Session 1

NEURAL CIRCUITS OF COMPULSIVE REWARD-SEEKING

Kay Tye

MIT

New Innovator Award, 2013

Obesity and diabetes are among the greatest preventable threats to human health, and overconsumption of sucrose has been thought to be the leading preventable cause. While legislative efforts have been made to restrict sales of sugary drinks, these approaches have failed and the demand for sugary consumables has remained high. Despite these widely-appreciated issues, the neural mechanisms underlying compulsive sucrose-seeking behaviors are poorly understood. We have investigated the neural circuits linking the lateral hypothalamus (LH; a brain region important for feeding, homeostasis and reward processing) to the ventral tegmental area (VTA; the site of midbrain dopamine neurons implicated in reward processing). We used cutting-edge approaches such as photoidentification of LH-VTA neurons to show that these neurons encoded the learned response of reward-seeking behavior. Based on the concept that reward-seeking behaviors repeated many times can become habits which can then lead to compulsive behavior, we decided to test the ability of LH-VTA neurons to drive compulsive sucrose-consumption (seeking sucrose in the face of a negative consequence). Indeed, activating LH-VTA neurons promoted, while inhibiting these neurons suppressed, compulsive sucrose-seeking -- without altering hunger-driven feeding. We then investigated the circuit mechanism for how this occurred. Specifically, we demonstrated that activating GABAergic LH inputs to the VTA disinhibited VTA dopamine neurons, thereby increasing dopamine release in the ventral striatum. We are now in the process of determining whether closed-loop manipulations can induce plasticity and alter the probability of transitioning into compulsive reward-seeking behavioral states.

THE LIQUID NUCLEOLUS

Cliff Brangwynne

Princeton University

New Innovator Award, 2013

In this talk I will discuss our work on the physicochemical assembly and properties of the nucleolus, the largest structure within the cell nucleus. This work has important implications for human health: nucleoli can be thought of as factories for ribosome biogenesis, and since the ribosome is the key protein translation machine, nucleoli are intimately linked to cell growth and size control, which are dysregulated in cancer. Our work has revealed that nucleoli represent phase-separated liquids, which condense around ribosomal DNA loci. A phase transition model explains many features of nucleolar assembly, including the internal subcompartments of the nucleolus, which we've shown arise from multi-phase immiscibility. I will also discuss our new "Optodroplet" approaches, which uses light to enable spatiotemporal control of phase transitions within living cells. We are now using Optodroplet

variants to quantitatively map intracellular phase diagrams, and examine the role of liquid phase immiscibility in nucleolar assembly, and various other aspects of nuclear organization.

LIPID METABOLISM AND THE REGULATION OF CHRONIC INFLAMMATION

Augusto Ochoa, Maria Sanchez-Pino, Amir Al Khami, Matthew Dean, Jovanny Zabaleta, Dorota Wyczechowska, Luis Del Valle

Louisiana State University Health Cancer Center

Transformative Research Award, 2013

Developing a protective immune response is a well-coordinated process where antigen-presenting cells (APC) capture, process and present antigen to effector T and B cells (adaptive immunity). In turn T and B cells develop effector functions (cytokine production, cytotoxic function or specific antibody production) associated with a protective immune response that eliminates the offending (or invasive) agent. Recent findings have demonstrated that the type and function of immune cells have characteristic metabolic signatures associated with their function. Resting lymphoid (B and T cells) and myeloid (macrophages, dendritic cells and granulocytes) cells use low levels of glucose oxidative phosphorylation to support "housekeeping" functions. Upon antigen stimulation they rapidly upregulate glycolysis and oxygen to produce high levels of ATP and nucleotides required for cell proliferation and effector functions. In an effort to understand the transformation of a protective immune response into a chronic inflammatory response, we studied the metabolic characteristic of immune cells in cancer and allergic diseases such as asthma. Our data demonstrates that upon infiltrating the hypoxic, hypoglycemic and acidic microenvironment present in tumors or in a site of chronic inflammation, myeloid cells undergo a dramatic metabolic reprogramming from the use of glucose to fatty acid oxidation. This is also characterized by an increase in mitochondrial mass, and increased oxygen consumption rate and activation of lipid metabolism enzymes and pathways. It is further associated with the activation of immunosuppressive mechanisms that inhibit protective anti-tumor responses, promote angiogenesis and protect tumors from immune destruction. This distinct metabolic reprogramming has provided new mechanisms to understand the basis for immune dysfunction in cancer and other chronic inflammatory conditions, and identified new pathways that can be targeted to treat these diseases. These findings will also allow us to further classify certain immune mediated diseases by their metabolic characteristics. Thus future personalized medicine may not only include genetic testing to identify and prescribe drugs that target specific genes, but also metabolic characterization of the inflammatory process to initiate specific therapies that modulate the chronic inflammatory process and promote instead a protective immune response.

Session 2

MONITORING CELL-CELL INTERACTIONS IN VIVO BY INTERCELLULAR ENZYMATIC LABELING

Gabriel Victora

Rockefeller University

Early Independence Award, 2012

Interaction between different cell types is essential for many biological processes, including immunity, embryonic development, and neuronal signaling. Dynamic immune cell interactions in vivo have been investigated primarily by intravital multiphoton microscopy using fluorescent reporter mice, an approach which is technically challenging, difficult to quantitate-especially when dealing with short-lived interactions- and not suitable for isolation of interacting cells for downstream analysis. We present a complementary quantitative approach that uses bacterial sortase labeling across immune synapses to identify interactions between cells within living animals, generating a signal that can be readily detected by flow cytometry. We call this approach to labeling “kiss-and-run” interactions between immune cells Labeling Immune Partnerships by SorTagging Intercellular Contacts (LIPSTIC). We use LIPSTIC to show that interactions between dendritic cells (DCs) and CD4+ T cells during T cell priming in vivo occur in two distinct modalities: an early, cognate stage when CD40-CD40L interactions occur specifically between T cells and antigen-loaded DCs, and a later, non-cognate stage when these interactions no longer require T cell receptor (TCR) engagement. Thus, LIPSTIC allows direct measurement of dynamic cell-cell interactions both in vitro and in vivo. Given its flexibility for use with different receptor ligand pairs and a range of detectable labels, we expect that this approach will be of use to any field of biology interested in quantifying intercellular communication.

