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Abstract Book

Oral Presentation Abstracts

Monday, December 7, 2015

Session 1

1.1

Compressed Ultrafast Photography: World's Fastest 2D Receive-Only Camera Captures Light Propagation at Light Speed

Award Type: Pioneer Award

Award Year: 2012

Awardees: Lihong Wang, Washington University in St. Louis

Presenter: Lihong Wang

Capturing transient scenes at a high imaging speed has been pursued by photographers for centuries, tracing back to Muybridge's 1878 recording of a horse in motion and Mach's 1887 photography of a supersonic bullet. However, not until the late 20th century were breakthroughs achieved in demonstrating ultrahigh speed imaging (>100 thousand). In particular, the introduction of electronic imaging sensors, such as the charge-coupled device (CCD) and complementary metal-oxide-semiconductor (CMOS), revolutionized high-speed photography, enabling acquisition rates up to millions of fps. Despite these sensors' widespread impact, further increasing frame rates using CCD or CMOS is fundamentally limited by their on-chip storage and electronic readout speed. Here we demonstrate a two-dimensional (2D) dynamic imaging technique, compressed ultrafast photography (CUP), which can capture non-repetitive time-evolving events at up to 100 billion fps. Compared with existing ultrafast imaging techniques, CUP has a prominent advantage of measuring an x, y, t (x, y, spatial coordinates; t, time) scene with a single camera snapshot, thereby allowing observation of transient events occurring on a time scale down to tens of picoseconds. Further, akin to traditional photography, CUP is receive-only—avoiding specialized active illumination required by other single-shot ultrafast imagers. As a result, CUP can image a variety of luminescent—such as fluorescent or bioluminescent—objects. Using CUP, we visualize four fundamental physical phenomena with single laser shots only: laser pulse reflection, refraction, photon racing in two media, and faster-than-light propagation of non-information. Given CUP's capability, we expect it to find widespread applications in both fundamental and applied sciences including biomedical research.

Publication

1. Nature 516, 74 (Dec. 4, 2014)

1.2

Illuminating Bacterial Signaling with RNA-Based Biosensors

Award Type: New Innovator Award

Award Year: 2011

Awardees: Ming C. Hammond, University of California, Berkeley

Presenter: Ming C. Hammond

Bacteria are both our friend and foe. They reside on our bodies and are ubiquitous in natural and manmade environments. To thrive in these different niches, bacteria adapt their physiology and lifestyle in response to other members of the microbial fauna, to host cells, and to abiotic surfaces. These changes underlie important interactions such as commensal and competitive relationships in microbial communities, pathogenesis leading to infectious disease, and beneficial effects of bacteria on animals, plants, and the environment. However, the signaling pathways that link environmental and chemical cues to the regulation of bacterial physiology are still incompletely understood. In the case of signaling mediated by second messengers, a major roadblock has been the difficulty in observing changes in the levels of these signaling molecules in live cells. As supported by the New Innovator Award, we invented a new class of fluorescent biosensors based on riboswitch scaffolds, which are among the first imaging tools for studying the temporal and spatial dynamics of cyclic dinucleotide signaling. We applied these biosensors to make several biological discoveries, including identifying components of a newfound signaling pathway that regulates surface attachment, which is required for bacteria to occupy and colonize different niches.

1.3

Cryo-EM Structure of the BK Ion Channel in a Lipid Membrane

Award Type: Transformative Research Award

Award Year: 2010

Awardees: Liguo Wang, University of Washington

Presenter: Liguo Wang

The large-conductance voltage- and Ca2+-activated potassium (BK) channel has many physiological roles including the control of firing patterns in neurons, the modulation of the tone of blood vessels and the regulation of heart rate. Among ion channels, it has served as a model system because of its remarkable ion-permeation properties and its accessibility for studies of allosteric control of gating.

As shown in both structural and functional studies, the lipid membrane environment has played an essential role for membrane proteins. To restore the lipid membrane environment, BK channels were reconstituted into liposomes (lipid vesicles) for both functional and structural studies. In 2009, we have reported the full-length BK structure in lipid environment at a resolution of 1.7nm using "random spherically constrained" (RSC) single-particle Cryo-EM method. Recent breakthrough in hardware and the optimization of image collection and processing techniques make it possible to achieve a higher resolution. Here, we present the BK structure in a lipid membrane in the closed state with a greatly improved resolution. The helices in the transmembrane region can be clearly identified, and the helix S0 is identified to be next to helices S2 and S3.

1.4

MOZART: High-Resolution Optical Molecular Imaging System

Award Type: Early Independence Award

Award Year: 2012

Awardees: Adam de la Zerda, PhD, Stanford University

Abstract Author(s): Adam de la Zerda, PhD, Stanford University

Presenter: Adam de la Zerda, PhD

We have developed a new high-resolution optical molecular imaging system, we call MOZART. It is the only imaging technique that is capable of providing real-time imaging of living tissue with single-cell spatial resolution over a large 3D imaging area of 2 mm3. This is achieved through innovations in nanoparticle synthesis, nanoparticle biofunctionalization, and image reconstruction algorithms. We have synthesized large gold nanorods (LGNR) with a 30-fold greater intensity per particle than conventional GNRs, and functionalized them for biological applications. We have demonstrated an imaging sensitivity of 40 nanoparticles per imaging voxel in living mice – approximately 1000-fold greater than other imaging modalities, such as photoacoustic contrast or fluorescence imaging. In this presentation, we describe the fundamental operation and application of MOZART. We show 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 lymph vessel drainage, including observing the functional states of individual lymphangions and valves in a lymphatic network. MOZART provides a promising platform for in vivo imaging of cellular expression of cancer biomarkers, visualizing intercellular signaling among 100 million cells to assess drug response or disease progression, such as may be associated with cancer metastasis. Following the presentation, attendees can view MOZART images on a 3D holographic display.

2.1

Regulatory Protein Translation in the Human Nucleus

Award Type: New Innovator Award

Award Year: 2011

Awardees: Steven T. Kosak, Department of Cell and Molecular BiologyFeinberg School of Medicine, Northwestern University

Presenter: Steven T. Kosak

Abstract Author(s): Cogswell A.1, Fanslow D.1, Garza-Gongora A.1, Smith E.D.1, and Kosak S.T.1

1 Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.

Unlike the compartmentalization of cytoplasmic functions into membrane-bound organelles, activities within the nucleus are contained in non-membranous assemblies, or nuclear bodies (NBs). Protein synthesis in the nucleus was first reported over fifty years ago, and recent studies present strong and convincing evidence that nuclear translation does indeed occur. Although compelling, these studies have not addressed the functional significance of nuclear translation. Our investigation supports the idea of nuclear translation and indicates that protein synthesis in the nucleus is re-distributed to sites outside of the nucleolus under conditions of stress. These sites co-localize with the much studied but enigmatic promyelocytic leukemia (PML) nuclear bodies. Prior research suggests that PML bodies are involved in the stress response, protein homeostasis, and apoptosis. Interestingly, several components of the translation machinery including ribosomal proteins, eIF3, eIF4E, and elongation factor 1 are found in PML bodies, and eIF4E is required for their proper assembly. Our research suggests that PML bodies function as a site of protein quality control in the nucleus, particularly under stress. Moreover, we demonstrate that the nuclear aggregates characteristic of neurodegenerative disease is the result of this novel regulatory strategy being overwhelmed by aberrant mRNAs and misfolded proteins. We suggest that a variety of neurodegenerative diseases may be treated through the manipulation of PML body function.

2.2

Ether-Linked Phospholipids Modulate Stress Response in C. elegans

Award Type: Early Independence Award

Award Year: 2015

Awardees: Carissa Perez Olsen, Fred Hutchinson Cancer Research Center

Presenter: Carissa Perez Olsen

Ether-linked phospholipids including plasmalogens are a unique class of membrane lipids that are prevalent in eukaryotes but whose biological function is not yet understood. The vinyl-ether bond in plasmalogens is proposed to serve an important antioxidant function, and, here, we use Caenorhabditis elegans to explore the role of these ether-lipids in responding to oxidative stress. Using high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS), we mapped the distribution of ether-linked phospholipids in the nematode and find that they represent nearly 10% of the membrane lipids. In fact, over half of the phosphatidylethanolamine population contains ether bonds, similar to the content of many mammalian tissues. Next, we characterized the ether-linked lipid synthesis pathway through homology with the mammalian enzymes and find that we can eliminate the production of these lipids with RNAi against three major synthesis genes including fard-1, a fatty acyl-CoA reductase. After establishing C. elegans as a model for studying plasmalogens, we looked for changes in the abundance of ether-linked phospholipids in animals exposed to oxidative stress and found an active upregulation of their production in control animals. Adult-only RNAi of fard-1 results in abrogation of the stress-induced ether lipid increase. Not only are fard-1 RNAi animals are sensitive to stress, but they also fail to remodel both ether-linked and typical ester-linked membrane lipids in response to oxidative insults. Taken together, we find that ether-linked lipids are a critical component of membrane remodeling in response to oxidative stress and that their depletion may contribute to membrane dysfunction and disease.

2.3

Conservation of a Fundamental Pathway of Stress Resistance from Worms to Man

Award Type: Pioneer Award

Award Year: 2010

Awardees: Bruce Yankner, Harvard Medical School,

Presenter: Bruce A. Yankner

Human neurons are functional over an entire lifetime, yet the mechanisms that preserve function and protect against neurodegeneration during aging are unknown. We recently showed that induction of the repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST/NRSF) is a universal feature of normal aging in human cortical and hippocampal neurons. REST function is lost, however, in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Chromatin immunoprecipitation with deep sequencing (ChIP-seq) and expression analysis show that REST represses genes that promote cell death and AD pathology, and induces the expression of stress response genes. Moreover, REST potently protects neurons from oxidative stress and amyloid β-protein (Aβ) toxicity, and conditional deletion of REST in the mouse brain leads to age-related neurodegeneration. To explore the evolutionary conservation of the REST stress resistance pathway, we investigated C. elegans orthologs of the REST co-repressor complex, the suppressor of presenilin signaling (spr) family of genes. Worms bearing mutations in these genes exhibit increased vulnerability to oxidative, thermal, genotoxic, and proteotoxic stress. Furthermore, loss of function mutations in the C. elegans REST ortholog spr-4 increase neuronal cell death and pathologic protein aggregation in worm models of AD and Huntington's disease. Finally, by utilizing a recently derived CRISPR-CAS9 transcriptional activator system, we show that activation of the endogenous spr-4 gene significantly increases lifespan. These results suggest that REST is a component of a conserved stress resistance pathway that protects against neurodegeneration and contributes to the regulation of organismal aging. This work was supported by an NIH Director's Pioneer Award (DP1OD006849).

3.1

piRNA Biogenesis and Trans-Generational Epigenetic Inheritance

Award Type: New Innovator Award

Award Year: 2010

Awardees: Alexei Aravin, California Institute of Technology

Abstract Author(s): Alexei Aravin, California Institute of Technology

Presenter: Alexei Aravin

Small non-coding RNAs called piRNAs serve as the sequence-specific guides for an adaptable immune system that represses transposable elements in germ cells of Metazoa. In Drosophila the adaptation of the piRNA pathway to novel transposons is believed to occur when active transposons integrate into piRNA clusters, special genomic regions, which encode piRNA precursors. However, transposons carry termination signals that have a potential to disrupt transcription of piRNA clusters calling for the mechanism to prevent premature termination. The RDC complex is enriched on chromatin of dual-strand piRNA clusters and required for transcription of piRNA precursors. Here we dissected the function of RDC complex and show that its effector component, Cuff protein, prevents premature termination of RNA polymerase II by two distinct mechanisms. First, Cuff prevents cleavage of nascent RNA at poly(A) sites. Second, if processing does occur, Cuff stabilizes the 5'-monophosphorylated transcripts that are formed downstream of the cleavage site, by protecting them from degradation by the exonuclease Rat1. Our findings identify Rat1 as a new player in the piRNA pathway that works as a suppressor of transposon silencing and reveal a conceptually novel mechanism of transcriptional control through inhibition of termination.

3.2

Uniting Major Constituents of the Genome: A Novel Function of the PIwi-piRNA Pathway in the Germline

Award Type: Pioneer Award

Award Year: 2010

Awardees: Haifan Lin, Yale University

Abstract Author(s): Toshiaki Watanabe, Yale University

Presenter: Haifan Lin

The eukaryotic genome has vast intergenic regions containing transposons, pseudogenes, repetitive sequences, and noncoding genes that produce numerous long non-coding RNAs (lncRNAs) and PIWI-interacting RNAs (piRNAs). Yet the functions of the intergenic regions remain largely unknown. In mammals, a unique set of piRNAs, pachytene piRNAs, is abundantly expressed in the germline in late spermatocytes and early spermatids. Recently, we showed that piRNAs derived from transposons and pseudogenes mediate the degradation of a large number of mRNAs and lncRNAs in mouse late spermatocytes. In particular, they have a large impact on the lncRNA transcriptome, as a quarter of lncRNAs expressed in late spermatocytes are upregulated in mice deficient in piRNA pathway. Furthermore, our genomic and in vivo functional analyses revealed that retrotransposon sequences are frequently found in the 3' UTR of mRNAs that are targeted by piRNAs for degradation. Similarly, the degradation of spermatogenic cell-specific lncRNAs by piRNAs is mediated by retrotransposon sequences. Moreover, we have shown that pseudogenes regulate mRNA stability via the piRNA pathway. The degradation of mRNAs and lncRNAs by piRNAs requires MIWI and, at least in part, depends on its slicer activity. Together, these findings reveal a highly complex and global RNA regulatory network through which transposons and pseudogenes regulate target mRNA and lncRNA stability via the piRNA pathway to promote meiosis-spermiogenesis transition.

3.3

Apoptosis during Fetal Development Eliminates Clonally-Related Germ Cells

Award Type: New Innovator Award

Award Year: 2010

Awardees: Diana Laird, UCSF

Abstract Author(s): Daniel H. Nguyen, UCSF

Presenter: Diana Laird

The ability to pass on genetic information to the next generation requires that a germ cell successfully navigate a developmental gauntlet from specification through maturation into a gamete-producing cell. In mice, a significant portion of germ cells fails during this process and is eliminated through developmentally programmed waves of apoptosis during the fetal period and then again postnatally. The basis for this apoptosis, as well as its effects on the composition of the developing germline, remains poorly understood. We investigated the spatial distribution of male germ cell apoptosis in mice during the fetal wave (e12.5-e15.5) using wholemount imaging and showed mathematically that it occurs nonrandomly in highly localized clusters. We found no evidence for localized environmental factors contributing to this distribution, and the persistence of clustered germ cell apoptosis in a mutant that lacks specialized intercellular bridges formed by incomplete cytokinesis of fetal germ cells indicates that apoptosis operates independently on the basis of inherent properties of each cell. To determine if apoptotic clusters were clonally-related, we undertook random multicolor labeling with a lineage-specific drug-inducible Cre and the Rainbow and Confetti reporter alleles. After inducing labeling at the conclusion of germ cell migration (e10.5), we observed at e13.5 that clusters of apoptotic germ cells always shared the same color and hence derived from the same clonal ancestor. Multicolor labeling also facilitates a comparative perspective on clonal development and how clonal differences alter germ cell composition; variance in clone size suggests that clonal development is individualistic and uncoordinated across compartments. Our results suggest that germ cell clones have distinct developmental fates and are consistent with the function of scheduled apoptosis as a quality control mechanism in targeting specific clones for elimination.

3.4

Identification of Molecular Signature in Cystic Fibrosis Using Serum-Based Functional Genomics

Award Type: New Innovator Award

Award Year: 2010

Awardees: Hara Levy, Human Molecular Genetics Stanley Manne Children's Research Institute Ann and Robert H Lurie Children's Hospital of Chicago Department of Pediatrics, Section of Pediatric Pulmonary Medicine

Presenter: Hara Levy

Genomic technologies, including transcriptomics, offer unprecedented opportunities to advance our understanding of how environmental, genetic, and epigenetic factors modify disease progression. Importantly, there is no consistent phenotype-genotype correlation in cystic fibrosis (CF), perhaps due to protein-activity thresholds, modifier genes, and/or system dynamics. We have therefore validated an integrative genomics approach to assess the extracellular milieu associated with CF, an inherited, multisystem disease. In the present work supported by grant DP2 OD007031 (Integration of Genomics with Genetics—Molecular Phenotypes for CF Lung Disease), we analyzed plasma-induced transcriptional profiles in CF patients and correlated these data with common outcome measures including CFTR genotype, mutation class pancreatic function, pulmonary function, and infection status; we performed similar analyses in age-matched healthy controls. Our methodology, which utilizes serum-induced transcription in peripheral blood mononuclear cells functioning as reporter cells, consistently identified a small number of genes that are unique to CF patients and that correlate with disease severity. These genes, many of which are involved in immune and transcriptional regulation, are promising candidates for future therapeutic targeting.

This molecular signature has the potential to serve as a non-invasive clinical method for monitoring disease progression, clinical phenotypes, and response to treatment in CF. Our study's overall goal was to determine whether such a patient-based model system better captured disease complexity in CF than other approaches and could extended to other chronic lung diseases. Our gene-expression data discriminated between CF cases and controls, correlated with lung-function phenotypes, and identified new CF molecular phenotypes. Continued involvement with CF newborn screening programs will ensure the critical longitudinal follow-up of young patients necessary for determining whether profiles are established early in life and are correlated with the molecular signatures uncovered by our mononuclear cell-based protocol and validated with NanoString, RNAseq, and miRNA analyses. Our findings therefore lay the foundation for the inclusion of gene-expression arrays in real-time clinical settings, perhaps leading to the development of novel prognostic tools for CF and other lung disorders.

Session 4

4.1

Seek, Destroy and Heal: Disease-Responsive Nanoparticles as In Vivo Targeted Delivery Systems

Award Type: New Innovator Award

Award Year: 2011

Awardees: Nathan Gianneschi, University of California, San Diego

Presenter: Nathan Gianneschi

Nanoparticle targeting strategies have largely relied on the use of surface conjugated ligands designed to bind overexpressed cell-membrane receptors associated with a given cell-type. We envisioned a targeting strategy that would lead to an active accumulation of nanoparticles by virtue of a supramolecular assembly event specific to tumor tissue, occurring in response to a specific signal. For this purpose, we utilize enzymes as stimuli, rather than other recognition events, because they are uniquely capable of propagating a signal via catalytic amplification. We will describe the preparation of highly functionalized polymer scaffolds, their development as in vivo probes and their utility as a multimodal imaging platform and as drug carriers capable of targeting selectively to tumor tissue having efficacy in treating cancer. This approach represent an entirely novel direction in tissue targeting with promise in the treatment of cancer where current formulations have limited therapeutic effect due to toxicity and off-target accumulation.

4.2

Shrink Induced Manufacturing Platform for Low-Cost Diagnostics (SIMPL-CD)

Award Type: New Innovator Award

Award Year: 2010

Awardees: Michelle Khine, UC Irvine

Presenter: Michelle Khine

The challenge of traditional 'top down' micro- and nano-fabrication lies in the difficulties and costs associated with patterning at such high resolution. To make such promising technology – which could enable pervasive health monitoring and disease detection/surveillance – more pervasive, there is a critical need to develop a manufacturing approach such that prototypes as well as complete devices cost only pennies. Instead of relying on traditional fabrication techniques largely inherited from the semiconductor industry, my lab leverages the inherent heat-induced relaxation of pre-stressed thermoplastic sheets: commodity shrink-wrap film. We pattern at the large scale and achieve our desired structures by controlled shrinkage down to 5% of the original, patterned sizes. This enables us to beat the limit of resolution inherent to traditional 'top-down' manufacturing approaches.

With these tunable shape memory polymers, compatible with roll-to-roll as well as with standard lithographic processing, we can robustly integrate extremely high resolution, high surface area, and high aspect ratio nanostructures directly into our microsystems. Importantly, when the underlying polymer substrate relaxes and 'shrinks', a stiffer deposited thin film cannot and therefore buckles. We can control the buckling and therefore create nanostructures of deterministic sizes and patterns. Interestingly, our metallic nanostructures that self-assemble due to the stiffness mismatch between the thin metal film deposited on the retracting plastic sheet have demonstrated unprecedented electromagnetic field enhancements.

Our ultra-rapid fabrication approach therefore results in field-compatible plastic based microfluidic systems with integrated nanostructures for robust signal amplification. This design-on-demand approach to create a suite of custom biomedical tools for low cost diagnostics including sample prep with magnetic nanotraps, embedded on-chip electrodes, microlens arrays, surface enhanced sensing substrates, and flexible electronics. We have developed a process to lift-off the unique nanostructured patterns from the shrink plastic and to transfer them into other materials. This allows us to create truly conformal, high resolution epidermal electronics that move with the skin.

Our suite of tools has allowed us to create a platform to engage and to teach modern science and engineering concepts to the next generation of inventors through our outreach program, A Hundred Tiny Hands. Motivated by the facts that: > 67% of all engineers received their PhDs in the US are not US citizens, only 18% of working engineers are female, and that the US is ranked 52nd in STEM education, we clearly recognized that we need to come up with a better way to attract and retain our young scientists and engineers. Using the technology we have developed in our lab, we have developed Inventors Toolboxes for children as young as 5 years old to learn fundamental engineering concepts.

4.3

Nanoscale Tools to Advance Biomedical Frontiers

Award Type: Pioneer Award

Award Year: 2015

Awardees: Michael Roukes, Caltech

Presenter: Michael Roukes

I will describe two main projects carried out with support from my Pioneer Award:

1.   Integrated Neurophotonics. In 2011, I banded together with with five other U.S. scientists from different disciplines, outlined a vision [1], and we then managed to convince the Obama administration of the unprecedented opportunity for launching a coordinated, large-scale effort to map brain activity. This culminated in the U.S. BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies), which was launched in 2013. Our perspective was predicated, in part, on the current level of maturity of diverse fields of nanotechnology that can now be coalesced to realize powerful new tools for neuroscience. I will highlight our own development of the new field of integrated neurophotonics for realizing this vision. It leverages advances in integrated nanophotonics, optical reporters and effectors for neural recording and stimulation, and our recent developments in multisite neural nanoprobes for electrophysiology based on silicon large-scale integration.

2.   Native Single-Molecule Analysis. We have employed NEMS (nanoelectromechanical systems) to realize a new form of mass spectrometry (MS) with single molecule sensitivity [2], and have demonstrated the analysis of individual large-mass biomolecular complexes, one-by-one, in real-time [3]. I will describe its prospects in the field of native MS, which focuses upon the topological investigation of intact protein complexes with a theoretically unrestricted mass range. Recently, we have developed an enhanced approach that greatly extends the capabilities of NEMS-MS by enabling imaging the spatial mass distribution of individual analytes – in real time, and with molecular-scale resolution – when they adsorb onto a NEMS resonator [4]. This new approach is somewhat akin to Ion Mobility Spectrometry, which provides rotationally-averaged molecular collision cross sections (CCS) – however, it is superior in providing single-molecule resolution, without rotationally averaging the CCS. NEMS-based single molecule analysis offers the prospect of direct stratification of generically-heterogeneous mixtures of protein complexes without arduous up-front sample preparation protocols, which will find important applications in analyzing complex protein assemblies, individual organelles and viruses, and membrane proteins.

[1] Alivisatos AP, et al., The Brain Activity Map project and the challenge of functional connectomics. Neuron 74, 970-4 (2012).

[2] Naik, AK, et al., Nature Nanotechnology 4, 445–450 (2009).

[3] Hanay, MS, et al., Nature Nanotechnology, 7, 602-608 (2012).

[4] Hanay, MS, et al., Nature Nanotechnology 10, 339-344 (2015).

