor piRNAs were present or active in somatic tissues. Our research overturns this accepted knowledge by finding widespread PIWIL expression in multiple somatic tissues of the mouse in three male biological replicates in whole brain, hippocampus, heart, kidney, liver and testes, and one female biological replicate in ovary. When using only high quality RNA (RIS score > 7.0), the housekeeping gene, beta actin, averaged a 27.91 cycle threshold (CT) across all somatic tissues with a range from 23 CT to 33 CT. Average PIWIL 1 CT across all tissues was 31.29, ranging from 25 to undetectable with the highest expression in testes and the lowest detectable expression in brain. PIWIL 2 displayed an average 35.19 CT value across all tissues, mirroring PIWIL 1 expression with the highest expression level in testes and the lowest detectable expression level in brain. PIWIL 4 mirrored PIWIL1 in having a high relative expression averaging 32.03 CT value across all tissues. PIWIL 4, however, had the highest expression in testes and lowest detectable expression in heart. Overall, we detected PIWIL expression in brain, hippocampus, heart, kidney, liver in males and females as well as in testes and ovary. While no tissue exhibited expression of any PIWIL as high as testes, the considerable somatic PIWIL gene expression within the mouse strongly suggests piRNA function in the soma of adults. Ongoing work on PIWIL protein expression as well as piRNA-seq will identify the number, type, and tissue-specificity of the piRNA expressed across somatic tissues, and will serve as further evidence of piRNA function in non-germ line cells. The preliminary research shown here is essential for the development of in vivo piRNA targeted DNA methylation approaches applicable to the wider research and therapeutic communities.

1.16

CONTROLLING CELLULAR FATE THROUGH GLYCAN ENGINEERING

New Innovator Award, 2015

Kamil Godula

University of California, San Diego, La Jolla, CA, USA

The glycocalyx is a complex biological interface rich in carbohydrates (or glycans) and with intricate nanoscale organization that separates cells from the outside world. The purpose of cell surface glycans is to regulate intracellular signaling events as well as to mediate the interactions of cells with the outside environment. While the functional roles of individual glycan structures within the glycocalyx have begun to emerge, very little is known about how their three-dimensional presentation influences biological events. Our lab has developed a new family of nanoscale glycomaterials that mimic the structure and function of various cell surface glycoconjugates. These glycomaterials can be used to modulate growth factor interaction at the cell-matrix interface to drive gene expression and differentiation or to orchestrate multicellular signaling events, such as synaptic receptor clustering, to promote the bottom up assembly of artificial tissues.

1.17

TARGETING PERSONALIZED NANOMEDICINES TO THE TUMOR MICROENVIRONMENT

New Innovator Award, 2012

Daniel A Heller

Memorial Sloan-Kettering Cancer Center, New York, NY, USA; Weill Cornell Medical College, New York, NY, USA

Disseminated tumors are poorly accessible to nanoscale drug delivery systems because of the vascular barrier, which attenuates extravasation at the tumor site. We investigated P-selectin, a molecule expressed on activated vasculature that facilitates metastasis by arresting tumor cells at the endothelium, for its potential to treat tumors using the same mechanism to arrest nanomedicines at the tumor endothelium (Shamay, et. al., *Sci Trans Med*, 2016). We developed a nanoparticle drug carrier platform using a fucosylated polysaccharide with nanomolar affinity to P-selectin. The nanoparticles targeted the tumor microenvironment to localize targeted therapies at the tumor site and away from healthy tissues to obviate dose-limiting toxicities and concomitantly improve therapeutic index. We found that the nanoparticles targeted MEK and PI3K inhibitors to tumor sites in both primary and metastatic models, resulting in superior anti-tumor efficacy and the striking reduction of toxicities. In tumors devoid of P-selectin, we found that ionizing radiation guided the nanoparticles to the disease site by inducing P-selectin expression, suggesting a potential strategy to target disparate drug classes to almost any solid tumor.

1.18

CLINICAL AND TRANSLATIONAL APPROACHES TO COGNITIVE IMPAIRMENTS IN BREAST CANCER

New Innovator Award, 2014

Michelle Janelsins, Ph.D., M.P.H.
University of Rochester, Rochester, NY, USA

While chemotherapy has greatly improved survival for cancer patients, the side effects of this treatment can lead to substantial detrimental effects on quality of life that can be debilitating. Cancer- and chemotherapy-related cognitive impairment (CRCI) is characterized by difficulty in memory, attention, concentration and executive function. CRCI is most pronounced and severe during chemotherapy (in up to 80% of patients), however, it can last for years following treatment in up to 35% of survivors. With over 13 million cancer survivors in the US, it is estimated that up to 4 million survivors could be living with long-lasting effects of CRCI. CRCI is particularly significant because long-term cognitive impairment can develop, CRCI negatively impacts quality of life, and CRCI can affect treatment adherence. Little is known about the biological mechanisms contributing to CRCI development, though studies suggest that increased inflammation may be involved.

This research involves a novel combination of animal modeling and human research to address the role of inflammation in CRCI, and also uses animal modeling to develop interventions that will lead to clinical research studies. We have developed a clinically relevant CRCI mouse breast cancer tumor model (E0771) with adriamycin and cytoxan chemotherapy to study the effects of cancer and chemotherapy on memory function, as well as the contributing role of key cytokine pathways that contribute to neurotoxicity involved in CRCI. We are also longitudinally assessing CRCI in breast cancer patients receiving chemotherapy and contributing cytokine and related genetic factors.

Our preliminary data suggest that both cancer and chemotherapy impact cognitive function in a clinically relevant breast cancer mouse model. Our memory assessment used in our mouse study is translatable to the human study. Ongoing work includes the assessment of the role of cancer and cytokine factors that play a role in CRCI in mice and humans. Additionally, we are planning pre-clinical studies to assess physical activity interventions in mice that may be applicable to human research.

1.19

TRANSFORMING OUR UNDERSTANDING OF EUKARYOTIC GENE FUNCTIONS THROUGH CHEMICAL GENETICS IN THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

New Innovator Award, 2015

Martin C. Jonikas
Princeton University, Princeton, NJ, USA

Abstract author: Friedrich Fauser
Princeton University, Princeton, NJ, USA

Presented by Martin C. Jonikas

Thousands of genomes have been sequenced, but the functions of most of the genes that they encode remain largely unknown. My lab aims to transform our ability to engineer biology by developing broadly applicable tools that dramatically accelerate the study of uncharacterized genes. In this New Innovator project, we are developing chemical genetics tools to assign functions to thousands of uncharacterized genes in the green alga *Chlamydomonas reinhardtii*, a powerful model system central to studies of ciliary biogenesis and motility, centrosomes, photosynthesis, electron transport, inter-organelle communication and optogenetics.

In a major step towards our goal, we have generated an indexed and mapped library of 60,000 mutants covering 80% of all *Chlamydomonas* genes. The library is available to the community, and to date more than 1,000 mutants have been distributed to more than 100 laboratories worldwide.

We designed this library to be compatible with high-throughput chemical genetics approaches. The abundance of individual mutants can be tracked in pools through deep sequencing of unique DNA barcodes. We have demonstrated the ability to perform 50 genome-wide mutant screens in the span of 4 weeks, and are preparing for 250 additional screens in the near future. Mutants with similar phenotypic profiles are typically disrupted in genes involved in the same pathway and can be clustered accordingly. In a proof-of-concept study, we screened the *Chlamydomonas* library for mutants deficient in photosynthesis, the algal carbon concentration mechanism, and several DNA repair pathways. In these screens, dozens of homologs of well-characterized genes appear as hits, as do dozens of uncharacterized genes. The characterization of these new factors will give transformative insights into multi-gene pathways across the tree of life.

1.20

CRISPR/CAS9-MEDIATED ENGINEERING OF CLINICALLY RELEVANT MUTATIONS INTO THE ENDOGENOUS LOCI OF PRIMARY HUMAN MELANOCYTES REVEALS NOVEL ROLES FOR BRAF AND CDKN2A DURING MELANOMA PROGRESSION

Early Independence Award, 2014

Robert L Judson

Department of Dermatology, University of California, San Francisco, San Francisco, CA, USA

If allowed to reach advanced stages, the mortality rate of cutaneous melanoma is high despite the recent development of targeted therapies and immunotherapies. Yet, if detected at earlier stages, melanoma is curable, highlighting the need for better methods of early detection and prevention. Roughly 30% of melanomas are derived from benign precursors called nevi (common moles) providing an opportunity for early intervention of a large subset of melanomas. Interestingly, the majority of both malignant melanomas and benign nevi are driven by activating mutations in the MAP kinase signaling pathway, most commonly BRAF^{V600E}. The cellular and molecular biology that differentiates BRAF^{V600E}-driven nevi from subsequent melanoma initiation is poorly understood. Current models suggest that BRAF^{V600E} induces senescence through INK4A activation and subsequent loss of INK4A overcomes this growth arrest. Although loss of the INK4A genetic locus, CDKN2A, is indeed a frequent acquired mutation during melanoma progression, this model remains controversial due to conflicting clinical and experimental observations. The cellular consequences of BRAF^{V600E} mutations and its relationship with CDKN2A deletion remain poorly defined due to a lack of appropriate model systems for studying these early processes. Here we report the engineering of BRAF and CDKN2A mutations into the endogenous loci of primary human melanocytes using CRISPR/Cas9-mediated homology directed repair. This novel model system allows for the direct comparison of normal human melanocytes to isogenic sibling cells harboring clinically-relevant mutations. Contrary to the well-documented observation that exogenously over-expressed BRAF^{V600E} triggers oncogene-induced senescence in melanocytes, we find that introduction of the point mutation into the endogenous locus induces a persistent increase in proliferation and that exogenous-BRAF^{V600E}-induced senescence occurs independent of CDKN2A. Regarding their effect on proliferation, endogenous BRAF^{V600E} and CDKN2A^{DEL} exhibit a synergistic relationship, instead of the presumed epistatic relationship. We further used our engineered melanocytes to investigate novel oncogenic phenotypes induced by these mutations and find that INK4A actively inhibits melanocyte motility and invasion through post-translational regulation of the MITF-BRN2 axis. We confirm the role of INK4A as a regulator of motility and invasion using patient derived melanoma lines, staining of primary lesions, and exome and mRNA sequencing of matched clinical samples. Together our data support an alternative model for melanoma initiation whereby BRAF^{V600E} induces persistent, but slow, cellular proliferation controlled in nevi by external factors and that CDKN2A^{DEL} induces cell invasion and is synergistic with BRAF^{V600E} in the induction of more rapid cell growth. Confirmation of this model would give rise to novel approaches for early melanoma detection and prevention.

1.21

EYE DEVELOPMENT AND PHOTORECEPTOR DIFFERENTIATION IN THE SQUID *DORYTEUTHIS PEALEII*

Early Independence Award, 2016

Kristen Koenig
Harvard University, Cambridge, MA, USA

Understanding the evolution of complex organ systems is an important aspect of understanding the evolution of diversity. The visual system is particularly compelling because of the high level of morphological diversity and complexity in photoreceptive organs across the Metazoa. Our research interest is to better understand visual system evolution from a developmental perspective. We have established the squid, *Doryteuthis pealeii*, as a lophotrochozoan model for complex eye development. Utilizing histological, transcriptomic and molecular assays we characterize eye formation in *Doryteuthis pealeii*. Through lineage tracing and gene expression analyses, we demonstrate that cells expressing Pax and Six genes incorporate into the lens, cornea and iris tissue, suggesting a convergent involvement in lens formation. We identify the sole source of retinal tissue and functional assays demonstrate that Notch signaling is required for photoreceptor cell differentiation and retina organization. These assays support a conserved role for notch signaling in neurogenesis in the cephalopod eye.

1.22

ION MOBILITY MASS SPECTROMETRY OF INTACT MEMBRANE PROTEIN-LIPID COMPLEXES

New Innovator Award, 2016

Arthur Laganowsky
Texas A&M University, College Station, TX, USA

Membrane proteins are embedded in the biological membrane where they intimately interact with lipids. The chemically diverse lipid environment can modulate the structure and function of membrane proteins, including a number of membrane proteins that require specific lipid interactions for their function. However, the thermodynamics governing the molecular recognition and interaction of lipids with membrane proteins is poorly understood. Ion mobility mass spectrometry (IM-MS) of intact protein complexes, or native IM-MS, is an emerging technology to probe membrane protein complexes and their interactions with lipids and other molecules. Unlike other biophysical approaches, IM-MS can resolve and interrogate individual lipid binding events and coupled with an apparatus to control temperature, determine binding thermodynamic parameters, such as for protein-lipid interactions. We validated our new approach using three soluble protein-ligand systems and obtained similar results to those using isothermal titration calorimetry and surface plasmon resonance. We also determined for the first time the thermodynamics of individual lipid binding to the ammonia channel (AmtB), an integral membrane protein from *Escherichia coli*. Remarkably, we observed distinct thermodynamic signatures for the binding of different lipids and entropy-enthalpy compensation for binding lipids of variable chain length. Additionally, using a mutant form of AmtB that abolishes a specific phosphatidylglycerol (PG) binding site, we observed distinct changes in the thermodynamic signatures for binding PG, implying these signatures can identify key residues involved in specific lipid binding and potentially differentiate between specific lipid binding sites. In summary, native IM-MS provides unprecedented insight into individual lipid binding events to membrane proteins that would otherwise remain intractable using other biophysical approaches.

1.23

DNA-ORIGAMI TEMPLATED MEMBRANE STRUCTURE AND DYNAMICS

New Innovator Award, 2014

Chenxiang Lin

*Department of Cell Biology, Yale University School of Medicine, Nanobiology Institute, Yale University,
New Haven, CT, USA*

Lipid-bilayer membranes form barriers to define the boundaries of a cell and its subcellular compartments. With the help of membrane-associating molecules, they undergo dramatic structural changes during the life cycle of cells and mediate complex reactions that are vital to cell division, growth and communication. Inspired by such elegance in nature, bioengineers and synthetic biologists have aspired to build artificial membranes to recapitulate the cellular membrane structure and dynamics. In addition, such *in vitro* preparations provide a complexity-reduced system for cell biologists and biophysicists to study functional interactions between membranes and their associating molecules. Despite the advanced chemical and physical methods that are now available to manipulate and observe lipid membranes, two technical challenges have hampered our ability to construct a completely artificial system that mimics natural membrane systems. First, it has been difficult to produce large quantities of mono-dispersed lipid vesicles with well-defined structure (size & shape). Second, it has been challenging to regulate the membrane dynamics (fusion, budding, etc.) in a programmable way.