ENGINEERING NEXT-GENERATION T CELLS FOR CANCER IMMUNOTHERAPY

Yvonne Chen

University of California, Los Angeles

Early Independence Award, 2012

The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of next-generation T cells that can target multiple cancer antigens and resist immunosuppression, thereby increasing the robustness of therapeutic T cells against tumor defense mechanisms. Specifically, I will discuss the development of multi-input receptors and T cells that can interrogate intracellular antigens. I will also discuss the engineering of T cells that can effectively convert TGF-beta from a potent immunosuppressive cytokine into a T-cell stimulant. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

ADVANCES IN GENOME EDITING TECHNOLOGIES

Feng Zhang

Broad Institute of MIT and Harvard

Pioneer Award, 2012

In the five years since the initial demonstration of mammalian genome editing using the Cas9 enzyme, the molecular scissors of the microbial adaptive immune CRISPR system, a number of advancements in genome editing technology have been made with astounding speed. Cas9 has been leveraged for a range of genome manipulation tools, including gene activation and repression as well as modulation of chromatin and DNA modifications. Additional DNA-targeting Cas enzymes have been discovered, broadening the possible targeting space within the human genome and offering greater activity in other species. More recently, RNA-targeting Cas enzymes have been discovered, expanding CRISPR-mediated technologies into the realm of the transcriptome modulation. We have characterized a number of these novel enzymes, known as Cas13, and identified orthologs that work in mammalian cells with high activity and specificity. We have shown that Cas13 can be used to knock down endogenous transcripts as well as serve as a programmable RNA-binding platform. Additionally, we engineered a fusion between Cas13 and the adenine deaminase ADAR to achieve RNA Editing for Precise A-to-I Replacement (REPAIR). We showed that REPAIR has the potential to correct single-base pathogenic mutations at the transcriptional level. REPAIR may be a powerful therapeutic for diseases that affect cell types and tissues not amenable to DNA-based gene therapies, such as neurons and other post-mitotic cells. We are continuing to explore microbial diversity to find new enzymes and systems that can be adapted for use as molecular biology tools and novel therapeutics.

Session 3

THERAPEUTIC INTERFERING PARTICLES (TIPS): DEVELOPMENT OF A RESISTANCE-PROOF, TRANSMISSIBLE ANTIVIRAL FOR RESOURCE-LIMITED SETTINGS

Leor Weinberger

University of California- San Francisco, Gladstone Institutes

Pioneer Award, 2013

Infectious disease control faces significant challenges including: how to therapeutically target the highest-risk populations, circumvent behavioral barriers, and surmount persistence and resistance mechanisms. In particular, HIV-1 contol efforts in the developing world are stymied by these barriers. I will review our development of an antiviral that can overcome these challenges by ‘piggybacking’ on viral replication. These engineered molecular parasites of viruses, termed ‘Therapeutic Interfering Particles’ (TIPs), steal replication resources from the wild-type virus thereby depriving viruses of crucial replication machinery and reducing viral loads and transmission potential. As obligate parasites, TIPs can co-adapt and transmit via the same risk factors and transmission routes as wild-type virus, automatically reaching high-risk 'superspreader' populations, to substantially limit viral transmission even in resource-poor settings. I will present our progress developing TIPs for HIV-1.

PHASE-BASED CONTROL OF LOCOMOTION FOR HIGH-PERFORMANCE PROSTHESES AND ORTHOSES

Robert Gregg

University of Texas at Dallas, University of Texas Southwestern Medical Center

New Innovator Award, 2013

The human gait cycle is typically viewed as a periodic sequence of events over time, starting with heel contact during initial stance and ending with knee extension during late swing. This convention has informed the design of control strategies for powered prosthetic legs, which almost universally switch between several distinct controllers throughout the gait cycle. This paradigm requires substantial time and effort to tune many controller parameters to each patient. Moreover, temporal representations of gait cannot accommodate disturbances that push the gait cycle forward or backward (i.e., changes in phase), making it difficult to 1) study non-steady human locomotion and 2) control wearable robots in synchrony with human users. We instead propose that the progression through the gait cycle can be continuously represented by a mechanical phase variable. The concept of a phase variable has been successfully used to control the progression of leg joints in dynamic walking robots that can walk, run, and climb stairs. However, it was unclear which variable, if any, could robustly represent phase for non-steady human locomotion. This talk will share the results of a perturbation study with 10 able-bodied human subjects, observing that thigh motion can represent the progression of leg joint patterns with very high correlation. A thigh-based phase variable was then used to synchronize the joint patterns of a powered knee-ankle prosthesis with the motion of the human user, enabling volitional activities such as forward/backward stepping, clearing an obstacle, and kicking a ball. The user-synchronization also enabled four above-knee amputee subjects to walk at variable speeds up to 1.56 m/s and inclines up to 9 deg. Normative adjustments in prosthetic joint work were observed as speed increased. Amputee compensations were reduced when using the powered prosthesis compared to the conventional prosthesis. Extensions of this approach to powered orthoses will also be presented.