4.4

An Accommodative Contact Lens for Presbyopic Correction

Award Type: New Innovator Award

Award Year: 2011

Awardees: Hongrui Jiang, University of Wisconsin-Madison

Presenter: Hongrui Jiang

Presbyopia is the most common ocular affliction and presents an extraordinary public health issue. Our goal is to correct presbyopia by developing a new type of contact lens called an accommodative contact lens (ACL) that incorporates a tunable lens for accommodation and devices to convert light energy to electricity and store it in situ for the operation. We first demonstrate different types of flexible lenses based on electrowetting on dielectrics, dielectrophoretic force, and Fresnel zone plates. These lenses are fabricated onto soft polymers for ultimate integration and embedment into contact lenses. We then report on light energy harvesting devices that can simultaneously achieve storage within the same single device structure. Our approach is to combine capacitive storage with dye-sensitized solar cells (DSSCs). To improve the charge storage capacity, we developed a novel hydrothermal process to prepare porous hierarchically nanostructured tungsten trioxide (WO_3), and then applied WO_3 to fabricate flexible supercapacitors as a storage device. Compared with traditional carbon electrodes, WO_3 nanomaterials significantly enhanced energy storage capability. In order to improve the light-harvesting efficiency of our device, we introduced a light-trapping structure in the photoelectrode via a femtosecond laser ablation technique. The processed photoelectrode was then used to fabricate DSSCs to enhance the photon-harvesting efficiency (η) by up to 13.5%. We also developed an all-optical approach to enhance the photosensitivity of the overall imaging device by realizing micro-scale light concentrators. Lastly, we report on a fabrication platform to integrate the accommodative liquid lens, control electronics, and energy harvesting and storage device into the soft contact lens for presbyopic correction.

5.1

Mapping the Human Toxome by Systems Toxicology

Award Type: Transformative Research Award

Award Year: 2011

Awardees: Thomas Hartung, Johns Hopkins University

Presenter: Thomas Hartung

Technological advances allow high-resolution biological phenotyping of the responses of cells and organisms for the elucidation of mechanisms of toxicity; these include the various omics, high-throughput and high-content technologies. Some of these information-rich tools have the potential to provide a molecular understanding of toxicological mechanisms and are the focal point of our transformative research project. The NIH Project "Mapping the Human Toxome by Systems Toxicology" (NIEHS grant R01ES020750) is a collaboration of Johns Hopkins Bloomberg School of Public Health, Brown University, The Hamner Institute, Georgetown University, U.S. EPA National Center for Computational Toxicology and Agilent; it aims to establish a workflow for the systematic identification and annotation of pathways of toxicity (PoT). A Human Toxome knowledge database and its governance shall be established. This shall represent a point of reference for the toxicology for the 21st century. The project combines untargeted mass spectrum-based metabolomics and gene array-based transcriptomics with bioinformatics. The pilot model is estrogenic response of MCF-7 cells, a well-established test for endocrine disruption.

In this presentation, we cover the recent developments in systems biology and toxicology in terms of computational tools necessary to cope with the flow of information-rich omics datasets. In particular, the bioinformatics tools for pathways of toxicity deduction represent a core deliverable and a real challenge. A number of contributions to quality and standardization of cell systems, omics technologies and bioinformatics will also be addressed. In addition, we developed frameworks and concepts for pathway annotation, validation and sharing. Generally, the presentation will present the general framework on how to use the new toxicity testing paradigm to create a Human Toxome and how this can change the paradigm of establishing safety and risk of substances. This demonstrates the value of systems approaches in toxicology and risk assessment.

5.2

Metabolic Control of Early Mammalian Development

Award Type: Pioneer Award

Award Year: 2012

Awardees: Utpal Banerjee, UCLA

Abstract Author(s): Raghavendra Nagaraj, UCLA

Presenter: Utpal Banerjee

Recent studies point to an essential role for intermediary metabolism in transcriptional control of gene expression. The transcriptional input of metabolism occurs at the level of epigenetic modifications of histone and non-histone proteins in the nucleus and is directed by metabolites derived from the tricarboxylic acid (TCA) cycle. In the mouse pre-implantation embryo, the transcriptional activation of the genome of the zygote (zygotic genome activation (ZGA), has been shown to require global epigenetic changes during 2-cell stage. Interestingly, at this stage   a majority of the mitochondria of the early embryo are inactive and glycolysis is blocked. This is surprising since metabolic quietness seems incompatible with genome-wide remodeling. Embryos are dependent on pyruvate for development beyond 2-cell stage. Our results show that pyruvate is essential for zygotic genome activation and its lack in the culture medium results in global changes in multiple epigenetic marks. We find that a number of enzymes belonging to or associated with the TCA cycle that generate essential metabolites, are transiently localized to the nucleus during ZGA, thus enabling the synthesis of essential metabolites such as acetyl-CoA and alpha-ketoglutarate for global epigenetic reprogramming. These enzymes return to the mitochondrion following ZGA. Our studies further show the nuclear translocation of TCA cycle enzymes in the early embryos is pyruvate dependent, and requires O-linked glycosylation and HSP90 chaperon. Inhibition of OGT, an enzyme which O-glycosylates proteins, recapitulates the phenotypes seen upon pyruvate withdrawal, and prevents the translocation TCA cycle proteins to the nucleus. Finally, nuclear localization of a mitochondrial enzyme associated with the activation of the TCA cycle also showed nuclear localization in human embryos. Instead of the 2-cell stage seen in mouse, this nuclear localization occurred in 4-cell and 8-cell stages. Amazingly, this later stage is coincident with embryonic genome activation in humans. This work has long-term implications for research on obesity related infertility, cancer and stem cell function.

5.3

Extending Caenorhabditis elegans Lifespan by Extending the Duration of Young Adulthood

Award Type: New Innovator Award

Award Year: 2011

Awardees: Michael Petrascheck, TSRI

Abstract Author(s): Sunitha Rangaraju, Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

Presenter: Michael Petrascheck

Interventions that extend lifespan are generally thought to slow the course of aging across the lifetime of an organism. An alternative would be that some longevity mechanisms act only during specific periods of life thus extending lifespan by extending the duration of a specific period. However, as a molecular description of physiological age remains elusive, it has not been possible to distinguish these possibilities. We found that aging causes a profound loss of synchronized gene expression that is evolutionarily conserved from worms to humans. This loss of transcriptional synchrony, which we have termed "transcriptional drift", parallels the age-associated loss of homeostatic capacity and provides a quantifiable measure for physiological age. Measuring transcriptional drift in C. elegans allowed us to dissociate physiological age from chronological age, and revealed that pharmacological inhibition of serotonergic signals extends lifespan by exclusively prolonging the duration of young adulthood. Thus, our results show that aging mechanisms exist that exclusively act during certain periods of time and that lifespan can be extended by specifically extending a period in life.

5.4

Rapafucins, a New Type of Natural Product-Inspired Macrocycles as Chemical Probes and Drug Leads

Award Type: Pioneer Award

Award Year: 2010

Awardees: Jun Liu, Johns Hopkins School of Medicine

Abstract Author(s): Sam Hong, Zufeng Guo, Jingxin Wang, Wukun Liu

Presenter: Jun Liu

The natural products rapamycin along with FK506 and cyclosporine share a unique and extraordinary mode of action. They work by inducing the dimerization of an abundant cellular immunophilin and their respective targets, mTOR or calcineurin. Inspired by these natural products, we have designed a hybrid combinatorial macrocycle library by borrowing the FKBP-binding domain of rapamycin to serve both as a scaffold to present combinatorial peptide and non-peptides and as an embedded universal tag for all macrocycles, and named them rapafucins. We have synthesized a 45,000-compound rapafucin library and screened it for new biological activities in a number of cell- and target-based assays. We have identified potent inhibitors of diverse cellular processes from cell proliferation to signal transduction. Mechanistic deconvolution has revealed distinct molecular targets for some of hits with potential implications in treating a variety of human diseases from ischemic reperfusion injury to cancer. Together, these results suggest that rapafucins may serve as a promising new source of small molecule probes and therapeutic drug leads.

5.5

Chemical Probes for Histidine Kinase Protein Profiling and Inhibitor Discovery

Award Type: New Innovator Award

Award Year: 2011

Awardees: Erin Carlson, University of Minnesota

Presenter: Erin Carlson

Adaptive bacterial signaling is largely regulated by two-component systems (TCSs). Comprised of histidine kinases (HKs) and response regulators (RRs), these phosphorylation cascades result in myriad intracellular responses that include various pathogenic mechanisms. Despite their abundance in Gram-negative, Gram-positive, and Mycobacterial organisms, little is known about global TCS regulation. We are working to address this deficiency by generation of probes to profile HK autophosphorylation activity. Significantly, we have identified the first non-radioactive activity-based probe for HK signaling, BODIPY-FL-ATP-gamma-S, a fluorescent adenosine triphosphate (ATP)-based probe that exploits thiophosphorylation to afford greater stability and thus more facile detection of protein activation. This and other ATP-gamma-S-based probes are in development for the profiling of HK activity from bacterial proteomes. We also seek to inhibit TCS signaling networks and thereby severely attenuate bacteria's ability to cause infection. We have targeted the highly conserved ATP-binding domain present across the HK superfamily. An ADP-based probe enabled us to pursue new inhibitor scaffolds through the execution of a high-throughput screen of ~75,000 diverse small molecules and natural product extracts. As a result, nine lead compounds have been identified for deactivation of multiple HKs. Scaffolds identified in these studies contribute to the foundation for HK inhibitor development, supporting a new class of antibiotics with a novel mechanism of action. Together, our chemical probes will continue to advance our current understanding of global HK regulation and inhibition.

6.1

Improving Resilience to Infectious Diseases

Award Type: Pioneer Award

Award Year: 2011

Awardees: David S. Schneider, Stanford

Presenter: David S. Schneider

Our goal is to increase the resilience of infected patients to improve both their chances and rates of recovery. We plan to limit the severity of infectious diseases by improving the balance between pathogenesis and recovery mechanisms, which will lead to treatments that limit antibiotic use. In a typical infection, host parameters (for example pathogen load, immune cells, health) rise and fall in a defined order. Though this is simple to study in model organisms, one difficulty we have in assessing patients is that we need to diagnose and treat individuals when they enter the clinic and can't measure these time series. We reasoned that we could reconstruct the paths patients take through "disease space" if we plotted health parameters against each other instead of against time; by choosing parameters that were out of phase with each other we could identify looping disease curves, which provides two insights:

1. Loops serve as maps where each point uniquely plots a patient's progress along the disease path. These loops let us order cross sectional data so that we can compare patients at the same stage of infection rather than lumped together. We've developed methods to measure deviations from these paths that identified at risk individuals and those with extreme resilience. We demonstrate this using a mouse-malaria model to identify factors leading to increased pathology. By applying these techniques to human infections to we identified resilient individuals.

2. Patients move in a unidirectional manner through these disease maps, suggesting that there are checkpoints; once a patient passes a checkpoint, they have to complete the entire infection cycle. By identifying these checkpoints we hope to be able to short circuit the disease process to limit illness. We demonstrate that by avoiding the first checkpoint in a model malaria infection, mice recover without ever becoming sick.

These approaches provide a foundation for new diagnostic visualizations and analyses that will improve our ability to improve the outcomes of infections.

6.2

From Zebrafish to Humans: Reprogramming the Host Response to Tuberculosis

Award Type: New Innovator Award

Award Year: 2011

Awardees: David Tobin, Duke University School of Medicine Department of Molecular Genetics and Microbiology,

Presenter: David Tobin

Pathogenic mycobacteria, including Mycobacterium tuberculosis, induce formation of characteristic immune aggregates called granulomas. Using a zebrafish-mycobacterial infection model, we have defined host processes, including granuloma formation, angiogenesis and modulation of host eicosanoid balance, that influence disease outcome. Using intravital microscopy in the transparent larval zebrafish, we show that granuloma formation is intimately associated with angiogenesis. The initiation of angiogenesis in turn coincides with the generation of local hypoxia and transcriptional induction of the canonical pro-angiogenic molecule VEGFA. Pharmacological inhibition of the VEGF pathway suppresses granuloma-associated angiogenesis, reduces infection burden and limits dissemination. Moreover, anti-angiogenic therapies synergize with the first-line anti-tubercular antibiotic rifampicin as well as with the antibiotic metronidazole, which targets hypoxic bacterial populations. Our data suggest that mycobacteria induce granuloma-associated angiogenesis, which promotes mycobacterial growth and increases spread of infection to new tissue sites. These findings suggest that anti-angiogenic agents, now being used in cancer regimens, may provide a new approach to treating TB. In addition, we have identified other host pathways that mediate granuloma formation in the zebrafish that we show are conserved in human granulomas during infection with Mycobacterium tuberculosis.

6.3

Reviving the Apicoplast as an Anti-Malarial Drug Target

Award Type: Early Independence Award

Award Year: 2012

Awardees: Ellen Yeh, Stanford Medical School

Abstract Author(s): Katie Amberg-Johnson,

Presenter: Ellen Yeh

The discovery of a non-photosynthetic plastid, the apicoplast, in Plasmodium spp parasites and other pathogenic protozoa promised the rapid identification of new parasite-specific drug targets in this unique organelle. In the intervening 20 years, the initial excitement has been tempered by the reality and challenges of drug discovery. A subset of apicoplast inhibitors that target housekeeping functions result in a peculiar "delayed death" phenotype with resultant slow drug onset. The apicoplast was also found to have very limited metabolic function during symptomatic blood-stage Plasmodium infection. These features significantly limit the utility of known drug targets in the apicoplast. Meanwhile, new apicoplast drug targets are difficult to validate given the significant gaps in our knowledge of organelle biology. To overcome these challenges, we developed a novel chemical rescue screen that opens new avenues for identification of specific apicoplast inhibitors and validation of apicoplast drug targets. We used this chemical rescue screen to identify a new inhibitor with a novel mode of action targeting apicoplast biogenesis.

6.4

Natural Pseudotyping of HIV-1 Facilitates Infection of Female Primary Genital Tract Epithelial Cells Promoting Vaginal Transmission

Award Type: Pioneer Award

Award Year: 2011

Awardees: James E.K. Hildreth, PhD, MD, Meharry Medical College University of California, Davis,

Abstract Author(s): James E.K. Hildreth, Meharry Medical College, University of California, Davis

Presenter: James E.K. Hildreth, PhD, MD

The devastating spread of HIV in young females in some countries is out of proportion to the overall risk of infection based on well characterized factors. There is an urgent need to identify the additional factors which may be driving sexual transmission of the virus. The regions with high prevalence of HIV-1 are often also highly endemic for the human T-lymphotropic virus 1(HTLV-1). Here, we demonstrated that co-infection of HIV-1 and HTLV-1 in CD4+ T lymphocytes resulted in occurrence of HTLV-1 glycoproteins pseudotyped HIV-1 (a process we called natural pseudotying), which was able to directly infect primary female lower genital tract (FLGT) epithelial cells via both cell-free and cell-associated infection. The HIV-1 infection was neutralized by antibodies against HTLV-1 envelope protein (Env) but not by the antibody against HIV-1 Env, confirming that HIV-1 infection of FLGT epithelial cells was mediated through HTLV-1 Env. Active HIV-1 replication in FLGT epithelial cells was blocked by HIV protease inhibitors and RT inhibitors. However, in cells co-infected by both HIV-1 and HTLV-1, RT inhibitors did not block HIV-1 replication. The infected FLGT epithelial cells were able to spread HIV-1 to natural target T cells or to uninfected epithelial cells via additional rounds of natural pseudotyping. We have obtained evidence that natural pseudotyping also occurs when HIV-1 co-infects T cells with HTLV-2 as well. Our results support the hypothesis that natural pseudotyping of HIV-1 by HTLV-1 and HTLV-2 could result in direct infection genital tract epithelial cells thereby drastically increasing the likelihood of sexual transmission in women. Furthermore, our results suggest that treatment for HIV-1 in persons co-infected with HTLV-1 could be ineffective due to resistance to RT inhibitors. This phenomenon could also confound efforts to prevent virus spread through treatment of infected individuals.

6.5

Exploiting Social Interaction in New Therapies against Pathogenic Bacteria

Award Type: New Innovator Award

Award Year: 2015

Awardees: Joao Xavier, Memorial Sloan Kettering Cancer Center

Presenter: Joao Xavier

Antibiotics are our main line of defense against bacterial infections but antibiotic use faces a global threat of emerging resistance. Understanding how bacteria interact with each other within communities can open new therapeutic avenues that exploit these social interactions against pathogens. Enabled by the New Innovator Award, my laboratory has made important findings on microbial social evolution, an exciting new field that is shedding light on the fascinating social lives of microbes. In this talk I will describe how we dissect the molecular mechanisms underlying a bacterial decision to cooperate within communities using novel computational and experimental tools. I will explain how we are applying the same concepts to investigate social interaction in the gut microbiome, and how that enables finding new microbial mechanisms of resistance against host colonization by clinically-relevant pathogens. We investigate the detrimental effects of antibiotics on the gut microbiome of bone marrow transplant patients, revealing key microbes and interactions that protect against infection by Clostridium difficile, a rising microbial threat.

Session 7

7.1

Harnessing Neuroplasticity with Computer-Presented and Physical Brain-Training Exercises: Medical and Educational Outcomes

Award Type: Transformative Research Award

Award Year: 2011

Awardees: Bruce E. Wexler, Yale University; James Leckman, Yale University,

Presenter: Bruce E. Wexler

ADHD affects 5% of children with immediate consequences of poor school performance, low self-esteem and problematic family and social relationships, and long term consequences of dropping out of school, under and unemployment, drug abuse and physical accidents. Available pharmacotherapy is palliative rather than curative, has limited long term benefits and is associated with problematic side effects in some children. Brain imaging shows that in children with ADHD attention regulating systems in the temporal and parietal lobes and prefrontal cortex develop more slowly and less fully, and activation in these areas during executive function tasks is similar to that in younger typically developing children. These underdeveloped brain regions, functional neural systems, and associated compromised executive cognitive functions, provide a neurocognitive target for brain exercises designed to harness neuroplasticity and promote activity-dependent enhancement of neural development and function. We created computer-presented brain exercises to activate the neural target substrate through multiple cognitive operations that together are called Executive Functions. The programs have 100s of difficulty levels and real-time data-driven individualization of training to optimize the balance between reward and challenge. We created a parallel program of physical exercises with cognitive demands that activate the same neural target but in the context of whole body activity and social interaction. The computer and physical exercises were done in school settings, and we added an established classroom group-level intervention to help create an environment conducive for the computer and physical exercises, and itself further engage executive process of self-regulation. This multi-dimensional program constituted an intensive intervention for a neurodevelopmental disorder during the period of active neurodevelopment. Although the originally planned ANOVA on half the data set (i.e., excluding the data from controls who then received the intervention) showed no significant effects of the intervention, variance in outcome scores was very high in both active and control subjects indicative of the sensitivity of symptoms to many life factors and heterogeneity of the symptom-based diagnostic category. In exploratory analyses we found evidence suggesting that a subset of 25-50% of the children with ADHD improve both in clinical symptoms and cognitive function from the intervention. Moreover, responders differed significantly from non-responders in clinical characteristic at study entry. In other work during the study period with this same approach, we found strong gains in cognitive function and math and reading achievement in large samples of school children, and recovery in 80% of geriatric depressed patients who had failed to respond to medication.

7.2

Neural Circuits Underlying Operant Learning in Larval Zebrafish

Award Type: Pioneer Award

Award Year: 2011

Awardees: Florian Engert, Harvard

Presenter: Florian Engert

During operant conditioning, animals learn to respond to stimuli with actions that lead to favorable outcomes. Recordings in mammals have uncovered neural signals that link stimulus, action, and outcome, but a limited number of recording sites have hindered the comprehensive discovery of learning signals. Here, we use brain-wide functional imaging in larval zebrafish to screen more than 100,000 neurons while animals learn to terminate a heat stimulus with a directional tail movement. We identify neurons comprising two major classes: class 1 consists of action-selective neurons that encode the direction of heat-evoked tail movements seconds before and after their execution. Class 2 consists of neurons that encode outcome prediction and prediction error. This class includes both positive relief prediction signals that are enhanced by learning, and negative relief prediction signals that are suppressed by learning. These positive and negative relief prediction signals not only have opposing patterns of activity but are also found on opposing sides of the habenula. Strikingly, both outcome-predictive and action-selective signals are correlated with natural variability in learning performance across animals. This study provides the first comprehensive survey of the neural dynamics during learning, suggests that lateralized neuronal activity contributes to operant conditioning, and raises novel hypotheses about the roles of action-selective and outcome-prediction neurons.

7.3

Manipulating Memory through Cholinergic Signaling in the Brain

Award Type: Pioneer Award

Award Year: 2010

Awardees: Lorna W. Role, SUNY Stony Brook

Presenter: Lorna W. Role

Emotionally salient experiences - both positive and negative – potently affect the memories we make and how we recall these memories. Healthy individuals "learn from experience" - incorporating events into memory in a manner that permits ensuing recall in a context and/or cue relevant fashion. Conditions of neuropsychiatric vulnerability or disease severely alter the acquisition and recall of salient experiences. Affected individuals may be unable to attend to, engage in or recollect emotionally significant occasions (e.g. as in Alzheimer's disease) while others experience events in a manner that results in highly distorted recall (e.g. PTSD). Acetylcholine plays a critical, but poorly understood role in this process. My DP1 was to test the efficacy of manipulating cholinergic signaling on memory formation and retention.

We first examined the contribution of endogenous cholinergic signalling to the acquisition and extinction of fear-related memory by optogenetic labelling and photo-stimulation of cholinergic input to the basal lateral amygdala (BLA). Stimulation of cholinergic terminal fields within the BLA in awake-behaving mice during training in a cued fear-conditioning paradigm slowed the extinction of learned fear. Inhibition of cholinergic activity during training reduced the acquisition of learned fear behaviours, rendering the animals essentially fearless. Circuit mechanisms underlying the behavioural effects of cholinergic signalling in the BLA were assessed by in vivo and ex vivo electrophysiological recording and, most recently, by engram mapping. Overall, our studies support an essential role of cholinergic modulation of BLA circuits in the inscription and retention of fear memories.

7.4

Hypothalamic AgRP Neurons are Determinants of Healthy Lifespan and Higher Brain Functions

Award Type: Pioneer Award

Award Year: 2010

Awardees: Tamas Horvath, Yale University

Presenter: Tamas Horvath

Results generated in support with the NIH Director's Pioneer award unmasked that the hypothalamus is a key regulator of the adaptation of the central nervous system (CNS) to the changing environment in support of survival. Specifically, a subset of hypothalamic neurons responsible for hunger were found to act as upstream regulators of brain regions classically considered as master determinants of CNS function, such as the cortex and hippocampus, while also controlling peripheral tissue functions, including the immune system and the bone. This novel regulatory role of these hypothalamic neurons in coordinating complex behaviors with autonomic functions mandates that the conceptual framework to understand impairment of brain functions and that of peripheral tissues need reconsideration to better serve the sick.

7.5

Engineered Perception of Complex Bionic Hand Movements through Kinesthetic Illusions

Award Type: Transformative Research Award

Award Year: 2013

Awardees: Paul D. Marasco, Cleveland Clinic

Abstract Author(s): Paul D. Marasco, Laboratory for Bionic Integration, Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue / ND20, Cleveland, OH USA 44195, Advanced Platform Technology Center of Excellence, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, 10701 East Boulevard 151 W/APT, Cleveland, OH USA 44106

Presenter: Paul D. Marasco

As humans, we have an innate ability to interact with our environment and navigate our surroundings with grace and fluidity. Spatial awareness is the cornerstone of purposeful movement yet individuals with amputation are denied this sense when using artificial limbs. Kinesthesia, the sense of body movement, allows us to feel the activity of our extremities without looking at them. A principal reason older cable-actuated prosthetic split-hook grippers and body-powered elbows still see widespread use is because joint movements can be intuitively felt through the cable system. In comparison, motorized prosthetic arms and hands lack meaningful sensory feedback and must be carefully watched at all times to perform even simple tasks, much to the detriment of efficient control or multi-tasking. The inability to provide useful movement feedback is a major roadblock to effective prosthetic limb control and prevents full realization of the impressive advancements made towards a new generation of advanced dexterous robotic prostheses. Here, we move prosthetic feedback into a new perceptual cognitive framework and provide physiologically relevant active kinesthetic sensation for advanced bi-directionally integrated dexterous prosthetic hands. We show that it is possible to engineer perception of specific illusionary hand movements with functionally relevant grip conformations for human amputees. This was accomplished by reassigning the sensory-neural structure of the remaining post-amputation musculature and projecting hand conformation specific perceptual movement illusions to the missing hand. We then systematically generated active complex synergistic cognitive grip-conformation percepts and mapped them directly to the movement of commercially-available dexterous robotic prosthetic hands; thus providing simultaneous real-time volitional motor control and kinesthetic sensory feedback for prosthetic limbs.