Here we present our progress in resolving these technical limitations. Our approach is to use self-assembled DNA nanostructures as templates to guide the assembly of lipid bilayers and transduce the programmable feature of the DNA nanostructures to the templated vesicles. Specifically, we show the assembly, arrangement, and remodeling of liposomes with designer geometry: all of which are exquisitely controlled by a set of modular, reconfigurable DNA nanocages. Unlike previous methods that rely on trial and error to tune vesicle shape, here the geometry of a liposome is directly programmed into its DNA cage. Tubular and toroidal shapes, among others, are transcribed from DNA cages to liposomes with high fidelity, giving rise to membrane curvatures present in cells yet previously difficult to construct *in vitro*. Moreover, the conformational changes of DNA cages drive membrane fusion and bending with predictable outcomes, opening up opportunities for the systematic study of membrane mechanics.

1.24

IMMUNOPHENOTYPING OF HUMAN HYPERTENSION USING SINGLE-CELL MULTIPLEX MASS CYTOMETRY TO IDENTIFY NOVEL THERAPEUTIC TARGETS

New Innovator Award, 2016

Meena Madhur

Vanderbilt University Medical Center, Nashville, TN, USA

Hypertension is a major global health concern. In the U.S. alone, one-third of all adults have hypertension and this rises to two-thirds by the age of 60. Hypertension is a key contributor to morbidity and mortality from stroke, myocardial infarction, heart failure, peripheral vascular disease, and chronic kidney disease. Unfortunately, despite current treatment, blood pressure remains poorly controlled in approximately 50% of individuals with this disease. Even when blood pressure is reasonably controlled, hypertension is associated with increased cardiovascular risk. There is emerging evidence from our lab and others that this residual risk and the end-organ effects of hypertension are largely mediated by inflammation. For example, mice that are deficient in T cells, B cells, monocytes/macrophages, or one of a number of pro-inflammatory cytokines exhibit blunted hypertension and reduced end-organ dysfunction in response to hypertensive stimuli. Yet most of these studies have been conducted in experimental animals, particularly rodents, with limited studies examining the effect of inflammation in human hypertension. Moreover, global or even partial immunosuppression in humans is not without risk. To address this problem, the first goal of this proposal is to utilize a cutting-edge single cell multiplex mass cytometry time of flight (CyTOF) approach to profile the immune cells in the peripheral blood of normotensive and hypertensive humans to identify unique and possibly rare subpopulations that are altered in human hypertension. In mass cytometry, antibodies to extracellular and intracellular targets are conjugated to rare earth metal isotopes that are detected by a mass spectrometer. As obesity and aging are intricately associated with hypertension, our study population will include people with a range of body mass indices and age (35 to 75) to determine the extent to which observed hypertensive changes in immune profiles correlate with obesity and aging. The second goal is to isolate these novel or altered cell populations through flow sorting and further characterize them by RNA-sequencing, cytokine production, and/or deep sequencing of T cell receptors in the case of T lymphocytes. Mapping the immunological landscape of hypertension, obesity, and aging promises to lead to new diagnostic, prognostic, and therapeutic strategies to treat human hypertension and limit the associated end-organ damage from this chronic, widespread disease without inducing global immunosuppression.

1.25

3D PRINTED NANO-BIONIC ORGANS

New Innovator Award, 2014

Michael McAlpine

University of Minnesota, Minneapolis, MN, USA

The development of approaches for multidimensional integration of functional electronic components with biological tissue and organs could have tremendous impact in regenerative medicine, smart prosthetics, and restorative health. However, current electronic devices and systems are inherently two dimensional and rigid, thus prohibiting seamless meshing with three-dimensional, soft biology. The ability to three-dimensionally interweave biological tissue with functional electronics could enable the creation of bionic organs for alleviating impairments or restoring loss of critical function. Current electronics are inherently two-dimensional, preventing seamless integration with biology, as the processes and materials used to create synthetic tissue constructs vs. conventional electronic devices are very different. Here, we present a novel strategy for overcoming these difficulties via additive manufacturing of biological cells with various classes of functional electronic nanomaterials. Recently, we have generated a functional bionic ear via 3D printing of a cell-seeded hydrogel matrix in the precise anatomic geometry of a human ear, along with an intertwined conducting polymer consisting of infused silver nanoparticles. This allowed for the *in vitro* culturing of cartilage tissue around an inductive coil antenna in the ear, which subsequently connects to cochlea-shaped electrodes. The printed ear exhibits enhanced auditory sensing for radio frequency reception, and complementary left and right ears can listen to stereo music. Here, we propose extending this approach to new functionalities – such as ultrasonic acoustic reception and vasculature – and bionic organs, including bionic eyes and a bionic nose. Overall, our approach presents a disruptive and paradigm-shifting new method to intricately merge biology and electronics via 3D printing. The work outlined here thus constitutes a novel, highly interdisciplinary investigation to addressing outstanding questions in the generation of bionic organs, and we anticipate that this work will represent a paradigm-shift in dynamic tissue engineering, regenerative medicine, as well as 3D interweaving of functional electronics into biological systems.

1.26

IF JOHN SNOW HAD A SMART PHONE: EVALUATION OF A DECISION-SUPPORT TOOL FOR DIARRHEAL DISEASE OUTBREAK MANAGEMENT

Early Independence Award, 2014

Eric J. Nelson

University of Florida, Gainesville, FL, USA; Stanford University School of Medicine, Stanford, CA, USA

Abstract authors: Eric J. Nelson, M.D. Ph.D.,^{1,2} F. Haque,^{3,4} R. L. Ball,² S. Khatun,³ M. Ahmed,³ Md. J. Chisti,⁴ S. A. Sarker,⁴ S. Maples,² D. Pieri,⁵ T. Vardhan,⁶ C. Sarnquist,² N. Federspiel,² Md. W. Rahman,^{3,4} J. R. Andrews,² M. Rahman^{3,4}

¹*University of Florida, Gainesville, FL, USA;* ²*Stanford University School of Medicine, Stanford, CA, USA;* ³*Institute of Epidemiology, Disease Control, and Research, Dhaka, Bangladesh;* ⁴*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh;* ⁵*Independent Technology Developer, San Francisco, CA, USA;* ⁶*Beehyv Software Solutions Inc., Hyderabad, India*

Presented by Eric J. Nelson

The emergence of mobile technology offers new opportunities to improve clinical guideline adherence in resource-limited settings. We conducted a clinical pilot study in rural Bangladesh to evaluate the impact of a smartphone adaptation of the World Health Organization diarrheal disease management guidelines, including a modality for age-based weight estimation. The development of the software was guided by end-user input and was then evaluated at a resource-limited district and sub-district hospital in rural Bangladesh during the fall 2015 cholera season; these hospitals lack scales which necessitated weight estimation. The study consisted of a 6-week pre-intervention and 6-week intervention period with a 10-day post-discharge follow-up. Standard-of-care was maintained throughout the study with the exception that admitting clinicians used the tool during the intervention. Inclusion criteria were patients two months of age and older with uncomplicated diarrheal disease. The primary outcome was adherence to guidelines for prescriptions of intravenous (IV) fluids, antibiotics and zinc. A total of 841 patients were enrolled (325 pre-intervention; 516 intervention). During the intervention, the proportion of prescriptions for IV fluids significantly decreased at the district and sub-district hospitals (both $p < 0.001$) with odds ratios (ORs) of 0.22 and 0.002, respectively. However, when IV fluids were prescribed, the volume better adhered to recommendations. The proportion of prescriptions for the recommended antibiotic azithromycin significantly increased ($p < 0.001$ district; $p = 0.035$ sub-district) with ORs of 46 (district) and 50 (sub-district) while prescriptions for other antibiotics that were not recommended decreased (e.g. ciprofloxacin). Zinc adherence also increased. This study identified opportunities to improve clinical care, including better dehydration assessment, weight estimation, and fluid/ antibiotic selection. These findings demonstrate that a smartphone-based tool can significantly improve guideline adherence. This study should serve as a catalyst for a randomized controlled trial to expand on the findings and address study limitations.

1.27

CELL SIGNALING IN CONTROL OF REGENERATIVE GROWTH

New Innovator Award, 2013

Christian Petersen

Northwestern University, Evanston, IL, USA

Pluripotent stem cells offer great hopes for regenerative medicine, but it remains a significant challenge to finely orchestrate their activity in order to form organs identical to functional adult tissues. Animals capable of tissue regeneration can perfectly re-establish their form after diverse injuries, suggesting such abilities rely critically on robust patterning to instruct the behavior of progenitors for tissue production, but the underlying molecular mechanisms remain enigmatic. Planarian flatworms have emerged as a model organism to study whole-body regeneration mediated by adult pluripotent stem cells. Our studies have used this organism to identify the cell signaling and regulatory principles that allow restoration of a body axis truncated by injury. Using the head-to-tail body axis of planarians as a model, we identify a canonical Wnt/beta-catenin signaling pathway mediated by asymmetric expression of the Wnt inhibitor *notum* that responds to tissue orientation at the wound site and polarizes the identity of the axis termini in regeneration. Downstream of this early decision step, a stem-cell-dependent pathway activated by injury-induced expression of Zic-family transcription factors forms a Wnt inhibitory organizing center needed for head outgrowth after decapitation. Furthermore, we find a cohort of regionally expressed genes as candidates that define the identity of pre-existing tissues independent of stem cell activity. Among these, a pathway involving three genes expressed in overlapping body-wide gradients regulate tissue identity along regions of the head-tail axis: *wntP-2*, a noncanonical Wnt signaling co-receptor *ptk7*, and a conserved FGFR-like tyrosine kinase-deficient cell-surface protein. These analyses suggest that natural mechanisms of regeneration involve early injury-induced directional cues used in axis polarization, the use of stem cells to create tissue organizing centers needed for blastema outgrowth, and constitutive expression gradients of signaling molecules used to restore regional identity along an amputated axis. Together, these analyses seek to uncover the regulatory logic underlying regenerative growth.

1.28

DYNAMICS OF OCT4 SIGNALING IN HUMAN EMBRYONIC STEM CELLS

New Innovator Award, 2016

Jeremy Purvis

The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

The choice between self-renewal and differentiation is central to the biology of human stem cells, but it is not clear how an individual cell makes this fate decision. Here, we used time-lapse fluorescence microscopy to visualize differentiation of single human embryonic stem cells to trophoblast—the first differentiation decision during mammalian development. We found that expression of the pluripotency factor OCT4 is heritable from mother cell to daughter cell but disproportionately distributed during cell division. Daughter cells that received a larger share of maternal OCT4 showed persistently high levels of OCT4, more frequent bursts in OCT4 expression, shorter cell cycle durations, and a reduced capacity to differentiate. These results support a model in which asymmetric inheritance of OCT4 replenishes the pluripotent population faster than producing cells that are competent to differentiate. More fundamentally, our work reveals a single-cell mechanism that controls the fate choice between self-renewal and differentiation in human embryonic stem cells.

1.29

COMPUTING THE ANKLE-BRACHIAL INDEX WITH COMPUTATIONAL FLUID DYNAMICS

Early Independence Award, 2014

Amanda Randles
Duke University, Durham, NC, USA

Peripheral artery disease (PAD), in which narrowing and blockage of peripheral arteries reduces blood flow to the extremities of the body, is associated with a six-fold increase in mortality risk from cardiovascular disease. PAD is diagnosed by computing the ankle-brachial index (ABI), a metric relating blood pressure in the ankles and upper arms. With parallel computing, we use 3D computational fluid dynamics to simulate flow in a complete, patient-derived arterial system and compute the ABI. The simulations employ a massively parallel CFD application, HARVEY, designed for large-scale hemodynamic simulations and based on the lattice Boltzmann method. Simulations were conducted on Vulcan, a Blue Gene/Q supercomputer at Lawrence Livermore National Laboratory with 393,216 cores. We consider the dependence of ABI on simulation resolution and find adequate numerical convergence at 50 microns. The influence of body posture on ABI is investigated by incorporating gravitational forces corresponding to supine and standing body positions. Additionally, we consider the influence of an aortic coarctation, which imposes the same hemodynamic compromise on the peripheral arteries as PAD, and observe the expected decrease in ABI.

1.30

COMPARATIVE GENETIC SCREENS IN HUMAN CELLS REVEAL NEW REGULATORY MECHANISMS IN WNT SIGNALING

New Innovator Award, 2012

Rajat Rohatgi

Stanford University School of Medicine, Stanford, CA, USA

Abstract author: Andres M. Lebensohn

Stanford University School of Medicine, Stanford, CA, USA

Presented by Rajat Rohatgi

The comprehensive understanding of cellular signaling pathways remains a challenge due to multiple layers of regulation that may become evident only when the pathway is probed at different levels or critical nodes are eliminated. To discover regulatory mechanisms in canonical WNT signaling, we conducted a systematic forward genetic analysis through reporter-based screens in haploid human cells. Comparison of screens for negative, sensitizing and positive regulators of WNT signaling, mediators of R-spondin-dependent signaling and suppressors of constitutive signaling induced by loss of the tumor suppressor APC or casein kinase 1alpha uncovered new regulatory features at all levels of the pathway. These include a requirement for the transcription factor TFAP4, a role for the DAX domain of AXIN2 in controlling beta-catenin specific activity, a contribution of GPI anchor biosynthetic enzymes and glypicans to R-spondin-potentiated signaling, and two different mechanisms that regulate signaling when distinct components of the beta-catenin destruction complex are lost.