MEMBRANE DISRUPTION AND THE MOLECULAR BASIS OF ANESTHESIA AND MECHANOSENSATION

Scott Hansen, Nicholas Petersen, Arif Pavel

The Scripps Research Institute

New Innovator Award, 2012

Inhaled anesthetics are a chemically diverse collection of hydrophobic molecules that robustly activate TWIK related K+ channel (TREK-1) and reversibly induce loss of consciousness. For many anesthetics (but not all), lipophilicity is the single most significant indicator of potency. This observation is known as the Overton-Mayer correlation and led research to focus on the membrane as the target of anesthesia. Despite a hundred years of investigation, no definitive membrane delimitated mechanism has emerged for anesthesia. Here we employ super resolution imaging (dSTORM) and electrophysiology to show a two-step membrane delimited process in TREK-1 channel activation. First, anesthetics disrupt lipid rafts and displace the enzyme phospholipase D (PLD2). PLD2, a palmitoylated protein, translocates out of the rafts where it finds its substrate PC and activates TREK-1 through local production of anionic lipid. Mechanical force also disrupts lipid rafts. We show, in a biological membrane, TREK-1 sensitivity to mechanical force requires PLD and raft disruption identical to the mechanism of anesthetics activation. Many important signaling molecules are palmitoylated and may similarly be susceptible to translocation in response to raft disruption by anesthetics or mechanical force.

REGULATORY PROGRAMS CONTROLLING REGENERATIVE GROWTH

Chris Petersen

Northwestern University

New Innovator Award, 2013

Selected organisms have evolved natural solutions to the problem of repairing injured organs with stem cells and offer informative models for understanding regenerative repair. Using functional genomics, we have investigated the molecular mechanisms underlying the extraordinary repair ability of the planarian Schmidtea mediterranea, which uses pluripotent adult stem cells to regenerate an entirely new body after any injury, even decapitation. Our analysis of this system has uncovered molecules and principles of regenerative repair in animals. First, planarians activate injury-induced factors that use canonical Wnt signaling to probe tissue polarization near the wound site and determine subsequent responses. Next, the organism deploys a specialized program of injury-induced stem cell differentiation to produce post-embryonic organizing centers that direct subsequent outgrowth. Axis-wide constitutive gradients of Wnt and FGFRL factors form a patterning "coordinate system" that controls information about the presence/absence of regions necessary to replace. Regeneration re-scales this coordinate system after axis truncation, and regeneration is completed through a combination of new tissue production and the ability of pre-existing tissues to absorb migratory organ progenitors. Using information about the overall mechanism and RNAi screens, we identify methods to enhance the regeneration of selected tissues. Unraveling the regulatory logic of regenerative growth will be an important step forward in understanding and modifying natural regenerative abilities.

INHIBITION MECHANISM IN EPITHELIAL SODIUM CHANNEL REVEALED BY CRYO-EM

Isabelle Baconguis[a], Sigrid Noreng[b], Arpita Bharadwaj[b], Craig Yoshioka[b]

[a]Vollum Institute, [b]OHSU

Early Independence Award, 2013

The epithelial sodium channel (ENaC), a protease-sensitive integral membrane protein that transports Na+ across tight epithelia, plays a central role in ion homeostasis and thus in regulating extracellular fluid volume. ENaC mutations cause Liddle's syndrome and pseudohypoaldosteronism type 1 (PHA-1), diseases that are the consequence of ENaC hyperactivity and hypoactivity, respectively. While those genetic diseases are rare, more subtle ENaC polymorphisms may contribute to essential hypertension, and ENaC activation appears to play a central role in hypertension and volume retention resulting from common disease processes and from drugs. Understanding the molecular basis for the function of these channels is crucial, not only for elucidating their roles in physiological and pathophysiological processes but also for providing blueprints for future therapeutic strategies. We focused our efforts in elucidating the mechanism by which ENaC works by using complementary biochemical and biophysical techniques, and especially single-particle cryo-electron microscopy. We integrated the recent advances in software and hardware in single-particle cryo-EM and harnessed the robust technology of monoclonal antibodies to determine the first structure of ENaC.

Session 4

CAN YOU SEE A THOUGHT? NEURONAL ENSEMBLES AS EMERGENT UNITS OF CORTICAL FUNCTION

Rafael Yuste

Columbia University

Pioneer Award, 2013

Since the time of Cajal and Sherrington, it has been traditional assumed that the individual neurons are the basic units of cortical function. At the same time, it is possible that coactive groups of neurons, i.e. neuronal ensembles, could represent emergent building blocks of neural circuits. I will review our efforts to test this hypothesis, by characterizing the phenomenology, mechanisms and potential functional and pathological roles of neuronal ensembles in the primary visual cortex of mice, using optical methods to image and manipulate ensembles during behavioral tasks. Our results are consistent with the hypothesis that neuronal ensembles are building blocks of perceptual states.

SPATIAL ANALYSIS OF MOLECULAR FEATURES WITH DNA-PAINT SUPER-RESOLUTION MICROSCOPY AND A BIOCHEMICAL DNA ”NANOSCOPE”

Peng Yin

Harvard University

Transformative Research Award, 2013

Analysis of the spatial arrangement of molecular features is important for biological study. I will describe two techniques based on dynamic DNA nanotechnology. The first technique uses DNA-PAINT, a super-resolution fluorescence microscopy technique that exploits programmable transient oligonucleotide hybridization. Compared with alternative super-resolution techniques, DNA-PAINT is resistant to photobleaching, and has independently and precisely tunable blinking frequency and duration, which collectively enables ultra-high resolution imaging of dense targets packed on a 5 nm x 5 nm grid (Dai, Jungmann, Yin, Nature Nanotechnology, 2016, 11:798). In addition to optical imaging methods, I will describe a biochemical assay (a DNA "nanoscope") that records nanostructure features in situ and for later readout. Based on an auto-cycling proximity recording mechanism, the DNA "nanoscope" continuously and repeatedly produces proximity records of any nearby pairs of DNA-barcoded probes without altering the probes themselves, which enables the computational reconstruction of the spatial arrangement of the underlying targets (Schaus, Woo, Xuan, Chen, and Yin, Nature Communications, 2017, 8:696). I will also discuss current and prospective biological applications of DNA-PAINT and DNA nanoscope, including single-cell nucleus architecture and single-cell proteomics.