8.1

The Nuclear Periphery Acts as a Regulator of Recombinatorial Potential

Award Type: New Innovator Award

Award Year: 2011

Awardees: Megan C. King, Yale School of Medicine

Presenter: Megan C. King

Repetitive regions of the genome are prone to recombination and thus can lead to genome instability. However, such recombination reactions can also drive molecular diversity and adaptation. Little is known about cellular mechanisms that control the recombinatorial potential of repetitive DNA. Since many repetitive DNA elements are preferentially associated with the inner nuclear membrane, we hypothesized that their association with the nuclear periphery may serve as an input to genome stability by regulating the likelihood that recombination occurs. To test this concept, we examined recombination in the repeat-rich cell surface adhesin genes of the fission yeast, S. pombe. This gene family is repetitive intragenically (repeat modules encode a repetitive peptide domain) and intergenically (related genes reside at distinct genomic loci). We show that the adhesin genes are associated with the nuclear envelope; this association is dependent on the function of proteins bound to proximal transposons and LTRs. Using assays designed to measure intragenic and intergenic recombination, we find that recombination rates are sensitive to internal repeat number but not transcriptional status. Further, disrupting their nuclear envelope tethers weakened the association of adhesin genes with the nuclear periphery and led to an increase in recombination rate. Using live-cell microscopy, we find that factors that promote strand invasion (Rad51 and Rad52) are displaced from processed DNA double-strand breaks specifically at the nuclear periphery, leading to repair by single-strand annealing and alternative pathways. Importantly, inducing cell stress through a variety of mechanisms leads to the release of adhesin genes from the nuclear periphery and the concomitant increase in recombination. Take together, our data suggest that the recombinatorial potential of repetitive elements can be tuned by their nuclear compartmentalization, which may provide a mechanism to balance genome integrity with adaptation in response to a changing environment. This fundamental mechanism may also contribute to pathogen evasion of the host immune system.

8.2

Reading and Writing Genomes in 3-D: Hacking the CTCF Code

Award Type: New Innovator Award

Award Year: 2011

Awardees: Erez Lieberman Aiden, Baylor College of Medicine Rice University,

Presenter: Erez Lieberman Aiden

Stretched out from end-to-end, the human genome – a sequence of 3 billion chemical letters inscribed in a molecule called DNA – is over 2 meters long. Famously, short stretches of DNA fold into a double helix, which wind around histone proteins to form the 10nm fiber. But what about longer pieces? Does the genome's fold influence function? How does the information contained in such an ultra-dense packing even remain accessible?

In this talk, I describe our work developing 'Hi-C' (Lieberman-Aiden et al., Science, 2009; Aiden, Science, 2011) and more recently 'in-situ Hi-C' (Rao & Huntley et al., Cell, 2014), which use proximity ligation to transform pairs of physically adjacent DNA loci into chimeric DNA sequences. Sequencing a library of such chimeras makes it possible to create genome-wide maps of physical contacts between pairs of loci, revealing features of genome folding in 3D.

Next, I will describe recent work using in situ Hi-C to construct haploid and diploid maps of nine cell types. The densest, in human lymphoblastoid cells, contains 4.9 billion contacts, achieving 1 kb resolution. We find that genomes are partitioned into contact domains (median length, 185 kb), which are associated with distinct patterns of histone marks and segregate into six subcompartments. We identify ~10,000 loops. These loops frequently link promoters and enhancers, correlate with gene activation, and show conservation across cell types and species. Loop anchors typically occur at domain boundaries and bind the protein CTCF. The CTCF motifs at loop anchors occur predominantly (>90%) in a convergent orientation, with the asymmetric motifs "facing" one another.

Next, I will discuss the biophysical mechanism that underlies chromatin looping. Specifically, our data is consistent with the formation of loops by extrusion (Sanborn & Rao et al., PNAS, 2015). In fact, in many cases, the local structure of Hi-C maps may be predicted in silico based on patterns of CTCF binding and an extrusion-based model.

Finally, I will show that by modifying CTCF motifs using CRISPR, we can reliably add, move, and delete loops and domains. Thus, it possible not only to "read" the genome's 3D architecture, but also to write it.

8.3

The Spatial Organization of Transcription

Award Type: New Innovator Award

Award Year: 2011

Awardees: Arjun Raj, University of Pennsylvania

Presenter: Arjun Raj

Nuclear organization and chromosome structure is thought to influence transcription, but it has been difficult to measure this relationship directly. Using multiplex in situ hybridization techniques, we show that chromosomal translocations can influence transcription at the whole chromosome level, and show evidence for long-range transcriptional interactions. We also show by direct visualization that long non-coding RNAs can influence transcription locally by interacting with nearby genes. We end with some preliminary data using expansion microscopy to allow us to visualize the details of transcriptional foci.

8.4

In Situ Imaging of Genome & Transcriptome in Single Cells

Award Type: Transformative Research Award

Award Year: 2010

Awardees: Xiaowei Zhuang, Harvard University Howard Hughes Medical Institute; Sunney Xie, Harvard University

Presenter: Xiaowei Zhuang

Transcriptome-wide analyses of the abundance and spatial organization of RNAs in single cells promise to transform our understanding in many areas of biology, such as the mechanism of gene regulation, the heterogeneous behavior of cells, and the development and maintenance of cell fate. Single-molecule imaging allows for counting and mapping of RNA molecules in individual cells; however, the number of RNA species that can be simultaneously imaged has been limited. To overcome this challenge, we recently developed multiplexed error-robust fluorescent in situ hybridization (MERFISH)1, which allows numerous RNA species to be quantified in a spatially resolved manner in single cells in situ. This massively multiplexed imaging approach enables unique analyses based on the variation and correlation of copy numbers and spatial distributions of a large number of RNA species within single cells, facilitating the delineation of gene regulatory networks and in situ identification of cell types. In this talk, I will present the technology development and applications of MERFISH1.

I will also present a super-resolution study of the three-dimensional (3D) structure of genome in single cells2. Metazoan genomes are spatially organized at multiple scales, from individual nucleosomes to whole chromosome territories. At the intermediate scale that encompasses the sizes of genes, gene clusters and gene regulatory domains, the 3D organization of DNA is implicated in many regulatory mechanisms, but understanding this organization remains a major challenge. We used 3D super-resolution imaging to determine the structural organization of chromatin in three major epigenetic states, transcriptionally active, constitutively inactive, and Polycomb-repressed states, and obtained structural information that are not previously obtainable by conventional imaging or biochemical approaches. Our super-resolution chromatin images revealed both common principles of chromatin folding and striking differences in the structural organization of chromatin in different epigenetic states with direct implications on gene regulation2.

References:

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2. A. N. Boettiger, B. Bintu, J. R. Moffitt, S. Wang, B. J. Beliveau, G. Fudenberg, M. Imakaev, L. A. Mirny, C. -t. Wu, X. Zhuang. Super-resolution imaging reveals distinct chromatin folding for different epigenetic domains. Nature (in press).

Poster Presentation Abstracts

1.1

Regulatory Ribosomal Ubiquitylation Communicates Protein Homeostasis Stress to the Translation Machinery

Award Type: New Innovator Award

Award Year: 2015

Awardees: Eric Bennett, University of California San Diego

Abstract Author: Eric Bennett, UCSD

Presenter: Eric Bennett

Maintaining proper protein homeostasis upon genetic and environmental challenges is a critical cellular function. Defects in protein homeostasis have been implicated in a number of human aging-related pathologies ranging from cancer to neurodegenerative disease. The ubiquitin-proteasome system (UPS) assists in the destruction of misfolded, misassembled, and improperly translated proteins. The importance of this quality control (QC) function is highlighted by the observation that 10-30% of newly synthesized proteins undergo UPS-dependent degradation. Misfolded and poorly translated proteins that accumulate upon aging act to decrease the fidelity of the already error-prone translation reaction and impair protein degradation capacity. The age-dependent accumulation of burdensome QC products also typifies many cancers that display elevated protein synthesis rates. The accumulation of genetic alterations observed during carcinogenesis further contributes to protein homeostasis dysfunction, thus exposing a targetable vulnerability within tumors. Taken together, defining and designing molecular strategies aimed at either limiting the production of erroneous translation products or elevating protein quality control capacity has the potential to transform our ability to combat a wide array of aging related disorders. Towards this goal, we have employed quantitative proteomic technologies to identify critical targets of the ubiquitin proteasome system upon challenges to protein homeostasis. Surprisingly, we have found that regulatory, non-degradative ubiquitylation is widespread and the vast majority of the targets for this non-canonical modification are completely uncharacterized. Our recent research has shown that the ribosome and other components of the translation machinery are among the most prevalent cellular targets for non-degradative ubiquitylation. We have demonstrated that a subset of proteins within the small 40S ribosomal subunit become ubiquitylated in a site-specific manner in response to protein homeostasis stress. These ubiquitylation events are conserved from yeast to man and are required for cellular resistance to proteotoxic stress. Regulatory ribosomal ubiquitylation (RRub) represents a new axis of translation control that can be leveraged and examined within the context of protein homeostasis disorders.

1.2

Increasing Healthspan by Rapid and Transient Telomere Extension

Award Type: Transformative Research Award

Award Year: 2012

Awardees: Helen M. Blau, Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine

Abstract Author(s): Helen M. Blau, Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine

Presenter: Helen M. Blau

Short telomeres are implicated in age-related and genetic disorders including cardiovascular disease, immunosenescence, and diabetes. Telomeres comprise repetitive TTAGGG sequences at chromosome ends and are essential for the preservation of genome integrity. Short telomeres can result in genomic instability and DNA damage response resulting in senescence or apoptosis. Telomeres are extended by the enzyme complex comprising telomerase reverse transcriptase (TERT), mutations in which are found in a spectrum of diseases, the telomeropathies. We have shown that short telomeres underlie the fatal cardiomyopathy of Duchenne muscular dystrophy (1,2). Telomere extension has been proposed as a means to improve cell culture and tissue engineering, and to treat disease. However, telomere extension by non-viral, non-integrating methods remains inefficient and viral delivery can lead to immortalization. Our goal is to extend telomeres using a nucleoside-modified mRNA that is advantageous therapeutically as it is transient and minimally immunogenic. We have determined that delivery of modified mRNA encoding TERT to diverse human cell types increases telomerase activity transiently (24-48 h) and rapidly extends telomeres (3, 4). Successive transfections over four days extended telomeres up to 0.9 kb in a cell type-specific manner conferring up to 28±1.5 additional population doublings, and an additional transfection after a refractory period conferred as much proliferative capacity as the first transfection. There is a major unmet need for methods to deliver modified mRNA in a targeted manner in vivo. We have tested a broad range of existing nucleic acid delivery vehicles and methods for their ability to deliver modified mRNA in mice, including nanoparticles, cationic lipids, HDL, sonoporation, and hydrodynamic injection with little success. Recently, we have made substantial progress in developing exosomes as delivery vehicles. Exosomes are 50-150 nm vesicles that naturally carry mRNA between cells in a targeted manner, cross biological membranes, and release their contents directly into the cytoplasm, avoiding the lysosomal degradative pathway. Our transient telomere extension technology is enabling unprecedented augmentation of cell proliferative capacity. Additionally, targeted exosomes loaded with biomolecules represent a fundamental advance that will enable delivery of modified mRNA for telomere extension and other therapeutic applications.

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(2) Mourkioti, F. et al. Role of telomere dysfunction in cardiac failure in Duchenne muscular dystrophy. Nat. Cell Biol. 15, 895–904 (2013).
(3) Ramunas, J. et al. "Transient delivery of modified mRNA encoding TERT rapidly extends telomeres in human cells." The FASEB Journal 29.5 (2015): 1930-1939.
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1.3

Epigenetic Regulation of Social Behavior in Ants

Award Type: New Innovator Award

Award Year: 2014

Awardees: Roberto Bonasio, University of Pennsylvania

Presenter: Roberto Bonasio

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. As a first step, we obtained gene expression profiles for Harpegnathos brains before, during, and after their transition to queen status and identified key regulatory genes. In addition, we have exploited the fact that any individual, regardless of caste, has reproductive potential, to develop technologies that will establish Harpegnathos as a true genetic model system for the investigation of social behavior in a tractable organism.

1.4

Trans-Generational Effects of Social Learning

Award Type: Pioneer Award

Award Year: 2015

Awardees: Giovanni Bosco, Geisel School of Medicine at Dartmouth Department of Genetics,

Presenter: Giovanni Bosco

Can the social experiences of parents be inherited by their offspring? One of the most exciting findings of modern molecular genetics has been that the information encoded in our DNA cannot completely explain heritability of complex traits. This "missing heritability" is now being intensely studied in every living system. At the same time we are now rediscovering how a mind-body connection through which cognitive experiences can have profound effects on physiology and health. However, the possibility that social cognitive experiences, or state-of-mind, can contribute to heritability is relatively unexplored at the level of molecular genetics. In social animals, groups of individuals share information about the environment and about themselves, and these social interactions also can result in dramatic physiological changes. Because the physiological consequences of social interactions can be long-lasting it raises the exciting possibility that socially learned behaviors affecting the mind become epigenetically embedded and could result in trans-generational effects. How social experiences epigenetically reprogram germline cells in order to transmit information to subsequent generations is terra incognita. Using the Drosophila fruit fly model this project seeks to elucidate the molecular and neurogenetic mechanisms of how social experiences result in inheritance of specific behaviors through multiple generations. Epigenetic regulation of social behavior in ants

1.5

Hunting Viral Receptors Using Haploid Cells

Award Type: New Innovator Award

Award Year: 2012

Awardees: Jan Carette, Stanford University

Abstract Author(s): Pillay, S., Stanford University

Presenter: Jan Carette

Viruses have evolved intricate mechanisms to gain entry into the host cell. Identification of critical receptors has enabled insights into virus particle internalization, host and tissue tropism, as well as viral pathogenesis. We have developed the use of forward genetic screens in human haploid cells for virus receptor discovery. An attractive target for this approach is adeno-associated virus (AAV) because it is the leading vector for gene therapy applications and its receptor has proven to be elusive using traditional methods. Here we use an unbiased, haploid genetic screen to identify critical players in AAV serotype 2 (AAV2) infection. The most significantly enriched gene of the screen encoded an uncharacterized type-I transmembrane protein. We characterize this as a protein capable of rapidly endocytosing from the plasma membrane and trafficking to the trans-Golgi network. We show that it directly binds to AAV2 particles and that antibodies efficiently block AAV2 infection. Moreover, genetic ablation of this gene renders a wide range of mammalian cell types highly resistant to AAV2 infection. The importance of this gene for in vivo gene delivery is further underscored by the robust resistance of knockout mice to AAV infection. Collectively, the data indicate that we identified a universal receptor involved in AAV infection.

1.6

An Input-Output Relation between RNA Polymerase II Clustering and Gene Output in Living Cells

Award Type: New Innovator Award

Award Year: 2014

Awardees: Ibrahim Cissé, MIT

Presenter: Ibrahim Cissé

We developed a live cell super-resolution approach to uncover the correlation between mRNA synthesis and the dynamics of RNA Polymerase II (Pol II) clusters at a gene locus. For endogenous β-actin genes in mouse embryonic fibroblasts, we observe that short (~8 s) Pol II clusters correlate with basal mRNA output. With serum stimulation, a stereotyped increase in Pol II cluster lifetime correlates with the proportionate increase in mRNAs synthesized minutes later. An additional burst of mRNA synthesis can be induced, at will, with a drug that stalls then releases Pol II clustering. Our findings unveil that transient clustering of Pol II constitutes a pre-transcriptional regulatory event which dynamically controls gene expression output.

1.7

Suppression of Mitotic Holliday-Junction Resolvases Promotes Crossover Assurance in Mouse Meiosis

Award Type: New Innovator Award

Award Year: 2015

Awardees: Francesca Cole, University of Texas MD Anderson Cancer Center

Presenter: Francesca Cole

Meiotic recombination in mammals is induced by a large number of programmed DNA double-strand breaks. Each break is repaired as either a crossover (CO), which involves exchange of chromosome arms, or a noncrossover (NCO), which is a patch-like repair. Recombination leading to both NCOs and COs promotes homolog pairing and COs are required to physically tether homologs to ensure accurate chromosome segregation. Multiple pathways are thought to generate COs and NCOs, however, investigation of the crosstalk between these pathways is stymied by the difficulty of NCO detection. To gain a more sophisticated perspective of mammalian recombination mechanisms, we combine cytological techniques to detect recombination intermediates with comprehensive, analysis of recombination outcomes at highly polymorphic hotspots. We have previously shown that NCOs are ten-fold more frequent than COs at the A3 hotspot and this ratio mirrors the global break to CO frequency. However, A3 is located on Chr1, the longest in the mouse genome. By contrast, we report that recombination at the 59.5 hotspot, which is located on the shortest autosome, is highly enriched for COs resulting in a 1:1 ratio of NCOs to COs. To investigate how CO frequency is enriched at 59.5, we examine the dependence of recombination on the crossover-specific Holliday-junction resolvase complex, comprised of the endonuclease MutLγ (MLH1/MLH3). In the absence of MLH3 and similar to published reports, COs are dramatically down regulated at A3 and are formed at the frequency expected based upon the number of chiasmata observed in mice lacking MutLγ activity. Holliday-junction resolvases that also act in mitosis are thought to generate the MLH3-independent COs. In marked contrast to A3, CO recombination at 59.5 is entirely dependent upon MLH3. However, this requirement for MLH3 is not absolute and during juvenile meiosis, COs are formed at the expected frequency. These results suggest that mitotic resolvases, which likely generate a substantial fraction of NCOs, are actively suppressed at 59.5 during adult meiosis. As such, suppression of mitotic resolvases works to promote CO formation at the expense of NCOs. Consistent with this hypothesis, juvenile mice have fewer MLH1 foci that mark sites of MutLγ-dependent COs and subsequent reduction in crossover assurance. Further, we determine that resolvase suppression is dependent upon testosterone responsiveness and is likely the root cause of the long known, but little understood increased risk of aneuploidies in children of pubescent males.

1.8

Immune Regulation of Early Carcinogenesis

Award Type: Early Independence Award

Award Year: 2015

Awardees: Shadmehr Demehri, Massachusetts General Hospital

Abstract Author(s): Trevor Cunningham, Massachusetts General Hospital

Presenter: Shadmehr (Shawn) Demehri

The field of cancer immunology has made substantial advances in recent years by deciphering the role of the tumor infiltrating CD8+ cytotoxic T lymphocytes (CTLs) in attacking cancer cells, which have led to promising new cancer immunotherapeutics. The current immunotherapeutic approaches, however, are largely designed to boost the anti-tumor immune response that has already formed against late-stage metastatic cancers. Therefore, the current cancer immunotherapies like immune checkpoint blockade, which rely on a pre-existing CTL infiltrate in the tumor for their effects, are proven ineffective to treat cancers that frequently lack a significant anti-tumor immune infiltrate, especially during the early in-situ phases of their development. In order to expand the potential of cancer immunotherapy, our laboratory studies the pathways that lead to immune system activation against early phases of cancer development. Devising a mechanism to activate the immune system against early-stage cancers has clear immunopreventive implications by directly blocking the cancer promotion and immunotherapeutic benefits by potentiating the immunity against late disease.

1.9

Using a Disease-Affected Cell to Synthesize Its Own Drug

Award Type: Pioneer Award

Award Year: 2015

Awardees: Matthew Disney, The Scripps Research Institute

Presenter: Matthew Disney

One of the major challenges in biomedical research is to leverage advances in genome sequencing into lead therapeutic modalities to treat human disease. This precision medicine approach holds great promise to not only advance patient-specific therapeutics, but to provide highly selective chemical probes of function to study disease biology. We describe an innovative precision therapeutic approach to custom synthesize highly selective and potent lead therapeutics in only disease-affected cells and tissues by using a disease-causing gene product as a catalyst. That is, the disease-affected cell serves as a reaction vessel and a disease-causing RNA as a catalyst to allow for the synthesis of its own treatment. This is in contrast to traditional precision medicine approaches in which both healthy and disease-affected cells are exposed to the therapeutic, potentially causing toxicity due to binding off-targets. This technology will be applied to develop compounds to treat and study microsatellite disorders that affect millions of people worldwide and have no known cure. We describe application of these approaches towards an incurable form of muscular dystrophy, myotonic dystrophy.

1.10

Progress towards Essential Gene Discovery in the Malaria Parasite Plasmodium falciparum

Award Type: New Innovator Award

Award Year: 2013

Awardees: Jeffrey Dvorin, Boston Children's Hospital, Harvard Medical School,

Presenter: Jeffrey Dvorin

Human malaria is a leading cause of death and disease worldwide, resulting in nearly one million deaths each year. The most severe forms of malaria result from infection by the *Plasmodium falciparum* parasite, which causes the vast majority of malaria in Africa. A molecular understanding of the fundamental biological process of *P. falciparum* replication will provide the necessary tools to develop new anti-malarial therapeutics. Although the genome of P. falciparum has been fully sequenced, the function of more than half of the 5,300 genes in the parasite remains unknown. Many of the genes with unknown function have little or no homology with characterized genes from other organisms. Therefore, existing molecular genetic and bioinformatics techniques cannot be used to efficiently determine the function of many of the genes in the parasite. Furthermore, existing technologies cannot predict which genes are essential for survival of the parasite. We hypothesize that these essential genes, and the proteins that they encode, will be attractive targets for the rational design of new anti-malarial therapeutics.

A forward-genetic system to investigate the function of essential genes does not exist currently. We are developing two forward-genetic methods to identify which genes are essential for the blood-stage *of P. falciparum* and to functionally characterize these essential genes. In our first technique, we are refining an inducible transposon system to allow saturating mutagenesis of the parasite genome. This technique will produce a list of essential genes, but is unlikely to provide the function of these genes. In our second method, we have developed a robust high throughput system to identify temperature-sensitive mutations in essential parasite genes. We are able to screen nearly 10,000 individual clones per attempt. The long-term objectives and public health implications of these studies are to identify novel targets for new anti-malarial therapeutics. This long-term goal will be achieved as a direct result of our identification of novel essential genes in *P. falciparum* parasites.

1.11

Engineering Robust Ionotropic Activators for Brain-Wide Manipulation of Neurons

Award Type: New Innovator Award

Award Year: 2015

Awardees: Andrew Ellington, University of Texas at Austin

Presenter: Jimmy Gollihar

The ability to manipulate defined neuron populations is central to in vivo investigations of brain circuitry. The main requirement for a genetically encoded neuronal actuator is that it be absent from the central nervous system, so that only neurons expressing the actuator are sensitized to the subsequent optical or chemical stimulus. While many metabotropic and ionotropic actuators have been devised, the drawbacks of each have created the impetus for alternatives. Building on our combined expertise in actuator design and directed evolution, we are developing and testing a new protein engineering platform for generating orthogonal receptor/ligand pairs to activate distinct genetically-defined neuron populations in vivo and in vitro. Significantly, our orthogonal receptor/ligand engineering scheme, in which each binding partner can be optimized to fit the other, will build upon the favorable kinetics and desensitization properties of the receptor class while ensuring orthogonality through ligand optimization. Using the Molecular Operating Environment of protein/ligand design tools, we have identified amino acid substitutions to three model ligands as a proof-of-principle of our computational design approach. In parallel, we have adapted the directed evolution technique pioneered in the Ellington laboratory, Compartmentalized Partnered Replication (CPR), to engineer orthogonal purinergic receptor-ligand pairs by optimizing yeast functional selection circuits. Using alternating rounds of positive and negative selection, we envision libraries that converge on a small number of unique solutions for each ligand analog. We are confident that our synthetic purinergic activator (SPArk) will usher in a new era of cell and circuit level interrogations of the brain's emergent properties.