1.31

CIRCUIT MECHANISM OF A NOVEL RETINAL GANGLION CELL WITH NON-CANONICAL RECEPTIVE FIELD STRUCTURE

New Innovator Award, 2015

Gregory Schwartz

Northwestern University, Evanston, IL, USA

Center-surround antagonism is the canonical model to describe receptive fields (RFs) of visual neurons, including retinal ganglion cells (RGCs). We describe a newly identified RGC type in mouse, called the ON delayed (OND) RGC, with RF properties that deviate from center-surround organization. Responding with an unusually long latency to light stimulation, OND RGCs respond earlier as the visual stimulus increases in size. Furthermore, OND RGCs are excited by light falling far beyond their dendrites. We unravel details of the circuit mechanisms behind these phenomena, revealing new roles for inhibition in controlling both temporal and spatial RF properties. The non-canonic RF properties of the OND RGC – integration of long temporal and large spatial scales – make it well suited to encode a slowly varying, global property of the visual scene. The exquisite sensitivity of OND RGCs to spatial blur in across a large region of visual space suggests a role in the image focus control circuit, revealing a new potential target for clinical interventions in myopia.

1.32

MODULATION OF PDGFRA AND IDH1 ONCOGENES BY TOP2 IS ASSOCIATED WITH ITS PROMOTER LOCALIZATION IN GLIOMAS

Early Independence Award, 2015

Adam M. Sonabend

Department of Neurosurgery and Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA

Gliomas are brain tumors with a remarkable heterogeneous gene expression pattern. Epigenetic factors contribute to the regulation of these transcriptional profiles, yet these mechanisms are not well understood. TOP2A/B are helicases implicated in transcriptional regulation in other contexts, and regulate transcription by inducing double-strand DNA breaks and enhancing chromatin accessibility. TOP2B has been shown to interact with CTCF, a transcription factor capable of binding heterochromatin. Here we hypothesize that TOP2A/B regulate the transcription of oncogenes in specific subsets of gliomas. We hypothesize that CTCF plays a role in recruiting TOP2A/B to these loci.

We used proneural glioma cell lines with IDH1^{R132H} mutation (BT142) and IDH1^{wt} (TS543) as well as a mesenchymal glioma cell line (SNB19). We performed ChIP-qPCR in the presence of etoposide to stabilize TOP2A/B genomic binding. qRT-PCR was used to evaluate gene expression +/- ICRF-193, a pharmacologic TOP2A/B inhibitor. Inhibition was confirmed by decatenation assays. A T-test showing $p < 0.05$ was considered significant.

PDGFRA and IDH1, two genes that play roles as oncogenes in proneural gliomas were highly expressed on TS543 and BT142, compared to SNB19 ($p < 0.05$). For these two genes, CTCF, TOP2A and TOP2B genomic binding was enriched in the promoter/intron 1 compared to IgG control in all cases.

Decatenation assays showed that TOP2A/B enzymatic activity was inhibited following treatment with ICRF-193 in all glioma cell lines. In the case of PDGFRA and IDH1, TOP2B binding to the promoter/intron 1 was associated with a decrease in expression following TOP2A/B inhibition with ICRF-193 in all cell lines. IDH1 decreased 5-fold in BT142 ($p < 0.05$) and 22-fold in TS543 ($p < 0.0001$) whereas PDGFRA decreased 3.8-fold in BT-142 ($p < 0.05$) and 6.8-fold in TS543 ($p < 0.05$), in spite of the fact that PDGFRA is amplified in the latter cell line.

As opposed to PDGFRA and IDH1, the expression of the tumor suppressor genes Rb1 and p53 was low and similar in all cell lines. TOP2B binding was only enriched in the Rb1 promoter of SNB19. Similar to the case of PDGFRA and IDH1, TOP2B binding on the Rb1 promoter was associated with a decrease in expression following TOP2A/B inhibition. p53 promoter binding by CTCF, TOP2A, and TOP2B was enriched in BT142 and TS543 proneural glioma cell lines, but only CTCF binding was found on SNB19. As opposed to the other genes, the expression of p53 was not altered by TOP2A/B inhibition.

Our results suggest a role for epigenetic regulation of transcription by TOP2 in gliomas. This is supported by its localization on promoters of oncogenes, and the associated decrease in expression of these genes following pharmacological inhibition. Targeting of TOP2 might allow for the simultaneous therapeutic modulation of multiple oncogenes, exploiting the epigenetic mechanisms underlying chromatin structure in cancer.

1.33

OPTOGENETIC ACTIVATION OF CHOLINERGIC NEURONS IN THE PPT OR LDT INDUCES REM SLEEP

Transformative Research Award, 2012

Emery Brown,^{1,2} Ken Solt,¹ Ed Boyden,² and Matthew Wilson²

¹*Massachusetts General Hospital, Boston, MA, USA;* ²*Massachusetts Institute of Technology, Cambridge, MA, USA*

Presented by Christa Van Dort

Massachusetts Institute of Technology, Cambridge, MA, USA; Massachusetts General Hospital, Boston, MA, USA

Rapid eye movement (REM) sleep is a critical component of restful sleep, yet the mechanisms that control REM sleep remain incompletely understood. Brainstem cholinergic neurons have long been implicated in REM sleep regulation. However, lesions of this area across species (cat, rat, mouse) have had varying effects on the resulting amounts of REM sleep. In addition, the area contains heterogeneous cell types making it difficult to determine the contribution of each subpopulation of neurons to overall REM sleep regulation. These challenges have led to a debate about the importance of cholinergic neurons in REM sleep regulation. Therefore, this study aimed to clarify the role of cholinergic neurons in the brainstem pedunculopontine tegmentum (PPT) and laterodorsal tegmentum (LDT) in REM sleep generation. We found that selective optogenetic activation of cholinergic neurons in the PPT or LDT during NREM sleep increased the number of REM sleep episodes but did not change REM sleep episode duration. The induced REM sleep state closely resembles natural REM sleep. Therefore, activation of cholinergic neurons in the PPT or LDT during NREM sleep was sufficient to induce REM sleep in mice. Our data demonstrate that brainstem cholinergic neurons remain important modulators of REM sleep and clarify their role in REM sleep initiation.

1.34

ADIPOCYTES IN THE MELANOMA MICROENVIRONMENT

New Innovator Award, 2013

Richard Mark White

Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract authors: Maomao Zhang,¹ Sanjeethan Baksh,² Theresa Simon-Vermot,¹ Isabelle Kim,¹
Travis Hollman,^{1,3} and Richard Mark White¹

¹*Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA;*

²*Tri-Institutional M.D.-Ph.D. Program, Weill Cornell Medical College, New York, NY, USA;* ³*Department of Pathology, Memorial Sloan Kettering Cancer Center New York, NY, USA*

Presented by Maomao Zhang

The success of immunotherapy in melanoma underscores the capacity for the tumor microenvironment (TME) to have a dominant effect on tumor progression. The role of other TME cell types in melanoma are relatively unexplored, though they could provide novel therapeutic targets. Using the BRAF;p53 zebrafish model of melanoma, we have uncovered a role for microenvironmental adipocytes as mediators of melanoma progression. Melanoma cells in subcutaneous sites show a 50% increase in lipid content compared to parental cells. This is accompanied by increased expression of the fatty acid transporter proteins FATP2 and FATP6, suggesting that the increase in lipids is derived from exogenous uptake from the surrounding microenvironment. The melanoma cells growing at subcutaneous sites grow in direct contact with adipocytes, raising the hypothesis that TME adipocytes are the source of these exogenous lipids. Consistent with this, both fish and human melanoma cells co-cultured with 3T3-L1 adipocytes also have a 50% increase in total lipid content, and fluorescently labeled fatty acids can be transferred directly from adipocytes to melanoma cells. Co-culture of melanoma cells with adipocytes is accompanied by a significant growth advantage measured by pH3 staining, along with an increase in invasiveness. These effects can be blocked by the small molecule FATP-transport inhibitor lipofermata, which inhibits both lipid uptake as well as melanoma cell viability. RNA-seq of melanoma cells in the presence of adipocytes reveals a striking dysregulation of lipid metabolism accompanied by increased synthesis of extracellular matrix-associated genes, suggesting that the melanoma cells reshape the local microenvironment in the presence of adipocytes. Taken together, these data suggest that TME adipocytes play an important role in melanoma growth, acting as a rich source of lipids for melanoma, and that uptake of these lipids is mediated by the FATP fatty-acid transport proteins

1.35

CULLING THE HUMAN GENOME OF DISEASE VARIANTS USING ULTRACONSERVED ELEMENTS

Transformative Research Award, 2016

Ting (C.-ting) Wu¹ and Michael E. Talkowski^{1,2,3}

¹Harvard Medical School, Boston, MA, USA; ²Massachusetts General Hospital, Boston, MA, USA;

³Broad Institute, Cambridge, MA, USA

Presented by Ting (C.-ting) Wu

Mammalian ultraconserved elements (UCEs) are DNA sequences that have been maintained at the highest (100%) level of conservation for hundreds of millions of years. Because neither protein coding regions, nor regulatory regions, nor transcription factor binding sites require such conservation, the mere existence of UCEs has been a long debated conundrum. Breaking rank from popular models, we have proposed that ultraconservation can be explained if the maternal and paternal copies of each UCE pair and then undergo comparison, such that discrepancies in sequence or copy number, as well as rearrangements that compromise pairing, result in loss of fitness via disease or reduced fertility. Such a mechanism would contribute to the maintenance of genome integrity and, intriguingly, our studies of copy number variants (CNVs) are consistent with UCEs acting at the cellular level to cull cells with deleterious rearrangements. Our presentation will present our most recent analyses to determine whether UCEs respond to changes in gene dosage or, as our model predicts, to disruptions of the capacity of homologous genomic regions to pair. Whereas our previous studies focused on cancers, our most recent work focuses on neurodevelopmental disorders, including autism. We will conclude with speculations about how UCEs may embody a strategy for fighting diseases associated with genome rearrangements, including those arising from radiation-induced damage during long-term travel in space.

Brief summary of research of Pioneer Award: We have developed three new single-cell technologies for imaging the genome *in situ*, all of which use the oligonucleotide-based Oligopaint approach for fluorescent in situ hybridization (FISH). Two of the technologies, OligoSTORM and OligoDNA-PAINT, combine Oligopaints with the single-molecule super-resolution methodologies of, respectively, STORM (in collaboration with the laboratory of Xiaowei Zhuang) and DNA-PAINT (in collaboration with the laboratory of Peng Yin) to achieve ≤ 20 nm resolution images (Beliveau, Joyce, et al. 2012 PMC3535588; Beliveau, Boettiger, et al. 2015 PMC4430122). The third harnesses single nucleotide polymorphisms (SNPs) to enable Oligopaints to distinguish maternal and paternal homologous chromosomes. We have applied these technologies to mammalian and *Drosophila* cells, discovering intriguing organizational themes for different types of chromatin (Boettiger et al. 2016 PMC4905822; Wang et al. 2016 PMID: 27445307).

1.36

PROPAGATING HUMANIZED BLT MICE FOR THE STUDY OF HUMAN IMMUNOLOGY AND IMMUNOTHERAPY

New Innovator Award, 2014

Lili Yang

University of California, Los Angeles, Los Angeles, CA, USA

The humanized BLT (bone marrow-liver-thymus) mouse model harbors a nearly complete human immune system, therefore providing a powerful tool to study human immunology and immunotherapy. However, its application is greatly limited by the restricted supply of human CD34⁺ hematopoietic stem cells and fetal thymus tissues that are needed to generate these mice. The restriction is especially significant for the study of human immune systems with special genetic traits, such as certain HLA (human leukocyte antigen) haplotypes or monogene deficiencies. In order to circumvent this critical limitation, we have developed a method to quickly propagate established BLT mice. Through secondary transfer of bone marrow cells and human thymus implants from BLT mice into NSG (NOD/SCID/IL-2R γ ^{-/-}) recipient mice, we were able to expand one primary BLT mouse into a colony of 4-5 proBLT (propagated BLT) mice in 6-8 weeks. These proBLT mice reconstituted human immune cells, including T cells, at levels comparable to that of their primary BLT donor mouse. They also faithfully inherited the human immune cell genetic traits from their donor BLT mouse, such as the HLA-A2 haplotype that is of special interest for studying HLA-A2 restricted human T cell immunotherapies. Moreover, an EGFP reporter gene engineered into the human immune system was stably passed from BLT to proBLT mice, making proBLT mice suitable for studying human immune cell gene therapy. This method provides an opportunity to overcome a critical hurdle to utilizing the BLT humanized mouse model and enables its more widespread use as a valuable pre-clinical research tool.

1.37

IMPLANTABLE CARDIAC POWER GENERATION USING FLEXIBLE 3D POROUS THIN FILMS

Transformative Research Award, 2016

John X. J. Zhang

*Thayer School of Engineering, Dartmouth College, Hanover, NH, USA; Dartmouth-Hitchcock
Medical Center, Lebanon, NH, USA*

Abstract authors: Dajing Chen,¹ Nan Hu,¹ Zi Chen,¹ Marc D. Feldman,² and John X. J. Zhang^{1,3}

*¹Thayer School of Engineering, Dartmouth College, Hanover, NH, USA; ²The University of Texas Health Science
Center, San Antonio, TX, USA; ³Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA*

Presented by John X. J. Zhang

Batteries for automatic implantable cardiac defibrillators (AICDs) typically need to be replaced every 5-7 years, whereas the average post-implantation longevity of AICD recipients with congestive heart failure (CHF) has increased to over 15 years. This mismatch poses a significant and ever-growing clinical and economic burden, since replacing the battery requires surgical intervention. Reducing the number of replacement surgeries will both prevent morbidity and lower costs. In the U.S. alone, AICD battery replacement costs billions of dollars each year, and reducing or eliminating these costs is clearly an imperative with health care reform. An innovative solution to increase AICD battery lifetimes is to harness the robust intrinsic energy of the heart and convert it to electrical power. Few successful studies on implantable energy generators have been reported however, and current piezoelectric generators are unsuitable for implantable applications due to low energy density or poor biocompatibility. In our preliminary studies, we have demonstrated that increasing the porosity of poly(vinylidene fluoride) (PVDF) structures increases their compressibility, resulting in higher piezoelectric efficiency.