MHC GENOTYPE SHAPES THE ONCOGENIC MUTATIONAL LANDSCAPE

Hannah Carter

University of California, San Diego

Early Independence Award, 2013

Significant insights into tumorigenesis have been gained by characterizing the extensive somatic alterations that arise during cancer and uncovering rare inherited mutations that lead to early onset cancer syndromes. However, little is understood about the role of genetic background in “sporadic” adulthood cancers. We have demonstrated that somatic evolution of a tumor is influenced by inherited polymorphisms. The genomic region encoding the Major Histocompatibility Complex Class (MHC) is one of the most variable regions in the human population. MHC molecules expose peptide fragments on the cell surface, allowing T-Cell elimination of cells contaminated by foreign peptide. Although this system has evolved as a defense against microbial and viral agents, MHC can also trigger elimination of cells harboring mutant peptides (neoantigens) in cancer. Each individual carries multiple MHC alleles that define the set of peptides that can be effectively presented for immune surveillance. We hypothesized that individual variation in MHC could create personal gaps in immune surveillance, generating individual-specific susceptibility for cells to acquire specific oncogenic mutations. To test this hypothesis, we developed residue-centric patient presentation scores for MHC class I and II molecules and applied them to 1,018 recurrent oncogenic mutations in 9,176 cancer patients. This analysis uncovered a clear signature of immune selection acting on tumors; cancer-causing mutations were more likely to be observed when a patient’s genotype-based scores suggested poor MHC-based presentation. Mutations that were poorly presented by most patients were more likely to reach high frequency among tumors. Individual coverage of driver mutations by MHC-based presentation was also found to be a determinant of age at diagnosis. Thus the landscape of oncogenic mutations observed in clinically diagnosed tumors is shaped by MHC genotype-restricted immunoediting during tumor formation, and individual MHC genotype provides information about the mutations likely to emerge in tumors that develop later in life.

SPATIAL PROTEOMICS IN LIVING CELLS VIA PROXIMITY LABELING

Alice Ting

Stanford University

Transformative Research Award, 2013

Scientists are routinely interested in identifying the protein interaction partners of molecules of interest, or the protein residents of a cellular subcompartment of interest. Traditional methods to address these questions, such as immunoprecipitation and biochemical fractionation, frequently lose weak interaction partners and pick up contaminants. Many organelles such as the synaptic cleft and P-bodies are also impossible to purify. With TR01 support, our laboratory developed an alternative approach, based on enzyme-catalyzed proximity labeling. A promiscuous labeling enzyme, such as APEX peroxidase or TurboID ligase, is genetically targeted to a protein or cellular region of interest. Addition of a small molecule substrate results in catalytic generation of a reactive biotin species that covalently tags endogenous proteins within a few nanometers of APEX or TurboID. I will describe the development and characterization of the methodology, as well as its application to a variety of systems, such as the mitochondria, synaptic cleft, and mitochondria-ER contact sites.

Session 5

SUPER-RESOLUTION IMAGING OF TRANSCRIPTION IN LIVING MAMMALIAN CELLS

Ibrahim Cissé

Massacheusetts Institute of Technology

New Innovator Award, 2014

Gene activation is thought to involve a multistep process whereby transcription factors bind to distal enhancer sites and recruit the Mediator complex which contacts the pre-initiating RNA Polymerase II (Pol II) complex assembled at the start site of the gene. The interaction of Mediator and Pol II has yet to be observed in the nucleus of living cells and the dynamics of this interaction are not yet elucidated. Here we use quantitative live cell super-resolution and light sheet imaging to study the organization and dynamics of endogenous Mediator and Pol II directly in living mouse embryonic stem cells. In addition to forming transient clusters with average lifetimes of 11.1 (± 0.9) s, and 12.1 (± 1.4) s respectively, Mediator and Pol II also form large and stable clusters in stem cells (~15 stable clusters per cell). The large and stable Mediator and Pol II clusters gradually disappear within hours after induction of stem cell differentiation. Mediator and Pol II colocalize in the large clusters. Inhibition of Brd4 bromodomains necessary for enhancer association eliminates both Mediator and Pol II stable clusters, and inhibition of transcription elongation selectively eliminates stable Pol II but not stable Mediator clusters. Tracking of Mediator and Pol II stable clusters suggests they are chromatin associated and they coalesce upon contact, a property associated with phase separated droplets. We conclude that Mediator and Pol II associate in diffraction-sized condensates with a defined lifetime dependent on active transcription in living stem cells.

ACTIVATION OF DENTATE GYRUS MEMORY TRACES RESCUES AGE-RELATED COGNITIVE DECLINE

Christine Denny

Columbia University, Research Foundation for Mental Hygiene, Inc. (RFMH)

Early Independence Award, 2013

Age-related cognitive decline affects numerous types of mnemonic capabilities, including pattern separation, the ability to distinguish between two similar cues. We sought to understand this cognitive impairment by identifying and manipulating memory traces to improve behavior. In aged mice, pattern separation impairments, particularly the failure to distinguish between mildly aversive and neutral contexts that are otherwise similar, were accompanied by decreased immediate early gene (IEG) expression in the dentate gyrus (DG) and CA3 hippocampal subregions. Optogenetically stimulating DG neural ensembles representing the neutral context memory trace decreased fear generalization and facilitated contextual discrimination, resulting in improved pattern separation in young and aged mice. Furthermore, chronically stimulating the neutral memory trace improved long-term pattern separation and increased DG IEG expression in aged mice. Together, our data suggest that activating DG memory

traces is sufficient to improve pattern separation and points to DG manipulation as a target to correct age-related memory deficits.

NANOPARTICLE-BASED THERAPIES FOR TRAUMATIC BRAIN INJURY, IS IT FEASIBLE?