1.12

Bottom-Up Engineering of the Heart Using Developmentally-Inspired Protein Scaffolds

Award Type: New Innovator Award

Award Year: 2012

Awardees: Adam Feinberg, Carnegie Mellon University

Presenter: Adam Feinberg

The extracellular matrix (ECM) is a nanofibrillar network of proteins such as collagen and other molecules that physically integrates cells into tissues and acts as an insoluble, mechanosensitive signaling network. Recent work has demonstrated that decellularized organs can serve as scaffolds to regrow tissues by providing instructive ECM cues for cells. But this top-down approach requires an existing organ to be decellularized first. We asked, why not build the ECM from the bottom-up just like cells do during embryogenesis or wound healing? To do this, we have developed a biomimetic, surface-initiated assembly process that recapitulates how cells naturally build the ECM in tissues. We are using these ECM nanofibers to engineer scaffolds for cardiac tissue engineering that mimic the ECM structure and composition in the embryonic heart, using developmental biology as a design template. Further, we are also developing new 3D bioprinting techniques to create larger structures that incorporate more intricate anatomy. Together, these approaches provide a reductionist system where complexity can be engineered back into the matrix system, which we are exploiting as a tissue engineering platform and basic science tool.

1.13

Watching Rods Form Out of Spheres – Short Axis Sensing by MreB Orients Cell Wall Synthesis Allowing Robust Rod Shaped Growth and Recovery

Award Type: New Innovator Award

Award Year: 2014

Awardees: Ethan Garner, Harvard / Molecular and Cellular Biology, Harvard / Center for Systems Biology,

Abstract Author(s): Carl Wivagg, Harvard / Molecular and Cellular Biology

Presenter: Ethan Garner

Rod shaped bacteria elongate by the action of cell-wall synthetic complexes that move circumferentially around the cell width. These motions that are thought to reflect the insertion of new cell wall material. These synthetic complexes, located on the outside of the cell, are linked to MreB filaments bound to the cytoplasmic membrane surface. Each filament/enzyme complex moves around the rod independently, with adjacent complexes moving in opposing directions.

To understand how the independent, disconnected motions of these filament/enzyme complexes are able to orient their motions around 90 degrees to the long axis of the bacteria, we observed their dynamics as we deformed and reformed bacteria from rods into spheres. To cause this transition, we modulate the levels of teichoic acids and PBP2, each titrating the width and the rigidity (straightness) of the rod shaped Bacillus subtilis. We find that as we decrease cell rigidity, the cellular width increases, up to a point at which rod shape fails. We find that the motions of MreB are circumferentially organized in rods of all widths, yet become anisotropic and disorganized in spheres. By confining these disorganized cells in chambers near the width of normal cells, we see that MreB aligns its motion along this externally imposed axis, indicating that the elongation system can sense the aspect ratio of the cell. This orientation also occurs in the absence of filament motion or cell wall, and is also observed from purified MreB assembled inside liposomes, indicating this orientation is dependent upon filaments alone.

We can watch how this system reorganizes as we convert spheres back into rods by suddenly increasing the magnesium or teichoic acid levels. In isolated cells, we observe the orientation and shape transitions occur by spheres emitting rods from one point, suggesting a local, not global, reorganization of growth, a result corroborated with staining of sites of new cell wall growth. The initially emitted rods are near the normal bacterial width, suggesting that this machinery is tuned to both sense and propagate a given cellular radius.

These results suggest that the elongation machinery encodes an intrinsic sensor of cell width, one that creates rod shape by orienting their motion of synthesis along given curvatures. We suggest that feedback between curvature, orientation of synthesis, and cell wall stability provides a robust mechanism to initiate and maintain rod shaped growth, independent of the preexisting shape or cell wall organization. This mechanism would allow cells to form and grow as rods based on local rules rather than long-range organization.

1.14

Towards the Total Synthesis of the Human Mammary Gland

Award Type: New Innovator Award

Award Year: 2013

Awardees: Zev J Gartner, UCSF

Presenter: Zev Gartner

Reconstituting tissues from their cellular building blocks facilitates the modeling of morphogenesis, homeostasis and disease in vitro. Here we describe DNA-programmed assembly of cells (DPAC), a method to reconstitute the multicellular organization of organoid-like tissues having programmed size, shape, composition and spatial heterogeneity. DPAC uses dissociated cells that are chemically functionalized with degradable oligonucleotide 'Velcro', allowing rapid, specific and reversible cell adhesion to other surfaces coated with complementary DNA sequences. DNA-patterned substrates function as removable and adhesive templates, and layer-by-layer DNA-programmed assembly builds arrays of tissues into the third dimension above the template. DNase releases completed arrays of organoid-like microtissues from the template concomitant with full embedding in a variety of extracellular matrix (ECM) gels. DPAC positions subpopulations of cells with single-cell spatial resolution and generates cultures several centimeters long. We used DPAC to explore the impact of ECM composition, heterotypic cell-cell interactions and patterns of signaling heterogeneity on collective cell behaviors. We are currently using DPAC to build towards the "total synthesis" of a functional human mammary gland from dissociated cells.

1.15

An Optical Investigation of Olfaction in the Drosophila Larva

Award Type: New Innovator Award

Award Year: 2015

Awardees: Marc Gershow, NYU

Abstract Author(s): Marc Gershow, NYU

Presenter: Marc Gershow

The Gershow lab studies study the olfactory behaviors of the Drosophila larva. To move towards or away from an odor, the Drosophila larva uses sensory input to drive motor output. In this process, it modulates a number of behaviors, e.g. moving forward, stopping, and sweeping the head to one side or the other. We seek to understand the rules by which the larva changes its behavior in response to odor input in order to move towards a goal, and we seek to understand how these rules are encoded in the structure of the larva's nervous system and effected by the correlated activities of its neurons.

We used light activated ion channels to introduce noise into odor receptor neurons, measured the resulting perturbations in behavior, and determined the rules by which the larva transforms activity from individual neurons into strategic decisions[1]. Natural odors stimulate multiple receptors simultaneously; to begin understanding how the brain interprets these signals, we will measure how the presence of a natural odor changes the meaning of activity in individual odor receptor neurons. We will apply these methods to learn how the larva makes decisions on the basis of activity in other neurons throughout its olfactory circuit.

Although we have a sophisticated understanding of individual neurons, we are far from understanding how connected groups of neurons (neural circuits) work together to process information. The Drosophila larva has few neurons but performs complex behaviors, making it an ideal model in which to investigate the operating principles of neural circuits. Advances in genetics, protein engineering, and microscopy allow us to optically activate, suppress, and visualize the activity of these neurons through the larva's transparent cuticle. In addition to our experiments measuring behaviors evoked by activating neurons, we will directly measure the larva's neural responses to odor using optical microscopy.

The promise of the larva, a small crawling animal with a transparent skin, is that we can visualize neural activity in freely behaving animals, but in practice, we do not have a microscope capable of keeping up with a moving animal. We will develop a microscope that can track individual neurons in a moving larva. Using acousto-optic elements, we will raster the microscope's focal spot rapidly around the location of the neuron. Using field-programmable gate arrays, we will read out the fluorescent intensity at each point around the target neuron and update our estimate of the neuron's location with sub-millisecond latencies. Using this microscope, we will be able to "read the larva's mind" as it goes about its business, allowing us to link together sensory input, neural activity, and evoked behavior in order to understand the function of neural circuits.

[1] Gepner R, Mihovilovic Skanata M, Bernat N, Kaplow M, & Gershow M (2015) Computations underlying Drosophila photo-taxis, odor-taxis, and multi-sensory integration. eLife e06229

1.16

Natural Control of Lifespan

Award Type: Pioneer Award

Award Year: 2013

Awardees: Vadim Gladyshev, Brigham and Women's Hospital, Harvard Medical School

Presenter: Vadim Gladyshev

Understanding the mechanisms that control lifespan is among the most challenging biological problems. Many complex human diseases are associated with aging, which is both the most significant risk factor and the process that drives the development of these diseases. It is clear that the aging process and the maximum lifespan of species can be regulated and adjusted. For instance, mammals are characterized by >100-fold difference in lifespan, which can both increase and decrease during evolution. We employ this diversity in mammalian lifespan and the associated life-history traits to shed light on the mechanisms that regulate species lifespan. For this, we utilize methods of comparative genomics to sequence and examine the genomes of exceptionally long-lived species and carry out analysis of lifespan across a panel of mammals. We sequenced the genomes of several mammals with exceptional lifespan, including the naked mole rat, the Damaraland mole rat, and the Brandt's bat, and identified genes that contribute to their longevity. We also work with the longest lived mammal, the bowhead whale. In addition, we applied RNAseq and metabolite profiling approaches to characterize the molecular basis for adaptations associated longevity across a large cohort of mammals. These studies identify both species-specific and common mammalian processes involving various pathways and provide the insights into how Nature changes species lifespan. It is our hope that a better understanding of molecular mechanisms of mammalian lifespan control will lead to a better understanding of human diseases of aging.

1.17

Controlling Stem Cell Differentiation by Chemical Editing of Glycan Signals at the Cell-Matrix Interface

Award Type: New Innovator Award

Award Year: 2015

Awardees: Kamil Godula, University of California, San Diego

Presenter: Kamil Godula

Stem cells have the remarkable ability to develop into a range of functional cell types of the adult body that can be used to repair damaged or diseased tissues. Among the regulators that control the direction of stem cell differentiation are glycosaminoglycans (GAGs). These highly sulfated polysaccharides presented on cell surface proteins orchestrate the formation of active growth factor-receptor complexes and initiate signaling associated with gene expression and cell specification. The spatial organization of the negatively charged groups in GAGs is believed to encode their specificity for individual growth factors. Precise tailoring of these structures on the surfaces of stem cells may open new opportunities for enhancing their differentiation toward cell types with therapeutic potential. In the absence of molecular biology tools to manipulate endogenous GAGs in living cells, our laboratory has focused on developing materials approaches to control the function of these glycans. We have generated synthetic nanoscale mimetics of GAGs and a microarray platform to evaluate their interactions with growth factors. We have established chemical methods to introduce these materials into membranes of embryonic stem cells, where they assumed the functions of native GAGs and promoted differentiation toward neural cell types. Our most current efforts focus on integrating this glycan engineering approach with the application of small molecule inhibitors of GAG biosynthesis enzymes to more precisely edit endogenous GAG signals to eventually gain control over this important component of developmental signaling.

1.18

Identifying Pathways for Motor Variability in the Mammalian Brain

Award Type: New Innovator Award

Award Year: 2015

Awardees: Jesse Goldberg, Cornell University

Presenter: Jesse Goldberg

Deficits in movement initiation, control and variability constitute the core dysfunctions of neurological disease, but we still don't know how these processes are implemented in the brain. The main obstacle is the sheer complexity of brain pathways for movement. The mammalian motor system is a distributed group of neural circuits, which are in turn comprised of complex microcircuits and specific cell types. Because we don't know how these small circuit elements influence behavior, current treatments lack effectiveness and specificity. To address this problem, we developed a panel of new technologies that will allow us to define how previously inaccessible microcircuits control motor behavior. First, we invented a touch-sensing joystick that quantifies mouse forelimb trajectories with unprecedented (micron-millisecond) spatiotemporal resolution. Second, we incorporate this joystick into automated, computer-controlled homecages that perform real-time behavioral analysis and high-throughput behavioral training. Third, we devise a new way of doing high-throughput optogenetics in untethered mice using newly available red-shifted opsins. Finally, we demonstrate for the first time that mice can learn complex center-out forelimb tasks similar to ones long used in primates. By establishing a new, sophisticated motor learning paradigm in mice - a tractable model system with powerful genetic tools – we are now poised to selectively manipulate neural activity in large batches of behaving animals. First, we will perform projection-specific optogenetic silencing to determine how each of fourteen pathways converging on mouse forelimb motor cortex controls movement initiation and variability in the joystick trajectories. Next, we will use Cre-transgenic mouse lines to test how distinct layers inside forelimb cortex differentially control these processes. For both of these experiments, real-time behavioral analysis will enable optogenetic manipulations to be time-locked to specific task events and animal postures, as well as at distinct stages of skill learning. In summary, the proposed work combines unprecedented readout of motor output with unprecedented tools for manipulating previously inaccessible parts of the mammalian motor system. Our new behavioral and experimental paradigm will identify yet-to-be discovered circuits controlling movement initiation, variability and learning. If successful, it will no longer be so mysterious where tremors, dystonias, akinesias and choreas come from. We will be able to point to specific pathways and cell types positioned to cause specific deficits, which in turn will provide a roadmap towards the next generation of more targeted therapies.

1.19

Rapid, Multiscale Sensing Using Acoustic Detection Mechanisms

Award Type: New Innovator Award

Award Year: 2014

Awardees: Andrew Goodwin, University of Colorado Boulder

Abstract Author(s): Andrew P. Goodwin,

Presenter: Andrew P. Goodwin

The overall goal of this work is to design a new class of in vitro and in vivo acoustic biosensors based on the interactions of biomolecules with the surfaces of soft and hard colloidal particles. A technology that could provide a convenient, inexpensive, and portable method for detecting biomolecules without sample manipulation would provide new avenues for measuring both systemic and localized biomolecular concentrations in many different environments and media. For in vitro detection, an in-solution acoustic sensor would obviate the need for sample processing and washing steps, be scalable from microfluidics to batch process, and possess almost no background. For in vivo imaging, development of a contrast agent that can respond to levels of specific soluble biomarkers in a localized environment would provide considerable power to a ubiquitous imaging modality.

In order to create acoustic biosensors, we have focused on developing tools to study the effect of surface and interfacial chemical structures on ultrasound contrast. Microbubbles are the most effective ultrasound contrast agents because their compressibility allows generation of nonlinear echoes in response to the incident ultrasound pulse. We use high intensity focused ultrasound (HIFU) to create transient bubbles that can sensitize ultrasound energy. In one approach, we have utilized perfluorocarbon droplets. Perfluorocarbon droplets are relatively stable and can be prepared ~ 200 nm in diameter for preferential extravasation into tumor tissue. We have shown that simple changes in the structure of the stabilizing ligands can have a profound effect on the resultant ultrasound contrast. Second, we have created highly porous silica nanoparticles that can store large amounts of air at sizes far smaller than the practical limit for bubbles. The particles can be scaled down to ~100 nm and show stable ultrasound contrast not ordinarily found in soft colloidal agents.

Finally, in order to convert these approaches into in-solution and in vivo biosensors, we have developed volatile liquid droplets that change their ultrasound contrast specifically in response to interactions with biomolecules. Interaction of plane wave sound with droplets causes refraction into points of constructive interference, thereby allowing detection of even low-order aggregates. This mechanism of detection was utilized to sense a model biomarker streptavidin down to 100 fM, and current studies are focusing on both adapting these sensors for in vivo validation and developing more complex pulse programs for analyte quantification in vivo.

1.20

Nanotechnologies for Biomedical Imaging and Optical Sensors

Award Type: New Innovator Award

Award Year: 2012

Awardees: Daniel A. Heller, Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College,

Presenter: Daniel A. Heller

Two major promising applications of nanotechnology towards the treatment of cancer are the control over molecular binding sites and the detection of binding phenomena, which could lead to better therapies, earlier cancer detection, and better tools for cancer research. For these pursuits, new methods are needed to quantify disease biomarkers and other bioanalytes. The real-time and spatially-resolved detection and identification of analytes in biological media present important goals for next-generation nanoscale sensors. To this end, we employ the intrinsic near-infrared fluorescence of single-walled carbon nanotubes. The emission of semiconducting nanotubes is photostable yet sensitive to the immediate environment. Analyte identification is achieved by modulation of the nanotube's spectral response, resulting in distinct optical fingerprints. The responses can be spatially mapped in live cells and tissues, and measured in real-time with sensitivity down to the single-molecule level, facilitating unprecedented bioanalytical studies.

1.21

Enabling Cholesterol Catabolism in Human Cells: Lessons from Nature

Award Type: Transformative Research Award

Award Year: 2011

Awardees: Richard E. Honkanen, University of South Alabama

Presenter: Richard E. Honkanen

Cardiovascular disease (CVD) is a leading cause of death in the US, killing >370,000 people yearly. CVD is complex and can originate from different aberrations in lipid metabolism. However, nearly all forms of CVD are associated with elevated plasma lipoproteins (principally LDLs) often in combination with low levels of high-density lipoproteins (HDLs). For many people, CVD is a progressive disease that is largely undetected until an event (i.e. myocardial infarction) occurs in the later stages of disease. Therefore, current therapies focus on preventing a second event (or a primary event in high risk individuals) by reducing LDL-cholesterol. At a biochemical level, the inability of human cells to degrade the cholestane ring of cholesterol is a fundamental component of CVD. More precisely, if human had the ability to degrade cholesterol, macrophages would not become engorged with cholesterol/cholesterol esters and elicit the maladaptive immune response that leads to the onset and progression of atherosclerosis. The observation that Mycobacteria survival in human macrophages is aided by their ability to use phagosome cholesterol as a source of carbon and energy lead us to a novel hypothesis: genes encoding bacterial ring opening enzymes can be humanized and used to transform human monocyte derived macrophages, enabling the degradation of phagosome-cholesterol. To test our hypothesis, we humanized key enzymes, measured their activities, and assessed the stability and toxicity of the products produced in Hep3B cells. As predicted the activity of both 3-ketosteroid- Δ1-dehydrogenase (KSTD) and 3-ketosteroid-9α-hydroxylase (KshA/B) are critical for ring opening. Cholestenone, produced by cholesterol dehydrogenase (CD), and 9-hydroxyderivatives, generated by KshA/B, require KSTD for further catabolism, and the build up of cholestenone is toxic. To increase KSTD activity, we engineered and introduced a P450/Fdx/Fdr fusion protein that removes the cholesterol side chain. Without the side-chain, human 3β-hydroxysteroid dehydrogenase can replace bacterial CD, eliminating cholestenone buildup. The expression of our multi-enzyme system is coordinated by that addition of viral "ribosomal skipping" sequence between proteins. This eliminates the need for multiple promoters. Ring opening was achieved, but flux in human cells was not ideal. In bacteria, intermediates are "funneled" through two phospholipid membranes, with catabolizing enzymes imbedded in, sequestered between, or associated with the membranes. To mimic this environment, some enzymes were further engineered for expression in/around the mitochondria. This greatly improved flux. A transcription activator-like effector nuclease (TALENs) targeting the AAVS1 locus in the human genome has been constructed, and this year we hope to document the catabolism of cholesterol originating from LDLs provided to macrophages produced following differentiation of the genetically modified monocytes.

1.22

Clinical and Translational Approaches to Cognitive Impairments in Breast Cancer

Award Type: New Innovator Award

Award Year: 2014

Awardees: Michelle Janelsins, University of Rochester

Abstract Author(s): Michelle Janelsins, University of Rochester

Presenter: Michelle Janelsins

Background: 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. 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. Methods: 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 are currently developing a clinically relevant mouse model of CRCI with a breast tumor model. Additionally, we are conducting longitudinal human studies assessing cognitive change over time in breast cancer patients treated with chemotherapy compared to controls as well as investigating key neuro-immune pathways that contribute to neurotoxicity. Results and Outcomes: This presentation will provide an update on the activities from the first year of the New Innovator Award. We are fine-tuning our CRCI mouse model and beginning the longitudinal human study. We will build upon recent research from Dr. Janelsins' laboratory identifying changes longitudinally in memory and executive function in breast cancer patients receiving chemotherapy as well as the identification of correlations of cognitive declines with IL-1β, MCP-1 and sTNFR1. The goal of this work is to understand the role of inflammation and other neuro-immune factors in CRCI (in humans), develop a clinically relevant animal model of CRCI, and to develop, test, and optimize interventions for CRCI in animal models in order to move them into clinical research protocols.

1.23

Communal Feedback as an Innovative Alternative to Skin Self-Exam

Award Type: New Innovator Award

Award Year: 2015

Awardees: Jakob D. Jensen, University of Utah

Abstract Author(s): Jakob D. Jensen, University of Utah

Presenter: Jakob D. Jensen

In 2014, the American Academy of Dermatology highlighted my skin self-examination (SSE) research as a promising direction for further study. There is a clear need for innovative SSE research as skin cancer rates have increased steadily over the past 30 years. SSE is the advocated approach for detecting skin cancer – for example, most melanomas are initially detected by patients – but two problems undermine its efficacy: (1) validation studies have found low agreement between patient SSE classifications and dermatological examinations and (2) attempts to improve lay ability to perform SSE have produced marginal gains. Given the drawbacks of traditional SSE, I propose to study an innovative alternative: communal feedback. Communal feedback is the use of collective effort to perform a task typically carried out by a single agent. It is especially effective when a single agent has low reliability at a task as the pattern of the group is more predictive than a single-user. Communal feedback occurs when the results of collective effort are conveyed back to the individual. For example, contestants on game shows use communal feedback when they "ask the audience" for help. When participants "ask the audience" for help, they receive feedback in the form of poll data showing what percent of the group supports a given answer. The participant then has to decide if the pattern of the group reflects reality. As an initial test of this strategy in skin cancer control, my lab trained 500 adults to identify suspicious moles using the ABCDEs. Following training, the participants were shown high resolution images of 40 moles (9 of which were clinically diagnosed melanomas) and asked to circle those they found suspicious. Consistent with the collective effort approach, the pattern of the group was more predictive of melanoma (sensitivity = .90, specificity = .72) than the average individual user (sensitivity = .58, specificity = .81). Specifically, if 19% of participants (or more) identified a mole as suspicious, then collective effort correctly identified 90% of melanomas, and correctly classified 72% of non-melanomas. Through the New Innovator grant, I propose to carry out four studies validating and translating communal feedback as an approach to skin cancer control. Communication researchers have argued that individuals can benefit from communal feedback. Communal feedback is an innovative strategy that attempts to build on the potential of new communication technology. Critics have noted that 21st century research has largely failed to harness new communication technology for public good.

1.24

Elucidating the Protein Homeostasis Network in Disease States of Human Cells by Next-Generation Functional Genomics

Award Type: New Innovator Award

Award Year: 2015

Awardees: Martin Kampmann, UCSF

Presenter: Martin Kampmann

A functional proteome is of paramount importance for all cells and organisms. The cellular pathways involved in maintaining the integrity of the proteome are collectively referred to as the proteostasis network. This network adapts dynamically to meet the requirements of the cell and is also rewired in a range of disease states, including cancer and neurodegenerative disease, making it a promising therapeutic target. However, the dynamic, context-dependent nature and size of the proteostasis network present a formidable challenge that cannot be addressed using traditional approaches. To understand how the proteostasis network functions in normal and disease states, and to pinpoint nodes that are effective targets for therapeutic intervention, a systems approach is called for. We are establishing such an approach by integrating two breakthrough technologies that we recently developed: Genetic interactions maps in mammalian cells, which reveal cellular pathways, and genome-wide CRISPR-based gain- and loss-of-function screens which yield rich, complementary insights into gene function. We are extending our strategy to FACS-based screening of cellular phenotypes monitored by fluorescent reporters. Taken together, these innovations will enable the generation of context-dependent, multi-phenotype, gain- and loss-of-function genetic interaction maps. Our long-term goal is to use this technology and other innovative approaches to understand the proteostasis network in normal and disease contexts and to harness its therapeutic potential.

1.25

Targeting the Evolution of Antibiotic Resistance

Award Type: New Innovator Award

Award Year: 2012

Awardees: Rahul M. Kohli, University of Pennsylvania

Presenter: Rahul M. Kohli

Bacteria possess a remarkable ability to rapidly adapt and evolve in response to antibiotics. Acquired antibiotic resistance can arise by multiple mechanisms, but commonly involves altering the target site of the drug, enzymatically inactivating the drug, or preventing the drug from accessing its target. These mechanisms often involve new genetic changes in the pathogen leading to heritable resistance. This recognition underscores the importance of understanding how such genetic changes can arise. We are interested in exploring how the bacterial DNA damage response or SOS pathway contributes to genomic diversification and acquired antibiotic resistance. Focusing on the key regulator of this pathway, LexA, we have demonstrated how the kinetics of this stress response regulator have evolved to maximize fitness, while also facilitating diversification and escape under antibiotic stress. Recognizing the importance of this pathway to antibiotic resistance, we have also performed a high throughput screen which has revealed lead inhibitors that antagonize the SOS response. Overall, our work aims to elucidate the key role of the SOS pathway in acquired resistance and demonstrate the feasibility of perturbing stress responses to potentiate our current antibiotic arsenal.