The hypothesis of this new transformative research award is that flexible and conformable porous PVDF polymer films embedded inside AICD leads, or as stand-alone leads, can convert the mechanical motion of the heart into electrical energy by exploiting the high piezoelectricity efficiency of the PVDF film. In our specific aims, we will first develop flexible micro-power generators made of porous PVDF layers that can be interfaced with current AICD lead technology. Secondly, we will design computational models for porous PVDF structures and cardiac energy harvesting devices to allow for optimal design and power efficiency. In parallel, two types of bistable structures fabricated through strain engineering will be explored as energy harvesting devices, and their performance will be optimized using computer simulations. Thirdly, in vitro quantification and testing of the micro-power generator in an animal model of canine will be carried out to evaluate the clinical potential of our approach. Our research will support the development of a broad class of tunable porous nanomaterial networks capable of high efficient energy conversion, with potentially far-reaching applications in biomedical engineering.

Tuesday, December 6, 2016

2.1

EXOGENOUS LIPID UPTAKE INDUCES METABOLIC AND FUNCTIONAL REPROGRAMMING IN CANCER-ASSOCIATED MYELOID-DERIVED SUPPRESSOR CELLS

Transformative Research Award, 2014

Augusto C. Ochoa

Louisiana State University Health Sciences Center, New Orleans, LA, USA

Abstract authors: Amir A. Al-Khami, Liqin Zheng, Paulo C. Rodriguez, and Augusto C. Ochoa
Louisiana State University Health Sciences Center, New Orleans, LA

Presented by Amir A. Al-Khami

Myeloid-derived suppressor cells (MDSC) play an essential role in protecting tumor cells from chemotherapy and immunotherapy. Our recent reports showed that activation of fatty acid oxidation (FAO) metabolically supported MDSC function in tumors and that inhibition of FAO triggered T cell-mediated anti-tumor effects. Herein, we sought to determine the role of the exogenous lipid uptake in the metabolic and functional reprogramming occurring in tumor MDSC. We report that lipid content and uptake were significantly higher in tumor-infiltrating MDSC relative to splenic MDSC and immature myeloid cells (iMC), via the up-regulation of lipid transport receptors. Notably, exogenous lipids, including unsaturated fatty acids and the triacylglycerol-rich lipoproteins VLDL and LDL, favored the metabolic switch and development of highly immunosuppressive MDSC phenotype that was dependent on nitric oxide (NO) and peroxynitrite (PNT) production. Tumor-derived factors, such as G-CSF and GM-CSF, and a subsequent activation of STAT3 and STAT5 signaling were critical for the metabolic and functional reprogramming in MDSC. Emphasizing the role of lipid uptake in the tumor microenvironment, we observed a delayed tumor growth in mice lacking the scavenger receptors CD36. CD36 deletion substantially inhibited the oxidative metabolism and regulatory function of tumor-infiltrating MDSC compared with wild-type MDSC. Of interest, human tumor-associated MDSC expressed appreciable levels of CD36, and external lipids significantly promoted the suppressive function of human peripheral blood stem cell-derived MDSC. Our data suggest that lipid uptake and accumulation could have a profound effect on MDSC metabolic and functional polarization in the tumor microenvironment, thus providing a novel approach to enhance the immunotherapy of cancer in addition to other chronic inflammatory diseases.

2.2

BIOLOGIC HARD DRIVES TO RECONSTRUCT THE PRENATAL AND EARLY CHILDHOOD ENVIRONMENT

New Innovator Award, 2014

Manish Arora

Icahn School of Medicine at Mt. Sinai, New York, NY, USA

Environmental stressors experienced during fetal and early childhood development may alter life-long health trajectories. Two major barriers have prevented us from characterizing the early life environment in human populations. First, it is not possible to routinely sample fetal tissues in human populations, and maternal biomarkers do not accurately measure fetal exposures for all environmental stressors due to variability in placental regulation. Second, prospective cohort studies provide the strongest evidence for environmental risk factors, but for low frequency diseases this design is problematic due to the need for large sample sizes. For a condition with a frequency less than 1% and long latency (e.g. Parkinson's, amyotrophic lateral sclerosis, or schizophrenia), thousands of participants need to be prospectively studied for decades from conception to adulthood.

My team and I have embarked on the development of Biologic Hard Drives – massively multiplexed analyses that use specific human tissues (deciduous and permanent teeth, for example) to quantify environmental stressors (and the biologic response to those stressors) at weekly temporal resolution from fetal life to adulthood. Using these methods, the microspatial architecture of tissues is mapped with nuclear beam methods to reveal information on several thousand exposures over the life course. We have applied these methods in combination with high-dimensional mathematical approaches to uncover early life determinants of conditions such as autism spectrum disorders (ASD) and amyotrophic lateral sclerosis (ALS).

The results of our studies reveal complex interactions between environmental factors, and importantly, show that these interactions are present at discrete developmental periods (critical windows of susceptibility). For late onset conditions such as ALS, identifying early life risk factors to characterize windows of susceptibility offers the opportunity to create 'windows of opportunity' because many environmental factors are preventable or modifiable. Overall, this presentation will highlight the advances in laboratory methods and mathematical modeling, and their application to population-based studies on autism and ALS.

2.3

HIGH-SENSITIVITY NMR AT ROOM TEMPERATURE FOR MOLECULAR STRUCTURE AND DYNAMICS

New Innovator Award, 2014

Alexander B. Barnes

Washington University in St. Louis, St. Louis, MO, USA

I will show our progress towards implementing Dynamic Nuclear Polarization (DNP) at room temperature to increase the sensitivity of solid-state NMR by more than a factor of 200 under physiological conditions. Solid-state NMR (SSNMR) is unique in its ability to measure interatomic distances and characterize molecular dynamics within amyloid fibrils and complex heterogeneous environments such as membranes and even intact cells. The drawback of SSNMR is inherently weak sensitivity. NMR sensitivity can be increased by transferring signal intensity from electron paramagnetic resonance (EPR) to NMR in a process known as Dynamic Nuclear Polarization (DNP). However, with current DNP methodology, experiments must be performed at cryogenic temperatures. We have improved the capability of high-frequency DNP instrumentation by implementing high-power microwave sources with fast frequency tuning. This improved microwave control will permit the extension of DNP SSNMR to room temperature, yielding the following advantages over existing cryogenic DNP methodology: studies will be performed under physiological conditions, molecular dynamics (motion) can be determined experimentally, improved spectral resolution (sharper NMR lines), broader dissemination by removing the need for cryogenic infrastructure. Non-cryogenic DNP spectrometers will enable structural and molecular dynamics studies of every protein, molecule, and chemical architecture of interest to the biological sciences. Structural characterization of amyloid fibrils and membrane proteins will particularly benefit from improved SSNMR sensitivity at room temperature. We have made considerable progress in the following innovations that will be required to achieve significant DNP enhancements of SSNMR at room temperature: high-power fast frequency tunable microwaves sources at 200 GHz (gyrotrons), frequency swept time-domain DNP at high magnetic fields, electron-nuclear (hyperfine) decoupling to reduce paramagnetic relaxation effects (PREs), microwave irradiation strategies to reduce sample heating.

2.4

EPIGENOMICS OF T CELL ACTIVATION AND T CELL MEMORY

New Innovator Award, 2015

Artem Barski

Cincinnati Children's Hospital, Cincinnati, OH, USA

The heterogeneity within the CD4+ effector/memory T-cell compartment is critical for our ability to deal with diverse pathogens. Dedicated populations of CD4+ T helper cells are required for promoting immune defense against intracellular infections (Th1 cells), helminth infections (Th2 cells), and fungal infections (Th17 cells). On the other hand, each of these differentiated states is associated with human disease: Th1 and Th17 cells can promote autoimmunity, while Th2 cells can promote allergy and asthma. Thus, understanding and learning to exploit the mechanisms that underlie lineage choice is vital for understanding and treatment of immunological and infectious diseases. The importance of epigenetic regulation for T-cell differentiation is becoming more and more clear. Profiling epigenetic modifications in naïve and memory T cells has shown that the rapid recall ability of memory T cells is associated with gene poising as indicated by the presence of positive chromatin modifications at promoters and enhancers of these genes. These results support the hypothesis that the rapid recall ability of memory T cells is encoded in their epigenome. My laboratory is interested in how T cell epigenome is written during T cell differentiation and whether modifying the epigenome can change T cell phenotype.

2.5

EFFECTS OF HOME ENVIRONMENT AND SEMANTIC STRUCTURE ON EARLY LEXICAL DEVELOPMENT

Early Independence Award, 2014

Elika Bergelson
Duke University, Durham, NC, USA

While infants' vocabularies grow remarkably fast (Bergelson & Swingley, 2012; Dale & Fenson, 1996; Fernald et al, 1998), it is unclear what causes improved comprehension with age. We explore whether advances in early word comprehension seem better explained by changing input data, i.e. parents talking more or differently to older infants, or by changes in the learner, i.e. cognitive growth and linguistic exposure. These possibilities have different implications for how to best support early language-learning for at-risk children. Here we contrast these explanations using early-learned nouns as a test-case, combining in-lab and at-home measures in a yearlong longitudinal sample ($n = 44$). We probed infants' word comprehension across two contexts (semantically related and semantically distinct word-pairs) at 3 time-points (6, 12, and 18 months) using eyetracking to quantify infants' fixations to named object on a visual display. We further analyzed the input statistics of the tested words in a corpus of monthly daylong audio-recordings and hour-long video-recordings of these same children at home. We asked whether (1) in-lab comprehension is modulated by age and semantic context and (2) home exposure to the tested words changes month-to-month. No extant datasets combine such environmental and behavioral data within the same infants. Mixed-effects models and non-parametric tests indicated that 12- and 18-months exhibited above-chance performance across semantic contexts, but 6-m.o.s only did so in the semantically-distinct condition. 78% of infants attained positive means ($M = .064$, $p = .00043$ by Binomial Test). These results suggest that even infants' earliest words are part of interconnected lexical networks; relations between words affect comprehension for infants, as for adults. Corpus analyses revealed high and consistent rates of exposure to the tested words: on an average recording infants heard 50-100% of our tested words, with multiple instances of each. Tested words were heard from ~ 2 speakers, and the words' referents were available and attended to $\sim 60\%$ of the time infants heard them said aloud. There was no evidence for changing exposure over time, ruling out a 'changing data' account. Indeed, infants' word exposure was incredibly stable over time. Infants' improvement from 6-18 months does not seem to reflect relatively more word-instances over time, more talker-variability, or different exposure conditions. Rather, improvement seems to stem from an accrual of similar learning instances, and growing differentiation of related concepts. This in turn is compatible with several accounts. On a "more data" account, data accrual increases learning. On a "better learner" account, linguistic, cognitive, and social gains let older infants take better advantage of the same kind of data. We explore how to adjudicate between these accounts, and to unpack what the variability in home and lab measures can tell us about early language development.

2.6

RESISTANCE IS FUTILE: HOW PHAGES COOPERATE TO BEAT CRISPR-CAS IMMUNITY

Early Independence Award, 2015

Joseph Bondy-Denomy
University of California San Francisco, San Francisco, CA, USA

CRISPR-Cas systems (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated genes) are adaptive immune systems that many bacteria employ to defend against phage attack. By acquiring small fragments of the phage genome into a CRISPR “memory” locus, the bacterial cell can generate small RNA-loaded CRISPR-Cas complexes that use base-pairing to guide a Cas nuclease to destroy a foreign target. A common foreign target of CRISPR-Cas systems are bacterial viruses called phages and interestingly, CRISPR inhibitor proteins are produced by phages infecting *Pseudomonas aeruginosa* (PA). These “anti-CRISPR” proteins inhibit the PA CRISPR-Cas system either by preventing DNA target recognition or DNA target cleavage. In this study, we aim to understand the ecological and evolutionary consequences of anti-CRISPR deployment during phage infection. Surprisingly, we find that anti-CRISPR proteins are easily overwhelmed by increased CRISPR activity, and when there are a low number of phages per cell (low MOI), anti-CRISPRs are ineffective. This demonstrates that anti-CRISPR activity alone is not sufficient to fully protect a single phage during infection. How then can phages use anti-CRISPRs to overcome CRISPR-Cas immunity? By isolating lysogenic strains possessing integrated phages (prophages) that required anti-CRISPR proteins to establish lysogeny, we were surprised to find that CRISPR activity was selecting for multi-copy prophage integration. These multi-copy lysogens arise from phage co-infections, suggesting that multiple phages infecting the same cell can defeat CRISPR immunity while a single phage cannot. In support of this model, we demonstrate that viral infection at higher MOI (although less than one phage per cell) is sufficient to inhibit CRISPR immunity and support phage proliferation. We hypothesize that non-random co-infecting phages provide a sufficient concentration of anti-CRISPR molecules to inhibit CRISPR immunity, which facilitates phage survival and replication in the lytic cycle or multi-copy prophage integration in the lysogenic cycle.

2.7

ACTIVITY-BASED PROBES FOR SELECTIVE IMAGING OF AN ESSENTIAL PBP IN *STREPTOCOCCUS PNEUMONIAE*

New Innovator Award, 2011

Erin Carlson

University of Minnesota, Minneapolis, MN, USA

The cell wall integrity of bacteria is dependent upon a complex polymeric structure, the peptidoglycan (PG). Inhibition of PG biosynthesis has yielded many antibacterial agents and opportunities remain for the identification of novel drug targets through a more complete understanding of the multi-protein molecular machines that dictate cell wall construction. One of the major mechanisms by which existing antibacterial agents act is inhibition of the penicillin-binding proteins (PBPs), enzymes involved in PG polymerization and crosslinking. Despite this importance, the discrete functions of individual PBP homologs have been difficult to determine. Historically, study of bacterial cell wall biosynthesis has focused on the investigation of genetically-modified organisms, including fluorescent protein-tagged proteins. More recently, chemical probes such as the fluorescent D-amino acids (FDAAs) have been utilized to image nascent peptidoglycan. While valuable, these techniques cannot provide the complete picture, as they do not enable investigation of catalytic activity. To accomplish this goal, small molecules that selectively target individual PBPs in an activity-dependent fashion are required. We have identified a novel privileged scaffold for generation of PBP-selective probes and utilized these compounds for imaging of PBP2x in *Streptococcus pneumoniae*. PBP2x performs crucial roles in septal PG synthesis and is a critical target for the β -lactam antibiotics. We found that PBP2x activity is present both at the septal center and as a surrounding ring, which could not be detected by previous approaches, highlighting a critical strength of our PBP-selective strategy.