Sarah Stabenfeldt[a], Vimala Bharadwaj[a], Chen Wu[b], Ishitha Jagadish[a], Rachel Rowe[bc], Trent Anderson[b], Jonathan Lifshitz[bcd], P. David Adelson[bc], Vikram Kodibagkar[a]

[a]Arizona State University, [b]University of Arizona, College of Medicine-Phoenix, [c]BARROW Neurological Institute at Phoenix Children's Hospital, [d]Phoenix Veteran Affairs Healthcare System

New Innovator Award, 2014

Given the high incidence of traumatic brain injuries (TBI) and limitations of current therapeutic regimens, there is a dire need for effective therapeutic strategies. Nanoparticle (NP)-based materials have gained significant traction for pharmacological and diagnostic applications. Over two-dozen NP systems have been approved by the US FDA for the clinic in recent years. NP applications for brain disorders are typically limited by the inability to cross the tightly regulated blood-brain-barrier (BBB). However, seminal studies using small molecule tracers have established BBB breakdown after TBI and report two transient phases for BBB disruption. The first phase peaks within 1-6hrs after TBI while the second phase emerges around 3days post-injury. We hypothesized that these transient BBB breaches may provide opportunities for NP-based therapeutics. Therefore, this study assessed the spatiotemporal distribution of intravenously delivered NPs, based on size, using three different pre-clinical mouse models of TBI. An array of NPs ranging from 20nm to 500nm, each with unique fluorescent spectra, was injected systemically into mice at different time points post-injury (immediate to 7days). We established that NPs sizes up to 500nm accumulated in the brain injured tissue at acute time points post-injury, directly correlated with BBB breakdown. Peak accumulation occurred within 1h-6h post-injury depending on injury type (focal vs diffuse). Notably, maximal accumulation was observed with the 40nm NPs across all models where disruption of the BBB occurred. Collectively, these studies provided critical insight into NP design and delivery (NP size and delivery time window post-TBI) as well as motivation for pursuing NP-based therapies and diagnostics for TBI.

NATURAL EXPERIMENTS IN HEALTH CARE

Anupam Jena

Harvard Medical School

Early Independence Award, 2013

Natural experiments in health care are naturally occurring events that allow researchers to understand the quasi-experimental effects of health care interventions and policies, where prospectively designed, controlled trials would be infeasible. For example, most policy evaluations are not implemented and evaluated as randomized trials and so quasi-experimental methods are needed to understand whether policies achieve their intended effects. This presentation will describe several natural experiments that I have analyzed through my Early Independence Award, with the goal of demonstrating the importance of creativity and rigorous methodology in identifying and evaluating these experiments. For example, how do patient care and outcomes change for cardiac patients who are hospitalized during the dates of

national cardiology meetings? What happens to patient outcomes when hospital inspectors (specifically, the Joint Commission) make unannounced accreditation visits to U.S. hospitals? Why is it that cardiac mortality increases on dates of major U.S. marathons for older Americans who live close to marathon routes? The presentation will highlight these and other counter-intuitive findings with the aim of helping the audience better understand how natural experiments that occur around us can tell us something about what works and doesn't work in health care.

Session 6

OPTICAL TOOLS FOR ANALYZING AND REPAIRING COMPLEX BIOLOGICAL SYSTEMS

Ed Boyden

MIT

Pioneer Award, 2013

Understanding and repairing complex biological systems, such as the brain, requires new technologies that enable such systems to be observed and controlled with great precision, across extended spatial and temporal scales. We are discovering new molecular principles that are leading to such technologies. For example, we recently discovered that it was possible to physically magnify biological specimens manyfold, in an even way, by embedding them in dense swellable polymers, mechanically homogenizing the specimens, and then adding water to isotropically swell the specimens. In this method, which we call expansion microscopy (ExM), we enable scalable, inexpensive diffraction-limited microscopes to do large-volume nanoscopy, in a multiplexed fashion- important, for example, for brain mapping. I will discuss recent directions we have taken, including the optimization of expansion microscopy for human specimens, the development of iterated versions that enable extremely precise imaging, and the adaptation of multiplexed readout strategies into the ExM context. As another example, we discovered that microbial opsins, genetically expressed in neurons, could enable their electrical activities to be precisely driven or silenced in response to millisecond timescale pulses of light. These tools, called optogenetic tools, are enabling causal assessment of the contribution of defined neurons to behaviors and pathologies in a wide variety of basic science settings. I will discuss how these tools are now enabling millisecond-precise, single cell control in intact mammalian brain circuits. Finally, we have developed new methods of directed evolution that enable multidimensional protein evolution in mammalian cells, and discovered mutant forms of optogenetic tools that enable precision fluorescent imaging of the high-speed voltage of neurons in the living brains of multiple species. We share all these tools freely, and aim to integrate the use of these tools so as to lead to comprehensive understandings of neural circuits.

EARLY WARNING SYSTEMS FOR CHILDHOOD AND ADULT DISEASE

Manish Arora

Icahn School of Medicine at Mount Sinai

New Innovator Award, 2014

According to the developmental origins of health and disease hypothesis, many disorders originate via environmental exposures in fetal and early postnatal life. Such early life environmental exposures can alter the developmental trajectory by disrupting the homeostasis of one or more systems, and in doing so produce identifiable biochemical signatures characteristic of the disease process. However, due to technological barriers many disorders are not detected until overt clinical or biochemical signs appear in adulthood, at which point it is no longer possible to meaningfully alter the course of development or disease. A new paradigm is proposed that will overcome these barriers to detect disease years before current clinical and biochemical tests. By doing so we will be able to predict, and hopefully prevent and treat diseases decades before any clinical signs. Central to our proposal is an underappreciated characteristic of many human physiologic processes- they commonly exhibit highly temporally resolved biochemical rhythms (or cycles) when at homeostasis. The idea of biochemical rhythms in itself is not revolutionary; sleep cycles, body temperature, cortisol rhythms, and menstrual cycles are all examples of the rhythmic nature of human physiology operating at various intervals. Medical testing, however, seldom considers rhythmicity. We propose to develop not just a novel technology that analyzes dynamic rhythmicity of key biochemical pathways during fetal development and childhood to accurately detect marked and sustained deviations from homeostasis that would be prognostic of later-life disease onset, but also a new framework for understanding development not solely as a linear trajectory but as interconnected rhythmic processes embedded within the growth trajectory. Data are presented from multinational studies conducted by the PI that extend from childhood disorders such as autism to adult onset disorders such as ALS or Lou Gehrig's disease.