1.26

Analysis of How Quantitative Cellular Network Variation Impacts Tumor Progression

Award Type: New Innovator Award

Award Year: 2014

Awardees: Pamela Kreeger, UW-Madison/Biomedical Engineering

Presenter: Pamela Kreeger

In many tumors, mutations in genes impact protein functions (i.e., loss or gain of function); when these mutations impact proteins in cellular pathways that control key processes such as proliferation and invasion, they provide potential drug targets to slow tumor progression. In contrast, other tumors such as high-grade serous ovarian cancer (HGSOC) do not have a significant number of such mutations, and instead have subtle changes in the relative levels of multiple proteins distributed across the cellular network, setting up cellular networks that are qualitatively the same (i.e., nearly all components are present and have normal functionality) but quantitatively very different. This research program seeks to analyze this new paradigm in order to address the hypothesis that at each stage of progression in HGSOC specific quantitative changes in the cell network influence the likelihood of progression.

To address this hypothesis, we are developing in vitro culture systems that mimic the in vivo environment of HGSOC. In particular, we are developing models of the ovarian surface epithelium and ovarian inclusion cysts to determine how these microenvironments influence the likelihood of successful metastasis from the initial lesion on the fallopian tube. In future work, we will utilize genetically-manipulated HGSOC cells to perturb expression of different cellular proteins to examine how these changes interact with the different microenvironments to influence disease progression. As a first step, we have utilized the OVCAR5 cell line and computational models to examine how changes in IGF2R and IGFBPs influence cell proliferation in response to IGF ligands. Through these studies, we hope to significantly improve our understanding of HGSOC progression and identify potential therapeutic strategies.

1.27

Orthogonal Replication for Rapid Evolution and Synthetic Biology

Award Type: New Innovator Award

Award Year: 2015

Awardees: Chang Liu, UC Irvine

Presenter: Chang Liu

We recently developed an orthogonal DNA replication (OrthoRep) system in the yeast Saccharomyces cerevisiae. OrthoRep exploits an unusual selfish DNA element consisting of a DNA plasmid–DNA polymerase pair that stably replicates in the cytoplasm of yeast. The spatial and mechanistic isolation of OrthoRep from genomic replication may have fundamental significance for biomolecular engineering and synthetic biology. For biomolecular engineering, it offers a platform for rapid targeted evolution where genes encoded on the OrthoRep system undergo accelerated evolution without any increase in the genomic mutation rate. For synthetic biology, OrthoRep is a platform that should enable the bottom-up construction of synthetic genetic and replication systems in vivo.

1.28

Modulating Plasma Membrane Phosphatidylserine Exchange Controls Innate Immune Responses by Microglia

Award Type: New Innovator Award

Award Year: 2012

Awardees: Axel Nimmerjahn, Salk Institute for Biological Studies

Abstract Author(s): Yusuf Tufail, Waitt Advanced Biophotonics Center, Salk Institute for Biological Studies

Presenter: Axel Nimmerjahn

Microglia are the intrinsic immune sentinels of the central nervous system. Their activation restricts tissue injury and pathogen spread, but in some settings, including viral infection, this response can contribute to cell death and disease. Identifying mechanisms that control microglial responses is therefore an important objective. Using replication-incompetent adenovirus 5 (Ad5)-based vectors as a model, we investigated the mechanisms through which microglia recognize and respond to Ad5-transduced cells. Transgenic, immunohisto-chemical, molecular biology, and fluorescence imaging approaches revealed that phosphatidylserine (PtdSer) exposure on the outer leaflet of transduced cells triggers their TAM receptor-dependent engulfment by microglia. Modulation of the phospholipid transporter activity required for PtdSer externalization provided long-term protection of transgene-expressing cells from microglial phagocytosis. Our study identifies PtdSer transport as a target through which the innate immune response to viral vectors, and potentially other stimuli, may be controlled.

1.29

Characterizing Lymphatic Micrometastases

Award Type: New Innovator Award

Award Year: 2011

Awardees: Timothy P. Padera, Massachusetts General Hospital, Harvard Medical School,

Presenter: Timothy P. Padera

Metastasis remains the major cause of cancer mortality, but breakthroughs in our understanding of the molecular and cellular mechanisms regulating metastasis have yet to be broadly translated into improved survival rates in patients with metastatic disease. The challenge is how to treat cancer cells that have spread to lymph nodes or distant organs in order to prevent their growth and ideally eradicate them from the body. Most cancer therapies are developed against the primary tumor growing in its native microenvironment. However, it is clear that the local microenvironment in which tumor cells grow greatly affects the growth rate, metabolism, vascularization, and ultimately the response to therapeutic intervention. For instance, antiangiogenic therapy to date has failed to improve overall survival in cancer patients when used in the adjuvant setting. The presence of lymph node metastases dictate treatment decisions, however their reliance on angiogenesis for growth has not been described. We have developed a novel chronic lymph node window (CLNW) model to facilitate new discoveries in the growth and spread of lymph node metastases. Using the CLNW in multiple models of spontaneous lymphatic metastases in mice, we reveal the surprising lack of sprouting angiogenesis during metastatic growth, despite the presence of hypoxia in some lesions. Treatment with two different antiangiogenic therapies showed no effect on the growth or vascular density of lymph node metastases in our models. We confirmed these findings in clinical specimens. Further, we show that lymph node metastasis do not originate from a single cancer cell, but from multiple metastatic cancer cells. We are currently exploring the transcriptional changes that occur when a cancer cells spontaneously metastasizes from the primary tumor to the lymph node. With these data we hope to understand the mechanisms necessary for cancer cells to survive in the lymph node microenvironment, including avoiding the immune system.

1.30

Maternal Neutralizing Antibodies Protect Against Severe Fetal Outcome in a Novel Nonhuman Primate Model of Congenital Cytomegalovirus Infection

Award Type: New Innovator Award

Award Year: 2012

Awardees: Sallie Permar, Duke University Medical Center

Abstract Author(s): Kristy Bialas, Duke University Medical School

Presenter: Sallie Permar

Elucidation of maternal immune correlates of protection against congenital cytomegalovirus (CMV) is necessary to inform future vaccine design. Here, we present a novel rhesus macaque model of placental rhesus CMV (rhCMV) transmission and use it to dissect determinants of protection against congenital transmission following primary maternal rhCMV infection. In this model, asymptomatic intrauterine infection was observed following i.v. rhCMV inoculation during the early second trimester in two of three rhCMV-seronegative pregnant females. In contrast, fetal loss or infant CMV-associated sequelae occurred in four rhCMV-seronegative pregnant macaques that were CD4+ T-cell depleted at the time of inoculation. Animals that received the CD4+ T-cell-depleting antibody also exhibited higher plasma and amniotic fluid viral loads and delayed production of autologous neutralizing antibodies compared with immunocompetent monkeys. To determine whether maternal neutralizing antibodies can protect against severe fetal outcome following maternal CMV infection, CD4+ T cell depleted rhCMV seronegative dams were passively infused with hyperimmune globulin from rhCMV seropositive monkeys prior to i.v. rhCMV challenge. In fact, passive IgG infusion protected against fetal loss in three of three dams, but did not protect against rhCMV transmission. Thus, maternal CD4+ T-cell immunity and maternal antibody responses during primary rhCMV infection are important to prevention of severe CMV-associated fetal disease and should be targeted by maternal CMV vaccine strategies.

1.31

Cell Signaling in Control of Regenerative Growth

Award Type: New Innovator Award

Award Year: 2013

Awardees: Christian Petersen, Northwestern University

Presenter: Christian Petersen

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 noncanoncial 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.

1.32

Mechanical Superresolution: Imaging Structure, Chemistry, Forces, and Voltage across Biomolecules and Cells

Award Type: New Innovator Award

Award Year: 2013

Awardees: Ozgur Sahin, Columbia University

Presenter: Ozgur Sahin

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 voltage in synaptic terminals, all non-invasively and without using labels. We will present how chemical identities and physiological signals are transduced into readily detectable mechanical signatures, and illustrate the potential of these microscopes with challenging imaging problems in structural biology, mechanobiology, and neuroscience.

1.33

Control of the Neonatal Septisome and Hydrocephalus in Sub-Saharan Africa

Award Type: Pioneer Award

Award Year: 2014

Awardees: Steven Schiff, Penn State University

Presenter: Steven Schiff

The majority of the world's hydrocephalus of infancy is likely related to prior neonatal sepsis. Nevertheless, the microbial origins of neonatal sepsis remain largely uncharacterized in the developing world. Similarly, the routes of these infections remain uncharacterized. The number of infants affected is so large that this is in effect a neurosurgical epidemic for which there is no effective surgical option at the population level.

In this project, we are characterizing neonatal sepsis at regional hospitals throughout Uganda, and through exhaustive follow-up, identifying the sentinel cases that go on to develop hydrocephalus. Most bacteria cannot be grown in laboratories, and substantially fewer are recovered in the laboratories of the developing world than in the industrialized countries. We are employing next-generation DNA and RNA sequencing technologies to perform a broad screen for all microorganisms present in such infants – what we term the Neonatal Septisome. We seek the additional characterization of sequential and polymicrobial co-infections, especial the interaction of viruses and bacteria, that may underlie these conditions. We further seek a proteomic assessment of the characteristics of the host immune response to stratify the unifying factors of this syndrome when the causal agents are disparate.

In recent work, we have recovered a bacterial agent through culture in less than 1/3 of cases of neonatal sepsis, but through genomic sequencing have uncovered a substantial number of leptospirosis cases where the bacterial agent cannot be recovered in diagnostic laboratories. Such infections are enzootic with the domestic animals that these infants are in close contact with. We are comparing genomic sequences from postinfectious hydrocephalic infants with age matched control populations presenting with congenital non-postinfectious origins of their hydrocephalus. We have uncovered a strong climate link to seasonal rainfall to neurosurgical case numbers.

Our long-term goal is a model-based feedback control strategy seeking a rational, and optimal, framework to better treat and prevent neonatal sepsis and postinfectious hydrocephalus in developing countries, and in particular sub-Saharan Africa, where such infections and their sequelae remain out of control. This model-based framework will readily adapt to other regions of the developing world, with the potential for a substantial impact on global infant health.

1.34

Cardinal Orientation Selectivity is Represented by Two Distinct Ganglion Cell Types in Mouse Retina

Award Type: New Innovator Award

Award Year: 2015

Awardees: Gregory Schwartz, Northwestern University

Abstract Author(s): Amurta Nath,

Presenter: Gregory Schwartz

Orientation selectivity (OS) is a prominent and well-studied feature of early visual processing in mammals, but recent work has brought into question the circuit location at which OS is first computed. While both classic and modern work has identified an OS mechanism in selective wiring from lateral geniculate nucleus (LGN) to primary visual cortex, OS responses have now been found upstream of cortex in mouse LGN and superior colliculus, suggesting a possible origin in the retina. We have identified two novel types of OS ganglion cells in the mouse retina that are highly selective for horizontal and vertical cardinal orientations. Reconstructions of the dendritic trees of these OS ganglion cells and measurements of their synaptic conductances offer insights into the mechanism of the OS computation at the earliest stage of the visual system.

1.35

Development of Combination Immunotherapies for Atherosclerotic Inflammation

Award Type: New Innovator Award

Award Year: 2015

Awardees: Evan Scott, Northwestern University

Abstract Author(s): Evan Scott, Northwestern University

Presenter: Evan Scott

Cardiovascular disease (CVD) continues to be the leading cause of death in the developed world and is a considerable economic burden. A principle cause of CVD is atherosclerosis, an immunologically complex inflammatory condition within the intima of arterial vessel walls. Current clinical treatments for atherosclerosis focus on lowering serum levels of low density lipoprotein (LDL) using therapeutics such as statins, administration of antithrombotic drugs, and surgical intervention. A critical weakness of current therapeutic strategies is cell-mediated inflammation. We intend to address this weakness by modulating specific inflammatory cell subsets that function as systemic and local sources of inflammation during the progression of atherosclerosis.

Recent advances in nanotechnology and immunology now permit the rational design of targeted nanomaterials for the systematic probing and modulation of individual immune cell populations for either therapeutic or investigative purposes. We hypothesize that nanomaterials designed to modulate systemic sources of inflammation as well as local inflammation within atherosclerotic lesions can synergize with statin therapy. Our objective is therefore to develop nanomaterial-based immunotherapies that will enhance current CVD therapies by eliciting both a systemic and local atheroprotective anti-inflammatory immunoregulatory profile. This proposal is "high risk" in that our nanomaterials have not been previously tested in models of atherosclerosis. But, if successful, these formulations will be significantly "high reward" by serving as a proof-of-concept for the rational design of nanomaterials for immunotherapeutic treatment of CVD.

Our nanomaterials consist of custom block copolymers engineered to self-assemble into virus-like nanostructures for targeting of specific inflammatory cell populations. These materials will be tagged with a near infrared fluorescence (NIRF) imaging agent for in vivo real time imaging of targeted cells. We have therefore begun our studies by loading the non-toxic, FDA-approved near infrared fluorophore indocyanine green (ICG, or cardiogreen) into diverse poly (ethylene glycol)-bl-poly (propylene sulfide) (PEG-bl-PPS) nanostructures. The loading method and concentration were optimized to maximize ICG fluorescence and minimize self-quenching effects. ICG-loaded PEG-bl-PPS nanostructures were then administered by tail vein injection into mice for assessment of their in vivo biodistributions. While ICG has a half-life of only 2 to 3 minutes and is rapidly cleared by the kidneys and liver, ICG-nanocarriers remained in circulation for days, completely avoiding the liver during the first 2 hours and instead strongly targeting the spleen. These results are extremely promising, as splenic targeting is ideal for stimulation and inhibition of inflammatory cells that have systemic influences over the progression of CVD.

1.36

Age-Dependent Clonal Enrichment of Pathogenic Mutations in the Male Germline

Award Type: New Innovator Award

Award Year: 2013

Awardees: Marco Seandel, Weill Cornell Medical College

Abstract Author(s): Marco Seandel, Weill Cornell Medical College

Presenter: Marco Seandel

A subset of monogenic disorders (e.g., Apert and Noonan syndromes) and polygenic disorders (e.g., autism, schizophrenia, and some cancers) are more common in those born to older fathers. This association has been referred to as the paternal age effect (PAE). Driving this phenomenon, positive selection of mutant spermatogonial stem cells (SSCs) in the testis may enable enrichment of mutant alleles, as we have shown for the Apert syndrome S252W mutation. The goals of this project are to address the mechanisms of specific disease-associated mutations and develop protocols to discover and rapidly validate novel mutations. Here, we are address the cellular and biochemical pathways that enhance competitiveness of SSCs in a genetically mosaic setting. We employ adult SSCs in culture, in conjunction with transplantation in vivo as experimental models, to uncover the functions of specific candidate alleles found in patients. To explain why certain disorders but not others appear to be driven by paternal age, we are investigating how normal SSCs limit self renewal-associated signaling, to prevent premature cellular differentiation and oncogenesis. The experiments described herein support common mechanisms for PAE-associated disorders and show that PAE diseases could be considered prototypical stem cell-based disorders. By improving our understanding of the cellular basis for PAE pathogenesis and the roles of discrete mutations, experimental manipulation of SSCs could lead directly to novel therapeutic strategies for patients.

1.37

A New High-Throughput Platform for the Discovery of Therapeutic Molecules

Award Type: New Innovator Award
Award Year: 2015

Awardees: Mohammad Seyedsayamdost, Princeton University

Presenter: Mohammad Seyedsayamdost

Multidrug-resistant bacterial pathogens represent one of the most serious health threats to our society today. Over 55% of our clinical antibiotics are derived from naturally-occurring molecules that are synthesized by innocuous soil bacteria. These bacteria harbor numerous dedicated biosynthetic gene clusters that in a stepwise fashion generate bioactive molecules from simple building blocks. However, the majority of these gene clusters are not expressed under normal growth conditions, and methods that activate them would have a profound impact on drug discovery. We have devised a new strategy for activating these so-called 'silent' biosynthetic gene clusters. Our method combines high-throughput screening and genetic reporter fusions to induce silent gene clusters by over 2 orders of magnitude. Future applications of this methodology promise to uncover new and potentially therapeutic small molecules.

1.38

Parasympathetic Neural Remodeling in the Setting of Myocardial Infarction and Electrical Stabilization by Vagal Nerve Stimulation

Award Type: New Innovator Award

Award Year: 2015

Awardees: Marmar Vaseghi, UCLA Cardiac Arrhythmia Center

Presenter: Marmar Vaseghi

The autonomic nervous system plays an important role in genesis and maintenance of arrhythmias in the setting of cardiac pathology. Specifically, sympathetic activation and parasympathetic dysfunction are known to occur the setting of myocardial infarction and increase the risk of sudden cardiac death. Neural remodeling both at the central and end-organ level combined with a heterogeneous myocardial substrate of viable myocytes and fibrosis along border zones regions can create the alterations in conduction velocity and repolarization required to initiate and maintain arrhythmias. We hypothesized that the parasympathetic dysfunction observed in the setting of myocardial infarction is due to decreased central parasympathetic drive, and use a combination of molecular, neural recording, and electrophysiological techniques to assess the end organ effects of this dysfunction. We have found that myocardial acetylcholine levels are not significantly decreased in the setting of myocardial infarction while the activity of parasympathetic neurons in the intrinsic cardiac ganglia is altered, with a decrease in basal activity of neurons that normally respond to parasympathetic activation. This data suggests that central cardio-motor drive to the heart is reduced while the end-organ neurotransmitters are intact. Detailed in-vivo electrophysiological recordings indicate that vagal nerve stimulation can overcome the decrease in parasympathetic tone, and stabilize the most pro-arrhythmic and heterogeneous regions, the border zones of infarcts, reducing inducibility of life-threatening ventricular arrhythmias in-vivo. These studies provide the insight required to subsequently evaluate changes in the central nervous system as well as afferent neural transmission that occur with myocardial infarction. A better understanding of the etiology of this decrease in central parasympathetic drive can lead to more specific therapeutic targets, beyond neural stimulation, to prevent or reverse pathological cardiac and neural remodeling.

1.39

Novel Signal Transduction Complexes as New Targets for Drug Discovery

Award Type: Pioneer Award

Award Year: 2015

Awardees: Hao Wu, Boston Children's Hospital, Harvard Medical School

Presenter: Hao Wu

Formation of location-specific, higher-order signaling complexes, now called supramolecular organizing centers (SMOCs), is an almost universal feature of innate immune signaling. SMOC-mediated signal transduction is distinct from classical signal transduction, in which a chain reaction of ligand-induced conformational changes, enzyme activation and second messenger production leads to signal transmission and amplification. SMOCs illustrate important principles involving cooperativity, signal amplification, threshold behavior and time delay of response, as well as proximity-driven allosteric enzyme activation, spatial and temporal control of activation and termination, and reduction of biological noise. These key signaling concepts are at the forefront of modern signal transduction theory, and understanding them at a rigorous molecular, structural and cell biological level would transform how we approach innate immunity, at both basic and applied levels. We intend to investigate a subset of these concepts that directly guide the development of attractive new models for targeted drug discovery, using fresh ideas and methodologies.

Innate immunity is critically important for host-defense and inflammation, and its dysregulation underlies many human diseases, including genetic disorders, cancer, gout, psoriasis, lupus, multiple sclerosis, neurodegenerative diseases, diabetes, ulcerative colitis and Crohn's disease, just to name a few. We propose that SMOCs provide a previously untapped druggable proteome as they offer opportunities for dominant-negative, rather than competitive inhibition as a result of the cooperativity in their assembly. These target sites may include polymerization interfaces on the oligomerization domains, SMOC-induced, intrinsically weak interactions required for allosteric enzyme activation, and potential SMOC-cytoskeleton interactions required for SMOC formation in cells. The principles learned from these studies will further inform strategies for modulating the assembly of higher-order complexes in other biological processes.

1.40

Single-Molecule Super-Resolution in Situ Imaging of Chromosomal DNA and Haplotype Visualization Using Oligopaints

Award Type: Pioneer Award

Award Year: 2012

Awardees: Ting (C.-ting) Wu, Harvard Medical School; Brian J. Beliveau, Harvard Medical School

Presenter: Ting (C.-ting) Wu

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We report two new single-cell technologies for imaging the genome, both using the bioinformatically-designed, oligonucleotide-based Oligopaint approach for fluorescent in situ hybridization (FISH). The first uses the STORM and DNA-PAINT single-molecule super-resolution methodologies to yield ≤20 nm resolution images of chromosomal DNA. The second harnesses single nucleotide polymorphisms (SNPs) 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. In brief, these new technologies enhance the capacity of researchers to query the impact of chromosome organization and positioning on genome function with an unprecedented level of resolution.

1.41

De Novo Biosynthesis of Terminal Alkyne-Tagged Natural Products and Applications

Award Type: New Innovator Award

Award Year: 2015

Awardees: Wenjun Zhang, UC Berkeley

Presenter: Wenjun Zhang

Natural products and their derivatives have been rich sources for drug discovery; it has been estimated that more than 60% of the drugs that are in the market derive from natural sources. However, during the last three decades, research aimed at exploiting natural products for drug discovery has seriously declined, in part due to our limited capability in new natural product discovery and analogue production. Innovative technology for natural product purification, quantification, overproduction, and diversification is urgently needed to make a paradigm shift in the field of natural product research and to promote natural product-based drug discovery and development. As tagging natural products can address these challenging aspects of natural product research, our lab seeks to develop a novel strategy that overcomes the limitations of known methods for tagging natural products with a bioorthogonal functionality. To this end, we recently developed a new platform to de novo biosynthesize terminal alkyne-tagged natural products by characterizing and engineering the novel terminal alkyne synthetic biology that living systems offer. In addition to directly producing orthogonally functionalized natural product analogues that can be subjected to facile chemical modification for drug screening, our work may significantly advance the field of natural product research by enabling the visualization, enrichment, quantification, and mode of action study of natural products through coupling with diverse azido analytical handles. For example, using a fluorogenic probe, a natural product-based, quantitative, high-throughput screening method based on this tagging strategy was established, serving as a useful tool for evolving natural product biosynthetic enzymes that can efficiently function in specific pathways to produce natural product analogues in high titers. Furthermore, since azide-alkyne click chemistry has recently emerged as one of the most powerful tools in drug discovery and chemical biology, our findings open the door to numerous biological applications in which in situ enzymatic generation of a terminal alkyne is required or preferred.

1.42

Nanomaterial Integrated Microfluidic Devices for Virus Analysis

Award Type: New Innovator Award

Award Year: 2012

Awardees: Siyang Zheng, The Pennsylvania State University

Presenter: Siyang Zheng

Outbreaks of viral infectious diseases are often devastating, causing illness, disability, death and massive economic loss. Viral infectious diseases can spread rapid and affecting a large population. The high mutation rate of viruses are especially challenging for disease control and surveillance. Point-of-care technologies using microfluidic principles and integrated with nanomaterials promise to meet these challenges of future analysis of viral infectious diseases by providing cost-effective and sometimes enabling technologies with extraordinary portability, high sensitivity, and fast processing speed. Recently we developed several technologies of nanomaterial integrated microfluidic devices for physical virus enrichment from complex samples. High performance virus enrichment was shown to significantly improve downstream virus detection, identification and discovery.