2.8

COMBATING ANTIGEN ESCAPE WITH CD19/CD20 BISPECIFIC CAR-T CELL THERAPY

Early Independence Award, 2012

Yvonne Y. Chen

University of California, Los Angeles, Los Angeles, CA, USA

T cells expressing chimeric antigen receptors (CARs) specific for the B-cell marker CD19 have shown impressive results in the treatment of B-cell malignancies. However, multiple clinical trials have also demonstrated the vulnerability of single-input CD19 CAR-T cell therapy to antigen escape, in which patients relapse with the emergence of CD19 tumor cells. Here, we report on the rational design and systematic optimization of bispecific CAR-T cells that trigger robust cytotoxicity against target cells expressing either CD19 or CD20. We demonstrate that optimized bi-specific CARs can rapidly eliminate large, engrafted tumors *in vivo*. We further demonstrate the bi-specific CAR-T cells' ability to prevent tumor escape while single-input CD19 CAR-T cells succumb to the selective expansion of CD19 mutant cells, which are shown to arise spontaneously in engrafted mice. The CD19-OR-CD20 CAR is fully compatible with existing T cell manufacturing procedures and implementable by current clinical protocols. These results present an effective solution to the challenge of antigen escape in CD19 CAR T cell therapy, and they highlight the utility of structure-based rational design in the development of receptors with higher-level complexity.

2.9

HIGH-RESOLUTION PROFILING OF ANTI-HIV IMMUNE RESPONSES USING PAIRED ANTIBODY HEAVY AND LIGHT CHAIN SEQUENCING

Early Independence Award, 2016

Brandon DeKosky

*Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA;
University of Kansas, Lawrence, KS, USA*

The induction of HIV-1 broadly neutralizing antibodies (bNAbs) is a major goal of HIV vaccine development, and methods for antibody lineage characterization are essential to understand HIV broadly neutralizing responses. However, antibody molecules are encoded by two distinct chains mRNA strands, and conventional NextGen sequencing fails to identify native pairings from individual lymphocytes. We are applying recently-invented technologies for high-throughput sequencing of complete antibodies (i.e., paired heavy and light chains) to accelerate HIV vaccine design. We developed a new approach that leverages focused genetic priming to enrich bNAb lineage frequency in sequence data and enable the robust analysis of bNAb lineages from longitudinal patient samples. We used targeted lineage priming to characterize VRC34-class bNAbs that bind to the HIV fusion peptide, obtaining >2,000 VRC34 lineage sequence reads comprising > 10 new antibodies from HIV broadly neutralizing donor N123. We are extending these methods to analyze recently developed HIV fusion-peptide-targeting vaccines.

We are also leveraging high-throughput paired antibody sequencing to evaluate candidate immunogens for induction of HIV-1 CD4 binding site (CD4bs)-specific bNab precursors. In a transgenic mouse model encoding the human VH1-2 antibody gene which is necessary for VRC01-class bNAbs, we found that an eOD-GT8 antigen elicited robust VRC01-class precursors with 200 fold increased prevalence compared to unimmunized control mice. We are analyzing the antibody repertoire responding to the eOD-GT8 immunogen to better understand how we can further manipulate antibody responses to HIV-1 neutralization-sensitive epitopes in this mouse model. This work represents the first quantitative repertoire scale data demonstrating vaccine-based induction of broadly neutralizing precursor antibodies in mice with diverse precursor repertoires. These quantitative antibody repertoire analyses are providing high-throughput, high-resolution information to understand and improve the current generation of HIV vaccines.

2.10

CHEMICAL BIOLOGY APPROACHES TO CONTROLLING AND UNDERSTANDING THE INNATE IMMUNE SYSTEM

New Innovator Award, 2013

Aaron Esser-Kahn
University of California, Irvine, Irvine, CA, USA

Foreign pathogens are detected through a series of receptors on antigen presenting cells. These receptors are synergistically activated by multiple pathogen associated molecular patterns. Chemical biology has a large role to play in the coordinated design of vaccines and synthetic activators of the immune system. At the same time, chemical tools can help us understand the critical elements that help produce the best immune responses. We report on our development of chemical and polymeric tools for interacting with the immune system. Our methods involve the bioconjugation of multiple different PAMPs to polymeric scaffolds. These synergistic PAMP scaffolds have been used in active vaccination. Additionally, we will report on our work coupling these synergistic combinations to cell-surface and other antigen rich environments. We also report on our attempts to control the innate immune system using light to guide responses.

2.11

RESTORING HIGH-ACUITY VISION USING INHIBITORY NEURON TRANSPLANTATION

New Innovator Award, 2013

Sunil Gandhi

University of California, Irvine, Irvine, CA, USA

The adult brain lacks the capacity to rewire itself that is abundant during critical periods in juvenile development. In the visual cortex, the maturation of inhibitory neurons is responsible for opening a critical period for the development of high acuity vision. We have discovered that the transplantation of embryonic inhibitory neurons into adult mouse visual cortex creates a new critical period for binocular vision. Functional imaging of transplanted interneurons reveals that these cells wire into the host visual system following an intrinsically determined developmental program. The transplanted inhibitory neurons reactivate juvenile cortical plasticity when the donor animal's critical period would have occurred. Depriving mice of vision during the normal critical period produces a permanent impairment of high acuity vision that models human amblyopia. We find in both neurophysiological and behavioral measures that transplantation completely reverses visual acuity deficits in amblyopic mice. Using in vitro and in vivo mapping of inhibitory circuits, we find that transplanted cells contribute a powerful, disinhibitory microcircuit to the host visual cortex. The new circuit responds rapidly to visual deprivation and depends critically on Neuregulin-1/ErbB4 signaling, a key pathway implicated in schizophrenia. These transplantation experiments open up a new avenue for understanding the mechanisms of juvenile brain plasticity. Furthermore, our approach to reactivating juvenile plasticity in the adult cortex may lead to novel therapeutics for brain injury, neurodegeneration and neurodevelopmental disorders such as schizophrenia.

2.12

AUTOSOMAL MONOALLELIC EXPRESSION AS A MECHANISM OF VARIATION BETWEEN INDIVIDUALS

Transformative Research Award, 2014

Alexander Gimelbrant
Dana-Farber Cancer Institute, Boston, MA, USA

We and others have recently shown that a large fraction of human autosomal genes are subject to monoallelic expression (MAE). Such genes can be expressed from a single allele in one cell, and from the other allele in a neighboring cell in the same individual. Similar to X-inactivation, the random initial choice of the active allele is mitotically stable in the clonal cell progeny.

Studies of clonally stable epigenetic phenomena, such as X-inactivation, have shown that they can lead to variability in clonal composition between individuals, and hence to significant variation in phenotype. We performed the first systematic analysis of variability associated with human MAE genes at two scales: quantitative variation between individuals in transcript abundance and genetic variation in MAE genes in human populations.

To assess the contribution of MAE genes to expression variability across individuals relative to biallelically expressed (BAE) genes, we took advantage of the GTEx dataset—a large-scale gene expression RNA-seq dataset measuring expression in multiple primary human tissues. We classified genes as MAE and BAE using the specific chromatin signature we described recently. In all tested tissues, variance between individuals was consistently and significantly higher for MAE genes compared to expression-matched sets of BAE genes.

A major open question is how MAE interacts with evolutionary processes that affect these genes. It has been suggested that MAE genes would be less genetically diverse because deleterious alleles would be effectively hemizygous in numerous cells. Using recent large-scale resequencing datasets, we systematically assessed the variation in MAE genes in human populations. In contrast to the expectations, MAE genes have dramatically higher nucleotide diversity in all analyzed populations. The elevated diversity of MAE genes is due to several factors. Most surprisingly, several lines of evidence suggest that MAE genes are enriched with genes under balancing selection.

These findings relate parameters of human genetic variation to epigenetic mechanisms of transcriptional control, and point to MAE as a significant source of biological variability at different scales.

2.13

NEW TOOLS FOR UNDERSTANDING THE BLOOD-BRAIN BARRIER

Pioneer Award, 2014

Chenghua Gu

Department of Neurobiology, Harvard Medical School, Harvard University, Boston, MA, USA

Abstract author: Benjamin Andreone

Department of Neurobiology, Harvard Medical School, Harvard University, Boston, MA, USA

Presented by Chenghua Gu

The central nervous system (CNS) requires a tightly controlled environment free of toxins and pathogens to provide the proper chemical conditions for synaptic transmission. This environment is maintained by the 'blood brain barrier' (BBB), which is composed of highly specialized blood vessels whose endothelium display specialized tight junctions and unusually low rates of transcellular vesicular transport (transcytosis). While BBB breakdown has recently been associated with various neurological disorders, an intact BBB also poses a major obstacle for drug delivery to the CNS. Little progress has been made on manipulating the BBB due to a significant knowledge gap in understanding how BBB function is regulated and identifying the essential molecular constituents governing its processes. This limited understanding has also thwarted our ability to therapeutically manipulate the BBB.

The major impediment to understanding the BBB is identifying its essential constituent and unraveling the mechanism by which these key regulators control BBB function. However, the current *in vitro* models rely on fully differentiated endothelial cells, which already contain unique properties that prevent their use in reconstitution studies. Similarly, the main technique to study the BBB has been EM, however its static snapshots do not provide information on active and dynamic vesicular transport, directionality, or their specific routes to allow investigators to interrogate the key molecular mechanisms that regulate BBB integrity.

Recently, we mapped the precise timing of BBB formation and then identified molecules with possible roles in BBB function from simple transcriptome comparisons between CNS and peripheral endothelial cells. Surprisingly, we also found that instead of a physical buildup or disruption of structurally important tight junctions as previously thought, transcytosis regulation seems to be the more likely the major mechanism underlying BBB integrity. In characterizing these developmental properties, I realized that these findings are just the tip of the iceberg and that truly fundamental questions remain in identifying the core pathway and understand how they regulate BBB function. New tools thus are needed for understanding the BBB. Here we propose first to develop a new stem cell-based system to allow reconstitution of a functional BBB *in vitro*, and then to develop a genetic-optical system for monitoring the functional integrity of the BBB *in vivo* at subcellular resolution in real time. This integrated approach will address fundamental questions about the regulation of the BBB, which will then lead to more effective therapeutic strategies and specific targets for BBB restoration and manipulation.

2.14

PATERNAL HISTONES AND THEIR ROLE IN DEVELOPMENT

New Innovator Award, 2016

Sue Hammoud

University of Michigan, Ann Arbor, MI, USA

The epigenome consists of several types of chemical modifications on either histone proteins or DNA. In somatic cells, these modifications are maintained across mitotic divisions, whereas in the germline and embryo, the epigenome is reset to ensure proper development and to prevent the trans-generational inheritance of acquired epigenetic information. Despite the two waves of genome-wide reprogramming during mammalian development, the inheritance of epigenetic information has been observed in various organisms, including humans. These findings raise a fundamental question: is epigenetic information in gametes transmitted from generation to generation and, if so, what are the consequences of trans-generational epigenetic inheritance?

The epigenetic contribution from the paternal genome is considered limited since the sperm genome is largely depleted of histones. The small amounts of histones retained in sperm were believed to be remnants of incomplete histone-to-protamine exchange. However, my studies revealed that key developmental gene promoters in sperm are DNA hypomethylated and are enriched for both active and repressive histone modifications: a “poised” chromatin state presumed to be specific to a totipotent/pluripotent cell. However, our findings demonstrated that competency for totipotency is already embedded in sperm chromatin, and possibly inherited through the paternal lineage, therefore, challenging prior notions and expanding the breadth of paternal contribution to the developing embryo beyond the DNA sequence. Furthermore, subsequent studies have shown that the epigenetic modifications retained in sperm chromatin are distinct from those present in the oocyte. Whether these retained parent-of-origin epigenetic marks (apart from imprinted genes) are instructive for early embryonic development or the first cell fate determination events remains unknown.

This proposal aims to address two fundamental questions in biology: (1) What is the biological function and significance of retained histones and their attendant modifications in sperm, and (2) Is the preservation of the parent-of-origin epigenetic marks (apart from imprinted genes) important for early development and the first cell fate determination events or are these marks merely a remnant of the paternal or maternal origin of the genomes? To address these questions and others, we propose to develop exciting, cutting edge cell biology and molecular genetic tools that will enable the visualization, tracking, and temporal control of paternal histones in the developing embryo. Through these studies we hope to uncover whether the modified nucleosomes retained in sperm chromatin are inherited and instructive for development, therefore, providing a molecular mechanism for transgenerational inheritance, a notion supported by observation, yet without a clear molecular explanation.

2.15

MAPPING THE HUMAN TOXOME BY SYSTEMS TOXICOLOGY

Transformative Research Award, 2011

Thomas Hartung

Johns Hopkins University, Baltimore, MD, USA

The 21st century vision for toxicology involves a transition away from high-dose animal studies to *in vitro* and computational models. This transition requires mapping Pathways of Toxicity (PoT) by understanding how *in vitro* systems respond to chemical perturbation.

The Human Toxome Project, funded as an NIH Transformative Research Award since 2011 (“Mapping the Human Toxome by Systems Toxicology,” NIEHS R01ES020750), is focused on developing the concepts and the means for deducing, validating, and sharing molecular PoT as a collaboration of Johns Hopkins University, Brown University, the Hamner Institute, Georgetown University, the U.S. Environmental Protection Agency, and Agilent Technologies, Inc. Using the test case of estrogenic endocrine disruption, the responses of MCF-7 human breast cancer cells are being phenotyped by transcriptomics and mass-spectroscopy-based metabolomics.