THE ROLE OF MEMBRANE CURVATURE IN TOPOGRAPHY-INDUCED INTRACELLULAR SIGNALING

Bianxiao Cui

Stanford University, Department of Chemistry

New Innovator Award, 2012

The interaction between the cell membrane and the substrate surface is crucial for many biological and medical applications such as in vitro cell culture and in vivo medical implants. We are interested in exploring nanotechnology and novel materials to improve the membrane-surface interactions. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. Vertical nanopillars deform the plasma membrane inwards and induce membrane curvature when the cell engulfs them, leading to a reduction of the membrane-substrate gap distance. We found that the high membrane curvature induced by vertical nanopillars significantly affects the distribution of curvature-sensitive proteins and stimulates several cellular processes in live cells. Our studies show a strong interplay between biological cells and nanoscale-featured surfaces, which is an essential consideration for future development of interfacing devices.

USING NEUROSCIENCE TO PROMOTE HEALTH BEHAVIOR CHANGE

Emily Falk

University of Pennsylvania

New Innovator Award, 2012

Can neuroscience dramatically improve our ability to design health communications? Our New Innovator Award allowed us to make substantial advances in using neural responses in small groups of people to forecast individual behavior change and the spread of ideas outside of the lab, as well as population level behaviors that go beyond the individuals whose brains are scanned. I will also describe recent research linking brain activity to behavior outside of the lab that incorporates social network measurements and suggest new questions at the intersection of brains and social networks.

CARDIAC MICROENVIRONMENT SUPERSEDES DEVELOPMENTAL ORIGIN FOR FIBROBLAST-TO-CARDIOMYOCYTE REPROGRAMMING

Reza Ardehali, Sara Ranjbarvaziri, Ben Van Handel

UCLA

New Innovator Award, 2014

Direct reprogramming of cardiac fibroblasts (CFbs) to cardiomyocytes is a promising strategy to regenerate damaged myocardium from endogenous fibroblasts and potentially minimize fibrotic tissue. Several studies have reported the remarkable tendency of fibroblasts to be reprogrammed more efficiently to induced cardiomyocytes (iCMs) in vivo, particularly in the context of cardiac injury, when compared to in vitro reprogramming. Despite the therapeutic promises, questions remain regarding how the heterogeneity of CFbs may influence cardiac reprogramming efficiency. It is not entirely known whether there exists a distinct subset of CFbs in vivo with a predisposition towards reprogramming to cardiomyocytes. Furthermore, it is possible that injury may induce cues in a subpopulation of CFbs and make them more susceptible to conversion to iCMs. Herein, we employed genetic fate-mapping and transplantation studies to show that the majority of CFbs originate from a shared mesodermal ancestor as cardiomyocytes while a minority emerges from neural crest-derived precursors. Using single-cell imaging, we demonstrated that developmental heterogeneity of CFbs partially influences their anatomical distribution within the heart. Additionally, we found that regardless of their developmental origin, CFbs are able to be successfully converted to beating iCMs through in vitro direct reprogramming. We also show that despite invoking similar levels of proliferation and activation, cardiac injury induces a temporary re-expression of early developmental genes in CFbs that is dependent on their lineage of origin. When compared to fibroblasts of identical developmental origin from extra-cardiac organs, CFbs generated iCMs with higher efficiency and intrinsically maintain open chromatin at key cardiac transcription factors, suggesting a link between the microenvironment and gene regulation that may influence fate conversion more than developmental origin. These data underscore the importance of the tissue of residence in relation to direct reprogramming to iCMs, which may be crucial for the development of targeted therapies to promote cardiac repair.

Session 7

IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS COUNTER-IMMUNE FACTORS USING TN-SEQ

Anna Tischler, Sarah B. Namugenyi, Alisha Aagesen, Nagendra Palani, Kenneth Beckman

University of Minnesota

New Innovator Award, 2013

Mycobacterium tuberculosis can survive in the lungs of immune-competent hosts for decades despite stimulating adaptive immune responses, suggesting that the bacteria have evolved mechanisms to dampen or evade host immunity. To identify bacterial "counter-immune" factors that are specifically involved in evading macrophage activation mediated by the cytokine interferon-gamma (IFN-γ), we employed transposon (Tn) mutagenesis combined with massively parallel Illumina sequencing (Tn-seq). We created an arrayed library of *M. tuberculosis* Tn mutants and sequenced the library by an orthogonal pooling approach to map the location of individual Tn mutants. We screened this library for Tn insertion mutants with differential ability to replicate in the lungs and spleens of three immune-deficient strains of mice: IFN-$\gamma^{-/-}$ mice, NOS2$^{-/-}$ mice that lack the IFN-γ inducible nitric oxide synthase, and Irgm1$^{-/-}$ mice that lack a IFN-γ-regulated GTPase involved in phagosome acidification. To avoid stochastic loss of mutants due to colonization bottlenecks in the lung, we screened the Tn mutants in pools of limited complexity (~94 Tn mutants per pool). Screening of 12 pools of mutants identified numerous *M. tuberculosis* pathways involved in resistance to IFN-γ-dependent immune mechanisms in the lung. Individual retesting of select Tn mutants recovered from the arrayed library by aerosol infection to confirm their attenuation is in progress. The *M. tuberculosis* factors we identified that counteract IFN-γ dependent immune responses could be targets for development of novel therapeutic interventions that would sensitize the bacteria to host immunity.