1.43

Understanding Tipping Points in Biology

Award Type: New Innovator Award

Award Year: 2012

Awardees: Jeff Gore, MIT

Presenter: Jeff Gore

Natural 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 the collapse of fisheries in response to over-fishing. Given that these population transitions can have substantial economic and 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 the fluctuations of the population size can serve as an early warning signal that the population is close to collapse [1,5]. In addition, we have demonstrated that the emergence of spatial patterns can be used to anticipate an impending collapse [2]. Given the universal nature of these sudden transitions, the behavior explored here may be relevant to tipping points in other complex systems [7]. For example, we have recently demonstrated that phenotypic memory in cells loses resilience to environmental perturbations near such a critical transition [8].

[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] Korolev et al, Nature Reviews Cancer (2014)
[8] Axelrod et al, eLife (2015)

2.1

Direct Observation of Force-Induced Conformational Transitions in F-Actin

Award Type: Early Independence Award

Award Year: 2013

Awardees: Gregory M. Alushin, NHLBI / NIH

Abstract Author(s): Pinar S. Gurel, NHLBI / NIH

Presenter: Gregory M. Alushin

The ability of a cell to sense and respond to the mechanical properties of its environment ("mechanosense") influences many core cellular processes, including division, migration, differentiation, and survival. Dysregulation of mechanosensory pathways has recently been implicated in malignant transformation, tumorigenesis, and metastasis, highlighting this process as a key element of cancer progression. At the foundation of this behavior lies an intricately coordinated contractile network consisting of the actin cytoskeleton, myosin motor proteins, and their myriad binding partners; however, we currently lack a basic understanding of the underlying molecular mechanisms connecting actin to cellular mechanosensation. Actin filaments are flexible polymers that can adopt multiple conformational states, and recent evidence suggests that forces applied to filaments can influence interactions with binding partners. Here, we explore how mechanical stimuli alter the actin filament structural landscape and how this may influence downstream interactions, potentially serving as an initial signal in mechanotransduction. We have developed a novel reconstitution system to place actin filaments under tension suitable for functional biophysical studies with fluorescence microscopy and high-resolution structural studies with cryo-electron microscopy (cryo-EM). Using a modified gliding assay, we find that the combined activity of two surface-immobilized myosin motor proteins which move in opposite directions induces mechanical strain in filaments, evidenced by increased straightening at the micron scale and breakage in the presence of ATP. In contrast, either myosin alone produces processive gliding of filaments. Cryo-EM micrographs demonstrate global straightening of filaments at the nanometer scale in the presence of ATP and reveal the appearance of a novel architectural feature at discrete sites along filaments. High-resolution studies are currently underway to investigate the conformational changes produced in actin in detail. Resolving these structures will provide unprecedented insight into the molecular mechanisms of mechanosensation and ultimately advance the development of targeted therapeutics against specific actin conformational states.

2.2

Molecular Vulnerabilities for Higher Cognitive Disorders in the Newly Evolved Primate Association Cortex

Award Type: Pioneer Award

Award Year: 2013

Awardees: Amy Arnsten, Yale Medical School

Presenter: Amy F.T. Arnsten

Disorders of higher cognition such as Alzheimer's disease (AD) and schizophrenia primarily afflict the newly evolved association cortices, with little effect on the primary sensory cortices. What makes the association cortices so vulnerable, and the sensory cortex so resilient? We have been exploring the hypothesis that the molecular signaling pathways modulating higher cortical connections have evolved to be fundamentally different from those found in the evolutionarily older, sensory cortices, and that dysregulation of these signaling pathways following genetic or environmental insults predisposes these higher circuits to dysfunction and degeneration. We have been using immunoelectron microscopy (immunoEM) and iontophoresis coupled with neuronal recordings in cognitively-engaged monkeys to compare the primary visual cortex (V1) to the newly evolved dorsolateral prefrontal cortex (dlPFC). This work must be done in nonhuman primates, as rodents do not have dlPFC. We have focused on cAMP-protein kinase A (PKA) intracellular signaling pathways, as they are increased by stress exposure, and become disinhibited in the aged dlPFC. We are finding that primate V1 is regulated in a classical manner, where cAMP-protein kinase A (PKA) signaling proteins are concentrated in mushroom spines and especially in pre-synaptic terminals, where they enhance glutamate release, strengthen neural connections and increase neuronal firing. In contrast, cAMP-PKA signaling in the dlPFC weakens neural connections and decreases neuronal firing by opening K+ channels near network connections. Thus, increased cAMP-PKA signaling during stress exposure weakens higher cortical functions but strengthens more primitive circuits. Importantly, we see evidence of cAMP-PKA phosphorylation of tau - where they lead to increased phosphorylation of tau, an early step in Alzheimer's-type degeneration- in the aged dlPFC, but not in the aged V1. We hope that this research will transform our view of cognitive disorders, revealing key vulnerabilities that will provide informed therapeutic targets for prevention and treatment of these crippling, complex diseases.

2.3

Specialized Ribosomes: A New Frontier in Gene Regulation, Organismal Biology, & Evolution

Award Type: New Innovator Award

Award Year: 2011

Awardees: Maria Barna, Departments of Developmental Biology & Genetics, Stanford University, Stanford

Presenter: Maria Barna

The central dogma of molecular biology has for decades served as an explanation for the flow of genetic information within a biological system. In so far as the normal flow of biological information from mRNA to protein, the ribosome has been perceived to decode the genome with essentially machine-like precision; serving as an integral but largely passive participant in the synthesis of all effector proteins across all kingdoms of life. Our research has fundamentally changed this view, by demonstrating that not all of the millions of ribosomes within each cell are the same and that ribosome heterogeneity provides a novel means for diversity of the proteins that can be produced in specific cells, tissues, and organisms. Collectively, we term this additional layer of gene regulation as a "ribocode", whereby heterogeneity in ribosomes composition enables specialized ribosomes to be tuned to translating specific subsets of mRNAs. To decipher the fundamental rules for a ribocode to gene regulation will require the development of novel technologies and paradigms to bring critical understanding to an important new step to gene expression. I will present our work centered on providing a roadmap for the characterization of ribosome composition at a single cell level and during cellular differentiation. We employed a highly quantitative mass spectrometry-based approach to precisely quantify the abundance of each ribosomal protein (RP) belonging to actively translating ribosomes within embryonic stem cells. This led to the identification of a subset of ribosomes that are heterogeneous for RP composition. To further address the functional role of ribosome heterogeneity in translational control of the mammalian genome, we employed CRISPR/Cas9 to endogenously tag select RPs to further purify heterogeneous ribosome populations. We then developed an adapted ribosome profiling method to precisely quantify and characterize the nature of mRNAs translated by distinct heterogeneous ribosomes genome-wide. This led to the identification of subpools of transcripts, critical for key cellular processes including cell signaling, metabolism, growth, proliferation and survival, which are selectively translated by specific types of ribosomes. Most remarkably, there are specific metabolic pathways where almost every single component is selectively translated by specialized ribosomes demarcated by a single RP. I will further present recent findings on the mechanisms by which ribosome-mediated control of gene expression is encoded by structured RNA regulons within 5'UTRs. Together, these studies reveal a critical link between ribosome heterogeneity and specialized translational control of the mammalian genome, which adds a new layer of control to the post-transcriptional circuitry of gene regulation.

2.4

High-Sensitivity NMR at Room Temperature for Molecular Structure and Dynamics

Award Type: New Innovator Award

Award Year: 2015

Awardees: Alexander B. Barnes, Washington University in St. Louis

Presenter: Alexander B. Barnes

This research proposes to develop solid state Dynamic Nuclear Polarization (DNP) to achieve NMR signal enhancements of >200 at room temperature, representing a significant advancement over currently employed DNP spectrometers operating at 100 Kelvin. Solid state NMR is well suited to probe atomic level structure and molecular dynamics of membrane proteins and amyloid fibrils. However, low inherent sensitivity limits solid state NMR measurements. Sensitivity from electron paramagnetic resonance (EPR) can be transferred to NMR to boost signals by more than two orders of magnitude in a process known as DNP. These ultra-sensitive DNP experiments currently require samples to be frozen to below 100 Kelvin. Performing NMR based structural biology at cryogenic temperatures has significant drawbacks including perturbing molecular structure and a loss of spectral resolution. We propose to implement DNP at room temperature with new fast frequency tuning microwave sources (gyrotrons) to enable time domain DNP. These new gyrotrons will have an irradiation bandwidth of >600 MHz compared to currently available sources of 1 MHz, resulting in much better EPR control. We will also design new microwave resonance structures to improve microwave penetration, while reducing microwave heating. The new DNP technology and methodology will be demonstrated with structural and molecular dynamic studies of activators bound to Protein Kinase C regulatory domains. Achieving NMR sensitivity gains of >200 at room temperature will greatly expand the scope and precision of NMR based structural biology.

2.5

A Ketogenic Diet Rescues Hippocampal Memory Defects in a Mouse Model of Kabuki Syndrome

Award Type: Early Independence Award

Award Year: 2013

Awardees: Hans Tomas Bjornsson, Johns Hopkins University, School of Medicine

Abstract Author(s): Joel S. Benjamin, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, Predoctoral Training Program in Human Genetics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Presenter: Hans Tomas Bjornsson

Kabuki syndrome (KS) is a rare intellectual disability syndrome caused by mutations in two genes (KMT2D and KDM6A) involved in chromatin opening. We have previously shown that an agent that favors chromatin opening, the histone deacetylase inhibitor AR-42, ameliorates an ongoing deficiency of adult neurogenesis in the granule cell layer of the dentate gyrus, and recovers hippocampal memory defects in a mouse model of KS (Kmt2d+/βGeo). Here we report that treatment with a ketogenic diet elevates beta-hydroxybutyrate (BHB), an endogenous HDACi, and thereby modulates H3Ac and H3K4me3 in the granule cell layer of the dentate gyrus, with concomitant rescue of both the neurogenesis defect and hippocampal memory abnormalities seen in Kmt2d+/βGeo mice; similar effects were observed upon exogenous administration of BHB. These data suggest that dietary modulation of epigenetic modifications through elevation of BHB may be a feasible treatment strategy for the intellectual disability seen in Kabuki syndrome and related disorders.

2.6

Multiple Mechanisms for CRISPR-Cas Inhibition by Anti-CRISPR Proteins

Award Type: Early Independence Award

Award Year: 2015

Awardees: Joseph Bondy-Denomy, UCSF

Presenter: Joseph Bondy-Denomy

The battle for survival between bacteria and the viruses that infect them (phages) has led to the evolution of many bacterial defense systems and corresponding phage-encoded antagonists. Previously, we identified the first examples of proteins produced by phages that inhibit bacterial CRISPR-Cas immune systems. Thus far, twelve distinct genes have been identified that inactivate the Type I-F or I-E systems of Pseudomonas aeruginosa, but their mechanism of action is unclear. Here, we show that three different Type I-F anti-CRISPR (ACR) proteins operate through unique mechanisms. Biochemical experiments revealed that two ACR proteins bind to distinct regions of the Type I-F CRISPR-Cas (Csy) complex, utilizing different Cas proteins as binding partners. Binding of either of the two ACR proteins to the Csy complex inhibits the ability of the complex to bind to dsDNA targets through distinct mechanisms. A third ACR formed a stable interaction with Cas3, preventing the recruitment of this effector nuclease/helicase protein to the Csy complex. Experiments in P. aeruginosa revealed that by inhibiting the recruitment of Cas3, this ACR protein could repurpose the endogenous CRISPR-Cas system into a transcriptional repressor. These studies represent the first in vivo and biochemical analyses of proteins that modulate the activity of a CRISPR-Cas system and provide insight into Type I CRISPR-Cas mechanisms. These data also imply the independent evolution of different anti-CRISPR proteins and highlights the strong evolutionary pressures imposed on phages by CRISPR-Cas systems.

2.7

Expansion Microscopy: Towards Comprehensive in Situ Biomolecular Imaging

Award Type: Pioneer Award

Award Year: 2013

Awardees: Ed Boyden, MIT

Abstract Author(s): Ed Boyden, MIT

Presenter: Ed Boyden

For centuries, lenses have been used to magnify images of biological specimens. Recently, our group discovered that it was possible to physically magnify biological specimens -- such as a cell line, a brain circuit, or a human tumor biopsy -- by embedding the specimen in a dense swellable polymer, anchoring key biomolecules or labels to the polymer, mechanically homogenizing the sample, and then swelling the polymer so that anchored molecules are moved apart from one another. We call this method expansion microscopy, or ExM (Science 347(6221):543-548), and it is rapidly gaining adoption because it enables the imaging of 3-D samples with nanoscale precision, in a scalable fashion, on conventional optics. Here I discuss another advantage of ExM: by moving all the anchored molecules apart from one another, it creates room around the anchored molecules, thus enabling new kinds of in situ biochemical assay to be performed, in an in vitro-like environment. We have been working to enable in situ sequencing of DNA and RNA to be performed in expanded samples (in collaboration with the Church lab at Harvard), to enable multiplexed hybridization against nucleic acids to be performed for multiplexed RNA readout (in collaboration with the Raj lab at UPenn), and to enable multiplexed proteomic readout to be performed. In combination with new expansion microscopy chemistries that enable physical magnification of ~20-fold in linear dimension, as well as chemistries which are extremely easy to apply by end users, ExM may help fulfill the need for a technology suite that enables comprehensive and simultaneous genomic, epigenomic, transcriptomic, and proteomic imaging throughout cells and tissues, for the systematic mapping of fundamental building blocks and mechanisms in complex biological systems.

2.8

A Small Molecule Mimic of the Kinase Suppressor of Ras Phenotype Antagonizes MAPK Complexes and Signaling

Award Type: New Innovator Award

Award Year: 2013

Awardees: Arvin Dar, Icahn School of Medicine at Mount Sinai

Presenter: Arvin Dar

Kinases catalyse the phosphorylation of target substrates on hydroxyl-containing residues as a means to nucleate multi-component complexes or to stabilize unique conformational states. Pseudokinases constitute a subclass of these enzymes that were originally predicted as inactive based on mutations of key conserved active site residues. Like the active kinases, pseudokinases appear to play important functional roles in signal transduction pathways and several pseudokinases have been shown to be important mediators of diseases, including cancer and diabetes.

Many pseudokinases share homology inside and outside of the kinase domain with catalytically active kinases. Examples include Kinase Suppressor of Ras (KSR1/2), the pseudokinase relatives within the RAF-family of kinases. And Her3, the pseudokinase member of the EGFR family. In both examples, the pseudokinase has been demonstrated to allosterically stimulate the activity of their active ancestral partners through the formation of higher-order complexes. The ability to allosterically modulate active signaling kinases appears to be a dedicated feature of several pseudokinases that overlaps or appears redundant with the ability of active kinases to modulate themselves. For example, KSR forms a high affinity dimer with RAF related kinases and this drives RAF catalytic activation. However, RAF may dimerize with itself to drive its own activation. Clinical inhibitors that bind to RAF are thought to exploit the ability of RAF to regulate itself, leading to activation, rather than inhibition, of RAF when dimeric. Similarly, Her3 dimerizes with EGFR through the formation of an asymmetric dimer, but EGFR appears able to form this dimer in the absence of Her3 as well. In this case, the clinical kinase inhibitor bosutinib, has been shown to function as an agonist through direct modulation of Her3-EGFR complexes.

Because pseudokinase function can be redundant with the scaffolding abilities of active kinases, it has been difficult to dissect their precise roles in biology using conventional genetic or biochemical techniques. Small molecules that could antagonize pseudokinase dependent activities would be valuable tools that could be used to functionally annotate the biology and therefore pharmacology of this class of proteins. In particular, direct small molecule antagonists would allow one to disable pseudokinase structure and function on very short timescales and could also provide new leads for therapeutic development. I will describe my laboratories work on developing small molecule modulators of KSR. In particular, I will describe biochemical and cell based approaches used to identify KSR antagonists and furthermore X-ray crystallographic analysis of a lead compound bound to a KSR associated complex.

2.9

Development of piRNAs for Target-Specific Methylation

Award Type: Transformative Research Award

Award Year: 2015

Awardees: Dana C. Dolinoy, University of Michigan School of Public Health

Abstract Author(s): Christopher Faulk, University of Minnesota Department of Animal Sciences

Presenter: Dana C. Dolinoy

Epigenetic changes to DNA are associated with age, disease, and environmental influences. Currently existing methods of modifying DNA methylation act in a global and non-specific manner. For example, existing targeted approaches rely on creating transgenic animals or gene therapy, and therefore face a difficult path for translation to the clinic. Thus, the goal of our 2015 Transformative Research Award is to develop gene-specific and easy to administer technology for targeted epigenetic manipulation. Specifically, our project seeks to develop a suite of tools, based on the Piwi-interacting RNA (piRNA) system, to accurately induce DNA methylation of targeted loci in adult tissues, applicable to all mammals. PIWI-interacting RNAs (piRNAs) represent a fascinating adaptive mechanism and a potentially "ready made" tool for innovation in gene-specific repression. Originally piRNAs and their associated proteins were thought to be expressed only in germ cells, however, mounting evidence finds the expression of piRNAs in somatic tissues as diverse as brain and kidney in animals from sea slug to mouse to macaque. Activating the piRNA pathway in adult mammals holds promise in closing the gap between basic research and human application. The crucial difference in silencing by piRNAs compared to widely used miRNA/siRNA treatment is that piRNAs offer sensitive sequence specificity and induce DNA methylation. We will use this class of RNA to develop the technology to target specific genes and loci for stable, mitotically heritable, silencing at pre-determined genomic locations. While much is known about global silencing by piRNAs in germ cells, much less is known about their activity in adult somatic tissues. Our studies will validate their use as a technological platform for targeted epigenetics in any gene for all mammalian species. Choice of model organism is critical in the testing of potential epigenetic therapeutics. For these studies, we will use the Agouti viable yellow mouse, which varies in coat color concomitantly with DNA methylation at a single locus. Induced methylation targeted to the Avy transposon will provide direct visual semi-quantitative evidence of systemic molecular silencing at this locus. Additional experiments will verify the site specificity and degree of silencing. Further, we will adapt the piRNA suppression system in the soma to target genic regions as well as transposons. The research generated will provide sorely needed evidence clarifying the roles and activity of piRNA in somatic tissues of mammals and will be used to develop piRNA targeted methylation for the wider research and therapeutic communities.

2.10

Elucidating in Vivo Regulation of Adipocyte Stem Cell Activity

Award Type: New Innovator Award

Award Year: 2010

Awardees: Brian Feldman, Stanford University

Abstract Author(s): Brian Feldman, Stanford University

Presenter: Brian Feldman

A great deal of our understanding of the process of adipogenesis has come from in vitro studies on cultured cell lines and adipose stromal fractions, focusing on the differentiation of committed preadipocytes into mature lipid-laden adipocytes. These studies have provided a wealth of knowledge, including the elegant characterization of a cascade of mostly transcription factors that propels preadipocytes through the differentiation process into mature adipocytes. However, there is still a large knowledge gap in our understanding about the systemic factors that trigger the adipogenesis cascade in vivo. We have identified a paracrine and endocrine relay system regulating adipocyte stem cell activity in vivo. Using these in vivo approaches, we discovered that this pathway connects systemic changes with the cell intrinsic differentiation program in endogenous adipocyte stem cells by functioning as a toggle switch regulating a differentiation versus proliferation decision. We found this toggle switch coordinates the adipose tissue response to physiological changes in dietary cues. Finally, we discovered that this signal is present and functionally conserved in humans, indicating relevance for physiological adipose tissue homeostasis and the pathogenesis of obesity.

2.11

DNA Variants that Are Reported as Pathogenic for Arrhythmogenic Cardiomyopathy Are Highly Prevalent and Show Minimal Association with Heart Disease: A Study in 31,036 Participants Who Underwent Opportunistic Whole Exome Sequencing

Award Type: Early Independence Award

Award Year: 2012

Awardees: Brandon K. Fornwalt, MD, PhD, Geisinger Health System

Abstract Author(s): Brandon K. Fornwalt, MD, PhD, Geisinger Health System

Presenter: Brandon K. Fornwalt, MD, PhD

Background Arrhythmogenic cardiomyopathy (ACM) is a primary heart disease with a partially elucidated genetic etiology. The American College of Medical Genetics and Genomics has recommended clinical follow-up of incidentally identified ACM variants that are classified as pathogenic or likely pathogenic. This study sought to characterize the prevalence of ACM-associated variant-carriers and their related electronic health record phenotypes in a population of unselected patients undergoing whole exome sequencing.

Methods The MyCode™ Biorepository of 31,036 whole-exome sequences was reviewed to identify individuals with a variant reported as pathogenic or likely pathogenic for ACM in any of 8 genes. A total of 227 (0.73%) subjects met this criterion. Electronic health records data for this group were compared to controls (matched 5:1) lacking known ACM variants.

Results The prevalence of "known" pathogenic or likely pathogenic variants in genes associated with ACM was 0.73%. None of the 227 subjects identified with a pathogenic or likely pathogenic variant had a clinical diagnosis of ACM despite an average of 12 years of longitudinal electronic health record data. The collective phenotype of the variant-positive cohort had few differences compared to a control group lacking known ACM genetic variants, while demonstrating significantly less disease compared to a group of confirmed ACM cases (Figure 1).

Conclusions The prevalence of reported pathogenic or likely pathogenic variants associated with arrythmogenic cardiomyopathy exceeds reported disease prevalence by an order of magnitude. In variant-positive subjects, the electronic health record showed no ACM diagnoses and little evidence of heart disease, suggesting that the positive-predictive value for classic ACM in individuals with incidental positive genetic findings is low. Targeted clinical phenotyping of variant-positive individuals is needed to investigate subclinical disease.

2.12

Characterizing the Metabolic Stress Response in Hepatocellular Carcinoma Cells Surviving Severe Ischemia Using Dynamic Nuclear Polarization Carbon-13 MR Spectroscopy

Award Type: Early Independence Award

Award Year: 2015

Awardees: Terence P. Gade, Penn Image-Guided Interventions Lab, University of Pennsylvania,

Abstract Author(s): Terence Gade, University of Pennsylvania

Presenter: Terence P. Gade

Purpose: Cancer cells have demonstrated the ability to adapt their defining growth program to heterogeneous and often harsh microenvironments. Herein, we examine the associated alterations in hepatocellular carcinoma cellular metabolism that enable their survival under severe ischemia, an essential first step in the development of targeted therapies.

Materials and Methods: Growth and cell cycle kinetic profiles of cells cultured from a rat model of hepatoma (HCC) were studied under standard and ischemic conditions (1% O2, 1% serum, 1 mM glucose). Protein and mRNA expression of the metabolic stress response (MSR) was measured by Western blot and quantitative polymerase chain reaction, respectively. Metabolic alterations were measured with in vitro Carbon-13 nuclear polarization DNP-NMRS nuclear magnetic resonance spectroscopy of perfused cells under ischemia and standard growth conditions.

Results: HCC cells surviving ischemia demonstrated a 38% increase in quiescent cell fraction compared to standard conditions (p=0.0001). Surviving cells showed activation of the MSR on Western blot analysis. This was underscored by in vitro NMRS demonstrating metabolic alterations including abrogation of lipogenesis and protein synthesis as well as a 4-fold increase in glycolytic flux measured as conversion of pyruvate to lactate(p<0.05). Surviving cells under perfusion were found to have a more than 5-fold reduction in ATP production and a 16-fold reduction in oxygen utilization as well as a 50% decrease in glycolytic flux using DNP-NMRS (p<0.05).

Conclusion: Cells surviving severe ischemia demonstrated altered cell cycle kinetics with activation of the MSR and associated metabolic alterations. In vitro DNP-NMRS enabled real time imaging of ischemia-induced alterations in cellular metabolism.