The three main achievements of the Human Toxome project were—

- The Human Toxome Collaboratorium, a shared computational environment hosted on third-party cloud services, which allows a multi-omic computational collaboration within a consortium (*Front Pharmacol* 2016 6:322). The Collaboratorium represents a novel distribution method that could increase the reproducibility and reusability of results from similar large, multi-omic studies.
- Second, a number of contributions to quality control and standardization of cell systems, omics technologies and bioinformatics will be addressed. In particular, in this part we will show that common recommendations for cell line authentication, annotation and quality control fall short addressing genetic heterogeneity in cell lines and that this heterogeneity can have serious consequences for reproducibility of experiments as shown by morphology, estrogenic growth dose-response, whole genome gene expression and untargeted mass-spectroscopy metabolomics for MCF-7 cells. These findings underscore the need for additional quality assurance in Good Cell Culture Practice and cell characterization, especially using other methods such as Comparative Genomic Hybridization to reveal possible genomic heterogeneity and genetic drifts within cell lines (*Nature’s Scientific Reports* 2016 6:28994; *ALTEX* 2015 32(4):319-26; *J Appl Toxicol* 2013 33(12):1365-83).
- The third part will focus on an information-dependent enrichment analysis that reveals time-dependent transcriptional regulation of the estrogen PoT by information-dependent enrichment analysis—an algorithm and software which has been developed within this project. Uncovering transcription factors/signaling networks responsible for gene expression patterns is essential for defining pathways of toxicity, and ultimately, for determining the chemical modes of action through which a toxicant acts (*Arch Toxicol* 2016, *in press*).

Taken together, this project has developed a workflow for PoT identification and advanced the quality assurance of cell models and omics approaches used, which opens up for systematic mapping of the human toxome.

2.16

HIBERNATION: INSIGHTS FROM COMPARATIVE GENOMICS OF ZAPUS SPECIES

Early Independence Award, 2015

William J. Israelsen

The University of Texas Southwestern Medical Center, Dallas, TX, USA

Hibernating mammals employ unique metabolic strategies to survive harsh conditions. They become obese prior to the onset of winter, then conserve their stored energy during the months-long fast of hibernation by slowing metabolism and becoming deeply hypothermic. These extreme hibernation phenotypes may provide insights into control of metabolism and have applications in medicine, but little is known about the molecular mechanisms or genetic underpinnings of hibernation. We have begun using the meadow jumping mouse (*Zapus hudsonius*) as a laboratory hibernation model. We generated whole genome sequences for 7 meadow jumping mice and 7 western jumping mice (*Zapus princeps*), a closely related hibernator. *Z. hudsonius* uses photoperiod as the cue to initiate fall fattening, while *Z. princeps* ignores photoperiod and prepares for hibernation based on food availability. Preliminary comparative genomic analysis reveals a subset of ~ 1,700 proteins that are significantly diverged between the species. These diverged proteins are enriched for genes involved in pheromone sensing and reproduction, immunity, and metabolism. Analysis of individual genes yields insights into speciation and may reveal candidates that are involved in the differences between the hibernation phenotypes of the two species.

2.17

INTRINSICALLY DISORDERED PROTEINS DRIVE THE EMERGENCE AND INHERITANCE OF BIOLOGICAL TRAITS

New Innovator Award, 2015

Daniel Jarosz

Stanford University, Stanford, CA, USA

Prions are a paradigm-shifting mechanism of inheritance in which phenotypes are encoded by self-templating protein conformations rather than nucleic acids. Here, we examine the breadth of protein-based inheritance across the yeast proteome by assessing the ability of nearly every open reading frame to induce heritable traits. Transient overexpression of nearly 50 proteins created traits that remained heritable long after their expression returned to normal. These traits were beneficial, had prion-like patterns of inheritance, were common in wild yeasts, and could be transmitted to naive cells with protein alone. Most inducing proteins were not known prions and did not form amyloid. Instead, they are highly enriched in nucleic acid binding proteins with large intrinsically disordered domains that have been widely conserved across evolution, including in humans. Thus, our data establish a common type of protein-based inheritance through which intrinsically disordered proteins can drive the emergence of new traits and adaptive opportunities.

2.18

TARGETING THE SOS PATHWAY TO COMBAT THE EVOLUTION OF ANTIBIOTIC RESISTANCE

New Innovator Award, 2012

Rahul M. Kohli

Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Bacteria possess a remarkable ability to rapidly adapt and evolve in response to antibiotics. The SOS pathway, a widely conserved DNA damage stress response in bacteria, is activated by many antibiotics and has been shown to play a central role in promoting survival and the evolution of resistance under antibiotic stress. As a result, targeting the SOS response has been proposed as an adjuvant strategy to revitalize our current antibiotic arsenal. The SOS pathway is under the control of a key dual-functional repressor and protease, LexA, whose self-cleavage marks the start of the SOS response. Focusing on LexA, we have characterized the determinants of self-cleavage by deep positional scanning of residues involved in auto-proteolysis. Our biochemical studies reveal the mechanisms regulating the induction of the SOS response and highlight a series of mutations that can either slow or accelerate self-cleavage. By exploiting such rate-altering mutants, we have generated *E. coli* strains that span the full spectrum of SOS activities, from a response that is constitutively off to one that is constitutively on. We have systematically analyzed the strains under a wide range of antimicrobials by comparing the mean inhibitory concentrations (MICs) and induced mutation rates for each drug-strain combination. This analysis provides support for multiple adjuvant strategies, while also suggesting that the combination of an SOS inhibitor with a DNA damaging antibiotic could offer the best potential for lowering MICs and decreasing acquired drug resistance. Building on this comprehensive assessment of how to target the SOS response, we have made significant advances in exploring the feasibility of targeting evolution with small molecules, including a high throughput screen on a library of more than 1.8 million compounds that has yielded potential leads. These studies offer fundamental insights into *how* antibiotic resistance can arise under antimicrobial stress and offer a novel means to potentiate our current antibiotic arsenal.

2.19

A NEW PARADIGM IN NANOMEDICINE: CAN STRUCTURAL INTERIORS OF NANOPARTICLES REGULATE CELLULAR DELIVERY?

New Innovator Award, 2016

Cecilia Leal

*Frederick Seitz Materials Research Laboratory, University of Illinois at Urbana-Champaign,
Urbana, IL, USA*

RNAi technology has been recently subjected to remarkable improvements in efficacy and viability due to chemical modulations of short nucleic acids and/or *via* their packaging into nanoparticles. At present there is no FDA approved system for RNAi technology in humans. The design of the next generation of carriers of RNAi inducing species requires a deep understanding of how nanoparticle's physicochemical properties truly impart biological stability and efficiency. For example, we now know that nanoparticles need to be sterically stabilized in order to meet adequate biodistribution profiles. At present, targeting, uptake, and in particular endosomal escape are amongst the most critical challenges impairing the delivery of RNAi activators (e.g. siRNA and miRNA). The disruption of endosomes in order to release nanoparticle cargo requires membrane transformations (for example pore formation) that cost significant elastic energy. Nanoparticle size and shape have been identified as relevant parameters impacting tissue accumulation and cellular uptake. In this work we propose that nanoparticle structural interiors are a novel handle to regulate endosomal escape. Lipid nanoparticles (LNP) have been long recognized as promising siRNA delivery vectors however virtually all studies are locked to the concept of using liposome formulations. We will instill a new direction of LNP design where nanoparticle interiors will be sculpted to comprise highly ordered networks of membranes with high surface-to-volume ratios and intrinsic membrane properties prone to disrupt endosomal membranes at minimal energetic cost. We will demonstrate a microfluidic device as the enabling technology to produce such particles and present electron microscopy combined with Small Angle X-ray Scattering to carefully characterize these particles. This insight on particle structure will be conducted in parallel with functional studies of siRNA delivery and gene knockdown to a variety of cell lines, including neurons and stem cells that are known to be hard to transfect. It is our goal to underpin the correlation between nanoparticle internal structures with the dynamic process of siRNA cellular delivery and enable the next generation of highly efficient RNAi nanomedicine.

2.20

DNA METHYLATION ON N6 ADENINE

New Innovator Award, 2016

Eric Lieberman Greer

Boston Children's Hospital Boston, Harvard Medical School, Boston, MA, USA

In mammalian cells, DNA methylation on the fifth position of cytosine (5mC) plays an important role as an epigenetic mark. However, DNA methylation was considered to be absent in *C. elegans* because of the lack of detectable 5mC, as well as homologs of the cytosine DNA methyltransferases. Here, using multiple approaches, we demonstrate the presence of adenine N⁶-methylation (6mA) in *C. elegans* DNA. We further demonstrate that this modification increases transgenerationally in a paradigm of epigenetic inheritance. Importantly, we identify enzymes that regulate 6mA levels and control the epigenetic inheritance of longevity and fertility phenotypes associated with the loss of the H3K4me2 demethylase *spr-5*. Together, these data identify a DNA modification in *C. elegans* and raise the exciting possibility that 6mA may be a carrier of heritable epigenetic information in eukaryotes.

2.21

STRETCHABLE, BIODEGRADABLE, AND SELF-HEALING SEMICONDUCTORS FOR WEARABLE AND IMPLANTABLE SENSORS

New Innovator Award, 2015

Darren J. Lipomi

Department of NanoEngineering, University of California, La Jolla, San Diego, CA, USA

This project aims to create a new class of organic semiconductors for applications in wearable and implantable healthcare that—in contrast to all other research on “electronic skin”—will actually have properties inspired by biological tissue: extreme elasticity, biodegradability, and the ability to self-heal. 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. Seamless integration with soft, biodegradable, and self-healing tissue has thus not yet been realized. In Phase I of this project, we will develop a modular synthetic methodology based on segmented polymerization of semiconducting segments and biodegradable elastomeric segments. Phase II will characterize the properties of this new class of materials, which will be the first polymeric semiconductors to have the mechanical properties of human tissue, the first known semiconductors capable of self-repair, and the first organic semiconductors that can degrade under physiological conditions into biocompatible byproducts, which will be established in a rat model. Phase III will use the synthetic materials as transducers of chemical, biomolecular, mechanical, and electrical signals in several modalities as proof-of-concept devices, including skin-like pressure sensors for instrumented prostheses, biochemical sensors for wearable health monitors, and photodetectors for artificial retinas. Phase III will culminate in the demonstration of an implantable epidural pressure sensor for continuous monitoring of intracranial pressure (ICP). The long-term goal of this research is to endow these devices with the capability of wireless power and telemetry. The strength of the proposal is its vertically integrated strategy that combines molecular engineering and synthetic chemistry with determination of biodegradability and biocompatibility, the fabrication of devices, and their use in detecting physiological signals relevant to a range of diseases. In the first year of the award, my group has made significant progress toward Phase I, that is, synthesizing semiconducting polymers exhibiting skin-like deformability and the chemical functional groups that should permit biodegradability. We have also developed a new type of biocompatible signal transducer based on metallic nanoislands on the surface of graphene. Using the materials developed in our laboratory, we have developed a glove-like sensor that can transmit gestures and biophysical data to a computer for decoding.

2.22

PROBING CYSTEINE POSTTRANSLATIONAL MODIFICATIONS

New Innovator Award, 2014

Brent Martin

University of Michigan, Ann Arbor, MI, USA

Radical species are an unavoidable consequence of respiration and the environment, and are tightly buffered by small molecule antioxidants and redox detoxifying enzymes. Oxidative stress emerges when an imbalance develops between the levels of reactive oxygen species and the cell's ability to readily eliminate the reactive intermediates or to repair the resulting damage. Aberrant oxidative signaling is perhaps one of the most important factors contributing to aging, neurodegeneration, heart disease, diabetes, and cancer. Despite the central role of oxidative stress in human health, our ability to study the precise mechanisms of such modifications is hampered by a lack of selective chemical and analytical methods. Here, we present a series of innovative chemical approaches to study oxidative damage across experimental scales, from live-cell imaging to in vivo imaging, in combination with proteome-wide quantitative profiling of oxidative post-translational modifications.

2.22

VARIANT SURFACE GLYCOPROTEIN EXPRESSION AND DIVERSIFICATION IN THE PROTOZOAN PARASITE *TRYPANOSOMA BRUCEI*

Early Independence Award, 2016

Monica Mugnier

Johns Hopkins University, Baltimore, MD, USA

African sleeping sickness, or human African trypanosomiasis, is a devastating human disease affecting sub-Saharan Africa. It is fatal if left untreated, existing drugs are extremely toxic, and the economic burden of sleeping sickness, along with its zoonotic counterpart, *nagana*, is estimated to be at least 1.5 billion USD per year. The causative agent of both human and animal trypanosomiasis is *Trypanosoma brucei*, a protozoan parasite covered by a dense variant surface glycoprotein (VSG) coat. This extracellular parasite evades elimination by its mammalian hosts' immune systems through antigenic variation of its VSG coat: it "switches" the expressed VSG, taking advantage of a genomic repertoire of ~2000 VSG-encoding genes. Despite the fact the antigenic variation is the major mechanism of immune evasion in *T. brucei*, its dynamics during infection are poorly understood. We have developed a method, termed VSG-seq, for quantitatively examining the diversity of expressed VSGs in any population of trypanosomes, whether in the lab or in the field. Our experiments reveal more dynamic and nuanced host-parasite interactions than previously expected. Most notably, parasite populations *in vivo* express an extremely diverse set of VSG-encoding genes, and the number of expressed VSGs over 30 days of infection can approach as much as one-third of the functional VSG repertoire within the parasite genome. This highlights an interesting problem: if the existing genomic repertoire can be used up in a matter of months, how can an infection be sustained for years in the field? The only way the parasite can sustain such long infections is by diversifying its genome-encoded VSG repertoire, both through the mutation of individual VSGs and recombination among sets of VSGs. With this in mind, we are currently exploring the mechanisms driving these diversification processes in order to understand how the parasite sustains the chronic infections observed in the wild.