MICRO/NANO ENGINEERING FOR LIQUID BIOPSY AND CANCER THERAPEUTICS

Siyang Zheng

The Pennsylvania State University

New Innovator Award, 2012

Micro/nano engineering is an important tool to investigate biological system and provide new solutions for healthcare challenges. In the past five years or so, my group has been focusing on developing micro/nano technology solutions for cancer diagnosis and therapeutics. Liquid biopsy refers to a suite of new technologies, including circulating tumor cells (CTCs), circulating free DNA (cfDNA) and extracellular vesicles (EVs), that can diagnose cancer from peripheral blood samples. To isolate rare CTCs from cancer patients, we invented a microfabricated flexible micro spring array (FMSA) device. It can filter 7.5 ml unfixed whole blood within 5 minutes and achieve 90% CTC capture efficiency and 99.99% leukocyte removal. The captured CTCs can be identified by immunocytochemistry on-chip and single CTC isolated for genetic analysis. EVs are sub-micrometer lipid membrane enclosed vesicles released by almost all the cells. They are discovered as messengers for intercellular communication. We invented a lipid nanoprobe (LNP) technology that efficiently isolates EVs from blood plasma in 15 minutes. This is a nanomaterial-based approach that utilizes an engineered lipid probe to label membrane-bounded vesicles and functionalized magnetic nanoparticles to rapidly capture labeled vesicles. Double stranded

DNA fragments can be extracted from isolated EVs and pathogenic mutations can be detected from plasma samples of cancer patients. On the other hand, flexible design of nanoparticles equips them with properties and functions for precise drug delivery. We designed nanoparticles that load therapeutic proteins efficiently, protect the proteins during systemic delivery, and offload the cargos inside cells. To exploit the unique features of EVs as natural intercellular messengers, we developed methods to envelop nanoparticles with EV membranes for targeted drug delivery and enhanced circulation time. The methods have been validated both in vitro and in animal models.

TARGET-GUIDED GENOME MINING OF NATURAL PRODUCTS

Yi Tang

UCLA

Pioneer Award, 2012

Bioactive natural products have evolved to inhibit specific cellular targets and have served as lead molecules for health and agricultural applications for the last century. The post-genomics era has brought a renaissance in natural product discovery using synthetic biology tools. However, compared to traditional bioactivity-guided approaches, genome mining of natural products with specific and potent biological activities remains challenging. Our approach is based on the coclustering of a self-resistance gene in the natural product biosynthetic gene cluster, which serves as a window to potential biological activity of the encoded compound. Here we present the application of this methodology towards discovery of natural products of new modes of action. One example includes the discovery of a highly potent herbicide lead that targets a critical metabolic enzyme that is required for plant survival. The self-resistance gene was validated to be insensitive to the natural product and was deployed as a transgene in establishment of plants that are resistant to the herbicide. This powerful herbicide-resistance gene combination complements urgent efforts in overcoming weed resistance. Other examples of discovery will also be presented to demonstrate the potential of using a resistance-gene directed approach in discovery of highly specific natural products for diverse applications.

EXPANDED POTENCY OF AN ANTIVIRAL TRANSCRIPTION FACTOR IN BATS

John Schoggins

University of Texas Southwestern Medical Center

New Innovator Award, 2014

Bats as a species host a rich variety of zoonotic viruses, often without displaying overt disease symptoms. The mechanisms underlying this unique viral resistance remain unclear. Several lines of evidence point to potentially unique contributions from the bat innate immune system. A key antiviral pathway in mammals is the interferon (IFN) response. IFN elicits a broad cellular transcriptional program that culminates in the induction of hundreds of interferon-stimulated genes (ISGs), many of which encode proteins with antiviral functions. In this study, we compared the antiviral activity of over 70 ISG human-bat ortholog pairs to identify potential differences in individual effector function. We identified the bat transcription factor IRF7 as a potent and broad-acting antiviral molecule that, unlike human IRF7, suppresses viral infection without previous activation. We show that bat IRF7 uniquely induces a

large subset of inhibitory ISGs independently of IFN signaling, which leads to robust suppression of infection by multiple viruses including flaviviruses, alphaviruses, and rhabdoviruses. Mechanistically, we show that bat IRF7 is readily able to translocate to the nucleus and binds the promoters of ISGs. This property of IRF7 constitutes a major difference between bat and human ISG regulation and may provide an additional layer of antiviral protection not typically observed in humans.

Session 8

EPIGENETICS OF SOCIAL BEHAVIOR IN ANTS

Roberto Bonasio

University of Pennsylvania

New Innovator Award, 2014

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 that corazonin is 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, and transcriptional regulation in general, is shown to play a causal role in caste determination and caste-specific behavior in a social organism.

OPTICAL CONTROL OF PROTEIN ACTIVITIES BY ENGINEERED PHOTODISSOCIABLE FLUORESCENT PROTEIN DOMAINS

Michael Lin, Xin Zhou, Linlin Fan, Xinzhi Zhou, Hokyung Chung

Stanford University

Pioneer Award, 2013

A generalizable method for optical control of proteins would enable fine interrogation or engineering of biochemical pathways and cellular functions. In this study, we design, build, and apply synthetic single-chain protein architectures for bidirectional and cofactor-independent control of protein activity with visible light. We first engineered a cofactor-free dimeric protein that reversibly photodissociates in cyan light and photoassociates in violet light. We then found that fusing two copies of this domain, pdDronpa, at rationally identified locations in target proteins could create single-chain proteins with photoswitchable activity. As protein kinases regulate virtually every aspect of eukaryotic cellular function by phosphorylating their substrates with high spatial and temporal precision, we first created photoswitchable (ps) variants of the kinases MEK1, MEK2, Raf1, and Cdk5. Using psMEK1, we established an all-optical cell-based assay for screening inhibitors specifically targeting MEK and ERK. Using the ability to activate psRaf1 in short time windows, we uncovered a previously unknown direct and rapid inhibitory feedback loop from ERK to MEK1. Using psCdk5, we established that Cdk5 activity was required only after neuronal differentiation for proper synaptic vesicle localization in living animals. In addition to kinases, we also used pdDronpa for single-chain photoswitchable Cas9 variants, successfully controlling S. pyogenes Cas9 for gene editing and transcriptional regulation and, for the first time, creating a photocontrollable form of S. aureus Cas9. Our results demonstrate that engineered photodissociable fluorescent protein domains enables a general method for optical control of protein activity that is generalizable, self-reporting, and requires no exogenous cofactors.