2.13

Safety Signal Learning as a Novel Mechanism for Fear Reduction during Adolescence

Award Type: Early Independence Award

Award Year: 2015

Awardees: Dylan G. Gee, Weill Cornell Medical College

Presenter: Dylan G. Gee

Frontoamygdala circuitry and the ability to extinguish fear undergo dynamic changes across normative development. Translational studies in mice and humans have demonstrated a period of diminished cued fear extinction during adolescence, raising the question of whether adolescents may benefit from efforts to optimize fear reduction through novel mechanisms that bypass prefrontally-mediated extinction processes. Rodent studies have shown that safety signals effectively reduce anxiety to threat and prevent the development of new fears. Because they provide a context for the conditioned stimulus (CS), safety cues may rely on hippocampal projections to frontoamygdala circuitry and thus be particularly useful during adolescence. The present fMRI study examined the development of safety signal learning across childhood and adolescence (7-17 years old). Though children and adolescents both showed robust hippocampal activation to the CS paired with the safety cue, only adolescents showed increased prefrontal activation and downregulation of amygdala reactivity that was associated with behavioral evidence of safety learning. Our findings suggest that safety signals may be a powerful way to reduce fear during this developmental window. This study is expected to have important implications for optimizing treatments for anxiety in youth based on the biological state of the developing brain.

2.14

Fully Implantable, Soft, Stretchable Optoelectronics Systems for Wireless Optogenetics

Award Type: Transformative Research Award

Award Year: 2012

Awardees: Robert W. Gereau, Washington University School of Medicine; John A. Rogers, University of Illinois, ; Michael R. Bruchas, Washington University,

Abstract Author(s): Daniel S. Brenner, Washington University

Presenter: Robert W. Gereau

The introduction of optogenetics, a technique that allows rapid and temporally specific optical control of neuronal activity via targeted expression and activation of light-sensitive proteins, has dramatically accelerated the process of mapping complex neural circuits and determining their function. Traditionally, optogenetics has required remote light sources and fiber optic delivery schemes that impose significant physical constraints on natural behaviors and thus limit utility in typical animal behavioral studies. Even recently described wireless, tether-free systems demand rigid fixtures and external components that mount to mechanically stable skeletal features (e.g. the skull) in configurations that leave fragile electrical devices vulnerable to physical damage and also limit access to non-cranial regions of the anatomy. Here, we report technologies that combine soft, compliant neural interfaces with fully-implantable, stretchable wireless power and control systems to achieve chronic optogenetic modulation of nearly any region of the nervous system including the spinal cord and peripheral nerves, in freely behaving animals. Engineering design options range from stretchable appliques that interface directly with peripheral nerves to conforming filaments that insert into the narrow confines of the mouse spinal epidural space. Behavioral studies demonstrate the utility of these devices in the modulation of pain behavior, and provide evidence for their widespread use in neuroscience research and potential clinical applications of optogenetics outside the brain.

2.15

Promoting Bone Formation through the SHN3 Pathway

Award Type: Early Independence Award

Award Year: 2015

Awardees: Matthew Greenblatt, Weill Cornell Medical College

Presenter: Matthew Greenblatt

Half of all women will experience a skeletal fracture due to osteoporosis, or low bone mass, during their lifetime, with approximately the same number of women dying each year from osteoporotic fractures as die from breast cancer. To address this clinical need, we must ultimately identify new molecular pathways that regulate the differentiation and bone formation capacity of osteoblasts, the sole bone forming cell lineage.

We have discovered that the adaptor protein Schnurri-3 (SHN3, also HIVEP3) is an inhibitor of bone formation, as SHN3-deficient mice display a progressive increase in bone mass due to augmented osteoblast activity. Subsequently, we identified that SHN3 controls bone mass and osteoblast differentiation by suppressing the ability of activated ERK to phosphorylate a subset of it substrates. These findings suggest that inhibiting the SHN3 pathway is a promising approach to treat metabolic bone disorders.

Here, we report advancing our understanding of SHN3 function in several key areas. First, we demonstrate that SHN3 deficient mice show a relative protection in the murine ovariectomy model of post-menopausal osteoporosis, suggesting that inhibition of the SHN3 pathway is likely to have utility in treating common forms of osteoporosis. Next, we have confirmed that SHN3 function is intrinsic to osteoblasts, as conditional deletion of a Shn3 alelle in osteoblasts recapitulates the germline null allele phenotype, and SHN3-deficient osteoblast precursors adoptively transferred to secondary hosts display increased mineralization capacity. Lastly, we report that SHN3 acts in osteoblasts to regulate skeletal vasculogenesis, which may be an important component of the overall ability of SHN3 to promote bone formation. This functional interface between osteoblasts and endothelial cells may provide new therapeutic opportunities to promote bone formation.

2.16

High-Performance Control of Powered Prosthetic Legs with Human-Inspired Phase Variables

Award Type: New Innovator Award

Award Year: 2013

Awardees: Robert Gregg, University of Texas at Dallas University of Texas Southwestern Medical Center,

Abstract Author(s): Robert Gregg, University of Texas at Dallas, University of Texas Southwestern Medical Center

Presenter: Robert Gregg

The gait cycle is typically viewed as a periodic sequence of discrete events, 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 and exoskeletons, which almost universally switch between several distinct control modes throughout the gait cycle. However, this methodology is not robust to perturbations that push the gait cycle forward or backwards, preventing active prostheses from responding in harmony with the human user. Instead of discretely representing the phases of gait, a continuous representation could parameterize a nonlinear control strategy for larger portions, or the entirety, of the gait cycle. In particular, the concept of a mechanical phase variable has been widely successful in controlling the progression of leg joints in dynamic walking robots. However, it is unclear what phase variables, if any, can robustly represent the phase of human locomotion. This talk will share a recent perturbation study with 10 able-bodied human subjects, observing a mechanical variable that robustly parameterizes leg joint patterns over the entire gait cycle (with correlation coefficients between 0.95 and 0.997). A unified prosthetic leg control strategy is then designed around this phase variable to synchronize prosthetic joint patterns with the location of the human body. The clinical viability of this approach is demonstrated by experiments with human amputee subjects walking on a powered knee-ankle prosthesis at variable speeds.

2.17

Detection and Functional Characterization of Prion-Like Protein Self-Assembly

Award Type: Early Independence Award

Award Year: 2011

Awardees: Randal Halfmann, Stowers Institute for Medical Research

Abstract Author(s): Randal Halfmann, Stowers Institute for Medical Research

Presenter: Randal Halfmann

Prion-like switches have been proposed to functionally encode molecular memories and to transduce cellular signals. We seek to explore the breadth of biological effects mediated by prion-like self assembly and to decipher the rules that govern it. We do so by investigating prion-like proteins from two extremes of conformation space: intrinsically disordered regions commonly involved in gene regulation, and globular death domains involved with mammalian innate immunity and programmed cell death. Our findings with these proteins establish a general role for prion formation in cell fate determination. First, we have discovered that prions formed by certain low complexity transcription factors in budding yeast act as environmentally-responsive epigenetic determinants of multicellularity. We have further found that the different growth forms produced by prion switching exhibit frequency-dependent fitness interactions that drive primitive metabolic divisions of labor. Second, we are revealing a class of mammalian death domain superfamily proteins that function via prion formation to commit cells to the execution of pre-programmed signaling responses. Finally, we have developed a powerful new method that enables high throuput detection and quantification of prion-like self assembly, which we are now using to explore the full breadth of prion-mediated processes and uncover the rules that govern them.

2.18

Decoding Water Dynamics and Interaction Landscape of Proteins

Award Type: New Innovator Award

Award Year: 2011

Awardees: Songi Han, UCSB, Dept of Chemistry and Biochemistry, UCSB, Dept of Chemical Engineering,

Presenter: Songi Han

My group invented Overhauser dynamic nuclear polarization (ODNP)-amplified NMR relaxometry of water protons for highly localized measurements of hydration dynamics on the surface and interface of biological systems. Interest in the functional role of water in its interactions with biological surfaces, though deep and longstanding, has often been subject of contention, due in part to the lack of experimental techniques that can directly measure the dynamic surface water landscape of dilute biomolecular systems under ambient solution conditions and with site- and surface-specificity— precisely the operating condition and capability of ODNP 1H-relaxometry. After developing the ODNP methodology from the ground up, by building a dual, electron and nuclear spin magnetic resonance instrumentation, we applied this methodology to gain insight into the early stages of protein fibrilization.

Amyloid fibril formation is a key process accompanying many neurodegenerative diseases, where oligomers formed at the early stages of aggregation have been thought to play a key role in disease effects, but their studies are challenging. We employ site-specific measurements of surface water diffusion, protein segmental dynamics and inter-strand packing to track the process of early tau protein aggregation. Our study reveals that tau aggregation is accompanied by a dramatic conformational transformation within minutes of initiating aggregation, followed by the formation of dynamic and diffusible, yet partially structured, oligomer assemblies and their gradual rearrangement into stable aggregate species with b-sheet signatures. Our findings suggest that therapeutic intervention to fibril formation may focus on disrupting earliest aggregation events in the solution state.

2.19

Transduction of Mechanical Force in a Tension Free Membrane

Award Type: New Innovator Award

Award Year: 2013

Awardees: Scott B Hansen, The Scripps Research Institute, Dept. of Molecular Therapeutics, Jupiter FL 3345

Presenter: Scott B. Hansen

Mechanosensation provides two of the 5 fundamental senses integral to human sensation-touch and hearing. Surprisingly the seemingly simple sense of "touch" lacks a satisfactory molecular description. How do molecules in a cell sense and transduce mechanical force. Mechanical force appears to perturb the plasma membrane, but the identity of the molecules accelerated by force and the molecular basis for transferring this energy into a biological signal remains unclear. We propose a lipid mixing theory of mechanosensation where lipids spontaneously partition laterally in the plasma membrane to form ordered lipid micro domains and mechanical force disrupts the ordered domains causing mixing and the release of the micro domain content. We show disruption of raft lipids generates signaling molecules leading to ion channel activation and mechano transduction. We conclude ordered lipid domains are integral sensors in the mechano transduction pathway.

2.20

Next-Generation Targeted Proteomics Reaches Single-Cell Resolution

Award Type: New Innovator Award

Award Year: 2010

Awardees: Amy E. Herr, Ph.D., University of California, Berkeley

Presenter: Amy E. Herr, Ph.D.

Biology is becoming quantitative. Technology advances have driven a genomics revolution with sweeping impact on our understanding of life processes. Yet, when researchers and clinicians seek to measure unmodified, endogenous proteins, the immunoassay remains the de facto standard. While certainly powerful in assays ranging from ELISA to immunohistochemistry to mass cytometry, numerous protein targets lack an antibody probe with adequate specificity. Insufficient immunoprobe specificity translates into less-than-ideal assays for protein isoforms, complexes, and even multiplexing (owing to background cross-reactivity). To this end, I will detail our New Innovator efforts to introduce new microengineering design strategies for critical multi-stage protein assays -- where selectivity is enhanced by concatenating multiple independent assays. New tunable photopatterned materials for switchable function will be described, as will microfluidic architectures for seamless integration of discrete stages, and multiplexed readouts for quantitation. As specific case studies, I will discuss a spectrum of translational measurements my group is making possible: from near-patient diagnostics for HIV confirmation to biomarker validation of cancer signaling isoforms to single-cell Western blotting of pathway activation during neural stem cell differentiation. Performance and operational gains will be discussed, as well as a forward-looking view on where the tools can make an impact. Ultimately, our work infuses protein measurement advances into the biological and biomedical sciences.

2.21

Versatile Protein Tagging in Cells Using Split Fluorescent Protein

Award Type: New Innovator Award

Award Year: 2010

Awardees: Bo Huang, Department of Pharmaceutical Chemistry, UCSF

Presenter: Bo Huang

To label proteins in live mammalian cells, we developed two small epitope tags based on self-complementing split fluorescent proteins. The two tags, GFP11 and sfCherry11 were derived from the 11th β-strand of super-folder GFP and sfCherry . The small size of FP11-tags enables their cloning-free insertion into endogenous genomic loci via CRISPR-mediated homology-directed repair, making it possible to generate libraries of mammalian cells with fluorescently tagged proteins. Tandem arrangement FP11-tags allowed proportional enhancement of fluorescence signal in tracking intraflagellar transport particles, or reduction of photobleaching for live microtubule imaging. Finally, we showed the utility of tandem GFP11-tag in scaffolding protein oligomerization. These experiments illustrated the versatility of FP11-tag as a labeling tool as well as a multimerization-control tool for both imaging and non-imaging applications.

2.22

The Meadow Jumping Mouse: A Novel Hibernation Model

Award Type: Early Independence Award

Award Year: 2015

Awardees: William Israelsen, UT Southwestern Medical Center

Presenter: William J. Israelsen

Hibernating mammals exhibit great metabolic flexibility. They transition from a lean summer baseline to a severely obese state in preparation for hibernation. Then, during hibernation in cold climates, they enter a state of metabolic torpor and profound hypothermia and begin a months-long fast. The reversible transition from lean to obese in hibernators may hold lessons for human obesity, and an understanding of how hibernators slow their metabolic rate by >95% may lead to advances in surgery, organ storage, or emergency and battlefield medicine. However, the mechanisms controlling these metabolic changes are not well understood, and past efforts have been limited by shortfalls of existing model organisms. The goal of this research has been to establish the meadow jumping mouse (Zapus hudsonius) as a convenient laboratory model of hibernation. Meadow jumping mice are true hibernators; they have a short generation time and can be induced to fatten up and hibernate regardless of outside season. We have established a breeding colony using wild-caught meadow jumping mice and demonstrated control of the hibernation phenotype in the laboratory. Work to sequence the meadow jumping mouse genome is ongoing, and we expect that current experimental efforts will yield new information about the genetic and molecular bases of metabolic regulation during hibernation.

2.23

Characterization of Matrix Metalloproteinases in Cavitary Lesions in a Tuberculosis Murine Model

Award Type: Transformative Research Award

Award Year: 2015

Awardees: Sanjay K. Jain, Johns Hopkins University School of Medicine

Abstract Author(s): Alvaro A. Ordonez*, Rokeya Tasneen*, Supriya Pokkali, Paul J. Converse, Mariah Klunk, Eric L. Nuermberger#, Sanjay K. Jain#

Presenter: Sanjay K. Jain

Mice are widely used to study the pathogenesis of tuberculosis (TB) and efficacy of therapeutic agents. However, standard mouse strains do not develop caseous necrosis or cavities, key features of human TB associated with transmission, poor treatment outcomes and resistance. The mechanism of matrix destruction resulting in cavitation is not well defined but matrix metalloproteinases (MMPs) are thought to play a significant role. Occasional observations of cavities in mice prompted us to use high resolution computer tomography (CT) to evaluate cavity formation and characterize it further.

C3HeB/FeJ mice were aerosol infected with Mycobacterium tuberculosis. A cohort of these animals received combination drug treatment with rifampin, isoniazid and pyrazinamide to evaluate the effects of treatment on cavity formation. Live M. tuberculosis-infected mice were imaged within a sealed bio-containment bed to comply with biosafety level-3 (BSL-3) containment. Each animal was weighed and imaged using a NanoSPECT/CT small animal imager. Images were reconstructed using VivoQuant 1.23. After imaging, mice were sacrificed to collect tissues for histology and immunohistochemistry staining.

Initial pulmonary bacterial burden (day 1 post-infection) was 2.00 ± 0.13 log10 colony forming units (CFU). At 2-4 weeks post-infection, consolidations were observed by CT in the lung fields of infected animals. In some animals the central area of these consolidations underwent liquefaction and was evacuated to form a cavity within the lesions. Cavitation was evident by CT and confirmed by gross pathology and histology in 61% of treated and 60% of untreated M. tuberculosis-infected mice. CT imaging and subsequent post-mortem histological analyses clearly demonstrated that the necrotic contents of the liquefying granuloma are expelled into the airways, as in human TB. Macrophages (CD11b+) were immunoreactive for MMP-7 and MMP-9 in the cavity wall. MMP-7 and MMP-9 activity co-localized with infected lung areas and decreased with therapy.

Serial pulmonary CT imaging was able to non-invasively identify cavity formation in live M. tuberculosis-infected mice, and provided novel insights into the pathogenesis of cavity formation. This is the first systematic demonstration of cavity formation and MMPs driven matrix remodeling in a mouse model of TB. C3HeB/FeJ mice display key pathological features of human TB and could therefore serve as an important preclinical model for the development of novel therapeutics.

*there 2 authors contributed equally, #these 2 authors contributed equally.

2.24

Protein-based Molecular Memories in Gene Regulation, Disease, and Development

Award Type: New Innovator Award

Award Year: 2015

Awardees: Daniel Jarosz, Stanford University

Presenter: Daniel Jarosz

During their lifetimes, individuals commonly experience transient changes in gene expression as a result of different environmental stimuli. These responses are classically thought to have no heritable influence once they decay. However, we have recently discovered that such environmental stimuli frequently induce self-perpetuating changes in protein conformations. This occurs most commonly in proteins that regulate information flow: transcription factors and RNA binding proteins. These self-templating changes in protein conformation can be broadly defined as 'prions,' although their structures do not usually match the cross-beta sheet amyloids of the archetypical prion PrP. However, like known prions, corresponding changes in protein function are heritable from one generation to the next without any change to the genome. In this sense, such protein-based inheritance represents and extreme form of epigenetics. We have begun to identify and characterize these epigenetic elements in diverse eukaryotic proteomes and investigate their influence on disease, development, and evolution. Our results provide a mechanistic understanding of a form of inheritance that is quasi-Lamarckian in character, but firmly rooted in a Darwinian framework of mutation and natural selection. Lessons learned will also provide much needed insight into mechanisms of pathological and beneficial protein aggregation alike, and how they can be modulated therapeutically.

2.25

Direct Gradient Photolithography of Photodegradable Hydrogels with Patterned Stiffness Control with Sub-Micron Resolution

Award Type: New Innovator Award

Award Year: 2011

Awardees: Andrea M. Kasko, University of California, Los Angeles

Presenter: Andrea M. Kasko

Cell response to matrix mechanics is a well-recognized phenomenon; however, the ability to spatially pattern matrix stiffness with a high degree of control has been difficult to attain. This study describes the use of maskless photolithography as a flexible process for direct, non-contact gradient patterning of photodegradable hydrogels with custom graphics. Any input gray scale image can be used to directly chart hydrogel crosslink density as a function of spatial position. Hydrogels can be patterned with submicron resolution, where length-scales within a single substrate span several orders of magnitude. A quantitative relationship between input grayscale image pixel intensity and output gel stiffness is validated, allowing for direct gradient patterning. Such physical gradient hydrogel constructs are rapidly produced in a highly controlled fashion with measured stiffness ranges and length-scales that are physiologically relevant. Mechano-sensitive cell lines can be cultured on these physical gradient matrices where their behavior can be observed in a highly controlled and repeatable environment. Mesenchymal stem cells cultured on these physical gradients matrices congregate and align orthogonal to the gradient direction along iso-degraded lines. This approach results in a robust and high throughput platform to answer key questions about cell response in heterogeneous physical environments.

2.26

DNA-Origami Templated Membrane Structure and Dynamics

Award Type: New Innovator Award

Award Year: 2014

Awardees: Chenxiang Lin, Yale University

Presenter: Chenxiang Lin

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 vesicular transport processes and mediate complex reactions that are vital to cell division, growth and death. Inspired by such elegance in nature, bioengineers and synthetic biologists have aspired to build artificial membranes to mimic the vesicular transport machineries. 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, fission, etc.) in a programmable way.

Here we present our lab's recent effort (in collaboration with William Shih lab and James Rothman lab) to resolve these technical limitations. Our approach is to use self-assembled DNA nanostructures as templates to guide the assembly of lipid bilayers and the membrane associating proteins, and transduce the programmable feature of the DNA nanostructures to the templated vesicles. First, we show our ability to manufacture DNA-templated vesicles with defined size (Figure 1) and shape. Second, we present a programmable DNA-origami platform that organizes SNAREs for membrane fusion.

2.27

Life, Death, and Function: The Primate-Specific Long Non-Coding RNA Transcriptome

Award Type: New Innovator Award

Award Year: 2014

Awardees: Leonard Lipovich, Wayne State University

Abstract Author(s): Leonard Lipovich, Wayne State University

Presenter: Leonard Lipovich

BACKGROUND. The post-genomic era has changed our view of RNA. Not a mere messenger in the "Central Dogma of Molecular Biology" flow of information from DNA to protein, RNA is now known to have essential cellular roles independent of protein-coding potential. Less than one-third of the approximately 60,000 human genes are protein-coding. Traditionally, protein-coding genes conserved in evolution were thought responsible for most functional outcomes in normal cells and disease. LncRNA genes lack sequence conservation, even between closely related species. This motivates my disruptive, paradigm-shifting question: Do lncRNAs contribute to the molecular basis of primate uniqueness, and to the tantalizing question of what it means to be human? Is human cancer, to an extent, a primate-specific disease caused by non-conserved lncRNAs? Testing my hypothesis that non-conserved lncRNAs regulate cell growth and cell death, this project profiles a major nuclear hormone receptor pathway in human breast cancer: the estrogen receptor pathway. Estrogen, a proliferative hormone, turns on proliferative, and turns off cell-death-inducing, genes upon binding and activating its receptor, a transcription factor that undergoes nuclear translocation.

RESULTS. In the human MCF7 estrogen receptor alpha (ERα) positive cell line, a key breast cancer model, we identified 127 estrogen-regulated lncRNAs. Knockdown of 17 estrogen-induced and overexpression of 11 estrogen-repressed lncRNAs reduced cell growth and increased cell death, as expected. These lncRNAs exhibited primate-specific exonic sequences, splice sites, and polyadenylation signals. Knockdown of our top estrogen-induced lncRNA killed cancer but not normal cells, implying that targeting a primate-specific lncRNA may leave normal tissue unharmed. Our top estrogen-repressed lncRNA reduced ERK phosphorylation in ERα positive but not negative cancer cells. Hence, non-conserved lncRNAs may regulate the conserved MAP kinase cancer pathway. We used Stellaris RNA-FISH to show this lncRNA as cytoplasmic. Are long non-coding RNAs really non-coding? We were the first to answer this question, documenting rare translation of lncRNAs in human cells (Bánfai et al 2012, Genome Research). By protein mass spectrometry in estrogen-stimulated and unstimulated MCF7 cells, we now for the first time show hormone-responsive and hormone-independent translation of 58 lncRNAs. Ribo-seq experiments to validate ribosome association are underway, and we are applying third-generation full-length Pacific Biosciences RNAseq to the estrogen-responsive lncRNAome.

CONCLUSION. We reveal primate-specific RNA genes that contribute to breast cancer pathogenesis in humans. These lncRNAs represent novel therapeutic targets that, unlike driver mutations in conserved protein-coding genes, are unlikely to be critical to the health of normal cells.

2.28

Constructing Mechanosensitive Vesicles as Artificial Platelets

Award Type: New Innovator Award

Award Year: 2014

Awardees: Allen Liu, University of Michigan/Department of Mechanical Engineering University of Michigan/Department of Biomedical Engineering, University of Michigan/Cell and Molecular Biology

Abstract Author(s): Kenneth Kwun Yin Ho, University of Michigan/Department of Mechanical Engineering

Presenter: Allen Liu

The field of synthetic biology has recently emerged as a result of achieving a critical mass in our knowledge of biology. While many biological molecules and systems are still too complex to be rationally designed de novo, the continued efforts in isolation and characterization of individual biological components offer the possibility of integrating them into biologically inspired devices that exhibit novel functionalities. As a first step in this direction, we seek to construct artificial platelets using lipid bilayer vesicles as a platform and emulating the activation of natural platelets by reconstituting the externalization of phosphatidylserine (PS) to the external membrane leaflet, a key signature of activated platelets. We have developed a mammalian cell free expression system that is encapsulated in double emulsion droplets generated using capillary droplet microfluidics. Using this combination of techniques, we are generating vesicles containing mechanosensitive channels of large conductance (MscL) in which an increase in membrane tension due to shear stress in the blood would trigger MscL opening. We have devised a microfluidic micropipette array device for characterizing the mechanosensitive vesicles. With the activation of MscL, we hope to reconstitute SNARE-mediated calcium dependent membrane fusion to allow PS exposure, which will subsequently trigger thrombin generation and clot formation.