2.23

A NEW STRATEGY TO DISRUPT PROTEIN-PROTEIN INTERACTIONS IN EUKARYOTIC CELLS

Pioneer Award, 2012

Hide Ploegh

Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Now in the final year of this NIH Pioneer Award we can look back on having indeed transformed my laboratory into a focused effort that exploits the unique properties of camelid-derived single domain antibody fragments (also called VHHs or nanobodies), which was an entirely new area of pursuit at the time of application. As originally proposed, we have been able to use intracellular expression of VHHs to perturb protein-protein interactions. This has been particularly informative in the case of influenza virus and vesicular stomatitis virus. We found antibodies against the nucleoprotein complexes of these viruses that strongly inhibit replication.

Taking advantage of the fact that VHHs serve as crystallization chaperones, we could map, at atomic resolution, the epitopes recognized and provide a plausible explanation for their inhibitory properties. In an independent line of work we generated VHHs against proteins of immunological interest, and used these to improve immunogenicity towards payloads covalently linked to the appropriate VHH. We next exploited the specificity and small size of these VHHs to generate a novel class of imaging agents that allowed us to track anti-tumor responses non-invasively by positron emission tomography. The quality of the images obtained exceeds by far what can be accomplished with conventional full-size antibodies and has enabled the prediction of the response in mice to a therapeutic antibody that blocks an immunological checkpoint, CTLA4.

2.24

AMYLOID BETA DEPOSITION PRECEDES TAU TANGLE FORMATION IN AUTOSOMAL-DOMINANT ALZHEIMER'S DISEASE

Early Independence Award, 2014

Yakeel Quiroz

Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; Grupo de Neurociencias, Universidad de Antioquia, Medellin, Antioquia, Colombia; Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA, USA

Examining rare families with autosomal dominant mutations that cause early-onset Alzheimer's disease (AD) provides a unique model for studying the trajectory of AD-related pathology, especially in the preclinical stages. We used PET imaging to characterize the relation between amyloid burden and tau accumulation in the brains of young *PSEN1* E280A mutation carriers and non-carriers from a Colombian kindred with autosomal dominant AD (ADAD). We hypothesized that amyloid beta deposition precedes tau tangle formation both within and beyond the medial temporal lobe in ADAD.

Eight presymptomatic carriers of the *PSEN1* mutation (mean age 32 years), two carriers with mild cognitive impairment, and ten age-matched non-carriers underwent tau and amyloid imaging. [C11] PiB PET cerebral-to-cerebellar DVRs and [F18] AV1451-PET cerebral-to-cerebellar SUVRs were compared based on mutation status and clinical status. Both PiB and AV1451 utilized structural ROIs as defined by Freesurfer.

Compared with non-carriers, presymptomatic mutation carriers had higher mean cortical PiB DVRs ($p < .001$), and higher [18F]AV1451 SUVRs in medial temporal lobe regions, including entorhinal cortex ($p < .03$), hippocampus ($p < .03$) and parahippocampal gyrus ($p < .03$). Presymptomatic carriers also showed trending higher tau uptake levels in inferior temporal lobe ($p = .07$).

Findings are consistent with the hypothesis that *PSEN1* E280A mutation carriers express amyloid deposition before tau deposition. Studying amyloid and tau concomitantly in ADAD is a promising method for clarifying the temporal relationship between the emergence of these two cardinal pathologies in AD.

2.25

RATIONAL DESIGN OF CYTOMEGALOVIRUS-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T CELLS FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

New Innovator Award, 2012

Laura A. Johnson

University of Pennsylvania Center for Cellular Immunotherapies, Philadelphia, PA, USA

Presented by Jesse L. Rodriguez

University of Pennsylvania Philadelphia, PA, USA

Glioblastoma multiforme (GBM) is the most common, and most deadly primary brain tumor. GBM is an incurable disease with few therapeutic options. Cytomegalovirus (CMV) can be detected in up to 90% of GBM tumor samples but not the surrounding normal brain tissue. The role of CMV as a tumor-promoting virus is poorly understood but its presence in the tumor presents a novel approach to developing a therapy for GBM by re-directing T cells to target CMV. To test for the presence of CMV in GBM we used a sensitive method of nested RT-PCR. Forty unique primary GBM samples were tested for expression of CMV gene UL55/glycoprotein B (gB). Each GBM sample was subtyped by its distinct molecular alterations and then screened for CMV. The RT-PCR results revealed that CMV was found in eighteen of the forty tumor samples (45%), and was preferentially expressed in the proneural subtype of GBM (8 out of 8 tumors, 100%). Given the presence of the virus in the tumor, we wanted to engineer T-cells to recognize the CMV gB antigen. Chimeric antigen receptors (CARs) are synthetic gene constructs that fuse an antibody recognition domain to the signaling domain of a T cell receptor. CARs confer the ability to re-direct T cells to recognize virtually any antigen on the surface of a cell. We designed a CAR to recognize the CMV surface antigen gB. In vitro testing of various CAR constructs revealed the ability of transduced CAR T cells to specifically respond to gB antigen-expressing target cells. Moreover, optimization of the gB CAR using a short spacer domain enhanced CAR T cell recognition of a gB-expressing human glioma cell line, U87gB. In vivo, gB CARs were able to treat established GBM tumors in a xenograft mouse model. It was important to demonstrate whether gB CAR T cells could recognize primary GBM tumors in addition to established cell lines. In in vitro co-cultures of gB CAR T cells against the human GBM explant, D270, gB CAR T cells were able to recognize and exhibit anti-tumor function against primary GBM. CMV gB CAR redirected T cells were able to recognize tumors bearing extremely low antigen levels, and could recognize low levels of ligand undetectable by mAb staining and flow cytometry. These results suggest that CAR T cells may be effective in recognizing extremely low abundance antigens, and taken together, the results of this study show the feasibility of using gB CAR T cells as a platform to target CMV in GBM tumors to treat patients with GBM. Ultimately, the goal of this study is translate these findings into clinical trials.

2.26

“BOTTOM-UP” PROFILING OF INTERACTING CELLULAR SYSTEMS

New Innovator Award, 2015

Alex K. Shalek

Massachusetts Institute of Technology, Cambridge, MA, USA

Abstract author: Aleth Gaillard

Massachusetts Institute of Technology, Cambridge, MA, USA; Broad Institute of MIT and Harvard, Cambridge, MA, USA; Ragon Institute of Massachusetts General Hospital, MIT, and Harvard University, Cambridge, MA, USA

Presented by Alex K. Shalek

We consist of trillions of interacting cells, but our understanding of how they work together is limited. This is because we have traditionally divided organisms from the “top-down” into broad cell types or iteratively-refined “homogeneous” subsets and then studied each such population separately. Yet recent studies have shown that even “identical” cells can exhibit functionally important differences and that cellular behaviors are strongly influenced by both the microenvironment and cellular interactions. Unfortunately, our inability to thoroughly measure and analyze each of these influences within the context of a complex system has limited our ability to grasp how proper immune function is achieved.

To address this and related issues, we are leveraging recent advances in nanotechnology and molecular biology to develop broadly applicable platforms for systematically manipulating and deeply profiling many interacting single cells, so that we can uncover how they collectively perform systems-level behaviors. More specifically, we are building and deploying five innovative core technologies to enable (a) culturing and monitoring individual cells in isolation; (b) examining specific single cells within an ensemble; (c) performing targeted manipulations; (d) detecting many different types of molecular entities in the same cell (e.g., RNA and protein); and (e) profiling genome-wide gene expression in many single cells, *in vitro* and *ex-vivo*. Collectively, the proposed work will help identify the cellular players and the strategies they use to execute systems-level behaviors, radically altering our understanding of cellular response, communication, disease, and therapeutics, as well as enabling us to design and build functionality for therapeutic aims.

2.27

TUMOR-SUPPRESSIVE EFFECTS OF ANEUPLOIDY

Early Independence Award, 2015

Jason Sheltzer

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

Chromosomal instability and whole-chromosome aneuploidy are hallmarks of human malignancies. The prevalence of chromosome segregation errors in cancer – first noted more than 100 years ago – has led to the widespread belief that aneuploidy plays a crucial role in tumor development. In this work, we describe the first systematic test of this hypothesis. We transduced congenic euploid and trisomic fibroblasts with 14 different oncogenes or oncogene combinations, thereby creating genetically-matched cancer cell lines that differ only in karyotype. Surprisingly, nearly all aneuploid cell lines divided slowly *in vitro*, formed few colonies in soft agar, and grew poorly as xenografts, relative to matched euploid lines. Similar results were obtained when comparing a near-diploid human colorectal cancer cell line with derivatives of that line that harbored extra chromosomes, and when comparing chromosomally-stable and chromosomally-unstable primary cell lines. Only a few aneuploid lines grew at close to wild-type levels, and no aneuploid line exhibited greater tumorigenic capabilities than its euploid counterpart. These results demonstrate that rather than promoting tumorigenesis, aneuploidy can very often function as a tumor suppressor. Moreover, our results suggest one potential way that cancers can overcome the tumor suppressive effects of aneuploidy: upon prolonged culture *in vitro* or *in vivo*, cell lines with simple aneuploidies developed recurrent chromosomal aberrations that were absent from their euploid counterparts and that were associated with enhanced growth. Thus, the genome-destabilizing effects of single-chromosome aneuploidy may facilitate the evolution of balanced, high-complexity karyotypes that are frequently found in advanced malignancies.

2.28

DIFFERENTIAL STOICHIOMETRY AMONG CORE RIBOSOMAL PROTEINS

New Innovator Award, 2016

Nikolai Slavov

Northeastern University, Boston, MA, USA

Understanding the regulation and structure of ribosomes is essential to understanding protein synthesis and its dysregulation in disease. While ribosomes are believed to have a fixed stoichiometry among their core ribosomal proteins (RPs), some experiments suggest a more variable composition. Testing such variability requires direct and precise quantification of RPs. We used mass spectrometry to directly quantify RPs across monosomes and polysomes of mouse embryonic stem cells (ESC) and budding yeast. Our data show that the stoichiometry among core RPs in wild-type yeast cells and ESC depends both on the growth conditions and on the number of ribosomes bound per mRNA. Furthermore, we find that the fitness of cells with a deleted RP-gene is inversely proportional to the enrichment of the corresponding RP in polysomes. Together, our findings support the existence of ribosomes with distinct protein composition and physiological function.

2.29

THE ANALGESIC EFFECTS OF PERIAQUEDUCTAL GRAY DOPAMINE NEURONS

Transformative Research Award, 2012

Emery N. Brown,^{1,2} Ken Solt,^{1,2} Ed Boyden,² and Matthew Wilson²

¹Massachusetts General Hospital, Boston, MA, USA; ²Massachusetts Institute of Technology, Cambridge, MA, USA

Presented by Norman Taylor

Drugs which modulate neural dopamine (DA) produce significant analgesia, while also preventing opioid-induced side effects of nausea, respiratory depression and sedation. Despite their potential, DA modulating agents have never been used clinically to treat pain, possibly due to a lack of understanding about their mechanism of action. We hypothesized that DA neurons in the periaqueductal gray (PAG) powerfully modulate pain and are important participants in descending pain inhibition. To test this hypothesis, we used DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to see if selective stimulation of DA neurons in the PAG could produce analgesia in an inflammatory pain model.

DREADDs are G-protein coupled receptors engineered to be selectively activated by the ligand Clozapine N-Oxide (CNO). Male, adult DAT-cre mice received bilateral injections of adeno-associated virus carrying an excitatory DREADDs construct (hM3Dq) into the ventral lateral PAG. Control mice were similarly prepared, but received a construct lacking the hM3Dq receptor. After 4 weeks to allow stable viral transfection, thermal hyperalgesia was measured by injecting a carrageenan solution into a single hind paw and measuring the time latency for paw withdrawal upon thermal stimulation. Viral expression and localization were confirmed using immunohistochemistry at the end of the study. D-amphetamine is a clinically available drug which modulates neural DA levels, and provides a translational approach to examine the clinical relevance of exciting DA neurons in the PAG. We subsequently treated carrageenan-induced hind limb pain in adult male C57BL/6 mice with intraperitoneal (ip) D-amphetamine, and compared the analgesic effect with morphine treated mice.

We found that following ip CNO injection in control mice ($n = 8$), paw withdrawal latency in the carrageenan injected paw was significantly decreased at 2.4 ± 0.7 s compared with 8.0 ± 1.5 s in the non-injected paw, indicating significant thermal hyperalgesia. Animals with DREADD activation of vIPAG DA neurons by CNO ($n = 9$) showed no significant difference in paw withdrawal latencies between treated (8.2 ± 1.7 s) and untreated paws (9.3 ± 1.6 s), indicating that DA neuron activation in the PAG prevented inflammation-induced thermal hyperalgesia. Histologic examination of neural tissue following the experiments confirmed DREADD viral expression in PAG DA neurons.

A dose dependent increase in paw withdrawal latencies was observed with ip D-amphetamine treatment. 6 mg/kg of D-amphetamine was as effective as 3 mg/kg of morphine in eliminating hind paw pain in carrageenan induced inflammation, suggesting a powerful analgesic role for DA modulating drugs.

In summary, selective activation of DA neurons in the vIPAG as well as systemic administration of D-amphetamine produced profound analgesia in an inflammatory pain model. DA modulating agents may represent a novel new treatment for pain.

2.30

SUBCELLULAR-SCALE SILICON FOR BIOELECTRIC INTERFACES

New Innovator Award, 2016

Bozhi Tian

The University of Chicago, Chicago, IL, USA

Silicon-based materials exhibit biocompatibility, biodegradability as well as a spectrum of important electrical, optical, thermal and mechanical properties, leading to their potential applications in biophysical or biomedical research. However, existing forms of silicon (Si) materials have been primarily focused on one-dimensional (1D) nanowires and two-dimensional (2D) membranes. Si with three-dimensional (3D) mesoscale features has been an emerging class of materials with potentially unique physical properties. Here we introduce a biocompatible and degradable mesostructured form of silicon with multi-scale structural and chemical heterogeneities. The material was synthesized using mesoporous silica as a template through a chemical vapor deposition process. It has an amorphous atomic structure, an ordered nanowire-based framework and random sub-micrometer voids, and shows an average Young's modulus that is 2–3 orders of magnitude smaller than that of single-crystalline silicon. In addition, we used the heterogeneous silicon mesostructures to design a lipid-bilayer-supported bioelectric interface that is remotely controlled and temporally transient, and that permits non-genetic and subcellular optical modulation of the electrophysiology dynamics in single dorsal root ganglia neurons. Our findings suggest that the biomimetic expansion of silicon into heterogeneous and deformable forms can open up opportunities in extracellular biomaterial or bioelectric systems.