SYNAPTOMES AND SYNAPTIC PROTEIN TRANSCRIPTOMES OF MOUSE AND MAN

Stephen Smith[ab], Randall Burns[c], Richard Weinberg[d], Kristina Micheva[b], William Seeley[e], James Trimmer[e], Guillermo Sapiro[g], Joshua Vogelstein[c], Dan Madison[b], Ed Lein[a]

[a]Allen Institute for Brain Science, [b]Stanford University, [c]Johns Hopkins University, [d]University of North Carolina, [e]UC San Francisco, [f]UC Davis, [g]Duke University

Transformative Research Award, 2014

Chemical synapses are the brain's principle cell-cell signaling component. They are highly complex, plastic, strongly modulated and deeply diverse entities, and their corresponding molecular complexity and diversity is fundamental to all neural circuit development, plasticity and function. Moreover, many or most neurodevelopmental, psychiatric and neurodegenerative disorders are rooted in abnormalities of the brain's vast and highly heterogeneous chemical synapse populations. Unfortunately, such disorders are poorly understood and difficult to diagnose, prevent and treat because we lack adequate tools to measure these diverse synapse populations and because too many of the limited tools in use today can be applied only to experimental animals such as mice. We have assembled an interdisciplinary consortium comprising neurobiologists, clinicians, statisticians and computer scientists to develop an end-to-end "synaptomic" analysis pipeline based on both fluorescence and electron modes of array tomography (AT). This novel workflow has enabled measurement, analysis and modeling of heterogeneous synapse and neuromodulatory fiber populations with unprecedented rigor and precision, in both mouse and human specimens.

The recent availability of deep and plentiful single-cell RNA-seq data and corresponding neuron-type taxonomies now provides powerful new guidance for our AT-based explorations of synaptic protein

diversity. We'll discuss ways a powerful new synergy of transcriptomic and AT proteometric methods is transforming our abilities to fathom synapse molecular diversity in the context of the brain's highly heterogeneous synaptic network.

STEM CELL-ENGINEERED INVARIANT NATURAL KILLER T CELLS FOR CANCER IMMUNOTHERAPY

Lili Yang, Yanni Zhu, Drake Smith, Victor Jiaji Yu, Charlie Yanruide Li, Alice Yang Zhou

UCLA

New Innovator Award, 2014

Invariant natural killer T (iNKT) cells comprise a small population of αβ T lymphocytes. They bridge the innate and adaptive immune systems and mediate strong and rapid responses to many diseases, including cancer, infections, allergies and autoimmunity. However, the study of iNKT cell biology and the therapeutic applications of these cells are greatly limited by their small numbers (~0.001-1% in mouse and human blood). Preciously, we demonstrated that we could genetically engineer hematopoietic stem cells (HSCs) to produce iNKT cells in mice, and that the engineered HSC-iNKT cells could effectively control tumor growth in a mouse melanoma lung metastasis model. Here we report that using a humanized BLT (human bone marrow-liver-thymus engrafted) mouse model, we proved that we could also engineer human CD34+ HSCs to produce human iNKT cells. These human HSC-iNKT cells displayed a typical iNKT cell phenotype, and showed potent anti-tumor efficacy in multiple human tumor xenograft mouse models, including a multiple myeloma (MM) metastasis model and a melanoma solid tumor model. These results highlight the therapeutic potential of HSC-engineered iNKT cell therapy and encourage its clinical development. Because iNKT cells can target tumor independent of MHC- and tumor antigen-restrictions, if successful, the HSC-iNKT therapy has the potential to become a general immunotherapy for treating a broad range of cancers and a large population of cancer patients.

MICROBES REGULATE THE DEVELOPMENT OF ANTI-BLOOD GROUP ANTIBODIES

Sean Stowell

Emory University

Early Independence Award, 2014

Anti-ABO(H) antibodies represent one of the most significant barriers to transfusion and transplantation. However, despite the discovery of ABO(H) blood group antigens and corresponding anti-ABO(H) antibodies over a century ago, the mechanism through which these antibodies form remains relatively unknown. This lack of understanding largely reflects a lack of suitable models capable of defining key players that impact anti-ABO(H) antibody formation. To overcome this limitation, we generated a mouse model that lacks the mouse blood group B disaccharide (B^{dis}). Using this model, we demonstrated that B^{dis} negative recipients spontaneously develop varying levels of anti-B^{dis} antibodies within the first few weeks of life, similar to humans. To determine whether environmental exposure may impact anti-B^{dis} antibody formation, we specifically housed B^{dis} negative recipients in sterile conditions to determine whether microbial exposure specifically impacts anti-B^{dis} antibody induction. Remarkably, B^{dis} negative recipients housed in sterile conditions failed to form any detectable anti-B^{dis} antibodies for over a year, strongly suggesting that microbial exposure may actually be a requirement for naturally occurring anti-

B^{dis} antibody development. Consistent with this, exposure of adult B^{dis} negative recipients housed in sterile conditions to blood group positive microbes isolated from conventionally housed B^{dis} negative recipients induced anti-B^{dis} antibody sufficient to readily clear B^{dis} positive red blood cells. In contrast, exposure to blood group negative microbes failed to produce any detectable anti-B^{dis} antibody. Taken together, these results demonstrate a vital role for microbial exposure on the development of anti-B^{dis} antibody in blood group negative mice and provide valuable insight into the mechanism of anti-blood group antibody formation in blood group negative individuals.

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