2.29

Genome-Scale Screens for Toxoplasma Gene Function using CRISPR/Cas9

Award Type: Early Independence Award

Award Year: 2013

Awardees: Sebastian Lourido, Whitehead Institute

Abstract Author(s): Saima M Sidik, Whitehead Institute

Presenter: Sebastian Lourido

Apicomplexans encode vast numbers of completely uncharacterized genes. Their distance from other eukaryotic lineages makes homology to model organisms difficult and often misleading. Despite the wealth of molecular tools available to manipulate apicomplexan genomes, the lack of RNAi in these organisms has precluded high-throughput functional genetic screens. We recently established the use of CRISPR/Cas9 as a flexible and efficient tool to edit the Toxoplasma gondii genome. In this system, the Cas9 nuclease is targeted by a small guide RNA (sgRNA) with 20 bp of homology to the gene of interest. The resulting double stranded DNA break is typically repaired by non-homologous end-joining in T. gondii, frequently disrupting the targeted gene. In mammalian cells, pooled screens have been performed using libraries of sgRNAs targeting each gene in the genome. Depletion of certain mutants can be tracked by using the sgRNAs as barcodes. We have built a library that targets each of the ~8200 genes of T. gondii with 10 different sgRNAs per gene. By introducing this library into Cas9-expressing parasites and monitoring the population over time, we determine the contribution of each gene to T. gondii replication in human fibroblasts. We also demonstrate the power of this approach in a positive selection screen using FUDR, which accurately identifies loss of UPRT as a mechanism of drug resistance. Our work reveals the utility of this technology in investigating the function of parasite genes and presents the first comprehensive functional analysis of any apicomplexan genome.

2.30

Cryo-EM Reveals a Novel Octameric Integrase Structure for ß-Retroviral Intasome Function

Award Type: Early Independence Award

Award Year: 2015

Awardees: Dmitry Lyumkis, The Salk Institute for Biological Studies

Abstract Author(s): Allison Ballandras-Colas, Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Department of Medicine, Harvard Medical School

Presenter: Dmitry Lyumkis

22. Retroviral integrase (IN) catalyzes the integration of viral DNA (vDNA) into host DNA, which is an essential step in the lifecycle of all retroviruses. Prior structural characterization of IN-vDNA complexes, or intasomes, from the spumavirus prototype foamy virus (PFV) revealed a functional IN tetramer, and it is generally believed that intasomes derived from other retroviral genera will employ tetrameric IN. However, the intasomes of orthoretroviruses, which include all known pathogenic species, have not been characterized structurally. Using single-particle cryo-electron microscopy (cryo-EM) and X-ray crystallography, we determine here an unexpected octameric IN architecture for the β-retrovirus mouse mammary tumor virus (MMTV) intasome. The structure is composed of two core IN dimers, which interact with the vDNA ends and structurally mimic the PFV IN tetramer, and two flanking IN dimers. Contrary to the belief that tetrameric IN components are sufficient to catalyze integration, the C-terminal domains (CTDs) of the flanking IN dimers interact with the vDNA and contribute to IN strand transfer activity. The IN octamer solves a conundrum for the α- and β-retroviruses by providing critical CTDs to the core intasome structure that cannot be provided in cis due to evolutionarily restrictive catalytic core domain (CCD)-CTD linker regions. The octameric architecture of the MMTV intasome provides a new paradigm for the structural basis of retroviral DNA integration.

2.31

Multiscale Approaches to Map Oxidative Stress

Award Type: New Innovator Award

Award Year: 2014

Awardees: Brent Martin, University of Michigan

Presenter: Brent Martin

Aberrant oxidative signaling is perhaps one of the most important factors contributing to aging, neurodegeneration, heart disease, diabetes, and cancer. Cysteine S-nitrosation and S-sulfination are naturally occurring post-translational modifications (PTMs) induced by redox stress. My group has found that sulfinic acids and nitrosothiols react to form a stable thiosulfonate bond. We have now leveraged this reactivity with sulfinate-linked probes to enrich and annotate hundreds of endogenous S-nitrosated proteins. In parallel, S-nitrosothiol-linked probes enable enrichment and detection of a similar number of endogenous S-sulfinated proteins, demonstrating a direct, bi-directional method to profile select redox cysteine modifications. Through a multi-step quantitative mass spectrometry assay, we assigned proteins as targets of both nitrosative and/or oxidative damage, and identified privileged biochemical pathways selective for each modification from cells and tissue homogenates. In addition, I will present a new methodology for the selective enrichment of sulfinic acids based on orthogonal alkylation reagents. Finally, I will present a new class of ratiometric fluorescent probes for live-cell imaging and 19F-NMR of protein sulfenylation in vivo, and describe a simple solution to bypass aldehyde cross-reactivity common to current methods. Despite the central role of oxidative stress in human health, our ability to study the precise mechanisms of such modifications has been hampered by a lack of selective chemical and analytical methods. In this presentation I will present recent progress that directly addresses this gap, and provides a series of chemical approaches for in-depth annotation of the in vivo targets of oxidative and nitrosative stress.

2.32

3-D Printed Nano-Bionic Organs

Award Type: New Innovator Award

Award Year: 2014

Awardees: Michael McAlpine, University of Minnesota

Presenter: Michael McAlpine

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.

2.33

The Foundations and Repair of Cognitive Control in Human Brain Networks

Award Type: Early Independence Award

Award Year: 2015

Awardees: John D. Medaglia, University of Pennsylvania

Presenter: John D. Medaglia

Cognitive control is essential to numerous daily functions ranging from playing sports to maintaining conversations. Cognitive control is exquisitely sensitive to neurological and mental disorders. Innovations in network neuroscience are rapidly clarifying the properties of brain structural and functional networks that underlie cognitive control, but major frontiers remain. In particular, the mechanisms of cognitive control in the human brain are poorly understood. Understanding these mechanisms at the level of brain networks is a particularly salient challenge with broad-reaching translational implications. Here, we hypothesize that cognitive control relies on dynamical mechanisms akin to networks studied in engineering, and we leverage recent developments in network control theory to provide a mechanistic basis of human cognitive control in large-scale structural brain networks. We describe our research involving the integration of structural, functional, and cognitive data using novel behavioral and brain stimulation paradigms. We conclude by describing a new framework designed to scale these findings from basic science to applied translational strategies for cognitive dysfunction.

2.34

RTK Bypass Resistance Requires Complimentary Pathway Reactivation

Award Type: Early Independence Award

Award Year: 2014

Awardees: Aaron S. Meyer, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Tech

Presenter: Aaron S. Meyer

The efficacy of receptor tyrosine kinase (RTK)-targeted therapies is limited by the development of resistance. Combination therapies targeting resistance mechanisms can considerably improve effectiveness, but require identification of the relevant mechanism at hand. A common form of resistance is bypass signaling wherein RTKs not targeted by an inhibitor can direct reactivation of pathways essential for survival. While this mechanism of resistance is well appreciated, overcoming it with combination therapies will require methods to prognostically identify which receptor is driving resistance. Here, we apply a combined experimental- and modeling-based approach attempting to identify a set of pathway reactivation essential for RTK-mediated bypass resistance. Differences in the complement of signaling provided by particular RTKs means that these receptors differ qualitatively in the ability to confer resistance despite commonalities in the pathways essential for survival. These results provide necessary information for the precise prognostic design of therapeutic combinations and for evaluating therapeutic efficacy.

2.35

Inhibition of Fatty Acid Oxidation Modulates Immunosuppressive Functions of Myeloid-Derived Suppressor Cells and Enhances Cancer Therapies

Award Type: Transformative Research Award

Award Year: 2014

Awardees: Augusto C. Ochoa, Louisiana State University Health Sciences Center, New Orleans, Louisiana

Presenter: Amir A. Al-Khami

Myeloid-derived suppressor cells (MDSC) promote tumor growth by inhibiting T cell immunity and supporting malignant cell proliferation and migration. The therapeutic potential of blocking MDSC in tumors has been limited by their heterogeneity, plasticity, and resistance to various chemotherapy agents. Recent studies have highlighted the role of energy metabolic pathways in the differentiation and function of immune cells; however, the metabolic characteristics regulating MDSC remain unclear. We aimed to determine the energy metabolic pathway(s) used by MDSC, establish its impact on MDSC function, and test whether its inhibition blocks MDSC and enhances anti-tumor therapies. Using several murine tumor models, we found that tumor-infiltrating MDSC (T-MDSC) increased fatty acid uptake and activated fatty acid oxidation (FAO). This was accompanied by an increased mitochondrial mass, upregulation of key FAO enzymes, and increased oxygen consumption rate. Pharmacological inhibition of FAO blocked immune inhibitory pathways and functions in T-MDSC and decreased their production of inhibitory cytokines. FAO inhibition alone significantly delayed tumor growth in a T cell-dependent manner and enhanced the anti-tumor effect of adoptive T cell therapy. Furthermore, FAO inhibition combined with low-dose chemotherapy completely inhibited T-MDSC immunosuppressive effects and induced a significant anti-tumor effect. Interestingly, a similar increase in fatty acid uptake and expression of FAO-related enzymes was found in human MDSC in peripheral blood and tumors. These results support the possibility of testing FAO inhibition as a novel approach to block MDSC and enhance various cancer therapies.

2.36

Next-Generation Drug Discovery

Award Type: New Innovator Award

Award Year: 2011

Awardees: Brian Paegel, The Scripps Research Institute

Presenter: Brian Paegel

The NIH Molecular Libraries Program (MLP) was founded to translate the discoveries of the Human Genome Project into therapeutics. With gene sequences (and thereby target identities) in hand, the only obstacle to discovery was access to high-throughout screening (HTS) technology. A decade of discovery produced hundreds of probes — highly selective small molecules that modulate cellular function — but the Genome Project's promise of proteome-wide drug discovery remains out of reach because centralized compound screening bears the same cost and infrastructure burdens of millennial DNA sequencing centers. We are building a next-generation drug discovery platform to eliminate the need for HTS centers. We have developed DNA-encoded solid-phase synthesis to generate ultra-miniaturized compound libraries and engineered microfluidic instrumentation for miniaturizing automated screening. An integrated circuit loads individual microscopic compound library beads into picoliter-scale droplets of assay reagent. Compound attached to the bead via photolabile linker is released into the droplet in a UV dose-dependent fashion. The dosed droplets are then incubated, evaluated for activity using laser-induced confocal fluorescence detection, and sorted for PCR amplification and high-throughput sequencing. To demonstrate the feasibility of the platform, we synthesized a modest (~100k compounds) DNA-encoded library of aspartic protease inhibitors, developed an HIV protease activity assay, and demonstrated high-throughput dose-response screening. Not only are the molecular libraries and screening technology deployable in any laboratory setting, but dose-response screening will generate whole-library structure activity relationship profiles. The unprecedented molecular detail of these data will yield portfolios of new leads and replenish the pipeline of therapeutics, especially those targeting rapidly-evolving bacterial and viral pathogens.

2.37

A Massively Parallel Model of Hemodynamics in the Human Circulatory System

Award Type: Early Independence Award

Award Year: 2014

Awardees: Amanda Randles, Duke University

Abstract Author(s): Amanda Randles, Duke University

Presenter: Amanda Randles

The potential impact of blood flow simulations on the diagnosis and treatment of patients suffering from vascular disease is tremendous. Empowering models of the full arterial tree can provide insight into diseases such as arterial hypertension and enables the study of the influence of local factors on global hemodynamics. We present a new, highly scalable implementation of the Lattice Boltzmann method which addresses key challenges such as multiscale coupling, limited memory capacity and bandwidth, and robust load balancing in complex geometries. We demonstrate the strong scaling of a three-dimensional, high-resolution simulation of hemodynamics in the systemic arterial tree on 1,572,864 cores of Blue Gene/Q. Faster calculation of flow in full arterial networks enables unprecedented risk stratification on a per-patient basis. In pursuit of this goal, we have introduced computational advances that significantly reduce time-to-solution for biofluidic simulations. In this talk, I will discuss the development of HARVEY, a parallel fluid dynamics application designed to model hemodynamics in patient-specific geometries. I will cover the methods introduced to reduce the overall time-to-solution and enable near-linear strong scaling on the IBM Blue Gene/Q supercomputer. Finally, I will present the expansion of the scope of projects to address not only vascular diseases, but also treatment planning and the movement of circulating tumor cells in the bloodstream.

2.38

"Bottom-up" Profiling of Interacting Cellular Systems

Award Type: New Innovator Award

Award Year: 2012

Awardees: Alex K. Shalek, IMES & Chemistry, MIT Ragon Institute, Broad Institute

Abstract Author(s): Alex K. Shalek, IMES & Chemistry, MIT, Ragon Institute, Broad Institute

Presenter: 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. Illustratively, for immune dendritic cells (DCs) and T cells – collectively responsible for recognizing pathogens and inducing adaptive immune responses – cellular subtype, signaling milieu, and physical contacts all impact the balance between proinflammatory and regulatory responses. 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.

Here, I will discuss our efforts to leverage recent advances in nanotechnology and biological chemistry to develop broadly applicable platforms for systematically manipulating and deeply profiling many interacting single cells so that we can dissect how they work from the "bottom-up". Using the mouse immune system as our model, we are developing five broadly-applicable, core, cross-disciplinary platforms to: (a) culture and monitor individual cells in isolation; (b) examine specific single cells within an ensemble; (c) perform targeted manipulations; (d) detect many different types of molecular entities in the same cell (e.g., RNA and protein); and, (e) profile, genome-wide, gene expression in many single cells, in vitro and ex-vivo. Collectively, we hope that our 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.

2.39

Tracking RNA Populations Using Efficient and Reversible Covalent Chemistry

Award Type: New Innovator Award

Award Year: 2014

Awardees: Matthew D. Simon, Yale University Chemical Biology Institute, Department of Mol Biochem & Biophys

Presenter: Matthew D. Simon

In order to track distinct RNA populations using reversible chemistry, we developed a chemical method to label and purify 4-thiouridine (s4U) -containing RNA. Methanethiolsulfonate (MTS) reagents form disulfide bonds with s4U efficiently, and are superior to the commonly used HPDP-biotin. MTS-biotin leads to high yields and enrichment of s4U-RNA allowing the use of s4U-labeling to study global miRNA turnover in proliferating system using cultured human cells, and without impacting global miRNA levels or inhibiting the miRNA processing machinery. This MTS-chemistry enhances various methods that depend on tracking different populations of RNA such as 4-thiouridine-tagging (TU-tagging) to study tissue-specific transcription and dynamic transcriptome analysis (DTA) for monitoring RNA turnover.

2.40

Synaptomes of Mouse and Man: High-Throughput Array Tomography Methods for Cortical Synapse Taxonomy

Award Type: Transformative Research Award

Award Year: 2014

Awardees: Stephen J. Smith, Allen Institute for Brain Science; Randal Burns, Johns Hopkins University,

Presenter: Stephen J. Smith

Many neurodevelopmental, psychiatric and neurodegenerative disorders are rooted in abnormalities of the brain's vast and diverse synapse populations. Unfortunately, such disorders are poorly understood and difficult to diagnose, prevent and treat because we lack adequate tools to measure these synapse populations, and because too many of today's limited tools can be applied only to experimental animals such as mice. A multidisciplinary consortium of investigators at the Allen Institute, Johns Hopkins, Duke, UNC, UCSF and UC Davis, supported by a Transformative Research Award, is developing synaptomic resources proposed here to answer needs for faster and more precise analysis of heterogeneous synapse populations in both humans and experimental animals to leverage the lessons from animal research much more effectively to the improvement of human mental health. One of our main goals is to develop a synapse taxonomies relating molecular and structural synapse diversity to diverse parent neuron types, and to use such taxonomies for systematic and quantitative comparison of mouse and human cerebral cortex.

Our experimental work is based on array tomography, a super-resolution histological imaging method that allows combination of highly multiplexed immunofluorescence microscopy with voxel-conjugate electron microscopy. We are using array tomography to measure synaptic proteins with single-synapse resolution in situ, while surveying local-circuit-scale volumes of human and mouse cortex comprising many millions of synapses. Here we report progress in automating and accelerating array tomographic image acquisition as needed to reliably resolve individual synapses over volumes large enough to encompass complete neuronal arbors. This, in turn, will allow us to begin relating synapse diversity to presynaptic and postsynaptic parent neuron diversity, in both mouse and human cortex, as a path toward cell-biologically principled synapse taxonomies.

2.41

Fluorescent Biosensors for Imaging Neurotransmitters: Observing Synapses in Action

Award Type: New Innovator Award

Award Year: 2014

Awardees: Lin Tian, University of California, Davis

Presenter: Lin Tian

One of the greatest challenges in neuroscience is to decipher the logic of the neural circuitry and link it to learning, memory, and behavior. Neural circuitry is a dynamic network that incorporates neuronal activity at a variety of spatial and temporal scales. Therefore, analysis of neural circuitry demands broad and dense sampling of neuronal activity across time and brain structures. Recent breakthroughs in modern microscope and protein based fluorescence sensors have brought this goal within reach. For example, application of genetically encoded calcium indicators, such as GCaMP family, combined with two-photon microscopy, has facilitated the large-scale recording of neural activity in a genetically-identified population at multiple time scales in awake, behaving animals. These applications have greatly advanced our understanding of the dynamics of neural circuitry and its control of behavior—a critical first step toward understanding complex brain function.

Building upon the momentum of calcium imaging, the immediate need to accelerate further analyses of the dynamics of neural circuitry is to develop a broader suite of optical sensors to expand the kinds of neuronal activity that can be measured. One particular area of interest is synaptic transmission, a critical event of information processing in the brain that is difficult to access with the optical tools currently available. There are two key questions that need to be addressed before we can develop a dynamic picture of synaptic transmission. First, we must understand how synaptic connectivity is linked to its activity; second, we must determine how different types of neurotransmitters balance with each other in a defined circuitry. Here we have developed two classes of novel protein-based fluorescent sensors to enable monitoring of synaptic transmission from these two different angles. A broad application of these sensors will enable neuroscientists to obtain a comprehensive view of both excitatory and inhibitory synapses in action at the cellular, tissue, and whole-animal level.

2.42

Elucidating Mechanisms of Vertebrate Limb Regeneration

Award Type: New Innovator Award

Award Year: 2015

Awardees: Jessica L. Whited, Harvard Medical School Brigham & Women's Hospital,

Presenter: Jessica L. Whited

Humans and other mammals have extremely limited regenerative capabilities in key parts such as limbs. Nearly two million Americans are currently living with the consequences of having undergone limb amputation due to injury or disease, and this number is expected to rise. Exceedingly few biological therapeutics have been devised to address this problem. For the ultimate goal of limb regeneration to become feasible, a blueprint for natural regeneration of a morphologically and functionally similar structure, composed of similar cell types, is a valuable resource. Such a blueprint exists with axolotl salamanders, whose limbs are anatomically similar to human limbs, but they can be completely regenerated throughout life. This regeneration has been known for centuries, but much of the mechanistic detail at the molecular level has remained elusive. We are taking an a priori approach to discovering the transcripts which fuel axolotl limb regeneration. Using single-cell RNA-seq and differential gene expression analysis, we have thus far identified transcripts which define three major cell types in the regenerating portion of the limb (wound epidermis, blastema/progenitor cells, and blood cells). We are now investigating the transcripts that distinguish cell subtypes within these broad classes, and we are mapping the expression of these transcripts across both limb regeneration and limb development. Simultaneously, we have investigated the function of particular factors, and we have identified some mechanisms underlying limb regeneration. Collectively, this project will provide a much-needed molecular and cellular framework for understanding total vertebrate limb regeneration, informing future hypotheses and investigation aimed at understanding why mammalian limb regeneration is so limited.

2.43

Genetic Engineering of Hematopoietic Stem Cells to Generate Invariant Natural Killer T Cells

Award Type: New Innovator Award

Award Year: 2014

Awardees: Lili Yang, University of California, Los Angeles

Abstract Author(s): Drake J. Smith, UCLA, MIMG Department, BSCRC

Presenter: Lili Yang

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 in vivo (~0.01-1% in mouse and human blood). Here, we report a new method to generate large numbers of iNKT cells in mice through T cell receptor (TCR) gene engineering of hematopoietic stem cells (HSCs). We showed that iNKT TCR-engineered HSCs could generate a clonal population of iNKT cells. These HSC-engineered iNKT cells displayed the typical iNKT cell phenotype and functionality. They followed a two-stage developmental path, first in thymus and then in the periphery, resembling that of endogenous iNKT cells. When tested in a mouse melanoma lung metastasis model, the HSC-engineered iNKT cells effectively protected mice from tumor metastasis. This method provides a powerful and high-throughput tool to investigate the in vivo development and functionality of clonal iNKT cells in mice. More importantly, this method takes advantage of the self-renewal and longevity of HSCs to generate a long-term supply of engineered iNKT cells, thus opening up a new avenue for iNKT cell-based immunotherapy.

2.44

Graphene as a Novel Tool for Cell Membrane Manipulation and Regulation of Neurotransmission

Award Type: New Innovator Award

Award Year: 2011

Awardees: Qi Zhang, Vanderbilt University

Presenter: Qi Zhang

Recent interdisciplinary advances have made possible new technologies for active control of neurotransmission down to the cellular level. New methods for precise synaptic manipulation would aid in furthering understanding of the integration of synaptic and cellular signaling processes. Advances in nanoscale materials fabrication have made possible new classes of materials well-suited for addressing this challenge. However, the biological effects of these materials are still poorly understood. We provide biophysical evidence that graphene interacts with plasma membrane cholesterol, through which it promotes presynaptic neurotransmitter release while sparing postsynaptic receptors. Using optical and electrical approaches, we further demonstrate that cholesterol-dependent increases in synaptic vesicle pool size, release probability, and recycling rate are the underlying mechanisms. We then demonstrate that this interaction may be leveraged to enhance transmembrane receptor signaling responses via membrane nanodomains. By bio-functionalizing graphene derivatives, it is thus possible to selectively manipulate different synaptic apparatuses, a first step toward achieving active regulation of neurotransmission at the synaptic level.

2.45

Cortical Hierarchy Underlies Preferential Connectivity Disturbances in Schizophrenia

Award Type: Early Independence Award

Award Year: 2015

Awardees: Alan Anticevic, Yale University

Abstract Author(s): Genevieve Yanh, Yale University

Presenter: Alan Anticevic

Schizophrenia (SCZ) is a neuropsychiatric illness associated with abnormal neural connectivity. In particular, patients show prefrontal cortex (PFC) hypo-connectivity, assessed by computing blood-oxygen level-dependent (BOLD) signal correlations. However, recent studies reveal elevated BOLD signal variance in SCZ, which may impact correlations, computed as covariance normalized by variance. We hypothesized that functional connectivity (using covariance) may be elevated in SCZ, but that this may occur in the context of elevated signal variance. Further, we hypothesized that preferential PFC effects may intrinsically arise from the information-processing hierarchy, with corresponding physiological consequences, which we tested via biophysically-grounded computational modeling.

We conducted resting-state fMRI in 161 SCZ and 164 matched healthy subjects, assessing group differences in connectivity and BOLD variance. Both voxel-wise and network-level analyses were performed. To mechanistically inform fMRI findings, we used a biophysically grounded neural network model to simulate a well-known synaptic hypothesis of SCZ pathology—namely excitation/inhibition (E/I) imbalance, and analyzed resulting in silico 'BOLD' signals.

Empirically, we observed hyper-connectivity in PFC and other associative regions in SCZ, with concurrent increases in BOLD variance. These effects were absent in a comparison group of bipolar patients (N=73). In initial simulations of increasing E/I imbalance, we observed global elevations in covariance and variance of model-generated BOLD signals. To investigate our empirical associative effects, we extended our model to reflect known differences in associative vs. sensory neuronal dynamics. This extended model reproduced preferential associative effects, and predicted that covariance and variance elevations would be positively correlated, which we confirmed empirically.

Collectively, we show that elevations in BOLD covariance and variance in chronic SCZ co-occur and are strongly related phenomena. Thus, some connectivity elevations may not be fully captured by correlation measures that normalize connectivity by variance. Hypo-connectivity seen in previous studies may be reconciled with our findings by considering the effect of elevated variance in reducing correlation-based measures. We also computationally demonstrate how a common cellular-level mechanism can produce elevations in covariance and variance of BOLD signals. Further, we show how this global perturbation can produce preferential effects in associative regions due to neural differences arising from the intrinsic functional cortical hierarchy.