2.31

LARGE COPY NUMBER VARIANTS IN THE HUMAN GENOME ARE ASSOCIATED WITH COMMON PSYCHIATRIC DISORDERS AND CAN BE ANALYZED ON THE MOLECULAR LEVEL IN NEURONAL MODELS BASED ON INDUCED PLURIPOTENT STEM CELLS

New Innovator Award, 2012

Alexander E. Urban, Ph.D.

Department of Psychiatry and Behavioral Sciences and Department of Genetics, Stanford University, Stanford, CA, USA

One very exciting discovery in human genetics of the past decade is that there is a group of large genomic copy number variants (large CNVs) that are strongly associated with abnormal brain development, and with complex and poorly understood neuropsychiatric disorders such as schizophrenia and the autism spectrum disorders (ASDs).

These large CNVs are deletions or duplications of stretches of the genome sequence up to several million base pairs long. They are enticing points of entry for the analysis of the strong but complex and poorly resolved molecular genetics of these common disorders that are still very often intractable.

However, most questions remain unanswered regarding how the large CNVs affect changes on the molecular level that lead to aberrant brain development and the clinical phenotype. The large CNVs often affect the copy number of several dozen genes and the resulting effects are most likely very complex as well as specific to cell type and developmental time point.

The use of induced pluripotent stem cells (iPSCs) makes it possible to create neurons in culture that are derived from patients with neuropsychiatric phenotypes who carry a large neuropsychiatric CNV in the sequence of their genomes. The molecular effects of the large CNV can then be studied in the relevant cell type, along the trajectory of cellular differentiation, and by integrating multiple layers of genomic and epigenomic activity and regulation.

Under the NIH Director's New Innovator Award we have established a cohort of iPSC lines from neuropsychiatric patients (as well as matched controls) that carry a large CNV in one of the three most prominent large-CNV loci for schizophrenia and the ASDs – on chromosomes 22q11, 15q13 or 16p11 (for 16p11 we have both deletion and duplication CNVs in the cohort).

Using these iPSCs and tissue culture differentiation models with the neuronal state as the endpoint we have generated, and are in the process of analyzing and integrating, extensive and highly granulated genomics and epigenomics data sets for gene expression and several markers of chromatin states, as well as whole-genome sequencing maps that catalog all potential genetic modifiers of the large CNV.

Already we can observe that the genes within the large CNVs are affected in a cell-type specific manner, but also that there are transcriptome-wide, cell-type specific network effects – which are to some extent converging across the different large CNVs. Furthermore there are also multi-layered effects on the epigenomic level (e.g. on chromatin condensation and chromosome conformations), again at the large CNV locus itself but also across regional and long distances in the cellular nucleus. Testable hypotheses

as to how the large neuropsychiatric CNVs are molecularly connected to the clinical phenotype are emerging as a result of these analyses and will be discussed.

2.32

NEURODATA SYNAPTOME PROJECT: TOWARD AN AT-BASED PLATFORM FOR SINGLE SYNAPSE ANALYSIS OF DIVERSE CNS SYNAPSE POPULATIONS

Transformative Research Award, 2014

Stephen Smith,¹ Randal Burns,² Guillermo Sapiro,³ and Ed Lein¹

¹*Allen Institute for Brain Science, Seattle, WA, USA;* ²*Johns Hopkins University, Baltimore, MD, USA;*

³*Duke University, Durham, NC, USA*

Presented by Joshua T. Vogelstein

Johns Hopkins University, Baltimore, MD, USA

The NeuroData Synaptome Project (NDSP) is an effort led by a consortium of investigators at the Allen Institute, Johns Hopkins University, UNC Chapel Hill, Duke, UC Davis and UC San Francisco aimed at building broadly accessible foundations for single-synapse analysis of diverse CNS synapse populations. The aims of the NDSP include: (1) improve methods for preparing specimens of mouse and human neocortex for ATomo analysis, (2) expand and validate synapse-relevant antibody panels, (3) develop faster and more automated ATomo imaging methods, (4) deploy advanced petascale image database methods, (5) improve methods for automated synaptomic analysis of ATomo image data, (6) develop cell-biologically principled taxonomies of mouse and human synapse types, and (7) establish web portals for sharing of methods, data, taxonomies, and other resources across the broad synapse biology community. Preliminary results are available here: <http://synaptomes.neurodata>.

2.33

CHIRAL TRACTION FORCES OBSERVED ON 2D GEOMETRICALLY DEFINED SURFACES

New Innovator Award, 2014

Leo Q. Wan

Rensselaer Polytechnic Institute, Troy, NY, USA

Abstract author: Kathryn E. Worley

Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA

Presented by Leo Q. Wan

Collective cell migration helps to facilitate the formation of the asymmetric vertebrate body plan through specific cell-cell and cell-surface interactions. Here we examine how variation in these interactions affects cellular biomechanical properties which ultimately influence their ability to establish chiral bias. Chiral bias is polarity in the left-right axis and this third axis along with the apicobasal and front-rear axes form a complete division of living organisms. Previous work has shown that traction forces are important for collective cell migration. We therefore propose that there exist chiral traction forces responsible for the establishment and maintenance of collective chiral behaviors of cells. As a first step, we have chosen to examine the relationships between chiral bias in cell alignment, cell migration and the generation of traction forces, and study how these relationships are altered through disruption of cell-cell adhesions. Epithelial, endothelial and myoblast cell types were seeded separately onto micropatterned rings on polyacrylamide gels of known stiffness. Live cell imaging of each cell type with and without disruption of cell-cell adhesions, through EGTA treatment, allowed for a comprehensive evaluation of cell alignment, migration velocity and traction force generation. We have found phenotype specific chiral bias in alignment, migration rate and traction force generation for MDCK, HUVEC and C2C12 cell types, with biases of slightly more counterclockwise, almost entirely clockwise and almost entirely counterclockwise, respectively. Treatment with EGTA showed the most distinct influence on the epithelial MDCK cells, resulting in a loss of cell alignment and an almost complete loss of chiral force transmission through the cell sheet. A thorough understanding of these relationships allows for a more complete quantification of inherent chiral bias and allows for the importance of calcium dependent cell-cell adhesions for various cell types to be determined. This knowledge can be used in the future to more accurately classify variations in chiral bias, as well as to create more realistic models for both *in vitro* and *in vivo* collective migration.

2.34

IDENTIFYING ROADBLOCKS TO REGENERATION BY REPEAT DEPLOYMENT OF THE LIMB REGENERATION PROGRAM

New Innovator Award, 2015

Jessica L. Whited

Harvard Medical School, Brigham & Women's Hospital, Boston, MA, USA

Abstract author: Donald M. Bryant

Harvard Medical School, Brigham & Women's Hospital, Boston, MA, USA

Presented by Jessica L. Whited

Axolotl salamanders are powerful models for understanding how regeneration of complex body parts can be achieved. Factors identified as key for normal axolotl regeneration can be examined in mammals to determine if they exhibit altered activity in this context and if experimentally modulating them can improve regenerative outcomes. We sought to determine if we could experimentally compromise the axolotl's ability to regenerate limbs and, if so, what molecular changes might underlie their inability to regenerate. We challenged axolotls to repeated amputation, allowing sufficient time between amputations to fully regenerate limbs. We found that repeated limb amputation at the same site severely compromised the ability to regenerate. The data points to a defect in the initiation stage of regeneration. We compared the transcriptional profiles of cells at the tip of the limb at three days post-amputation from compromised animals versus their sibling controls undergoing their first regenerative event. We found many examples of transcripts that are aberrantly upregulated in the compromised animals that might be considered antagonistic to regeneration. We will present data demonstrating the sufficiency of one factor, Amphiregulin, to antagonize normal regeneration in naive animals and discuss its published roles in mammalian pathologies, emphasizing the possible relation to regeneration. We also discovered the inverse class of transcripts that are more highly expressed in controls versus compromised animals. Future studies will examine the function of these genes in normal regeneration and mechanisms whereby animals cannot fully activate them following repeated amputation.

2.35

CELLULAR AND MOLECULAR CHARACTERIZATION OF THE BREATHING PACEMAKER

Early Independence Award, 2016

Kevin Yackle^{1,2} and Mark Krasnow^{2,3}

¹University of California, San Francisco, San Francisco, CA, USA; ²Stanford University, Stanford, CA, USA; ³Howard Hughes Medical Institute, Stanford, CA, USA

Presented by Kevin Yackle

There are two critical pacemakers for life: the cardiac pacemaker and the breathing pacemaker, the preBötzinger Complex (preBötC). The preBötC is a cluster of ~3000 neurons in the brainstem that are cyclically active, with each burst of activity initiating a breath. In contrast to the cardiac pacemaker, the molecular and cellular basis of breathing rhythm generation remains unknown, as do diseases associated with it, such as central sleep apnea and sudden infant death. The prevailing model of preBötC rhythm generation, called the ‘group-pacemaker’ model, proposes that each breath is triggered by an emergent preBötC network phenomena. An important assumption of this model is that there are not dedicated breath-initiating neurons. However, based on the observed variety of preBötC neuron firing patterns, including ones that fire just before each breath (pre-inspiratory), I hypothesized that preBötC neurons are molecularly and functionally distinct. I performed a full genome *in silico in situ* hybridization screen to identify mRNA transcripts specifically expressed within the preBötC and subsequently identified more than 70 molecularly distinct preBötC neural types by the combinatorial expression 22 markers. I then show that small numbers of molecularly defined neural types have distinct and interesting roles in breathing. For example, ~50 preBötC neurons that express the neuropeptides Somatostatin (Sst) and *cocaine and amphetamine related transcript* (Cart) are required to prolong the length of expiration of calm, hypoxic and hypercapnic breaths. I propose that small numbers of molecularly distinct preBötC neurons have important, dedicated and specific functions in breathing and that among the remaining more than 70 cell types are specific cell types that generates the pace of breathing and propose to identify and characterize them in this research proposal below. I plan to comprehensively molecularly map preBötC cell types with single cell gene expression analysis and subsequently molecularly define candidate breath-initiating neurons by their anticipated activity during breathing (pre-inspiratory) and their autonomous, rhythmic activity *in vitro* (pacemaker activity). Lastly, I will identify candidate pacemakers by their proposed connectivity to multiple preBötC neural types I identified in my Ph.D. which either modulate or are modulated by the proposed breath-initiating neurons. I predict that these approaches will converge on the same preBötC subtypes and I will then use intersectional genetic strategies to test if the identified neurons have breathing pacemaker properties: autonomous rhythmic activity, pre-inspiratory activity, ability to initiate a breath, and requirement for breathing.

2.36

MECHANO-RESPONSIVE STEM CELLS TO TARGET CANCER METASTASES THROUGH BIOPHYSICAL CUES

New Innovator Award, 2014

Weian Zhao

University of California, Irvine, Irvine, CA, USA

Cancer metastases are responsible for more than 90% of cancer deaths, however no current effective therapeutics directly and specifically targets them. The unique mechanical properties of metastatic niche offer an intriguing target for the development of treatments selectively targeting metastases. Systemically infused mesenchymal stem cells (MSCs) preferentially home to tumors. Besides, it has been established that tissue mechanical properties regulate MSC differentiation by driving expression of certain genes. We hypothesize that increased matrix stiffness is an essential property of the metastatic niche that can be targeted with MSC-based, mechano-responsive therapies. Here we present, by targeting the mechano-environment of the metastatic niche, a new methodology for the treatment of cancer metastases, using promoter-driven, MSC-based vectors, named as mechano-responsive cell system (MRCS). Our data suggest that the MRCS homes to and targets cancer metastasis responding to specific mechanical microenvironment to deliver therapeutics, including cytosine deaminase (CD) that locally activates prodrug to kill cancer with minimal side-effects. Compared to MSCs expressing CD constitutively, MRCS treats metastatic breast cancer with reduced deleterious effects and more effective outcome. Our technology could be broadly applicable to therapies targeting abnormal tissue stiffness including fibrotic diseases.

Author List

[Ahern, Jennifer](#)
[Al-Khami, Amir](#)
[Allbritton, Nancy](#)
[Allen, Christopher](#)
[Aller, Stephen](#)
[Alushin, Gregory](#)
[Anticevic, Alan](#)
[Arora, Manish](#)
[Auguste, Debra](#)
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[Bass, Brenda](#)
[Basu, Uttiya](#)
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[Blau, Helen](#)
[Bonasio, Roberto](#)
[Bondy-Denomy, Joseph](#)
[Bruening, Meg](#)
[Brunet, Anne](#)
[Cai, Long](#)
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[Carter, Hannah](#)
[Chen, Yvonne](#)
[Cole, Francesca](#)
[Cox, Jeffery](#)
[de la Zerda, Adam](#)
[DeKosky, Brandon](#)
[Dethlefsen, Les](#)
[Dong, P. Duc Si](#)
[Esser-Kahn, Aaron](#)
[Faulk, Christopher](#)
[Fraser, James](#)
[Gandhi, Sunil](#)
[Gimelbrant, Alexander](#)
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[Gore, Jeff](#)
[Gu, Chenghua](#)
[Hammoud, Sue](#)
[Han, Songi](#)
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[Kohli, Rahul](#)
[Kupper, Thomas](#)
[Laganowsky, Arthur](#)
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[Lieberman Greer, Eric](#)
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[Mugnier, Monica](#)
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[Sahin, Ozgur](#)
[Schwartz, Gregory](#)
[Shalek, Alex](#)
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[Siapas, Thanos](#)
[Singh Gill, Harvinder](#)
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[Smolke, Christina](#)
[Solt, Ken](#)
[Sonabend, Adam](#)
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[Tian, Bozhi](#)
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[Urban, Alexander](#)
[Van Dort, Christa](#)
[Vogelstein, Joshua](#)
[Wan, Leo](#)
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